BIO-EVALUATION OF LARVICIDAL PLANTS FROM THE COASTAL PARTS OF KENYA

By

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A thesis submitted in partial fulfillment for the degree of Master of Science of Kenyatta University

SEPTEMBER 2003

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Declaration by Candidate

This thesis is my original work and has not been presented for any other degree in any other university.

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List of abbreviations

CC	Column Chromatography
$CDCl_3$	Deuterated Chloroform
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethylene
DEET	Diethyl-*m*-toluamide
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethylsulphoxide
EIMS	Electron Impact Mass Spectrometry
eV	Electron Volt
GDP	Gross Domestic Product
HETCOR	Heteronuclear Correlation
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
HPLC	High Performance Liquid Chromatography
Hz	Hertz
IR	Infrared
KEFRI	Kenya Forestry Research Institute
LC	Lethal Concentration
MHz	Megahertz
m/z	Mass to Charge ratio
NOE	Nuclear Overhauser Enhancement
PTLC	Preparative Thin Layer Chromatography
TLC	Thin Layer Chromatography
UoN	University of Nairobi
www	World Wide Web

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Finally I wish to extend my sincere gratitude to my family especially my parents for their moral and financial support without which I would not have come this far.

Dedication

This thesis is dedicated to my parents Jane and Alfred in affection and gratitude

Abstract

Malaria is responsible for significant morbidity and mortality, particularly among children in sub-Saharan Africa. In this area, estimates of symptomatic malaria range from one to five episodes per child per year, while deaths attributable to malaria are estimated at between 0.5 to 2.0 million, annually. Annual global clinical cases are estimated at 300-500 million with 90% of the cases in sub-Saharan Africa. Malaria remains intractable throughout most of its range, in part due to the evolution of behaviours maximizing contact of some *Anopheles* vectors with the human host while minimizing the risk of mosquito mortality. There is rapid development of insect-resistance to conventional insecticides. Virtually all areas where the disease is endemic have seen drug-resistant strains of the malaria parasite emerge. Meanwhile, a vaccine for malaria is proving elusive. The situation of malaria worldwide is therefore one of deterioration and desperation. Therefore there is need to search for new and ecologically acceptable methods for the control of the malaria vector. Plants have provided a wide range of useful chemicals such as insecticides, pharmaceuticals, nutraceuticals, dyes, pesticides and other agro-chemicals. Many plants have been reported to have larvicidal activity against mosquitoes. Larvicides of plant origin are preferable because they are biodegradable and generally target-specific.

Larval control using plant-derived compounds is a measure that could be adopted to arrest the insects before they develop into disease transmitting adults. It is easier to control the insects at the larval stage when they are most accessible, concentrated within specific habitats and less mobile. This research set out to examine the larvicidal activity of extracts of various plants from coastal parts of Kenya. *Zanthoxylum paracanthum, Z. holstiana* and *Derris trifolia* showed the highest larvicidal activity. Activity of up to 2 ppm was recorded in *Derris trifolia* dichloromethane extract. Extracts from these plants were subjected to bio-assay guided fractionation and isolation of 12 larvicidal compounds from the three plants and three compounds are being reported for the first time. The isolated compounds were assayed against 3rd instar *Anopheles gambiae* larvae and LC_{50} values ranging between 1.8 and 93.3 ppm were recorded.

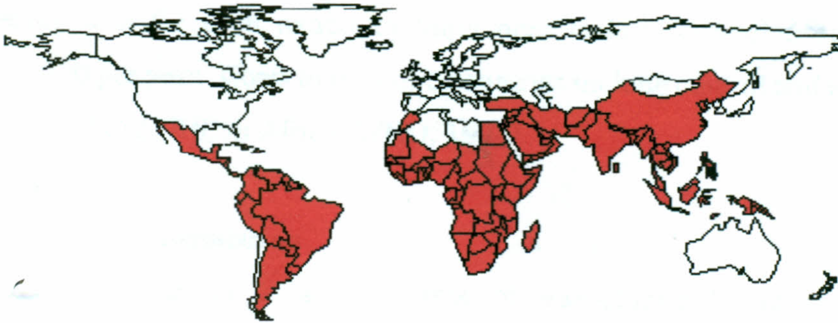
CHAPTER ONE

INTRODUCTION

1.1 Malaria prevalence

Malaria, a preventable but often fatal disease, affects more than 90 countries, inhabited by more than 2.4 billion people (40% of the world's population) (WHO, 2000; WHO, 1998; Cattani *et al.*, 1993). Malaria is considered endemic in 105 countries throughout the tropics and warm temperate zones (Fig. 1). However, the most affected region is sub-Saharan Africa (McGinn, 2002). It is the most important tropical parasitic disease (Cattani *et al.*, 1993). Globally, the prevalence of the disease is estimated at 300-500 million clinical cases each year, with more than 90% of the cases in sub-Saharan Africa (WHO, 1998). Deaths due to malaria are estimated at 1-3 million annually, most of them infants or young children in Africa, especially in remote rural areas with poor access to health services (Collins and Paskewitz, 1995a; WHO, 2000). Other high-risk groups include pregnant women, non-immune travelers, refugees, displaced persons and labourers entering endemic areas.

Fig. 1: Current distribution of indigenous malaria (WHO, 1998).



Although the geographical area affected by malaria has shrunk considerably over the past 50 years, control is becoming more difficult and gains are being eroded. This can be attributed to economic developments or agricultural activities that change land use such as road construction, creation of dams, irrigation schemes, mining, commercial tree cropping and deforestation. These result in an increase in breeding sites (Lindsay and Martens, 1998; WHO, 1998). Climatic changes resulting from "global warming" and other events such as *el nino* weather

phenomenon also play their role in increasing risk of the disease (WHO, 1998). The emergence of insecticide and drug resistance makes the situation even more desperate (WHO, 1999).

1.2 Economic costs

Malaria is a major public health problem and an important obstacle to economic development in most developing countries, particularly in Africa. Disease costs in terms of the burden on the health systems and loss of economic activity are enormous. Globally, it accounts for 10-30% of all hospital admissions and is responsible for 15-25% of all deaths of children under the age of five (WHO, 1998). In Africa, where malaria reaches a peak at harvest time and hits young adults, a single bout of the disease causes loss of 10 working days (Anon., 2000).

Malaria undermines investment in education since large amounts of development funds are channeled to disease prevention and treatment. In highly endemic areas, the learning capacity of 35-60% of all school children may be impaired (WHO, 1998). As a broad social burden, malaria is estimated to cost Africa $3-12 billion annually (McGinn, 2002). In Africa, the average cost for each nation to implement malaria control programmes is estimated at > $300,000 per year. Costs to endemic countries include control and lost working days–estimated to be 1-5 % of GDP in Africa (WHO, 1998).

1.3 Malaria transmission

With the exception of a few cases of transplacental and blood transfusion-associated transmission, malaria parasites are exclusively transmitted by female adult mosquitoes of the genus *Anopheles* (Collins and Paskewitz, 1995). Mosquitoes (Culicidae) are a family of about 3500 species within the order Diptera. They are classified into three sub-families: Toxorhynchitinae, Anophilinae and Culicinae (Clements, 1992). Mosquitoes are found throughout the world except in places that are permanently frozen. Three quarters of all mosquito species live in the humid tropics and sub-tropics, where the warm moist climate is favourable for rapid development and adult survival. All vectors of human malaria belong to the genus *Anopheles*. Some 60 species of *Anopheles* are important vectors of human malaria. The three most efficient vectors are *An. gambiae* Giles *sensu stricto* (subsequently referred to as *An. gambiae*), *An. arabiensis* and *An. funestus*. These species are widely distributed in tropical

Africa (Manson-Bhar and Bell, 1987). *An. gambiae* is the principal vector of malaria parasite in East Africa and is probably the world's most efficient (Service, 1986). It is an ubiquitous species that breeds in rainwater puddles, borrow pits, river pools or quiet backwaters (Wigglesworth, 1976). Since anopheline mosquitoes are the only known vectors of malaria, manipulation of their adult populations may directly impact on malaria transmission.

1.4 Mosquito life cycle

Like other flies, mosquitoes exhibit complete metamorphosis – the juvenile form passes through both larval and pupal stages (Clements, 1992). Female mosquitoes lay some 50 to 500 eggs on water or sites that will be flooded. These hatch into larvae within 1-7 days. Larval development takes seven days for tropical mosquitoes and the pupal stage lasts 2-3 days before adult emergence. Males emerge earlier to ensure sexual competence by the time females emerge. Both sexes feed on plant juices from nectaries, rotting fruit and honeydew. Anopheline and culicine females have a requirement for protein, from which they develop large batches of eggs and hence engorge on vertebrate blood for that purpose (Clements, 1992). It is during the blood feeding that malaria parasites from an infected mosquito are injected into healthy individuals. Knowledge of the mosquito life cycle may help in designing appropriate control strategies that target vulnerable stages of development.

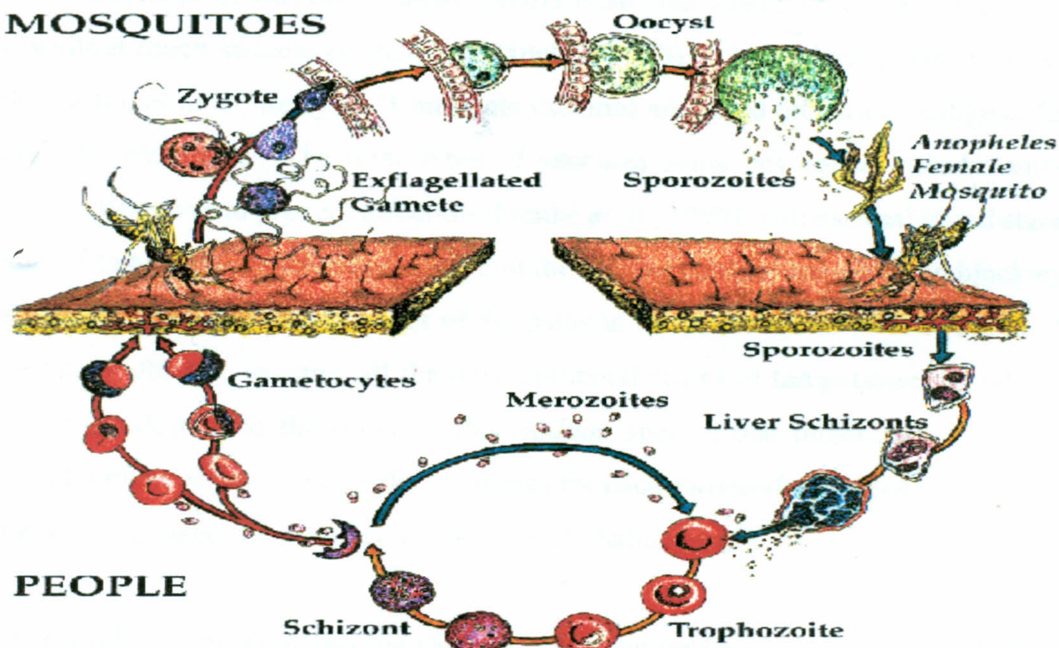
1.5 The malaria parasite

There are four species of human malaria parasites: *Plasmodium vivax, P. ovale, P. malariae* and *P. falciparum*. *P. falciparum* is the most virulent species and predominates in Africa, eastern Asia, Oceania and the Amazons (WHO, 1997).

The life cycle of malaria parasite is relatively complex, requiring passage through several organs in two very different hosts (Fig. 2). Malaria sporozoites may be injected when an infected female takes a blood meal, although a substantial proportion of blood feeding by infected mosquitoes does not result in transmission of sporozoites (Rosenberg *et al.*, 1990; Beier *et al.*, 1991a,b; Ponnudurai *et al.*, 1991). Successfully transmitted sporozoites enter the liver parenchymal cells where they develop and undergo a form of asexual replication – schizogony. Eventually, the schizonts burst to release thousands of merozoites into the bloodstream, from

where they enter red blood cells (Bruce-Chwatt, 1985). Once inside the erythrocyte, parasites begin to grow by ingesting haemoglobin and other nutrients. Eventually, these growing trophozoites undergo schizogony on a more modest scale than in the liver. The bursting of infected red blood cells is associated with the classic malaria paroxysm of chills and fever. Released merozoites infect other red blood cells. Eventually some of them differentiate into the pre-sexual form (gametocyte) of which there are two kinds, male and female. When a mosquito secures gametocytemic blood meal from man, the merozoites differentiate into gametes inside the mosquito midgut where fertilization occurs. Soon after fertilization, the gametes (ookinetes) migrate through the peritrophic membrane and the midgut epithelium and attach to the outer wall of the gut where it becomes an oocyst (Shahabuddin and Kaslow, 1994). The oocysts develop and divide asexually into thousands of haploid sporozoites (Pringle, 1965; Rosenberg and Rungsiwongse, 1991). A proportion of the sporozoites make their way into the mosquito's salivary glands ready for re-infection of the vertebrate host. Knowledge of the parasite life cycle is important in the design of effective anti-malarial vaccines and chemotherapeutic or chemoprophylactic agents.

Fig 2: Life cycle of *Plasmodium falciparum*



Malaria can assume many manifestations in an individual depending on parasite species and the pattern of transmission. *P. malariae* and *P. ovale* infections cause little morbidity and almost no mortality while *P. vivax* infections are more severe and debilitating but are usually self-limiting in healthy individuals. *P. falciparum* infections are always life threatening in non-immune individuals. In areas highly endemic to *P. falciparum*, the entomological inoculation rates (EIRs) can exceed several hundred infective bites per year resulting in severe anemia in infants. It is the principal manifestation and a major contributor to mortality (Beier *et al.*, 1990). Where EIRs are lower and transmission is less stable or highly seasonal, more fulminant clinical manifestations such as cerebral malaria are typical (Collins and Paskewitz, 1995).

1.6 Malaria control strategies

These encompass a variety of measures that may protect against infection or the development of disease in infected individuals.

1.6.1 Vaccination

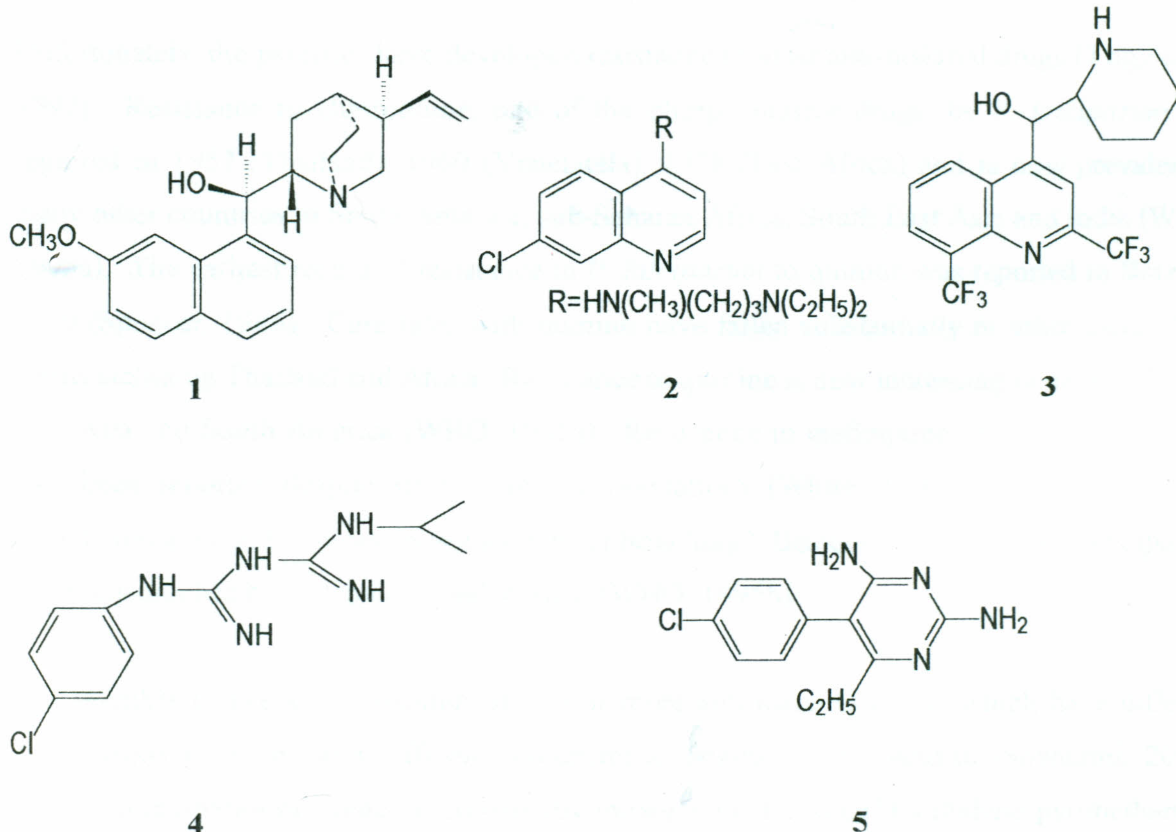
The development of vaccines against malaria is an area where a lot of effort has been directed but without much success (Cohen and Mitchel, 1978). The malaria parasite has a number of different stages in its life cycle. Candidate vaccines are based on various antigens derived from these different stages. The three types of vaccines being developed include: anti-sporozoite vaccines, designed to prevent infection (Franke *et al.*, 1999); anti-asexual blood stage vaccines, designed to prevent severe manifestation of the disease, and the transmission blocking vaccines, designed to arrest the development of the parasite in the mosquito (WHO, 1998). In order to develop an effective vaccine, all the developmental stages of the parasite should be taken into account in addition to the different *Plasmodium* species that infect man. All these factors, coupled with the existence of different strains for each *Plasmodium* species, have made the task of developing malaria vaccine complicated and challenging.

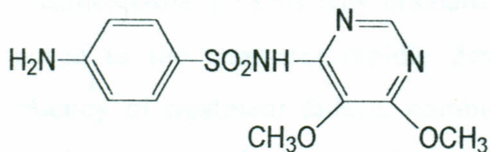
A chemically synthesized vaccine (SPf66) has undergone trials in children between ages of 1-15years. Its protective efficacy was found to be 30% in Tanzania and 9% in Thailand in contrast to its lack of protection in Gambia. However, the vaccine has been found to cause mild erythema, induration and tenderness at site of injection (Migasena *et al.*, 1997; Nosten *et al.*,

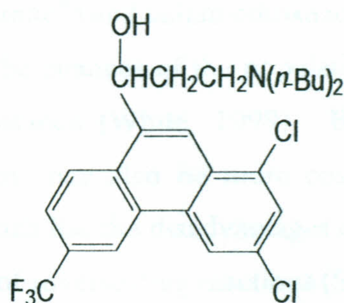
1996; D'Alessandro *et al.*, 1995; Alonso *et al.*, 1994). The most recently developed vaccine that has been tried on humans with promising results is the RTS, S/AS02, which showed 47% protective efficacy in Gambia. Although its protection is incomplete and short-lived, further tests of the vaccine are continuing in Mozambique (Bojang *et al.*, 2001). The absence of an effective vaccine has warranted the investigation of alternative methods of protection such as chemoprophylaxis and chemotherapy.

1.6.2 Chemotherapy

This can either be prophylactic or curative. Some drugs give little protection against infection, but are effective in treatment, while others serve as both prophylactic and curative drugs (Service, 1986). Examples of anti-malarial drugs include quinine (**1**), chloroquine (**2**) and analogs like mefloquine (Lariam®) (**3**), proguanil (**4**), pyrimethamine (**5**), sulfadoxine (**6**), halofantrine (**7**), artemisinin (**8**), chlorotetracycline (**9**) and tetracycline (**10**). A group of French researchers recently reported encouraging results for a new anti-malarial drug, G25. The drug when administered to aotus monkeys cleared the parasites from their systems (McGinn, 2002).







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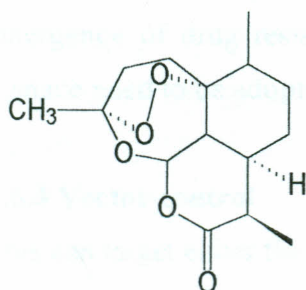
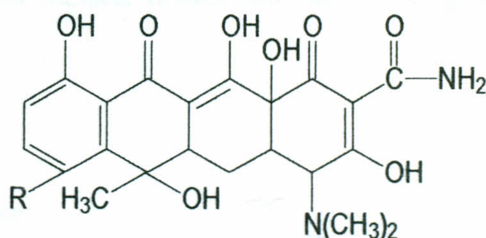




8 **9** R=Cl, **10** R=H

Unfortunately, the parasites have developed resistance to most anti-malarial drugs (Trigg *et al.*, 1997). Resistance to chloroquine, one of the cheap curative drugs, by *P. falciparum* was reported in 1957 (Thailand), 1960 (Venezuela), 1978 (East Africa) and is now prevalent in many other countries in South America, sub-Saharan Africa, South East Asia and India (WHO, 1995a). The earliest record of resistance in *P. falciparum* to quinine was reported in Brazil in 1910 (Spencer, 1985). Cure rates with quinine have fallen substantially in other parts of the world including Thailand and Africa. Resistance to quinine is now increasing in parts of South-East Asia and South America (WHO, 1995a). Resistance to mefloquine by *P. falciparum* has also been reported despite strict drug use regulations (White, 1999). Newer drugs like artemisinin are still free from resistance but for how long? Besides, resistance of *P. falciparum* to artemisinin has been demonstrated *in-vitro* (WHO, 1995b).

It is possible to use a combination of two or more anti-malarial drugs, which have different mechanisms of action and different bio-chemical targets in the parasite (Sowumni, 2002). Typical combinations include sulfadoxine/pyrimethamine (Fansidar®), sulfalene-pyrimethamine

(Metakelfin®), atovaquone-proguanil chloride (Malarone®) and sulfamethoxazole-trimethoprim (Co-trimoxazole®). This may dramatically reduce the chances of the populations of parasites exposed to the treatment rapidly developing resistance (White, 1999). By reducing the frequency of treatment failure, combination therapy may also be more cost effective than monotherapy in the long-term. However, this approach has the disadvantages of increasing the immediate costs of treatment and increasing the risk of adverse drug reactions (Sowumni, 2002). Besides, resistance of *P. falciparum* has been reported to drug combinations like Fansidar® (sulfadoxine-pyrimethamine combination) (Markwalder and Meyer, 1982). Continued emergence of drug resistant strains of malaria means that new strategies of combating the menace need to be adopted.

1.6.3 Vector control

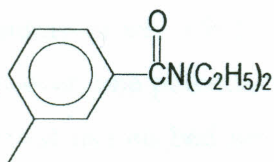
This can target either the adult or larvae.

1.6.3.1 Adult control

This may involve personal protection, use of insecticides (adulticides) and genetic modification.

1.6.3.1.1 Personal Protection

Personal protection may be of critical importance in its effect on mosquito biting. Repellents reduce biting intensity (Deither, 1947; Gupta and Rutledge, 1994) and can be a very useful short-term strategy to reduce contact with disease vectors. The most effective repellent is *N, N*-diethyl-*m*-toluamide (DEET) (**11**). It is the most widely used synthetic repellent for application to the skin (Beroza, 1970). It is effective against fleas, mosquitoes, jiggers, ticks, deer and sand flies among others. When applied to the skin its effect lasts for 3-8 hours (Beroza, 1970). Other synthetic repellents that have been used to control mosquito bites include dimethylphthalate (DMP) (**12**) whose effect lasts for approximately 2 hours (Service, 1986) and *n*-butyl-5, 6-dihydro-1, 4-pyrone-2-carboxylate (**13**).



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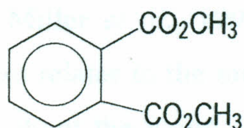

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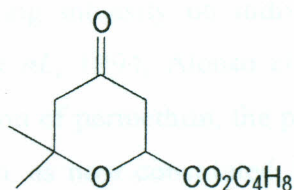

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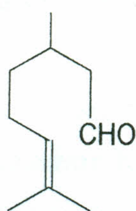
Use has also been made of essential oils extracted from plants. These include citronellal (**14**), camphor (**15**), *p*-menthane-3,8-diol (**16**), nepetalactone (**17**), geraniol (**18**) and linalool (**19**) (Curtis *et al.*, 1987; Dethier, 1947; Granett, 1940). Natural pyrethroids like pyrethrin I (**20**) have also been found to be effective mosquito repellents (Kumar, 1984).



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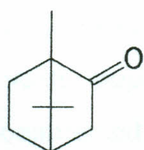

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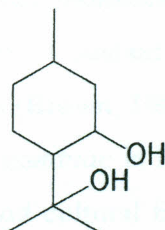

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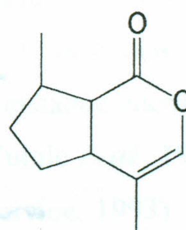

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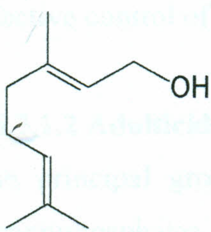

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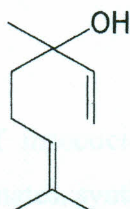

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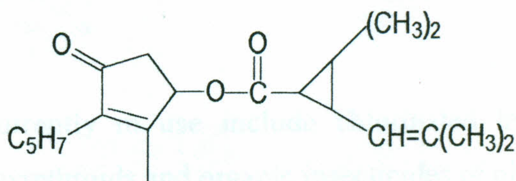

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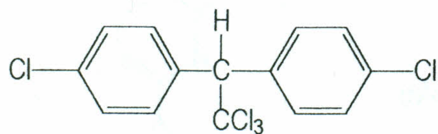
With few exceptions, essential oils are volatile thus providing only transient protection. Repetitive application of the repellents may be necessary if reasonable protection is required. Synthetic pyrethroids have improved activity and stability and reduced costs hence enhanced affordability.

Insecticide-treated bed nets are effective in reducing biting intensity on individual users (Lindsay *et al.,* 1991; 1992; Miller *et al.*, 1991; Stich *et al.*, 1994; Alonso *et al.*, 1991). However, one potential problem relates to the mode of action of permethrin, the pyrethroid in greatest use on bed nets throughout the world. Permethrin, as now constituted, has a major deterrent effect on house entry by anophelines (Lindsay *et al.*, 1991; Miller *et al.*, 1991; Curtis, 1992). It is possible that mosquitoes deterred from entering a house with a permethrin-treated bed net may search elsewhere for a blood meal, which could increase malaria challenge to unprotected members of the community (Miller *et al.*, 1991). An important shortcoming of most insecticide treated bed nets is washing which significantly reduces their efficacy (Luxemburger *et al.*, 1994). Bed nets also do not hold up in areas of consistently high humidity, lasting only about a year in Thailand (Luxemberger *et al.*, 1994). Besides, lack of compliance and improper use of bed nets is yet another shortcoming. In addition other mosquito species may bite before bedtime or during the day. Constant exposure to insecticides may also select for resistance in target vector populations (Brown, 1986). For instance, increased resistance to permethrin has been documented in *An. gambiae* in Kenya (Vulule *et al.*, 1994). Finally, bed nets use may be constrained by social and cultural factors (Service, 1993). Bed net use and ownership is uncommon in endemic areas, particularly in Africa, where it is usually associated with high densities of pest mosquitoes. Other complimentary methods are therefore required for effective control of mosquitoes and hence malaria.

1.6.3.1.2 Adulticides

The principal groups of insecticides currently in use include chlorinated hydrocarbons, organophosphates, carbamates, synthetic pyrethroids and organic insecticides of plant origin.

Chlorinated hydrocarbons are probably the most widely used group of insecticides. They are among the more persistent insecticides and the effects last long. Traces accumulate in the bodies of many vertebrates, including man, and this has caused concern over their long-term effects. Besides there is also reported resistance to these insecticides (Kumar, 1984). They generally kill by contact but can also act as stomach poisons and in a few cases as fumigants (Palchick, 1996). Examples include DDT **(21)**, lindane **(22)**, chlordane **(23)**, dieldrin **(24)** and toxapane ($C_{10}H_{10}Cl_8$).



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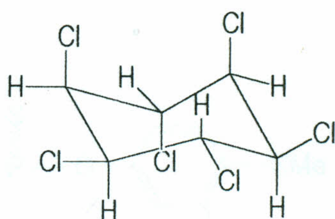

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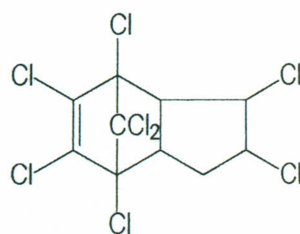

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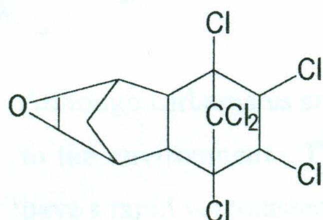

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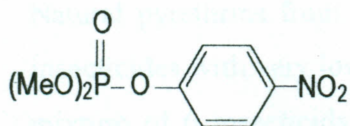
Organophosphates are esters or organic salts of phosphoric acid or its derivatives. They are highly toxic to mammals and other non-target organisms and are much less stable than organochlorines. They can degrade in the presence of water by hydrolysis, chemical alterations by reaction with oxygen, through heat and bacterial action (Palchick, 1996). Some possess systemic properties while others are fumigants. Examples include methylparathion (**25**), malathion (**26**), parathion (**27**), diazinon (**28**) and fenthion (**29**) (Kumar, 1984). Unfortunately, insects have developed resistance to many of these insecticides (Motoyama *et al.*, 1977).



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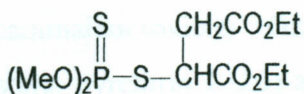

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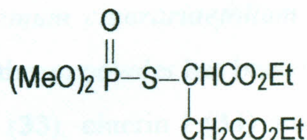

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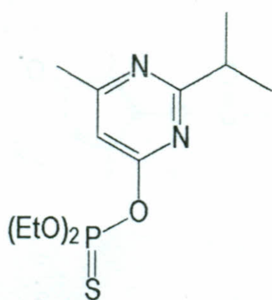
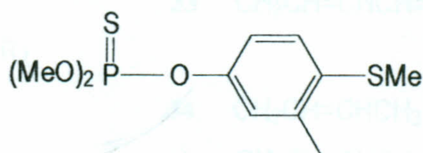
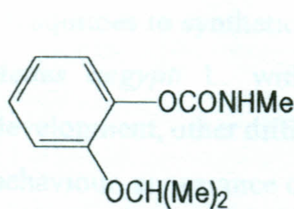
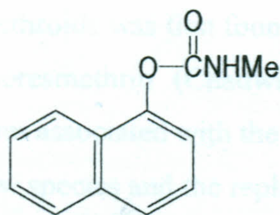


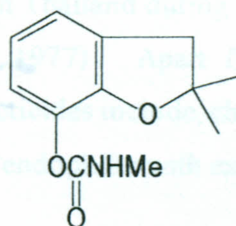


28 **29**

Although carbamates are generally stable, they easily breakdown in formulations when exposed to the environment. They are inexpensive and relatively broad-spectrum insecticides. They have a rapid yet transient poisoning effect in mammals. Resistance to these insecticides has also been reported (Brown, 1971). Examples include, propoxur (**30**), carbaryl (**31**) and carbofuran (**32**).

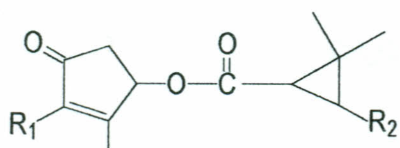






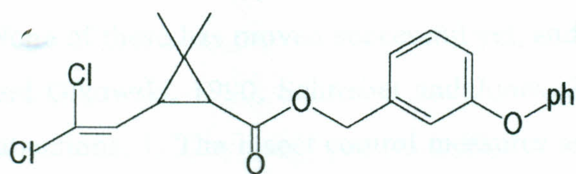
30 **31** **32**

Natural pyrethrins from the flower heads of *Chrysanthemum cinerariaefolium* are powerful insecticides with very low mammalian toxicity. The pyrethrum powder has been shown to be a mixture of 6 insecticidal esters: pyrethrin I (**20**) and II (**33**), cinerin I (**34**) and II (**35**), and jasmolin I (**36**) and II (**37**) (Elliott and Janes, 1979). Although the use of natural pyrethrins has depended on the rapid knock down of insects, their application has been restricted due to lack of prolonged residual action and cost (Kumar, 1984).



	R_1	R_2
33	$CH_2CH{=}CHCH{=}CH_2$	$CH{=}C(CH_3)COOCH_3$
34	$CH_2CH{=}CHCH_3$	$CH{=}C(CH_3)_2$
35	$CH_2CH{=}CHCH_3$	$CH{=}C(CH_3)COOCH_3$
36	$CH_2CH{=}CHCH_2CH_3$	$CH{=}C(CH_3)_2$
37	$CH_2CH{=}CHCH_2CH_3$	$CH{=}C(CH_3)COOCH_3$

This has led to development of synthetic pyrethroids such as permethrin (NRDC 143) (**38**) (Elliott *et al.*, 1973; Beach *et al.*, 1993) and decamethrin (NRDC 161) (**39**) (Elliott *et al.*, 1974). They have enhanced photostability and diminished mammalian toxicity. Resistance of mosquitoes to synthetic pyrethroids was first found in 1974 in Thailand during trials to control *Aedes aegypti* L. with bioresmethrin (Chadwick *et al.*, 1977). Apart from resistance development, other difficulties associated with the use of insecticides include, changes in vector behaviour, appearance of new species and the replacement of endophilic with exophilic species (Gutsevich *et al.*, 1974).



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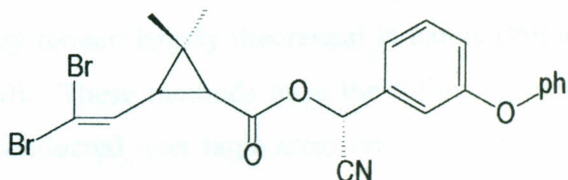

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Other insecticides of plant origin are rotenone (**40**) from the roots of *Derris*, *Lonchocarpus*, *Tephrosia* and *Milletia* species, ryanodine from the ground stem wood of *Ryania speciosa* and nicotine from *Nicotania* sp. and the Russian weed *Anabasis aphylla* (Kumar, 1984).

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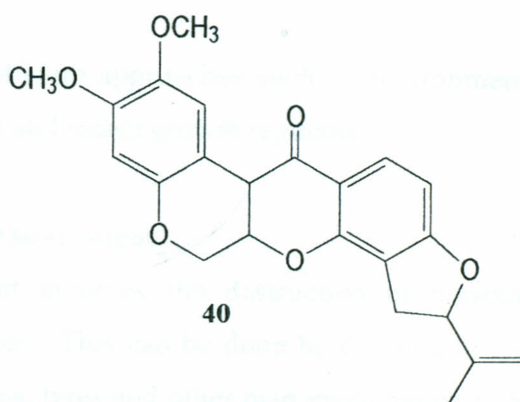


These insecticides have almost no residual action and persistence (rotenone and ryanodine have low persistence). Their toxicity to non-target organisms including man and photo instability has limited their widespread use as insecticides (Kumar, 1984).

1.6.3.1.3 Genetic modification

This approach involves the use of genetically impaired insects to limit reproduction and survival of their own species in natural populations. Such insects are mass produced in the laboratory and released among wild populations in the field so that mating with normal insects will either not result in an offspring or lead to reduced fitness (sterility, failure to adapt properly to the environment) of the progeny (Cutkomp, 1967). Genetic approaches include the sterile-insect release method, chromosomal translocations, hybrid sterility and cytoplasmic incompatibility. None of these has proven successful yet, and they remain largely theoretical in nature (Popiel and Olkowski, 1990; Schrieber and Jones, 2000). These methods have the following main attractions: 1. The insect control measures are conducted over large areas under professional supervision thereby avoiding mistakes or omissions by individuals; 2. They have the potential of increasing efficiency as the densities of target populations decline. Insecticidal control measures usually become less cost-effective as the target population decreases towards the economic threshold, below which it becomes uneconomical to employ insecticides at all; 3. Genetic control is specific and avoids undesirable effects on other organisms. No residues are involved and other adverse effects associated with the use of insecticides are avoided; and 4. Once established it may cause dramatic economic savings by completely eliminating the insect (Cutkomp, 1967).

1.6.3.2 Larval control

Larval control may take different approaches such as environmental management, biological control, chemical larvicides and insect growth regulators.

1.6.3.2.1 Environmental management

Environmental management involves the destruction of mosquito breeding habitats thus targeting the immature stages. This can be done by draining of marshes, filling of ponds and removing abandoned tin cans, tyres and other man-made habitats. Sometimes, a habitat can be altered to make it unsuitable for breeding (by removing overhanging vegetation to eliminate shade-loving mosquitoes). Stream breeding mosquitoes can be controlled by 'flushing' (MacDonald, 1939). Siphons are built into streams, which periodically release a large volume of water. The larvae migrating to the margins of the stream are stranded as the flow subsides. Suitable bushes can also be planted along grassy edged streams to eliminate *An. minimus* (Ramsay, 1930). But care is needed to ensure that such changes do not attract other species. Besides, these strategies are labour-intensive and costly.

1.6.3.2.2 Biological control

Biological control implies the use of predators, parasites or pathogens. For a pathogen to be considered a bio-control agent it must have: high virulence in target species in the field, be harmless to non-target species including beneficial organisms and vertebrates, be easily produced and stored for long periods without loss of virulence, have the capability of acting rapidly against the target species and be resistant to harmful factors in the environment such as solar radiation, dessication, heat, changes in pH, among others (Kumar, 1984). The best-known biological control agent is the top minnow or mosquito fish, *Gambusia affinis*, a native of southern USA, which has now been introduced into many tropical and sub-tropical countries to control mosquito larvae (Meisch, 1985). Another fish, the guppy *Poecilia (Lebistes) reticulata* is also used (Service, 1986). Among the parasite-based mosquito control methods, one of the most successful is use of the nematode, *Romanomermis culicivorax*, but it is not effective against all species of mosquito larvae (Service, 1986). There are numerous viruses, bacteria, protozoa and fungi that parasitize mosquito larvae but only a few have shown some potential as bio-control agents. The most promising is *Bacillus thuringiensis* var. *israelensis* (*Bti*) (or

serotype H-14), which also destroys larval simulds (Service, 1986). *Bti* is extremely safe, having no adverse effects on beneficial insects, man, pets and other animals (Fisher and Rosmer, 1959). Commercial preparations (often termed biotic chemicals) of *Bti* like Dipel®, Thuricide®, HPC®, Biotrol® and Bactospeine P.M.®, have been successfully used against several agricultural pests (Kumar, 1984). *Bti* disrupts the midgut lining of mosquito and black fly larvae. *B. thuringiensis* and *B. sphaericus* are also effective against mosquito larvae (Palchick, 1996).

Numerous species of fungi attack and kill insects in nature. Field trials of *Beauveria* spp. have been found effective against a number of insect pests. Pathogenic fungi of the genus *Entomophthora* are also well known (Kumar, 1984). Laboratory tests of a fungus *Tolypocladium clindrosporum* against *Ae. aegypti*, *Ae. vexans* and *Ae. triseriatus*, gave interesting results. Field application has not been undertaken hence stability of this fungus under normal environmental conditions has not been established (Goettel, 1987; Nadeau and Boisvert, 1994).

The disadvantage of biological control methods is that they require a good understanding of the population dynamics of the vector. Besides, they are slow acting and therefore unsuitable in emergencies such as disease epidemics, where insecticides are more appropriate intervention tools (Service, 1986).

1.6.3.2.3 Larvicides

The classical method of killing mosquito larvae is by applying oil to the water surface (Wigglesworth, 1976). The oil is usually applied as a spray to form a layer 15-20 µm thick. A little of the oil enters the tracheal system of the larvae and toxic aromatic hydrocarbons affect the nervous system. However, the oil film on water surface is likely to prevent free exchange of oxygen between water surface and the free air and can lead to suffocation of other organisms in the water (Murray, 1936; 1938; 1939).

Synthetic inorganic compounds such as Paris green {$Cu(C_2H_3O)_2.3Cu(AsO_2)_2$} and metarsenite {$Cu(AsO_2)_2$} have also been used as larvicides (Metcalf *et al.*, 1962). Paris green is used to control larvae in water that has much vegetation. A cloud containing 1-2% of Paris green is

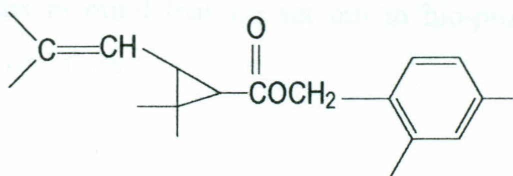
blown over the water in a vehicle of dry dust. Anopheline larvae feed at the water surface and are poisoned by the floating particles (Wigglesworth, 1976). However, inorganic larvicides are highly toxic to most aquatic organisms and plants because of relatively large amounts of water-soluble arsenic and copper in them. Nevertheless, these have been observed to lack resistance compared to other existing larvicides over time (Kirk and Othmer, 1981).

Chlorinated synthetic organic compounds like lindane **(22)**, dieldrin **(24)**, chlordane **(23)**, heptachlor or DDT **(21)** have also been used as larvicides (Kirk and Othmer, 1981). However, they are non-bio-degradable and therefore accumulate in the biotic chain. Resistance to organochlorines has been reported in mosquito larvae (Brooks, 1974).

Synthetic organophosphates like malathion **(26)** have also been used as larvicides. These compounds kill non-target aquatic life but are bio-degradable and hence do not accumulate in the environment (Kirk and Othmer, 1981). Resistance to organophosphates has been reported in mosquito larvae (Matsumura and Brown, 1961).

Baygon®, a methylcarbamate insecticide has also found use as a mosquito larvicide. Unfortunately, resistance has been reported in larvae of various mosquito species such as *Culex pipiens fatigans* and *An. albimanus* (Kuhr and Dorough, 1976).

Dimethrin **(43)**, a synthetic pyrethroid has been used as a mosquito larvicide. It has outstanding mammalian safety and has been used as a larvicide that is safe for use in potable waters such as rain barrels and cisterns (Kirk and Othmer, 1981) to rid them of larvae.



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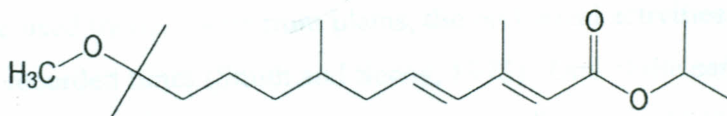
Insect growth regulators (IGRs) are compounds that act on the highly species-specific insect hormonal systems that control moulting and metamorphosis. They have the advantage of low mammalian toxicity; some disadvantages are species specificity, time required to kill and poor

stability. An example is methoprene (**44**), a synthetic juvenile hormone that inhibits the moulting process (Palchick, 1996). It has been used commercially as a mosquito larvicide and for horn-fly control (when fed to cattle) in manure (Kirk and Othmer, 1981).



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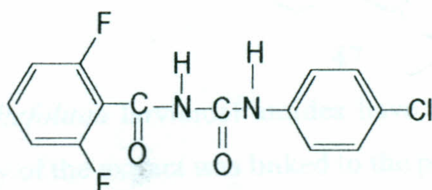
Chitin inhibitors interfere with the formation of chitin, a major constituent of the insect exo-skeleton. As vertebrates and most plants do not need chitin, these compounds are probably safe for humans, domestic animals, wildlife and plants. Diflubenzuron (**45**) is an example of a chitin inhibitor (Palchick, 1996).



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Plants have provided a wide range of chemicals such as insecticides, pharmaceuticals, nutraceuticals, agro-chemicals and dyes. Plant-derived insecticides include pyrethrins, nicotine and related alkaloids, rotenoids and ryanodine among others. The possibility of finding new potent plant-derived larvicides is high. The advantages would be their bio-degradability and target specifity. It is with this in mind that we set out to bio-prospect for phytochemical larvicides from the Kenyan coastal flora.

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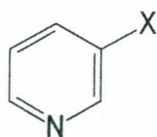
CHAPTER TWO

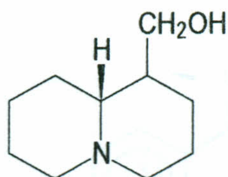
BIO-ORGANIC LARVICIDES

The pool of plants possessing insecticidal substances is enormous (Jacobson, 1975). Over 2000 species of plants are known that possess some insecticidal activity (Jacobson, 1989). The first insecticides to be used by man were from plants, the biological activities of which were known from the earliest recorded times (Smith and Secoy, 1975). One of the earliest reports of the use of plants against mosquito larvae is credited to Campbell *et al.* (1933) who found that plant alkaloids like nicotine (**46**), anabasine (**47**), methylanabasine and lupinine (**48**) extracted from the Russian weed, *Anabasis aphylla*, killed larvae of *Culex pipiens, Cx. territans,* and *Cx. quinquefasciatus*.



46 X=N-methyl pyrolidine

48 X=Piperidine



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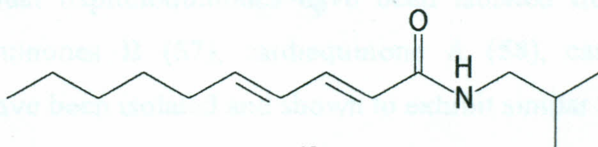
From the plant *Achillea millefolium* larvicidal amides have been isolated (Bohlmann *et al.*, 1974). The larvicidal activity of the extract was linked to the presence of *N*-2-methylpropyl- (*E*, *E*)-2,4-decadieneamide (**49**).



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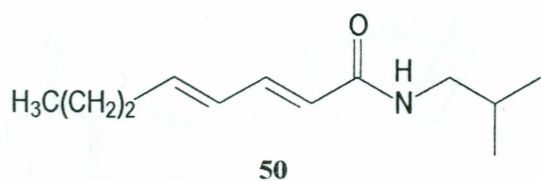
Other long chain amides with larvicidal activity have been reported. Among them are *N*-isobutyl 2*E*, 4*E*-octadieneamide (**50**) from *Fagara macrophylla* (Rutaceae) which exhibited activity against *Cx. pipiens* larvae (Kubo *et al.*, 1984) and *N*-isobutyl-2*E*, 4*E*, 8*E*, 10*Z*-dodeca-2,4,8,10-tetraeneamide (**51**) from *Spilanthes mauritiana* (Compositae) which showed activity against *Ae. aegypti* larvae (Jondiko *et al.*, 1986).



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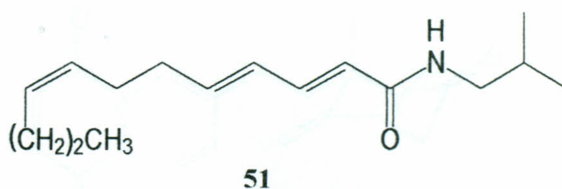

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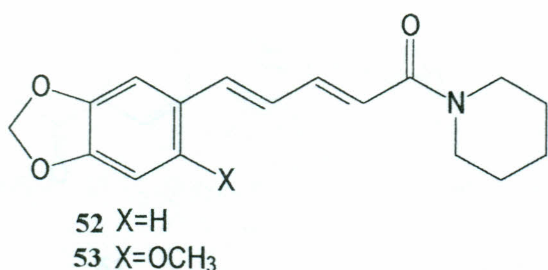
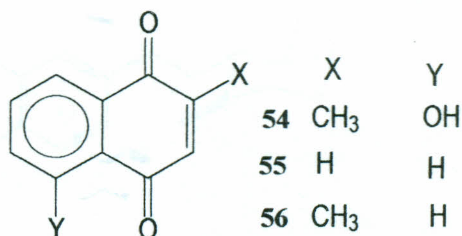
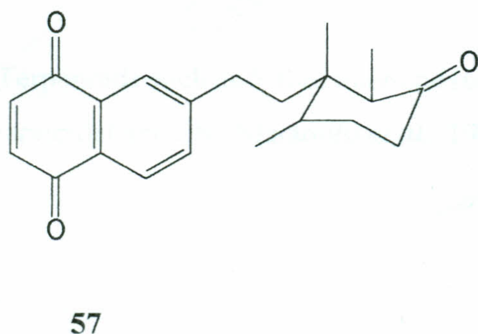
Piperine (**52**) and wisanine (**53**) isolated from *Piper guineense* are alkaloids, which have been found to be effective on *Ae. aegypti* larvae (Addae-Mensah and Achieng, 1986). Plumbagin (**54**), a naphthoquinone isolated from *Plumbago zeylanica* (Plumbiginaceae), also exhibits larvicidal activity (Hassanali and Lwande, 1989). Structural variants of plumbagin such as juglone (**55**) and 2-methyl-1, 4-naphthoquinone (**56**) also possess larvicidal effects (Hassanali and Lwande, 1989).





Anti-fungal and larvicidal naphthoquinones have been isolated from the roots of *Cordia corassavica.* Cardiaquinones B (**57**), cardiaquinone A (**58**), cardiaquinone J (**59**) and cardiaquinone K (**60**) have been isolated and shown to exhibit similar larvicidal activity against the larvae of *Ae. aegypti* (Ioset, 2000).



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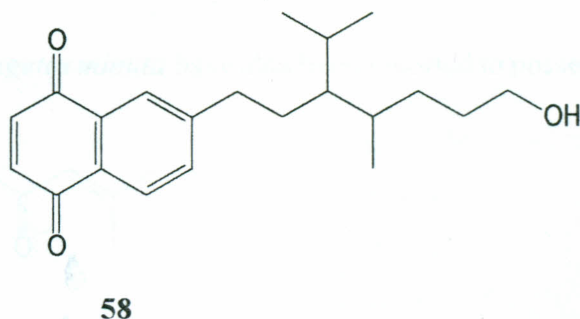

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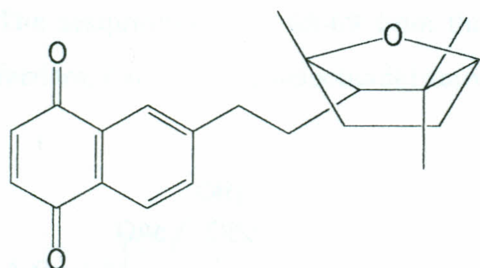
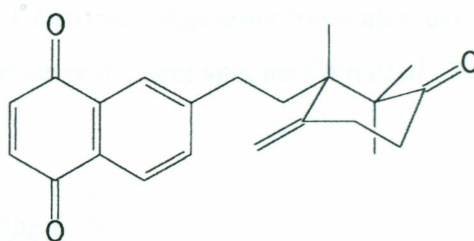
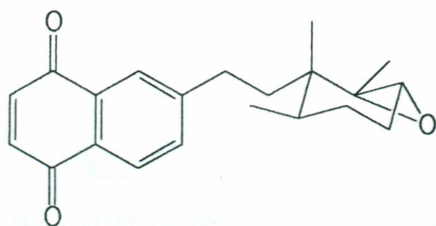
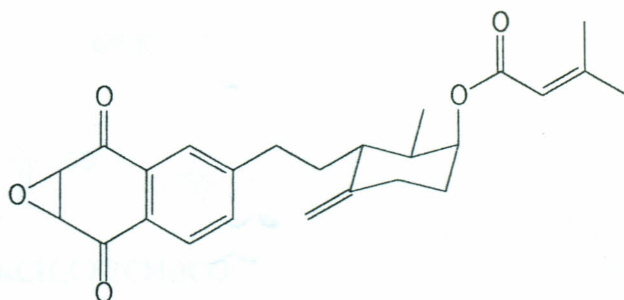




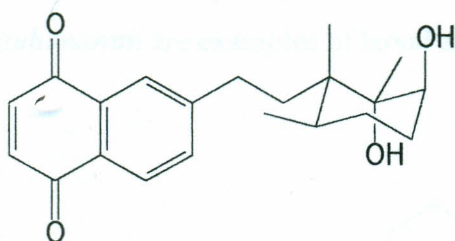
59 **60**

The closely related compounds cardiaquinone E (**61**), cardiaquinone F (**62**) and cardiaquinone G (**63**) isolated from the roots of *C. linnaei* have also shown activity against the larvae of *Ae. aegypti* (Ioset *et al.*, 1998).





61 **62**



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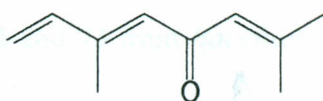
Terpenoids such as 5-*E*-ocimenone (**64**) from *Tagetes minuta* have also been reported to possess larvicidal activity (Maradufu *et al.*, 1978).



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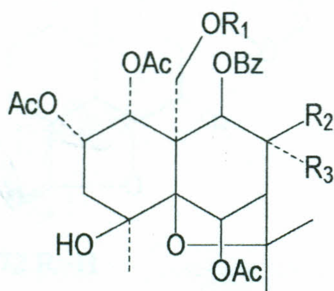
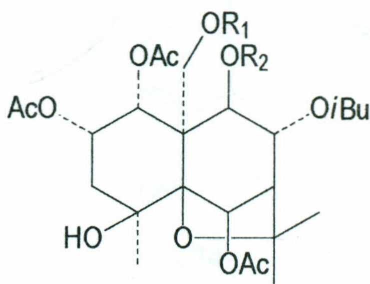
The sesquiterpenoids **65-69** from the roots of *Celastrus angulatus* have also exhibited anti-feedant, narcotic and insecticidal activity against several insect species (Wu *et al*., 2001).





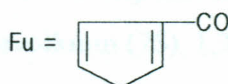
65 R_1=Ac, R_2=OAc, R_3=H

66 R_1=*i*-Bu, R_2=H, R_3=Ofu

67 R_1=*i*-Pet, R_2=Fu

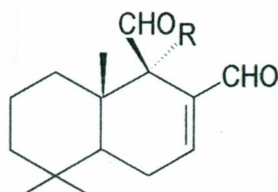
68 R_1=*i*-Bu, R_2=Bz

69 R_1=*i*-Pet, R_2=Bz



i-Bu = $(CH_3)_2CHCO$ *i*-Pet = $CH_3CH_2CH(CH_3)CO$

The drimane sesquiterpenoids, warburganal **(70)** and polygodial **(71)**, isolated from *Warburgia stuhlmannii* are examples of larval anti-feedant substances (Kubo *et al*., 1976; 1977).



70 R=OH

71 R=H

Many *neo*-clerodane diterpenoids with anti-feedant activity against *Tenebrio molitor* larvae have been isolated from plants in the families Compositae (Asteraceae) and Labiatae (Lamiaceae). Examples include **72** and **73** from *Baccharis crispa* and **74** from *B. rethinodes* (Sosa *et al*., 1994).

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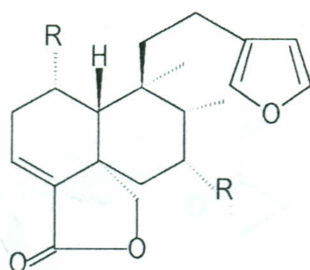
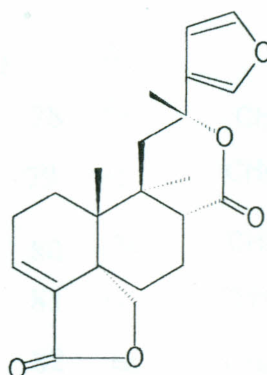


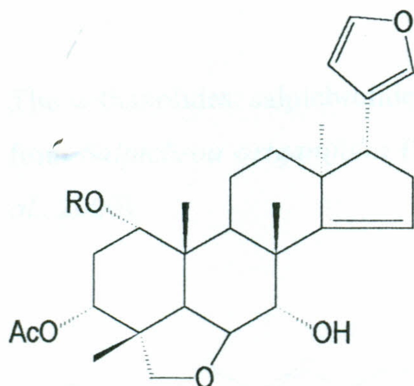


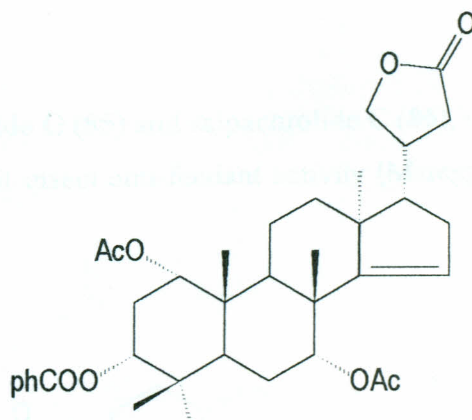
72 R=H

73 R=OH

74

Melia volkensii (Meliaceae) fruit kernel extracts have been found to have acute toxicity and growth inhibiton on *Ae. aegypti* (Mwangi and Rembold, 1987; 1988) and *An. arabiensis* larvae (Mwangi and Mukiama, 1988). Several triterpenoids isolated from this plant have been reported to exhibit larvicidal activity. Meliavolkinin (**75**), 1,3-diacetylvilasinin (**76**) and melianin B (77) have been reported to possess larvicidal activity against *Ae. aegypti* (Rogers *et al.*, 1998).



