# PHYTOCHEMICAL AND ANTIPLASMODIAL INVESTIGATION OF *RHAMNUS PRINOIDES* AND *KNIPHOFIA FOLIOSA*


**BY**

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**A THESIS SUBMITED IN PARTIAL FULFILMENT OF THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY OF THE UNIVERSITY OF NAIROBI**

**2010**

## DECLARATION

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

.....

**Meron Gebru**

**This thesis has been submitted for examination with our approval as supervisors**

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# DEDICATION

THIS THESIS IS DEDICATED TO AFEWORKI ABRAHAM AND HIS FAMILY

THEIR ADVICE, LOVE AND SUPPORT MADE ME WHO I AM TODAY

YOU WILL ALWAYS BE IN MY HEART

MERON

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# LIST OF ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| *m/z* | Mass to Charge ratio |
| HMQC | Heteronuclear multiple quantum coherence ($^{1}J_{CH}$) |
| HMBC | Hetronuclear multiple bond correlation ($^{2}J_{CH}$, $^{3}J_{CH}$) |
| COSY | Correlated spectroscopy |
| HRMS | High resolution mass spectroscopy |
| NOESY | Nuclear overhauser and exchange spectroscopy |
| NMR | Nuclear magnetic resonance |
| 1D | One dimension analysis |
| 2D | Two dimension analysis |
| MS | Mass spectroscopy |
| UV | Ultra violet |
| $\lambda_{max}$ | Maximum wavelength of absorption |
| nm | Nanometer |
| MHz | Mega hertz |
| Hz | Hertz |
| *J* | Coupling constant |
| *s* | Singlet |
| *d* | Doublet |
| *dd* | Double of a doublet |
| *ddd* | Doublet of a doublet of a doublet |
| *t* | Triplet |
| TLC | Thin layer chromatography |
| PTLC | Preparative thin layer chromatography |
| $IC_{50}$ | Concentration of 50% inhibition |

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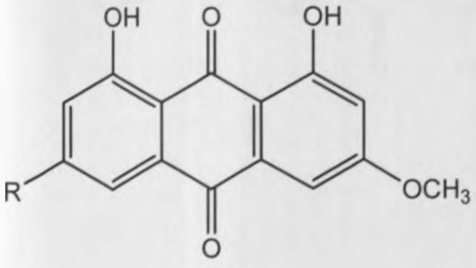
# ABSTRACT

The dried and ground whole root of *Rhamnus prinoides* (Rhamnaceae) were exhaustively extracted using dichloromethane/methanol (1:1) by cold percolation. The crude extract was subjected to chromatographic separations on oxalic acid impregnated silica gel, Sephadex LH-20 and preparative TLC, which resulted in the isolation of six compounds. The structures of the isolated compounds were determined using spectroscopic methods including UV, $^{1}H$ and $^{13}C$ NMR, COSY, NOESY, HMBC and HMQC and where necessary, by comparison with authentic samples. These were three anthraquinones [chrysophanol (**1**), physcion (**2**) and emodin (**3**)], a flavonol [rhamnazin (**4**)] and two naphthalenic derivatives [β-sorigenin (**5**) and geshoidin (**6**)].

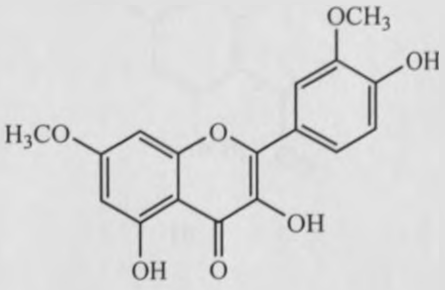
The rhizomes of *Kniphofia foliosa* (Asphodelaceae) were dried, ground and extracted using dichloromethane/methanol (1:1) by cold percolation. Chromatographic separation led to the isolation of three monomeric anthraquinones [chrysophanol (**1**), islandicin (**7**) and laccaic acid D (**8**)], a dimeric anthraquinone [chryslandicin (**9**)], a phenylanthraquinone [knipholone (**10**)], two dimeric phenylanthraquinones [joziknipholone A (**11**) and joziknipholone B (**12**)], a tetrameric phenylanthrone [Jozi-joziknipholone anthrone (**13**)], and a benzoic acid derivative [3,4-dihydroxybenzoic acid (**14**)]. The structures of these compounds were also determined using spectroscopic techniques.

The tetrameric phenylanthrone Jozi-joziknipholone anthrone (**13**) isolated from *Kniphofia foliosa* in this study is the first and the only example of a tetrameric phenylantraquinone. Furthermore, this is only the second report on the occurrence of the two dimeric phenylanthraquinones [joziknipholone A (**11**) and joziknipholone B (**12**)] in nature. Laccaic acid D (**8**) is reported here for the first time from the family Asphodelaceae.

The *in-vitro* antiplasmodial activities of the isolated compounds were performed against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. The naphthalenic derivative Geshoidin (**6**) from *Rhamnus prinoides* showed an $IC_{50}$ value of 4.0 ± 0.9 μM and 0.4 ± 0.2 μM against the chloroquine sensitive (D6) and chloroquine resistant (W2) strains of *Plasmodium falciparum*. The dimeric anthraquinone **9** [$IC_{50}$ = 6.5 μM (W2)], the phenylanthraquinone **10** [$IC_{50}$ = 10.4 ± 2.4 μM (W2), 23.3 ± 0.1 μM (D6)], the two dimeric phenylanthraquinones **11** [$IC_{50}$ = 0.4 ± 0.01 μM (W2), 0.2 μM (K1)], **12** [$IC_{50}$ = 3.3 ± 0.91 μM (W2), 0.3 μM ($K_2$)] and the tetrameric phenylanthrone **13** [$IC_{50}$ = 0.3 μM (K1)] showed good to potent antiplasmodial activities. The antimicrobial activities of the isolated compounds were also tested, but no significant activity was observed for any of the compounds tested.




| | R |
|---|---|
| 1 | H |
| 2 | $OCH_3$ |
| 3 | OH |

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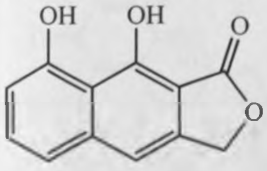


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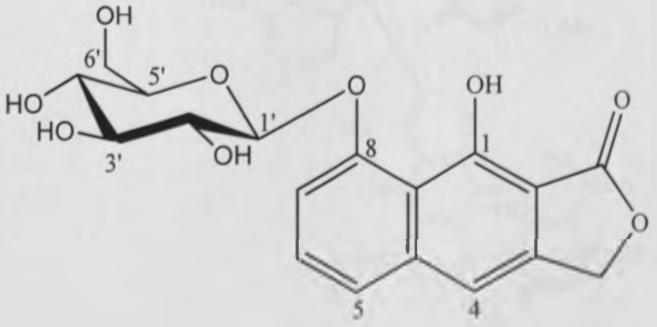


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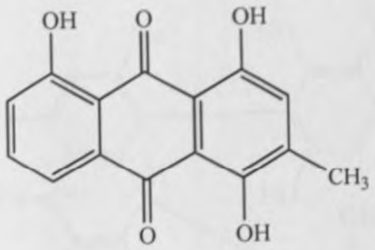

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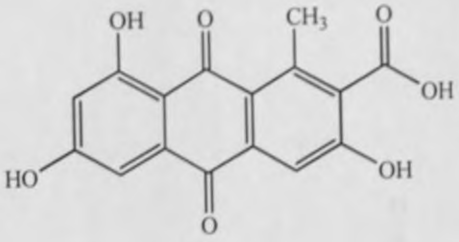

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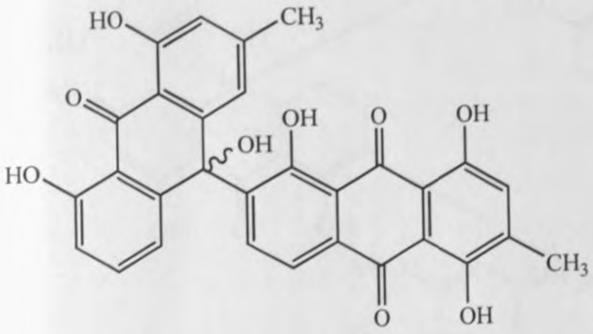

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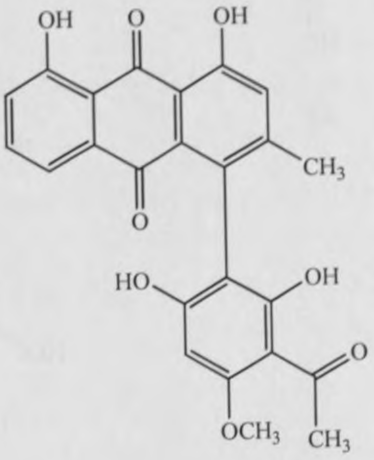

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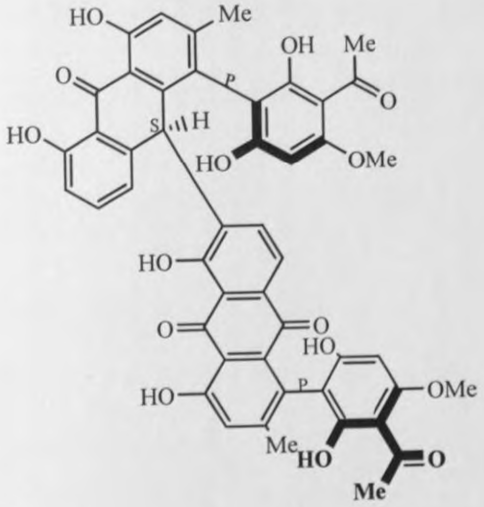

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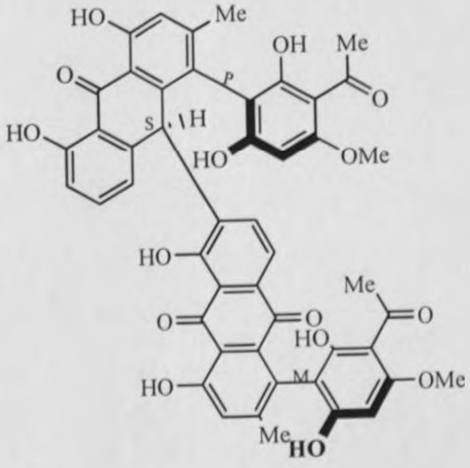

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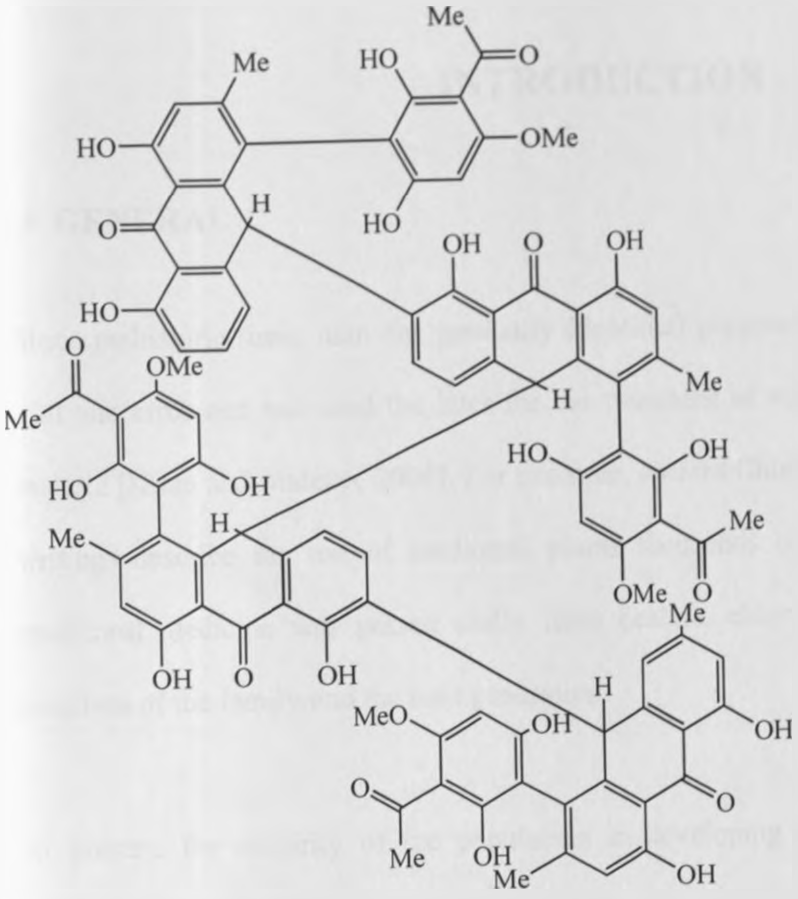

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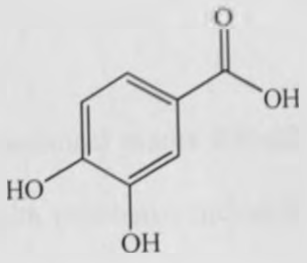

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# CHAPTER 1

# INTRODUCTION

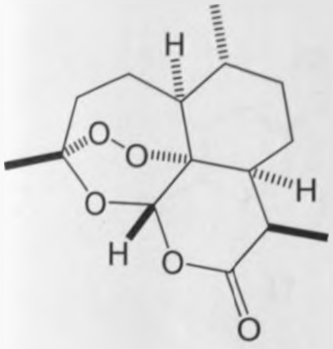
## 1.0 GENERAL

Since prehistoric time, man has gradually identified poisonous and medicinal plants through trial and error and has used the later for the treatment of various health problems, including malaria [Kitua and Malebo, 2004]. For example, ancient Chinese, Indian and Egyptian papyrus writings describe the use of medicinal plants thousands of years ago. This knowledge of traditional medicine was passed orally from healers, elders, herbalist and parents to some members of the family and the next generation.

At present, the majority of the population in developing countries depends on traditional medicine to meet some of the primary health care needs owing to the high cost of western pharmaceuticals and health care practices. Cultural and spiritual acceptance of traditional medicine is also an important factor for the wide use of medicinal plants around the world.

Based on the knowledge accumulated over centuries, plant extracts continue to be used for the treatment of various infectious and chronic diseases in many societies. Furthermore, traditional medicinal knowledge is serving as a base line for the development of new drugs. For example, the antimalarial drug artemisinin (**15**) isolated from *Artemisia annua* commonly known as "*qinghao*" in Chinese herbal medicine has been used traditionally for the treatment of fever and

malaria for almost 2000 years [Dewick, 2002; Derese, 2004]. The genus *Taxus* which is the source of the antitumor agent taxol (paclitaxel, **16**) had been used for the treatment of cancer in the Indian ayurvedic medicine for a long time [Mitscher, 2007].



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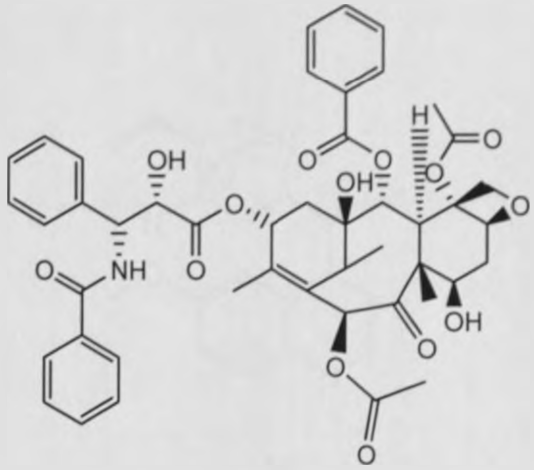

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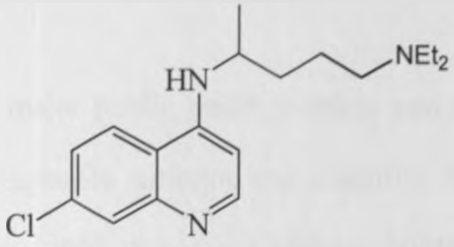
Malaria is a complex and life threatening vector borne infectious disease that has afflicted human beings since antiquity. It is among the world's worst killers particularly in sub-Saharan Africa. It is caused by the protozoan *Plasmodium* species and is transmitted from the salivary gland during the bite of the female *Anopheles* mosquito, which multiplies in the victim's liver and infects the red blood cells. Of the 40 different species of the *Anopheles* mosquito, *Anopheles gambiae* is the most difficult to control and is the predominant species in Africa [World malaria report, 2005].

Malaria, which causes about one to two million deaths per year in Africa, is considered as one of the most serious tropical diseases [Njoroge and Bussmann, 2006]. The major problem associated with the prevention and treatment of malaria is the spread of resistance of *Plasmodium* species to the available first-line anti-malarial drugs such as chloroquine (**17**); and the development of

resistant mosquito to conventional insecticides. Therefore, development of new drugs or drug combination therapy is required for the prevention and treatment of this infectious disease; preferably, drugs with a unique mode of action or with different chemical compositions from those currently in use.

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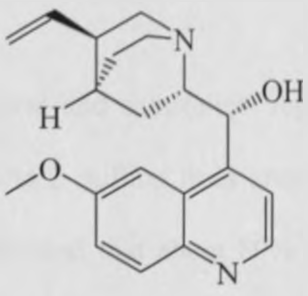


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Most of the drugs used for the treatment of malaria are derived from plants used by indigenous societies in different parts of the world. For example the alkaloid quinine (**18**), first discovered from the South American plant *Cinchona* (Rubiaceae) has been used as an antimalarial agent for a long time and has saved many lives for the past 300 years [Dewick, 2002]. In recent years, the sesquiterpene lactone artemisinin (**15**) from the Chinese herbal remedy *Artemisia annua* (Compositeae/Asteraceae) has been found to be effective against the chloroquine-resistant *Plasmodium falciparum* [Dewick, 2002]. These active plant ingredients often have served as molecular templates for the development of synthetic antimalarials that are safe and more effective than the mother molecules.

In Africa, the enormously rich biodiversity has allowed the use of a variety of plants for the treatment of malaria in different societies. Among these are *Rhamnus prinoides* (Rhamnaceae),

*Rhamnus staddo* (Rhamnaceae), *Albezia gummifera* (Mimosaceae), *Vernonia lasiopus* (Compositeae) and *Toddalia astiatica* (Rutaceae) [Muregi *et al.*, 2007].

## 1.1 PROBLEM STATEMENT

Malaria is a major public health problem mainly in tropical and subtropical regions. Besides causing unimaginable suffering and disability, it costs up to 2 million lives annually [Njoroge and Bussmann, 2006; Kitua and Malebo, 2004]. It is estimated that about 80% of all clinical cases of malaria occur in tropical African countries and about 90% of the people residing in this region carry the parasite [Kitua and Malebo, 2004]. The chemotherapy of malaria has become challenging due to the increasing resistance of *Plasmodium* species to the first line anti-malarial drugs such as chloroquine (**17**). Therefore, investigation of medicinal plants for potential antimalarial agents has to continue in search of new, potent and safe antimalarial drug templates.

## 1.2 JUSTIFICATION

Traditionally used plants for the treatment of malaria have played a great role in the development of antimalarial drugs. The two most important and effective antimalarial drugs quinine (**18**) and artemisinin (**15**) were obtained from plants that have been used traditionally for the treatment of malaria. Based on the molecular framework of these two compounds, a number of more effective and safe synthetic derivatives have been developed. Therefore, plants used traditionally remain important sources of new and more potent antimalarial drugs.

*Rhamnus prinoides* and *Kniphofia foliosa* are among the traditionally used plants for the treatment of malaria in Eastern Africa. The leaves and root bark extract of the former and the rhizome and the leave extracts of the later have shown promising *in vivo* and *in vitro* antiplasmodial activities, respectively [Muregi *et al.*, 2007, Wube *et al.*, 2005]. However, the compounds responsible for the antimalarial activity have not yet been isolated and identified. Therefore, it is worthwhile to isolate the metabolites of these plants and investigate their antiplasmodial activities.

## 1.3 OBJECTIVES

### 1.3.1 GENERAL OBJECTIVE

To isolate and characterize antiplasmodial compounds from the roots of *Rhamnus prinoides* and the rhizomes of *Kniphofia foliosa*.

### 1.3.2 SPECIFIC OBJECTIVES

- To isolate the constituents of the roots of *Rhamnus prinoides* and the rhizomes of *Kniphofia foliosa* using chromatographic methods.
- To characterize the structures of the isolated compounds by the use of spectroscopic methods.
- To establish the antiplasmodial activities of the crude extracts and isolated compounds of the two plants.

- To perform structural modification of some of the isolated compounds where necessary.

# CHAPTER 2

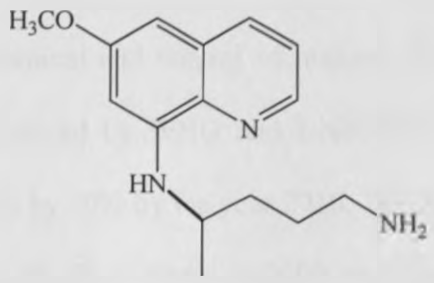
# LITERATURE REVIEW

## 2.1 BACKGROUND ON MALARIA

Malaria, the Italian word for 'bad air' has greatly influenced human history and has been noted for more than 4000 years [Sherman, 1998]. Malaria, an endemic and deadly disease is distributed in 101 countries, 45 of these are in sub-Saharan Africa [Casteel, 2003]. Malaria which had a worldwide distribution in the early 18$^{th}$ century had been a major public health problem [Sherman, 1998]. However, at present it is mainly concentrated in the world's poorest countries particularly in areas with social, political and economic instabilities [Trigg and Kondrachine, 1998].

Of the approximately 100 *Plasmodium* species, only four cause malaria in humans while the others affect birds, monkeys, livestock, rodents and reptiles [Lemke, 2002]. *P. falciparum*, *P. malariae, P. ovale* and *P. vivax* are the species that have an effect on humans. Among these *P. falciparum* is the most fatal, as it develops to the cerebral stage of the malaria rapidly and is responsible for most of the morbidities and mortalities in Africa [Casteel, 2003]. Although, infection with *P. vivax* is also common in temperate regions, it is rarely fatal, but can remain dormant in the liver and cause recurring and debilitating infection [Casteel, 2003]. Both *P. falciparum* and *P. vivax* have shown drug resistance to the commonly used first-line antimalarial drugs such as chloroquine (**17**). Even though the resistance differs with geographical distribution, *P. falciparum* has shown resistance to almost all anti-malarial drugs including the new

artemisinin based combination therapy; whereas incidents of *P. vivax* resistance to chloroquine (**17**) and primaquine (**19**) is common [Bloland, 2001]. The major reason for the development of such resistance is the indiscriminate use of anti-malarial drugs and the close similarities of chemical structures of most of the drugs in use. Although the loss of life due to malaria is avoidable and preventable, the development of drug-resistant strains of the parasite and the resistance of the mosquito vector to insecticides has become the greatest challenge in the control of the disease.

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The World Health Organization estimates that between 1.5 and 2.7 million people, out of the 300-500 million cases of malaria infection, die every year [Wube *et al.*, 2005; Casteel, 2003]. The majority of deaths occur in children under the age of 5 years in Africa, south of Sahara [Casteel, 2003; Africa malaria report, 2003]. Besides the disease episodes and deaths in Africa, malaria also causes anemia in children and pregnant women, undesirable birth outcomes such as spontaneous abortion, stillbirth, premature delivery and low birth weight, and overall maternal and child mortality [World malaria report, 2005].

The global economic burden of malaria is enormous and is more prominent in poor countries with fewer resources. Countries with endemic malaria are estimated to experience loss of

economic growth as high as 1.3% per year [World malaria report, 2005]. In addition, malaria causes loss in agricultural productivity and school absenteeism in children, permanent neurological, developmental and other damages which severely restrain investment and economic growth [Sachs and Malaney, 2002; Malaney *et al.*, 2004; Trigg and Kongrachine, 1998].

With the aim of reducing the mortality and morbidity due to malaria, several initiatives have been launched that are playing a major role in the establishment of goals, indicators and targets for the prevention, treatment and control of malaria. One such program is Roll Back Malaria (RBM) which was launched by WHO and UNICEF in 1998. It was aimed at reducing the mortality due to malaria by 50% by the year 2010. The RBM which was supported by the Abuja Declaration by African Heads of States in 2000 made commitment to intensify efforts to halve the malaria mortality in Africa by 2010. Another program which was initiated by the World Health Assembly in 2005 planned to ensure a reduction in the burden of malaria by at least 50% by 2010 and by 75% by 2015. These goals are to be achieved by using long lasting insecticidal bed nets, artemisinin based combination therapy and indoor residual spraying of insecticides along with developing new drugs mainly from traditionally used plants [World malaria report, 2009].

One of the research strategies towards the development of new drugs involves the evaluation and validation of antimalarial traditional medicines since plants offer a good opportunity for the discovery and development of effective, affordable and alternative drugs for the prevention and treatment of malaria.

## 2.1.1 LIFE CYCLE OF MALARIA

The malaria parasite which has a complex life cycle spreads by infecting both humans and the female *Anopheles* mosquito which is the definitive host. In humans, the parasite undergoes asexual cycle [Rang *et al.*, 2007]. The sporozoites are injected into the blood stream of the human host by the bite of the infected mosquito. After about an hour, the sporozoites travel to the liver where they grow and asexually multiply in the liver cell to form merozoites before infecting the red blood cell [Faust *et al.*, 1970]. At this stage, commonly known as the pre-erythrocytic stage the host is asymptomatic [Casteel, 2003]. After a variable period of time depending on the *Plasmodium* species, the merozoites are released as the liver cell raptures and re-enter the blood circulation to invade the red blood cell (erythrocytes) [Rang *et al.*, 2007]. In case of *P. vivax* and *P. ovale*, some of the sporozoites which are responsible for the relapse of the disease remain in the liver and differentiate into hypnozoites or the dormant non-dividing stage [Fujioka and Aikawa, 2002]. Once the merozoites invade the red blood cell, they develop to the motile trophozoites [Casteel, 2003] where the nucleus divides asexually to produce schizonts containing several nuclei. In the erythrocytic stage, the schizont undergoes division inside the red blood cell and the successive broods of mononucleated merozoites are released along with the parasites waste and cell debris as the erythrocyte raptures and continue the cycle by invading other blood cells [Casteel, 2003]. These toxins (parasite waste and the cell debris) that are released to the victim's body are responsible for the periodic cycles of fever and chill which are the common symptoms of malaria [Casteel, 2003]. Some of the merozoites in the erythrocyte differentiate sexually into male and female gametocytes which are latent in human [Casteel, 2003]. As the erythrocyte containing the gametocytes do not rapture, the sexual forms of the

parasite will be available in the blood stream of the infected individual when the mosquito takes its blood meal.

In the mosquito, only the sexual phase of the malarial life cycle takes place and can take 10-20 days [Bloland and Williams, 2003]. The sporozoites are ingested by the mosquito during a blood meal from an infected human. Provided favorable conditions such as ambient temperature, humidity and rainfall, the male and female gametocytes fuse inside the gut of the mosquito producing the male and female gametes [Lemke, 2002]. The diploid zygotes differentiate into oocysts on the outside wall of the mosquito's stomach which then undergo repeated mitotic division resulting in the formation of the sporozoites [Lemke, 2002]. These sporozoites migrate to the salivary gland of the mosquito and are injected into the blood stream of another human during blood meal, beginning the life cycle of the parasite again [Dick and Parrish, 2007].

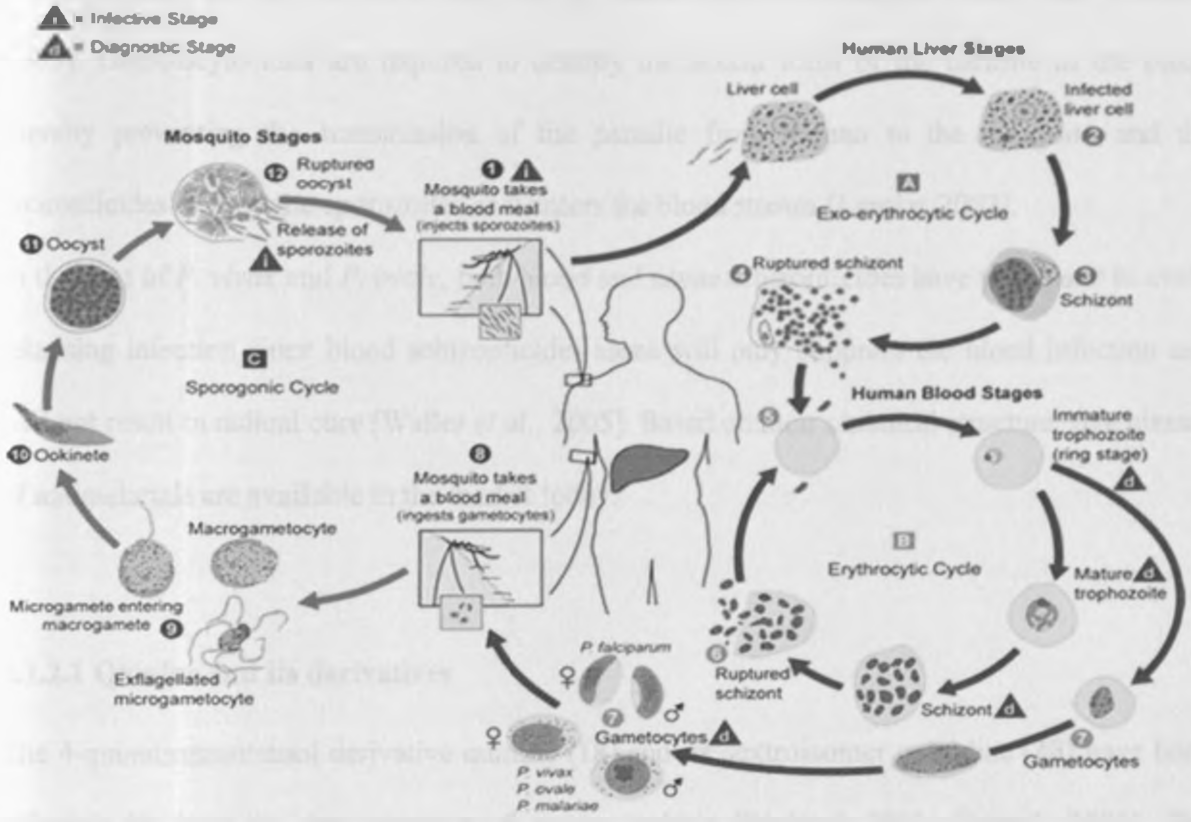



Fig 2.1 Life cycle of malaria (Source: www.dpd.cdc.gov/dpdx/html/imagelibrary/malaria)

### 2.1.2 CHEMOTHERAPY OF MALARIA

Generally, antimalarial drugs are classified as per the site at which they terminate the life cycle of the parasite [Rang *et al.*, 2007]. The efficacy of these drugs is measured by the clearance time of both the fever and the parasite [Casteel, 2003]. The majority of the drugs used for the prevention and treatment of malaria target the parasite either in the erythrocytic stage or the exo-erythrocytic stage. The blood schizonticides are used to cease the erythrocytic stage i.e. inside the red blood cells thereby treating acute attack to provide clinical cure [Rang *et al.*, 2007]. These drugs are used to cure or suppress relapse of *P. falciparum* and *P. malariae* as there is

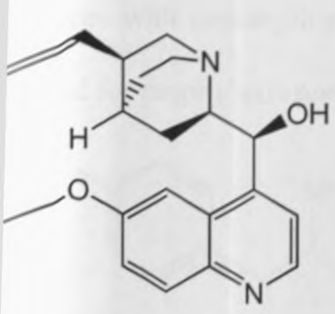
neither reinfection nor relapse from the liver. The tissue schizonticide eradicates the liver stage of the parasite thus preventing re-entry to the blood stream resulting in radical cure [Casteel, 2003]. Gametocytocides are required to destroy the sexual form of the parasite in the blood thereby preventing the transmission of the parasite from human to the mosquito and the sporonticides destroy the sporozoites as it enters the blood stream [Lemke, 2002].

In the case of *P. vivax* and *P. ovale*, both blood and tissue schizonticides have to be used to avoid relapsing infection since blood schizonticides alone will only suppress the blood infection and will not result in radical cure [Waller *et al.*, 2005]. Based on their chemical structure, five classes of antimalarials are available in the market today.

#### 2.1.2.1 Quinine and its derivatives

The 4-quinolinemethanol derivative quinine (**18**) and its dextroisomer quinidine (**20**) have been effective for long for the treatment of severe malaria [Bloland, 2001; Casteel, 2003]. The difference in the stereochemistry of the two drugs is responsible for the difference in potency making quinidine (**20**) more active than quinine (**18**) [Casteel, 2003]. Quinine (**18**) has been the drug of choice for the treatment of chloroquine-resistant strains of *P. falciparum* [Lemke, 2002]. The two synthetic 4-aminoquinoline derivatives of quinine, chloroquine (**17**) and amodiaquine (**21**) have been among the most widely used antimalarial drugs [Bloland, 2001]. Chloroquine, first synthesised in 1934, has been the drug of choice for the prophylaxis and treatment of all types of malaria for long since it is cheap and effective with few side effects when taken in the dose prescribed for malaria [Bloland, 2001]. Unfortunately, *P. falciparum* and some strains of *P. vivax* have developed resistance against this drug and have reduced its use. On the other hand,

Amodiaquine (**21**), a structural analog of chloroquine [Goldsmith, 1983] is as effective as chloroquine. Even though amodiaquine was withdrawn from the market due to its side effects, it is being reintroduced in areas where chloroquine-resistant strains of *Plasmodium* exists [Rang *et al.*, 2007]. Presently, it is used against the chloroquine-resistant strains of *P. falciparum* particularly in combination with either artesunate or sulphadoxine/pyrimethamine [Rosenthal, 2009]. All the above drugs are blood schizonticides, however chloroquine (**17**) and quinine (**18**) exhibit moderate gametocidal activity against the human *Plasmodium* strains except *P. falciparum* [Rosenthal, 2009]. The only antimalarial with tissue schizonticidal activity is the 8-aminoquinolines derivative primaquine (**19**) [Neal, 2009]. Although this drug has no activity against the erythrocytic stage, it has gametocidal activity against the four *Plasmodium* species [Lemke, 2002]. In order to potentiate its activity, it is used in combination with either chloroquine or quinine for the treatment of *P. vivax* and *P. ovale* infection thereby providing radical cure [Goldsmith, 1983]. Primaquine can also be used as a chemo-prophylactic agent in cases where mefloquine cannot be used [Rosenthal, 2009]. Mefloquine (**22**) another quinoline methanol derivative of quinine is a blood schizonticide and is effective for the prophylaxis (in areas with chloroquine-resistance) and treatment of multidrug resistant strains of *P. falciparum* and *P. vivax* [Bloland, 2001; Harvey *et al.*, 2009].

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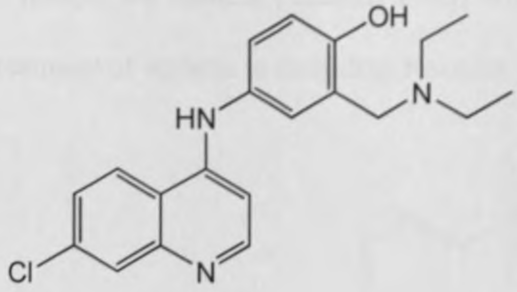


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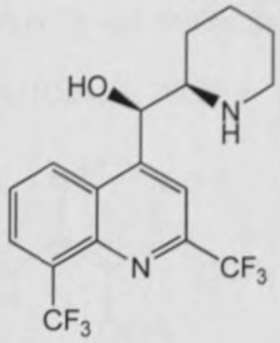


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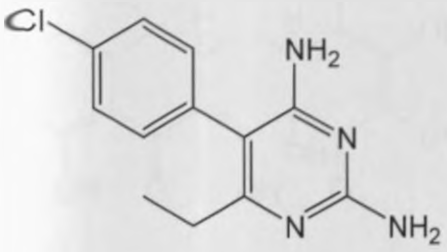
### 2.1.2.2. Antifolate combination drugs

These drugs are combination of dihydrofolate reductase inhibitors and the long acting sulfa drugs (sulfonamides and sulfones) which are competitive inhibitors of dihydropteroate synthetase an enzyme responsible for the synthesis of dihydropteroate from hydroxymethyldihydropterin [Casteel, 2003; Harvey *et al.*, 2009]. As both these class of drugs influence the same pathway at different sites on the parasite, they have synergistic effect when used in combination thereby blocking the synthesis of tetrahydrofolate [Lemke, 2002; Rang *et al.*, 2007]. The advantage of antifolate combination drugs is that they can be effective even in the presence of resistance to the individual components [Bloland, 2001]. The most common combination is the 2,4 diaminopyrimidine pyrimethamine (**23**) and the sulfonamide sulphadoxine (**24**) [Rosenthal, 2009; Casteel, 2003]. This combination drug can be used for the treatment of non-severe *P. falciparum* infection [Bloland, 2001]. Pyrimethamine which is used for radical cure has both blood schizonticidal and sporocidal activity [Harvey *et al.*, 2009]. Another combination of antifolates introduced recently is the biguanide chlorproguanil (**25**) with the sulfone drug dapsone (**26**). Besides being cheaper than pyrimethamine/sulphadoxine, it is safe and effective in

places with uncomplicated *P. falciparum* malaria [Casteel, 2003]. However, it can neither be used for prophylaxis nor for treatment of malaria in multidrug resistant areas [Casteel, 2003].



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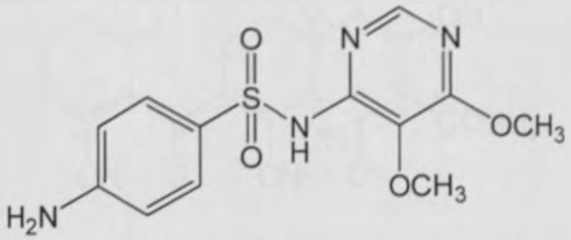

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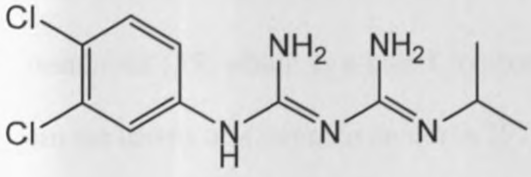

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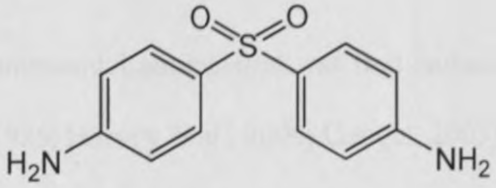

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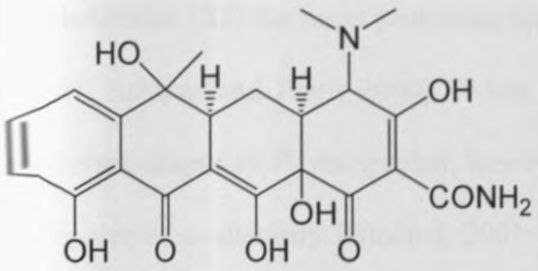
### 2.1.2.3 Antibiotics

Tetracycline (**27**) and its deoxyderivative doxycycline (**28**) are blood schizonticides which act by inhibiting the synthesis of protein in the ribosomes of the parasite [Vaidya, 2005; Casteel, 2003]. They are the most common antimalarial antibiotics used for prophylaxis and treatment of malaria [Bloland, 2001]. Since these drugs are slow acting, they are used in combination with fast acting antimalarials such as quinine in areas with multidrug resistant strains as well as where the activity of quinine is declining [Casteel, 2003; Bloland, 2001].

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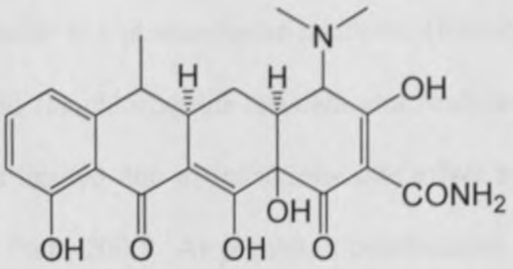


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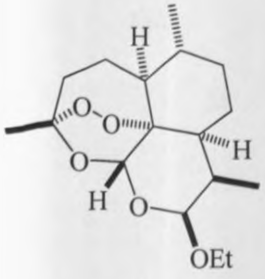
### 2.1.2.4 Artemisinin and derivatives

Artemisinin (**15**) which is a blood schizonticidal and gametocidal antimalarial was first isolated from the leaves of *Artemisia annua* in 1971 [Klayman, 1985; Harvey *et al.*, 2009; Casteel, 2003]. It has been the drug of choice for the treatment of multidrug resistant *P. falciparum* and cerebral malaria [Rang *et al.*, 2007]. Unlike most antimalarials which have nitrogen containing hetrocyclic ring system, artemisinin and its derivatives, arteether (**29**), artemether (**30**) and artesunate (**31**), are endoperoxide bearing sesquiterpene lactones [Klayman, 1985]. In order to increase the potency and avoid development of drug resistance these drugs are used in combination with long lasting antimalarials such as mefloquine (**22**) [Bloland, 2001].

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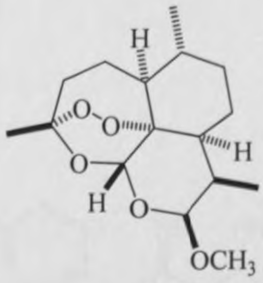


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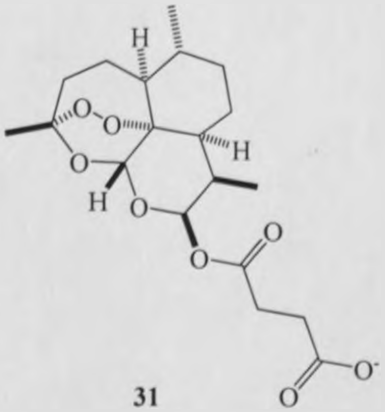


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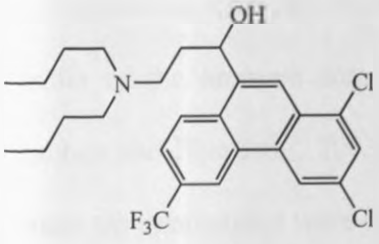
### 2.1.2.5 Miscellaneous compounds

Halofantrine (**32**) the most promising blood schizonticide is a phenanthrene-methanol [Bloland, 2001; Scholar and Pratt, 2000]. It was recommended for chloroquine-resistant and multidrug resistant strains of *P. falciparum*, however its use is limited due to its serious side effect and irregular bioavailability [Bloland, 2001; Scholar and Pratt, 2000]. At present, a combination of the hydroxynaphthoquinone atovaquone (**33**) and the biguanide derivative proguanil (**34**) is used for prophylaxis and treatment of multi-drug resistant *P. falciparum* [Bloland, 2001]. Atovaquone has both tissue and erythrocytic schizonticidal activity whereas proguanil (**34**) is a dihydrofolate reductase inhibitor [Rosenthal, 2009]. Although atovaquone alone had a fast clearance of fever and the asexual form of the parasite, it cannot be used single-handedly due to the development of recrudescence [Viadya, 2001]. However, when combined with proguanil (**34**) increased potency as well as synergistic activity against *P. falciparum* malaria has been observed [Viadya, 2001; Muraleedharan and Avery, 2007].

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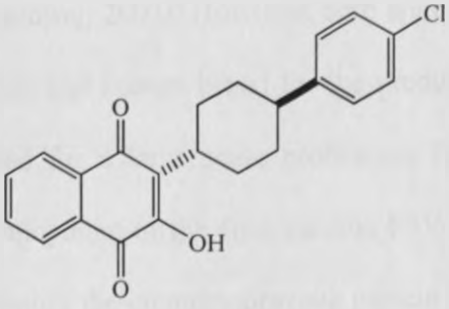


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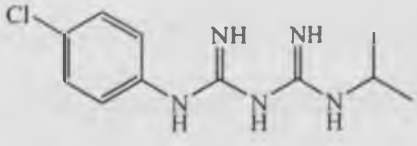


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## 1.3 VACCINE DEVELOPMENT

Even though a number of drugs are available for the prophylaxis and treatment of malaria, the mortality and morbidity due to malaria is increasing. This is mainly due to the development of drug resistant parasites and insecticide resistant mosquitoes. Besides the continuous use of existing tools, the eradication of malaria from sub-Saharan Africa will require the development of new drugs as well as a vaccine [Butler, 2009]. Vaccines are effective mode of control since they are comparatively cheap, easy to administer and in most cases have reduced the spread and burden of most infectious diseases.

The development of vaccine for malaria which started in the early 1980's has been a challenge for long [Maher, 2009]. Theoretically, inoculation of irradiated sporozoites, the form of the parasite which is introduced to the blood circulation of humans during the bite of the mosquito as well as the transfer of immunoglobulin G from semi-immune individuals would have shown long term protection [Maher, 2009; Chauhan and Bhardwaj, 2003]. However, both are impractical, as the irradiated sporozoites require live mosquitoes and human blood for the production and the transfer of the immune sera cannot be attained for a large scale production [Maher, 2009; Chauhan and Bhardwaj, 2003]. During the development of the first vaccine FSV-1, antibodies against the sporozoites were used in order to identify the circumsporozoite protein and clone the relevant gene. The practical application of this vaccine was not feasible as it protected only one person in six. The recently developed malaria vaccine RTS,S which is the descendent of FSV-1 is in the late stage of trial [Maher, 2009]. This vaccine is the first to show promising safety and significant protection (i.e. 30% protections) in children aged 1- 4 in Mozambique in 2004 and will soon undergo further trials in seven African countries including Kenya [Maher, 2009].

Although RTS,S will not completely protect infection, the onset of the disease will be reduced. Some indicators predict it might diminish levels of severe malaria by 50% which is enough to give infants and small children a better chance of survival during their most vulnerable age [Maher, 2009].

### 2.1.4 DRUG RESISTANCE

One of the major factors that have hindered the control of malaria is drug resistance. The resistance developed by the currently available antimalarial drugs is summarized in Table 2.1. Irrational uses of drugs, lack of compliance as well as substandard and counterfeit drugs are the major factors that have contributed to the development of resistance. Currently, *P. falciparum* has developed resistance against almost all the available antimalarial drugs including the new antimalarial artemisinin and its derivatives [Casteel, 2003]. On the other hand, chloroquine and primaquine resistant strains of *P. vivax* have been reported [Casteel, 2003]. Drug resistance can be reduced by the use of combination drug therapy, avoiding unnecessary use of drugs particularly new drugs, in areas where resistance have not been reported for the previously used drugs and if possible introducing direct observation treatment as in the case of tuberculosis. However, the most important and long lasting approach for the eradication and treatment of drug resistant malaria would be searching for safe, affordable and effective antimalarial drugs.

**Table 2.1:** Drug resistance developed by some of the available antimalarial drugs

| Antimalarial drugs | *Plasmodium* strain | Resistance first reported year | Resistance reported Areas | Reference |
|---|---|---|---|---|
| Quinine (**18**)<br><br>Quinidine (**20**) | *P. falciparum* | 1910 | Brazil, Brazil-Bolivia border, Thai-Cambodian border, Thailand, Vietnam, Southeast Asia, Western Oceania | ICMR bulletin, 2008; Gregson and Plowe, 2005; Bloland, 2001; |
| Chloroquine (**17**) | *P. falciparum* | 1960 | S. America Southeast Asia and Oceania, East Asia, Africa and Indian subcontinent | ICMR bulletin, 2008; Bloland, 2001 |
| | *P. vivax* | 1989 | Indonesia, Papua New Guinea, Myanmar, Thailand, Borneo, India, Brazil | ICMR bulletin, 2008; Mendis *et al.*, 2001 |
| Amodiaquine (**21**) (Cross resistance with Chloroquine (**17**) | *P. falciparum* | - | Areas where chloroquine resistance is high | Ochong *et al.*, 2003; Bloland, 2001 |
| Proguanil (**34**) | *P. falciparum* | 1949 | South-east Asia, Brazil | Gregson and Plowe, 2005 |
| Sulphadoxine (**24**) Pyrimethamine (**23**) | *P. falciparum* | 1967 | Southeast Asia and Oceania, South America, Africa and East Asia | ICMR bulletin, 2008; Bloland, 2001 |
| Mefloquine (**22**) | *P. falciparum* | 1981 | Thailand, Southeast Asia and Oceania , Amazon region of South America, Western Africa | ICMR bulletin, 2008; Bloland, 2001 |
| Atovaquone (**33**) | *P. falciparum* | 1996 | Areas where multidrug resistant falciparum is reported | ICMR bulletin, 2008 |
| Artemisinin (**15**) | *P. falciparum* | - | Thailand-Cambodian border | World malaria report, 2009 |

**Table 2.1:** Drug resistance developed by some of the available antimalarial drugs

| **Antimalarial drugs** | ***Plasmodium* strain** | **Resistance first reported year** | **Resistance reported Areas** | **Reference** |
|---|---|---|---|---|
| Quinine (**18**)<br><br>Quinidine (**20**) | *P. falciparum* | 1910 | Brazil, Brazil-Bolivia border, Thai-Cambodian border, Thailand, Vietnam, Southeast Asia, Western Oceania | ICMR bulletin, 2008; Gregson and Plowe, 2005; Bloland, 2001; |
| Chloroquine (**17**) | *P. falciparum* | 1960 | S. America Southeast Asia and Oceania, East Asia, Africa and Indian subcontinent | ICMR bulletin, 2008; Bloland, 2001 |
| | *P. vivax* | 1989 | Indonesia, Papua New Guinea, Myanmar, Thailand, Borneo, India, Brazil | ICMR bulletin, 2008; Mendis *et al.*, 2001 |
| Amodiaquine (**21**) (Cross resistance with Chloroquine (**17**) | *P. falciparum* | - | Areas where chloroquine resistance is high | Ochong *et al.*, 2003; Bloland, 2001 |
| Proguanil (**34**) | *P. falciparum* | 1949 | South-east Asia, Brazil | Gregson and Plowe, 2005 |
| Sulphadoxine (**24**) /pyrimethamine (**23**) | *P. falciparum* | 1967 | Southeast Asia and Oceania, South America, Africa and East Asia | ICMR bulletin, 2008; Bloland, 2001 |
| Mefloquine (**22**) | *P. falciparum* | 1981 | Thailand, Southeast Asia and Oceania , Amazon region of South America, Western Africa | ICMR bulletin, 2008; Bloland, 2001 |
| Atovaquone (**33**) | *P. falciparum* | 1996 | Areas where multidrug resistant falciparum is reported | ICMR bulletin, 2008 |
| Artemisinin (**15**) | *P. falciparum* | - | Thailand-Cambodian border | World malaria report, 2009 |

## 2.2 BOTANICAL INFORMATION

The genera *Rhamnus* and *Kniphofia* belong to the family Rhamnaceae and Aspodelaceae, respectively.

### 2.2.1 THE FAMILY RHAMNACEAE

The Rhamnaceae commonly known as the Buckthorn family is a large family of flowering plants in the order Rhamnales [Medan and Schirarend, 2004]. It comprises of 59 genera and about 900 species, most of which are small trees, scrambling shrubs and frequently climbers, the majority of which have strong, hooked spines [Evans, 1996; Dharani, 2002]. The family is widely distributed throughout the world mainly in the tropical, subtropical and temperate regions [Preston and Braham, 2002; Medan and Schirarend, 2004]. The leaves are simple and stipulate, alternate or opposite [Dharani, 2002]. In many genera, the leaves are modified in to spines. The inconspicuous flowers are small with four or five petals and are mostly white or greenish in colour and rarely yellow, pink, red or blue [Preston and Braham, 2002; Medan and Schirarend, 2004]. The flower bloom mainly in spring and summer with the exception of *Rhamnus* which flowers in winter and the genus *Colletia* throughout the cold season [Medan and Schirarend, 2004]. The one-seeded fruits are drupaceous [Dharani, 2002; Medan and Schirarend, 2004].

The family is known to have economic importance; for example, the genus *Rhamnus* is used as medicine and dye and the genus *Ziziphus* have fruits that are edible [Preston and Braham, 2002].

### 2.1.1.1 The genus *Rhamnus*

The genus *Rhamnus* which comprises about 150 species of shrubs and small trees occurs both in temperate and tropical countries [Evans, 1996]. It is evergreen or deciduous plant and is resistant to frost [Kristen, 2001]. The simple leaves are either alternate or sub-opposite. The hermaphrodite small flowers are weakly scented [Preston and Braham, 2002; Medan and Schirarend, 2004]. The fruits are drupaceous and contain two to four cartilaginous nuts [Preston and Braham, 2002]. There are only two species of *Rhamnus* in Africa, *Rhamnus prinoides* and *Rhamnus staddo*, both of which are found in Kenya.

### 2.2.1.2 *Rhamnus prinoides*

The African dogwood, *Rhamnus prinoides* L'Herit also known as *Rhamnus pauciflorus* or *Rhamnus celifollius* is a climbing shrub or small tree that grows up to 4 m high [Schmidt *et al.*, 2002]. It is prevalent on grasslands, forest margins and at the side of streams and is known to be frost resistant [Johnson and Johnson, 2002]. In Africa, it is widely distributed from South Africa to Ethiopia and to Angola and grows at an altitude between 1400 m and 3200 m above sea level [Edwards, 1991; Schmidt *et al.*, 2002]. The leaves which are glossy and dark green from above and pale green below are simple and alternate [Schmidt *et al.*, 2002]. The green inconspicuous flowers appear as small clusters in the leaf axils and bloom between October and January [Schmidt *et al.*, 2002]. The round drupe fruits appear between November and June and are red to purple in colour [Kristen, 2001; Schmidt *et al.*, 2002].

In Ethiopia, where the plant is commonly known as *Gesho*, it is widely cultivated particularly in the Northern part of the country besides being a natural constituent in the forests [Edwards,

1991]. The dried leaves and stem of *Gesho* are used as a bittering principle in the preparation of the local alcoholic beverages *Tella* and *Tej*. Ethnomedicinally, in Ethiopia the fruits are used for the treatment of ring worm infections [Abegaz *et al.*, 1999].

### 2.2.2 THE FAMILY ASPHODELACEAE

The family Asphodelaceae which comprises of 15 genera and about 780 species is widely distributed in the temperate, tropical and subtropical regions of the Old World [Smith and Van Wyk, 1998]. The family is characterized by small to medium sized perennial rhizomatous herbs that is rarely bulbous, shrubs or pachycaul trees that are mostly found in arid environment particularly in the Southern part of Africa [Jussien, 2004]. The roots are yellowish in colour due to the presence of anthraquinones and are fibrous, often succulent and in some genera with velamen [Smith and Van Wyk, 1998; Whitehouse, 2002]. The leaves which appear as vascular bunch surrounding an inner mucilaginous region are lanceolate to linear or subulate, terete, frequently succulent and exist in a basal rosette or a short woody stem [Smith and Van Wyk, 1998; Whitehouse, 2002; Jussien, 2004]. The family has spike, racemous or paniculate inflorescence on a stalk that arise from the axils of the leaf close to the hub of the rosette [Llamas, 2003; Jussien, 2004]. The flowers are bisexual and have bright colours i.e. red, orange, and yellow and white [Smith and Van wyk, 1998]. The fruits appear as loculicidal capsule with the seeds enclosed in a dull brown or grayish black aril [Whitehouse, 2002]. . Economically, besides having ornamental value the family especially the genus *Aloe* is used in medical and cosmetic industries [Smith and Van Wyk, 1998].

### 2.2.2.1 The genus *Kniphofia*

The genus *Kniphofia* named after the German botanist Johann Hieronymus Kniphof belongs to the subfamily Asphodeloidea [Armitage, 2000]. The genus is common among horticulturists and is entirely African (main land) with only three species occuring outside Africa [Ramdhani, 2006] i.e. *Kniphofia pallidiflora*, *Kniphofia ankaratrensis* and *Kniphofia sumarae*, where the first two are indigenous to Madagascar and the later to Yemen [Bringmann *et al.*, 2008a]. Due to their tall scarlet or red flowers the genus is commonly known as 'red hot pokers' and encompasses about 70 species [Bosch, 2008; Bringmann *et al.*, 2008a]. It is widely distributed in the Eastern and Southern part of Africa, of which about 47 of them occur in Southern Africa [Droop, 1986; Bringmann *et al.*, 2008a]

*Kniphofia* are perennial, rhizomatous herbs, rarely with aerial stem [Smith and Van Wyk, 1998]. With few exceptions which favour dry conditions with good drainage, most species grow in mountainous grasslands, alongside tributaries and in moist and swampy grounds [Bringmann *et al.*, 2008a; Anisko, 2008]. The leaves which are usually kneeled with smooth to serrulate margins are mostly rosulate, basal, long and linear, that narrows gradually to the apex [Whitehouse, 2002]. They have elongated inflorescences with a dense raceme on a simple erect peduncle [Whitehouse, 2002]. The small tubular flowers are bisexual with tantalizing colours ranging from white, yellow, lime green to various shades of red that are more conspicuous at the apex of the inflorescence producing a bicolourous appearance [Whitehouse, 2002]. The fruits are globose to spherical capsules and house seeds that have fleshy endosperm, usually flattened, acutely three angled or winged [Whitehouse, 2002; Rhamdani, 2006].

#### 2.2.2.2 *Kniphofia foliosa*

Of the seven species found in Ethiopia, *Kniphofia foliosa* is one of the five endemic species to Ethiopia [Bosch, 2008]. It is a perennial herb widely distributed along roadsides, grasslands, hills and on mountains at altitudes between 2050 and 4000 m particularly in the Bale mountains [Philips and Carillet, 2006]. Mostly, it is stemless with thick erect rhizomes [Bosch, 2008]. The simple, linear to lanceolate leaves have basal rosette with kneeled apex and have finely toothed margins [Bosch, 2008]. The inflorescence appears as a long terminal raceme and is compactly flowered [Bosch, 2008]. The hermaphrodite flowers which bloom from May to October and from December to January are long and funnel shaped, gradually broadening from the base to the mouth [Philips and Carillet, 2006; Bosch, 2008]. The ovoid capsule shaped fruits are brown to black in colour with few seeds [Bosch, 2008]. The slightly flattened 3-angled seeds are grey-black in colour [Bosch, 2008]. The rhizomes which are considered to be edible are used medicinally by the Bale people of Ethiopia and to exterminate endoparasites in cattles [Bosch, 2008].

## 2.3 ETHNOMEDICINAL USES OF THE GENUS *RHAMNUS*

The different species of the genus *Rhamnus* have spiritual and medicinal values in many societies throughout the world. For instance, in the Southern part of Africa, where *Rhamnus prinoides* is most popular, the plant is used traditionally for the preparation of charms for bewitching as well as protection against witchcraft, for protection against lightening and as good luck charisma for hunters [Schmidt et al., 2002; Mwangi, 2005]. In Kenya, where the plant is believed to have wide medicinal use, it is a major ingredient in the preparation of the *"muteta soup"*; a soup prepared by many local communities as an appetizer [Mwangi, 2005]. Some of the ethno- medicinal uses of the genus are listed in table 2.2.

**Table 2.2**: Ethnomedicinal uses of *Rhamnus* species

| Species | Plant part | Used for/as | Reference |
|---|---|---|---|
| ***R. alaternus*** | Young branches<br><br>Fresh leaves | Jaundice | Dafni *et al.*, 1984 |
| | Dried bark | Purgative | Abou-Chaar and Shamlian, 1980 |
| | Leaves, stem | Hypertension and cold | Martinez-Lirola *et al.*, 1996 |
| | Part not mentioned | Prevention and treatment of liver disease, hepatitis, inflammation | Saad *et al.*, 2008 |
| ***R. alnifolia*** | Whole plant | Antidote, dermatological aid, back pains | Moerman, 2004 |
| | Bark | Blood purifier, tonic, physic, sedative in children, laxative | |
| ***R. alnifolia*** | Roots | Gonorrhea | Moerman, 2004 |
| | Inner bark | Cathartic | |

**Table 2.2:** Ethnomedicinal uses of *Rhamnus* species cont....

| Species | Plant part | Used for/as | Reference |
|---|---|---|---|
| ***R. cathartica*** | Branches | Analgesic, anti-inflammatory, stomach ulcer, externally for cuts (as a compress) | Zevin *et al.*, 1997 |
| | Fruits | Purgative, chronic constipation, hemorrhoids, anal irritation | |
| ***R. cathartica*** | Bark | Gastritis due to hyperacidity, analgesic, anti-inflammatory | Zevin *et al.*, 1997 |
| | Fruits | Skin cancer | Spiridonov, 2008 |
| | Bark (boiled in ale) | Jaundice | Page, 1999 |
| | Bark | Cathartic, itch, sore and inflammed eye | Moerman, 2004 |
| | Fruits | Cathartic | |
| ***R. crenata*** | Roots or root bark | To clear away heat, remove dampness, destroy intestinal worms, detoxify body | Yang, 2003 |
| ***R. crocea*** | Bark | As an analgesic in intestinal soreness, as blood medicine, to relieve cough | Moerman, 2004 |
| | Whole plant | To relieve headache, rheumatism, used for boils and carbuncles, for stomach disorders, spleen and liver stimulant | |
| | Roots | Used by women for blood shortage, diuretics, laxative, gonorrhea, as an analgesic in intestinal soreness, as blood medicine, to relieve cough | |
| ***R. davurica*** | Fruits | To remove heat, diuretic, to cease stagnancy, to kill parasites | Yang, 2003 |
| | Bark | To release noxious heat and pathogenic wind | |
| ***R. frangula*** | Bark | Cathartic | Belkin and Fitzgerald, 1952 |
| | Dried stem bark | Diabetics | Tucakov, 1978 |

**Table 2.2:** Ethnomedicinal uses of *Rhamnus* species cont.....

| Species | Plant part | Used for/as | Reference |
|---|---|---|---|
| | Dried stem bark | Skin irritation, Cathartic | Anon, 1931 |
| | Dried stem bark | Constipation | Lokar and Poldini, 1988 |
| ***R. glandulosa*** | Leaves | Antiviral | Silva *et al.*, 1997 |
| ***R. heterophylla*** | Roots, branch leaves | To remove heat from blood, to cease bleeding | Yang, 2003 |
| ***R. japonica*** | Dried bark | Antifungal | Ito and Ota, 1951 |
| ***R. prinoides*** | Dried entire plant | Sprains | Chhabra *et al.*, 1991 |
| | Dried leaves | Sedatives | Chhabra *et al.*, 1991 |
| | Dried leaves | treatment of pneumonia, gonorrhea, colic and rheumatism | Chhabra and Uiso, 1991 |
| | Dried roots | Blood purifier, pneumonia, gonorrhea. Colic (mixed with *E. abyssinica*) and rheumatism | Chhabra *et al.*, 1991 |
| | Leaves, Root bark | Malaria | Muregi *et al.*, 2007 |
| | Fruits | Ring worm infection | Abegaz *et al.*, 1999 |
| | | Fungal and ring worm infection | Abegaz and Dagne, 1988 |
| | Whole plant | Pneumonia, malaria | Schmidt *et al.*, 2002 |
| | Part not specified | Malaria | Kuria *et al.*, 2001 |
| | Roots with the bark of *Erythrina abyssinica* | Colics | Kokwaro, 1993 |
| | Roots | Gonorrhoea, rheumatism, eradicate intestinal worms | |
| | Roots | Indigestion, gonorrhea, malaria and rheumatism | Beentje,1994 |
| | Dried bark | Treatment of spleen, emetic and purgative, tonic and astringent | Libster, 2002 |

**Table 2.2:** Ethnomedicinal uses of *Rhamnus* species cont....

| Species | Plant part | Used for/as | Reference |
|---|---|---|---|
| ***R. prinoides*** | Bark | Digestive complaint, tonic laxative for habitual constipation, hemorrhoids | Small and Catling, 1999 |
| ***R. purshiana*** | Parts not mentioned | Constipation, cancer | Stargrove *et al.*, 2008 |
| | Bark | Laxative, body cleansing tonic particularly for intestinal worms | Page, 1999 |
| ***R. serrata*** | Part not specified | To stop bloody bowels, to cure dysentry | Ortiz de Montellano, 1975 |
| ***R. staddo*** | Shade dried root | Used for fertility control | Desta, 1994 |
| | Dried root bark | Malaria and fever | Gakunju *et al.*, 1995 |
| | Roots | Women for fertility, venereal disease | Kokwaro, 1993 |
| | Fresh leaves | Cold | Yineger *et al.*, 2008 |
| ***R. virgata*** | Fruits | Emetic | Rana and Datt, 1997 |
| | Fruits | Spleen infection | |
| | Fruits | Purgative | |

## 2.4 ETHNOMEDICINAL USES OF THE GENUS *KNIPHOFIA*

Although the genus is widely recognized for its ornamental value owing to their colourful flowers, the use of the genus in traditional medicine is limited to few species which is summarized in table 2.3.

