**UNIVERSITY OF NAIROBI**

**COLLEGE OF PHYSICAL AND BIOLOGICAL SCIENCES**

**DEPARTMENT OF CHEMISTRY**

# PHYTOCHEMICAL INVESTIGATION OF THE STEM BARK AND THE LEAVES OF *TECLEA SIMPLICIFOLIA* FOR ANALGESIC ACTIVITY

**BY**

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**I56/63228/10**

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**2015**

## DECLARATION

I declare that this thesis is my original work and has never been presented elsewhere for examination, award of a degree or publication. In areas where other people's work has been used, it has been properly acknowledged and referenced in accordance with the University of Nairobi requirements.

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# DEDICATION

This thesis is dedicated to:

My parents Mr and Mrs. Charles Mugane,

My husband, Moses Karani and my daughter, Valerie Ikol.

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# LIST OF ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| COSY | Correlation Spectroscopy |
| HMBC | Heteronuclear Multiple Bond Correlation |
| HMQC | Heteronuclear Multiple Quantum Correlation |
| MHz | Mega Hertz |
| HZ | Hertz |
| J | Coupling constant |
| *s* | Singlet |
| *d* | Doublet |
| *t* | Triplet |
| TLC | Thin Layer Chromatography |
| PTLC | Preparative Thin Layer Chromatography |
| NMR | Nuclear Magnetic Resonance |
| NOESY | Nucleur Overhauser and Exchange Spectroscopy |
| NOE | Nucleur Overhauser effect |

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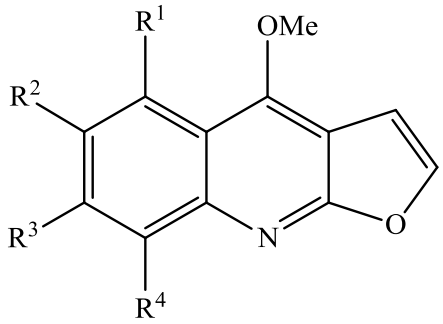
# ABSTRACT

Pain, acute or chronic, has been part of mankind throughout history; therefore, pain alleviation continues to occupy the minds of many health practitioners and researchers. The high cost and the scarcity of pain relieving drugs remains a challenge in many developing nations and hence herbal remedies have been used as alternatives. *Teclea simplicifolia* is one of the plants that have been used to treat pain in traditional medicine in Kenya, but no phytochemical studies have been conducted to determine the chemical constituents for analgesic activities. The aim of this study therefore, was to isolate and characterize secondary metabolites from the stem bark and the leaves of *Teclea simplicifolia* and determine their analgesic activities in Swiss albino mice.

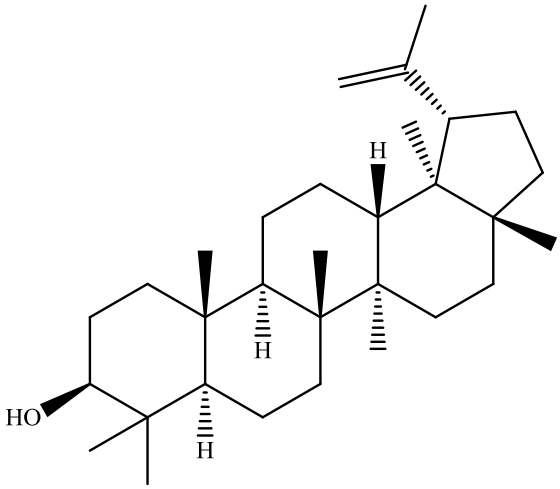
The stem bark and the leaves of *Teclea simplicifolia* were extracted with $CH_2Cl_2/CH_3OH$ (1:1) and tested for analgesic properties using the tail flick method on mice. The extracts showed significant activities, $p<0.05$. Chromatographic separation of the stem bark extract led to the isolation of five compounds. These were characterized using NMR ($^{1}H$, $^{13}C$, COSY, NOEDIFF, and NOESY) spectroscopy as the quinoline alkaloids, maculine (**1**), flindersiamine (**2)**, kokusaginine (**3)**, maculosidine (**4** ), 4,5,6,7-tetramethoxyfuro[2,3-b]quinoline **(5),** and the triterpene derivative, lupeol **(7**). Similar treatment of the leaves extract led to the identification of nobiline (**6)** and maculine (**1).**

The pure compounds, maculine and maculosidine were evaluated for the analgesic activity and showed significant activity ($p<0.05$) comparable to aspirin which is a mild pain killer. This study has therefore explained the use of *Teclea simplicifoilia* in traditional medicine for pain treatment.




| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| **1** | H | $OCH_2O$ | | H |
| **2** | H | $OCH_2O$ | | OMe |
| **3** | H | OMe | OMe | H |
| **4** | H | OMe | H | OMe |
| **5** | OMe | OMe | OMe | H |
| **6** | H |  | OMe | H |



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# CHAPTER ONE

## 1.0 INTRODUCTION

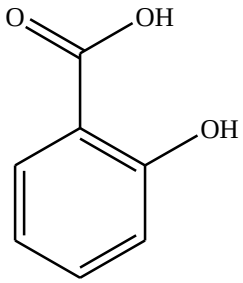
## 1.1 GENERAL

Medicinal plants have always been of great importance to mankind in preventing and curing of various diseases (Okpuzor *et al*., 2008). Globally, nearly three quarters of drugs are derived from plants (Mustaffa *et al*., 2010). In Sub-Saharan Africa, over 80% of the population depends largely on plant based medicine in meeting their basic health care needs (WHO, 2008). The heavy reliance on herbal remedies has increased due to resistance of microbial pathogens to the existing convectional drugs (WHO, 2014). This is mainly due to the fact that, when resistance is reported on first line antimicrobials, alternative second and third line drugs which are usually expensive have to be deployed (Aliero and Ibrahim, 2012). In the developing countries these alternative drugs are neither readily available nor affordable and thus medicinal plants have been used as replacements (Aliero and Ibrahim, 2012).

The medicinal properties of plants are due to chemical compounds that they synthesize. These chemicals, also referred to as secondary metabolites, are responsible for their diverse biological activities (Khan *et al*., 2011). Consequently, there is an increased focus on medicinal plants by pharmaceutical industry over the past decades as potential sources of lead compounds for drug development (Khan *et al*., 2011; Severino *et al*., 2011). Phytochemists recognize that these plant species contain diverse classes of natural products which possess various biological activities; the most abundant of which are alkaloids, flavanoids, tannins, glycosides, phenolic compounds and terpenoids (Severino *et al*., 2011).

Africa is highly endowed with medicinal plants; for example, in East Africa there are over 1,200 documented medicinal plants from a plant population of over 10,000 species (Kokwaro, 1976). The family Rutaceae is among the largest taxa of flowering plants. This family is widely distributed throughout the continent and is commonly used in traditional medicine (Kokwaro, 2009). Phytochemical studies carried out on this family indicate that it is highly rich in secondary metabolites (Rajkumar *et al*., 2014). Plants of the family Rutaceae are known to contain different classes of compounds of which quinoline and acridone alkaloids stand out (Kubitzki *et al*., 2011). In addition, species from this family have diverse biological activities such as larvicidal, antimicrobial, anti-oxidant, anti-allergic, antifeedant, anti-inflammatory, analgesic, antipyretic and anticancer (Tiwary *et al*., 2007; Negi *et al*., 2011; Peneluc *et al*., 2009).

Pain is one of the oldest medical conditions recognized and has been part of mankind throughout history (Bourke, 2011). Medicinal plants have been used for thousands of years in relieving pain (Stalker, 2013). For example, opiates derived from opium which is obtained from a dried extract of unripe seedpods of poppy plant (*Papaver somniferum*) have been used for centuries in treating pain (Bourland, 2011). In addition, the willow bark has been used to treat many different kinds of pain, such as rheumatic pain, back pain, toothache, headache, and menstrual cramps (Highfield and Kemper, 1999). The pain relieving activities have been associated with the chemical compounds present in these plants. In the 1800s salicylic acid (**8**), a plant metabolite from which aspirin is derived, was known to relieve pain (Stalker, 2013).The active substance morphine (**9**) present in opium, is a powerful painkiller and has been widely used in alleviating pain both moderate and severe cases since it is safe and effective (GAPRI, 2010). The main undermining factor of morphine is that it is addictive.

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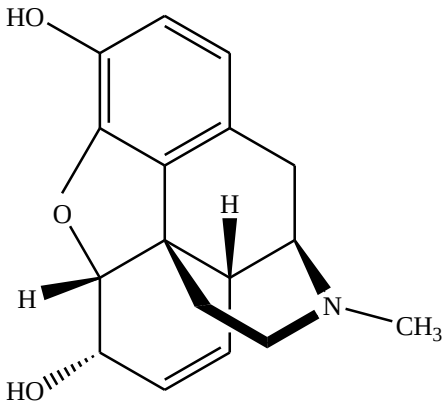


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*Teclea* species (Rutaceae) have widely been used to treat pain in many cultures (Adnan *et al*., 2001). The leaves and the stem bark of *Teclea nobilis*, are used in reduction of pain and fever in Ethiopia (Yenesew and Dagne, 1988), whereas, the leaves of *Teclea simplicifolia* are used by the Samburu community in Kenya for treating pain conditions (Beentje, 1994). Despite their wide use in treating painful conditions by different communities, a number of these species have not undergone even initial screening, leave alone detailed phytochemical investigations to determine their efficacy and toxicity levels (Midiwo *et al*., 2005). There is hence need for more studies to ascertain the use of these plants and their toxicity levels. It is in this light that this study aimed at conducting phytochemical and analgesic investigation on the stem bark and the leaves of *Teclea simplicifolia* currently referred to as *Vepris simplicifolia* (Breteller, 1995).

## 1.2 PROBLEM STATEMENT

Pain control is one of the greatest challenges that individuals continue to face in developing nations when seeking medical attention (Mercola, 2013). This is due to the fact that analgesic drugs remain scarce and inaccessible in these regions (Goltz *et al*., 2013). As result, approximately, 5 billion people living in developing nations have limited access to antipain

medicines; this number includes 5.5 million patients suffering from chronic ailments such as terminal cancer and HIV/AIDS (GAPRI, 2010). Over 2.9 million terminal cases are reported annually as a result of unrelieved pain (GAPRI, 2010). In order to mitigate these problems, there is need to develop alternative pain relieving drugs, that are cheap and readily available.

## 1.3 JUSTIFICATION

Plants are directly used as medicines by a majority of cultures around the world with over 80% of the world`s population continuing to rely heavily on herbal remedies, especially in Africa and Asia (WHO, 2008). In the modern society, plants have been a starting point for countless drugs used in the market today (Allison, 2006). Many researchers have shown that natural products from plants and other organisms have been the most promising source of lead structures in the development of new drugs (Allison, 2006). For example, analgesic drugs such as opiates that are derived from medicinal plants have been used for decades in the treatment of both severe and moderate pain. In addition, drugs such as morphine, derived from opium are powerful painkillers and used in the treatment of both acute and chronic pain (WHO, 2004b).

Plant species belonging to the genus *Teclea* have widely been used in treatment of pain in many cultures for example; *Teclea nobilis* is used in treating pain and fever in Ethiopia (Yenesew and Dagne, 1988). The use of these species in pain treatment has been supported by the analgesic studies of some species that have shown significant activity with no cytotoxic effects (Adnan *et al*., 2001; Mascolo *et al.,* 1988). These properties have been associated with the presence of quinolines alkaloids in these species. The leaves of *Teclea simplicifolia* have been reported to contain quinolines alkaloids but no study has been done to determine whether these quinolines

could have analgesic activity (Wondimu *et al*., 1988). Therefore, the crude extracts of the stem bark and the leaves of *Teclea simplicifolia* as well as the isolated compounds were tested for analgesic activities in this study.

## 1.4 OBJECTIVES

### 1.4.1 General objective

To isolate and characterize secondary metabolites from the stem bark and the leaves of *Teclea simplicifolia* and determine their analgesic activities in swiss albino mice.

### 1.4.2 Specific objectives

The specific objectives of this study were to:

1. Determine the analgesic activities of the crude extracts of the stem bark and the leaves of *Teclea simplicifolia* in Swiss albino mice;
2. Isolate and characterize the chemical constituents of the stem bark and the leaves of *Teclea simplicifolia;*
3. Establish the analgesic activities of the isolated compounds in Swiss albino mice.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 PAIN

Pain is an unpleasant feeling that is triggered in the nervous system as a result of actual or potential tissue damage (Leknes and Tracey, 2010). Pain alleviation has therefore, preoccupied health care management and research for many years. It is mainly classified into two categories; acute and chronic pain. Acute pain can be intense but is short lived and is easier to control since medication and rest are often effective treatments; while chronic pain may resist treatment and prolong for years causing hopelessness and anxiety (Wells *et al*., 2008). There are many causes of pain and some of which include cuts, surgical procedures and chronic ailments. In chronic ailments such as cancer and HIV/AIDS, pain manifests itself as a second symptom after fever (WHO, 2004a).

According to the Global Access to Pain Relief Initiative (GAPRI, 2010), majority of patients suffer from unrelieved pain due to a number of barriers that prevent them from accessing proper pain treatment (GAPRI, 2010). One of the major barriers is the lack or high cost of pain relieving drugs (Goltz, *et al*., 2013). This problem is mainly encountered in developing nations, because internationally recommended pain relieving drugs are scarce and not easily accessible (Goltz *et al*., 2013). In addition, the current antipain drugs have side effects such as sedation, tolerance, physical dependence and gastrointestinal complications which can lead to gastric bleeding. This has led to seeking alternative treatment to counter these challenges. Medicinal plants have been one of the areas of interest because they are readily available and affordable in rural communities.

### 2.1.1 Pain management

Analgesics are mainly drugs that are used to manage pain (Jha, 2014). These drugs are classified into three: simple analgesics such as paracetamol, non-steroidal anti-inflammatory drugs and opioid analgesics. The mode of action of these drugs varies from one category to another because they target pains at different points along the pain pathway (Reddi *et al*., 2014). The exact mode of action for paracetamol has not been determined but it has been speculated that it acts centrally on the brain than peripherally on nerve endings (Jha, 2014). Aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) intercept the feeling of pain at the source (Reddi *et al*., 2014). This is because they inhibit the cyclo-oxygenase enzymes (COX-1 and COX-2) hence preventing arachidonic acid metabolism leading to a decrease in prostaglandin and thromboxane release (Kumar *et al*., 2014). Prostaglandins are responsible for induction of pain and inflammation. Opioid analgesics act at bonding receptor sites to the brain hence the signal of pain does not reach the brain (Chan, 2008). These are actually recommended in relieving severe pain even though they are addictive.

## 2.2 THE RUTACEAE FAMILY

Rutaceae is a family of flowering plants placed in the order Sapindales and is commonly referred to as Citrus or Rue family (Groppo *et al*., 2008). It is a large family consisting of 158 genera and 1,900 species that have diverse morphological features (Bayer *et al*., 2009). This family is largely known for its economic importance since many species are sources of foods, spices, essential oils, herbal medicines, horticultural items and pharmaceuticals (Ling *et al*., 2009). The plants in this family are distributed worldwide, mainly in tropical and temperate regions with a greater diversity in South Africa and Australia (Groppo *et al*., 2008). Most species are shrubs and trees, a few are herbs which are sometimes armed with spines and prickles. A distinct

characteristic of this family is the presence of glands containing aromatic oils on the stems, leaves, flowers and fruits (Beentje, 1994). Generally, the leaves are opposite and compound, while flowers mainly divide into four or five parts (Beentje, 1994).

The family is known for its extraordinarily array of secondary metabolites such as alkaloids, flavonoids, coumarins, limonoids and lignans (Groppo *et al*., 2008). It comprises of a wide range of alkaloids making it one of the most chemically versatile plant families (Price, 1963). One unique feature of the family is the elaboration of quinoline and acridone alkaloids derived from anthranilic acid and these are restricted to the Rutaceae (Kubitzki *et al*., 2011). The metabolites of this family possess a wide spectrum of biological activities with some of them having proven medically useful (Holmstedt *et al*., 1979; Moraes *et al*., 2003).

The classification within the family at the intra and infra generic level is complex and has undergone several changes. Traditionally, the family has been classified into three subfamilies i.e. the Rutoideae, the Toddalioideae and the Aurantioideae (Dagne *et al*., 1988). The changes in classification were based on morphological and chemical characteristic studies that have shown significant relationships within and among groups of its genera (Groppo *et al*., 2008). For example, close affinities between the genera of Rutoideae and Toddalioideae were observed and hence challenging the separation of these two subfamilies (Hartley, 2001). As a result, genera such as *Vepris* and *Teclea* have been shown to have close morphological characteristics and thus have been merged; therefore, species such as *Teclea simplicifolia* investigated in this study are now referred to as *Vepris simplicifolia*. In order to compare with previous phytochemical studies on *Teclea* species in this study the name *Teclea simplicifolia* has been retained.

## 2.3 THE GENUS *TECLEA*

*Teclea* is one of the genera that constitute the Rutaceae family (Beentje, 1994). About 30 species of *Teclea* are found in Africa the majority of which are trees and shrubs (Kokwaro, 1982). Six species are found in Kenya namely: *T. amaniensis, T. grandifolia, T. hanangensis, T. nobilis, T. simplicifolia, T. trichocarpa* (Beentje, 1994).

### 2.3.1 Botanical information on *Teclea simplicifolia*

*Teclea simplicifolia* is a shrub or medium-sized tree of 2-9 m (Kokwaro, 1982). It is an evergreen plant with a smooth bark, yellow-green flowers and orange or red fruits (Beentje, 1994). This plant is also widely distributed in the tropical Eastern Africa regions such as Kenya, Uganda, Ethiopia and Tanzania (Kokwaro, 1982). Fig 1 shows the picture of *Teclea simplicifolia* plant.

- *Fig 1:* Picture of *Teclea simplicifolia* (GreenPlantSwap, 2015)

## 2.4 ETHNOBOTANICAL USES OF *TECLEA* SPECIES

A number of *Teclea* species are widely used by various communities in treating a range of ailments. In Kenya, herbalists in the Akamba community use *T. trichocarpa* roots in the treatment of malaria (Mwangi *et al*., 2010); while in Ethiopia, the bark and leaves of *T. nobilis* are used as analgesics (Yenesew and Dagne., 1988). *Teclea simplicifolia* has several uses such as treatment of malaria by the Maasai community in Kenya, while the wood of the plant is used in making roof beams, walking sticks and bows (Beentje, 1994). Table 1 shows the ethno-medical uses of some *Teclea* species.

**Table 1: Ethnobotanical uses of some *Teclea* species**

| SPECIES | PLANT PART | AILMENT | REFERENCE |
|---|---|---|---|
| *T. trichocarpa* | Roots<br>Leaves | Malaria<br>Fever | Mwangi *et al*., 2010 |
| *T. nobilis* | Roots<br><br>Leaves<br>Stem bark | Rheumatism, athritis and pneumonia<br>Fever and malaria<br>Gonorrhea and pain | Kokwaro, 2009<br><br>Lacroix *et al*., 2012<br>Adnan *et al*., 2001 |
| *T. simplicifolia* | Bark and leaves<br>Leaves | Malaria and hepatitis<br>Pleurisy | Kokwaro, 2009 |
| *T. pilosa* | Bark | Heart pain | Kokwaro, 1993 |

## 2.5 BIOLOGICAL ACTIVITIES OF *TECLEA* SPECIES

There are various biological activities that have been reported from this genus. The essential oils of the leaves of *Teclea nobilis* showed significant analgesic and antipyretic activity in mice (Al-Rehaily, 2001). The crude extracts and lupeol isolated from *Teclea nobilis* also showed anti-inflammatory activity on rats without causing apparent deleterious effects (Al-Rehaily *et al*., 2001; Mascolo *et al*., 1988; Adnan *et al*., 2001). *Teclea trichocarpa* was reported to have

significant antiplasmodial, antifungal, antibacterial activities. Insect antifeedant activity against the African army worm (*Spodoptera exempta*) has also been reported for this plant (Muriithi *et al*., 2002; Lwande *et al*., 1983). The antiplasmodial, antibacterial and antifungal activities of maculine and kolbisine of *Teclea afzelii* have been documented (Wansi *et al*., 2010).

## 2.6 PHYTOCHEMISTRY OF *TECLEA* SPECIES

The genus *Teclea* has been reported to contain diverse classes of secondary metabolites such as quinolines alkaloids, acridone alkaloids, triterpenes, and flavonoid glycosides (Al-Rehaily *et al*., 2002).

### 2.6.1 Alkaloids

#### 2.6.1.1 Quinoline Alkaloids

Quinoline alkaloids belong to a class of alkaloids that have a bicyclic system, whereby a benzene and a pyridine ring are fused together; and a number of them can undergo prenylation and cyclization giving rise to furoquinoline alkaloids (Hoffman, 2003). Figure 2 shows the basic skeleton of quinoline alkaloids.

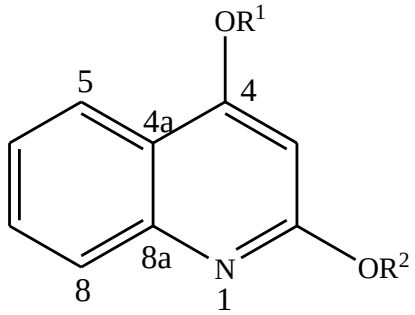
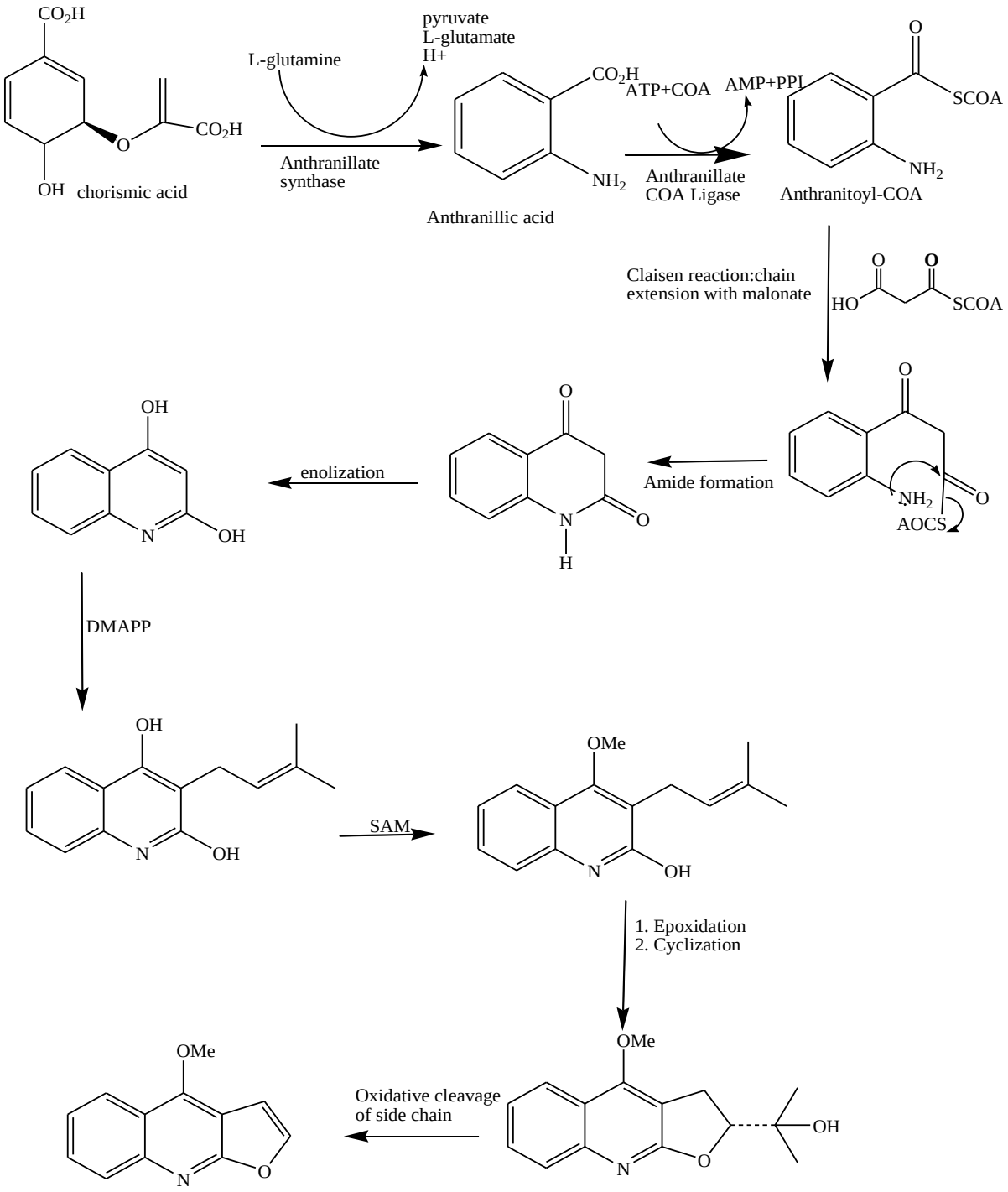



Fig 2: The basic structure of quinoline alkaloids

The majority of these alkaloids are known to occur in the Rutaceae family. Scheme 1 shows the biosynthetic process in which quinoline alkaloids are derived from chorismic acid.



