UNIVERSITY OF NAIROBI

DEPARTMENT OF CHEMISTRY

PHYTOCHEMICAL INVESTIGATION OF SELECTED *TEPHROSIA* SPECIES FOR ANTIPLASMODIAL AND ANTI-INFLAMMATORY PRINCIPLES

BY

YOSEPH ATILAW ASSEFA

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A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD) IN CHEMISTRY AT THE UNIVERSITY OF NAIROBI

2018

DECLARATION

I declare that this PhD thesis is my original work and has not been submitted anywhere for any examination, award of a degree or publication.

Signature _____ Date 10/8/2018

YOSEPH ATILAW ASSEFA (BSc, MSc)

(I80/92721/2013)

This PhD thesis is submitted to the University of Nairobi with our approval as research supervisors:

	Signature	Date
PROF. ABIY YENESEW DEPARTMENT OF CHEMISTRY UNIVERSITY OF NAIROBI	_____	10/8/2018
DR. ALBERT NDAKALA DEPARTMENT OF CHEMISTRY UNIVERSITY OF NAIROBI	_____	10/8/2018
DR. HELLEN KARIUKI DEPARTMENT OF MEDICAL PHYSIOLOGY UNIVERSITY OF NAIROBI	_____	10/8/2018

DEDICATION

This work is dedicated to my devoted loving wife Sinidu Mekonnen, I am very lucky for having you in my life. I also dedicate this work to my entire family (Atilaw, Tilaye, Mahindera, Fiker, Hiwot, Saba, Sami, Selam, Hanna and my son Betremariam).

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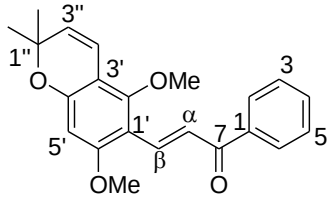
ABSTRACT

Malaria is one of the major diseases affecting human beings, whose symptoms include pain and inflammation. It is caused by five *Plasmodium* species of which *P. falciparum* and *P. vivax* are the important causative agents of human malaria. From the time *P. falciparum* showed evidence of resistance towards the most recently introduced antimalarial drugs; the degree of malaria infection has increased. Malaria causes inflammation and pain; some of the drugs used to treat these conditions have side effects while others are not affordable to the majority the third world countries. Therefore, there is an urgent need for new antimalarial and anti-inflammatory agents that lack side-effects and resistance associated with the currently used drugs. In this study, six *Tephrosia* species; *T. aequilata, T. elata, T. noctiflora, T. pumila, T. purpurea* subsp. *leptostachya* and *T. rhodesica* were investigated with the aim of identifying their antiplasmodial and anti-inflamatory principles. The stem bark, roots and leaves of these plants were collected, dried, ground and extracted with dichloromethane/methanol (1:1) by cold percolation at room temperature. The crude extracts of these plants were subjected to column chromatography on silica gel. Based on the TLC profile of the fractions, further purification was done using Sephadex LH-20, preparative HPLC, preparative TLC, and crystallization techniques. As a result, from the six *Tephrosia* species, fifty compounds were isolated and three derivatives prepared. Thirteen of the isolated compounds are new. From the roots of *T. aequilata,* four new compounds **[**aequichalcone A (**168**), aequichalcone B (**169**), aequichalcone C (**170**) and 3,4:8,9-dimethylenedioxy-6a,11a-pterocarpene (**167**)] and seven known compounds were isolated. From the seedpods and leaves of *T. elata*, a total of nineteen compounds were isolated and characterized. The roots of *T. rhodesica* gave twenty one compounds, of which five are new compounds [rhodimmer (**183**), rhocarpin (**188**), rhodiflavan A (**191**), rhodiflavan B (**192**), rhodiflavan C (**193**)). From stem of *T. purpurea* spp. *leptostachya*, four new compounds [*(E)*-5-hydroxy-tephrostachin (**198**), purleptone (**194**), *(E)*-5-oxo- anhydrotephrostachin (**195**) and terpurlepflavone (**199**)] and seven previously reported compounds were isolated. From the stem of *T. noctiflora*, three compounds, two of which are flavonoids were isolated and characterized. Three compounds were isolated and characterized from the aerial part of *T. pumila.* Characterization of the iolated compounds was done using a combination of spectroscopic techniques including, UV, 1D-NMR (^{1}H-NMR, ^{13}C-NMR, DEPT), 2D (HMBC, HSQC, COSY) and MS. The crude extracts and the isolated compounds were evaluated for antiplasmodial activities against the chloroquine-sensitive 3D7 and D6 strains of *P. falciparum.* Among the pure compounds *(E)*-5-hydroxy-tephrostachin (**198**) (IC_{50} 1.7$\pm$0.1 μM) was the most active against the chloroquine-sensitive (D6) strain with a much lower cytotoxicity, (IC_{50} > 21 μM) against four cell-lines. Aequichalcone C (**168**), (IC_{50} 2.48 ± 0.22 μM), obovatachalcone (**147**) (IC_{50} 4.23 ± 1.11 μM) and praecansone B (**146**) (4.14 ± 0.26 μM) showed good activity against the 3D7 strain. Rhodiflavan A (**191**) showed good (3.6 ± 1.0 μM) and moderate (6.5 ± 0.9 μM) activity against the 3D7 and D6 strains, respectively. Anti-inflammatory and anti-nociceptive activities for some of the extracts and pure compounds were also evaluated using the formalin test. Tachrosin (**41**) reduced pain by 50.4% (in the early phase) and 49.2% (in the late phase). Kaempferitrin (**200**) reduced pain by 49% (in the early phase) and 44% (in the later phase). The reference drug diclofenac, reduced pain by 53.4% (in the early phase) and 62% (in the late phase). Overall, from this study 50 compounds including 13 new flavonoids were isolated from the selected *Tephrosia* species and some of these new flavonoids showed significant antiplasmodial and anti-

inflammatory activities. Further studies should be directed at testing the flavonoids of *Tephrosia* species for *in vivo* antiplsmodial activity and toxicity.



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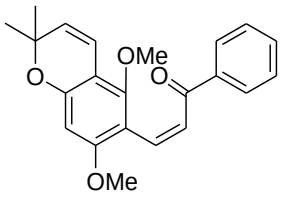

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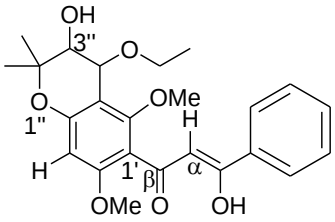

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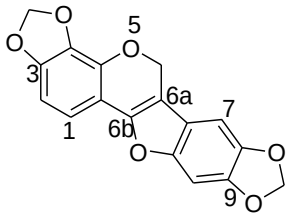

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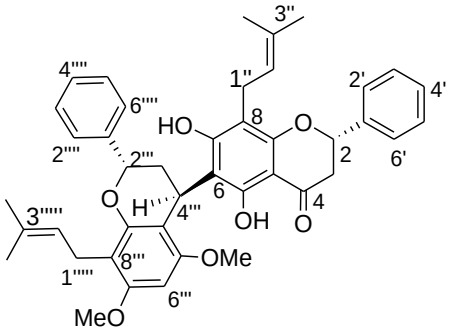

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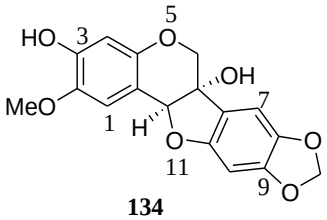

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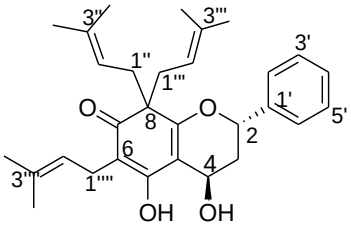

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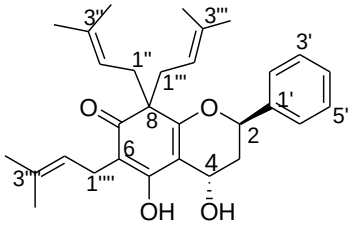

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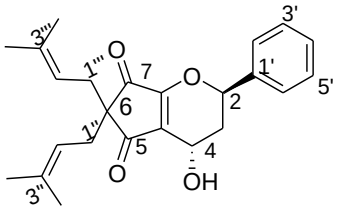

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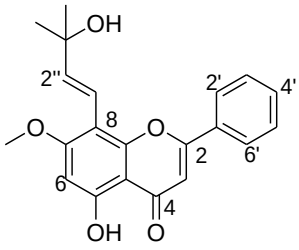

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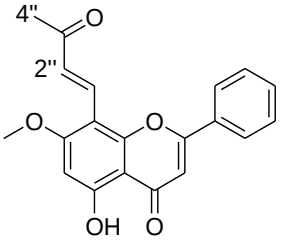

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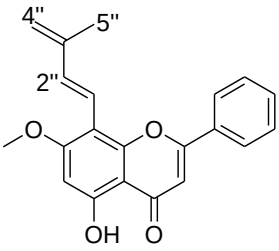

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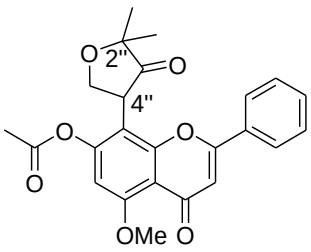

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List of publications from this work

1. **Yoseph Atilaw,** Lois Muiva-Mutisya, Matthias Heydenreich,Vicky M. Avery , Máté Erdélyi, Sandra Duffy, and Abiy Yenesew: *Three Chalconoids and a Pterocarpene from the Roots of Tephrosia aequilata* **.** *Molecules* 2017, *22*(2), 318.

2. **Yoseph Atilaw**, Albert Ndakala, Lois Muiva-Mutisya, Redemptah Yeda, Hoseah M. Akala, Yu J.Wu, Vincent K.W.Wong, Máté Erdélyi and Paolo Coghi, Abiy Yenesew. *Four Prenylflavone Derivatives with Antiplasmodial Activities from the Stem of Tephrosia purpurea* subsp. *leptostachya Molecules 2017, 22, 1514.*

3. **Yoseph Atilaw**, Albert Ndakala, Matthias Heydenreich, Máté Erdélyi and Abiy Yenesew: *New Prenylflavonoids, Biflavanoids and pterocarpane from the Roots of Tephrosia rhodesica. Manuscript 2018, xxxxxx.*

4. **Yoseph Atilaw**, Albert Ndakala, Hellen Kariuki, Abiy Yenesew Antinociceptive and Anti-inflammatory properties of the stem crude extract and the isolated compounds of *Tephrosia purpurea* subsp. *leptostachya; Manuscript 2018, xxxxxx*

Paper presentations

1. **Yoseph Atilaw,** Lois Muiva-Mutisya, Matthias Heydenreich,Vicky M. Avery , Máté Erdélyi, Sandra Duffy, and Abiy Yenesew: *Three Chalconoids and a Pterocarpene from the Roots of Tephrosia aequilata.*

-University of Nairobi, Department of Chemistry, Nairobi, (Kenya)

-University of Gothenburg, Department of Chemistry, Gothenburg, (Sweden)

-University of Potsdam, department of Chemistry, Potsdam (Germany).

-Researcher Link Workshop Exploring Kenyan Biodiversity, Naivasha, (Kenya).

2. Yoseph Atilaw, Albert Ndakala, Lois Muiva-Mutisya, Redemptah Yeda, Hoseah M. Akala, Yu J.Wu, Vincent K.W.Wong, Máté Erdélyi and Paolo Coghi, Abiy Yenesew. *Four Prenylflavone Derivatives with Antiplasmodial Activities from the Stem of Tephrosia purpurea subsp. leptostachya.* University of Gothenburg, Department of Chemistry, Gothenburg (Sweden)

3. **Yoseph Atilaw**, Lois Muiva-Mutisya, Albert Ndakala, Matthias Heydenreich, Máté Erdélyi and Abiy Yenesew: *New Prenylflavonoids, Biflavanoids* and pterocarpane from the *Roots of Tephrosia rhodesica.* Uppsala University, Department of Chemistry and Molecular Biology, Uppsala, (Sweden)

Poster Presentations

1. Researcher Link Workshop Exploring Kenyan Biodiversity: Chemistry, Pharmacology and Commercialization, Naivasha, Kenya

2. Kenya Chemical Society 9th international conference, Nairobi, Kenya.

List of Abbreviations

COSY	Correlation Spectroscopy
CD	Circular Dichroism
d	doublet
dd	doublet of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
ESIMS	Electron Spray Ionization Mass spectrometry
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Coherence
HPLC	High performance liquid chromatography
Hz	Hertz
IC_{50}	Concentration of 50 % Inhibition
MS	Mass Spectroscopy
M	Multiplet
m/z	Mass to charge ratio
NOESY	Nuclear Overhauser and Exchange Spectroscopy
NMR	Nuclear Magnetic Resonance
1D NMR	One Dimensional Nuclear Magnetic Resonance
2D NMR	Two Dimensional Nuclear Magnetic Resonance
PTLC	Preparative Thin Layer Chromatography
s	Singlet
TLC	Thin Layer Chromatography
t	Triplet
UV	Ultra Violet
WHO	World Health Organization
δ	Chemical shift

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CHAPTER 1: INTRODUCTION

1.1: Background Information

Despite the advancement of modern medicine, traditional medicines continue to be the major source of therapy for the majority of the global population. In Asian and African countries, 80% of the populations depend on traditional medicines for their primary healthcare needs. In Africa, traditional medicine is ancient and has remained an essential part of the culture and traditions of the people (Fennell *et al.*, 2004). The extended use of these medicines for thousands of years allowed accumulation of empirical knowledge of their utility, which demands adequate evaluation of efficacy, safety and mechanism of action (Calixto, 2005).

According to the WHO 2014 report, traditional medicine includes "approaches, knowledge, health practices and beliefs incorporating animal, plant and mineral-based medicines, manual techniques, spiritual therapies and exercises, when applied in combination or singularly to treat, diagnose and prevent illnesses or maintain well-being". Herbal medications are prepared by extracting components from different parts of a particular plant species or as mixtures of extracts from different species. Extracts are prepared in the form of decoctions and concoctions, infusions for oral consumption, enemas and inhalations, or paste for tropical applications on surface lesions including painful swellings and fractures (Gurib-Fakim, 2006; Kokwaro, 2009).

The active ingredients in these medicinal plants are usually small organic molecules collectively referred to as 'Natural Products'. These are also referenced to as 'Secondary Metabolites' and belong to different classes, such as alkaloids, saponins, tannins, alkenyl phenols, glycol-alkaloids, flavonoids, sesquiterpenes, lactones, terpenoids, phorbol esters, phytosteroids and

polyphenols among others (Gurib-Fakim, 2006). The search for chemotherapeutic agents from plants has been practiced for a long time. Many of these natural products are of great structural diversity with biological activity which has resulted in the development of new drugs. In fact, more than 50% of the drugs in clinical use are natural products or their derivatives (Gurib-Fakim, 2006).

Several secondary metabolites from medicinal plants have been used directly for treatment of different disorders including infectious, inflammatory, parasitic, neurological, cardiovascular, metabolic, oncological and pain-related diseases and/or as templates in the development of new synthetic drugs (Bakhotmah and Alzahrani, 2010; Harvey, 2000).

Malaria is one of the main diseases affecting human beings and whose symptoms include pain and inflammation. It is caused by five *Plasmodium* species (*P. falciparum, P. vivax, P. malariae, P. ovale and P. knowlesi*). Among these species, *P. falciparum* and *P. vivax* are the major cause of human malaria. In Africa, *P. falciparum* is the most common cause of malaria than *P. vivax.* Malaria is transmitted to humans through the bites of the female mosquitoes of the genus *Anopheles*. Malaria is still the predominant cause of mortality in the world, resulting in about a million deaths annually. The larger number of these cases occur in Africa (sub-Saharan) countries (88%) followed by South-East Asia Region (10%) (Batista *et al.*, 2009; Duffy and Avery, 2012; Pohlit *et al.*, 2013; WHO, 2015b).

Inflammation and pain are associated with malaria and treatment strategies also include treatment of these symptoms. Inflammation is a non-specific, localized immune reaction of the organism, which tries to localize the pathogen agent. Many consider the syndrome as a self-defense mechanism. The main stimuli of inflammation are physical damage, microbial invasion,

immune reaction and ultra-violet irradiation. The common signs of inflammation are swelling, local redness, heat, pain and loss of function (Gautam and Jachak, 2009b). These symptoms are mainly treated with non-steroidal anti-inflammatory drugs (NSAIDs) or steroidal drugs, which have been proven effective, but could also, have negative side effects (Odabasoglu *et al.*, 2006).

Pain is a symptom of a disease resulting in emotional changes and tissue damage. The need to treat pain along with inactions cannot be over emphasized. In this regard, analgesics and local anesthetics have been developed from compounds isolated from higher plants. Analgesics are mainly divided into two classes: Opiate receptor agonists' and non-steroidal anti-inflammatory drugs (NSAIDs). The former may lead to drug dependency even though they are effective against various forms of pains (Guimaraes *et al.*, 2013; Tchimene *et al.*, 2013).

Medicinal plants continue to be an attractive source of antimalarial and analgesic drugs and phytomedicine. Therefore, in this study six selected plants from *Tephrosia* species (*T. aequilata, T. elata, T. noctiflora, T. pumila, T. purpurea* spp. *leptostachya* and *T. rhodsica*) were investigated to determine the antiplasmodial, anti-inflammatory and analgesic effects of their extracts and identify the compounds responsible for these properties.

1.2: Statement of the Problem

The resistance of *P. falciparum* to antimalarial drugs appears to be growing with time leading to an increase in malaria infection. According to WHO, 214 million new malaria cases were reported in the year 2015 (WHO, 2015b). Therefore, there is a crucial need for new antimalarial and anti-inflammatory agents that lack the side-effects and resistance associated with the currently used drugs. Inflammation and pain are associated with malaria and are becoming

common in the aging population throughout the world. Besides being expensive the clinically used anti-inflammatory drugs have side effects. Some of the most common side effects caused by NSAIDs are respiratory depression, gastric irritation that may lead to gastric bleeding, stomach upset or bleeding in the stomach and potential for addiction, platelet dysfunction and possible kidney or liver damage. Likewise, corticosteroid drugs that have been used for treatment of rheumatoid arthritis, have adverse effects including glaucoma, growth arrest, hypertension, hyperglycemia, increased susceptibility to infection, muscular weakness, osteoporosis, psychiatric disturbances, and Cushing's habitus (appearance with rounded face, supraclavicular hump, narrow mouth, obesity of the trunk with relatively thin limbs).

1.3: Objectives of the Study

1.3.1. General objective

The main objective of this study was to identify antiplasmodial and anti-inflammatory principles from six *Tephrosia* (Leguminosae) species.

1.3.2. Specific objectives

The specific objectives of this study were to:

i. Establish the antiplasmodial and anti-inflammatory activities of the crude extracts from *Tephrosia aequilata, T. elata, T. noctiflora, , T. pumila, T. purpurea* spp. *leptostachya* and *T. rhodesica,*
ii. Identify the constituents of the selected plants,
iii. Establish the antiplasmodial and anti-inflammatory activities of the isolated compounds,
iv. Enhance the antiplasmodial and anti-inflammatory activities of the most active compounds through structure modification.

1.4: Justification and Significance

Since ancient times, traditional medicines have been used for the treatment of various disorders including inflammation and malaria. Several plants from the family Fabaceae including *Tephrosia* species have exhibited potential to reduce inflammation and to control malaria. Previous phytochemical studies on *Tephrosia* species have showed the presence of compounds with a wide range of bioactivities; such as antimicrobial, antioxidant and antiplasmodial (Muiva-Mutisya *et al.*, 2014; Tarus *et al.*, 2002). The crude extracts of some of these plants showed good to moderate antiplasmodial and anti-inflammatory activities. However, most of the compounds responsible for the antiplasmodial and anti-inflammatory activities have not been identified.

CHAPTER 2: LITERATURE REVIEW

2.1: Malaria

Malaria is a deadly parasitic disease of tropical and subtropical regions of the world, endemic in low and lower-middle income countries (WHO, 2014b). It is caused by five *Plasmodium* species (*P. falciparum, P. vivax, P. malariae, P. ovale and P. knowlesi*). Malaria affects mainly children and pregnant women due to their low level of immunity (Duffy and Avery, 2012). According to the recent WHO malaria report (WHO, 2016), the number of malaria cases and mortality reduced by 41% and 50% between 2000 and 2015, respectively. Most notably, the death of children under the age of five decreased by 60% (from 723,000 deaths in 2000 to 306,000 deaths in 2015) (WHO, 2015b, 2016). Despite this decline, malaria is still killing a child under age five every 2 minutes in sub-Saharan Africa (WHO, 2014b, 2015b, 2016).

Malaria is diagnosed by clinical symptoms and microscopic examination of blood. Clinically malaria is known to cause fever which may be accompanied by chills and rigor, hyperpyrexia, brain damage, acute respiratory tract infection, gastrointestinal, decreased cognition, myalgia (muscle pain), miscarriages, diarrhea, anaemia and irreversible disabilities (Flannery *et al.*, 2013; WHO, 2015a).

2.2: Malaria Interventions

Malaria can be prevented and treated by cost-effective interventions including vector control, chemoprevention, and case management (Figure 2.1). Vector control reduces the transmission of parasites from human to mosquitos and then back to humans. This is achieved mainly through the use of insecticide-treated mosquito nets (ITNs), larval control and indoor residual spraying

(IRS) using pyrethroids and non-pyrethroid insecticides. Chemoprevention which suppresses blood-stage infection in humans is particularly effective in young children and pregnant women. Case management involves detecting malaria parasite in human blood, diagnosis and treating the infection (Hemingway and Bates, 2003; WHO, 2015b).

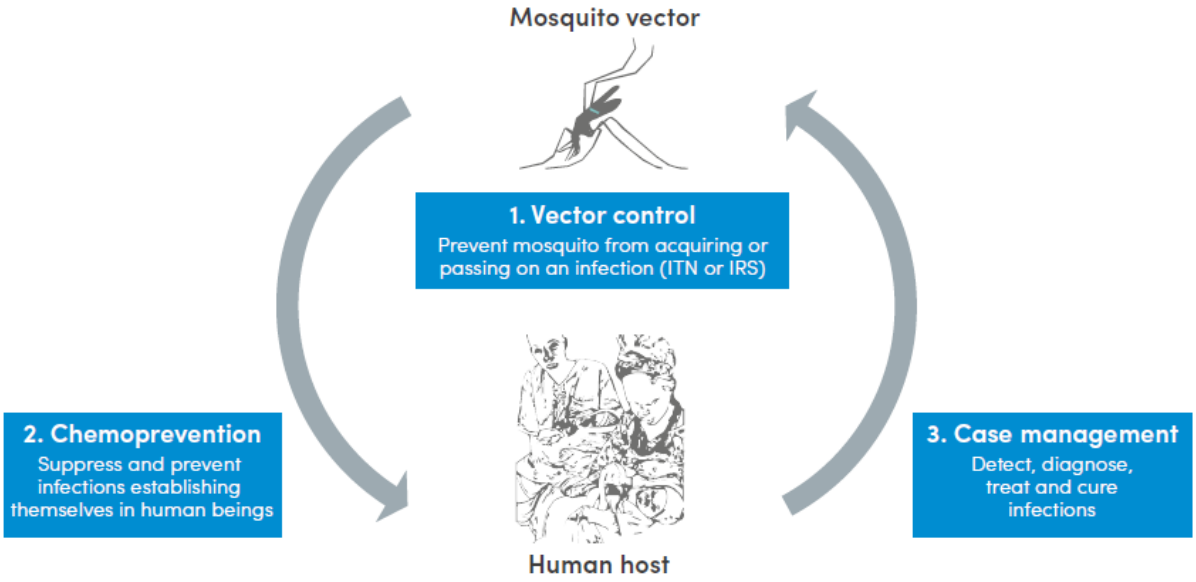


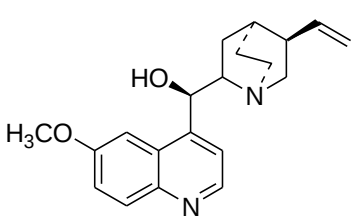
Figure 2.1: Main strategies to prevent and treat malaria (WHO, 2015b)

2.3. Antimalarial Drugs

Malaria interventions are used to prevent malaria before affecting individuals, but if the person is already infected with malaria, there is a need to use antimalarial drugs to treat the patient. The antimalarial drugs are principally used to eliminate the erythrocytic and hepatic stages of the plasmodium parasite. There are several classes of antimalarial drugs depending on their core chemical structure, of which the most commonly used are listed in Table 2.1. Medicinal plants are the main sources of antimalarial drugs currently in use. The first antimalarial drug quinine (**1**) was isolated from the stem bark of *Cinchona succuriba* in the 17th century by Caventou and

Pelletier, and it has been the only antimalarial drug for decades. It acts on the blood stages of the plasmodium parasite life cycle. Quinine is still in use for the treatment of uncomplicated malaria cases (Biamonte *et al.*, 2013; Cui *et al.*, 2015; Pohlit *et al.*, 2013).

Chloroquine was one of the first synthetic antimalarial drugs modeled along the structure of quinine. It is used for the treatment of malaria and also as prophylaxis. It was an effective, safe and affordable drug until resistance developed in the early 1960s by some strains of *Plasmodium falciparum* parasites. Chloroquine is still the first line prescribed drug for the treatment of *P.vivax* malaria cases in most regions even if resistance has developed by *P. falciparum*. The mode of action of chloroquine is similar to that of quinine which acts on the blood stage of the parasite life cycle (Cui *et al.*, 2015; Schlitzer, 2008).



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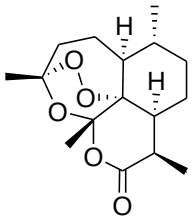

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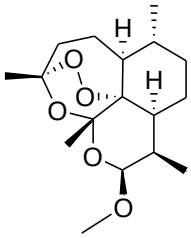

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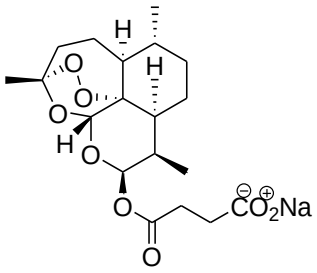

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Artemisinin (**2**) is also a plant based drug isolated from the Chinese medicinal plant *Artemisia annua.* Some derivatives such as artemether (**3**) and artesunate (**4**) are derived from it and were formulated to drugs. The derivatives are more potent than the parent drug. Artemisinin and its derivatives are used for the treatment of severe and drug resistant malaria cases. The mode of actions of these drugs is by the formation of iron-catalysed free radicals followed by alkylation of heme and consequently damaging the membrane of the parasite (Mishra *et al.*, 2009; Pohlit *et al.*, 2013; Schlitzer, 2008).

Table 2.1: Antimalarial drugs

Chemical group	Antimalarial drugs
4-Aminoquinolines	Chloroquine, amodiaquine, piperaquine
Amino-alcohols	Quinine, quinidine, mefloquine, halofantrine, lumefantrine
Sulphonamides/sulphones	Sulfadoxine, sulfalene, dapsone
Biguanides	Proguanil, chlorproguanil
Diaminopyrimidine	Pyrimethamine
8-Aminoquinoline	Primaquine
Sesquiterpene lactones	Artemisinin, arteether, artemether, artesunate, dihydroartemisin
Naphthoquinone	Atovaquone
Antibiotics	Azithromycin, clindamycin, doxycycline, tetracycline

Currently, Artemisinin-based Combination Therapy (ACT) is the first-line drug recommended for the treatment of malaria. The importance of combining artemisinin based drugs with other antimalarial drugs is to improve effectiveness of the treatment and delay resistance by the parasite. The commonly used ACTs are: artesunate-amodiaquine, artesunate sulfadoxine-pyrimethamine, artesunate-mefloquine and artemether-lumefantrine and dihydroartemisinin-piperaquine.

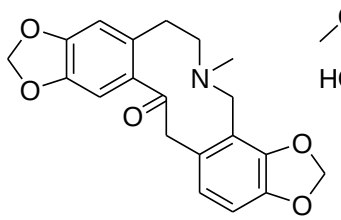
2.4: Antimalarial Drug Resistance

One of the challenges that been encountered in the efforts to control malaria is the development of resistance of parasites to the commonly used drugs. Also, mosquito populations have also developed resistance to insecticides (Menard and Dondorp, 2017). The antimalarial drug resistance is brought about by the ability of a plasmodium parasite strain to persist and multiply despite the absorption and administration of medicine given in equal to or higher doses than those commonly recommended, but within a tolerance of the subject. It is alarming that almost all of the antimalarial drugs (Table 2.1.)

developed to date have eventually succumbed to resistance. The first plasmodium resistance to quinine (**1**) was observed in 1910 in Southern America and Asia; then to chloroquine in the 1970s and 1980s in Africa (Farooq and Mahajan, 2004). Recently, strains of *P. falciparum* which have developed resistance to artemisinin (**2**) and its derivatives artemether (**3**) and artesunate (**4**) have been detected in five countries in the Greater Mekong Sub-region (GMS): Viet Nam, Thailand, Myanmar, Lao People's Democratic Republic, and Cambodia (WHO, 2015b). Mass antimalarial drug administration (MDA) and use of substandard and counterfeit drugs are the key causes of drug resistance. To avoid resistance, adequate dosage and right administration of the drugs in combination therapy, like artemisinin-based combination therapy(ACT), have been recommended (Menard and Dondorp, 2017; Mishra *et al.*, 2009).

2.5: Phytochemicals with Antiplasmodial Activities

Several potent anti-malarial secondary metabolites from plants have been reported. Alkaloids, flavonoids, quinones, anthraquinones, and terpenoids are the major class of these compounds. Alkaloids isolated from *Corydalis calliantha* (Papaveraceae) protopine (**5**) ($IC_{50,}$ TM4 = 4.25 ± 0.69 μM; K1= 4.29 ± 1.24 μM) and cheilanthifoline (**6**) ($IC_{50,}$ TM4 = 2.78 ± 0.39 μM; K1= 3.76 ± 1.00 μM) showed promising *in vitro* antiplasmodial activities against *P. falciparum*, for both wild type (TM4) and multidrug-resistant (K1) strains. Cassiarin A (**7**), an alkaloid isolated from the leaves of *Cassia siamea* (Leguminosae), had inhibitory effects against *P. falciparum* with an IC_{50} value of 0.02 μM. Further, the *in vivo* antimalarial activity test, cassiarin A displayed high activity with an ED_{50} value of 0.17 μM (Nogueira and Lopes, 2011; Wangchuk *et al.*, 2010).



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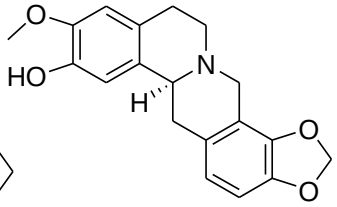

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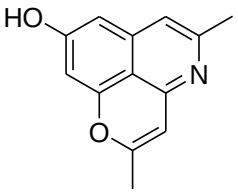

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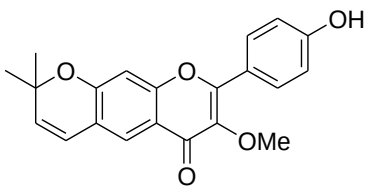
Among the flavonoids reported with promising antimalarial activities include; the flavone 3-methoxycarpachromene (**8**), isolated from *Pistacia atlantica* (Anacardiaceae), showed an IC_{50} value of 3.4 μM toward the *P. falciparum* K1 strain (Nogueira and Lopes, 2011); isochamaejasmin (**9**), a biflavonoid isolated from *Ormocarpum kirkii* (Leguminosae), showed moderate antiplasmodial activity ($IC_{50,}$ 7.3 ± 3.8 μM) against *P. falciparum* K1 strain with relatively low cytotoxicity ($CC_{50,}$ 29.0 ± 10.9 μM) (Dhooghe *et al.*, 2010); 2'-hydroxy-3,4,4',5,6'-pentamethoxychalcone (**10**), isolated from *Neoraputia magnifica* (Rutaceae), showed good antiplasmodial activity against the *P. falciparum* 3D7 strain with an IC_{50} value of 6.9 μM; 3',4' -methylenedioxy-7,8-(2",2'-dimethylpyrano)-flavone (**11**) and 3,4-Methylenedioxy-2'-methoxy-6",6"-dimethylchromeno[2",3":4',3']-*b*-oxochalcone (**12**), isolated from *Lonchocarpus subglaucescens* (Leguminosae), exhibited antiplasmodial activity against the *P. falciparum* 3D7strain with IC_{50} values of 7.6 and 9.5 μM, respectively (Santos *et al.*, 2009) .



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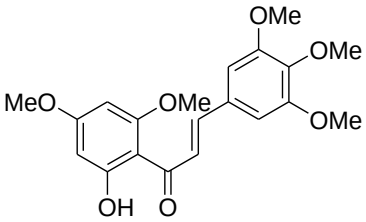

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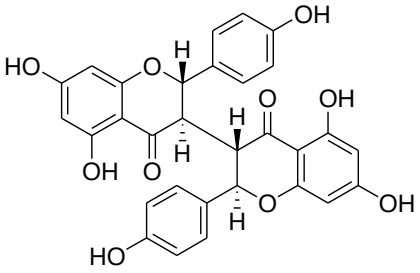

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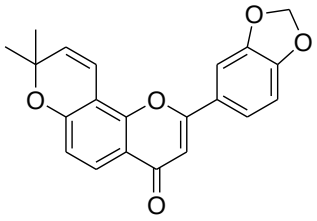

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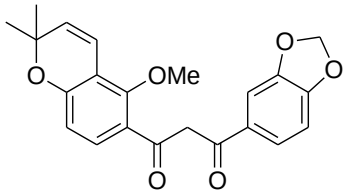

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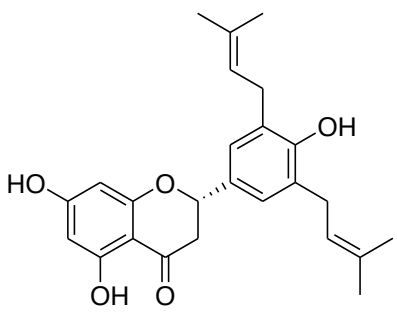
Flavonoids isolated from the stem and root bark of *Erythrina abyssinica* (Leguminosae) showed good to moderate antiplasmodial activities against chloroquine sensitive (D6), and chloroquine-resistant (W2) strains of *P. falciparum* (Yenesew *et al.*, 2003b; Yenesew *et al.*, 2004): abyssinone V (**13**) [$IC_{50,}$ D6 = 4.9 ± 0.8 μM; W2 = 6.1 ± 1.3 μM], sigmoidin A (**14**) [D6 = 5.8 ± 0.6 μM; W2 = 5.9 ± 1.1 μM], abyssinin III (**15**) [$IC_{50,}$ D6 = 5.8 ± 1.1 μM; W2 = 5.2 ± 1.7 μM], and abyssinone IV(**16**) [$IC_{50,}$ D6 = 5.4 ± 1.5 μM; W2 = 5.9 ± 1.8 μM]; Erythrabssin-II (**17**) [$IC_{50,}$ D6 = 8.1 ± 1.4 μM; W2 = 6.5 ± 0.6 μM].



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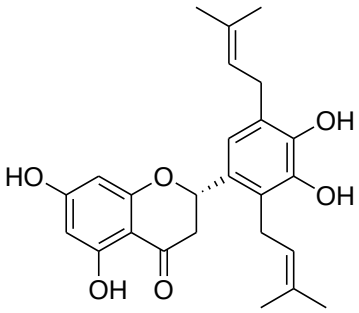

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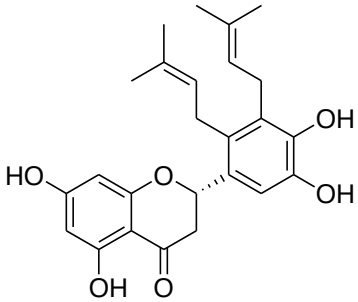

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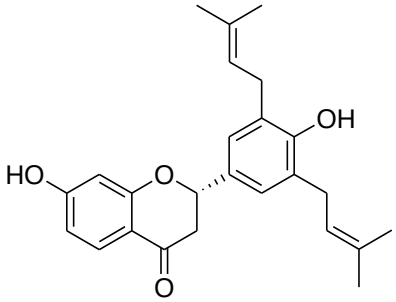

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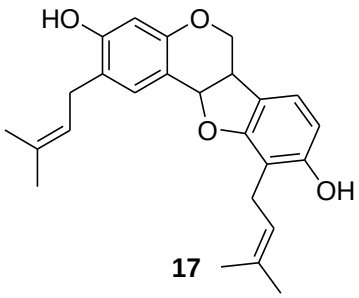

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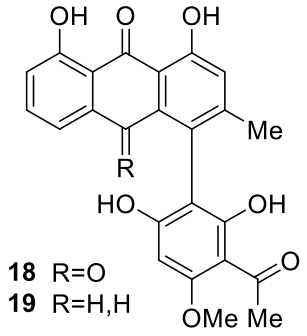
Quinones, including anthraquinones with marked antiplasmodial activity have also been identified from different plants. Knipholone (**18**) (IC_{50}, K1=1.06; NF54 =1.70 μg/mL) isolated from roots of *Bulbine capitata* **(**Asphodelaceae) and knipholone anthrone (**19**) (IC_{50}, K1= 0.38; NF54 = 0.42 μg/mL) isolated from *Kniphophia foliosa* **(**Asphodelaceae) showed excellent activity against chloroquine-resistant K1 and the chloroquine-sensitive NF54 strains of *P. falciparum* (Bringmann *et al.*, 1999).



18 R=O
19 R=H,H

2.6: Inflammation and Pain

The primary reason why sick people seek medical attention is that they experience some discomfort like inflammation and pain. Inflammation is the local bodily response to tissue injury or infection which can be caused by pathogens, microbial infection, physical injury, irritant or corrosive chemicals. It is categorized into acute and chronic inflammation. Acute inflammation is the early reaction of the body to tissue injury; whereas chronic inflammation is the subsequent reaction of a body that may occur if the stimulus or injury cannot be removed or when the damaged tissue has not recovered (Ann *et al.*, 2009; Gautam and Jachak, 2009a).

The two common pathways to inflammation are arachidonic acid (AA)-dependent and arachidonic acid (AA)-independent pathways. The arachidonic acid-dependent pathway may include cyclooxygenase (COX), lipoxygenase (LOX) and phospholipase A2 (PLA2) as mediators. In contrast, the arachidonic acid (AA)-independent pathway includes nitric oxide synthase (NOS), NF-*k*B, peroxisome proliferator-activated receptor (PPAR) and NSAID-activated gen-1 (NAG-1). The products from both pathways are considered to be important mediators in the control of inflammation (Issa *et al.*, 2006; Yoon and Baek, 2005).

Pain is widely accepted as one of the most important indicators of the quality of life since it impairs the individual's ability to perform daily activities. Pain is symptomatic of some form of dysfunction in the body. Pain can arise from two different stimuli, the actual or potential damage. Pain is also a subjective event and cannot truly be measured by an objective observer (IASP, 1979).

Pain can also be designated as acute or chronic depending on the duration. Acute pain has a predictable end and typically lasts less than 30 days. The cause of this kind of pain can be easily

identified and treated. Medication may be useful to stop or decrease pain and also to speed-up the remedial process by shortening the period of the injury (Conn, 2005; Rowe *et al.*, 2006). Chronic pain persists longer than the temporal course of natural healing from a particular type of injury or disease process (Hansen, 2005). The common effects of chronic pain may include tense muscles, lack of energy, limited mobility, anger and anxiety, change in appetite and depression (DiSantostefano, 2011; Turk and Okifuji, 2001).

2.7: Anti-inflammatory and Anti-pain Drugs

Patients use different methods to get relief from the suffering caused by inflammation or pain. The method of treating pain ranges from the use of traditional concoctions to conventional drugs such as Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), opioids, and paracetamol (**21**). Medications that relieve pain are called analgesics, also known as painkillers. This term applies to any member of the group of drugs used to relieve pain and to achieve analgesia - painless state. The analgesic drugs may act on the central nervous or peripheral system. The pain relief induced by analgesics occurs either by interfering with the brain's interpretation of the signals or blocking pain signals going to the brain. However, many non-drug treatments can also be used for medication. There are several painkillers, and they include aspirin (**20**), paracetamol (**21**), narcotic drugs such as morphine (**22**), the NSAIDs like the salicylates, synthetic drugs with narcotic properties such as tramadol (Schneider, 2006; Donna, 2007).

2.7.1. Over the Counter Drugs

Aspirin (**20**) discovered more than a century ago, remains one of the most widely used pain relievers in the world. It relieves fever, headaches, minor to moderate pain and swelling. It can cause stomach upset or bleeding in the stomach, and it also causes kidney failure if it is taken for

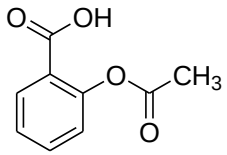
an extended period of time, or in high doses (Belay *et al.*, 1999; Liebschutz *et al.*, 2014). Paracetamol (**21**) is used alone for mild to moderate pain. It is also combined with other types of pain medications for serious pain. However, it does not reduce swelling and has side effects (can cause liver and kidney damage) if taken in excessive doses (Liebschutz *et al.*, 2014)

2.7.2. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) is a group of medicines that work similar to aspirin (**20**) by relieving both inflammation and pain. Celecoxib, ibuprofen, and naproxen are examples belonging to this group of drugs. They normally relieve mild to moderate pain, fever, headache, and swelling. Stomach upset or bleeding in stomach, and kidney, or liver damage are the common side effects of these drugs (Belay *et al.*, 1999; Liebschutz *et al.*, 2014)

2.7.3. Opioid Medications

Opioids such as morphine (**22**), oxycodone and others are strong drugs used to treat moderate to severe pain. Opioids come in a variety of forms and strength; some act fast, but the effect does not last long, while others provide long-lasting pain relief. Opioids usually produce side effects such as stomach upset, sleepiness, constipation and potential for addiction (Liebschutz *et al.*, 2014). Morphine (**22**) was isolated from opium (*Papaver somniferum* (Papaveraceae)) by a German pharmacist Friedrich Serturner in 1803. It was used extensively as a painkiller during the American Civil War. Consequently, many soldiers became addicted. It works through the body's natural pain-killing mechanisms, preventing pain messages from reaching the brain (Chalise, 2015).



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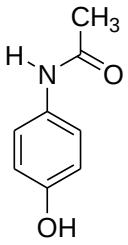

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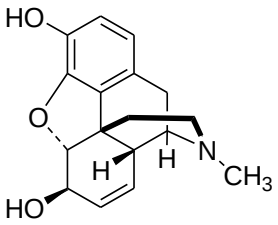

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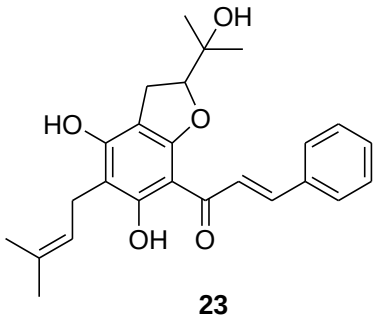
2.8: Phytochemicals with Anti-inflammatory Activities

A wide range of phytochemicals including flavonoids, phenolics, alkaloids, and terpenoids showed anti-inflammatory activities. For instance, some flavonoids in addition to anti-oxidative activities (Dorta *et al.*, 2008), exhibit anti-inflammatory activity *in vivo* and *in-vitro* models of both acute and chronic inflammation (Tanwar and Modgil, 2012). They inhibit the COX (cyclooxygenase) and LOX (lipooxygenase) enzymes (Miller, 1996). Cedrediprenone (**23**), a chalcone isolated from the seed of *Cedrelopsis grevei* (Ptaeroxylaceae), showed anti-inflammatory activity by scavenging superoxide anions in a cell free system (IC_{50} of 0.2 µg/mL) and the luminol-enhanced chemiluminescence of reactive oxygen metabolites generated by human polymorphonuclear leucocytes activated with opsonized zymosan (IC_{50} of 8.1 µg/mL) (Koorbanally *et al.*, 2003).

The isoflavone griffonianone D (**24**) was isolated from *Millettia griffoniana* (Leguminosae) and tested for TPA-induced ear edema activity on mice and showed 77% inhibition at a dose of 0.25mg/ear (Yankep *et al.*, 2003). Warangalone (**25**), an isoflavone isolated from the bark of *Erythrina addisoniae* (Leguminosae), showed 68% inhibition at 5 mg/kg concentration on phospholipase A2 (PLA2)-acute mouse paw edema anti-inflammatory test (Talla *et al.*, 2003). Erycristagallin (**26**), isolated from the root of *E. mildbraedii* (Leguminosae), when tested in

different models of inflammation, exhibited a strong inhibition (94% at 0.25 mg/ear) in the 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced acute ear edema test in mice. (Njamen *et al.*, 2003).



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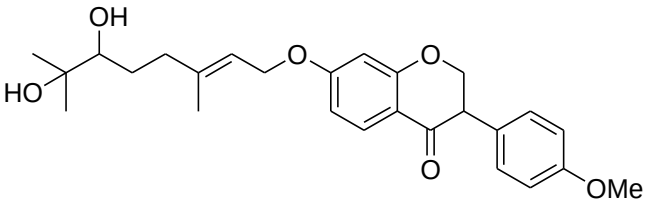

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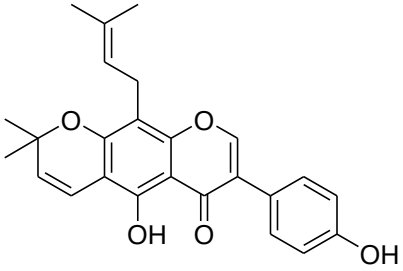

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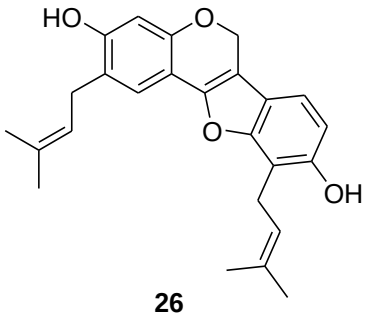

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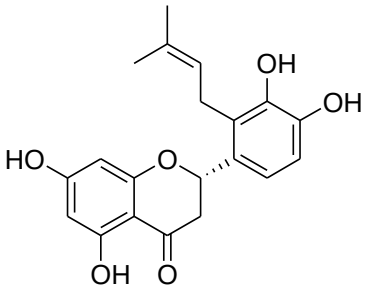
Sigmoidins A (**14**) and B (**27**) were also effective against TPA-induced ear edema, showing 89% and 83% inhibition, respectively, at a dose of 0.25 mg/ear. These compounds occur in *Erythrina* species (Njamen *et al.*, 2004).

Comalada *et al*. (2006) reported the effects of some of the common flavonoids on LPS-induced TNFa release in bone marrow-derived macrophages anti-inflammatory assay. Kaempferol (**28**) (17.1 ± 1.3 μM), chrysin (**29**) (23.6 ± 4.6 μM), daidzein (**30**) (14.1 ± 1.8 μM) and hesperetin (31) (24.1 ± 1.3 μM) showed significant inhibition of TNFα at 25 μM concentration (Comalada *et al.*, 2006).



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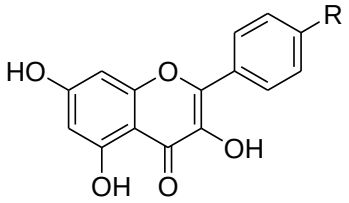
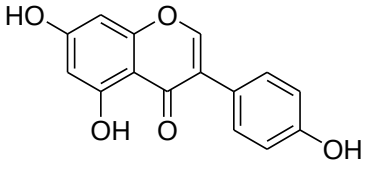


28 R= OH
29 R= H



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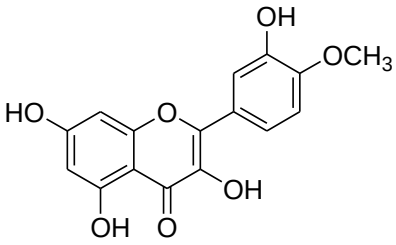
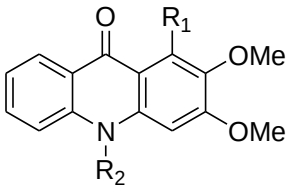
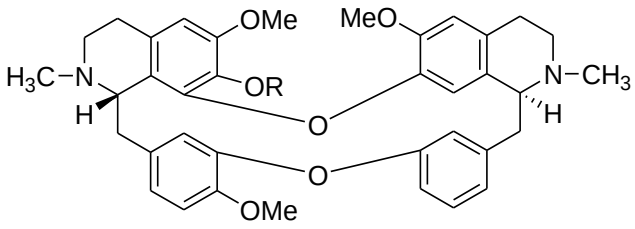


31

Alkaloids with anti-inflammatory activity reported from different plants include toddaliopsins A (**32**), B (**33**), C (**34**), and D (**35**), isolated from the leaves *Toddaliopsis bremekampii (*Rutaceae). When these compounds were tested i*n vitro* on zymosan-activated human polymorphonuclear leucocytes, in a chemoluminescence assay system, they showed moderate activity with mean IC_{50} values of 27.3, 48.3, 4.21, and 79.1 µg/mL, respectively (Naidoo *et al.*, 2005). The bisbenzyl isoquinoline alkaloids, fangchinoline (**36**) and tetrandrine (**37**), isolated from the roots of *Stephania tetrandrae* (Menispermaceae), exhibited anti-inflammatory effects, causing 23% and 24% inhibitions of croton oil-induced ear edema, at a dose of 20 mg/kg. respectively (Choi *et al.*, 2000).



32 R^1 = OMe, R^2= H
33 R^1 = OMe, R^2= CH_2OCOCH_3
34 R^1 = OH, R^2= CH_2OCOCH_3
35 R^1 = OMe, R^2= CH_2OCH_3



36 R= H
37 R= CH_3

2.9: The Family Leguminosae

The plant species investigated in this study belong to the family Leguminosae, sub-family Papilionoideae. The Leguminosae family comprises of around 19,500 species in 750 genera. It is the third largest family after orchids (Orchidaceae) and asters (Asteraceae). This family is divided into three subfamilies Caesalpinoideae, Mimosoideae and Papilionoideae. These subfamilies are similar in the fruit being a legume but different in the flower structures (Doyle and Luckow, 2003). The family is dispersed in all terrestrial habitats occupied by plants especially in the tropics where the family probably originated. It is diversely distributed in the tropical rain forests and the dry forests of the Americas and Africa as well as temperate plains, woodlands, and deserts (Wanda *et al*., 2015).

2.9.1. The subfamily Papilionoideae

The Papilionoideae subfamily differs from the other two subfamilies of Leguminosae (Caesalpinoideae and Mimosoideae) by the presence of papilionoid flowers and a hilar valve in the seeds. The Papilionoideae comprises of 28 tribes, 480 genera and 13,800 species which are trees, shrubs and herbs. *Astragalus* (1,200 species), *Adesmia* (250 species), *Crotalaria* (300 species), *Desmodium* (300 species), *Erythrina* (200 species) *Indigofera* (700 species), *Trifolium*

(200 species) and *Tephrosia* (400 species) are among the big genera in this subfamily (Murer-Grimes, 1997). These genera are distributed in the tropical and subtropical region of the world.

2.9.2 The genus Tephrosia

The genus *Tephrosia* is an enormous pantropical genus which includes more than 400 species; of these species, 35 are found in India, 30 are native to South America, and 110 originate in Africa, and 30 of these in Kenya. *Tephrosia* has important traditional uses especially in East Africa. This genus is characterized by pinnate leaves (fern-like), without stipels, with subequal calyx teeth, sessile (lacking stalks) ovary, upcurved style, long pods flattened and dehiscent. *T. aequilata*, *T. elata*, *T. holstii*, *T. interrupta*, *T. noctiflora*, *T. paucijuga*, *T. pentaphylla*, *T. pumila*, *T. purpurea*, *T. villosa* among others are native *Tephrosia* species in East Africa (Gillett, 1958; Kantachot *et al.*, 2014).

2.9.2.1. Tephrosia noctiflora

Local name: CHIBALAZI (Digo), KABAZI (Bondei, Shambaa, Zigua)

Morphologically, a shrubby herb up to 1.3 m tall with spreading branches, sometimes annual. Stems and branches with appressed grayish hairs, especially when young. An irregular, short-lived perennial; leaflets 15-25; flowers variable in color but often whitish with a purple center, in fatal pseudo-racemens, rarely also in upper leaf axils; standard densely brown-silky outside, 8-12 mm long; pod pale with dark edges, 5 cm (Agnew and Agnew, 1994).

Distribution— Native to Africa, India, Peninsular Malaysia, Sri Lanka, Taiwan, West Java, Sabah.

Ethnomedicinal use: - The roots are chewed as a cough remedy, and water is drunk at the same time. The roots have a strong taste and also used as an emetic.

2.9.2.2. Tephrosia pumila

Local name: CHIBALAZI (Digo), XAANO HAY EHI (Iraqw)

Morphologically, a sprawling woody annual or short-lived perennial, with 7-13 leaflets, each about 22x7 mm; flowers purple in short open racemes, with the standard under 8 mm long; pod 4 mm long (Agnew and Agnew, 1994).

Photo: *Tephrosia pumila* (Photo taken by: Lois Muiva-Mutisya)

Distribution- Angola, Botswana, Burundi, DRC Ethiopia, Kenya, Ghana, India, Malawi, Mozambique, Madagascar, Pakistan Rwanda, Somalia, Sudan, South Africa, Malaysia, Indonesia, Philippines, Thailand, Tanzania, Togo, Uganda, Zambia, Zimbabwe.

Ethnomedicinal use:- The roots are chewed as a remedy for cold in the chest. The vapour from the boiling of the plant is used as an inhalant for babies suffering from colds in the head with running nose. The boiled roots and the infusion are taken in broth as a cure for venereal diseases. Normally, one glass of the infusion is mixed with one glass of broth.

2.9.2.3. Tephrosia aequilata

Local name: MSHUHU (Pare)

Morphologically, a softly woody shrub, less than 2 m high, rarely taller or a small tree up to 5m.; leaflets 13-21. 40 x15 mm, all the same size, rounded, straight-edged or notched at base, dark above, pale below, veins visible but not prominent; flowers purple, in dense terminal, nearly stalkless subglobose white or brown-hairy inflorescences; standard silky, 13-17 mm long; pod 45 mm, light brown (Agnew and Shirley, 1994; Beentje, 1994).

Photo: *Tephrosia aequilata* (Photo taken by: Lois Muiva-Mutisya)

Distribution- Ethiopia; Somalia; Sudan; Kenya

Ethnomedicinal use: - Roots chewed with salt as a cure for venereal diseases.

2.9.2.4. Tephrosia elata

Morphologically, it is a short-leaved bushy perennial shrub. Leaflets grey-green, often deeply notched at apex, flowers pink or purple, lateral veins beneath, visible and longer dense terminal racemes, standard golden hairy, pods 55 x 5 mm, 14 - 16 mm long; ascending or erect, with dense light brown hairs (Agnew and Agnew, 1994; Beentje *et al.*, 1994).

Photo: *Tephrosia elata* (Photo taken by: Lois Muiva-Mutisya)

Distribution- Ethiopia; Somalia; Sudan; Kenya

2.9.2.5. Tephrosia purpurea subspecies leptostachya

Local name: KAPODOZ (Giriama), LUDUMIO (Sukuma)

An erect or more often spreading, annual or short-leaved perennial; leaflets 9-17, 22 x 8 mm; flowers reddish-purple or pink, in slender, lax, leaf-opposed pseudo-racemes and in upper leaf axils; standard white-hairy outside, about 8 mm long; pod about 35 mm long, 6-9 seeded (Agnew and Agnew, 1994; Beentje *et al.*, 1994).

Photo: *Tephrosia purpurea* ssp *leptostachya* (Photo taken by: Lois Muiva-Mutisya)

Distribution- Ethiopia; Kenya; Somalia; Sudan

Ethnomedicinal use: - Roots used as a medicine for stomach pains. A decoction of the roots and leaves is used as a purgative. Leaves are used for snake bite and headache.

2.9.2.6. Tephrosia rhodesica

Morphologically, much-branched small shrub, usually less than 2 m tall. A short-leaved perennial; leaflets 11-19, 25x9 mm; flowers pink or mauve in rather dense terminal pseudo-racemes and also often in upper axils; standard long-hairy, about 11 mm long; pod pale hairy, with pale margins (Agnew and Agnew, 1994; Beentje *et al.*, 1994).

Photo: *Tephrosia rhodesica* (Photo taken by: Lois Muiva-Mutisya)

Distribution- Ethiopia, Kenya, Somalia, Sudan, Zimbabwe

2.10: Ethnomedical Information of the Genus *Tephrosia*

Tephrosia species are traditionally used in traditional medicine practice for curing different illnesses, as a fish poison and as insecticide (Roy *et al.*, 1986b; Tarus *et al.*, 2002). The traditional uses of some of the *Tephrosia* species are summarized in Table 2.2.

Table 2.2: Ethnomedical uses of some *Tephrosia* species.

Tephrosia species	Ethnomedical use	Reference
T. aequilata	Roots are used to treat venereal diseases when chewed with salt.	(Kokwaro, 2009; Tarus *et al.*, 2002)
T. apollinea	Aerial part is used to treat cough, earache, nasal and bronchitis congestion, wounds and bone fractures	(Ammar *et al.*, 2013)
T. elata	Roots chewed as a treatment for stomach pain, fever, and general weakness	(Muiva *et al.*, 2009)
T. calophylla	Roots are used for the treatment of diabetes and the leaf extracts used for treating ulcers, inflammation, and microbial infections.	(Parine *et al.*, 2015)
T. holstii	Roots are used for curing stomach pain and general weakness.	(Beentje *et al.*, 1994)
T. linearis	Boiled leaves are used to relieve baby illnesses	(Kokwaro, 2009)
T. interrupta	The roasted and ground roots are mixed with a little salt and are used to cure cough. Leaf infusion is used for treating sore throat and also used to cure poultry fever.	(Kokwaro, 2009)
T. noctiflora	Roots are chewed to relive cough and as emetic.	(Kokwaro, 2009)
T. paucijuga	Dried and ground roots and leaves are used to treat wounds.	(Kokwaro, 2009)
T. purpurea	Roots, stems, and leaf are used to cure gastroduodenal disorders, elephantitis, flactulance, haemmaroids, asthma, bronchitis, anemia, dysmenorrhea, chronic fever, boils, pimples, gingivitis, stomach pains, as a purgative also used in skin disorders.	(Chinniah *et al.*, 2009; Dalwadi *et al.*, 2014; Kalume *et al.*, 2012; Kokwaro, 2009)
T. pentaphylla	The roots are chewed to relieve pain in the throat and cold in the chest.	(Kokwaro, 2009)
T. pumila	The infusion of the boiled roots is used for the treatment of venereal diseases. The roots are also chewed as a medicine for cold in the	(Ganapaty *et al.*, 2008a; Kokwaro,

	chest.	2009)
T. obovata	The seeds are used to catch fish	(Chen *et al.*, 1978a)
T. unzflora	To treat poisonous bites	(Abreu and Luis, 1996)
T. villosa	The roots are boiled and mixed with milk to treat liver problems and relieve irritating pain, as well as to treat respiratory problems.	(Kokwaro, 2009; Mirutse *et al.*, 2009; Vijayan *et al.*, 2012)
T. vogelii	The seeds are used as purgative, fish poison and pesticide. The roots are boiled and mixed with milk and used to treat liver problems and to relieve irritating pain and also to treat constipation. The leaves are used to treat scabies, yaws, eradication of ticks and fleas on livestock and poultry.	(Dzenda *et al.*, 2015; Kalume *et al.*, 2012; Kokwaro, 2009; Li *et al.*, 2015; Stevenson *et al.*, 2012)

2.11: Biological Activities of the Genus *Tephrosia*

Several *Tephrosia* species have been investigated for their biological activities and showed different types of activities (Table 2.3.). The different parts of *T. aequilata, T. villosa* and *T. vogelii* were found to possess antimicrobial activities (Balakrishnan *et al.*, 2007; Tarus *et al.*, 2002; Wanga *et al.*, 2007). The seed pods of *T. elata* showed antiplasmodial activity. *T. sinapou* and *T. spinosa* showed anti-inflammatory activities (Chakradhar *et al.*, 2005; Martinez *et al.*, 2012). The extract of *T. purpurea* exhibited anti-oxidant, antimicrobial, antibacterial, antileishmanial, anti-inflammatory, anticancer, hepatoprotective and antidiabetic activities (Choudhary, 2007; Gupta *et al.*, 2008; Hussain *et al.*, 2012; Pavana *et al.*, 2007; Shah *et al.*, 2011; Sharma *et al.*, 2003).

Table 2.3: Selected *Tephrosia* species and their biological activities

Tephrosia species	Plant part	Biological activity	References
T. aequilata	Roots	Antimicrobial and parasitic activities	(Tarus *et al.*, 2002)
T. bracteolata	Leaves	Antipyretic activity	(Onaolapo *et al.*, 2009)
T. calophylla	Roots	Hepatoprotective, antihyperlipidemic	(Adinarayana *et al.*, 2009; Mohan, 2011)
T. deflexa	Seeds	Antibacterial	(Kare *et al.*, 2006)
T. elata	seed pods	Antiplasmodial	(Muiva *et al.*, 2009)
T. hidebrandtii	Roots	Antifeedant	(Lwande *et al.*, 1986b)
T. linearis	Roots	Antibacterial	(Ratsimamanga-Urverg *et al.*, 1994)
T. pumila	Roots	Antiprotozoal, cytotoxicity	(Ganapaty *et al.*, 2008a; Ganapaty *et al*., 2008b; Ganapaty *et al*., 2009)
T. purpurea	Roots	Antioxidant, antimicrobial, Antibacterial, antileishmanial, anti-inflammatory, anticancer, hepatoprotective activity, antidiabetic	(Choudhary, 2007; Gupta *et al.*, 2008; Hussain *et al.*, 2012; Pavana *et al.*, 2007; Shah *et al.*, 2011; Sharma *et al.*, 2003)
T. sinapou	Roots	anti-inflammatory activity	(Martinez *et al.*, 2012)
T. spinosa	Aerial parts	anti-inflammatory activity	(Chakradhar *et al.*, 2005)
T. toxicaria	Roots	Anticancer activity	(Jang *et al.*, 2003)
T. villosa	-	Anti-hyperglycemic activity	(Balakrishnan *et al.*, 2007)
T. vogelii	Roots and leaves	Antimicrobial activity	(Wanga *et al.*, 2007)

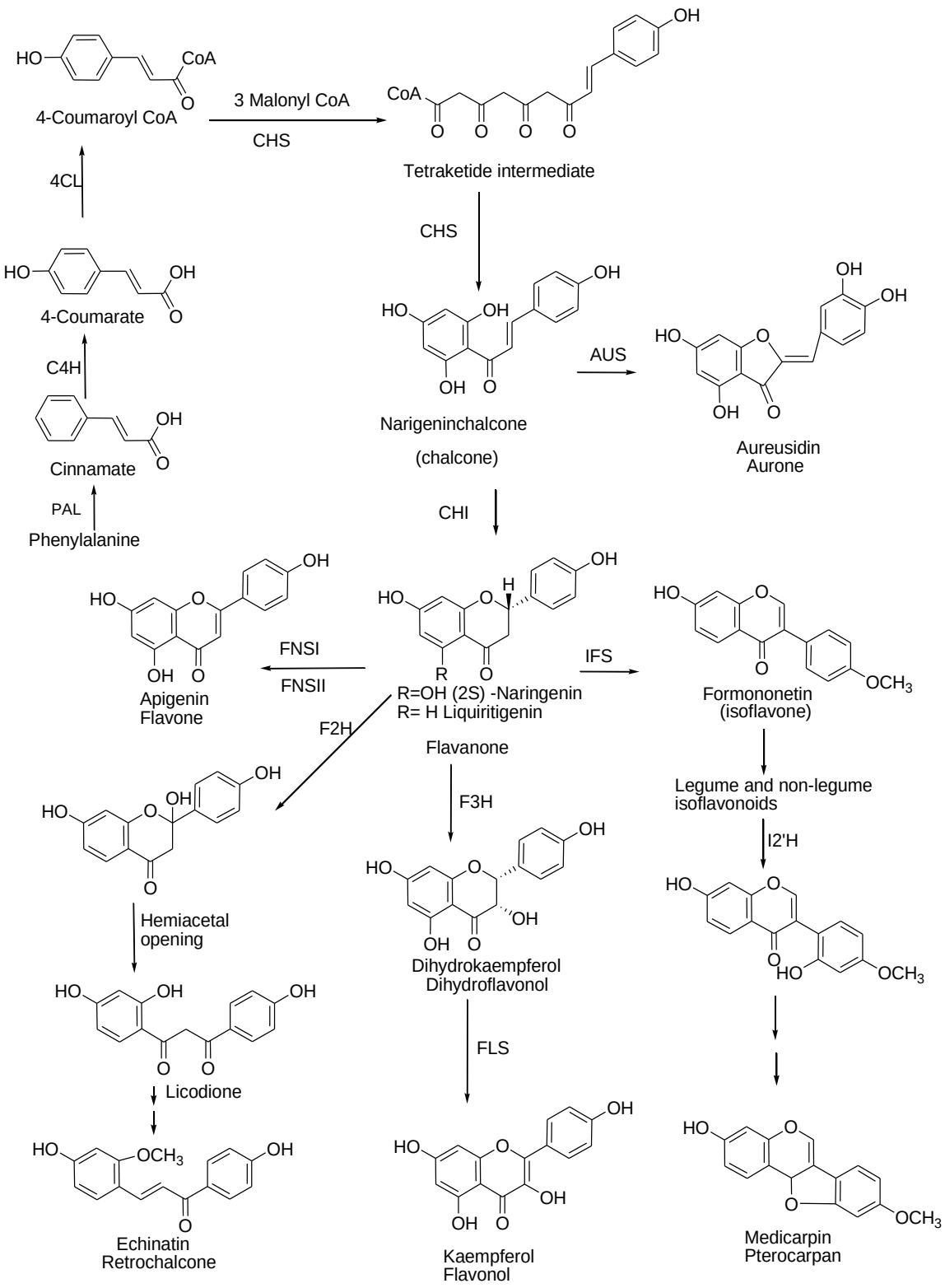
2.12: Biosynthesis of Flavonoids

The biosynthesis of flavonoids involves two metabolic pathways, the shikimate and phenylpropanoids pathways. The flavonoid skeleton is initially formed through the phenylpropanoid pathway, where the aromatic amino acid phenylalanine is converted into 4-coumaroyl-CoA (Scheme 1). Upon incorporation of three malonyl CoA groups a tetraketide 4-coumaroyl CoA intermediate is formed which upon aromatization results in the formation of a chalcone. (Dewick, 2002; Saito *et al.*, 2013; Winkel-Shirley, 2001).

The first subclass of flavonoids chalcone is formed in the initial step of the biosynthesis of flavonoids. Chalcone synthase (CHS) is the specific enzyme for the synthesis of narigeninchalcone or isoliquiritigenin chalcone by synthesis of *p*-hydroxycoumaroyl CoA with three molecules of malonyl-CoA to form a tetraketide intermediate which is cyclized into a hydroxylated aromatic ring system to form a chalcone scaffold (Dewick, 2002; Ferreyra *et al.*, 2012).

The other subclasses of flavonoids are formed in various ways from the chalcone skeleton. The enzymes chalcone isomerase (CHI) catalyzes the stereospecific isomerization of chalcone into their corresponding flavanones; form naringenin and liquiritigenin (flavanones) from Narigeninchalcone and isoliquiritigenin chalcone, respectively. An aurone (aureusidin) is formed by the enzyme aurone synthase (AUS). The enzyme isoflavone synthetase (IFS) forms isoflavonoids (formononetin) from flavanone through two steps: formation of 2-hydroxyisoflavanone through the 2-hydroxylation and aryl migration of flavanone substrates, followed by a dehydration step to the corresponding isoflavone derivative. The various subclasses of flavonoid are formed from the basic skeleton of flavonoids depending on the

specific group enzymes. The Scheme 1 below summarizes the biosynthesis of some subclasses of flavonoids (Ferreyra *et al.*, 2012; Winkel-Shirley, 2001).



Scheme 1. Biosynthetic pathways of flavonoids (Akashi *et al.*, 1999; Dewick, 2002; Winkel-Shirley, 2001)

2.13: Phytochemical Information on the Genus *Tephrosia*

Several *Tephrosia* species have been investigated for their secondary metabolites. As a result, different classes of compounds, such as sterols, terpenoids, triterpenoids, sesquiterpenes, and flavonoids have been isolated. Among these, flavonoids are the major class of compounds; more than two hundred flavonoids have been reported from fifty *Tephrosia* species.

2.13.1. Flavonoids

The most common subclasses of flavonoids that were isolated from this genus are flavanones, flavones, flavans, flavens, isoflavones, flavanol, chalcones, rotenoids and pterocarpans.

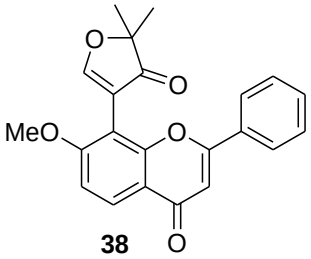
2.13.1.1. Flavones of Tephrosia

Flavones are among the major compounds of the genus *Tephrosia.* They are known for having an additional ring at C-7/ C-8. All of the reported flavones from this genus showed the same pattern of lack of oxygenation on B-ring, prenylation at C-8 and few were prenylated at C-6. Tachrosin (**41**) being the first flavone isolated from *T. polystachyoides* with a modified prenyl group (Smalberger *et al.*, 1971). Staohyoidin (**42**) and tephrodin (**43**), isolated from *T. polystachyoides* contain a unique modified prenyl group (Vleggaar *et al.*, 1972). The flavones so far reported from *Tephrosia* are listed in Table 2.4. Most of these compounds were reported from *T. apollinea, T. purpurea,* and *T. polystachyoides.*

Table 2.4: Flavones of *Tephrosia*

Compound	Species	Reference
Tephroglabrin (**38**)	*T. purpurea* (RT)	(Pelter *et al.*, 1981)
Tepurindiol (**39**)	*T. purpurea* (RT)	(Pelter *et al.*, 1981)
Glabratephrin (**40**)	*T. apollinea* (SD)	(Waterman and Khalid, 1980a)
Tachrosin (**41**)	*T. polystachyoides* (RT)	(Smalberger *et al.*, 1971)
Staohyoidin (**42**)	*T. polystachyoides*	(Vleggaar *et al.*, 1972)
Tephrodin (**43**)	*T. polystachyoides*	(Vleggaar *et al.*, 1972)
Semiglabrin (**44**)	*T. semiglabra, T. apollinea*	(Smalberger *et al.*, 1973; Vleggaar *et al.*, 1978)
Semiglabrinol (**45**)	*T. semiglabra, T. apollinea*	(Smalberger *et al.*, 1973; Waterman and Khalid, 1980b)
Tephrostachin (**46**)	*T. polystachyoides*	(Vleggaar *et al.*, 1973)
Emoroidone (**47**)	*T. emoroides* (RT)	(Machocho *et al.*, 1995)
Tephroapollin C (**48**)	*T. apollinea* (AP)	(El-Razek *et al.*, 2007)
Tephroapollin D (**49**)	*T. apollinea* (AP)	(El-Razek *et al.*, 2007)
Tephroapollin E (**50**)	*T. apollinea* (AP)	(El-Razek *et al.*, 2007)
Tephroapollin F (**51**)	*T. apollinea* (AP)	(El-Razek *et al.*, 2007)
Tephroapollin G (**52**)	*T. apollinea* (AP)	(El-Razek *et al.*, 2007)
Tephropurpulin A (**53**)	*T. apollinea* (AP)	(Khalafallah *et al.*, 2009)
Polystachin (**54**)	*T. polystachya*	(Vleggaar *et al.*, 1978)
Hookerianin (**55**)	*T. hookeriana* (RT)	(Prabhakar *et al.*, 1996)
Apollinine (**56**)	*T. purpurea* (SD)	(Waterman and Khalid, 1980a)
Terpurinflavone (**57**)	*T. purpurea* (ST)	(Juma *et al.*, 2011)
Demethylapollinin 7-*O*-*β*-D-Glucopyranoside (**58**)	*T. cinerea*	(Maldini *et al*., 2011)
Isopongaflavone (**59**)	*T. candida* (SD)	(Chibber and Dutt, 1981)

AP (Aerial part), SD (Seed pods), ST (Stem), RT (Roots)



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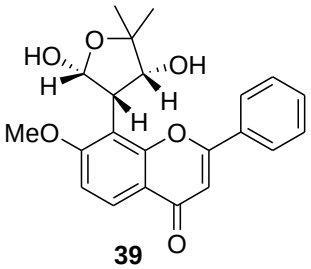

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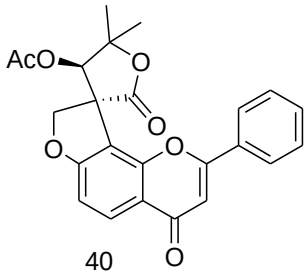

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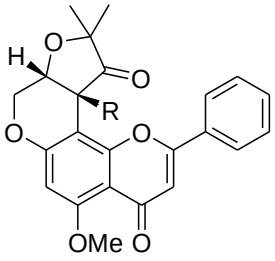
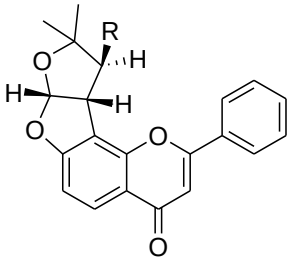
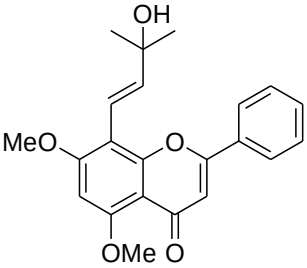


42 R = H
43 R = OH



44 R = OAc
45 R = OH



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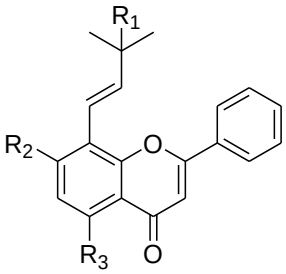
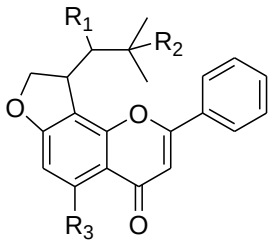
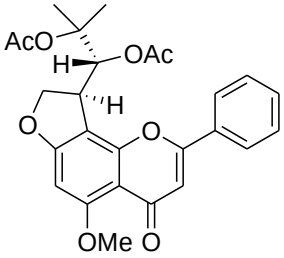


47 R^1 = OH, R^2 = OH, R^3 = OMe
48 R^1 = OH, R^2 = OMe, R^3 = H
49 R^1 = OMe, R^2 = OMe, R^3 = H



50 R^1 = OH, R^2 = OH, R^3 = H
51 R^1 = OAc, R^2 = OH, R^3 = H
52 R^1 = OAc, R^2 = OAc, R^3 = H
53 R^1 = OAc, R^2 = OH, R^3 = OH



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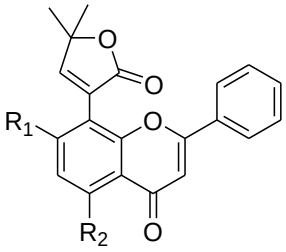
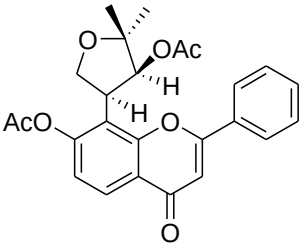


55 R^1 = OMe, R^2 = OMe
56 R^1 = OMe, R^2 = H
58 R^1 = O-β-D-Gluc, R^2 = H



57

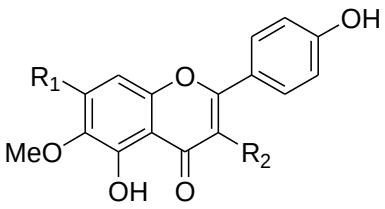
2.13.1.2. Flavonols of Tephrosia

All the flavonols isolated from *T. vogelii* are glycosides, with the same substitution pattern, involving rhamnoside at position C-3 and C-7. 6-Hydroxykaempferol 4'-methyl ether (**64**), candidol (**65**), candirone (**66**) and 7-ethoxy-3,3',4'-trihydroxyflavone (**67**) have been isolated from *T. candida* and *T. procumbens* without glycoside substituent. Table 2.5 shows some of the flavonols isolated from the genus *Tephrosia*.

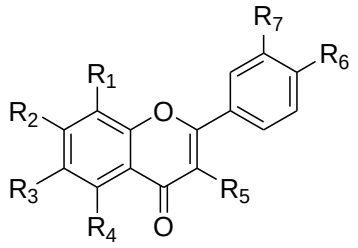
Table 2.5: Flavonols of *Tephrosia*

Compound	Species	Reference
6-Hydroxykaempferol 6-methyl ether 3-*O*-*α*-rhamno-pyranosyl(7→6)-*β*-galactopyranoside-7-*O*-*α*-rhamno-pyranoside (**60**)	*T. vogelii* (LF)	(Stevenson *et al.*, 2012)
6-Hydroxykaempferol 6-methyl ether 3-*O*-*α*-rhamno-pyranosyl(1→2)[*α*-rhamnopyranosyl(1→6)-*β*-galacto-pyranoside (**61**)	*T. vogelii* (LF)	(Stevenson *et al.*, 2012)
6-Hydroxykaempferol 6-methyl ether 3-*O*-*α*-rhamno-pyranosyl(1→2)[*α*-rhamnopyranosyl(1→ 6)]-*β*-galacto-pyranoside-7-*O*-*α*-rhamnopyranoside (**62**)	*T. vogelii* (LF)	(Stevenson *et al.*, 2012)
6-Hydroxykaempferol 6-methyl ether 3-*O*-*α*-rhamnopyranosyl (1→2)[(3-*O*-E-feruloyl)-*α*-rhamnopyranosyl(1→6)]-*β*-galacto-pyranosides (**63**)	*T. vogelii* (LF)	(Stevenson *et al.*, 2012)
6-Hydroxykaempferol 4'-methyl ether (**64**)	*T. candida* (SD)	(Dutt and Chibber, 1983)
Candidol (**65**)	*T. candida* (SD)	(Dutt and Chibber, 1983)
Candirone (**66**)	*T. candida*	(Horie *et al.*, 1994)
7-Ethoxy-3,3',4'-trihydroxyflavone (**67**)	*T. procumbens* (RT)	(Venkataratnam *et al.*, 1987)

SD (Seed pods), LF (Leaves), RT (Roots)



60 R^1 = *O*-(α-Rha), R^2 = *O*-(α-Rha-(1-2)-[*O*-(α–Rha-(1-6)]-β-Gal-
61 R^1 = *O*-(α-Rha), R^2 = *O*-(α-Rha-(1-6)]-β-Gal-
62 R^1 = OH, R^2 = *O*-(α-Rha-(1-2)-[*O*-(α-Rha-(1-6)]-β-Gal-
63 R^1 = OH, R^2 = *O*-(α-Rha-(1-2)-[(3-*O*-E-feruloyl)-α-Rha-(1-6)]-β-Gal-



	R^1	R^2	R^3	R^4	R^5	R^6	R^7
64	H	ORha	OH	OH	ORha	OMe	H
65	H	OMe	OMe	OMe	OH	OH	H
66	OMe	H	OMe	OH	OMe	OH	H
67	H	OEt	H	H	OH	OH	OH

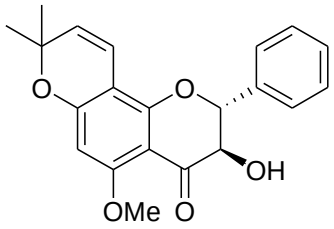
2.13.1.3. Flavanonols of Tephrosia

A small number of flavanonols have been isolated from the genus *Tephrosia*. (2*R*,3*R*)-3-Hydroxy-5-methoxy-6",6"-dimethylpyrano-[2",3":7,8]flavanone (**68**) was reported from the leaf part of *T. vogelii* (Stevenson *et al.,* 2012). Lupinifolinol (**69**) and lupinifolinol triacetate (**70**) were isolated from *T. lupinifolia* (Smalberger *et al.,* 1975). Table 2.6 gives some examples of these flavanonols.

Table 2.6: Flavanonols of *Tephrosia*

Compound	Species	Reference
(2*R*,3*R*)-3-Hydroxy-5-methoxy-6",6"-dimethylpyrano-[2",3":7,8]flavanone (**68**)	*T. vogelii* (LF)	(Stevenson *et al.*, 2012)
Lupinifolinol (**69**)	*T. lupinifolia* (RT)	(Smalberger *et al.*, 1975)
Lupinifolinol triacetate (**70**)	*T. lupinifolia* (RT)	(Smalberger *et al.*, 1975)

LF (Leaves), RT (Roots)



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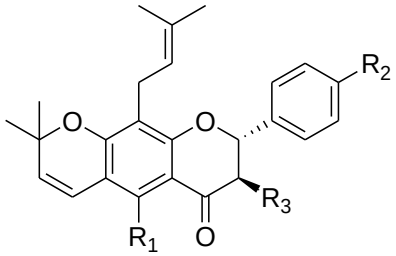


69 R^1 = OH, R^2 = OH, R^3 = OH
70 R^1 = OAc, R^2 = OAc, R^3 = OAc

2.13.1.4. Flavanones of Tephrosia

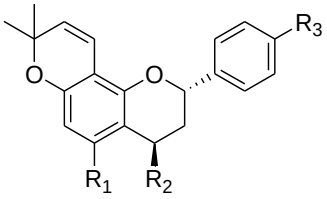
Flavanones are major compounds in the genus *Tephrosia*. More than fifty flavanones have been reported from the genus *Tephrosia*. Most of the flavanones isolated from this genus are prenylated at C-8 in ring-A. (2*S*)-5-Methoxy-6",6"-dimethy1-4",5"-dihydrocyclopropa-[4",5"]furano[2",3":7,8] flavanone (**85**) isolated from *T. villosa* has an uncommon modified prenyl group (Stevenson *et al.*, 2012). A biflavonoid tepicanol A (**108**) was isolated from *T. tepicana* (Gómez-Garibay *et al.*, 1997). The reported flavanones are summarized in Table 2.7.

Table 2.7: Flavanones of *Tephrosia*

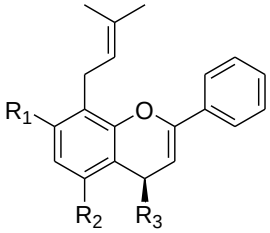
Compound	Species	Reference
(2*S*)-4'-Hydroxy-5-methoxy-6",6"-dimethylpyrano[2",3":7,8]-flavanone (**71**)	*T. vogelii* (LF)	(Stevenson *et al.*, 2012)
Obovatin (**72**)	*T. obovata* (WP)	(Yuh-Lin *et al.*, 1978)
Obovatin methyl ether (**73**)	*T. obovata* (WP)	(Yuh-Lin *et al.*, 1978)
Methylhildardtol B (**74**)	*T. hildebrandtii* (RT)	(Lwande *et al*., 1987)
Hildgardtol B (**75**)	*T. hildebrandtii* (RT)	(Lwande *et al*., 1987)
(2S)-7-Hydroxy-5-methoxy-8-prenylflavanone (**76**)	*T. vogelii* (LF)	(Stevenson *et al.*, 2012)
Tephrinone (**77**)	*T. villosa* (RT)	(Rao and Srimannarayana, 1981)
5,7-Dimethoxy-8-prenylflavan (**78**)	*T. madrensis*	
Tephrowatsin A (**79**)	*T. watsoniana* (RT)	(Gómez *et al.*, 1985b)
Tephrocandidin A (**80**)	*T. candida* (AP)	(Hegazy *et al.*, 2011)
Tephrocandidin B (**81**)	*T. candida* (AP)	(Hegazy *et al.*, 2011)
Falciformin (**82**)	*T. falciformis*	(Khan *et al.*, 1986)
(2S)-5,7-Dimethoxy-8-(3-methylbut-1,3-dienyl)flavanone (**83**)	*T. villosa* (LF)	(Stevenson *et al.*, 2012)
Tephroleocarpin B (**84**)	*T. leiocarpa* (RT)	(Gómez *et al.*, 1985b)
(2S)-5-Methoxy-6",6"-dimethy1-4",5"-dihydrocyclopropa-[4",5"]furano[2",3":7,8] flavanone (**85**)	*T. villosa* (LF)	(Stevenson *et al.*, 2012)
(+)-Tephrorin A (**86**)	*T. purpurea* (WP)	(Chang *et al.*, 2000b)
Tephroapollin A (**87**)	*T. apollinea* (AP)	(El-Razek *et al.*, 2007)
Tephroapollin B (**88**)	*T. apollinea* (AP)	(El-Razek *et al.*, 2007)
Quercetol C (**89**)	*T. quercetorum* (RT)	(Gómez-Garibay *et al.*, 1988)
Tephroleocarpin A (**90**)	*T. leiocarpa* (RT)	(Go´mez-Garibay *et al.*, 1991)

Lupinifolin (**91**)	*T. lupinifolia* (RT)	(Smalberger *et al.*, 1974)
5,4'-*O*,*O*-Dimethyl-lupinifolin (**92**)	*T. lupinifolia* (RT)	(Smalberger *et al.*, 1974)
Lupinifolin diacelate (**93**)	*T. lupinifolia* (RT)	(Smalberger *et al.*, 1974)
Hildgardtene (**94**)	*T. hildebrandtii* (RT)	(Monache *et al.*, 1986)
Methylhildgardtol A (**95**)	*T. hildebrandtii* (RT)	(Monache *et al.*, 1986)
Hildgardtol A (**96**)	*T. hildebrandtii* (RT)	(Monache *et al.*, 1986)
Candidone (**97**)	*T. candida* (ST and LF)	(Roy *et al.*, 1986b)
Quercetol B (**98**)	*T. quercetorum* (RT)	(Gomez-Garibay *et al.*, 1988)
Quercetol A (**99**)	*T. quercetorum* (RT)	(Gomez-Garibay *et al.*, 1988)
Tephrowatsin B (**100**)	*T. watsoniana* (RT)	(Gómez *et al.*, 1985b)
Tephrowatsin C (**101**)	*T. watsoniana* (RT)	(Gómez *et al.*, 1985b)
Nitenin (**102**)	*T. nitens* (RT)	(Gomez *et al.*, 1984)
Tephrowatsin D (**103**)	*T. watsoniana* (RT)	(Gómez *et al.*, 1985b)
Tephrowatsin E (**104**)	*T. watsoniana* (RT)	(Gómez *et al.*, 1985b)
Spinoflavanone A (**105**)	*T. spinosa* (RT)	(Rao and Prasad, 1992)
Spinoflavanone B (**106**)	*T. spinosa* (RT)	(Rao and Prasad, 1992)
Maxima flavanone A (**107**)	*T. maxima* (RT)	(Venkata Rao *et al.*, 1994)
Tepicanol A (**108**)	*T. tepicana* (RT)	(Gómez-Garibay *et al.*, 1997)

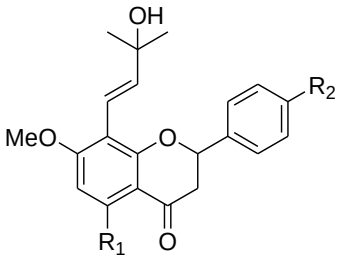
AP (Aerial part), SD (Seed pods), ST (Stem), RT (Roots), LF (Leaves)



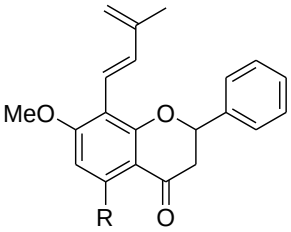
71 R^1 = OMe, R^2 = O, R^3 = OH
72 R^1 = OH, R^2 = O, R^3 = H
74 R^1 = OMe, R^2 = OMe, R^3 = H
75 R^1 = OMe, R^2 = OH, R^3 = H



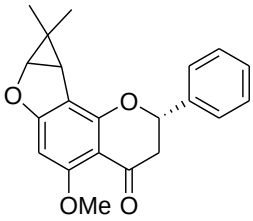
76 R^1 = OH, R^2 = OMe, R^3 = O
78 R^1 = OMe, R^2 = OMe, R^3 = H
79 R^1 = OMe, R^2 = OMe, R^3 = OH



80 R^1 = OMe, R^2 = H
81 R^1 = OMe, R^2 = OH
82 R^1 = H, R^2 = H



83 R = OMe
84 R = OH



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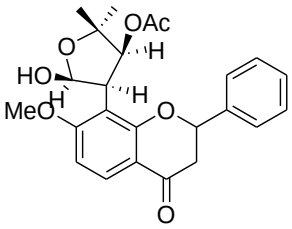

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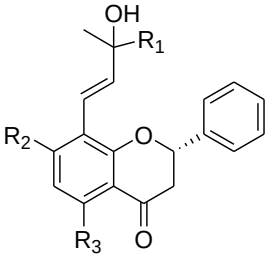
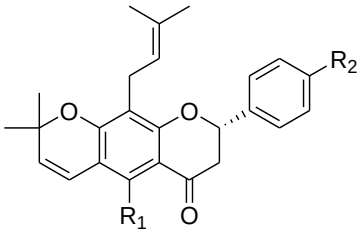
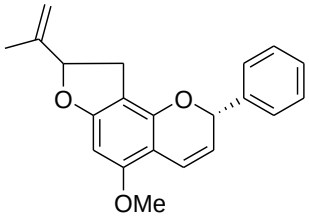


87 R^1 = Me, R^2 = OH, R^3 = H
88 R^1 = CH_2OAc, R^2 = OH, R^3= H
89 R^1 = OH, R^2 = OMe, R^3 = OMe
90 R^1 = Me,R^2 = OMe, R^3 = OH



91 R^1 = R^2 = OH
92 R^1 = R^2 = OMe
93 R^1 = R^2 = OAc



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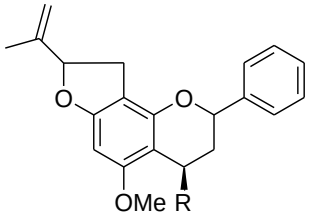
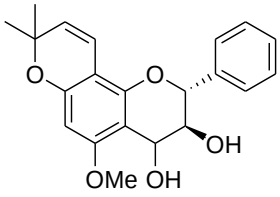


95 R = OMe
96 R = OH



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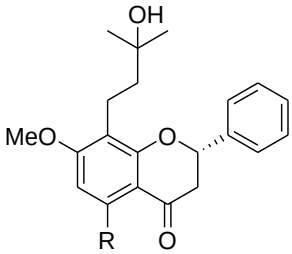
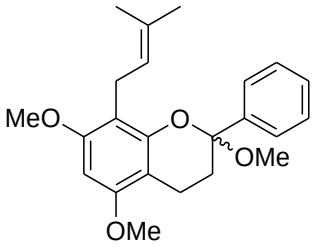


101 R = OH
102 R = OMe



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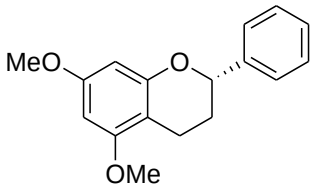

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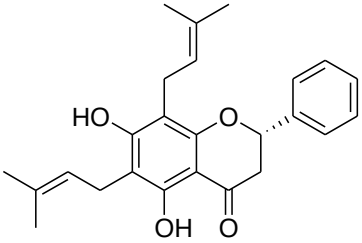

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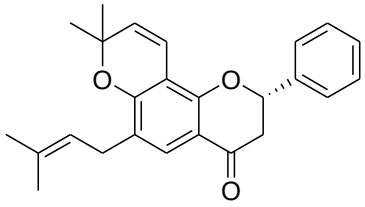

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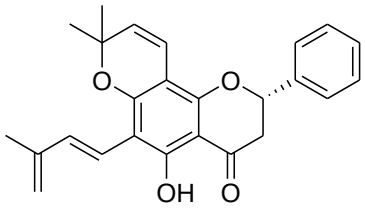

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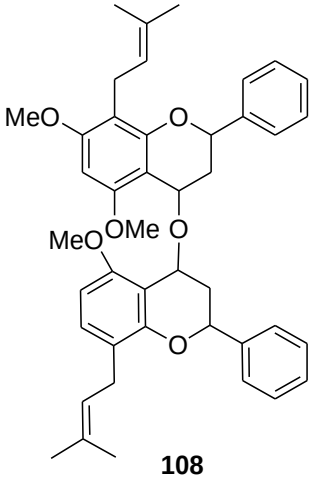


108

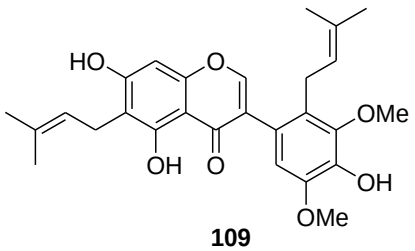
2.13.1.5. Isoflavones of Tephrosia

Examples of isoflavones isolated from genus Tephrosia are listed in Table 2.8. Most of these compounds have oxygenation at C-4'. Pumilaisoflavones A (**116**) and C (**117**), isolated from the seed pods of *T. pumila* have prenyl substitution on rings-A- and B (Yenesew *et al.*, 1989). The isoflavones isolated from *T. maxima,* maxima isoflavone F (**110**), maxima isoflavone G (**111**) and viridiflorin (**112**) contain unusual substitution pattern on B-ring (Gómez *et al.*, 1985a; Rao *et al.*, 1984). *T. pumila* and *T. maxima* are reported as a main source of isoflavones from this genus.

Table 2.8: Isoflavones of *Tephrosia*

Compound	Species	Reference
7,4'-Dihydroxy-3',5'-dimethoxyisoflavone (**109**)	*T. purpurea* (WP)	(Chang *et al.*, 1997)
Maxima isoflavone F (**110**)	*T. maxima* (RT)	(Rao *et al.*, 1984)
Maxima isoflavone G (**111**)	*T. maxima* (RT)	(Gómez *et al.*, 1985a; Rao *et al.*, 1984)
Viridiflorin (**112**)	*T. viridiflora* (RT)	(Gómez *et al.*, 1985a; Rao *et al.*, 1984)
Maxima isoflavone D (**113**)	*T. maxima* (RT)	(Rao *et al.*, 1984)
Maxima isoflavone E (**114**)	*T. maxima* (RT)	(Rao *et al.*, 1984)
Barbigerone (**115**)	*T. barbigera* (SD)	(Vilain, 1980)
Pumilaisoflavone A (**116**)	*T. pumila* (SD)	(Dagne *et al.*, 1988)
Pumilaisoflavone C (**117**)	*T. pumila* (SD)	(Yenesew *et al.*, 1989)

WP (whole part), AP (Aerial part), SD (Seed pods), ST (Stem), RT (Roots), LF (Leaves)



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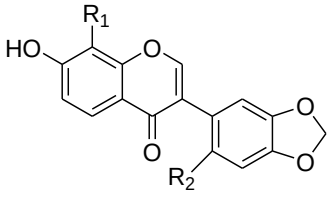
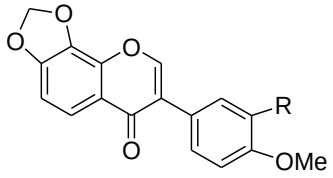
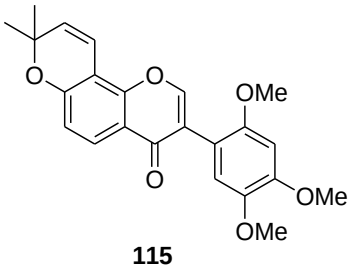


110 R^1 = OMe, R^2 = H
111 R^1 = R^2 = OMe
112 R^1 = H, R^2 = OMe



113 R = OMe
114 R = H



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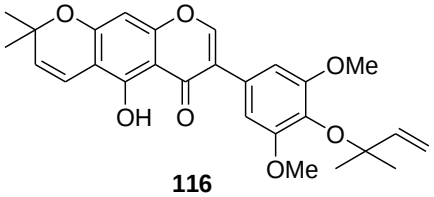


116

2.13.1.6. Rotenoids of Tephrosia

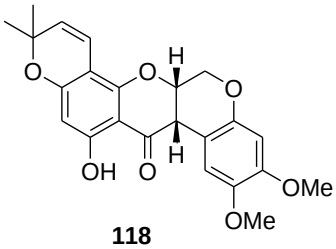
The rotenoids reported from this genus are characterized by having methoxy substituents at C-2 and C-3 on ring-A. Modified prenyl group at C-8 and hydroxylation at C-12a is common among the rotenoids of *Tephrosia*. Villosone (**123**) and 12a-hydroxyrotenone (**129**) are oxygenated at C-6. Dihydrostemonal (**125**) and 6-acetoxydihydrostemonal (**127**), reported from the seed pods of

T. pentaphylla, contain methoxy group at C-8, which is not common in this genus. The common rotenoids isolated from this genus are listed in Table 2.9. These rotenoids are mostly isolated from *T. villosa.*

Table 2.9: Rotenoids of *Tephrosia*

Compound	Species	Reference
Toxicarol (**118**)	*T. toxicaria* (RT)	(Stevenson *et al.*, 2012)
Villosinol (**119**)	*T. villosa* (RT)	(Sarma *et al.*, 1976)
Villosol (**120**)	*T. villosa* (RT)	(Sarma *et al.*, 1976)
Villosin (**121**)	*T. villoss* (RT)	(Krupadanam *et al.*, 1977)
Villol (**122**)	*T. villoss* (RT)	(Krupadanam *et al.*, 1977)
Villosone (**123**)	*T. villoss* (RT)	(Krupadanam *et al.*, 1977)
Villinol (**124**)	*T. villoss* (RT)	(Krupadanam *et al.*, 1977)
Dihydrostemonal (**125**)	*T. pentaphylla* (SD)	(Dagne *et al.*, 1989)
9-Demethyldihydrostemonal (**126**)	*T. pentaphylla* (SD)	(Dagne *et al.*, 1989)
6-Acetoxydihydrostemonal (**127**)	*T. pentaphylla* (SD)	(Dagne *et al.*, 1989)
12a-Dehydro-6-hydroxysumatrol (**128**)	*T. villosa* (RT)	(Prashant and Krupadanam, 1993)
12a-Hydroxyrotenone (**129**)	*T. uniflora* (ST)	(Abreu and Maria, 1996)
12a-Hydroxy-*β*-toxicarol (**130**)	*T. candida* (RT)	(Andrei *et al.*, 1997)

SD (Seed pods), ST (Stem), RT (Roots)



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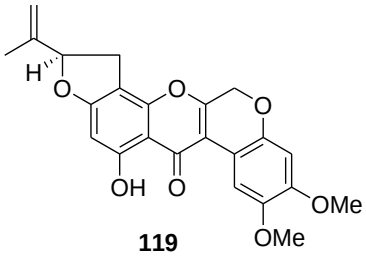

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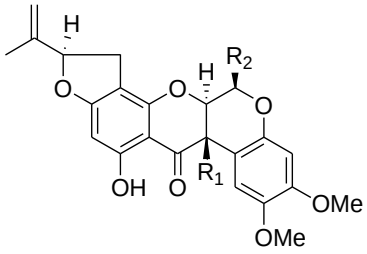
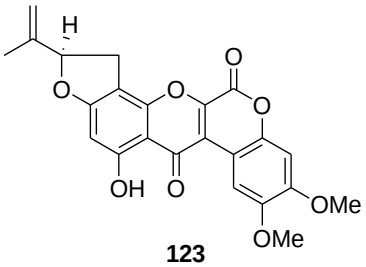


120 R^1 = OH, R^2 = H
121 R^1 = H, R^2 = OH
122 R^1 = OH, R^2 = OH



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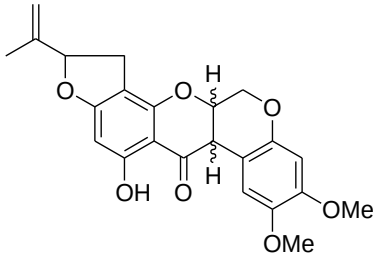

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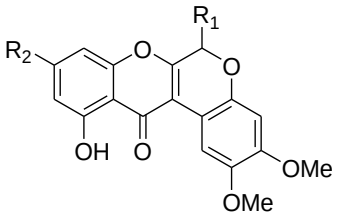
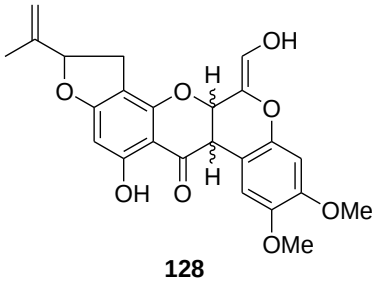


125 R^1 = OH, R^2 = OMe
126 R^1 = OH, R^2 = OH
127 R^1 = OAc, R^2 = OMe



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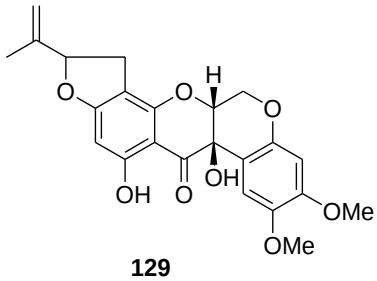

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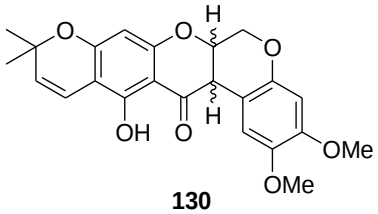


130

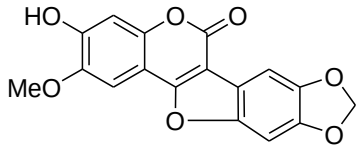
2.13.1.7. Pterocarpanes of Tephrosia

The pterocarpanes reported from the genus *Tephrosia* are well known for C-5 deoxygenation and B-ring hydroxylation or methylenedioxy formation at C-8/9. Examples of some of the pterocarpans reported form *Tephrosia* are listed below (Table 2.10).

Table 2.10: Pterocarpanes of *Tephrosia*

Compound	Species	Reference
Tephrosol (**131**)	*T. villosa* (RT)	(Rao and Srimannarayana, 1980)
2-Methoxy-3,9-dihydroxy coumestone (**132**)	*T. hamiltonii* (RT)	(Rajani and Sarma, 1988)
Tephrocarpin (**133**)	*T. bidwilli* (LF)	(Ingham and Markham, 1980)
Hildecarpin (**134**)	*T. hildebrandtii* (RT)	(Lwande *et al.*, 1987a)
3,4:8,9-Dimethylenedioxypterocarpan (**135**)	*T. aequilata* (RT)	(Tarus *et al.*, 2002)
Hildecarpidin (**136**)	*T. hildebrandtii* (RT)	(Lwande *et al.*, 1987a)
Tephcalostan (**137**)	*T. calophylla* (RT)	(Gunasekar *et al.*, 2003)

RT (Roots), LF (Leaves)



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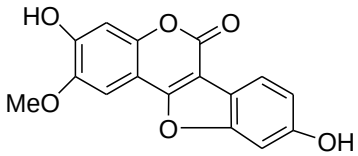

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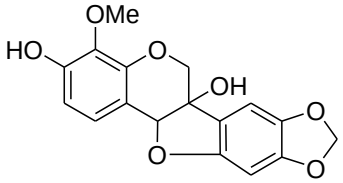

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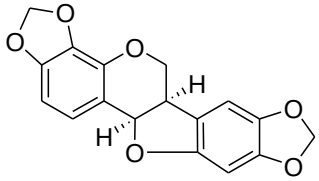

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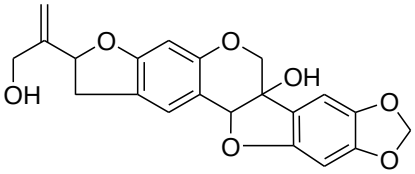

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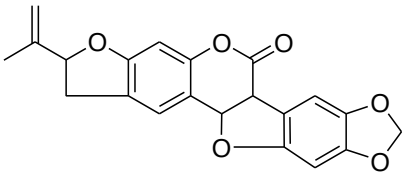


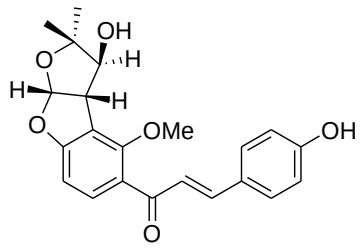
137

2.13.1.8. Chalconoids of Tephrosia

Chalconoids are among the main constituents of the genus *Tephrosia*. More than twenty chalcones have been reported. These chalcones are characterized by having a prenyl group mainly in ring-A at C-3' and/or C-5' and oxygenated at C-2'. The majority of these chalcones do not have a hydroxyl group at C-4 on ring-B which is a common feature in other chalcones. *T. purpurea* is one of the major sources of these chalcones. (+)-Tephrosone (**138**) and (+)-tephropurpurin (**139**) have unusual modified prenyl substituent at C-3'/4'. Some of these compounds are summarized in Table 2.11.

Table 2.11: Chalcones of *Tephrosia*

Compound	Species	Reference
(+)-Tephrosone (**138**)	*T. purpurea* (WP)	(Chang *et al.*, 2000b)
(+)-Tephropurpurin (**139**)	*T. purpurea* (WP)	(Chang *et al.*, 1997)
O-Methylpongamol (**140**)	*T. purpurea* (RT)	(Pelter *et al.*, 1981)
Purpuritenin (**141**)	*T. purpurea* (SD)	(Sinha *et al.*, 1982)
Candidachalcone (**142**)	*T. candida* (AP)	(Hegazy *et al.*, 2011)
(*S*)-Elatadihydrochalcone (**143**)	*T. elata* (SD)	(Muiva *et al.*, 2009)
2',6'-Dimethoxy-4',5'-(2"2"dimethyl)-pyranochalcone (**144**)	*T. pulcherrima* (RT)	(Ganapaty *et al.*, 2008b)
Praecansone A (**145**)	*T. praecans* (SD)	(Camele *et al.*, 1980a)
Praecansone B (**146**)	*T. praecans* (SD)	(Camele *et al.*, 1980a)
Obovatachalcone (**147**)	*T. obovata* (RT)	(Chen *et al.*, 1978a)
Oaxacacin (**148**)	*T. woodii* (RT)	(Dominguez *et al.*, 1983)
6'-Demethoxypraecansone B (**149**)	*T. purpurea* (RT)	(Rao and Raju, 1984)
Tephrone (**150**)	*T. candida* (SD)	(Chibber and Dutt, 1982)
Spinochalcone A (**151**)	*T. spinosa* (RT)	(Rao and Prasad, 1992)
3',5'-Diisopentenyl-2',4'-dihydroxychalcone (**152**)	*T. spinosa* (RT)	(Sharma and Rao, 1992)
Tunicatachalcone (**153**)	*T. tunicate* (RT)	(Andrei *et al.*, 2000)
Epoxyobovatachalcone (**154**)	*T. carrollii* (RT)	(Gómez-Garibay *et al.*, 2001)
2',6'-Dihydroxy-3'-prenyl-4'-methoxy-β-hydroxychalcone (**155**)	*T. major* (RT)	(Gomez-Garibay *et al.*, 2002)



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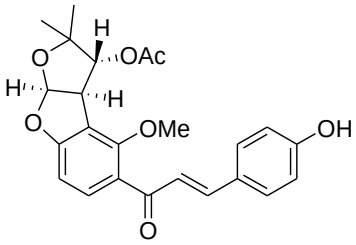

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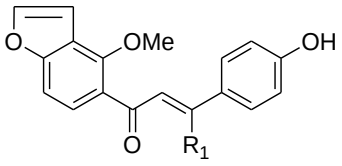
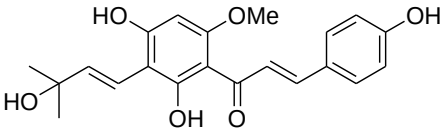


140 R^1 = OMe, R^2 = H
141 R^1 = H, R^2 = OMe



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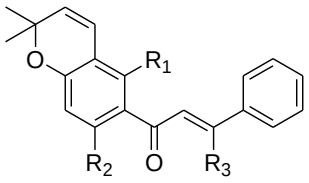
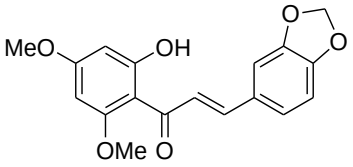


144 R^1 = OMe, R^2 = OMe, R^3 = H
148 R^1 = OMe, R^2 = OH, R^3 = H
149 R^1 = OMe, R^2 = H, R^3 = OH



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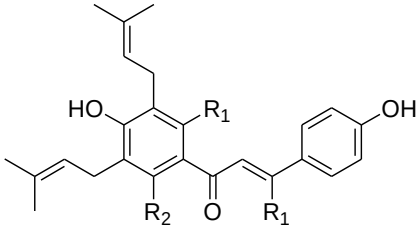
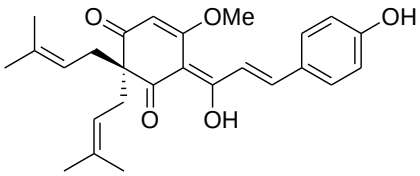


151 R^1 = H, R^2 = OH
152 R^1 = OH, R^2 = H



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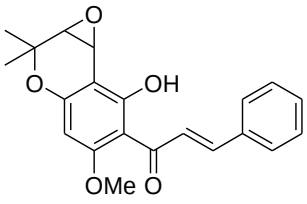

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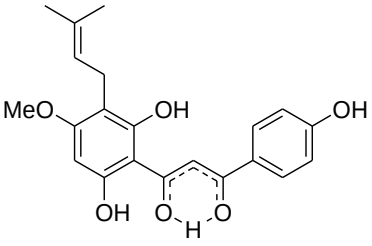


154

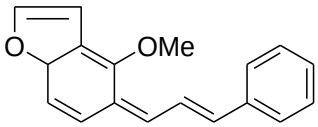
2.13.1.9. Other Flavonoids of Tephrosia

Table 2.12 illustrates some examples of compounds with unusual flavonoid skeleton that have been reported from the genus *Tephrosia*.