**Table 2.3:** Ethnomedicinal uses of *Kniphofia* species

| Species | Plant part | Used for/as | Reference |
|---|---|---|---|
| ***K. buchananii*** | Plant infusion | Snake deterrents, chest ailments | Ramdhani, 2006; Bringmann *et al.*, 2008a |
| ***K. caulescens*** | Part not specified | Charm against lightening | Ramdhani, 2006 |
| ***K. foliosa*** | Roots | Abdominal cramp and ache | Wube *et al.*, 2005; Philips and Carillet, 2006; Bosch, 2008; Bringmann *et al.*, 2008a |
| | | Wound healing | Wube *et al.*, 2005 |
| ***K. isoetifolia*** | Roots | Gonorrhea, hepatitis B | Yineger *et al.*, 2008 |
| ***K. laxiflora*** | Plant infusion | Snake deterrents; chest aliments | Ramdhani, 2006; Bringmann *et al.*, 2008a |
| ***K. linearifolia*** | Roots | To treat infertility in women | Bosch, 2008 |
| ***K. parviflora*** | Plant infusion | Snake deterrents, chest ailment | Ramdhani, 2006; Bringmann *et al.*, 2008a |
| ***K. ritualis*** | Part not specified | Shoulder pains | Ramdhani, 2006 |
| ***K. rooperi*** | Plant infusion | Chest aliments, snake deterrent | Ramdhani, 2006; Bringmann *et al.*, 2008a |
| ***K. uvaria*** | Part not specified | Included in enemas, administered for painful menstruation and to treat infertility | Ramdhani, 2006 |

## 2.5 BIOLOGICAL ACTIVITY OF THE GENUS *RHAMNUS*

In addition to its wide use as laxative, the genus *Rhamnus* has shown a wide range of biological activities which are summarized in table 2.4. It is worth to note that the two African species, *Rhamnus prinoides* and *Rhamnus staddo* have shown to possess antimalarial activity.

**Table 2.4:** Biological activity of some species of *Rhamnus*

| Species | Plant part | Biological activity | References |
|---|---|---|---|
| ***R. alaternus*** | Part not specified | Anti-proliferative (Human leukemia K562 cells) | Ammar *et al.*, 2008 |
| | Roots, leaves | Radical scavenging activity, antioxidant effect, antimutagenic activity | |
| ***R. frangula*** | Part not specified | Anti-leukemic activity (P-338 lymphocytic leukemia) | Kupchan and Karim, 1976 |
| ***R. nakaharai*** | Stem bark | Anti-platelet effect (Arachidonic acid and collagen-induced platelet aggregation) | Lin *et al.*, 1995 |
| ***R. nepalensis*** | Fruits | Cytotoxic activity (KB cells) | Mai *et al.*, 2001 |
| ***R. prinoides*** | Flowers, leaves, stems | Antimutagenic activity | Wall *et al.*, 1988 |
| | Leaves | Antibacterial activity, | Chhabra and uiso, 1991 |
| | | insecticide activity | Van Puyvelde *et al.*, 1985 |
| | Root bark | Cytotoxic activity | Koch *et al.*, 2005 |
| | Leaves, Roots | Antimalarial | Kuria *et al.*, 2001 |
| | Leaves, Root bark | Antimalarial | Muregi *et al.*, 2007 |
| ***R. staddo*** | Shade dried leaves | Taenicide activity | Desta, 1995 |
| | Part not specified | Antimalarial activity | Kuria *et al.*, 2001 |
| | Shade dried roots | Anti-implantation activity, uterine stimulant effect | Desta, 1994 |
| | Root bark | Cytotoxic activity | Koch *et al.*, 2005 |
| | Leaves, Root bark | Antimalarial | Muregi *et al.*, 2007 |

## 2.6 BIOLOGICAL INFORMATION ON *KNIPHOFIA* SPECIES

Some of the compounds isolated from *Kniphofia foliosa* have shown promising antimalraial activity. The diverse biological activity of the compounds isolated is summarized in table 2.5.

**Table 2.5:** Biological activity of compounds isolated from *Kniphofia foliosa*

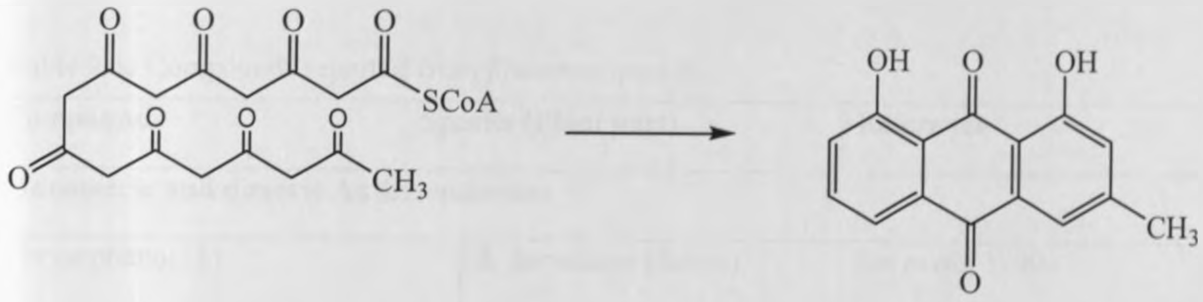
| Biological activity | Compound | Reference |
|---|---|---|
| Antioxidant activity | Knipholone anthrone (**76**) | Habtemariam, 2007; Bringhmann *et al.*, 2008a |
| Antiprotozoal activity, radical scavenging effect against DPPH | Knipholone anthrone (**76**) | Habtemariam, 2007 |
| Antitumour activities (HSC-2 cells) | Knipholone (**9**), isoknipholone (**74**) | Bringhmann *et al.*, 2008a |
| Antimalarial (K1 and NF54 strains of *Plasmodium falciparum*) | Isoknipholone (**74**), knipholone anthrone (**76**) | |
| Inhibition of the growth of *P. falciparum* | Chryslandicin (**8**) | Wube *et al.*, 2005 |
| Inhibition of leukotriene (treatment of asthma and other inflammatory diseases),free radical scavenging, lipid peroxidation inhibitor | Knipholone (**9**) | Wube *et al.*, 2006 |

## 2.7 PHYTOCHEMISTRY OF THE RHAMNACEAE AND ASPHODELACEAE

Quinones are the principal secondary metabolites found in both the Rhamnaceae and Asphodelaceae. They are coloured compounds that are widely distributed in higher plants, fungi, lichen and insects and are responsible for pigments in some organisms [Adzet and Camarasa, 1996; Harborne *et al.*, 1999]. Depending on the aromatic rings they contain, quinones can be classified as benzoquinones such as embelin; naphtoquinones e.g. juglone and anthraquinones like aloe-emodin. Anthraquinones are the largest group of plant quinones and are found concealed in the bark, heartwood, roots or leaves [Harborne *et al.*, 1999]. Both Rhamnaceae (particularly the genus *Rhamnus*) and Asphodelaceae are among the families known to contain a wide array of anthraquinones that have been used for long, both medicinally and commercially. By 1995, over 300 naturally occurring anthraquinones had been identified [Hao *et al.*, 1995]. Some studies associate the production of anthraquinone in plants with the production of fruits and seeds [Miller, 1973].

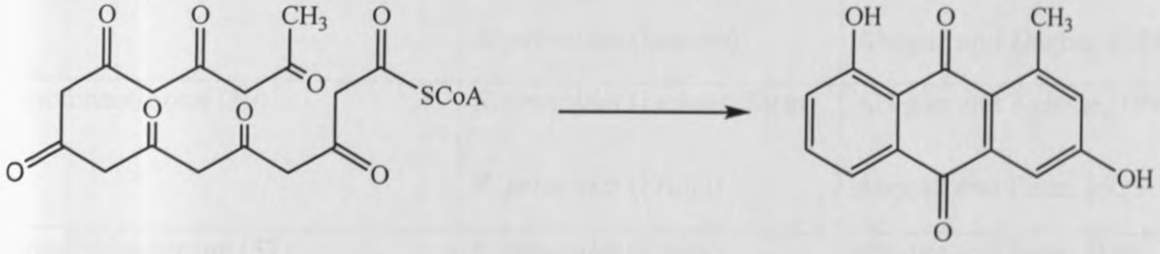
Medicinally, anthraquinones have shown to possess anti-inflammatory, wound healing, analgesic, antipyretic, antimicrobial and antitumor properties [Leu *et al.*, 2008]. The *O*- or *C*-anthraquinone glycosides found in *Senna*, *Cascara*, *Frangula*, *Rhubarab* and *Aloe* are known to possess laxative and purgative activities [Dewick, 2002]. In addition, the anthraquinones isolated from *Hemerocallis fulva* were found to inhibit the proliferation of breast, central nervous system, colon and lung cancer cells [Cseke, *et al.*, 2006]. Though the combined free anthraquinones had some purgative activity, the presence of water soluble glycosides potentiates their therapeutic use. Commercially, natural and synthetic anthraquinones are assimilated as colorants in food, drug, cosmetics, hair dye and as dyestuff in textile manufacturing industries [Hao *et al.*, 1995].

Biosynthetically, anthraquinones found in the families Rhamnaceae and Asphodelaceae are derived through the polyketide pathway where the anthraquinone skeleton is derived by cyclization of the octaketide chain. Folding of the octaketide chain can take place in two ways. The 1,8-dihydroxy-3-methylanthraquinones such as chrysophanol (**1**) are obtained through the customary folding (Scheme 1) where as the rare type of folding (Scheme 2) which is mainly observed in the *Aloe* species (Asphodelaceae) results in the formation of 3,8-dihydroxy-1-methylanthraquinones with the typical example being aloesaponarin II (**35**).




**1**

**Scheme 1:** Formation of 1,8-dihydroxy-3-methylanthraquinone from octaketide chain




**35**

**Scheme 2:** Formation of 3,8-dihydroxy-1-methylanthraquinone from octaketide chain

### 2.7.1 PHYTOCHEMICAL INFORMATION ON *RHAMNUS* SPECIES

Phytochemically, the family Rhamnaceae consist calcium oxalate, tannins, flavanols, leucoanthocyanins and certain alkaloids [Medan and Schirarend, 2004]. The different classes of compounds reported from the genus *Rhamnus* include anthraquinones, alkaloids, coumarins, flavonols, sterols, triterpenes, tannins and miscellaneous lactones. Anthraquinones, anthranols and their glycosides are restricted to the genus *Rhamnus* and are known to possess laxative property [Evans, 1996; Medan and Schirarend, 2004]. The different compounds isolated from the genus *Rhamnus* are summarized in table 2.6 below.

**Table 2.6:** Compounds reported from *Rhamnus* species

| **Compounds** | **Species (Plant part)** | **Reference** |
|---|---|---|
| **Monomeric and dimeric Anthraquinones** | | |
| Chrysophanol (**1**) | *R. formosana* (Roots) | Lin *et al.*, 1990 |
| | *R. prinoides* (Leaves, Stem) | Abegaz and Kebede, 1995 |
| Emodin (**3**) | *R. formosana* (Roots) | Lin *et al.*, 1990 |
| | *R. prinoides* (Fruits) | Abegaz and Peter, 1995 |
| | *R. prinoides* (Leaves) | Abegaz and Dagne, 1988 |
| Emodinanthrone (**36**) | *R. prinoides* (Leaves, Stem) | Abegaz and Kebede, 1995 |
| | *R. prinoides* (Fruits) | Abegaz and Peter, 1995 |
| Emodinbianthrone (**37**) | *R. prinoides* (Fruits) | Abegaz and Peter, 1995 |
| Physcion (**2**) | *R. formosana* (Roots) | Lin *et al.*, 1990 |
| | *R. prinoides* (Leaves) | Abegaz and Dagne, 1988 |
| | *R. prinoides* (Leaves, Stem) | Abegaz and Kebede, 1995 |

**Table 2.6:** Compounds reported from *Rhamnus* species cont...

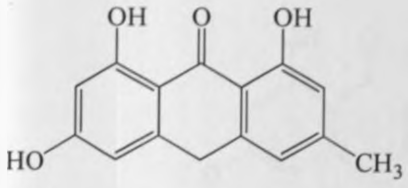
| Compounds | Species (Plant part) | Reference |
|---|---|---|
| **Anthraquinone glycosides** | | |
| Emodin 6-*O*-L-rhamnoide (**38**) | *R. libanoticus* (Bark) | Coskun et al., 1990 |
| Emodinanthrone-6-*O*-rhamnopyranoside-2',3',4'-triacetate (**39**) | *R. prinoides* (Fruits) | Abegaz and Peter, 1995 |
| Frangulin B (**40**) | *R. formosana* (Roots) | Lin *et al.*, 1990 |
| Glucofrangulin A (**41**) | *R. prinoides* (Fruits) | Bezabih and Abegaz, 1998 |
| Physcion 8-*O*-β-rutinoside (**42**) | *R.libanoticus* (Bark) | Coskun *et al.*, 1990 |
| 1,6,8- trihydroxy-3-methylanthraquinone 1-*O*-rhamnosyl (1→2) glucoside (**43**) | *R. formosana* (Roots) | Lin *et al.*, 1991 |
| 1,8-dihydroxy-6-methoxy-3-methyl anthraquinones 8-*O*-rhamnosyl-(1→2)-glucoside (**44**) | *R. formosana* (Roots) | Lin *et al.*, 1990 |
| **Anthrone rhamnoside** | | |
| Prinoidin (**45**) | *R. prinoides* (fruits) | Abegaz and Peter, 1995; Abegaz and Kebede, 1995 |
| **Napthalenic derivatives** | | |
| Geshoidin (β-sorigenin-8-*O*-β-D-glucoside) (**6**) | *R. prinoides* (Leaves and stem) | Abegaz and Kebede, 1995 |
| Musizin (**46**) | *R. prinoides* (Leaves and stem) | |
| β-sorigenin (**5**) | *R. prinoides* (Leaves and stem) | |
| β-sorigenin-1-*O*- β-glucoside (**47**) | *R. wightii* | |
| α-sorinin (**48**) | *R. pallasii, R. japonicus* | |

**Table 2.6:** Compounds reported from *Rhamnus* species cont...

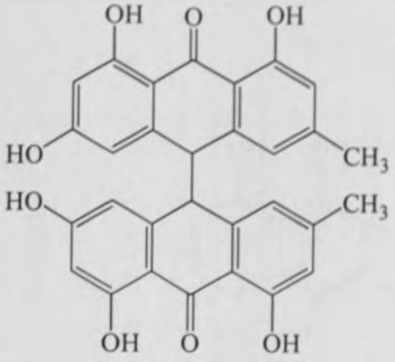
| Compounds | Species (Plant part) | Reference |
|---|---|---|
| **Flavanol glycosides** | | |
| Kaempferol-3-*O*-β-rhamninoside (**49**) | *R. petiolaris* (Dried fruits) | Ozipek *et al.*, 1994 |
| Rhamnazin 3-*O*-[L-rhamnopyranosyl(1→4)-L-rhamnopyranosyl (1→6)-β-D-galactopyranoside (rhamnazin-3-isorhamninoside) (**50**) | *R formosana* (Roots) | Lin *et al.*,1991 |
| Rhamnazin 3-*O*-β-rhamnoside (**51**) | *R. petiolaris* (Dried fruits) | Riess- Maurer and Wagner, 1982 |
| Rhamnetin 3-*O*-(3''''-*O*-*p*-coumaroyl)-β - rhamninoside (**52**) | *R. petiolaris* (Berries) | Ozipek *et al.*, 1994 |
| Rhamnocitrin 3-*O*-isorhamninoside (**53**) | *R. formosana* (Roots) | Lin *et al.*,1991 |
| Rhamnocitrin -3-*O*-rutinoside (**54**) | *R. lycioides* (Leaves) | Khalifa *et al.*, 2001 |
| 8-O-β-D-glucoside kaempferol (**55**) | *R. libanoticus* (Bark) | Coskun *et al.*, 1990 |
| **Flavonoids** | | |
| Quercetin (**56**) | *R. prinoides* (Leaves and stem) | |
| Quercitrin (**57**) | *R. petiolaris* (Dried fruits) | Ozipek *et al.*, 1994 |
| Rhamnazin (**4**) | *R. prinoides* (fruits, Leaves) | Abegaz and Peter, 1995 |
| Rhamnetin (**58**) | *R. prinoides* (Leaves and stem) | Abegaz and Kebede, 1995 |
| Rhamnocitrin (**59**) | *R. prinoides* (Leaves and stem) | Abegaz and Kebede, 1995 |
| Xanthorhamnin B (Rhamnetin 3-*O*-(3'''-*O*-4-coumaroyl) rhamniniside (**60**) | *R. petiolaris* (Dried fruits) | Ozipek *et al.*, 1994 |
| 3-*O*-Methylquercetin (**61**) | *R. prinoides* (Leaves and stem) | Abegaz and Kebede, 1995 |

**Table 2.6:** Compounds reported from *Rhamnus* species cont...

| **Steroids** | | |
|---|---|---|
| Stigmasterol-β-D-glycoside (**62**) | *R. formosana* (Roots) | Lin *et al.*, 1990 |
| β-sitosterol (**63**) | *R. formosana* (Roots) | Lin *et al.*, 1990 |



**36**



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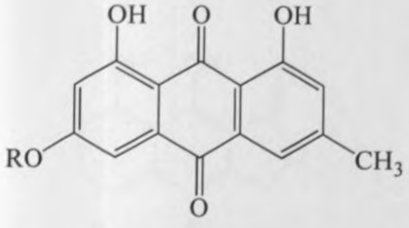
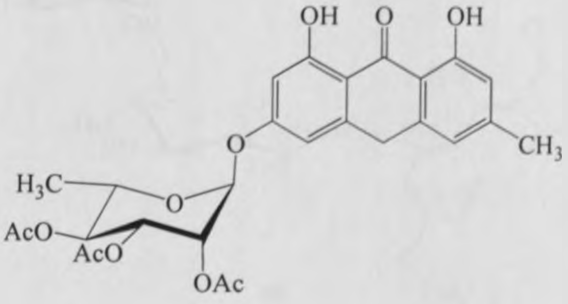


R = L - Rhamnoside

**38**



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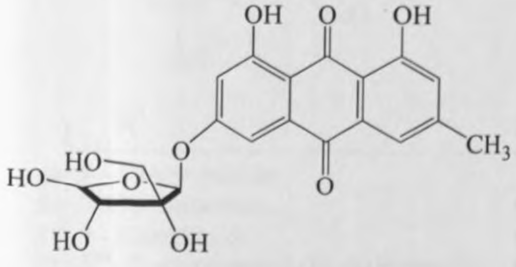

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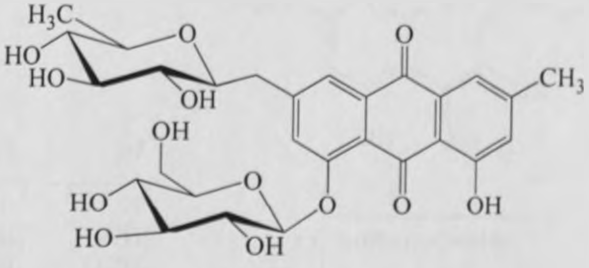
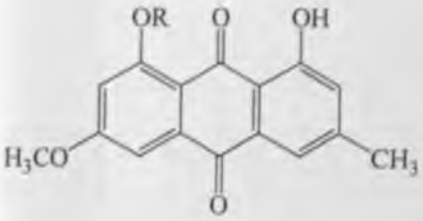


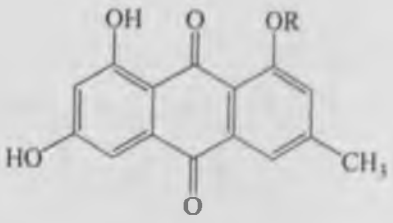
**41**



R

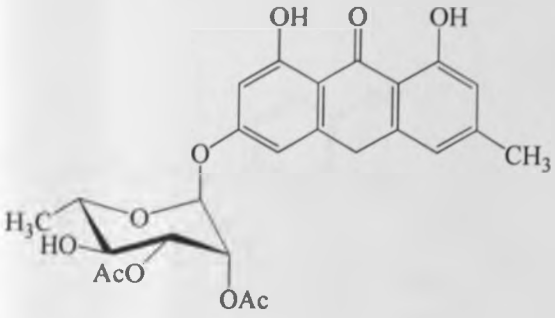
42 β - Rutinoside

44 Rhamnosyl - (1→2) glucoside



R = Rhamnosyl - (1→2) glucoside

43



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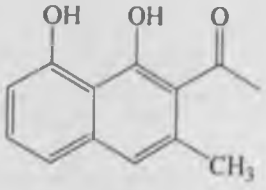

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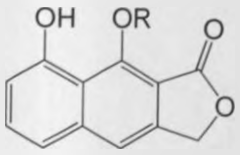
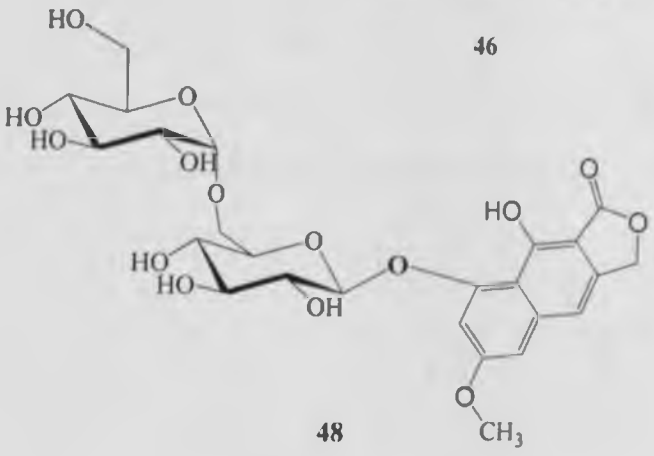
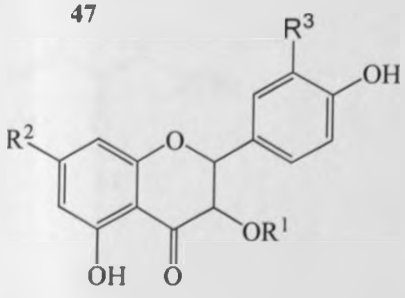


R = β – Glucoside

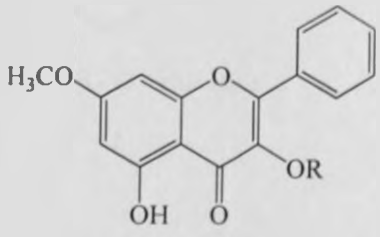
47



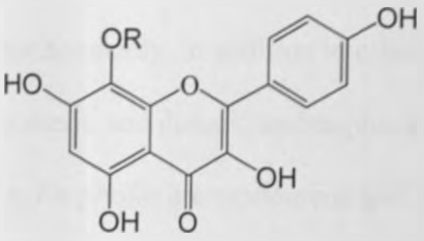
48



| | R¹ | R² | R³ |
|---|---|---|---|
| 49 | β – Rhamninoside | OH | H |
| 50 | Isorhamninoside | $OCH_3$ | $OCH_3$ |
| 51 | β - Rhamnoside | $OCH_3$ | $OCH_3$ |
| 52 | 3""- O -p- coumaryl- β–rhamninoside | $OCH_3$ | H |

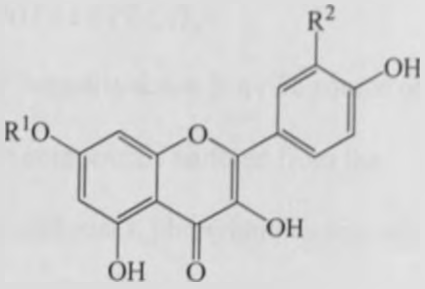


| | R |
|---|---|
| 53 | Isorhamninoside |
| 54 | Rutinoside |

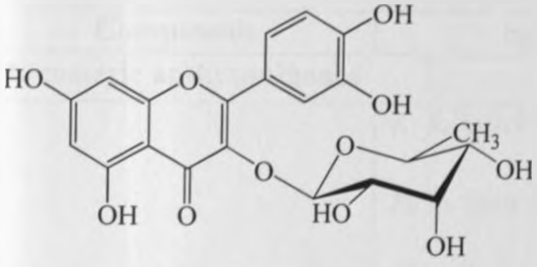


R = β–D-Glucoside

**55**



| | $R^1$ | $R^2$ |
|---|---|---|
| **56** | H | OH |
| **58** | $CH_3$ | H |



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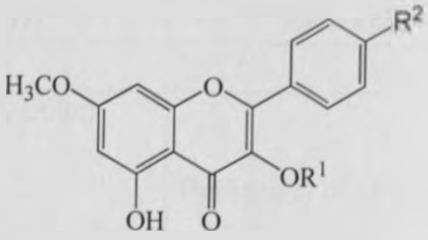
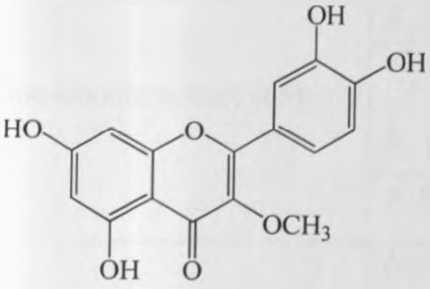
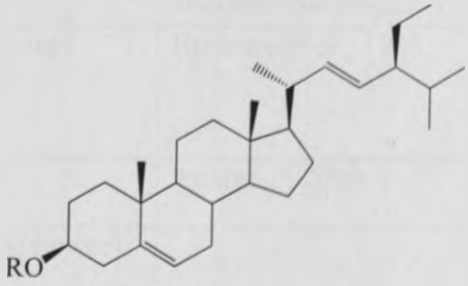


| | $R^1$ | $R^2$ |
|---|---|---|
| **59** | H | H |
| **60** | 3-O - (3‴-O - 4- coumaroyl) rhamniniside | OH |

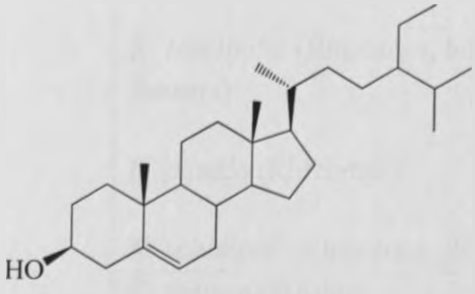


**61**



R = β - D - glucoside

**62**



**63**

### 2.7.2 PHYTOCHEMICAL INFORMATION ON *KNIPHOFIA* SPECIES

Phytochemically, in addition to other compounds, the family Asphodelaceae is a rich source of monomeric and dimeric anthraquinones. The major classes of compounds isolated from the genus *Kniphofia* are monomeric and dimeric anthraquinones, anthrones, phenylanthraquinones and oxanthrones. The sources of these compounds are summarized in table 2.7 below.

**Table 2.7:** Compounds reported from *Kniphofia* species

| Compounds | Species (plant part) | Reference |
|---|---|---|
| **Monomeric anthraquinones** | | |
| Aloe-emodin (**64**) | *K. foliosa* (Leaves, flowers, fruits)<br>*K. insignis* (Flowers)<br>*K. isoetifolia* (Flowers)<br>*K. schimperi* (Flowers) | Berhanu *et al.*, 1986 |
| | *K.thomsonii* (Root) | Achieng', 2009 |
| Aloe-emodin acetate (**65**) | *K.foliosa* (Leaves) | Berhanu and Dagne, 1984 |
| | *K. foliosa* (Flower, leaves, fruits)<br>*K. isoetifolia* (Flowers) | Berhanu *et al.*, 1986 |
| | *K. thomsonii* (Root) | Achieng', 2009 |
| Chrysophanol (**1**) | *K. foliosa* (Rhizomes, leaves, flower)<br>*K. insignis* (Rhizomes)<br>*K. isoetifolia* (Rhizomes, leaves, flowers)<br>*K. pumila* (Rhizomes)<br>*K. schimperi* (Rhizomes, flower) | Berhanu *et al.*, 1986 |
| | *K. thomsonii*(Root) | Achieng', 2009 |

**Table 2.7:** Compounds reported from *Kniphofia* species cont....

| Compounds | Species (plant part) | Reference |
|---|---|---|
| Chrysophanic acid (**66**) | *K. caulescens* (Root) | Yenesew *et al.*, 1988 |
| | *K. foliosa* (Leaves) | Berhanu and Dagne, 1984; Berhanu *et al.*, 1986 |
| | *K. foliosa* (Rhizomes) | Berhanu *et al.*, 1986 |
| | *K. foliosa* (Root, fruits) | Yenesew *et al.*, 1988 |
| | *K. insignis* (Rhizomes) | Berhanu *et al.*, 1986 |
| | *K. isoetifolia* (Flowers, Leaves, Rhizomes) | Berhanu *et al.*, 1986 |
| | *K. linearifolia* (Root) | Yenesew *et al.*, 1988 |
| | *K. pumila* (Rhizomes) | Berhanu *et al.*, 1986 |
| | *K. reynolds* (Root) | Yenesew *et al.*, 1988 |
| | *K. schimperi* (Flowers, Rhizomes) | Berhanu *et al.*, 1986 |
| Islandicin (**7**) | *K.foliosa* (Rhizomes, root) | Yenesew *et al.*, 1988 |
| | *K. foliosa* (Rhizomes, leaves, flowers)<br>*K. insignis* (Rhizomes)<br>*K. isoetifolia* (Rhizomes)<br>*K. pumila* (Rhizomes)<br>*K. schimperi* (Rhizomes) | Berhanu *et al.*, 1986 |
| | *K. linearifolia* (Root) | Yenesew *et al.*, 1988 |
| | *K. reynolds* (Root) | Yenesew *et al.*, 1988 |
| | *K. thomsonii* (Root) | Achieng', 2009 |
| Physcion (**2**) | *K. thomsonii* (Root) | Achieng', 2009 |
| **Dimeric anthraquinones** | | |
| Asphodelin (**67**) | K. albescens (Root) | Van Wyk *et al.*, 1995 |
| | K. linearifolia (Root) | |
| Chrysalodin (**68**) | *K. foliosa* (Leaves) | Dagne *et al.*, 1987 |
| Chryslandicin (**8**) | *K. caulescens* (Root) | Yenesew *et al.*, 1988 |
| | *K.foliosa* (Root) | Yenesew *et al.*, 1988; Wube *et al.*, 2005 |
| | *K. linearifolia* (Root) | Yenesew *et al.*, 1988 |

**Table 2.7:** Compounds reported from *Kniphofia* species cont…

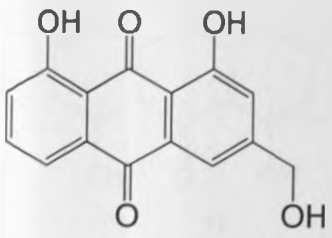
| Compounds | Species (plant part) | Reference |
|---|---|---|
| Kniphofine (**69**) | *K. foliosa* (Rhizomes)<br>*K. insignis* (Rhizomes)<br>*K. isoetifolia* (Rhizomes)<br>*K. pumila* (Rhizomes)<br>*K. schimperi* (Rhizomes) | Berhanu *et al.*, 1985 |
| 10-Hydroxy-10-(chrysophanol-7'-yl)-chrysophanol anthrone (**70**) | *K.foliosa* (Root) | Wube *et al.*, 2005 |
| | *K. thomsonii* (Root) | Achieng', 2009 |
| 10, 10'-Bichrysophanolanthrone (**71**) | *K. thomsonii* (Root) | Achieng', 2009 |
| 10-Hydroxy-10-(chrysophanol-7'-yl)-aloe-emodin anthrone (**72**) | *K. thomsonii* (Root) | Achieng', 2009 |
| 10-hydroxy-10-(islandicin-7'-yl)-aloe-emodin anthrone (**73**) | *K. thomsonii* (Root) | Achieng', 2009 |
| **Phenyl anthraquinones and anthrones** | | |
| Isoknipholone (**74**) | *K.foliosa* (Stem) | Yenesew *et al.*, 1994 |
| Isoknipholone anthrone (**75**) | *K.foliosa* (Stem) | Yenesew *et al.*, 1994 |
| Knipholone anthrone (**76**) | K. foliosa (Stem) | Dagne and Yenesew (1993); Yenesew *et al.*, 1994 |
| Knipholone (**9**) | *K. albescens* (Root)<br>*K. brachystachya* (Root) | Dagne and Yenesew, 1993 |
| | *K. foliosa* (Root) | Dagne and Yenesew, 1993 |
| | *K. foliosa* (Stem) | Yenesew *et al.*, 1994 |
| | *K. foliosa* (Leaves) | Berhanu and Dagne, 1984 |
| | *K. foliosa* (Root) | Yenesew *et al.*, 1988 |
| | *K. foliosa* (Root) | Dagne and Steglich, 1984 |

**Table 2.7:** Compounds reported from *Kniphofia* species cont…

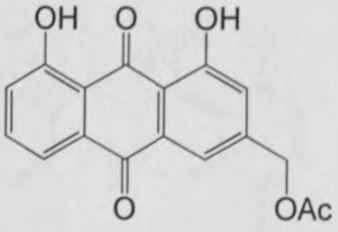
| Compounds | Species (plant part) | Reference |
|---|---|---|
| Knipholone (**9**) | *K. foliosa* (Rhizomes, leaves, flowers, fruits)<br>*K. insignis* (Rhizomes)<br>*K. isoetifolia* (Rhizomes)<br>*K. pumila* (Rhizomes, Flowers)<br>*K. schimperi* (Rhizomes) | Berhanu *et al.* 1986 |
| | *K. acraea* (Roots)<br>*K. caulescens* (Root)<br>*K. flammula* (Root)<br>*K. linearifolia* (Root) | Yenesew *et al.*, 1988 |
| | *K. thomsonii* (Root) | Achieng', 2009 |
| **Oxanthrones** | | |
| Foliosone (**77**)<br>Isofoliosone (**78**) | *K.foliosa* (Stem) | Yenesew *et al.*, 1994 |
| **Miscellaneous compounds** | | |
| Aloesaponol III (**79**) | *K. foliosa* (Stem) | Yenesew *et al.*, 1994 |
| Aloesaponol III-8-methyl ether (**80**) | *K. foliosa* (Stem) | Yenesew *et al.*, 1994 |
| Citric acid (**81**) | *K. burchelli* (Leaves) | Van Rheede Van Oudtshoorn, 1964 |
| **Miscellaneous compounds continued…** | | |
| Flavoglucin (**82**) | *K. thomsonii* (Root) | Achieng', 2009 |
| Malic acid (**83**) | *K. burchelli* (Leaves) | Van Rheede Van Oudtshoorn, 1964 |

**Table 2.7:** Compounds reported from *Kniphofia* species cont…

| Compounds | Species (plant part) | Reference |
|---|---|---|
| Quinic acid (**84**) | *K. uvaria* (Leaves) | Yoshida *et al.*, 1975 |
| Shikimic acid (**85**) | | |
| 3''',4'''- Dehydroflavoglaucin (**86**) | *K. thomsonii* (Root) | Achieng', 2009 |
| 2-Acetyl-1-hydroxy-8-methoxy-3-methylnaphthalene (**87**) | *K. foliosa* (Roots) | Wube *et al.*, 2005 |
| 4,6-Dihydroxy-2-methoxyacetophenone (**88**) | *K. foliosa* (Stem) | Yenesew *et al.*, 1994 |




**64**

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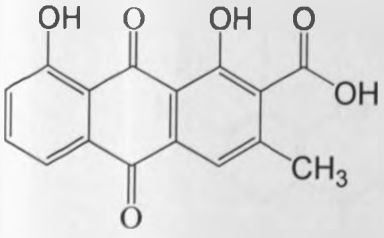
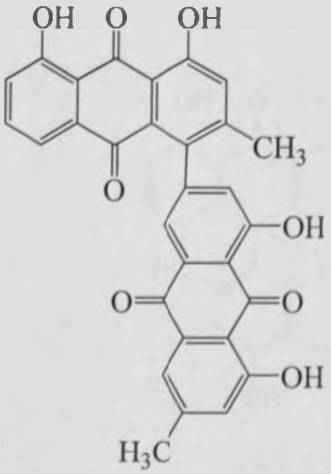
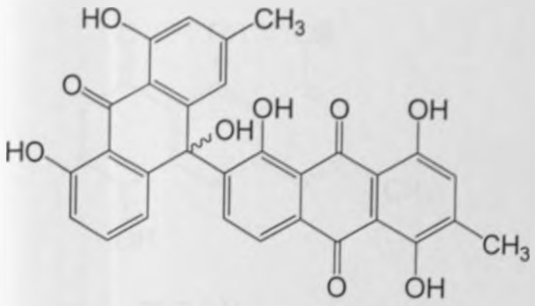



**66**




**67**



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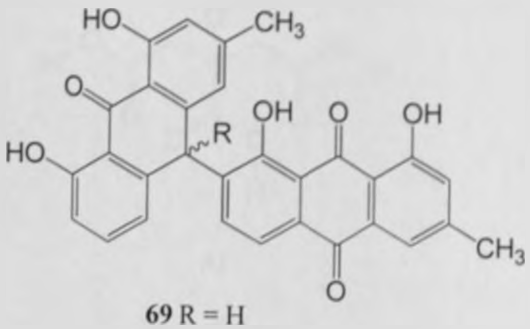
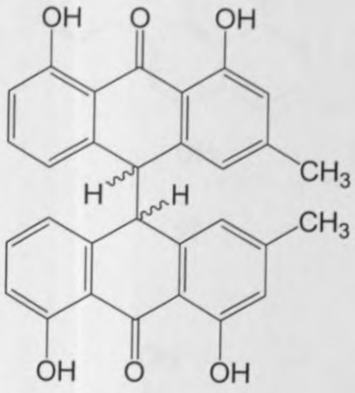


**69** R = H
**70** R = OH



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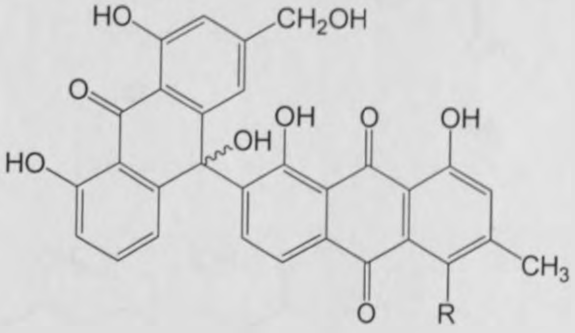
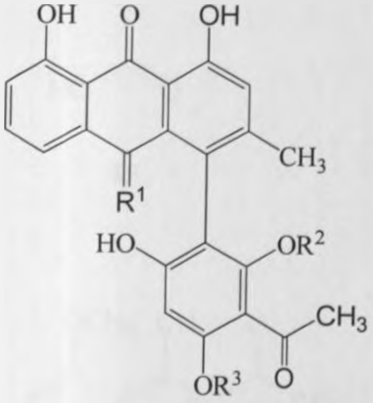
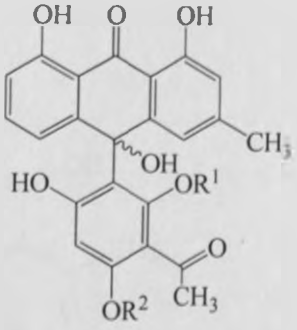


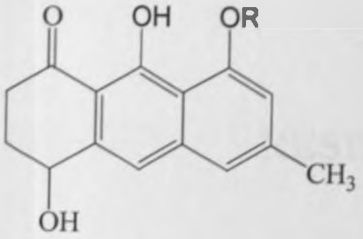
**72** R= H
**73** R= OH



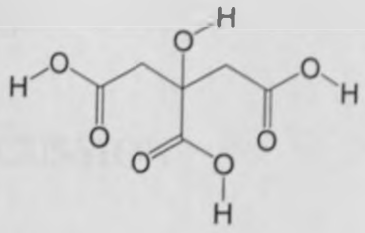
| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **74** | O | $CH_3$ | H |
| **75** | H, H | $CH_3$ | H |
| **76** | H, H | H | $CH_3$ |



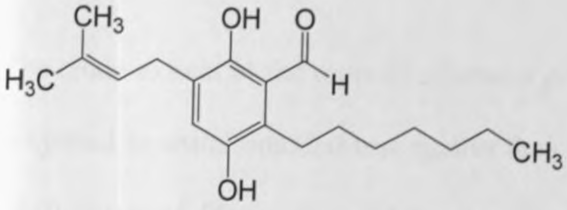
| | $R^1$ | $R^2$ |
|---|---|---|
| **77** | H | $CH_3$ |
| **78** | $CH_3$ | H |



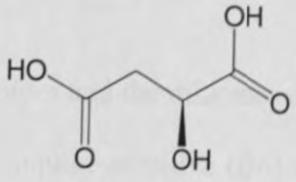
**79** R = H
**80** R = $CH_3$



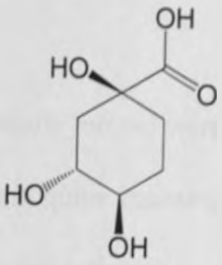
**81**



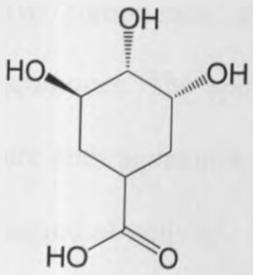
**82**



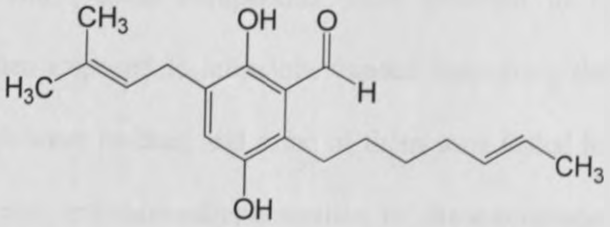
**83**



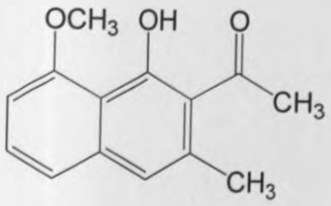
**84**



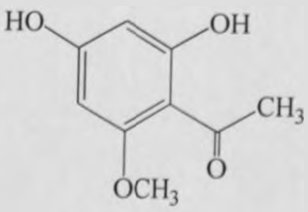
**85**



**86**



**87**



**88**

# CHAPTER 3

# RESULTS AND DISCUSSION

## 3.1 PRELIMINARY TEST

The crude extract of the roots of *Rhamnus prinoides* and the rhizomes of *Kniphofia foliosa* were subjected to antiplasmodial test against the chloroquine sensitive (D6) and chloroquine resistant (W2) strains of *Plasmodium falciparum*. The extracts showed potent antiplasmodial activity.

TLC analyses of the crude extracts showed the presence of coloured and UV (254 and 366 nm) sensitive compounds. Based on chemotaxonomy, these compounds were assumed to be anthraquinones. The spots changed colour when exposed to ammonia vapour supporting that these are anthraquinones. The major compounds were isolated and some of them were tested for antiplasmodial activity. The characterization and antiplasmodial activities of the compounds isolated from *Rhamnus prinoides* and *Kniphofia foliosa* are discussed below.

## 3.2 CHARACTERIZATION OF COMPOUNDS

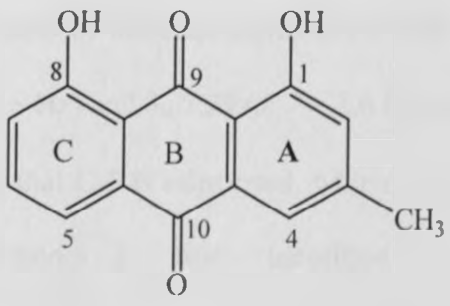
### 3.2.1 CHARACTERIZATION OF COMPOUNDS ISOLATED FROM *RHAMNUS PRINOIDES*

The air dried and ground roots of *Rhamnus prinoides* were exhaustively extracted using dichloromethane/methanol (1:1) by cold percolation at room temperature. The crude extract was then subjected to chromatographic separation which led to the isolation of three anthraquinones, a flavanol and two naphthalenic derivatives. The compounds were characterized using spectroscopic techniques and by comparing with authentic samples in some cases.

#### 3.2.1.1 ANTHRAQUINONES

##### 3.2.1.1.1 Chrysophanol (1)

Compound **1** was isolated as dark yellow amorphous powder with an $R_f$ value of 0.57 (50% dichloromethane in hexane). Upon exposure to ammonia, the yellow spot on TLC changed to red. Compound **1** was identified as 1,8-dihydroxy-3-methylanthracene-9,10-dione trivial name chrysophanol by direct comparison with authentic sample.



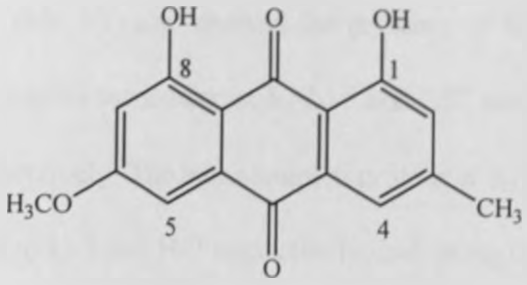

**1**

Compound **1** is widely distributed in plants mainly in the families Asphodelaceae, Rhamnaceae, Rubiaceae and Polygonaceae [Dewick, 2002]. It has also been isolated from the marine annelid *Urechis unicinctus* [DNP, 2009]. Compound **1** is known to possess antimicrobial as well as cathartic activity [DNP, 2009].

**3.2.1.1.2 Physcion (2)**

Compound **2** was isolated as yellow amorphous powder with $R_f$ value of 0.4 (40% dichloromethane in hexane). The yellow spot on TLC changed to red upon exposure to ammonia. In the $^1$H NMR spectrum, two chelated hydroxyl protons at C-1 ($\delta_C$ 166/163) and C-8 ($\delta_C$ 163/166) resonated downfield at $\delta_H$ 12.15 and 12.03 which is characteristic of 1,8-dihydroxyanthraquinone derivatives. In addition, the $^1$H NMR spectrum (Table 3.1) showed the presence of four aromatic protons ($\delta_H$ 7.17, 7.60, 7.29 and 6.81), a methyl ($\delta_H$ 2.31) and a methoxy at $\delta_H$ 4.02.

Signals of two mutually *meta*-coupling protons which were also exhibiting long range coupling with the neighboring methyl group at $\delta_H$ 7.17 (*bd*, $J$ = 1.6 Hz) and $\delta_H$ 7.60 (*bd*, $J$ = 0.6 Hz) were assigned to H-2 and H-4, respectively with the methyl group being at C-3 of ring A, which is in agreement with the biosynthesis of anthraquinones. Two other *meta*-coupling protons which resonated at $\delta_H$ 6.81 (*d*, $J$ = 2.6 Hz) and $\delta_H$ 7.29 (*d*, $J$ = 2.6 Hz) were assigned to H-5 and H-7 of ring C respectively, requiring that C-6 is substituted, which in this case is a methoxy group ($\delta_H$ 4.02). Therefore, compound **2** was identified as 1,8-dihydroxy-3-methyl-6-methoxyanthraquinone, trivial name physcion.



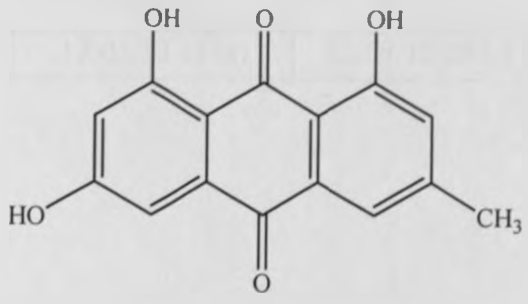

**2**

Compound **2** is produced by *Aspergillus* and *Penicillium* [DNP, 2009] and is also widely distributed in lichens and higher plants such as *Rumex* [Fairbairn and El-Muhtadi, 1972]. It has also been isolated from the marine annelid *Urechis unicintus* [DNP, 2009]. It has been reported from the pods of *Senna didymobotrya* [Alemayehu *et al.*, 1996] and was also isolated for the first time from the genus *Kniphofia* i.e. *Kniphofia thomsonii* [Achieng', 2009]. Compound **2** has exhibited antimicrobial and cathartic properties [DNP, 2009]. Physcion isolated from *Rheum emodi* and *Polygonum cuspidatum* exhibited antifungal and tyrosinase inhibitor property, respectively [Agarwal *et al.*, 2000; Leu *et al.*, 2008].

### 3.2.1.1.3 Emodin (3)

Compound **3** was isolated as orange needle like crystals with $R_f$ value of 0.47 (40% acetone in dichloromethane). Upon exposure to ammonia vapour, the orange spot on TLC changed to reddish brown. In the $^1$H NMR spectrum, the presence of two deshielded protons at $\delta_H$ 12.20 and 12.08 were due to the chelated hydroxyl protons at C-1 and C-8, and the biosynthetically expected aromatic methyl ($\delta_H$ 2.31) at position C-3 suggest that compound **3** has a 1,8 dihydroxy-3-methyl anthraquinone skeleton.

The $^1$H NMR spectrum (Table 3.1) also showed the presence of four aromatic protons, two of which appeared as broad singlets resonating at $\delta_H$ 7.15 and 7.57 and were assigned to protons at C-2 and C-4 of ring A, respectively. The *meta*-coupled protons at $\delta_H$ 7.26 (*d*, *J* = 2.4) and $\delta_H$ 6.67 (*d*, *J* = 2.40) were assigned to H-5 and H-7 respectively, indicating that a hydroxyl substituent, is present at C-6. Compound **3,** was therefore, identified as 6-methyl-1,3,8-trihydroxy anthraquinone (trivial name emodin).




**3**

Compound **3** is widely distributed in higher plants such as *Polygonum cuspidatum*, *Rhamnus purshiana*, *Senna didymobotrya*, and *Rumex* among others [DNP, 2009; Alemayehu *et al.*, 1996; Fairbairn and El-Muhtadi, 1972; Dewick, 2002]. It has also been isolated from *Penicillium*. *Aspergillus* and *Anixiella micropetrusa* [DNP, 2009]. It is known to possess antimicrobial, antineoplastic, cathartic and monoamine oxidase inhibitory property [DNP, 2009]. In addition, emodin which is the predominant anthraquinone in *Polygonum cuspidatum* has shown chemo-preventive effect on skin carcinogenesis in addition to its throsinase inhibition property [Leu *et al.*, 2008].

**Table 3.1:** $^1$H (200 MHz) NMR data for compounds **2** and **3** (Acetone –$d_6$)

| Carbon No. | Compound | |
|---|---|---|
| | **2** $\delta_H$ (*m*, *J* in Hz) | **3** $\delta_H$ (*m*, *J* in Hz) |
| 2 | 7.17 (*brs*) | 7.15 (*brs*) |
| 3 | - | - |
| 4 | 7.60 (*brs*) | 7.57 (*brs*) |
| 5 | 7.29 (*d*, 2.6) | 7.26 (*d*, 2.4) |
| 7 | 6.81 (*d*, 2.6) | 6.67 (*d*, 2.4) |
| 1-OH | 12.15/12.03 (*s*) | 12.20/12.08 (*s*) |
| 3-Me | 2.31 (*brs*) | 2.31 (*brs*) |
| 6-OMe | 4.02 (*s*) | - |
| 8-OH | 12.03/12.15 (*s*) | 12.08/12.20 (*s*) |

### 3.2.1.2 FLAVONOL

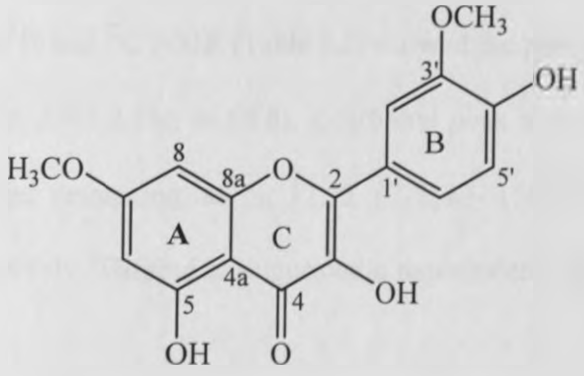
#### 3.2.1.2.1 Rhamnazin (4)

Compound **4** was isolated as pale yellow amorphous solid with $R_f$ value of 0.20 (100% dichloromethane). The UV spectrum showed absorption maxima at 370 and 253 nm suggesting **4** to be a flavonoid (Valesi et al., 1972). The NMR data displayed a singlet at $\delta_H$ 12.14 due to a chelated hydroxyl group at C-5 and an upfield shifted carbonyl group which is conjugated. The $^1$H NMR also showed two sharp singlets at $\delta_H$ 3.93 and 3.94 due to methoxyl groups.

The $^1$H NMR further showed five aromatic protons which exhibited an AX and AXY spin system. The former was observed due to *meta* coupled protons that resonated at $\delta_H$ 6.33 and $\delta_H$ 6.72 (*d*, $J$ = 2.2 Hz) which were assigned to H-6 and H-8 of ring A, respectively. The shielding of these protons indicated that C-5 and C-7 of this ring are oxygenated as expected biogenetically. In addition, in the $^{13}$C NMR, the chemical shift value of C-8 ($\delta_C$ 92.2) is 5 ppm upfield than C-6 which is the case among flavonols [Agrawal *et al.*, 1989].

On the other hand, an AXY pattern was observed due to the three aromatic protons of a disubstituted ring B. These protons which resonated at $\delta_H$ 7.02 (*d*, *J* = 8.6 Hz), $\delta_H$ 7.85 (*dd*, *J* = 8.6, 2 Hz) and $\delta_H$ 7.92 (*d*, *J* = 2 Hz) were assigned to C-2', C-5' and C-6', respectively showing that C-3' and C-4' of this ring are substituted. By comparing the NMR and UV data with literature, the two methoxy groups which resonated at $\delta_C$ 55.8 were assigned to C-7 and C-3', respectively whereas the two hydroxyl groups were placed at C-3 and C-4' [Valesi *et al.*, 1972]. From the above data, although other possible isomers could not be fully eliminated, compound **4** which was previously isolated from other *Rhamnus* species and other plant parts of *Rhamnus prinoides* was identified as 3,4',5-trihydroxy-3',7-dimethoxyflavone trivial name rhamnazin (**4**).

Rhamnazin, a compound known to possess cytotoxic activity, has also been isolated from *Retama sphaerocarpa*, *Larrea cuneifolia*, and from *Cistus* species [Valesi *et al*, 1972; DNP, 2009].




**4**

**Table 3.2:** $^{1}H$ (200 MHz) NMR data of compound **4** (Acetone-$d_6$)

| Carbon No. | $^{1}H$ $\delta_H$ (*m*, *J* in Hz) |
|---|---|
| 6 | 6.33 (*d*, 2.2) |
| 8 | 6.72 (*d*, 2.2) |
| 2' | 7.92 (*d*, 2) |
| 5' | 7.02 (*d*, 8.6) |
| 6' | 7.85 (*dd*, 8.6, 2) |
| 7-OMe | 3.93 (*s*) |
| 3'-OMe | 3.94 (*s*) |
| 5-OH | 12.14 (*s*) |

## 3.2.1.3 NAPHTHALENIC DERIVATIVES

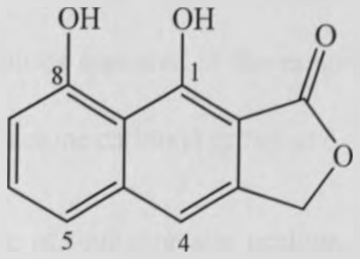
### 3.2.1.3.1 *β*-Sorigenin (5)

Compound **5** was isolated as a brown amorphous solid with green fluoresce under UV light (366 nm) and had an $R_f$ value of 0.6 (EtOAc/MeOH/$H_2O$ 7.5:1.5:1.0). The UV spectrum showed $\lambda_{max}$ at 384nm, while the $^{1}H$ and $^{13}C$ NMR (Table 3.3) showed the presence of a deshielded methylene protons at $\delta_H$ 5.39 (*d*, $J$ = 1.2 Hz; $\delta_C$ 68.8), a carbonyl peak at $\delta_C$ 169.3 of the lactone ring, and two hydroxyl groups resonating at $\delta_H$ 12.92 (C-1, $\delta_C$ 156.6/156.1) and $\delta_H$ 10.41 (C-8, $\delta_C$ 156.1/156.6), respectively. These data suggested a naphthalenic lactone derivative [Abegaz and Kebede, 1995].

The $^{1}H$ NMR further displayed three aromatic protons with ABX spin system at $\delta_H$ 7.44 (*dd*, $J$ = 7.8, 0.6 Hz), 7.47 (*t*, $J$ = 7.8 Hz) and 6.93 (*dd*, $J$ = 7.2, 0.6 Hz) which were assigned to protons at C-5, C-6 and C-7, respectively of a C-1, C-2, C-3 and C-8 substituted naphthalene. The fourth aromatic proton which resonated as broad singlet ($\delta_H$ 7.41, $\delta_C$ 120.2) due to long range coupling

with the neighboring methylene lactone protons was assigned to the proton at C-4. Compound **5** was therefore identified as 8,9-dihydroxynaphtho[2,3-c] furan-1 (3H)-one trivial name *β*–sorigenin.

Compound **5** has been previously isolated from the leaves and fruits of *Rhamnus prinoides* [Abegaz and Kebede, 1995; Abegaz and Peter, 1995] and from the stem bark of *Rhamnus wightii* [Pepalla *et al.*, 1991]. However, this is the first report from the roots of *Rhamnus prinoides*.




**5**

**Table 3.3:** $^{1}H$ (600 MHz) and $^{13}C$ (50 MHz) NMR data of compound **5** (Acetone-$d_6$)

| Carbon | $^{1}H$ $\delta_H$ (*m*, *J* in Hz) | $^{13}C$ | HMBC |
|---|---|---|---|
| 1 | - | 156.6[a] | |
| 2 | - | 114.2 | |
| 3 | - | 142.7 | |
| 4 | 7.41 (*s*) | 111.5 | $CH_2$, C-8a, C-2, C-5 |
| 4a | - | 140.7 | |
| 5 | 7.44 (*d*, 8.1) | 120.2 | C-7, C-5 |
| 6 | 7.49 (*t*, 7.8) | 130.4 | C-4a, C-8 |
| 7 | 6.92 (*dd*, 7.2, 0.6) | 109.6 | C-5, C-8 |
| 8 | - | 156.1[a] | |
| 8a | - | 105.3 | |
| 1-OH | 10.65 (*s*) | - | |
| 8-OH | - | - | |
| Lactone-$CH_2$ | 5.39 (*d*, 1.2) | 68.8 | C-8a, C-4, C-3, C-2, CO |
| Lactone- CO | - | 169.3 | |

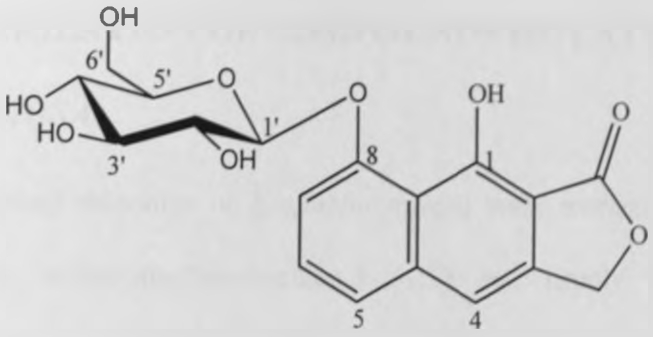
[a]Interchangeable

### 3.2.1.3.2 Geshoidin (6)

Compound **6** was isolated as a white amorphous powder with purple fluoresces under UV light (366 nm). It had an $R_f$ value of 0.43 (EtoAc/MeOH/$H_2O$ 7.5:1.5:1.0). The UV spectrum showed absorption bands at $\lambda_{max}$ 299 and 352 nm characteristic of a naphthalenic lactone. The $^1H$ NMR data (Table 3.4) showed a signal at $\delta_H$ 10.46 indicating the presence of a chelated hydroxyl group at C-1 ($\delta_C$ 156.0). The deshielded methylene lactone resonated at $\delta_H$ 5.38 ($\delta_C$ 60.7) as a singlet while the signals for the sugar protons appeared in the range of $\delta_H$ 3.25 - 5.18. In addition, the $^{13}C$ NMR showed a signal for the lactone carboxyl group at $\delta_C$ 168.2.