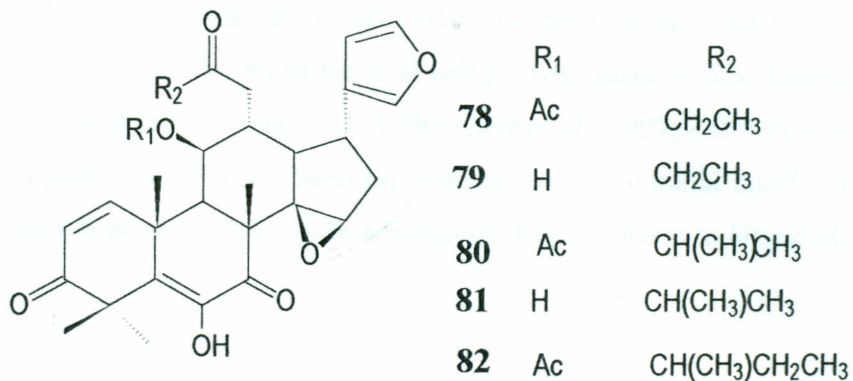


75 R=PhCO

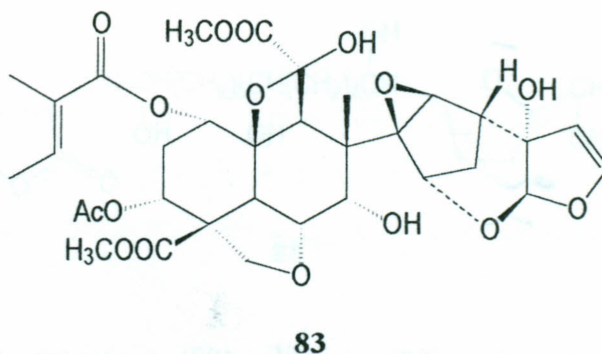
76 R=Ac

77

Tetranorterpenoids (**78-82**) from *Trichilia pallida* (Meliaceae) have anti-feedant activity against larvae of Lepidoptera (Simmonds *et al.*, 2001)

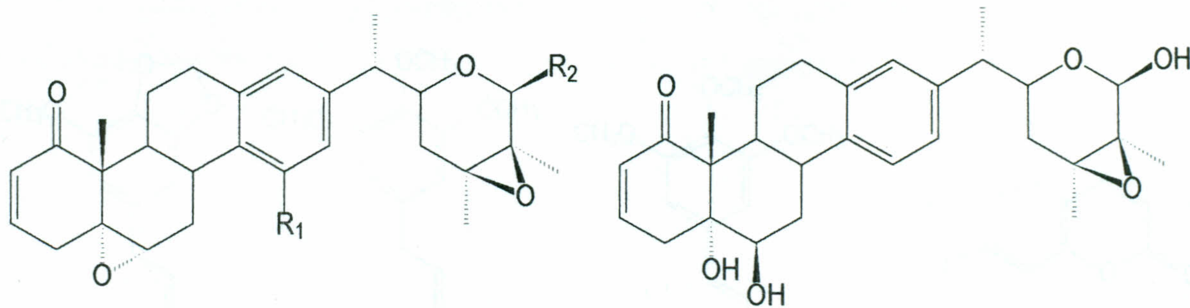


Azadirachtin (**83**), a limonoid isolated from the neem tree (*Azadirachta indica* A. Juss), has been reported to have larvicidal activity against various mosquito species (Zebitz, 1984; 1986). It also disrupts the growth and metamorphosis of insects by interfering with the production of ecdysone and juvenile hormones (Chamagne *et al.*, 1989).



83

The withanolides, salpichrolide A (**84**), salpachrolide G (**85**) and salpachrolide C (**86**), isolated from *Salpichroa origanifolia* (Solanaceae), exhibit insect anti-feedant activity (Mareggiani *et al.*, 2000).

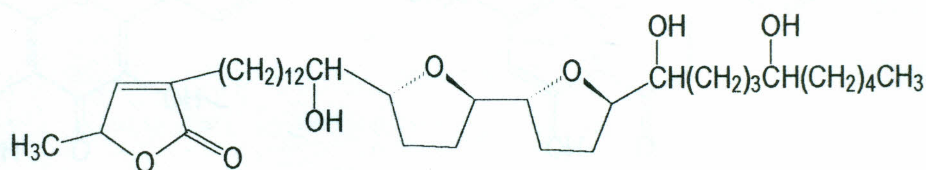


84 $R_1 = H, R_2 = OH$

85 $R_1 = OH, R_2 = OH$

86

Annonaceous acetogenins, a family of secondary metabolites isolated from the plant family Annonaceae, are potent inhibitors of tumoral cell growth, insecticides, fungicides, acaricides, anti-parasitics and herbicides (Zeng *et al*., 1996; Cave *et al*., 1997; Zafra-Polo *et al*., 1998; Alali *et al*., 1999). Squamocin (**87**) has been reported to exhibit ovicidal and larvicidal activity in *Drosophila* (Kawazu *et al*., 1989), while annonacin (**88**) was toxic to larvae of *Ae. aegypti* (He *et al*., 1997).



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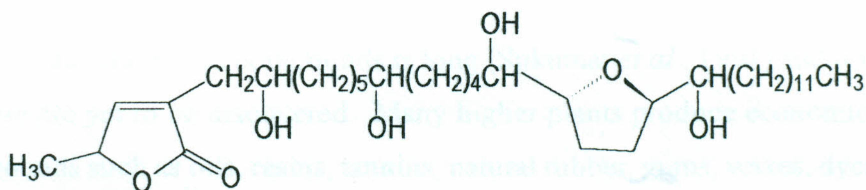
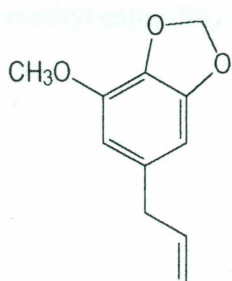
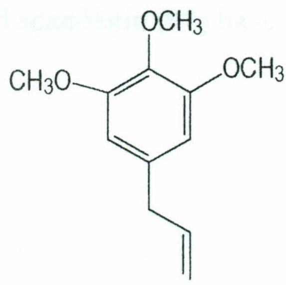
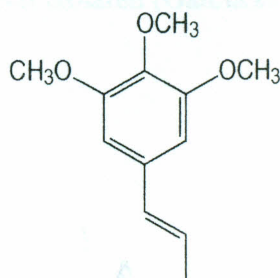


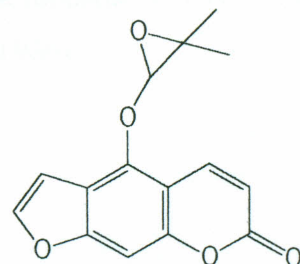
88

The phenylpropanoids, myristicin (**89**), elemicin (**90**) and *trans*-isoelemicin (**91**) and a furanocoumarin, oxypeucedanin (**92**), isolated from the leaves of *Diplolophium buchananii* (Umbelliferae), have been shown to exhibit larvicidal activity against *Ae. aegypti* larvae (Marston *et al*., 1995).



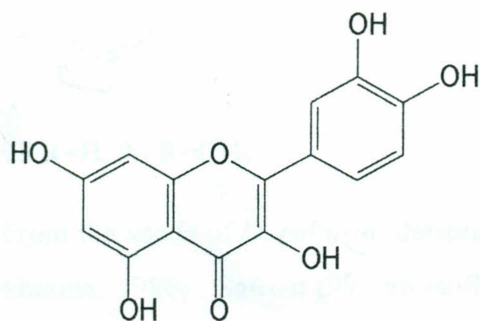






89 **90** **91** **92**

Flavonoids such as quercetin (**93**) and 2′, 6′-dihydroxy-4′-methoxydihydrochalcone (**94**) isolated from *Polygonum senegalense* (Polygonaceae) have been reported to possess larvicidal activity on *Ae. aegypti* larvae (Gikonyo *et al.*, 1998).



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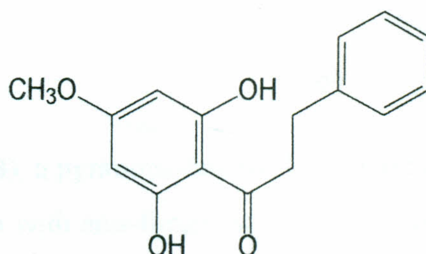


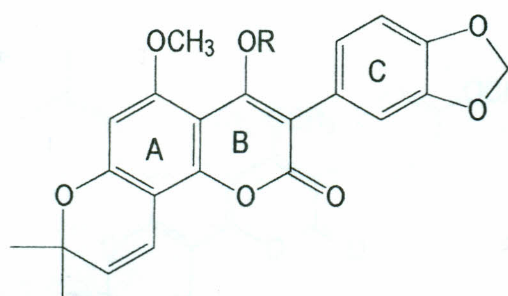
94

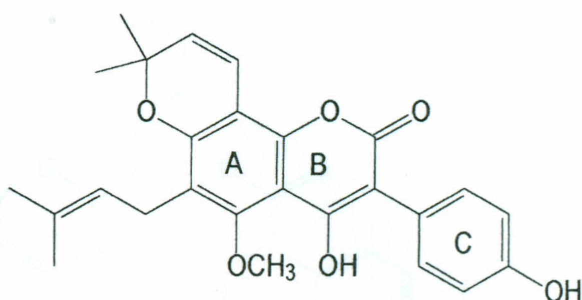
The list of larvicidal plants and compounds is long (Sukumar *et al.*, 1991) and it is believed that many more are yet to be discovered. Many higher plants produce economically important organic compounds such as oils, resins, tannins, natural rubber, gums, waxes, dyes, flavours and fragrances, pharmaceuticals, nutraceuticals, insecticides and pesticides.

2.1 The genus *Derris*

This genus belongs to the tribe Tephrosieae of the Leguminosae family and the sub-family Papilionoidae. Species of this tribe are noted for the profuse production of ring A prenylated flavonoids and isoflavonoids (Garcia *et al.*, 1986).

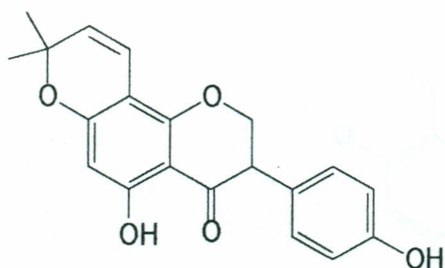
From the roots of *Derris spruceana*, the angular pyranocoumarins like robustin (**95**), robustin methyl ester (**96**) and scandenin (**97**) have been isolated (Garcia *et al.*, 1986).

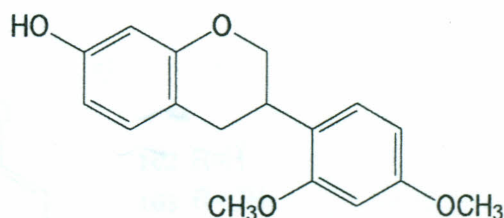




95 R=H, **96** R=CH_3 **97**

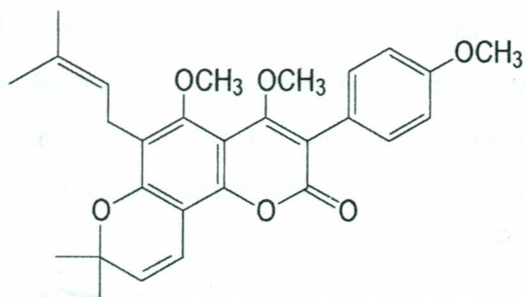
From the seeds of *D. robusta*, derrone (**98**), a pyranoisoflavone has been isolated (Chibber and Sharma, 1980). Sativin (**99**), an isoflavan with anti-fungal activity, has been isolated from the leaves of *D. amazonica* (Harbone and Baxter, 1993).

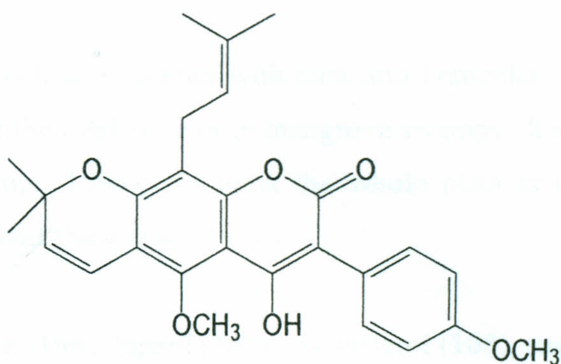




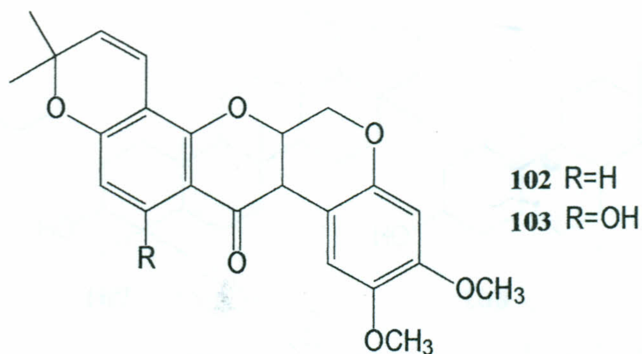
98 **99**

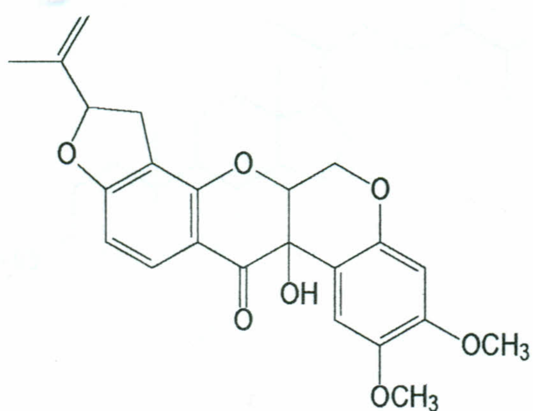
From *D. scandens*, a 4-oxygenated coumarin, 4,4′-di-*O*-methylscandenin (**100**) (Estevez-Braun and Gonzalez, 1997) and lonchocarpenin (**101**), a 3-aryl-4-hydroxycoumarin (Harbone and Baxter, 1993), have been isolated.

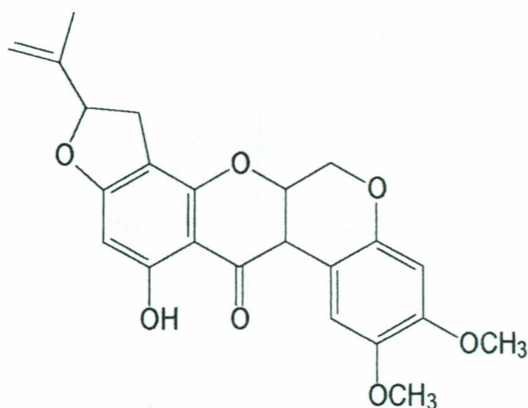




Rotenoids such as deguelin (**102**), rotenone (**40**) and toxicarol (**103**), from *D. elliptica*, 12a-hydroxyrotenone (**104**) from *D. urucu* and sumatrol (**105**) from *D. malaccensis* have been isolated, with all of them exhibiting insecticidal activity (Harbone and Baxter, 1993).



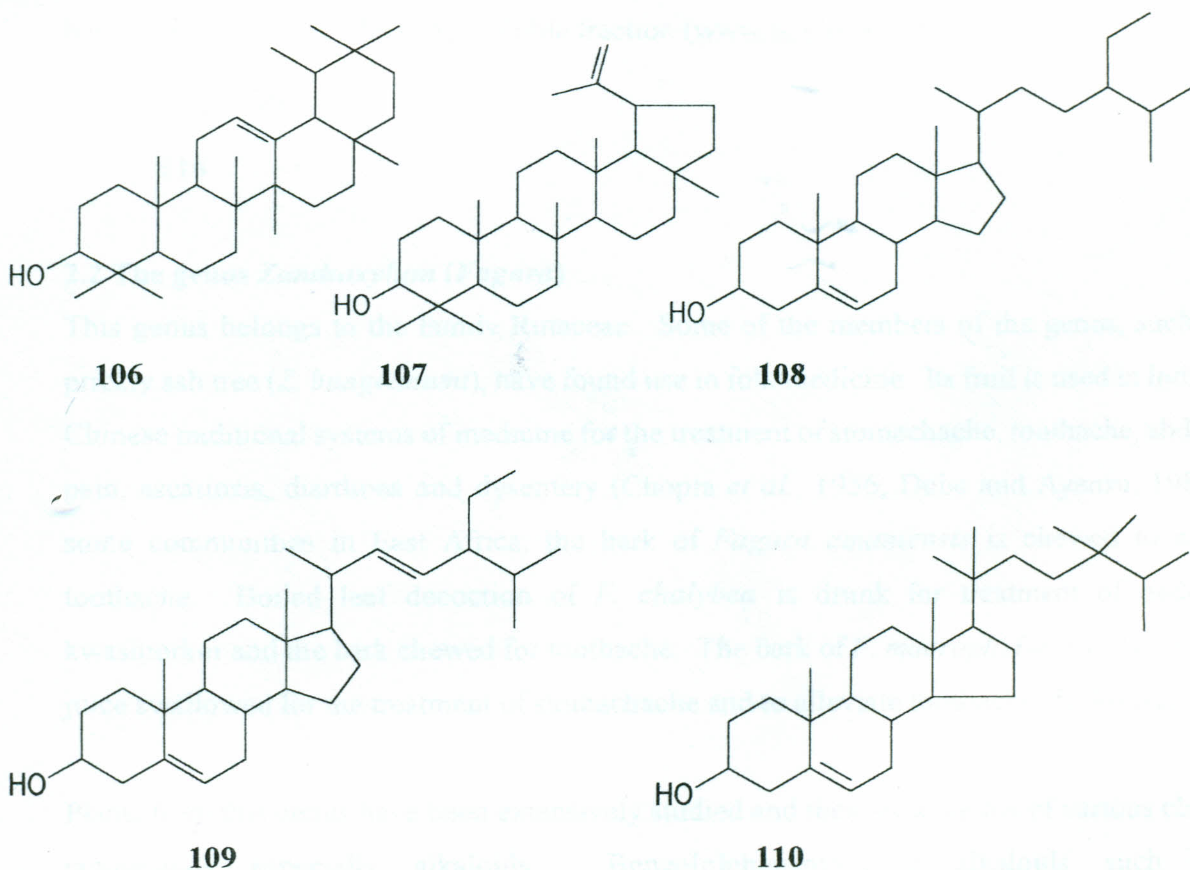


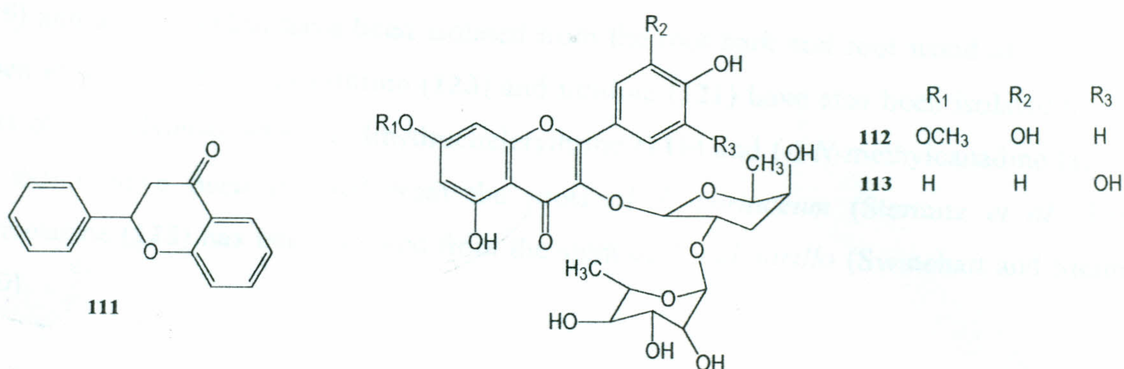


2.1.1 *Derris trifolia*

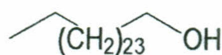
This is a liana with twining branches, which is evergreen with bark and branchlets that have raised lenticels. It grows in riverines near the tidal zone or in mangrove swamps. It is referred to as 'mkuburuku' by the Swahili (Beentje, 1994). In India the whole plant is used as a stimulant, anti-spasmodic and counter-irritant (Nair *et al.*, 1986).

From the leaves of *Derris trifolia* β-amyrin **(106)**, lupeol **(107)**, β-sitosterol **(108)**, stigmasterol **(109)**, campesterol **(110)**, flavanone **(111)** (www.sc.chula.ac.th), rhamnetin 3-*O*-β-neohesperidoside **(112)** and quercetin 3-*O*-β-neohesperidoside **(113)** have been isolated (Nair *et al.*, 1986).





From the ground stem root of *D. trifolia*, hexacosanol (**114**), lupeol (**107**), stigmasterol (**109**) and long chain fatty acids of molecular formulae $C_{26}H_{52}O_2$, $C_{28}H_{56}O_2$ and $C_{30}H_{60}O_2$ have been isolated. In addition, K^+, Na^+, Mg^{2+}, Zn^{2+}, Fe^{2+}, Cl^-, amino acids, glucose, fructose and sucrose have been detected in the water soluble fraction (www.sc.chula.ac.th).



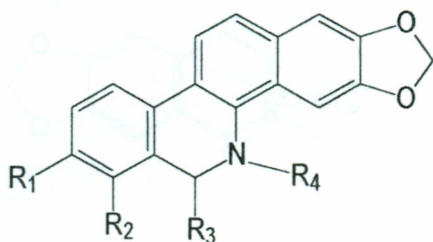
114

2.2 The genus *Zanthoxylum* (*Fagara*)

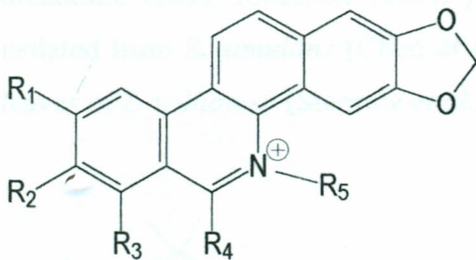
This genus belongs to the family Rutaceae. Some of the members of the genus, such as the prickly ash tree (*Z. bungeanumi*), have found use in folk medicine. Its fruit is used in Indian and Chinese traditional systems of medicine for the treatment of stomachache, toothache, abdominal pain, ascariosis, diarrhoea and dysentery (Chopra *et al.*, 1956; Dube and Ayensu, 1985). In some communities in East Africa, the bark of *Fagara amaniensis* is chewed to alleviate toothache. Boiled leaf decoction of *F. chalybea* is drunk for treatment of oedema in kwashiorkor and the bark chewed for toothache. The bark of *F. macrophylla* is chewed and the juice swallowed for the treatment of stomachache and to alleviate toothache (Kokwaro, 1976).

Plants from this genus have been extensively studied and they are a source of various classes of compounds especially alkaloids. Benzo[c]phenanthridine alkaloids such as 6-methylnorchelerythrine (**115**), dihydrochelerythrine (**116**), boccolline (**117**), 8-acetonyldihydrochelerythrine (**118**), oxychelerythrine (**119**), decarine (**120**), nitidine (**121**), norchelerythrine (**122**), chelerythrine (**123**), 6-methylhydrochelerythrine (**124**), arnottianamide

(**125**) and avicine (**126**) have been isolated from the root bark and root wood of *Z. simulans* (Chen *et al.*, 1994). Chelerythrine (**123**) and nitidine (**121**) have also been isolated from the roots of *Z. williamsii* whereas dihydrochelerythrine (**116**) and (-)-*N*-methylcanadine (**127**), as the iodide have been isolated from the roots of *Z. coriaceum* (Stermitz *et al.*, 1980). Tembetarine (**128**) has been isolated from the stem of *Z. culantrillo* (Swinehart and Stermitz, 1980).



	R_1	R_2	R_3	R_4
115	OCH_3	OCH_3	CH_3	CH_3
116	OCH_3	OCH_3	H	CH_3
117	OCH_3	OCH_3	CH_2OH	CH_3
118	OCH_3	OCH_3	CH_2COCH_3	CH_3
119	OCH_3	OCH_3	O	CH_3
120	OH	OCH_3	H	_



	R_1	R_2	R_3	R_4	R_5
121	OCH_3	OCH_3	H	H	CH_3
122	H	H	H	H	_
123	H	OCH_3	OCH_3	H	CH_3
124	H	H	H	CH_3	CH_3

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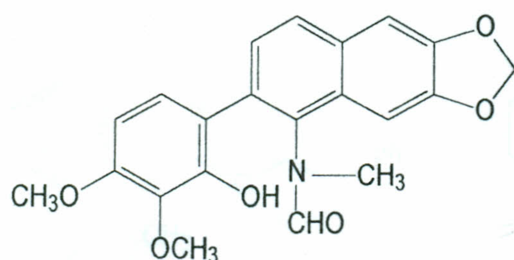

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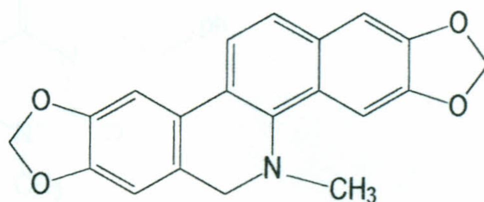

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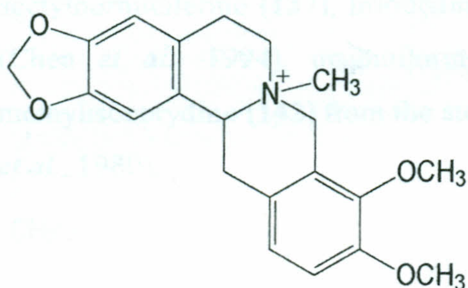

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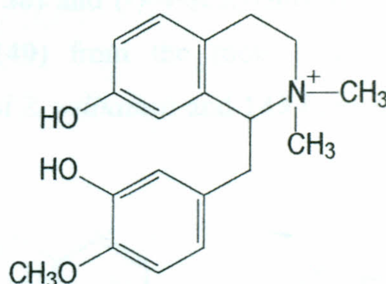
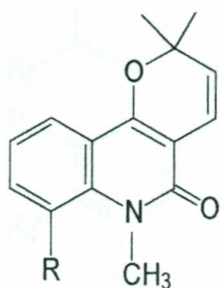
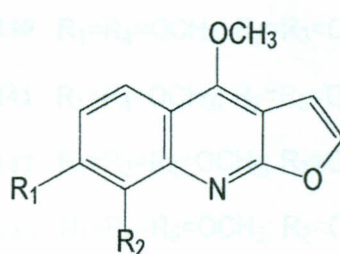


128

Quinoline alkaloids such as *N*-methylflindersine (**129**), 8-methoxy-*N*-methylflindersine (**130**), dictamine (**131**), robustine (**132**), γ-fagarine (**133**) and skimmianine (**134**) have also been isolated from *Z. simulans* (Chen *et al.*, 1994). Skimmianine has also been isolated from the leaves of *Z. williamsii* (Stermitz *et al.*, 1980).

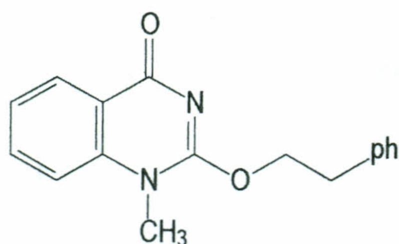


129 R=OCH_3
130 R=H



	R_1	R_2
131	H	H
132	H	OH
133	H	OCH_3
134	OCH_3	OCH_3

4-Quinazoline alkaloids such as 1-methyl-3-(2′-phenylethyl)-*1H,3H*-quinazoline-2,4-dione (**135**) and 1-methyl-3-[2′-(4″-methoxyphenyl)ethyl]-*1H,3H*-quinazoline-2,4-dione (**136**) have been isolated from the seed husks of *Z. arborescens* (Dreyer and Brenner, 1980).



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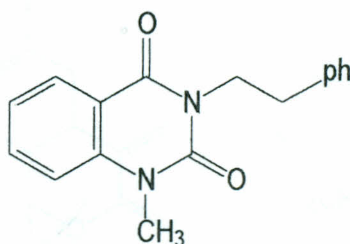
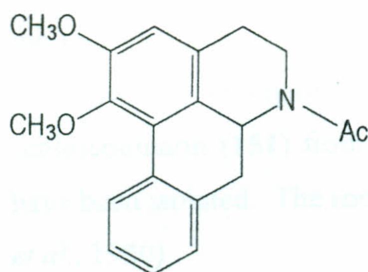


136

Aporphine alkaloids have also been isolated from members of this genus. These include (-)-*N*-acetylnornuciferine (**137**), liriodenine (**138**) and (-)-*N*-acetylanonaine (**139**) from *Z. simulans* (Chen *et al.*, 1994), magnoflorine (**140**) from the roots, and laurifoline (**141**), (-)-*N*-methylisocorydine (**142**) from the stem of *Z. williamsii* and **143** from *Z. tingoassuiba* (Stermitz *et al.*, 1980).



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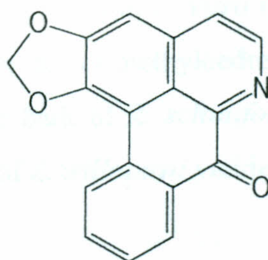

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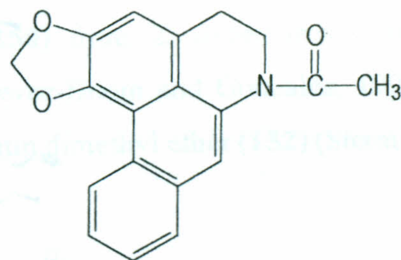

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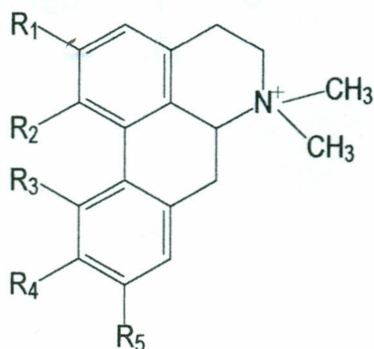


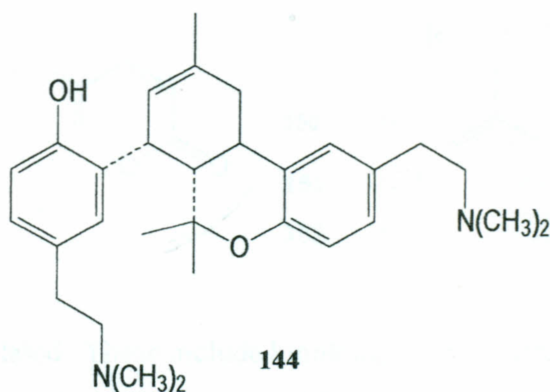
140 $R_1=R_4=OCH_3$; $R_2=R_3=OH$; $R_5=H$

141 $R_1=R_4=OCH_3$; $R_2=R_5=OH$; $R_3=H$

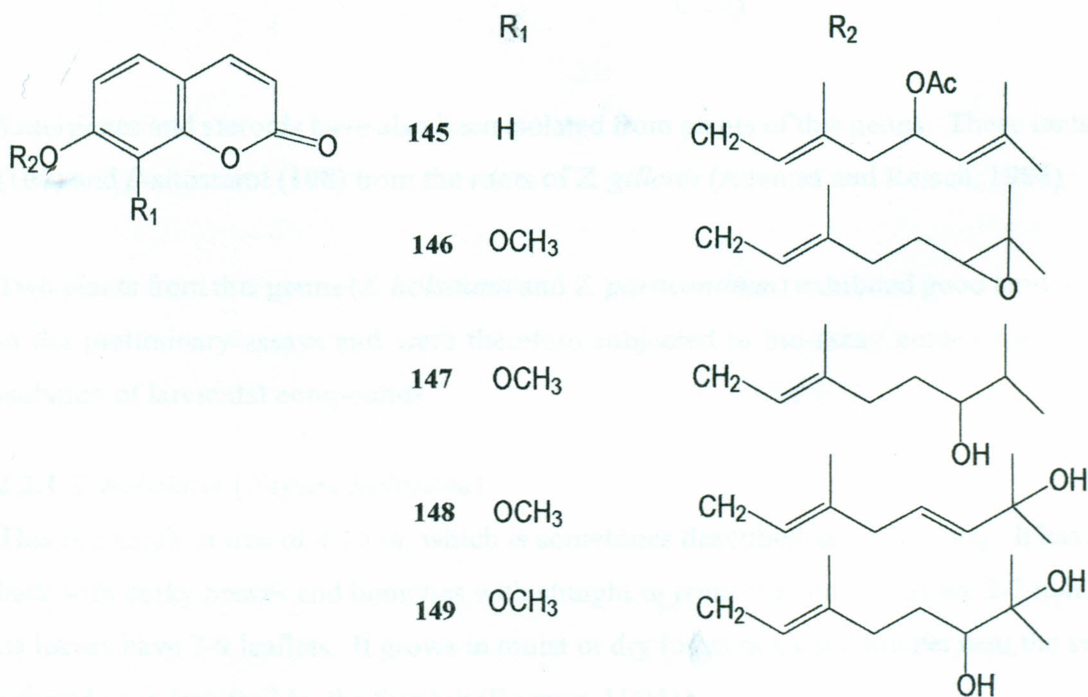
142 $R_1=R_2=R_4=OCH_3$; $R_3=OH$; $R_5=H$

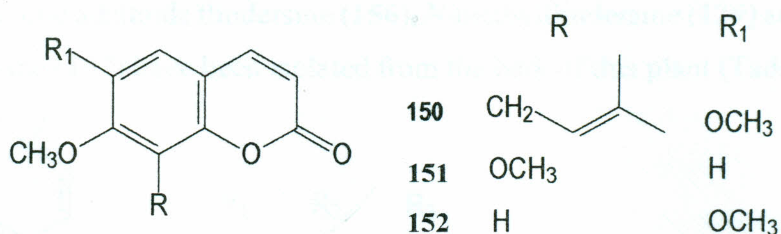
143 $R_1=R_4=R_5=OCH_3$; $R_2=OH$; $R_3=H$

From the leaves of *Z. punctatum*, alfileramine (**144**), a bishordeninyl terpene alkaloid has been isolated (Caolo and Stermitz, 1979; Swinehart and Stermitz, 1980).

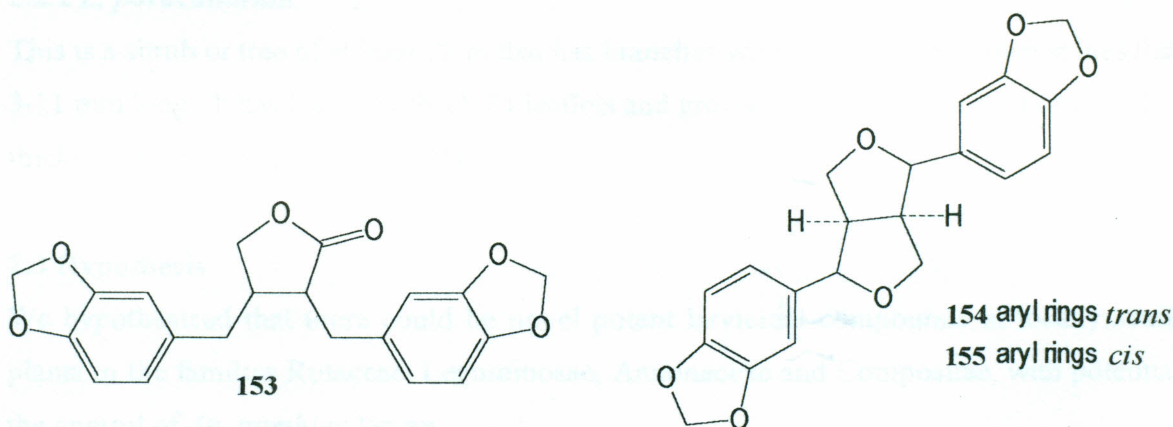


Coumarins have also been isolated from members of this genus. These include 7-oxygenated coumarins like acetoxyaurapten (**145**) and 7,8-dioxygenated coumarins such as epoxycollinin (**146**), schinallylol (**147**), schinilenol (**148**) and schinindiol (**149**) isolated from the bark of *Z. schinifolium*. Acetoxyaurapten (**145**) and schinallylol (**147**) have been shown to exhibit inhibitory activity on platelet aggregation *in vitro* (Estevez-Braun and Gonzalez, 1997). 6,7-Dioxygenated coumarins such as *O*-methylcedrelopsin (**150**) from *Z. usambarense* and schinicoumarin (**151**) from the bark of *Z. schinifolium* (Estevez-Braun and Gonzalez, 1997) have been isolated. The roots of *Z. williamsii* yielded esculentin dimethyl ether (**152**) (Stermitz *et al.*, 1980).





Lignans have also been isolated. These include hinokinin (**153**) isolated from *Z. simulans* (Chen *et al*., 1994), (+)-asaranin (**154**) and (+)-sesamin (**155**) from the roots of *Z. williamsii* (Stermitz *et al*., 1980).



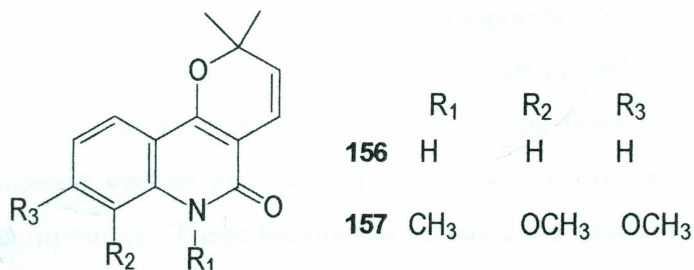
Triterpenes and steroids have also been isolated from plants of this genus. These include lupeol (**107**) and *β*-sitosterol (**108**) from the roots of *Z. gillettii* (Adenisa and Reisch, 1988).

Two plants from this genus (*Z. holstiana* and *Z. paracanthum*) exhibited good larvicidal activity in the preliminary assays and were therefore subjected to bio-assay guided fractionation and isolation of larvicidal compounds.

2.2.1 *Z. holstiana* (*Fagara holtziana*)

This is a shrub or tree of 4-15 m, which is sometimes described as scrumbling. It has a grey bark with corky bosses and branches with straight or recurved spines that are 2-7 mm long and its leaves have 7-9 leaflets. It grows in moist or dry forest or closed thicket near the sea and is referred to as 'mjafari' by the Swahili (Beentje, 1994).

The pyranoquinolone alkaloids flindersine (**156**), *N*-methylflindersine (**129**) and 7,8-dimethoxy-*N*-methylflindersine (**157**) have been isolated from the bark of this plant (Tadao *et al*., 1995).



	R1	R2	R3
156	H	H	H
157	CH_3	OCH_3	OCH_3

2.2.2 *Z. paracanthum*

This is a shrub or tree of at least 10 m that has branches with straight or upturned spines that are 3-11 mm long. It has leaves with 11-23 leaflets and grows in moist or dry forest or closed thicket near the sea (Beentje, 1994).

2.3 Hypothesis

We hypothesised that there could be novel potent larvicidal compounds in locally available plants in the families Rutaceae, Leguminosae, Annonaceae and Compositae, with potential for the control of *An. gambiae* larvae.

2.4 Objectives

The general objective was to screen, isolate and characterize larvicidal compounds from plants in the families Rutaceae, Leguminosae, Annonaceae and Compositae (Asteraceae).

The specific objectives were: (i) to extract potential larvicidal plants and screen the extracts by high throughput bio-assay for larvicidal activity; (ii) fractionate the larvicidal extracts and bio-assay the fractions; and (iii) isolate larvicidal compounds and characterize them by spectroscopic techniques.

2.5 Justification

Despite advances in control and treatment methods, malaria is the most widespread of all tropical diseases and one of the most lethal. It remains endemic in most tropical countries and in the northern sub-tropical zone. With the emergence of drug resistant *Plasmodium* parasites

and increasing cost of drugs, there is need for employment of other methods to control malaria. One of the methods employed more vigorously is vector control via insecticides that kill the mosquito at various stages of development. Synthetic organic insecticides, although highly efficacious against target species, are detrimental to a variety of animal life including man. In addition to adverse environmental effects from the use of conventional insecticides, major disease vectors and pest species have become physiologically resistant to many of these compounds. These factors have created the need for environmentally safe, bio-degradable and target-specific insecticides against mosquitoes.

Plants synthesize a dazzling array of compounds with various structural types, which exhibit an almost equal array of biological activities. Various plant compounds affect insects differently. Pyrethrins and nicotine affect nerve axons and synapses. Some of these compounds have already been exploited commercially, while others offer a unique opportunity as sources and models of new insect control agents. There is urgent need to exploit plant sources for natural compounds that will be eco-safe and target-specific. This might ease the malaria burden and generate income for the rural communities where such plants can be cultivated on a large scale for commercial exploitation.

CHAPTER THREE

LARVICIDAL ACTIVITY STUDIES

3.1 Preliminary larvicidal assays

The preliminary larvicidal assay of 4 extracts (hexane, dichloromethane, ethyl acetate and methanol) was carried out at 100 ppm for the 9 plants (*Zanthoxylum paracanthum* (bark), *Z. holstiana* (stem bark), *Z. chalybeum* (stem bark), *Derris trifolia* (root bark), *Monondora grandieri* (stem bark), *Mkilua fragrans* (stem bark), *Isolana cauliflora* (stem bark), *Bidens schimperi* (root) and *Salsola monoica* (leaves and stem bark)) and those that showed activity were assayed further at lower concentration ranges (10-1 ppm) (Tables 1-4).

Table 1: Percentage mortality of 3rd instar *An. gambiae* larvae in aqueous medium treated with hexane extracts

	% Mortality											
Time (h)	24				48				72			
Conc. (ppm)	100	10	5	2	100	10	5	2	100	10	5	2
Z. paracanthum	100	100	100	32	-	-	-	32	-	-	-	40
Z. holstiana	100	100	82	0	-	-	90	0	-	-	100	94
Z. chalybeum	100	100	64	28	-	-	80	36	-	-	88	40
D. trifolia	100	88	30	24	-	92	50	40	-	100	68	62
M. grandieri	100	100	68	-	-	-	82	-	-	-	100	-
M. fragrans	80	44	-	-	88	68	-	-	92	76	-	-
I. cauliflora	100	88	32	-	-	92	64	-	-	100	68	-
B. schimperi	96	0	-	-	100	0	-	-	-	0	-	-
S. monoica (b)	92	0	-	-	100	4	-	-	-	4	-	-
S. monoica (l)	0	-	-	-	0	-	-	-	26	-	-	-

Table 2: Percentage mortality of 3rd instar *An. gambiae* larvae in aqueous medium treated with CH_2Cl_2 extracts

%Mortality

Time (h)	24					48					72				
Conc. (ppm)	100	10	5	2	1	100	10	5	2	1	100	10	5	2	1
Z. paracanthum	100	100	82	20	-	-	-	90	22	-	-	-	90	24	-
Z. holstiana	100	100	90	6	-	-	-	92	6	-	-	-	94	6	-
Z. chalybeum	100	100	72	40	-	-	-	84	44	-	-	-	100	64	-
D. trifolia	100	100	96	74	18	-	-	98	82	34	-	-	98	92	34
M. grandieri	100	36	-	-	-	-	52	-	-	-	-	72	-	-	-
M. fragrans	0	-	-	-	-	16	-	-	-	-	16	-	-	-	-
I. cauliflora	32	-	-	-	-	44	-	-	-	-	56	-	-	-	-
B. schimperi	92	4	-	-	-	100	4	-	-	-	-	4	-	-	-
S. monoica (b)	6	-	-	-	-	30	-	-	-	-	72	-	-	-	-
S. monoica (l)	0	-	-	-	-	26	-	-	-	-	26	-	-	-	-

Table 3: Percentage mortality of 3rd instar *An. gambiae* larvae in aqueous medium treated with EtOAc extracts

% Mortality

Time (h)	24				48				72			
Conc. (ppm)	100	10	5	2	100	10	5	2	100	10	5	2
Z. paracanthum	100	100	72	10	-	-	76	10	-	-	82	12
Z. holstiana	100	100	88	14	-	-	90	14	-	-	92	14
Z. chalybeum	100	100	100	60	-	-	-	72	-	-	-	80
D. trifolia	100	100	70	46	-	-	82	54	-	-	98	66
M. grandieri	12	-	-	-	20	-	-	-	28	-	-	-
M. fragrans	92	0	-	-	100	0	-	-	-	0	-	-
I. cauliflora	12	-	-	-	48	-	-	-	52	-	-	-
B. schimperi	94	0	-	-	100	0	-	-	-	0	-	-
S. monoica (b)	32	-	-	-	66	-	-	-	90	-	-	-
S. monoica (l)	0	-	-	-	48	-	-	-	52	-	-	-

Table 4: Percentage mortality of 3rd instar *An gambiae* larvae in aqueous medium treated with MeOH extracts

	% Mortality								
Time (h)	24			48			72		
Conc. (ppm)	100	10	5	100	10	5	100	10	5
Z. paracanthum	100	72	24	-	100	40	-	-	54
Z. holstiana	100	52	12	-	80	48	-	92	52
Z. chalybeum	100	100	32	-	-	40	-	-	64
D. trifolia	96	60	-	100	76	-	-	76	-
M. grandieri	32	-	-	44	-	-	48	-	-
M. fragrans	52	-	-	76	-	-	76	-	-
I. cauliflora	84	40	16	100	72	36	-	88	48
B. schimperi	82	0	-	100	0	-	-	0	-
S. monoica (b)	2	-	-	50	-	-	68	-	-
S. monoica (l)	2	-	-	2	-	-	2	-	-

b-bark, l-leaves

The methanol extracts exhibited the lowest activity for all the plants. Three extracts with the highest activity were selected for further detailed bio-assays. These comprised CH_2Cl_2 extracts of *Z. paracanthum* and *D. trifolia* and EtOAc extract of *Z. holstiana*. The bio-assay data was subjected to linear regression analysis (Busvine, 1971; Finney, 1971) (Tables 5-9).

Table 5: Probit analysis of larvicidal data for *Z. paracanthum* CH_2Cl_2 extract

	Log dose +1	% Mortality			Empirical probit		
Conc (ppm)/time (h)		24	48	72	24	48	72
10	2	100	-	-	-	-	-
5	1.7	82	90	90	5.92	6.28	6.28
2	1.3	20	22	24	4.16	4.23	4.29

The regression equations obtained were $y=4.4x-1.56$, $y=5.125x-2.4325$ and $y=4.975x-2.1775$ after 24, 48 and 72 hours, respectively.

Table 6: Probit analysis of the larvicidal data for *Z. holstiana* EtOAc extract

	Log dose +1	% Mortality			Empirical probit		
Conc (ppm)/time (h)		24	48	72	24	48	72
10	2	100	-	-	-	-	-
5	1.7	88	90	92	6.18	6.28	6.41
2	1.3	14	14	14	3.92	3.92	3.92

The regression equations obtained were y=5.65x-3.425, y=5.9x-3.75 and y=6.225x-4.1725 after 24, 48 and 72 hours, respectively.

Table 7: Probit analysis of larvicidal data for *D. trifolia* CH_2Cl_2 extract

	Log dose +1	% Mortality			Empirical probit		
Conc (ppm)/time (h)		24	48	72	24	48	72
10	2	100	-	-	-	-	-
5	1.7	96	98	98	6.75	7.05	7.05
2	1.3	74	82	92	5.64	5.92	6.41
1	1	18	34	38	4.08	4.59	4.69

The regression equations obtained were y=3.7581x+0.4792, y=3.477x+1.2173 and y=3.2757x+1.6824 after 24, 48 and 72 hours, respectively.

From the regression lines obtained the LC_{90}, LC_{50} and LC_{25} of the extracts were calculated (Table 8).

Table 8: LC of *Z. paracanthum*, *Z. holstiana* and *D. trifolia* extracts

	Lethal concentration (ppm)								
	LC_{25}			LC_{50}			LC_{90}		
Time (h)	24	48	72	24	48	72	24	48	72
Z. paracanthum (CH_2Cl_2)	2.18	2.09	2.03	3.10	2.82	2.77	6.05	5.01	5.01
Z. holstiana (EtOAc)	2.36	2.34	2.32	3.10	3.04	2.98	5.22	5.01	4.78
D. trifolia (CH_2Cl_2)	1.06	0.79	0.65	1.60	1.22	1.03	3.50	2.86	2.53

D. trifolia CH_2Cl_2 extract exhibited the highest larvicidal activity after 24 hours while the activities of *Z. paracanthum* (CH_2Cl_2 extract) and *Z. holstiana* (EtOAc extract) were the same after 24 hours. The three extracts were therefore fractionated by column chromatography on silica gel and the fractions re-evaluated for their larvicidal activity on 3rd instar *An. gambiae* larvae.

3.2 Fractionation of *D. trifolia* CH_2Cl_2 extract and bio-assay of fractions

The dichloromethane extract of *D. trifolia* was subjected to column chromatography on silica gel into 77 fractions with an *n*-hexane-ethyl acetate gradient (100:0 →0:100). Based on the R_f values, they were pooled into 15 fractions (F_{1D}-F_{15D}). Fractions F_{1D}, F_{2D}, F_{5D}, F_{6D}, F_{8D}, F_{9D} and F_{10D} were available in adequate amounts and thus were assayed at 10 ppm against 3rd instar *An. gambiae* larvae (Table 9). F_{1D}, F_{2D}, F_{5D} and F_{10D} showed no larvicidal activity at 10 ppm. F_{6D} showed 2% mortality at 48 and 72 hours. F_{8D} and F_{9D} exhibited 94 and 100% mortality after 24 and 72 hours, respectively. They were assayed at lower concentrations to determine the LC_{90}, LC_{50} and LC_{25} values. F_{3D}, F_{4D}, F_{7D} F_{11D} and F_{12D} were available in very minute amounts and therefore could not be assayed. F_{13D}, F_{14D} and F_{15D} were insoluble in acetone and only slightly soluble in ethanol or water (the bio-assay solvents).

Table 9: % Mortality of 3rd instar *An. gambiae* larvae in *D. trifolia* dichloromethane extract fractions at 10 ppm

	% Mortality						
Time (h)	F_{1D}	F_{2D}	F_{5D}	F_{6D}	F_{8D}	F_{9D}	F_{10D}
24	0	0	0	0	94	94	0
48	0	0	0	2	100	100	0
72	0	0	0	2	-	-	0

3.3 Detailed larvicidal assay of pure compounds from *D. trifolia* dichloromethane extract

F_{1D} afforded 11.3 mg of D_1, which was found to be impure by NMR analysis. It was recrystallised in acetone to give D_{1A} (1.5 mg) that turned out to be insoluble in $CDCl_3$, DMSO and acetone. The compound was therefore not assayed due to inadequate quantities. F_{5D} was cleaned with ether to yield 20 mg of D_2. Through preparative thin layer chromatography (35%

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ethyl acetate in hexane, SiO_2), 5.0 mg of D_{3A} was obtained from F_{6D}. Similarly, 65.4 mg of F_{10D} gave D_4 (4.1 mg) through preparative thin layer chromatography (30% hexane in ethyl acetate, SiO_2). However, D_4 was impure and was processed further by PTLC. Through preparative thin layer chromatography, 20.4 mg of D_7 was obtained from F_{8D} and F_{9D}. D_7 was assayed against *An. gambiae* and data subjected to probit analysis (Table 10).

Table 10: % Mortality of 3rd instar *An. gambiae* larvae in D_7 solutions and probit analysis

Conc (ppm)	Log dose +1	% Mortality ± SD			Empirical probit		
		24h	48h	72h	24h	48h	72h
10	2	100	-	-	-	-	-
5	1.7	100	-	-	-	-	-
2	1.3	94±0.55	94±0.55	96±0.55	6.55	6.55	6.75
1	1	43.33±1.52	53.33±1.52	56.67±2.08	4.82	5.08	5.18
0.75	0.9	26.67±2.52	33.33±3.06	40.0±3.0	4.39	4.56	4.75
0.5	0.7	26.67±0.58	30.0±1.0	30.0±1.0	4.39	4.48	4.48
0.25	0.4	0	5.0±0.71	10.0±0	-	3.96	3.72

The regression equations obtained were $y=3.8013x+1.3312$, $y=2.7372x+2.572$ and $y=3.2239x+2.2035$ after 24, 48 and 72 hours, respectively.

From the regression equations obtained, the LC_{90}, LC_{50} and LC_{25} values after 24, 48 and 72 hours were calculated (Table 11).

Table 11: LC of D_7 for *An. gambiae*

	Lethal concentration (ppm)		
Time (h)	LC_{25}	LC_{50}	LC_{90}
24	0.6	1.8	2.3
48	0.4	0.8	2.0
72	0.5	0.7	1.8

Since the other compounds (D_2, D_{3A} and D_4) were obtained in low yield and had not shown any larvicidal activity at 10 ppm they were not assayed further.

3.4 Fractionation of *Z. holstiana* ethyl acetate extract and bio-assay of fractions

The ethyl acetate extract was fractionated by column chromatography, on silica gel with an *n*-hexane-ethyl acetate gradient (100:0→0:100), to give 33 fractions which were pooled into 12 fractions (F_{1H}-F_{12H}) based on R_f values. F_{1H}, F_{2H}, F_{3H}, F_{4H}, F_{8H}, F_{9H} and F_{10H} were obtained in substantial amounts. F_{8H}, F_{9H} and F_{10H} were not soluble in acetone or water. They were only slightly soluble in ethanol and hence could not be assayed. F_{5H}, F_{6H} and F_{7H} were obtained in small quantities and therefore were not assayed. F_{1H}, F_{2H}, F_{3H} and F_{4H} were assayed against 3rd instar *An. gambiae* larvae and mortality monitored after 24, 48 and 72 hours. At 10 ppm, F_{3H} exhibited 100% mortality after 24 hours (Table 12). F_{4H} exhibited 54% mortality after 72 hours whereas F_{1H} and F_{2H} exhibited no larvicidal activity at this concentration.

Table12: % Mortality of 3rd instar *An. gambiae* larvae in *Z. holstiana* ethyl acetate extract fractions (10 ppm)

	% Mortality			
Time (h)	F_{1H}	F_{2H}	F_{3H}	F_{4H}
24	0	0	100	26
48	0	0	-	50
72	0	0	-	54

F_{2H} was cleaned with ether and 82.4 mg of H_1 obtained. By preparative thin layer chromatography (30% ethyl acetate in hexane, SiO_2), F_{3H} afforded H_5 (30.0 mg), H_6 (12.2 mg) and H_7 (18.7 mg). F_{7H} gave H_2 (2.5 mg). H_4 (4.2 mg) was obtained from F_{6H}. F_{1H} and F_{4H} were a mixture of compounds, which proved difficult to separate. F_{10H} afforded 4.3 mg of H_3.

3.5 Larvicidal assays of pure compounds from *Z. holstiana* ethyl acetate extract

H_2, H_4 and H_6 were available in inadequate amounts and hence could not be assayed. H_1, H_5 and H_7 were assayed further against 3rd instar *An. gambiae* larvae (Table 13). H_1 exhibited no larvicidal activity at the highest concentration assayed (50 ppm). At 10 ppm, H_5 exhibited

100% mortality after 48 hours while the same mortality was achieved after 72 hours at 5 ppm (Table 13). At 2.5 ppm, H_5 exhibited 6.67, 30 and 43.3% mortality after 24, 48 and 72 hours, respectively. H_7 exhibited 100% mortality at 10 and 5 ppm after 24 and 72 hours, respectively (Table 13). Mortality of 86% was obtained at 2.5 ppm after 72 hours.

Table 13: % Mortality of 3rd instar *An. gambiae* larvae in H_5 and H_7 solutions and probit analysis

	Conc. (ppm)	Log dose +1	Mortality (%) ± SD			Empirical probit		
Time (h)			24	48	72	24	48	72
H_5	10	2	56.7±2.08	100		5.18	-	
	5	1.7	26.67±3.06	63.33±0.67	100	4.39	5.33	-
	2.5	1.4	6.67±0.67	30.0±1.0	43.3±1.53	3.52	4.48	4.82
	2	1.3	3.3±0.58	20.0±0	30.0±1.0	3.12	4.16	4.48
	1	1	3.3±0.58	6.67±0.67	20.0±0	3.12	3.52	4.16
H_7	10	2	100	-	-	-	-	-
	5	1.7	88±0.84	90±0.67	100	6.18	6.28	
	2.5	1.4	56±1.0	70±2.49	86±0.67	5.15	5.52	6.08
	2	1.3	16±0.47	24±0.89	36±1.0	4.01	4.29	4.64
	1	1	0	0	0	-	-	-
	0.5	0.7	0	0	0	-	-	-

The regression equations were obtained for H_5 as $y=2.2425x+0.5471$, $y=2.598x+0.8652$ and $y=1.5154x+2.6177$ after 24, 48 and 72 hours, respectively. Similarly the equations obtained for H_7 were: $y=4.9654x-2.1692$, $y=4.4115x-1.1069$ and $y=14.4x-14.08$ after 24, 48 and 72 hours, respectively. From the equations the LC of H_5 and H_7 were calculated (Table 14).