Table 2.12: Other Flavonoids of *Tephrosia*

Compound	Species	Reference
Purpureamethied (**156**)	*T. purpurea* (RT)	(Sinha *et al.*, 1982)
Calophione A (**157**)	*T. calophylla* (RT)	(Ganapaty *et al.*, 2009)
Tephrospirolactone (**158**)	*T. candida* (RT)	(Andreia *et al.*, 2002)
Tephrospiroketone I (**159**)	*T. candida* (RT)	(Andreia *et al.*, 2002)
Tephrospiroketone II (**160**)	*T. candida* (RT)	(Andreia *et al.*, 2002)

RT (Roots)



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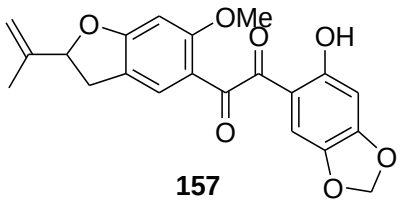

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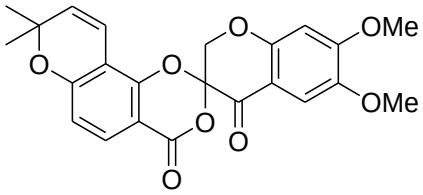

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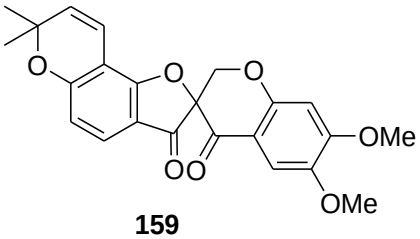

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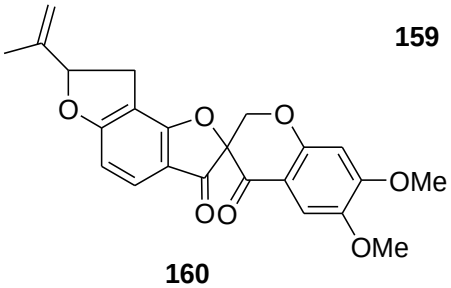


160

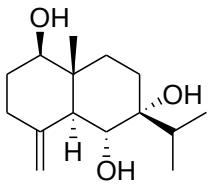
2.13.2. Triterpenoid and Sesquiterpenes of Tephrosia

Very few triterpenoids and sesquiterpenes are reported from *Tephrosia* species. These compounds are presented in Table 2.13.

Table 2.13: Triterpenoid and Sesquiterpenes of *Tephrosia*

Compound	Species	Reference
1β-Hydroxy-6,7α-dihydroxyeudesm-4(15)-ene (**161**)	*T. candida* (AP)	(Hegazy *et al.*, 2011)
1β,6α,10α-Guai-4(15)-ene-6,7,10-triol (**162**)	*T. vogelii* (LF)	
Linkitriol (**163**)	*T. purpurea* (RT)	(Khalafalah *et al.*, 2010)
Oleanolic acid (**164**)	*T. strigosa* (AP)	

AP (Aerial part), RT (Roots), LF (Leaves)



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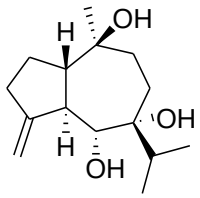

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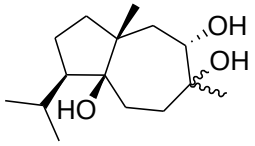

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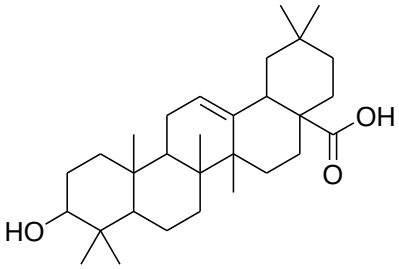

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2.14: Biological Activities of Isolated Compounds from the Genus T*ephrosia*

Phytochemical investigations have been carried out on the *Tephrosia* species to isolate the active compounds based on their ethnomedical uses and the biological activities of their crude extracts. Some of the compounds that are responsible for the biological activities are flavanones,

flavonols, flavones, chalcones, pterocarpans, and rotenoids. These compounds have shown diverse activities, such as antiplasmodial, antifeedant, antileiashmanial, estrogenic, antitumor and antimicrobial activities (Table 2.14). For instance; Candidachalcone (**142**) isolated from *T. candida* showed estrogenic activity (IC_{50} = 80 μM) (Hegazy *et al.*, 2011). Calophione A (**157**) isolated from *T. calphylla* exhibited significant cytotoxicity (IC_{50} 5.00 (RAW) and 2.9 μM (HT-29)) against mouse macrophage cells (RAW) and Colon cancer cell lines (HT-29) (Ganapaty *et al.*, 2009). (+)-Tephrorin A (**86**), (+)-tephrosone (**138**), (+)-tephropurpurin (**139**), and 7,4'-dihydroxy-3',5'-dimethoxyisoflavone (**109**) isolated from *T. purpurea* displayed cancer chemopreventive properties (Chang *et al.*, 2000a; Chang *et al.*, 1997).

2',6'-Dimethoxy-4',5'-(2",2"-dimethyl)-pyranochalcone (**144**) isolated from *T. pulcherrima* displayed significant antimicrobial activity against a sequence of micro-organisms (Ganapaty *et al.*, 2008b). (*S*)-Elatadihydrochalcone (**143**) isolated from *T. elata* showed good antiplasmodial activity against D6 strain (chloroquine-sensitive) (IC_{50} = 2.8 ± 0.3 μg/mL) and chloroquine-resistant W2 (IC_{50} = 5.5 ± 0.3 μg/mL) strains of *P. falciparum* (Muiva *et al.*, 2009). Terpurinflavone (**57**) isolated from *T. purpurea* showed antiplasmodial activity against the D6 (IC_{50} = 3.12 ± 0.28 μM) and W2 (IC_{50} = 6.26 ± 2.66 μM) strains of *Plasmodium falciparum* (Juma *et al.*, 2011). Hildecarpin (**134**) isolated from *T. hildebrandtii* displayed insect antifeedant activity against the legume pod-borer *Maruca testulalis*, and pest of cowpea *Vigna* (Lwande *et al.*, 1986b; Lwande *et al.*, 1986c).

Table 2.14: Biological activities of some compounds isolated from *Tephrosia* species

Compounds	Biological activity	*Tephrosia* species	References
Candidachalcone (**142**)	Estrogenic	*T. candida*	(Hegazy *et al.*, 2011)
Calophione A (**157**)	Antitumor	*T. calphylla*	(Ganapaty *et al.*, 2009)
Tephcalostans B	Antitumor		
Tephcalostans C			
Tephcalostans D			
(+)-Tephrorin A (**86**)	Cancer chemopreventive	*T. purpurea*	(Chang *et al.*, 2000a)
(+)-Tephrosone (**138**)			
(+)-Tephropurpurin (**139**)	Cancer chemopreventive	*T. purpurea*	(Chang *et al.*, 1997)
7,4'-Dihydroxy-3',5'-dimethoxyisoflavone (**109**)			
2',6'-Dimethoxy-4',5'-(2",2"-dimethyl)-pyranochalcone (**144**)	Antimicrobial	*T. pulcherrima*	(Ganapaty *et al.*, 2008b)
3,4:8,9-Dimethylenedioxypterocarpan (**135**)	Antimicrobial	*T. aequilata*	(Tarus *et al.*, 2002)
Hildecarpin (**134**)	Antifeedant and antifungal	*T. hildebrandtii*	(Lwande *et al.*, 1986b; Lwande *et al.*, 1986c)
Terpurinflavone (**57**)	Antiplasmodial	*T. purpurea*	(Juma *et al.*, 2011)
Obovatin (**72**)	Piscicidal	*T. obovata*	(Yuh-Lin *et al.*, 1978)
Obovatin methyl ether (**73**)			
(*S*)-elatadihydrochalcone (**143**)	Antiplasmodial	*T. elata*	(Muiva *et al.*, 2009)
Tephrinone (**77**)	Antiprotozoal	*T. pumila*	(Ganapaty *et al.*, 2008a)
Emoroidenone	Antifeedant	*T. emoroides*	(Machocho *et al.*, 1995)

CHAPTER 3: MATERIALS AND METHODS

3.1: General Experimental Procedures

TLC was carried out on Merck pre-coated silica gel 60 F254 plates. Melting points were acquired on a Büchi Melting point B-545 Switzerland apparatus. UV spectra were recorded from a Specord S600 (Analytik Jena AG) spectrophotometer. CD experiments were run on a Jasco J-715 spectropolarimeter and Optical rotations were measured on Perkin Elmer 341-LC. NMR spectra were acquired on 600 and 800 MHz Bruker Avance III HD spectrometers, using the residual solvent peaks as reference. EI-MS were obtained on a Micromass GC-TOFmicro mass spectrometer (Micromass, Wythenshawe, Waters Inc. UK), using direct inlet, and 70 eV ionization voltage. Column chromatography was run on silica gel 60 (70-230 mesh). Gel filtration was done on Sephadex LH-20. Preparative HPLC was carried out on a Waters 600E instrument using the Chromulan (Pikron Ltd) software and an RP C8 Kromasil® (250 mm x 55 mm) column with a MeOH/H_2O solvent system.

3.2: Plant Materials

The *Tephrosia* species used in this study were collected from Kilungu hills in Makueni County, Kenya as shown in Table 3.1. The plant specimens were identified by Mr. Patrick C. Mutiso of the School of Biological Sciences, the University of Nairobi, where vouchers specimen were deposited.

Table 3.1: Plant collection details

***Tephrosia* species**	**Plant part**	**Time of collection**	**Voucher number**
T. aequilata	Roots	May 2013	Mutiso - 839/May 2010
T. elata	Leaves and seedpods	May 2010	Mutiso - 839/May 2010
T. noctiflora	Stem	May 2009	Mutiso - 837/May 2009
T. pumila	Aerial part	May 2009	Mutiso - 841/May 2009
T. purpurea	Stem	April 2014	Mutiso - 840/April 2014
T. rhodesica	Root	July 2015	Mutiso - 842/July 2015

3.3: Extraction and Isolation of Compounds

3.3.1: Extraction and isolation of compounds from the stems of Tephrosia noctiflora

The air dried stem (300 g) of *T. noctiflora* was ground and extracted with CH_2Cl_2/MeOH (1:1) (5 x 1 L) by percolation at room temperature. The extract was concentrated *in vacuo* on a rotary evaporator to yield 30 g of a dark yellow paste. A portion of the extract (28 g) was subjected to column chromatography over silica gel (200 g) eluting with *n*-hexane containing increasing amounts of EtOAc. The eluent with 1% EtOAc in *n*-hexane gave tephrowatsin B (**100)** (10 mg) after column chromatography on Sephadex LH-20 (CH_2Cl_2:MeOH (1:1)). Elution with 3-5% EtOAc in *n*-hexane gave a white precipitate of tephrinone (**77)** (200 mg) which was recrystallized from CH_2Cl_2/MeOH (1:1). The eluent with 30% EtOAc in *n*-hexane was washed with MeOH to give a white amorphous solid of D-pinitol (**165)** (1500 mg).

3.3.2: Extraction and Isolation of Compounds from the Aerial Part of Tephrosia pumila

The air dried and ground aerial part of *T. pumila* (500 g) was extracted with CH_2Cl_2/MeOH (1:1), (5 x 1.5 L) by cold percolation at room temperature. The extract was filtered and the

solvent removed under vacuum using a rotary evaporator at 50 ^{0}C to yield a dark green oily paste (50 g). The extract was diluted with methanol and extracted with *n*-hexane to remove the fat. The methanol layer (35 g) was subjected to column chromatography on silica gel (400 g) eluting with *n*-hexane containing increasing percentages of EtOAc. The fraction which eluted with 3% EtOAc in *n*-hexane yielded *E*-praecansone A **(145)** (20 mg), 5% EtOAc in *n*-hexane yielded 8-*O*-methylretusin **(166)** (10 mg) and 7- 10 % EtOAc in *n*-hexane yielded Pumilaisoflavone C **(117)** (10 mg).

3.3.3: Extraction and Isolation of Compounds from the Roots of Tephrosia aequilata

The air dried and ground roots of *T. aequilata* (2 kg) were extracted with CH_2Cl_2/MeOH (1:1), (5 x 1.5 L) by percolation at room temperature. The extract was filtered and the solvent removed under vacuum using a rotary evaporator at 50 ^{0}C to yield a dark brown oily paste (120 g). The extract was diluted with methanol and extracted with *n*-hexane. The methanol layer (80 g) was subjected to column chromatography on silica gel (600 g) eluting with *n*-hexane containing increasing percentages of EtOAc. The fraction which eluted with 1% EtOAc in *n*-hexane was washed with acetone to yield 3,4:8,9-dimethylenedioxy-dihydropterocarpan (**167)** (100 mg) and the mother liquor was subjected to column chromatography on Sephadex LH-20 (CH_2Cl_2/MeOH; 1:1) to yield tephrowatsin B (**100)** (5 mg) (Go´mez *et al.*, 1985). The fraction that was eluted with 3% EtOAc in *n*-hexane was further subjected to column chromatography on silica gel (120 g) eluting with 1% EtOAc in *n*-hexane to yield obovatachalcone (**147)** (20 mg), 5% EtOAc in hexane yielded praecansone B (**146)** (900 mg) and 7- 10% EtOAc in *n*-hexane to yield *E*- praecansone A (**145**) (100 mg).

The fractions that eluted with 5 – 7% EtOAc in *n*-hexane were combined and purified using preparative HPLC (MeOH/H_2O, gradient elution) to give *E*-2',6'-dimethoxy-3',4'-(2",2"-dimethyl)pyranoretrochalcone (**168**) (20 mg) and *Z*-2',6'-dimethoxy-3',4'-(2",2"-dimethyl) pyranoretrochalcone (**169**) (25 mg).

The fraction which was eluted with 7% EtOAc was applied to Sephadex LH-20 (CH_2Cl_2/CH_3OH; 1:1) and the major fraction was further purified by PTLC (5% EtOAc in *n*-hexane) to yield 3",4"-*cis*-4"-ethoxy-3"-hydroxypraecansone B (**170**) (15 mg). The fraction which eluted with 10% EtOAc was further purified by PTLC to give candidone (**97**) (10 mg).

The fractions that were eluted with 15 - 20% EtOAc in *n*-hexane were combined and subjected to Sephadex LH-20 column chromatography to give isopongaflavone (**59**, 1.2 g). Crystallization of the fraction that eluted with 50% EtOAc in methanol yielded a white amorphous solid of *β*-sitosterol-3-*O*-glucoside (**171**) (50 mg).

3.3.4: Extraction and Isolation of Compounds from the Seedpods of Tephrosia elata

The air dried and ground seedpods of *T. elata* (2 kg) were extracted with CH_2Cl_2/MeOH (1:1), (5 x 1.5 L) by percolation at room temperature. The extract was filtered and the solvent removed under vacuum using a rotary evaporator at 50 ^{0}C to yield a dark brown paste (80 g). The extract was diluted with methanol and extracted with *n*-hexane. The methanol layer (50 g) was subjected to column chromatography on silica gel (400 g) eluting with *n*-hexane containing increasing percentages of EtOAc.

The fraction which eluted with 1% EtOAc in *n*-hexane was subjected to column chromatography on Sephadex LH-20 (CH_2Cl_2/MeOH; 1:1) to yield obovatachalcone (**147**) (20 mg) and obovatin

methyl ether (**73**) (5 mg). The fraction that was eluted with 3% EtOAc in *n*-hexane was further subjected to column chromatography on silica gel (120 g) eluting with 1% EtOAc in *n*-hexane to yield (*S)*-elatadihydrochalcone (**143**) (20 mg), 5% EtOAc in *n*-hexane yielded xanthohumol C (**172**), (10 mg) and 7 - 10% EtOAc in *n*-hexane yielded 8-*O*-methylretusin (**166**) (15 mg).

The fractions that eluted with 5 – 7% EtOAc in *n*-hexane were combined and purified using preparative HPLC (MeOH/H_2O, gradient elution) to give tephrosin (**173**) (30 mg) and deguelin (**174)** (20 mg). The fractions that eluted with 15 - 20 % EtOAc in *n*-hexane were combined and subjected to Sephadex LH-20 column chromatography to give isopongaflavone (**59**) (400 mg).

3.3.5: Extraction and Isolation of Compounds from the Leaves of Tephrosia elata

The air dried and ground leaves of *T. elata* (1 kg) were extracted with CH_2Cl_2/MeOH (1:1), (5 x 1.5 L) by percolation at room temperature. The extract was filtered and concentrated under vacuum using a rotary evaporator at 50 ^{0}C to yield a dark brownish oily paste (75 g). The extract was diluted with methanol and extracted with *n*-hexane to remove the fat. The methanol layer (35 g) was subjected to column chromatography on silica gel (300 g) eluting with *n*-hexane containing increasing percentages of EtOAc. The fraction eluted with 3% EtOAc in *iso*-hexane was purified by column chromatography on Sephadex LH-20 column (eluent: CH_2Cl_2/MeOH; 1:1) to give tephrolecarpin A (**175**) **(**20 mg) and obovatin methyl ether (**73**) (25 mg). The eluent with 5% EtOAc in *iso*-hexane was first subjected to a column chromatography on a Sephadex LH-20 (CH_2Cl_2/MeOH; 1:1) followed by preparative HPLC (MeOH/H_2O, gradient elution) to give maackiain (**176**) (15 mg) and deguelin (**174**) (10 mg). Elution with 6% EtOAc in *iso*-hexane gave a white solid which was recrystallized from CH_2Cl_2/MeOH (1:1) to give isopongaflavone (**59**) **(**500 mg). Further elution with 10% EtOAc in *iso*-hexane gave cinnamic acid (**177)** (10 mg),

the eluent with 12% EtOAc in *iso*-hexane gave salicylic acid (**178**) (20 mg) and the 50% EtOAc in *iso*-hexane eluent gave quercetin (**179**) (30 mg), kaemferol (**180**) (20 mg) and apigenin (**181**) (20 mg).

3.3.6: Extraction and Isolation of Compounds from the Root of Tephrosia rhodesica

The air dried roots (2 kg) of *T. rhodesica* were ground and extracted with CH_2Cl_2/MeOH (1:1) (3 x 2 L) by percolation at room temperature to yield 70 g of a dark brown paste. A 37 g portion of the extract was subjected to column chromatography over silica gel (400 g) eluting with *iso*-hexane containing increasing amounts of EtOAc. The fraction eluted with 1% EtOAc in *iso*-hexane was purified by preparative HPLC (MeOH/H_2O, gradient elution) to give tephrowatsin B (**100**) (20 mg). The elution with 3% EtOAc in *iso*-hexane was purified by column chromatography on Sephadex LH-20 column (eluent: CH_2Cl_2/MeOH; 1:1) to give tephrinone (**77**) (200 mg) and glabranin (**182**) (100 mg). The eluent with 5% EtOAc in *iso*-hexane purified by column chromatography on Sephadex LH-20 column (eluent: CH_2Cl_2/MeOH; 1:1) followed by recrystallization from CH_2Cl_2/MeOH (1:1) to give rhodimmer (**183**) (15 mg). Elution with 6% EtOAc in *iso*-hexane was subjected to column chromatography on Sephadex LH-20 (CH_2Cl_2:MeOH; 1:1) to give quercetol B (**98**) (300 mg). The elution product of 7% EtOAc in *iso*-hexane was further purified by column chromatography on Sephadex LH-20 column (eluent: CH_2Cl_2/MeOH; 1:1) to give maackiain (**175**) (20 mg), 6a-hydroxymaackiain (**184**) (10 mg) and pisatin (**185**) (15 mg). The eluent with 9 - 10% with EtOAc in *iso*-hexane were combined and purified by column chromatography on a Sephadex LH-20 column (eluent: CH_2Cl_2/MeOH; 1:1). Then by preparative HPLC (MeOH/H_2O, gradient elution) to give tephrosin (**173**, 10 mg), rotenone (**186**, 15 mg), 6-hydroxyrotenone (**187**, 10 mg), 12a-hydroxyrotenone (**129**, 10 mg), rhocarpin (**188,** 10 mg), hildecarpin (**134,** 10 mg) and 3-hydroxy-2-methoxy-8-9-

methylenedioxypterocarpene (**189**, 15 mg). Elution with 12% EtOAc in *iso*-hexane gave isoliquirtigenin (**190**) (15 mg**)** and elution with 20% EtOAc in *iso*-hexane gave D-pinitol (**165**) (900 mg). The fraction eluted with 8% EtOAc in *iso*-hexane was further subjected to column chromatography on silica gel (200 g) to give tephrowatsin A (**79**) (10 mg) **191** (rhodiflavan A, 50 mg), **192** (rhodiflavan B, 20 mg) and **193** (rhodiflavan C, 15 mg) with 3%, 5% and 10% EtOAc in *iso*-hexane. These compounds were further purified by preparative HPLC (MeOH/H_2O, gradient elution).

3.3.7: Extraction and isolation of compounds from the stem of Tephrosia purpurea spp. leptostachya

The air dried stem (2 kg) of *T. purpurea* spp. *leptostachya* was ground and extracted with CH_2Cl_2/MeOH (1:1) (3 x 2 L) by percolation at room temperature. The extract was concentrated *in vacuo* to yield 80 g of a dark yellow paste. A portion of the extract (31 g) was subjected to column chromatography over silica gel (300 g) eluting with *iso*-hexane containing increasing amounts of EtOAc. The fraction eluted with 3% EtOAc in *n*-hexane was further purified by column chromatography on Sephadex LH-20 column (eluent: CH_2Cl_2/MeOH; 1:1) to give purleptone (**194)** (20 mg) and (*E*)-5-oxo-anhydrotephrostachin (**195**) (25 mg). The eluent with 5% EtOAc in *iso*-hexane was further subjected to column chromatography on Sephadex LH-20 (CH_2Cl_2:MeOH; 1:1) followed by preparative HPLC (MeOH/H_2O, gradient elution) to give derrone (**196**) (30 mg), glabranin (**182**) (50 mg), obovatin methyl ether (**73**) (50 mg) and genistein (**197**) (50 mg). Elution with 6% EtOAc in *iso*-hexane gave a yellow solid which was recrystallized from CH_2Cl_2/MeOH (1:1) to give (*E*)-5-hydroxytephrostachin (**198**) **(**500 mg). Further elution with 8% EtOAc in *n*-hexane gave terpurlepflavone (**199**) (70 mg); the elution with 9% EtOAc in *iso*-hexane gave tachrosin (**41**) (200 mg) and the 10% EtOAc in *iso*-hexane

eluent gave kaempferitrin (**200**) (100 mg). The fraction eluting with 15% EtOAc in *iso*-hexane gave D-pinitol (**165**) (500 mg).

3.4: Structure Modification

3.4.1: Pyrazoline Derivative of Praecansone B (146)

Praecansone B (**146**) (100 mg) was dissolved in 10 ml of ethanol and 5 ml hydrazine hydrate was added drop wise. The mixture was then heated to reflux for 12 hour. The reaction mixture was concentrated in vacuo, diluted with water (10 ml) and extracted into CH_2Cl_2 (3 x 15 ml). The CH_2Cl_2 layer was concentrated to dryness to give a white amorphous solid pyrazopraecansone B (**201)** (75 mg).

3.4.2: Pyrazoline Derivative of Isopongaflavone (59)

Pyrazoisopongaflavone (**202) (**85 mg) was prepared from isopongaflavone (**59**) following the same procedure described in Section 3.4.1.

3.4.3: Guanidine Derivative of Isopongaflavone (59)

Isopongaflavone (100 mg) was mixed with guanidinium chloride (100 mg) and dissolved in 20 ml of ethanol. Catalytic amount of KOH was added and the mixture heated to reflux for 12 hrs. The mixture was transferred to a 250 ml beaker into which crushed ice was poured. A few drops of HCl were added. The precipitate that formed was filtered out and purified with column chromatography to give Guanidinoipongaflavone (**204) (**25 mg).

3.5: Antinociceptive and Anti-inflammatory Tests

3. 5.1: Sensorimotor Test

The sedative effects or nonspecific muscle relaxant of the plant extracts and isolated compounds on the mice was evaluated using sensorimotor apparatus. This test was done before and after one hour of the treatment of the mice with extracts, isolated compounds, standard drugs and vehicle. The mice were placed on the apparatus for 20 seconds to test their sensorimotor function (Kariuki, 2013).

3. 5.2: Formalin Test

The formalin test was carried out as described by (Hunskaar *et al.*, 1985). All the animals (mice) were injected with the treatments, after thirty minutes 0.1 ml of 5% formalin was administrated in the subplantar region of the left hind paw of the mice to induce nociceptive behavior of lifting, licking and biting. Two different periods of rigorous lifting, licking and biting activity were identified and recorded separately. The early phase response (Phase I) was recorded immediately after formalin injected and lasting for 5 minutes. Recording the late phase response (Phase II) started 20 minutes after formalin injection and lasted for 10 minutes (Mwangi *et al.*, 2015; Rosland *et al.*, 1990).

3.6: ***Plasmodium falciparum Culture***

In vitro parasite culture of the *P. falciparum* strains 3D7 was maintained in RPMI with 10 mM Hepes (Life Technologies), 50 μg/mL hypoxanthine (Sigma) and 5% human serum from male AB plasma and 2.5 mg/mL AlbuMAX II® (Life Technologies). Human 0+ erythrocytes were obtained from the Australian Red Cross Blood Service (Agreement No: 13-04QLD-09). The

parasites were maintained at 2-8 % parasitaemia (% P) at 5% haematocrit (% H), and incubated at 37 °C, 5 % CO_2, 5 % O_2, 90 % N_2 and 95 % humidity.

3.7: *Plasmodium falciparum* Growth Inhibition Assay

A previously developed, well-established asexual *P. falciparum* imaging assay was used to determine parasite growth inhibition according to the procedure described by Duffy and Avery (Duffy and Avery, 2012). Briefly, 2% or 3% parasite (3D7 or Dd2) and 0.3% hematocrit in a total assay volume of 50 μL were incubated in the presence of compounds for 72 h at 37 °C and 5% CO_2, in poly-D-lysine-coated Cell Carrier Imaging plates. After incubation, plates were stained with DAPI (6,4′-diamidino-2-phenylindole) in the presence of saponin and Triton X-100 and incubated for a further 5 h at room temperature in the dark before imaging on the OPERA HTS confocal imaging system (Smalberger *et al.*, 1974). The digital images obtained were then analyzed using the PerkinElmer Acapella spot detection software, where spots fulfilling the criteria established for a stained parasite were counted. The percent inhibitions of parasite replication were then calculated using DMSO and artemisinin control data.

Human red blood cells for plasmodium culture were provided by the Australian Red Cross Blood Bank in accordance with their routine MTA for nonclinical blood product supply. All work undertaken is covered by the following approval from the Griffith University Biosafety and Human Ethics Committee (GU ref no. ESK/03/12/HREC).

3.8: Cytotoxicity Assay

All tested compounds were dissolved in DMSO at a final concentration of 50 mmol/L and stored at -20^{o}C before use. Cytotoxicity was assessed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5.0 mg/ml) assay as previously described by Wong et al., (2013). Briefly, 4×10^{3} cells per well were seeded in 96-well plates before drug treatments. After overnight culture, the cells were then exposed to different concentrations of selected compounds (0.039-100 μmol/L) for 72 h. Cells without drug treatment were used as control. Subsequently, MTT (10 μL) solution was added to each well and incubated at 37^{o}C for 4 h followed by the addition of 100 μL solubilization buffer (10% SDS in 0.01 mol/L HCl) and overnight incubation. A_{570} nm was then determined in each well on the next day. The percentage of cell viability was calculated using the following formula: Cell viability (%) = $A_{treated}/A_{control}\times100$. Data were obtained from three independent experiments and the standard error has been calculated.

3.9: Physical and Spectroscopic Properties of the Isolated and Modified Compounds

Tephrowatsin B ***(100)***

Brown paste. UV (CH_2Cl_2) λ_{max}: 230 and 290 nm. CD (CH_2Cl_2) λ nm (Δε; $M^{-1}cm^{-1}$): $(-16.6)_{296}$; $(67.11)_{237}$; $(-49.9)_{220}$. ^{1}H (Table 4.1). ^{13}C NMR (Table 4.2). LC-ESI-MS: $[M+H]^{+}$ at *m/z* 339.3.

Tephrinone ***(77)***

White crystals. UV (CH_2Cl_2) λ_{max}: 230 and 290 nm. CD (CH_2Cl_2) λ nm (Δε; $M^{-1}cm^{-1}$): $(12.8)_{314}$; $(-56.76)_{291}$; $(51.6)_{228}$; $(-70.5)_{215}$. $[\alpha]_D$ -74.77^{o} (*c* 0.001, CH_2Cl_2). ^{1}H (Table 4.1). ^{13}C NMR (Table 4.2). LC-ESI-MS: $[M+H]^{+}$ at *m/z* 337.8.

D-Pinitol ***(*****165*****)***

White crystals. Mp 195-197 ^{o}C. ^{1}H and ^{13}C NMR (Table 4.3) are in agreement with the literature (Gao *et al.,* 2015; Raya-Gonzalez *et al.*, 2008). LC-ESI-MS: $[M+H]^+$ at *m/z* 339.3.

E-Praecansone A **(145)**

Yellow oily substance. UV (CH_2Cl_2) λ_{max}: 230 and 280 nm. ^{1}H and ^{13}C NMR (Table 4.9). LC-ESI-MS: $[M+H]^+$ at *m/z* 381.5.

8-O-Methylretusin **(165)**

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 230 and 250. ^{1}H and ^{13}C NMR (Table 4.31). LC-ESI-MS: $[M+H]^+$ at *m/z* 299.7.

Pumilaisoflavone C **(117)**

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 230 and 280 nm. ^{1}H and ^{13}C NMR (Table 4.5). LC-ESI-MS: $[M+H]^+$ at *m/z* 467.2.

3,4:8,9-Dimethylenedioxypterocarpene **(167)**

Colorless crystal. Mp 198-200 ^{o}C; UV (CH_2Cl_2) λ_{max}: 225, 337, 353 nm. ^{1}H and ^{13}C NMR (Table 4.8). EIMS *m/z* (rel. int.) 397 $[M]^+$ (100), 325 (23), 383 (20), 297 (15). HRMS $[M]^+$ *m/z* 310.0512 $C_{17}H_{10}O_6$ (Calculated: 310.0477).

E-2',6'-Dimethoxy-4',5'-(2'',2''-dimethyl)pyranoretrochalcone (**168)**

Yellow paste. UV (CH_2Cl_2) λ_{max}: 240, 290 and 370 nm. ^{1}H and ^{13}C NMR (Table 4.6). ESIMS *m/z* 351.7 $[M + H]^+$. HRMS $[M]^+$ *m/z* 350.1506 $C_{22}H_{22}O_4$ (Calculated: 350.1518).

Z-2',6'-Dimethoxy-4',5'-(2'',2''-dimethyl)pyranoretrochalcone **(169)**

Colorless paste. UV (CH_2Cl_2) λ_{max}: 245 nm. ^{1}H and ^{13}C NMR (Table 4.6). EIMS *m/z* (rel. int.) 397 $[M]^+$ (100), 325 (23), 383 (20), 297 (15). HRMS $[M]^+$ *m/z* 351.1586 $C_{22}H_{22}O_4$ (Calculated: 351.1596).

3'',4''-cis-4''-Ethoxy-3''-hydroxypraecansone B **(170)**

Yellowish oil. UV (CH_2Cl_2) λ_{max}: 225, 334 nm. CD (CH_2Cl_2) λ nm ($\Delta\varepsilon$; $M^{-1}cm^{-1}$): $(-3.7)_{403}$; $(0.9)_{297}$; $(2.4)_{209}$. $[\alpha]_D$ -18.87° (c 0.001, CH_2Cl_2). ^1H and ^{13}C NMR (Table 4.7). EIMS *m/z* (rel. int.) 397 $[M]^+$ (100), 325 (23), 383 (20), 297 (15). HRMS $[M]^+$ *m/z* 429.1905 $C_{24}H_{28}O_7$ (Calculated: 429.1913).

Obovatachalcone **(147)**

Orange crystal. UV (CH_2Cl_2) λ_{max}: 230, 300 and 350 nm. ^1H and ^{13}C NMR (Table 4.12). LC-ESI-MS: $[M+H]^+$ at *m/z* 337.9.

Praecansone B **(146)**

Yellow oily substance. UV (CH_2Cl_2) λ_{max}: 230 and 310 nm. ^1H and ^{13}C NMR (Table 4.9). LC-ESI-MS: $[M+H]^+$ at *m/z* 368.5.

Candidone **(97)**

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 240, 290 and 370 nm. . ^1H (Table 4.1). ^{13}C NMR (Table 4.2). LC-ESI-MS: $[M+H]^+$ at *m/z* 353.6.

Isopongaflavone **(59)**

White crystal. UV (CH_2Cl_2) λ_{max}: 230, 270 and 340 nm. ^1H and ^{13}C NMR (Table 4.10). LC-ESI-MS: $[M+H]^+$ at *m/z* 335.3.

*β-Sitosterol-3-O-glucoside **(171)***

White amorphous solid. **^{1}H NMR** (DMSO-d_6, 600 MHz): δ_H 8.8 (3H, *s*, H-27), 8.7 (3H, s, H-26), 5.47 (1H, *s*, H-6), 5.35 (1H, *dd*, *J* =12.5 Hz, H-23), 5.03 (1H, *dd*, *J*= 12.5 Hz, H-22), 3.86 (1H, *m*, H-3), 1.24 (3H, *m*, H-19), 1.0 (3H, *d*, *J* =6.5 Hz, H-21), 0.97 (3H, *t*, *J* =7.1 Hz, H-29), 0.85 (3H, *s*, H-18), 5.06 (H-1`), 4.58 (H-6`*β*), 4.43 (H-6`α), 4.30 (H-3`), 4.03 (H-4`), 4.07 (H-2`), 3.97 (H-5`). **^{13}C NMR** (DMSO-d_6, 150 MHz): δ_C 36.8 (C-1), 31.3 (C-2), 77.5 (C-3), 38.2 (C-4),

141.1 (C-5), 121.9 (C-6), 31.4 (C-7), 29.2 (C-8), 49.5 (C-9), 36.8 (C-10), 22.5 (C-11), 40.0 (C-12), 41.7 (C-13), 56.2 (C-14), 25.3 (C-15), 28.6 (C-16), 55.4 (C-17), 11.7 (C-18), 19.1 (C-19), 36.2 (C-20), 18.8 (C-21), 35.4 (C-22), 27.8 (C-23), 45.1 (C-24), 29.2 (C-25), 19.1 (C-26), 19.1 (C-27), 23.8 (C-28), 11.8 (C-29), 100.7 (C-1`), 73.4 (C-2`), 76.8 (C-3`), 70.0 (C-4`), 74.1 (C-5`),61.0 (C-6`). ^{13}C NMR (Table 4.11).

Obovatin methyl ether **(73)**

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 230 and 280 nm. ^{1}H and ^{13}C NMR (Table 4.10). LC-ESI-MS: $[M+H]^+$ at *m/z* 337.6.

(S)-Elatadihydrochalcone **(143)**

Yellow oily substance. UV (CH_2Cl_2) λ_{max}: 230, 270 and 300 nm. ^{1}H and ^{13}C NMR (Table 4.12). $[\alpha]_D$ +17.35^o (c 0.001, CH_2Cl_2). CD (CH_2Cl_2) λ nm ($\Delta\varepsilon$; $M^{-1}cm^{-1}$): $(11)_{311}$; $(-27)_{297}$; $(32)_{289}$; $(-55)_{272}$; $(55)_{263}$. LC-ESI-MS: $[M+H]^+$ at *m/z* 355.5.

Xanthohumol C **(172)**

Yellow oily substance. UV (CH_2Cl_2) λ_{max}: 230, 270 and 300 nm. ^{1}H and ^{13}C NMR (Table 4.12).LC-ESI-MS: $[M+H]^+$ at *m/z* 393.3.

Tephrosin **(173)**

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 230 and 280 nm. ^{1}H and ^{13}C NMR (Table 4.13). LC-ESI-MS: $[M+H]^+$ at *m/z* 411.4.

Deguelin **(174)**

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 230 and 280 nm. ^{1}H and ^{13}C NMR (Table 4.13). LC-ESI-MS: $[M+H]^+$ at *m/z* 395.2.

Tephrolecarpin A **(175)**

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 230 and 270 nm. ^1H (Table 4.1). ^{13}C NMR (Table 4.2).

Maackiain **(176)**

Colourless amorphous solid. UV (CH_2Cl_2) λ_{max}: 230, 280 and 310 nm. ^1H and ^{13}C NMR (Table 4.18). LC-ESI-MS: $[M+H]^+$ at *m/z* 385.2.

Cinnamic acid **(177)**

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 230 and 300 nm. ^1H and ^{13}C NMR (Table 4.14). LC-ESI-MS: $[M+H]^+$ at *m/z* 165.3.

Salicylic acid **(178)**

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 230 and 310 nm. ^1H and ^{13}C NMR (Table 4.15). LC-ESI-MS: $[M+H]^+$ at *m/z* 139.3.

Quercitin **(179)**

White substance. UV (CH_2Cl_2) λ_{max}: 210, 250 and 270 nm. ^1H and ^{13}C NMR (Table 4.16). LC-ESI-MS: $[M+H]^+$ at *m/z* 303.5.

Kaemferol **(180)**

White substance. UV (CH_2Cl_2) λ_{max}: 260 and 360 nm. ^1H and ^{13}C NMR (Table 4.16). LC-ESI-MS: $[M+H]^+$ at *m/z* 387.2.

Apigenin **(181)**

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 230 and 270 nm. ^1H and ^{13}C NMR (Table 4.16). LC-ESI-MS: $[M+H]^+$ at *m/z* 371.6.

Glabranin **(182)**

White crystal. UV (CH_2Cl_2) λ_{max}: 230 and 290 nm. ^1H and ^{13}C NMR (Table 4.1 and 4.2). LC-ESI-MS: $[M+H]^+$ at *m/z* 325.6.

*Rhodimmer **(183)***

White crystal. UV (CH_2Cl_2) λ_{max}: 230, 290 and 350 nm.. CD (MeOH) λnm(Δε; $M^{-1}cm^{-1}$): $(-40.36)_{292}$; $(-5.24)_{263}$; $(-23.33)_{249}$; $(50.08)_{235}$; $(-56.36)_{220}$; $(62.23)_{212.}$ $[\alpha]_D^{20}$ +13.40° (c 0.001, MeOH) ^1H and ^{13}C NMR (Table 4.17). ^1H (Table 4.1). ^{13}C NMR (Table 4.2). HRMS $[M]^+$ at *m/z* 660.3095 $C_{42}H_{44}O_7$ (Calculated: 660.3087).

*Quercetol B **(98)***

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 240, 270 and 300 nm. ^1H and ^{13}C NMR (Table 4.20). LC-ESI-MS: *m/z* 369.5 $[M+H]^+$.

*6a-Hydroxymaackiain **(184)***

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 230, 280, 310 and 340 nm. ^1H (Table 4.18). ^{13}C NMR (Table 4.19). LC-ESI-MS: *m/z* 383.6 $[M+H]^+$.

*Pisatin **(185)***

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 230, 280 and 310 nm^1H (Table 4.18). ^{13}C NMR (Table 4.19). LC-ESI-MS: *m/z* 314.4 $[M+H]^+$.

*Rotenone **(186)***

Colourless amorphous solid. UV (CH_2Cl_2) λ_{max}: 240 and 300 nm. ^1H and ^{13}C NMR (Table 4.24). LC-ESI-MS: $[M+H]^+$ at *m/z* 395.3.

*6-Hydroxyrotenone **(187)***

Colourless amorphous solid. UV (CH_2Cl_2) λ_{max} : 230 and 290 nm. ^1H and ^{13}C NMR (Table 4.24). LC-ESI-MS: $[M+H]^+$ at *m/z* 411.4.

*12a-Hydroxyrotenone **(129)***

Colourless amorphous solid. UV (CH_2Cl_2) λ_{max}: 230 and 300 nm. ^1H and ^{13}C NMR (Table 4.24). LC-ESI-MS: $[M+H]^+$ at *m/z* 411.4.

*Rhocarpin **(188)***

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 230, 290, 310 and 350 nm. ^{1}H and ^{13}C NMR (Table 4.25). LC-ESI-MS: $[M+H]^+$ at *m/z* 369.1. HRMS $[M]^+$ *m/z* 369.1367 $C_{22}H_{22}O_4$ (Calculated: 369.1338).

*Hidecarpine **(134)***

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 230 and 300 nm. ^{1}H (Table 4.18). ^{13}C NMR (Table 4.19). .LC-ESI-MS: $[M+H]^+$ at *m/z* 330.9.

*3-hydroxy-2-methoxy-8-9-methylenedioxypterocarpene **(189)***

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 230 and 280 nm. ^{1}H (Table 4.18). ^{13}C NMR (Table 4.19). LC-ESI-MS: $[M+H]^+$ at *m/z* 313.5.

*Isoliquirtigenin **(190)***

Colourless amorphous solid. UV (CH_2Cl_2) λ_{max}: 230 and 260 nm. ^{1}H and ^{13}C NMR (Table 4.24). LC-ESI-MS: $[M+H]^+$ at *m/z* 357.5.

*Tephrowatsin A **(79)***

Yellow oily substance. UV (CH_2Cl_2) λ_{max}: 230 and 270 nm. ^{1}H (Table 4.1). ^{13}C NMR (Table 4.2). LC-ESI-MS: $[M+H]^+$ at *m/z* 355.5.

*Rhodiflavan A **(191)***

Yellow oily substance. UV (CH_2Cl_2) λ_{max}: 230 and 270 nm. CD (MeOH) λnm ($\Delta\varepsilon$; $M^{-1}cm^{-1}$): $(89.0)_{316}$; $(-32.21)_{284}$; $(91.80)_{248}$; $(-26.26)_{226}$; $(70.0)_{218}$; $(80.5)_{210.}$ $[\alpha]_D$ $+90.0^o$ (c 0.001, CH_2Cl_2). ^{1}H and ^{13}C NMR (Table 4.25). HRMS $[M]^+$ *m/z* 784.4202 $C_{50}H_{56}O_8$ (Calculated: M+ H 784.3974).

*Rhodiflavan B **(192)***

Yellow oily substance. UV (CH_2Cl_2) λ_{max}: 240 and 290 nm. CD (MeOH) λnm (Δε; $M^{-1}cm^{-1}$): $(-28.25)_{305}$; $(45.45)_{260}$; $(-109.45)_{223}$; $(-26.26)_{226}$; $(70.0)_{218}$; $(80.5)_{210.}$ $[\alpha]_D$ +17.5° (c 0.001, CH_2Cl_2). ^1H and ^{13}C NMR (Table 4.26). HRMS $[M]^+$ *m/z* 462.2693 $C_{30}H_{38}O4$ (Calculated: M+ H 463.2848).

Rhodiflavan C ***(193)***

Yellow oily substance. UV (CH_2Cl_2) λ_{max}: 230 and 280 nm. CD (MeOH) λnm (Δε; $M^{-1}cm^{-1}$): $(199.73)_{297}$; $(-113.29)_{275}$; $(106.11)_{225.}$ $[\alpha]_D$ +66.1° (c 0.001, CH_2Cl_2). ^1H and ^{13}C NMR (Table 4.27). HRMS $[M]^+$ *m/z* 381.2062 (Cal.), $C_{24}H_{28}O_4$ (Calculated: M+ H 381.2066).

Purleptone ***(194)***

Colourless amorphous solid. UV (CH_2Cl_2) λ_{max}: 230, 290 nm. ^1H and ^{13}C NMR (Table 4.28). EIMS *m/z* (rel. int.) 337 $[M]^+$ (100). HRMS $[M]^+$ *m/z* 336.0980 $C_{20}H_{16}O_5$ (Calculated: 336.0998).

(E)-5-oxo-anhydrotephrostachin ***(195)***

Colourless amorphous solid. UV (CH_2Cl_2) λ_{max}: 230, 280 nm. ^1H and ^{13}C NMR (Table 4.28). EIMS *m/z* (rel. int.) 336.1276 $[M]^+$. HRMS $[M]^+$ *m/z* 335.1227 $C_{22}H_{22}O_4$ (Calculated: 335.1283).

Derrone ***(196)***

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 230 and 270 nm. ^1H and ^{13}C NMR (Table 4.30). LC-ESI-MS: $[M+H]^+$ at *m/z* 337.5.

Genistein ***(197)***

White amorphous solid. UV (CH_2Cl_2) λ_{max}: 230 and 280 nm. ^1H and ^{13}C NMR (Table 4.31). LC-ESI-MS: $[M+H]^+$ at *m/z* 381.5.

(E)-5-Hydroxytephrostachin ***(198)***

Yellow crystals (CH_2Cl_2/MeOH; 1:1). Mp 160-162 ^oC. UV (CH_2Cl_2) λ_{max}: 230, 270 nm. ^1H and ^{13}C NMR (Table 4.28). EIMS *m/z* (rel. int.) 353.6 $[M]^+$ (100). HRMS $[M]^+$ *m/z* 352.1315 $C_{21}H_{20}O_5$ (Calculated: 352.1311).

Terpurlepflavone ***(199)***

White amorphous solid. Mp 210-214 ^oC UV (CH_2Cl_2) λ_{max}: 230, 260 nm and 310 nm. CD (MeOH) λ nm ($\Delta\varepsilon$; $M^{-1}cm^{-1}$): $(122.83)_{121}$; $(-58.17)_{112.}$ $[\alpha]_D^{20}$ +14.00^o (c 0.001, MeOH). ^1H and ^{13}C NMR (Table 4.29). EIMS *m/z* (rel. int.) 424.1500 $[M]^+$. HRMS $[M]^+$ *m/z* 423.1465 $C_{24}H_{22}O_7$ (Calculated: 423.1444).

Tachrosin ***(41)***

White crystal. UV (CH_2Cl_2) λ_{max}: 230, 260 and 320 nm. ^1H and ^{13}C NMR (Table 4.32). LC-ESI-MS: $[M+H]^+$ at *m/z* 393.2.

Kaempferitrin ***(200)***

White crystal. UV (CH_2Cl_2) λ_{max}: 230, 260 and 320 nm. ^1H and ^{13}C NMR (Table 4.33). LC-ESI-MS: $[M+H]^+$ at *m/z* 579.7.

Pyrazopraecansone B ***(201)***

White amorphous solid. ^1H and ^{13}C NMR (Table 4.34). HRMS $[M]^+$ m/z 364.1778 $C_{22}H_{24}O_3N_2$. (Calculated: 364.1787).

Pyrazoisopongaflavone ***(202)***

White amorphous solid. ^1H and ^{13}C NMR (Table 4.34). HRMS $[M]^+$ *m/z* 350.1630 $C_{21}H_{22}O_3N_2$. (Calculated: 350.1640).

Guanidinoisopongaflavone ***(203)***

White amorphous solid. ^{1}H and ^{13}C NMR (Table 4.35). HRMS $[M]^{+}$ m/z 375.1573 $C_{22}H_{21}O_3N_3$. (Calculated: 375.1556).

CHAPTER 4: RESULTS AND DISCUSSION

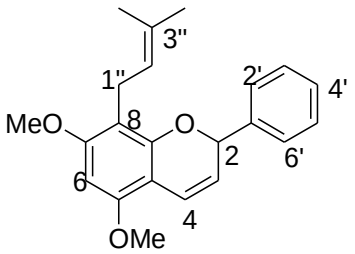
4.1: Characterization of Isolated Compounds

4.1.1: Compounds from Tephrosia noctiflora

From the stems of *T. noctiflora* three compounds, including two flavonoids and one cyclohexane derivative were isolated and characterized. All these compounds are reported for the first time from this species.

4.1.1.1: Tephrowatsin B (100)

Compound **100** was obtained as a brown paste and identified as a flavene derivative based on UV spectrum (λ_{max} = 230 and 290 nm), ^{1}H (δ_H 5.83 for H-2, 5.66 for H-3 and 6.82 for H-4) and ^{13}C (δ_C 76.5 for C-2, 120.0 for C-3 and 119.0 for C-4) NMR data (Table 4.1). The HMBC spectrum showed a clear J^3 coupling of H-4 with C-2 and C-5; and H-3 with C-2, C-4a, C-8a and C-1' which confirm the suggestion that the compound is a flavene derivative. The LC-ESI-MS spectrum that showed a $[M+H]^+$ peak at *m/z* 337.8 together with the ^{1}H and ^{13}C NMR data (Table 4.1) is consistent with the molecular formula $C_{22}H_{24}O_3$.



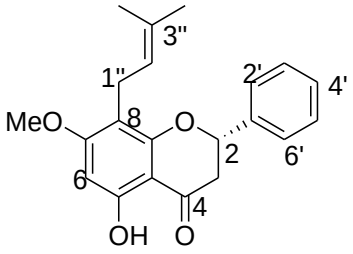
100

In the ^{1}H NMR spectrum, in addition to the two olefinic protons at δ_H 5.66 for H-3, δ_H 6.82 for H-4, the presence of a third olefinic signal at δ_H 5.05 for H-2" (δ_C 123.1 for C-2") along with signals at δ_H 3.2 for CH_2-1" (δ_C 21.7), δ_C 130.7 for C-3", δ_H 1.35 for Me-4" (δ_C 25.7) and δ_H

1.45 for Me-5" (δ_C 17.7) indicated the presence of a prenyl group. The NMR also showed the presence of two methoxy groups (Table 4.1). Three sets of multiplet protons resonating at δ_H 7.43 *m* (H-2'/6, δ_C 126.5), 7.33 *m* (H-3'/5', δ_C 129.1) and 7.28 *m* (H-4', δ_C 131.9) together with the corresponding carbon signals (Table 4.1) is consistent with an unsubstituted ring-B. In the ^{1}H NMR spectrum, only one aromatic proton (δ_H 6.82) was observed in ring-A, which means the two methoxy groups should be placed at C-5 and C-7 (on biogenetic basis, and confirmed by HMBC correlations), while the prenyl is located either at C-8 (compound **100**) or C-6 (compound **100a**). The singlet at δ_H 6.82 was assigned to H-6 because of its HMBC correlation with C-4 (δ_C 119.0), C-4a (δ_C 104.6), C-5 (δ_C 153.8), C-7 (δ_C 158.5) and C-8 (δ_C 110.4) which is consistent with structure **100**. From the above spectral data and by comparison with literature, compound **100** was identified as 5,7-dimethoxy-8-(3-methylbut-2-enyl)-2-phenyl-2H-chromene (trivial name tephrowatsin B). This is the first report from *T. noctiflora* but previously this compound was reported from *T. watsoniana* (Gómez *et al.*, 1985b).

4.1.1.2. Tephrinone (77)

Compound **77** was isolated as white crystals. The UV (λ_{max} = 230 and 290 nm), ^{1}H NMR (δ_H 5.46 for H-3, 3.10 and 2.90 for H-2) and ^{13}C NMR (δ_C 78.7 for C-2, 43.3 for C-3 and 196.3 for C-4) spectral data suggested that compound **77** is a flavanone derivative. The molecular formula $C_{21}H_{20}O_5$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 339.3) together with the ^{1}H and ^{13}C NMR data (Table 4.1).



77

The NMR spectral

data (δ_H 7.43 for H-4', δ_H 7.47 for H-3'/H-5', δ_H 7.51 for H-2'/'H-6'; δ_C 138.9 for C-1', 126.0 for C-2'/6', 128.1 for C-3'/5' and 128.8 for C-4') indicated that ring-B is unsubstituted. The ^{1}H NMR spectrum showed a deshielded singlet proton at δ_H 12.17 for OH-5 which is hydrogen-bonded to the carbonyl on C-4 (δ_C, 196.34), and showed HMBC correlation with C-5 (δ_C 162.5), C-6 (δ_C 92.3), and C-4a (δ_C 102.8). The presence of a methoxy (δ_H 3.89, δ_C 55.9) and prenyl groups is also evident from the NMR spectra (Table 4.1) which are also placed in ring-A. With the methoxy being at C-7 based on biogenetic considerations, the prenyl could either be at C-6 or C-8. ^{1}H NMR spectrum further showed two more singlet protons for H-6 and for OMe-7. The HMBC correlations of the singlet at δ_H 6.14 (H-6) with C-4a, C-5, C-7 and C-8, and CH_2-1" with C-8, C-8a, C-7, C-3" and C-2" supported the placements of the prenyl group at C-8. Hence this compound (**77**) was characterized as 5-hydroxy-7-methoxy-6-prenylflavone, a compound (trivial name tephrinone), previously reported from *T. villosa* (Rao and Srimannarayana, 1981) and *T. pumila* (Ganapaty *et al.*, 2008a). The configuration at C-2 was established as *S* from the CD spectrum (Figure 4.1) which showed a positive Cotton effect at 314 nm and a negative one effect at 291 nm (Smalberger *et al.*, 1974).

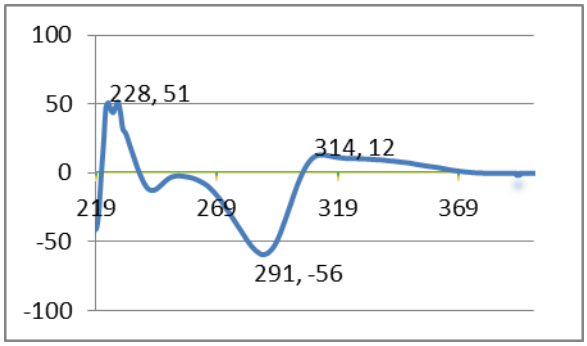


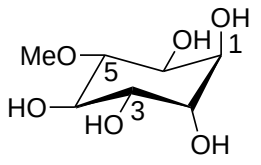
Figure 4.1: CD spectrum of (**S**)-tephrinone (**77**).

Table 4.1. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **100** and **77** in $CDCl_3$

Position	**100**			**77**		
	δ_C (ppm)	δ_H (ppm), *m*, (*J* in Hz)	HMBC correlations	δ_C (ppm)	δ_H (ppm), *m*,(*J* in Hz)	HMBC correlations
2	76.5	5.83 *dd* (1.7, 3.8)	C-4,C-1',C-2'/6', C-8a	78.7	5.41 *dd* (3.1, 12.8)	
3	120.0	5.66 *dd* (3.7, 10.0)	C-2,C-4a ,C-1',C-8a	43.5	3.05 *dd* (12.8, 17.1) 2.85 *dd* (3.1, 17.1)	C-2, C-4, C-4a ,C-1' C-4, C-1'
4	119.0	6.82*dd* (1.7, 10.0)	C-2,C-4a ,C-5,C-8a	196.3		
4a	104.6			102.9		
5	153.8			162.6		
5(OH)					12.13 *s*	C-5, C-6, C-4a
6	88.3	6.04 *s*	C-4, C-4a, C-5, C-7, C-8	92.5	6.10 *s*	C-4a, C-5, C-7, C-8
7	158.5			165.7		
8	110.4			109.0		
8a	151.9			158.7		
1'	141.0	-		138.9	-	
2'/6'	126.8	7.43	C-2, C-2'/6', C-3'/5'	126.0	7.51	C-2, C-2'/6', C-3'/5'
3'/5'	128.3	7.33	C-3'/5', C-1'	128.7	7.47	C-3'/5', C-1'
4'	127.9	7.28	C-2'/6'	128.5	7.43	C-2'/6'
1''	21.6	3.20 *bt*	C-8, C-8a, C-7, C-3", C-2"	21.6	3.27 *bt*	C-8, C-8a, C-7, C-3", C-2"
2''	123.1	5.05 *btt*	C-1"**,** 4"-Me, 5"-Me	122.4	5.14 *btt*	C-1"**,** 4"-Me, 5"-Me
3''	130.6			131.4		
4''-Me	25.7	1.35	C-3", C-2"	17.7	1.62	C-3", C-2"
5''-Me	17.6	1.45		25.8	1.65	
OMe (C-5)	55.7	3.82 *s*	C-5			
OMe (C-7)	55.6	3.81 *s*	C-7	55.9	3.86 *s*	C-7

4.1.1.3. D-Pinitol (165)

Compound **165** was isolated as a white amorphous solid. The NMR data (Table 4.2) suggested that the compound is a cyclohexane derivative. The ^{1}H NMR spectra showed seven sets of peaks in the aliphatic region and the ^{13}C NMR spectra revealed these protons are attached to oxygenated carbon atoms. H-1 [δ_H 2.59 (*m*); δ_C 70.9] coupled with H-6 [δ_H 2.59 (*m*); δ_C 70.6], H-2 [δ_H 2.44 (*dd, J* = 2.3, 9.73 Hz); δ_C 69.2] coupled with H-3 [δ_H 2.39 (*dd, J* = 2.3, 9.70 Hz); δ_C 69.7] and H-4 [δ_H 2.28 (*dd, J* = 9.5, 9.5 Hz); δ_C 71.5] coupled with H-5 [δ_H 1.95 (*dd, J* = 9.5, 9.5 Hz); δ_C 82.1]. These couplings are supported by the HH-COSY spectrum (Appendix **3C**). The placement of the methoxy group at C-5 was confirmed from the HMBC spectra. The methoxy group (δ_H 2.31 *s*) showed an HMBC correlation with C-5 (δ_C 82.1). H-5 (δ_H 1.95) showed an HMBC correlation with C-3 (δ_C 69.7), C-4 (δ_C 71.5) and OMe-5 (δ_C 58.1). Furthermore, H-3 (δ_H 2.39) and H-4 (δ_H 2.44) showed an HMBC correlation with C-5 (δ_C 82.1) and C-3 (δ_C 69.7), and C-5 (δ_C 82.1), respectively. Direct comparison of the above data with the literature identified compound **165** as D-pinitol (Gao *et al.*, 2015; Raya-Gonzalez *et al.*, 2008). This is the first report of D-pinitol from *Tephrosia* species but has previously been isolated from several plant species.



165

Table 4.2. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **165**, acquired in $CDCl_3$

Position	**165**		
	δ_C (ppm)	δ_H (ppm), *m*, (*J* in Hz)	HMBC correlations
1	70.9	2.59 *m*	
2	69.2	2.44 *dd* (2.3, 9.7)	
3	69.7	2.39 *dd* (2.3, 9.7)	C-5
4	71.5	2.28 *dd* (9.5, 9.5)	C-3, C-5,
5	82.1	1.95 *dd* (9.5, 9.5)	C-3, C-4, OMe-5
6	70.6	2.59 *m*	
OMe-5	58.1	2.31 *s*	C-5

4.1.2: Compounds from **Tephrosia pumila**

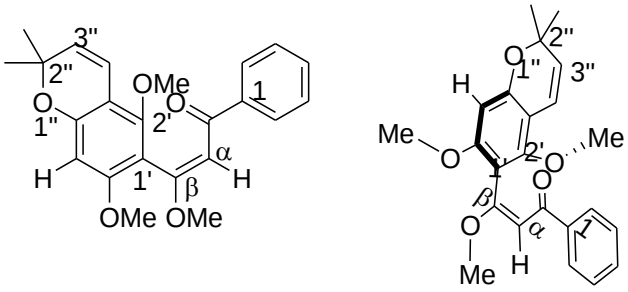
Two isoflavonoids, one *retro*-chalcone and one flavone were isolated and characterized from the aerial part of *T. pumila.*

4.1.2.1: (E)-Praecansone A (145)

Compound **145** was isolated as a yellow oily substance. The molecular formula $C_{25}H_{24}O_5$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 381.5) together with the ^{1}H and ^{13}C NMR data (Table 4.3). The UV spectrum (λ_{max} 230 and 280 nm) along with NMR spectra (Table 4.3) suggesting that compound **145** is a *retro*-chalcone (Dagne and Yenesew, 1990).

The ^{1}H NMR spectrum showed three multiplet signals of the unsubstituted ring-A; for H-2/6 (δ_H 7.86), H-3/5 (δ_H 7.43) and H-4 (δ_H 7.45). H-2/6 showed HMBC correlation with C-7 (δ_C 189.4), C-4 (δ_C 131.6) and C-3/5 (δ_C 128.1), is in agreement that compound **145** is a *retro*-chalcone. The presence of three methoxy and a 2,2-dimethylchromene substituents on a *retro*-chalcone skeleton was apparent from the NMR spectra (Table 4.3). In addition, in the ^{1}H NMR

spectrum, a singlet resonating at δ_H 6.48 showed HMBC correlation with C-7, C-9 and C-1' and hence was assigned to H–α, whereas C-9 is substituted with a methoxy group (δ_H 3.92; δ_C 56.1). The remaining two methoxy group were placed at C-2' and C-6' while the 2,2-dimethyl group at c-3'/C-4' based on HMBC correlations (Table 4.3). NOESY interaction of H–α with 9-OMe and H-2/6 is consistent with the geometry across C-α and C-β (C–9) double bond to be (*E*)-configured (Dagne and Yenesew, 1990; Tarus *et al.*, 2002). From the forgoing discussion and direct comparison of the spectroscopic data with literature, compound **145** was identified as (*E*)-praecansone A, previously isolated from the seed pods of *T. pumila* (Dagne and Yenesew, 1990) and the roots *T. aequilata* (Tarus *et al*., 2002).



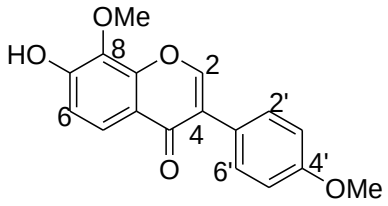
145

Table 4.3. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **145** and **146**, CD_2Cl_2

Position	**145**			**146**		
	δ_C (ppm)	δ_H (ppm), *m*, (*J* in Hz)	HMBC correlations	δ_C (ppm)	δ_H (ppm), *m*, (*J* in Hz)	HMBC correlations
1	139.9			135.10		
2/6	127.6	7.89 *m*	C-3/5, C-4, C-7	127.0	8.01 *m*	C-2/6, C-4, C-7
3/5	128.1	7.45 *m*	C-2/6, C-1	128.7	7.53 *m*	C-1, C-3/5
4	131.6	7.53 *m*	C-3/5, C-2/6	132.2	7.45 *m*	C-3/5
7(C=O)	189.4			182.0		
α	101.1	6.48 *s*	C-9, C-7, C-1'	100.6	6.62 *s*	C-1, C-7, C-9
C-9	165.8			188.3		
1'	112.1			114.4		
2'	154.7			158.5		
3'	107.6			108.2		
4'	155.6			156.6		
5'	95.9	6.21 *s*	C-1',C-3', C-4', C-6', C-9	96.2	6.38 *s*	C-1', C-4', C-5'
6'	157.9			155.3		
2''	76.5			76.9		
3''	127.1	5.57 *d*, (10.0)	C-2", C-3', 2"-Me_2	127.9	5.67 *d*, (10.0)	C-3', C-2"
4''	116.7	6.53 *d*, (10.0)	C-2', C-3', C-4', C-2"	116.5	6.64 *d*, (10.0)	C-3', C-4', C-2"
2''-Me_2	27.65 27.78	1.49 *s* 1.47 *s*	C-2", C-3"	27.7	1.55 *s*	C-2", C-3"
OMe (C-2')	62.14	3.74 *s*		63.12	3.89 *s*	C-2'
OMe (C-6')	55.88	3.71 *s*		55.97	3.85 *s*	C-6'
OMe (C-9)	56.13	3.92 *s*				

4.1.2.2: 8-O-Methylretusin (166)

Compound **166** was isolated as an amorphous solid. The molecular formula $C_{17}H_{14}O_5$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 299.0) together with the ^1H and ^{13}C NMR data (Table 4.4). The UV spectrum (λ_{max} 230, 250 and 310 nm) along with the singlet proton peak at δ_H 8.05 ppm in the ^1H NMR spectrum with its corresponding oxygenated carbon peak resonating at δ_C 151.6 ppm revealed that compound **166** is an isoflavone derivative. This is further confirmed by the HMBC spectrum, where H-2 (δ_H 8.05 ppm) showed correlation with C-3 (δ_C 124.1 ppm), C-4 (δ_C 175.5 ppm), and C-8a (δ_C 150.0 ppm). That this isoflavone is tri-oxygenated with a hydroxy and methoxy groups was evident from the NMR spectra (Table 4.4).



166

The ^1H NMR spectrum further showed a low field doublet at δ_H 7.92 ppm assigned to H-5 *ortho*-coupled to H–6 (δ_H 7.08 ppm), of ring A which is substituted at C-7 and C-8. The HMBC correlation of H–5 (δ_H 7.92 ppm) with C-4 (δ_C 175.5 ppm) and C-8a (δ_C 150.0 ppm) is consistent with assigning the *ortho*-coupled protons to H-5 and H-6. An AA'XX' spin system, resonating at δ_H 7.52 ppm (H-2'/6') and δ_H 7.02 ppm (H–3'/5') is consistent with 4'-oxygenated ring B. Of the two methoxy groups, the ^{13}C NMR resonance of one of them, δ_C 61.9 (δ_H 4.11), is deshielded and requires it is di-o*rtho*-substituted placing it at C-8. The second methoxy (δ_H 3.88,δ_C 55.2 was placed at C-4') on the basis of HMBC correlation of the methoxy protons and the ring-B aromatic protons with C-4'. Hence on the basis of these spectroscopic evidence and comparison

of the data with literature, compound **166** was identified as 8-*O*-methylretusin (Jurd *et al.*, 1972; Puebla *et al.*, 2010). This is the first report of its isolation from *Tephrosia* species.

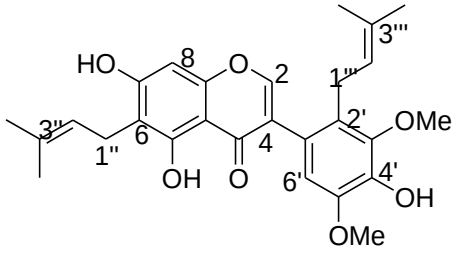
Table 4.4. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **166**, CDCl3

Position			
	δ_C (ppm)	δ_H (ppm), *m*, (*J* in Hz)	HMBC correlations
1		-	
2	151.6	8.05 *s*	C-3, C-4, C-8a
3	124.1		
4(C=O)	175.5		
4a	119.0		
5	121.5	7.95 *d* (8.2)	C-4, C-8a
6	113.7	7.08 *d* (8.2)	C-8, C-4a, C-7
7	153.0		
8	134.0		
8a	150.0		
1'	124.5	-	
2'/6'	130.1	7.52 *d* (8.2)	
3'/5'	113.7	7.02 *d* (8.2)	
4'	159.4	-	
OMe (C-8)	61.9	4.11 *s*	C-8
OMe (C-4')	55.2	3.88 *s*	C-4'

4.1.2.3: Pumilaisoflavone C (117)

Compound **117** was isolated as an amorphous solid. The molecular formula $C_{27}H_{30}O_7$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 467.20) together with the ^{1}H and ^{13}C NMR data (Table 4.5). Compound **117** was identified as an isoflavonoid derivative from the ^{1}H NMR signal at δ_H 7.96 (1H, *s*) for H-2 and the ^{13}C NMR signals for ring-C carbon; atoms δ_C 154.8 (C-

2), δ_C 120.2 (C-3) and δ_C 181.7 (C-4) ppm. The HMBC correlation of H-2 with C-3, C-4, C-8a (δ_C 155.9) and C-1' (δ_C 114.6) is in agreement with an isoflavone skeleton.



117

The presence of two prenyl, three hydroxy (one of which is hydrogen-bonded) and two methoxy groups were evident from the ^{1}H and ^{13}C NMR spectra (Table 4.4). The hydrogen-bonded hydroxy proton (δ_H 13.23, 5-OH *s*) showed HMBC cross peak with C-5 (δ_C 159.1), C-4a (δ_C 105.7) and C-6 (δ_C 110.3). The methylene protons, CH_2-1'' (δ_H 3.47) of one of the prenyl group also showed HMBC correlation with C-5 and C-7 allowing the placement of this prenyl group at C-6. The only ring A proton resonating at δ_H 6.42 ppm was then assigned to H-8 with its corresponding carbon C-8 appearing at δ_C 93.7. In agreement with this, the HMBC spectrum showed correlation of H-8 with C-6 (δ_C 110. 3 ppm), C-7 (δ_C 161.1 ppm), C-4a (δ_C 105.7 ppm), C-8a (δ_C 155.9 ppm) and a W-coupling with C-4 (δ_C 181. 7 ppm).

Ring B bears the second prenyl group, a hydroxy and two methoxy groups. The chemical shift values of the oxygenated carbon atoms indicated 1,2,3-trioxygenation which in this case is C-3', C-4' and C-5' oxygenation, and the prenyl at C-1'. The singlet aromatic peak at δ_H 6.19 can then be assigned to H-6'. This proton showed correlation with C-1' (δ_C 114.6), C-3 (δ_C 120.2), C-5' (δ_C 142.8), C-4' (δ_C 144.6) and C-3' (δ_C 151.0). The placement of the second prenyl group C-2' was confirmed from the HMBC correlations of H-1''' (δ_H 3.44 ppm) with C-3' (δ_C 151.0 ppm).

With careful comparison of the above spectroscopic data with literature, compound **117** was identified as pumilaisoflavone C, previously reported from the seed pods of *Tephrosia pumila* (Yenesew *et al.*, 1989). This is only the second report on the occurrence of compound **177** in nature.

Table 4.5. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **117**, CD_2Cl_2

Position	δ_C (ppm)	δ_H (ppm), *m*, (*J* in Hz)	HMBC correlations
1		-	
2	154.8	7.96 *s*	C-3, C-4, C-8a, C-1'
3	120.2		
4(C=O)	181.7		
4a	105.7		
5	159.1		
6	110.3		
7	161.1		
8	93.7	6.42 *s*	C-4(W), C-4a, C-6, , C-7, C-8a
8a	155.9		
4–OH		13.23 *s*	C-4, C-4a, C-6
1'	114.6	-	
2'	110.3	-	
3'	151.0	-	
4'	144.6	-	
5'	142.8		
6'	111.2	6.19 *s*	C-3, C-1', C-5', C-4', C-3'
1''	21.5	3.47 *m*	C-5, C-6, C-2", C-3"
1'''	23.3	3.44 *m*	C-2'", C-3'", C-4', C-3'
2''	121.5	5.29 *m*	
2'''	122.4	5.29 *m*	
3''	134.6		
3'''	131.2		
3''-Me	17.5		
3''-Me	17.6		
3'''-Me	25.3		
3'''-Me	25.5		
OMe (C-3')	61.2	3.53 *s*	
OMe (C-5')	56.2	3.90 *s*	

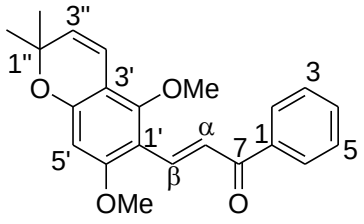
4.1.3: *Compounds from* Tephrosia aequilata

Extraction of the air dried roots of *T. aequilata* with CH_2Cl_2/CH_3OH (1:1) at room temperature, followed by chromatographic separation afforded eleven compounds. Of these obovatin methyl ether (**73**) (Gomez-Garibay *et al.*, 1988), obovatachalcone (**147**) (Roy *et al.*, 1986b), praecansone B (**146**) (Camele et al., 1980b), *Z*-praecansone A (**145**) (Camele *et al.*, 1980b), candidone (**79**) (Roy *et al.*, 1986b), isopongaflavone (**59**) (Khalid and Waterman, 1981; Parmar and Jain, 1988) and β-sitostrol-3-*O*-glucoside (**171**) are known, while four compounds **167-170** are new.

4.1.3.1: E-2',6'-Dimethoxy-4',5'-(2'',2''-dimethyl)pyranoretrochalcone (168)

Compound **168** was isolated as a yellow paste showing UV absorption maxima at 240, 290 and 370 nm, typical of a chalconoid chromophore (Peng *et al.*, 2013). Based on HRESIMS analysis ($[M+H]^+$ obs *m/z* 350.1506, calcd 350.1518), and ^1H and ^{13}C NMR spectral data (Table 4.6) the molecular formula $C_{22}H_{22}O_4$ was assigned. The ^1H NMR signals observed at δ_H 7.96 (*d, J* = 16.0 Hz) and δ_H 8.15 (*d, J* = 16.0 Hz) correspond to the H-α and H-β, respectively, of a chalconoid skeleton possessing *E*-geometry. The corresponding C-α (δ_C 122.8) and C-β (δ_C 136.2) were identified from the HSQC spectrum. The presence of two methoxy and a 2,2-dimethylpyrano substituents were evident from the NMR spectra (Table 4.6). Of the two methoxy functionalities observed, the ^{13}C NMR signal of one was deshielded (δ_C 62.2) suggesting *diortho*-substitution. This methoxy group (δ_H 3.77) showed NOE correlation to H-β (δ_H 8.15) and H-4" (δ_H 6.55), and was accordingly placed at C-2'. The second methoxy group (δ_H 3.88, δ_C 55.8) showed NOE correlation with the aromatic singlet δ_H 6.25 (H-5'), and was hence placed at C-6', supported by the HMBC correlations of H-5' (δ_H 6.25) with C-1' (δ_C 110.5), C-2' (δ_C 161.2), C-3' (δ_C 108.2), and C-4' (δ_C 157.0). The HMBC correlations of H-α (δ_H 7.96) with C-1' (δ_C 110.5), C=O (δ_C

191.9) and those of H-β (δ_H 8.15) with C-6' (δ_C 157.7), C-2' (δ_C 161.2), C-α (δ_C 122.8) and C=O (δ_C 191.9) suggested that compound **168** is a retrochalcone (Ayabe and Furuya, 1981; Kajiyama *et al.*, 1992; Saitoh and Shibata, 1975; Saitoh *et al.*, 1975). The high chemical shift of protons H-2/6 of ring A (δ_H 8.01), which showed HMBC correlation with the carbonyl carbon (δ_C 191.9), and the lack of any NOE between H-2/6 (δ_H 8.01) and H-β (δ_H 8.15) suggested that the carbonyl is adjacent to the A ring (Karé *et al.*, 2006). This ring is unsubstituted, as indicated by the COSY correlations connecting the H-2/6 (δ_H 8.01), H-3/5 (δ_H 7.47) and H-6 (δ_H 7.53) spin system. Connection of the 2,2-dimethylpyranoretro group (C ring) to the B ring *via* the bridging C-3' and C-4' atoms was revealed by the HMBC correlations of H-4" (δ_H 6.55) with C-3' (δ_C 108.2) and C-4' (δ_C 157.0), and by that of H-3" (δ_H 5.55) with C-3' (δ_C 108.2). It was further confirmed by the NOE of H-4" (δ_H 6.55) and MeO-2' (δ_H 3.77). The HMBC correlations of H-3" (δ_H 5.55) with Me-2" (δ_C 28.1) and C-2" (δ_C 76.6) along with the NOE of H-3" (δ_H 5.55) with Me-2" (δ_H 1.44) defined the constitution of the C ring. Thus on the basis of its spectroscopic data, compound **168** was characterized as *E*-2',6'-dimethoxy-4',5'-(2",2"-dimethyl)pyranoretrochalcone, and was assigned the trivial name aeqchalcone A.



168

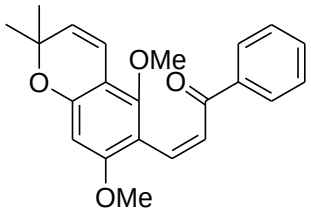
4.1.3.2: Z-2',6'-Dimethoxy-4',5'-(2'',2''-dimethyl)pyranoretrochalcone (169)

Compound **169** was isolated as a colorless paste, and was assigned the molecular formula $C_{22}H_{22}O_4$ based on HRESIMS ($[M+H]^+$ *m/z* obs 351.1586, calcd 351.1596) and NMR (Table 4.6) analyses. Similar to compound **168**, the NMR signals δ_H 6.94 (*d, J* = 12.6 Hz) and δ_H 6.57 (*d, J* = 12.6 Hz) corresponding to H-α and H-β, respectively, suggested a chalconoid skeleton with a *Z*-double bond configuration. Ring B of **169** was observed to be comparable to that of **168**, with two methoxy groups at C-2' (δ_H 3.67, δ_C 61.8) and C-6' (δ_H 3.47, δ_C 54.9), and a 2,2-dimethylchromene ring C connected to ring B *via* the bridging C-3' (δ_C 107.7) and C-4' (δ_C 155.2) atoms. The substitution pattern of ring C was confirmed by HMBC and NOESY correlations as described above for **168**. Ring A of **169** was unsubstituted, and thus the only difference between **168** and **169** was the geometry of their α,β-double bond, reflected by the $^3J_{H\alpha H\beta}$ = 16.0 Hz *vs* 12.6 Hz, and the strong NOE of H-α and H-β observed for **169** but not for **168**. Therefore, compound **169** was characterized as *Z*-2',6'-dimethoxy-4',5'-(2",2"-dimethyl) pyranoretrochalcone, and was given the trivial name aeqchalcone B.

Despite being geometrical isomers at one double bond, the chemical shifts of **168** and **169** are substantially different. Particularly, H-α (δ_H 7.96) and H-β (δ_H 8.15) of the *E*-isomer **168** are deshielded as compared to those of the Z-isomer (H-α δ_H 6.94; H-β δ_H 6.57). Moreover, the carbonyl of **169** is deshielded (δ_C 194.38) as compared to that of compound **168** (δ_C 191.8). These data suggest that due to steric crowding the α,β-unsaturated carbonyl system of **169** is distorted and does not possess coplanar aromatic rings decreasing the extent of conjugation. The shielding of OMe-2' (δ_H 3.47) and OMe-6' (δ_H 3.67) of **169** further indicates that ring B is most likely perpendicular to the α,β–unsaturated system, and accordingly the methoxy groups

experience the anisotropic effect of the α,β−unsaturated carbonyl system. Compound **169** is colorless and shows only a benzenoid absorption band at λ_{max} 237 nm, while compound **168** is yellow and possesses the characteristic UV spectrum of chalconoids with λ_{max} at 240, 290 and 370 nm, further corroborating the above hypothesis. Such distortion was earlier reported for preacansone A (Colegate *et al.*, 1992; Dagne and Yenesew, 1990) and for methyltepanone (Steven *et al.*, 1992).

Upon standing at room temperature in acetone-d_6 solution for days, compound **168** was observed by ^{1}H NMR to slowly convert to compound **169**. Consequently, we cannot rule-out that **169** may have been formed during the extraction and separation process. Similar phenomenon has been observed for the retrochalonoids preacansone A and methyltepanone isolated from *Tephrosia pumila* (Dagne *et al*., 1990) and *Ellipeia cuneijblia* (Annonaceae) (Steven *et al.*, 1992) .



169

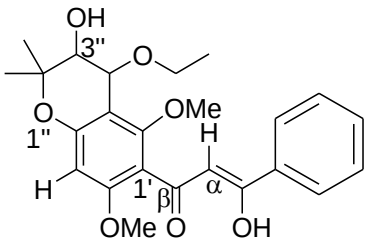
Table 4.6. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **168** & **169**, CD_2Cl_2

position	**168**				**169**			
	δ_C	δ_H, *m,* (*J* in Hz)	HMBC	NOE	δ_C	δ_H, *m,* (*J* in Hz)	HMBC	NOE
1	139.0				137.6			
2/6	128.7	8.01 *dd* (7.7, 1.4)	C-3/5, C-4, C-7		128.7	7. 86 *m*	C-3/5, C-4, C-7	H-α, H-3/5
3/5	128.0	7.47 *dd* (7.7, 7.7)	C-1, C-2/6		128.0	7.34 *m*	C-1, C-2/6,	
4	132.2	7.53 *tt* (7.7, 1.4)	C-2/6, C-3/5		132.1	7.431 *m*	C-2/6, C-3/5	
7	192.0				194.4			
α	122.8	7.96 *d* (16.0)	C-7, C-1'		127.3	6.57 *d* (12.6)	C-1', C-7	H-β
β	136. 1	8.15 *d* (16.0)	C-α, C-7, C-6', C-2'	OMe -6'	130.0	6.94 *d* (12.6)	C-1', C-2', C-6', C-7	H-α
1'	110.5				111.4			
2'	161.2				155.0			
3'	108.2				107.7			
4'	157.0				155.2			
5'	96.4	6.25 *s*	C-1', C-2', C-3', C-4'	OMe-6'	96.0	6.01 *s*	C-1', C-3', C-4', C-6'	OMe-6'
6'	157.7				157.6			
2''	77.0				76.6			
3''	128.4	5.55 *d*, (9.9)	C-2", C-3', 2"-Me_2	2"-Me_2	127.3	5.44 *d* (10.0)	C-2", C-3', 2"-Me_2	H-4"
4''	116.5	6.55 *d*, (9.9)	C-2', C-3', C-4', C-2"	OMe-2'	116.8	6.41 *d* (10.0)	C-2', C-3', C-4', C-2"	H-3", OMe -6'
2''-Me_2	28.1	1.44 *s*	C-2", C-3"		27.9	1.37 *s*	C-2", C-3"	
OMe -2'	62.3	3.77 *s*	C-2'	H-3'	54.9	3.47 *s*	C-2'	
OMe -6'	55.9	3.88 *s*	C-6'	H-4", H-α, H–β	61.8	3.67 *s*	C-6'	H-α, H–β

4.1.3.3: 3'',4''-cis-4''-Ethoxy 3''-hydroxypraecansone B (170)

Compound **170** was isolated as a yellow paste, and was assigned the molecular formula $C_{24}H_{28}O_7$ based on HRESIMS ($[M+H]^+$ obs *m/z* 429.1905, calcd 429.1913) and NMR analyses (Table 4.7). It showed UV absorption at λ_{max} 225 and 334 nm, which along with its NMR data suggested it to be a chalconoid derivative as well. The high similarity of its NMR spectra with those of praecansone B (**146**)(Camele *et al.*, 1980a) suggested **170** to be a *β*-hydroxychalcone. Its H-*α,* olefinic proton (δ_H 6.57), showed HMBC correlation with C-1 (δ_C 134.9), C- 1' (δ_C 114.6) and C-9 (δ_C 188.1). Based on the arguments described for **168** above, ring A of **170** was assumed to be unsubstituted. Its ring B was substituted with two methoxy groups at C-2' (δ_H 3.87, δ_C 62.6) and C-6' (δ_H 3.82, δ_C 55.9), as revealed by the HMBC correlations of H-5' (δ_H 6.27) of this ring with C-1' (114.6), C-3' (107.4), C-4' (155.8), C-6' (158.7) and the NOE observed between H-5' (δ_H 6.27) and MeO-6' (δ_H 3.82). In contrast to the structurally closely related compound **146** that possesses a 2,2-dimethylchromene ring C, that of **170** is saturated and substituted. Thus, protons H-3" and H-4" of **170** are not olefinic, but showed ^{1}H NMR signals at δ_H 3.86 and δ_H 4.40, respectively. The chemical shift of these along with that of the corresponding carbon signals at δ_C 70.2 (C-3") and δ_C 72.6 (C-4") suggested that both are oxygenated. Whereas C-3" (δ_C 70.2) was substituted with a hydroxy group, C-4" (δ_C 72.6) beared an ethoxy functionality (δ_H 3.75, 2H, *q*; δ_C 64.9; δ_H 1.24, 3H, *t*; δ_C 15.3). The placement of the ethoxy group at C-4" was based on the HMBC correlation of its oxymethylene protons (δ_H 3.75) with C-4" (δ_C 155.8) and that of H-4" (δ_H 4.40) with C-2' (δ_C 160.2). The *gauche* coupling (J = 2.8 Hz) of H-3" (δ_H 3.86) and H-4" (δ_H 4.40) revealed their *cis* configuration. Ethoxy substitution is unusual among natural products, yet **170** is not the first to possess a 4"-ethoxy-3"-

hydroxydihydropyran ring (Parsons *et al.*, 1994). On the basis of the above spectroscpic data, and by comparison with that of praecansone B (**146**), compound **170** was characterized as 3",4"-*cis*-4"-ethoxy-3"-hydroxypraecansone B and was given the trivial name eaqchalcone C.



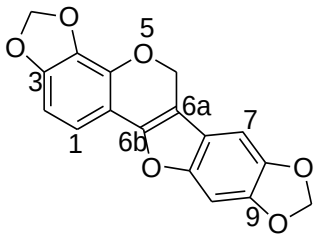
170

Table 4.7. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **170**, CD_2Cl_2

Position	δ_C	δ_H, *m*, (*J* in Hz)	HMBC	NOESY
1	135.0	-		
2/6	127.0	7. 97 *m*	C-2/6, C4, C-7	H-8
3/5	128.6	7.52 *m*	C-2/6, C-1	
4	132.2	7.59 *m*	C-1, C-3/5, C-2/6	
7	182.2			
8*(α)*	100.6	6.57 *s*	C-1, C-1', C-7, C-9,	
9*(β)*	188.1			
1'	114.6			
2'	160.2			
3'	107.2			
4'	155.8			
5'	95.9	6.27 *s*	C-1',C-3', C-4', C-6', C-9, C-4"	OMe-6'
6'	158.7			
2''	77.5			
3''	70.3	3.86 *d* (2.8)	C-4"	2"-Me_2
4''	72.8	4.40 *d*, (2.8)	C-2', C-3', C-4', C-2", C-3", C-2'"	
2'''	64.8	3.75 *m*	C-3'",C-4"	
3'''	15.3	1.25 *t* (7.0, 14.0)	C-2'"	
2''-Me_2	24.8 23.3	1.47 *s* 1.49 *s*	C-2", C-3"	
OMe-2'	62.6	3.87 *s*		
OMe-6'	55.9	3.82 *s*		
OH-9		16.37		

4.1.3.4: 3,4:8,9-Dimethylenedioxypterocarpene (167)

Compound **167** was isolated as an amorphous solid, and was assigned the molecular formula $C_{17}H_{10}O_6$ based on HRESIMS ($[M+H]^+$ *m/z* obs 310.0512, calcd 310.0472) and NMR (Table 4.8) analyses. It showed characteristic UV (λ_{max} 225, 337 and 353 nm), ^{1}H NMR (δ_H 5.59, *s*, CH_2-6) and ^{13}C NMR (δ_C 65.8, CH_2-6; δ_C 119.0, C-6a; δ_C 147.0, C-11a) features for a pterocarpene skeleton (Oberholzer *et al.*, 1976;Yenesew *et al.*, 2003a). Its NMR spectra indicated the presence of two methylenedioxy groups (δ_H 6.02, δ_C 101.7 and δ_H 6.04, δ_C 101.8), connected at the bridging C-3 and C-4, and C-8 and C-9 of the pterocarpene skeleton, as revealed by the HMBC correlations of 3,4-OCH_2O (δ_H 6.02) to C-3 (δ_C 149.5) and C-4 (δ_C 134.3) and 8,9-OCH_2O (δ_H 6.04) to C-8 (δ_C 144.1) and C-9 (δ_C 146.0). Moreover, the two *ortho*-coupled (*J* = 8.0 Hz) aromatic protons at δ_H 7.02 and δ_H 6.55, and the two *para*-oriented aromatic protons at δ_H 7.07 and δ_H 6.81 indicated that rings A and D are disubstituted. The substitution pattern of ring A was determined based on the HMBC correlation of H-1 (δ_H 7.02) with C-11a (δ_C 147.0) and the oxygenated C-3 (δ_C 149.5) along with the *ortho*-coupling of H-1 (δ_H 7.02) and H-2 (δ_H 6.55), which is consistent with the HMBC-based placement (*vide supra*) of the methylenedioxy group at C-3 (δ_C 149.5) and C-4 (δ_C 134.3). The *para*-orientation of the aromatic protons H-7 (δ_H 7.07) and H-10 (δ_H 6.81) of ring D is consistent with the second methylenedioxy group being placed at C-8 (δ_C 144.1) and C-9 (δ_C 146.0). Assignation of the carbons of rings B and C was based on the HMBC correlations of H-1 (δ_H 7.02), H-6 (δ_H 5.59), H-7 (δ_H 7.07) and H-10 (δ_H 6.81) (Table 4.8). On the basis of the above spectroscopic evidence, this new compound (**167)** was characterized as 3,4:8,9-dimethylenedioxypterocarpene.



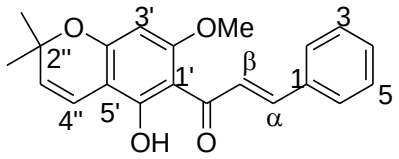
167

Table 4.8. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **167**, CD_2Cl_2

Position	δ_C	δ_H, *m,* (*J* in Hz)	HMBC
1	113.3	6.98 *d* (8.0)	C-3, C-4a, C-11a
2	101.8	6.50 *d* (8.0)	C-3, C-4, C-11b,
3	149.5		
4	134.5		
4a	137.0		
6	65.8	5.54 *s*	C-4a, C-6a, C-6b, C-11a, C-11b (w)
6a	119.0		
6b	107.3		
7	93.8	7.02 *s*	C-6a, C-8, C-9, C-10a
8	144.9		
9	146.1		
10	97.3	6.76 *s*	C-6b, C-7 (w), C-8, C-9, C-10a
10a	150.3		
11a	147.0		
11b	112.5		
3,4-OCH_2O	101.7	6.00 *s*	C-3, C-4
8,9-OCH_2O	101.8	5.97 *s*	C-8, C-9

4.1.3.5: Obovatachalcone (147)

Compound **147** was isolated as orange crystals. The molecular formula $C_{21}H_{20}O_4$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 337.90) together with the ^1H and ^{13}C NMR data (Table 4.9). The UV spectrum (λ_{max} 230, 300 and 350 nm) along with the proton signals resonating at δ_H 7.88 ppm (H-α, *d*, *J* = 15.6 Hz) and δ_H 7.78 ppm (H-β, *d*, *J* = 15.6 Hz) in the ^1H NMR spectrum and the carbonyl group resonating at δ_C 192.7 ppm in ^{13}C NMR spectrum revealed that compound **147** is a chalcone. This was further supported by HMBC correlations of H-α (δ_H 7.88 ppm) with C-7, C-β and C-1', and H-β (δ_H 7.78 ppm) with C-1, C-2/6, C-α and C-7. The presence of hydrogen-bonded hydroxy (δ_H 14.53 ppm), a methoxy (δ_H 3.90, δ_C 55.9) and 2.2-dimethylchromene substituents was evident from NMR spectra (Table 4.9).



147

The only singlet aromatic proton at δ_H 5.92 ppm, (δ_C 91.5 ppm) showed HMBC correlation with C-1' (δ_C 106.1 ppm), C-3' (δ_C 102.9 ppm), C-4' (δ_C 160.4 ppm) and C-6' (δ_C 162.5 ppm) and was assigned to H-5'. In the NOESY spectrum, this proton showed a correlation with the methoxy signal placing the methoxy at C-6'. With the hydrogen bonded hydroxyl group being at C-2 (which showed HMBC correlation with C-1' (δ_C 106.1 ppm), C-2' (δ_C 162.6 ppm) and C-3' (δ_C 102.9 ppm) the 2,2-dimethylpyrano group should be at C-3'/4'. The identity of compound **147**, trivial name obovatachalcone was confirmed from the X-ray single crystal structure (Figure 4.1) and comparison of the spectroscopic data with literature (Chen *et al.*, 1978b).

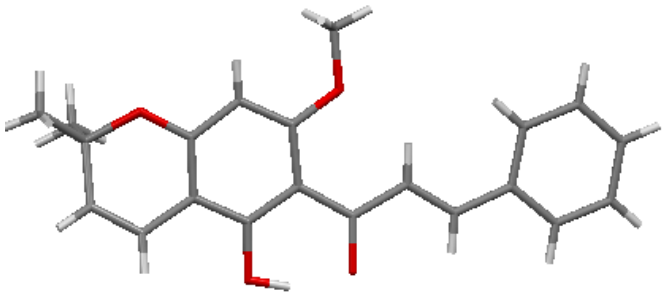


Figure 4.2: X-ray single crystal structure of compound **147**

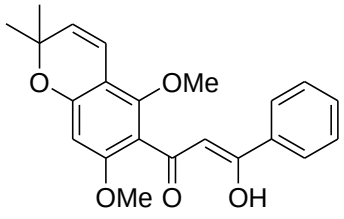
Table 4.9. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **147**, CD_2Cl_2

Position	**147**			
	δ_C (ppm)	δ_H (ppm), *m*, (*J* in Hz)	HMBC correlations	NOESY
1	135.5			
2/6	128.3	7.59 *m*	C-3/5, C4, C-7	
3/5	128.8	7.41 *m*	C-2/6, C-1	
4	130.0	7.40 *m*	C-3/5, C-2/6	
7(C=O)	192.6			
α	127.6	7.88 *d* (15.6)	C-7, C-1', C-β	
β	142.15	7.78 *d* (15.6)	C-4', C-2', , C-1' , C-α, C-7	
1'	106.1			
2'	162.6			
3'	102.9			
4'	160.3			
5'	91.5	5.93 *s*	C-1',C-3', C-4', C-6'	6'-OMe
6'	162.5			
2''	78.2	-		
3''	125.3	5.46 *d* (10.0)	C-2", C-3', 2"-Me_2	C-4", 2"-Me_2
4''	116.0	6.68 *d* (10.0)	C-2', C-3', C-4', C-2"	C-3"
2''-Me_2	28.4	1.45 *s*	C-2", C-3"	
6'-OMe	55.8	3.90 *s*	C-6'	
2'-OH		14.53 *s*	C-1', C-2', C-3'	

4.1.3.6: Praecansone B (146)

Compound **146** was isolated as a yellow oily substance. The molecular formula $C_{22}H_{22}O_5$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 368.5) together with the ^1H and ^{13}C NMR data (Table 4.3). The UV spectrum (λ_{max} 230 and 310 nm) along with NMR spectra (Table 4.3) suggest that compound **146** is a *retro*-chalcone derivative.

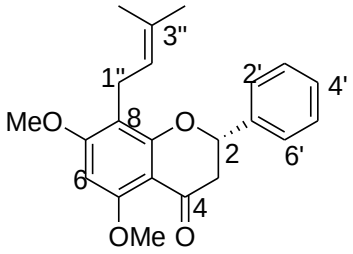
Careful analysis of the 1D and 2D NMR data (Table 4.3) of compound **146** allowed the identification of two methoxyl groups (δ_C 63.1, δ_H 3.89 *s*) and (δ_C 55.9 δ_H 3.85 *s*), an unsubstituted benzene ring [δ_H 8.01 (2H, *m*, for H-2/6), 7.53 (2H, *m*, H-3/5), 7.45 (1H, *m*, for H-4); δ_C 135.1 (for C-1); 127.0 (for C-2/6), 128.7 (for C-3/5) and 132.2 (for C-4), a 2,2-dimethylbenzo[*β*]pyrano moiety [δ_H 5.67 (1H, *d*, *J* = 10.0 Hz, for H-3"), 6.64 (1H *d*, *J* =10.0 Hz, for H-4") and 1.55 (6H, *s*, for 2"-$(CH_3)_2$)] and a hydroxy group (7-OH, δ_C 182.0 (for C-7) with a strong hydrogen bond to a carbonyl group (δ_C, 188.3, for C-9) that exists in Keto-Enol tautomer. The HMBC spectrum (Table 4.3) established the placement of the substituents in ring B, including the position of the hydroxy group at C-7. This led to the identification of compound **146** as 3',4'-(2",2"-dimethlylpyrano)-2',6'-methoxy–*β*–hydroxychalcone, trivial name praecansone B. This compound has been previously identified from *Tephrosia praecans*, *T. pumila* and *T. aequilata* (Camele *et al.*, 1980; Dagne *et al.*, 1988; Tarus *et al.*, 2002).



146

4.1.3.7: Candidone (97)

Compound **97** was isolated as a white amorphous solid. The UV (λ_{max} = 240, 290 and 370 nm), ^{1}H NMR [δ_H 5.39 (1H, *dd, J*=12.5, 3.0 Hz, for H-2), 2.76 (1H, *dd, J*=16.3, 12.5 Hz, for H-2ax), 2.96 (1H, *dd, J*=16.3, 3.1 Hz, for H-3eq)] and ^{13}C NMR [δ_C 78.6 (for C-2), 45.5 (for C-3), and 189.0 (for C-4)] spectral data showed that compound **97** is a flavanone derivative. The molecular formula $C_{22}H_{24}O_4$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 353.6) together with the ^{1}H and ^{13}C (Table 4.10) NMR data.



97

The ^{1}H NMR spectrum further revealed the presence of two methoxy groups (δ_H 3.53*, s,* for OMe-5 and 3.67, s*,* for OMe-7), a prenyl side chain ($\delta_{H,}$ 3.28, *bt,* for H-1"; 5.15, *btt*, for H-2"; 1.63 and 1.64, s, for 3"-Me_2), a singlet at δ_H 6.15 for H-6 and an unsubstituted ring-A [δ_H 7.47 (2H, *m*, for H-2'/6'), 7.41 (2H, *m*, for H-3'/5'), 7.35 (1H, *m*, for H-4') with their corresponding carbon peaks resonating at δ_C 139.9 (for C-1'); 125.9 (for C-2'/6'), 128.5 (for C-3'/5') and 128.15 (for C-4)]. The placement of the substitutions was established from the HMBC spectrum; H-6 (δ_H 6.15) showed a ^{3}J coupling with C-7 (δ_C 163.3) and C-4a (δ_C 105.8); ^{2}J coupling with C-5 (δ_C 160.6) and C-7 (δ_C 163.3), H-1" (δ_H 3.28) showed a ^{3}J coupling with C-8a (δ_C 160.8), C-7 (δ_C 163.3) and C-3"(δ_C 131.1); ^{2}J coupling with C-8 (δ_C 110.8) and C-2" (δ_C 122.4). The identity of compound **97**, trivial name candidone, was confirmed through comparison of the above spectroscopic data with literature (Roy *et al.*, 1986a).

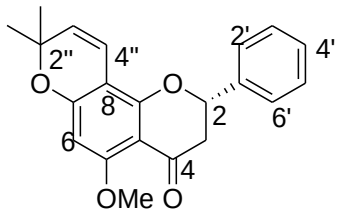
Table 4.10. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **97**, CD_2Cl_2

Position	**97**		
	δ_C (ppm)	δ_H (ppm), *m*, (*J* in Hz)	HMBC correlations
1		-	
2	78.6	5.39 *m*	C-3, C-4, C-8a, C-2′/6′, C-1′
3	45.7	2.76 *m* 2.96 *m*	C-4, C-4a ,C-1′ C-2, C-4, C-1′
4(C=O)	189.0		
4a	105.8		
5	160.6		
6	88.8	6.15 *s*	C-4, C-4a, C-5, C-7, C-8, C-1"
7	163.3		
8	110.0		
8a	160.8		
1′	139.9	-	
2′/6′	125.9	7.47	C-2, C-2′/6′, C-3′/5′
3′/5′	128.5	7.41	C-3′/5′, C-1′
4′	128.2	7.35	C-2′/6′
1″	21.7	3.28 *bt*	C-8-, C-8a, C-7, C-3", C-2"
2″	122.4	5.15 *btt*	C-1", 3"-Me_2
3″	131.1		
3″-Me_2	17.4 25.4	1.63 1.64	C-3", C-2"
OMe (C-5)	55.8	3.89 *s*	C-5
OMe (C-7)	55.6	3.89 *s*	C-7

4.1.3.8: Obovatin methyl ether (73)

Compound **73** was isolated as white crystals. The UV (λ_{max} = 230 and 280 nm), ^{1}H NMR [δ_H 5.47 for H-2 (*dd, J*=1.4, 13.0 Hz), 2.80 (*dd, J*=1.4, 2.9 Hz) and 3.00 (*dd, J*=2.9, 13.3 Hz) for H-3] and ^{13}C NMR (δ_C 78.9 for C-2, 45.6 for C-3 and 188.4 for C-4) spectral data were consistent with flavanone skeleton. The molecular formula $C_{21}H_{20}O_4$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 337.6) together with the ^{1}H and ^{13}C NMR data (Table 4.11). The NMR data

(Table 4.11) further showed the presence of 2,2-dimethylchromene ring, a methoxyl group and an unsubsitiuted ring B. In the HMBC spectrum the aromatic singlet at δ_H 6.11 (H-6) showed a HMBC correlation with C-4a (δ_C 105.5), C-5 (δ_C 162.0), C-7 (δ_C 159.8), C-8 (δ_C 102.8) and a W-coupling with C-4. H-4 (δ_H 6.64 *d, J*=10 Hz) showed HMBC correlation with C-8 (δ_C 102.8), C-2" (δ_C 77.9), C-8a (δ_C 158.7). These correlations are in agreement with the placement of 2,2-dimethylchromene ring at C-7/8 and the methoxyl group at C-5. Finally, through comparison of the spectroscopic data of compound **73** with literature, this compound was identified as obovatin methyl ether, a compound which has been isolated from several *Tephrosia* species (Chen *et al.*, 1978b; Muiva *et al.*, 2009; Stevenson *et al.*, 2012).

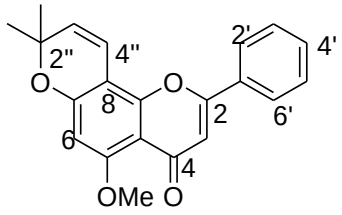


73

4.1.3.9: Isopongaflavone (59)

Compound **59** was isolated as white crystals. The UV (λ_{max} = 240, 290 and 340 nm), ^{1}H NMR (δ_H 6.58 for H-3) and ^{13}C NMR (δ_C 160.0 for C-2, 108.0 for C-3 and 177.0 for C-4) spectral data suggested that the compound is a flavone derivative. The molecular formula $C_{21}H_{18}O_4$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 353.3) together with the ^{1}H and ^{13}C NMR data (Table 4.11). The NMR data (Table 4.10) of compound **59** is comparable with the flavanone **73,** except that this compound is a flavone derivative where C-2 (δ_C 160.0) and C-3 (δ_C 108.0 are sp^2 hybridized). Furthermore, the HMBC spectrum showed a correlation of H-3 (δ_H 6.58, *s*) with C-2 (δ_C 160.0), C-4 (δ_C 177.0), C-4a(δ_C 108.5) and C-1'(δ_C 131.6) in agreement with the above

suggestion. The presence of a 2,2-dimethylchromene ring at C-7/C-8, a methoxyl group at C-5 in ring A and an unsubsitiuted ring B were evident in the NMR spectra (Table 4.11).



59

In addition to the NMR evidence discussed above, the identity of this compound was confirmed by the X-ray single crystal structure analysis (Figure 4.2), which also confirmed the position of the 2,2-dimethylchromene ring as being angular to the methoxy group. Compound **59** was therefore identified as isopongaflavone previously isolated from *Tephrosia Bracteolata* (Khalid and Waterman, 1981).

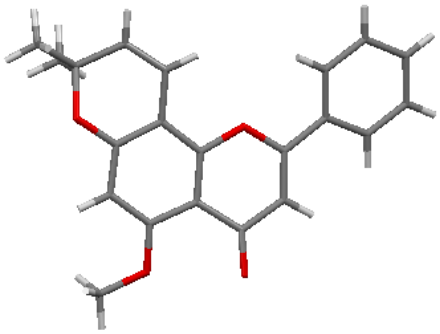


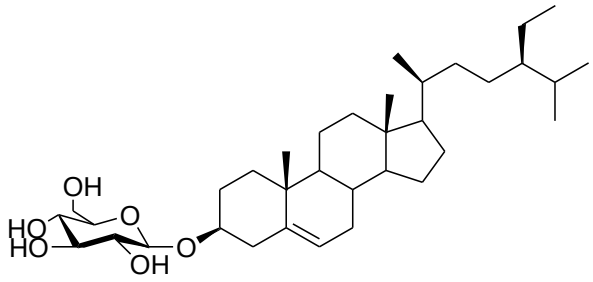
Figure 4.3: X-ray single crystal struture of compound **59**

Table 4.11. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **73** and **59**, CD_2Cl_2

Position	**73**			**59**		
	δ_C (ppm)	δ_H (ppm), *m*, (*J* in Hz)	HMBC correlations	δ_C (ppm)	δ_H (ppm), *m*, (*J* in Hz)	HMBC correlations
2	77.8	5.33 *dd* (2.9, 13.3)	C-3, C-4, C-8a, C-2'/6', C-1'	160.0		
3	44.5	2.80 *dd* (1.5, 13.1) 3.00 *dd* (1.5, 2.9)	C-4, C-4a ,C-1' C-2, C-4, C-1'	108.0	6.58 *s*	C-2, C-4, C-4a ,C-1'
4(C=O)	188.3			177.0		
4a	104.5			108.5		
5	161.1			160.5		
6	92.7	5.97 *s*	C-4, C-4a, C-5, C-7, C-8	96.6	6.33 *s*	C-4, C-4a, C-5, C-7, C-8
7	159.0			157.9		
8	101.8			102.7		
8a	157.7			153.9		
1'	137.8	-		131.6	-	
2'/6'	124.8	7.37	C-2, C-2'/6', C-3'/5'	125.8	7.88	C-2, C-1', C-2'/6', C-3'/5', C-4'
3'/5'	127.6	7.33	C-3'/5', C-1'	128.9	7.51	C-3'/5', C-1'
4'	127.4	7.28	C-2'/6'	131.1	7.41	
2''	77.9			78.0		
3''	125.2	5.38 *d*	C-1", C-8, 3"-Me_2	127.5	5.66 *d* (10.0)	C-8, C-2", 2"-Me_2
4''	114.9	6.51 *d*	C-8, C-2", C-8a	115.1	6.88 *d* (10.0)	C-7, C-8 C-8a, C-2"
2''-Me_2	27.4 27.1	1.37 1.36	C-3", C-2"	27.8	1.49 *s*	C-3", C-2", 2"-Me_2
OMe (C-5)	55.1	3.80 *s*	C-5	56.8	3.90 *s*	C-5

4.1.3.10: β–Sitosterol-3-O-glucoside (171)

Compound **171** was isolated as a white amorphous solid. The ^{13}C-NMR (Table 4.12) showed thirty five peaks of which twenty nine belong to a steroid skeleton; and six belong to a glucopyranosyl moiety (δ_C 100.7, 76.8, 76.7, 73.4, 70.05 and 61.08). The presence of two olefinic carbons (δ_C 141.4 and 121.8) was also observed, while the rest were sp^3 carbons. From the HSQC experiment, compound **171** has nine methines, eleven methylenes, six methyl, and three quaternary carbon atoms. The glucopyranosyl was placed at C-3 with *β*-linkage as shown from the coupling constant (7.8 Hz) of the anomeric proton. Based on comparison of the spectroscopic data with literature, this compound was identified as *β*-sitosterol-3-*O*-glucoside (**171)** (Martin *et al.,* 2000).



171

Table 4.11. ^{13}C (150 MHz) NMR data for compound **171**, DMSO-d_6

Carbon No	**171**		**Carbon No**	**171**
1	36.8		**19**	19.1
2	31.3		**20**	36.2
3	77.5		**21**	18.8
4	38.2		**22**	35.4
5	141.1		**23**	27.8
6	121.9		**24**	45.1
7	31.4		**25**	29.2
8	29.2		**26**	19.1
9	49.5		**27**	19.7
10	36.8		**28**	23.8
11	22.5		**29**	11.8
12	40.0		**1'**	100.7
13	41.7		**2'**	73.4
14	56.2		**3'**	76.8
15	25.3		**4'**	70.0
16	28.6		**5'**	74.1
17	55.4		**6'**	61.0
18	11.7			

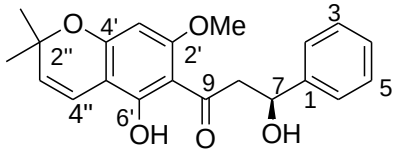
4.1.4. Compounds from Tephrosia elata

4.1.4.1. Compounds from the seedpods of T. elata

The air dried and ground seedpods of *T. elata* were extracted with dichloromethane/methanol (1:1) by cold percolation. Chromatographic separation resulted in the isolation of eight known flavonoids. Obovatachalcone (**147**), (*S*)-elatadihydrochalcone (**143**), xanthohumol C (**172**), Isopongaflavone (**59**), obovatin methyl ether (**73**), 8-*O*-methylretusin (**166**), tephrosin (**173**) and deguelin (**174**).