The $^1H$ NMR showed the presence of four aromatic protons, three of which exhibited an ABX spin system resonating at $\delta_H$ 7.66 (*d*, $J$ = 7.8 Hz), 7.59 (*dd*, $J$ = 8.4, 7.8 Hz) and 7.43 (*dd*, $J$ = 7.2, 1.2 Hz) which were assigned to H-5, H-6 and H-7, respectively while the signal for the fourth aromatic proton which appeared as a singlet at $\delta_H$ 7.46 was assigned to H-4. The structure was further confirmed using the DEPT spectrum which indicated the presence of two $CH_2$, nine CH and seven quaternary carbons. The presence of the glucose moiety was confirmed using the $^{13}C$ NMR which showed six carbons ($\delta_C$ 68.1- 102.9) in the region typical of a glucose unit. By comparing the NMR data with literature [Abegaz and Kebede, 1995], compound **6** was identified as β-sorigenin-8-*O*- *β*–D-glucoside (trivial name geshoidin).

Compound **6** was previously isolated from the leaves of *Rhamnus prinoides* [Abegaz and Kebede, 1995], but this is the first report from the roots of this plant.




**6**

**Table 3.4:** $^{1}H$ (600MHz) and $^{13}C$ NMR (125 MHz) data of compound **6** (DMSO-$d_6$)

| Carbon No. | $^{1}H$ $\delta_H$ (*m*, *J* in Hz) | $^{13}C$ |
|---|---|---|
| 1 | - | 156.0 |
| 2 | - | 114.3 |
| 3 | - | 139.6 |
| 4 | 7.46 (*s*) | 111.1 |
| 4a | - | 143.1 |
| 5 | 7.66 (*d* , 7.8) | 123.2 |
| 6 | 7.59 (*dd* ,8.4, 7.8) | 129.8 |
| 7 | 7.43 (*dd* , 7.2, 1.2) | 111.2 |
| 8 | - | 155.4 |
| 8a | - | 106.0 |
| Lactone- $CH_2$ | 5.38 (*s*) | 60.7 |
| Lactone-CO | - | 168.2 |
| 1' | 5.15 (*d*, 7.8) | 102.9 |
| 2' | 3.45 (*t*, 9, 7.8) | 73.4 |
| 3' | 3.37 (*t*, 9) | 77.9 |
| 4' | 3.24 (*t*, 9) | 69.8 |
| 5' | 3.49 (*ddd*, 9.6, 6.6,1.8) | 76.2 |
| 6'a | 3.54 (*dd*, 12, 6) | 68.1 |
| 6'b | 3.79 (*dd*, 12, 1.8) | |

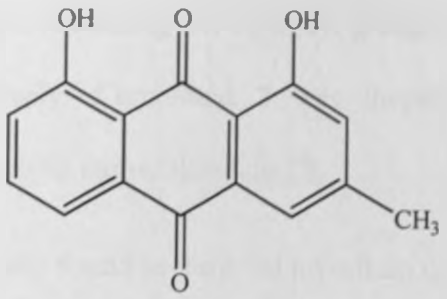
## 3.2.2 CHARACTERIZATION OF COMPOUNDS ISOLATED FROM *KNIPHOFIA FOLIOSA*

The air dried and ground rhizomes of *Kniphofia foliosa* were extracted exhaustively using acetone followed by dichloromethane/methanol (1:1) and finally with methanol. The dichloromethane/methanol and methanol crude extracts were combined and partitioned between ethylacetate and water. The ethyl acetate layer was subjected to column chromatography on oxalic acid impregnated silica gel. This led to the isolation of three monomeric anthraquinones, a monomeric phenylanthraquinone, a dimeric anthraquinone and a benzoic acid derivative. The acetone extract was also partitioned between dichloromethane and water. The dichloromethane extract formed a yellow precipitate after sometimes which was taken in a mixture of acetone and methanol. This extract was then subjected to column chromatography on oxalic acid impregnated silica gel which led to the isolation of two dimeric phenylanthraquinones and a tetrameric phenylanthrone, in addition to the compounds isolated from the first extract. These compounds were characterized using spectroscopic methods.

### 3.2.2.1 Monomeric Anthraquinones

#### 3.2.2.1.1 Chrysophanol (1)

Compound **1** was isolated as a dark yellow amorphous powder with $R_f$ value of 0.57 (20% EtOAc in hexane). Compound **1** was identified by direct comparison with an authentic sample as in section 3.3.1.1.1 1,8-dihydroxy-3-methylanthracene-9,10-dione trivial name chrysophanol (**1**).




**1**

**3.2.2.1.2 Islandicin (7)**

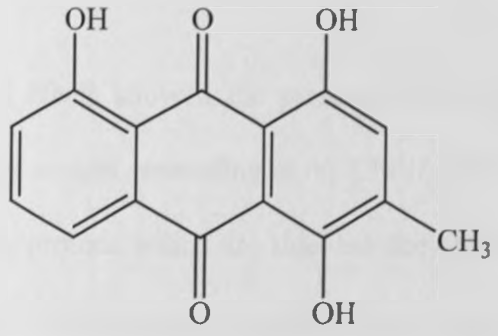
Compound **7** was isolated as a red amorphous powder with $R_f$ value of 0.59 (10% EtOAc in hexane). The red spot on TLC changed to purple on exposure to ammonia vapour. The UV spectrum of compound **7** showed absorption maxima at 493, 431, 401 and 282 nm due to the presence of three hydroxyl substituents. This is a characteristic feature of either a 1,4,8-trihydroxy- or 1,5,8-trihydroxy-anthraquinones [Achieng', 2009].

The $^1$H NMR spectrum showed the presence of three chelated hydroxyl protons at $\delta_H$ 13.49, 12.33 and 12.29 which were attributable to C-4 (or C-5), C-1 and C-8 of an anthraquinone skeleton. The biogenetically expected aromatic methyl group at C-3 of ring A resonated at $\delta_H$ 2.38 (*d*, $J$ = 1 Hz) and appeared as doublet due to long range coupling with the neighboring proton at C-2.

In the aromatic region, the presence of four protons were evident, three of which exhibited an ABX spin system resonating at $\delta_H$ 7.89 (*dd*, $J$ = 7.6, 1.4 Hz), 7.69 (*t*, $J$ = 8.4, 7.6 Hz) and 7.31 (*dd*, $J$ = 8.4, 1.2 Hz) corresponding to H-5, H-6 and H-7 of ring C, respectively. This indicated that one of the hydroxyl groups is at C-4 of ring A. The fourth aromatic proton which appeared as a broad singlet ($\delta_H$ 7.16 *bs*) due to long range coupling with the methyl group at C-3 was

assigned to H-2 with the remaining two hydroxyl groups being placed at C-1 and C-8 of ring A and ring C, respectively. Compound 7 was therefore identified as 1,4,8-trihydroxy-3-methylanthraquinone, trivial name islandicin (7).

Compound 7 is commonly found in the dried mycelium of *Penicillium islandicum* and the lichen *Asahinea chrysantha* [Mishchenko *et al.*, 1980; Berhanu *et al.*, 1986]. In higher plants, it has been previously isolated from the heartwood of *Maesopsis eminii*, the fruits of *Bulbine abyssinica*, the roots of *K. thomsonii*, the rhizomes of *K. foliosa*, *K. insignis*, *K. isoetifolia*, *K. pumila* and *K. schimperi*, and also from the leaves and flowers of *K. foliosa* [Cumming and Thomson, 1970; Berhanu *et al.*, 1986; Wanjohi, 2005; Achieng', 2009].




7

**Table 3.5:** $^1$H NMR (600 MHz) data of compound 7 ($CDCl_3$)

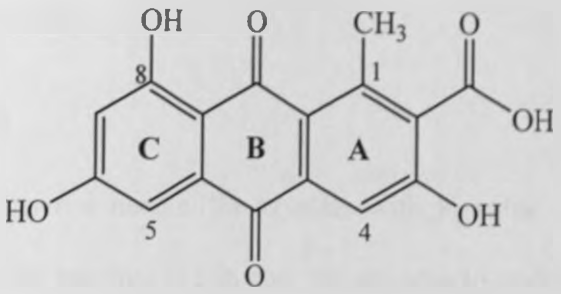
| Carbon No. | $^1$H $\delta_H$ (*m*, *J* in Hz) |
|---|---|
| 2 | 7.16 (*brs*) |
| 5 | 7.89 (*dd* , 7.6, 1.4) |
| 6 | 7.69 (*t* , 8.4, 7.6) |
| 7 | 7.30 (*dd*, 8.4, 1.2) |
| 3-$CH_3$ | 2.38 (*d* ,1.0) |
| 1-OH | 12.29 (*s*) |
| 4-OH | 13.49 (*s*) |
| 8-OH | 12.33 (*s*) |

### 3.2.2.1.3 Laccaic acid D (8)

Compound **8** was isolated as dark yellow amorphous solid with $R_f$ value of 0.15 (5% acetone in dichloromethane). The UV absorption maxima at 431, 287 and 221 nm is typical of a 3,8-dihydroxy-1-methylanthraquinone [Wanjohi, 2005]. The $^1$H NMR (300 MHz) data showed signals at $\delta_H$ 13.18 due to chelation of the hydroxyl proton at C-8 and a broad singlet was also observed at $\delta_H$ 10.43 due to hydroxyl proton at C-6. A deshielded methyl signal was observed at $\delta_H$ 2.82. In addition the $^{13}$C NMR (125 MHz) showed the carbonyl carbon peaks at $\delta_C$ 190.6 (C-9) and $\delta_C$ 183.5 (C-10) of the anthraquinone skeleton. This NMR data is in agreement with 3,8-dihydroxy-1-methyl anthraquinone derived by folding of the octaketide chain in the unusual way (Scheme 2).

Furthermore, the $^1$H NMR showed the presence of two aromatic protons in ring C, which exhibited an AX spin system resonating at $\delta_H$ 7.18 *d* ($J$ = 2.4 Hz) and $\delta_H$ 6.66 *d* ($J$ = 2.7 Hz). These *meta* coupling protons which are shielded due to the presence of neighboring hydroxyl groups at C-6 and C-8 were assigned to H-5 ($\delta_C$ 108.7) and H-7 ($\delta_C$ 110.1), respectively. The third aromatic proton appeared as a singlet at $\delta_H$ 7.71 and was assigned to H-4 ($\delta_C$ 114.0) of ring A. The biogenetically expected methyl at C-1 of ring A resonated at $\delta_H$ 2.82 ($\delta_C$ 21.0). Therefore compound **8** was identified as 3,6,8-trihydroxy-1-methyl-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (trivial name laccaic acid D).

This is the first report of laccaic acid D from the family Asphodelaceae. Compound **8** which is used as a natural food colourant has been previously isolated from the insect *Kermes ilicis*. From higher plants, it has been isolated from *Butea monosperma* and *Zizyphus mauritiana*, from the rhizomes of *Rhubarb* and from *Senna* species [DNP, 2009].




**8**

**Table 3.6:** $^{1}H$ (300 MHz), $^{13}C$ (125 MHz) NMR data (Acetone-$d_6$) together with HMBC correlation for compound **8**

| Carbon No | $^{1}H$ NMR $\delta_H$ (*m*, *J* in Hz) | $^{13}C$ NMR | HMBC |
|---|---|---|---|
| 1 | - | 131.6 | - |
| 2 | - | 143.0 | - |
| 3 | - | 160.7 | - |
| 4 | 7.71 (*s*) | 114.0 | C-1a, C-10 |
| 4a | - | 138.7 | - |
| 5 | 7.18 (*d*, 2.4) | 108.7 | C-7, C-8a, C-10 |
| 5a | - | 136.3 | - |
| 6 | - | 167.0 | - |
| 7 | 6.66 (*d*, 2.7) | 110.1 | C-5, C-8, C-8a |
| 8 | - | 167.0 | - |
| 8a | - | 112.6 | - |
| 9 | - | 190.2 | - |
| 10 | - | 183.5 | - |
| 1-Me | 2.82 (*s*) | 21.0 | C-1a, C-2 |
| 3-OH | 13.18 (*s*) | - | - |
| 8-OH | 10.43 (*brs*) | - | C-7, C-8, C-8a |
| COOH | | 169.4 | |

*Not observed

### 3.2.2.2 Dimeric Anthraquinones

#### 3.2.2.2.1 Chryslandicin (9)

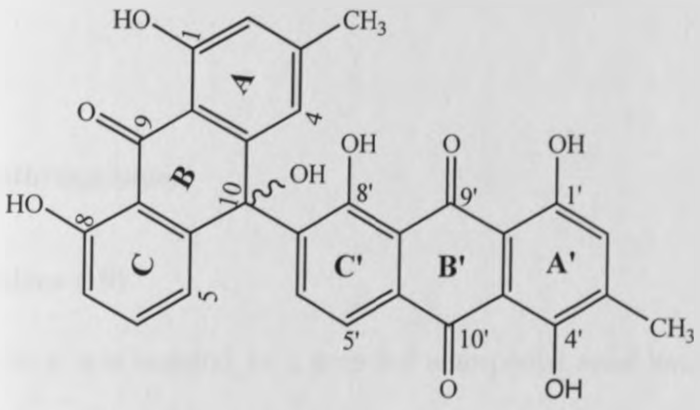
Compound **9** was isolated as red needle-like crystals with $R_f$ value of 0.42 (20% EtOAc in hexane). It showed absorption maxima at 288 and 383 nm (due to anthrone) and 494 nm (due to anthraquinone) in the UV spectrum. Furthermore, the $^1$H NMR (Table 3.7) showed the presence of five highly deshielded proton signals resonating at $\delta_H$ 13.48, 12.42, 12.33, 12.31 and 12.07 due to the presence of chelated hydroxyl groups. These data suggested that this compound is a dimer of an anthraquinone and anthrone units. In addition, the presence of two aromatic methyl protons which resonated at 2.25 (C-3) and 2.35 (C-3') supported that the compound is a dimeric anthraquinone derivative.

The $^1$H NMR data of one half of the molecule showed *meta*-coupled protons at $\delta_H$ 6.61 (*d*, $J$ = 1.2 Hz) and 6.77 (*d*, $J$ = 1.2 Hz). These signals were assigned to protons at C-2 and C-4, respectively with the biogenetically expected methyl being at C-3. In addition, an ABX spin system was observed for three aromatic protons which resonated at $\delta_H$ 6.94 (*dd*, $J$ = 8.2, 1.2 Hz), 7.40 (*t*, $J$ = 8.2, 8.0 Hz) and 6.79 (*dd*, $J$ = 7.6, 1.2 Hz) and this is characteristic of proton at C-5, C-6 and C-7 of the chrysophanol anthrone moiety, leaving C-10 as the point of attachment to the other half of the molecule.

For the other half of the molecule, the presence of the biogenetically expected aromatic methyl at C-3' ($\delta_H$ 2.35) and a singlet at $\delta_H$ 7.08 (C-2') suggested that C-4' is substituted with a hydroxyl group. The NMR pattern of this half of the molecule was found to be similar with that observed for compound **7** except for the absence of an ABX pattern for ring C. Instead, a pair of

deshielded *ortho*-coupled protons with AX pattern was observed, indicating that the point of attachment in this half of the molecule is at C-7'. The deshielded *ortho*-coupled protons at $\delta_H$ 8.65 (*d*, *J* = 8.0 Hz) and $\delta_H$ 8.05 (*d*, *J* = 8.0 Hz) were assigned to H-5' and H-6'. Therefore, compound **9** which is composed of chrysophanol anthrone and islandicin moieties was identified as 10-hydroxy-10-(islandicin-7'-yl)-chrysophanol anthrone, trivial name chryslandicin (**9**). The identity of this compound was further confirmed by direct comparison with authentic sample. The absolute configuration at C-10 has not been determined.

Compound **9** previously isolated from the roots of *Kniphofia foliosa* have shown potent antiplasmodial activity [Wube *et al.*, 2005]. It has also been isolated from the roots of *Kniphofia thomsonii* [Achieng', 2009].




**9**

**Table 3.7:** $^1$H NMR (200 MHz) data of compound **9** ($CDCl_3$)

| Carbon No. | $^1$H $\delta_H$ (*m*, *J* in Hz) |
|---|---|
| 2 | 6.61 (*d*, 1.2) |
| 4 | 6.77 (*d*, 1.2) |
| 5 | 6.94 (*dd*, 8.2, 1.2) |
| 6 | 7.40 (*t*, 8.2) |
| 7 | 6.79 (*dd*, 7.6, 1.2) |
| 2' | 7.08 (*s*) |
| 5' | 8.65 (*d*, 8) |
| 6' | 8.05 (*d*, 8) |
| 3-$CH_3$ | 2.25 (*s*) |
| 3'-$CH_3$ | 2.35 (*s*) |
| 1-OH | 12.07 (*s*) |
| 8-OH | 12.31 (*s*) |
| 1'-OH | 12.33 (*s*) |
| 4'-OH | 13.48 (*s*) |
| 8'-OH | 12.42 (*s*) |

### 3.2.2.3 Phenylanthraquinone

#### 3.2.2.3.1 Knipholone (10)

Compound **10** which was isolated as a deep red amorphous solid had an $R_f$ value of 0.6 (5% acetone in dichlorormethane). The orange spot on TLC plate changed to red on exposure to ammonia vapour which is typical of quinones. The UV ($\lambda_{max}$ 279 and 435 nm) suggested an anthraquinone skeleton. In the $^1$H NMR spectrum, two downfield shifted signals at $\delta_H$ 12.47 and 11.96 were observed due to chelated hydroxyl protons at C-1 and C-8 of an anthraquinone moiety.

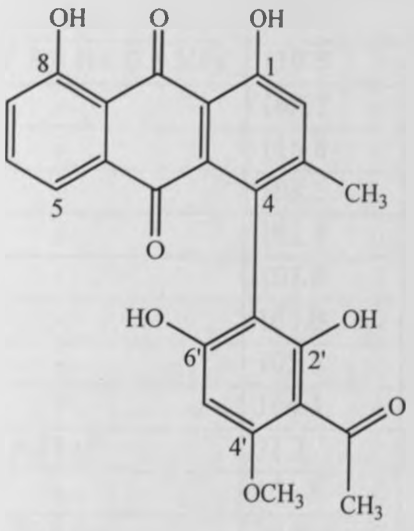
Comparison of the $^1$H NMR data with literature showed one half of the compound to be a chrysophanol moiety with the aromatic protons of ring C (H-5, H-6 and H-7) exhibiting an ABX spin system and resonating at $\delta_H$ 7.54 (*dd*, *J* = 7.4, 1.0 Hz), 7.74 (*t*, *J* = 8.2, 7.6 Hz) and 7.29 (*dd*, *J* = 8.8, 0.8 Hz), respectively. Although *meta* coupled protons were expected for ring A, only a singlet was observed at $\delta_H$ 7.31 and was assigned to H-2 with the methyl group ($\delta_H$ 2.18) at C-3 as expected biogenetically. This implies that C-4 ($\delta_C$ 132.4) is the point of attachment with aromatic substituent whose presence was evident from the NMR spectrum.

The $^1$H NMR data for the aromatic substituent in the compound showed a downfield shifted singlet ($\delta_H$ 14.20) due to the chelated hydroxyl proton, an acetyl proton ($\delta_H$ 2.73) at C-3', a sharp singlet at $\delta_H$ 3.97 due to a methoxy group showing that the substituent is acetylphloroglucinol methyl ether. The $^{13}$C NMR spectrum (Table 3.8) is consistent with such substituent. In order to fix the position of the methoxyl group, the NMR data of compound **10** was compared (Table 3.8) with that reported in literature [Yenesew *et al.*, 1994]. The NMR chemical shift position of the methoxyl group is similar to that reported for knipholone (OMe at C-4') rather than isoknipholone (OMe at C-2'). Therefore, compound **10** was identified as 1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methylanthraquinone, trivial name knipholone (**10**).

The absolute configuration of compound **10** at the biaryl axis was determined by Bringmann *et al.*, (2007) using time dependent DFT and DFT/MRCI circular dichromism calculations and was found to be *P*-configured. It is interesting to note that the optical rotation of compound **10** ($[\alpha]_D$ +80° in $CHCl_3$) is much smaller than its precursor knipholoneanthrone ($[\alpha]_D$ +200° in $CH_3CO$)

[Bringmann *et al.*, 2008a]. It is possible that compound **10** was isolated as scalmic mixture of *P* - and *M* –configuration rather than enantiomerically pure *P*.

Compound **10** is widely distributed in the genus *Kniphofia*, *Bulbine* and *Bulbinella* and possesses antiplasmodial and antitumor activities [Bringmann *et al.*, 2008a]. It was also found to be a good inhibitor of leukotriene formation [Wube *et al.*, 2006]. The only report of compound **10** outside the family Asphodelaceae is from the pods of *Senna didymobotrya* (Leguminosae) [Alemayehu *et al.*, 1996].




**10**

**Table 3.8:** $^{1}H$ (200 MHz) and $^{13}C$ (50 MHz) NMR data of compound **10** (Acetone-$d_6$) along with literature values (Yenesew *et al.*, 1994) of knipholone and isoknipholone

| Carbon No. | $^{1}H$ NMR $\delta_H$ (*m*, *J* in Hz) **10** | $^{13}C$ NMR **10** | $^{1}H$ NMR ($CDCl_3$) $\delta_H$ (*m*) **Knipholone** (Yenesew *et al.*, 1994) | $^{1}H$ NMR ($CDCl_3$) $\delta_H$ (*m*) **Isoknipholone** (Yenesew *et al.*, 1994) |
|---|---|---|---|---|
| 1 | - | 162.0 | | |
| 1a | - | 115.1 | | |
| 2 | 7.31 (*s*) | 123.5 | 7.32 (*brs*) | 7.31 (*brs*) |
| 3 | - | 152.4 | | |
| 4 | - | 132.4 | | |
| 4a | - | 135.0 | | |
| 5 | 7.54 (*dd*, 7.4 Hz, 1 Hz) | 124.8 | 7.56 (*brd*) | 7.63 (*brd*) |
| 5a | - | 128.4 | | |
| 6 | 7.74 (*t*, 8.2 Hz, 7.6 Hz) | 137.5 | 7.75 (*t*) | 7.64 (*t*) |
| 7 | 7.28 (*dd*, 8.8 Hz, 0.8 Hz) | 119.5 | 7.30 (*brd*) | 7.28 (*brd*) |
| 8 | - | 162.7 | | |
| 8a | - | 115.8 | | |
| 9 | - | 198.5 | | |
| 10 | - | 182.1 | | |
| 1' | - | 107.8 | | |
| 2' | - | 163.0 | | |
| 3' | - | 105.5 | | |
| 4' | - | 164.1 | | |
| 5' | 6.23 (*s*) | 91.2 | 6.24 (*s*) | 6.34 (*s*) |
| 6' | - | 161.5 | | |
| Me-3 | 2.18 (*s*) | 20.3 | 2.17 (*s*) | 2.19 (*s*) |
| $OCH_3$-4' | 3.97 (*s*) | 55.4 | 3.98 (*s*) | - |
| $OCH_3$-2' | - | - | - | 3.33 (*s*) |
| $COCH_3$-3' | 2.73 (*s*) | 32.5 | 2.62 (*s*) | 2.65 (*s*) |
| OH-1 | 12.47/11.96 (*s*) | - | 12.53/12.00 (*s*) | 12.61/12.00 (*s*) |
| OH-8 | 11.96/12.47 (*s*) | - | 12.00/12.53 (*s*) | 12.00/12.61 (*s*) |
| OH-2' | 14.20 (*s*) | - | 14.22 (*s*) | 13.47 (*s*) |
| CO-3' | - | 206.0 | | |

### 3.2.2.4 Dimeric Phenylanthraquinones

#### 3.2.2.4.1 Joziknipholone A (11)

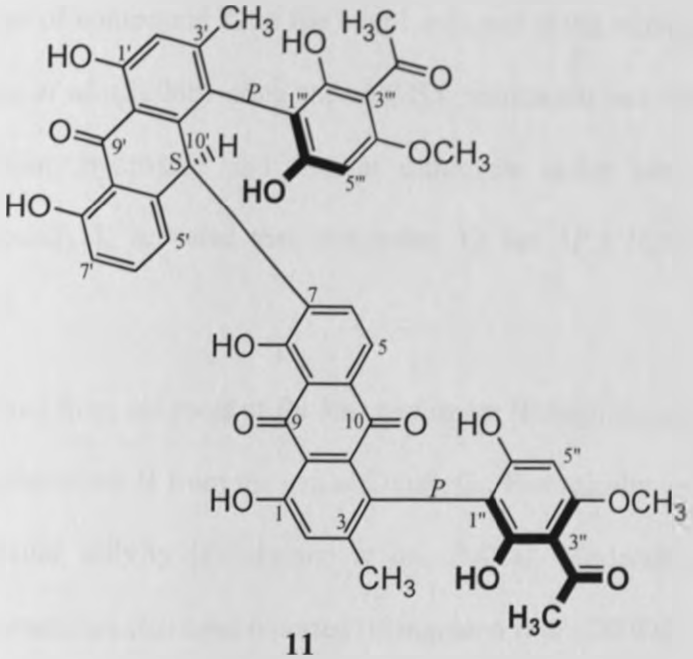
Compound **11** was isolated as an orange amorphous powder with $R_f$ value of 0.19 (5% methanol in dichloromethane). The UV ($\lambda_{max}$ at 278, 293, 385, 412 and 444 nm) is typical of an anthraquinone and anthrone skeleton [Bringmann *et al.*, 2008b]. In the $^1H$ and $^{13}C$ NMR spectra, the presence of six chelated hydroxyl protons ($\delta_H$ 11.95, 12.21, 12.47, 12.60, 14.07 and 14.45) and five carbonyls ($\delta_C$ 182.7, 193.3, 194.6, 204.1 and 204.1) indicated that the compound is a dimeric phenylanthraquinone derivative. Further analysis of the NMR data showed a pattern similar to that of compound **10.** However, in this case signals were doubled i.e. two aromatic methyls ($\delta_H$ 1.99, 2.13), two methoxyls ($\delta_H$ 3.73, 3.93) and two acetyl groups ($\delta_H$ 2.62, 2.72) which supported that the compound is a phenylanthraquinone dimer, possibly a dimer of knipholone and knipholone anthrone.

In one half of the molecule, the $^1H$ NMR data of a chrysophanol (**1**) portion exhibited an AX spin pattern instead of the usual ABX spin system for the protons in ring C. These *ortho* coupled protons were assigned to H-5 [$\delta_H$ 7.24 (*d*, $J$ = 7.9 Hz)] and H-6 [$\delta_H$ 6.91 (*d*, $J$= 8.0 Hz)] and indicated that C-7 ($\delta_C$ 139.0) is the point of linkage to the other portion of the molecule. A singlet at $\delta_H$ 7.29 was assigned to H-2 with the methyl group ($\delta_H$ 2.13) being at C-3 as expected biogenetically. The NMR data of this half of the molecule was found to be closely related to that of compound **10** except for the absence of a signal for the proton at C-7. Therefore, one half of the molecule was found to be the phenylanthraquinone knipholone (**10**).

The other half of the molecule exhibited an ABX pattern for the protons in ring C of a chrysophanol anthrone moiety. These protons which resonated at $\delta_H$ 6.87 (br*d*, $J$ = 7.5 Hz), 7.33 (*t*, $J$ = 8.0 Hz) and 6.81 (*d*, $J$ = 8.2 Hz) were assigned to H-5', H-6' and H-7', respectively. A singlet at $\delta_H$ 6.98 was observed due to the proton at C-2' with the biogenetically expected methyl group being at C-3' ($\delta_H$ 1.99, $\delta_C$ 21.0). The absence of a proton signal for H-4 indicated that C-4 is the point of linkage of the chrysophanol anthrone moiety to acetophloroglucinol unit (Table 3.9). A singlet at $\delta_H$ 5.96 ($\delta_C$ 37.5) was observed which indicated that this half of the molecule is a phenylanthrone and was assigned to H-10' of the chrysophanol anthrone moiety. When comparing the NMR data of this portion in compound **11** with literature [Dagne and Yenesew, 1993], it showed similar characteristic features to those of knipholone anthrone except for the upfield shift of the proton at C-5''' ($\delta_H$ 5.58 in **11** vs $\delta_H$ 6.30 in knipholone anthrone) and the downfield shifted proton at C-10' ($\delta_H$ 5.96 in **11** vs $\delta_H$ 4.07 in knipholone anthrone) [Dagne and Yenesew, 1993]. From this it was concluded that the phenylanthrone knipholone anthrone is the second half of the molecule with C-10' being the point of attachment to the other half of the molecule. Hence, compound **11** is 7,10'-knipholone-knipholone anthrone. TLC comparison of this compound with an authentic sample revealed that compound **11** is indeed joziknipholone A. The absolute configuration of compound **11** at the biaryl axis as well as the stereogenic centre (C-10') was determined by Bringmann *et al.*, (2008b) using reductive cleavage, NOESY correlation and quantum chemical circular dichromism calculation.

This compound which had been previously isolated from the roots of *Bulbine frutescens* and has shown promising antiplasmodial and moderate antitumor activity [Bringmann *et al.*, 2008a]. This

is only the second report on the occurrence of this compound in nature and the first report from the genus *Kniphofia*.



**11**

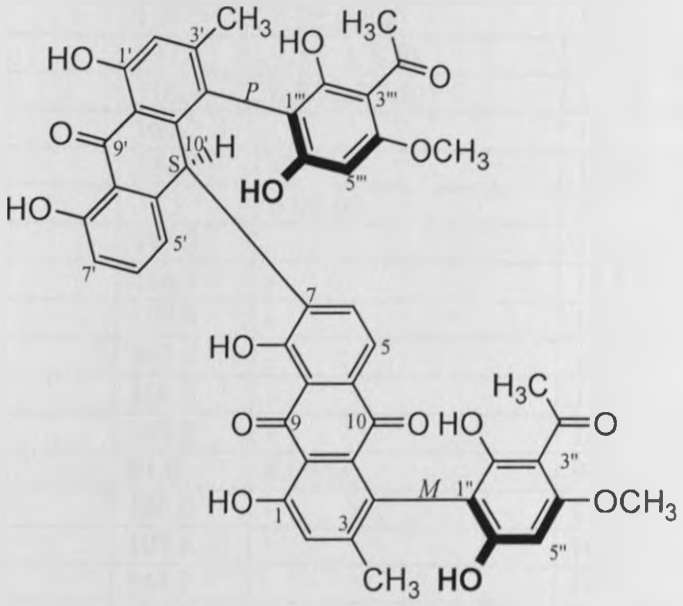
### 3.2.2.4.2 Joziknipholone B (12)

Compound **12** was isolated as an orange amorphous powder and had an $R_f$ value of 0.29 (5% methanol in dichloromethane). The UV ($\lambda_{max}$ at 219, 267, 291, 351 and 439 nm) suggested that it is a dimer of an anthraquinone and an anthrone moiety. The $^{1}H$ and $^{13}C$ NMR which was similar to that of compound **11** showed the presence of six chelated protons ($\delta_H$ 14.45, 14.11, 12.23, 12.58, 12.01 and 12.51) and five carbonyls ($\delta_C$ 182.5, 193.3, 194.6, 204.1 and 204.3). This indicated that compound **12** was also a dimer of knipholone and knipholone anthrone linked at C-7 ($\delta_C$ 139.6) and C-10' ($\delta_C$ 37.3) positions (Table 3.9). By direct TLC comparison with an

authentic sample, compound **12** was identified as the atropdiasteromer of compound **11**, joziknipholone B.

The absolute configuration of compound **12** at the biaryl axis and at the stereogenic center was determined by Bringmann *et al.* (2008b) using the NOESY correlation and reductive cleavage using degassed 5% sodium hydroxide and sodium dithionate under inert condition. CD calculations as in compound **11**, revealed that compound **12** has 4*P*,4'*M*,10'*S* configuration [Bringmann *et al.*, 2008b].

Although previously isolated from the roots of *Bulbine frutescens* [Bringmann *et al.*, 2008b], this is the first report of joziknipholone B from the genus *Kniphofia*. Biologically, compound **12** has shown potent antiplasmodial activity [Bringmann *et al.*, 2008a]. Moderate activity against murine leukemic lymphoblasts has also been reported [Bringmann *et al.*, 2008a].




**12**

**Table 3.9:** $^{1}H$ (600 MHz) and $^{13}C$ (150 MHz) NMR data of compounds **11** and **12** ($CDCl_3$)

| Carbon | 11 | | 12 | |
|---|---|---|---|---|
| | $^{1}H$ $\delta_H$ (*m*, *J* in Hz) | $^{13}C$ | $^{1}H$ $\delta_H$ (*m*, *J* in Hz) | $^{13}C$ |
| 1 | - | 163.5 | - | 163.7 |
| 2 | 7.79 (s) | 126.0 | 7.30 (*s*) | 125.9 |
| 3 | - | 153.3 | - | 153.4 |
| 4 | - | 126.2 | - | 126.1 |
| 5 | 7.24 (*d*, 7.9) | 120.3 | 7.24 (*d*, 7.9) | 120.4 |
| 6 | 6.91 (*d*, 8.0) | 136.4 | 6.90 (*d*, 7.9) | 136.1 |
| 7 | - | 139.0 | - | 139.6 |
| 8 | - | 159.1 | - | 159.1 |
| 9 | - | 193.3 | - | 193.3 |
| 10 | - | 182.7 | - | 182.5 |
| 11 | - | 133.3 | - | 133.2 |
| 12 | - | 116.1 | - | 115.9 |
| 13 | - | 115.7 | - | 115.7 |
| 14 | - | 133.4 | - | 133.2 |
| 1' | - | 163.6 | - | 163.6 |
| 1'a | - | 115.7 | - | 115.7 |
| 2' | 6.98 (*s*) | 118.8 | 6.97 (*s*) | 118.8 |
| 3' | - | 150.8 | - | 151.0 |
| 4' | - | 121.7 | - | 121.9 |
| 4'a | - | 140.1 | - | 142.9 |
| 5' | 6.87 (br *d*) | 120.0 | 6.92 (br *d*) | 119.9 |
| 6' | 7.33 (*t*, 8.0) | 137.4 | 7.32 (*t*, 8.0) | 137.4 |
| 7' | 6.81 (*d*, 8.2) | 116.3 | 6.80 (*d*, 7.9) | 116.3 |
| 8' | - | 163.2 | - | 163.2 |
| 9' | - | 194.6 | - | 194.6 |
| 10' | 5.96 (*s*) | 37.5 | 6.06 (*s*) | 37.3 |
| 11' | - | 145.7 | - | 147.1 |
| 12' | - | 114.7 | - | 114.7 |
| 1'' | - | 107.6 | - | 107.6 |
| 2'' | - | 163.9 | - | 163.9 |
| 3'' | - | 106.5 | - | 106.5 |
| 4'' | - | 163.5 | - | 163.6 |
| 5'' | 6.12 (*s*) | 91.0 | 6.09 (*s*) | 91.1 |
| 6'' | - | 160.0 | - | 159.8 |
| 1''' | - | 105.6 | - | 106.9 |
| 2''' | - | 164.7 | - | 164.7 |
| 3''' | - | 106.9 | - | 106.9 |
| 4''' | - | 164.6 | - | 164.5 |
| 5''' | 5.58 (*s*) | 90.2 | 5.57 (*s*) | 90.2 |
| 6''' | - | 161.0 | - | 160.9 |

Table 3.9: $^{1}H$ (600MHz) and $^{13}C$ (150MHz) NMR data of compounds **11** and **12** ($CDCl_3$) cont....

| Carbon | 11 | | 12 | |
|---|---|---|---|---|
| | $^{1}H$ ($\delta_H$, *m*, *J* in HZ) | $^{13}C$ | $^{1}H$ ($\delta_H$, *m*, *J* in HZ) | $^{13}C$ |
| 3-$CH_3$ | 2.13 (*s*) | 21.2 | 2.13 (*s*) | 21.3 |
| 3'-$CH_3$ | 1.99 (*s*) | 21.0 | 1.99 (*s*) | 21.1 |
| 4''-$OCH_3$ | 3.93 (*s*) | 56.2 | 3.92 (*s*) | 56.2 |
| 4'''-$OCH_3$ | 3.73 (*s*) | 56.2 | 3.69 (*s*) | 56.2 |
| 3''-$COCH_3$ | 2.62 (*s*) | 33.5 | 2.63 (*s*) | 33.5 |
| 3'''-$COCH_3$ | 2.72 (*s*) | 33.5 | 2.71 (*s*) | 33.5 |
| 3''-$COCH_3$ | - | 204.1 | - | 204.3 |
| 3'''-$COCH_3$ | - | 204.1 | - | 204.1 |
| 1-OH | 12.47 (*s*) | - | 12.51 (*s*) | - |
| 8-OH | 11.95 (*s*) | - | 12.01 (*s*) | - |
| 1'-OH | 12.60 (*s*) | - | 12.58 (*s*) | - |
| 8'-OH | 12.21 (*s*) | - | 12.23 (*s*) | - |
| 2''-OH | 14.07 (*s*) | - | 14.11 (*s*) | - |
| 2'''-OH | 14.45 (*s*) | - | 14.45 (*s*) | - |

### 3.2.2.5 Tetrameric Phenylanthrone

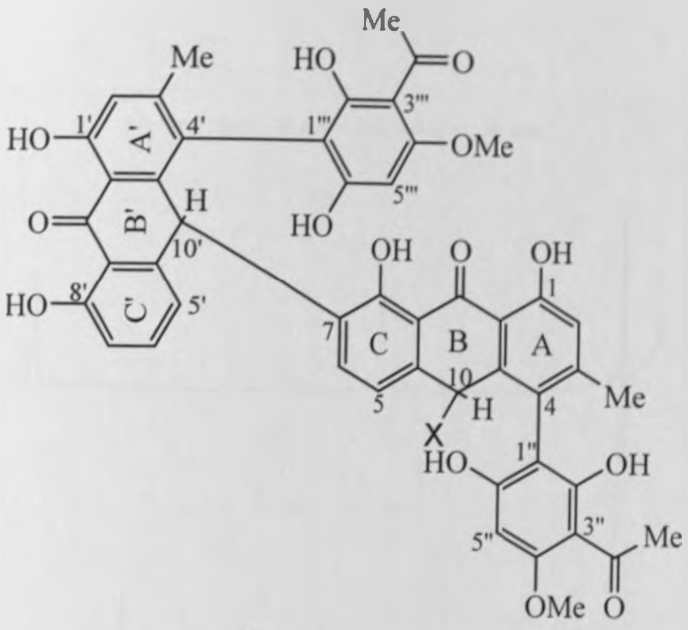
#### 3.2.2.5.1 Jozi-joziknipholone anthrone (13)

Compound **13** was isolated as a yellow amorphous solid with an $R_f$ value of 0.20 (5% methanol in dichloromethane). The $^{1}H$ NMR spectrum was similar to that of compound **11** showing six chelated hydroxyl protons ($\delta_H$ 14.46, 14.05, 12.68, 12.43, 12.01 and 10.72), two aromatic methyls ($\delta_H$ 1.74, 1.92), two methoxyls ($\delta_H$ 2.96, 3.87) and two acetyl groups ($\delta_H$ 2.52, 2.68). However, in the $^{13}C$ NMR only four carbonyls resonating at $\delta_C$ 205.5, 202.8, 194.8 and 192.1 were observed. From this it was deduced that compound **13** could be a dimeric phenylanthrone derivative.

The $^{1}H$ NMR spectrum for the aromatic region was in agreement with the pattern expected for a dimeric phenylanthrone. An AX spin system for the two protons in ring A on the chrysophanol

moiety of one half of the molecule was observed. These *meta* coupled protons which resonated at $\delta_H$ 5.88 (*d*, *J* = 7.8 Hz) and $\delta_H$ 6.39 (*d*, *J* = 8.0 Hz) were assigned to H-5 and H-6, respectively indicating that C-7 ($\delta_C$ 132.1) is the point of attachment to one of the phenylanthrone moieties. In addition, the expected two singlets at $\delta_H$ 6.35 and 5.98 were observed due to the protons at C-2 of the chrysophanol moiety and C-5'' of the acetophloroglucinol unit, respectively. However, instead of a methylene group as in knipholone anthrone, an unpredictably upfield shifted methine ($\delta_H$ 4.47, $\delta_C$ 51.7) was observed for H-10 of ring B.

For the other half of the molecule the anticipated ABX pattern was observed for the protons in ring C of the chrysophanol anthrone unit. These protons which resonated at $\delta_H$ 7.41 (*d*, *J* = 7.6 Hz), $\delta_H$ 7.78 (*t*, *J* = 8.0 Hz) and $\delta_H$ 6.99 (*d*, *J* = 8.2 Hz) were assigned to H-5', H-6' and H-7', respectively. The biosynthetically expected methyl at C-3' of ring A resonated at $\delta_H$ 1.92 ($\delta_C$ 20.4) with the proton at C-2' appearing at $\delta_H$ 6.88 in the $^1$H NMR spectrum. The other signals were identical to that observed for the first half of the molecule with the exception of the downfield shifted signal of H-10' ($\delta_H$ 6.27, $\delta_C$ 37.4) (as compared to the other half) indicating that it is the point of attachment to the other half of the molecule. This was confirmed using HMBC which showed correlation between H-10' with C-6 as well as H-10' with C-8. From this it was assumed that compound **13** was a dimeric phenylanthrone, i.e. 7-10' knipholone anthrone-knipholone anthrone with a substituent (X) at C-10 which was unknown at this stage (13a).




**13a**

The identity of the substituent X was elusive as there were no additional NMR signals to account for. In the HMBC spectrum unusual correlation peak between $\delta_H$ 4.47 (H-10) and $\delta_C$ 51.7 (C-10) was observed which prompted the generation of 1D HMQC spectrum. Interestingly, in the 1D HMQC spectrum (Figure 3.1), a $^3J$ correlation peak between $\delta_H$ 4.47 (H-10) with $\delta_C$ 51.7 (assigned to C-10) indicated that there must be a carbon atom which is three bonds away and yet chemically equivalent with C-10. This pointed out the possibility of **13** being a tetrameric phenylanthrone or a dimer of joziknipholone anthrone.

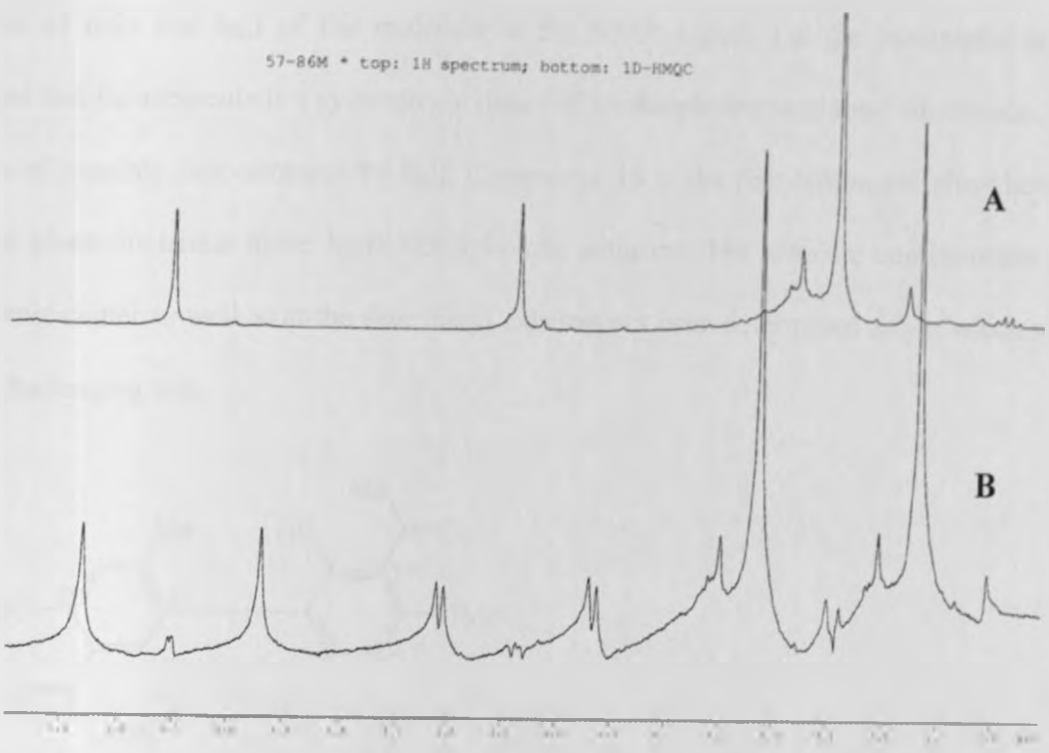
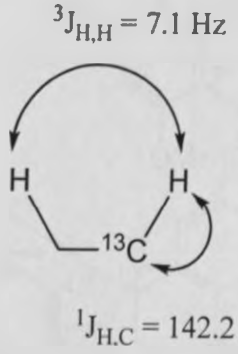
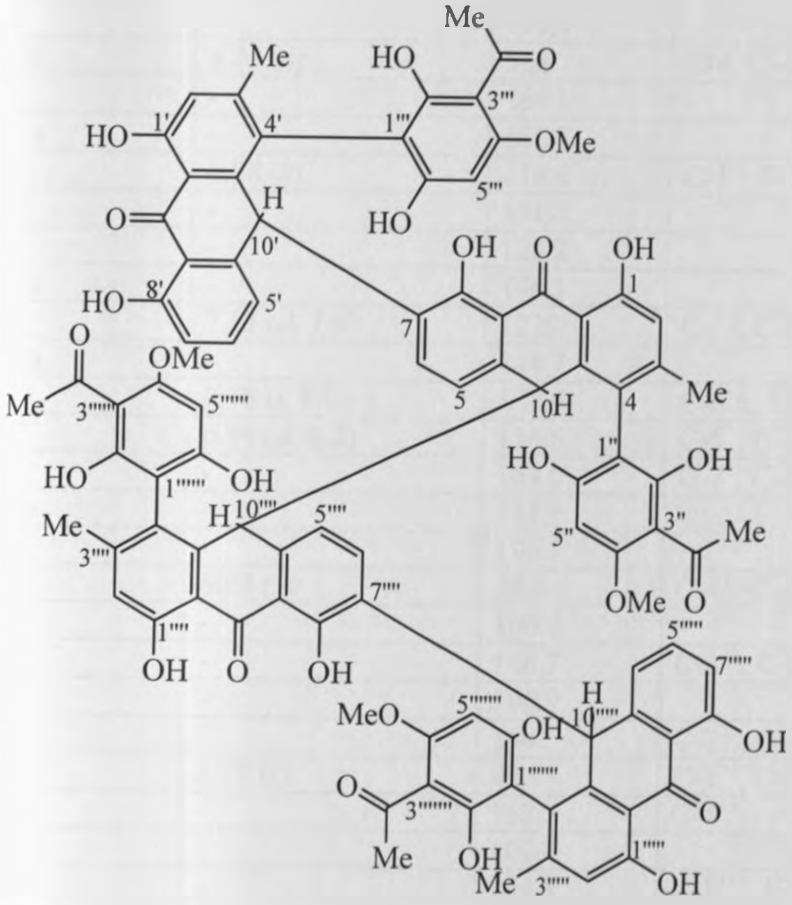



Fig. 3.1 Partial $^{1}H$ NMR (A) and 1D-HMQC (B) spectra of **13**




Indeed, the HRMS-ESI of this compound showed an [M+Na] peak at *m/z* 1697.42643 corresponding to the molecular formula of $C_{96}H_{74}O_{28}$. From the 1D HMQC of compound **13**, coupling and HMBC correlation between $\delta_H$ 4.47 from one half of the molecule and $\delta_C$ 51.7 from the other half of the molecule is consistent with a tetrameric phenylanthrone containing four

knipholone anthrone units with C7 - C10', C10 - C10'''' and C7'''' - C10''''' linkage. The presence of only one half of the molecule in the NMR signals i.e. the joziknipholone part indicated that the molecule is a symmetrical dimer of joziknipholone anthrone which reduced the number of possible stereoisomers by half. Compound **13** is the first tetrameric phenylanthrone and was given the trivial name Jozi-joziknipholone anthrone. The absolute configuration at the stereogenic center as well as at the four biaryl axis has not been determined as yet which will be a very challenging task.




**13**

**Table 3.10**: $^{1}H$ (500 MHz) and $^{13}C$ (75 MHz) NMR data of compounds **13** (Acetone-$d_6$)

| CARBON | $^{1}H$ $\delta_H$ (*m*, *J* in HZ) | $^{13}C$ | HMBC |
|---|---|---|---|
| 1 | - | 162.8 | |
| 1a | - | 116.4 | |
| 2 | 6.36 (*s*) | 118.3 | C-1, C-1a, C-4, 3-$CH_3$ |
| 3 | - | 149.4 | |
| 4 | - | 125.2 | |
| 4a | - | 141.4 | |
| 5 | 5.88 (*d*, 7.8) | 122.7 | C-6, C-7, C-8a |
| 5a | - | 146.5 | |
| 6 | 6.39 (*d*, 8.0) | 133.5 | C-8, C-8a |
| 7 | - | 133.4 | |
| 8 | - | 159.5 | C-6, C-7, C-8a |
| 8a | - | 117.7 | |
| 9 | - | 193.4 | |
| 10 | 4.48 (*s*) | 53.0 | C-4, C-4a, C-5, C-8a |
| 1' | - | 164.1 | |
| 1'a | - | 116.7 | |
| 2' | 6.88 (*s*) | 118.6 | C-1', C-1'a, C-4',3'-$CH_3$ |
| 3' | - | 151.7 | |
| 4' | - | 125.2 | |
| 4'a | - | 148.1 | |
| 5' | 7.41 (*d*, 7.6) | 122.4 | C-7', C-8'a, C-10' |
| 5'a | - | 148.7 | |
| 6' | 7.78 (*t*, 8.0) | 138.4 | C-5'a, C-8' |
| 7' | 6.99 (*d*, 8.2) | 116.8 | C-5', C-8', C-8'a |
| 8' | - | 164.0 | C-7', C-8'a |
| 8'a | - | 115.5 | |
| 9' | - | 196.1 | |
| 10' | 6.27 (*s*) | 38.8 | C-4', C-4'a, C-5', C-5'a, C-6, C-8, C-8'a |
| 1'' | - | 106.4 | |
| 2'' | - | 166.7 | C-1'', C-3'' |
| 3'' | - | 106.9 | |
| 4'' | - | 164.5 | C-2'', C-4'' |
| 5'' | 5.98 (*s*) | 92.1 | C-1'', C-3'', C-4'', C-6'' |
| 6'' | - | 162.5 | C-1'' |
| 1''' | - | 107.4 | |
| 2''' | - | 164.9 | C-1''', C-3''' |
| 3''' | - | 106.9 | |
| 4''' | - | 164.3 | C-2''', C-4''' |
| 5''' | 5.05 (*s*) | 91.5 | C-1''', C-3''', C-6''' |
| 6''' | - | 164.5 | |
| 3-$CH_3$ | 1.74 (*s*) | 21.6 | C-2, C-3, C-4 |

**Table 3.10**: $^{1}H$ (500 MHz) and $^{13}C$ (75 MHz) NMR data of compounds **13** (Acetone-$d_6$) cont....

| CARBON | $^{1}H$ $\delta_H$ (*m*, *J* in HZ) | $^{13}C$ | HMBC |
|---|---|---|---|
| 3'-$CH_3$ | 1.99 (*s*) | 21.7 | C-2', C-3', C-4' |
| 4''-$OCH_3$ | 3.88 (*s*) | 56.4 | |
| 4'''-$OCH_3$ | 2.99 (*s*) | 55.9 | |
| 3''-$COCH_3$ | 2.62 (*s*) | 33.8 | C-3'' |
| 3'''-$COCH_3$ | 2.53 (*s*) | 33.7 | C-3''' |
| 3''-$COCH_3$ | - | 204.1 | |
| 3'''-$COCH_3$ | - | 204.1 | |
| 1-OH | 11.99 (*s*) | - | C-1, C-1a, C-2 |
| 8-OH | 10.71 (*s*) | - | C-7, C-8, C-8a |
| 1'-OH | 12.66 (*s*) | - | C-1', C-1'a, C-2' |
| 8'-OH | 12.42 (*s*) | - | C-7', C-8', C-8'a |
| 2''-OH | 14.03 (*s*) | - | C-2'' |
| 6''-OH | 9.1 (*s*) | - | C-1'' |
| 2'''-OH | 14.44 (*s*) | - | C-2''' |
| 6'''-OH | 7.3 (*s*) | - | C-5''' |

### 3.2.2.6 Miscellaneous compounds

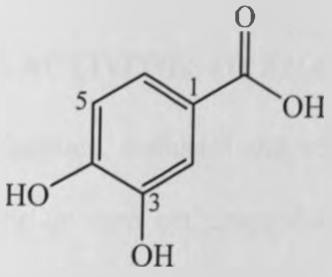
#### 3.2.2.6.1 3,4-Dihydroxybenzoic acid (14)

Compound **14** was isolated as a mixture with compound **8**. Compound **14** changed to brown on TLC plate after exposure to air and had an $R_f$ value of 0.13 (5% acetone in dichloromethane). The $^{13}C$ NMR indicates the presence of seven carbon atoms including a carbonyl at $\delta_C$ 165.6 due to the carboxylic acid substituent at C-1. Two downfield shifted signals at $\delta_C$ 160.1 and $\delta_C$ 151.4 were observed as a result of the two hydroxyl substitutents at C-3 and C-4, respectively.

The $^{1}H$ NMR data showed the presence of an AXY spin system corresponding to three aromatic protons which resonated at $\delta_H$ 7.53 *d* (*J* = 2.1 Hz), $\delta_H$ 6.90 *d* (*J* = 8.4 Hz) and $\delta_H$ 7.48 *dd* (*J* = 8.1

Hz, 2.1 Hz) which were assigned to H-2 ($\delta_C$ 118.3), H-5 ($\delta_C$ 116.2) and H-6 ($\delta_C$ 124.3), respectively. Therefore compound **14** was identified as 3,4-dihydroxybenzoic acid.

This is the first report on the occurrence of compound **14** in the family Asphodelaceae. However, it has been previously isolated from the genus *Fagopyrum* and *Alnus* as well as from some species of *Allium* [DNP, 2009]. Biologically, it inhibits low density lipoprotein oxidation and platelet aggregation [DNP, 2009]. It also possesses antioxidant, free radical scavenging as well as some cytostatic activity [DNP, 2009].




**14**

**Table 3.11**: $^{1}$H (300 MHz) and $^{13}$C (50 MHz) NMR data (acetone-$d_6$) together with HMBC correlation for compound **14**

| Carbon No | $^{1}$H NMR $\delta_H$ (*m*, *J* in Hz) | $^{13}$C NMR | HMBC |
|---|---|---|---|
| 1 | - | 146.2 | |
| 2 | 7.53 (*d*, 2.1) | 118.2 | C-1, C-6 |
| 3 | - | 160.1 | |
| 4 | - | 151.4 | |
| 5 | 6.90 (*d*, 8.4) | 116.4 | C-6 |
| 6 | 7.48 (*dd*, 8.1, 2.1) | 124.2 | C-2, C-4 |
| COOH-1 | - | 165.6 | |

## 3.3 BIOLOGICAL ACTIVITIES

### 3.3.1 ANTIPLASMODIAL ACTIVITIES

The crude extracts of the rhizomes of *Kniphofia foliosa* as well as some of the isolated compounds of both *Rhamnus prinoides* and *Kniphofia foliosa* were tested for their antiplasmodial activity against the chloroquine sensitive (D6) and chloroquine resistant (W2) strains of *Plasmodium falciparum*. Chloroquine and mefloquine were used as a positive control.

#### 3.3.1.1 ANTIPLASMODIAL ACTIVITIES OF *RHAMNUS PRINOIDES*

The hexane, chloroform, ethylacetate, methanol and water extracts of the root bark of *Rhamnus prinoides* were tested for their *in vitro* antiplasmodial activity against the K39 (chloroquine sensitive) *Plasmodium falciparum* strain isolated from a patient [Muregi *et al.*, 2003]. Among the extracts, the methanolic extract showed the highest activity with an $IC_{50}$ value of 15.1 µg/mL [Muregi *et al.*, 2003]. The antiplasmodial activity of the methanolic extract was further tested against the ENT30 (chloroquine resistant, isolated from a patient), V1/S (chloroquine resistant, standard) and NF54 (chloroquine sensitive, standard) strains of *Plasmodium falciparum* and had an $IC_{50}$ value of 23.2 µg/mL, 29.2 µg/mL and greater than 62.5 µg/mL, respectively [Muregi *et al.*, 2003]. In addition, the *in vivo* antiplasmodial activites of the crude methanolic extracts of the leaves and root bark of *Rhamnus prinoides* against *Plasmodium berghei* NK65 strain was tested in mice [Muregi *et al.*, 2007]. The extracts showed 43.9% (leaves) and 34.1% (root) supression of the parasite in mice after 4 day of infection, whereas 20% of the mice treated with the methanolic extract of the root bark survived on the ninth day of infection [Muregi *et al.*, 2007].

In this study, the naphthalenic lactone glycoside, geshoidin (**6**) isolated from this plant was tested for its antiplasmodial activity against the chloroquine sensitive (D6) and chloroquine resistant (W2) strains of *P. falciparum*. A potent antiplasmodial activity with an $IC_{50}$ value of 4.0 ± 0.9 µM (D6) and 0.4 ± 0.2 µM (W2) was observed. Geshoidin (**6**) being the major compound in the methanol extract, it is possible that this compound could be responsible for the observed antiplasmodial activity of the methanolic extract of the plant. The antiplasmodial activity of geshoidin (**6**) is reported here for the first time.

### 3.3.1.2 ANTIPLASMODIAL ACTIVITIES OF *KNIPHOFIA FOLIOSA*

The antiplasmodial activities of the ethyl acetate extract of the rhizomes of *Kniphofia foliosa* against the chloroquine sensitive (D6) and the chloroquine resistant (W2) strains of *Plasmodium falciparum* were tested. The crude extract showed strong *in vitro* antiplasmodial activity, an $IC_{50}$ value of 4.7 ± 0.5 µg/mL and 4.1 ± 0.8 µg/mL for the D6 and W2 strains, respectively. In addition, the antiplasmodial activities of some of the isolated compounds were also evaluated and showed promising activities, particularly the phenylanthraquinone, dimeric anthraquinone, dimeric phenylanthraquinones and the terameric phenylanthraquinone, with the latter two showing high inhibition of the malaria parasite (table 3.13).

Although the individual monomers, chrysophanol and islandicin showed no significant activity, the dimeric anthraquinone 10-hydroxy-10-(islandicin-7'-yl)-chrysophanol anthrone (**9**) which is composed of the two monomers showed an $IC_{50}$ value of 6.5 µM against the chloroquine resistant (W2) strain of *Plasmodium falciparum* [Achieng', 2009]. Also, the antiplasmodial

activity of compound **9** against the choloroquine sensitive 3D7 strain of *Plasmodium falciparum* with an $IC_{50}$ value of 1.0μM has been reported [Wube et al., 2005].

Similarly, the monomers chrysophanol and acetophloroglucinol showed no antiplasmodial activity. However, the phenylanthraquinone knipholone (**10**) which is composed of these two monomers showed good activity against the chloroquine resistant (W2) ($IC_{50}$ 10.4 ± 2.4 μM) and moderate activity against the chloroquine sensitive (D6) strains ($IC_{50}$ 23.3 ± 0.1 μM) of *P. falciparum*. A high inhibition of the growth of the malaria parasite against the chloroquine sensitive (NF54) and chloroquine resistant (K1) strains was observed for compound **10** with an $IC_{50}$ value of 3.9 μM and 2.4 μM, respectively [Bringmann et al., 1999]. In another study, the antiplasmodial activity of compound **10** against the chloroquine sensitive NF54 strain of *Plasmodium falciparum* was found to be 3.4 μM [Wube et al., 2005]. In 2008, Bringmann *et al.* reported the antiplasmodial activity of knipholone to be 1.5 μM and 2.1 μM against the K1 and NF54 strains, respectively [Bringmann *et al.*, 2008a]. The variation of the activity in the different studies may be due to the enantiomeric purity of the knipholone being tested. In addition, compound **10** has comparatively higher antiplasmodial activity against the chloroquine resistant strains of *Plasmodium falciparum*.

Since the monomers (knipholone 10.4 ± 2.4 μM and knipholone anthrone 7.5 ± 0.1 μM against W2) forming the dimeric phenylanthraquinones were active, some activity was expected for the dimeric and tetrameric phenylanthrones. The dimeric phenylanthraquinone showed better antiplasmodial activity than the respective monomers. Joziknipholone A (**11**) and its atropisomer, joziknipholone B (**12**) displayed an $IC_{50}$ value of 0.4 ± 0.01 μM and 3.3 ± 0.9 μM against the chloroquine resistant W2 strain, respectively. Similarly potent activities were

observed for the two dimeric phenyl anthraquinones **11** and **12** against the K1 strain (0.2 μM and 0.3μM).