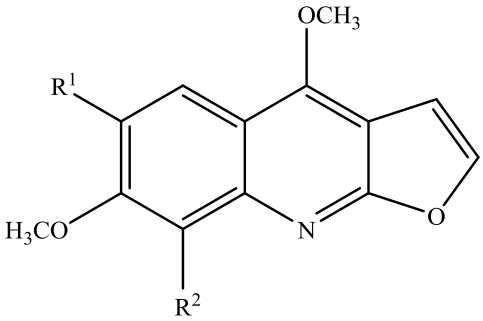

Scheme 1: Biosynthesis of Quinoline alkaloids (Cordell, 1981)

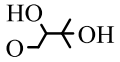
Table 2: Quinoline alkaloids isolated from various *Teclea* species

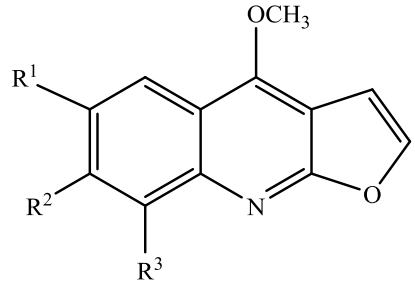
| NAME | SPECIES | PLANT PART | REFERENCE |
|---|---|---|---|
| Kokusaginine **(10**) | *T. afzelii* | Stem bark | Wansi *et al*., 2010 |
| | *T. nobilis* | Aerial parts | Al-Rehaily *et al*., 2003 |
| | *T. ouabanguiensis* | Stem bark | Ayafor and Okogun, 1982 |
| Skimmianine **(11)** | *T. nobilis* | Leaves | Yenesew and Dagne., 1988 |
| | *T. simplicifolia* | Leaves | Wondimu *et al*.,1988 |
| | *T. trichocarpa* | Leaves | Mwangi *et al*., 2010 |
| | *T. gerrardii* | Stem bark | Coombes *et al*., 2009 |
| Tecleanatalensine A (**12)** | *T. natalensis* | Leaves | Tarus *et al*., 2005 |
| Tecleanatalensine B (**13)** | *T. natalensis* | Leaves | Tarus *et al*., 2005 |
| Montrifoline **(14)** | *T. nobilis* | Leaves | Yenesew and Dagne., 1988 |
| | *T. simplicifolia* | Leaves | Wondimu *et al*., 1988 |
| | *T. afzelii* | Stem bark | Wansi *et al*., 2010 |
| | *T. ouabanguiensis* | Stem bark | Ayafor and Okogun, 1982 |
| Tecleabine (**15)** | *T. nobilis* | Aerial parts | Al-Rehaily *et al*., 2003 |
| Tecleoxine **(16)** | *T. nobilis* | Aerial parts | Al-Rehaily *et al*., 2003 |
| Isotecleoxine **(17)** | *T. nobilis* | Aerial parts | Al-Rehaily *et al*., 2003 |
| Methylnkolbisine (**18)** | *T. nobilis* | Aerial parts | Al-Rehaily *et al*., 2003 |
| Chlorodesnkolbisine (**19)** | *T. nobilis* | Aerial parts | Al-Rehaily *et al*., 2003 |
| Nobiline **(20)** | *T. nobilis* | Leaves | Yenesew and Dagne., 1988 |
| | | Aerial parts | Al-Rehaily *et al*., 2003 |
| Tecleaverdoornine **(21)** | *T. afzelii* | Stem bark | Wansi *et al*., 2010 |
| | *T. ouabanguiensis* | Stem bark | Ayafor and Okogun, 1982 |
| Maculine **(22)** | *T. nobilis* | Leaves | Yenesew and Dagne., 1988 |
| | *T. afzelii* | Stem bark | Wansi *et al*., 2010 |
| Flindersiamine (**23)** | *T. nobilis* | Leaves | Yenesew and Dagne., 1988 |
| | *T. natalensis* | Leaves | Tarus *et al*., 2005 |
| | *T. ouabanguiensis* | Stem bark | Ayafor and Okogun, 1982 |
| Tecleine **(24)** | *T. verdoorniana* | Stem bark | Ayafor and Okogun, 1982 |

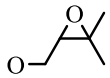
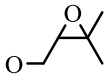
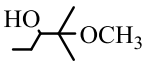
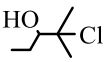
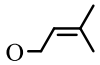
Table 2 continued…

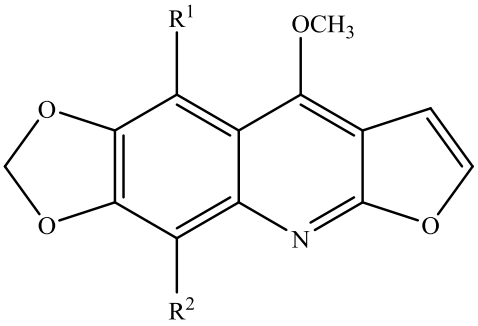
| NAME | SPECIES | PLANT PART | REFERENCE |
|---|---|---|---|
| Tecleaverdine **(25)** | *T. verdoorniana* | Stem bark | Ayafor and Okogun, 1982 |
| Tecleamine (**26)** | *T. ouabanguiensis* | Stem bark | Ayafor and Okogun, 1982 |
| Dictamnine **(27)** | *T. natalensis* | Leaves | Tarus *et al*., 2005 |
| Pteleine (**28)** | *T. nobilis* | Aerial parts | Al-Rehaily *et al*., 2003 |
| Ribalinine **(29)** | *T. nobilis*<br>*T. simplicifolia* | Leaves<br>Leaves | Yenesew and Dagne., 1988<br>Wondimu *et al*., 1988 |
| Isoplatydesmine (**30)** | *T. nobilis*<br>*T. simplicifolia* | Leaves<br>leaves | Yenesew and Dagne., 1988<br>Wondimu *et al*., 1988 |
| Edulinine (**31)** | *T. nobilis*<br>*T. simplicifolia* | Leaves<br>Leaves | Yenesew and Dagne, 1988<br>Wondimu *et al*., 1988 |
| Haplopine-3,3`-dimethylallyether (**32)** | *T. nobilis* | Aerial parts | Al-Rehaily *et al*., 2003 |
| Anhydroevoxine **(33)** | *T. nobilis* | Aerial parts | Al-Rehaily *et al*., 2003 |
| Evoxine (**34)** | *T. boiviniaana*, | Leaves | Vaquette *et al*., 1978 |
| Acetylmontrifoline **(35)** | *T. nobilis* | Fruits | Lacroix *et al*., 2012 |
| 8-[(3-methyl-2-butenyl)oxy]-4,7dimethoxyfuro[2,3-*b*]quinoline (**36)** | *T. natalensis* | Leaves | Tarus *et al*., 2005 |
| Kolbisine (**37)** | *T. afzelii* | Stem bark | Kuete *et al*., 2008 |

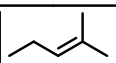
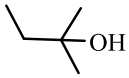


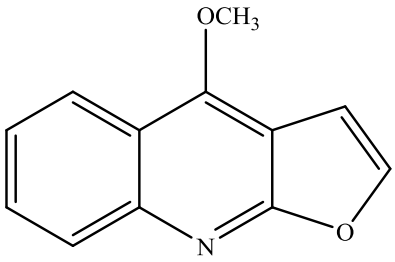
| | $R^1$ | $R^2$ |
|---|---|---|
| **10** | OMe | H |
| **11** | H | OMe |
| **12** |  | H |
| **13** |  | H |
| **14** |  | H |



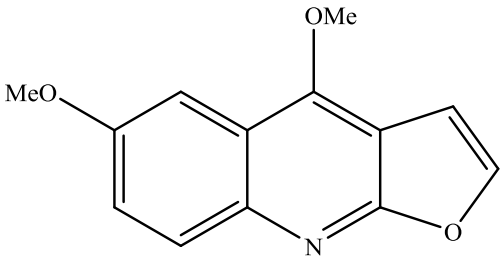
| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **15** | $OCH_3$ | H |  |
| **16** |  | $OCH_3$ | H |
| **17** | $OCH_3$ |  | H |
| **18** |  | $OCH_3$ | H |
| **19** |  | $OCH_3$ | H |
| **20** | $OCH_3$ |  | H |



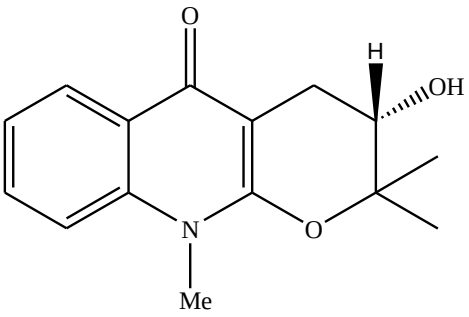
| | $R^1$ | $R^2$ |
|---|---|---|
| **21** |  | OH |
| **22** | H | H |
| **23** | H | OMe |
| **24** | H | OH |
| **25** |  | OH |
| **26** | H |  |



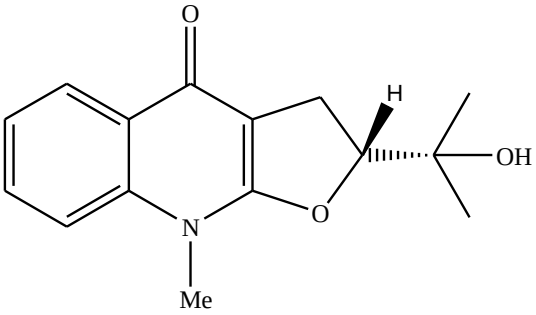
**27**



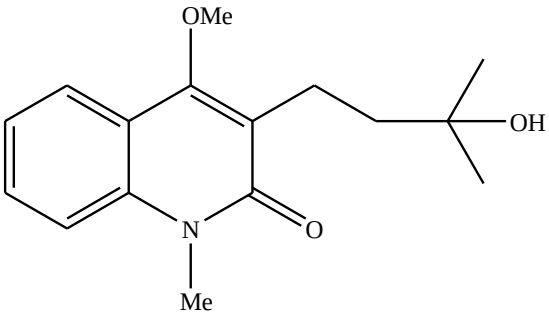
**28**



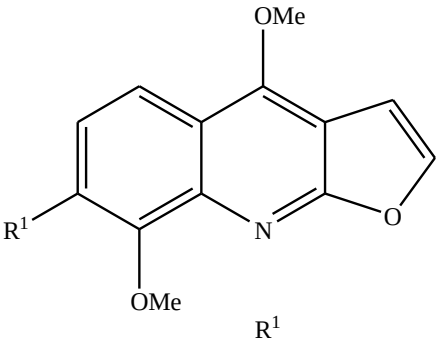

**29**

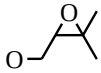
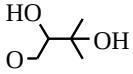


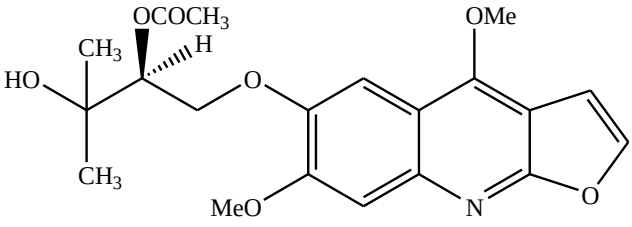

**30**



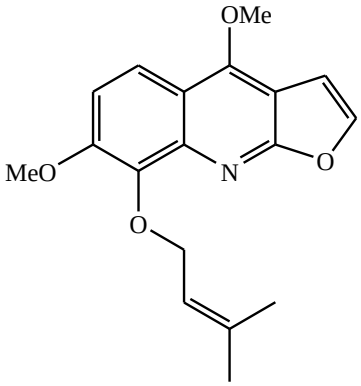

**31**



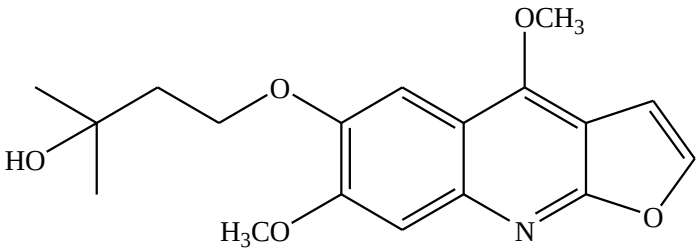

| | $R^1$ |
|---|---|
| **32** |  |
| **33** |  |
| **34** |  |




**35**




**36**




**37**

### 2.6.1.2 Acridone Alkaloids

Acridone alkaloids are mainly ketones of a parent tricyclic molecular-skeleton having an N-atom at the position-10 and a keto group at the position-9 (Tsassi *et al*., 2011). They are small group of alkaloids that are only known to occur in the Rutaceae family (Tsassi *et al*., 2011). These compounds possess a variety of biological activities such as antimalarial, antiviral, antibiotic and antitumor properties (Dos Santos *et al*., 2009; Gurrala *et al*., 2013). Acridone alkaloids are also known to resemble quinolines since they are both from a common biosynthetic precursor, anthranillic acid. Figure 3 shows the basic structure of acridone alkaloids (Gurrala *et al*., 2013).

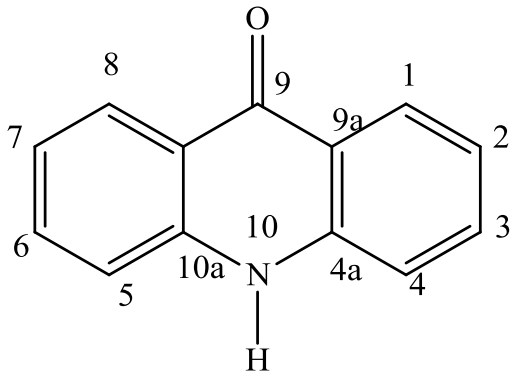
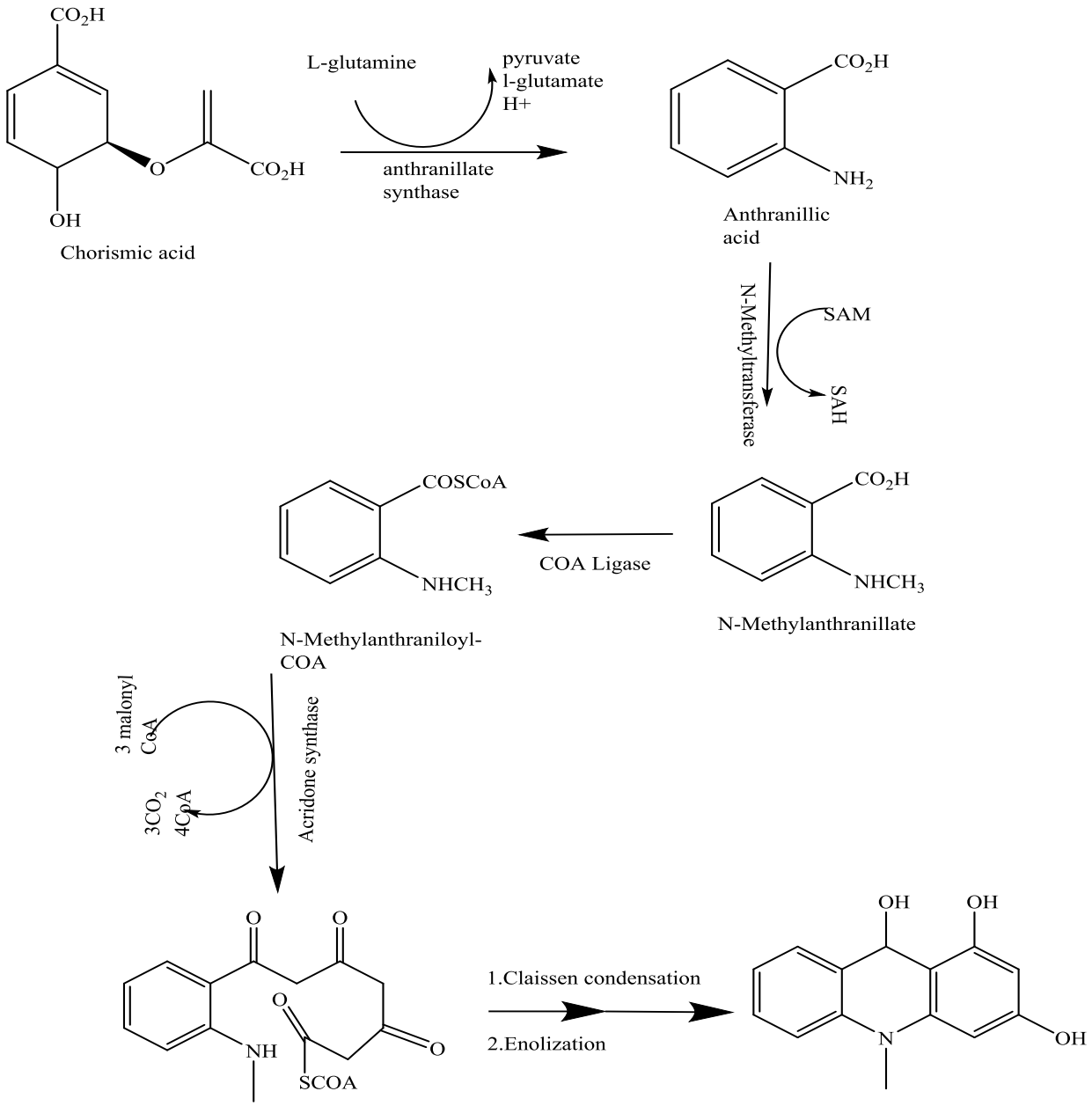



Fig 3: The basic structure of acridone alkaloids

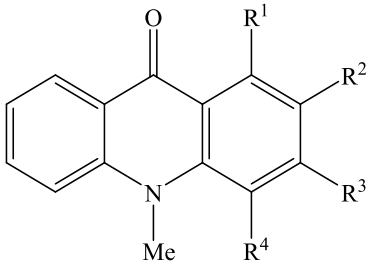
Scheme 2 below is a summary of the biosynthetic process through which acridone alkaloids are derived from chorismic acid.



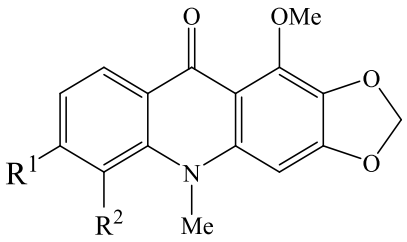

Scheme 2: Biosynthesis of acridone alkaloids (Maier *et al*., 1990)

Table 3: Acridone alkaloids isolated from *Teclea* species

| NAME | SPECIES | PLANT PART | REFERENCE |
|---|---|---|---|
| Melicopicine **(38)** | *T. trichocarpa* | Bark | Lwande *et al*., 1983 |
| | *T. gerrardii* | Fruit | Coombes *et al*., 2009 |
| | *T. natalensis* | Bark | Tarus *et al*., 2005 |
| 1,2,3-Trimethoxy-N-methylacridone (**39)** | *T. gerrardii* | Fruit | Coombes *et al*., 2009 |
| Normelicopicine **(40)** | *T. trichocarpa* | Leaves | Mwangi *et al*., 2010 |
| Arborinine (**41)** | *T. trichocarpa* | Leaves | Mwangi *et al*., 2010 |
| | *T. gerrardii* | Bark | Waffo *et al*., 2007 |
| | *T. natalensis* | Bark | Tarus *et al*., 2005 |
| Tegerrardin A (**42)** | *T. gerrardii* | Bark | Waffo *et al*., 2007 |
| Tegerrardin B **(43)** | *T. gerrardii* | Bark | Waffo *et al*., 2007 |
| Tecleanthine (**44)** | *T. boiviniaana* | Leaves | Vaquette *et al*., 1978 |
| | *T. natalensis* | Bark | Tarus *et al*., 2005 |
| 6 –Methoxy tecleanthine (**45)** | *T. boiviniaana,* | Leaves | Vaquette *et al*., 1978 |
| Evoxanthine (**46)** | *T. boiviniaana* | Leaves | Vaquette *et al*., 1978 |
| | *T. natalensis* | Bark | Tarus *et al*., 2005 |




| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| **38** | OMe | OMe | OMe | OMe |
| **39** | OMe | OMe | OMe | H |
| **40** | OH | OMe | OMe | OMe |
| **41** | OH | OMe | OMe | H |
| **42** | OMe | H | OH | H |
| **43** | O⁄=\ | H | OH | H |



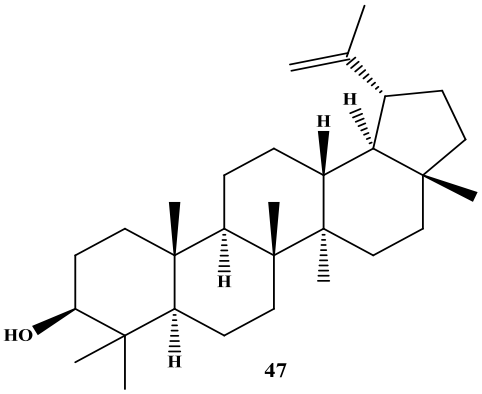

| | $R^1$ | $R^2$ |
|---|---|---|
| **44** | H | OMe |
| **45** | OMe | OMe |
| **46** | H | H |

### 2.6.2 Terpenoids

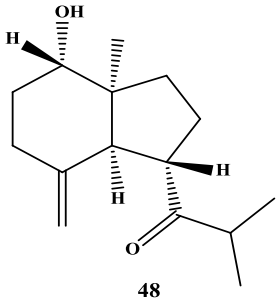
*Teclea* species have been reported to possess terpenoids mainly the triterpenoids and sesquiterpenes (Kuete *et al*., 2008). Sesquiterpenes are class of compounds that are made up of fifteen carbons atoms and are assembled from three isoprenoid units, while triterpenoids contain thirty carbon atoms. Limonoids which are triterpene derivatives have also been reported from this genus. In table 4 some of the terpenoids that have been isolated from this genus are listed.

Table 4: Terpenoids isolated from the *Teclea* species

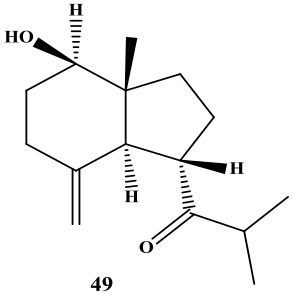
| NAME | SPECIES | PLANT PART | REFERENCE |
|---|---|---|---|
| Lupeol (**47**) | *T. afzelii*<br>*T. nobilis* | Stem bark<br>Leaves | Kuete *et al*., 2008<br>Al-Rehaily *et al*., 2001 |
| Teclenone A (**48**) | *T. nobilis* | Aerial parts | Al-Rehaily *et al*., 2002 |
| Teclenone B (**49**) | *T. nobilis* | Aerial parts | Al-Rehaily *et al*., 2002 |




47




48

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# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

## 3.1 INSTRUMENTATION

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

## 3.2 CHROMATOGRAPHIC METHODS

Chromatographic techniques that were employed in the separation procedure included column chromatography on normal silica gel 60G (Merck, 70-230 mesh) and Sephadex LH-20. In order to monitor the separation of compounds, analytical thin layer chromatography (TLC) pre-coated plates were used (silica gel 60 $F_{254}$ (Merck). To qualitatively determine presence or absence of compounds the TLC plates were visualized under ultraviolet (254 and 366 nm) light, exposed to iodine vapor or sprayed with Dragendorff reagent.

For purification, preparative thin layer chromatography (PTLC) was used in which preparative TLC plates (20×20 cm) were prepared from a slurry containing silica gel (13 g) and water (33 ml). The plates were left to dry at room temperature then activated for an hour at 110$^{o}$C. After applying the sample, the plate was developed using a suitable solvent system while monitoring under ultraviolet light (254 and 366 nm) for band detection.

## 3.3 PLANT MATERIAL COLLECTION

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

## 3.4 EXTRACTION AND ISOLATION OF COMPOUNDS

### 3.4.1 *Teclea simplicifolia* (stem bark)

The stem bark of *Teclea simplicifolia* was dried under a shade and then ground into powder. The powdered material (2.0 kg) was extracted thrice using $CH_2Cl_2$/$CH_3OH$ (1:1) by cold percolation and then with $CH_3OH$. The two extracts were combined and partitioned between $CH_2Cl_2$ and water (1:1). The aqueous layer was further partitioned between EtOAc and water (1:1). The organic extracts were combined (50 g) and were subjected to column chromatography packed with 500 g of silica gel. Gradient elution with n-hexane containing increasing amounts of EtOAc afforded 25 fractions (labeled **A-Y**).

Fraction B (eluted with n-hexane) was crystallized (from n-hexane/$CH_2Cl_2$) to yield compound **7** (10 mg). Similarly, fraction J (eluted with 4% EtOAc) was crystallized (from n-hexane/$CH_2Cl_2$) to yield compound **1** (6 mg). Fractions M-Q (eluted with 8 % EtOAc) were combined and purified using column chromatography on Sephadex LH 20 [eluted with $CH_2Cl_2$/MeOH (1:1)] then column chromatography on silica gel, [gradient elution starting with ($CH_2Cl_2$/n-hexane (1:1)] yielding compound **2** (4 mg). Similar treatment of fractions R (eluted with 10% EtOAc), T (eluted 12% EtOAc) and W (eluted with 15%EtOAc**)** yielded compound **3** (4 mg), **4** (5 mg) and **5** (2 mg**)** respectively.

3.4.2 Physical and spectroscopic data for the compounds isolated from the stem bark of *Teclea simplicifolia*

Maculine **(1) -** Appendix 1

Colorless crystals**.** $^{1}$H NMR (200 MHz, $CDCl_3$): δ 7.55 (1H, *d*, J=3 Hz, H-2), 7.01 (1H, *d*, J=3 Hz, H-3), 7.50 (1H, *s*, H-5), 7.23 (1H, *s*, H-8), 4.39 (3H, *s*, $OCH_3$), 6.07 (2H, *s*, $OCH_2O$). $^{13}$C NMR (50 MHz, $CDCl_3$): δ142.8 (C-2), 104.7 (C-3), 102.7 (C-3a), 156.2 (C-4), 114.5 (C-4a), 104.7 (C-5), 150.9 (C-6), 146.3 (C-7), 98.2 (C-8), 144.0 (C-8a), 163.3 (C-9a), 59.1 ($OCH_3$), 101.82 **(**$OCH_2O$).

Flindersiamine **(2)** - Appendix 2

White amorphous solid. $^{1}$H NMR (200 MHz, $CDCl_3$): δ 7.54 (1H, *d*, J=3 Hz, H-2), 6.98 (1H, *d*, J=3Hz, H-3), 7.22 (1H, *s*, H-5), 4.36 (3H, *s*, 4-$OCH_3$), 4.23 (3H, *s*, 8-$OCH_3$), 6.03 (2H, *s*, $OCH_2O$). $^{13}$C NMR (50 MHz, $CDCl_3$): δ143.2 (C-2), 104.6 (C-3), 103.1 (C-3a), 156.3 (C-4), 115.2(C-4a), 92.6 (C-5), 138.2 (C-6), 137.9 (C-7), 146.9 (C-8), 136.6 (C-8a), 162.8 (C-9a), 60.8 (4-OMe), 59.1 (8-OMe), 101.7 ($OCH_2O$).

Kokusaginine **(3) -** Appendix 3

Colorless crystals. $^{1}$H NMR (200 MHz, $CDCl_3$): δ7.57 (1H, *d*, J=3 Hz, H-2), 7.04 (1H, *d*, J=3 Hz, H-3), 7.45 (1H, *s*, H-5), 7.33 (1H, *s*, H-8), 4.44 (3H, *s*, 4-$OCH_3$), 4.02 (3H, *s*, 6-$OCH_3$), 4.02 (3H, *s*, 7-$OCH_3$). $^{13}$C NMR (50 MHz, $CDCl_3$): δ142.7 (C-2), 106.9 (C-3), 102.5 (C-3a), 155.8 (C-4), 113.2 (C-4a), 104.9 (C-5), 152.8 (C-6), 148.0 (C-7), 100.42 (C-8), 142.8 (C-8a), 162.6 (C-9a), 59.1 (4-OMe), 56.2 (6-OMe), 56.2 (7-OMe).

Maculosidine **(4) -** Appendix 4

White amorphous solid. $^{1}$H NMR (200 MHz, $CDCl_3$): δ7.62 (1H, *d*, J=2.4 Hz, H-2) , 7.04 (1H, *d*, J=2.4 Hz, H-3), 6.7 (1H, *d*, J= 2.6 Hz, H-5), 7.08 (1H, *d* , J=2.6 Hz ,H-7), 4.42 (3H, *s*, 4-$OCH_3$), 4.02 (3H, *s*, 6-$OCH_3$), 3.9 (3H, *s*, 8-$OCH_3$). $^{13}$C NMR (50 MHz, $CDCl_3$): δ144.1 (C-2), 101.6 (C-3), 119.8 (C-3a), 156.4 (C-4), 119.8 (C-4a), 91.7 (C-5), 155.9 (C-6), 104.5 (C-7), 155.7 (C-8),134.0 (C-8a), 162.2 (C-9a), 59.1 (4-OMe), 56.2 (6-OMe), 55.7 (8-OMe).

4, 5,6,7-Tetramethoxyfuro[2, 3-b]quinoline **(5) -**Appendix 5

White amorphous solid. $^{1}$H NMR (200 MHz, $CDCl_3$): δ7.54 (1H, *d*, J=2.8 Hz ,H-2), 6.94 ( 1H, *d*, J=2.8 Hz, H-3), 7.23 (1H, *s*, H-8), 4.38 (3H, *s*, 4-$OCH_3$) ,4.09 (3H, *s*, 5-$OCH_3$), 3.99 (3H, *s*, 6-$OCH_3$) ,3.93 (3H, *s* ,7-$OCH_3$). $^{13}$C NMR (50 MHz, $CDCl_3$): δ 143.5 (C-2), 104.7(C-3), 114.4 (C-3a), 115.6 (C-4a), 96.1 (C-8), 62.2 (4-OMe), 61.6 (5-OMe), 59.1 (6-OMe), 56.1 (7-OMe).

Lupeol **(7)** – Appendix 7-(Chepkirui, 2012)

White crystals**.** $^{1}$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).

### 3.4.3 *Teclea simplicifolia* (leaves)

The leaves of *Teclea simplicifolia* was dried under a shade and then ground into powder. The powdered material (4.0 kg) was extracted thrice using $CH_2Cl_2/CH_3OH$ (1:1) by cold percolation and then with $CH_3OH$. The combined crude extract was partitioned between $CH_2Cl_2$ and water (1:1). The aqueous layer was further partitioned between EtOAc and water (1:1). The combined extract (80 g) was subjected to column chromatography packed with 800 g of silica gel. Gradient elution with n-hexane containing increasing amounts of EtOAc afforded 55 fractions.