4.1.4.1.1. (S)-Elatadihydrochalcone (143)

Compound **143** was isolated as a yellow oily substance. The molecular formula $C_{21}H_{23}O_5$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 355.5) together with the NMR data (Table 4.12). The UV spectrum (λ_{max} 230, 270 and 300 nm) along with ^{1}H NMR ([δ_H 3.45, *dd*, *J* = 3.0, 18.0 Hz, H-8) and 3.34, (*dd*, *J* = 9.0, 18.0 Hz), and H-7(*β*), δ_H 5.28 (*dd*, *J* = 3.0, 9.0 Hz)) and ^{13}C NMR (C-9 δ_C 204.2, C-8 δ_C 52.7, C-7(*β*), δ_C 70.2) data suggested that compound **143** is a *β*–hydroxydihydrochalcone derivative. The placement of the hydroxy group at C-7 (δ_C 70.2) was established from the down-field ^{1}H NMR chemical shift value of H-7 (δ_H 5.21, *dd J*= 3.0, 9.0 Hz) (Muiva *et al.*, 2009). In agreement with this H-7 (δ_H 5.21 *dd*) showed an HMBC correlation with C-2/6 (δ_C 124.9). Also H-2/6 (δ_H 7.35 *m*) showed a J^3 correlation with C-7 (δ_C 70.2).



143

The NMR data (Table 4.12) of compound **143** further revealed the presence of a 2,2-dimethylchromene ring, a methoxy group, unsubstituted ring-A and additional hydroxy groups. The hydroxy group (δ_H 13.90 *s*) which form an intra-molecular hydrogen bond with the carbonyl at C-9 (δ_C 204.1) showed an HMBC correlation with C-1' (δ_C 105.5), C-2'(δ_C 161.8) and C-3'(δ_C 102.8). The cross peak of the methoxy group with H-5' (δ_H 5.80 *s*) in the NOESY spectrum suggested the placement of 2,2-dimethylchromene ring adjacent to the hydroxy group (δ_H 13.90 *s*). In support of this, H-4" (δ_H 6.58, *d J*=10.1 Hz) showed an HMBC cross peak with C-4' (δ_C 160.6) and C-2" (δ_C 77.1). Furthermore, H-3" (δ_H 5.39) showed an HMBC correlation with C-3' (δ_C 102.8) and H-2" (δ_H 1.37 *s*) with C-2" (δ_C 77.1) and C-3" (δ_C 125.4). Through detailed analysis of the above data and direct comparison with literature, compound **143** was identified as elatadihydrochalcone (Muiva *et al*., 2009). The absolute configuration at C-9 was established as *S* from the ECD spectrum which showed a positive Cotton effect at 314 and negative one at 296 nm (Figure 4.4), as previously reported (Muiva *et al*., 2009; Nel *et al*., 1999). This is the second report on the occurrence of compound **143** in *T. elata*.

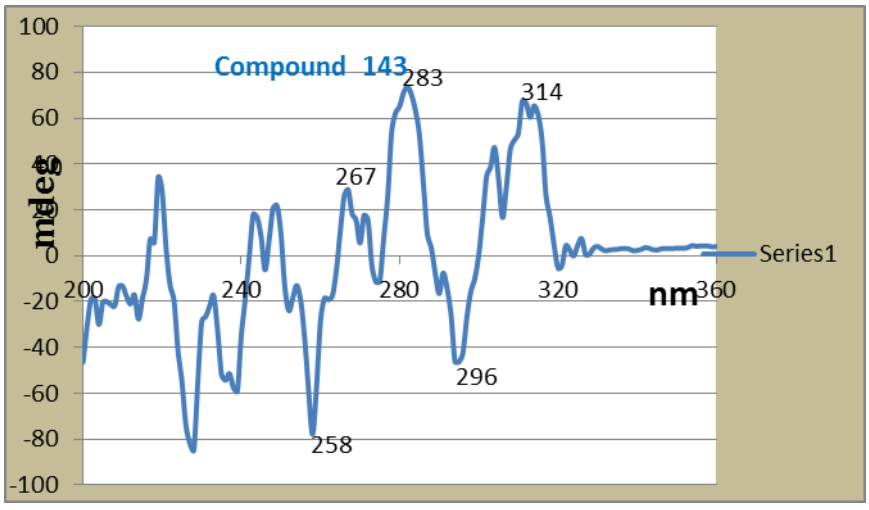
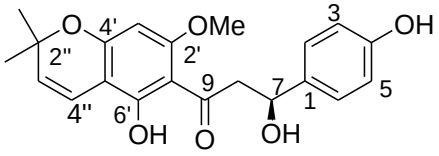


Figure 4.4: CD spectrum of compound **143**

4.1.4.1.2. Xanthohumol C (172)

Compound **172** was isolated as a yellow oily substance. The molecular formula $C_{21}H_{20}O_5$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 353.3) together with the ^1H and ^{13}C NMR data (Table 4.12). The UV spectrum (λ_{max} 230, 270 and 300 nm) along with NMR data (Table 4.12) indicated a chalcone derivative. Compound **172** has similar skeleton with that of obovatachalcone (compound **147**). The difference is in the presence of an additional hydroxy group in compound **172** on C-4 (δ_C 157.4). In agreement with this, the ^1H NMR spectrum showed an AA'XX' spin system for ring-A protons, vis H-2/6 and H-3/5. The nature of this ring was confirmed by the HMBC correlation of H-2/6 (δ_H 7.52) with C-3/5 (115.8), C-4 (δ_C 157.4) and C-7(δ_C 142.1), and H-3/5 with C-2/6 (δ_C 130.2) and C-1 (δ_C 128.5). Comparison of the above spectroscopic data with literature confirmed the identity of this compound as xanthohumol C. This compound was previously reported from *Humulus lupulus. L* (Cannabaceae) (Dresel *et al.*, 2015; Nookandeh *et al.*, 2004), however this is the first report from the genus *Tephrosia.*



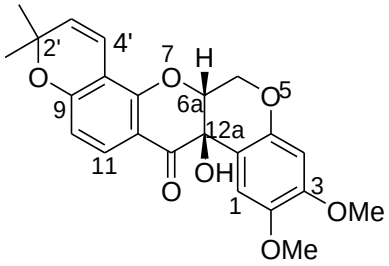
172

Table 4.13. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **143** and **172**, $CDCl_3$

Position	**143**			**172**		
	δ_C	δ_H *m* (*J* in Hz)	HMBC	δ_C	δ_H (ppm), *m*, (*J* in Hz)	HMBC
1	142.2			128.5		
2/6	124.9	7.35 *m*	C-1, C-4, C-3/5, C-7(β)	130.2	7.52 *d* (8.5)	C-3/5, C4, C-7(β)
3/5	127.4	7.30 *m*	C-1, C-2/6, C-4	115.8	6.86 *d* (8.5)	C-1, C-2/6
4	126.4	7.22 *m*	C-2/6, C-3/5	157.4		
7(β)	70.1	5.21 *dd* (3.0, 9.0)	C-2/6	142.1	7.76 *s*	C-1', C-2', C-4', C-7(β), C-8(α)
8(α)	52.6	3.38 *dd* (3.0,18.0)	C-1, C-7(β), C-9	125.3	7.76 *s*	C-1', C-7(β)
		3.28 *dd* (9.0,18.0)	C-1, C-7(β), C-9			
9(C=O)	204.0			192.6		
1'	105.5			106.0		
2'	161.8			162.5		
3'	102.7			103.0		
4'	160.5			160.1		
5'	91.2	5.80 *s*	C-1', C-3', C-4', C-6', C-9	91.5	5.93 *s*	C-1',C-3', C-4', C-6'
6'	162.9			162.5		
2''	77.1			78.20		
3''	125.4	5.39 *d* (10.1)	C-3'	125.3	5.46 *d*, (9.9)	C-2", C-3', 2"-Me_2
4''	115.7	6.58 *d* (10.1)	C-2", C-4'	116.1	6.69 *d* (9.9)	C-2', C-2",C-3', C-4'
2''-Me_2	28.4	1.37 *s*	C-2", C-3"	28.3	1.46 *s*	C-2", C-3"
6'-OMe	55.6	3.72 *s*	C-6'	55.8	3.92 *s*	C-6'
2'-OH		13.90 *s*	C-1', C-2', C-3'		14.61 *s*	C-1', C-2', C-3'

4.1.4.1.3. Tephrosin (173)

Compound **173** was isolated as an amorphous solid. The molecular formula $C_{23}H_{22}O_7$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 411.4) together with the ^1H and ^{13}C NMR data (Table 4.13). The UV spectrum (λ_{max} 240, 270 and 290 nm) along with the ^1H NMR spectrum which exhibited the presence of a doublet of doublets signal at δ_H 4.57 (*J* = 2.5, 1.1 Hz) assigned to H-6a, and HMBC correlation with C-6 (δ_C 63.8), C-12a (δ_C 67.4) and C-1a (δ_C 108.6) indicated that compound **173** is a 12a-hydroxyrotenoid derivative.



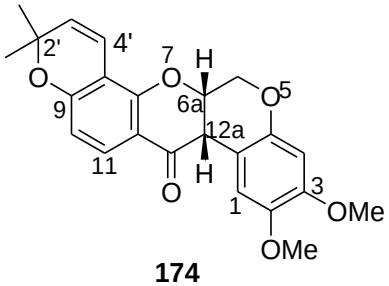
173

The ^1H and ^{13}C (Table 4.13) NMR spectra further showed the presence of a 2,2-dimethylpyran and two methoxyl substituents on the 12a-hydroxyrotenoid skeleton. The ^1H NMR displayed *ortho* coupled (*d, J*= 8.7 Hz) aromatic protons at δ_H 6.47 and 7.73, assigned to H-10 and H-11 respectively. The HMBC correlations of H-10 (with C-8 and C-9) and H-11 (with C-9) support the placement of the 2,2-dimethylpyran group at C-8/C-9, with the oxygen at C-9. Furthermore the placement of the two methoxyl groups on C-2 and C-3 was confirmed by the HMBC correlation (Table 4.13) of the two *para*-oriented aromatic protons of H-1 (δ_H 6.56, *s*) and H-4 (δ_H 6.48, *s*). As in other reported rotenoids, the ^1H NMR established the *cis*-B/C ring junction. The absolute configuration of this compound (6a*R*,12a*R*) was established from the ECD spectrum (Figure 4.6) which showed a negative Cotton effect at 338 nm, and the negative specific rotation ($[\alpha]_D$ -9.6^0) (Yenesew, 1997). Thus, based on the above spectroscopic data, the compound was identified as tephrosin. This compound has been reported from several plant species of the

family leguminosae (Ahmad *et al.*, 1999; Deyou *et al.*, 2015) however, this is the first report from *T. elata*.

4.1.4.1.4. Deguelin (174)

Compound **174** was isolated as an amorphous solid. The molecular formula $C_{23}H_{22}O_6$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 395.2) together with the ^1H and ^{13}C NMR data (Table 4.13). The UV spectrum (λ_{max} 240, 270 and 290 nm) along with the NMR data (Table 4.13) suggested that compound **174** is a rotenoid derivative with a 2,2-dimethylchromene and two methoxyl substituents.



174

As in compound **173**, the ^1H NMR spectrum of **174** displayed two *para*-oriented aromatic protons for H-1 (6.79, *s*) and H-4 (δ_H 6.45, *s*) in ring A; and two *ortho* coupling aromatic protons for H-10 (δ_H 6.48 *d, J* = 8.7 Hz) and H-11 (δ_H 7.75 *d, J* = 8.7 Hz) in ring D. Furthermore, the placement of 2,2-dimethylchromene at C-8/C-9 and the methoxyl groups at C-2 and C-3 was confirmed from the HMBC data (Table 4.13). The *cis* configuration at the B/C ring junction was established from the similarity of the chemical shift value of H-1 (δ_H 6.79 *s*) to previously reported rotenoids. The absolute configuration (6a*S*,12a*S*) was established from the ECD spectrum (Yenesew, 1997) (Figure 4.5). Therefore, this compound was identified as deguelin (**174**). This compound was earlier reported from the seedpods of *T. elata* (Muiva et al., 2009).

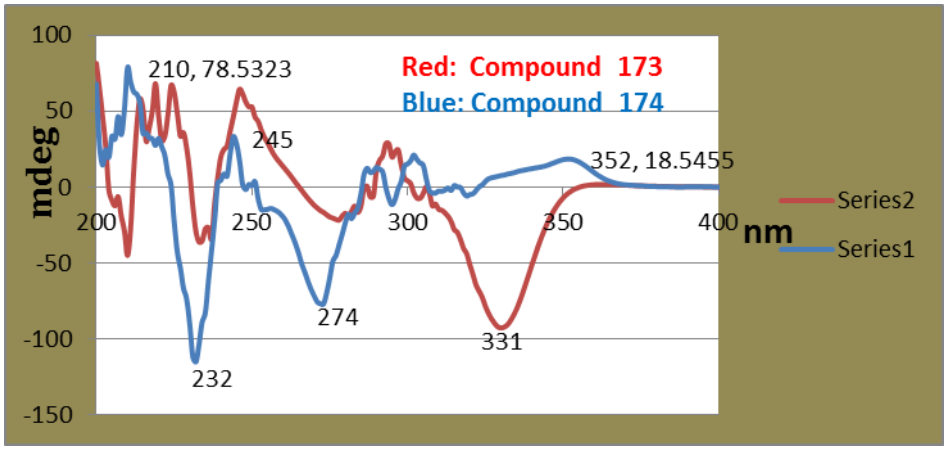


Figure 4.5: CD spectra of compound **173** and **174**

Table 4.14. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **173** and **174**, $CDCl_3$

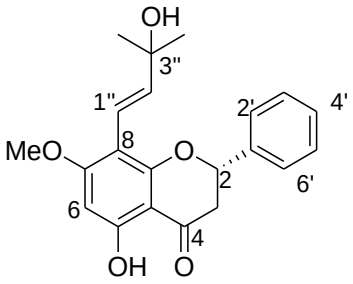
Position	**173**			**174**		
	δ_C	δ_H , *m*, (*J* in Hz)	HMBC	δ_C	δ_H, *m*, (*J* in Hz)	HMBC
1	109.3	6.56 *s*	C-2, C-3, C-1a, C-4a, C-12a	110.3	6.79 *s*	C-2, C-3, C-1a, C-4a, C-12a
1a	108.6			104.7		
2	143.9			143.8		
3	151.0			149.4		
4	101.0	6.48 *s*	C-2, C-3, C-1a, C-4a, C-12a	100.9	6.45 *s*	C-2, C-3, C-1a, C-4a, C-12a
4a	148.3			147.3		
6	63.8	4.63 *dd* (12.1, 2.5) 4.50 *dd* (12.1, 1.1)	C-12a, C-6a, C-4a C-6a, C-7a	66.2	4.64 *dd* (12.0, 3.0) 4.19 *d* (12.0)	C-12a, C-6a, C-4a, C-12 C-12a, C-6a, C-7a
6a	76.2	4.57 *dd* (2.5, 1.1)	C-6, C-12a, C-1a	72.4	4.92 *m*	C-1a
7a	156.6			156.9		
8	109.1			109.1		
9	160.7			160.0		
10	111.8	6.47 *d* (8.7)	C-8, C-9, C-11a	111.4	6.48 *d* (8.7)	C-8, C-9, C-11a
11	128.5	7.73 *d* (8.7)	C-7a, C-9, C-12	128.5	7.75 *d* (8.7)	C-7a, C-9, C-12
11a	111.0			112.7		
C=O	191.3			189.2		
12a	67.4			44.3	3.84 *d* (4.2)	C-1, C-12, C-1a, C-4a
2'	78.0			77.6		
3'	115.4	6.60 *d* (10.2)	C-8, C-5', C-4'	115.7	6.65 *d* (10.2)	C-8, C-6', C-6', Me_2
4'	128.8	5.56 *d* (10.2)	C-8, C-2',C-5', C-6'	128.6	5.56 *d* (10.2)	C-8, C-9, C-7a, C-6'
5'	28.3	1.39 *s*	C-2', C-6', C-4'	28.1	1.39 *s*	
6'	28.5	1.45 *s*	C-2', C-6', C-4'	28.4	1.45 *s*	
2-OMe	56.4	3.73 *s*	C-2	56.2	3.77 *s*	
3-OMe	55.8	3.82 *s*	C-3	55.8	3.81 *s*	

4.1.4.2. Compounds from the leaves of T. elata

The air dried and ground leaves of *T. elata* was extracted with dichloromethane/methanol (1:1) by cold percolation. Chromatographic separation of the extract led to the isolation of tephrolecarpin A (**175**), obovatin methyl ether (**73**), maackiain (**176**), deguelin (**174**), isopongaflavone (**59**), coumaric acid (**177**), salicylic acid (**178**), quercetin (**179**), kaempferol (**180**), apigenin (**181**)

4.1.4.2.1. Tephrolecarpin A (175)

Compound **175** was isolated as yellow crystals. The molecular formula $C_{21}H_{22}O_5$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 355.1) together with the ^1H and ^{13}C NMR data (Table 4.14). The UV (λ_{max} 230 and 270 nm), ^1H NMR (δ_H 5.29 for H-2 (*dd* , *J*=12, 3 Hz) and δ_H 3.35 (*dd* , *J*=17, 12 Hz), 3.44 (*dd* , *J*=17, 3 Hz) for H-3) and ^{13}C NMR (δ_C 70.2 for C-2, 52.7 for C-3 and 204.2 for C-4) spectral data suggested that the compound is a flavanone derivative.



175

The NMR spectrum showed the presence of unsubstituted ring-B (δ_H = 7.30 for H-4', δ_H = 7.37 for H-3'/H-5', δ_H = 7.42 for H-2'/'H-6'; δ_C = 143.3 for C-1', 125.9 for C-2'/6', 128.5 for C-3'/5' and 127.4 for C-4'), a methoxy (δ_H 3.79, δ_C 55.7), an OH which form a hydrogen bond (δ_H 13.79) and a 2-methylbut-3-en-2-ol substituent. This compound has similar structural skeleton with that

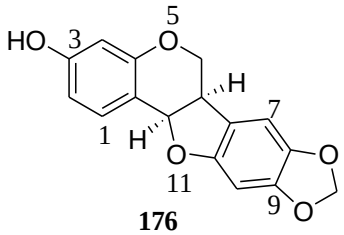
of compound **77** except for the nature of the C_5 group at C-8. In compound **175** this group is substituted with OH at C-3" (δ_C 78.4) and a double bond between C-1" (δ_C 115.8) and C-2" (δ_C 125.5). The HMBC correlations of H-1" (δ_H 6.66, *d, J* = 10.0 Hz) and H-2" (δ_H 5.82, *d, J* = 10.0 Hz) with C-3" (δ_C 78.4) supported the placement of the OH at position C-3". Therefore this compound (**175**) was characterized as (*S*)-tephrolecarpin A, previously reported from *Tephrosia leiocarpa* (Go´mez-Garibay *et al.*, 1991) but this being the first report from *T.elata*

Table 4.15. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **175**, $CDCl_3$

Position	δ_C	δ_H, *m, (J* in Hz)	HMBC
2	70.2	5.29 *dd* (12, 3)	C-3, C-4, C-1', C-2', C-6'
3	52.7	3.44 *dd* (17, 12) 3.35 *dd* (17, 3)	C-2, C-4, C-1'
4	204.2		
4a	105.6		
5	160.7		
5-OH		13.97 *s*	C-4a, C-5, C-6
6	91.3	5.87 *s*	C-4a, C-5, C-7, C-8
7	162.9		
8	105.3		
8a	161.9		
1'	143.3		
2',6'	125.9	7.42 *m*	C-2, C-4', C- 2', C-6'
3',5'	128.5	7.37 *m*	C-1', C-3', C-5'
4'	127.4	7.30 *m*	C-2', C-6'
1"	115.8	6.66, *d* (10.0)	C-7, C-8a, C-2", C-3"
2"	125.5	5.82, *d* (10.0)	C-8, C-3", C-4", C-5"
3"	78.4		
4",5"	28.4	1.44	C-2", C-3", C-5", C-4"
7-OMe	55.7	3.79	C-7

4.1.4.2.2. Maackiain (176)

Compound **176** was isolated as an amorphous solid, and was assigned the molecular formula $C_{16}H_{12}O_5$ based on LC-ESI-MS data ($[M+H]^+$ at *m/z* 385.2) together with the ^1H and ^{13}C NMR data (Table 4.15). It showed characteristic UV (λ_{max} 230, 380 and 310 nm), ^1H NMR (δ_H 3.64 *d*, *J*=11.0, 11.0 Hz and 4.22 *d*, *J*=11.0, 5.1 Hz for CH_2-6; δ_H 3.48 *ddd*, *J*=11.0, 6.8, 5.0 Hz for H-6a; δ_H 6.47 *d*, *J*=11.0 Hz for H-11a) and ^{13}C NMR (δ_C 66.4 for C-6, 40.1 for C-6a, 78.4 for C-11a) features for a pterocarpan skeleton. The ^1H NMR spectrum of compound **176** further pointed out the presence of three protons in an AXY spin system on ring-A assigned to H-1 (δ_H 7.37), H-2 (δ_H 6.55) and H-4 (δ_H 6.42).The NMR spectra also revealed the presence of a methylenedioxy group (δ_H 5.92 and 5.90; δ_C 101.3) on ring-D; connected to C-8 (δ_C 141.7) and C-9 (δ_C 148.1), which was established by the HMBC correlations of OCH_2O protons (δ_H 5.90 and δ_H 5.92) with C-8 (δ_C 141.7) and C-9 (δ_C 148.1). Furthermore two *para*-oriented aromatic protons of ring-D were assigned to H-7 (δ_H 6.72) and H-10 (δ_H 6.43). The HMBC correlations of H-7 (δ_H 6.72) with C-6a (δ_C 40.14), C-8 (δ_C 141.7), C-9 (δ_C 148.1) and C-10a (δ_C 154.21); and H-10 (δ_H 6.43) with C-8 (δ_C 141.7), C-9 (δ_C 148.1), C-6b (δ_C 40.14), C-10a (δ_C 154.21) confirmed the identity of ring-D.



176

The absolute configuration of the B/C ring junction (6a*R*, 11a*R*)was established from the ECD spectrum (Figure 4.6), showing a positive Cotton effect at 309 nm and a negative one at 241 nm

which is consistent with pervious data (Marco *et al.*, 2017). Therefore, this compound was identified as 6a*R*, 11a*R*- maackiain, previously reported from different plant species of the family Leguminosae (Chang *et al.*, 1997), however this is the first report from *T. elata*.

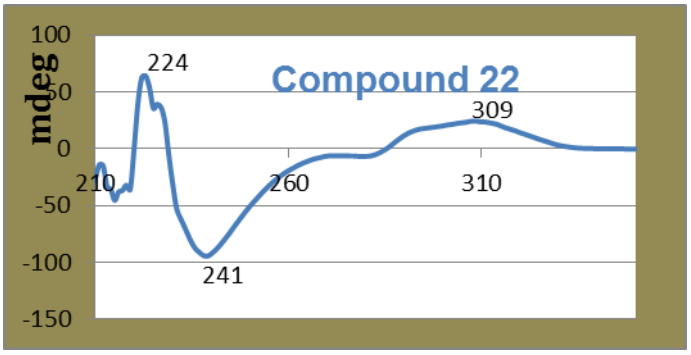


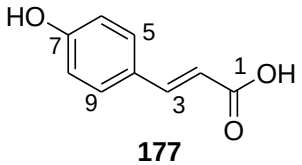
Figure 4.6: ECD spectrum of compound **176**

Table 4.16. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **176**, $CDCl_3$

Position	δ_C	δ_H, *m*, (*J* in Hz)	HMBC
1	132.1	7.36 *d* (8.4)	C-3, C-11a, C-4a
2	109.7	6.55 *dd* (8.4, 2.5)	C-11b, C-4
3	157.0		
4	103.6	6.42 *d* (2.5)	C-2, C-3, C-11b, C-4a
4a	156.6		
6	66.4	3.64 *dd* (11.0, 11.0)	C-4a, C-6b, C-11a
		4.22 *dd* (11.0, 5.1)	C-4a, C-6a, C-6b, C-11a
6a	40.1	3.48 *ddd* (11.0, 6.8, 5.0)	C-4a, C-6, C-6b, C-10a
6b	117.9		
7	104.7	6.72 *s*	C-6a, C-8, C-9, C-10a
8	141.7		
9	148.1		
10	93.8	6.43 *s*	C-8, C-9, C-6b, C-10a
10a	154.2		
11a	78.4	6.47 *d* (6.8)	
11b	112.6		
8,9-OCH_2O	101.3	5.92 *d* (1.4) 5.90 *d* (1.4)	C-8, C-9

4.1.4.2.3. Coumaric acid (177)

Compound **177** was isolated as a white amorphous solid, and the molecular formula $C_9H_8O_3$ was assigned based on LC-ESI-MS data ($[M+H]^+$ at *m/z* 165.3) together with the ^1H and ^{13}C NMR data (Table 4.16). It showed UV absorption (λ_{max} 230 and 300 nm), which along with its ^{13}C NMR data (C-1 (δ_C 169.6) and C-7 (δ_C 159.7)) suggested a coumaric acid derivative.



177

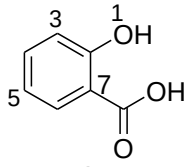
The ^{13}C NMR revealed the presence of a carboxylic carbon (C-1 (δ_C 169.6)) and an oxygenated quaternary aromatic carbon (C-7 (δ_C 159.7)). The ^1H NMR spectrum also showed an AA'XX' aromatic protons assigned to H-5/9 (δ_H 7.46 *d, J* = 8.6 Hz) and H-6/8 (δ_H 6.82 *d, J* = 8.6 Hz) as established from the HMBC correlation of H-5/9 (δ_H 7.46) with C-3 (δ_C 145.2), C-5/9 (δ_C 129.6), C-7 (δ_C 159.7), C-6/8 (δ_C 115.4) and H-6/8 (δ_H 6.82) correlation with C-4 (δ_C 125.8), C-7 (δ_C 159.7), C-6/8 (δ_C 115.4). In addition the ^1H NMR showed two *trans*-oriented olefinc protons assigned to H-2 (δ_H 6.30 *d, J* = 15.9 Hz) and H-3 (δ_H 7.62 *d, J* = 15.9 Hz). Therefore, comparison of the above spectroscopic data with published literature led to the identification of compound **177** as (*E)-p*-coumaric acid (Dresel *et al.*, 2015). This compound is a precursor to different classes of natural products including flavonoids. Although it is expected to occur widely in this genus, surprisingly this is the first report in *Tephrosia* species.

Table 4.17. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **177**, $CDCl_3$

Position	δ_C	δ_H , *m*, (*J* in Hz)	HMBC
1	169.6		
2	114.2	6.30 *d* (15.9)	C-1, C-3, C-4
3	145.2	7.62 *d* (15.9)	C-1, C-2, C-4, C-5/9
4	125.8		
5/9	129.6	7.46 *d* (8.6)	C-3, C-5/9, C-7, C-6/8
6/8	115.4	6.82 *d* (8.6)	C-4, C-7, C-6/8
7	159.7		

4.1.4.2.4. Salicylic acid (178)

Compound **178** was isolated as a white amorphous solid, and the molecular formula $C_7H_6O_3$ was assigned based on LC-ESI-MS data ($[M+H]^+$ at *m/z* 139.3) together with the ^{1}H and ^{13}C NMR data (Table 4.17). Based on comparison of the ^{1}H NMR (δ_H 7.01, *dd*, *J* = 3, 8 Hz, H-3; 7.01, *dd*, *J* = 3, 8 Hz, H-4, 7.01, *dd*, *J* = 3, 8 Hz H-5, 7.01, *dd*, *J* = 3, 8 Hz H-6) and ^{13}C NMR (C-2 δ_C 161.4, C-3 δ_C 117.5 C-4 δ_C 136.2 δ_C C-8, δ_C 172.5) data with published data led the identification of compound **24** as salicylic acid. The identity of this compound was confirmed by HMBC correlations (Table 4.17). The compound has been reported from several plants (Pierpoint, 1994), but this is the first report from *Tephrosia* species.



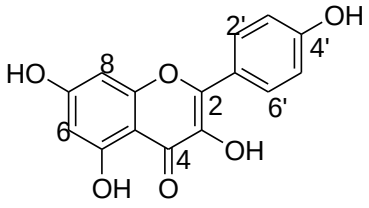
178

Table 4.18. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **178**, DMSO-d_6

Position	δ_C	δ_H , *m*, (*J* in Hz)	HMBC
2	161.4		
3	117.5	7.01 *d* (15.8)	C-2, C-4, C-5, C-7
4	136.2	7.56	C-2, C-3, C-5, C-6
5	119.5	6.98 *d* (8.5)	C-3, C-4, C-6, C-7
6	130.5	7.86 *d* (8.5)	C-2, C-4, C-5, C-7, C-8
7	113.5		
8	172.5		

4.1.4.2.5. Kaempferol (179)

Compound **179** was isolated as a yellow amorphous solid. The molecular formula $C_{15}H_{10}O_6$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 287.2) together with the ^{1}H and ^{13}C NMR data (Table 4.18). The UV spectrum (λ_{max} 210, 250 and 270 nm) along with ^{13}C NMR (δ_C 145.7 for C-2, 135.7 for C-3 and 175.7 for C-4) and absence of a signal for H-3 in the ^{1}H NMR spectral data suggested a flavonol derivative.



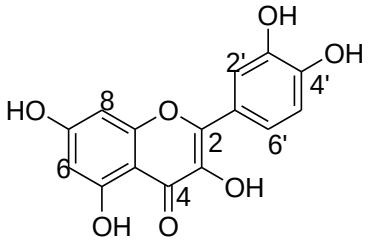
179

The ^{1}H NMR displayed an AA'XX' spin system at δ_H 8.11 (*d, J* = 8.9 Hz) for H-2'/6' and at δ_H 6.93 (*d, J* = 8.9 Hz) for H-3'/5' of ring-B. The signal at δ_H 6.93 showed an HMBC correlation with C-1' (δ_C 122.3) and the signal at δ_H 8.11 with C-2 (δ_C 145.7) and C-4' (159.2). This is in support of the placement of the hydroxyl group at C-4'. Furthermore, the ^{1}H NMR spectrum

exhibited a *meta*-coupling protons at δ_H 6.20 (*d, J* = 2.0 Hz, for H-6) and δ_H 6.42 (*d, J* = 2.0 Hz, for H-8) or consistent with C-5 and C-7 oxygenated ring-A. In the HMBC spectrum, H-6 showed correlation with C-4a (103.14), C-5 (161.9), C-7(164.2), C-8 (93.1) and H-8 with C-4a (103.14), C-7(164.2), C-8 (93.1) and C-8a (156.8) allowing the assignment of the carbon atoms in this ring. Based on the above spectroscopic data and comparison with literature, compound **179** was identified as Kaempferol. It is widely reported from several plants but this is the first report form *T. elata.*

4.1.4.2.6. Quercetin (180)

Compound **180** was isolated as a yellow amorphous solid. The molecular formula $C_{15}H_{10}O_7$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 303.5) together with the ^1H and ^{13}C NMR data (Table 4.18). The UV spectrum (λ_{max} 210, 260 and 360 nm) along with ^{13}C NMR (δ_C 145.6 for C-2, 135.7 for C-3 and 175.7 for C-4) and absence of the signal for H-3 in the ^1H NMR spectral data suggested a flavonol derivative.



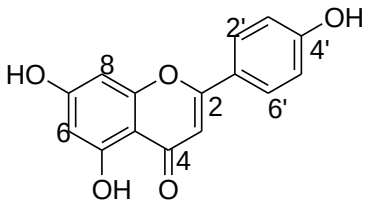
180

The NMR spectra of compound **180** is similar that of compound **179**. The only difference observed is that the AA'XX' spin system in compound **179** is replaced by an AXY spin system which indicated a 3',4'-dihydroxylated ring-B in compound **180.** The placement of the second hydroxy group at position C-3' (δ_C 144.8) was confirmed by the HMBC correlation of H-5' (δ_H

6.90 *d*, *J*= 8.4 Hz) with C-1' (δ_C 122.7), C-3' (δ_C 144.8) and C-4' (δ_C 147.4), and also the HMBC correlation of H-2' (δ_H 7.75 *d*, *J*=2.16 Hz) with C-2 (δ_C 145.6), C-4' (δ_C 147.4) and C-6' (δ_C 120.3). Therefore, compound **180** was characterized as quercetin, This is the first report from *T. elata* but previously reported from *T. purpurea* (Touqeer *et al*., 2013).

4.1.4.2.7. Apigenin (181)

Compound **181** was isolated as a yellow amorphous solid. The molecular formula $C_{15}H_{10}O_5$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 271.6) together with the ^1H and ^{13}C NMR data (Table 4.18). The UV spectrum (λ_{max} 230 and 270 nm) along with the ^1H NMR (δ_H 6.62 for H-3) and ^{13}C NMR (δ_C 164.9 for C-2, 102.4 for C-3 and 175.9 for C-4) spectral data suggested that compound **181** is a flavone derivative.



181

Similar to compound **179**, the ^1H NMR spectrum of compound **181** exhibited an AA'XX'-spin system for H-2'/6' and H-3'/5', and *meta* coupling protons for H-6 and H-8. The only singlet proton signal at δ_H 6.62 in the ^1H NMR spectrum was for H-3. In agreement with this assignment, H-3 showed HMBC correlation with C-2 (δ_C 164.9), C-4a (δ_C 103.9) and C-1' (δ_C 121.9). Thus analysis of the above spectroscopic data and in comparison with published literature led to the identification of compound **181** as apigenin. This is the first report of apigenin in the genus *Tephrosia* but it has been reported from a number of plant species (Seigler, 1998).

Table 4.19. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **179**, **180** and **181**, CD_3OD

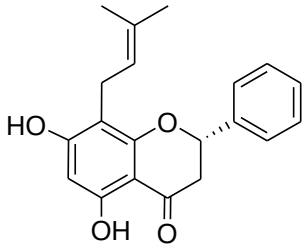
Position	**179**			**180**			**181**		
	δ_C	δ_H , *m, (J* in Hz)	HMBC	δ_C	δ_H, *m, (J* in Hz)	HMBC	δ_C	δ_H, *m, (J* in Hz)	HMBC
2	145.7			145.6			164.9		
3	135.7			135.8			102.4	6.62 *s*	C-2, C-4a, C-1'
4	176.0			175.9			175.9		
4a	103.1			103.1			103.9		
5	161.9			161.1			161.8		
6	97.9	6.20 *d* (2.1)	C-4a, C-5, C-7, C-8	97.8	6.20 *d* (2.1)	C-4a, C-5, C-7, C-8	98.7	6.24 *d* (2.1)	C-4a, C-5, C-7, C-8
7	164.2			164.2			164.6		
8	93.1	6.42 *d* (2.1)	C-4a, C-7, C-8, C-8a	93.0	6.41 *d* (2.1)	C-4a, C-7, C-8, C-8a	93.6	6.48 *d* (2.1)	C-4a, C-7, C-8, C-8a
8a	156.8			156.8			158.0		
1'	122.3			122.7			121.8		
2'	129.3	8.11 *d* (8.9)	C-2, C-4', C- 2', C-6'	114.6	7.75 *d* (2.2)	C-2, C-4', C- 2', C-6'	128.1	7.88 *d* (8.7)	C-2, C-4', C- 2', C-6'
3'	114.9	6.93 *d* (8.9)	C-1', C-3', C-5'	144.8			115.6	6.96 *d* (8.7)	C-1', C-3', C-5'
4'	159.2			147.4			161.4		
5'	114.9	6.93 *d* (8.9)	C-1', C-3', C-5'	114.8	6.90 *d* (8.4)	C- 3', C-1', C-4'	115.6	6.96 *d* (8.7)	C-1', C-3', C-5'
6'	129.3	8.11 *d* (8.9)	C-2, C-4', C- 2', C-6'	120.3	7.65 *dd* (8.4, 2.2)	C- 2', C-3', C-4'	128.1	7.88 *d* (8.7)	C-2, C-4', C- 2', C-6'

4.1.5. Compounds from **Tephrosia rhodesica**

The roots of *Tephrosia rhodesica* was investigated in similar way as the other plants described in this thesis and yielded five new compounds (rhodimer (**183**), rhocarpin (**188**), rhodiflavan A (**191**), rhodiflavan B (**192**) and rhodiflavan C (**193**)) and sixteen known compounds (Tephrowatsin **B** (**100**), tephrinone (**77**), glabranin (**182**), quercetol **B** (**98**), maackiain (**176**), pisatin (**185**), 6a-hydroxymaackiain (**184**), tephrosin (**173**), rotenone (**186**), 6-hydroxyrotenone (**187**), 12-hydroxyrotenone (**129**), hildecarpin (**134**), 3-hydroxy-2-methoxy-8-9-methylenedioxypterocarpene (**189**), isoliquirtigenin (**190**), D-pinitol (**165**) and tephrowastin A (**79**))

4.1.5.1. Glabranin (182)

Compound **182** was isolated as white crystals. The molecular formula $C_{20}H_{20}O_4$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 325.6) together with the ^1H and ^{13}C NMR data (Table 4.20). The UV (λ_{max} = 230, 290 and 340 nm) along with ^1H (δ_H 5.42 for H-2, δ_H 3.03 and 2.85 for H-3) and ^{13}C (δ_C 78.9 for C-2, 43.5 for C-3 and 196.2 for C-4) NMR spectral data suggested that compound **182** is a flavanone derivative. In support of this, the oxymethine proton signal at δ_H 5.42 (*dd J*=3.1, 13.0 Hz) for H-2 which coupled with H-3 (δ_H 3.05 (*dd J*=13.0, 17.1 Hz) and 2.85 (*dd J*=3.1, 17.1Hz) showed an HMBC correlation with C-4 (δ_C 196.2), C-8a (δ_C 159.6), C-1' (δ_C 138.7) and C-2'/6' (δ_C 125.9).



182

The NMR spectra further revealed the presence of unsubstituted ring-B (δ_C = 138.7 for C-1'; δ_C 125.9, δ_H 7.46 for C-2'/6'; δ_C 128.8, δ_H 7.45 for C-3'/5'; δ_C 128.6, δ_H 7.39 for H-4'), two hydroxyl and a prenyl group (Table 4.20).

In ring-A, the ^{1}H NMR spectrum exhibited a singlet aromatic proton at δ_H 6.03 which showed HMBC correlation with C-5 (δ_C 162.2), C-7 (δ_C 163.7), C-8 (δ_C 109.0) and C-4a (δ_C 103.2) allowing the assignment of this signal to H-6. The two hydroxyl groups were then placed at C-5 (δ_H 12.0 *s*) and C-7 and the prenyl at C-8 of ring-A. The substitution pattern in this ring was confirmed from the HMBC spectrum (Table 4.20). The absolute configuration at C-2 was determined as (*S*) from the ECD spectrum (Figure 4.7) which showed a positive Cotton effect at 330 nm and a negative one at 286 nm, consistent with previously reported flavanones (Slade *et al.*, 2005; Sun *et al.*, 2017). Therefore, based on the above spectroscopic data this compound was identified as glabranin, a compound previously reported from *Helichrysum hypocephalum* (Asteraceae) (Bohlmann and Abraham, 1979). However; this is the first report from the genus *Tephrosia*.

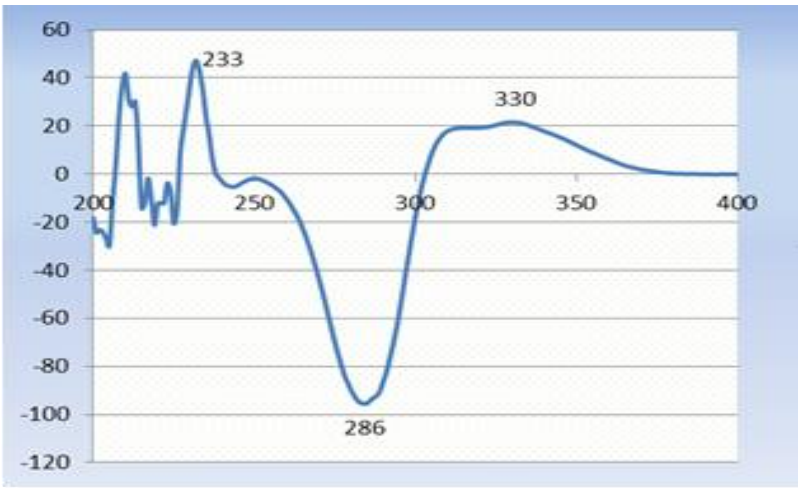
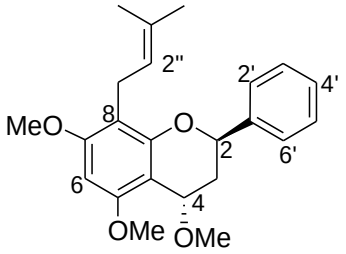


Figure 4.7: CD spectrum of compound **182**

4.1.5.2. Quercetol B (98)

Compound **98** was isolated as an oily paste. The molecular formula $C_{23}H_{28}O_4$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 369.7) together with the ^1H and ^{13}C NMR data (Table 4.20). The UV (λ_{max} = 240, 270 and 300 nm) along with ^1H (δ_H 5.24 for H-2, δ_H 1.8 and 2.32 for H-3, and H-4 δ_H 4.56) and ^{13}C (δ_C 72.9 for C-2, 34.9 for C-3 and 67.6 for C-4) NMR spectral data suggested that compound **98** is a flavan derivative. The NMR data (Table 4.20) of this compound showed the presence of three methoxy groups, unsubstituted ring-B, and a prenyl group. The placement of the two methoxy groups at C-5 (δ_C 157.3) and C-7 (δ_C 158.4) was established based on HMBC correlations (Table 4.20) and biogenetic considerations. The third methoxy group was placed at C-4 (δ_C 67.6) on the basis of HMBC correlation of H-4 (δ_H 4.56) with 4-OMe and 4-OMe with C-4. In addition, H-4 (δ_H 4.56) showed HMBC correlations with C-2 (δ_C 72.9), C-4a (δ_C 103.9), C-5 (δ_C 157.3) and C-8a (δ_C 153.8). Furthermore, H-2 (δ_H 3.03) showed HMBC correlation with C-4 (δ_C 67.6), C-3(δ_C 34.9), C-1'(δ_C 142.0) and C-2'/6'(δ_C 126.1) which confirmed the above suggestion.

The absolute configuration (2*R*, 4*S*) of this compound was determined from the ECD spectrum (Figure 4.8) and is consistent with previously reported flavan (Pouget *et al.*, 2000; Slade *et al.*, 2005). Therefore, comparing the above data with published literature led the identification of compound **98** as (2*R*, 4*S*)-quercetol B, which was previously reported from *T. quercetorum* (Gómez-Garibay *et al.*, 1988) but this is the first report from *T. rhodesica*.

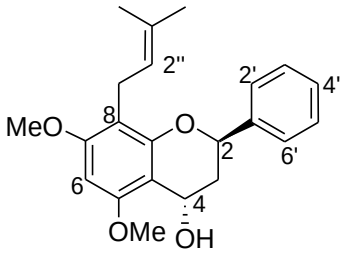


98

4.1.5.3. Tephrowatsin A (79)

Compound **79** was isolated as an oily paste. The molecular formula $C_{22}H_{26}O_4$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 355.4) together with the ^1H and ^{13}C NMR data (Table 4.20). The UV (λ_{max} = 230 and 270 nm) along with ^1H (δ_H 5.16 for H-2, δ_H 1.96 and 2.28 for H-3, and H-4 δ_H 5.01) and ^{13}C (δ_C 73.2 for C-2, 37.9 for C-3 and 59.7 for C-4) NMR spectral data suggested that compound **79** has a flavan skeleton. The NMR data (Table 4.20) of compound **79** displayed close similarities with compound **98**. As in compound **98**, ring A of compound **79** is substituted with a prenyl group at C-8 and with methoxyl groups at C-5 and C-7, and ring-B is unsubstituted (Table 4.20). In support of this the ^1H NMR spectrum showed two methoxy groups at δ_H 3.39 (5-OMe) and 3.89 (7-OMe). The only difference is that, the substituent on ring-C at C-4 is a hydroxy group in compound **79**, instead of a methoxy group in as in compound **98.** The absolute configuration (2*R*, 4*S*) of this compound was also determined from the ECD data (Figure 4.8) which was consistent with previously reported

flavan (Pouget *et al.*, 2000; Slade *et al.*, 2005). Thus, this compound was identified as (2*R*, 4*S*)-tephrowatsin A, which was previously reported from *T. watsoniana* (Gómez *et al.*, 1985b).



79

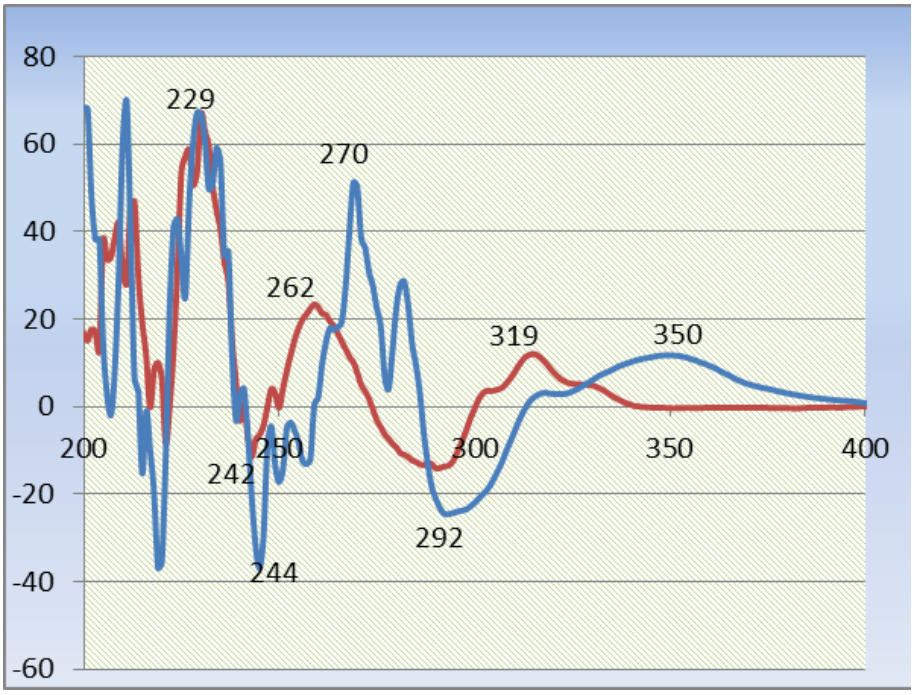


Figure 4.8: CD spectra of compound **79** (Red) and **98** (Blue)

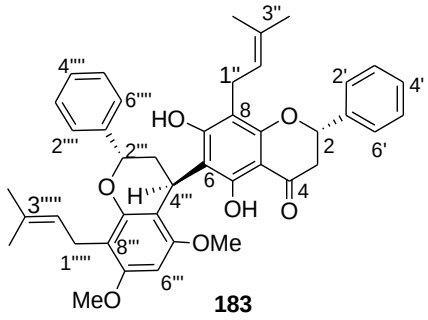
Table 4.20. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **182**, **98** and **79** ($CDCl_3$)

Position	**182**			**98**			**79**		
	δ_C	δ_H, *m*, (*J* in Hz)	HMBC	δ_C	δ_H, *m*, (*J* in Hz)	HMBC	δ_C	δ_H , *m*, (*J* in Hz)	HMBC
2	78.9	5.42 *dd* (3.1, 13.0)	C-4,C-1',C-2'/6', C-8a	72.9	5.24 *dd* (2.1, 12.4)	C-4,C-1',C-2'/6', C-3	73.2	5.16 *dd* (1.9, 12.3)	C-4,C-1',C-2'/6'
3	43.5	3.05 *dd* (13.0, 17.1) 2.85 *dd*(3.1, 17.1)	C-2, C-4, C-1' C-4, C-4a, C-1'	34.9	1.8 *ddd* (3.0, 12.5, 14.1) 2.31*ddd* (2.3, 2.3, 14.2)	C-2 C-4, C-4a	37.9	1.96 *ddd* (3.9, 12.5, 14.3) 2.28 *ddd* (1.9, 1.9, 14.3)	C-2 C-4, C-4a
4	196.2			67.6	4.56 *dd* (2.7, 2.7)	C-2,C-4a ,C-5,C-8a, 4-OMe	59.7	5.01 *ddd* (1.8, 1.8, 3.8)	C-2,C-4a ,C-5,C-8a
4a	103.2			103.9			106.2		
5	162.2			157.3			157.0		
5-OH		12.0 *s*	C-5, C-6, C-4a						
6	96.9	6.03 *s*	C-4a, C-5, C-7, C-8	87.9	6.13 *s*	C-4a, C-7, C-8	87.8	6.15 *s*	C-4a, C-5, C-7, C-8
7	163.7			158.4			158.3		
8	109.0			110.1			110.5		
8a	159.6			153.8			153.6		
1'	138.7			142.0			141.6		
2'/6'	125.9	7.46	C-2, C-2'/6', C-3'/5'	126.1	7.47 *m*	C-2, C-2'/6', C-3'/5'	126.0	7.49 *m*	C-2, C-2'/6', C-3'/5'
3'/5'	128.8	7.45	C-1', C-3'/5'	128.3	7.38 *m*	C-3'/5', C-1'	128.3	7.39 *m*	C-3'/5', C-1'
4'	128.6	7.39	C-2'/6'	127.5	7.31 *m*	C-2'/6'	127.6	7.32 *m*	C-2'/6'
1"	21.8	3.33 *bt*	C-7, C-8, C-8a, C-3", C-2"	21.8	3.28 *m*	C-8, C-8a, C-7, C-3", C-2"	21.9	3.30 *m*	C-8, C-8a, C-7, C-3", C-2"
2"	121.6	5.22 *btt*	C-1", 3"-Me_2	123.5	5.19 *ddt* (1.51, 5.67, 8.71)	C-1", 4", 5"	123.2	5.21 *ddt* (2.9, 5.9, 7.4)	C-1", 4", 5"

3"	134.9			130.4			130.8		
4"	17.8	1.73	C-3", C-2"	25.8	1.61 *d* (1.57)	C-3", C-2", 5"	25.9	1.64 *s*	C-3", C-2", 5"
5"	25.8	1.73		17.7	1.63 *d* (1.89)	C-3", C-2", 4"	17.8	1.64 *s*	C-3", C-2", 4"
4-OMe				56.2	3.48 *s*	C-4			
5-OMe				55.9	3.83 *s*	C-5	55.5	3.89 *s*	C-5
7-OMe				55.7	3.86 *s*	C-7	55.9	3.85 *s*	C-7

4.1.5.4. Rhodimmer (183)

Compound **183** was isolated as white crystals. The molecular formula $C_{42}H_{44}O_7$ was established from HRMS, exhibiting a molecular ion peak at *m/z* 660.3095, together with the ^1H and ^{13}C NMR data (Table 4.21). The UV (λ_{max} 230, 290 and 350 nm), ^1H NMR (δ_H 5.43 *dd* *J*=2.9, 13.1 Hz for H-2; δ_H 5.06 *dd* *J*=2.0, 11.5 Hz for H-2'''; δ_H 2.84 *dd* *J*=2.9, 17.1 Hz and 3.07 *dd* *J*=13.1, 17.1Hz for H-3; δ_H 2.18 *dd* *J*=2.1, 6.0, 10.8 Hz and 2.31 *dd* *J*=2.0, 2.1, 14.0 Hz for H-3'''; and δ_H 4.66 *dd* *J*=1.9, 5.8 Hz for H-4''') and ^{13}C NMR (δ_C 78.6 for C-2, 75.4 for C-2''', 43.7 for C-3, 36.9 for C-3''', 196.3 for C-4 and 26.8 for C-4''') spectral data suggested that compound **183** is a flavanone-flavan dimer.



183

The NMR data (Table 4.21) of compound **183** showed the presence of two sets of unsubstituted aromatic rings (δ_C = 139.1 for C-1'; δ_C 125.9 (δ_H 7.47) for C-2'/6'; δ_C 128.7, (δ_H 7.43) for C-3'/5'; δ_C 128.5, (δ_H 7.39) for C-4'; δ_C = 141.4 for C-1''''; δ_C 126.0 (δ_H 7.38) for C-2''''/6''''; δ_C 128.3 (δ_H 7.34) for C-3''''/5''''; δ_C 127.6 (δ_H 7.29) for C-4''''), two sets of prenyl groups (δ_C = 21.8 (δ_H 3.18 *bt*) for C-1''; δ_C 122.5 (δ_H 5.51 *bt*) for C-2''; δ_C 131.6 for C-3''; δ_C 17.8 (δ_H 1.57 *s*) for C-4''; δ_C 25.8 (δ_H 1.65 *s*) for C-5''; δ_C 21.9 (δ_H 3.35 *bt*) for C-1''''; δ_C 123.2 (δ_H 5.24 *bt*) for C-2''''; δ_C 130.8 for C-3''''; δ_C 17.8 (δ_H 1.66 *s*) for C-4''''; δ_C 25.9 (δ_H 1.68 *s*) for C-5'''') , two hydroxyl (δ_H

12.61 *s* for 5-OH; δ_H 6.80 *s* for 7-OH) and two methoxy groups (δ_C 55.9 (δ_H 3.73 *s*) for 5'''-OMe; δ_C 55.9 (δ_H 3.73 *s*) for 7'''-OMe) which is consistent with the biflavanone units. The substitutions pattern of these units were deduced based on the HMBC correlation and biogenetic consideration. The linkage of the two units at C-6 and C-4''' was established from the HMBC spectrum. In agreement with this H-4''' (δ_H 4.66 (*dd J*=1.9, 5.8 Hz) showed HMBC correlation with C-2'''(δ_C 75.4), C-3'''(δ_C 36.9), C-4'''a(δ_C 102.8), C-5'''(δ_C 157.5), C-8'''a(δ_C 154.3), C-5(δ_C 159.0), C-6(δ_C 109.8) and C-7(δ_C 162.8) clearly showed the linkage of the two units. In addition to this, H-3'''(δ_H 2.18 *dt* (2.1, 6.0, 10.8 Hz); 2.31*dt* (2.0, 2.1, 14.0 Hz)) showed HMBC correlation with C-1''''(δ_C 141.4), C-4'''(δ_C 26.6), C-4'''a (δ_C 102.8) and C-6(δ_C 109.8). Furthermore the connectivity of the two units was supported by the NOE interaction of H-2''' with 7-OH in the NOESY spectrum.

The absolute configuration (2'''*S*, 4'''*R*) was established from the X-ray structure (Figure 4.9) of compound **183.** In support of this the ECD (Figure 4.10) spectrum showed positive cotton effect at 314 nm and negative effect at 292 nm (Pouget *et al.*, 2000; Slade *et al.*, 2005; Sun *et al.*, 2017). In addition, it exhibited a positive specific rotation $[\alpha]_D^{20}$ +13.40^o (c 0.001, MeOH). Thus, based on the above spectroscpic data this new compound was characterized as (2'''*S*,4'''*R*)- 6-(3''',4'''-dihydro-5''',7'''-dimethoxy-8'''-(3''''-methylbut-2''''-enyl)-2'''-phenyl-2'''H-chromen-4'''-yl)-5,7-dihydroxy-8-(3-methylbut-2-enyl)-2-phenylchroman-4-one and given a trival name rhodimmer.

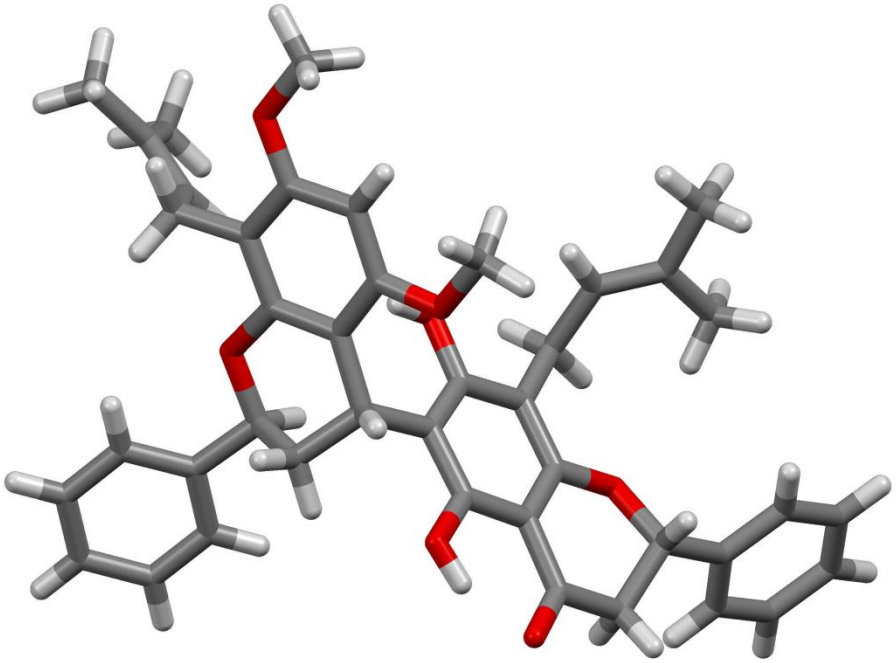


Figure 4.9: X-ray single crystal struture of compound **183**

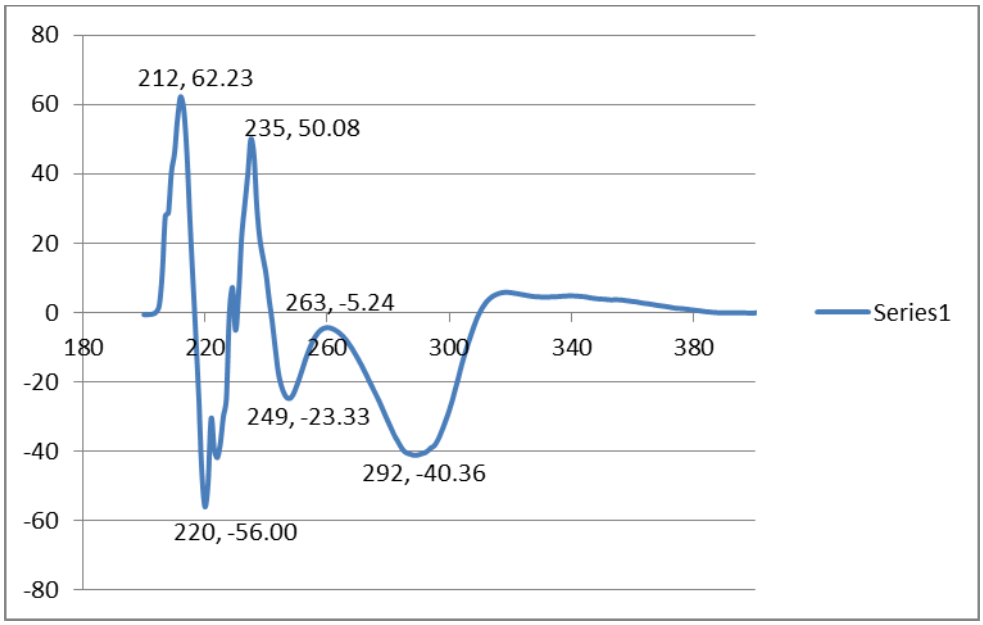


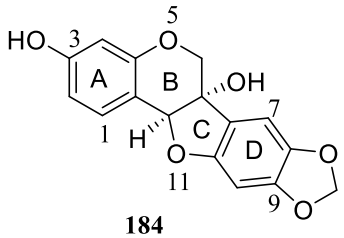
Figure 4.10: CD spectrum of compound **183**

Table 4.21. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **29**, $CDCl_3$

Position	δ_C	δ_H , *m*, (*J* in Hz)	HMBC	Position	δ_C	δ_H , *m*, (*J* in Hz)	HMBC	NOESY
2	78.6	5.43 *dd* (2.9, 13.1)	C-4,C-1',C-2'/6', C-8a	2'''	75.4	5.06 *dd* (2.0, 11.5)	C-4''',C-1'''',C-2''''/6'''', C-8a	C-3, C-3
3	43.7	2.84 *dd* (2.9, 17.1) 3.07 *dd* (13.1, 17.1)	C-2, C-4, C-1' C-4, C-4a, C-1'	3'''	36.9	2.18 *dt* (2.1, 6.0, 10.8) 2.31*dt* (2.0, 2.1, 14.0)	C-1'''', C-4''', C-6 C-4''', C-4'''a, C-6	
4	196.3			4'''	26.8	4.66 *dd* (1.9, 5.8)	C-2''', C-3''', C-4'''a, C-5''', C-8'''a, C-5 C-6, C-7	C-3
4a	100.4			4'''a	102.8			
5	159.3			5'''	157.5			
6	109.8			6'''	88.4	6.17 *s*	C-4''', C-4'''a, C-5''', C-7''',C-8''', C-1'''' w	5'''-OMe 7-'''OMe
7	162.8			7'''	158.6			
8	108.1			8'''	110.5			
8a	157.6			8'''a	154.3			
1'	139.1			1''''	141.4			
2'/6'	125.9	7.47	C-2, C-2'/6', C-3'/5'	2''''/6''''	126.0	7.38	C-2''', C-2''''/6'''', C-3''''/5''''	C-2 C-2'''
3'/5'	128.7	7.43	C-1', C-3'/5'	3''''/5''''	128.3	7.34	C-3''''/5'''', C-1''''	
4'	128.5	7.39	C-2'/6'	4''''	127.6	7.29	C-2''''/6''''	
1''	21.85	3.18 *bt*	C-7, C-8, C-8a, C-3'', C-2''	1'''''	21.9	3.35 *bt*	C-8''', C-8'''a, C-7''', C-3''''', C-2'''''	
2''	122.5	5.15 *bt*	C-1'', C-4'', C-5''	2'''''	123.2	5.24 *bt*	C-1''''', C-4''''', C-5'''''	C-1'''''
3''	131.6			3'''''	130.8			
4''	17.8	1.57 *s*	C-3'', C-2'', C-5''	4'''''	17.8	1.66 *s*	C-2''''', C-3''''', C-5'''''	
5''	25.8	1.65 *s*	C-3'', C-2'', C-4''	5'''''	25.9	1.68 *s*	C-2''''', C-3''''', C-4'''''	
5-OH		12.61 *s*	C-5, C-6, C-4a, C-4,	5'''-OMe	55.9	3.73 *s*	C-5	
7-OH		6.80 *s*	C-6, C-8, C-8a, C-7, C-4a	7'''-OMe	55.8	3.87 *s*	C-7'''	C-2

4.1.5.5. 6a-Hydroxymaackiain (184)

Compound **184** was isolated as an amorphous solid and was assigned the molecular formula $C_{16}H_{12}O_6$ based on LC-ESI-MS data ($[M+H]^+$ at *m/z* 301.2) together with the ^1H and ^{13}C NMR data (Table 4.22). It showed characteristic UV (λ_{max} 230, 380, 310 and 340 nm), ^1H NMR (δ_H 4.0 *d J*=11.6 Hz and 4.18 *d J*=11.6 Hz for CH_2-6; δ_H 5.27 for H-11a) and ^{13}C NMR (δ_C 69.5 for C-6, 77.2 for C-6a, 84.7 for C-11a) features for a 6a-hydroxypterocarpan skeleton.



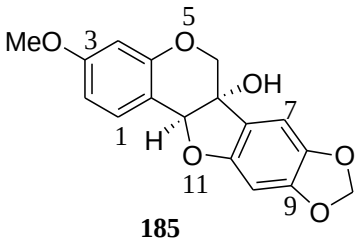
184

Compound **184** has similar spectroscopic features with that of maackiain (**176**) (section 4.1.4.2.2). Similar to that of maackiain (**176**), the ^1H NMR spectrum (Table 4.22) of compound **184** showed the presence of an AXY spin system in ring-A [assigned to H-1 (δ_H 7.35 *d J*=8.4 Hz), H-2 (δ_H 6.57 *dd J*=8.4, 2.5 Hz)) and H-3 (δ_H 6.41 *d J*=2.5 Hz)] and two *para*-oriented aromatic protons in ring-D [assigned to H-7 (δ_H 6.80 *s*) and H-10 (δ_H 6.40)]. The only difference observed was the presence of a hydroxyl group at C-6a (δ_C 77.2) in compound **184**. The HMBC correlations of H-11a (δ_H 5.27) and CH_2-6 (δ_H 4.0 *d J*=11.6 Hz and 4.18 *d J*=11.6 Hz) with C-6a (δ_C 77.2) confirmed the above suggestion. The absolute configuration at the B/C-ring junction was determined from the ECD spectrum (Figure 4.11) which showed a negative Cotton effect at ca. 240 nm is consistence with 6a*R*, 11a*R* absolute configuration (Goel *et al.*, 2013; Marco *et al.*, 2017). Therefore, based on the above spectroscopic data and comparison with literature data, compound **184** was identified as (6a*R*, 11a*R*)-6a-hydroxymaackiain. It has been previously

isolated from different plants such as *Trifolium pretense* (Leguminosae) (Bilton *et al.*, 1976) and *Derris laxiflora* (Leguminosae) (Chien *et al.*, 2016). However, this is the first report from the genus *Tephrosia*.

4.1.5.6. Pisatin (185)

Compound **185** was isolated as an amorphous solid, and was assigned the molecular formula $C_{17}H_{14}O_6$ based on LC-ESI-MS data ($[M+H]^+$ at *m/z* 315.7) together with the ^1H and ^{13}C NMR data (Table 4.22). It showed characteristic UV (λ_{max} 230, 380 and 310 nm), ^1H NMR (δ_H 4.0 *d* *J*=11.6 Hz and 4.17 *d* *J*=11.6 Hz for CH_2-6; δ_H 5.27 *s* for H-11a) and ^{13}C NMR (δ_C 69.6 for C-6, 77.0 for C-6a, 84.7 for C-11a) features for a 6a-hydroxypterocarpan skeleton.



185

The NMR data (Table 4.22) of compound **185** are similar to those of compound **184**. The only difference observed was that the hydroxy group at C-3 in compound **184** was replaced by a methoxy group in compound **185**. The HMBC cross peak of H-1 (δ_H 8.55 *d* *J*=8.5 Hz) and H-4 (δ_H 6.44 *d* *J*=2.5 Hz) with C-3 (δ_C 161.0) is in support of the above suggestion. As in compound **184**, the absolute configuration of the B/C-ring junction of this compound was determined from the ECD spectrum and is consistent with 6a*R*, 11a*R* absolute configuration (Figure 4.11). Thus, based on the spectroscopic data and comparison with literature, compound **185** was identified as (6a*R*, 11a*R*)-pisatin. This compound has been previously reported from several plants including

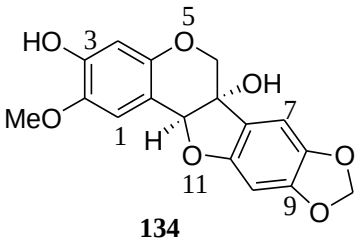
Tephrosia bidwilli (Ingham and Markham, 1980) and *T. candida* (Hegazy *et al.*, 2011). However, this is the first report from *T. rhodesica.*

Table 4.22. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **184** and **185**, $CDCl_3$

Position	**184**			**185**		
	δ_C	δ_H, *m*, (*J* in Hz)		δ_C	δ_H, *m*, (*J* in Hz)	
1	132.2	7.35 *d* (8.4)	C-3, C-11a, C-4a, C-1’	131.8	8.55 *d* (8.5)	C-3, C-11a, C-4a, C-1’
2	110.4	6.57 *d* (8.4, 2.5)	C-11b, C-4	121.81	6.64 *d* (8.6, 2.5)	C-11b, C-4
3	157.1			161.0		
4	103.7	6.41 *d* (2.5)	C-2, C-3, C-11b, C--4a	101.6	6.44 *d* (2.5)	C-2, C-3, C-11b, C--4a
4a	155.7			155.8		
6	69.5	4.00 *d* (11.6)	C-4a, C-6b, C-11a	69.6	4.00 *d* (11.6)	C-4a, C-6b, C-11a
		4.18 *d* (11.6)	C-4a, C-6a, C-6b, C-11a		4.17 *d* (11.6)	C-4a, C-6a, C-6b, C-11a
6a	77.2			77.0		
6b	118.8			119.0		
7	103.0	6.80 *s*	C-6a, C-8, C-9, C-10a	103.0	6.7 *s*	C-6a, C-8, C-9, C-10a
8	142.3			142.3		
9	149.8			149.8		
10	94.2	6.40 *s*	C-8, C-9, C-6b, C-10a	94.1	6.38 *s*	C-8, C-9, C-6b, C-10a
10a	154.5			154.4		
11a	84.7	5.27 *s*	C-1, C-4a, C-6, C-6a, C-11b, C-10a	84.8	5.27 *s*	C-1, C-4a, C-6, C-6a, C-11b, C-10a
11b	112.6			112.1		
8,9-OCH_2O	101.5	5.91 *d* (1.4) 5.94 *d* (1.4)		101.5	5.89 *d* (1.6) 5.93 *d* (1.5)	
OMe (C-3)				55.3	3.77 *s*	C-3

4.1.5.7. Hildecarpin (134)

Compound **134** was isolated as an amorphous solid, and was assigned the molecular formula $C_{17}H_{14}O_6$ based on LC-ESI-MS data ($[M+H]^+$ at *m/z* 331.1) together with the ^1H and ^{13}C NMR data (Table 4. 23). It showed characteristic UV (λ_{max} 230, 300 nm), ^1H NMR (δ_H 4.03 *d J*=11.4 Hz and 4.06 *d J*=11.4 Hz for CH_2-6; δ_H 5.27 *s* for H-11a) and ^{13}C NMR (δ_C 69.6 for C-6, 77.0 for C-6a, 84.7 for C-11a) features for a 6a-hydroxypterocarpan skeleton.

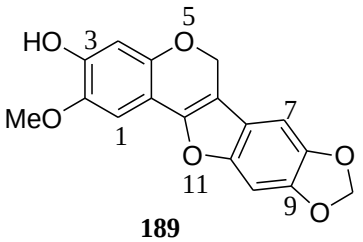


134

The ^1H NMR (Table 4.23) spectrum of compound **134** displayed two sets of *para*-oriented aromatic protons assigned to H-1 (δ_H 6.99 *s*), H-3 (δ_H 6.39 *s*), H-7 (δ_H 6.88 *s*) and H-10 (δ_H 6.38 *s*). Further, the NMR data (Table 4. 23) showed the presence of methoxy, hydroxy and methylenedioxy groups. The placement of the methoxy group at C-2 was established from the NOESY spectrum which showed a cross peak of H-1 (δ_H 6.99 *s*) with 3-OMe (δ_H 3.85 *s*). With the methylenedioxy group being in ring-D, the hydroxy group is placed at C-3. Based on this spectroscopic data compound **134** was identified as (6a*R*, 11a*R*)-hildecarpin. The absolute configuration of the B/C-ring junction of this compound was also determined from the ECD data as (6a*R*, 11a*R*) (Figure 4.11). This is the first report of compound **134** from *T. rhodesica*, but it has been earlier reported form the roots of *T. hildebrandtii* (Lwande *et al.*, 1987b).

4.1.5.8. 3-Hydroxy-2-methoxy-8-9-methylenedioxypterocarpene (189)

Compound **189** was isolated as an amorphous solid, and was assigned the molecular formula $C_{17}H_{14}O_6$ based on LC-ESI-MS data ($[M+H]^+$ at *m/z* 331.1) together with the ^1H and ^{13}C NMR data (Table 4. 23). It showed characteristic UV (λ_{max} 230, 300 nm), ^1H NMR (δ_H 5.46 *s* for CH_2-6) and ^{13}C NMR (δ_C 69.6 for C-6, 77.0 for C-6a, 147.9 for C-11a) for a pterocarpene skeleton.



189

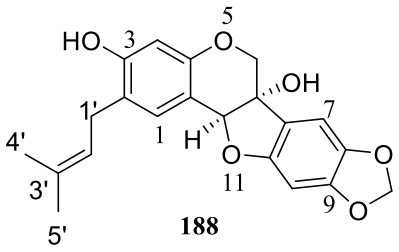
The NMR data (Table 4.23) of compound **189** revealed the presence of two pairs of *para*-oriented aromatic protons, methoxy, hydroxy and methylenedioxy groups as in compound **134.** Unlike that of **134** which contains a hydroxy group at C-6a, compound **189** has a double bond at ring-B/C junction, hence it is a pterocarpene. This was confirmed by the HMBC correlation of H-1 (δ_H 6.99) *s* and H-6 (δ_H 5.45) with C-11a (δ_C 147.8). The placement of the methoxy group at C-2 was established from the NOESY spectrum which showed a cross peak of H-1 (δ_H 6.99 *s*) with 3-OMe (δ_H 3.93 *s*). With the methylenedioxy group being in ring-D, the hydroxy group is placed at C-3. The complete substitution pattern was established based on the 1D and 2D HMBC NMR data. Thus, comparing the above data with published literature led to the identification of compound **189** as 3-hydroxy-2-methoxy-8-9-methylenedioxypterocarpene. This is the first report of compound **189** in nature, but it has been reported as a dehydration product of hildecarpin (Lwande *et al.*, 1986a).

Table 4.23. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **134** and **189**, $CDCl_3$

Position	**134**			**189**		
	δ_C	δ_H , *m*, (*J* in Hz)	HMBC	δ_C	δ_H , *m*, (*J* in Hz)	HMBC
1	113.7	6.99 *s*	C-3, C-11a, C-4a, C-1'	102.8	6.99 *s*	C-3, C-11a, C-4a, C-1'
2	143.4			141.6		
3	148.3			145.5		
4	103.6	6.39 *s*	C-2, C-3, C-11b, C-4a	103.9	6.57 *s*	C-2, C-3, C-11b, C-4a
4a	149.9			148.4		
6	69.9	4.03 *d* (11.4)	C-4a, C-6b, C-11a	65.1	5.46 *s*	C-4a, C-6a, C-11a
		4.06 *d* (11.4)	C-4a, C-6a, C-6b, C-11a			
6a	77.1			119.2		
6b	120.7			106.6		
7	103.9	6.88 *s*	C-6a, C-8, C-9, C-10a	93.9	7.01 *s*	C-6a, C-8, C-9, C-10a
8	142.6			144.7		
9	150.0			145.5		
10	93.8	6.38 *s*	C-8, C-9, C-6b, C-10a	97.3	6.74 *s*	C-8, C-9, C-6b, C-10a
10a	155.0			150.3		
11a	85.8	5.27 *s*	C-1, C-4a, C-6, C-6a, C-11b, C-10a	147.8		
11b	111.9			108.4		
8,9-OCH_2O	102.3	5.96 *d* (1.12) 5.92 *d* (1.14)	C-8, C-9	101.3	6.00 *s*	C-8, C-9
4-OMe	56.7	3.86 *s*		56.5	3.93 *s*	

4.1.5.9. Rhocarpin (188)

Compound **188** was isolated as an amorphous solid, and was assigned the molecular formula $C_{17}H_{10}O_6$ based on HRESIMS ($[M+H]^+$ *m/z* obs 369.1367, calcd 369.1338) and NMR (Table 4.24) analyses. It showed characteristic UV (λ_{max} 230, 190, 310 and 350 nm), ^{1}H NMR (δ_H 3.95 *d J*=11.5 Hz and 4.15 *d J*=11.5 Hz for CH_2-6; δ_H 5.25 *s* for H-11a) and ^{13}C NMR (δ_C 69.6 for C-6, 77.0 for C-6a, 84.7 for C-11a) for a 6a-hydroxypterocarpan skeleton.



188

The NMR data (Table 4.23) of compound **188** indicated the presence of a prenyl group (^{1}H NMR δ_H 3.32, *d, J*=7.2 Hz for H-1'; δ_H 5.31, *m*, for H-2'; δ_H 1.78, *s*, for H-4' and H-5'; ^{13}C NMR δ_C 29.2 for C-1'; δ_C 29.2 for C-2'; δ_C 135.8 for C-3'; δ_C 17.9 for C-4'; δ_C 25.8 for C-5'), hydroxyl and methylenedioxy group (δ_H 5.91 *d J*=1.4 Hz and 5.94 *d J* 1.4 Hz; δ_C 101.5). The prenyl group was placed at C-2 as revealed by the HMBC correlation of H-1 (δ_H 7.20 *s*) with C-1' (δ_C 29.2) and H-1' (δ_H 3.32 *d J*=7.2 Hz) with C-1(δ_C 131.8) and C-3 (δ_C 155.8). In agreement with the above discussion the placement of the prenyl group at C-2, H-1 showed NOE cross peak with CH_2-1' in the NOESY spectrum. With the hydroxyl group being at C-3 (from biogenetic consideration) the presence of two singlets at δ_H 7.20 *s* for H-1 (δ_C 131.8 for C-1) and δ_H 6.40 *s* (δ_C 104.0 for C-4), are consistent with C-2 (prenyl) and C-3 (hydroxyl) substituted ring A. In ring D, two para oriented proton signals at δ_H 6.80 *s* (δ_C 103.0 for C-7) and δ_H 6.81 *s* (δ_C 94.2 for C-10) would place the methylenedioxy group at C-8/C-9. The complete assignment of this compound was

established based on 1D (^{1}H and ^{13}C) and 2D (HHCOSY, NOESY, HSQC, and HMBC) NMR data. The absolute configuration at the B/C- ring junction was determined as 6a*R*, 11a*R* based on the ECD spectrum (Figure 4.11) which exhibited a positive Cotton effect at 309 nm and a negative one at 241 nm (Goel *et al.*, 2013). Based on the above spectroscopic data this new compound (**188)** was characterized as (6a*R*, 11a*R*)-3-hydroxy-2-prenyl-8-9-methylenedioxypterocarpan and was given a trivial name rhocarpin.

Table 4.24. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **188**, $CDCl_3$

Position	**188**		
	δ_C	δ_H, *m*, (*J* in Hz)	HMBC correlations
1	131.8	7.20 *s*	C-3, C-11a, C-4a, C-1'
2	121.8		
3	155.8		
4	104.0	6.40 *s*	C-2, C-3, C-11b, C--4a
4a	154.1		
6	69.65	3.95 *d* (11.5)	C-4a, C-6b, C-11a
		4.15 *d* (11.5)	C-4a, C-6a, C-6b, C-11a,
6a	77.2		
6b	119.0		
7	103.0	6.80 *s*	C-6a, C-8, C-9, C-10a
8	142.4		
9	149.7		
10	94.2	6.81 *s*	C-8, C-9, C-6b, C-10a
10a	149.7		
11a	84.8	5.25 *s*	C-1, C-4a, C-6, C-6a, C-11b, C-10a
11b	112.1		
8,9-OCH_2O	101.5	5.91 *d* (1.4) 5.94 *d* (1.4)	C-8, C-9
1'	29.2	3.32, *d*, (7.2)	C-1,C-3, C-2',C-3'
2'	121.8	5.31 *m*	C-4',C-5', C-1'
3'	135.8		
4'	17.9	1.78 *s*	C-2',C-3',C-5'
5'	25.8	1.78 *s*	C-2',C-3',C-4'

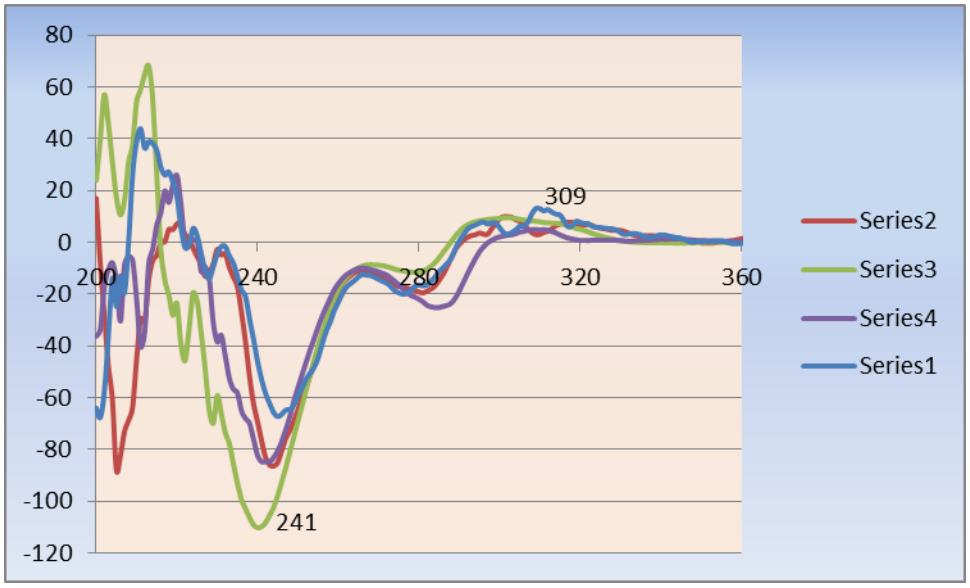
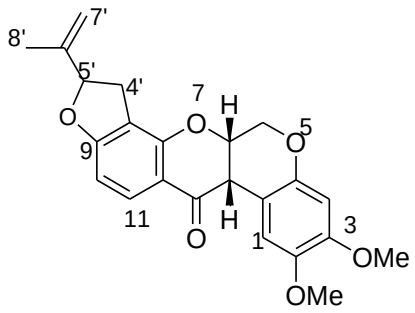


Figure 4.11: ECD spectra of compound **184**(Blue), **185**(Purple), **134** (Green) and **189** (Red)

4.1.5.10. Rotenone (186)

Compound **186** was isolated as colourless amorphous solid. The molecular formula $C_{23}H_{22}O_7$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 395.3) together with the ^1H and ^{13}C NMR data (Table 4.25). The UV spectrum (λ_{max} 240 and 300 nm) along with ^1H NMR (δ_H 4.61, *dd*, *J*=3.1, 12.2 Hz, and 4.18, *dd*, *J*= 1.1, 12. 2 Hz for CH_2-6; δ_H 4.93, m, for H-6a; and δ_H 3.84, *d*, *J*=4.1Hz for H-12a) ^{13}C NMR (δ_C 66.3 for C-6, δ_C 72.2 for C-6a, δ_C 44.6 for C-12a and δ_C 188.9 for C-12) suggested that compound **186** is a rotenoid derivative.



186

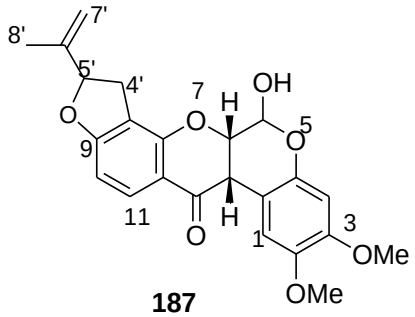
The ^1H and ^{13}C NMR data (Table 4.25) of compound **186** displayed similar pattern to those of deguelin (Section 4.1.4), with the only difference being that the 2,2-dimethylchromene ring in deguelin is replaced by 2-(prop-1-en-2-yl)tetrahydrofuran group in compound **186** [δ_H 2.96 (*dd*, 15.7, 8.1, for H-4a', 3.32, (1H, *dd*, *J* = 15.6, 9.8 for H-4b', 5.24, *t J* = 9.0, 9.0 for H-5', 1.77 (3H, *s*, Me-8'), 4.93 and 5.09 (2H, *bs*, for the terminal methylene); [δ_C 31.2, (C-4') 87.8, (C-5') 143.0, (C-6'),17.1 (C-7') and 112.5 (C-8'). The placement of the 2-(prop-1-en-2-yl) tetrahydrofuran group at C-8/C-9 was confirmed from the HMBC correlations of H-10 (δ_H 6.51 *d J*= 8.5 Hz) and H-11 (δ_H 7.84 *d J*= 8.5 Hz) with C-8 (δ_C 167.3) and C-9 (δ_C 104.8). In support of this H-11 showed HMBC correlations with C-7a (δ_C 157.9) and C-12 (δ_C 188.9). Further, the complete chemical assignment of compound **186** was carried out using 1D (^1H and

^{13}C) and 2D (HH-COSY, HSQC, and HMBC) NMR (Table 4.25). The resonance for H-1 at δ_H 6.77 ppm is consistent with *cis* configuration of the B/C-ring junction (Yenesew, 1997). The absolute configuration at the B-C ring junction (6a*S*, 12a*S*) was determined from the ECD spectrum (Figure 4.12) that showed a positive Cotton effect at 354 nm and negative one at 318 nm similar, to previous reports on rotenoids of this family (Slade *et al.*, 2005). Therefore, the compound was identified as the rotenoid rotenone (**186**), previously reported from several *Tephrosia* species (Ganapaty *et al.*, 2008a; Muiva-Mutisya *et al.*, 2014; Venkataratnam *et al.*, 1987). However, this is the first report from *T. rhodesica.*

4.1.5.11. 6-Hydroxyrotenone (187)

Compound **187** was isolated as an amorphous solid. The molecular formula $C_{23}H_{22}O_7$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 411.4) together with the ^{1}H and ^{13}C NMR data (Table 4.25). The UV spectrum (λ_{max} 240, 270 and 290 nm) along with NMR data (Table 4.24) indicated that compound **187** is a rotenoid derivative. The spectroscopic data of compound **187** is similar to that of compound **186;** the major difference was that compound **187** is oxygenated at C-6 as shown from the ^{1}H (δ_H 5.78 *d J*=2.6) and ^{13}C (δ_C 90.0) NMR data and also from the HMBC correlation of H-6 with C-4a (δ_C 144.1), C-6a (δ_C 73.1) C-12 (δ_C 188.7) and C-12a (δ_C 40.5) (Table 4.25). The alpha orientation of H-6 proton was established from the coupling constant $J_{H\text{-}6,6a}$=2.6 Hz and the absolute configuration at the B/C-ring junction was established as (6a*S*, 12a*S*) from the ECD spectrum (Figure 4.12) consistent with previously reported rotenoid (Slade *et al.*, 2005; Yenesew, 1997). Therefore based on the spectroscopic data and comparison with literature compound **187** was identified as 6-

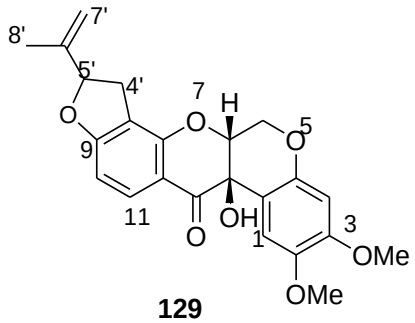
hydroxyrotenone, previously reported from *T. pentaphylla* (Dagne *et al*., 1989) but this is the first report form *T. rhodesica*.



187

4.1.5.12. 12a-Hydroxyrotenone (129)

Compound **129** was isolated as an amorphous solid. The molecular formula $C_{23}H_{22}O_7$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 411.4) together with the ^1H and ^{13}C NMR data (Table 4.25). The UV spectrum (λ_{max} 240, 270 and 290 nm) along with NMR data (Table 4.24) suggested that compound **129** is a 12a-hydroxyrotenoid derivative.



129

The NMR spectra features of compound **129** are similar to those of compound **186.** The major difference observed is that compound **129** has a hydroxy group at C-12a (δ_C 67.5). The placement of this hydroxy group at position C-12a was confirmed by the HMBC correlation of H-1 (δ_H 6.55 *s*), H-4 (δ_H 6.48 *s*) and H-6 (δ_H 4.60 *dd J*=2.4, 11.8 Hz; 4.49 *dd J*=1.0, 11.8) with

C-12a (δ_C 67.5). With H-1 resonating at δ_H 6.55 ppm in the ^{1}H NMR established the *cis* configuration at the B/C-ring junction. The absolute configuration (6a*S*,12a*S*) was determined from the ECD data (Figure 4.12) which showed a negative Cotton effect 334 nm and a positive one at 300 nm similar to earlier reported 12a-hydroxyrotenoids (Slade *et al.*, 2005). Therefore, based on this spectroscopic data compound **129** was characterized as 12a-hydroxyrotenone. It has been previously isolated from different *Tephrosia* species including *T. candida* (Parmar *et al.*, 1988), *T. uniflora* (Abreu and Luis, 1996) and *T. pentaphylla* (Dagne *et al.*, 1989). However, this is the first report of 12a-hydroxyrotenone form *T. rhodesica*.

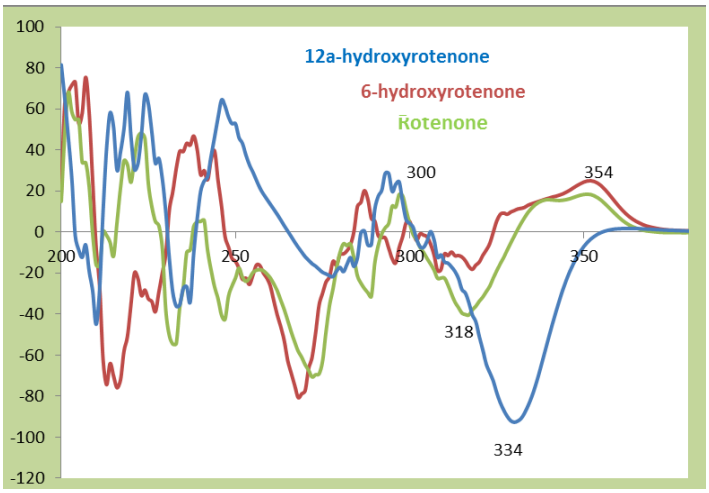


Figure 4.12: CD spectra of compound **186** (Red), **187**(Blue) and **129**(Green)

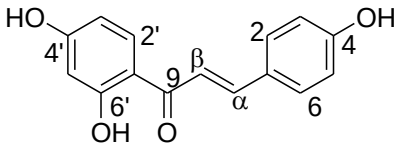
Table 4.25. ^{1}H (800 MHz), ^{13}C (200 MHz) and HMBC NMR data for compound **186**, **187**, and **129**, $CDCl_3$

Position	**186**			**187**			**129**		
	δ_C	δ_H ,*m*, (*J* in Hz)	HMBC	δ_C	δ_H ,*m*, (*J* in Hz)	HMBC	δ_C	δ_H , *m*, (*J* in Hz)	HMBC
1	110.3	6.77 *s*	C-2, C-3, C-1a, C-4a, C-12a	109.9	6.77 *s*	C-2, C-3, C-1a, C-4a, C-12a	109.2	6.55 *s*	C-2, C-3, C-1a, C-4a, C-12a
1a	104.8			105.0			108.7		
2	143.8			144.2			143.9		
3	149.4			149.5			151.1		
4	100.8	6.45 *s*	C-2, C-3, C-1a, C-4a, C-12a	101.4	6.47 *s*	C-2, C-3, C-1a, C-4a, C-12a	101.0	6.48 *s*	C-2, C-3, C-1a, C-4a, C-12a
4a	147.3			144.1			148.3		
6	66.2	4.61 *dd* (3.11, 12.1)	C-12a, C-6a, C-7a	90.0	5.78 *d* (2.6)	C-12a, C-6a, C-4a, C-12	63.8	4.60 *dd* (2.4, 12.1)	C-12a, C-6a, C-7a
		4.18 *dd* (1.1, 12.2)	C-12a, C-6a, C-4a, C-12					4.49 *dd* (1.0, 11.8)	C-12a, C-6a, C-4a, C-12
6a	72.2	4.93 *m*	C-1a, C-6a, C-12	73.1	4.82 *dd* (2.6, 3.8)	C-12a, C-6a, C-4a, C-12	76.0	4.58 *m*	C-1a, C-6a, C-12
7a	157.9			157.3			157.5		
8	112.9			112.8			113.2		
9	167.3			167.3			168.0		
10	104.8	6.51 *d* (8.5)	C-8, C-9	105.0	6.51 *d* (8.5)	C-8, C-9, C-11a	105.3	6.48 *d* (8.6)	C-8, C-9
11	129.9	7.84 *d* (8.5)	C-7a, C-8, C-9, C-12	130.0	7.85 *d* (8.5)	C-7a, C-9, C-12	130.0	7.83 *d* (8.5)	C-7a, C-8, C-9, C-12
11a	113.3			114.1			111.7		
C=O	188.9			188.7			191.0		

12a	44.6	3.84 *d* (4.1)	C-1, C-12, C-1a, C-4a	40.5	3.93 *dd* (1.1, 3.9)	C-1, C-12, C-1a, C-4a	67.5		
4'	31.2	2.96 *dd* (15.7, 8.1)	C-2', C-5', C-4', C-7a, C-8, C-9	31.2	2.93 *dd* (15.6, 8.1)	C-2', C-5', C-4', C-7a, C-8, C-9	31.1	2.94 *dd* (15.7, 8.1)	C-2', C-5', C-4', C-7a, C-8, C-9
		3.32 *dd* (15.6, 9.8)	C-2', C-5', C-4', C-7a, C-8, C-9		3.28 *dd* (15.7, 9.8)	C -2', C-5', C-4', C-7a, C-8, C-9		3.29 *dd* (15.7, 9.8)	C-2', C-5', C-4', C-7a, C-8, C-9
5'	87.8	5.24 *t* (9.0, 9.0)	C-3', C-4', C-5', C-6', C-9	87.8	5.22 *t* (9.00, 9.0)	C-6',C-3', C-4', C-5', C-8, C-9,	87.9	5.24 *t* (9.00, 9.00)	C-3', C-4', C-5', C-6', C-9
6'	143.0			142.9			142.8		
7'	112.5	4.93 *bs*	C-6',C-2'	112.6	4.93 *bs*	C-6',C-2'	112.7	4.94 *bs*	C-6',C-2'
		5.07 *bs*	C-6',C-2', C-4'		5.07 *bs*	C-6',C-2', C-4'		5.07 *bs*	C-6',C-2', C-4'
8'	17.1	1.77 *s*	C-2', C-4', C-5'	17.1	1.76 *s*	C-2', C-4', C-5'	17.0	1.76 *s*	C-2', C-4', C-5'
2-OMe	56.3	3.77 *s*	C-2	56.2	3.76 *s*	C-2	56.3	3.73 *s*	C-2
3-OMe	55.8	3.81 *s*	C-3	55.9	3.81 *s*	C-3	55.8	3.82 *s*	C-3

4.1.5.13. Isoliquirtigenin (190)

Compound **190** was isolated as a yellow oily substance. The molecular formula $C_{15}H_{12}O_4$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 257.5) together with the ^1H and ^{13}C NMR data (Table 4.26). The UV spectrum (λ_{max} 230, 300 and 360 nm) along with NMR data (Table 4.25) suggested that compound **190** is a chalcone derivative.



190

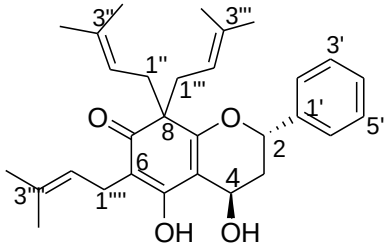
The ^1H NMR (Table 4.26) spectrum displayed *trans*-oriented olefinic protons for H-α (δ_H 7.81 *d* *J*=15.3 Hz) and H-β (δ_H 7.57 *d* *J*=15.3 Hz). The signal at δ_H 7.81 showed an HMBC correlation with C-1 (δ_C 127.2), C-2/6 (δ_C 131.2), C-8 (δ_C 118.1) and C-9 (δ_C 192.5) and the signal at δ_H 7.57 showed HMBC correlations with C-1 (δ_C 127.2), C-7 (δ_C 144.5) and C-9 (δ_C 192.5) which is in agreement with the above suggestion. Furthermore, the ^1H NMR spectrum showed AA'XX'-spin system at δ_H 7.65 (*d, J* = 8.6 Hz) for H-2/6 and at δ_H 6.88 (*d, J* = 8.6 Hz) for H-3/5; and AXY spin system at δ_H 7.98 (*d, J* = 8.8 Hz) for H-2', δ_H 6.43 (*dd, J* = 2.4, 8.8 Hz) for H-3' and δ_H 6.34 (*d, J* = 2.4 Hz) for H-5'. The placement of the hydroxyl groups at C-4' and C-6' was established from the HMBC correlations and biogenetic considerations. Thus, based on the spectroscopic evidence and by comparison with published data (Jang *et al.*, 2003), compound **190** was identified as isoliquiritigenin. It has been isolated from different plants species including the genus *Tephrosia* (*T. toxicaria*) (Jang *et al.*, 2003), however this is the first report from the roots of *T. rhodesica*.

Table 4.26. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **190**, $CDCl_3$

Position	δ_C	δ_H, *m*, (*J* in Hz)	HMBC
1	127.2		
2/6	131.2	7.65 *d* (8.6)	C-2/6, C-4, C-α
3/5	116.3	6.88 *d* (8.6)	C-1, C-3/5, C-4
6	160.0		
α	144.5	7.81 *d* (15.3)	C-1, C-2/6, C-β, C-9
β	118.1	7.57 *d* (15.3)	C-1, C-α, C-9
9	192.5		
1'	114.1		
2'	132.8	7.98 *d* (8.8)	C-9, C-1', C-4', C-6'
3'	108.1	6.43 *dd* (2.4, 8.8)	C-1', C-5'
4'	164.6		
5'	103.2	6.34 *d* (2.4)	C-3', C-1', C-4', C-6'
6'	166.7		
OH-6'		13.49 *s*	C-1', C-5',C- 6'

4.1.5.14. Rhodiflavan A (191)

Compound **191** was isolated as an oily paste. The molecular formula $C_{30}H_{38}O_4$ was established from HRMS, exhibiting a molecular ion peak at *m/z* 423.1465, together with the ^{1}H and ^{13}C NMR data (Table 4.27). The UV (λ_{max} = 240 and 290 nm) along with ^{1}H (δ_H 5.22, *dd*, *J*=12.6, 2.3 Hz) for H-2), δ_H 1.8 (*ddd, J*=14.6, 12.4, 4.2 Hz for H-3_{ax}) and 2.31 (*dt*, *J*=14.6, 1.9, 1.9 Hz, H-3_{eq}), and δ_H 4.56 (*m*, for H-4) and ^{13}C (δ_C 72.9 for C-2, 34.9 for C-3 and 67.6 for C-4) NMR spectral data suggested that compound **191** is a flavan derivative. The typical flavan ring-C hydroxyl group at C-4 (δ_C 67.6) was confirmed from HMBC correlation of H-4 (δ_H 4.99, *m*) with C-2 (76.1), C-3 (37.0), C-5 (194.7), C-4a (114.9) and C-8a (171.0).



191

The NMR data (Table 4.27) showed that ring-B is unsubstituted. In ring-A, there is presence of three sets of prenyl groups (Table 4.27), a ring which also contains a carbonyl (δ_C 206.4) and hydroxyl (δ_C 194.7). From biogenetic considerations, the carbonyl and the hydroxyl group should be located at C-5 and C-7, leaving C-6 and C-8 the sites where the three prenyl groups are located. That two of the prenyl groups are located on the same carbon atom(C-8) was established from the ^{3}J HMBC correlations of the olefinic protons of the prenyl groups. H-2'' (δ_H 5.03 *m*) and H-2''' (δ_H 4.66 *m*) with C-8. The third prenyl group was fixed at C-6 from the HMBC correlation of H-2'''' (δ_H 4.99 *m*) with C-6. In support of this H-1'' and H-1''' showed HMBC correlations with C-7 (δ_C 206.4) and C-8a (δ_C 171.0) also H-1'''' showed correlation

with C-5 (δ_C 194.7). This showed that ring-A is fully substituted. The absolute configuration (2*R*,4*S*) was determined from the ECD spectrum (Figure 4.13) which showed a negative Cotton effect at 300 nm, a positive one at 257 nm (Pouget *et al.*, 2000; Sun *et al.*, 2017). Therefore, based on the above spectroscopic data, this new compound was characterized as (2*S*,4*R*)-3,4-dihydro-4,5-dihydroxy-6,8,8-tris(3-methylbut-2-enyl)-2-phenyl-2H-chromen-7(8H)-one and given a trivial name rhodiflavan A.

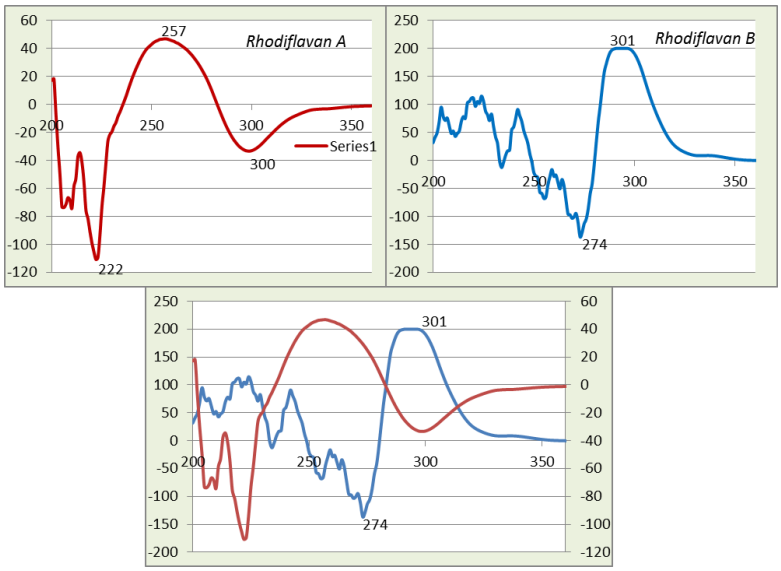
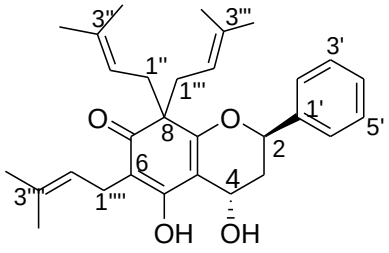


Figure 4.13: CD spectra of compound **191** (Red) and **192** (Blue)

4.1.5.15. Rhodiflavan B (192)

Compound **193** was isolated as an oily paste. The molecular formula $C_{30}H_{38}O_4$ was established from HRMS, exhibiting a molecular ion peak at *m/z* 423.1465, together with the ^{1}H and ^{13}C NMR data (Table 4.27). The UV (λ_{max} = 240 and 290 nm) along with ^{1}H (δ_H 5.24 (*dd*, *J*=12.6, 2.3 Hz) for H-2; δ_H 2.00 (*ddd, J*=14.6, 12.5, 3.6 Hz) and 2.30 (*ddd, J*=14.6,2.3,2.3 Hz) for H-3; and δ_H 4.55 (*dd J*=2.0,3.6 Hz) for H-4) and ^{13}C (δ_C 76.2 for C-2, 36.5 for C-3 and 59.5 for C-4) NMR spectral data suggested that compound **192** is a flavan derivative. The spectroscopic features of this compound are similar to those of compound **191** and the only difference was the configuration at C-2 and C-4 as shown from ECD spectrum (Figure 4.13) which is showed a positive Cotton effect at 301 nm and negative one at 257 nm for compound **193**, which is opposite from that of compound **191**. This led to the identification of the two compounds as enantiomer (Pouget *et al.*, 2000; Sun *et al.*, 2017). Thus, the absolute configuration of compound **192** was determined as 2*R*, 4*S*. Therefore this compound was characterized as (2*R*,4*S*)-3,4-dihydro-4,5-dihydroxy-6,8,8-tris(3-methylbut-2-enyl)-2-phenyl-2H-chromen-7(8H)-one. A trivial name rhodiflavan B was given for the compound.



192

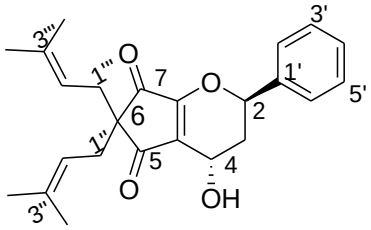
Table 4.27. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **191** and **192**, $CDCl_3$

191				**192**		
Position	δ_C	δ_H, *m*, (*J* in Hz)	HMBC	δ_C	δ_H, *m*, (*J* in Hz)	HMBC
2	76.1	5.22, *dd* (12.6, 2.3)	C-3,C-4, C-1', C-2', C-6'	76.2	5.24, *dd (*12.6, 2.3)	C-3,C-4, C-1', C-2', C-6'
3	37.0	2.31, *dt* (14.6,1.9,1.9)	C-4, C-4a	36.5	2.30, *dt* (14.6,2.3, 2.3)	C-4, C-4a
		1.80, *ddd* (14.6, 12.4, 4.2)	C-1', C-2		2.00, *ddd* (14.6, 12.5,3.6)	C-2, C-1'
4	57.4	4.99, *m*	C-2,C-3,C-5, C-4a, C-8a	59.5	4.55, *dd* (2.0,3.6)	C-2, C-4a, C-8a, C-5
4a	114.9			114.2		
5	194.7			197.0		
6	83.3			83.4		
7	206.4			207.2		
8	58.3			58.3		
8a	171.0			169.5		
1'	138.9			139.2		
2',6'	125.9	7.40 *m*	C-2, C- 2',C-4', C-6'	125.9	7.40 *m*	C-2, C-4', C- 2', C-6'
3',5'	128.7	7.43 *m*	C-1', C-3', C-5'	128.7	7.43 *m*	C-1', C-3', C-5'
4'	128.5	7.39 *m*	C-1', C-2', C-6'	128.5	7.39 *m*	C-1', C-2', C-6'
1"	35.0	2.81, *dd* (14.2, 9.2)	C-7, C-8, C-8a, C-2", C-3", C-1'"	34.5	2.89, *dd* (14.2, 9.8)	C-7, C-8, C-8a, C-2", C-3", C-1'"
		2.60, *dd* (6.3, 14.2)	C-7, C-8, C-8a, C-2", C-3", C-1'"		2.59, *m*	C-7, C-8, C-8a, C-2", C-3", C-1'"
1'"	38.5	2.77, *m*	C-7, C-8, C-8a, C-2'", C-3'", C-1"	38.5	2.69, *dd* (13.5, 7.3)	C-7, C-8, C-8a, C-2'", C-3'", C-1"
		2.46 *dd* (13.7, 8.2)	C-7, C-8, C-8a, C-2'", C-3'", C-1"		2.43, *dd* (13.5, 8.7)	C-7, C-8, C-8a, C-2'", C-3'", C-1"

1""	38.5	2.71, *dd* (14.9, 6.3)	C-6, C-5, C-2""	38.2	2.63 *dd* (14.7, 8.7)	C-6, C-5, C-2""
		2.25 *dd* (15.0, 5.7)	C-6, C-5, C-2""		2.18 *m*	C-6, C-5, C-2""
2"	118.8	5.03 *m*	C-1", C-4", C-5"	118.8	5.13 *m*	C-8, C-1", C-4", C-5"
2"'	117.4	4.66, *m*	C-8, C-1"', C-4"', C-5"'	117.4	4.71, *m*	C-8, C-1"', C-4"', C-5"'
2""	115.6	4.99 *m*	C-6, C-4"",C-5""	115.5	4.97 *m*	C-6, C-4"",C-5""
3"	136.0			136.0		
3"'	136.9			136.9		
3""	136.9			136.9		
4"	26.0	1.64 *s*	C-1", C-2", C-5"	18.0	1.61	C-1", C-2", C-5"
4"'	18.1	1.52 *s*	C-1"', C-2"', C-5"'	17.8	1.52	C-1"', C-2"', C-5"'
4""	26.0	1.71 *s*	C-2"",C-3"", C-5""	25.9	1.71	C-2"",C-3"", C-5""
5"	18.1	1.59 *s*	C-1", C-2", C-4"'	26.0	1.68	C-1", C-2", C-4"'
5"'	25.8	1.55 *s*	C-1", C-2", C-4"'	25.7	1.56	C-1", C-2", C-4"'
5""	17.9	1.55 *s*	C-2"",C-3"",C-4""	17.9	1.54	C-2"",C-3"",C-4""

4.1.5.16. Rhodiflavan C (193)

Compound **193** was isolated as an oily paste. The molecular formula $C_{24}H_{28}O_4$ was established from HRMS, exhibiting a molecular ion peak at *m/z* 381.2062 (Cal. 381.2066), together with the ^1H and ^{13}C NMR data (Table 4.28). The UV (λ_{max} = 230 and 270 nm) along with ^1H (δ_H 5.45 (*dd*, *J*=12.3, 2.2 Hz, for H-2); δ_H 2.02 (*ddd*, *J*=14.9, 12.3, 3.5 Hz for H-3_{ax}) and 2.29 (*dt*, *J*=14.8, 2.3 Hz for H-3_{eq}); and δ_H 4.80 (*dd J*=3.4, 2.3 Hz) for H-4) and ^{13}C (δ_C 78.8 for C-2, 37.0 for C-3 and 55.6 for C-4) NMR spectral data suggested that compound **193** is a flavan derivative.