The tetrameric phenylanthrone, jozi-joziknipholone anthrone (**13**) which is composed of two dimeric phenylanthraquinone showed comparable antiplasmodial activity, with an $IC_{50}$ value of 0.3 μM against the chloroquine resistant (K1) strain of *P. falciparum*, as with those observed for the dimeric phenylanthraquinones. When trying to correlate the observed antiplasmodial activity with the structure of the compounds tested, it was noted that the activity of the compounds increases with the size of the compounds. The antiplasmodial activities of the crude extract as well as some of the isolated compounds are summarized in table 3.12.

**Table 3.12**: *In vitro* antiplasmodial activity of some of the isolated compounds of *Kniphofia foliosa*

| Compounds | *Plasmodial strain* | $IC_{50}$ μM | References |
|---|---|---|---|
| Chryslandicin (**9**) | W2 | 6.5 | Achieng', 2009 |
| | 3D7 | 1.0 | Wube *et al.*, 2005 |
| Knipholone (**10**) | D6 | 23.3 | |
| | W2 | 10.4 | |
| | NF54 | 3.9 | Bringmann *et al.*, 1999 |
| | K1 | 2.4 | |
| | NF54 | 3.4 | Wube *et al.*, 2005 |
| | K1 | 1.5 | Bringmann *et al.*, 2008a |
| | NF54 | 2.1 | |

Table 3.12: *In vitro* antiplasmodial activity of some of the isolated compounds of *Kniphofia foliosa* cont.....

| Compounds | *Plasmodial strain* | IC50 µM | References |
|---|---|---|---|
| Joziknipholone A (**11**) | W2 | 0.4 | |
| | K1 | 0.2 | Bringmann *et al.*, 2008b |
| Joziknipholone B (**12**) | W2 | 3.3 | |
| | K1 | 0.3 | Bringmann *et al.*, 2008b |
| Jozi-joziknipholone anthrone (**13**) | K1 | 0.3 | |

### 3.3.2 ANTIMICROBIAL ACTIVITY

The crude extracts as well as some of the isolated compounds of *Rhamnus prinoides* as well as *Kniphofia foliosa* were tested against certain bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*) and fungi (*Candida albicans*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes*). The crude extracts as well as the isolated compounds of both plants did not show any inhibition at a concentration of 0.12 mg/disc.

## 3.4 Conclusion

- From the roots of *Rhamnus prinoides* six compounds, chrysophanol, physcion, emodin, rhamnazin, β-sorigenin and geshoidin were isolated and characterised. This is the first report on the occurrence of these compounds from the roots of this plant.

- Geshoidin has been identified as the antiplasmodial principle in *Rhamnus prinoides*, a plant which is widely used traditionally to treat malaria.

- The rhizomes of *Kniphofia foliosa* yielded nine compounds, chrysophanol, islandicin, laccaic acid, chryslandicin, knipholone, joziknipholone A, joziknipholone B, Jozi-joziknipholone anthrone and 3,4-dihydroxybenzoic acid. One of these, Jozi-joziknipholone anthrone is the first tetrameric phenylanthrone. This is only the second report on the occurrence of joziknipholone A and joziknipholone B in nature having being reported recently from the roots of *Bulbine frutescens*.

- The crude extract of *Kniphofia foliosa* as well as some of the isolated compounds showed good antiplasmodial activity. Phenylanthraquinones and anthraquinone dimers may serve as lead structures for the development of antimalarial drugs.

## 3.5 Recommendation

- *In vivo* antiplasmodial test should be carried out for the naphthalenic derivative, geshoidin isolated from *Rhamnus prinoides*.
- *In vivo* antiplasmodial test on the phenylanthraquinones and anthraquinone dimer is recommended.
- Further phytochemical investigation of *Kniphofia foliosa* using inert chromatographic condition may result in the identification of more novel and active compounds
- The stereochemistry at the biaryl axis as well as at the stereogenic center should be determined for the tetrameric phenylantraquinone Jozi-joziknipholone anthrone.
- The absolute configuration of the dimeric anthraquinone chryslandicin at C-10 should also be determined.
- Phytochemical investigation of other *Kniphofia* species as well as other genera in the family be carried out to determine the chemotaxonomic relation in the family as well as for more novel and biologically active compounds.

# CHAPTER 4

# EXPERIMENTAL

## 4.1 General

The $^{1}H$ (200, 300, 500, 600 MHz) and $^{13}C$ (50, 75, 125, 150 MHz) were acquired using Varian-Mercury and Bruker instrument using TMS as the internal standard. Homonuclear correlation spectroscopy (COSY), Hetronuclear multiple quantum correlation (HMQC) and Hetronuclear multiple bond correlation (HMBC) spectra were obtained using the standard Bruker software. The Pye-Unicam SPS 150 spectrophotometer was employed to acquire the UV/Vis spectra. Solvents were distilled prior for use for extraction and chromatographic separation.

## 4.2 Chromatographic conditions

Column chromatography was carried out using 3% oxalic acid impregnated silica gel 60G (Merck 70-230 mesh) and Sephadex LH-20. Analytical TLC using silica gel 60 F254 pre-coated plates Merck were used to monitor the purity of compounds. UV light (254 and 366 nm) along with ammonia and iodine vapours were used for detection of spots on TLC plates. Merck 60 $PF_{254}$ silica gel was used as adsorbent for preparative thin layer chromatography (PTLC).

**4.3 TLC solvent systems**

The TLC solvent systems used for in the study were

Hexane/Acetone ; 7:3 (Solvent system 1)

$CH_2Cl_2$/ methanol ; 9.8:0.2 (Solvent system 2)

EtOAc/methanol/$H_2O$; 7.5:1.5:1 (Solvent system 3)

Hexane/$CH_2Cl_2$ ; 1:1 (solvent system 4)

$CH_2Cl_2$/Acetone; 9.5:0.5 (Solvent system 5)

Hexane/EtOAc; 7:3 (Solvent system 6)

$CH_2Cl_2$, 100% (Solvent system 7)

$CH_2Cl_2$/Acetone, 9:1 (Solvent system 8)

$CH_2Cl_2$/MeOH, 20:1 (Solvent system 9)

$CH_2Cl_2$/MeOH, 9.5:0.5 (Solvent system 10)

Hexane/EtOAc, 9.6:0.4 (Solvent system 11)

Hexane/EtOAc, 9:1 (Solvent system 12)

Hexane/EtOAc, 8:2 (Solvent system 13)

## 4.4 PLANT MATERIAL

### 4.4.1 *Rhamnus prinoides*

The whole root of *Rhamnus prinoides* were collected in July 2007 from Meru, Kenya. The plant specimen was identified by Mr. S. G. Mathenge of the University of Nairobi Herbarium, Department of Botany.

### 4.4.2 *Kniphofia foliosa*

The underground stems (rhizomes) of *Kniphofia foliosa* was collected from the Addis Ababa University botanical garden in August, 2008. For authentication, refer Yenesew *et al*., 1994

# 5 EXTRACTION AND ISOLATION OF COMPOUNDS

## 5.1 *RHAMNUS PRINOIDES*

### 5.1.1 EXTRACTION AND ISOLATION FROM THE WHOLE ROOTS

The air dried and ground whole root (819 g) of *Rhamnus prinoides* were extracted using dichloromethane/methanol (1:1) by cold exhaustive percolation. The crude extract (93.7 g) was obtained after removal of the solvent, a portion (87.3 g) of which was subjected to column chromatography using oxalic acid impregnated silica gel (400 g). Gradient elution with n-hexane containing increasing amount of acetone and finally 100% methanol afforded eight major fractions labeled A-H.

Column chromatography of fraction A on oxalic acid impregnated silica gel (80 g) (eluting with 1%, 2% and 4% ethyl acetate in hexane) and purification using preparative TLC yielded chrysophanol (**1**, 5.8 mg) and physcion (**2**, 6.4 mg).

Fraction B of the first column was also subjected to column chromatography on oxalic acid impregnated silica gel and eluting with 1% ethylacetate in hexane afforded crystals of emodin (**3**, 95.5 mg).

Fractions C and D were combined and also chromatographed on oxalic acid impregnated silica gel and eluted with 1% ethyl acetate in n-hexane. Purification of the fractions using preparative thin layer chromatography afforded emodin (**3**, 95.5 mg) and rhamnazin (**4**, 7.0 mg).

The polar four fractions (E-H) crystallized and formed copious amount of crystals, the solutions of which were filtered under sanction and left overnight. Fraction G gave β-sorigenin (**5**, 43.7 mg) where as the crystals from fractions E, F and H yielded geshoidin (**6**, 2.7 g) (β-sorigenin-8-*O*-β-D-glucoside).

### 4.5.1.2 PHYSICAL AND SPECTROSCOPIC PROPERTIES OF COMPOUNDS OF THE ROOTS OF *R. PRINOIDES*

**Chrysophanol (1)**

Dark yellow amorphous powder, melting point 195 – 197 $^{0}$C: UV ($\lambda_{max}$): 270, 288 and 430nm

**Physcion (2)**

Yellow amorphous powder. **$^1$H NMR** (acetone-$d_6$, 200 MHz): $\delta_H$ 7.17 (1H, *s*, H-2), 7.60 (1H, *s*, H-4), 7.29 (1H, *d*, *J* = 2.6 Hz, H-5), 6.81 (1H, *d*, *J* = 2.6 Hz, H-7), 2.31 (3H, *s*, Me-3), 12.03 (OH-1/OH-8), 12.15 (OH-8/OH-1).

**Emodin (3)**

Orange needle like crystals. **$^1$H NMR** (acetone-$d_6$, 200 MHz): $\delta_H$ 7.15 (1H, *s*, H-2), 7.54 (1H, *s*, H-4), 7.26 (1H, *d*, *J* = 2.4 Hz, H-5), 6.67 (1H, *d*, *J* = 2.4 Hz, H-7), 2.31 (3H, *s*, Me-3), 12.08 (OH-1/OH-8), 12.20 (OH-8/OH-1).

**Rhamnazin (4)**

Pale yellow amorphous solid. **$^1$H NMR** (acetone-$d_6$, 200 MHz): $\delta_H$ 6.33 (1H, *d*, *J* = 2.2Hz, H-6), 6.72 (1H, *d*, *J* = 2.2 Hz, H-8), 7.02 (1H, *d*, *J* = 8.6Hz, H-5'), 7.85 (1H, *dd*, *J* = 8.6, 2 Hz, H-6'), 7.92 (1H, *d*, *J* = 2 Hz, H-2'), 3.93 (3H, *s*, OMe-7), 3.94 (3H, *s*, OMe-3'), 12.14 (OH-5).

**β-Sorigenin (5)**

Brown amorphous solid. UV $\lambda_{max}$ 384 (MeOH), 315, 277 (MeOH + NaOH). **$^1$H NMR** (acetone-$d_6$, 200 MHz): $\delta_H$ 7.41 (1H, *s*, H-4 Hz), 7.44 (1H, *dd*, 7.8, 0.6 Hz, H-5), 7.49 (1H, *t*, 7.8 Hz, H-6), 6.93 (1H, *dd*, 7.2, 0.6 Hz, H-7), 5.39 (2H, *d*, 1.2 Hz, lactone $CH_2$). **$^{13}$C NMR** (50 MHz): $\delta_C$ 156.6 (C-1), 114.2 (C-2), 142.7 (C-3), 120.2 (C-4), 140.7 (C-4a), 111.5 (C-5), 130.4 (C-6), 109.6 (C-7), 156.1 (C-8), 105.3 (C-8a), 68.8 (lactone $CH_2$), 169.3 (lactone CO).

**Geshoidin (6)**

Faint yellow amorphous powder. UV $\lambda_{max}$ 352, 299 (MeOH), 322, 275 (MeOH + NaOH). **$^1$H NMR** (DMSO, 200 MHz): $\delta_H$ 7.46 (1H, *s*, H-4), 7.66 (1H, *d*, *J* = 7.8Hz, H-5), 7.59 (1H, *t*, *J* = 8.4 Hz, H-6), 7.43 (1H, *dd*, *J* = 7.5, 1.2 Hz, H-7), 5.38 (2H, *s*, lactone $CH_2$), 5.15 (1H, *d*, *J* = 7.8 Hz, H-1'), 3.45 (1H, *t*, *J* = 9 Hz, H-2'), 3.37 (1H, *t*, *J* = 9 Hz, H-3'), 3.24 (1H, *t*, *J* = 9 Hz, H-4'), 3.49 (1H, *ddd*, *J* = 9.6, 6.6, 1.8 Hz, H-5'), 3.54 (1H, *dd*, *J* = 12.0, 6.0 Hz, H-6'a), 3.79 (1H, *dd*, *J* = 12, 1.8 Hz, H-6'b). **$^{13}$C NMR** (125 MHz): $\delta_C$ 156 (C-1)114.3 (C-2), 139.6 (C-3), 111.1 (C-4), 143.1 (C-4a), 123.2 (C-5), 129.8 (C-6), 111.2 (C-7), 155.4 (C-8), 106.0 (C-8a), 60.7 (lactone $CH_2$), 168.2 (lactone CO), 102.9 (C-1'), 73.4 (C-2'), 77.9 (C-3'), 69.8 (C-4'), 76.2 (C-5'), 68.1 (C-6').

### *4.5.2 KNIPHOFIA FOLIOSA*

#### 4.5.2.1 EXTRACTION AND ISOLATION FROM THE RHIZOMES

The air dried and ground underground stems (rhizomes) of *Kniphofia foliosa* was extracted using acetone followed by dichloromethane/methanol (1:1) and finally with methanol. The dichloromethane/methanol extract was partitioned between ethyl acetate and water. The ethyl acetate layer (27.2 g) was fractionated by column chromatography on oxalic acid impregnated silica gel (400 g) and eluted with mixtures of hexane and ethyl acetate with increasing polarity. The fraction eluted with 1% ethyl acetate in hexane after column chromatography on Sephadex LH-20 gave dark yellow amorphous solid chrysophanol (**1**, 10 mg) and needle like crystals of islandicin (**7**, 21.1 mg). Column chromatography on Sephadex LH-20 of the fraction eluted with 2% and 4% ethyl acetate in hexane gave chryslandicin (**9**, 20.3 mg). Column chromatography on

Sephadex LH-20 of the fraction eluted with 8% ethyl acetate in hexane gave knipholone (**10**. 275.0 mg). The fractions eluted with 8-15% ethyl acetate in hexane after column chromatography on Sephadex LH-20 (dichloromethane/methanol 1:1) gave laccaic acid (**8**. 21.5 mg) and 3,4-dihydroxybenzoic acid (**14**, 21.5 mg).

The acetone extract was partitioned between dichloromethane and water. After some time the dichloromethane extract formed a yellow precipitate which was taken up in a mixture of acetone and methanol. The crude extract (7.69 g) was then subjected to column chromatography on oxalic acid impregnated silica gel (300g) and was eluted with hexane containing increasing amount of dichloromethane followed by dichloromethane with increasing amount of acetone. Fractions eluted with 20% of dichloromethane in hexane gave chrysophanol (**1**) and islandicin (7). Fractions eluted with 50-90 % dichloromethane in hexane gave knipholone (**10**). The fractions eluted with 100% dichloromethane and 1% acetone in dichloromethane after column chromatography on Sephadex LH-20 (dichloromethane/methanol 1:1) followed by purification using preparative thin layer chromatography (silica gel, 5% methanol in dichloromethane) gave joziknipholone A (**11**, 38.4 mg) and joziknipholone B (**12**, 23.0 mg). Column chromatography on Sephadex LH-20 (dichloromethane/methanol 1:1) followed by purification on preparative thin layer chromatography (silica gel, 5% methanol in dichloromethane) of the fractions eluted with 12-25% acetone gave Jozi-joziknipholone anthrone (**13**, 44.4 mg).

### 4.5.2.2 PHYSICAL AND SPECTROSCOPIC PROPERTIES OF COMPOUNDS OF THE RHIZOMES OF *K. FOLIOSA*

**Islandicin (7)**

Red amorphous powder. UV $\lambda_{max}$ 493, 431, 401, 282 (MeOH), 600, 554, 523, 416, 281 (MeOH + NaOH). **$^1$H NMR** ($CDCl_3$, 200 MHz): $\delta_H$ 7.16 (1H, *br s*, H-2), 7.89 (1H, *dd*, *J* = 7.6, 1.4 Hz, H-5), 7.69 (1H, *t*, *J* = 8.4 Hz, H-6), 7.30, (1H, *dd*, *J* = 8.4, 1.2 Hz, H-7), 2.38 (3H, *d*, *J* = 1Hz, Me-3), 12.29 (OH-1), 12.33 (OH-8), 13.49 (OH-4).

**Laccaic acid (8)**

Dark yellow amorphous solid. **$^1$H NMR** (acetone-$d_6$, 300 MHz): $\delta_H$ 7.71 (1H, *s*, H-4), 7.18 (1H, *d*, *J* = 2.4Hz, H-5), 6.66 (1H, *d*, *J* = 2.7 Hz, H-7), 2.82 (3H, *s*, Me-1), 13.18 (OH-3), 10.43 (OH-8). **$^{13}$C NMR** (300 MHz): $\delta_C$ 131.6 (C-1a), 143.0 (C-2), 160.7 (C-3), 114.0 (C-4), 138.7 (C-4a), 108.7 (C-5), 136.3 (C-5a), 110.1 (C-7), 167.0 (C-8), 112.6 (C-8a), 169.4 (C-9), 183.5 (C-10), 21.0 (CH3-1), 190.2 (COOH).

**Chryslandicin (9)**

Red crystals. UV $\lambda_{max}$ 494, 383, 288 (MeOH), 595, 557, 420, 316, 282 (MeOH + NaOH). **$^1$H NMR** ($CDCl_3$, 200 MHz): $\delta_H$ 6.61 (1H, *d*, *J* = 1.2 Hz, H-2), 6.77 (1H, *d*, *J* = 1.2 Hz, H-4), 6.94 (1H, *dd*, *J* = 8.2, 1.2 Hz), 7.40 (1H, *t*, 8.2 Hz, H-6), 6.79 (1H, *dd*, *J* = 7.6, 1.2 Hz, H-7), 7.08 (1H, *s*, H-2'), 8.65 (1H, *d*, *J* = 8Hz, H-5'), 8.05 (1H, *d*, *J* = 8Hz, H-6'), 2.25 (3H, *s*, Me-3), 2.35 (3H, *s*, Me-3'), 12.07 (OH-1), 12.31 (OH-8), 12.33 (OH-1'), 13.48 (OH-4'), 12.42 (OH-8').

**Knipholone (10)**

Deep red amorphous powder. UV $\lambda_{max}$ 435, 279 (MeOH), 483, 308 (MeOH + NaOH). **$^1$H NMR** (acetone-$d_6$, 200 MHz): $\delta_H$ 7.31 (1H, *s*, H-2), 7.54 (1H, *dd*, *J* = 7.4, 1 Hz, H-5), 7.74 (1H, *t*, *J* = 8.2 Hz, H6), 7.28 (1H, *dd*, *J* = 8.8, 0.8 Hz, H-7), 6.23 (1H, *s*, H-5'), 2.18 (3H, *s*, Me-3), 14.20 (OH-2'), 2.73, (3H, *s*, COMe-3'), 3.97 (3H, *s*, OMe-4'). **$^{13}$C NMR** (50MHz): $\delta_C$ 162 (C-1), 115.1 (C-1a), 123.5 (C-2), 152.4 (C-3), 132.4 (C-4), 135.0 (C-4a), 124.8 (C-5), 128.4 (C-5a), 137.5 (C-6), 119.5 (C-7), 162.7 (C-8), 115.8 (C-8a), 198.5 (C-9), 182.1 (C-10), 107.8 (C-1'), 163.0 (C-2'), 105.5 (C-3'), 164.1 (C-4'), 91.2 (C-5'), 161.5 (C-6'), 20.3 (Me-3), 32.5 (COMe-3'), 55.4 (OMe-4'), 206.0 (CO-3').

**Joziknipholone A (11)**

Orange amorphous powder. **$^1$H NMR** ($CDCl_3$, 600 MHz): $\delta_H$ 7.29 (1H, *s*, H-2), 7.24 (1H, *d*, *J* = 7.9 Hz, H-5), 6.91 (1H, *d*, *J* = 8.0 Hz, H-6), 6.98 (1H, *s*, H-2'), 6.87 (1H, *brd*, H-5'), 7.33 (1H, *t*, *J* = 8.0 Hz, H-6'), 6.81 (1H, *d*, *J* = 8.2 Hz, H-7'), 5.96 (1H, *s*, H-10'), 6.12 (1H, *s*, H-5''), 5.58 (1H, *s*, H-5'''), 2.13 (3H, *s*, Me-3), 1.99 (3H, *s*, Me-3'), 3.93 (3H, *s*, OMe-4''), 3.73 (3H, *s*, OMe-4'''), 2.62 (3H, *s*, COMe-3''), 2.72 (3H, *s*, COMe-3'''), 12.47 (OH-1), 11.95 (OH-8), 12.60 (OH-1'), 12.21 (OH-8'), 14.07 (OH-2''), 14.45 (OH-2'''). **$^{13}$C NMR** (150 MHz): $\delta_C$ 163.5 (C-1), 126.0 (C-2), 153.3 (C-3), 126.2 (C-4), 120.3 (C-5), 136.4 (C-6), 139.0 (C-7), 159.1 (C-8), 193.3 (C-9), 182.7 (C-10), 133.3 (C-11), 116.1 (C-12), 115.7 (C-13), 133.4 (C-14), 163.6 (C-1'), 115.7 (C-1'a), 118.8 (C-2'), 150.8 (C-3'), 121.7 (C-4'), 140.1 (C-4'a), 120.0 (C-5'), 137.4 (C-6'), 116.3 (C-7'), 163.2 (C-8'), 194.6 (C-9'), 37.5 (C-10'), 145.7 (C-11'), 114.7 (C-12'), 107.6 (C-1''), 163.9 (C-2''), 106.5 (C-3''), 163.5 (C-4''), 91.0 (C-5''), 160.0 (C-6''), 105.6 (C-1'''), 164.7 (C-2'''), 106.9 (C-3'''), 164.6 (C-4'''), 90.2 (C-5'''), 161.0 (C-6'''), 21.2 (Me-3), 21.0

(Me-3'), 56.2 (OMe-4''), 56.2 (OMe-3'), 33.5 (COMe-3''), 33.5 (COMe-3'''), 204.1 (COMe-3''), 204.1 (COMe-3''').

**Joziknipholone B (12)**

Orange amorphous powder. **$^1$H NMR** ($CDCl_3$, 600 MHz): $\delta_H$ 7.30 (1H, s, H-2), 7.24 (1H, *d*, *J* = 7.9 Hz, H-5), 6.90 (1H, *d*, *J* = 7.9 Hz, H-6), 6.97 (1H, *s*, H-2'), 6.92 (1H, *brd*, H-5'), 7.32 (1H, *t*, *J* = 8.0 Hz, H-6'), 6.80 (1H, *d*, *J* = 7.9 Hz, H-7'), 6.06 (1H, *s*, H-10'), 6.09 (1H, *s*, H-5''), 5.57 (1H, *s*, H-5'''), 2.13 (3H, *s*, Me-3), 1.99 (3H, *s*, Me-3'), 3.92 (3H, *s*, OMe-4''), 3.69 (3H, *s*, OMe-4'''), 2.63 (3H, *s*, COMe-3''), 2.71 (3H, *s*, COMe-3'''), 12.51 (OH-1), 12.01 (OH-8), 12.58 (OH-1'), 12.23 (OH-8'), 14.11 (OH-2''), 14.45 (OH-2'''). **$^{13}$C NMR** (150 MHz): $\delta_C$ 163.7 (C-1), 125.9 (C-2), 153.4 (C-3), 126.1 (C-4), 120.4 (C-5), 136.1 (C-6), 139.6 (C-7), 159.1 (C-8), 193.3 (C-9), 182.5 (C-10), 133.2 (C-11), 115.9 (C-12), 115.7 (C-13), 133.2 (C-14), 163.6 (C-1'), 115.7 (C-1'a), 118.8 (C-2'), 151.0 (C-3'), 121.9 (C-4'), 142.9 (C-4'a), 119.9 (C-5'), 137.4 (C-6'), 116.2 (C-7'), 163.2 (C-8'), 194.6 (C-9'), 37.3 (C-10'), 147.1 (C-11'), 114.7 (C-12'), 107.6 (C-1''), 163.9 (C-2''), 106.5 (C-3''), 163.6 (C-4''), 91.1 (C-5''), 159.8 (C-6''), 106.9 (C-1'''), 164.7 (C-2'''), 106.9 (C-3'''), 164.5 (C-4'''), 90.2 (C-5'''), 160.9 (C-6'''), 21.3 (Me-3), 21.1 (Me-3'), 56.2 (OMe-4''), 56.2 (OMe-3'), 33.5 (COMe-3''), 33.5 (COMe-3'''), 204.3 (COMe-3''), 204.1 (COMe-3''').

**Jozi-joziknipholone anthrone (13)**

Yellow amorphous solid. **$^1$H NMR** (acetone-$d_6$, 500 MHz): $\delta_H$ 6.36 (1H, *s*, H-2), 5.88 (1H, *d*, *J* = 7.8 Hz, H-5), 6.39 (1H, *d*, *J* = 8.0 Hz, H-6), 4.48 (1H, *s*, H-10), 6.88 (1H, *s*, H-2'), 7.41 (1H, *d*, *J* = 7.6 Hz, H-5'), 7.78 (1H, *t*, *J* = 8.0 Hz, H-6'), 6.99 (1H, *d*, *J* = 8.2 Hz, H-7'), 6.27 (1H, *s*, H-

10'), 5.98 (1H, *s*, H-5''), 5.05 (1H, *s*, H-5'''), 1.74 (3H, *s*, Me-3), 1.99 (3H, *s*, Me-3'), 3.88 (3H, *s*, OMe-4''), 2.99 (3H, *s*, OMe-4'''), 2.62 (3H, *s*, COMe-3''), 2.53 (3H, *s*, COMe-3'''), 11.99 (OH-1), 10.71 (OH-8), 12.66 (OH-1'), 12.42 (OH-8'), 14.03 (OH-2''), 14.44 (OH-2'''), 9.1 (OH-6''), 7.3 (OH-6'''). **$^{13}$C NMR** (Acetone-$d_6$, 125 MHz): $\delta_C$ 162.8 (C-1), 116.4 (C-1a), 118.3 (C-2), 149.4 (C-3), 125.2 (C-4), 141.4 (C-4a), 122.7 (C-5), 146.5 (C-5a), 133.5 (C-6), 133.4 (C-7), 159.5 (C-8), 117.7 (C-8a), 193.4 (C-9), 53.0 (C-10), 164.1 (C-1'), 116.7 (C-1'a), 118.6 (C-2'), 151.7 (C-3'), 125.2 (C-4'), 148.1 (C-4'a), 122.4 (C-5'), 148.7 (C-5'a), 138.4 (C-6'), 116.8 (C-7'), 164.0 (C-8'), 115.5 (C-8'a), 196.1 (C-9'), 38.8 (C-10'), 106.4 (C-1''), 166.7 (C-2''), 106.9 (C-3''), 164.5 (C-4''), 92.1 (C-5''), 162.5 (C-6''), 107.4 (C-1'''), 164.9 (C-2'''), 106.9 (C-3'''), 164.3 (C-4'''), 91.5 (C-5'''), 164.5 (C-6'''), 21.6 (Me-3), 21.7 (Me-3'), 56.4 (OMe-4''), 55.9 (OMe-4'''), 33.8 (COMe-3''), 33.7 (COMe-3'''), 204.1 (COMe-3''/COMe-3'''), 204.1 (COMe-3'''/COMe-3'').

**3,4-dihydroxybenzoic acid (14)**

Dark yellow amorphous solid. **$^{1}$H NMR** (acetone-$d_6$, 300 MHz): $\delta_H$ 7.53 (1H, *d*, *J* = 2.1 Hz, H-2), 6.90 (1H, *d*, *J* = 8.4 Hz, H-5), 7.48 (1H, *dd*, *J* = 8.1, 2.1 Hz, H-6). **$^{13}$C NMR** (300 MHz): $\delta_C$ 146.2 (C-1), 118.2 (C-2), 160.1 (C-3), 151.4 (C-4), 116.4 (C-5), 124.2 (C-6), 165.6 (COOH). This compound was isolated as a mixure with compound **8**.

## 4.6 Preparation of derivatives

### 4.6.1 Hydrolysis of Geshoidin (6)

50 mg of geshoidin (6) was placed in a round bottomed flask containing 10ml of $MeOH/H_2SO_4$ (3N) and the mixture was refluxed for 10 h. After evaporating the methanol, 2 ml of cold water was added and the precipitate was extracted using dichloromethane. The extracted sample was found to be similar (co-TLC) to the aglycone β-sorigenin (5).

## 4.7Antiplasmodial test

This test was performed by Mr. Hoseah Akala of the United States Army Medical Research Unit- Kenya, Walter Reed project, Kisumu.

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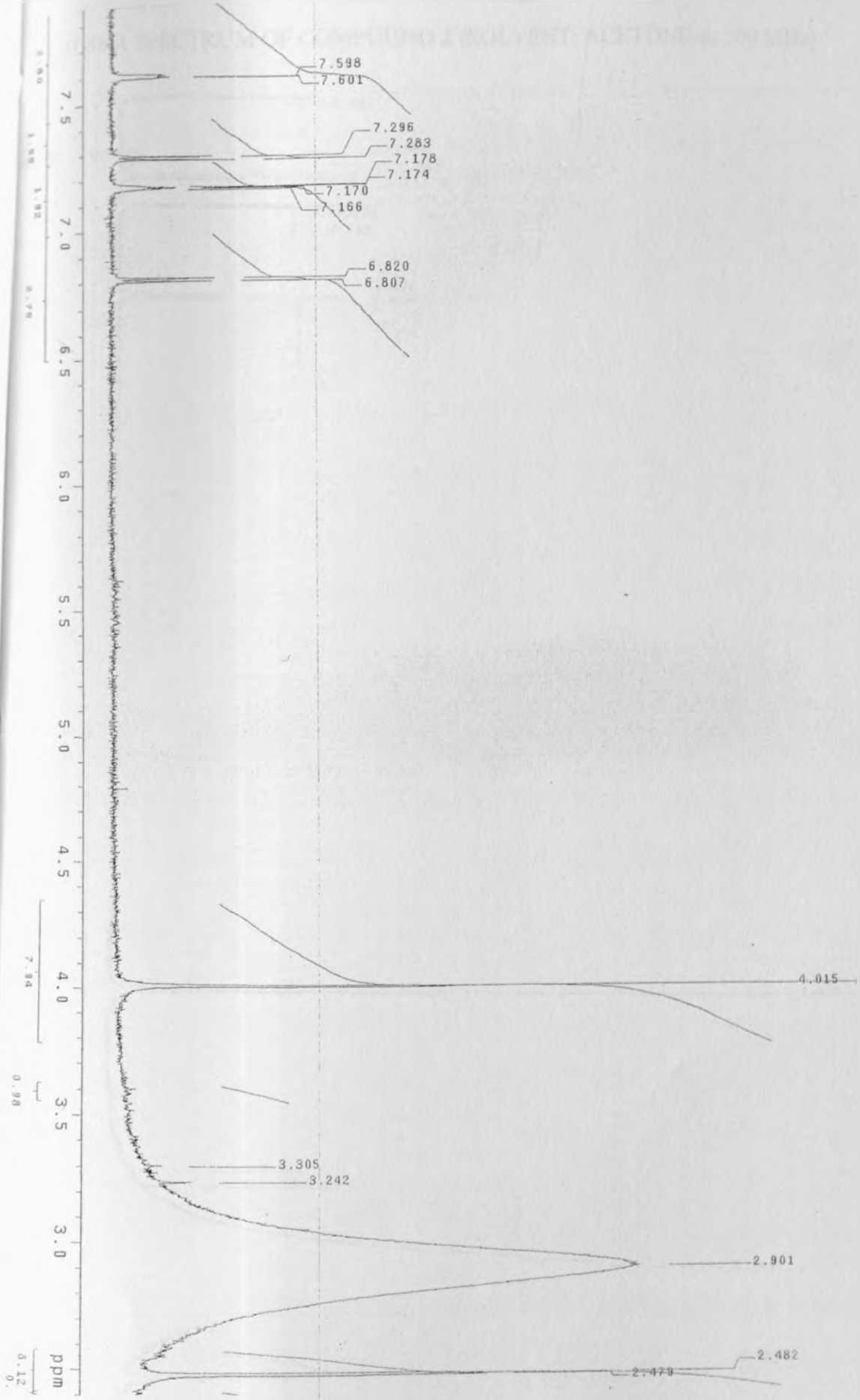
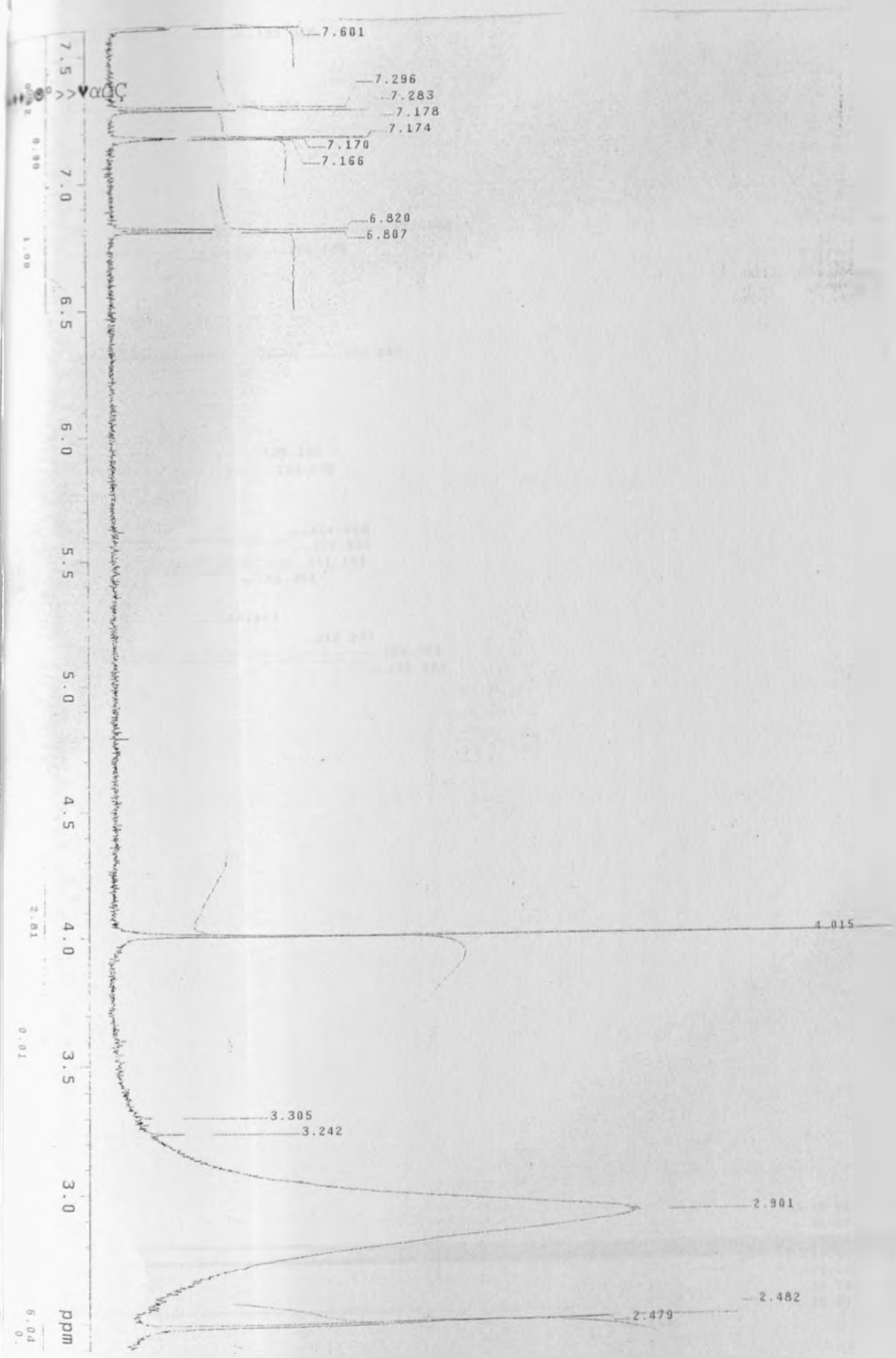
SPECTRA FOR COMPOUND **2**

$^1$H NMR SPECTRUM OF COMPOUND **2** (SOLVENT: ACETONE-$d_6$ 200 MHz)



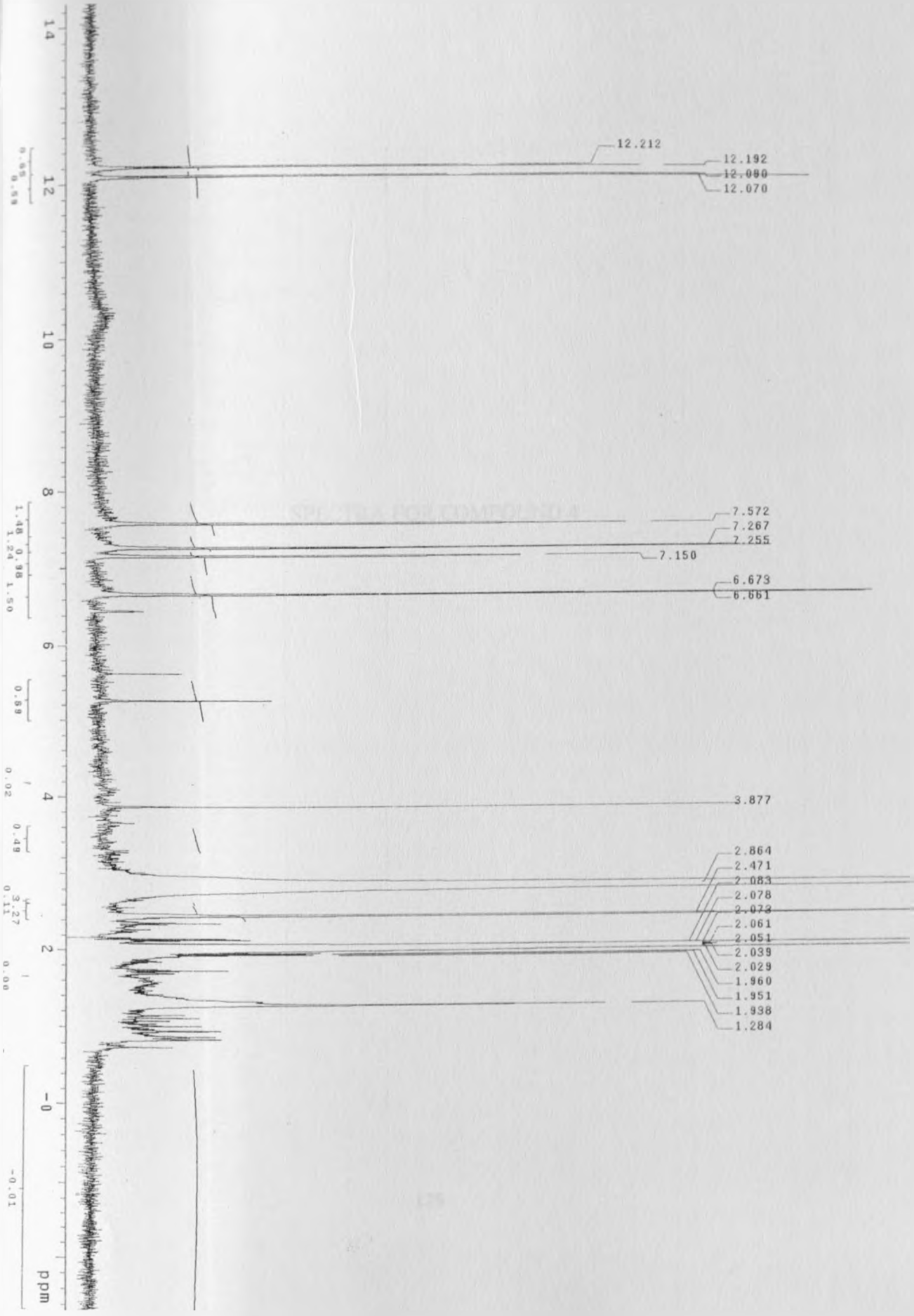
$^1$H NMR SPECTRUM OF COMPOUND **2** (SOLVENT: ACETONE-$d_6$ 200 MHz)



# $^{13}C$ NMR SPECTRUM OF COMPOUND **2** (SOLVENT: ACETONE-$d_6$, 200 MH

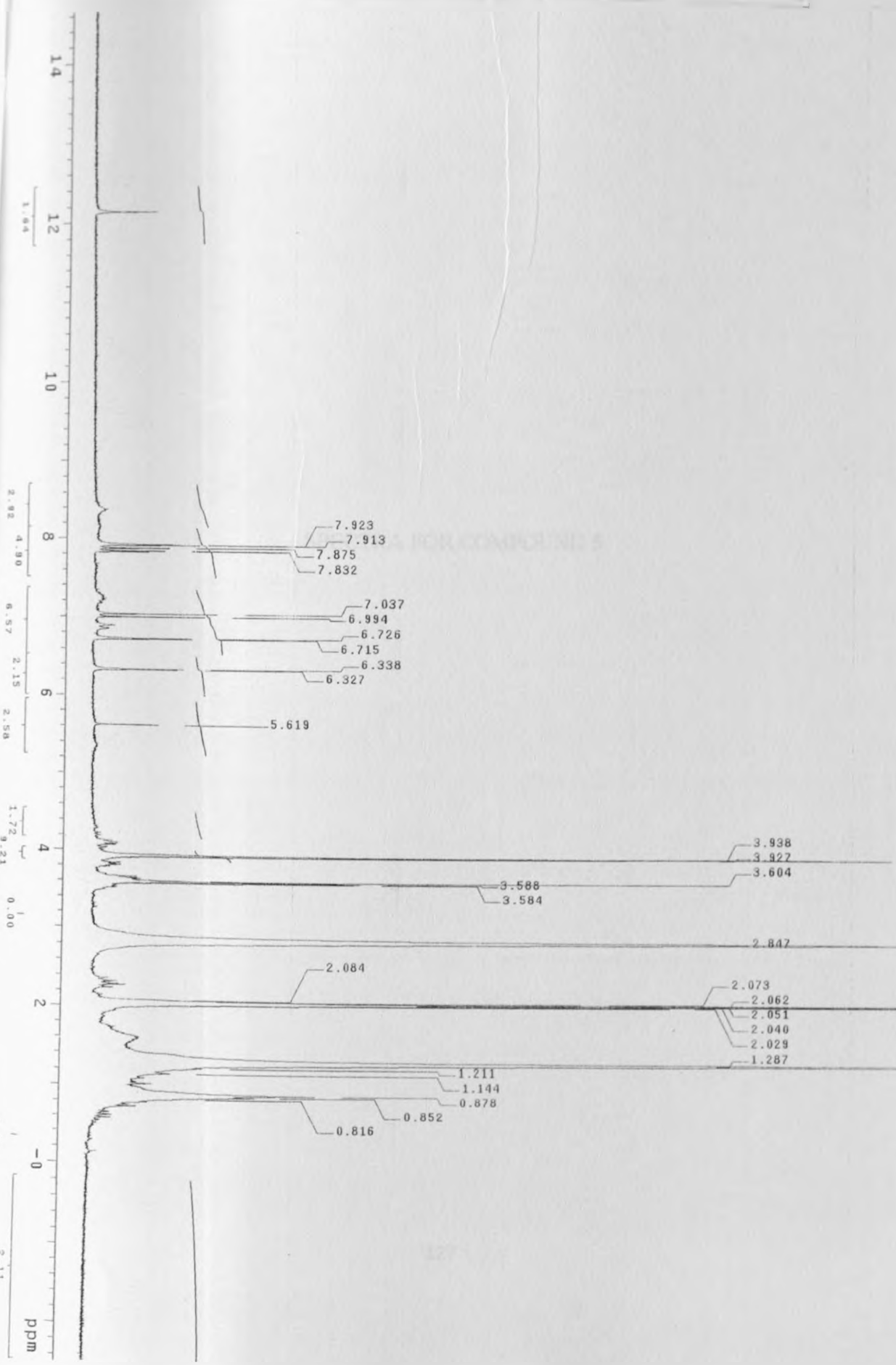
SPECTRA FOR COMPOUND **3**

# $^1$H NMR SPECTRUM FOR COMPOUND 3 (SOLVENT: ACETONE-$d_6$, 200 MHz)



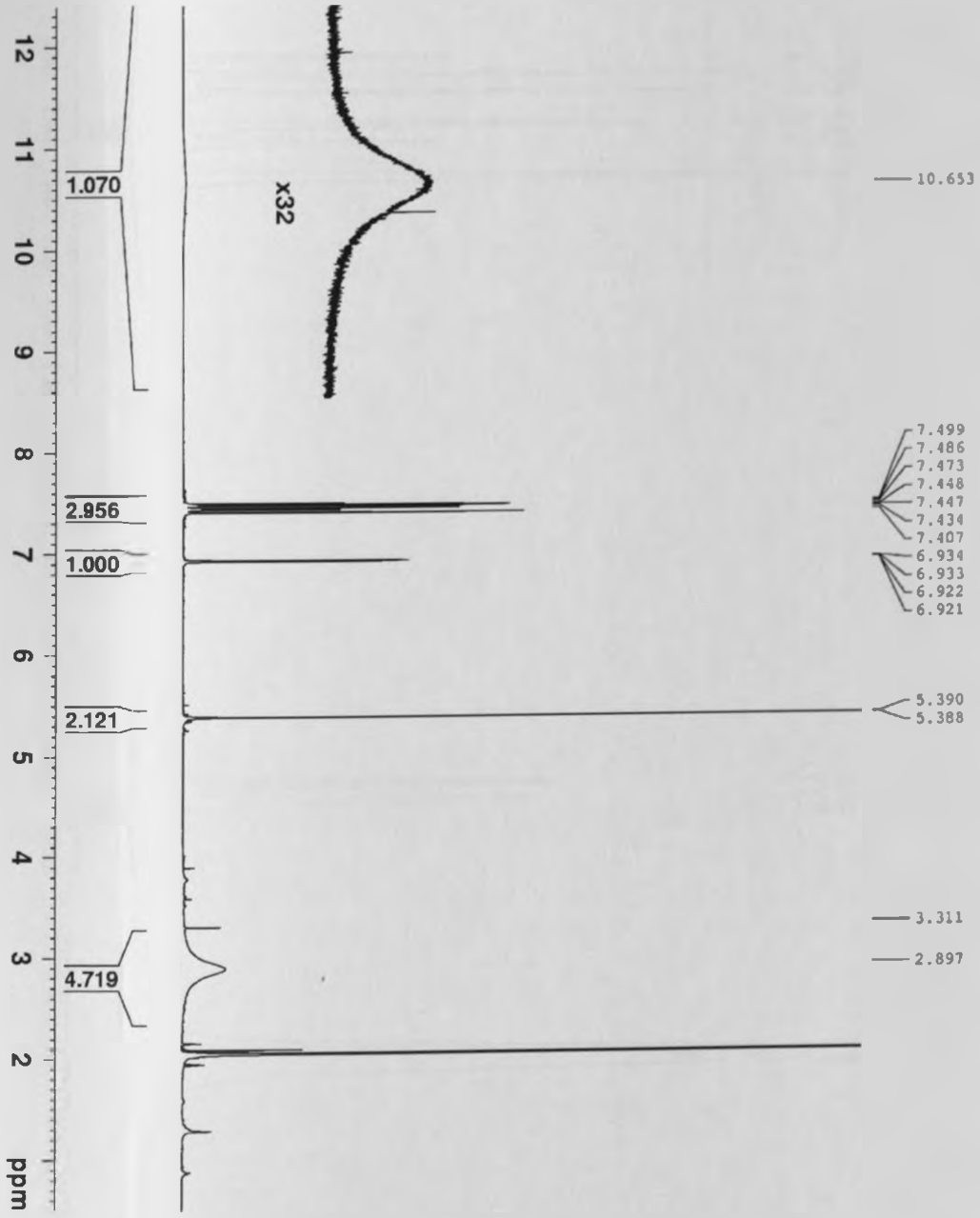
SPECTRA FOR COMPOUND 4

H NMR SPECTRUM FOR COMPOUND 4 (SOLVENT: ACETONE-$d_6$ 200 MHz)

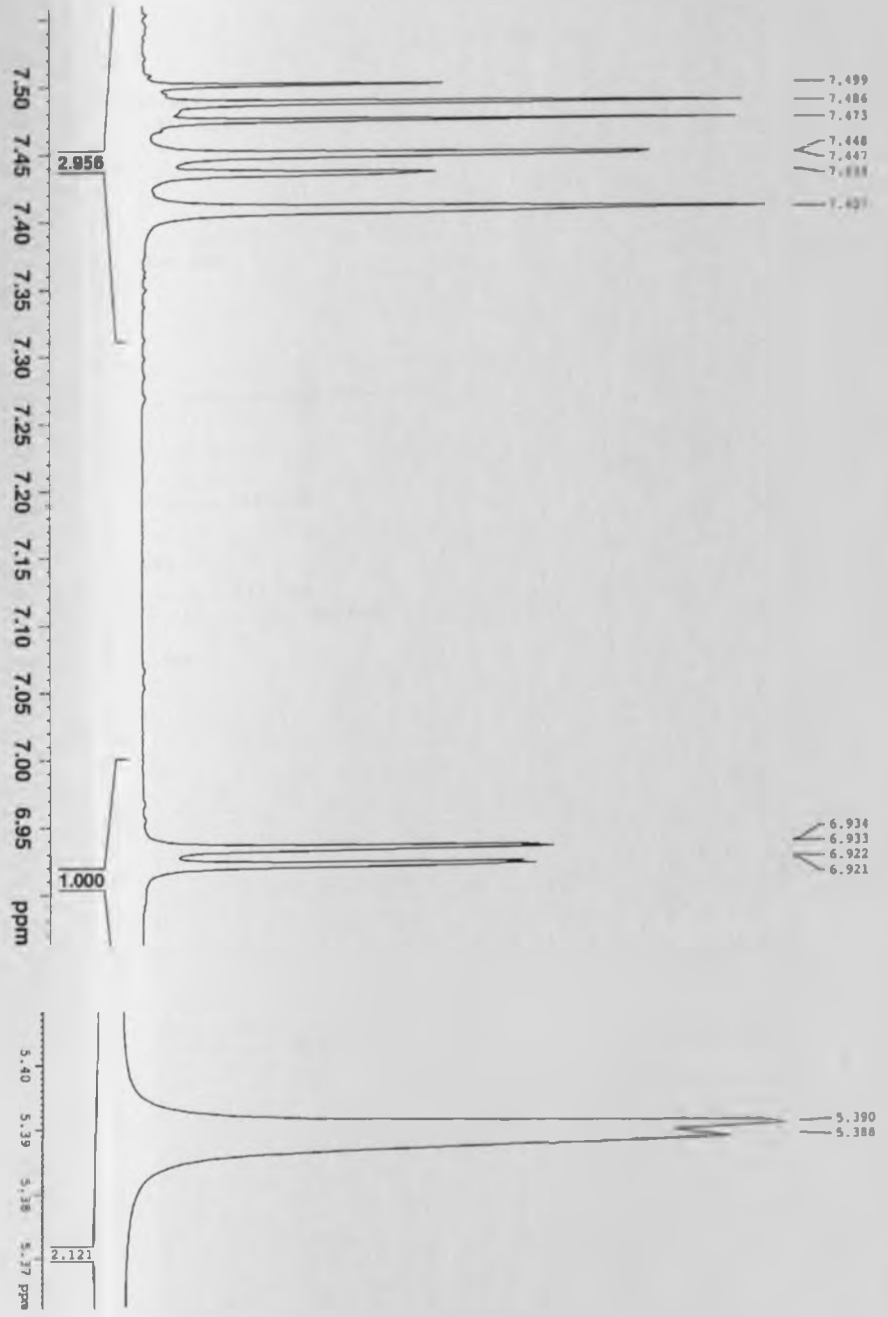


SPECTRA FOR COMPOUND **5**

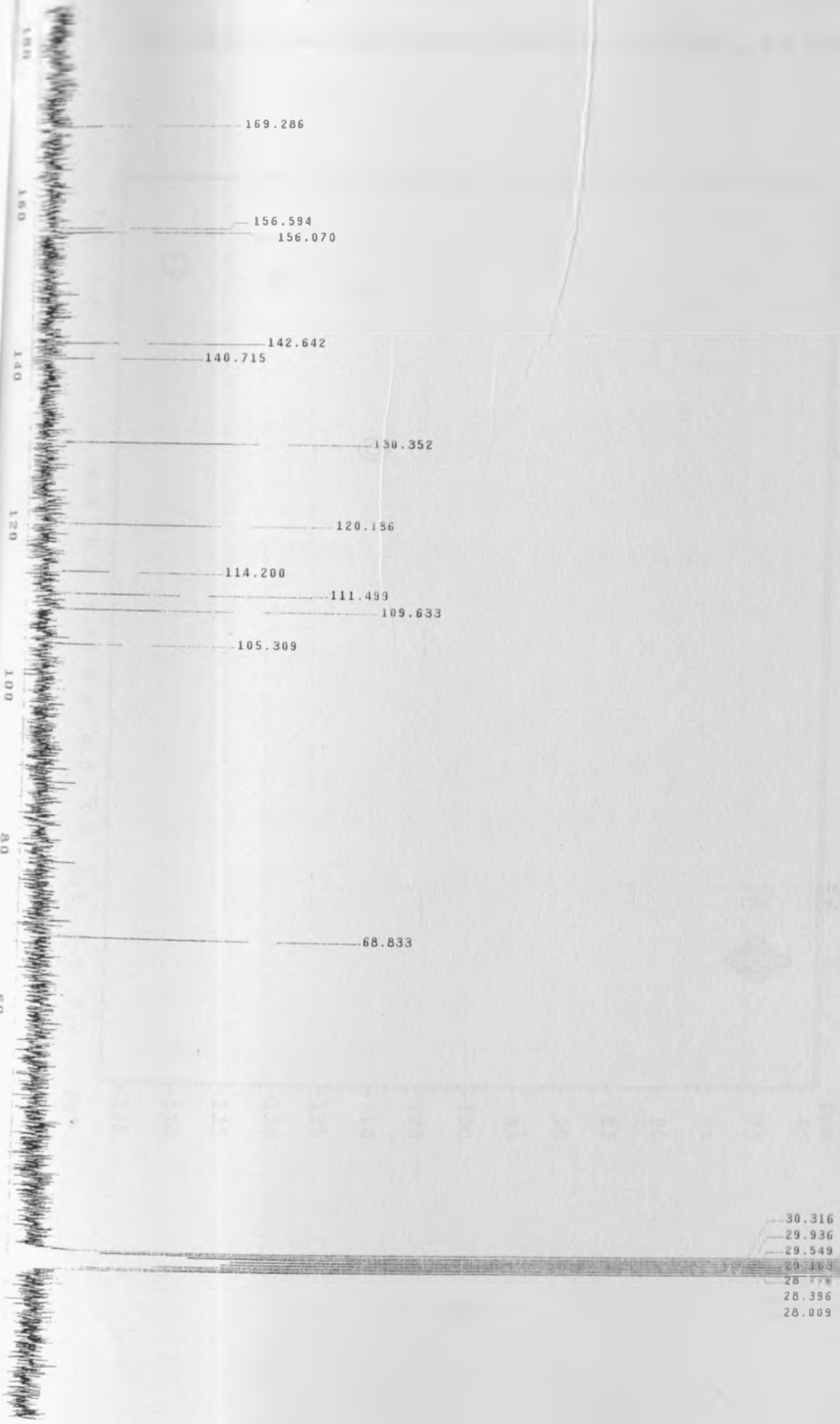
$^{1}$H NMR SPECTRUM FOR COMPOUND **5** (SOLVENT: ACETONE-$d_6$, 600 MHz)



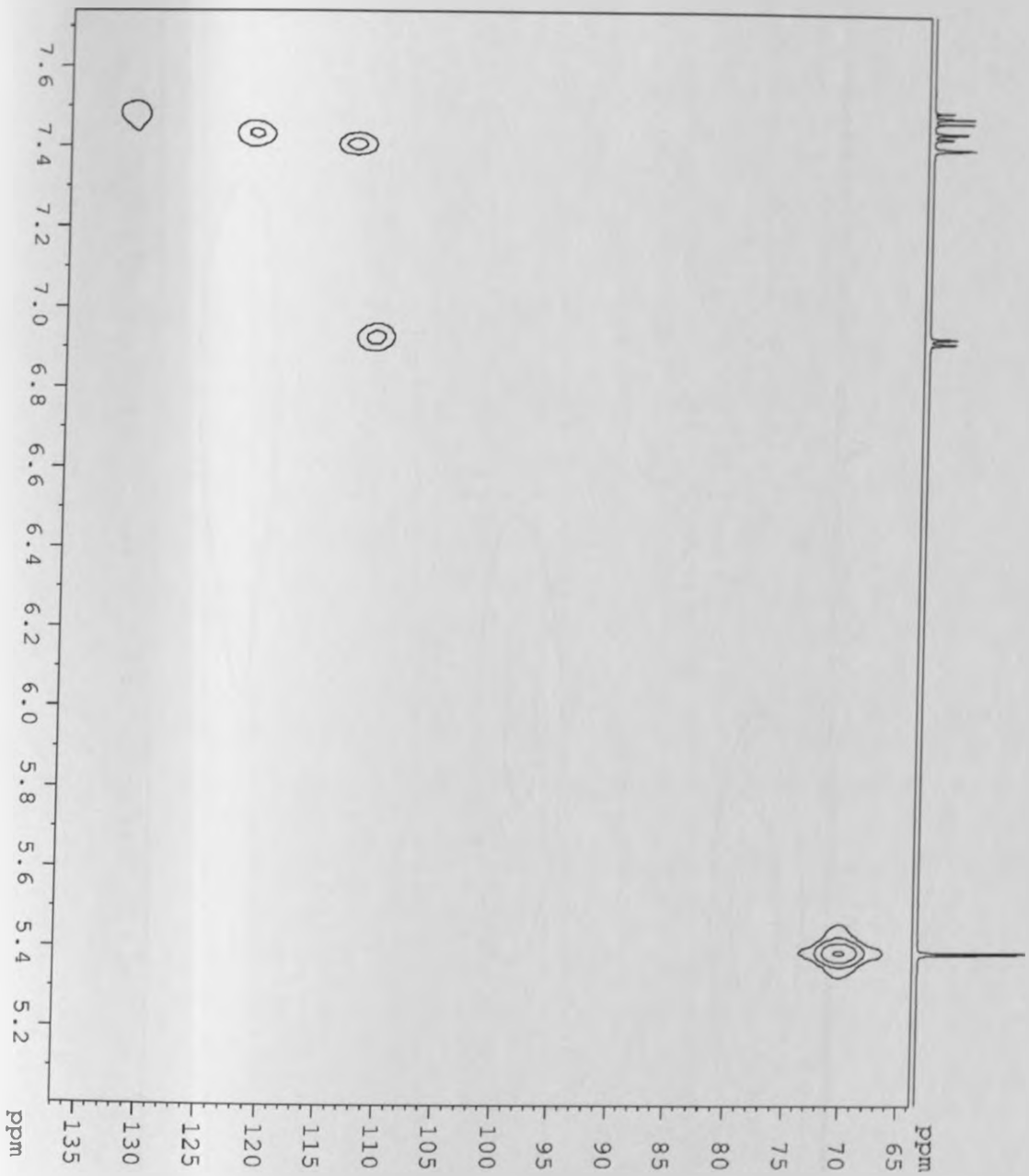
## $^1$H NMR SPECTRUM FOR COMPOUND **5** (SOLVENT: ACETONE-$d_6$, 600 MHz)



