PHYTOCHEMICAL ANALYSIS OF SIX *PENTAS* SPECIES FOR ANTIPLASMODIAL PRINCIPLES

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PhD Thesis by

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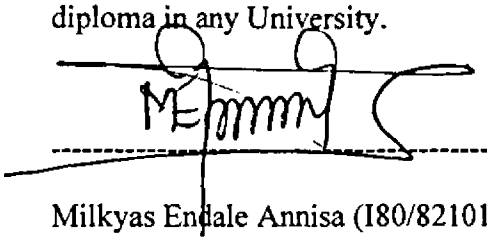
A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY AT THE UNIVERSITY OF NAIROBI

2012

DECLARATION

This is my original work and has never been presented for award of any degree or diploma in any University.



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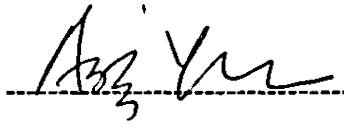

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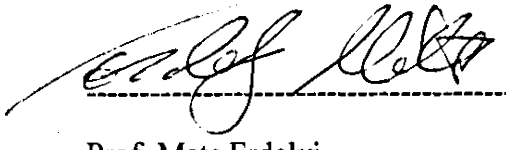

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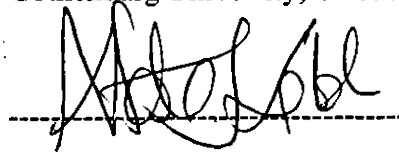

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DEDICATION

This doctoral thesis is dedicated to my family, colleagues and friends in various parts of the world who have never failed to give me all types of support.

"You are truly the best. May the almighty God bless you all!"

ACKNOWLEDGEMENTS

I would like to thank my supervisors Prof. Abiy Yenesew, Dr. Albert Ndakala, Dr. Martin Mbugua and Prof. Mate Erdelyi for their in depth supervision and persistent support of all kinds throughout the PhD study period. I owe you a big thank you!

I have benefited a lot from the tutelage of several mentors over the years. Of these, I have appreciation to Prof. Abiy's commitment to research. I have usually walked out of his office feeling more motivated towards the project than when I walked in and I feel fortunate to have had a research supervisor who had taken the word "supervisor" to heart.

I would like to express my sincere gratitude to all academic, technical and support staff of the Department of Chemistry, University of Nairobi for their assistance throughout the study period. In particular, I would like to thank Dr. Solomon Derese for his scientific support, encouragement and accommodation arrangement. Prof. Jacob Midiwo, executive secretary of NAPRECA and Dr. Amir O. Yusuf, chairman of Department of Chemistry, are greatly acknowledged for their assistance in many ways and encouragement. Mr. Hosea Akala of the United States Army Medical Research Unit-Kenya and Mrs. Beatrice Irungu Kimani of the Kenya Medical Research Institute (KEMRI) are deeply acknowledged for carrying out *in vitro* and *in vivo* antiplamodial tests respectively. Mr. Patrick Chalo Mutiso of the Department of Botany, School of Biological Sciences, University of Nairobi, is sincerely acknowledged for identification of the plant materials. I would like to thank Prof. Mate Erdelyi and his group members, in particular Ms. Annabel Ekberg and Dr. Sven Arenz, Department of Chemistry of University of Gothenburg, Sweden, for the hospitality and help they accorded me during the research visit to Sweden. Swedish Institute is earnestly acknowledged for the financial support during my stay in Sweden. Prof. Per

Sunnerhagen and Dr. John Patrick of Department of Cell and Molecular Biology, University of Gothenburg are greatly acknowledged for the cytotoxicity tests. Thanks are due to all past and present members of the Natural Product Chemistry Research group of the Department of Chemistry of University of Nairobi where I have learnt a lot from the inspiring atmosphere.

I cannot say enough thank you to my hero and love - my wife Yeshewahareg Feyisa, for her continuous support and encouragement all along. My lovely son, Yafet, and daughter, Amen, are my inspiration. Although they are not yet old enough to read and understand what I am doing and writing at the moment, several times Yafet asked me why I stay away from him for so long. Yafet, this is the result of all those years I have been away from you. I wish them to do more than what I have done and succeed where I have failed! My parents have always a special place in my life. I cannot thank you enough my mom, W/ro Abaynesh Geleta, my father the late Master Technician Endale Annisa, and my elder brother, Abel Endale, for being good and loving family to me. If I possess any good qualities, it is all because of you. My brother, Engineer Ermias Endale, and sister, Tirsit Endale, I owe you a big thank you for your support and love all along. Thank you all for teaching me that all aspects of success in life can be achieved if it is done step by step with endurance, hard work and commitment. I should not finish without expressing my gratitude to all those who helped me in one way or another to successfully complete my PhD study. Thank you for all your support, comfort and the good times I have shared with you. Last but not least, I would like to thank German Academic Exchange Service (DAAD) for the PhD scholarship given to me through Natural Product Research Network for Eastern and Central Africa (NAPRECA). Thank You!

ABSTRACT

Malaria, caused by the protozoan parasites of the genus *Plasmodium,* is a major disease in the tropical and subtropical regions of the world. Out of the yearly 300-500 million clinical episodes, 1.5-2.7 million are lethal. To date, a large number of herbal remedies are used to treat malaria and manage related fever. Nevertheless, efficacies of most of these plants have not been proven or the active components identified. In an effort to address the problem of malaria and the associated complications, six *Pentas* species: *P. bussei, P. lanceolata, P. longiflora, P. micrantha, P. parvifolia* and *P. suswaensis* were phytochemically investigated. Fractionation of the extracts was carried out using a combination of chromatographic methods including column chromatography on oxalic acid impregnated silica gel, preparative High Performance Liquid Chromatography (HPLC), Medium Pressure Liquid Chromatography (MPLC) and sephadex LH-20. Characterization of the pure compounds was done using spectroscopic techniques: mainly Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy (MS) assisted by Infra Red Spectroscopy (IR), Ultra Violet Spectroscopy (UV) and Circular Dichroism (CD). The distribution of the isolated compounds in the roots of the six *Pentas* species was studied using analytical HPLC and TLC.

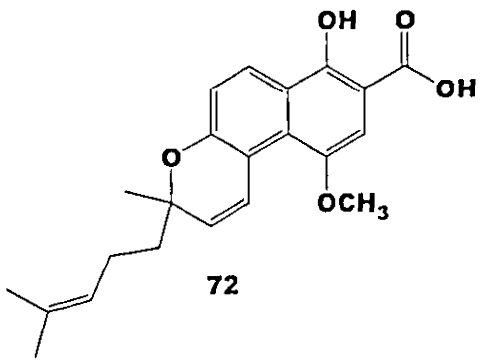
A total of fourty one compounds categorized as naphthalene derivatives, anthraquinones and pyranonaphthoquinones were included in this thesis of which thirty one natural compounds including seven new natural products (**70-73, 78, 82, 86**) and ten new synthetic derivatives (**87-96**). This is the first report on the occurrence of eight anthraquinones and one polyoxygenated naphthalene derivative from the genus *Pentas.*

Four new naphthalene derivatives (**70-73**) were isolated from *P. bussei* and *P. parvifolia*. Anthraquinones occur in the roots of *P. lanceolata, P. suswaensis* and *P. micrantha;* eleven of which including a new anthraquinone, 5,6-dihydroxydamnacanthol (**78**), were isolated from the roots of *P. lanceolata*. The new anthraquinone 5,6-dihydroxydamnacanthol (**78**) was isolated from *P. suswaensis* along with twelve known anthraquonones and anthraquinone glycosides. Two new anthraquinones (5,6-dihydroxydamnacanthol (**78**) and 5,6-dihydroxylucidin-ω-methyl ether (**82**) were isolated from the roots of *P. micrantha* together with six known anthraquinones. Two pyranonaphthoquinones, pentalongin (**33**) and psychorubrin (**80**), were isolated from the roots of *P. longiflora* together with the naphthalene derivative mollugin (**34**). Overall, anthraquinones having carbon (CH_3, CH_2OH, CHO, CH_2OCH_3) substitution at C-2 were found to be the major constituents of *P. lanceolata, P. micrantha* and *P. suswaensis;* whereas pyranonaphthaquinones and naphthalene derivatives were found to be the major constituents *P. longiflora, P. parvifolia* and *P. bussei*.

The plant extracts and the isolated compounds were tested for antiplasmodial activity against chloroquine sensitive (D6) and chloroquine resistant (W2) clones. Cytotoxicity of the pure compounds was also done on MCF-7 human breast cancer cells. Significant antiplasmodial activity (IC_{50}) was observed in the root extracts of *P. lanceolata* [1.33 μg/mL against D6, 2.55 μg/mL against W2], *P. micrantha* [4.00 μg/mL against D6, 3.37 μg/mL against W2], *P. longiflora* [0.93 μg/mL against D6, 0.99 μg/mL against W2], anthraquinones [5-31 μg/mL against D6 and W2], naphthalene derivatives [7.45-44.50 μg/mL against D6 and W2], and pentalongin (**33**) and psychorubrin (**80**) [< 1 μg/mL against D6 and W2]. Rubiadin-3-*O*-primveroside (**52**) showed synergestic effect in

combination with chloroquine *in vitro*. The tested compounds also exhibited different degrees of cytoxicity (LD_{50}) as follows: naphthalene derivatives (**56**, **70-73**) [≥ 22.3 μg/mL] and pyranonaphthoquinones [0.80 μg/mL for pentalongin (**33**) and 0.89 μg/mL for psychorubrin (**80**)]. The excellent antiplasmodial activity observed by the two pyranaphthaquinones (**33**, **80**) is accompanied by comparably high cytotoxicity makes their direct application as antimalarial agents virtually impossible.

Microwave assisted syntheses of ten new (**87-96**) C_4-aryl, C_4-bromide and C_4-chloride substituted analogues of rubiadin (**49**) and rubiadin-1-methyl ether (**51**) were achieved by Suzuki-Miyaura, nickel catalyzed halogen exchange and Sonogashira cross-coupling reactions. Improvement in the *in vitro* antiplasmodial activity (IC_{50}) was observed for 4-phenylrubiadin (**89**) [4.96 μg/mL against D6 and 13.97 μg/mL against W2] and 4-(*p*-methoxyphenyl)rubiadin-1-methyl ether (**91**) [7.55 μg/mL against D6 and 14.2 μg/mL against W2] as compared to the parent anthraquinone.



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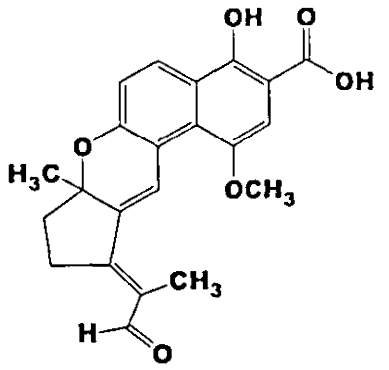

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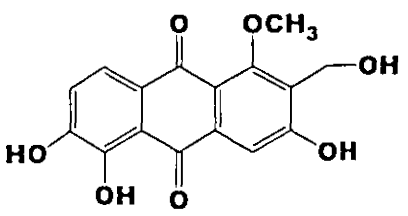

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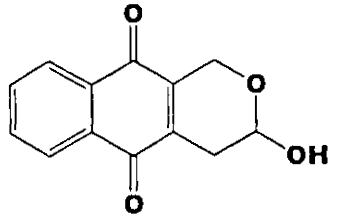

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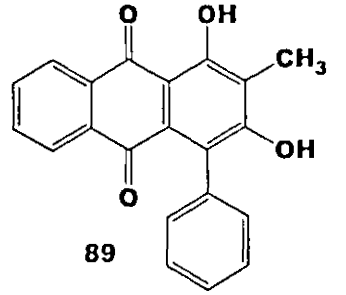

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LIST OF ABBREVIATIONS

ACT	Artemisinin-based Combination Therapy
COSY	Correlation Spectroscopy
CQ	Chloroquine
DEPT	Distortionless Enhancement by Polarization Transfer
ED_{50}	Effective Dose which inhibit 50% of the test organism
ESI-MS	Electron Spray Ionization Mass Spectrometry
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
IC_{50}	50% Inhibition Concentration
MPLC	Medium Pressure Liquid Chromatography
MS	Mass Spectroscopy
NOESY	Nuclear Overhauser and Exchange Spectroscopy
1D NMR	One Dimensional Nuclear Magnetic Resonance
2D NMR	Two Dimensional Nuclear Magnetic Resonance
PTLC	Preparative Thin Layer Chromatography
TLC	Thin Layer Chromatography
OPD	Out Patient Department
UV	Ultra Violet
λ_{max}	Maximum wavelength of absorption
nm	Nanometer
MHz	Mega Hertz
J	Coupling constant
s	Singlet
d	Doublet
dd	Doublet of a doublet
ddd	Doublet of a doublet of a doublet
t	Triplet

CHAPTER ONE

INTRODUCTION

1. General Introduction

Throughout history, mankind has always been interested in naturally occurring compounds from prebiotic, microbial, plants and animals sources. Various plants and insects produce compounds whose taste, color and odor could be used for various purposes. Many naturaly occurring metabolites such as plant hormones, serve a regulatory role, while others function as chemical defenses against pests and herbivores. Some compounds play the role of chemical messengers, such as sex-attractants (pheromones) in insects, terrestrial and marine animals and humans (Bahar, 2008).

Medicinal plants have a long history of use throughout the world. Traditional medicine is the cornerstone of healthcare systems for about 80% of the population residing in the developing countries (Akerele, 1984). Initially these medicines were in the form of crude extracts contained in herbal formulations like teas, tinctures, poultices and powders (Balunas and Kinghorn, 2005). With development of chemical separation techniques and pharmacological testing, the medicines in modern times are in the form of active compounds isolated from the plants, or their synthetic analogues. Currently, the search for new chemotherapeutic agents has been expanded to the whole biodiversity: animals especially insects and arthropods, plants, microorganisms, as well as marine organisms.

In Africa, the use of traditional medicine has persisted over the years while in the west in the last few decades there is an upsurge of interest in traditional medicine and other alternative forms of healthcare (Zhang, 1996; Craker, 1999). Kenya has its share of the

use of traditional medicine (Nyamwaya, 1995). The information about medicinal plants from Kenya has been documented by Kokwaro (2009). Altough proportion of the population which uses traditional medicine is not clearly established, it is estimated that up to 75% of the people in Kenya have used traditional medicine at one time or another (Maneno and Mwaniza, 1991).

Malaria is one of the major endemic parasitic diseases in Kenya, tropical and sub-tropical countries of the world. Its aetiological agents are protozoa of the genus *Plasmodium*. It is responsible for over 1 million deaths globally each year and over 500 million cases with approximately 3.2 billion people are presently at risk. The emergence of chloroquine-resistant strains of *Plasmodium falciparum*, the most deadly species of malaria parasites, the resistance of the vector (*Anopheles* spp.) to insecticides, poverty and lack of quality healthcare, are the main causes for the increase of malaria morbidity and mortality (WHO, 2005a).

Over 80% of malaria deaths occur in Africa and 15% in Asia (Dolabela *et al.*, 2008). In America, 14% of the population is at risk although the mortality is relatively low in this region. Brazil reports approximately 40% of the total number of malaria cases in the Americas, of which almost 99% occurs in the Amazon region, where 12% of the population of the country lives. An increase in the number of cases began in the 1980s and a peak of 610,878 cases was reported in 2000 (Dolabela *et al.*, 2008).

One of the major factors for the increase in the global burden of malaria is the development of resistance of the parasite to most of the available drugs. Chloroquine (CQ) was once considered as the most effective and safe drug, while at the same time

being the least expensive and the most affordable drug for the treatment of malaria in developing world. World Health Organization (WHO) recommended using combination therapy to overcome the development of drug resistance. The drugs that are recommended under this new scheme are too expensive and not affordable to the poor population of the developing world (WHO, 2009).

The global economic burden of malaria is enormous and more prominent in poor countries with inadequate resources. Countries with endemic malaria are estimated to experience losses of economic growth as high as 1.3% per year (WHO, 2009). In addition, malaria causes reduced agricultural productivity, school absenteeism for children, permanent neurological, developmental and other damages which severely curtail economic growth (Sachs and Malaney, 2002).

The resistance of *P. falciparum* to chloroquine is based on the parasites ability to produce gluthathione transferase (GST) enzyme which binds heme more effectively than chloroquine. This assumption is consistent with the observation that resistant parasites have developed a new way of detoxifying heme as opposed to the non-resistant parasites which convert heme to the dimeric haemozoin (malaria pigment) (Egan, 2004). The need for new drugs, preferably with new mode of action, is therefore strongly felt.

1.1 Statement of the Problem

Malaria is a major public health problem in tropical and subtropical regions. Besides causing great suffering and morbidity, it costs up to 2 million lives annually (Njoroge and Bussmann, 2006). There is vast biomedical knowledge on the cause, prevention, treatment and control of malaria. Despite this, malaria remains a public health problem

because of the rapid development of resistance to antimalarial drugs. Increasing *P. falciparum* resistance to chloroquine first, then to sulphadoxine/pyrimethamine (SP) has led East African countries, including Kenya, to revise their treatment policy and adopt an artemisinin-based combination therapy (ACT) as the first line drugs for treatment of uncomplicated malaria (WHO, 2001). However, ACT is expensive and is not readily accessible to the rural population in Kenya. Furthermore, in the event that resistance to ACT spreads, there are no alternative drugs at the moment. Therefore, there is urgent need for alternative, effective and affordable antimalarial drugs.

1.2 Justification of the study

In Africa, indigenous plants still play important role in malaria treatment (Gessler *et al.*, 1994; Benoit-Vical *et al.*, 1998). Taking into account that the existing first line antimalarial drugs were derived from plants, there is still potential of isolating lead compounds from plants, especially from traditional medicinal plants that are empirically used to treat malaria.

In Kenya, there are several medicinal plants including those in the genus *Pentas* that are traditionally used to treat malaria. Koch *et al.*, (2005) evaluated 21 medicinal plants collected from Maasai land of Kenya using *in vitro* antimalarial assays. *Pentas* species were among those showing good antiplasmodial activity (IC_{50} < 10 μg/mL). The compounds responsible for antiplasmodial activity in these plants have not reported. The only phytochemical information on *Pentas* species from Kenya were on *P. longiflora* (El-Hady *et al.*, 2002) and *P. bussei* (Bukuru *et al.*, 2002; Bukuru *et al.*, 2003). It is therefore important that the *Pentas* species from Kenya be investigated for the secondary metabolites and tested for antiplasmodial and cytotoxicity activity. Consequently,

motivated by the screening results of the crude extracts of six *Pentas* species and lack of phytochemical information on most *Pentas* species, we undertook the isolation, characterization, and confirmation of the antiplasmodial activity of secondary metabolites from the *Pentas* species in Kenya.

1.3 Objectives

1.3.1 General Objectives

The overall objective of this study was to isolate and characterize antiplasmodial compounds from *Pentas* species from Kenya.

1.3.2 Specific Objectives

1. To isolate secondary metabolites from *Pentas longiflora*, *P. lanceolata*, *P. suswaensis*, *P. micrantha*, *P. bussei* and *P. parvifolia*.
2. To elucidate the chemical structures of the secondary metabolites isolated from these six *Pentas* species from Kenya.
3. To establish the antiplasmodial activity of the plant extracts and isolated compounds.
4. To establish the cytotoxicity of the secondary metabolites.
5. To undertake structural modification of isolated compounds to improve their antiplasmodial activity.

CHAPTER TWO

LITERATURE REVIEW

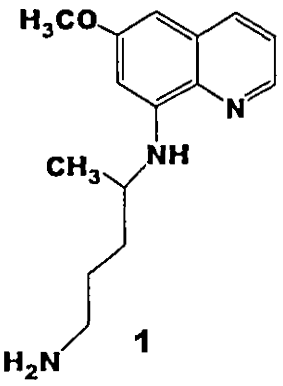
2.1 Malaria Problem

Malaria is a major public health problem in the world and continues to afflict human beings especially the poor. It is endemic in 105 countries within the tropical and sub-tropical regions. Between 300 to 500 million clinical episodes of malaria and 1.5 to 2.7 million deaths worldwide are reported every year. Of these, 90% occur in tropical sub-saharan region of Africa, where malaria is the leading cause of mortality in children under five years of age. Besides young children and pregnant women are among the most affected by the disease (WHO, 2009).

Although effective interventions against malaria are available, the burden persists, largely because most people at risk of contacting malaria infection are either unaware of the existence of interventions or do not have access to these interventions for various reasons, including affordability of the treatment or prevention (WHO, 2005a,b). Consequently, the greatest burden of malarial disease and death is borne by the poor in the developing countries, whose population has the least access to interventions against the disease.

Treatment of malaria is becoming more complicated partly because of the emergence of drug-resistant strains of *P. falciparum*, the parasite that causes the most deadly form of the disease. As a result, there is a great need for new and improved chemotherapy and prophylaxis agents to control malaria. A structurally diverse group of therapeutic agents collectively referred to as oxidant drugs holds the promise of effective treatment of multiple drug-resistant *Plasmodium* parasites (Vennerstrom and Eaton, 1988). These

drugs act to render parasites (or their host cells) more susceptible to attack by oxygen radicals or cause enhanced production of oxygen radicals inside parasitized erythrocytes (Golenser *et al.*, 1991). Some drugs in this class that are currently in use include primaquine (1) and artemisinin (2) (Bates *et al.*, 1990; Hong *et al.*, 1994; Guttman and Ehrlich, 1891).



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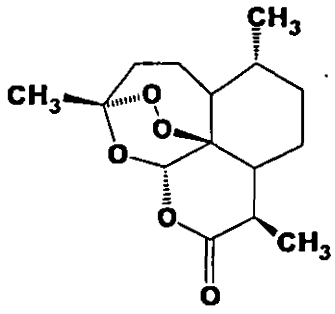

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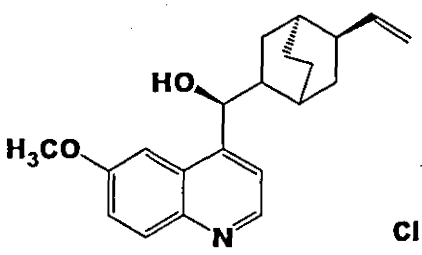

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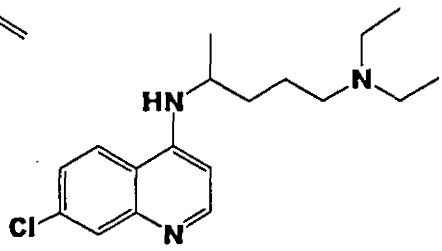

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Most of the drugs used for the treatment of malaria are derived from plants used by indigenous communities in different parts of the world. For example the alkaloid quinine (3), first discovered from the South American plant *Cinchona* species (Rubiaceae), has been used as an antimalarial agent for the past 300 years and has saved many lives during this period (Dewick, 2002). However, natural quinine has been gradually replaced by synthetic drugs such as chloroquine (4) leading decline in the use of quinine (3). However, in recent years, with increasing emergence of drug-resistant strains of malaria, quinine (3) is once again showing promise for use in the treatment of complicated

malaria. Recently, the sesquiterpene lactone artemisinin (2) from the Chinese herbal remedy *Artemisia annua* (Compositeae/Asteraceae) was found to be effective against chloroquine-resistant *P. falciparum* (Dewick, 2002). The active plant ingredients of *Artemisia annua* have served as molecular templates for the development of synthetic antimalarials that are safe and more effective than the parent molecules. The search for other antimalarial lead structures from plants has continued ever since the discovery of quinine (3) and catalyzed by the isolation of artemisinin (2).

2.2 Oxidative Stress in Malaria

During malaria infection, both the host and the parasite are under oxidative stress occasioned by increased production of reactive oxygen species (ROS, such as superoxide anion and the hydroxyl radical) by activated neutropils in the host and during degradation of the hemoglobin in the parasite (Postma *et al.*, 1996). Such oxidative stress lead to tissue damage and are associated with several pathological phenomenon, such as celebral and pulmonary oedema, poor eyesight, atherosclerosis, cardiac ischemies, rheumatic disease and cancer (Bahorun *et al.*, 1994).

ROS are also produced by the host immune system with the intention of suffocating the parasite, as the parasite is highly vulnerable to oxidative burden (Kawazu *et al.*, 2008). At the same time malaria parasites are equipped with anti-oxidant defenses that are meant to establish redox equilibrium for their survival. Such defense mechanisms are the ones targeted in malaria parasite control strategies, for example an anti-malarial like chloroquine (4) is known to act by increasing the production of ROS (Taoufiq *et al.*, 2008).

2.3 Malaria Control

Currently the main approaches to combat the malaria problem include: biological control of malaria mosquito, vaccine development, insecticides and chemotherapy.

2.3.1 Biological control

Biological methods of the vector control use natural enemies of mosquitoes and biological toxins to restrain the vector inhabitants. The major biological control strategies are predators, predominantly fish and the bacterial pathogens *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* (Walker, 2002). Other promising organisms include fungal pathogens, nematodes and the aquatic plant *Azolla* (Walker, 2002; Walker 2007). Larvivorous fish have been used for mosquito control for several years of which *Gambusia*, *Guppies*, *Tilapia* and *Carp*, among others, feed on the aquatic larval stages thereby decreasing the abundance of mosquitoes (WHO, 2003). Fish are a safe and inexpensive malaria vector control alternative that can be easily introduced in defined breeding sites (WHO, 2003; Howard *et al.*, 2007).

Several plants are used as repellents of mosquitoes. Some involve the use of either live-potted plants or thermal expulsion from a source of heat (Seyoum *et al.*, 2003). Products of the neem tree shown to exhibit a wide range of effects on mosquitoes. Neem oil extracted from its seeds has repellent properties and has been effectively used as a biolarvicide for anopheline mosquito control (Okumu *et al.*, 2007). *Citronella* is among the most commonly used herbal insect repellents. Its efficacy is comparable to that of the chemical repellent DEET (*N,N*-Diethyl-3-methylbenzamide), but it provides shorter protection time (Fradin, 1998). Protozoa, nematodes, fungi and the aquatic plant *Azolla*

have all shown promise as a means of controlling mosquito populations under experimental conditions (Pérez-Pacheco *et al.*, 2005).

2.3.2 Vaccine development

Relatively few malaria vaccine candidates have progressed to clinical and field trials to date. Much of the research activity over the past 15 years has focused on the identification of unmodified parasite antigens to be formulated in traditional adjuvants such as alum. The approach as new approaches to producing modified antigens together with the advent new strategies such as DNA vaccines and novel adjuvants for human use. In this regard scientists continue their effort to search for effective and affordable malaria vaccine (Engers and Godal, 1998).

The ultimate goal of developing an effective vaccine against malaria is to devise a method for *in vitro* propagation of the parasites (Trager and Jensen, 1976). The main use of cultures in relation to development of malaria vaccines is in identifying target antigens for both the asexual erythrocytic stages and for those of the sexual stages (Kaslow *et al.*, 1992). They have also been used for *in vitro* assessment of immunity, especially to test for the antibodies that inhibit merozoite invasion. In addition, the cultures have been used to supply gametocytes to infect mosquitoes. The mosquitoes in turn are used for both studies on transmission blocking immunity and to infect volunteers in clinical vaccination trials.

2.3.3 Insecticides

Since the 1990s, insecticide-treated bed nets (ITNs) have been regarded as the most powerful malaria vector control tool. Two categories are available: conventional insecticide treated nets and long lasting insecticide treated nets (LLTNs). Only pyrethroid insecticides are recommended for use in ITNs. Concern about the sustained effectiveness of ITNs due to pyrethroid resistant vectors was sparked by a study in Benin where ITNs lost their efficacy (Guessan, 2007). Twelve insecticides are recommended by WHO in vector control for indoor residual spraying (IRS), with DDT and pyrethroids thought to be the most cost-effective. The recommended insecticides are broadly classified in to four: Organochlorine, Organophosphates, carbamate and pyrethroid. DDT is the only approved insecticide from Organochlorine class. Organophosphate insecticides constitute: Fenitrothion, malathion, and pirimiphos-methyl. Carbamte class of insecticides constitute: propoxure and bendiocarb. Pyrethroid class of insecticides constitute: alpha-cypermethrin, cyfluthrin, deltamethrin, etofenprox, lambda-cyhalothrin and bifenthrin (WHO, 2008). Resistance to DDT and pyrethroids is widespread and cross-resistance between these chemical classes severely limits the choice of insecticide (WHO, 2008).

2.3.4 Chemotherapy

Chemotheraphy continues to be the major component of malaria control and has been adopted by the World Health Organization as a sustainable and realistic approach (Gupta and Singla, 2007).

2.3.4.1 Antimalarial drugs

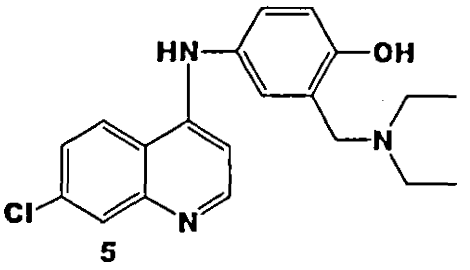
Some of the existing antimalarial drugs are described below in relation to their chemical structure and biological activity.

2.3.4.1.1 *Cinchona* alkaloids

The first antimalarial drug is quinine (**3**), an alkaloid derived from *Cinchona* bark. It has a chemical structure composed of the quinoline ring (with a methoxy group at C-6 position), the quinclidine complex attached with the vinyl group and the connecting link in the form of a hydroxylated methylene group. However, due to the problem of drug resistance and undesirable side effects, it is now only used for treating mutltiple drug-resistant and severe *P. falciparum* malaria (Ernest and Mokuolu, 2005). The mechanism of resistance to quinine (**3**) is still not understood (Foley and Tilley, 1998).

2.3.4.1.2 4-Aminoquinolines

Among the several 4-aminoquinolines available for malaria theraphy, chloroquine (**4**) proved to be the most effective. Amodiaquine (**5**) has a structural formula in which the alkylamino side chain is replaced by anilion group. Its antimalarial activity is equal to that of chloroquine (**4**) but amodiaquine (**5**) appears to be marginally more active on chloroquine resistant strains of *P. falciparum*. The mode of action of chloroquine (**4**) seems to be related to the accumulation of this weak base in the acidic lysosome that binds to ferriprotoporphyrin-IX, thereby preventing detoxification of the parasite by polymerization and thus killing the parasite (Bray, 1999). It acts through accumulation inside the acidic vesicles of the parasite raising the vesicular pH and interfering with degradation of hemoglobin by parasitic lysosomes (Gupta and Singla, 2007).



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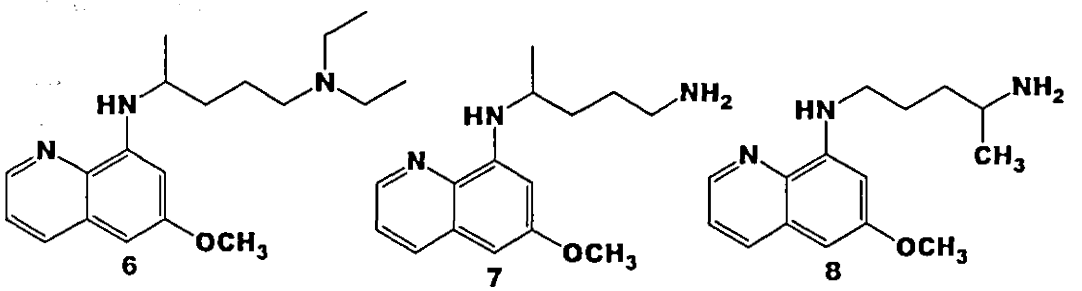
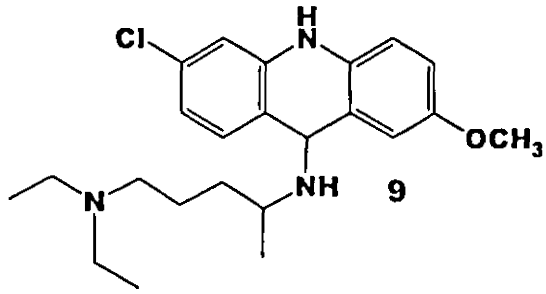
2.3.4.1.3 8-Aminoquinolines

The search for effective synthetic antimalarials led to replacement one of the methyl groups of methylene blue by the dialkylaminoalkyl side chain which led to the preparation of pamaquine (6). In this first synthetic antimalarial 6-methoxyquinoline is combined with the basic side chain. Other antimalarial drugs in this category include premaquine (7) and quinocide (8) (Grewal, 1981).



2.3.4.1.4 9-Aminoacridines

The introduction of the basic dialkyl aminoalkyl chain in the acridine nucleus resulted in the preparation of mepacrine (9) (Valdés, 2011). Mepacrine (9) contains the same alkyl side chain as Chloroquine (4) but differs from the latter in having acridine skeleton instead of quinoline nucleus and lack a methoxy group. It is obsolete today and not available in the market.



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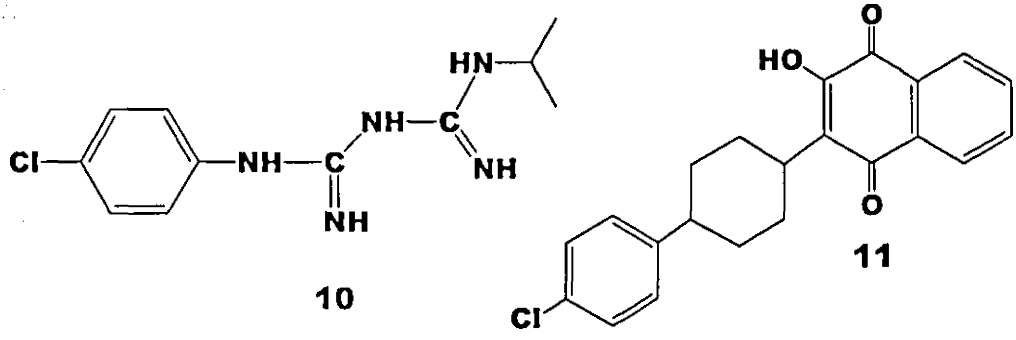
2.3.4.1.5 Biguanides

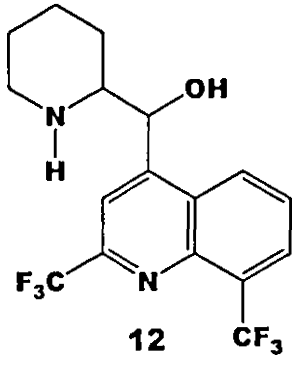
Analogues where the phenyl ring linked to a simple isopropylamino group (-NH-$CH(CH_3)_2$) through two amidine groups were shown to be active. The highest antiplasmodial activity was found in proguanil (**10**) in which a biguanide chain has a chlorophenyl ring and a simple alkyl group. Proguanil (**10**) acts by inhibiting the plasmodial dihydrofolate reductase (DHFR) enzyme (Gupta and Singla, 2007). The drug Malarone® is a combination of proguanil (**10**) and atovaquone (**11**) released in 1998. Atovaquone (**11**) acts by inhibiting parasite mitochondrial electron-transport (Robert *et al.*, 2001).



2.3.4.1.6 Quinoline methanols

Mefloquine (**12**), structurally related to quinine (**3**), was effective against malaria resistant compared to other forms of treatment when it was first introduced and because of its long half life was a good prophylactic. Neverthless, its use has declined due to

resistance developed and undesirable side effects. Its long half-life (14-21 days) has contributed to the rapid development of resistance (Robert *et al.*, 2001).



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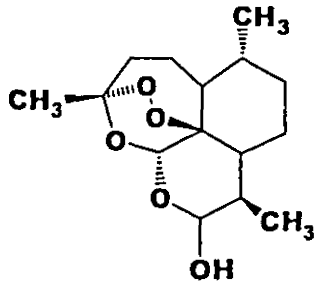
2.3.4.1.7 Artemisinins

The artemisinin group of compounds includes artemisinin (**2**), isolated from *Artemisia annua*, and its semisynthetic derivatives, the reduced lactol, dihydroartemisinin (**13**), the oil-soluble artemether (**14**) and arteether (**15**) and the water-soluble derivative artesunate (**16**) (Bray *et al.*, 2005). All of which are effective against both asexual and sexual blood-stage parasites. Their mode of action is mediated by a unique structural component, the endoperoxide bridge. The target is controversial but recent evidence suggests that an Fe^{2+} activated form of the drug potently inhibits PfATP6, a key parasite Ca^{2+} transporter in the parasite (Eckstein-Ludwig *et al.*, 2003).



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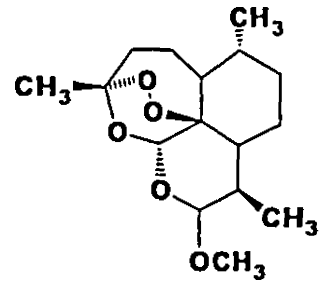

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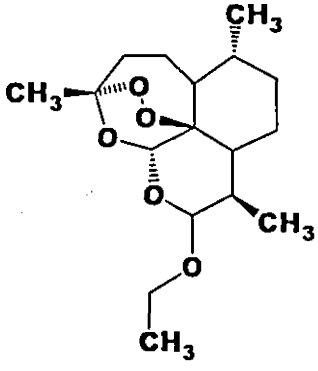

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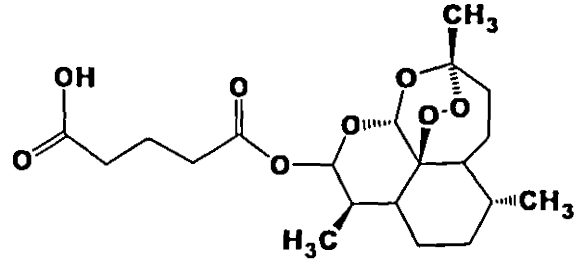

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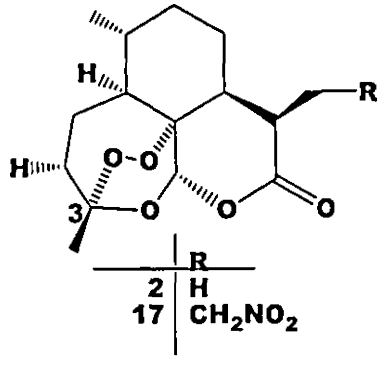
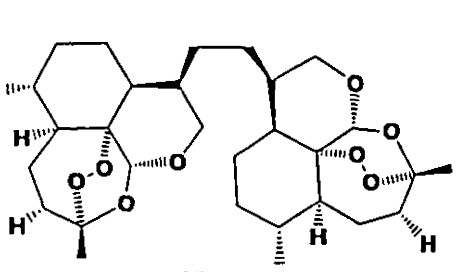
Artemisinin (2) and its derivatives (**13-16**) are toxic to malaria parasites *in vitro* at sub-nanomolar concentrations, whereas micromolar concentrations are required for toxicity to mammalian cells (Robert *et al.*, 2001). One reason for this selectivity is the enhanced uptake of the drug by the parasite: *P. falciparum*-infected erythrocytes concentrate (^{3}H)-dihydroartemisinin and (^{14}C)-artemisinin to more than 100-fold higher concentration than do uninfected erythrocytes (Robert *et al.*, 2001). Furthermore, artemisinin is hydrophobic and localized in specific parasite membranes (Meshnick *et al.*, 1996). Deoxyartemisinin lacking endoperoxide bridge is devoid of antimalarial activity and hence this peroxide function within the parasite is assumed to be the key factor of the pharmacological activity (Klayman, 1985).

Artemisinin and its derivatives appear to be the best alternative for the treatment of severe malaria (Robert *et al.*, 2001) and artemether (**14**) has been included in the WHO list of essential drugs for the treatment of severe multiple drug-resistant malaria (WHO, 2008). The high antimalarial activity of artemisinin (**2**) has prompted extensive efforts to synthesize more potent analogs. Artemisinin analogs (**13-16**) modified at C-3 and C-13 were prepared by Han *et al.* (2001) from artemisinic acid. Among these analogs, 13-nitromethylartemisinin (**17**) produced activity comparable to artemisinin (IC_{50} 0.68 ng/mL) while 13-(1-Nitroethyl)artemisinin was 20-fold less active.



Posner *et al.* (2003) examined antimalarial potencies of dimers **18-20** against chloroquine-sensitive *P. falciparum* (NF 54) parasites. The most potent dimer (**18**) (ED_{50}= 2.4nM), while isonicotinate N-oxide dimer (**20**, ED_{50}= 0.53nM) was more effective than clinically used sodium artesunate (ED_{50} = 1.5nM) via both oral and intravenous administration (Posner *et al.*, 2003).



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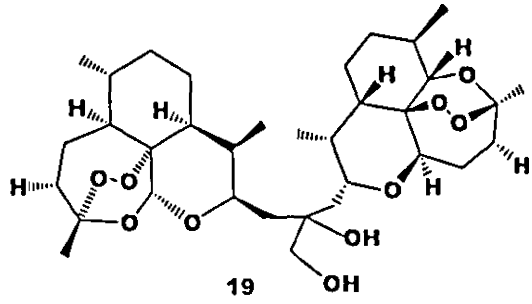

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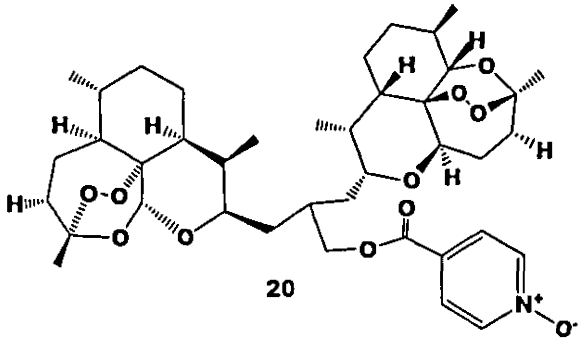

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2.3.4.2 Drug resistance

In vitro test have been applied to a large number of clinical isolates to verify the prevalence of drug-resistant malaria (Ringwald *et al.*, 1996). Cultures have been useful in attempts to determine the genetic and biochemical basis for drug resistance. Egan (2004) argue that the resistance of *P. falciparum* to chloroquine is based on the parasites ability to produce gluthathione transferase (GST) enzyme which binds heme more effectively than chloroquine. This assumption is consistent with the observation that resistant parasites have developed a new way of detoxifying heme as opposed to the non-resistant parasites which convert heme to the dimeric haemozoin (malaria pigment) (Egan, 2004). Future research will be directed towards addressing this issue as it is now possible to produce sufficient quantities of the enzyme using *E. coli*, isolate, purify it and then conduct binding studies (Abegaz *et al.*, 2007).

2.3.4.3 Ethnopharmacological approach

A number of studies have applied an ethnobotanical approach to investigate plants from malaria endemic areas in the search for novel antimalarial drugs (Gessler *et al.*, 1994; Kraft *et al.*, 2003; Soh and Benort, 2007). The most prominent examples are the quinoline-based antimalarials modelled on quinine (3), isolated from the bark of the Peruvian *Cinchona* tree; and the endoperoxide-based antimalarials, artemisinin (2), isolated from the Chinese herbal medicine *Artemisia annua* (Camacho *et al.*, 2000). In light of this historic success and the fact that most indigenous people living in malaria endemic areas use traditional medicines to fight the disease, there is every possibility that ethnopharmacological approaches could lead to new antimalarial agents (Phillipson and Wright, 1991a). The development of sustaining continuous cultures of *P. falciparum* (Trager and Jensen, 1976) and subsequent *in vitro* assays (Desjardins *et al.*, 1979; Geary *et al.*, 1983; Makler *et al.*, 1995) made it possible to screen plant extracts for antiplasmodial activity and use bioassay-guided fractionation to isolate active principles (Schwikkard and Van Heerden, 2002).

The preferred and most effective treatments for malaria today are artemisinin-based combination therapies (ACT). The current cost of a three-day course of drugs containing artemisinin is US$2.40, which places it out of reach for people in many countries where the disease is most prevalent (Handbook of Best Practices: Executive Guide/CS 49, 2009). Reducing the price would make the treatment more widely accessible. Artemisinin (2) is currently extracted from the worm wood plant, which is supplied by farmers in Vietnam and China (and more recently, Africa). Seasonality and availability of the plant contribute to the high price of the drug. Plant sources of the chemical are variable and

crop shortages contribute to increased cost. Chemical synthesis of the molecule would require 30 to 40 steps and is therefore impractical on a commercial scale (Martin *et al.*, 2003).

2.3.4.4 Iron chelation therapy

A considerable number of iron (III) chelators, designed for purposes other than treating malaria, have antimalarial activity *in vitro*, apparently through the mechanism of withholding iron from vital metabolic pathways of the intra-erythrocytic parasite. Several of these agents also have antimalarial activity in animal models of plasmodial infection. Evidence is now available that iron chelation therapy with desferrioxamine (DFO) has clinical activity in both uncomplicated and severe malaria in humans. It has been suggested to further advance knowledge on the iron metabolism of the malaria parasite in order to develop iron chelators specifically designed for the treatment of malaria (George *et al.*, 1999).

2.4 Artemisinin Combination Theraphy (ACT)

The principle of combination theraphy was derived from the treatment of tuberculosis, leprosy and bacterial infections where drugs are combined to stem the risk of resistance development. Combination therapy to treat malaria uses two or more blood schizonticidal drugs with independent mode of actions and different biochemical targets in the parasite. The combination therapy is classified into Artemisinin combination theraphy (ACT) and Sulfadoxine/Pyrimethamine (SP) based combinations. The current WHO recommended treatment policy include artemisinin combination theraphy (ACT). As of 2004, 32 countries adopted one of these WHO combination therapies. About 14 countries in Africa have adopted the artemisinin combination theraphy as first line or second line drug of

which Kenya is one of them. The rate at which malaria parasite is becoming resistant to many drugs with increasing treatment failure and increasing case fatality, has necessitated a therapeutic paradigm shift from monotheraphy to combination theraphy. This strategy is likely to become the bride to be embraced by many more countries in the near future (Ernest and Mokuolu, 2005; WHO, 2008).

2.5 Quinones in malaria theraphy

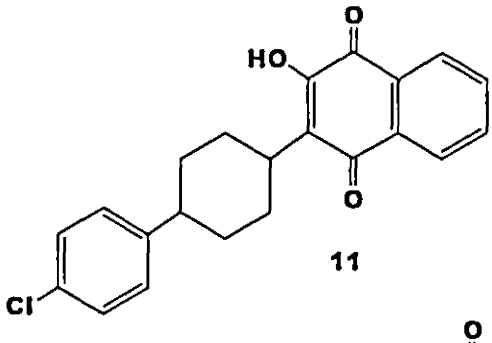
The extensive use and efficacy of the plant based drugs quinoline and artemisinin antimalarials led scientists to explore more on the two class of compounds. Several quinone antimalarials have also been investigated. The chemistry and biochemistry of synthetic and naturally occurring antimalarial quinones is summarized below.

2.5.1 Naphthoquinones

The naphthoquinone derivative, atovaquone (**11**), was reported as an effective antimalarial drug against the multiple drug-resistant parasite with a novel mechanism (Fry and Pudney, 1992). It was demonstrated that certain 2-hydroxy-3-alkyl-naphthoquinones inhibtited the growth of *P. vivae* by targeting the respiratory and carbohydrate cycles in the parasite (Wendel, 1946). Following these reports, several clinical trials were conducted on quinones between 1940's and 1960's with little success. The lack of success could be attributed to two main factors: the poor absorption and rapid metabolism of those compounds within the human body (Fieser, 1948). In retrospect, these assays were incapable of predicting the antimalarial efficacy of the quinones in humans. The advent of *in vitro* test systems using human parasites (*P. faciparum*) afforded opportunities to conduct more meaningful studies on the relationship between chemical structures and clinical activity. This resulted in a new phase of reinvestigation

of antimalarial activity of naphthaquinones towards *P. falciparum* in the 1980's and which confirmed several quinones as being active towards *P. falciparum* (IC_{50} < 1 nM). However, only atovaquone (**11**) was formed to be non toxic to human liver microsomes (Hudson *et al.*, 1991). Atovaquone (**11**) inhibits mitochondrial electron transport selectively at the level of ubiquinone-cytochrome oxidoreducatse (Fry and Pudeny, 1992) as well as causing the collapse of the electro-potential across the micochondrial membrane of malaria parasite (Gupta and Singla, 2007). Unfortunately, this compound was found to be metabolically unstable and despite the high activity against malaria parasites, it could not be used for long time as a single agent. In fact, when used as a single agent resistance to atovaquinone (**11**) rose rapidly prompting its combination with proguanil (**10**) and marketed under trade name Malarone® (Vaidya and Mather, 2000).

Phytochemical analysis on rootbark of *Kigelia pinnata* led to the isolation of antiplasmodial naphthoquinone, 2-(1-hydroxyethyl)naphtho(2,3-*b*)furan-4,9-dione (**21**) (IC_{50} 627 nM (K1), 718 nM (T9-96) strains of *P. falciparum*) and isopinnatal (**22**) (Weiss *et al.*, 2000). The mode of action of these naphthoquinones appears to be through the inhibition of mitochondrial electron transport and respiratory chain by reducing oxygen consumption similar to that of atovaquone (**11**). The lower activity of plumbagin (**23**, IC_{50} = 0.27 μM) isolated from the roots of *Nepenthes thorelii* than compound **24** was suggested to be due to its annealed ring that decreases electron movement. Its possibility to function as an electron carrier or to trigger a radical formation at the quinone structure is reduced by the presence of an hydroxyl group. This prevents a possible induction of a parasitocidal oxidative stress resulting in lower efficacy (Likhitwitayawuid *et al.*, 1998a).



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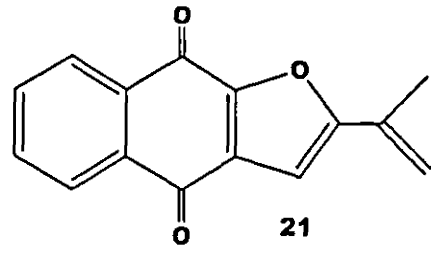

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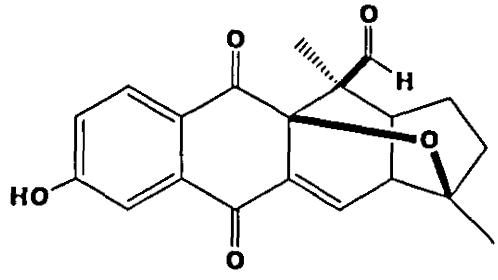

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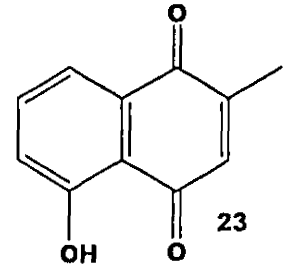

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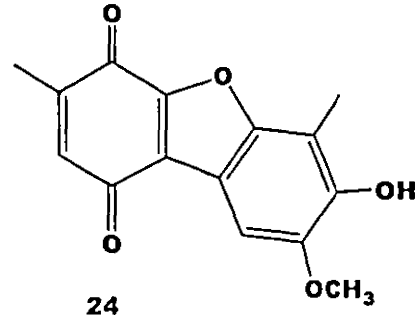

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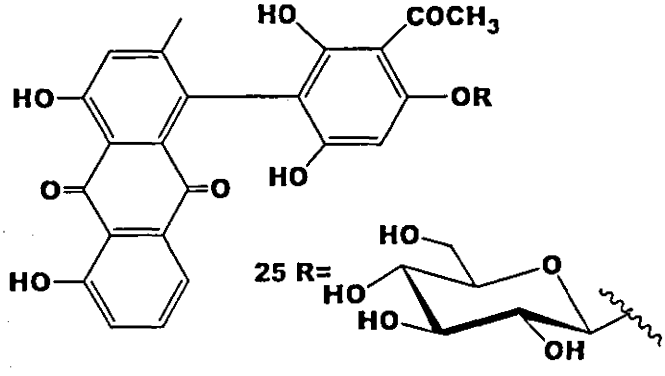
2.5.2 Anthraquinones

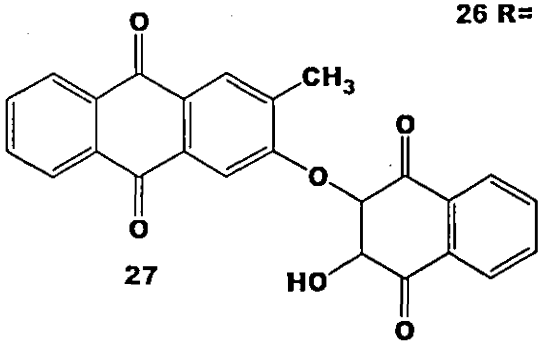
The biological activities of anthraquinone and anthrones are very diverse. These compounds are very reactive and have broad pharmacological activities (Teuscher and Lindequist, 1994). Anthrones can easily form anthrone anion and these anions can lead to anthrone radicals and hydroxyl radicals (Hayden *et al.*, 1994). Anthrones have several effects on mitochondria like inhibition of oxygen uptake or inhibition of ATP (Fuchs *et al.*, 1990). Because of the toxicity of anthrones and anthraquinones, different organisms use them for defense. In insects, anthraquinones may function as defensive devise against various natural enemies. Because of their antimicrobial acitivty they protect insects from

attack by bacteria and fungi (Izhaki, 2002). Anthraquinones also show antiviral effects (Barnard *et al.*, 1992; Semple *et al.*, 2001).

The phenylanthraquinones demethylknipholone glycoside (**25**) and knipholone (**26**) isolated from *Bulbine frutescens*, showed antiplasmodial activities (IC_{50} 0.67 and 0.41 µg/mL, respectively), whose activity appears to be associated essentially with the entire molecular array of a phenylanthraquinone including the stereogenic axis (Abegaz *et al.*, 2002). Newbouldiaquinone A (**27**), a naphthaquinone-anthraquinone pigment coupled via an oxygen bridge, isolated from *Newbouldia laevis*, moderately suppressed growth of *P. falciparum*, *in vitro* (Eyong *et al.*, 2006). Laurent *et al.* (2006) isolated xestoquinone (**28**) from marine sponge, *Xestospongia*, which inhibited Pfnek-1 (IC_{50} 1.1 µM), but was inactive towards PfPK7 and PfGSK-3.153.



26 R= CH_3



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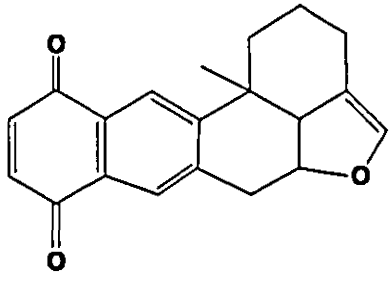

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2.6 Mechanism of action of quinones as antimalarials

Hudson (1993) postulated that in mammalian cells ubiquinone-linked dehydrogenase was involved in energy generation *via* the synthesis of ATP, while oxidation of dihydro-ortate to orate with dihydro-orotate dehydrogenase (DHOD) is central to pyrimidine biosynthesis. Since malaria parasite are homolactate fermenters, the involvement of ubiquinone with ATP formation should be minimal. In contrast, DHOD is a key enzyme in plasmodial metabolism as malaria parasites, unlike mammals rely excusively on *de novo* synthesis of pyrimidines to satisfy their nucleoside requirements (Gutteridge *et al.*, 1979). Studies have shown that the plasmodial enzyme is susceptible to inhibition by quinones which disrupts pyrimidine biosynthesis without altering ATP levels (Hammond *et al.*, 1985).

Atovoquone (**11**) was found to bind selectively and strongly to the ubiquol-cytochrome reductase region of the respiratory chain (Complex III). The compound was found to be 2000 times more active in this system (ED_{50} 1.7 nM) than the corresponding one from rat liver mitochondria (Fry and Pudney, 1992). This suggests that the selective antimalarial effect of atovoquone (**11**) resulted from specific blockage of pyrimidine biosynthesis *via* inhibition of the parasite respiratory system at Complex III. This mechanism of action is different from the other antimalarial agents.

2.7 Synergism and Positive Interactions

Several plants belonging to over 160 families were reported to be tradionally used for treatment of malaria (Rasoanaivo *et al.*, 2011). In most cases, it has not been possible to isolate active constituents from active extracts. Several explanations have been proposed for this, such as the poor quality of ethnopharmacological studies, plant material

processing, preclinical laboratory protocols which are often very different from local practices, inadequate fractionation processes, degradation of active constituents during fractionation and poor biological models to demonstrate activity. Neverthless, one hypothesis that has not been extensively exploited in conventional antimalarial therapy is the synergistic interaction or multifactorial effects between compounds present in herbal extracts (Gilbert *et al.*, 2003).

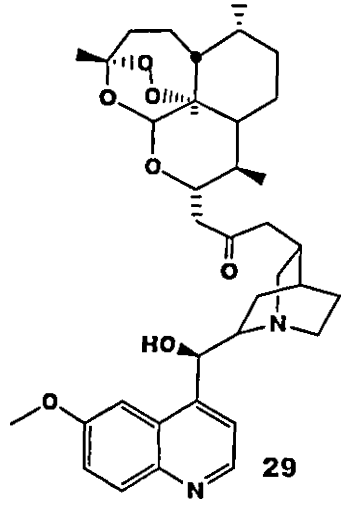
Synergy or potentiation means that the effect of the combination is greater than the sum of the individual effects. One definition of what counts as significant synergy is for at least a two-fold increase in activity (Σ FIC < 0.5) (Fidock *et al.*, 2004). In pharmacokinetic synergy, substances with little or no activity on the causative agent assist the main active principle to reach the target by improving bioavailablity or by decreasing metabolism and excretion. Other positive interactions include complementary mechanisms such as immunomodulation, reversal of resistance and modulation of adverse effects.

2.7.1 Pharmacodynamic synergy

Pharmacodynamic synergy results from enhancement of action when two drugs are directed at a similar receptor target or physiological system. A good example of this process can be seen in the constituents of *Senna*. Sennocide A and C, separately have similar laxative action but a mixture of these two compounds in a ratio of 7:3 doubles the laxative effect (Kisa *et al.*, 1981).

In view of the reported antimalarial synergism between artemisinins with other endoperoxides and quinine, John *et al.* (2007) examined the antimalarial efficacy of a

covalently linked artemisinin-quinine hybrid (**29**) in which the vinyl functionality of quinine was modified to allow for the attachment of dihydroartemisinin. The study found that artemisinin-quinine hybrid (**29**) has superior activity to that of artemisinin (**2**) alone, quinine (**3**) alone, or a 1:1 mixture of artemisinin (**2**) and quinine (**3**). The artemisinin-quinine hybrid (**29**) had potent antimalarial activity in which *P. falciparum* 3D7 was inhibited by much lower concentrations of the hybrid than of quinine (**3**) or artemisinin (**2**) alone, suggesting that the actions of both moieties were conserved.



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2.7.2 Pharmacokinetic synergy

This results from alteration of the process of drug absorption, distribution, biotransformation (metabolism) or elimination. An example of pharmacokinetic synergy is the simultaneous ingestion of vitamin C to improve the absorption of iron (Teucher *et al.*, 2004).

2.8 Plants and natural products in malaria chemotheraphy

Plants have been used in the fight against malaria since time immemorial. In Kenya and other developing countries, the list of traditional medicinal plants used in treatment of

malaria is documented in several books and other publications (Kokowaro, 2010; Koch *et al.*, 2005). In addition to their use in traditional medicine, some plants *Chinchona* species and *Artemisia annua* are sources of some commonly used antimalarial drugs like quinine (3) and artemisinin (2). The synthetically modified analogues of these compounds have also contributed to modern treatment of malaria.

A survey conducted on the use of herbal medicines in the treatment of malaria in central Kenya revealed fifty eight species distributed in fifty four genera and thirty three families. Of these, the Rubiaceae had the highest number of representatives (Grace and Rainer, 2006).

2.9 Botanical Information

2.9.1 Rubiaceae

It is one of the six largest angiosperm families according to the number of genera and species. Less than 20% of the genera are herbaceous while twenty nine of the thirty eight currently accepted tribes are predominantly woody (Robbercht, 1988). In East Africa, there are 100 genera and about 600 species belonging to Rubiaceae (Verdcourt, 1976).

Several rubiaceous plant species are widely used in African traditional medicine (Kokowaro, 2010). *Rubia cordifolia* is widespread in tropical and southern Africa. In the Cape Province (South Africa), a decoction of the leaf and root is used as a remedy for pleurisy and other inflammatory conditions of the chest (Watt and Breyer-Brandwijk, 1962). Evaluation of selected medicinal plants in Kenya and some parts of Africa indicate that the family Rubiaceae is one of few families that have attracted attention for phytochemical analysis due to the low toxicity and significant antiplasmodial activity of

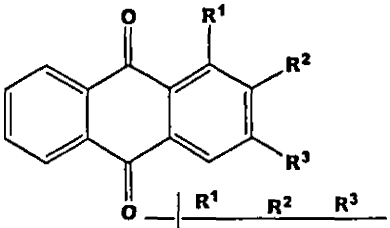
the crude extracts. Table 2.1 summarizes some of the activity shown by selected plants from Rubiaceae.

Table 2.1: *In vitro* antiplasmodial test results of selected medicinal plants from Rubiaceae family.

Plant Species	Plant part	Strain	IC_{50} value		Reference
Pentas longiflora	root	K39	20.4 ± 0.1		Wanyoike *et al.*, 2004
		V1/s	25.8 ± 2.3		
		M24	14.1 ± 1.0		
	leaves	K39	24.3 ± 1.1		
		V1/s	24.3 ± 1.1		
		M24	24.3 ± 1.1		
Pentas bussei	whole plant part	K1	Water	> 100	Irungu *et al.*, 2007
			CH_2Cl_2	ND	
			CH_3OH	35.2	
		NF54	Water	> 100	
			CH_2Cl_2	ND	
			CH_3OH	40.5	
Tarena greveolens	stem bark	K1	Water	> 100	Irungu *et al.*, 2007
			CH_2Cl_2	ND	
			CH_3OH	40.7	
		NF54	Water	> 100	
			CH_2Cl_2	ND	
			CH_3OH	52.6	
Canthium setosum	areal part	3D7	CH_3OH	6.21 ± 1.10	Weniger *et al.*, 2004
			CH_2Cl_2	2.77 ± 2.29	
		K1	CH_3OH	>20	
			CH_2Cl_2	4.80 ± 0.05	
Pavetta corymbosa	areal part	3D7	CH_3OH	>20	Weniger *et al.*, 2004
			CH_2Cl_2	>20	
		K1	CH_3OH	17.50 ± 2.25	
			CH_2Cl_2	5.54 ± 0.79	

Several plants of the family have been subjected to phytochemical analysis. *Morinda lucida* is widely used in Africa as antiasthma, antibronchitis and antiseptic (Boullard, 2001), diuretic, purgative and antipyretic, antimalarial, astringent and antiulcer (Abbiw, 1990). It also exhibits hypotensive and sedative effects (Barre and Wirtheimer, 1962). However, the stem wood alcoholic extract exhibited a remarkably strong hypertensive effect (Sandberg and Cronlund, 1982). The plant extract also inhibited *Plasmodium*

falciparum significantly *in vitro* (Gbeassor *et al.*, 1989). The antimalarial (Koumaglo *et al.*, 1992; Sittie *et al.*, 1999) and the antileishmanial (Yff *et al.*, 2002) activities of the stem bark and the roots of *Morinda lucida* have been attributed to the constituent anthraquinones (30-32).



	R^1	R^2	R^3
30	H	CHO	OH
31	OCH_3	CHO	OH
32	OCH_3	CH_3	OH

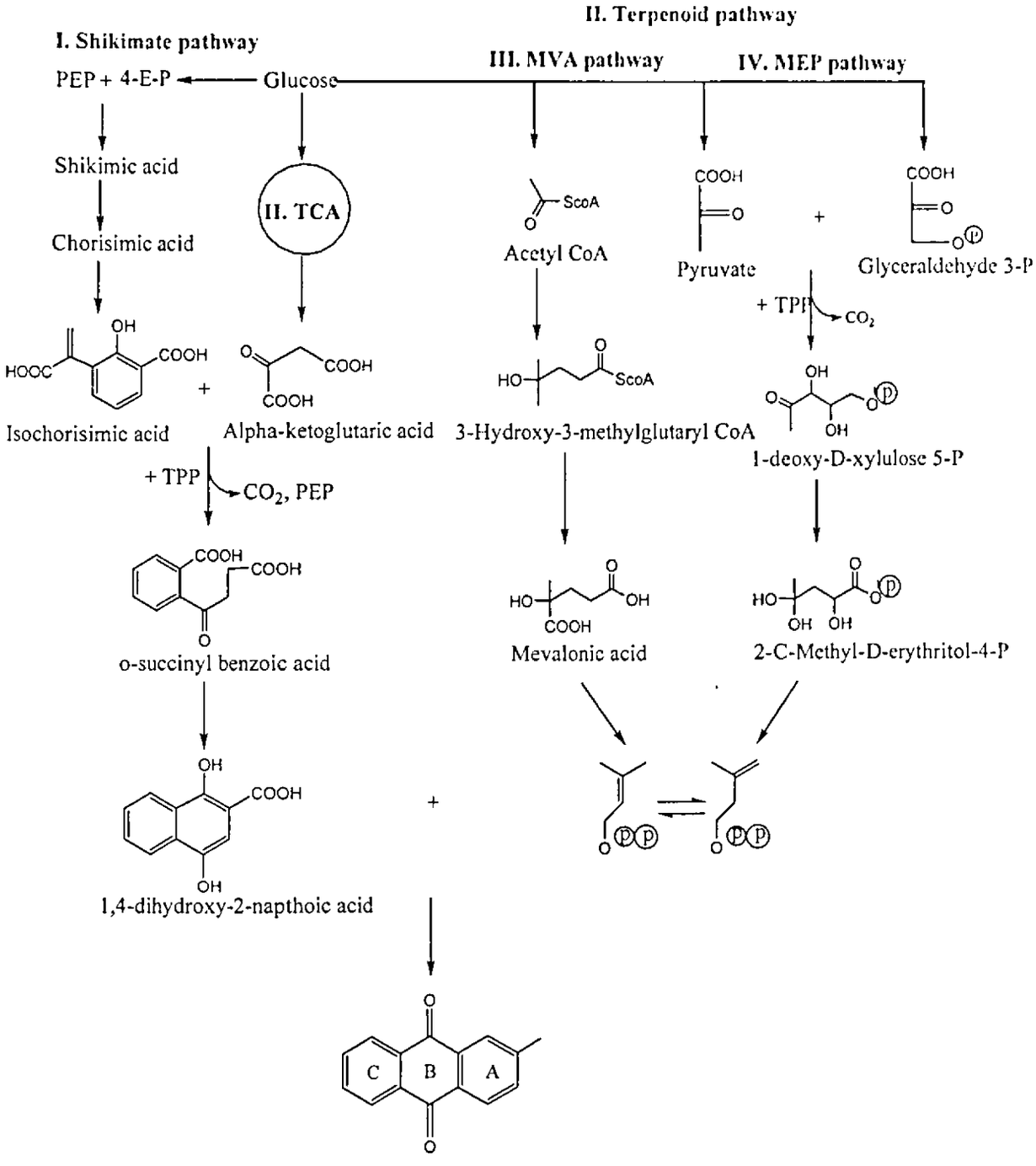
Pentanisia prunelloides Walp is widely used medicinal plant. In southern Africa, the plant has been used for a long time for the treatment of rheumatism, fever, venereal diseases, and as an anthelminthic agent in infants. It is also used for relieving pain in the chest (Watt and Breyer-Brandwijk, 1962). The aqueous, ethanolic and ethyl acetate extracts of the leaves and roots of *Pentanisia prunelloides* showed cyclooxygenase-1 inhibition in an anti-inflammatory assay, together with inhibition of replication of the influenza A virus (Yff *et al.*, 2002). A decoction of the plant also exhibited direct stomach muscle relaxation activity on rat uterus and ileum preparation (Kaido *et al.*, 1997).

2.9.2 Biosynthesis of anthraquinones in Rubiaceae

Anthraquinones are secondary metabolites occurring in bacteria, fungi, lichens, and higher plants (Thomson, 1976; Muzychina 1998). In higher plants, they are found in many families including Verbenaceae (Muzychina, 1998), Bignoniaceae (Burnett and Thomson, 1967), Rhamnaceae (Tripathi *et al.*, 1979), Leguminosae (Bhattacharjee, 2000)

and Rubiaceae (Burnett and Thomson, 1968). Several quinones (Itokawa *et al.*, 1993; Koyama *et al.*, 1992) and anthracene derivatives have been reported from Rubiaceae (Chang *et al.*, 2000).

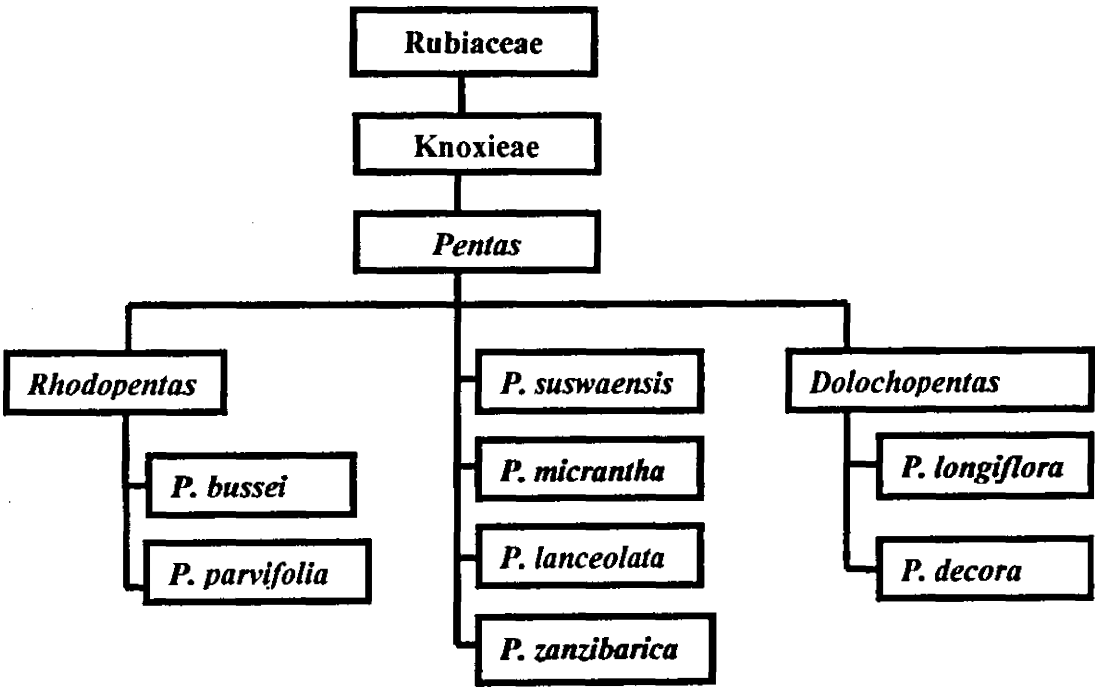
It has been established that the anthraquinones originate from a variety of precursors and pathways (Inouye, 1988). There are two main biosynthetic pathways leading to anthraquinones in higher plants: the polyketide pathway (Van den Berg and Labadie, 1989) and the chorismate/O-succinylbenzoic acid pathway (Leistner, 1985), the latter occurs in the Rubiaceae (Scheme 1). Rings B and C are derived from shikimic acid, α-keto glutarate *via* o-succinyl benzoate, whereas ring A is derived from mevalonic acid, possibly *via* isopentenyl pyrophosphate (IPP)/3,3-dimethylallyl pyrophosphate (DMAPP) (Han *et al.*, 2001).



Scheme 2.1: Biosynthetic pathway leading to anthraquinones in Rubiaceae (Han *et al.*, 2001): E-4-P=erythrose 4-phosphate, P=phosphate residue, PEP=phosphoenolpyruvate, TCA = tricarboxylic acid, TPP=thiamine diphosphate

2.10 *Pentas*

The systematics of the genus *Pentas* species, focusing on the Kenya species, is summarized in Figure below (Kårehed and Bremer, 2007). The genus comprises about 40 species, widely distributed throughout tropical Africa from West Africa and Somali Republic to Angola and Natal (South Africa), also in tropical Arabia, Madagascar and Comoro Islands (Verdcourt *et al.*, 1976). In Kenya, there are eleven species distributed as summarized in table 2.2. Of these, *P. suswaensis* and *P. decora* are indigenous to Kenya.



Scheme 2.2: Classification of the genus *Pentas* (Kårehed and Bremer, 2007)

Table 2.2: Geographical distribution of *Pentas* species in Kenya.

S. No	Species	Region	Reference
1.	*Pentas arvensis*	Kakamega, Mumias	Agnew, 1974
2.	*Pentas bussei*	Coast Province	Agnew, 1974 Bukuru, 2002 Beentjie, 1994
3.	*Pentas decora*	Mt Elgon, Cheranganyi, Aberdares, Kitale, Mumias, Kisii	Agnew, 1974
4.	*Pentas hindsiodes*	Machakos, Taita	Agnew, 1974
5.	*Pentas lanceolata*	Mau, Loita, Abedares, Mt Kenya, Nyambene, Kitale, Mumias, Kisii, Kisumu, Baringo, Narok, and Nairobi, (altitude of 1520-3000 m)	Agnew, 1974 Bukuru, 2002
6.	*Pentas longiflora*	Mt Elgon, Cheranganyi, Aberdares, Kitale, Mumias, Kisii, Baringo, Tinderet, Mau, Narok, Machakos, Kajiado	Agnew, 1974
7.	*Pentas parvifolia*	Mumias, Kisii, Baringo, Narok, Nanyuki, Embu, Machakos, Kajiado, and Nairobi (altitude of 650-2400 m)	Agnew, 1974 Bukuru, 2002
8.	*Pentas pubiflora*	Mt Elgon, Cheranganyi, Tinderet, Mau, Nyambene, Aberdares, Mumias, Kisii, Narok, Kitale	Agnew, 1974
9.	*Pentas schimeriana*	Mt Elgon, Cheranganyi, Mau, Loita, Aberdares, Tinderet	Agnew, 1974 Beentje, 1994
10.	*Pentas suswaensis*	Mt Suswa	Agnew, 1974
11.	*Pentas zanzibarica*	Mau, Loita, Abedares, Nyambene, Kitale, Narok, Machakos, Nairobi, Kajiado, Mt Kenya	Agnew, 1974

2.10.1 Ethnobotanical information on *Pentas*

Pentas species are widely used by local communities as medicinal plants. According to Kokwaro (2009), the roots of *P. decora* are used as a cure for pimples (the roots are normally pounded, mixed with some ghee and rubbed on pimples). *Pentas hindsioides* is used as a remedy for scabies (pounded roots and leaves are soaked in warm water for bathing), while *P. micrantha* is used as a cough remedy (fresh roots chewed or boiled or pounded and soaked in water and the infusion drunk). The juice of the pounded leaves of

P. zanzibarica, mixed with a little water, is drunk as a drastic purgative, whereas a decoction of the roots is taken as a remedy for gonorrhea and syphilis or given to children as a tonic (Kokwaro, 2009). Among the Digo in Kenya, where it is called "Mdobe" or "Mudobe" in the local dialect, a decoction of the roots of *P. bussei* is taken as a remedy against gonorrhea, syphilis and dysentery (Kokwaro, 2009). The roots of *P. longiflora* are used as a cure for tapeworm, itchy rashes and pimples. A decoction of the roots is mixed with milk and taken as a cure for malaria. In Rwanda, the powdered roots of this plant mixed with butter, is used as an ointment to treat skin diseases like scabies and pityriasis versicolor (Kokwaro, 2009; Van Puyvelde *et al.*, 1985). A decoction of the roots of *P. purpurea*, mixed with sugar cane, is used by Tanzanian women to initiate menstruation; whereas the juice of the plant is taken as a remedy for headache, fever and rheumatic pains. The juice from the leaves of another *Pentas* species, mixed with a small amount of water, is taken as a drastic purgative in East Africa (Watt and Breyer-Brandwijk, 1962). The ethnobotanical information of East African *Pentas* species is summarized in table 2:3.

Table 2.3: Ethnobotanical Information on *Pentas* species available in East Africa

S. No	Species	Uses	Country (local place)	References
1	*Pentas bussei*	Decoction of roots for gonorrhea, syphilis and dysentery.	Kenya (Digo)	Kokwaro, 2009 Beentije, 1994 Bukuru *et al.*, 2002
2	*Pentas decora*	Roots are cure for pimples. They are grounded, mixed with some ghee and rubbed on the pimples.	Tanzania (Shambaa)	Kokwaro, 2009
		Leaf extract rubbed on ring worms	Uganda	Ahumuza and Kirimuhuzya, 2011
3	*Pentas hindsiodes*	Used as a remedy for scabis. Roots and leaves are pounded and soaked in water for bathing.	Tanzania (pare)	Kokwaro, 2009
4	*Pentas lanceolata*	Root bark boiled and drunk for malaria, depression. Boil with Iseketek bark, olchani-enyokie, and olkokola; boil all day until turns pink/yellow and thick; drink three cups for arthritis and venereal disease	Kenya (Maasai name: Olkilaki-olkerr)	Koch *et al.*, 2005
		Root and leaf used to treat lymphadenitis by topical and oral routes	Ethiopia	Giday *et al.*, 2009
5	*Pentas longifolia*	Roots are used as cure for tape worm. Also used for itchy rashes and pimples. Decoction of roots is mixed with milk and taken as cure for malaria, but causes acute diarrhea and acts as purgative. Also used to treat scabies and skin pityriasis versicolor.	Tanzania (Shambaa and pare)	Kokwaro, 2009 El-Hady *et al.*, 2002 Claessens *et al.*, 2006
6	*Pentas micrantha*	Fresh roots are chewed, boiled or pounded and soaked in water and infusion drunk as a cough remedy.	Tanzania (Zamaramo)	Kokwaro, 2009
7	*Pentas zanzibarica*	Juice of the pounded leaves are mixed with water and drunk as purgative. Decoction of the roots is taken as remedy for gonorrhea and syphilis. Leaves and roots are used to make a form of tea which is given to children as a tonic.	Kenya (Digo) Tanazania (Shambaa)	Kokwaro, 2009

2.10.2 *Pentas* as potential source of antiplasmodial compounds

In Tanzania, decoction of roots of *P. longiflora* mixed with milk is taken as cure for malaria (El-Hady *et al.*, 2002; Claessens *et al.*, 2006). In Kenya, various parts of the plant are used to heal various diseases including malaria (Table 2.3). Preliminary antiplasmodial assay of the crude extracts of *Pentas longiflora* (Wanyoike *et al*, 2004), *P. bussei* (Irungu *et al.*, 2007), and *P. lanceolata* (Koch *et al.*, 2005) revealed good to moderate activity (IC_{50} in μg/mL) 35.2-40.5, 20.4-25.8 and 5.15 respectively on various strains of *Plasmodium falciparum*.

The *in vitro* antiplasmodial test results of the aforementioned three species coupled with their wide traditional uses in various parts of East Africa (Koch *et al.*, 2005; Kokwaro, 2009; Beentije, 1994) justifies the identification of the genus as a potential source of antiplasmodial compounds or lead chemical structures which may be used as templates for the development of new antimalarial drugs.

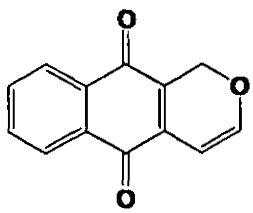
2.10.3 Phytochemistry of the Genus *Pentas*

The phytochemical information of *Pentas* species is limited in the available literature. So far the chemical constituents of *P. longiflora* (El-Hady, 2002), *P. bussei* (Bukuru *et al.*, 2002), *P. lanceloata* (Bukuru *et al.*, 2002; Gbeassor *et al.*, 1989) and *P. zanzibarica* (Kusamba *et al.*, 1993) have been studied. The phytochemical interest on these plants appear to be related to their wide spread use in traditional medicine, particularly in Africa (La Barre and Wirtheimer, 1962; Bates *et al.*, 1990; Abbiw, 1990).

2.10.3.1 Chemical constituents of *Pentas longiflora*

Pentas longifolia has been found to be a good source of quinones (El-hady, 1999; Van Puyvelde *et al.*, 1985), such as pentalongin (**33**) and mollugin (**34**). Pentalongin (**33**) exhibit an antifungal activity against *Pityrosporum ovale*, antibacterial activity against freshwater blue-green bacterium *Anabaena cylindrical*, algicidal activity against freshwater green algae *Chemydomonas sphagmophilla* var. *dysosmos* and *Chlorella vulgaris*, and marine algae *Phaeodactylum tricornutum* and *Porphyridium purpureum* (Kokwaro, 2009; Van Puyvelde, 1985; El-Hady, 1999). The compound has served as a "lead" chemical structure for the synthesis of new antibiotic compounds (Claessens *et al.*, 2007; Bukuru *et al.*, 2002).

Mollugin (**34**) was previously isolated from the root bark of *Pentas longiflora* (Van Puyvelde *et al.*, 1985; Hari *et al.*, 1991). The compound was earlier isolated from two other rubiaceous plant species, *Galium molluga* (Schildknecht *et al.*, 1976) and *Rubia cordifolia* (Itokawa *et al.*, 1983).



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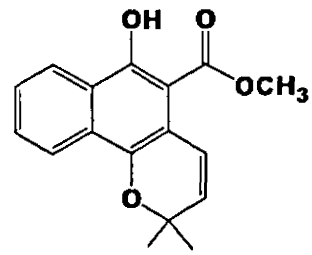

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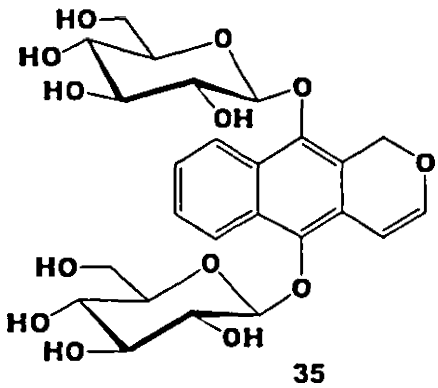
Pentalonginhydroquinone diglycoside (harounoside) (**35**) (Harouna *et al.*, 1995) was isolated from *Mitracarpus scaber* Zucc (Rubiaceae), a plant used in African traditional medicine (Niger) as an antifungal and antiparasitic agent (Adjanohoun *et al.*, 1985).



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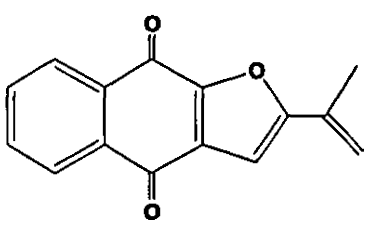
Compounds that are structurally related to pentalongin (**33**) with furanonaphthoquinone skeleton (**36-39**) were isolated from *Tabebuia ochracea,* belonging to the Bignoniaceae family. The furanonaphthoquinones have been assessed for antimalarial activity, and some of them showed promising activity *in vitro* and *in vivo* activities against both *P. falciparum* and *P. berghei*, respectively (Weiss *et al.*, 2000).



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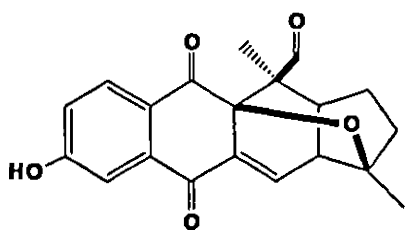

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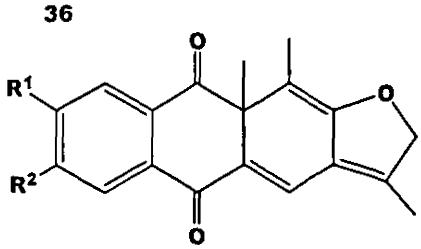
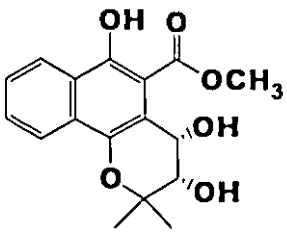


	R^1	R^2
38	H	OH
39	OH	H

Phytochemical studies on the roots of *P. longiflora* led to the isolation of cis-3,4-dihydroxy-3,4-dihydromollugin (**40**), pentalongin (**33**), mollugin (**34**), *trans*-3,4-dihydroxy-3,4-dihydromollugin (**41**), 3-hydroxymollugin (**42**), 3-methoxymollugin (**43**), methyl-3-prenyl-1,4-naphthoquinone-2-carboxylate (**44**), methyl-2,3-epoxy-3-prenyl-1,4-naphthoquinone-2-carboxylate (**45**), (3α,3'α,4β,4'β)-3,3')-dimethoxy-*cis*-(4,4'-bis(3,4,5,10-tetrahydro-1H-naphtho(2,3-c)pyran))-5,5',10,10'-tetraone (**46**), and

scopoletin (47) (El-Hady, 2002). A similar phytochemical study done by Van Puyvelde *et al.*, (1998) on the same plant yielded isagarin (48).



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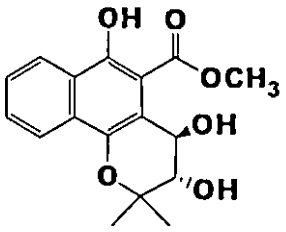

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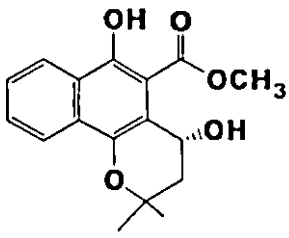

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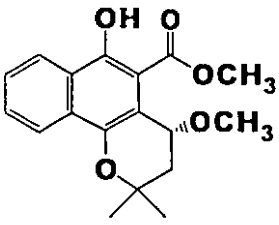

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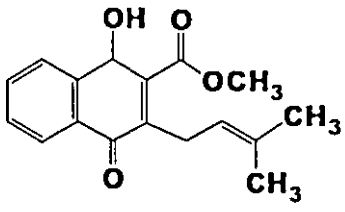

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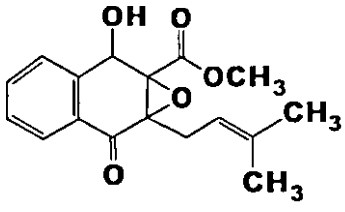

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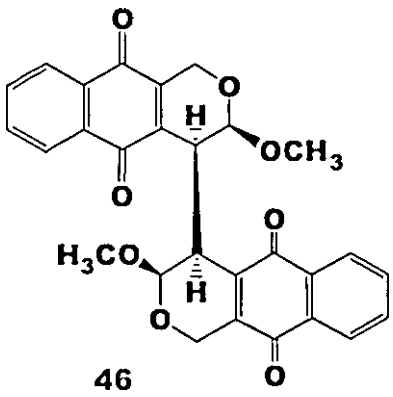

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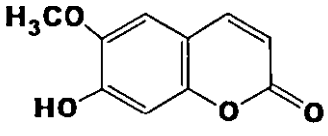

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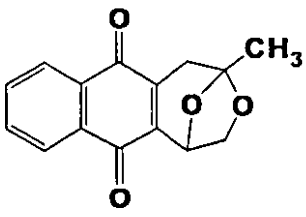
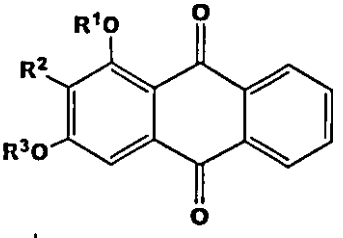


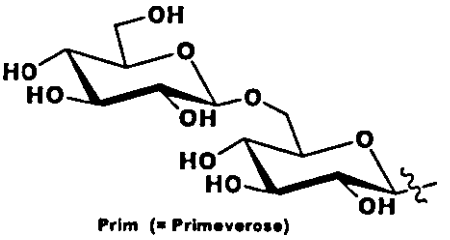
48

2.10.3.2 Chemical constituents of *Pentas zanzibarica*

The methanol extract of the stem of *Pentas zanzibarica*, from Congo, yielded anthraquinones: rubiadin (49), damnacanthal (50) and rubiadin-1-methyl ether (51), together with the anthraquinone glycosides rubiadin-3-*O*-β-primeveroside (52), rubiadin-

1-methyl ether 3-*O*-β-primeveroside (53), lucidin-3-*O*-β-primeveroside (54) and damnacanthol-3-*O*-β-primeveroside (55) (Kusamba *et al.*, 1993).



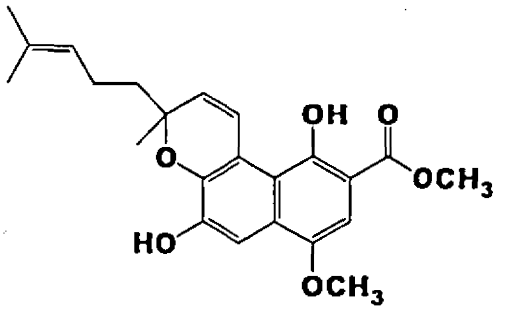


	R^1	R^2	R^3
49	H	CH_3	H
50	CH3	CHO	H
51	CH_3	CH_3	H
52	H	CH_3	Prim
53	CH_3	CH_3	Prim
54	H	CH_2OH	Prim
55	CH_3	CH_2OH	Prim

2.10.3.3 Chemical constituents of *Pentas bussei*

A homoprenylated benzochromene (56) was isolated from the roots of *Pentas bussei* along with anthraquinone glycosides: rubiadin-1-methyl ether-3-*O*-β-primeveroside (53), lucidin-3-*O*-β-primeveroside (54), damnacanthol-3-*O*-β-primeveroside (55) and the ubiquitous β-stigmasterol (57) (Bukuru, 2003).

Another intresting compound isolated from this plant is compounds 58 which contains a cyclol moiety (dihydropyran, a cyclopentane, and cyclobutane ring) derived from cyclization of a geranylgeraniol group with an adjacent hydroxyl attached to the dihydronaphthoquinone skeleton. A cyclol unit occurs in natural phenolic compounds such as cannabinoids like cannabicyclol from *Cannabis sativa* (Jefferie and Worth, 1973, Vinayak *et al.*, 1984, Rashid *et al.*, 1992).



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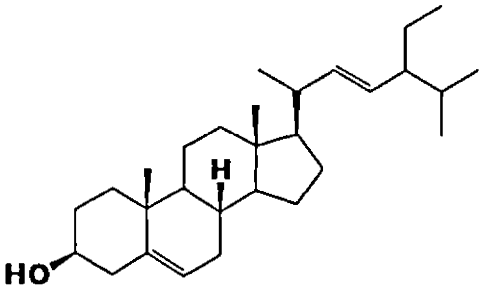

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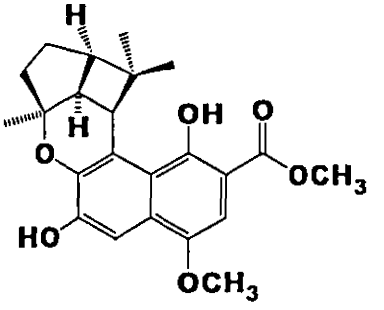
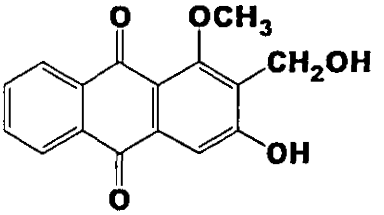


58

2.10.3.4 Chemical constituents of *Pentas lanceolata*

Fractionation of the dichloromethane, ethyl acetate and methanol extracts of the roots of *P. lanceolata* resulted in the isolation of five anthraquinones: rubiadin (49), rubiadin-1-methyl ether (51), damnacanthol (59), lucidin-ω- methyl ether (60) and damnacanthol-3-*O*-methyl ether (61) together with the anthraquinone glycoside rubiadin-1-methyl ether-3-*O*-β-primeveroside (53). The *n*-hexane extract also afforded the steroid β-stigmasterol (57) (Bukuru *et al.*, 2002).



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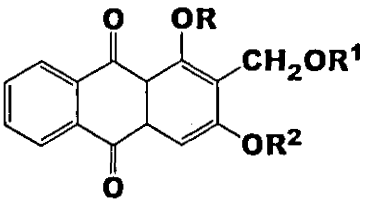
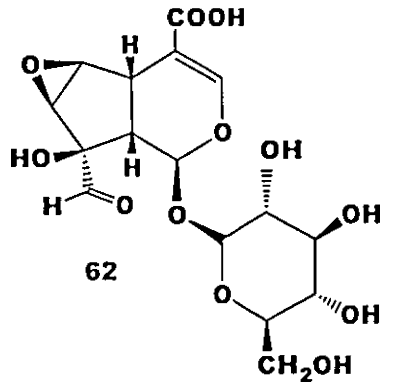


	R	R^1	R^2
60	H	CH_3	H
61	CH_3	H	CH_3

From the aerial parts of *P. lanceolata*, seven iridoids of which five of them are new were isolated and charachterized. These are: tudoside (**62**), 13*R*-*epi*-gaertneroside (**63**), 13*R*-*epi*epoxygaertneroside (**64**) and a mixture of *E*-uenfoside (**65**) and *Z*-uenfoside (**66**) (Jan *et al.*, 2007).



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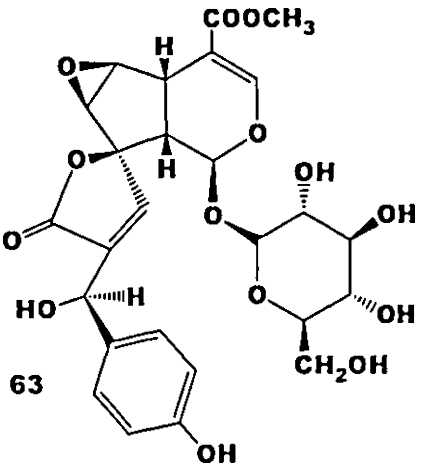

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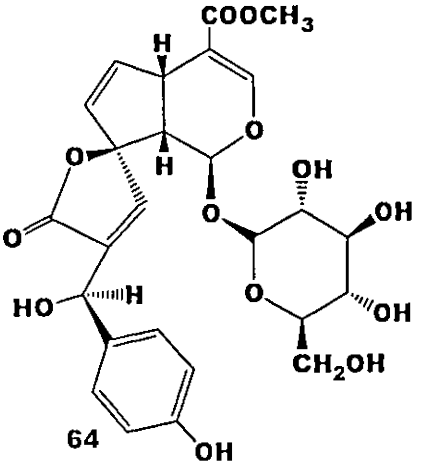

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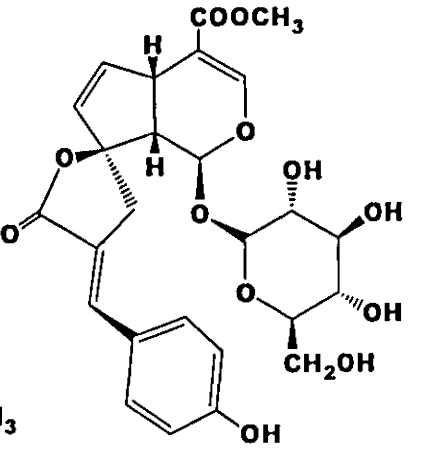

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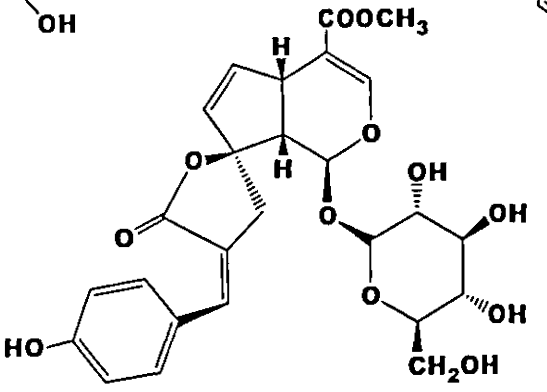

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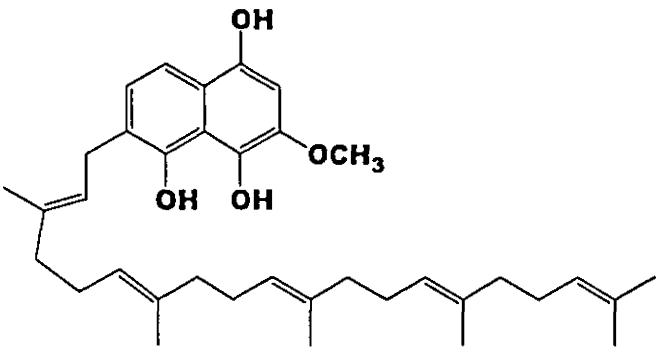
2.10.3.5 Chemical constituents of *Pentas parvifolia*

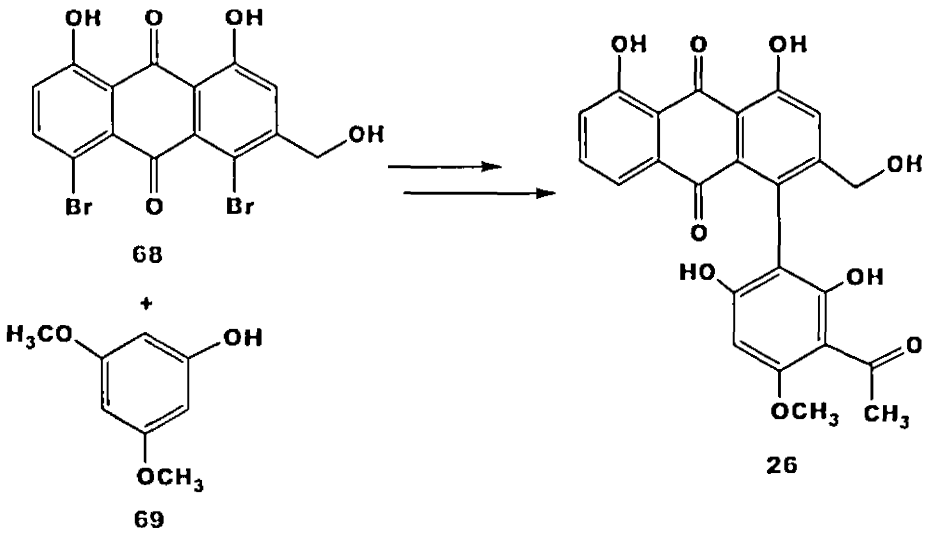
Pentas parvifolia has not been investigated in detail. The only report is available by Bukuru *et al.* (2002) reported the isolation of 1,4,5-trihydroxy-3-methoxy-6-(3',7',11',15',19'-pentamethyleicosa-2',6',10',14',18'-pentaenyl) naphthalene (**67**) from the ethyl acetate extract of the root.



67

2.11 Synthesis of phenylanthraquinones

Knipholone (**26**), isolated from the roots of *Kniphofia foliosa*, showed considerable antiplasmodial activity (IC_{50}, 1.06 μM (K1 strain), 1.70 μM (NF 54 strain)) with low cytoxicity (Bringmann *et al.*, 1999). The presence of two moieties in the structure of knipholone (**26**), *i.e.*, an anthraquinone (viz. chrysophanol) and a phenyl (viz. xanthoxylline), represents one of the rare "true" examples of a constitutionally unsymmetric natural biaryl. In subsequent years, the same group reported the application of 'lactone concept' as the first atropo-enantioselective total synthesis of (+)-knipholone (**26**) (Bringmann *et al.*, 2002). The successful construction of regio- and stereoselective biaryl axis was made by coupling compounds **68** and **69** (scheme 2.2).



Scheme 2.3: Synthesis of knopholone (**26**) (Bringmann *et al.*, 2002)

2.12 Application of microwaves technology in organic synthesis

High-speed microwave chemistry has attracted a considerable amount of attention in the past two decades with new and innovative applications in organic/peptide synthesis, polymer chemistry, material sciences, nanotechnology and biochemical processes continuously being reported in literature. In many instances, the use of microwave dielectric heating has been shown to dramatically reduce processing times, increase product yields, and enhance product purities or material properties compared to conventionally processed experiments (Kappe, 2004). Currently, many academic and industrial research groups are using microwave assisted organic synthesis as a forefront technology for rapid reaction optimization, efficient synthesis of new chemical entities, or discovering and probing new chemical reactivity.

2.12.1 Principles of microwave irradiation

The wavelength (λ) of a microwave is related to the frequency (f) (2.45 GHz) and speed (c) *via* eqn. (1). The frequency indicates the number of oscillations of the electric or magnetic field in one second.

$$\lambda_0 = c/f \qquad (1)$$

The mechanism by which matter absorbs microwave energy is called dielectric heating (Mingos and Baghurst, 1997). The mobility of the dipoles and the ability to orient them according to the direction of the electric field is an important property. The orientation of the dipoles changes with the magnitude and the direction of the electric field. Molecules that have a permanent dipole moment are able to align themselves through rotation completely or at least partly with the direction of the field. Molecules can rotate in time with field frequencies of 106 Hz in gases or liquids (Gabriel *et al.*, 1998). However, they cannot follow the inversion of the electric field at an indefinite time. Phase shifts and dielectric losses are the results. In this case, besides the dielectric coefficient (permittivity), the size (mass) of the excited molecules is also relevant.

Field energy is transferred to the medium and electrical energy is converted into kinetic or thermal energy. Molecular friction is often cited as a model for this behaviour. For numerous polar substances, dielectric losses are observed in the microwave range (Gabriel *et al.*, 1998). The fast changing electric field of the microwave radiation leads to a rotation of the water molecules. Due to this process, "internal friction" takes place in the polar medium, which leads to a direct and almost even heating of the reaction mixture.

Because the change in the polarity of the electric field is faster than the rotation of the water molecules around its dipole centre, a phase shift results and energy is absorbed from the electric field. Reflections and refractions on local boundaries yield "hot spots" and may result in a "super-heating" effect (Stadler and Kappe, 2001). This effect can be described best as local overheating and is comparable to the delayed boiling of overheated liquids under conventional conditions. This effect is characteristically found only in unstirred solutions.

2.12.2 Microwave technology in organic synthesis

In many of the published examples, microwave heating has been shown to dramatically reduce reaction times, increase product yields and enhance product purities by reducing unwanted side reactions compared to conventional heating methods. The advantages of this enabling technology have, more recently, been exploited in the context of multistep total synthesis (Baxendale *et al.*, 2002) and medicinal chemistry/drug discovery (Larhed and Hallberg, 2001; Wilson and Roth, 2002), and have additionally penetrated related fields such as polymer synthesis (Bogdal and Prociak, 2007), material sciences (Wang *et al.*, 2004), nanotechnology (Langa and De la Cruz, 2007) and biochemical processes (Rejasse *et al.*, 2007).

Recently, an alternative method for performing microwave assisted organic reactions, termed "enhanced microwave synthesis" (EMS) has been introduced (Hayes and Collins, 2004). By externally cooling the reaction vessel with compressed air, while simultaneously administering microwave irradiation, more energy can be directly applied to the reaction mixture. In "Conventional Microwave Synthesis" (CMS), the initial microwave power is high, increasing the bulk temperature (Tb) to the desired set point

very quickly. However, upon reaching this temperature, the microwave power decreases or shuts off completely in order to maintain the desired bulk temperature without exceeding it. When microwave irradiation is off, classical thermal chemistry takes over, losing the full advantage of microwave-accelerated synthesis.

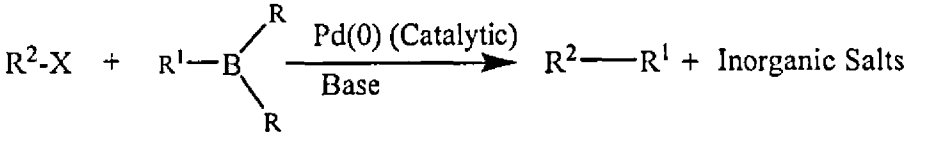
Microwave enhancement of chemical reactions will only take place during application of microwave energy (Perreux *et al.*, 2002). This source of energy will directly activate the molecules in a chemical reaction; therefore, it is not desirable to suppress its application. EMS ensures that a high, constant level of microwave energy is applied and simultaneous cooling enables a greater amount of microwave energy to be introduced into a reaction, while keeping the reaction temperature low. This results in significantly greater yields and cleaner reactions.