193

The NMR data (Table 4.28) displayed the presence of an unsubstituted ring-B [(δ_C = 137.9 for C-1'; δ_C 126.3 (δ_H 7.4) for C-2'/6'; δ_C 128.9, (δ_H 7.46) for C-3'/5'; δ_C 129.1, (δ_H 7.43) for C-4'). Ring-A appears to be fully substituted as there is no aromatic proton observed. Two carbonyl groups resonating at δ_C 188.6 (for C-5) and at δ_C 203.4 (for C-7), and two prenyl groups (δ_C = 32.8 (δ_H 2.51 (*dd*) and 2.42 (*dd*)) for C-1"; δ_C 116.9 (δ_H 4.86 *m*) for C-2"; δ_C 136.6 for C-3"; δ_C 25.9 (δ_H 1.61 *s*) for C-4"; δ_C 17.9 (δ_H 1.57 *s*) for C-5"; δ_C 21.9 (δ_H 3.35 (*dd*) and 2.44 (*dd*)) for C-1'"; δ_C 123.2 (δ_H 4.86 *m*) for C-2'"; δ_C 136.6 for C-3'"; δ_C 25.8 (δ_H 1.61 *s*) for C-4'"; δ_C 17.6 (δ_H 1.56 *s*) for C-5'") could only be located in this ring. In the HMBC spectrum, correlation of H-2" and H-2'" with C-6 revealed that the two pernyl groups attached on the same carbon atom (C-6). The methylene protons of one prenyl group showed HMBC correlation to the methylene

carbon atom of the second prenyl group and *vise versa*, supporting that these are attached to the same carbon atom (C-6). Both methylene groups also showed HMBC correlation to the carbonyl resonance, (δ_C 188.6) and C-7 (δ_C 203.4), and also with C-6 (δ_C 52.9), allowing the placement of carbonyl groups at C-5 and C-7 of ring-A, which unusually is a five-membered ring. The MS (M^+ at *m/z* 381), NMR data and the UV spectrum (λ_{max} 230, 270 nm) which only showed a benzenoid band is consistent with such structure. Furthermore, in support of this H-4 (δ_H 4.80 (*dd* *J*=3.4, 2.3Hz)) showed a cross peak with C-5 (δ_C 188.6). The absolute configuration was determined as (2*R*,4*S*) from the ECD spectrum (Figure 4.14) which showed a positive Cotton effect at 314 nm, a negative one at 284 and a positive at 245 nm (Pouget *et al.*, 2000; Sun *et al.*, 2017). Therefore, based on the above spectroscopic data, this unique compound was characterized as (2*R*,4*S*)-3,4-dihydro-4-hydroxy-6,6-bis(3-methylbut-2-enyl)-2-phenylcyclopenta[b]pyran-5,7(2H,6H)-dione and was given a trivial name rhodiflavan C.

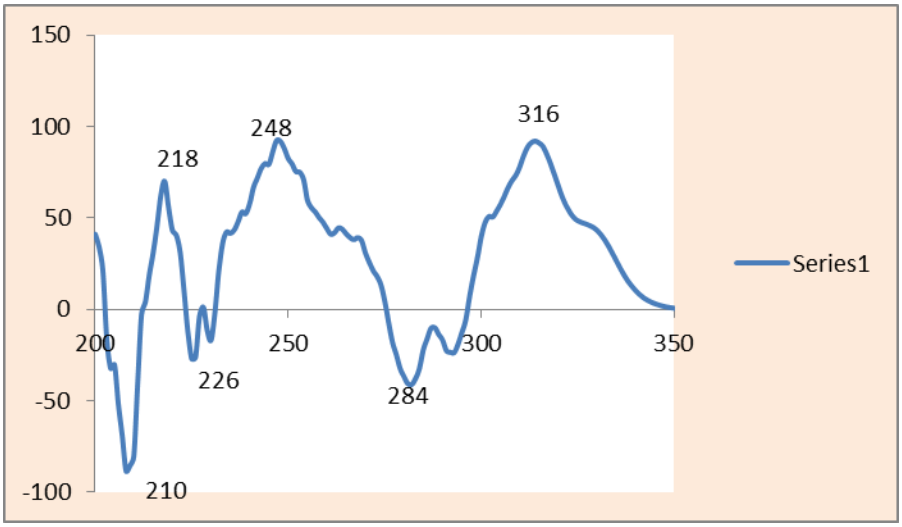


Figure 4.14: CD spectrum of compound **193**

Table 4.28. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **193**, $CDCl_3$

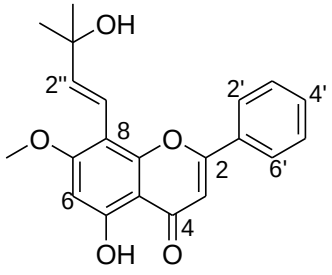
Position	δ_C	δ_H , *m*, (*J* in Hz)	HMBC
2	78.8	5.45 *dd (*12.3, 2.2*)*	C-3,C-4, C-1', C-2'/C-6'
3	37.0	2.29 *dt* (14.8, 2.3)	C-4, C-4a
		2.02 *ddd* (14.9, 12.3, 3.5)	C-2, C-1'
4	55.6	4.86 *m*	C-2, C-5, C-4a, C-8a
4a	125.9		
5	188.6		
6	52.9		
7	203.4		
7a	184.5		
1'	137.9		
2',6'	126.3	7.40 *m*	C-2, C- 2', C-4', C-6'
3',5'	128.9	7.46 *m*	C-1', C- 2', C-3', C-5', C-6'
4'	129.1	7.43 *m*	C-2', C-6', C-3', C-5'
1"	32.8	2.51, *dd* (14.3, 7.2)	C-5, C-6, C-7, C-8, C-8a, C-2", C-3", C-1'"
		2.42, *dd* (14.2, 7.9)	C-5, C-6, C-7, C-8, C-8a, C-2", C-3", C-1'"
1'"	32.6	2.56, *dd* (14.3, 7.2)	C-5, C-6, C-7, C-8, C-8a, C-2'", C-3'", C-1"
		2.44, *dd* (14.2, 7.9)	C-5, C-6, C-7, C-8, C-8a, C-1", C-2'", C-3'"
2"	116.9	4.86, *m*	C-8, C-1", C-4", C-5"
2'"	116.9	4.86, *m*	C-8, C-1'", C-4'", C-5'"
3"	136.6		
3'"	136.8		
4"	25.9	1.61	C-1", C-2", C-5"
4'"	25.9	1.61	C-1", C-2", C-4'"
5"	17.9	1.57	C-1'", C-2'", C-5'"
5'"	17.8	1.56	C-1", C-2", C-4'"

4.1.6. *Compounds from* Tephrosia purpurea

Extraction of the air dried stems of *T. purpurea* ssp. *leptostachya* with CH_2Cl_2/MeOH (1:1) at room temperature followed by a combination of chromatographic separations gave four new compounds: *(E)*-5-hydroxy-tephrostachin (**198**), purleptone (**194**), *(E)*-5-oxo-anhydrotephrostachin (**195**), terpurlepflavone (**199**) and seven known compounds; derrone (**48)** (Lin *et al.*, 2016), glabranin (**182)** (Yuldashev *et al.*, 2000), obovatin methyl ether (**73**) (Chen *et al.*, 1978a), genistein (**197**) (Gao *et al.*, 2016), tachrosin **(41)** (Smalberger *et al.*, 1971), kaempferitrin **(200)** (Yin *et al.*, 2014) and D-pintol **(165)**.

4.1.6.1. (E)-5-Hydroxy-tephrostachin (198)

Compound **198** was isolated as yellow crystals. The molecular formula $C_{21}H_{20}O_5$ was established from HRMS exhibiting a molecular ion peak at *m/z* 352.1315 together with the ^1H and ^{13}C NMR data (Table 28). The UV (λ_{max} = 230, 270 310 nm), ^1H NMR (δ_H 6.67 for H-3) and ^{13}C NMR (δ_C 164.2 for C-2, 105.5 for C-3 and 182.9 for C-3) spectral data suggested that the compound is a flavone derivative. The HMBC correlation of H-3 (δ_H 6.67) with C-2 (δ_C 164.2), C-4 (δ_C 182.9) and C-4a (δ_C 105.2) was further evidence that this compound is indeed a flavone derivative.



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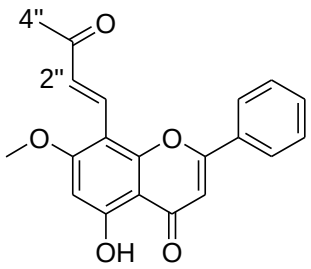
In addition, the NMR spectra showed the presence of a methoxy (δ_H 3.92; δ_C 56.1), a hydrogen bonded hydroxy group (δ_H 13.08) and a 2-methylbut-3-en-2-ol (Table 4.28) substituent on the flavone skeleton.

Three sets of mutually coupled protons resonating at δ_H 7.91 *m* (H-2′/6'), 7.52 *m* (H-3′/5') and 7.55 *m* (H-4') [with the corresponding carbon atoms resonating at δ_C 126.5 (C-2′/6'), 129.1 (C-3′/5') and 131.9 (C-4')] are assigned to ring-B of this flavone which is unsubstituted. In ring-A, the ^{1}H NMR spectral data of compound **198** showed the presence of a singlet at δ_H 6.40 (δ_C 95.3) indicating that this ring is trisubstituted with methoxyl (at C-7), a hydrogen bonded hydroxyl group (at C-5) and (*E*)-2-methylbut-3-en-2-ol groups. The HMBC correlation of the singlet at δ_H 6.40 with C-4a (δ_C 105.2), C-5 (δ_C 161.3), C-7 (δ_C 163.1) and C-8 (δ_C 105.3) allowed the assignment of this signal to H-6. With the methoxy group (δ_H 3.92, δ_C 56.1) being at C-7 (δ_C 163.1) and the hydrogen bonded hydroxyl group (δ_H 13.08) at C-5-OH), [showing HMBC correlation with C-4a (δ_C 105.2), C-5 (δ_C 161.3) and C-6 (δ_C 95.3)], the 2-methylbut-3-en-2-ol group could only be placed at C-8. This was confirmed by the HMBC spectrum which showed correlation of one of the olefinic protons, H-1" (δ_H 6.85), with C-7 and C-8a. The large coupling constant (*J* = 16.5 Hz) between (H-1" (δ_H 6.85) and H-2" ((δ_H 6.70) suggested an *E*-configuration for the double bond of 2-methylbut-3-en-2-ol group (Khalid and Waterman, 1981). Therefore compound **198** was characterized as (*E*)-5-hydroxy-8-(3-hydroxy-3-methylbut-1-en-1-yl)-7-methoxy-2-phenyl-4H-chromen-4-one. Compound **198** has similar structure with *trans*-tephrostachin (Khalid and Waterman, 1981) except the presence of a hydroxyl group at position **5**, and hence given the trivial name (*E)*-5-hydroxy-tephrostachin.

4.1.6.2. Purleptone (194)

Compound **194** was isolated as a colourless amorphous solid. The molecular formula $C_{20}H_{16}O_5$ was established from HRMS spectrum exhibiting a molecular ion peak at *m/z* 336.0980 together with the ^{1}H and ^{13}C NMR data (Table 4.28). The UV spectrum (λ_{max} = 230, 290 and 330 nm) along with NMR spectra (Table 4.28) suggested that the compound has a flavone skeleton.



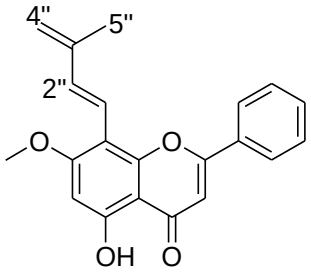
194

The ^{1}H and ^{13}C NMR spectra (Table 4.28) of compound **194** showed close similarities with compound **198**. As in compound **198**, the B ring of compound **194** is unsubstituted, while ring A is trisubstituted with hydroxyl at C-5 and a methoxyl at C-7 and a modified prenyl group at C-8 (Table 4.28). The nature of the substituent at C-8 is however different. In the ^{1}H NMR spectrum of compound **194**, the presence of *trans*-oriented and mutually coupled (J = 16.4 Hz) olefinic protons were also observed, but in this case the protons are down-field shifted (δ_H 8.08 for H-1" and δ_H 7.18 for H-2"). Furthermore, only one methyl signal, which is down-field-shifted (δ_H 2.41; δ_C 27.8) was observed. In the ^{13}C NMR spectrum, an additional carbonyl signal (δ_C 199.1) showing HMBC correlation with H-1" (δ_H 8.08) and H-2" (δ_H 7.18) and the methyl signal (δ_H 2.41) indicated that the substituent at C-8 is (*E*)-but-3-en-2-one group as in (2S)-5-hydroxy-7-

methoxy-8-[(*E*)-3-oxo-1-butenyl]flavanone (Jang *et al.*, 2003) and erylivingstone F (Bedane *et al.*, 2016) . There are some reports with such modified prenyl group but this is the second report from *Tephrosia* species. Based on the above spectroscopic data, compound **194** was characterized as (*E*)-5-hydroxy-7-methoxy-8-(3-oxobut-1-en-1-yl)-2-phenyl-4H-chromen-4-one and given a trivial name purleptone.

4.1.6.3. (E)-5-Oxo-anhydrotephrostachin (195)

Compound **195** ($[M]^+$ *m/z* 335.1227, $C_{21}H_{18}O_4$) is also a flavone derivative (λ_{max} = 230, 280 and 310 nm) whose ^1H and ^{13}C NMR spectra (Table 4.28) showed close similarities with those of compound **198**. This flavone also has unsubstituted B ring, while ring A has a hydroxyl at C-5, a methoxyl at C-7 and a prenyl derivative at C-8. This substituent is (*E*)-3-methylbuta-1,3-dien-1-yl as suggested by the ^1H and ^{13}C NMR spectra (Table 4.28).



195

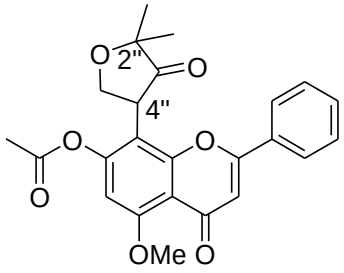
The identification of this group was confirmed from HMBC correlations of CH_2-4" (δ_H 5.10) with C-2", C-3", C-5"; the placement of this group at C-8 was established from HMBC correlation of H-2" with C-8, C-3", C-4", C-5"; and H-5" with C-2", C-3", C-4". In agreement with the above correlation H-1" also showed an HMBC correlation with C-7, C-8a, C-2", C-3". Compound **195** was therefore characterized as (*E*)-5-hydroxy-7-methoxy-8-(3-methylbuta-1,3-dien-1-yl)-2-phenyl-4H-chromen-4-one and given the trivial name *(E)*-5-oxo-anhydrotephrostachin by relating it to anhydrotephrostachin (Khalid and Waterman, 1981).

Table 4.28. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR spectroscopic data for compounds **198**, **194** and **195**, $CDCl_3$

	198			**194**			**195**		
Position	δ_C	δ_H , *m*, (*J* in Hz)	HMBC	δ_C	δ_H, *m*, (*J* in Hz)	HMBC	δ_C	δ_H, *m*, (*J* in Hz)	HMBC
2	164.2			164.6			164.2		
3	105.5	6.57 *s*	C-2, C-4, C-4a, C-1’	106.2	6.74 *s*	C-2, C-4, C-4a, C-1'	105.5	6.71 *s*	C-2, C-4, C-4a, C-1'
4	182.9			182.6			183.0		
4a	105.2			105.4			105.3		
5	161.3			164.2			161.4		
5-OH		13.08 *s*	C-4a, C-5, C-6		13.41 *s*	C-4a, C-5, C-6		13.11 *s*	C-4a, C-5, C-6
6	95.3	6.40 *s*	C-4a, C-5, C-7, C-8	95.6	6.40 *s*	C-4a, C-5, C-7, C-8	95.4	6.45 *s*	C-4a, C-5, C-7, C-8
7	163.1			165.0			163.2		
8	105.3			103.4			106.0		
8a	154.1			156.0			154.2		
1'	131.5			131.5			131.5		
2',6'	126.5	7.91 *m*	C-2, C-4', C- 2', C-6'	126.5	7.92 *m*	C-2, C-4', C- 2', C-6'	126.4	7.93 *m*	C-2, C-4', C- 2', C-6'
3',5'	129.1	7.52 *m*	C-1', C-3', C-5'	129.4	7.59 *m*	C-1', C-3', C-5'	129.2	7.54 *m*	C-1', C-3', C-5'
4'	131.9	7.55 *m*	C-2', C-6’	132.2	7.59 *m*	C-2', C-6'	132.0	7.56 *m*	C-2', C-6'
1"	114.9	6.85, *d* (16.47)	C-7, C-8a, C-2", C-3"	132.0	8.06, *d* (16.43)	C-7, C-8a, C-2", C-3"	117.5	6.83, *d* (16.50)	C-7, C-8a, C-2", C-3"
2"	141.3	6.70, *d* (16.47)	C-8, C-3", 3"-Me_2	128.8	7.18, *d* (16.43)	C-8, C-3", C-4"	135.4	6.29, *d* (16.50)	C-8, C-3", C-4", C-5"
3"	71.5			199.1			142.9		
3"-Me_2	30.0	1.50	C-2", C-3", 3"-Me_2						
4"				27.8	2.41	C-2", C-3"	116.8	5.10 *s*	C-2", C-3", C-5"
5"							18.2	2.06 *s*	C-2", C-3", C-4"
7-OMe	56.1	3.92	C-7	56.4	4.01	C-7	56.2	3.97	C-7

4.1.6.4. Terpurlepflavone (199)

Compound **199** was isolated as a white amorphous solid. The molecular formula $C_{24}H_{22}O_7$ was established from HRMS, exhibiting a molecular ion peak at *m/z* 423.1465, together with the ^{1}H and ^{13}C NMR data (Table 4.29). The UV spectrum (λ max = 230 nm 260 nm and 310 nm) along with NMR spectra (Table 4.29) suggested once again that the compound has a flavone skeleton.



199

The NMR spectra (Table 4.29) revealed the presence of an unsubstituted B-ring (δ_H 7.70 [(H-2'/6' *m*); δ_C 126.3], δ_H 7.45 [(H-3'/5' *m*); δ_C 128.7] and δ_H 7.49 [(H-4' *m*); δ_C 131.1]), a methoxyl (δ_H 3.96, δ_C 56.7, at C-5), an acetate (δ_H 2.11; (δ_C 21.4, 170.0, at C-7) groups and a modified prenyl group in the form of tetrahydrofuran ring (at C-8) as in terpurinflavone (Juma *et al.*, 2011) or tephroglabrin (Waterman and Khalid, 1980b). The presence of an additional carbonyl (δ_C 206.1) and two germinal methyl groups (δ_H 1.57 and 1.65; δ_C 24.0 and 23.9) and three mutually coupled protons at δ_H 4.95 (*dd*, *J* = 6.1, 10.2 Hz), 4.90 (*dd*, *J* = 6.1, 8.8) and 4.84 (*dd*, *J* = 6.1, 8.8 Hz) showed that the substituent at C-8 is 5,5-dimethyl-4-oxo-tetrahydrofuran-3-yl group. In agreement with this H-2", H-4", H-5" and 2"-$(Me)_2$ showed HMBC correlation to the carbonyl carbon (δ_C 206.1, C-3"). The HMBC correlation of H-4" (δ_H 4.95) with C-7; H-6 (δ_H 6.41) with C-4a, C-5, C-7 and C-8, and the only OMe with C-5 (δ_C 162.9) confirmed the substitution pattern in this ring. The coupling constant (*J*= 10.2) between H-4" and H-5"

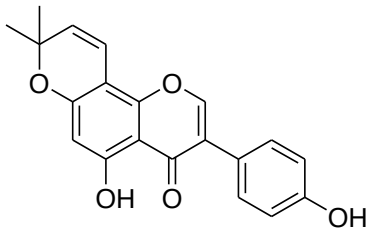
indicated that the orientation of these two protons is in axial position. Based on the above spectroscopic data compound **199** was characterized as 8-(5,5-dimethyl-4-oxotetrahydrofuran-3-yl)-5-methoxy-4-oxo-2-phenyl-4H-chromen-7-yl acetate and given a trivial name terpurlepflavone.

Table.4.29. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **199**, $CDCl_3$

Position	δ_C	δ_H, *m*, (*J* in Hz)	HMBC
2	160.6		
3	110.1	6.55 *s*	C-2, C-4, C-4a, C-1'
4	177.2		
4a	109.1		
5	162.9		
6	91.1	6.41 *s*	C-4a, C-5, C-7, C-8
7	166.4		
8	103.9		
8a	155.0		
1'	131.8		
2',6'	126.4	7.70 *m*	C-2, C-4', C- 2', C-6'
3',5'	128.8	7.45 *m*	C-1', C-3', C-5'
4'	131.1	7.49 *m*	C-2', C-6'
2"	83.9		
3"	206.2		
4"	47.8	4.95 *dd* (10.2, 6.1)	C-7, C-8, C-8a, C-2", C-3", C-5"
5"	75.8	4.90 *dd* (10.3, 8.8)	C-7, C-8, C-3", C-4"
		4.84 *dd* (6.1, 8.8)	C-7, C-8, C-3'', C-4''
2"-Me	24.0	1.57 *s*	C-2", C-3", 2"-Me
2"-Me	24.0	1.65 *s*	C-2", C-3", 2"-Me
5-OMe	56.7	3.96 *s*	C-5
7-COMe	170.0		
7-COMe	21.5	2.11	7-COMe

4.1.6.5. Derrone (196)

Compound **196** was isolated as a white amorphous solid. The molecular formula $C_{20}H_{16}O_5$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 337.1) together with the ^1H and ^{13}C NMR data (Table 4.30). The UV spectrum (λ_{max} 230 and 270 nm) along with the singlet proton peak at δ_H 7.84 ppm in the ^1H NMR spectrum with its corresponding oxygenated carbon peak resonating at δ_C 152.5 ppm revealed that compound **196** is an isoflavone derivative.



196

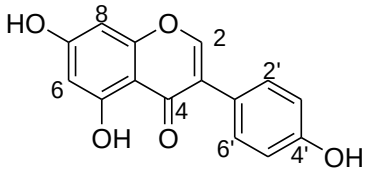
In support of the isoflavone skeleton, the singlet proton peak at δ_H 7.84 ppm (H-2) showed HMBC correlations with C-3 (δ_C 122.7), C-4 (δ_C 180.0, C-8a (δ_C 159.6 and C-1' (δ_C 122.7). The ^1H NMR spectra clearly unveiled the presence of an AA'XX' spin system (δ_H 7.38 (H-2'/6') and 6.85 (H-3'/5')) which is characteristic for C-4' oxygenated substituent ring-B. Further the ^1H NMR showed the presence of a singlet proton at δ_H 6.25 (H-6) and 2,2-dimethylchromene ring (C-2" δ_C 78.1; C-3" δ_C 127.5 (δ_H 5.68 *d* (10.0)); C-4" δ_C 114.5 (δ_H 6.7 *d* (10.0)) and C-2"-Me_2 δ_C 28.2 (δ_H 1.48 *s*). The placement of the 2",2"-dimethylchromene ring was based on the HMBC correlation of H-6 with C-4a (δ_C 106.0), C-5 (δ_C 163.7), C-7 (δ_C 162.2), C-8 (δ_C 101.7) and H-4" with C-7 (δ_C 162.2), C-8 (δ_C 101.7), C-8a (δ_C 159.6), C-2"(δ_C 78.1). Therefore from the above spectroscopic data compound **196** was identified as derrone, previously reported from *Derris robusta* (Chibber and Sharma, 1980). This however is the first report form *Tephrosia* species.

Table 4.30. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **196**, $CDCl_3$

Position	δ_C	δ_H, *m*, (*J* in Hz)	HMBC
2	152.5	7.84 *s*	C-3, C-4, C-8a, C-1'
3	122.7		
4	180.0		
4a	106.0		
5	163.7		
5-OH		12.95 *s*	
6	100.4	6.26 *s*	C-4a, C-5, C-7, C-8
7	162.2		
8	101.7		
8a	159.6		
1'	122.7		
2'/6'	130.3	7.38 *d* (8.7)	C-3, C-4'
3'/5'	115.7	6.85 *d* (8.7)	C-1', C-4'
4'	156.1		
2"	78.1		
3"	127.5	5.68 *d* (10.0)	C-8, C-2", 2"-Me_2
4"	114.5	6.7 *d* (10.0)	C-7, C-8, C-8a, C-2"
2"-Me_2	28.2	1.48 *s*	C-3", C-2"

4.1.6.6. Genistein (197)

Compound **197** was isolated as an amorphous solid. The molecular formula $C_{15}H_{10}O_5$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 271.6) together with the ^1H and ^{13}C NMR data (Table 4.31). This compound was also identified as an isoflavone derivative based on the UV (λ_{max} 230, 270, 330 nm), ^1H NMR (δ_H 8.00 *s*, H-2) and ^{13}C NMR (δ_C 154.8, for C-2, 120.2, for C-3) and δ_C 181.7 for C-4) spectra.



197

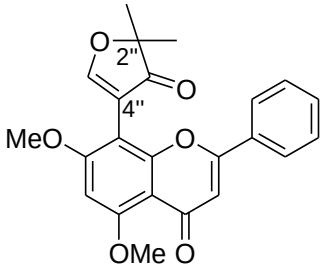
The ^1H NMR showed the presence of two *meta*-coupled aromatic protons at δ_H 6.26 (H–6) and δ_H 6.39 (H-8) for ring-A protons, with their corresponding carbon atom signals at δ_C 99.2 (C-6) and δ_C 93.9 (C-8). The HMBC correlations of H–6 with C-4a (δ_C 104.9), C-5 (δ_C 163.7), C-7 (δ_C 165.1), C-8 (δ_C 93.9) and H–8 with C-4a (δ_C 104.9), C-6 (δ_C 99.2), C-7 (δ_C 165.1), C-8a (δ_C 158.3) influenced the placements of hydroxyl groups at C-5 and C-7 on ring A. Furthermore the ^1H NMR displayed an AA'XX' spin system attributed to H-2'/6' (7.41 *d J*= 8.6) and H-3'/5' (6.90 *d J*= 8.6) suggesting oxygenating at C-4' on ring-B. Thus, based on these spectroscopic data and comparison with literature data, compound **197** was identified as genistein (Jang *et al.*, 2003). This is the first report of genistein from *T.purpurea* ssp. *leptostachya*.

Table 4.31. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **197**, $CDCl_3$

Position	**197**		
	δ_C	δ_H , *m, J in* Hz	HMBC
2	153.9	8.00 *s*	C-3, C-4, C-8a, C-1'
3	122.9		
4	180.8		
4a	104.9		
5	163.7		
5-OH		12.95 *s*	
6	99.2	6.26 *d* (2.1*)*	C-4a, C-5, C-7, C-8
7	165.1		
8	93.9	6.39 *d* (2.1)	C-4a, C-6, C-7, C-8a
8a	158.3		
1'	122.6		
2',6'	130.0	7.41 *d* (8.6)	C-3, C-4', C- 2', C-6'
3',5'	115.1	6.90 *d* (8.6)	C-1', C-3', C-4', C-5'
4'	157.1		

4.1.6.7. Tachrosin (41)

Compound **41** was isolated as a white solid. The molecular formula $C_{23}H_{20}O_6$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 393.2) together with the ^1H and ^{13}C NMR data (Table 4.32). The UV spectrum (λ_{max} 230, 260 and 320 nm) along with ^1H NMR (δ_H 6.68, *s*, for H-3); ^{13}C NMR (δ_C 177.8, for C-4) (Table 4.29) suggested a flavone derivative.



41

The NMR data of compound **41** revealed the presence of an unsubsitiuted ring-B [(δ_C 131.8 (for C-1'), δ_C 126.2, δ_H 7.71 for C-2'/6'), δ_C 128.7, δ_H 7.41 for C-3'/5'), δ_C 131.1, δ_H 7.45 for C-4')], two methoxy groups (δ_C 56.1, δ_H 4.01 for 5-OMe and δ_C 56.5, δ_H 3.92 for 7-OMe) and a modified prenyl group in the form of 2,2-dimethylfuran-3(2H)-one ring (δ_C 88.1 for C-2", δ_C 204.5 for C-3", δ_C 109.5 for C-4", δ_C 175.6 for C-5", δ_C 23.1 for C-2"-Me_2).

The ^1H NMR displayed a singlet aromatic proton at δ_H 6.45 (H-6) which showed a HMBC correlation with C-4, C-4a, C-5, C-7, C-8 in support of the placement of the methoxy groups at C-5 and C-7. In addition, the proton spectrum exhibited a downfield singlet proton at δ_H 8.31 (assigned for H-5"), a chemical shift value that is consistent with an olefinic proton α to oxygen and β to a carbonyl.

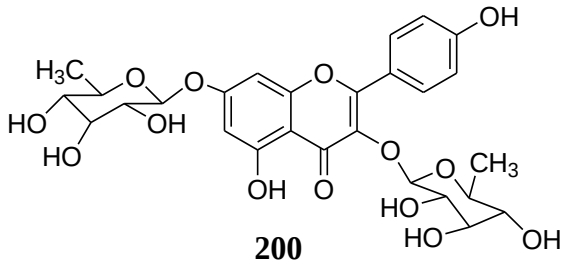
Therefore, based on the above spectroscopic data and comparison with literature led to the identification of compound **41** as tachrosin, previously isolated from different *Tephrosia* species including *T. purpurea* (Chen *et al.*, 2014; Muiva-Mutisya *et al.*, 2014; Pelter *et al.*, 1981). However; this is the first report from the subspecies *T.purpurea* ssp. *leptostachya*.

Table 4.32. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **41**, $CDCl_3$

Position	δ_C	δ_H, *m*, *J*=Hz	HMBC
2	161.1		
3	108.9	6.63 *s*	C-2, C-4, C-4a, C-1'
4	177.8		
4a	109.1		
5	161.2		
6	91.6	6.45 *s*	C-4, C-4a, C-5, C-7, C-8
7	161.5		
8	98.3		
8a	156.8		
1'	131.8		
2',6'	126.2	7.71 *m*	C-2, C-4', C- 2', C-6'
3',5'	128.7	7.41 *m*	C-1', C-3', C-5'
4'	131.1	7.45 *m*	C-2', C-6'
2"	88.1		
3"	204.5		
4"	109.5		
5"	175.6	8.31 *s*	C-2", C-3", C-4"
2"-Me_2	23.1	1.57 *s*	C-3",C-2"
5-OMe	56.1	4.01	C-5
7-OMe	56.5	3.92	C-7

4.1.6.8. Kaempferitrin (200)

Compound **200** was isolated as a white solid. The molecular formula $C_{27}H_{30}O_{14}$ was established from LC-ESI-MS data ($[M+H]^+$ at *m/z* 579.7) together with the ^1H and ^{13}C NMR data (Table 4.33). The UV (λ_{max} 230, 260 and 320 nm) along with the NMR (δ_C 158.4 for C-2, 135.1 for C-3 and 178.4 for C-4) spectral data suggested that compound **200** has a flavonol skeleton. The NMR features of compound **200** are similar to those of compound **179,** with the main difference being compound **200** is glycosylated at C-3 and C-7.



200

The ^1H NMR spectrum of compound **200** showed characteristic anomeric peaks at δ_H 5.58 for H-2" and δ_H 5.42 for H-2"' while the appearance of two methyl groups (δ_H 1.15 for 6"-Me (d *J* = 6.2) and δ_H 0.82 for 6"'-Me (d *J*=5.8)) suggested the two glycosides to be rhamnoside moieties. The ^3J HMBC correlation of H-2" with C-7 and H-2"' with C-3 was the basis for the placement of the two rhamnoside moieties at C-3 (δ_C 135.1) and C-7 (δ_C 162.1). Furthermore, as in compound **179**, it displayed an AA'XX'-spin system (δ_H 7.81 (*d, J* = 8.7 Hz) for H-2'/6' and at δ_H 6.96 (*d, J* = 8.2 Hz) for H-3'/5') for ring-B and an AX spin system δ_H 6.74 (*d, J* = 2.2 Hz) for H-6 and δ_H 6.48 (*d J* = 2.2 Hz for H-8) for ring-A. The complete assignment of compound **200** was established based on 1D and 2D NMR analysis and comparison with literature. Therefore, compound **200** was identified as kaempferol-3,7,*O,α*–L-dirhamnoside common name

kaempferitrin. It has been reported from several plants such as *Justicia spicigera* (Acanthaceae) and *Consolida armeniaca* (Ranunculaceae) (Cassani *et al.*, 2014; Jorge *et al.*, 2004; Pizzolatti *et al.*, 2003), however this is the first report from *Tephrosia* species.

Table 4.33. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **200**, $CDCl_3$

Position			
	δ_C	δ_H , *m*, (*J* in Hz)	HMBC
1			
2	158.4		
3	135.1		
4(C=O)	178.4		
4a	106.2		
5	161.6		
5(OH)		12.61 *s*	C-4a, C-6, C-5
6	94.2	6.74 *d* (2.2)	C-4a, C-6, C-8a, C-7
7	162.1		
8	99.1	6.48 *d* (2.2)	C-6, C-4a, C-7
8a	156.7		
1'	120.9	-	
2'/6’	130.6	7.81 *d* (8.7)	C-3'/5', C-2'/6’, C-4'
3'/5'	115.2	6.96 *d* (8.2)	C-3'/5', C-1', C-4'
4'	160.4	-	
2"	98.4	5.58 *s*	C-7
3"	70.3	3.86 *bd*	
4"	71.1	3.16 *m*	
5"	72.0	3.33 *t*	
6"	70.5	3.44 *m*	
2"'	102.1	5.42 s	C-3
3"'	70.5	4.00 *bd*	
4"'	70.8	3.50 *bd*	
5"'	70.7	3.66 *bd*	
6"'	71.6	3.15 *m*	
6"-Me	18.4	1.15 *d* (6.2)	
6"'-Me	17.9	0.82 *d* (5.8)	

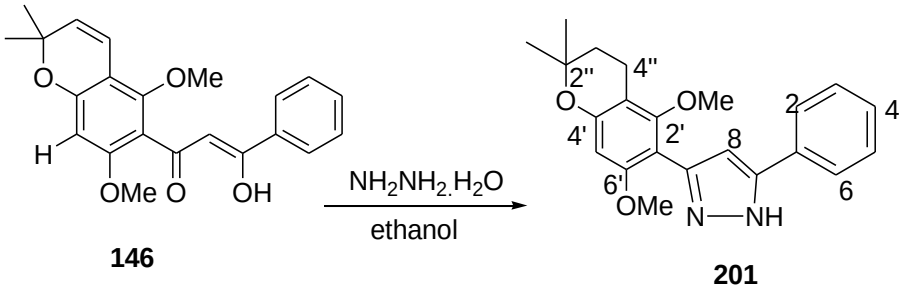
4.2. Structural Modification of Selected Flavones

4.2.1. Pyrazole Derivatives

Compound **146** (praecansone B) and compound **59** (Isopongaflavone) were treated with hydrazine hydrate in the presence of ethanol to give pyrazopraecansone B (**201**) and pyrazoisopongaflavone (**202**).

4.2.1.1. Pyrazopraecansone B (201)

Compound **201** was prepared from praecansone B (**146**) as white solid. The molecular formula C_{22} H_{24} N_2O_3 was established based on HRESIMS ($[M+H]^+$ *m/z* obs 364.1778, calcd 364.1788) and NMR data(Table 4.34). The UV spectrum (λ_{max} 230, 270 and 310 nm) along with NMR spectra (Table 4.34) suggested that compound **201** is a pyrazole derivative.



146 **201**

The NMR data (Table 4.34) of compound **201** displayed the presence of an unsubstituted phenyl group [δ_H 7.93 (2H, *m*, for H-2/6), 7.42 (2H, *m*, H-3/5), 7.30 (1H, *m*, for H-4); δ_C 134.5 (for C-1); 125.3 (for C-2/6), 128.4 (for C-3/5) and 127.0 (for C-4) and two methoxyl groups on C-2' (δ_C 55.3, δ_H 3.89 *s*) and on C-6' (δ_C 59.3 δ_H 3.62 *s*) like that of compound **146**. The ^{13}C NMR spectrum further showed the presence of two quaternary carbons at δ_C 150.1 and δ_C 137.0 which are assigned for C-7 and C-9 of the pyrazole ring. The formation of the pyrazole ring was further confirmed from the HMBC correlation H-8 (δ_H 7.07 *s*) with C-7 (δ_C 150.1) and C-9 (δ_C 137.0).

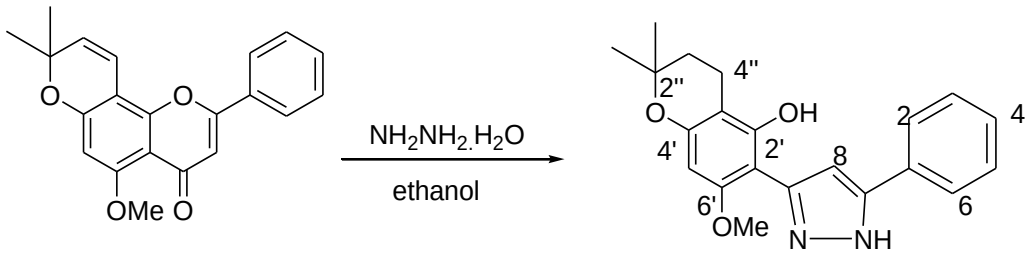
In support of the above suggestion H-2/6 (δ_H 7.93) and H-5'(δ_H 6.33) showed HMBC correlation with C-7(δ_C 150.1) and C-9 (δ_C 137.0) respectively. Based on the above spectroscopic data this new pyrazole derivative was characterized as pyrazopraecansone B (**201**).

Table 4.34. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **201**, Acetone

Position	**201**		
	δ_C (ppm)	δ_H (ppm), *m*, (*J* in Hz)	HMBC correlations
1	134.5	-	
2/6	125.3	7.93 *m*	C-2/6, C-4, C-7
3/5	128.4	7.42 *m*	C-1, C3/5
4	127.0	7.30 *m*	C-2/6
7	150.1		
8	102.8	7.07 *s*	C-7, C-9
9	137.0		
1'	104.4		
2'	155.6		
3'	107.7		
4'	157.2		
5'	96.6	6.33 *s*	C-1', C-3', C-4', C-6', C-9(W)
6'	156.8		
2"	74.5		
3"	16.8	2.77 *t*	C-3', C-2", C-4"
4"	32.1	1.84 *t*,	C-3', C-4', C-2"
2"-Me_2	26.03	1.36 *s*	C-2", C-3"
2'-OMe	55.3	3.89 *s*	C-2'
6'-OMe	59.3	3.62 *s*	C-6'

4.2.1.2. Pyrazoisopongaflavone (202)

Compound **202** was prepared from isopongaflavone (**59**) as an amorphous solid. The molecular formula $C_{21}H_{22}N_2O_3$ was established based on HRESIMS ($[M+H]^+$ *m/z* obs 350.1640, calcd 350.1630) and NMR (Table 4.35) analyses. The NMR data (Table 4.35) of compound **202** showed the presence of a methoxyl group (δ_C 54.1, δ_H 3.94 *s*), an unsubstituted phenyl group [δ_H 7.86 (2H, *m*, for H-2/6), 7.52 (2H, *m*, H-3/5), 7.43 (1H, *m*, for H-4); δ_C 129.6 (for C-1); 125.5 (for C-2/6), 128.9 (for C-3/5) and 128.5 (for C-4). The formation of the pyrazole ring was confirmed from the HMBC correlation H-8 (δ_H 7.07 *s*) with C-7 (δ_C 150.1) and C-9 (δ_C 137.0). Based on the above spectroscopic data of this new pyrazole derivative characterized as pyrazoisopongaflavone (**202**).



59 **202**

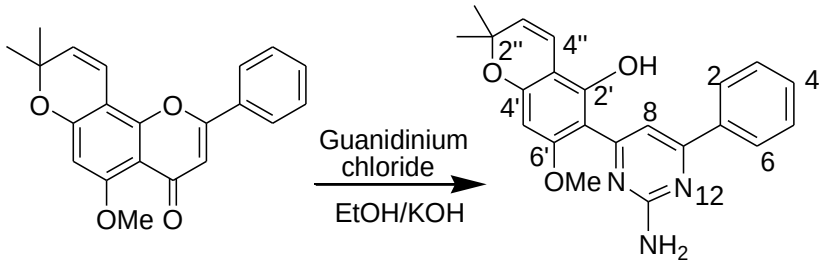
Table 4.35. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **202**, Acetone

Position	**202**		
	δ_C (ppm)	δ_H (ppm), *m*, (*J* in Hz)	HMBC correlations
1	129.6		
2/6	125.5	7.86 *m*	C-2/6, C-4, C-7
3/5	128.9	7.52 *m*	C-1, C3/5
4	128.5	7.43 *m*	C-2/6
7	150.5		
8	103.2	7.37 *s*	C-7, C-9
9	142.5		
1'	98.7		
2'	154.6		
3'	101.2		
4'	156.3		
5'	91.3	6.03 *s*	C-1', C-3', C-4', C-6', C-9
6'	157.2		
2"	73.8		
3"	32.2	1.81 *t*	C-3', C-2", C-4"
4"	16.7	2.66 *t*	C-3', C-4', C-2"
2"-Me_2	26.1	1.33 *s*	C-2", C-3"
6'-OMe	54.8	3.94 *s*	C-6'

4.2.2. Guanidine Derivative

4.2.2.1. Guanidinoisopongaflavone (203)

Compound **203** was isolated as an amorphous solid, and was assigned the molecular formula $C_{22}H_{21}O_3N_3$ based on HRESIMS ($[M+H]^+$ *m/z* obs 375.1573, calcd 375.1583) and NMR (Table 4.36) analyses.



59 **203**

The 1D and 2D NMR data (Table 4.36) of compound **203** displayed the presence of one methoxy groups (δ_C 63.1, δ_H 3.89 *s*) and (δ_C 55.9 δ_H 3.85 *s*), an unsubstituted ring B [δ_H 8.01 (2H, *m*, for H-2/6), 7.53 (2H, *m*, H-3/5), 7.45 (1H, *m*, for H-4); δ_C 135.1 (for C-1); 127.0 (for C-2/6), 128.7 (for C-3/5) and 132.2 (for C-4) like that of compound **146**. The presence of the guanidine ring was further confirmed from the HMBC correlation H-8 (δ_H 7.07 *s*) with C-7 (δ_C 166.9) and C-9 (δ_C 165.6). Based on the above spectroscopic data compound **203** was characterized as guanidinoisopongaflavone (**203**)

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Table 4.36. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound **203,** Acetone

Position	**203**		
	^{13}C (ppm)	^{1}H (ppm)	HMBC
1	139.6	-	
2/6	128.6	8.13 (2H, m)	C-7, C-4, C-2
3/5	130.1	7.52 (2H, m)	C-1, C- 2, C- 3
4	131.9	7.51 (1H, m)	
7	166.9	-	
8	106.9	8.16 (1H, s)	C-1', C-7, C-1
9	165.6	-	
11	162.3 (*br*)		
1'	102.8	-	
2'	160.7 (*br*)	-	
3'	104.9	-	
4'	158.4	-	
5'	93.0	6.07 (1H, s)	C-3', C-1', C-6'(w), C-4'
6'	163.2	-	
2"	78.3	-	
3"	126.7	5.52 (1H, d, 9.9)	C-2",C-3', C- 2"-Me_2
4"	118.3	6.69 (1H, d, 9.9)	C-2", C-3' (w), C-2', C- 4'
2"-Me_2	26.1	1.33 s	C-2", C-3"
6'-OMe	57.0	3.99 (3H, s)	C-6'
2'-OH		15.49 (1H, s)	
NH_2		6.53 (2H, brs)	

4.3. Biological Activity

4.3.1. In-Vitro Antiplasmodial Activity

The crude extracts of *Tephrosia aequilata* (roots) and *T. rhodesica* (roots) were tested for *in vitro* antiplasmodial activity against the chloroquine-sensitive (3D7) strain of *Plasmodium falciparum.* The crude extracts exhibited 100% growth inhibition at 10 μg/ml. Some of the isolated compounds from these plants belonging to different classes of flavonoids, chalcones, flavanones, flavans were also evaluated for antiplasmodial activity (Table 4.37). Among these classes, chalcones were the most active compounds, with 3",4"-*cis*-4"-ethoxy-3"-hydroxypraecansone B **(170)** (IC_{50} 2.48 ± 0.22 μM) showing the highest activity followed by praecansone B (**146**) (IC_{50} 4.14 ± 0.26 μM) and obovatachalcone (**147**)(IC_{50} 4.23 ± 1.11 μM) which also previously showed good activity (Batista *et al.*, 2009; Mishra *et al.*, 2009). The activities of these chalcones were in similar range as for licochalcone A (IC_{50} 4.17 ± 1.60 μM against the 3D7 strain), a retrochalcone which is also known for its *in vivo* antimalarial activity and for enhancing the activity of artemisinin in *vitro* (Mishra *et al.*, 2009; Ziegler *et al.*, 2004). The other tested chalcones *E*-praecansone A **(145)** (6.45 ± 0.48 μM), *E*-2',6'-dimethoxy-4',5'-(2",2"-dimethyl)pyranoretrochalcone (**168)** (9.20 ± 1.42 μM), and *Z*-2',6'-dimethoxy-4',5'-(2",2"-dimethyl)pyranoretrochalcone **(169)** (9.75 ± 0.81 μM) also showed moderate activity (Batista *et al.*, 2009).

Obovatin methyl ether **(73),** a flavanone reported from several *Tephrosia* species, was also tested and showed good activity (IC_{50} 3.69 ± 0.34 μM) (Batista *et al.*, 2009). The level of activity observed here against the chloroquine-sensitive (3D7) strain is similar to what has been

reported by Muiva *et al.,*(2009) against the D6 (IC_{50} 3.8 ± 0.3 μM) and W2 (4.40± 0.6 μM) strains.

The flavans, quercetol B **(98)**, rhodiflavan A **(191)**, rhodiflavan C **(193)**, rhodiflavan B **(192)** and tephrowatsin A **(79)** showed moderate activity (Batista *et al.*, 2009); among which the highest activity was exhibited by rhodiflavan B (IC_{50} =5.72 ± 1.91 μM). The pterocarpans, pisatin **(185)** (IC_{50} =5.88 ± 1.46 μM) and rhocarpin **(188)** (IC_{50} =10.25 ± 0.21μM), exhibited moderate activity (Batista *et al.*, 2009), while 3,4:8,9-dimethylenedioxypterocarpene **(167)** showed no activity at 40 μM.

The flavone isopongaflavone **(59)** (IC_{50} =8.19 ± 1.48 μM) and the rotenone, 6-hydroxyrotenone **(187)** (IC_{50} =8.67 ± 2.6 μM) exhibited moderate activity (Batista *et al.*, 2009). Flavones, including some isolated from *Tephrosia* species have been reported to show antiplasmodial activities.

Table 4. 37. *In vitro* antiplasmodial activities of isolated compounds and crude extracts against 3D7 strains of *P. falciparum*.

Compounds	IC_{50}, μM
Chalcones	
E-2',6'-Dimethoxy-4',5'-(2",2"-dimethyl)pyranoretrochalcone (**168)**	9.20 ± 1.42
Z-2',6'-Dimethoxy-4',5'-(2",2"-dimethyl)pyranoretrochalcone **(169)**	9.75 ± 0.81
3",4"-*cis*-4"-Ethoxy-3"-hydroxypraecansone B **(170)**	2.48 ± 0.22
Obovatachalcone **(147)**	4.23 ± 1.11
Praecansone B **(146)**	4.14 ± 0.26
E-Praecansone A **(145)**	6.45 ± 0.48
Flavanone	
Obovatin methyl ether **(73)**	3.69 ± 0.34
Pterocarpanes	
Rhocarpin **(188)**	10.25 ± 0.21
Pisatin **(185)**	5.88 ± 1.46
3,4:8,9-Dimethylenedioxypterocarpene **(167)**	> 40
Flavans	
Rhodiflavan A **(191)**	7.32 ± 1.87
Rhodiflavan B **(192)**	5.72 ± 1.91
Rhodiflavan C **(193)**	7.0 ± 2.4
Quercetol B **(98)**	7.45 ± 0.32
Tephrowatsin A **(79)**	14.55 ± 0.78
Flavone	
Isopongaflavone **(59)**	8.19 ± 1.48
Rotenoid	
6-Hydroxyrotenone **(187)**	8.67 ± 2.6
Crude extracts	
T. aequilata (root) *	100% active at 10 μg/ml
T. rhodesica (root)*	100% active at 10 μg/ml
Standards	
Chloroquine	0.0047
Artesunate	0.00067

Crude extract *

Some of the flavones isolated from the stem of *T.purpurea* ssp. *leptostachya* were also tested for antiplasmodial activity against the D6 strain of *Plasmodium falciparum* (Table 4.38). Among

these, (*E*)-5-hydroxytephrostachin (**198**) showed very good activity, (IC_{50} 1.7 ± 0.1 μM) (Batista *et al.*, 2009), while terpurlepflavone (**199**) and tachrosin (**41**) showed weak antiplasmodial activity against the D6 strain of *Plasmodium falciparum*. The compounds were also tested for cytotoxicity against two normal and two cancerous cell-lines (Table 4.35). Most of these compounds did not show cytotoxicity (IC_{50} > 100 μM) while compound **198** showed IC_{50} between 21-100 μM against these cell-lines, which is still significatly lower than its antiplasmodial activity with a selectivity index >12.

Table 4.38. *In vitro* antiplasmodial activity and cytototoxicity of compounds (IC_{50}, μM).

Compounds	Antiplasmodial activity against *P. falciparum*	Cytotoxicity			
	D6	LO2*	BEAS*	A549**	HepG2**
(E)-5-Hydroxytephrostachin **(198)**	1.7±0.1	21.7±4.8	24.5±2.7	76.1±2.9	>100
Purleptone **(194)**	NT	>100	>100	>100	>100
Terpurlepflavone **(199)**	14.8±3.2	>100	>100	>100	>100
Tachrosin **(41)**	27.1±3.2	>100	>100	>100	>100
Chloroquine	0.037±0.003				
Artesunate-Mefloquine	0.075±0.006				

*Normal cell: LO2, Immortal human hepatic cell line; BEAS, Lung/bronchus cell line (epithelial virus transformed);

**Cancer cell: A549, adenocarcinomic human alveolar basal epithelial cells; HepG2, human liver cancer cell line.

NT = Not Tested.

4.3.2. In-Vivo Anti-inflammatory and Antinociceptive Activity

The crude extracts of *T. rhodesica* (roots) and *T. purpurea* ssp. *leptostachya* (Stem) and some of the isolated compounds along with some derivatives were tested for *in-vivo* anti-inflammatory and anti-nocieptive activities using formalin induced test. In the formalin induced test, the periods which a mouse takes in lifting, licking and biting the injected paw was considered as a response for the induced pain and this period was recorded. Two phases (periods) were recorded: the first five minutes after formalin injection was considered as the early phase which is caused by direct chemical stimulation of nociceptors. The next fifteen to thirty minutes was considered as late phase, which is caused by the release of inflammatory mediators after formalin injection.

The collected data was analysed using analysis of variance (ANOVA) method followed by Tukey's *post-hoc* test. The values with $P < 0.05$ were considered to be significant. The crude extracts and the compounds tested showed reduction of pain in anti-nocieptive activity in both early and late phases (Table 4.39). Among the tested compounds pyrazopraecansone B (**201)** (Early (63%) and late (53%)), isopongaflavone (**59**) (early phase: 58%, and late phase: 51%) and tachrosin **(41)** (early phase: 50.4 %, and late phase: 49.2 %) showed a high significant ($p<0.001$) decrease in pain in both phases. It is worth noting that pyrazopraecansone B (**201)** was the most active compound and its activity is comparable to the standard drug diclofenac (Table 4.39).

Table 4.39. *In vivo* antinociceptive activities of isolated compounds and crude extracts

Treatment	Dose (mg/kg)	Time spent in pain behavior in seconds(% pain reduction)	
		Early phase (0-5min)	Late phase (15-30min)
Vehicle	0	72.2 ± 17.05	37.8 ± 13.29
(*E*)-5-Hydroxytephrostachin **(198)**	50	40 ± 5.7 (44.6 %)	36.8 ± 3.76 (2.6 %)
D-Pinitol **(165)**	50	37.6 ± 6.69 (47.9 %)	29.8 ± 5.97 (21.1 %)
Kaempferitrin **(200)**	50	36.8 ± 4.43 (49 %)	21.2 ± 7.91 (44 %)
Tachrosin **(41)**	50 ***	35.8 ± 11.84 (50.4 %)	19.2 ± 7.32 (49.2 %)
Tephrinone (**77**)	50	44.0 ± 8.51 (39 %)	30.8 ± 8.70 (18.5 %)
Rhodiflavan C (**193**)	50	46.1 ± 8.27 (36 %)	37.2 ± 4.76 (2 %)
Rhodiflavan A (**191**)	50	38.6 ± 6.84 (47%)	29.2 ± 6.14 (23 %)
Isopongaflavone (**59**)	50 ***	30.6 ± 5.85 (58 %)	18.6 ± 4.03 (51 %)
Pyrazoisopongaflavone (**202**)	50	48.6 ± 11.37 (33 %)	24.6 ± 5.54 (35 %)
Pyrazopraecansone B (**201**)	50 ***	26.4 ± 6.80 (63 %)	17.8 ± 2.77 (53 %)
*T. rhodesica**	100	48.2 ± 5.87 (34%)	32.4 ± 9.63 (14.2%)
*T. purpurea ssp. Leptostachya**	100	36.8 ± 2.77 (49%)	20.0 ± 4.35 (47%)
Diclofenac	25	33.6 ± 11.41 (53.4%)	14.4 ± 11.58 (62%)

*Crude extracts; *** p<0.001

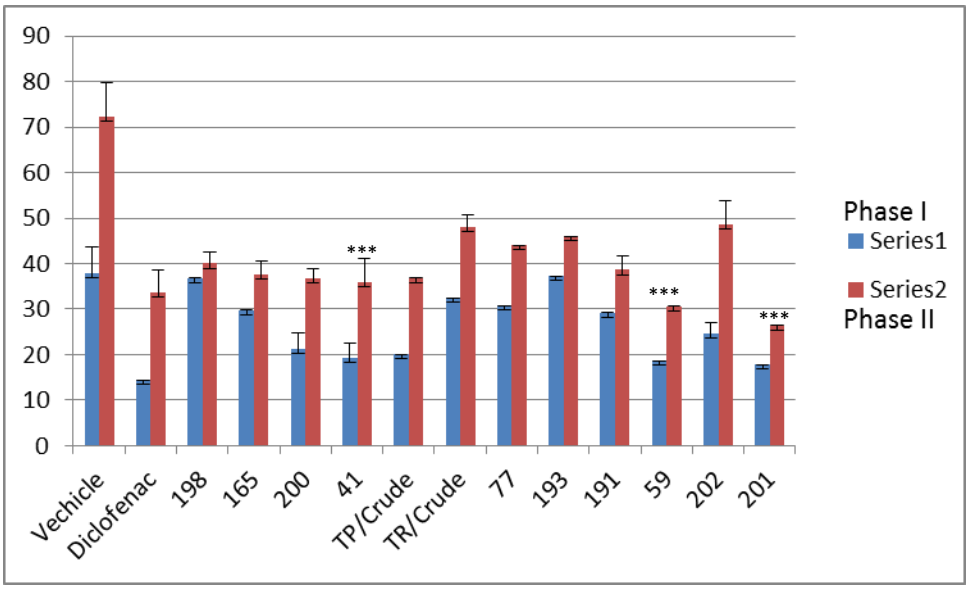

Figure 4.15: Effects of the tested compounds, crude extracts and diclofenac in early and late phase of formalin test.

The anti-inflammatory activity of the crude extracts and the isolated compounds was also measured using the formalin test and showed reduction of hind paw circumference compared with the vehicle (Table 4.40). Among the tested compounds, kaempferitrin **(200)** (30%), tephrinone (**77**) (25.6%) and rhodiflavan C (**193**) (25.5%) showed comparable reduction of the hind paw circumference to diclofenac (at 25 mg/kg 23 %). However the rest of the tested compounds did not exhibited significant reduction at a dose of 50 mg/kg.

Table 4.40. *In vivo* anti-inflammatory activities of isolated compounds and crude extracts

Treatment	Dose (mg/kg)	Size of hind paw circumference (% reduction)
Vehicle		1.224 ± 0.212
(*E*)-5-Hydroxytephrostachin **(198)**	50	1.014 ± 0.118 (17%)
D-Pinitol **(165)**	50	1.002 ± 0.218 (18%)
Kaempferitrin **(200)**	50	0.854 ± 0.221 (30%)
Tachrosin **(41)**	50	0.966 ± 0.359 (21%)
Tephrinone (**77**)	50	0.91 ± 0.279 (26%)
Rhodiflavan C (**193**)	50	0.912 ± 0.355 (26%)
Rhodiflavan A (**191**)	50	1.078 ± 0.076 (12%)
Isopongaflavone (**59**)	50	0.986 ± 0.258 (19%)
Pyrazoisopongaflavone (**202)**	50	1.064 ± 0.111 (13%)
pyrazopraecansone B (**201)**	50	1.062 ± 0.150 (13%)
Crude extract *T. rhodesica*	100	1.166 ± 0.476 (21%)
Crude extract *T. purpurea*	100	0.968 ± 0.061 (5%)
Diclofenac	25	0.936 ± 0.118 (23%)

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In this study, six selected *Tephrosia* species were investigated and fifty compounds isolated and characterized. The conclusions drawn from this study are outlined below:

From the roots of *T. aequilata* four new compounds **(**aequichalcone A (**161**), aequichalcone B (**162**), aequichalcone C (**163**) and 3,4:8,9-dimethylenedioxy-6a,11a-pterocarpene (**164**)), along with seven known compounds were isolated. From the seedpods and leaf of *T. elata*, a total of nineteen compounds were isolated and characterized.

The roots of *T. rhodesica* gave twenty one compounds, including five new compounds (rhodimmer (**183**), rhocarpin (**190**), rhodiflavan A (**191**), rhodiflavan B (**192**), rhodiflavan C (**193**)). From the air dried stem of *T. purpurea* subsp. *leptostachya*, four new compounds (*(E)*-5-hydroxy-tephrostachin (**198**), purleptone (**194**), *(E)*-5-oxo- anhydrotephrostachin (**195**) and terpurlepflavone (**199**)) and seven known compounds were isolated.

From the stem of *T. noctiflora* four compounds, including two flavonoids were isolated and characterized. Four flavonoids were isolated and characterized from the aerial part of *T. pumila.*

The crude extracts and the isolated compounds were evaluated for antiplasmodial activities against the chloroquine-sensitive 3D7 and D6 strains of *P. falciparum*. Among the tested compounds (*E)*-5-hydroxytephrostachin (**198**) was the most active against the chloroquine-sensitive (D6) strain with a much lower cytotoxicity, while aequichalcone C (**170**) was the most active against the chloroquine-sensitive (3D7) strains.

The crude extracts of *T. rhodesica* and *T. purpurea* ssp. *leptostachya*) and some of the pure compounds were evaluated for anti-inflammatory and anti-nociceptive activities. Among the tested compounds pyrazopraecansone B (**201)** showed a very significant decrease in pain in both phases (63% and 53%) ($p<0.001$) followed by isopongaflavone (**59**) and tachrosin **(41)** which showed a significant decrease in pain compared to the diclofenac.

Two pyrazole (pyrazopraecansone B **(201)** and pyrazoisopongaflavone **(202)**) and one guanidine (guanidinoipongaflavone (**203)**) derivatives were prepared and were evaluated for anti-inflammatory and anti-nociceptive activities.

Overall from this study novel flavonoids were isolated from the studied *Tephrosia* species and some of these new flavonoids showed significant antiplasmodial and anti-inflammatory activities.

5.2 Recommendations

Furhter to the conclusions drawn, I make the following recommendations:

1. Further phytochemical investigation and HPLC profiling on sub species of *T. purpurea* should be done to establish their taxonomic relationships.
2. Further phytochemical investigation on the seedpods, leaves and stem parts of *T. rhodesica* should be examined in order to assess whether these plant parts also produce unique compounds as the roots of this plant.
3. Further phytochemical investigation on the fresh collections of *T. noctiflora* and *T. pumila* should be done to see if these plants have more compounds.
4. Further studies should be directed at testing the flavonoids of *Tephrosia* species for *in vivo* antiplsmodial activity and toxicity.
5. Further anti-inflammatory and anti-nociceptive activities should be done in different doses using animal and cell models.
6. The biosynthetic pathway of *Rhodiflavan C* (**193**) should be studied and X-Ray data should be generated.

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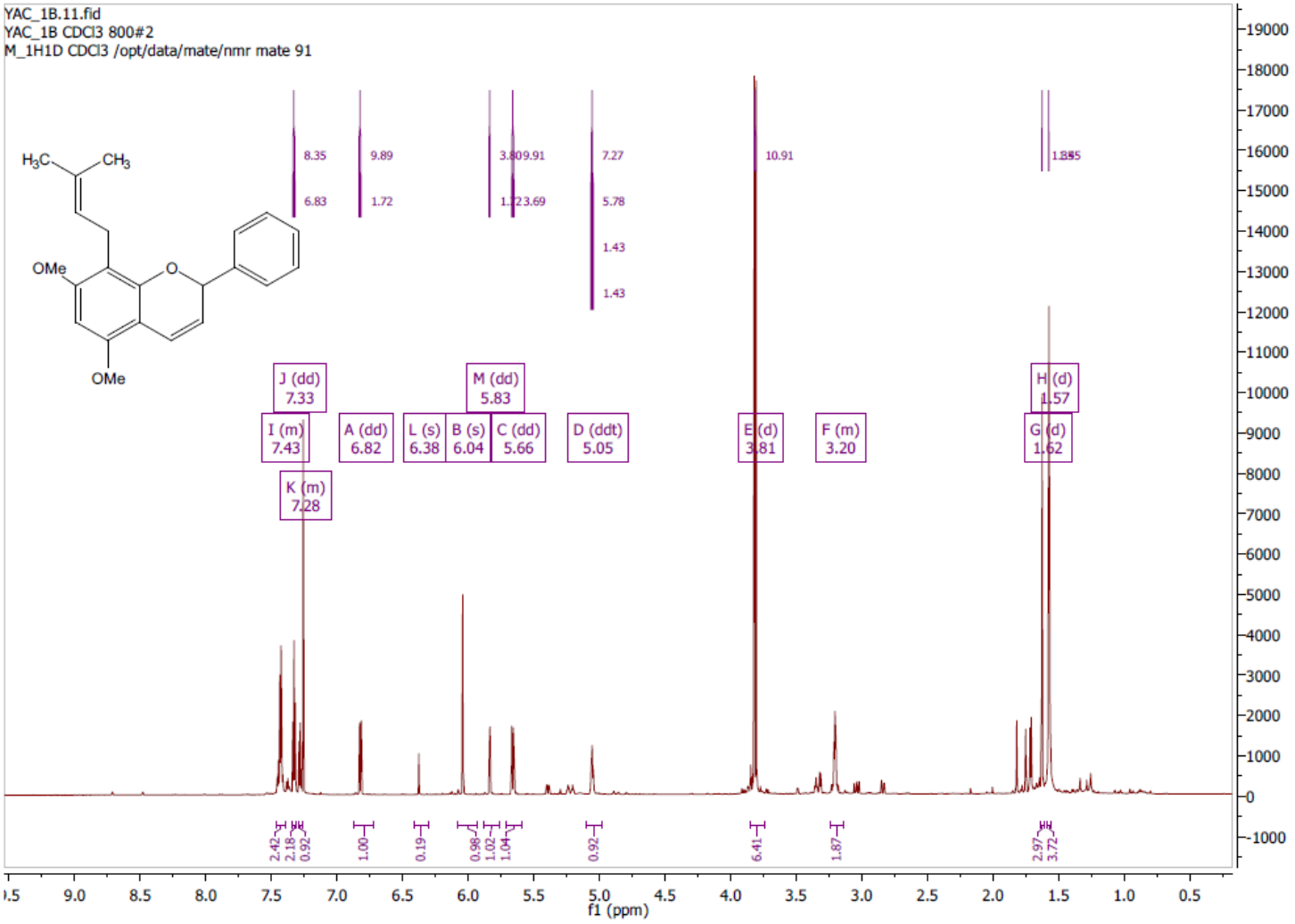
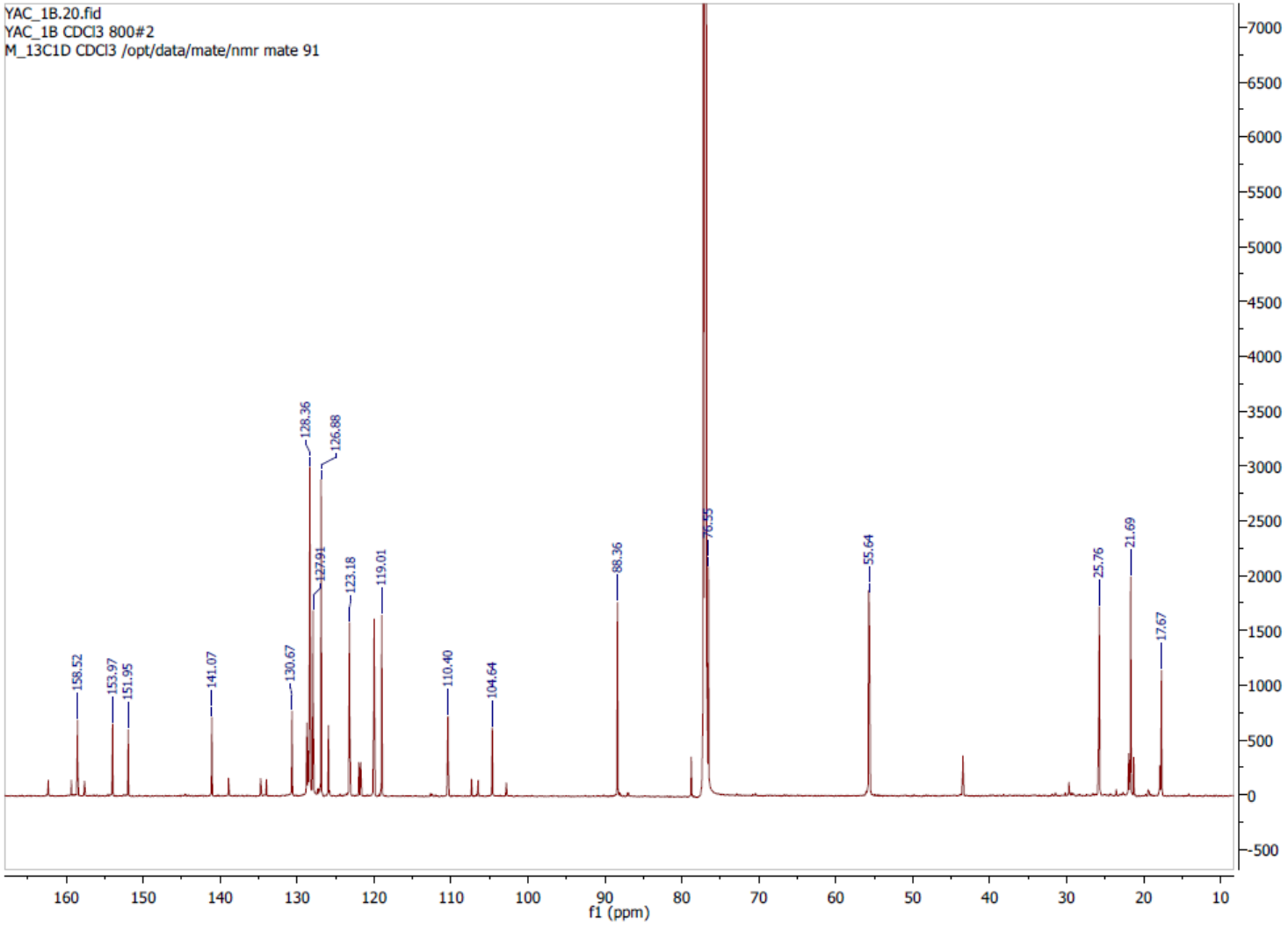
APPENDICES

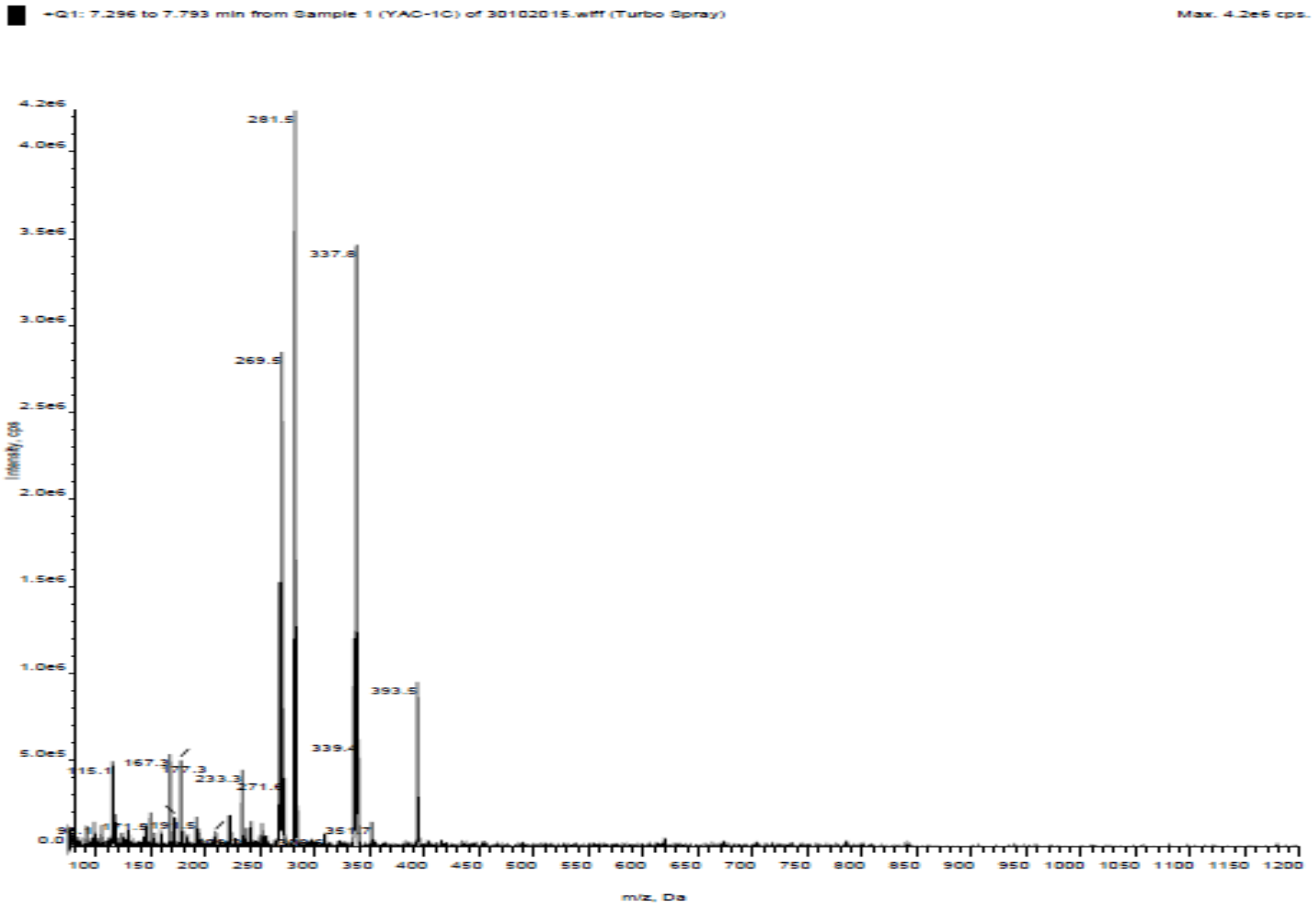
APPENDIX 1A: ^{1}H NMR Spectrum of Tephrowatsin B (100) (800 MHz; $CDCl_3$)



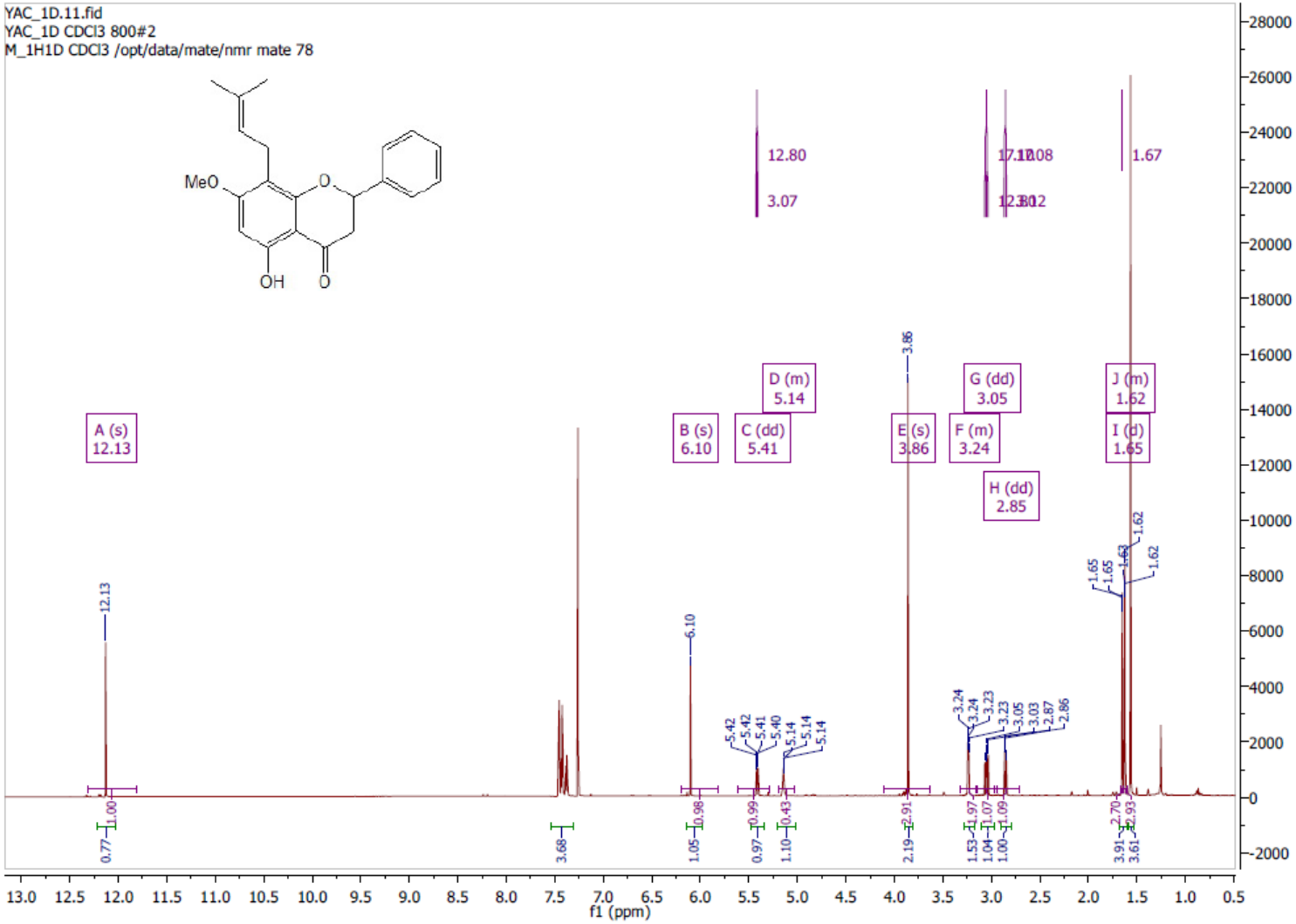
APPENDIX 1B: ^{13}C NMR Spectrum of Tephrowatsin B (100) (200 MHz; $CDCl_3$)



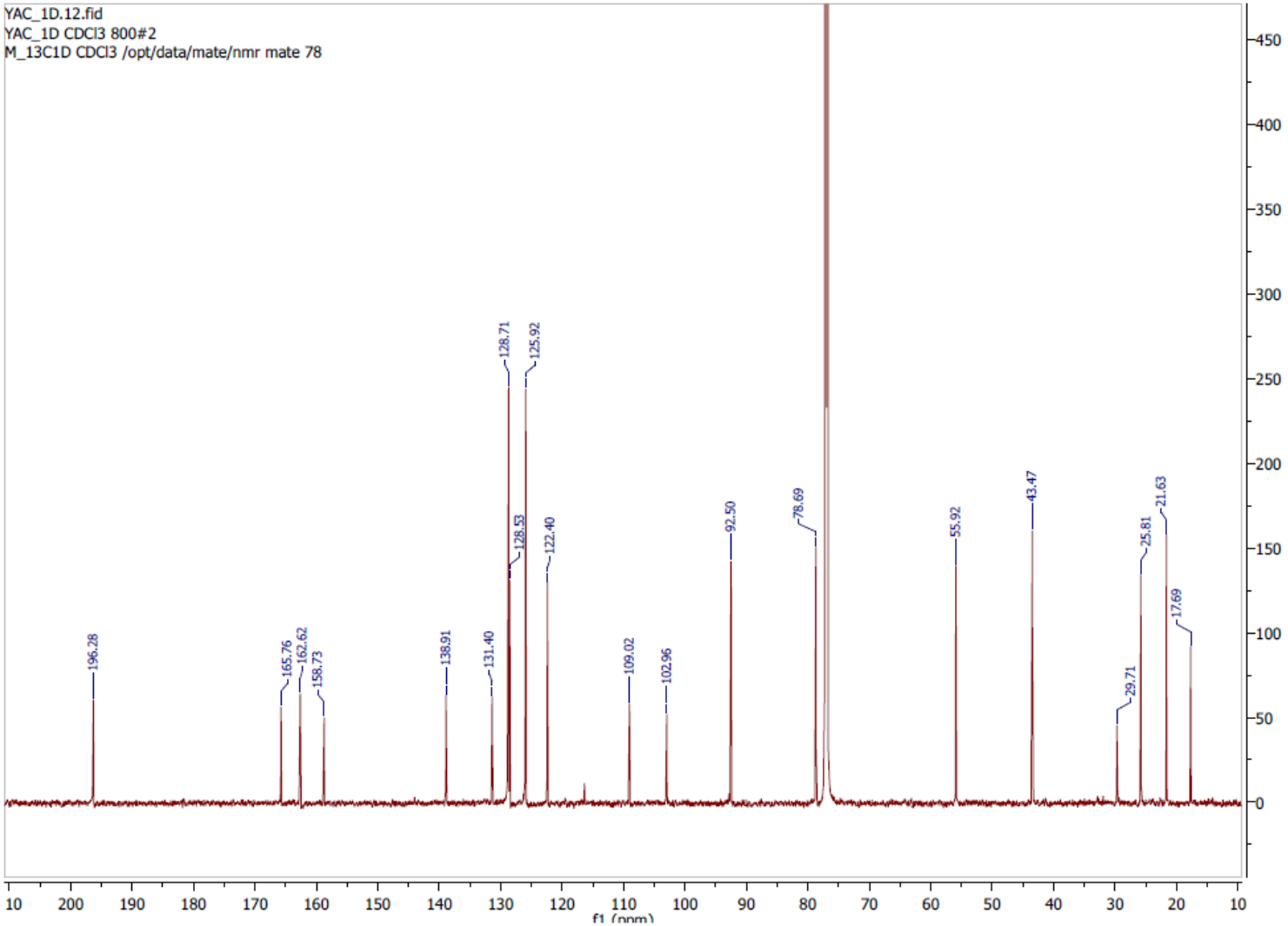
APPENDIX 1C: LCMS Spectrum of Tephrowatsin B (100)



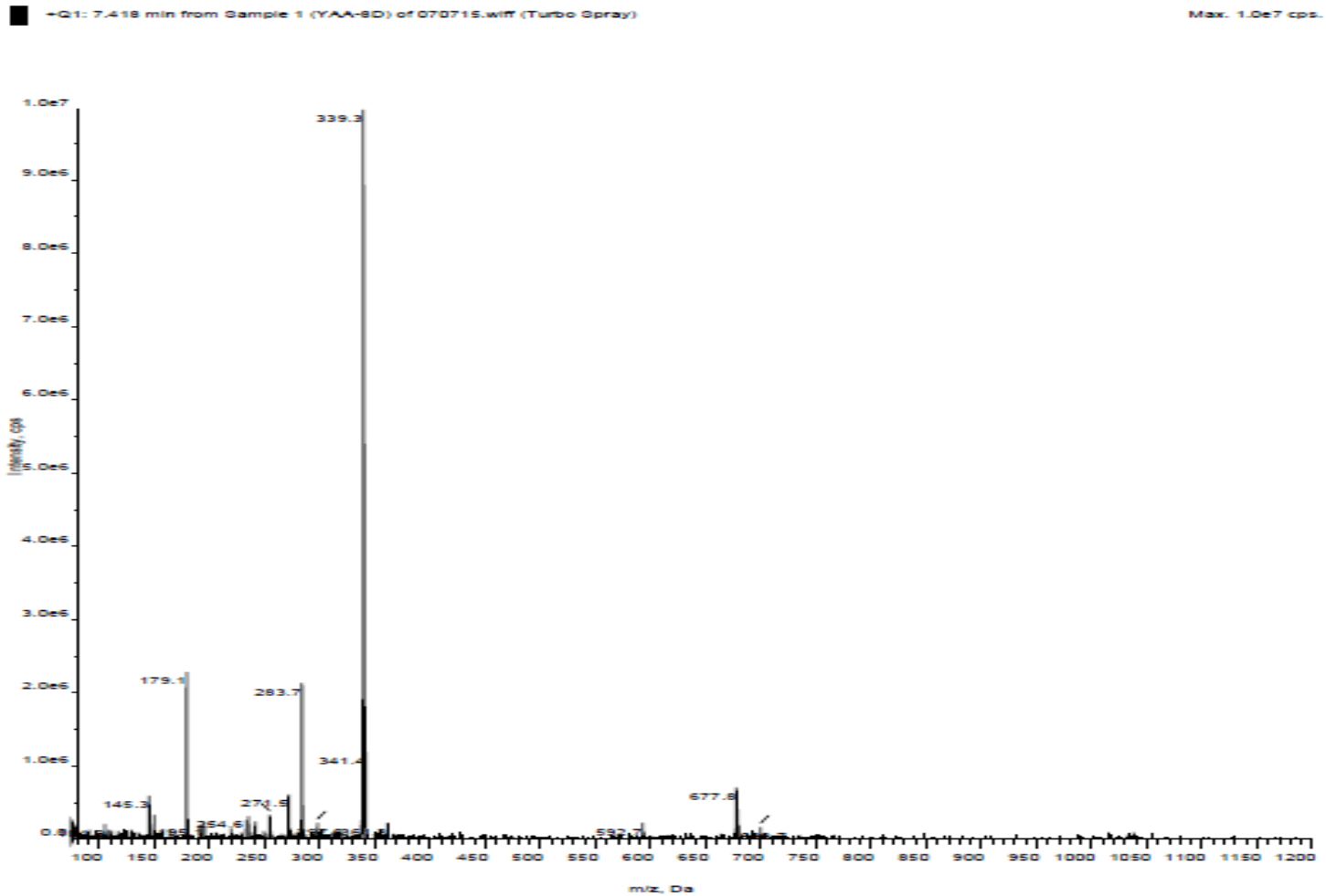
APPENDIX 2A: ^{1}H NMR Spectrum of Tephrodine (77) (800 MHz; $CDCl_3$)



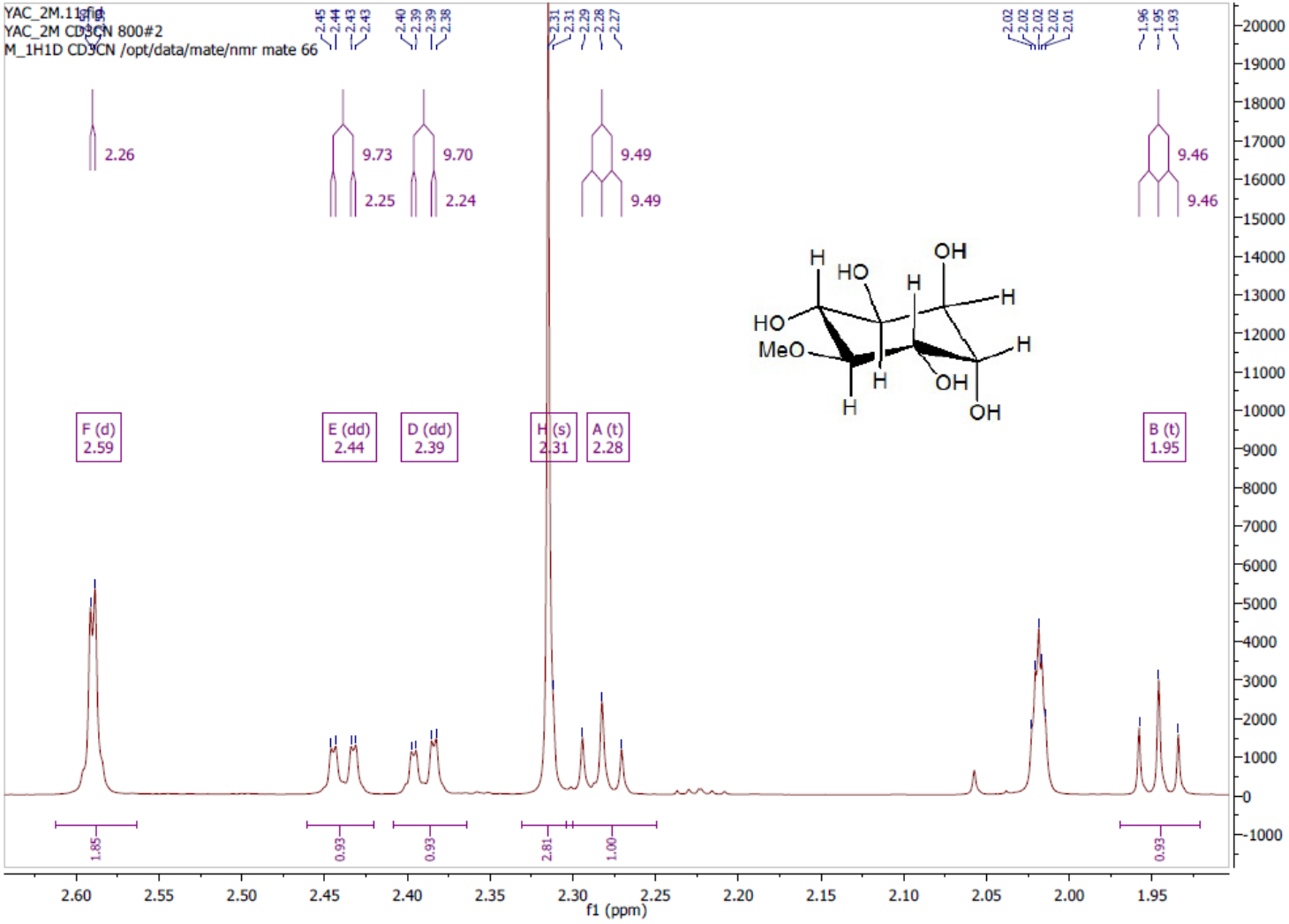
APPENDIX 2B: ^{13}C NMR Spectrum of Tephrodine (77) (200 MHz; $CDCl_3$)



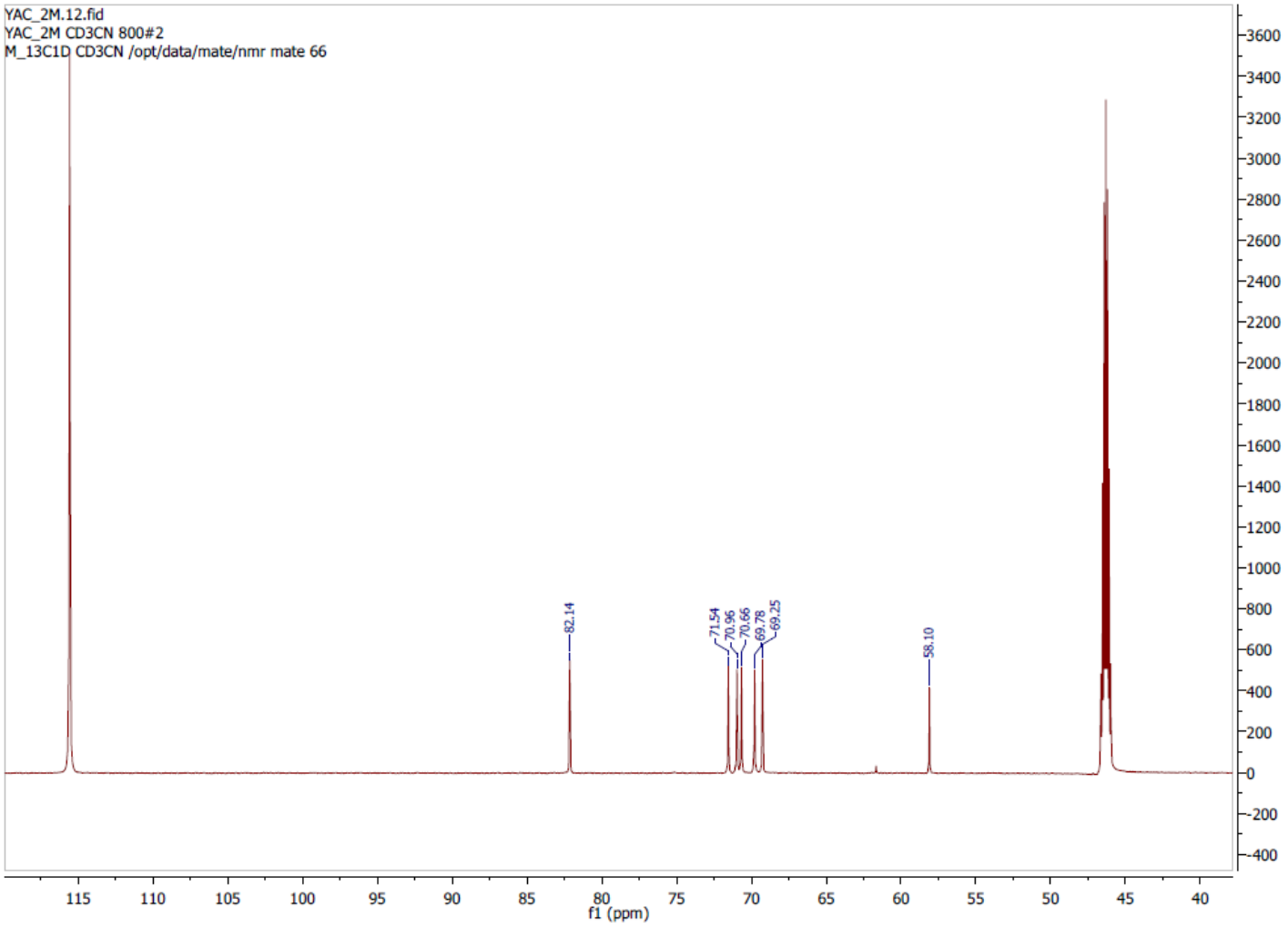
APPENDIX 2C: LCMS Spectrum of Tephrodine (77)



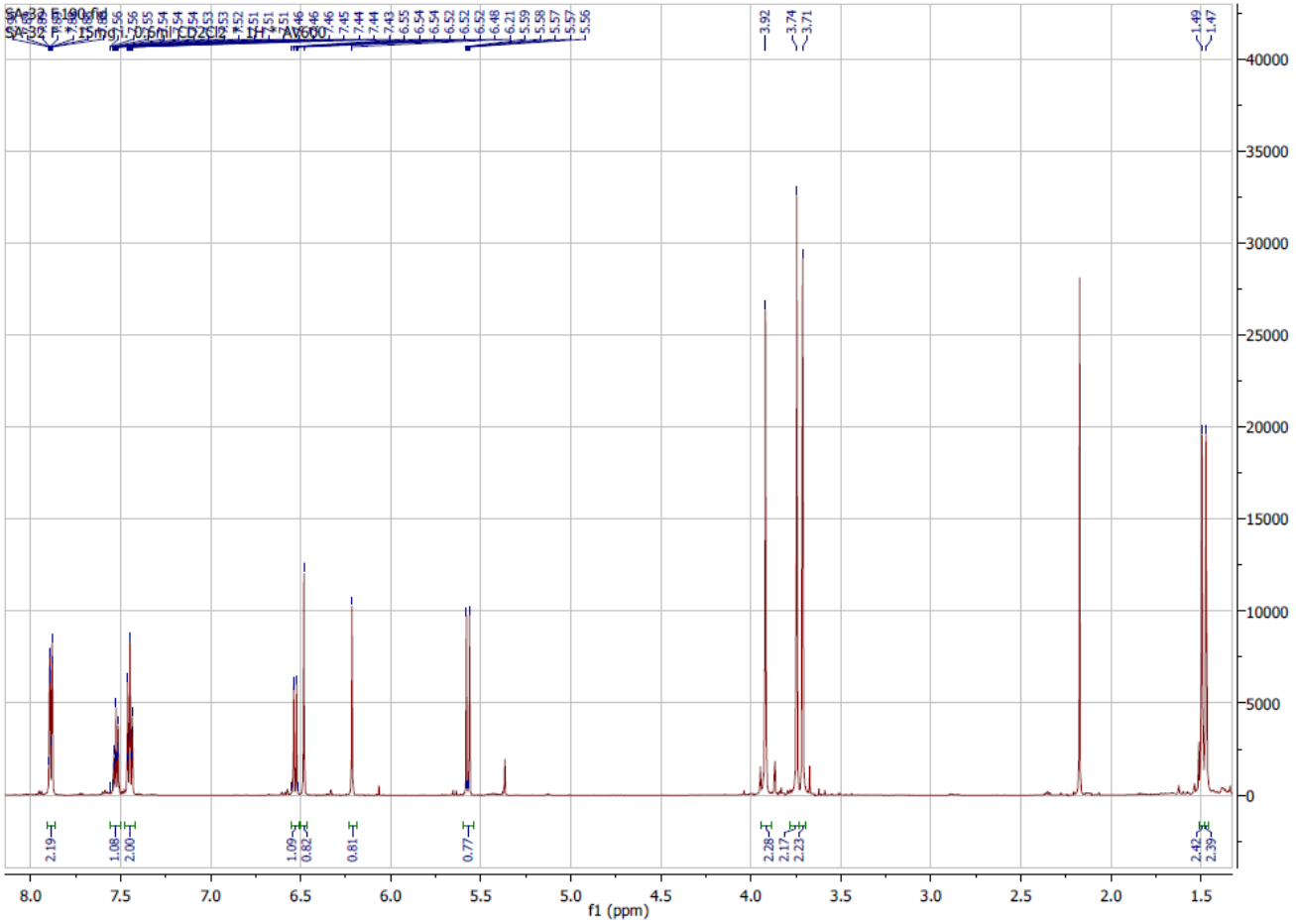
APPENDIX 3A: ^{1}H NMR Spectrum of D-pinitol (165) (800 MHz; CD_3CN)



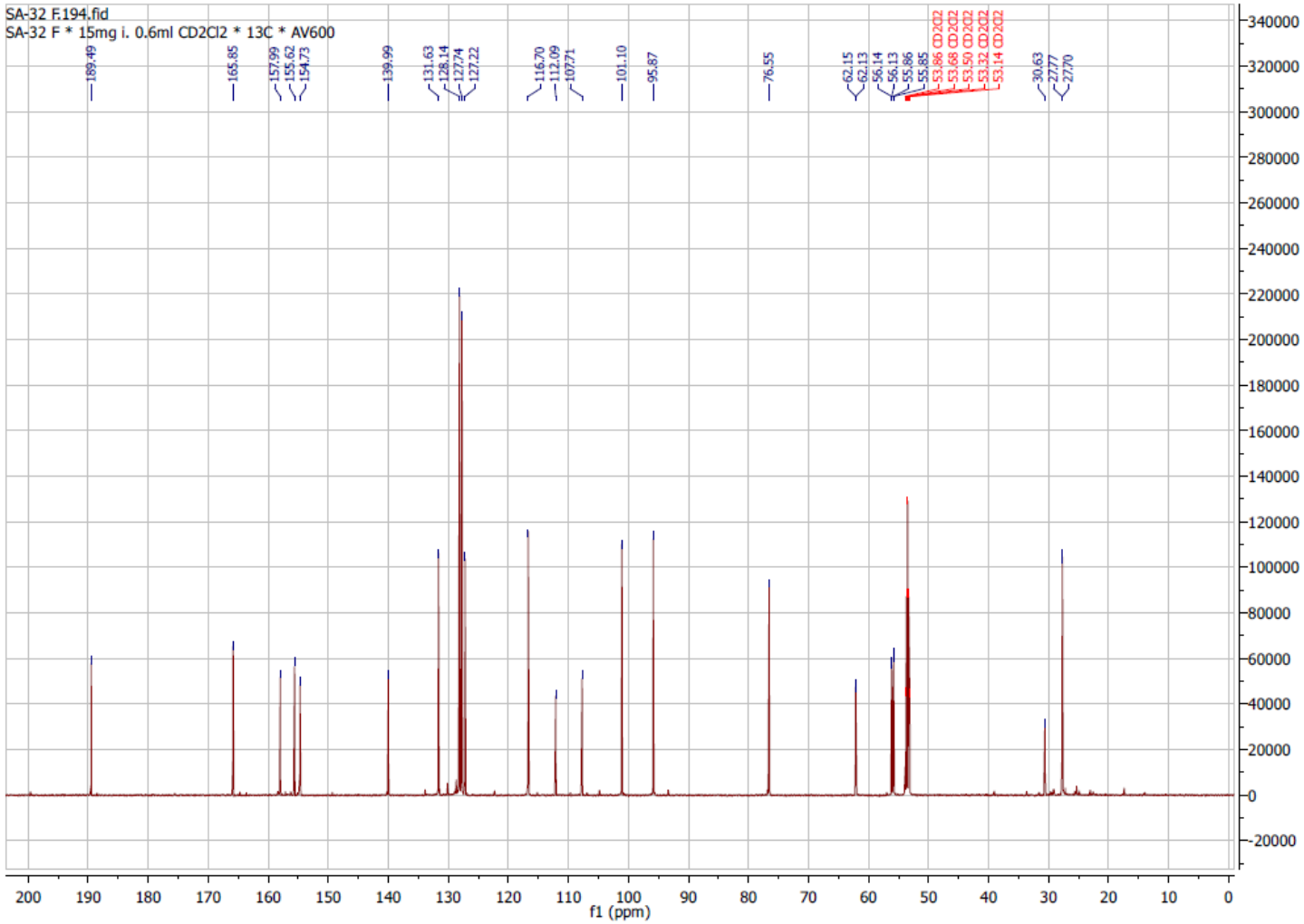
APPENDIX 3B: ^{13}C NMR Spectrum of D-pinitol (165) (200 MHz; CD_3CN)



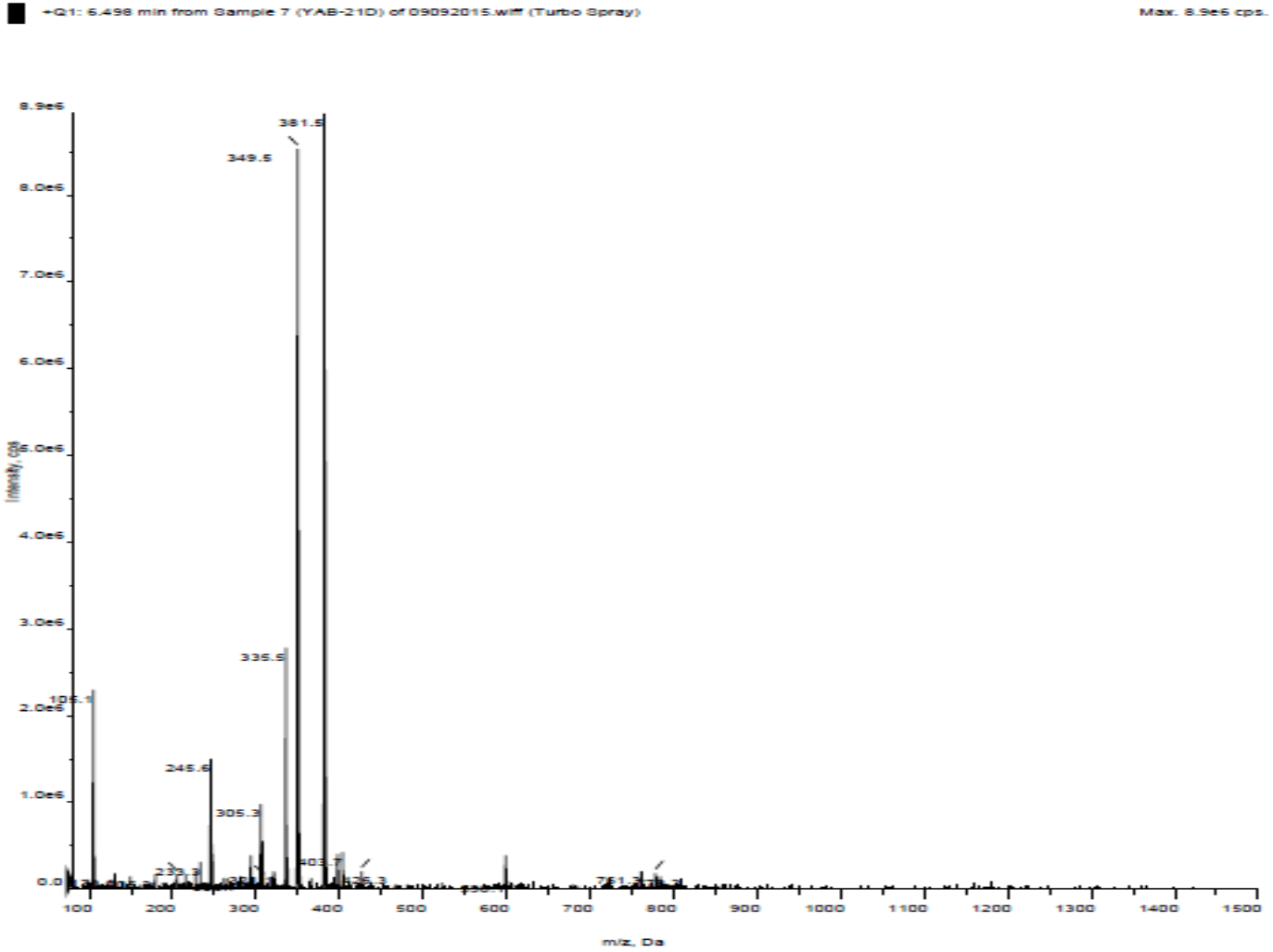
APPENDIX 4A: ^{1}H NMR Spectrum of (E)-Praecansone A (145) (600 MHz; CD_2Cl_2)



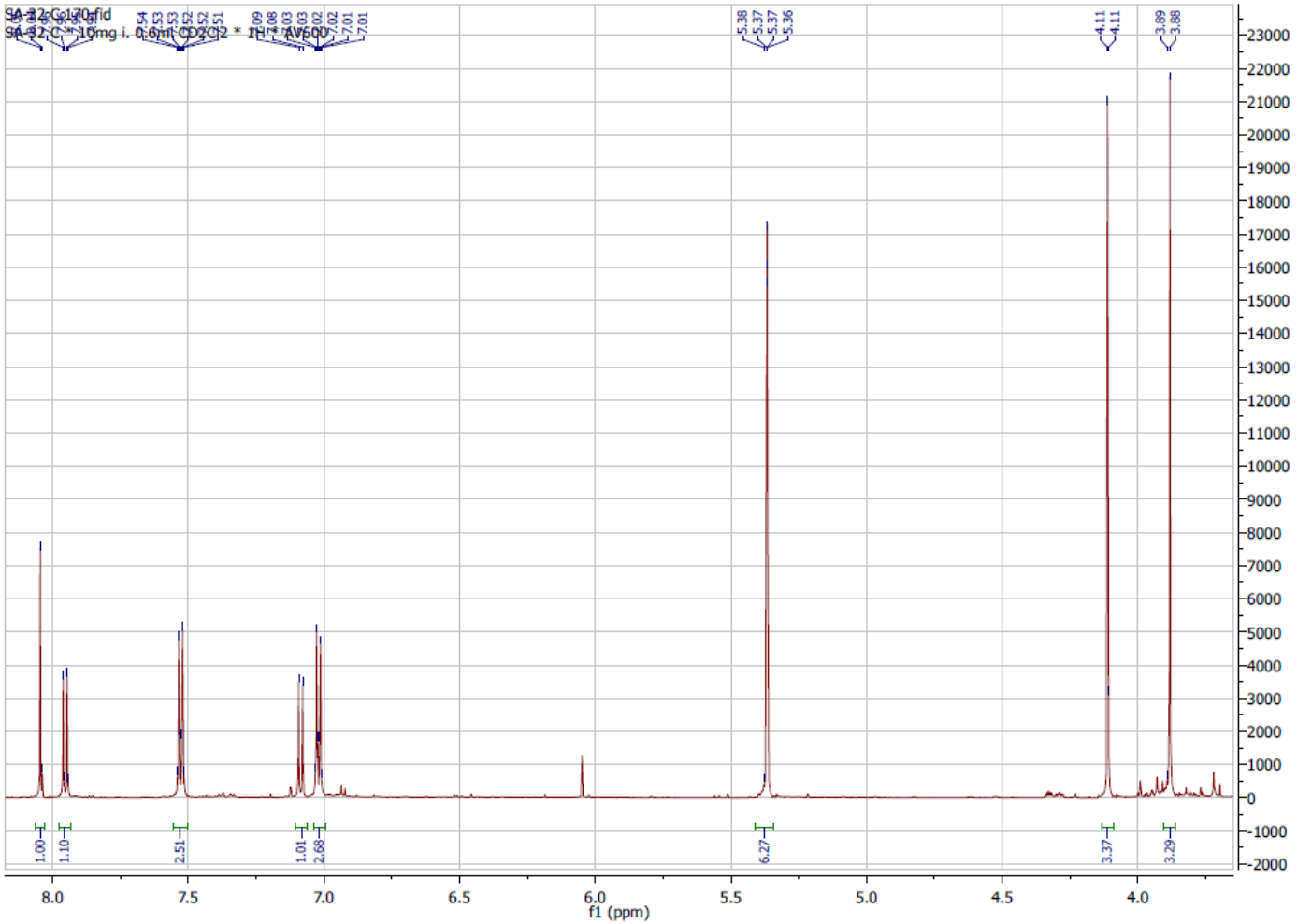
APPENDIX 4B: ^{13}C NMR Spectrum of (E)-Praecansone A (145) (150 MHz; CD_2Cl_2)



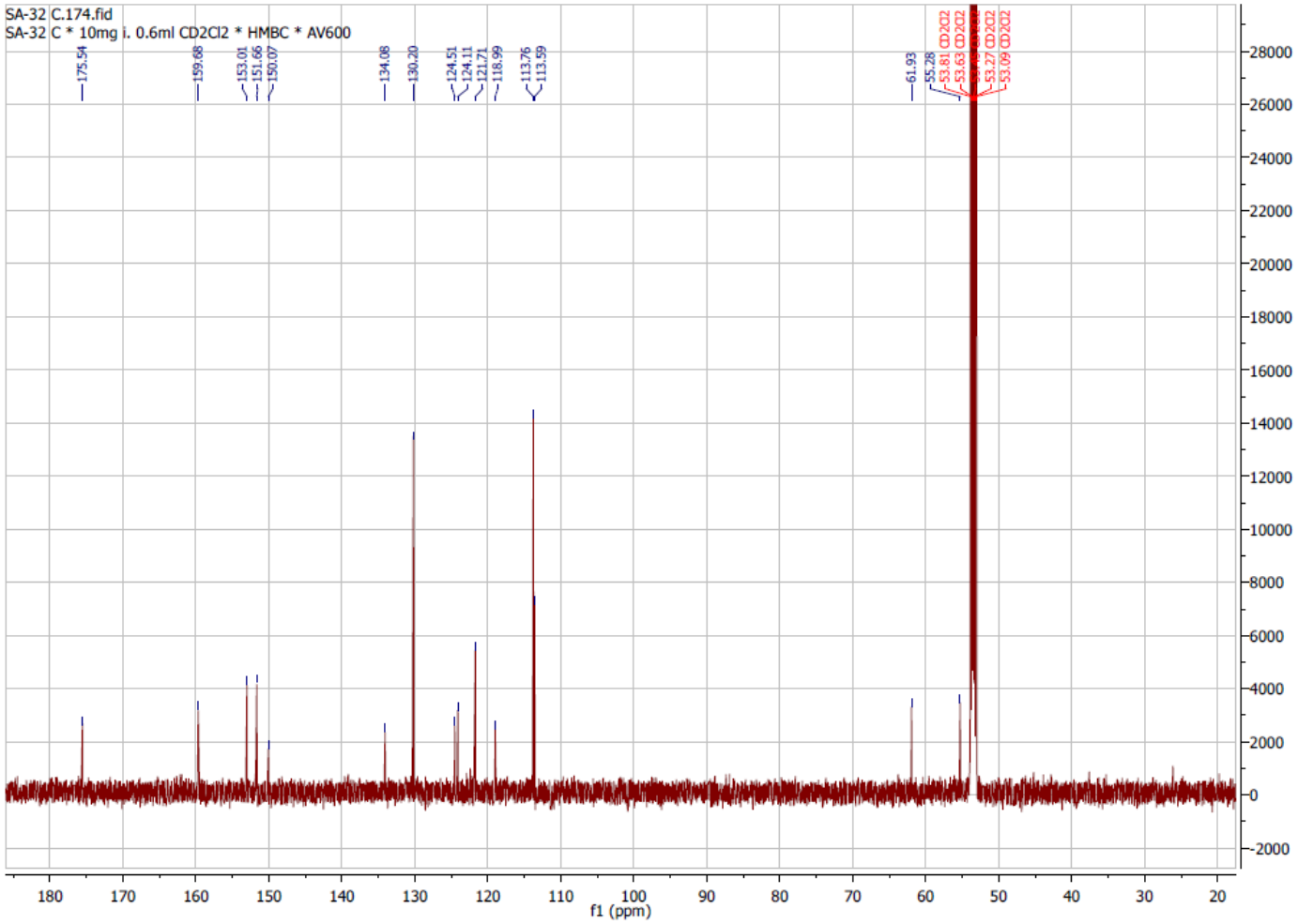
APPENDIX 4C: LCMS Spectrum of (E)-Praecansone A (145)