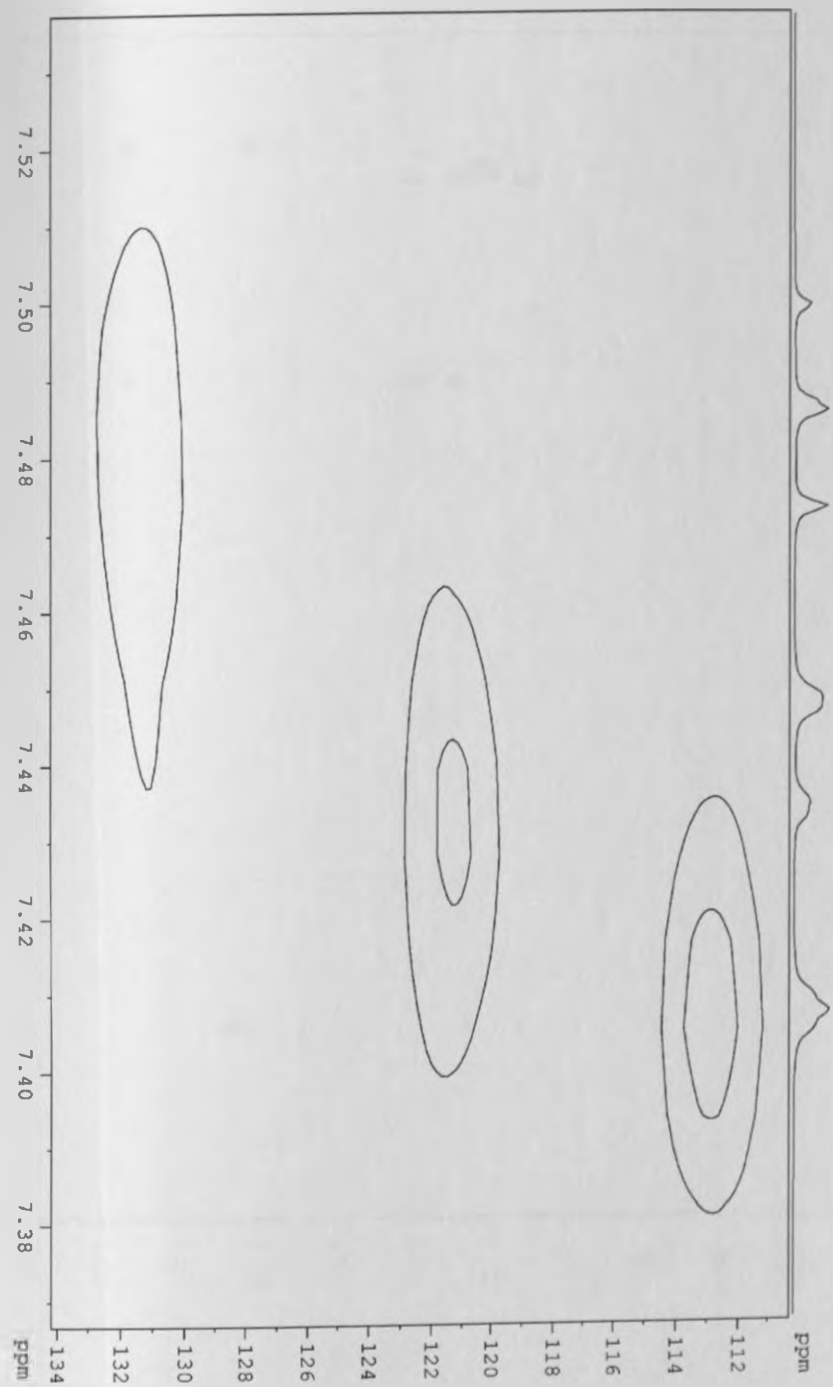
$^{13}$C NMR SPECTRUM FOR COMPOUND **5** (SOL[illegible]NT: ACETONE-$d_6$, 50MHz)



HMQC SPECTRUM COMPOUND **5** (SOLVENT: ACETONE-$d_6$, 600 MHz)



HMQC SPECTRUM OF COMPOUND **5** (SOLVENT: ACETONE-$d_6$, 600 MHz



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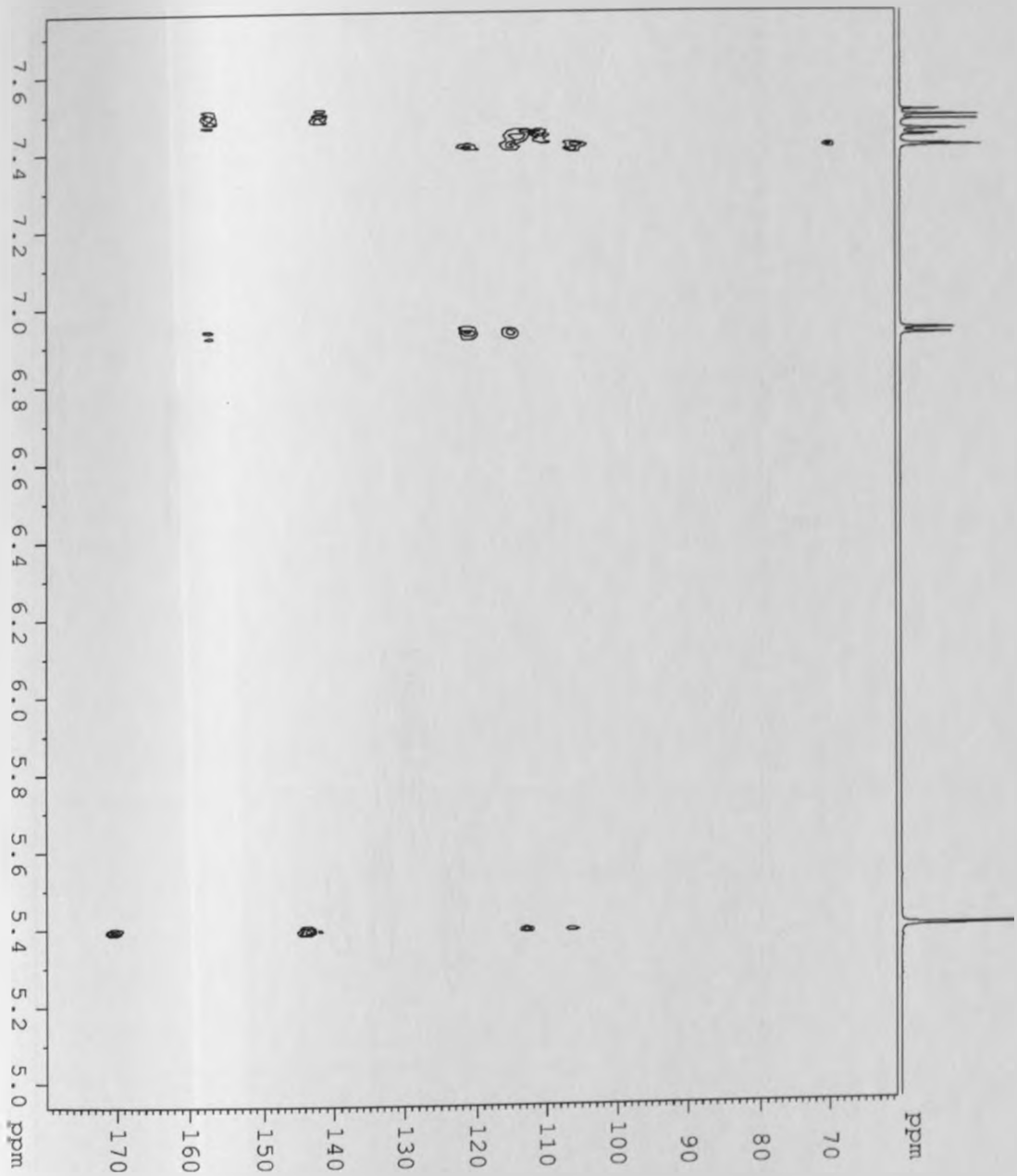

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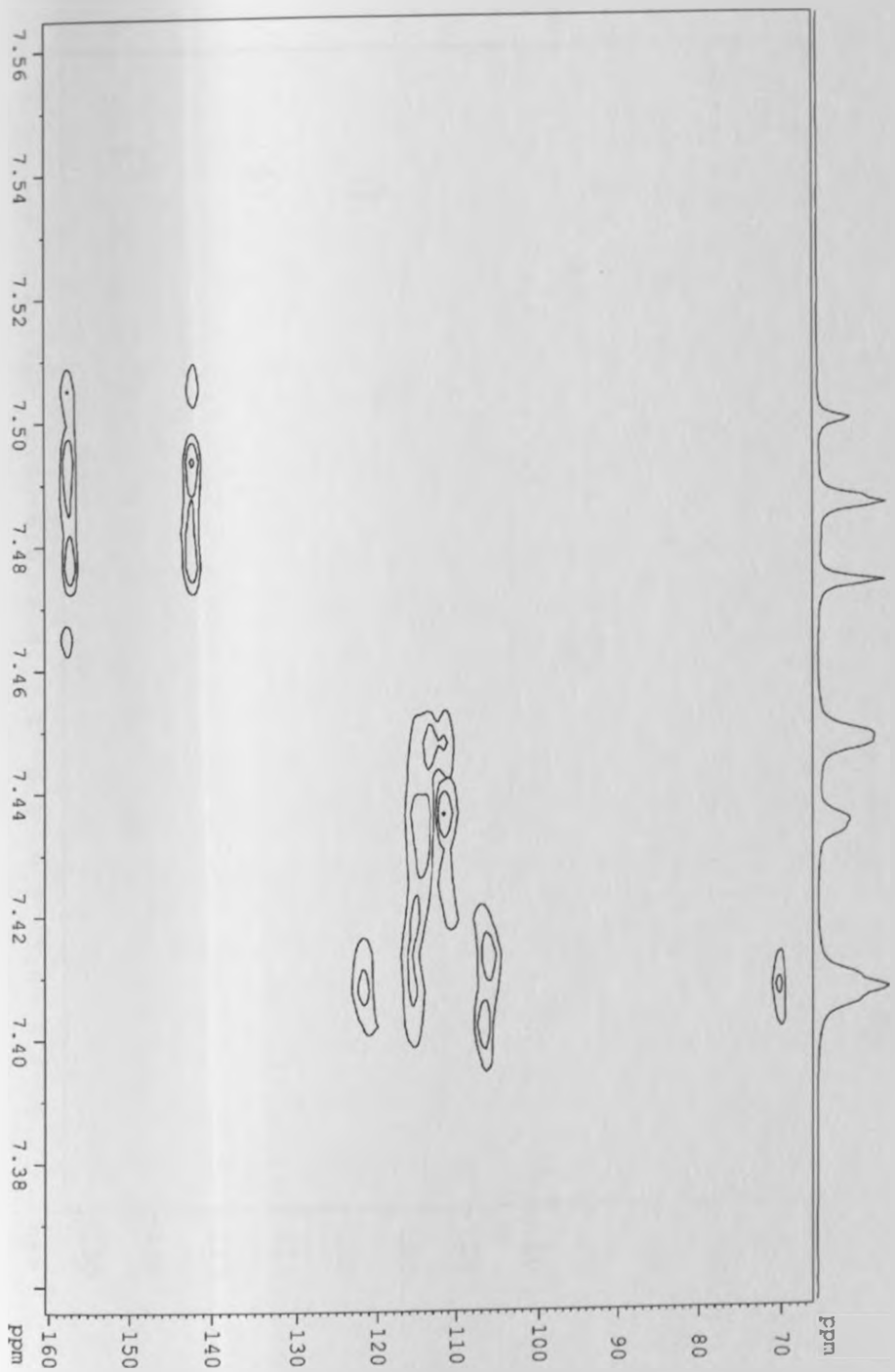

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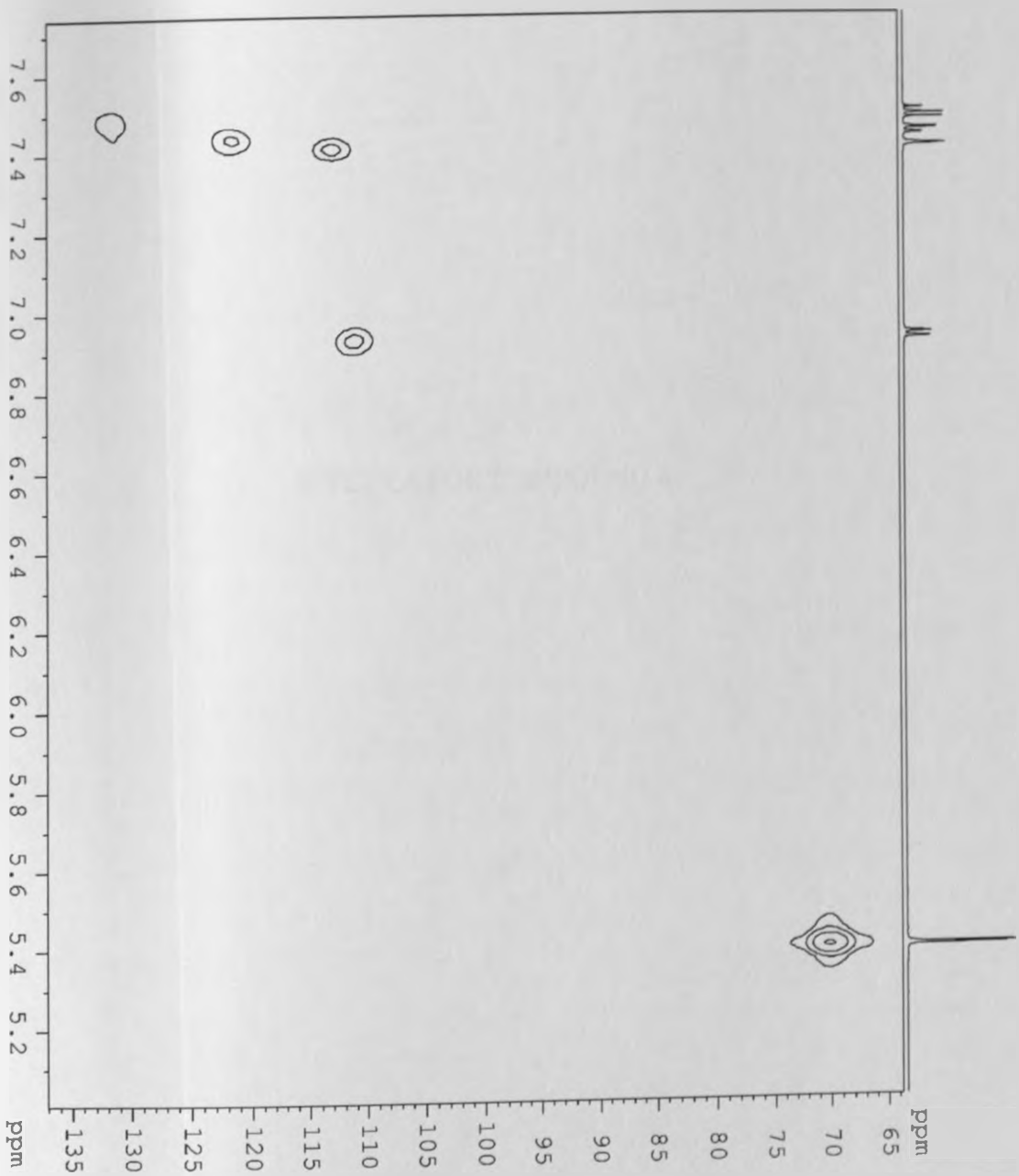
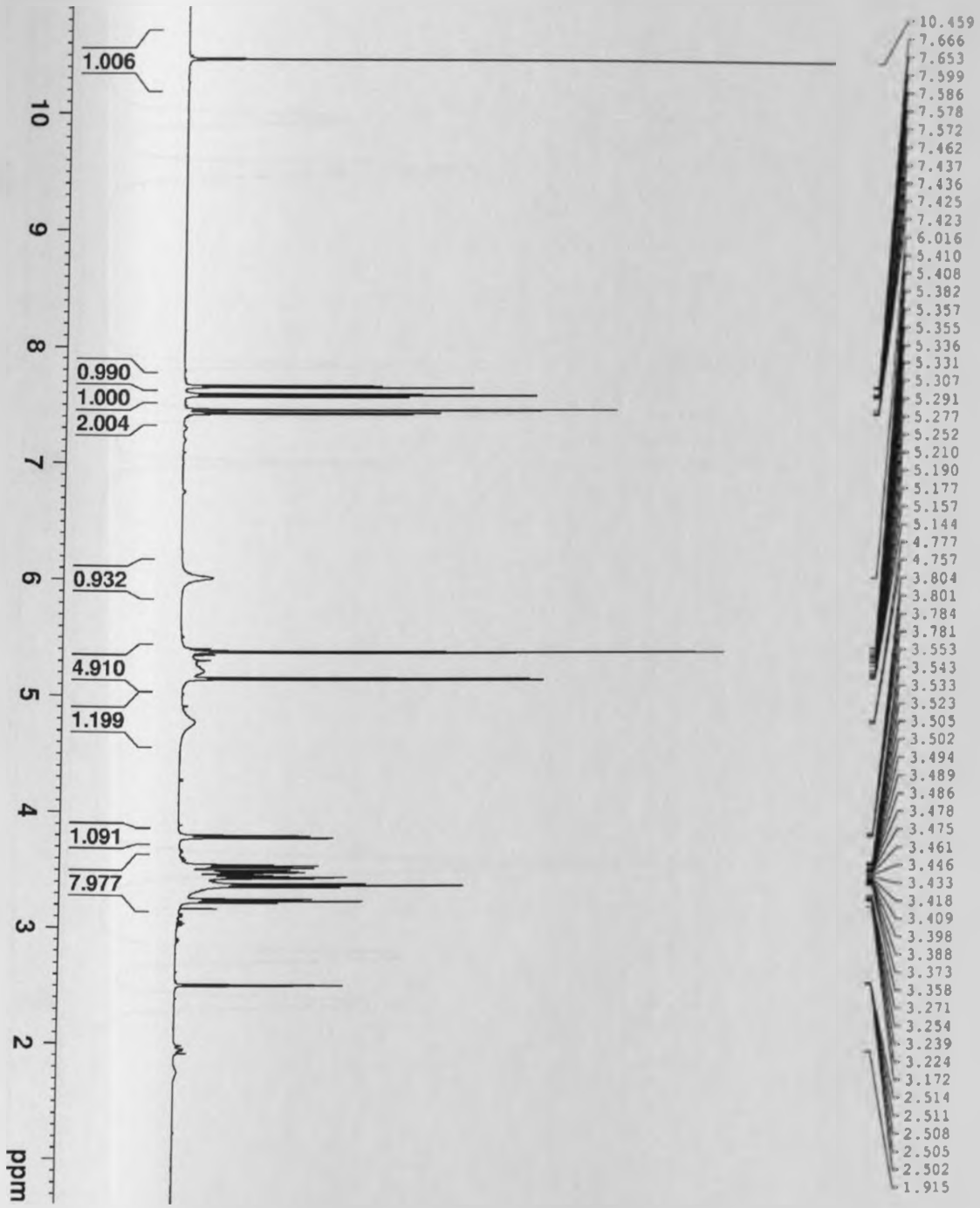
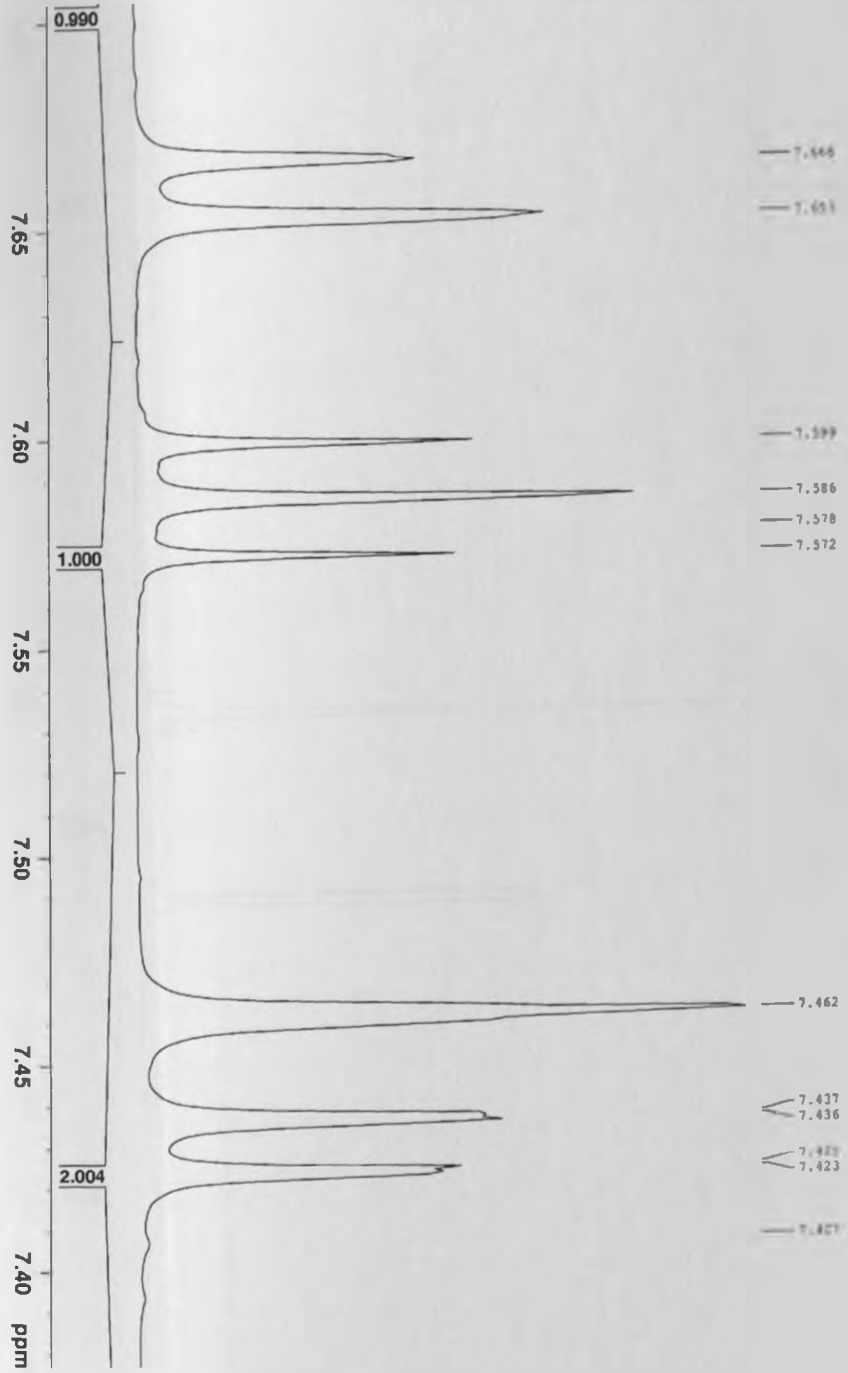


SPECTRA FOR COMPOUND **6**

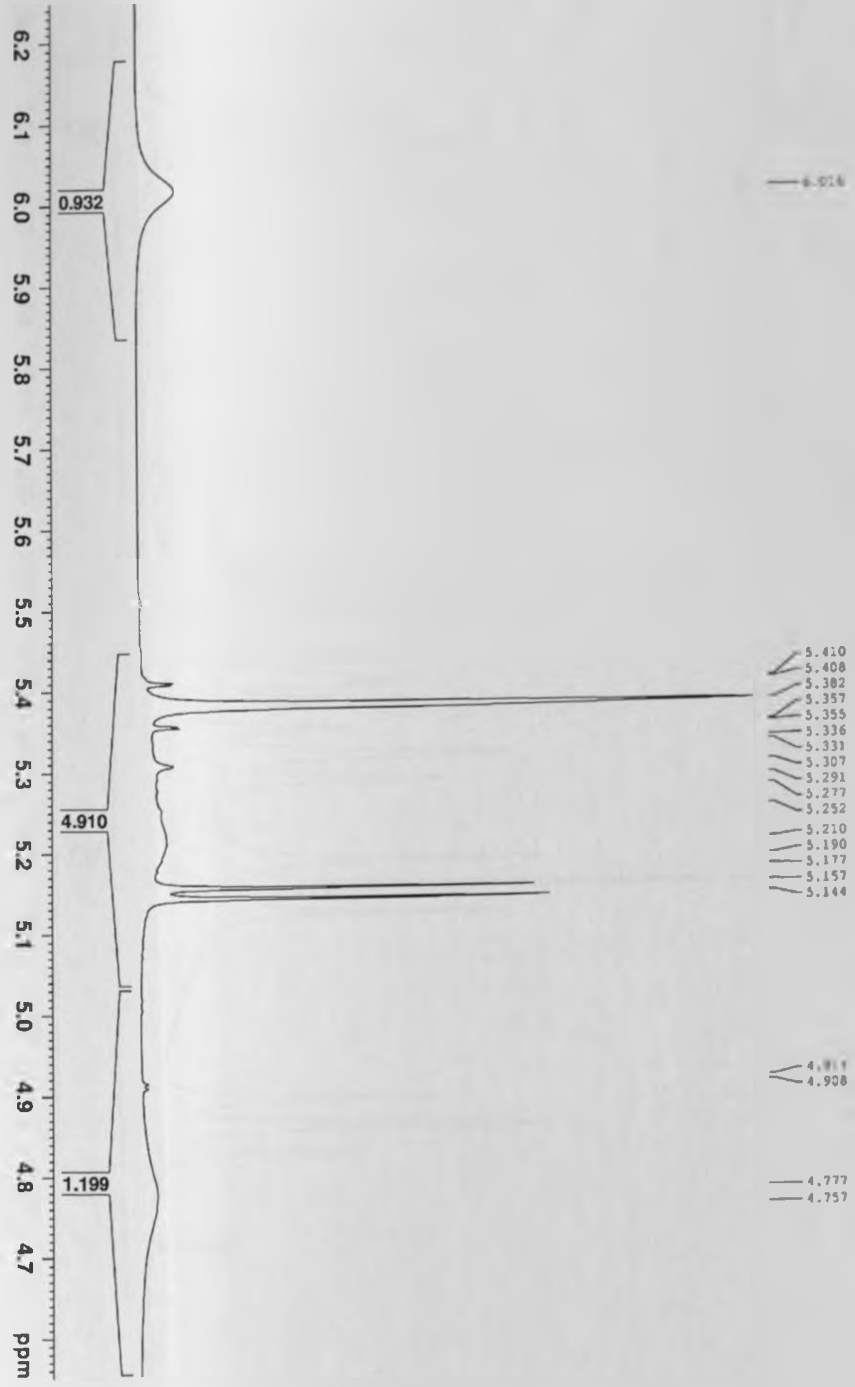
1H NMR SPECTRUM FOR COMPOUND **6** (SOLVENT: DMSO 600 MHz)



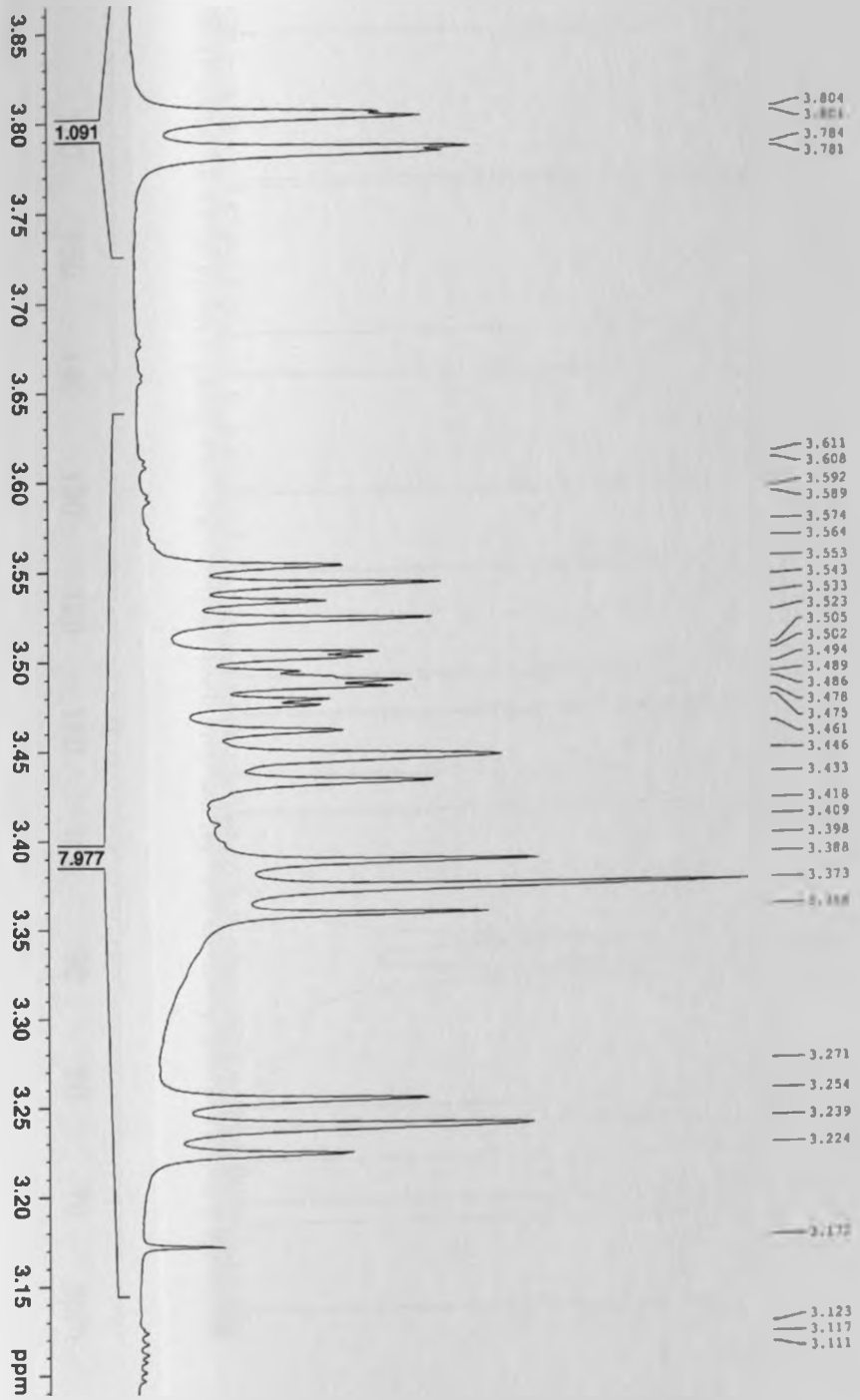
$^{1}$H NMR SPECTRUM FOR COMPOUND **6** (SOLVENT: DMSO 600 MHz)



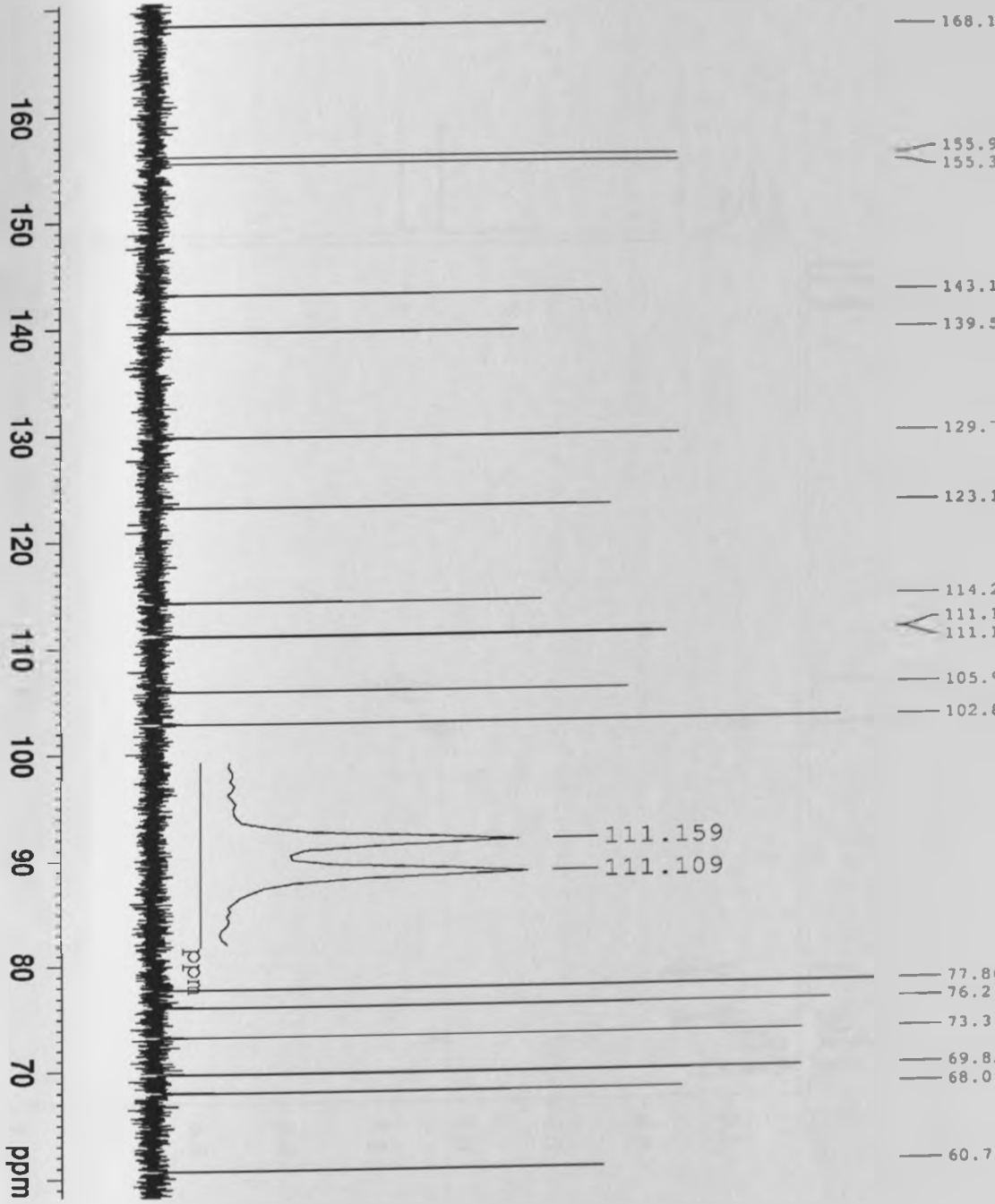
$^{1}$H NMR SPECTRUM FOR COMPOUND **6** (SOLVENT: DMSO 600 MHz)



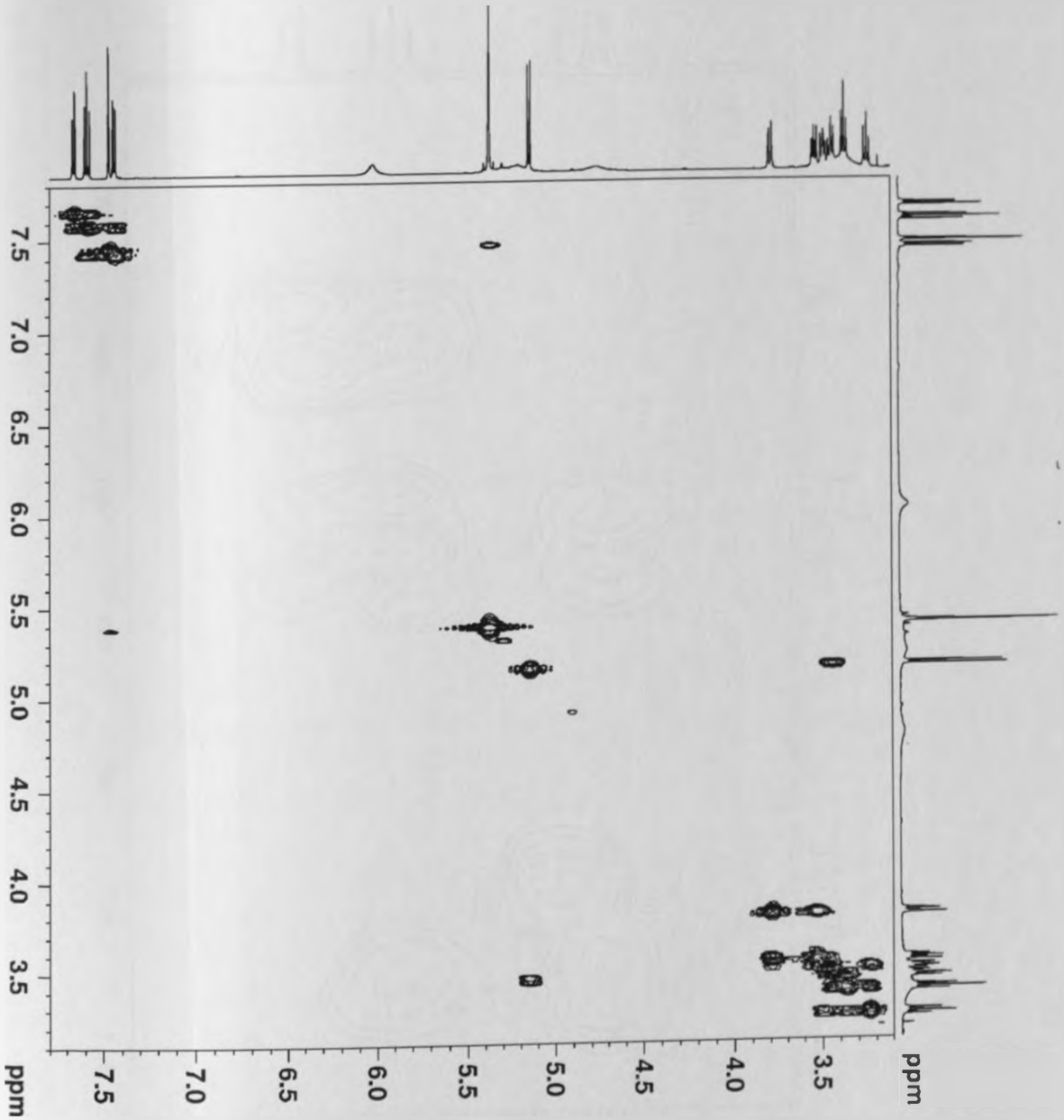
## $^1$H NMR SPECTRUM FOR COMPOUND **6** (SOLVENT: DMSO 600 MHz)



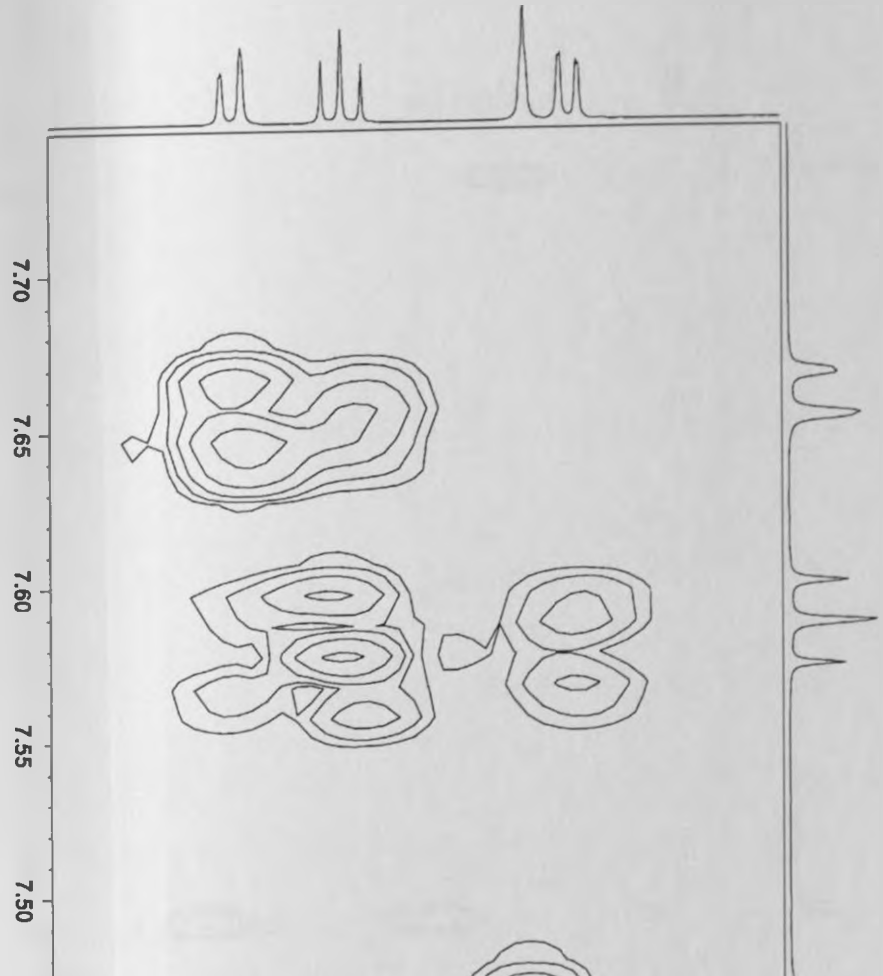
$^{13}C$ NMR SPECTRUM FOR COMPOUND **6** (SOLVENT: DMSO 150 MHz)



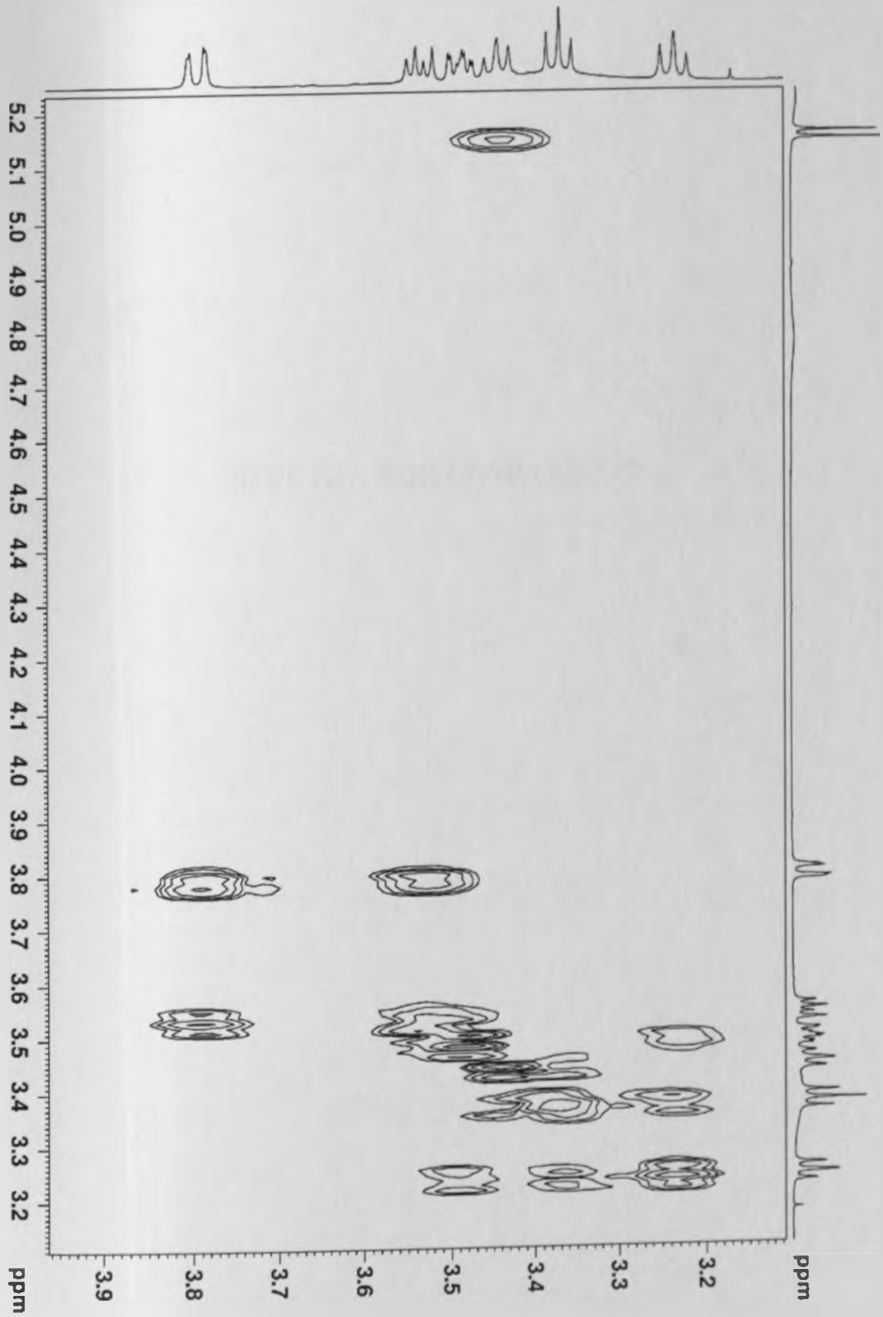
COSY SPECTRUM FOR COMPOUND **6**



COSY SPECTRUM FOR COMPOUND **6**

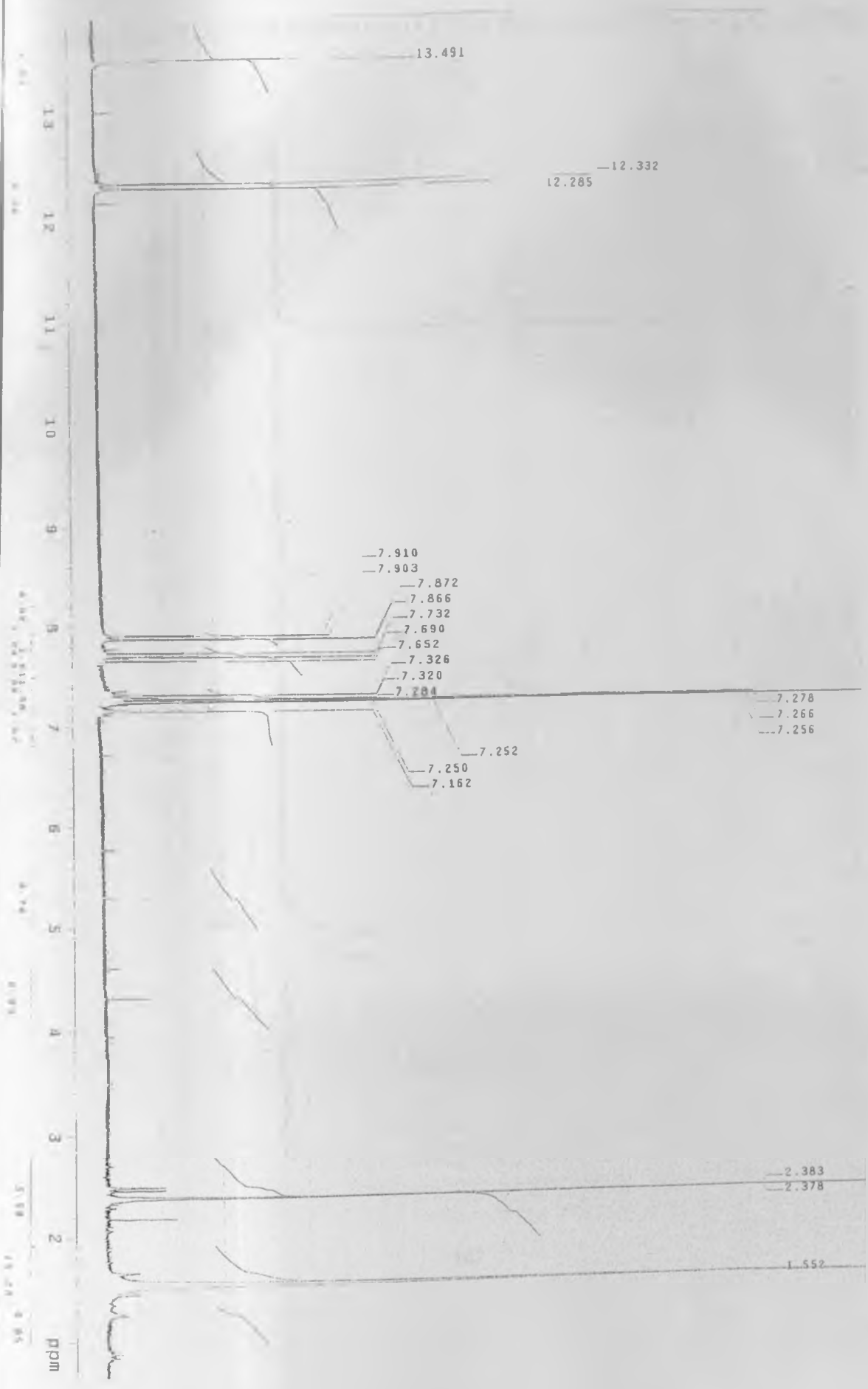


COSY SPECTRUM FOR COMPOUND **6**



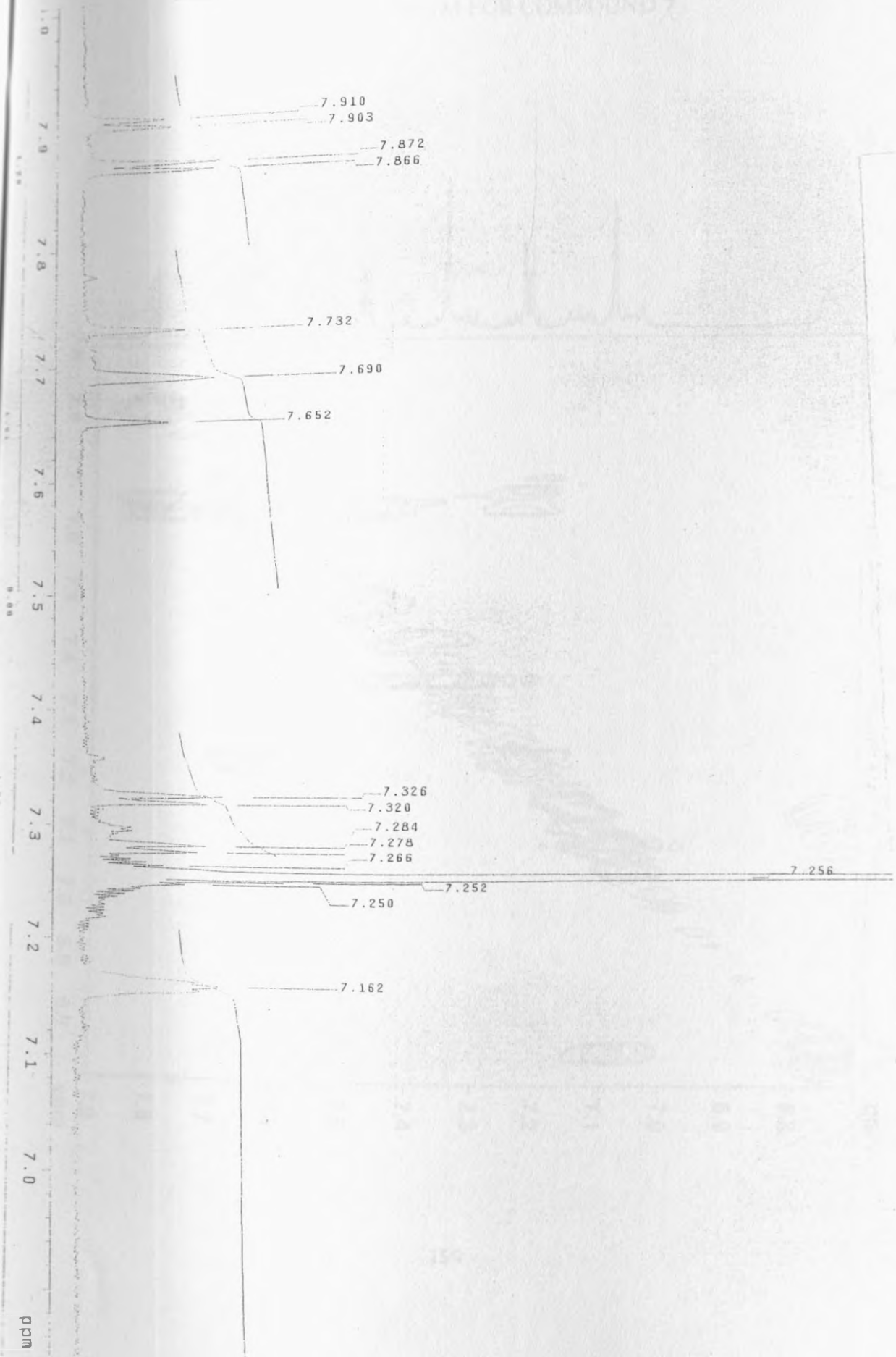
SPECTRA FOR COMPOUND 7

# $^1$H NMR SPECTRUM OF COMPOUND 7 (SOLVENT: $CDCl_3$ 200 MHz)

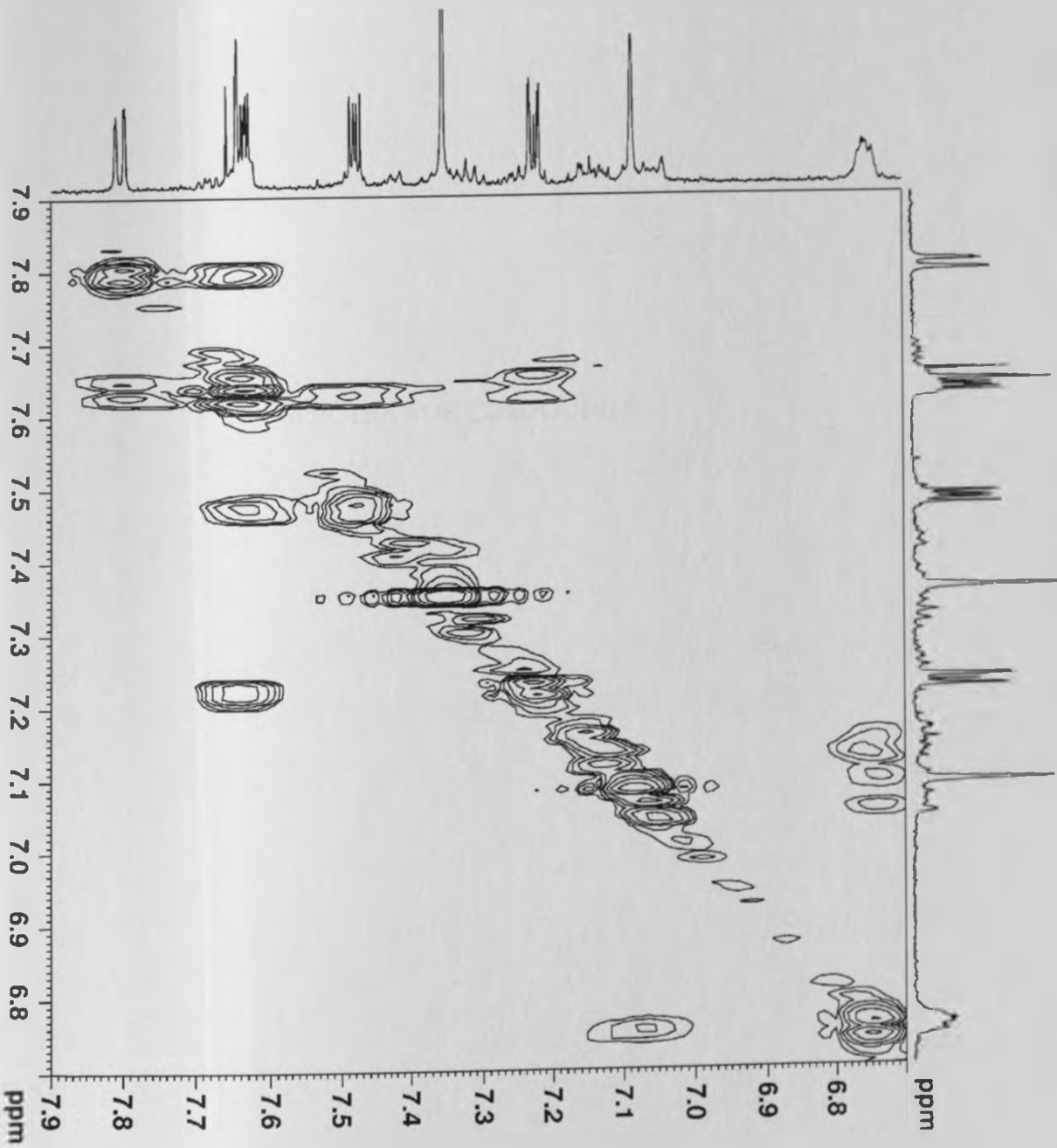


$^1$HNMR SPECTRUM FOR COMPOUND **7** (SOLVENT: ACETONE-$d_6$ + $CDCl_3$ 600MHz)

# $^1$H NMR SPECTRUM OF COMPOUND 7 (SOLVENT: $CDCl_3$ 200 MHz)

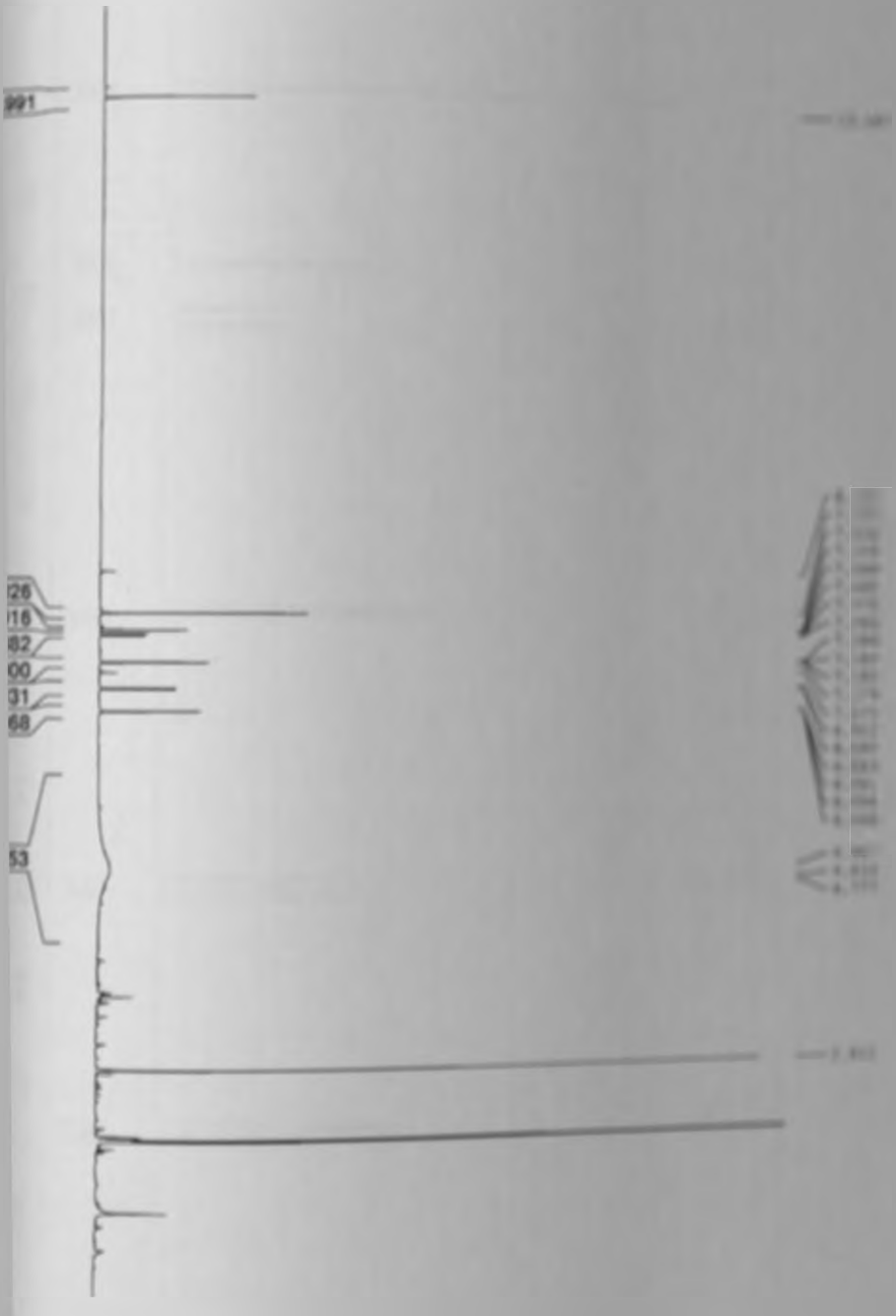


COSY SPECTRUM FOR COMPOUND 7

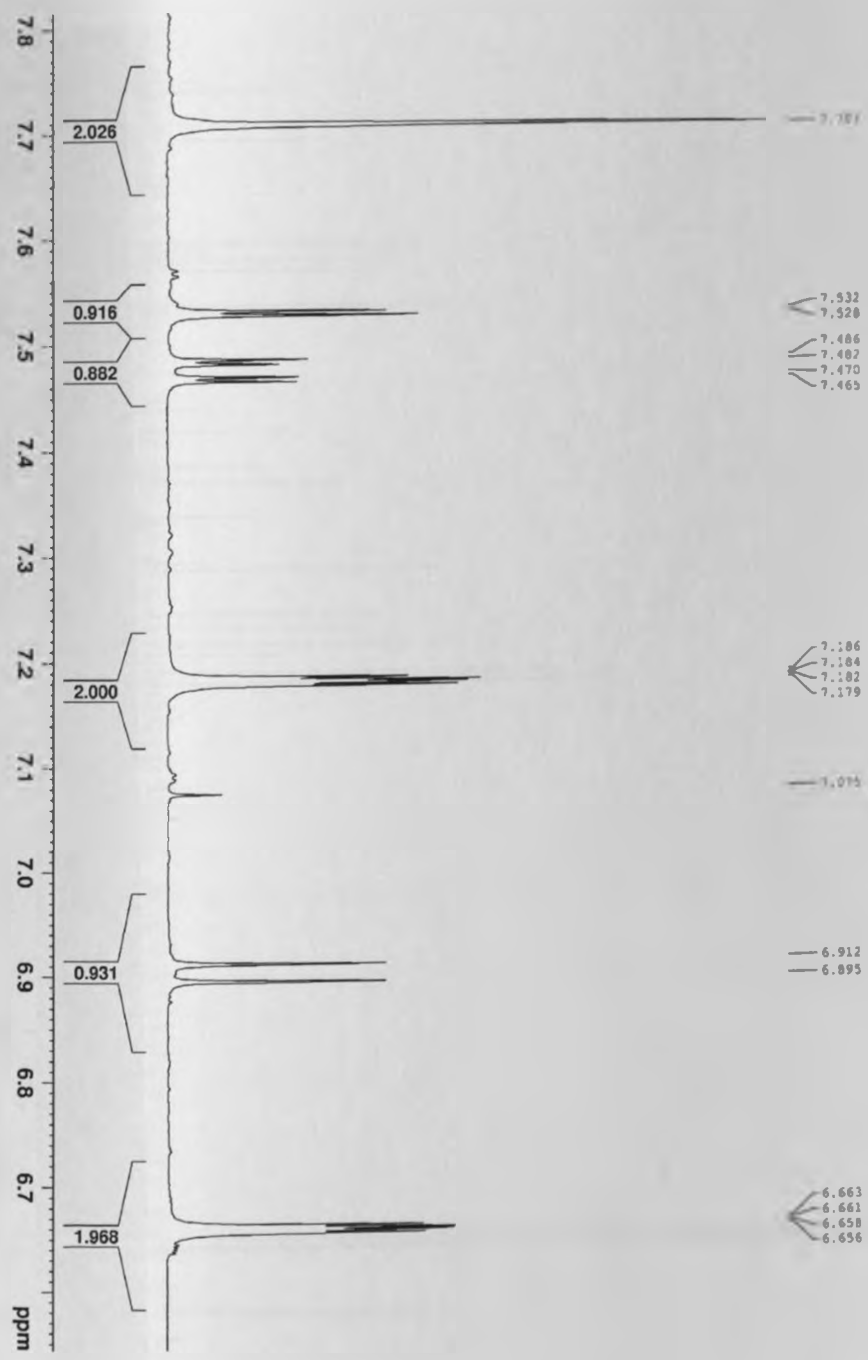


SPECTRA FOR COMPOUND 8

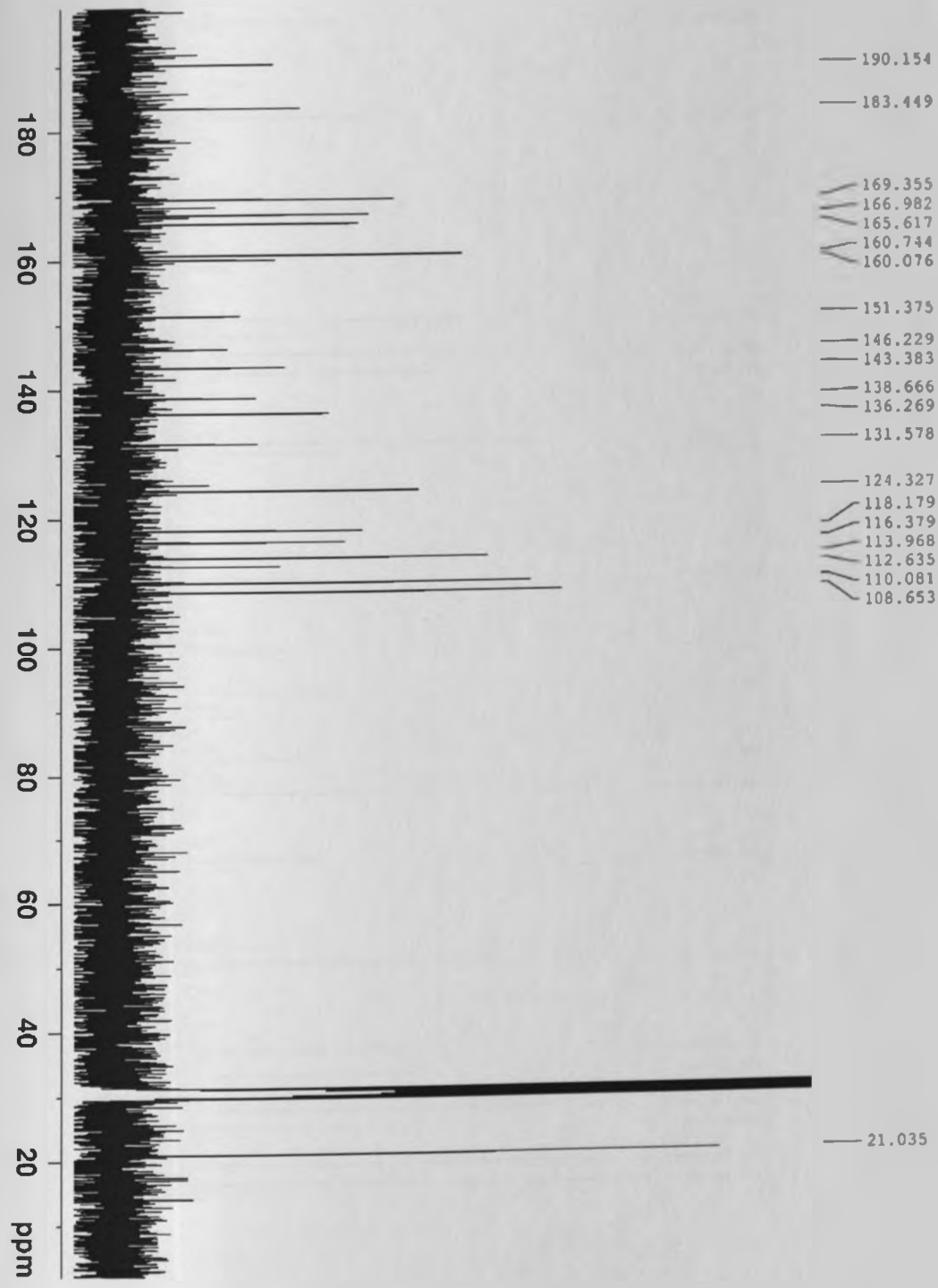
R SPECTRUM FOR COMPOUND 8 (SOLVENT: ACETONE-d, 500 MHz)