Table 14: LC of H_5 and H_7 for *An. gambiae*

	Lethal concentration (ppm)								
	LC_{25}			LC_{50}			LC_{90}		
Time (h)	24	48	72	24	48	72	24	48	72
H_5	4.9	2.2	1.4	9.7	3.9	3.7	36.0	12.1	26.1
H_7	2.0	1.7	1.9	2.8	2.4	2.1	5.0	4.7	2.6

3.6 Fractionation of *Z. paracanthum* dichloromethane extract and bio-assay of fractions

Dichloromethane extract was subjected to column chromatography on silica gel with an` *n*-hexane-ethyl acetate gradient (100:0→0:100) to give 249 fractions, which were pooled into 18 fractions (F_{1P}-F_{18P}) based on their R_f values. F_{1P}, F_{3P}, F_{5P}, F_{6P}, F_{7P}, F_{8P}, F_{9P}, F_{10P}, F_{11P}, F_{12P}, F_{15P} and F_{16P} were of substantial amounts and hence were assayed against 3rd instar *An. gambiae* larvae at 10 ppm (Table 15). F_{1P}, F_{3P}, F_{5P} and F_{16P} exhibited no larvicidal activity at 10 ppm after 72 hours whereas fractions F_{9P}, F_{10P}, F_{11P}, F_{12P} and F_{15P} exhibited 30, 50, 20, 34 and 10% mortality, respectively, after 72 hours at the same concentration. F_{7P} and F_{8P} exhibited 100% mortality after 24 hours while F_{6P} exhibited 84% mortality after 72 hours at 10 ppm. These were subjected to further bio-assay at 5 and 1 ppm.

Table 15: % Mortality of 3rd instar *An. gambiae* larvae in *Z. paracanthum* ethyl acetate extract fractions

Time (h)	% Mortality													
	F_{6P}			F_{7P}			F_{8P}			F_{9P}	F_{10P}	F_{11P}	F_{12P}	F_{15P}
Conc (ppm)	10	5	1	10	5	1	10	5	1	10	10	10	10	10
24	60	28	0	100	100	14	100	100	36	24	20	6	10	0
48	68	36	4	-	-	18	-	-	40	30	26	16	24	3.3
72	84	48	10	-	-	24	-	-	42	30	50	20	34	10

F_{6P} was cleaned with ether and B_1 (145 mg) obtained. F_{11P} afforded B_{2A} (8.0 mg) through PTLC although it still had contaminants. F_{5P} was also cleaned with ether and B_3 (7.5 mg) obtained. B_1 and B_3 were found to be the same compound through spectral analysis. F_{12P} and F_{10P} were cleaned with ether to give B_4 (51.4 mg) obtained. F_{14P} was cleaned with ether to give B_5 (3.0

mg). B_6 (10.0 mg) that was found to be similar to B_{2A} was obtained from F_{9P} on cleaning with ether. B_{7A} (8 mg) was obtained from F_{8P} through preparative TLC. F_{4P} gave B_8 (9.8 mg), which was found to be similar to B_1 and B_3. B_{10} (1.6 mg) was obtained from F_{8P} through preparative TLC and was found to be similar to B_1, B_3 and B_8. F_{11P} gave B_9 (3.6 mg) by preparative TLC. F_{17P} afforded B_{11} (1.2 mg).

3.7 Larvicidal assays of pure compounds from *Z. paracanthum* ethyl acetate extract

B_5 was not available in sufficient amounts for bio-assay. B_1, B_4, B_5, B_6 and B_{7A} were assayed against 3rd instar *An. gambiae* larvae (Table 16) and the data, except for B_6, subjected to regression analysis (Busvine, 1971). The data for B_6 could not be subjected to regression analysis due to inadequate points.

Table 16: % Mortality of 3rd instar *An. gambiae* larvae in B_1, B_4, and B_6, and B_{7A} solutions and probit analysis

	Conc. (ppm)	LogDose +1	Mortality (%) ± SD			Empirical probit		
Time (h)			24	48	72	24	48	72
B_1	20	2.3	16.67±0.58	76.67±0.58	93.33±0.58	4.05	5.74	6.48
	10	2	6.67±0.58	30.0±0	43.53±0.58	3.52	4.48	4.85
	5	1.7	0	6.67±0.33	6.67±0.33	-	3.52	3.52
B_4	20	2.3	78±1.64	92±0.84	100	5.77	6.41	-
	15	2.2	76±1.67	90±1.0	92±0.67	5.71	6.28	6.41
	10	2	10±1.0	24±1.75	34±2.51	3.72	4.29	4.59
B_6	10	2	0	16±0.55	20±0.70	-	4.01	4.61
	5	1.7	0	0	0	-	-	-
B_{7A}	5	1.7	76±1.75	92±1.0	100	5.71	6.41	-
	2.5	1.4	64±0.89	68±0.89	92±0.67	5.36	5.47	6.41
	2	1.3	10±1.0	32±0.70	40±2.51	3.72	4.53	4.75

From probit analysis, the regression equations of B_1 were found to be $y=1.7667x-0.0133$, $y=3.7x-2.82$ and $y=4.9333x-4.9267$ after 24, 48 and 72 hours, respectively. Similarly, the

regression equations for B_4 were found to be y=7.2786x-10.704, y=7.4786x-10.544 and y=9.1x-13.61 after 24, 48 and 72 hours, respectively. Finally, the regression equations for B_{7A} were found to be y=4.0962x-1.0777, y=4.3385x-0.8931 and y=16.6x-16.83 after 24, 48 and 72 hours, respectively. From the regression equations the LC_{25}, LC_{50} and LC_{90} values were calculated (Table 17).

Table 17: LC of B_1, B_4, and B_{7A} for *An. gambiae*

	Lethal concentration (ppm)								
	LC_{25}			LC_{50}			LC_{90}		
Time (h)	24	48	72	24	48	72	24	48	72
B_1	28.7	8.6	7.5	68.8	13.0	10.3	364.9	28.8	18.7
B_4	11.6	9.8	9.4	14.4	12.0	11.1	21.6	17.8	15.3
B_{7A}	2.1	1.6	1.9	3.1	2.3	2.1	6.3	4.5	2.5

CHAPTER FOUR

STRUCTURAL ELUCIDATION

4.1 Structural elucidation of compounds isolated from *Zanthoxylum paracanthum*

B_1 (155)

B_1 was isolated as white crystals (145 mg, R_f 0.76, silica gel, 1:1 *n*-hexane-ethyl acetate) and melting point 121-123 ^{0}C. IR revealed peaks at 1498 and 1489 (aromatic C-H stretching), 1377 (epoxide C-O stretching), 1095 (C-O stretching suggesting presence of a cyclic ether) 1059 and 1037 cm^{-1} (out of plane C-H deformation). UV λ_{max} (MeOH) 235 and 284 nm. ^{1}H NMR revealed 6 signals resulting from 9 protons (Table 18).

Table 18: ^{1}H NMR ($CDCl_3$) data for B_1 (**155**)

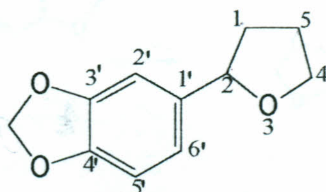
Chemical shift (δ)	Multiplicity	J (Hz)	Integral	COSY
3.05	br d	1.4	1H	3.86, 4.71, 4.23
3.86	dd	3.7, 9.1	1H	4.23
4.23	dd	7.2, 9.1	1H	3.05, 3.86
4.71	d	3.7	1H	3.05
5.95	s	-	2H	-
6.79	s	-	2H	-
6.85	s	-	1H	-

The doublet at δ 3.05 suggested a tertiary centre or the presence of nitrogen whereas the signals at δ 3.86, 4.23 and 4.71 suggested oxygenation at those carbons. The signal at δ 5.95 (2H, s) suggested a methylenedioxide moiety. There were also aromatic protons at δ 6.79 (2H, s) and 6.85 (1H, s). The ^{1}H-^{1}H COSY spectrum revealed coupling between the signals at δ 3.05 with those at δ 3.86, 4.23 and 4.71 and also between that at δ 3.86 and 4.23. This suggested that the proton resonating at δ 3.05 is in between those at 3.86, 4.23 and 4.71. The ^{13}C NMR spectrum revealed 10 signals (Table 19)

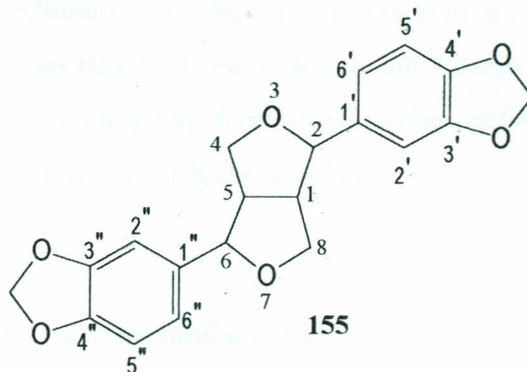
Table 19: ^{13}C NMR ($CDCl_3$) data for B_1 (**155**)

Chemical shift (δ)	DEPT	Chemical shift (δ)	DEPT
54.5	CH	108.4	CH
71.9	CH_2	119.6	CH
86.0	CH	135.2	C
101.3	CH_2	147.3	C
106.7	CH	148.2	C

From DEPT, three signals at δ 148.2, 147.3 and 135.2 were shown to be aromatic and quaternary. The signals at δ 148.2 and 147.3 suggested oxygenation on the aromatic ring. Protonated aromatic carbons were revealed at δ 119.6, 108.4 and 106.7. The methylene carbon at δ 101.3 suggested presence of a methylenedioxy group. There was also a methylene signal at δ 71.9, which suggested oxygenation at this carbon. The remaining 2 signals were at δ 86.0 and 54.5 with that 86.0 suggesting oxygenation while that at 54.5 suggesting a tertiary carbon. From the NMR data a methylenedioxyphenylpropanoid skeleton below was suggested.

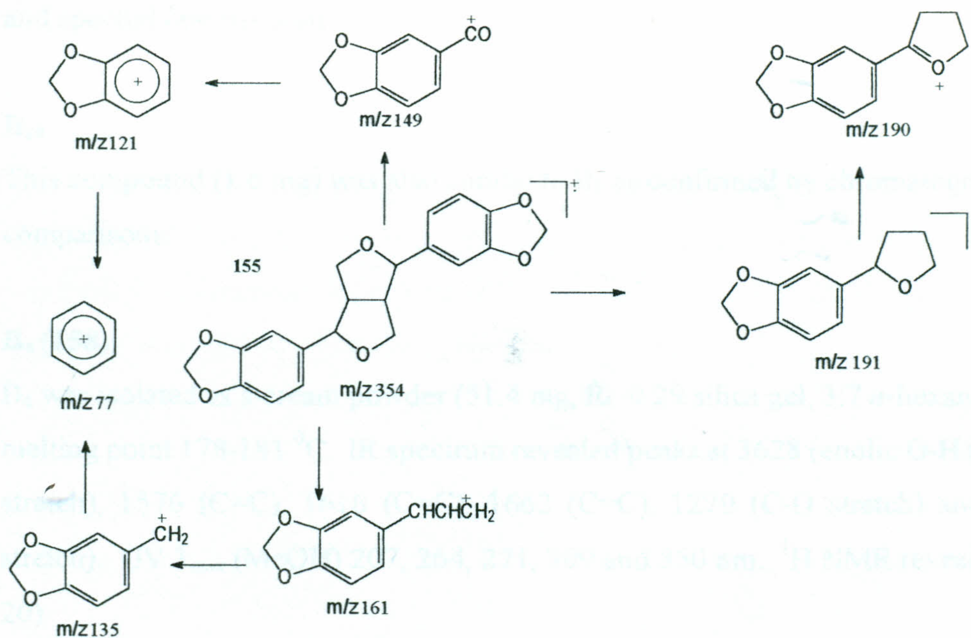


EIMS revealed a molecular ion (M^+) at m/z 354 that was consistent with the molecular formula $C_{20}H_{18}O_6$ with a double bond equivalence (DBE) of 12. This suggested some kind of dimerisation of the methylenedioxyphenylpropanoid skeleton and symmetry. The structure sesamine (**155**) was thus proposed.



The suggested mass spectral fragmentation pattern is given in scheme 1.

Scheme 1: Mass spectral fragmentation pattern for sesamine (**155**)



The spectral data obtained was in agreement with that published by Pelter *et al.* (1976) and Anjaneulu *et al.* (1977). Sesamine is widely distributed in plants and occurs in *Sesamum indicum* seed oil (Pedaliaceae), *Zanthoxylum acanthapodium* (Rutaceae) bark, *Piper longum* (Piperaceae) seeds, *Paulownia tomentosa* (Scrophulariaceae) wood, *Zanthoxylum piperitum* bark, *Ruta montana* (Rutaceae) leaves, *Magnolia mutabilis* (Magnoliaceae), *Asarum sieboldii* (Aristolochiaceae) roots, *Eleutherococcus senticosus* (Araliaceae) (Harbone and Baxter, 1993) and *Magnolia kobus* (Magnoliaceae) (Kamikado *et al.*, 1975). It has been shown to be a

growth-inhibiting substance for *Bombyx mori* larvae (Kamikado *et al.*, 1975). It is also a known insecticide synergist (Harbone and Baxter, 1993; MacRae and Towers, 1984). The compound was recently isolated in the research group from *Zanthoxylum gillettii* (Okinyo, 2002) and shown to have mild larvicidal activity towards *An. gambiae*.

B_3

This compound (7.5 mg) was found to be similar to B_1 according to the chromatographic and spectral data obtained.

B_8

This compound (9.8 mg) was also found to be similar to B_1 as confirmed by chromatographic and spectral comparisons.

B_{10}

This compound (1.6 mg) was also similar to B_1 as confirmed by chromatographic and spectral comparisons.

B_4 (158)

B_4 was isolated as a cream powder (51.4 mg, R_f 0.29 silica gel, 3:7 *n*-hexane-ethyl acetate) and melting point 178-181 ^{0}C. IR spectrum revealed peaks at 3628 (enolic O-H stretch), 1703 (C=O stretch), 1576 (C=C), 1616 (C=C), 1662 (C=C), 1279 (C-O stretch) and 1653 cm^{-1} (C=N stretch). UV λ_{max} (MeOH) 207, 264, 271, 309 and 350 nm. ^{1}H NMR revealed 8 signals (Table 20)

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Table 20: ^{1}H NMR ($CDCl_3$) data for B_4 (**158**)

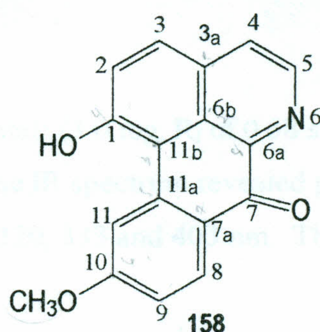
Chemical shift (δ)	Multiplicity	J (Hz)	Integral	COSY	NOE
3.99	s	-	3H	-	7.08, 8.22
6.96	d	9.9	1H	8.01	-
7.08	dd	2.5, 8.7	1H	7.97, 8.22	3.99
7.86	d	5.0	1H	8.75	-
7.97	d	8.7	1H	7.08	-
8.01	d	9.9	1H	6.96	-
8.22	d	2.5	1H	7.08	3.99
8.75	d	5.0	1H	7.86	-

The singlet upfield at δ 3.99 suggested the presence of a methoxy group. Three pairs of coupled protons were visible at δ 6.96 (1H, J=9.9 Hz), 8.01 (1H, J=9.9 Hz); 7.08 (1H, J=2.5, 8.7 Hz), 7.97 (1H, J=8.7 Hz) and 7.86 (1H, J=5.0 Hz), 8.75 (1H, J=5.0 Hz) with the last pair suggesting presence of an isoquinoline ring. There was also long range coupling between 8.22 (1H, J=2.5 Hz) and 7.08 (1H, J=2.5, 8.7 Hz). ^{1}H-^{1}H COSY spectrum revealed coupling between the proton resonating at δ 6.96 and 8.01; 7.08, 7.97 and 8.22; 8.22 and 7.08; 7.86 and 8.75. The ^{13}C NMR spectrum revealed 15 discernible signals (Table 21).

Table 21: ^{13}C NMR ($CDCl_3$) data for B_4 (**158**)

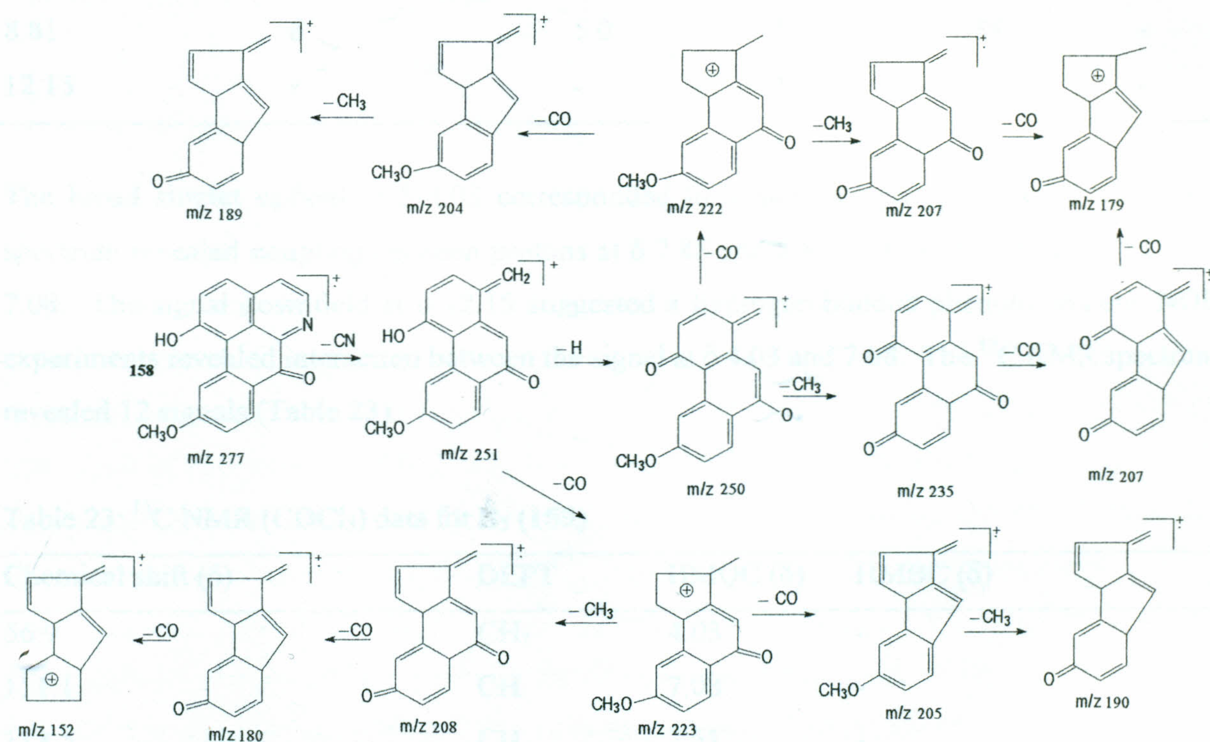
Chemical shift (δ)	DEPT	HMQC (δ)	HMBC (δ)
56.00	CH_3	3.98	-
101.2	CH	8.22	7.08
114.4	CH	7.08	8.22
115.5	CH	7.86	8.75
116.8	C	-	7.08, 8.22
123.6	CH	7.97	-
129.0	CH	6.96	-
131.1	C	-	8.75
132.2	C	-	7.86, 8.01
134.5	C	-	6.96, 8.75
138.8	CH	8.01	7.86
141.6	C	-	7.97
144.6	CH	8.75	7.86
159.4	C	-	6.96, 8.01
162.8	C	-	7.97

The signal at δ 56.0 suggested the presence of a methoxy group as confirmed by DEPT. The methine signals at δ 101.2, 114.4, 115.5, 123.6, 129.0, 138.8 and 144.6 suggested the presence of protonated aromatic carbons, while those at δ 141.6, 159.4 and 162.8 suggested oxygenation at these carbons. From the NMR data, the structure **(158)** was proposed.



The expected molecular ion of m/z 277 was not observed but instead EIMS revealed a base peak at m/z 250, which corresponded to loss of HCN from the molecular ion. The peak at m/z 251 could be explained by loss of CN from the molecular ion. A further loss of CO from this ion resulted in the ion at m/z 223. Other peaks were observed at m/z 249 (18%), 235 (8%), 221 (34%), 207 (65%), 180 (12%), 179 (71%), 164 (11%) and 152 (23%). The suggested fragmentation pattern is presented in Scheme 2.

Scheme 2: Mass spectral fragmentation pattern for B_4 **(158)**



B_5 (159)

B_5 was isolated as fine orange crystals (3.0 mg, R_f of 0.58 silica gel, 3:7 *n*-hexane-ethyl acetate) and melting point 192-195 ^{0}C. The IR spectrum revealed peaks at 3600, 1710, 1667 and 1610 cm^{-1}. UV λ_{max} (MeOH) 212, 261, 320, 333 and 403 nm. The ^{1}HNMR revealed 8 signals (Table 22).

Table 22: ^{1}HNMR ($CDCl_3$) data for B_5 **(159)**

Chemical shift (δ)	Multiplicity	J (Hz)	Integral	COSY	NOE
4.03	s	-	3H	-	7.08
7.06	d	9.8	1H	8.15	-
7.08	d	8.8	1H	7.51	4.03
7.51	d	8.8	1H	7.06	-
7.88	d	5.0	1H	8.81	-
8.15	d	9.8	1H	7.06	-
8.81	d	5.0	1H	7.88	-
12.15	s	-	1H	-	-

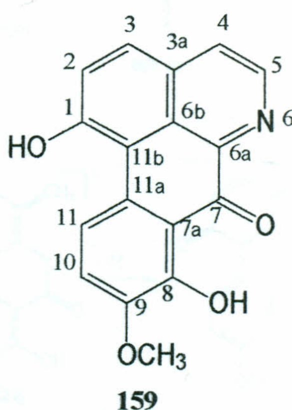
The broad singlet upfield at δ 4.03 corresponded to a methoxy group. The ^{1}H-^{1}H COSY spectrum revealed coupling between protons at δ 7.88 and 8.81; 7.06 and 8.15, and 7.51; and 7.08. The signal downfield at δ 12.15 suggested a hydrogen-bonded phenolic system. NOE experiments revealed interaction between the signal at δ 4.03 and 7.08. The ^{13}C NMR spectrum revealed 12 signals (Table 23).

Table 23: ^{13}C NMR ($CDCl_3$) data for B_5 **(159)**

Chemical shift (δ)	DEPT	HMQC (δ)	HMBC (δ)
56.9	CH_3	4.03	-
111.1	CH	7.08	-
113.5	CH	7.51	-
116.1	CH	7.88	8.81
119.5	C	-	7.06, 7.88
126.7	CH	7.06	-
128.4	C	-	7.51, 7.06
136.9	C	-	7.08, 12.15
141.1	CH	8.15	-
146.9	CH	8.81	7.88
151.7	C	-	7.51, 12.15, 4.03, 7.08
160.4	C	-	8.15

The signal at δ 56.8 suggested the presence of a methoxy group. The signals at δ 111.1, 113.5, 116.1, 126.7, 141.1 and 146.9 corresponded to protonated aromatic carbons whereas those at 151.7 and 160.4 suggested the presence of oxygenated carbons.

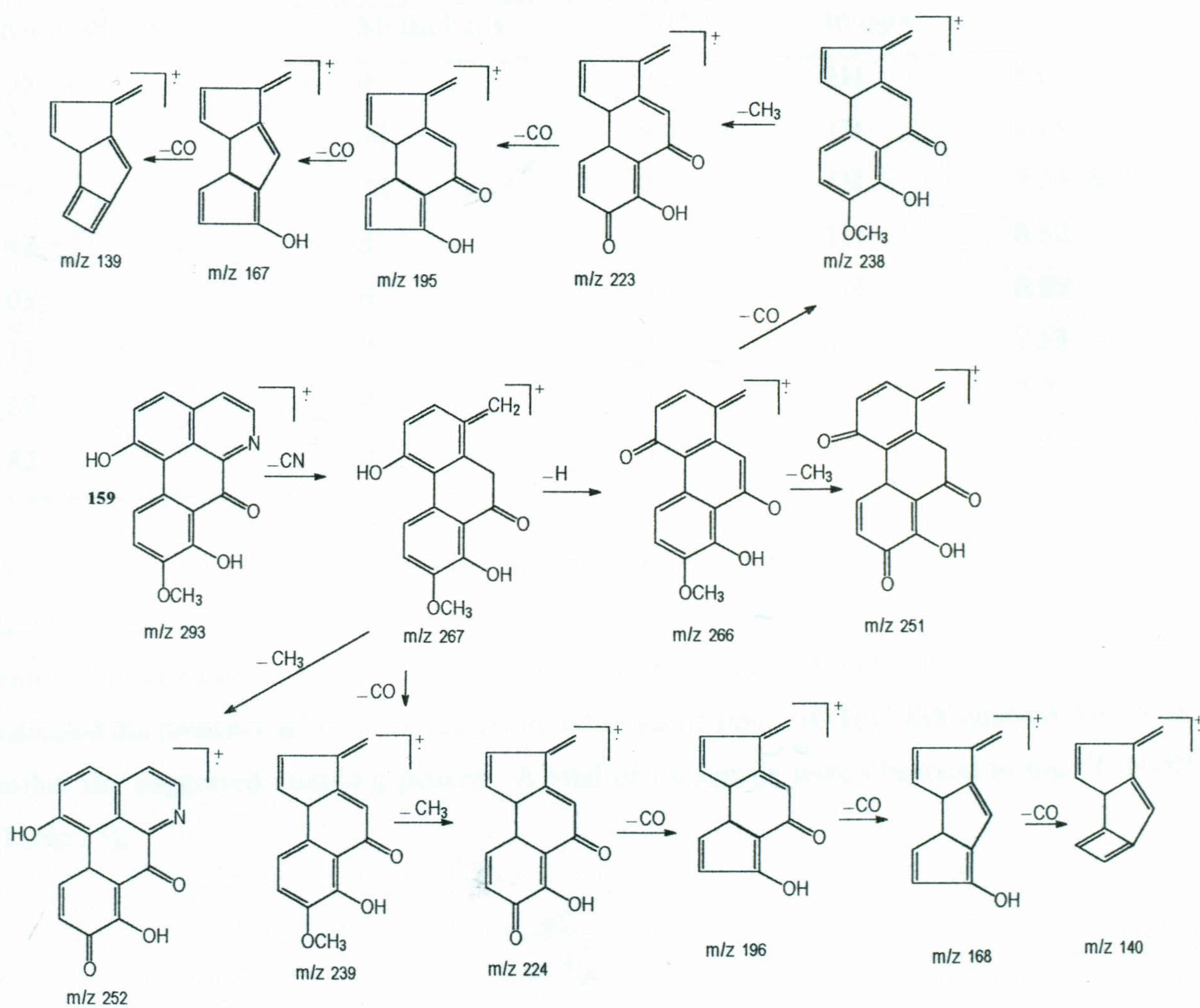
From NMR data the structure (**159**) was suggested.



159

The expected molecular ion of m/z 293 was not observed but instead EIMS revealed a base peak of m/z 266, which corresponded to loss of HCN from the molecular ion. A further loss of CH_3 from this ion resulted in the ion of m/z 251. Loss of CO from the m/z 266 gave the ion of m/z 238. Loss of CH_3 from m/z 238 resulted in ion of m/z 223, which on losing 3xCO resulted in the ions of m/z 195, 167 and 139, respectively. On the other hand, loss of CN from the molecular ion resulted in the ion of m/z 267. A further loss of CO from this ion resulted in the ion of m/z 239. Loss of CH_3 from m/z 267 gave the ion of m/z 252. A loss of CH_3 from m/z 239 gave the ion at m/z 224, which was followed by consecutive loss of 3xCO to give ions of m/z 196, 166 and 138, respectively. The suggested fragmentation pattern is given in Scheme 3.

Scheme 3: Mass spectral fragmentation pattern for B_5 **(159)**



B_6 (160)

B_6 was isolated as orange crystals (10 mg, R_f 0.43 silica gel, 3:7 *n*-hexane:ethyl acetate) and had a melting point of 161-164 ^{0}C. The IR spectrum revealed peaks at 3628 (O-H stretch), 1703 (C=O stretch), 1647 and 1635 (C=C stretch) cm^{-1}. UV λ_{max} (MeOH) 213, 242, 247, 253, 297 and 378 nm. The ^{1}H NMR revealed 8 signals (Table 24).

Table 24: ^{1}H NMR ($CDCl_3$) data for B_6 **(160)**

Chemical shift (δ)	Multiplicity	J (Hz)	Integral	COSY (δ)
6.99	d	9.8	1H	8.03
7.53	dd	8.0, 7.5	1H	8.11, 7.71
7.71	dd	7.6, 7.5	1H	7.53, 8.11
7.97	d	5.0	1H	8.82
8.03	d	9.8	1H	6.99
8.11	d	8.0	1H	7.53
8.67	d	7.6	1H	7.71
8.82	d	5.0	1H	7.97

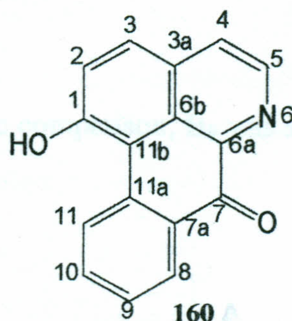
There was a pair of coupled doublets at δ 8.03 (1H, J=9.8 Hz) and 6.99 (1H, J=9.8 Hz). Another pair of coupled doublets at δ 8.82 (1H, J=5.0 Hz) and 7.97 (1H, J=5.0 Hz) indicated aromatic protons in an isoquinoline skeleton. The two double doublets at δ 7.71 and 7.53 indicated the presence of an *ortho*-disubstituted aromatic ring. ^{1}H-^{1}H COSY analysis confirmed further the suggested coupling pattern. A total of 14 signals were observed in the ^{13}C NMR (Table 25).

Table 25: ^{13}C NMR ($CDCl_3$) data for B_6 (**160**)

Chemical shift (δ)	DEPT	HETCOR (δ)	HMQC (δ)	HMBC (δ)
116.4	CH	7.97	7.97	8.82
117.2	CH	8.67	8.67	7.53
122.6	CH	8.11	8.11	7.71
124.3	C	-	-	7.53, 8.67
125.6	CH	7.53	7.53	8.67
128.8	CH	6.99	6.99	-
130.2	C	-	-	8.82
130.8	CH	7.71	7.71	8.11
131.9	C	-	-	8.03, 7.97
136.1	C	-	-	6.99, 8.82
139.3	C	-	-	8.11, 7.71
139.5	CH	8.03	8.03	7.97
145.7	CH	8.82	8.82	7.97
159.4	C	-	-	8.03, 6.99

The signal at δ 159.4 indicated the presence of an oxygenated carbon. Methine carbon signal at δ 145.7 and 139.5 suggested the presence of an isoquinoline skeleton. The 4 methine signals resonating at δ 130.8, 122.6, 128.8 and 117.3 were due to an *ortho*-disubstituted aromatic ring as observed in DEPT, HETCOR, HMQC and HMBC.

From NMR the structure (**160**) was suggested.



160

The expected molecular ion at m/z 247 was not observed but instead EIMS revealed a peak at

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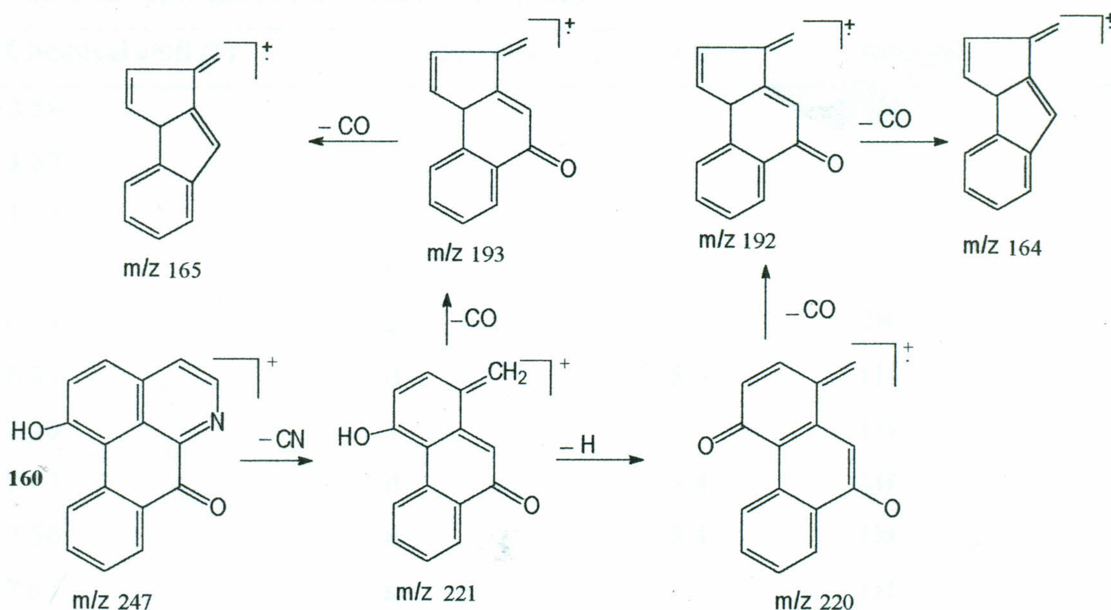
m/z 220 that corresponds to loss of HCN. Further loss of CO led to the base peak at m/z 192, which on losing a CO gave a peak at m/z 164. On the other hand loss of CN from the molecular ion led to m/z 221 which on losing CO resulted in the peak at m/z 193, that further lost a CO to give m/z 165. The expected molecular ion was consistent with the formula $C_{16}H_9O_2N$ with a DBE of 13. The suggested fragmentation pattern is presented in Scheme 4.

Scheme 4: Mass spectral fragmentation pattern for B_6 **(160)**



B_2

This compound (8.0 mg) was contaminated with fatty material (δ 1.2-1.3) but it was shown to be the same as B_6 through TLC and NMR (^{1}H and ^{13}C) spectral comparison.

B_9

From spectral and chromatographic comparison, B_9 was shown to be similar to B_6 although it also had some contaminants.

B_{11}

This compound was found to be insoluble in the available solvents used for NMR analysis and was also available in small amounts (1.6 mg) so it was not characterized.

4.2 Structural elucidation of the compounds isolated from *Zanthhoxylum holstiana*

H_1 (116)

H_1 was isolated as a white crystalline solid (82.4 mg, R_f of 0.81 silica gel, 3:7 ethyl acetate-hexane) and melting point 162-165 ^{0}C. The IR spectrum revealed signals at 3505, 2978, 1273, 1248, 1217 819 cm^{-1}. UV λ_{max} (MeOH) 200, 235 and 284 nm. The ^{1}H NMR spectrum revealed 11 signals (Table 26).

Table 26: ^{1}H NMR ($CDCl_3$) data for H_1 **(116)**

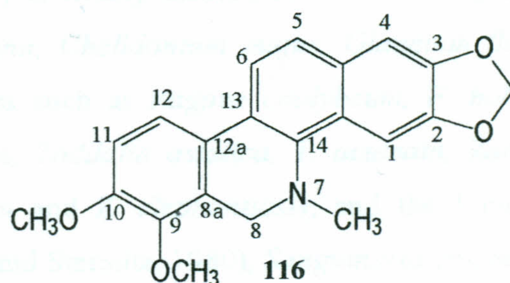
Chemical shift (δ)	Multiplicity	J (Hz)	Integral
2.59	s	-	3H
3.87	s	-	3H
3.92	s	-	3H
4.29	s	-	2H
6.04	s	-	2H
6.93	d	8.5	1H
7.10	s	-	1H
7.47	d	8.4	1H
7.50	d	8.4	1H
7.67	s	-	1H
7.69	d	8.5	1H

Two pairs of coupled protons were visible at δ 6.93 (1H, J=8.5 Hz) and 7.69 (1H, J=8.5 Hz); δ 7.47 (1H, J=8.4 Hz) and 7.50 (1H, J=8.4 Hz). These corresponded to two pairs of isolated aromatic protons. Two singlets at δ 7.10 (1H) and 7.67 (1H) were assigned to two isolated aromatic protons. The three singlets at δ 2.59 (3H), 3.87 (3H) and 3.92 (3H) were assigned to two methoxy groups and a methyl attached to nitrogen, respectively. The signals at δ 4.29 and 6.04 were assigned to a methylene group attached to nitrogen and a methylenedioxy group, respectively. A total of 20 signals were revealed in the ^{13}C NMR (Table 27).

Table 27: ^{13}C NMR ($CDCl_3$) data for H_1 (**116**)

Chemical shift (δ)	DEPT	Chemical shift (δ)	DEPT
41.4	CH_3	124.1	C
48.7	CH_2	126.0	C
55.8	CH_3	126.1	C
61.1	CH_3	126.2	C
100.6	CH_2	130.6	C
104.2	CH	142.5	C
110.7	CH	145.8	C
118.6	CH	147.2	C
120.0	CH	147.8	C
123.6	CH	152.0	C

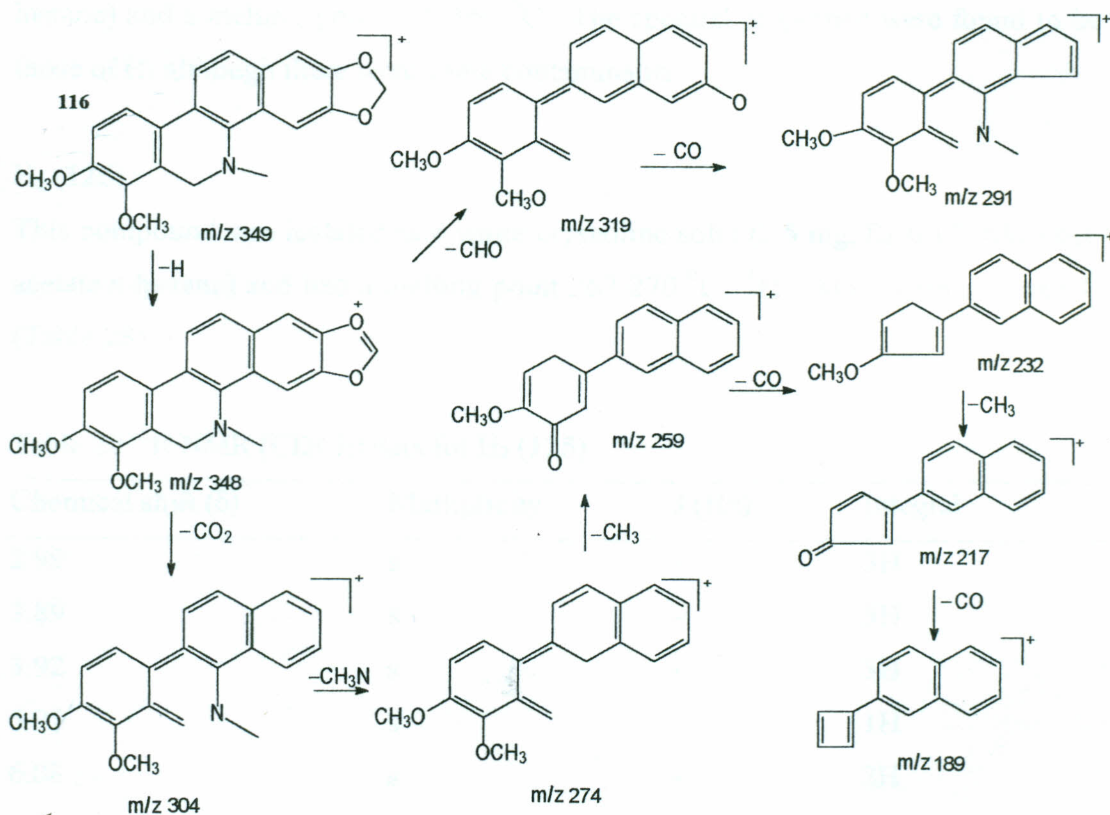
The signal at δ 48.7 and 100.6 confirmed the presence of nitrogen attached to a methylene group and a methylenedioxy groups, respectively. The signals at δ 104.2, 110.7, 118.6, 120.0 and 123.6 were due to methine carbons. The other 10 signals were from quaternary carbons. The signals at δ 152.0, 147.8, 147.2, 145.8 and 142.5 suggested aromatic carbons attached to electron withdrawing groups such as oxygen or nitrogen. The methyl signals at δ 55.8 and 61.1 confirmed the presence of methoxy groups. The methyl signal at 41.4 confirmed its attachment to a nitrogen atom. From the NMR spectrum, the structure dihydrochelerythrine (**116**) was proposed.



EIMS revealed a base peak at m/z 349 that corresponded to the molecular formula $C_{21}H_{19}O_4N$ with a DBE of 13. A loss of CHO from the molecular ion gave rise to the ion at m/z 319, which

on losing CO gave an ion at m/z 191. On the other hand loss of CO_2 from the molecular ion led to the ion of m/z 304, which on losing CH_3N resulted in ion at m/z 274. The fragmentation pattern is summarized in scheme 5.

Scheme 5: Mass spectral fragmentation pattern for dihydrochelerythrine (**116**)



Dihydrochelerythrine (**116**) is widely distributed in the family Papaveraceae, including the species *Argemone mexicana, Chelidonium majus, Glaucium flavum* var. *vestitum* and *G. vitellium*; Rutaceae species such as *Fagara chalybeum, F. holstii, F. semiarticulatum, F. rubescens, F. macrophylla, Toddalia asiatica, T. aculeata, Zanthoxylum tsihanimposa, Z. senegalense, Z. coriaceum* and *Z. elephantiasis*; and the Fumariaceae species *Corydalis ledebouriana*, (Swinehart and Stermitz, 1980), *Sanguinaria canadensis* (Arakawa *et al.*, 1992) and *Bocconia arbocea* (Navarro and Delgado, 1999). The compound has been known to exhibit a variety of biological activities such as anti-microbial, anti-feedant, cytotoxic and anti-tumour

effects (Navarro and Delgado, 1999). The spectral data obtained above was comparable to that published by Shafiee *et al.* (1979), Chen *et al.* (1994) and Guinaudeau and Bruneton (1993).

H_4 (116)

This compound was isolated as a white solid (4.2 mg, R_f 0.81 silica gel, 3:7 ethyl acetate-*n*-hexane) and a melting point 158-160 ^{0}C. The spectral properties were found to be similar to those of H_1 although there were some contaminants.

H_2 (125)

This compound was isolated as a white crystalline solid (2.5 mg, R_f 0.12, silica gel, 3:7, ethyl acetate:*n*-hexane) and had a melting point 267-270 ^{0}C. ^{1}H NMR analysis revealed 12 signals (Table 28).

Table 28: ^{1}H NMR ($CDCl_3$) data for H_2 (**125**)

Chemical shift (δ)	Multiplicity	J (Hz)	Integral
2.99	s	-	3H
3.89	s	-	3H
3.92	s	-	3H
5.95	s	-	1H
6.08	s	-	2H
6.53	d	8.7	1H
6.80	d	8.7	1H
7.07	s	-	1H
7.19	s	-	1H
7.30	d	8.3	1H
7.72	d	8.3	1H
8.15	s	-	1H

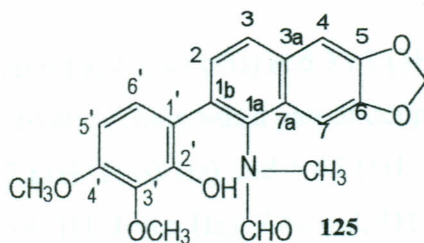
The three singlets at δ 2.99 (3H) 3.89 (3H) and 3.92 (3H) were assigned to a methyl group attached to a nitrogen and two methoxy groups respectively. The singlet at δ 5.95 (1H) was due to a hydroxyl group whereas that at δ 6.08 (s, 2H) was assigned to a methylenedioxy group.

Two pairs of coupled aromatic protons were visible at δ 6.53 (d, 1H, J=8.7 Hz), 6.80 (d, 1H, J=8.7 Hz), 7.30 (d, 1H, J=8.3 Hz) and 7.72 (d, 1H, J=8.3 Hz). This suggested two pairs of isolated aromatic protons. The two singlets at δ 7.07 (IH) and 7.19 (1H) resulted from two isolated aromatic protons. The singlet at δ 8.15 (1H) suggested the presence of an aldehyde type proton. The ^{13}C NMR revealed 19 signals (Table 29).

Table 29: ^{13}C NMR ($CDCl_3$) data for H_2 (**125**)

Chemical shift (δ)	DEPT	Chemical shift (δ)	DEPT
33.1	CH_3	127.2	CH
55.9	CH_3	127.3	CH
61.2	CH_3	131.1	C
99.2	C	133.2	C
101.4	CH_2	135.4	C
103.8	CH	146.5	C
104.3	CH	147.9	C
118.4	CH	149.1	C
125.0	CH	164.3	CH
151.8	C		

The signal at δ 164.3 suggested the presence of a carbonyl carbon to which hydrogen was attached. The signals at δ 151.8, 149.1, 147.9 and 146.5 were assigned to oxygenated carbons on the aromatic ring. Signals at δ 103.8, 104.3, 118.4, 125.0, 127.2 and 127.3 were due to protonated aromatic carbons whereas that at δ 101.4 confirmed further the presence of a methylenedioxide moiety. From the NMR data, arnottianamide (**125**) was proposed.



Arnottianamide has been isolated from the leaves *Zanthoxylum schinifolium* (Cheng *et al.*, 2002), *Argemone mexicana, Toddalia asiatica* (Sharma *et al.*, 1980), root bark and root wood of

Z. simulans (Chen *et al.*, 1994), root and pericarp of *Z. rubescens* (Adenisa, 1986), rootand bark of *Z. gillettii* (Adenisa and Reisch, 1988; Okinyo, 2002), *Xanthoxylum caspidatum* and *X. arnottianum* (Ishii and Ishikawa, 1976). The spectroscopic data obtained was comparable to that published by Adenisa (1986), Adenisa and Reisch (1988) and Ishii and Ishikawa (1976).

H_3

This compound was isolated as a white solid (4.3 mg) but its analysis could not be done because it was insoluble in the available NMR solvents.

H_5 (129)

H_5 was isolated as a cream-yellow wax (30.0 mg, R_f 0.33, silica gel, 3:7 ethyl acetate:*n*-hexane) and had a melting point of 82-84 ^{0}C. The IR spectrum revealed signals at 1705 and 1211 cm^{-1}. UV λ_{max} (MeOH) 216, 235, 331, 345 and 361 nm. The ^{1}H NMR revealed 8 signals (Table 30).

Table 30: ^{1}H NMR ($CDCl_3$) data for H_5 **(129)**

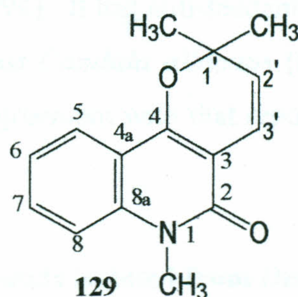
Chemical shift (δ)	Multiplicity	J (Hz)	Integral
1.43	s	-	6H
3.61	s	-	3H
5.45	d	10	1H
6.67	d	10	1H
7.14	dd	6.6, 7.3	1H
7.20	d	6.6	1H
7.46	dd	8.1, 7.3	1H
7.88	d	8.1	1H

The three singlets upfield at δ 1.43 (6H) and 3.61 (3H) represented two shielded methyl groups and *N*-methyl, respectively. There was a pair of coupled aromatic or olefinic protons appearing as doublets at δ 6.67 (1H, J=10 Hz) and 5.45 (1H, J=10 Hz). The signals at δ 7.14 (dd, 1H, J=6.6, 7.3 Hz), 7.20 (d, 1H, J=6.6 Hz), 7.46 (dd, 1H, J=8.1, 7.3 Hz) and 7.88 (d, 1H, J=8.1 Hz) suggested an *ortho*-di-substituted aromatic system. The ^{13}C NMR analysis revealed 14 signals while DEPT analysis revealed 2 methyl and 6 methine groups (Table 31).

Table 31: ^{13}CNMR ($CDCl_3$) data for H_5 (**129**)

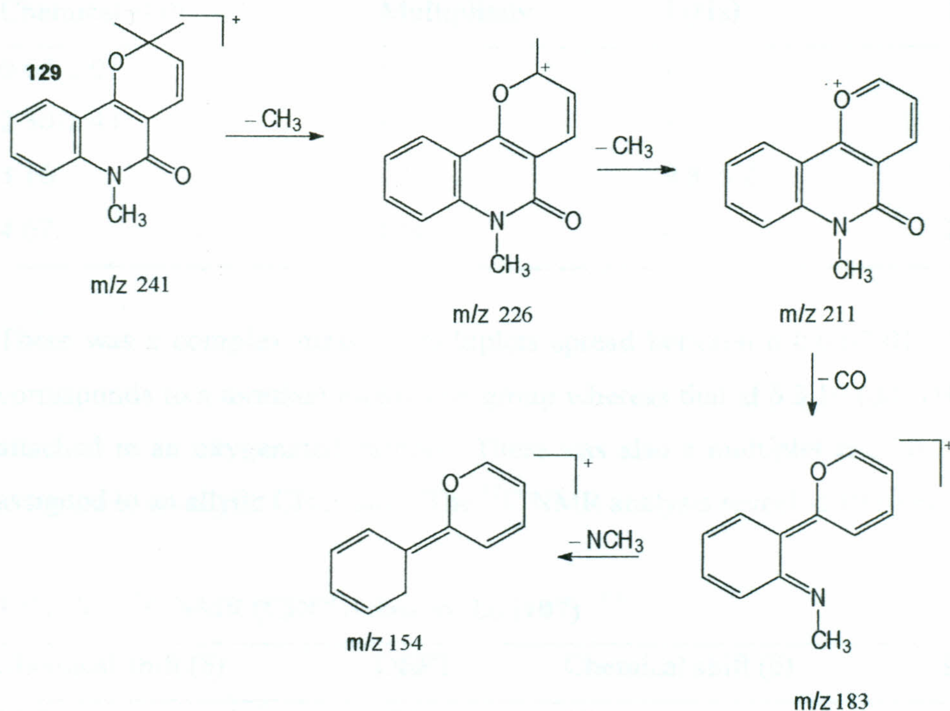
Chemical shift (δ)	DEPT	Chemical shift (δ)	DEPT
28.3	CH_3	121.6	CH
28.3	CH_3	122.9	CH
29.4	CH_3	126.2	CH
78.7	C	130.7	CH
105.7	C	139.1	C
113.9	CH	155.0	C
115.9	C	160.8	C
117.8	CH		

Two signals at δ 28.3 and that at 29.3 confirmed the presence of 2 methyl signals resonating at the same field strength and a methyl group attached to nitrogen, respectively. The peak at δ 78.7 suggested the presence of an oxygenated tertiary carbon. Methine signals at δ 113.9, 117.8, 121.6, 122.9, 126.2 and 130.7 confirmed the presence of olefinic or aromatic carbons. The signals at δ 155.0 and 160.8 suggested the presence of nitrogen attached to an aromatic ring and a carbonyl group, respectively. From NMR analysis, *N*-methylflindersine (**129**) was proposed.



EIMS revealed the molecular ion at m/z 241, which was consistent with the formula $C_{15}H_{15}NO_2$ with a DBE of 9. The loss of CH_3 led to the base peak at m/z 226. A further loss of CH_3 resulted in the ion at m/z 211, which lost CO to give the peak at m/z 183. Loss of NCH_3 from this ion resulted in the peak at m/z 154. The proposed fragmentation pattern is presented in Scheme 6.

Scheme 6: Mass spectral fragmentation pattern for *N*-methylflindersine (**129**)



N-methylflindersine (**129**) has been isolated from *Ptelea trifoliate, Xylocarpus granatum, Atalantia roxburghiana, Fagara chalybeum* (Harborne and Baxter, 1993) and *Zanthoxylum simulans* (Rutaceae) (Chen *et al.*, 1994). It has anti-feedant activity against beetles and shows anti-microbial action against the yeast *Candida albicans* (Harborne and Baxter, 1993). The spectroscopic data obtained was in agreement with that reported by Ishii *et al.* (1991) and Gray (1993).

4.3 Structural elucidation of compounds isolated from *Derris trifolia*

D_2 (107)

This compound was isolated as a white crystalline solid, (20.0 mg, R_f 0.76, silica gel, 3:7 ethyl acetate:*n*-hexane) and had a melting point of 213-215 ^{0}C. UV λ_{max} (MeOH) 201 nm. IR 3227 (O-H stretch) and 3092 (C-H stretch), 1670 (C=C stretch), 1377 (C-H deformation) cm^{-1}. The ^{1}H NMR spectrum revealed 4 regions (Table 32).

Table 32: ^{1}H NMR ($CDCl_3$) for D_2 (**107**)

Chemical shift (δ)	Multiplicity	J (Hz)	Integral
0.64-2.01	m	-	46H
2.30-2.43	m	-	1H
3.16	dd	9.8, 5.2	1H
4.67	br s	-	2H

There was a complex mass of multiplets spread between δ 0.64-2.01. The signal at δ 4.67 corresponds to a terminal methylene group whereas that at δ 3.16 (dd, 1H) suggested a proton attached to an oxygenated carbon. There was also a multiplet at 2.30-2.43 (1H), which was assigned to an allylic CH group. The ^{13}C NMR analysis revealed 30 signals (Table 33).

Table 33: ^{13}C NMR ($CDCl_3$) data for D_2 (**107**)

Chemical shift (δ)	DEPT	Chemical shift (δ)	DEPT
14.5	CH_3	37.1	C
15.4	CH_3	38.0	CH
16.0	CH_3	38.7	CH_2
16.1	CH_3	38.8	CH_2
18.0	CH_3	40.0	C
18.3	CH_2	40.8	C
19.3	CH_3	42.8	C
20.9	CH_2	43.0	C
25.1	CH_2	48.0	CH
27.4	CH_2	48.3	CH
28.0	CH_3	50.4	CH
29.7	CH_2	55.2	CH
29.7	CH_2	79.0	CH
34.2	CH_2	109.3	CH_2
35.5	CH_2	151.0	C

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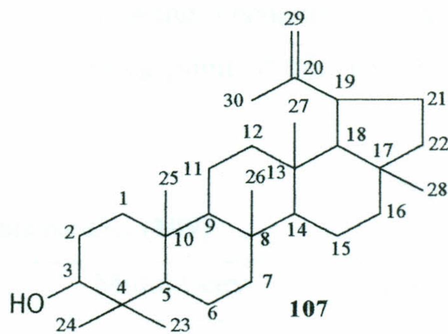
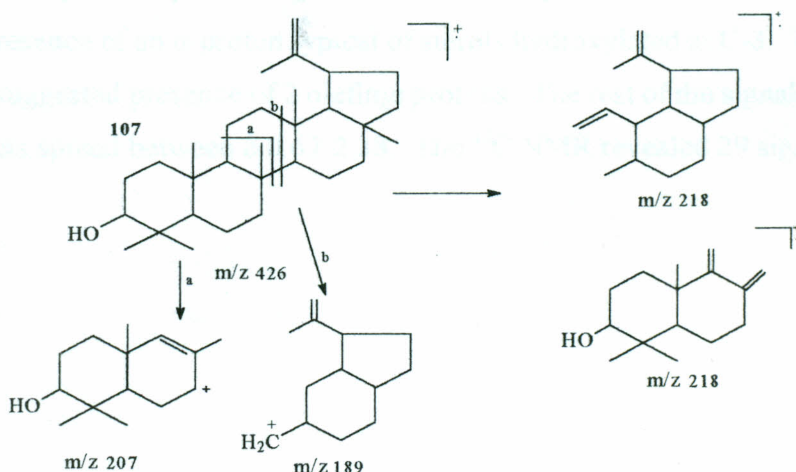
The methylene carbon signal at δ 109.3 was confirmed by DEPT and hence the presence of a terminal group. The signal at δ 79.0 indicated the presence of a carbinol carbon. Presence of 7 methyl groups was confirmed by DEPT analysis thus suggesting a triterpene skeleton. Besides, presence of 11 CH_2, 6 CH and 6 quaternary centers was confirmed by DEPT analysis. From NMR analysis, lupeol (**107**) was proposed.



EIMS revealed the molecular ion peak at m/z value 426, which corresponded to the molecular formula $C_{30}H_{50}O$ with a DBE of 6. The fragmentation pattern is presented in Scheme 7 to account for the observed MS peaks.