The fractions A-C (eluted with 5% EtOAc) were combined and purified by column chromatography on Sephadex LH 20 ($CH_2Cl_2$/ MeOH, 1:1) to give compound **6** (5 mg). Similarly, fractions F- H (eluted with 7% EtOAc) were combined and purified by column chromatography on Sephadex LH 20 ($CH_2Cl_2$/MeOH, 1:1) and using PTLC (2% EtOAc in n-hexane) to yield compound **1** (3 mg).

### 3.4.4 Physical and spectroscopic data for the compounds isolated from the leaves of *Teclea simplicifolia*

Nobiline **(6) –** Appendix 6

White amorphous powder. $^1$H NMR (600 MHz,$CDCl_3$): δ 7.50 (1H, *d*, H-2), 6.97 (1H, *d*, H-3*)*, 7.40 (1H, *s*, H-5), 7.28 (1H, *s, H-8*), 4.37 (3H, *s*, 4-OMe) ,3.94 (3H, *s* ,6 -OMe), 4.68 (2H, *d*, H-1`), 5.53 (1H, *t*, H-2`), 1.69 (3H, *s*, H-4`), 1.72 (3H, *s*, H-5`). $^{13}$C NMR (600 MHz, $CDCl_3$): 142.5 (C-2), 104.6 (C-3), 102.1 (C-3a), 155.6 (C-4), 112.9 (C-4a), 107.7 (C-5), 148.1 (C-6),151.9 (C-7), 121.5 (C-8), 142.4 (C-8a), 163.1 (C-9a), 58.9 (4-OMe), 65.7 (C-1'), 119.2 (C-2'),138.2 (C-3`), 18.3 (C-4`), 25.4 (C-5`).

Maculine **(1)**

Colorless crystals. $^{1}H$ NMR (600 MHz, $CDCl_3$): δ 7.62 (1H, *d*, J=6 Hz, H-2), 7.13 (1H, *d*, J=6 Hz, H-3), 7.56 (IH, *s*, H-5), 7.29 (1H, *s*, H-8), 6.13 (2H, *s*, -$OCH_2O$-).

## 3.5 ANALGESIC ACTIVITY TESTS

### 3.5.1 Preparation of test solutions

The tests solutions were prepared by dissolving the drugs in saline solution; to a concentration of 200 mg/kg for both crudes while the pure compounds and aspirin at constant concentration of 50 mg/kg.

### 3.5.2 The experimental animals and sampling

In order to perform this experiment, adult Swiss albino mice weighing between 23-28 g were used. These animals were obtained from the animal house of the Department of Medical Physiology, University of Nairobi. The animals were housed in cages with food and water *ad libitum*. The animal house was maintained at room temperatures and with controlled lighting (12 h light/dark cycles). Prior to the experiment day, training on handling of equipment and the animals was done, therefore, laboratory animal care guidelines were followed throughout the experiment. Animals were acclimatized to the laboratory for two hours before testing and were used only once during the protocol. These tests were done during daytime in the Medical physiology laboratory with ambient illumination and temperature similar to the animal house. Each experimental unit comprised of a treated group of six animals and a control groups with similar number of animals.

The mice were randomly picked and carefully placed on a bench, using the left hand the mouse was held by the loose skin on the dorsal side of the neck turned up to expose the ventral side

while holding the tail with the left little finger. 0.2 ml of the sample solution (200mg/kg for the crudes and 50mg/kg for pure compounds and aspirin) or vehicle (normal saline) was injected intraperitoneally using 1 ml syringe and left for one hour before the test (Davies et al., 1946). The animals were sacrificed immediately after the tests.

### 3.5.3 The Tail Flick Test

A radiant heat of an Ingress Intel Total Conversion (IITC) model 33 tail flick analgesiometer was used for this experiment. The test was based on the reaction of the mice on exposure to a heat stimulus that was applied to small area of its tail (Davies *et al*., 1946). The mouse under test was held in cylindrical plastic holder, placed horizontally, while a small tip of the tail was left exposed. Its tail was positioned in a straight manner along a channel, when the animal was in a quiet manner the machine was switched on. After some time, the animal withdrew its tail with a sudden and characteristic flick (Davies *et al*., 1946). The interval time was recorded with a stop watch and was determined to be the reaction time. The tests were done on all the mice pre-treated with the vehicle (negative control), crude extracts, pure compounds and aspirin (positive control). The reaction time for each was determined and recorded.

### 3.5.4. Statistical analysis

In the analysis of the analgesic data, the results were presented as a mean ± S.D (Standard Deviation). This was then followed by the univariate analysis, one way variance analysis i.e. ANOVA. Finally, a scheffe`s *post hoc* test was done, in which the difference in the control and the test values was considered to be of significant at $p<0.05$.

# CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSION

## 4.1 COMPOUNDS ISOLATED FROM STEM BARK OF *TECLEA SIMPLICIFOLIA*

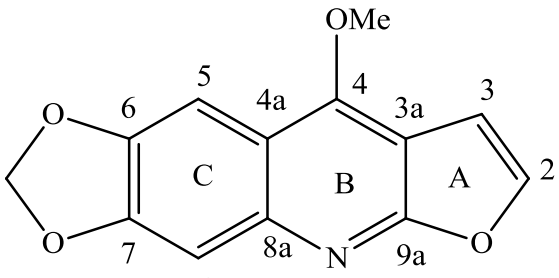
The stem bark of *Teclea simplicifolia* was extracted with $CH_2Cl_2/CH_3OH$ (1:1). The extract was then subjected to a combination of chromatographic techniques yielding six compounds comprising of five quinolines alkaloids and one terpenoid. The structures of these compounds were elucidated using NMR spectroscopic data and comparison with literature where report where available. The characterization of these compounds is discussed below.

### 4.1.1 Maculine (1)

Compound **1** was isolated as colorless crystals with an $R_f$ value of 0.34 (1% $CH_3OH$ in $CH_2Cl_2$). The spot on the TLC plate turned to orange when sprayed with Dragendorff reagent, indicating that it is an alkaloid. The $^{13}C$ NMR spectrum (Table 5 and appendix 1) showed signals at δc 156.2 (C-4), 163.4 (C-9a) and 144.1 (C-8a) which is consistent to a quinoline alkaloid skeleton (Adnan *et al*., 2001; Lacroix *et al*., 2012; Wondimu *et al*., 1988). Furthermore, the $^1H$ NMR spectrum showed the presence of a pair of mutually coupled doublets appearing at $\delta_H$ 7.55 (H-2) and 7.01 (*d, J*=3 Hz, H-3) with the corresponding carbon resonating at $\delta_C$ 142.8 (C-2) and 104.7 (C-3) which are characteristic of a furan ring in furoquinoline alkaloids (Yenesew and Dagne, 1988). The NMR spectrum in addition showed a downfield methoxy signal resonating at $\delta_H$ 4.39 and the corresponding carbon signal at $\delta_C$ 59.15 consistent for a 4-methoxyfuroquinoline alkaloids (Ayafor and Okogun, 1982).

The $^1H$ NMR spectrum further revealed two aromatic signals $\delta_H$ 7.50 (*s*) and 7.25 (*s*) an indication of *para*-oriented protons assigned to H-5 and H-8, respectively, in the furoquinoline

alkaloid skeleton which is substituted at C-6 and C-7. In addition, a downfield 2H singlet signal characteristic of a methylenedioxy substituent appearing at $\delta_H$ 6.07 ($\delta_C$ 101.82) was placed at C-6 ($\delta_C$ 150.9) and C-7 ($\delta_C$ 146.3). This compound was therefore identified as maculine (**1**)**.** It has previously been isolated from the leaves of *Teclea nobilis* (Yenesew and Dagne, 1988).




**1**

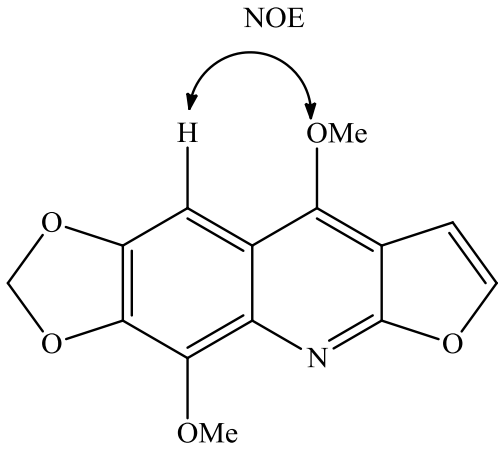
Table 5: $^{1}H$ (200 MHz) and $^{13}C$ (50 MHz) NMR data for maculine (**1**) in $CDCl_3$

| Position | $^{1}H$ ( δ in ppm, *J* in Hz) | $^{13}C$ |
|---|---|---|
| 2 | 7.55 (1H, *d*, *J*=3 Hz) | 142.8 |
| 3 | 7.01 (1H, *d*, *J*=3 Hz) | 104.7 |
| 3a | - | 102.7 |
| 4 | - | 156.2 |
| 4a | - | 114.5 |
| 5 | 7.50 (1H, *s*) | 104.7 |
| 6 | - | 150.9 |
| 7 | - | 146.3 |
| 8 | 7.23 (1H, *s*) | 98.2 |
| 8a | - | 144.1 |
| 9a | - | 163.4 |
| MeO-4 | 4.39 (3H, *s*) | 59.2 |
| $-OCH_2O-$ | 6.07(2H, *s*) | 101.8 |

### 4.1.2 Flindersiamine (2)

Compound **2** was isolated as white amorphous solid with an $R_f$ value of 0.46 (2% $CH_3OH$ in $CH_2Cl_2$). The spot on the TLC plate turned to orange when sprayed with Dragendorff reagent indicating that it is also an alkaloid. The compound was also shown to be a quinoline alkaloid due to the presence of carbon signals (Table 6 and appendix 6) at $\delta_c$ 156 (C-4), 162.8 (C-9a) and 136.1 (C-8a). The $^1$H NMR spectrum revealed the presence of a pair of coupled doublets as those in compound **1** resonating at $\delta_H$ 7.54 (H-2) and 6.98 (1H, *d*, *J*=3 Hz, H-3) the corresponding carbon atoms resonated at $\delta_C$ 143.2 and 104.6, respectively, characteristic of a furan ring in furoquinoline alkaloids. The $^1$H NMR spectrum revealed the presence of a methylenedioxy substituent resonating at $\delta_H$ 6.03 ($\delta_C$ 101. 7) assignable to C-6/C-7.

The NMR data of this compound was similar to compound **1** except that this compound had only one singlet aromatic proton ($\delta_H$ 7.22) whereas compound **1** had two singlets. Furthermore, the $^1$H NMR spectrum of this compound showed two methoxy substituents ($\delta_H$ 4.23 and 4.37 (*s*)) while compound **1** had only one methoxy substituent. The downfield methoxy resonating at $\delta_H$ 4.37 (*s*) was placed at C-4 as in compound **1**. The singlet aromatic signal was assigned to C-5 using NOEDIFF experiment whereby irradiation of the methoxy protons at C-4 resulted in signal enhancement on the aromatic proton signal. The other methoxy signal at $\delta_H$ 4.23 was therefore placed at C-8 causing up field shift signal of C-8a $\delta_c$ 136.1.From this spectroscopic data, the compound was identified as flindersamine (**2**). This compound has previously been reported from the leaves of *Teclea nobilis* (Yenesew and Dagne, 1988).




**2**

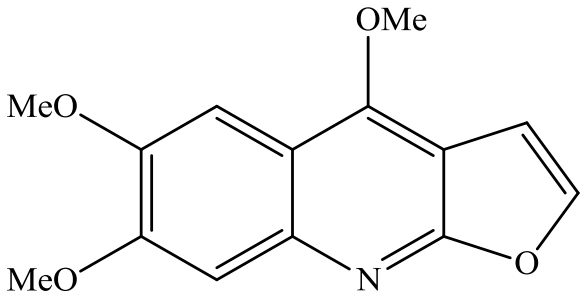
**Table 6: $^{1}$H (200 MHz) and $^{13}$C (50 MHz) NMR data for flindersiamine (2) in $CDCl_3$**

| **Position** | **$^{1}$H (δ in ppm, J in Hz)** | **$^{13}$C** |
|---|---|---|
| 2 | 7.54 (1H, *d*, *J*=3 Hz) | 143.2 |
| 3 | 6.98 (1H, *d*, *J*=3 Hz) | 104.6 |
| 3a | - | 103.9 |
| 4 | - | 156.2 |
| 4a | - | 115.1 |
| 5 | 7.22 (1H, *s*) | 92.6 |
| 6 | - | 138.2 |
| 7 | - | 137.9 |
| 8 | - | 146.9 |
| 8a | - | 136.1 |
| 9a | - | 162.8 |
| MeO-4 | 4.36( 3H, *s*) | 60.8 |
| MeO-8 | 4.23 (3H, *s*) | 59.1 |
| -$OCH_2O$- | 6.03 (2H, *s*) | 101.7 |

### 4.1.3 Kokusaginine (3)

Compound **3** was isolated as colorless crystals with an $R_f$ value of 0.42 (3% $CH_3OH$ in $CH_2Cl_2$). The spot on the TLC plate turned to orange when sprayed with Dragendorff reagent indicating it is also an alkaloid. As in compound **1** and **2**, the $^{13}C$ NMR (Table 7 and appendix 3) contained three carbon signals at $\delta_C$ 155.8 (C-4), 142.8 (C-8a) and 162.6 (C-9a) hence suggesting it is a quinoline alkaloid. The $^1H$ NMR revealed a pair of coupled doublets which resonated at $\delta_H$ 7.57 (H-2) and 7.04 (1H, *d, J*=3 Hz, H-3) also characteristic of the furan protons in furoquinoline alkaloids. In addition three methoxy signals resonating at 4.44 ($\delta_C$ 59.1) 4.02 ($\delta_C$ 56.2) and 4.02 ($\delta_C$ 56.2) were also revealed in the $^1H$ NMR spectrum. The presence of the downfield methoxy signal appearing at $\delta_H$ 4.44 was consistent for 4-methoxy furoquinoline alkaloids (Yenesew and Dagne, 1988).

Similar to compound **1,** the $^1H$ NMR spectrum also showed presence of two para-oriented protons resonating at $\delta_H$ 7.45 ($\delta_C$ 104.8) and 7.33 ($\delta_C$ 100.4) which were assigned to H-5 and H-8 in the furoquinoline alkaloids skeleton (Dreyer, 1980; Wansi *et al*., *2*010). The only difference between compound **1** and **3**, is that the methylenedioxy in compound **1** is replaced with two methoxy groups ($\delta_H$ 4.02 (6H), $\delta_C$ 56.2). Therefore, this compound was identified as kokusaginine (**3**). This compound had been previously reported from the stem bark of *Teclea afzelii* (Wansi *et al*., 2010).




**3**

**Table 7: $^{1}$H (200 MHz) and $^{13}$C (50 MHz) NMR data for kokusaginine (3) in $CDCl_3$**

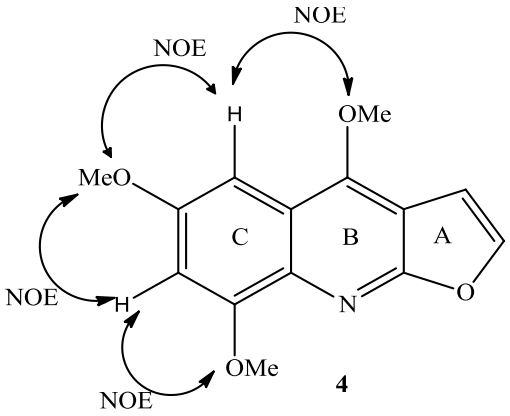
| Position | $^{1}$H (δ in ppm, J in Hz) | $^{13}$C |
|---|---|---|
| 2 | 7.57 (1H, *d*, *J*=3 Hz) | 142.7 |
| 3 | 7.04 (1H ,*d*, *J*=3 Hz) | 106.9 |
| 3a | - | 102.5 |
| 4 | - | 155.8 |
| 4a | - | 113.2 |
| 5 | 7.45 (1H, *s*) | 104.9 |
| 6 | - | 152.8 |
| 7 | - | 148.0 |
| 8 | 7.33 (1H, *s*) | 100.4 |
| 8a | - | 142.8 |
| 9a | - | 162.6 |
| MeO-4 | 4.44 (3H, *s*) | 59.1 |
| MeO-6 | 4.02 (3H, *s*) | 56.2 |
| MeO-7 | 4.02 (3H,*s*) | 56.2 |

### 4.1.4 Maculosidine (4)

Compound **4** was isolated as a white amorphous powder with an $R_f$ value of 0.42 (3% $CH_3OH$ in $CH_2Cl_2$) similar to that of compound **3**. This compound was UV active and was fluorescing at 245 nm unlike compound **3.** It was also assumed to be an alkaloid since on spraying with Dragendorffs reagent on TLC plate the colorless spot turned orange. The $^{13}C$ NMR (Table 8 and appendix 4) spectrum had three carbon signals which resonated at 156.4 (C-4), 134.0 (C-8a) and 162.2 (C-9a) characteristic of quinoline alkaloid. The $^{1}H$ NMR spectrum displayed a pair of coupled doublets which resonated at $\delta_H$ 7.62 (H-2) and 7.04 (*d, J*=2.4 Hz, H-3) which was again consistent to two furan protons in furoquinoline alkaloids where the corresponding carbons resonating at $\delta_C$ 144.1 (C-2) and 101.6 (C-3). The $^{1}H$ NMR spectrum further revealed the presence of three methoxy signals just like compound **3** which resonated $\delta_H$ 4.43 ($\delta_C$ 59.1), 4.02 ($\delta_C$ 56.3) and 3.92 ($\delta_C$ 55.7,the downfield methoxy signal at 4.43 was placed at C-4.

Despite the fact that compounds **3** and **4** had the same $R_f$ values and three methoxy substituent's, the distinctive feature between these two alkaloids was in the orientation of the two aromatic protons in ring C. In compound **3** the protons are *para* oriented while in compound **4** they are *meta*-coupled (Table 8) resonating at $\delta_H$ 7.08 and 6.7 (*d, J*=3 Hz). The placement of the protons was confirmed by NOEDIFF experiment; thus on irradiation of the methoxy group ($\delta_H$ 4.43) at C-4 causes enhancement of protons at $\delta_H$ 7.04 (H-3 furan proton) and $\delta_H$ 6.70 (aromatic proton). Therefore, indicating that the aromatic proton at $\delta_H$ 6.70 (IH, *d*, J=3 Hz) should be H-5 in ring C and hence the other proton at $\delta_H$ 7.08 (IH, *d, J*=3 Hz) is for a H-7 on the same ring. Similarly, on irradiation the methoxy at $\delta_H$ 4.02 ($\delta_C$ 56.3) led to enhancement of signals at $\delta_H$ 6.7 and 7.08 hence this methoxy was assigned to C-6 while the methoxy signal at $\delta_H$ 3.9 ($\delta_C$ 55.7) showed

enhancement of proton signal at $\delta_H$ 7.08 only hence placed at C-8. This assignment was further supported by the NOESY experiment. This compound was therefore identified as maculosidine (**4**), a compound that had been previously isolated from the roots of *Vepris uguenensis* (Cheplogoi *et al.,* 2008).




**4**

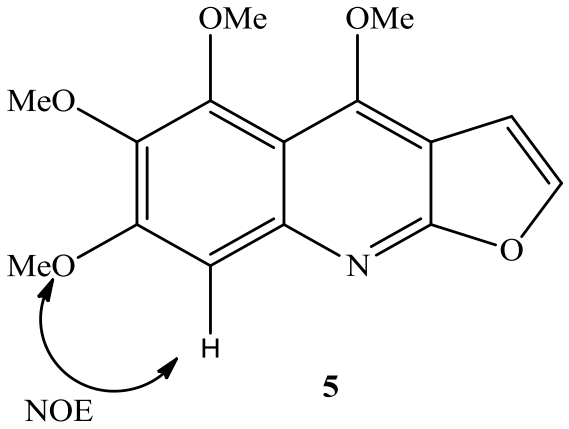
Table 8: $^{1}H$ (200 MHz) and $^{13}C$ (50 MHz) NMR data for maculosidine (**4**) in $CDCl_3$

| Position | $^{1}H$ ($\delta$ in ppm, J in Hz) | $^{13}C$ |
|---|---|---|
| 2 | 7.62 (1H, *d*, *J*=2.4 Hz) | 144.1 |
| 3 | 7.04 (1H, *d*, *J*=2.4 Hz) | 101.6 |
| 3a | - | 119.8 |
| 4 | - | 156.4 |
| 4a | - | 119.8 |
| 5 | 6.7 (1H, *d*, *J*=3 Hz) | 91.7 |
| 6 | - | 155.9 |
| 7 | 7.08 (1H, *d*, *J*=3 Hz) | 104.5 |
| 8 | - | 155.7 |
| 8a | - | 134.0 |
| 9a | - | 162.2 |
| MeO-4 | 4.42 (3H, *s*) | 59.2 |
| MeO-6 | 4.02 (3H, *s*) | 56.3 |
| MeO-8 | 3.90 (3H, *s*) | 55.7 |

### 4.1.5 4,5,6,7-Tetramethoxyfuro[2, 3-b]quinoline (5)

Compound **5** was isolated as a white amorphous solid and identified as an alkaloid by spraying with Dragendorrfs reagent where the TLC spot turned to orange. As in the other compounds, the NMR revealed that this compound is a 4-methoxyfuroquinoline alkaloid (Table 9 and appendix 5). In addition, a singlet characteristic for an aromatic proton with a chemical shift value of 7.23 was also revealed by the $^{I}$H NMR spectrum. The NMR of compound **5** is similar to those of compounds **3** and **4**.

The distinct feature between this compound and compounds **3** and **4** was the presence of an additional methoxy signals, hence the $^{1}$H NMR spectrum contained four methoxy signals resonating at $\delta_H$ 4.38 *(*$\delta_C$ 62.3*),* 4.09 *(*$\delta_C$ 61.7*),* 3.99 ($\delta_C$ 59.2) and 3.93 ($\delta_C$ 56.2). The methoxy group signal at $\delta_H$ 4.38 was characteristic of a methoxy group at C-4 for a 4-methoxyfuroquinoline alkaloid. Two of the remaining three methoxy groups were downfield shifted in the $^{13}$C NMR ($\delta_C$ 61.7, 59.2) showing that they are di-ortho-substituted while the third appeared within the normal range ($\delta_C$ 56.2). This is consistent with placing these methoxy groups at C-5, C-6 and C-7 or C-6, C-7 and C-8. The placement of the methoxy in compound **5** was on the basis of NOEDIFF experiment which on irradiation of the methoxy at $\delta_H$ 4.38 ($\delta_C$ 62.3) resulted in signal enhancement of the H-3 only. Therefore compound **5** was identified as 4,5,6,7-tetramethoxyfuro[2,3-b]quinoline which is a new compound. Unfortunately, the new compound underwent decomposition before any further analysis could be conducted.




**5**

Table 9: $^{1}H$ (200 MHz) and $^{13}C$ (50 MHz) NMR data for 4,5,6,7-tetramethoxyfuro[2,3-b]quinoline in $CDCl_3$

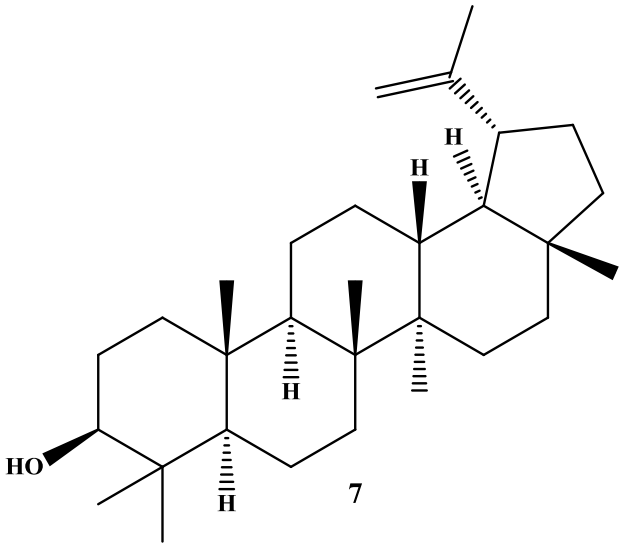
| Position | $^{1}H$ (δ in ppm, J in Hz) | $^{13}C$ |
|---|---|---|
| 2 | 7.54 ( 1H, *d, J*=2.8 Hz) | 143.5 |
| 3 | 6.94 ( 1H, *d, J*=2.8 Hz) | 104.7 |
| 3a | - | 114.4 |
| 4a | - | 115.6 |
| 8 | 7.23 (1H, *s*) | 96.1 |
| MeO-4 | 4.38 (3H, *s*) | 62.2 |
| MeO-5 | 4.09 (3H, *s*) | 61.6 |
| MeO-6 | 3.99 (3H, *s*) | 59.1 |
| MeO-7 | 3.93 (3H, *s*) | 56.1 |

### 4.1.6 Lupeol (7)

Compound **7** was not UV active (TLC at 254 and 366nm) and was isolated as white crystals. On examination of the $^{13}C$ NMR (appendix 7) spectrum there were thirty carbon signals which is a characteristic feature of triterpenes. The $^{13}C$ NMR/DEPT spectra displayed the presence of seven methyl carbons which resonated at $\delta_C$ 14.7, 16.1, 15.6, 18.2, 16.3, 19.5and 28.2 which was confirmed by the $^{1}H$ NMR spectrum which contained seven singlet signals at $\delta_H$ 0.95 ( Me-

23), 0.77( Me-24), 0.81 (Me-25), 1.01 ( Me-26), 0.93( Me-27), 0.77 ( Me-28) and 1.66 ( Me-30).

The $^{13}$C/ DEPT spectrum also showed ten methylene carbon atoms with the signals appearing at $\delta_C$ 18.5, 21.1, 25.3, 27.6, 29.9, 30.1, 34.5, 35.8, 38.9 and 40. Two of the methylene protons signals in the $^{1}$H NMR spectrum appeared at δ 4.55 for H-29a and 4.68 for H-29b and δ 3.17 for H-3. Presence of five methine carbons (resonating at $\delta_C$ 48.2, 48.5, 50.6, 55.5 and 38.3), olefinic cabons $\delta_C$ 151.2 and 109.5), quarternary carbon peaks (at $\delta_C$ 39.1, 37.4, 41.0, 43.0 and 43.2) and an oxymethine signal $\delta_C$ 79.2 for the C-3 were also apparent from $^{13}$C/DEPT spectrum. Using the data obtained and comparing with literature compound **8** was identified as lupeol (Al-Rehaily et al., 2001; Chepkirui, 2012).




**7**

## 4.2 COMPOUNDS ISOLATED FROM THE LEAVES OF *TECLEA SIMPLICIFOLIA*

The leaves of *Teclea simplicifolia* yielded 2 alkaloids. One of these compounds was identified as maculine (**1**) as discussed in section 4.1.1.

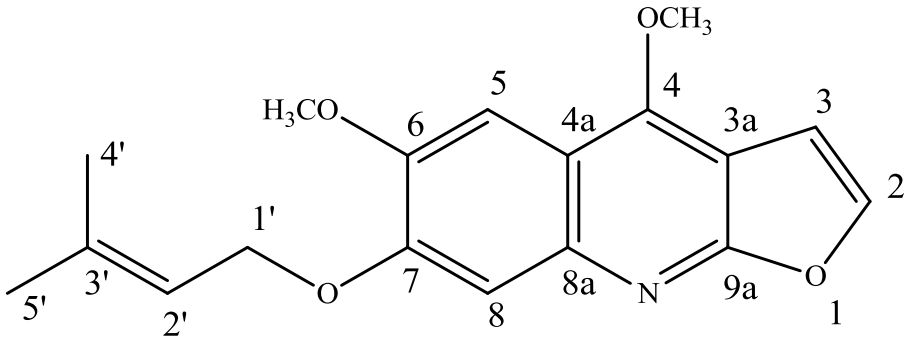
### 4.2.1 Nobiline (6)

Compound **6** was isolated as a white amorphous powder with $R_f$ value of 0.44 in 2% EtOAc in n-hexane. The colorless spot on the TLC plate turned to orange on spraying with Dragendoff reagent indicating it is an alkaloid. The $^{13}C$ NMR spectrum (Table 10 and appendix **6** ) showed signals at $\delta_C$ 155.6 (C-4a), 163.1 (C-9a) and 142.4 (C-8a) which are consistent with a quinoline skeleton (Adnan *et al*., 2003; Lacroix *et al*., 2012; Wondimu *et al*., 1998). Furthermore, the $^1H$ NMR displayed mutually coupled doublets which resonated at $\delta_H$ 7.50 and $\delta_H$ 6.97 (each 1H, J=2.8 Hz), characteristic of H-2 and H-3 of furan ring protons in a furoquinoline derivative (Ayafor and Okogun, 1982). The corresponding carbons atom resonated at $\delta_C$ 142.5 and $\delta_C$ 104.6 respectively. In addition, the spectrum also contained a downfield shifted methoxyl signal resonating $\delta_H$ 4.37, which is typical of methoxy group at C-4 in furoquinoline alkaloids, with the corresponding carbon atom resonating at $\delta_C$ 58.9.