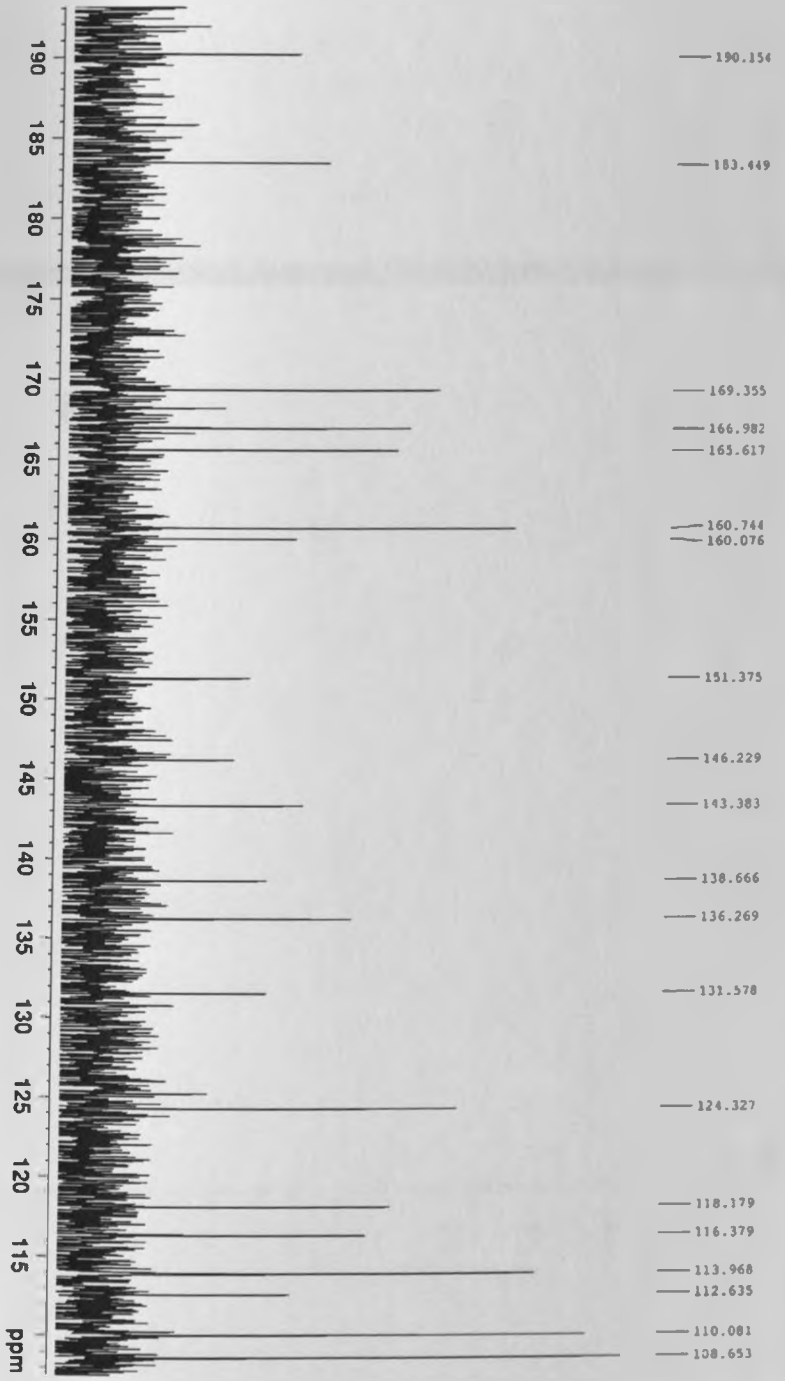
$^1$H NMR SPECTRUM FOR COMPOUND **8** (SOLVENT: ACETONE-$d_6$ 500 MHz)



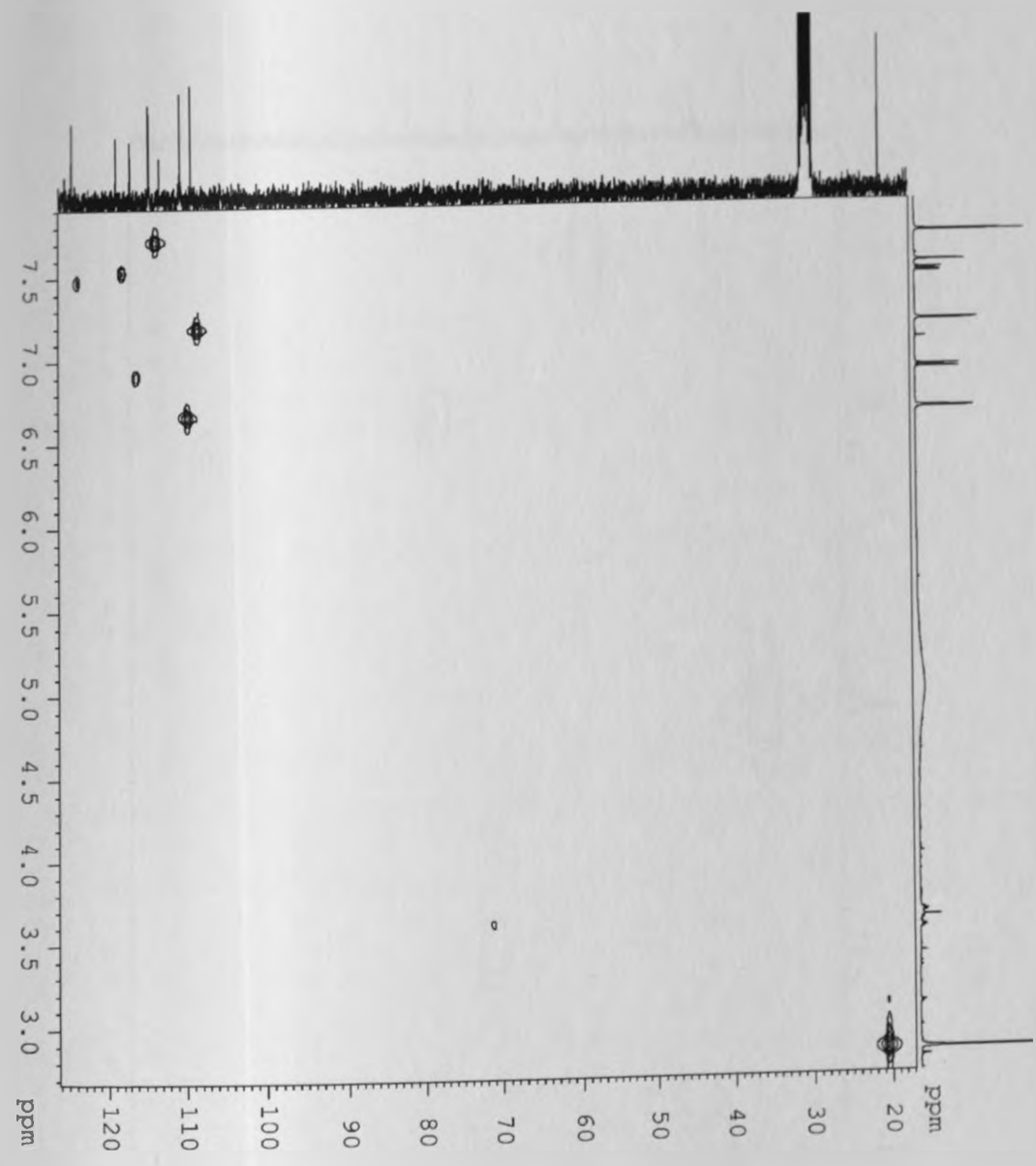
$^{13}C$ NMR SPECTRUM FOR COMPOUND **8** (SOLVENT: ACETONE-$d_6$ 125 MHz)



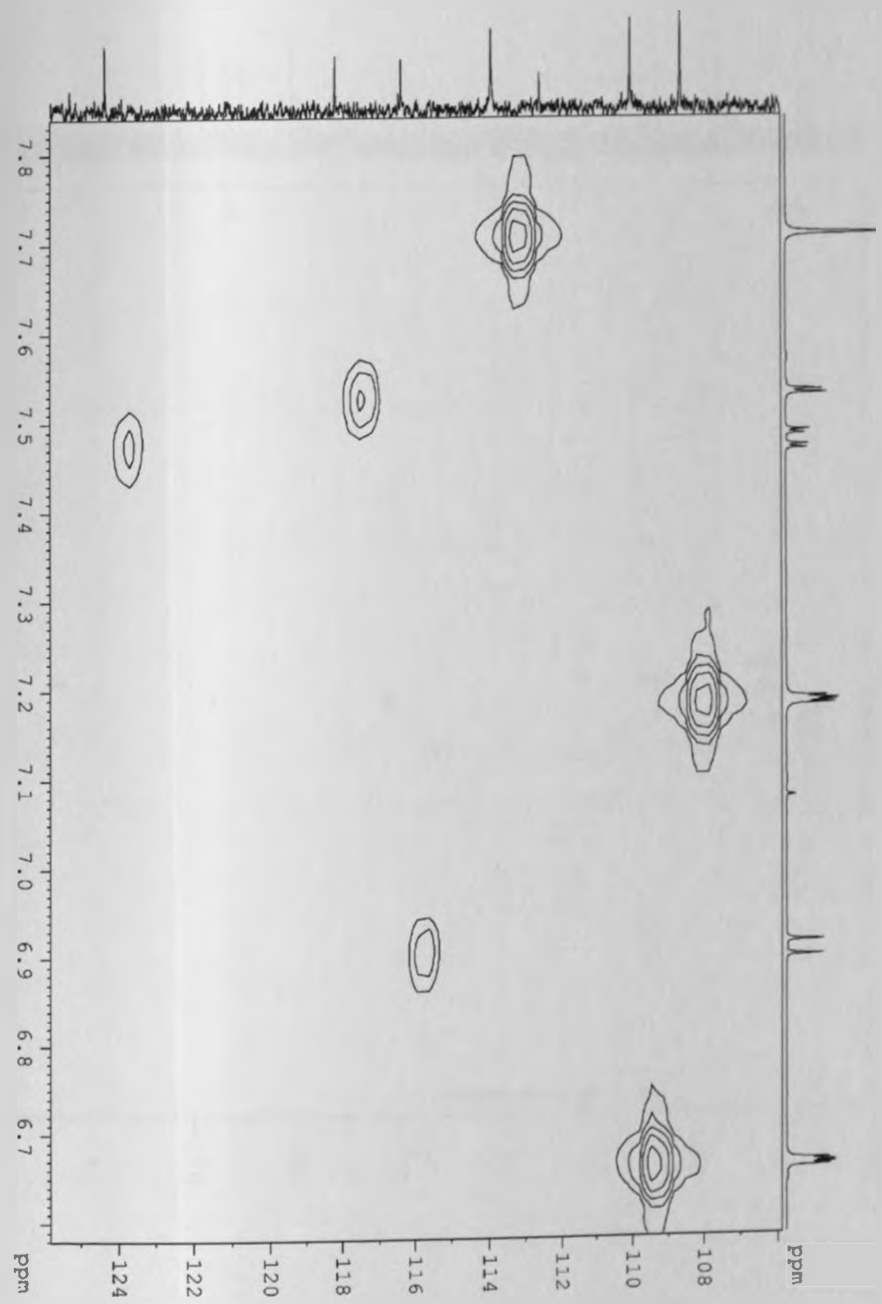
$^{13}$C NMR SPECTRUM FOR COMPOUND **8** (SOLVENT: ACETONE-$d_6$ 125 MHz)



HMQC SPECTRUM FOR COMPOUND **8** (SOLVENT: ACETONE-$d_6$ 500 MHz)

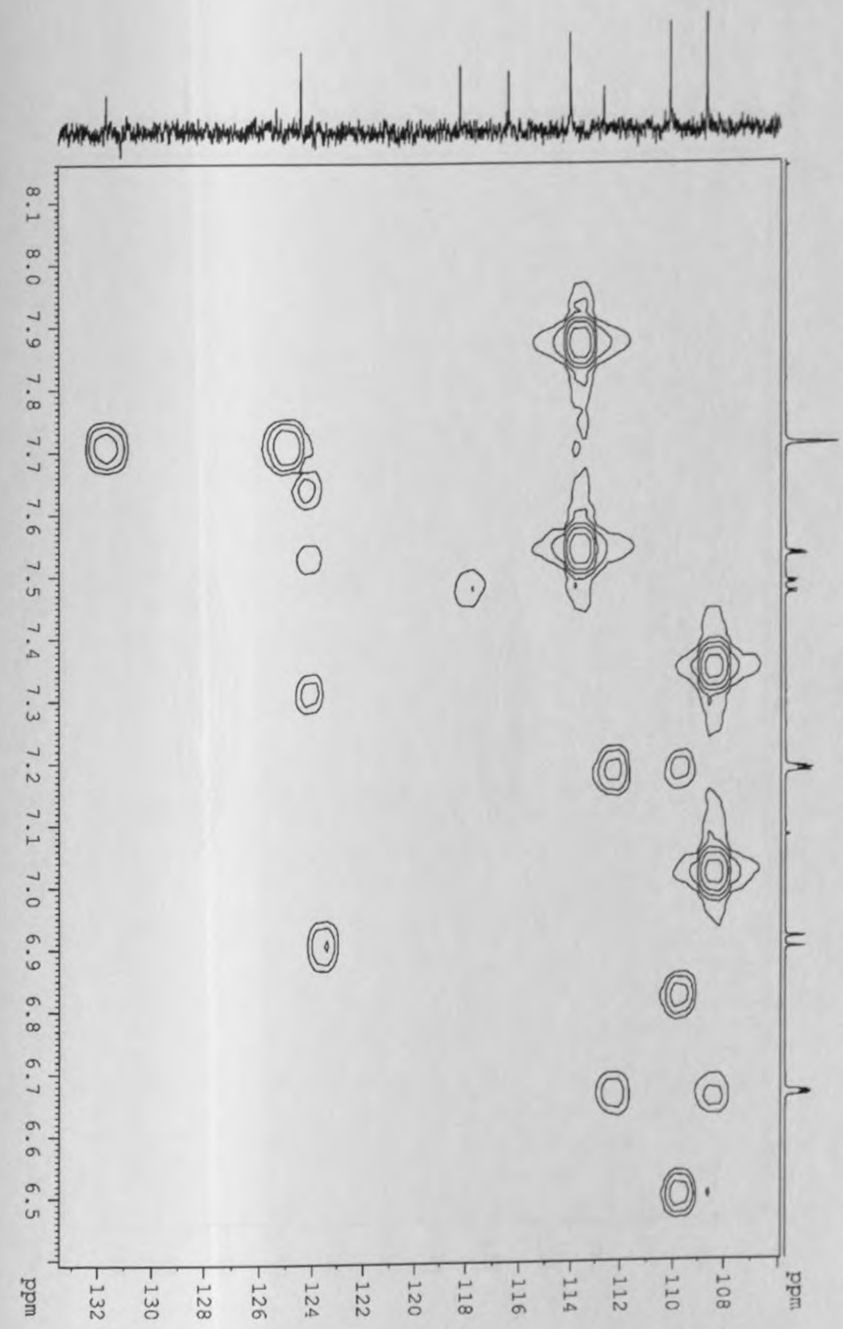


HMQC SPECTRUM FOR COMPOUND **8** (SOLVENT: ACETONE-$d_6$ 500 MHz)

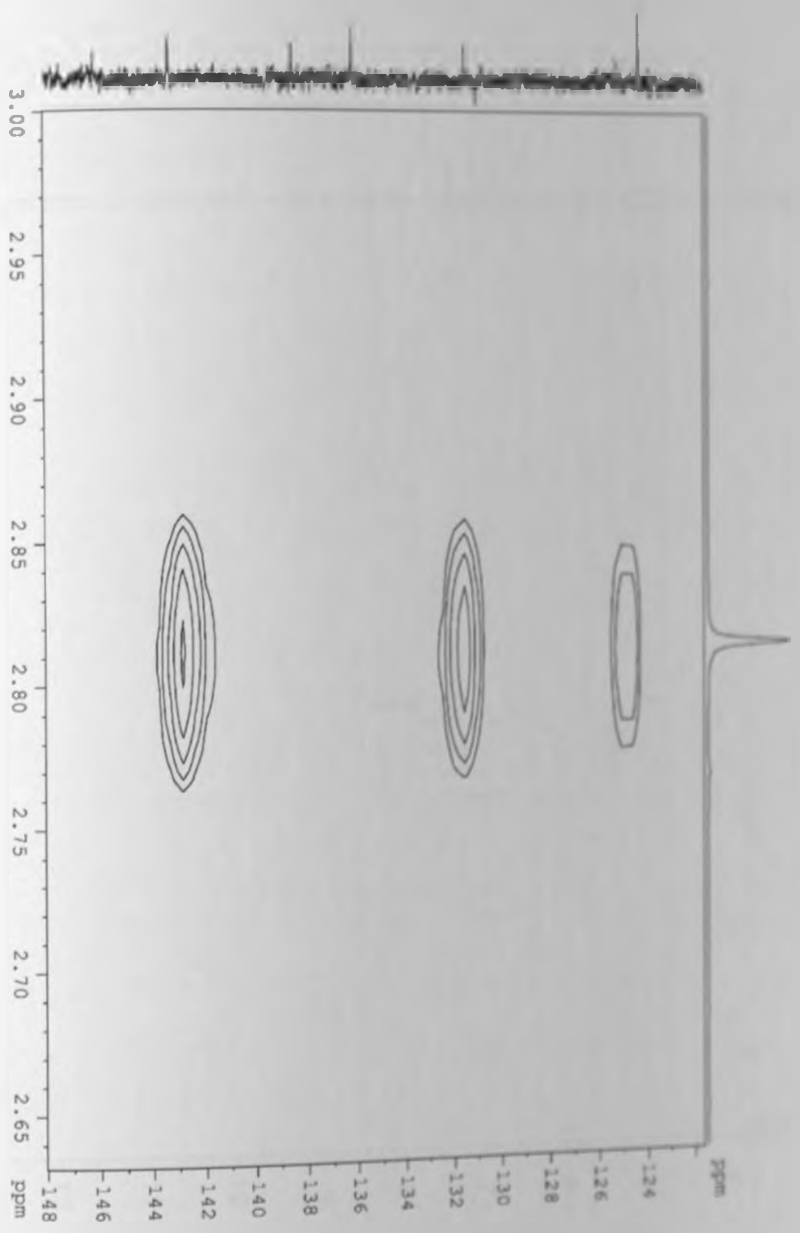


HMBC SPECTRUM FOR COMPOUND **8** (SOLVENT: ACETONE-$d_6$ 500 MHz)

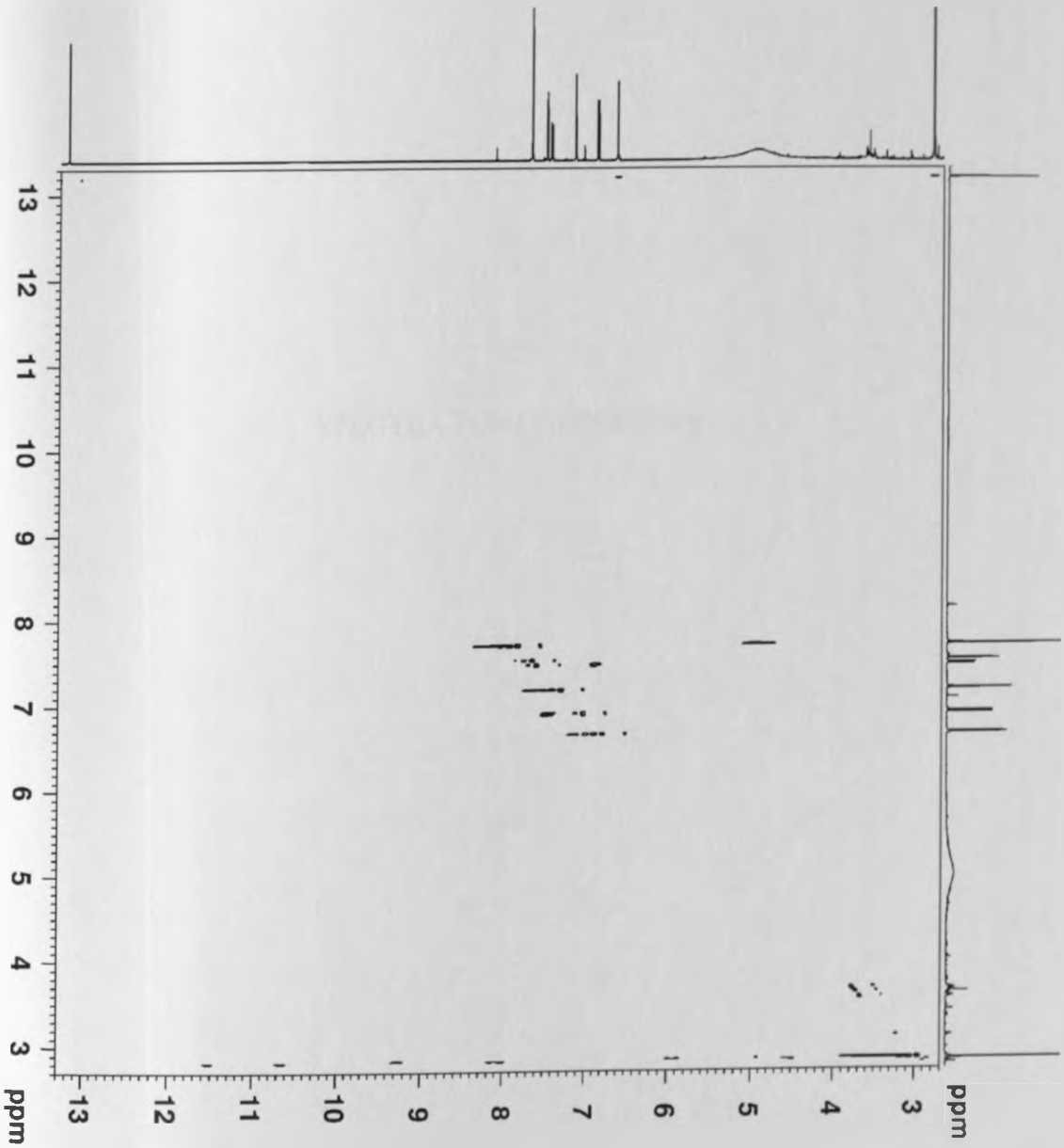
HMBC SPECTRUM FOR COMPOUND **8** (SOLVENT: ACETONE-$d_6$ 500 MHz)



HMBC SPECTRUM FOR COMPOUND **8** (SOLVENT: ACETONE-$d_6$, 500 MHz)

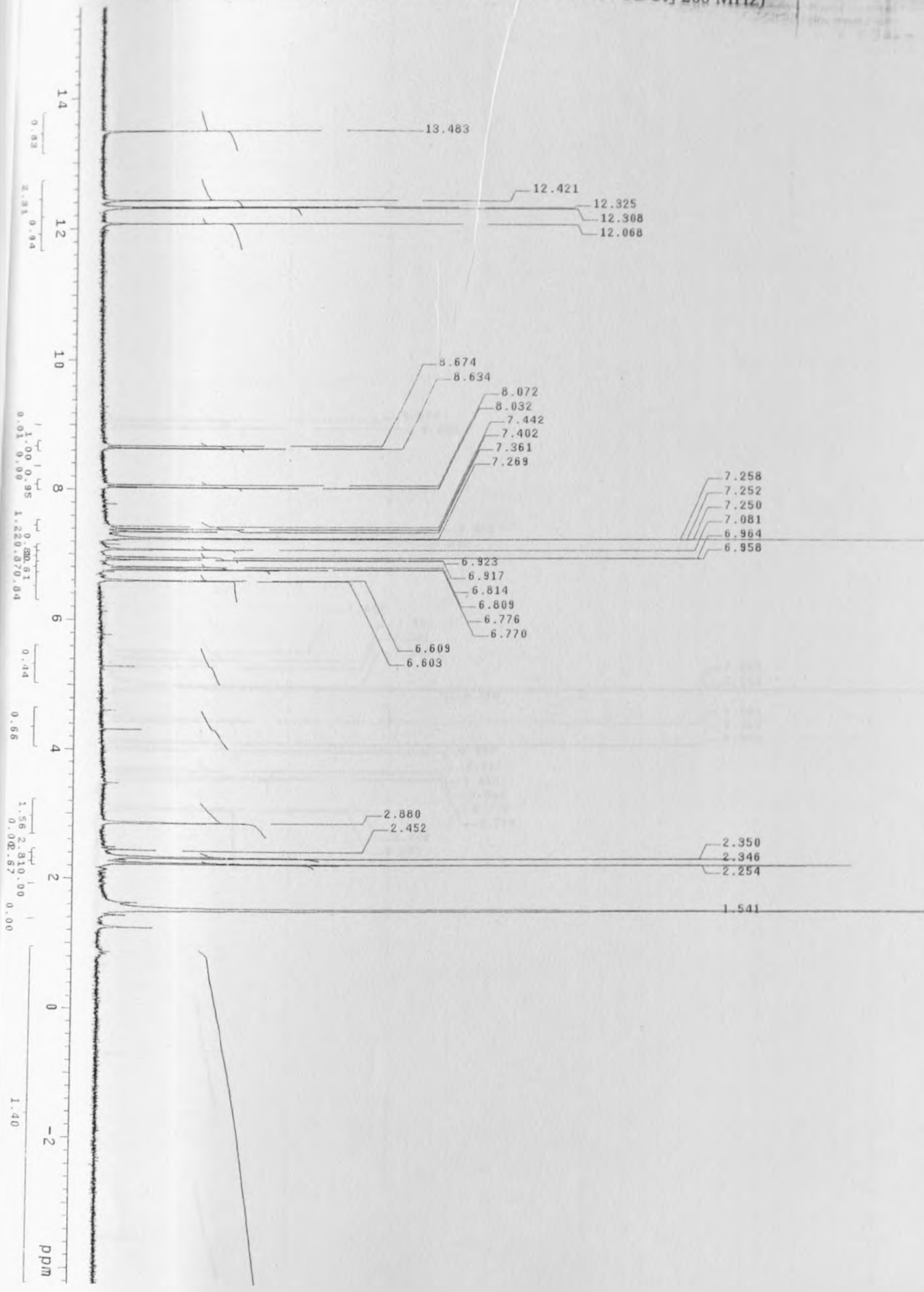


NOESY SPECTRUM FOR COMPOUND **8** (SOLVENT: ACETONE-$d_6$ 500 MHz)

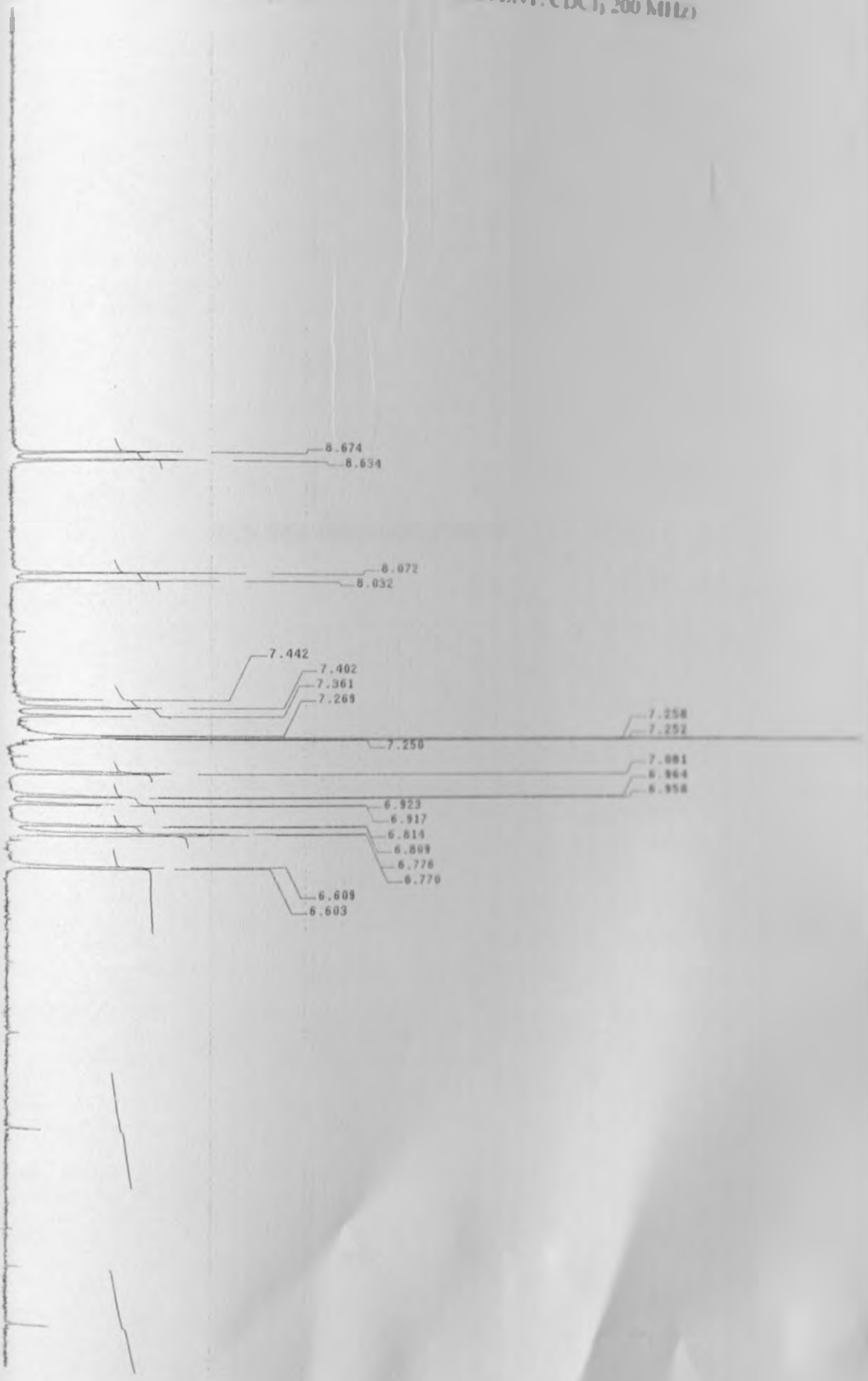


SPECTRA FOR COMPOUND **9**

$^1$HNMR SPECTRUM FOR COMPOUND **9** (SOLVENT: $CDCl_3$ 200 MHz)

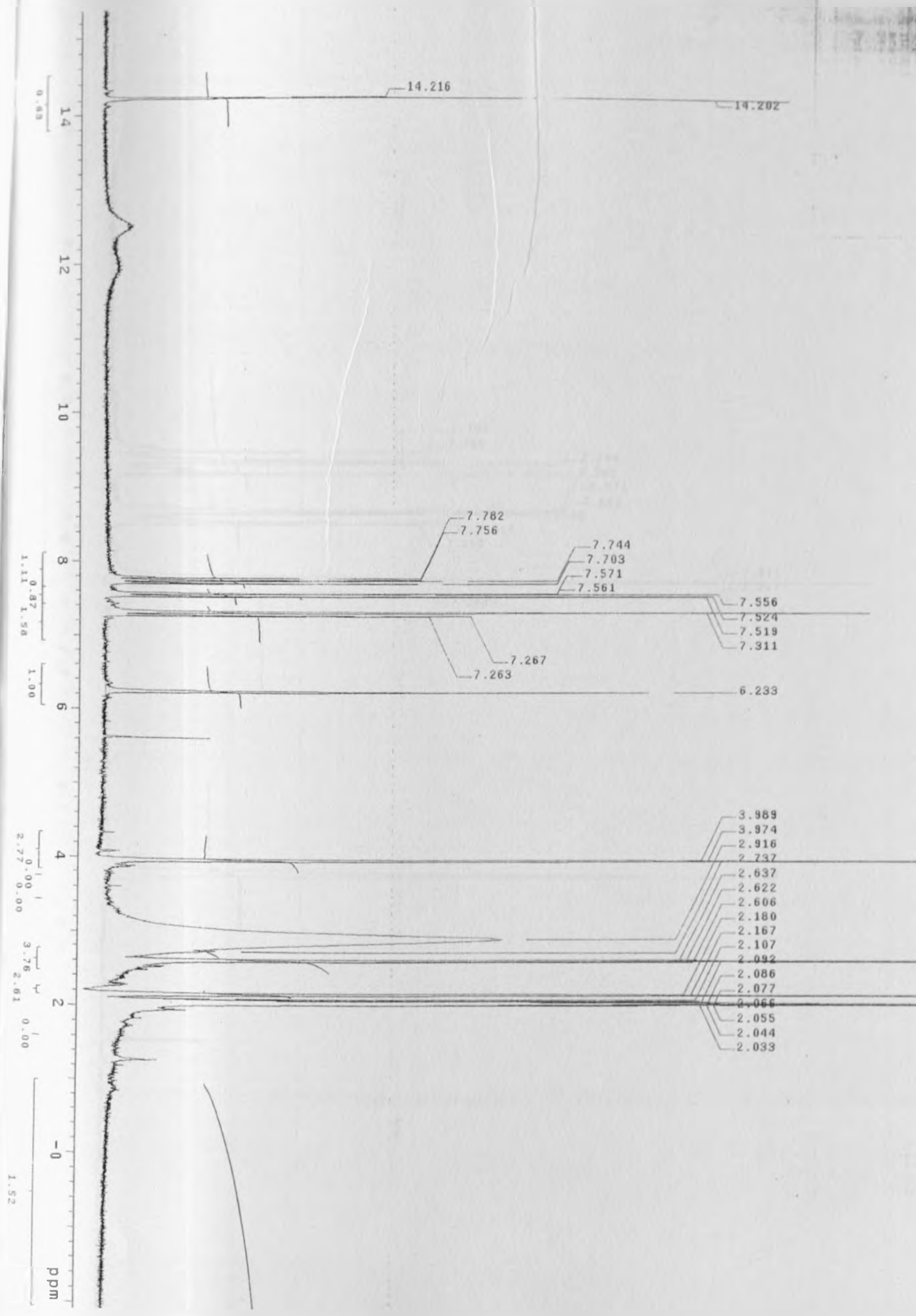


$^1$H NMR SPECTRUM FOR COMPOUND I (SOLVENT: CDCl$_3$, 200 MHz)

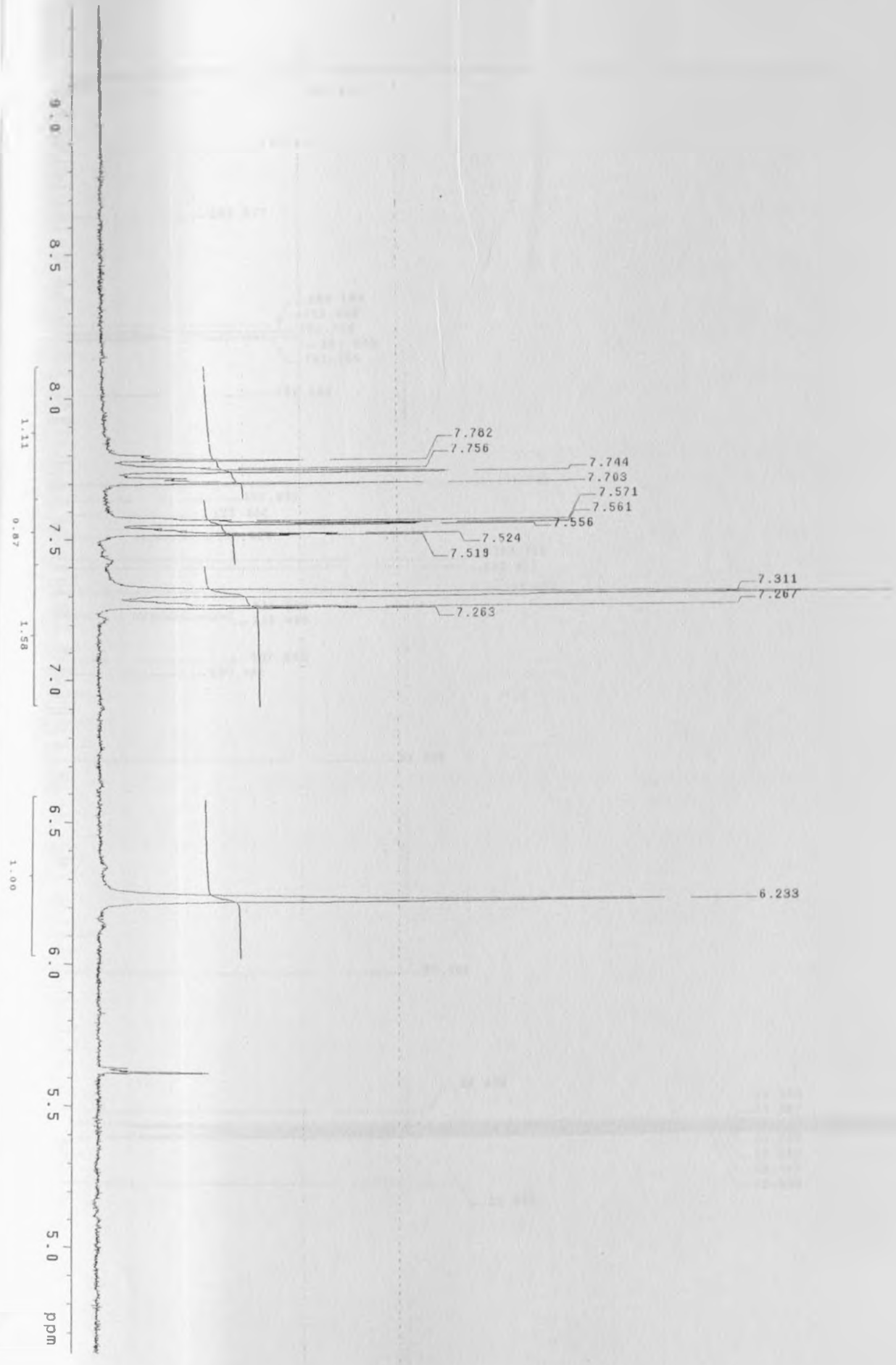


SPECTRA FOR COMPOUND **10**

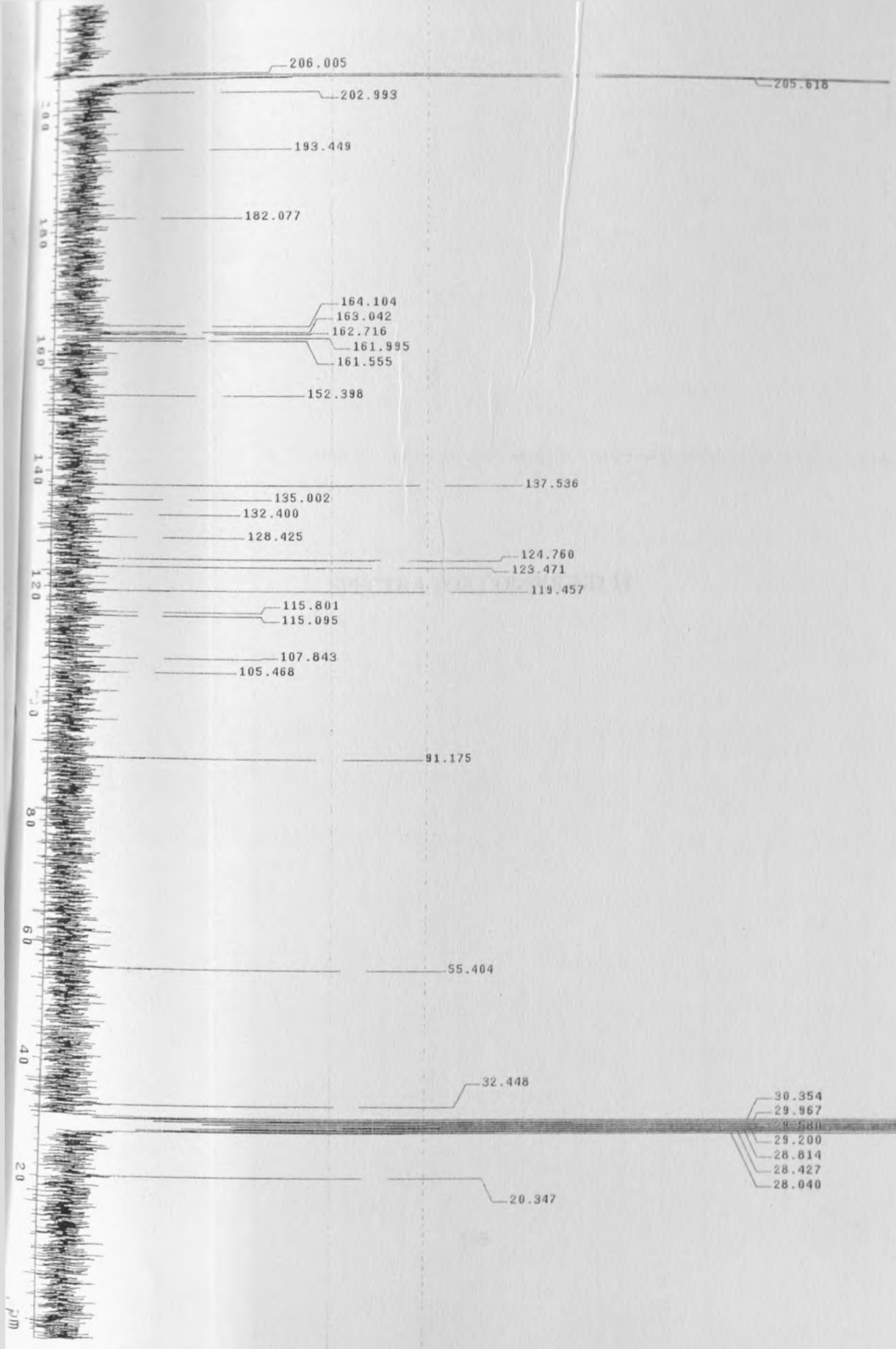
$^1$H NMR SPECTRUM FOR COMPOUND **10** (SOLVENT: ACETONE-$d_6$ 200 MHz)



# $^1$H NMR SPECTRUM FOR COMPOUND 10 (SOLVENT: ACETONE-$d_6$ 200 MHz)

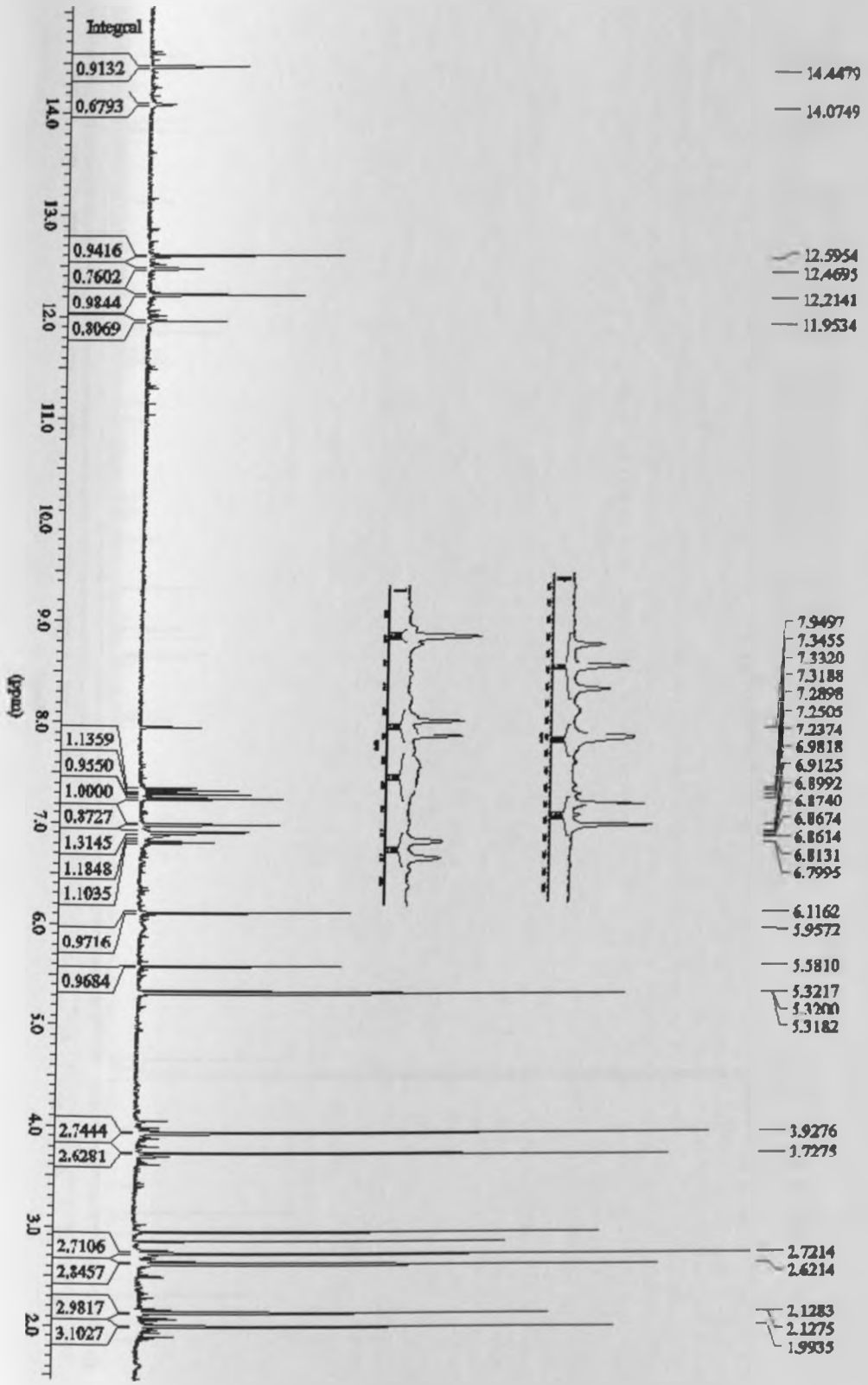


$^{13}C$ NMR SPECTRUM FOR COMPOUND **10** (SOLVENT: ACETONE-$d_6$ 50 MHz)

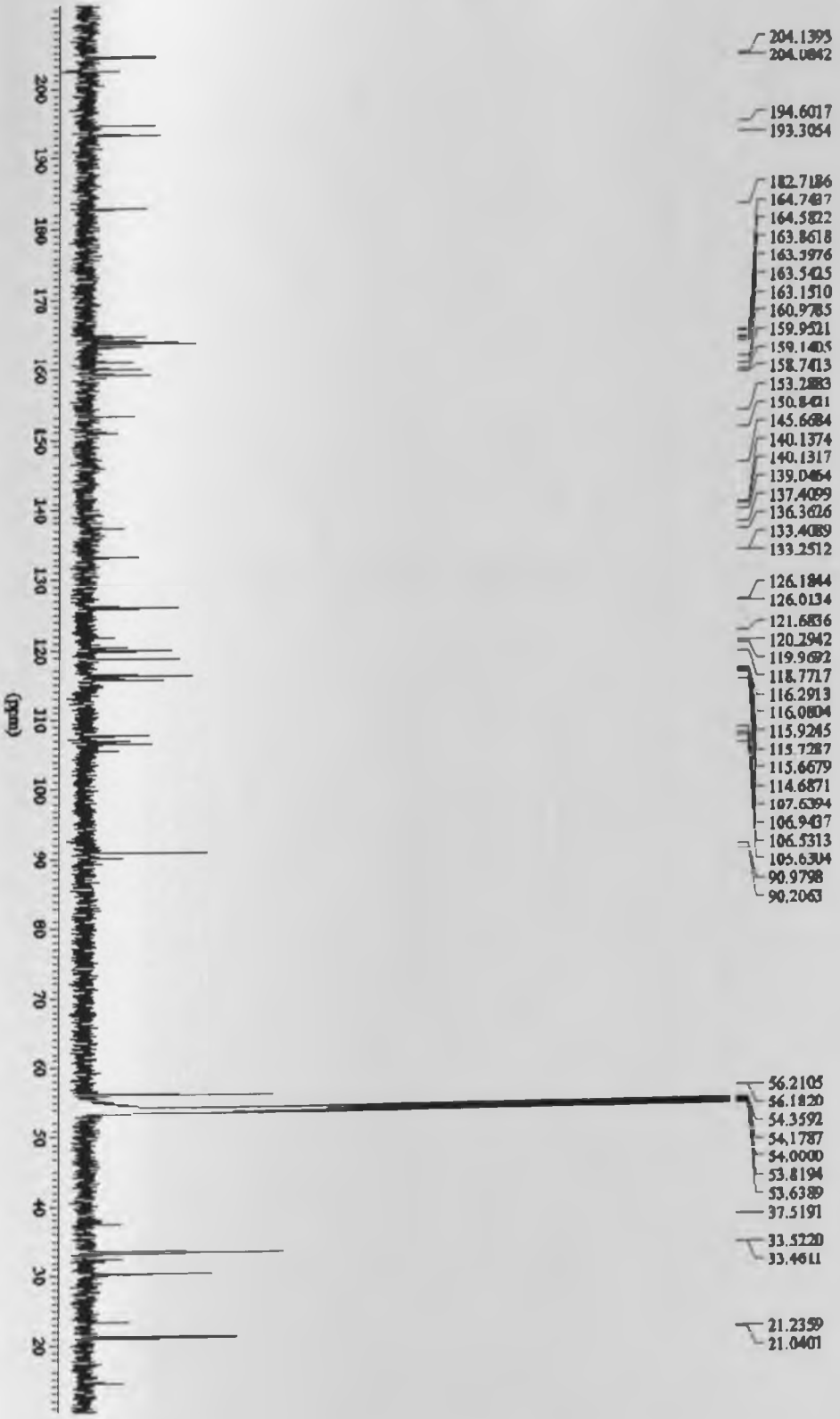


SPECTRA FOR COMPOUND **11**

$^{1}$H NMR SPECTRUM FOR COMPOUND **11** (SOLVENT: $CDCl_3$ 500 MHz)

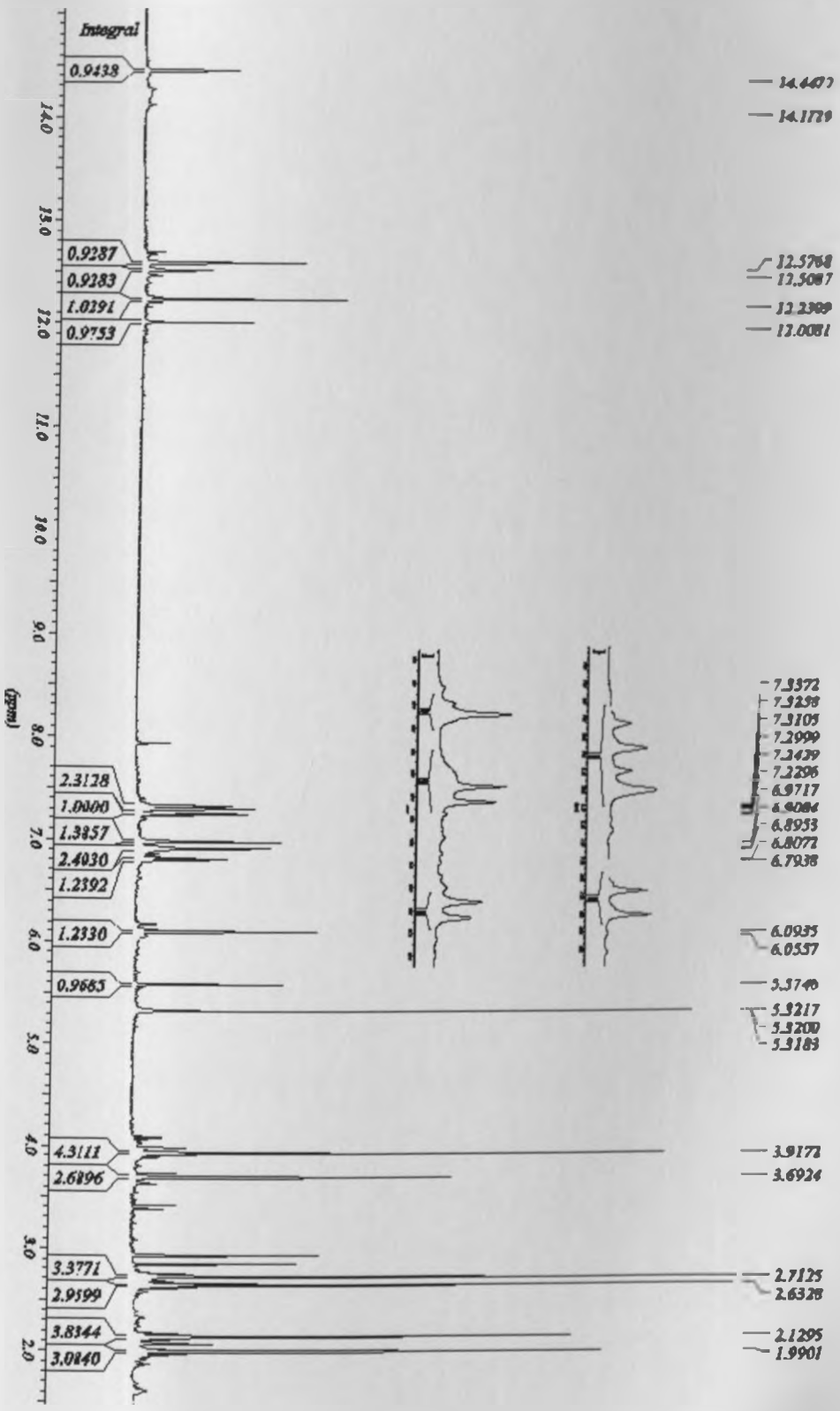


$^{13}C$ NMR SPECTRUM FOR COMPOUND **11** ($CDCl_3$ 125 MHZ)

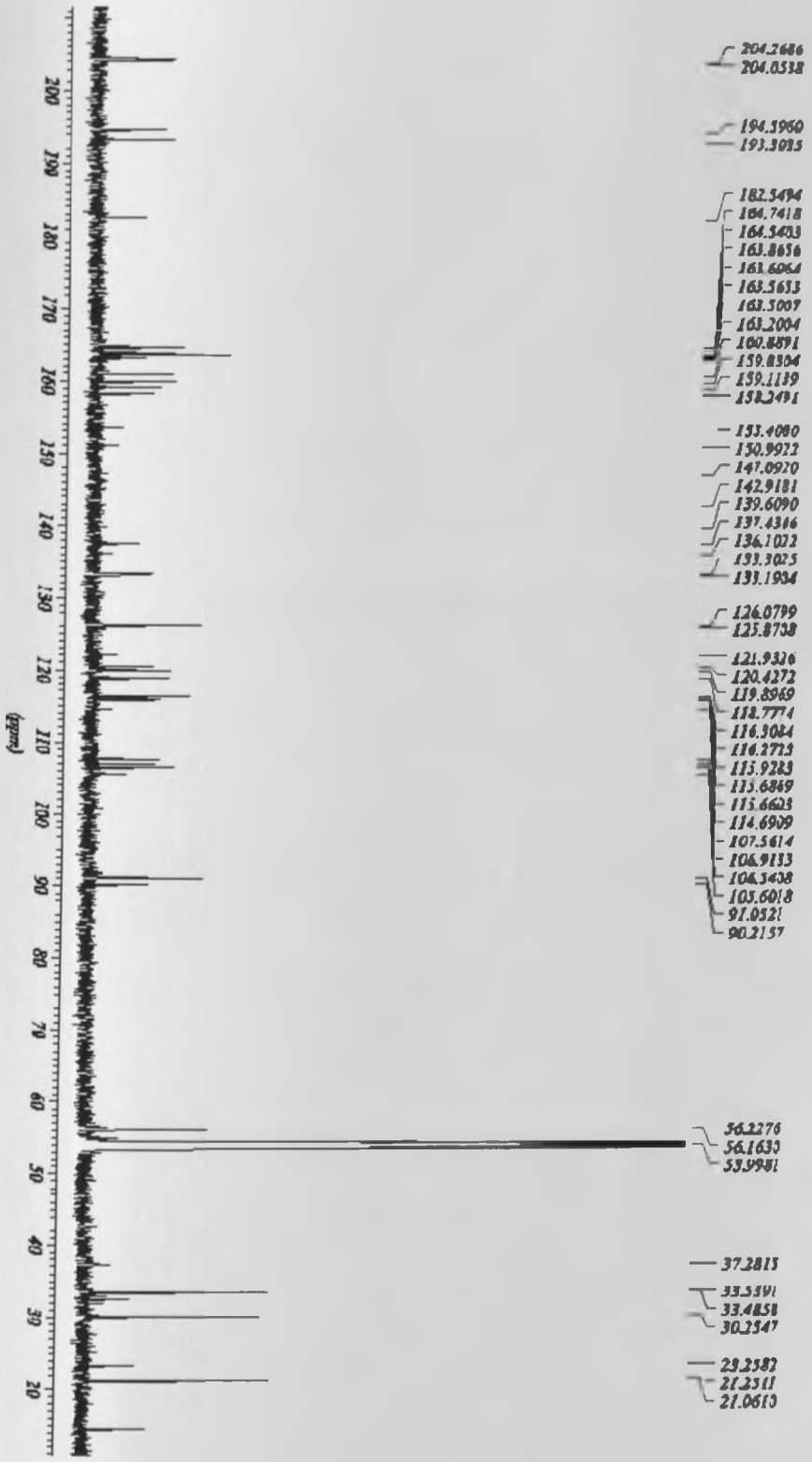


SPECTRA FOR COMPOUND **12**

$^{1}$H NMR SPECTUM FOR COMPOUND **12** (SOLVENT: $CDCl_3$ 500 MHz)