Scheme 7: Mass spectral fragmentation pattern for lupeol (**107**)



Lupeol occurs in the bark and leaves of many plants, especially of species in the Apocynaceae Ebenaceae Euphorbiaceae and Leguminosae. It is found in the bark of *Phyllanthus emblica* (Euphorbiaceae), *Lupinus luteus* (Leguminoseae) (Harborne and Baxter, 1993), *Zanthoxylum gillettii* (Rutaceae) (Okinyo, 2002). It has previously been isolated from this plant

(www.sc.chula.ac.th). It exhibits anti-tumour, anti-hyperglycaemic and hypotensive activities (Harborne and Baxter, 1993). The spectroscopic data obtained was in agreement with that published by Reynolds *et al.* (1986).

D_{3A} (109)

This compound was isolated as a white crystalline solid (3.4 mg, R_f 0.40, 1:3 ethyl acetate:hexane, SiO_2) and had a melting point of 162-165 ^{0}C. ^{1}H NMR revealed 4 regions (Table 34).

Table 34: ^{1}H NMR ($CDCl_3$) data for D_{3A} (**109**)

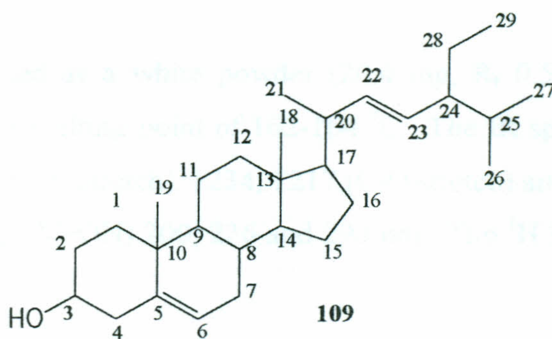
Chemical shift (δ)	Multiplicity	J (Hz)	Integral
0.67-2.38	m	-	44H
3.58	m	-	1H
5.01-5.12	m	-	2H
5.28	br d		1H

The signal at δ 5.28 (br d, 1H) was assigned to an olefinic proton whereas that at δ 3.58 (m, 1H) suggested the presence of an α-proton typical of sterols hydroxylated at C-3. The multiplet at δ 5.01-5.12 (2H) suggested presence of 2 olefinic protons. The rest of the signals were a complex mass of multiplets spread between δ 0.67-2.38. The ^{13}C NMR revealed 29 signals (Table 35).

Table 35: ^{13}C NMR ($CDCl_3$) data for D_{3A}. **(109)**

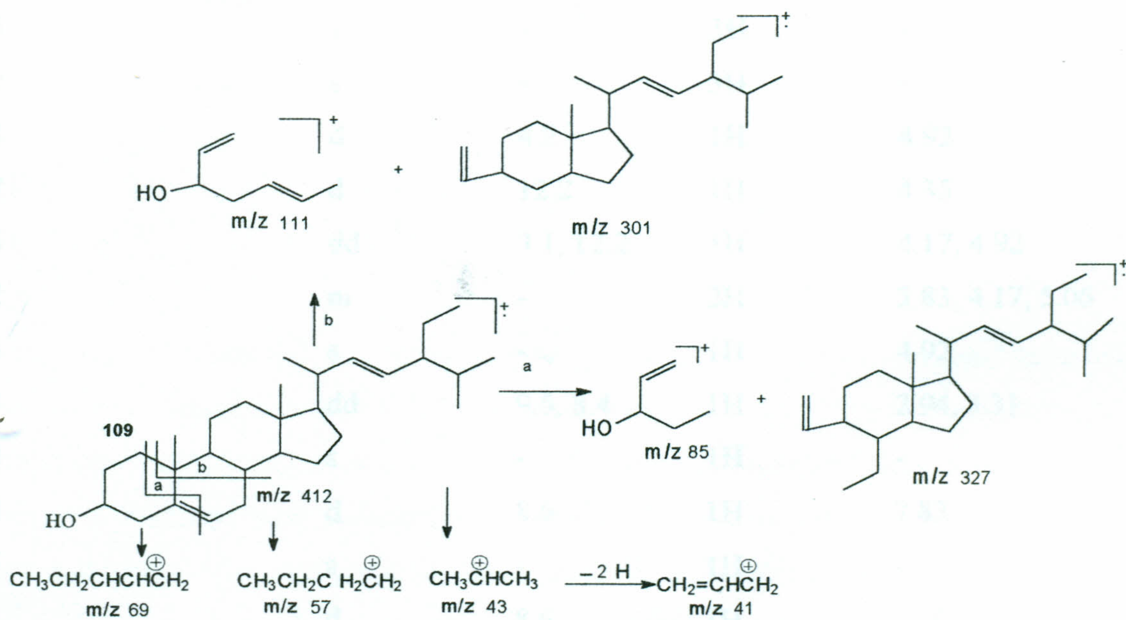
Chemical shift (δ)	DEPT	Chemical shift (δ)	DEPT
12.0	CH_3	36.6	C
12.2	CH_3	37.3	CH_2
18.9	CH_3	39.7	CH_2
19.1	CH_3	42.3	CH_2
19.5	CH_3	45.9	C
20.0	CH_3	50.1	CH
21.2	CH_2	51.3	CH
23.2	CH_2	56.0	CH
24.4	CH_2	56.8	CH
28.4	CH_2	71.8	CH
29.2	CH_2	121.6	CH
31.7	CH_2	129.1	CH
32.0	CH	138.2	CH
34.0	CH_2	140.6	C
36.2	CH		

Six methyl signals appeared at δ 12.0, 12.2, 18.9, 19.1, 19.5 and 20.0, as revealed by DEPT analysis. DEPT analysis also revealed 9 methylene carbon signals at δ 21.2, 23.2, 24.4, 28.4, 31.7, 34.0, 37.3, 39.7 and 42.3 and 11 methine carbon resonances at δ 29.2, 32.0, 36.2, 50.1, 51.3, 56.0, 56.8, 71.8, 121.6, 129.1 and 138.2. The remaining 3 signals at δ 36.6, 45.9 and 140.6 were due to quaternary carbons. From the NMR analysis, stigmasterol **(109)** was proposed.



EIMS revealed a molecular ion at m/z 412 (8%), a value that was consistent with the molecular formula $C_{29}H_{48}O$. Other peaks were observed at m/z 396 (7%), 119 (46%), 111 (43%), 69 (85%), 57 (100%) and 43 (97%). The proposed fragmentation pattern is as in Scheme 8.

Scheme 8: Mass spectral fragmentation pattern for stigmasterol (**109**)



Stigmasterol is found in many higher plants such as wheat germ, *Triticum,* sweetcorn, *Zea mays* where it plays a vital role in the membranes of plant cells (Harbone and Baxter, 1993). The spectral data obtained was in agreement with those published by Rubinstein *et al.* (1976) and Greca *et al.* (1990).

D_7 (40)

This compound was isolated as a white powder (20.4 mg, R_f 0.54, silica gel, 7:13, ethyl acetate:*n*-hexane) and had a melting point of 162-164 ^{0}C. The IR spectrum revealed peaks at 1674 (C=O stretch), 1647 (C=C stretch), 1234, 1217 (C-O stretch) and 1091 cm^{-1} (out of plane C-H deformation). UV λ_{max} (MeOH) 200, 236 and 293 nm. The ^{1}H NMR revealed 15 signals (Table 36).

Table 36: ^{1}H NMR ($CDCl_3$) data for D_7 (**40**)

Chemical shift (δ)	Multiplicity	J (Hz)	Integral	COSY
1.76	s	-	3H	-
2.94	dd	8.4, 16.0	1H	3.31, 5.23
3.31	dd	9.5, 16.0	1H	2.94, 5.23
3.75	s	-	3H	-
3.80	s	-	3H	-
3.83	d	4.2	1H	4.92
4.17	d	12.2	1H	4.35
4.35	dd	3.1, 12.2	1H	4.17, 4.92
4.92	m	-	2H	3.83, 4.17, 5.06
5.06	s	-	1H	4.92
5.23	dd	9.5, 8.4	1H	2.94, 3.31
6.44	s	-	1H	-
6.50	d	8.6	1H	7.83
6.75	s	-	1H	-
7.83	d	8.6	1H	6.50

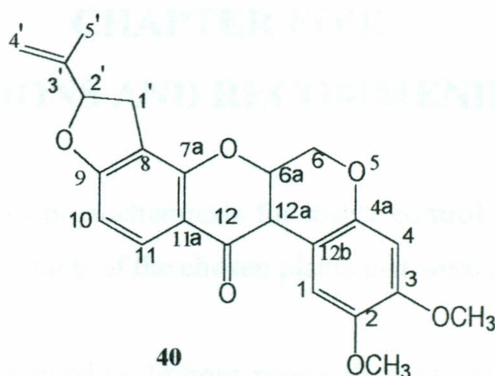
The spectrum showed two coupled aromatic doublets at δ 7.83 (1H, J=8.6 Hz) and 6.50 (1H, J=8.6 Hz). The singlets appearing at δ 6.75 and 6.44 suggested isolated aromatic protons. The signals at δ 4.92 (m, 2H) and 5.06 (s, 1H) corresponded to two protons of a terminal CH_2 group. The signals at δ 5.23 (dd, 1H, J=9.5, 8.4 Hz), 3.31 (dd, 1H, J=9.5, 16.0 Hz) and 2.94 (dd, 1H, J=8.4, 16.0 Hz) suggested 3 coupled protons two of which could be geminal (J=16.0 Hz). There was also a pair of coupled protons at δ 4.17 (d, 1H, J=12.2 Hz) and 4.35 (dd, 1H, J=3.1, 12.2

Hz). This suggested vicinal oxygenated protons. The ^{13}C NMR analysis revealed 23 signals (Table 37).

Table 37: ^{13}C NMR ($CDCl_3$) data for D_7 **(40)**

Chemical shift (δ)	DEPT	Chemical shift (δ)	DEPT
17.3	CH_3	112.5	CH_2
31.3	CH_2	112.9	C
44.6	CH	113.2	C
55.9	CH_3	129.9	CH
56.3	CH_3	142.8	C
66.3	CH_2	143.6	C
72.2	CH	147.1	C
87.8	CH	149.2	C
100.8	CH	157.7	C
104.7	C	167.1	C
104.8	CH	188.7	C
110.1	CH		

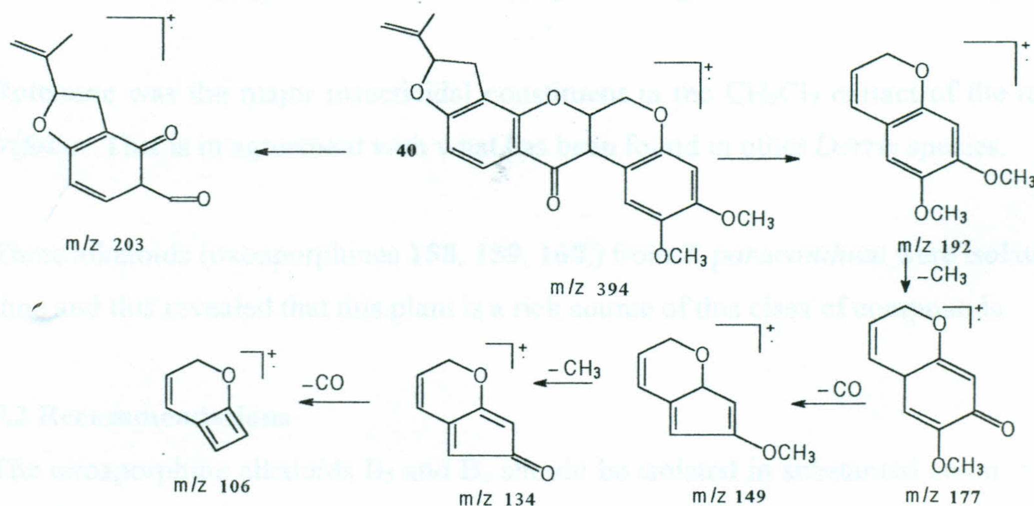
The signal at δ 17.3 was due to a methyl group. The signal at δ 44.6 corresponded to a methine carbon next to a quaternary centre. The two signals at δ 56.3 and 55.9 were found to be due to methoxy carbons. The signal at δ 66.3 suggested an oxygenated methylene carbon. The two signals at δ 72.2 and 87.8 indicated the presence of methine carbons attached to oxygen. Presence of a terminal methylene group was suggested by the signal at δ 112.5. The signals at δ 100.8, 104.8, 110.1 and 129.9 were due to aromatic carbons. The rest of the signals were due to quaternary carbons, with the signal at δ 188.7 suggesting the presence of a carbonyl group while those at δ 167.1, 157.7, 149.2, 147.1 and 143.6 indicated oxygenation at these positions on the aromatic ring. From ^{1}H and ^{13}C NMR, rotenone **(40)** was proposed.



40

EIMS revealed a molecular ion peak at m/z 394, which was consistent with the formula $C_{23}H_{22}O_6$. The base peak at m/z 192 was due to a retro-Diels-Alder fragmentation. The fragmentation pattern presented in scheme 9 was proposed to account for the observed MS peaks.

Scheme 9: Mass spectral fragmentation pattern for rotenone **(40)**



Rotenone occurs in *Derris, Tephrosia* and *Lonchocarpus*, and numerous other Leguminosae species (Harborne and Baxter, 1993). It is also found in the leaves of *Verbascum thapsus* (Scrophulariaceae) (Harborne and Baxter, 1993) and roots of *Millettia pachycarpa* (Singhal *et al.*, 1982). It has anti-protozoal and insecticidal activity (Harborne and Baxter, 1993). The obtained spectroscopic data was in agreement with the reported values (Singhal *et al.*, 1982; Crombie *et al.*, 1975).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Plants are a potential source of phytochemicals for insect control. This is evident from the preliminary bio-assays of the extracts of the chosen plants that were studied.

The larvicidal principles as screened in 24 hour assays were present mainly in the extracts of solvents of low polarity suggesting that they are not very polar. For most of the plants studied the CH_2Cl_2 and EtOAc extracts exhibited good larvicidal activity whereas the methanolic extracts were the least active.

The oxoaporphine alkaloid **158** from *Z. paracanthum* exhibited higher larvicidal activity against *An. gambiae* than the benzophenanthridine alkaloid, dihydrochelerythrine (**116**), from *Z. holstiana* that displayed no larvicidal activity at the highest concentration assayed (50 ppm).

Rotenone was the major insecticidal constituent in the CH_2Cl_2 extract of the root bark of *D. trifolia*. This is in agreement with what has been found in other *Derris* species.

Three alkaloids (oxoaporphines **158**, **159**, **160**,) from *Z. paracanthum* were isolated for the first time and this revealed that this plant is a rich source of this class of compounds.

5.2 Recommendations

The oxoaporphine alkaloids B_5 and B_6 should be isolated in substantial amounts to determine their larvicidal activity.

IGR properties of slow acting extracts such as dichloromethane and ethyl acetate extracts of *Salsola monoica* (bark) should be investigated further in order to isolate any bio-active principles.

Toxicological studies and semi-field trials of *n*-hexane, CH_2Cl_2, ethyl acetate and methanol extracts of *Z. paracanthum* (stem bark), *Z. holstiana* (stem bark), *Z. chalybeum* (stem bark), *D. trifolia* (root bark), *M. grandieri* (stem bark), should be carried out in order to evaluate their potential as larvicidal agents in malaria vector management.

The 2 active compounds from *Z. holstiana* (stem bark) ethyl acetate extract and *Z. paracanthum* (stem bark) dichloromethane extract that were not identified should be identified in future research.

CHAPTER SIX
EXPERIMENTAL

6.1 General procedures

All the glassware used was soaked in chromic acid, cleaned thoroughly with water, rinsed with distilled water, acetone and dried in the oven at 110 °C.

All solvents were supplied by Sigma-Aldrich or Merck at 97.5-99.9% purity and were used without any further purification.

Analytical TLC was performed on silica gel G_{60} F_{254} plates (5x10 cm x 0.20 mm thickness) with fluorescent indicator. The spots were visualized using multi-brand UV-254/366 nm lamp (UV GL-58). The plates were then sprayed with 2% sulphuric acid in methanol and kept in the oven at 110 °C until the spots appeared. Preparative TLC was done on silica gel G60 F_{254} plates (20 x 20 cm, thickness 0.25 mm) with fluorescent indicator.

Melting points of the isolated recrystallised compounds were determined on Sanyo Gallenkamp electronic melting point apparatus and are uncorrected.

Mass spectrometric (MS) analysis was performed on pure solid samples using the direct insertion probe (DIP) on a Fission Platform 11 Mass Spectrometer (ICIPE) operated at 70 eV and a mass range set at 38-800.

Nuclear magnetic resonance (NMR) spectra were obtained from Varian Gemini 200 (University of Nairobi), Bruker 300 and 600 MHz machines (University of Botswana). ^{1}H NMR spectra were run in $CDCl_3$. All the signals are reported as δ and the multiplicity: s-singlet, d-doublet, t-triplet, q-quartet and m-multiplet. The ^{13}C NMR spectra were run on the same machines at 50, 75 and 150 MHz, respectively and the multiplicity determined by DEPT experiments. Structural assignments are based on ^{1}H and ^{13}C chemical shifts, DEPT, HETCOR, HMQC and HMBC analysis.

Infrared (IR) spectra were obtained in nujol (liquid paraffin) from Shimadzu Fourier Transform (FT) Specrophotometer (KEFRI). Absorption bands were recorded in wave numbers (cm^{-1}) and transmittance (%).

Ultra violet (UV) spectra were obtained using a Beckman System Gold HPLC-UV with diode array detector (Module 168) and are reported in wavelength (nm) of absorption maxima.

6.2 Plant collection

The plants *Derris trifolia* (roots), *Zanthoxylum holstiana* (stem bark), *Z. paracanthum* (stem bark), *Z. chalybeum* (stem bark), *Monondora grandieri* (stem bark), *Mkilua fragrans* (stem bark), *Isolona cauliflora* (stem bark), *Salsola monoica* (leaves and stem bark) and *Bidens schimperi* (roots), were collected from coastal parts of Kenya (Ngomeni, Sabaki River delta, Diani, Shimba and Mrima Hills) (Table 38) and identified by Mr. Simon Mathenge of Botany Department, University of Nairobi. Sample specimens were deposited at the University Herbarium, Department of Botany for future reference. The collected parts were dried under shade for 14 days after which they were ground in readiness for extraction.

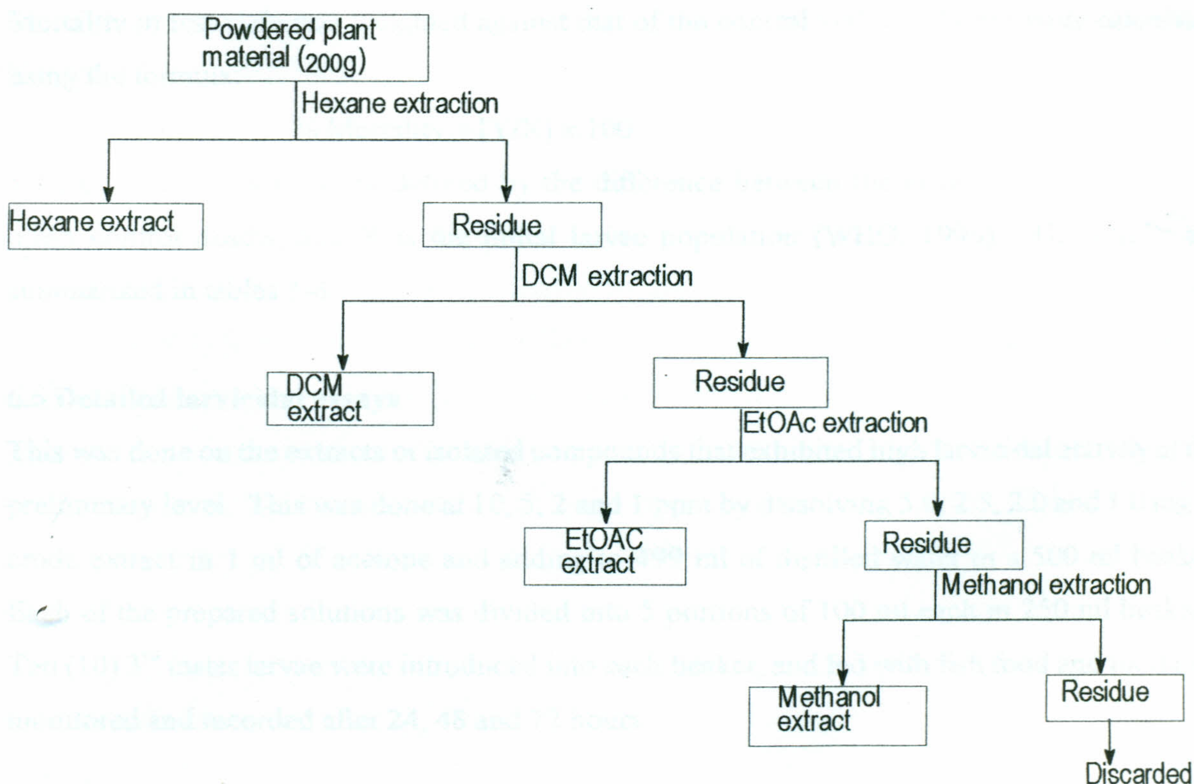
Table 38: Locations of collected plants

Plant	Location
Derris trifolia	Sabaki River Delta
Zanthoxylum paracanthum	Mrima Hills
Z. holstiana	Diani
Z. chalybeum	Diani
Monondora grandieri	Mrima Hills
Mkilua fragrans	Shimba Hills
Isolana cauliflora	Shimba Hills
Salsola monoica	Ngomeni
Bidens schimperi	Shimba Hills

6.3 Extraction

The powdered plant material (200 g) was subjected to sequential cold extraction using hexane, dichloromethane, ethyl acetate and methanol. The plant material (200 g) was soaked in 1litre of hexane for 3 days with constant stirring after which it was filtered to obtain a filtrate, which was concentrated *in vacuo* to give the extract and the residue soaked in CH_2Cl_2. The procedure was repeated with EtOAc and methanol (Scheme 10). The crude extracts obtained were assayed for larvicidal activity and the active ones subjected to further bioassay-guided fractionation.

Scheme 10: Extraction procedure for the plants



6.4 Larvicidal assays

The larvicidal assays were carried out using 3rd instar *An. gambiae* larvae reared at the ICIPE, Nairobi, mosquito insectary. The temperatures and humidity of the rearing and bio-assay rooms were maintained at 28 ^{0}C and 85%, respectively.

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6.5 Preliminary larvicidal assays

A stock solution was prepared by dissolving 10 mg of extract in 1 ml of acetone or absolute ethanol (for methanol extracts) and then adding 99 ml of distilled water to make 100 ml to achieve a concentration of 100 ppm. Each of the prepared solutions was divided into 5 portions of 20 ml each in 30 ml vials. Five 3rd instar *An. gambiae* larvae were introduced into each vial, fed with fish food and mortality monitored and recorded after 24, 48 and 72 hours. Control experiments were done by adding 1 ml of acetone into a 100 ml volumetric flask and then adding distilled water to the mark and the procedure above repeated.

Mortality in test vials was weighted against that of the control vials and % mortality calculated using the formula:

$$\% \text{ Mortality} = [Y/X] \times 100$$

Where, Y is the mean death defined by the difference between the mean test deaths and the mean control deaths, and X is the initial larvae population (WHO, 1996). The results are summarized in tables 1-4.

6.6 Detailed larvicidal assays

This was done on the extracts or isolated compounds that exhibited high larvicidal activity at the preliminary level. This was done at 10, 5, 2 and 1 ppm by dissolving 5.0, 2.5, 2.0 and 1.0 mg of crude extract in 1 ml of acetone and adding to 499 ml of distilled water in a 500 ml beaker. Each of the prepared solutions was divided into 5 portions of 100 ml each in 250 ml beakers. Ten (10) 3rd instar larvae were introduced into each beaker, and fed with fish food and mortality monitored and recorded after 24, 48 and 72 hours.

Probit analysis was done to determine the regression equations. This analysis was done using log dose and probit transformation of percentage mortality employing probit plane model by Busvine (1971). Probit transformations were plotted against log (+1) dose using excel programme. A regression equation was obtained and the lethal concentrations (LC) were calculated (Tables 5-8).

6.7 Bio-assay guided fractionation

The bio-active extracts were fractionated by column chromatography using Kiesegel G_{60} silica gel. All fractions were monitored using Kiesegel G_{60} F_{254} 5 x 10 cm analytical TLC plates with fluorescent indicator and those with similar compounds pooled together to give several portions. The locating agents were UV lamp (254 and 366 nm) and 2% concentrated sulphuric acid in methanol.

The fractions were assayed for larvicidal activity (Tables 9, 12, 15) and the bio-active ones subjected to further column or preparative thin layer chromatography and recrystallisation to obtain pure compounds.

The pure compounds obtained in substantial amounts were subjected to further bio-assay (Tables 10, 13, 16) and probit analysis used to calculate LC (Tables 11, 14, 17).

6.8 Isolation of compounds from *Derris trifolia*

Dichloromethane extract (6 g) was subjected to fractionation by column chromatography on silica gel with a hexane:ethyl acetate gradient (100:0 →0:100) giving 77 fractions which were pooled based on their R_f values and concentrated *in vacuo* to give 15 fractions (F_{1D}-F_{15D}). Through preparative thin layer chromatography, column chromatography and recrystallisation, Four compounds: D_{1A}, D_2, D_{3A} and D_7, were obtained in reasonable amounts with D_7 exhibiting high larvicidal activity (Tables 9-11). D_{1A} was insoluble in the solvents that were available for NMR analysis.

Lupeol (107) (D_2)

This compound was isolated as white powder (20.0 mg) from F_{5D} and its melting point was found to be 213-215 ^{0}C (lit. 215-216 ^{0}C, Reynolds *et al.*, 1986). UV λ_{max} (MeOH) 201 nm; IR ν_{max} 3227, 3092, 1670, 1377 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.64-2.01 (m, 46H), 2.30-2.43 (m, 1H, H-19), 3.16 (dd, 1H, H-3), 4.67 (s, 2H, H-29); ^{13}C NMR (200 MHz, $CDCl_3$) δ 14.5 (q, C-27), 15.4 (q, C-24), 16.0 (q, C-26), 16.0 (q, C-25), 18.0 (q, C-28), 18.3 (t, C-6), 19.3 (q, C-30), 20.9 (t, C-11), 25.1 (t, C-12), 27.4 (t, C-2), 28.0 (q, C-23), 29.7 (t, C-15), 29.7 (t, C-21), 34.2 (t, C-7), 35.5 (t, C-16), 37. 1 (s, C-10), 38.0 (d, C-13), 38.7 (t, C-1), 38.8 (t, C-4), 40.0 (s, C-22), 40.8 (s, C-8), 43.0 (s, C-14), 43.0 (s, C-17), 48.0 (d, C-19), 48.3 (d, C-18), 50.4 (d, C-9),

55.2 (d, C-5), 79.0 (d, C-3), 109.3 (t, C-29), 151.0 (s, C-20); EIMS m/z 426 (2%), 218 (7%), 189 (8%), 125(18%), 111 (38%), 109 (16%), 97 (78%), 83 (100%), 69 (86%). The spectral data obtained was comparable to that published by Reynolds *et al.* (1986).

Stigmasterol (109) (D_{3A})

This compound was isolated as a white solid (3.4 mg) from F_{6D} with a melting point of 162-165 ^{0}C (lit. 170 ^{0}C, Budavari, 1996); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.67-2.38 (m, 44H), 3.58 (m, 1H, H-3), 5.01-5.12 (m, 2H, H-22, H-23), 5.28 (br d, 1H, H-6); ^{13}C NMR (200 MHz, $CDCl_3$) δ 12.0 (q, C-18), 12.2 (q, C-29), 18.9 (q, C-19), 19.1 (q, C-27), 19.5 (q, C-26), 20.0 (q, C-21), 21.2 (t, C-11), 23.2 (t, C-15), 24.4 (t, C-28), 28.4 (t, C-16), 29.2 (d, C-25), 31.7 (t, C-2), 32.0 (d, C-8), 34.0 (t, C-7), 36.2 (d, C-20), 36.6 (s, C-10), 37.3 (t, C-1), 39.7 (t, C-12), 42.3 (t, C-4), 45.9 (s, C-13), 50.1 (d, C-9), 51.3 (d, C-24), 56.0 (d, C-17), 56.8 (d, C-14), 71.8 (d, C-3), 121.6 (d, C-6), 129. 1 (d, C-23), 138.2 (d, C-22), 140.6 (s, C-5); EIMS m/z 412 (8%), 396 (7%), 119 (46%), 111 (43%), 69 (85%), 57 (100%), 43 (97%). The spectral data obtained was comparable to that published by Rubinstein *et al.* (1976) and Greca *et al.* (1990).

Rotenone (40) (D_7)

This compound was isolated as a white crystalline solid (20.4 mg) from F_{8D} and F_{9D} and its melting point was found to be 162-164 ^{0}C (lit. 163^{0}C, Laforge and Smith, 1929; 162 ^{0}C, Singhal *et al.*, 1982). IR ν_{max} 1674, 1647, 1234, 1217 cm^{-1}; UV λ_{max} (MeOH) 200, 236, 293 nm; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.76 (s, 3H, H-5'), 2.94 (dd, 1H, J=8.4, 16.0 Hz, H-1'a), 3.31 (dd, 1H, J=9.5, 16.0 Hz, H-1'b), 3.75 (s, 3H, $-OCH_3$), 3.80 (s, 3H, $-OCH_3$), 3.83 (d, 1H, J=4.2 Hz, H-12a), 4. 17 (d, 1H, J=12.2 Hz, H-6_{ax}), 4.35 (dd, 1H, J=3.1, 12.2 Hz, H-6_{eq}), 4.92 (m, 2H, H-4'b superimposed on H-6a), 5.06 (s, 1H, H-4'a), 5.23 (dd, 1H, J=9.5, 8.4 Hz, H-2'), 6.44 (s, 1H, H-4), 6.50 (d, 1H, J=8.6 Hz, H-10), 6.75 (s, 1H, H-1), 7.83 (d, 1H, J=8.6 Hz, H-11); ^{1}H-^{1}H COSY revealed coupling between H-1'a (δ 2.94), H-1'b (δ 3.31) and H-2' (δ 5.23); H-6a (δ 4.92), H-12a (δ 3.83) and H-6_{eq} (δ 4.35); H-6_{ax} (δ 4.17) and H-6_{eq} (δ 4.35); H-6_{eq} (δ 4.35); H-4'b (δ 4.92) and H-4'a (δ 5.06); H-10 (δ 6.50) and H-11 (δ 7.83); ^{13}C NMR (200 MHz, $CDCl_3$) δ 17.3 (q, C-5'), 31.3 (t, C-1'), 44.6 (d, C-12a), 55.9 (q, $-OCH_3$), 56.3 (q, $-OCH_3$), 66.3 (t, C-6), 72. 2 (d, C-6a), 87.8 (d, C-2'), 100.8 (d, C-4), 104.7 (s, C-12b), 104.8 (s, C-10), 110.1 (d, C-1), 112.5 (t, C-4'), 112.9 (s, C-8), 113. 2 (s, C-11a), 129.9 (d, C-11), 142.8 (s, C-3'), 143.6 (s, C-2), 147. 1 (s,

C-4a), 149.2 (s, C-3), 157.7 (s, C-7a), 167.1 (s, C-9), 188.7 (s, C-12); EIMS m/z 394 (14%), 208 (4%), 203 (4%), 193 (17%), 192 (100%), 191 (42%), 177 (24%), 121 (13%), 106 (12%), 93 (12%), 77 (22%), 65 (16%). The spectral values obtained were comparable to those by Singhal *et al.* (1982) and Crombie *et al.* (1975).

Isolation of compounds from *Zanthoxylum holstiana*

Ethyl acetate extract (5 g) was subjected to fractionation by column chromatography on silica gel with a hexane-ethyl acetate gradient (100:0→0:100) giving 33 fractions which were pooled based on their R_f values and concentrated *in vacuo* to give 12 fractions (F_{1H}-F_{12H}). Through preparative thin layer chromatography and re-crystallisation, 4 compounds: H_1, H_2, H_5 and H_7 were obtained with H_5 and H_7 exhibiting high larvicidal activity (Table 14).

Dihydrochelerythrine (116) (H_1, H_4)

This compound was isolated as a cream crystalline solid (82.4 mg) from F_{2H} and had a melting point of 162-165 ^{0}C (lit. 163-166 ^{0}C, Shafiee *et al.*, 1979). IR ν_{max} 3505, 2978, 1273, 1248, 1217, 819 cm^{-1}; UV λ_{max} (MeOH) 200, 235, 284 nm; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.59 (s, 3H, -NCH_3), 3.87 (s, 3H, -OCH_3), 3.92 (s, 3H, -OCH_3), 4.29 (s, 2H, H-8), 6.04 (s, 2H, -OCH_2O-), 6.93 (d, 1H, J=8.5 Hz, H-11), 7.10 (s, 1H, H-4), 7.47 (d, 1H, J=8.4 Hz, H-5), 7.50 (d, 1H, J=8.4 Hz, H-6), 7.67 (s, 1H, H-1), 7.69 (d, 1H, J=8.5 Hz, H-12); ^{13}C NMR (200 MHz, $CDCl_3$) δ 41.4 (-NCH_3), 48.7 (t, C-8), 55.8 (q, -OMe), 61.1 (q, -OMe,), 100.7 (t, -OCH_2O-), 100.9 (d, C-1), 104.2 (d, C-4), 110.7 (d, C-11), 118.6 (d, C-5), 120.0 (d, C-12), 123.6 (d, C-6), 124.1 (s, C-13), 126.0 (s, C-4a), 126.1 (s, C-12a), 126.2 (s, C-8a), 130.6 (s, C-14a), 142.5 (s, C-3), 145.8 (s, C-2), 147.2 (s, C-10), 147.8 (s, C-9), 152.0 (s, C-14); EIMS m/z 349 (100%), 348 (85%), 332 (13%), 350 (23%), 319 (12%), 304 (12%), 290 (17%), 159 (25%), 137 (23%), 102 (38%), 88 (15%), 63 (24%). The spectral data obtained was comparable to that published by Shafiee *et al.* (1979), Chen *et al.* (1994) and Guinaudeau and Bruneton (1993).

Arnottianamide (125) (H_2)

This compound was isolated as a white crystalline solid (2.5 mg) from F_{7H} and it had a melting point of 265-267 ^{0}C (lit. 267-270 ^{0}C, Adenisa and Reisch, 1988; Ishii and Ishikawa, 1976).

^1H NMR (200 MHz, $CDCl_3$) δ 2.99 (s, 3H, $-NCH_3$), 3.89 (s, 3H, $-OCH_3$), 3.92 (s, 3H, $-OCH_3$), 5.95 (s, 1H, -OH), 6.08 (s, 2H, $-OCH_2O-$), 6.53 (d, 1H, J=8.7 Hz, H-6'), 6.80 (d, 1H, J=8.7 Hz, H-5'), 7.07 (s, 1H, H-7), 7.19 (s, 1H, H-4), 7.30 (d, 1H, J=8.3 Hz, H-3), 7.72 (d, 1H, J=8.3 Hz, H-2), 8.15 (s, 1H, -CHO); ^{13}C NMR (200 MHz, $CDCl_3$) δ 33.1 (q, -NMe), 55.9 (q, C-4', -OCH_3), 61.2 (q, C-3', $-OCH_3$), 99.2 (d, C-5'), 101.4 (t, $-OCH_2O-$), 103.8 (d, C-4), 104.3 (d, C-7), 118.4 (s, C-1'), 125.0 (d, C-3), 127.2 (d, C-2), 127.3 (d, C-6'), 131.1 (s, C-3a), 133.2 (s, C-6), 135.4 (s, C-5), 146.5 (s, C-2'), 147.9 (s, C-4'), 149.1 (s, C-3'), 151.8 (s, C-1a), 164.3 (s, CHO). The spectral values obtained were comparable to those published by Adenisa and Reisch (1988) and Ishii and Ishikawa (1976).

_N_-Methylflindersine (129) (H_5)

This compound was isolated as a cream wax (30.0 mg) from F_{3H} and its melting point was found to be 82-84 ^0C (lit. 84 ^0C, Budavari, 1996); IR ν_{max} 1705, 1211 cm^{-1}; UV λ_{max} (MeOH) 216, 235, 331, 345, 361 nm; ^1HNMR (200 MHz, $CDCl_3$) δ 1.43 (s, 6H, ($2CH_3$), 3.61 (s, 3H, $-NCH_3$), 5.45 (d, 1H, J=10 Hz, H-3'), 6.67 (d, IH, J=10 Hz, H-2'), 7.14 (dd, 1H, J=6.6, 7.3 Hz, H-7), 7.20 (d, 1H, d, J=6.6 Hz, H-8), 7.46 (dd, 1H, J=8.1, 7.3 Hz, H-6), 7.88 (d, 1H, J=8.1 Hz, H-5); ^{13}C NMR (200 MHz, $CDCl_3$) δ 28.3 (q, CH_3), 29.4 (q, $-NCH_3$), 78.7 (s, C-1') 105.7 (s, C-4a), 113.9 (d, C-2'), 115.9 (s, C-3), 117.8 (d, C-3'), 121.6 (d, C-3a), 122.9 (d, C-7), 126.2 (d, C-6), 130.7 (d, C-5), 139.1 (s, C-8), 155.0 (s, C-4), 160.8 (s, C-2); EIMS m/z 241 (33%), 227 (24%), 226 (100%), 183 (8%), 154 (7%), 132 (7%), 113 (29%), 104 (15%), 77 (46%), 51 (27%). The spectroscopic data obtained was in agreement with that reported by Ishii *et al.* (1991).

Isolation of compounds from _Zanthoxylum paracanthum_

The dichloromethane extract (6 g) was subjected to column chromatography as detailed above and a total of 249 fractions collected. These were pooled depending on their R_f values to give 18 fractions (F_{1P}-F_{18P}). The fractions were purified further and 11 compounds: B_1, B_2, B_3, B_4, B_5, B_6, B_{7A}, B_8, B_9, B_{10} and B_{11} obtained. B_1, B_3, B_8 and B_{10} were found to be similar from chromatographic and spectroscopic comparison. B_2, B_6 and B_9 were also found to be the same through spectral and chromatographic analysis. B_{11} was insoluble in the solvents that were available for NMR analysis, and was not identified.

Sesamin (155) (B_1, B_3, B_8, B_{10})

This compound was isolated as white crystals (145 mg) from F_{4P}, F_{5P}, F_{6P} and F_{8P} with a melting point of 121-123 ^{0}C (lit. 123-124 ^{0}C, Anjaneulu *et al.*, 1977; Pelter *et al.*, 1976). IR ν_{max} 1498, 1377, 1095 and 1037 cm^{-}1; UV λ_{max} (MeOH) 235 and 284 nm; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.05 (br d, 1H, J=1.4 Hz, H-1, H-5), 3.86 (dd, 1H, J=3.7, 9.1 Hz, H-4$_{eq}$, H-8$_{eq}$), 4.23 (dd, 1H, J=7.2, 9.1 Hz, H-4$_{ax}$, H-8$_{ax}$), 4.71 (d, 1H, J=3.7 Hz, H-2, H-6), 5.95 (s, 2H, -OCH_2O-), 6.79 (s, 2H, H-5", H-6", H-5', H-6'), 6.85 (s, 1H, H-2', H-2"); ^{1}H-^{1}H COSY revealed coupling between H-1, H-5 (δ 3.05), and H-2, H-6 (δ 4.71), H-4$_{ax}$, H-8$_{ax}$ (δ 4.23), H-4$_{eq}$, H-8$_{eq}$ (δ 3.86); H-4$_{ax}$, H-8$_{ax}$ (δ 4.23) and H-4$_{eq}$, H-8$_{eq}$ (δ 3.86); ^{13}C NMR (200 MHz, $CDCl_3$) δ 54.5 (d, C-1, C-5), 71.9 (t, C-4, C-8), 86.0 (d, C-2, C-6), 101.3 (t, -OCH_2O-), 106.7 (d, C-2', C-2"), 108.4 (d, C-5', C-5"), 119.6 (d, C-6', C-6"), 135.2 (s, C-1', C-1"), 147.3 (s, C-3', C-3"), 148.2 (s, C-4', C-4"); EIMS m/z 354 (9%), 203 (9%), 161 (28%), 150 (34%), 149 (100%), 135 (45%), 131 (34%), 121 (25%), 103 (25%), 77 (22%), 65 (29%). The spectral data obtained was in agreement with that published by Anjaneulu *et al.* (1977) and Pelter *et al.* (1976).

1-Hydroxy-10-methoxyooxoarpophine (158) (B_4)

This compound was isolated as a cream solid (51.4 mg) from F_{10P} and F_{12P} and its melting point found to be 178-181 ^{0}C. IR ν_{max} 3628, 1703, 1662, 1653, 1616, 1576, 1279 cm^{-1}; UV λ_{max} (MeOH) 207, 264, 271, 309, 350 nm; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.99 (s, 3H, -OCH_3), 6.96 (d, 1H, J=9.9 Hz, H-2), 7.08 (dd, 1H, J=2.5, 8.7 Hz, H-9), 7.86 (d, 1H, J=5.0 Hz, H-4), 7.97 (d, 1H, J=8.7 Hz, H-8), 8.01 (d, 1H, J=9.9 Hz, H-3), 8.22 (d, J=2.5 Hz, 1H, H-11), 8.75 (d, 1H, J=5.0 Hz, H-5); ^{1}H-^{1}H COSY revealed coupling between H-3 (δ 6.96) and H-2 (δ 8.01); H-9 (δ 7.08), H-8 (δ 7.97) and H-11 (δ 8.22); H-9 (δ 7.08) and H-11 (δ 8.22); H-4 (δ 7.86) and H-5 (δ 8.75); NOE revealed interactions between –OCH_3 (δ 3.99) with H-11 (δ 8.22) and H-9 (δ 7.08); ^{13}C NMR (200 MHz, $CDCl_3$) δ 56.0 (q, OCH_3), 101.2 (d, C-11), 114.4 (d, C-9), 115.5 (d, C-4), 116.8 (s, C-7a), 123.6 (d, C-8), 129.0 (d, C-2), 131.1 (s, 6a), 132.2 (s, 6b), 134.5 (s, 3a), 138.8 (d, C-3), 141.6 (s, C-11a), 144.6 (d, C-5), 159.4 (s, C-1), 162.8 (s, C-10); EIMS m/z 250 (100%), 251 (35%), 249 (18%), 235 (8%), 221 (34%), 207 (65%), 164 (11%), 153 (43%), 126 (27%). The structure was confirmed by COSY, NOE, HMBC and HMQC experiments (Table 20-21).

1,8-Dihydroxy-9-methoxyooxoarporphine (159) (B_5)

This compound was isolated as a fine orange crystal (3.0 mg) from F_{14P} and its melting point found to be 192-195 ^{0}C. IR ν_{max} 3600, 1710, 1667 and 1610 nm; UV λ_{max} (MeOH) 212, 261, 320, 333 and 403 nm; ^{1}H NMR (200 MHz, $CDCl_3$), δ 4.03 (s, 3H, $-OCH_3$), 7.06 (d, 1H, J=9.8 Hz, H-2), 7.08 (d, 1H, J=8.8 Hz H-10), 7.51 (d, 1H, J=8.8 Hz, H-11), 7.88 (d, 1H, J=5.0 Hz, H-4), 8.15 (d, 1H, J=9.8 Hz, H-3), 8.81 (d, 1H, J=5.0 Hz, H-5), 12.20 (s, 1H, -OH, H-8); ^{1}H-^{1}H COSY revealed coupling between H-2 (δ 7.06) and H-3 (δ 8.15), H-5 (δ 8.81) and H-4 (δ 7.88), H-10 (δ 7.08) and H-11 (δ 7.51); ^{13}C NMR (200 MHz, $CDCl_3$) δ 56.9 (q, OCH_3), 111.1 (d, C-10), 113.5 (d, C-11), 116.1 (d, C-4), 119.5 (s, C-3a), 126.7 (d, C-2), 128.4 (s, 11b), 136.9 (s, C-7a), 141.1 (d, C-3), 146.9 (d, C-5), 151.7 (s, C-9), 160.4 (s, C-1); EIMS m/z 266 (100%), 248 (58%), 237 (43%), 223 (83%), 195 (46%), 180 (15%), 167 (42%), 140 (54%). The structure was confirmed by COSY, NOE, DEPT, HMBC and HMQC experiments (Tables 22-23).

1-Hydroxyoxoarporphine (160) (B_6, B_2)

This compound was isolated as orange crystals (10.0 mg) from F_{9P} and its melting point found to be 161-164 ^{0}C. IR ν_{max} 3628, 1703, 1647, 1635 cm^{-1}; UV λ_{max} (MeOH) 213, 242, 247, 253, 297, 378 nm; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.99 (d, 1H, J=9.8 Hz, H-2), 7.53 (dd, 1H, J=8.0, 7.5 Hz, H-9), 7.71 (dd, 1H, J=7.6, 7.5 Hz, H-10), 7.97 (d, 1H, J=5.0 Hz, H-4), 8.03 (d, 1H, J=9.8 Hz, H-3), 8.11 (d, 1H, J=8.0 Hz, H-8), 8.67 (d, 1H, J=7.6 Hz, H-11), 8.82 (d, 1H, J=5.0 Hz, H-5); ^{1}H-^{1}H COSY revealed coupling between H-4 (δ 7.97) and H-5 (δ 8.82), H-3 (δ 8.03) and H-2 (δ 6.99), H-8 (δ 8.11) and H-9 (δ 7.53), H-11 (δ 8.67) and H-10 (δ 7.71), H-10 (δ 7.71) and H-9 (δ 7.53); ^{13}C NMR (200 MHz, $CDCl_3$) δ 116.4 (d, C-4), 117.2 (d, C-11), 122.6 (d, C-8), 124.3 (s, C-7a), 125.6 (d, C-9), 128.8 (d, C-2), 130.2 (s, C-6a), 130.8 (d, C-10), 131.9 (s, C-6b), 136.1 (s, C-3a), 139.3 (s, C-11a), 139.5 (d, C-3), 145.7 (d, C-5), 159.4 (s, C-1); EIMS m/z 221 (18%), 220 (98%), 192 (100%), 164 (27%), 139 (24%), 114 (28%), 96 (23%), 83 (28%). The structure was confirmed by COSY, DEPT, HMQC, HETCOR and HMBC experiments (Tables 24-25).

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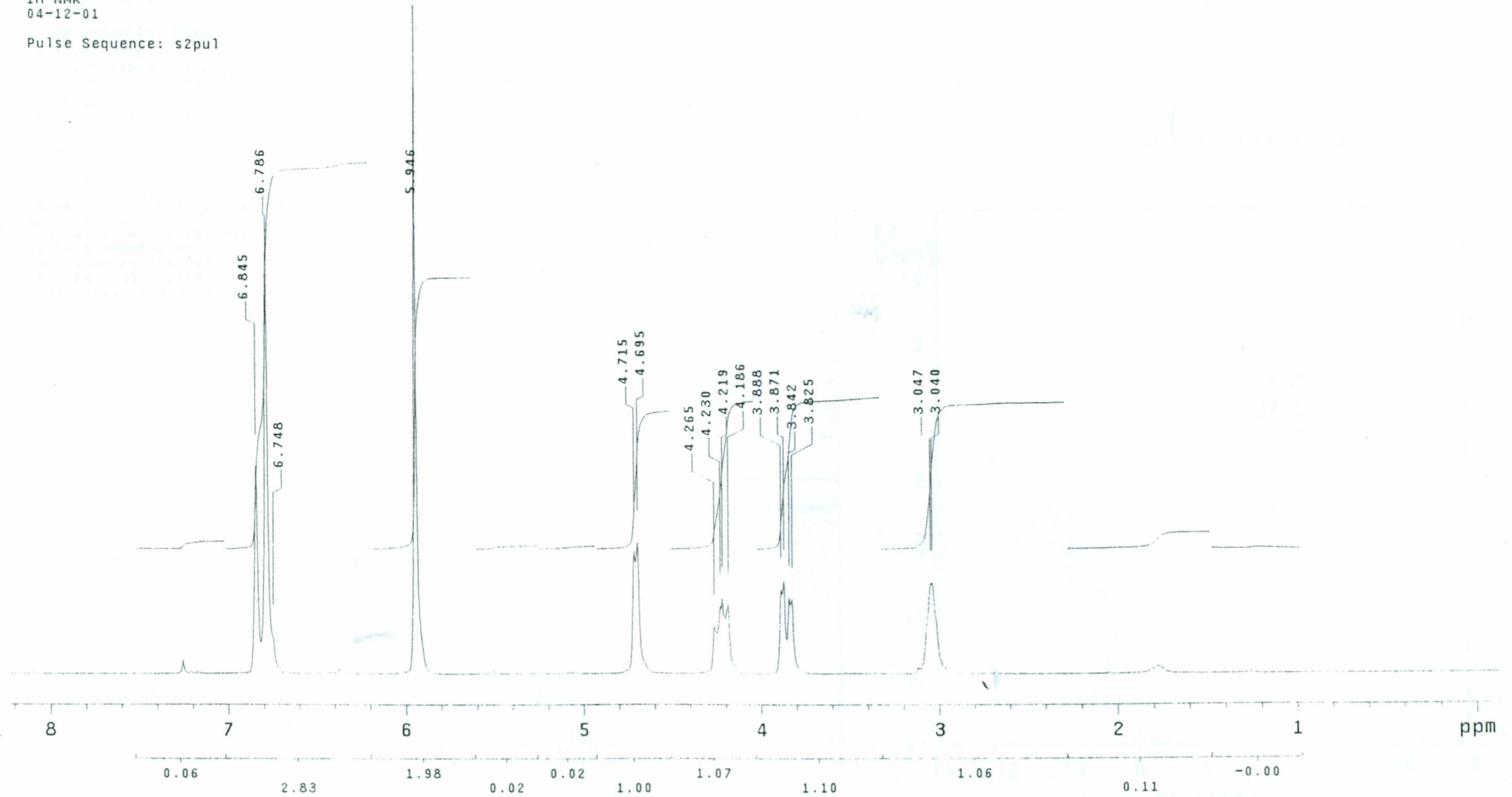
APPENDICES

^{1}H NMR Spectrum for B_1



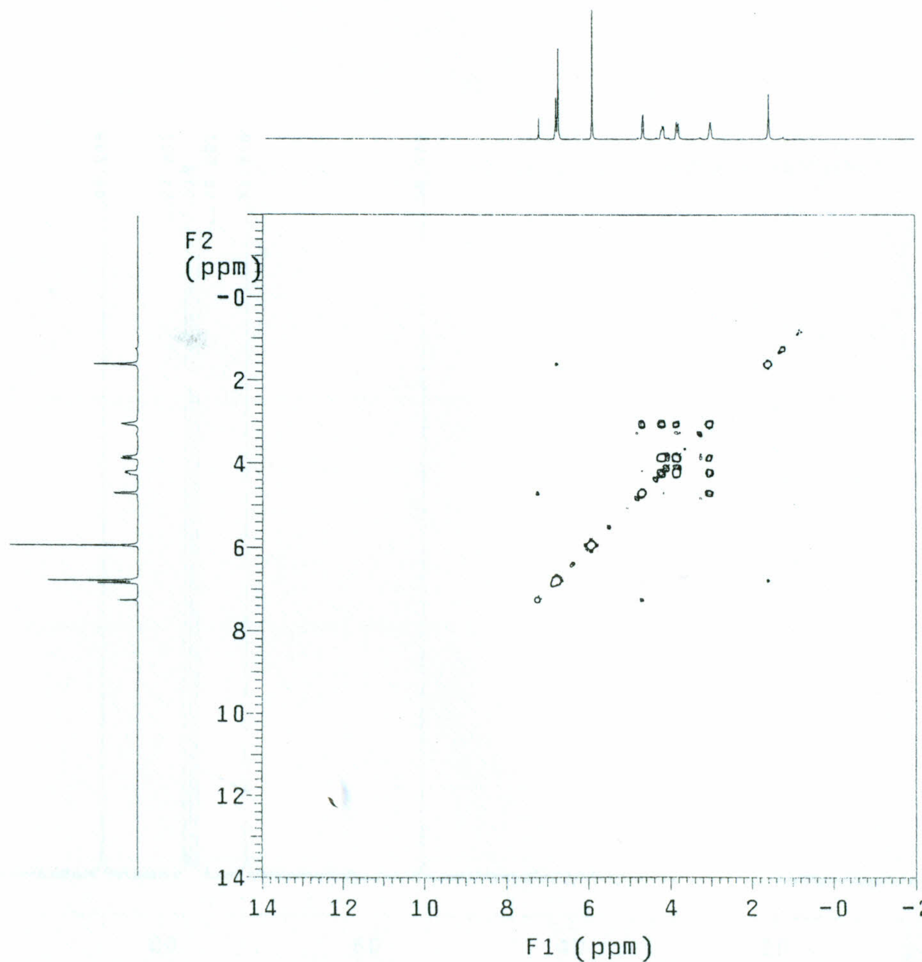
COSY Spectrum for B_1

Name: Fidelis Samita (KU)
Code: FS/B8/P
Weight: 9.8 mg
Solvent: CDCl3

Pulse Sequence: COSY

Solvent: CDCl3
Ambient temperature
Mercury-200 "uonnmr200"

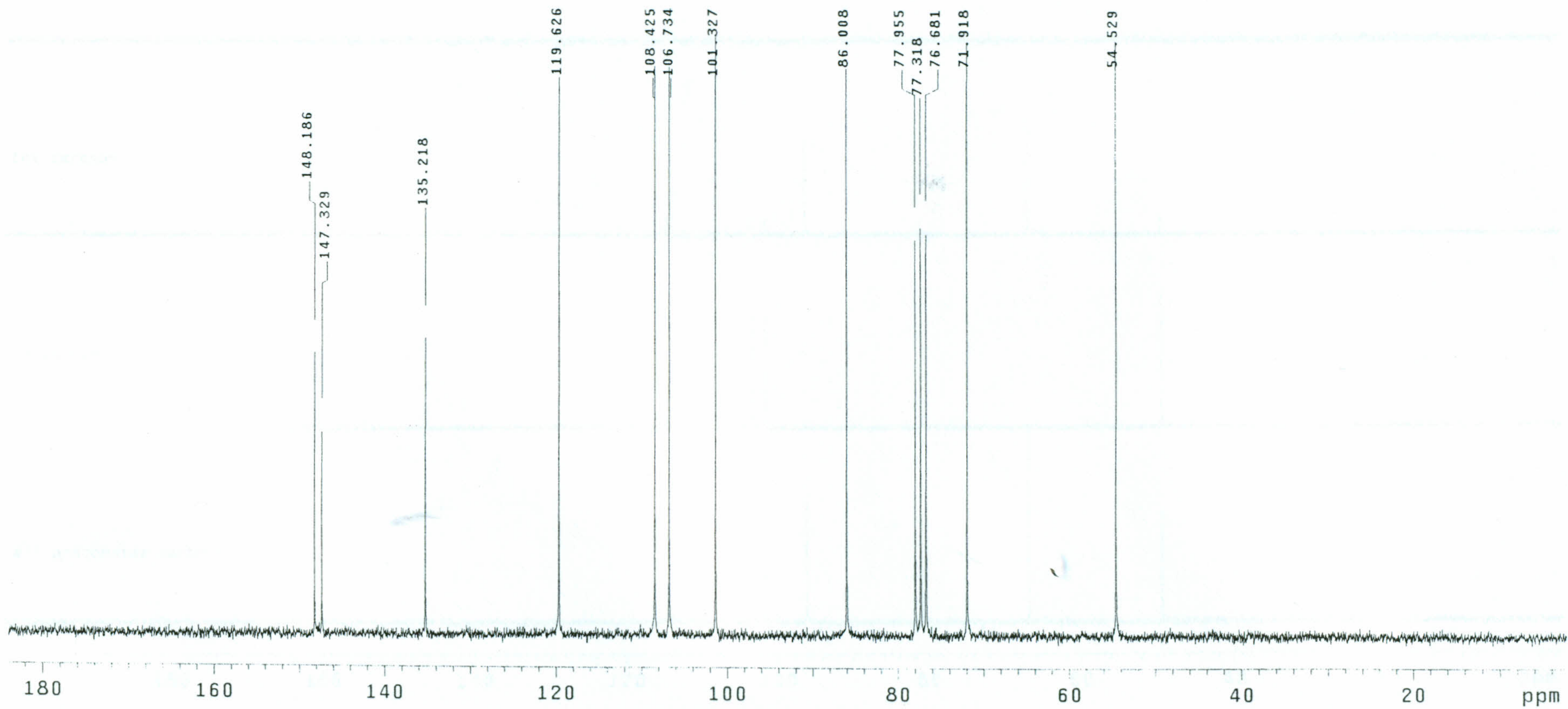
PULSE SEQUENCE: COSY
Relax. delay 1.000 sec
Acq. time 0.160 sec
Width 3200.9 Hz
2D Width 3200.9 Hz
4 repetitions
200 increments
OBSERVE H1, 200.0557687 MHz
DATA PROCESSING
Sq. sine bell 0.080 sec
F1 DATA PROCESSING
Sq. sine bell 0.062 sec
FT size 2048 x 2048
Total time 18 min, 5 sec