The NMR spectra also showed the presence of an additional methoxy ($\delta_H$ 3.94; δc 55.9) and prenyloxy (Table 10) substituents. The $^1H$ NMR spectrum also contained two singlets resonating at δH 7.40 and 7.28 characteristic of *para* oriented protons in an aromatic ring. These were assigned to H-5 and H-8 protons, respectively, of Ring C which is substituted at C-6 and C-7. From this data, this compound could either be tecleanatalensine B, whereby the methoxy group is placed at C-7 and the prenyloxy group at C-6 or nobiline, whereby the two substituents interchange positions (Tarus *et al*., 2005; Yenesew and Dagne, 1988). The HMBC spectrum

showed a $^{3}J$ correlation between $\delta_H$ 7.40 (H-5) with C-4 ($\delta_C$ 155.6), C-8a ($\delta_C$ 142.4) and C-7 ($\delta_C$ 151.9). In addition, there was a $^{3}J$ correlation between $\delta_H$ 4.37 (4-OMe) and C-4 ($\delta_C$ 155.6) hence confirming the placement of the methoxy group at C-4.

The HMBC further showed correlations between $\delta_H$ 3.94 and $\delta_C$ 148.1 while the oxymethylene at $\delta_H$ 4.68 (C-1`) with δc 151.9. These correlations ruled out that this compound was not tecleanatalensine B since the C-6 which is connected to prenyloxy resonates at $\delta_C$ 148.0 while C-7 connected to methoxy at $\delta_C$ 150.0 (Tarus *et al*., 2005). Therefore, the methoxy group was placed at C-6 while the prenyloxy substituent then was placed at C-7. Using this data and comparison with literature values, this compound was identified as nobiline (**6**). It has been previously isolated from the leaves of *Teclea nobilis* (Yenesew and Dagne, 1988).




**6**

Table 10: $^{1}H$ (600 MHz) and $^{13}C$ (150 MHz) NMR data for Nobiline (**6**) in $CDCl_3$

| POSITION | $^{1}H$ (J in Hz) | $^{13}C$ | HMBC ($^{2}J$,$^{3}J$) |
|---|---|---|---|
| 2 | 7.50 (1H, *d*, *J*=8 Hz) | 142.5 | C-3a, C-3, C-9a, |
| 3 | 6.97 (1H,*d*, *J*=8 HZ) | 104.6 | C-2, C-3a, C-9a |
| 3a | | 102.1 | |
| 4 | | 155.6 | |
| 4a | | 112.9 | |
| 5 | 7.40 (1H, *s*) | 100.2s | C-4, C-8a, C-7 |
| 6 | | 148.1 | |
| 7 | | 151.9 | |
| 8 | 7.28 (1H, *s*) | 107.6 | C-4a, C-8a, C-6, C-7 |
| 8a | | 142.4 | |
| 9a | | 163.1 | |
| 4-OMe | 4.37 (3H, *s*) | 58.9 | C-4 |
| 6-OMe | 3.94 (3H, *s*) | 55.9 | C-6 |
| 1` | 4.68 (2H, *d*, *J*=8Hz *)* | 65.6 | C-2`, C-3`,C-7 |
| 2` | 5.53 *(*1H, *d*, *J*=8Hz*)* | 119.2 | |
| 3’ | | 138.2 | |
| 4’ | 1.69 (3H, *s*) | 18.3 | C-2`, C-3`,C-5` |
| 5’ | 1.72 (3H, *s*) | 25.4 | C-2`, C-3`, C-4` |

## 4.3 ANALGESIC TEST RESULTS

The crude extracts of stem bark and the leaves of *Teclea simplicifolia* were tested as well as the compound maculine (**1)** and maculosidine **(4)**. The tail flick method on mice was used for this experiment. Data analysis was carried out using ANOVA (variance analysis) followed by Scheffe`s *post hoc* test. The difference between the experimental tests values and the control values were considered to be of significance at $p<0.05$. Table 11 shows the mean reaction time for all the experimental tests.

Table 11: Tail flick mean reaction time

| TEST SAMPLES | CONCENTRATION (mg/kg) | TAIL FLICK REACTION TIME IN SECONDS (MEAN±SD) |
|---|---|---|
| Vehicle (saline solution)-negative control | 0 | 1.75±0.27 |
| Stem bark crude | 200 | 4.25±0.88 |
| Leaves crudes | 200 | 4.50±0.45 |
| Maculine | 50 | 4.41±0.49 |
| Maculosidine | 50 | 4.75±0.52 |
| Aspirin (positive control) | 50 | 4.67±0.26 |
| Total | | 4.05±1.12 |

These results above were further compared using Scheffe`s *post hoc* test, i.e. each of the test sample was compared with others as illustrated in Table 12.

Table 12: Scheffe`s *post hoc* test (Multiple Comparisons)

| Dependent Variable: time Scheffe | | | | | | |
|---|---|---|---|---|---|---|
| (I) dose | (J) dose | Mean Difference (I-J) | Std. Error | P | 95% Confidence Interval | |
| | | | | | Lower Bound | Upper Bound |
| Vehicle | stem bark crude | -2.5000* | .30123 | **<0.0001** | -3.5721 | -1.4279 |
| | leaves crudes | -2.7500* | .30123 | **<0.0001** | -3.8221 | -1.6779 |
| | Masculine | -2.6667* | .30123 | **<0.0001** | -3.7388 | -1.5945 |
| | Maculosidine | -3.0000* | .30123 | **<0.0001** | -4.0721 | -1.9279 |
| | Aspirin | -2.9167* | .30123 | **<0.0001** | -3.9888 | -1.8445 |
| Stem bark crude | Vehicle | 2.5000* | .30123 | **<0.0001** | 1.4279 | 3.5721 |
| | leaves crudes | -.2500 | .30123 | .982 | -1.3221 | .8221 |
| | Masculine | -.1667 | .30123 | .997 | -1.2388 | .9055 |
| | Maculosidine | -.5000 | .30123 | .736 | -1.5721 | .5721 |
| | Aspirin | -.4167 | .30123 | .857 | -1.4888 | .6555 |
| Leaves crudes | Vehicle | 2.7500* | .30123 | **<0.0001** | 1.6779 | 3.8221 |
| | stem bark crude | .2500 | .30123 | .982 | -.8221 | 1.3221 |
| | Masculine | .0833 | .30123 | 1.000 | -.9888 | 1.1555 |
| | Maculosidine | -.2500 | .30123 | .982 | -1.3221 | .8221 |
| | Aspirin | -.1667 | .30123 | .997 | -1.2388 | .9055 |
| Maculine | Vehicle | 2.6667* | .30123 | **<0.0001** | 1.5945 | 3.7388 |
| | stem bark crude | .1667 | .30123 | .997 | -.9055 | 1.2388 |
| | leaves crudes | -.0833 | .30123 | 1.000 | -1.1555 | .9888 |
| | Maculosidine | -.3333 | .30123 | .939 | -1.4055 | .7388 |
| | Aspirin | -.2500 | .30123 | .982 | -1.3221 | .8221 |
| Maculosidine | Vehicle | 3.0000* | .30123 | **<0.0001** | 1.9279 | 4.0721 |
| | stem bark crude | .5000 | .30123 | .736 | -.5721 | 1.5721 |
| | leaves crudes | .2500 | .30123 | .982 | -.8221 | 1.3221 |
| | Masculine | .3333 | .30123 | .939 | -.7388 | 1.4055 |
| | Aspirin | .0833 | .30123 | 1.000 | -.9888 | 1.1555 |
| Aspirin | Vehicle | 2.9167* | .30123 | **<0.0001** | 1.8445 | 3.9888 |
| | stem bark crude | .4167 | .30123 | .857 | -.6555 | 1.4888 |
| | leaves crudes | .1667 | .30123 | .997 | -.9055 | 1.2388 |
| | Masculine | .2500 | .30123 | .982 | -.8221 | 1.3221 |
| | Maculosidine | -.0833 | .30123 | 1.000 | -1.1555 | .9888 |
| Based on observed means. The error term is Mean Square (Error) = .272. | | | | | | |
| *. The mean difference is significant at the 0.05 level in bold | | | | | | |

To evaluate the analgesic activity of *Teclea simplicifolia* on mice, the crude extracts of the stem bark and leaves were tested at a concentration of 200 mg/kg. In addition, two pure compounds maculine and maculosidine were tested at a concentration of 50 mg/kg. The reaction to thermal pain was longer in mice administered with maculosidine with a reaction time of 4.75±0.52 as compared to aspirin, 4.67±0.26. Furthermore, the crude extracts of the stem bark and the leaves showed a significant difference ($p<0.05$) at a reaction time of 4.25±0.88 and 4.50±0.45 respectively when compared with the vehicle treated group. Therefore, this supported the use of the plant in traditional practices for pain remedies.

In addition, there was a significant analgesic effect ($p<0.0001$) between the vehicle treated mice and those that were treated with maculine and maculosidine. However, there was no significant difference ($p>0.05$) between the analgesic effects of the crude extracts and the pure compounds hence supporting the claims that antipain properties is attributed to the presence of quinoline alkaloids in *Teclea* species. This hence suggests that maculine and maculosidine could be used as lead compounds in the development of more effective pain relieving drugs.

On comparison with values obtained from previous analgesic studies, there was a similarity in that the crude and compounds isolated from *Teclea* species have shown significant analgesic activity (Al-Rehaily *et al*., 2001). For example, the treatment of mice with MeCN, hexane extract and Lupeol from *Teclea nobilis* was shown to significantly increase the retention time of mice to the nociceptive stimuli, $p<0.05$ (Al-Rehaily *et al*.,2001).

# CHAPTER FIVE

## 5.0 CONCLUSIONS

The crude extracts of the stem bark and leaves of *Teclea simplicifolia* demonstrated significant analgesic activity. From the stem bark of this plant, the furoquinoline alkaloids maculine (**1**) flindersiamine (**2**), kokusaginine (**3**), maculosidine (**4**), 4,5,6,7-tetramethoxyfuro[2,3-b]quinolines (**5**) and triterpene lupeol (**7**) were identified. Maculine (**1**) and maculosidine (**4**) and other quinolines alkaloids are responsible for these activities, this is because there is no significant difference ($p>0.05$) between the analgesic activities of these two compounds when compared to the crudes. In addition maculine (**1)** and nobiline (**6**) were also identified from the leaves of this plant.

## 5.1 RECOMMENDATIONS

1. A more comprehensive phytochemical investigation of the leaves of *Teclea simplicifolia* should be done.
2. The analgesic activity of the crude extracts and other furoquinoline alkaloids at different concentrations should be evaluated.
3. The crude extracts and pure compounds should be evaluated for other biological activities such as antipyretic.
4. The mechanism of action for furoquinoline alkaloids should be investigated.

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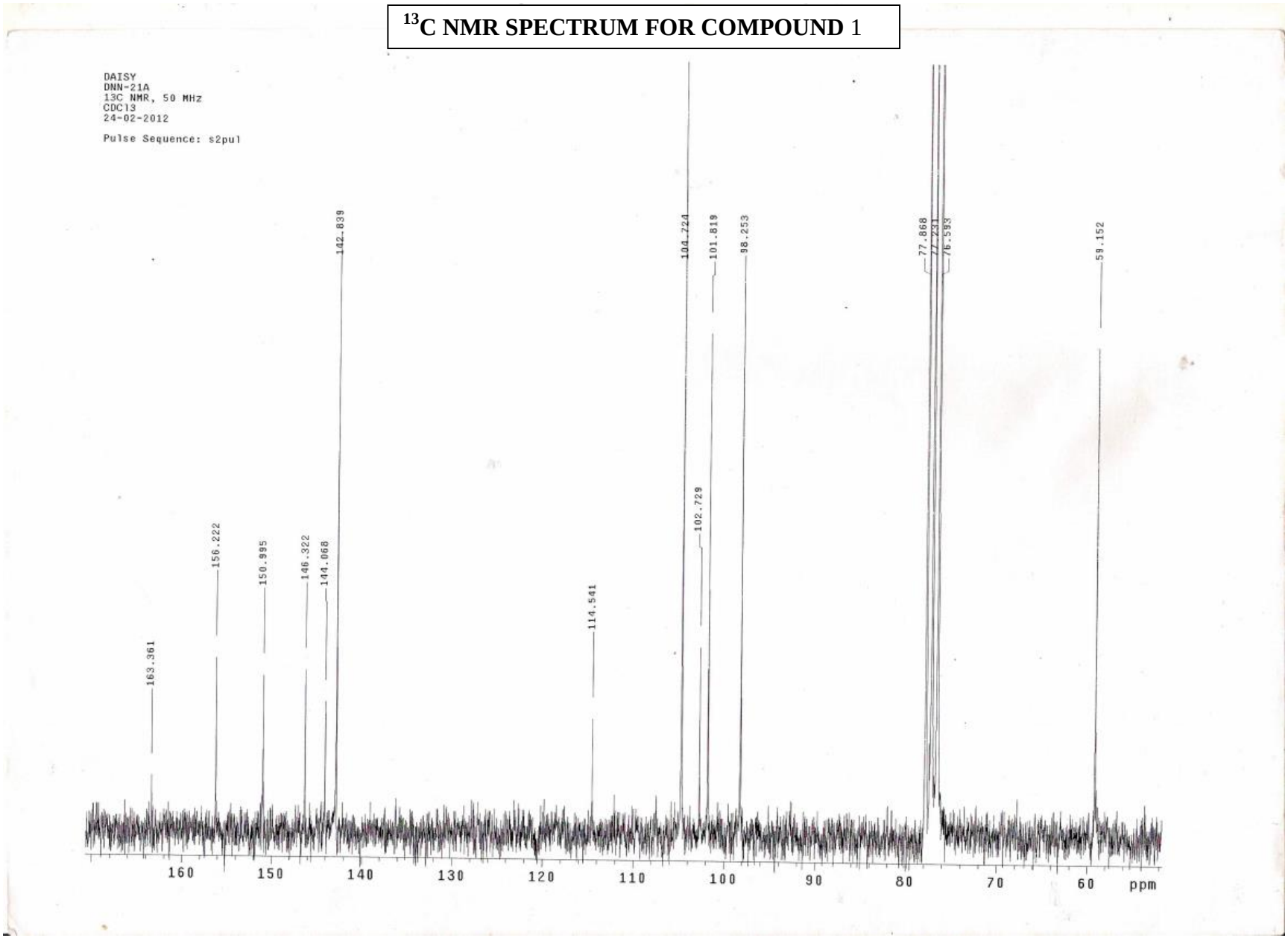
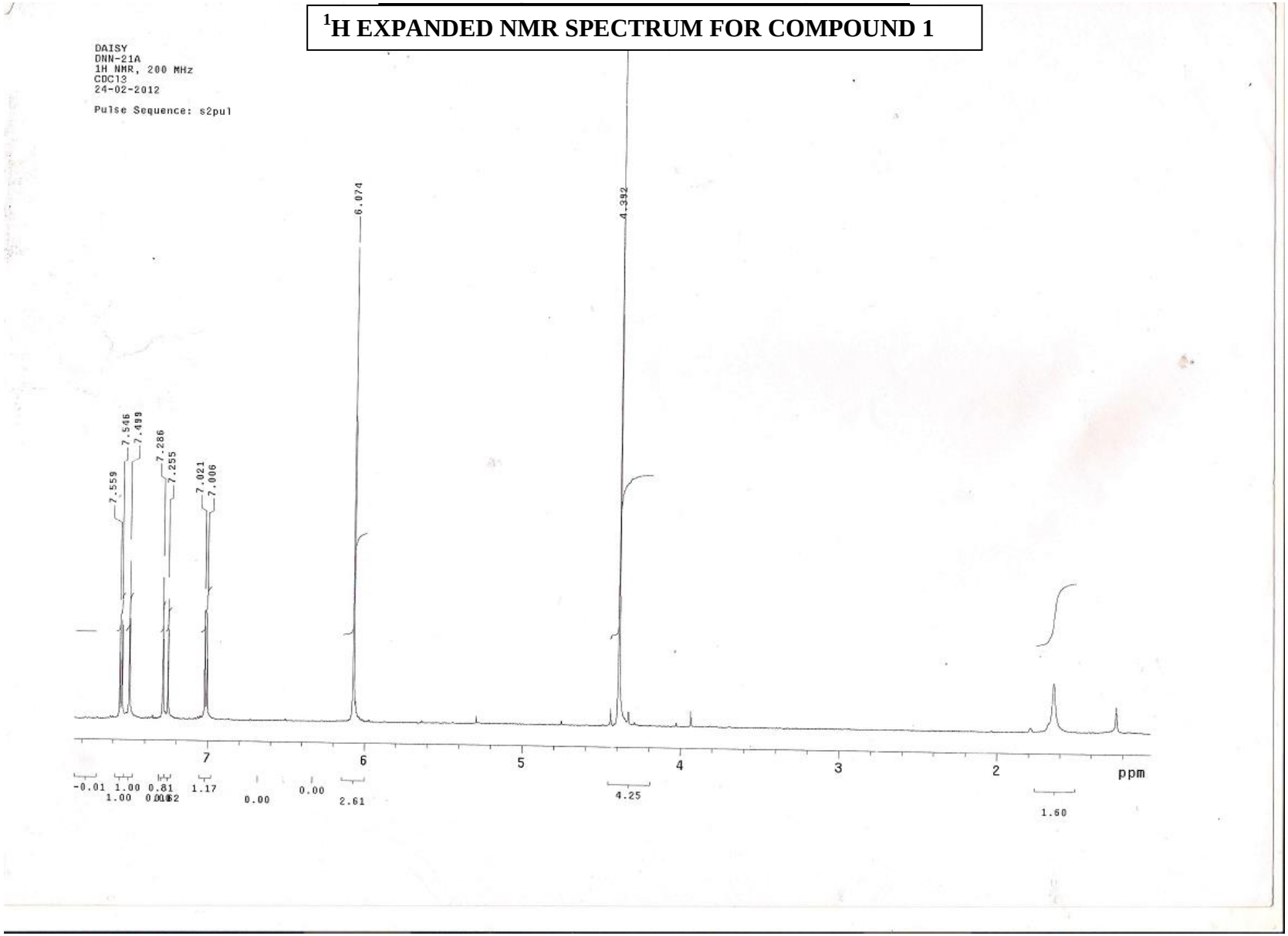
# APPENDICES