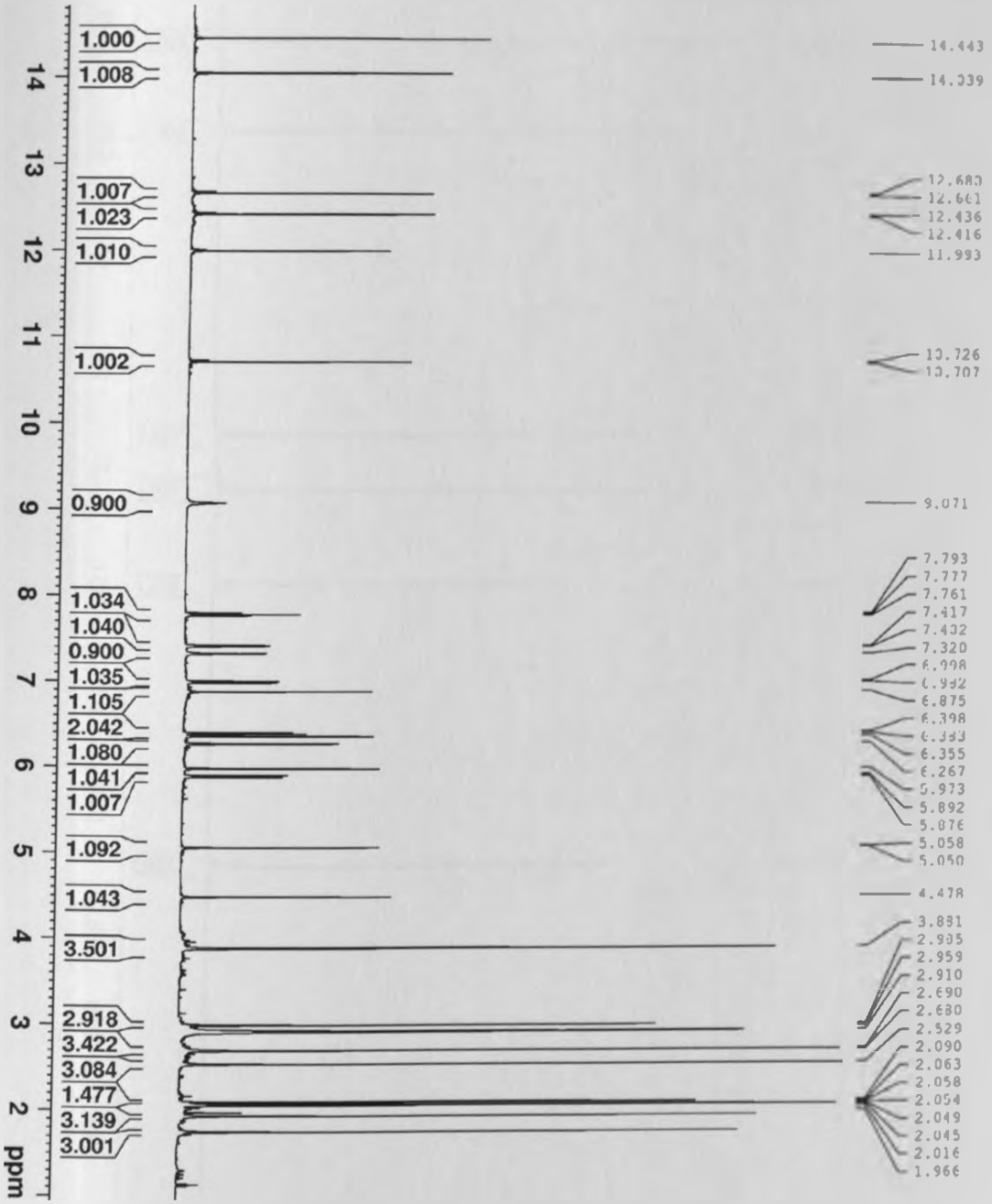


$^{13}$C NMR SPECTRUM FOR COMPOUND **12** (SOLVENT: $CDCl_3$ 125 MHz)

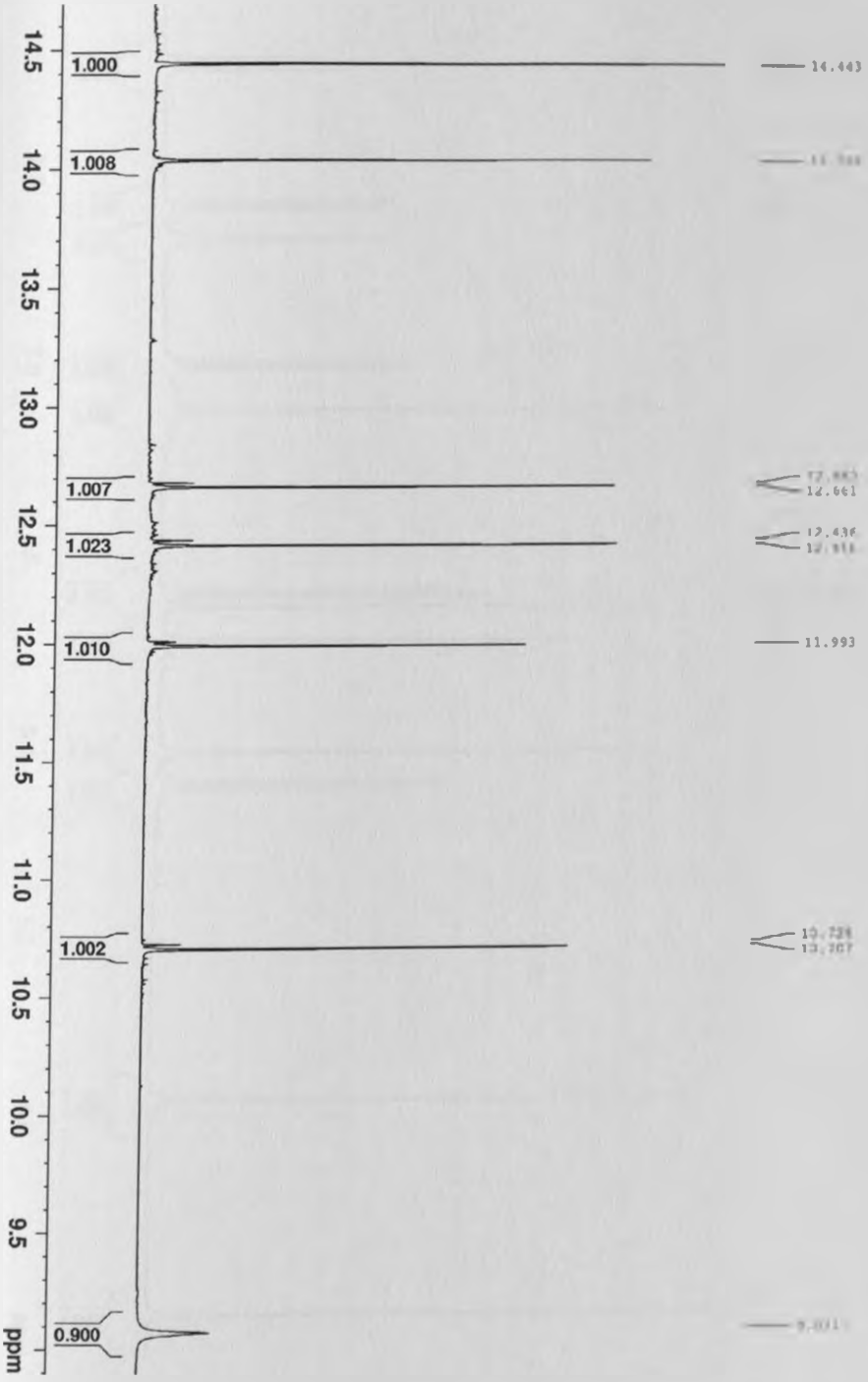


SPECTRA FOR COMPOUND **13**

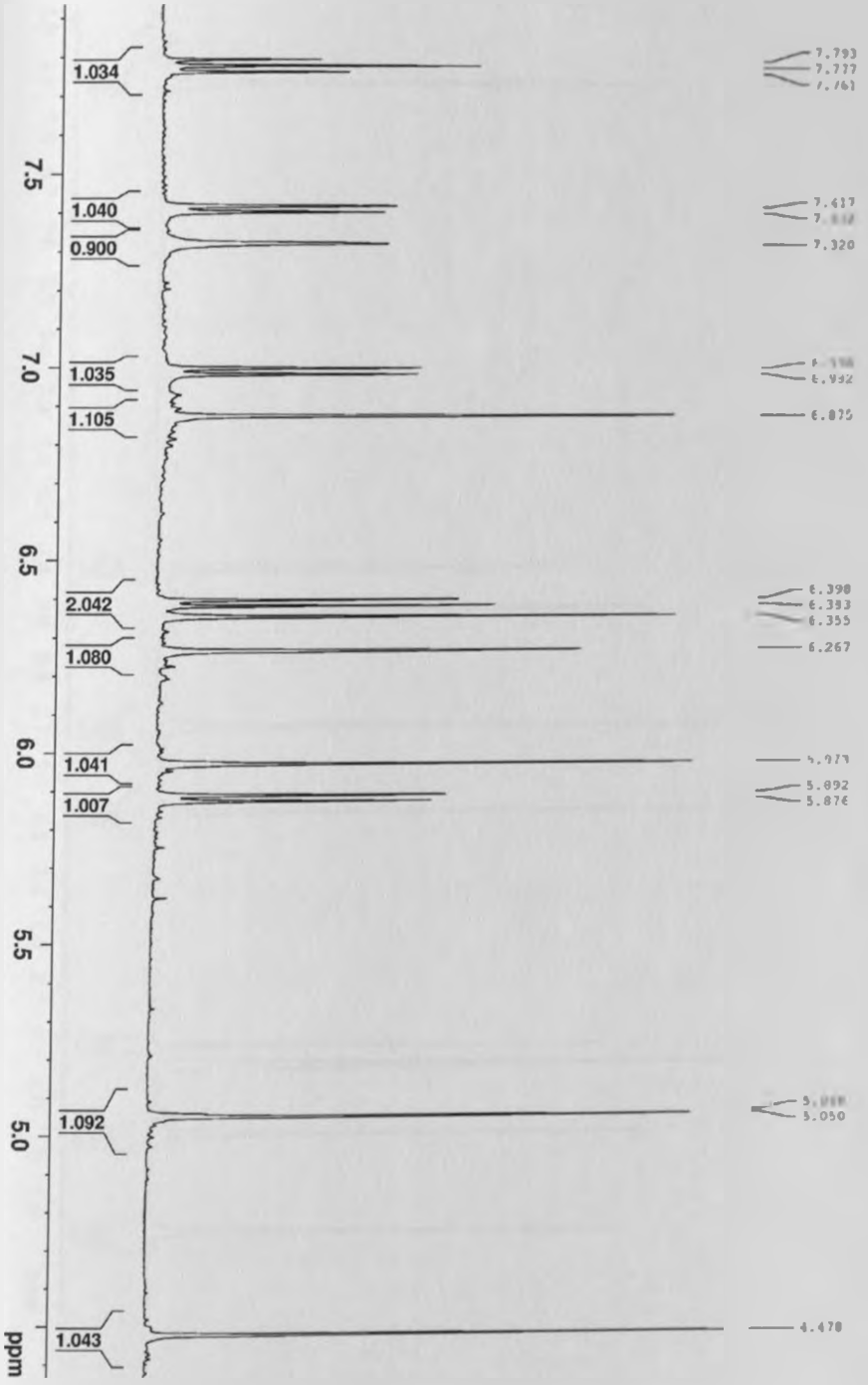
$^{1}$H NMR SPECTRUM FOR COMPOUND **13** (SOLVENT: ACETONE-$d_6$ 500 MHz)



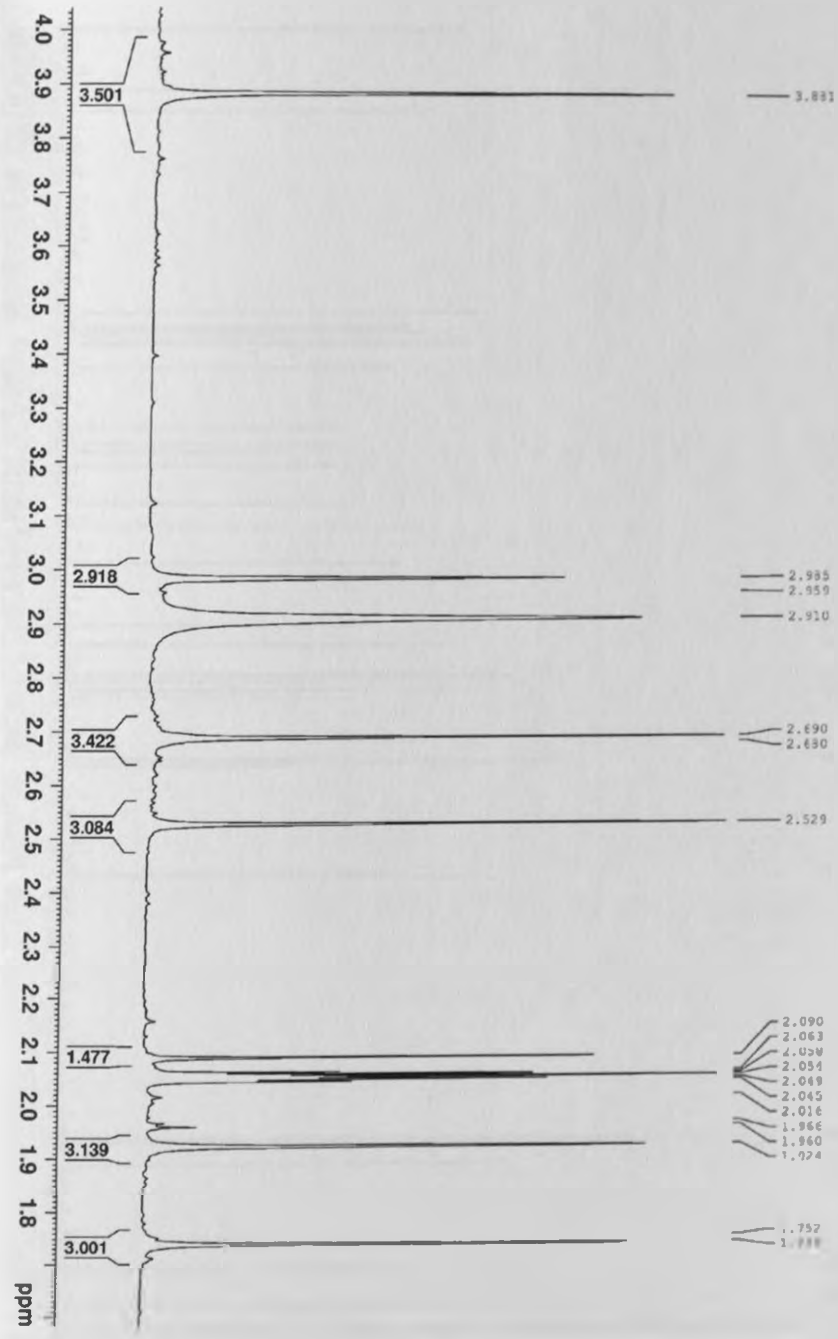
$^{1}$H NMR SPECTRUM FOR COMPOUND **13** (SOLVENT: ACETONE-$d_6$ 500 MHz)



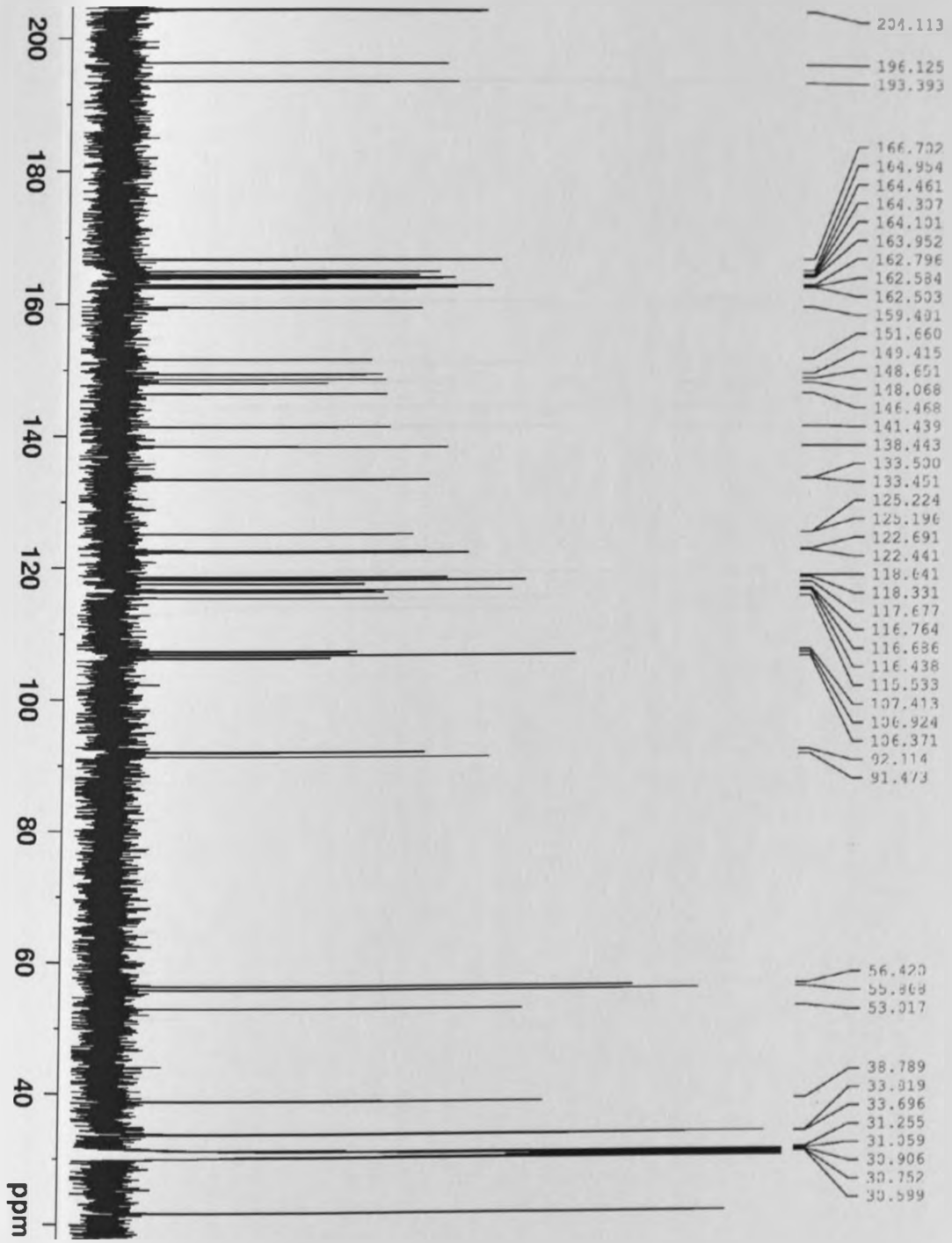
$^{1}$H NMR SPECTRUM FOR COMPOUND **13** (SOLVENT: ACETONE-$d_6$ 500 MHz)



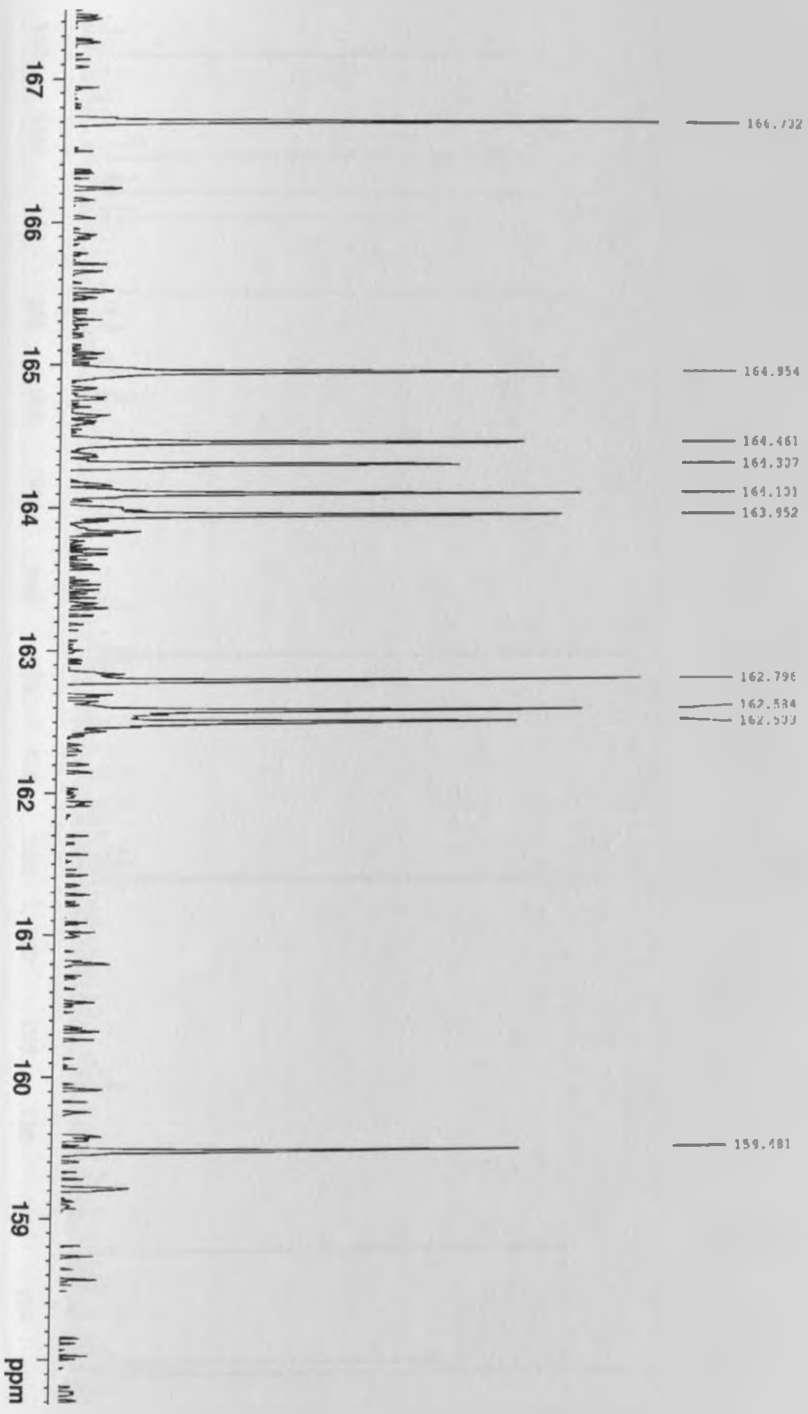
$^1$H NMR SPECTRUM FOR COMPOUND **13** (SOLVENT: ACETONE-$d_6$ 500 MHz)



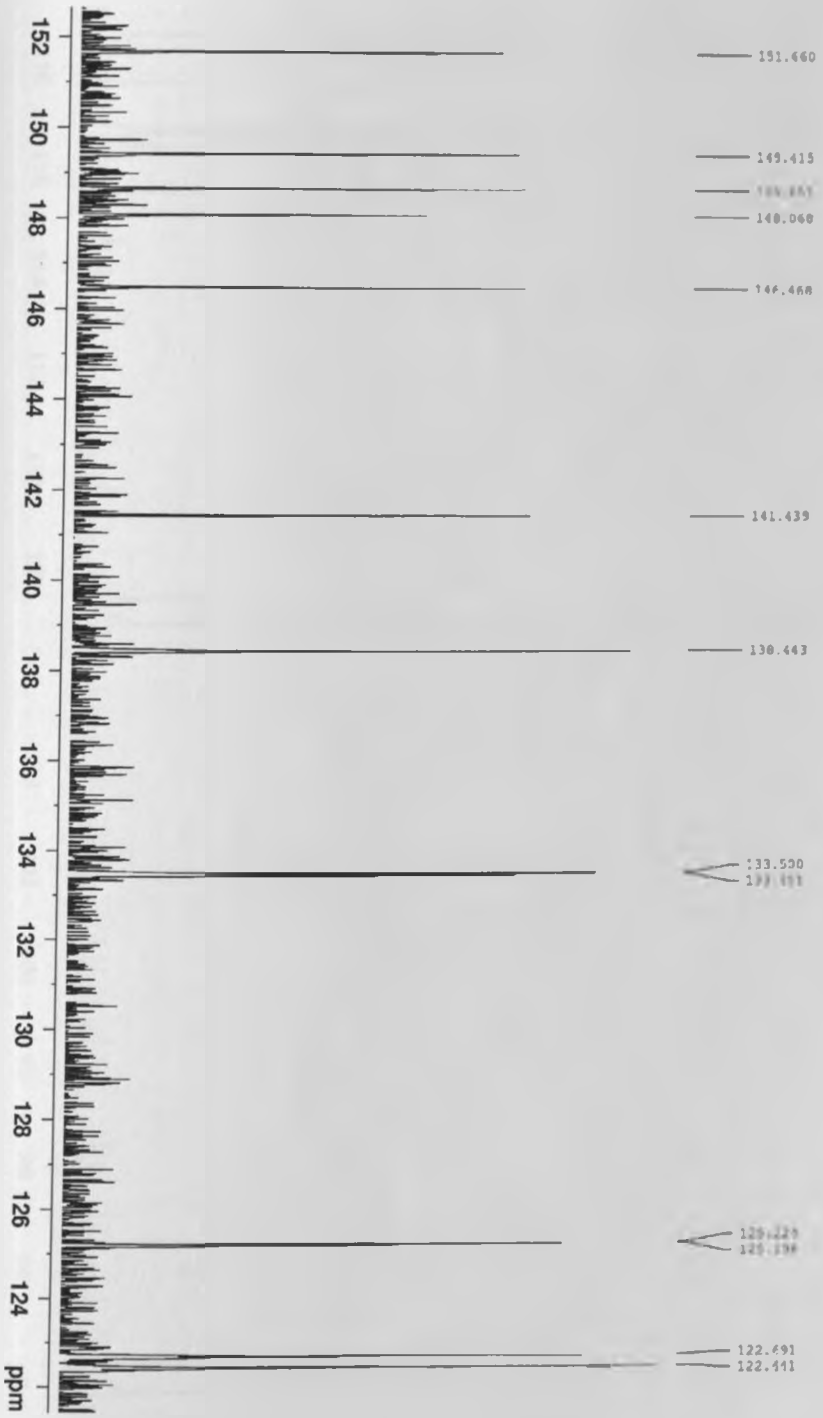
$^{13}$C NMR SPECTRUM FOR COMPOUND **13** (SOLVENT: ACETONE-$d_6$ 125 MHz)



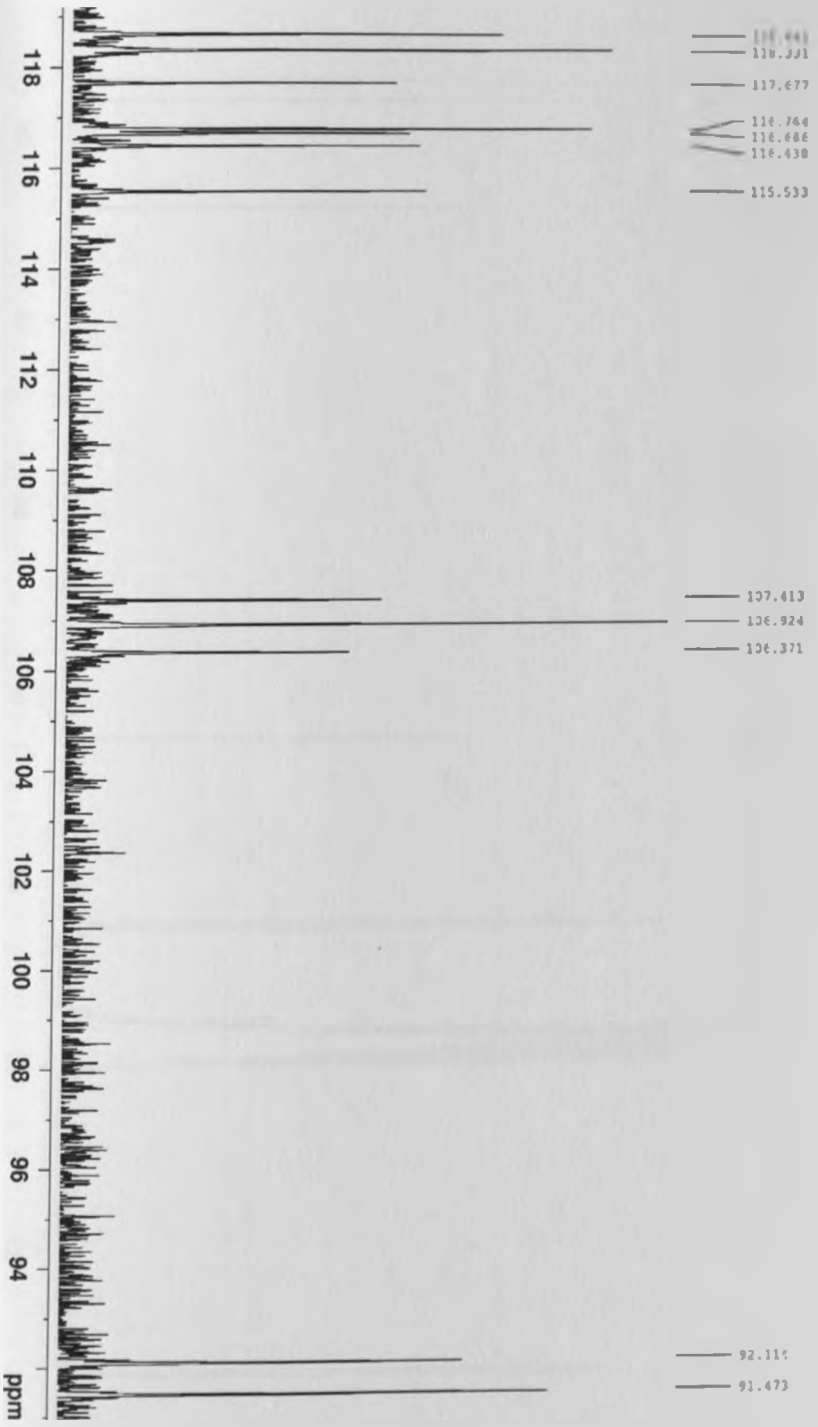
$^{13}C$ NMR SPECTRUM FOR COMPOUND **13** (SOLVENT: ACETONE-$d_6$ 125 MHz)



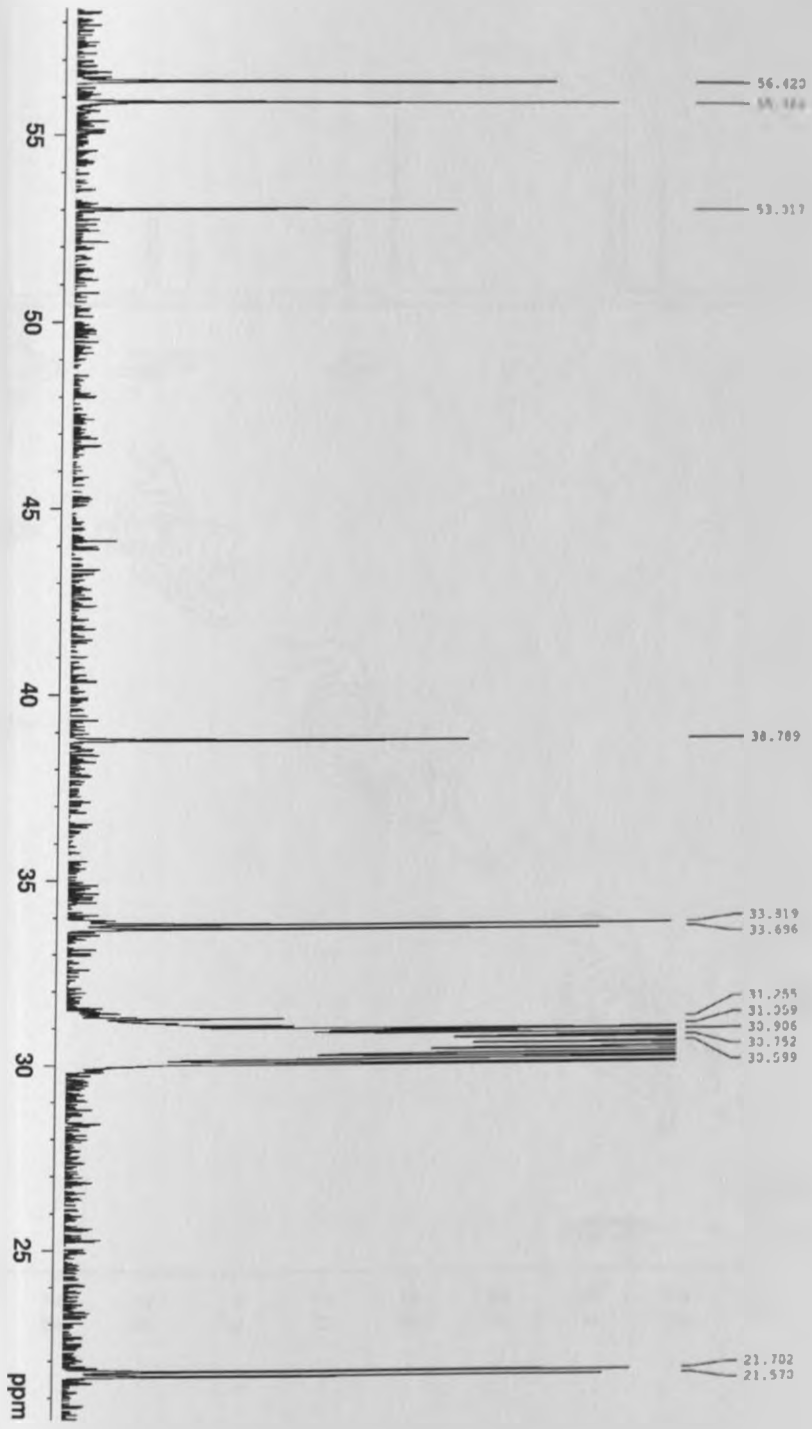
$^{13}$C NMR SPECTRUM FOR COMPOUND **13** (SOLVENT: ACETONE-$d_6$ 125 MHz)



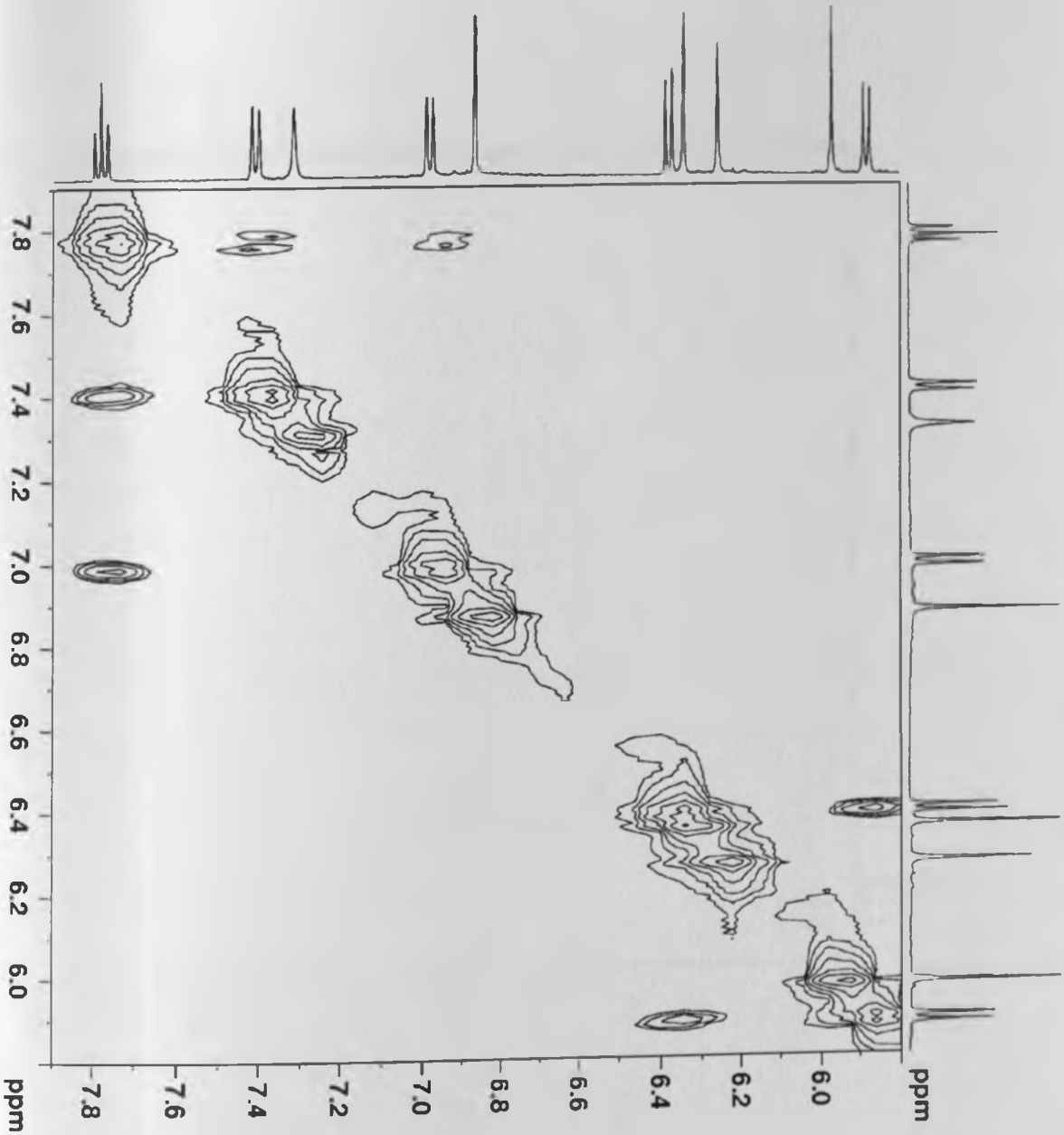
$^{13}$C NMR SPECTRUM FOR COMPOUND **13** (SOLVENT: ACETONE-$d_6$ 125 MHz)



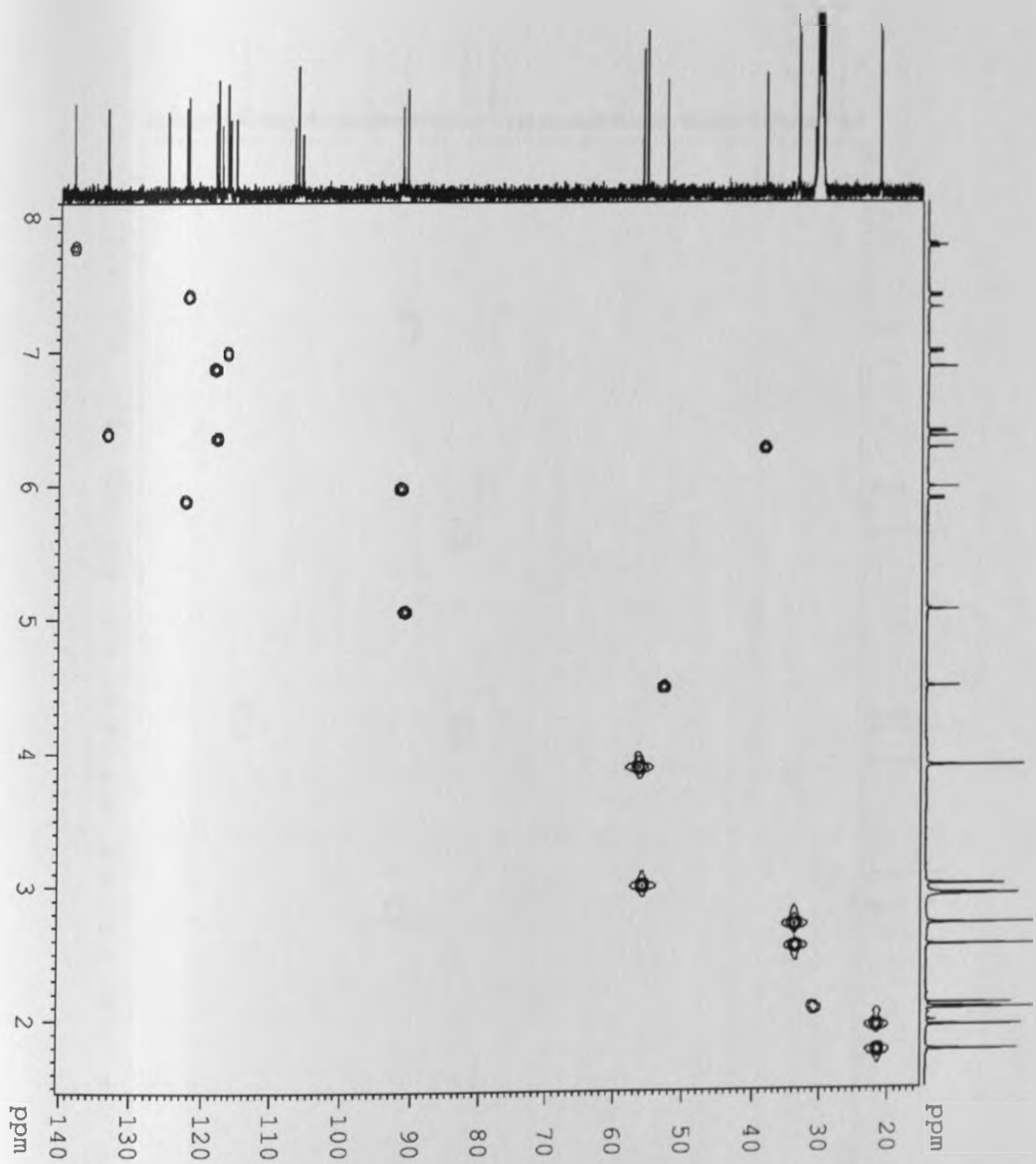
$^{13}$C NMR SPECTRUM FOR COMPOUND **13** (SOLVENT: ACETONE-$d_6$ 125 MHz)