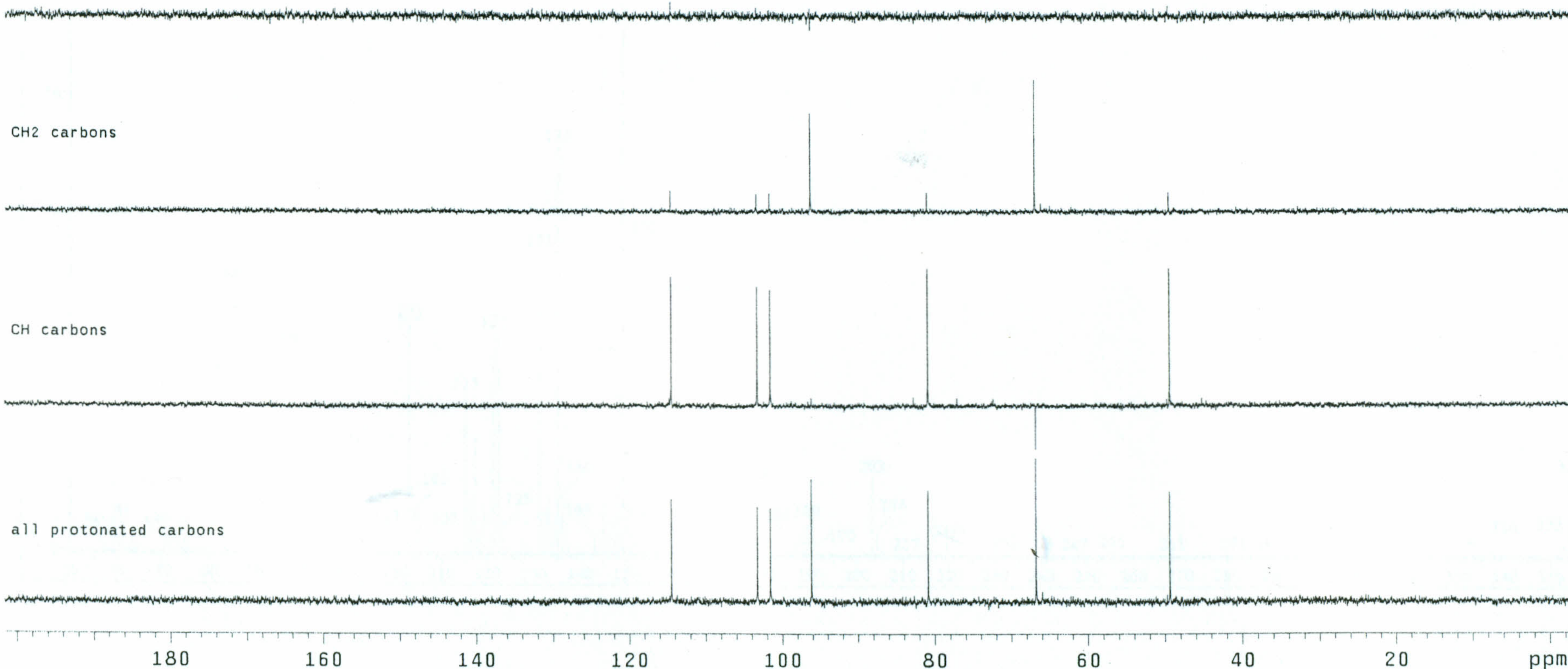
^{13}C NMR Spectrum for B_1

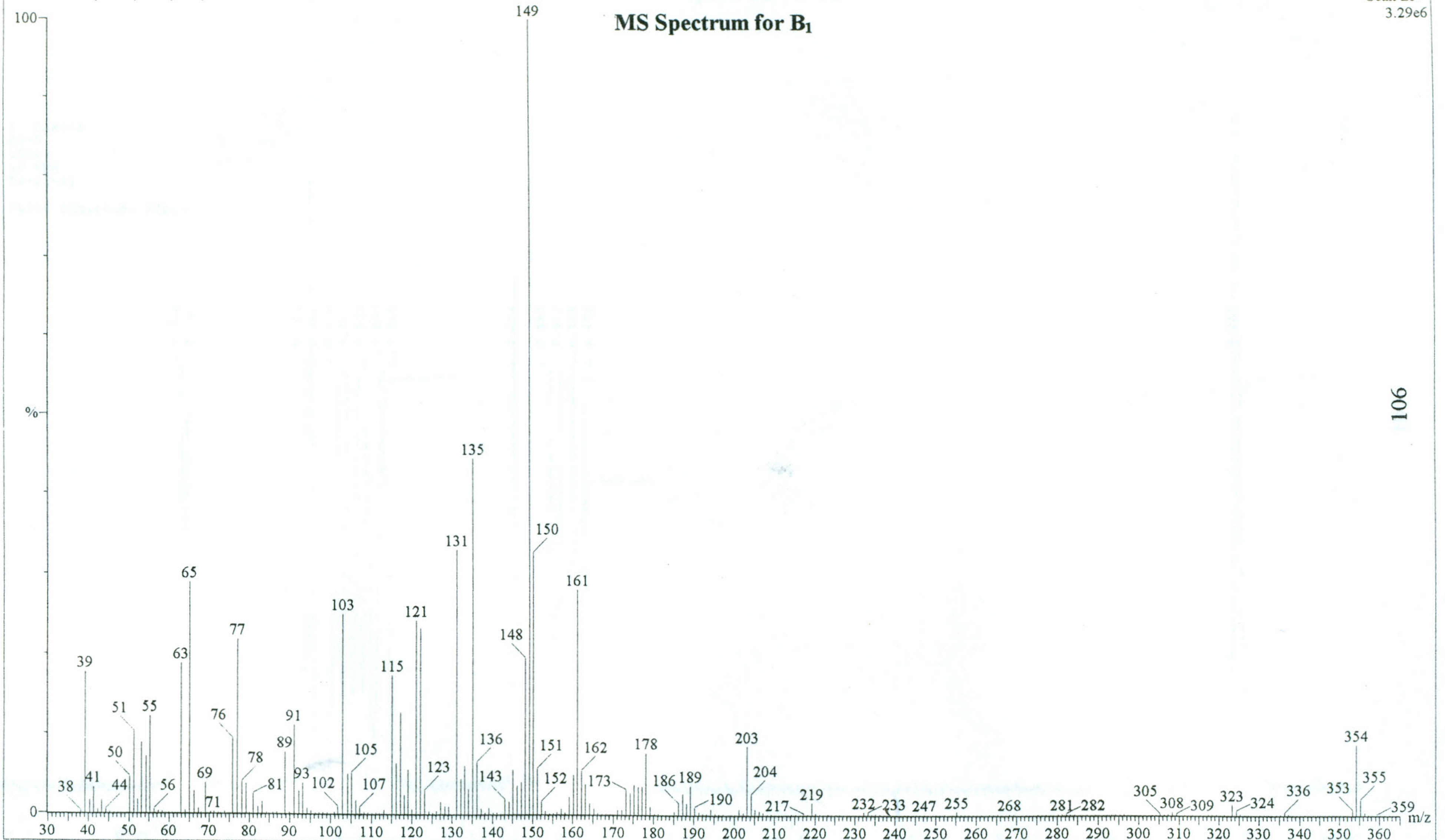
F. SAMITA
B1-P
CDCL3
13C NMR
04-12-01

Pulse Sequence: s2pul

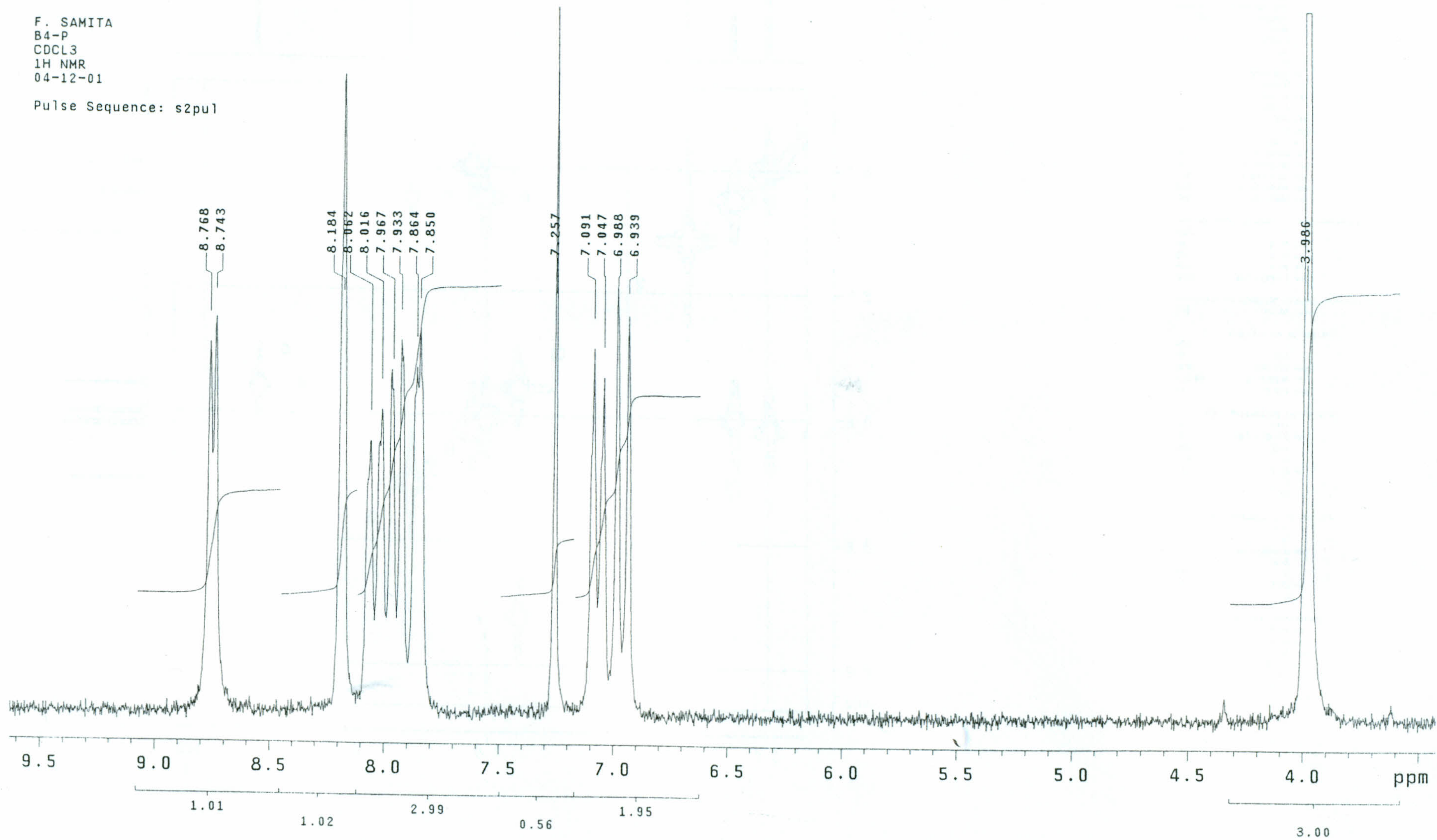


DEPT Spectrum for B_1

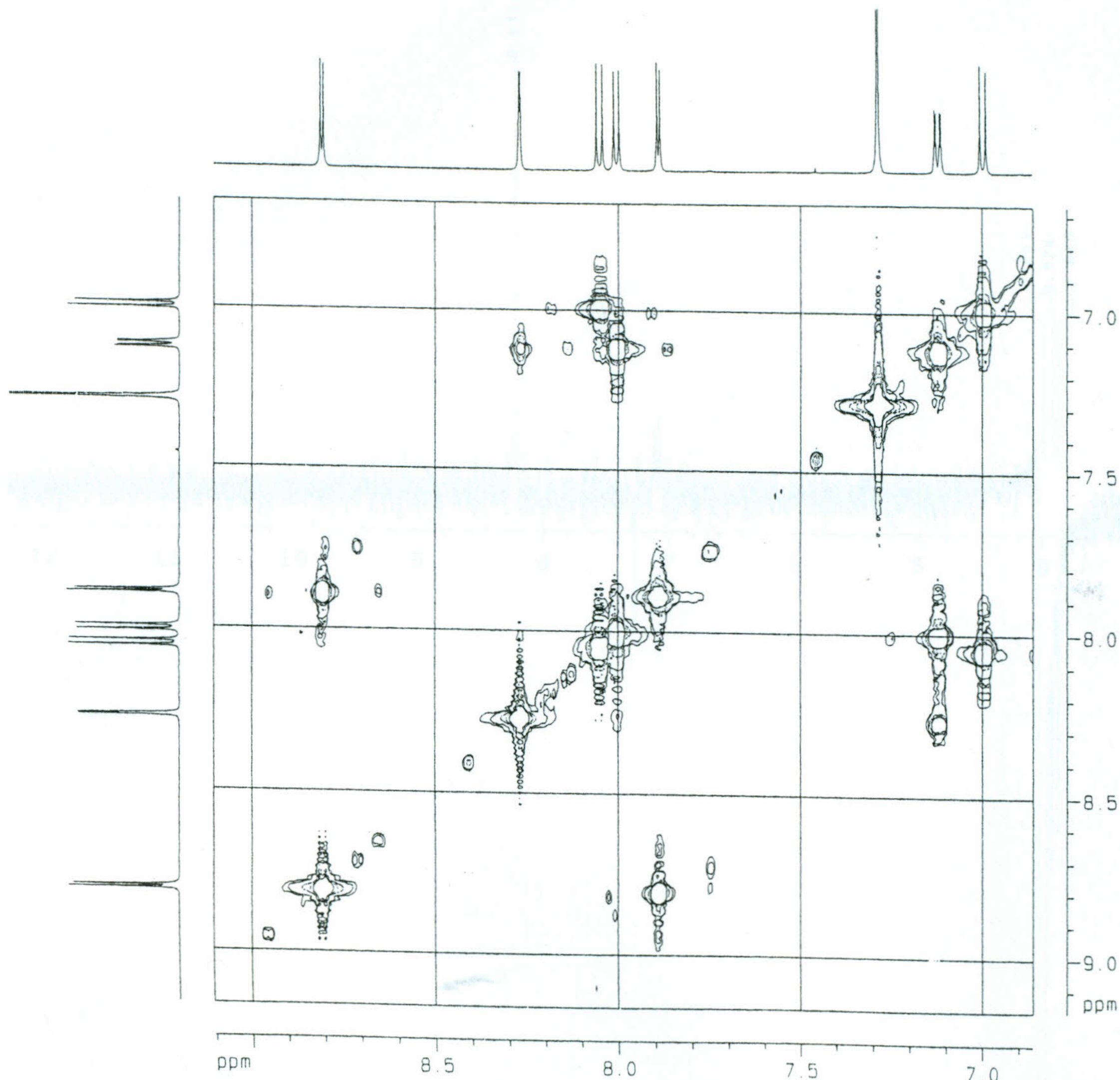



Ins: VG Platform II GC/LC-MS
BpM:149
FS B1 By Solid Probe
SF6602C 17 (0.592) Cm (6:21)
Date: 06-Jun-2002 Time: 11:15:02
BpI:3293632
Tic:24198938
Scan EI+
3.29e6
MS Spectrum for B1
%
m/z

^{1}H NMR Spectrum for B_4



COSY Spectrum for B_4



F5B4 COSY
University of Botswana
Chemistry Department
NABSA NMR Service

Current Data Parameters

NAME	f5b4
EXPNO	4
PROCNO	1

F2 - Acquisition Parameters

Date_	20021019
Time	11.05
INSTRUM	spect
PROBHD	5 mm SEI 1H-
PULPROG	cosygs
TD	2048
SOLVENT	CDCl3
NS	24
DS	8
SWH	5387.931 Hz
FIDRES	2.630826 Hz
AQ	0.1901044 sec
RG	1824.6
DW	92.800 usec
DE	6.00 usec
TE	300.0 K
D0	0.00000300 sec
D1	1.48689198 sec
D13	0.00000300 sec
D16	0.00010000 sec
IN0	0.00018560 sec

============ CHANNEL f1 ============

NUC1	1H
P0	8.40 usec
P1	8.40 usec
PL1	0.00 dB
SFO1	600.1329913 MHz

============ GRADIENT CHANNEL ========

P16	1000.00 usec

F1 - Acquisition parameters

ND0	1
TD	256
SFO1	600.133 MHz
FIDRES	21.046606 Hz
SW	8.978 ppm

F2 - Processing parameters

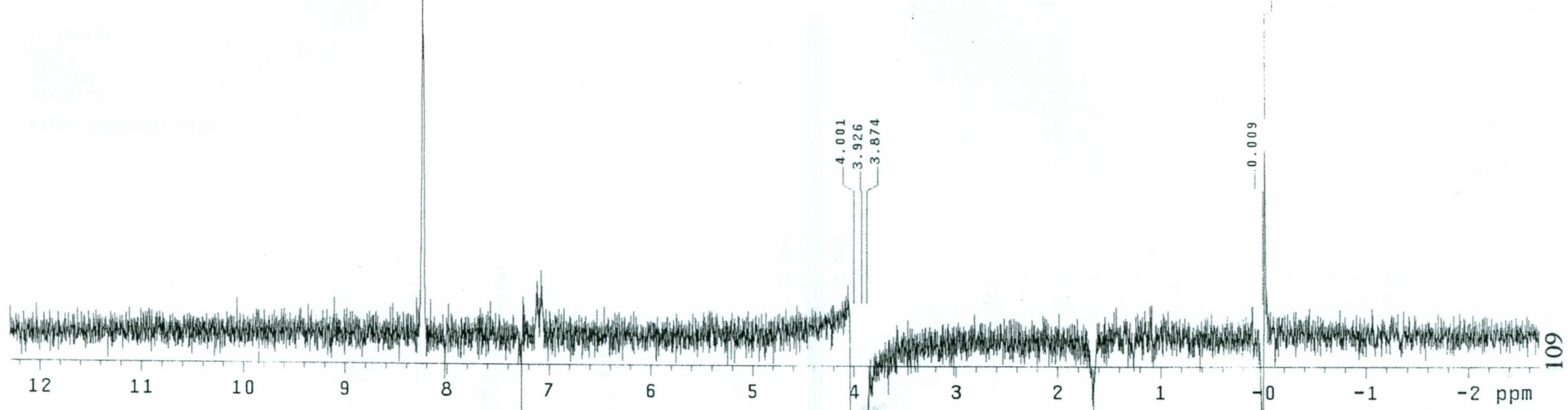
SI	1024
SF	600.1300000 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

F1 - Processing parameters

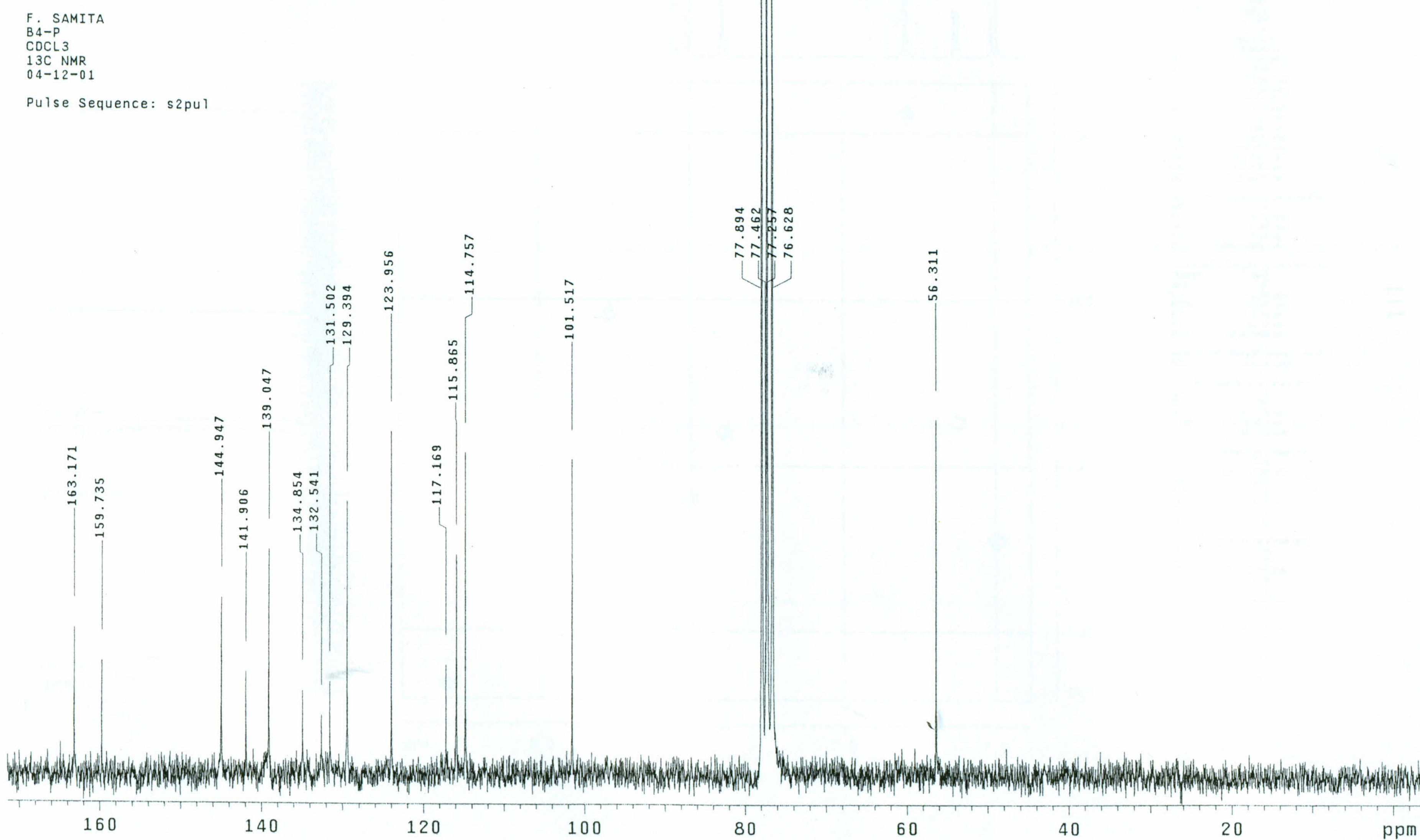
SI	1024
MC2	QF
SF	600.1300000 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	12.00 cm
CX1	12.00 cm
F2PLO	9.105 ppm
F2LO	5464.29 Hz
F2PHI	6.861 ppm
F2HI	4117.31 Hz
F1PLO	9.167 ppm
F1LO	5501.12 Hz
F1PHI	6.668 ppm
F1HI	4001.55 Hz
F2PPMCM	0.18704 ppm/cm
F2HZCM	112.24856 Hz/cm
F1PPMCM	0.20823 ppm/cm
F1HZCM	124.96423 Hz/cm

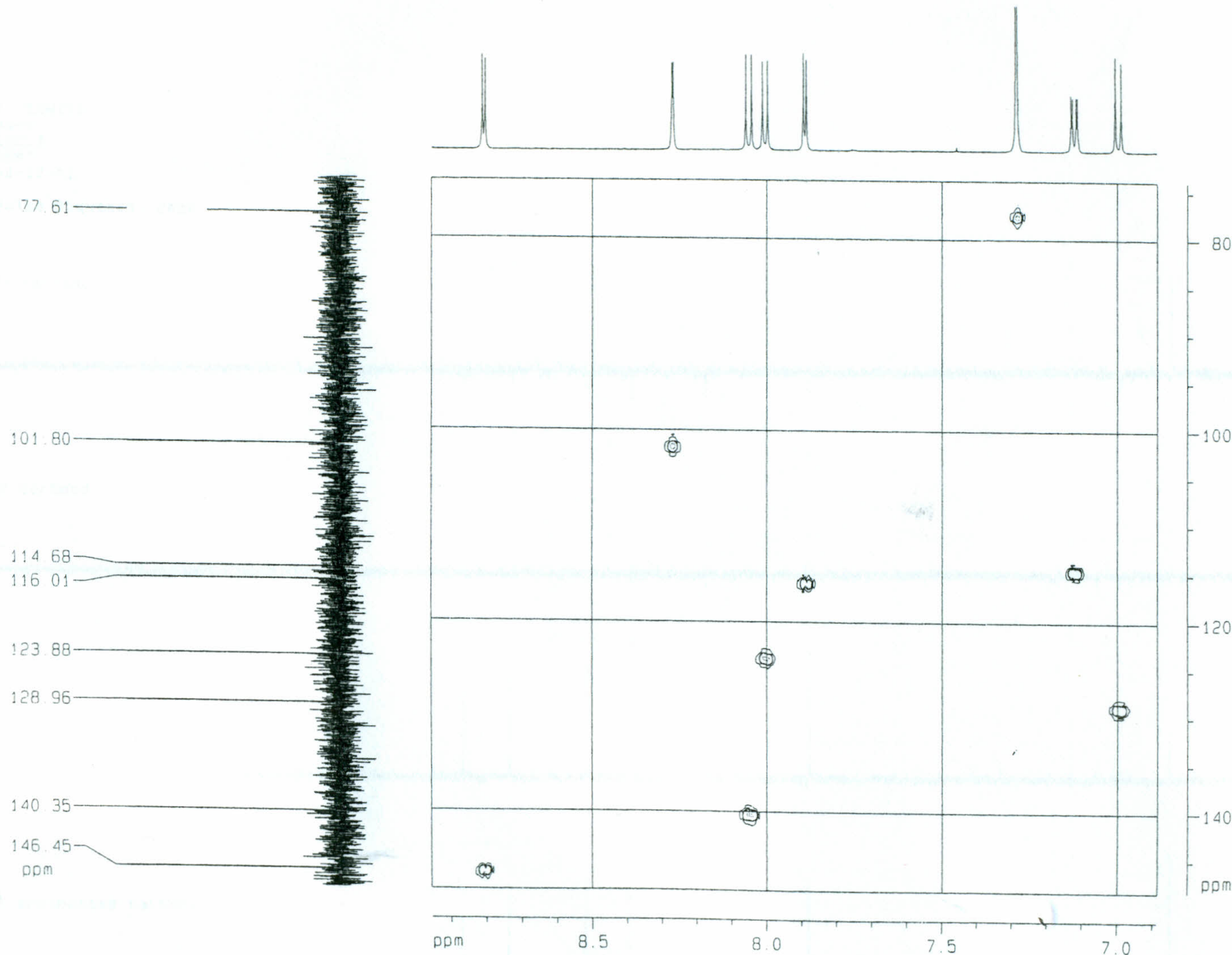

4.001
3.926
3.874
0.009
12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm

^{13}C NMR Spectrum for B_4



F5B4 HMQC
University of Botswana
Chemistry Department
NABSA NMR Service

HMQC Spectrum for B_4



Current Data Parameters
NAME 1504
EXPNO 6
PROCNO 1

F2 - Acquisition Parameters
Date_ 20021019
Time 18 02
INSTRUM spect
PROBHD 5 mm SEI 1H-
PULPROG inv4gs
TD 2048
SOLVENT CDCl3
NS 24
DS 8
SWH 5387 931 Hz
FIDRES 2 630826 Hz
AQ 0 1901044 sec
RG 26008
DW 92.800 usec
DE 6 00 usec
TE 300 0 K
D0 0 00000300 sec
D1 1 50000000 sec
D2 0 00345000 sec
D12 0 00002000 sec
D13 0 00000300 sec
D16 0 00010000 sec
d20 0 00242700 sec
IN0 0.00001490 sec

============ CHANNEL f1 ============
NUC1 1H
P1 8 40 usec
P2 16 80 usec
PL1 0 00 dB
SFO1 600 1329913 MHz

============ CHANNEL f2 ============
CPDPRG2 garp
NUC2 13C
P3 10.20 usec
PCPD2 65 00 usec
PL2 -1 00 dB
PL12 16 70 dB
SFO2 150 9177568 MHz

============ GRADIENT CHANNEL ========
P16 1000 00 usec

F1 - Acquisition parameters
ND0 2
TD 256
SFO1 150 9178 MHz
FIDRES 131 082214 Hz
SW 222 353 ppm

F2 - Processing parameters
SI 2048
SF 600 1300000 MHz
WDW SINE
SSB 0
LB 0 00 Hz
GB 0
PC 1 40

F1 - Processing parameters
SI 1024
MC2 QF
SF 150 9027490 MHz
WDW SINE
SSB 0
LB 0 00 Hz
GB 0

2D NMR plot parameters
CX2 12 00 cm
CX1 12 00 cm
F2PLO 8.956 ppm
F2LO 5374 84 Hz
F2PHI 6 887 ppm
F2HI 4133 09 Hz
F1PLO 148 315 ppm
F1LO 22381.14 Hz
F1PHI 73 828 ppm
F1HI 11140 84 Hz
F2PPMCM 0 17243 ppm/cm
F2HZCM 103 47915 Hz/cm
F1PPMCM 6 20725 ppm/cm
F1HZCM 936.69171 Hz/cm

DEPT Spectrum for B_4

F. SAMITA
B4-P
CDCL3
DEPT
04-12-01

Pulse Sequence: dept

CH3 carbons

CH2 carbons

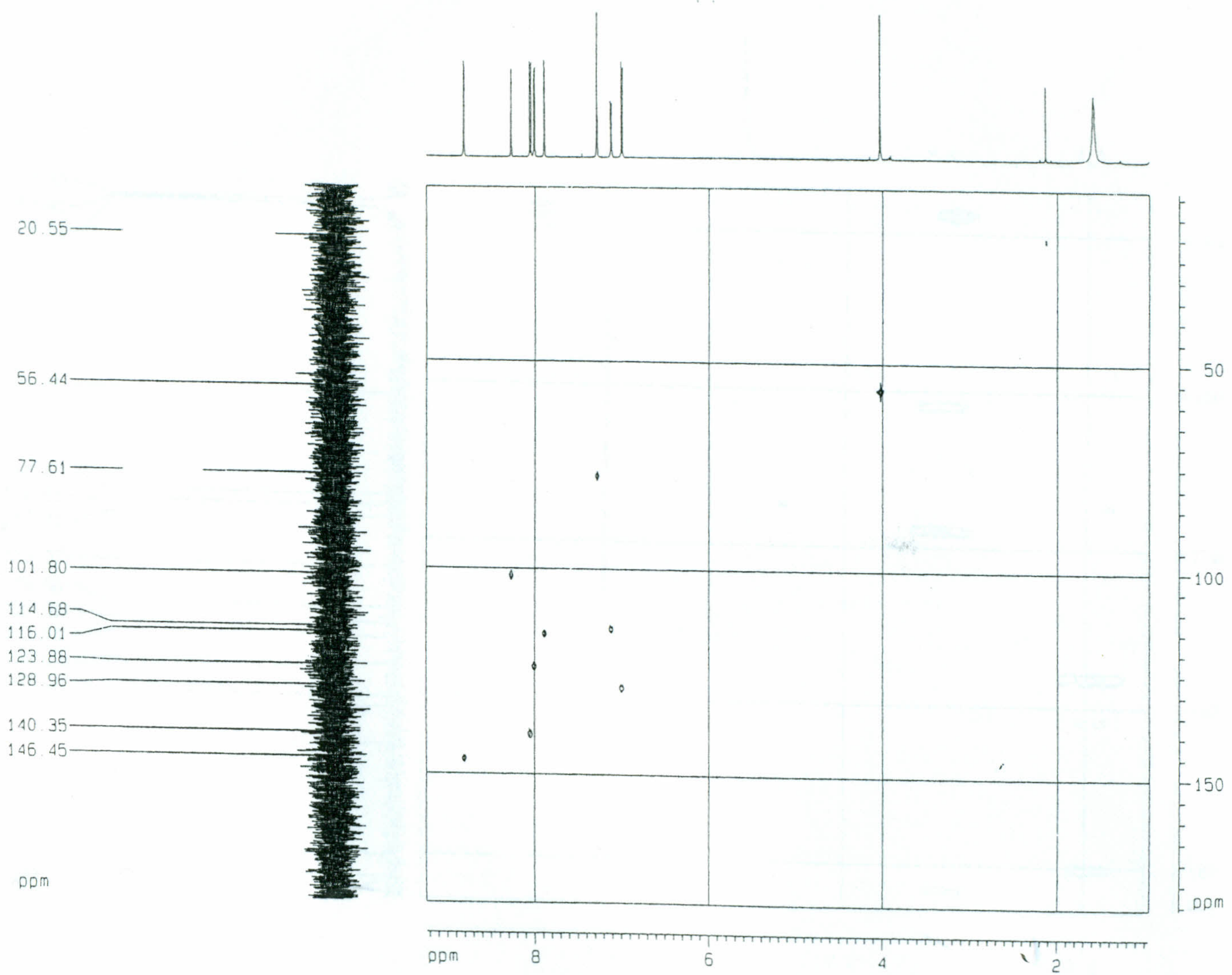
CH carbons

all protonated carbons

180 160 140 120 100 80 60 40 20 ppm

F5B4 HMQC
University of Botswana
Chemistry Department
NABSA NMR Service

HMQC Spectrum for B_4



Current Data Parameters

NAME	15b4
EXPNO	6
PROCNO	1

F2 - Acquisition Parameters

Date_	20021019
Time	18 02
INSTRUM	spect
PROBHD	5 mm SEI 1H-
PULPROG	inv4gs
TD	2048
SOLVENT	CDCl3
NS	24
DS	8
SWH	5387 931 Hz
FIDRES	2 630826 Hz
AQ	0 1901044 sec
RG	26008
DW	92.800 usec
DE	6 00 usec
TE	300 0 K
D0	0.00000300 sec
D1	1 50000000 sec
D2	0 00345000 sec
D12	0.00002000 sec
D13	0 00000300 sec
D16	0.00010000 sec
d20	0.00242700 sec
IN0	0 00001490 sec

************ CHANNEL f1 ************

NUC1	1H
P1	8 40 usec
P2	16 80 usec
PL1	0 00 dB
SFO1	600 1329913 MHz

************ CHANNEL f2 ************

CPDPRG2	garp
NUC2	13C
P3	10.20 usec
PCPD2	65.00 usec
PL2	-1.00 dB
PL12	16.70 dB
SFO2	150.9177568 MHz

************ GRADIENT CHANNEL ********

P16	1000.00 usec

F1 - Acquisition parameters

ND0	2
TD	256
SFO1	150 9178 MHz
FIDRES	131.082214 Hz
SW	222 353 ppm

F2 - Processing parameters

SI	2048
SF	600 1300000 MHz
WDW	SINE
SSB	0
LB	0 00 Hz
GB	0
PC	1 40

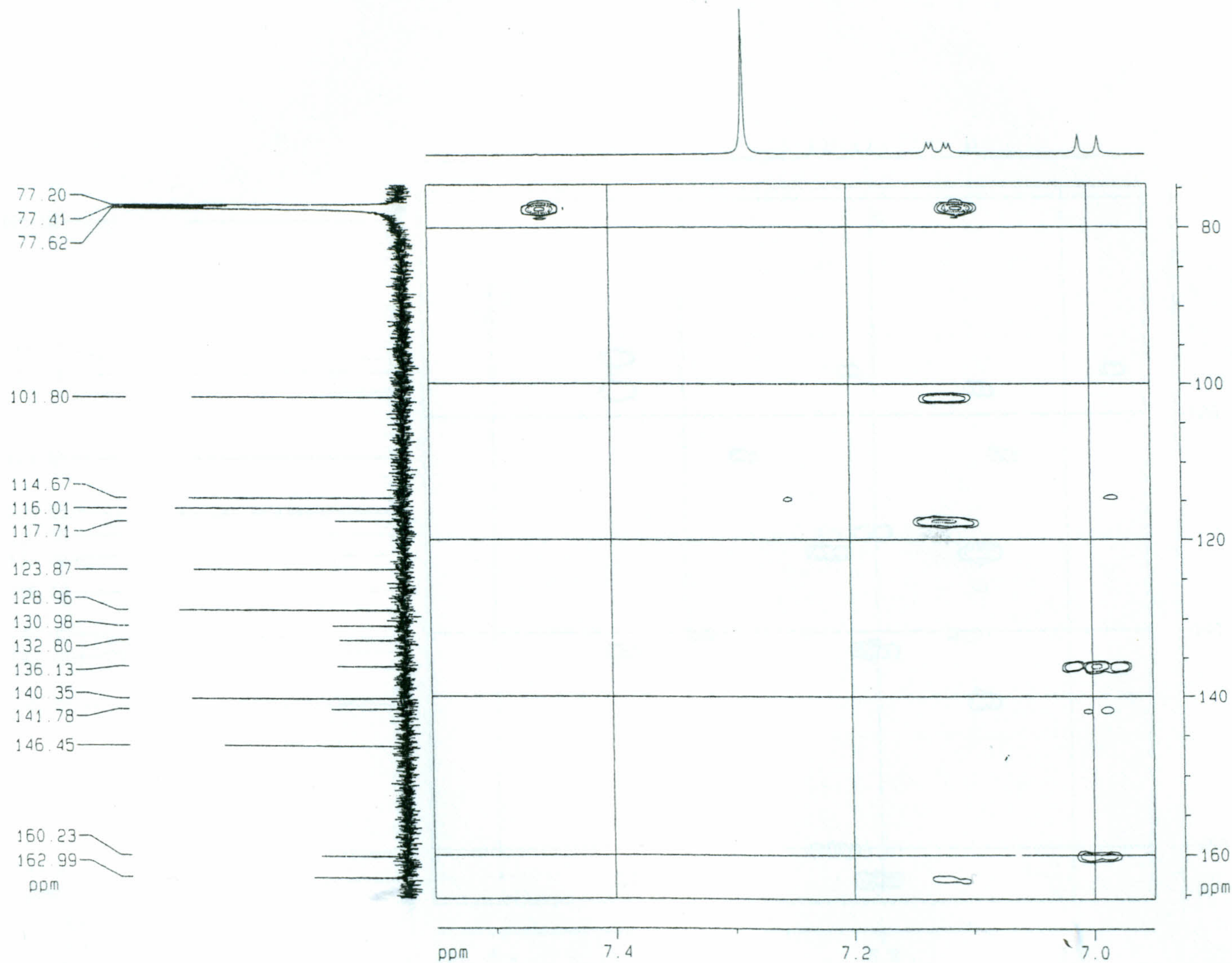
F1 - Processing parameters

SI	1024
MC2	QF
SF	150 9027490 MHz
WDW	SINE
SSB	0
LB	0 00 Hz
GB	0

2D NMR plot parameters

CX2	12.00 cm
CX1	12.00 cm
F2PLO	9.237 ppm
F2LO	5543 21 Hz
F2PHI	0 956 ppm
F2HI	573.58 Hz
F1PLO	180 672 ppm
F1LO	27263.96 Hz
F1PHI	8.462 ppm
F1HI	1276 91 Hz
F2PPMCM	0.69008 ppm/cm
F2HZCM	414 13583 Hz/cm
F1PPMCM	14.35088 ppm/cm
F1HZCM	2165.58765 Hz/cm

HMBC Spectrum for B_4



F5B4 HMBC
University of Botswana
Chemistry Department
NABSA NMR Service

Current Data Parameters

NAME	f5b4
EXPNO	5
PROCNO	1

F2 - Acquisition Parameters

Date_	20021019
Time	14.00
INSTRUM	spect
PROBHD	5 mm SEI 1H-
PULPROG	inv4gslplrnd
TD	2048
SOLVENT	CDCl3
NS	32
DS	16
SWH	5387.931 Hz
FIDRES	2.630826 Hz
AQ	0.1901044 sec
RG	16384
DW	92.800 usec
DE	6.00 usec
TE	300.0 K
D0	0.00000300 sec
D1	1.50000000 sec
D2	0.00345000 sec
D6	0.06500000 sec
D13	0.00000300 sec
D16	0.00010000 sec
IN0	0.00001490 sec

============ CHANNEL f1 ============

NUC1	1H
P1	8.40 usec
P2	16.80 usec
PL1	0.00 dB
SFO1	600.1336081 MHz

============ CHANNEL f2 ============

NUC2	13C
P3	10.20 usec
PL2	-1.00 dB
SFO2	150.9177568 MHz

============ GRADIENT CHANNEL ========

P16	1000.00 usec

F1 - Acquisition parameters

ND0	2
TD	256
SFO1	150.9178 MHz
FIDRES	131.082214 Hz
SW	222.353 ppm

F2 - Processing parameters

SI	2048
SF	600.1300000 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.40

F1 - Processing parameters

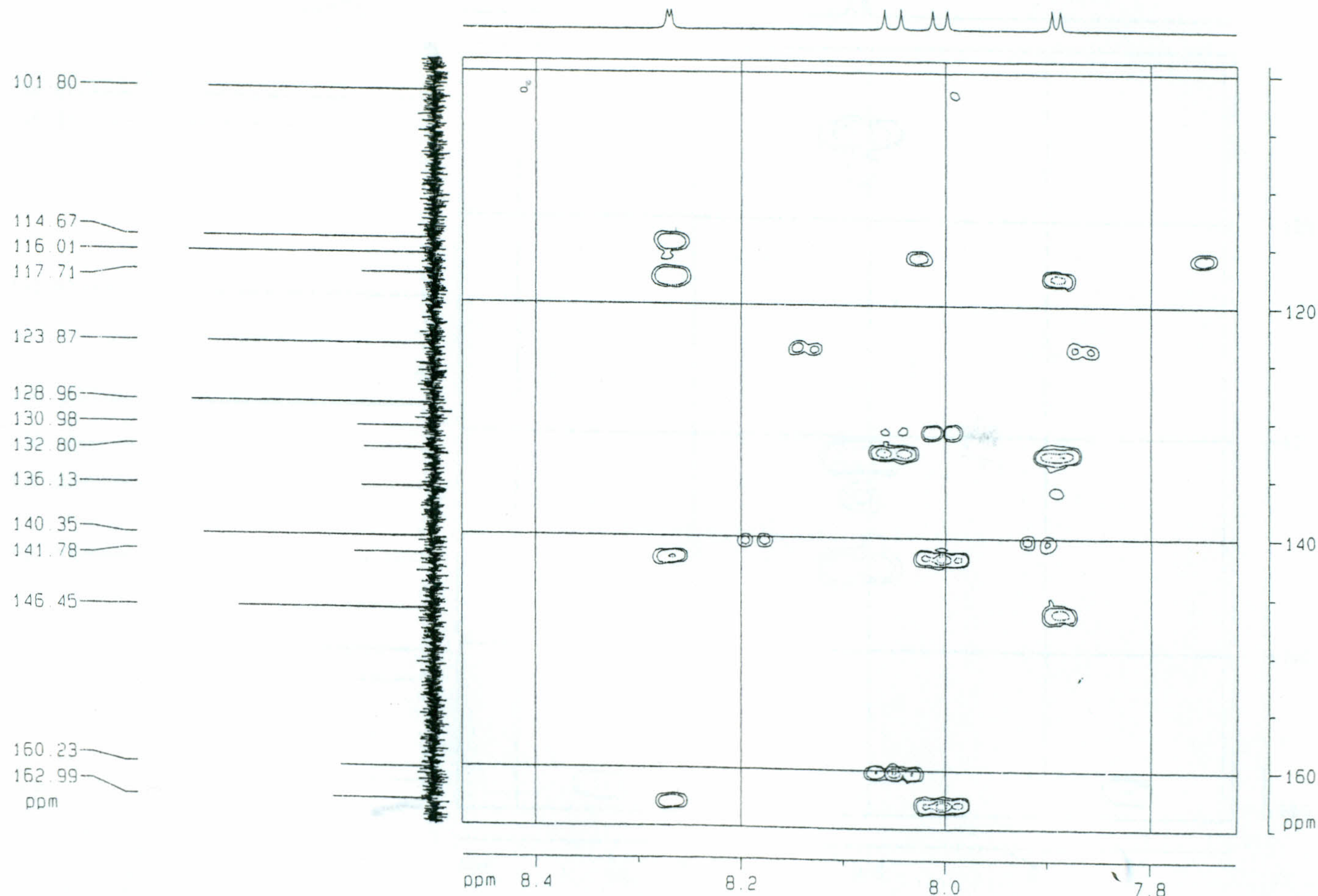
SI	1024
MC2	QF
SF	150.9027490 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	12.00 cm
CX1	12.00 cm
F2PLO	7.551 ppm
F2LO	4531.52 Hz
F2PHI	6.950 ppm
F2HI	4171.09 Hz
F1PLO	165.471 ppm
F1LO	24970.02 Hz
F1PHI	74.479 ppm
F1HI	11239.16 Hz
F2PPMCM	0.05005 ppm/cm
F2HZCM	30.03526 Hz/cm
F1PPMCM	7.58262 ppm/cm
F1HZCM	1144.23853 Hz/cm

KENYATTA UNIVERSITY LIBRARY

HMBC Spectrum for B_4



F5B4 HMBC
University of Botswana
Chemistry Department
NABSA NMR Service

Current Data Parameters

NAME	f5b4
EXPNO	5
PROCNO	1

F2 - Acquisition Parameters

Date_	20021019
Time	14.00
INSTRUM	spect
PROBHD	5 mm SEI 1H-
PULPROG	inv4gslplrnd
TD	2048
SOLVENT	CDCl3
NS	32
DS	16
SWH	5387.931 Hz
FIDRES	2.630826 Hz
AQ	0.1901044 sec
RG	16384
DW	92.800 usec
DE	6.00 usec
TE	300.0 K
D0	0.00000300 sec
D1	1.50000000 sec
D2	0.00345000 sec
D6	0.06500000 sec
D13	0.00000300 sec
D16	0.00010000 sec
IN0	0.00001490 sec

============ CHANNEL f1 ============

NUC1	1H
P1	8.40 usec
P2	16.80 usec
PL1	0.00 dB
SFO1	600.1336081 MHz

============ CHANNEL f2 ============

NUC2	13C
P3	10.20 usec
PL2	-1.00 dB
SFO2	150.9177568 MHz

============ GRADIENT CHANNEL ============

P16	1000.00 usec

F1 - Acquisition parameters

ND0	2
TD	256
SFO1	150.9178 MHz
FIDRES	131.082214 Hz
SW	222.353 ppm

F2 - Processing parameters

SI	2048
SF	600.1300000 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.40

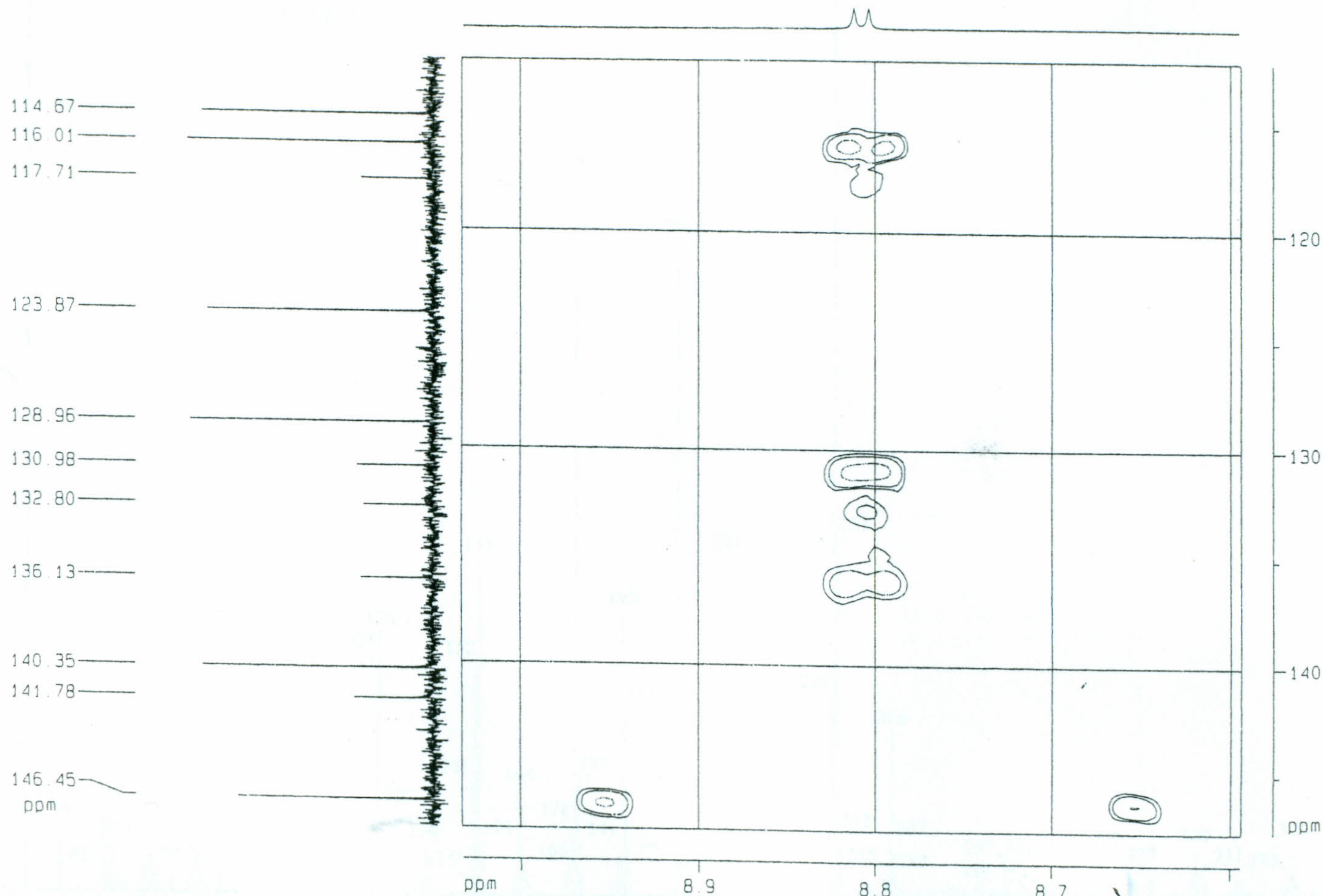
F1 - Processing parameters

SI	1024
MC2	QF
SF	150.9027490 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	12.00 cm
CX1	12.00 cm
F2PLO	8.471 ppm
F2LO	5083.99 Hz
F2PHI	7.717 ppm
F2HI	4631.49 Hz
F1PLO	165.037 ppm
F1LO	24904.48 Hz
F1PHI	99.019 ppm
F1HI	14942.23 Hz
F2PPMCM	0.06283 ppm/cm
F2HZCM	37.70851 Hz/cm
F1PPMCM	5.50147 ppm/cm
F1HZCM	830.10744 Hz/cm

HMBC Spectrum for B_4



F5B4 HMBC
University of Botswana
Chemistry Department
NABSA NMR Service

Current Data Parameters

NAME	f5b4
EXPNO	5
PROCNO	1

F2 - Acquisition Parameters

Date_	20021019
Time	14.00
INSTRUM	spect
PROBHD	5 mm SEI 1H-
PULPROG	inv4gslplrnd
TD	2048
SOLVENT	CDCl3
NS	32
DS	16
SWH	5387.931 Hz
FIDRES	2.630826 Hz
AQ	0.1901044 sec
RG	16384
DW	92.800 usec
DE	6.00 usec
TE	300.0 K
D0	0.00000300 sec
D1	1.50000000 sec
D2	0.00345000 sec
D6	0.06500000 sec
D13	0.00000300 sec
D16	0.00010000 sec
IN0	0.00001490 sec

************ CHANNEL f1 ************

NUC1	1H
P1	8.40 usec
P2	16.80 usec
PL1	0.00 dB
SFO1	600.1336081 MHz

************ CHANNEL f2 ************

NUC2	13C
P3	10.20 usec
PL2	-1.00 dB
SFO2	150.9177568 MHz

************ GRADIENT CHANNEL ********

P16	1000.00 usec

F1 - Acquisition parameters

ND0	2
TD	256
SFO1	150.9178 MHz
FIDRES	131.082214 Hz
SW	222.353 ppm

F2 - Processing parameters

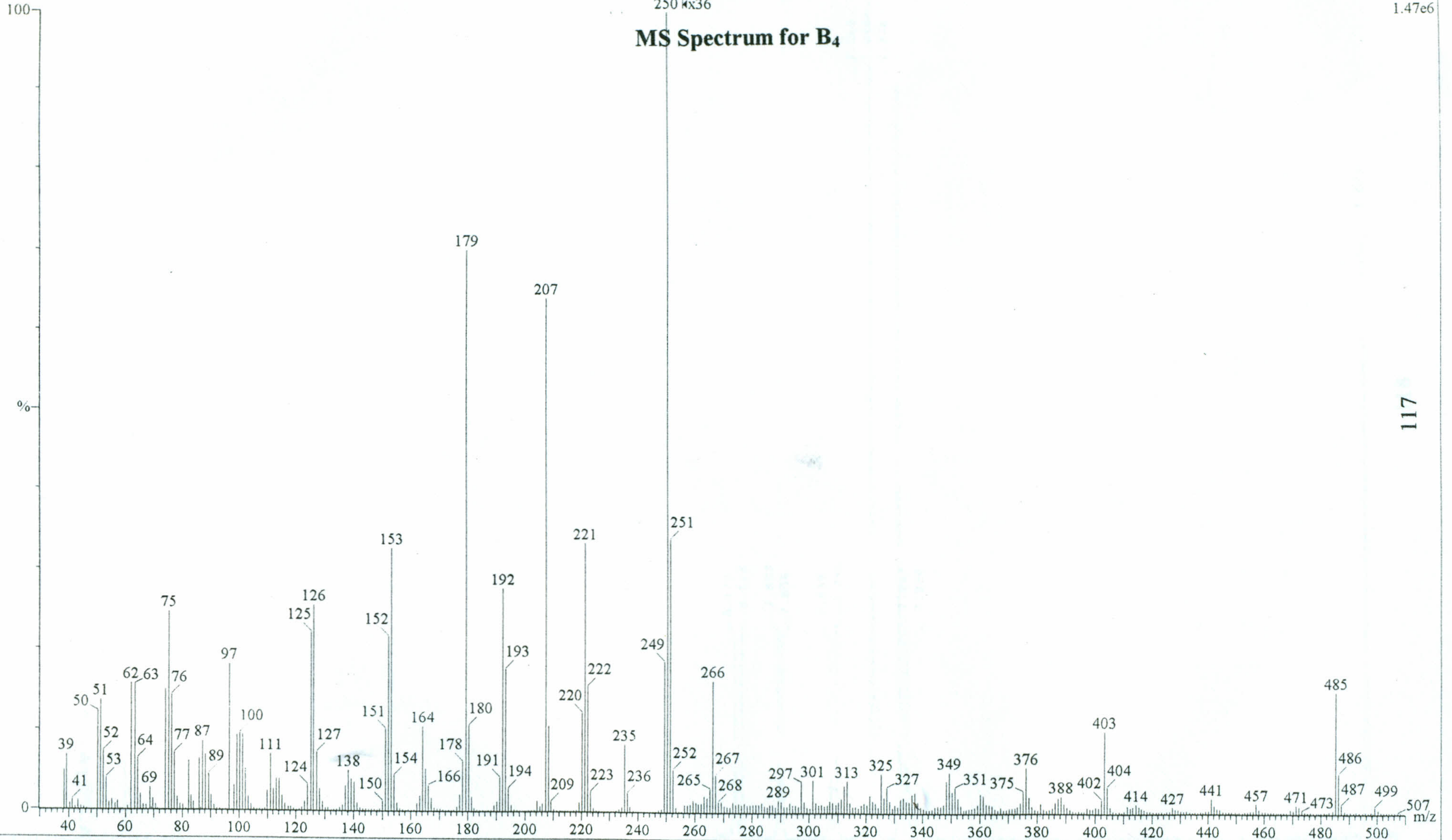
SI	2048
SF	600.1300000 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.40

F1 - Processing parameters

SI	1024
MC2	QF
SF	150.9027490 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