## APPENDIX I: SPECTRA FOR COMPOUND 1

## $^{13}$C NMR SPECTRUM FOR COMPOUND 1

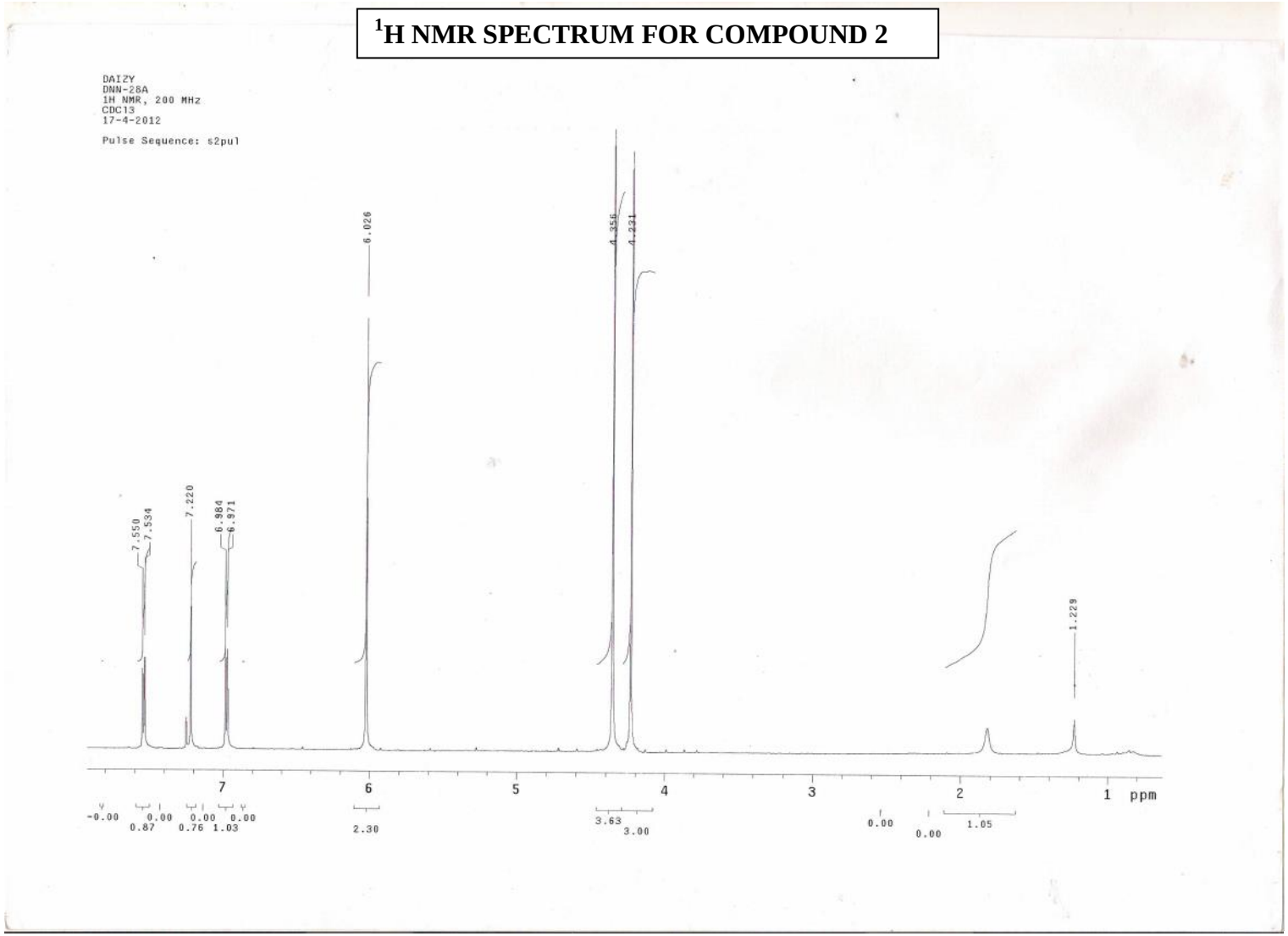


**$^{1}$H EXPANDED NMR SPECTRUM FOR COMPOUND 1**

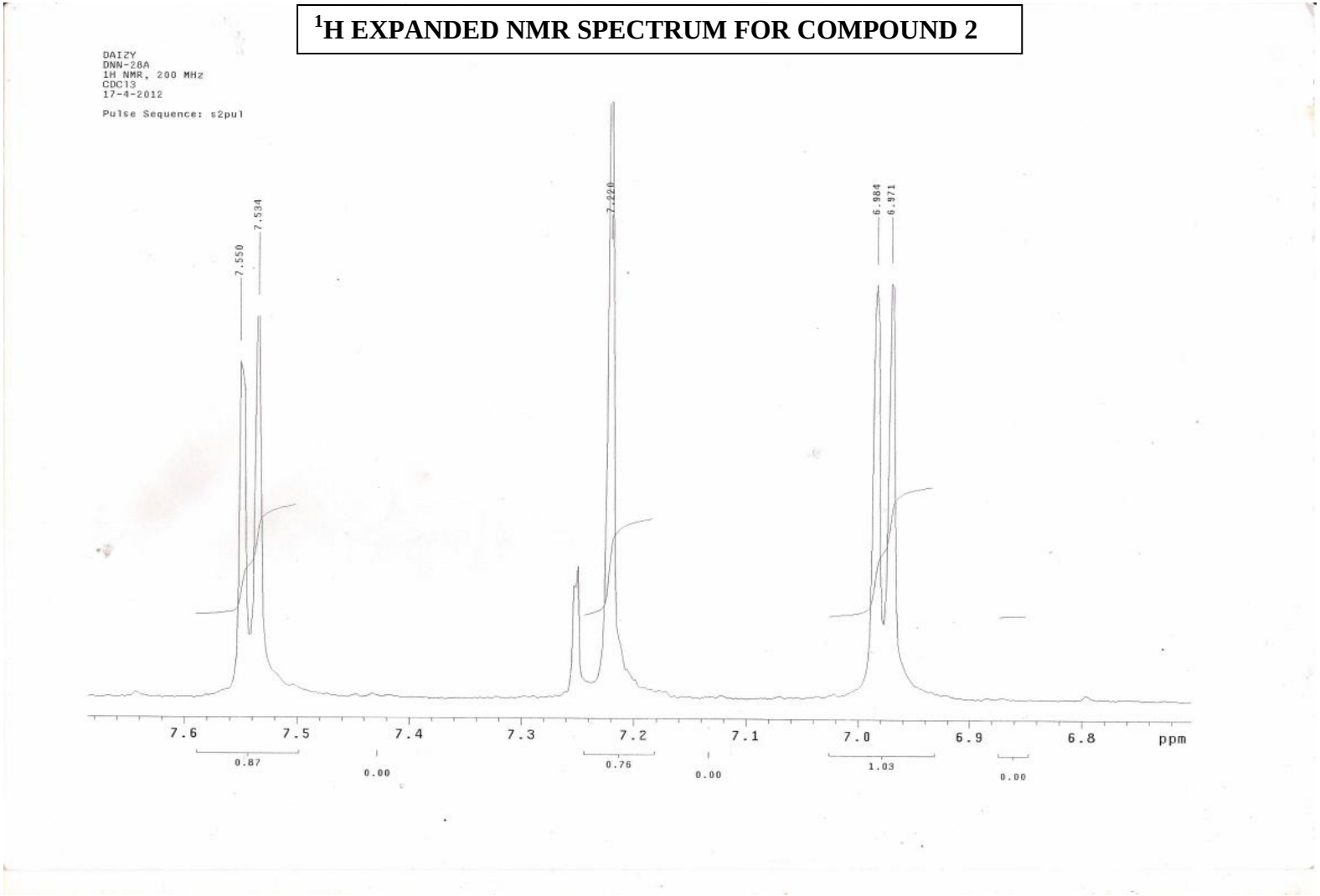


## APPENDIX 2: SPECTRA FOR COMPOUND 2

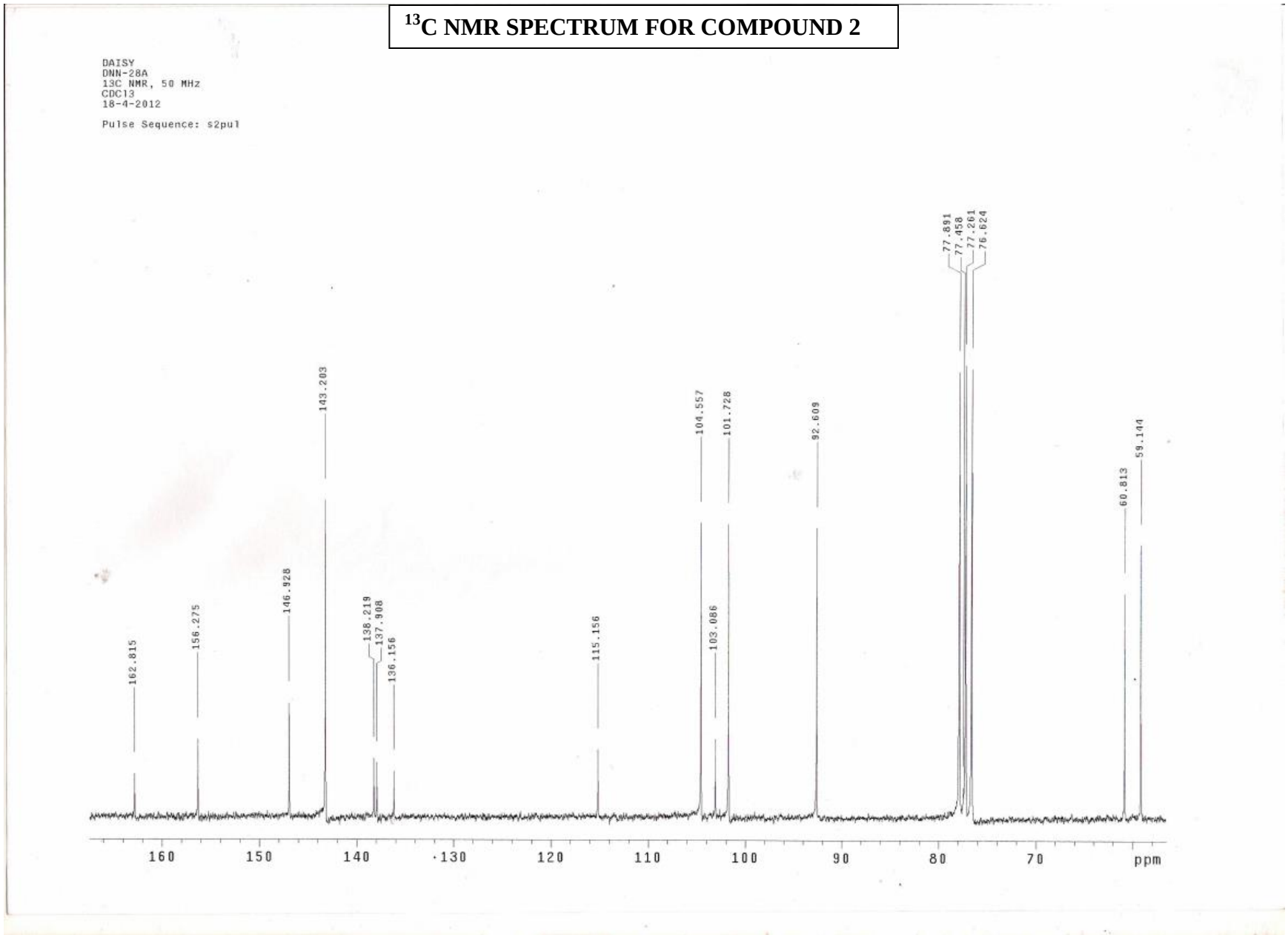
**$^{1}$H NMR SPECTRUM FOR COMPOUND 2**



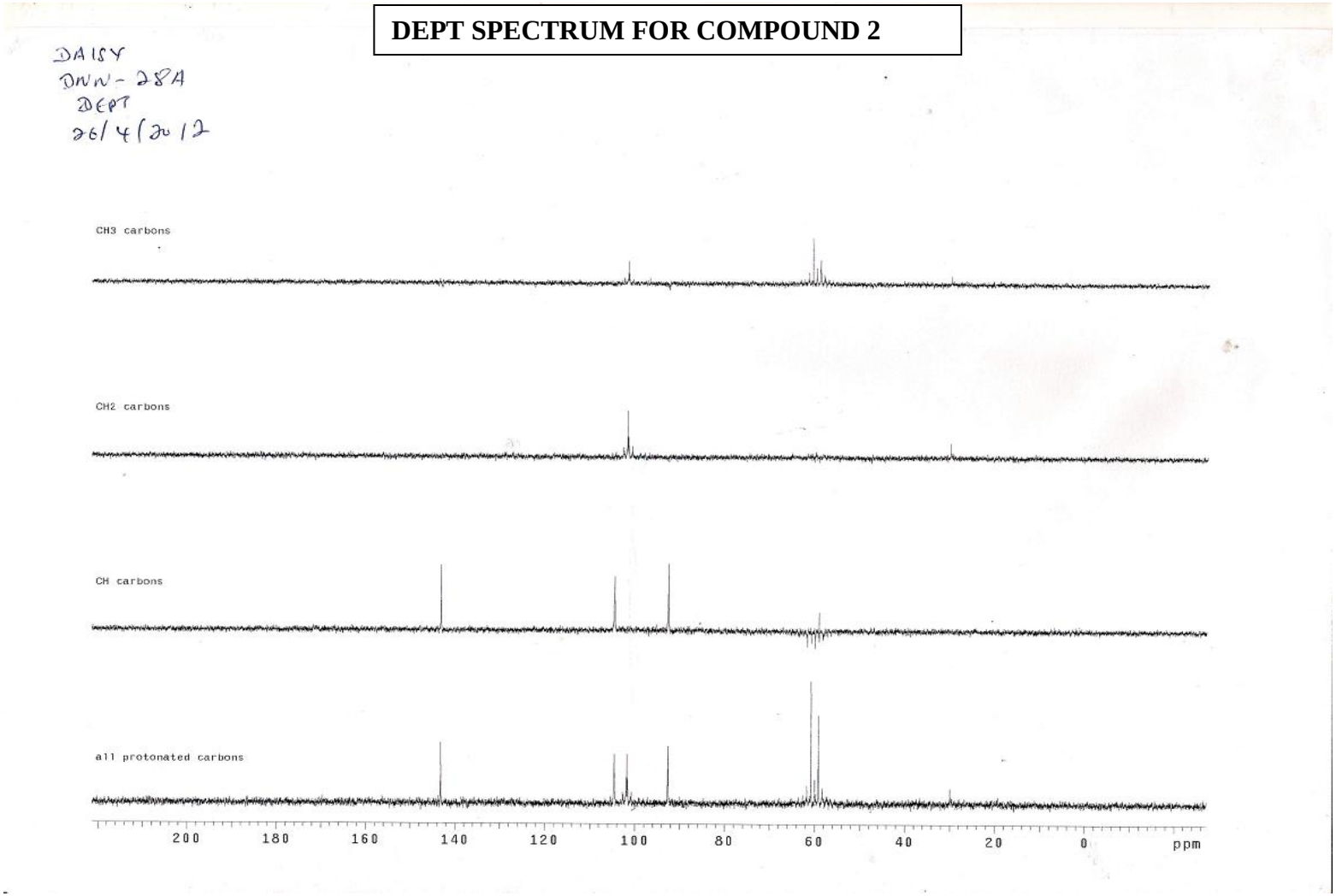
**$^{1}$H EXPANDED NMR SPECTRUM FOR COMPOUND 2**

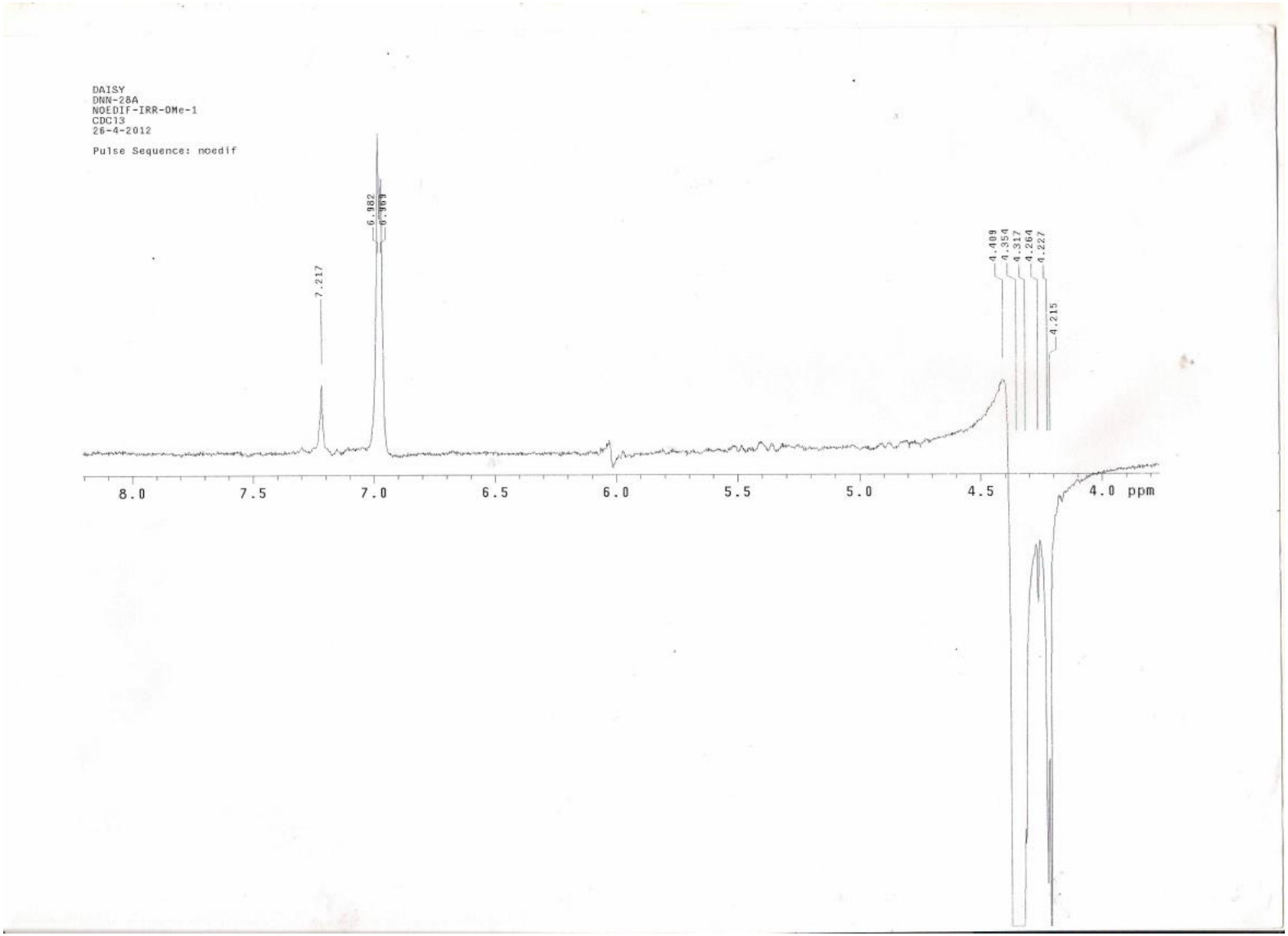


## $^{13}$C NMR SPECTRUM FOR COMPOUND 2

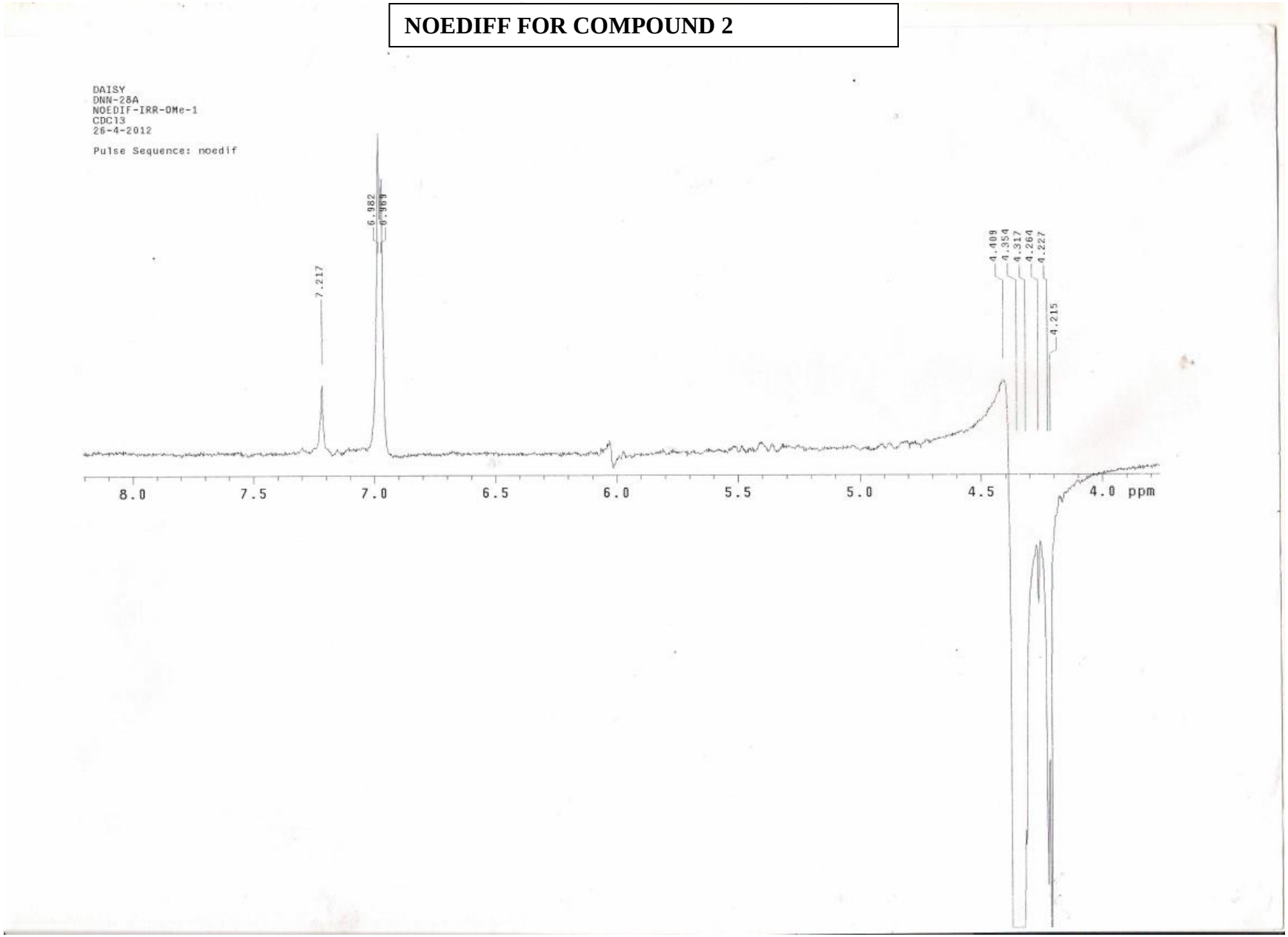


**DEPT SPECTRUM FOR COMPOUND 2**



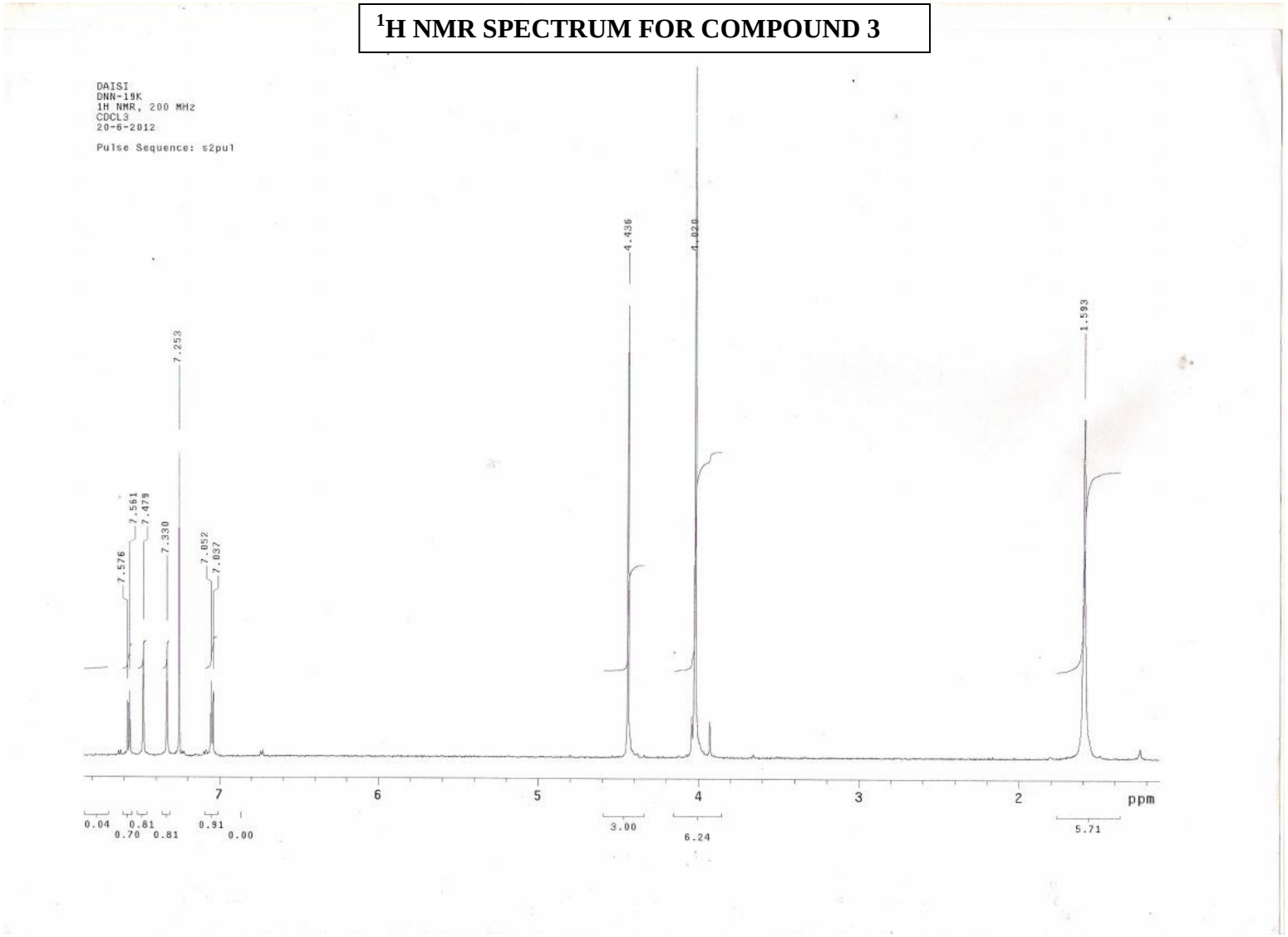

DAISY
DNN-28A
NOEDIF-IRR-OMe-1
CDCl3
26-4-2012
Pulse Sequence: noedif
7.217
6.982
6.969
4.409
4.354
4.317
4.264
4.227
4.215
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0 ppm

**NOEDIFF FOR COMPOUND 2**



## APPENDIX 3: SPECTRA FOR COMPOUND 3

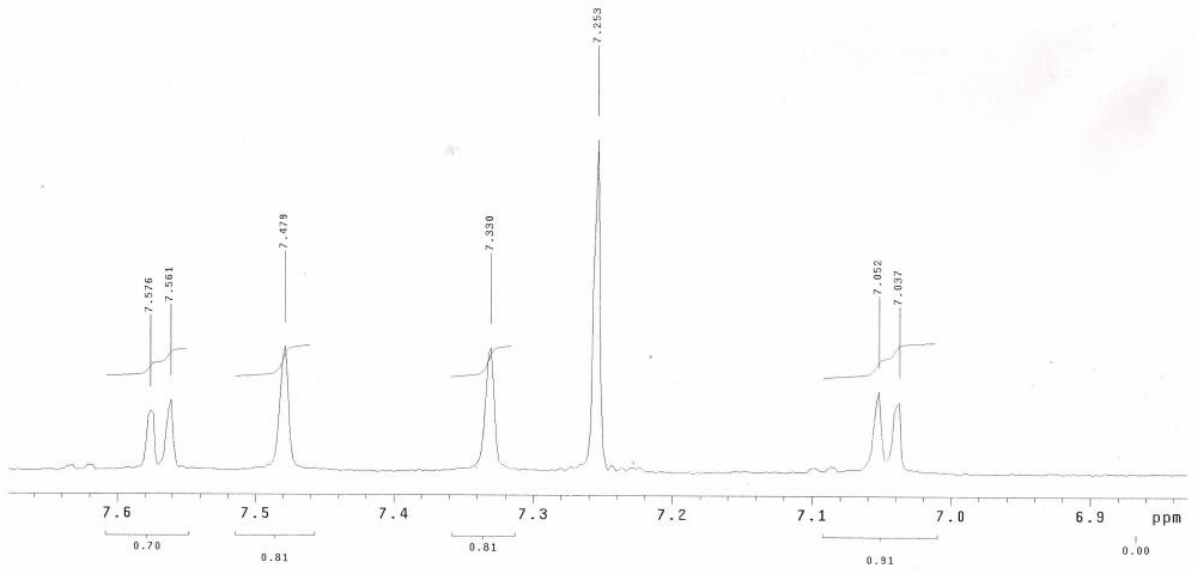
## $^{1}$H NMR SPECTRUM FOR COMPOUND 3



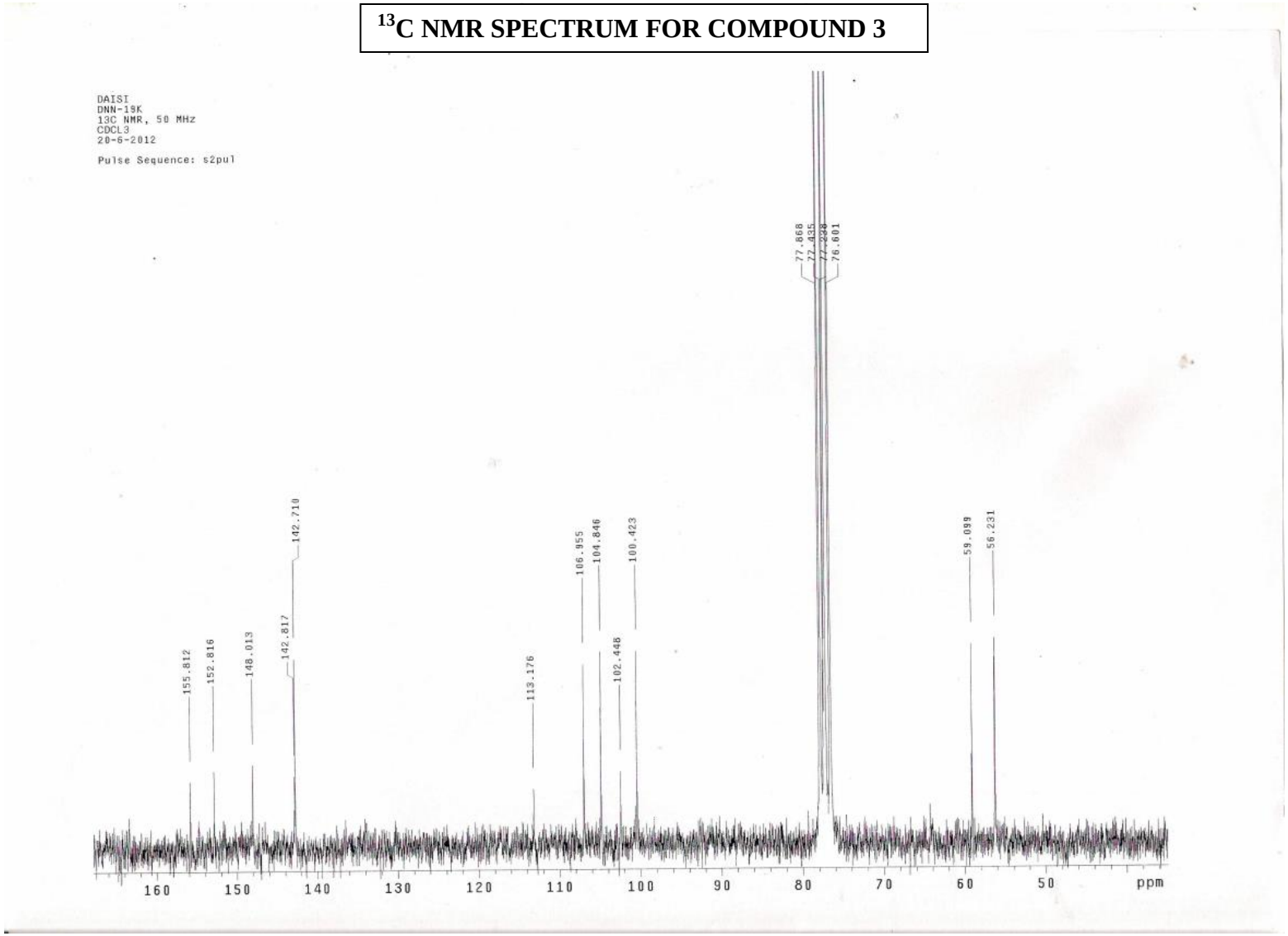
DAISI
DNN-19K
1H NMR, 200 MHz
CDCL3
20-6-2012

Pulse Sequence: s2pul

**$^{1}$H EXPANDED NMR SPECTRUM FOR COMPOUND 3**



## $^{13}$C NMR SPECTRUM FOR COMPOUND 3

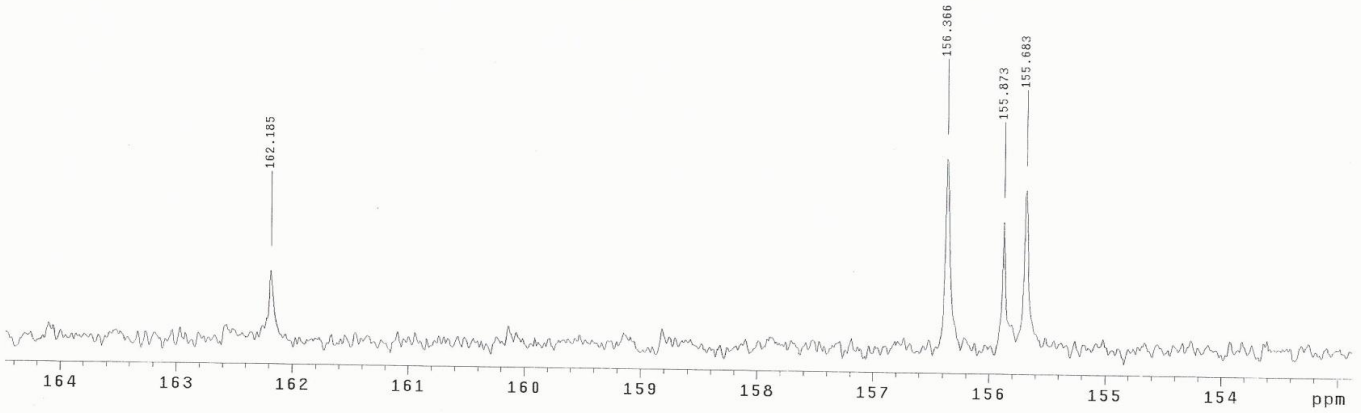


## APPENDIX 4: SPECTRA FOR COMPOUND 4

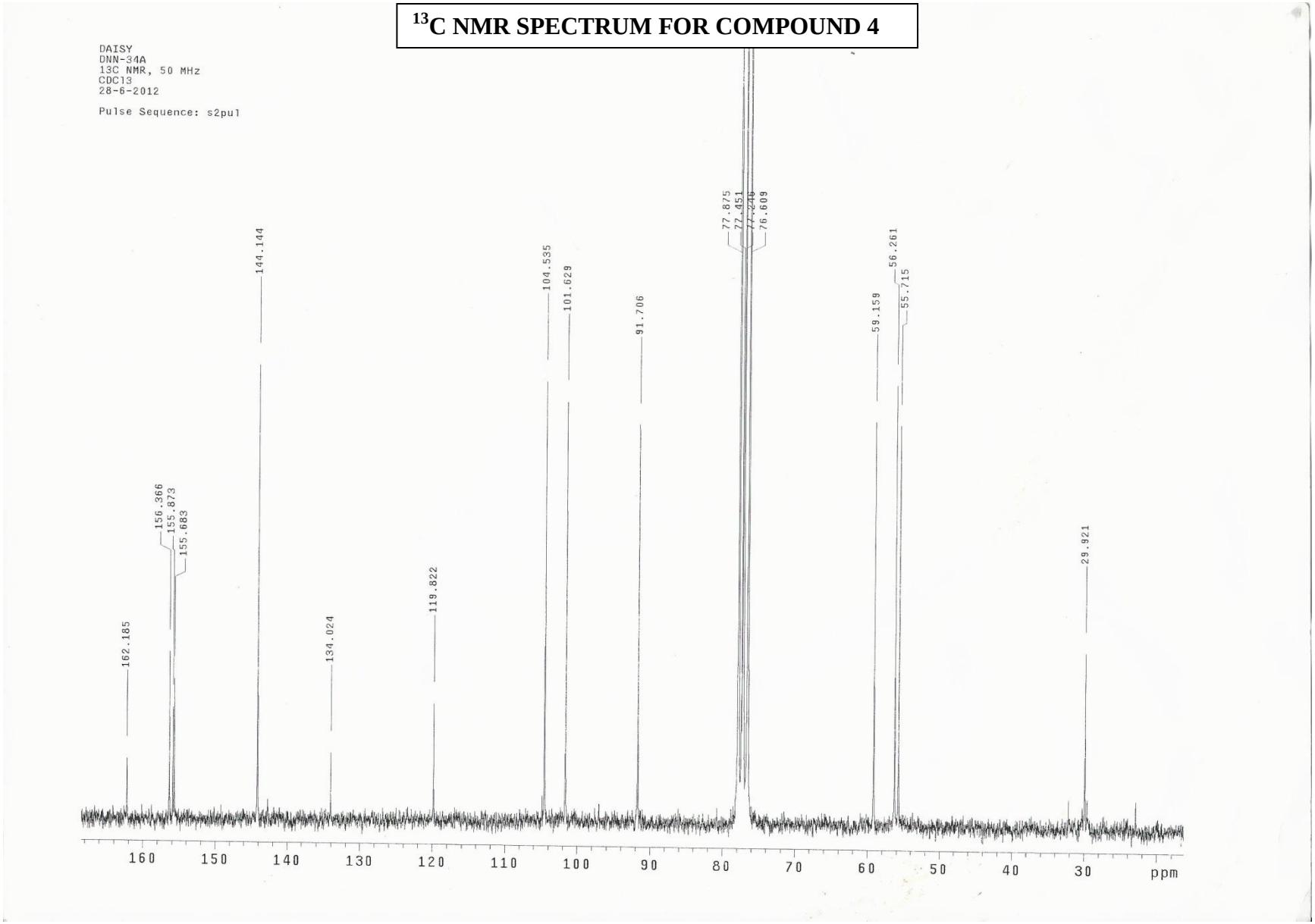
DAISY
DNN-34A
13C NMR, 50 MHz
CDCl3
28-6-2012