2.12.3 Microwave technology in metal catalized cross coupling reactions

Many transition metal-catalyzed carbon-carbon and carbon-hetero atom bond forming reactions typically need hours or days to reach completion and often require an inert atmosphere. It has been shown that many of those transformations can be enhanced significantly by employing microwave heating under sealed vessel conditions, in many cases without an inert atmosphere (Kappe, 2004). The use of homogeneous transition metal catalysts in conjunction with microwaves offers significant advantages in comparison with traditional heating methods, since the inverted temperature gradients under microwave conditions may lead to an increased lifetime of the catalyst by elimination of wall effects (Larhed and Hallberg, 1996a, b).

2.12.4 Microwave enhanced suzuki-miyaura cross coupling reaction

Suzuki-Miyaura coupling reaction is a palladium-catalyzed cross-coupling reaction reported by Suzuki and Miyaura in 1979. The reaction between organoboron compounds and organic halides or triflates provides a powerful method for the formation of carbon-carbon bonds. The coupling reaction offers several additional advantages, such as convenient method for preparation of biaryls, being largely unaffected by the presence of water, tolerating a broad range of functional groups, and proceeding regio- and stereo-selectively (scheme 2.3). Moreover, the inorganic by-product of the reaction is non-toxic and easily removed from the reaction mixture thereby making the Suzuki coupling suitable not only for laboratories but also for industrial processes. All kinds of carbon-boron bonds including (sp^3)C-B, (sp^2)C-B, and (sp)C-B bonds are employed as cross-coupling partners in the coupling reactions (Suzuki, 1998).



R^1 = Alkyl, allyl, alkenyl, alkynyl, aryl R= Alkyl, OH, O-Alkyl

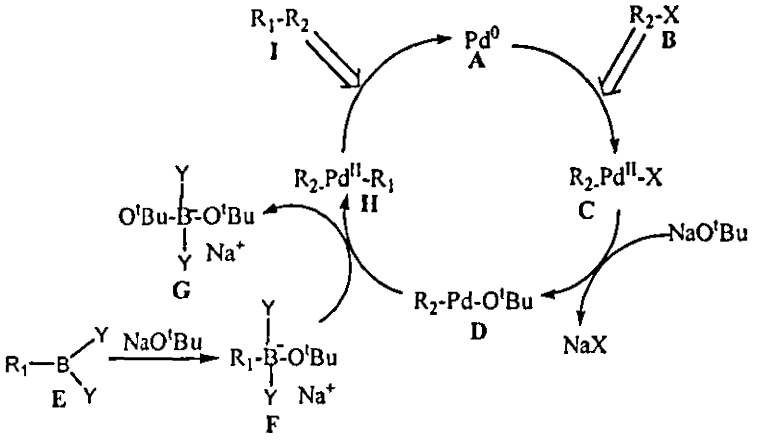
R^2 = Alkenyl, aryl, alkyl X= Cl, Br, I, OTf

Base = Sodium Carbonate, Sodium Hydroxide, M (O-alkyl), Potassium Phosphate tribasic

Scheme 2.3: General mechanism of suzuki-miyaura cross coupling reaction

The mechanism of the Suzuki reaction is best viewed from the perspective of the palladium catalyst. The first step is the oxidative addition of palladium to the halide **B** to form the organopalladium species **C**. Reaction with base gives intermediate **D**, which via transmetalation with the boron-ate complex **F** forms the organopalladium species **II**.

Reductive elimination of the desired product **I** restores the original palladium catalyst **A** as summerized in scheme 2.4.



Scheme 2.4: Mechanism of Suzuki-Miyaura reaction

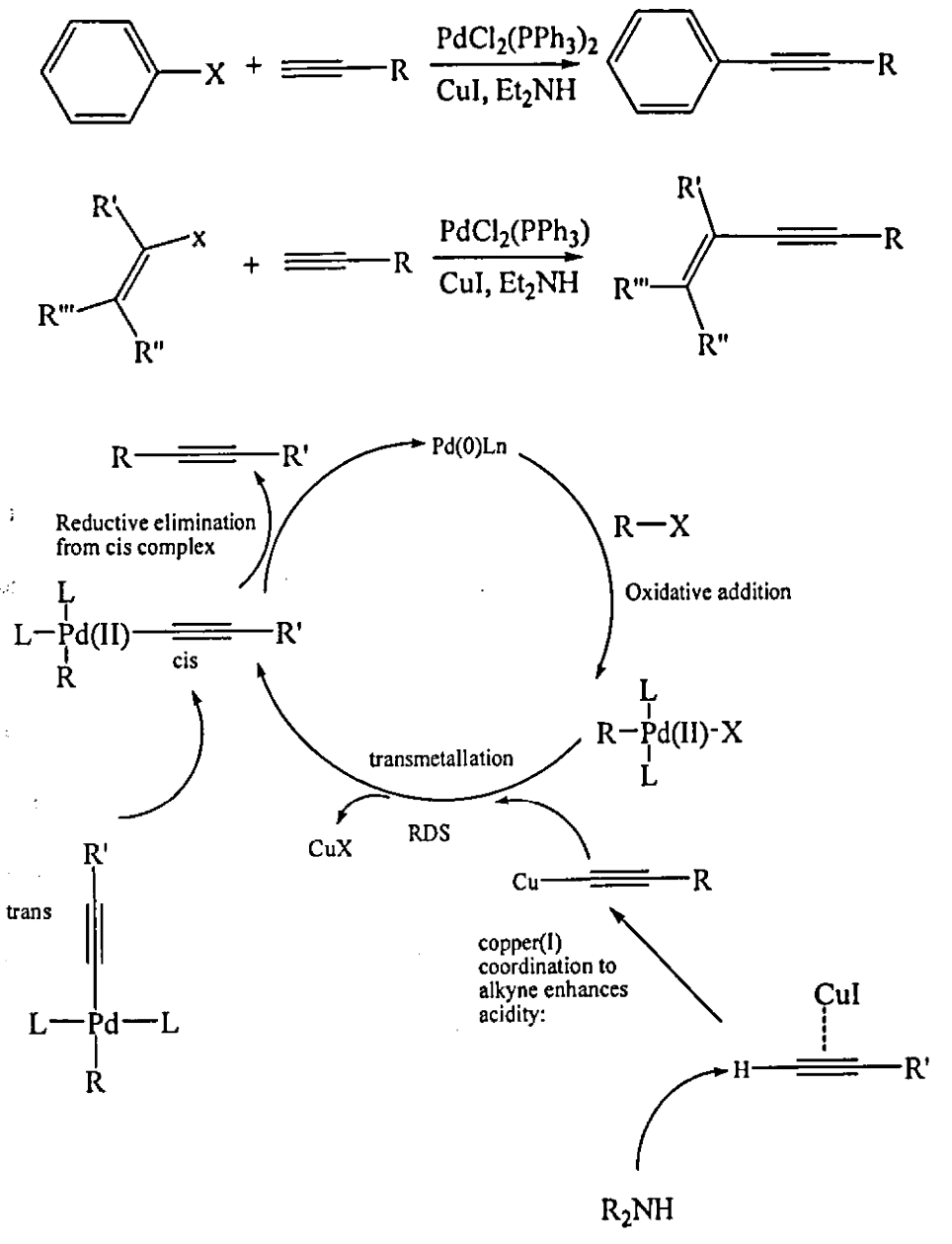
The first microwave-assisted Suzuki-Miyaura cross-coupling reaction was reported in 1996 (Larhed *et al.*, 1996a, Larhed *et al.*, 1996b). Since then the development of a simple, mild and environmentally more benign Suzuki-Miyaura reaction is still a hot topic. In particular that this procedure can be carried out in water medium has attracted considerable interest for the past decade (Lidstrom *et al.*, 2001).

2.12.5 Sonogashira cross coupling reaction

The Sonogashira reaction, a Pd-catalyzed cross-coupling between aryl halides and terminal alkynes, is a powerful tool for the synthesis of various aryl alkynes, although the use of copper salts, toxic phosphine ligands, amines and homogenous Pd catalysts are generally difficult to recover and reuse. The reaction mechanism is illustrated in scheme 2.5. Fast and solvent-less microwave assisted heterogeneous and homogeneous Sonogashira coupling involving aryl halides and acetylene moieties have been reported in the past decade (Kabalka *et al.*, 2002; Erdelyi *et al.*, 2001).

In recent years, a variety of modifications have been reported on this reaction and great progress has been made (Ruiz *et al.*, 2006). The most important modification is the elimination of the copper salt (Yi and Hua, 2006), which was used as co-catalyst since it could induce a Glaser-type homocoupling reaction of the terminal alkynes to diynes in the presence of oxidative agents or air (Siemsen *et al.*, 2000).

The use of water or aqueous solution represents one of the most economically and environmentally viable alternatives to organic solvents for metal-catalyzed reactions (Lubineau and Auge, 1999). Several examples of Pd-catalyzed Sonogashira reactions in aqueous media have been reported. However, many of these reactions are carried out in an aqueous-organic and in some case, special phosphine ligands and copper salts are required in order to reach high reaction efficiency. A copper-free $PdCl_2$-catalyzed Sonogashira coupling reaction of aryl iodides with terminal acetylenes was also reported in pure water (Liang *et al.*, 2005). Guan *et al.* (2007) recently reported palladium catalyzed Sonogashira reaction of aryl bromides with terminal alkynes catalyzed by air-stable and readily available $PdCl_2/PPh_3$ in pure water without adding any organic co-solvent and copper salt.



Scheme 2.6: Mechanism of sonogashira reaction

CHAPTER THREE

EXPERIMENTAL

3.1 General

Column chromatography was performed on oxalic acid impregnated silica gel. The silica gel was deactivated by mixing 2 kg of silica gel 60 (70-230 mesh) with 3% oxalic acid (30 g in 1 L water) and allowed to stand for 30 min, filtered and dried (reactivated) at 100°C for 45 min. TLC was done using silica gel 60 F254 (Merck) precoated plates. NMR analyses were carried out on Varian 800, 600, 500 and 200 MHz spectrometers. Structural assignment was performed based on gCOSY, gTOCSY, gNOESY, gHSQC, and gHMBC. ESI LC-MS was performed on a Perkin Elmer PE SCIEX API 150EX instrument equipped with a Turbolon spray ion source and a Gemini 5 mm C_{18} 110 Å HPLC column using a water-acetonitrile gradient (80:20 to 20:80). High-resolution mass spectral analysis (Q-TOF-MS) was performed at Stenhagen Analyslab AB, Gothenburg, Sweden. The compound purity was determined by analytical HPLC and TLC. Analytical HPLC was run on a Hewlett Packard Series 1050 HPLC using the software Chromulan (Pikron Ltd.), a Gemini 5-mm C18 110 Å HPLC column and a methanol-water mixture as the eluent. UV spectra were obtained in CH_3OH using a Hewlett- Packard 8453 spectrophotometer. Optical rotations were measured using a Perkin-Elmer 341 LC polarimeter. IR spectra (KBr disks) were recorded on a Perkin-Elmer 1725 FTIR spectrometer.

HPLC solvent system used for chemotaxonomy studies were as follows: gradient of acetonitrile/water (0.1 % formic acid), flow rate: 2.5 mL/minute, and 5 mm Gemini C_{18}

110 Å HPLC column. Plant extracts: isocratic solvent system of CH_3OH:H_2O (90:10) (0.1% formic acid) and 5mm hichrom C_8 column.

3.2 Plant materials

The roots of *P. bussei* were collected from Mombasa, coastal region of Kenya in July, 2010. The roots of *P. longiflora* were collected from Nandi East District, Kenya (Nandi Hills-Chebarus location) in August, 2009. The roots of *P. lanceolata* were collected from Ngong Forest, December, 2009. The roots of *P. micrantha* were collected from Mombasa, coastal region of Kenya in July, 2010. The root of *P. parvifolia* was collected from Western Kenya in June 2011. All the plant parts were collected with the help of Mr. Simon Mathenge and Mr. Patrick Chalo Mutiso of Department of Botany, School of Biological Sciences, University of Nairobi, Kenya. Voucher specimens are deposited at the Herbarium, School of Biological Sciences, University of Nairobi with voucher number MEA 2009/001 (*P. longiflora*), MEA 2009/002 (*P. lanceolata*), MEA 2010/003 (*P. bussei*), MEA 2010/004 (*P. micrantha*), MEA 2010/005 (*P. suswaensis*), and MEA 2010/006 (*P. parvifolia*). The plants were air dried under shade and pulverized using a Willy mill.

3.3 Extraction and isolation

3.3.1 General extraction method

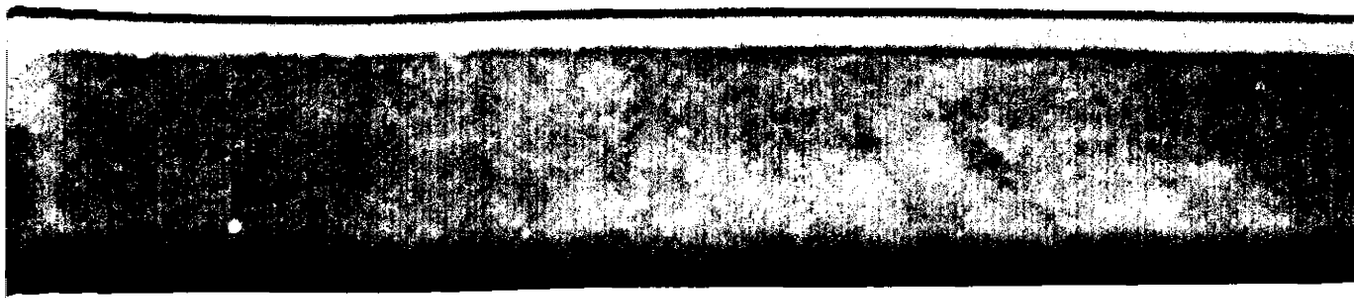
The ground roots of six *Pentas* species (0.6-1.6 kg) were extracted by cold percolation with CH_2Cl_2/CH_3OH (1:1) 4L three times for 24 h in each case. The extract was concentrated using a rotary evaporator to yield 4.8-11.8% (37-75g). The marc after CH_2Cl_2/CH_3OH (1:1) extraction was further extracted with CH_3OH (2.5 L) three times

for 24 h in each case and the extract was concentrated using a rotary evaporator to yield 6.0-11.6% (30-65g).

3.3.2 Extraction and isolation of compounds from the roots of *Pentas bussei*

The roots of *Pentas bussei* (1.0 kg) were extracted as per the procedure described above (Section 3.3.1) to yield 65 g (6.5%) brownish crude extract. A 64 g portion of the crude extract was subjected to column chromatography (80 cm length and 80 mm diameter, 450 g oxalic acid impregnated silica gel) with increasing gradient of ethyl acetate in *n*-hexane. Two hundred fractions (each *ca.* 150 mL) were collected. Fractions 15-20 (2% ethyl acetate in *n*-hexane) were combined and purified on sephadex LH-20 (eluent CH_2Cl_2/CH_3OH, 1:1) to give busseidihydroquinone A (**70**) (20 mg). Fractions 63-64 (eluent 5% ethyl acetate in *n*-hexane) were combined and purified by column chromatography (increasing gradient of ethyl acetate in *n*-hexane as eluent) to give busseidihydroquinone B (71) (40 mg). Fractions 75-79 (7% ethyl acetate in *n*-hexane as eluent) were combined and purified by column chromatography (increasing gradient of ethyl acetate in *n*-hexane as eluent) to give busseidihydroquinone C (**72**) (150 mg). Fractions 115-118 (12% ethyl acetate in *n*-hexane) were combined and purified on Sephadex LH-20 to give compound **56** (20 mg). Fractions 131-135 (18% ethyl acetate in *n*-hexane) were combined and purified using column chromatography (increasing gradient of ethyl acetate in *n*-hexane) to give busseidihydroquinone D (**73**) (25 mg).

The methanol extract (60 g, 6.0%) was subjected to column chromatography on oxalic acid impregnated silica gel with increasing gradient of methanol in dichloromethane. A total of 100 fractions (each *ca.* 150 mL) were collected. Fractions 10-15 (2% CH_3OH in CH_2Cl_2) were combined and purified by sephadex LH-20 to give busseidihydroquinone B



(**71**) (45 mg). Fractions 30-35 (3% CH_3OH in CH_2Cl_2) were combined and purified by column chromatography (eluent, increasing gradient of ethyl acetate in *n*-hexane) to give compounds **56** (15 mg) and busseidihydroquinone D (**73**) (35 mg).

3.3.3 Extraction and isolation of compounds from the roots of *Pentas lanceolata*

The roots (1.4 kg) of *P. lanceolata* were extracted as per the procedure described above (Section 3.3.1) to yield a brownish crude extract (57 g, 4.8%). The residue from the above extract was further extracted with CH_3OH to yield 100 g (7.1%) brownish crude extract. A 54 g portion of the crude CH_2Cl_2/CH_3OH (1:1) extract was subjected to column chromatography (420 g oxalic acid impregnated silica gel) with increasing gradient of ethyl acetate in *n*-hexane. A total of 550 fractions each 150 mL were collected. Fractions 10-13 (2% ethyl acetate in *n*-hexane) were combined and purified on sephadex LH-20 (eluent, CH_2Cl_2/CH_3OH; 1:1) to give tectoquinone (**74**) (40 mg). Fractions 15-25 (eluent, 3% ethyl acetate in *n*-hexane) were combined and purified by Sephadex LH-20 (eluent, CH_2Cl_2/CH_3OH; 1:1) to give rubiadin (**49**) (680 mg) and rubiadin-1-methyl ether (**51**) (50 mg). Fractions 30-35 (5% ethyl acetate in *n*-hexane as eluent) were combined and purified by column chromatography to give damnacanthal (**50**) (320 mg). Fractions 53-65 (7% ethyl acetate in *n*-hexane) were combined and purified by sephadex LH-20 (eluent, CH_2Cl_2/CH_3OH; 1:1) to give nordamnacanthal (**77**) (20 mg). Fractions 131-135 (18% ethyl acetate in *n*-hexane) were combined and purified using column chromatography on oxalic acid impregnated Silica gel) (increasing gradient of ethyl acetate in *n*-hexane) to give lucidin-ω-methyl ether (**60**) (50 mg). Fractions 400-405 (50% ethyl acetate in *n*-hexane) were combined and purified by MPLC (40% ethyl

acetate in *n*-hexane as eluent, flow rate of 30 mL/min) to give damnachantol (**59**) (50 mg).

The methanol extract (70 g) was subjected to column chromatography (420 g of oxalic acid impregnated silica gel) with increasing gradient of methanol in dichloromethane. A total of 100 fractions (each *ca.* 200 mL) were collected. Fractions 5-11 (100% dichloromethane) were combined and purified by Sephadex LH-20 (eluent, CH_2Cl_2/CH_3OH; 1:1) to give nordamnacanthal (**77**, 20 mg) and rubiadin-1-methyl ether (**51**, 18 mg). Fractions 21-25 (eluent, 1% of methanol in CH_2Cl_2) were combined and passed through sephadex LH-20 (eluent, CH_2Cl_2/CH_3OH; 1:1) to give damnachantol (**59**, 15 mg). Fractions 35-40 (eluent, 2% of methanol in CH_2Cl_2) were combined and passed through sephadex LH-20 (eluent, CH_2Cl_2/CH_3OH; 1:1) to give damnachantol-ω-methyl ether (77, 20 mg). Fractions 87-90 (5% CH_3OH in CH_2Cl_2) were combined and further purified using sephadex LH-20 (eluent, CH_2Cl_2/CH_3OH; 1:1) to give 5, 6-dihydroxydamnachantol (**78**, 40 mg). Fractions 105-110 (7% CH_3OH in CH_2Cl_2) were combined and purified by RP-HPLC (methanol/water as solvent system) to give rubiadin-1-methyl ether-3-*O*-primveroside (**53**) and rubiadin-3-*O*-primveroside (**52**).

3.3.4 Extraction and isolation from the roots of *Pentas longiflora*

The roots (0.8 kg) of *P. longiflora* were extracted as per the procedure described above (Section 3.3.1) to yield a brownish crude extract (50 g, 6.2%). A 45 g portion of the crude extract was subjected to column chromatography (80 cm length and 80 mm diameter, 350 g oxalic acid impregnated silica gel) with increasing gradient of acetone in *n*-hexane. Two hundred fractions (each *ca.* 200 mL) were collected. Fractions 15-17 (2% acetone in *n*-hexane) were further purified by sephadex LH-20 to give mollugin (**34**) (34 mg).

Fractions 18-25 (3% acetone in *n*-hexane) were further purified by column chromatography on oxalic acid impregnated silica gel (eluent 2% acetone in *n*-hexane) to give pentalongin (**33**) (40 mg). Fractions 90-112 (20% acetone in *n*-hexane) were combined and further purified by sephadex LH-20 (eluent CH_2Cl_2/CH_3OH; 1:1) to give psychorubrin (**80**) (150 mg).

3.3.5 Extraction and isolation of compounds from the roots of *Pentas micrantha*

The roots (0.8 kg) of *P. micrantha* were extracted as per the procedure described above (Section 3.3.1) to yield a brownish crude extract (55 g, 6.8%). A 53 g portion of the crude extract was subjected to column chromatography (80 cm length and 80 mm diameter, 500g oxalic acid impregnated silica gel) with increasing gradient of ethyl acetate in *n*-hexane. Two hundred fractions (each *ca.* 200 mL) were collected.

Purification of the fractions by column chromatography and sephadex LH-20 yielded eight anthraquinones and β-stigmasterol (**57**). Fractions 10-12 (1% ethyl acetate in *n*-hexane) were combined and purified on sephadex LH-20 to give tectoquinone (**74**) (15 mg). Fractions 15-20 (2 % ethyl acetate in *n*-hexane) were combined and purified on sephadex LH-20 (eluent, CH_2Cl_2/CH_3OH; 1:1) to give lucidin-ω-methyl ether (**60**) (80 mg). Fractions 75-79 (5% ethyl acetate in *n*-hexane as eluent) were combined and purified on sephadex LH-20 (eluent CH_2Cl_2/CH_3OH; 1:1) to give rubiadin-1-methyl ether (**51**) (25 mg). Fractions 100-103 (10% ethyl acetate in n-hexane) were combined to provide rubiadin (**49**) (25 mg), which was recrystalized from methanol. Fractions 115-118 (12 % ethyl acetate in *n*-hexane) were combined and purified on column chromatography with increasing gradient of ethyl acetate in *n*-hexane to give damnacanthol (**59**) (150 mg). Fractions 130-140 (18% ethyl acetate in *n*-hexane) were

combined and purified using column chromatography (increasing gradient of ethyl acetate in *n*-hexane) to give 3-methoxynordamnacanthal (**76**) (22 mg). Fractions 151-165 (20% ethyl acetate in *n*-hexane) were combined and purified using column chromatography (increasing gradient of ethyl acetate in *n*-hexane) to give 5, 6-dihydroxy lucidin-ω-methyl ether (**82**) (40 mg).

From the column chromatography of the methanol extract a total of one hundred fifty fractions were collected. Fraction 15-20 (2% methanol in dichloromethane) were combined and further purified with column chromatography isocratic (2% methanol in dichloromethane) to give 5, 6-dihydroxy damnacanthol (**59**) (40 mg). Fraction 35-43 (5% methanol in dichloromethane) were combined and purified by column chromatography (eluent, ethyl acetate in *n*-hexane) to give rubiadin (**49**) (60 mg) and rubiadin-1-methyl ether (**51**) (50 mg). Fraction 70-74 (7% methanol in dichloromethane) were combined and purified by Sephadex LH-20 twice to give 5, 6-dihydroxy lucidin-ω-methyl ether (**82**, 20 mg).

3.3.6 Extraction and isolation of compounds from the roots of *Pentas parvifolia*

The roots (600 g) of *P. parvifolia* were extracted as per the procedure described above (Section 3.3.1) to yield a brownish crude extract (71 g, 11.8%). A 70 g portion of the crude extract was subjected to column chromatography (80 cm length and 80 mm diameter, 500 g oxalic acid impregnated silica gel) with increasing gradient of ethyl acetate in *n*-hexane. Two hundred fractions (each *ca.* 200 mL) were collected.

Fractions 20-35 (4% ethyl acetate in *n*-hexane) were combined and purified by column chromatography (eluent, gradient of ethyl acetate in *n*-hexane) to give

busseidihyroquinone A (70) (220 mg). Fractions 55-60 (7% ethyl acetate in *n*-hexane) were combined and purified by column chromatography (eluent, gradient of ethyl acetate in *n*-hexane) to give busseidihyroquinone B (71) (34 mg). Fractions 62-70 (8% ethyl acetate in *n*-hexane) were combined and purified by sephadex LH-20 (eluent, gradient of ethyl acetate) to give busseidihyroquinone C (720) (150 mg). Fractions 105-115 (15% ethyl acetate in *n*-hexane) were combined and purified by column chromatography (eluent, ethyl acetate in *n*-hexane) to give busseidihyroquinone D (73) (20 mg). Fractions 120-123 (20% ethylacetate in *n*-hexane) were combined and purified by column chromatography (eluent, ethylacetate in *n*-hexane) to give compound 86 (15 mg).

3.3.7 Extraction and isolation of compounds from the roots of *Pentas Suswaensis*

The roots (600 g) of *P. suswaensis* were extracted as per the procedure described above (Section 3.3.1) to yield a brownish crude extract (45 g, 7.5%). A 44 g portion of the CH_2Cl_2/CH_3OH (1:1) crude extract was subjected to column chromatography (60 cm length and 60 mm diameter, 300 g oxalic acid impregnated silica gel) with increasing gradient of ethyl acetate in *n*-hexane. Two hundred fifty fractions (each *ca.* 150 mL) were collected and further purification of the fractions were done by RP-HPLC using either water/acetonitrile or water/methanol as an eluent.

Fractions 10-20 (2% ethyl acetate in *n*-hexane) were combined and purified by RP-HPLC (gradient of acetonitrile/water) to give lucidin-ω-methyl ether (60) (30 mg), rubiadin-1-methyl ether (51) (230 mg), and rubiadin (49) (150 mg). Fractions 25-55 (eluent, 3% ethyl acetate in *n*-hexane) were combined and purified by RP-HPLC (gradient of

acetonitrile/water) to give rubiadin (**49**) (280 mg) and damnacanthol (**59**) (50 mg). Fractions 40-55 (8% ethyl acetate in *n*-hexane as eluent) were combined and purified by RP-HPLC (gradient of acetonitrile/water) to give 5,6-dihydroxyrubiadin (**83**) (22 mg). Fractions 60-65 (10% ethyl acetate in *n*-hexane) were combined and purified by RP-HPLC (gradient of acetonitrile/water) to give 5,6-dihydroxyrubiadin-1-methyl ether (**84**) (20 mg). Fractions 131-135 (30% ethyl acetate in *n*-hexane) were combined and purified using RP-HPLC (gradient of acetonitrile/water) to give 5,6-dihydroxydamnacathol (**78**) (20 mg). Fractions 200-225 (45% ethyl acetate in *n*-hexane) were combined and purified by RP-HPLC (gradient of methanol/water) to give rubiadin-3-*O*-primveroside (**52**) (18 mg) and rubiadin-1-methyl ether-3-*O*-primveroside (**53**) (20 mg).

The methanol extract (70 g, 11.6%) was subjected to column chromatography (420 g of oxalic acid impregnated Silica gel) with increasing gradient of methanol in dichloromethane. A total of 150 fractions (each *ca.* 200 mL) were collected. Fractions 5-11 (2% methanol in dichloromethane) were combined and purified by RP-HPLC (eluent gradient methanol/water) to give lucidin-3-*O*-primveroside (**54**) (25 mg). Fractions 12-22 (4% methanol in dichlormethane) were combined and purified by RP-HPLC (eluent gradient methanol/water) to give damnacanthol-3-*O*-primveroside (**55**) (27mg). Fractions 70-90 (7% methanol in dichloromethane) were combined and purified by RP-HPLC (eluent methanol/water) to give 5,6-dihydroxydamnacathol-3-*O*-glucopyranose (**85**) (12 mg).

3.4 Antiplasmodial Activity

3.4.1 *In vitro* antiplasmodial assay

3.4.1.1 *Plasmodium falciparum* strains

The crude extracts and pure compounds were screened against two clones of *Plasmodium falciparum,* the Sierra Leone D6 chloroquine-sensitive and the Indochina W2 chloroquine-resistant clones (Juma *et al.*, 2011). The two were donated by the Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington DC. The strains were maintained aseptically based on the *in vitro* technique described by Trager and Jensen (1976) to attain replication robustness prior to assays.

3.4.1.2 Drugs

The reference antimalarial drugs, chloroquine and mefloquine having well-documented IC_{50} values were donated by the Walter Reed Army Institute of Research (WRAIR), Division of Experimental Therapeutics. The test samples pyranonaphthoquinones and dihydronapthoquinones were isolated from the roots of *Pentas longiflora, P. bussei* and *P. parvifolia* while the anthraquinones were isolated from the roots of *Pentas lanceolata, P. suswaensis* and *P. micrantha* as described above.

3.4.1.3 RPMI 1640 basic media preparation

RPMI 1640 basic media consisted of RPMI 1640 powder (10.4 grams; Invitrogen, Inc., Carlsbad, California, USA) combined with 2 g glucose (Sigma Inc., St Louis, Missouri, USA;) and 5.95 grams HEPES (Sigma Inc. St Louis, Missouri , USA) dissolved to homogeneity in 1 liter of de-ionized water and sterilized with a 0.2 µM filter.

3.4.1.4 Complete RPMI 1640 media preparation

Complete RPMI 1640 media, used for all parasite culture drug dilutions and drug testing, consisted of a mixture of RPMI 1640 basic media with 10% (v/v) pooled human plasma from blodd groups A, B and O, 3.2% (v/v) sodium bicarbonate (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) and 4 µg/mL hypoxanthine (Sigma Inc.). Complete RPMI 1640 media was stored at 4°C and used within 2 weeks.

3.4.1.5 Drug susceptibility assays

The SYBR green I-based *in vitro* drug susceptibility assay technique previously described (Smilkstein *et al.*, 2004) was used with modifications. Briefly, *P. falciparum* reference isolates in chloroquine-resistant W2 and chloroquine-sensitive D6 clones were first cultured at 6 % hematocrit, to reach 3 to 8% parasitemia for 7 to 30 days to establish parasite replication robustness. Prior to drug testing, the parasite culture was adjusted to 2% hematocrit and 1% parasitemia, and cultured in the presence of antimalarial drug aliquots in complete RPMI 1640.

3.4.1.6 Drug preparation

To prepare drug standards, chloroquine and mefloquine were dissolved in 70% ethanol at 1mg/mL. The test compounds were dissolved in 100% dimethyl sulfoxide at 10 mg/mL. The standard drugs were diluted in complete RPMI 1640 media to CQ 1000 ng/mL, MQ 250 ng/mL. Similarly, they were diluted to 50000 ng/mL starting concentrations. They were further subjected to twofold serial dilution across ten wells from CQ (1.953 to 1.000 ng/mL), MQ (0.488 to 250 ng/mL) and test compounds (97.7 to 50,000 ng/mL) using Biomek 2000.

3.4.1.7 *In vitro* drug assay

For drug assay, the ten varying serial twofold dilutions (10 concentrations and the first row were used as control wells) of the drugs (12.5 µl/well) were dispensed into 96-well micro-culture plates (Catalog # 167008; Nunc Inc., Roskilde, Denmark) by a semi-automated micro-dilution technique using Beckman 2000 laboratory work station. A total of 100 µl of cell medium mixture at 2% hematocrit and parasites at 1% (for reference isolates W2 & D6) was added to each well, and the plates incubated for 72 h in a gas mixture (5% CO_2, 5% O_2, and 90% N_2) at 37°C. The plates were subsequently freeze-thawed for haemolysis. For further haemolysis, 100 µl of lysis buffer (20 mM Tris (pH 7.5), 5 mM EDTA, 0.008% (w/v) saponin, and 0.08% (v/v) Triton X-100) containing SYBR green I (1x final concentration) were added directly to the plates and gently mixed by using the Beckman Coulter Biomek 2000 automated laboratory workstation (Beckman Coulter, Inc., Fullerton, CA). The plates were incubated for 5 to 15 min. at room temperature in the dark. Parasite growth inhibition was quantified by measuring the relative fluorescence units (RFU) per well of SYBR green I dye using the Tecan Genios Plus (Tecan US, Inc., Durham, NC) with excitation and emission wavelengths of 485 nm and 535 nm, respectively, with the gain set at 60.

3.4.2 *In vivo* antiplasmodial assay

The *in vivo* antiplasmodial activity was evaluated by the classical 4-day parasite suppressive test (David *et al*, 2004). Male Swiss mice (20-25 g) were inoculated intraperitoneally with 10^7 *P. berghei*-infected erythrocytes, resuspended in RPMI 1640 medium on day 0 to a volume of 0.2 mL. Fifty-four mice were divided into 9 groups. The first 4 groups received 25, 50, 100, and 200 mg/kg BW/d of aqueous extract,

respectively. The second 4 groups received 12.5, 25, 50, and 100 mg/kg BW/d of methanolic extract, respectively. The other group without compound was the control. Each dose of extract was administered once daily for 4 consecutive days, beginning on the day of infection, starting two hours after inoculation until day 3. The level of parasitemia as determined the day following the last treatment. The ED_{50}, which is the dose leading to 50% parasite growth inhibition compared to growth in the control, was evaluated from a plot of activity (expressed as a percentage of the activity in the control) versus the log dose.

3.5 Cytotoxicity assay

MCF-7 human breast cancer cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin and 100 µg/mL streptomycin at 37°C in humidified 5% CO_2. For cytotoxicity assays, cells were seeded in 96-well plates at optimal cell density to ensure exponential growth for the duration of the assay. After a 24 h pre-incubation, growth medium was replaced with experimental medium containing the appropriate drug concentrations or vehicle controls (0.1% or 1.0 % v/v DMSO). After 48 h incubation, cell viability was measured using PrestoBlue™ Cell Viability Reagent (Invitrogen Ab, Lidingö, Sweden) according to the manufacturer's instructions. Absorbance was measured at 570 with 600 nm as a reference wave length. Results were expressed as the mean ± S.E. for six replicates as a percentage of vehicle control (taken as 100%). Experiments were performed independently at least three times. Statistical analyses were performed using a two tailed student's t test. $\rho < 0.05$ was considered to be statistically significant (Endale *et al.*, 2012a).

3.6 Experimental Procedures for the Analogue Syntheses

3.6.1 Acetylation of psychorubrin (80) and rubiadin-1-methyl ether (51)

In a 50 mL round bottom flask, 20 mg (86.95 mmol) of psychorubrin (80) was dissolved in 3.5 mL acetic anhydride (34.2 mmol). Three drops of pyridine were added and the mixture was stirred for 48 h at room temperature (Hayashi *et al.*, 1987). TLC was done using 30% acetone in *n*-hexane to monitor the completion of the reaction. The reaction mixture was poured in to ice-water and stirred in an ice bath until the psychorubrin acetate (**81**) precipitated (18mg, 90% yield). A similar procedure was adopted to prepare rubaidin-1-methyl ether acetate (**75**) from rubiadin-1-methyl ether (**51**).

3.6.2 4-bromorubiadin (87) and 4-bromorubiadin-1-methyl ether (88)

To a solution of 400 mg of rubiadin (**49**) (1.54 mmol) in 50 mL of dry acetonitrile was added followed by 0.29 g (0.7 mmol) of *N*-bromo succinimide (NBS). The solution was stirred for 24 h. The reaction was monitored by TLC and analytical HPLC until all the starting material was completely consumed. The solvent was evaporated and the reaction mixture was purified using column chromatography (ethyl acetate in *n*-pentane as eluting solvent) to give **87** (352mg, 88% yeild). To a solution of 2.9 g (10.8 mmol) of rubiadin-1-methyl ether (**51**) in 200 mL of dry acetonitrile was added 2.01g (11.3 mmol) of *N*-bromo succinimide (NBS). The solution was stirred for 24 h. The reaction was monitored by TLC and analytical HPLC until all the starting material was completely converted (Tietze *et al.*, 2007). The solvent was evaporated and the reaction mixture was purified using column chromatography (ethyl acetate in *n*-pentane as eluting solvent) to give **88** (2.7g, 92% yeild).

3.6.3 Microwave assisted synthesis of novel series of 4-aryl substituted analogues of rubiadin (49) and rubiadin-1-methyl ether (51)

3.6.3.1 4-phenylrubiadin (89)

4-Bromo-rubiadin (87) (200 mg, 0.58 mmol) and phenylboronic acid (95.1 mg, 0.78 mmol) were suspended in a 1:1 mixture of dimethylformamide (DMF) and H_2O (20 mL) together with $NaHCO_3$ as the base (3 equivalents) and $PdCl_2(PPh_3)_2$ (5 mol%) and PPh_3(10 mol%) as a catalyst in a 20 mL sealed glass vial. The mixture was irradiated at 150 °C for 10 min using a maximum power level of 90W (Larhed *et al.*, 1996a, Larhed *et al.*, 1996b). The product was purified using column chromatography (silica gel eluting with 20% of ethyl acetate in *n*-pentane). The product (**89**) was isolated in 91% (180mg) yield.

3.6.3.2 4-(*p*-nitrophenyl)rubiadin-1-methyl ether (90)

4-Bromo-Rubiadin1-methyl ether (**87**) (200 mg, 0.58 mmol) and *p*-nitrophenyl boronic acid (130.2 mg, 0.78 mmol) were suspended in a 1:1 mixture of DMF and H_2O (20 mL) together with $NaHCO_3$ as the base (3 equivalent) and $PdCl_2(PPh_3)_2$ (5 mol%) and PPh_3(10 mol%) as a catalyst in a 20 mL sealed glass vial (Larhed *et al.*, 1996a, Larhed *et al.*, 1996b). The mixture was irradiated at 150°C for 10 min using a maximum power level of 90W and product was purified using column chromatography (silica 60) with 100% dichloromethane to give compound **90** (166 mg) in 83% yield.

3.6.3.3 4-(*p*-methoxyphenyl)rubiadin-1-methyl ether (91)

4-Bromo-Rubiadin-1-methyl ether (**88**) (200 mg, 0.58 mmol) and *p*-methoxyboronic acid (118.5 mg, 0.78 mmol) were suspended in a 1:1 mixture of DMF and H_2O (20 mL) together with $NaHCO_3$ as the base (3 equivalent) and $PdCl_2(PPh_3)_2$ (5 mol%) and

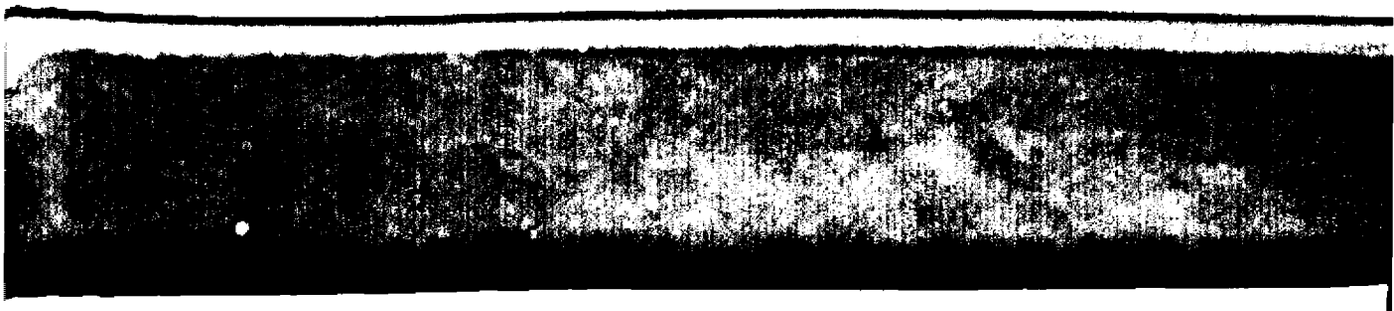
PPh_3(10 mol%) as a catalyst in a 20 mL sealed glass vial. The mixture was irradiated at 150°C for 15 min using a maximum power level of 90W (Larhed *et al.*, 1996a, Larhed *et al.*, 1996b). The product was purified using column chromatography (silica 60) with 20% of ethyl acetate in *n*-pentane. The product (**91**, 148 mg) was isolated in 86% yield.

3.6.3.4 4-phenylrubiadin-1-methyl ether (92)

4-Bromo-Rubiadin-1-methyl ether (**88**) (200 mg, 0.58 mmol) and phenylboronic acid (118.5 mg, 0.97 mmol) were suspended in a 1:1 mixture of DMF and H_2O (20 mL) together with $NaHCO_3$ as the base (3 equivalent) and $PdCl_2(PPh_3)_2$ (5 mol%) and PPh_3(10 mol%) as a catalyst in a 20 mL sealed glass vial. The mixture was irradiated at 150°C for 15 min using a maximum power level of 90W (Larhed *et al.*, 1996a, Larhed *et al.*, 1996b). The product was purified using column chromatography (silica 60) with 20% of ethyl acetate in *n*-pentane. The product (**92**, 150 mg) was isolated in 75% yield.

3.6.3.5 4-biphenylrubiadin-1-methyl ether (93)

4-Bromorubiadin-1-methyl ether (**88**) (200 mg, 0.58 mmol) and biphenylboronic acid (154.5mg, 0.78 mmol) were suspended in a 1:1 mixture of DMF and H_2O (20 mL) together with $NaHCO_3$ as the base (3 equivalent) and $PdCl_2(PPh_3)_2$ (5 mol%) and PPh_3(10 mol%) as a catalyst in a 20 mL sealed glass vial. The mixture was irradiated at 150°C for 10 min using a maximum power level of 90W (Larhed *et al.*, 1996a, Larhed *et al.*, 1996b). The product was purified using column chromatography (silica 60) with 20% of ethyl acetate in *n*-pentane. The product (**93**) was isolated in 85% (162 mg) yield.



3.6.3.6 4-(3',4',5'-trimethoxyphenyl)rubiadin-1-methyl ether (94)

4-Bromorubiadin-1-methyl ether (88) (100 mg, 0.29 mmol) and 3,4,5-trimethoxyphenylboronic acid (65, 0.30 mmol) were suspended in a 1:1 mixture of DMF and H_2O (20 mL) together with $NaHCO_3$ as the base (3 equivalent) and $PdCl_2(PPh_3)_2$ (5 mol%) and PPh_3(10 mol%) as a catalyst in a 20 mL sealed glass vial. The mixture was irradiated at 150°C for 10 min using a maximum power level of 90W (Larhed *et al.*, 1996a, Larhed *et al.*, 1996b). The product was purified using RP-HPLC (eluent: water/acetonitrile). The product (**94**) was isolated in 87% (87 mg) yield.

3.6.4.1 4-(Phenylethynyl)rubiadin (95)

4-Bromorubiadin (**87**) (150 mg, 0.45 mmol), $PdCl_2(PPh_3)_2$ (5 mol%), PPh_3 (10 mol%) and phenyl acetylene (0.5 mmol) were dissolved in a 20 mL of DMF together with diethyl amine as the base (0.75 mL, 5.42 mmol) and CuI (5 mol%) in a 20 mL sealed glass vial. The mixture was irradiated at 150°C for 35 min using a maximum power level of 90W. The product was purified using column chromatography (silica 70-230 mesh) with 100% dichloromethane. The product (**95**, 124mg) was isolated in 83% yield.

3.6.5 4-chlororubiadin-1-methyl ether (96) through halogen exchange reaction

4-Bromorubiadin-1-rubiadin methyl ether (**88**) (60 mg, 0.17 mmol) was mixed with $NiCl_2$ (38mg, 0.3mmol) and the mixture were dissolved in a 20 mL of DMF and irradiated at 150°C for 50 min using a maximum power level of 90W (Sheppard, 2009). The product was purified using using RP-HPLC (eluent: water/acetonitrile). The product (**96**, 55mg) was isolated in 92% yield.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Preliminary Screening of Six *Pentas* Extracts

Pentas species are among the most widely used plants to treat malaria in Kenya. The roots of six *Pentas* species from Kenya were separately extracted with CH_2Cl_2:CH_3OH (1:1) and tested for antiplasmodial activity against the D6 (CQ sensitive) and W2 (CQ resistant) strains of *Plasmodium falciparum*. Three of the *Pentas* species namely *P. lanceolata*, *P. longiflora* and *P. micrantha* showed good activity (< 4 µg/mL) for crude (CH_2Cl_2:CH_3OH, 1:1) extract (Table 4.1). The activity observed can be interpreted as good based on WHO guideline which states plant extracts with IC_{50} of less than 10 µg/mL as good activity where as those with IC_{50} of 11-50 µg/mL as moderately active (Fidock *et al.*, 2004).

Table 4:1: Antiplasmodial activity of the root extracts of six *Pentas* species

Plant (root)	Activity (IC_{50}, µg/mL)	
	D6	W2
P. bussei	49.04 ± 28.73	49.86 ±0.00
P. lanceolata	1.33 ± 0.15	2.55 ± 0.30
P. longiflora	0.99 ± 0.09	0.93 ± 0.16
P. micrantha	4.00 ± 1.86	3.37 ± 0.74
P. parvifolia	19.46 ± 2.05	14.23 ± 2.89
P. suswaenesis	12.11 ± 2.00	11.13 ± 1.62

All the six *Pentas* species were subjected to chromatographic fractionation which resulted in the isolation and characterization of dihydronaphthoquinones, pyranonaphthoquinones and anthraquinones as discussed below in detail.

4.2 Secondary Metabolites from *Pentas bussei*

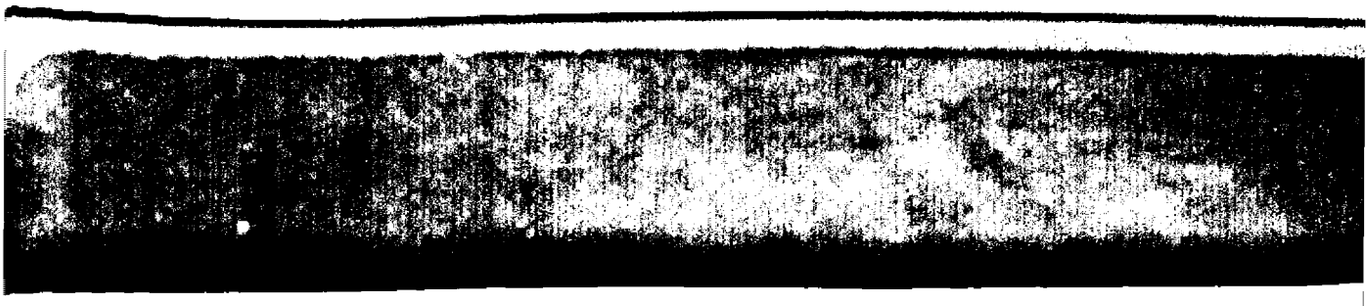
4.2.1 Dihydronaphthoquinones

Chromatographic separation of the root extracts of *P.bussei* led to the isolation and identification of five dihydronaphthaquinone derivatives (**56**, **70-73**) of which four (**70-73**) are new natural products (Endale *et al.*, 2012b). The known dihydronaphthaquinone derivative, methyl-5,10-dihydroxy-7-methoxy-3-(4-methyl-3-pentenyl)-3H-benzo(f) chromene-9-carboxylate (**56**) was also isolated as previous, from the root of the same plant (Bukuru *et al.*, 2003).

Figure 4.1: Photograph of *Pentas bussei* (Patrick Chalo Mutiso, Mombasa, July 2010)

4.2.1.1 Busseihydroquinone A (70)

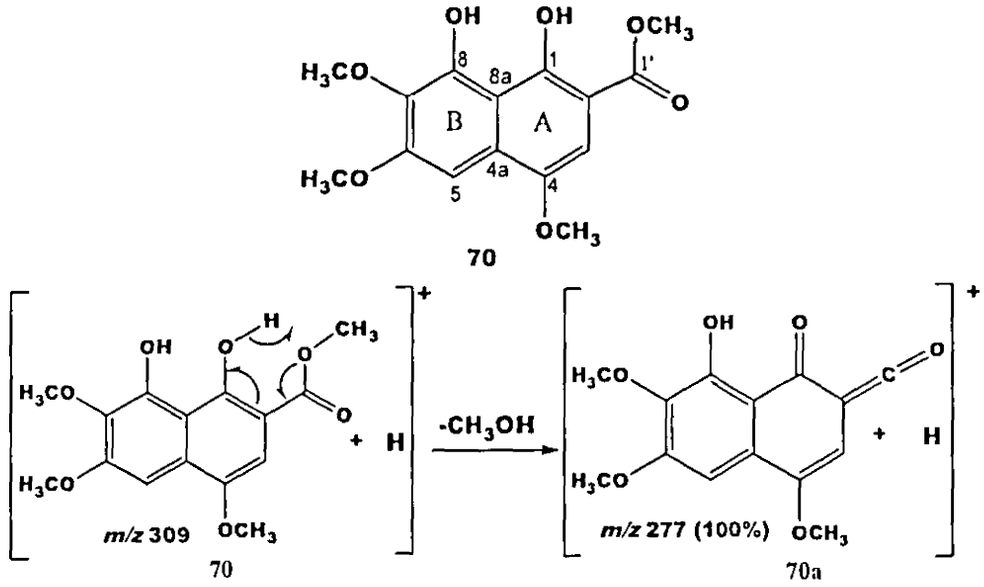
Busseihydroquinone A (**70**) was isolated as brown needle-like crystals from CH_3OH (mp 171.5 °C). The HRMS provided an exact mass of *m/z* 309.2300 $(M + H)^+$ suggesting a molecular formula of $C_{15}H_{16}O_7$. The UV (λ_{max} 250, 285 nm) and IR (ν_{max} 3412, 1637,



1618, 1522, 1400 cm^{-1}) spectra were compatible with the proposed naphtalene skeleton (Bukuru *et al.*, 2002; Silverstien, 1998). In agreement with this the ^{13}C NMR (Table 4.2) revealed ten sp^2 hybridized carbon atoms of naphthalene. Presence of six substituents: methyl ester (δ_C 173.5 for C=O, δ_C 52.4/δ_H 3.94 for OCH_3), three methoxyl (δ_H 3.91, 3.90, 3.76) and two hydroxyl (δ_H 9.70, 12.44) groups were evident from NMR (Tables 4.2) and MS data. The downfield chemical shift values of the hydroxyl groups indicated that both are involved in interamolecular hydrogen bonding suggesting the placement of the two hydroxyl groups at C-1 and C-8 positions and the methyl ester at C-2. The singlet aromatic proton at δ_H 6.94 showed HMBC correlation with two oxygenated quaternary carbons (δ_C 149.6 and δ_C 157.8) and the ester carbonyl carbon (δ_C 173.5), allowing its assignment to H-3 of ring A which is oxygentated at C-1 and C-4 with the methyl ester being at C-2. The methoxy at δ_H 3.90 showed HMBC correlation with oxygenated quaternary carbon at δ_C 157.8 and hence placed at C-4. The only other singlet proton is located in ring B which also contains the hydroxyl (at C-8) and two methoxyl groups (δ_C 55.9 and δ_C 60.8). The methoxyl signal at δ_C 60.8 is down field shifted indicating that it is di-*ortho* substituted and placed at C-7 being between hydroxyl (at C-8) and methoxyl (δ_C 55.9 at C-6); consequently the singlet at δ_H 7.05 is H-5.

The upfeild chemical shift of C-7 (δ_C 138.0) is in agreement of it being between two oxygenated sp^2 carbon atoms. The presence of vicinal hydroxyl groups and an ester carbonyl leads to a ketene ion ("*ortho* effect") after expulsion of methanol from the molecular ion peak $(M + H)^+$ (Silverstein, 1998). Consequently, the base peak (100%) displayed at *m/z* 277.1 is due to the fragment ketene ion (**70a**). This compound has not been previously reported was therefore characterized as methyl-1,8-dihydroxy-4,6,7-

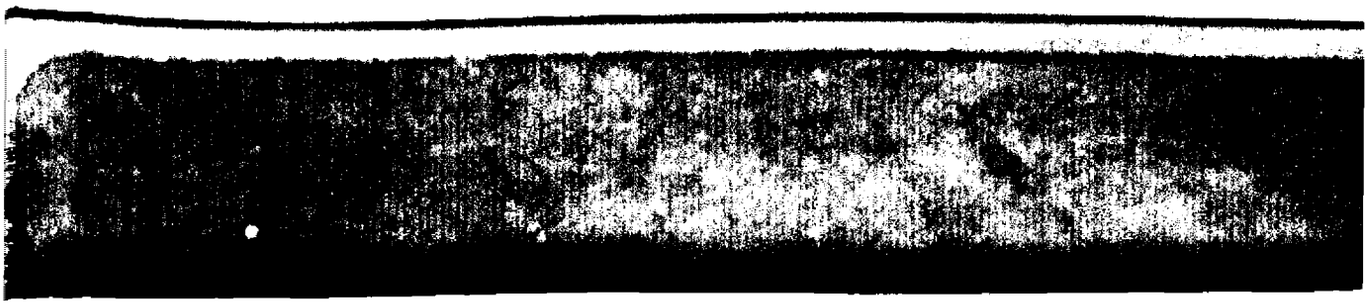
trimethoxy-2-naphthoate (70) and assigned the trivial name busseihydroquinone A (70) (Endale *et al.*, 2012b).



Scheme 4.1: Mass fragmentation of busseihydroquinone A (70) to ketene ion (70a)

Table 4.2: ^{1}H NMR/^{13}C NMR (600/150 MHz) and HMBC spectral data for Busseihydroquinone A (70) in DMSO-d_6.

Position	δ_H (J in Hz)	δ_C	HMBC (^2J, ^3J)	Position	δ_H (J in Hz)	δ_C	HMBC (^2J, ^3J)
1	-	157.8	-	6	-	158.6	-
2	-	130.2	-	6-OCH_3	3.76	55.9	C-6
3	6.89	99.97	C-1, 2, 4, 4a, 1'	7	-	138.0	-
4	-	149.6	-	8	-	158.0	-
4a	-	130.2	-	8a	-	113.1	-
5	7.05	96.6	C-4, 4a, 6, 8a, 7	8-OH	9.55	-	C-8
4-OCH_3	3.90	55.6	C-4	1-OH	12.44	-	C-1
7-OCH_3	3.91	60.8	C-7	2-$COOCH_3$	3.94	52.4	C-1'
				2-$COOCH_3$	-	173.5	-



4.2.1.2 Busseihydroquinone B (71)

Busseihydroquinone B (71) was isolated as a yellow powder. The HRMS spectrum revealed the molecular ion peak *m/z* 299.0996 $(M-H)^+$ attributed to the molecular formula $C_{17}H_{16}O_5$. The UV (λ_{max} 255, 265, 280 nm) and IR (ν_{max} 3547, 3415, 1637, 1618, 1522, 1399 cm^{-1}) absorptions indicated a naphthalene skeleton where ring A is substituted with hydroxyl at C-1 (δ_H 12.40), carboxylic acid at C-2 (δ_C 172.5), and methoxyl at C-4 (δ_H 3.86, δ_C 55.6) (Table 4.3). The HMBC spectrum showed correlation of H-3 with C-1, C-2, C-4, C-4a and the carboxylic acid carbonyl carbon, as well as the correlation of the methoxyl protons with C-4 confirmed the substitution pattern in this ring. In ring B the presence of two *ortho*-coupled protons at δ_H 7.11 (1H, *d*, J = 8.2 Hz) and δ_H 8.12 (1H, *d*, J = 8.2 Hz) suggested that di-substituted either at C-5 and C-6 or C-7 and C-8. HMBC correlation of the signal at δ_H 8.12 with C-1 (δ_C 154.9) showd that this signal is for H-8 (and hence the signal at δ_H 7.11 is for H-7). The coupling pattern confirmed the substituents in ring B is at C-5 and C-6. The substituent was identified as 2',2'-dimethylchromene group as evident from the ^1H [δ_H 7.64, *d*, J = 9.1 Hz, H-4' and δ_H 5.72, *d*, J = 9.1 Hz, H-3', δ_H 1.44 for 2',2'-$(CH_3)_2$] and ^{13}C NMR data (Table 4.3) spectrum. Busseihydroquinone B (71) was therefore characterized as 5,6-(2',2'-dimethylpyrano)-1-hydroxy-4-methoxy-2-naphthoic acid, has not been reported before. It was assigned the trivial name busseiydroquinone B (71) (Endale *et al.*, 2012b).

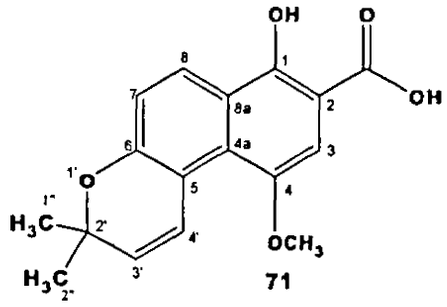


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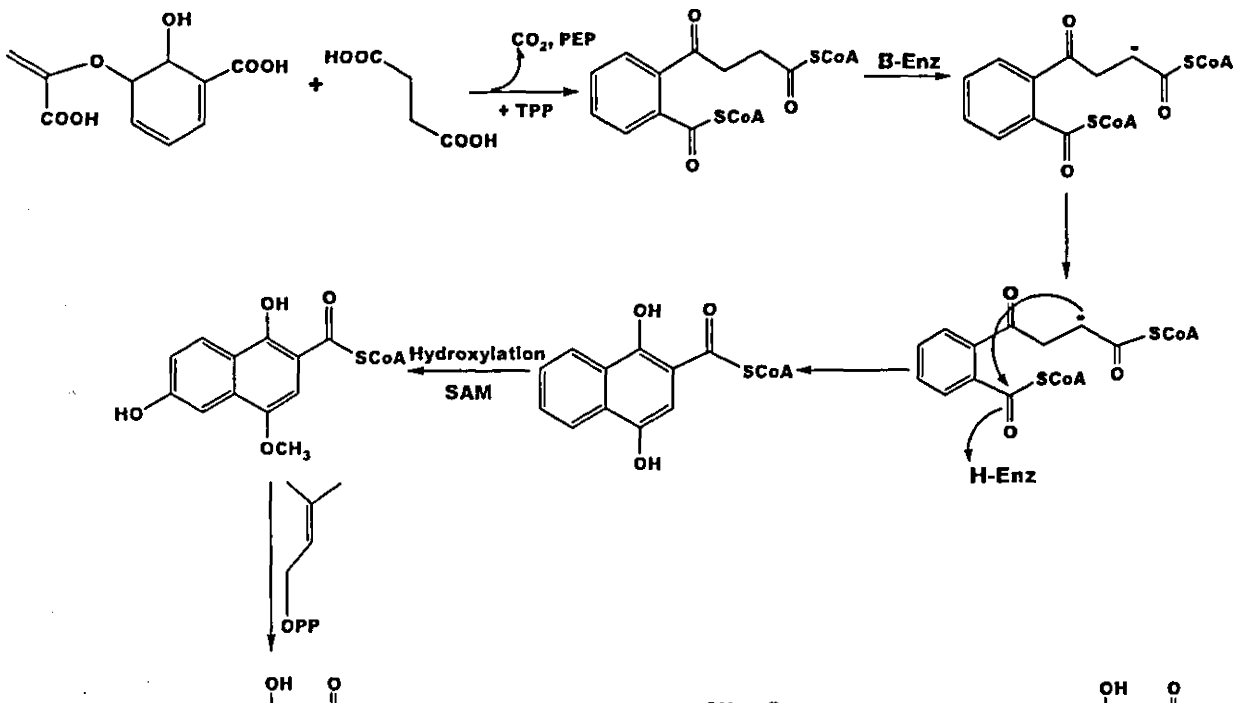
Table 4.3: ^{1}H (600 MHz), ^{13}C NMR (150 MHz) NMR and HMBC spectral data for Busseihydroquinone B (**71**) in DMSO-d_6

sition	δ_H (J in Hz)	δ_C	HMBC (^2J,^3J)	Position	δ_H (J in Hz)	δ_C	HMBC (^2J,^3J)
	-	154.9	-	**1''**	1.44 (*s*, 3H)	27.0	C-2', 2'',3'
	-	104.2	-	**2''**	1.44 (*s*, 3H)	27.0	C-2', 1'', 3'
	7.07 (*s*)	102.9	C-1, 2, 4, 4a, 2-COOH	**1-OH**	12.40	-	C-1
	-	148.5	-	**2-COOH**	-	172.5	-
	-	125.8	-	**4-OCH_3**	3.86	55.6	C-4
	-	114.3	-				
	-	154.2	-				
	7.11 (*d*, *J*=8.19)	118.3	C-5, 6, 8, 8a				
	8.12 (*d*, *J*=8.19)	124.9	C-1, 4a, 6, 7, 8a				
	-	120.8	-				
	-	75.3	-				
	5.68 (*d*, *J*=9.2)	128.2	C-1'', 2'', 2', 4'				
	7.64 (*d*, *J*=9.2)	122.1	C-2', 3', 5, 6, 4a				

Busseihydroquinone B (**71**) could have been biosynthesized via shikimic acid pathway and acetyl CoA as extender unit. Deprotonation followed by cylization to form aromatic system (ring A and B) occur as shown in the Scheme 4.2 below. Hydroxylation followed by prenylation and cyclization leads to pyran ring on ring B of the naphthalene skeleton.



Scheme 4.2: Proposed biogenesis pathway for busseihydroquinone B (**71**)

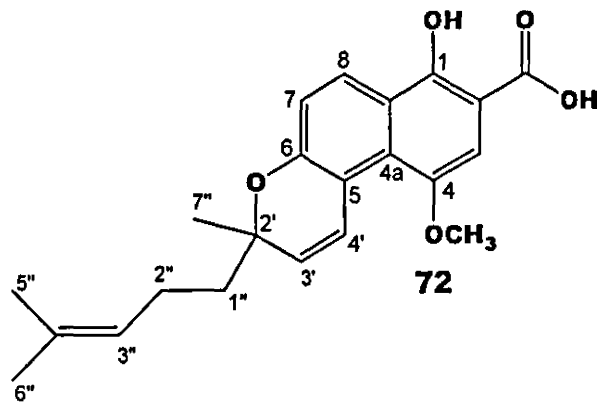
4.2.1.3 Busseihydroquinone C (72)

Busseihydroquinone C (**72**) was isolated as amorphous powder. The HRMS spectrum revealed the exact mass at *m/z* 369.1680 $(M+H)^+$ attributed to the molecular formula of $C_{22}H_{24}O_5$. The UV-VIS (λ_{max} 255, 275, 280), IR (ν_{max} 3550, 3413, 3236, 1638, 1617 cm^{-1}) absorptions and the ^1H and ^{13}C NMR spectral data (Table 4.4) indicated a naphthalene

skeleton as in busseidihydroquinones A (**70**) and B (**71**) (Silverstein, 1998). The NMR data (Table 4.4) suggested that ring A, as in busseidihydroquinones B (**71**), is substituted with hydroxyl at C-1 (δ_H 12.26), carboxylic acid at C-2 (δ_C 172.5), methoxyl at C-4 (δ_H 3.93, δ_C 55.8). The HMBC correlation observed between the singlet proton at δ_H 7.06 (*s*, H-3) with the carbonyl carbon at δ_C 172.5, δ_C 155.0 (C-1) and 148.5 (C-4) as well as the correlation of the methoxy protons (δ_H 3.93) with δ_C 148.5 (C-4) confirmed the substitution pattern in ring A. The location of the methoxy group at C-4 was also supported by its NOE interaction with H-3 from the gNOESY spectrum.

The ^{1}H NMR spectral data (Table 4.4) showed a pair of *ortho*-coupled doublets at δ_H 7.11 and δ_H 7.64 (J = 7.7 Hz) suggesting ring B of the naphthalene skeleton is disubstituted. The signal at δ_H 7.64 showed HMBC correlation with the oxygenated quaternary carbon at δ_C 155.0 (C-1) and hence was assigned to H-8. Consequently, the second doublet (δ_H 7.09) was assigned to H-7. Thus ring B is substituted at C-5 and C-6. In busseihydroquinone C (**72**), however, the substituent at these carbons is formed upon cyclization of a geranyl group at C-5 with the hydroxyl group at C-6 (δ_C 154.4). Thus a pair of mutually coupled doublets (δ_H 5.67 and δ_H 8.12, J = 9.11 Hz) for H-3' and H-4' respectively along with a sp^3 hybridized oxygenated quaternary carbon at δ_C 77.3 (C-2'), in the NMR spectra, are consistent with the presence of pyran ring at C-5/C-6. The HMBC correlation of the oxygenated quaternary carbon at δ_C 154.4 (C-6) with δ_H 7.09 (H-7) and δ_H 8.12 (H-8) as well as between H-3' and C-5 (δ_C 114.3) is consistent with a pyran substituted at C-5/C-6 positions of ring B. On the pyran ring, the ^{1}H and ^{13}C NMR spectra (Table 4.4) showed that there are a methyl (δ_H 1.45, δ_C 25.4) and a 4-methyl-pent-3-enyl substituents at C-2' of the pyran ring, which is expected as the result of cyclization

of geranyl group producing the pyran ring. NOE cross-peaks between H-3 and 4-OCH_3 as well as H-3' and 4-OCH_3 revealed the annulation of ring C (2,2-dimethylpyran) to ring B, forming a 3H-benzo[f]chromene skeleton. The NOESY and HMBC data (Supporting Information) allowed unambiguous assignment of all functionalities, including determination of the position of the dimethyl substituents of the C ring. Thus, by combination of the above spectroscopic evidence this new compound was characterized as 7,8-(2'-methylpyrano-2'-(4"-methyl-3"-pentenyl)-1-hydroxy-4-methoxy-2-naphthoic acid (**72**) for which the trivial name busseihydroquinone C is assigned (Endale *et al.*, 2012b).



72

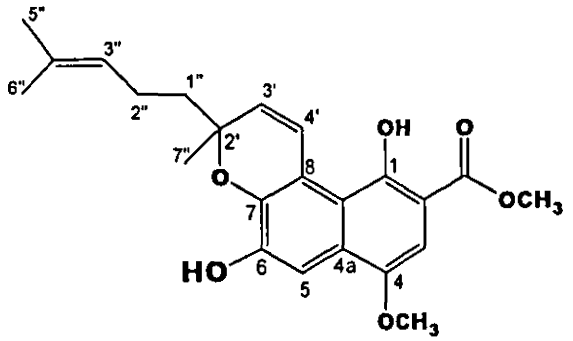
Table 4.4: ^{1}H (600 MHz) and ^{13}C (125 MHz, DMSO-d_6) NMR data along with HMBC correlations for busseihydroquinone C (**72**) in DMSO-d_6.

osition	δ_H (*J* in Hz)	δ_C	HMBC	Position	δ_H (*J* in Hz)	δ_C	HMBC
	-	155.0	-	1"	1.66-1.70, *m*	39.9	C-2", 7", 2', 3'
	-	102.9	-	2"	2.04-2.08, *m*	22.2	C-2', 1", 3"
	7.06	104.3	C-1, 2, 4, 4a, 2-COOH	3"	5.05, *t*	123.9	C-2", 5",6"
	-	148.5	-	4"	-	130.9	C-2", 3", 5",6"
a	-	124.9	-	5"	1.50, *s*	17.4	C-3", 6"
	-	114.3	-	6"	1.59, *s*	25.3	C-3", 5"
	-	154.4	-	7"	1.45, *s*	25.4	C-2', 1", 3'
	7.09 (*d*, *J*= 7.7)	118.2	C-5, 6, 8,8a	2-COOH	-	172.5	-
	7.69 (*d*, *J*= 7.7)	122.5	C-4a, 5,6, 7,8a	4-OCH_3	3.93, *s*	55.8	C-4
a	-	120.8	-	1-OH	12.26, *s*	-	C-1
'	-	77.3	-				
'	5.67 (*d*, *J*= 9.11)	127.3	C-1", 7", 2', 4'				
'	8.11 (*d*, *J*= 9.11)	125.9	C-2', 3', 7, 8, 8a				

4.2.1.4 Compound 56

Compound **56** was isolated as a yellow powder. The UV-VIS (λ_{max} 255, 272, 290) and the NMR spectral data (Table 4.5) once again revealed a naphthalene skeleton. The HRMS spectrum provided exact mass of *m/z* 399.1808 $(M + H)^+$, ESI-MS *m/z* 399.3, attributed to a molecular formula of $C_{23}H_{27}O_6$. Ring A was substituted with chelated hydroxyl (δ_H 12.26) at C-1, methyl ester (δ_H 3.94, δ_C 55.6 for CH_3; δ_C 174.6 for carbonyl) at C-2, methoxyl (δ_H 3.86, δ_C 58.7) at C-4. The location of the methoxyl at C-4 was established from the gNOESY spectrum where NOE interaction of the methoxyl protons with H-3 was observed. The HMBC correlation of H-3 with C-1, C-2, C-4 and the carbonyl of the methyl ester confirmed the substitution pattern in this ring. As in

busseihydronaphthoquinone C (72), the ^{1}H and ^{13}C NMR (Table 4.5) suggested that ring B was substituted with 2'-methyl-2'-(4'-methyl-3'-pentenyl)chromene at C-7/C-8. The singlet aromatic proton at δ_H 7.41 was assigned to H-5 as it showed HMBC correlation with C-4; which requires substituent at C-6 which is a hydroxyl group (δ_C 146.6). With the combination of the above spectroscopic evidence, the compound was identified as methyl-1,5-dihydroxy-4-methoxy-2'-methyl-2'-(4'-methyl-3'-pentenyl)-2'*H*-benzo(*f*)-chromene-2-carboxylate (**56**) previously reported from the roots of *Pentas bussei* (Bukuru *et al.*, 2002).



56

Table 4.5: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data along with HMBC correlations in compound **56** (DMSO-d_6)

Position	δ_H	δ_C	HMBC (^2J,^3J)	Position	δ_H	δ_C	HMBC (^2J,^3J)
	-	159.5	-	1''	1.66-1.70 (2H, *m*)	32.1	C-2', 3', 2", 3"
	-	127.1	-	2''	2.04-2.08 (2H, *m*)	28.5	C-2', 3",1"
	6.88, *s*	101.9	C-1, 2, 4, 4a, 2-COOH	3''	5.08 (1H, *t*)	120.6	C-1", 2", 5", 6"
	-	149.2	-	4''	-	131.5	-2", 3", 5", 6"
a	-	134.1	-	5''	1.51 (3H, *s*)	25.2	C-6", 3"
	7.41, *s*	105.7	C-4a, 6, 7	6''	1.60 (3H, *s*)	20.5	C-3", 5"
	-	146.6	C-4a, 5, 7	7''	1.39 (3H, *s*)	28.0	C-2', 1", 3'
	-	152.4	-	4-OCH_3	3.86, *s*	58.7	C-4
	-	131.5	-	2-$COOCH_3$	3.94, *s*	55.6	C-(2-COOH)
a	-	129.4	-	2-$COOCH_3$	-	174.6	-
*	-	80.2	-	1-OH	12.26, *s*	-	C-1
*	' (*d*, *J*=8.8)	125.7	C-2', 1", 7", 4', 8				
*	84 (*d*, *J*=8.8)	117.0	C-2', 3', 7, 8, 8a				

4.2.1.5 Busseihydroquinone D (73)

Busseihydroquinone D (**73**) was isolated as an orange solid. The HRMS revealed the molecular ion peak at *m/z* 379.1220 $(M-H)^+$ attributed to a molecular formula of $C_{22}H_{20}O_6$. The UV-VIS (λ_{max} 255, 275, 300), IR (ν_{max} 3550, 3413, 3236, 1654, 1638, 1618, 1522, 1450, 1400 cm^{-1}) absoprtions and the NMR spectral data (Table 4.6) revealed a naphthalene skeleton. Comparison of the ^{1}H and ^{13}C NMR spectra of **73** (Table 4.6) with those of busseihydroquinone C (**72**) showed identical ring A, substituted with chelated hydroxyl (δ_H 12.00) at C-1, carboxylic acid (δ_C 172.3) at C-2 and methoxyl (δ_H 3.88, δ_C 55.9) at C-4. The identity of this ring was confirmed once again from the HMBC

spectrum, where the singlet aromatic proton at δ_H 7.14 (H-3) showed HMBC correlations with two of the oxygenated quaternary carbons (at δ_C 154.9, 148.0), carboxylic acid carbonyl (δ_C 172.3) and two quaternary carbons (δ_C 105.5, δ_C 126.9). Furthermore, the methoxy group (δ_H 3.88) showed HMBC correlation with the oxygenated quaternary carbon at δ_C 148.0 confirming its placement at C-4 (Table 4.6). In ring B, the presence of two *ortho*-coupled protons at δ_H 7.21 and δ_H 8.22 (J = 8.7 Hz), indicated di-substitution in this ring. HMBC correlation of the doublet at δ_H 8.22 with δ_C 154.9 (C-1) allowed its assignment to H-8 placed which is *peri* to the hydroxyl group at C-1. This requires that the substituent in this ring be at C-5/6 with oxygenation at C-6 (δ_C 154.2). From the MS and ^{13}C NMR (δ_C 21.0, 81.9, 137.2, 126.4, 37.3, 25.9, 152.6, 129.9, 11.8, 191.7) the ten carbon atoms have not been accounted for, suggesting that the substituent at C-5/6 could be cyclized geranyl derivative with the initial cyclization product probably being 2'-methylpyrano-2'-(4"-methyl-3"-pentenyl), as in busseihydroquinone C (**72**) and the known compound **56**, which up on further modification gives busseihydroquinone D (**73**).

Similar biogenetic relationship has been proposed between the cyclol derivative **58** and **56**, both reported from this plant (Bukuru *et al.*, 2002, Bukuru *et al.*, 2003). The singlet at δ_H 8.27 showed HMBC correlation with C-4a (δ_C 126.5), C-5 (δ_C 115.5) and C-6 (δ_C 154.2) of ring B as well as with C-2' (δ_C 81.9) and a quaternary sp^2 hybridized carbon at δ_C 137.2 (C-3'), indicating the presence of a chromene substituent at C-5/6. Consequently, the singlet at δ_H 8.27 was assigned to H-4'. That H-4' appeared as a singlet and that C-3' is a quaternary carbon suggested substitution at C-2' and C-3' of the chromene ring as a result of further cyclization involving C-3' to form cyclopentane ring (Bukuru *et al.*, 2003). In this ring, the ^{1}H NMR spectrum showed two mutually coupled methylene groups

at δ_H 2.3 and 2.2 (δ_C 37.3) and 2.2 and 3.3 (δ_C 25.9) assignable to CH_2-1" and CH_2-2" respectively. These signals showed HMBC correlation with δ_C 81.9 (C-2') and 152.6 (C-3") confirming the identity of this ring. Additional NMR signals were observed at δ_H 9.98 and δ_C 191.7 for aldehyde; δ_C 129.9 for quaternary sp^2 carbon C-4" and δ_H 2.05 and δ_C 11.8 for methyl group C-5") showed the presence of 1-oxopropene-2-ylidene substituent at C-3" of the cyclopentane ring. The HMBC correlation between the methyl group (δ_H 2.05) and the aldehyde carbonyl carbon (δ_C 191.7) and an sp^2 quaternary carbon at δ_C 129.9 (C-4") coupled with the correlation between the aldehyde proton at δ_H 9.98 with C-3" (δ_C 152.6), C-4" (δ_C 129.9) and δ_C 5"-CH_3 (11.8) supported the proposed structure. The NOE interaction (Fig. 4.2), between H-4' with 4-OCH_3 confirmed that the carbon chain is attached at C-5 and oxygen at C-6. Further NOE interactions were observed between the 4-OCH_3 and 5"-CH_3 and also between the aldehyde proton with CH_2-2" establishing *E*-geometry at the 3'-4' double bond. The circular dichroism (CD) spectra did not show any observable cotton effects in the 250-600 nm region; neither was significant optical rotation observed (+ 0.9°C). This indicated that compound occur as racemic mixtures in the analyzed plant sample. Therefore this new compound was characterized as 2',3'-(3"-(1-oxopropene-2-ylidene)-cyclopentane)-1-hydroxy-4-methoxy-2'-methyl-3*H*-benzo(f)chromene-2-carboxylic acid (**73**) for which the trivial name busseihydroquinone D is assigned (Endale *et al.*, 2012b). It is likely that this compound was formed from busseihydroquinone C (**72**) through oxidation of the allylic methyl group followed by Michael-type cyclization as shown in scheme 4.3.

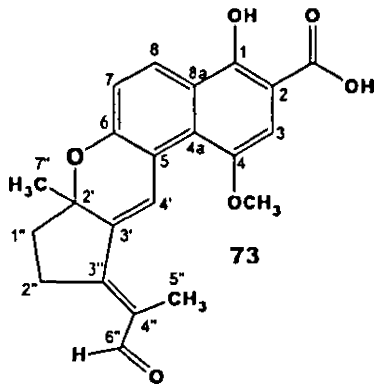
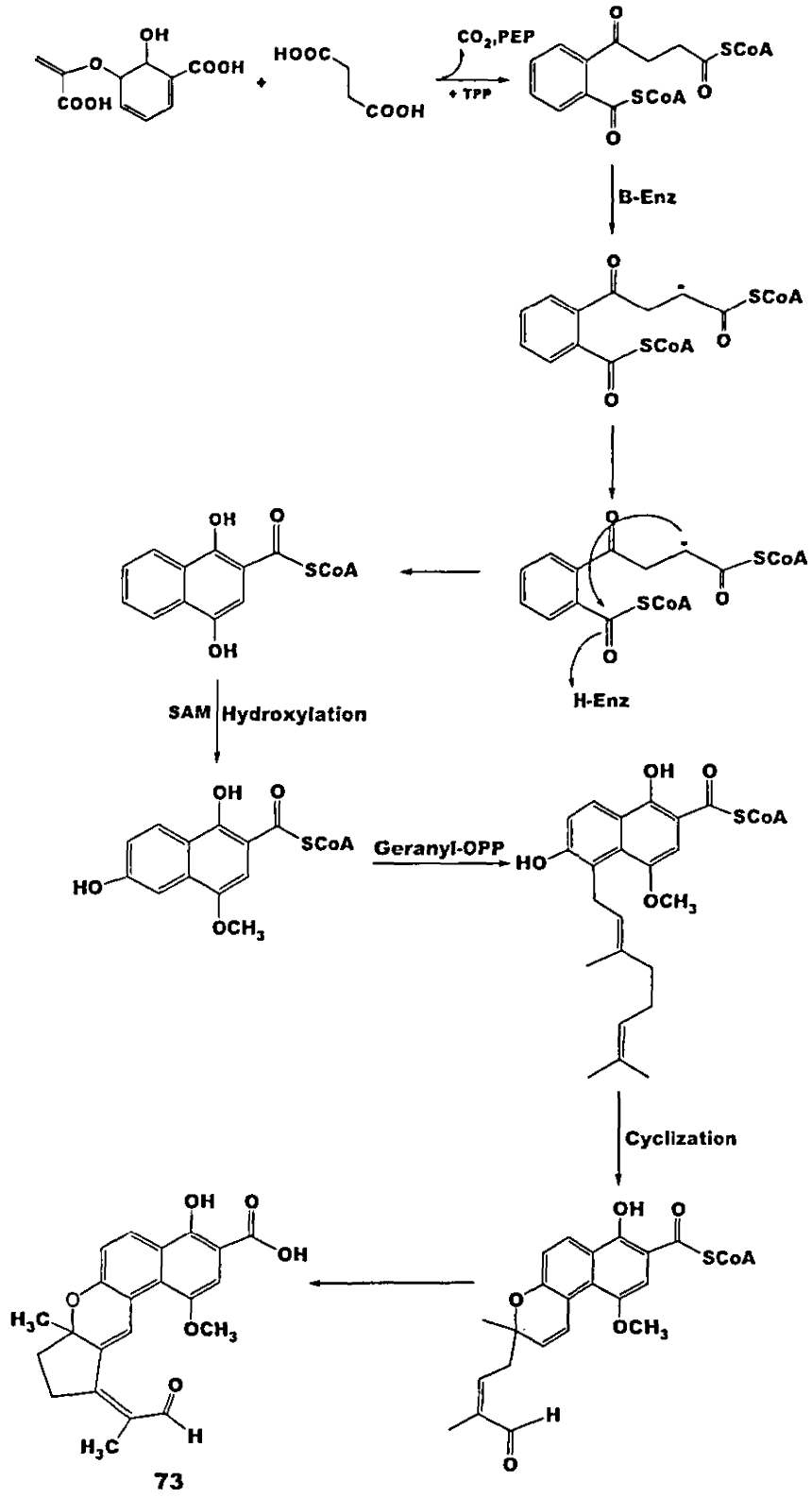


Table 4.6: ^{1}H (600 MHz), ^{13}C (125 MHz) NMR and HMBC data of busseihydroquinone (73) (DMSO-d_6)

Position	δ_H (J in Hz)	δ_C	HMBC (^2J, ^3J)
1	-	154.9	-
2	-	104.2	-
3	7.16, *s*	105.5	C-1,2, 4, 4a, 2-COOH
4	-	148.0	-
4a	-	126.5	-
5		115.5	-
6	-	154.2	-
7	7.23, *d* (J = 7.18)	118.1	C-5, 6, 8, 8a
8	8.23, *d* (J = 7.18)	126.9	C-7, 6, 8a, 4a
8a	-	121.2	-
2'	-	81.9	-
3'	-	137.2	-
4'	8.27, *s*	126.4	C-4a, 5, 6, 3', 2', 3"
1"	2.3, *m*	37.3	C-2', 2",7", 3"
	2.2, *m*		
2"	2.86, *m*	25.9	C-1", 3", 4"
3"	-	152.6	-
4"	-	129.9	-
5"	2.05, *s*	11.8	C-3", 4", 6"
6"	-	191.7	C-3", 4", 5"
7"	1.32, *s*	21.0	C-2', 3', 1"
2-COOH	-	172.4	-
4-OCH_3	3.89, *s*	55.9	C-4



Scheme 4.3: Possible biogenesis pathway towards busseihydroquinone D (73)

NOE interactions between H-4' and 4-OCH_3, H-5" and 4-OCH_3, H-5" and H-4', H-3 and 4-OCH_3 were used to acertain the position of the annulations to be at C-5/6 (Figure 4.2).

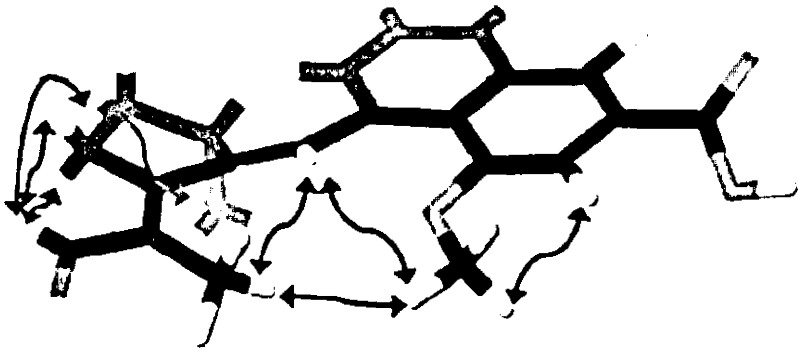


Figure.4.2: Key NOE interactions in the NOESY spectrum of busseihydroquinone D (**73**), mixing time 700ms, DMSO-d_6, 25°C, 800 MHz.

The phytochemical analysis on the roots of *P. bussei* afforded five compounds, one polyoxygenated naphthalene derivative (**70**) and four dihydronaphthoquinones (**56**, **71-73**). Four of the isolated compounds (**70-73**) have been found to be new natural products (Endale *et al.*, 2012b).

The compounds isolated from the roots of *Pentas bussei* are listed as follows (Figure 4.3).

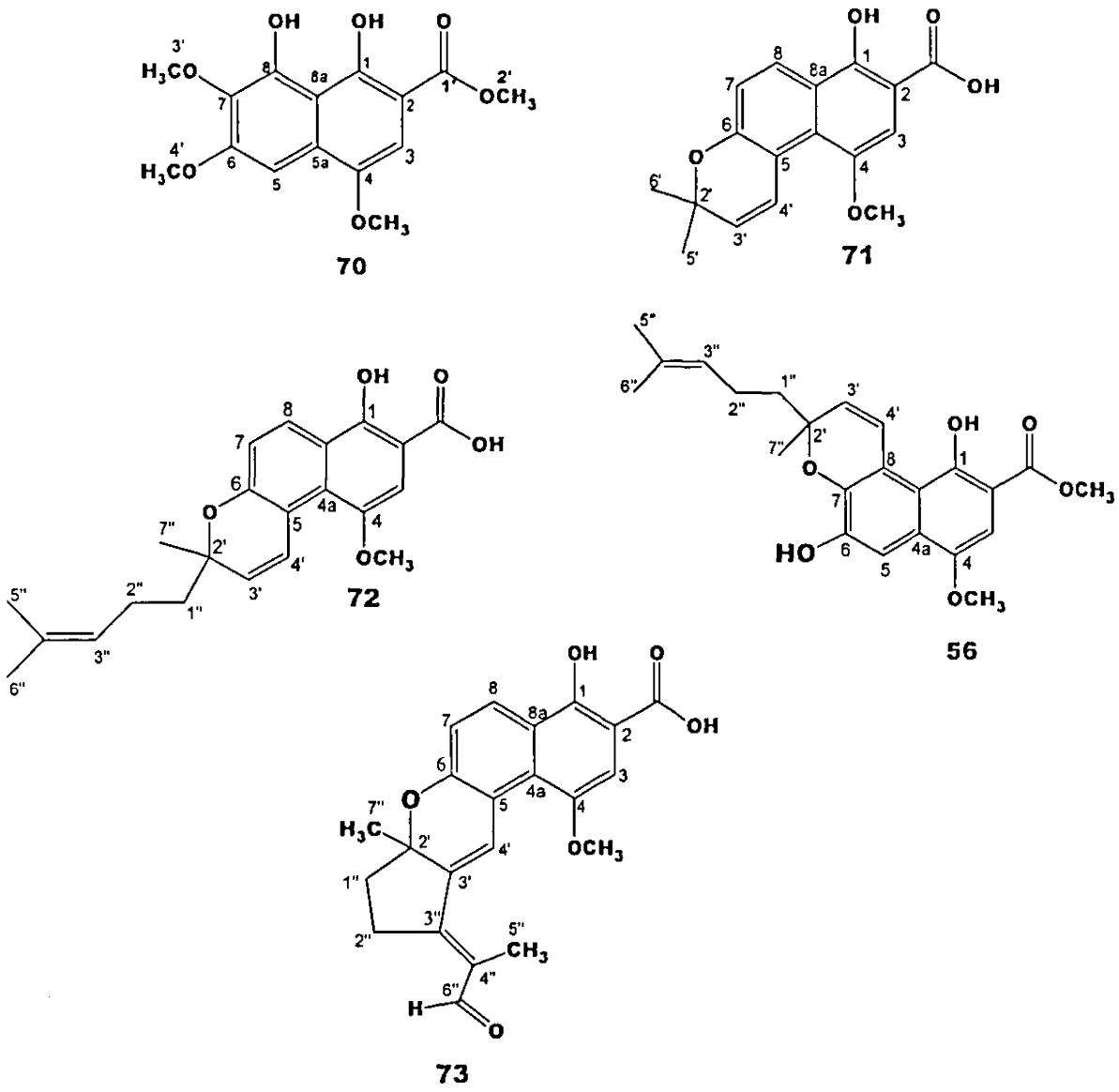


Figure 4.3: Dihydronaphthoquinones isolated from the roots of *Pentas bussei*

4.3 Secondary metabolites isolated from *Pentas lanceolata*

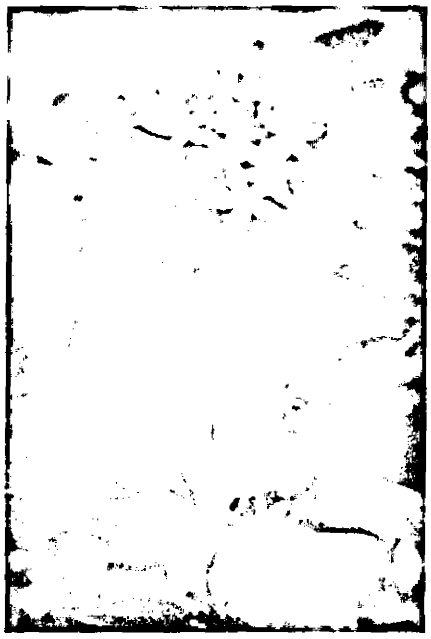


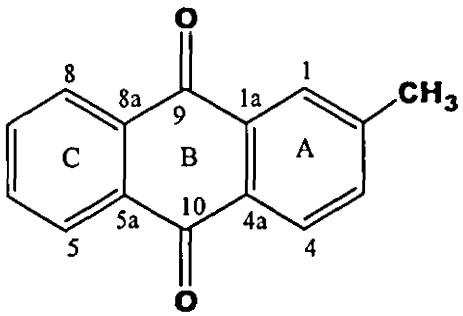
Figure 4.4: Photograph of *Pentas lanceolata* (Milkyas Endale, Nairobi, July 2010)

4.3.1 Anthraquinones and anthraquinone glycosides

Chromatographic separation of the roots of *Pentas lanceolata* led to the isolation and characterization of twelve anthraquinones of which one of them (**78**) is a new natural product. The known anthraquinones include: tectoquinone (**74**), rubiadin (**49**), rubiadin-1-methyl ether (**51**), lucidin-ω-methyl ether (**60**), nordamnacanthal (**76**), damnacanthal (**50**), damnacanthol (**59**), 5,6-dihydroxydamnacanthol (**79**), rubiadin-3-*O*-primerveroside (**52**), and rubiadin-1-methyl ether-3-*O*-primerveroside (**53**) and damnacanthol-ω-methyl ether (**77**). Damnacanthol (**59**), lucidin-ω-methyl ether (**60**) and nordamnacanthal (**76**) were isolated for the first time from the genus *Pentas* (Endale *et al.*, 2012a).

4.3.1.1 Tectoquinone (74)

Tectoquinone (74) was isolated as an orange solid. The ESI-MS provided a molecular ion peak of *m/z* 223.2 $(M + H)^+$, which was attributed to the molecular formula of $C_{15}H_{10}O_2$. The UV-VIS absorption maxima at 275 and 405 nm suggested the presence of an anthraquinone chromophore (Scott, 1964; Liu *et al.*, 2008). ^1H NMR spectral data (Table 4.7) revealed four aromatic protons with AA'XX' spin system at δ_H 8.39 (2H, *m*) and δ_H 7.87 (2H, *m*) suggesting ring C of the anthraquinone skeleton is unsubstituted (Rycroft *et al.*, 1997). Additional three non-equivalent aromatic protons with ABX spin system were observed at δ_H 8.29 (*d*, *J*= 8.1 Hz), δ_H 8.19 (*d*, *J*= 2.4 Hz), δ_H 7.80 (*dd*, *J*= 2.4, 8.1 Hz) suggesting that ring A of the anthraquinone is monosubstituted at C-2 with a methyl group (δ_H 2.62 (*s*, 3H)) as the other anthraquinones of Rubiaceae (Han *et al.*, 2001). In agreement with the ^1H NMR spectrum, the ^{13}C NMR spectrum (Table 4.8) showed the presence of fifteen carbons: seven aromatic methines (δ_C 135.3, 134.4, 134.2, 127.8, 127.5 and 127.7), two carbonyl carbons (δ_C 183.8 and δ_C 183.3), five aromatic quaternary carbons (δ_C 145.6, 133.9, 133.88, 133.7 and 131.6) and a methyl (δ_C 22.2) group. The compound was therefore identified as 2-methyl-9,10-anthraquinone (74) as reported earlier from *Rubia tinctorum* with the trivial name of tectoquinone (Sato *et al.*, 1992). This appears to be the first report from the genus *Pentas*.



74

Table 4.7: ^{1}H NMR spectral data (δ in ppm, *J* in Hz) of compounds **49-51**, **59-60** and **74***

Position	Tectoquinone (75)	Rubiadin (49)	Damnacanthal (50)	Rubaidin-1-methyl ether (51)	Damnacanthol (59)	Lucidin-ω-methyl ether (60)
1	8.19 (*d*, *J*= 2.4)	-	-	-	-	-
1a	-	-	-	-	-	-
2	-	-	-	-	-	-
3	7.80 (*dd*, *J*= 2.4, 8.1)	-	-	-	-	-
4	8.29 (*d*, *J*= 8.1)	7.11, *s*	7.49, *s*	7.46, *s*	7.70, *s*	7.33, *s*
4a	-	-	-	-	-	-
5	8.39 (*m*)	8.06 (*m*)	8.14 (*dd*, *J*= 7.9, 2.4)	8.06 (*m*)	8.10 (*dd*, *J*= 7.9, 2.4)	8.25 (*dd*, *J*= 1.4, 7.7)
5a	-	-	-	-	-	-
6	7.87 (*m*)	7.90 (*m*)	7.88 (*dd*, *J*= 7.9, 7.9)	7.85 (*m*)	7.83 (*dd*, *J*= 7.7, 7.7)	7.83 (*ddd*, *J*= 1.4, 7.7, 7.7)
7	7.87 (*m*)	7.90 (*m*)	7.88 (*dd*, *J*= 7.9, 7.9)	7.85 (*m*)	7.89 (*dd*, *J*= 7.7, 7.7)	7.89 (*ddd*, *J*= 1.4, 7.7, 7.7)
8	8.39 (*m*)	8.06 (*m*)	8.14 (*dd*, *J*= 7.9, 2.4)	8.06 (*m*)	8.15 (*dd*, *J*= 7.9, 2.4)	8.17 (*dd*, *J*= 0.8, 1.7, 7.7)
8a	-	-	-	-	-	-
9	-	-	-	-	-	-
10	-	-	-	-	-	-
11	2.62 (3H, *s*)	1.97 (3H, *s*)	10.4 (*s*, 1H)	2.15 (3H, *s*)	4.56 (*s*, 2H)	3.43 (*s*, 3H)
12	-	-	3.97 (*s*, 1H)	3.79 (3H, *s*)	3.85 (*s*, 3H)	4.54 (*s*, 2H)

*200 MHz spectrometer for **60** and **74** in $CDCl_3$, 800 MHz spectrometer for **49-51**, **59** in DMSO-d_6

Table 4.8: ^{13}C NMR spectral data (δ_C in ppm) of compounds **49-51**, **59-60** and **74***

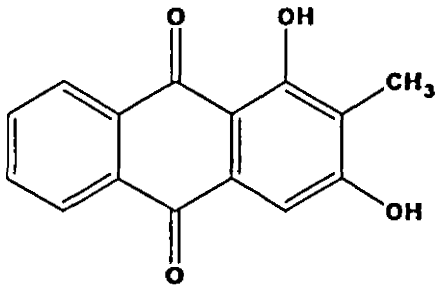
Position	Tectoquinone (74)	Rubiadin (49)	Damnacanthal (50)	Rubaidin-1-methyl ether (51)	Damnacanthol (59)	Lucidin-ω-methyl ether (60)
1	127.8	162.1	167.7	163.7	164.9	164.5
1a	133.9	108.8	120.8	120.9	121.0	109.8
2	145.6	117.2	123.7	129..2	132.0	117.5
3	135.3	162.7	168.3	164.6	165.2	165.0
4	127.7	107.3	113.9	112.1	112.9	108.5
4a	133.9	131.5	143.4	136.8	138.9	135.5
5	127.5	126.2	129.5	129.3	129.2	127.6
5a	133.7	132.8	135.6	135.2	135.3	135.3
6	134.4	134.3	136.7	136.3	136.5	133.6
7	134.2	134.2	138.1	137.6	137.7	133.8
8	127.5	126.5	129.9	129.8	129.9	127.2
8a	131.6	132.7	137.8	135.7	136.2	134.7
9	183.8	186.0	196.0	185.5	183.1	182.4
10	183.3	181.5	185.1	183.1	185.6	186.9
11	22.2	9.5	196.0	12.1	56.2	58.2
12	127.8	-	66.8	63.7	65.5	62.0

*50 MHz for **60** and **74** in $CDCl_3$, 200 MHz for **49-51** and **59** in DMSO-d_6; δ_C in ppm

4.3.1.2 Rubiadin (49)

Rubiadin (**49**) was isolated as yellow powder. The ESI-MS spectrum revealed a molecular ion peak *m/z* 255.6 $(M+H)^+$, attributed to $C_{15}H_{10}O_4$. The UV-VIS (CH_3OH) showed absorption maxima at 225, 240, 270, 280, 405 nm suggesting an anthraquinone chromophore (Scott, 1964). In comparison to tectoquinone (**74**), the ^1H NMR spectral data (Table 4.7) revealed a similar spin multiplicity pattern centered at δ_H 8.06 and 7.90, suggesting that ring C of the anthraquinone skeleton is unsubstituted. In ring A, the singlet aromatic proton at δ_H 7.11 showed HMBC correlation with C-2, C-3, C-4a and C-10 which is in agreement with its placement at C-4 of trisubstituted (at C-1, C-2, C-3) ring A of anthraquinone. The substituents in this ring being a methyl group (δ_H 1.97) and two hydroxyl groups, one of which is chelated (δ_H 13.80).

Based on the biogenetic consideration, which requires carbon substitution at C-2, the methyl group (δ_H 1.97) was placed at C-2. The chealated hydroxyl proton (δ_H 13.8) was unequivocally assigned to 1-OH while the second hydroxyl was placed at C-3 (δ_C 162.7). The ^{13}C NMR spectral data (Table 4.8) revealed the presence of fifteen carbons: two carbonyls (δ_C 186.0 and δ_C 181.5), five aromatic methine carbons (δ_C 134.3, 134.2, 126.5, 126.2 and 107.3), two oxygenated aromatic quaternary carbons (δ_C 162.1 and δ_C 162.7), five aromatic quaternary carbons (δ_C 132.8, δ_C 132.7, δ_C 131.5, δ_C 117.2 and δ_C 108.8) and a methyl carbon (δ_C 9.5). The compound was therefore identified as 2-methyl-1,3-dihydroxy-9,10-anthraquinone given trivial name rubiadin (**49**). These spectroscopic data are in agreement with the previous reports on the isolation of the compound from *Pentas zanzibarica* (Kusumba *et al.*, 1993) and *Galium verum* (Banthorpe and White, 1995).



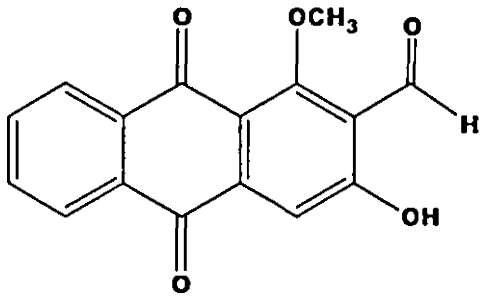
49

4.3.1.3 Damnacanthal (50)

Damnacanthal (**50**) was isolated as an orange powder. The ESI-MS spectrum provided a molecular ion peak *m/z* 305.3 $(M+Na)^+$, [ESI-MS at *m/z* 283.3 $(M+H)^+$] attributed to a molecular formula of $C_{16}H_{10}O_5$. Its UV-VIS (CH_3OH) spectrum showed absorption maxima at 230, 280, and 380 nm suggesting the presence of anthraquinone skeleton (Scott, 1964). The ^1H NMR (Table 4.7) revealed four aromatic protons at δ_H 7.88 and 8.14 having a similar spin multiplicity pattern observed in tectoquinone (**74**) suggesting that ring C of the anthraquinone skeleton is unsubstituted. The singlet aromatic proton observed at δ_H 7.49 showed HMBC correlation with C-4a, C-10 and C-3 which is in agreement with its placement at C-4, indicating that ring A of the anthraquinone skeleton is trisubstituted at C-1, C-2 and C-3 positions with methoxyl (δ_H 3.97, δ_C 66.8), formyl (δ_H 10.40; δ_C 196.0) and hydroxyl (δ_H 10.4, δ_C 168.3). The downfield chemical shift value of the methoxyl group (δ_C 66.8) is due to its di-*ortho* substitution and hence unequivocally placed at C-1 rather than C-3. This was supported by HMBC correlation of the methoxyl protons (δ_H 3.97) with oxygenated quaternary carbon at δ_C 168.3 (C-1). This implies that C-1 is substituted with hydroxyl group.

The position of the formyl group was ascertained by HMBC correlation between the aldehydic proton (δ_H 10.40) with quaternary carbon at δ_C 123.7 (C-2) which is in

agreement with its placement at C-2 as expected biogenetically. In agreement with this, the ^{13}C NMR spectral (Table 4.8) revealed the presence of sixteen carbons: three carbonyls (δ_C 196.0, δ_C 185.1 and δ_C 182.9), two oxygenated quaternary carbons (δ_C 168.3 and δ_C 167.7), five aromatic methine carbons (δ_C 138.1, 136.7, 129.9, 129.5 and 113.9), five non-oxygenated quaternary carbons (δ_C 143.4, 137.8, 120.8, 123.7 and 135.6) and a methoxy carbon (δ_C 66.8). The compound was therefore identified as 2-formyl-3-hydroxy-1-methoxy-9,10-anthraquinone, a compound known under the trivial name damnacanthal (**50**). The spectroscopic data was in agreement with the previous report on the isolation of this compound, from *Pentas zanzibarica* (Kusamba *et al.*, 1993).



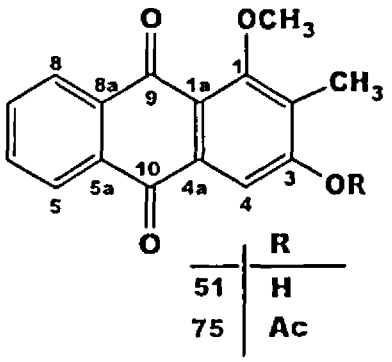
50

4.3.1.4 Rubiadin-1-methyl ether (51)

Rubiadin-1-methyl ether (**51**) was isolated as a yellow powder. The molecular formula was established by ESI-MS, *m/z* 291.3 $(M+Na)^+$, (ESI-MS, 269.6 $(M+H)^+$, to be $C_{16}H_{12}O_4$. The UV-VIS (CH_3OH) spectrum showed absorption maxima at 225, 265, 280, 360 nm suggesting the presence of an anthraquinone chromophore (Scott, 1964). The ^1H NMR spectral data (Table 4.7) showed signals of four aromatic protons at δ_H 8.06 (*m*) and 7.85 (*m*) having similar multiplicity pattern to that of tectoquinone (**74**), suggesting that ring C of the anthraquinone is unsubstituted. In ring A only one aromatic singlet proton was observed at δ_H 7.46 showing HMBC correlation with C-2, C-3, C-4a and C-

10 indicating that it is due to H-4. This ring is substituted with methyl (δ_H 2.15), methoxyl (δ_H 3.79) and hydroxyl (δ_H 11.0) groups. Based on the biogenetic consideration and HMBC data, the methyl group at δ_H 2.13 was placed at C-2. The methoxy group at δ_H 3.76, δ_C 63.7 was placed at C-1 based on the downfield chemical shift value, due its di-*ortho* substitution and the HMBC correlation with oxygenated quaternary aromatic carbon at δ_C 163.7. ^{13}C NMR spectral data (Table 4.8) revealed the presence of sixteen carbons: two carbonyls (δ_C 183.1 and δ_C 185.5), five aromatic methine carbons (δ_C 137.6, 136.3, 129.8, 129.3 and 112.1), two oxygenated aromatic quaternary carbons (δ_C 163.7 and 164.6), five non-oxygenated aromatic quaternary carbons (δ_C 129.2, 120.9, 136.8, 135.1 and 135.2), a methoxyl group (δ_C 63.7) and a methyl carbon (δ_C 12.1). The compound was therefore identified as 3-hydroxy-1-methoxy-2-methyl-9,10-anthraquinone (**51**).