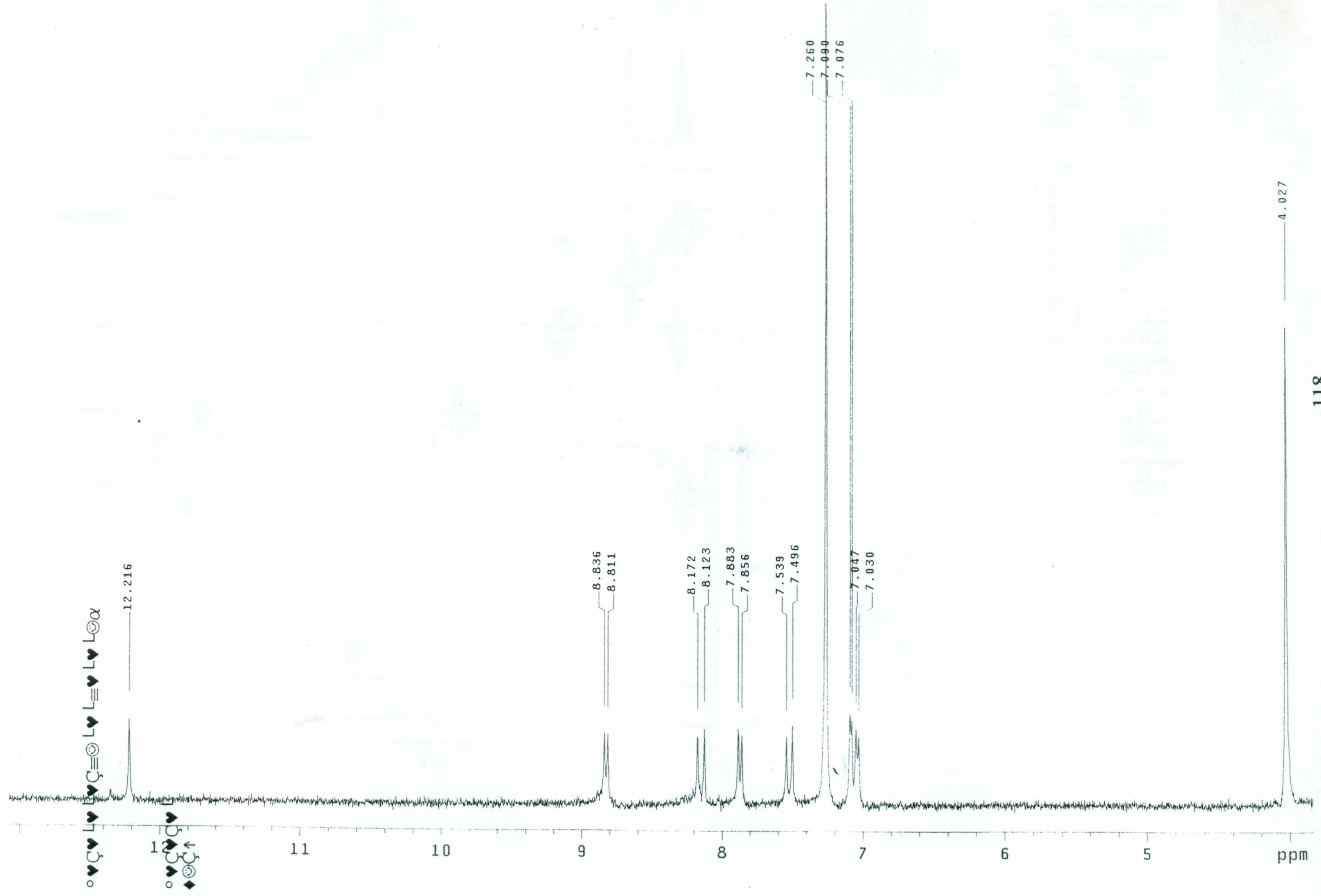
2D NMR plot parameters

CX2	12.00 cm
CX1	12.00 cm
F2PLO	9.033 ppm
F2LO	5420.73 Hz
F2PHI	8.594 ppm
F2HI	5157.65 Hz
F1PLO	147.664 ppm
F1LO	22282.83 Hz
F1PHI	112.049 ppm
F1HI	16908.46 Hz
F2PPMCM	0.03653 ppm/cm
F2HZCM	21.92357 Hz/cm
F1PPMCM	2.96790 ppm/cm
F1HZCM	447.86429 Hz/cm

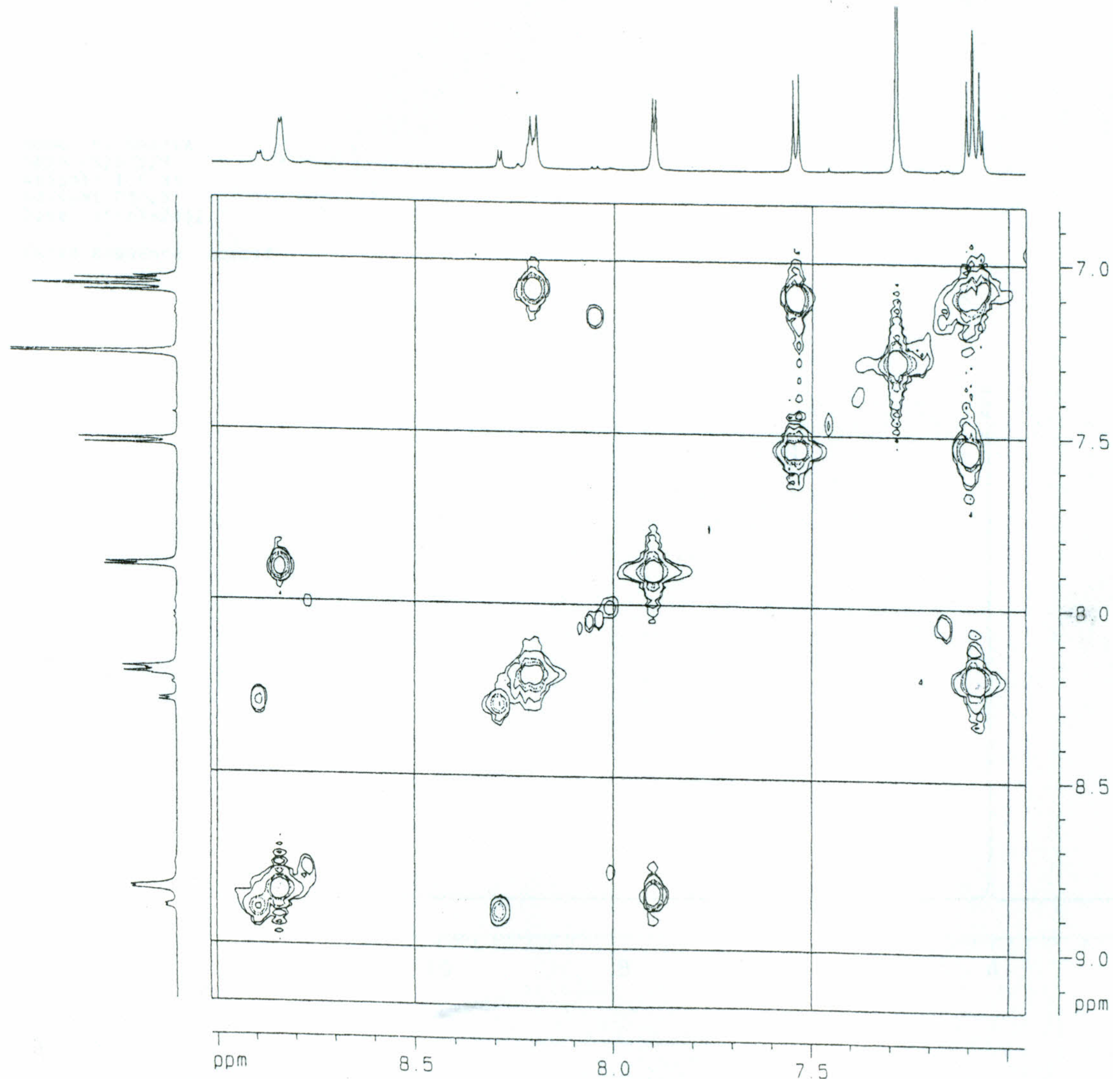

Ins: VG Platform II GC/LC-MS
BpM:250
Sample FS-B4 By Solid Probe
SF22702B 7 (0.261) Cm (3:8)
Date: 22-Jul-2002 Time: 16:38:34
BpI:1465686
Tic:13751672
Scan EI+
1.47e6
MS Spectrum for B4
250 x36
100
%
0
m/z
179
207
251
153
221
192
75
126
125
152
193
97
249
222
266
62 63
76
51
50
220
485
180
100
151
164
403
235
52
77 87
127
39
64
111
178
89
252
267
53
154
138
191
124
194
325
349
376
297 301 313
166
69
41
150
209
223
236
265
268
289
327
351 375
388 402
404
414
427
441
457
471
473
487
486
499
507
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500

Code: FS/B5/P
Weight: 15 mg
Solvent: CDCl3

Pulse Sequence: s2pul



COSY Spectrum for B_5



B5P COSY
University of Botswana
Chemistry Department
NABSA NMR Service

Current Data Parameters

NAME	b5o
EXPNO	4
PROCNO	1

F2 - Acquisition Parameters

Date_	20021016
Time	15.52
INSTRUM	spect
PROBHD	5 mm SEI 1H-
PULPROG	cosygs
TD	2048
SOLVENT	CDCl3
NS	24
DS	8
SWH	7716.049 Hz
FIDRES	3.767602 Hz
AQ	0.1327604 sec
RG	1024
DW	64.800 usec
DE	6.00 usec
TE	300.0 K
D0	0.00000300 sec
D1	1.48689198 sec
D13	0.00000300 sec
D16	0.00010000 sec
IN0	0.00012960 sec

============ CHANNEL f1 ============

NUC1	1H
P0	8.40 usec
P1	8.40 usec
PL1	0.00 dB
SFO1	600.1340164 MHz

============ GRADIENT CHANNEL ========

P16	1000.00 usec

F1 - Acquisition parameters

ND0	1
TD	256
SFO1	600.134 MHz
FIDRES	30.140818 Hz
SW	12.857 ppm

F2 - Processing parameters

SI	1024
SF	600.1300000 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

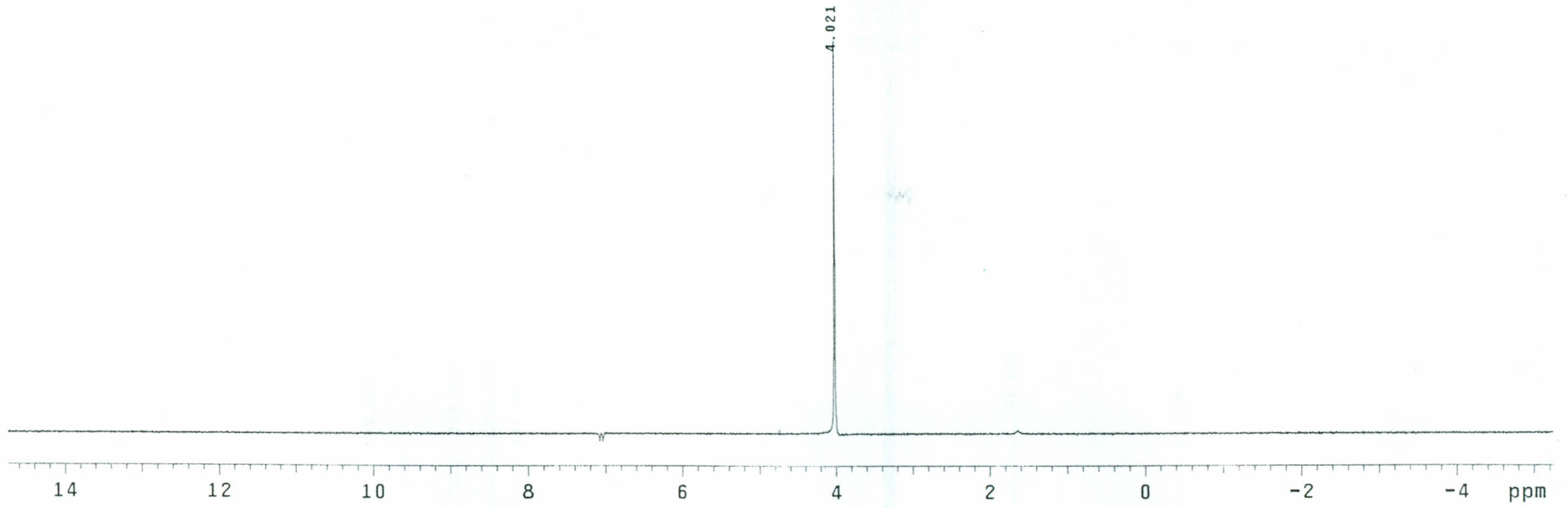
F1 - Processing parameters

SI	1024
MC2	QF
SF	600.1300000 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

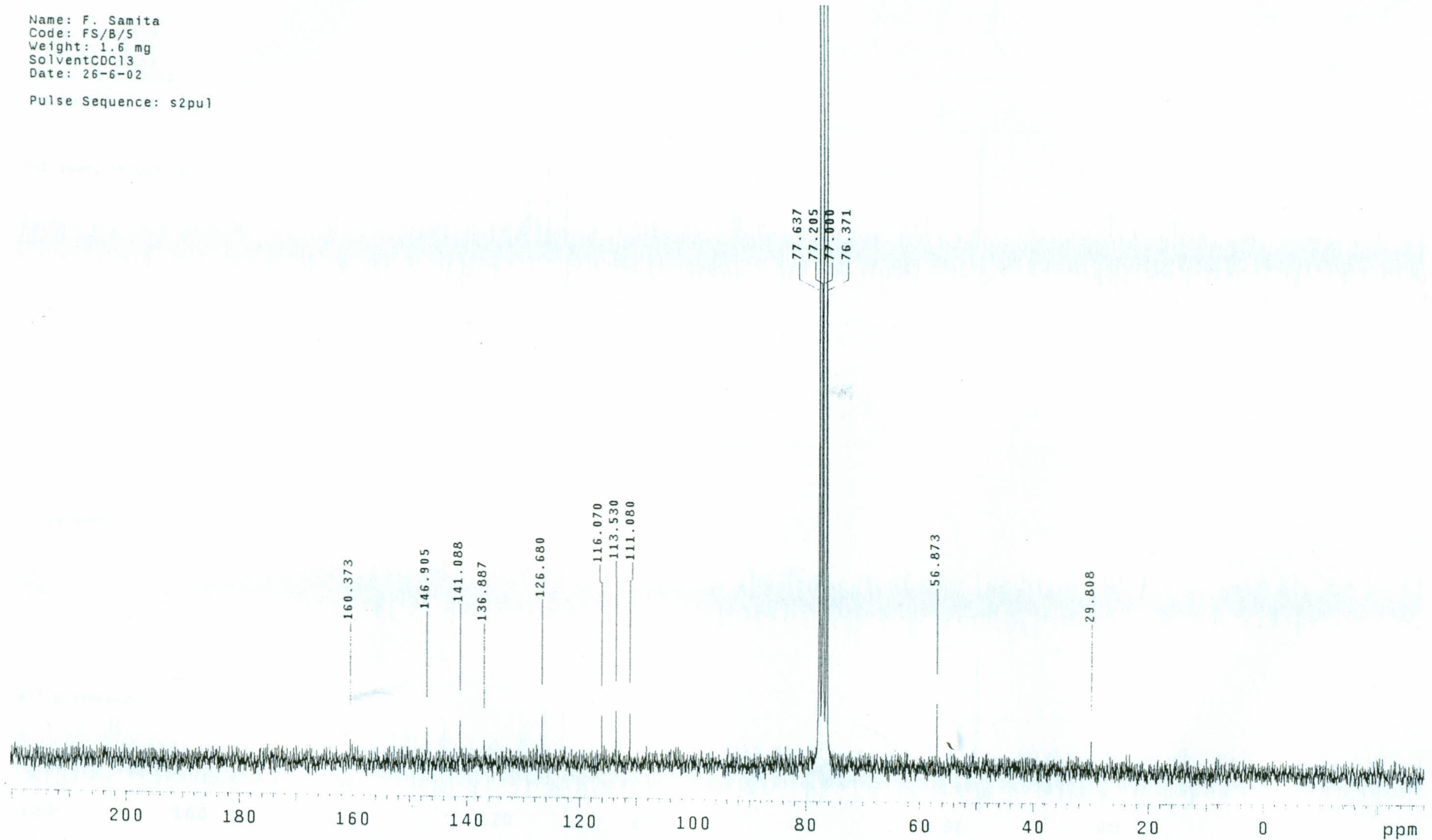
2D NMR plot parameters

CX2	12.00 cm
CX1	12.00 cm
F2PLO	9.015 ppm
F2LO	5410.41 Hz
F2PHI	6.956 ppm
F2HI	4174.63 Hz
F1PLO	9.166 ppm
F1LO	5500.83 Hz
F1PHI	6.831 ppm
F1HI	4099.28 Hz
F2PPMCM	0.17160 ppm/cm
F2HZCM	102.98113 Hz/cm
F1PPMCM	0.19462 ppm/cm
F1HZCM	116.79566 Hz/cm

NOE Spectrum for B_5



^{13}C NMR Spectrum for B_5



DEPT Spectrum for B_5

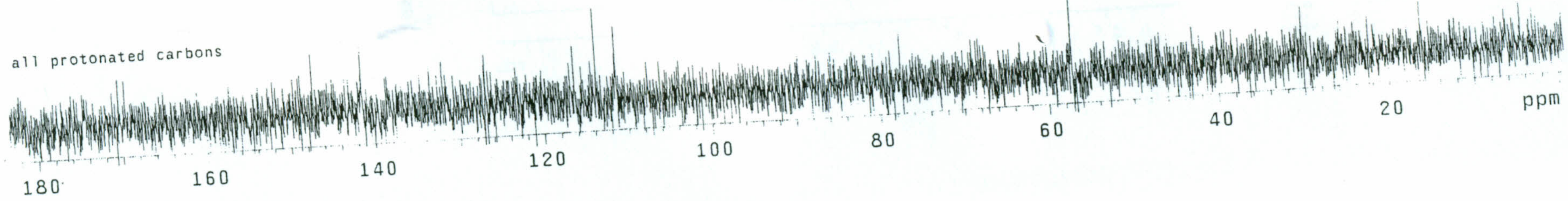
Name: F. samita
Code: FS/B/5/P
Weight: 1.6 mg
Solvent: CDCl3
Date: 16-07-2002

Pulse Sequence: dept

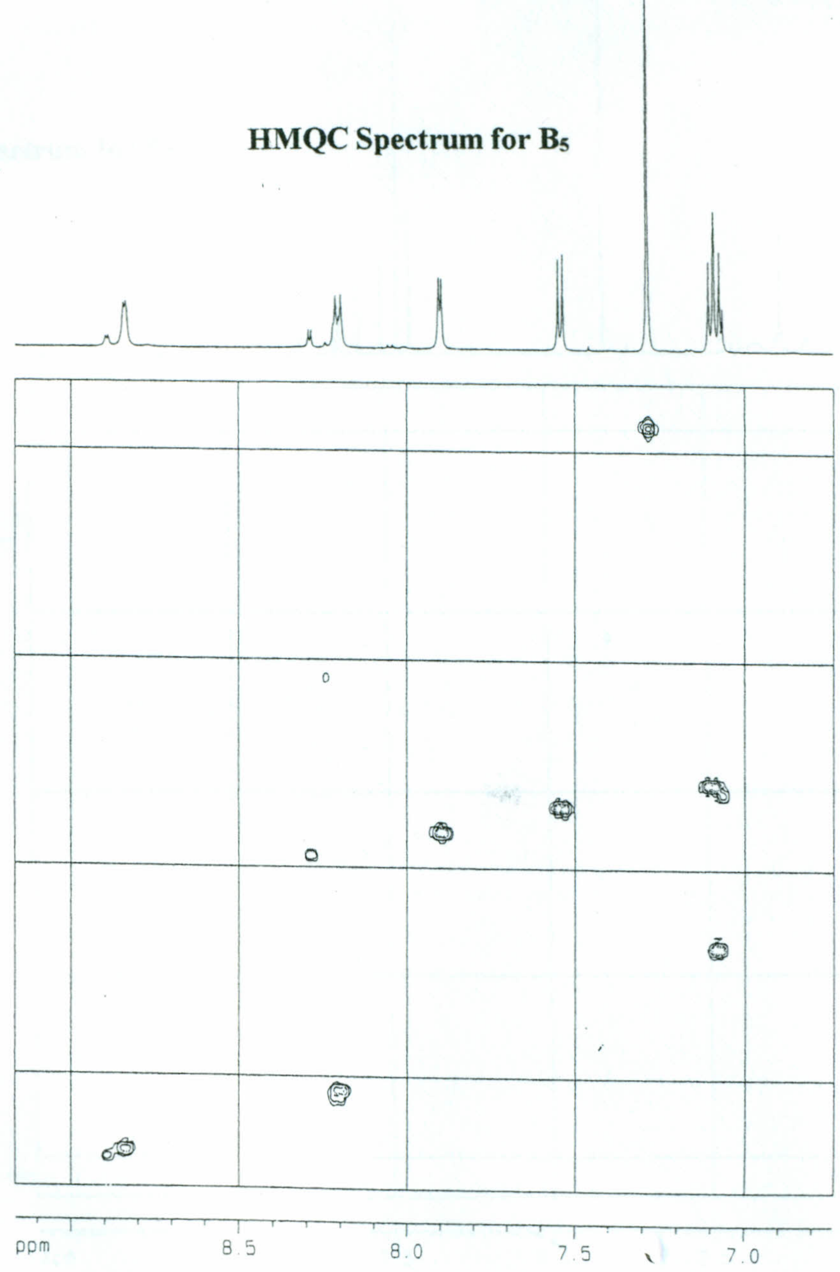
CH2 down, CH/CH3 up

CH carbons

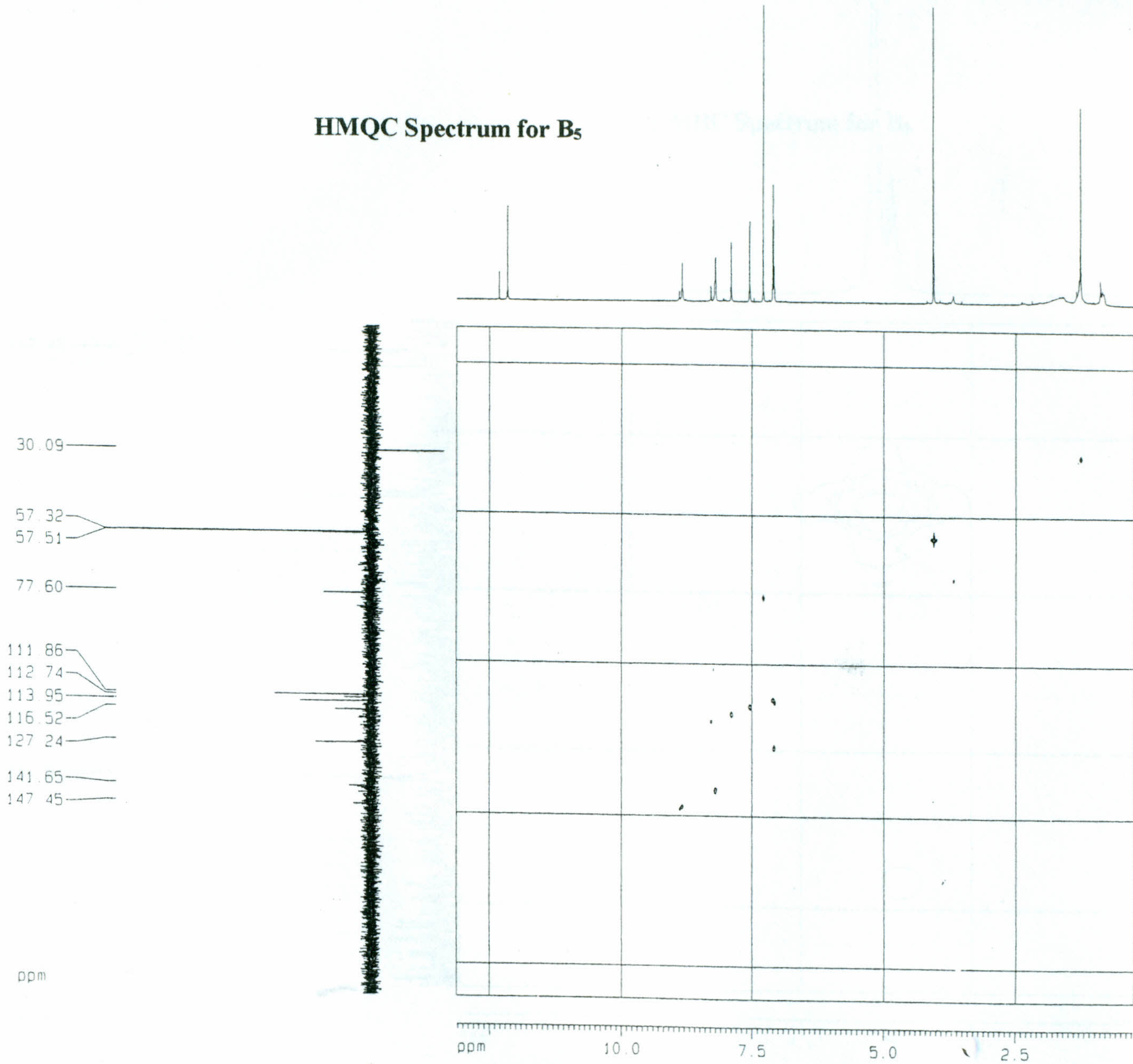
all protonated carbons



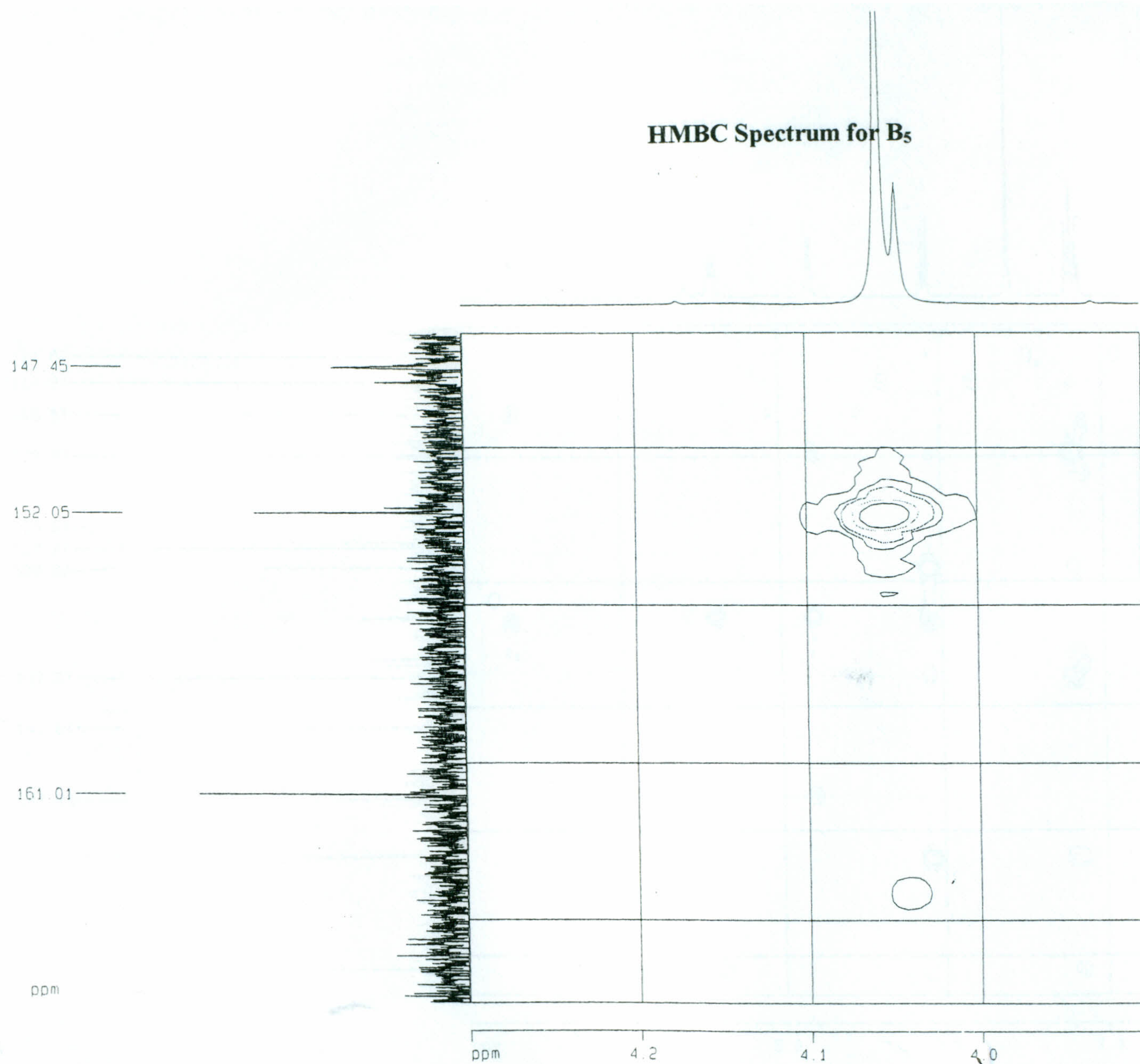
HMQC Spectrum for B_5



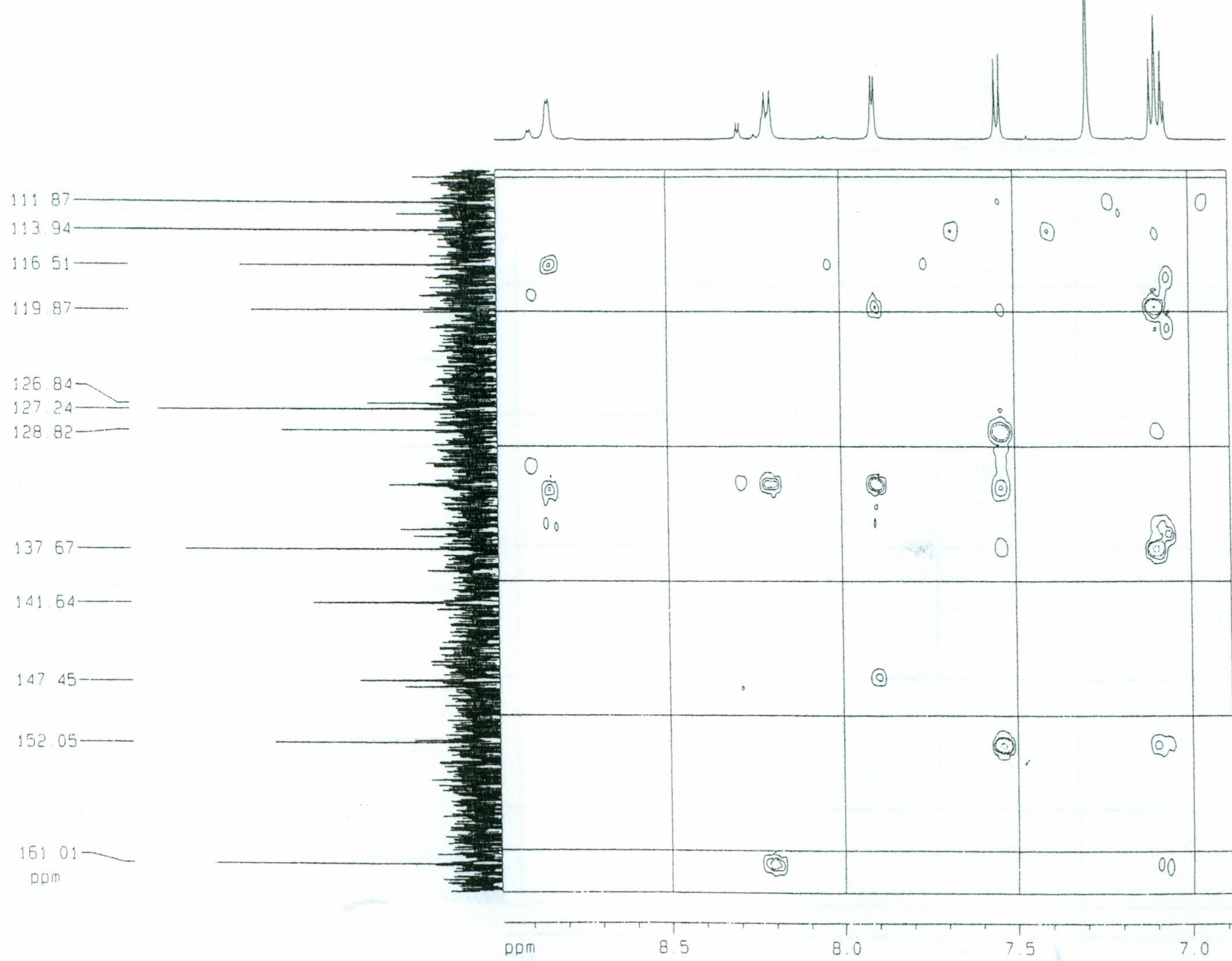
HMQC Spectrum for B_5



HMBC Spectrum for B_5



HMBC Spectrum for B_5



HMBC Spectrum for B_5

B5P HMBC
University of Botswana
Chemistry Department
NABSA NMR Service

Current Data Parameters
NAME b5p
EXPNO 5
PROCNO 1

F2 - Acquisition Parameters
Date_ 20021016
Time 18.41
INSTRUM spect
PROBHD 5 mm SEI 1H-
PULPROG inv4gslplrnd
TD 2048
SOLVENT CDCl3
NS 32
DS 16
SWH 7716.049 Hz
FIDRES 3.767602 Hz
AQ 0.1327604 sec
RG 29193
DW 64.800 usec
DE 6.00 usec
TE 300.0 K
D0 0.00000300 sec
D1 1.50000000 sec
D2 0.00345000 sec
D6 0.06500000 sec
D13 0.00000300 sec
D16 0.00010000 sec
IN0 0.00001490 sec

============ CHANNEL f1 ============
NUC1 1H
P1 8.40 usec
P2 16.80 usec
PL1 0.00 dB
SFO1 600.1340164 MHz

============ CHANNEL f2 ============
NUC2 13C
P3 10.20 usec
PL2 -1.00 dB
SFO2 150.9177568 MHz

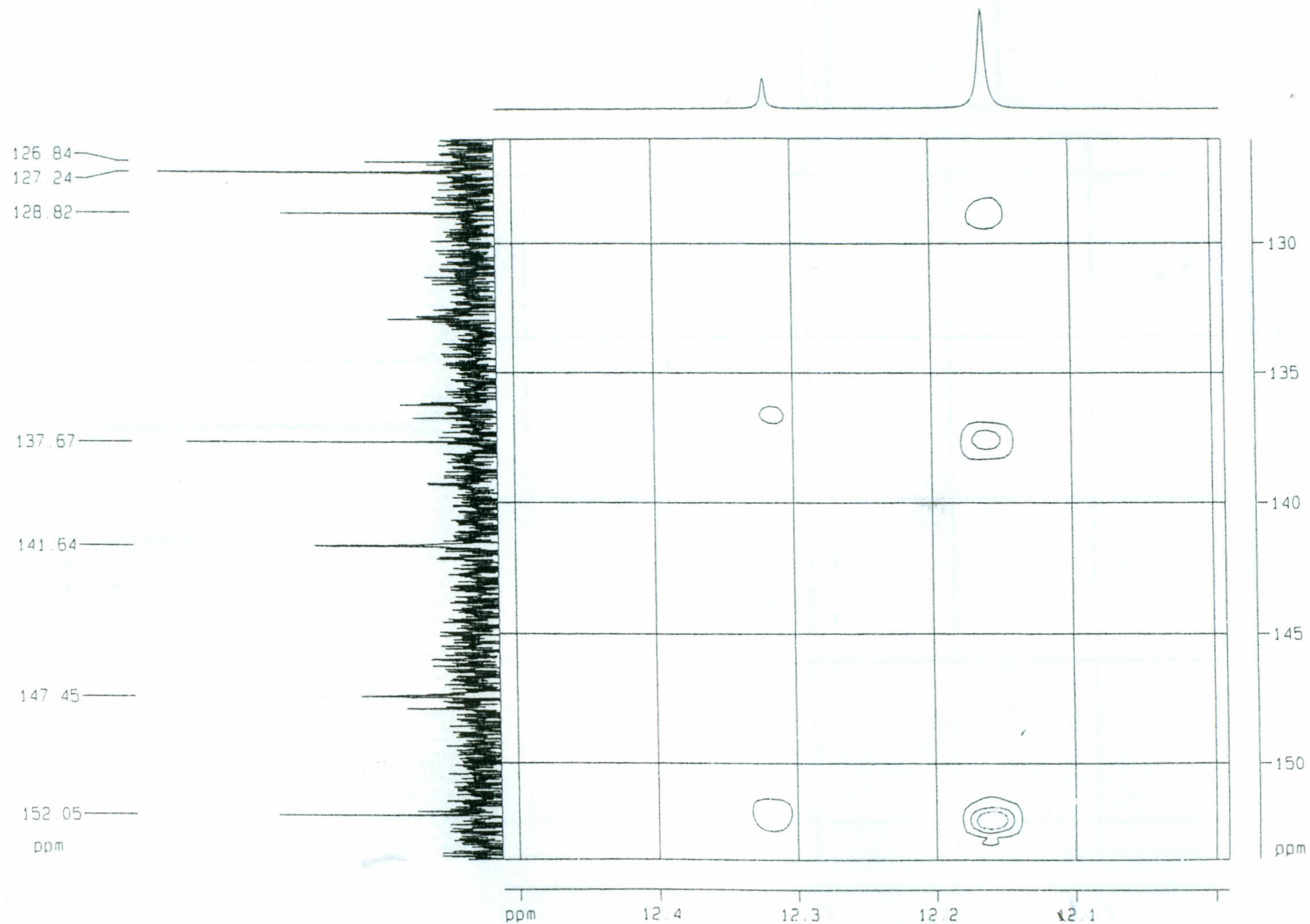
============ GRADIENT CHANNEL ============
P16 1000.00 usec

F1 - Acquisition parameters
ND0 2
TD 256
SFO1 150.9178 MHz
FIDRES 131.082214 Hz
SW 222.353 ppm

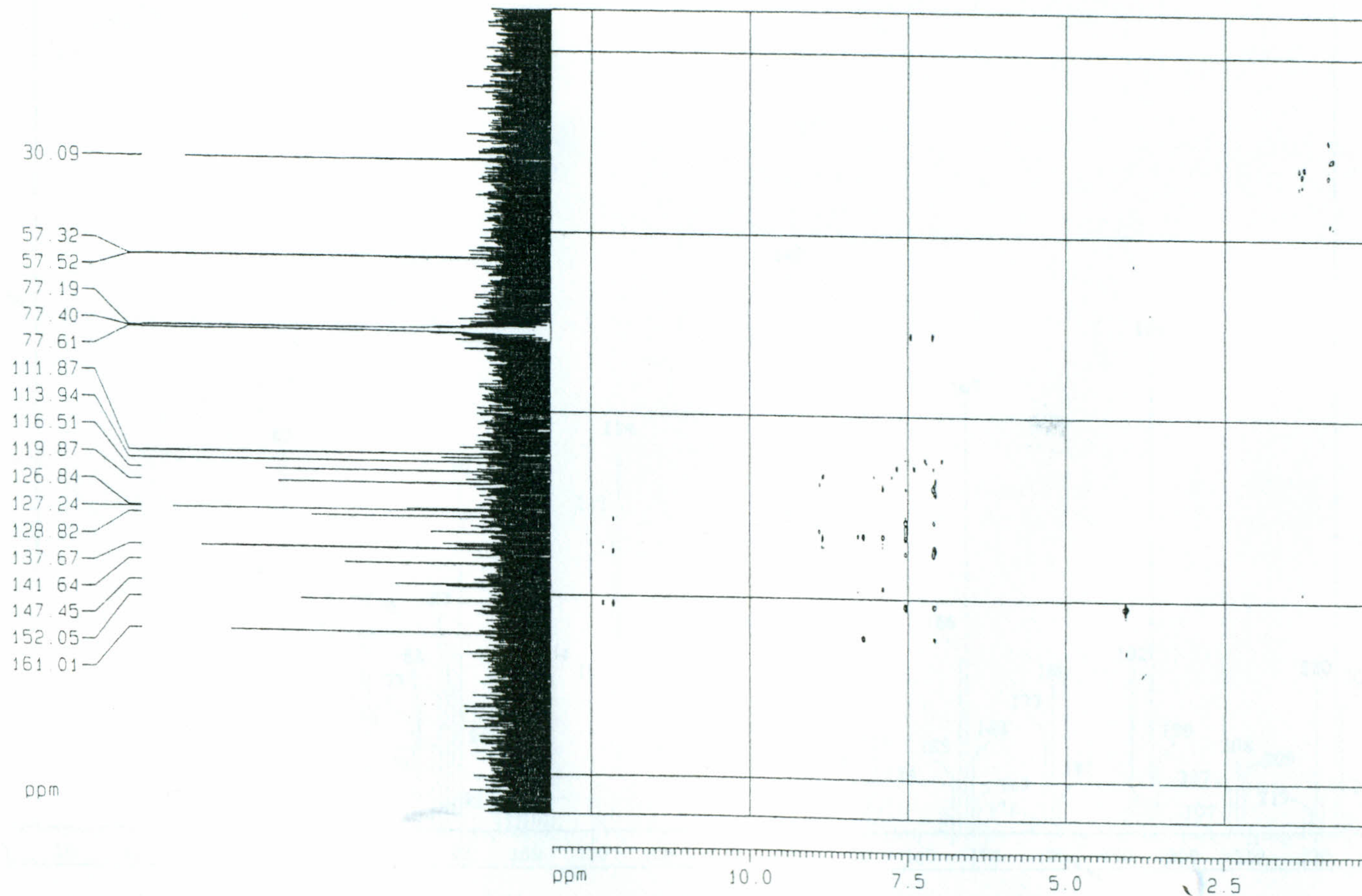
F2 - Processing parameters
SI 2048
SF 600.1300000 MHz
WDW SINE
SSB 0
LB 0.00 Hz
GB 0
PC 1.40

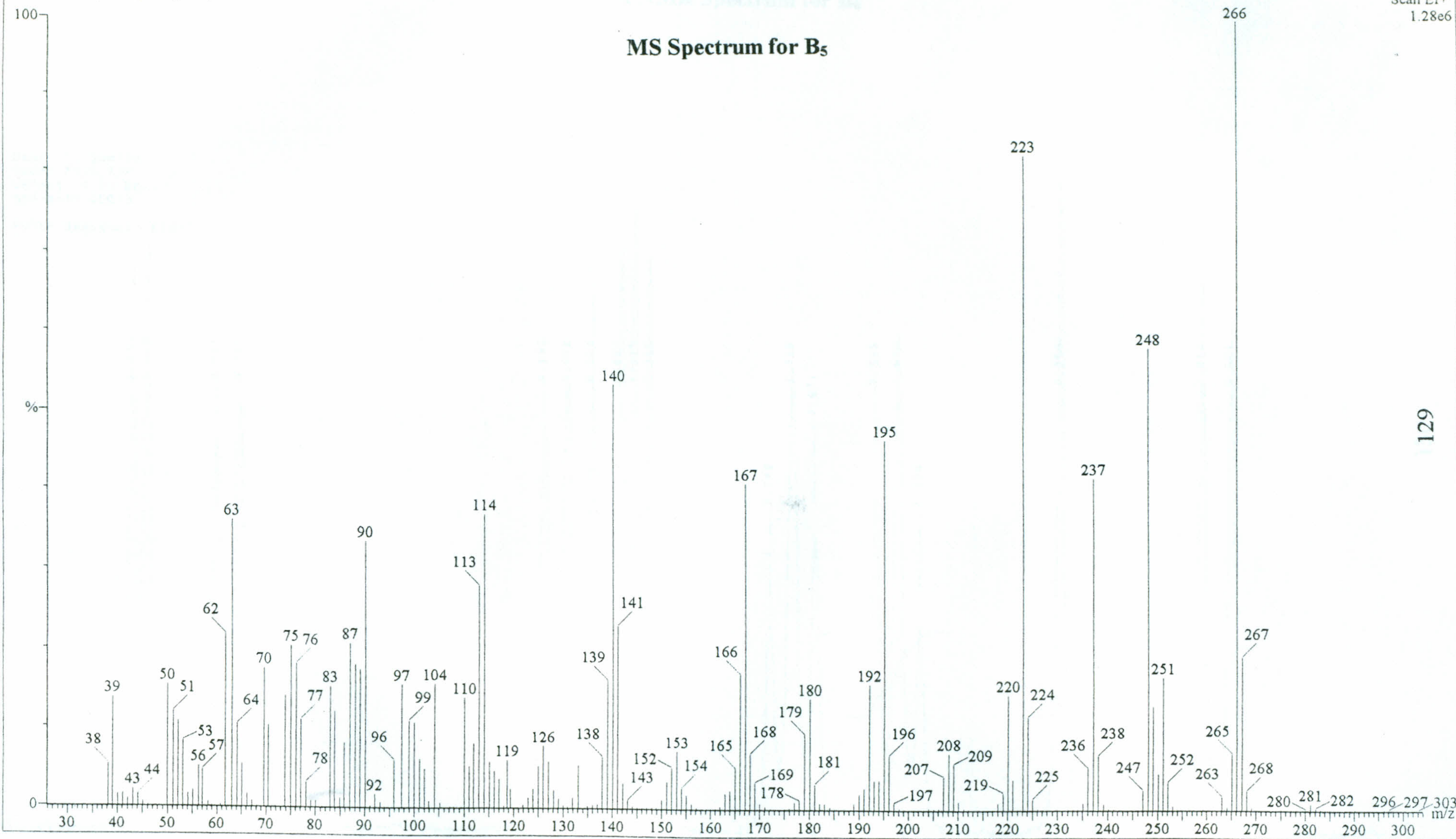
F1 - Processing parameters
SI 1024
MC2 QF
SF 150.9027490 MHz
WDW SINE
SSB 0
LB 0.00 Hz
GB 0

2D NMR plot parameters
CX2 12.00 cm
CX1 12.00 cm
F2PLO 12.512 ppm
F2LO 7508.96 Hz
F2PHI 11.991 ppm
F2HI 7196.25 Hz
F1PLO 153.744 ppm
F1LO 23200.41 Hz
F1PHI 125.947 ppm
F1HI 19005.78 Hz
F2PPMCM 0.04342 ppm/cm
F2HZCM 26.05927 Hz/cm
F1PPMCM 2.31641 ppm/cm
F1HZCM 349.55255 Hz/cm

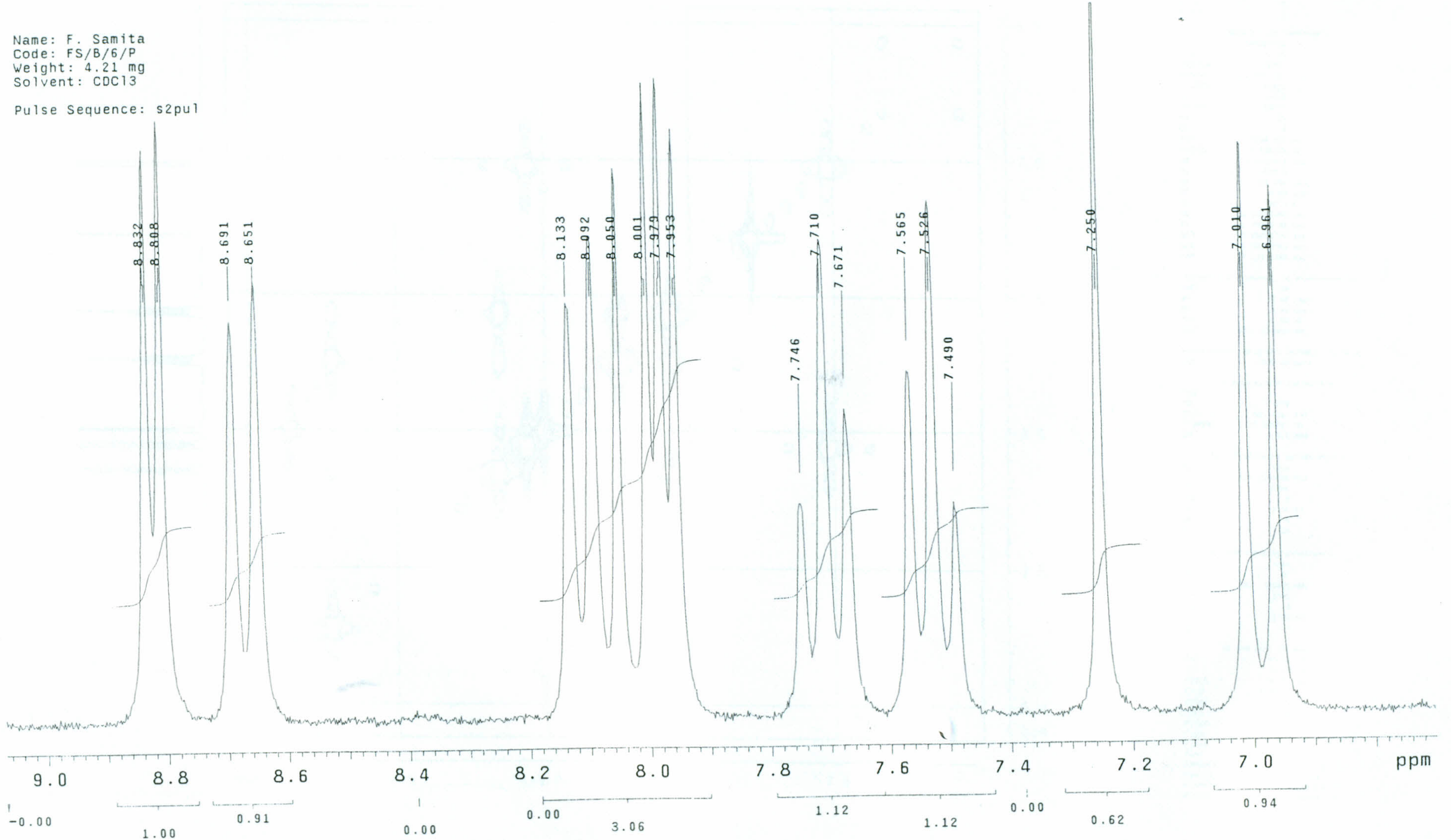


HMBC Spectrum for B_5

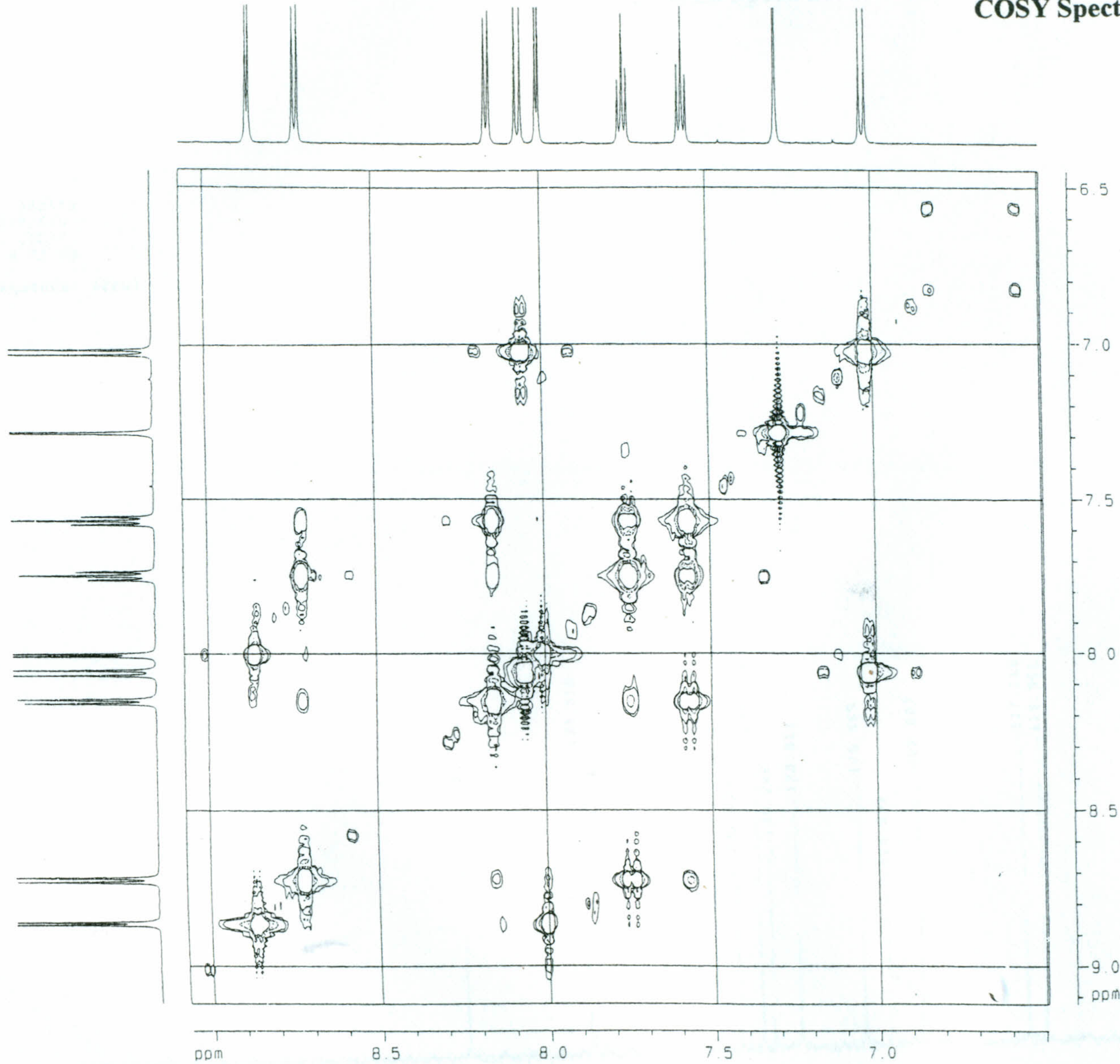



Ins: VG Platform II GC/LC-MS
BpM:266
FS B5 By Solid Probe
SF3602C 21 (0.725) Cm (5:21)
Date: 03-Jun-2002 Time: 11:39:10
BpI:1275317
Tic:17278864
Scan EI+
1.28e6
MS Spectrum for B5
100
%
0
266
223
248
140
195
237
167
114
63
90
113
141
62
267
75 76
87
70
139
166
83
39
50
51
97
104
99
110
77
64
180
192
220
224
251
179
168
196
238
265
53
96
126
138
236
208
209
153
165
119
57
56
78
152
154
181
207
247
252
268
263
43
44
92
143
169
225
219
178
197
280
281
282
296
297
303
m/z
30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300

^{1}H NMR Spectrum for B_6



COSY Spectrum for B_6



F5B6 COSY
University of Botswana
Chemistry Department
NABSA NMR Service

Current Data Parameters

NAME	1506
EXPNO	4
PROCNO	1

F2 - Acquisition Parameters

Date_	20021019
Time	21 15
INSTRUM	spect
PROBHD	5 mm SEI 1H-
PULPROG	cosygs
TD	2048
SOLVENT	CDCl3
NS	24
DS	8
SWH	5296 610 Hz
FIDRES	2 586236 Hz
AQ	0 1933812 sec
RG	1625 5
DW	94 400 usec
DE	6.00 usec
TE	300 0 K
D0	0 00000300 sec
D1	1.48689198 sec
D13	0 00000300 sec
D16	0.00010000 sec
IN0	0.00018880 sec