Pulse Sequence: s2pul

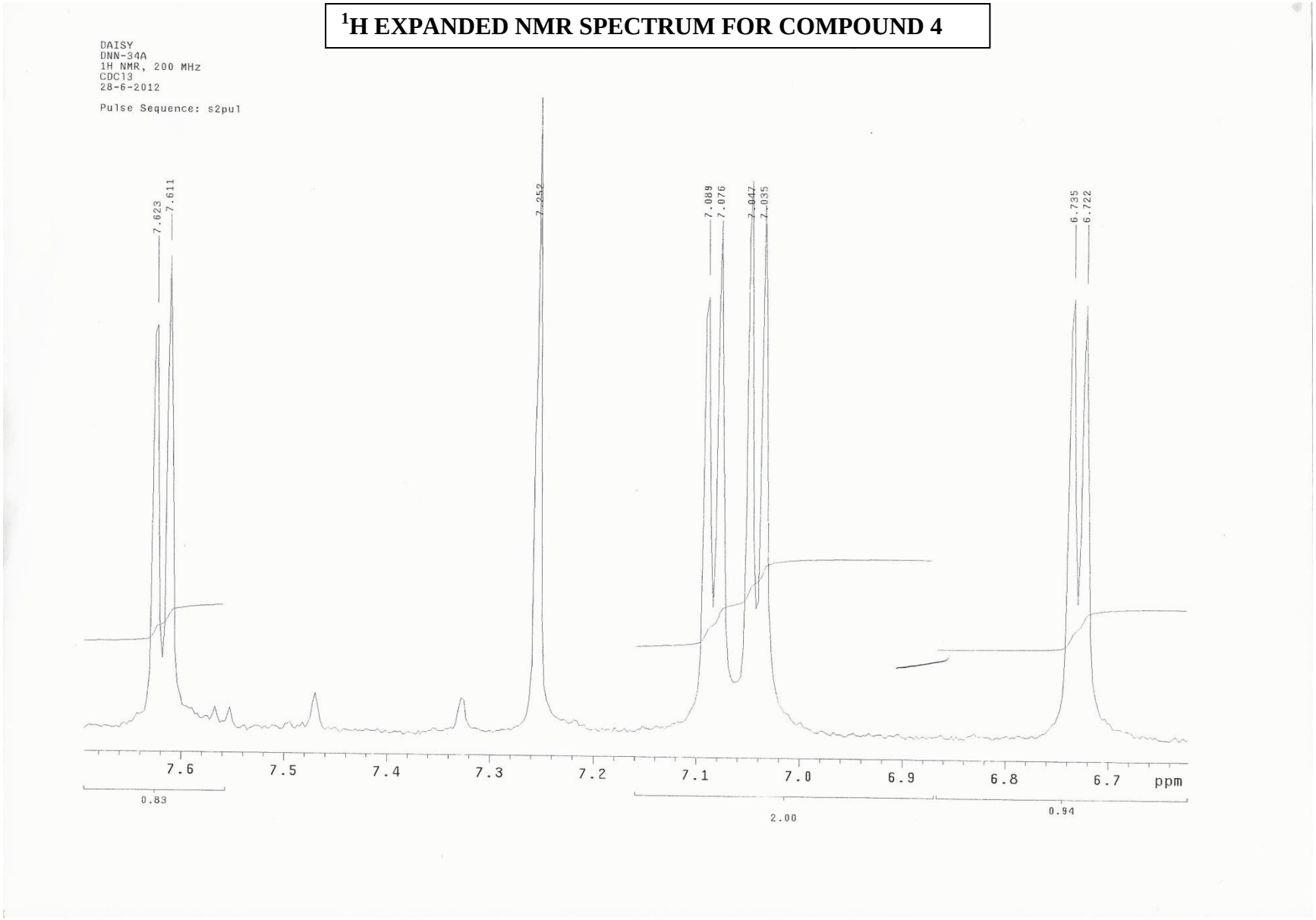
## $^{13}$C NMR SPECTRUM FPR COMPOUND 4



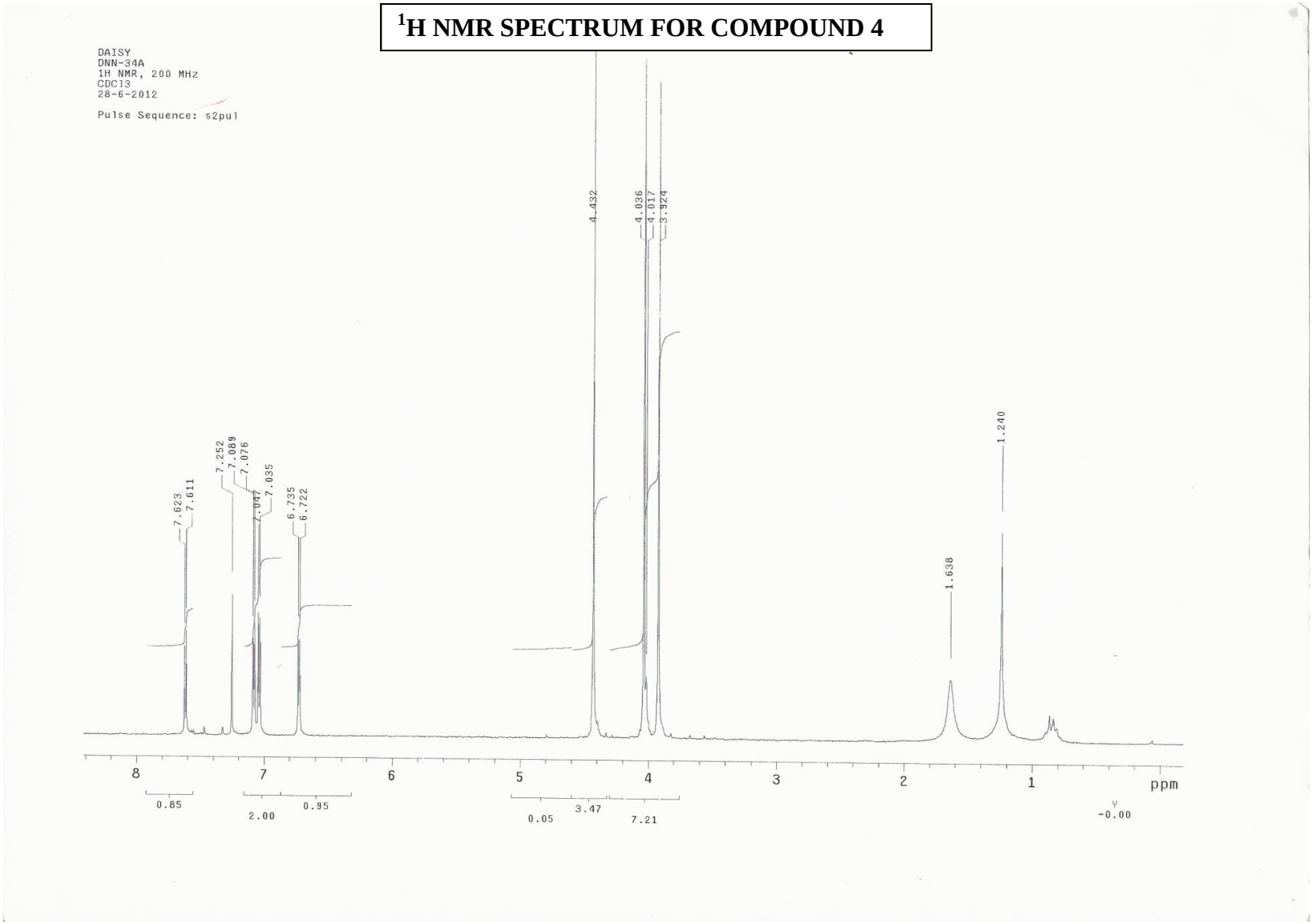
## $^{13}$C NMR SPECTRUM FOR COMPOUND 4



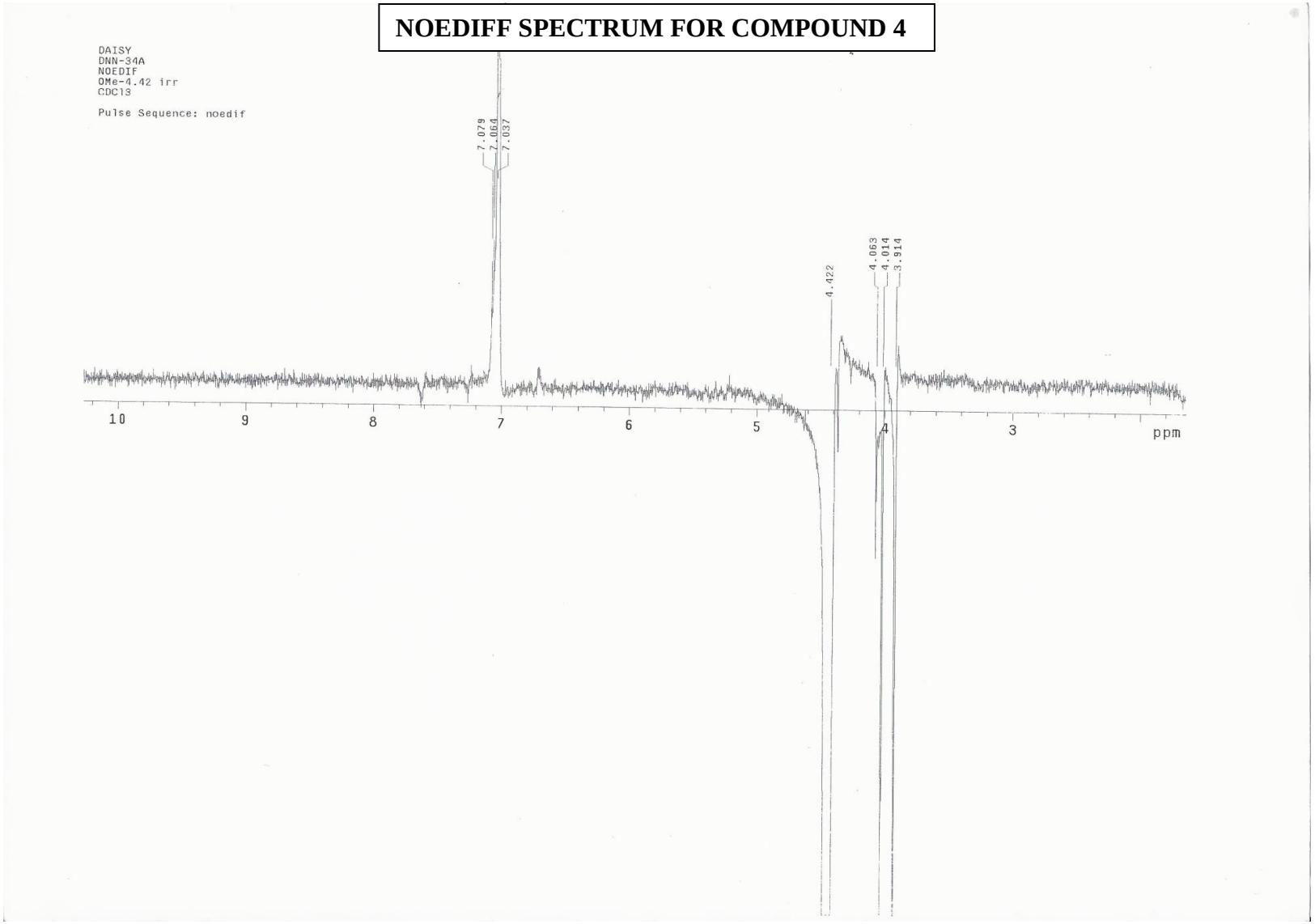
## $^{1}$H EXPANDED NMR SPECTRUM FOR COMPOUND 4



## $^{1}$H NMR SPECTRUM FOR COMPOUND 4



**NOEDIFF SPECTRUM FOR COMPOUND 4**

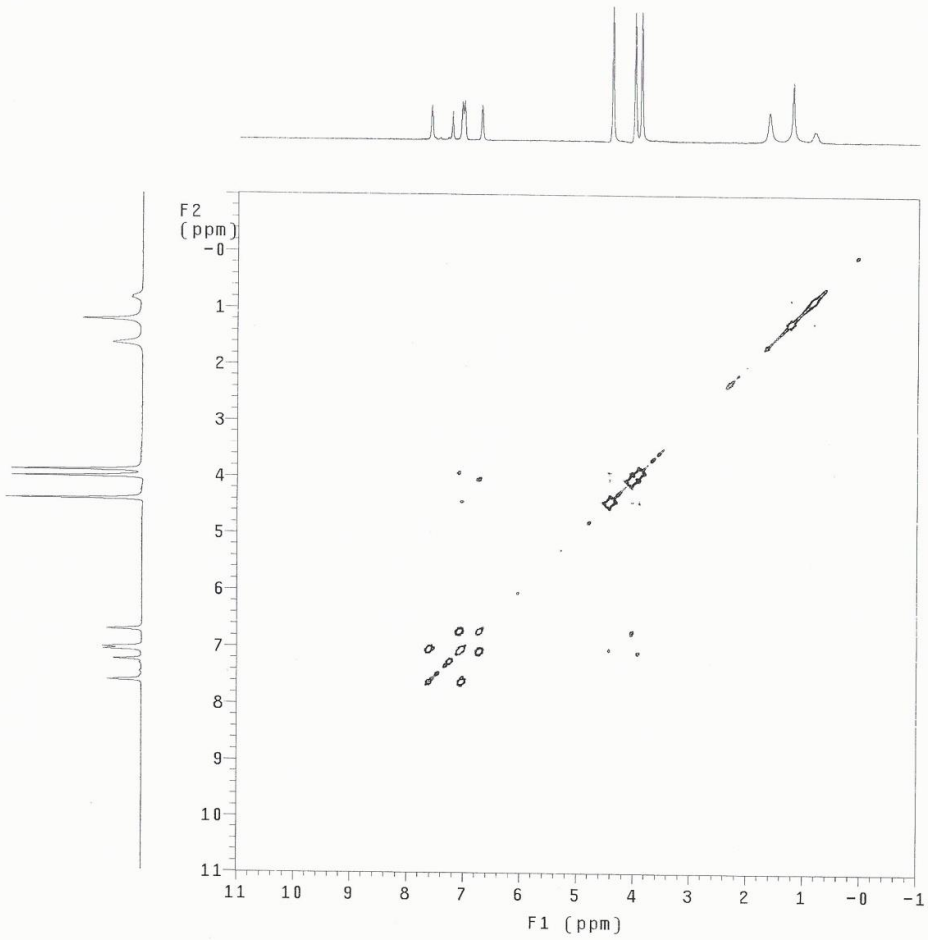


DAISY
DNN-34A

Pulse Sequence: COSY

Solvent: CDCl3
Ambient temperature
Mercury-200 "uonmr200"

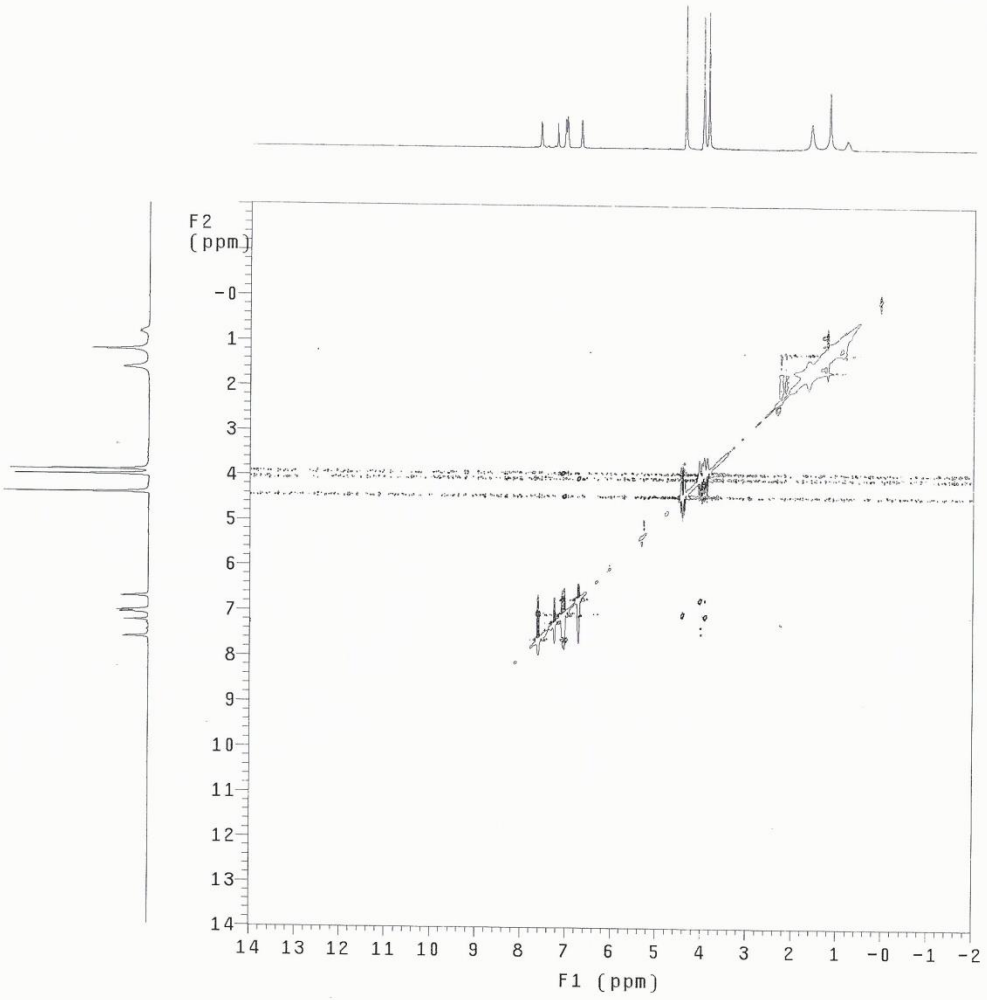
PULSE SEQUENCE: COSY
Relax. delay 1.000 sec
Acq. time 0.213 sec
Width 2399.7 Hz
2D Width 2399.7 Hz
8 repetitions
512 increments
OBSERVE H1, 199.9749908 MHz
DATA PROCESSING
Sq. sine bell 0.107 sec
F1 DATA PROCESSING
Sq. sine bell 0.213 sec
FT size 4096 x 4096
Total time 1 hr, 35 min, 38 sec



## COSY SPECTRUM FOR COMPOUND 4

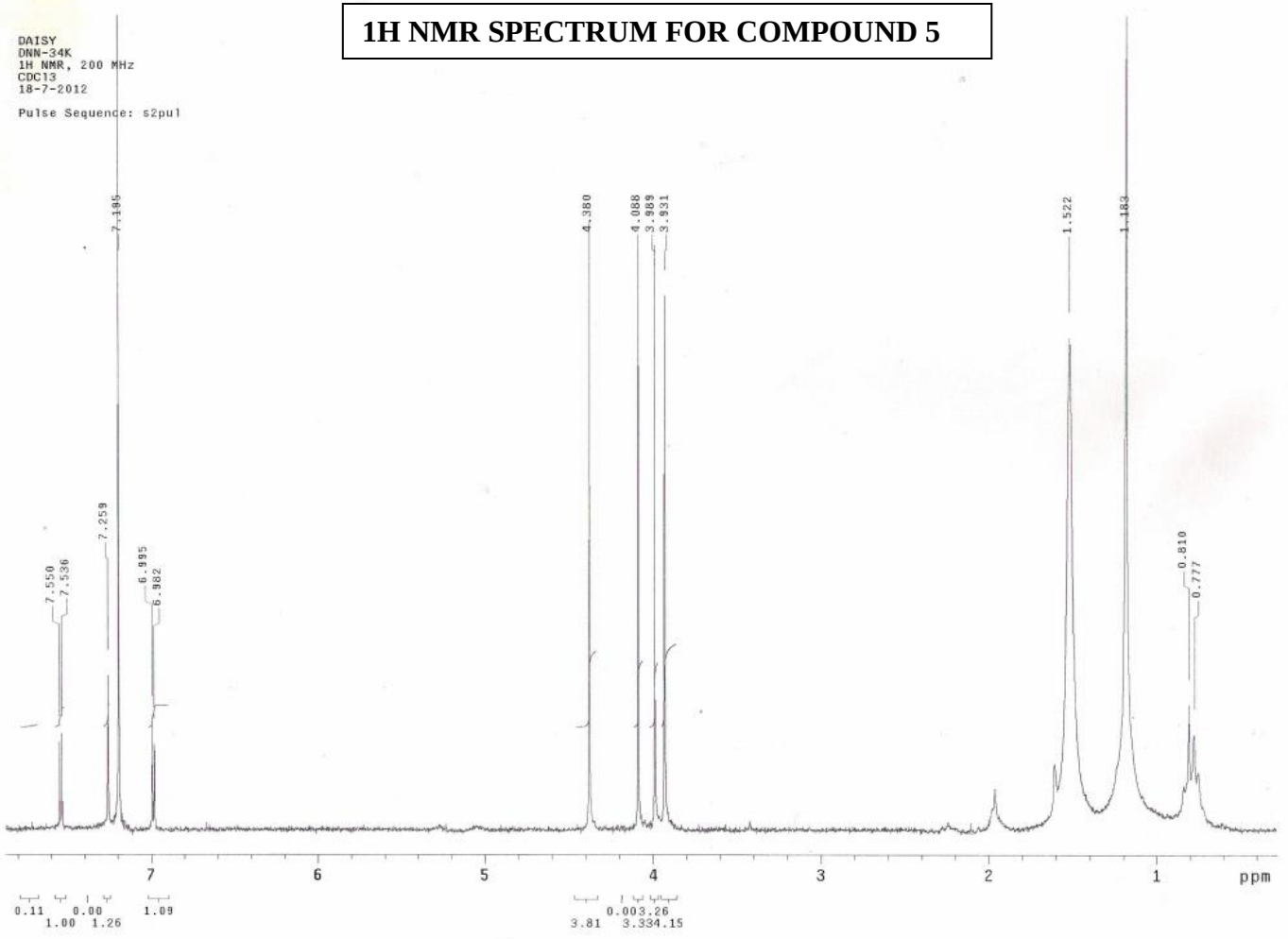
DAISY
DNN-34A

Pulse Sequence: NOESY
Solvent: CDCl3
Ambient temperature
Mercury-200 "uonnmr200"
PULSE SEQUENCE: NOESY
Relax. delay 3.000 sec
Mixing 0.500 sec
Acq. time 0.160 sec
Width 3199.6 Hz
2D Width 3199.6 Hz
32 repetitions
2 x 512 increments
OBSERVE H1, 199.9749908 MHz
DATA PROCESSING
Gauss apodization 0.074 sec
F1 DATA PROCESSING
Gauss apodization 0.148 sec
FT size 4096 x 4096
Total time 34 hr, 32 min, 2 sec