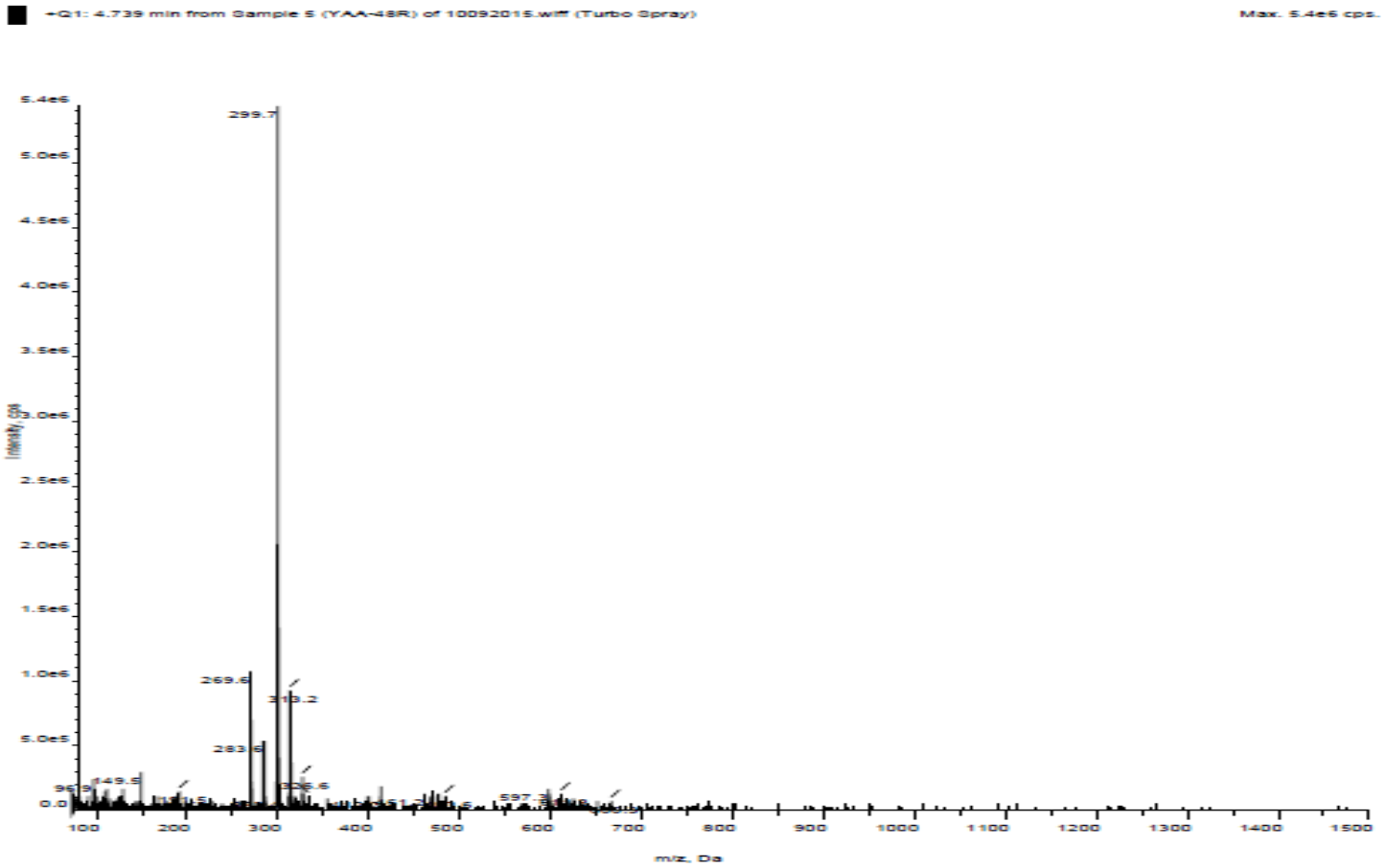
APPENDIX 5A: ^{1}H NMR Spectrum of 8-O-methylretusin (166) (600 MHz; CD_2Cl_2)



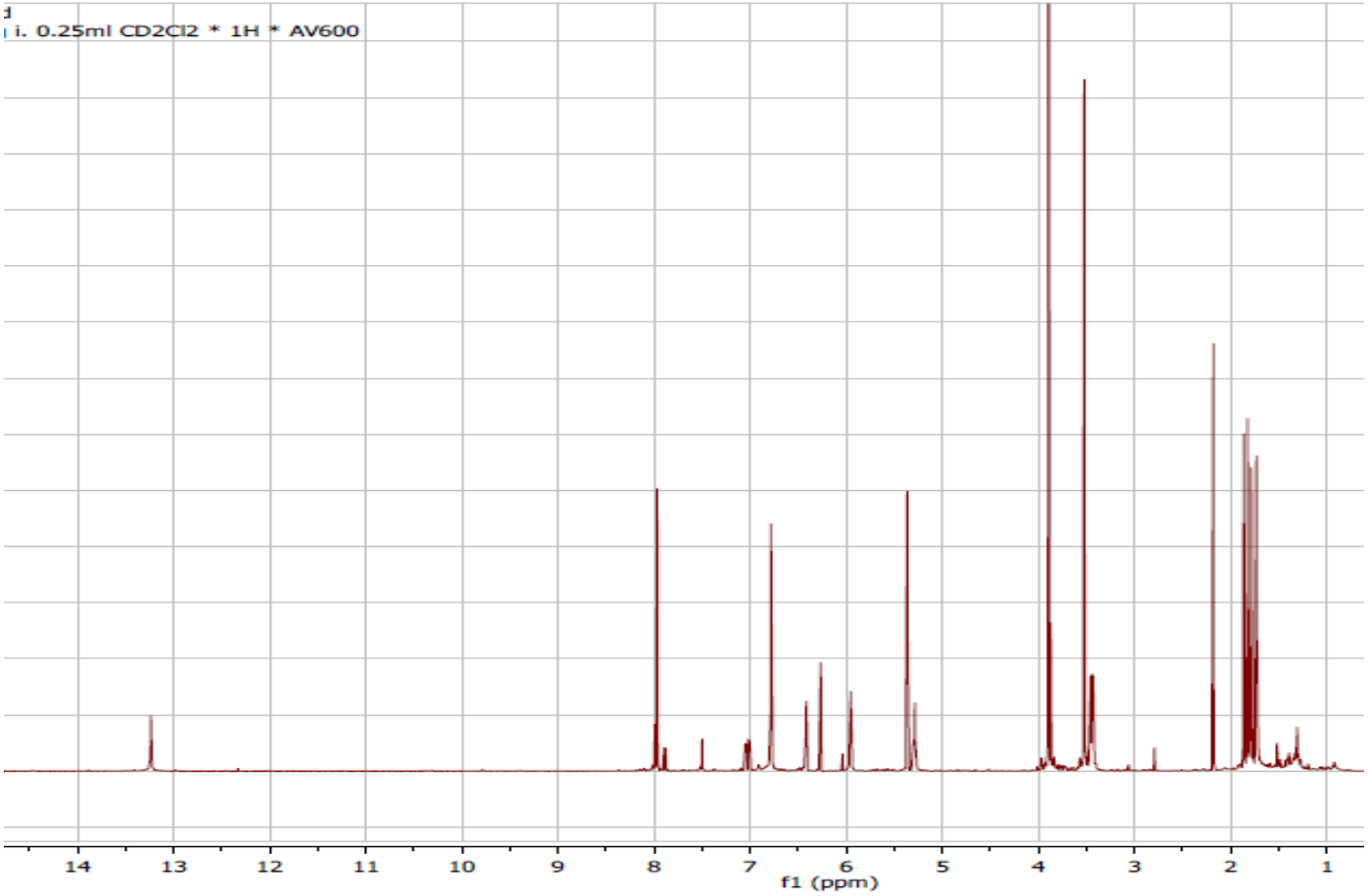
APPENDIX 5B: ^{13}C NMR Spectrum of 8-O-methylretusin (166) (150 MHz; CD_2Cl_2)



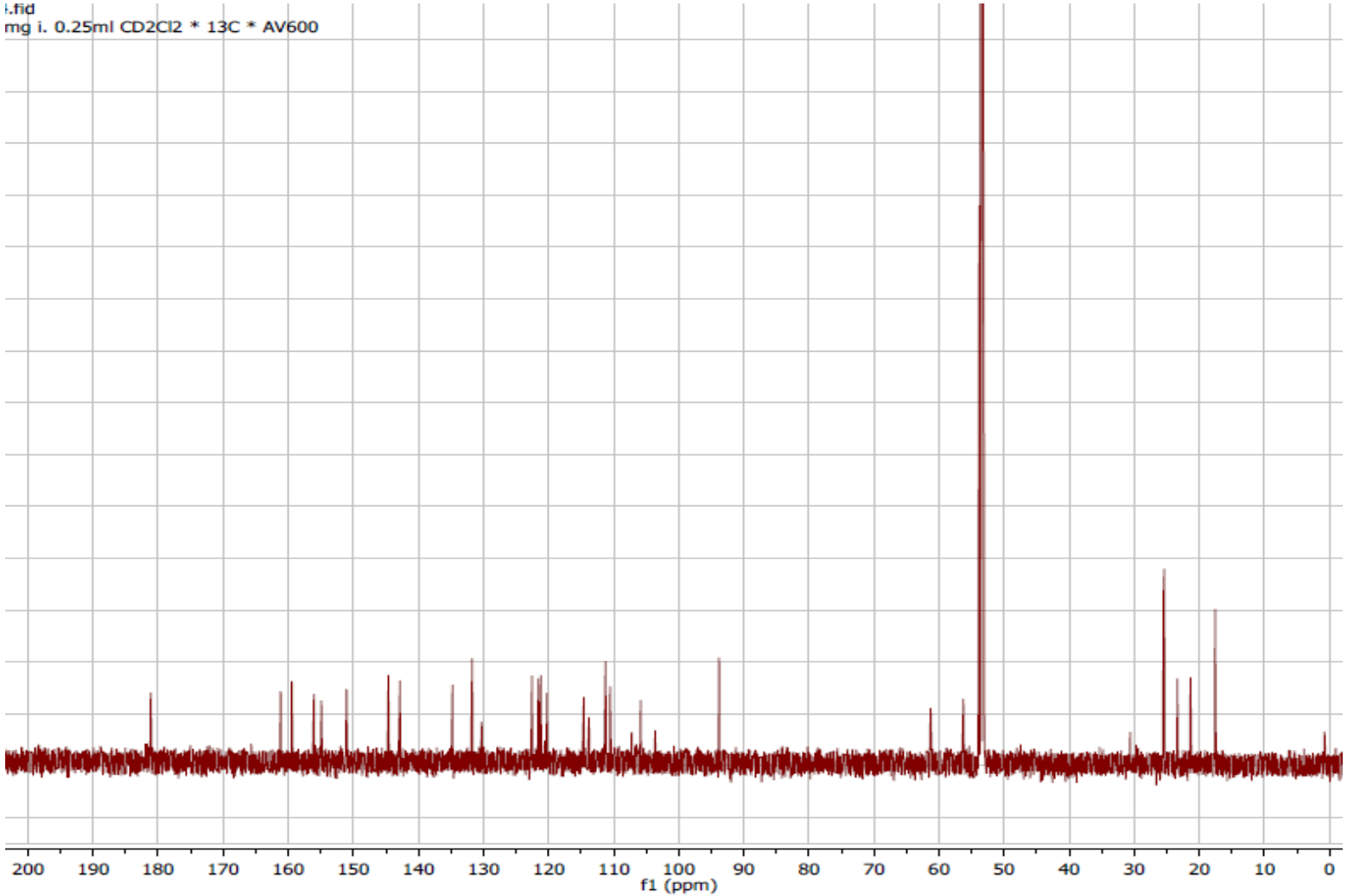
APPENDIX 5C: LCMS Spectrum of 8-O-methylretusin (166)



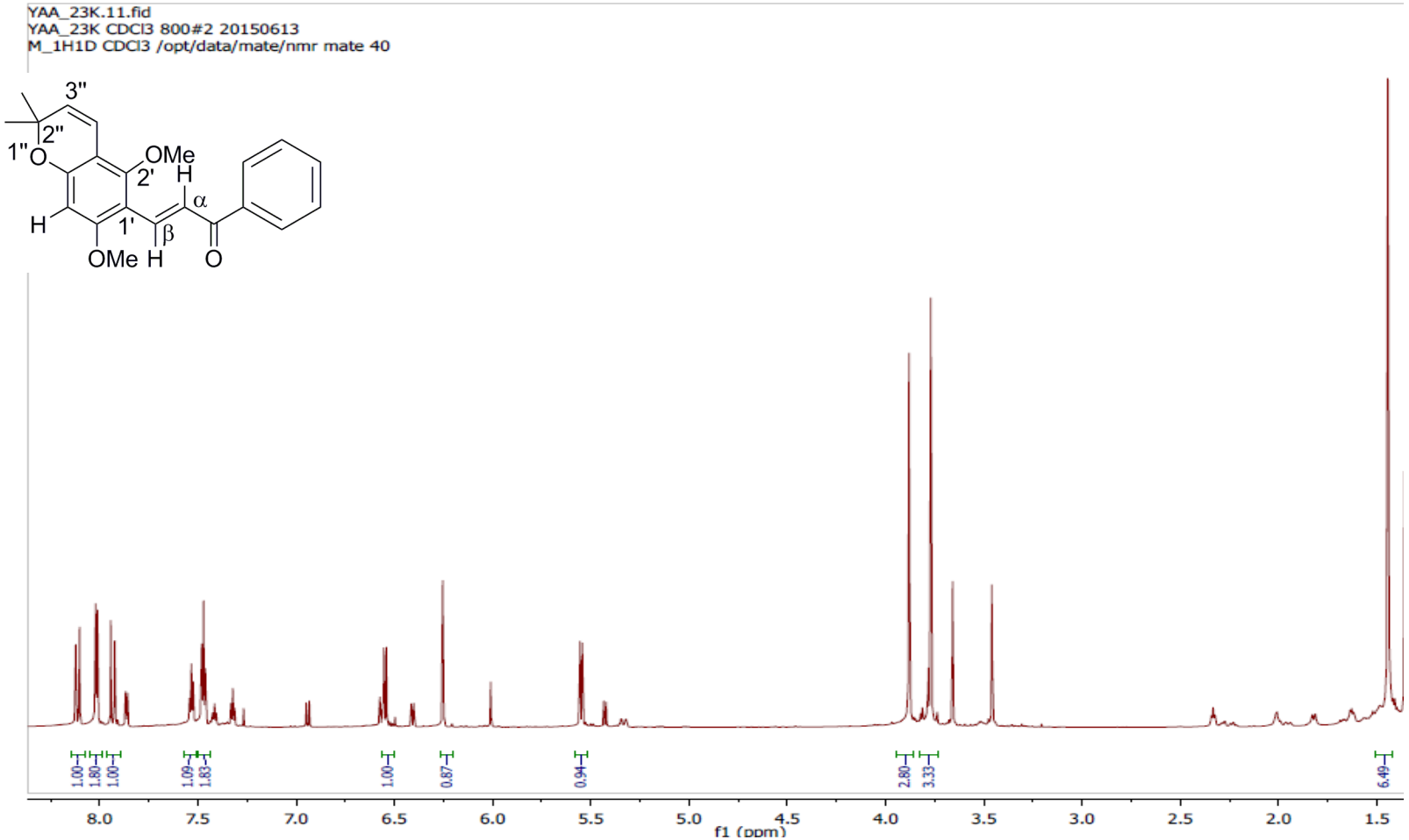
APPENDIX 6A: ^{1}H NMR Spectrum of Pumilaisoflavone C (117) (600 MHz; CD_2Cl_2)



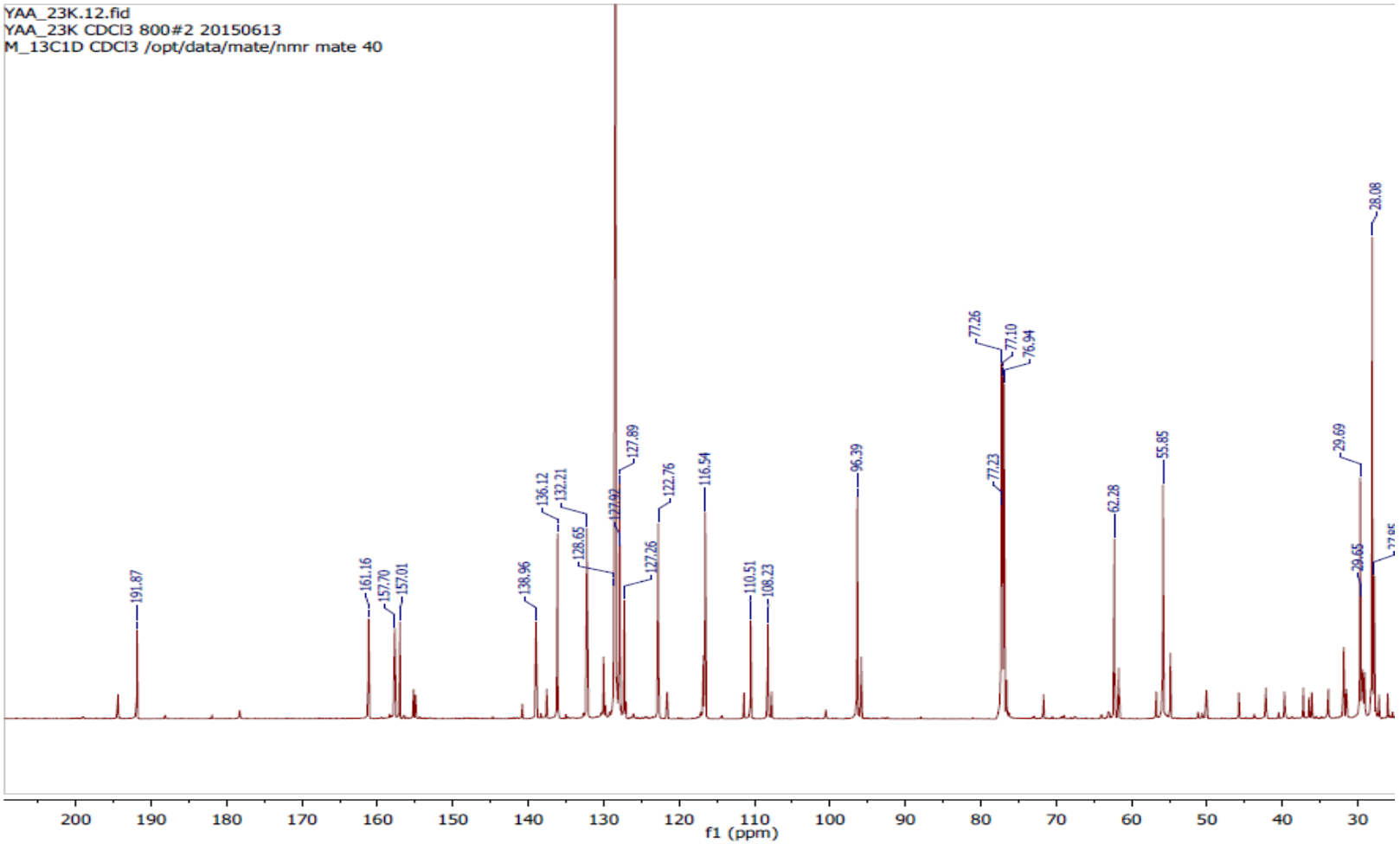
APPENDIX 6B: ^{13}C NMR Spectrum of Pumilaisoflavone C (117) (150 MHz; CD_2Cl_2)



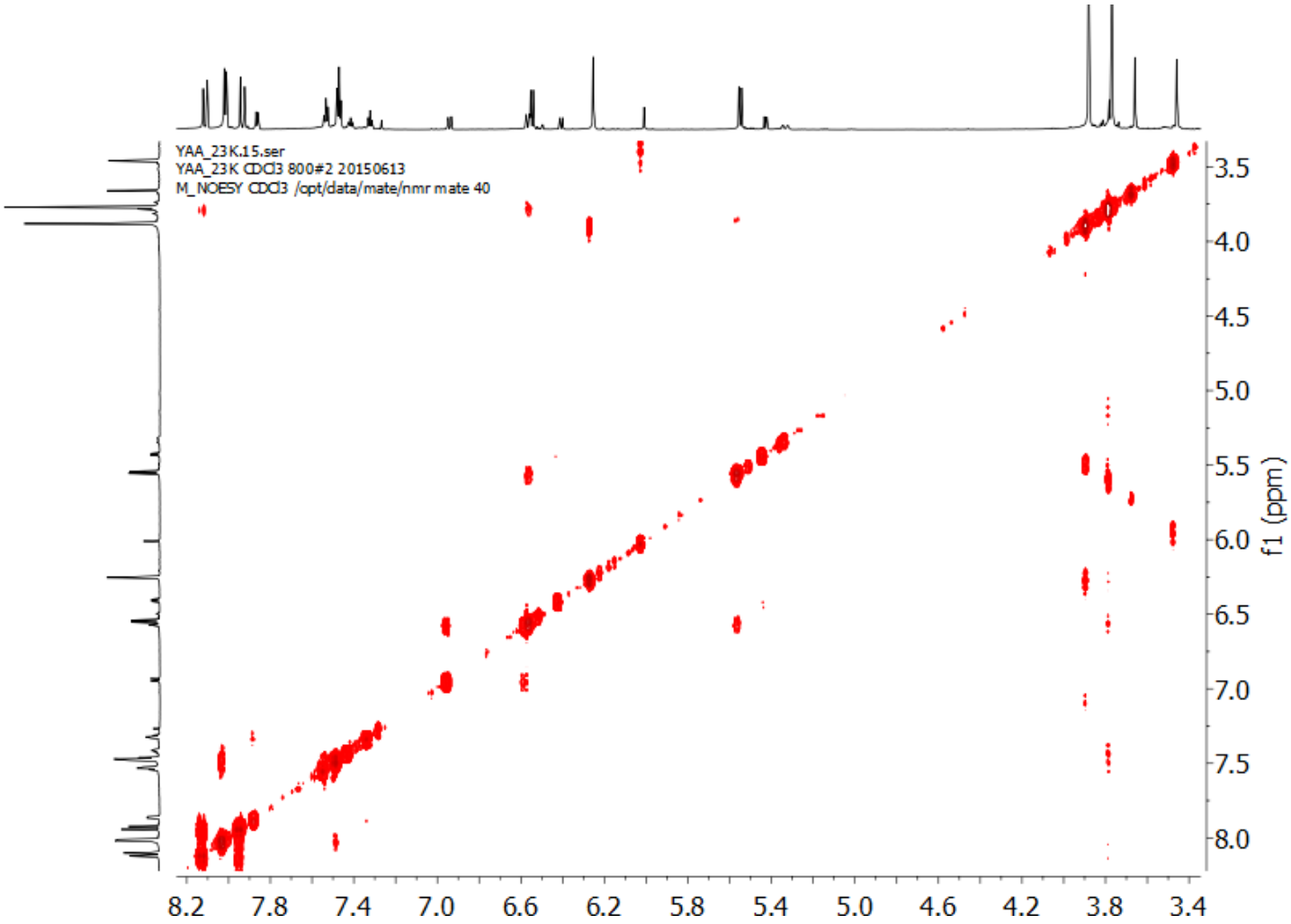
APPENDIX 7A: ^{1}H NMR Spectrum of Aeqchalcone A (168) (800 MHz; $CDCl_3$)



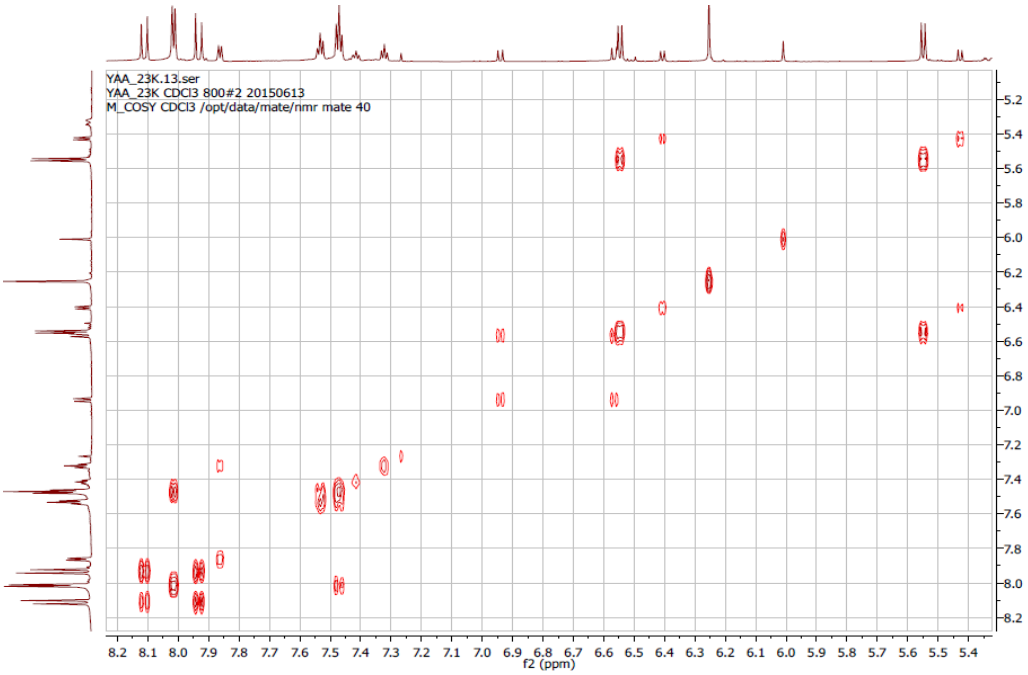
APPENDIX 7B: ^{13}C NMR Spectrum of Aeqchalcone A (168) (200 MHz; $CDCl_3$)



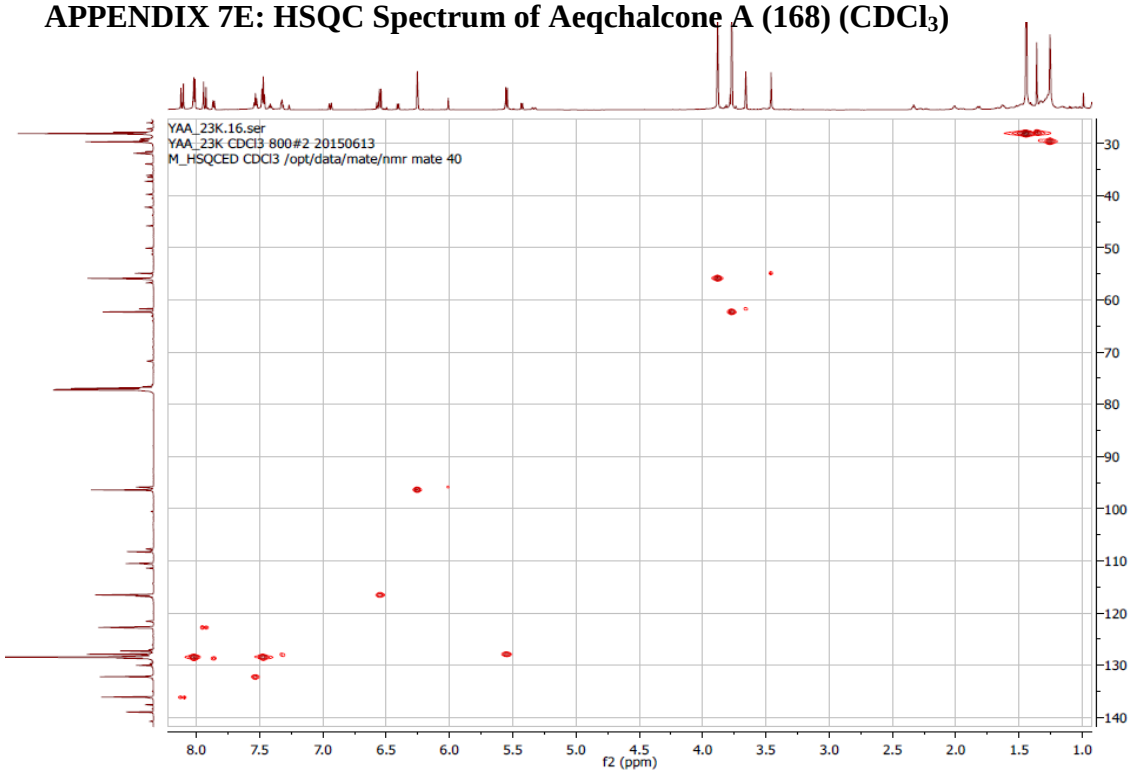
APPENDIX 7C: NOESY Spectrum of Aeqchalcone A (168) ($CDCl_3$)



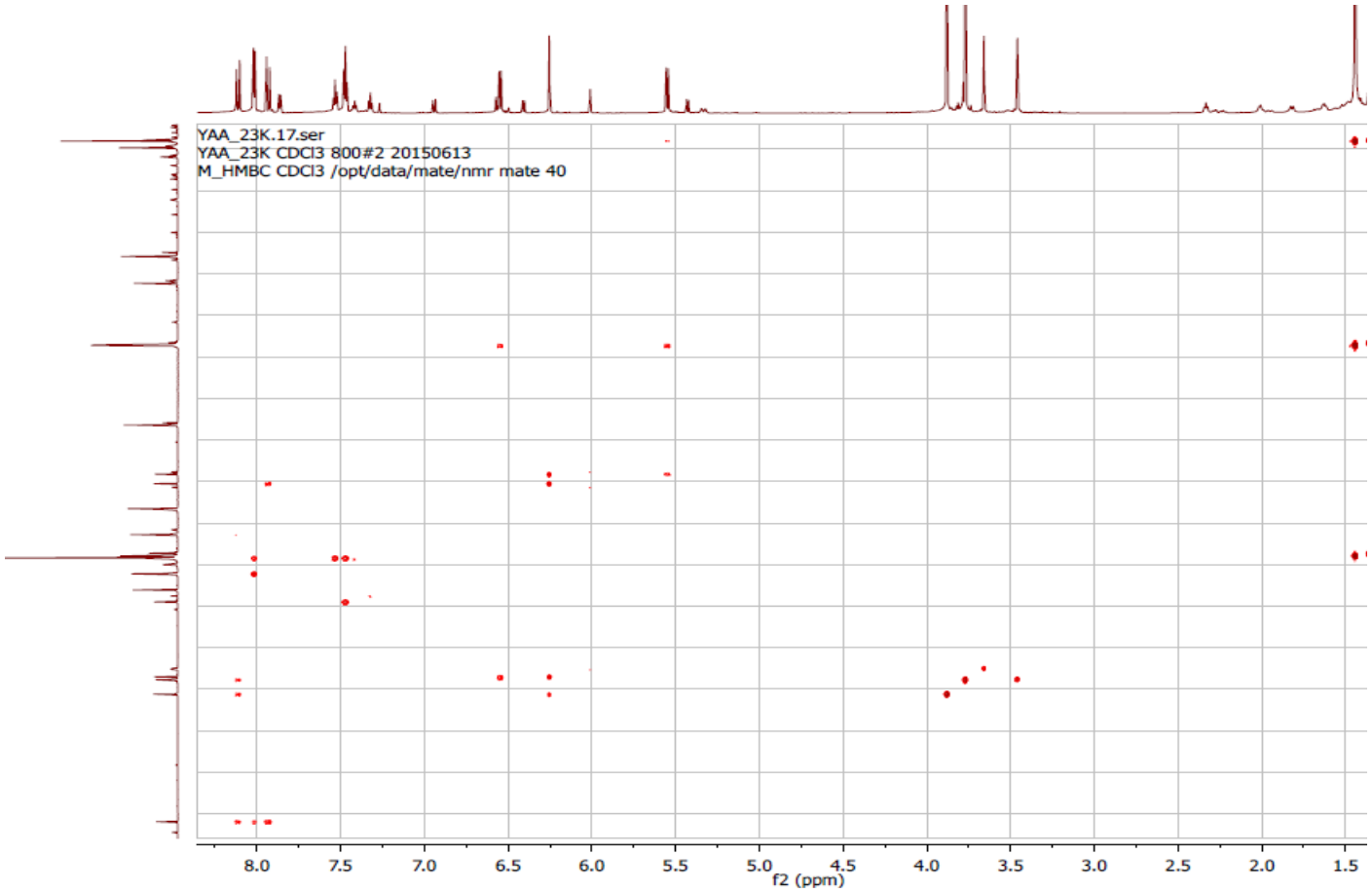
APPENDIX 7D: HH-COSY Spectrum of Aeqchalcone A (168) ($CDCl_3$)



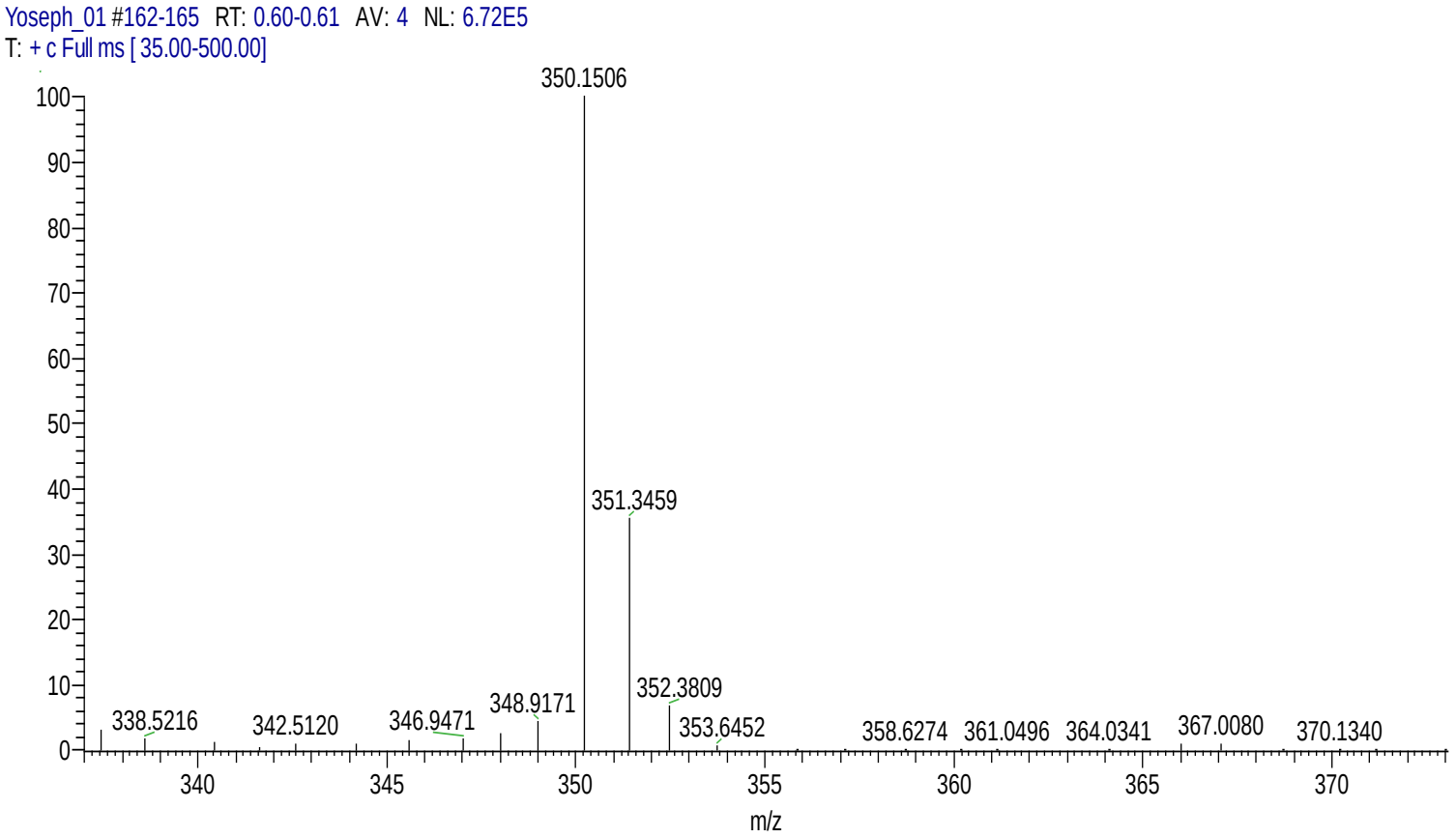
APPENDIX 7E: HSQC Spectrum of Aeqchalcone A (168) ($CDCl_3$)



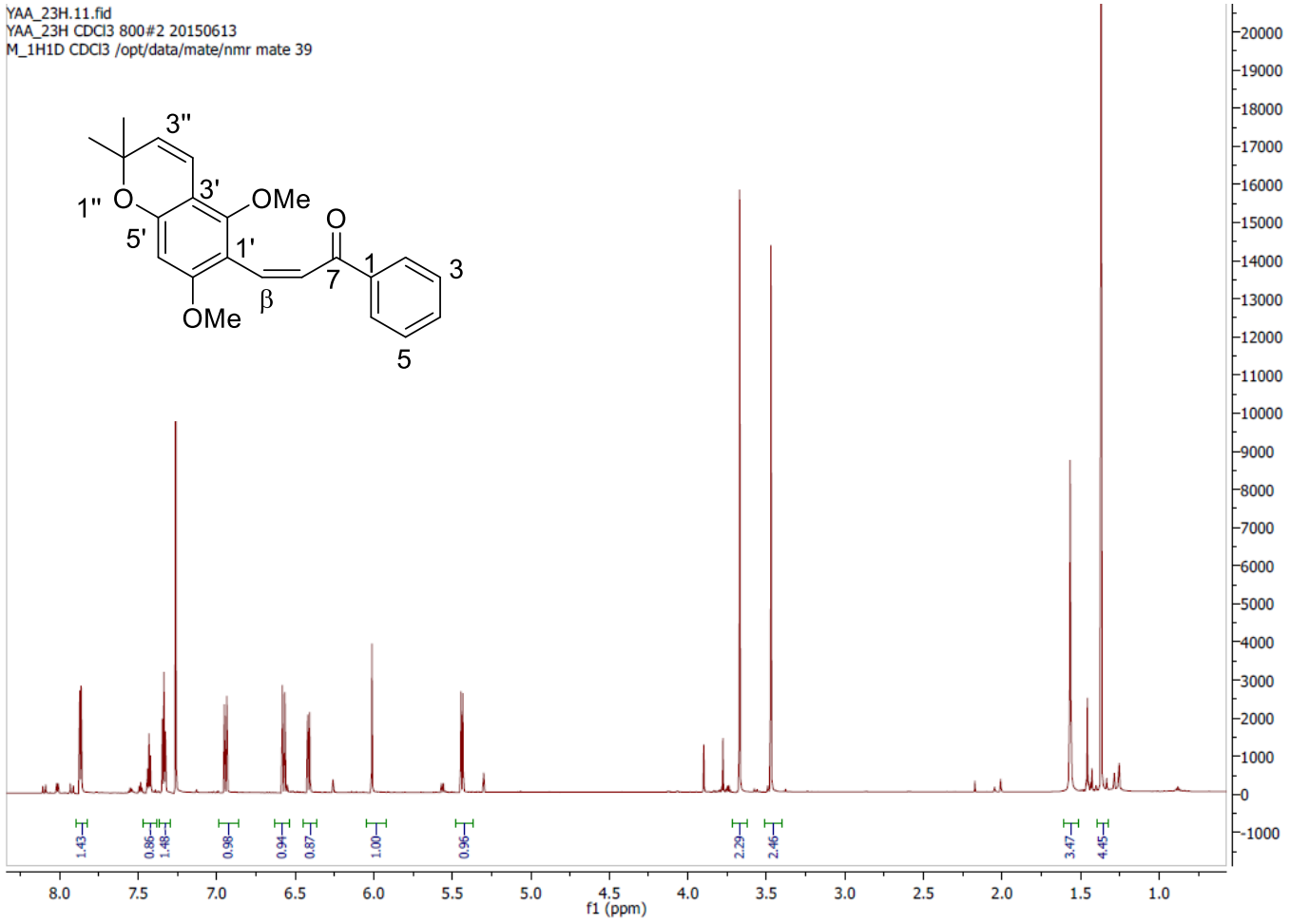
APPENDIX 7F: HMBC Spectrum of Aeqchalcone A (168) ($CDCl_3$)



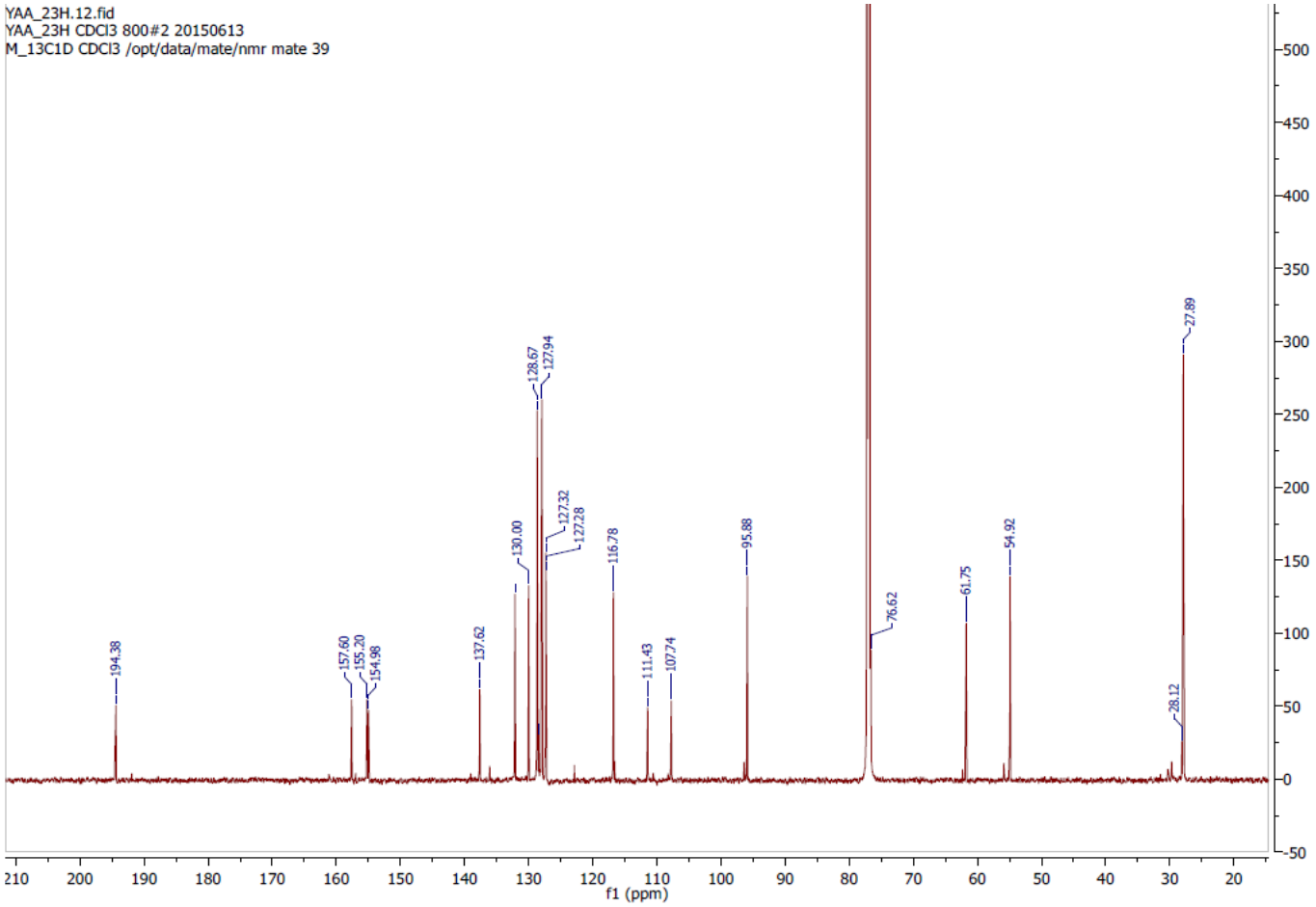
APPENDIX 7G: HRMS Spectrum of Aeqchalcone A (168) ($CDCl_3$)



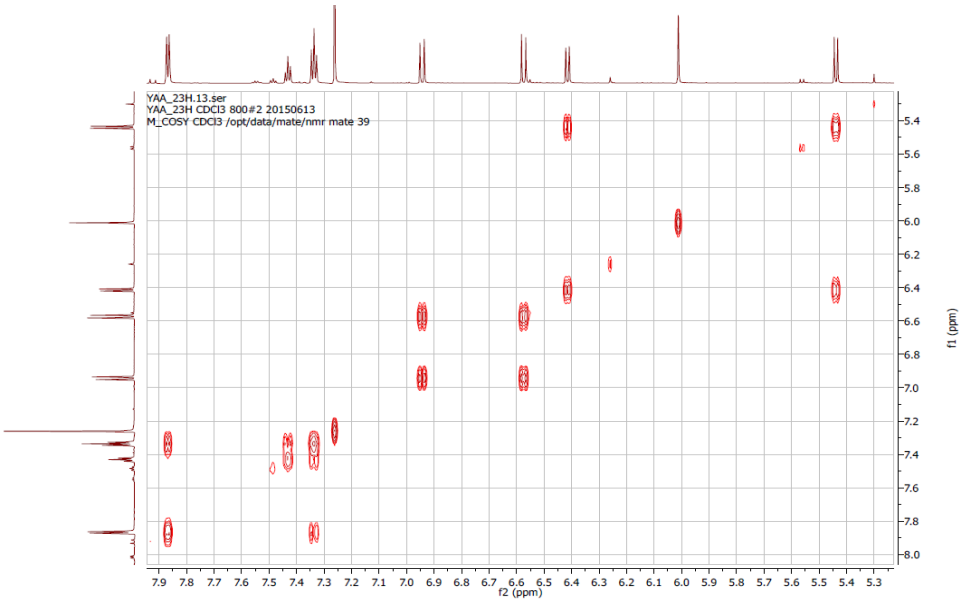
APPENDIX 8A: ^{1}H NMR Spectrum of Aeqchalcone B (169) (800 MHz; $CDCl_3$)



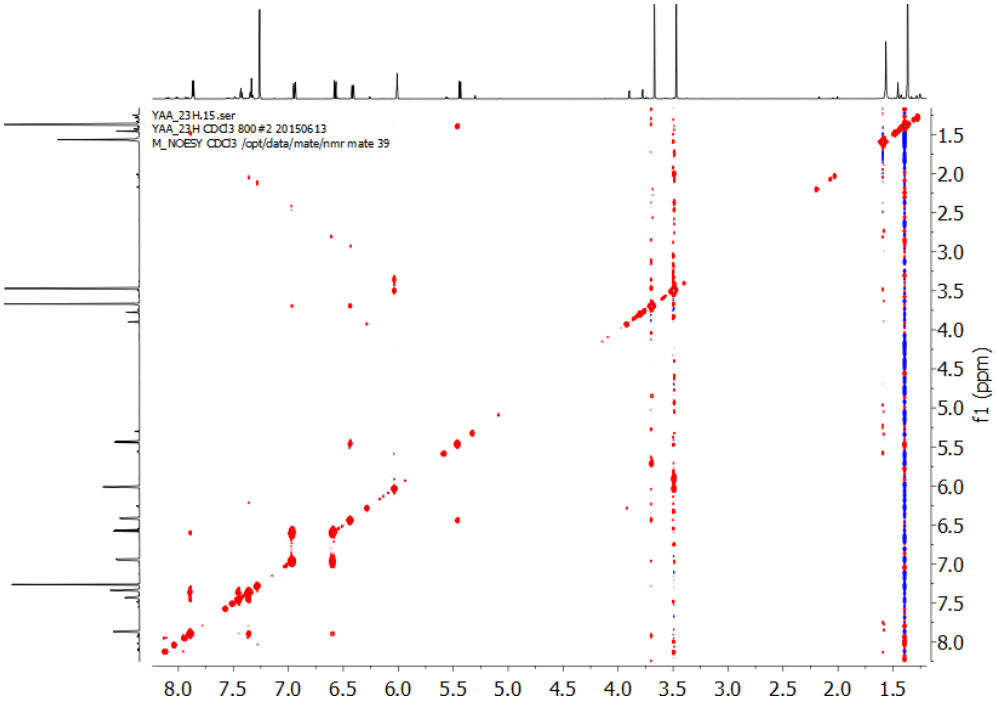
APPENDIX 8B: ^{13}C NMR Spectrum of Aeqchalcone B (169) (200 MHz; $CDCl_3$)



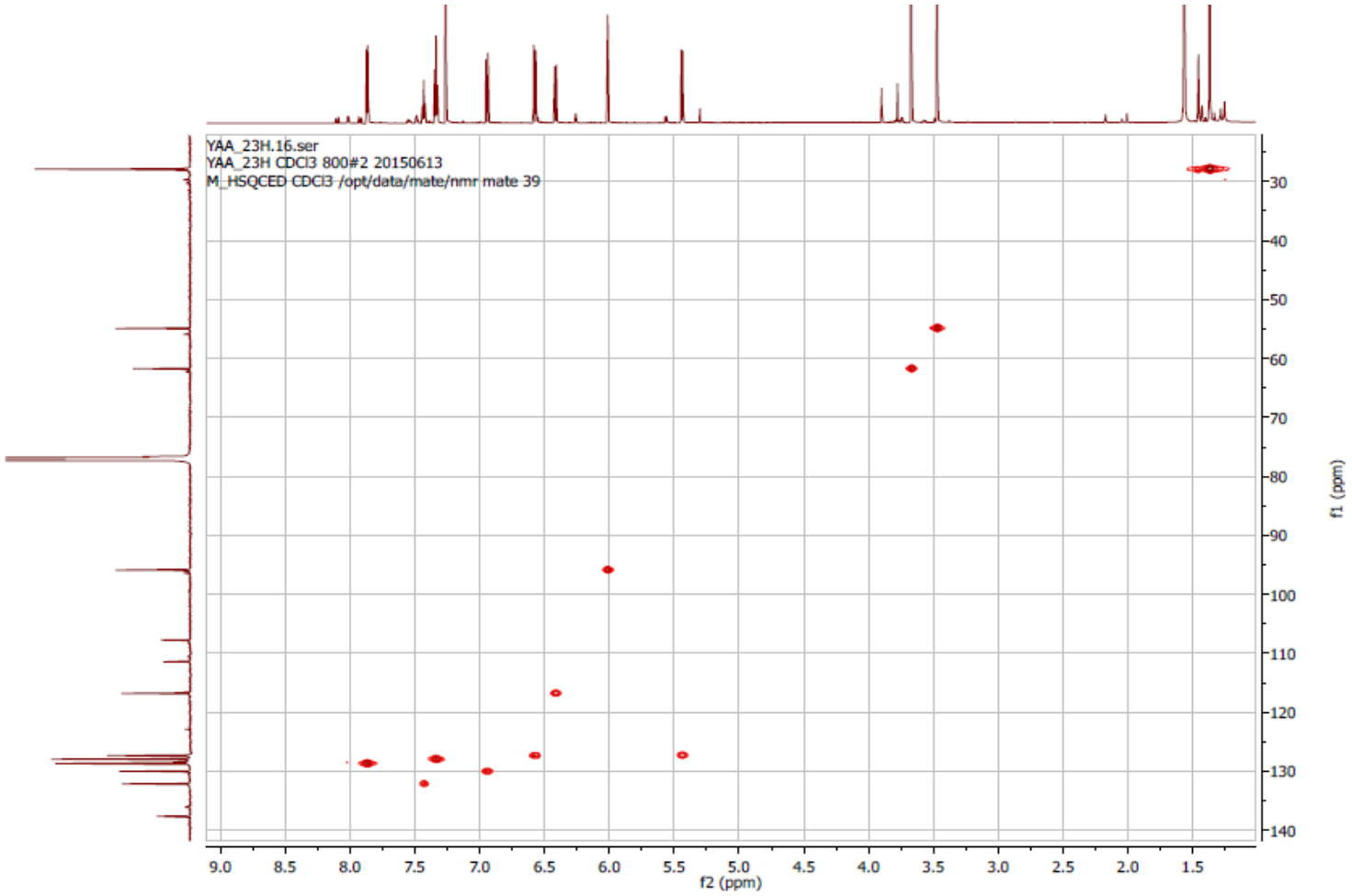
APPENDIX 8C: HH-COSY Spectrum of Aeqchalcone B (169) ($CDCl_3$)



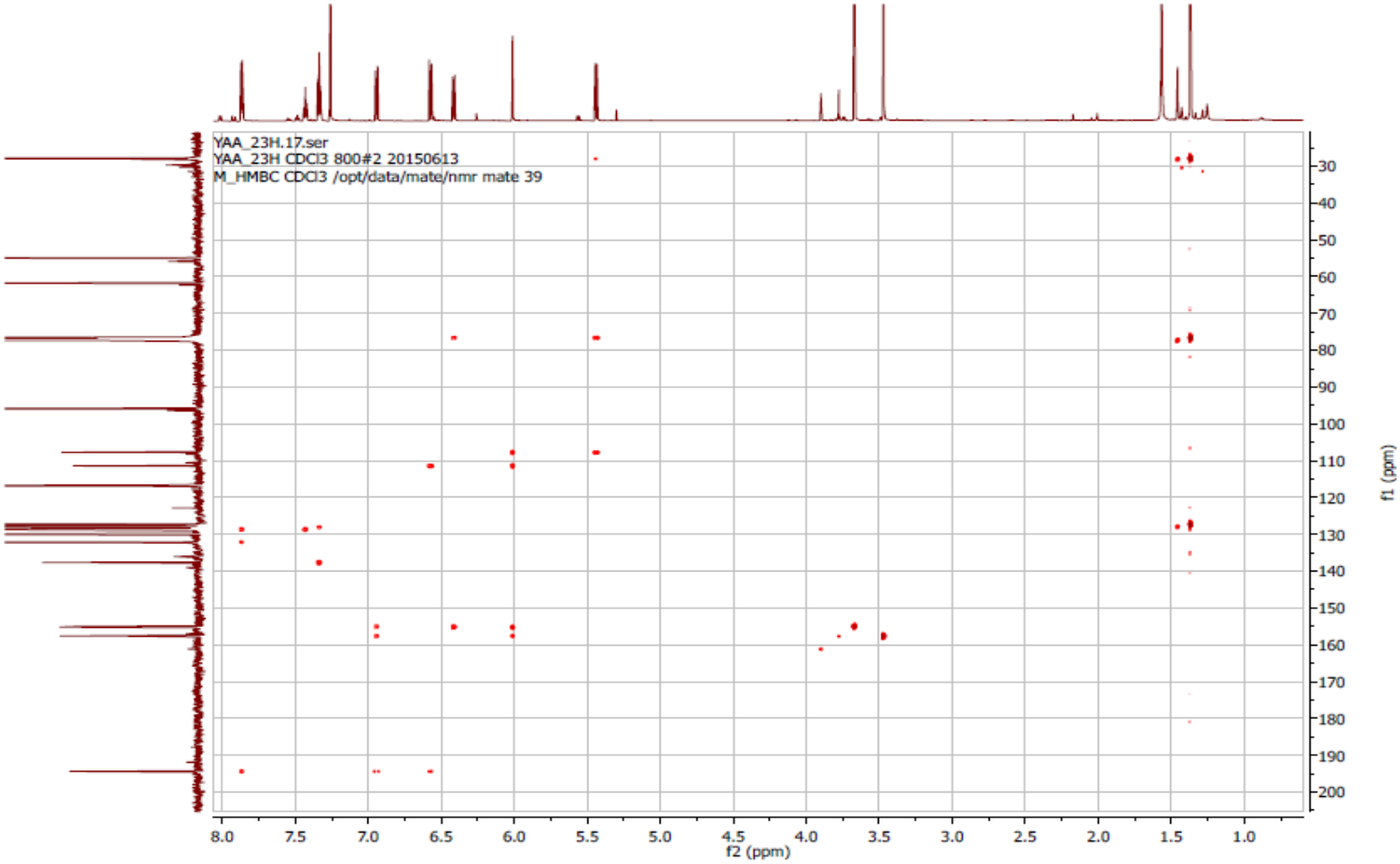
APPENDIX 8D: NOESY Spectrum of Aeqchalcone B (169) ($CDCl_3$)



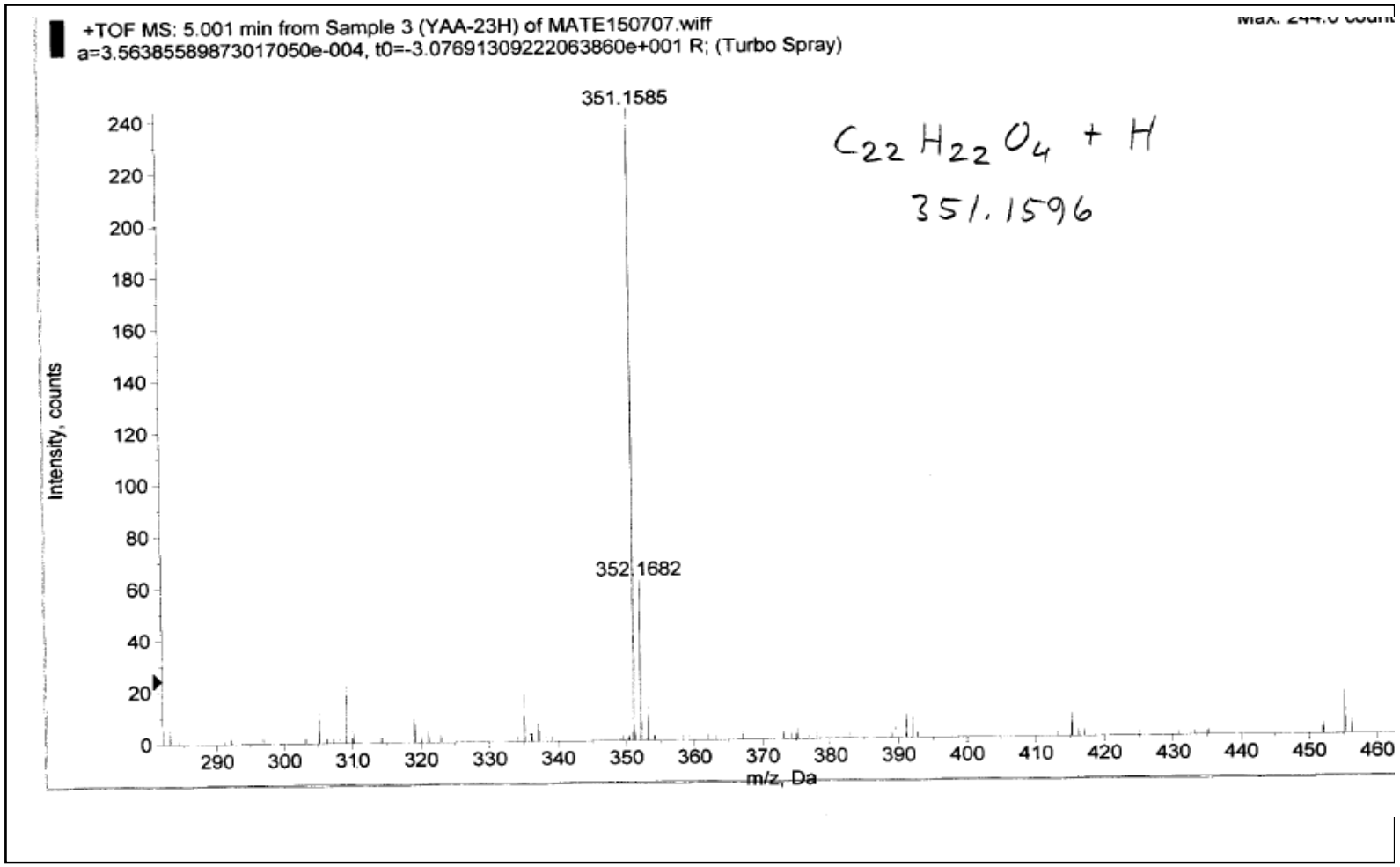
APPENDIX 8E: HSQC Spectrum of Aeqchalcone B (169) ($CDCl_3$)



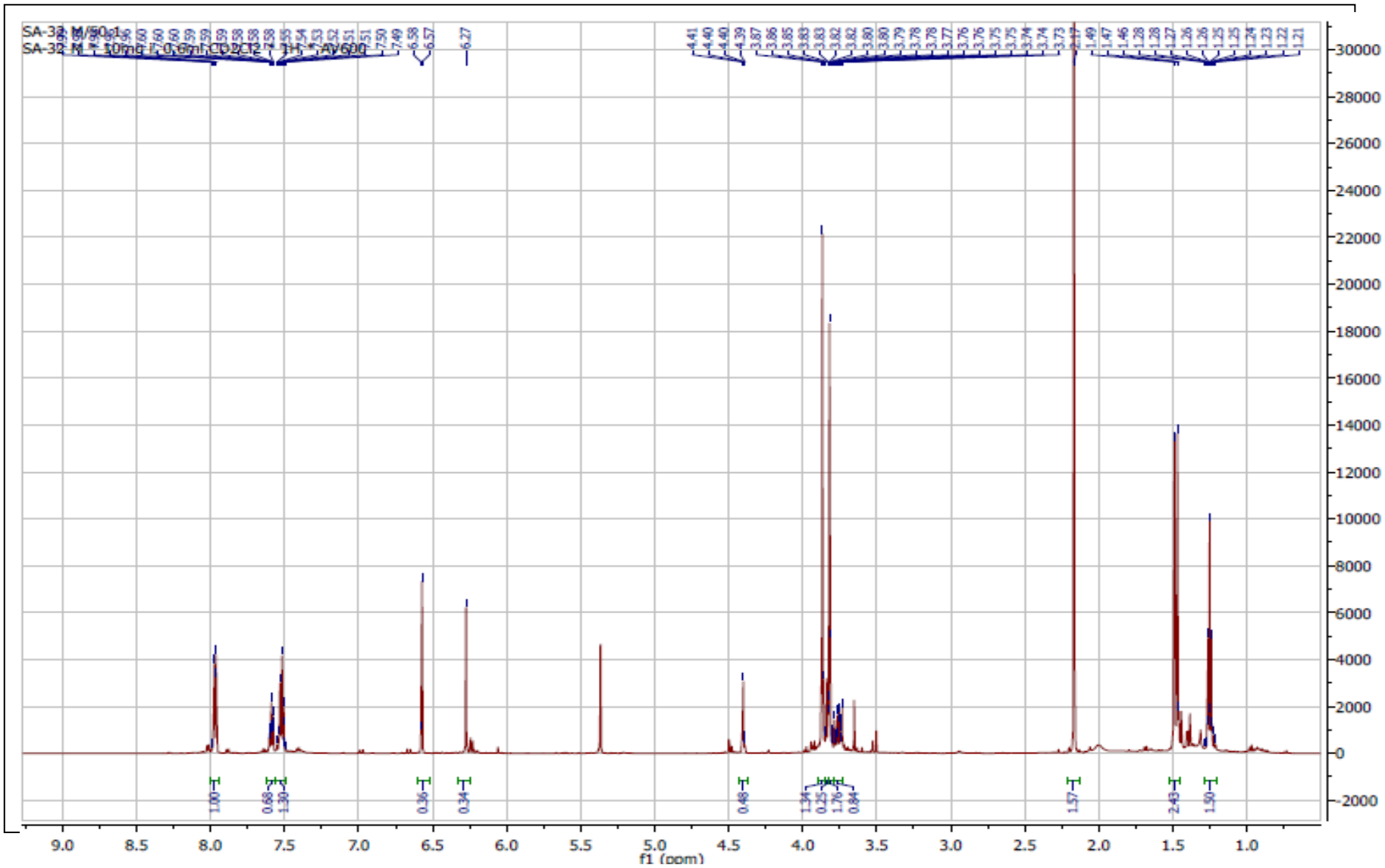
APPENDIX 8F: HMBC Spectrum of Aeqchalcone B (169) ($CDCl_3$)



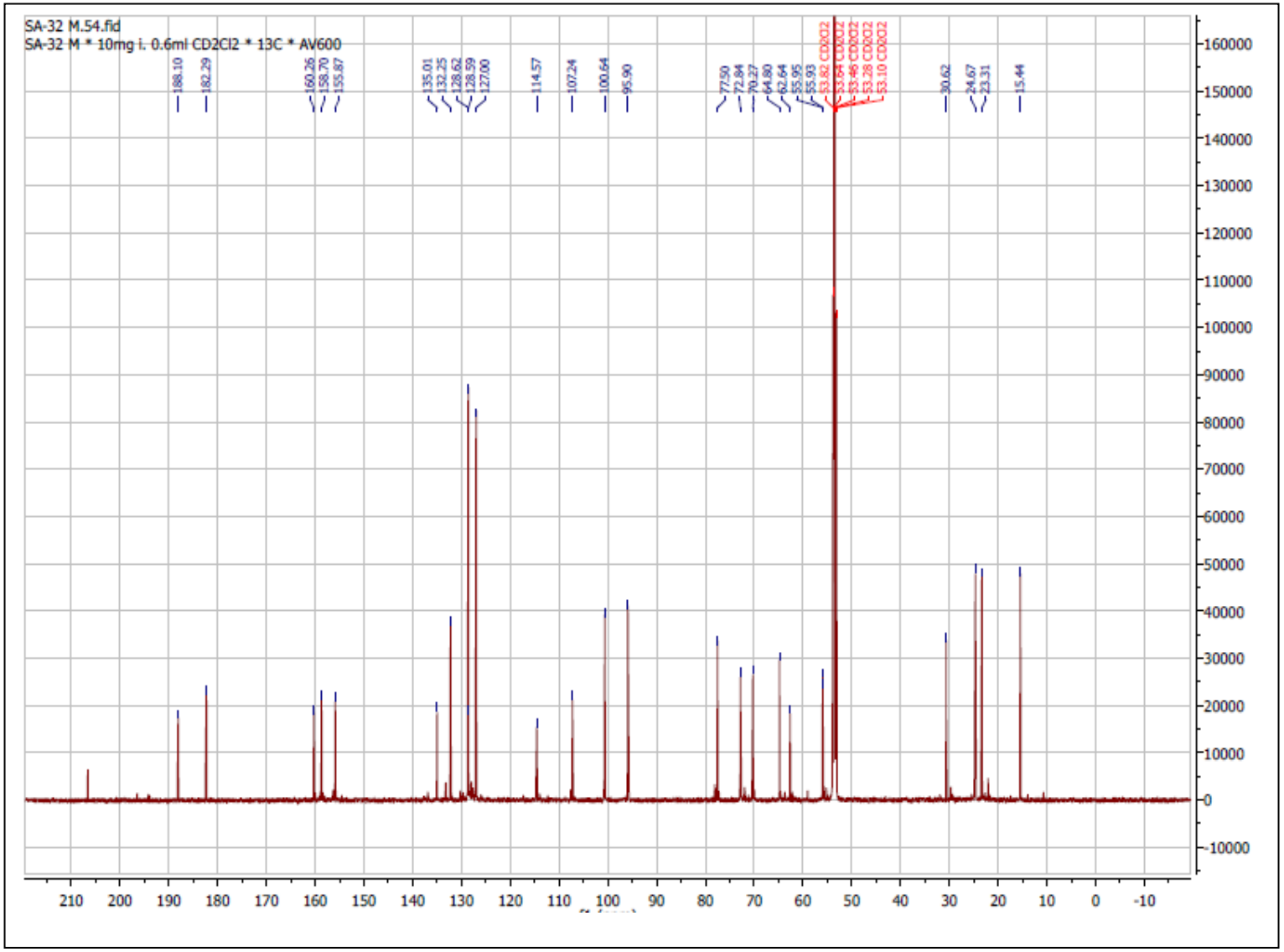
APPENDIX 8G: HRMS Spectrum of Aeqchalcone B (169) ($CDCl_3$)



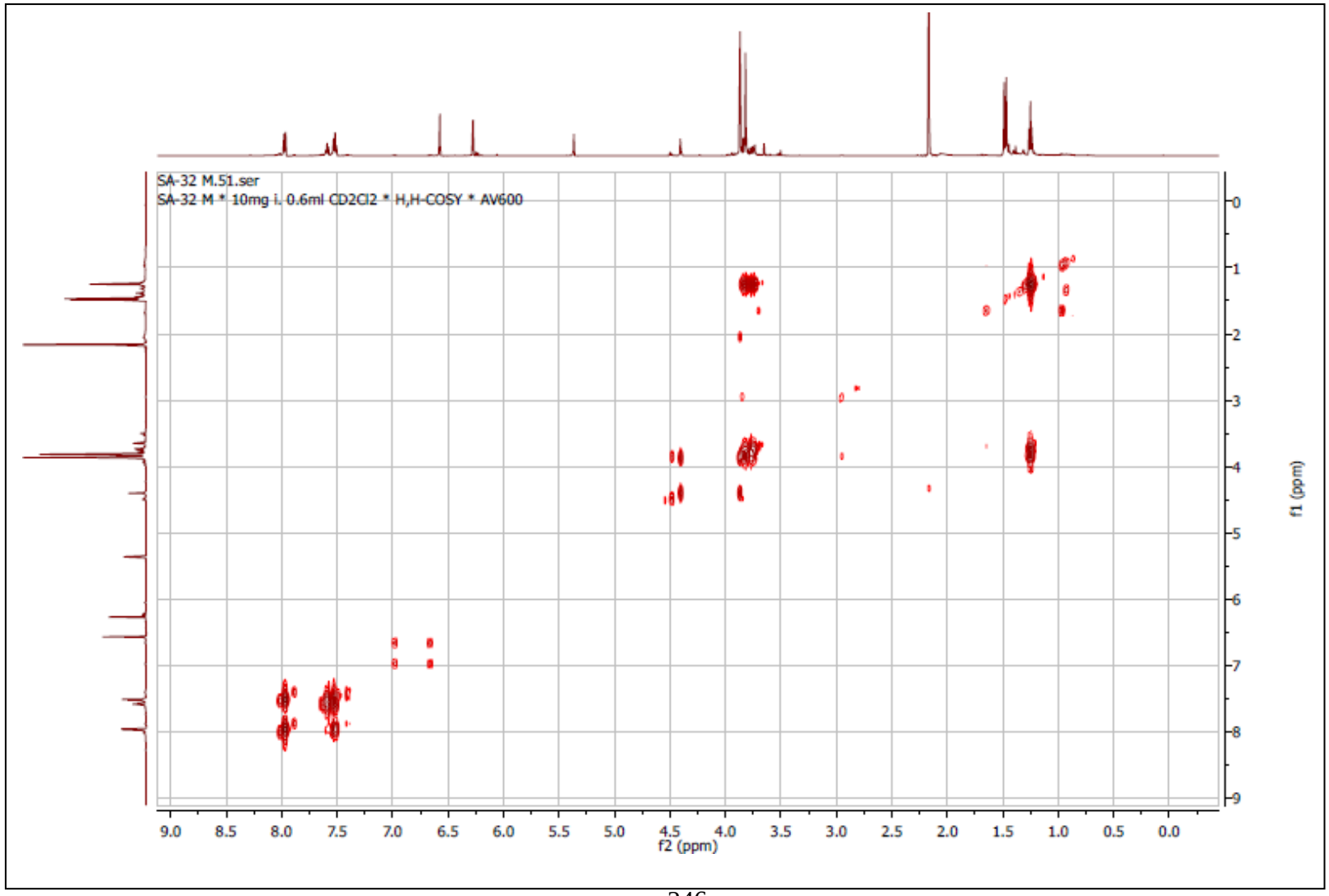
APPENDIX 9A: ^{1}H NMR Spectrum of Aeqchalcone C (170) (600 MHz; CD_2Cl_2)



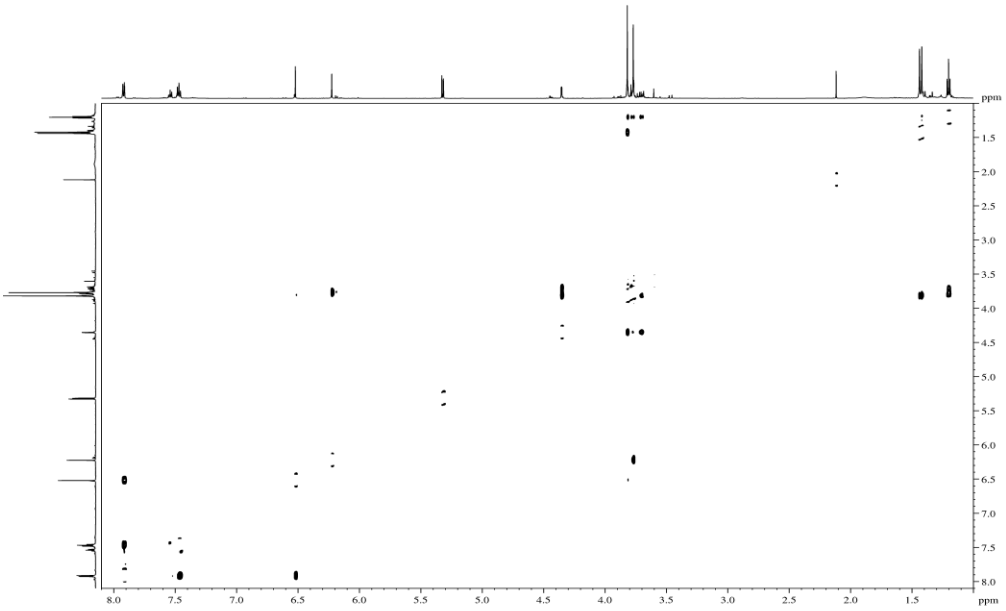
APPENDIX 9B: ^{13}C NMR Spectrum of Aeqchalcone C (170) (150 MHz; CD_2Cl_2)



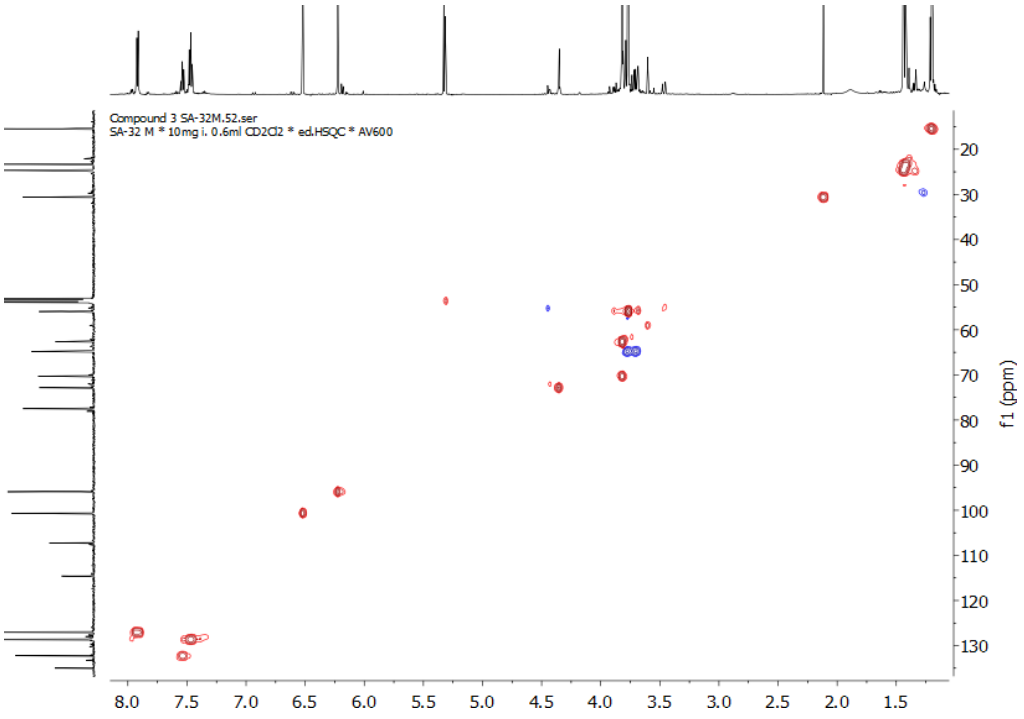
APPENDIX 9C: HH-COSY Spectrum of Aeqchalcone C (170) (CD_2Cl_2)



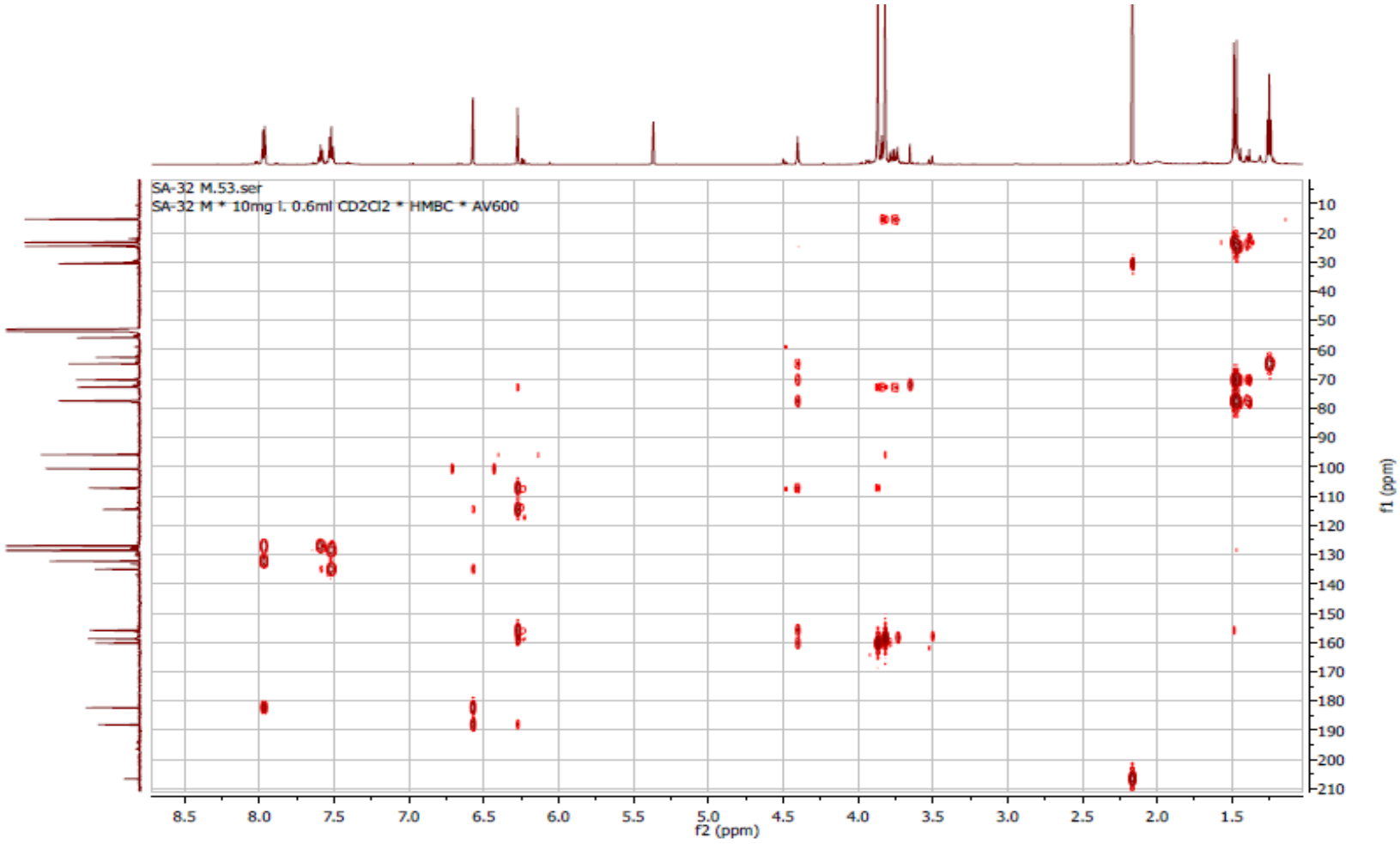
APPENDIX 9D: NOESY Spectrum of Aeqchalcone C (170) (CD_2Cl_2)



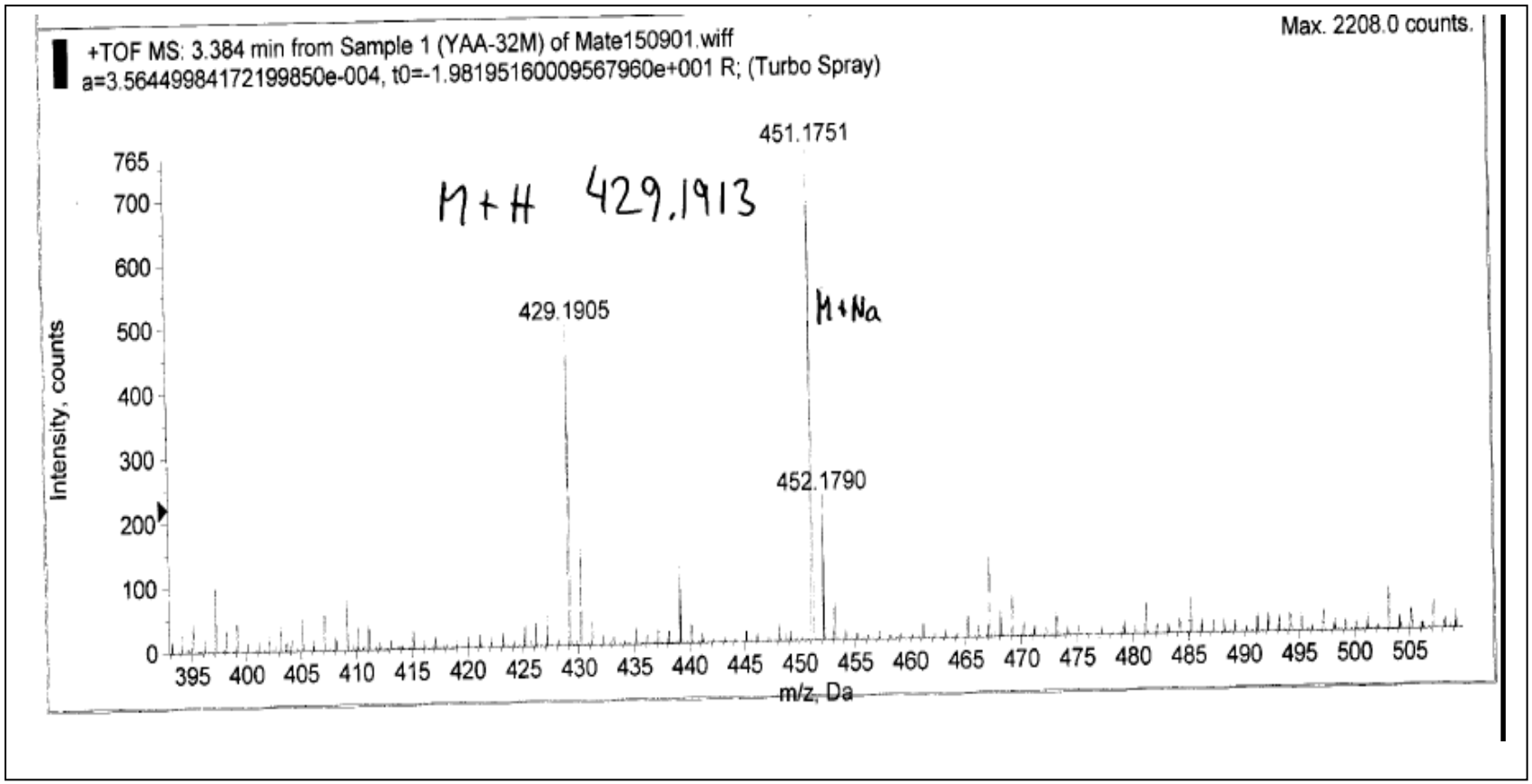
APPENDIX 9E: HSQC Spectrum of Aeqchalcone C (170) (CD_2Cl_2)



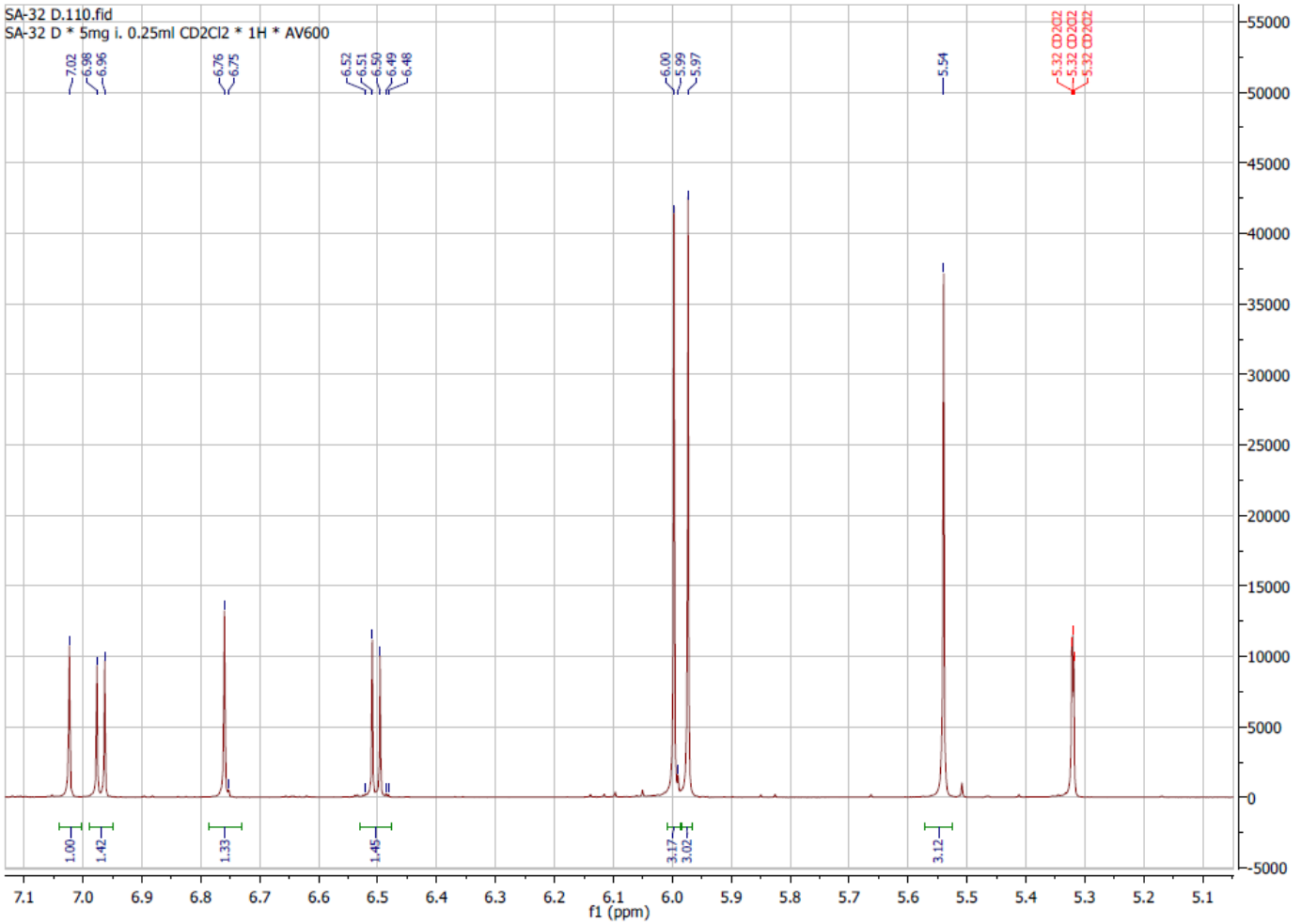
APPENDIX 9F: HMBC Spectrum of Aeqchalcone C (170) (CD_2Cl_2)



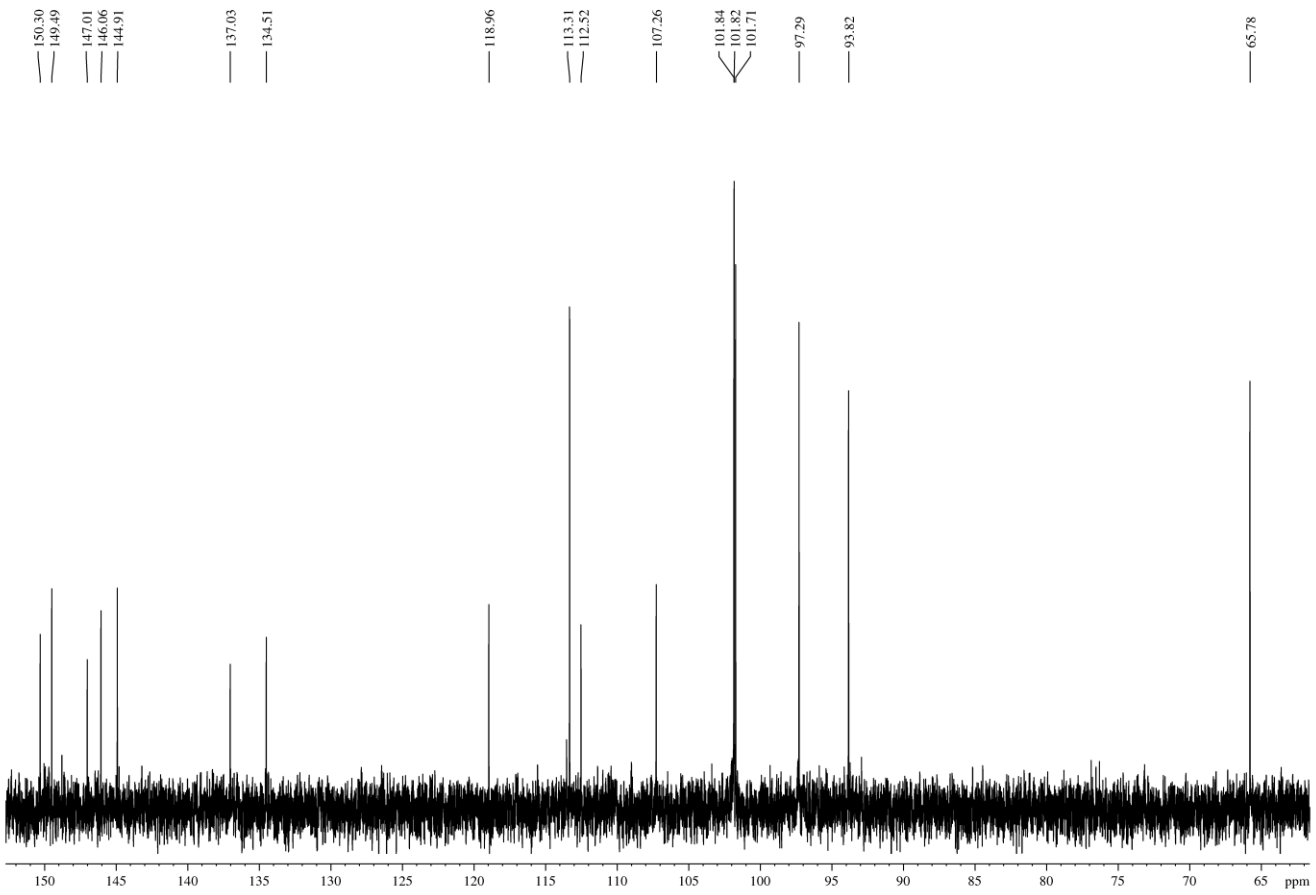
APPENDIX 9G: HRMS Spectrum of Aeqchalcone C (170)



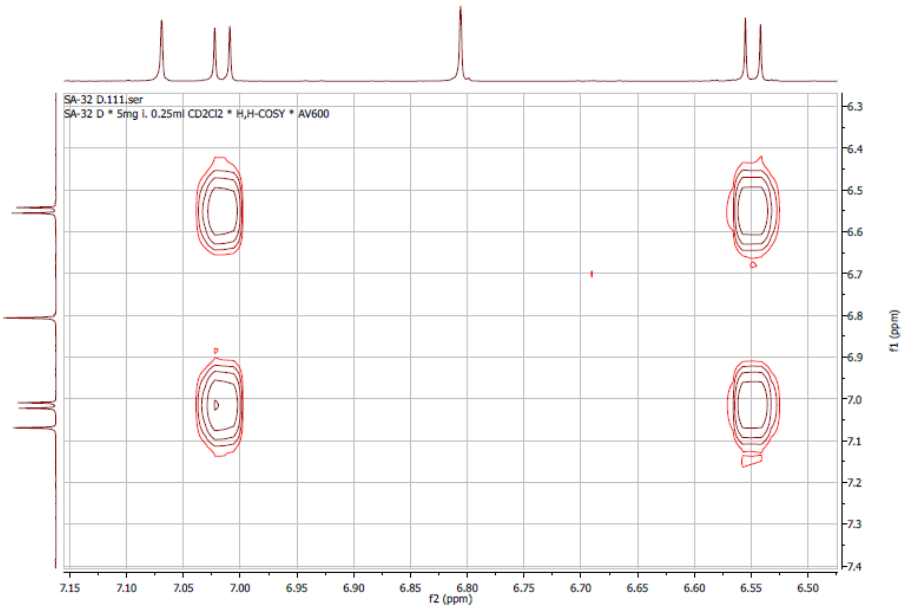
APPENDIX 10A: ^{1}H NMR Spectrum of *3,4:8,9-Dimethylenedioxypterocarpene (167)* (600 MHz; CD_2Cl_2)



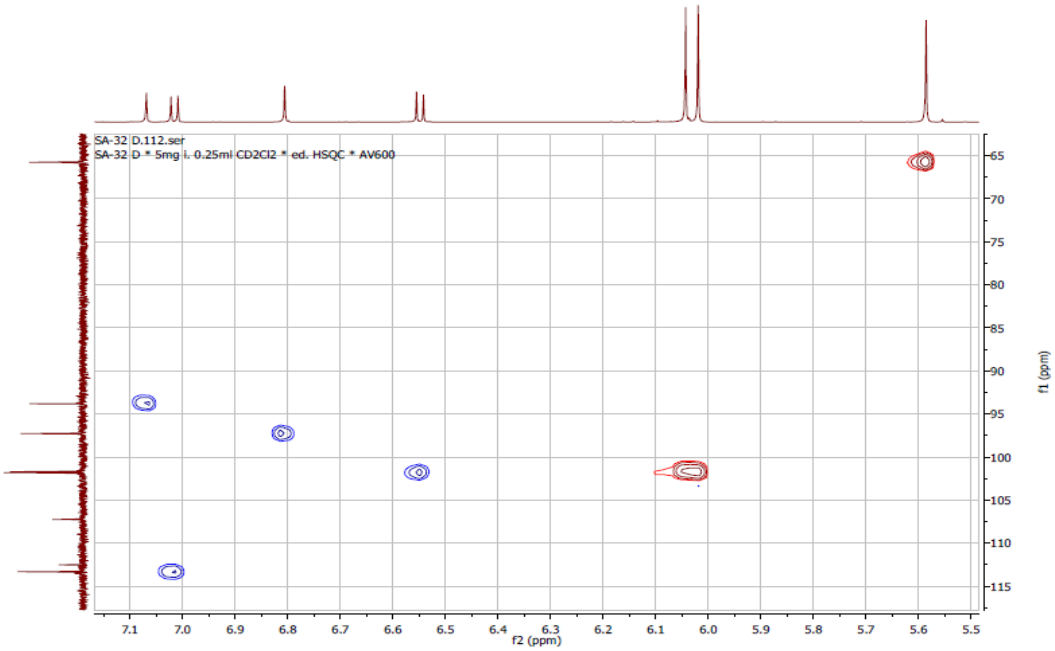
APPENDIX 10B: ^{13}C NMR Spectrum of *3,4:8,9-Dimethylenedioxypterocarpene (167)* (150 MHz; CD_2Cl_2)



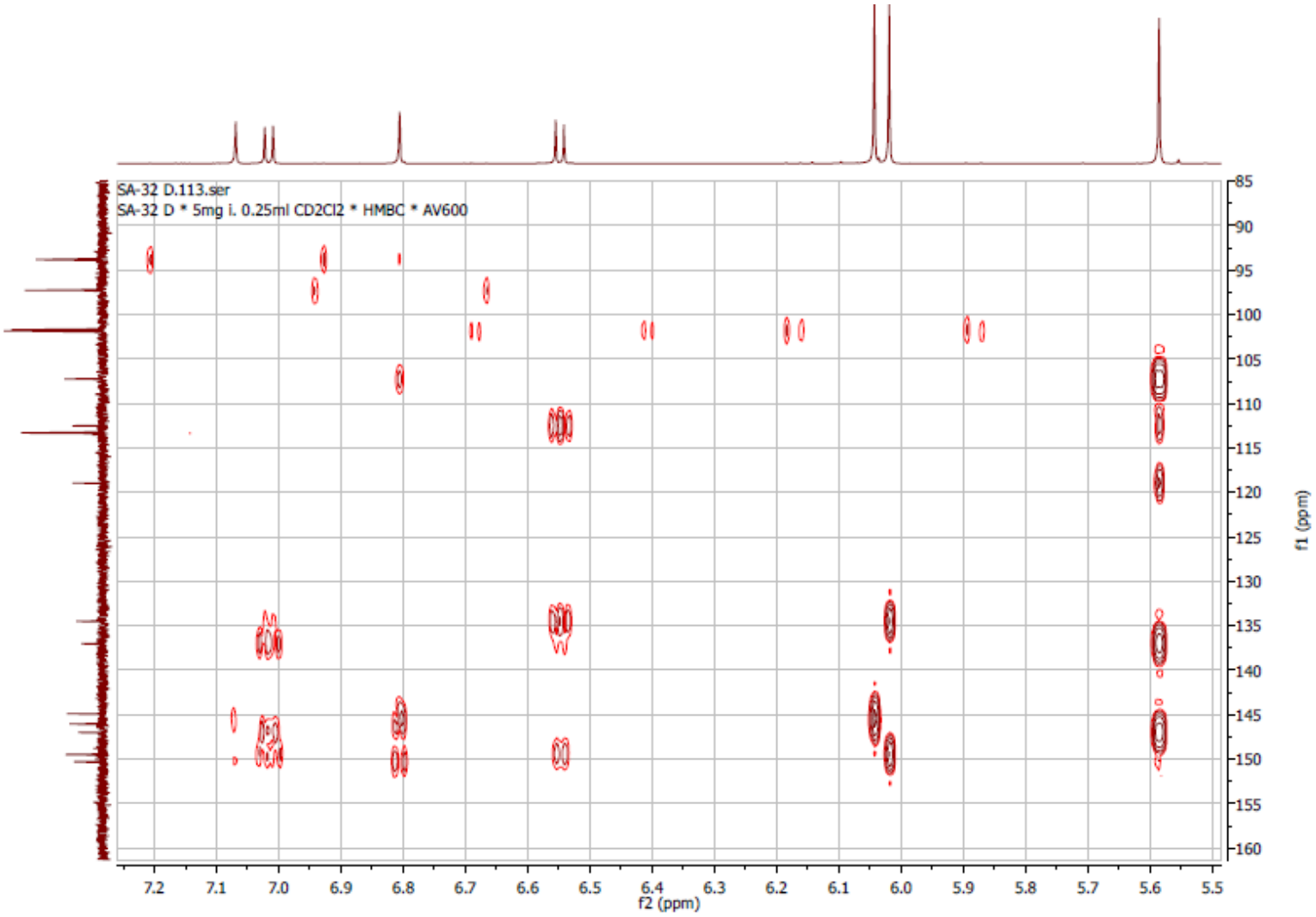
APPENDIX 10C: HH-COSY Spectrum of *3,4:8,9-Dimethylenedioxypterocarpene (167)* (CD_2Cl_2)



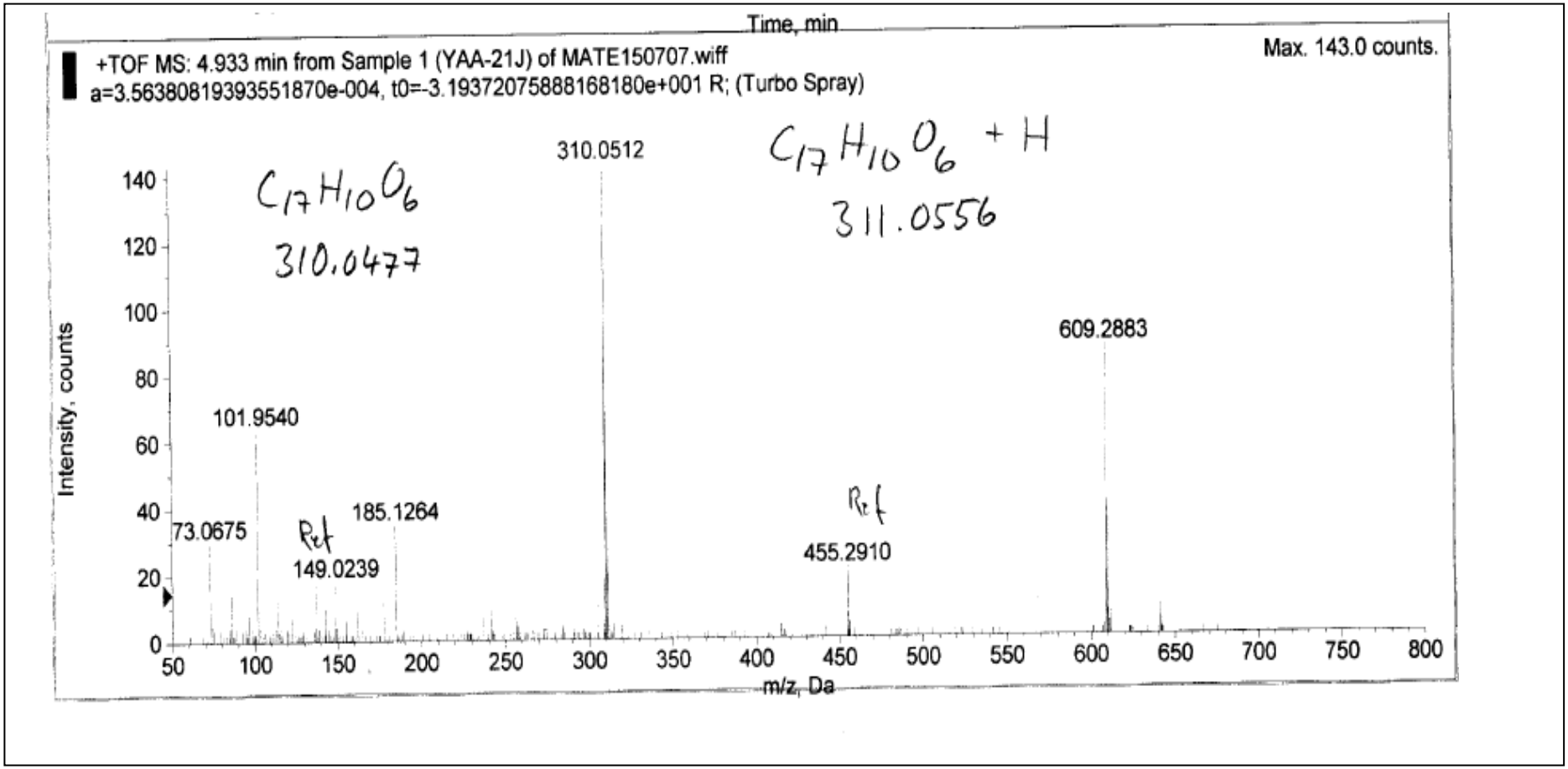
APPENDIX 10D: HSQC Spectrum of 3,4:8,9-Dimethylenedioxypterocarpene (167) (CD_2Cl_2)



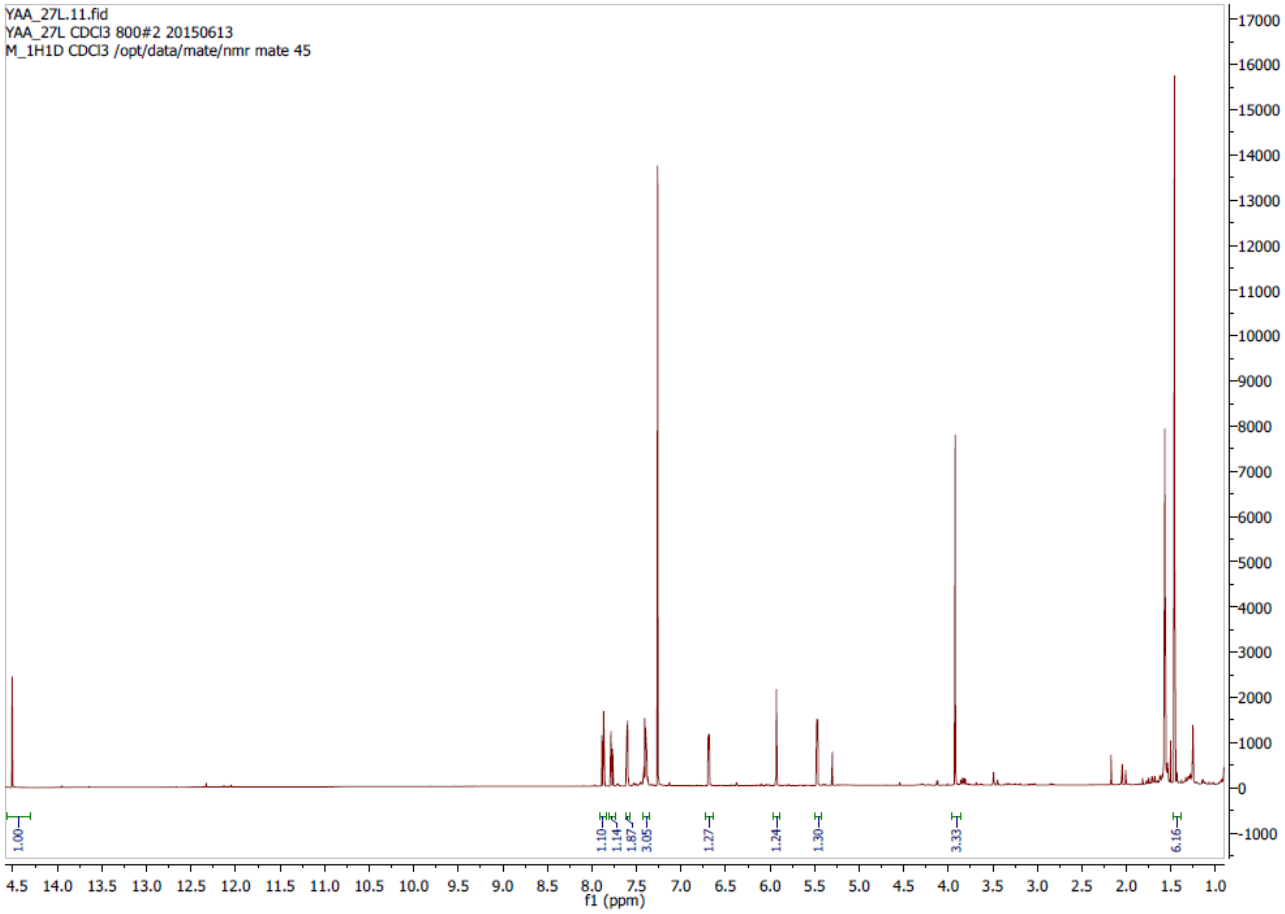
APPENDIX 10E: HMBC Spectrum of 3,4:8,9-Dimethylenedioxypterocarpene (167) (CD_2Cl_2)