The spectroscopic data are in agreement with the previous report on the isolation of this compound, under the trivial name rubiadin-1-methyl ether (**51**), from *Pentas zanzibarica* (Kusumba *et al.*, 1993). Acetylation of rubiadin-1-methyl ether (**51**) was conducted using the procedure described in section 3.6.1 (Hayashi *et al.*, 1987). Most of the NMR values for the acetate are identical with the starting material, rubiadin-1-methyl ether (**51**), except for the presence of the acetate carbonyl group at δ_C 175.2 and the methyl group at δ_H 2.24 further confirmed the structure to be rubiadin-1-methyl ether acetate (**75**).



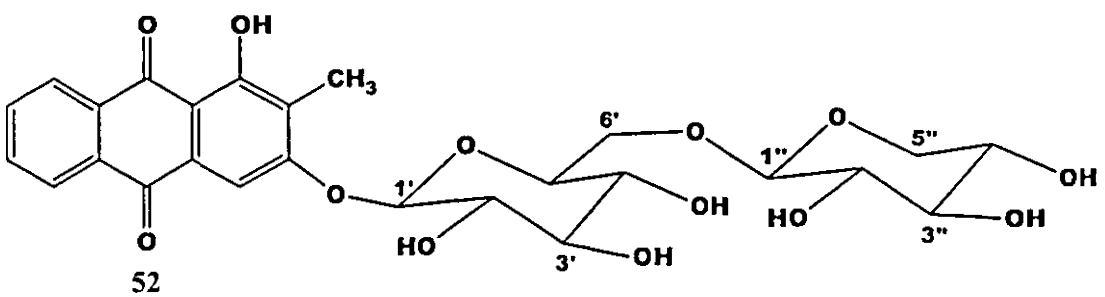
	R
51	H
75	Ac

4.3.1.5 Rubiadin-3-*O*-primveroside (52)

Rubiadin-3-*O*-primveroside (**52**) was isolated as a yellow powder. The ESI-MS provided a molecular formula at *m/z* 255.5 $(M\text{-sugar})^+$. The UV-VIS absorption maxima observed at 227, 260, 280, 355 nm suggests anthraquinone chromophore (Scott, 1964). ^1H NMR spectral data (Table 4.9) revealed four aromatic protons at δ_H 7.70 (*m*) and 8.31 (*m*) with a similar multiplicity pattern with that of tectoquinone (**74**), suggesting that ring C of this anthraquinone is unsubstituted. In ring A, the singlet aromatic proton at δ_H 7.77 (showing HMBC correlation with C-3, C-4a and C-2) and the methyl protons at δ_H 2.36 (showing HMBC correlation with C-1, C-2 and C-3) were observed enabling their assignment to C-4 and C-2, respectively. The ^{13}C NMR data (Table 4.10) suggested the presence of two carbonyl signals at δ_C 180.52 and 182.19, two oxygenated aromatic quaternary carbons at δ_C 160.0 and 159.78, and a methyl carbon at δ_C 9.22 which are all typical of an anthraquinone skeleton comparable to tectoquinone (**75**). In addition, ^1H NMR signals for glycoside moiety were observed with a characterstic pattern between δ_H 3.30-3.50 ppm, together with two clearly separated anomeric protons at δ_H 4.93 (*d*) and 5.84 (*d*) indicating the attached sugar is a disaccharide moiety. Furthermore, the ^{13}C NMR spectral data (Table 4.10) revealed eleven signals for the sugar moiety.

The ^{13}C NMR spectrum chemical shift value for one of the anomeric carbon (at δ_C 100.2) is typical of an anomeric carbon bonded to a *O*-aglycone in a phenolic glycoside and was assigned to C-1' of a β-D-glucopyranoside (Tori *et al.*, 1976). HMBC correlation of the anomeric proton H-1' with C-3 indicated that the disacharide is attached to C-3 with a β-D-glucopyranosidic linkage. The second anomeric carbon at δ_C 103.90 which was assigned to a xylosyl moiety (Table 4.10) showed HMBC correlation with the primary alcoholic C-6' of β-D-glucopyranoside (Kasai *et al.*, 1977). Comparison of the carbon chemical shift of the sugar moiety with literature data revealed that the signals at δ_C 67.92 assigned to C-6' of glucose was downfield shifted by 7 ppm from that of methyl-D-glucose (El-Emary and Backheet, 1998).

Thus, the terminal xylosyl moiety should be attached to the glycosyl part at C-6' through (1→6)-β-linkage. Moreover, the 1,2 diaxial coupling observed for the two anomeric protons H-1' and H-1" (J = 6.3 Hz) is consistent with a β-configuration for both anomeric linkages (Kasai *et al.*, 1977). From the spectral observations, the sugar moiety was deduced as being the primeverosyl (xylopyranosyl(1→6)-*O*-β-glucopyranosyl) moiety attached to the C-3 position of the anthraquinone. These spectral data are in full agreement with those reported for rubiadin-3-*O*-β-primveroside (52) isolated from *Pentas zanzibarica* (Kusamba *et al.*, 1993) and *Prismatomeris connate* (Rubiaceae) (Jing *et al.*, 2011).



52

Table 4.9: ^{1}H NMR data of rubiadin-3-*O*-β-primeveroside (**52**) (500 MHz, DMSO-d_6; this work) compared to literature (Kusamba *et al.*, 1993, 400 MHz, DMSO-d_6;): δ (ppm), *J* (Hz).

Position	Compound **52** (This work)	Compound **52** (Kusamba *et al.*, 1993)	Position	Compound **52** (This work)	Compound **52** (Kusamba *et al.*, 1993)
1	-	-	8	8.10 (*m*, 1H)	8.15 (*dd*, *J*=2,8)
2	-	-	8a	-	-
3	-	-	9	-	-
4	7.60, *s*	7.60, *s*	10	-	-
4a	-	-	CH_3-2	2.20 (*s*, 3H)	2.18 (*s*, 3H)
5	8.10 (*m*, 1H)	8.15 (*dd*, *J*=2,8)	1'	5.10 (*d*, 7.0)	5.15 (*d*, *J*=7.5)
5a	-	-	1"	4.10 (*d*, *J*=7.0)	4.12 (*d*, *J*=7.5)
6	7.80 (*m*, 1H)	7.89 (*dd*, *J*= 8,8)			
7	7.80 (*m*, 1H)	7.89 (*dd*, *J*=8,8)			

* For ^{1}H NMR of the sugar moiety only the anomeric values are shown

Table 4.10: ^{13}C NMR data of rubiadin-3-*O*-β-primeveroside (**52**) (125 MHz, DMSO-d_6; this work) with data from the literature (Kusamba *et al.*, 1993, 100 MHz, DMSO-d_6;): δ (ppm)

Position	Compound **52** (This work)	Compound **52** (Kusamba *et al.*, 1993)	Position	Compound **52** (This work)	Compound **52** (Kusamba *et al.*, 1993)
1	161.2	160.0	1'	100.3	100.4
2	120.7	129.2	2'	73.1	73.3
3	161.4	160.2	3'	76.3	76.3
4	110.9	108.4	4'	69.1	69.4
4a	132.0	132.2	5'	75.7	75.8
5	126.5	126.3	6'	68.1	68.2
5a	134.6	134.4	1''	104.1	104.1
6	132.8	133.7	2''	73.3	73.4
7	134.8	134.7	3''	76.4	76.5
8	126.8	126.8	4''	69.4	69.6
8a	133.1	134.0	5''	65.7	65.7
9	181.5	180.2			
10	187.2	182.4			
CH_3-2	8.2	9.2			

4.3.1.6 Rubiadin-1-methyl ether-3-*O*-primveroside (53)

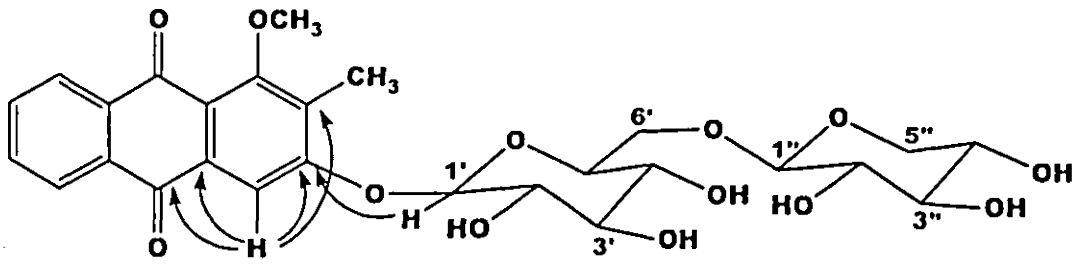
Rubiadin-1-methyl ether-3-*O*-primveroside (**53**) was isolated as a yellow powder. The ^{1}H NMR data (Table 4.11) revealed four aromatic protons at δ_H 7.95 (*m*) and 8.19-8.24 (*m*) with a similar multiplicity pattern to that of tectoquinone (**74**) suggesting that ring C of the anthraquinone skeleton is unsubstituted. The signal for the singlet aromatic proton at δ_H 7.49, a methoxyl (δ_H 3.81), a methyl group at δ_H 2.25, glycoside moiety between δ_H 3.00-6.00, and two distinct anomeric protons at δ_H 4.93 (*d*, J = 7.5Hz) and 5.15 (*d*, J = 7.5Hz) were observed. The ^{13}C NMR spectral data (Table 4.12) revealed the presence of two carbonyl signals at δ_C 184.6 and 190.2, two oxygenated aromatic quaternary carbons at δ_C 164.9 and 165.1, a methoxy group at δ_C 63.4, a methyl carbon at δ_C 12.3 and a sugar moiety at δ_C 53-107.

The anomeric carbon at δ_C 103.9 was attributed to C-1' which is typical of an anomeric carbon bonded to an *O*-aglycone in a phenolic glycoside (Tori *et al.*, 1976). The second anomeric carbons at δ_C 107.2 is indicative of a primary alcoholic β-D-glucopyranoside (Kasai *et al.*, 1977). Comparison of the carbon resonances with the literature data for sugar moieties revealed that the signals at δ_C 67.9 assigned to C-6' of glucopyranose were downfield shifted by 7 ppm from that of methyl-D-glucopyranose (El-Emary and Backheet, 1998). Thus, the terminal xylosyl moiety should be attached to the glycosyl part at C-6' through (1→6)-β-linkage. Moreover, the 1,2-diaxial coupling constant (J= 7.5 Hz) observed for the two anomeric protons H-1' and H-1", is consistent with a β-configuration of the anomeric linkages (Bubb, 2003). From the spectral observations, the sugar moiety was deduced to be primeverosyl (xylopyranosyl(1→6)-*O*-β-

glucopyranosyl) moiety, a disacharide of glucose and xylose displaying eleven carbon signals.

The disaccharide could be placed at C-1 or C-3. The placement of this group at C-1 was ruled out because of the downfield chemical shift of the methoxy carbon in the ^{13}C NMR spectrum (δ_C 63.4) suggests its di-*ortho* substitution and hence unequivocally placing the methoxyl group at C-1. The HMBC correlation of the methoxyl protons with one of the two oxygenated quaternary carbons (δ_C 164.9) enabeledd this signal to be assigned to C-1. Consequently, the second oxygenated quaternary carbon resonance at δ_C 165.1 was assigned to C-3. The HMBC correlation of the anomeric proton H-1' with this oxygenated quaternary carbon at δ_C 165.1 (C-3) confirmed the placement of the sugar moiety at C-3.

The HMBC correlation of the methyl protons at δ_H 2.25 with the quaternary carbon at δ_C 128.8 and biogenetic considerations (Han *et al.*, 2001) enabeled the placement of the methyl at C-2. The substitution pattern in ring A was confirmed from the HMBC correlation of the aromatic singlet proton at δ_H 7.46 (H-4) with C-2, C-3 and C-10 (Table 4.13). From the above spectroscopic evidence the compound was identified as rubaidin-1-methyl ether-3-*O*-β-primeveroside (**53**), which was previously reported from *Pentas zanzibarica* (Kusamba *et al.*, 1993) and *Prismatomeris connate* (Rubiaceae) (Jing *et al.*, 2011).



53

Table 4.11: ^{1}H NMR data of rubiadin-1-methyl ether-3-*O*-β-primeveroside (**53**) (500 MHz, DMSO-d_6) compared with literature.

Position	Compound **53** (This work)	Compound **53** (Kusamba *et al.*, 1993)	Position	Compound **53** (This work)	Compound **53** (Kusamba *et al.*, 1993)
1	-	-	8	8.19-8.24 (*m*)	8.15 (*dd*, *J*=2,8)
2	-	-	8a	-	-
3	-	-	9	-	-
4	7.49, *s*	7.60, *s*	10	-	
4a	-	-	CH_3-2	12.3	
5	8.19-8.24 (*m*)	8.15 (*dd*, *J*=2.0,8.0)	OCH_3-1	3.81 (*s*, 3H)	3.83 (*s*, 3H)
5a	-	-	1'	5.15 (*d*, *J*=7.5)	5.15 (*dd*, *J*=7.5)
6	7.95 (*m*)	7.89 (*dd*, *J*=8.0,8.0)	1"	4.10 (*d*, *J*=7.5)	4.12 (*dd*, *J*=7.5)
7	7.95 (*m*)	7.89 (*dd*, *J*=8.0,8.0)			

* For ^{1}H NMR of the sugar moiety only the values for anomeric protons are shown, δ_C in ppm, *J* in Hz

Table 4.12: ^{13}C NMR data of rubiadin-1-methyl ether-3-*O*-β-primeveroside (**53**) (125 MHz, DMSO-d_6) compared with literature.

Position	Compound **53** (This work)	Compound **53** (Kusamba *et al.*, 1993)	Position	Compound **53** (This work)	Compound **53** (Kusamba *et al.*, 1993)
1	164.9	160.0	OCH_3-1	63.4	61.0
2	129.3	129.2	1'	103.9	100.4
3	165.1	160.2	2'	76.3	73.3
4	109.5	108.4	3'	79.5	76.3
4a	130.1	132.2	4'	72.6	69.4
5	129.6	126.3	5'	78.8	75.8
5a	136.1	134.4	6'	71.1	68.2
6	135.9	133.7	1''	107.9	104.1
7	136.9	134.7	2''	76.4	73.4
8	129.3	126.8	3''	78.9	76.5
8a	137.8	134.0	4''	72.3	69.6
9	184.7	180.2	5''	68.7	65.7
10	190.2	182.4			
CH_3-2	12.3	12.3			

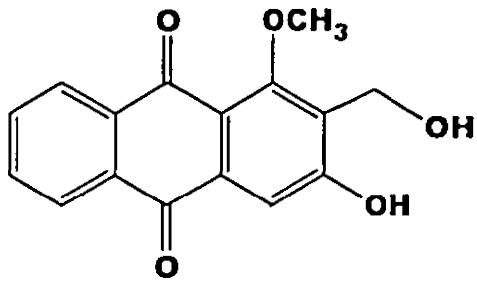
* δ_C in ppm

4.3.1.7 Damnacanthol (59)

Damnacanthol (**59**) was isolated as an orange powder. The ESI-MS provided a molecular ion peak at *m/z* 307.4 $(M+Na)^+$, 285.4 $(M+H)^+$, attributed to molecular formula of $C_{16}H_{10}O_5$. The UV-VIS (CH_3OH) spectrum showed absorption maxima at 225, 270, and 365 nm which indicates an anthraquinone skeleton (Scott, 1964). The ^1H NMR data (Table 4.7) revealed the presence of four aromatic protons at δ_H 8.10 (*m*) and 7.89 (*m*) having a similar splitting pattern with that of tectoquinone (**74**), suggesting that ring C of the anthraquinone is unsubstituted. An additional singlet aromatic proton was observed at δ_H 7.51 suggesting that ring A of this anthraquinone is trisubstituted. The singlet proton at δ_H 7.51 showed HMBC correlation with C-2, C-3, C-4a and C-10 enabling its assignment to H-4. One of the substituents in this ring is a methoxyl group (δ_H 3.85, δ_C 65.5) with a downfield chemical shift of the ^{13}C NMR methoxyl signal (δ_C 65.5) indicated its *peri* position to the carbonyl carbon (C-9) and hence in agreement with its location at C-1. The second substituent in this ring was identified an oxymethylene group (δ_H 4.56) and was placed at C-2 on the basis of biogenetic consideration (Han *et al.*, 2001) and HMBC correlations of its protons with C-1, C-2 and C-3, where the substituent at C-3 is hydroxyl group.

The ^{13}C NMR data (Table 4.8) revealed the presence of sixteen carbons: two carbonyls at δ_C 185.6 and 183.1, two oxygenated aromatic quaternary carbons at δ_C 165.2 and 164.9, five aromatic methines at δ_C 137.7, 136.5, 129.9, 129.2 and 112.9, five quaternary carbons at δ_C 138.9, 136.2, 135.3, 132.0 and 121.0, an oxymethylene carbon at δ_C 56.2 and a methoxyl group at δ_C 65.5. The compound was therefore identified as 3-hydroxy-1-methoxy-2-oxymethylene-9,10-anthraquinone, known by the trivial name damnacanthol

(59) previously reported from *Morinda angustifolia* (Xiang *et al.*, 2008). This is the first report of the compound from the genus *Pentas*.

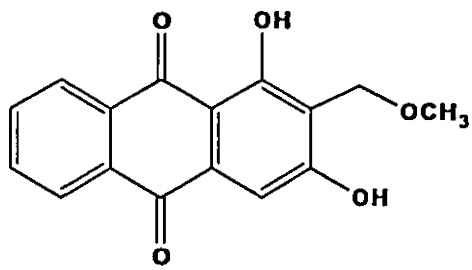


59

4.3.1.8 Lucidin-ω-methyl ether (60)

Lucidin-ω-methyl ether (**60**) was isolated as an orange solid. ESI-MS provided a $(M+H)^+$ *m/z* 285.3 attributed to molecular formula of $C_{16}H_{12}O_4$, The UV-VIS (CH_3OH) showed absorption maxima at 230, 280 and 410 nm suggesting an anthraquinone skeleton (Scott, 1964). The ^1H NMR spectral data (Table 4.7) revealed four aromatic protons centred at δ_H 8.17 (*m*) and 7.78 (*m*) suggesting that ring C of the anthraquinone skeleton is unsubstituted. In ring A, only one aromatic singlet proton was observed at δ_H 7.33 and was assigned to H-4 as established from its HMBC correlation with C-2, C-3, C-4a and C-10 of trisubstituted ring A. The substituents in this ring are oxymethylene (δ_H 4.54, δ_C 58.2) and two hydroxyl groups one of which is chelated (δ_H 13.31) and is placed at C-1. From biogenetic consideration the oxymethylene group is placed at C-2 and confirmed from the HMBC correlation of these protons with C-1, C-2 and C-3. A three proton singlet for the methyl group at δ_H 3.43 was also observed showing HMBC correlation with the oxymethyelene carbon at C-2 indicating that the substituent at C-2 is actually methoxymethylene ($-CH_2OCH_3$). From the ^{13}C NMR data (Table 4.8) chemical shift values of the ring A carbon atoms then the second hydroxyl group is placed at C-3.

The ^{13}C NMR data (Table 4.8) revealed the presence of sixteen carbons: two carbonyl carbons (δ_C 186.9 and 182.4), two oxygenated aromatic quaternary carbons (δ_C 165.0 and 164.5), five aromatic methines (δ_C 133.8, 133.6, 127.6, 127.2 and 108.5), five quaternary non-oxygenated carbons (δ_C 135.5, 135.3, 134.7, 117.5 and 109.8) and methoxymethylene (δ_C 62.0, C-12 and δ_C 58.2, C-11). The compound was therefore identified as 1,3-dihydroxy-2-methoxymethylene-9,10-anthraquinone (**60**). It has been previously isolated from *Putoria calabrica* (Calis *et al.*, 2002) and *Morinda citrifolia* (Leistner, 1985) under the trivial name lucidin-ω-methyl ether (**60**). However, this is the first report on its occurrence in the genus *Pentas*.

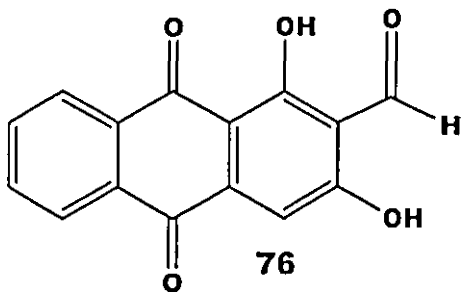


60

4.3.1.9 Nordamnacanthal (76)

Nordamnacanthal (**76**) was isolated as an orange powder. Its molecular formula was established by ESI-MS to be $C_{15}H_8O_5$ (*m/z* 269.2, $(M+H)^+$). The UV-VIS (CH_3OH) showed absorption maxima at 225, 250, 285 and 415 nm suggesting the presence of anthraquinone chromphore (Scott, 1964). The ^1H NMR spectral data (Table 4.13) revealed the presence of four aromatic protons at δ_H 8.16 and 7.96 with a similar splitting pattern with that of tectoquinone (**75**) suggesting that ring C of the anthraquinone skeleton is unsubstituted. A chelated hydroxyl group at δ_H 13.9, an aldehydic proton at δ_H 10.4, and a singlet aromatic proton at δ_H 7.17 were revealed in ^1H NMR spectrum.

The singlet proton at δ_H 7.17 showed HMBC correlation with C-2, C-3, C-4a and C-10 and hence unequivocally assigned to H-4 of ring A. The chelated hydroxyl proton (δ_H 13.9) was unequivocally placed at C-1 while the position of the formyl group was determined at C-2 from biogenetic considerations. This was confirmed from HMBC correlation of the aldehydic proton (δ_H 10.4) with quaternary carbon at δ_C 113.1 (C-2) of ring A which is also substituted with a hydroxyl group at C-3. The ^{13}C NMR spectral data (Table 4.13) showed the presence of fifteen carbons: three carbonyl (δ_C 190.6, δ_C 186.2 and δ_C 181.2), two oxygenated aromatic quaternary carbons (δ_C 167.4 and 166.6), five aromatic sp^2 carbons (δ_C 135.0, 134.9, 127.0, 126.8 and 108.1), five non-oxygenated aromatic quaternary carbons (δ_C 109.1, 132.9, 132.8, 138.4 and 113.1). The compound was therefore identified as 1,3-dihydroxy-2-formyl-9,10-anthraquinone (**76**), previously known by the trivial name nordamnacanthal. This compound has been previously isolated from *Morinda lucida* (Adesogan, 1973). However, this is the first report on its isolation from any *Pentas* species.



76

Table 4.13: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data of nordamnacanthal (**76**) and damnacanthol-11-*O*-methyl ether (**77**) in DMSO-d_6.

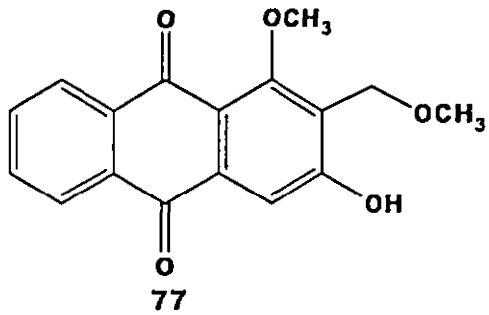
Position	Nordamnacanthal (76)		Damnacanthol-ω-methyl ether (77)	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	13.9 (*s*, 1-OH)		-	
		166.6		164.0
1a	-		-	
		109.1		121.0
2	-		-	
		113.1		129.1
3	-		-	
		167.4		164.7
4	7.17, *s*		7.46, *s*	
		108.1		112.1
4a	-		-	
		138.4		138.7
5	8.16 (*dd*, *J* = 7.8, 1.4)		7.90 (2H, *m*)	
		127.0		129.8
5a	-		-	
		132.9		135.1
6	7.96 (*dd*, *J* = 7.4, 7.4)		7.79 (2H, *m*)	
		135.0		136.8
7	7.93 (*dd*, *J* = 7.4, 7.4)		7.79 (2H, *m*)	
		134.9		136.4
8	8.22 (*dd*, *J* = 7.4, 7.4)		7.90 (2H, *m*)	
		126.8		129.1
8a	-		-	
		132.8		137.6
9	-		-	
		186.2		185.5
10	-		-	
		181.2		183.1
11	10.4, *s*		4.52 (*s*, 2H)	
		190.6		63.5
12	-		3.70 (*s*, 3H)	
		-		55.2
13	-		3.83 (*s*, 3H)	
		-		65.5

4.3.1.10 Damnacanthol-11-*O*-methyl ether (77)

Damnacanthol-11-*O*-methyl ether (77) was isolated as an orange powder. ESI-MS revealed molecular ion peak m/z 299.5 $(M+H)^+$ attributed to molecular formula of $C_{17}O_{14}O_5$. The ^1H NMR spectral data (Table 4.13) revealed that ring C of the anthraquinone is unsubstituted. The ^1H and ^{13}C NMR spectral data (Table 4.13) further showed the presence of an oxymethylene (δ_H 4.52, δ_C 63.5), two methoxyl groups (δ_H 3.82 and 3.70, δ_C 65.5 and δ_C 55.2) and a singlet proton at δ_H 7.46, suggesting that ring A is trisubstituted at C-1, C-2 and C-3 positions. The downfield ^{13}C NMR chemical shift value of one of the two methoxyl signals (δ_C 65.5) and the absence of chelated hydroxyl signal in the ^1H NMR spectrum, enabeled the location of one of the methoxyl groups at C-1. From biogenetic considerations then the oxymethylene was placed at C-2. The oxymethylene carbon (δ_C 63.5) showed HMBC correlation with the second methoxyl protons (δ_H 3.70; δ_C 55.2) confirming that the substituent at C-2 is methoxymethylene (-CH_2-O-CH_3) group. The singlet proton at δ_H 7.46 was assigned to H-4 based on HMBC spectrum enabling the assignment of the hydroxyl group to C-3.

In agreement with the above data, the ^{13}C NMR revealed the presence of seventeen carbon atoms constituting a methylene (δ_C 63.5), two methoxyl (δ_C 65.5 and δ_C 55.2), two carbonyl carbons (δ_C 185.7 and δ_C 183.1), two oxygenated quaternary centers (δ_C 164.7 and 164.0), five aromatic methines (δ_C 121.0, 129.1, 129.7, 131.9 and 135.1) and five non-oxygeneated quaternary centers (δ_C 121.1, 129.1, 138.7, 135.1 and 137.6). Based on the above spectroscopic evidence and comparison of the NMR data with literature values, the compound was identified as 3-hydroxy-1-methoxy-2-(methoxymethyl)-9,10-

anthraquinone (77) previously isolated from *Lasianthus acuminatissimus* (Rubiaceae) under the trivial name damnacanthol-11-*O*-methyl ether (Li *et al.*, 2006).

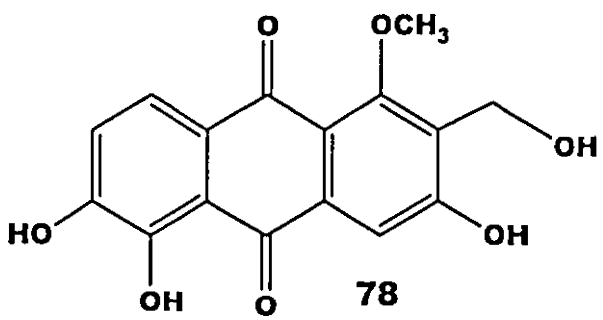


77

4.3.1.11 5,6-Dihydroxydamnacanthol (78)

5,6-Dihydroxydamnacanthol (78) was isolated as a red powder from the methanol extract of the *P. lanceolata*. The UV-VIS spectrum which showed absorption maxima at 218, 274, 308 and 424 nm is typical of 9,10-anthraquinone skeleton (Scott, 1964). The molecular formula was established as $C_{16}H_{12}O_7$ from EI-HRMS (*m/z* 317.0659, $M+H)^+$). The ^1H NMR spectral data (Table 4.14) revealed an aromatic singlet, a pair of *ortho*-coupling aromatic protons, a methoxyl and an oxymethylene substituents as well as two solvent accessible and one chelated (δ_H 12.40) hydroxyl groups. Furthermore, two carbonyl functionalities were revealed by ^{13}C NMR data (Table 4.14). HMBC correlation between the methoxy protons with C-1, the oxymethylene protons with C-1, C-2 and C-3 are consistent with the methoxyl, oxymethylene, and a hydroxyl substitution on ring A. The downfield chemical shift of the methoxyl group at δ_C 63.0 is indicative of di-*ortho* substitution allowing its placement at C-1 rather than C-3. Hence, in similarity to previously identified anthraquinones of the Rubiaceae where ring A is oxygenated at C-1 and C-3 and has the oxymethylene at C-2. The aromatic singlet at δ_H 7.52 (H-4) with HMBC correlation to the C-10 carbonyl (δ_C 189.2), indicating their *peri* position. The

high chemical shift of this carbonyl is indicative of a *peri* hydroxyl group at C-5, which is further confirmed by the HMBC correlation of the aromatic proton at δ_H 7.54 (*d*) to the carbonyl at δ_C 179.4 (C-9), but not with the one at δ_C 189.2 (C-10). These three bond heteronuclear correlations confirm the dihydroxy-substitution at C-5 and C-6 in ring C. From the above spectroscopic evidence the compound was identified as 3,5,6-trihydroxy-2-hydroxymethyl-1-methoxy-9,10-anthraquinone (**78**) for which the trivial name 5,6-dihydroxydamnacanthol is proposed. This compound has been reported as a new compound from this work (Endale *et al.*, 2012a). The assignment is in good agreement with that of the recently reported and closely-related 2-hydroxymethyl-1-methoxy-3,5,6-trihydroxyanthraquinone-3-*O*-*β*-glycopyranoside isolated from *Putoria calabrica* (Rubiaceae) (Calis *et al.*, 2002).



78

Table 4.14: ^{1}H (800 MHz, DMSO-d_6) and ^{13}C (125 MHz, DMSO-d_6) NMR data for 5,6-dihydroxydamnacantol (**78**).

Position	δ_H (J in Hz)	δ_C	HMBC (^2J, ^3J)
1	-	162.4	-
1a	-	116.4	-
2	-	126.1	-
3	-	162.3	-
4	7.52, *s*	110.3	C-1a, 2, 3, 4a, 10
4a	-	135.8	-
5	-	150.6	-
5a	-	118.8	-
6	-	151.8	-
7	7.18 (*d*, J=8.2)	121.1	C-5, 6, 8, 8a
8	7.54 (*d*, J=8.2)	121.8	C-6, 7, 9, 8a
8a	-	130.1	-
9	-	179.4	-
10	-	189.2	-
11	4.52 (*s*, 2H)	52.8	C-1, 2, 3
12	3.79 (*s*, 3H)	63.0	C-1
5-OH	12.4, *s*	-	C-5a, 6
11-OH	4.92	-	C-11

The compounds isolated from the CH_2Cl_2:CH_3OH (1:1) and CH_3OH extracts of the roots of *P. lanceolata* are listed below (figure 4.4).

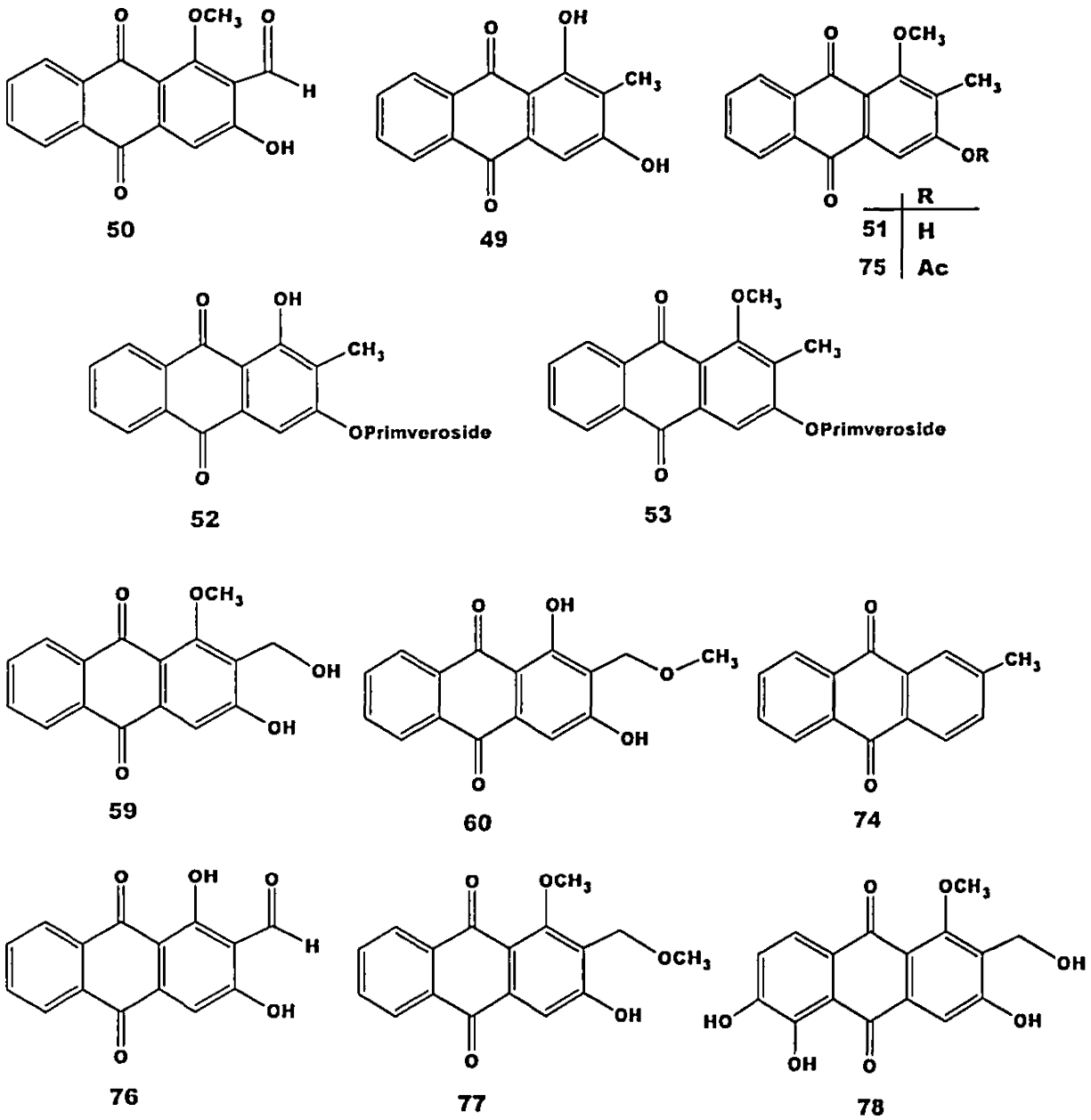


Figure 4.5: Anthraquinones isolated from the roots of *Pentas lanceolata*

4.4 Secondary metabolites isolated from *Pentas longiflora*

4.4.1 Pyranonaphthoquinones and dihydronaphthoquinone derivative

Figure 4.6: Photograph of *Pentas longiflora* (Prof. Mate Erdelyi, October 2011)

Chromatographic separation of the root extracts of *P. longiflora* led to the isolation and identification of two pyranonaphthaquinones (**33**, **80**), one dihydronaphthaquinone (**34**) and benzoic acid (**79**). The characterization of these compounds is discussed below.

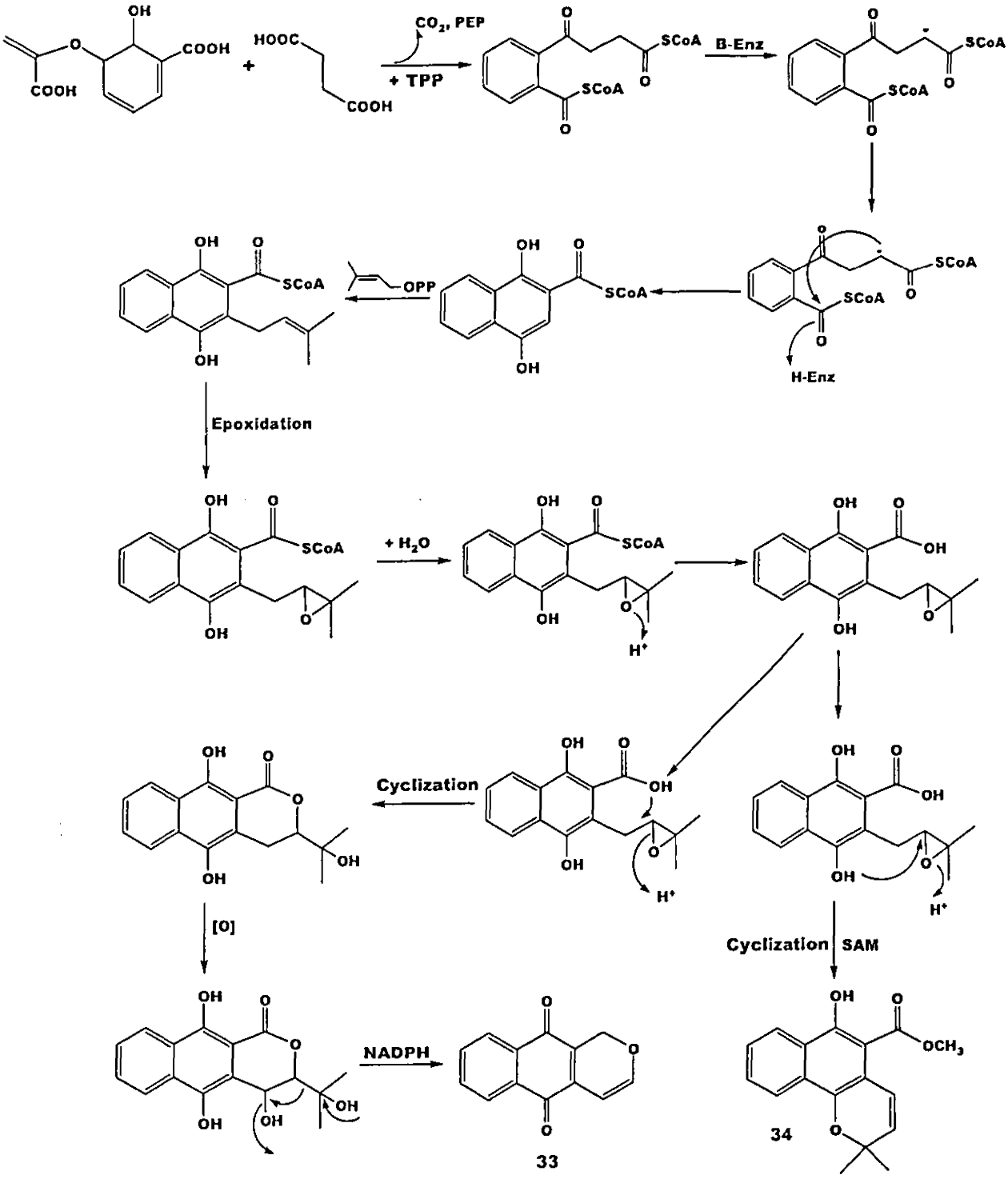
4.4.1.1 Pentalongin (33)

Pentalongin (**33**) was isolated as red crystals. ESI-MS provided a molecular ion peak of *m/z* 213.1 $(M+H)^+$, suggesting the molecular formula as $C_{13}H_8O_3$. The UV-VIS showed absorption maxima (λ_{max}, CH_3OH) 203, 259, 308 nm suggesting a naphthoquinone skeleton (Scott, 1964). The ^1H NMR spectral data (Table 4.15) revealed aromatic protons at δ_H 8.02 (*m*) and 7.85 9 (*m*) suggesting the presence of unsusbstituted aromatic ring C as part of the skeleton. The downfield chemical shift of the two methyelene protons at δ_H 5.11 suggests attachment to oxygen. Two olefinic protons coupling to each other were observed at δ_H 7.12 (*d*, J = 8.2 Hz) and 6.07 (*d*, J = 8.2 Hz) corresponding to the two olefinic protons in ring A. The ^{13}C NMR spectral data (Table 4.15) revealed the presence of thirteen carbons: four quaternary centers (δ_C 124.6, 131.8, 132.6 and 136.8), two

carbonyl carbons (δ_C 182.5 and 182.0), four aromatic methine carbons (δ_C 126.3, 126.8, 133.6 and 134.2) and two olefinic carbons (δ_C 98.3 and 154.5). The downfield chemical shift of the olefinic carbon (δ_C 154.52) and the methylene (δ_C 62.4) is in agreement with their position adjacent to an oxygen atom. The spectral data of the compound is in agreement with that reported for pentalongin (**33**) (El-hady, 1999) and confirmed the structure of the compound to be 10*b*-hydroxy-3-methoxy-2*a*,3,6,10*b*-tetrahydro-2*H*,5*H*-furo(2,3,4-*ed*)naphtho-(2,3-*c*)pyran-6-one (**33**) previously isolated from the same plant (El-hady, 1999) and synthesized (Kesteleyn *et al.*, 1999a; Kesteleyn *et al.*, 1999b). Pentalongin (**33**) could have been biosynthesized via shikimic acid pathway. Deprotonation followed by cylization to form aromatic rings A and B occur as shown in the scheme 4.4. Prenylation followed by epoxidation and cyclization forms the pyran ring.

Table 4.15: ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR data of pentalongin (**33**) in $CDCl_3$

Position	δ_H (*J* in Hz)	δ_C
1	5.11, *s*	62.4
1a	-	136.8
3	7.12 (1H, *d*, *J*=8.2)	154.5
4	6.07 (1H, *d*, *J*=8.2)	98.3
4a	-	124.6
5	8.02, *m*	133.2
5a	-	132.6
6	7.85, *m*	126.3
7	7.85, *m*	126.8
8	8.02, *m*	133.7
8a	-	131.8
9	-	182.5
10	-	182.0

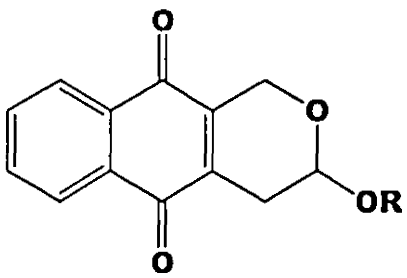


Scheme 4.4: Proposed biogenesis pathway for pentalongin (33) and mollugin (34)

4.4.1.2 Psychorubrin (80) and psychorubrin acetate (81)

Psychorubrin (80) was isolated as a yellow powder. The ESI-MS revealed a molecular ion peak of *m/z* 231.3 $(M+H)^+$ which is attributed to the molecular formula $C_{13}H_{10}O_4$. The UV-VIS (CH_3OH) showed λ_{max} at 204, 246, 263, 269, 333 nm suggesting naphthoquinone skeleton (Scott, 1964). ^1H NMR spectral data (Table 4.16) revealed four aromatic protons with a similar spin pattern as in pentalongin (**33**) suggesting the presence of unsusbstituted aromatic ring as part of the skeleton. The presence of a hemiacetal proton (δ_H 5.50, *m*), an oxymethylene group (δ_H 4.69, δ_H 4.82) and methylene group (δ_H 2.73, 2.85) were also observed from the ^1H NMR spectrum. The downfield chemical shift of the two methylene protons suggested attachment to oxygen. From the COSY spectrum the methylene protons at δ_H 2.73 and 2.85 showed correlation with the hemiacetal proton at δ_H 5.50 and the methylene protons at δ_H 4.69 and 4.82. The ^{13}C NMR spectral data (Table 4.16) revealed the presence of thirteen carbons: four quaternary carbons (δ_C 124.6, 131.8, 132.7 and 136.8), two carbonyl carbons (δ_C 182.5 and 182.0), four aromatic methine carbons (δ_C 126.3, 126.8, 133.7 and 134.2) and two olefinic carbons (δ_C 98.3 and 154.5). The downfield chemical shift of one of the methylene carbon at δ_C 53.9 indicates its adjacent position to an oxygen atom. The spectral data of the compound is in agreement with that of literature reported for psychorubrin (**80**) (Hayashi *et al.*, 1987) confirming the structure to be 3-hydroxy-3,4-dihydro-1*H*-benzo(g)isochromene-5,10-dione (**81**) which was isolated from *Psychotria rubra* (Hayashi *et al.*, 1987) and subsequently from *Mitracarpus scaber* (Moulis *et al.*, 1992). However, this is the first report of the isolation of the compound from *Pentas* species.

Acetylation of psychorubrin (**80**) was conducted to affirm the identified structure using the procedure described in section 3.6.1 (Hayashi *et al.*, 1987). Most of the NMR chemical shift values are identical to the starting material, psychorubrin (**80**), except for the presence of the acetate carbonyl at δ_C 169.6 and methyl group at δ_H 2.20; δ_C 21.2 which confirmed the chemical structure to be psychorubrin acetate (**81**).



	R
80	H
81	Ac

Table 4.16: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data of psychorubrin (**80**) and ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR data of psychorubrin acetate (**81**) in $CDCl_3$.

	Psychorubrin (80)			**Psychorubrin acetate (81)**	
Position	**δ_H (*J* in Hz)**	**δ_C**	**Position**	**δ_H (*J* in Hz)**	**δ_C**
1	4.78 (*dt*, 1H, *J*=3.5, 18.6 Hz) 4.82 (*dt*, 1H, *J*=3.5, 18.6 Hz)	53.9	**1**	4.66 (*dt*, 1H, *J*=3.5, 18.6 Hz) 4.85 (*dt*, 1H, *J*=3.5, 18.6 Hz)	58.2
1a	-	128.3	**1a**	-	140.9
3	5.49 (1H, *t*)	86.9	**3**	6.4 (1H, *t*)	89.5
4	2.73 and 2.85 (1H each, *dm*, *J*=19.3 Hz)	24.2	**4**	2.83 (2H, *m*)	26.4
4a	-	137.5	**4a**	-	138.6
5	8.06-8.10 (*m*)	122.5	**5**	8.11 (*m*)	126.8
5a	-	130.2	**5a**	-	132.1
6	7.71-7.73 (*m*)	130.1	**6**	7.72 (*m*)	134.2
7	7.71-7.73 (*m*)	130.1	**7**	7.72 (*m*)	134.1
8	8.06-8.10 (*m*)	122.7	**8**	8.11 (*m*)	126.5
8a	-	135.5	**8a**	-	131.9
9	-	179.3	**9**	-	183.0
10	-	179.8	**10**	-	183.4
1''	-	-	**1''**	-	169.6
2''	-	-	**2''**	2.0 (3H, *s*)	21.2

4.4.1.3 Mollugin (34)

Mollugin (**34**) was isolated as yellow crystals (in CH_3OH). The ESI-MS revealed a molecular ion peak of *m/z* 285.5 $(M+H)^+$ which is attributed to a molecular formular $C_{17}H_{16}O_4$. The UV-VIS (CH_3OH) showed absorption maxima λ_{max} 204, 247, 268 and 273 nm suggested the presence of a napthalene skeleton (Scott, 1964). ^1H NMR data (Table 4.17) revealed aromatic protons at δ_H 8.17-8.37 (*dd*, *J*=7.8, 2.4, H-5,8) and δ_H 7.56 (2H, *m*, H-6,7) suggesting the presence of an unsusbstituted aromatic ring as part of the skeleton. The presence of methyl ester group was evident from the peak at δ_H 4.02. A downfield chemical shift of the hydroxyl proton at δ_H 12.17 is due to the *peri* position of the hydroxyl group to the ester carbonyl carbon and intramolecular hydrogen bonding. Two olefinic protons were observed at δ_H 7.11 (*d*, *J*= 10.2) and δ_H 5.67 (*d*, *J*= 10.2). One of the protons is shifted upfield suggesting the influence of the aromatic ring. The 1,1-dimethyl protons of the chromene ring were observed at δ_H 1.49 (*s*, 6H) and the integeration confirmed two equivalent methyl groups attached to a single carbon atom. The ^{13}C NMR spectral data (Table 4.17) revealed seventeen carbons; two oxygenated quaternary carbons (δ_C 156.7 and 141.8), one ester carbonyl (δ_C 172.7), four aromatic sp^2 carbons (δ_C 26.5, 129.6, 124.2 and 122.1), two olefinic carbons (δ_C 129.1 and 122.5), one oxygenated sp^3 carbon (δ_C 74.9), one ester methyl group (δ_C 52.5), four quaternary carbons (δ_C 102.4, 112.8, 125.3 and 129.2) and two methyls groups (δ_C 27.1). The spectral data of the compound is in full agreement with previously reported in the literature (Liu *et al.*, 2008) confirming the structure to be methyl 6-hydroxy-2,2-dimethylbenzo(*h*)chromene-5-carboxylate (**34**) known by trival name mollugin and

previously isolated from *Galium mollugo* (Heide *et al.*, 1981). The proposed biosynthetic route towards the compound is shown in scheme 4.4 above.

Table 4.17: ^{1}H (200 MHz) and ^{13}C (50 MHz) data of mollugin (34) in $CDCl_3$.

Position	δ_H (*J* in Hz)	δ_C
1	12.17 (*s*, 1-OH)	156.7
2	-	102.4
3	-	112.8
4	-	141.8
5	8.17-8.37 (*dd*, *J*=7.8, 2.4)	122.1
5a	-	126.5
6	7.56 (*m*)	124.2
7	7.56 (*m*)	125.3
8	8.17-8.37 (*dd*, *J*=7.8, 2.4)	122.5
8a	-	129.1
2'	-	74.9
3'	5.67 (1H, *d*, *J*=10.2 Hz)	134.2
4'	7.11 (1H, *d*, *J*=10.2 Hz)	119.5
1''	1.49 (6H, *s*)	27.1
2''	-	172.7
3''	4.02 (3H, *s*)	52.5

The compounds isolated from the roots of *P. longiflora* are presented here.

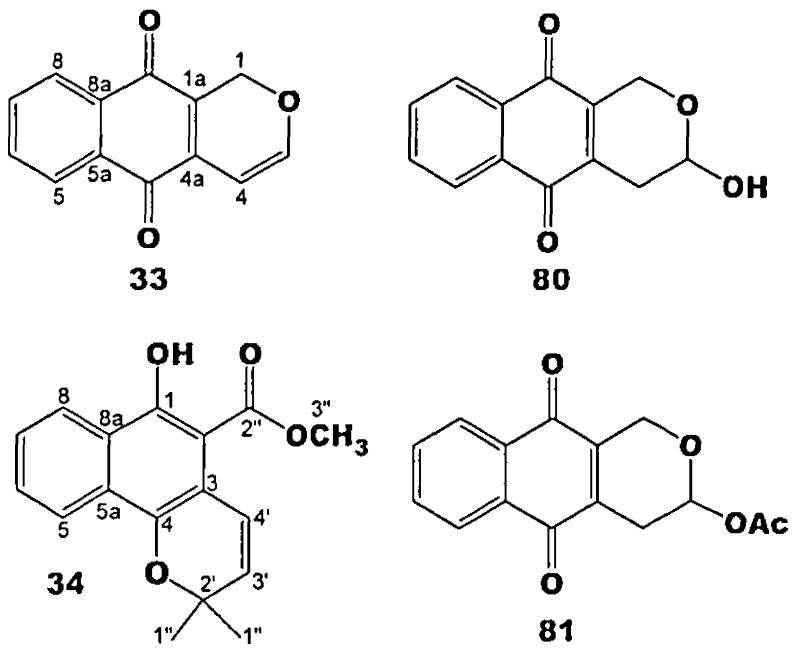


Figure 4.7: Pyranonaphthoquinone and dihydronaphthoquinone derivative from the roots of *P. longiflora*

4.5 Secondary metabolites isolated from *Pentas micrantha*

4.5.1 Anthraquinones

Figure 4.8: Photograph of *Pentas micrantha* (Patrick Chalo Mutiso, June 2010)

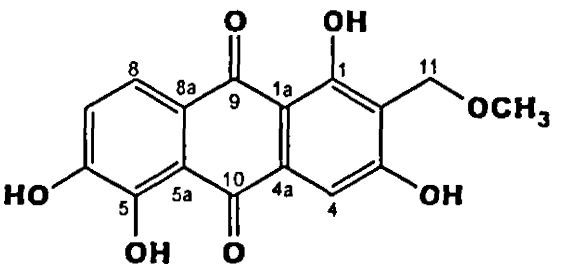
Chromatographic separation of the root of *P. micrantha* gave eight anthraquinones of which two of them 5,6-dihydroxylucidin-ω-methyl ether (**82**) and 5,6-dihydroxydamnacanthol (**78**) are new compounds. Rubiadin (**49**), damnacanthal (**50**), damnacanthol (**59**), lucidin-ω-methyl ether (**60**), tectoquinone (**74**), and 5,6-dihydroxydamnacanthol (**78**) have been identified as discussed earlier and the spectroscopic data of these compounds are in agreement with previous data from the roots of *P. lanceolata*. The characterization of the new compound, 5,6-dihydroxylucidin-ω-methyl ether (**82**), is discussed below.

4.5.1.1 5,6-Dihydroxylucidin-ω-methyl ether (82)

5,6-Dihydroxylucidin-ω-methyl ether (**82**) was isolated as a red powder. The HRMS provided a molecular ion peak at *m/z* 315.0571 $(M-H)^+$ attributed to the molecular formula of $C_{16}H_{12}O_7$. The UV-VIS spectrum revealed absorption maxima at λ_{max} 210, 270, 310, and 425 nm suggested a 9,10-anthraquinone skeleton (Scott, 1964). The ^1H NMR spectral data (Table 4.18) revealed an aromatic singlet at δ_H 7.26 and a pair of *ortho*-coupled aromatic protons at δ_H 7.77 and δ_H 7.31 (J= 8.1 Hz) suggesting that ring A and ring C are tri- and di-substituted. The presence of a methoxyl group (δ_H 3.26), an oxymethylene (δ_H 4.43) and two chelated hydroxyl groups (δ_H 13.29, δ_H 13.58) were evident from the ^1H NMR (Table 4.18) spectrum. The two chelated hydroxyl groups were placed at C-1 and C-5 from HMBC correlation of these hydroxyl protons with C-1 (δ_C 163.8) and C-5 (δ_C 151.4), respectively.

The HMBC correlations (Table 4.18) between the singlet proton at δ_H 7.26 with the carbonyl carbon at δ_C 185.2 (C-10), C-2 and C-3 is in agreement with this signal being for H-4 of a trisubstituted (at C-1, C-2 and C-3) ring A. The upfield chemical shift value of the methoxy protons (δ_H 3.26; 57.3, 11-OCH_3) coupled with its HMBC correlation with oxymethylene carbon (δ_C 60.7), suggested the presence of methoxymethylene (-CH_2-O-CH_3) which was placed at C-2 from biogeneic considerations and HMBC correlations (C_1-H_{11}, C_2-H_{11}, C_3-H_{11}). The second hydroxyl group in ring A was placed at C-3. In ring C, the HMBC correlation of one of the two *ortho*-coupled protons (δ_H 7.77) with the carbonyl carbon at δ_C 188.8 (C-9) agrees with its assignemt to H-8 and hence its coupling partner (δ_H 7.31) should be assigned to H-7. The compound was characterized as 1,3,5,6-tetrahydroxy-2-(methoxymethyl)-9,10-anthraquinone (**82**) for which the trivial

name 5,6-dihydroxylucidin-ω-methyl ether is proposed. This compound has not been reported before.



82

Table 4.18: The ^{1}H (800 MHz) and ^{13}C (125 MHz) NMR data for 5,6-dihydroxylucidin-ω-methyl ether (**82**) in DMSO-d_6

Position	δ_H (*J* in Hz)	δ_C	HMBC
1		163.8	-
1a	-	134.1	-
2	-	117.5	-
3	-	163.9	-
4	7.26, *s*	109.2	C-2, 3, 4a, 1a, 10
4a	-	134.1	-
5	-	151.4	-
5a	-	126.7	-
6	-	120.9	-
7	7.31 (*d*, *J*=8.1)	121.3	C-5, 6, 8, 8a
8	7.77 (*d*, *J*=8.1)	123.6	C-6, 7, 8a, 5a
8a	-	127.1	-
9	-	185.2	-
10	-	188.8	-
11	4.69 (*s*, 2H,CH_2-11)	60.7	C-1, 2, 3, 11-OCH_3
11-OCH_3	3.43 (*s*, 3H)	57.3	C-11
5-OH	13.29, *s*	-	C-5, 5a, 6
1-OH	13.58 , *s*		C-1, 2, 1a

The compounds isolated from the roots of *P. micrantha* are eight anthraquinones and a common sterol, stigmasterol (57), summarized in figure 4.8 below.

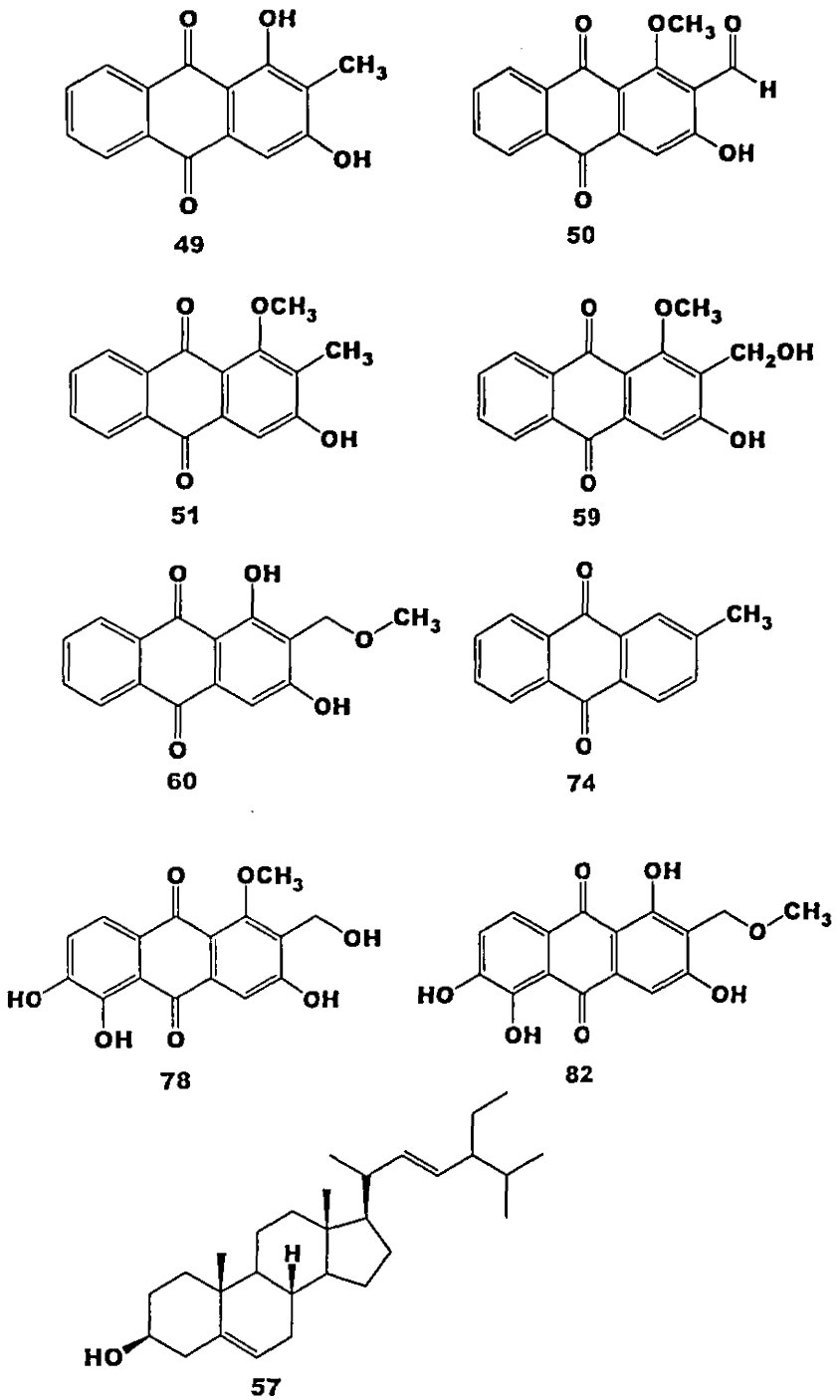
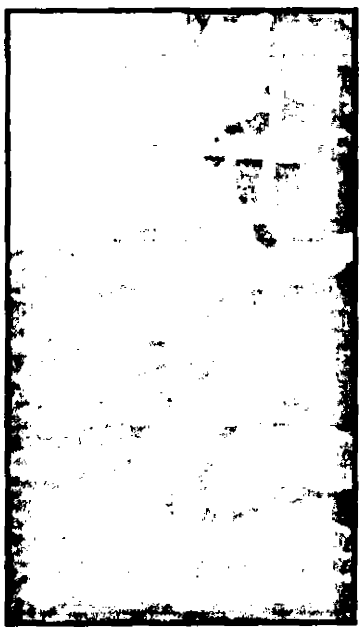


Figure 4.9: Anthraquinones isolated from the roots of *Pentas micrantha*

4.6 Secondary metabolites isolated from *Pentas suswaensis*

4.6.1 Anthraquinones



Figue 4.10: Photograph of *Pentas suswaensis* (Milkyas Endale, Mount suswa, June 2010)

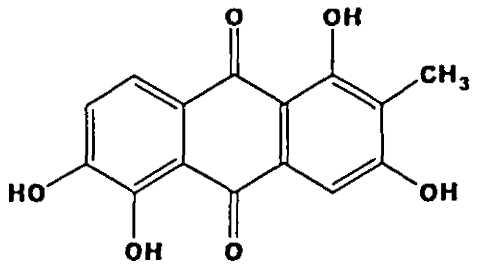
Chromatographic separation of the roots of *P. suswaensis* gave twelve anthraquinones of which, 5,6-dihydroxydamnacanthol (**78**), is a new compound. Four of the anthraquinones (**60**, **83-85**) are reported here for the first time from the genus *Pentas*. Rubiadin (**49**), Rubiadin-1-methyl ether (**51**), lucidin-ω-methyl ether (**60**), damnacanthol (**59**), and 5,6-dihydroxydamnacanthol (**78**) were identified as discussed earlier. The characterization of the other compounds are discussed below.

4.6.1.1 5,6-Dihydroxyrubiadin (83)

5,6-Dihydroxyrubiadin (**83**) was isolated as a reddish solid. The ^{1}H NMR data (Table 4.19) revealed a pair of *ortho*-coupled aromatic protons at δ_H 7.64 and 7.18 (*d*, J = 7.6 Hz) of disubstituted ring C of anthraquinone. One of these protons (δ_H 7.64) showed

HMBC correlation with the carbonyl carbon at δ_C 191.2 (C-9) and was assigned to H-8. Its coupling partner was therefore assigned to H-7. As in compound **78**, C-5 and C-6 are substituted with two hydroxyl groups. The ^{13}C NMR (Table 4.19) chemical shift of the aromatic carbons in this ring is consistent with C-5/C-6 oxygenation. Furthermore, in the ^{1}H NMR spectrum, the presence of two downfield shifted signals at δ_H 11.05 and δ_H 12.1 corresponding to chelated hydroxyl groups of this compound clearly indicated the presence of hydroxyl groups at C-5 of ring C and C-1 of ring A. The chemical shift values of the carbonyl groups (δ_C 187.9 and 191.2) indicated that both carbonyl groups are involved in hydrogen bonding.

Ring A is trisubstituted at C-1, C-2 and C-3, with the only aromatic proton (δ_H 7.28) being H-4, showing HMBC correlations with oxygenated (hydroxyl) quaternary carbon at δ_C 160.7 (C-3) and quaternary carbon δ_C 129.0 (C-2). The substituent at C-2 was determined as a methyl group (δ_H 2.06; δ_C 6.44) which is consistent from biogenetic considerations as well. HMBC correlation between the methyl protons and three quaternary carbons at δ_C 129.0 (C-2), 160.3 (C-1) and 160.7 (C-3) confirmed the substitution pattern in this ring. Thus, based on the above spectroscopic evidence the compound was characterized as 1,3,5,6-tetrahydroxy-2-methyl-9,10-anthraquinone (**83**), previously isolated from *Morinda citrifolia* (Kenichiro *et al.*, 1981) under the trivial name 5,6-dihyroxyrubiadin. However, this is the first report on the occurrence of the compound from any *Pentas* species.



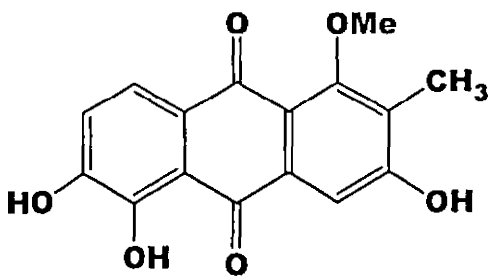
83

Table 4.19: ^{1}H (600 MHz) and ^{13}C (125 MHz,) NMR of 5,6-Dihydroxyrubiadin (**83**) and 5,6-Dihydroxyrubiadin-1-methyl ether (**84**): δ (ppm), *J* (Hz) in DMSO-d_6

Position	Compound 83		Position	Compound 84	
	δ_H (*J* in Hz)	δ_C		δ_H (*J* in Hz)	δ_C
1	-	160.3	1	-	165.0
1a	-	107.1	1a	-	107.1
2	-	129.0	2	-	119.6
3	10.3, *s*	160.7	3	10.5, *s*	164.5
4	7.28, *s*	110.1	4	7.51, *s*	112.7
4a	-	134.6	4a	-	137.4
5	-	153.6	5	-	153.8
5a	-	124.1	5a	-	124.2
6	-	154.3	6	-	155.1
7	7.18 (*d*, 1H, *J* = 7.6)	119.1	7	8 (*d*, 1H, *J* = 7.6)	119.8
8	7.65 (*d*, 1H, *J* = 7.6)	121.3	8	65 (*d*, 1H, *J* = 7.6)	124.3
8a	-	123.7	8a	-	130.8
9	-	187.9	9	-	182.8
10	-	191.2	10	-	192.6
1-OH	12.1, *s*	-	1-OH	12.1, *s*	
5-OH	11.05, *s*	-	5-OH	11.05, *s*	-
2-CH_3	2.06, *s*	6.44	2-CH_3	2.06, *s*	13.04
-	-	-	1-OCH_3	3.72 (*s*, 3H)	64.5

4.6.1.2 5,6-Dihydroxyrubiadin-1-methyl ether (84)

5,6-Dihydroxyrubiadin-1-methyl ether (**84**) was isolated as a red solid. Comparison of the ^{1}H and ^{13}C NMR data (Table 4.19) of this compound with those of **83** showed identical ring C substitution with both C-5 and C-6 being oxygenated. Ring A is also substituted at C-1, C-2 and C-3, with the only aromatic proton (δ_H 7.51) being H-4. ^{1}H NMR revealed that one of the two chelated hydroxyl resonance in **83** is replaced with methoxyl signals (δ_H 3.72; δ_C 64.5) in **84**. This suggested that C-1 or C-3 of **84** is substituted with methoxyl group. The downfield chemical shift of the methoxy group ($\geq$ 60 ppm) is attributed to di-*ortho* substitution and is in agreement with the methoxyl being at C-1 rather than C-3. Furthermore, the HMBC spectrum, correlation of the methoxyl protons with C-1 (δ_C 165.0), and methyl protons (δ_H 2.06) with C-1 (δ_C 165.0), C-2 (δ_C 119.6) and C-3 (δ_C 164.5) confirmed the substitution pattern in ring A. Based on the above spectroscopic evidence, the compound was identified as 3,5,6-trihydroxy-1-methoxy-2-methyl-9,10-anthraquinone (**84**), a compound previously isolated from *Putoria calabrica* (Gonzalez *et al.*, 1977), under the trival name of 5,6-dihydroxyrubiadin-1-methyl ether. This is only the second report on the isolation of this compound in nature and the first report from any *Pentas* species.

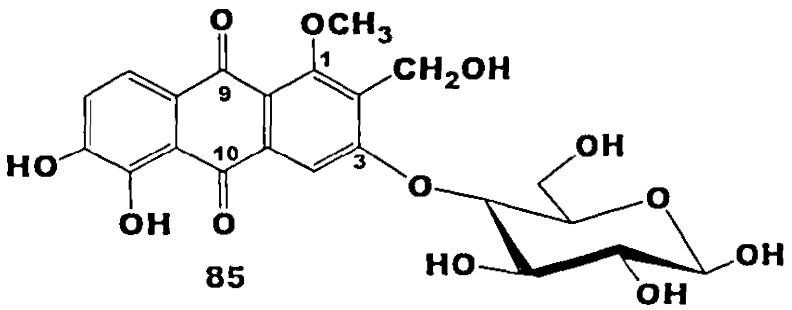


84

4.6.1.3 5,6-Dihydroxydamnacanthol-3-*O*-glucopyranoside (85)

5,6-Dihydroxydamnacanthol-3-*O*-glucopyranoside (**85**) was isolated as an orange solid. The ^{1}H and ^{13}C NMR spectral data (Table 4.20) revealed two hydroxyl (δ_C 153.1 and δ_C 154.6), methoxyl and oxymethylene substituents on an anthraquinone skeleton. Furthermore, the presence of a glucopyranosyl moiety was evident with the anomeric proton appearing at δ_H 5.13 along with the rest of the sugar signals (Table 4.20). In ring C, the presence of a pair of *ortho*-coupled aromatic protons at δ_H 7.19 and 7.57 (*d*, J = 8.2 Hz) and the ^{13}C NMR chemical shift values of the carbon atoms in this ring suggested C-5/C-6 oxygenation as in compounds **78**, **83** and **84**. Ring A therefore contains the methoxyl, oxymethylene and the glucopyranoxyl substituents. The singlet aromatic proton at δ_H 7.74 was assigned to H-4 of tri-substituted ring A. From biogenetic considerations and the ^{13}C NMR chemical shift values of the carbon atoms in this ring, oxygenation at C-1 and C-3 with carbon substitution (hydroxymethylene, δ_H 4.58) at C-2 was apparent as in the other anthraquinones of the *Pentas*species. In agreement with this, the singlet aromatic proton (δ_H 7.74) showed HMBC correlations with C-2 (δ_C 124.3), C-3 (δ_C 163.8) and with the carbonyl at δ_C 191.2 (C-10). The downfield chemical shift of the methoxy group (above 60 ppm) is attributed to the di-*ortho* substitution, and HMBC correlation of the methoxyl protons with C-1 enable the location of the methoxyl group at C-1. The glucopyranoxyl group could then be placed at C-3. In support of this, the anomeric proton of the sugar moiety (δ_H 5.13) showed HMBC correlation with the oxygenated quaternary carbon at δ_C 163.8 (C-3). Based on the above spectroscopic evidence, the compound was characterized as 5,6-dihydroxydamnacanthol-3-*O*-glucopyranoside (**85**), previously isolated from *Putoria calabria* (Calis *et al.*, 2002). The

NMR data for the compound are comparable to the literature information (Table 4.20). This is the first report on the isolation of this compound from any *Pentas* species.



85

Table 4.20: ^{1}H (800 MHz) and ^{13}C (125 MHz) NMR data of 5,6-dihydroxydamnacanthol-3-*O*-β-glucopyranose (**85**) compared with literature.

Position	This work		Colis *et al.*, 2002	
	δ_H	δ_C	δ_H	δ_C
1	-	163.5	-	160.6
1a	-	138.6	-	135.5
2	-	124.3	-	121.1
3	-	163.8	-	160.4
4	7.74, *s*	123.7	7.75, *s*	120.9
4a	-	135.5	-	132.2
5	-	153.1	-	150.2
5a	-	111.9	-	115.7
6	-	154.6	-	151.5
7	7.19 (*d*, *J*=8.2)	124.3	7.20 (*d*, *J*=8.0)	120.8
8	7.57 (*d*, *J*=8.2)	123.9	7.58 (*d*, *J*=8.0)	120.6
8a	-	128.3	-	125.2
9	-	182.1	-	179.0
10	-	191.2	-	187.9
1-OCH_3	3.81 (3H, *s*)	63.5	3.87 (3H, s)	62.6
2-CH_2OH	4.58 (2H, *m*)	55.1	4.60 (*m*, 2H)	51.9
1'	5.13 (*d*, *J*=7.6)	104.2	5.10 (*d*, *J*=7.8)	101.1
2'	3.33*	72.5	3.37*	73.3
3'	-	76.5	3.34*	76.0
4'	-	65.7	3.24*	69.4
5'	3.40*	80.4	3.40*	77.4
6'	3.55 (1H, *m*)	63.5	3.53* (*dd*, *J*=11, 4.5)	60.5
	3.70 (1H, *m*)		3.70* (*dd*, *J*=11, 2.0)	

* Multiplicity is unclear due to overlapping

4.6.1.4 Lucidin-3-*O*-β-primveroside (54)

Lucidin-3-*O*-β-primveroside (**54**) was isolated as an orange powder. The ^{1}H and ^{13}C NMR spectral data (Table 4.21) revealed that this compound is also an anthraquinone glycoside. The presence of four aromatic protons centered at δ_H 7.95 (*m*) and 8.20 (*m*)

displayed the same multiplicity pattern as tectoquinone (76) where ring C of the anthraquinone skeleton is unsubstituted. Ring A is trisubstituted at C-1, C-2 and C-3 as the other anthraquinones of this genus and the substituents being hydroxyl, hydroxymethylene and a sugar moiety, respectively. Thus the only aromatic proton (δ_H 7.47, *s*) was assigned to H-4. The hydroxyl group was placed at C-1 due to downfield chemical shift value (δ_H 12.40) being *peri* to the carbonyl carbon (C-9). The large difference in chemical shift value ($\Delta\delta$ 5.00 ppm) between the two carbonyl carbons C-9 (δ_C 190.0) and C-10 (δ_C 184.5) is due to the hydrogen bonding between the carbonyl carbon (C-9) and the *peri* hydroxyl group at C-1 confirming the location of the hydroxyl group at C-1. Based on biogenetic considerations, the oxymethylene group (δ_H 4.58, 4.66, two sets of mutually coupled protons, AB system (*d*, J = 12.0 Hz) was placed at C-2 of ring A.

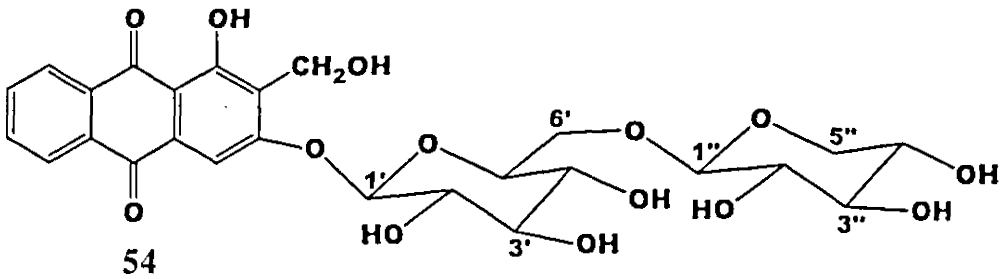
That the ^{1}H NMR spectrum (Table 4.21) revealed two anomeric protons at δ_H 4.90 (H-1"; δ_C 103.97, C-1") and δ_H 5.75 (H-1'; δ_C 100.6, C-1') suggested that the sugar is a disaccharide. By comparison with literature (Kusamba *et al.*, 1993) the disaccharide was readily identified as primverosyl moiety. This sugar could either be placed at C-3 or be attached to the oxymethylene moiety at C-2. The possibility of the primverosyl moiety being attached to the oxymethylene was ruled out by looking at the chemical shift value of the oxymethylene group (δ_H 4.58, 4.66; δ_C 54.0) which is typical of hydroxymethylene. When a sugar unit is attached to oxymethylene carbon the corresponding chemical shift of the carbon should be shifted to *ca.* 60 ppm, while the corresponding protons will be shifted higher field, *ca.* δ_H 4.70-4.90 (Kusamba *et al.*, 1993). Furthermore, the HMBC correlation of the anomeric proton with C-3 (δ_C 165.1)

confirmed the attachment of the primversoyl moiety to the oxygen at C-3. Based on the above spectral data, the compound was characterized as lucidin-3-*O*-β-primveroside (**54**), previously isolated from *Pentas zanzibarica* (Kusumba *et al.*, 1993), *Rubia tinctorum* (El-Emary and Backheet, 1998) and *Prismatomeris connate* (Rubiaceae) (Jing *et al.*, 2011).

Table 4.21: ^{1}H (800 MHz) and ^{13}C (125 MHz) NMR data of lucidin-3-*O*-β-primeveroside (**54**) (in DMSO-d_6) and comparison with literature.

Position	This work		Kusamba *et al.*, 1993	
	δ_H	δ_C	δ_H	δ_C
1	-	164.9	-	161.8
2	-	121.4	-	123.7
3	-	165.1	-	162.0
4	7.47, *s*	109.5	7.40, *s*	106.5
4a	-	135.9	-	132.6
5	8.20 (*m*)	126.7	8.12 (*dd*, *J*=2,8)	126.4
5a	-	129.6	-	133.7
6	7.95 (*m*)	135.8	7.88 (*dd*, *J*= 8,8)	134.7
7	7.95 (*m*)	135.9	7.88 (*dd*, *J*=8,8)	134.7
8	8.20 (*m*)	129.6	8.12 (*dd*, *J*=2,8)	126.8
8a	-	137.8	-	132.8
9	-	190.0	-	186.9
10	-	184.5	-	181.3
CH_2-OH	4.58 (*d*) & 4.66 (*d*, *J* = 12.0 Hz)	54.0	4.55 & 4.62 (*d*, *J* = 12.0 Hz)	51.0
1-OH	12.4, *s*	-		-
1'	5.12 (*d*, *J*=7.5)	103.8	5.12 (*d*, *J*=7.5)	100.9
2'		72.5		73.3
3'		76.3		75.9
4'		72.2		69.5
5'		78.9		76.4
6'		71.0		68.0
1''	4.15 (*d*, *J*=7.0)	107.1	4.12 (*d*, *J*=7.5)	104.1
2''		72.5		73.3
3''		78.7		75.8
4''		71.1		69.3
5''		68.6		65.6

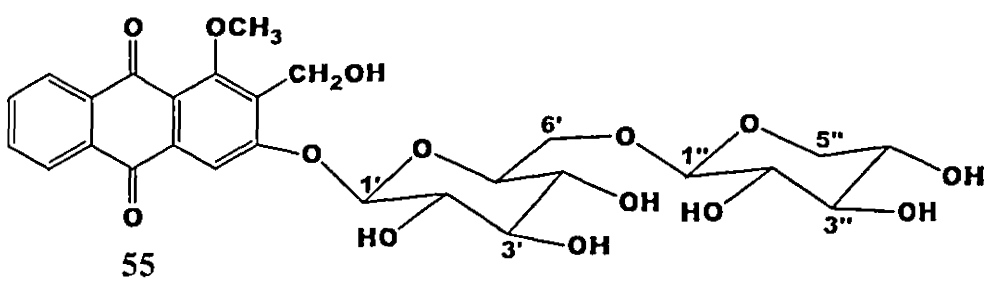
* For ^{1}H NMR of the sugar moiety only the anomeric values are shown, δ in ppm, *J* in Hz.



54

4.6.1.5 Damnacanthol-3-*O*-β-primveroside (55)

Damnacanthol-3-*O*-β-primveroside (**55**) was isolated as a yellow powder. Comparison of the ^{1}H and ^{13}C NMR spectral data (Table 4.22) to those of lucidin-3-*O*-β-primveroside (**54**) (Table 4.22) showed that the chelated hydroxyl group in **54** was replaced by methoxyl group (δ_H 3.88; δ_C 62.6) in **55**. This indicates that compound **55** has a methoxyl group at C-1 instead of a hydroxyl group. The ^{13}C NMR chemical shift of the methoxyl carbon (δ_C 62.6) is typical of a di-*ortho*-substitution confirming its location at C-1. The hydroxylmethylene group is at C-2 and *O*-primveroside group at C-3 as supported by the NMR spectral data (Table 4.22) and HMBC correlations as in **54**. The compound was identified as damnacanthol-3-*O*-primveroside (**55**), which had been previously isolated from *Pentas zanzibarica* (Kusamba *et al.*, 1993) and *Prismatomeris connate* (Rubiaceae) (Jing *et al.*, 2011).



55

Table 4.22: ^{1}H (800 MHz) and ^{13}C (125 MHz) NMR data of damnacanthol-3-*O*-β-primeveroside (55) (in DMSO-d_6) and comparison with the literature.

Position	This work		Kusamba *et al.*, 1993	
	δ_H	δ_C	δ_H	δ_C
1	-	163.7	-	160.7
1a	-	120.4	-	120.4
2	-	131.5	-	131.6
3	-	163.8	-	160.8
4	7.74, *s*	109.1	7.71, *s*	109.2
4a	-	131.9	-	132.0
5	8.05 (*m*)	126.1	8.14 (*dd*, *J*=2.0,8.0)	126.2
5a	-	135.6	-	135.8
6	7.88 (*m*)	133.5	7.88 (*dd*, *J*= 8.0,8.0)	133.6
7	7.88 (*m*)	134.2	7.88 (*dd*, *J*=8.0,8.0)	134.8
8	8.05 (*m*)	126.5	8.14 (*dd*, *J*=2.0,8.0)	126.7
8a	-	134.5	-	134.4
9	-	185.2	-	180.5
10	-	183.3	-	182.2
1-OCH_3	3.88 (3H, *s*)	62.6	3.87 (3H, *s*)	62.8
2-CH_2OH	4.62 (*d*, 2H, *J* = 12.0)	51.8	4.60 (*d*, 2H, *J* = 12.0)	52.0
1'	5.10 (*d*, *J*=7.5)	100.8	5.10 (*d*, *J*=7.5)	101.0
2'		73.2		73.3
3'		75.7		75.9
4'		69.3		69.5
5'		76.3		76.4
6'		67.9		68.0
1''	4.15 (*d*, *J*=7.5)	103.9	4.15 (*d*, *J*=7.5)	104.4
2''		73.1		73.4
3''		75.6		75.7
4''		69.1		69.2
5''		65.5		65.6

* For ^{1}H NMR of the sugar moiety only the anomeric values are shown, *J* in Hz, δ (ppm)

The compounds isolated from the roots of *P. suswaensis* are listed below (Fig: 4.10).

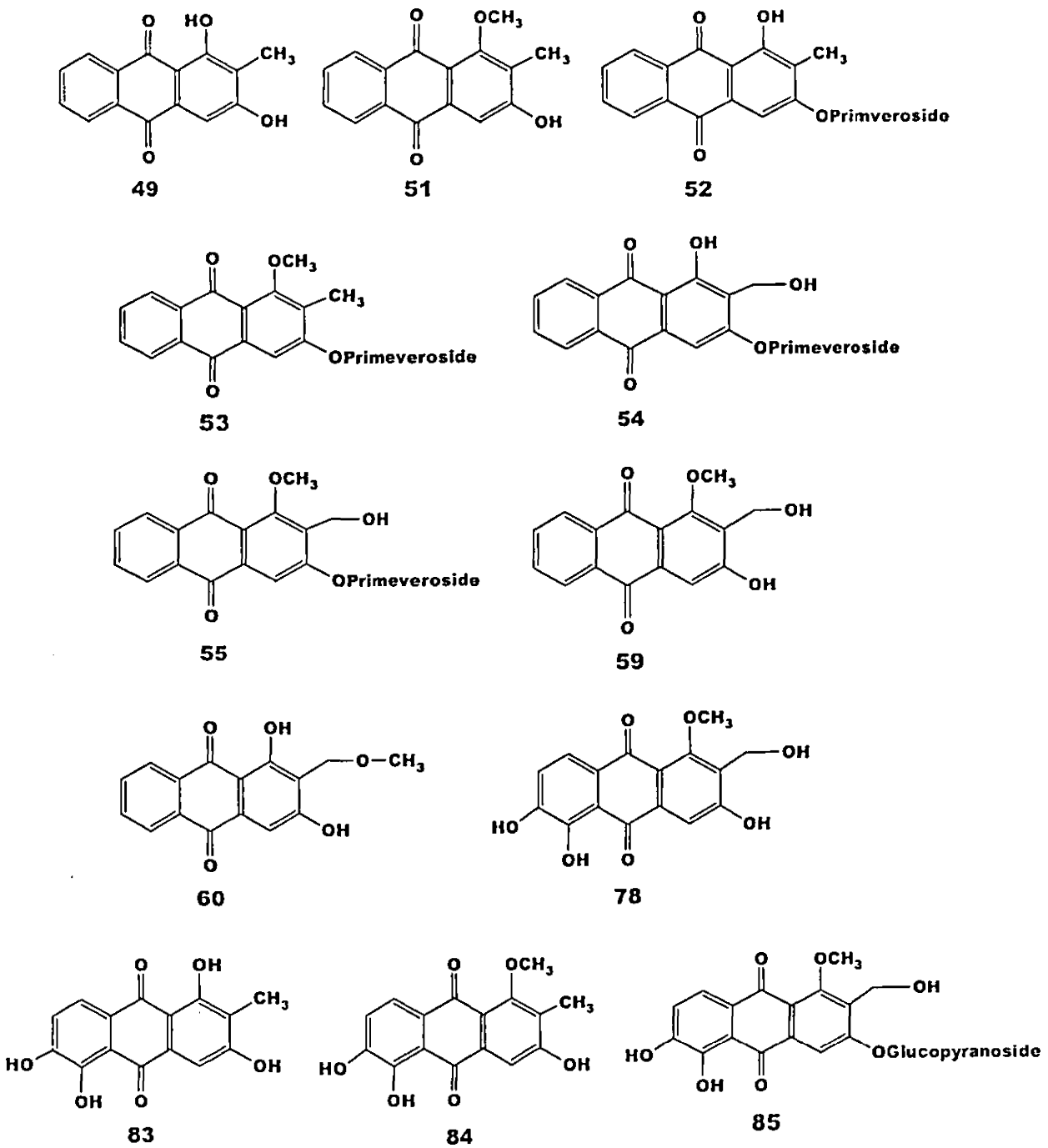


Figure 4.11: Anthraquinones isolated from the roots of *Pentas suswaensis*

4.7 Secondary metabolites isolated from *Pentas parvifolia*

4.7.1 Naphthalene derivatives isolated from the root of *Pentas parvifolia*

Figure 4.12: Photograph of *Pentas parvifolia* (Prof. Mate Erdelyi, October 2011)

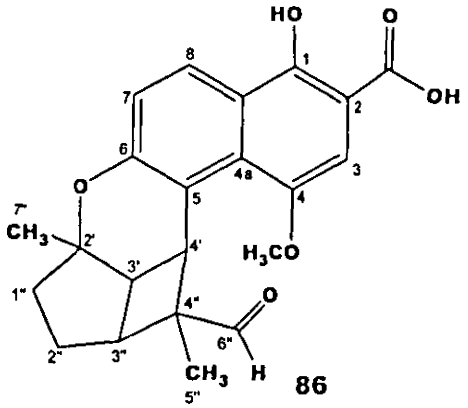
Chromatographic separation of the root of *Pentas parvifolia* gave five dihydronaphthaquinones of which four of the previously elaborated new compounds (**70-73**) were re-isolated and hence have been identified as discussed earlier. The characterization of the other compound is discussed below.

4.7.1 Compound 86

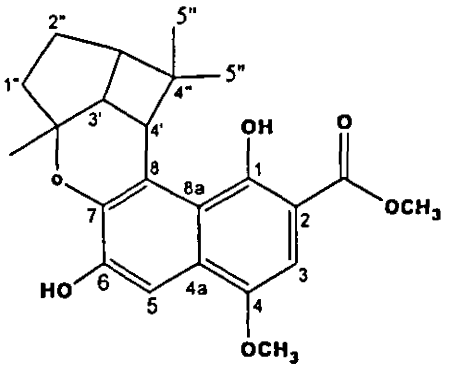
The compound **86** was isolated as an orange powder. As in **71-73** (Section 4.2), the ^{1}H and ^{13}C NMR data (Table 4.23) indicated that this compound is a naphthalene derivative. The ^{1}H and ^{13}C NMR spectra further revealed that ring A is trisubstituted with chelated hydroxyl (δ_H 12.01), carboxylic acid (δ_C 172.5) and methoxyl (δ_H 3.82; δ_C 55.5) groups, with the only aromatic proton (H-4) appearing as a singlet at δ_H 7.09. The HMBC correlation of this singlet (δ_H 7.09) with the carbonyl (δ_C 175.7), oxygenated quaternary carbons (δ_C 147.7 and 154.9) coupled with the correlation of the methoxyl at δ_H 3.82 with

the quaternary carbon (δc 147.7) suggests that ring A is substituted at C-1, C-2 and C-4 which is identical with the substitution pattern in **71-73**. The location of the hydroxyl group (δ_H 12.01) at C-1 is in agreement with its *peri* position to the carbonyl group at C-2. The methoxyl group can then be placed at C-4. In ring B, a pair of doublets appeared at δ_H 8.07 and 7.28 (J = 7.9 Hz), and suggested that this ring is substituted either at C-5/C-6 or C-7/C-8. The HMBC correlation of the proton at δ_H 8.07 (*d*, J=7.9 Hz) with the oxygenated quaternary carbon at δ_C 154.9 (C-1) suggested that this doublet be assigned to H-8 and its coupling partner (δ_H 7.28) be attributed to H-7. Consequently the substituents in ring B should be located at C-5/C-6. The ^1H and ^{13}C NMR (Table 4.23) data of the alicyclic system of compound **86** are in agreement with the assignment of the substituent at this position to a cyclol moiety constituting a cyclolnaphthoquinone as in **58** (Bukuru *et al.*, 2003). The only difference is that this compound has fewer methyl groups since one of the three methyl groups (6"-CH_3) is oxidized to an aldehyde (δ_C 204.9); as a consequence of which the sp^3 quaternary carbon C-4" is shifted downfield (δ_C 69.6). The C-5" methyl (δ_C 22.2) and C-3" methine (δ_C 40.4) were shifted downfield due to oxidation of the methyl 6"-CH_3 to aldehyde.

Bukuru *et al.* (2003) have suggested that the biosynthetic pathway leading to the cyclol dihydronaphthoquinones could be the same as for natural meroterpenoid coumarins (Crombie *et al.*, 1983). Oxidation of the methyl 6"-CH_3 in **72** followed by a concerted (2 + 2) *in vivo* cycloaddition of the dihydronaphthoquinone gives **86**. Alternatively, the concerted (2 + 2) cycloaddition take place first producing the dihydronaphthaquinone intermediate by a light-induced mechanism followed by oxidation of 6"-CH_3 to give **86** (Scheme 4.6).



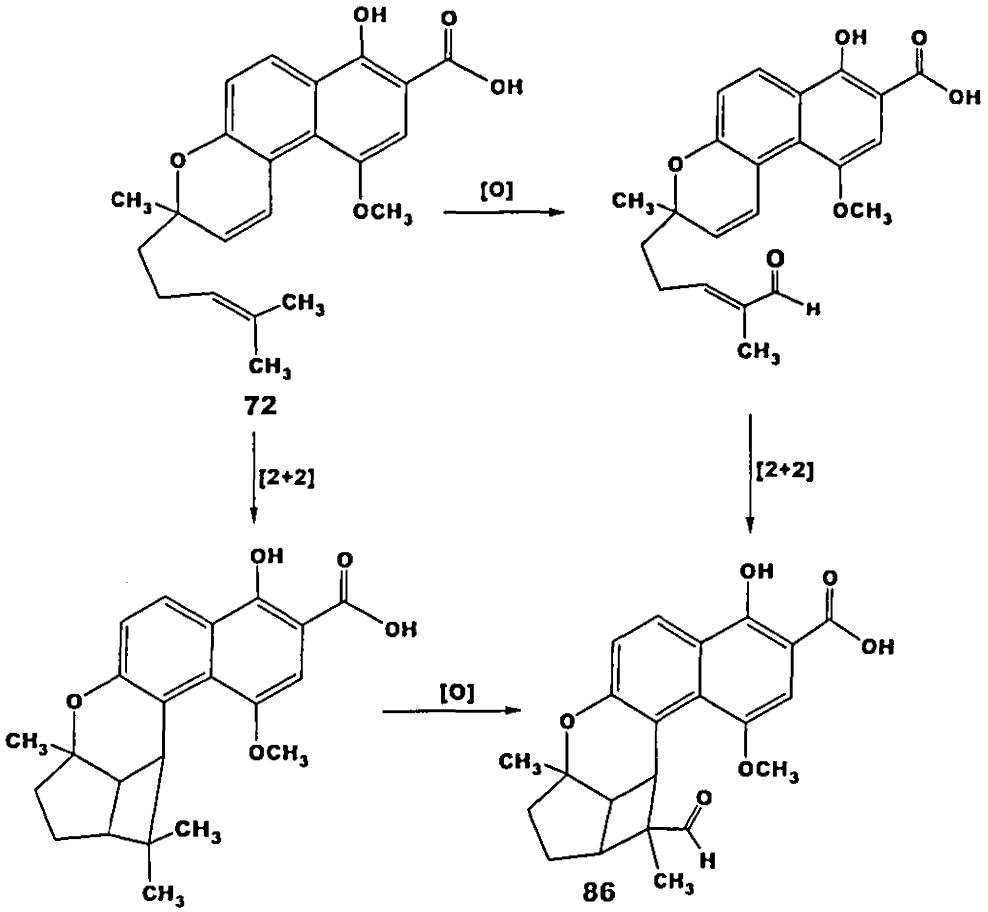
86



58

Table 4.23: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of compounds **58** and **86** (DMSO-d_6); δ in ppm, J in Hz

Position	86		58 (Bukuru *et al.*, 2003)	
	δ_H	δ_C	δ_H	δ_C
1	-	154.9	-	157.9
2	-	104.9	-	99.5
3	7.09 (*s*)	105.0	6.93 (*s*)	102.3
4	-	147.7	-	146.8
4a	-	117.6	-	119.1
5	-	125.4	-	123.1
6	-	151.9	-	141.5
7	7.28 (*d*, *J*=7.7)	117.6	-	148.9
8	8.07 (*d*, *J*=7.7)	121.3	7.60 (*s*)	102.7
8a	-	125.5	-	127.8
2'	-	81.9	-	85.4
4'	5.7 (*brs*)	37.6	4.57 (*d*, *J*= 9.57)	39.0
3'	2.8 (*m*)	47.7	2.7 (*dd*, *J*= 9.57, 9.24)	41.3
1''	1.9-2.06 (*m*)	37.6	1.64-1.80 (*m*) 2.00-2.07 (*m*)	40.6
2''	1.8-1.89 (*m*)	25.7	1.64-1.80 (*m*)	25.3
3''	1.99-2.08 (*m*)	40.4	2.49 (*m*)	46.7
4''	-	69.9	-	41.8
5''	1.25 (*s*)	22.2	1.49 (*s*)	34.0
6''	9.7 (*s*)	204.9	0.54 (*s*)	19.2
7''	1.00 (*s*)	22.0	1.36 (*s*)	25.6
4-OCH_3	3.82 (*s*)	55.5	3.87 (*s*)	55.8
2-$COO\underline{CH_3}$	-	-	3.96 (*s*)	52.1
2-$\underline{C}OOCH_3$	-	172.5	-	172.2



Scheme 4.5: Proposed biogenesis pathway for compound 86

The compounds isolated from the roots of *P. parvifolia* are listed below (Figure 4.13)

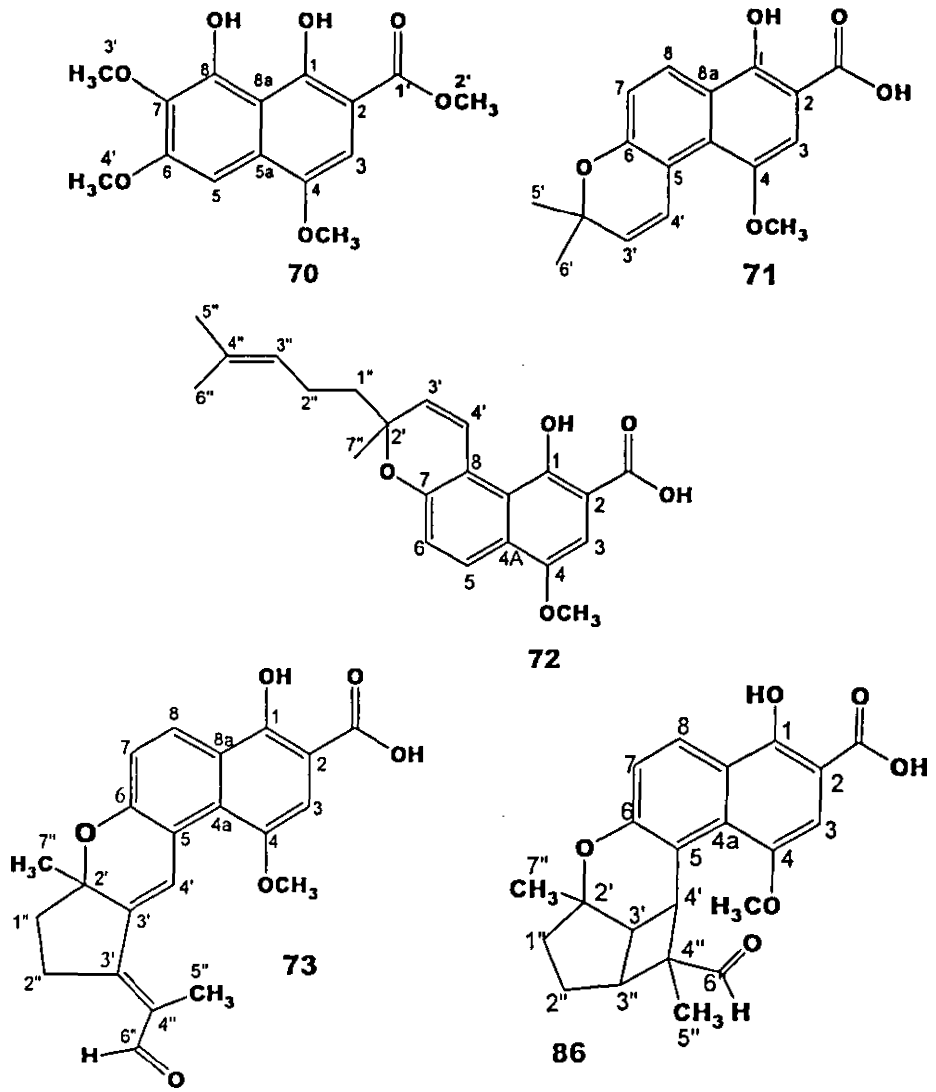


Figure 4.13: Dihydronaphthoquinone derivatives isolated from the roots of *Pentas parvifolia*

4.8 Biosynthesis of anthraquinones and naphthoquinones of the genus *Pentas*

There are two main biosynthetic pathways leading to anthraquinones in higher plants: the polyketide pathway (Van den Berg and Labadie, 1989) and the chorismate/O-succinylbenzoic acid pathway (Leistner, 1985), the latter occurs in Rubiaceae in which ring B and C of the anthraquinone skeleton are derived from shikimic acid, α-

ketoglutarate via o-succinylbenzoate, whereas ring A is derived from isopentenyl diphosphate (Han *et al.*, 2001). All (except tectoquinone (74)), anthraquinones isolated in the current work have a hydroxyl/methoxyl group at C-1 position, carbon substitution at C-2 position and hydroxyl/O-glycosyl at C-3 of ring A. Ring C in the majority of the anthraquinones of the family Rubiaceae remains unsubstituted. However, in the last decade anthraquinones having additional hydroxyl/alkoxyl groups at C-5 and C-6 of ring C have been reported from the family (Han *et al.*, 2001; Schripsema *et al.*, 1999; Endale *et al.*, 2012a; Calis *et al.*, 2002; Zhou *et al.*, 1993, Fraga *et al.*, 2009). Five anthraquinones bearing such pattern have been isolated under the current work (**78, 82-85**). The biogenetic route towards the anthraquinones of the genus *Pentas* appear to be inagreement (Scheme 2.1) with the one proposed by Han *et al.* (2001). The oxygenation at C-5/C-6 appears to have occurred at late stages of the biogenesis.

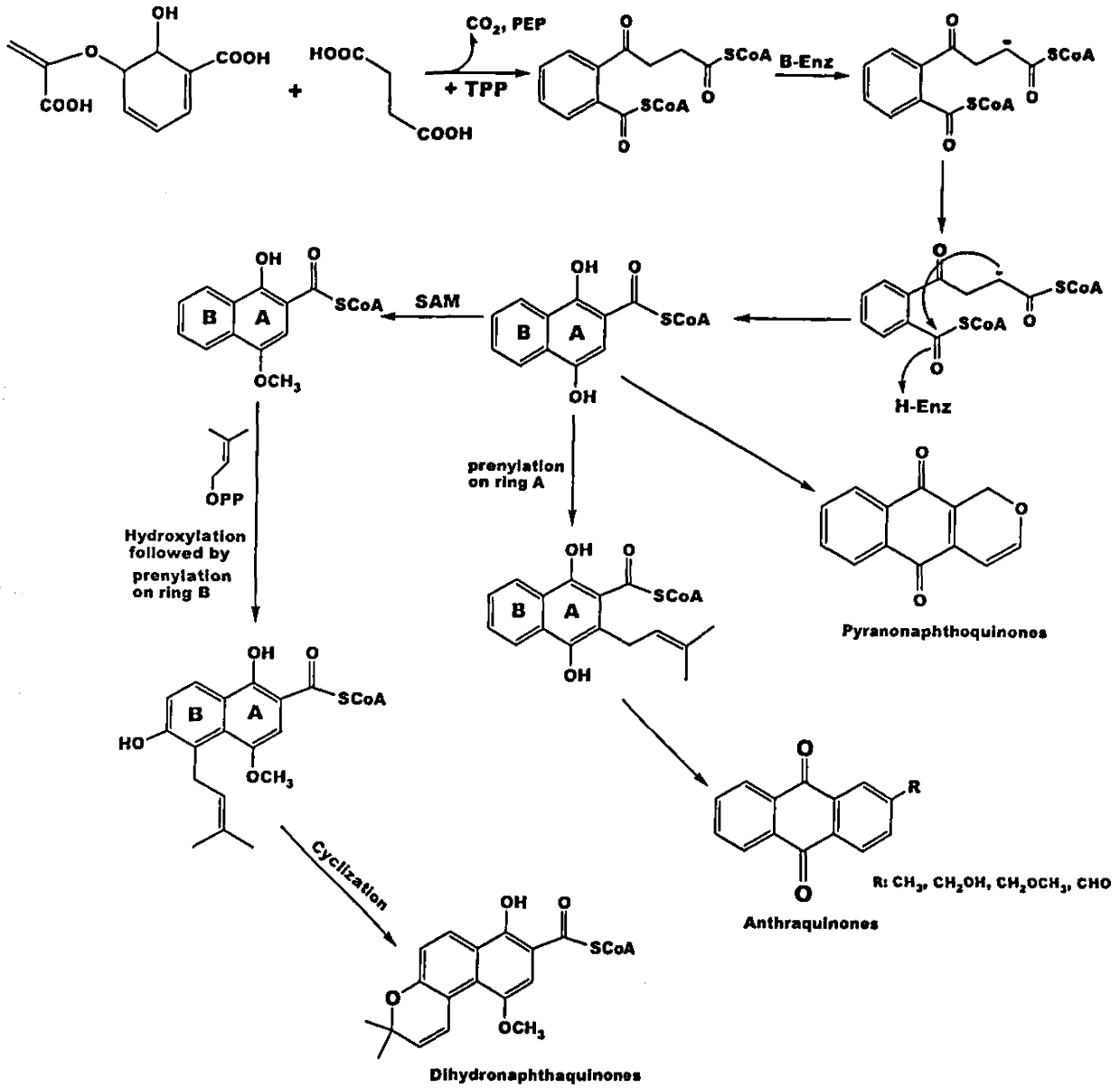
In the current study benzoic acid (**79**) was isolated from the roots of *P. longiflora*. Earlier studies suggest that one of the proposed biosynthesis schemes leading to benzoic acid (C6-C1) derivatives is shikimate/chorismate pathway, which is common to non-flavonoid phenolics (Wildermuth et *al.*, 2006; Budi Muljono *et al.*, 2002). The isolation of benzoic acid (**79**) in the root of *P. longiflora* is in agreement with the involvement of the phenylpropanoid precursor in the biosynthesis of the naphthalene derivatives and anthraquinones of this plant and by extension in the genus *Pentas* (Robbins *et al.*, 2003; Wildermuth *et al.*, 2006; Budi Muljono *et al.*, 2002).