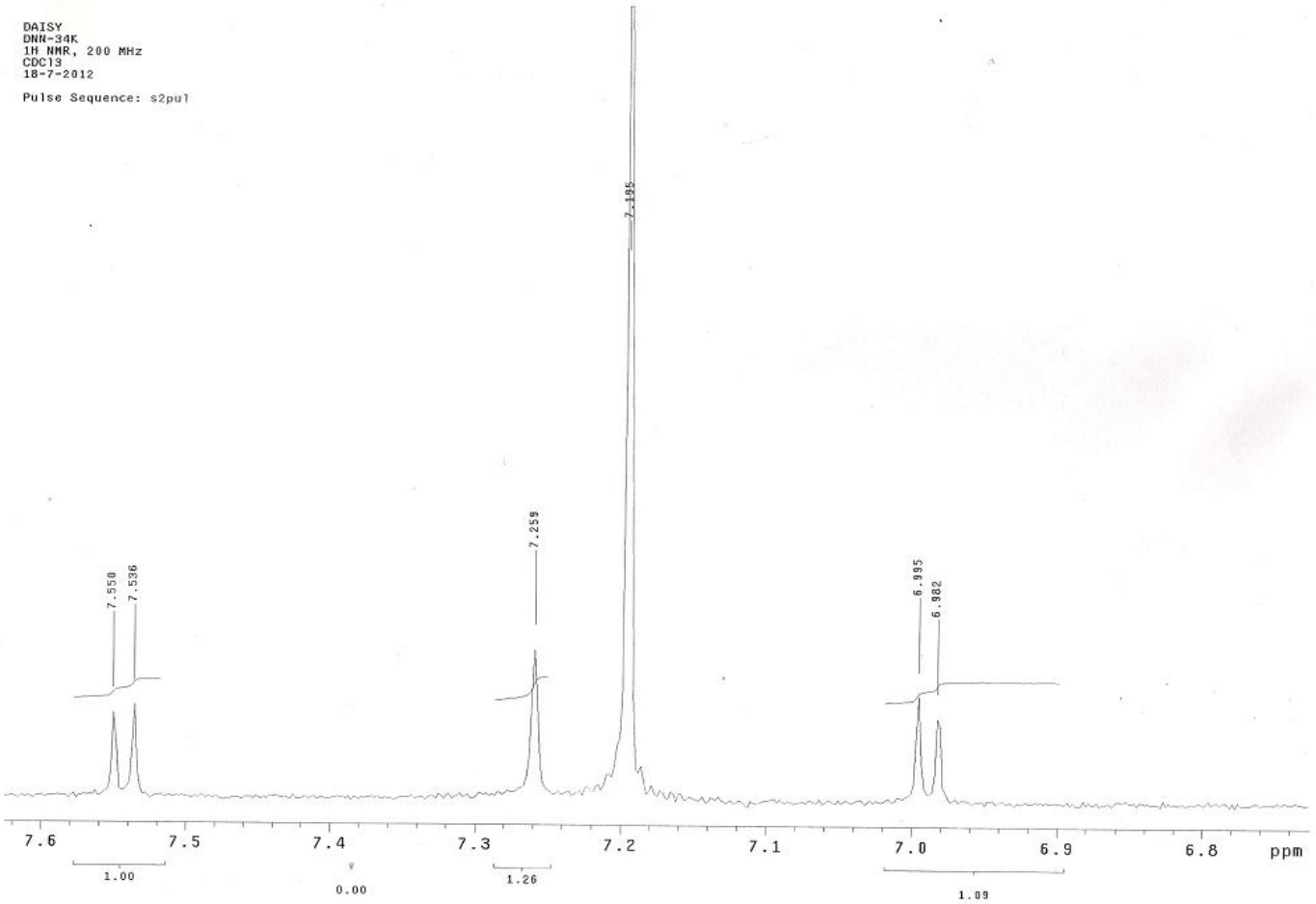


## APPENDIX 5: SPECTRA FOR COMPOUND 5

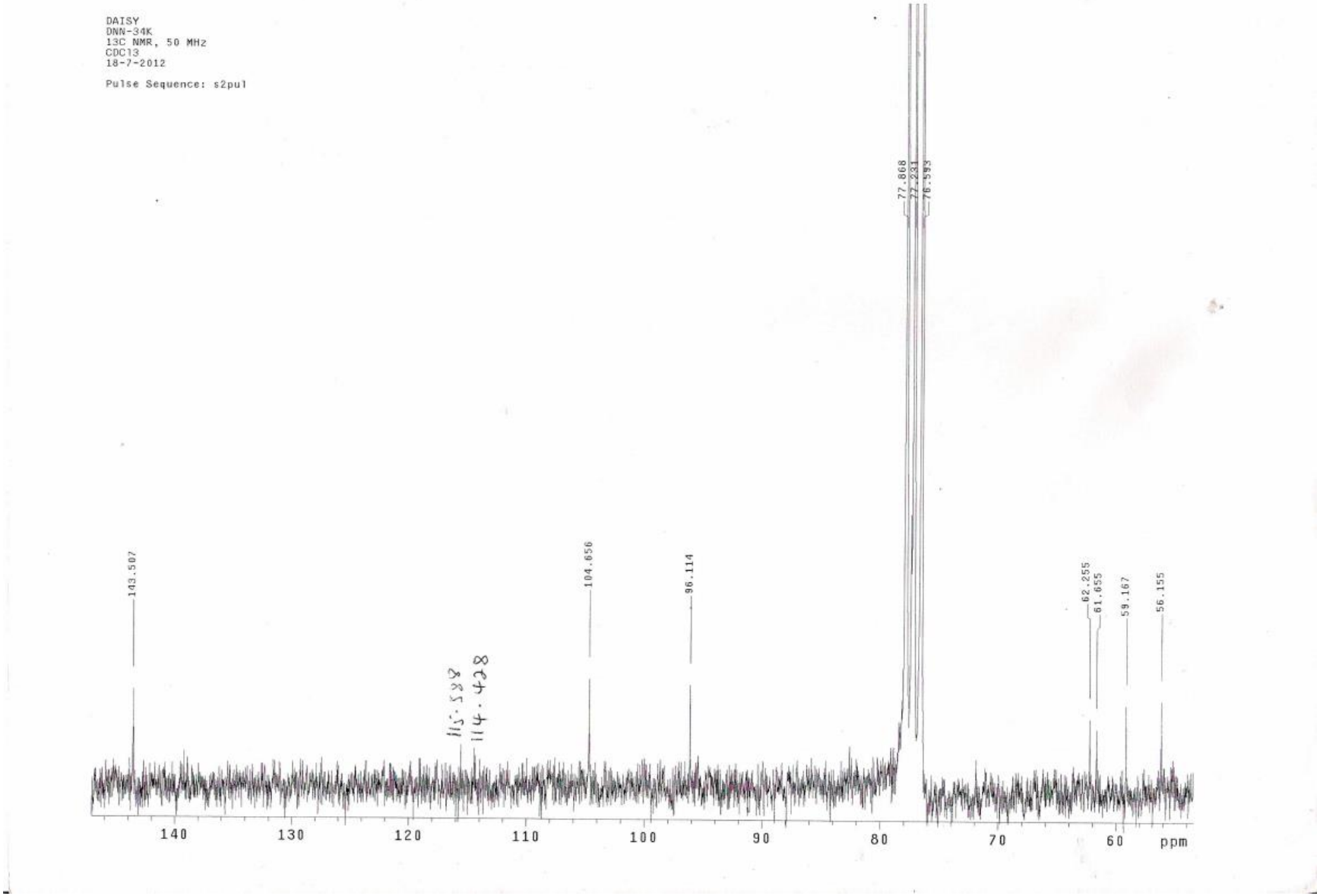
**1H NMR SPECTRUM FOR COMPOUND 5**



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



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

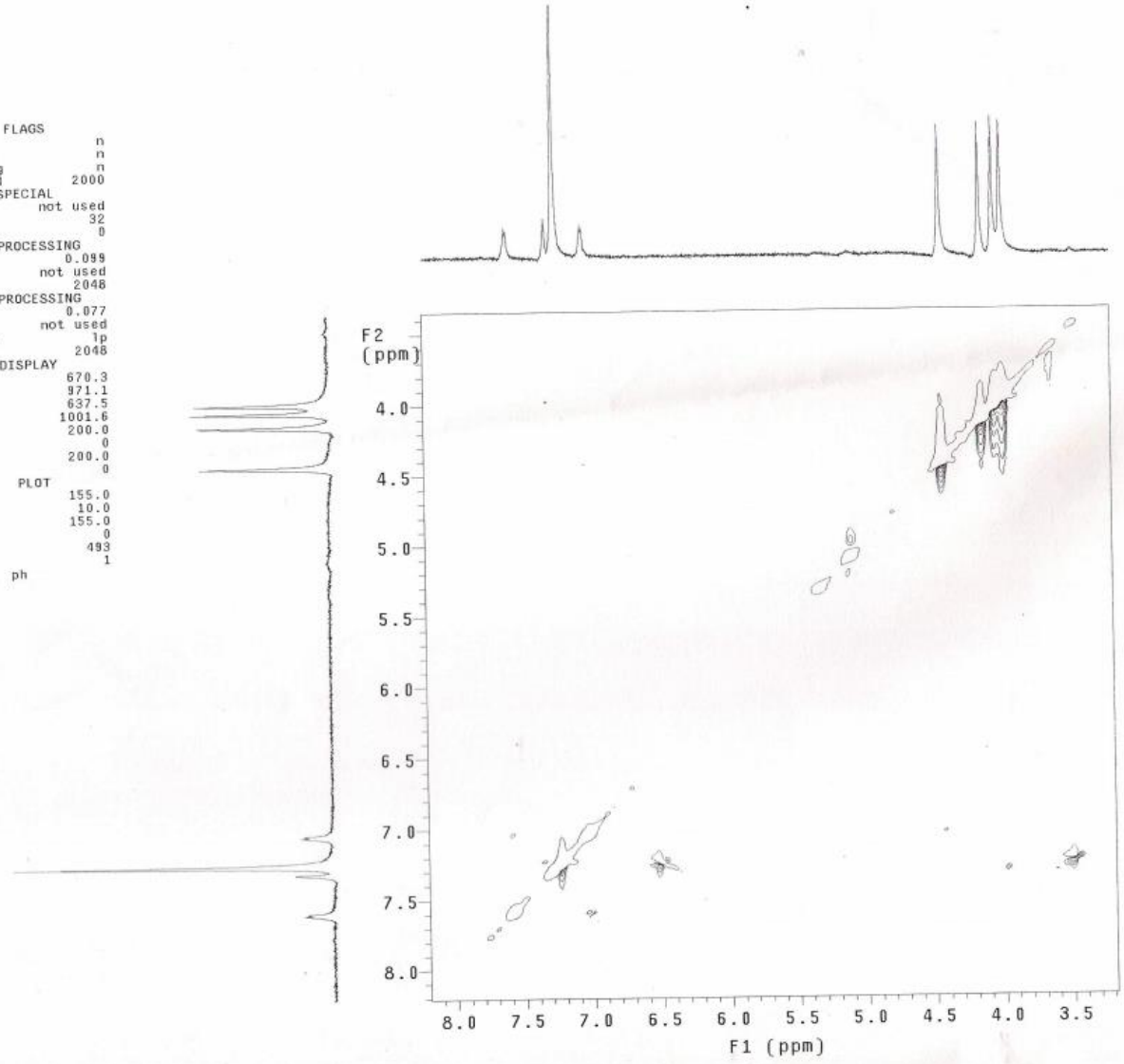


## NOESY SPECTRUM FOR COMPOUND 5

DAISY
DNN-34K

exp33 NOESY

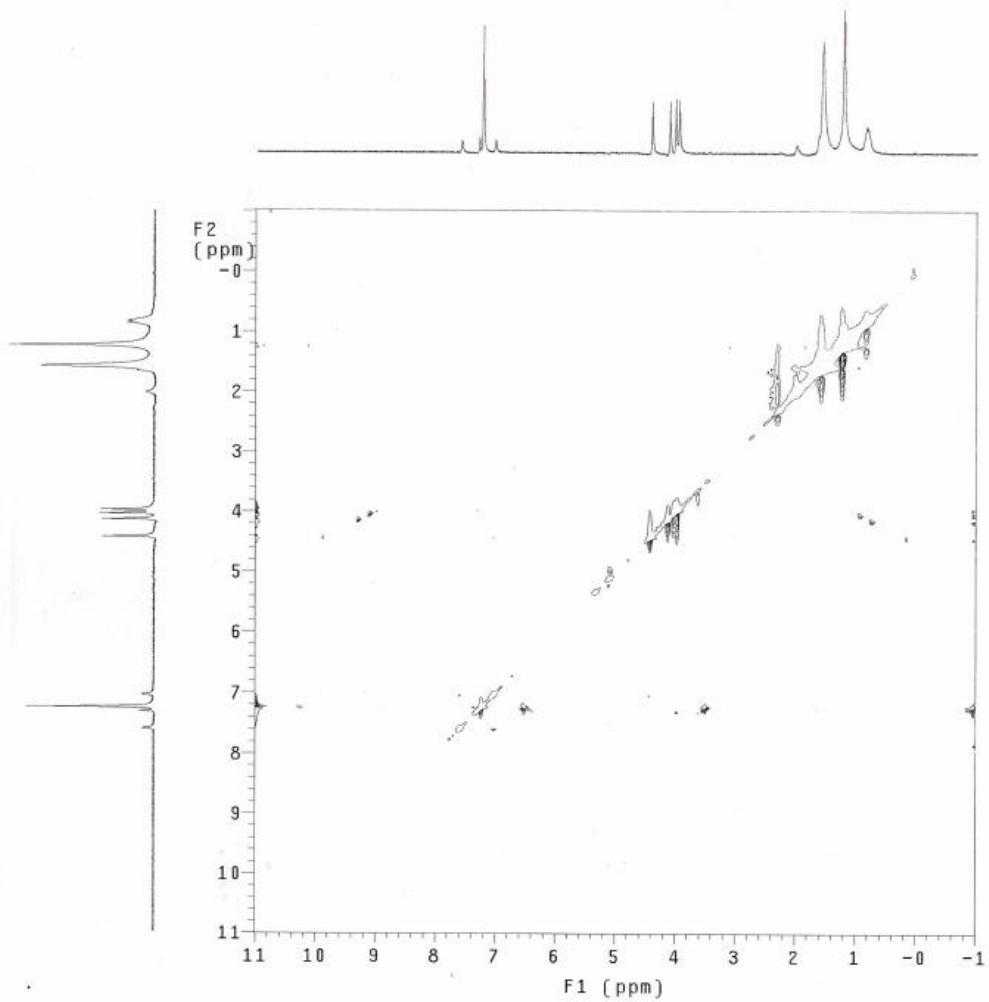
| SAMPLE | | FLAGS | |
|---|---|---|---|
| date | Jul 19 2012 | hs | n |
| solvent | CDCl3 | sspul | n |
| sample | DAISY-DNN-3~ 4K_19Jul2012 | PFGflg | n |
| | | hsglvl | 2000 |
| ACQUISITION | | SPECIAL | |
| sw | 2399.7 | temp | not used |
| at | 0.213 | gain | 32 |
| np | 1024 | spin | 0 |
| fb | 1400 | F2 PROCESSING | |
| ss | 32 | gf | 0.099 |
| d1 | 2.000 | gfs | not used |
| nt | 16 | fn | 2048 |
| 2D ACQUISITION | | F1 PROCESSING | |
| sw1 | 2399.7 | gf1 | 0.077 |
| ni | 200 | gfs1 | not used |
| TRANSMITTER | | proc1 | lp |
| tn | H1 | fn1 | 2048 |
| sfrq | 199.976 | DISPLAY | |
| tof | 41.1 | sp | 670.3 |
| tpwr | 54 | wp | 971.1 |
| pw | 17.500 | sp1 | 637.5 |
| NOESY | | wp1 | 1001.6 |
| mix | 0.200 | rf1 | 200.0 |
| PRESATURATION | | rfp | 0 |
| satmode | nnnn | rfl1 | 200.0 |
| satpwr | 0 | rfp1 | 0 |
| satdly | 0 | PLOT | |
| satfrq | 0 | wc | 155.0 |
| DECOUPLER | | sc | 10.0 |
| dn | C13 | wc2 | 155.0 |
| dm | nnn | sc2 | 0 |
| | | vs | 493 |
| | | th | 1 |
| | | ai ph | |



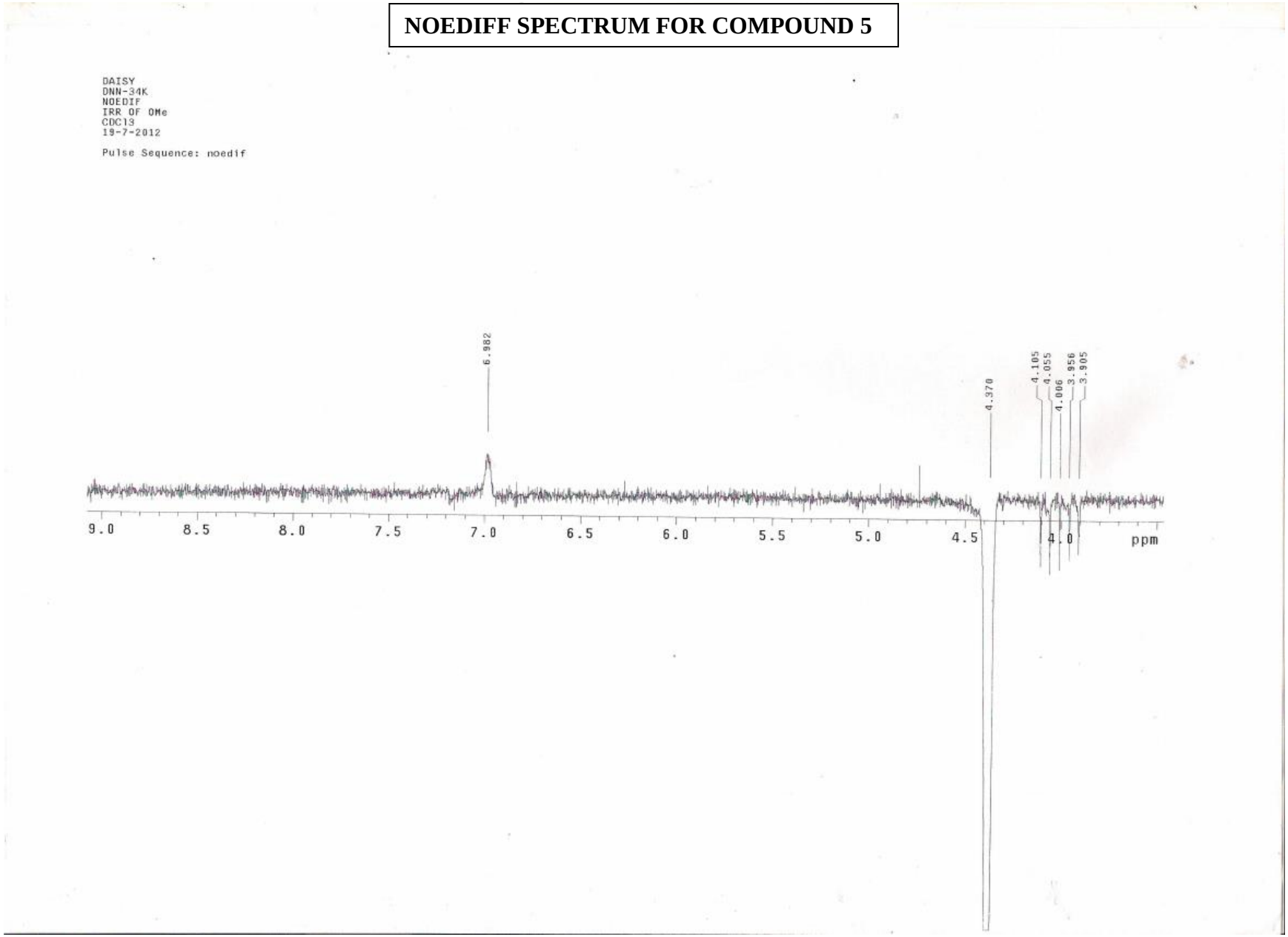
**NOESY SPECTRUM FOR COMPOUND 5**

DAISY
DNN-34K

Pulse Sequence: NOESY
Solvent: CDCl3
Ambient temperature
Mercury-200 "uonnmr200"
PULSE SEQUENCE: NOESY
Relax. delay 2.000 sec
Mixing 0.200 sec
Acq. time 0.213 sec
Width 2399.7 Hz
2D Width 2399.7 Hz
16 repetitions
2 x 200 increments
OBSERVE H1, 199.9749908 MHz
DATA PROCESSING
Gauss apodization 0.099 sec
F1 DATA PROCESSING
Gauss apodization 0.077 sec
FT size 2048 x 2048
Total time 4 hr, 28 min, 40 sec