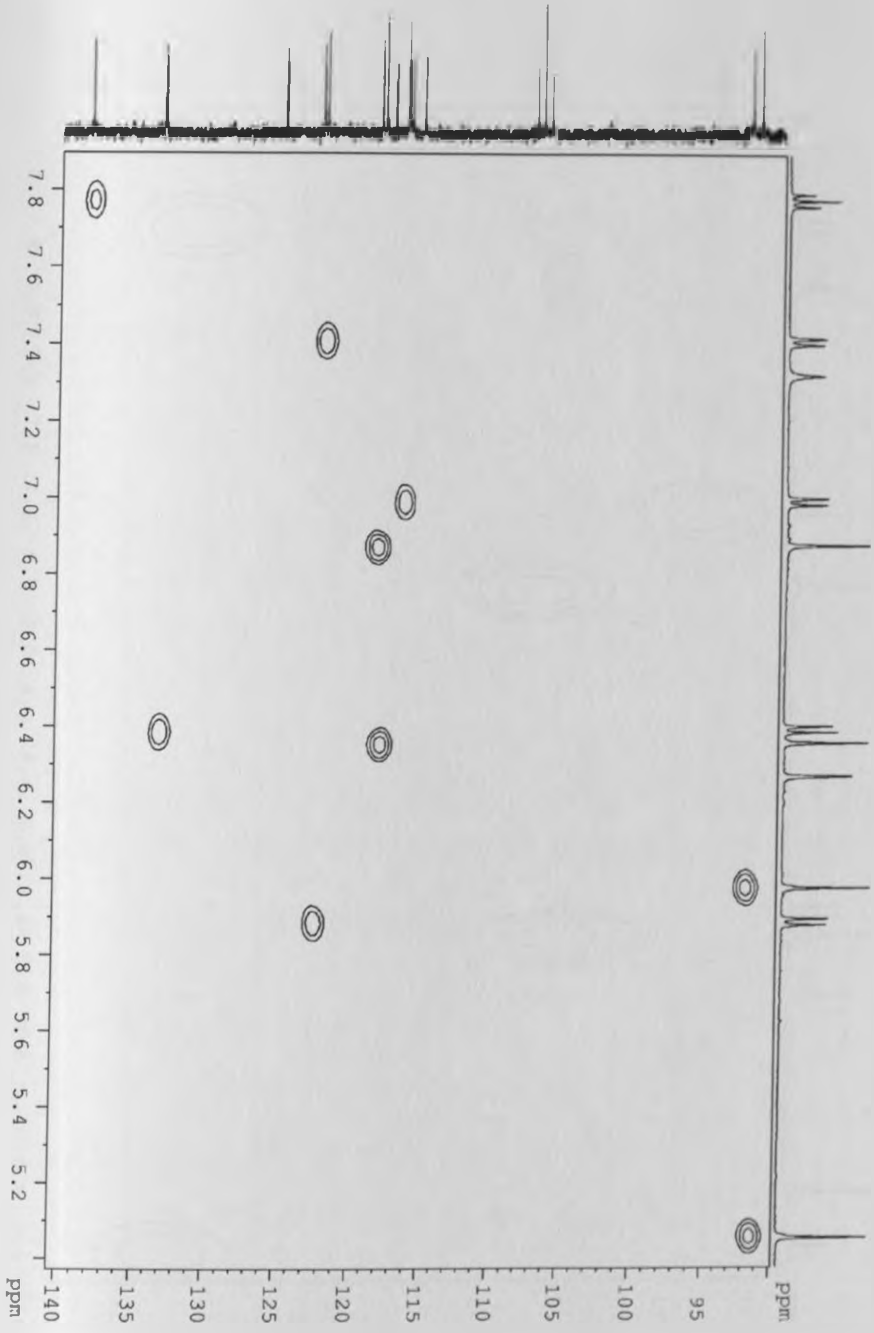
COSY SPECTRUM FOR COMPOUND **13**



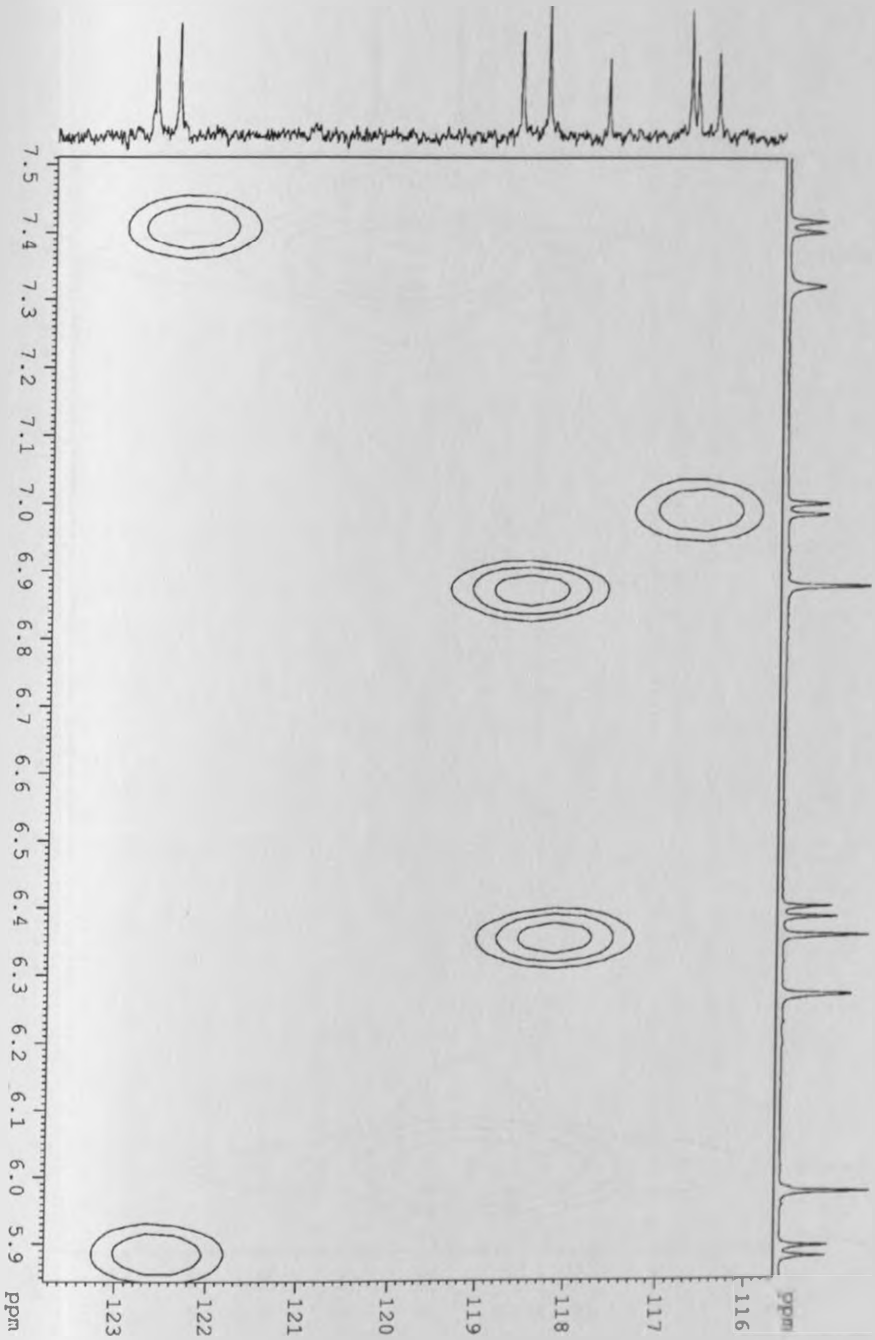
HMQC SPECTRUM FOR COMPOUND **13**



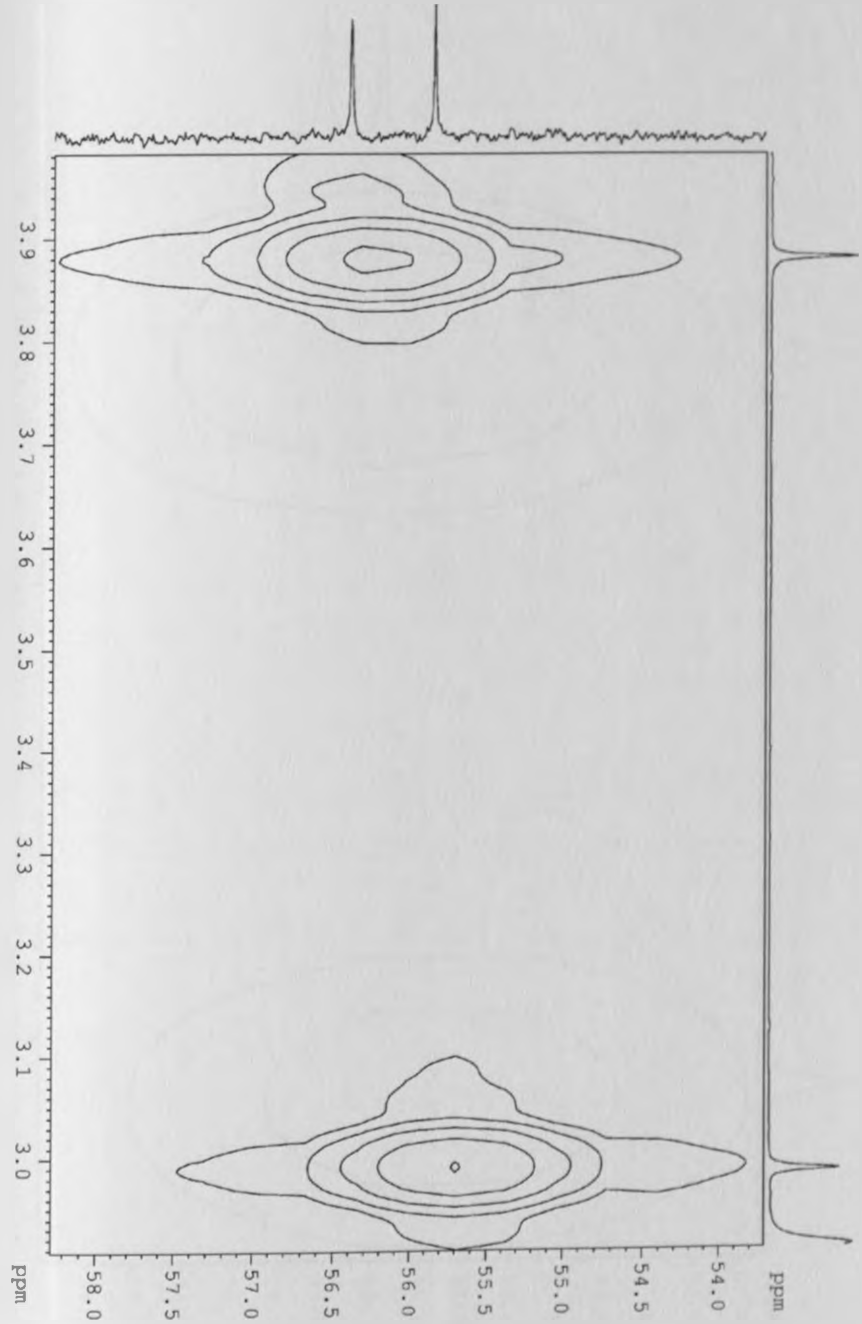
HMQC SPECTRUM FOR COMPOUND **13**



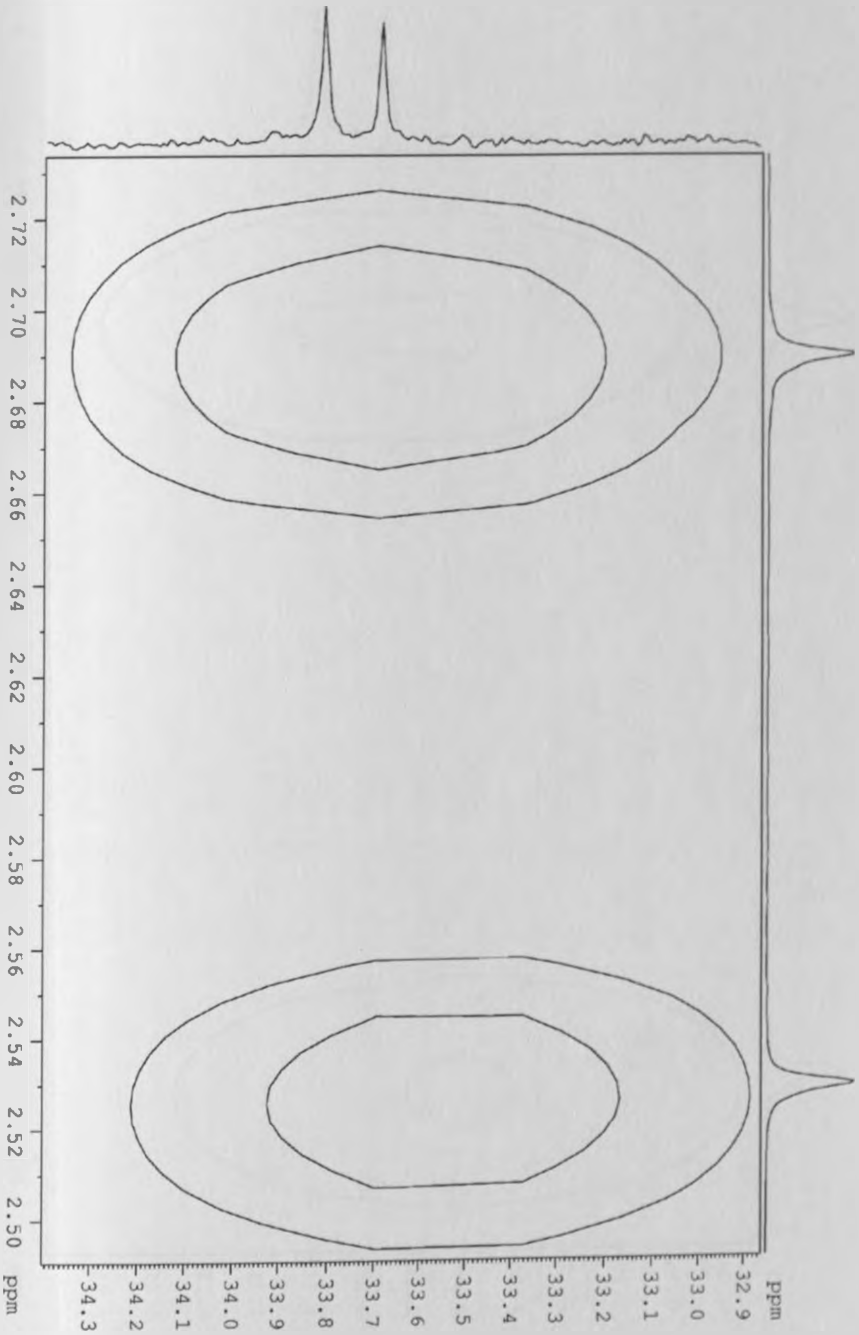
HMQC SPECTRUM FOR COMPOUND **13**



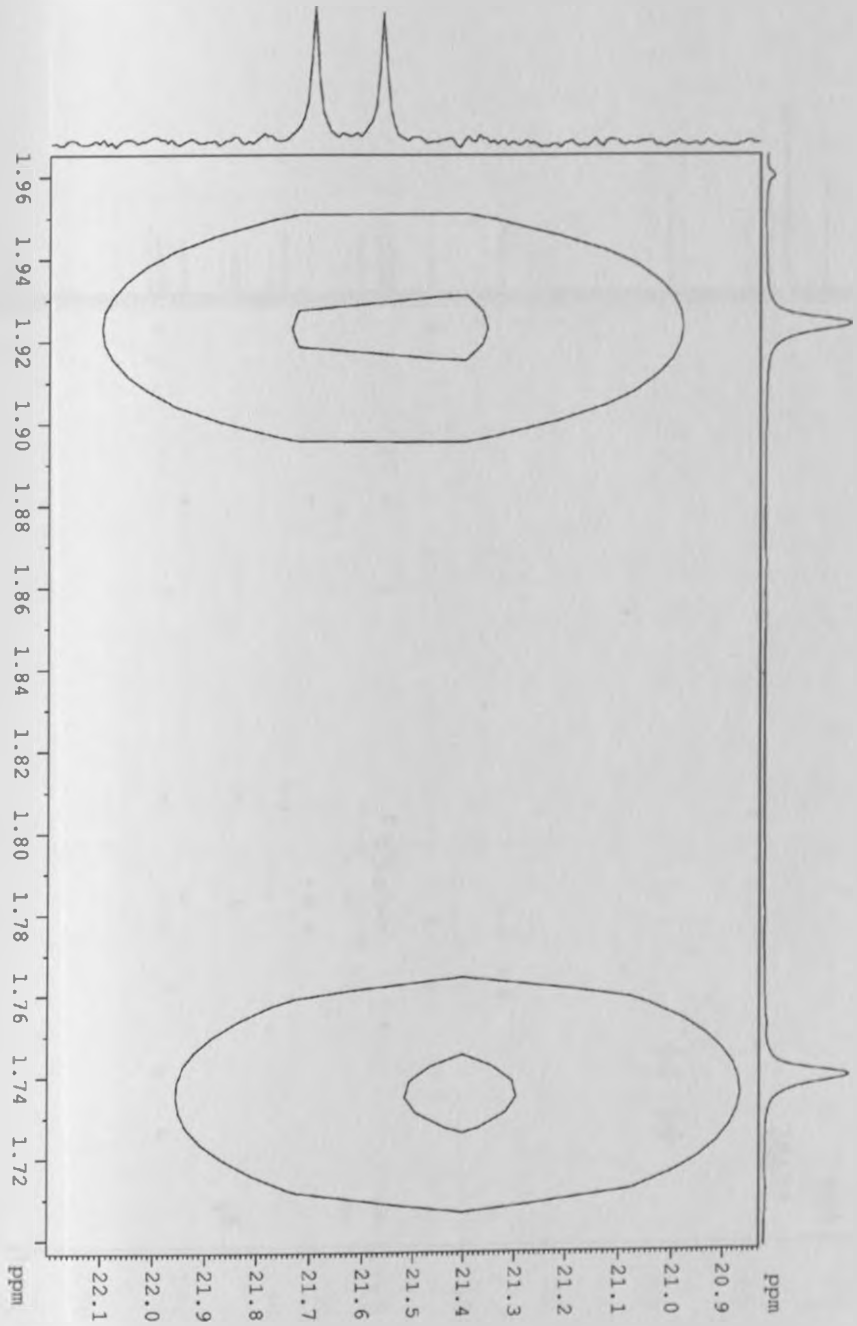
HMQC SPECTRUM FOR COMPOUND 13



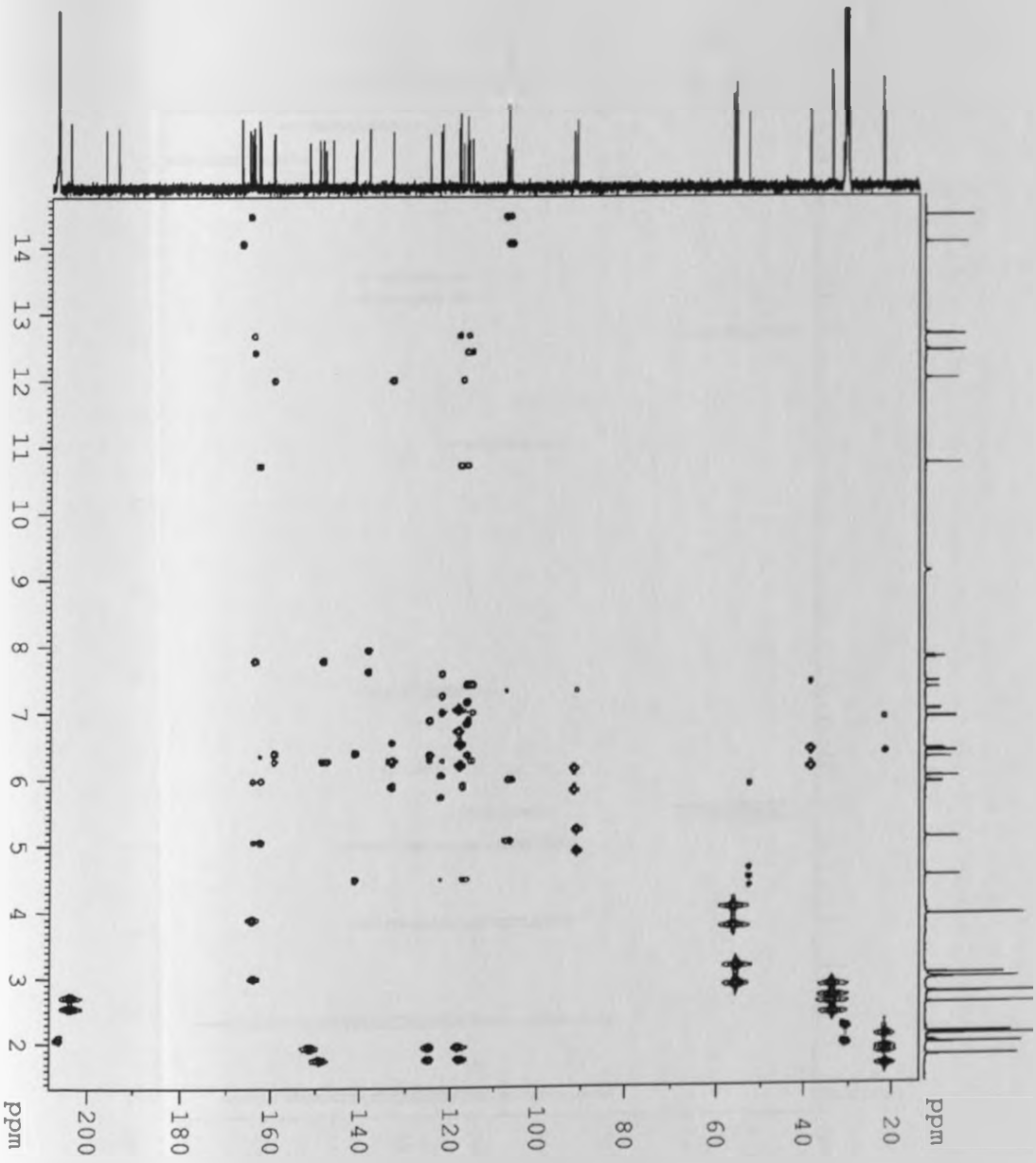
HMQC SPECTRUM FOR COMPOUND **13**



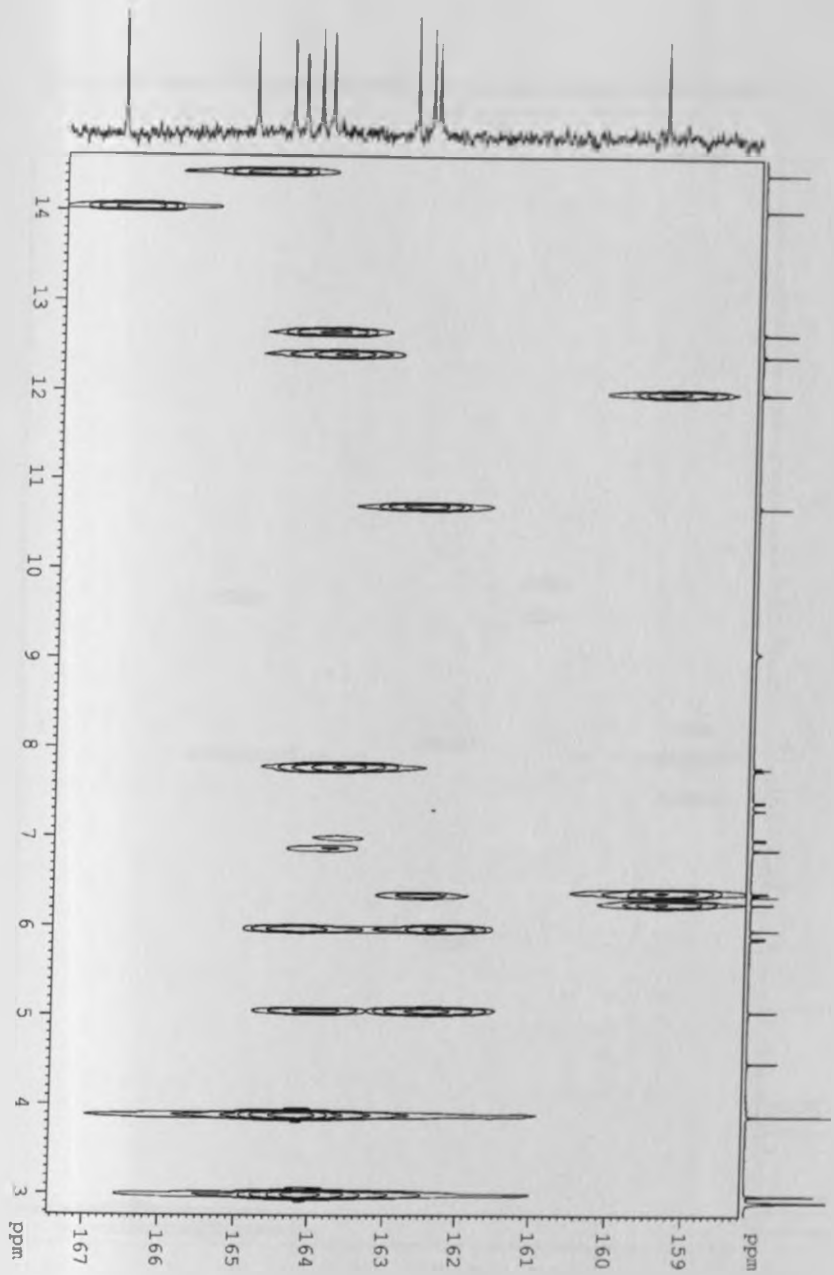
# HMQC SPECTRUM FOR COMPOUND **13**



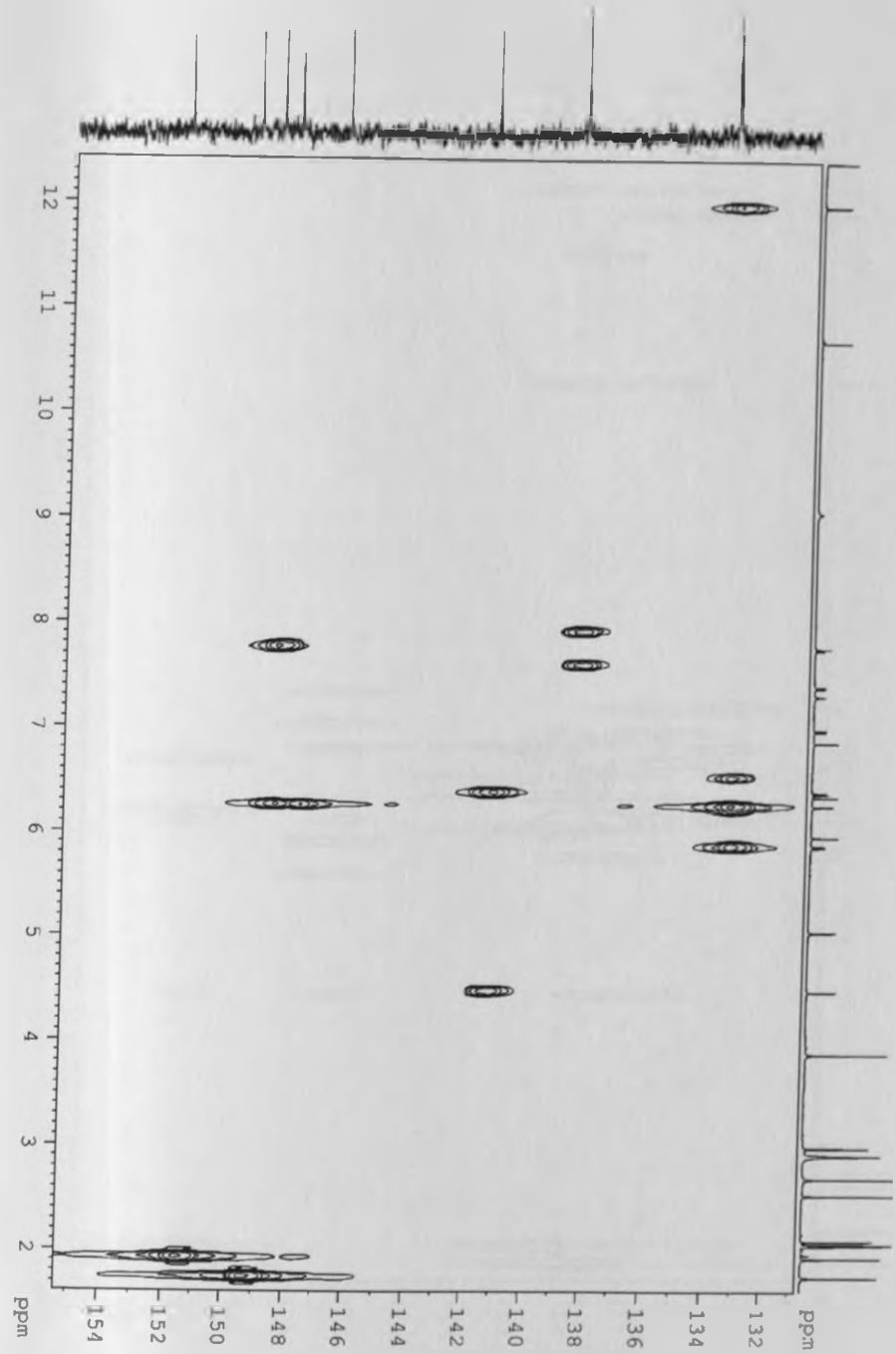
HMBC SPECTRUM FOR COMPOUND **13**



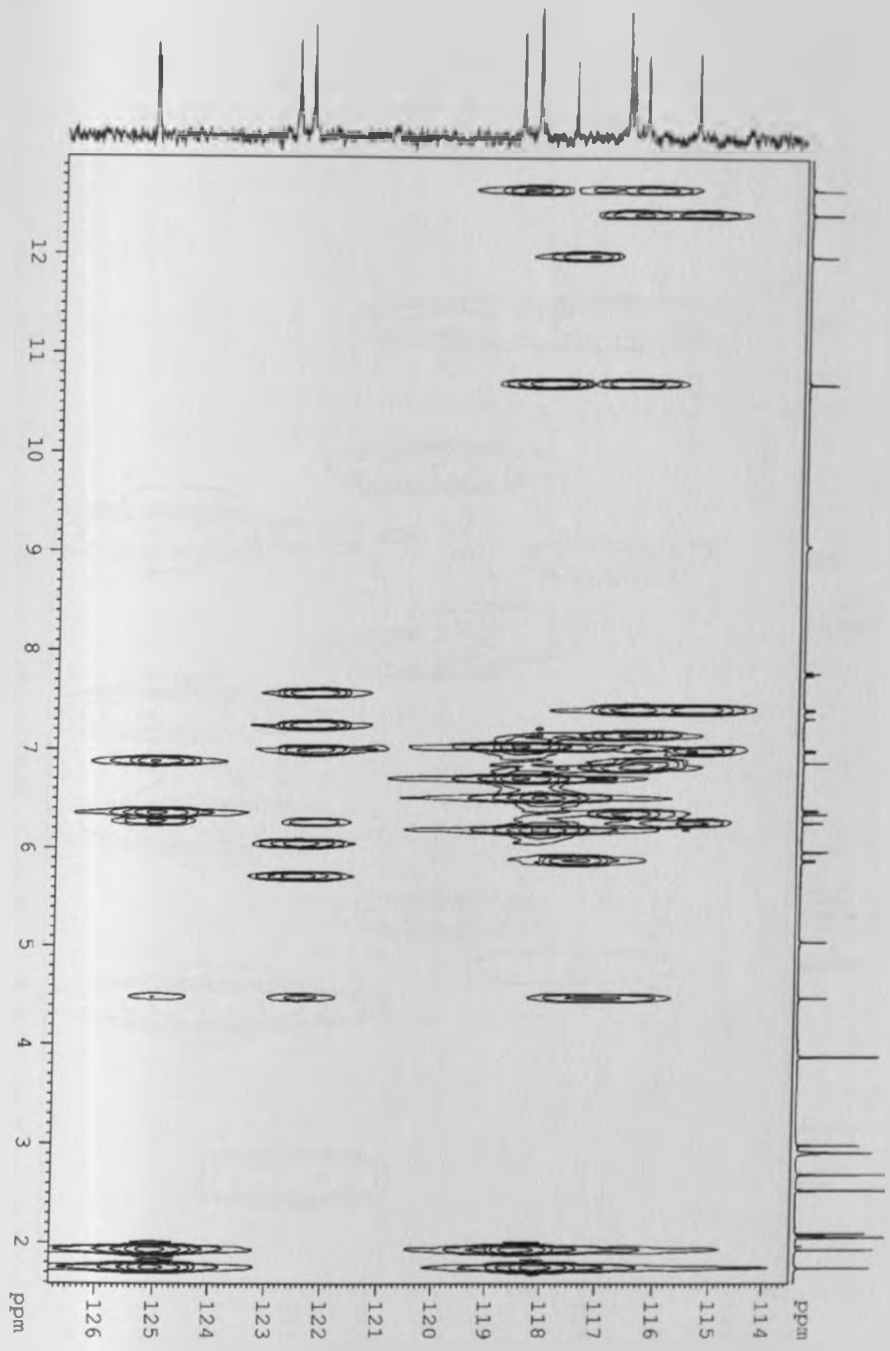
# HMBC SPECTRUM FOR COMPOUND 13



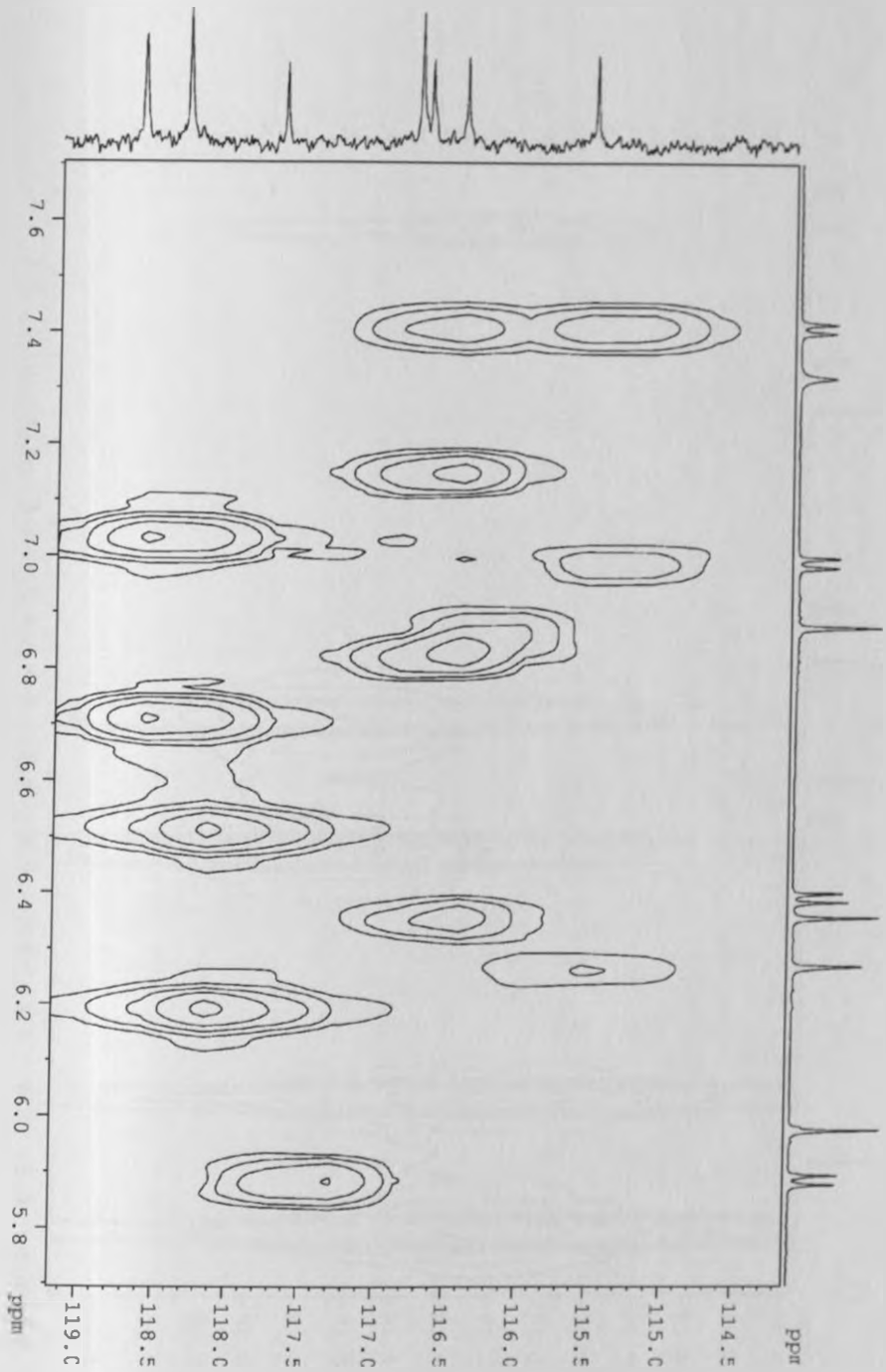
HMBC SPECTRUM FOR COMPOUND **13**



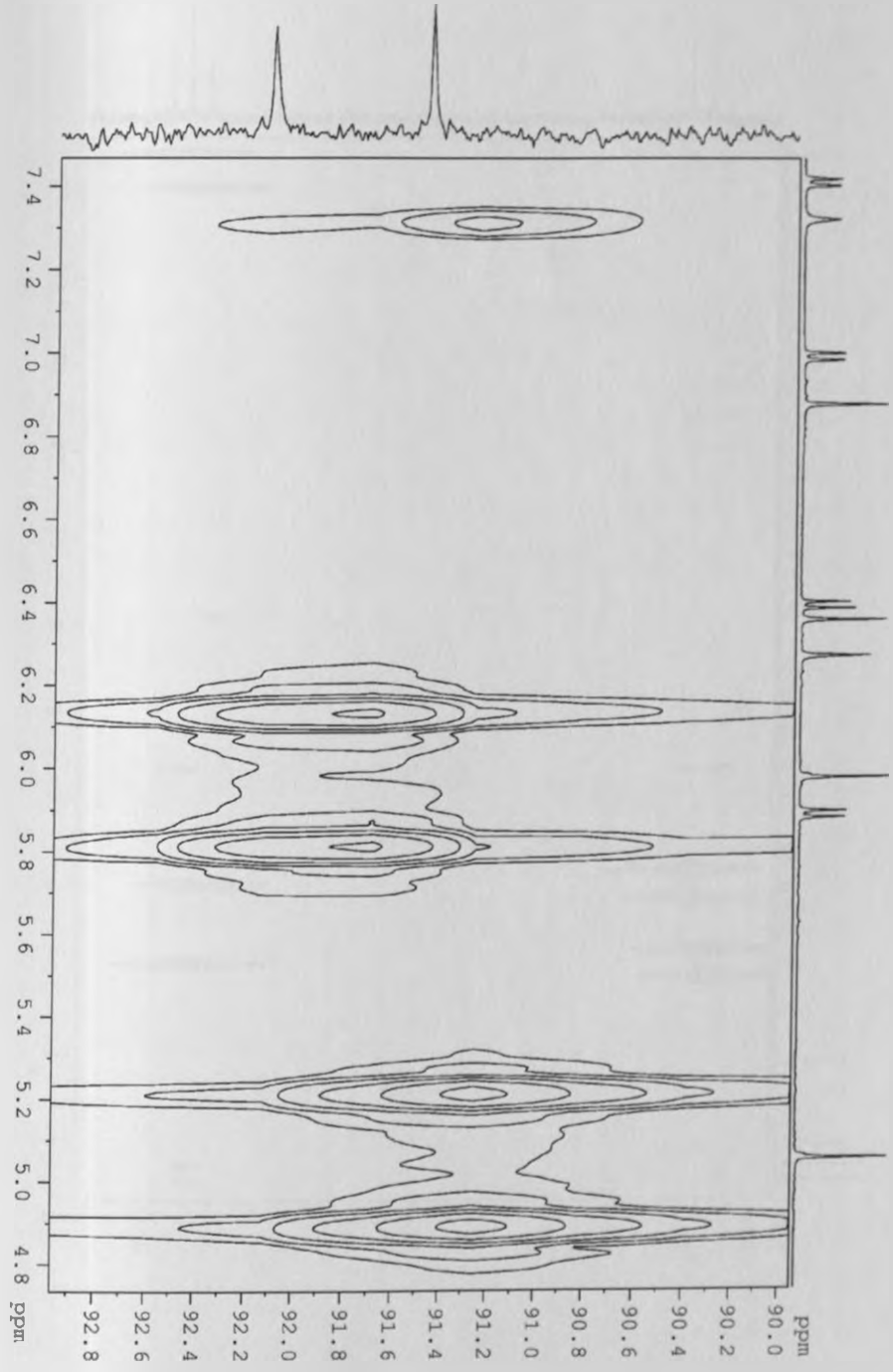
HMBC NMR SPECTRUM FOR COMPOUND **13**



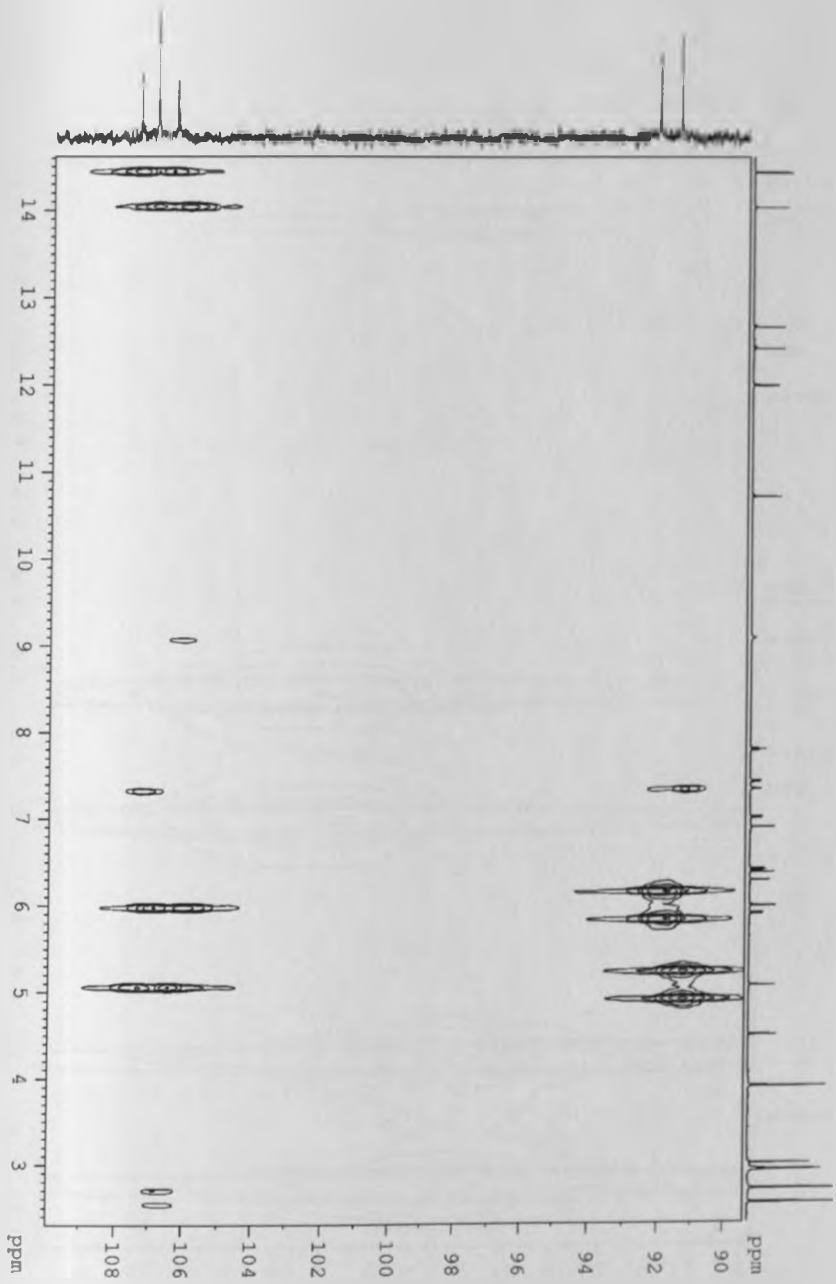
HMBC SPECTRUM FOR COMPOUND **13**



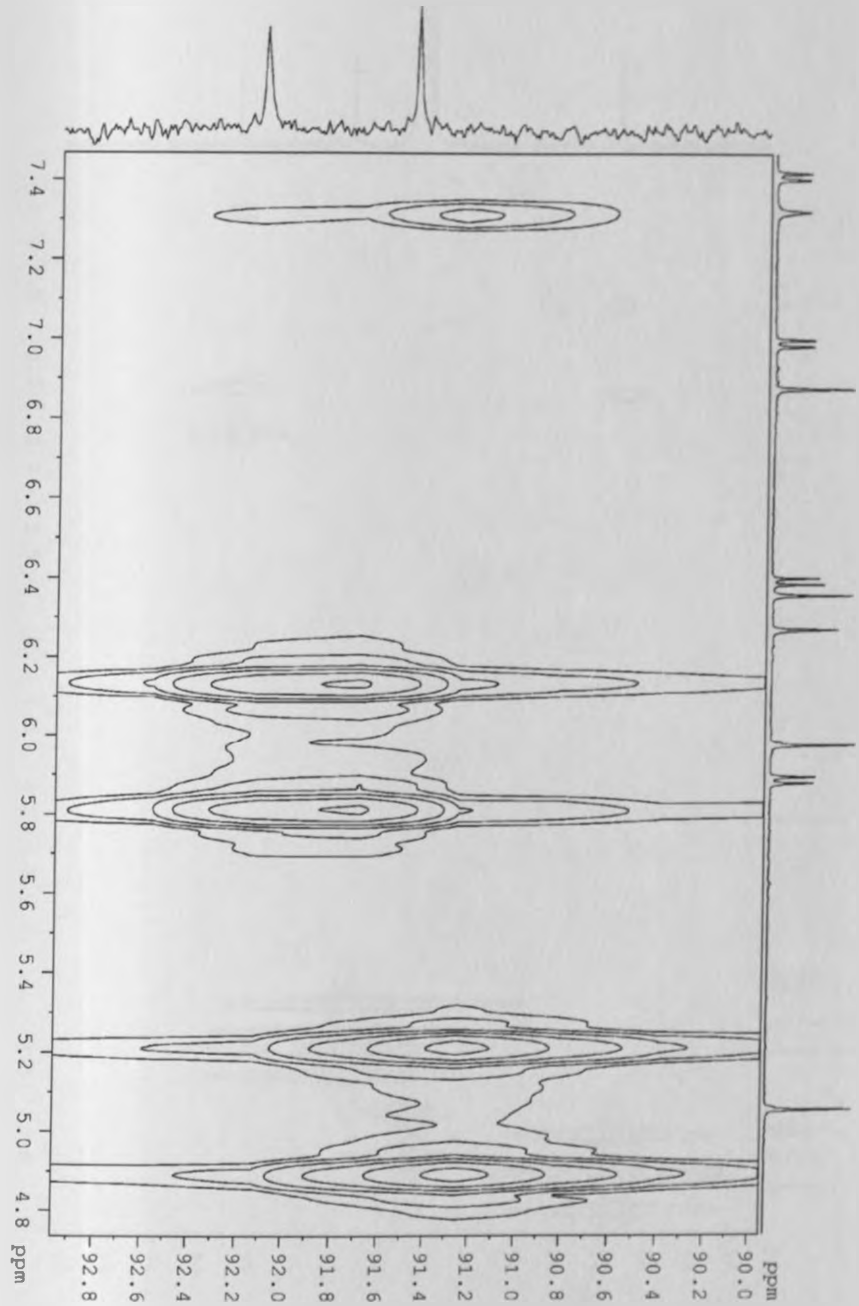
# HMBC SPECTRUM FOR COMPOUND **13**



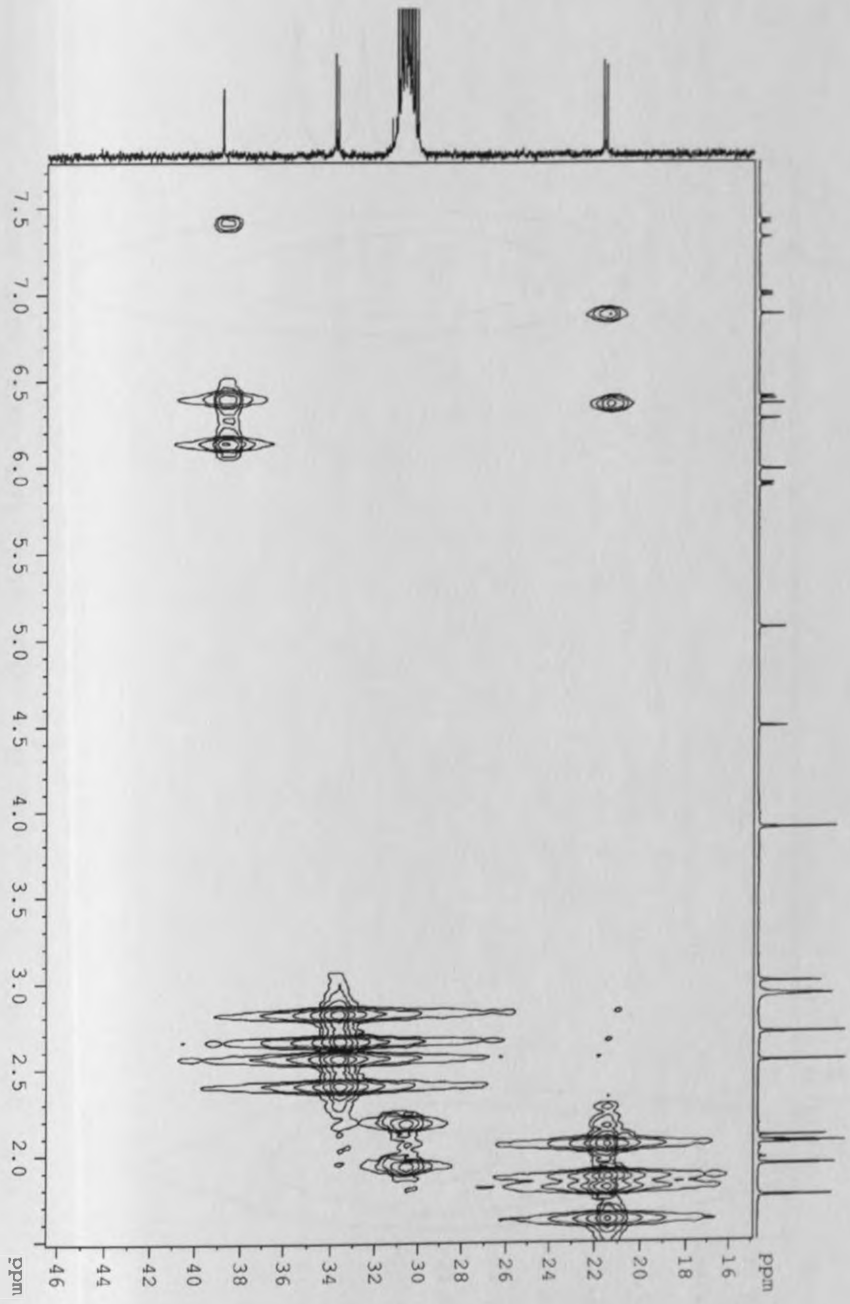
HMBC SPECTRUM FOR COMPOUND **13**



# HMBC SPECTRUM FOR COMPOUND **13**

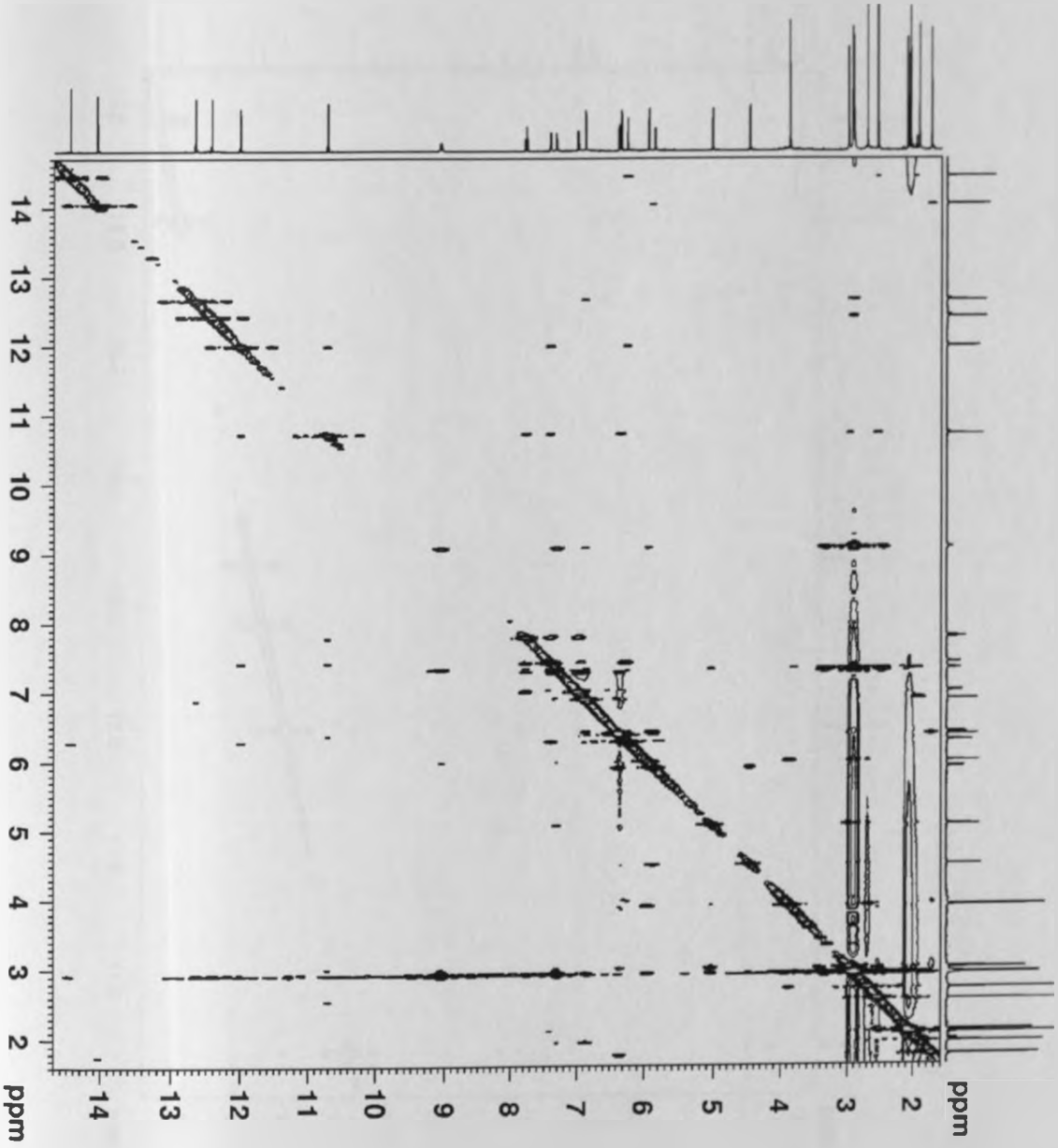


HMBC SPECTRUM FOR COMPOUND **13**

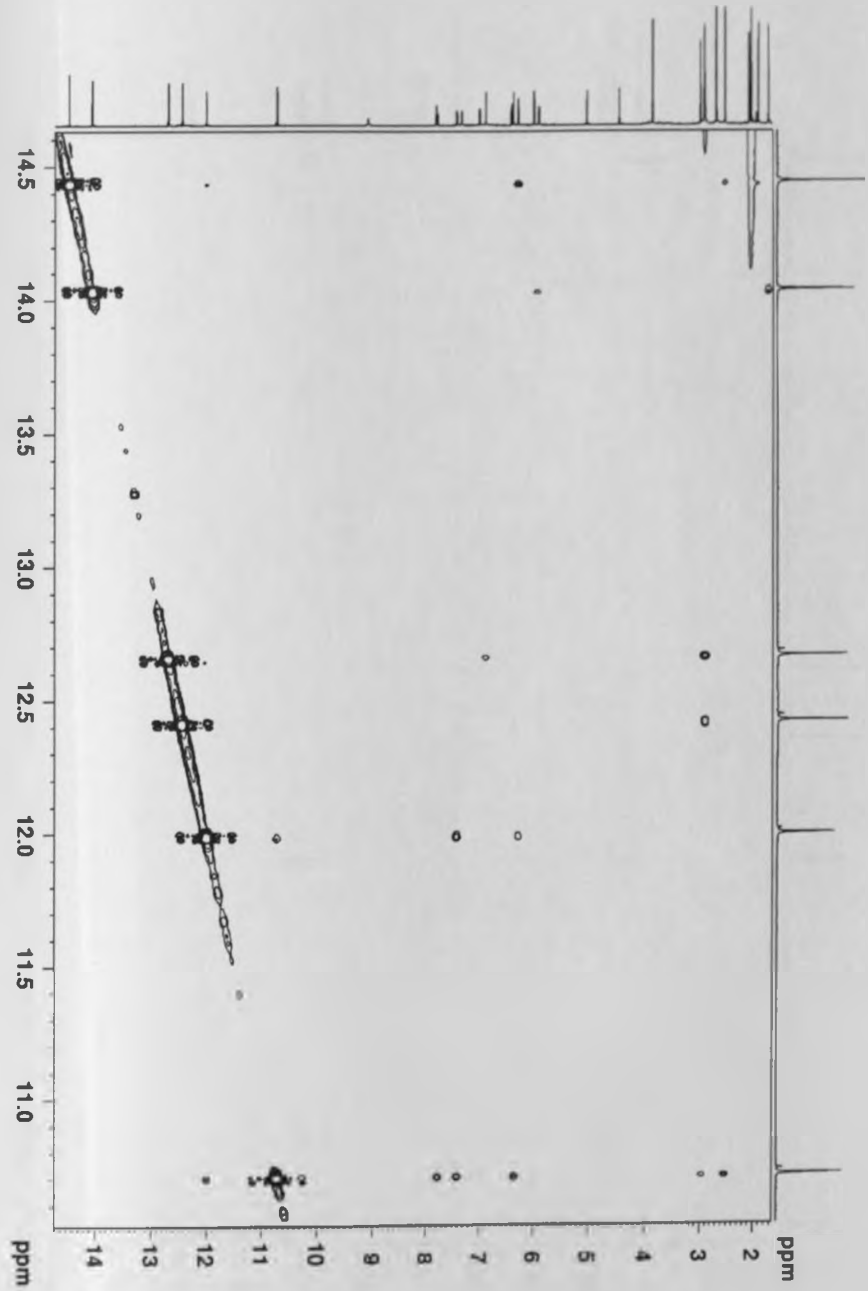


# HMBC SPECTRUM FOR COMPOUND 13

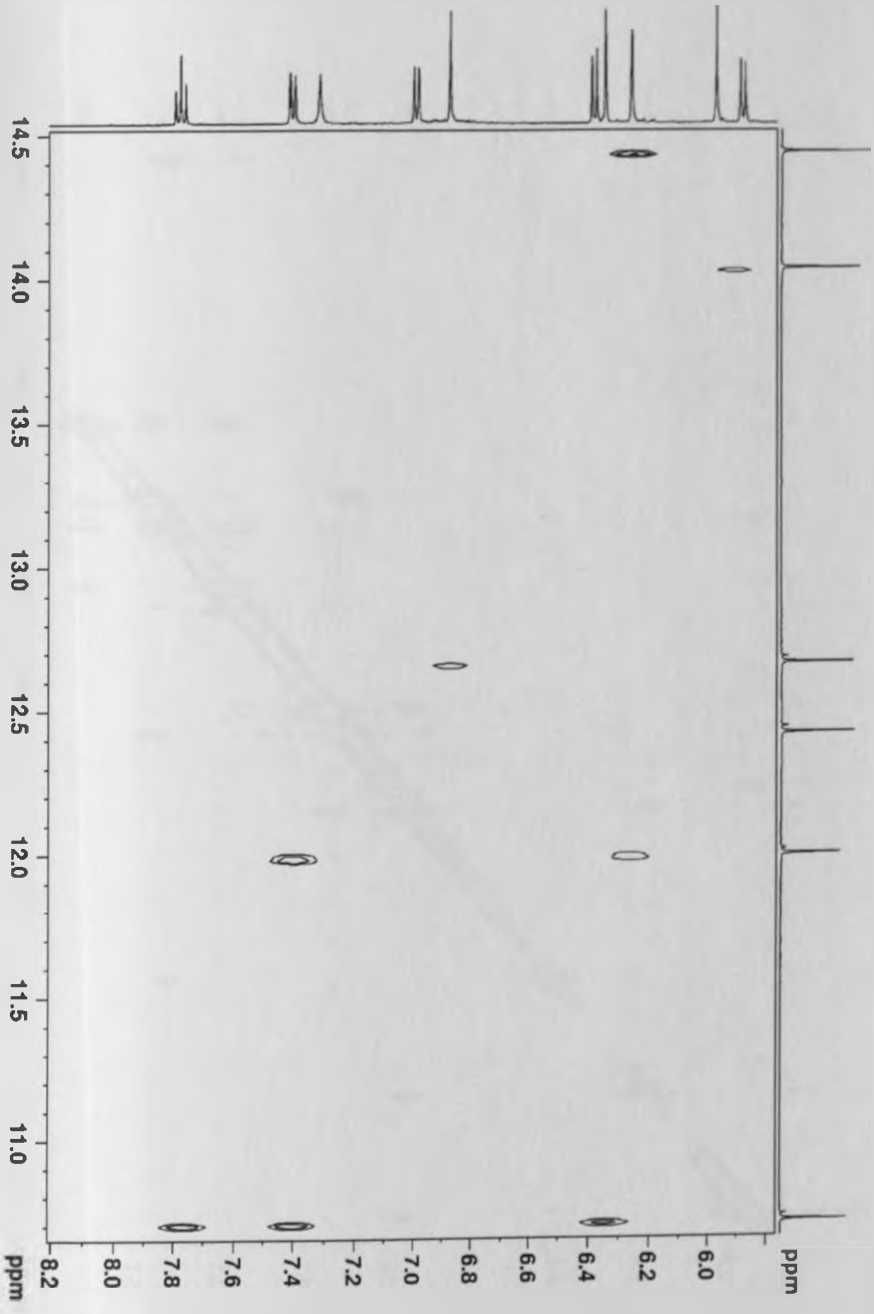
NOESY SPECTRUM FOR COMPOUND **13**



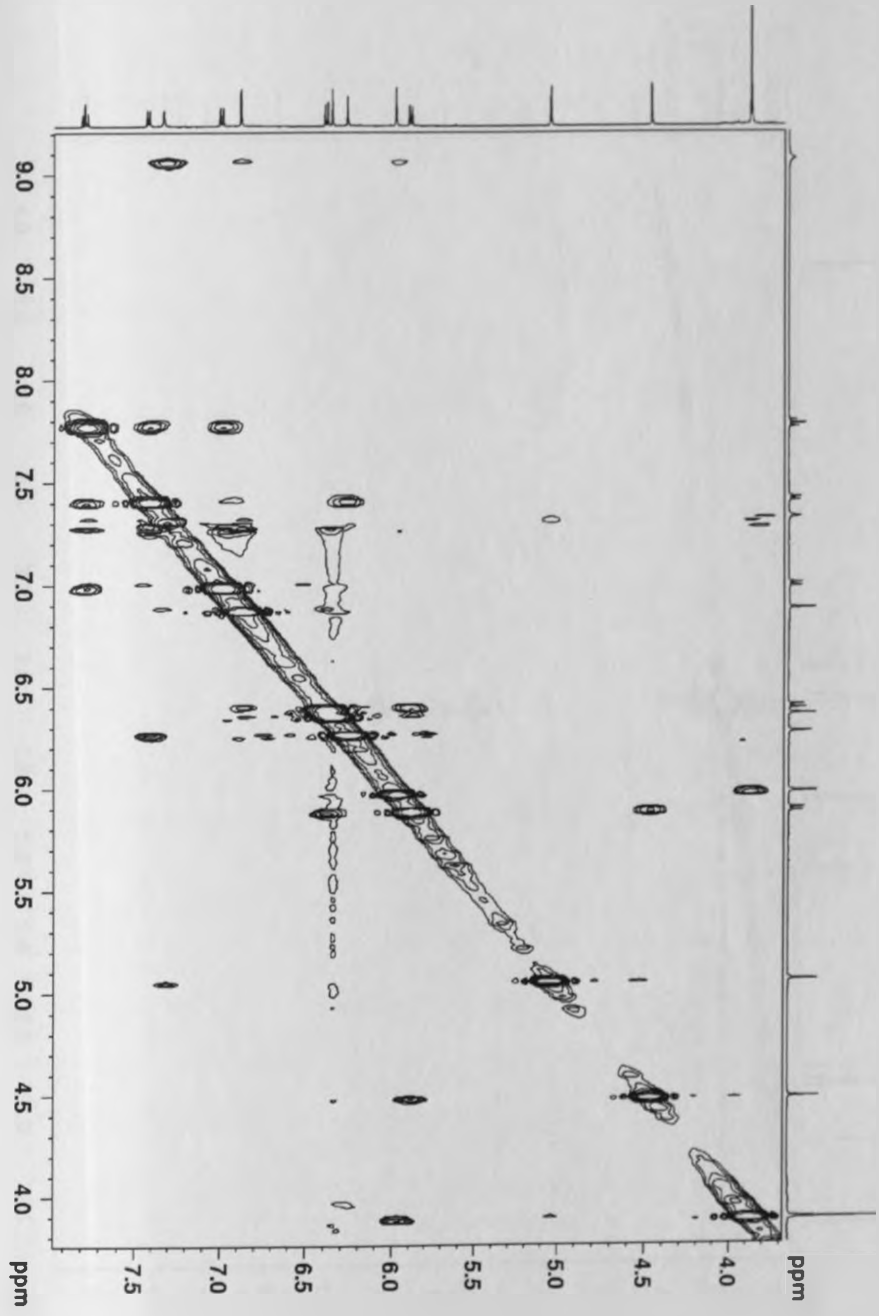
NOESY SPECTRUM FOR COMPOUND **13**



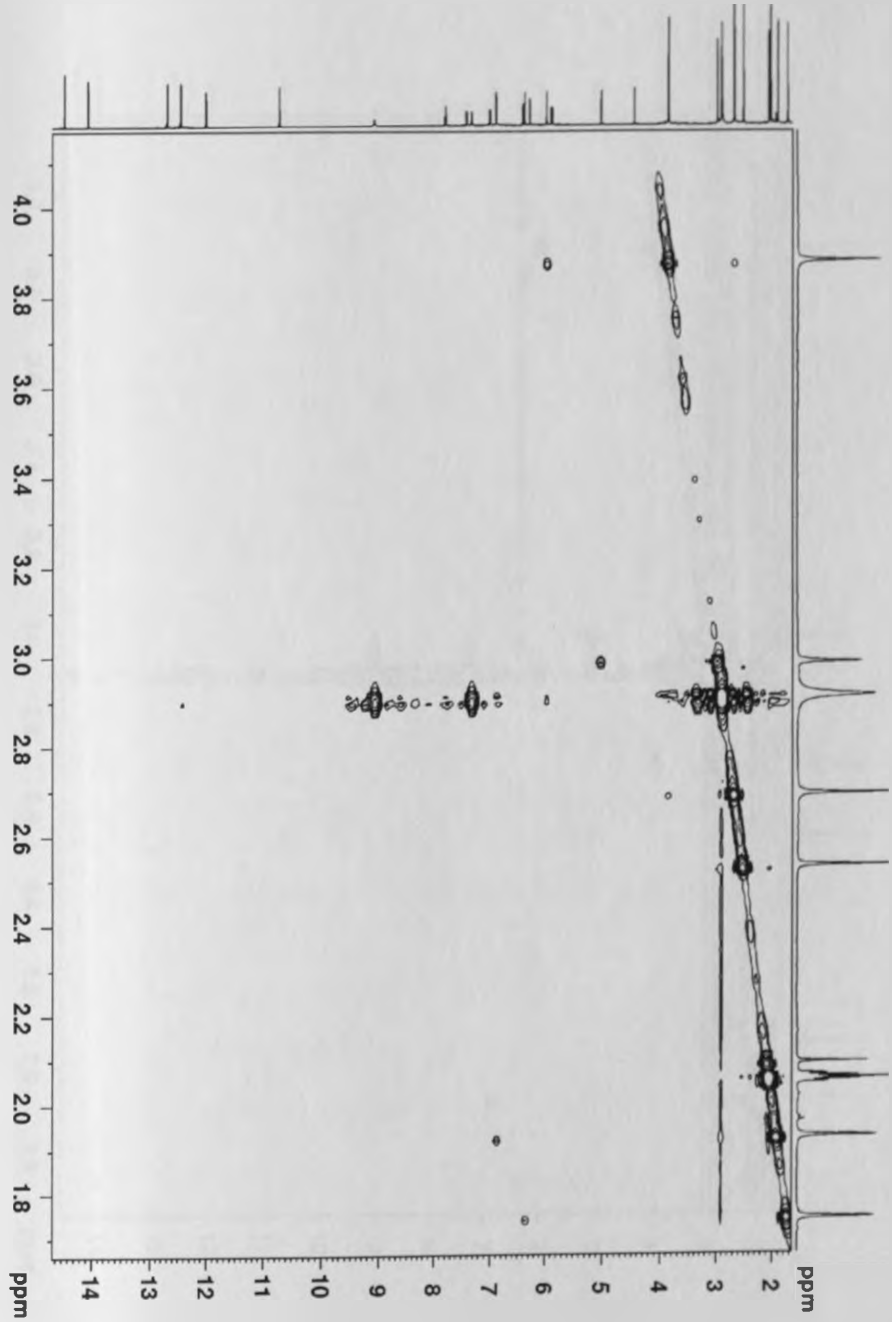
NOESY SPECTRUM FOR COMPOUND **13**



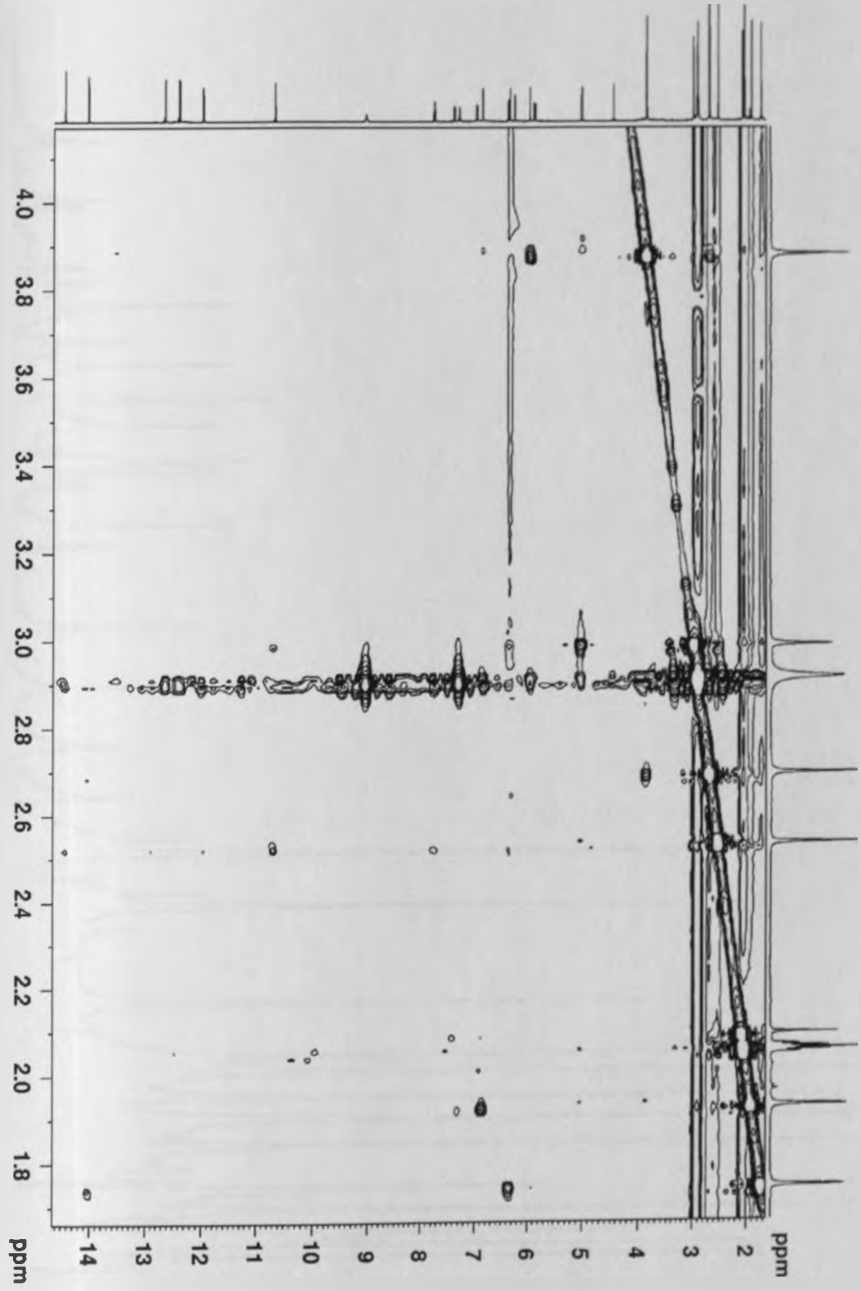
NOESY SPECTRUM FOR COMPOUND **13**



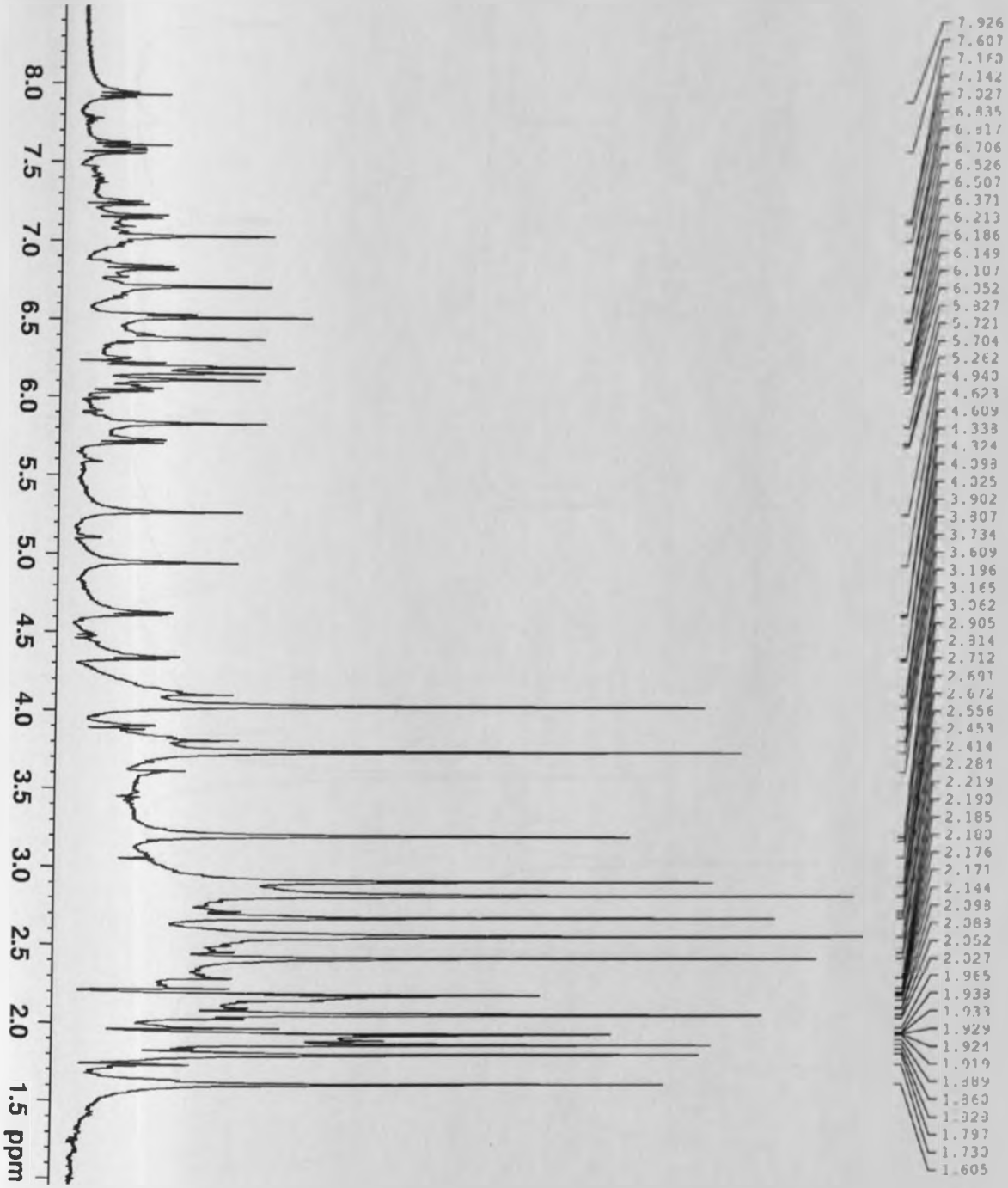
NOESY SPECTRUM FOR COMPOUND **13**



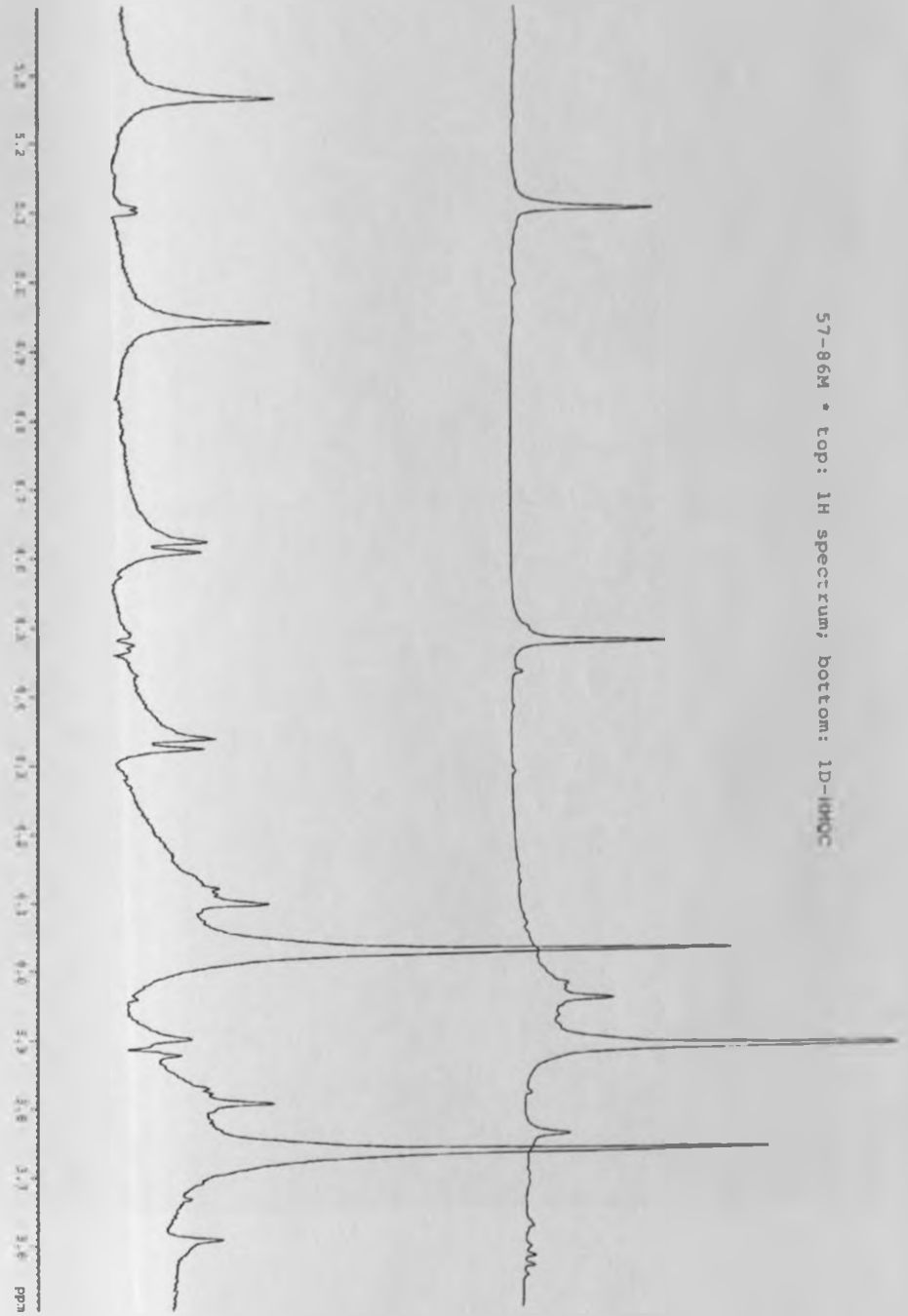
NOESY SPECTRUM FOR COMPOUND 13



# 1D HMQC SPECTRUM FOR COMPOUND **13**



# 1D HMQC SPECTRUM FOR COMPOUND **13**



MASS SPECTRUM FOR COMPOUND **13**