Originating from the biosynthetic pathways most anthraquinones of the Rubiaceae have hydroxyl, methoxyl and/or C-2 carbon substitution in ring A (Figure 2.1) (Schripsema *et al.*, 1999) and some carry additional hydroxyl and/or alkoxyl groups in ring C, mainly in

positions 5 and 6 (Han *et al.*, 2001, Endale *et al.*, 2012a). These latter oxygen atoms are introduced at a late stage of the biogenesis (Han *et al.*, 2001), which is shown for example for morindone, as reported form the cell cultures of *Morinda citrifolia* (Ee *et al.*, 2010) and for putorinoside A, isolated from *Putoria calabrica* (Gonzalez *et al.*, 1977). As a consequence of the biosynthetic pathway most, if not all, anthraquinones carry a carbon substituent at position 2 in ring A (Han *et al.*, 2001). One of the rare exceptions from the above rule is 2-ethoxy-1-hydroxyanthraquinone isolated from *Morinda citrifolia* (Ee *et al.*, 2009), a compound lacking carbon (CH_2, CHO, CH, etc.) substitution at C-2, which might be indicative for the existence of another biosynthetic pathway. We would like to emphasize that if carbon substitution is present in an anthraquinone derived from the family Rubiaceae, based on biogenesis (Han *et al.*, 2001), the currently accepted nomenclature assigns it unambiguously to position 2 in ring A. Not following the above convention (Osman *et al.*, 2010) may be perplexing in the evaluation of biosynthetic routes and bioactivities. Hence, the compounds named 1,2-dimethoxy-6-methyl-9,10-anthraquinone and 1-hydroxy-2-methoxy-6-methyl-9,10-anthraquinone (Osman *et al.*, 2010) should be correctly named as 5,6-dimethoxy-2-methyl-9,10-anthraquinone and 6-hydroxy-5-methoxy-2-methyl-9,10-anthraquinone.

Comparing the structural features of all compounds isolated from six *Pentas* species, we propose the following general biosynthesis route which explains the structural relationship between the chemical constituents as well as botanical grouping of respective species (Scheme 4.6). In this biosynthetic route, ring A and B of both anthraquinones and naphthalene derivatives are derived from shikimic acid, α-ketoglutarate via *o*-

succinylbenzoate, whereas ring C (for anthraquinone only) is derived from isopentenyl diphosphate, a universal building block for all isoprenoids (Han *et al.*, 2001).



Scheme 4.6: Summerized biogenesis pathway towards quinones of the genus *Pentas*

4.9 Chemotaxonomic significance of anthraquinones and naphthaquinones of the genus *Pentas*

Table 4.24 shows the distribution of eighteen quinones across six *Pentas* species, while Table 4.25 summarizes suggested chemotaxonomic marker compounds for some of the *Pentas* species. The phytochemical study on the roots extracts of *P. lanceolata*, *P. suswaensis* and *P. micrantha* gave C-2 alkyl ($CH_3/CH_2OH/CHO/CH_2OCH_3$) substituted anthraquinones of which three of the anthraquinones: rubiadin (**49**), rubiadin-1-methyl ether (**51**) and damnacanthol (**59**) were isolated from all the three species. However, C-2 formyl containing compounds such as damnacanthal (**50**) and nordamnacanthal (**76**) were isolated only from *P. lanceolata* and from *P. micrantha*. Nevertheless, none of the C-2 formyl containing anthraquinones were detected (TLC/HPLC) from *P. suswaensis*.

Dihydronaphthoquinones and pyranonaphthaquinone were isolated from the roots of *P. bussei*, *P. parvifolia* and *P. longiflora*. The pyranonaphthaquinones, pentalongin (**33**) and psychorubrin (**80**), were isolated only from *P. longiflora*; whereas dihydronaphthoquinone with complex heterocyclic ring system and oxygenation pattern were isolated only from *P. bussei* and *P. parvifolia*. A new polyoxygenated dihydronaphthoquinone (**70**) is reported for the first time from *P. bussei* and *P. parvifolia*. To the best of our knowledge, this is only the second report on the occurrence of highly oxygenated dihydronaphthoquinones in nature following the report from liverwort *Adelanthus decipiens* (Davi *et al.*, 1998). In the current phytochemical analysis, the cyclol containing compound (**86**) and its rearranged derivative (**73**) were isolated from the roots of *P. parvifolia* in addition to the previous report of a cyclol containing naphthalene derivative (**58**) from the roots of *P. bussei* (Bukuru *et al.*, 2003).

Pyranonaphthquinones such as pentalongin (**33**) and psychorubrin (**80**) can be considered as markers for *P. longiflora*, whereas dihydronaphthoquinones (**70-73**) can be considered as markers for *P. bussei* and *P. parvifolia*. 5,6-Dihydroxylucidin-ω-methyl ether (**82**) appear to be a marker compound for *P. micrantha*. In general, the presence of C-2 alkyl substituted anthraquinones are the characteristic constituents of *P. lanceolata, P. micrantha* and *P. suswaensis*. The absence of formyl substituted (at C-2) anthraquinones distinguish *P. suswaensis* from the other two species. Nordamnacanthal (**76**) can be taken as a chemotaxonomic marker for *P. lanceolata*.

Previous reports on the roots of *P. zanzibarica* showed that the plant contains anthraquinones and anthraquinone glycosides (Kusamba *et al.*, 1993). Such compounds have been isolated from *P. lanceolata* and *P. suswaensis* in the present study (Table 4.24) suggesting a close chemical connection among the three taxa (*P. suswaensis, P. micrantha* and *P. lanceolata*). This is in agreement with Agnew (1974) suggestion that *P. lanceolata* is botanically closely related to *P. suswaensis* and *P. zanzibarica*.

Table 4.24: Distribution of eighteen quinones isolated from the roots of six *Pentas* species

Compound	*P. bussei*	*P. longiflora*	*P. micrantha*	*P. suswaensis*	*P. lanceolata*	*P. parvifolia*	Literature
Pentalongin (33)	-	+	-	-	-	-	*Pentas longiflora* (Endale *et al.*, 2012a)
Mollugin (34)	-	+	-	-	-	-	*Pentas longiflora* (Endale *et al.*, 2012a)
Psychorubrin (80)	-	+	-	-	-	-	*Pentas longiflora* (Endale *et al.*, 2012a)
Rubiadin (49)	-	-	+	+	+	-	*Pentas lanceolata* (Endale *et al.*, 2012a)
Rubiadin-1-methyl ether (51)	-	-	+	+	+	-	
Nordamnacanthal (76)	-		-	-	+	-	
5,6-dihyroxylucidin-ω-methyl ether (82)	-	-	+	-	-	-	*Pentas micrantha* (Endale *et al.*, 2012c)
5,6-dihydroxyrubiadin (83)	-	-	-	+	-	-	
5,6-dihydroxyrubiadin-1-methyl ether (84)	-	-	-	+	-	-	
5,6-dihydroxydamnacantholglucopyranoside (85)	-	-	-	+	-	-	
5,6-dihydroxydamnacanthol (78)	-	-	+	+	+	-	*Pentas lanceolata* (Endale *et al.*, 2012a)
Damnacanthol (59)	-	-	+	+	+	-	*Pentas lanceolata* (Endale *et al.*, 2012a)
Lucidin-ω-methyl ether (60)	-	-	+	+	+	-	*Pentas lanceolata* (Endale *et al.*, 2012a)
Tectoquinone (74)	-	-	+	-	+	-	
Busseidihydroquinone A (70)	+	-	-	-	-	+	*Pentas bussei* (Endale *et al.*, 2012b)
Busseidihydroquinone B (71)	+	-	-	-	-	+	*Pentas bussei* (Endale *et al.*, 2012b)
Busseidihydroquinone C (72)	+	-	-	-	-	+	*Pentas bussei* (Endale *et al.*, 2012b)
Compound (56)	+	-	-	-	-	-	*Pentas bussei* (Endale *et al.*, 2012b)
Busseidihydroquinone D (73)	+	-	-	-	-	+	*Pentas bussei* (Endale *et al.*, 2012b)

Key: '+' indicates that the compound is present whereas '–' indicates that the compound is absent

Table 4.25: Marker compounds from six *Pentas* species

S.No	Species name	Marker compounds
1.	*Pentas longiflora*	**33, 34, 80**
2.	*Pentas lanceolata*	**76**
3.	*Pentas bussei*	**56, 70-73**
4.	*Pentas parvifolia*	**70-73, 86**
5.	*Pentas suswaensis*	**83-85**
6.	*Pentas micrantha*	**82**

4.9.1 HPLC analysis of the extracts of the root of *Pentas micrantha*

Analytical RP-HPLC comparison of three different (ethyl acetate, methanol and water) extracts of *P. micrantha* revealed that 5,6-dihydroxylucidin-ω-methyl ether (**82**), the new natural product, was only detected in methanol and ethyl acetate extracts but not in water extract whereas 5,6-dihydroxydamnacanthol (**78**) was detected in all three extracts (water, ethyl acetate and methanol). Rubiadin (**49**) was found to be a major component of the methanol extract (Figure 4:14).

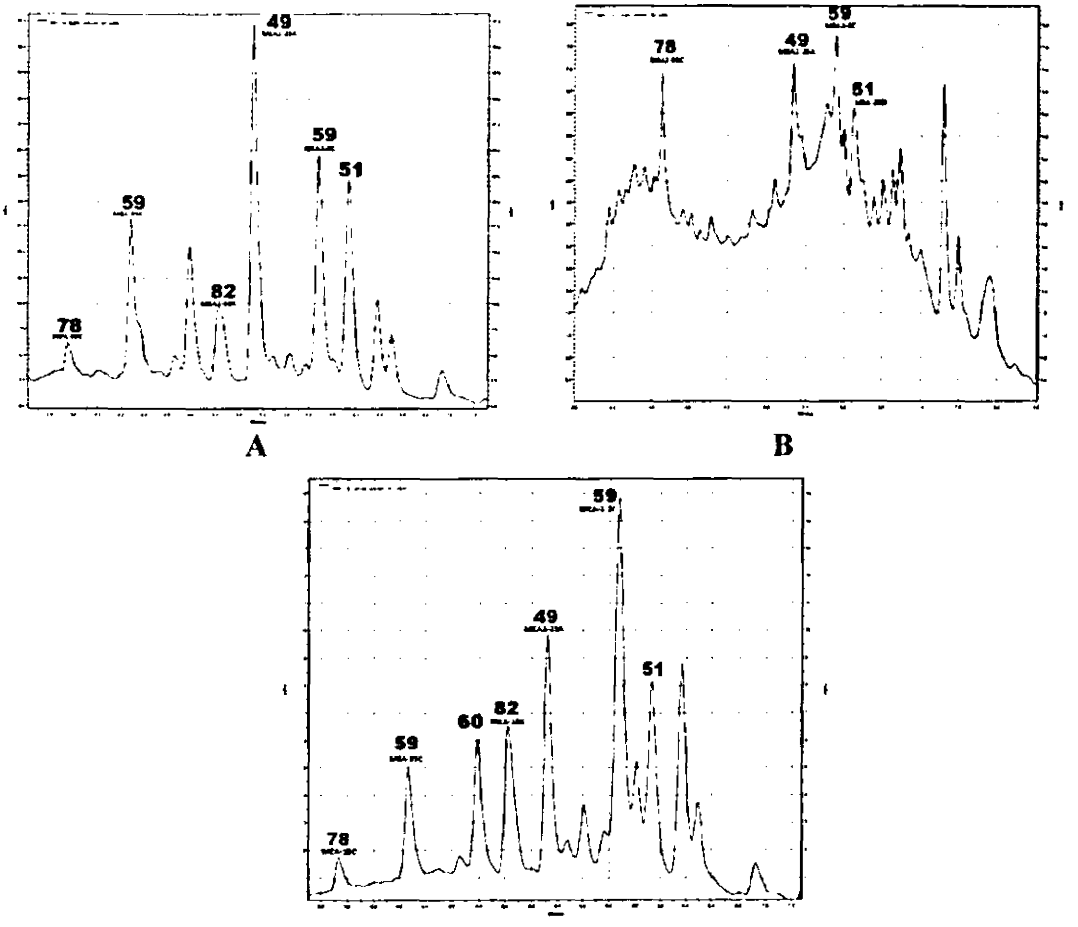


Figure 4.14: HPLC profile of methanol (**A**), water (**B**) and ethyl acetate (**C**) extracts of the roots of *Pentas micrantha*

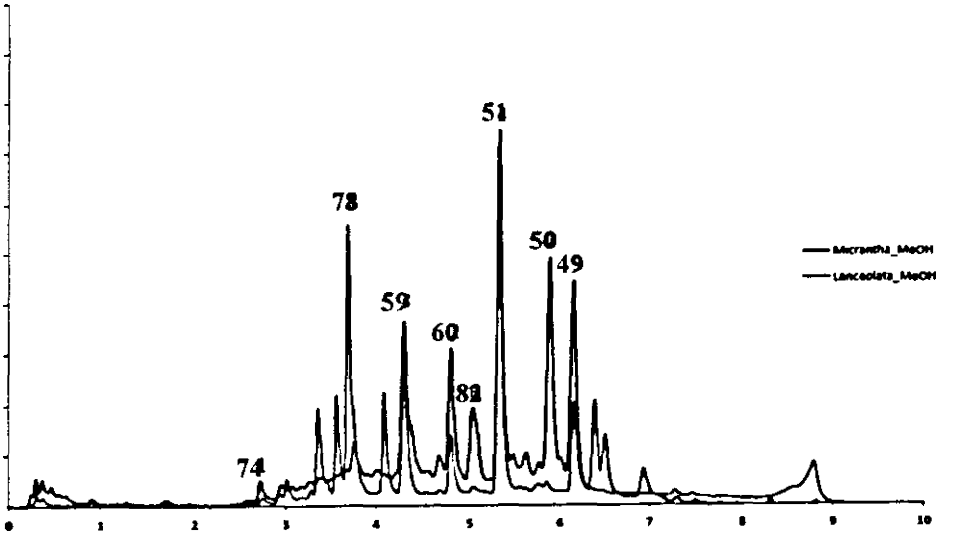


Figure 4.15: HPLC overlap of crude extracts of *Pentas micrantha* and *Pentas lanceolata*

4.9.2 HPLC analysis of the crude extracts of the root of *P. bussei*

Analytical RP-HPLC analysis of the root extract (CH_2Cl_2:CH_3OH, 1:1) of *P. bussei* is shown below (Fig 4.16). Busseihydroquinone B (**71**) is found to be the major compound in this extract. It is also to be noted that there are other peaks which have not been identified from the extracts of *P. micrantha* and *P. bussei*.

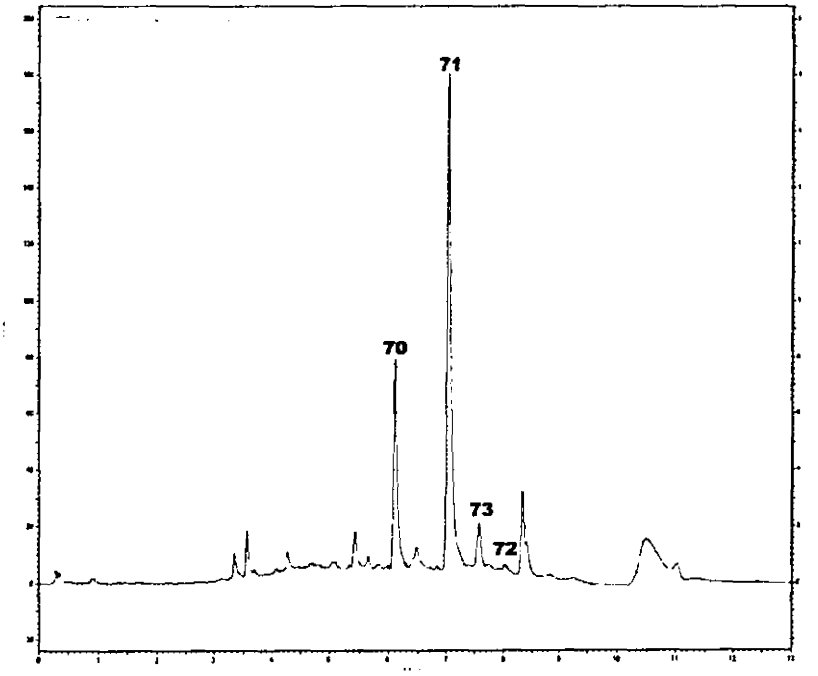


Figure 4.16: HPLC profile of CH_2Cl_2:CH_3OH (1:1) extract of the roots of *Pentas bussei*

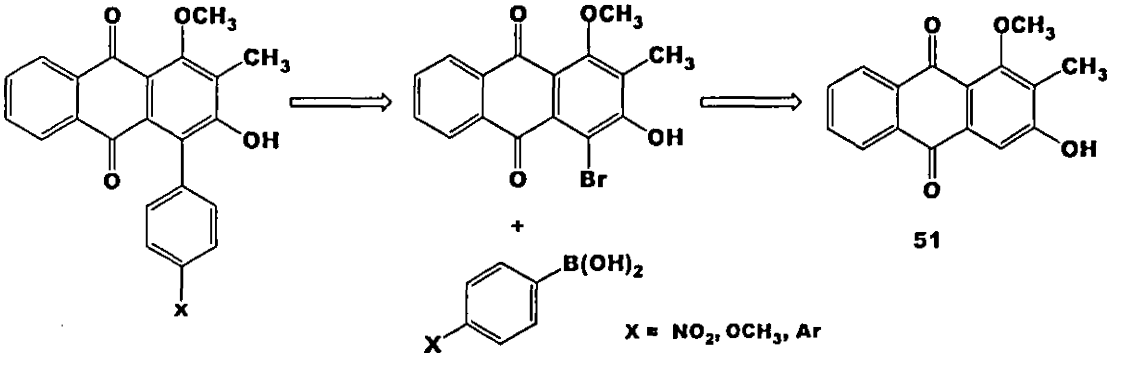
4.10 Synthesis of rubiadin (49) and rubiadin-1-methyl ether (51) analogues

The reduction of reaction time, instantaneous and uniform heating, carrying out solvent free reactions and possibility of parallel chemical reactions has proved as a bonanza for the researchers involved in drug discovery and development processes like high-speed combinatorial and medicinal chemistry. Some researchers have been working on the synthesis of 4-phenylanthraquinones (Bringmann *et al.*, 1997; Bringmann *et al.*, 2001; Bringmann *et al.*, 2002; Gautrot *et al.*, 2006). Motivated by the wide interest in synthesis of 4-phenylanthraquinones for antiplasmodial acvtity testing, a synthetic strategy towards

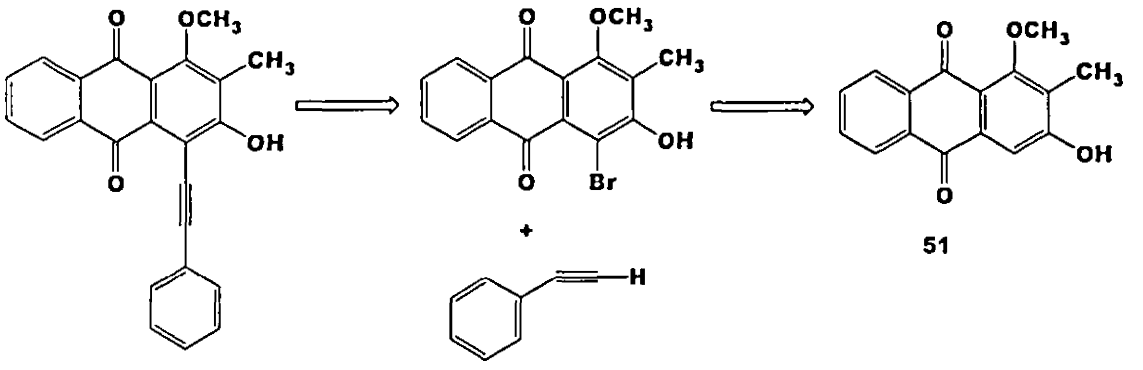
the synthesis of 4-aryl substituted analogues of rubiadin (49) and rubiadin-1-methyl ether (51) was designed and the antiplasmodial activity of the analogues were compared with the parent compounds.

4.9.1 Retrosynthetic route towards the synthesis of 4-phenyl substituted analogues of rubiadin (49) and rubiadin-1-methyl ether (51)

In order to have a versatile synthetic strategy towards aryl substituted anthraquinones, 4-bromorubiadin (87) and 4-bromorubiadin-1-methyl ether (88) were used as key intermediates which were subsequently transformed into target compounds using Suzuki-Miyaura and Sonogashira cross-coupling reactions (Erdelyi and Gogoll, 2001; Thiemann *et al.*, 2009). Retrosynthetic routes towards the synthesis of 4-aryl substituted analogues of rubiadin (49) and rubiadin-1-methyl ether (**51**) are shown in schemes 4.7 and 4.8.



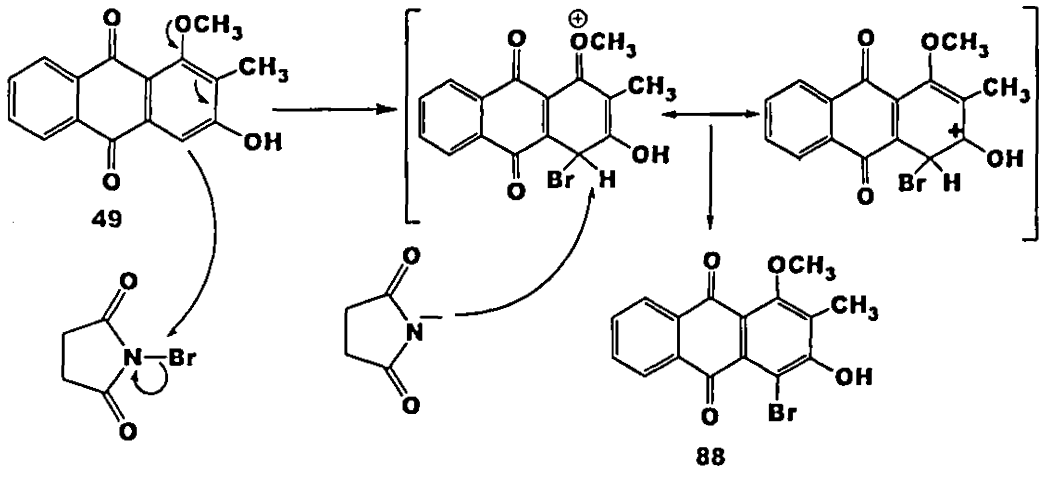
Scheme 4.7: Retrosynthetic scheme for 4-phenyl substituted analogues of rubiadin-1-methyl ether (**51**)



Scheme 4.8: Retrosynthetic scheme for synthesis of 4-alkynyl analogues of rubiadin-1-methyl ether (**51**)

4.9.2 Synthesis of 4-bromorubiadin (87) and 4-bromorubiadin-1-methyl ether (88)

Compounds **87** and **88** were synthesized following the procedure described in section 3.6.2 (Tietze *et al.*, 2007; Hong *et al.*, 2003). Using 1:1 mixture of *N*-bromo succinimide (NBS) and rubiadin (**49**) in dichloromethane (as a reaction solvent) gave low yield (35%). This is due to two possible competing reactions, *i.e.*, free radical pathway on the side chain methyl at C-2 and electrophilic aromatic substitution at C-4 of ring A. Nevertheless, using 5% excess of NBS to starting material and acetonitrile (as a reaction solvent) gave high yield of 4-bromorubiadin (**87**) (88%) and 4-bromorubiadin-1-methyl ether (**88**) (92%). The yields of the reaction can be explained in accordance with the previous findings (Hong *et al.*, 2003), on the use of NBS for bromination of activated aromatic rings. They proposed the side chain bromination follows a radical pathway, while aromatic nucleus brominations follow an electrophilic aromatic substitution reaction (Scheme 4.9). Low temperature and polar solvent facilitates the later reaction while non-polar solvent facilitates the former (Hong *et al.*, 2003).

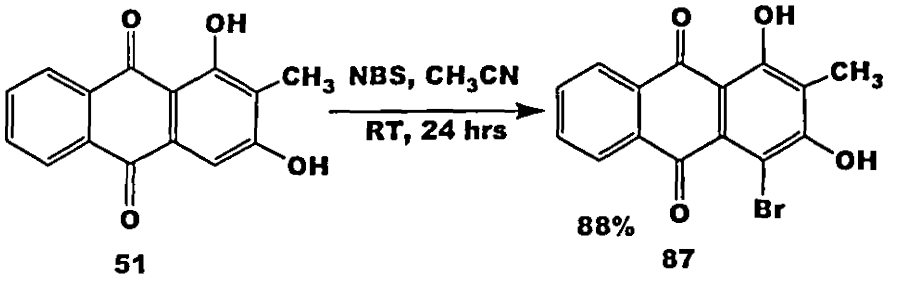


Scheme 4.9: Reaction mechanism for electrophilic aromatic substitution of rubiadin-1-methyl ether (**49**)

4.9.2.1 4-Bromorubiadin (87)

4-Bromorubiadin (**87**) was isolated as an orange powder. The ESI-MS spectrum revealed a molecular ion peak of *m/z* 331.2 $(M)^+$. The presence of two equivalent peaks in 1:1 ratio at *m/z* 331.2 $(M)^+$ and 333.2 $(M+2)^+$ supports the presence of one bromine substituent. The ^1H NMR data (Table 4.26) revealed four aromatic protons at δ_H 7.87-7.89 (*m*) and δ_H 8.10-8.16 (*m*) having a similar multiplicity pattern with the parent compound suggesting unsubstituted ring C was not affected by the bromination reaction. This agrees with the well known fact that an aromatic ring which is not activated does not undergo halogenation reaction. The singlet aromatic proton at δ_H 7.54 in the ^1H NMR spectrum of rubiadin (**49**) is not seen in the ^1H NMR spectrum of 4-bromorubiadin (**87**). A chelated hydroxyl proton was observed at δ_H 13.13 and the downfield chemical shift of the hydroxyl group is due to its *peri* position to the carbonyl (C-9) that enabeled its placement at C-1. The HMBC correlation between (C_2-H_{11}) enabeled the methyl group at δ_H 2.20 to be assigned to C-2 position. The ^{13}C NMR spectral data (Table 4.26) for **87** revealed fifteen carbons; two carbonyls (δ_C 186.3 and 181.1), two oxygenated aromatic quaternary centers (δ_C 161.8 and 159.5), four aromatic methines (δ_C 133.9, δ_C 133.6, δ_C

126.8 and 125.8), six quaternary carbons (δ_C 128.6, δ_C 118.5, δ_C 110.8, δ_C 131.7 and 134.7) and a methyl carbon at δ_C 9.4. Thus, based on the above spectroscopic evidence the compound was found to be 4-bromorubiadin (87) (Scheme 4.10).



Scheme 4.10: Synthesis of 4-bromorubiadin (87)

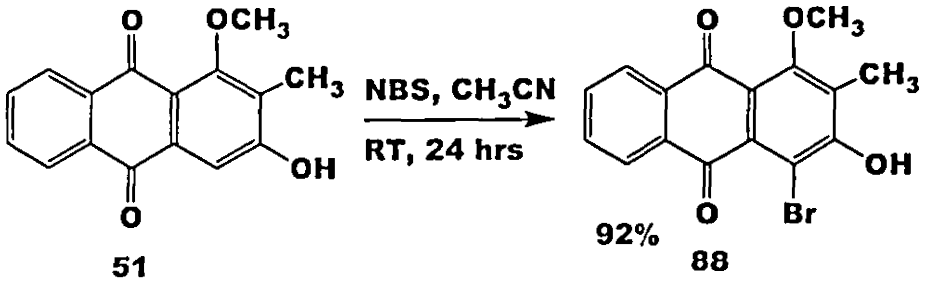
Table 4.26: ^1H (600 MHz) and ^{13}C (125 MHz) NMR data for 4-bromorubiadin (87) and 4-bromorubiadin-1-methyl ether (88) in DMSO-d_6

Position	87		Position	88	
	δ_H (J in Hz)	δ_C		δ_H (J in Hz)	δ_C
1	13.13 (*s*,1H,OH)	161.8	1	-	162.3
1a	-	118.5	1a	-	123.5
2	-	128.6	2	-	128.4
3	10.69 (*s*,1H,OH)	159.5	3	10.69 (*s*,1H,OH)	161.5
4	-	110.8	4	-	108.3
4a	-	129.0	4a	-	134.0
5	8.10-8.16 (2H, *m*)	126.8	5	8.11-8.15 (2H, *m*)	128.6
5a		134.7	5a	-	135.8
6	7.87-7.89 (*m*, 2H)	133.6	6	7.79-7.80 (2H, *m*)	136.2
7	7.87-7.89 (*m*, 2H)	133.9	7	7.79-7.80 (2H, *m*)	136.3
8	8.10-8.16 (2H, *m*)	125.8	8	8.11-8.15 (2H, *m*)	129.6
8a	-	131.7	8a	-	136.6
9	-	186.3	9	-	185.3
10	-	181.1	10	-	183.4
11	2.20 (*s*, 3H)	9.4	11	2.34 (3H, *s*)	11.2
-	-	-	1-OCH_3	3.89 (3H, *s*)	63.4

4.9.2.2 4-Bromorubiadin-1-methyl ether (88)

4-Bromorubiadin-1-methyl ether (88) was isolated as an orange powder. The HRMS spectrum revealed *m/z* 346.9919 $(M+H)^+$, attributed to a molecular formula of $C_{16}H_{11}BrO_4$. The presence of two equivalent peaks in the ESI-MS spectrum in 1:1 ratio at *m/z* 347.2 $(M)^+$ and 349.2 $(M+2)^+$ supports the presence of one bromine substituent. The ^1H NMR spectral data (Table 4.26) revealed signals of four aromatic protons at δ_H 7.87 (2H, *m*) and δ_H 8.11 (2H, *m*) suggesting that ring C of the starting material is not affected by the bromination reaction. The singlet aromatic proton at δ_H 7.50 observed in the ^1H NMR of the starting material (49) was absent in the spectrum of 4-bromorubiadin-1-methyl ether (88) confirming that the proton at C-4 of ring A was substituted by bromine. Methoxy signals were observed at δ_H 3.77 and δ_C 63.4 in the ^1H and ^{13}C NMR respectively. The downfield chemical shift of the methoxyl group (> 60 ppm) indicates its di-*ortho* substitution to one of the carbonyl carbon (C-9) and therefore inagreement with its assignment at C-1 position (Schripsema and Dagnino, 1996).

The HMBC correlation between C-2 and H-11 enabeled the methyl group at δ_H 2.18 to be assigned to C-2 position. The ^{13}C NMR spectral data (Table 4.26) revealed fifteen carbons: two carbonyl carbons at δ_C 185.3 and 183.4, two oxygenated aromatic quaternary carbons at δ_C 162.3 and 161.5, fivequaternary carbons at δ_C 136.6, 135.8, 128.4, 134.0 and 123.5, five aromatic methines at δ_C 108.5, 136.2, 136.3, 128.6 and 129.6, and a methyl carbon at δ_C 11.2. Thus, based on the above spectroscopic evidence the compound was found to be 4-bromorubiadin-1-methyl ether (88) (Scheme 4.11).



Scheme 4.11: Synthesis of 4-bromorubiadin-1-methyl ether (88)

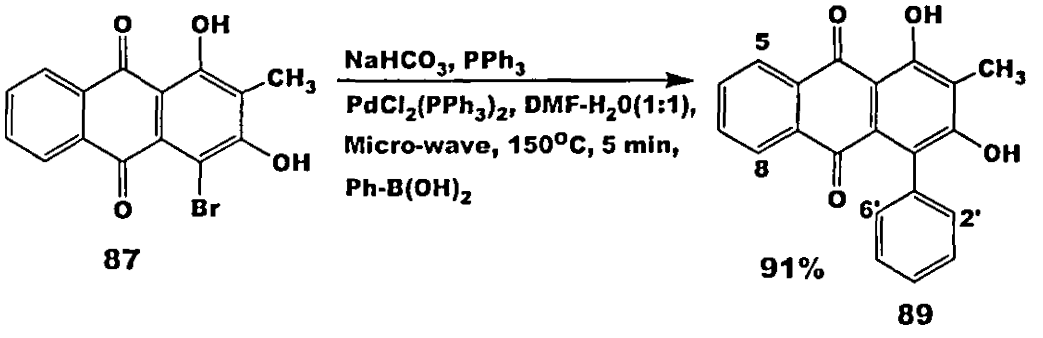
4.9.3 Mirowave assisted synthesis of 4-aryl substituted analogues of rubiadin (49) and rubiadin-1-methyl ether (51)

4.9.3.1 4-Phenylrubiadin (89)

4-Phenylrubiadin (89) was isolated as a yellow powder. The ESI-MS spectrum revealed the molecular ion peak of *m/z* 331.2 $(M+H)^+$. The ^1H NMR data (Table 4.27) revealed four aromatic protons at δ_H 7.61-7.67 (*dt*, 2H, H-6,7), 7.96-8.21 (*dd*, 2H, *J*= 7.8, 2.1, H-5,8) confirming unsubstituted ring C similar to the parent anthraquinone, 4-bromorubiadin (89). The presence of a chelated hydroxyl group at δ_H 13.75 confirmed hydroxyl group at C-1 and a methyl at δ_H 2.29 assigned to C-2 suggest a similar substitution pattern (at C-1 and C-2) to the parent anthraquinone.

The ^1H NMR data (Table 4.27) revealed aromatic protons at δ_H 7.52 (*dd*, 2H, H-1', 2', *J*= 8.0, 1.8), 7.47 (*dd*, 2H, H-3',4', *J*= 8.0, 1.8) and δ_H 7.19 (*m*, 1H, H-5') suggesting the presence of a mono substituted phenyl ring at C-4 position of ring A additional to the parent anthraquinone. The chemical shift of one of the oxygenated quaternary carbon (C-3) at δ_C 163.2 in the ^{13}C NMR spectrum of parent anthraquinone (87) shifted to δ_C 156.4 in ^{13}C NMR spectrum (Table 4.27) of 4-phenylrubiadin (89). This can be accounted due to the presence of phenyl group at C-4 bringing extra conjugation and there by increasing

the electron density at C-3 resulting in the upfeild chemical shift of the oxygenated quaternary carbon (C-3). Thus, based on the above spectroscopic evidence the compound was identified to be 4-phenylrubiadin (89) isolated in 91% yield (Scheme 4.12).



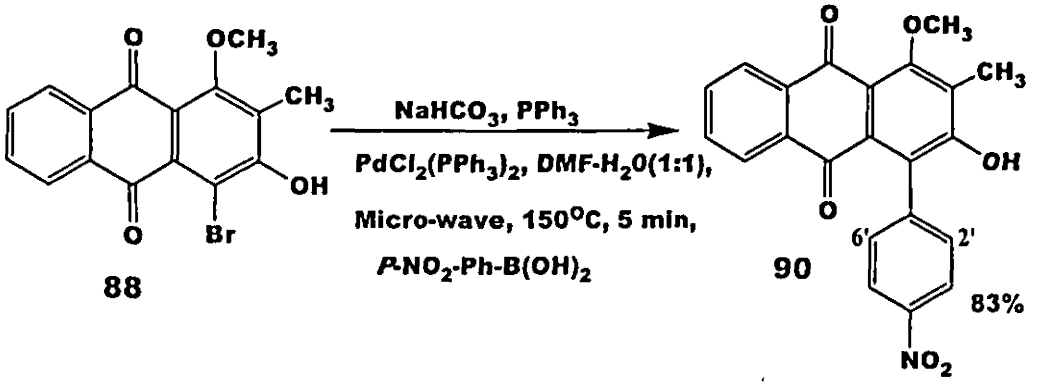
Scheme 4.12: Synthesis of 4-phenylrubiadin (89)

4.9.3.2 4-(*p*-Nitrophenyl)rubiadin-1-Methyl Ether (90)

4-(*p*-nitrophenyl)rubiadin-1-methyl ether (90) was isolated as an orange powder. The HRMS spectrum revealed a molecular ion peak at *m/z* 390.0978 $(M+H)^+$ (ESI-MS; *m/z* 390.7), attributed to molecular formula of $C_{22}H_{15}NO_6$. The ^1H NMR data (Table 4.27) revealed four aromatic protons at δ_H 7.96-8.21 (2H, *dd*, *J*= 8.2, 1.8 Hz, H-5,8) and 7.65-7.75 (2H, *dt*, 2H, H-6,7) suggesting unsubstituted ring C as in parent anthraquinone, 4-bromorubiadin-1-methyl ether (88). The presence of a methyl group at δ_H 2.35/δ_C 10.1 (at C-2) and a methoxy group at δ_H 3.85/δ_C 61.1 (C-1) are identical to the parent anthraquinone.

A set of ortho coupled aromatic protons at δ_H 8.44 (*dd*, *J*= 8.0, 1.8 Hz, H-2', 6') and δ_H 7.45 (*d*, *J*= 8.0, 1.8 Hz, H-3',5') were observed with AA'BB' spin system suggesting the presence a 1,4 substituted phenyl ring at C-4 position of ring A. The ^{13}C NMR spectrum (Table 4.27) revealed the presence of twenty two carbons: two carbonyl, two oxygenated aromatic quaternary centers, eight aromatic methines, seven non-oxygenated quaternary

carbons and a methyl groupg (Table 4.27). The chemical shift of one of the quaternary carbons (δ_C 146.8) is attributed to the quaternary aromatic carbon attached to nitro group. From the above spectroscopic evidence the compound was identified to be 4-(*p*-nitrophenyl)rubiadin-1-methyl ether (90) (Scheme 4.13).



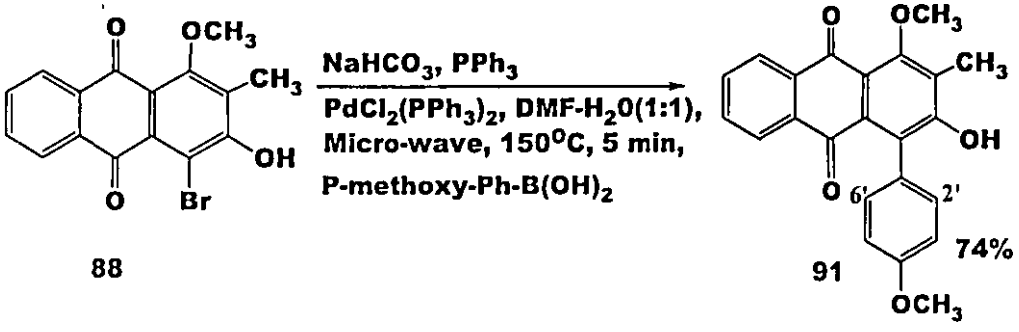
Scheme 4.13: Synthesis of 4-(p-Nitrophenyl)rubiadin-1-methyl ether (90)

4.9.3.3 4-(*p*-Methoxyphenyl)rubiadin-1-methyl ether (91)

4-(*p*-Methoxyphenyl)rubiadin-1-methyl ether (**91**) was isolated as a yellow powder. The HRMS spectrum revealed a molecular ion peak of 375.1232 $(M+H)^+$ (ESI-MS; *m/z* 375.5), attributed to molecular formula of $C_{23}H_{18}O_5$. The ^1H NMR data (Table 4.27) revealed four aromatic protons at δ_H 8.06-8.18 (2H, *dd*, *J*=8.0, 2.1 Hz, H-5,8) and δ_H 7.74-7.78 (2H, *dt*, H-6,7) suggesting the presence of unsubstituted ring C as in the parent anthraquinone, 4-bromorubiadin-1-methyl ether (**91**). The methyl group at δ_H 2.23 and a methoxy at δ_H 3.82/δ_C 64.0 suggest a similar substitution pattern (at C-1 and C-2) with the parent anthraquinone.

A pair doublets at δ_H 6.95 (2H, *dd*, *J*=7.6, 1.8 Hz, H-3',5') and δ_H 7.04 (2H, *dd*, *J*=7.6, 1.8 Hz, H-2',6') were observed having AA'BB' spin system suggesting the presence of a 1,4 substituted phenyl ring at C-4 position of ring A. Additional methoxy group (δ_H 3.82, 3H,

s) was observed and this suggests that the substituent at C-4 of the phenyl ring is a methoxy. Comparison with the parent anthraquinone, additional oxygenated quaternary carbon was observed in the ^{13}C-NMR data (Table 4.27) at δ_C 161.2 showing HMBC correlation with the methoxy protons at δ_H 3.83 (5'-OCH_3) and hence assigned to the carbon C-5'. Thus, based on the above spectroscopic evidence, the compound was identified to be 4-(*p*-methoxyphenyl)rubiadin-1-methyl ether (**91**).

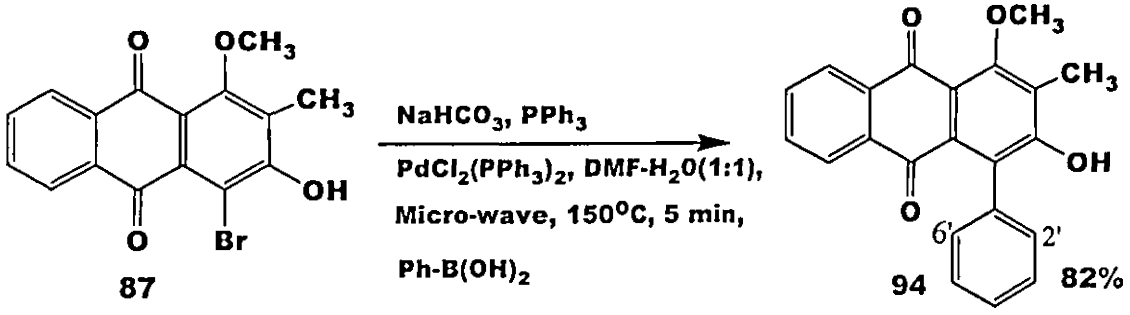


Scheme 4.14: Synthesis of 4-(p-Nitrophenyl)rubiadin-1-methyl ether (91)

4.9.3.4 4-phenylrubiadin-1-methyl ether (92)

4-phenylrubiadin-1-methyl ether (**92**) was isolated as an orange solid. The HRMS spectrum revealed a molecular ion peak at *m/z* 345.1127 $(M+H)^+$ (ESI-MS; *m/z* 345.3), attributed to molecular formula of $C_{22}H_{16}O_4$. The ^{1}H NMR (Table 4.27) revealed the four aromatic protons at δ_H 7.70-7.82 (*m*, 2H, H-6,7), δ_H 8.06 (*dd*, 1H, J = 8.0, 1.8 Hz, H-5) and δ_H 7.10 (*dd*, 1H, H-8) suggesting that ring C of anthraquinone is unsubstituted as in the NMR spectra of the parent anthraquinone. Similar to the parent anthraquinone, the presence of a methyl group at δ_H 2.21/δ_C 13.09 and a methoxyl group at δ_H 3.81/δ_C 63.9 were confirmed at positions C-1 and C-2 of ring A. However, the chemical shift of proton H-5 is deshilded compared to the parent anthraquinone suggesting that the substituent at C-4 after the reaction affected the checmial shift of H-5.

Additional to the NMR spectra of the parent anthraquinone, the presence of AA'XX' spin system containg was observed at δ_H 7.70-7.82 (*m*, 2H, H-2',6') and δ_H 7.30-7.38 (*m*, H-3',4', 5') suggesting the presence of a phenyl ring at C-4 of ring A. The ^{13}C NMR data (Table 4.27) is also in agreement with the presence of extra phenyl ring, compared to the parent anthraquinone, after the reaction. Thus, based on the above spectroscopic evidence, the compound was identified to be 4-phenylrubiadin-1-methyl ether (92).



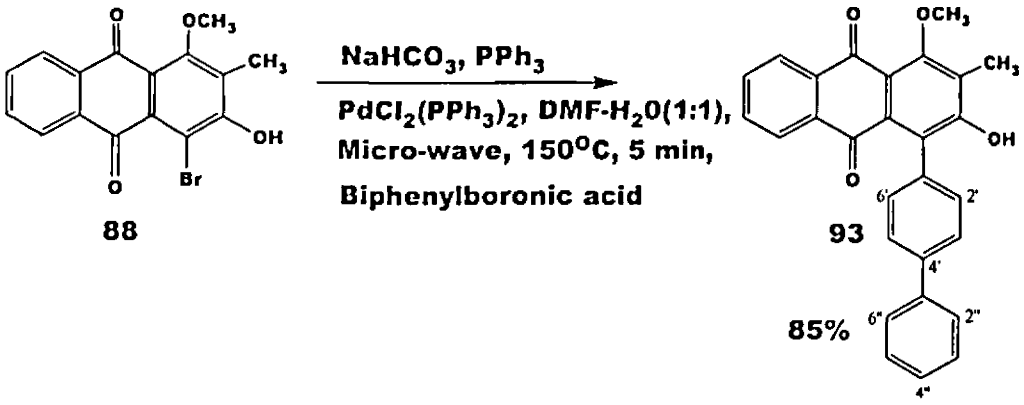
Scheme 4.15: Synthesis of 4-phenylrubiadin-1-methyl ether (92)

Table 4.27: ^{1}H (600 MHz) and ^{13}C (150 MHz) spectral data for compounds **89-92** in $CDCl_3$

Position	89		90		91		92	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	13.75 (*s*, 1-OH)	161.2	-	162.9	-	162.5	-	162.7
1a	-	124.2	-	125.9	-	129.7	-	128.9
2	-	116.4	-	128.8	-	122.3	-	124.3
3	10.69 (*s*,1H,OH)	156.4	-	162.3	-	162.6	-	162.5
4	-	108.5	-	123.0	-	129.0	-	121.7
4a	-	131.9	-	131.8	-	129.7	-	131.1
5	7.96-8.21 (*dd*, *J*=7.8, 2.1)	125.1	8.96-8.21 (*dd*, *J*= 8.2, 1.8)	126.2	8.06-8.18 (*dd*, *J*=8.0, 2.1)	136.1	8.06 (*dd*, *J*= 8.1, 1.8)	132.3
5a	-	130.8	-	134.2	-	136.9	-	130.3
6	7.61-7.67(*dt*)	132.6	7.65-7.75 (*dt*)	133.1	7.74-7.78 (*dt*)	132.1	7.70-7.82 (*dt*, 2H)	136.3
7	7.61-7.67 (*dt*)	131.6	7.65-7.75(*dt*)	133.5	7.74-7.78 (*dt*)	133.5	7.70-7.82 (*dt*, 2H)	136.5
8	7.96-8.21 (*dd*, *J*=7.8, 2.1)	125.1	7.96-8.21 (*dd*, *J*= 8.2, 1.8)	126.5	8.06-8.18 (*dd*, *J*=8.0, 2.1)	136.7	7.10 (*dd*, *J*= 8.1, 1.8)	135.1
8a	-	130.8	-	134.1	-	137.6	-	137.1
9	-	185.8	-	180.9	-	186.9	-	184.4
10	-	180.6	-	183.7	-	184.2	-	182.9
11	2.29 (*s*, 3H)	6.6	2.35 (*s*, 3H)	10.1	2.23 (*s*, 3H)	13.0	2.21 (*s*, 3H)	13.9
2'&6'	7.47(*dd*, *J*= 8.0, 1.8)	127.8	8.44 (*dd*, *J*= 8.0, 2.0)	130.8	7.04 (*dd*, *J*=7.6, 1.8)	116.2	7.95-7.97 (*dd*, *J*= 8.1, 1.8)	130.3
3'&5'	7.52(*dd*, *J*= 8.0, 1.8)	127.1	7.45 (*dd*, *J*= 8.0, 2.0)	123.2	6.94 (*dd*, *J*=7.6, 1.8)	128.7	7.74-7.76 (*m*, 2H)	129.3
4'	7.19 (*m*)	126.4	-	146.3	-	161.2	-	129.0
1-OCH_3	-	-	3.85, *s*	61.1	3.82, *s*	64.0	3.81, *s*	63.9
4'-OCH_3	-	-	-	-	3.83, *s*	58.0	-	-

4.9.3.5 4-Biphenylrubiadin-1-methyl ether (93)

4-Biphenylrubiadin-1-methyl ether (93) was isolated as a yellow compound. The HRMS spectrum revealed a molecular ion peak of *m/z* 421.1440 $(M+H)^+$ (ESI-MS; *m/z* 421.2), attributed to molecular formula of $C_{28}H_{20}O_4$. The ^1H NMR data (Table 4.28) revealed additional set of aromatic protons with AA'BB' spin pattern suggesting the presence of 1,4-substituted phenyl ring at δ_H 7.32 (2H, *dd*, *J*=8.0, 1.8, H-3',5') and 7.80 (2H, *dd*, *J*=8.0, 1.8, H-2',6'). The presence of additional aromatic protons at δ_H 7.69-7.72 (3H, *dt*, H-3", 4", 5") and 7.48-7.49 (2H, *dd*, *J*= 7.8, 2.0, H-2",6") suggest a biphenyl substituent at C-4 of ring A. The presence of AA'XX' ring system was observed at δ_H 8.22 (1H, *dd*, *J*=7.9, 1.8, H-5), 7.98 (1H, *dd*, *J*=7.9, 1.8, H-8), 7.38-7.40 (1H, *dd*, *J*=8.1, 2.0, H-7) and 7.61-7.64 (1H, *dd*, *J*=8.1, 2.0, H-6) suggesting unsubstituted ring C similar to the parent anthraquinone. The presence of a methyl proton (at δ_H 2.34; δ_C 10.0) and a methoxy group (at δ_H 3.98; δ_C 60.9) suggests a similar substitution pattern at C-1 and C-2 of ring A identical to the parent anthraquinone. The ^{13}C NMR (Table 4.28) revealed two carbonyl carbons (δ_C 183.8 and 181.1), two oxygenated quaternary carbons (δ_C 159.7 and 159.5) (identical to the parent anthraquinone), seven aromatic methines and nine quaternary aromatic carbons (not oxygenated, Table 4.28). Thus, based on the above spectroscopic evidence the compound was identified to be 4-(biphenyl)rubidin-1-methyl ether (**93**).



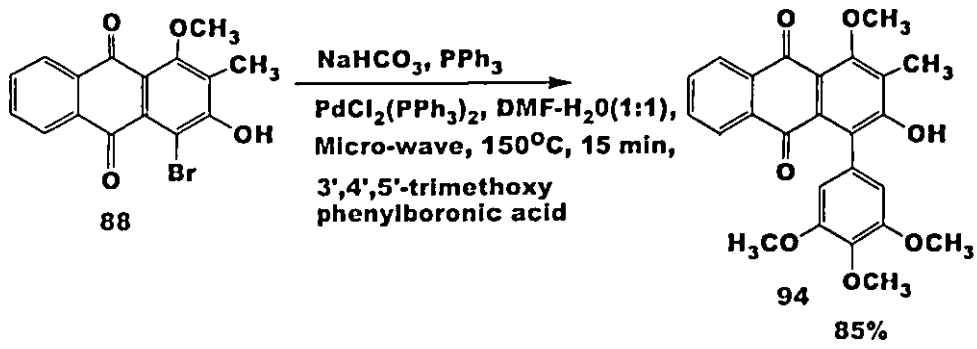
Scheme 4.16: Synthesis of 4-biphenylrubiadin-1-methyl ether (93)

Table 4.28: ^{1}H (800 MHz) and ^{13}C (125 MHz) NMR data for 4-biphenylrubiadin-1-methyl ether (93) in DMSO-d_6

Position	δ_H (J in Hz)	δ_C
1	-	159.7
1a	-	125.9
2	-	125.8
3	-	159.5
4	-	119.2
4a	-	130.4
5	8.22 (1H, *dd*, *J*=7.9, 1.8)	133.4
5a	-	132.0
6	7.61-7.64 (1H, *dd*, *J*=8.1, 2.0)	133.9
7	7.38-7.40 (1H, *dd*, *J*=8.1, 2.0)	133.9
8	7.98 (1H, *dd*, *J*=7.9, 1.8)	133.3
8a	-	136.9
9	-	183.8
10	-	181.1
11	2.34 (*s*, 3H)	10.0
1-OCH_3	3.98 (*s*, 3H)	60.9
2', 6'	7.80 (2H, *dd*, *J*=8.0, 1.8)	129.9
3',5'	7.32 (2H, *dd*, *J*=8.0, 1.8)	128.9
1'	-	138.0
4'	-	140.2
2'' & 6''	7.48-7.49 (2H, *dd*, *J*= 7.8, 2.0)	126.5
5'' & 3''	7.69-7.72 (*dt*, H-3'', 4'', 5'')	126.2
1''	-	126.7
4''	7.69-7.72 (*dt*, H-3'', 4'', 5'')	126.0

4.9.3.6 4-(3',4',5'-trimethoxyphenyl)rubiadin-1-methyl ether (94)

4-(3',4',5'-trimethoxyphenyl)rubiadin-1-methyl ether (**94**) was isolated as yellow solid. The HRMS spectrum revealed a molecular ion peak of *m/z* 435.1444 $(M+H)^+$ (ESI-MS; *m/z* 435.6), attributed to molecular formula of $C_{25}H_{22}O_7$. The ^1H NMR data (Table 4.29) revealed a singlet aromatic proton at δ_H 6.46 (2H, *s*, H-2',6') suggesting the presence of additional trisubstituted phenyl ring. The presence of AA'XX' spin system was sobserved at δ_H 8.14 (1H, *dd*, *J*=7.9, 1.8, H-5), 7.84 (1H, *dd*, *J*=7.9, 1.8, H-8), 7.65 (1H, *dt*, H-7) and 7.74 (1H, *dt*, H-6) suggesting the presence of unsubstituted ring C similar to the parent anthraquinone. The presence of a methyl proton (δ_H 2.24; δ_C 10.33) and four methoxy groups (δ_H 3.80/δ_C 56.2 (*s*, 6H); δ_H 3.85/δ_C 60.4 (*s*, 3H); δ_H 3.87/δ_C 61.2 (*s*, 3H)) are all evident from ^1H and ^{13}C NMR data (Table 4.29) suggesting there are three more extra methoxyl substituents, compared to parent anthraquinone. The singlet aromatic proton at δ_H 6.46 (2H, *s*) shows integration of two protons suggesting that the additional phenyl groups is substituted at C-3', C-4' and C-5' positions and hence the two aromatic protons become symmetrical displaying the same chemical shift. This suggests that the three extra methoxyl groups observed, compared to the parent anthraquinone, be placed at C-3', C-4' and C-5' positions of the C-4 phenyl group. The ^{13}C NMR (Table 4.29) revealed two carbonyl carbons (δ_C 181.6 and 184.3), five oxygenated quaternary carbons, six aromatic methines and seven quaternary aromatic carbons (not oxygenated, Table 4.29). Thus, based on the above spectroscopic evidence the compound was identified to be 4-(3',4',5'-trimethoxyphenyl)rubiadin-1-methyl ether (**94**)



Scheme 4.17: Synthesis of 4-(3',4',5'-trimethoxyphenyl)rubiadin-1-methyl ether (94)

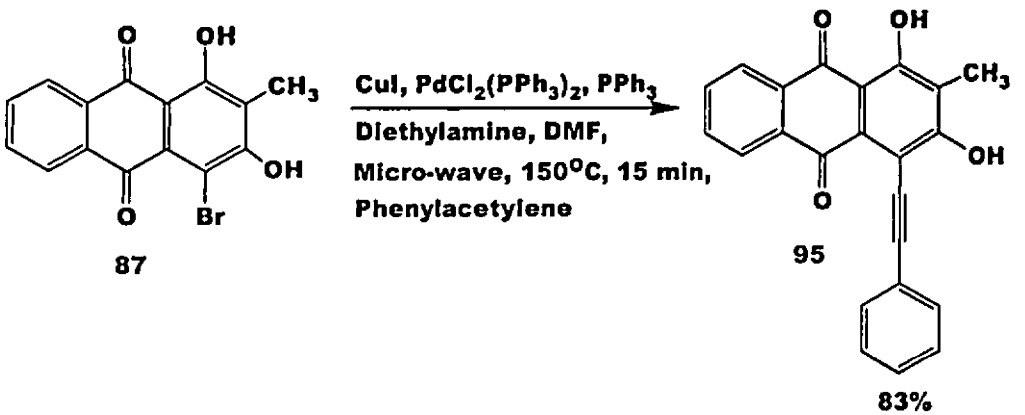
Table 4.29: ^{1}H (400 MHz) and ^{13}C (125 MHz) NMR data for 4-(3',4',5'-trimethoxyphenyl)rubiadin-1-methyl ether (94) in DMSO-d_6

Position	δ_H (J in Hz)	δ_C
1	-	162.5
1a	-	126.1
2	-	126.2
3	-	162.2
4	-	126.0
4a	-	132.2
5	8.14 (1H, *dd*, *J*=7.9, 1.8)	133.9
5a	-	134.1
6	7.74 (1H, *dt*)	133.5
7	7.65 (1H, *dt*)	133.5
8	7.84 (1H, *dd*, *J*=7.9, 1.8)	133.9
8a	-	134.4
9	-	184.2
10	-	181.2
11	2.34 (*s*, 3H)	13.1
1-OCH_3	3.87 (*s*, 3H)	61.2
2', 6'	6.46 (2H, *s*)	107.0
3',5'	-	155.2
1'	-	127.2
4'	-	136.7
3' & 5'-OCH_3	3.80 (*s*, 6H)	56.2
4'-OCH_3	3.85 (*s*, 3H)	60.3

4.9.3.7 4-(Phenylethynyl)rubiadin (95)

4-(Phenylethynyl)rubiadin (95) was isolated as an orange solid. The ^{1}H NMR (Table 4.30) revealed four aromatic protons at δ_H 7.61-7.67 (*dt*, H-6,7), δ_H 7.96 (*dd*, *J*= 8.0, 1.8, H-5) and δ_H 8.21 (*dd*, *J*= 8.0, 1.8, H-8) confirming the presence of unsubstituted ring C as in the starting anthraquinone. Further more, a methyl group at δ_H 2.57 (3H, *s*), a chelated hydroxyl group at δ_H 13.75 (*s*, 1H) and five additional aromatic protons (δ_H 7.47-7.51 (*dt*, H-4",6"), δ_H 7.18-7.19 (*dd*, *J*= 7.8, 1.8, H-1",3") were all evident from the ^{1}H NMR data (Table 4.30) confirming the presence of additional phenyl group. The ^{13}C NMR data (Table 4.30) revealed a methyl group at δ_C 27.3, two carbonyls (δ_C 184.6 and 179.3), two oxygenated quaternary centers (δ_C 161.1 and 154.9), nine aromatic quaternary carbons and nine aromatic methines (Table 4.30).

The chemical shift value of C-4 in the starting material becames more shielded from δ_C 113.9 to δ_C 109.3 in the product. The high electron density at C-4, due to the electron donating substituent at C-4, is responsible for the upfield chemical shift of C-4 observed. Moreover, the presence of aromatic quaternary carbon at δ_C 101.6 is a charachterstic peak for sp hybridized quaternary carbons (1', 2'). The chemical shift value of C-3 carbon becames more shielded from δ_C 163.2 to δ_C 154.9. This is due to the presence of a phenyl acethylene group at C-4 that brings extra conjugation and hence contributes to downfeild chemical shift value of oxygenated quaternary carbon C-3. Based on the above spectroscopic evidence, the compound was identified to be 4-phenylethynylrubiadin (95).



Scheme 4.18: Synthesis of 4-(phenylethynyl)rubiadin (95)

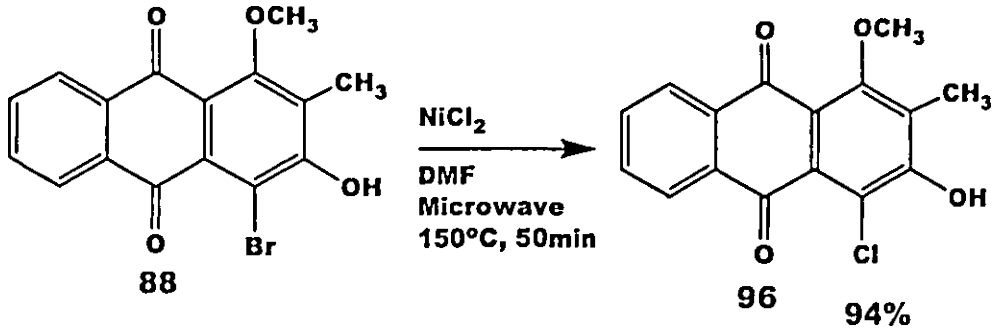
Table 4.30: The ^{1}H (800 MHz) and ^{13}C (125 MHz) data for 4-(Phenylethynyl)rubiadin (95) in $CDCl_3$

Position	δ_H	δ_C
1	-	161.1
1a	-	117.1
2	-	124.3
3	-	154.9
4	-	109.3
4a	-	132.4
5	7.96 (*dd*, *J*= 8.0, 1.8)	125.4
5a	-	130.3
6	7.61-7.67 (*dt*, H-6,7)	131.6
7	7.61-7.67 (*dt*, H-6,7)	131.8
8	8.21 (*dd*, *J*= 8.0, 1.8)	125.8
8a	-	131.2
9	-	184.6
10	-	179.3
11	2.57 (*s*, 3H)	27.3
1-OH	13.5, *s*	-
1' & 2'	-	101.6
2''	-	124.6
1'' & 3''	7.18-7.19 (*dd*, *J*= 7.8, 1.8)	125.5
4'' & 6''	7.47-7.51 (*m*, 3H,H-4'',5'', 6'')	124.3
5''	7.47-7.51 (*m*, 3H,H-4'',5'', 6'')	127.9

4.9.3.8 4-Chlororubiadin-1-methyl ether (96)

4-Chlororubiadin-1-methyl ether (96) was isolated as a yellow powder. The HRMS spectrum revealed a molecular ion peak of *m/z* 303.0424 $(M+H)^+$ (ESI-MS; *m/z* 303.4), attributed to molecular formula of $C_{16}H_{11}ClO_4$. The presence of two equivalent peaks in 2:1 ratio at *m/z* 303.2 $(M+H)^+$ and 305.5 $(M+2)^+$ supports the presence of one chlorine substituent. The ^1H NMR spectral data (Table 4.31) revealed signals of four aromatic protons at δ_H 7.64 (2H, *m*, H-6,7) and δ_H 8.11 (2H, *m*, H-5,8) suggesting that ring C of the starting material is was not affected by the halogen exchange reaction. Methoxy groups and methyl groups were observed at δ_H 3.83/δ_C 63.4 and δ_H 2.24/δ_C 11.0 in the ^1H and ^{13}C NMR (Table 4.31), respectively. The downfield chemical shift of the methoxyl group (> 60 ppm) indicates its di-*ortho* substitution to one of the carbonyl carbon (C-9) and therefore inagreement with its assignment at C-1 position (Schripsema and Dagnino, 1996).

The ^{13}C NMR spectral data (Table 4.31) revealed fifteen carbons: two carbonyl carbons at δ_C 185.3 and 183.4, two oxygenated aromatic quaternary carbons at δ_C 162.3 and 161.5, fivequaternary carbons at δ_C 136.6, 135.8, 128.4, 134.0 and 123.5, five aromatic methines at δ_C 108.5, 136.2, 136.3, 128.6 and 129.6, and a methyl carbon at δ_C 11.2. Thus, based on the above spectroscopic evidence the compound was found to be 4-bromorubiadin-1-methyl ether (88) (Scheme 4.11).



Scheme 4.19: Synthesis of 4-chlororubiadin-1-methyl ether (96)

Table 4.31: ^{1}H (400 MHz) and ^{13}C (125 MHz) NMR data for 4-chlororubiadin-1-methyl ether (96) in DMSO-d_6

Position	96	
	δ_H	δ_C
1	-	159.3
1a	-	126.8
2	-	120.7
3	10.4 (*s*,1H,OH)	158.6
4	-	106.4
4a	-	131.3
5	8.11 (2H, *m*, H-5,8)	126.0
5a	-	133.5
6	7.64 (2H, *m*, H-6,7)	133.4
7	7.64 (2H, *m*, H-6,7)	133.2
8	8.11 (2H, *m*, H-8)	125.8
8a	-	133.9
9	-	182.6
10	-	180.1
11	2.24 (3H, *s*)	10.5
1-OCH_3	3.83 (3H, *s*)	61.3

4.10 Biological assay

Over the years several natural products derived from prenylated naphthaquinones showed significant biological activity (Singh *et al.*, 2004). In the current work, two promising

antiplasmodial lead compounds, pentalongin (33) and psychorubrin (80), were isolated and tested (Table 4.32). Despite their promising activity against W2 and D6 strains of *P. falciparum*, the high cytotoxicity (LD_{50} < 1µg/mL, Table 4.32) of both compounds makes their direct application as antimalarial agents difficult.

4.10.1 *In vitro* antiplasmodial assay

The anthraquinones isolated from *P. lanceolata*, *P. micrantha* and *P. suswaensis* showed moderate to weak antiplasmodial activity (IC_{50} 5-32 µg/mL against D6 and W2, Table 4.32) with low cytotoxicity (Table 4.32) indicating the safer applicability of the anthraquinone containing indigenous decoction compared to that of the pyranonaphaquinones containing *P. longiflora*.

Table 4.32: *In vitro* antiplasmodial assay of plant extracts and compounds from *Pentas longiflora* and *Pentas lanceolata*

Sample (purity in %)	Antiplasmodial activity IC_{50} (µg/mL)		Cytotoxicity LD_{50} (µg/mL)	Selectivity Index	
	W2 clone (CQ-R)	D6 clone (CQ-S)		W2	D6
P. longiflora (extract)	0.93 ± 0.16	0.99 ± 0.09	-	-	-
Pentalongin (**33**, ≥ 98%)	0.27 ± 0.09	0.23± 0.08	0.80	2.96	3.48
Psychorubrin (**80**, ≥ 98%))	0.91 ± 0.15	0.82± 0.24	0.89	0.98	1.09
Mollugin (**34**, ≥ 95%)	10.22 ± 1.37	7.56±1.13	20.0	1.96	2.65
P. lanceolata (extract)	2.55 ± 0.30	1.33 ± 0.15	-	-	-
Tectoquinone (**75**, ≥ 98%)	10.78 ± 1.33	6.74 ± 1.73	> 100	> 9.27	>14.8
Rubiadin (**49**, ≥ 98%)	8.36 ± 2.19	5.47 ± 0.70	53.0	6.34	9.69
Rubiadin-1-methyl ether (**51**, ≥ 98%)	18.91± 0.39	12.08 ± 2.28	64.0	3.38	5.30
Nordamnacanthal (**77**, ≥ 99%)	9.33 ± 2.98	9.29 ± 0.00	51.0	5.47	5.49
Damnacanthal (**50**, ≥ 99%)	10.88 ± 2.09	7.67 ± 0.36	73.0	6.71	9.52
Lucidin-ω-methyl ether (**60**, ≥ 98%)	13.19 ± 2.15	12.08 ± 3.69	> 100	>7.58	> 8.28
Damnacanthol (**59**, ≥ 98%)	31.42 ± 2.32	16.07 ± 1.15	> 100	> 3.18	> 6.22
5,6-Dihydroxydamnacanthol (**78** > 99%)	19.33 ± 6.36	15.02 ± 4.28	> 100	> 5.17	> 6.66
Chloroquine	0.07 ± 0.01	0.01 ± 0.01	-		-
Mefloquine	0.004 ± 0.38	0.06 ± 0.04	-		-

Despite the traditional use of the roots of the *P. bussei* for malaria, the *in vitro* antiplasmodial results revealed that the dihydronaphthoquinones isolated from *P. bussei* and *P. parvifolia* showed moderate to weak antiplasmodial activity (IC_{50}, 7-45 µg/mL against both D6 and W2, Table 4.33) with low cytoxicity, while the crude extract was found to be inactive (49-50 µg/mL). The new compound, 5,6-dihydroxylucidin-ω-methyl ether (**82**), isolated from *P. micrantha* showed moderate antiplasmodial activity (IC_{50}, 5.36 µg/mL against D6 and 8.47 µg/mL against W2, Table 4.33) with low cytoxicity.

Table 4.33: *In vitro* antiplasmodial activity of plant extracts and compounds from *Pentas bussei*, *Pentas micrantha* and *Pentas parvifolia*

Sample (Purity in %)	Antiplasmodial activity IC_{50} (µg/mL)		Cytotoxicity LD_{50} (µg/mL)	Selectivity Index	
	D6 clone (CQ-S)	W2 clone (CQ-R)		D6	W2
P. bussei (CH_2Cl_2:CH_3OH, root)	49.04 ± 8.73	49.86 ± 0.00	-	-	-
P. micrantha (CH_2Cl_2:CH_3OH, root)	4.00 ± 1.86	3.37 ± 0.74	-	-	-
P. parvifolia (CH_2Cl_2:CH_3OH, root)	19.46 ± 2.05	11.13 ± 1.62	-	-	-
Bussidihydroquinone A (**70**) (> 99 %)	11.10 ± 3.52	44.50 ± 0.09	22.3	2.0	0.5
Bussidihydroquinone B (**71**) (> 99 %)	8.35 ± 3.07	37.92 ± 0.00	112.1	13.41	2.96
Bussidihydroquinone C (**72**) (> 99 %)	12.02 ± 2.99	22.12 ± 2.56	89.6	7.45	4.05
Compound 56 (> 99 %)	7.45 ± 2.13	30.45 ± 3.18	93.7	12.58	3.07
Bussidihydroquinone D (**73**) (> 99 %)	12.17 ± 3.87	33.86 ± 11.35	34.4	2.83	1.01
5,6-Dihydroxylucidin-ω-methyl ether (**82**)	5.36 ± 1.16	8.47 ± 0.72	> 100	> 18.6	> 11.8
Chloroquine (**4**)	0.002 ± 0.11	0.003 ± 0.31	-	-	-
Mefaloquine (**12**)	0.14 ± 0.01	0.14 ± 0.02	-	-	-

Improvement in the *in vitro* antiplasmodial activity was observed for 4-phenylrubiadin (**89**) (IC_{50} 4.96 µg/mL against D6 and 13.97 µg/mL against W2) and 4-(*p*-methoxyphenyl)rubiadin-1-methyl ether (**91**) (IC_{50} 7.55 µg/mL against D6 and 14.2 µg/mL against W2) as compared to the parent anthraquinones (Table 4.32).

Table 4.34: *In vitro* antiplasmodial activity of synthetic analogues of rubiadin (49) and rubiadin-1-methyl ether (51)

Sample	Antiplasmodial activity IC_{50} (µg/mL)	
	D6 clone (CQ-S)	W2 clone (CQ-R)
4-bromorubiadin (87)	14.27 ± 2.74	15.96 ± 0.47
4-bromorubiadin-1-methyl ether (88)	17.69 ± 7.19	21.69 ± 11.47
4-phenylrubiadin (89)	4.96 ± 0.28	13.97 ± 0.79
4-(*p*-nitrophenyl)rubiadin-1-methyl ether (90)	11.23 ± 2.84	24.06 ± 5.63
4-(*p*-methoxyphenyl)rubiadin-1-methyl ether (90)	7.55 ± 2.84	14.2 ± 0.59
4-(*p*-methoxyphenyl)rubiadin (92)	18.86 ± 9.67	12.99 ± 0.00
4-biphenylrubiadin-1-methyl ether (93)	10.83 ± 2.80	10.61 ± 2.13
4-(phenylethynyl)rubiadin (94)	10.65 ± 4.22	19.78 ± 0.38
Chloroquine (4)	0.002 ± 0.14	0.002 ± 0.41
Mefaloquine (12)	0.012 ± 0.00	0.16 ± 0.02

4.10.2 *In vivo* antiplasmodial assay

Results from the *in vivo* study showed that pentalongin (33) was moderately active *in vivo* against *P. berghei* in a dose-dependent manner with 37% parasite reduction (Table 4.33).

Table 4.35: *In vivo* antiplasmodial assay of pentalongin (33), psychorubrin (80) and extract of *Pentas longiflora*

Sample	Average parasitaemia	% Activity parasite reduction	Survival time in days
P. longiflora extract	24.42 ± 4.5	19.98	11.2 ± 4.4
Psychorubrin (80)	24.77 ± 4.9	18.81	14.4 ± 4.0
Pentalongin (33)	19.31 ± 8.9	37.00	12.4 ± 4.8
Chloroquine	0.56 ± 0.5	98.16	-
Untreated group	30.51 ± 5.8	-	10.8 ± 3.9

4.10.3 Cytotoxicity Assay

Table 4.36: Cytoxicity data of some of the isolated compounds

Compounds	LD_{50} (µg/mL)	95% confidence interval (µg/mL)
Pentalongin (**33**)	0.80	0.61-1.00
Psychorubrin (**80**)	0.89	0.45-1.80
Mollugin (**34**)	20.0	11.0-36.0
Tectoquinone (**74**)		No cytotoxicity at 100 µg/mL
Rubiadin (**49**)	53.0	23.0-119.0
Rubiadin-1-methyl-ether (**51**)	64.0	15.0-280.0
Nordamnachantal (**76**)	51.0	19.0-137.0
Damnachantal (**50**)	73.0	38.0-141.0
Lucidin-ω-methyl ether (**60**)		No cytotoxicity at 100 µg/mL
Damnacanthol (**59**)	129.0	59.0-282.0
5,6-Dihydroxydamnacanthol (**78**)		No cytotoxicity at 100 µg/mL
Bussidihydroquinone A (**70**)	89.6	51.2-157.0
Bussidihydroquinone B (**71**)	93.7	49.4-177.9
Bussidihydroquinone C (**72**)	34.4	17.9-66.1
Compound **56**	22.3	13.8- 37.7
Bussidihydroquinone D (**73**)	112.2	49.9-51.8

Confidence intervals for the cytotoxicity data (LD_{50} 95%)

4.10.4 *In vitro* antiplasmodial combinational assay with chloquine against *Plasmodium falciparum*

The susceptibility to a combination of some of the compounds with chloroquine were assessed against two parasite strains (D6 and W2) using the *in vitro* isotopic micro test. Of the compounds tested, a combination of rubiadin-3-*O*-primveroside (**52**) demonstrated synergetic effect *in vitro* with chloroquine (Fig. 4.17). The result obtained under the current study is comparable to a recent report on a similar work by Parquet *et al.*, (2010) based on combination assay of atorvastatin and quinine (**4**).

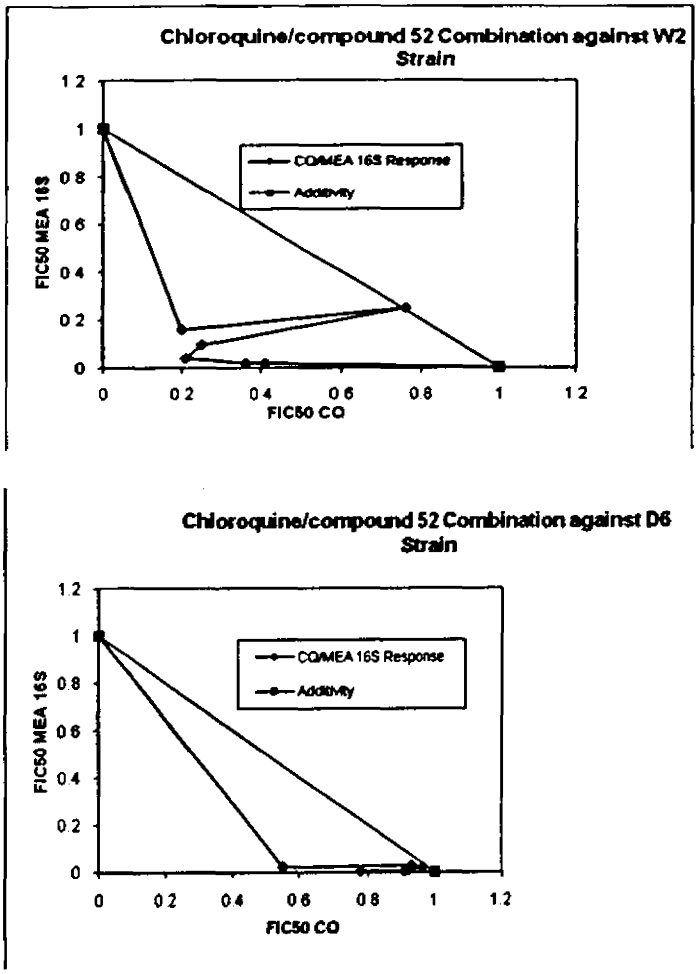


Figure 4.17: *In vitro* combinational assay of rubiaidn-3-*O*-β-primveroside (**52**) with chloroquine

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5. 1 CONCLUSION

- The roots of *Pentas* species showed strong (for *P. lanceolata*, *P. longiflora*, *P. micrantha*), moderate (for *P. suswaensis*) to weak (*P. bussei* and *P. parvofolia*) antiplasmodial activities against W2 and D6 strains of *P. falciparum*.

- The roots of *Pentas bussei* yielded five dihydronaphthaquinones of which four (**70-73**) are new natural products. The isolated compounds showed weak to moderate antiplasmodial activites.

- The roots of *Pentaslanceolata* afforded eleven anthraquinones one of which (**78**) is found to be a new natural product. In the antiplasmodial assay the anthraquinones showed moderate activities (5-31 μg/mL). One of the compounds, rubiadin-3-*O*-primveroside (**52**), showed synergetic effect with chloroquine *in vitro*.

- The roots of *Pentas longiflora* afforded two pyranonaphthoquinones (**33**, **80**) and a dihydronaphthaquinone derivative (**34**) of which the pyranonaphthaquinones are found to have excellent antiplasmodial activity (IC_{50} < 1 μg/mL).

- The roots of *Pentas micrantha* gave eight anthraquinones of which two (**78**, **82**) are new natural products. In the antiplasmodial assay the anthraquinones showed moderate activities.

- The roots of *Pentas suswaensis* yielded thirteen anthraquinones of which the new compound (**78**) was re-isolated. In the antiplasmodial assay the anthraquinones showed moderate activities.

- The roots of *Pentas parvifolia* afforded seven dihydronaphthaquinone derivatives of which four (**70-73**) have been re-isolated. In the antiplasmodial assay the dihydronaphthaquinones showed weak to moderate activities.

- Three of the *Pentas* species: *P. micrantha*, *P. lanceolata* and *P. suswaensis* constitute anthraquinones exclusively whereas the other three: *P. bussei*, *P. parvifolia* and *P. longiflora*, are found to be rich in dihydronaphthoquinone derivatives. The chemical profile appears to reflect the taxonomic/morphological groupings in these taxa.

- The anthraquinones and the dihydronaphthaquinone derivatives isolated from the *Pentas* species in this study appear to have been derived from the chorismate/O-succinyl benzoic acid pathway. The naphthalene derivatives are all oxygenated at C-1 and C-4, in the case of pentalongin (**33**) and psychorubrin (**80**) these are oxidized to produce 1,4-quinones. In other cases, methylation (alkylation in the case of **34**, **56**, **70-73**) prevents oxidation to the quinones. Additional modification through oxidation (in the case of **56**) and prenylation and geranylation produce complex derivatives such as dihydronaphthoquinones **73** and **86**.

- The pyranonaphthoquinones and some of the anthraquinones isolated from the roots of *Pentas lanceolata* and *Pentas longiflora* showed good to moderate antiplasmodial activities against the W2 and D6 strains of *Plasmodium falciparum* and an overall low cytotoxicity for anthraquinones.

- Ten new synthetic analogues of rubiadin (**49**) and rubiadin-1-methyl ether (**51**) were prepared using microwave assisted cross coupling reactions. Two of the synthetic analogues, 4-phenylrubiadin (**91**) and 4-(*p*-methoxyphenyl)rubiadin-1-methyl ether (**93**), showed improvement in activity compared to the parent anthraquinones.

5.2 Recommendation

- The molecular basis of the antiplasmodial activity of pyranonapthoquinones and anthraquinones is not firmly established and hence future work has to be done to fill this gap.
- Careful analysis of the antiplasmodial activity of anthraquinones and napthaquinones of the genus *Pentas* and their structure-activity relationship followed by rational synthetic modifications has potential for providing useful agents with low cytoxicity in the fight against malaria.
- Although there is close resemblance in chemical constituents between the species *Pentas bussei*, *P. parvifolia*, and *P. longiflora*, there has to be a more detailed molecular phylogenic study from the botanical perspective to supplement the phytochemical findings. The same should apply for *P. lanceolata*, *P. micrantha* and *P. suswaensis*.
- One of the strategies for evading the development of resistance to anti-malarials is the use of combination therapies. Thus, future studies need to be done on a combination of the compounds isolated from the genus *Pentas* with standard first line drugs *in vitro* and *in vivo*.

- Metabolic studies may be useful in understanding the high cytotoxicity of pyranonaphthaoquinones which showed excellent *in vitro* antiplasmodial activity and ways of lowering their cytoxicty need to be explored.
- The absolute configurations of some of the dihydronaphthoquinones should be determined
- In most cases the crude extracts are more active than any of the pure compounds. It is therefore intresting to find out if there is synergy between the isolated compounds or other more active compounds which should be isolated and identified.

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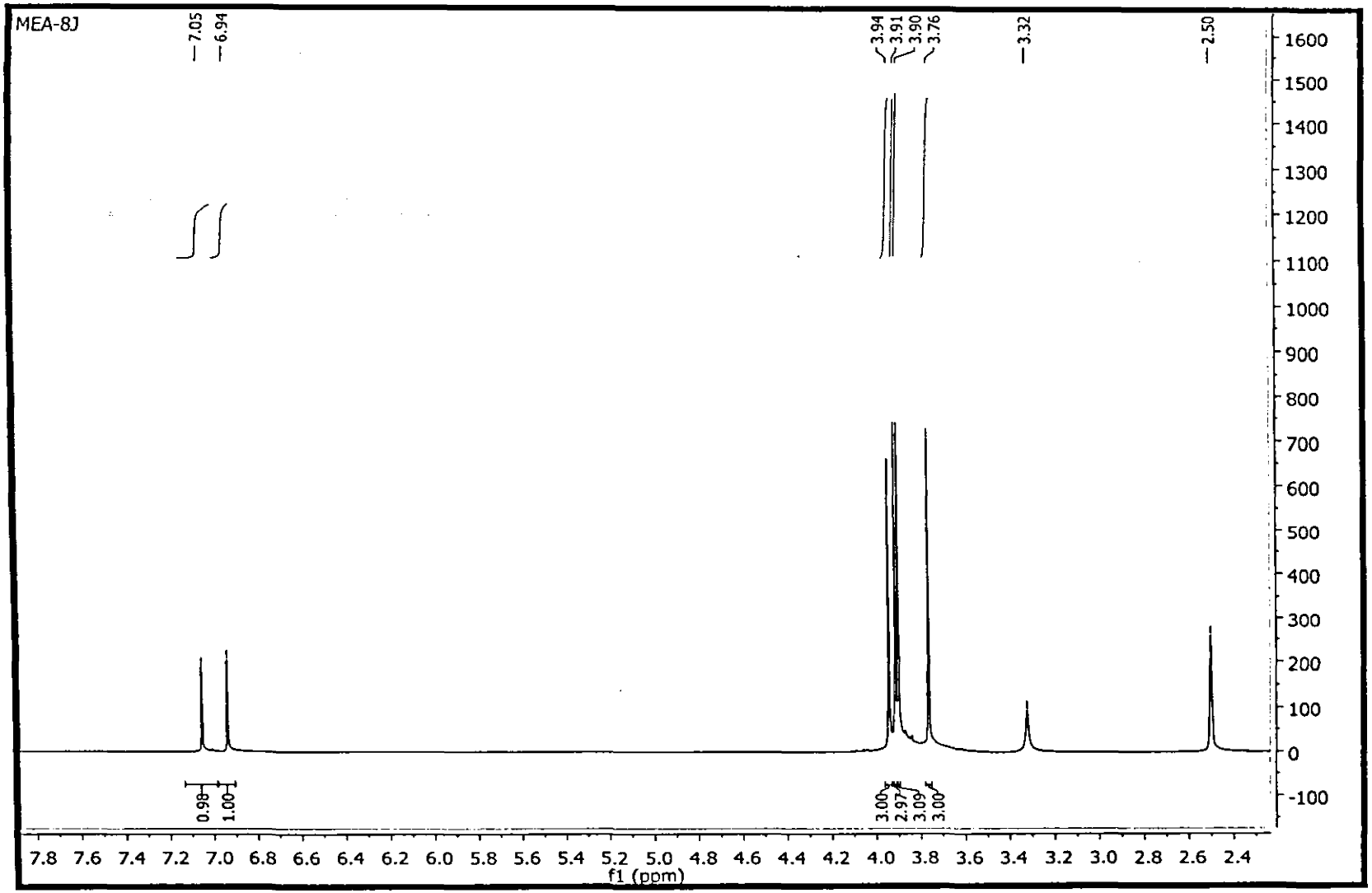
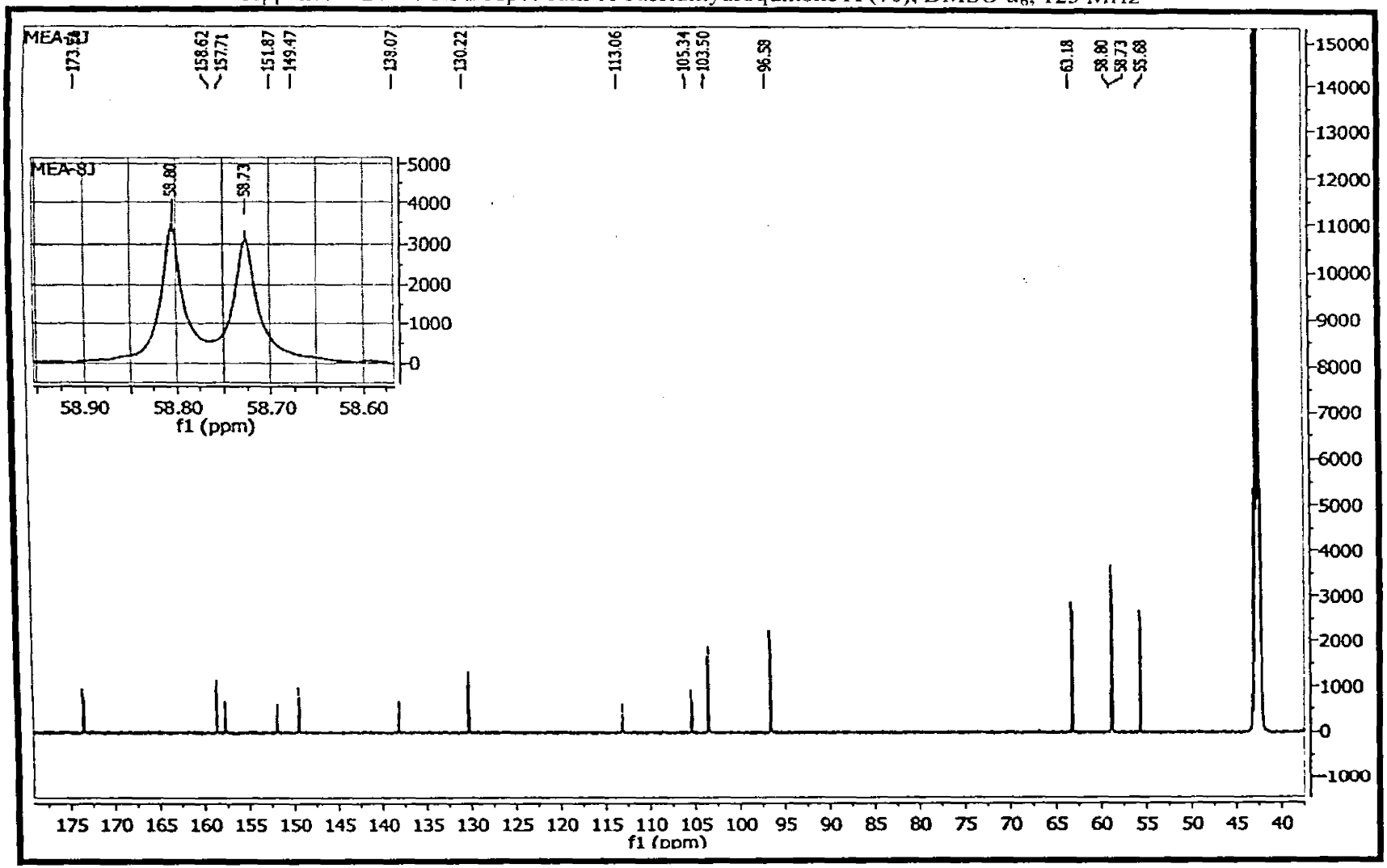
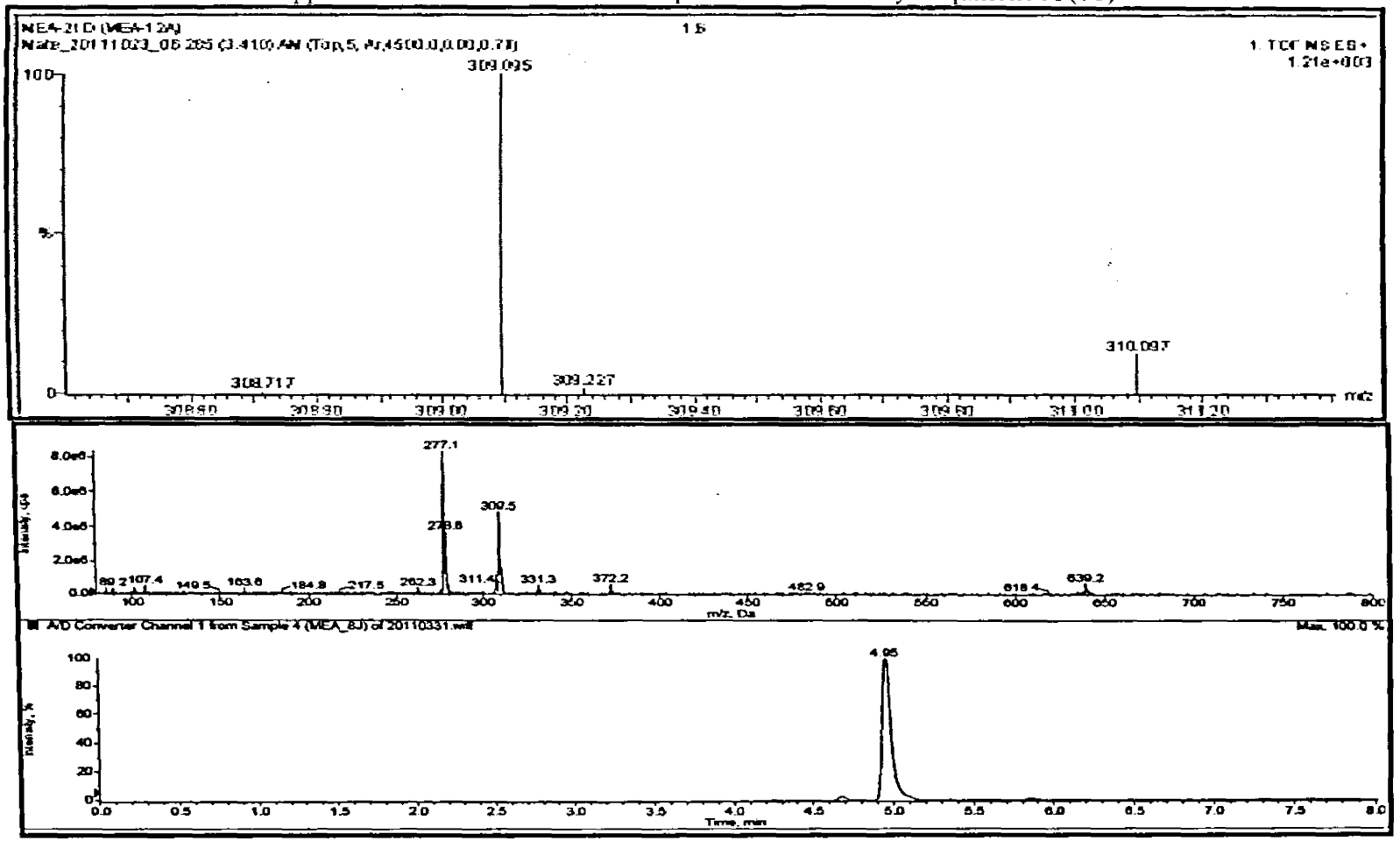
Appendix 1A: ^{1}H NMR spectrum of bussidihydroquinone A (70), DMSO-d_6, 800 MHz



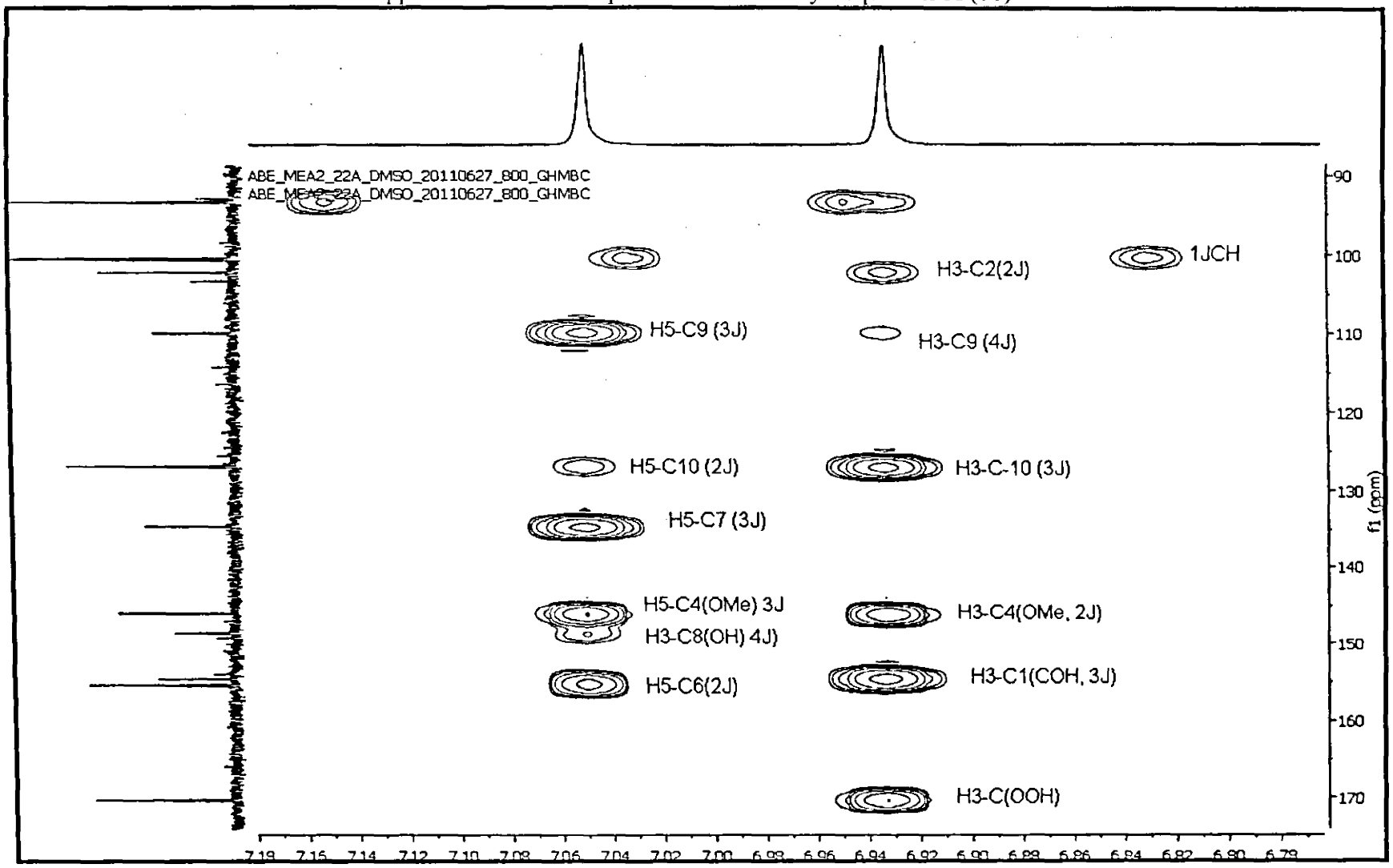
Appendix 1B: ^{13}C NMR spectrum of bussidihydroquinone A (70), DMSO-d_6, 125 MHz



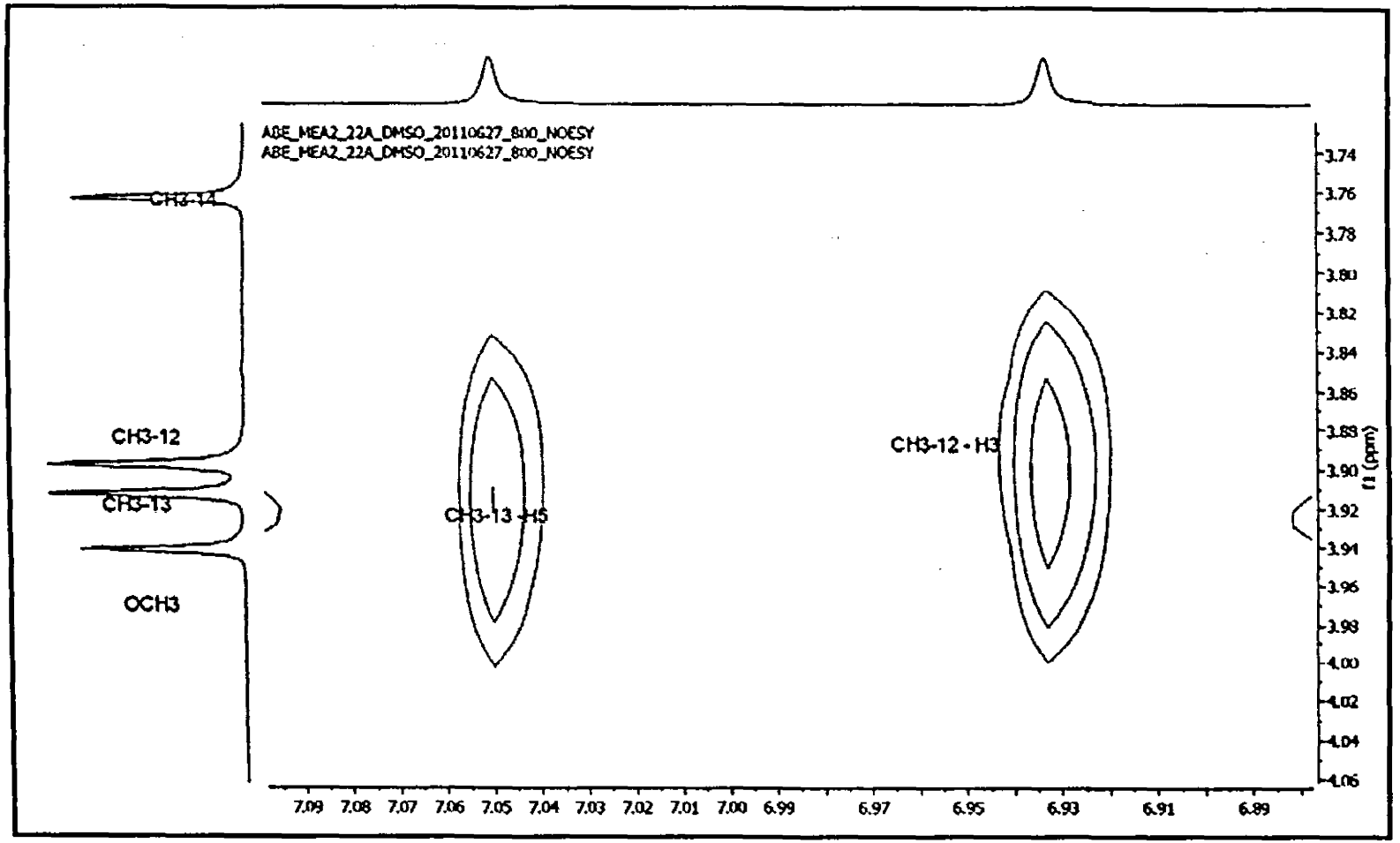
Appendix 1C: HRMS and ESI-MS spectrum of bussidihydroquinone A (70)



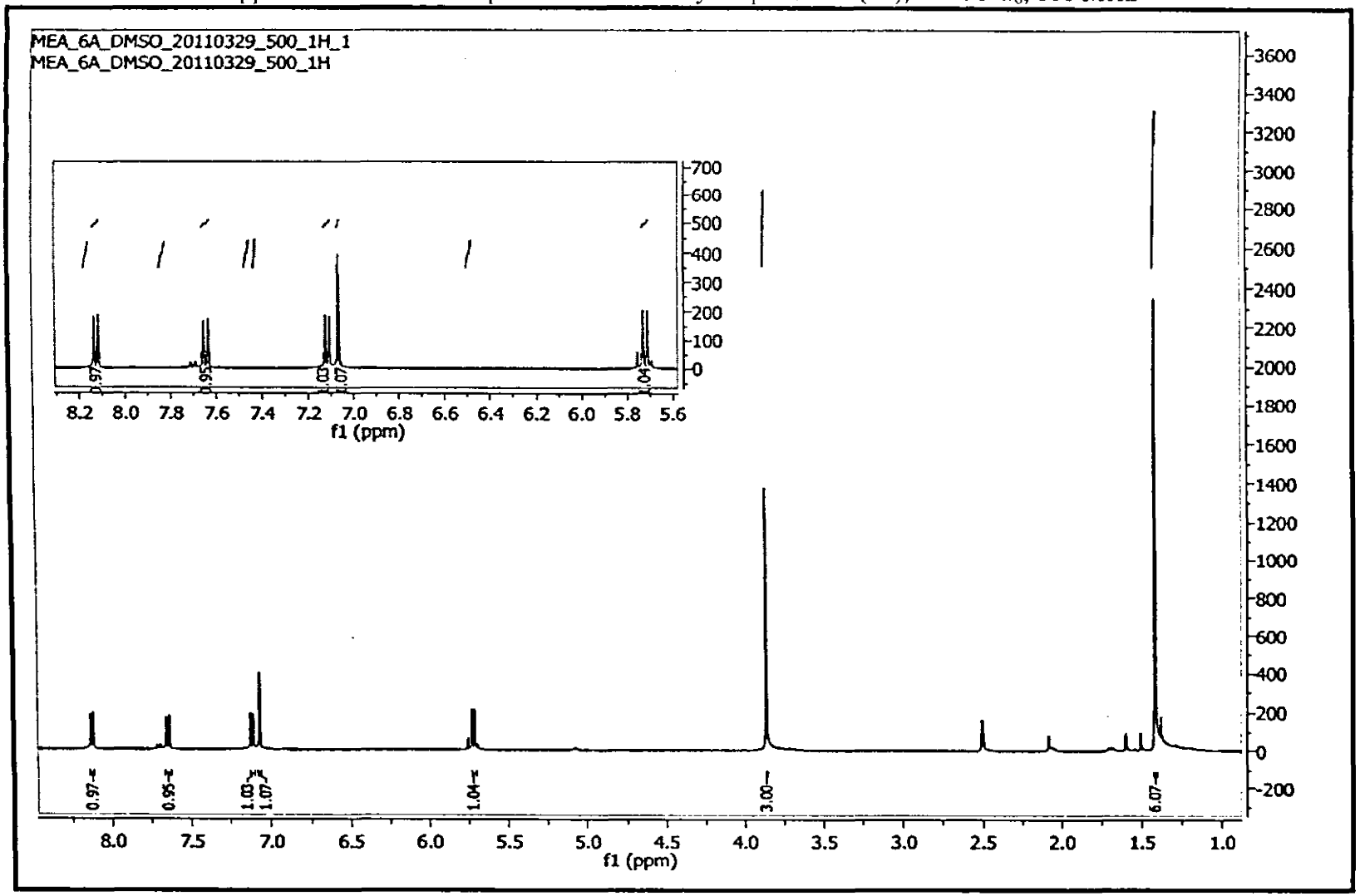
Appendix 1D: HMBC spectrum of bussidihydroquinone A (70)