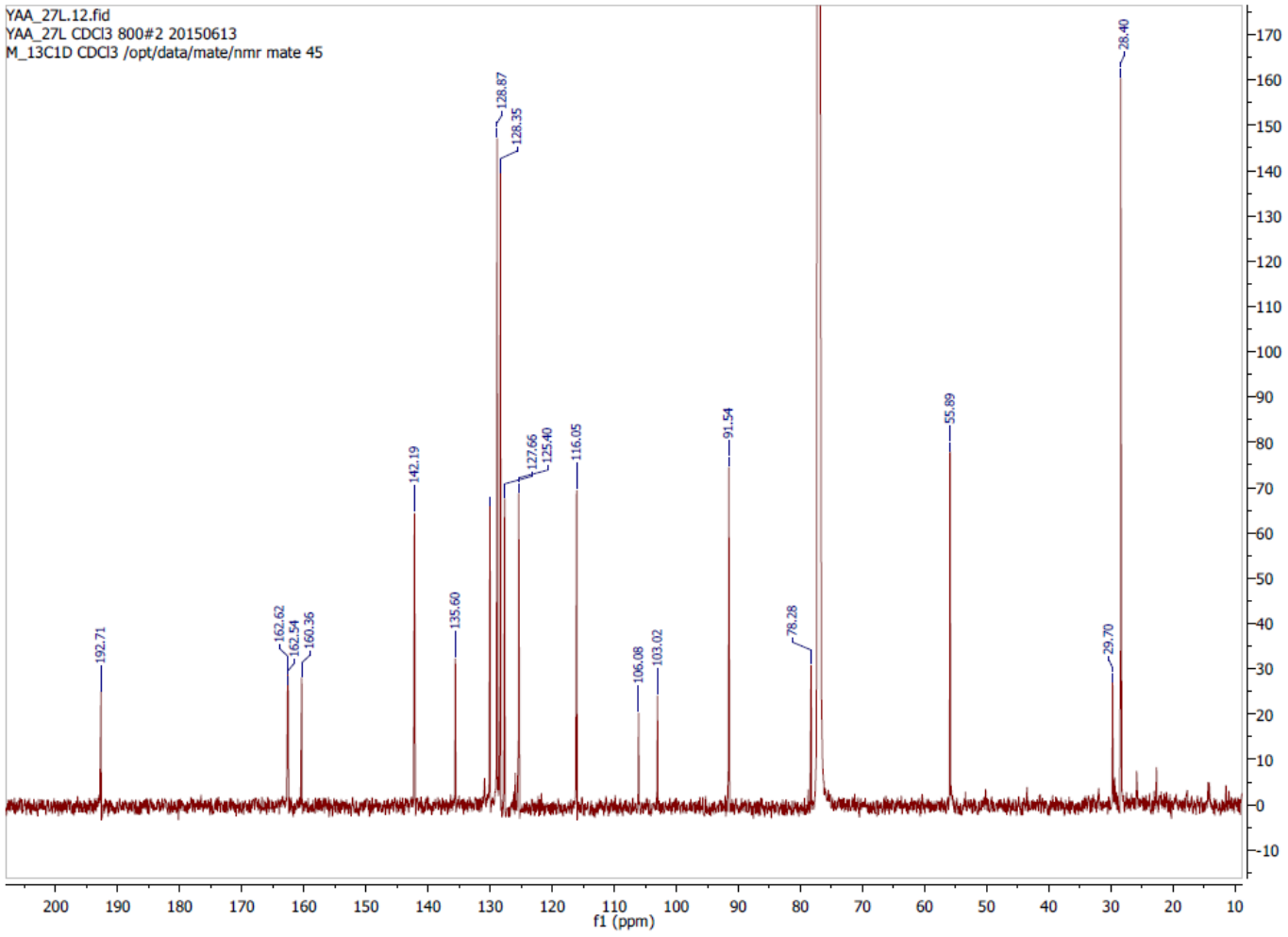
APPENDIX 10F: HRMS Spectrum of 3,4:8,9-Dimethylenedioxypterocarpene (167)



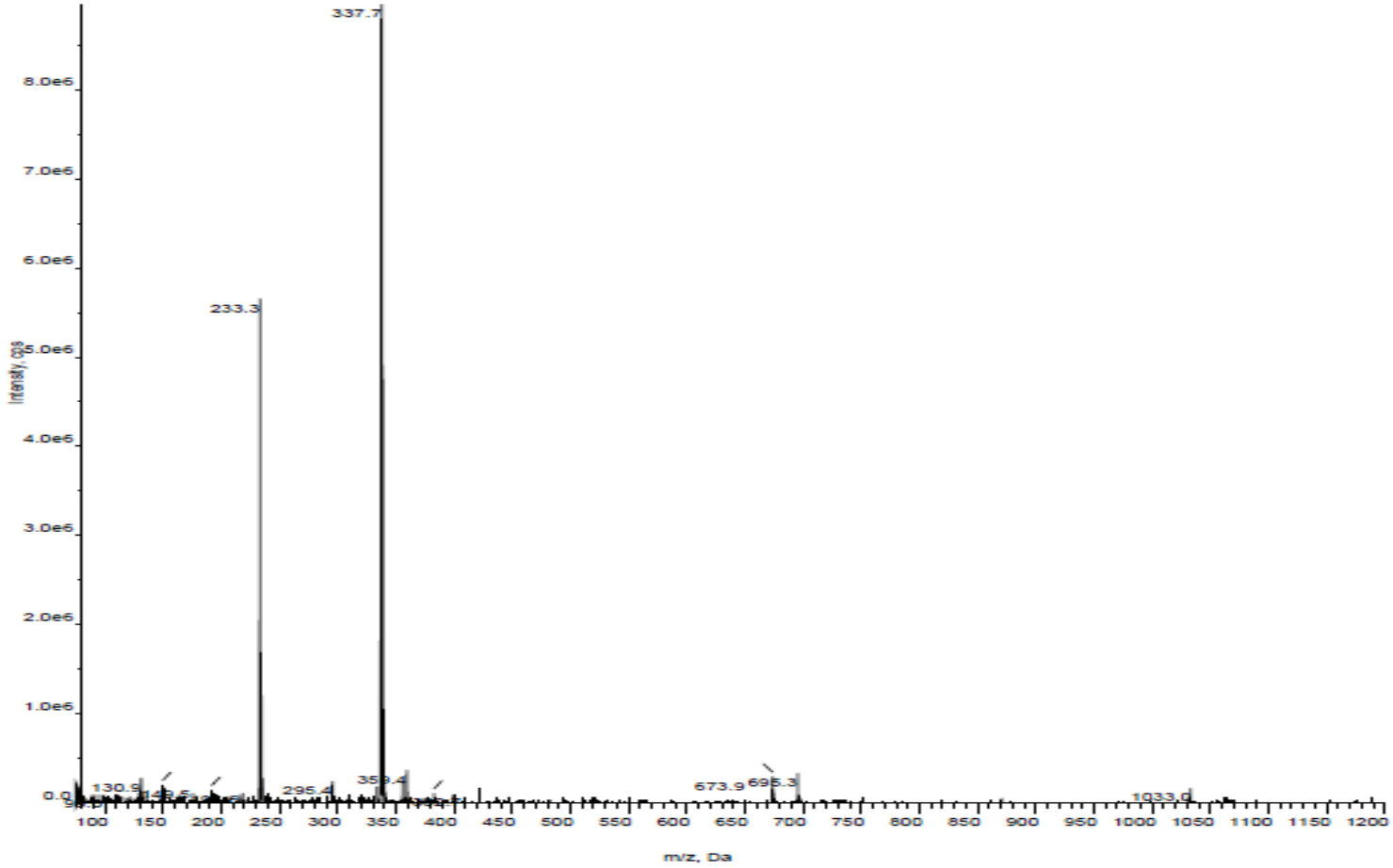
APPENDIX 11A: ^{1}H NMR Spectrum of Obovatachalcone (147) (800 MHz; $CDCl_3$)



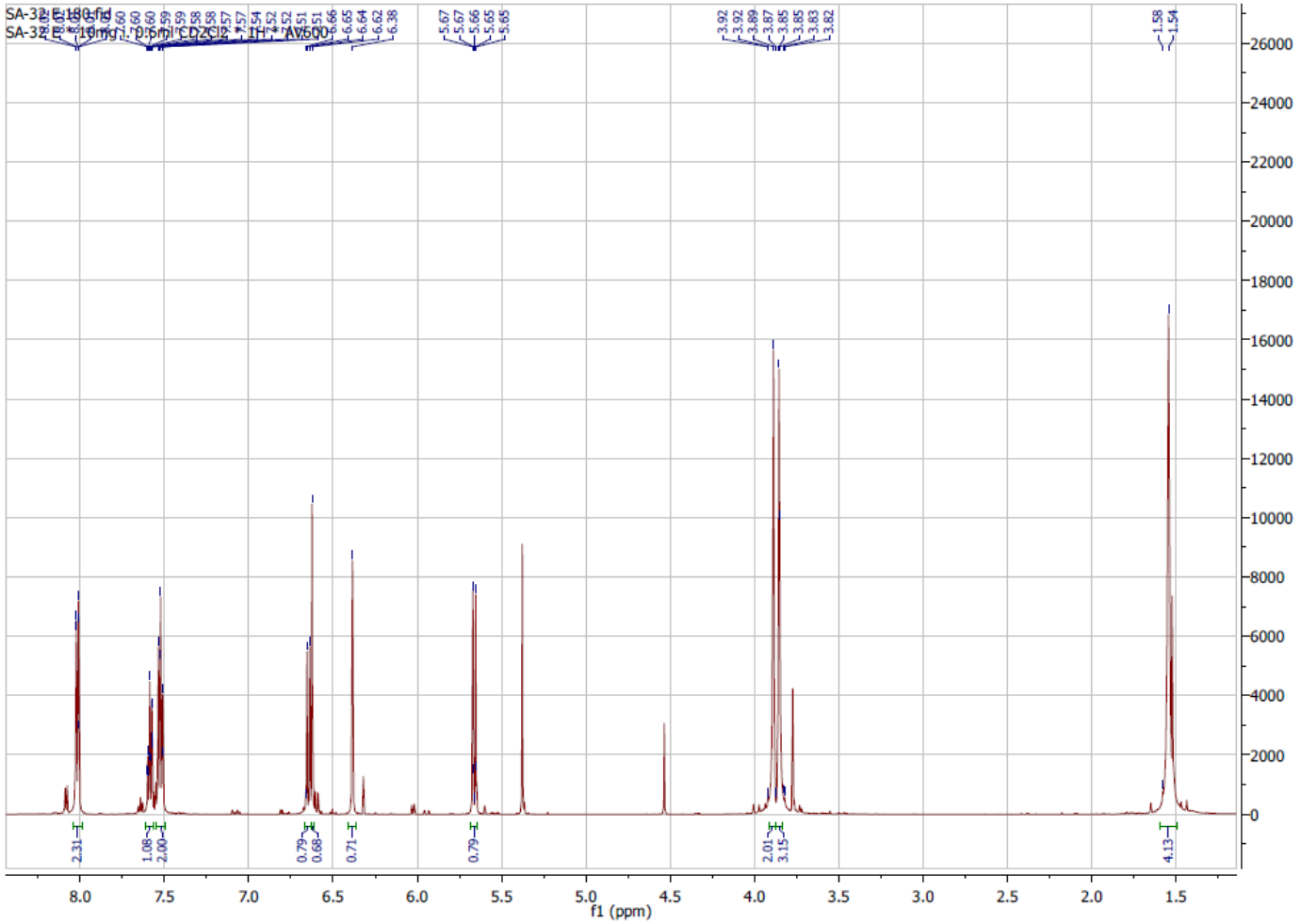
APPENDIX 11B: ^{13}C NMR Spectrum of Obovatachalcone (147) (200 MHz; $CDCl_3$)



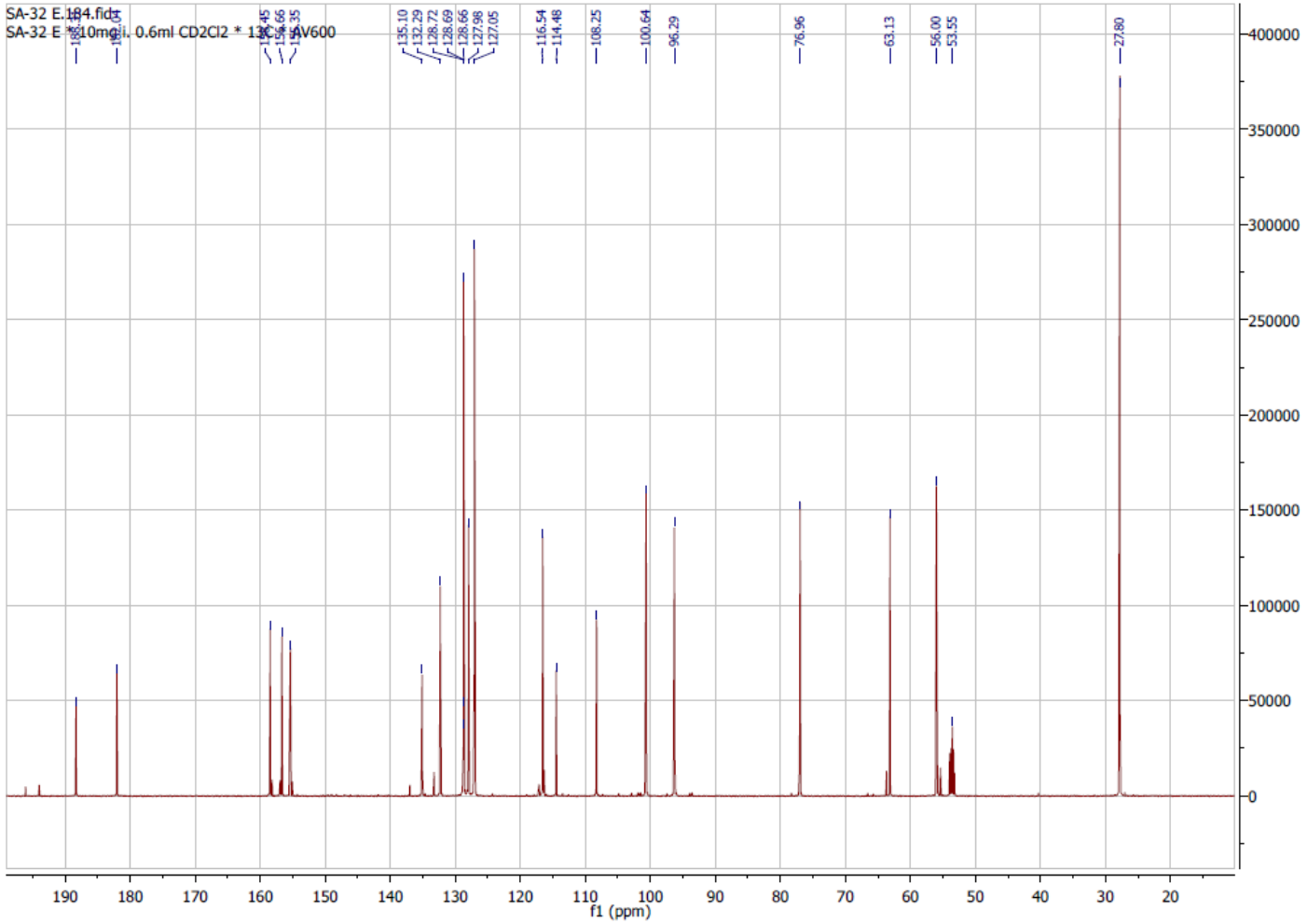
APPENDIX 11C: LCMS Spectrum of Obovatachalcone (147) ($CDCl_3$)



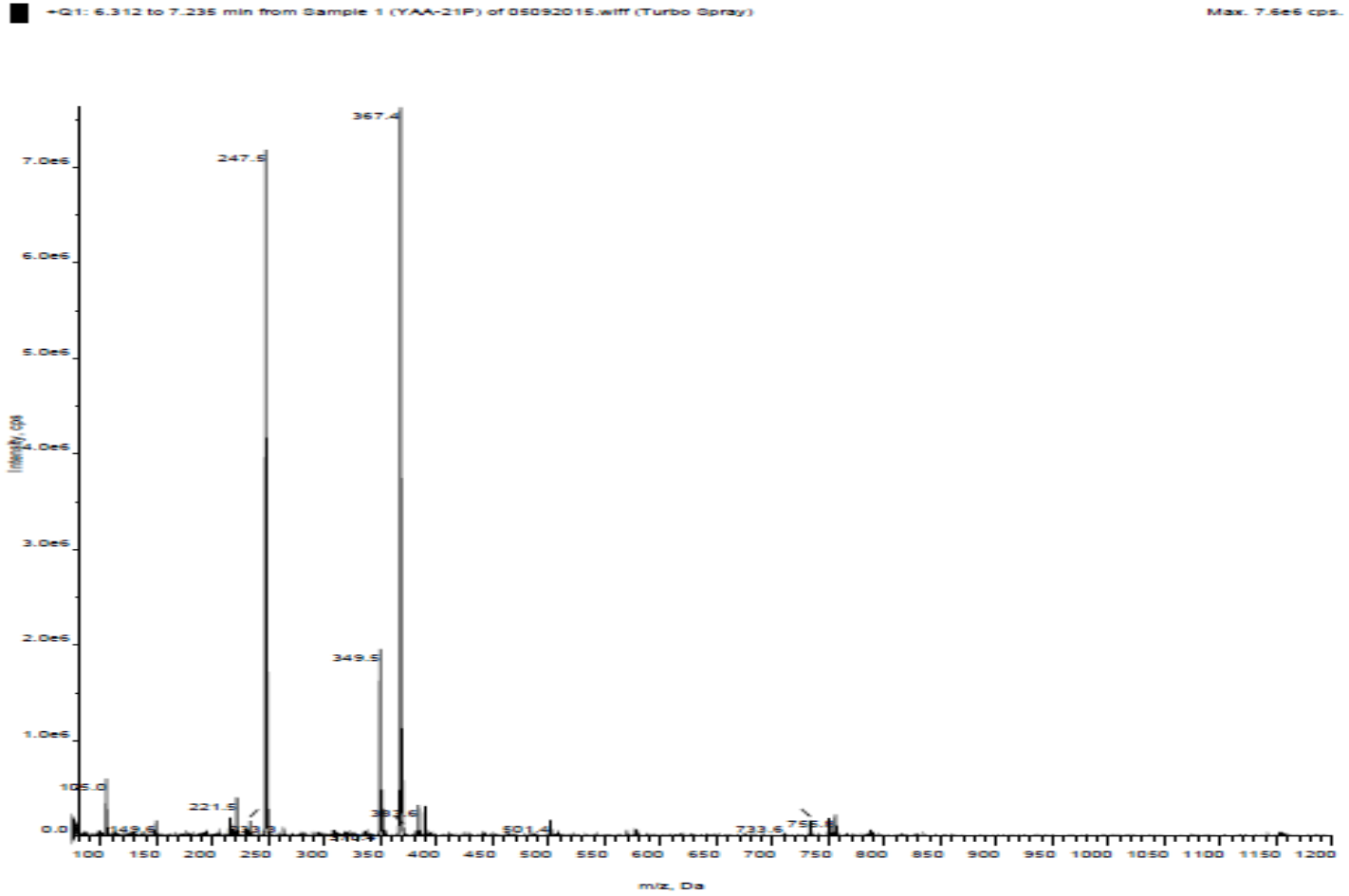
APPENDIX 12A: ^{1}H NMR Spectrum of Praecansone B (146) (600 MHz; CD_2Cl_2)



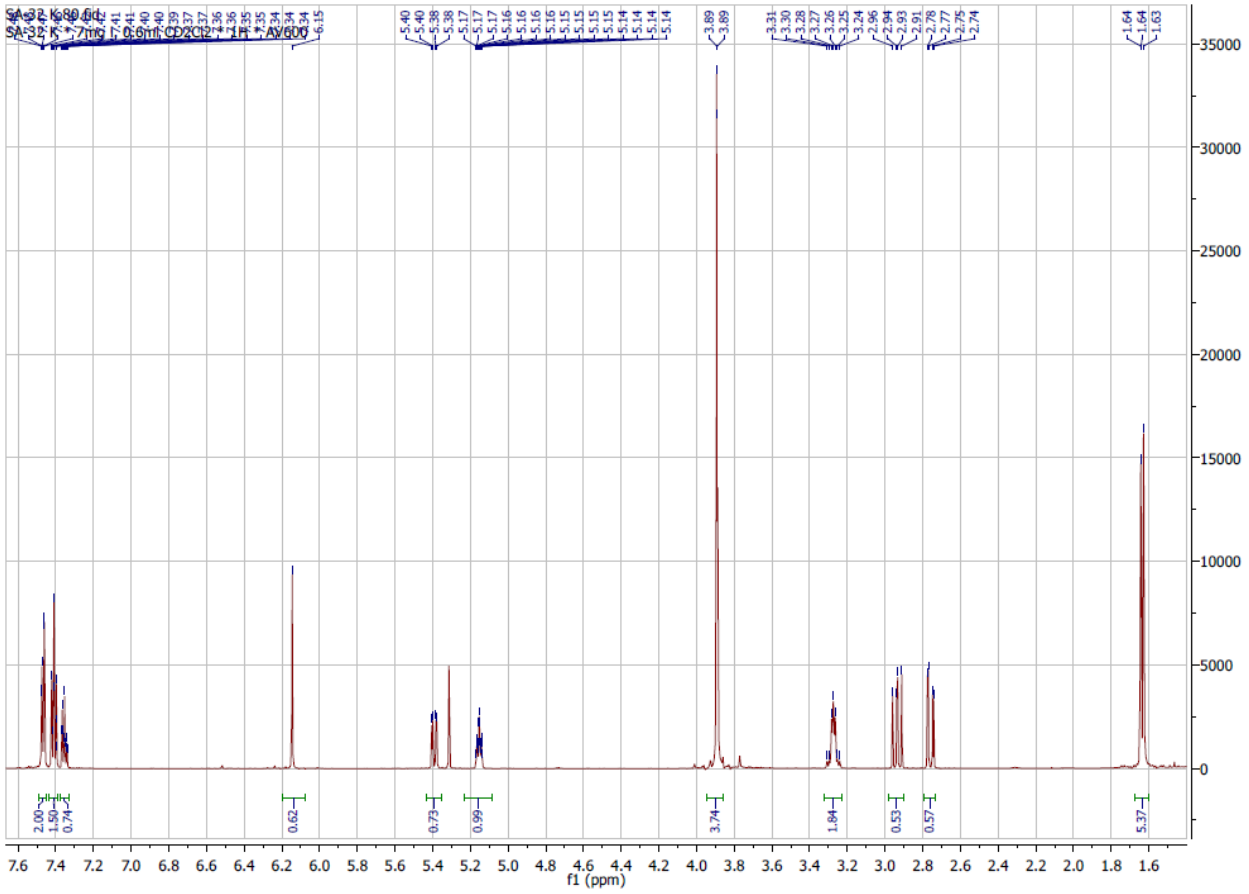
APPENDIX 12B: ^{13}C NMR Spectrum of Praecansone B (146) (150 MHz; CD_2Cl_2)



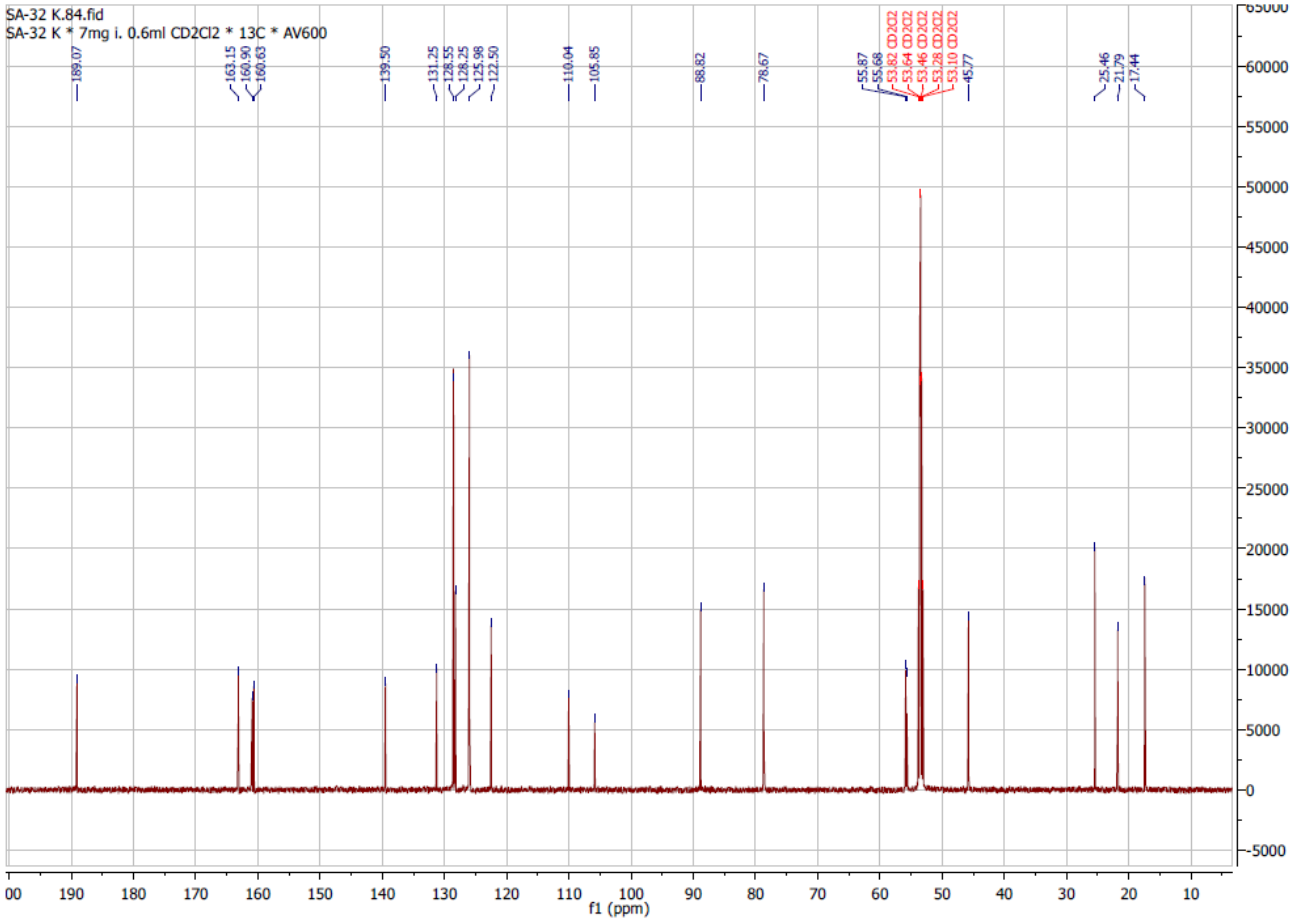
APPENDIX 12C: LCMS Spectrum of Praecansone B (146)



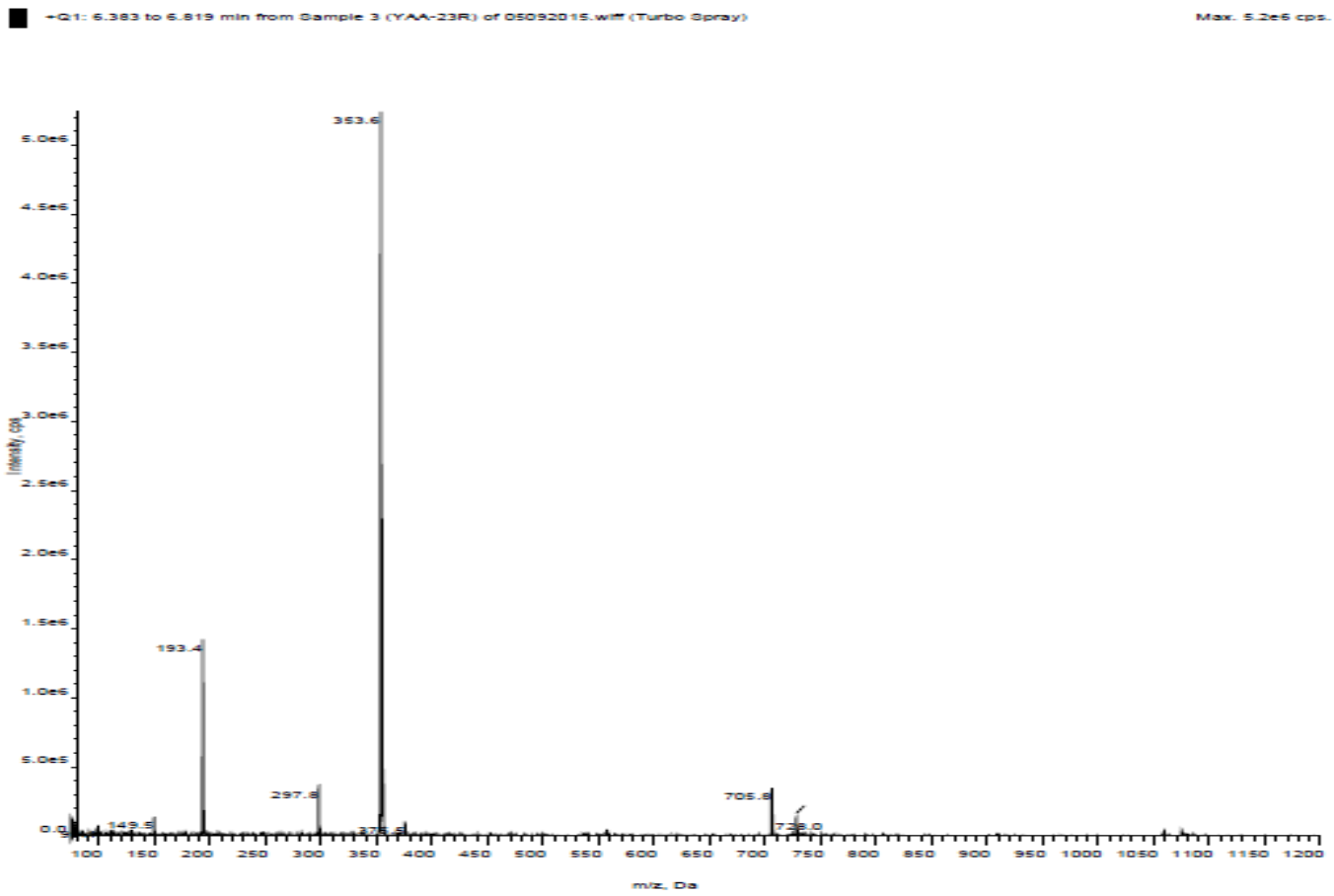
APPENDIX 13A: ^{1}H NMR Spectrum of Candidone (97) (600 MHz; CD_2Cl_2)



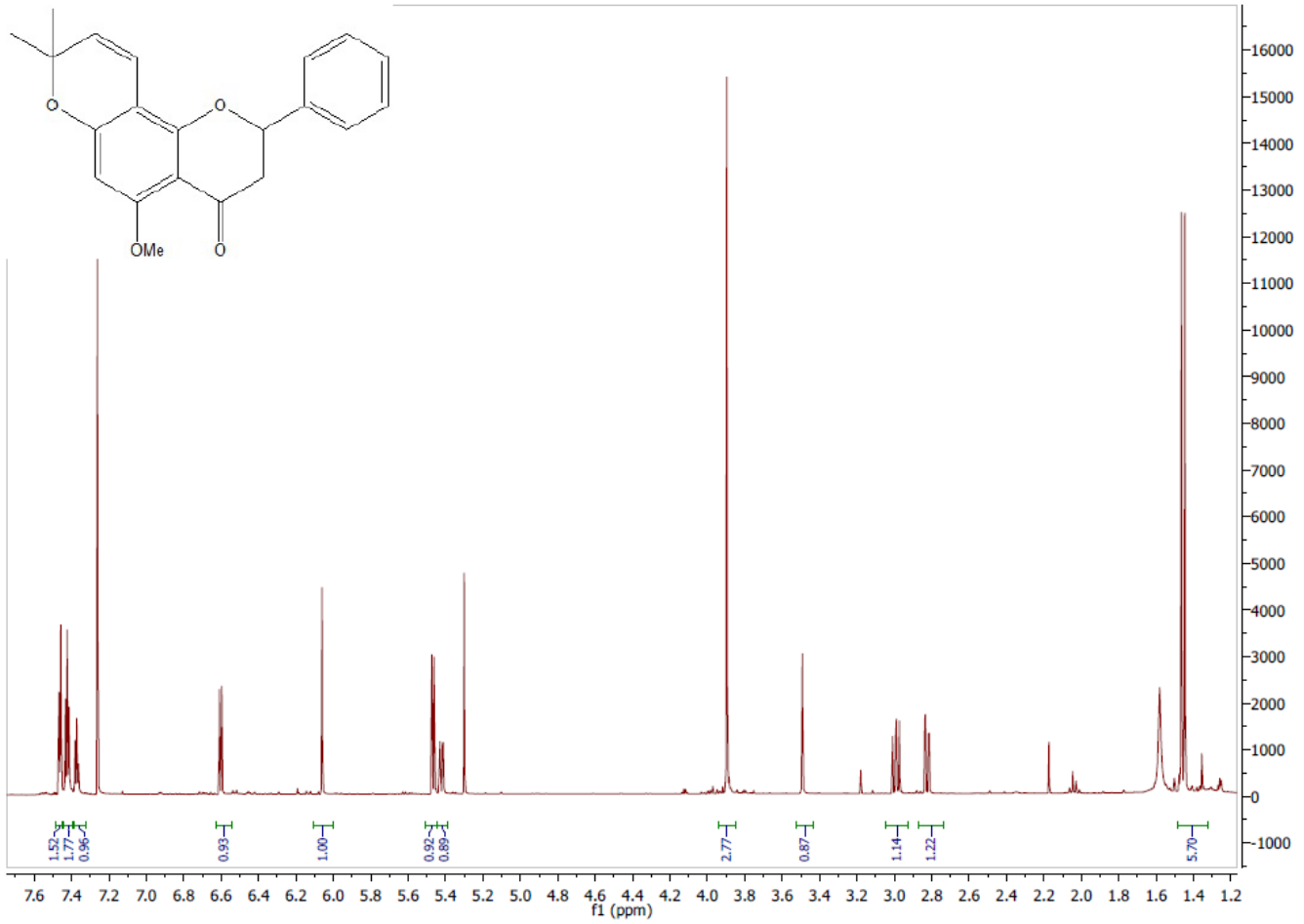
APPENDIX 13B: ^{13}C NMR Spectrum of Candidone (97) (150 MHz; CD_2Cl_2)



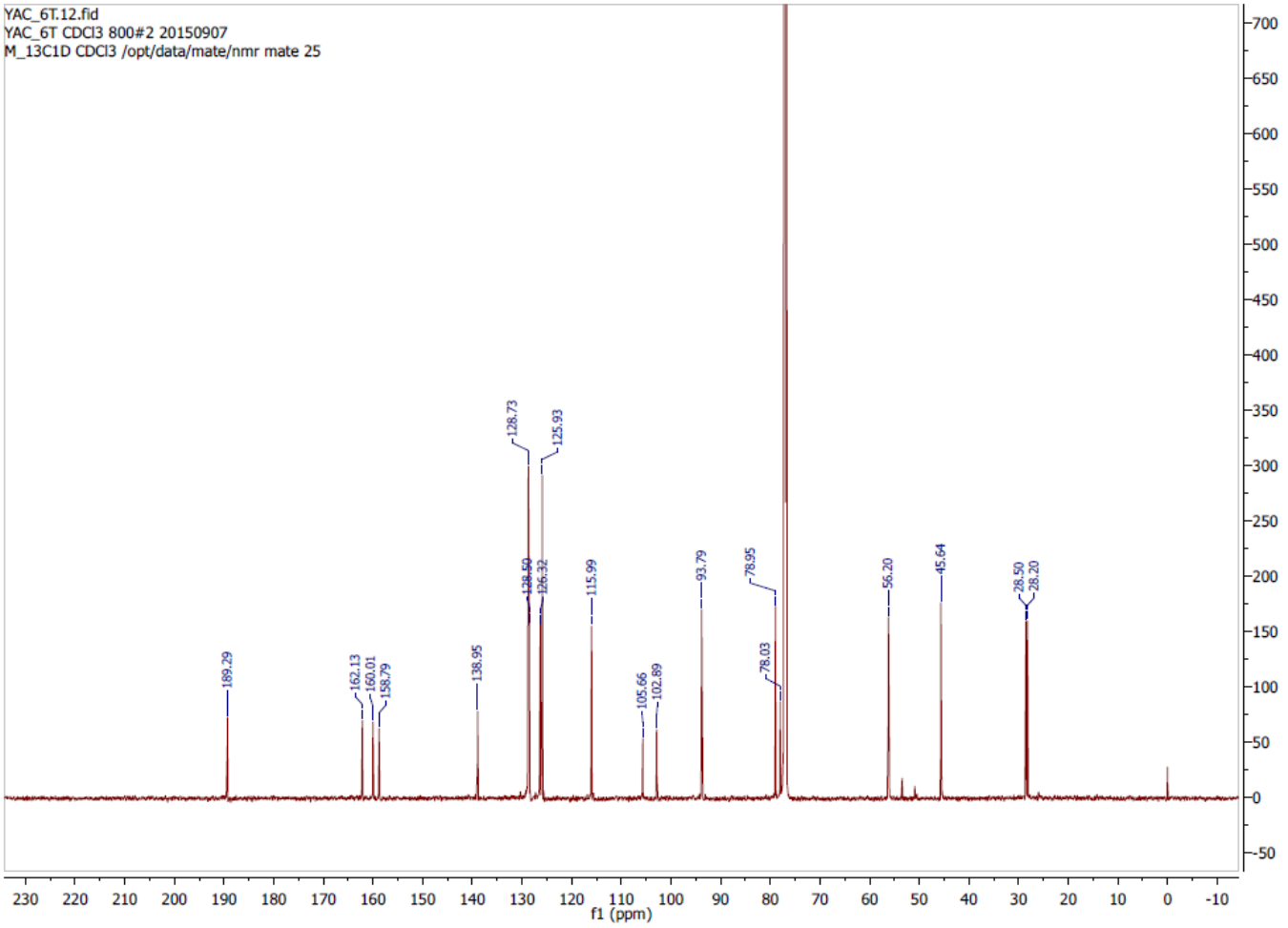
APPENDIX 13C: LCMS Spectrum of Candidone (97) (CD_2Cl_2)



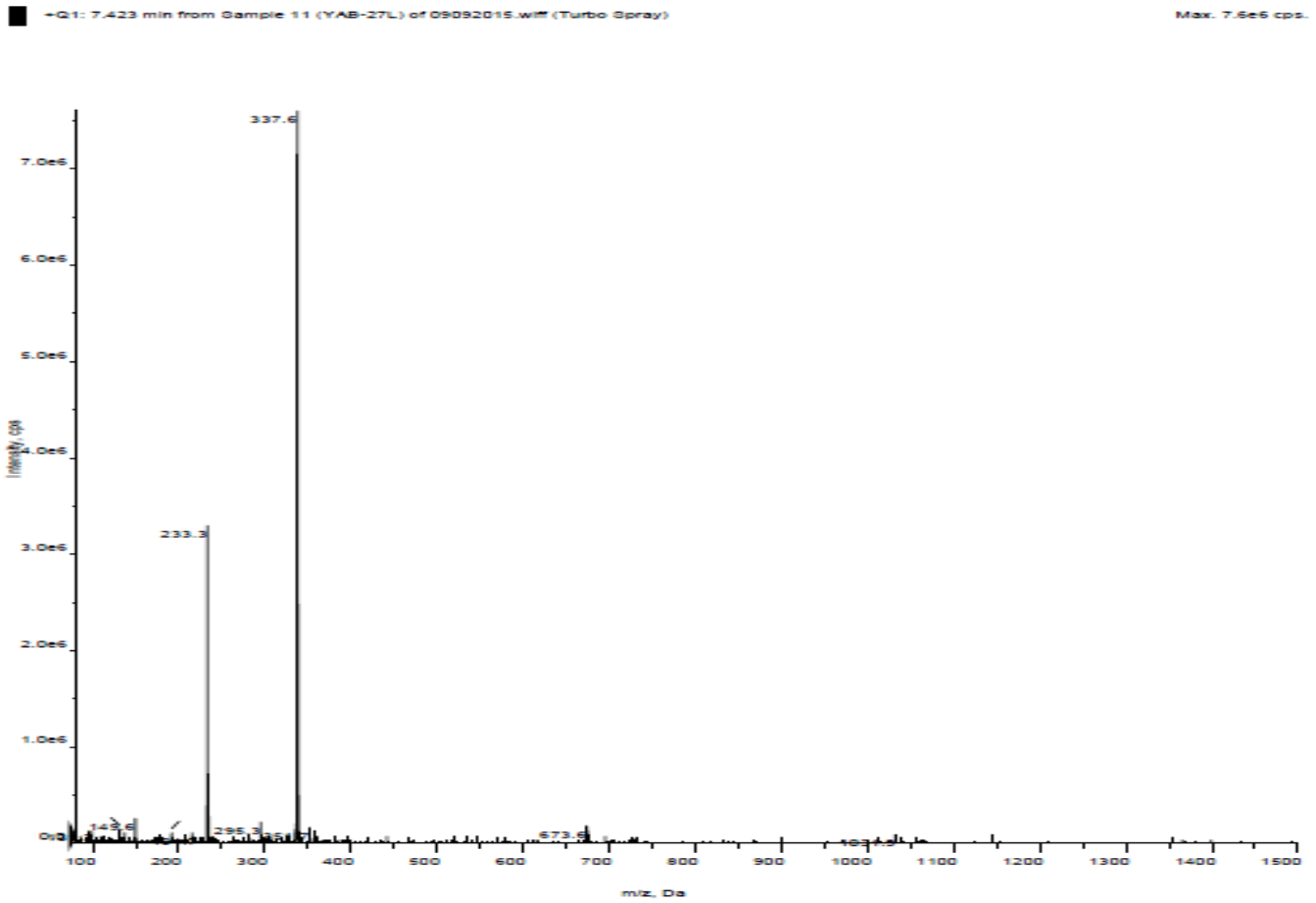
APPENDIX 14A: ^{1}H NMR Spectrum of Obovatin methyl ether (73) (800 MHz; $CDCl_3$)



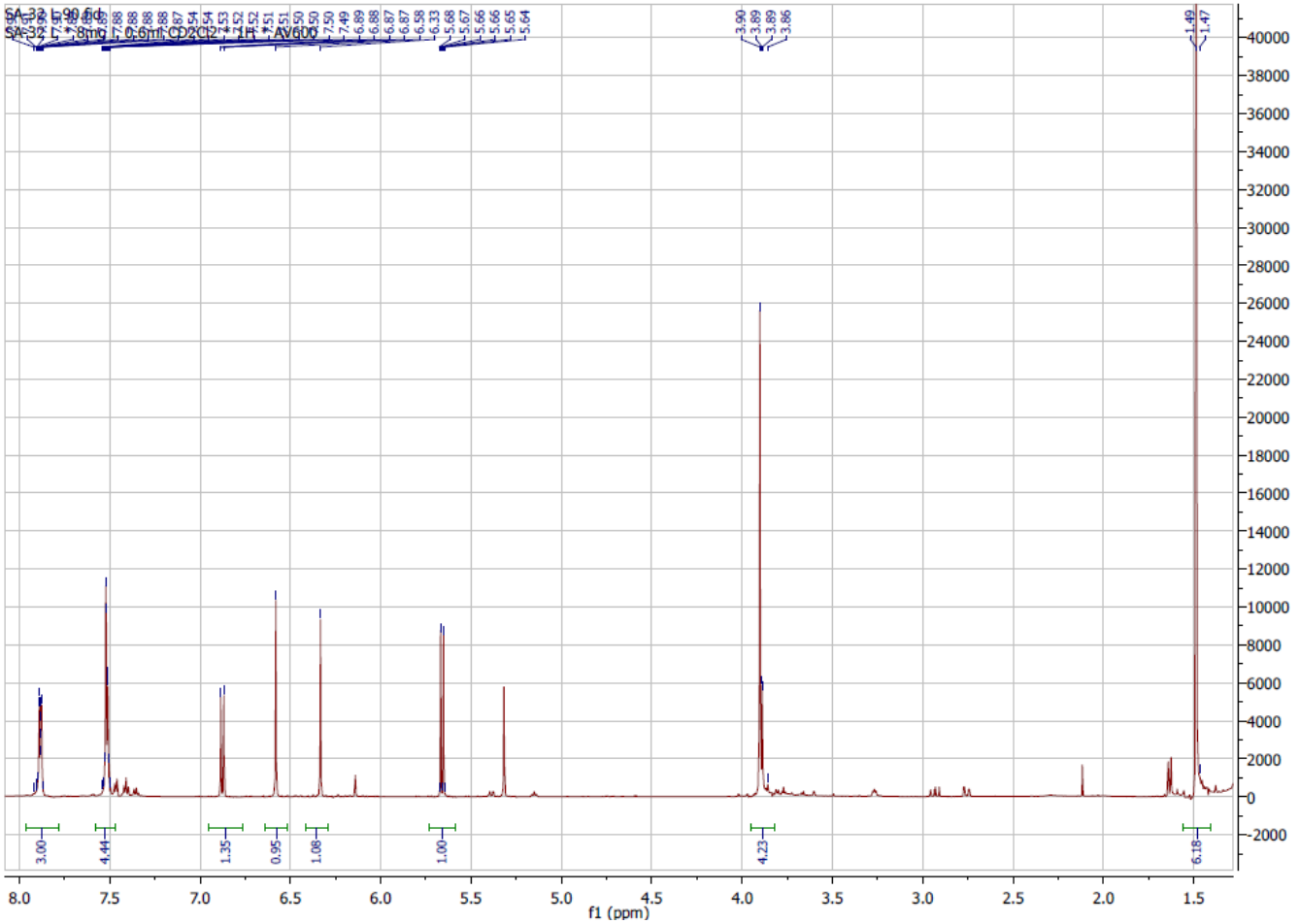
APPENDIX 14B: ^{13}C NMR Spectrum of Obovatin methyl ether (73) (200 MHz; $CDCl_3$)



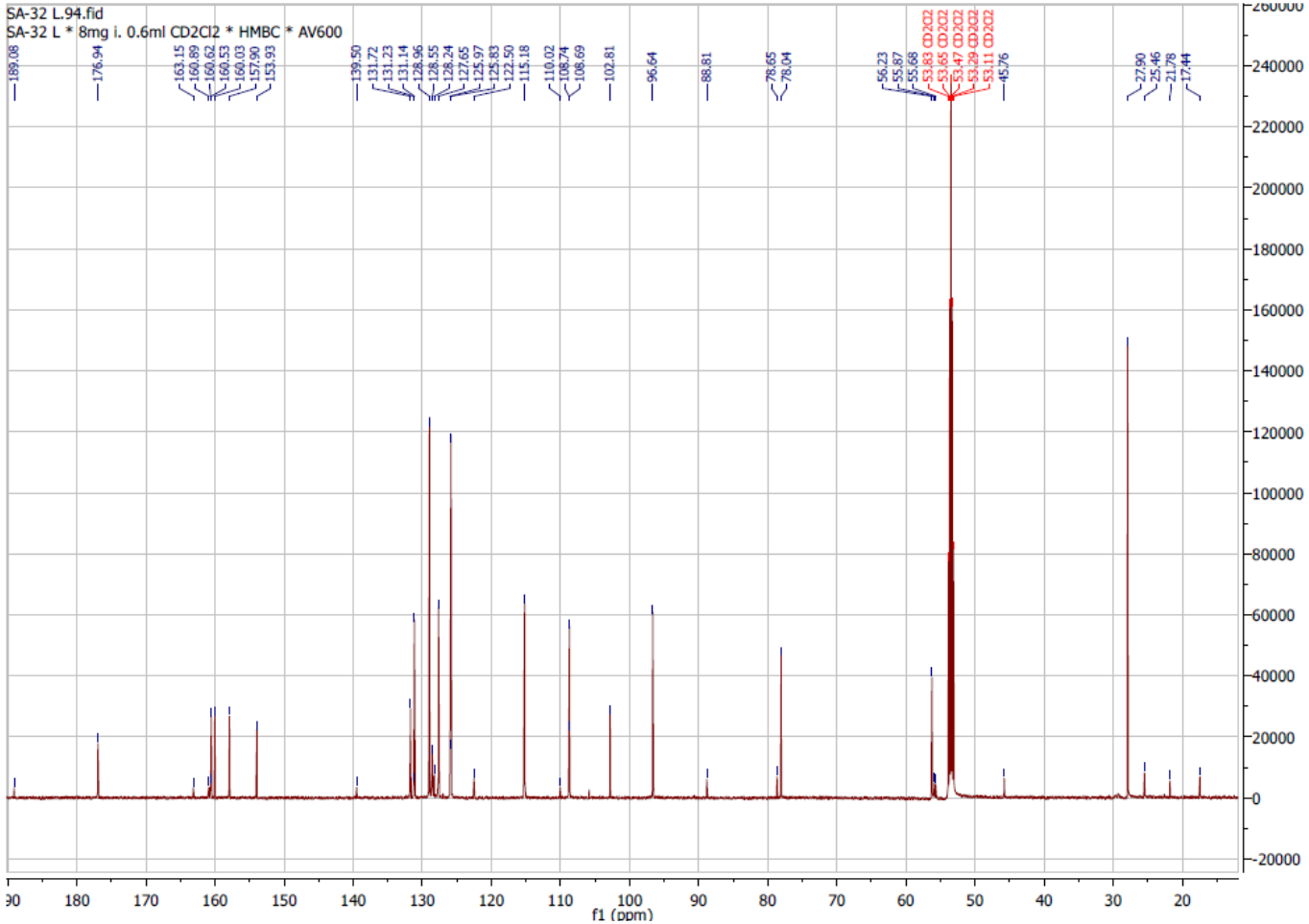
APPENDIX 14C: LCMS Spectrum of Obovatin methyl ether (73) ($CDCl_3$)



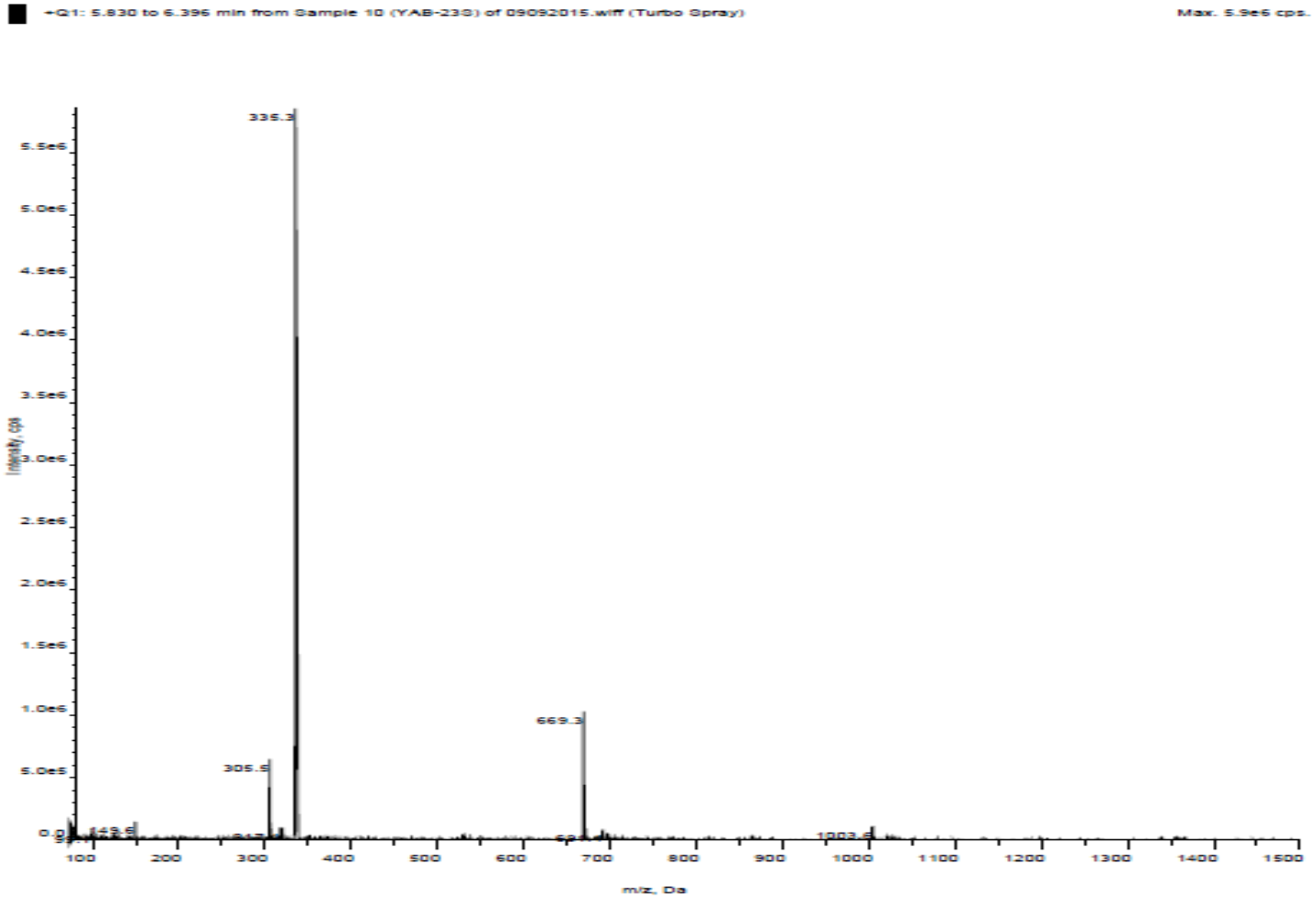
APPENDIX 15A: ^{1}H NMR Spectrum of Isopongaflavone (59) (600 MHz; CD_2Cl_2)



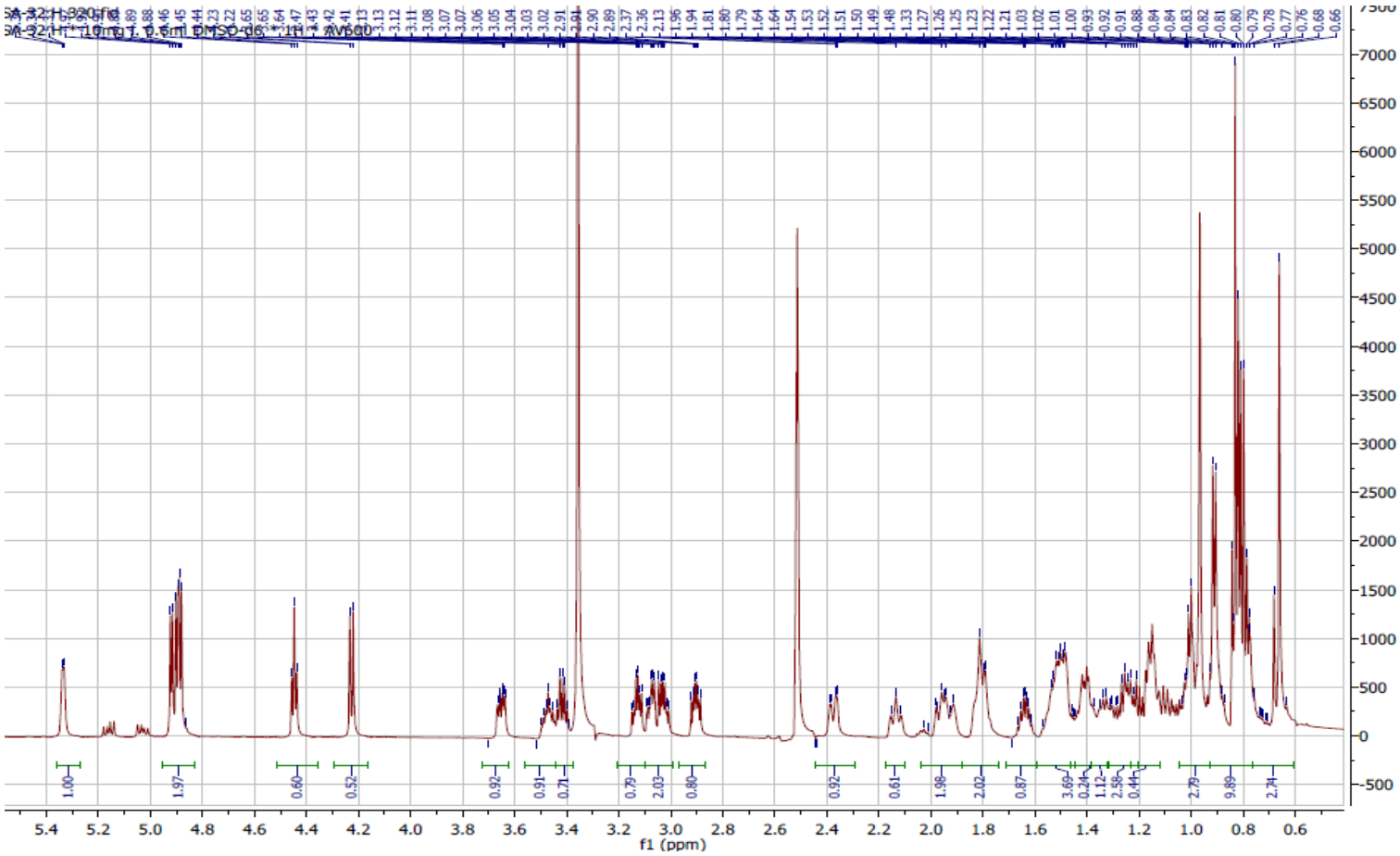
APPENDIX 15B: ^{13}C NMR Spectrum of Isopongaflavone (59) (150 MHz; CD_2Cl_2)



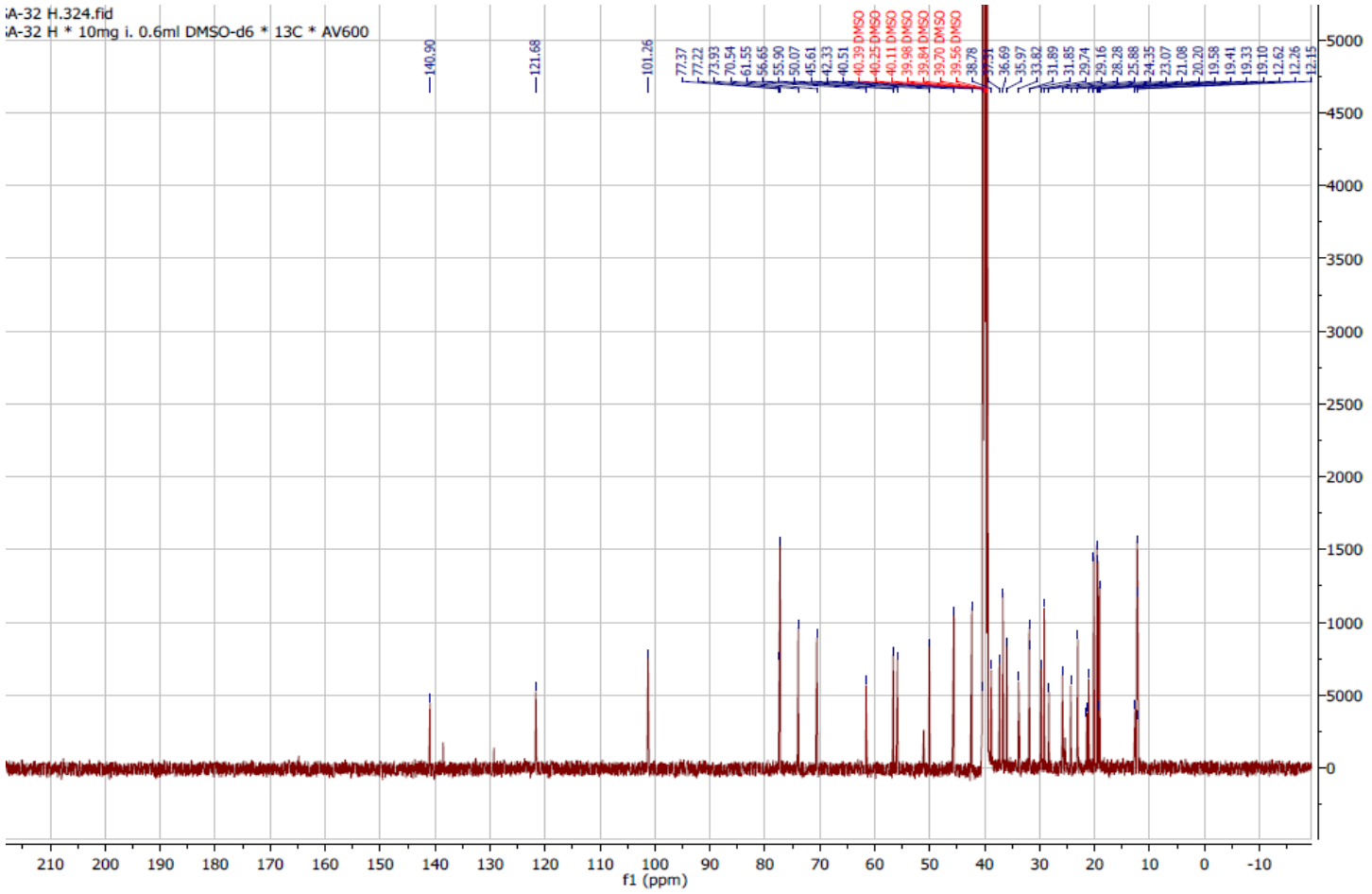
APPENDIX 15C: LCMS Spectrum of Isopongaflavone (59) (CD_2Cl_2)



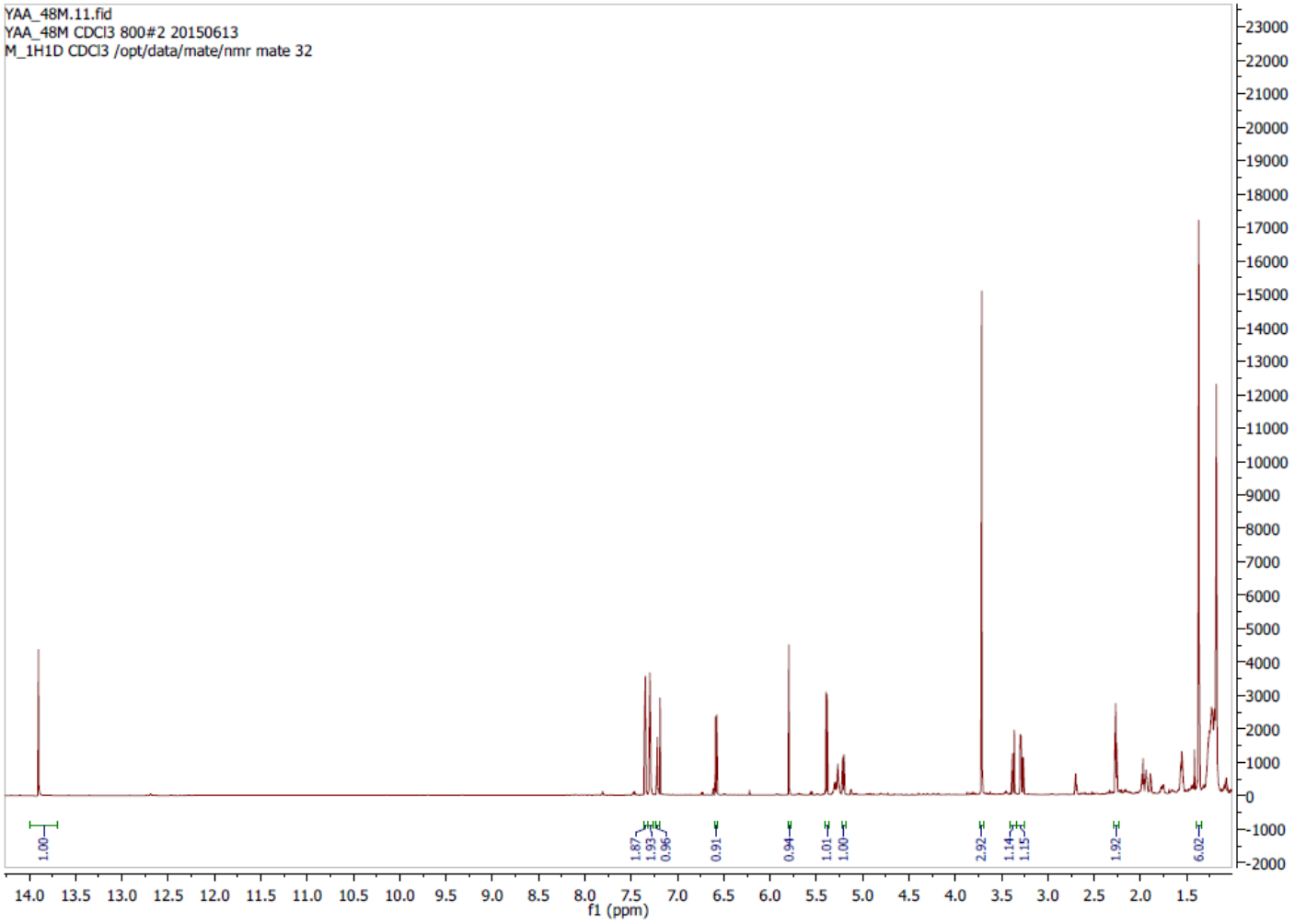
APPENDIX 16A: ^{1}H NMR Spectrum of □-sitostrol-3-O-glucoside (171) (600 MHz; CD_2Cl_2)



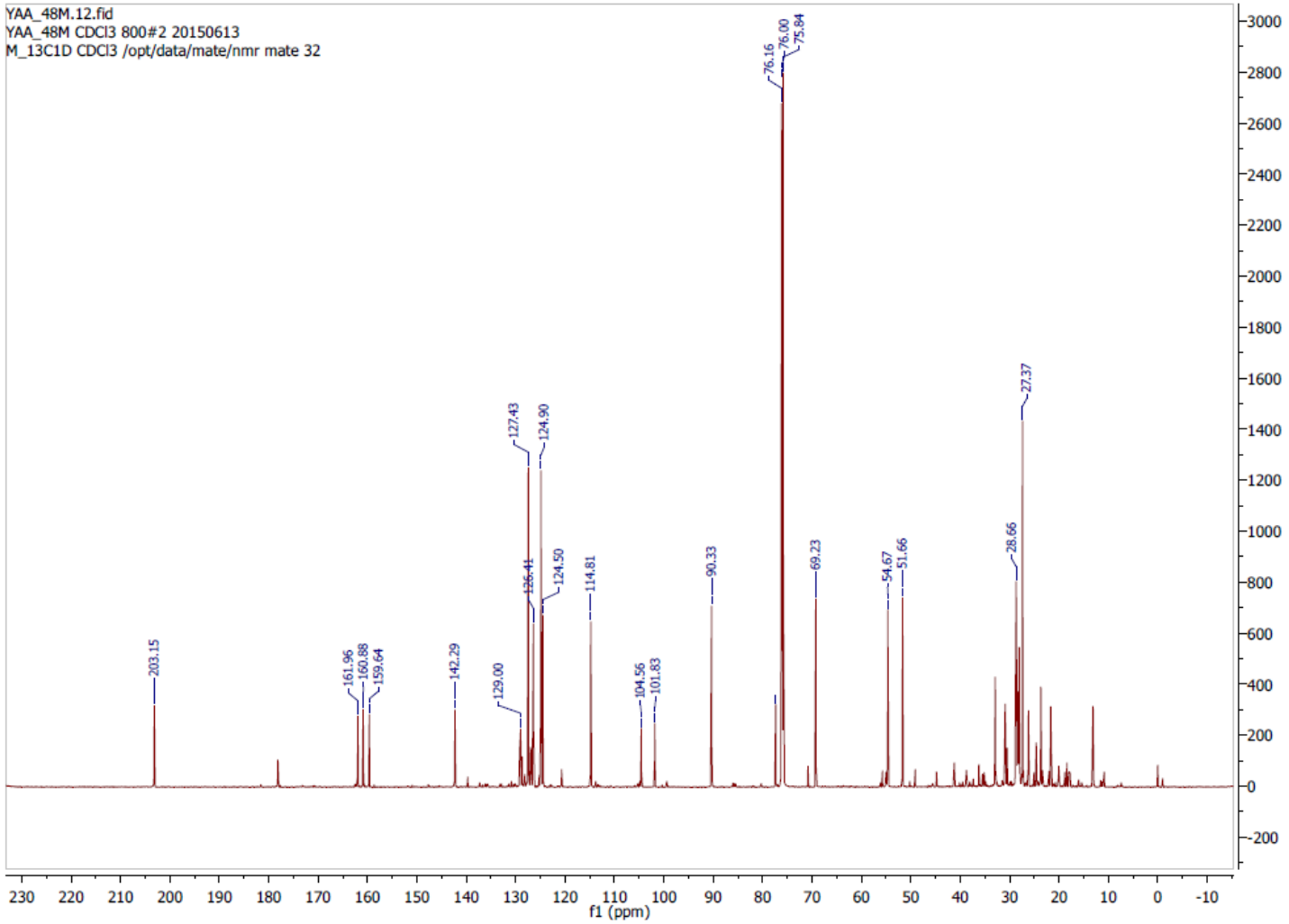
APPENDIX 16B: ^{1}H NMR Spectrum of β-sitostrol-3-O-glucoside (171) (150 MHz; CD_2Cl_2)



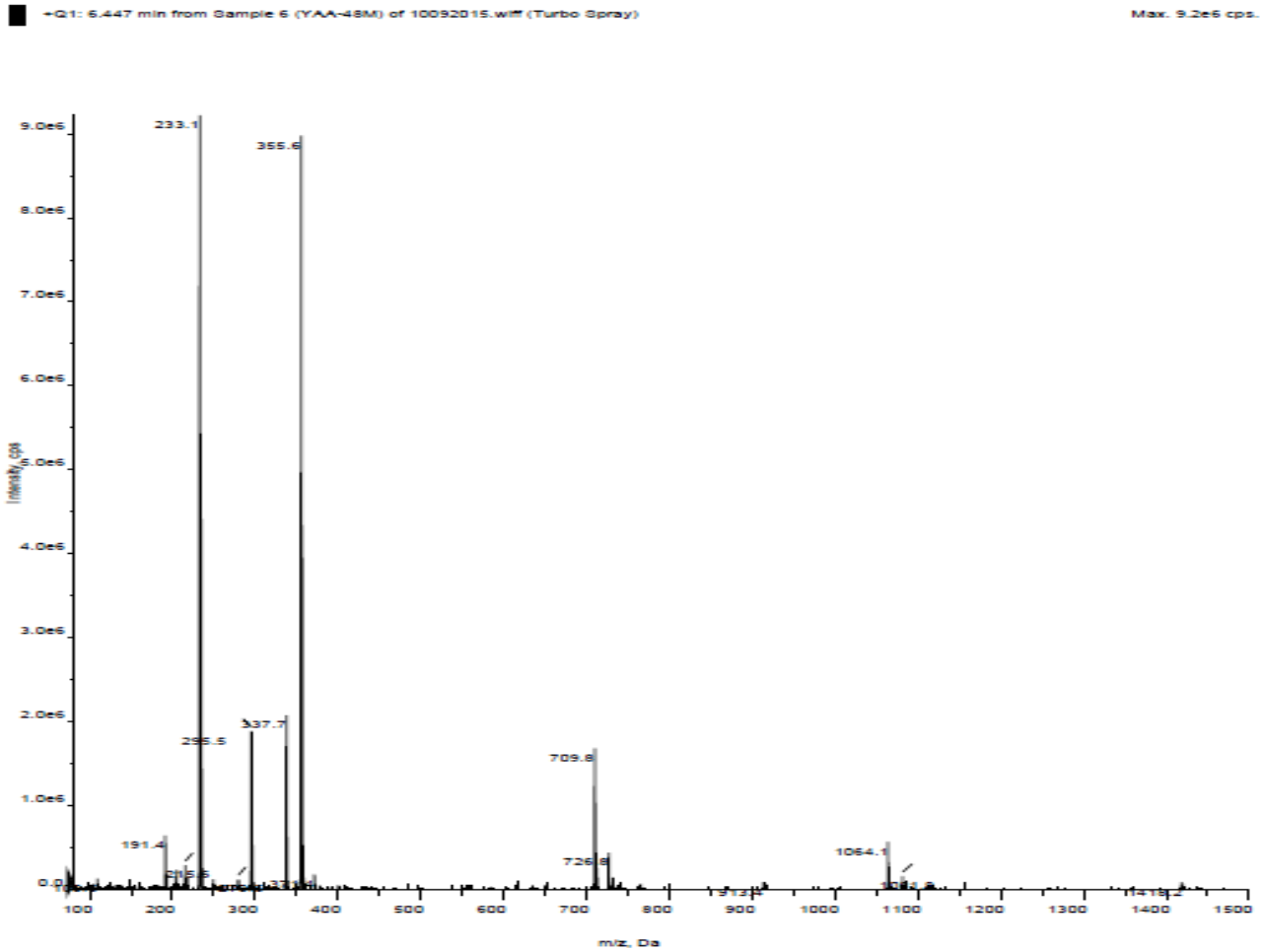
APPENDIX 17A: ^{1}H NMR Spectrum of (*S*)-Elatadihydrochalcone (143) (800 MHz; $CDCl_3$)



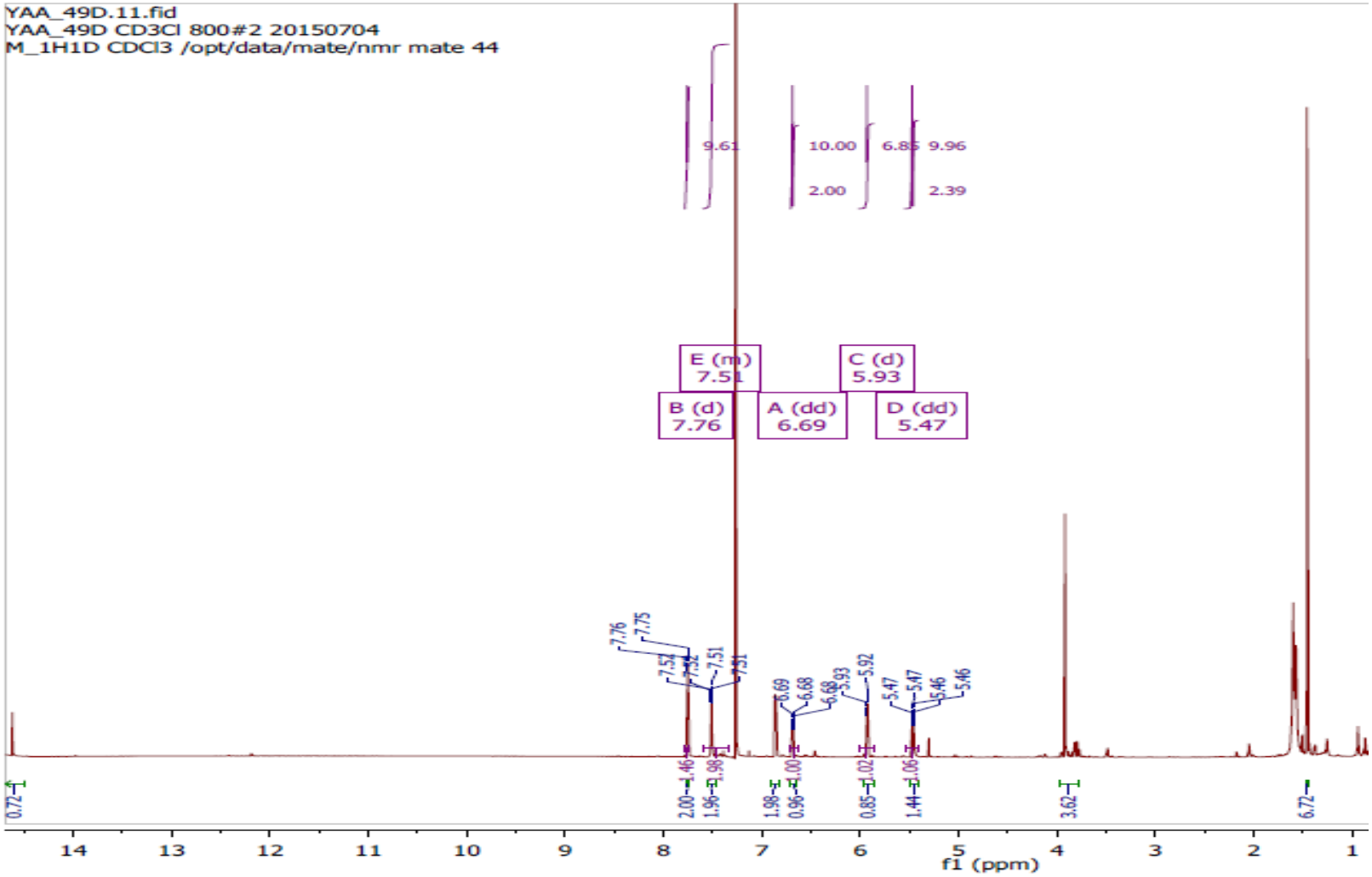
APPENDIX 17B: ^{13}C NMR Spectrum of (*S*)-Elatadihydrochalcone (143) (200 MHz; $CDCl_3$)



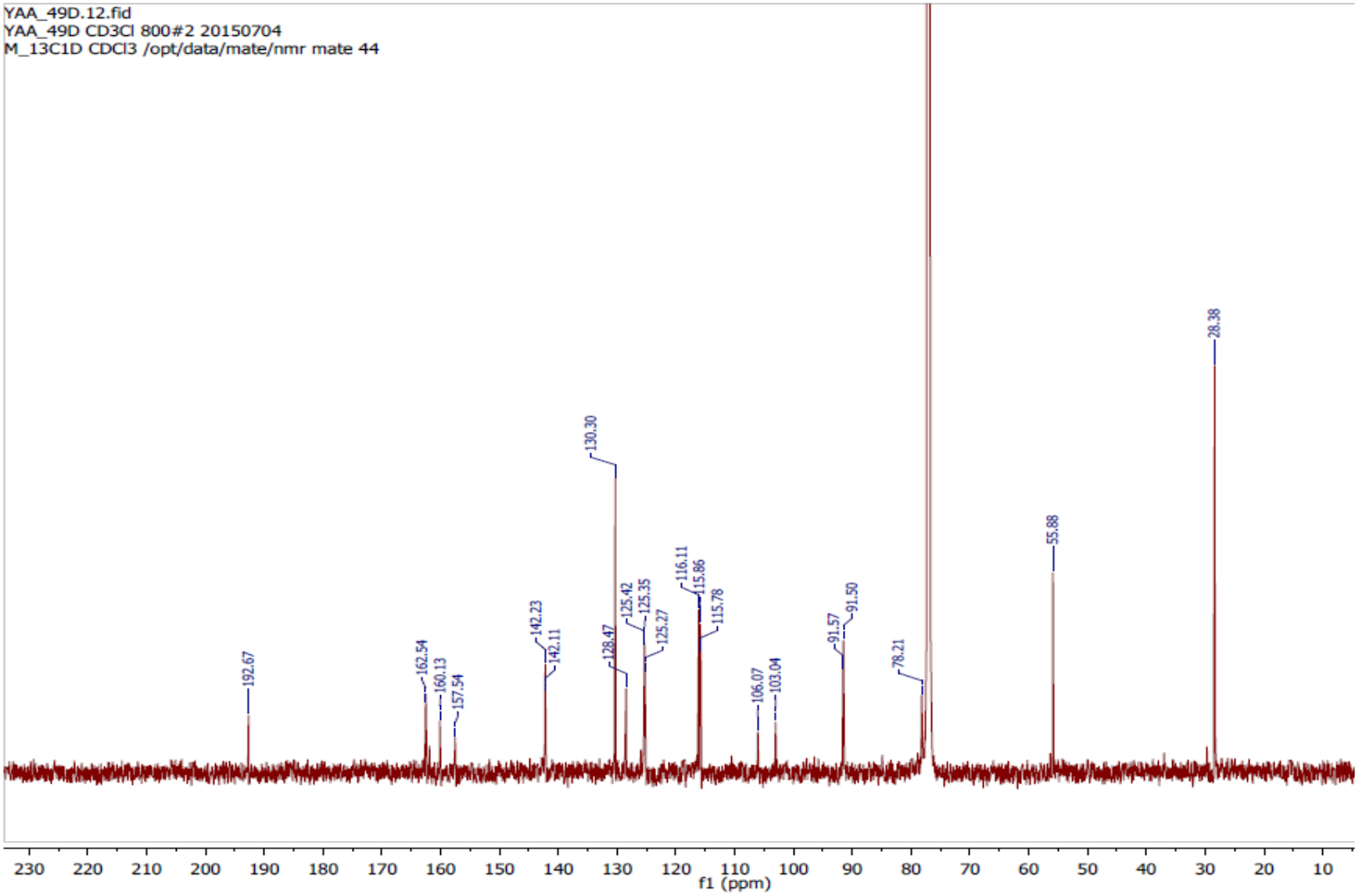
APPENDIX 17C: LCMS Spectrum of (*S*)-Elatadihydrochalcone (143)



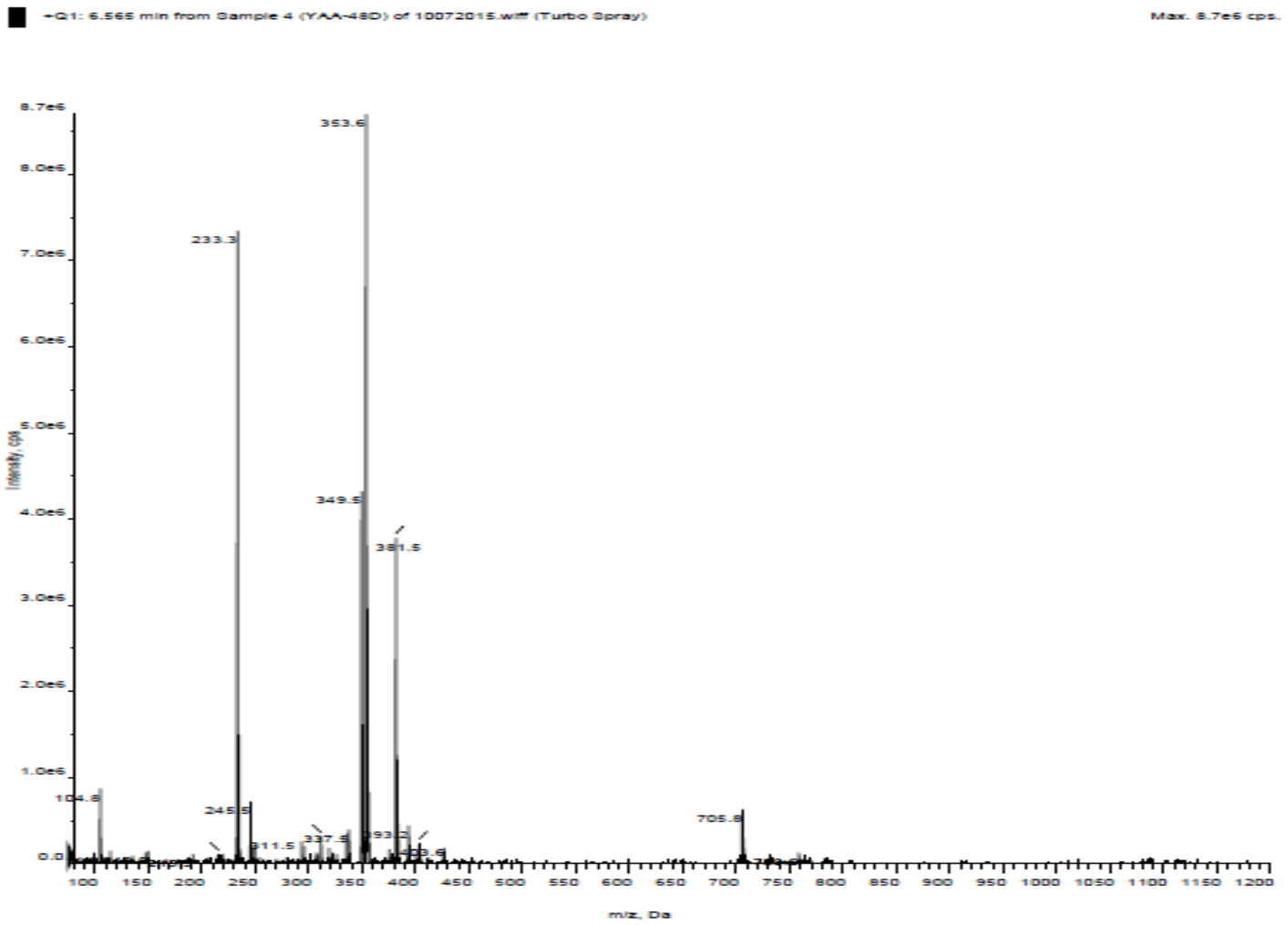
APPENDIX 18A: ^{1}H NMR Spectrum of Xanthohumol C (172) (800 MHz; $CDCl_3$)



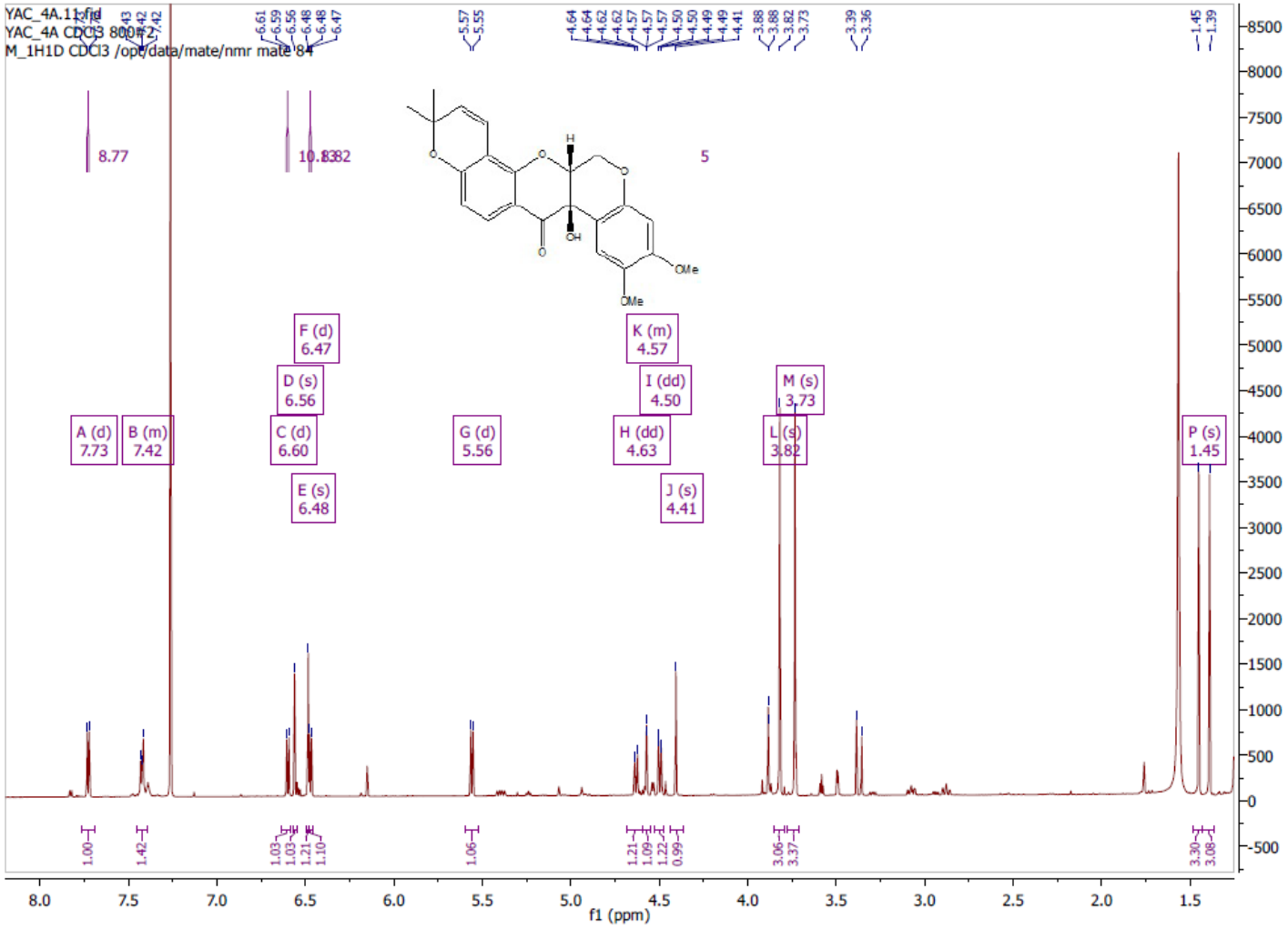
APPENDIX 18B: ^{13}C NMR Spectrum of Xanthohumol C (172) (200 MHz; $CDCl_3$)



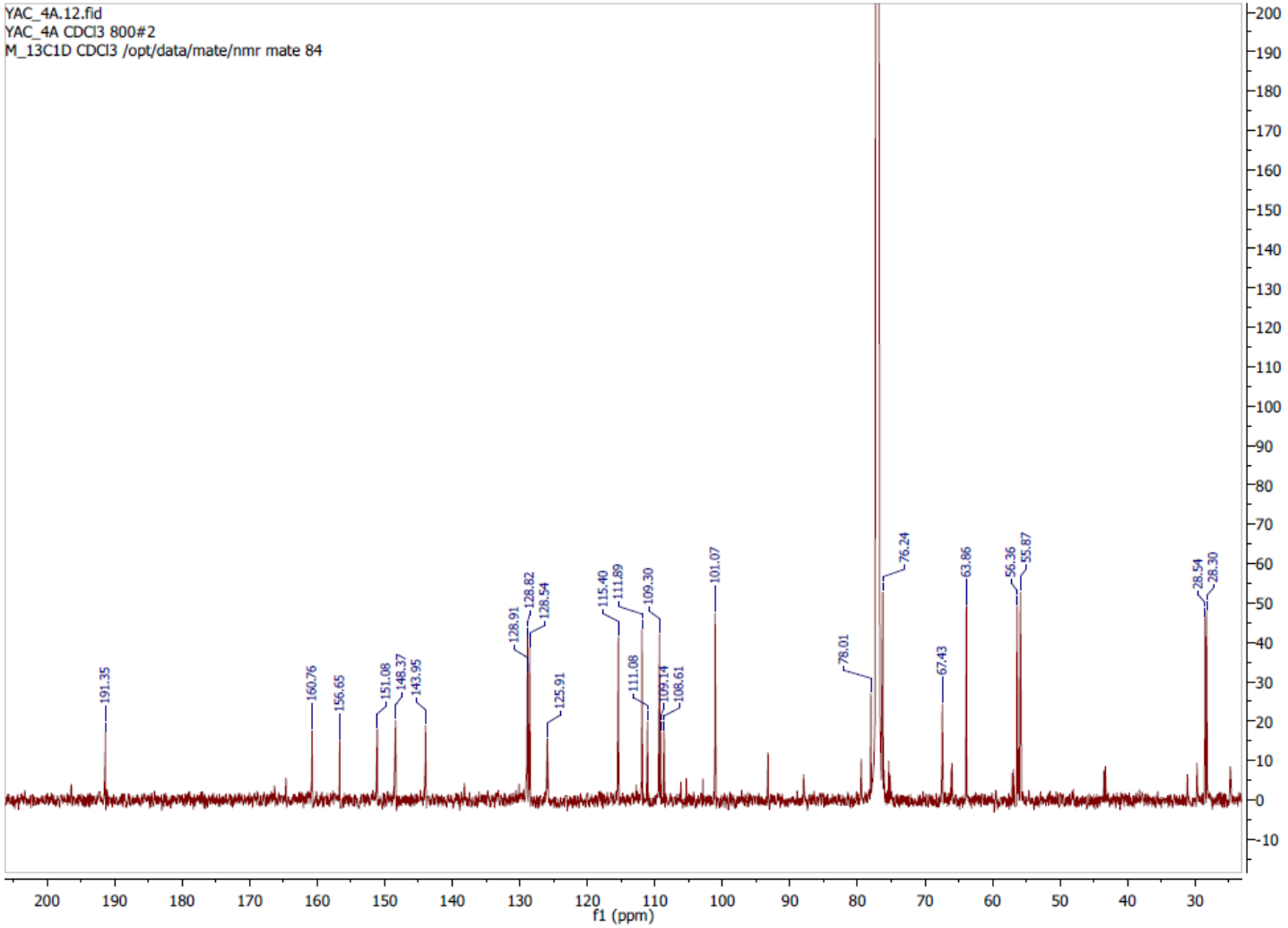
APPENDIX 18C: LCMS Spectrum of Xanthohumol C (172)



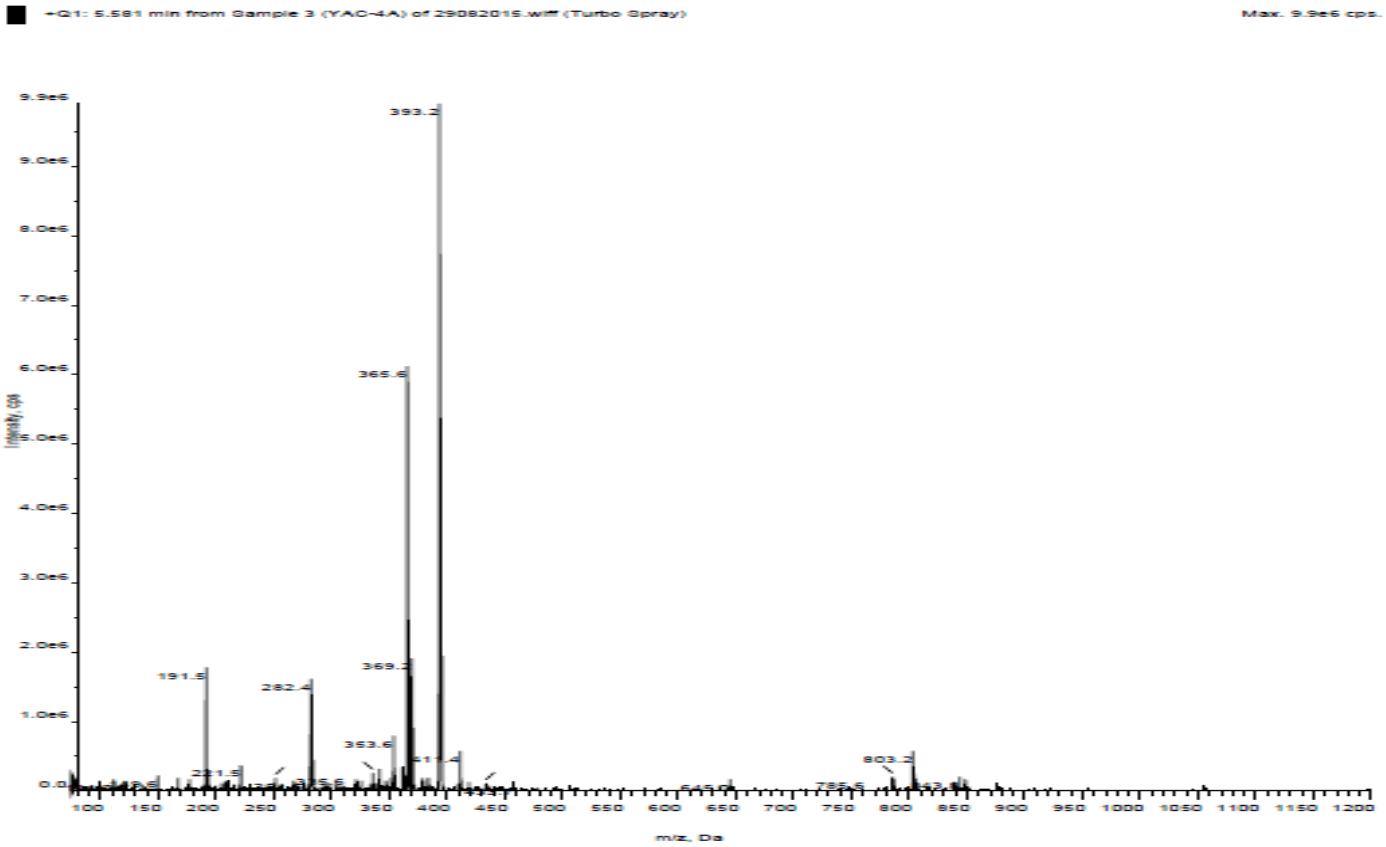
APPENDIX 19A: ^{1}H NMR Spectrum of Tephrosin (173) (800 MHz; $CDCl_3$)



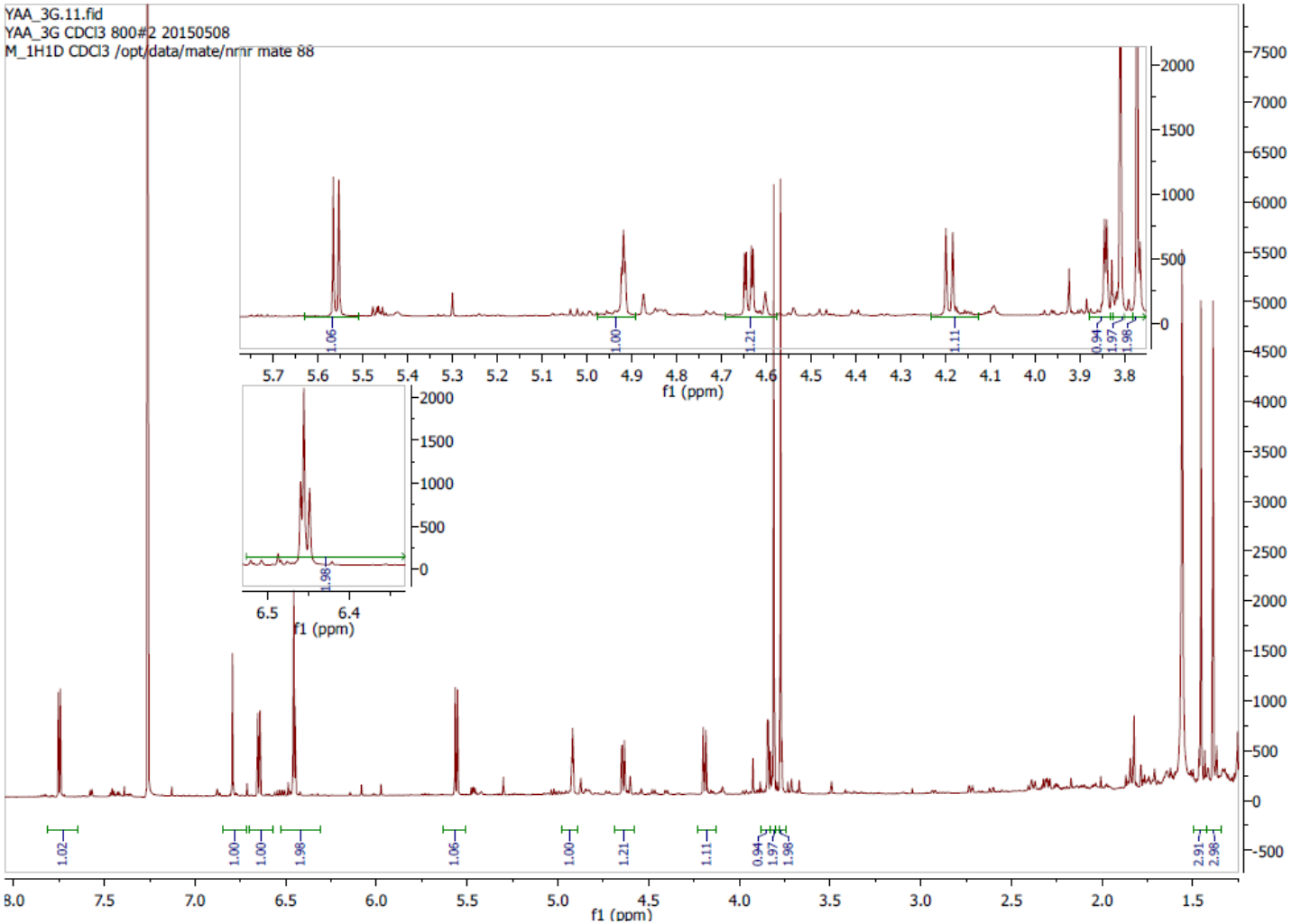
APPENDIX 19B: ^{13}C NMR Spectrum of Tephrosin (173) (200 MHz; $CDCl_3$)



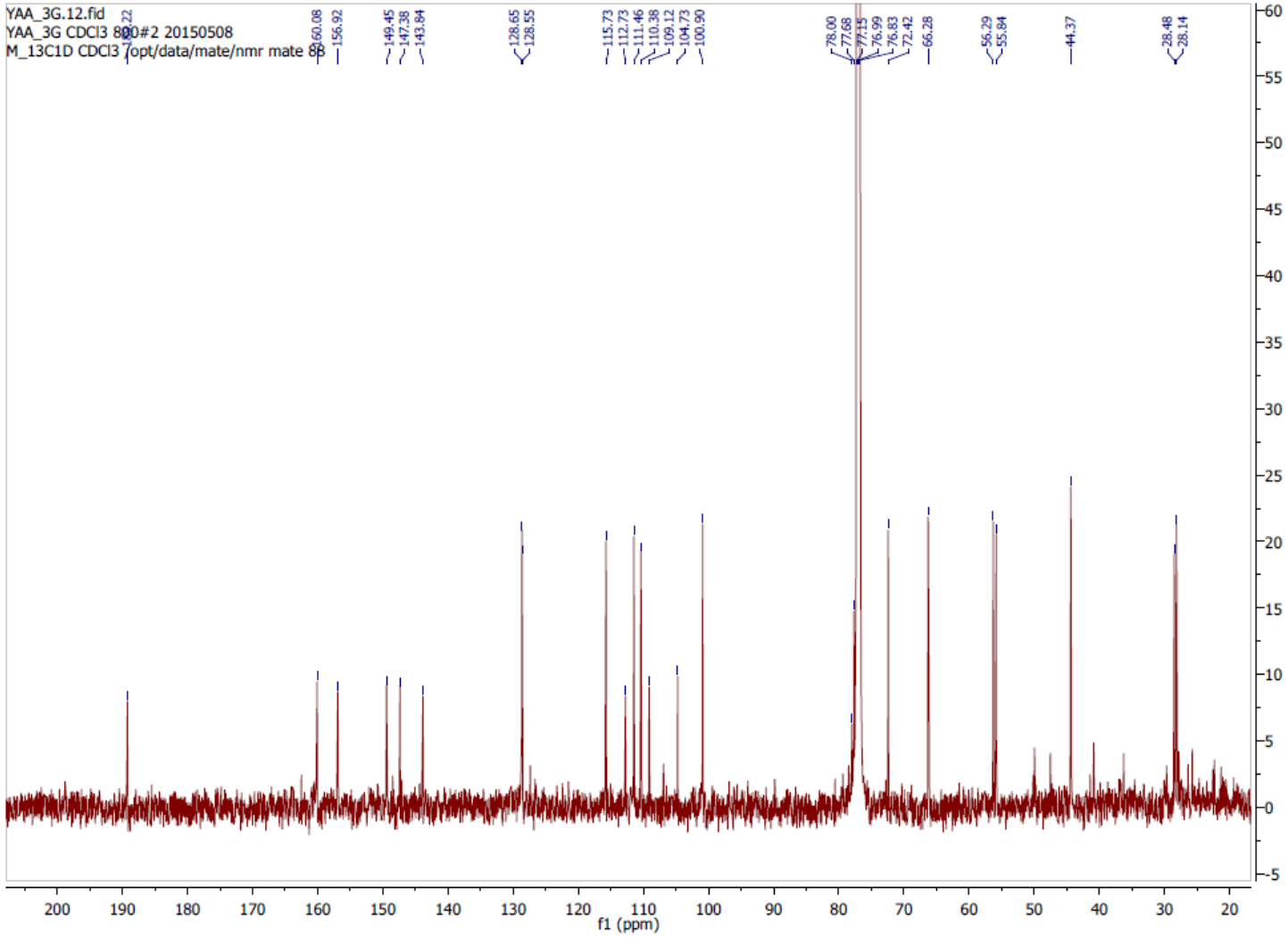
APPENDIX 19C: LCMS Spectrum of Tephrosin (173) ($CDCl_3$)



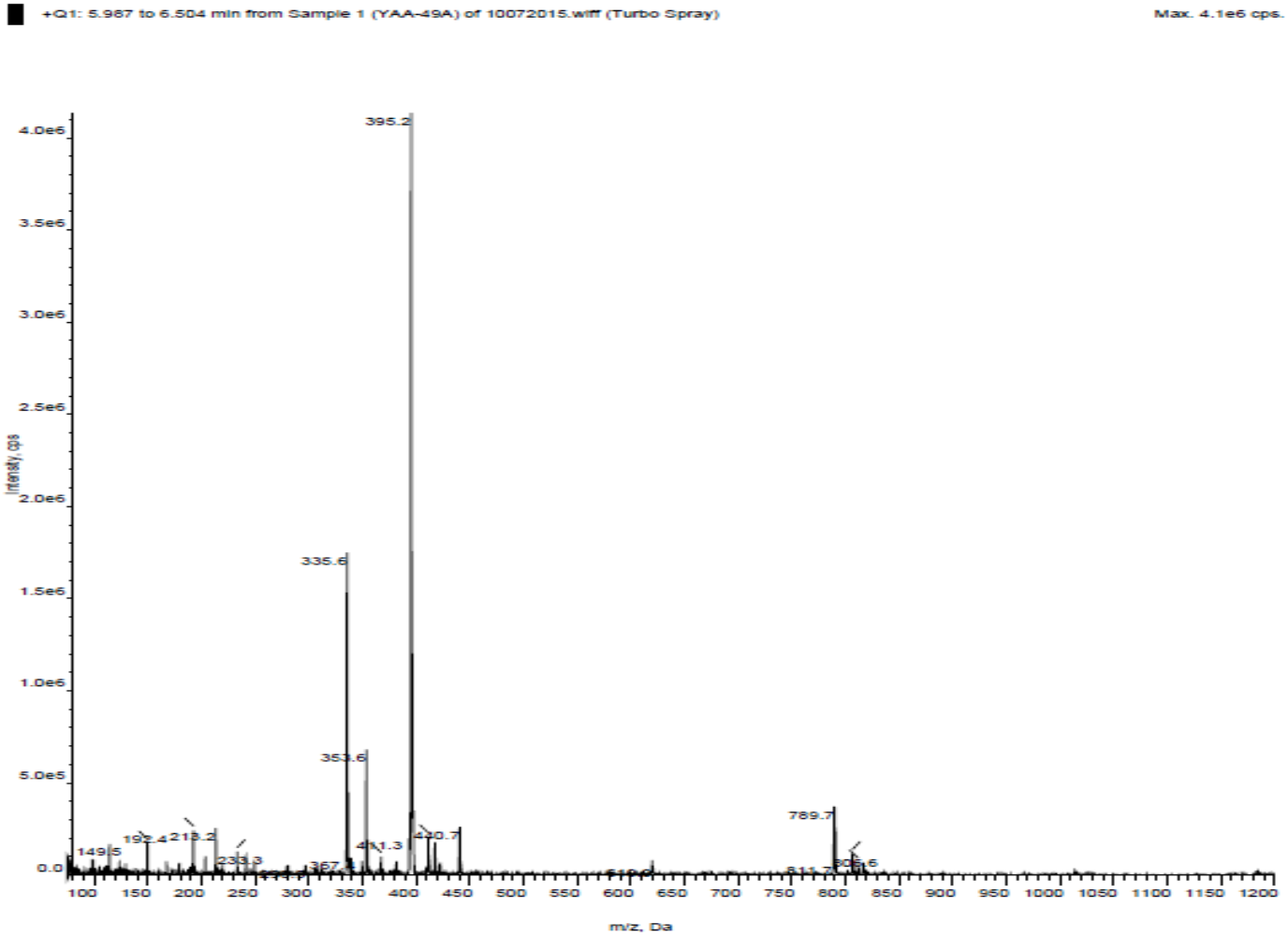
APPENDIX 20A: ^{1}H NMR Spectrum of Deguelin (174) (800 MHz; $CDCl_3$)