============ CHANNEL f1 ============

NUC1	1H
P0	8.40 usec
P1	8 40 usec
PL1	0.00 dB
SFO1	600 1330854 MHz

============ GRADIENT CHANNEL ========

P16	1000 00 usec

F1 - Acquisition parameters

ND0	1
TD	256
SFO1	600 1331 MHz
FIDRES	20 689884 Hz
SW	8 826 ppm

F2 - Processing parameters

SI	1024
SF	600 1300000 MHz
WDW	SINE
SSB	0
LB	0 00 Hz
GB	0
PC	1.00

F1 - Processing parameters

SI	1024
MC2	QF
SF	600 1300000 MHz
WDW	SINE
SSB	0
LB	0 00 Hz
GB	0

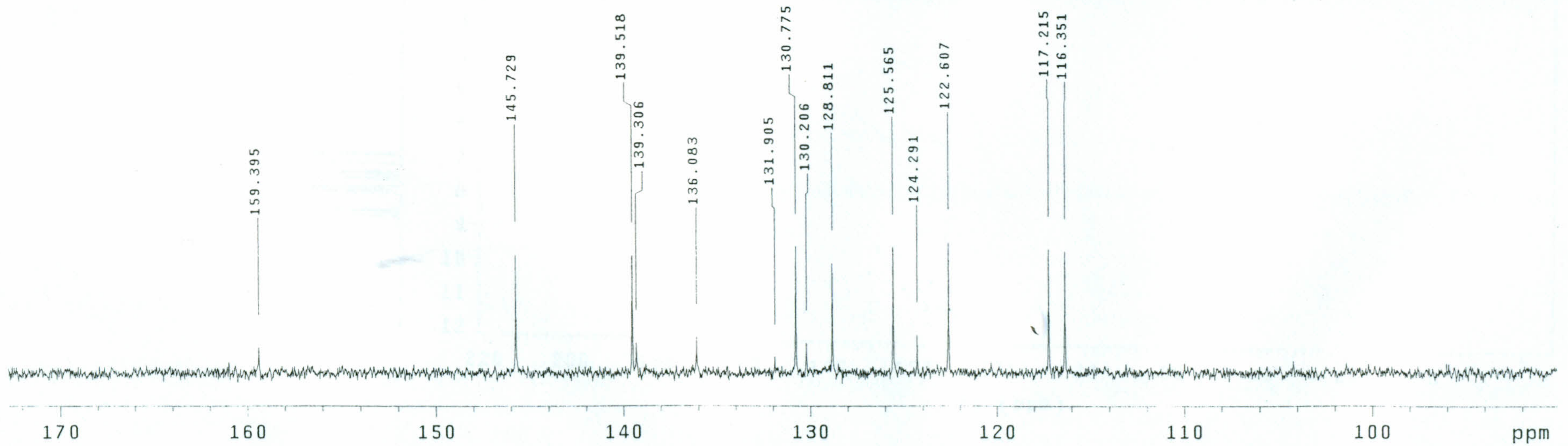
2D NMR plot parameters

CX2	15 00 cm
CX1	15 00 cm
F2PLO	9 071 ppm
F2LO	5444 01 Hz
F2PHI	6 494 ppm
F2HI	3897 44 Hz
F1PLO	9 123 ppm
F1LO	5475 04 Hz
F1PHI	6 443 ppm
F1HI	3866 40 Hz
F2PPMCM	0.17180 ppm/cm
F2HZCM	103.10459 Hz/cm
F1PPMCM	0 17870 ppm/cm
F1HZCM	107.24257 Hz/cm

^{13}C NMR Spectrum for B_6

Name: F. Samita
Code: FS/B/6/6
Solvent: CDCl3
Weight: 4.21 mg

Pulse Sequence: s2pul



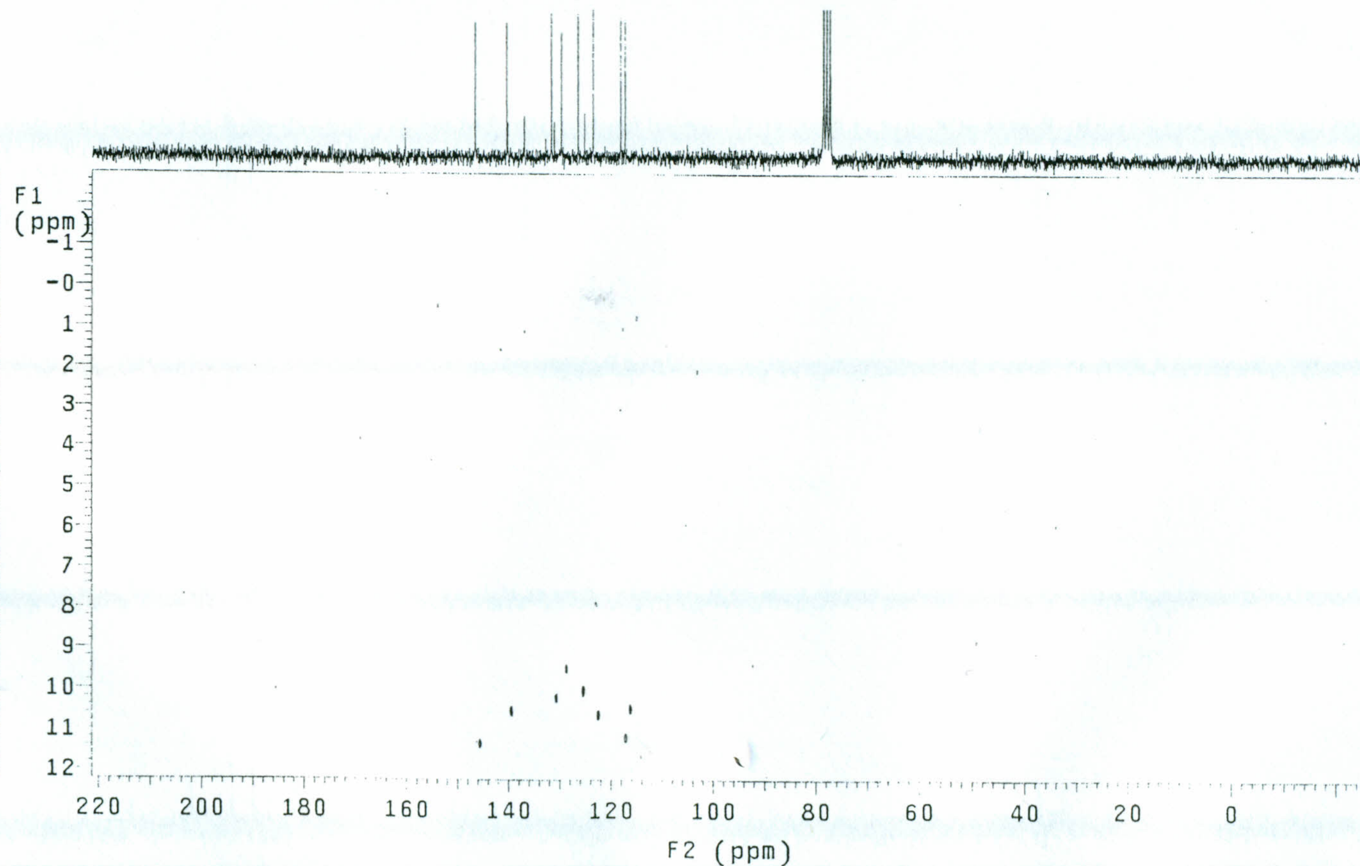
HETCOR Spectrum for B_6

Name: F. Samita
Code: FS/B/6/6
Solvent: CDCl3
Weight: 4.21 mg

Pulse Sequence: hetcor

Solvent: CDCl3
Ambient temperature
File: FS-B6-P-Hetcor
Mercury-200 "uonnmr200"

PULSE SEQUENCE: hetcor
Relax. delay 1.500 sec
Acq. time 0.082 sec
Width 12500.0 Hz
2D Width 3000.3 Hz
128 repetitions
128 increments
OBSERVE C13, 50.3040511 MHz
DECOUPLE H1, 200.0567133 MHz
Power 31 dB
on during acquisition
off during delay
WALTZ-16 modulated
DATA PROCESSING
Sine bell 0.041 sec
F1 DATA PROCESSING
Sine bell 0.021 sec
FT size 2048 x 1024
Total time 7 hr, 36 min, 32 sec



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DEPT Spectrum for B_6

Name: F Samita
Sample Code: FS/B/6/P
Solvent: CDCl3
Weight: 4.21 mg

Pulse Sequence: dept

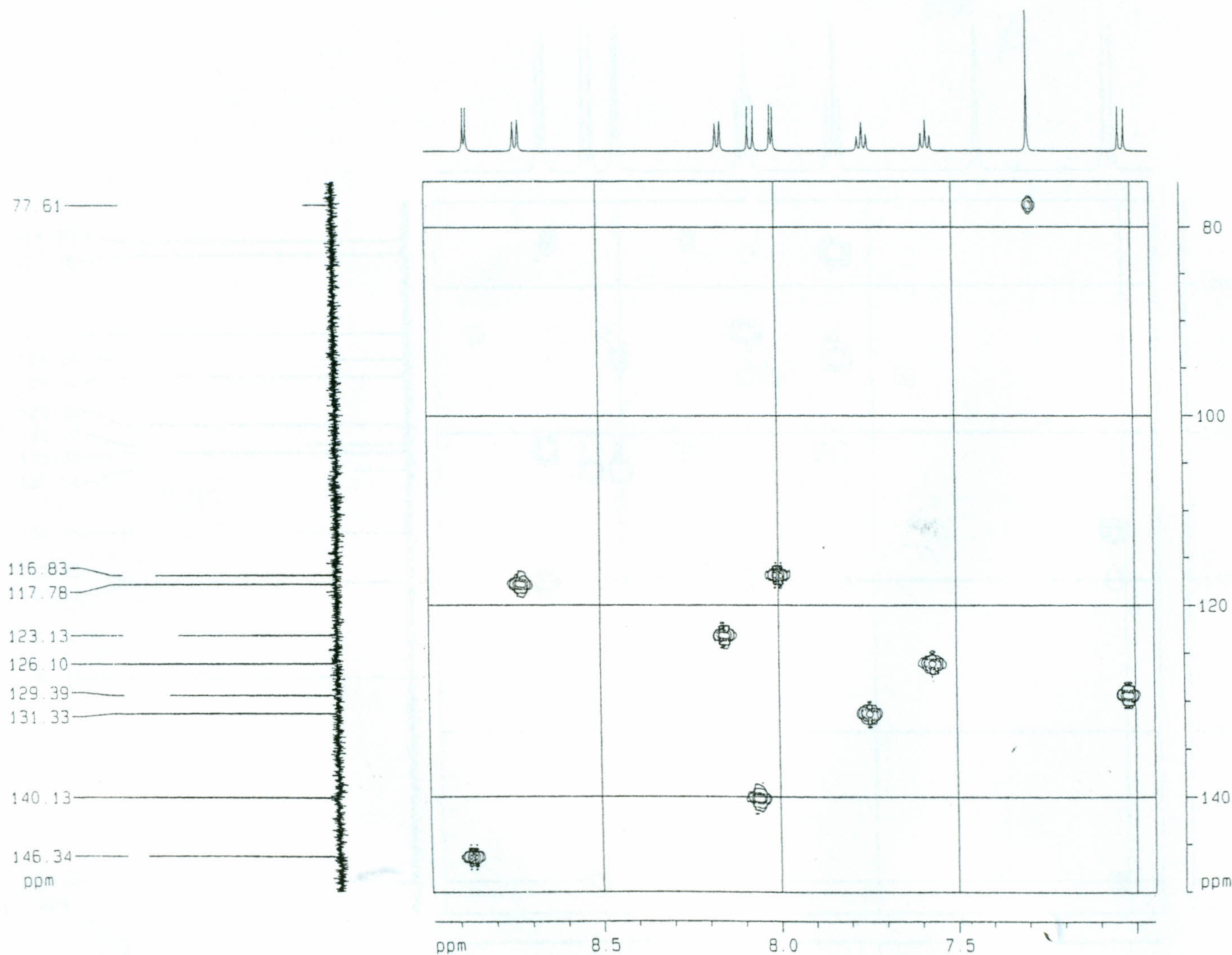
CH3 carbons

CH2 carbons

CH carbons

all protonated carbons

HMQC Spectrum for B_6



F586 HMQC
University of Botswana
Chemistry Department
NABSA NMR Service

Current Data Parameters

NAME	1506
EXPNO	6
PROCNO	1

F2 - Acquisition Parameters

Date_	20021020
Time	4 13
INSTRUM	spect
PROBHD	5 mm SEI 1H-
PULPROG	inv4gs
TD	2048
SOLVENT	CDCl3
NS	24
DS	8
SWH	5296 610 Hz
FIDRES	2 586236 Hz
AQ	0 1933812 sec
RG	26008
DW	94 400 usec
DE	6 00 usec
TE	300 0 K
D0	0 00000300 sec
D1	1 50000000 sec
D2	0 00345000 sec
D12	0 00002000 sec
D13	0 00000300 sec
D16	0.00010000 sec
d20	0 00242700 sec
IN0	0 00001490 sec

************ CHANNEL f1 ************

NUC1	1H
P1	8.40 usec
P2	16 80 usec
PL1	0.00 dB
SFO1	600.1330854 MHz

************ CHANNEL f2 ************

CPDPRG2	garp
NUC2	13C
P3	10 20 usec
PCPD2	65.00 usec
PL2	-1 00 dB
PL12	16 70 dB
SFO2	150 9177568 MHz

************ GRADIENT CHANNEL ********

P16	1000 00 usec

F1 - Acquisition parameters

ND0	2
TD	256
SFO1	150.9178 MHz
FIDRES	131.082214 Hz
SW	222 353 ppm

F2 - Processing parameters

SI	2048
SF	600.1300000 MHz
WDW	SINE
SSB	0
LB	0 00 Hz
GB	0
PC	1 40

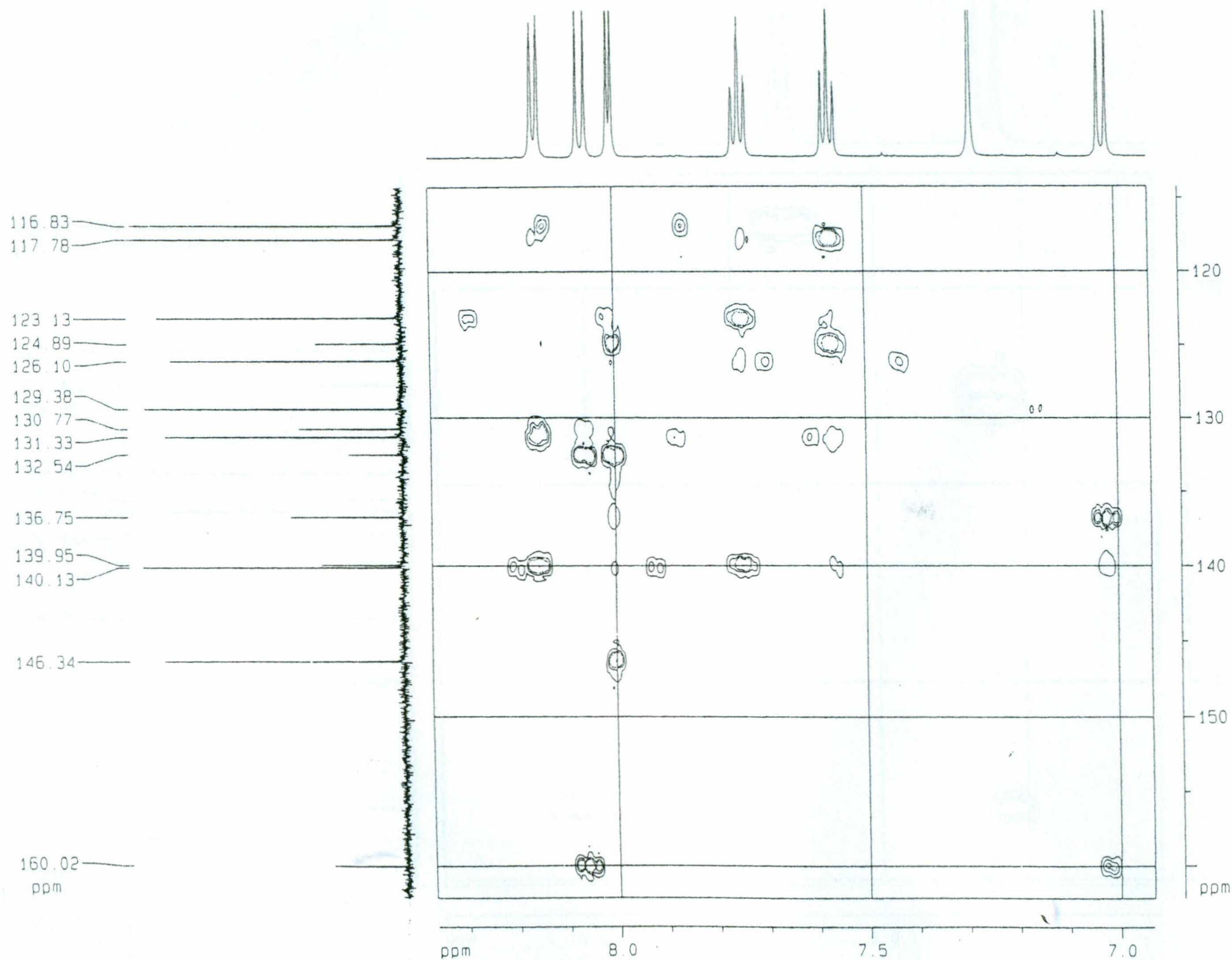
F1 - Processing parameters

SI	1024
MC2	QF
SF	150.9027490 MHz
WDW	SINE
SSB	0
LB	0 00 Hz
GB	0

2D NMR plot parameters

CX2	12 00 cm
CX1	12 00 cm
F2PLO	8 977 ppm
F2LO	5387 11 Hz
F2PHI	6 947 ppm
F2HI	4168 99 Hz
F1PLO	150.052 ppm
F1LO	22643 31 Hz
F1PHI	75 131 ppm
F1HI	11337 46 Hz
F2PPMCM	0 16915 ppm/cm
F2HZCM	101 50974 Hz/cm
F1PPMCM	6.24345 ppm/cm
F1HZCM	942 15344 Hz/cm

HMBC Spectrum for B_6



F586 HMBC
University of Botswana
Chemistry Department
NABSA NMR Service

Current Data Parameters

NAME	f586
EXPNO	5
PROCNO	1

F2 - Acquisition Parameters

Date_	20021020
Time	0.11
INSTRUM	spect
PROBHD	5 mm SEI 1H-
PULPROG	inv4gslplrnd
TD	2048
SOLVENT	CDCl3
NS	32
DS	16
SWH	5296.610 Hz
FIDRES	2.586236 Hz
AQ	0.1933812 sec
RG	26008
DW	94.400 usec
DE	6.00 usec
TE	300.0 K
D0	0.00000300 sec
D1	1.50000000 sec
D2	0.00345000 sec
D6	0.06500000 sec
D13	0.00000300 sec
D16	0.00010000 sec
IN0	0.00001490 sec

============ CHANNEL f1 ============

NUC1	1H
P1	8.40 usec
P2	16.80 usec
PL1	0.00 dB
SFO1	600.1330854 MHz

============ CHANNEL f2 ============

NUC2	13C
P3	10.20 usec
PL2	-1.00 dB
SFO2	150.9177568 MHz

============ GRADIENT CHANNEL ========

P16	1000.00 usec

F1 - Acquisition parameters

ND0	2
TD	256
SFO1	150.9178 MHz
FIDRES	131.082214 Hz
SW	222.353 ppm

F2 - Processing parameters

SI	2048
SF	600.1300000 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.40

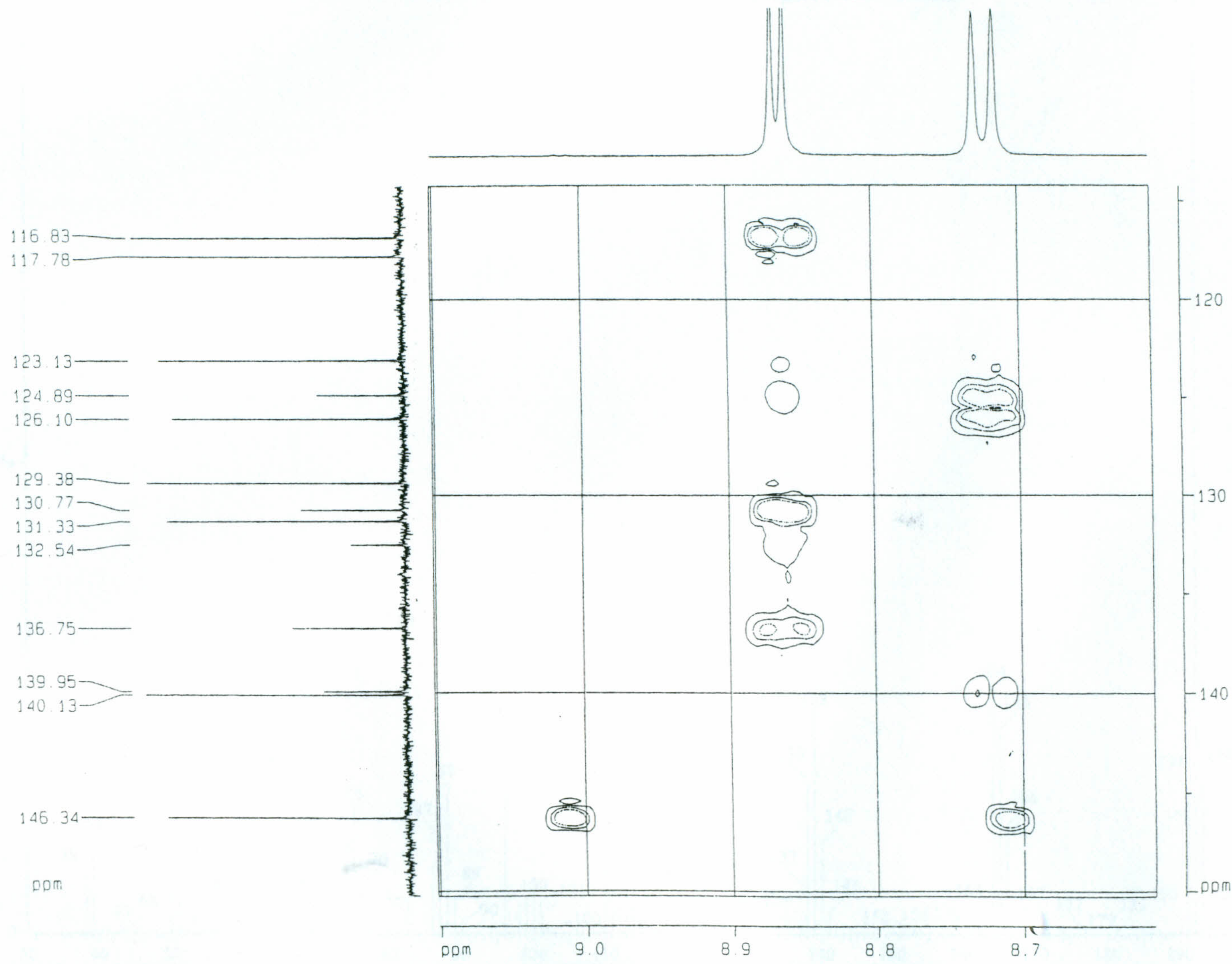
F1 - Processing parameters

SI	1024
MC2	QF
SF	150.9027490 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	12.00 cm
CX1	12.00 cm
F2PLO	8.365 ppm
F2LO	5019.86 Hz
F2PHI	6.934 ppm
F2HI	4161.23 Hz
F1PLO	162.213 ppm
F1LO	24478.46 Hz
F1PHI	114.220 ppm
F1HI	17236.16 Hz
F2PPMCM	0.11923 ppm/cm
F2HZCM	71.55252 Hz/cm
F1PPMCM	3.99943 ppm/cm

HMBC Spectrum for B_6



F5B6 HMBC
University of Botswana
Chemistry Department
NABSA NMR Service

Current Data Parameters
NAME 1506
EXPNO 5
PROCNO 1

F2 - Acquisition Parameters
Date_ 20021020
Time 0.11
INSTRUM spect
PROBHD 5 mm SEI 1H-
PULPROG inv4gslplrnd
TD 2048
SOLVENT CDCl3
NS 32
DS 16
SWH 5296.610 Hz
FIDRES 2.586236 Hz
AQ 0.1933812 sec
RG 26008
DW 94.400 usec
DE 6.00 usec
TE 300.0 K
D0 0.00000300 sec
D1 1.50000000 sec
D2 0.00345000 sec
D6 0.06500000 sec
D13 0.00000300 sec
D16 0.00010000 sec
IN0 0.00001490 sec

============ CHANNEL f1 ============
NUC1 1H
P1 8.40 usec
P2 16.80 usec
PL1 0.00 dB
SFO1 600.1330854 MHz

============ CHANNEL f2 ============
NUC2 13C
P3 10.20 usec
PL2 -1.00 dB
SFO2 150.9177568 MHz

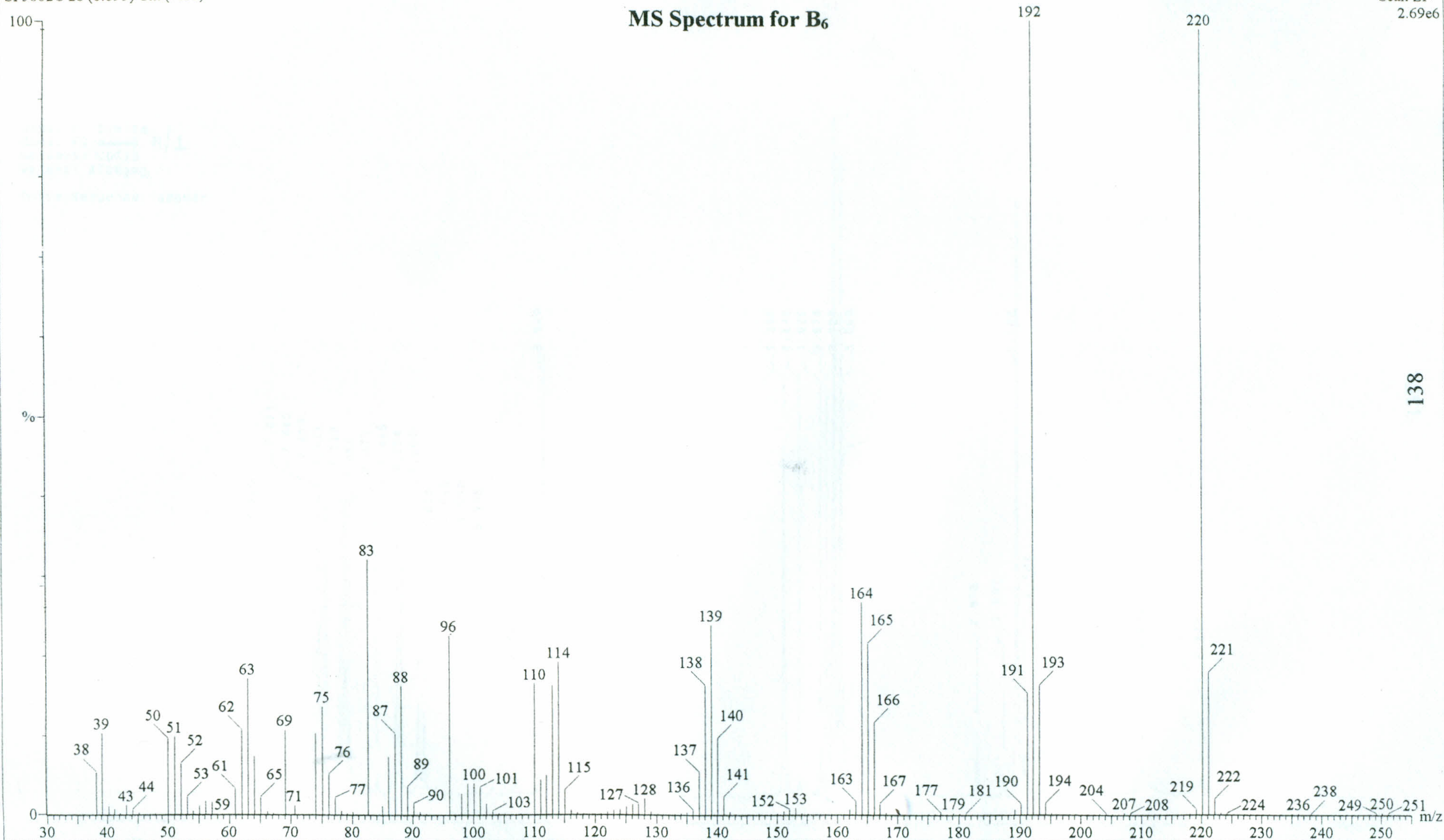
============ GRADIENT CHANNEL ========
P16 1000.00 usec

F1 - Acquisition parameters
ND0 2
TD 256
SFO1 150.9178 MHz
FIDRES 131.082214 Hz
SW 222.353 ppm

F2 - Processing parameters
SI 2048
SF 600.1300000 MHz
WDW SINE
SSB 0
LB 0.00 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 QF
SF 150.9027490 MHz
WDW SINE
SSB 0
LB 0.00 Hz
GB 0

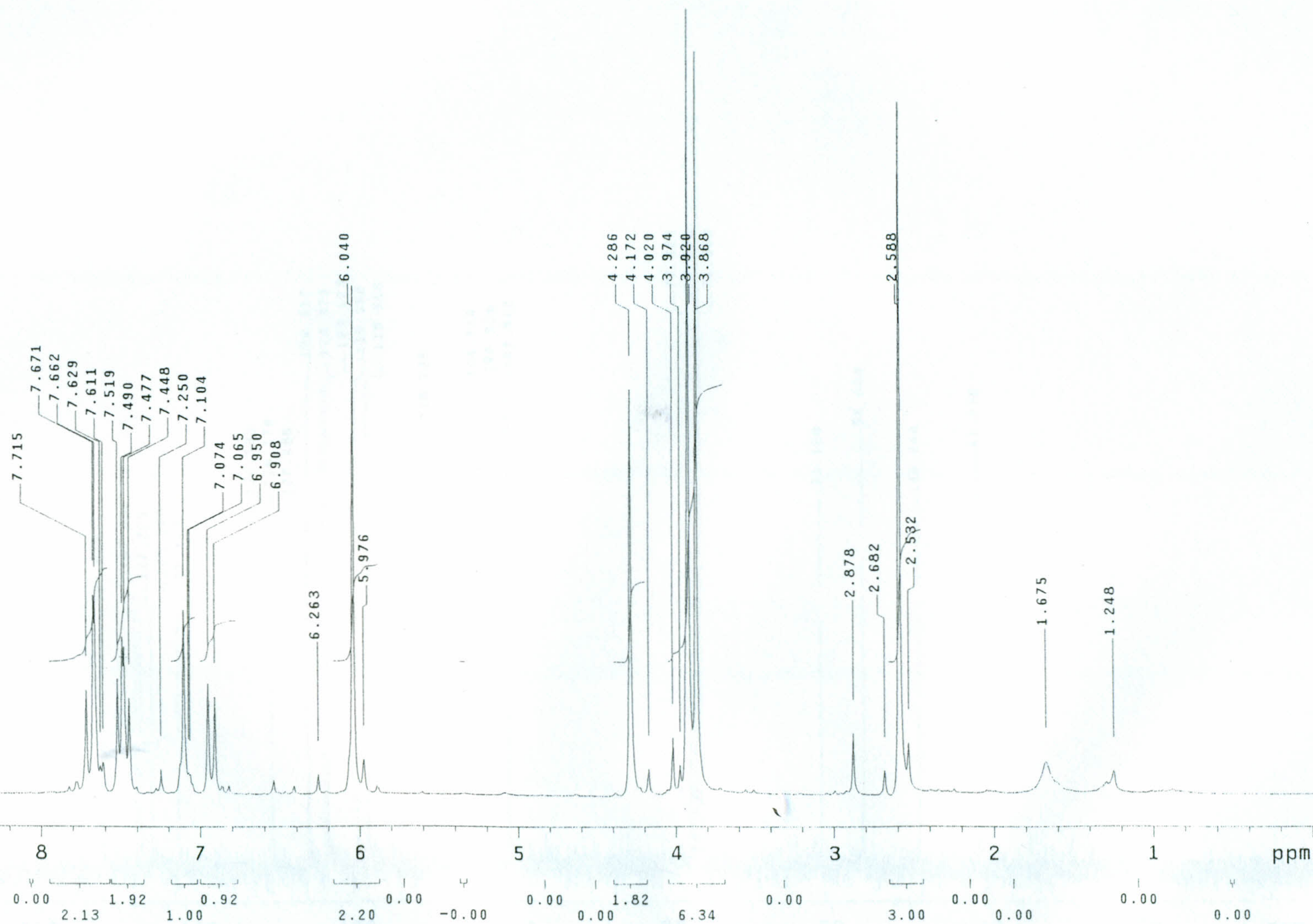
2D NMR plot parameters
CX2 12.00 cm
CX1 12.00 cm
F2PLO 9.102 ppm
F2LO 5462.11 Hz
F2PHI 8.610 ppm
F2HI 5167.28 Hz
F1PLO 150.269 ppm
F1LO 22676.08 Hz
F1PHI 114.220 ppm
F1HI 17236.17 Hz
F2PPMCM 0.04094 ppm/cm
F2HZCM 24.56925 Hz/cm
F1PPMCM 3.00409 ppm/cm
F1HZCM 453.32602 Hz/cm


Ins: VG Platform II GC/LC-MS
BpM:192
FS B6 By Solid Probe
SF5602C 26 (0.895) Cm (7:26)
Date: 05-Jun-2002 Time: 16:50:29
BpI:2694144
Tic:18825928
Scan EI+
2.69e6
MS Spectrum for B6
100
%
0
m/z
192
220
83
164
139
165
96
114
221
193
110
138
191
63
88
166
75
140
62
50
69
39
87
51
52
137
76
38
61
89
115
53
65
141
100
101
163
167
194
222
44
71
77
136
177
181
190
219
43
90
59
127
128
152
153
204
179
207
208
224
236
238
249
250
251
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250

^{1}H NMR Spectrum for H_1

Name: F. Samita
Code: FS/~~B/6/6~~ H/1
Solvent: CDCl3
Weight: 172mg

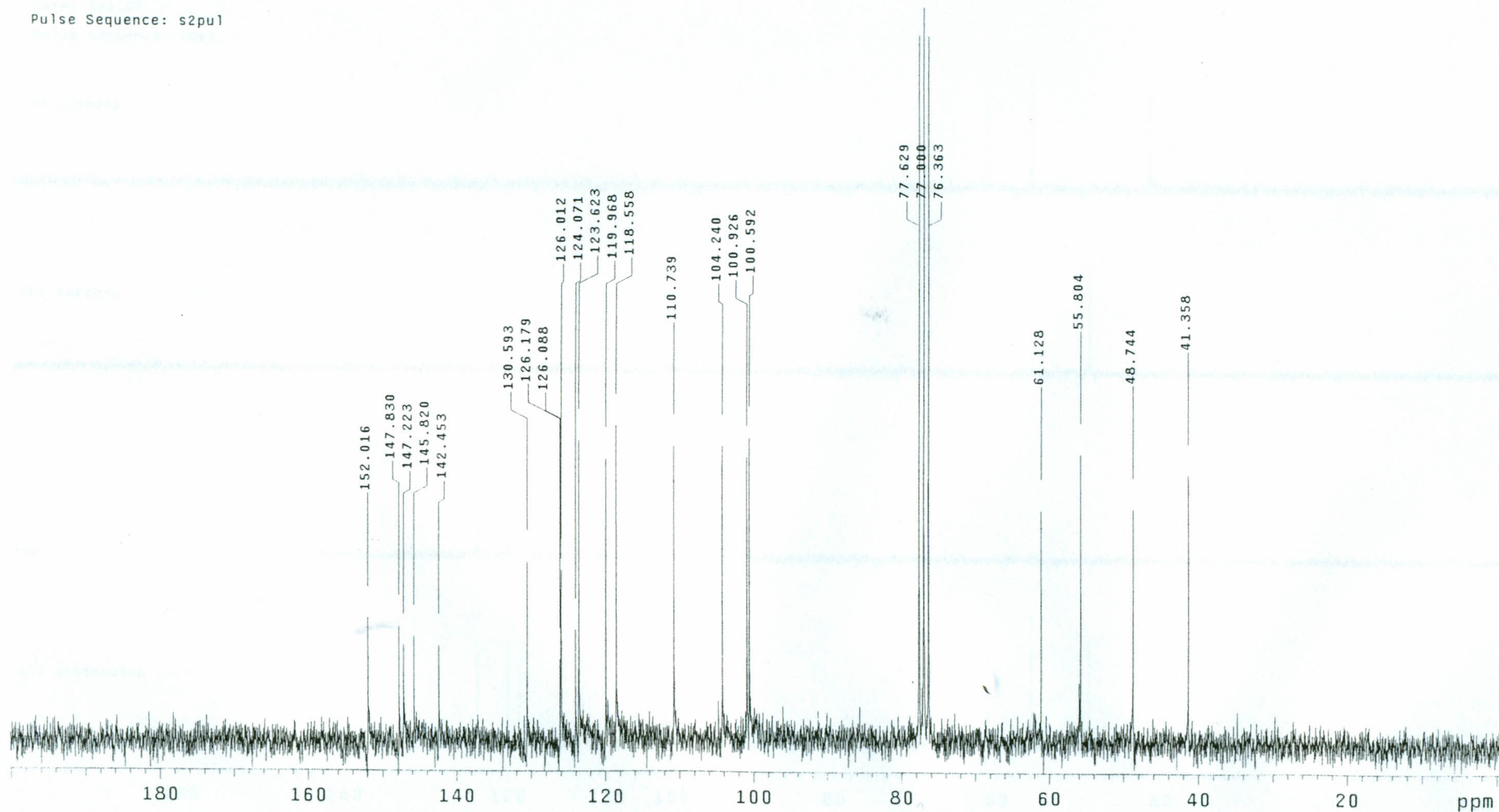
Pulse Sequence: s2pul



^{13}C NMR Spectrum for H_1

Name: F. Samita
Code: FS/H/1
Solvent: CDCl3
Weight: 17 mg

Pulse Sequence: s2pul



DEPT Spectrum for H_1

Name: F. Samita
Code: FS/H/1
Weight: 17 mg
Solvent CDCl3
Date: 4/6/02

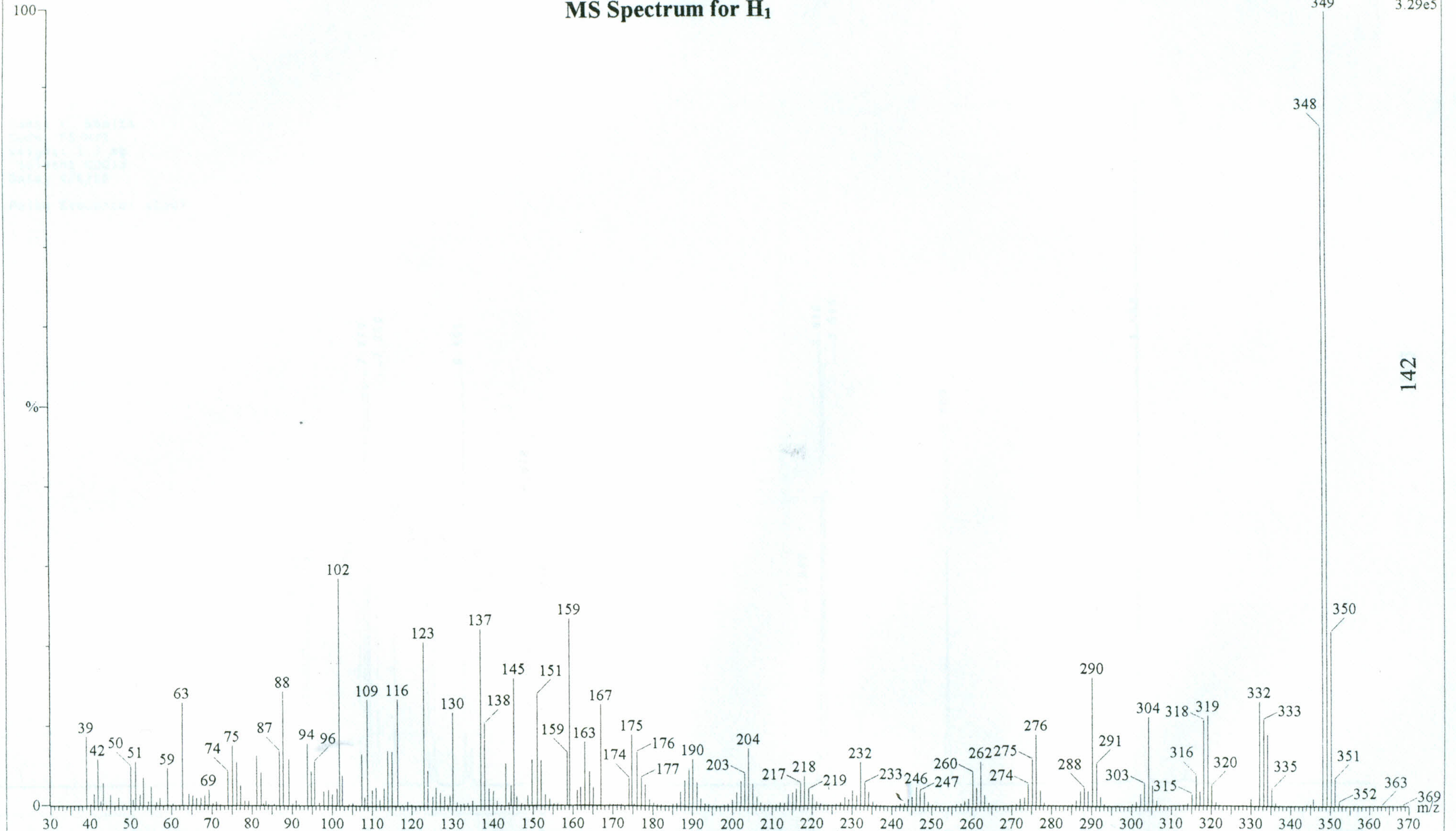
Pulse Sequence: dept

CH3 carbons

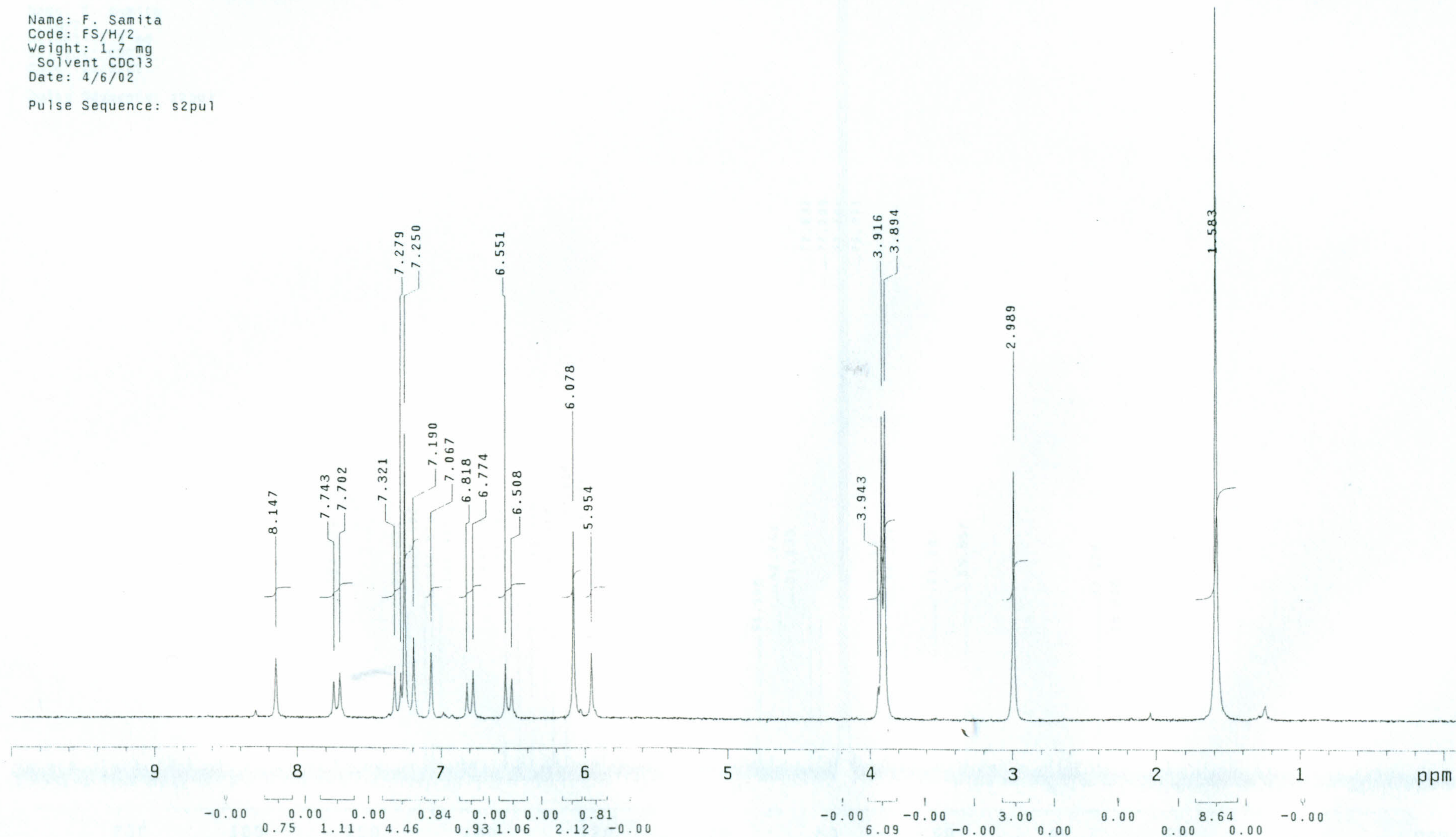
CH2 carbons

CH carbons

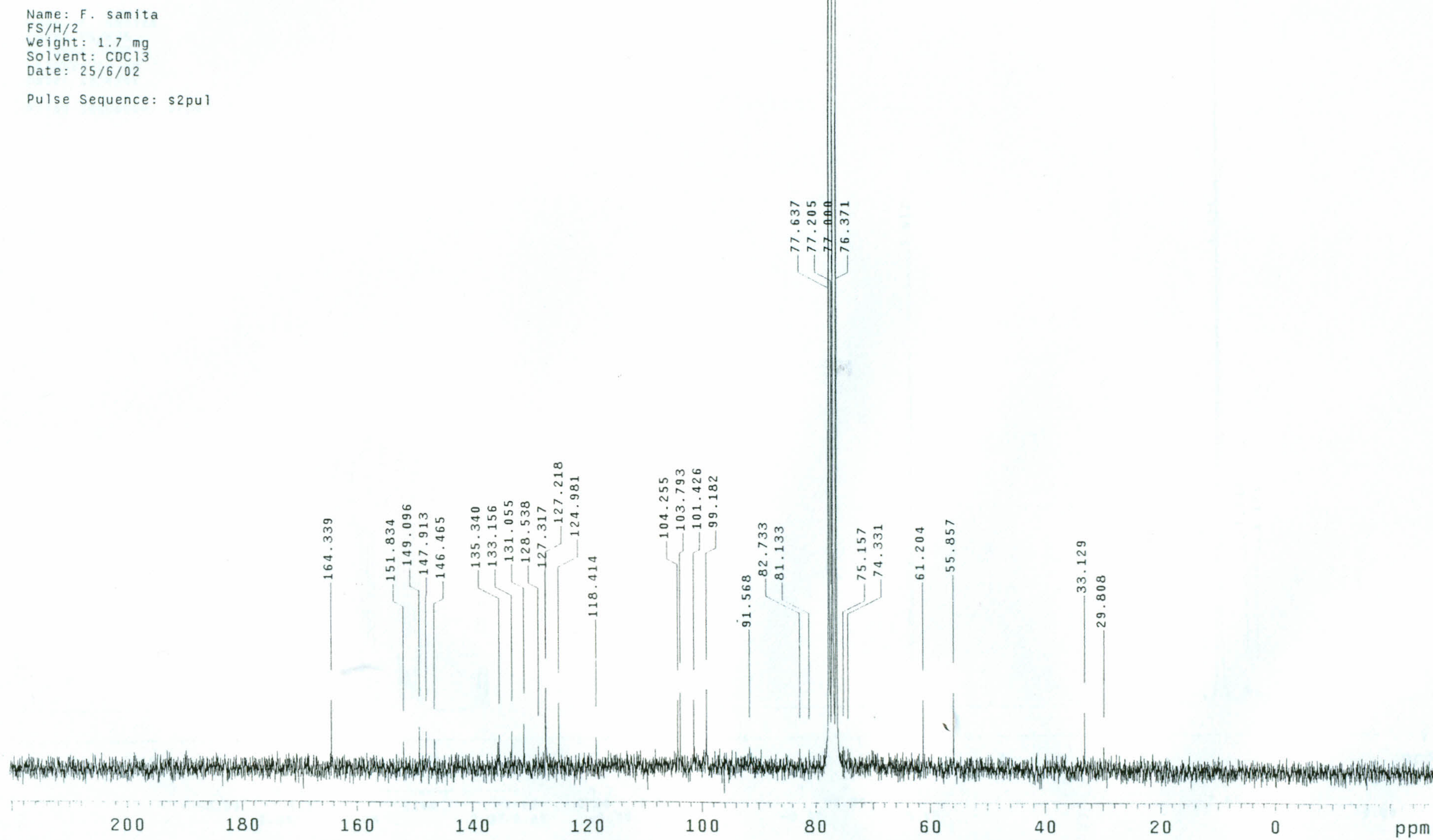
all protonated carbons


Ins: VG Platform II GC/LC-MS
BpM:349
FS H1 By Solid Probe
SF6602F 24 (0.825) Cm (10:25)
Date: 06-Jun-2002 Time: 16:41:01
BpI:329460
Tic:3010180
Scan EI+
3.29e5
MS Spectrum for H1
%
m/z