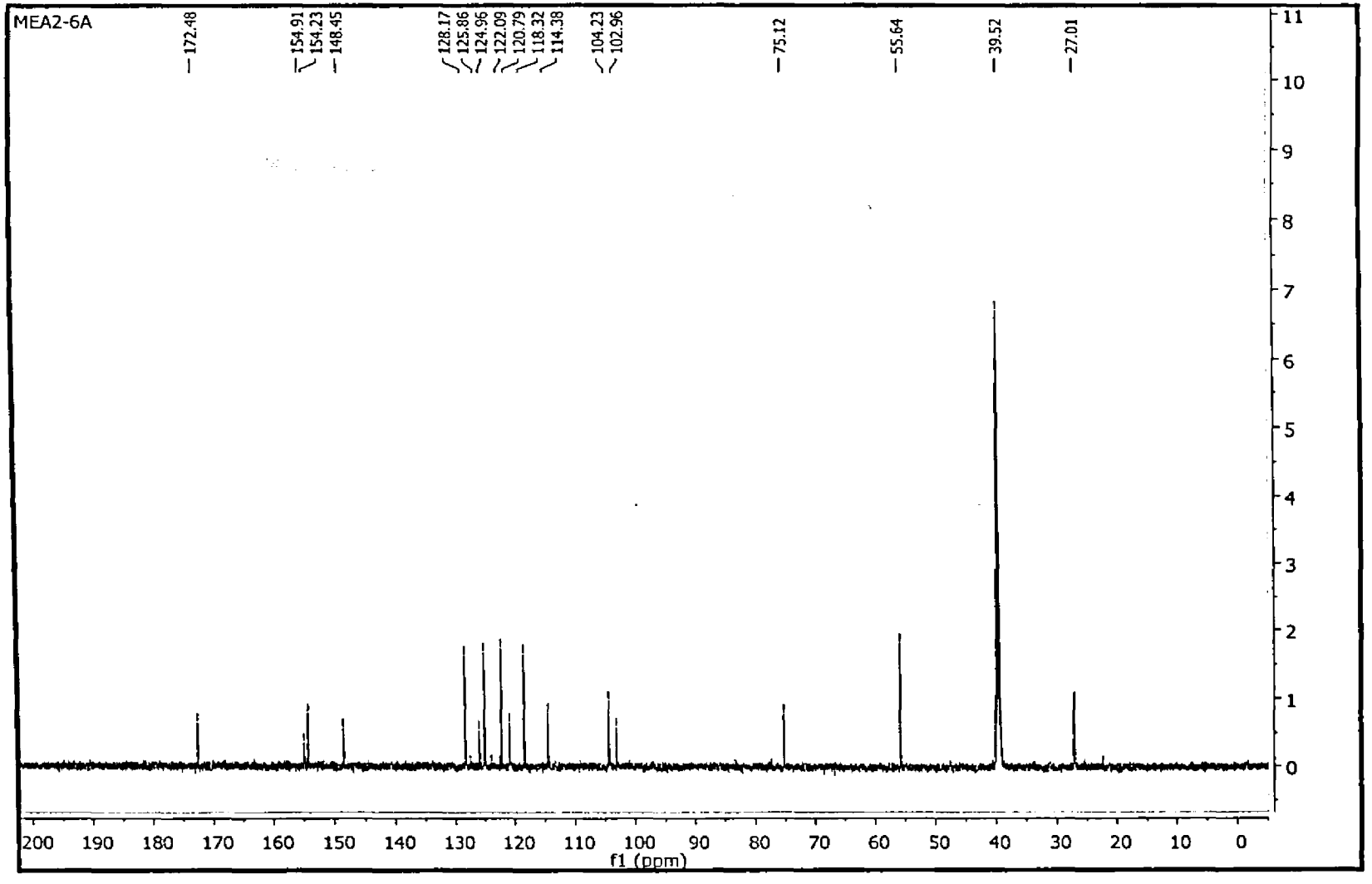
Appendix 1E: NOESY Spectrum of bussidihydroquinone A (70)



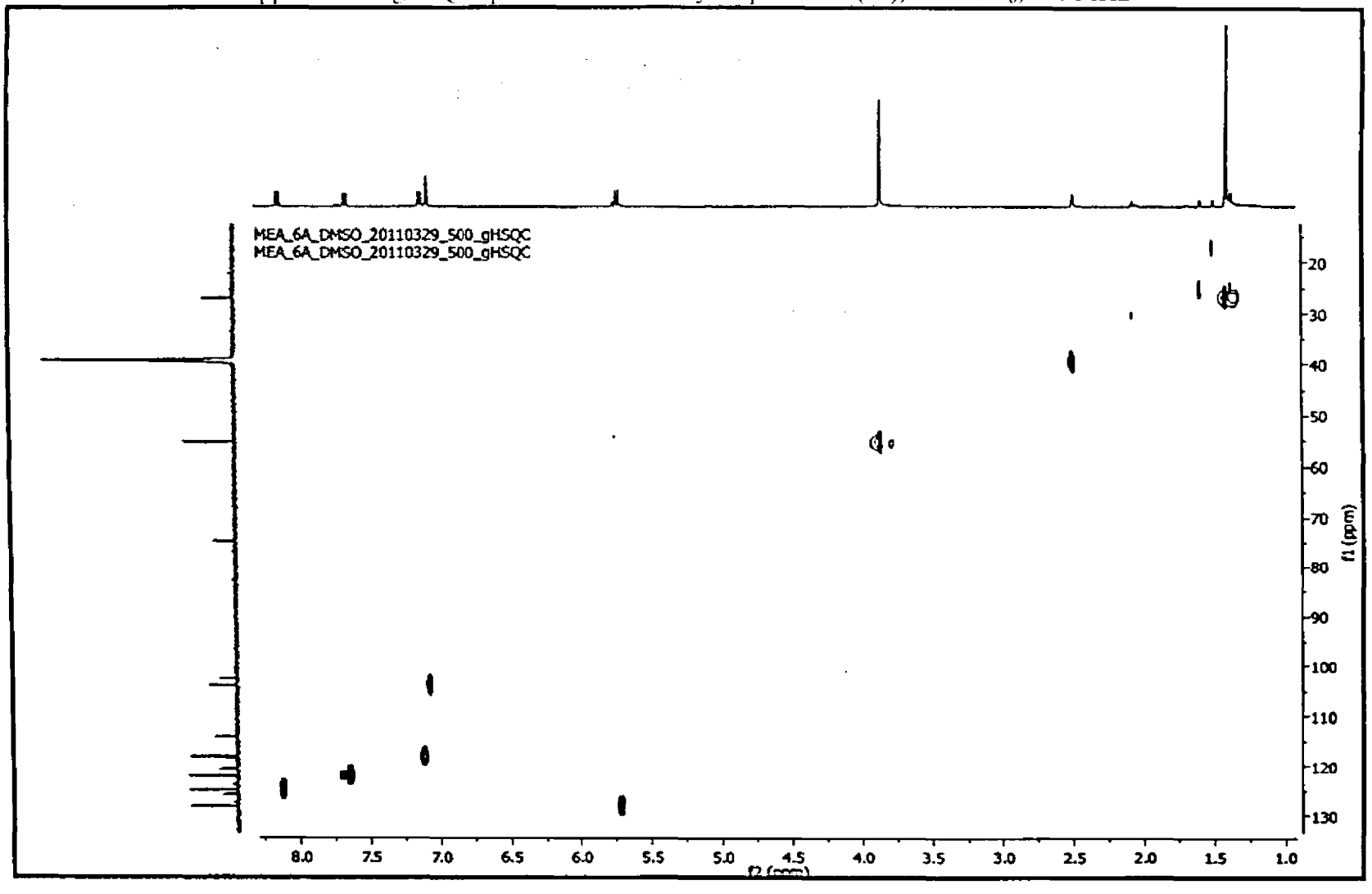
Appendix 2A: ^{1}H NMR spectrum of bussidihydroquinone B (**71**), DMSO-d_6, 800 MHz



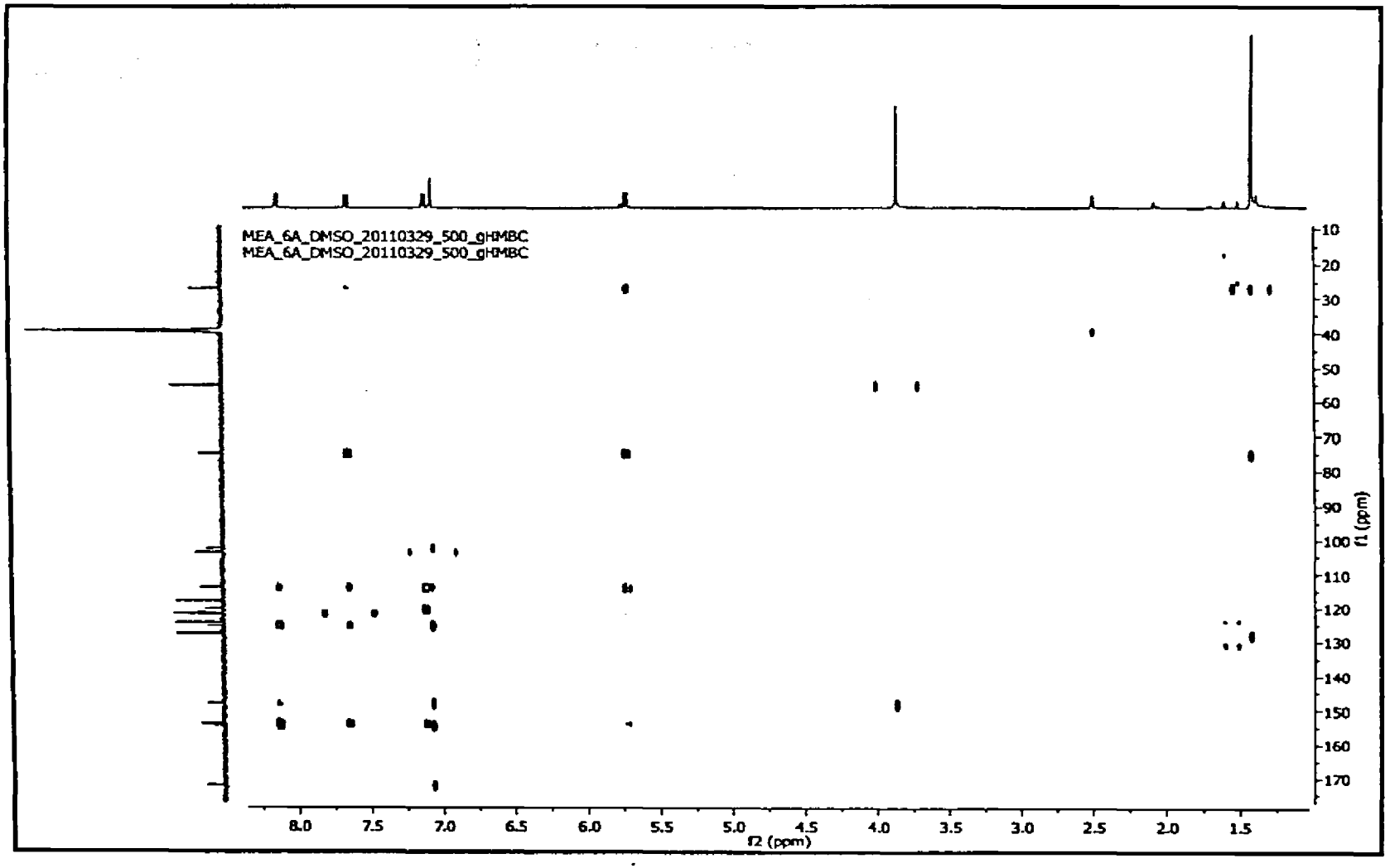
Appendix 2B: ^{13}C NMR spectrum of bussidihydroquinone B (**71**), DMSO-d_6, 200 MHz



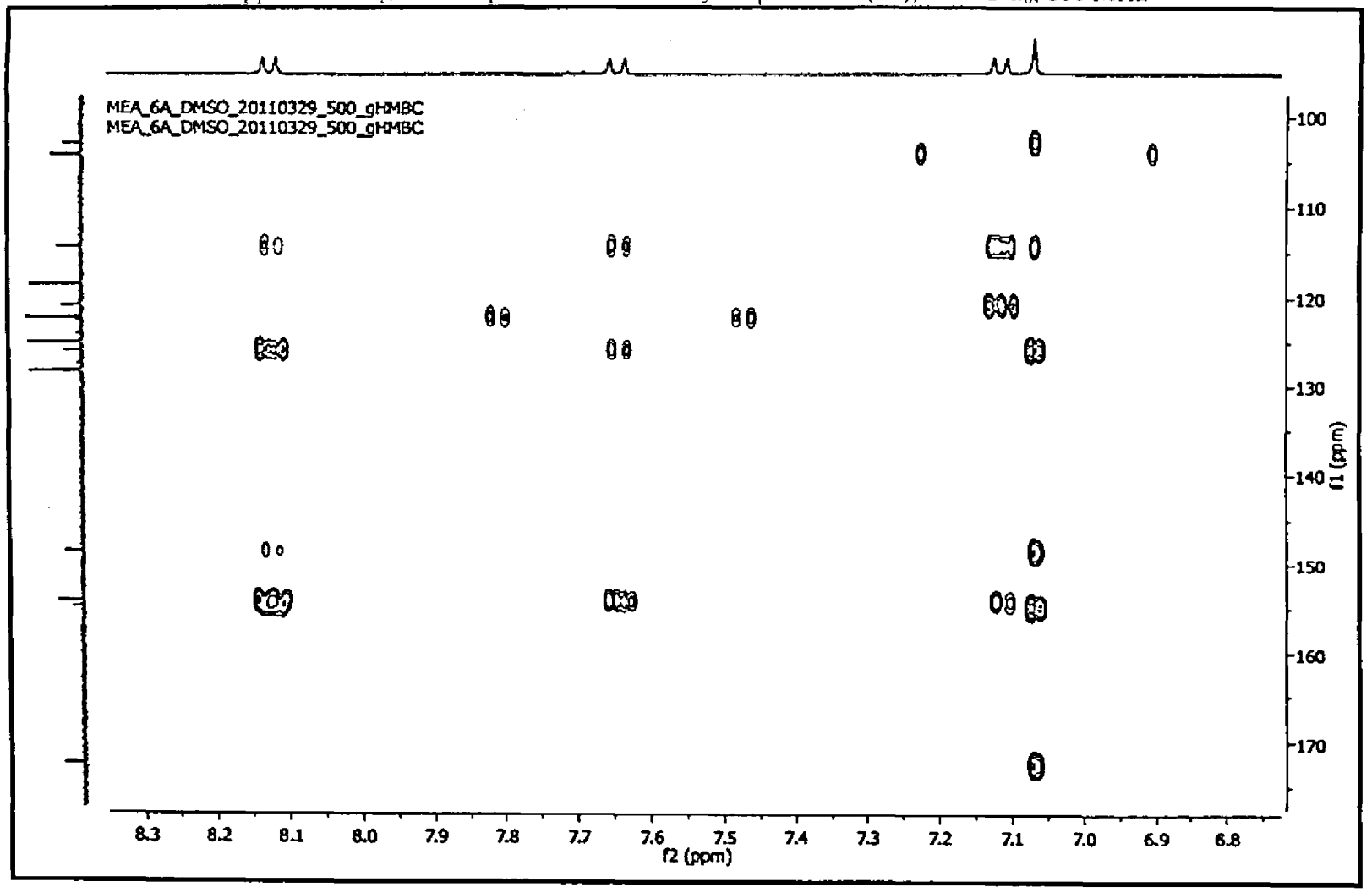
Appendix 2C: gHSQC spectrum of bussidihydroquinone B (71), DMSO-d_6, 800 MHz



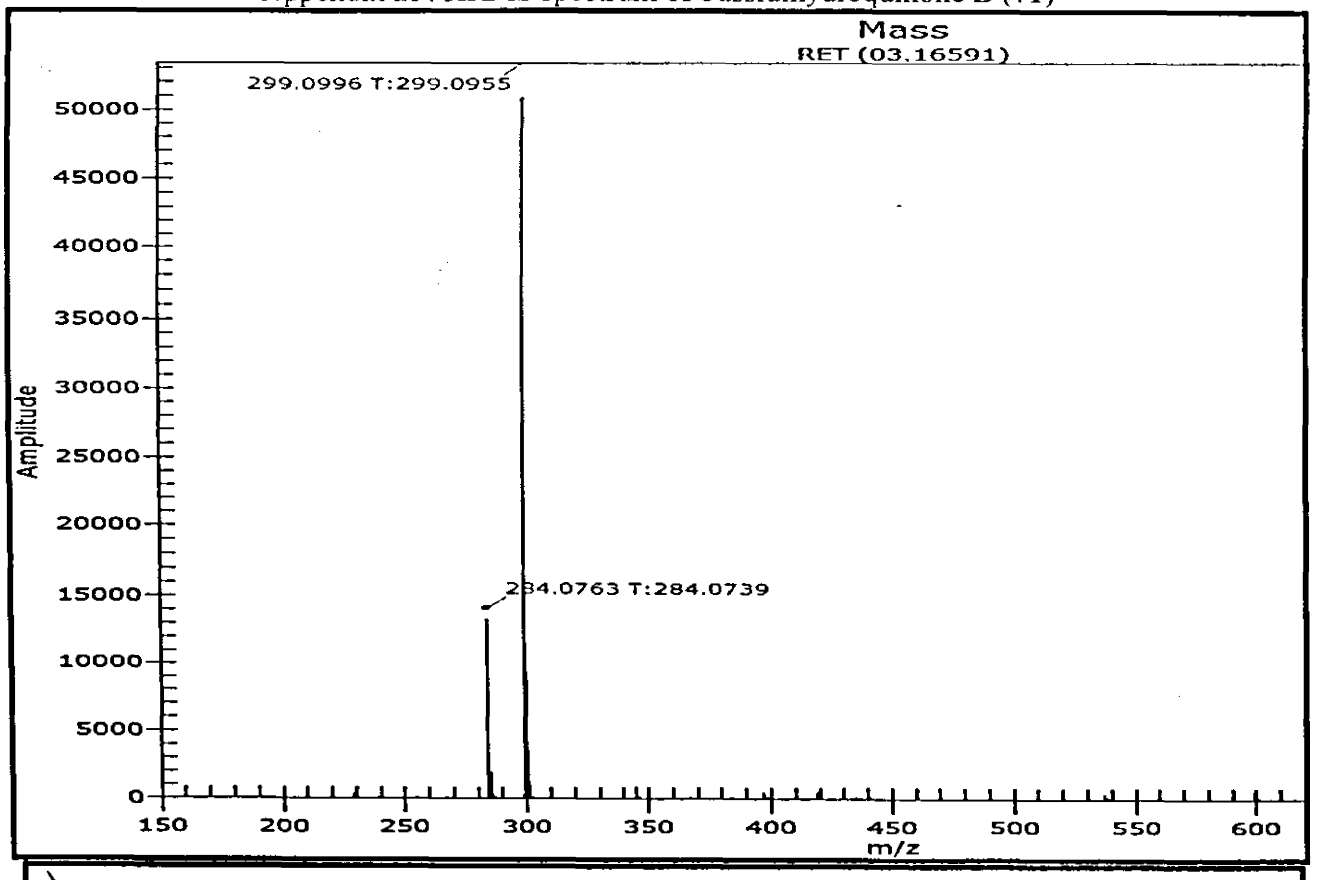
Appendix 2D: gHMBC spectrum of bussidihydroquinone B (**71**), DMSO-d_6, 800 MHz



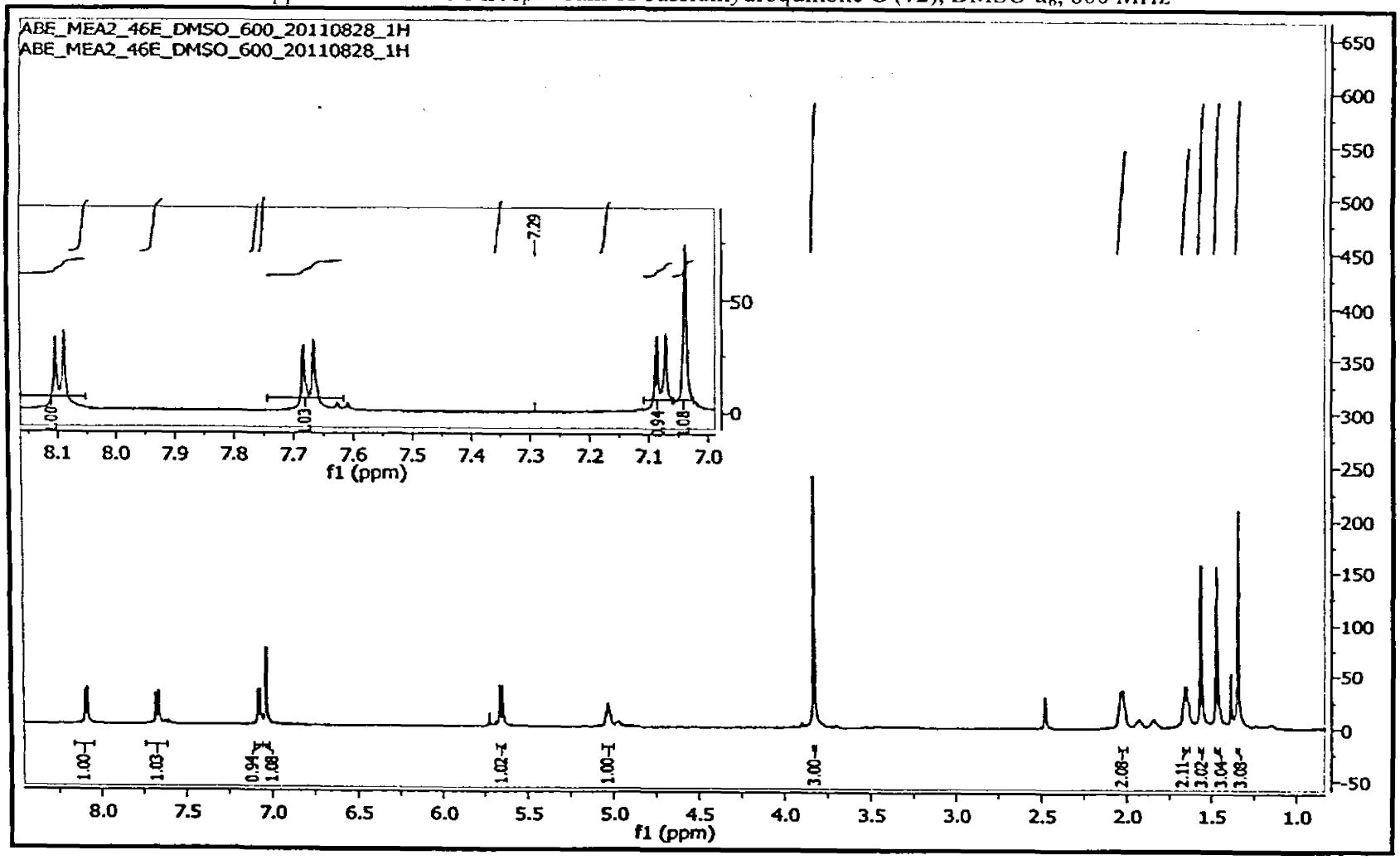
Appendix 2E: gHMBC spectrum of bussidihydroquinone B (71), DMSO-d_6, 800 MHz



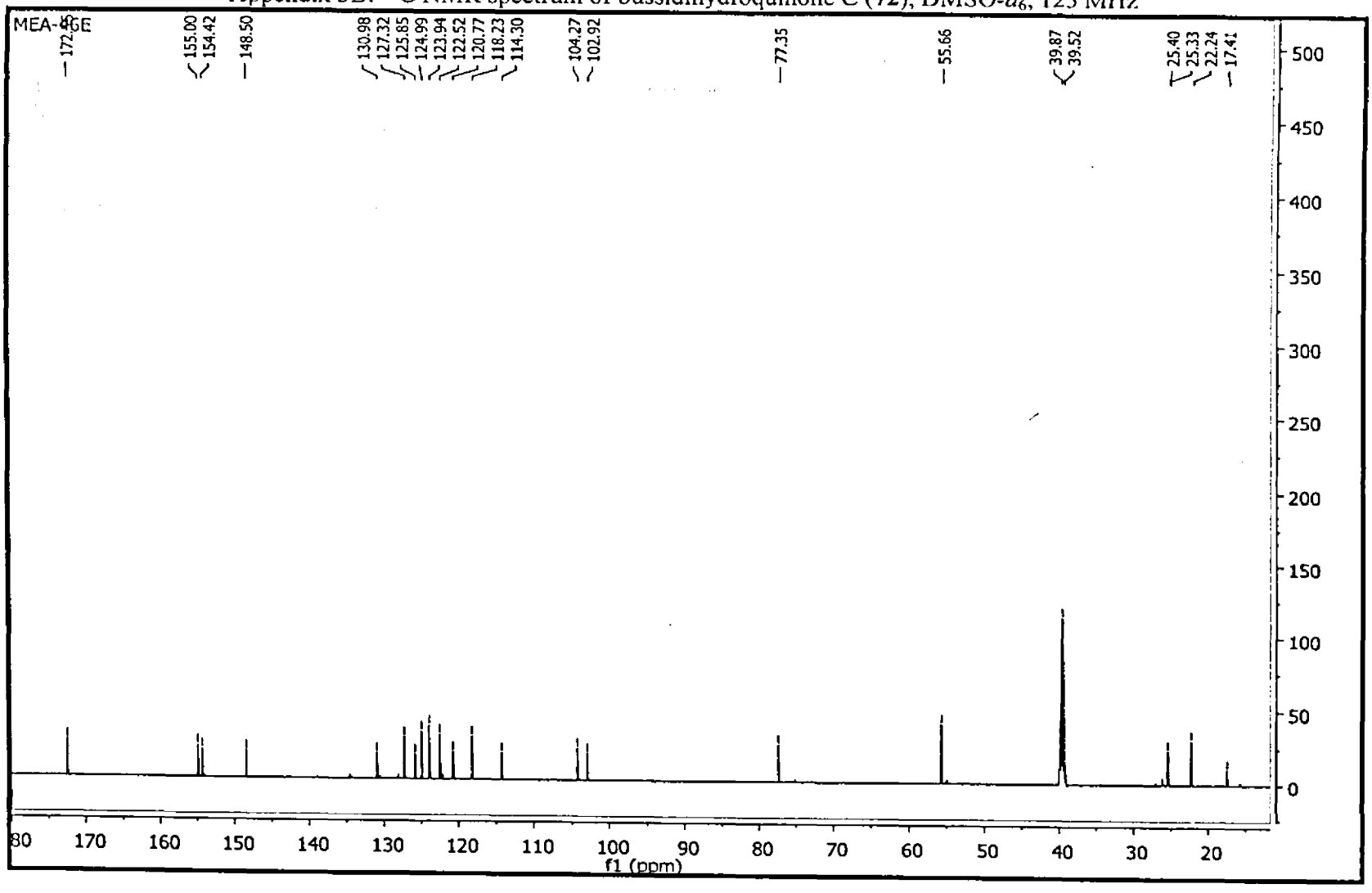
Appendix 2F: HRMS spectrum of bussidihydroquinone B (71)



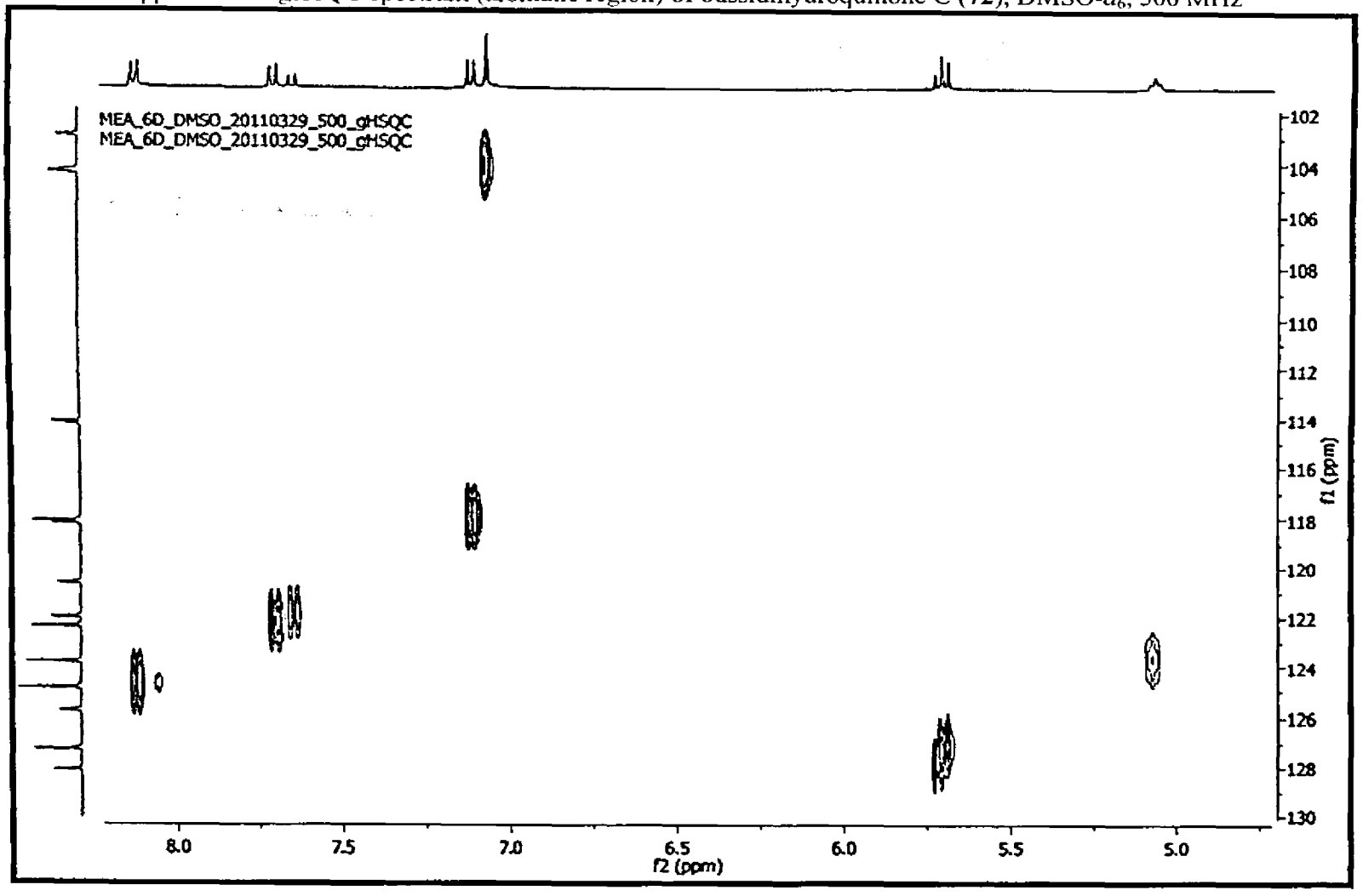
Appendix 3A: ^{1}H-NMR spectrum of bussidihydroquinone C (72), DMSO-d_6, 800 MHz



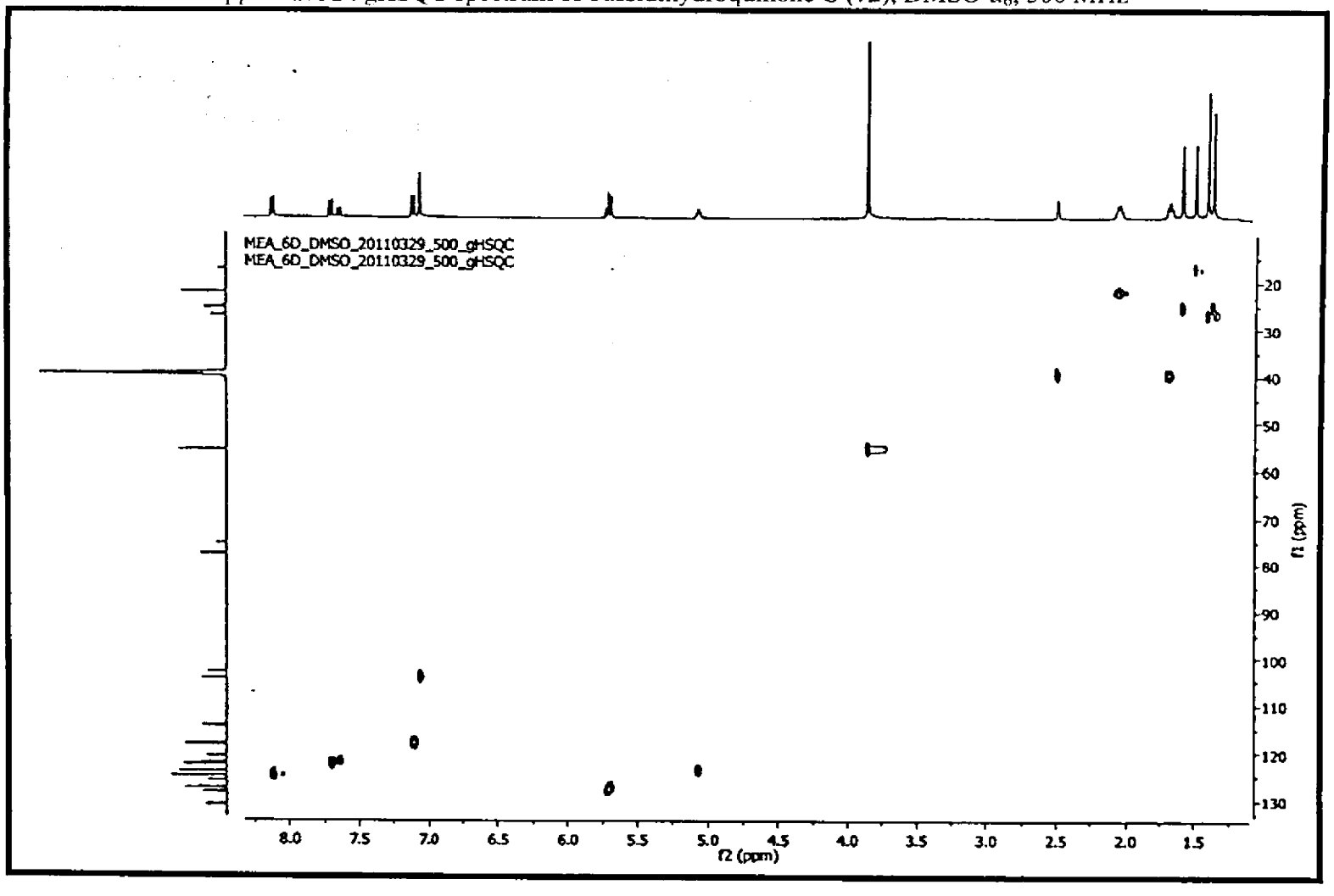
Appendix 3B: ^{13}C NMR spectrum of bussidihydroquinone C (72), DMSO-d_6, 125 MHz



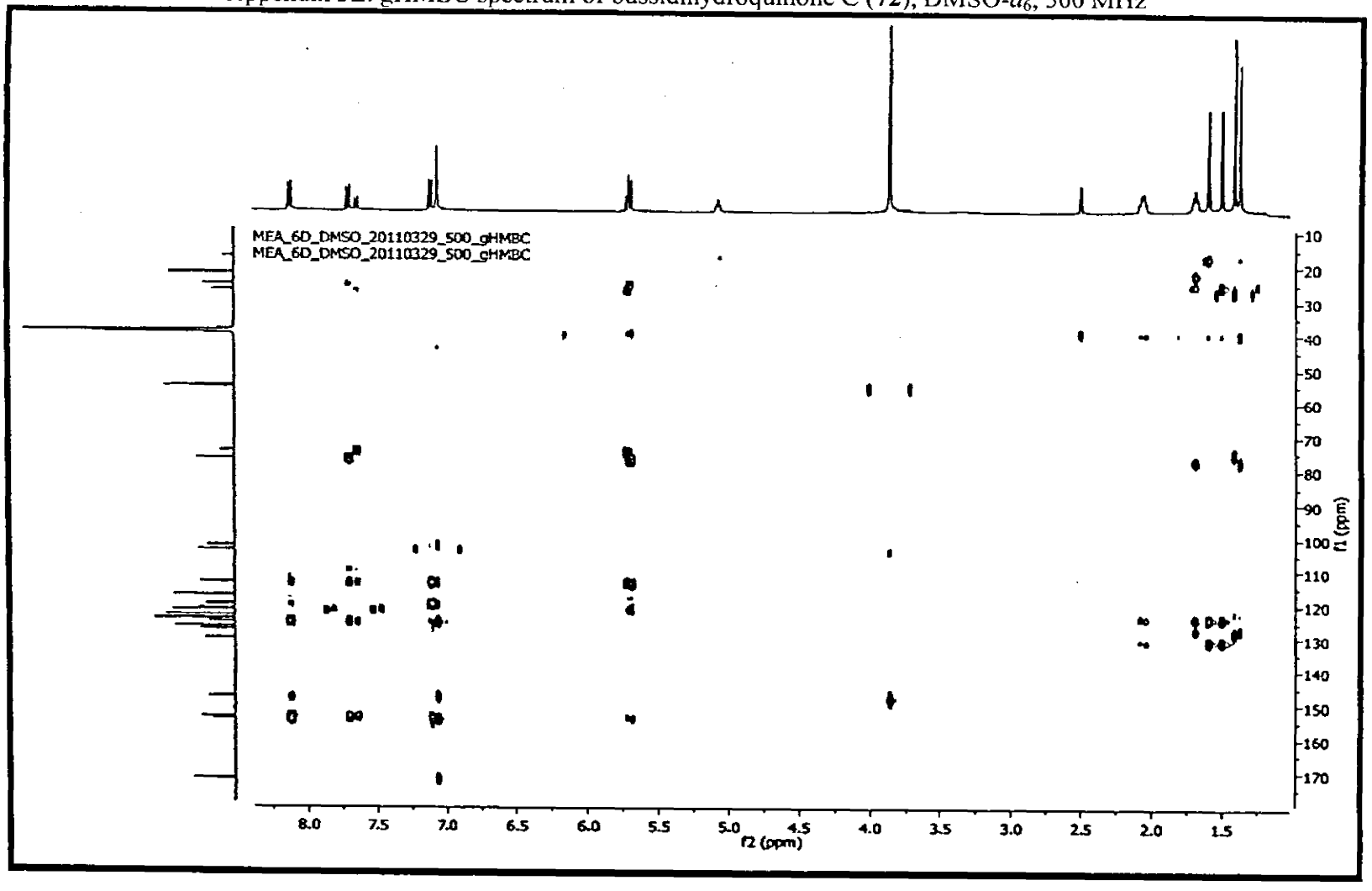
Appendix 3C: gHSQC spectrum (aromatic region) of bussidihydroquinone C (**72**), DMSO-d_6, 500 MHz



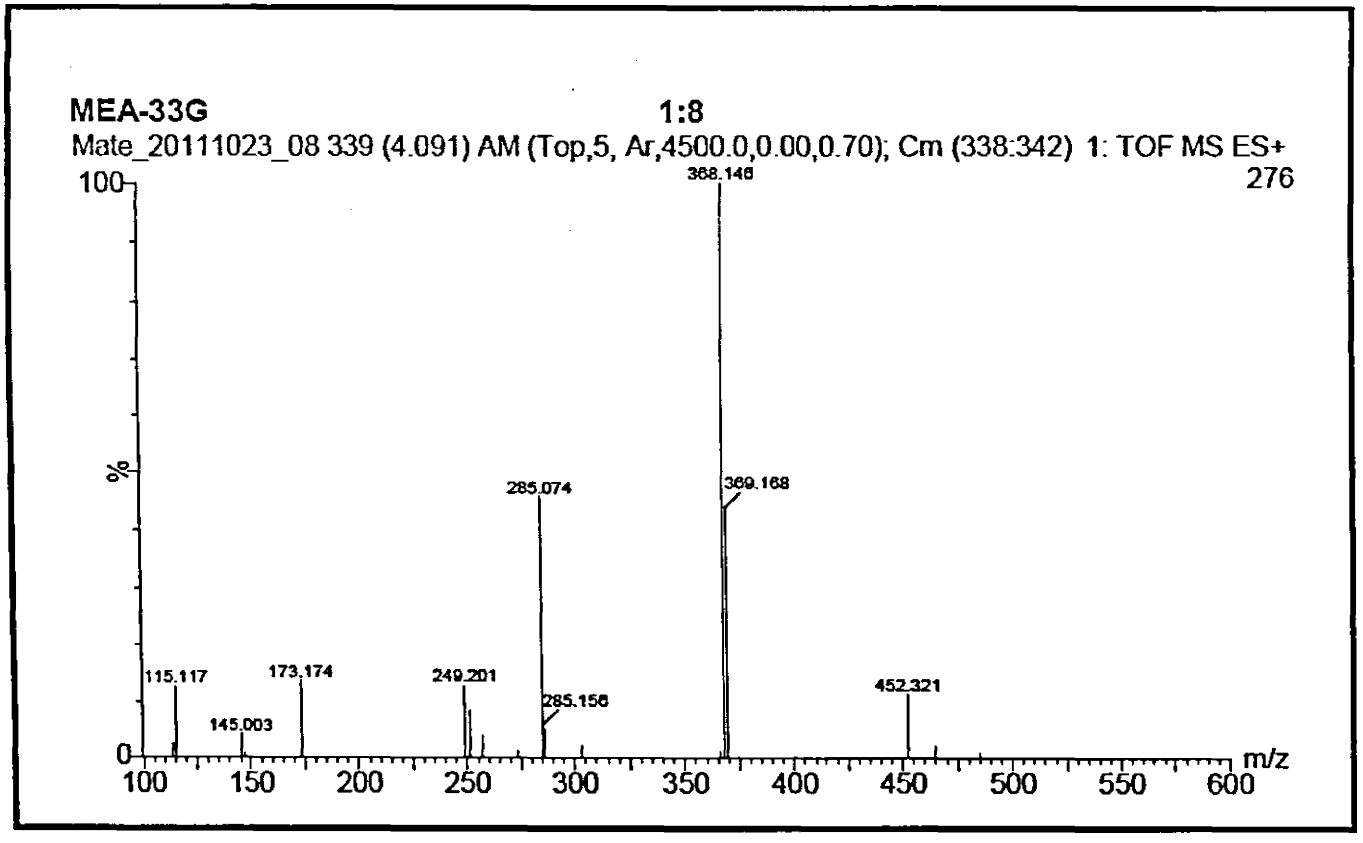
Appendix 3D: gHSQC spectrum of bussidihydroquinone C (72), DMSO-d_6, 500 MHz



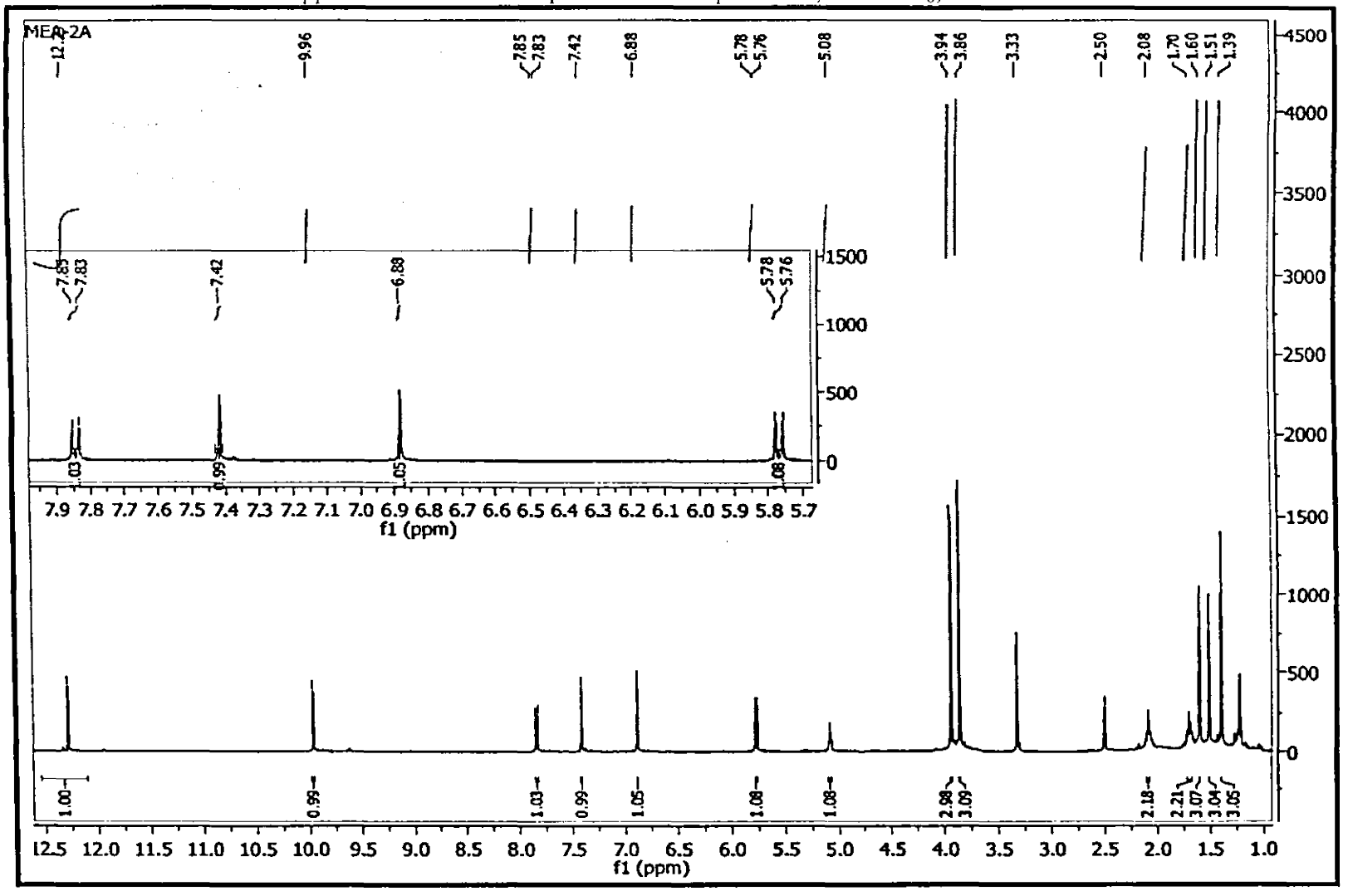
Appendix 3E: gHMBC spectrum of bussidihydroquinone C (72), DMSO-d_6, 500 MHz



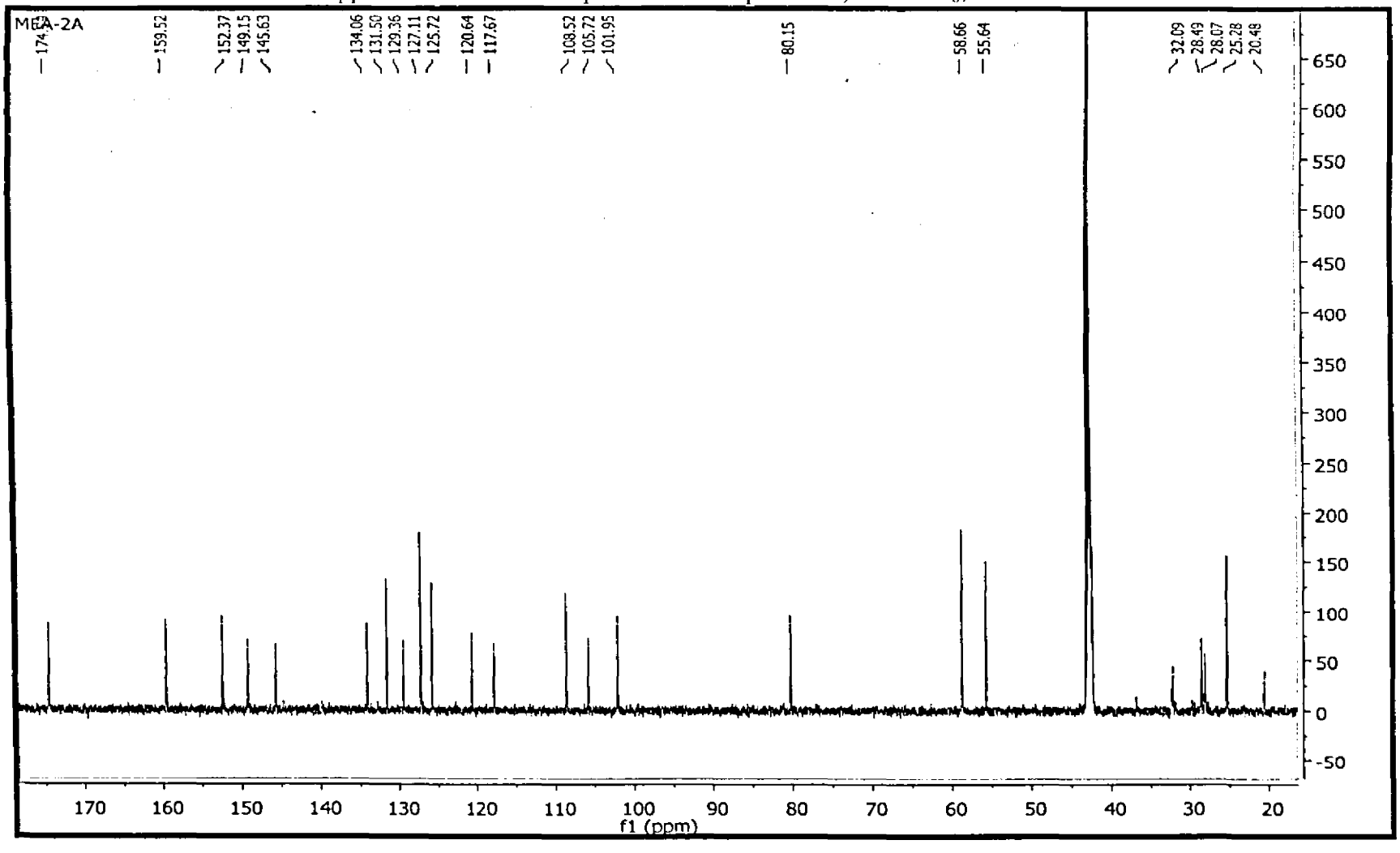
Appendix 3F: HRMS spectrum of bussidihydroquinone C (72)



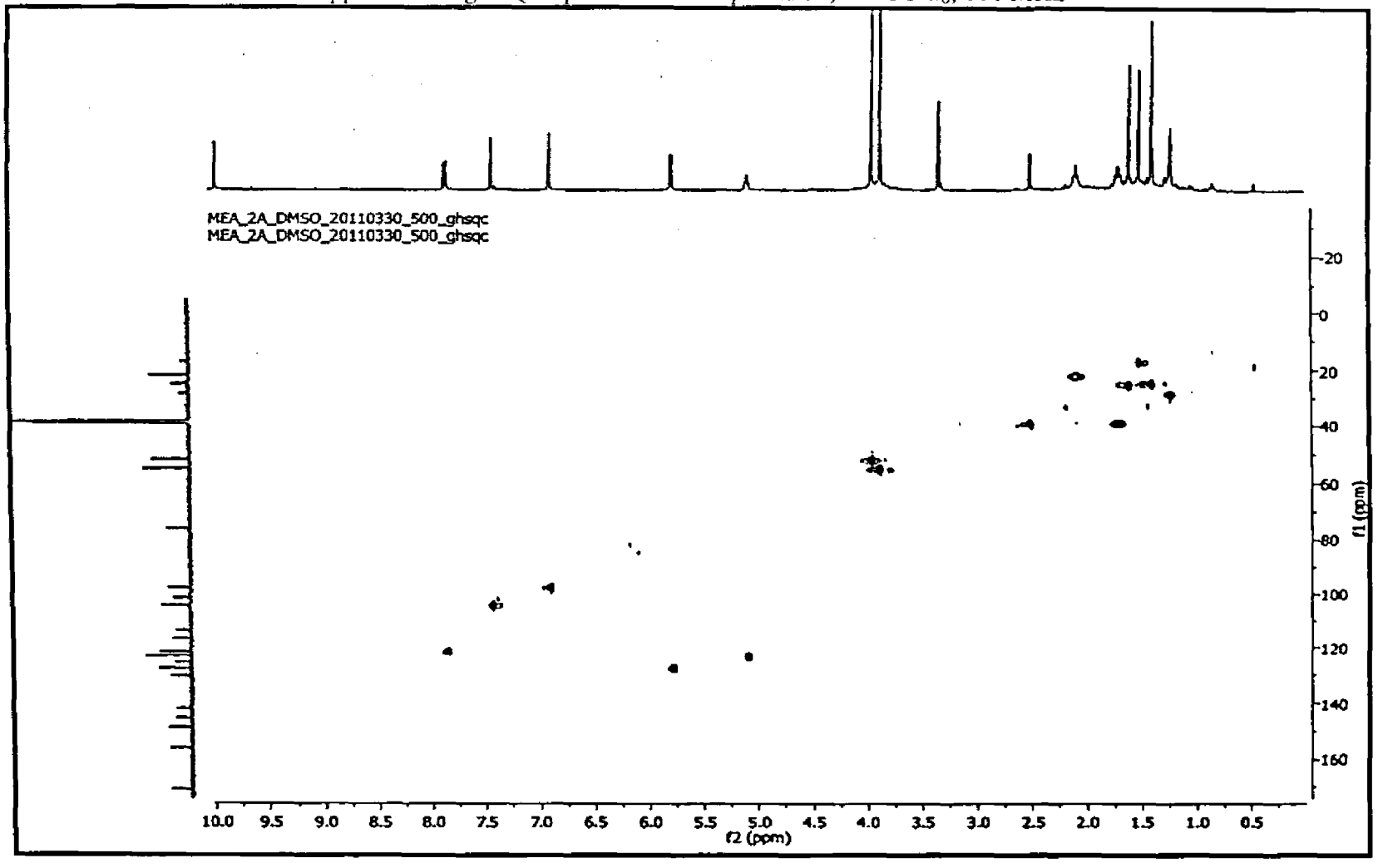
Appendix 4A: ^{1}H NMR spectrum of compound **56**, DMSO-d_6, 600 MHz



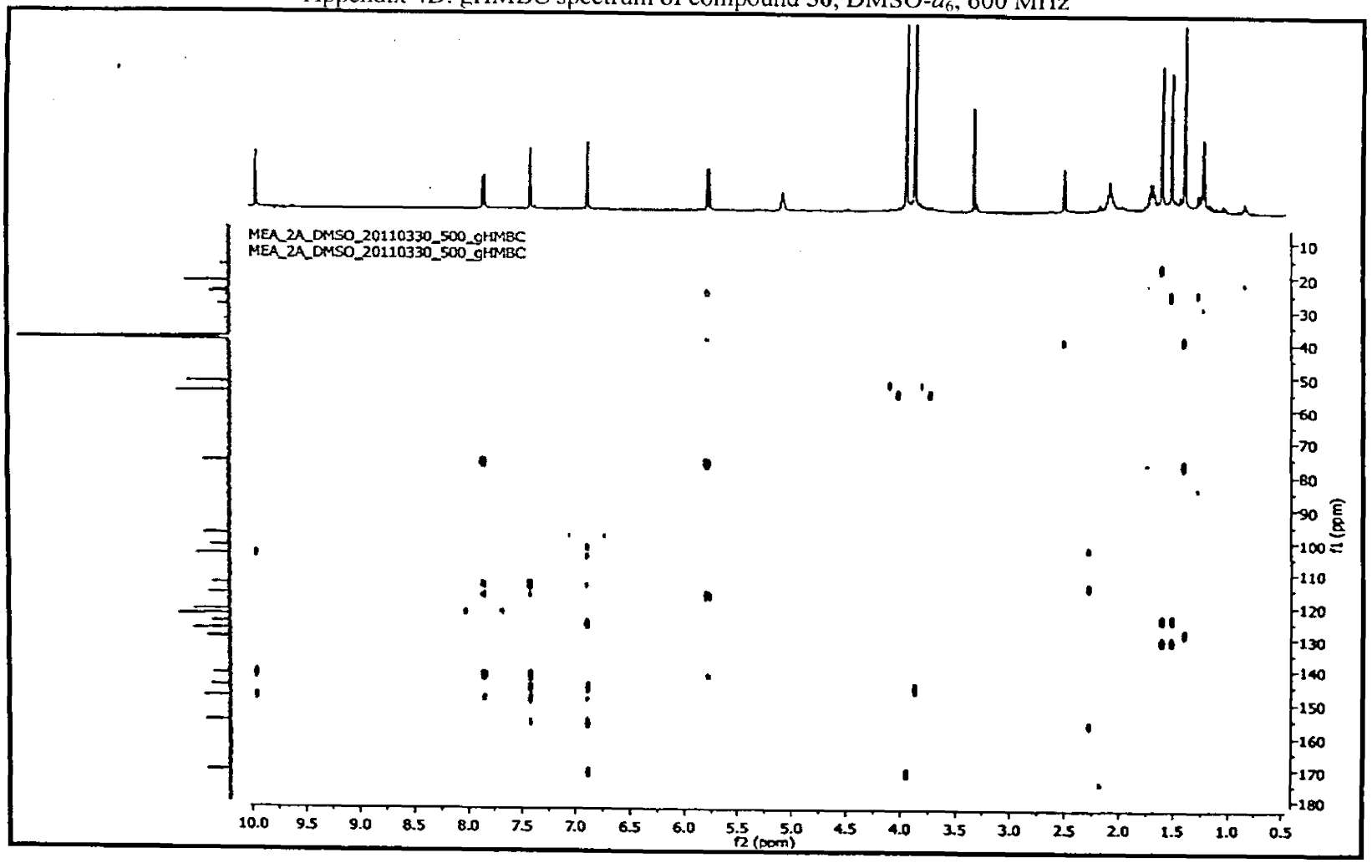
Appendix 4B: ^{13}C NMR spectrum of compound **56**, DMSO-d_6, 200 MHz



Appendix 4C: gHSQC spectrum of compound **56**, DMSO-d_6, 600 MHz



Appendix 4D: gHMBC spectrum of compound **56**, DMSO-d_6, 600 MHz



Appendix 4E: HRMS spectrum of compound **56**

Elemental Composition

File Edit View Process Help

Single Mass Analysis

Tolerance = 75.0 mDa / DBE: min = -5.0, max = 100.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions

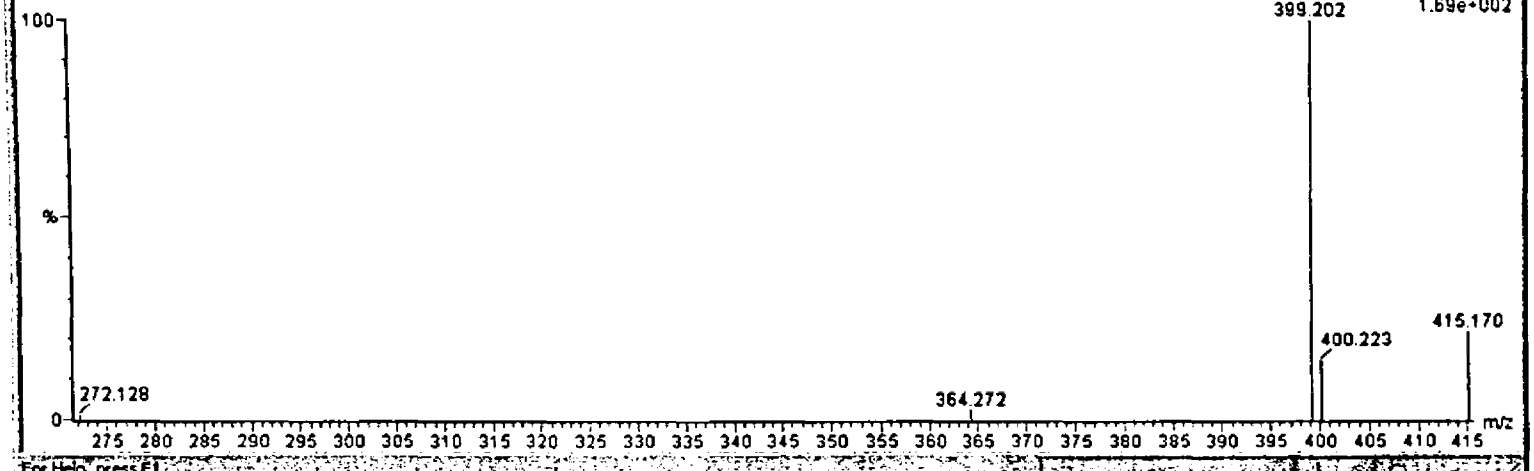
29 formula(e) evaluated with 11 results within limits (all results (up to 1000) for each mass)

Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	C	H	O
399.2023	399.2019	0.4	1.0	5.5	C20 H31 O8	3.0	20	31	8
	399.2171	-14.8	-37.1	9.5	C24 H31 O5	6.6	24	31	5
	399.2230	-20.7	-51.9	0.5	C17 H35 O10	2.4	17	35	10
	399.1808	21.5	53.9	10.5	C23 H27 O6	7.1	23	27	6
	399.2383	-36.0	-90.2	4.5	C21 H35 O7	7.5	21	35	7
	399.1655	36.8	92.2	6.5	C19 H27 O9	7.4	19	27	9
	399.2535	-51.2	-128.3	8.5	C25 H35 O4	15.1	25	35	4
	399.2594	-57.1	-143.0	-0.5	C18 H39 O9	11.9	18	39	9
	399.1444	57.9	145.0	11.5	C22 H23 O7	16.6	22	23	7

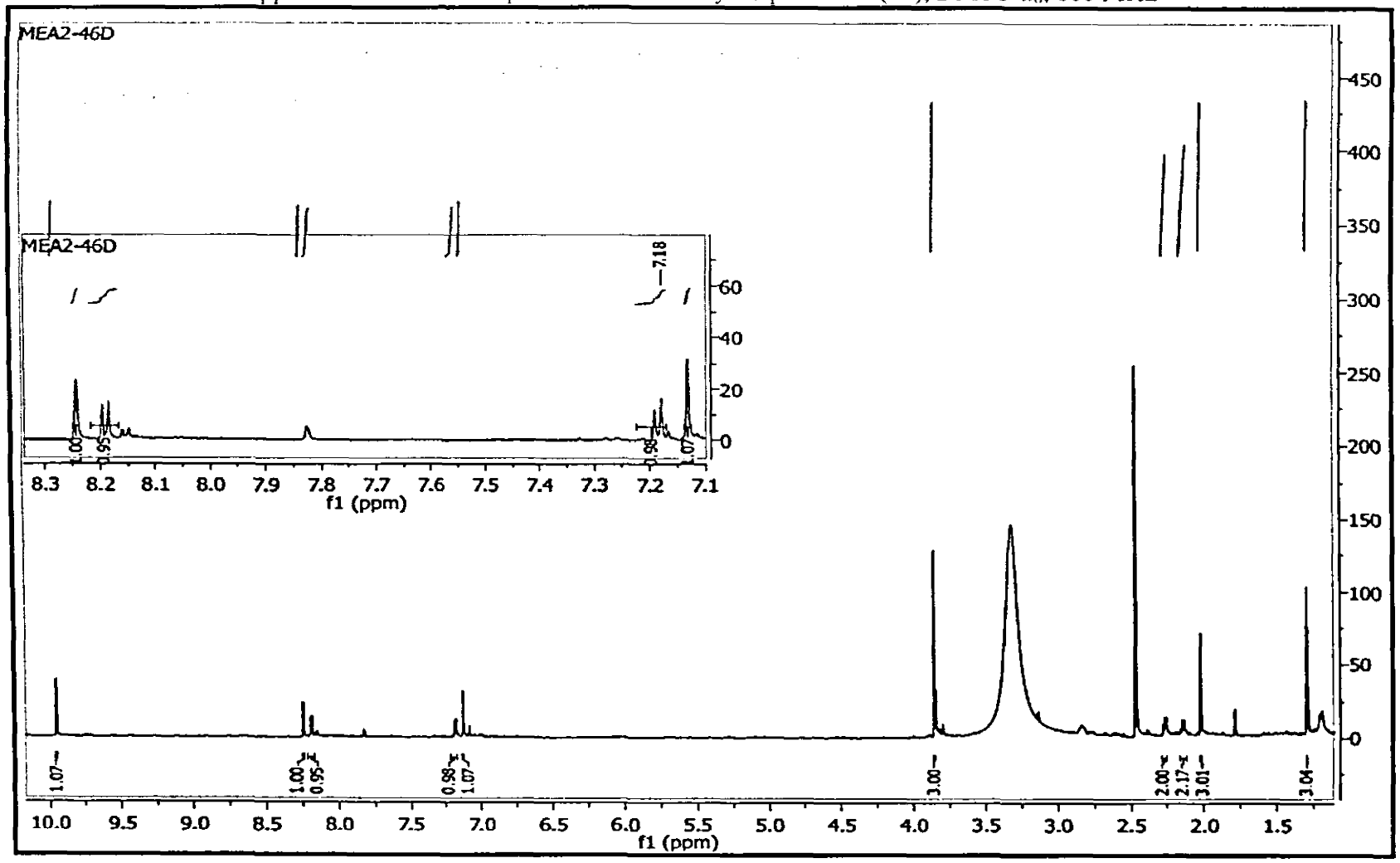
MEA-2A 1:2

Mate_20111023_02 303 (3.635) AM (Top,5, Ar,4500.0,0.00,0.70) 1: TOF MS ES+ 1.69e+002

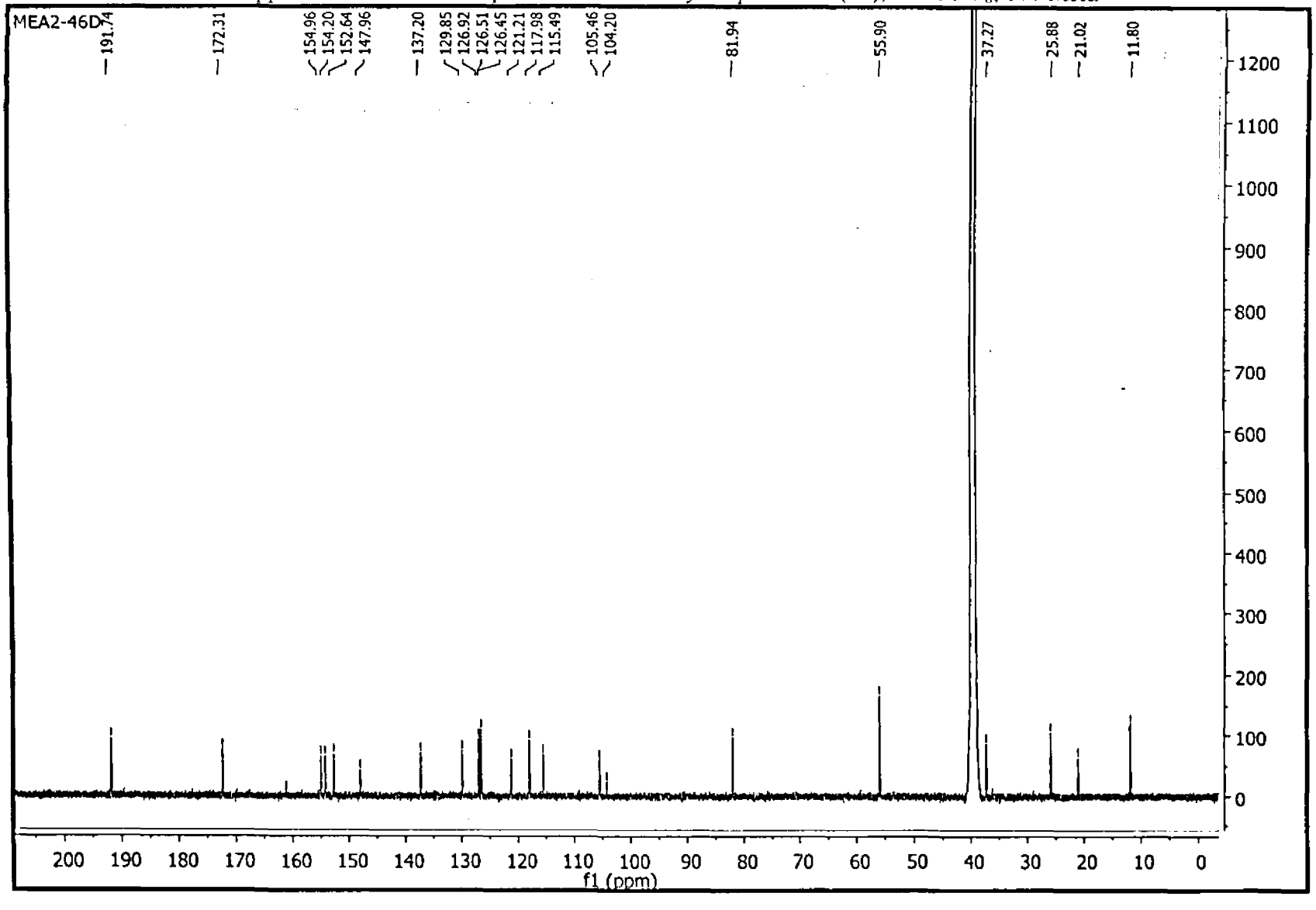


For Help, press F1

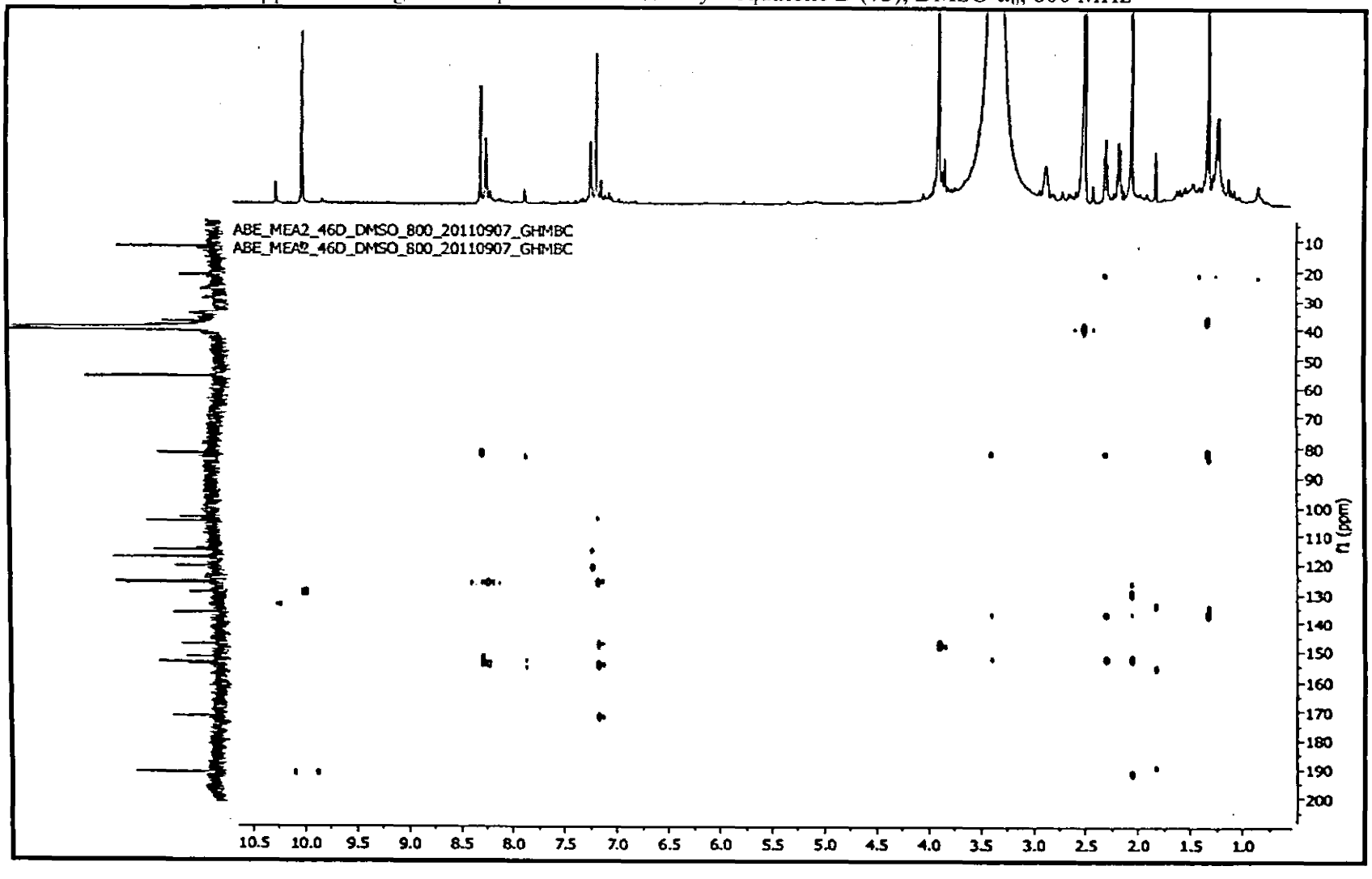
Appendix 5A: ^{1}H NMR spectrum of bussidihydroquinone D (**73**), DMSO-d_6, 800 MHz



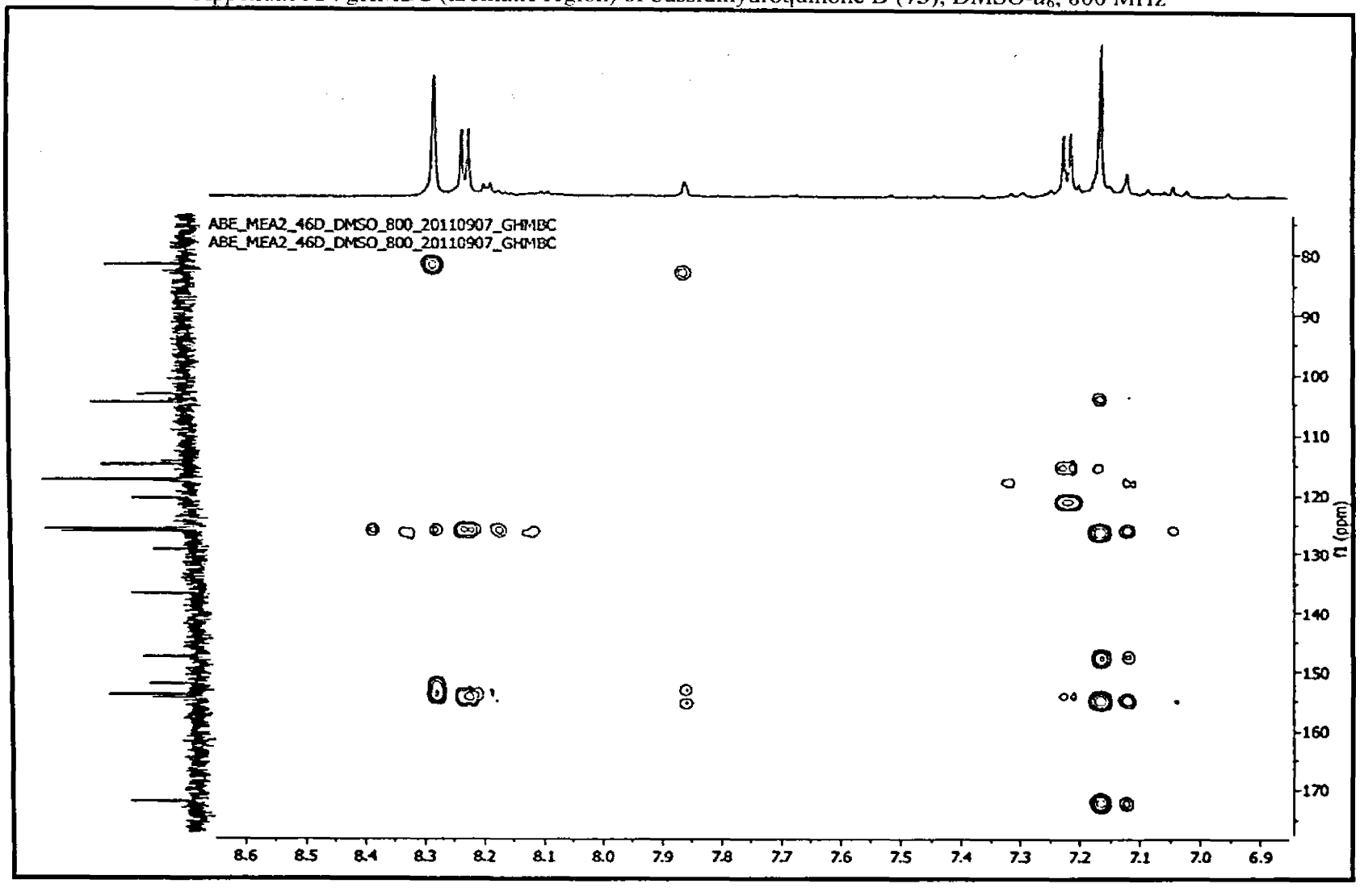
Appendix 5B: ^{13}C NMR spectrum of bussidihydroquinone D (**73**), DMSO-d_6, 800 MHz



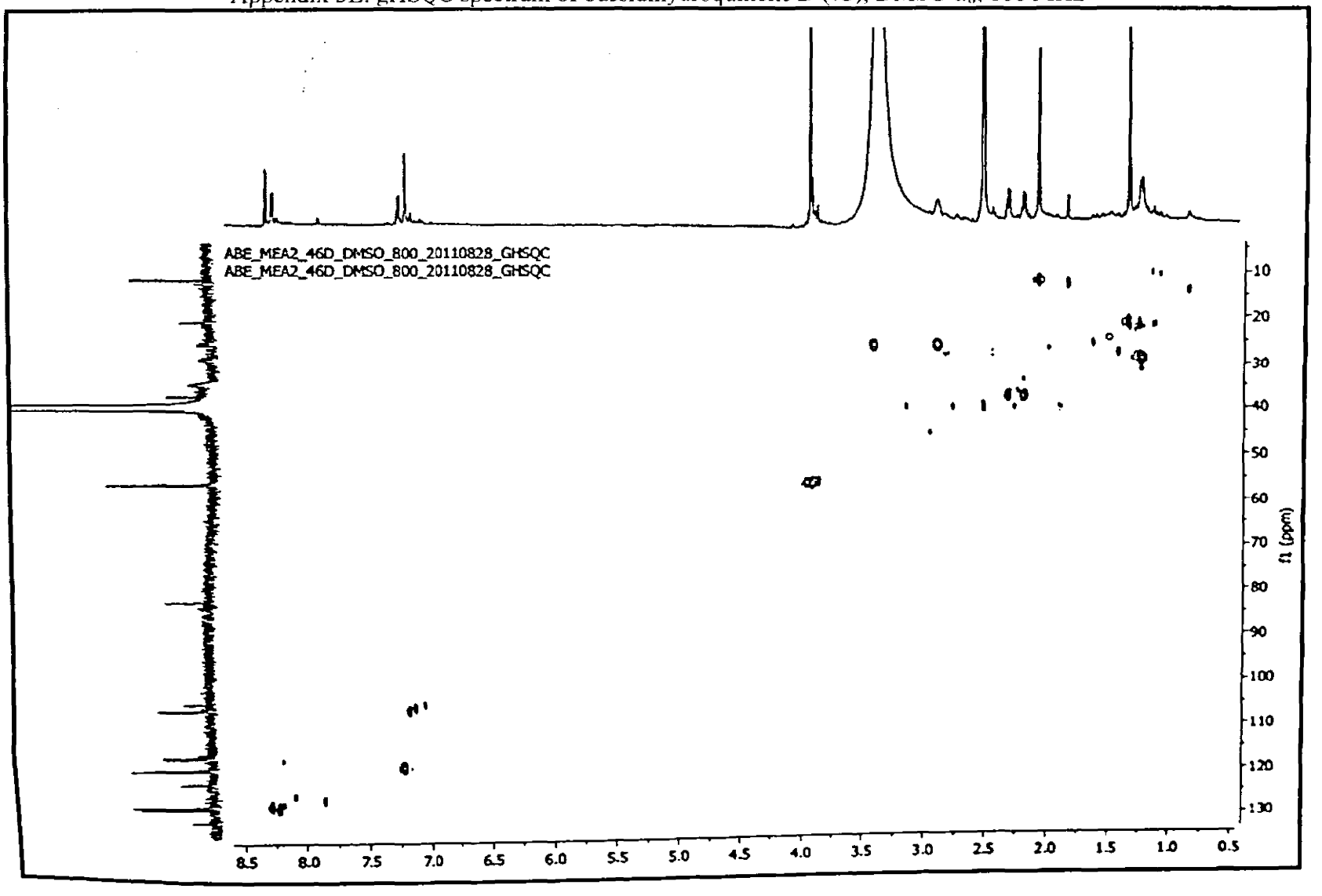
Appendix 5C: gHMBC spectrum of bussidihydroquinone D (**73**), DMSO-d_6, 800 MHz



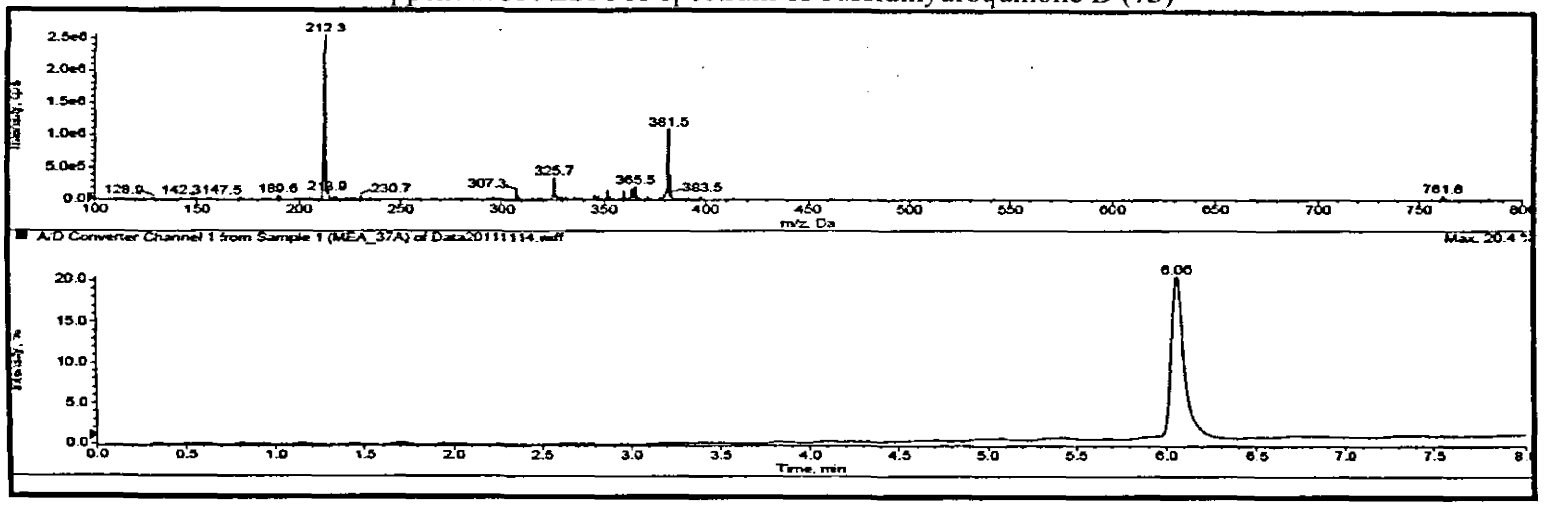
Appendix 5D: gHMBC (aromatic region) of bussidihydroquinone D (73), DMSO-d_6, 800 MHz



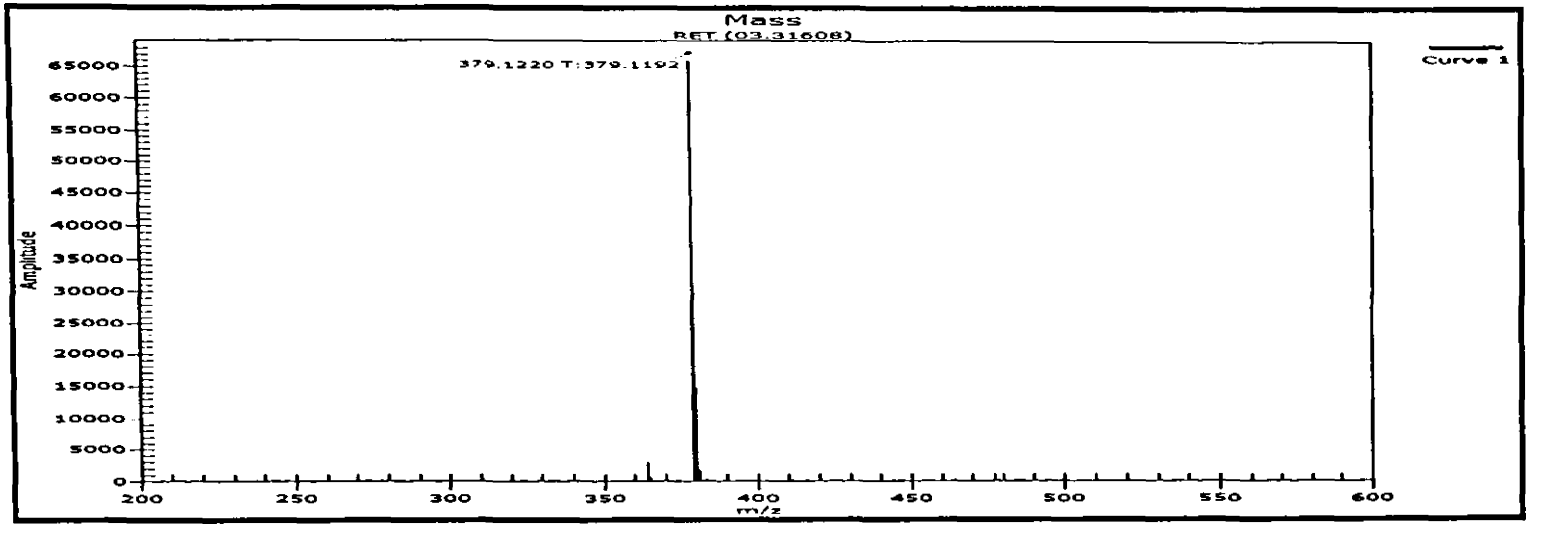
Appendix 5E: gHSQC spectrum of bussidihydroquinone D (73), DMSO-d_6, 800 MHz



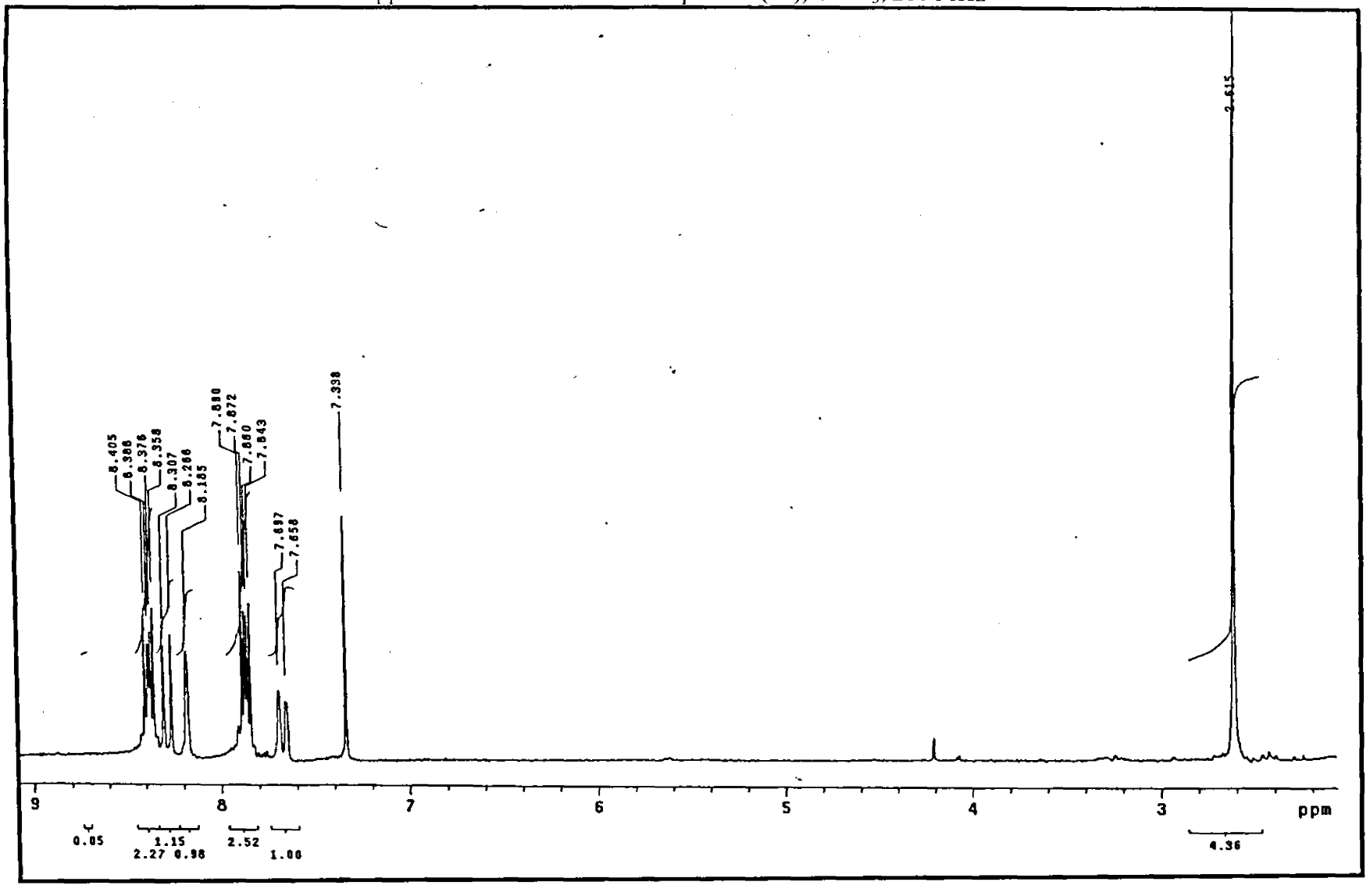
Appendix 5F. ESI-MS spectrum of bussidihydroquinone D (73)



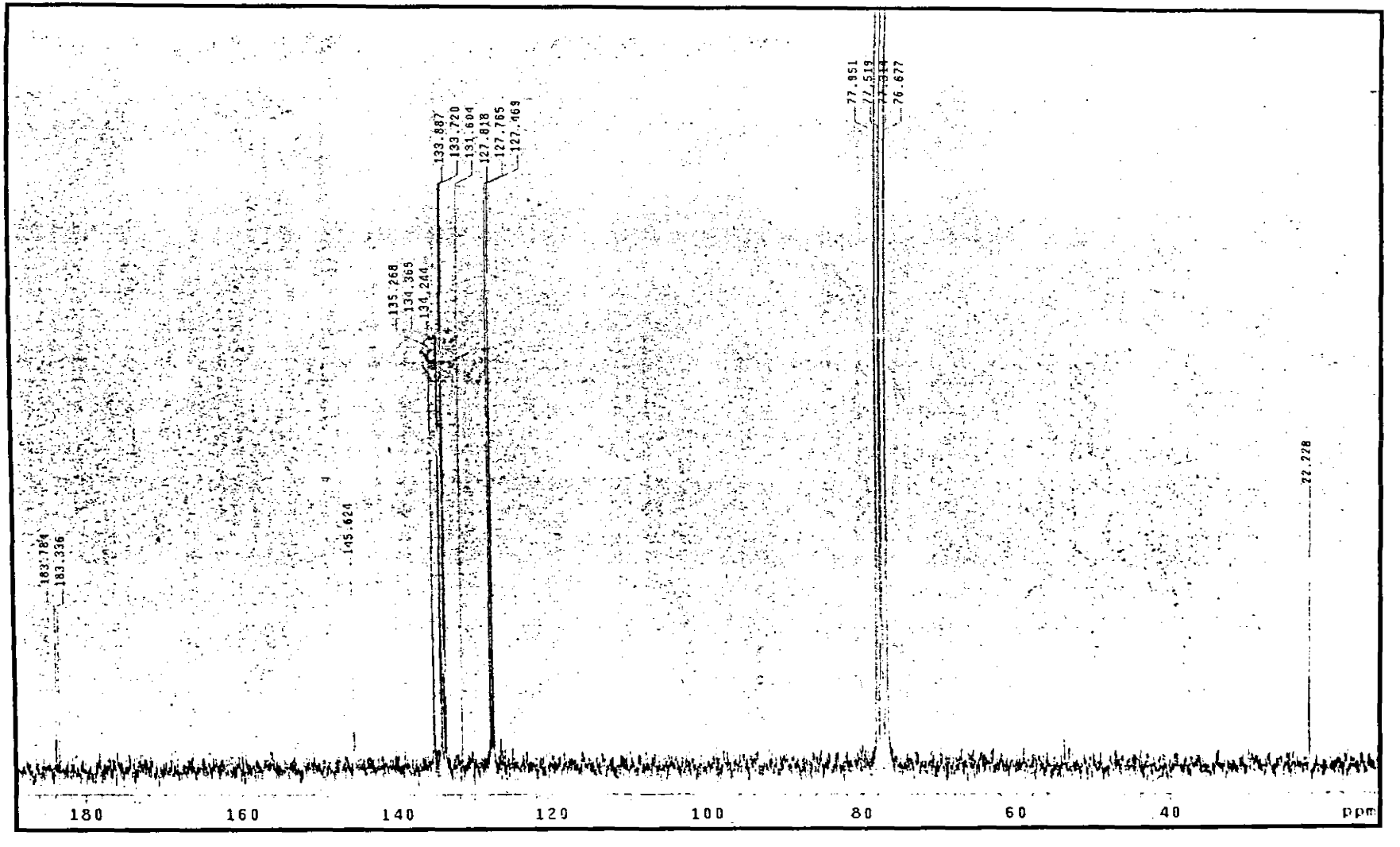
Appendix 5G. HRMS spectrum of bussidihydroquinone D (73)



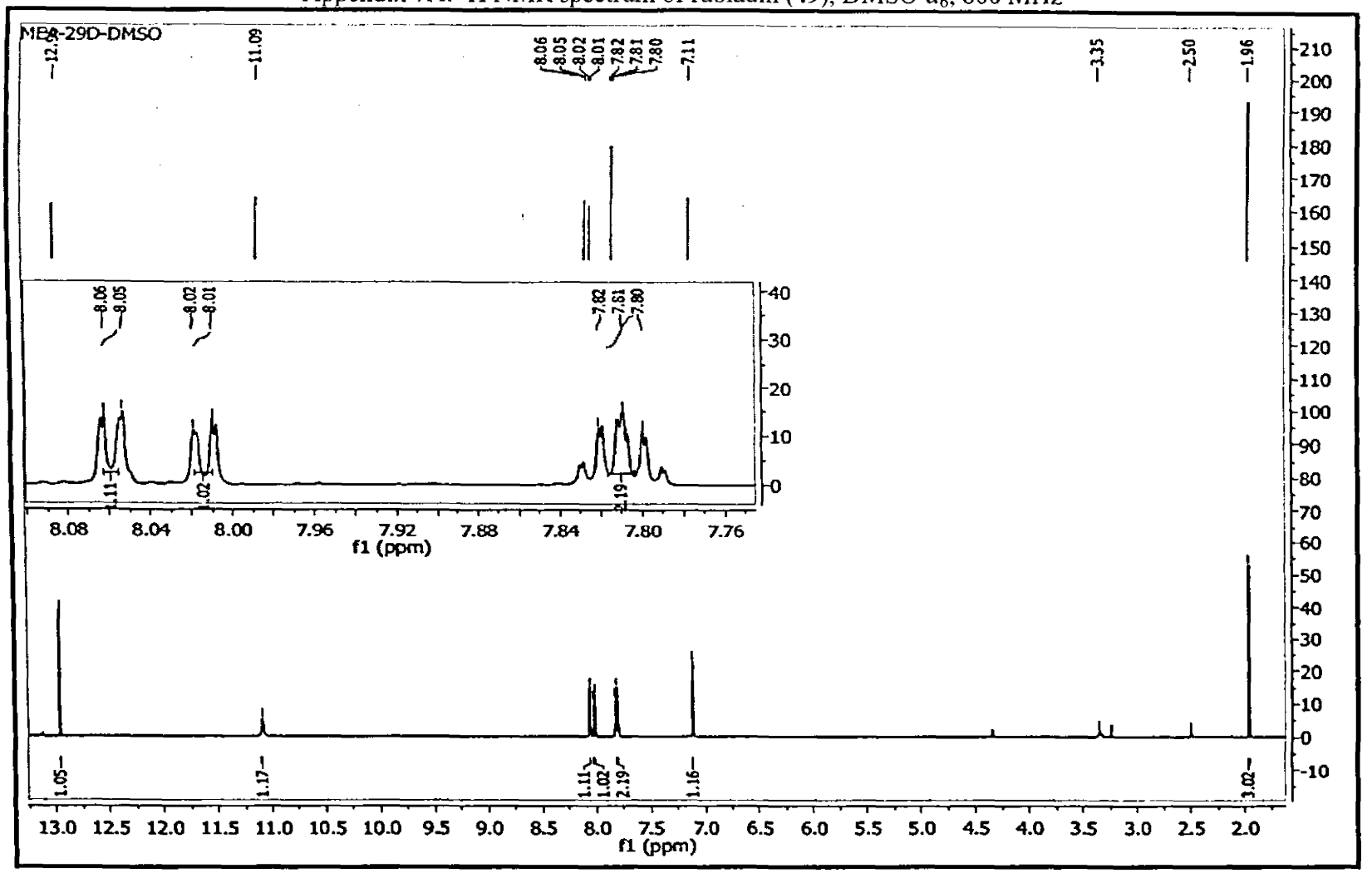
Appendix 6A: ^{1}H NMR of tectoquinone (74), $CDCl_3$, 200 MHz



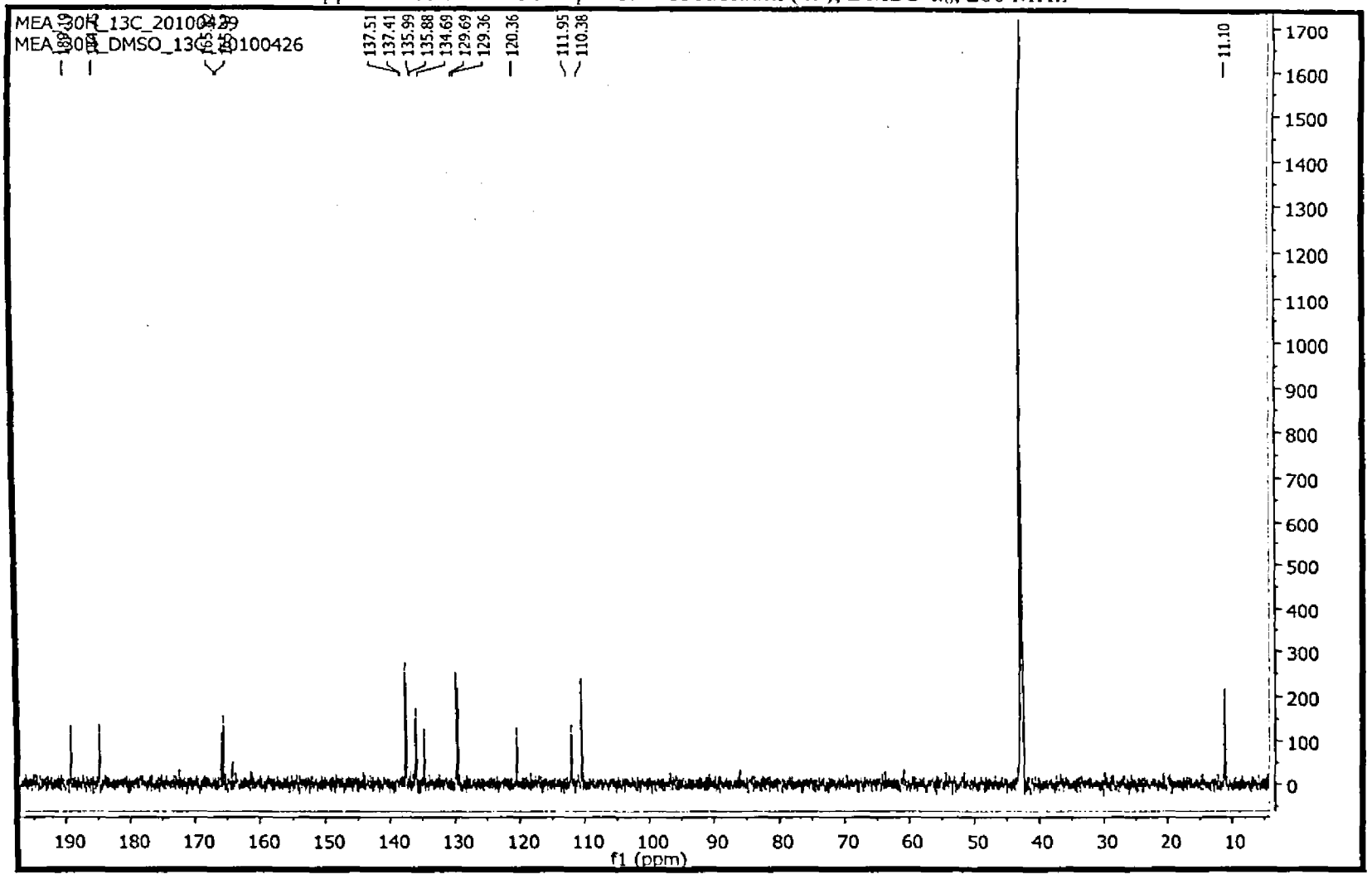
Appendix 6B: ^{13}C NMR of tectoquinone (74), $CDCl_3$, 50 MHz



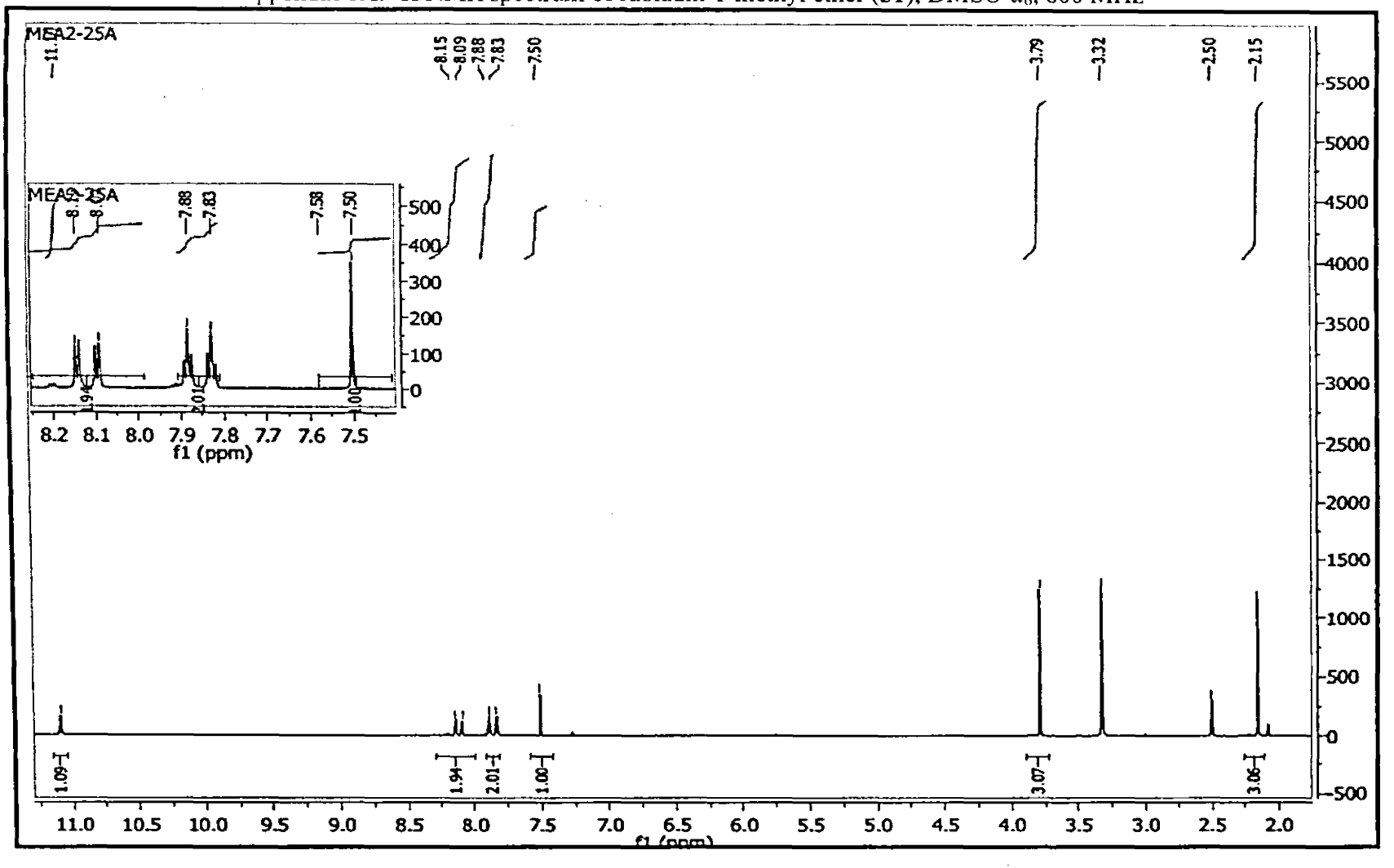
Appendix 7A: ^{1}H NMR spectrum of rubiadin (49), DMSO-d_6, 800 MHz