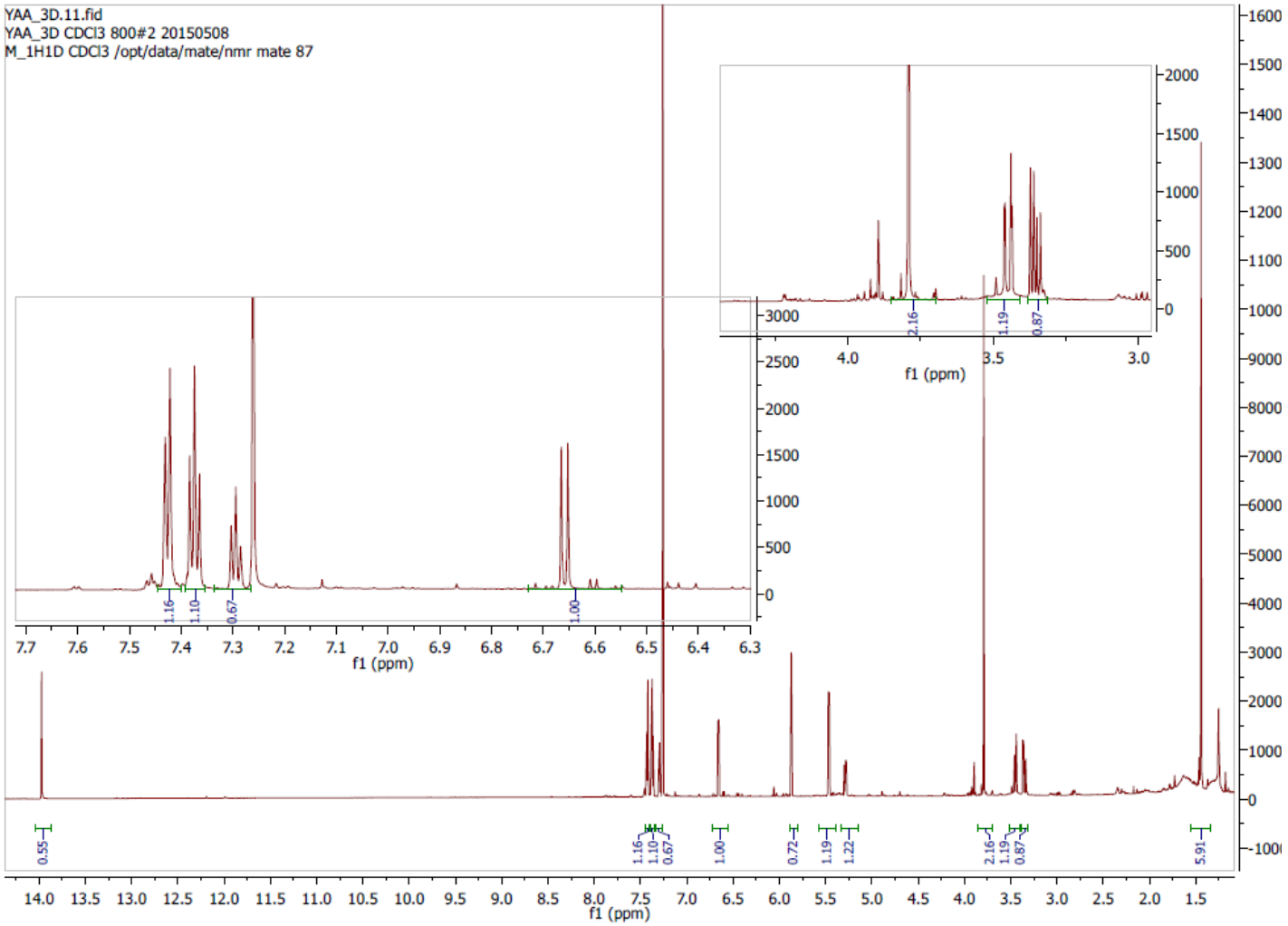
APPENDIX 20B: ^{13}C NMR Spectrum of Deguelin (174) (200 MHz; $CDCl_3$)



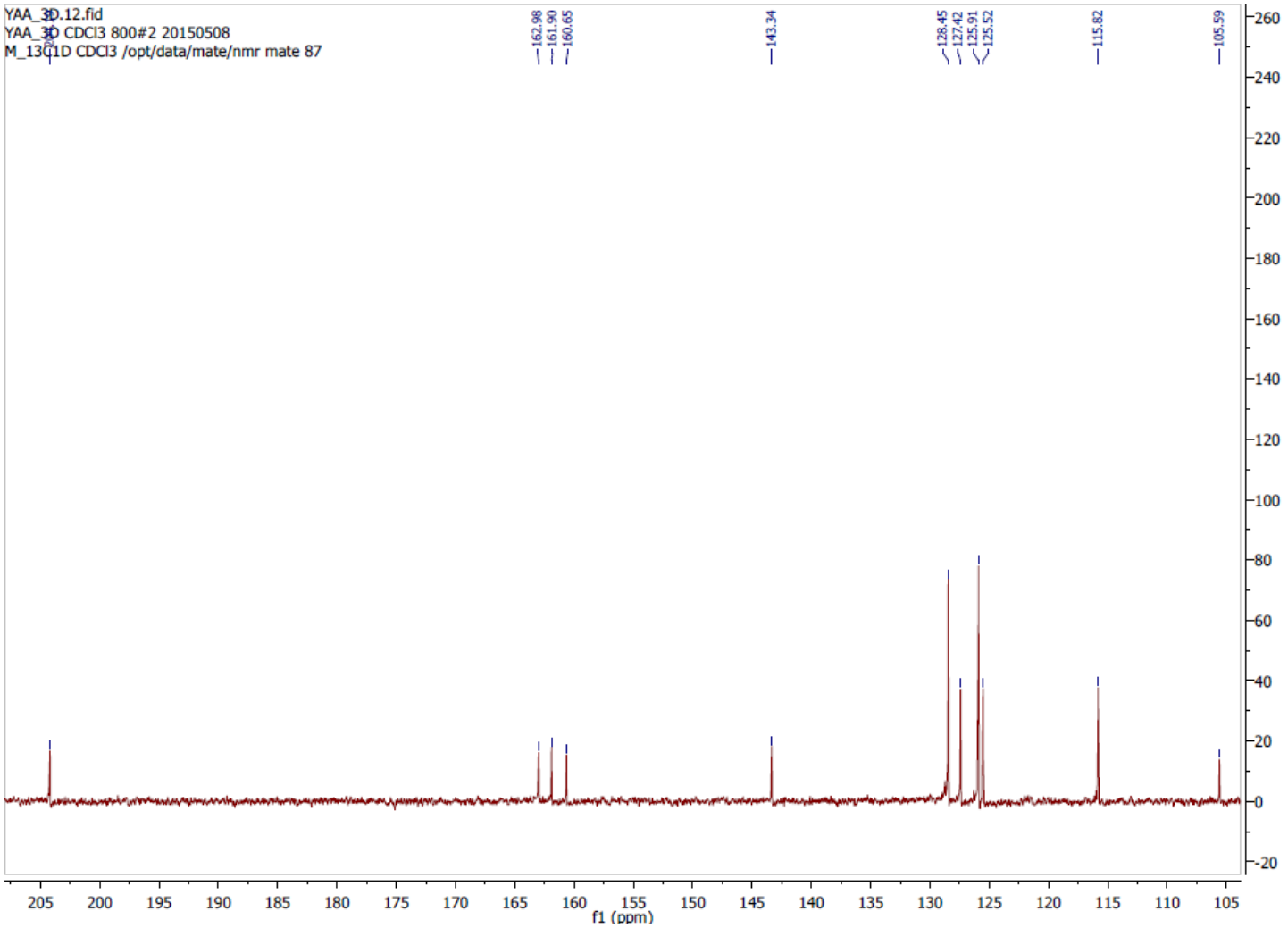
APPENDIX 20C: LCMS Spectrum of Deguelin (174) ($CDCl_3$)



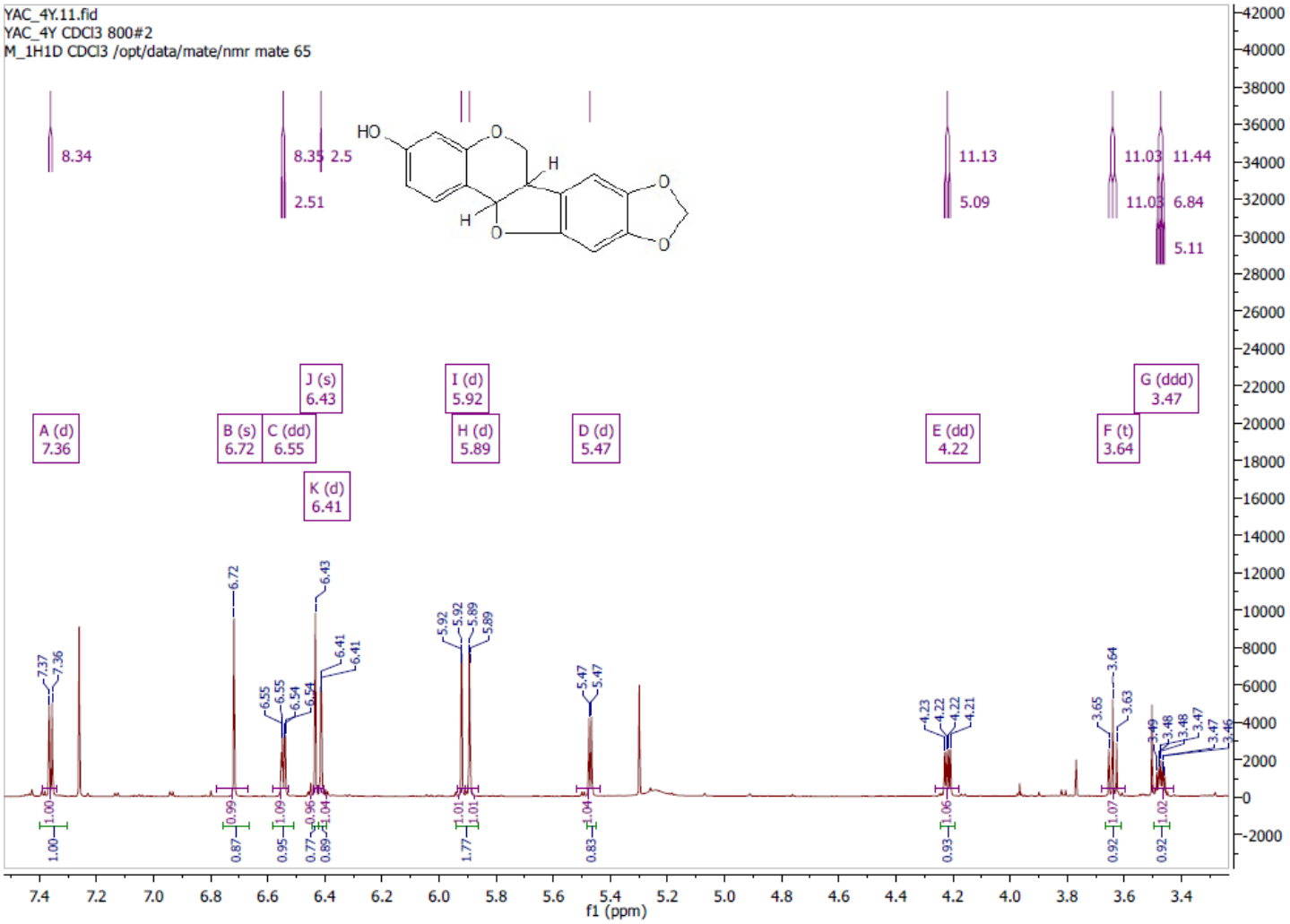
APPENDIX 21A: ^{1}H NMR Spectrum of Tephrolecarpin A(175) (800 MHz; $CDCl_3$)



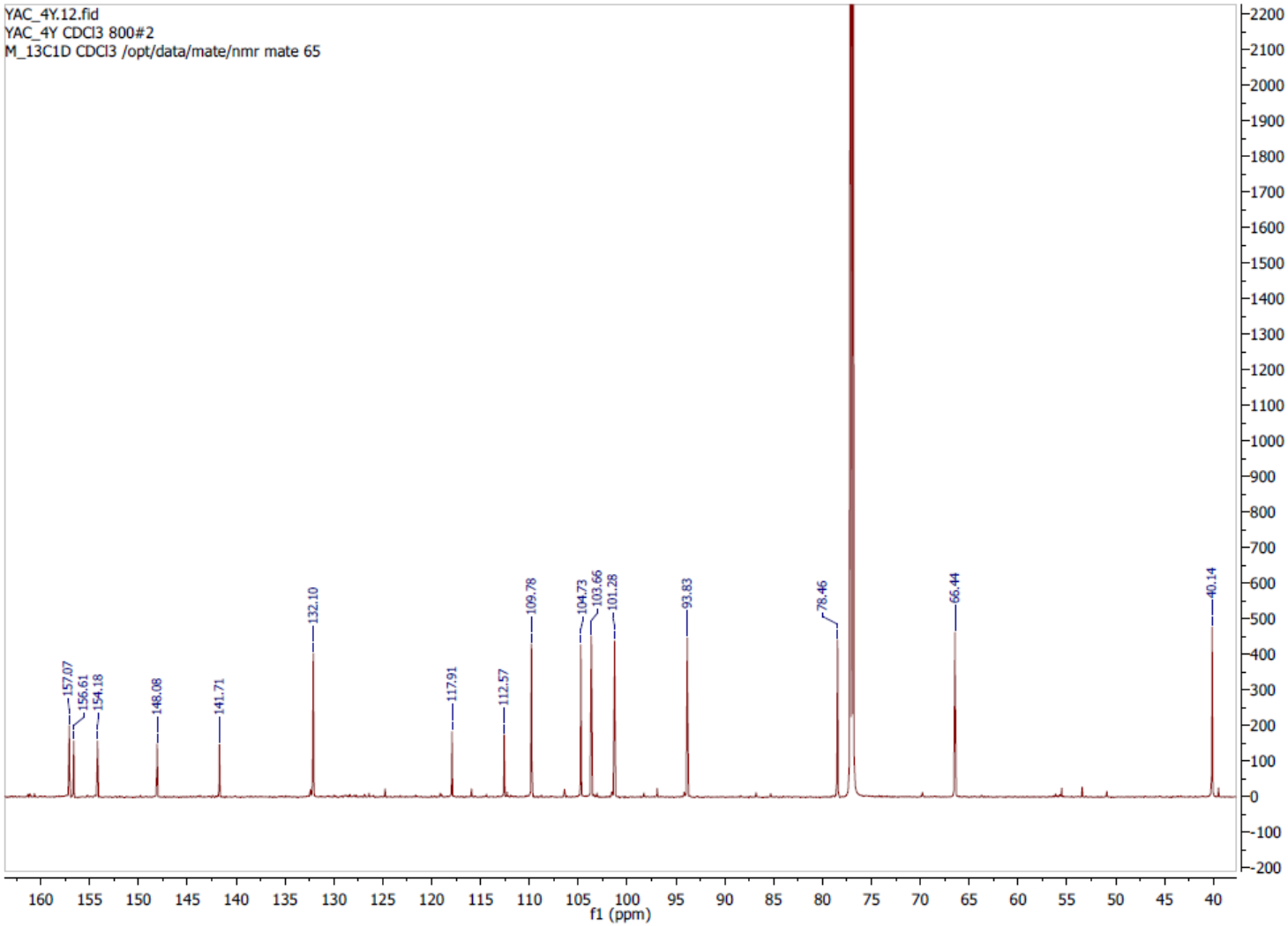
APPENDIX 21B: ^{13}C NMR Spectrum of Tephrolecarpin A(175) (200 MHz; $CDCl_3$)



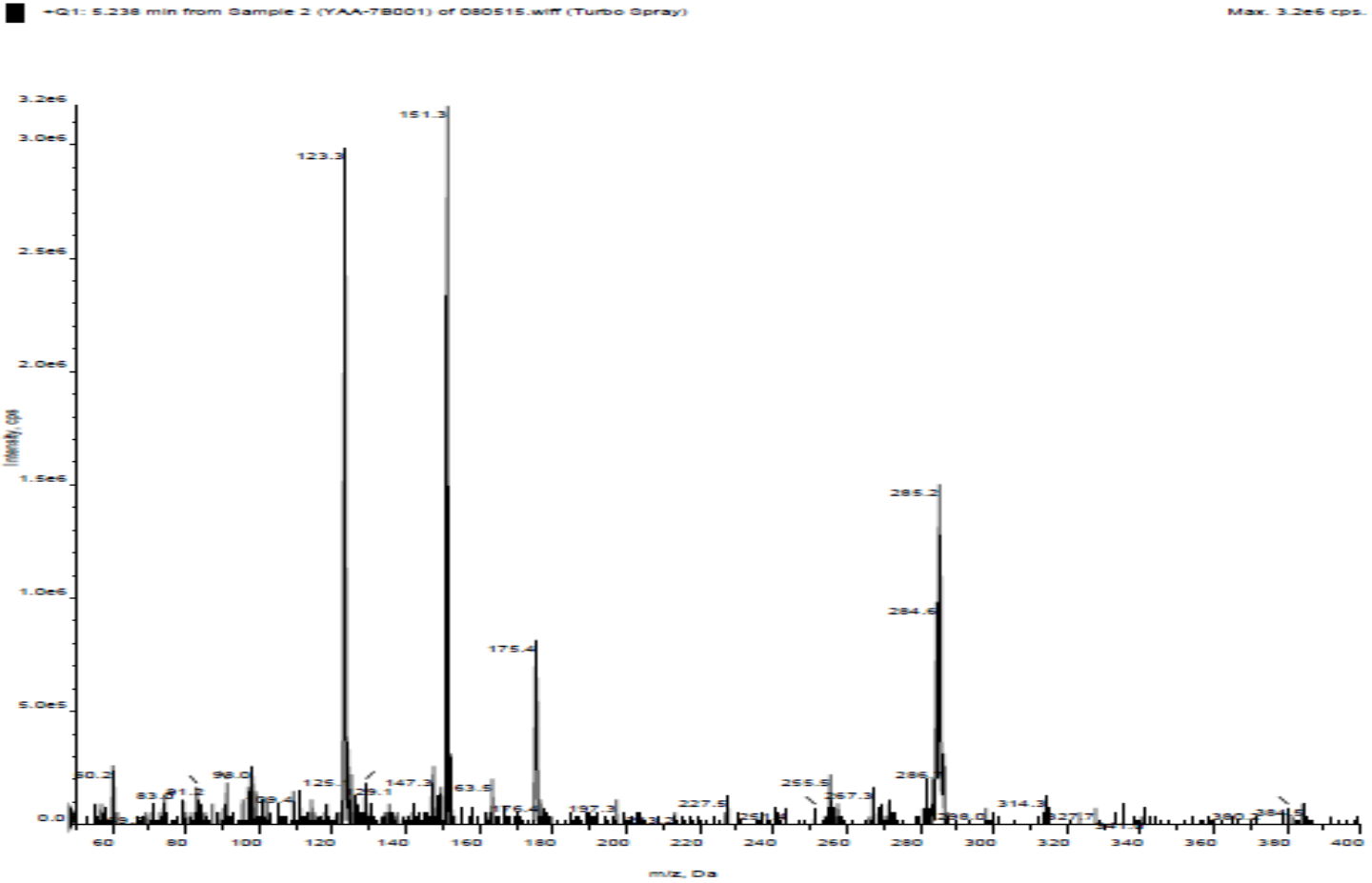
APPENDIX 22A: ^{1}H NMR Spectrum of Maackiain (176) (800 MHz; $CDCl_3$)



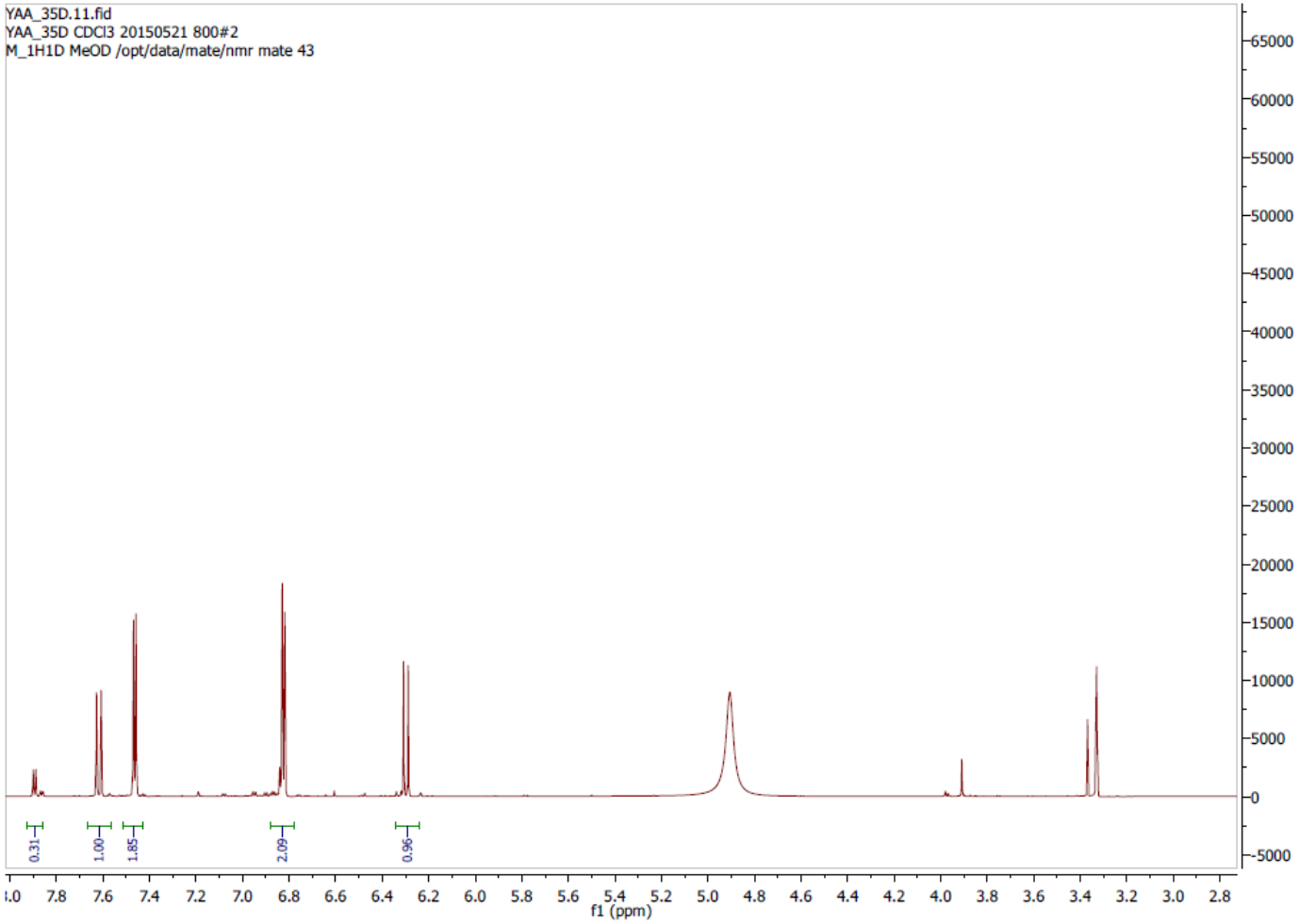
APPENDIX 22B: ^{13}C NMR Spectrum of Maackiain (176) (200 MHz; $CDCl_3$)



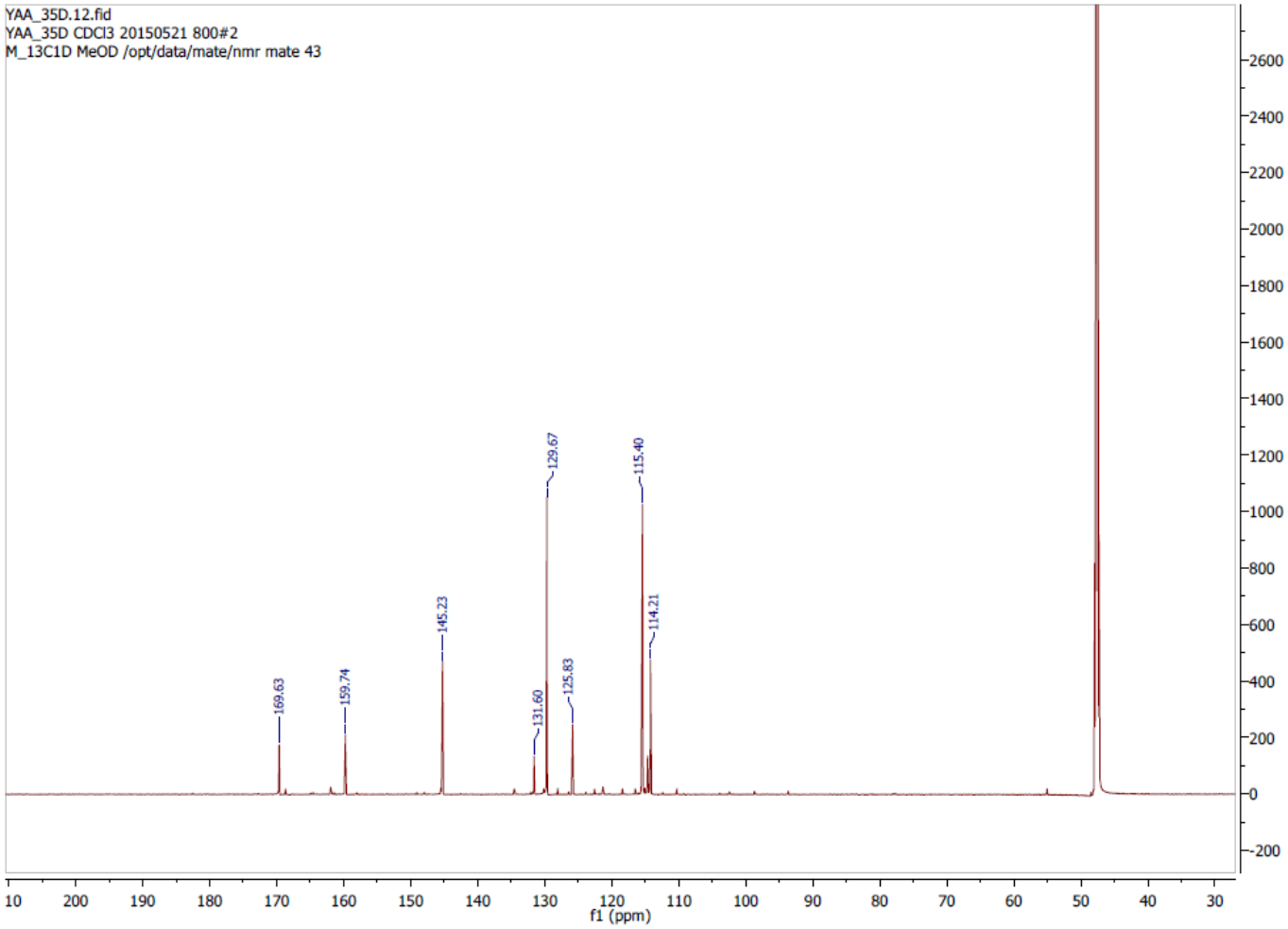
APPENDIX 22C: LCMS Spectrum of Maackiain (176)



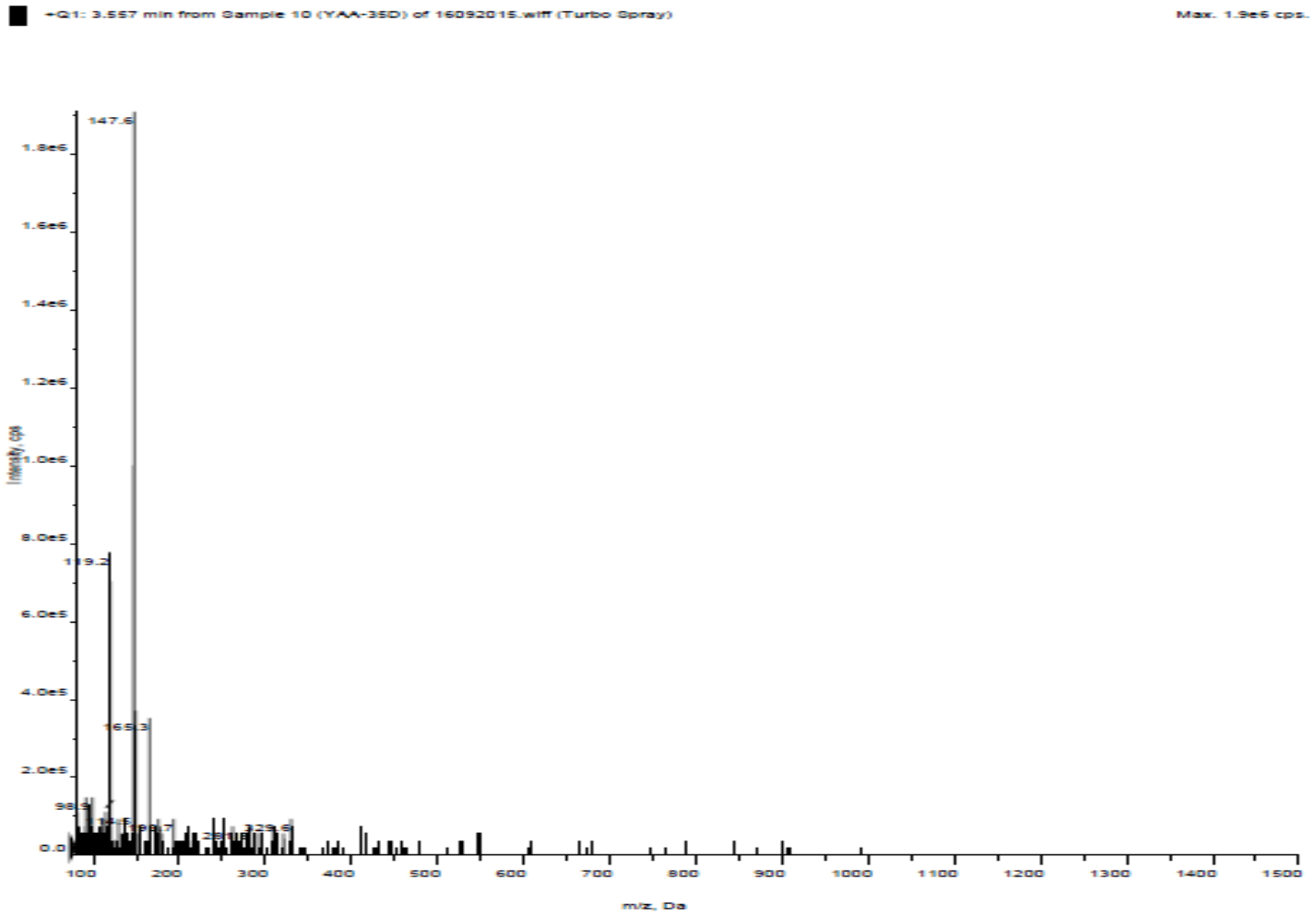
APPENDIX 23A: ^{1}H NMR Spectrum of Coumaric acid (177) (800 MHz; $CDCl_3$)



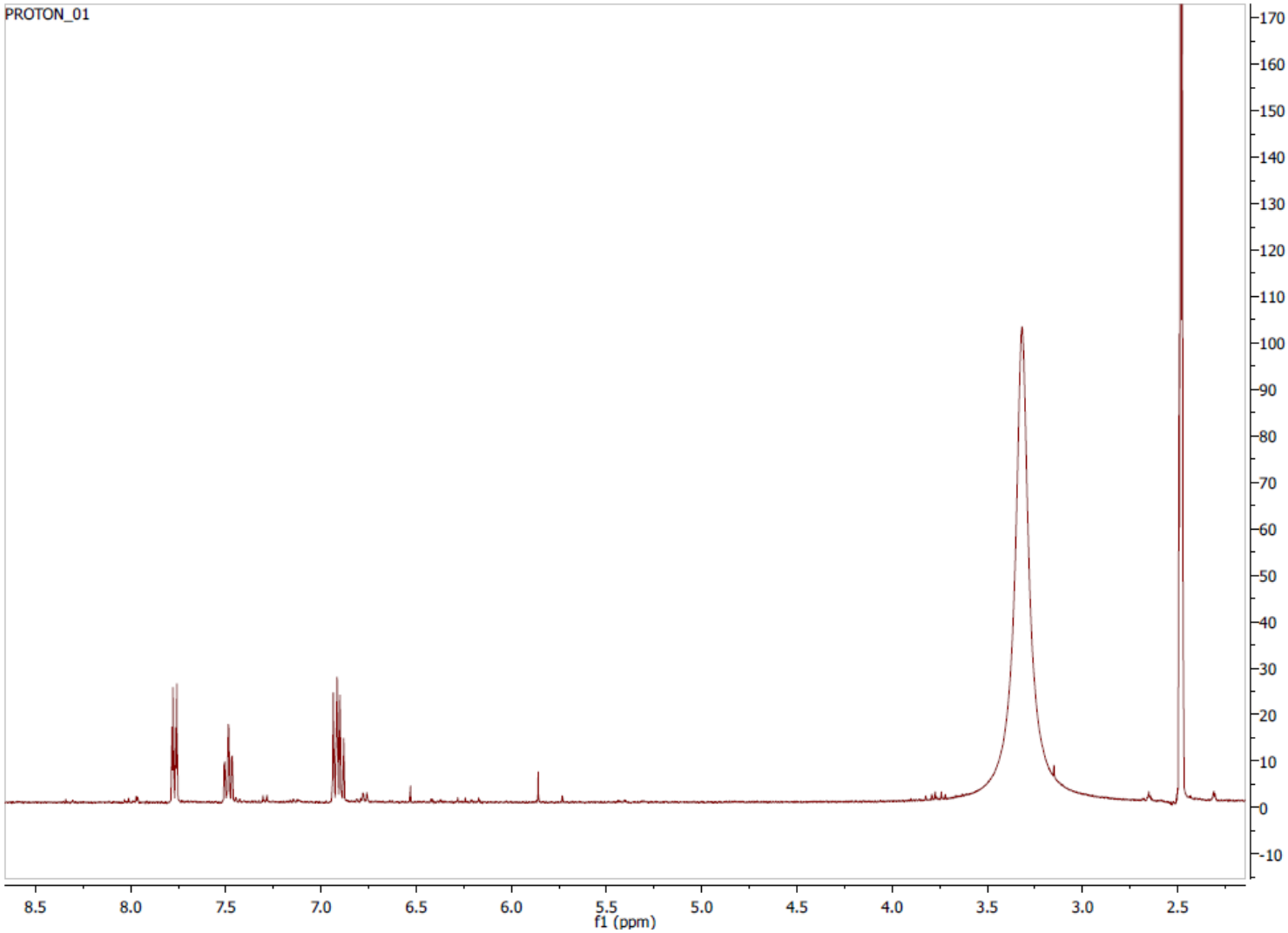
APPENDIX 23B: ^{13}C NMR Spectrum of Coumaric acid (177) (200 MHz; $CDCl_3$)



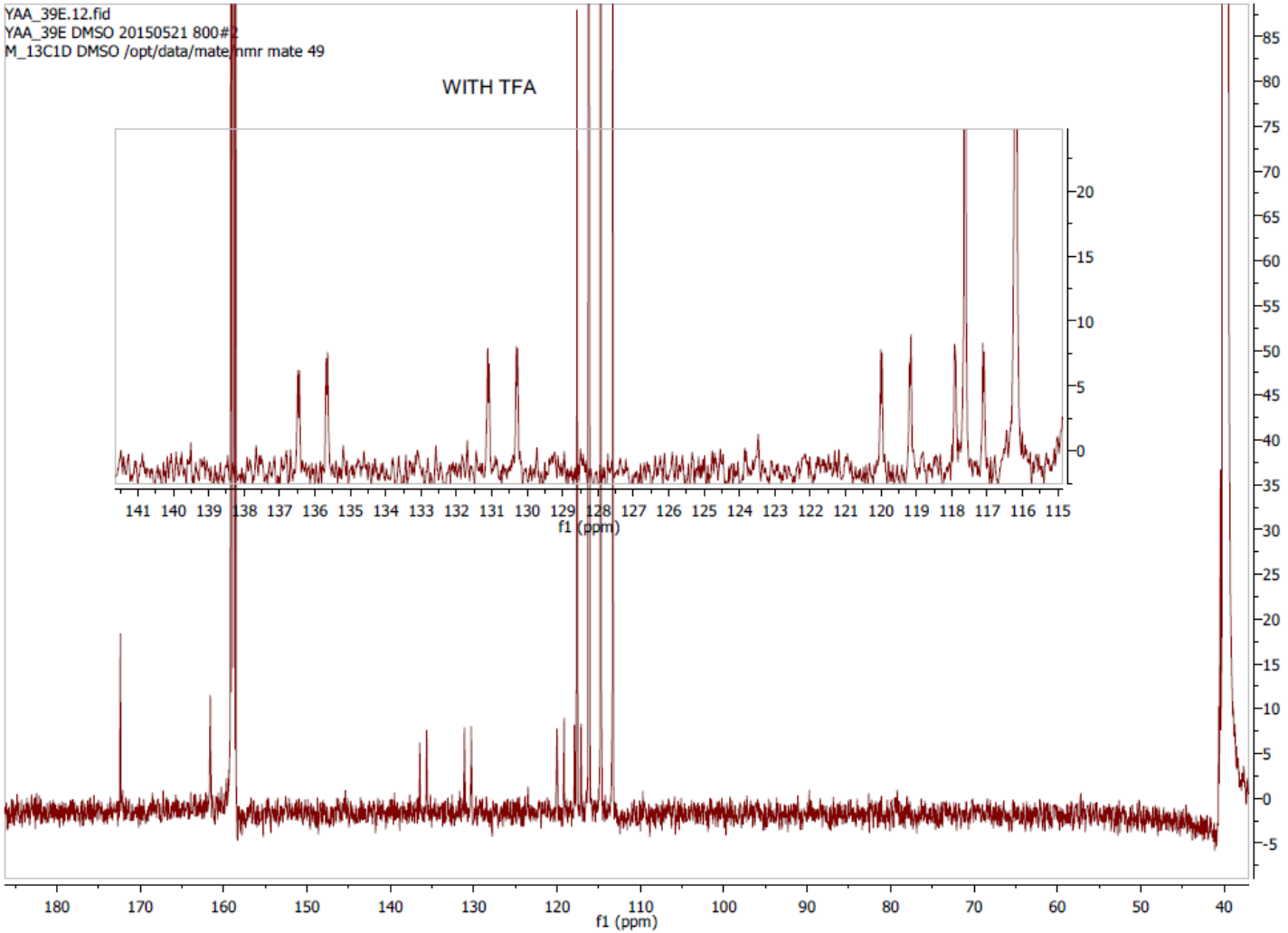
APPENDIX 23F: LCMS Spectrum of Coumaric acid (177) ($CDCl_3$)



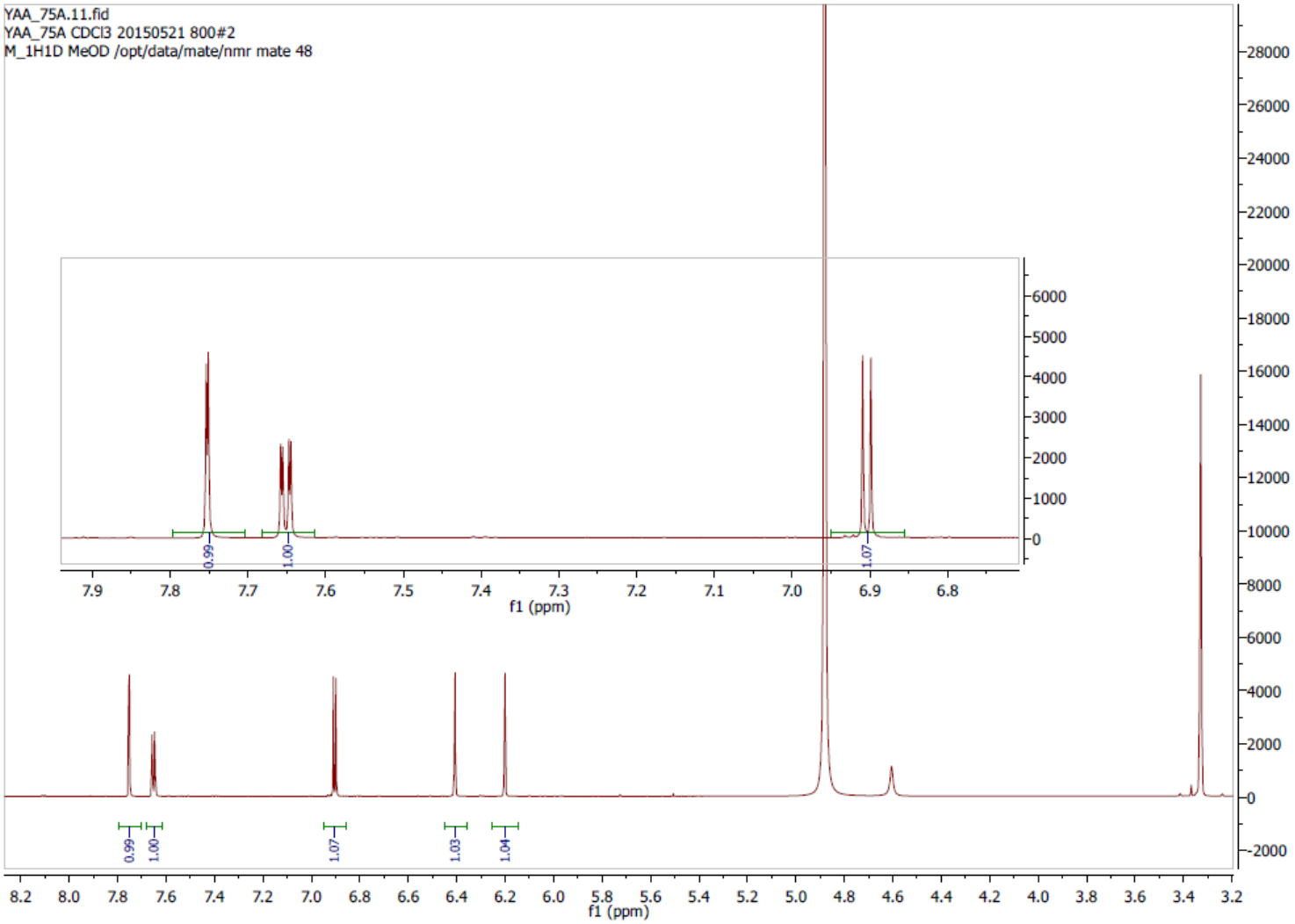
APPENDIX 24A: ^{1}H NMR Spectrum of Salicylic acid (178) (800 MHz; DMSO-d_6)



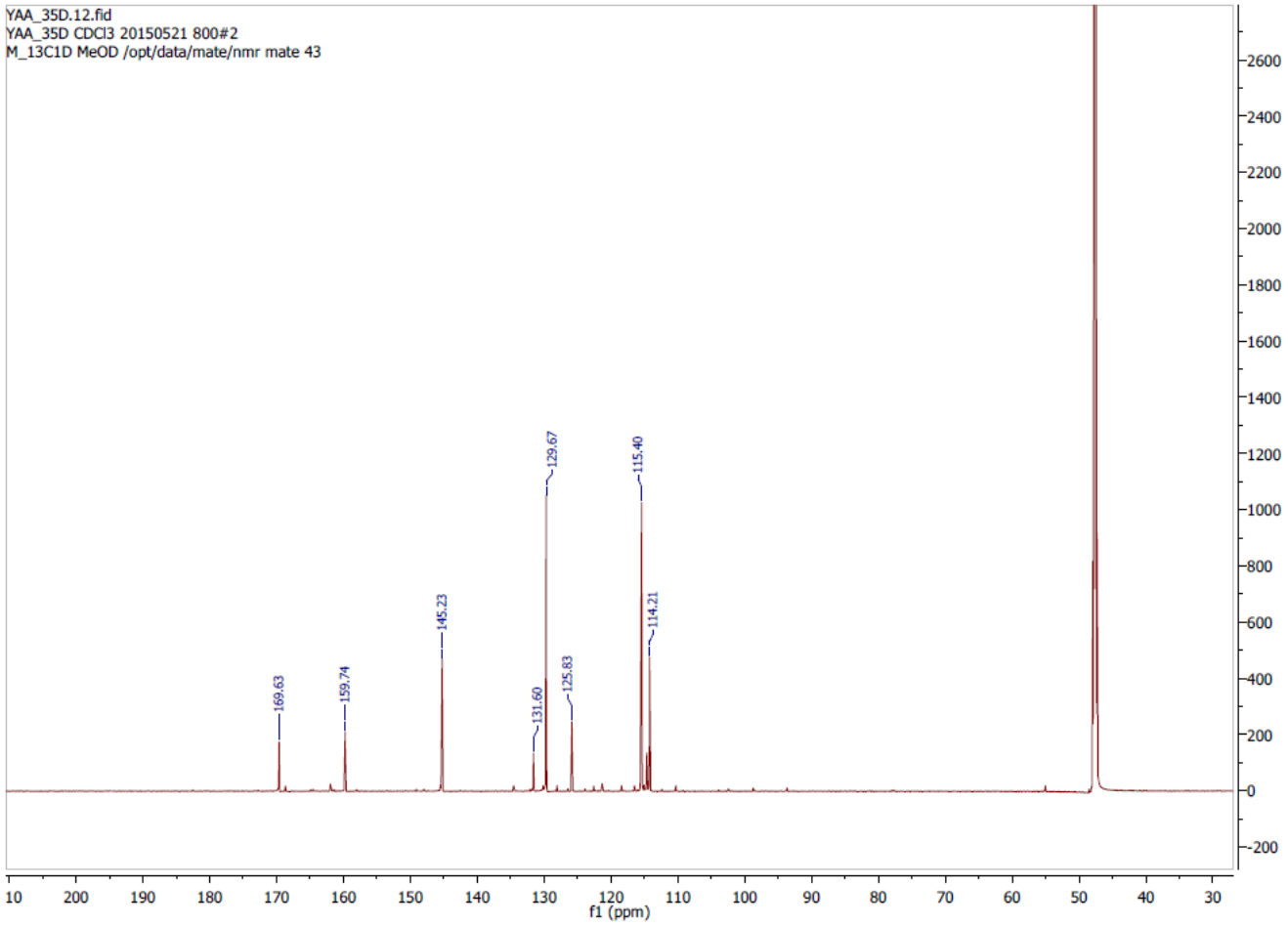
APPENDIX 24B: ^{13}C NMR Spectrum of Salicylic acid (178) (200 MHz; DMSO-d_6)



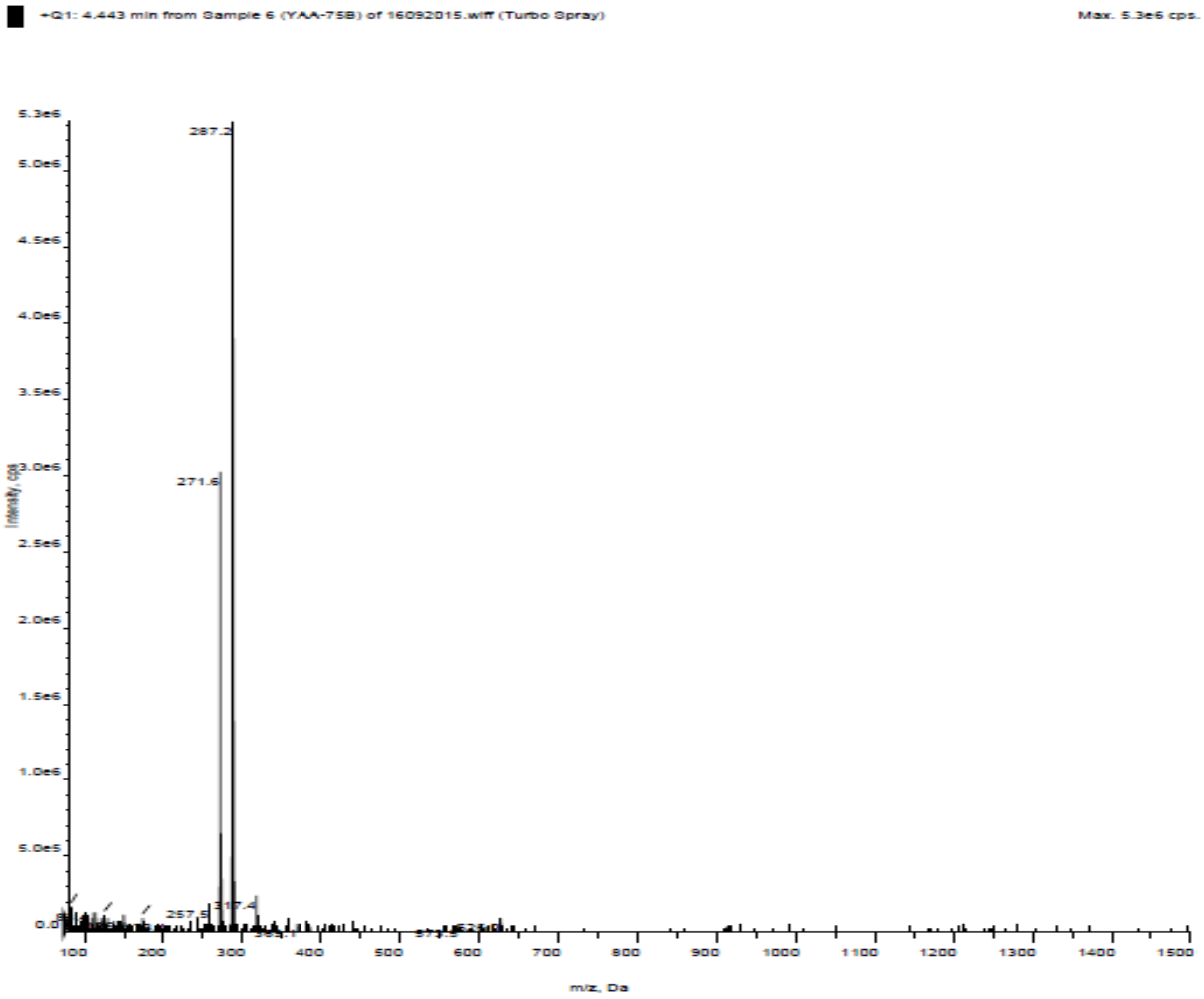
APPENDIX 25A: ^{1}H NMR Spectrum of Kaempferol (179) (800 MHz; MeOD)



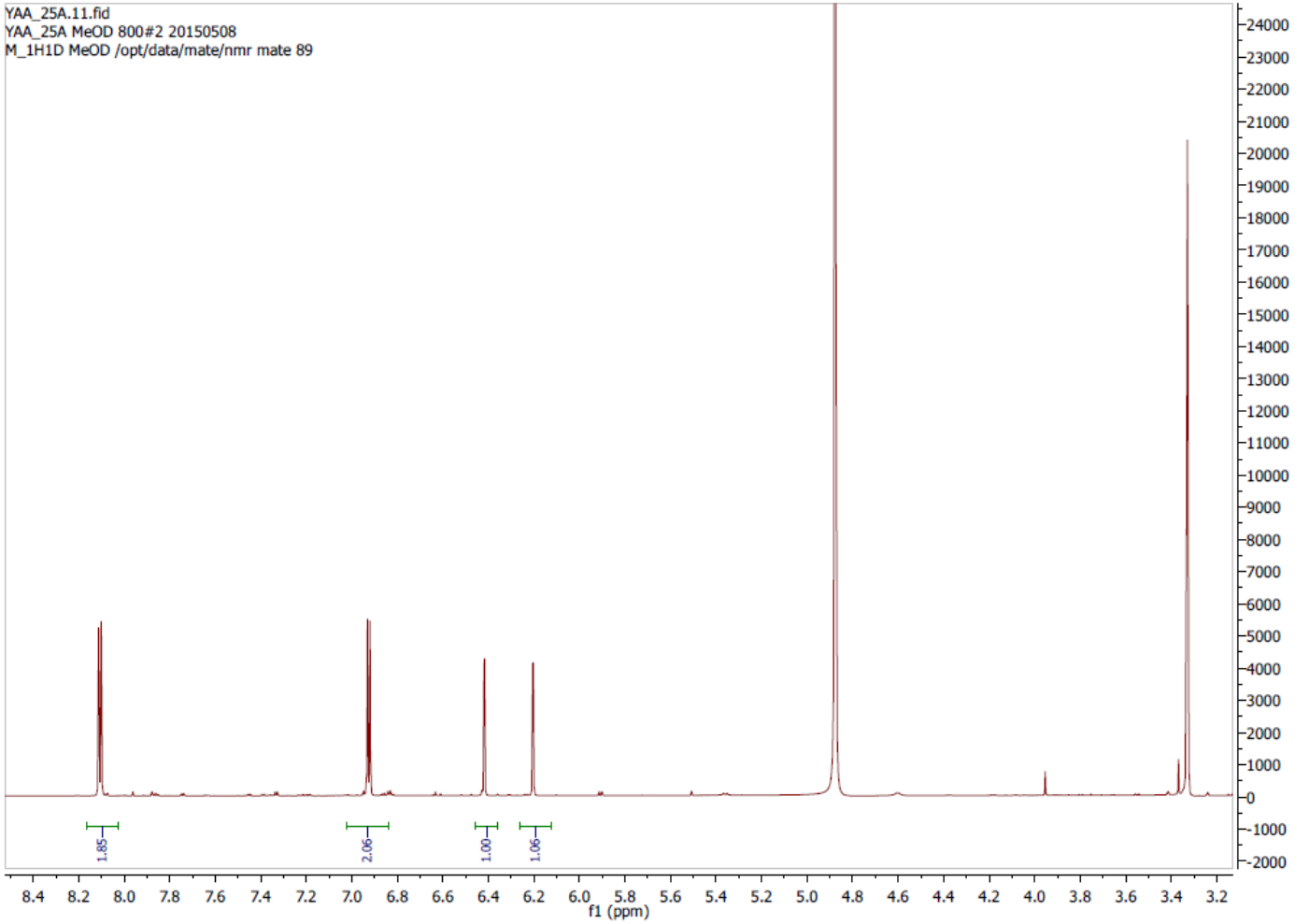
APPENDIX 25B: ^{13}C NMR Spectrum of Kaempferol (179) (200 MHz; MeOD)



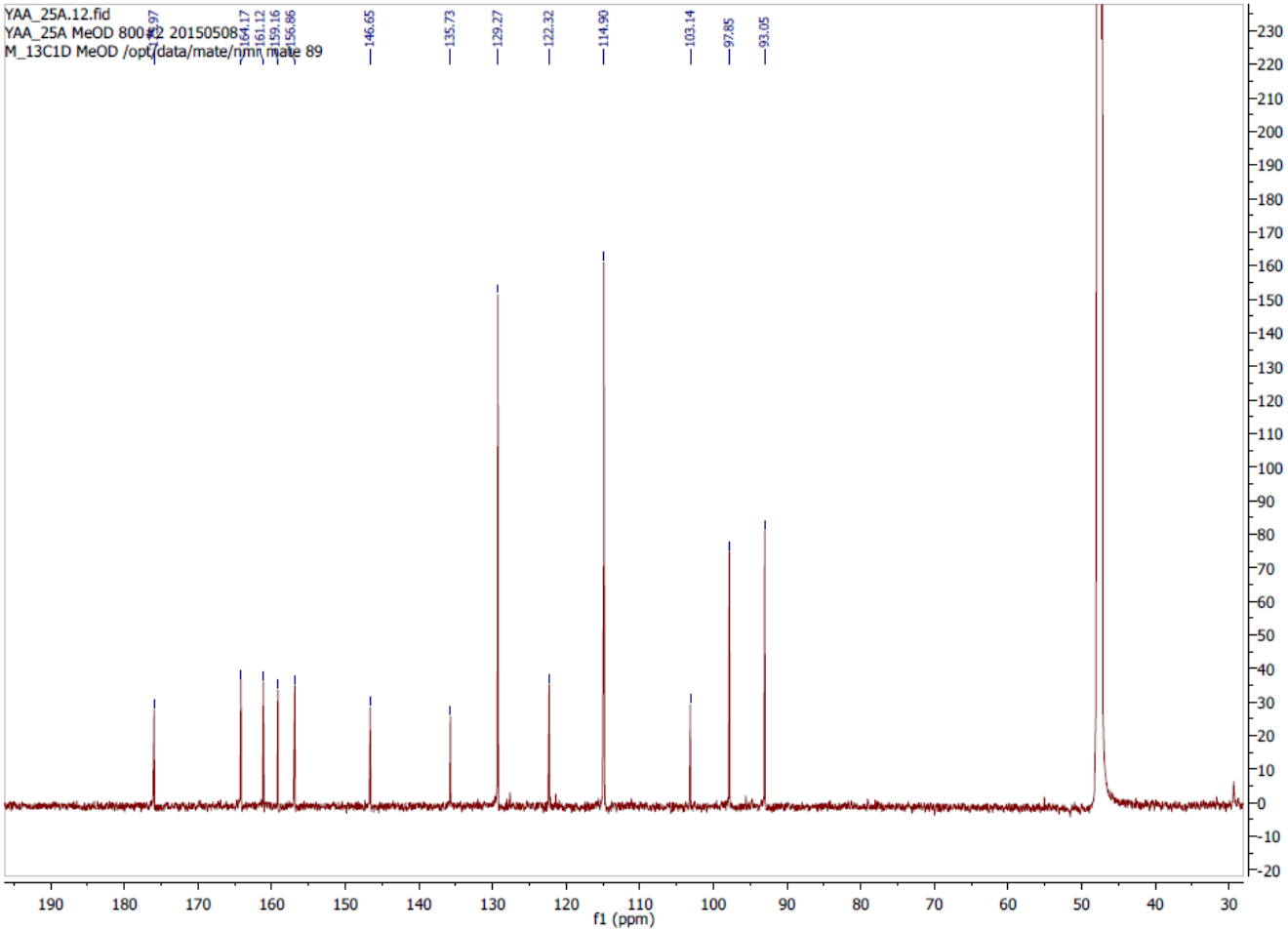
APPENDIX 25F: LCMS Spectrum of Kaempferol (179) (MeOD)



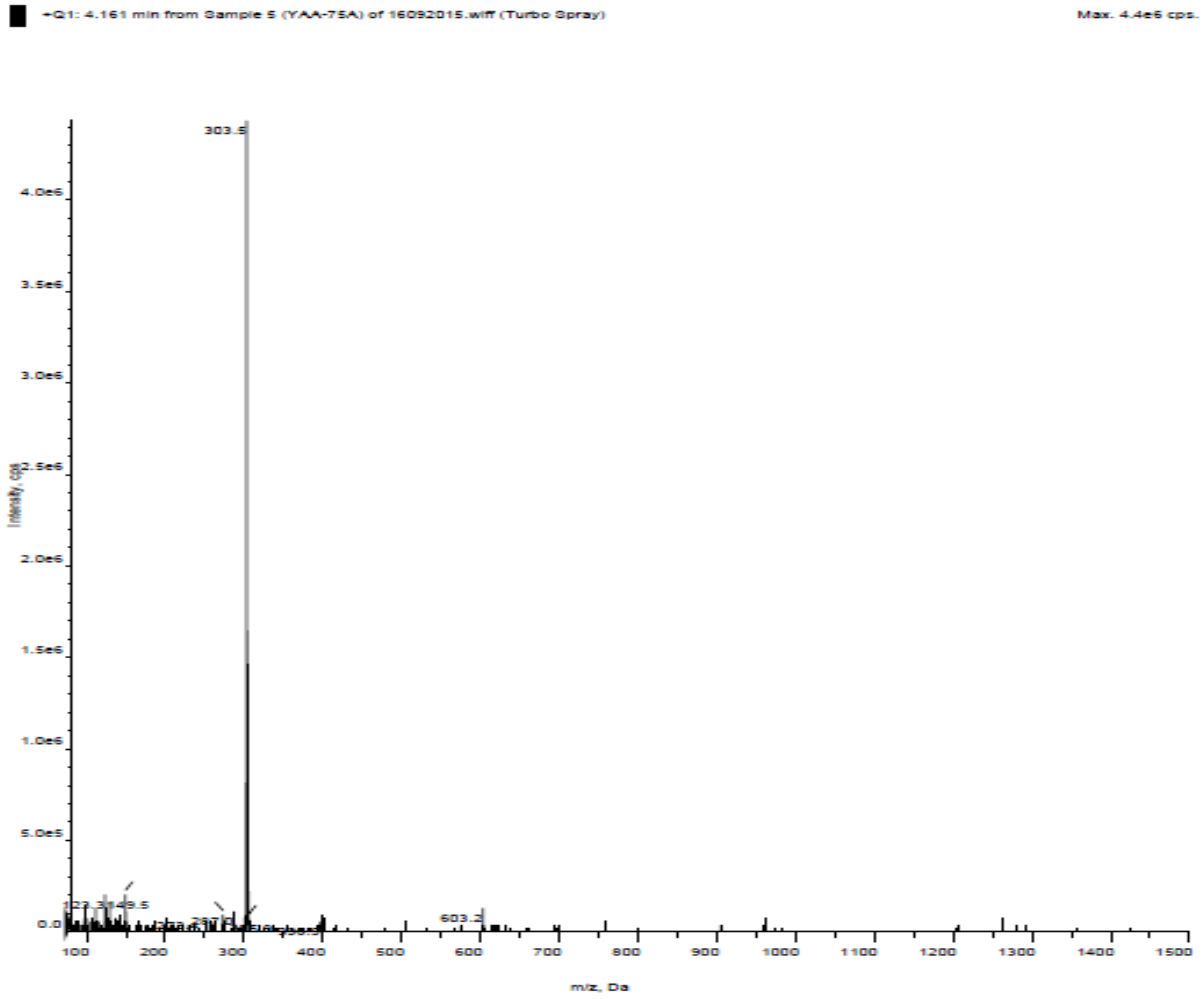
APPENDIX 26A: ^{1}H NMR Spectrum of Quercitin (180) (800 MHz; MeOD)



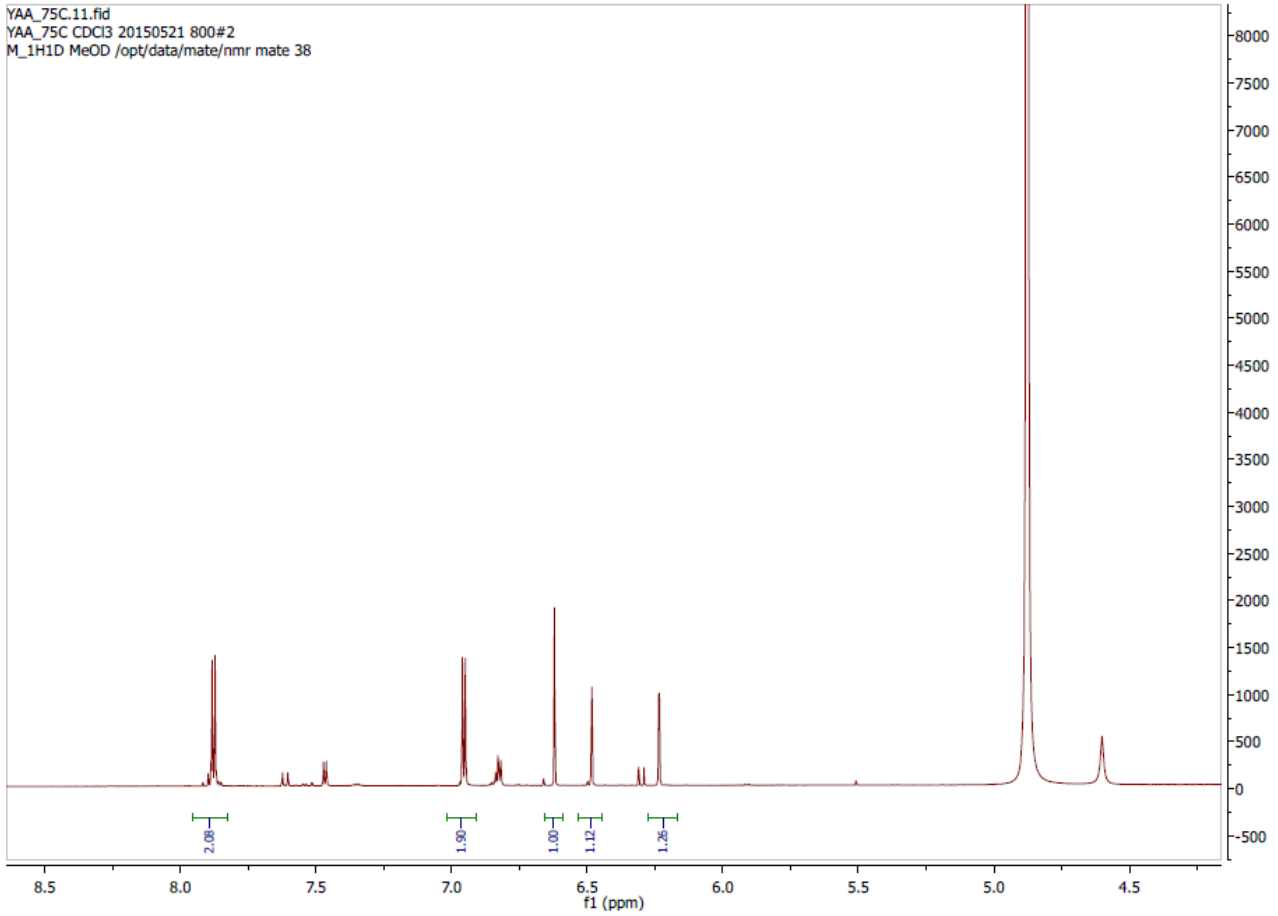
APPENDIX 26B: ^{13}C NMR Spectrum of Quercitin (180) (200 MHz; MeOD)



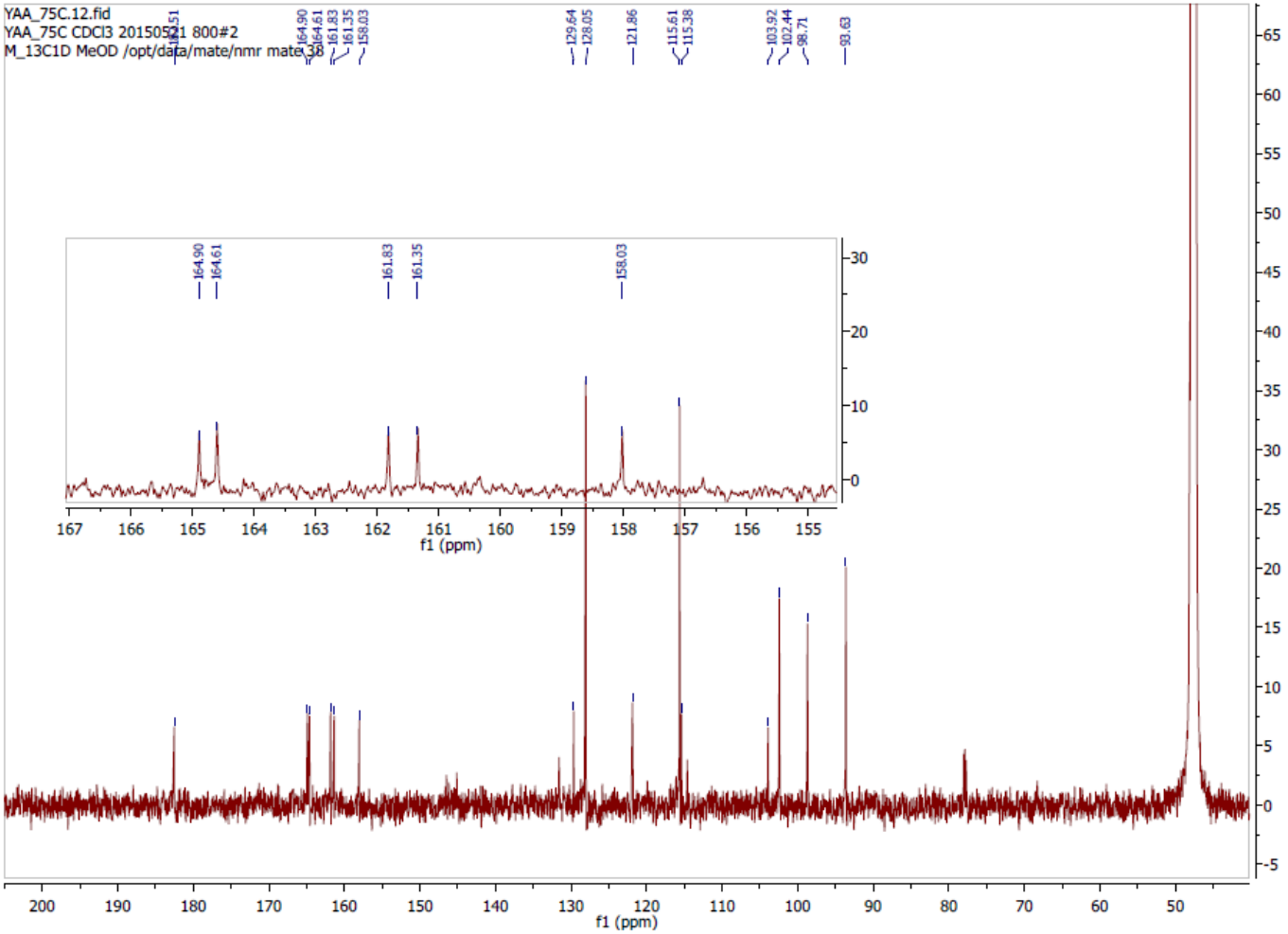
APPENDIX 26C: LCMS Spectrum of Quercitin (180) (MeOD)



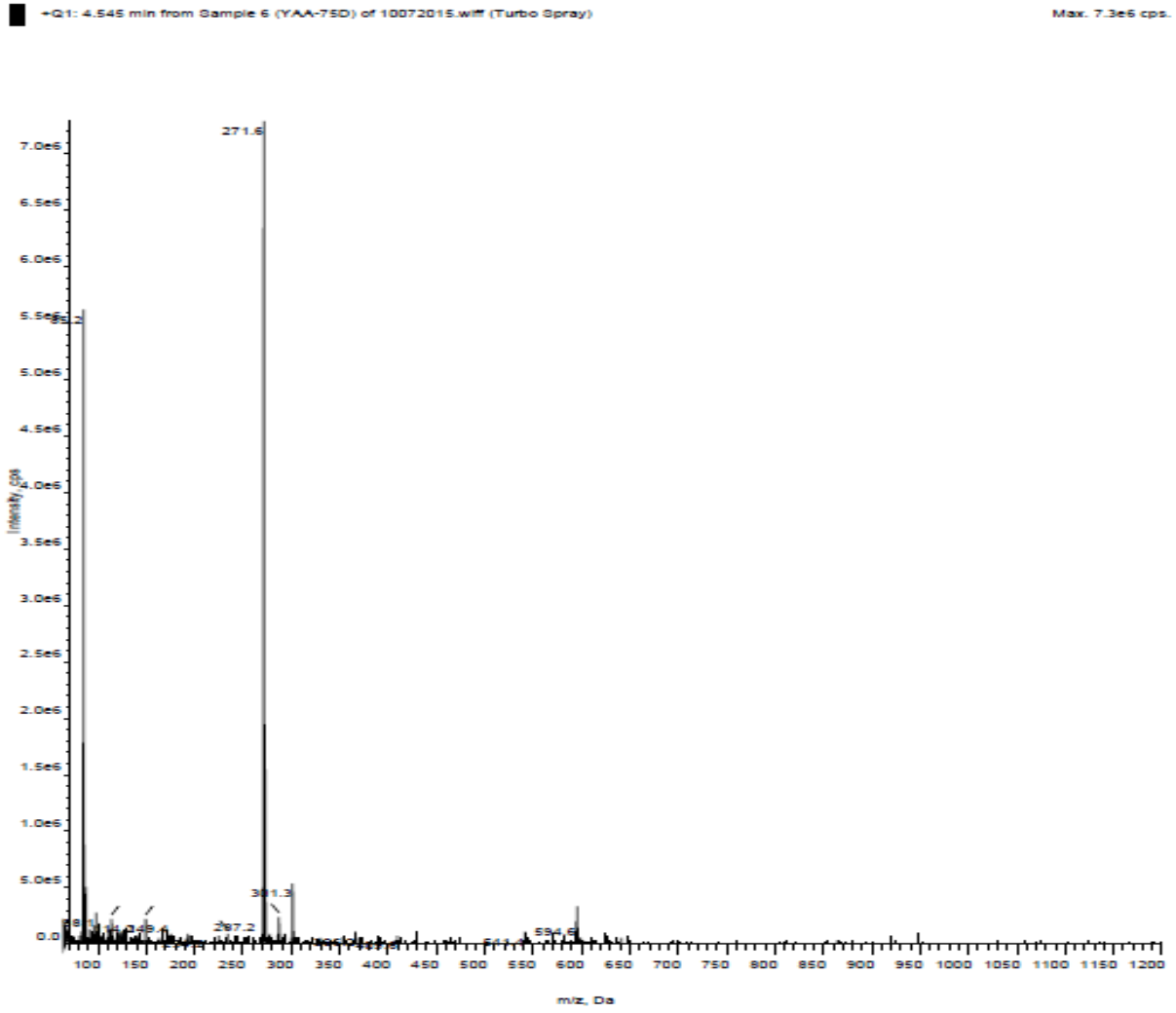
APPENDIX 27A: ^{1}H NMR Spectrum of Apigenin (181) (800 MHz; MeOD)



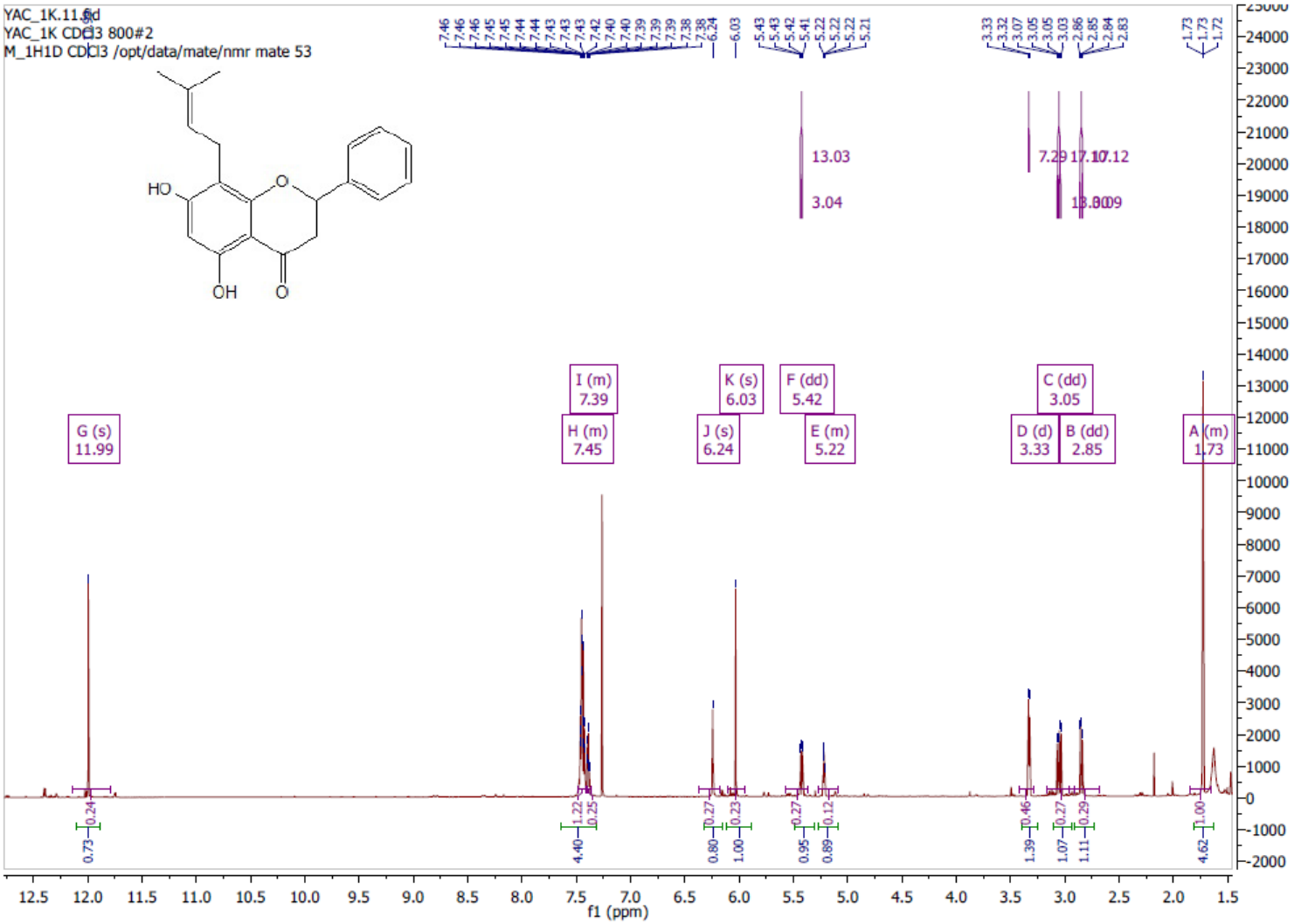
APPENDIX 27B: ^{13}C NMR Spectrum of Apigenin (181) (200 MHz; MeOD)



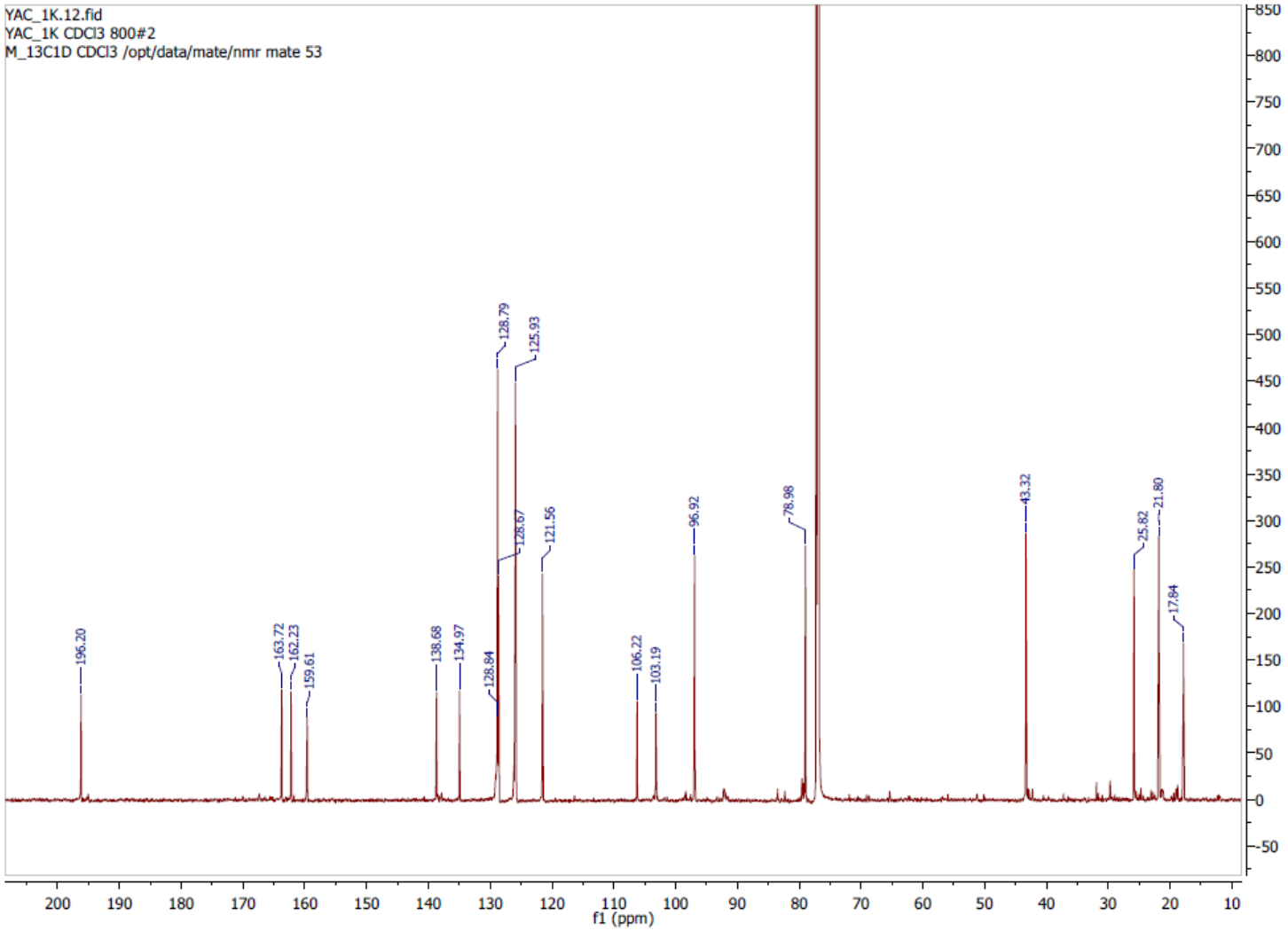
APPENDIX 27C: LCMS Spectrum of Apigenin (181)



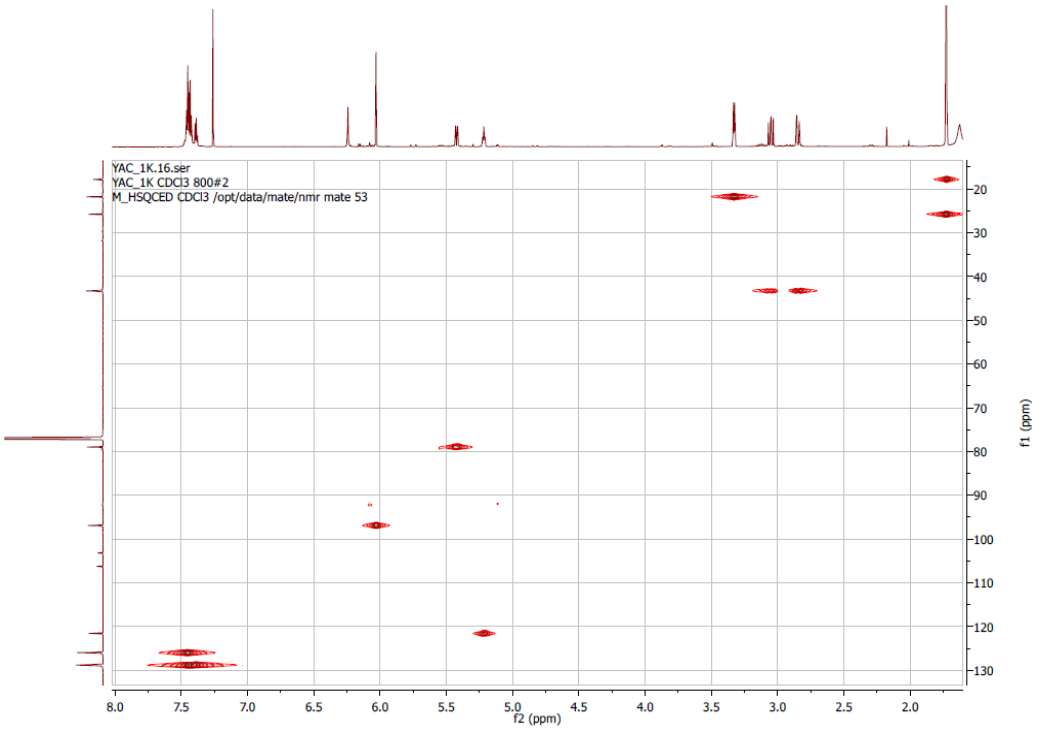
APPENDIX 28A: ^{1}H NMR Spectrum of Glabranin (182) (800 MHz; $CDCl_3$)



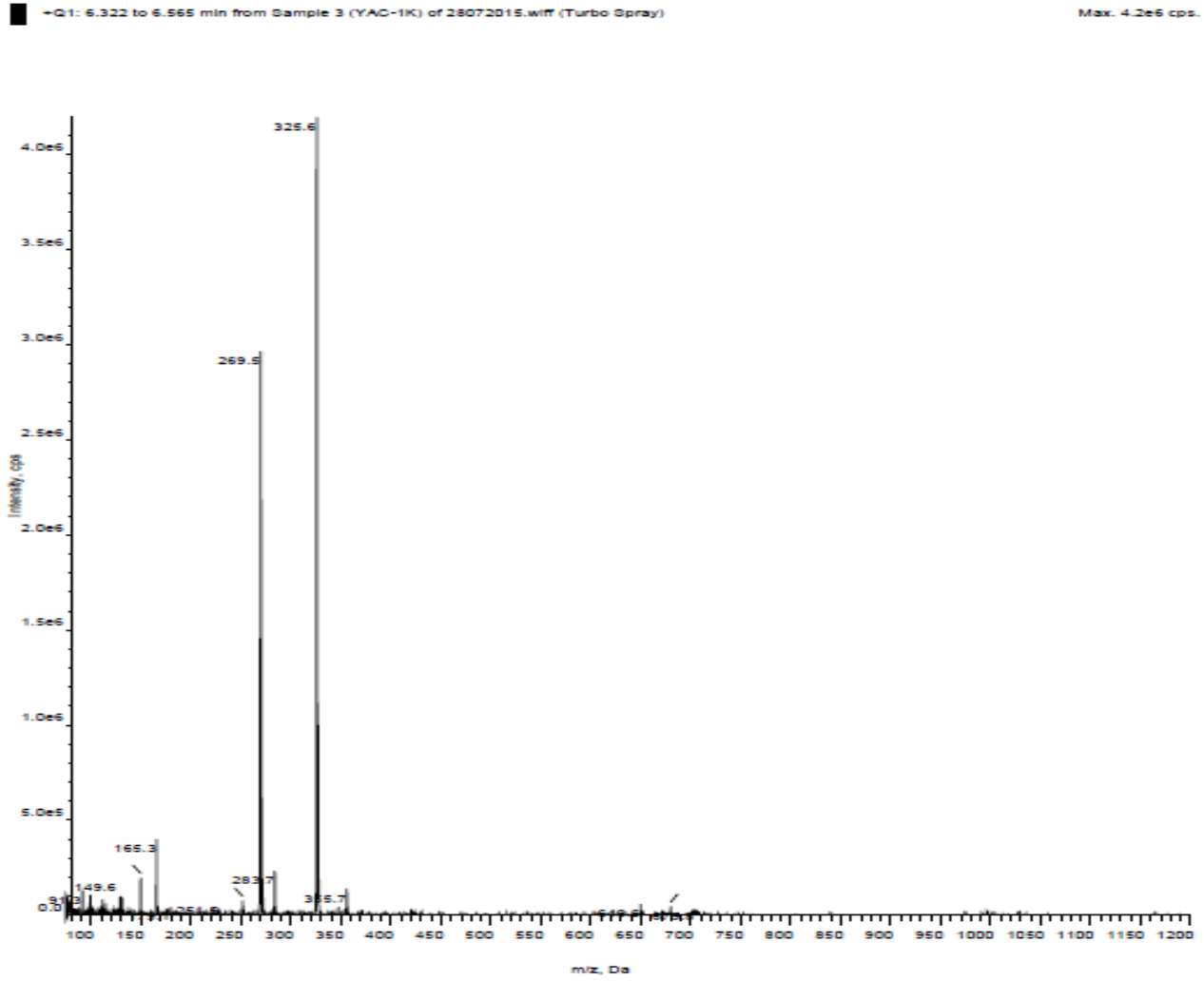
APPENDIX 28B: ^{13}C NMR Spectrum of Glabranin (182) (200 MHz; $CDCl_3$)



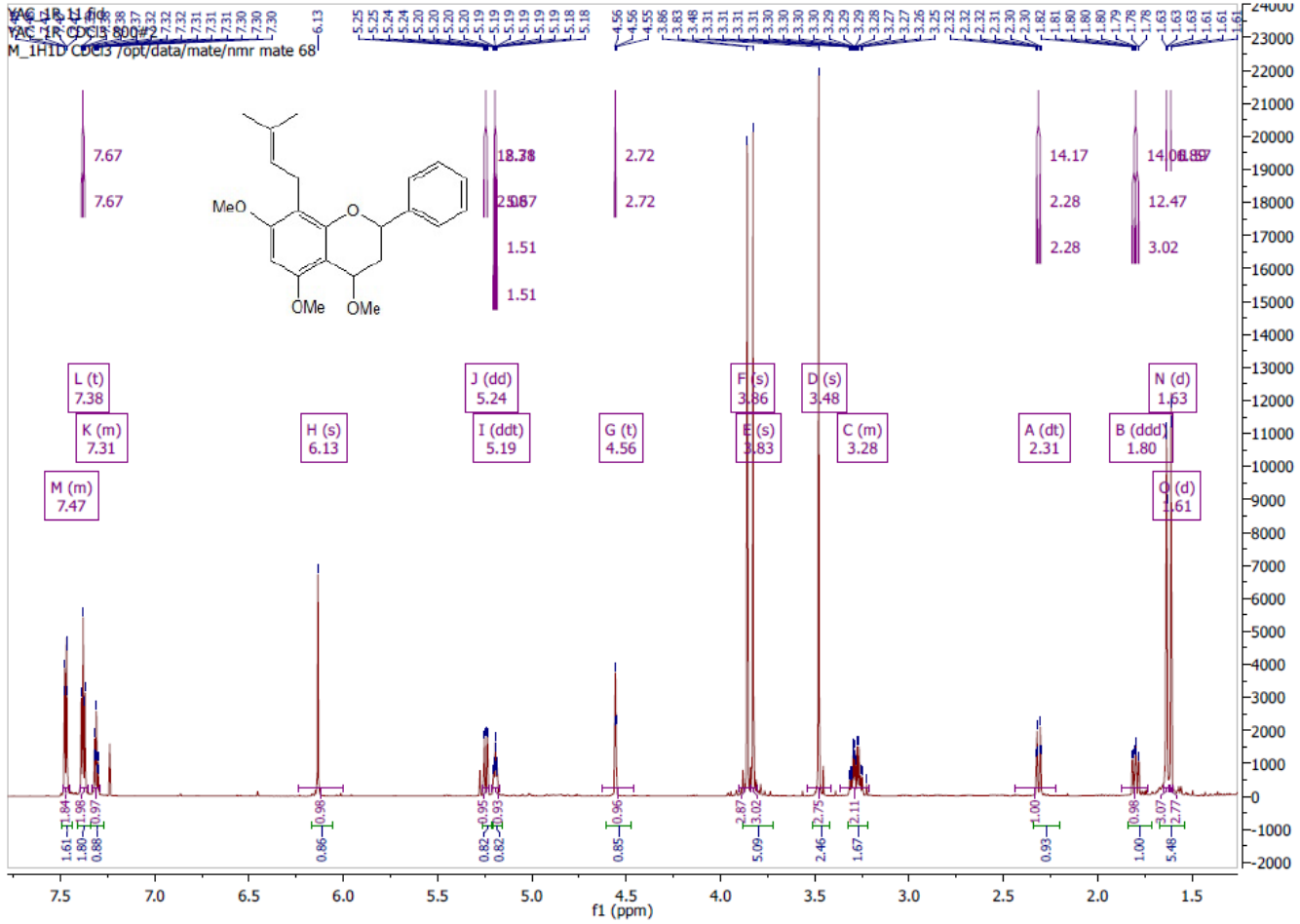
APPENDIX 28D: HSQC Spectrum of Glabranin (182) ($CDCl_3$)



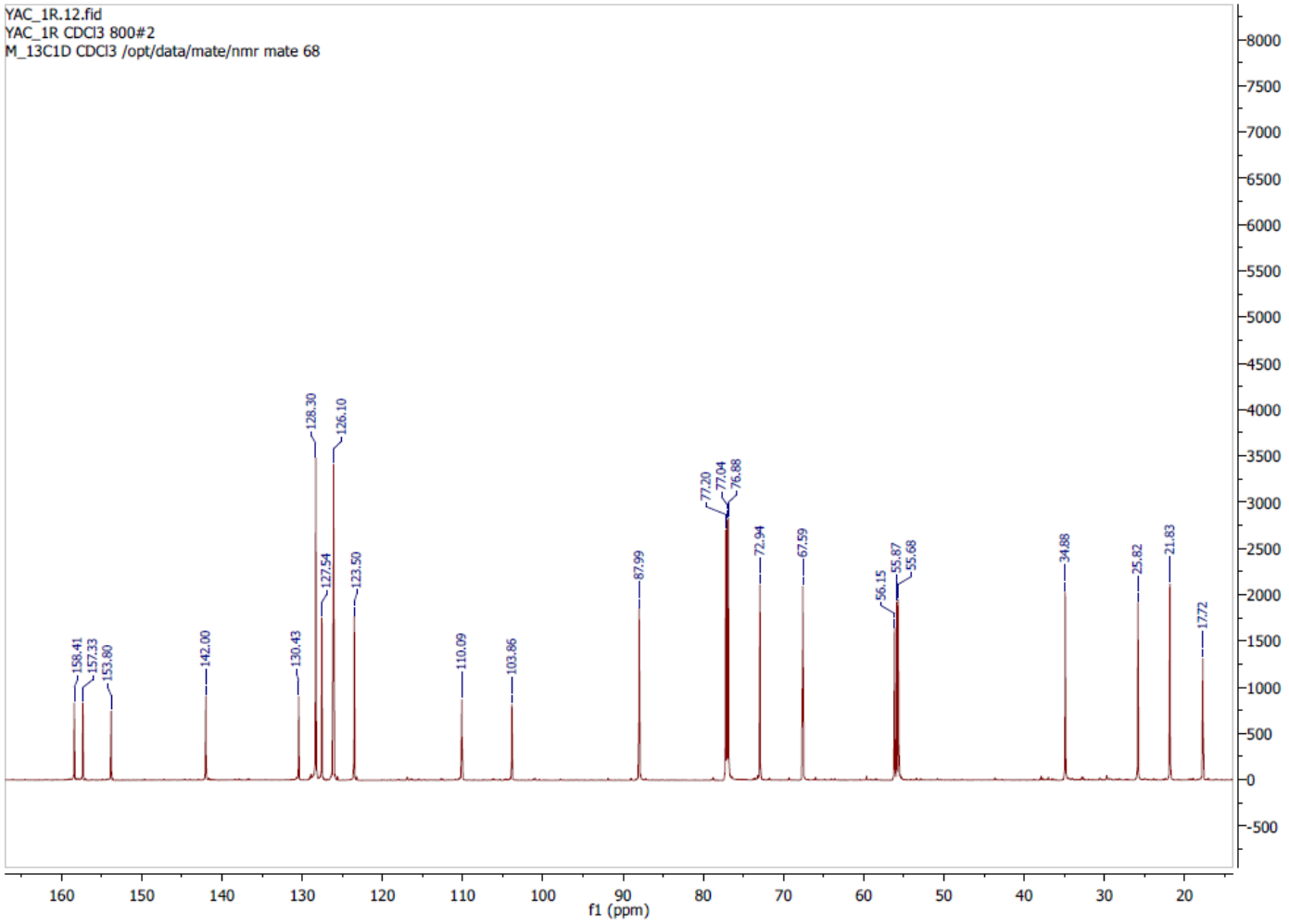
APPENDIX 28C: LCMS Spectrum of Glabranin (182)



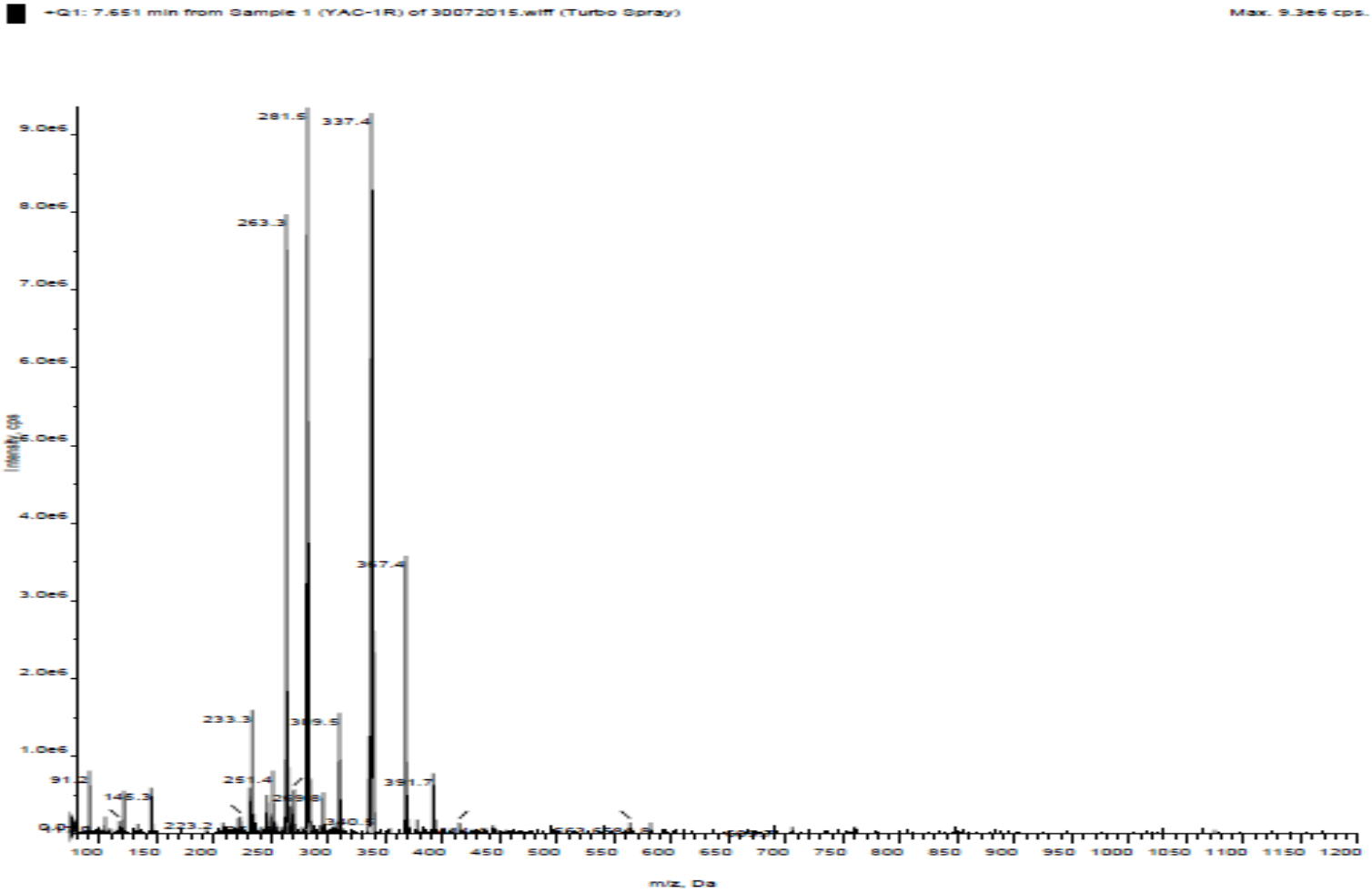
APPENDIX 29A: ^{1}H NMR Spectrum of Quercetol B (98) (800 MHz; $CDCl_3$)



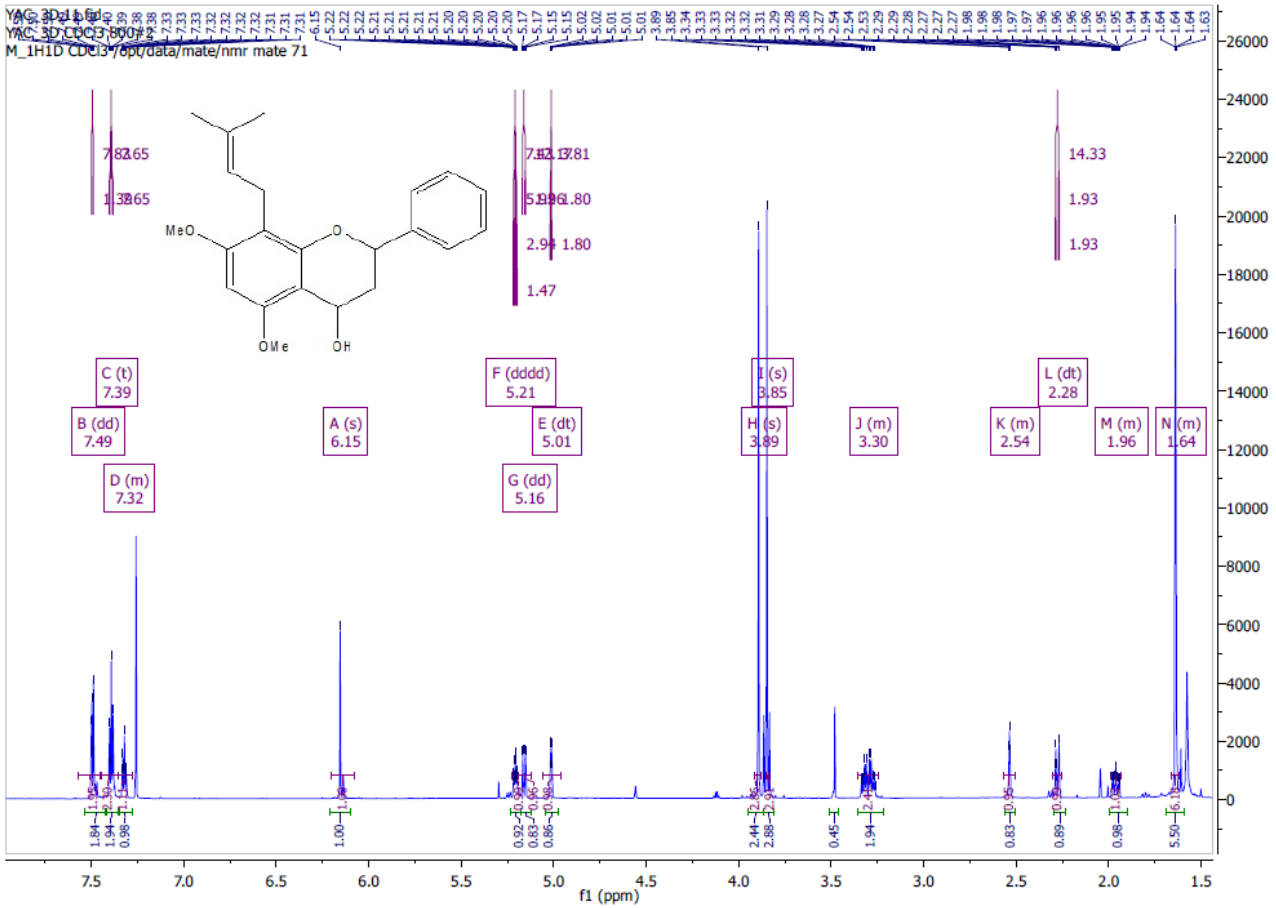
APPENDIX 29B: ^{13}C NMR Spectrum of Quercetol B (98) (200 MHz; $CDCl_3$)



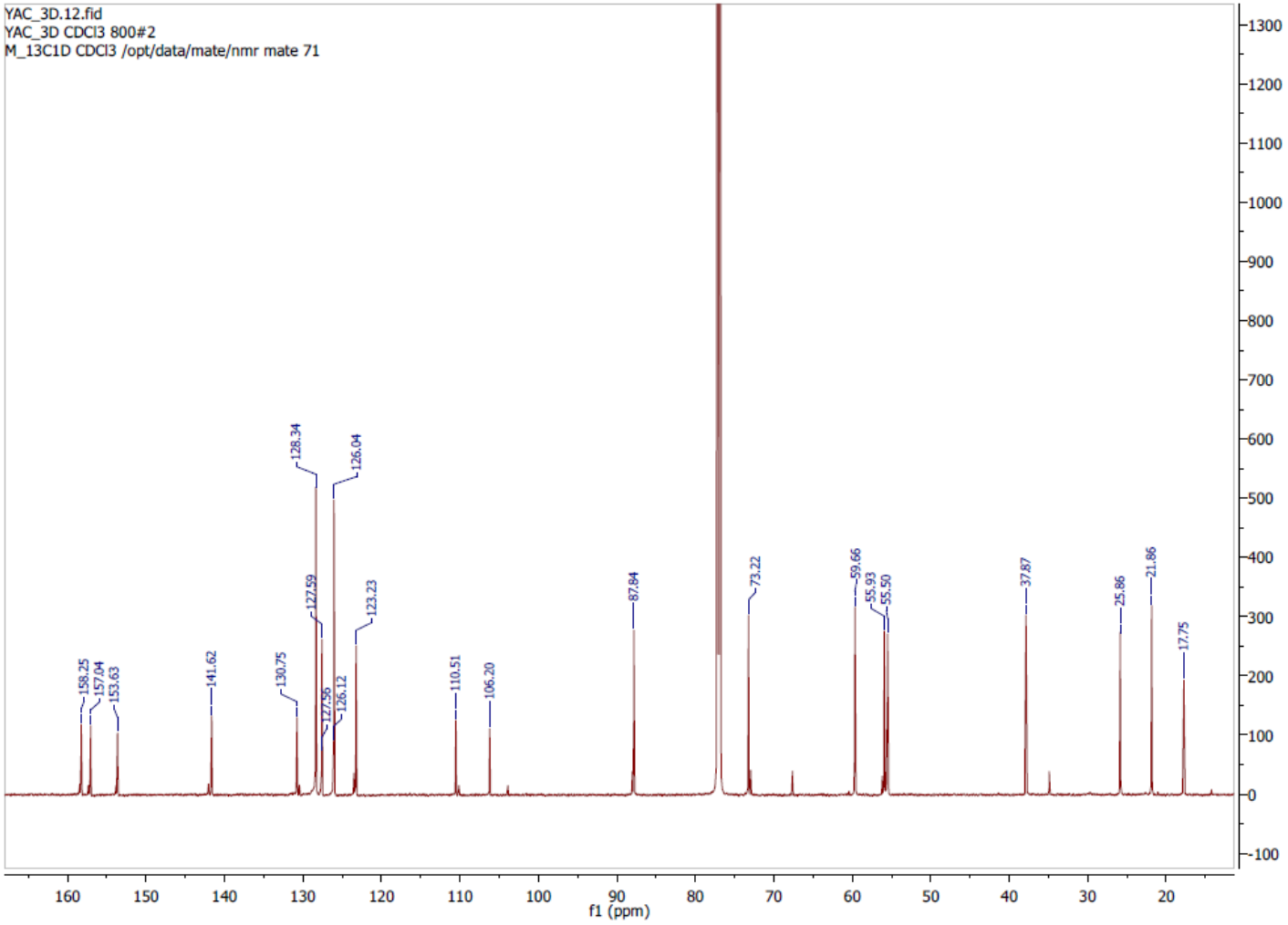
APPENDIX 29C: LCMS Spectrum of Quercetol B (98)



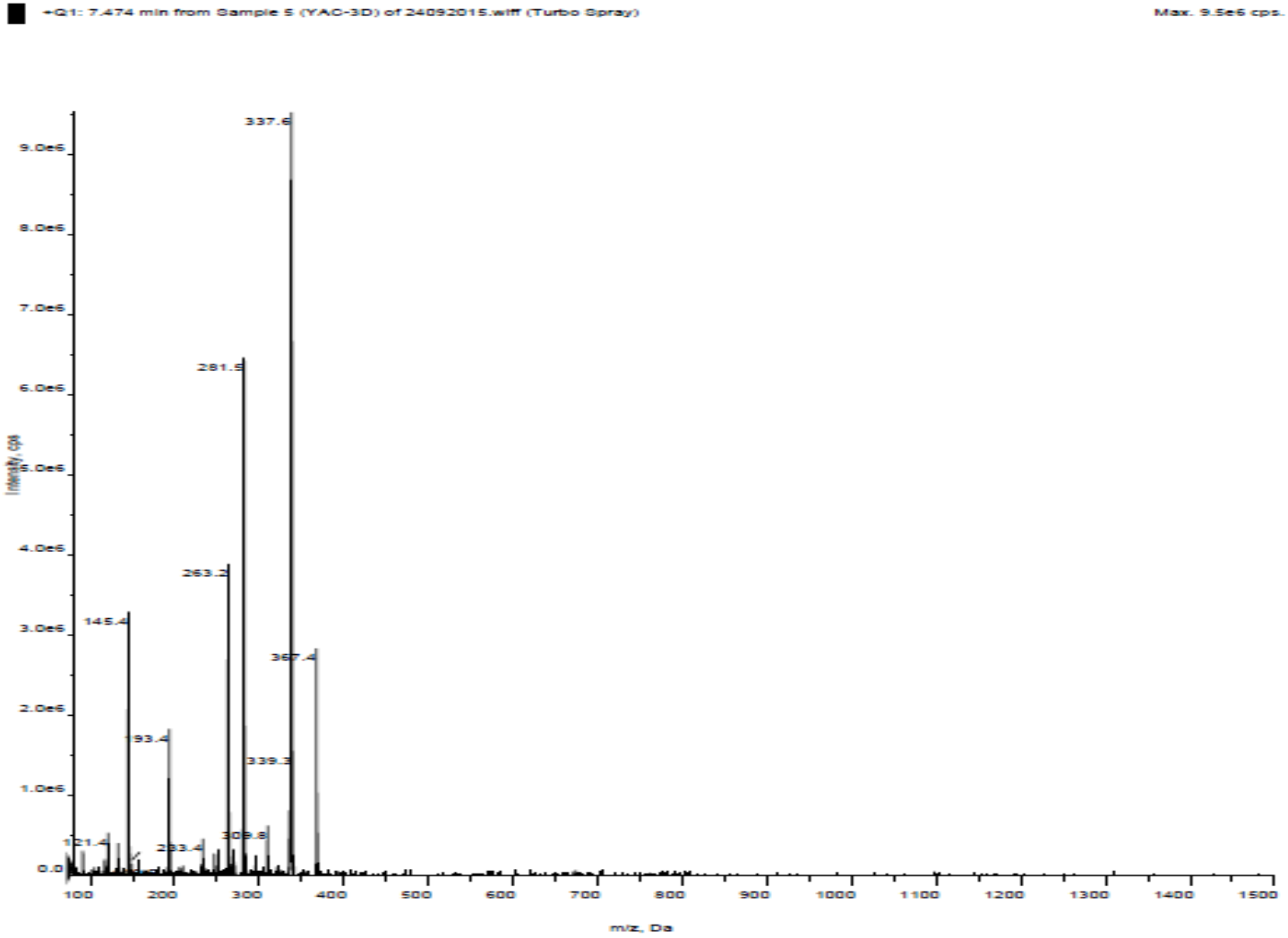
APPENDIX 30A: ^{1}H NMR Spectrum of *Tephrowatsin A* (79) (800 MHz; $CDCl_3$)