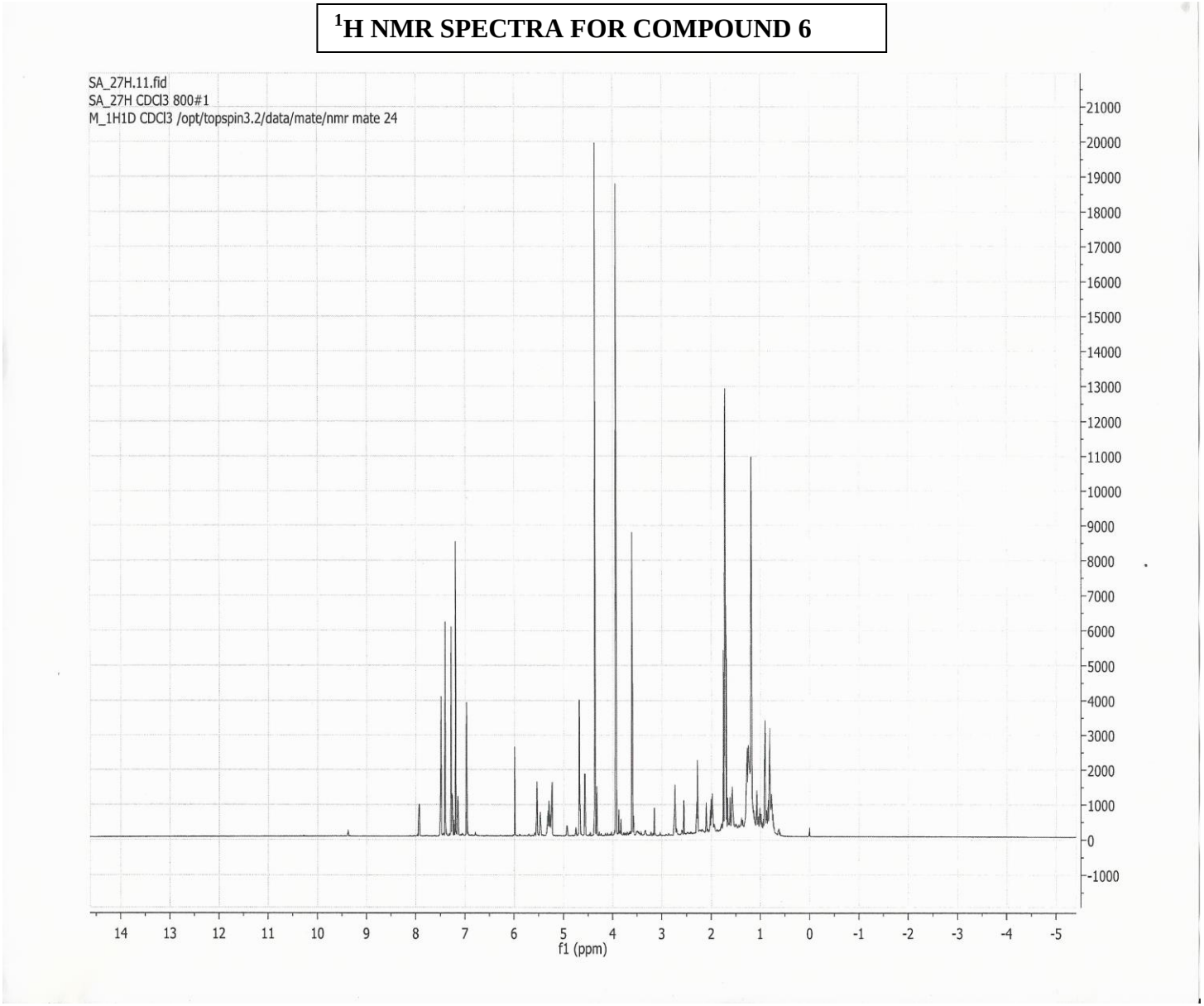


**NOEDIFF SPECTRUM FOR COMPOUND 5**

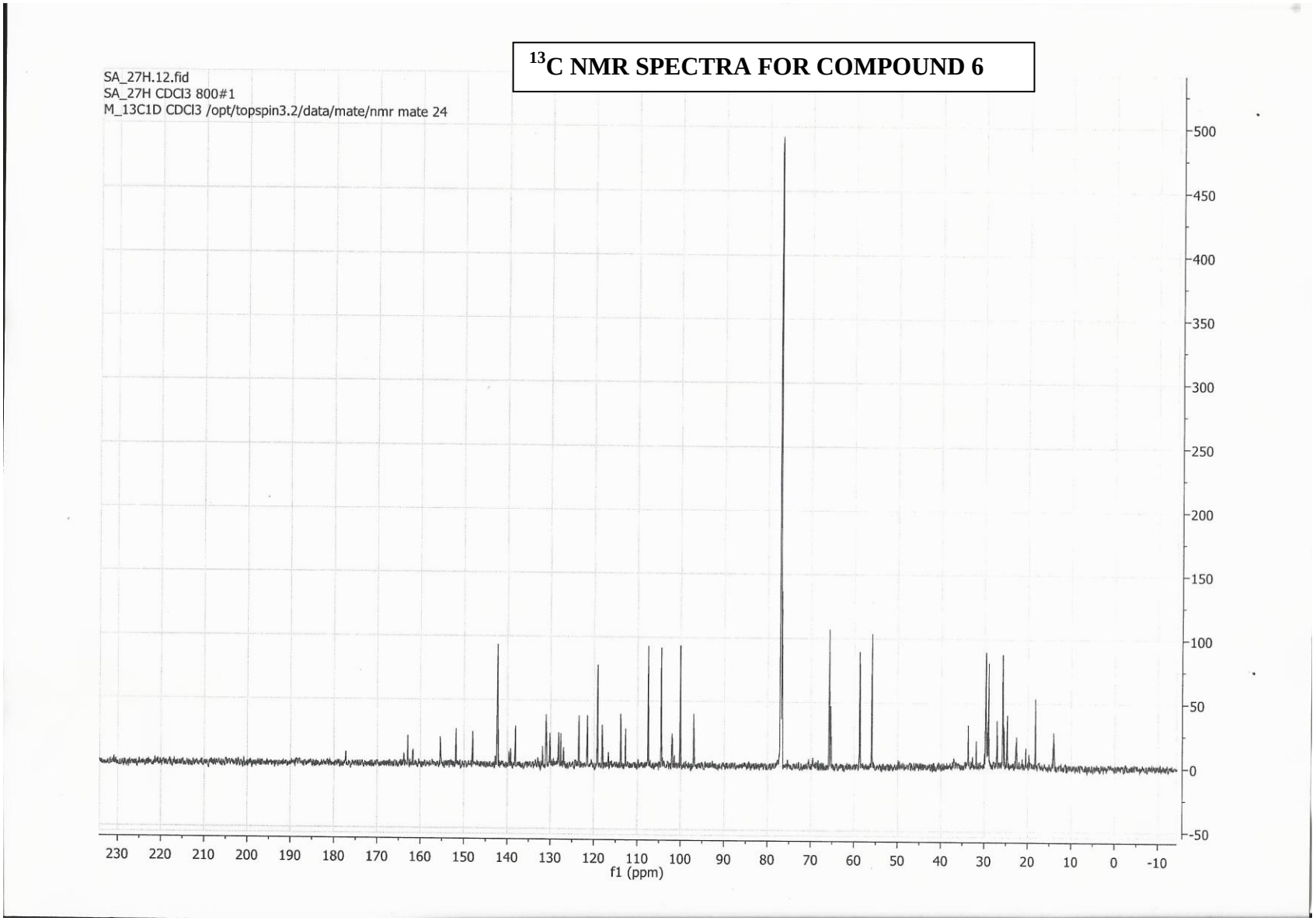


## APPENDIX 6: SPECTRA FOR COMPOUND 6

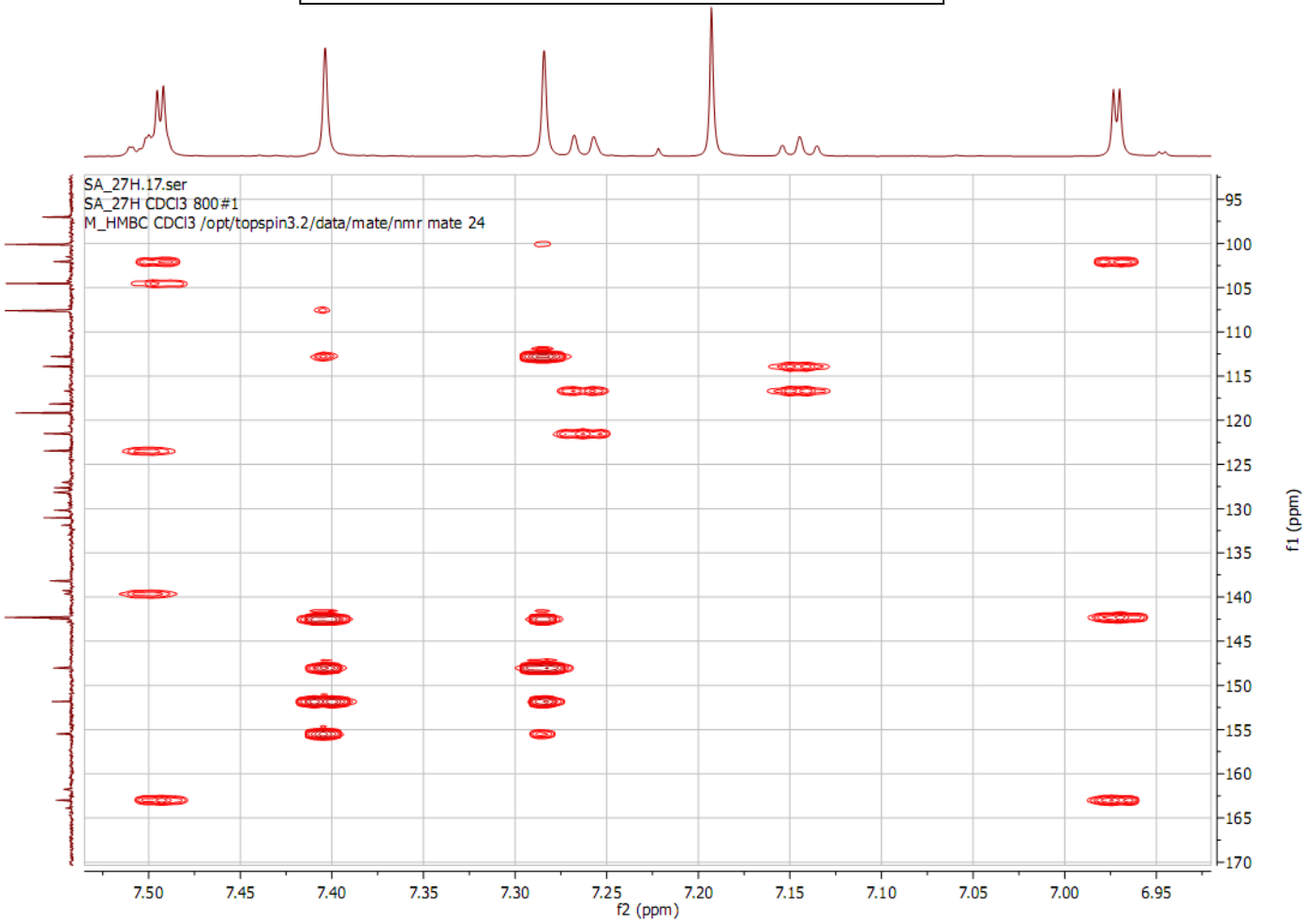
## $^{1}$H NMR SPECTRA FOR COMPOUND 6



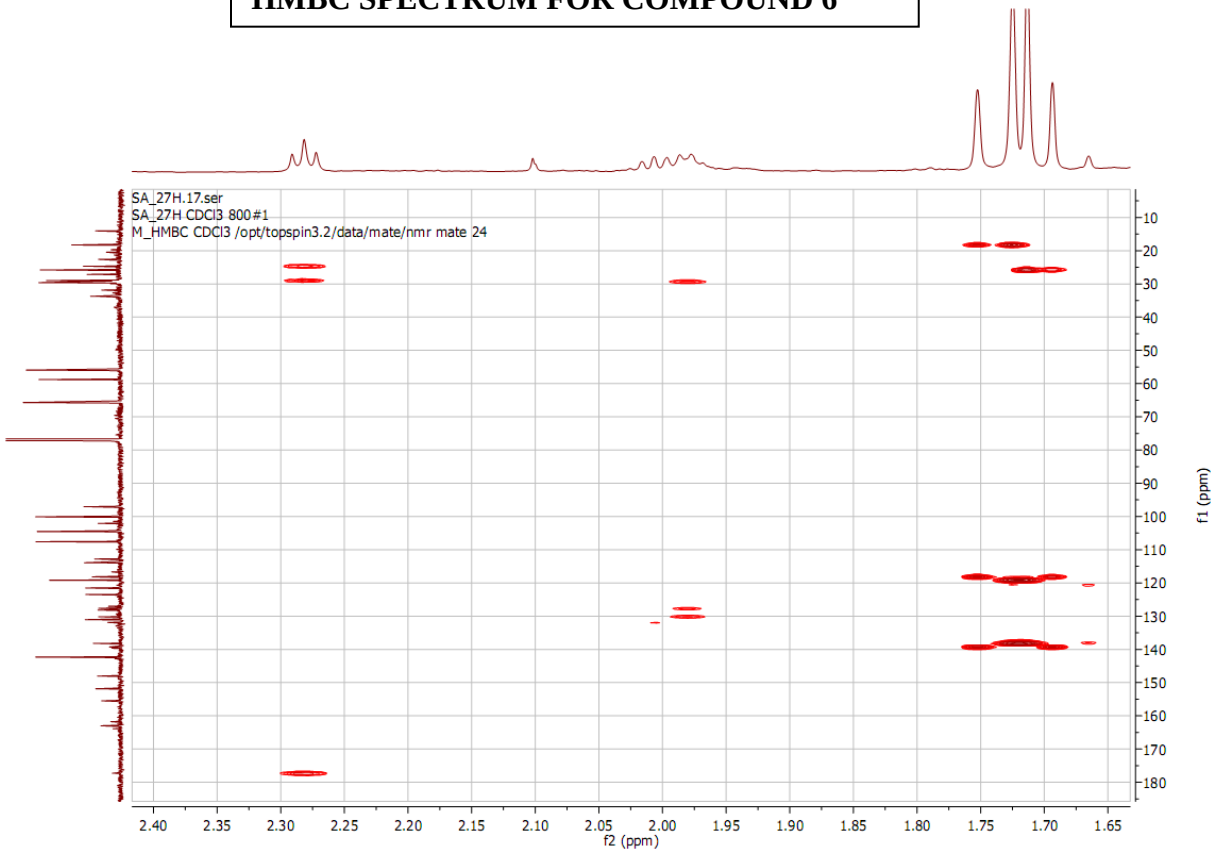
## $^{13}$C NMR SPECTRA FOR COMPOUND 6



**HMBC SPECTRUM FOR COMPOUND 6**

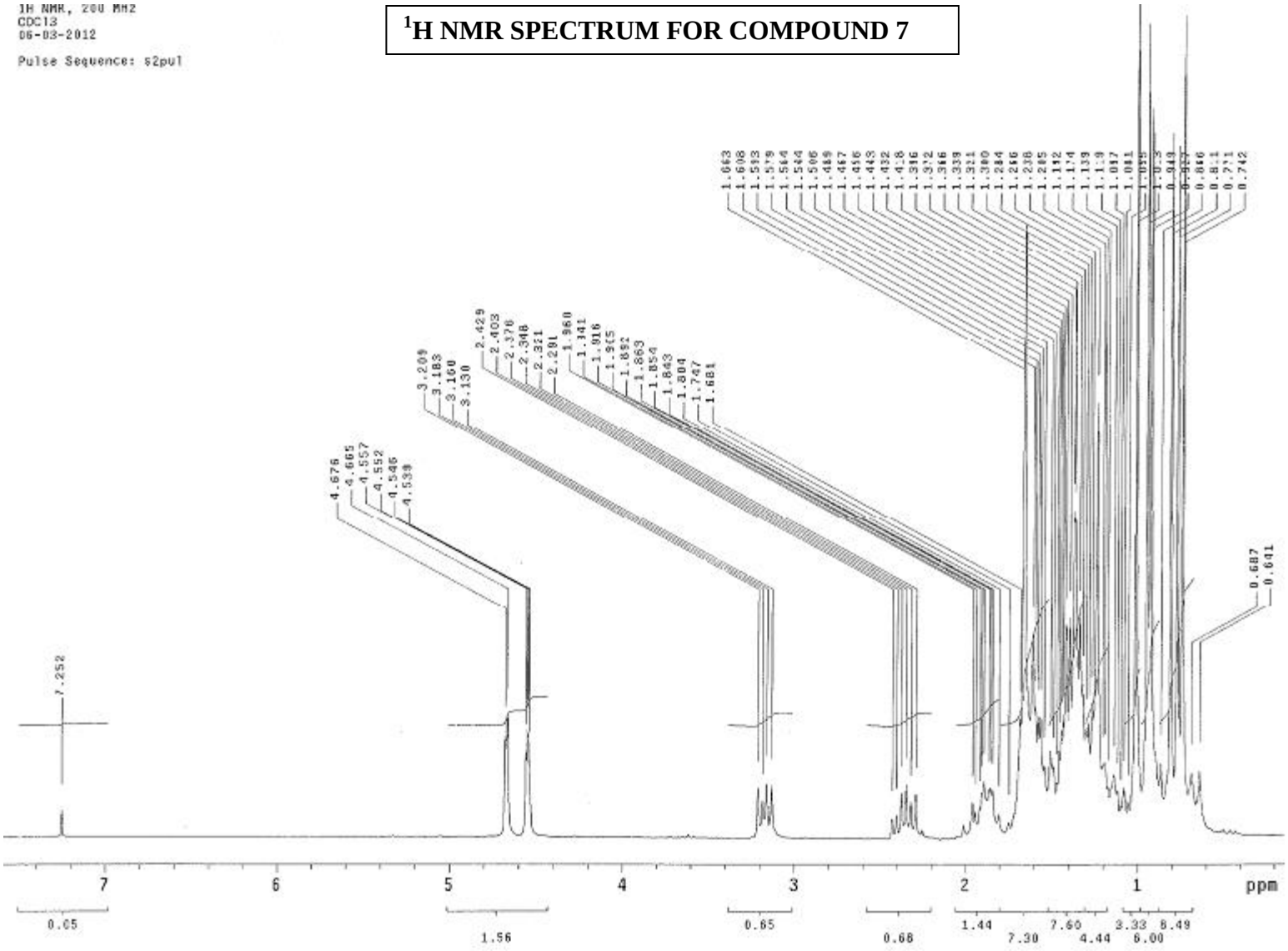


**HMBC SPECTRUM FOR COMPOUND 6**

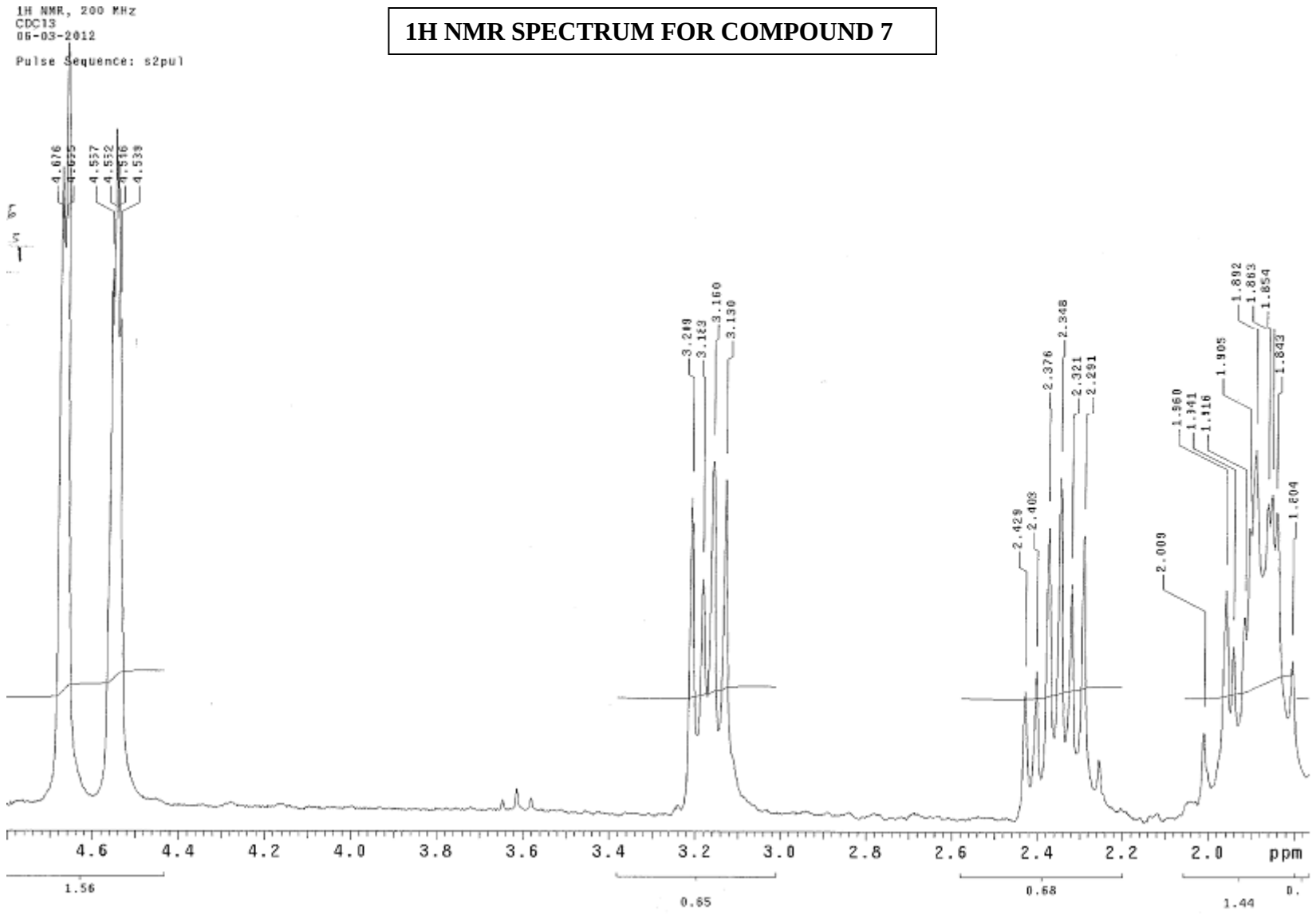


## APPENDIX 7: SPECTRA FOR COMPOUND 7

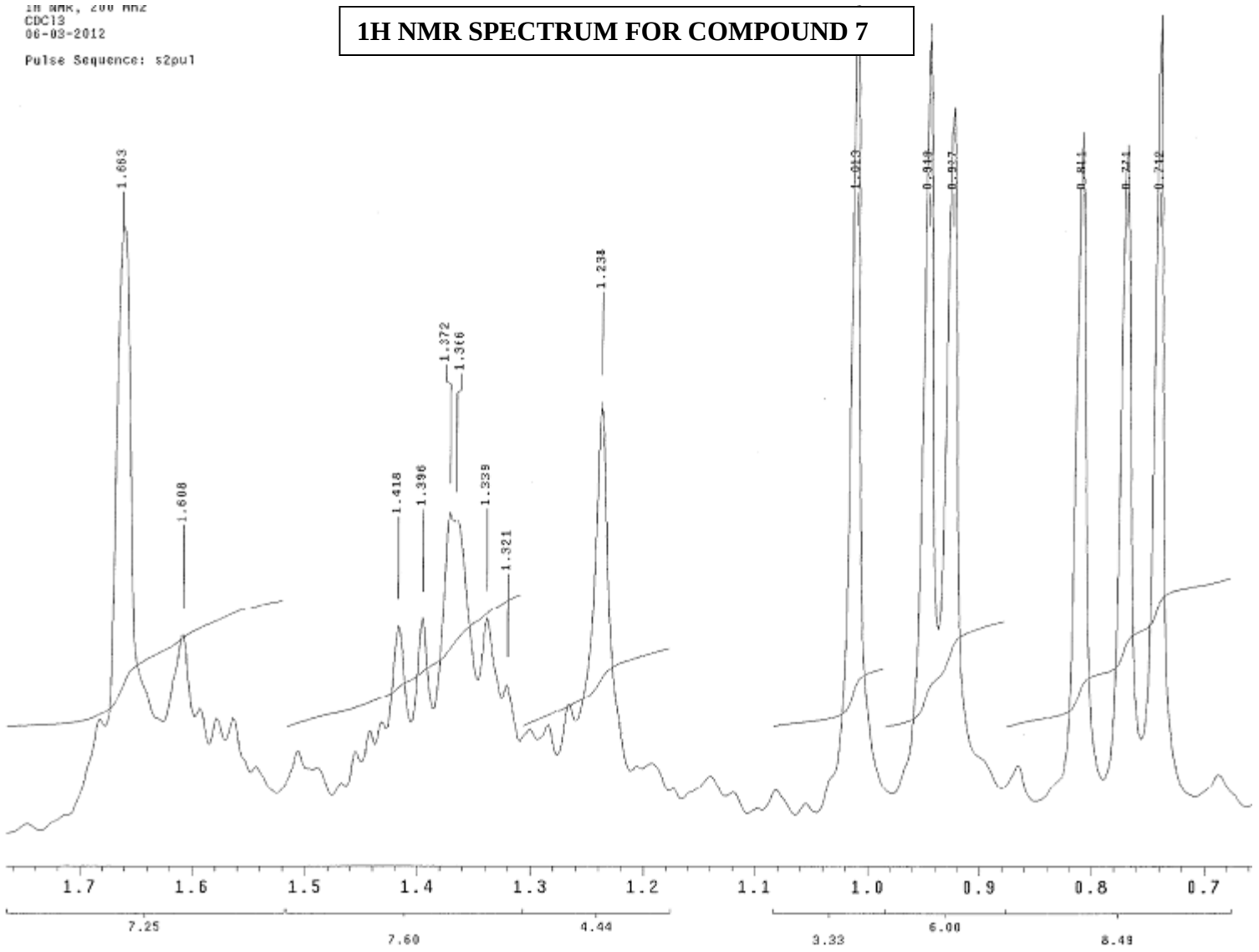
**$^{1}$H NMR SPECTRUM FOR COMPOUND 7**



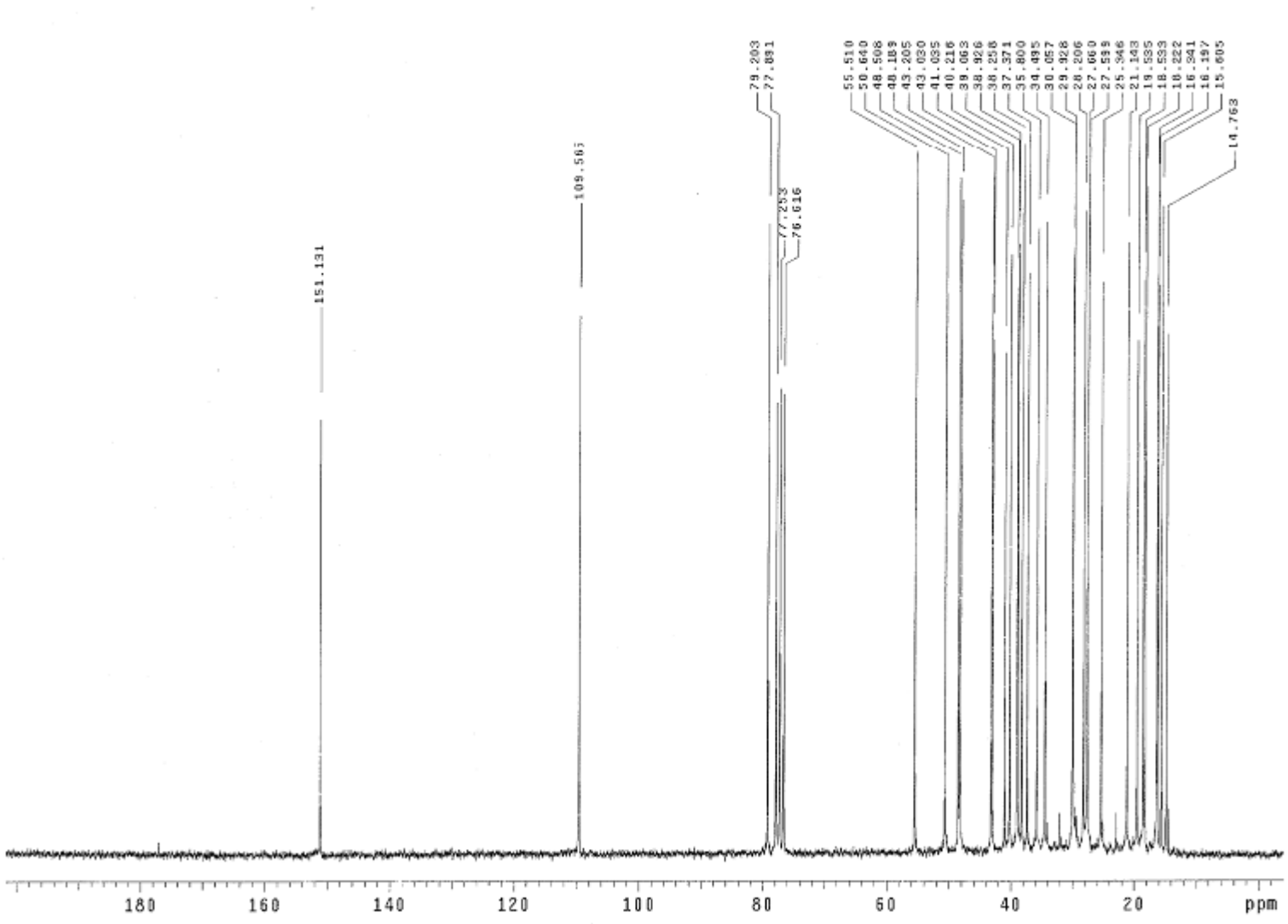
**1H NMR SPECTRUM FOR COMPOUND 7**



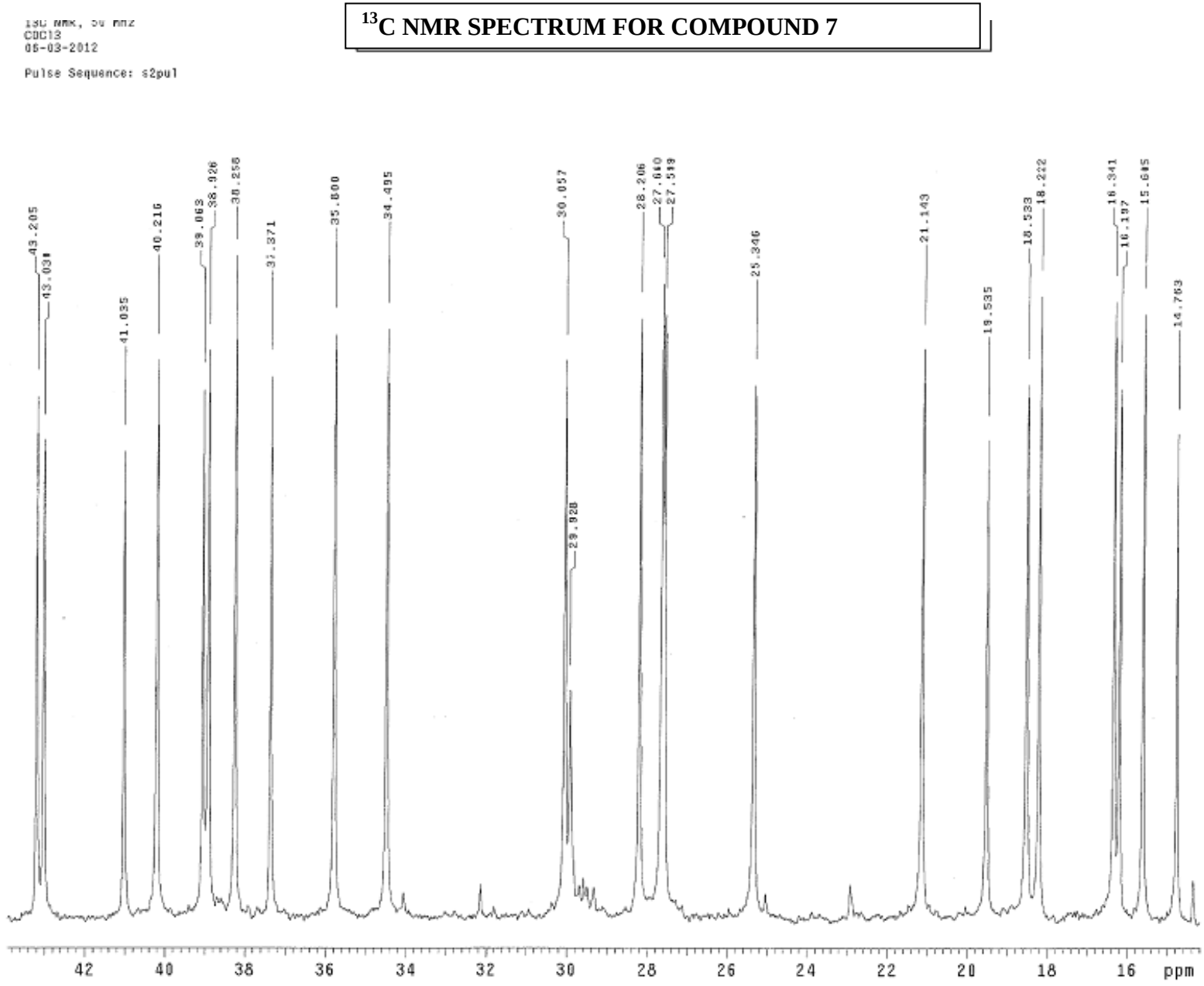
**1H NMR SPECTRUM FOR COMPOUND 7**



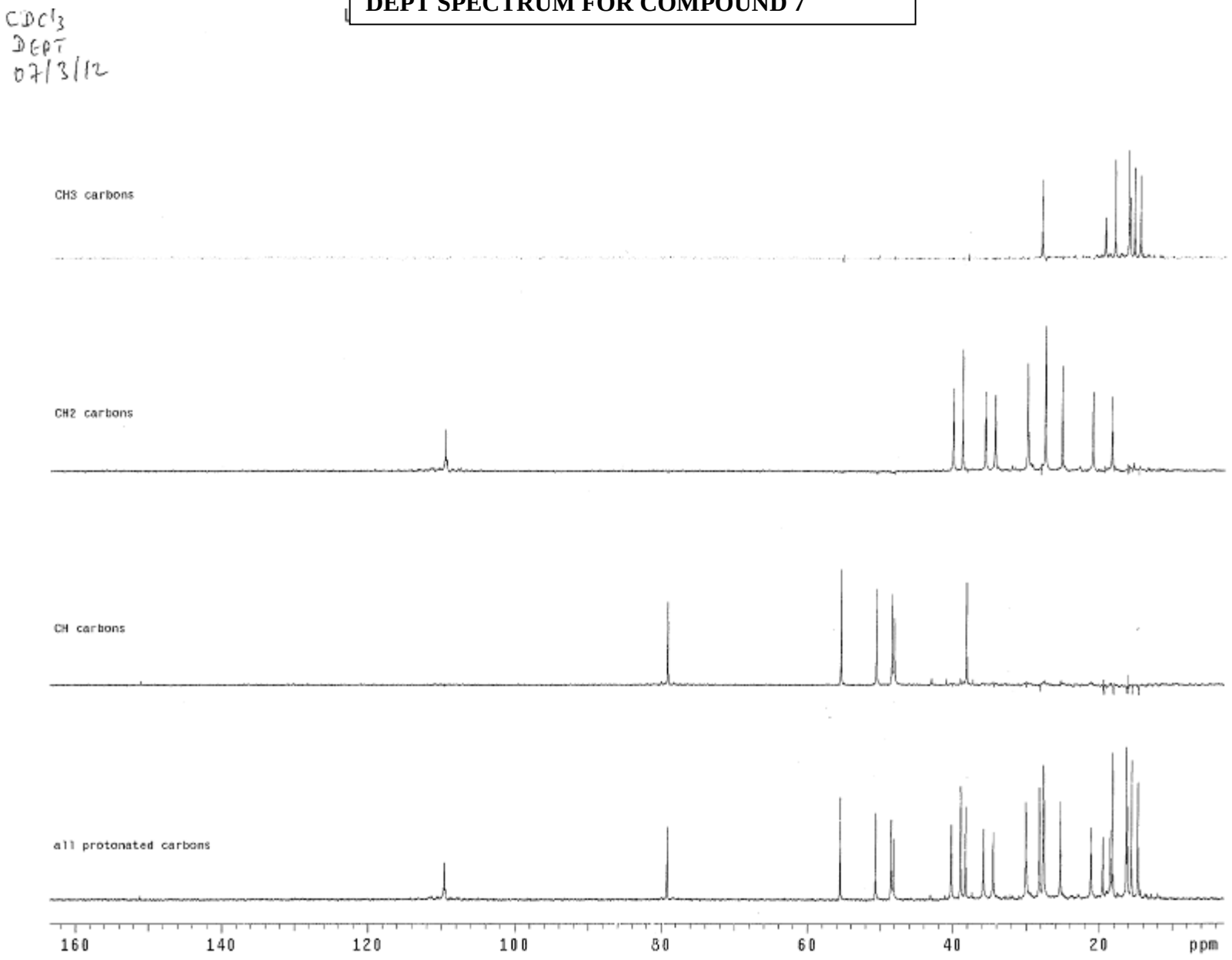
**$^{13}$C NMR SPECTRUM FOR COMPOUND 7**



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



**DEPT SPECTRUM FOR COMPOUND 7**



DEPT
07/3/12

**DEPT SPECTRUM FOR COMPOUND 7**