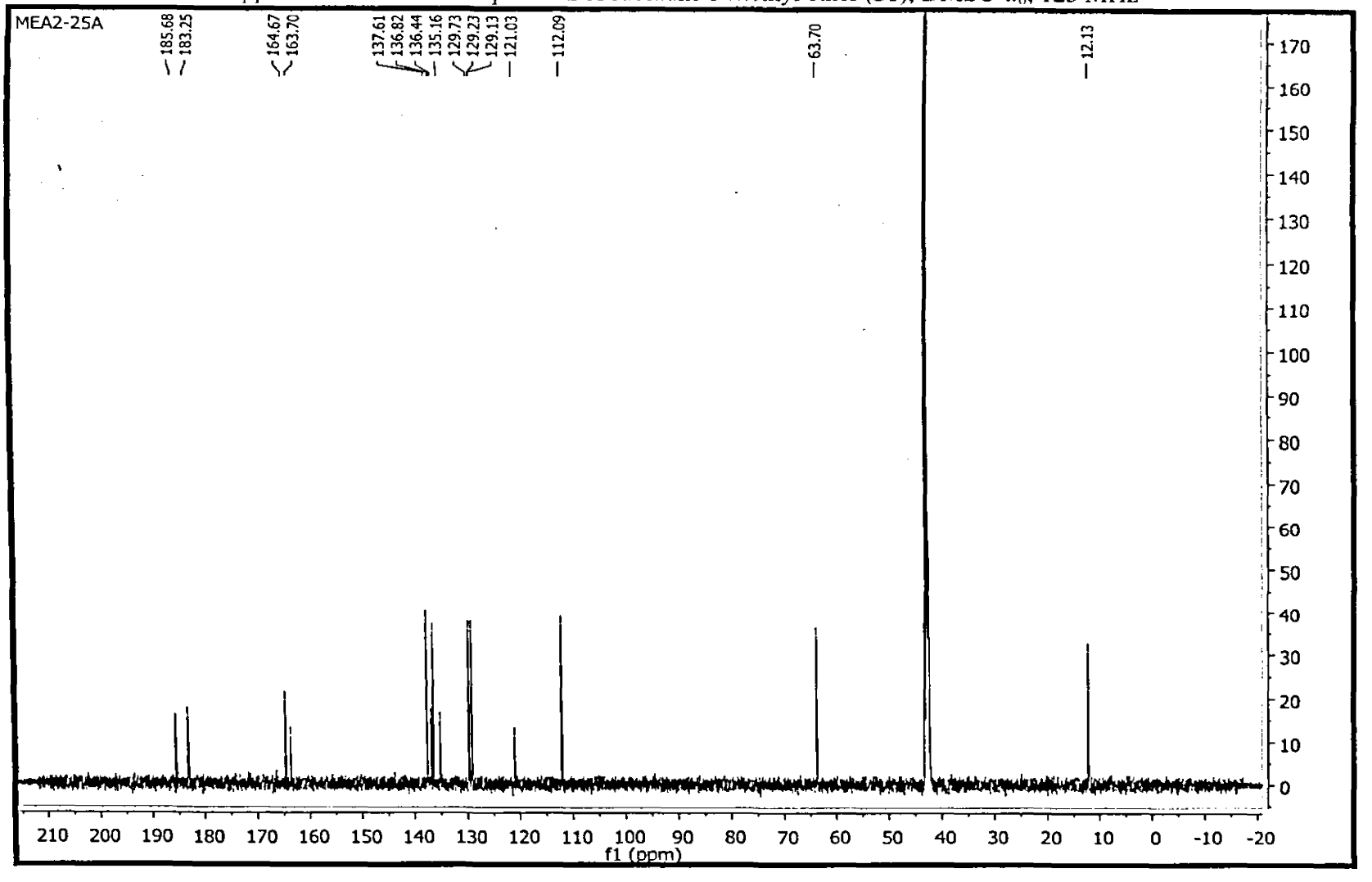
Appendix 7B: ^{13}C NMR spectrum of rubiadin (**49**), DMSO-d_6, 200 MHz



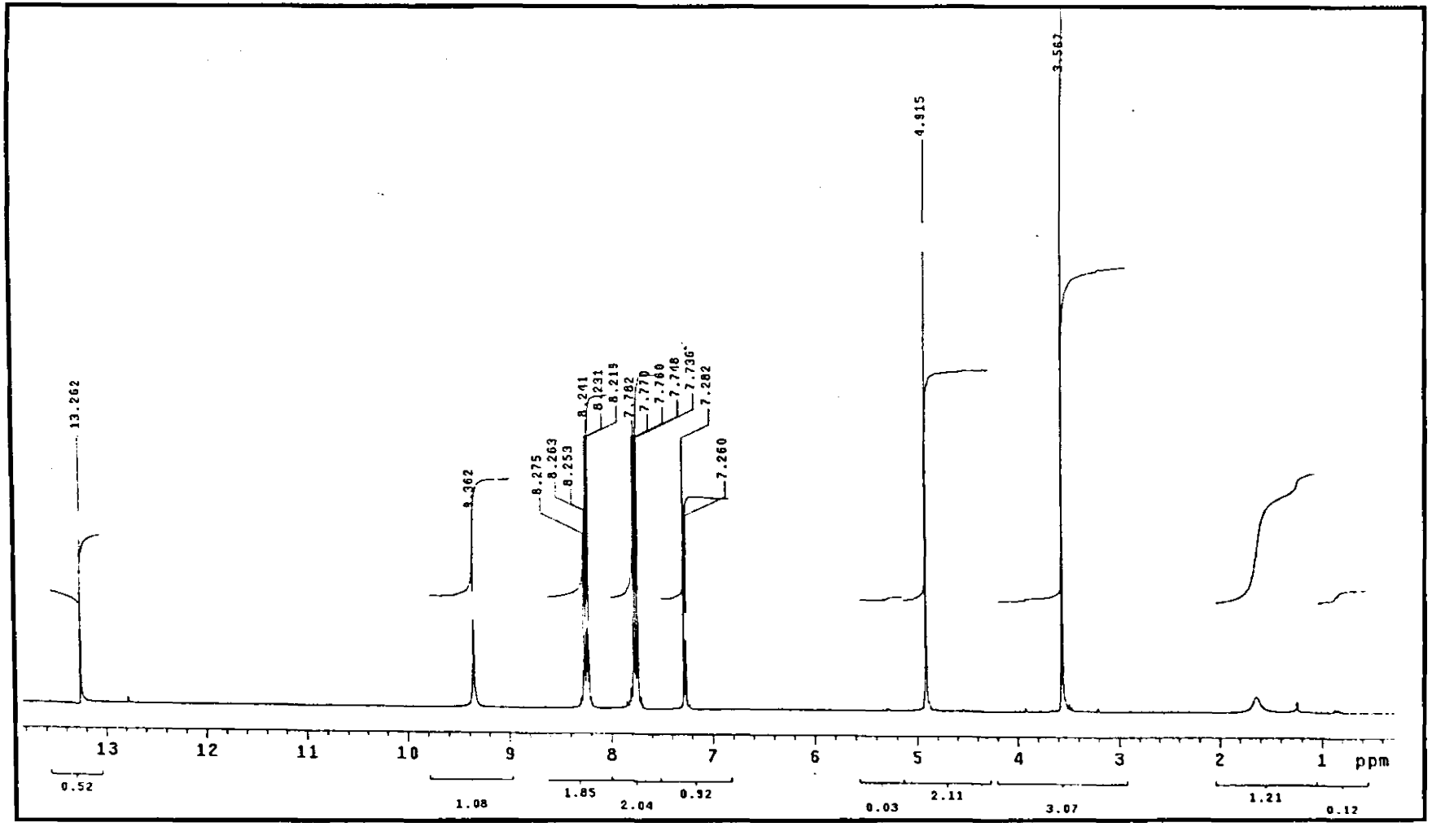
Appendix 8A: ^{1}H NMR spectrum of rubiadin-1-methyl ether (**51**), DMSO-d_6, 800 MHz



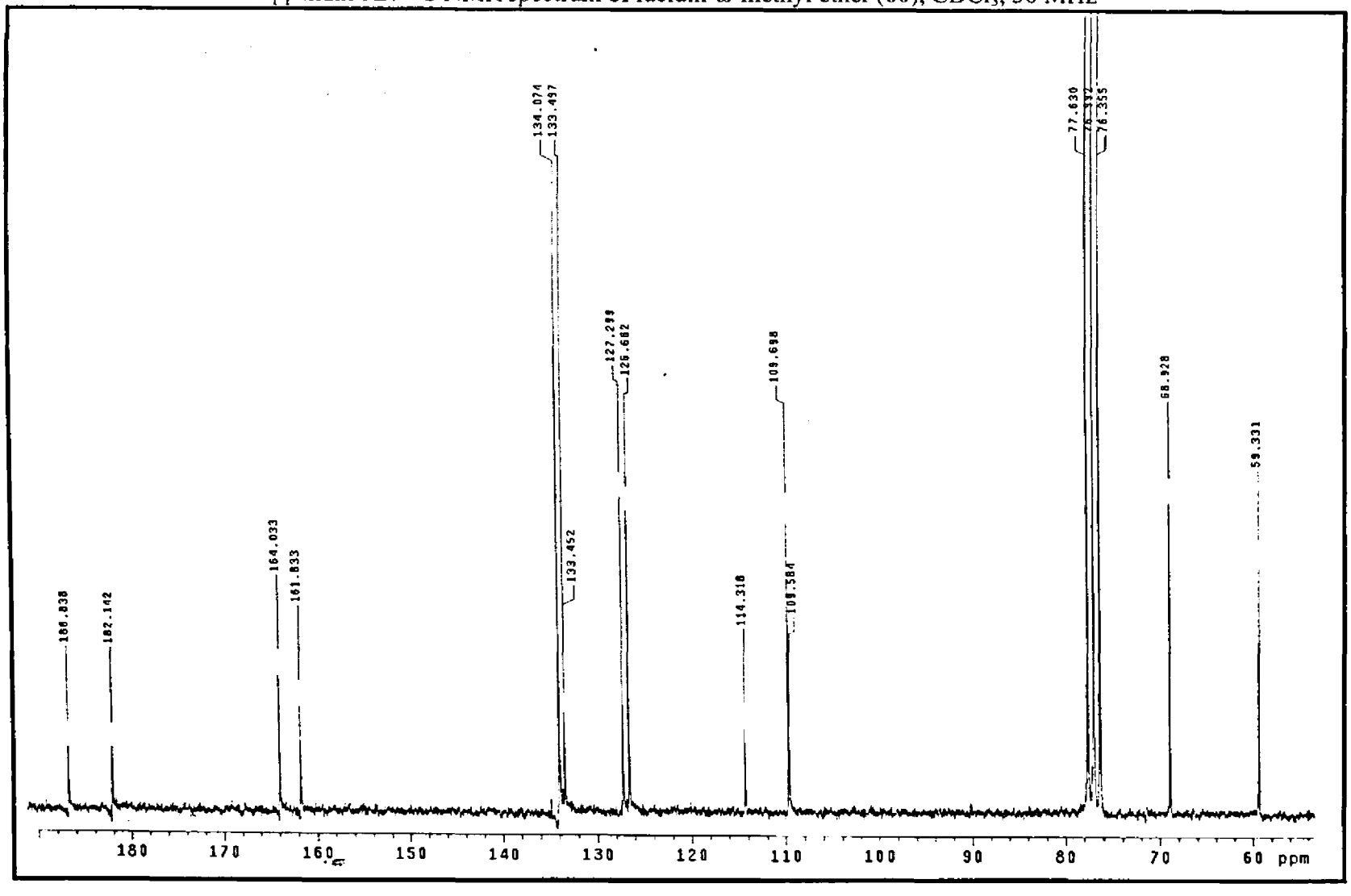
Appendix 8B: ^{13}C NMR spectrum of rubiadin-1-methyl ether (**51**), DMSO-d_6, 125 MHz



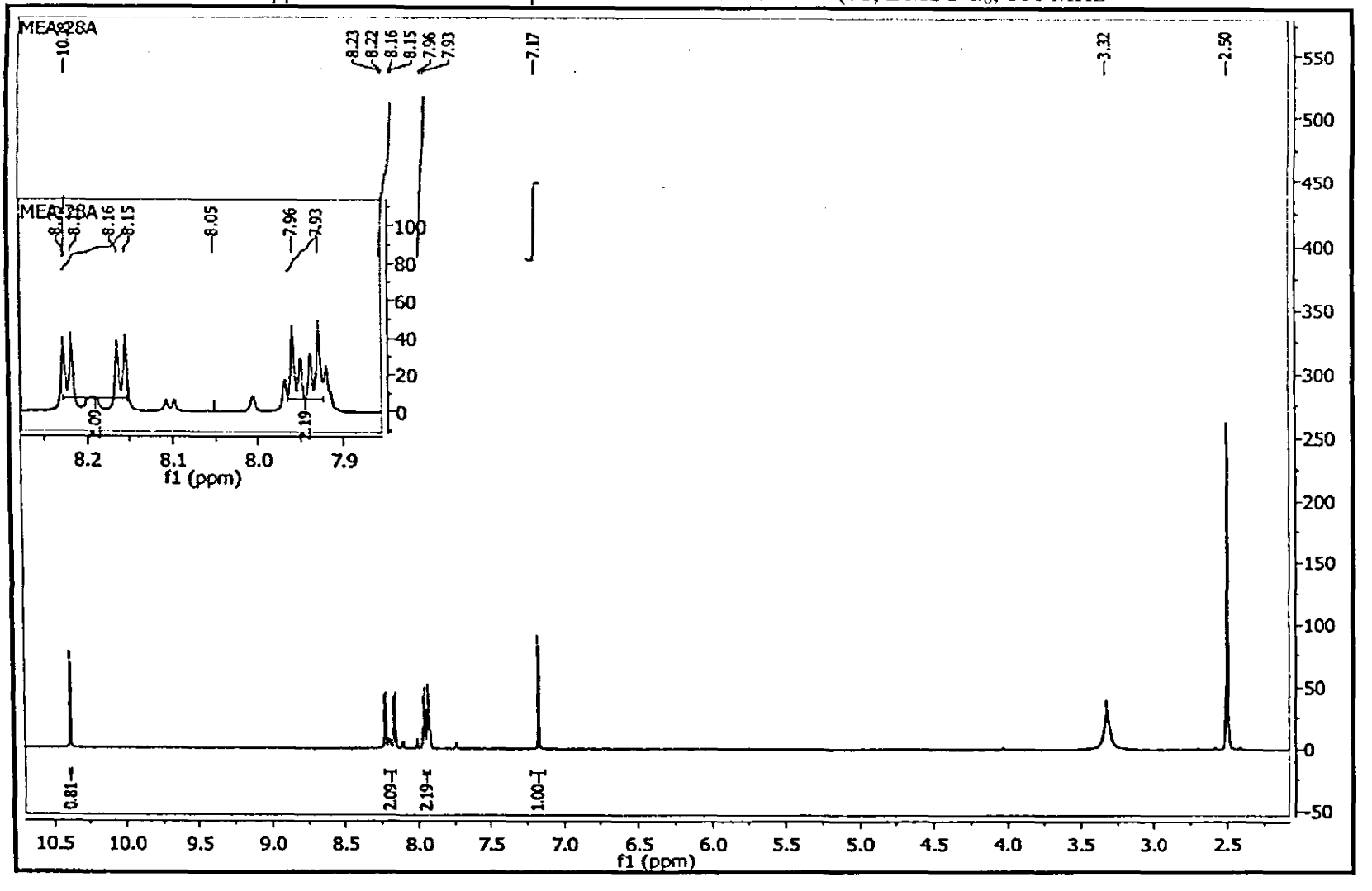
Appendix 9A: ^{1}H NMR spectrum of lucidin-ω-methyl ether (60), DMSO-d_6, 800 MHz



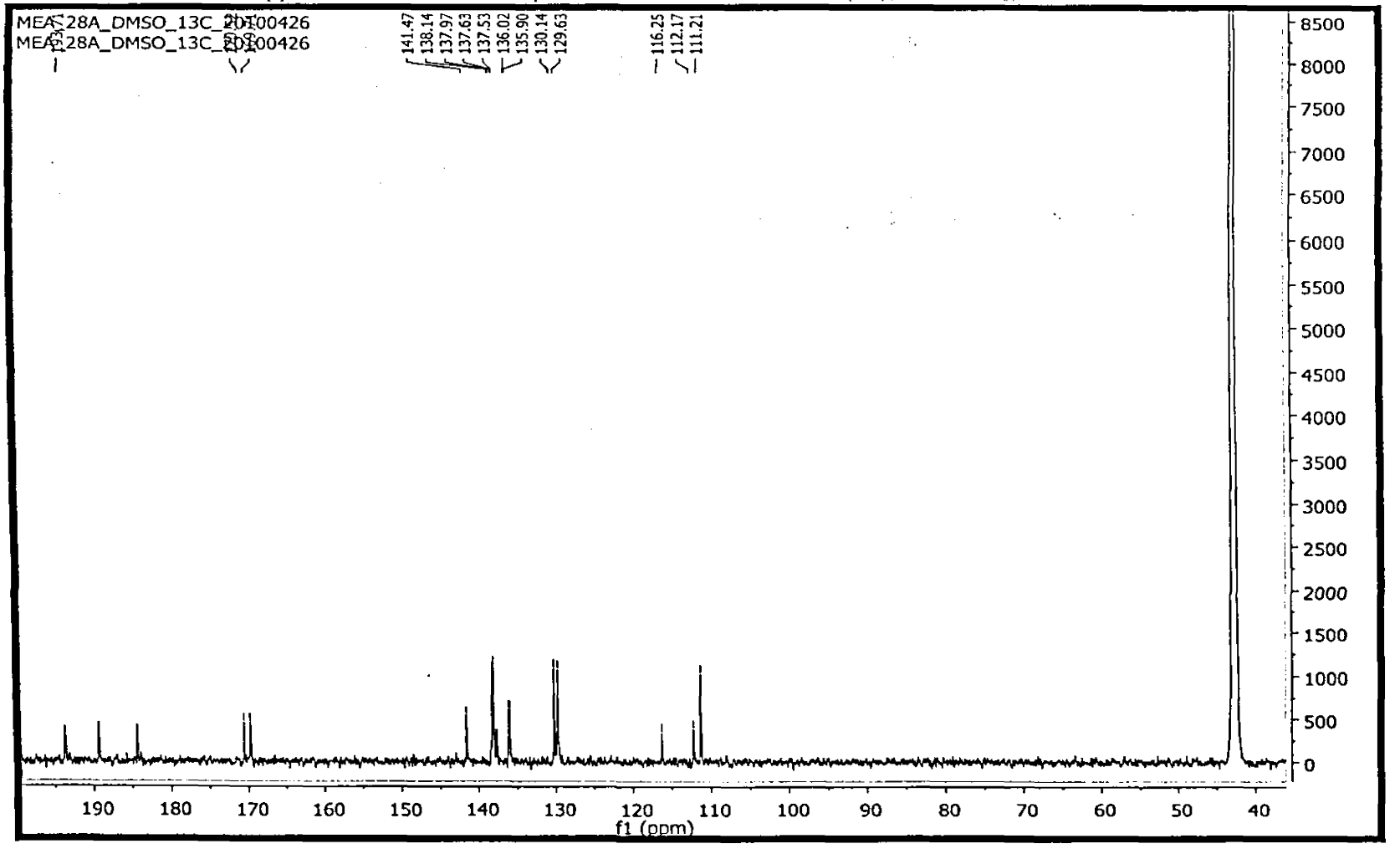
Appendix 9B: ^{13}C NMR spectrum of lucidin-ω-methyl ether (**60**), $CDCl_3$, 50 MHz



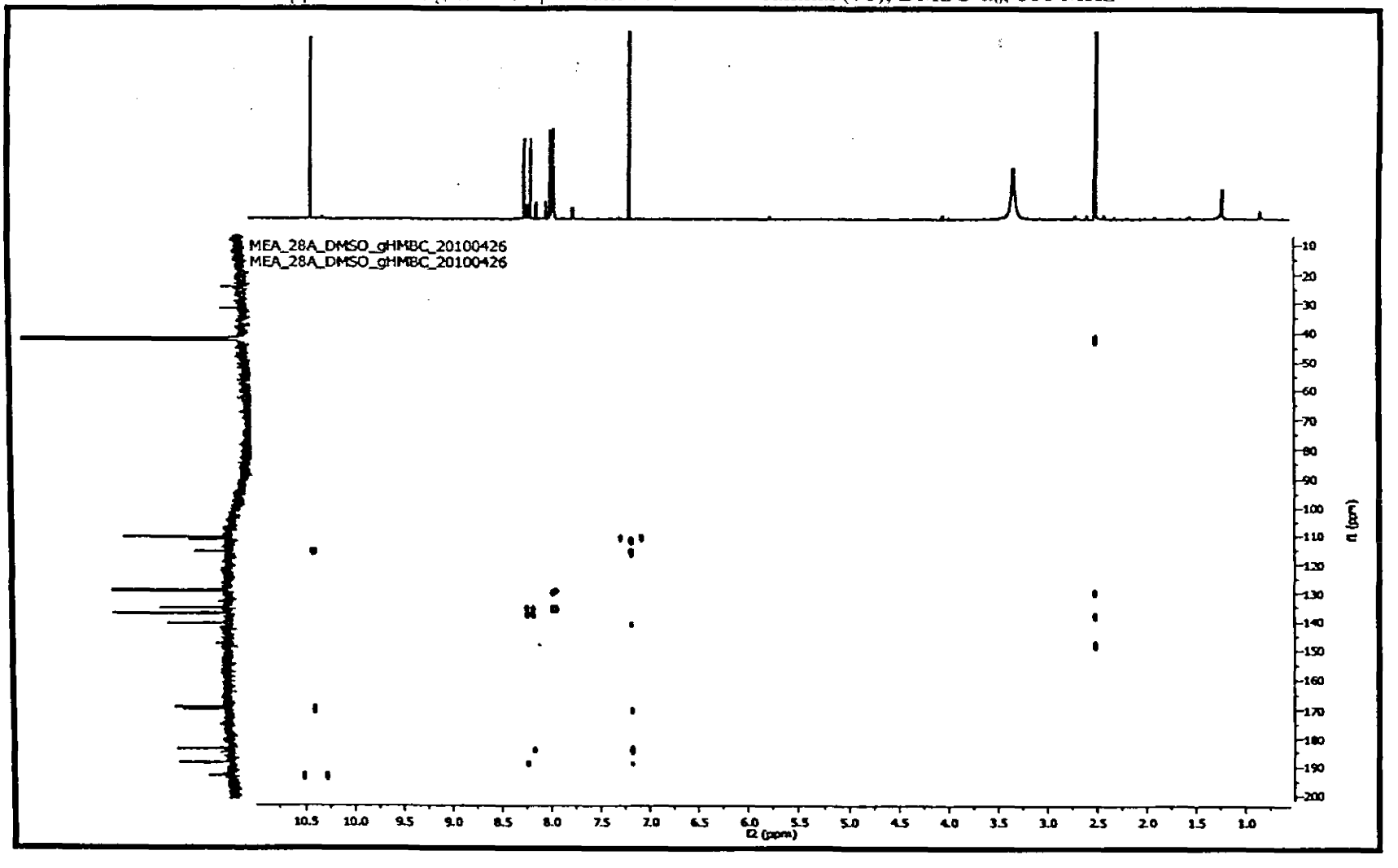
Appendix 10A: ^{1}H NMR spectrum of nordamnacanthal (**76**, DMSO-d_6, 800 MHz



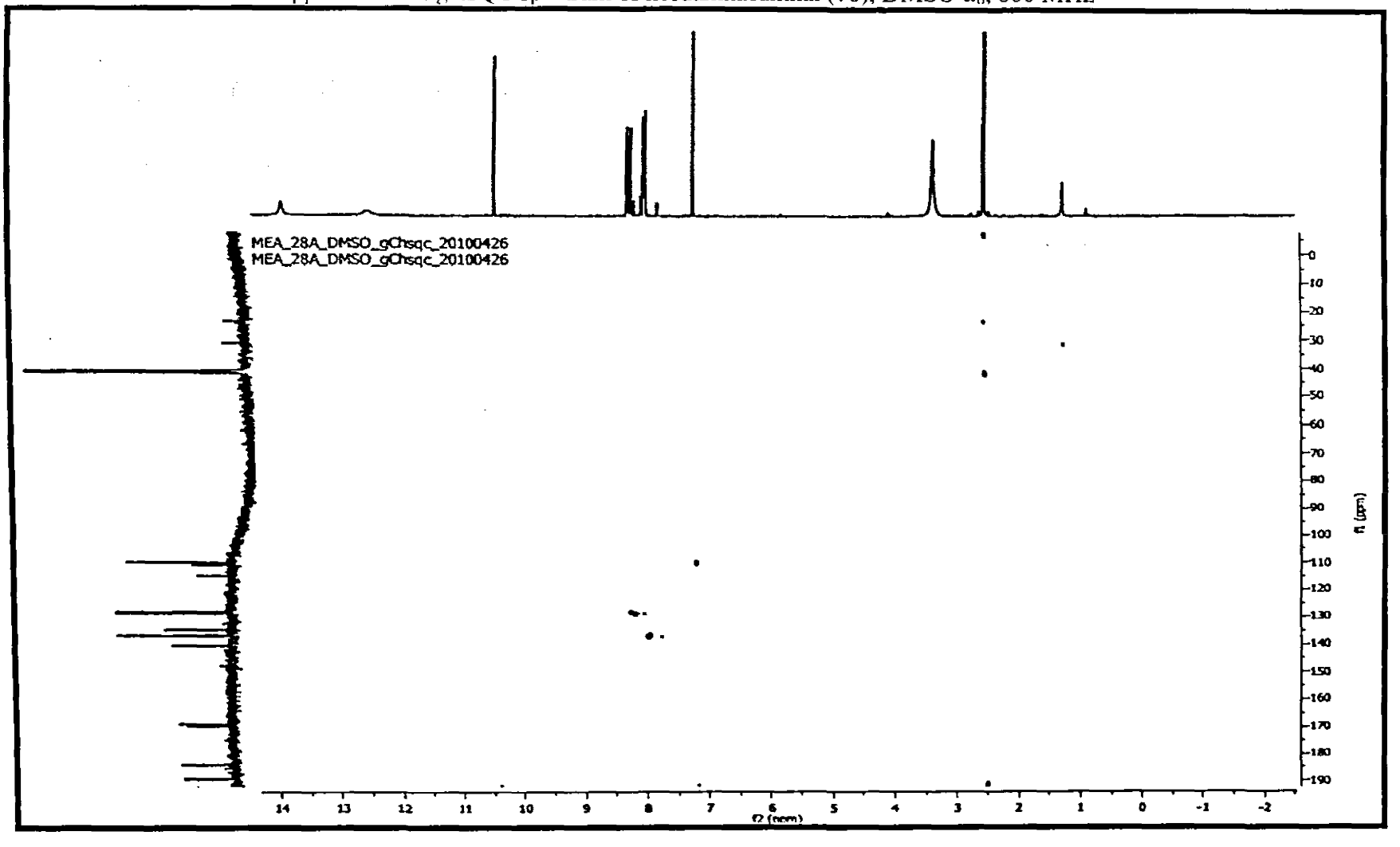
Appendix 10B: ^{13}C NMR spectrum of nordamnacanthal (76), DMSO-d_6, 150 MHz



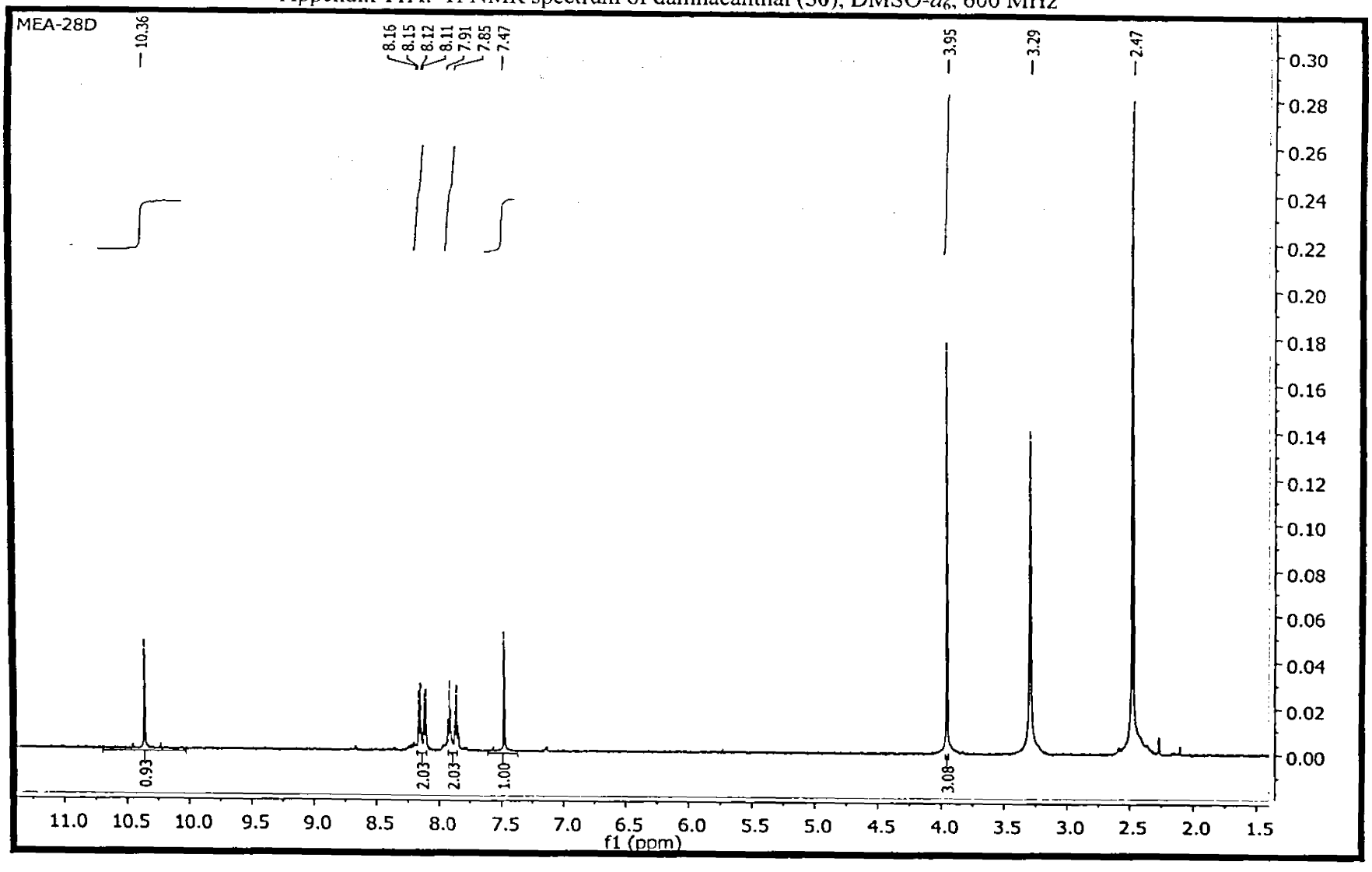
Appendix 10C: gHMBC spectrum of nordamnacanthal (**76**), DMSO-d_6, 800 MHz



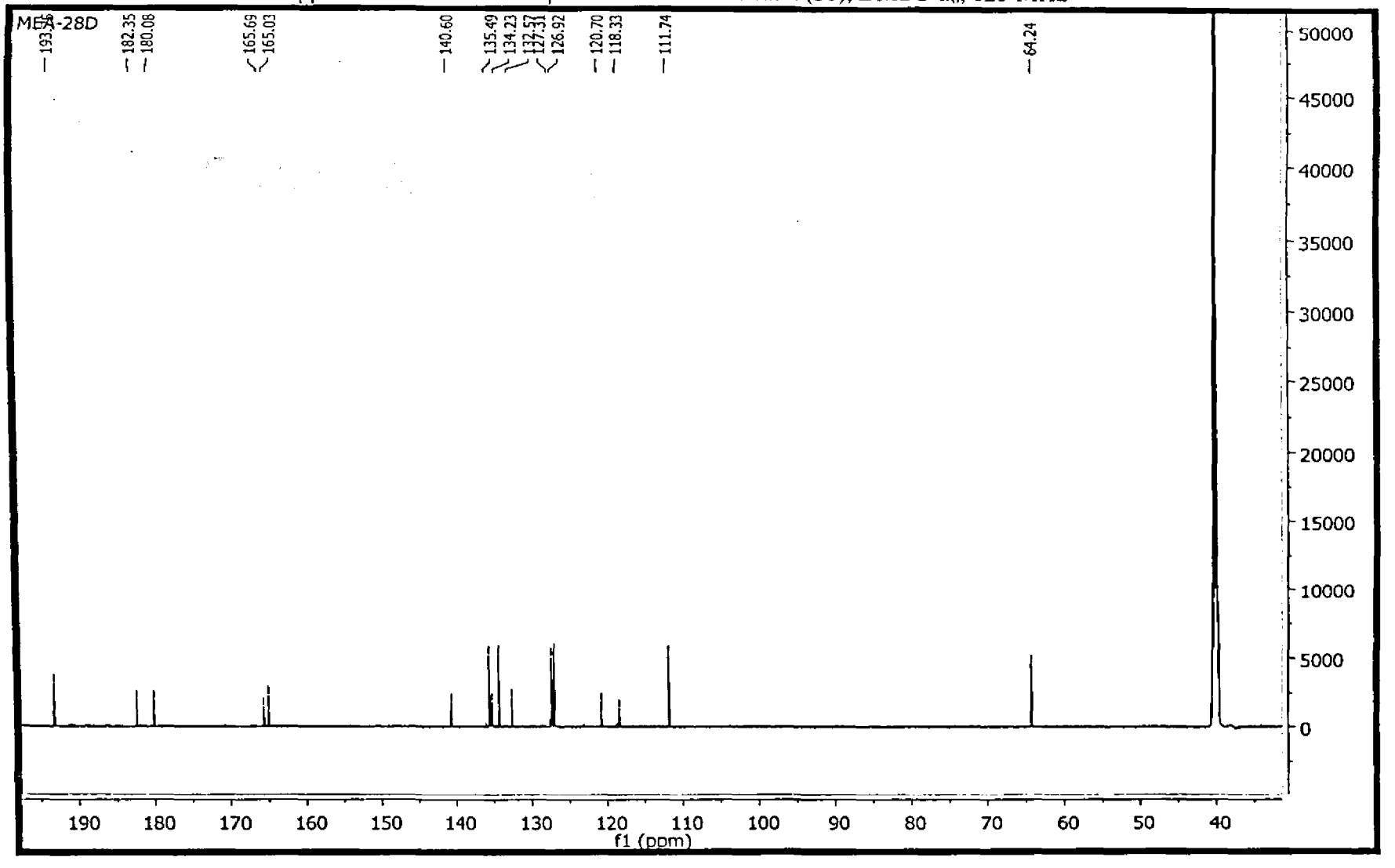
Appendix 10D: gHSQC spectrum of nordamnacanthal (**76**), DMSO-d_6, 800 MHz



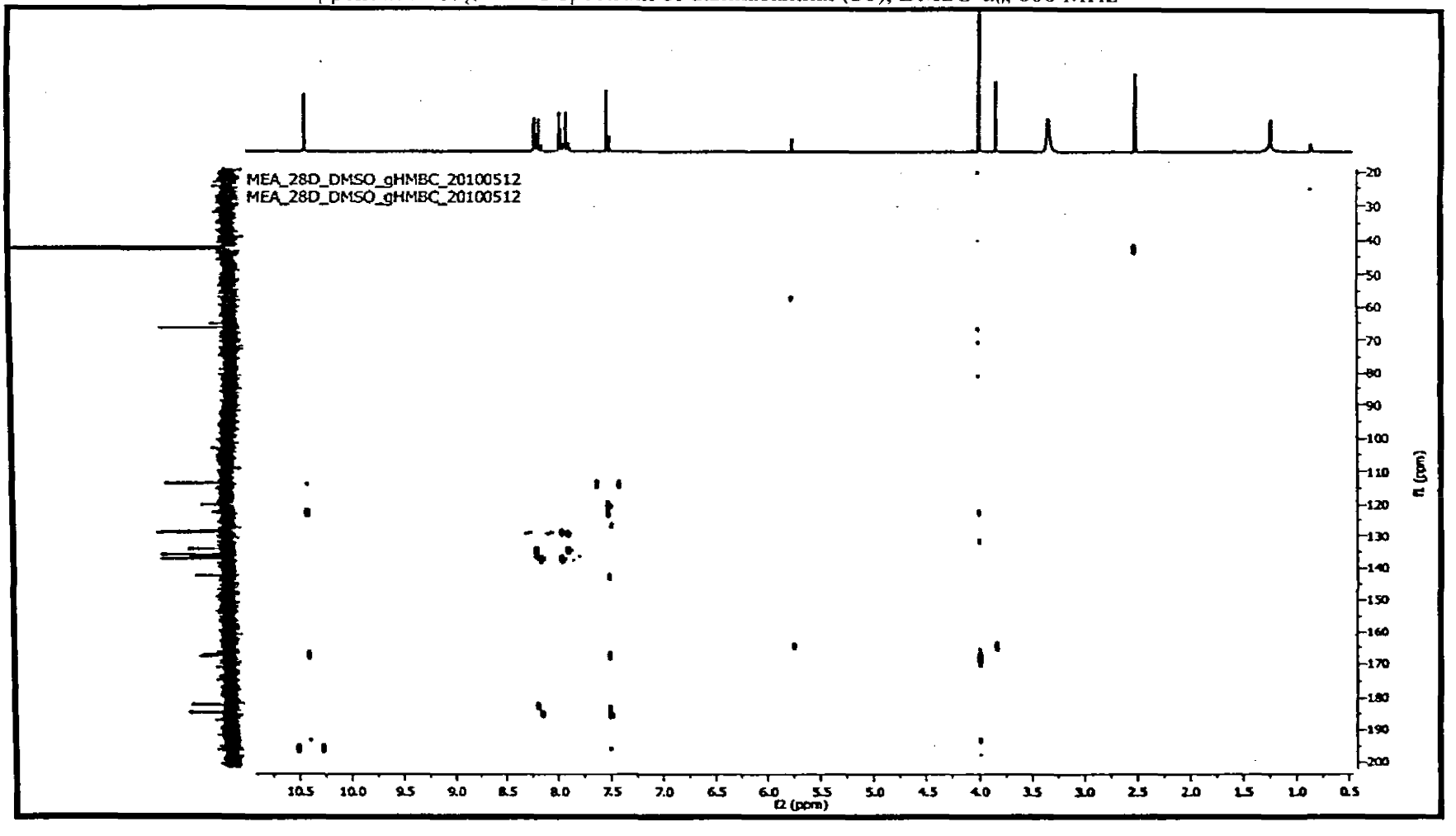
Appendix 11A: ^{1}H NMR spectrum of damnacanthal (**50**), DMSO-d_6, 600 MHz



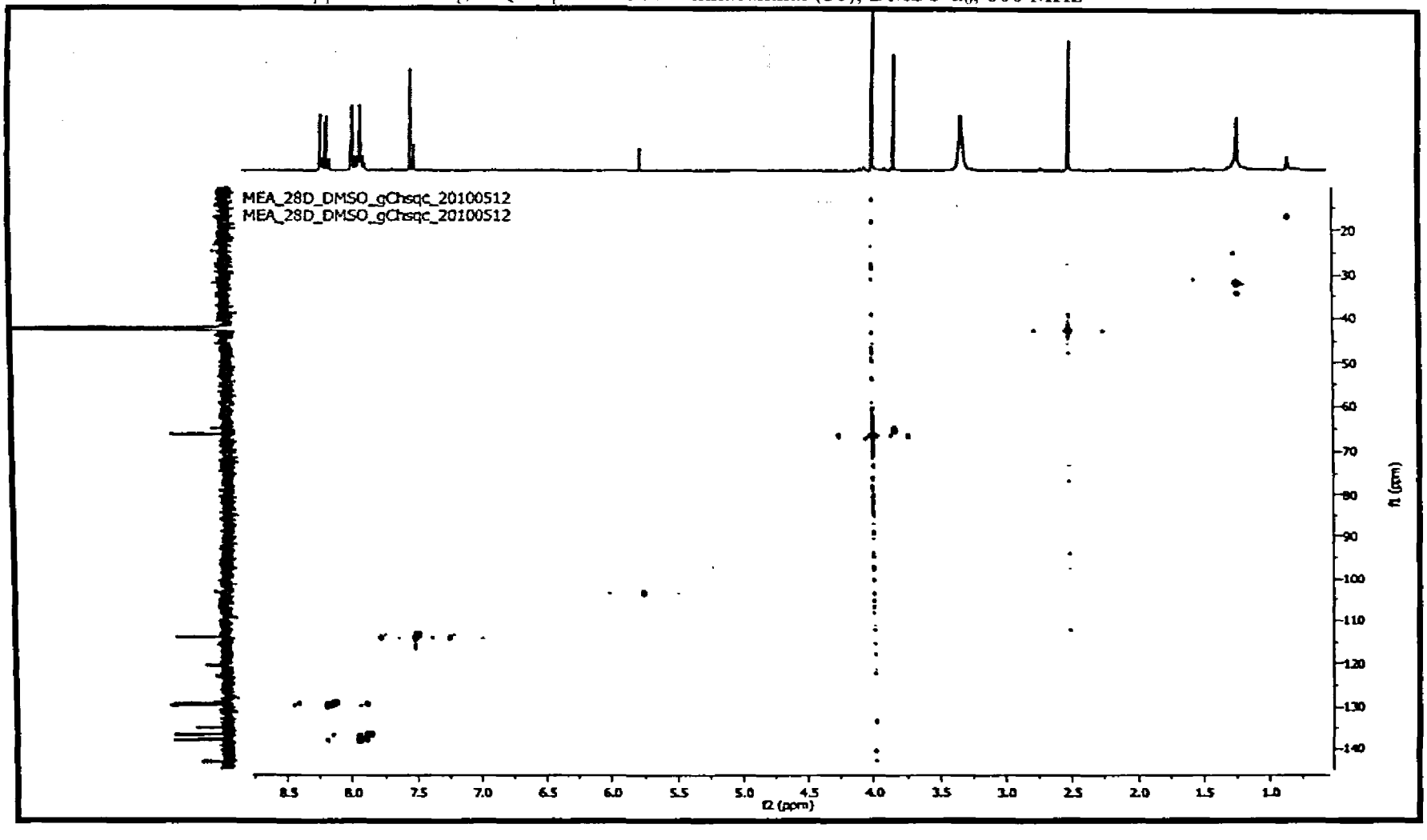
Appendix 11B: ^{13}C NMR spectrum of damnacanthal (**50**), DMSO-d_6, 125 MHz



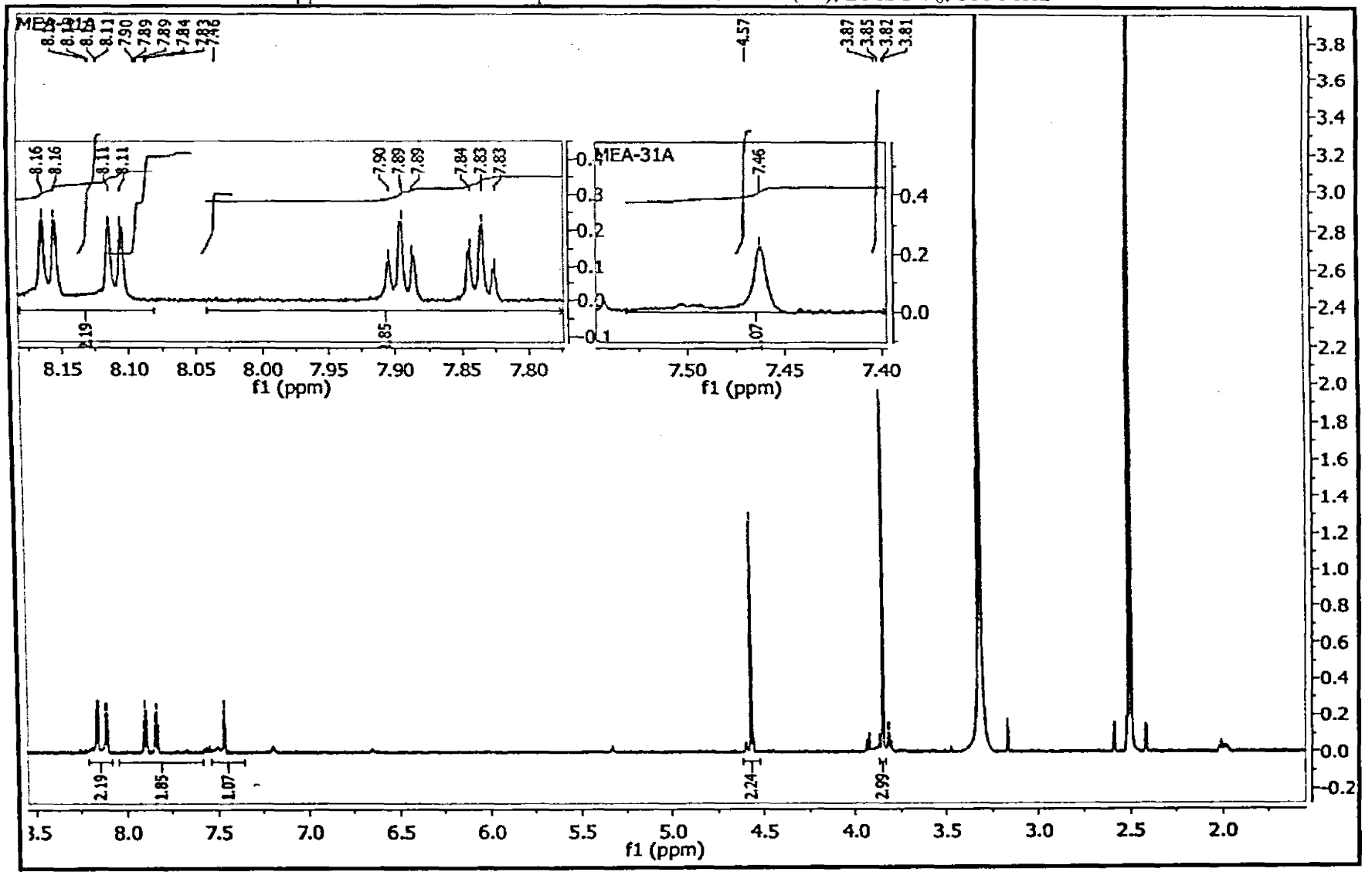
Appendix 11C: gHMBC spectrum of damnacanthal (**50**), DMSO-d_6, 600 MHz



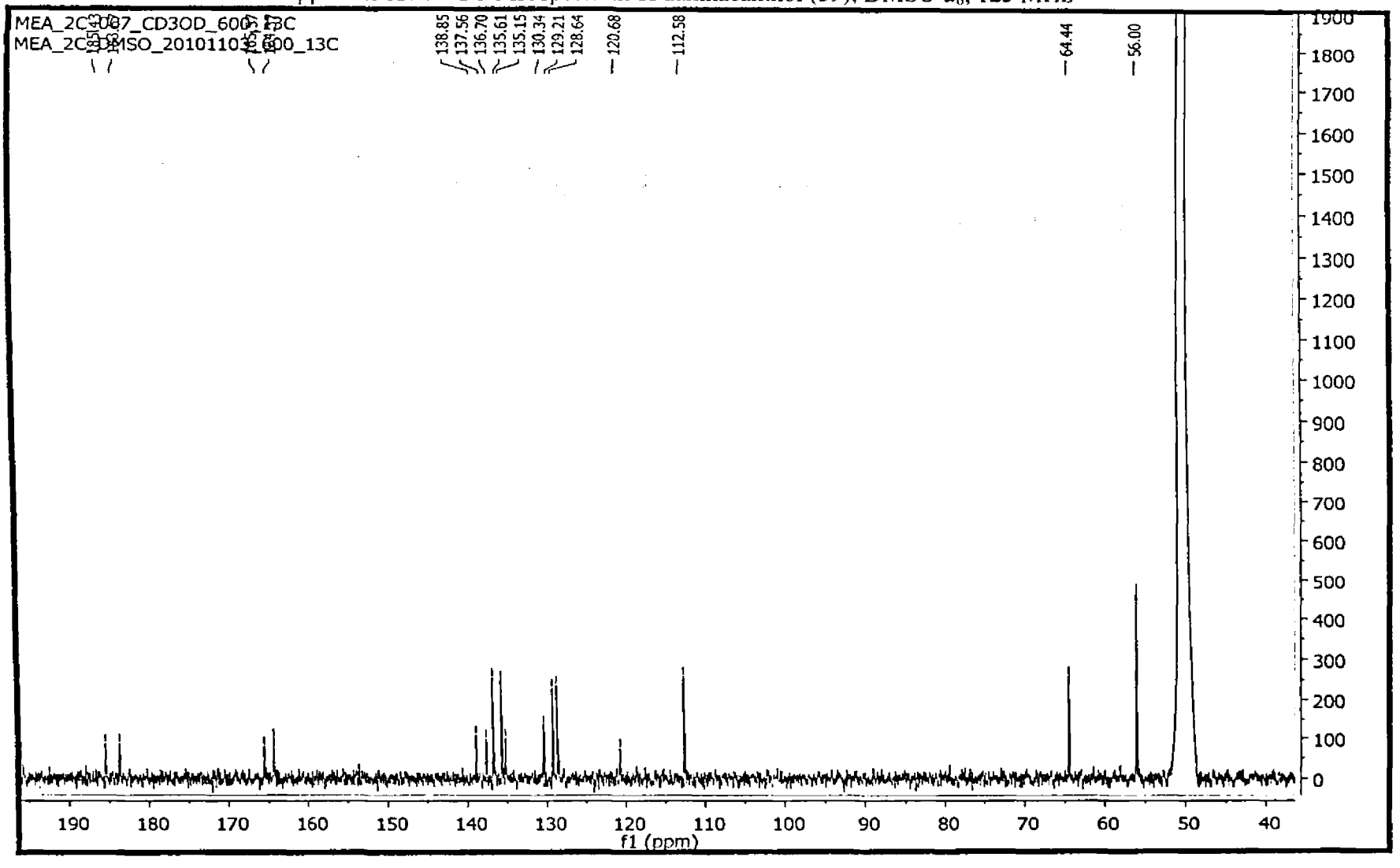
Appendix 11D: gHSQC spectrum of damnacanthal (**50**), DMSO-d_6, 600 MHz



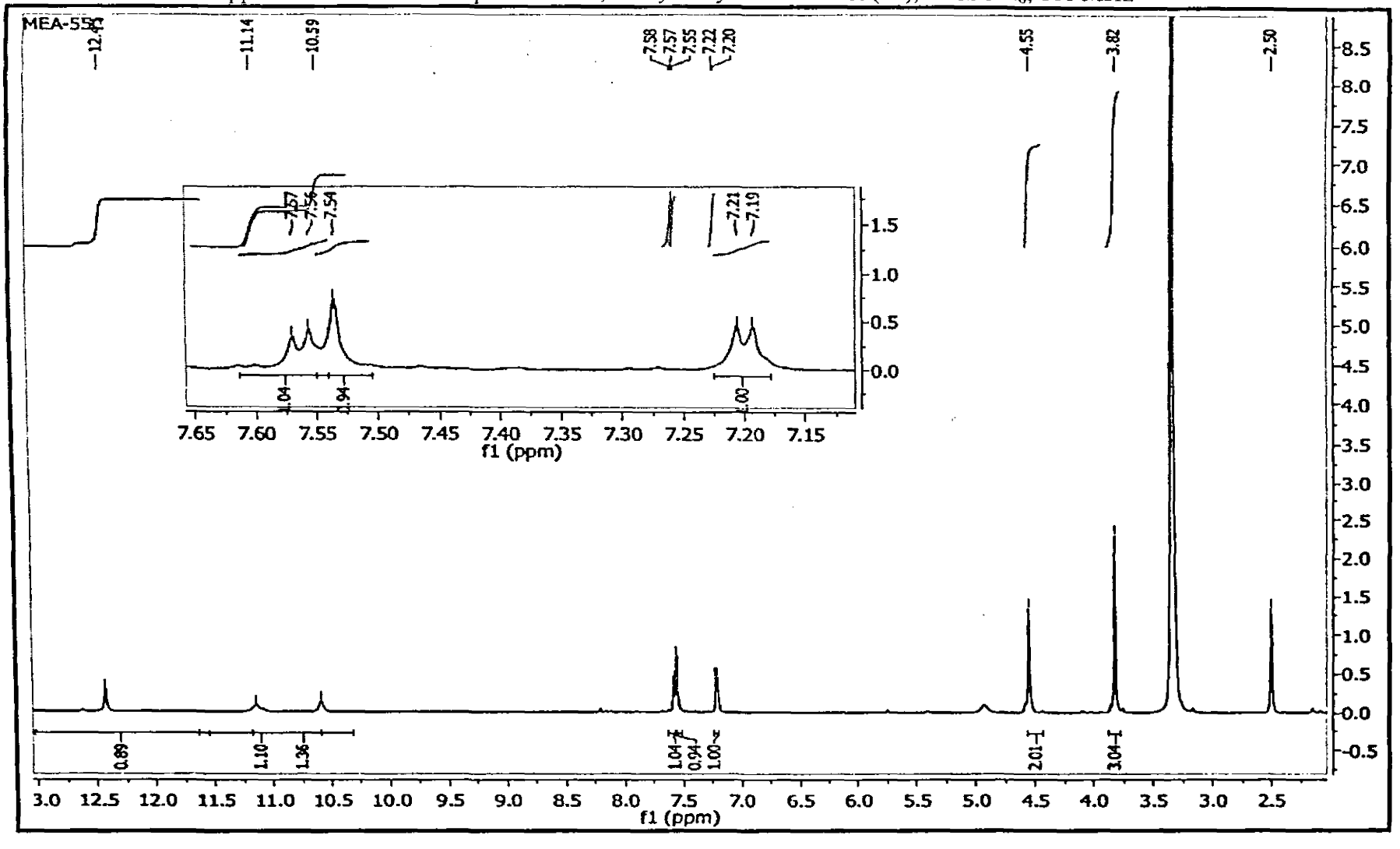
Appendix 12A: ^{1}H NMR spectrum of damnacanthol (59), DMSO-d_6, 600 MHz



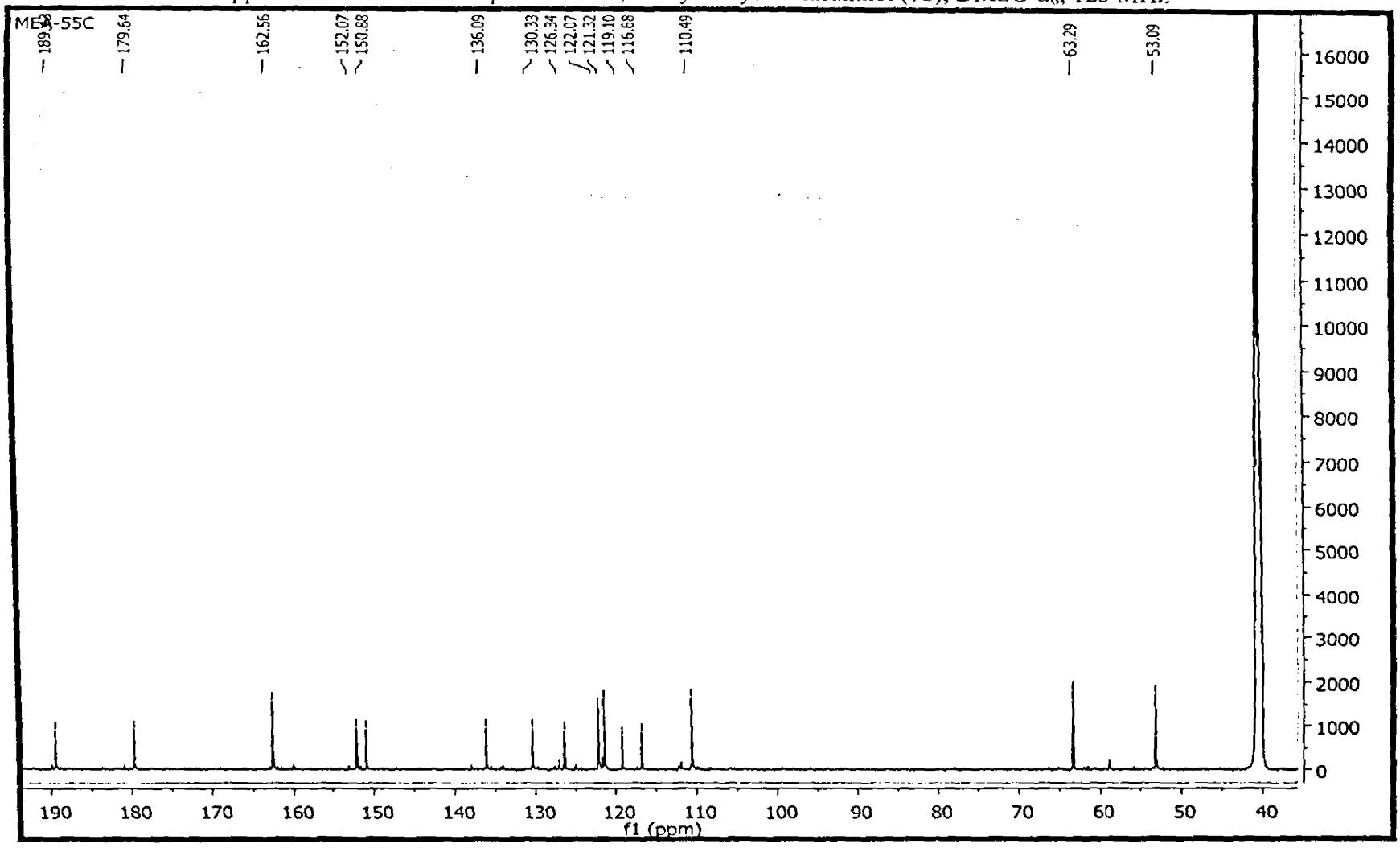
Appendix 12B: ^{13}C NMR spectrum of damnacanthol (**59**), DMSO-d_6, 125 MHz



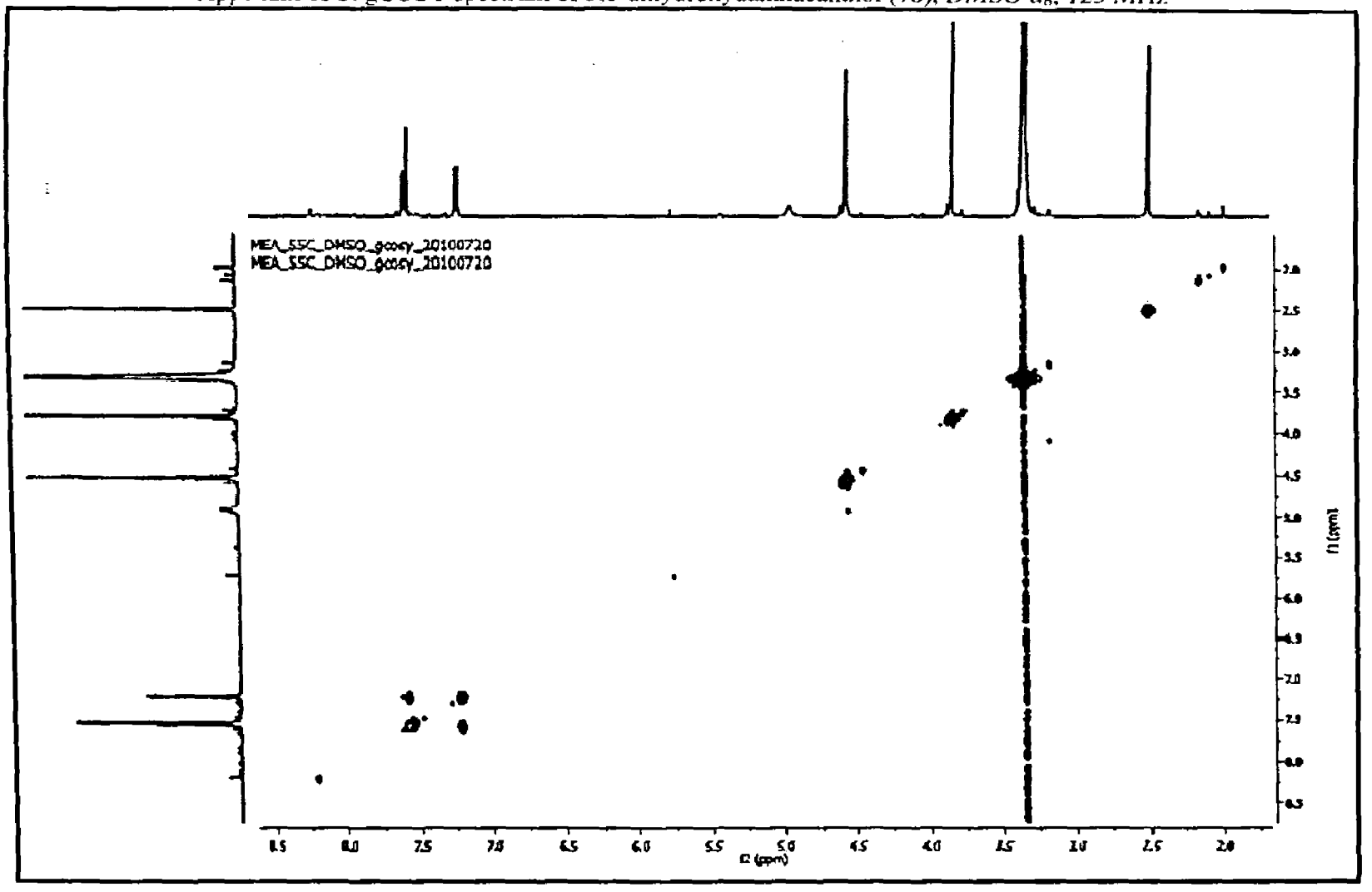
Appendix 13A: ^{1}H NMR spectrum of 5,6-dihydroxydamnacanthol (78), DMSO-d_6, 600 MHz



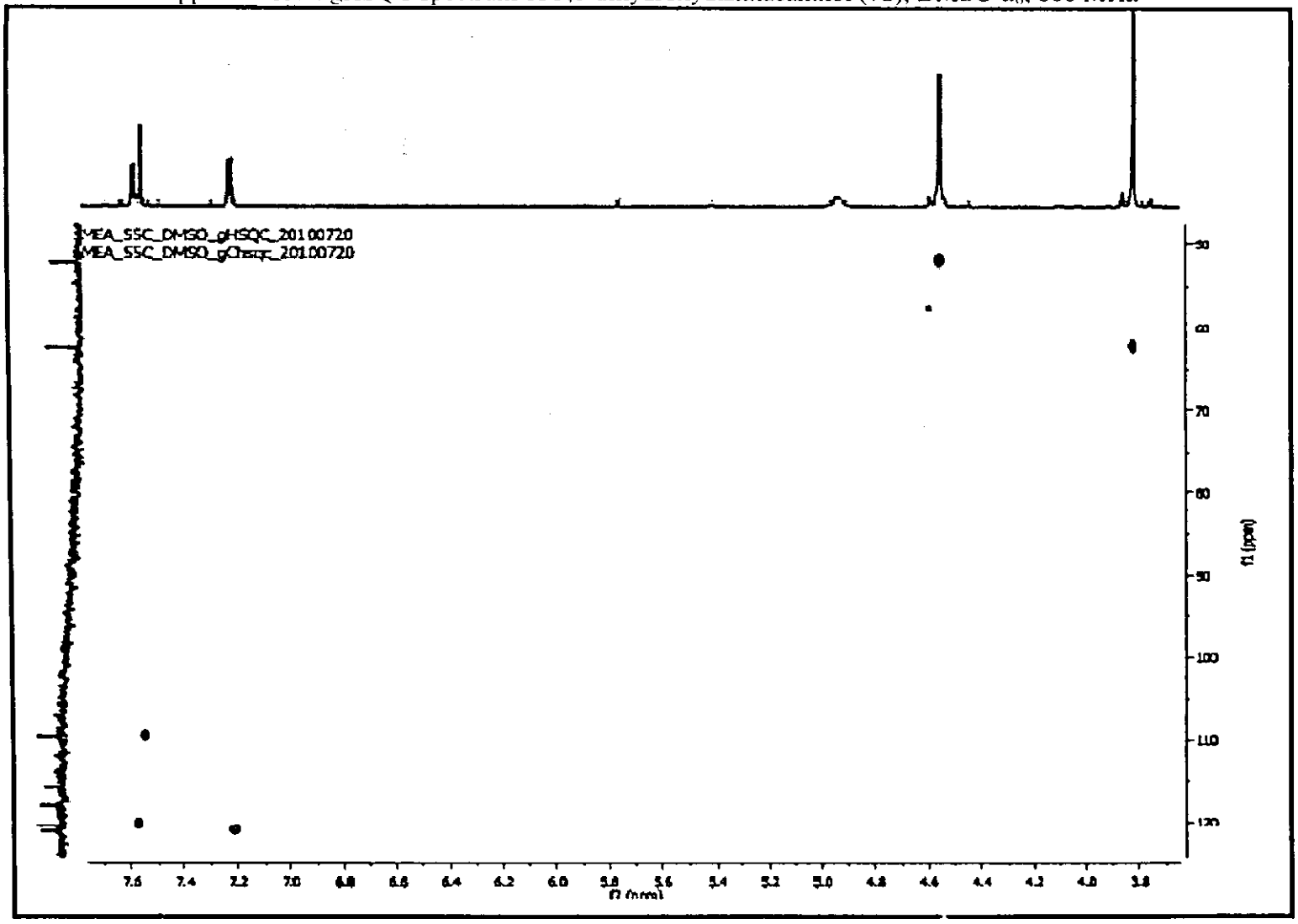
Appendix 13B: ^{13}C NMR spectrum of 5,6-dihydroxydamnacanthol (**78**), DMSO-d_6, 125 MHz



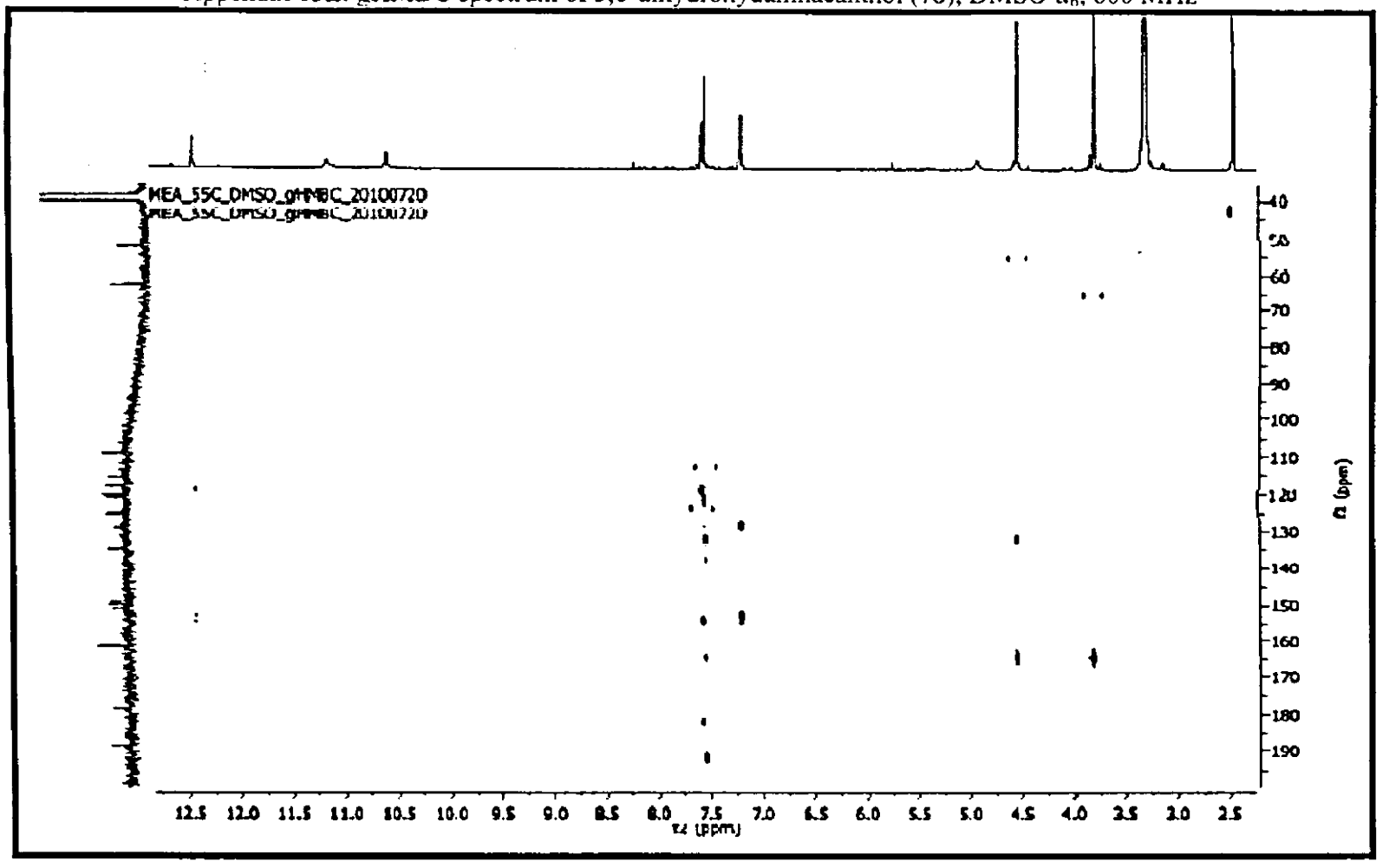
Appendix 13C: gCOSY spectrum of 5,6-dihydroxydamnacanthol (78), DMSO-d_6, 125 MHz



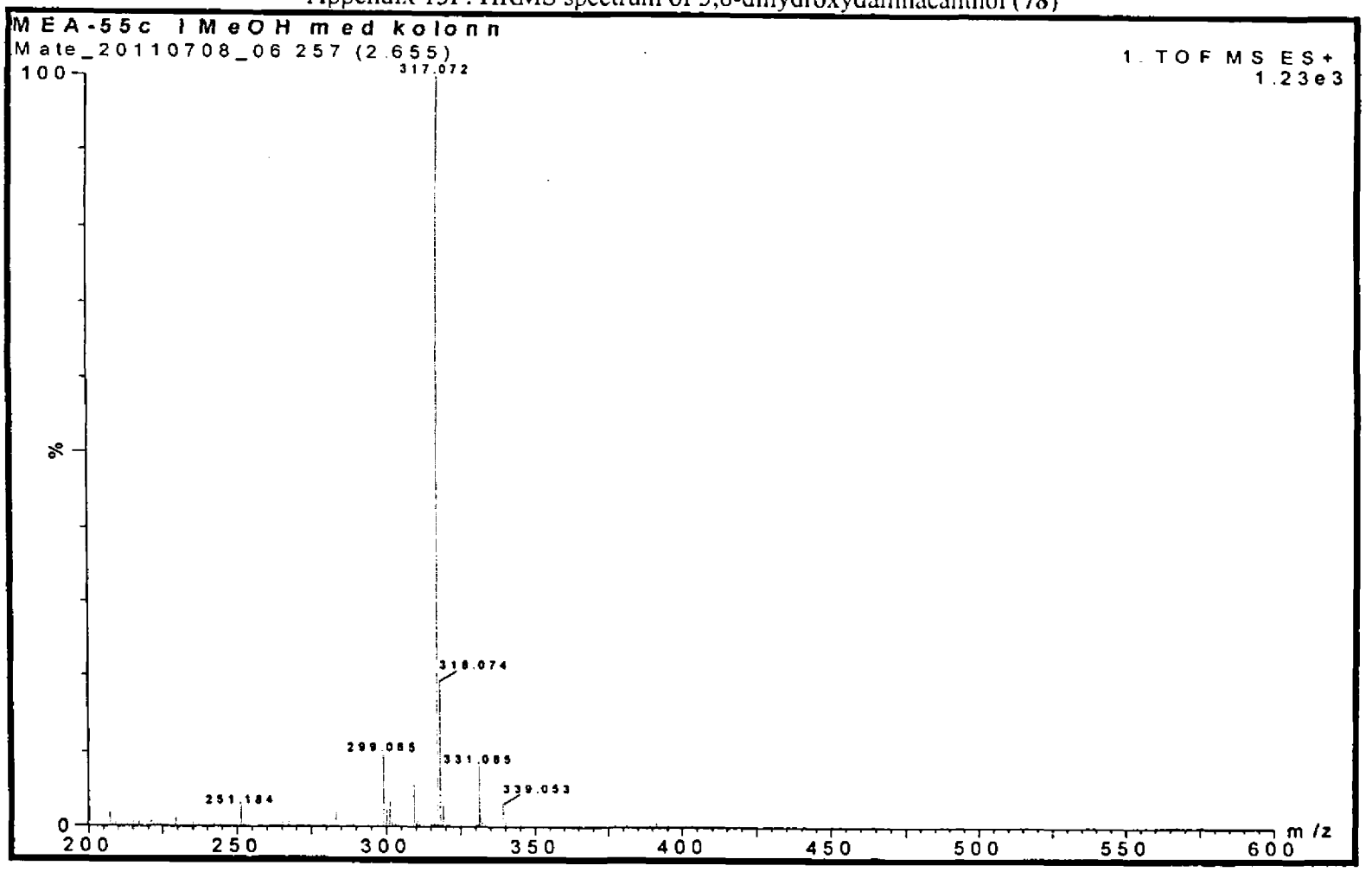
Appendix 13D: gHSQC spectrum of 5,6-dihydroxydamnacanthol (**78**), DMSO-d_6, 600 MHz



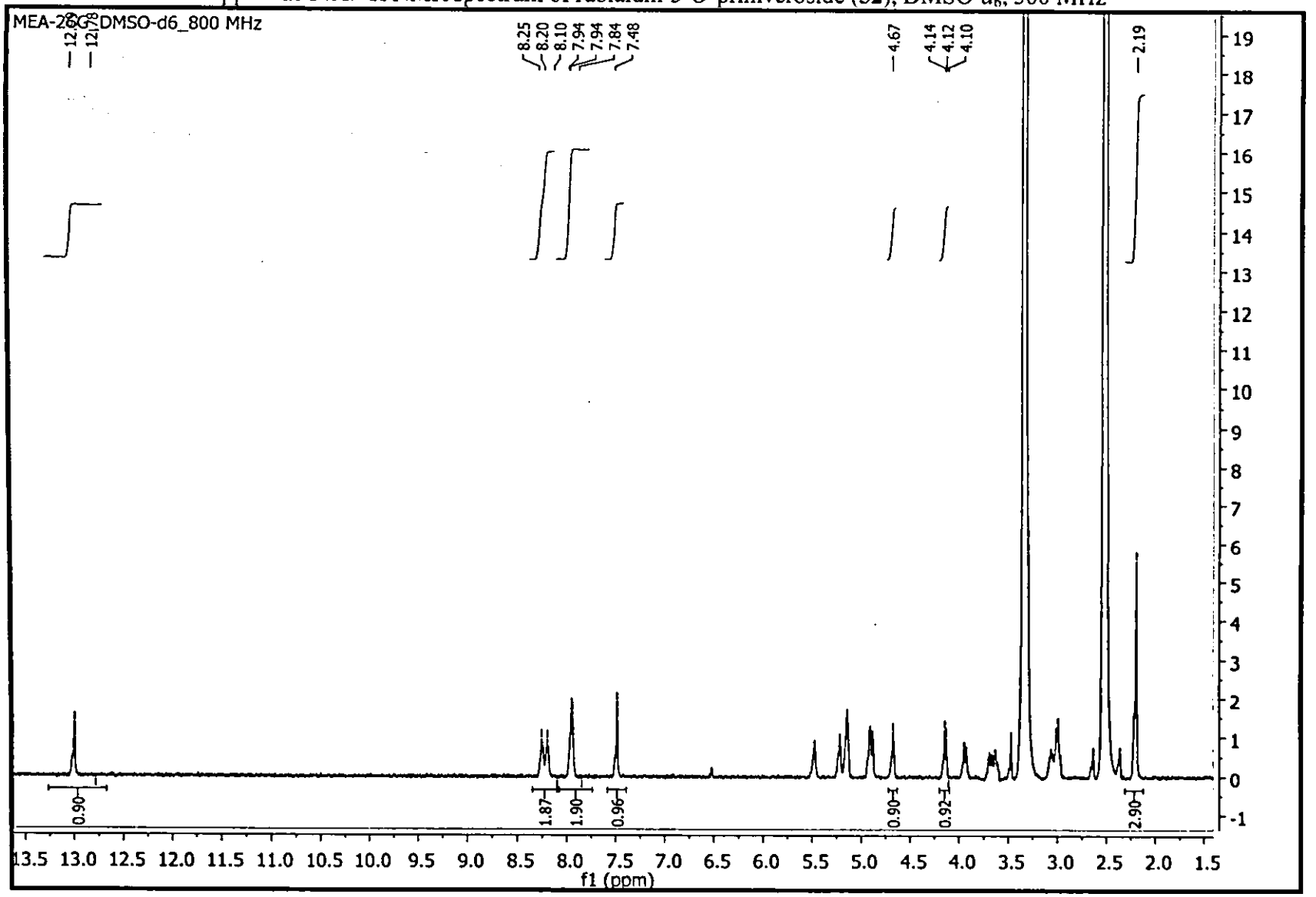
Appendix 13E: gHMBC spectrum of 5,6-dihydroxydamnacanthol (**78**), DMSO-d_6, 600 MHz



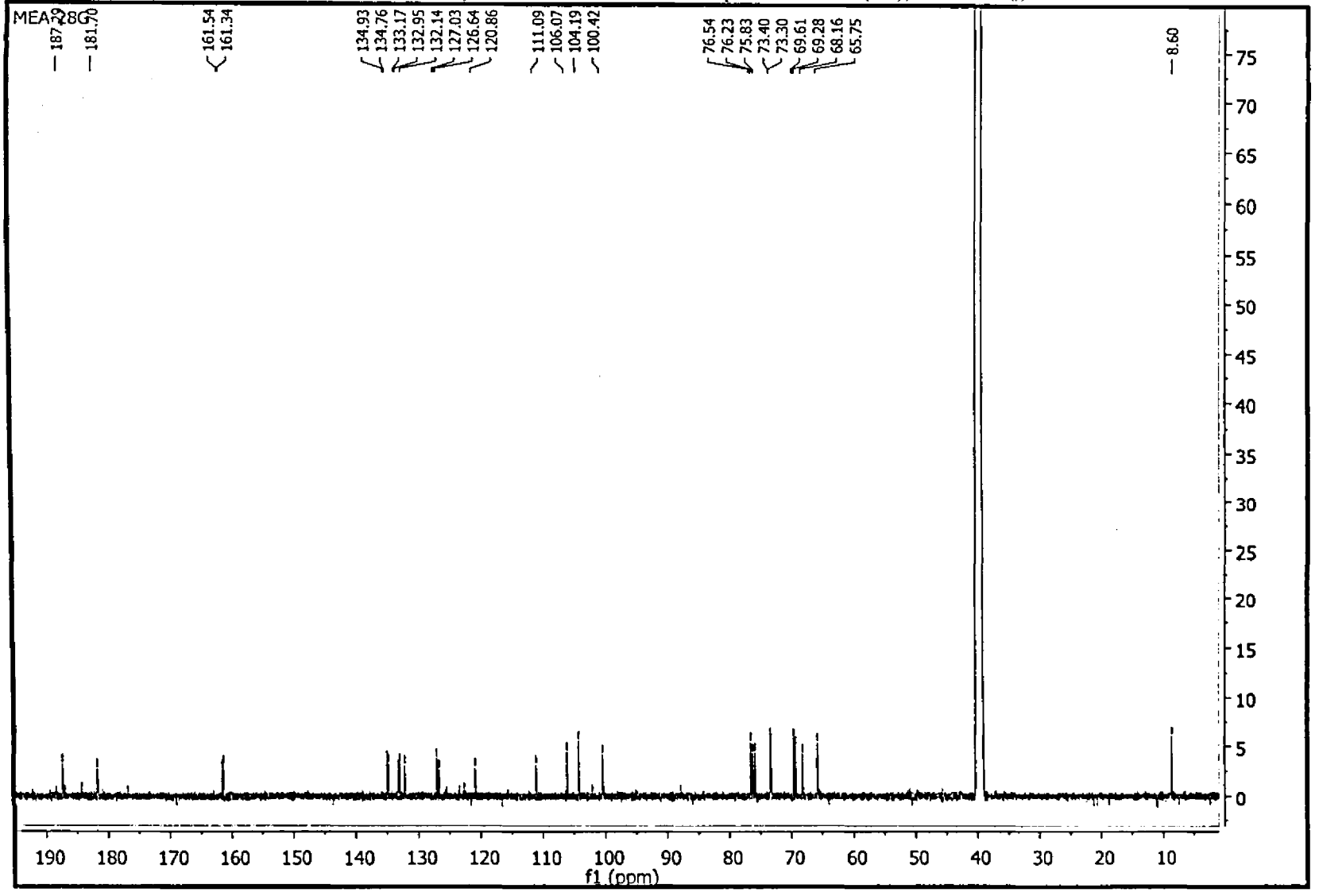
Appendix 13F: HRMS spectrum of 5,6-dihydroxydamnacanthol (78)



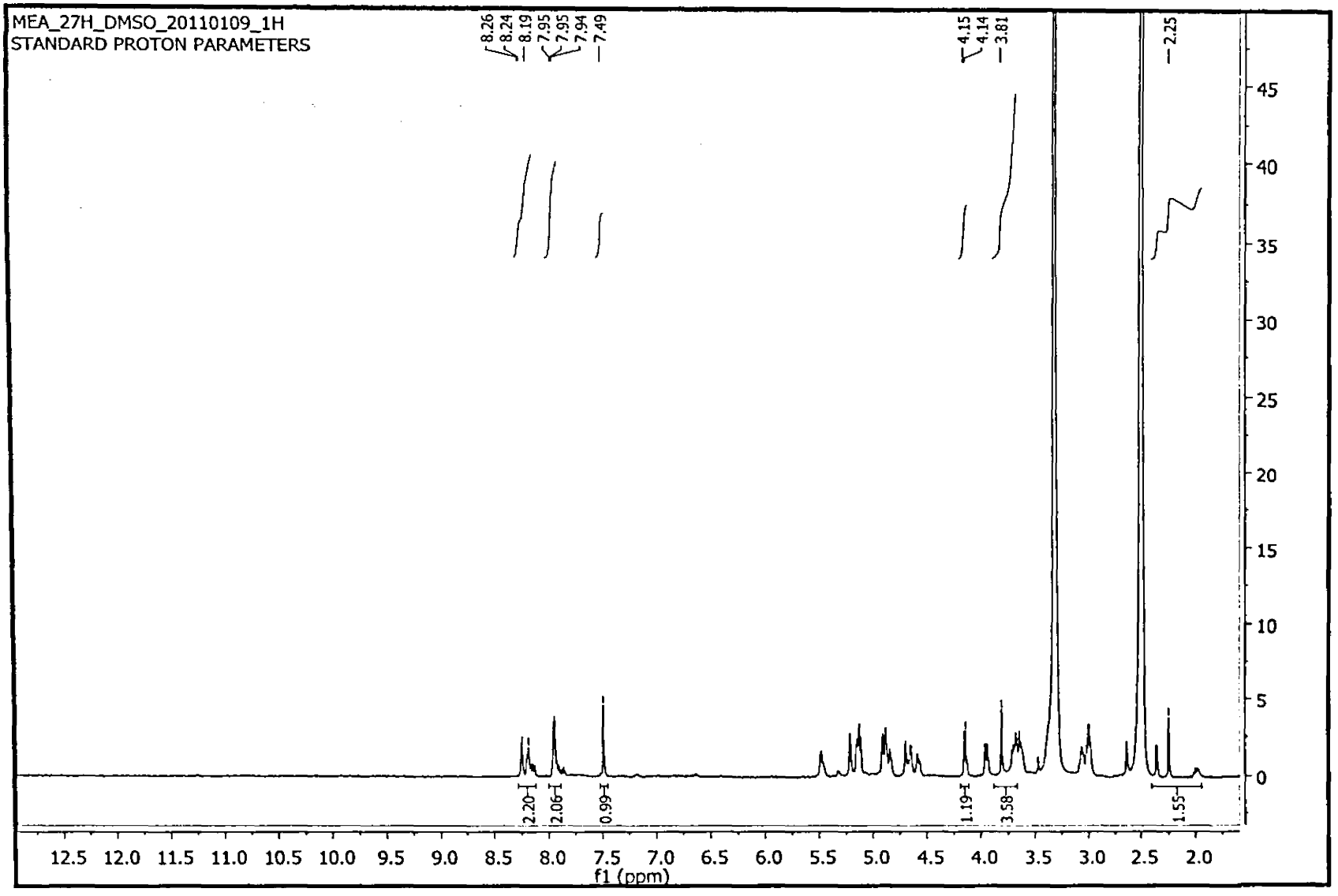
Appendix 14A: ^{1}H NMR spectrum of rubiaidin-3-*O*-primveroside (**52**), DMSO-d_6, 500 MHz



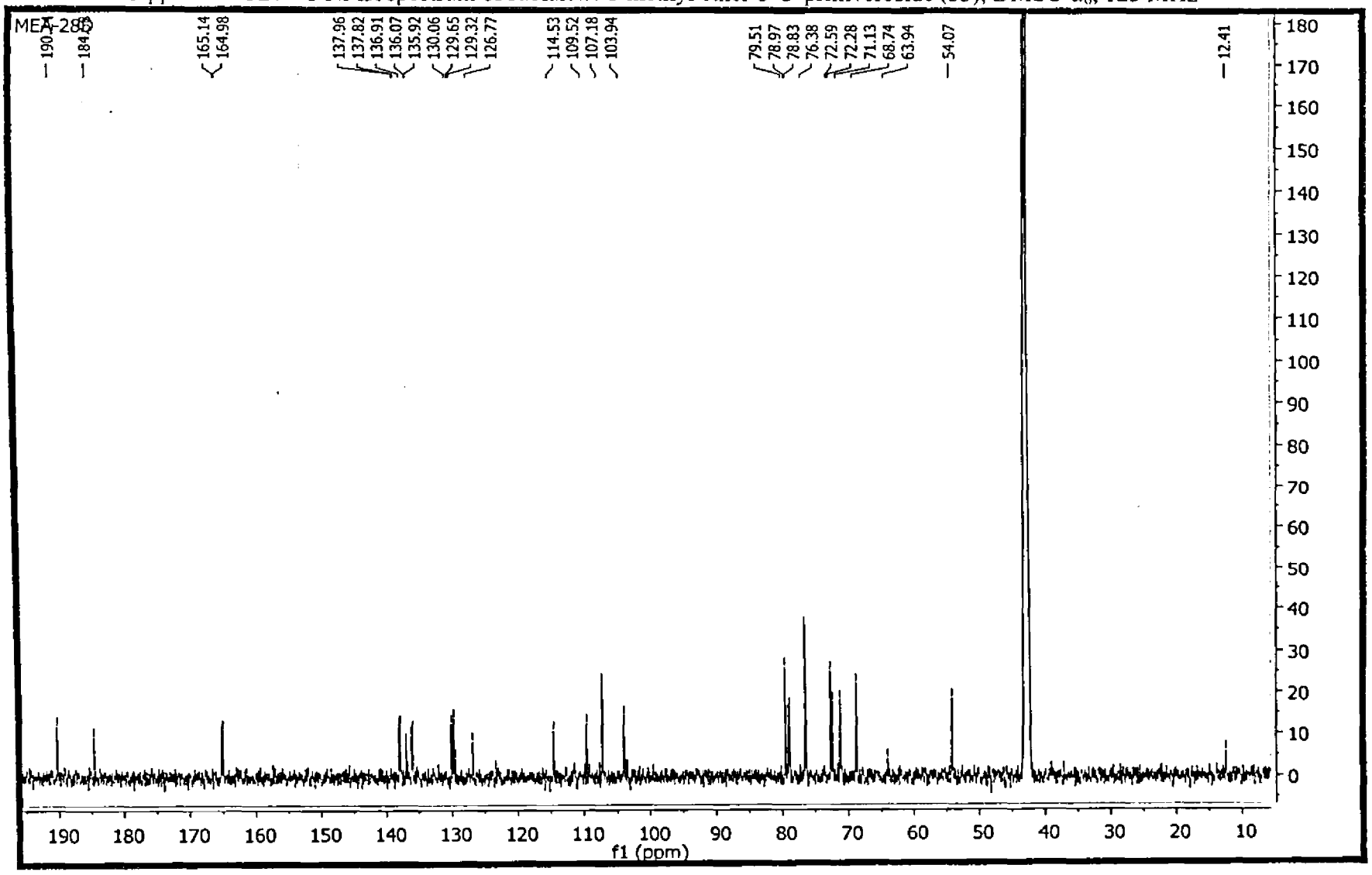
Appendix 14B: ^{13}C NMR spectrum of rubiaidin-3-*O*-primveroside (**52**), DMSO-d_6, 125 MHz



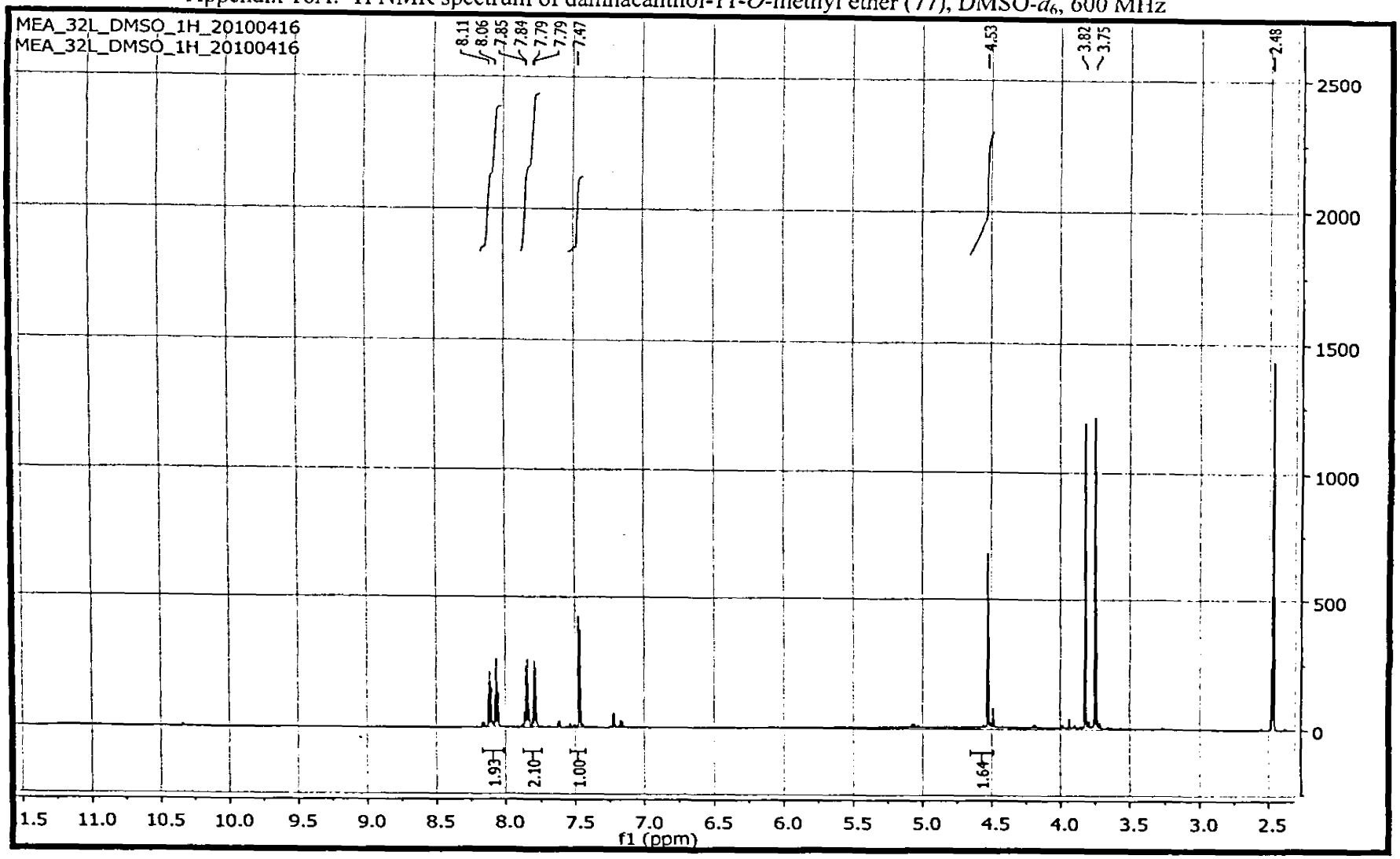
Appendix 15A: ^{1}H NMR spectrum of rubiaidin-1-methyl ether-3-*O*-primveroside (**53**), DMSO-d_6, 500 MHz



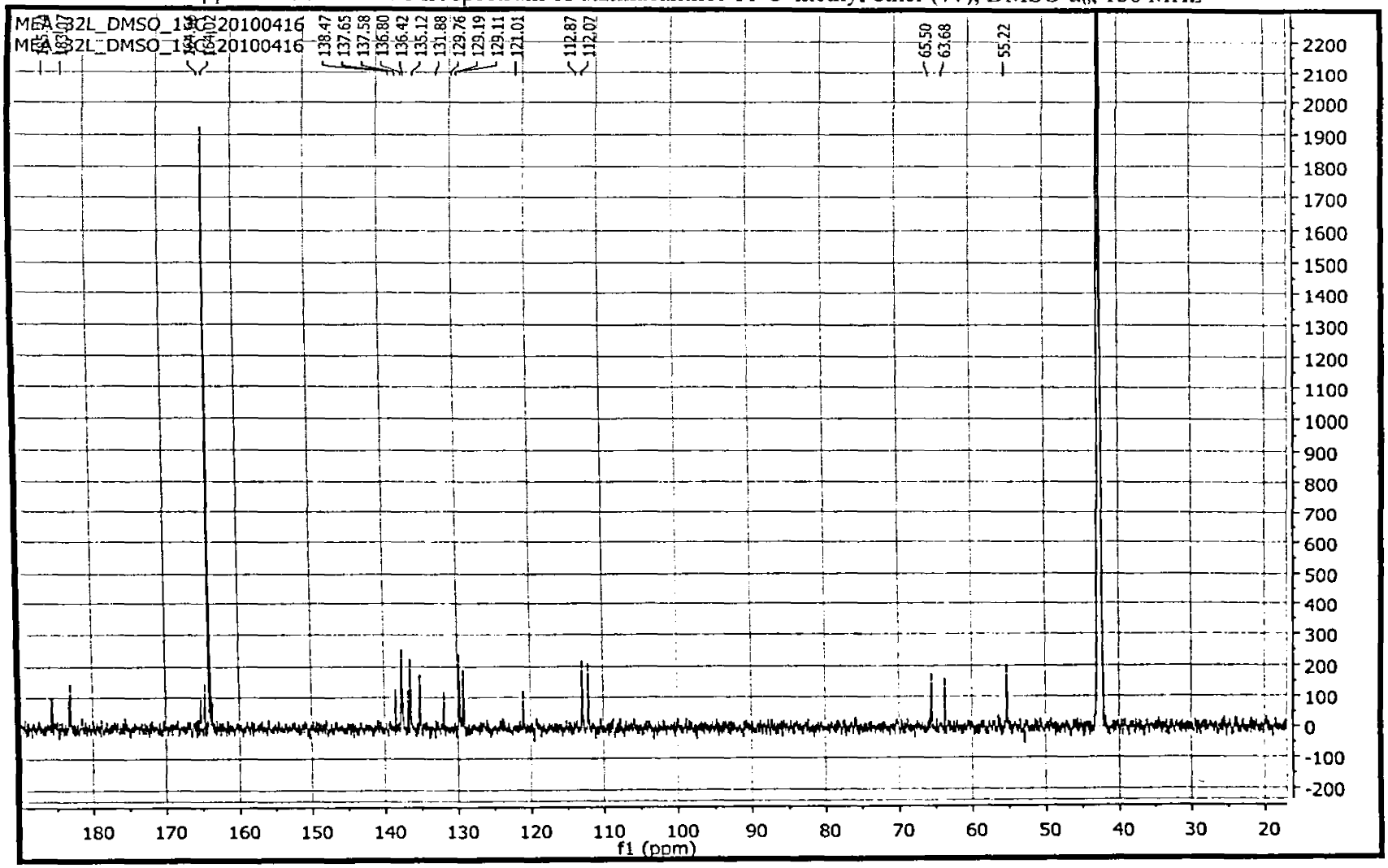
Appendix 15B: ^{13}C NMR spectrum of rubiaidin-1-methyl ether-3-*O*-primveroside (**53**), DMSO-d_6, 125 MHz



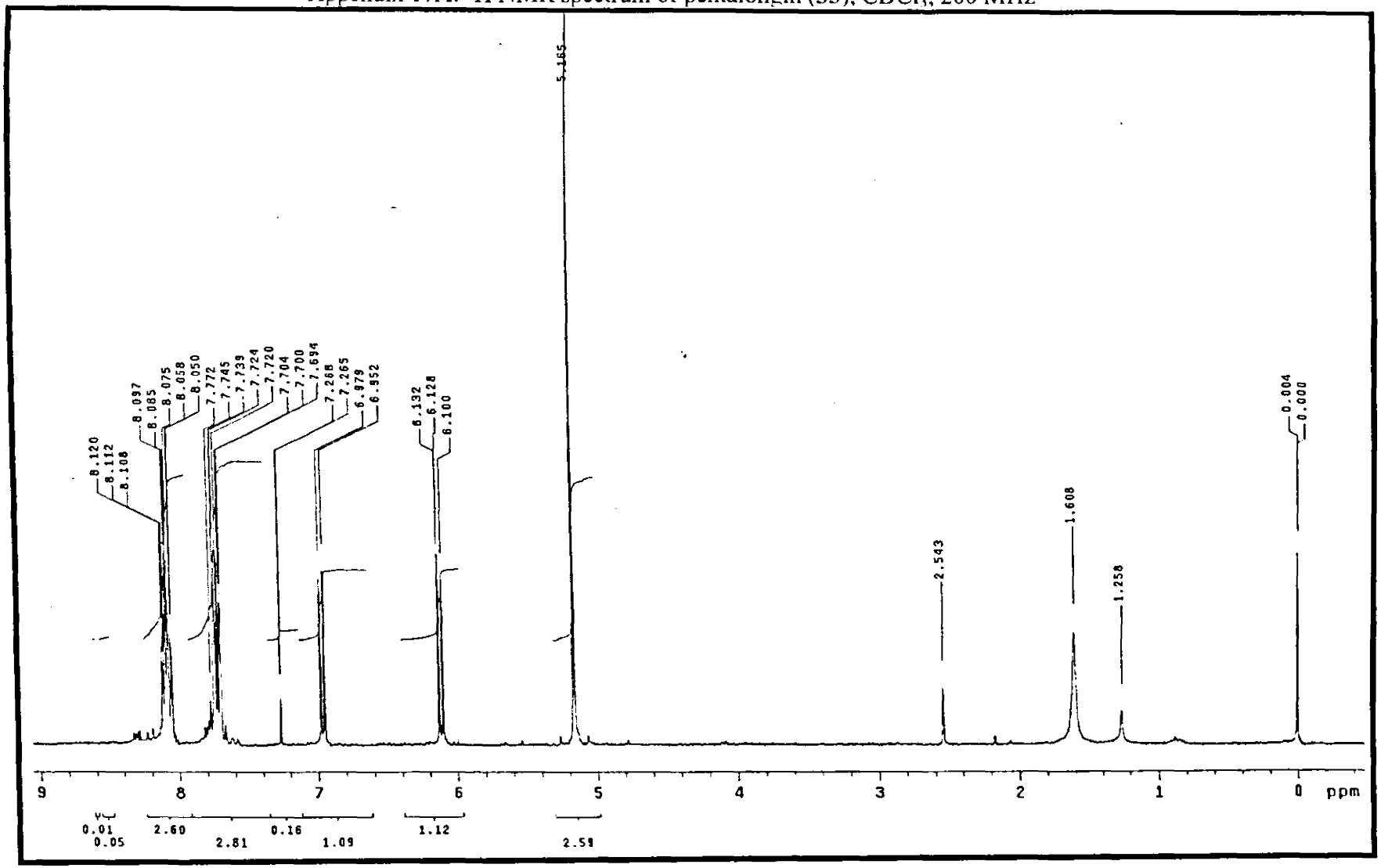
Appendix 16A: ^{1}H NMR spectrum of damnacanthol-11-*O*-methyl ether (77), DMSO-d_6, 600 MHz