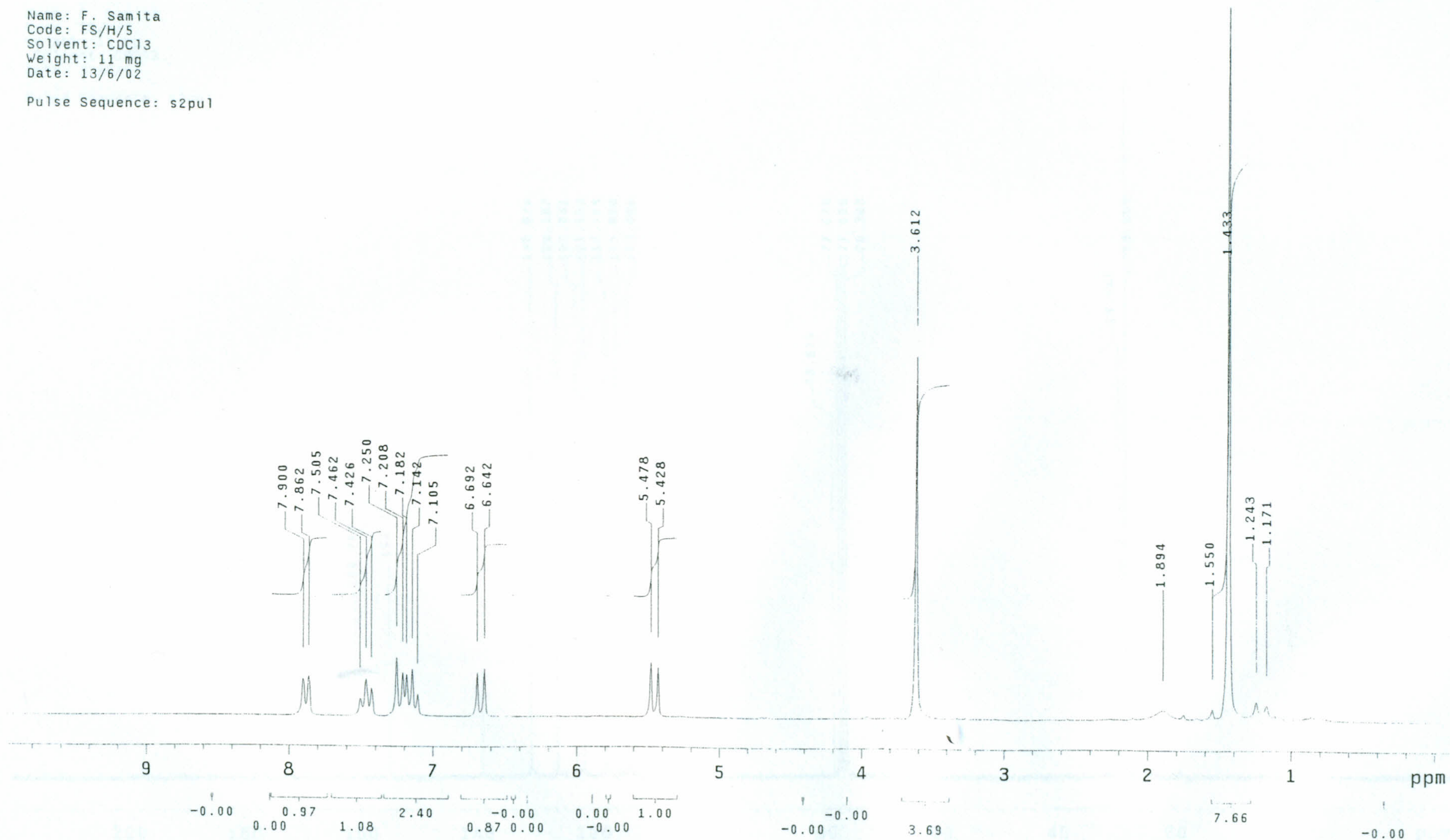
^1H NMR Spectrum for H_2



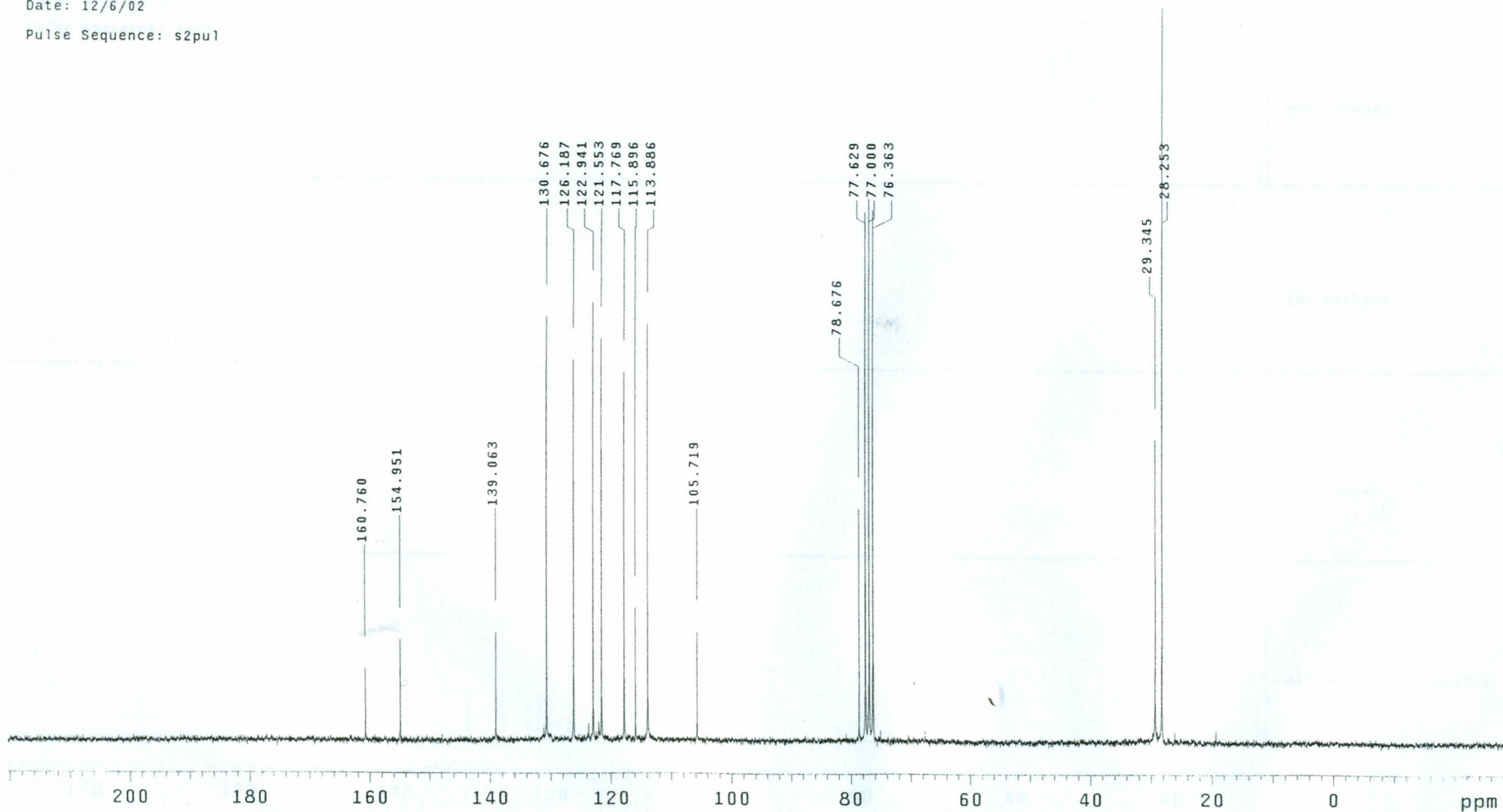
^{13}C NMR Spectrum for H_2



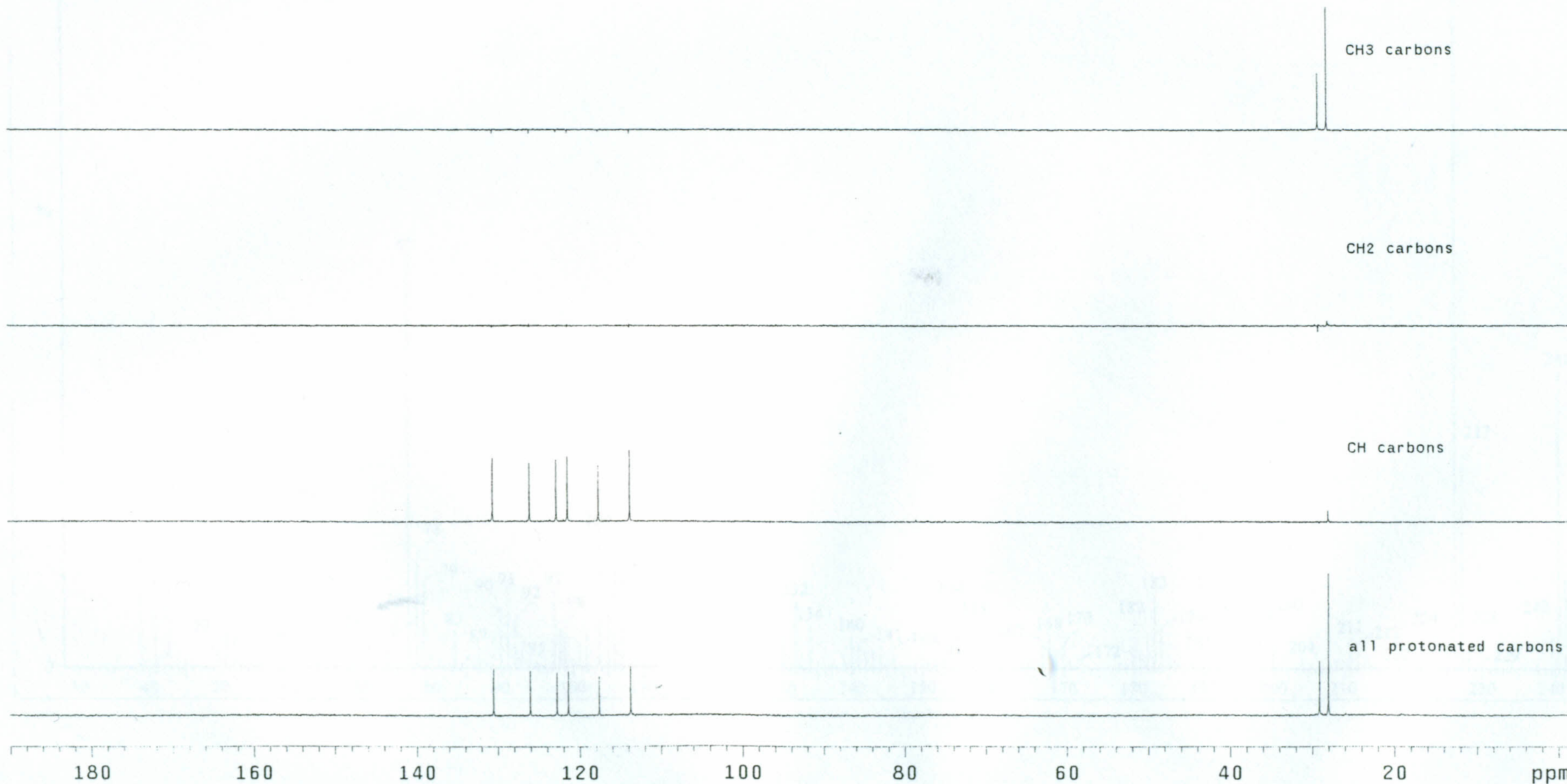
^{1}H NMR Spectrum for H_5

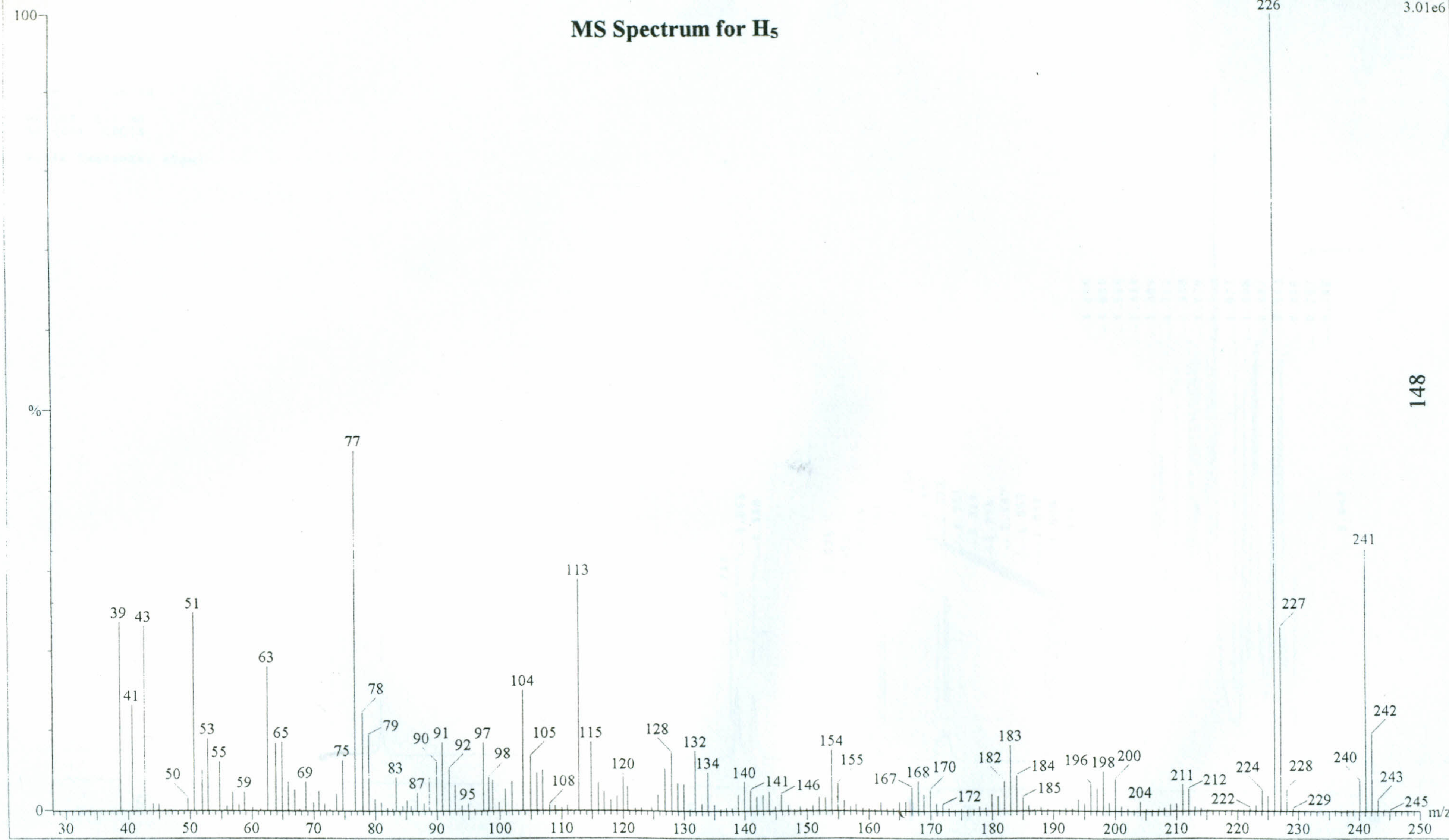


^{13}C NMR Spectrum for H_5



DEPT Spectrum for H_5





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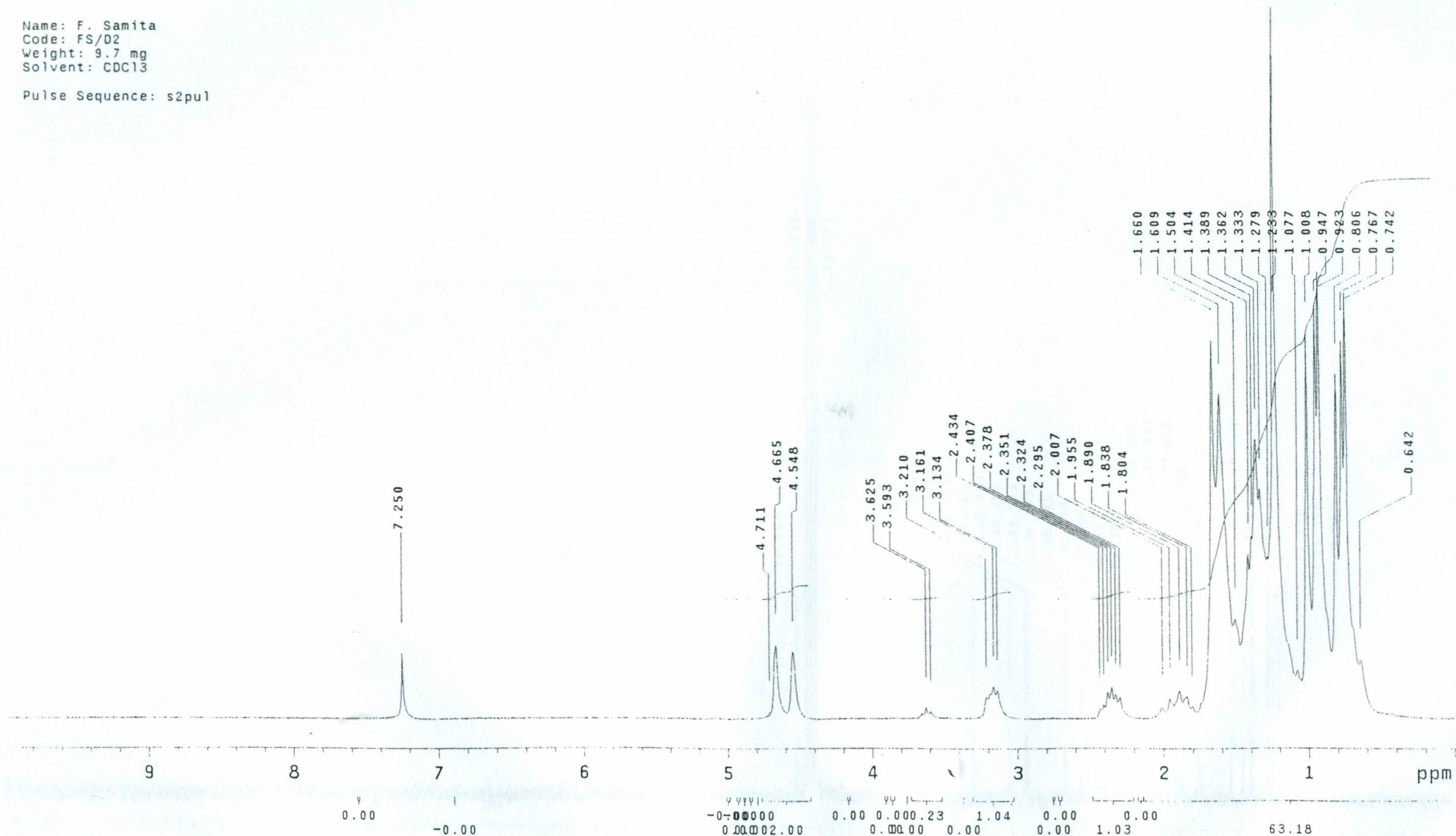
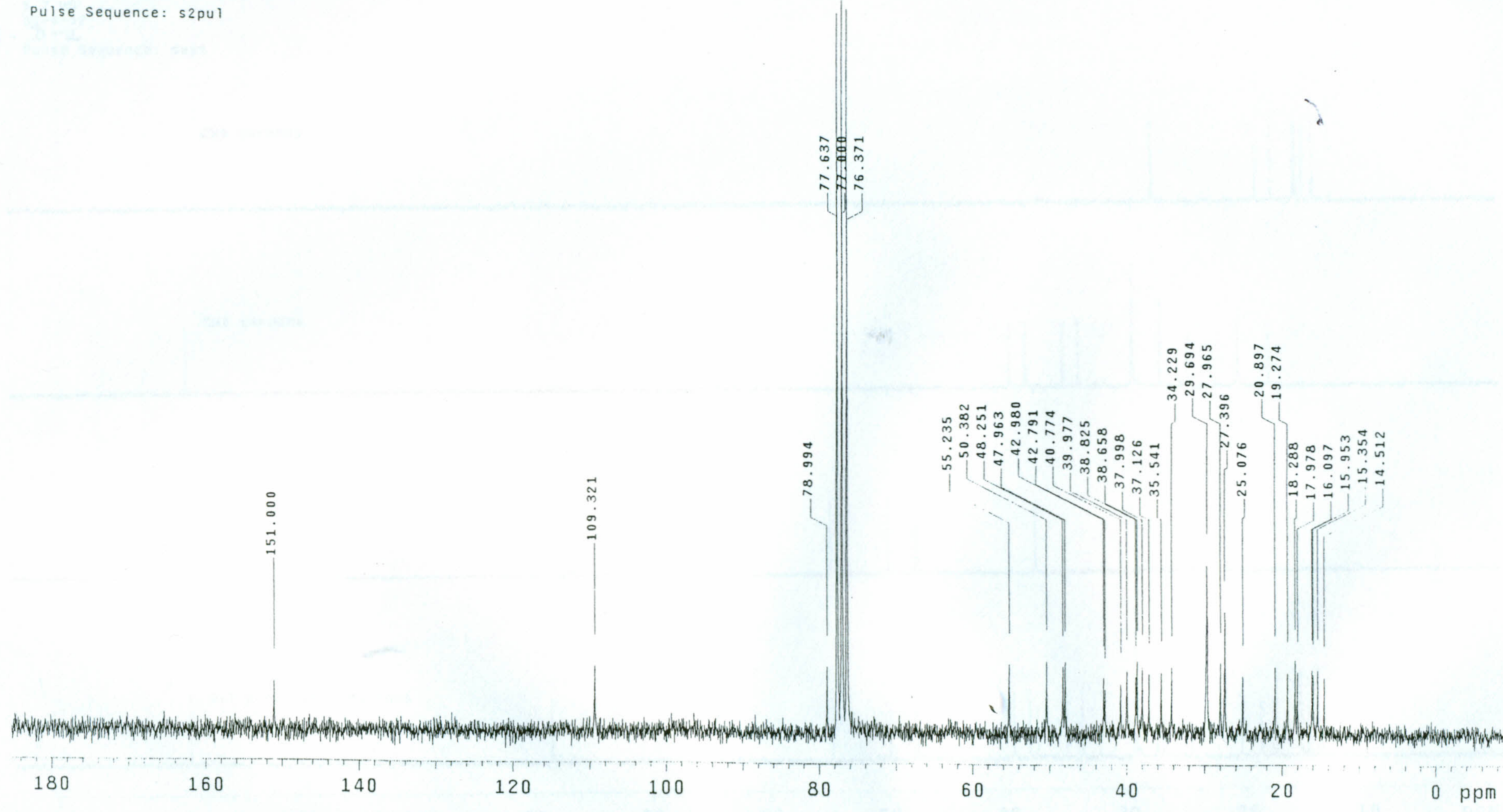
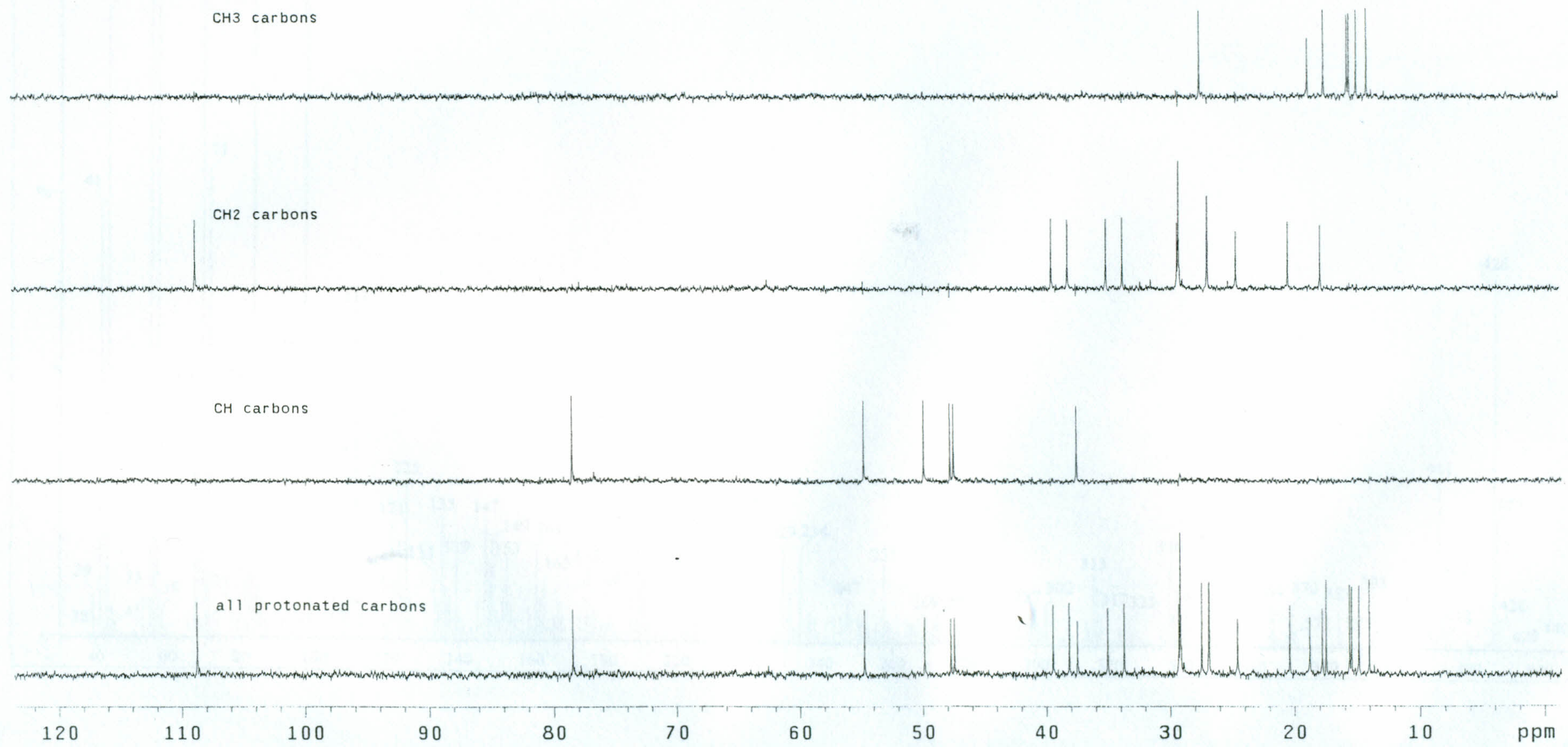
^{1}H NMR Spectrum for D_2

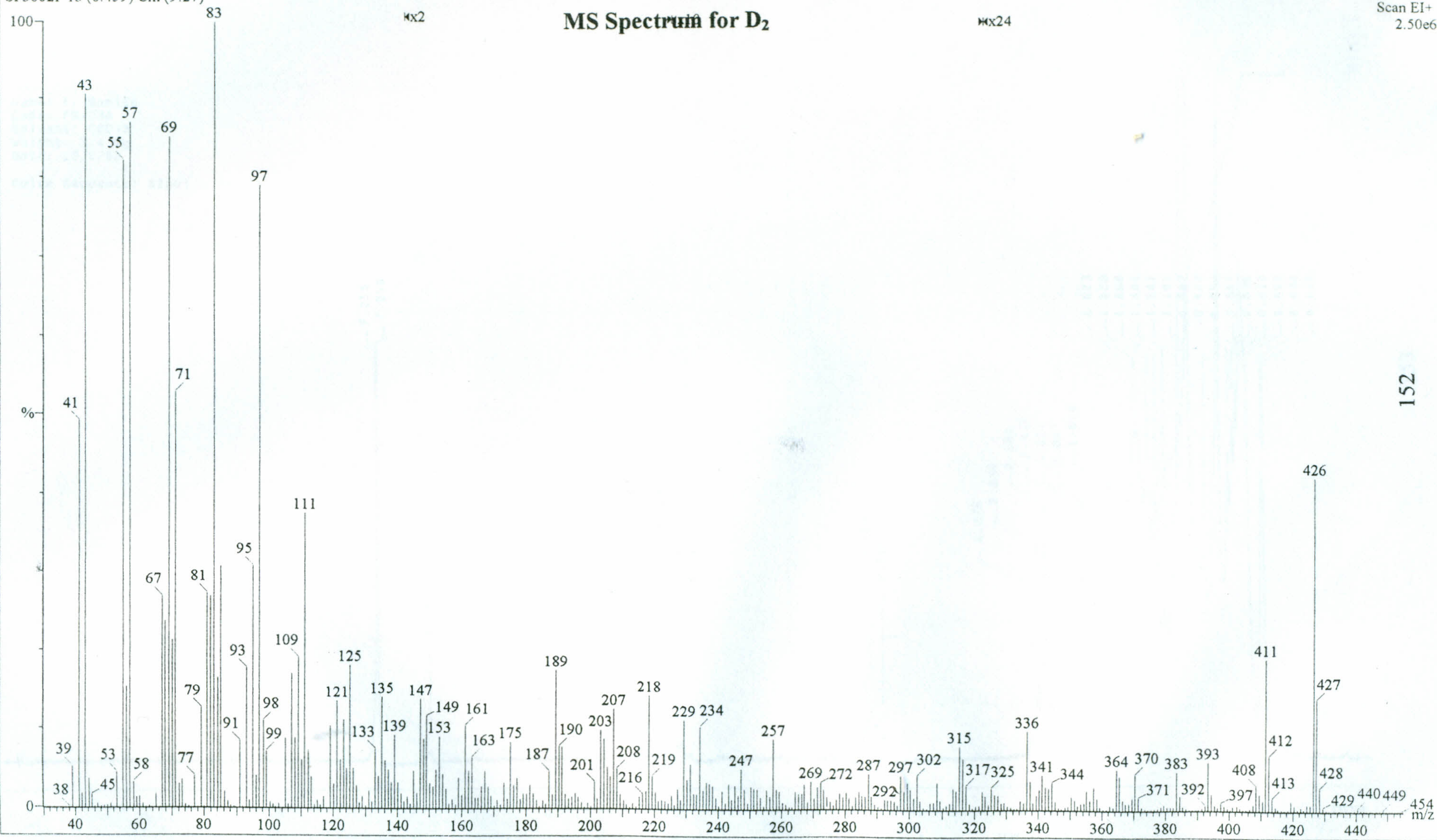


^{13}C NMR Spectrum for D_2



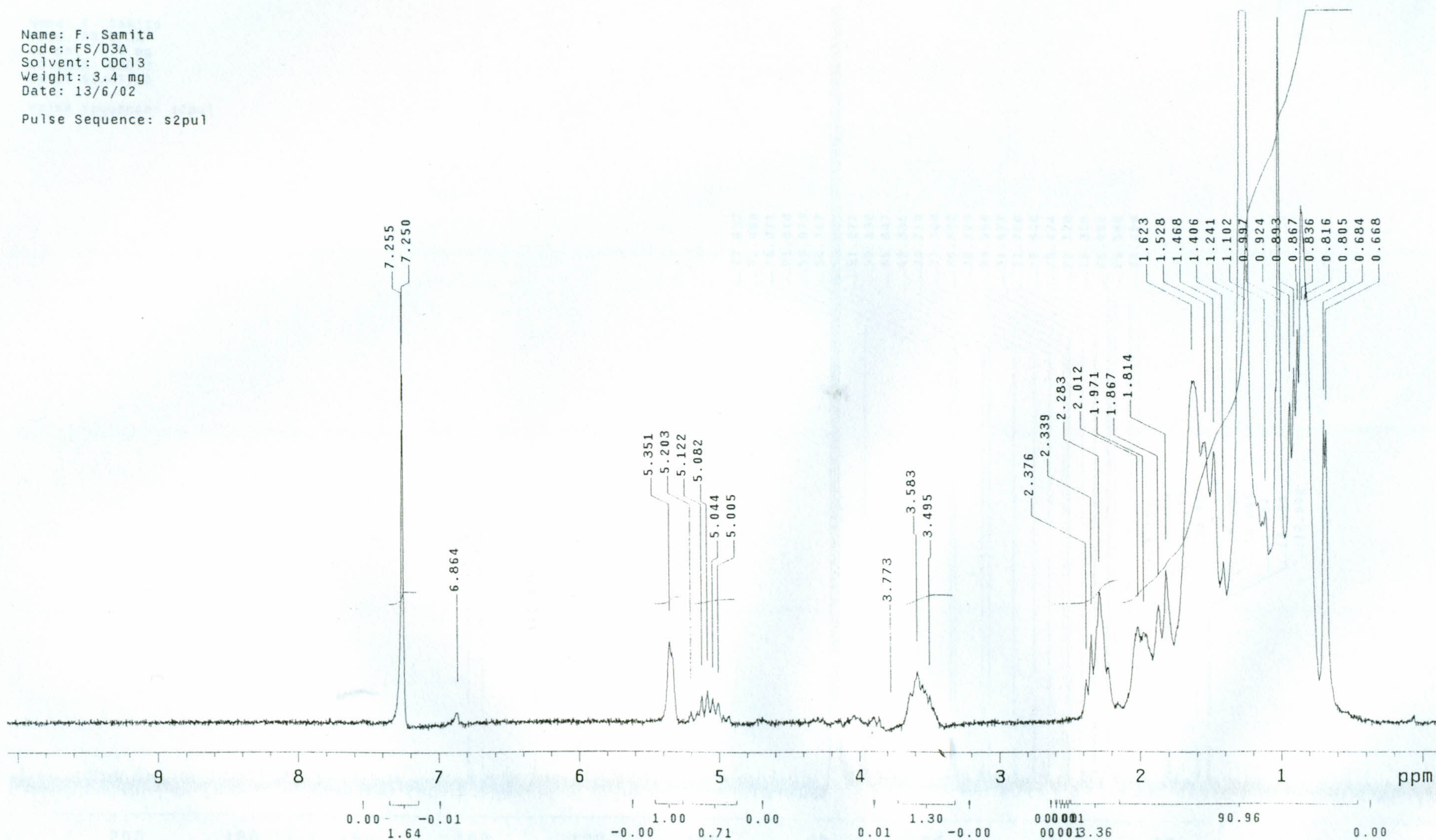
DEPT Spectrum for D_2



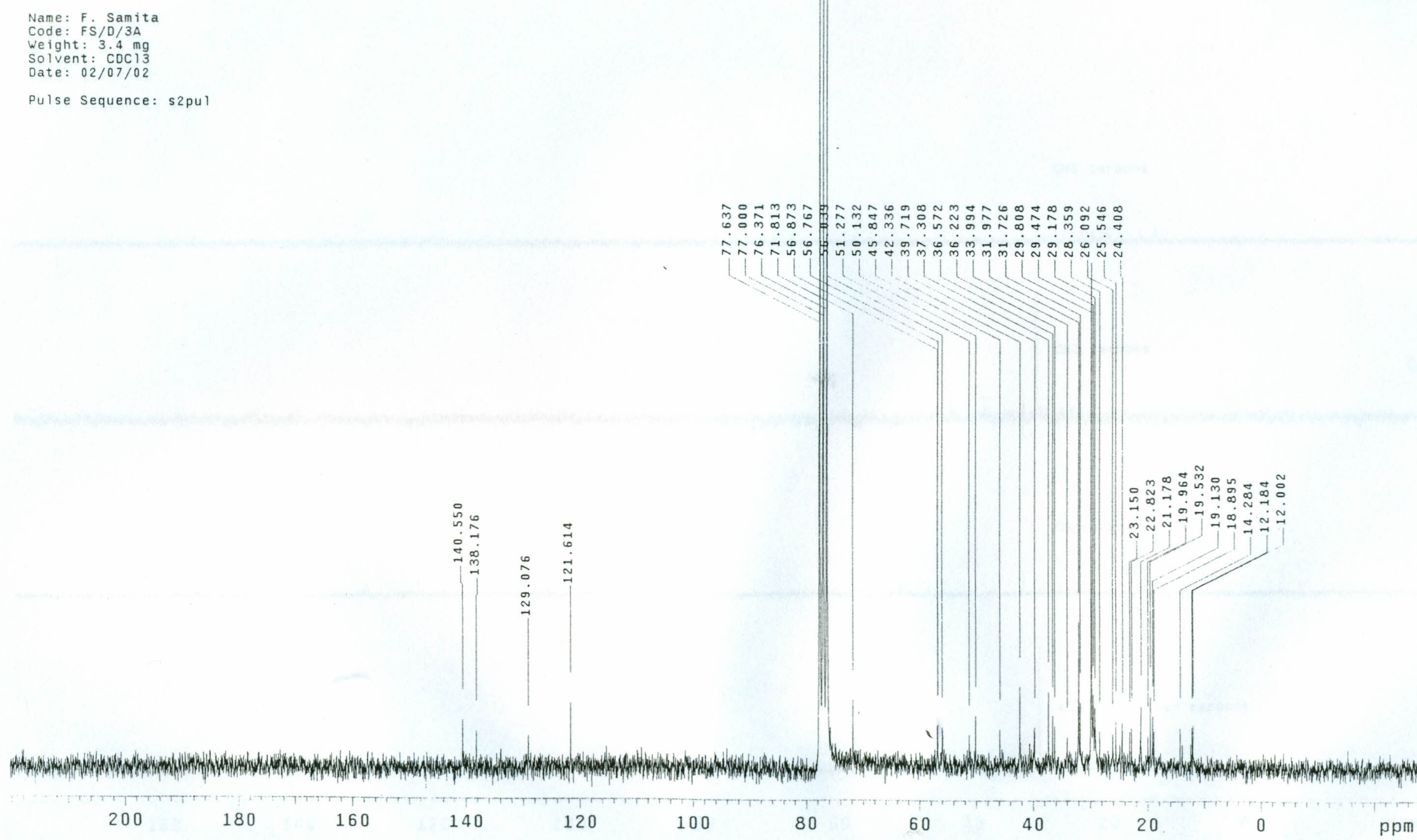


Ins: VG Platform II GC/LC-MS
BpM:83
FS D2 By Solid Probe
SF3602F 13 (0.459) Cm (9:27)
Date: 03-Jun-2002 Time: 18:47:12
BpI:2500123
Tic:34132224
MS Spectrum for D2
x2
x24
Scan EI+
2.50e6
100
%
0
m/z

^{1}H NMR Spectrum for D_{3A}



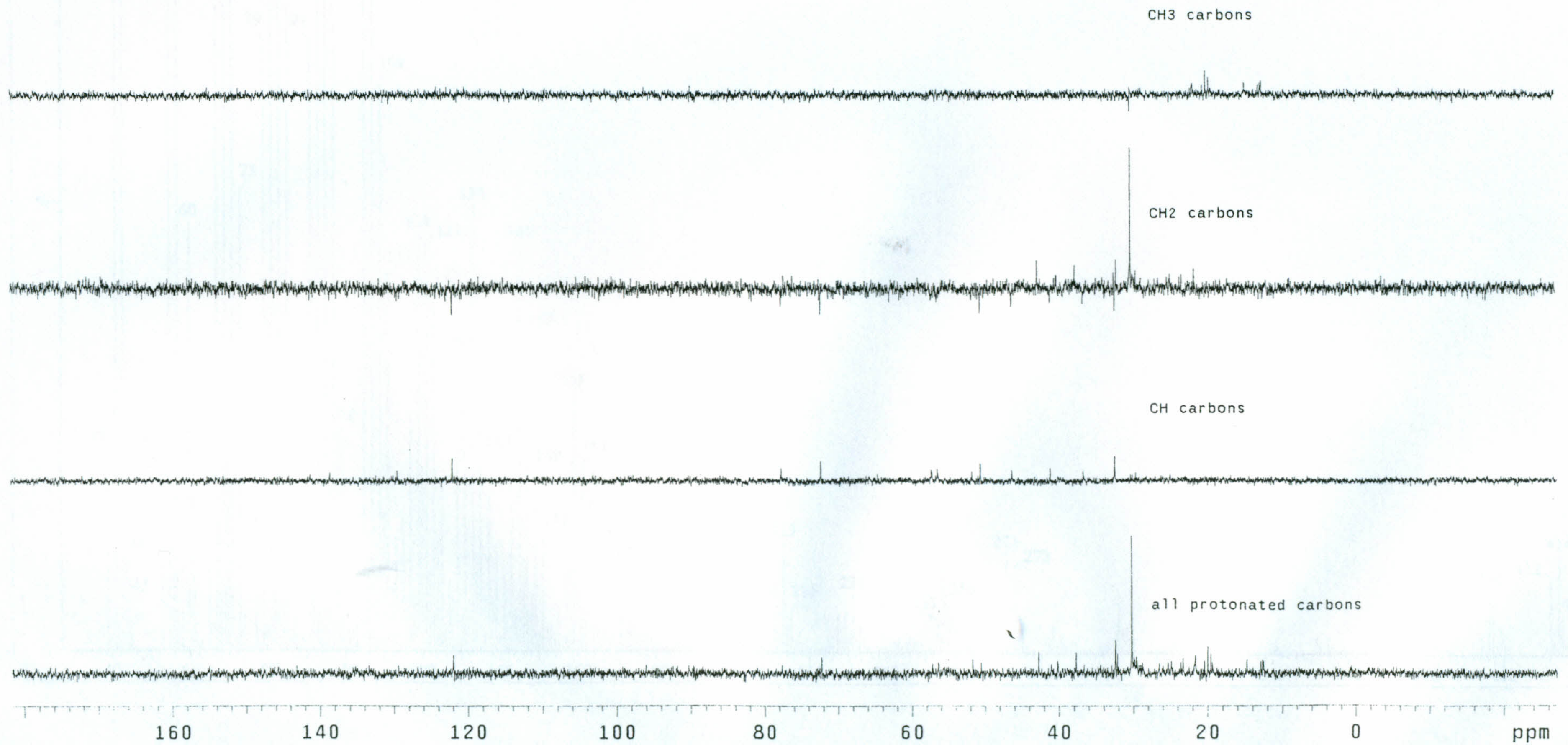
^{13}C NMR Spectrum for D_{3A}

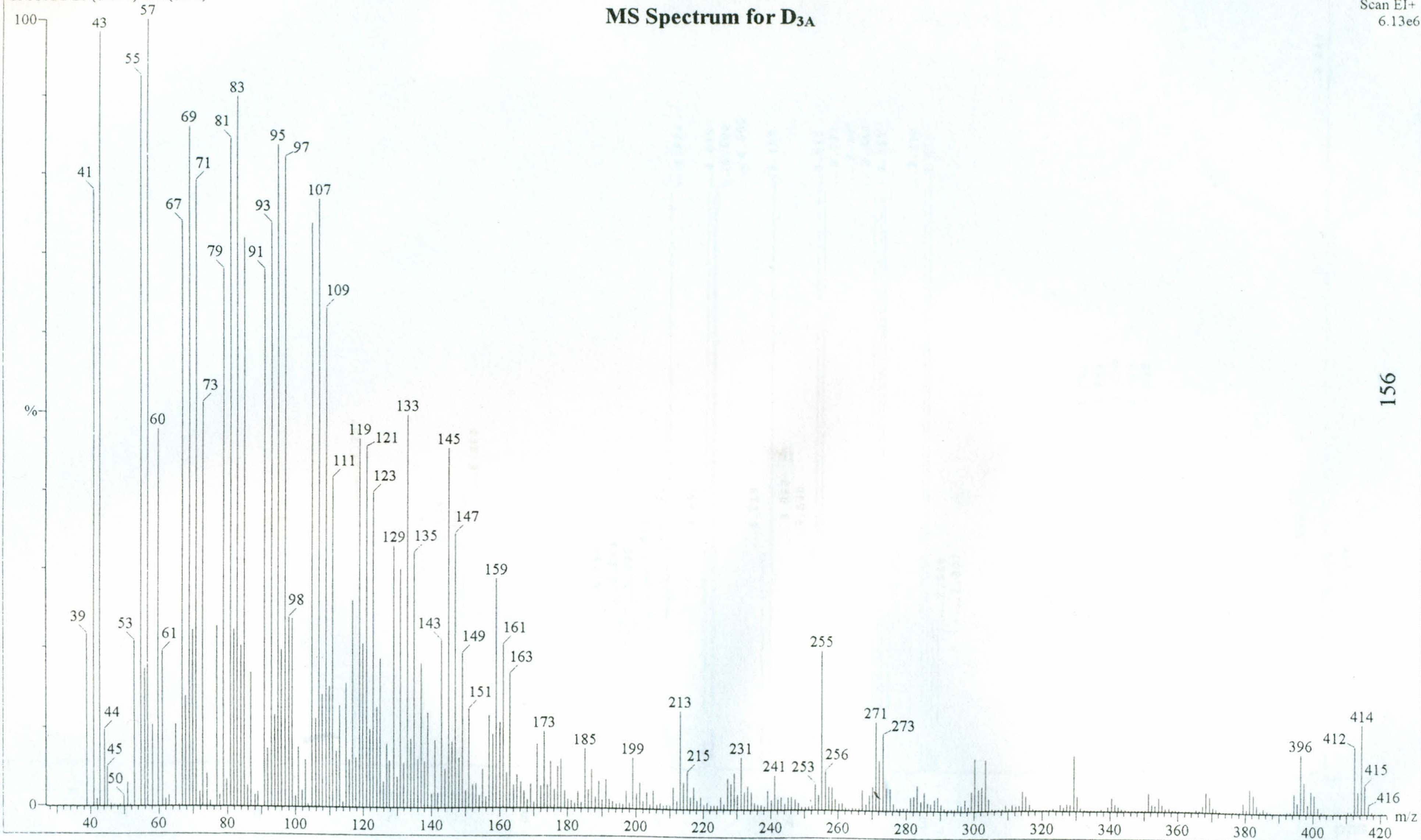


DEPT Spectrum for D_{3A}

Name: F. Samita
Code: FS/D/3A
Weight: 3.4 mg
Solvent: CDCl3
Date: 03-07-02

Pulse Sequence: dept





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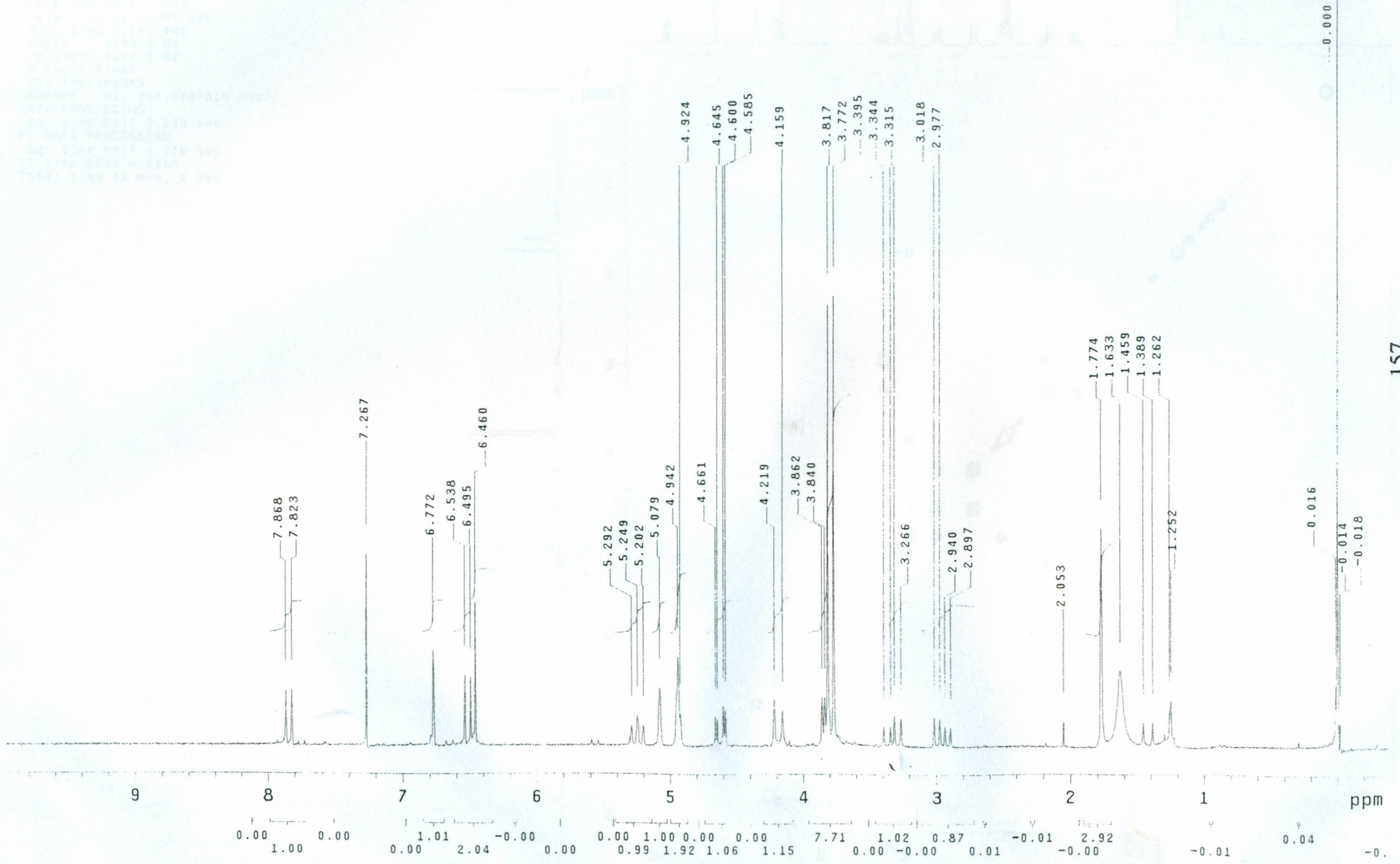
Onyango
CDl3
5mg
FS/D/7A
14-02-03

Pulse Sequence: s2pul

^{1}H NMR Spectrum for D_7

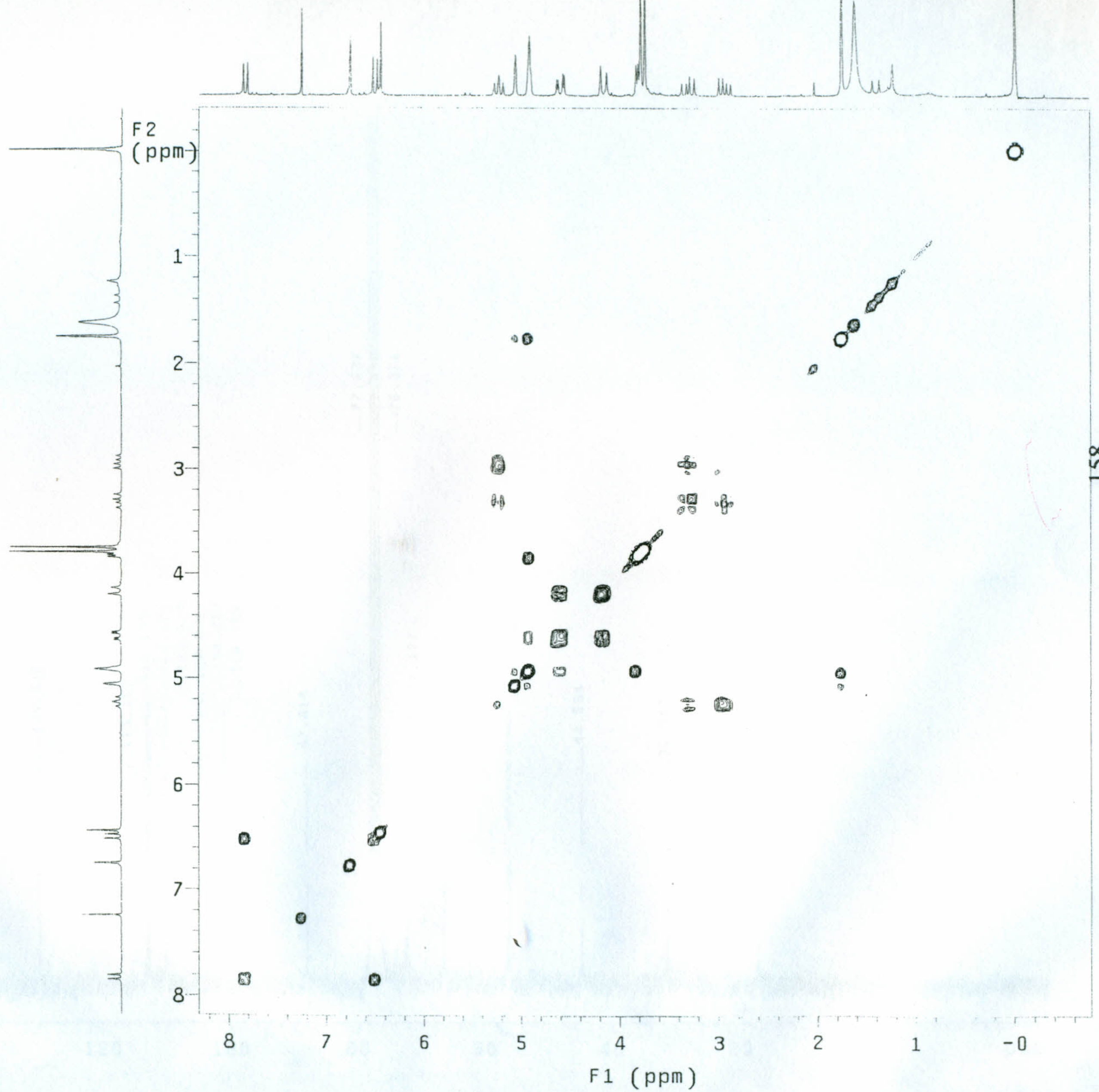


F. Samita
FS-D-7A
4.5 mg
CDCl3
11-2-03

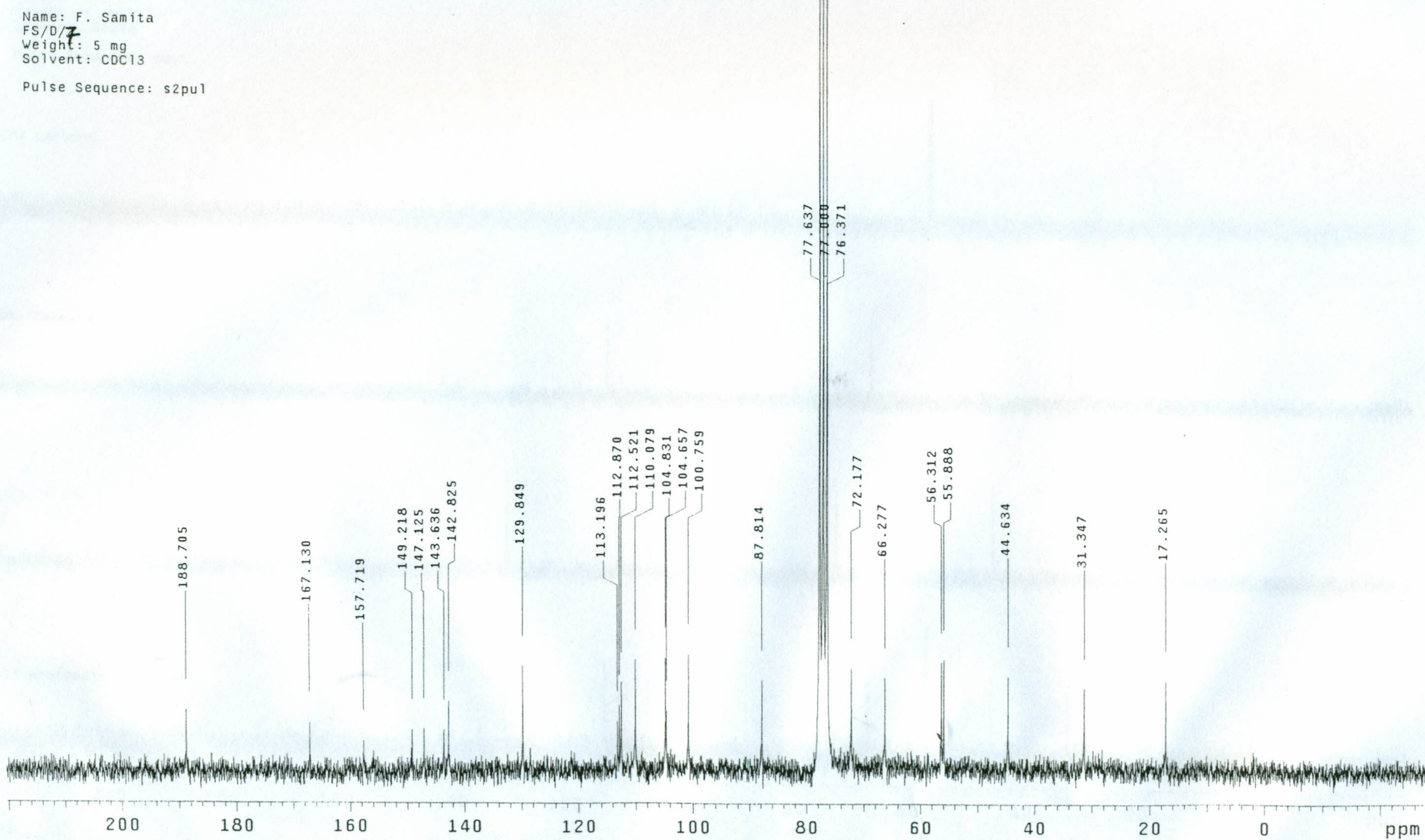
Pulse Sequence: COSY

Solvent: CDCl3
Ambient temperature
File: COSY
Mercury-200 "uonnmr200"

PULSE SEQUENCE: COSY
Relax. delay 1.000 sec
Acq. time 0.160 sec
Width 3200.8 Hz
2D Width 3200.8 Hz
8 repetitions
256 increments
OBSERVE H1, 200.0507826 MHz
DATA PROCESSING
Sq. sine bell 0.080 sec
F1 DATA PROCESSING
Sq. sine bell 0.080 sec
FT size 2048 x 2048
Total time 44 min, 6 sec



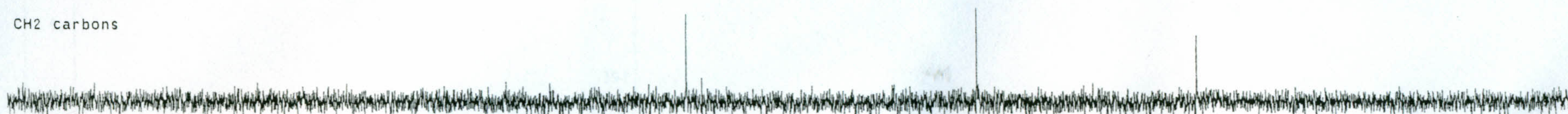
^{13}C NMR Spectrum for D_7

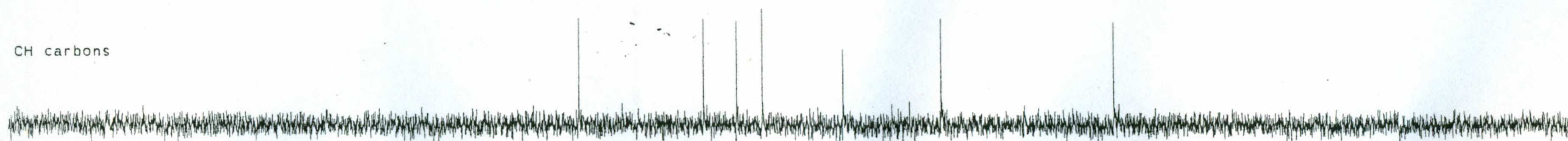


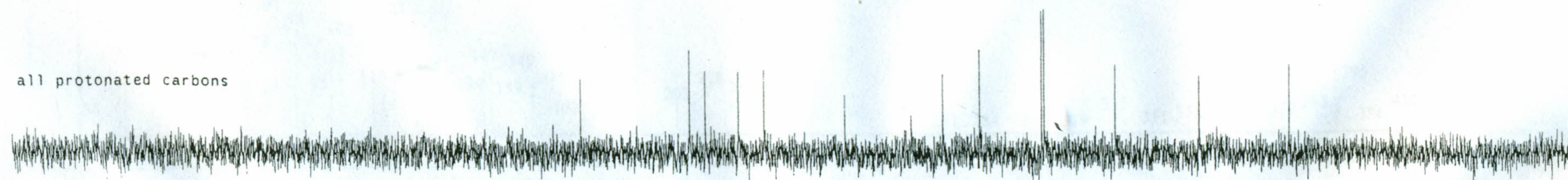
DEPT Spectrum for D_7

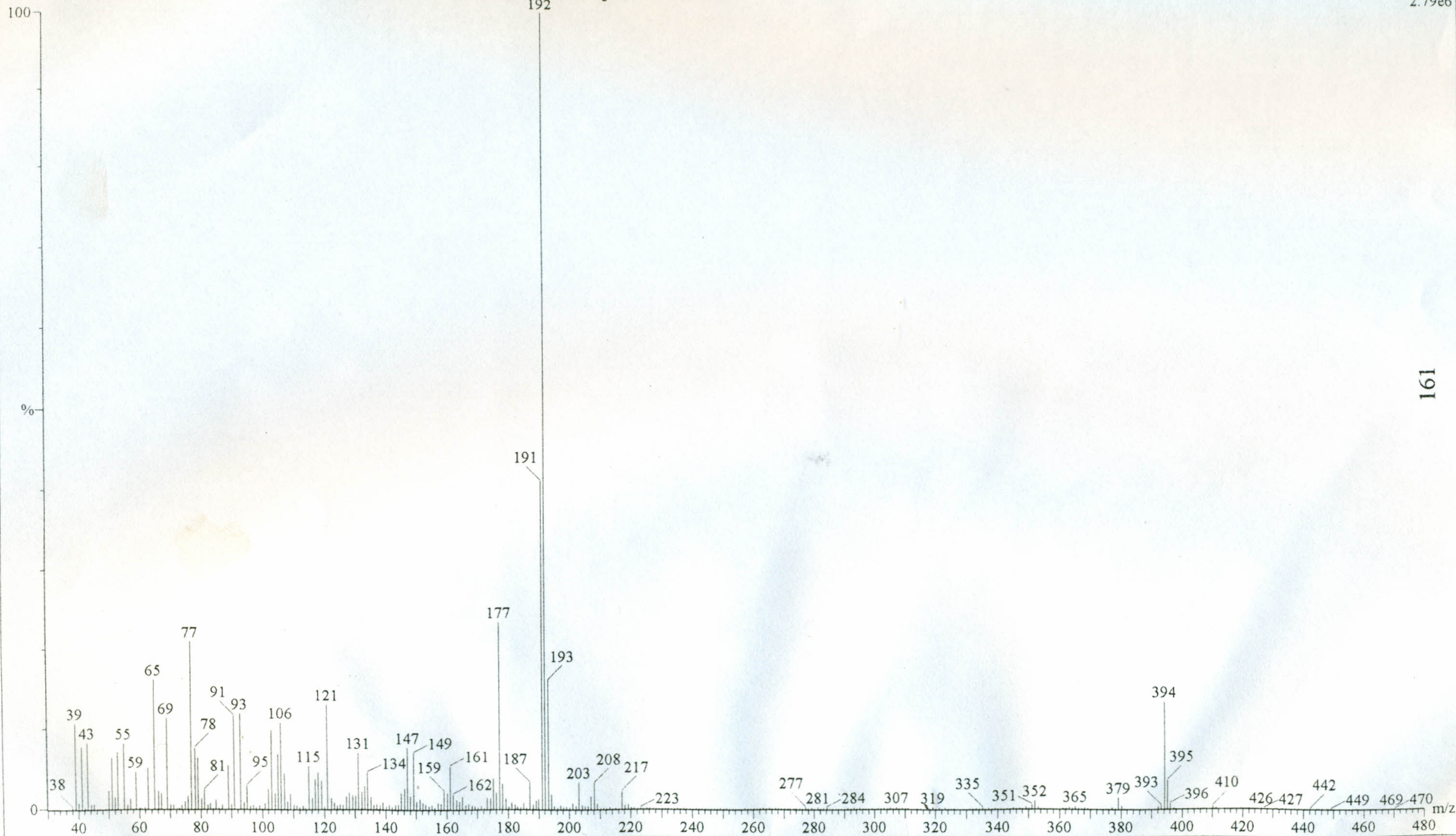
Name: F. Samita
FS/D/7
Weight: 5 mg
Solvent: CDCl3

Pulse Sequence: dept









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