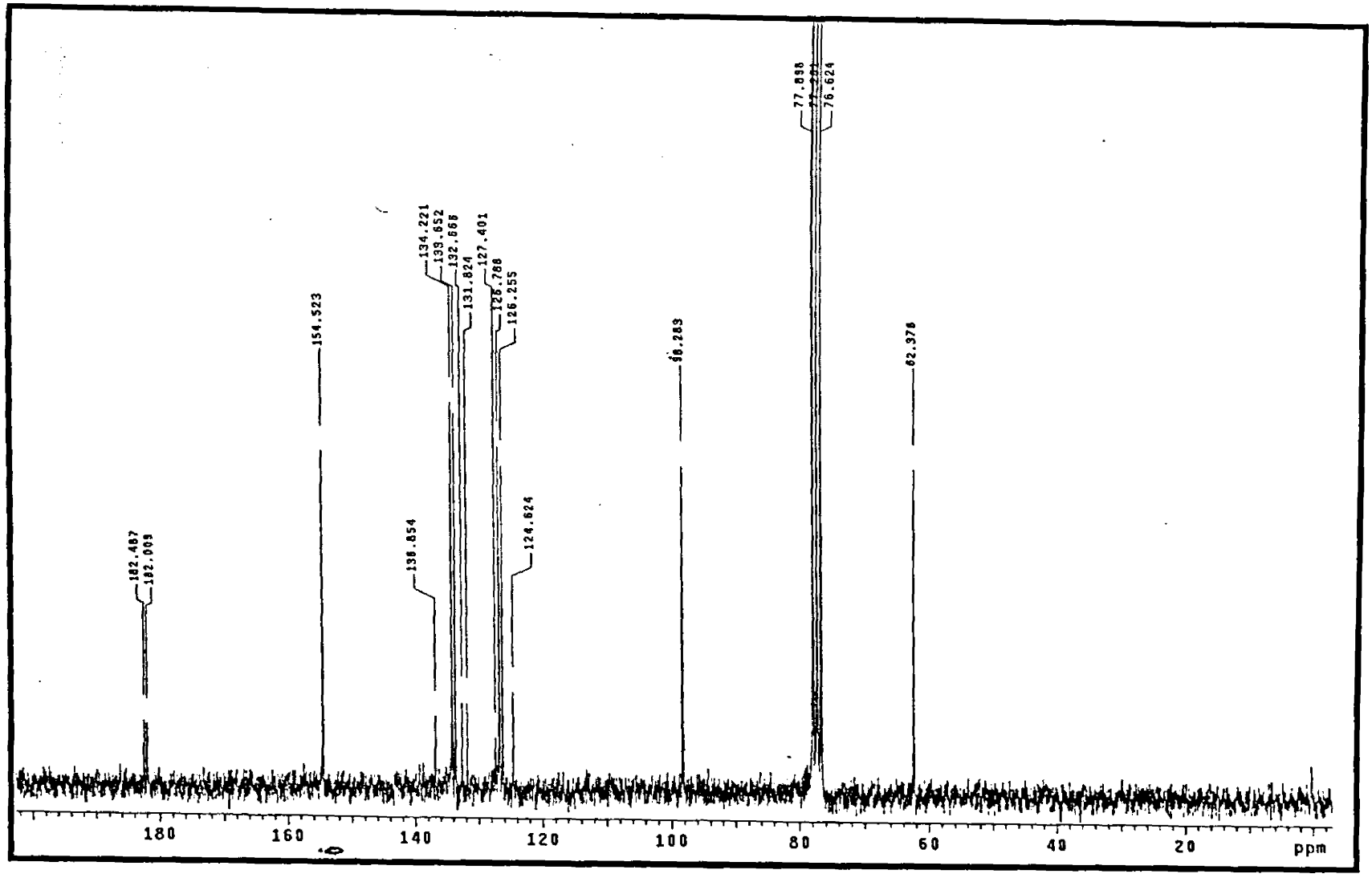
Appendix 16B: ^{13}C NMR spectrum of damnacanthol-11-*O*-methyl ether (77), DMSO-d_6, 150 MHz



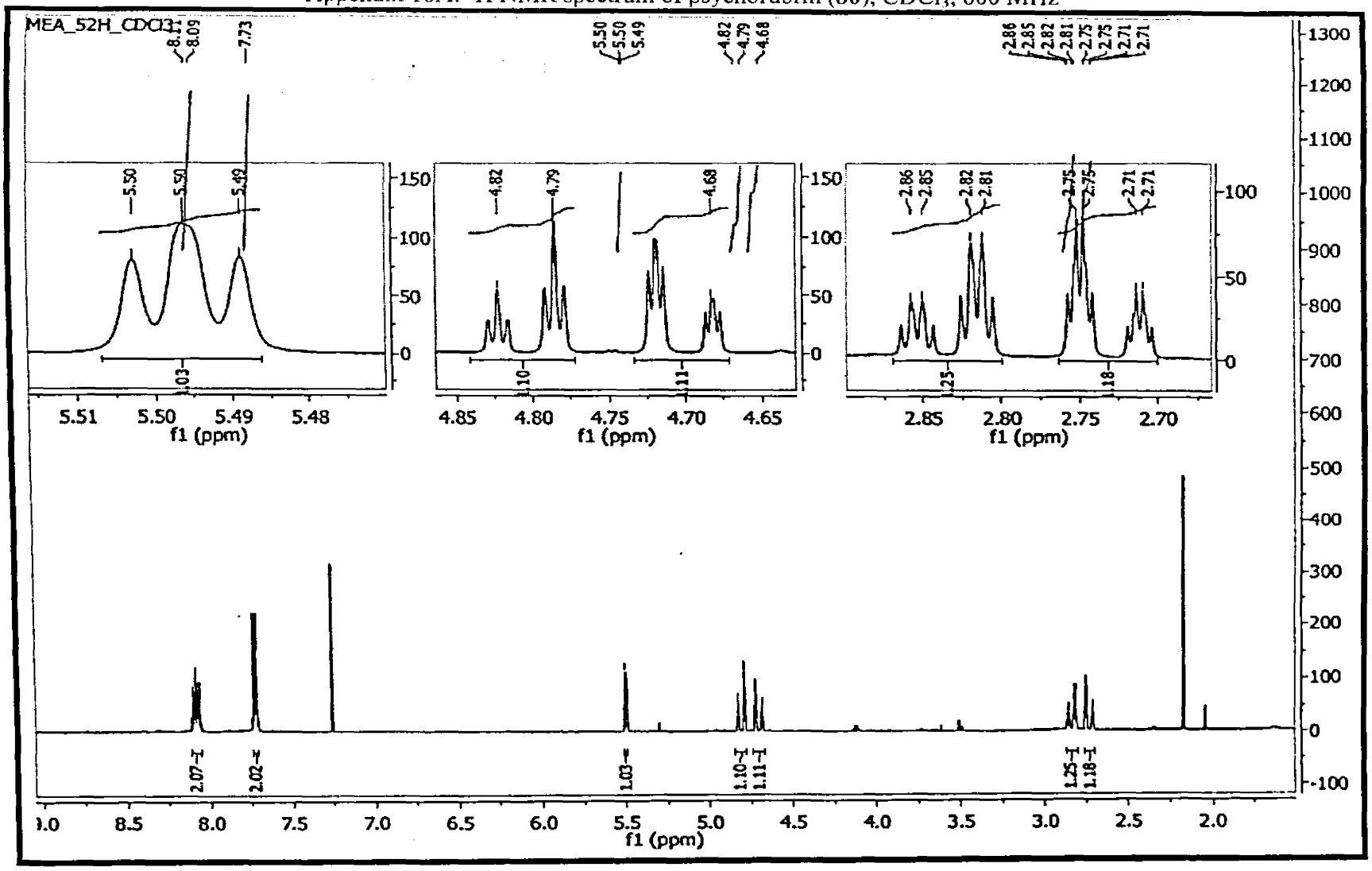
Appendix 17A: ^{1}H NMR spectrum of pentalongin (**33**), $CDCl_3$, 200 MHz



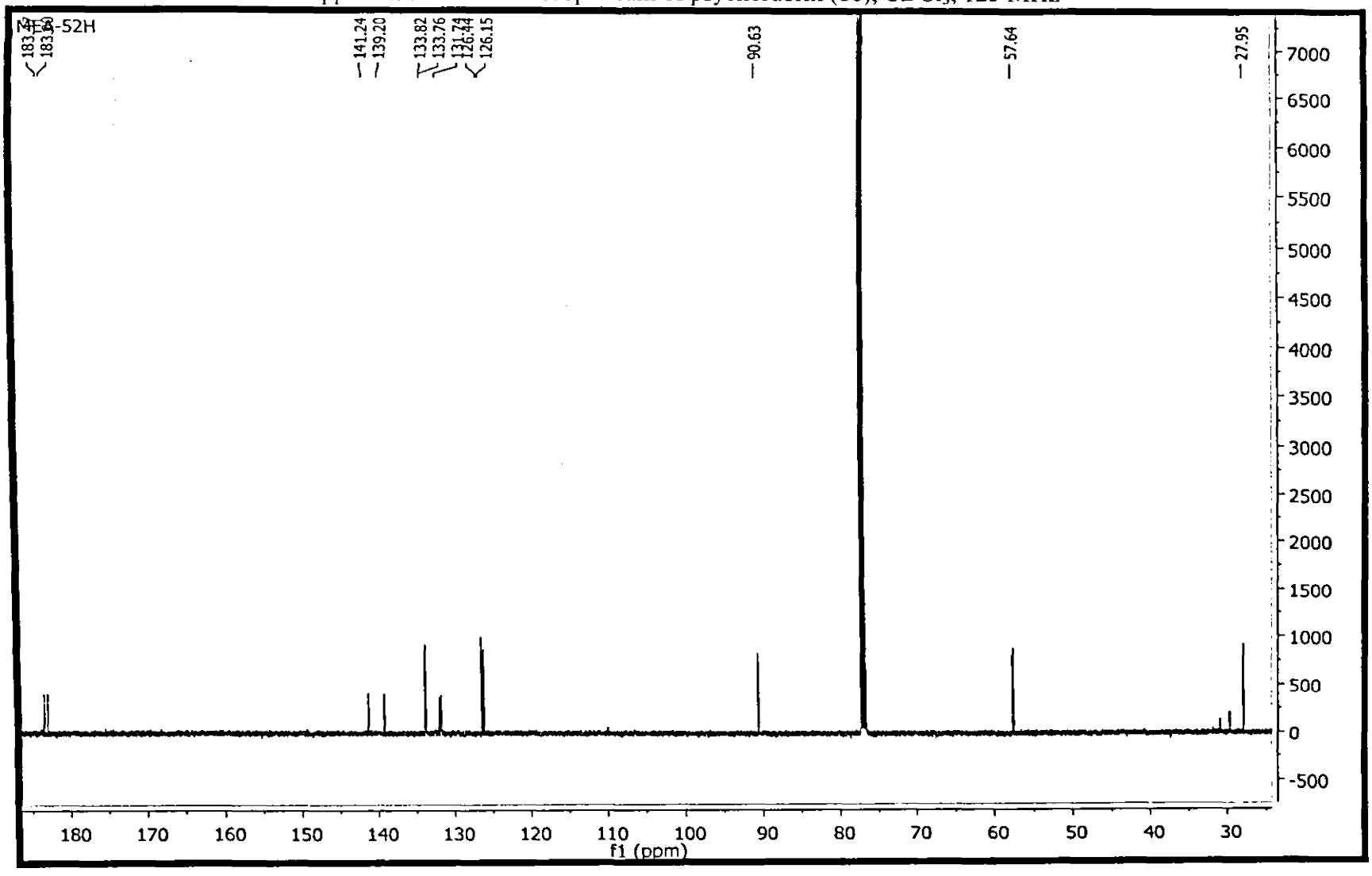
Appendix 17B: ^{13}C NMR spectrum of pentalongin (**33**), $CDCl_3$, 50 MHz



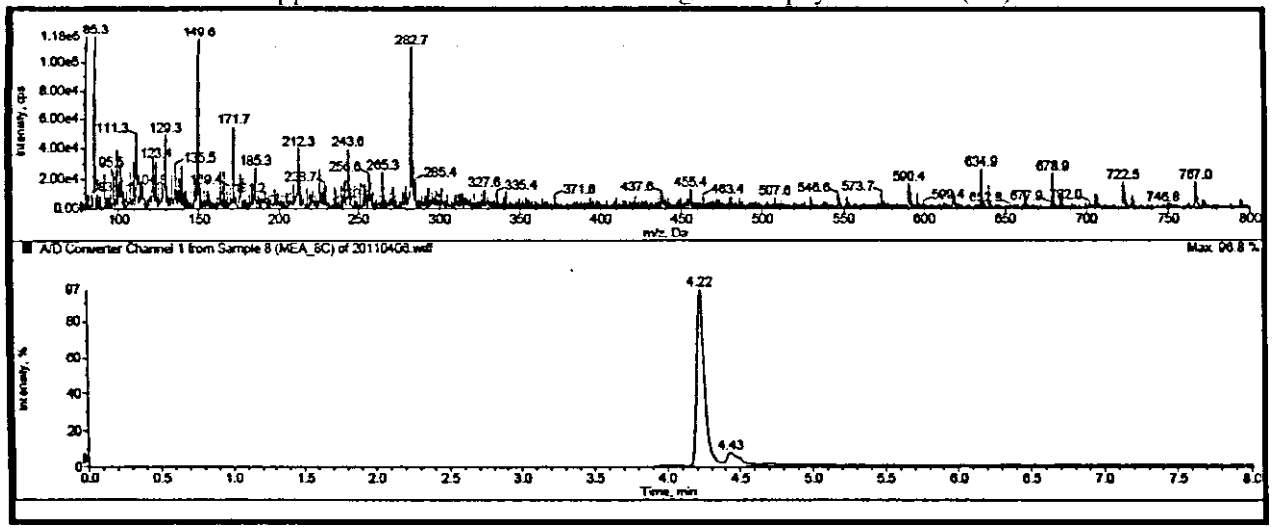
Appendix 18A: ^{1}H NMR spectrum of psychorubrin (80), $CDCl_3$, 800 MHz



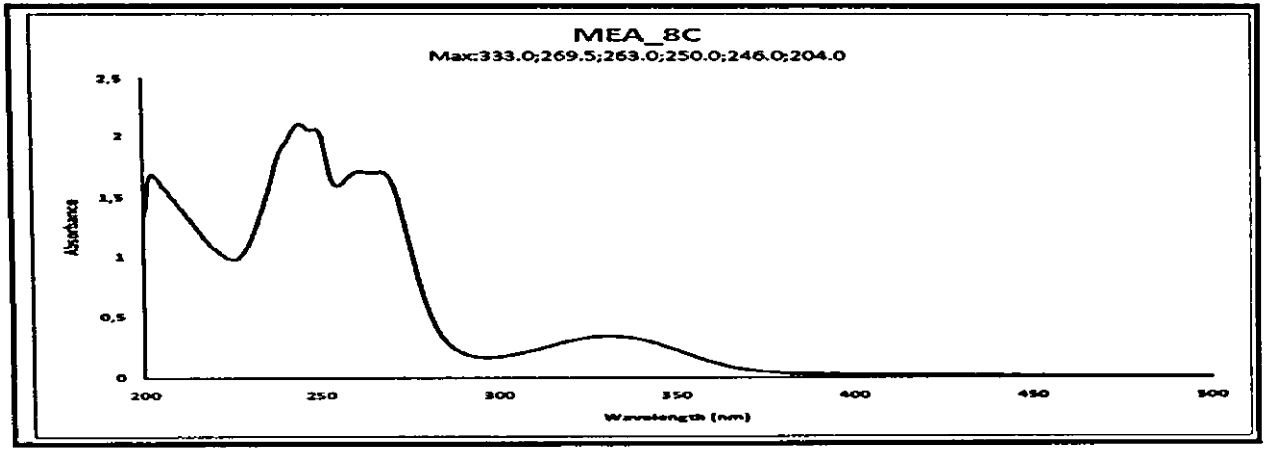
Appendix 18B: ^{13}C NMR spectrum of psychorubrin (**80**), $CDCl_3$, 125 MHz



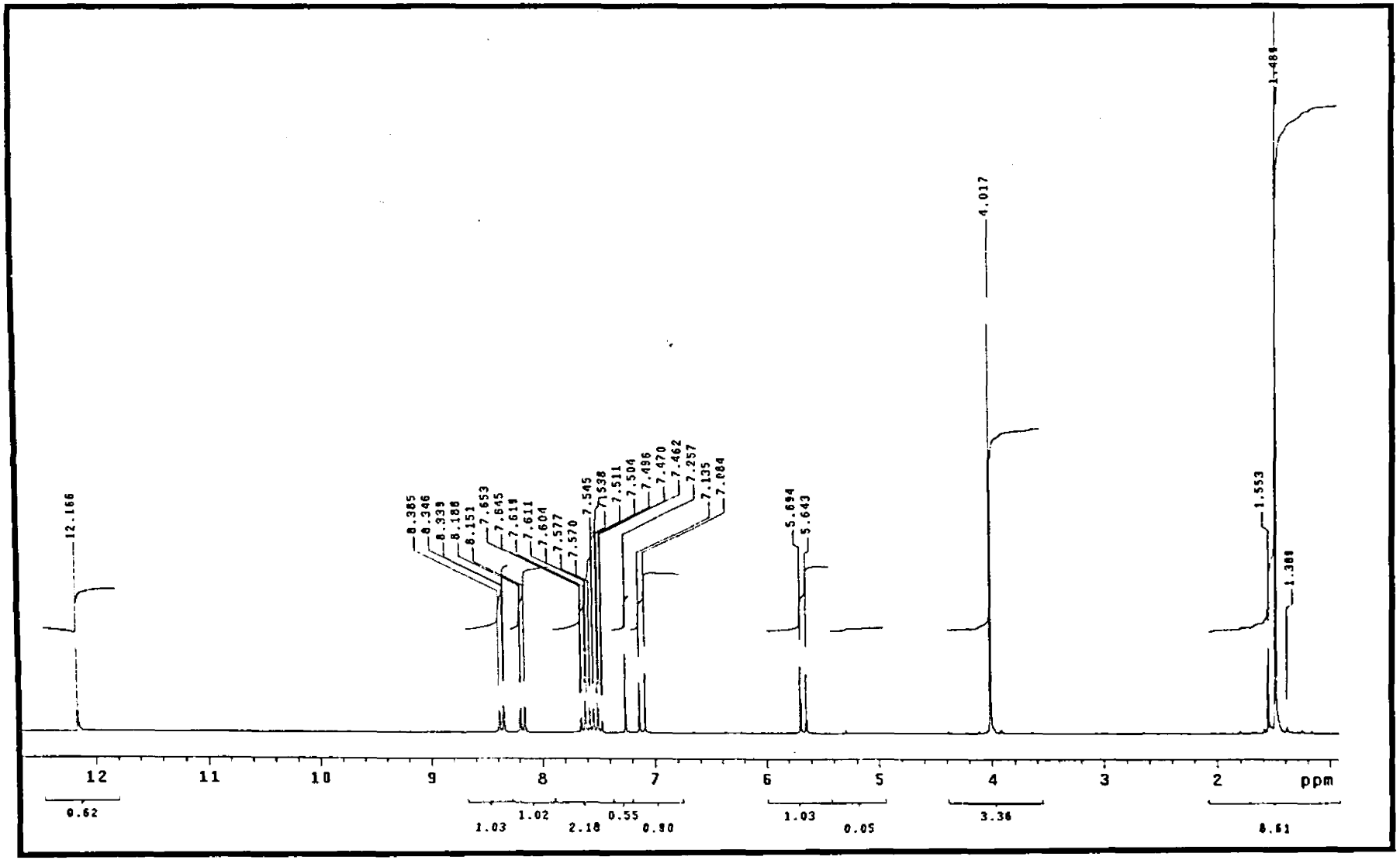
Appendix 18C: LC-MS chromatogram of psychorubrin (**80**)



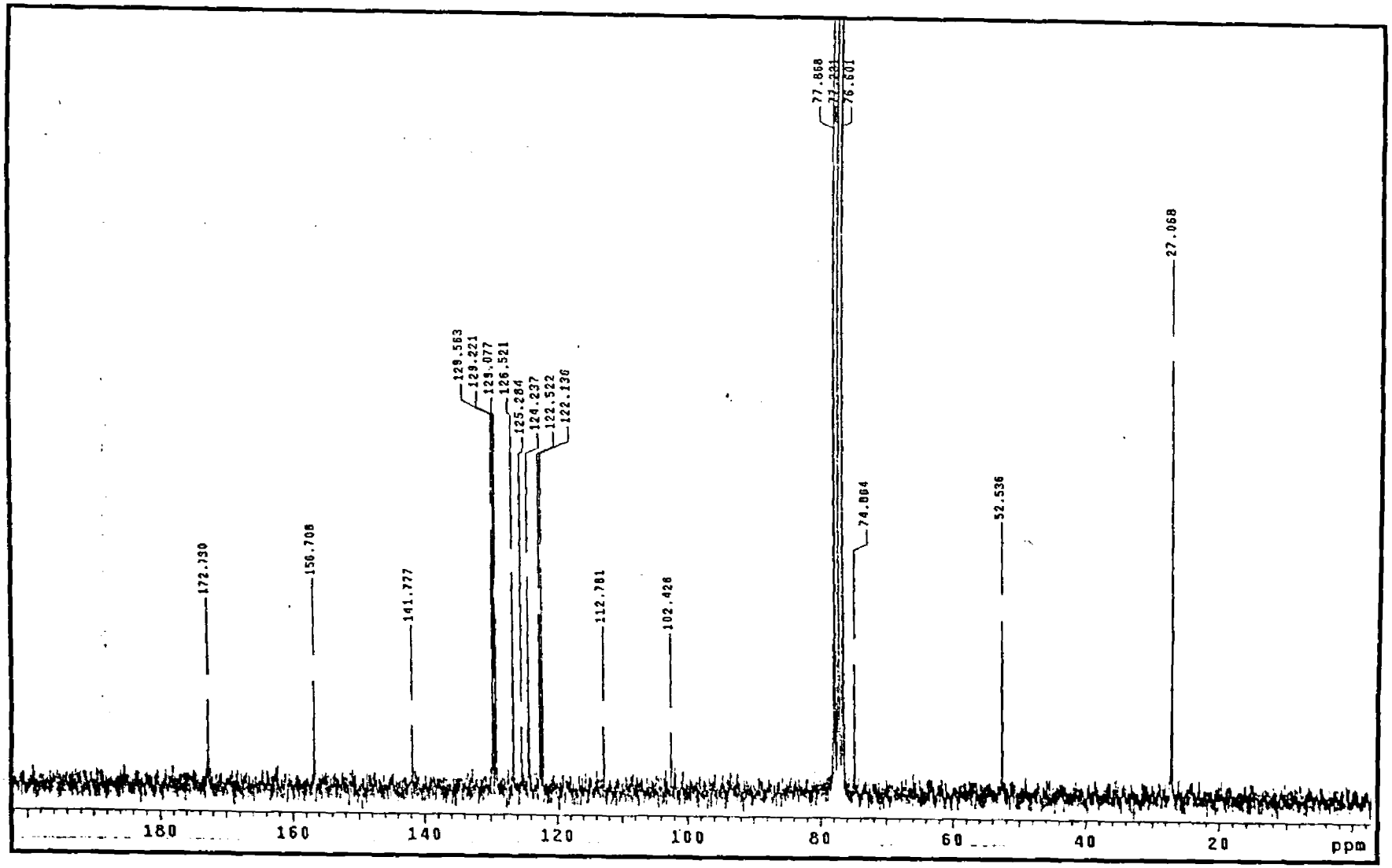
Appendix 18D: UV-Vis spectrum of psychorubrin (**80**)



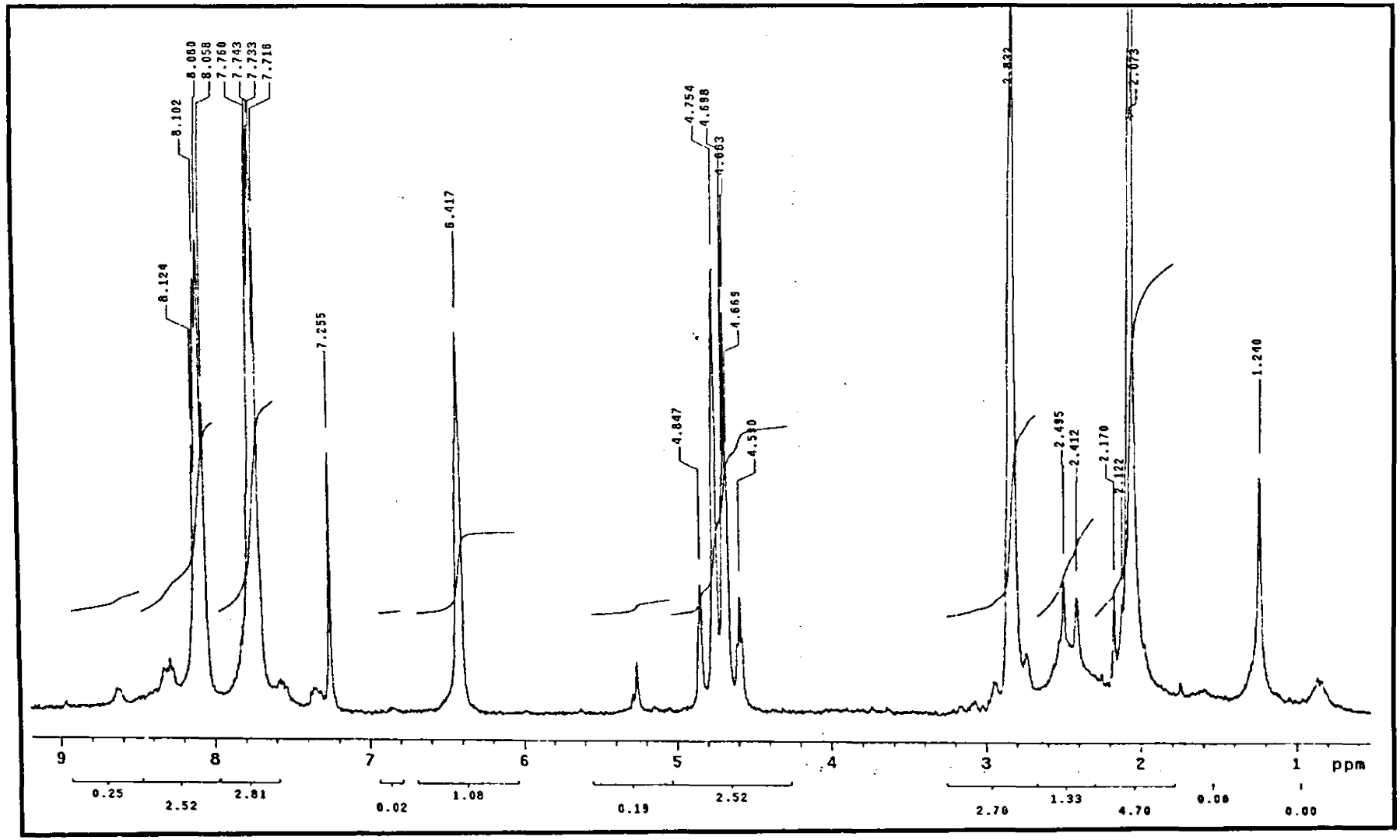
Appendix 19A: ^{1}H NMR spectrum of mollugin (**34**), $CDCl_3$, 200 MHz



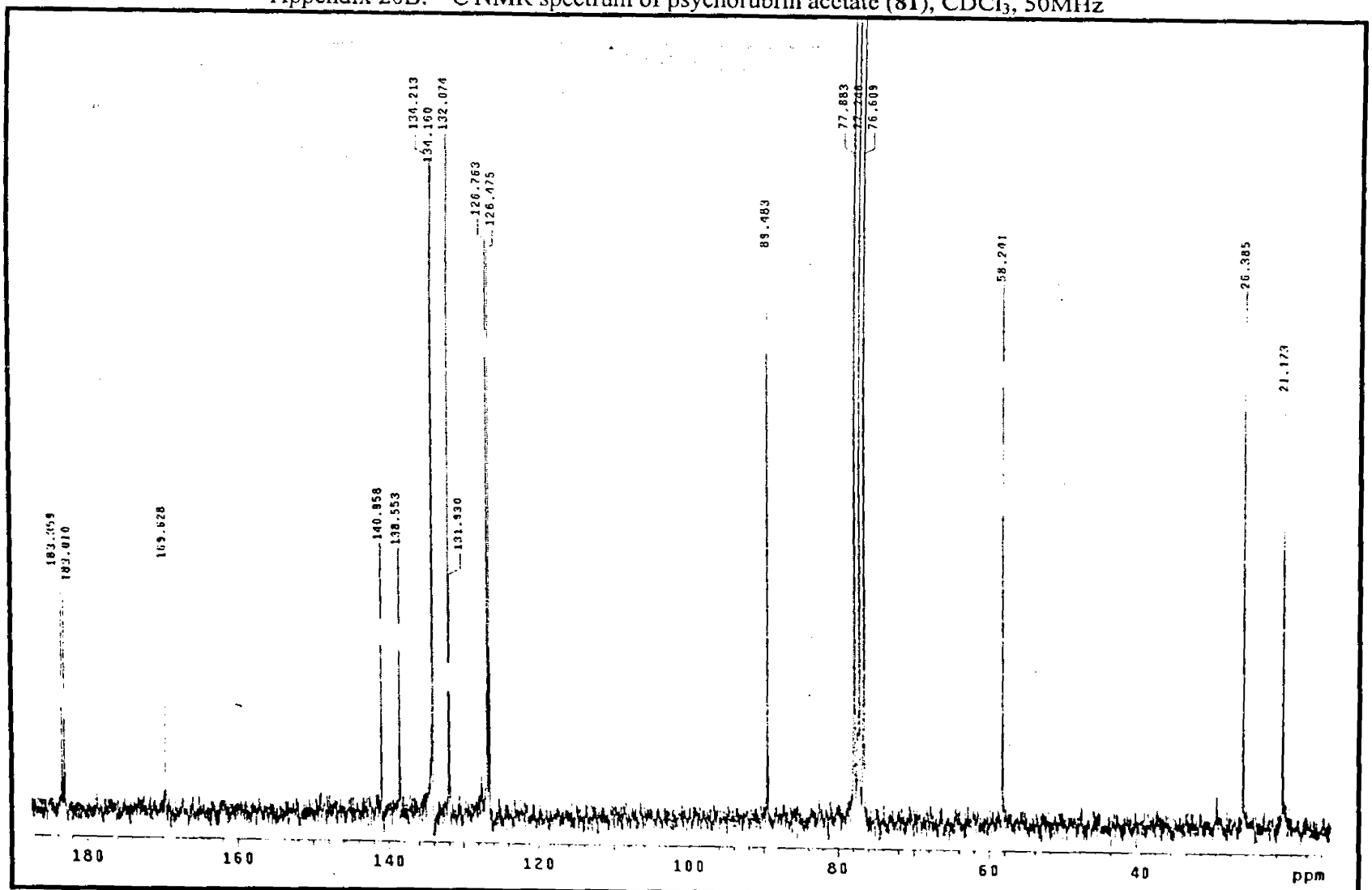
Appendix 19B: ^{13}C NMR spectrum of mollugin (**34**), $CDCl_3$, 200 MHz



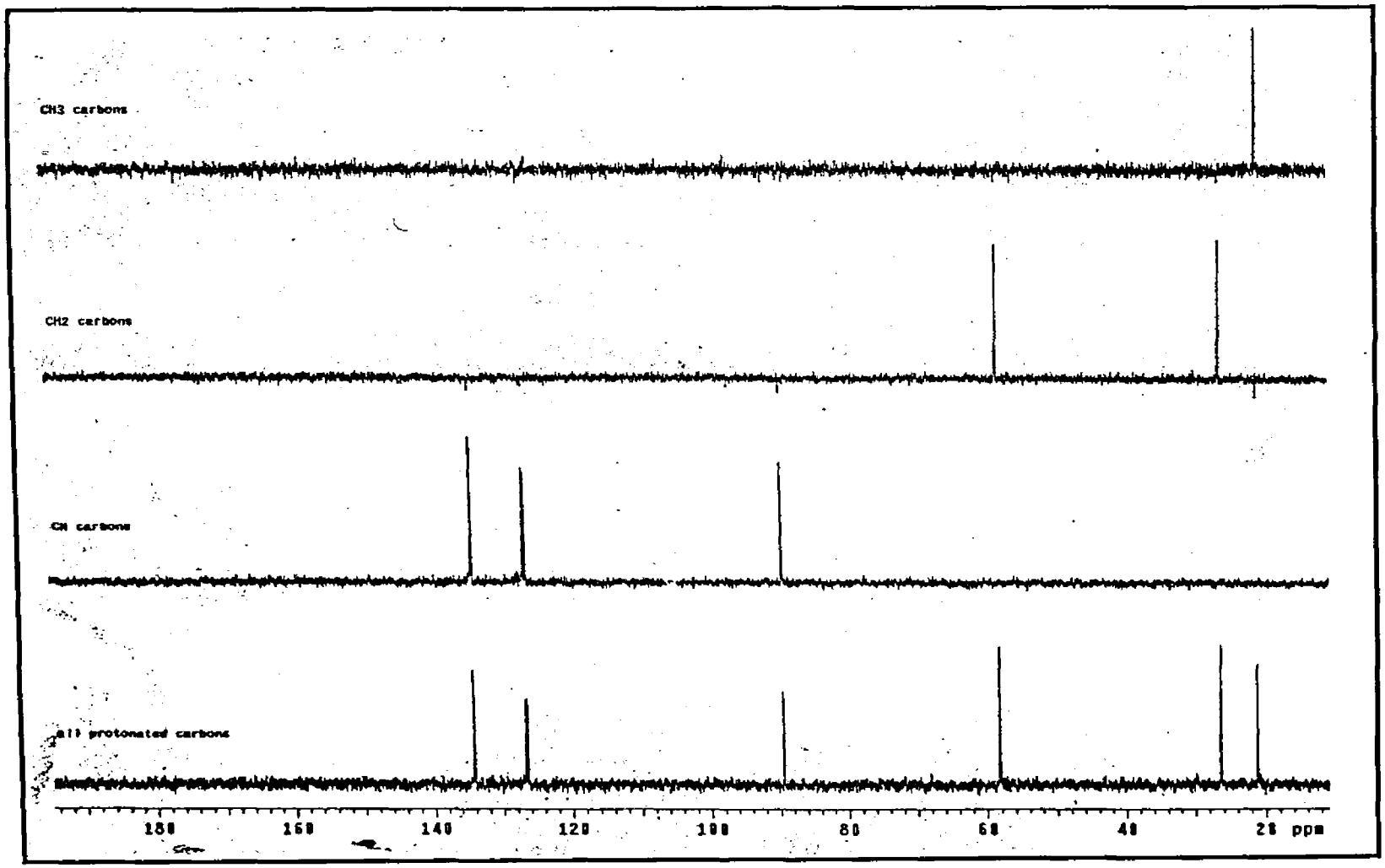
Appendix 20A: ^{1}H NMR spectrum of psychorubrin acetate (**81**), $CDCl_3$, 200 MHz



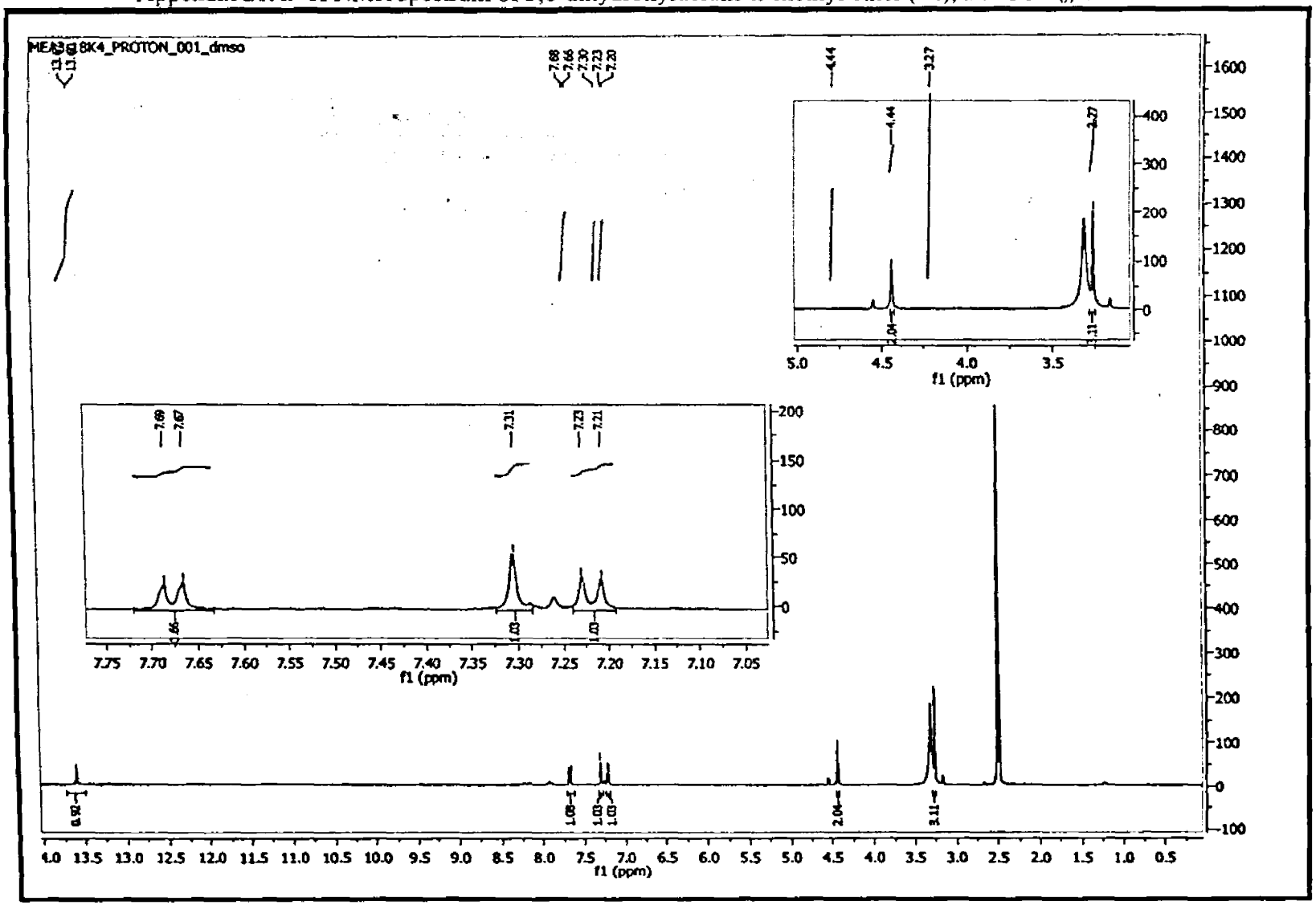
Appendix 20B: ^{13}C NMR spectrum of psychorubrin acetate (**81**), $CDCl_3$, 50MHz



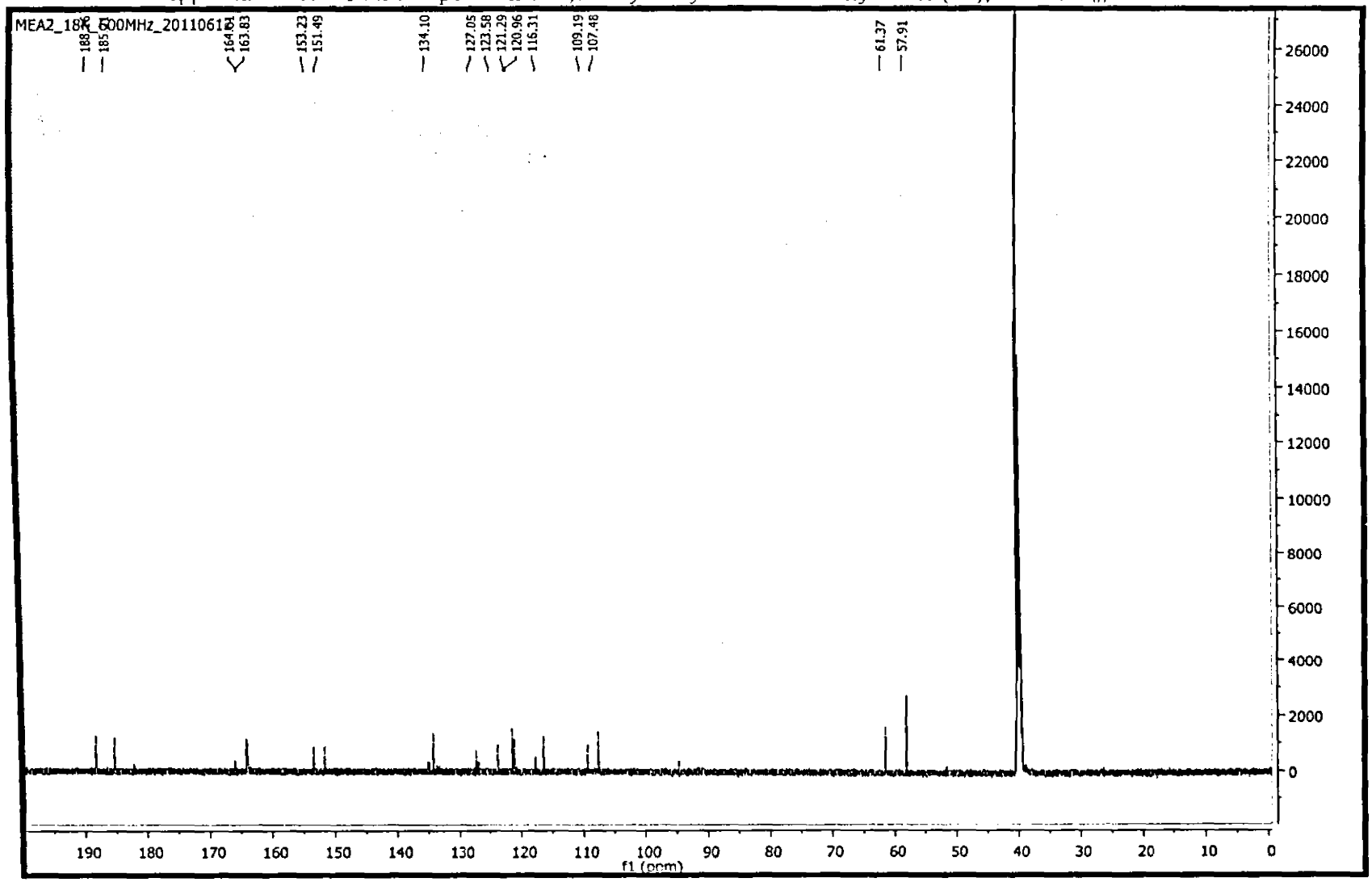
Appendix 20C: DEPT spectrum of psychorubrin acetate (**81**), $CDCl_3$, 50MHz



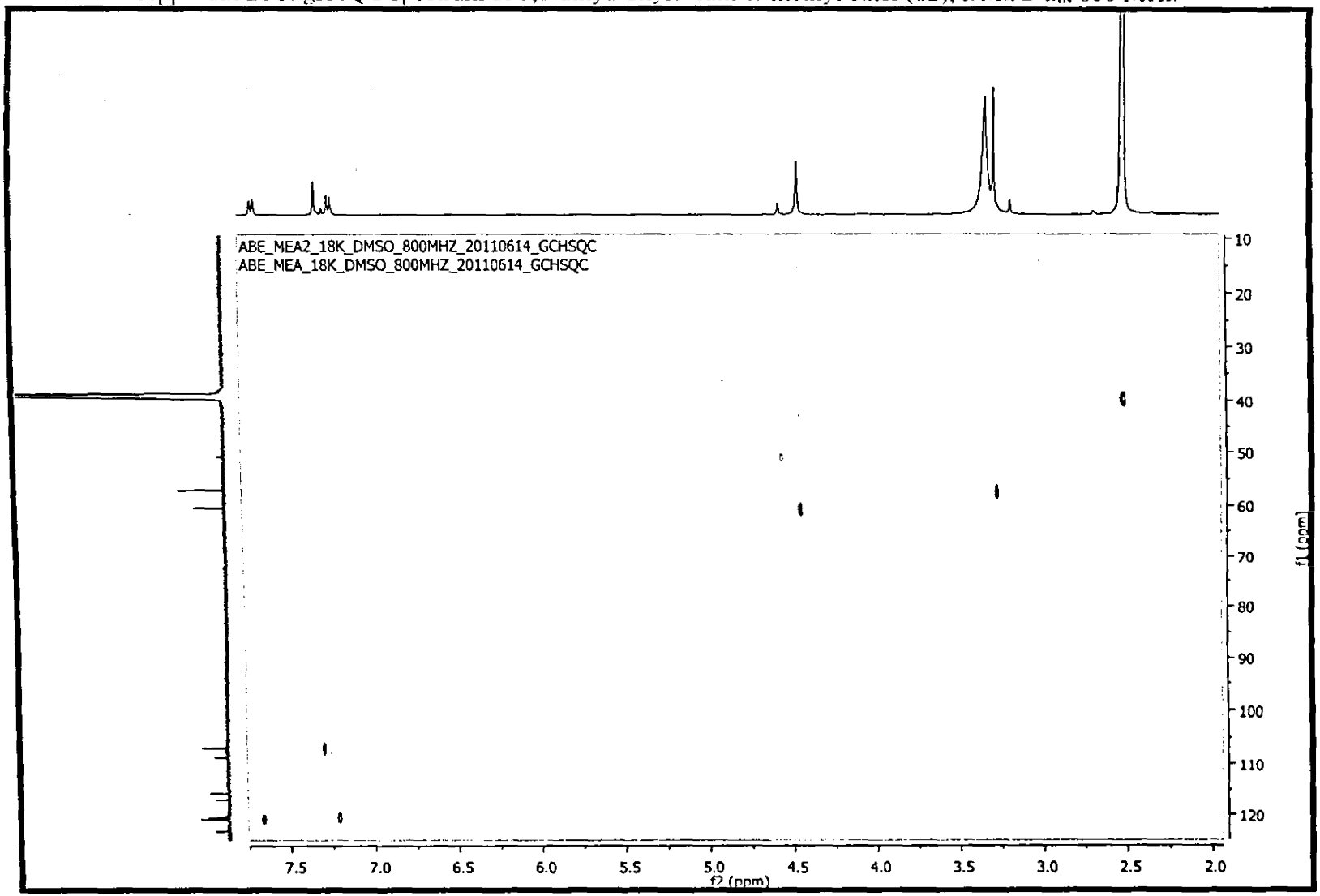
Appendix 21A: ^{1}H NMR spectrum of 5,6-dihydroxylucidin-ω-methyl ether (**82**), DMSO-d_6, 800 MHz



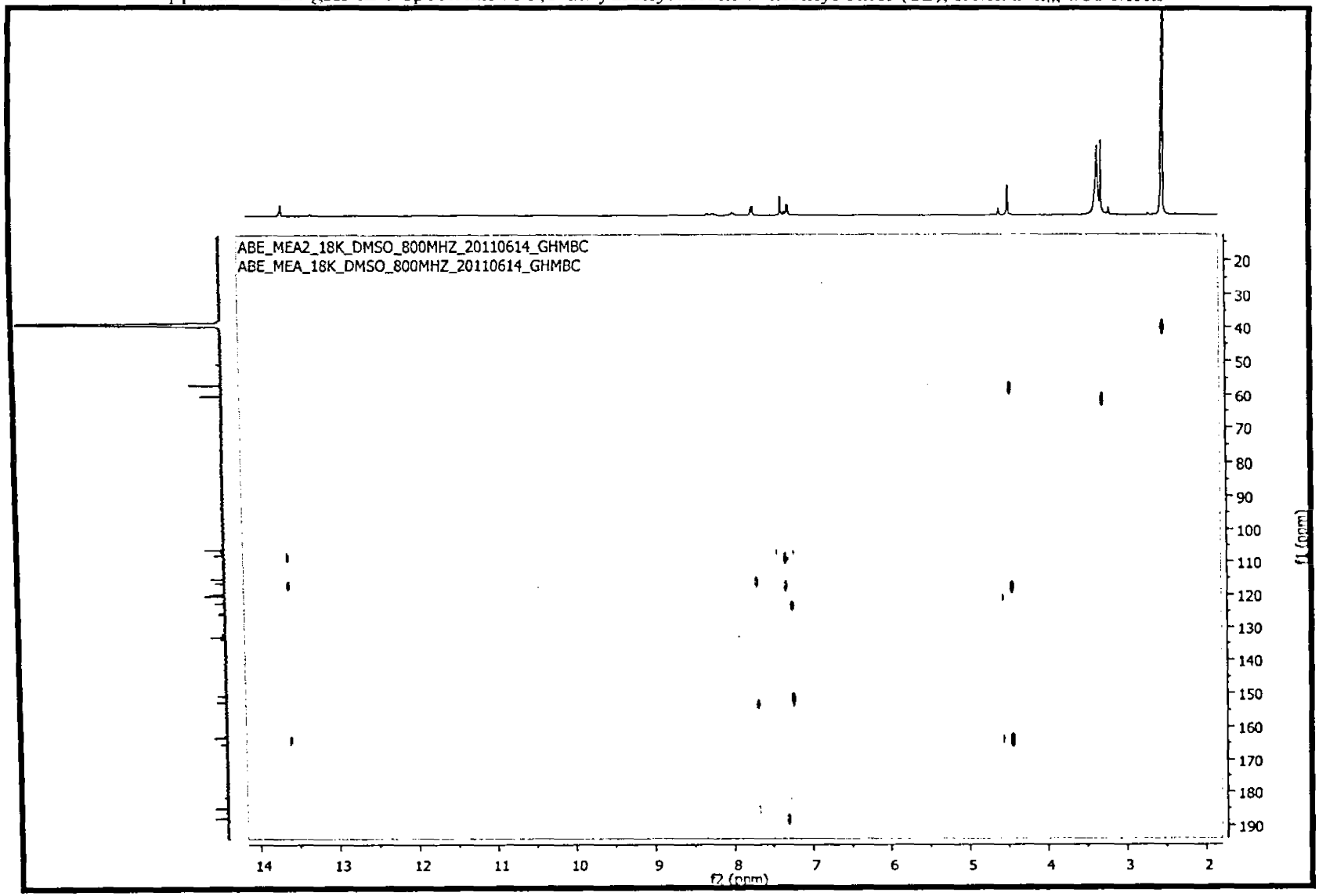
Appendix 21B: ^{13}C NMR spectrum of 5,6-dihydroxylucidin-ω-methyl ether (**82**), DMSO-d_6, 200 MHz



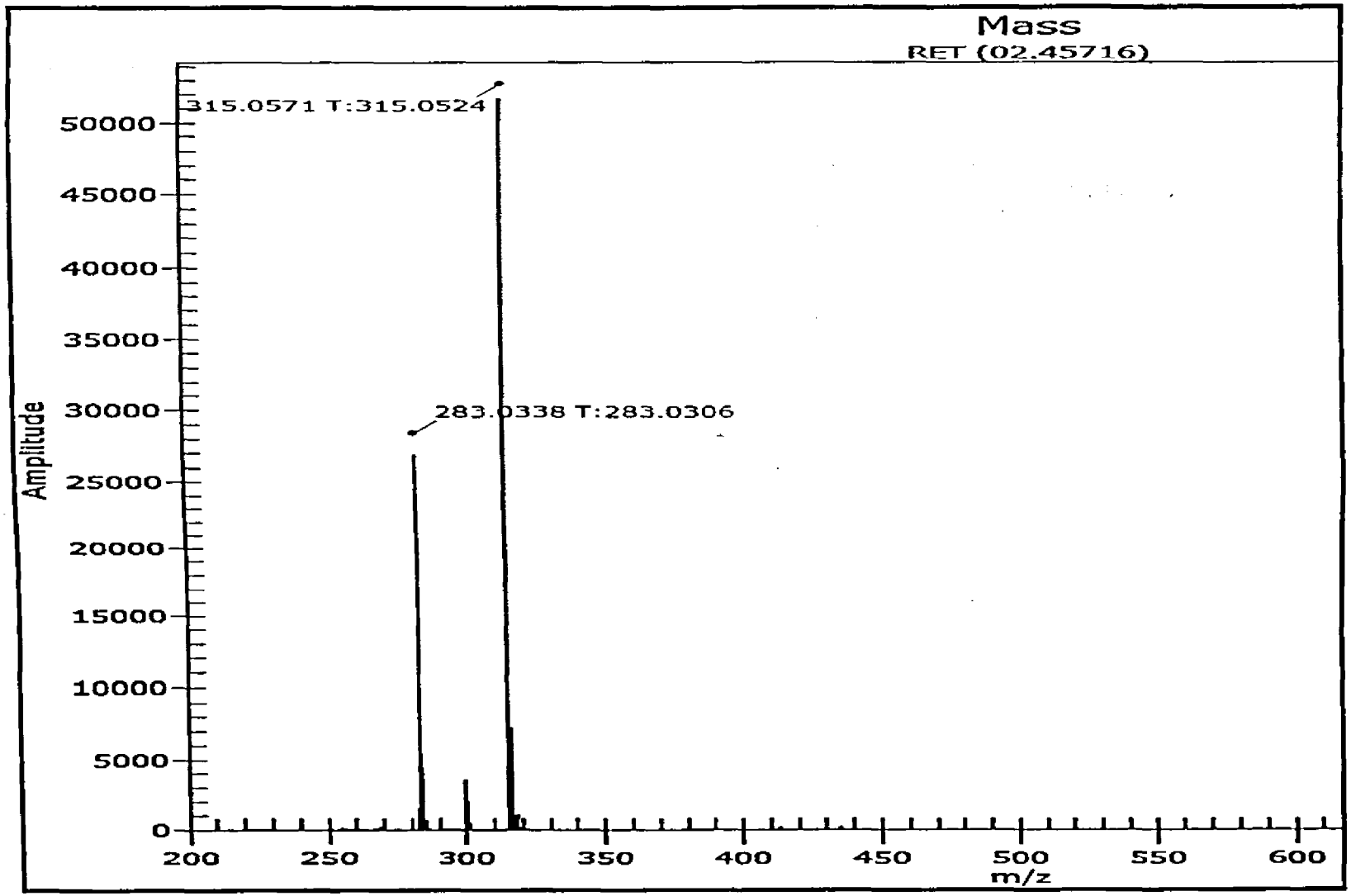
Appendix 21C: gHSQC spectrum of 5,6-dihydroxylucidin-ω-methyl ether (**82**), DMSO-d_6, 800 MHz



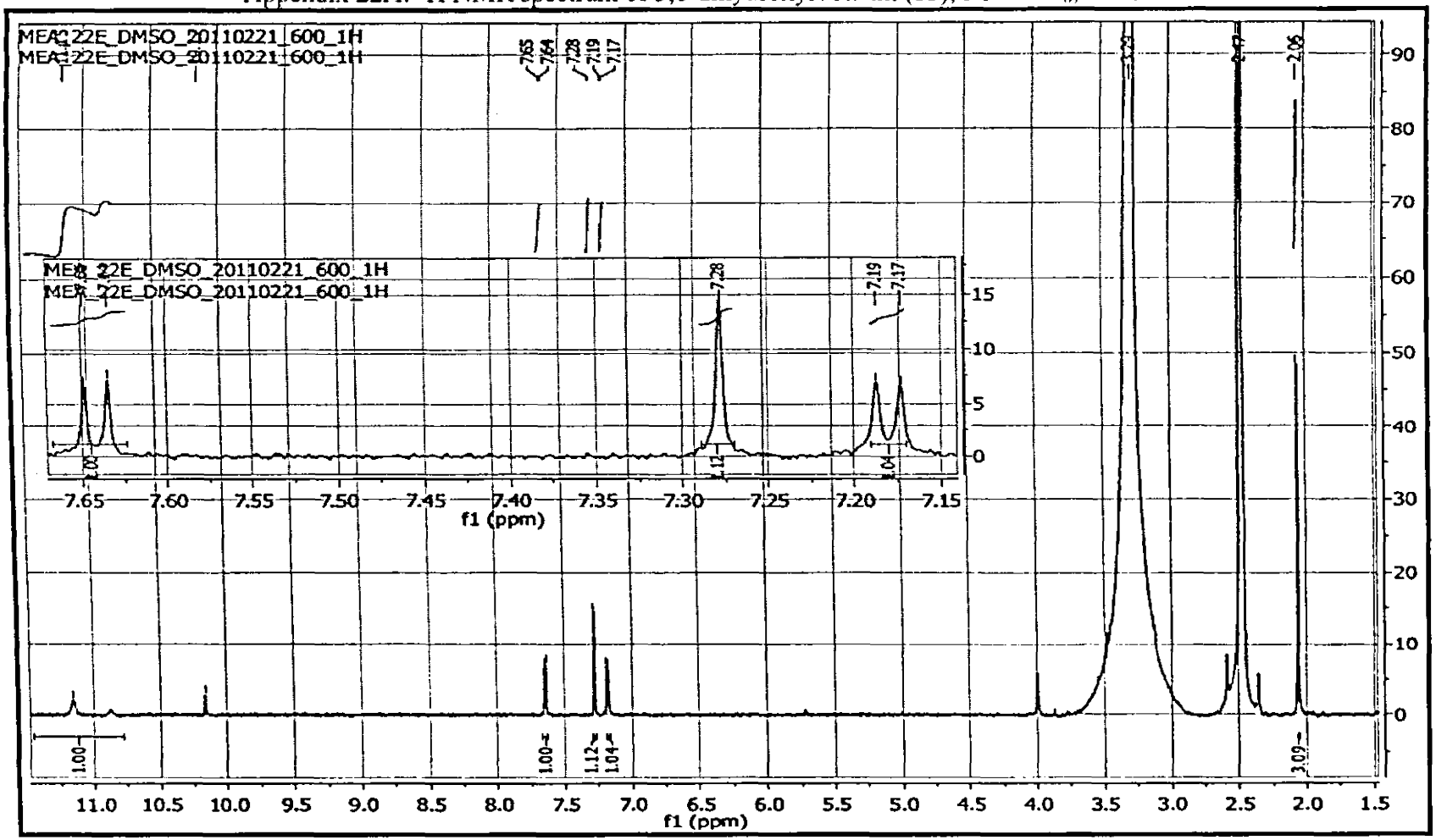
Appendix 21D: gHMBC spectrum of 5,6-dihydroxylucidin-ω-methyl ether (**82**), DMSO-d_6, 800 MHz



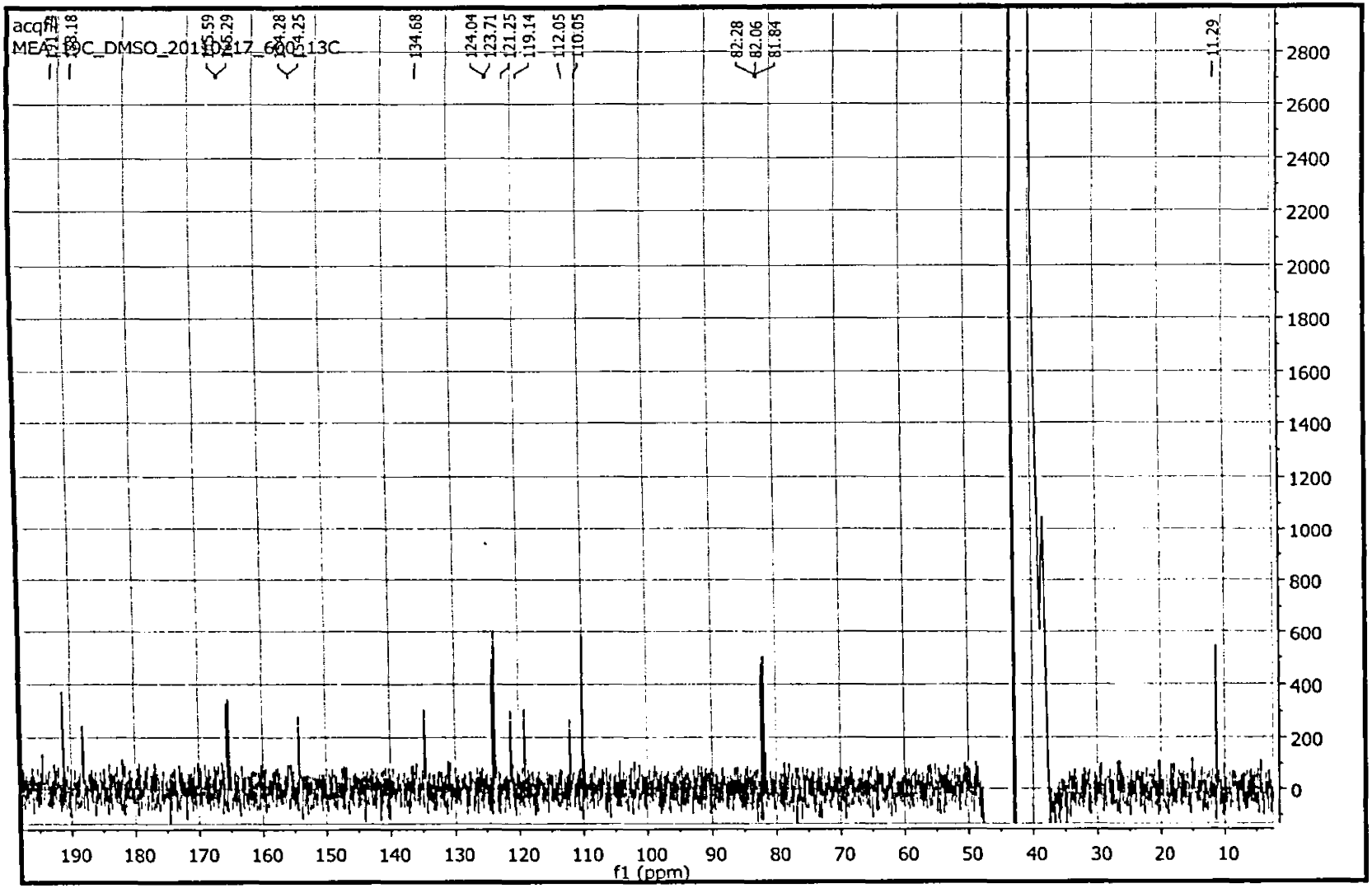
Appendix 21E: HRMS chromatogram of 5,6-dihydroxylucidin-ω-methyl ether (82)



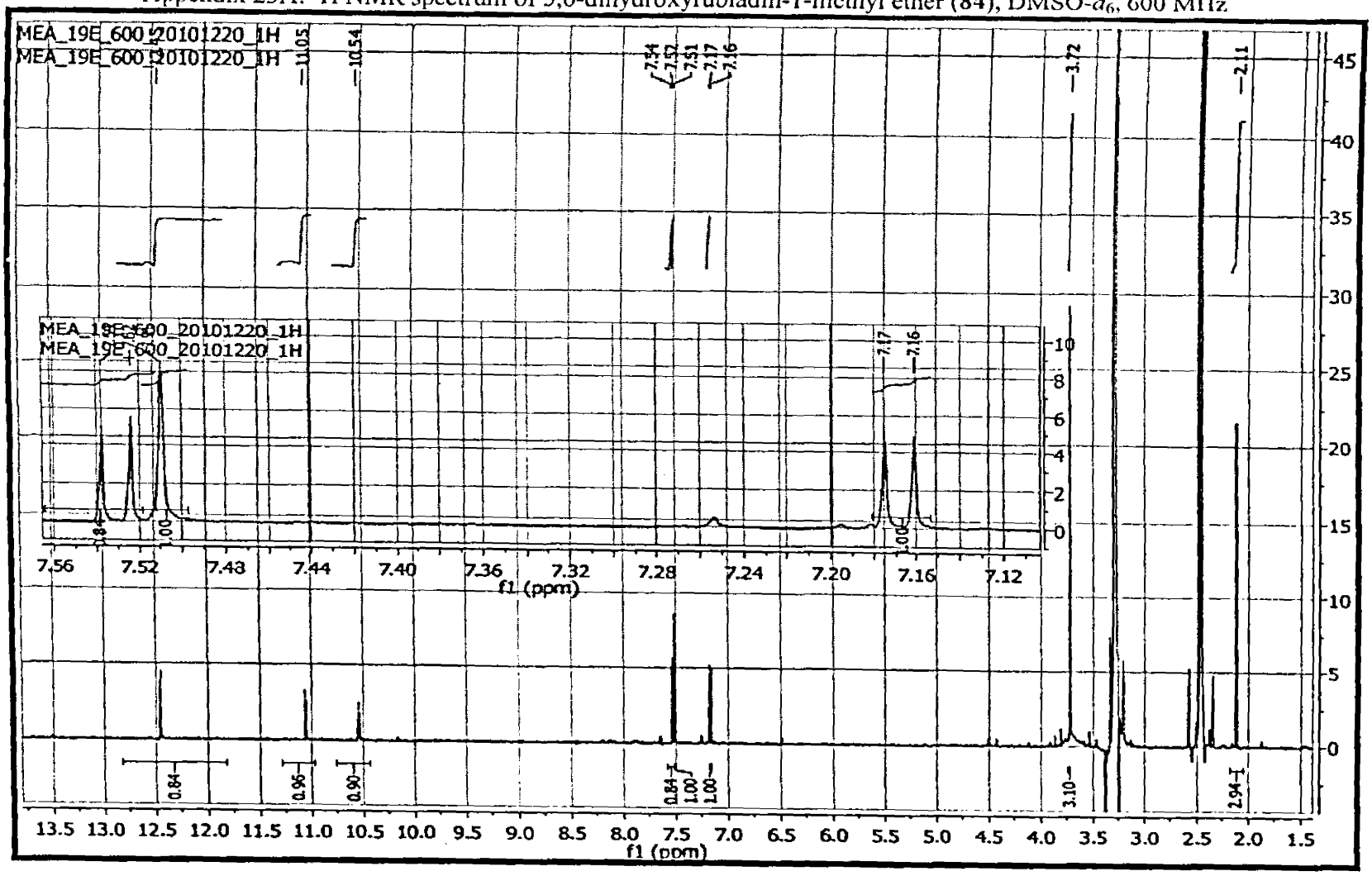
Appendix 22A: ^{1}H NMR spectrum of 5,6-dihydroxyrubiadin (**83**), DMSO-d_6, 600 MHz



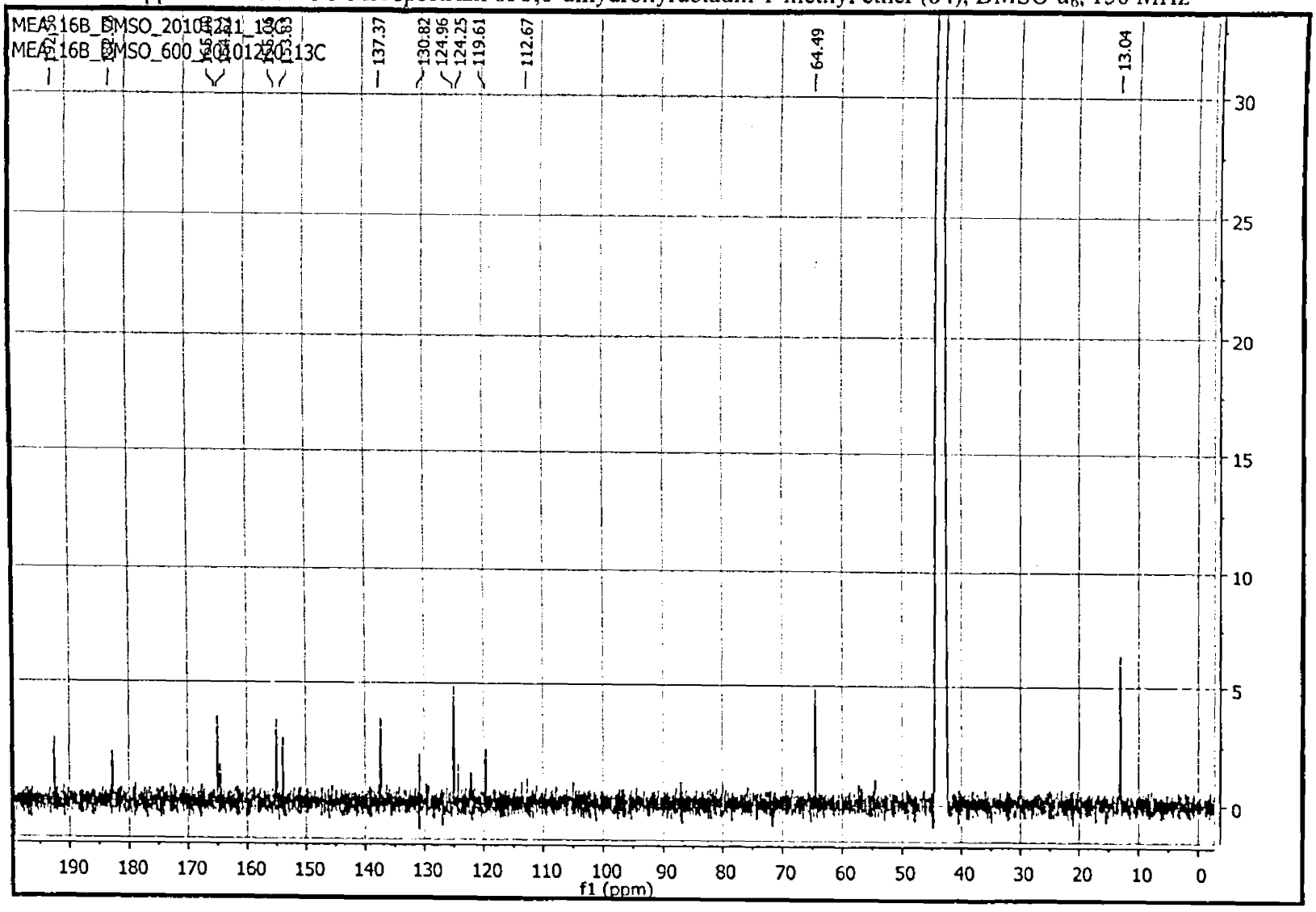
Appendix 22B: ^{13}C NMR spectrum of 5,6-dihydroxyrubiadin (**83**), DMSO-d_6, 800 MHz



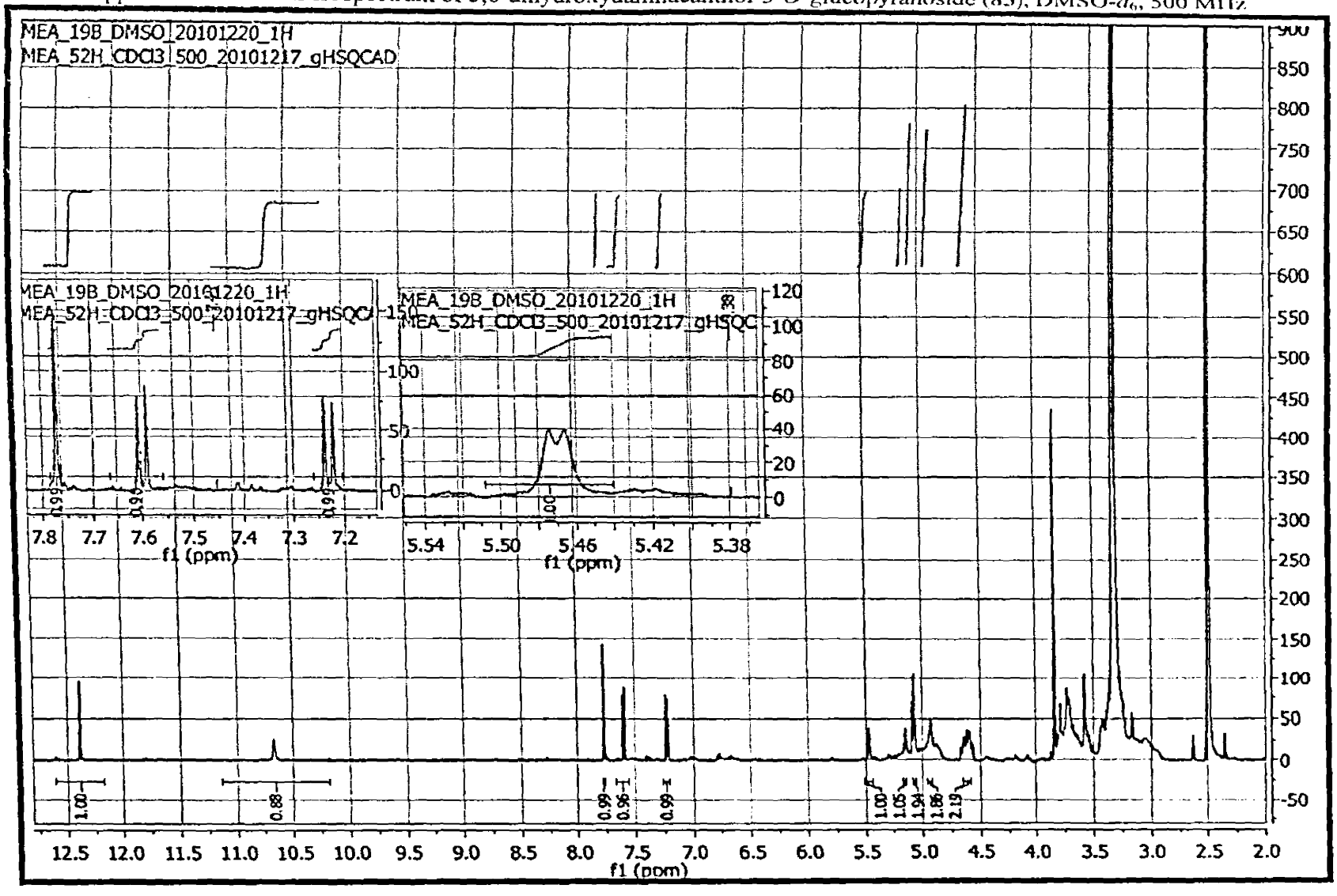
Appendix 23A: ^{1}H NMR spectrum of 5,6-dihydroxyrubiadin-1-methyl ether (84), DMSO-d_6, 600 MHz



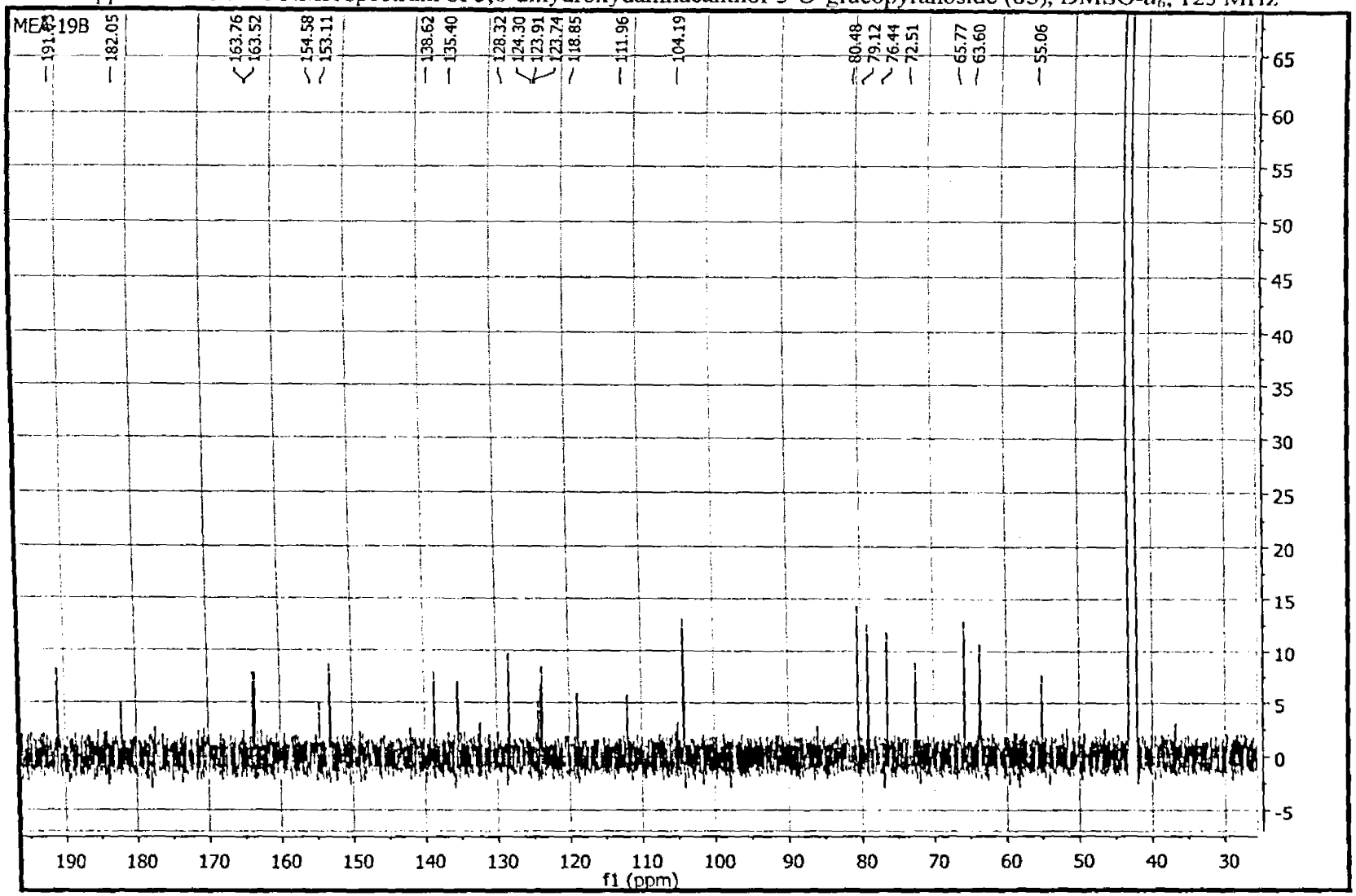
Appendix 23B: ^{13}C NMR spectrum of 5,6-dihydroxyrubiadin-1-methyl ether (84), DMSO-d_6, 150 MHz



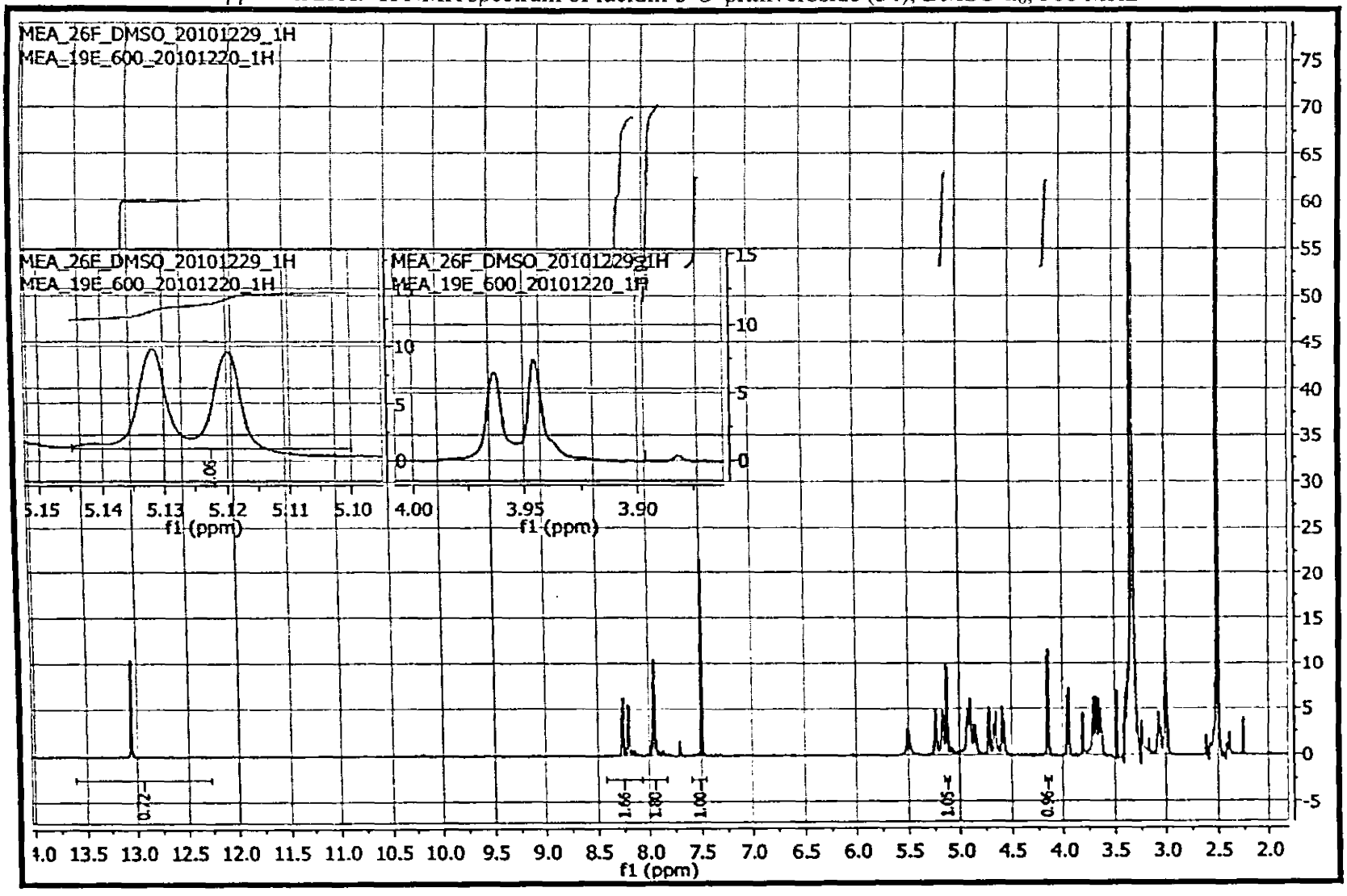
Appendix 24A: ^{1}H NMR spectrum of 5,6-dihydroxydamnacanthol-3-*O*-glucopyranoside (85), DMSO-d_6, 500 MHz



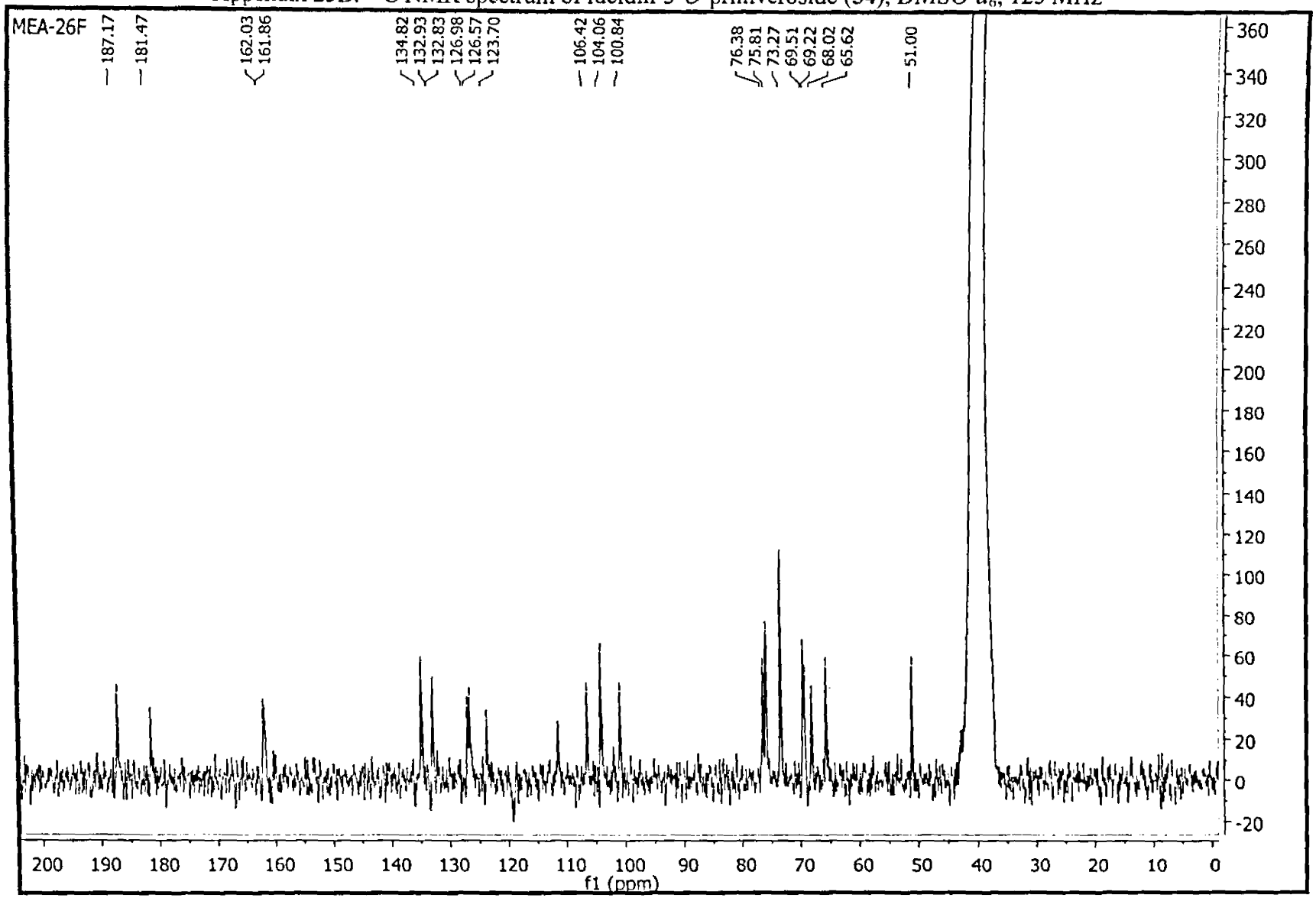
Appendix 24B: ^{13}C NMR spectrum of 5,6-dihydroxydamnacanthol-3-*O*-glucopyranoside (**85**), DMSO-d_6, 125 MHz



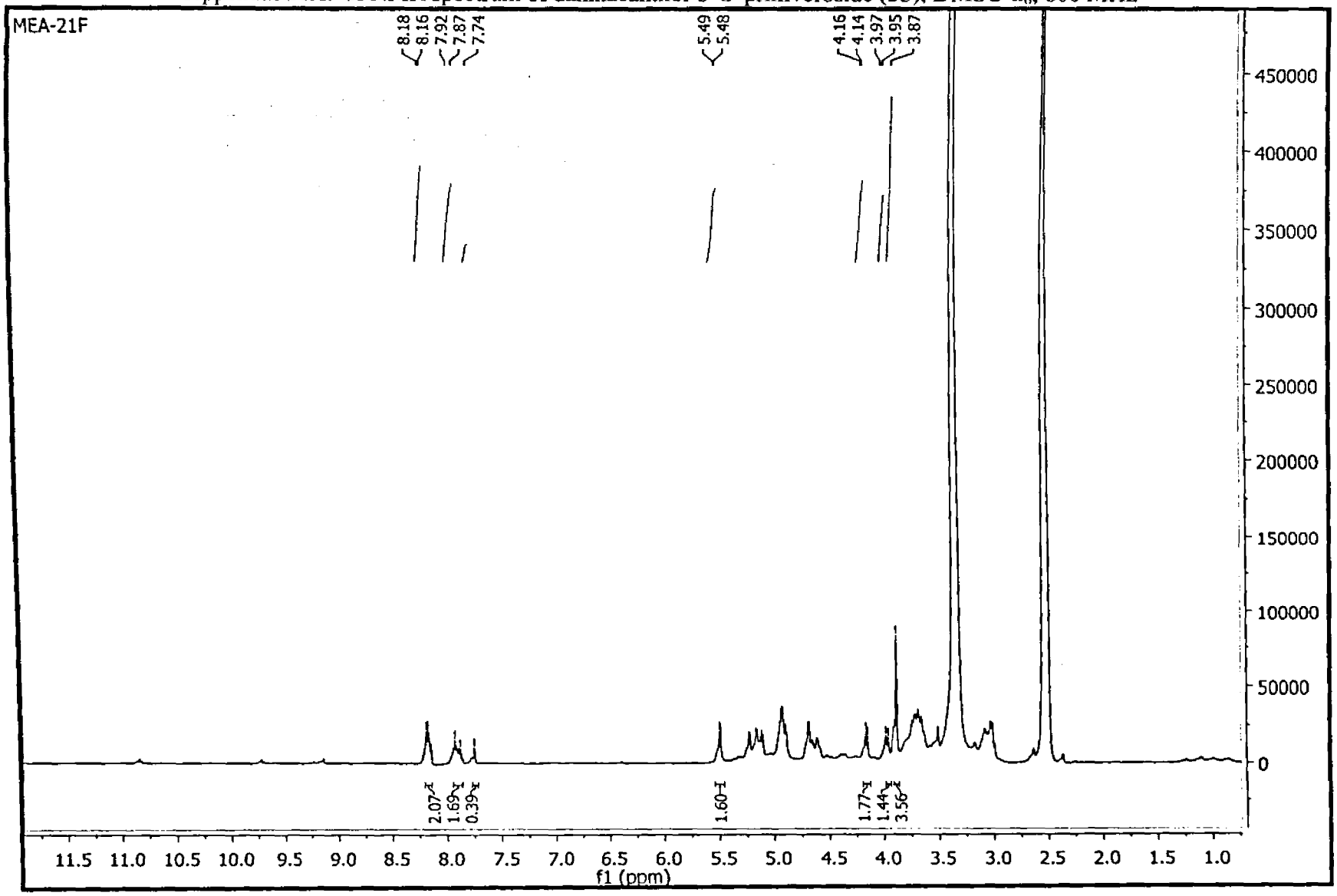
Appendix 25A: ^{1}H NMR spectrum of lucidin-3-*O*-primveroside (**54**), DMSO-d_6, 500 MHz



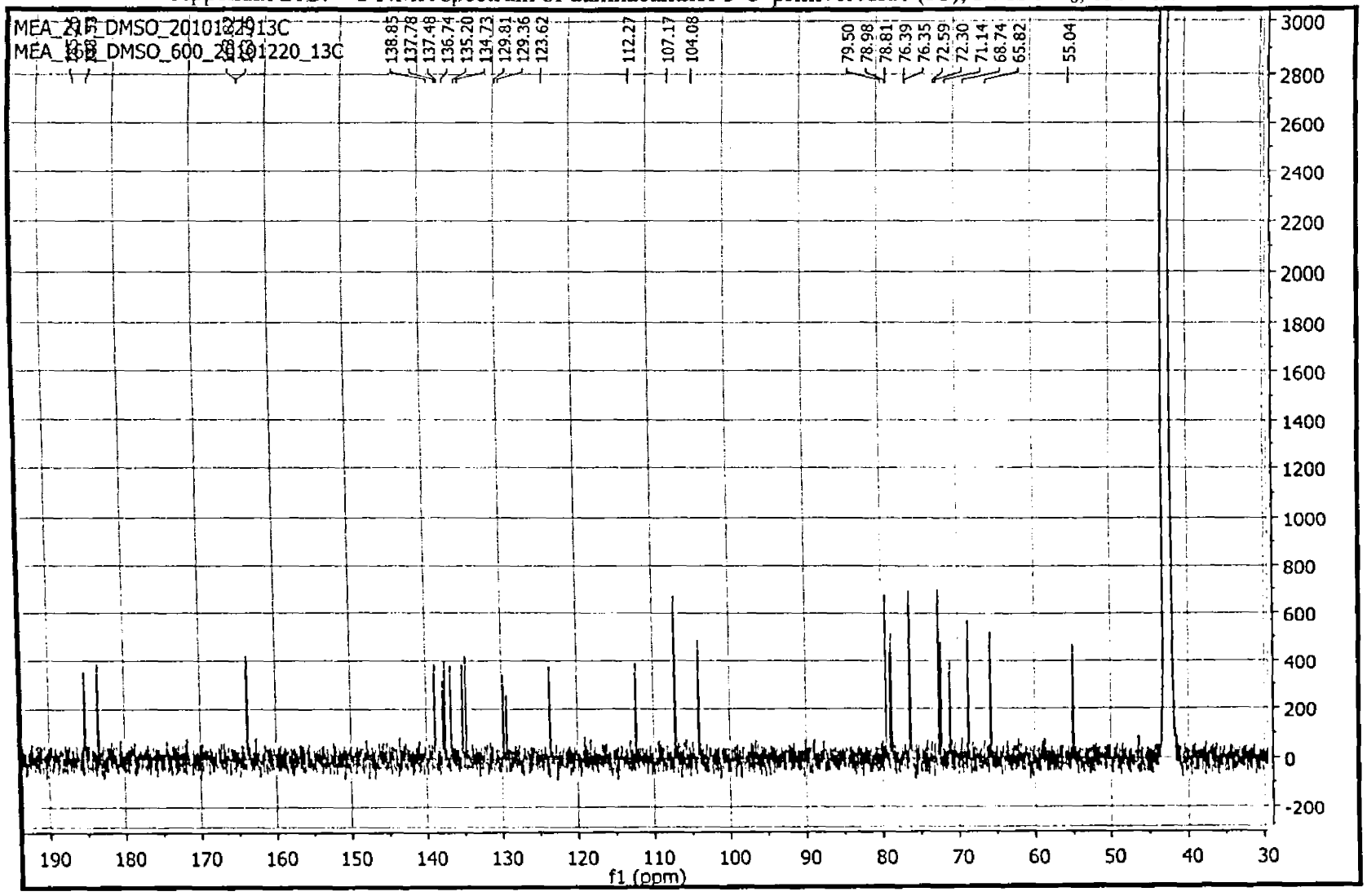
Appendix 25B: ^{13}C NMR spectrum of lucidin-3-*O*-primveroside (**54**), DMSO-d_6, 125 MHz



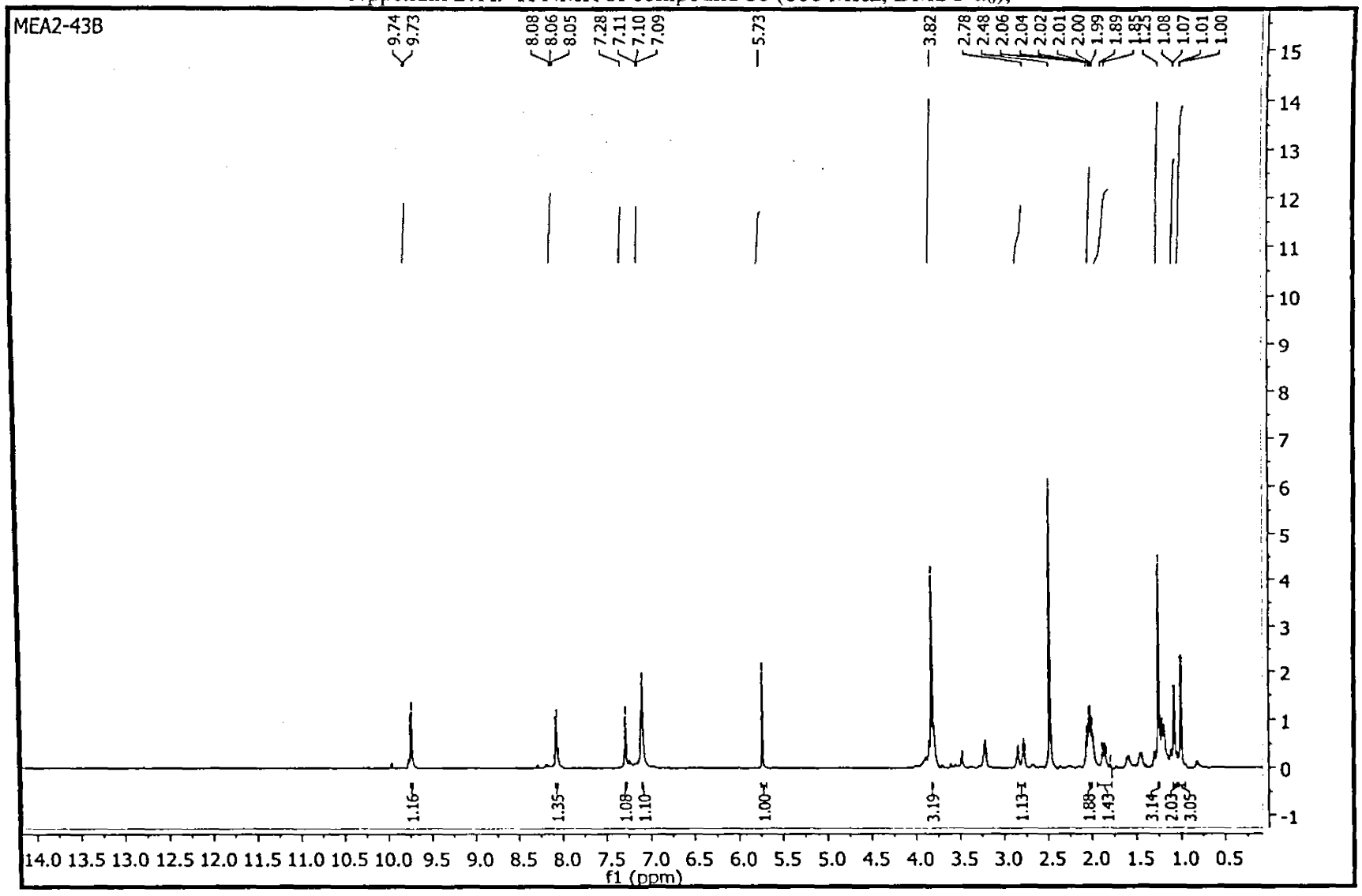
Appendix 26A: ^{1}H NMR spectrum of damnacanthol-3-*O*-primveroside (**55**), DMSO-d_6, 800 MHz



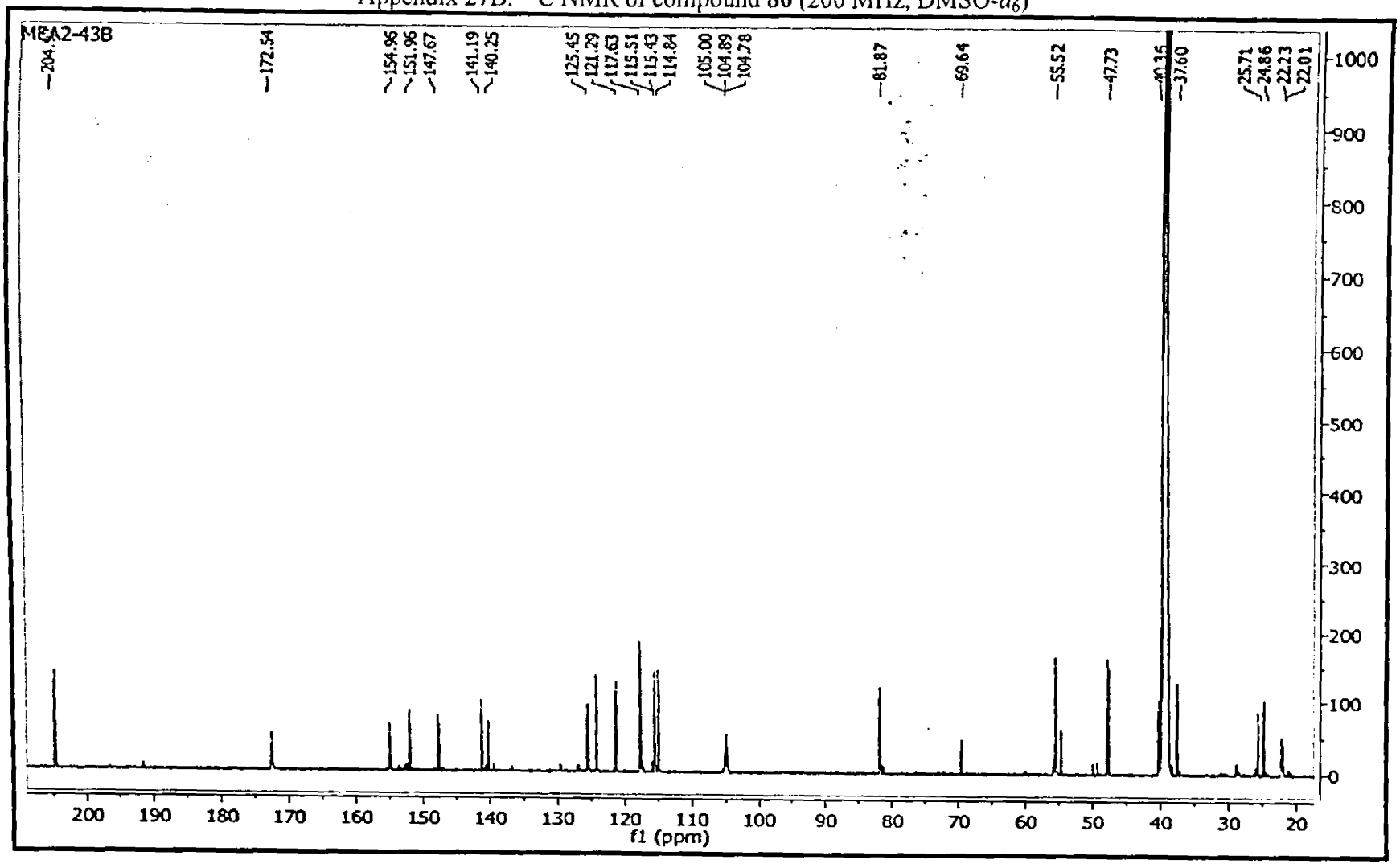
Appendix 26B: ^{13}C NMR spectrum of damnacanthol-3-*O*-primveroside (**55**), DMSO-d_6, 125 MHz



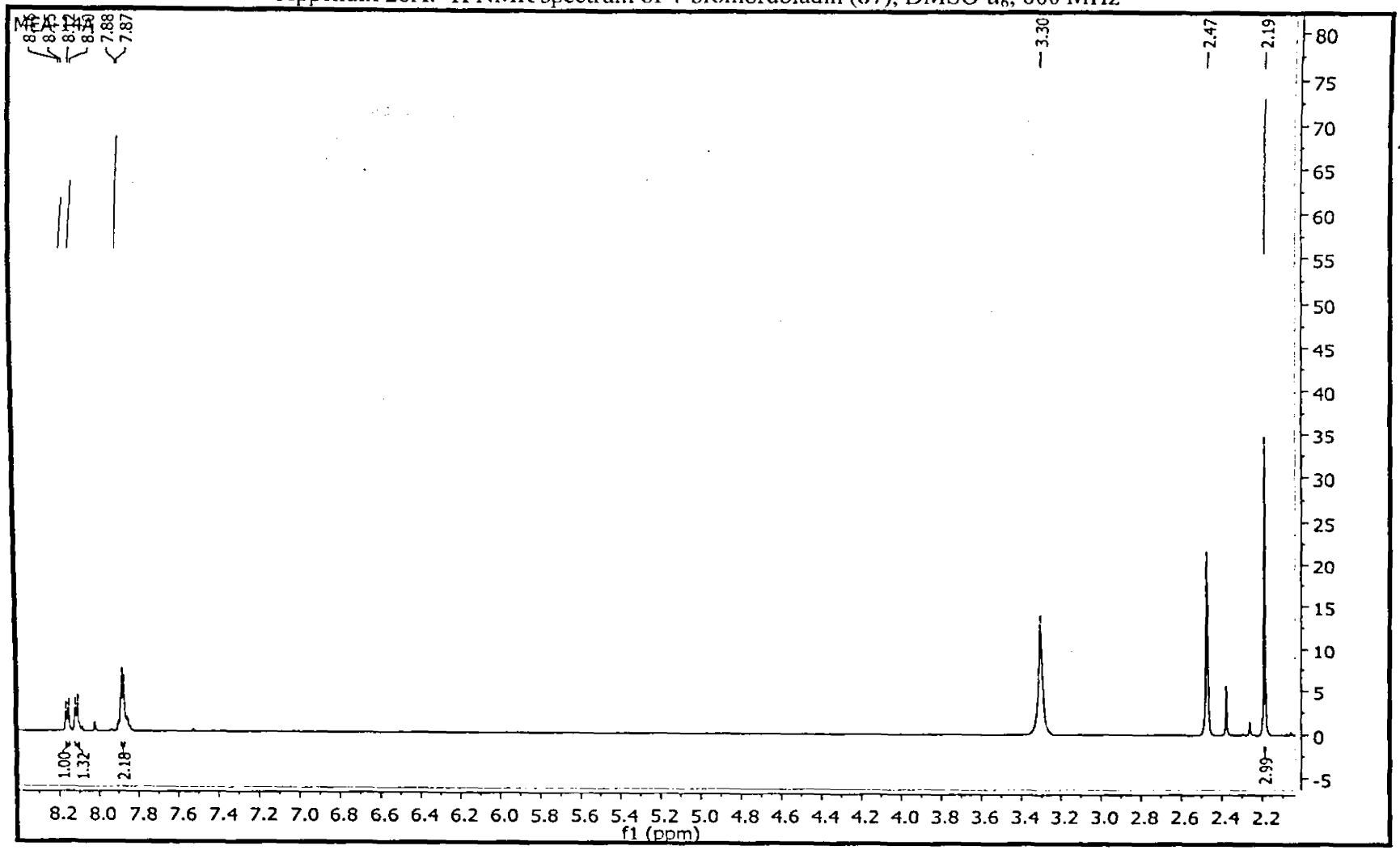
Appendix 27A. ^{1}H NMR of compound **86** (800 MHz, DMSO-d_6),



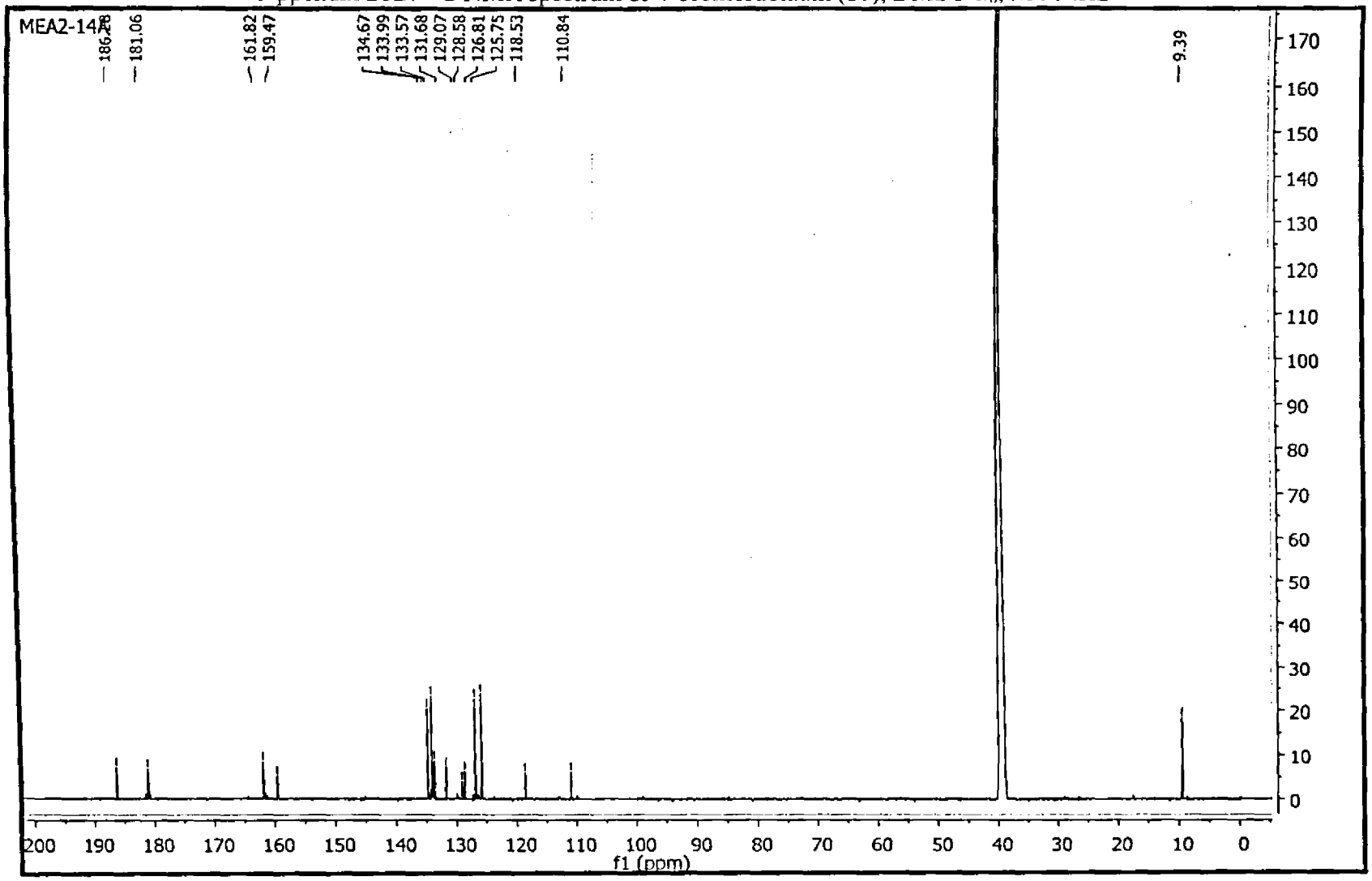
Appendix 27B. ^{13}C NMR of compound **86** (200 MHz, DMSO-d_6)



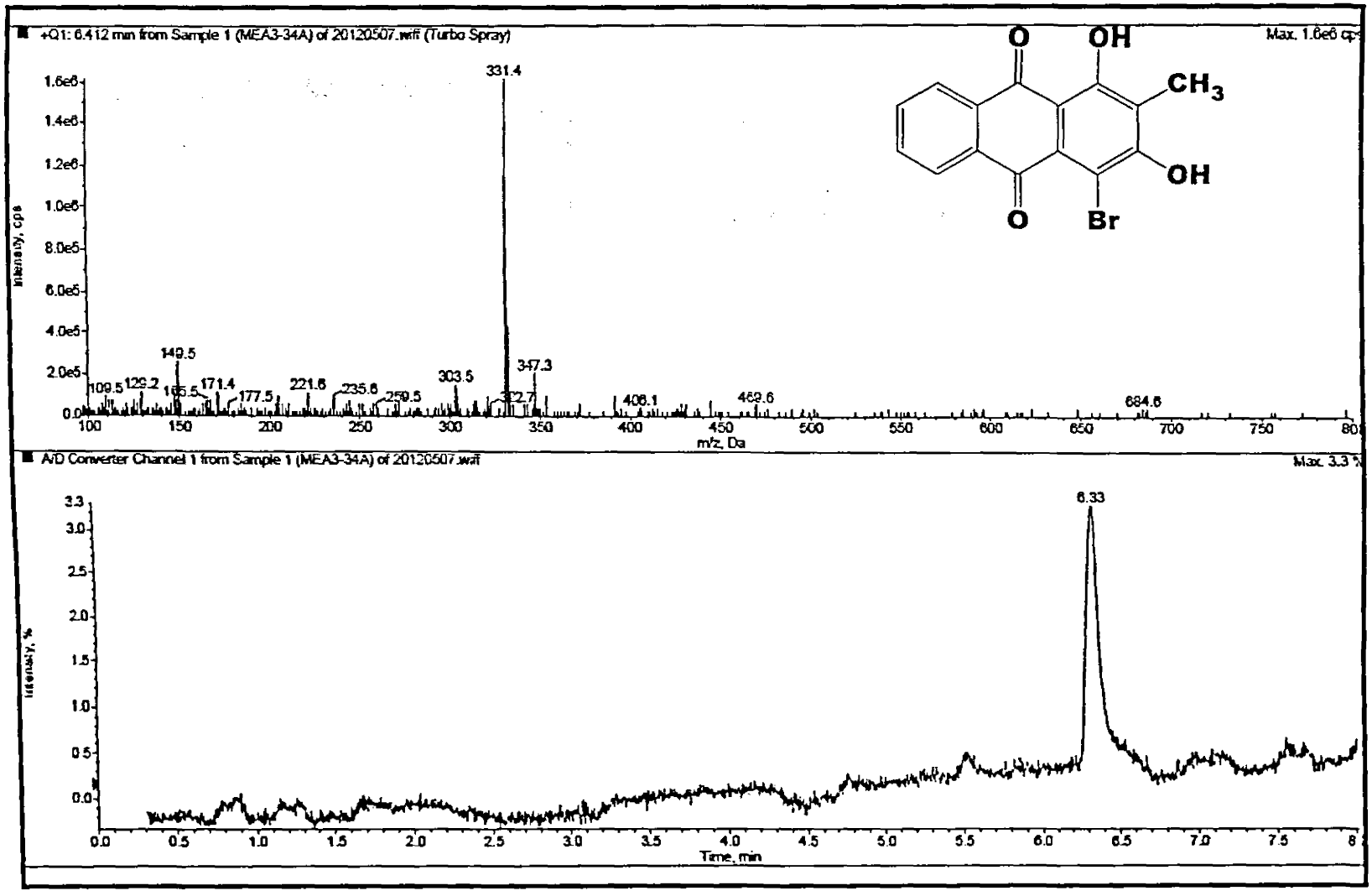
Appendix 28A: ^{1}H NMR spectrum of 4-bromorubiadin (87), DMSO-d_6, 800 MHz



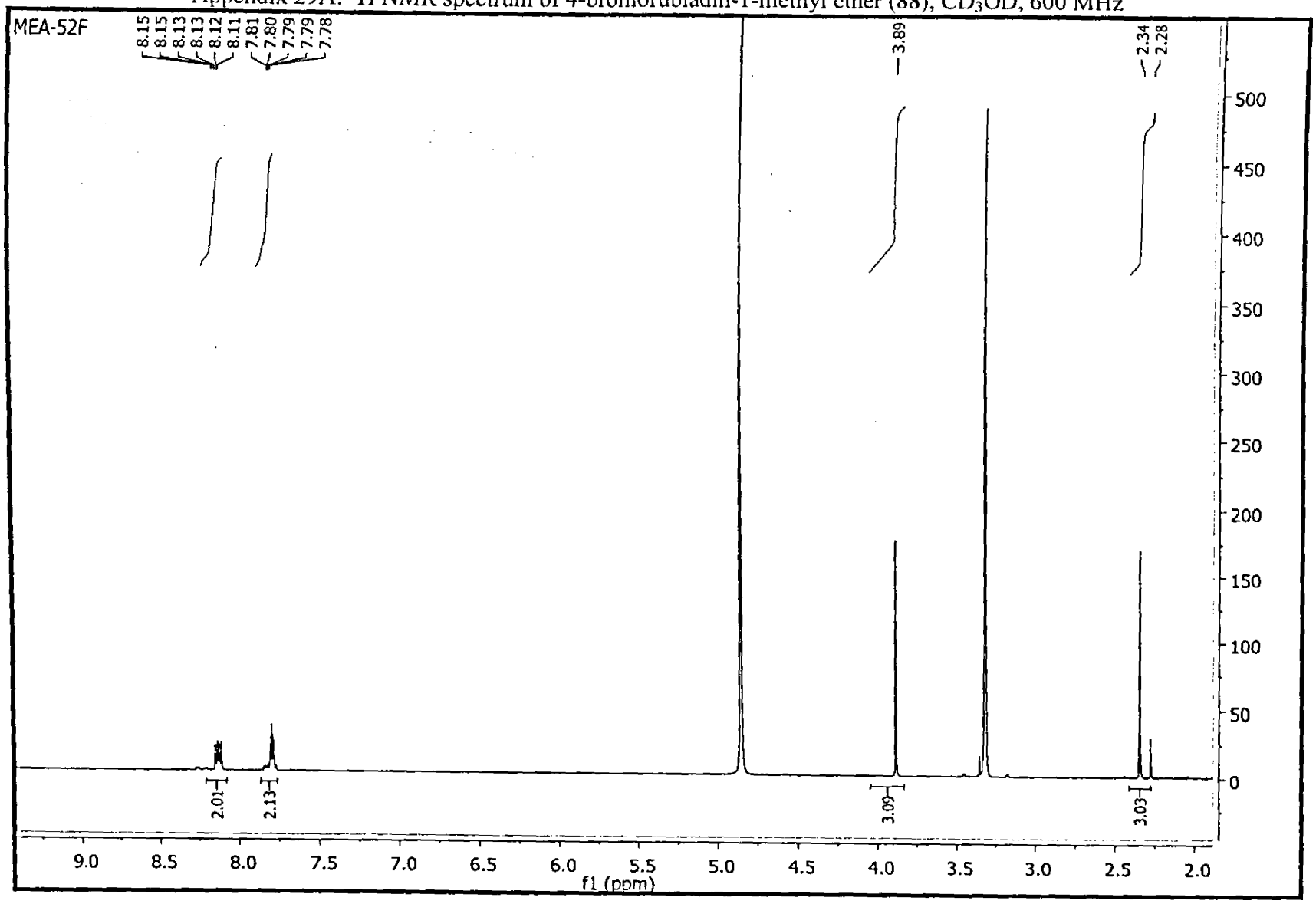
Appendix 28B: ^{13}C NMR spectrum of 4-bromorubiadin (**87**), DMSO-d_6, 200 MHz



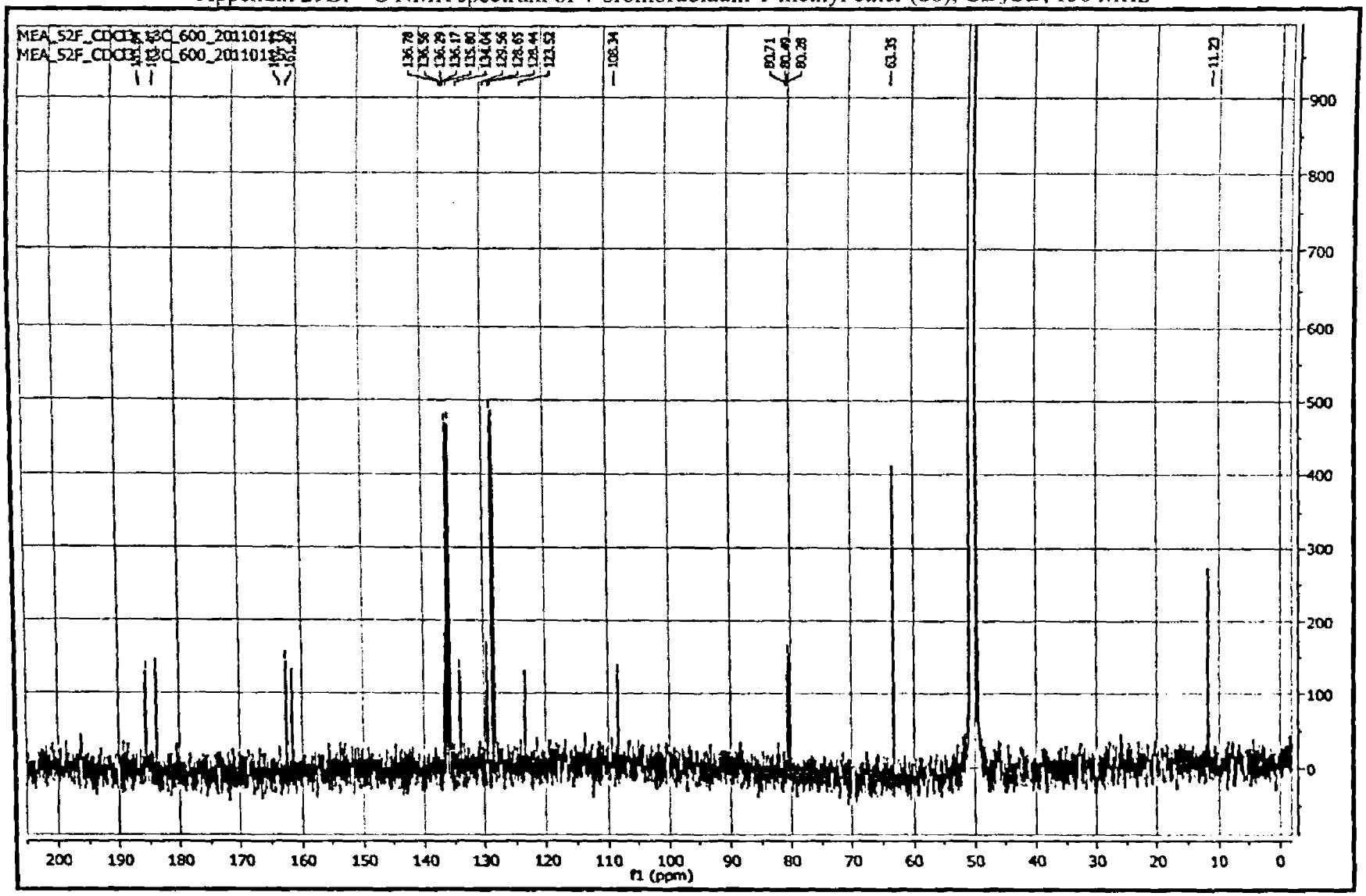
Appendix 28C: ESI-MS spectrum of 4-bromorubiadin (87)



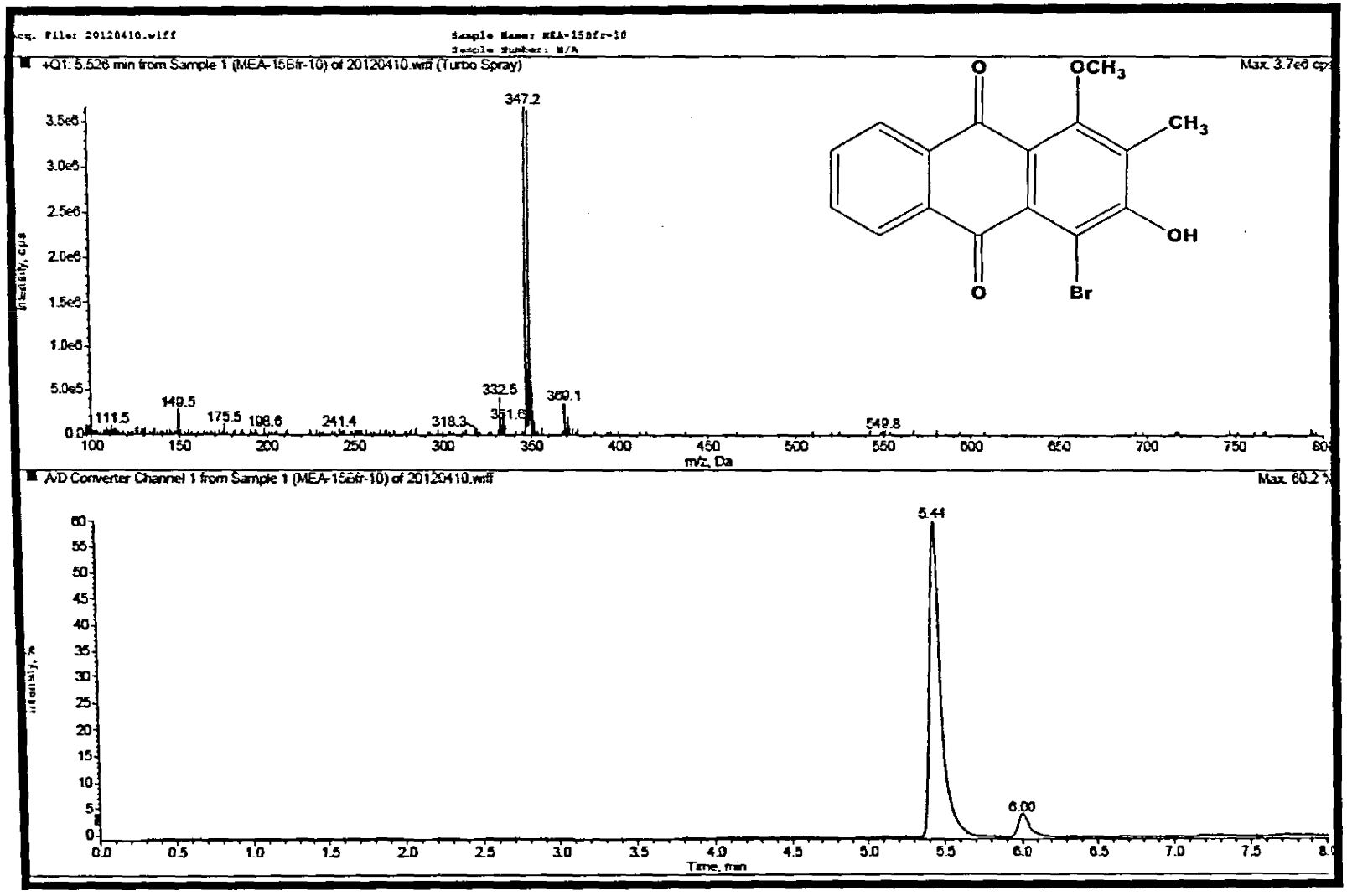
Appendix 29A: ^{1}H NMR spectrum of 4-bromorubiadin-1-methyl ether (**88**), CD_3OD, 600 MHz



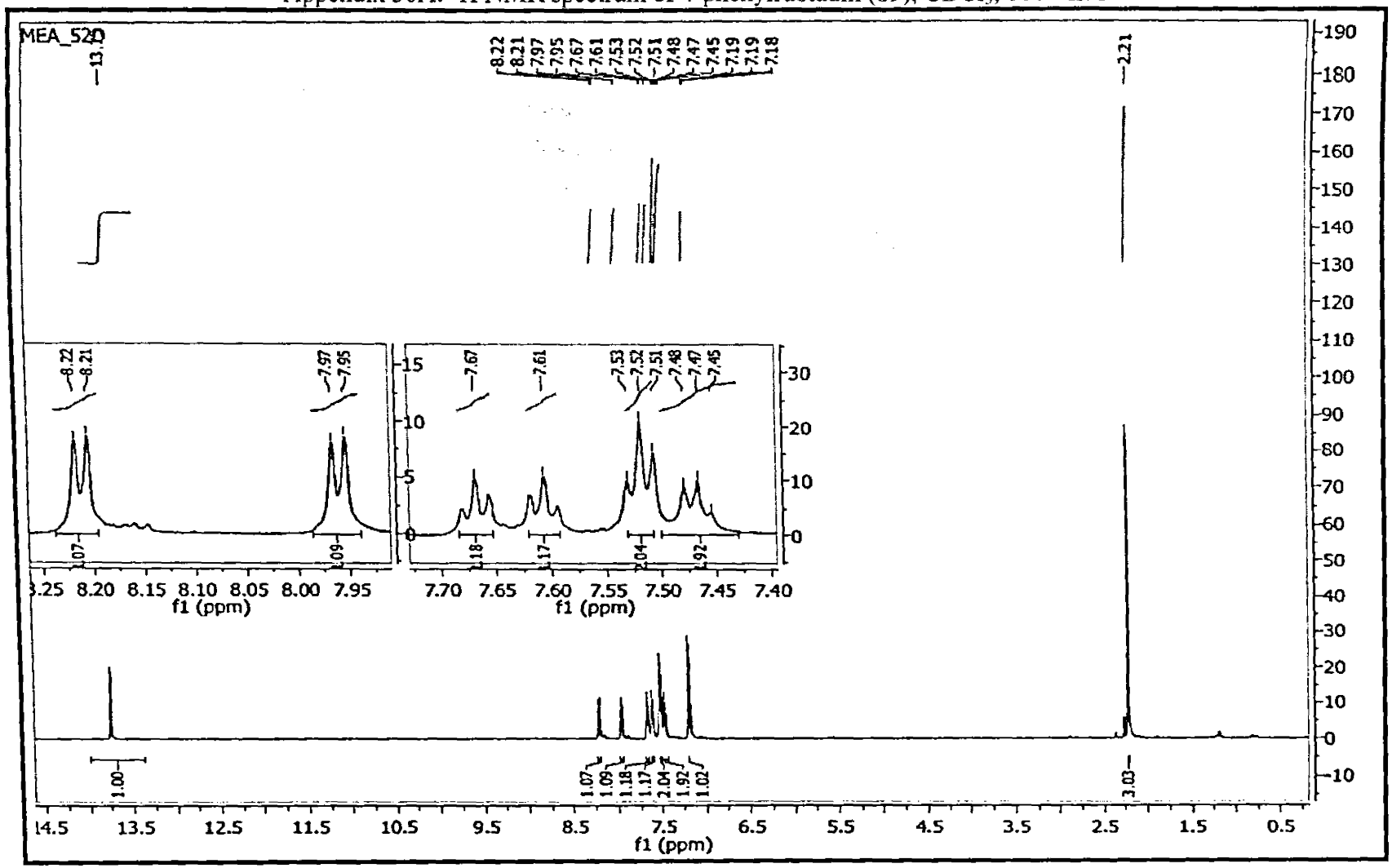
Appendix 29B: ^{13}C NMR spectrum of 4-bromorubiadin-1-methyl ether (88), CD_3OD, 150 MHz



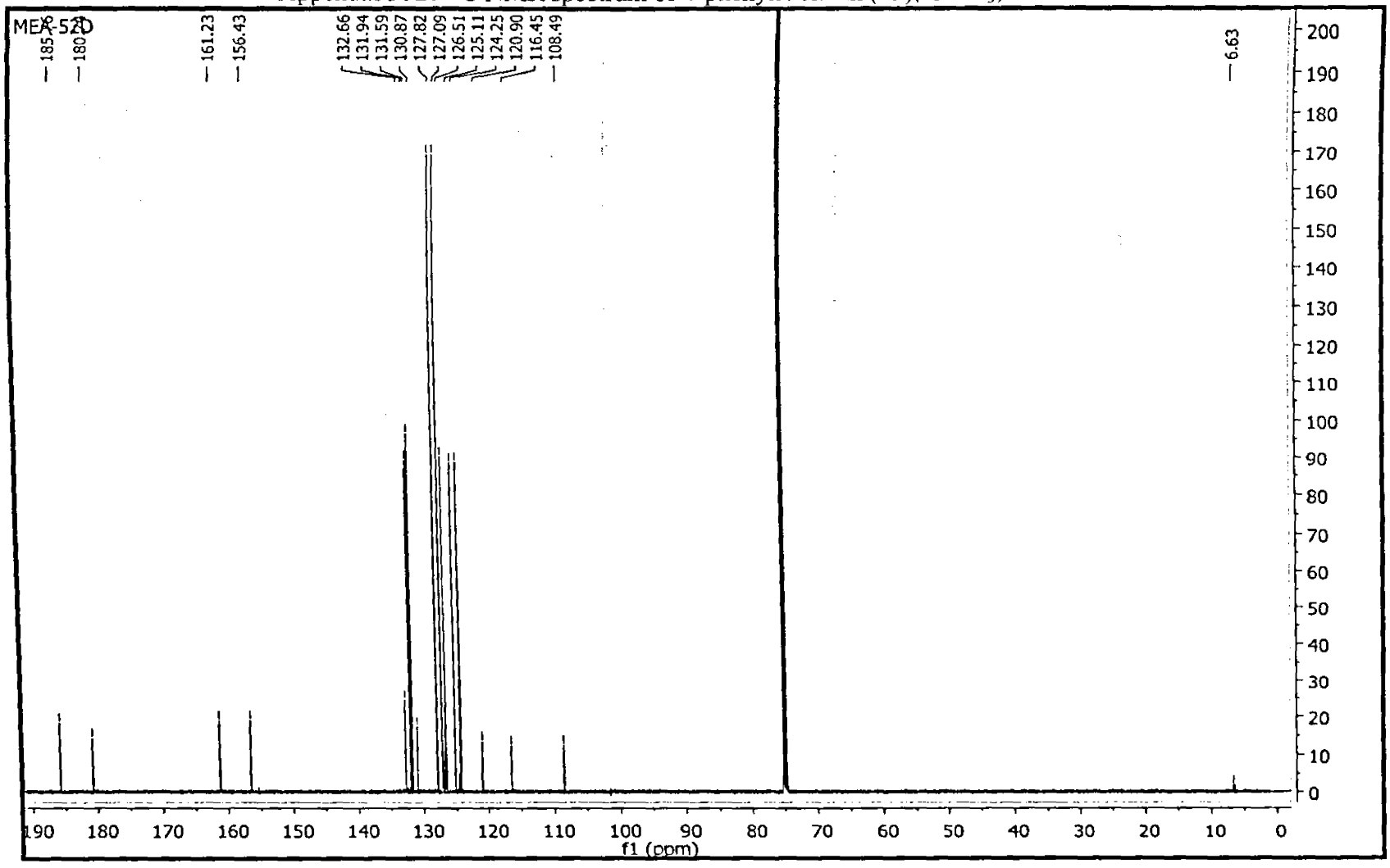
Appendix 29C: LC-MS chromatogram of 4-bromorubiadin-1-methyl ether (88)



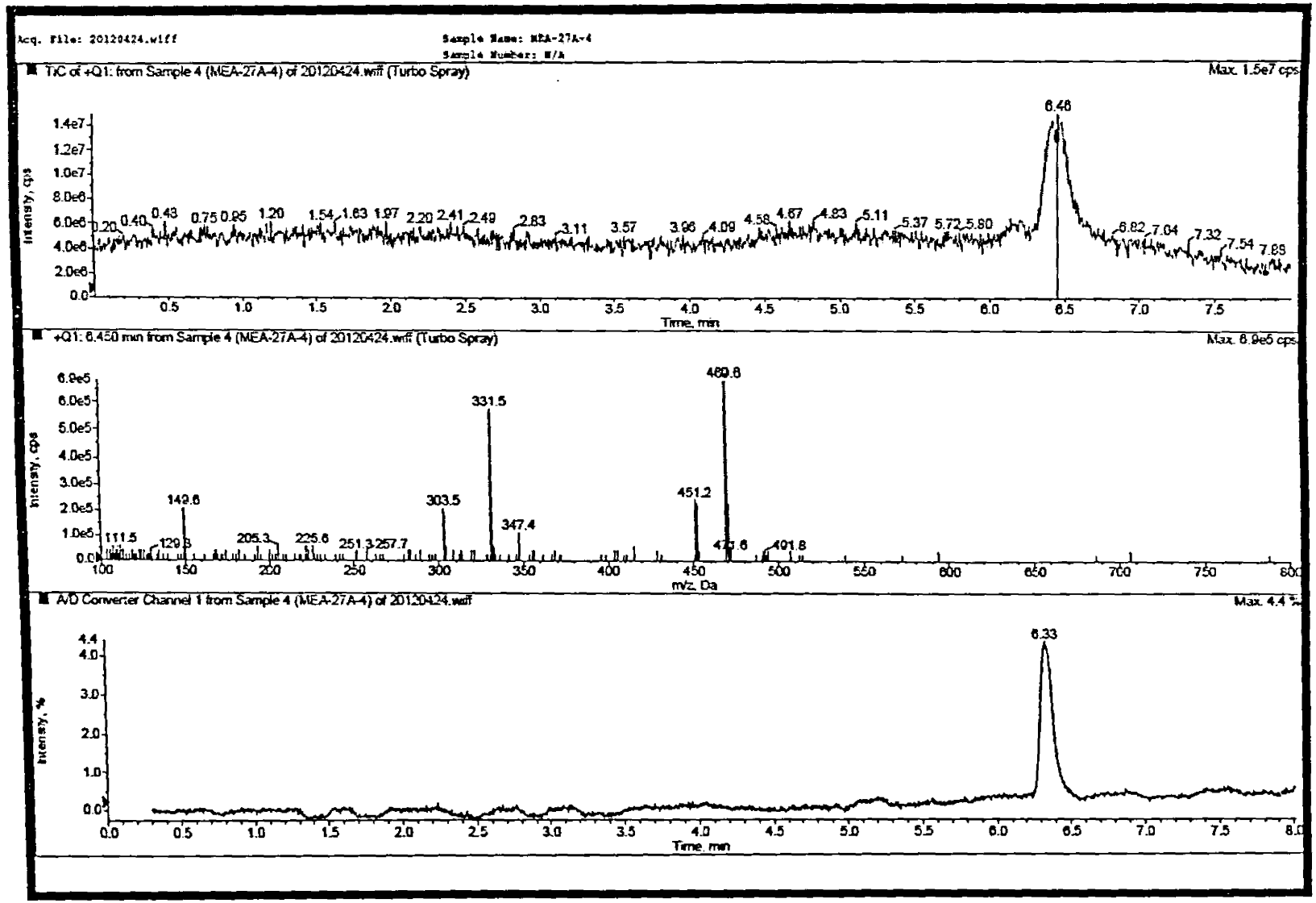
Appendix 30A: ^{1}H NMR spectrum of 4-phenylrubiadin (**89**), $CDCl_3$, 800 MHz



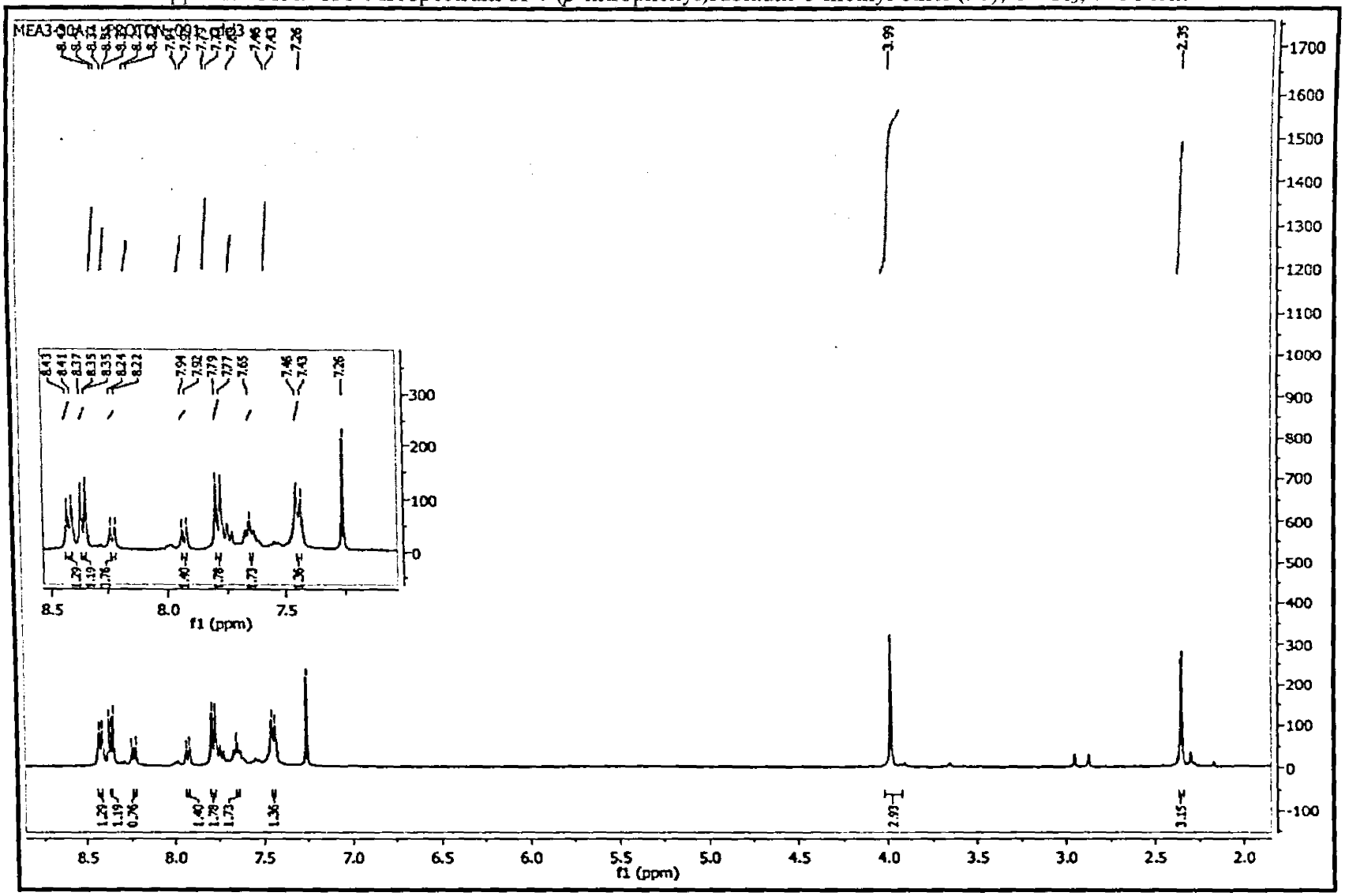
Appendix 30B: ^{13}C NMR spectrum of 4-phenylrubiadin (**89**), $CDCl_3$, 200 MHz



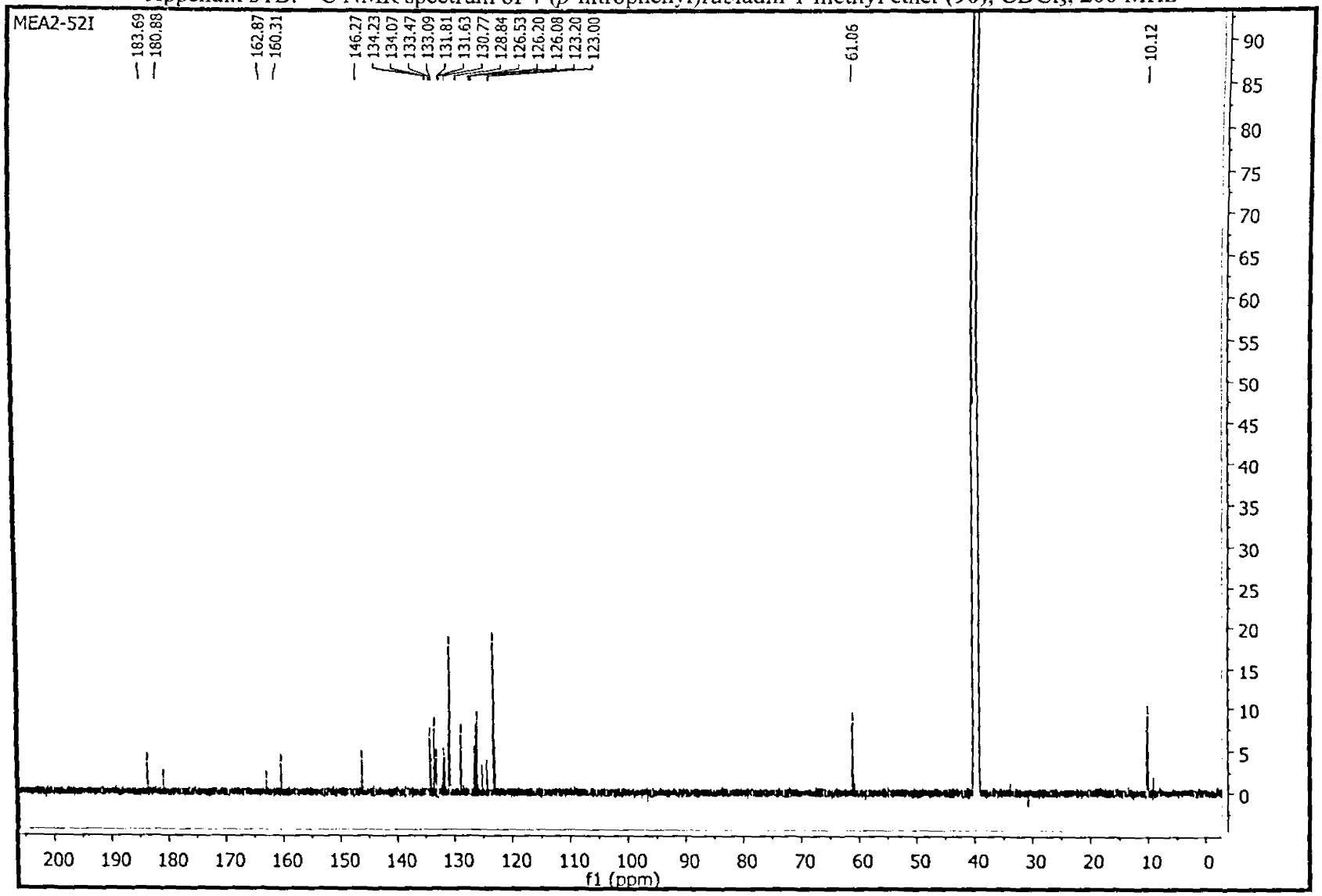
Appendix 30C: LC-MS spectrum of 4-phenylrubiadin (89)



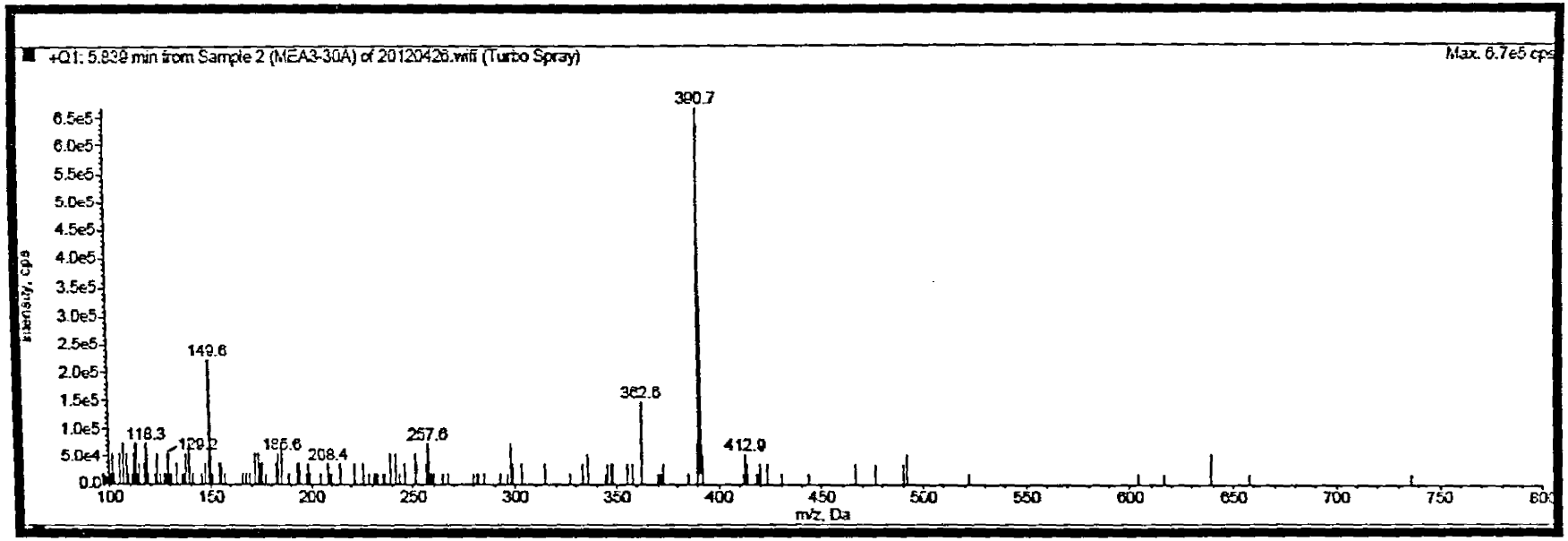
Appendix 31A: ^{1}H NMR spectrum of 4-(*p*-nitrophenyl)rubiadin-1-methyl ether (**90**), $CDCl_3$, 800 MHz



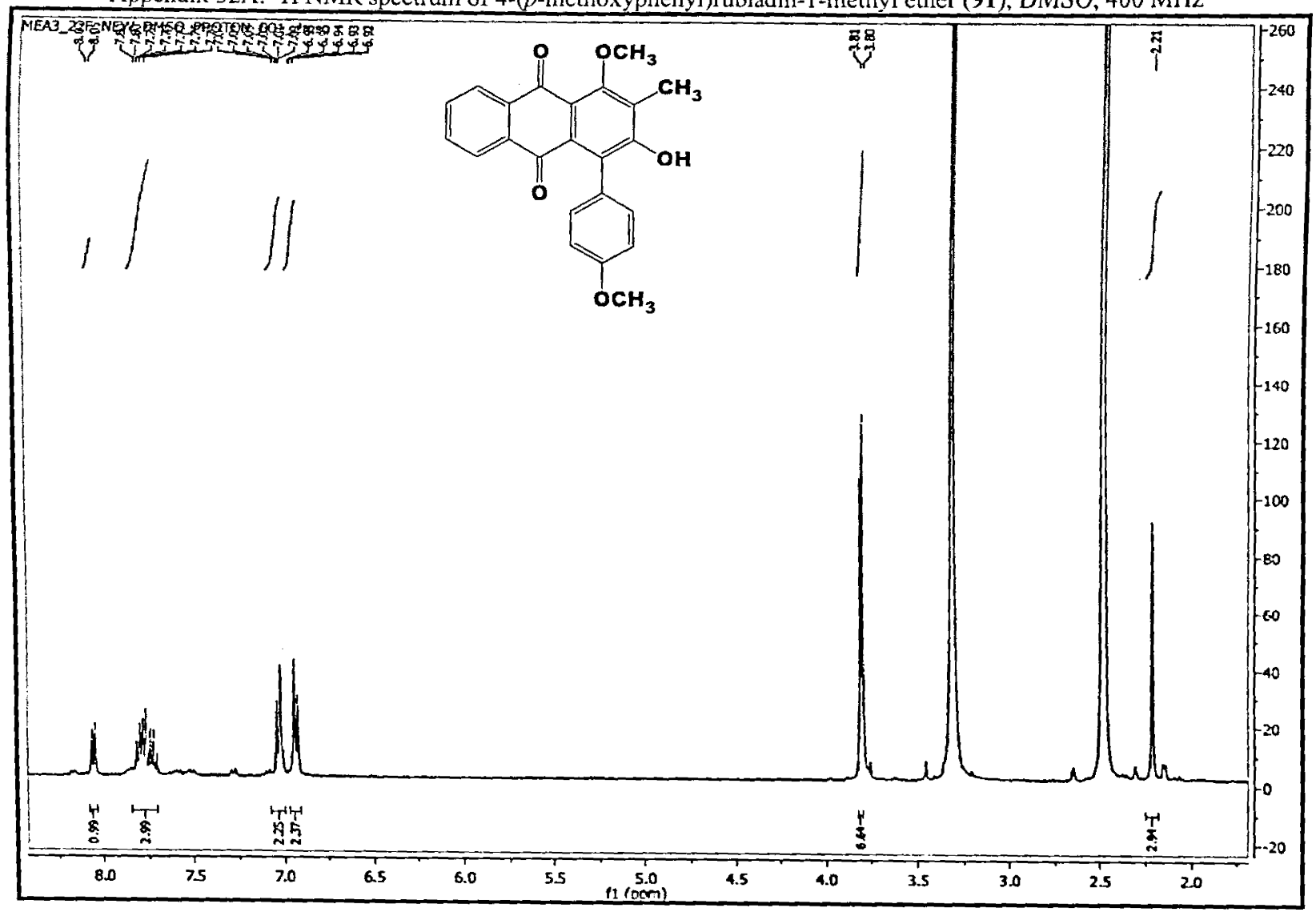
Appendix 31B: ^{13}C NMR spectrum of 4-(*p*-nitrophenyl)rubiadin-1-methyl ether (**90**), $CDCl_3$, 200 MHz



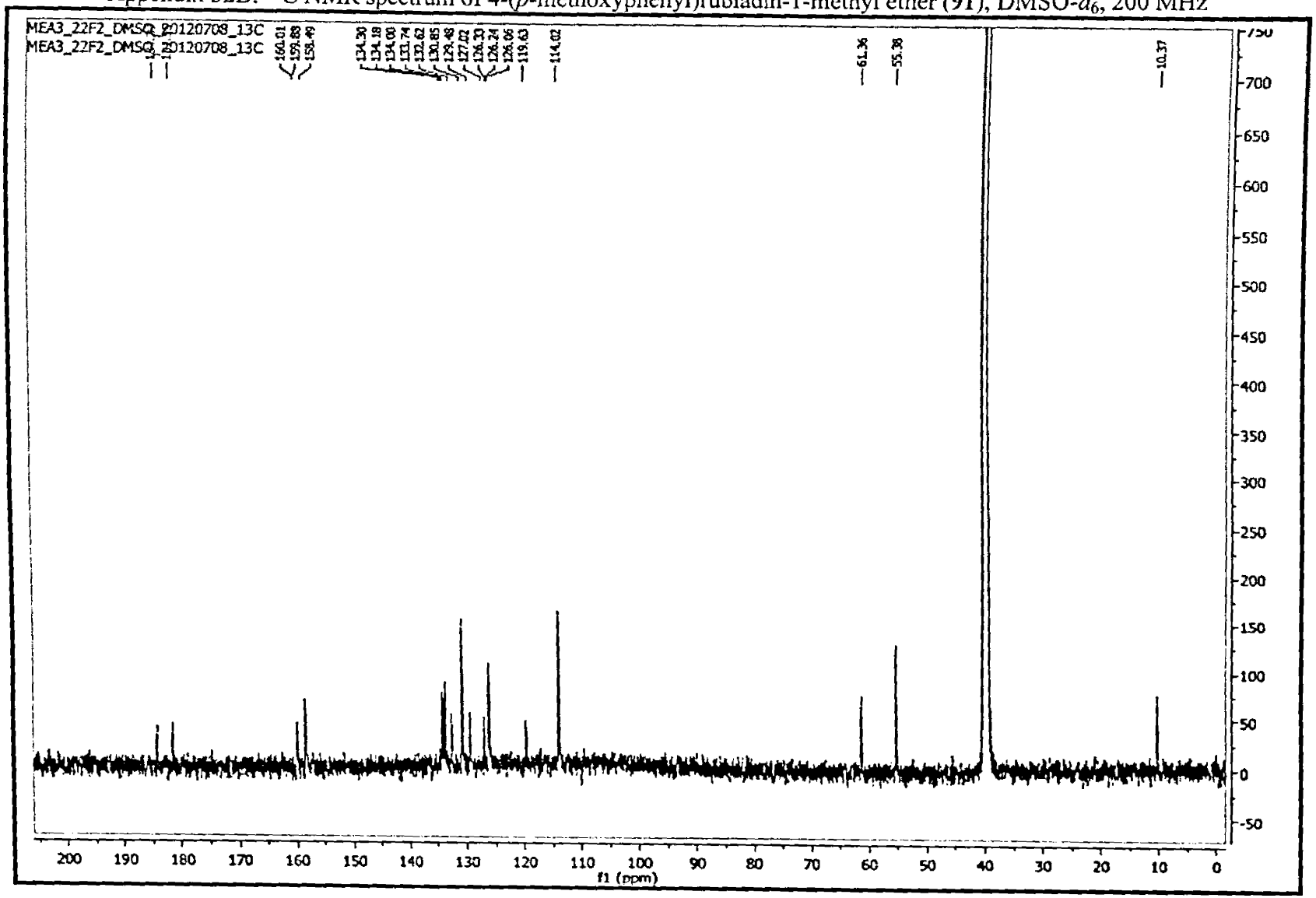
Appendix 31C: LC-MS spectrum of 4-(*p*-nitrophenyl)rubiadin-1-methyl ether (90)



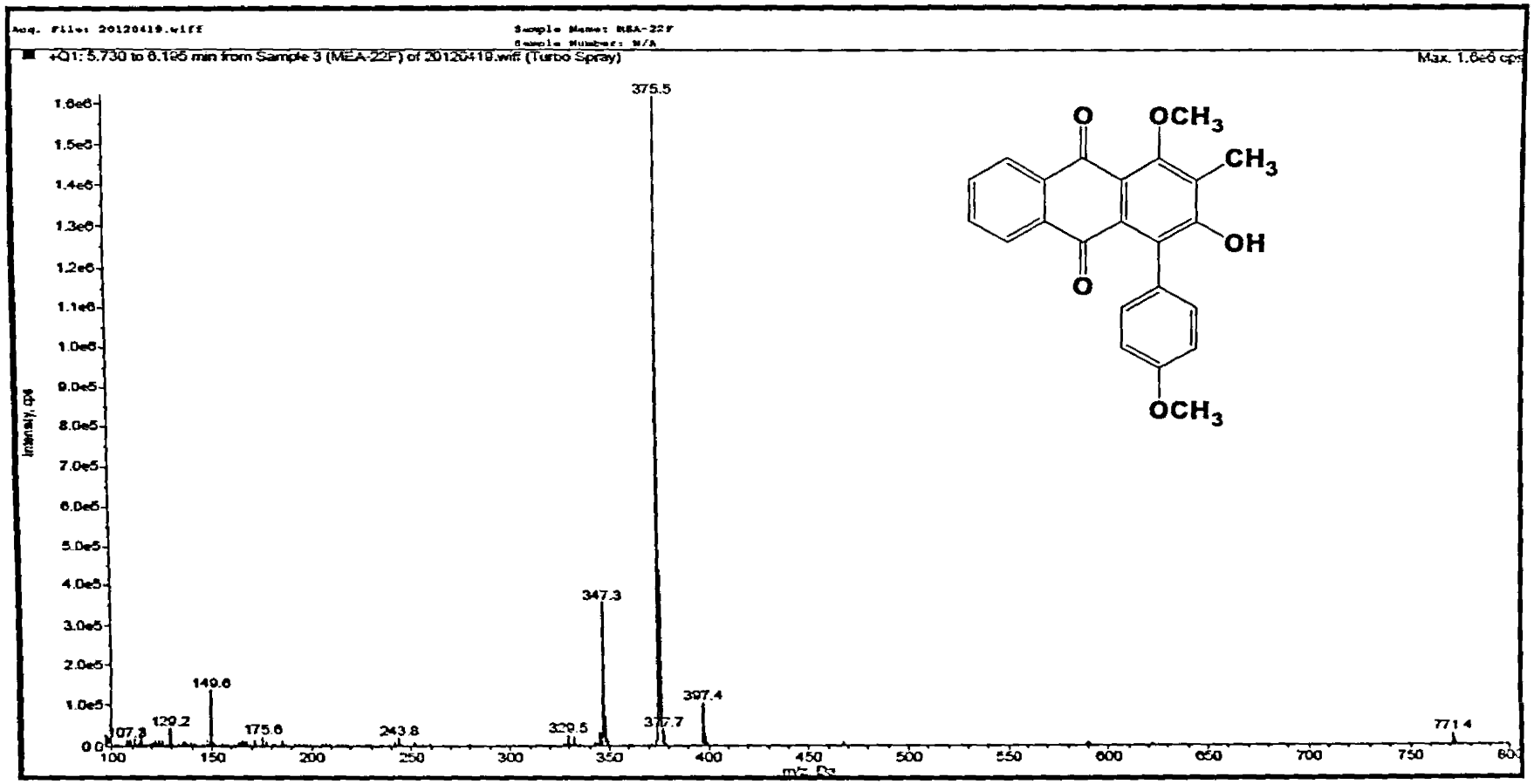
Appendix 32A: ^{1}H NMR spectrum of 4-(*p*-methoxyphenyl)rubiadin-1-methyl ether (**91**), DMSO, 400 MHz



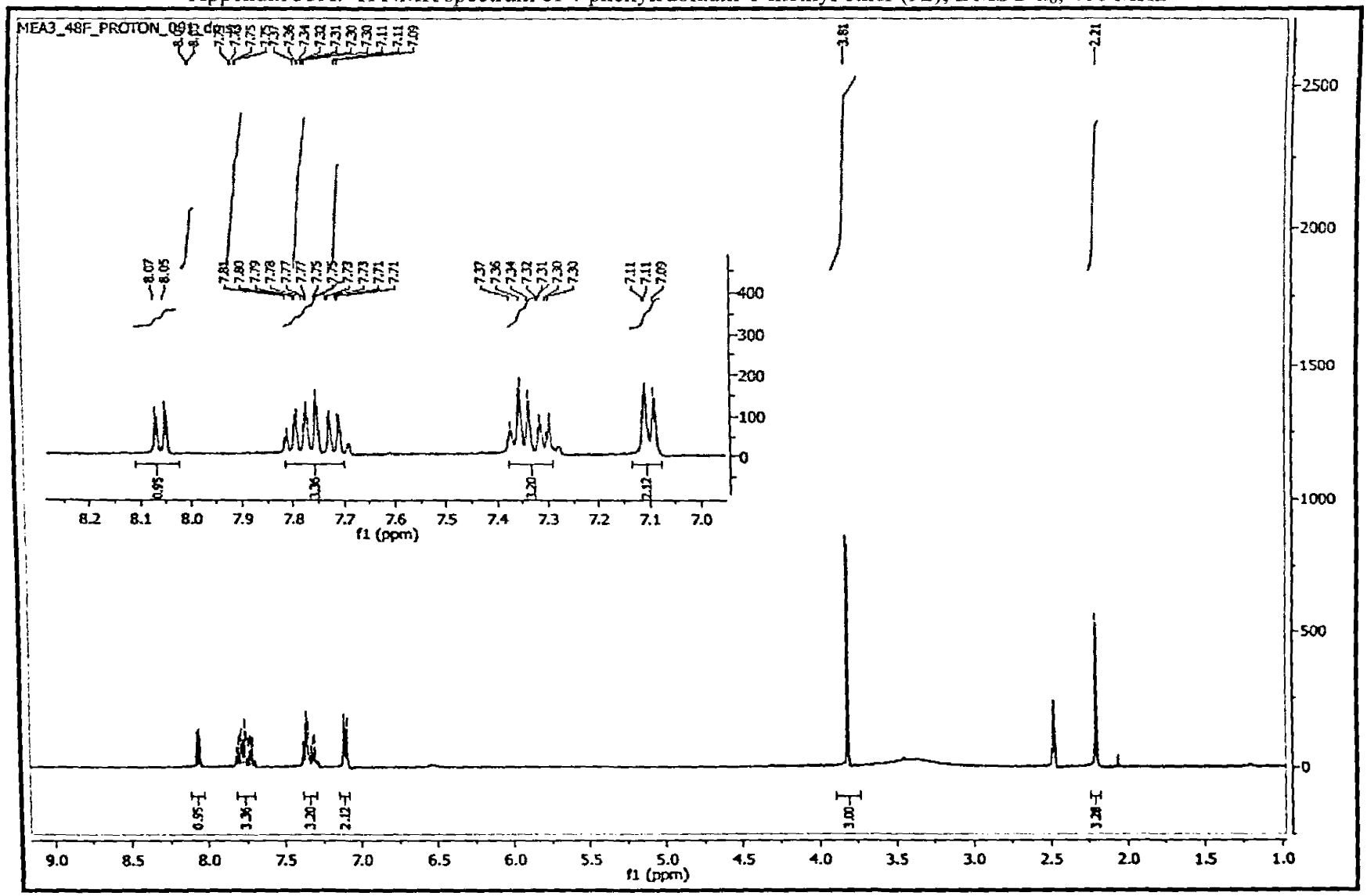
Appendix 32B: ^{13}C NMR spectrum of 4-(*p*-methoxyphenyl)rubiadin-1-methyl ether (**91**), DMSO-d_6, 200 MHz



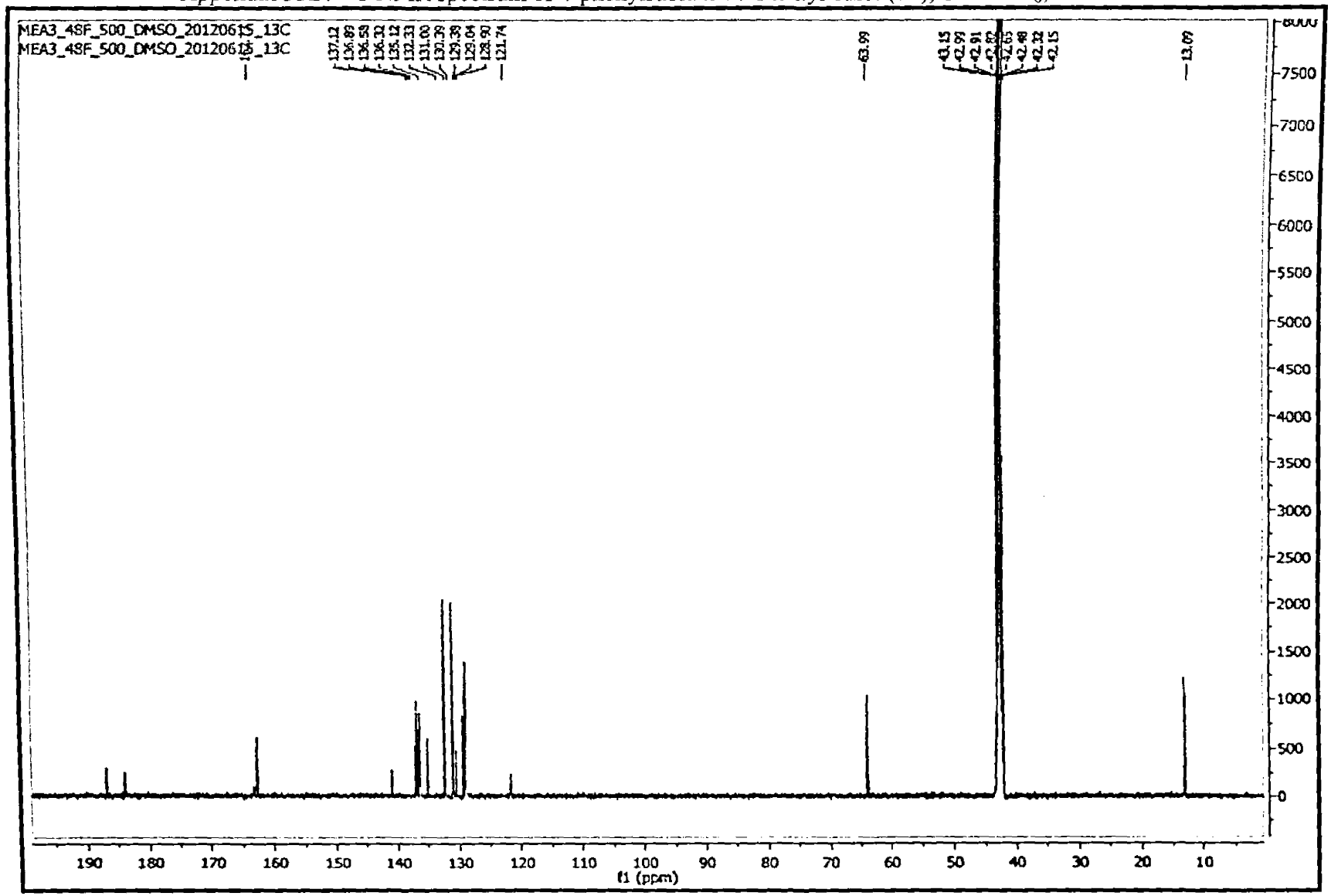
Appendix 32C: ESI-MS spectrum of 4-(*p*-methoxyphenyl)rubiadin-1-methyl ether (91)



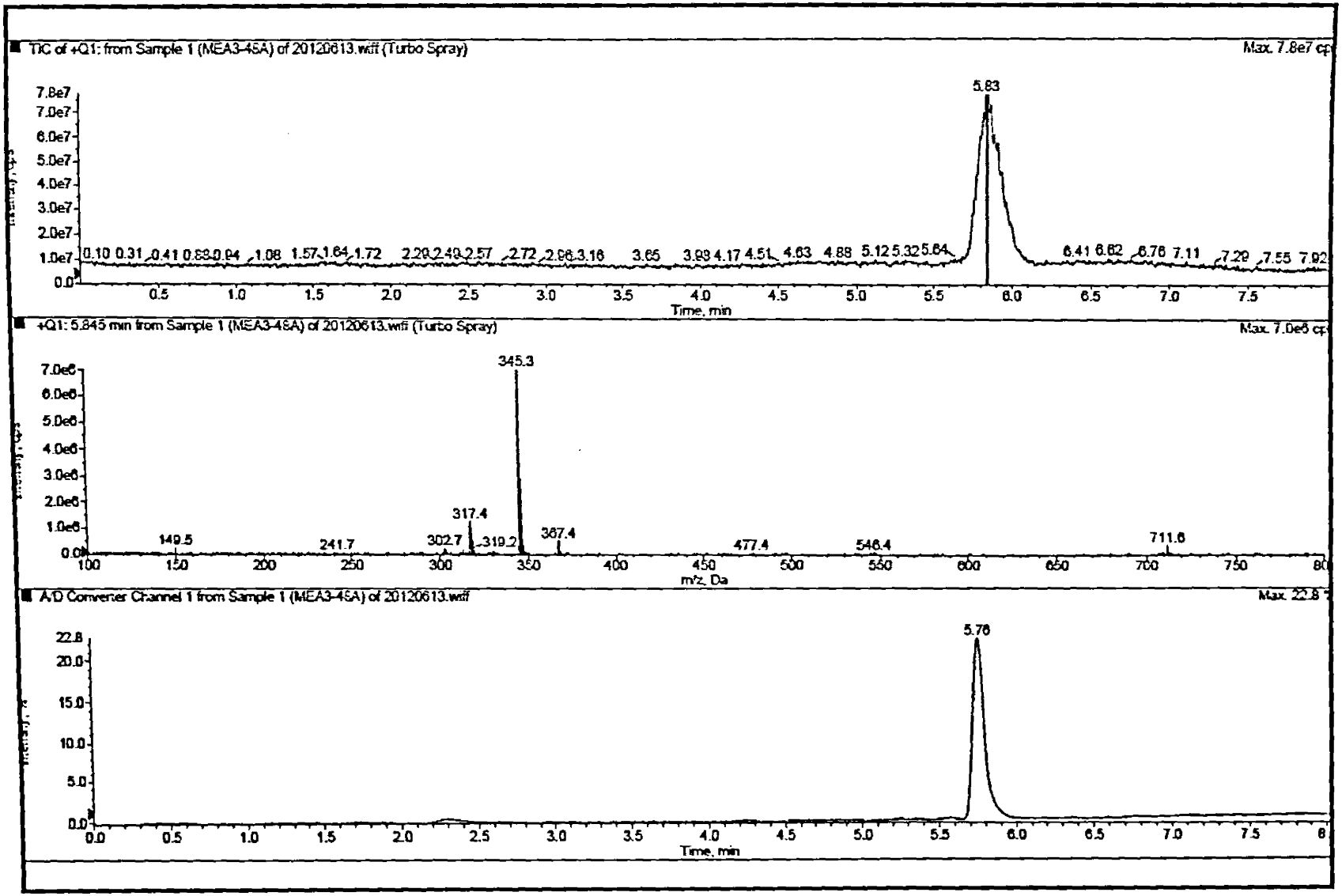
Appendix 33A: ^{1}H NMR spectrum of 4-phenylrubiadin-1-methyl ether (**92**), DMSO-d_6, 400 MHz



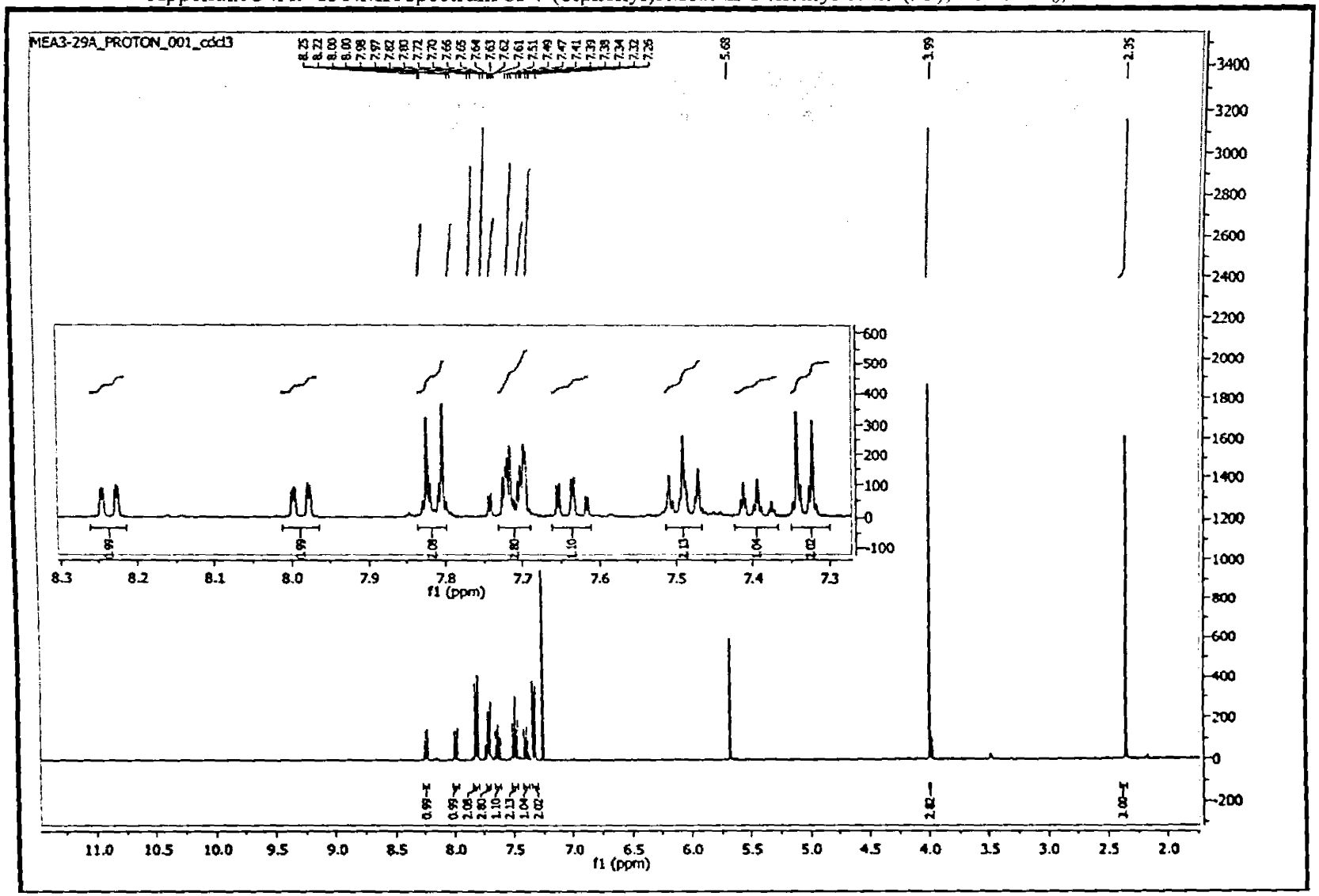
Appendix 33B: ^{13}C NMR spectrum of 4-phenylrubiadin-1-methyl ether (92), DMSO-d_6, 200 MHz



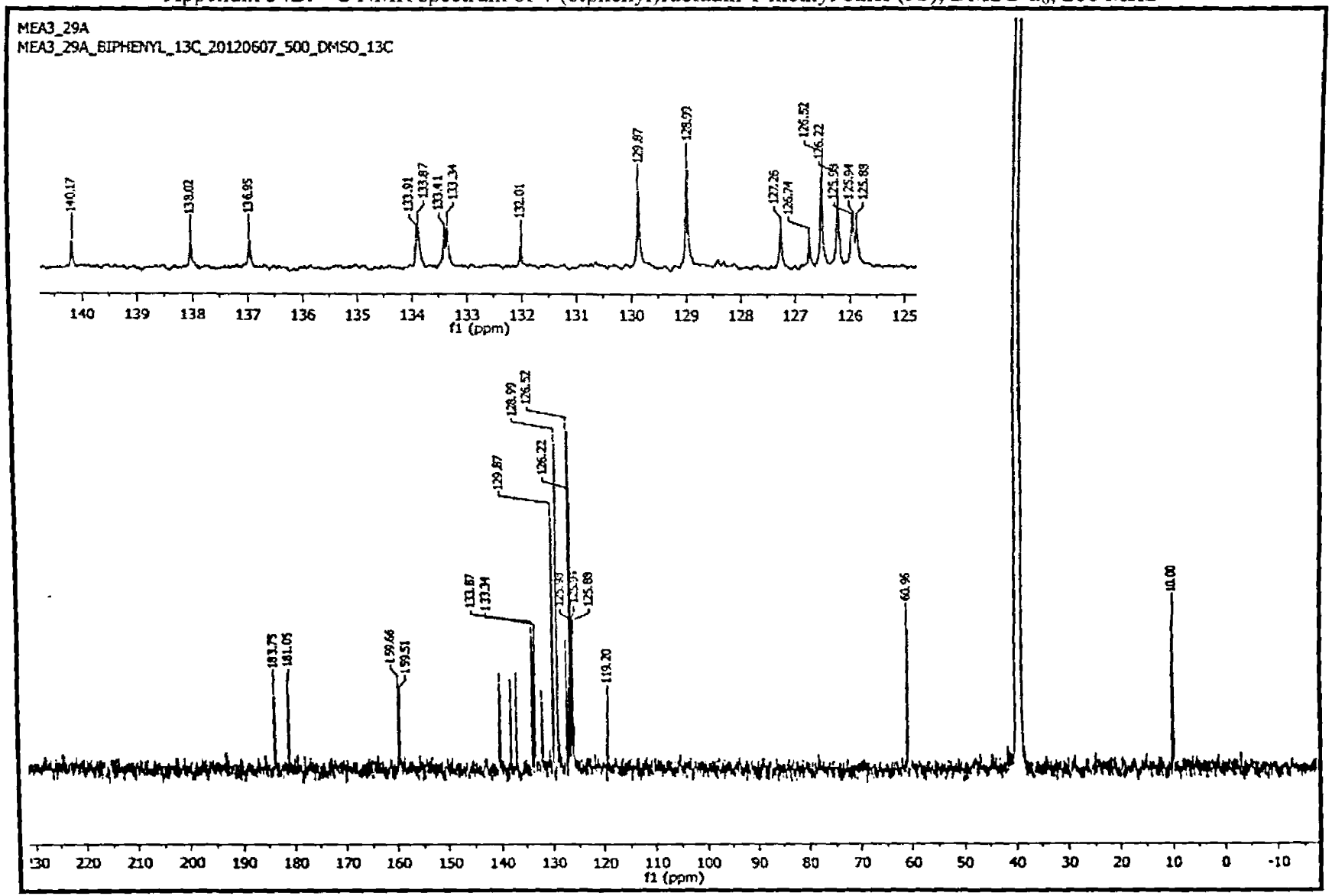
Appendix 33C: ESI-MS spectrum of 4-phenylrubiadin-1-methyl ether (92)



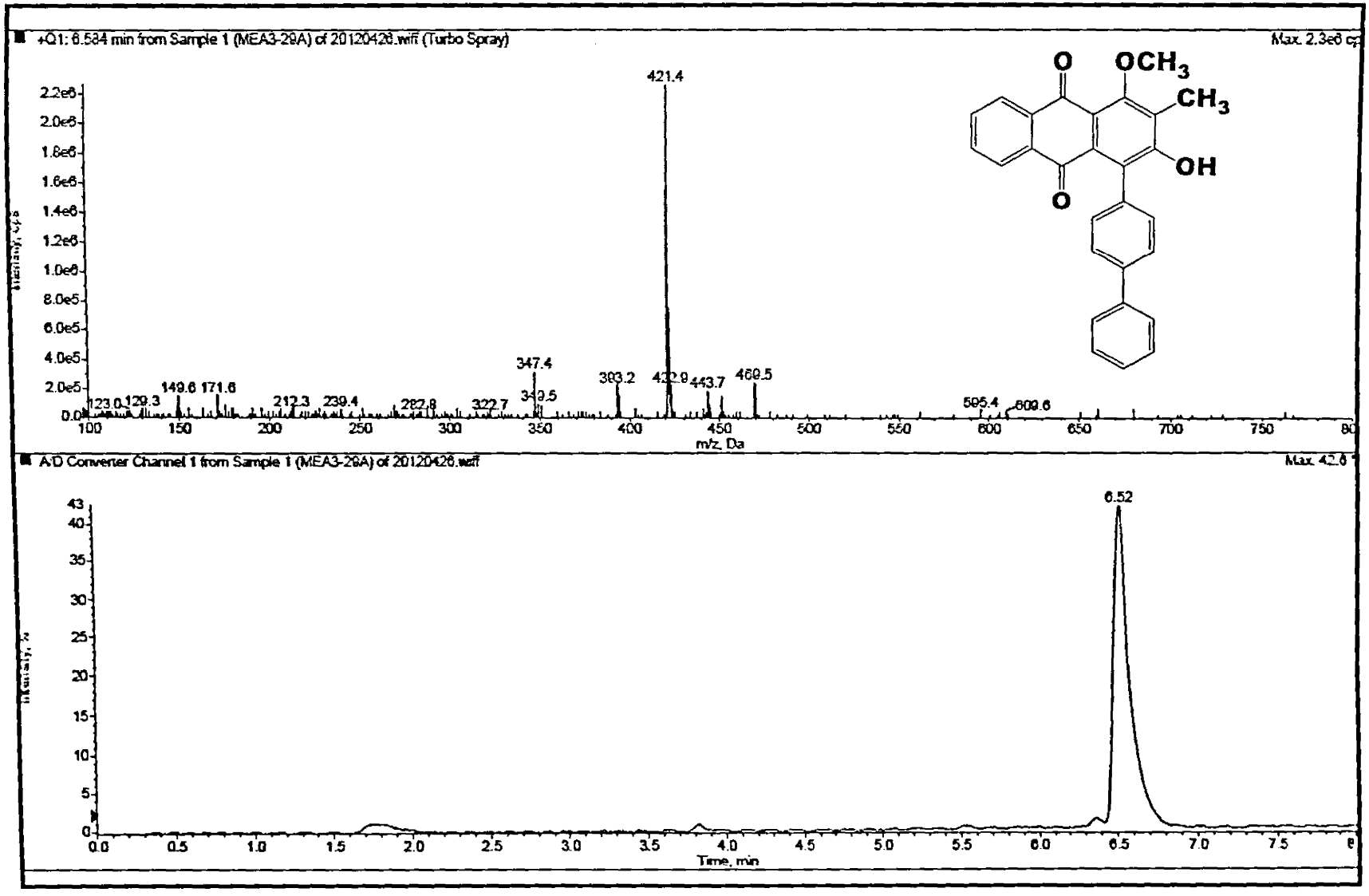
Appendix 34A: ^{1}H NMR spectrum of 4-(biphenyl)rubiadin-1-methyl ether (**93**), DMSO-d_6, 400 MHz



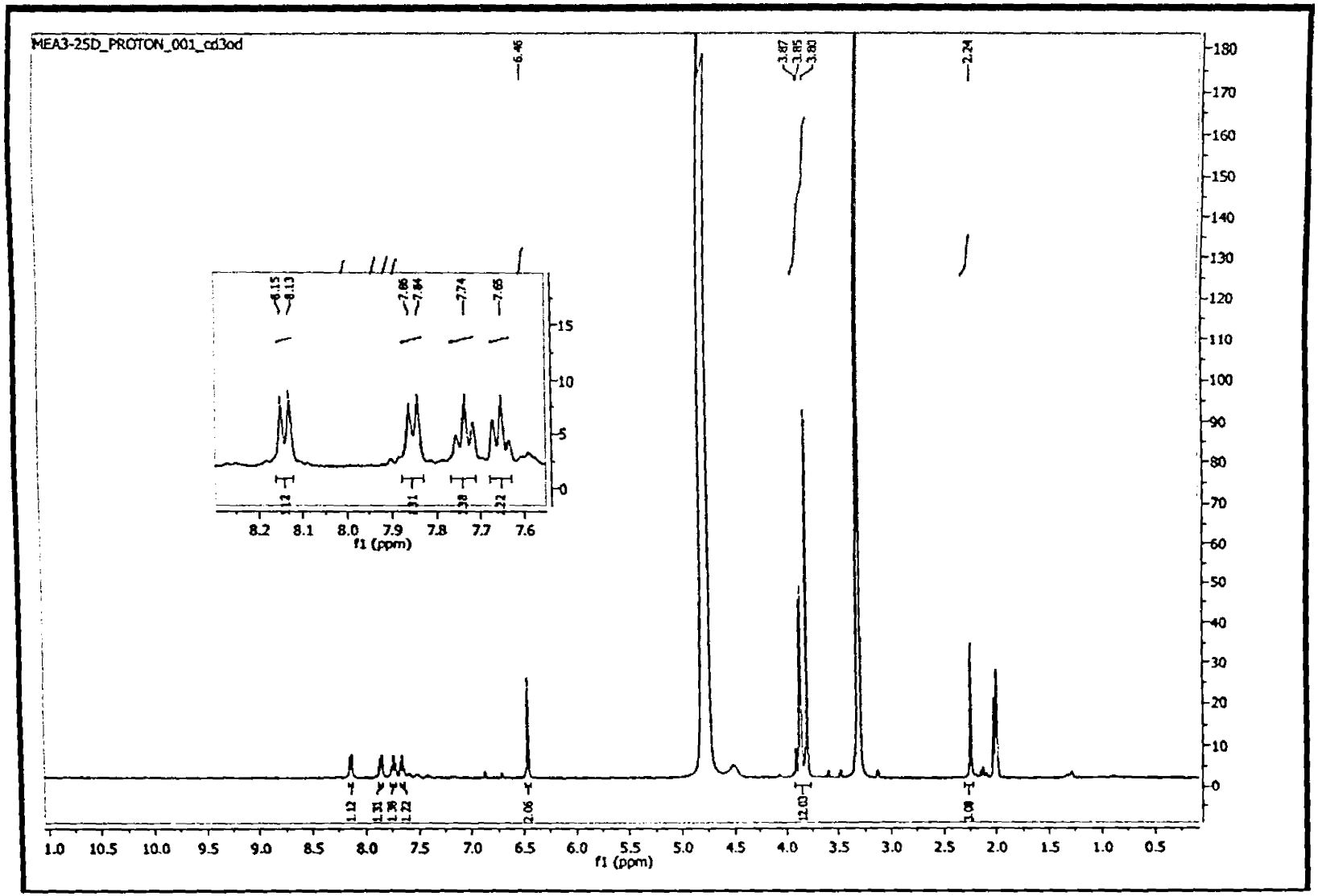
Appendix 34B: ^{13}C NMR spectrum of 4-(biphenyl)rubiadin-1-methyl ether (**93**), DMSO-d_6, 200 MHz



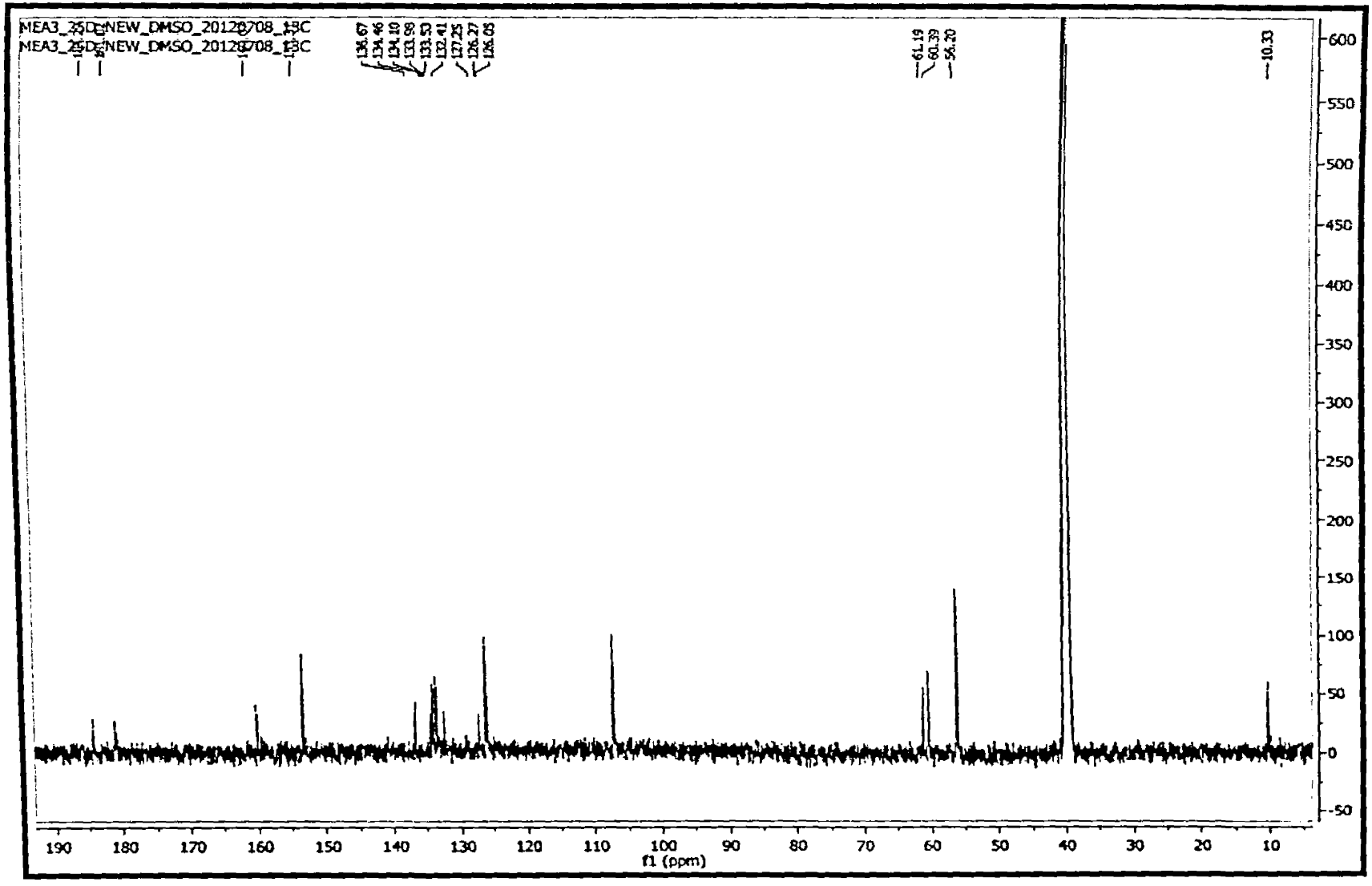
Appendix 34C: ESI-MS spectrum of 4-(biphenyl)rubiadin-1-methyl ether (93)



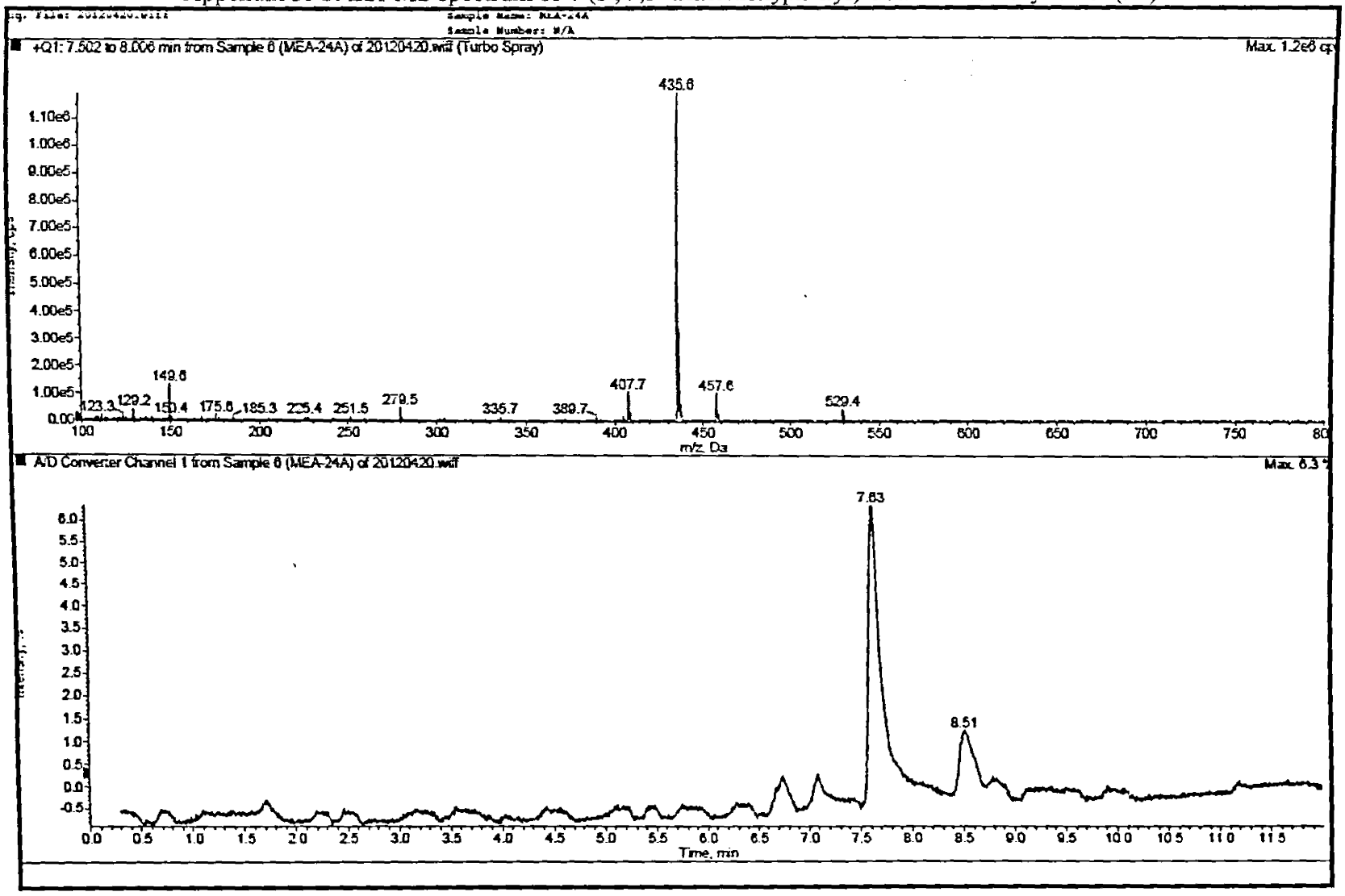
Appendix 35A: ^{1}H NMR spectrum of 4-(3',4',5'-trimethoxyphenyl)rubiadin-1-methyl ether (**94**), CD_3OH, 400 MHz



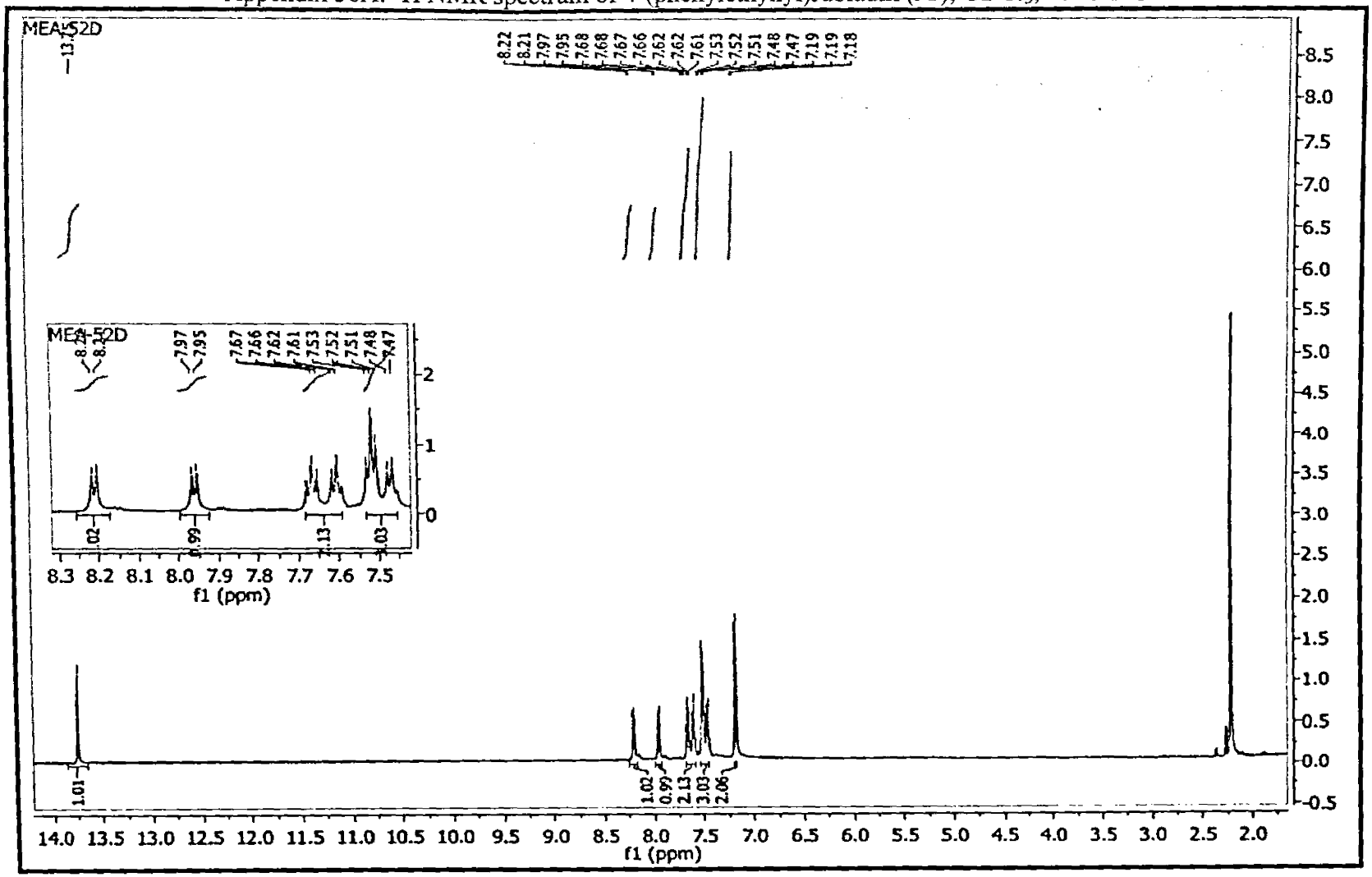
Appendix 35B: ^{13}C NMR spectrum of 4-(3',4',5'-trimethoxyphenyl)rubiadin-1-methyl ether (**94**), DMSO-d_6, 200 MHz



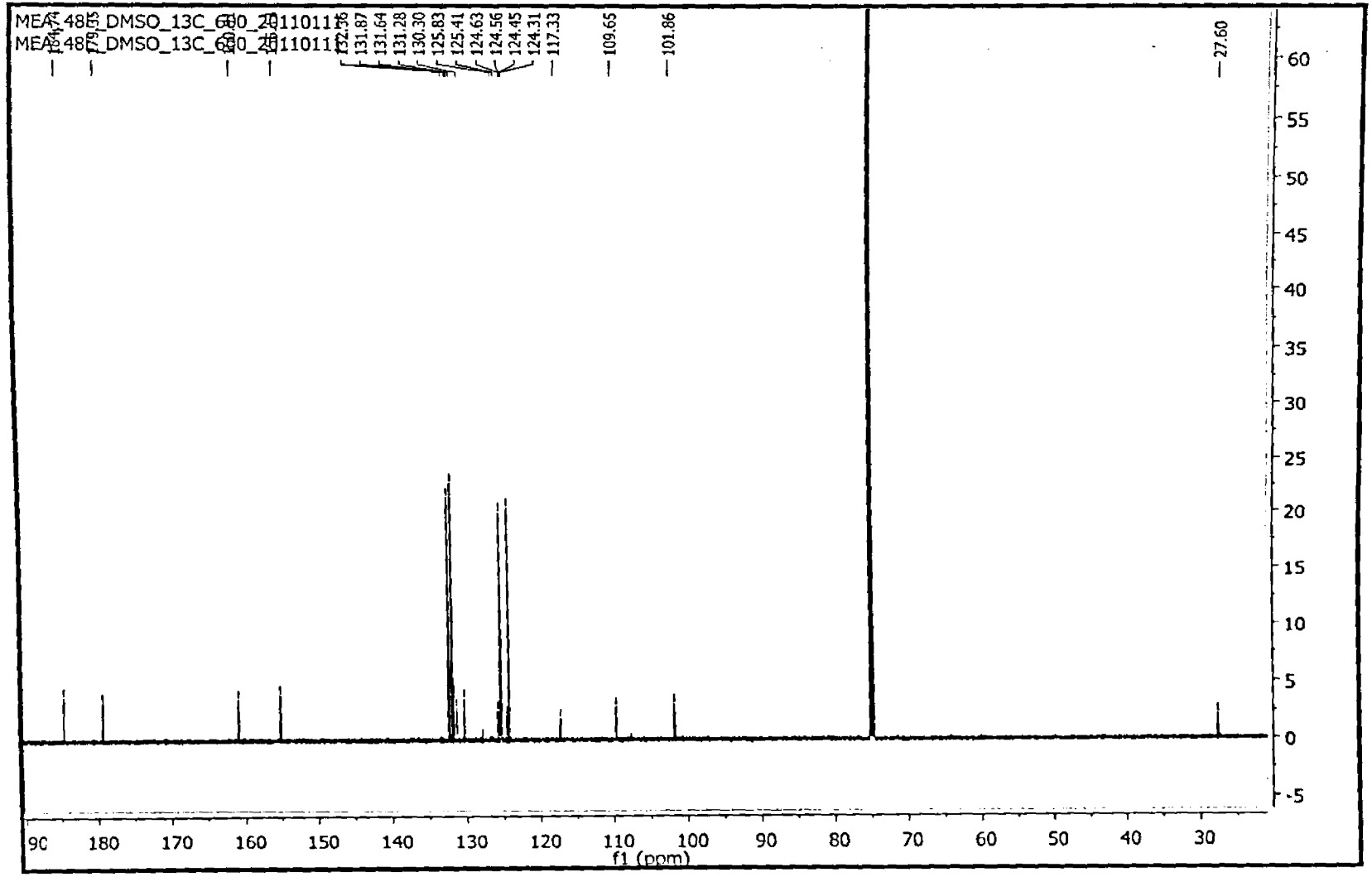
Appendix 35C: ESI-MS spectrum of 4-(3',4',5'-trimethoxyphenyl)rubiadin-1-methyl ether (94)



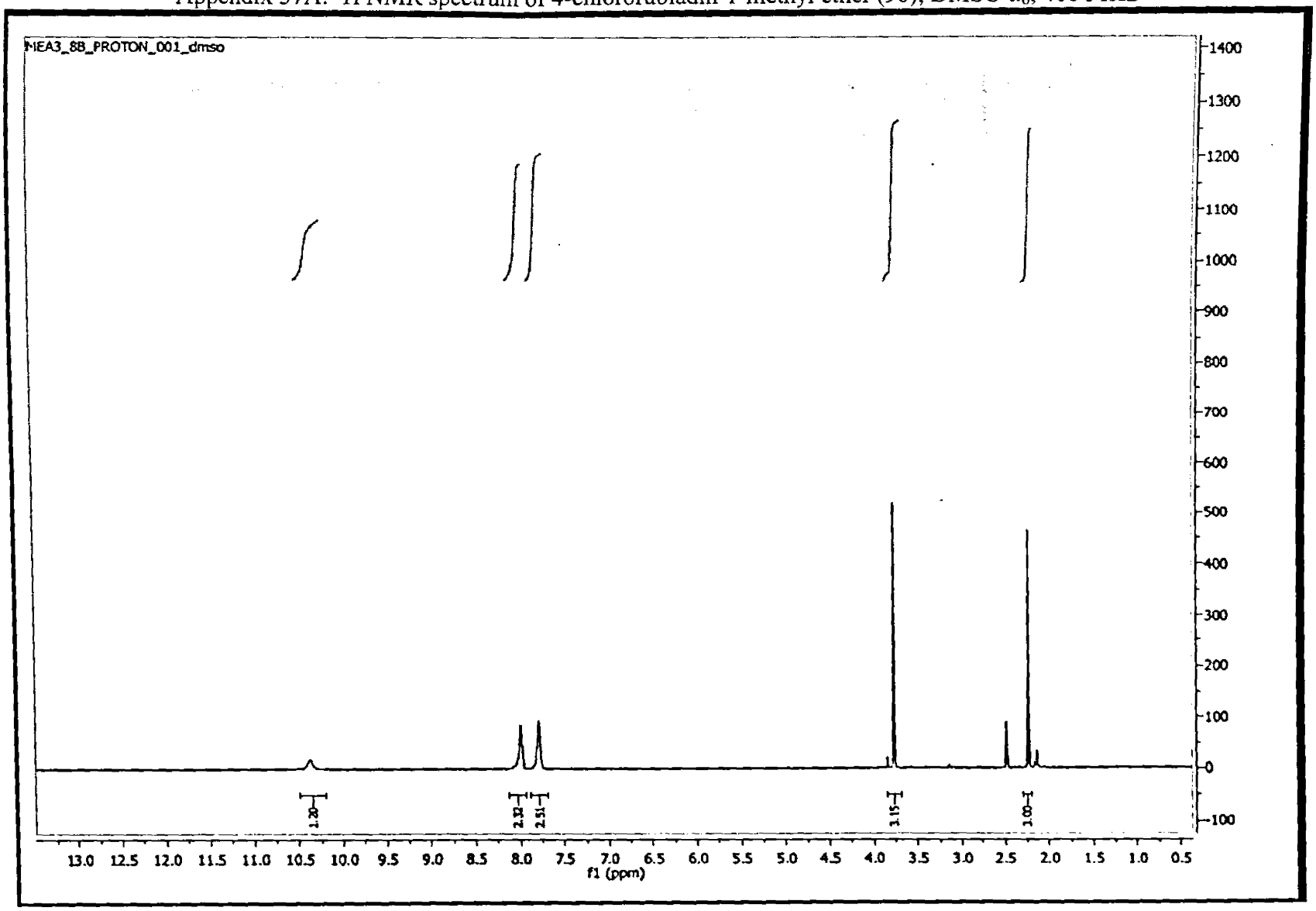
Appendix 36A: ^{1}H NMR spectrum of 4-(phenylethynyl)rubiadin (95), $CDCl_3$, 400 MHz



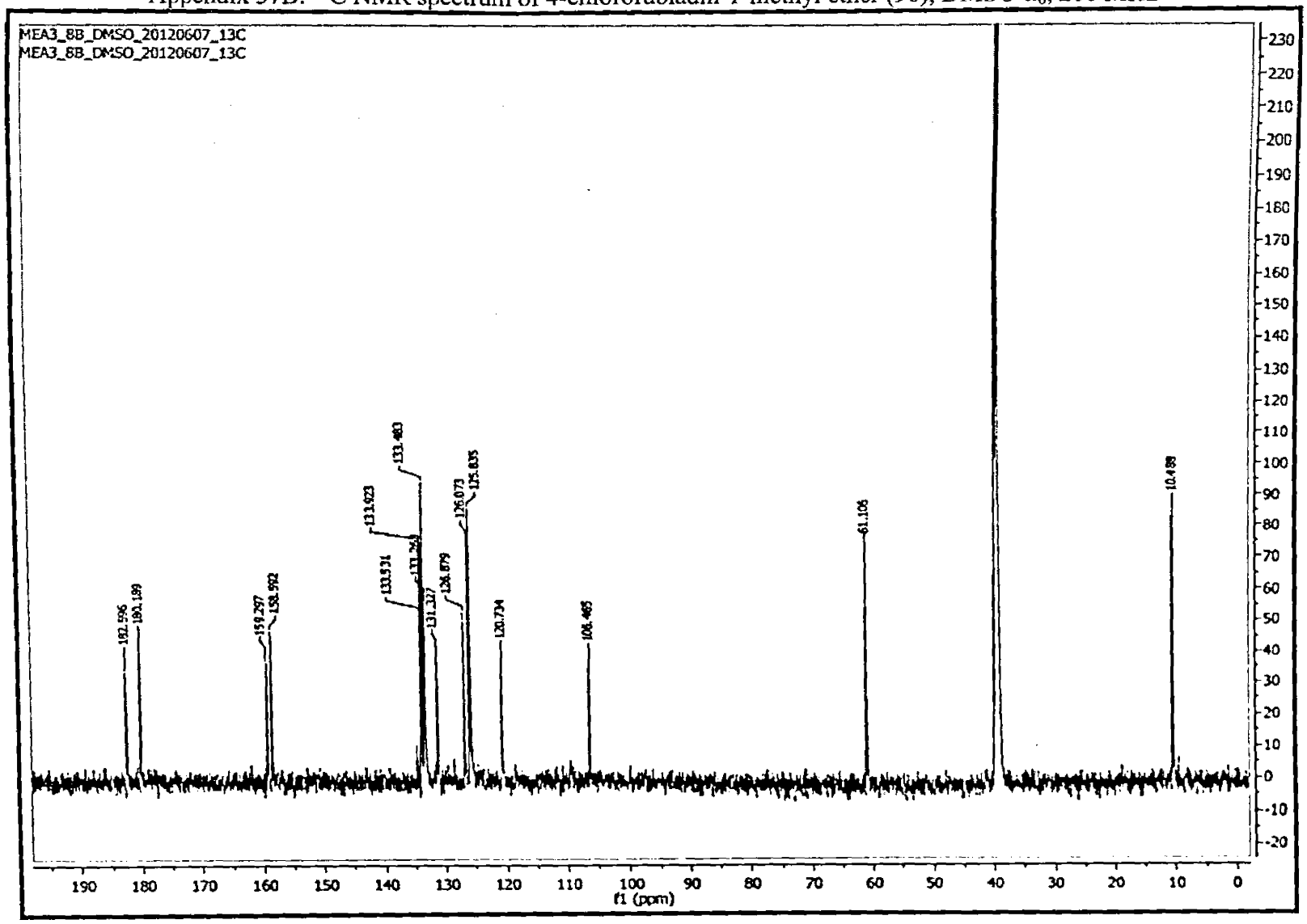
Appendix 36B: ^{13}C NMR spectrum of 4-(phenylethynyl)rubiadin (95), $CDCl_3$, 200 MHz



Appendix 37A: ^{1}H NMR spectrum of 4-chlororubiadin-1-methyl ether (96), DMSO-d_6, 400 MHz



Appendix 37B: ^{13}C NMR spectrum of 4-chlororubiadin-1-methyl ether (96), DMSO-d_6, 200 MHz



Appendix 37C: ESI-MS spectrum of of 4-chlororubiadin-1-methyl ether (96), DMSO-d_6