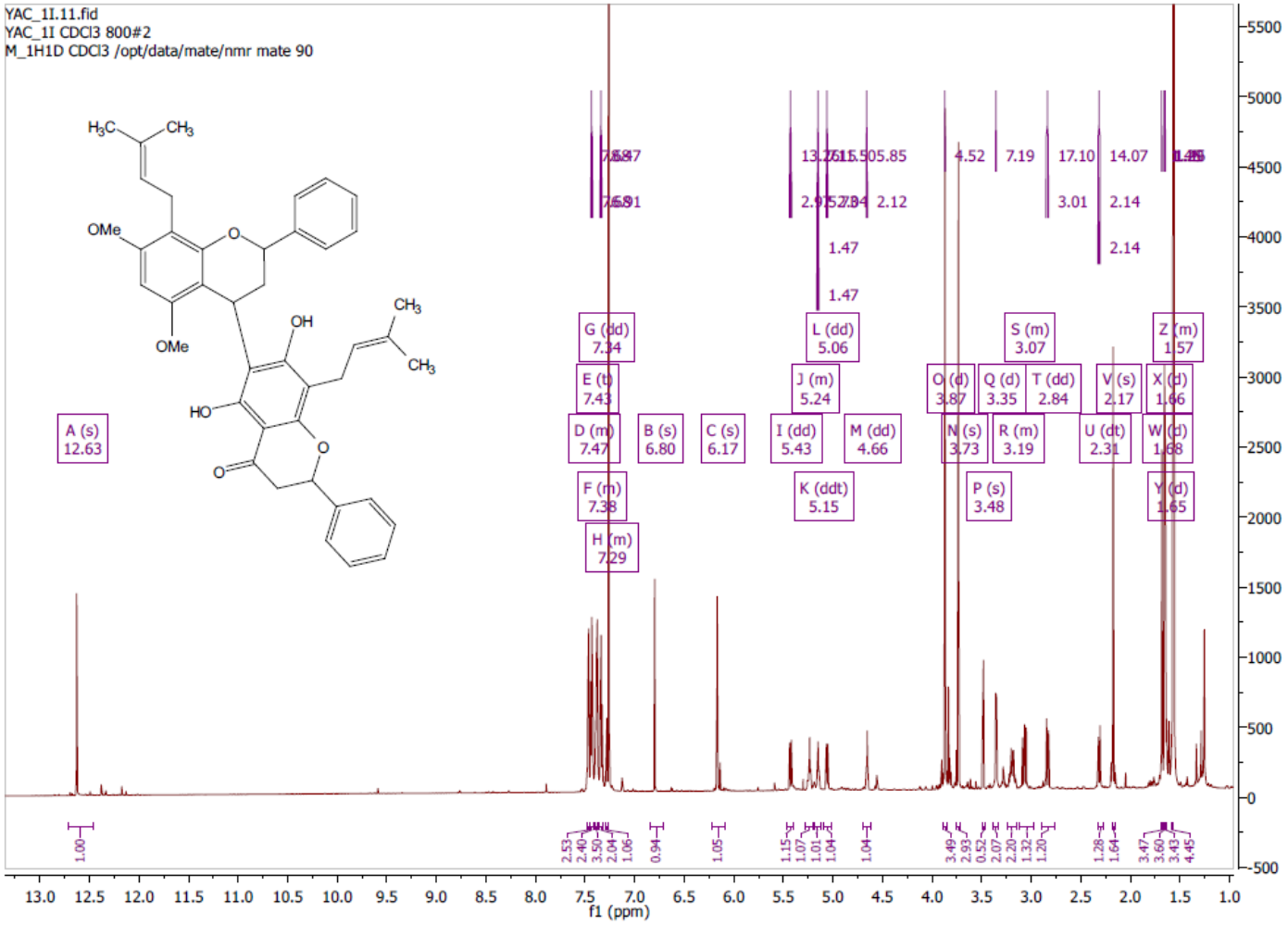
APPENDIX 30B: ^{13}C NMR Spectrum of *Tephrowatsin A* (79) (200 MHz; $CDCl_3$)



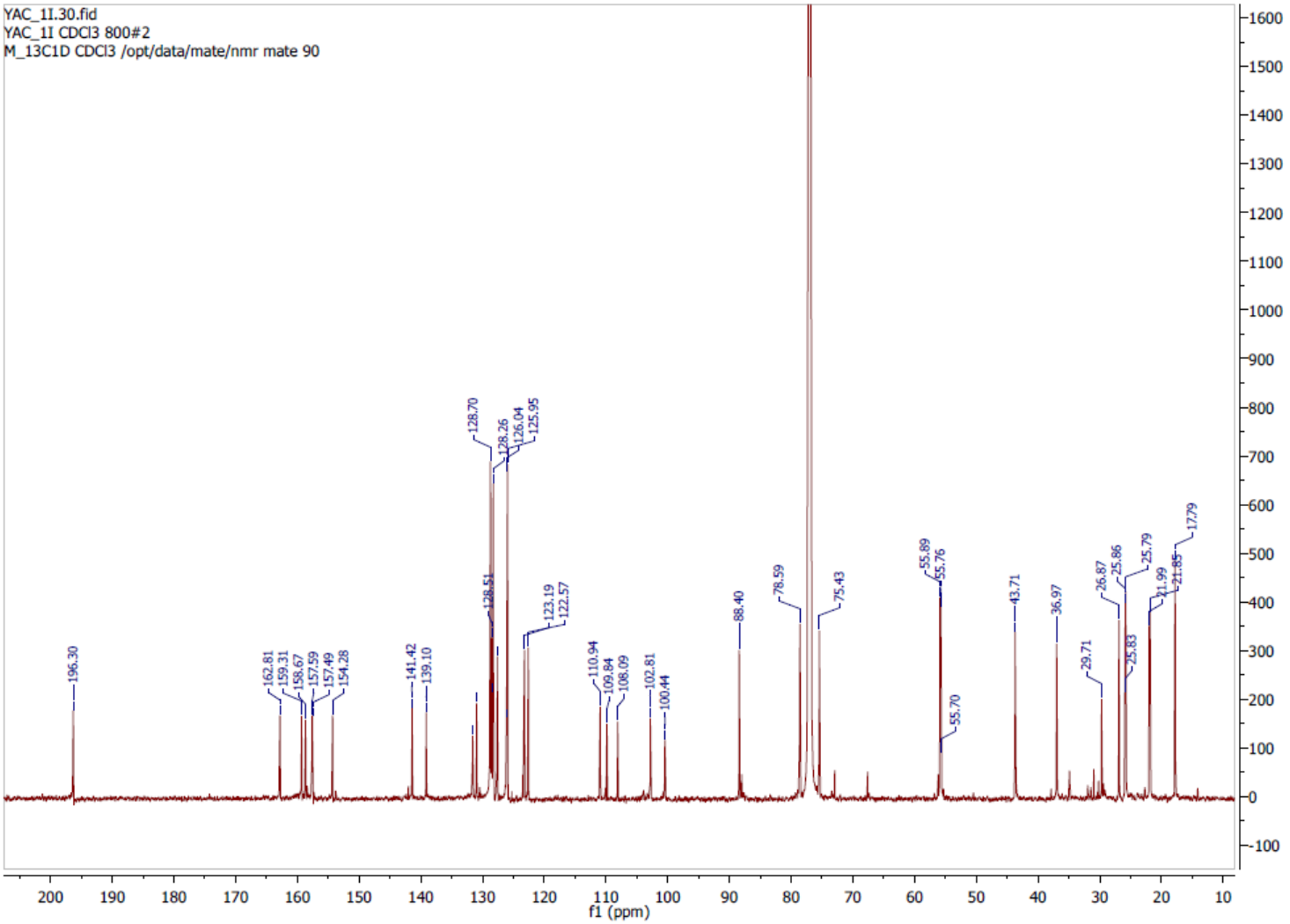
APPENDIX 30C: HMBC Spectrum of *Tephrowatsin A* (79) ($CDCl_3$)



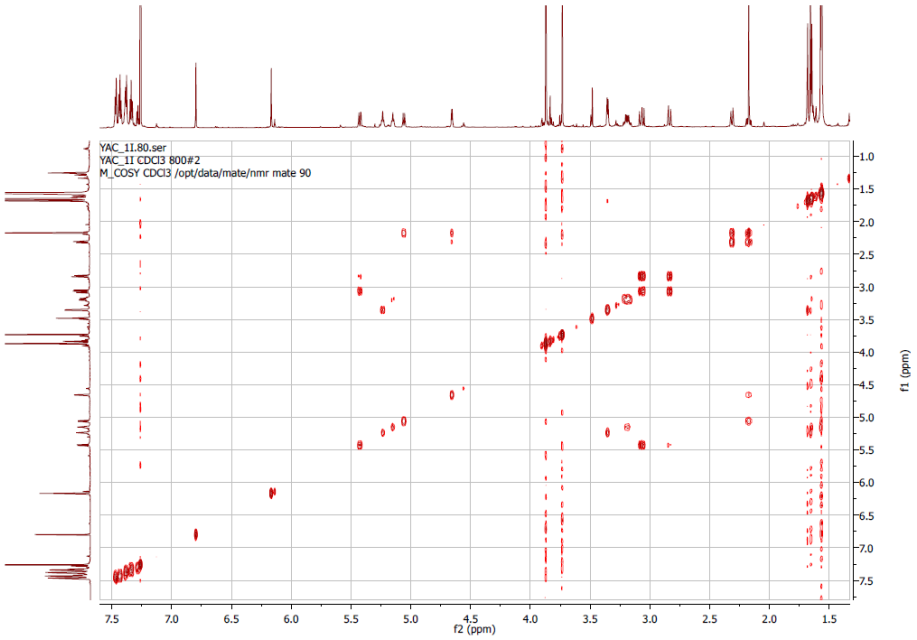
APPENDIX 30A: ^{1}H NMR Spectrum of Rhodimmer (183) (800 MHz; $CDCl_3$)



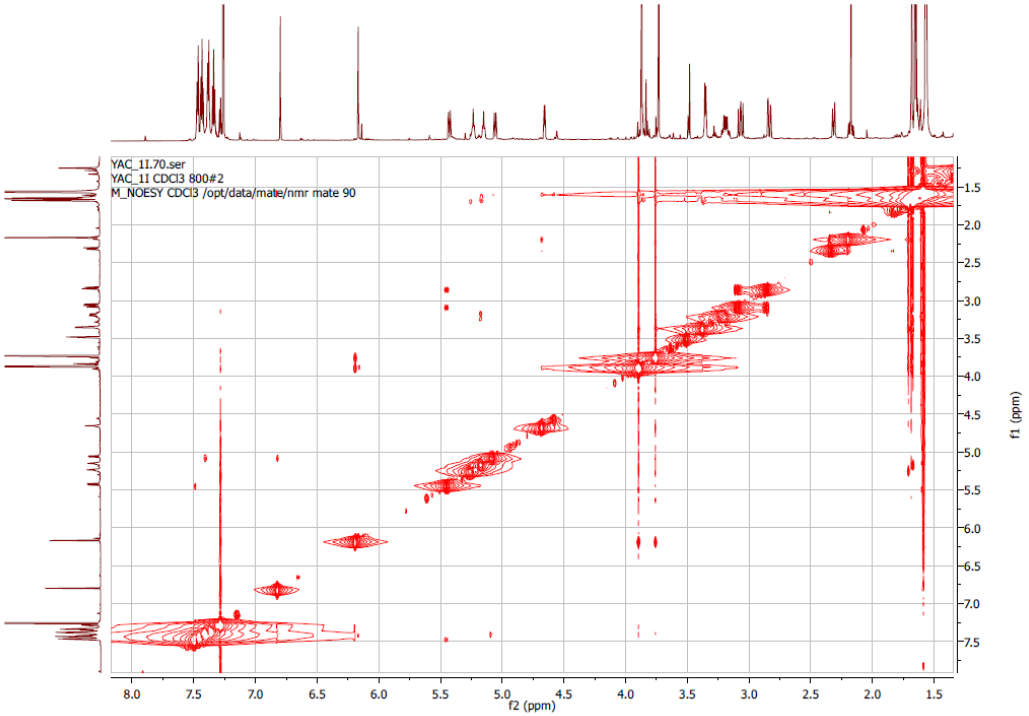
APPENDIX 30B: ^{13}C NMR Spectrum of Rhodimmer (183) (200 MHz; $CDCl_3$)



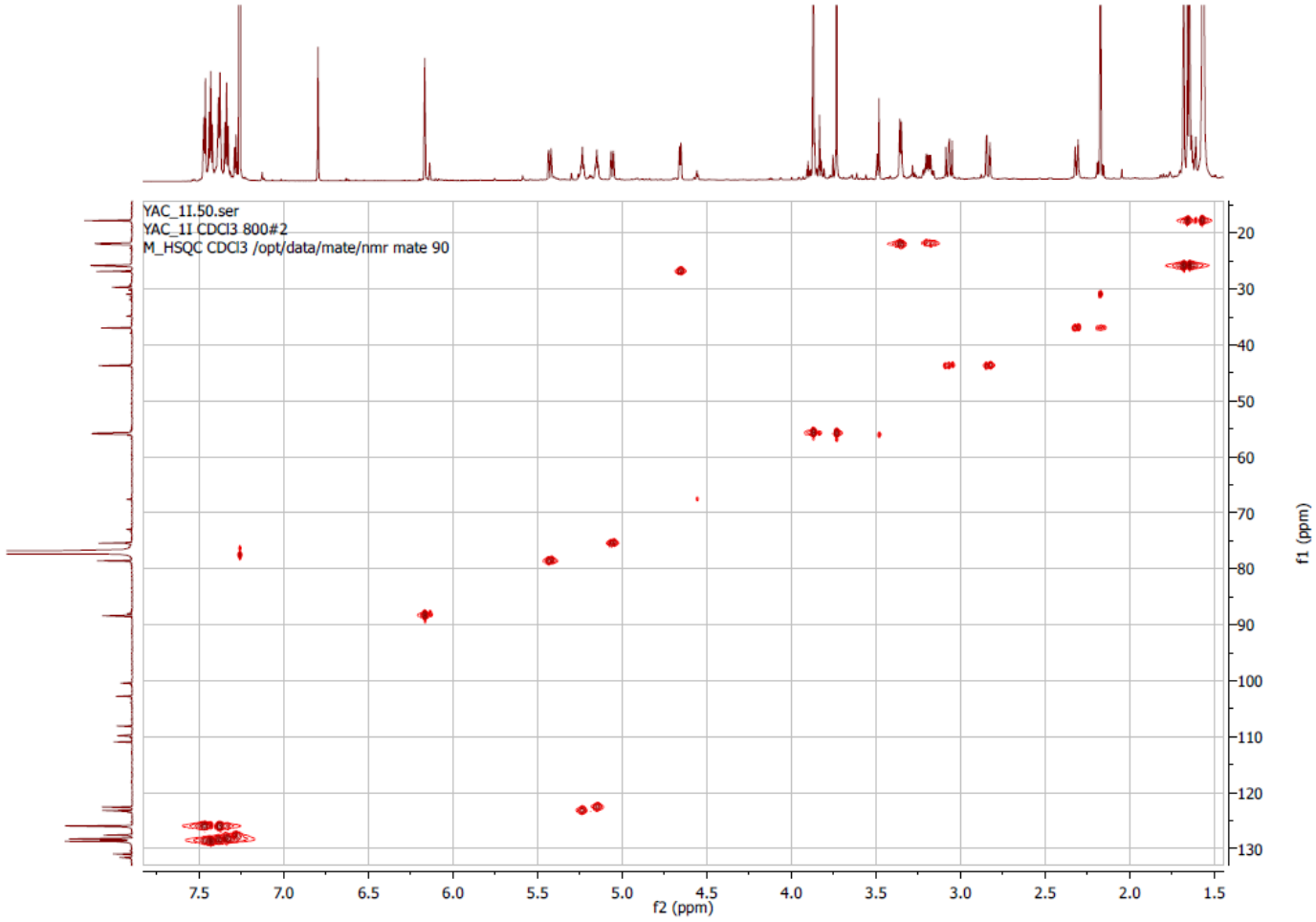
APPENDIX 30C: HH-COSY Spectrum of Rhodimmer (183) ($CDCl_3$)



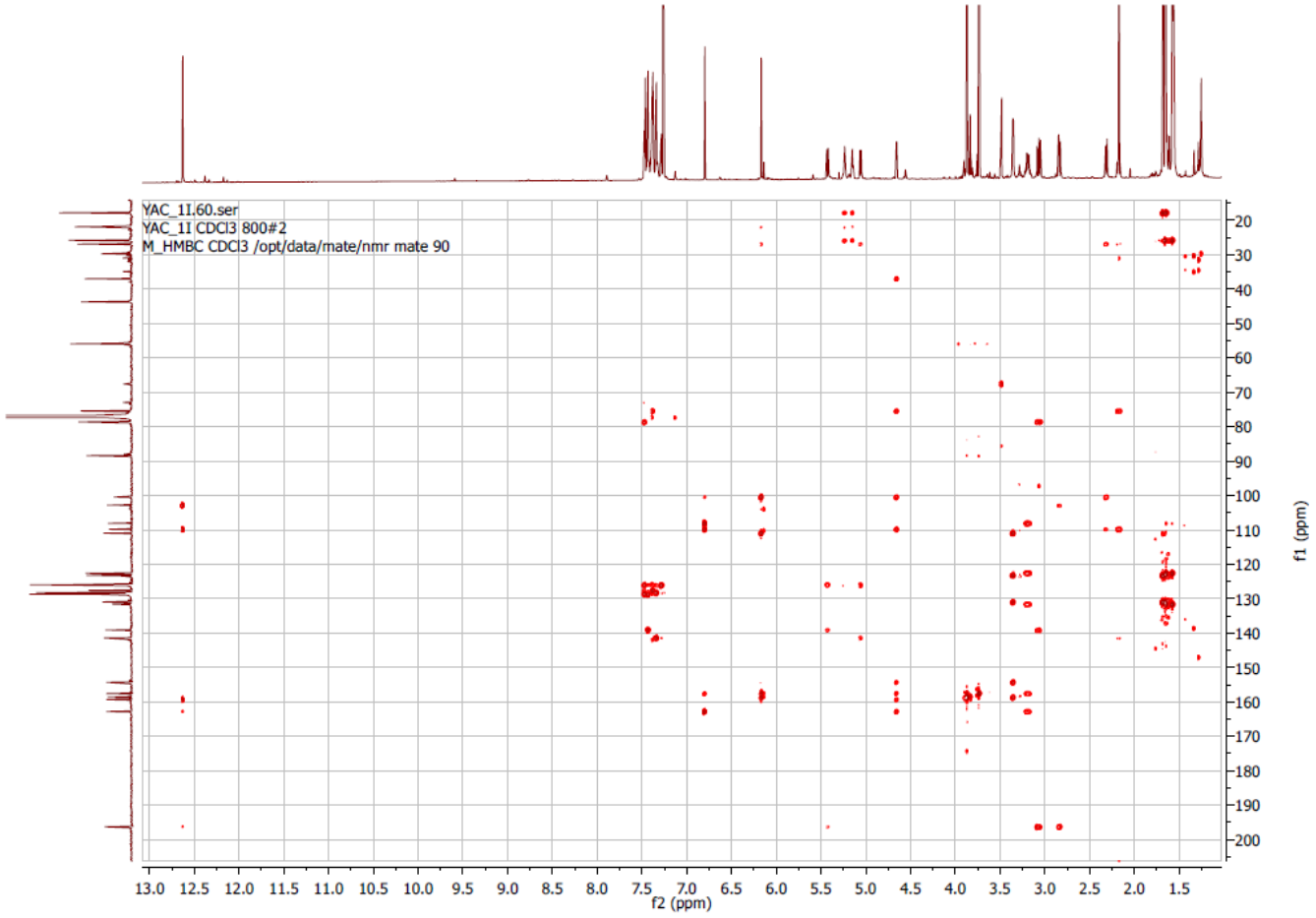
APPENDIX 30D: NOESY Spectrum of Rhodimmer (183) ($CDCl_3$)



APPENDIX 30E: HSQC Spectrum of Rhodimmer (183) ($CDCl_3$)

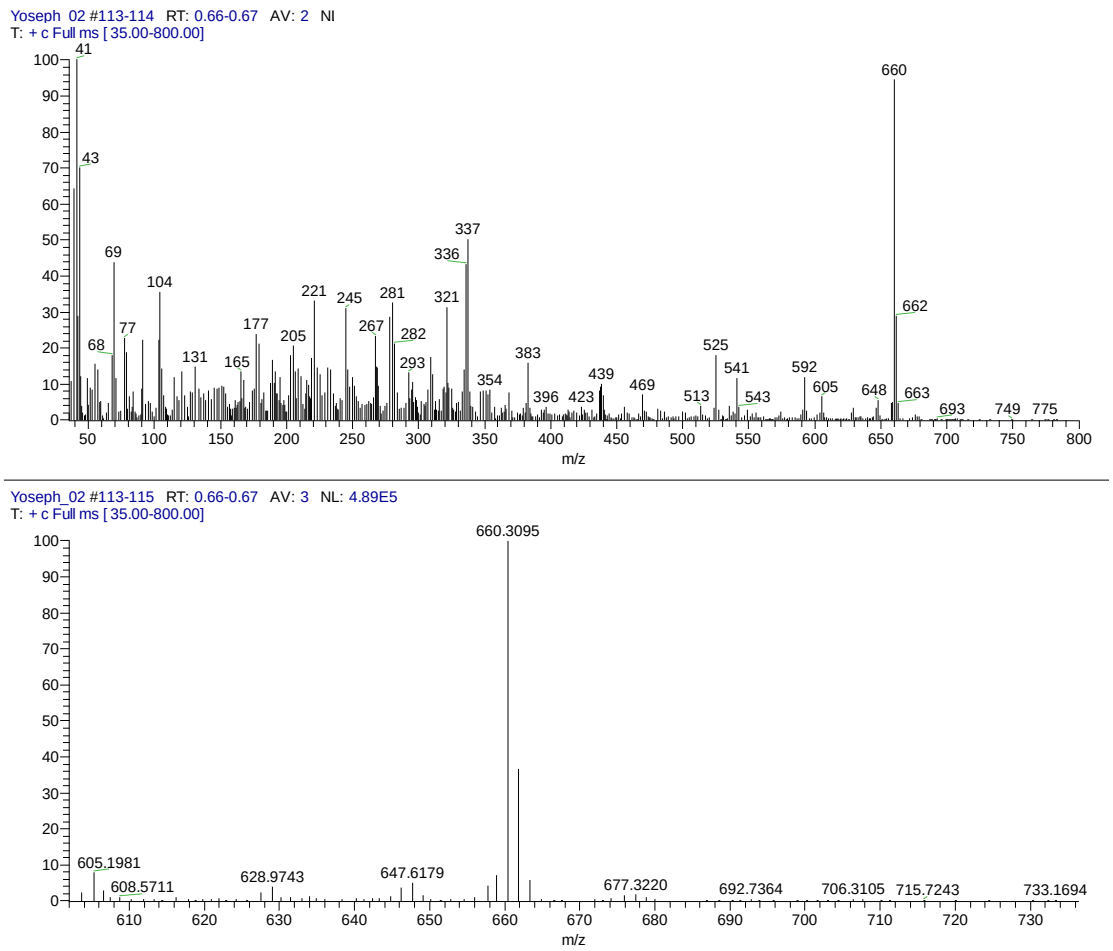


APPENDIX 30F: HMBC Spectrum of Rhodimmer (183) ($CDCl_3$)

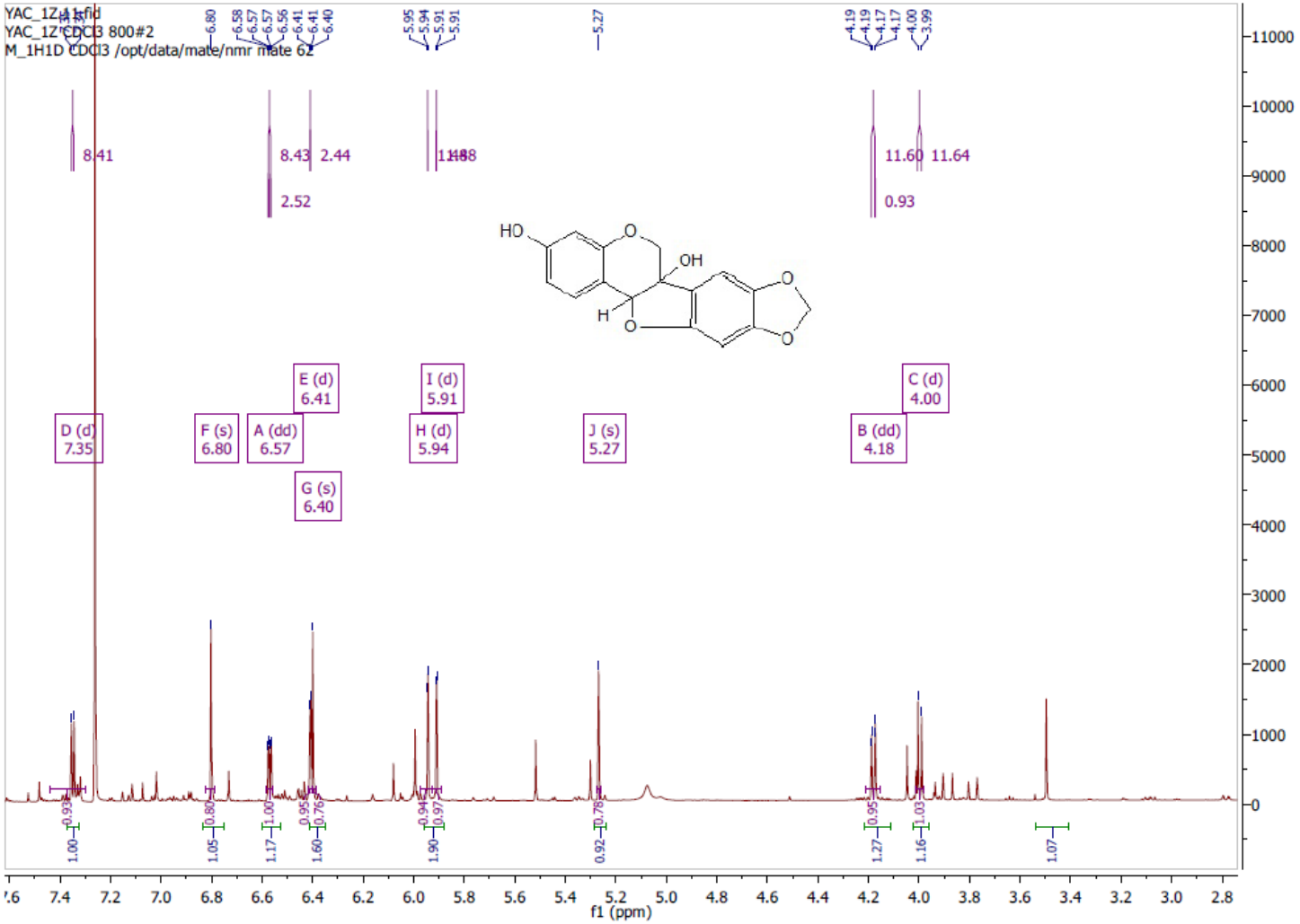


APPENDIX 30G: HRMS Spectrum of Rhodimmer (183)

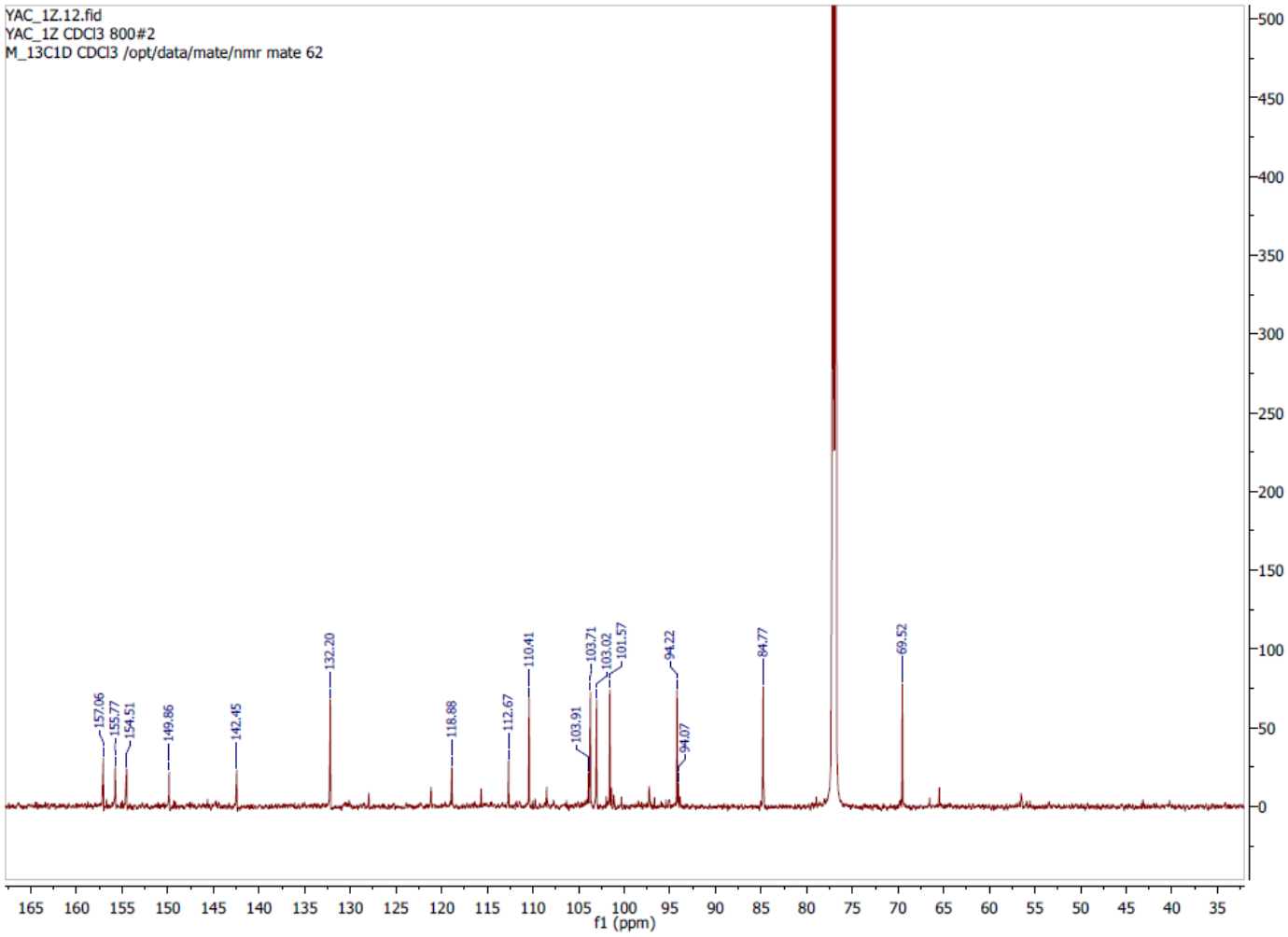
SA-53B



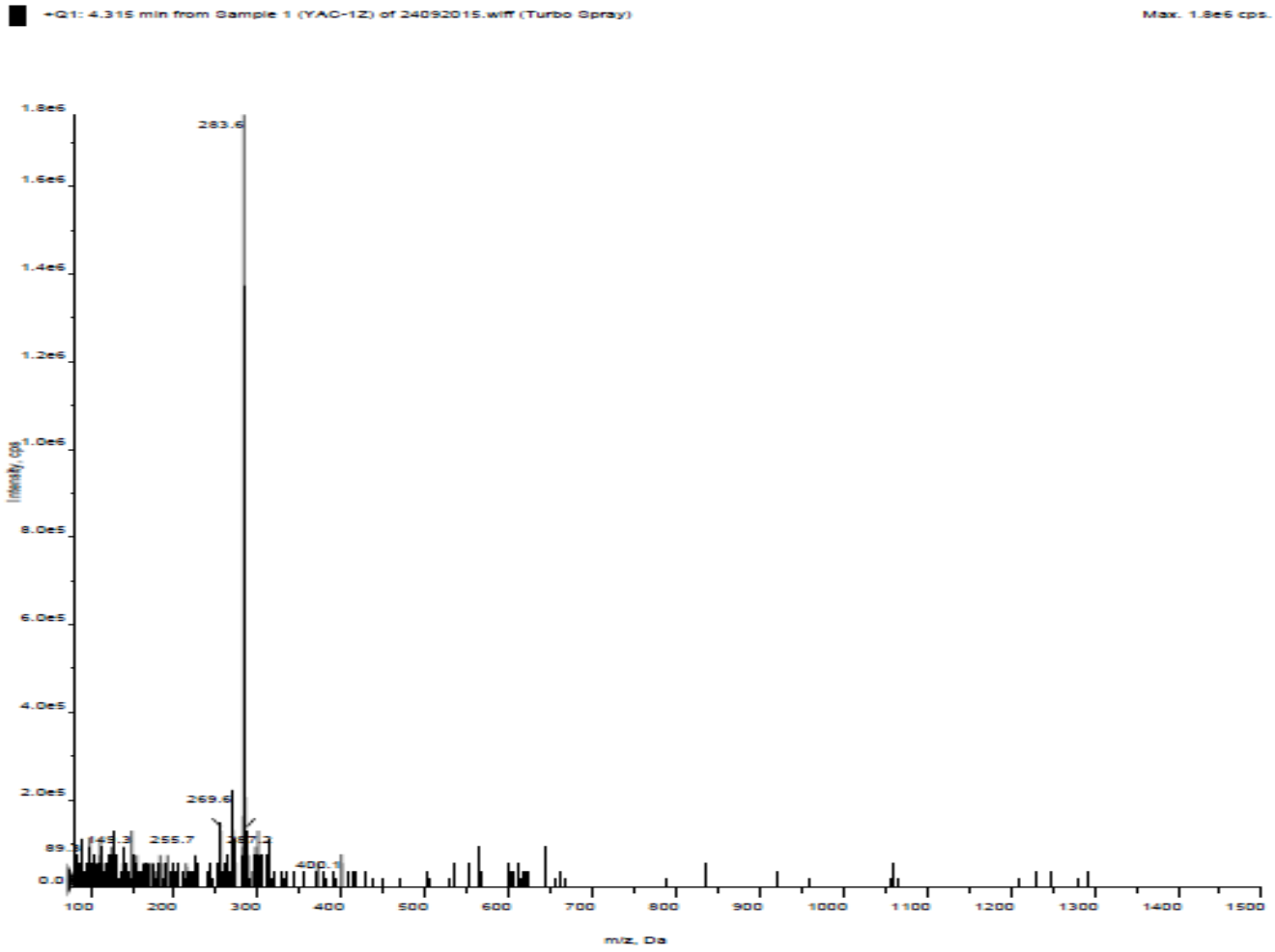
APPENDIX 31A: ^{1}H NMR Spectrum of 6a-hydroxymaackiain (184) (800 MHz; $CDCl_3$)



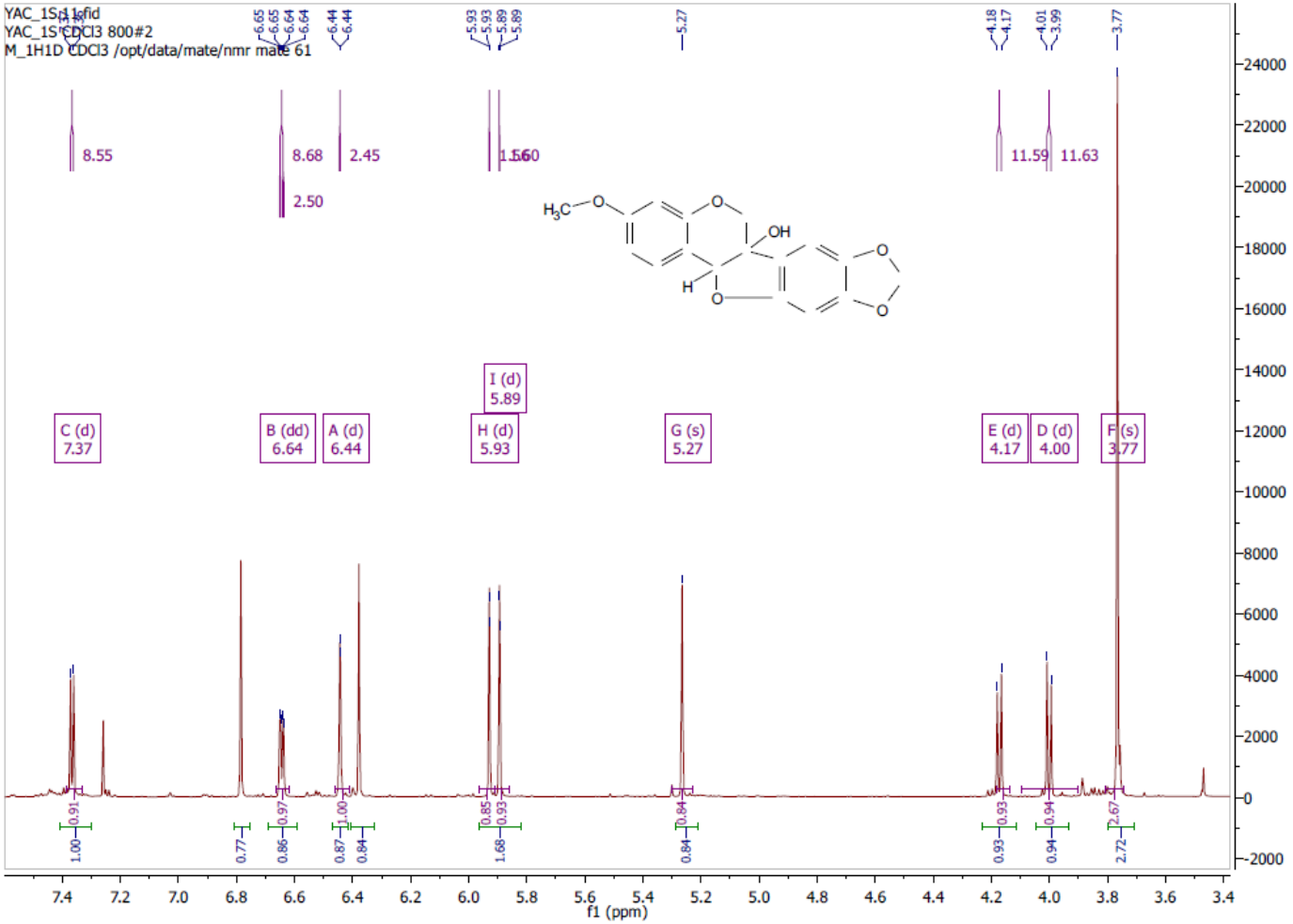
APPENDIX 31B: ^{13}C NMR Spectrum of 6a-hydroxymaackiain (184) (200 MHz; $CDCl_3$)



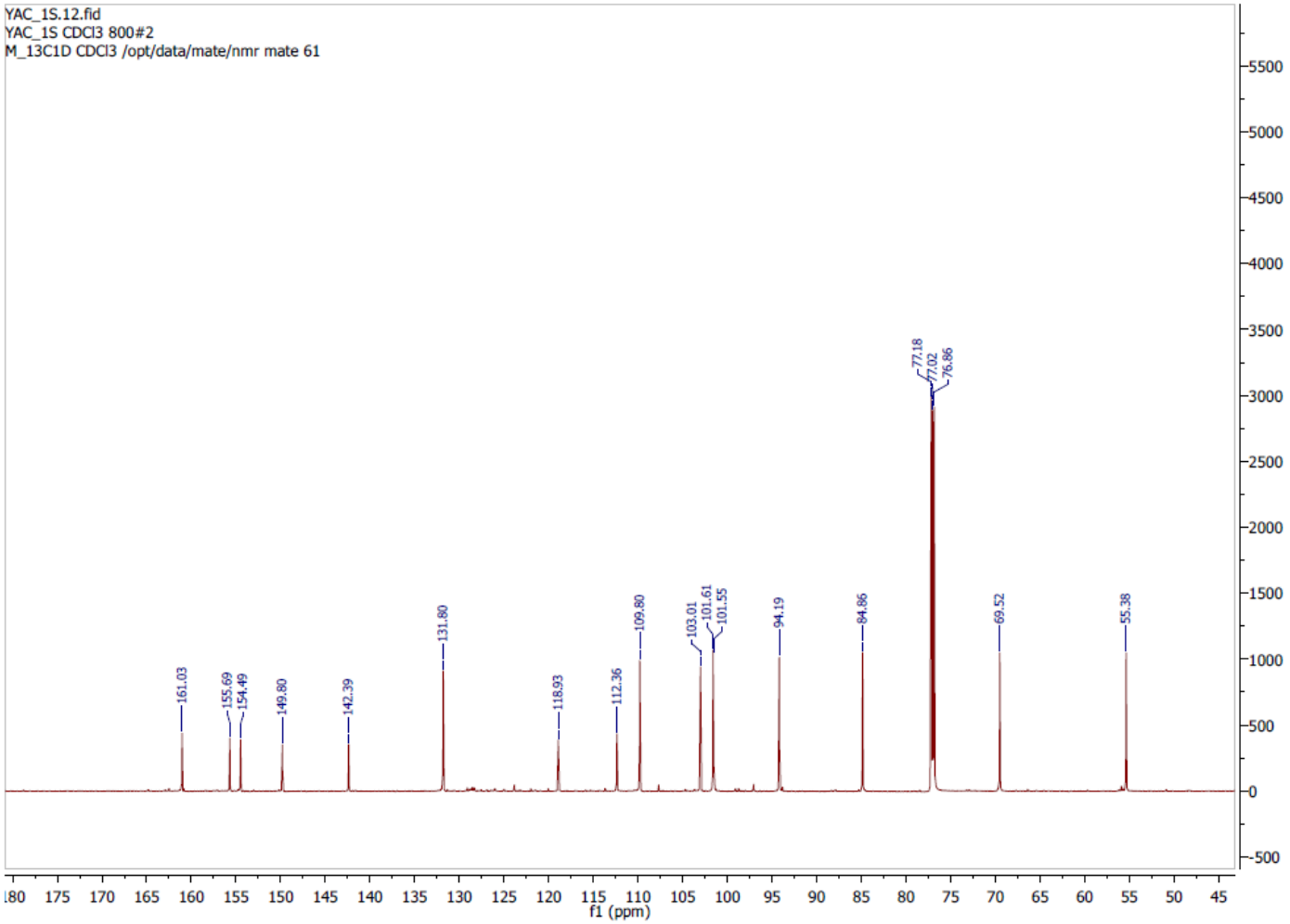
APPENDIX 31C: LCMS Spectrum of 6a-hydroxymaackiain (184) ($CDCl_3$)



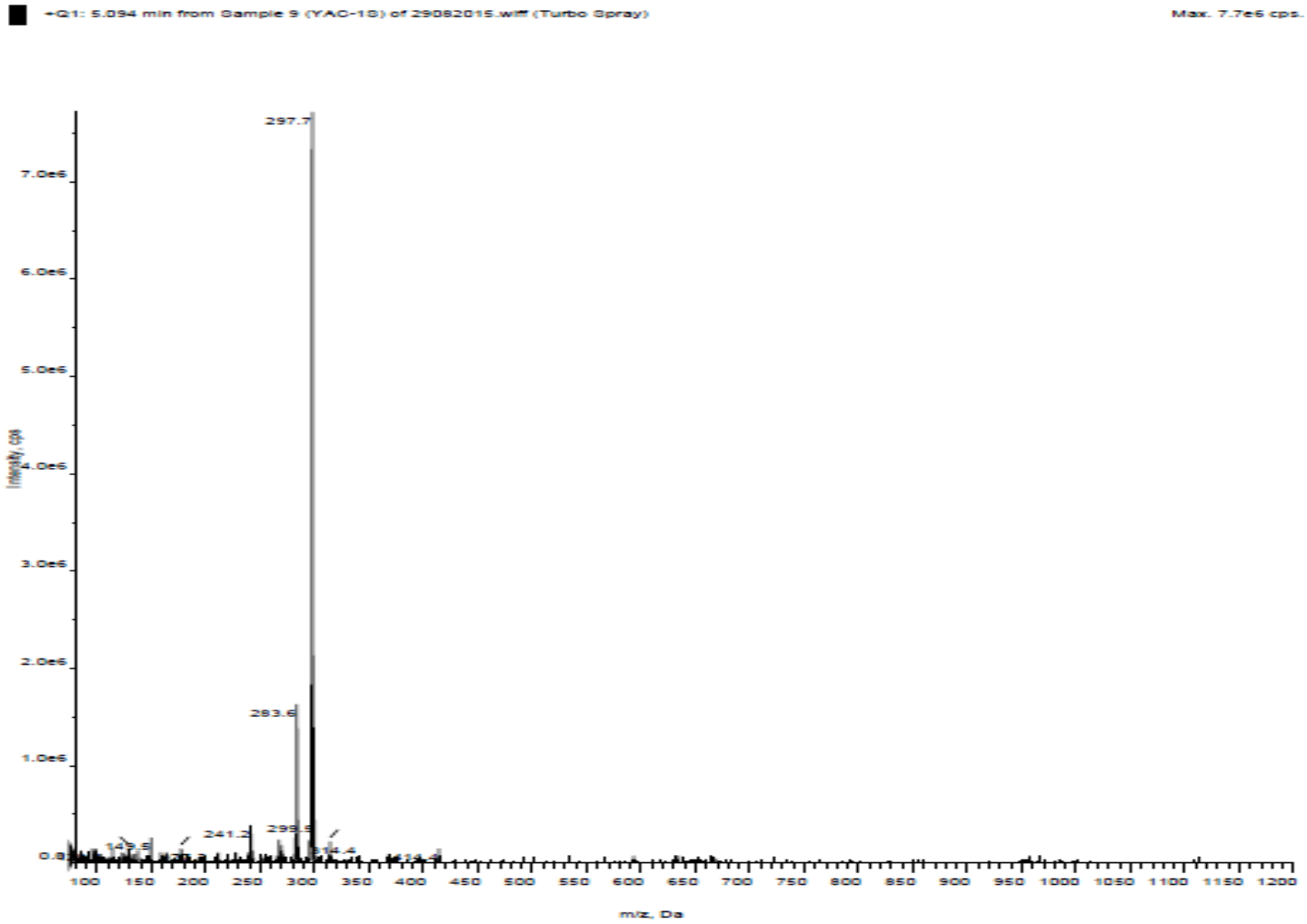
APPENDIX 32A: ^{1}H NMR Spectrum of Pisatin (185) (800 MHz; $CDCl_3$)



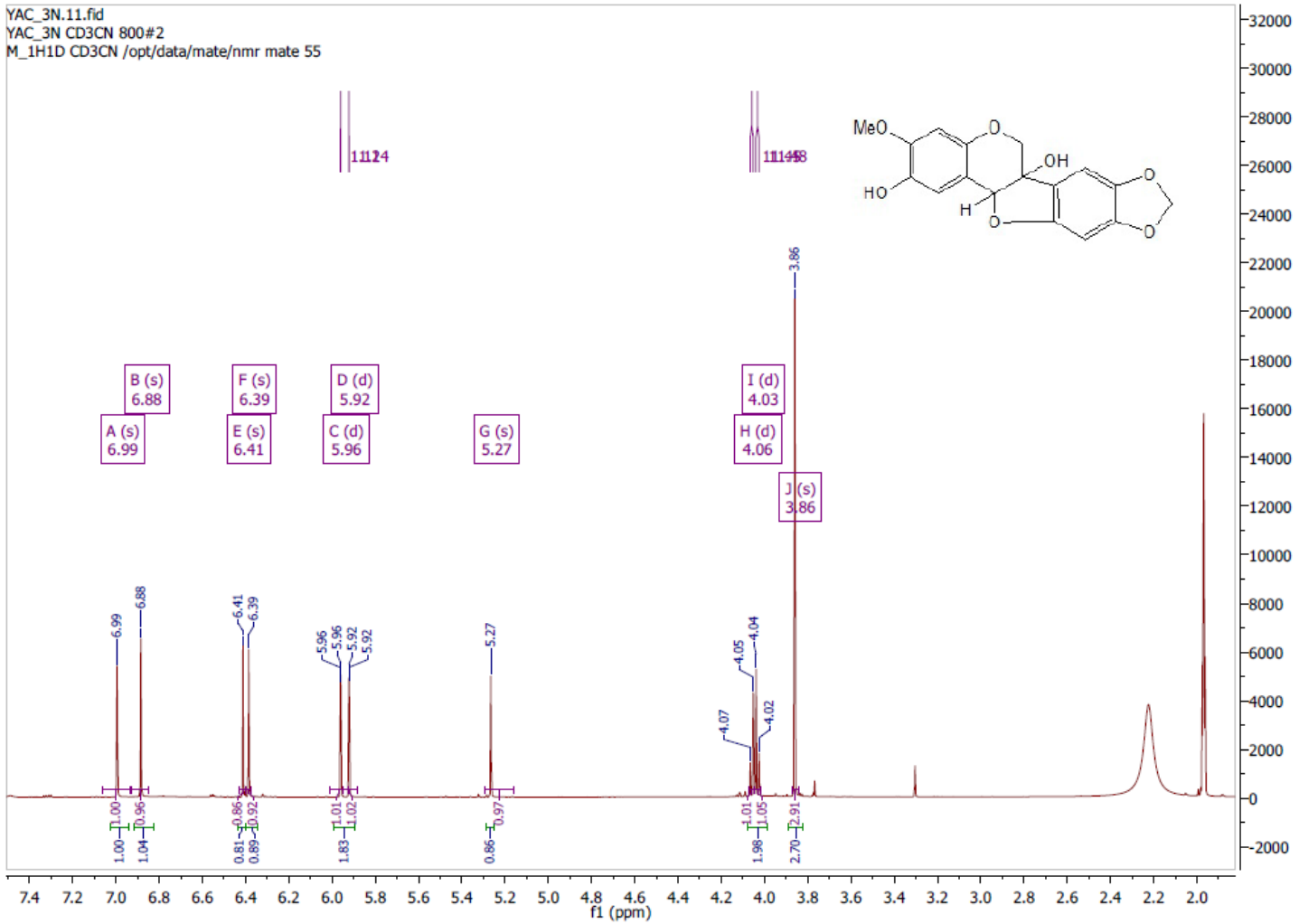
APPENDIX 32B: ^{13}C NMR Spectrum of Pisatin (185) (800 MHz; $CDCl_3$)



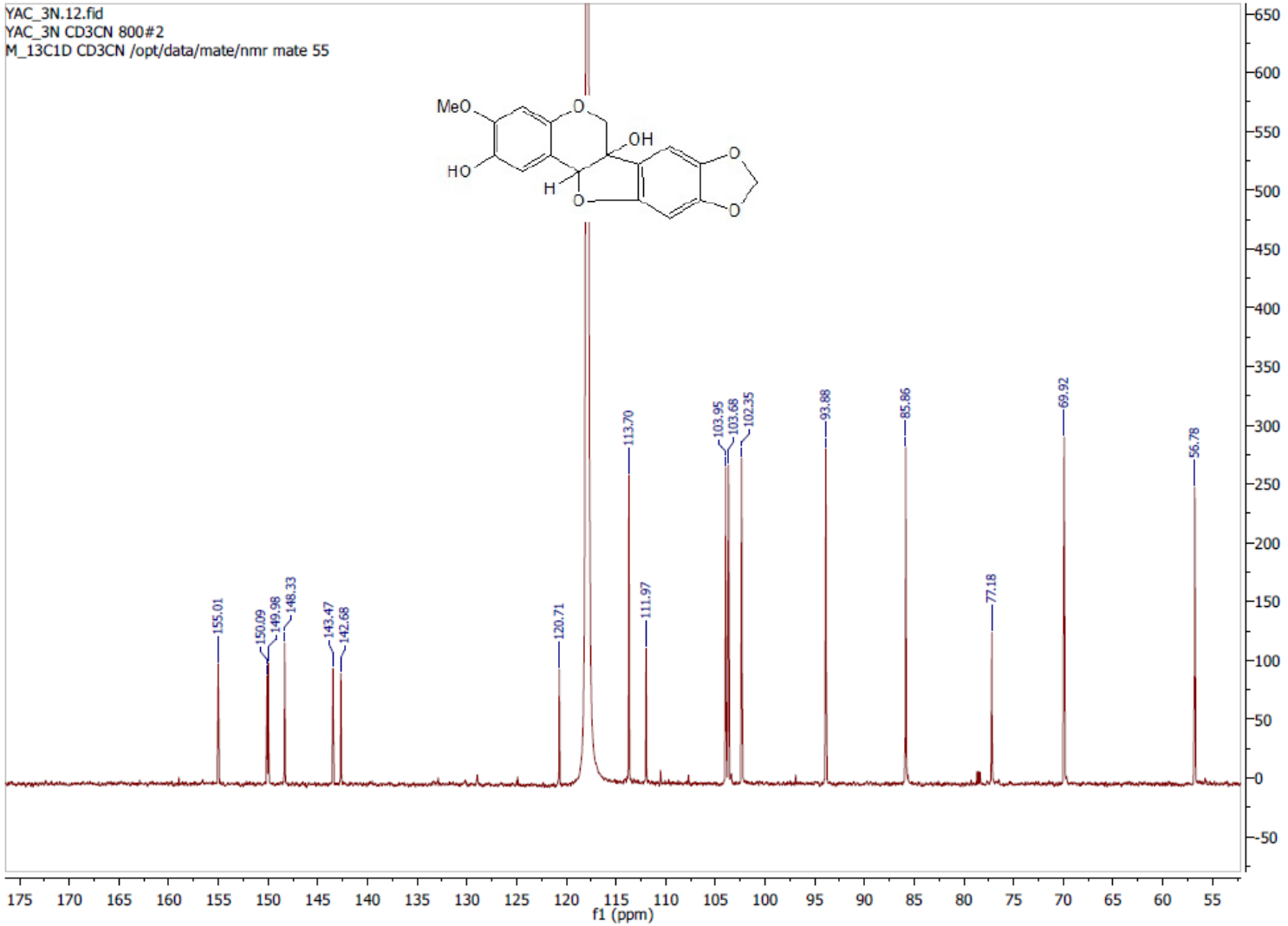
APPENDIX 32C: LCMS Spectrum of Pisatin (185) ($CDCl_3$)



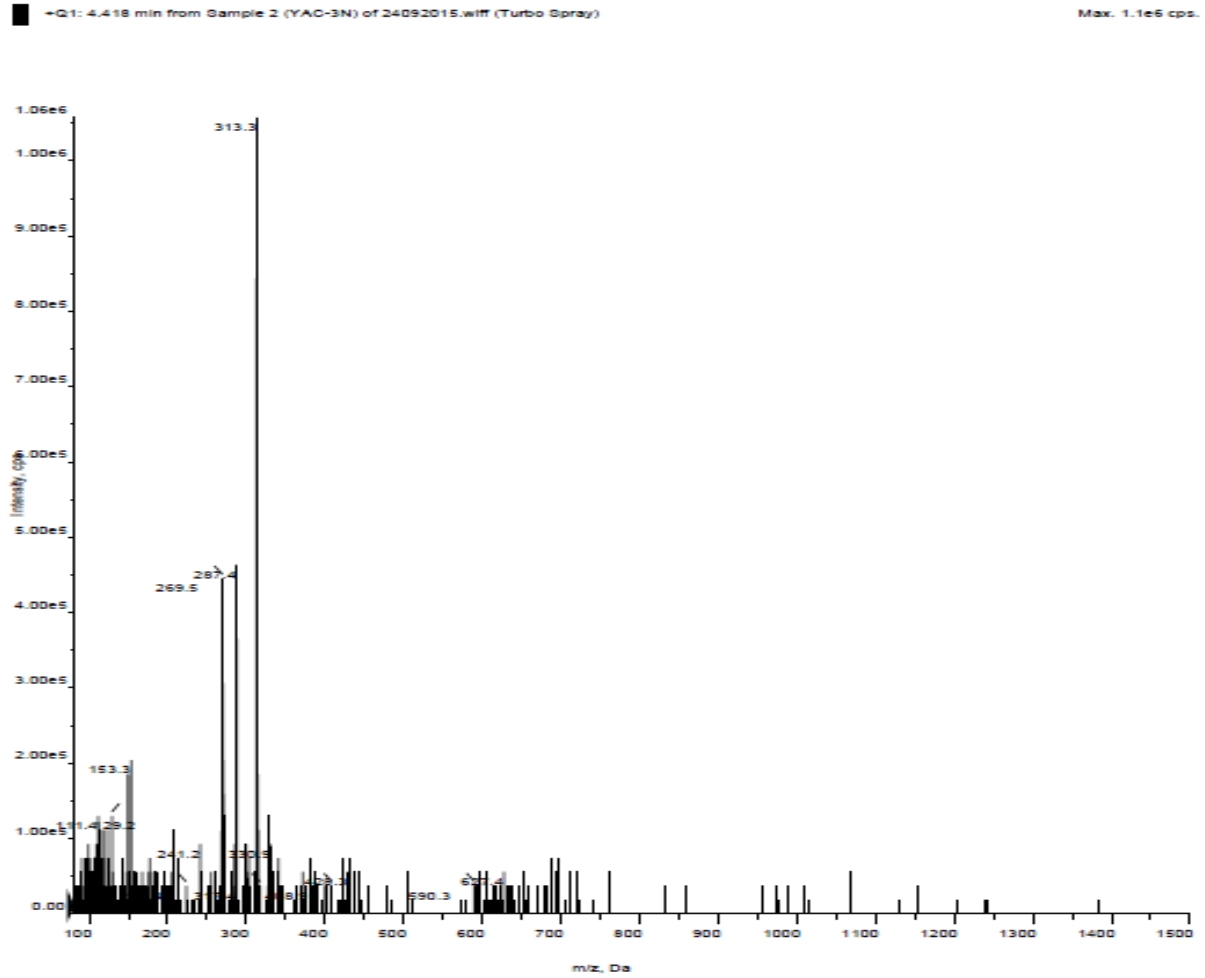
APPENDIX 33A: ^{1}H NMR Spectrum of Hildecarpin (134) (800 MHz; $CDCl_3$)



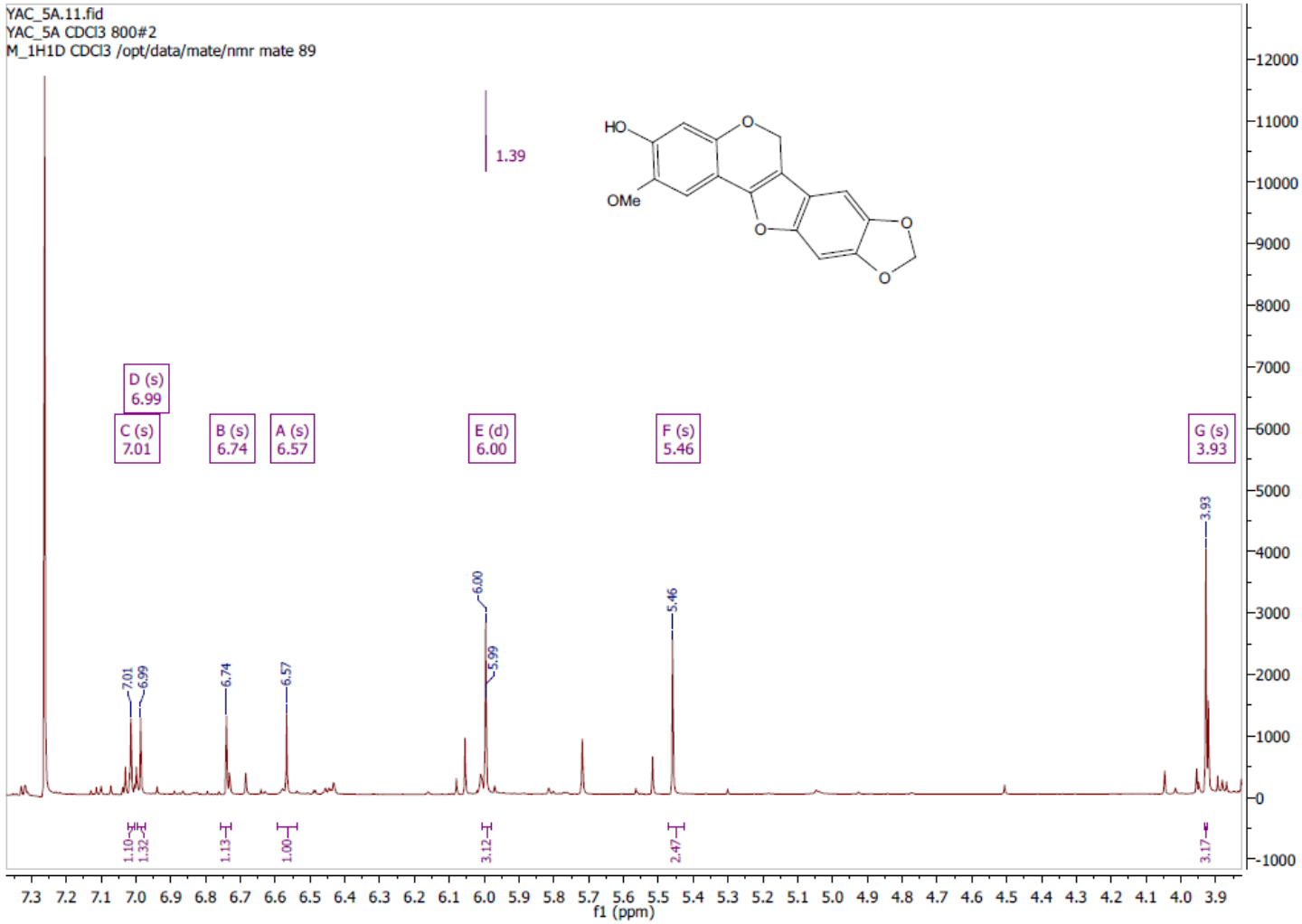
APPENDIX 33B: ^{13}C NMR Spectrum of Hildecarpin (134) (200 MHz; $CDCl_3$)



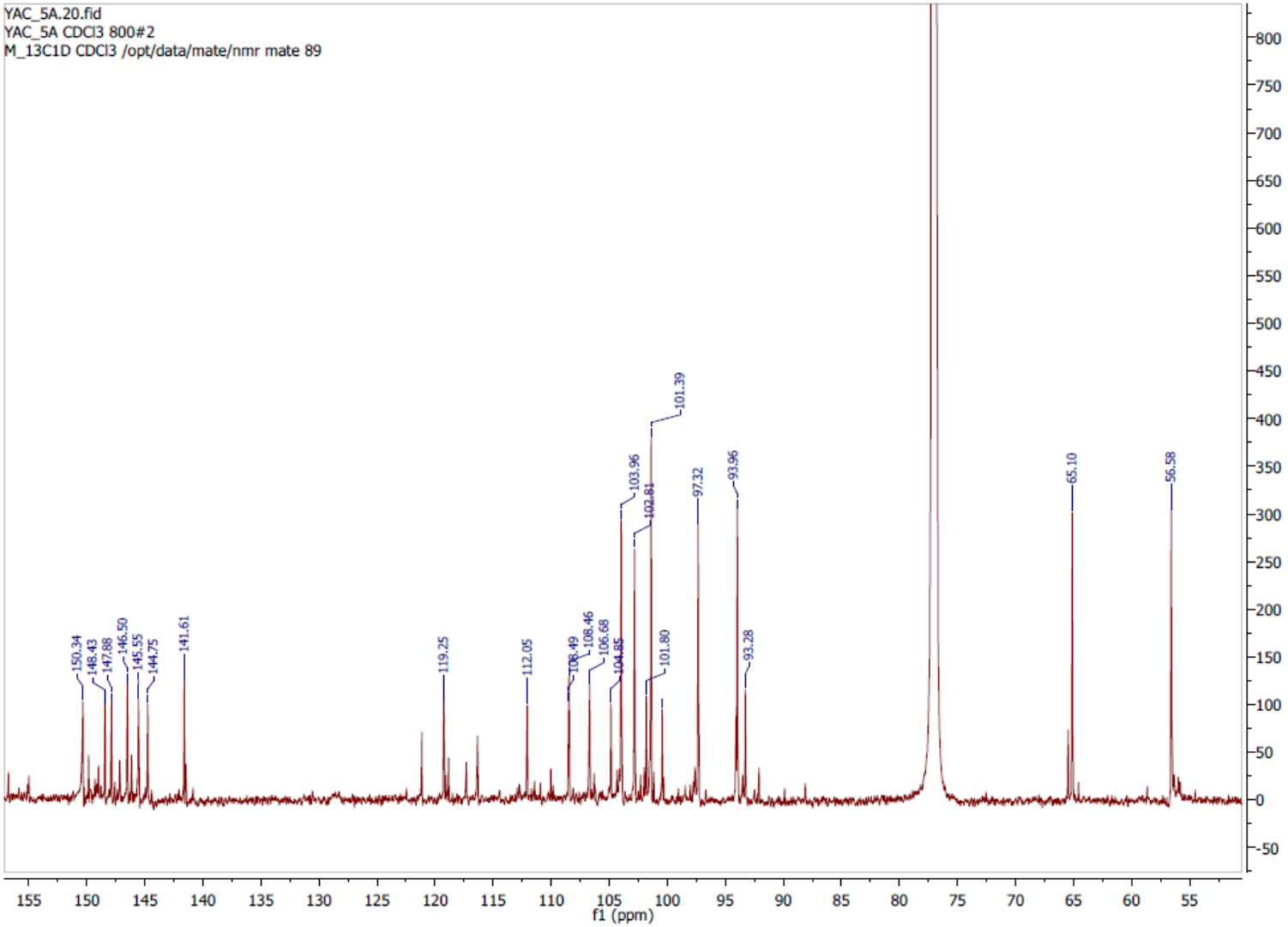
APPENDIX 33C: LCMS Spectrum of Hildecarpin (134) ($CDCl_3$)



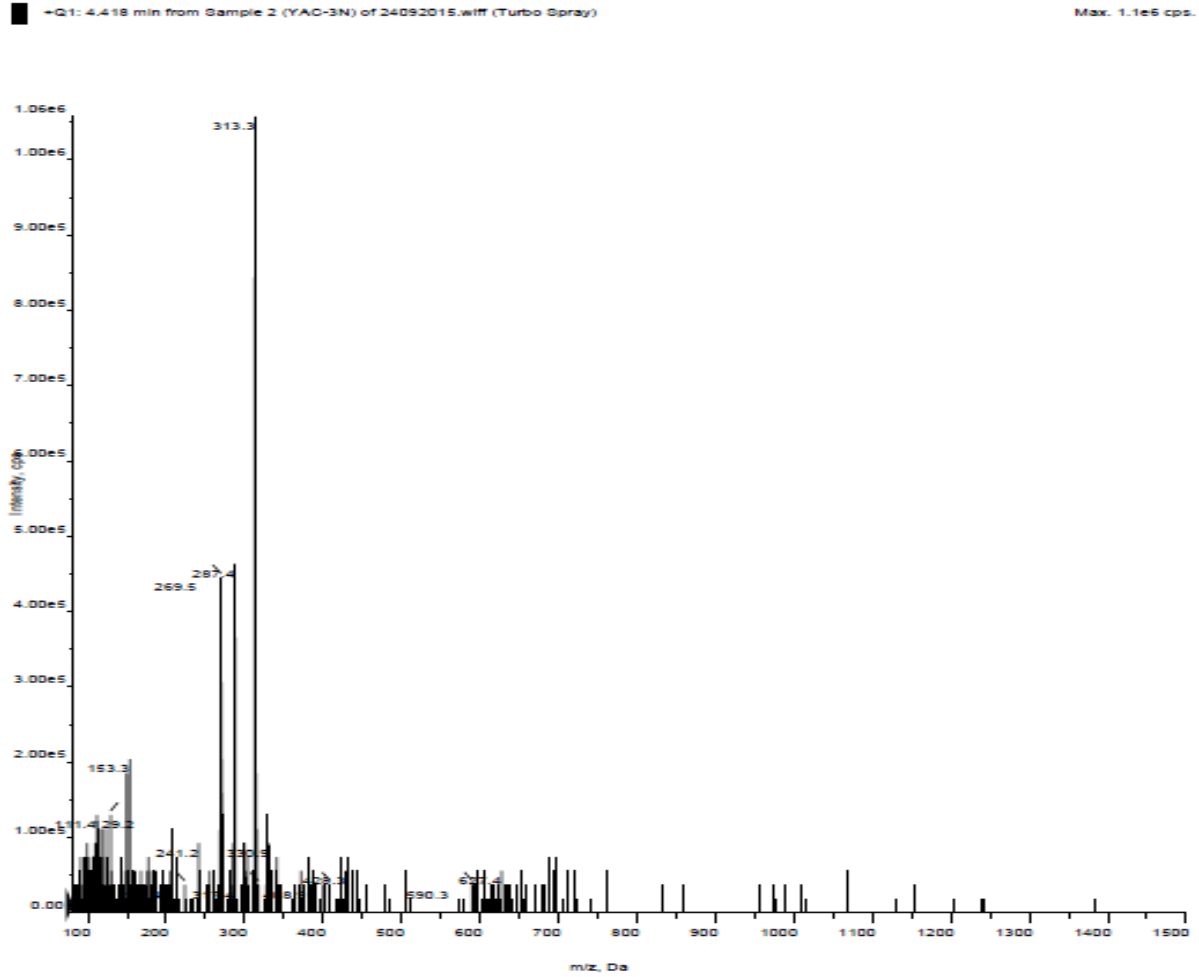
APPENDIX 34A: ^{1}H NMR Spectrum of 3-hydroxy-2-methoxy-8-9-methylenedioxypterocarpene (189) (800 MHz; $CDCl_3$)



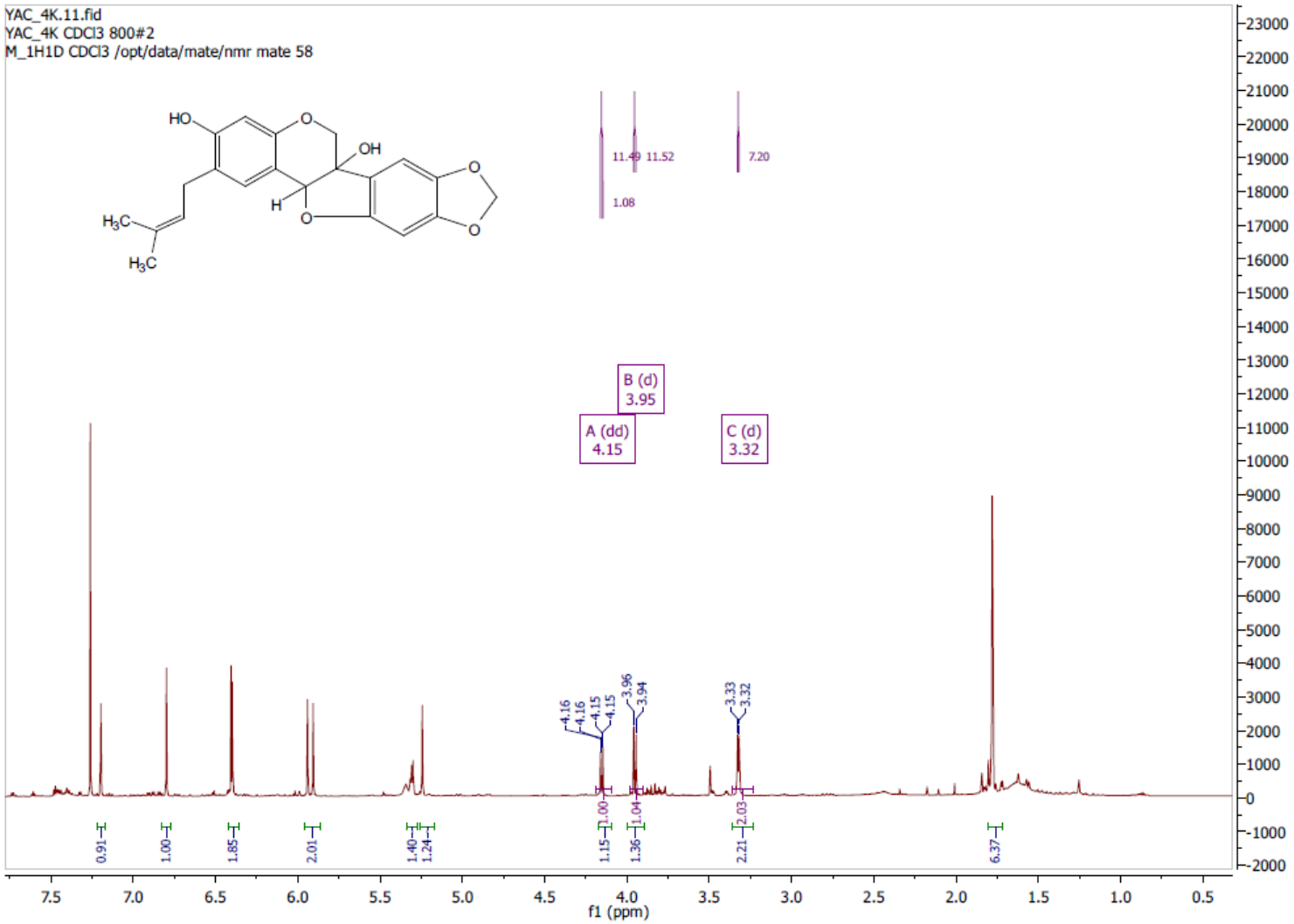
APPENDIX 34B: ^{13}C NMR Spectrum of 3-hydroxy-2-methoxy-8-9-methylenedioxypterocarpene (189) (200 MHz; $CDCl_3$)



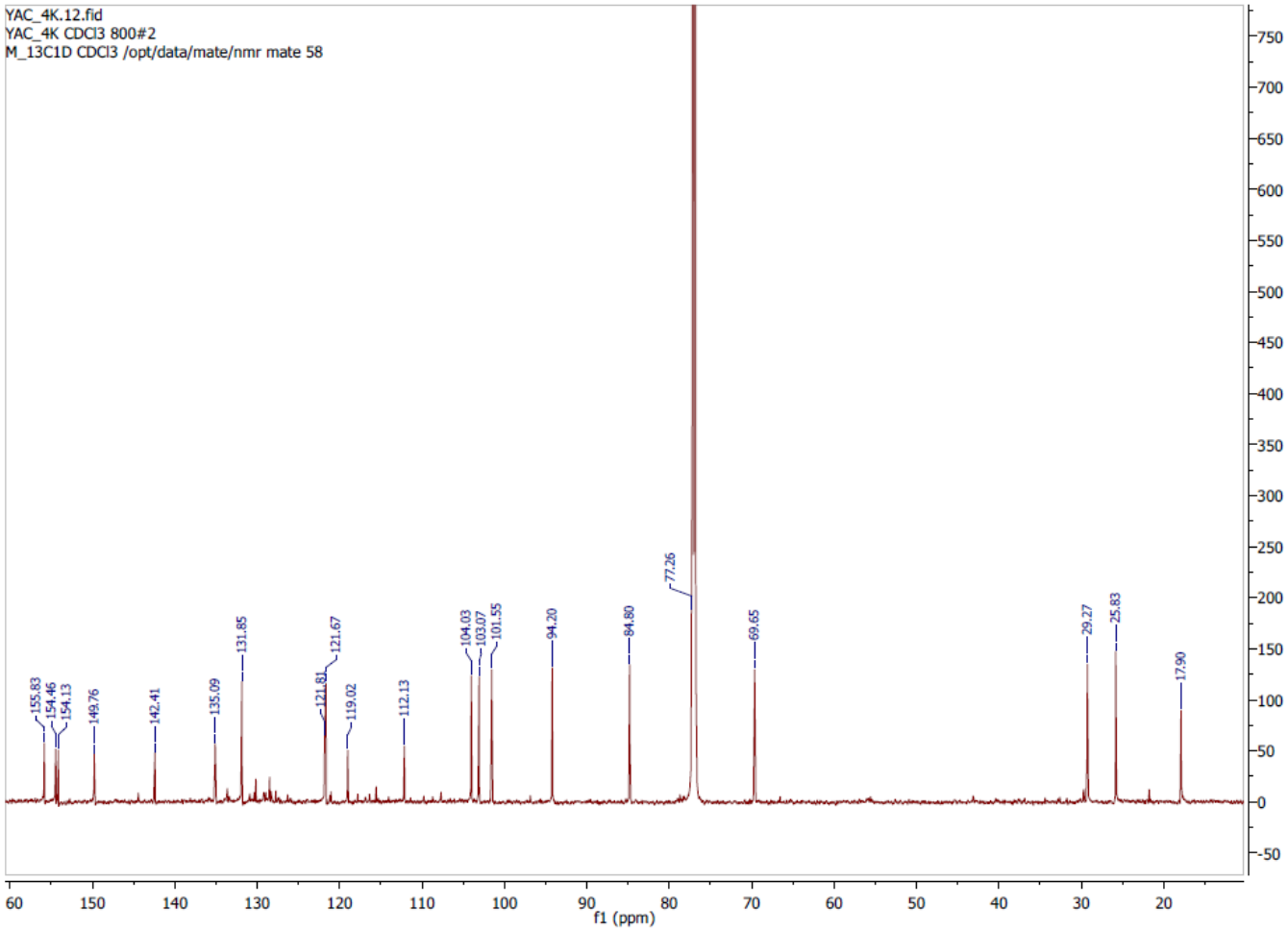
APPENDIX 34F: LCMS Spectrum of 3-hydroxy-2-methoxy-8-9-methylenedioxypterocarpene (189) ($CDCl_3$)



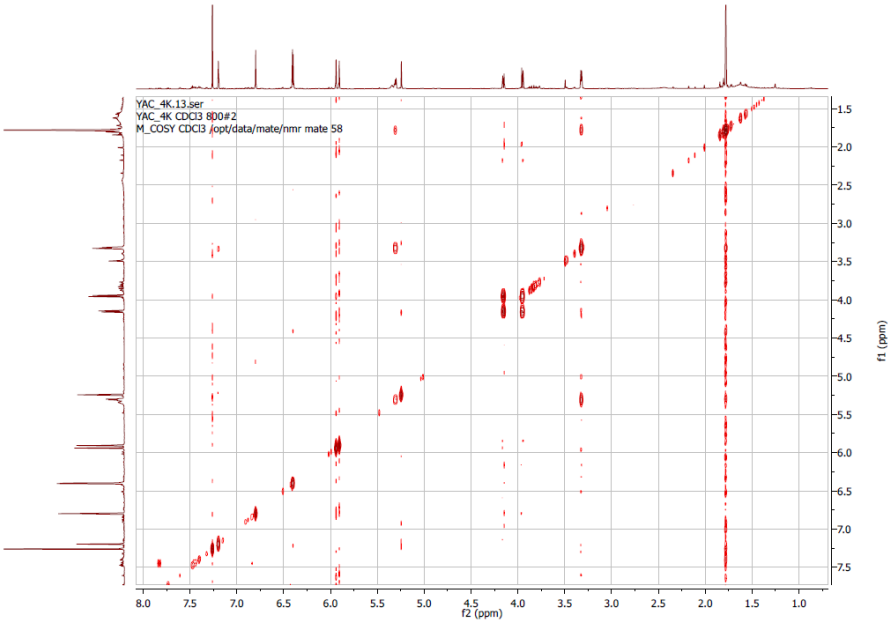
APPENDIX 35A: ^{1}H NMR Spectrum of Rhocarpin (188) (800 MHz; $CDCl_3$)



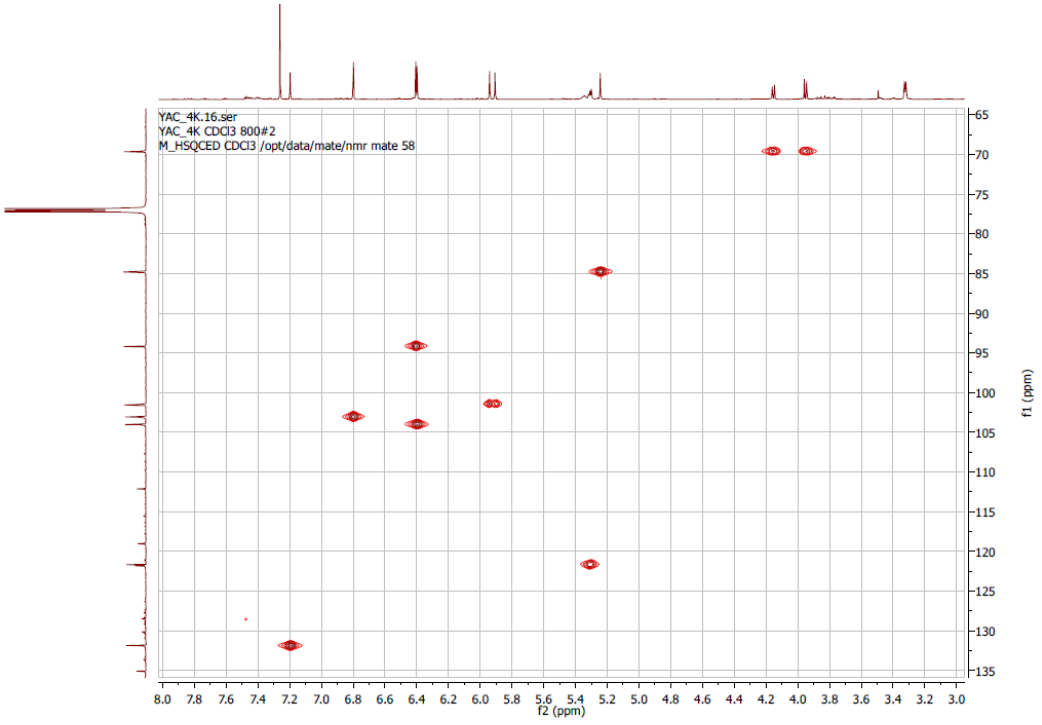
APPENDIX 35B: ^{13}C NMR Spectrum of Rhocarpin (188) (200 MHz; $CDCl_3$)



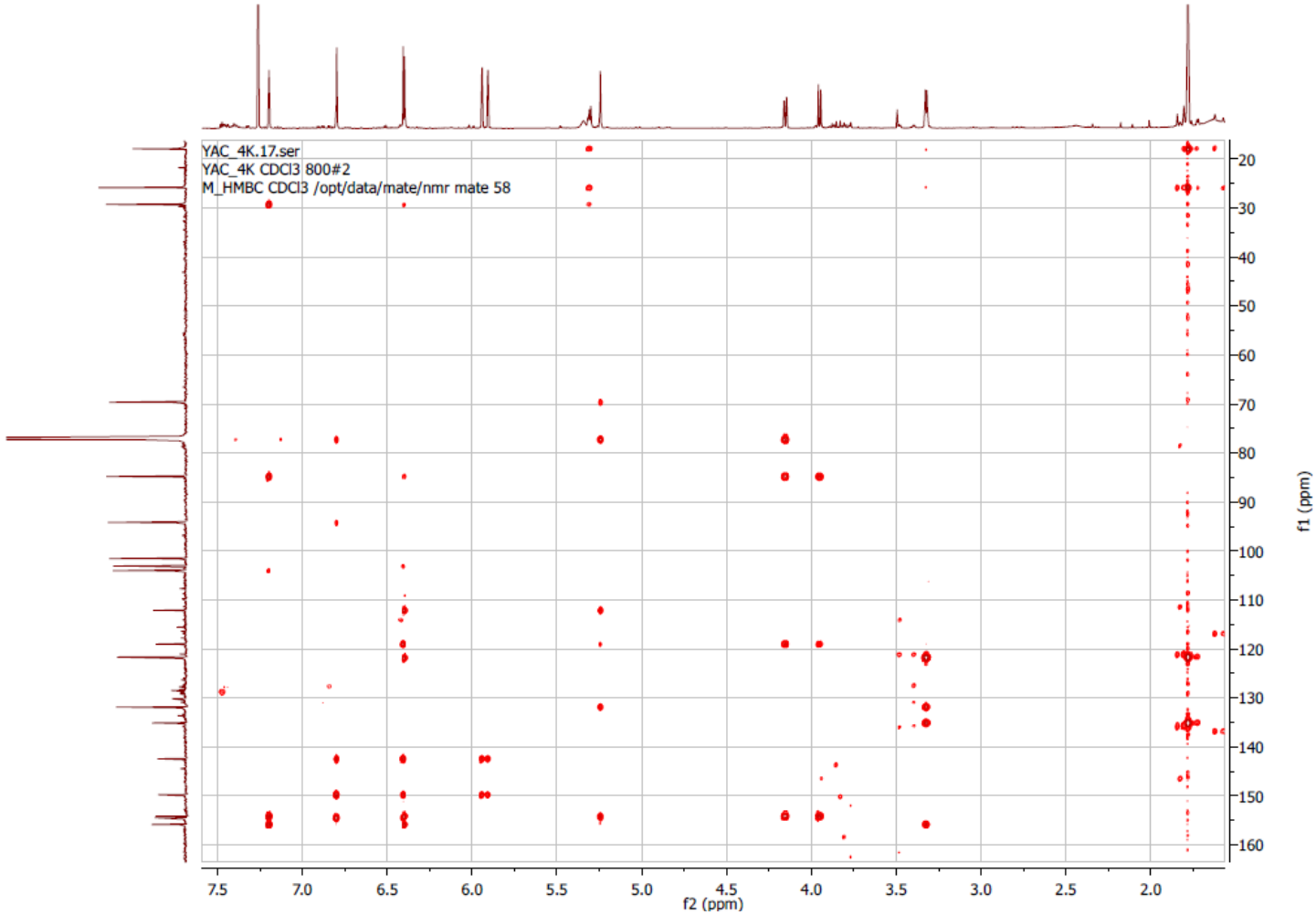
APPENDIX 35C: HH-COSY Spectrum of Rhocarpin (188) ($CDCl_3$)



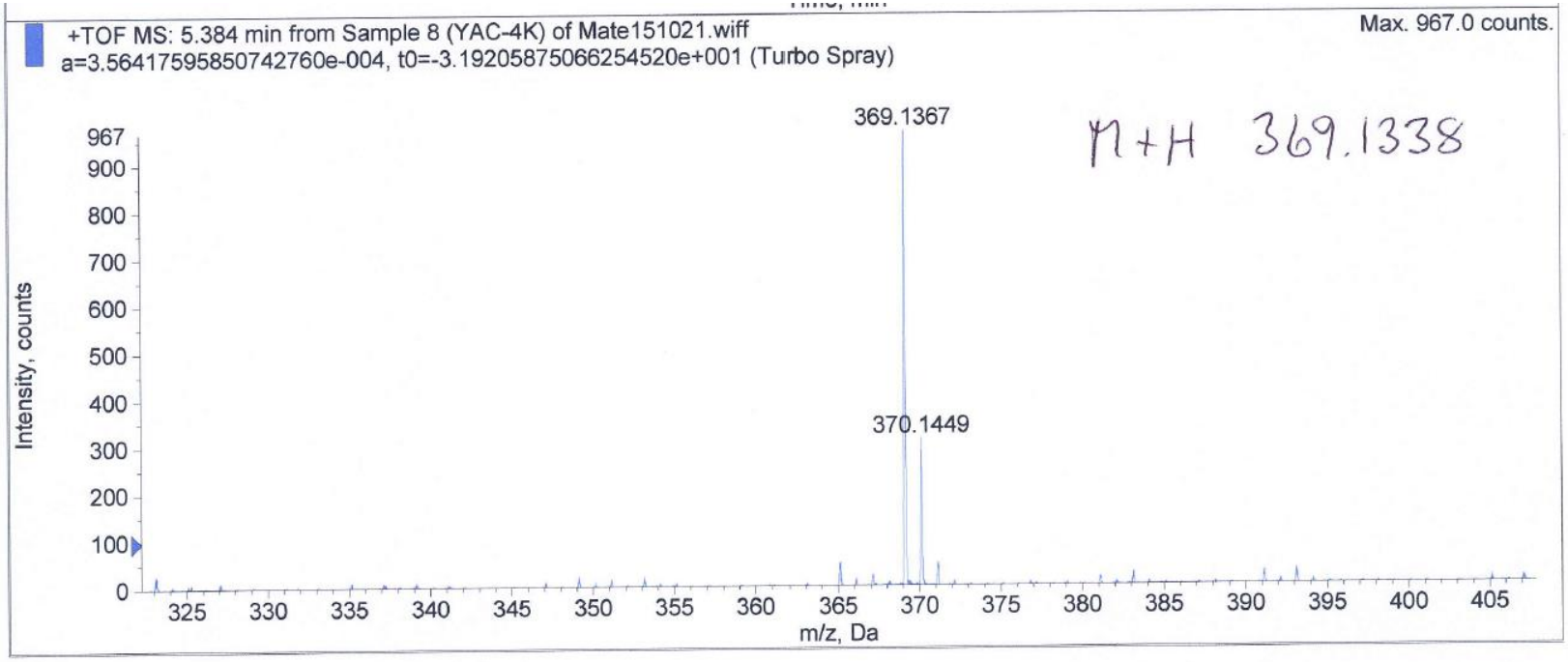
APPENDIX 35D: HSQC Spectrum of Rhocarpin (188) ($CDCl_3$)



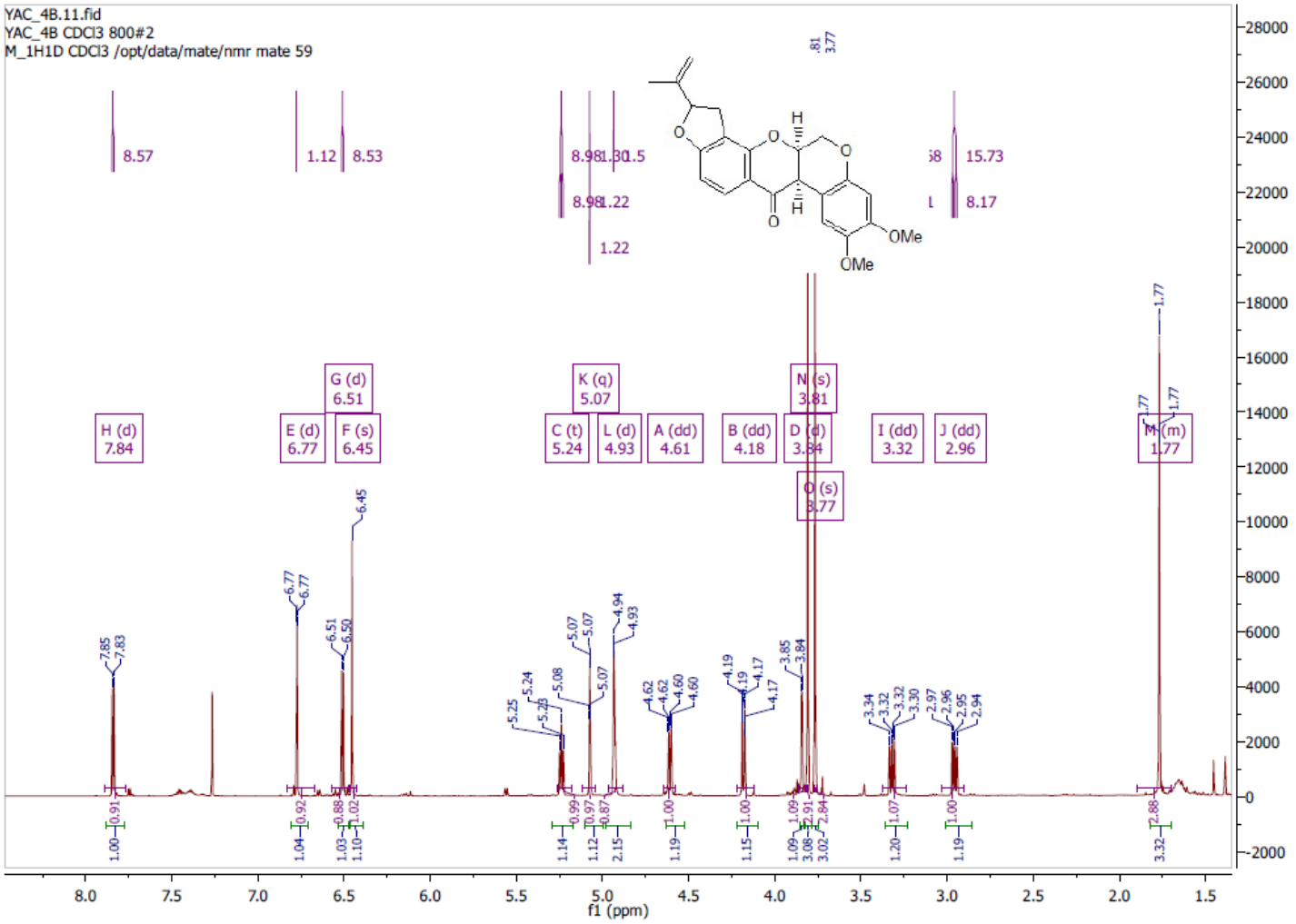
APPENDIX 35E: HMBC Spectrum of Rhocarpin (188) ($CDCl_3$)



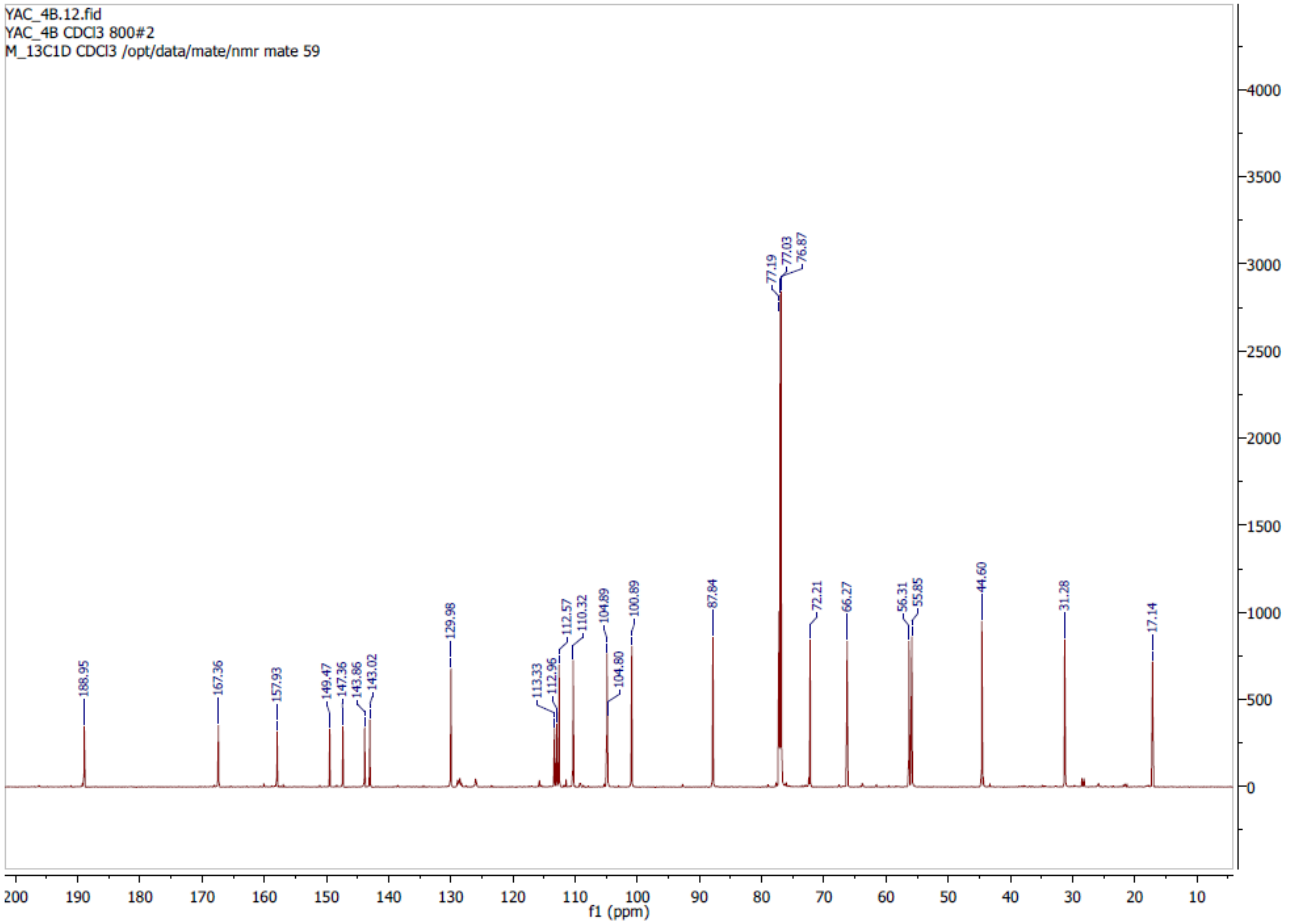
APPENDIX 35E: HRMS Spectrum of Rhocarpin (188) ($CDCl_3$)



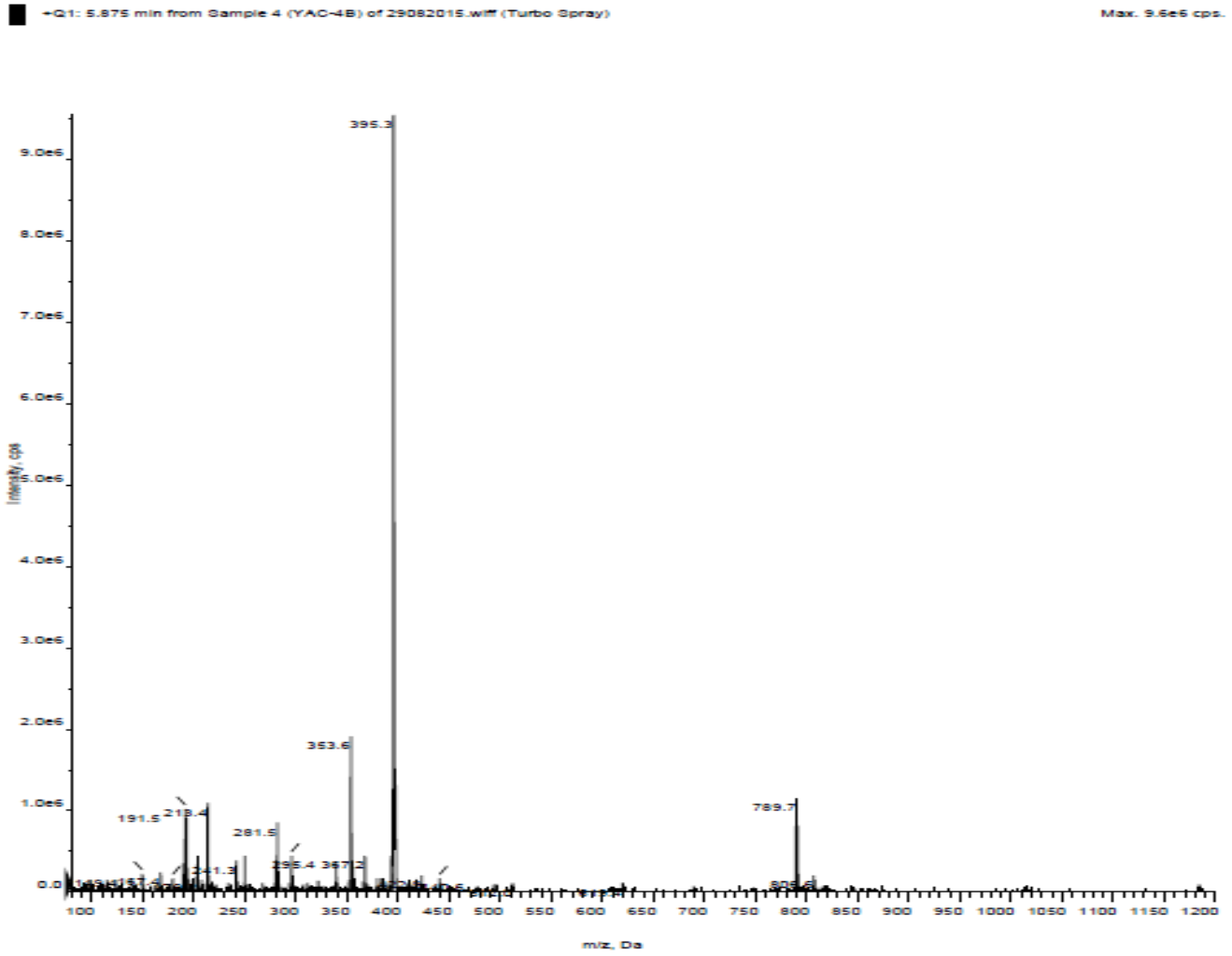
APPENDIX 36A: ^{1}H NMR Spectrum of Rotenone (186) (800 MHz; $CDCl_3$)



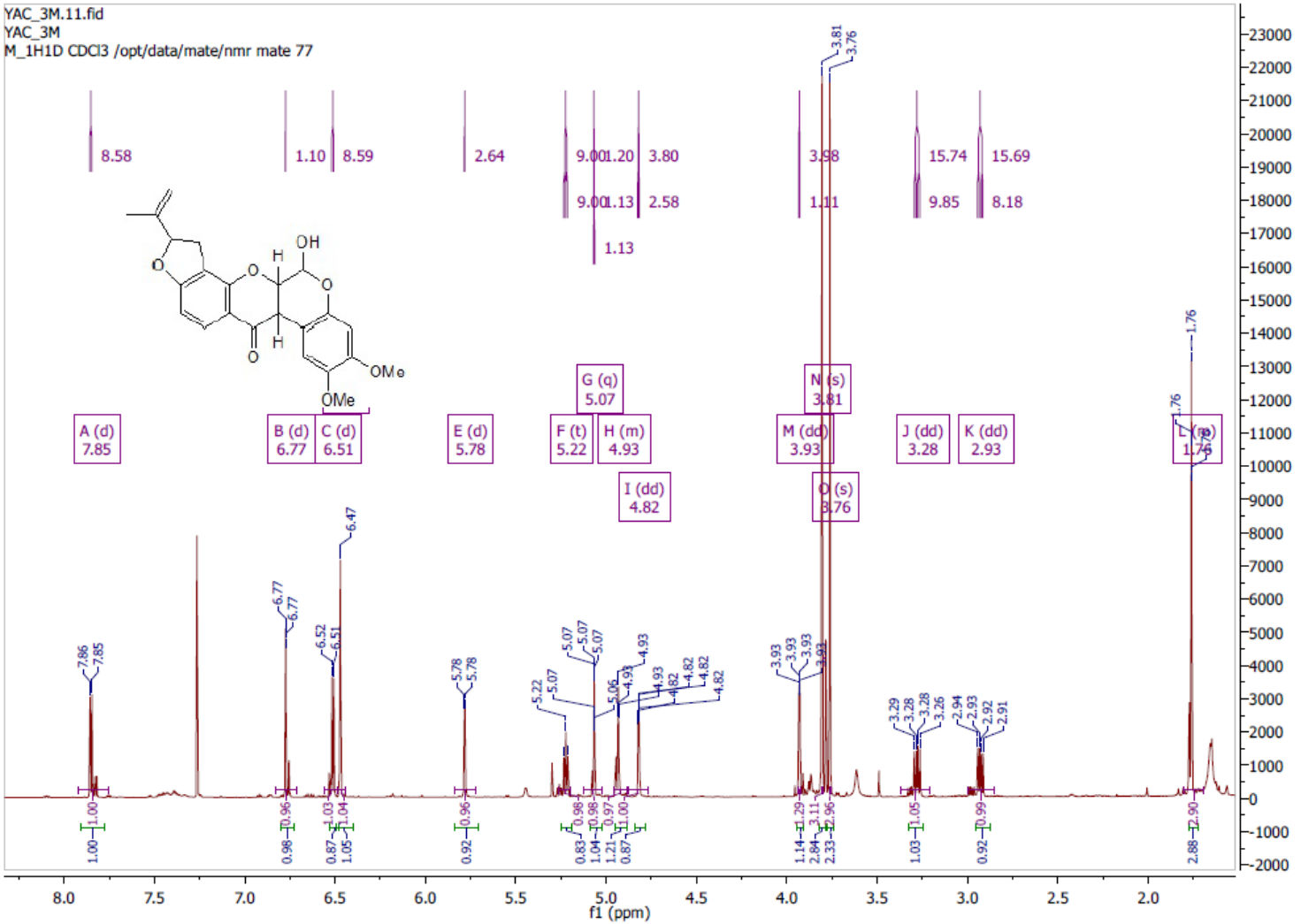
APPENDIX 36B: ^{13}C NMR Spectrum of Rotenone (186) (200 MHz; $CDCl_3$)



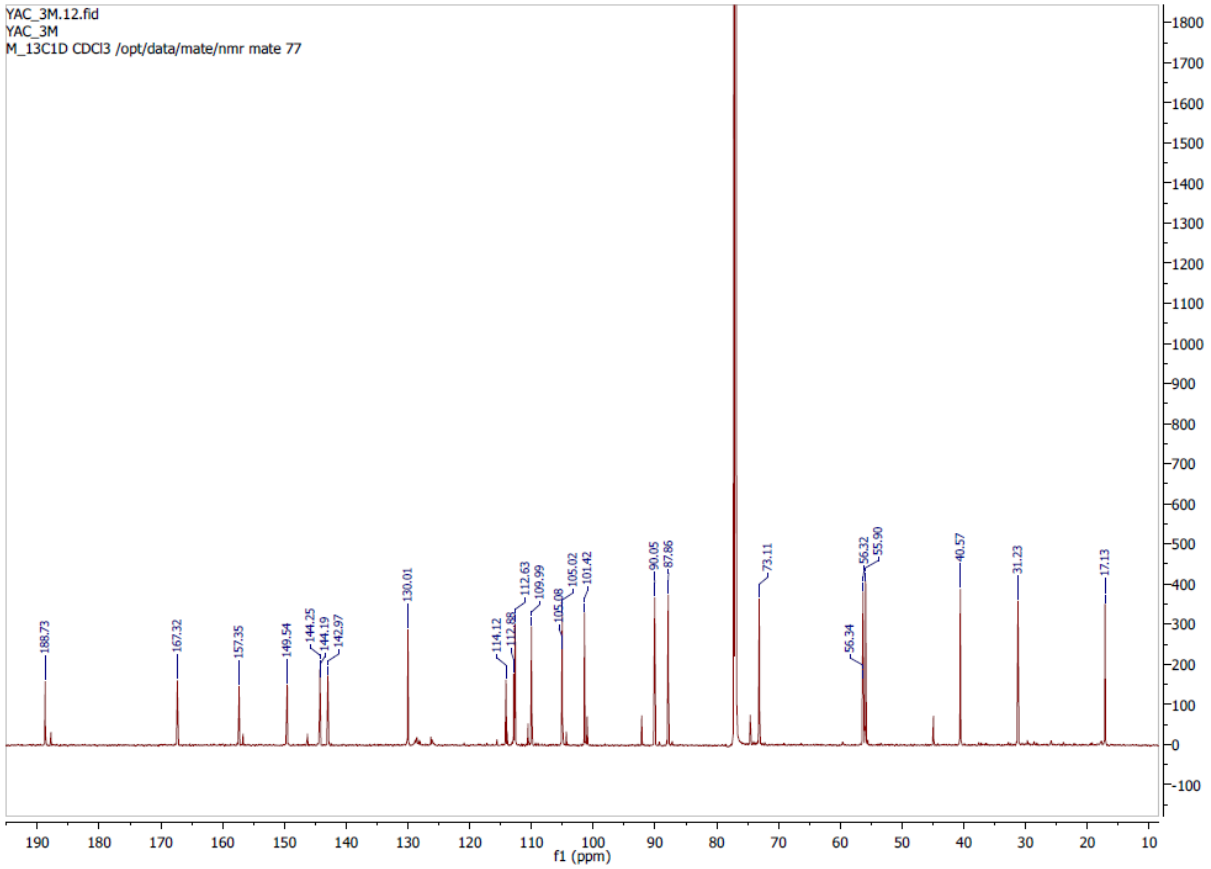
APPENDIX 36F: LCMS Spectrum of Rotenone (186) ($CDCl_3$)



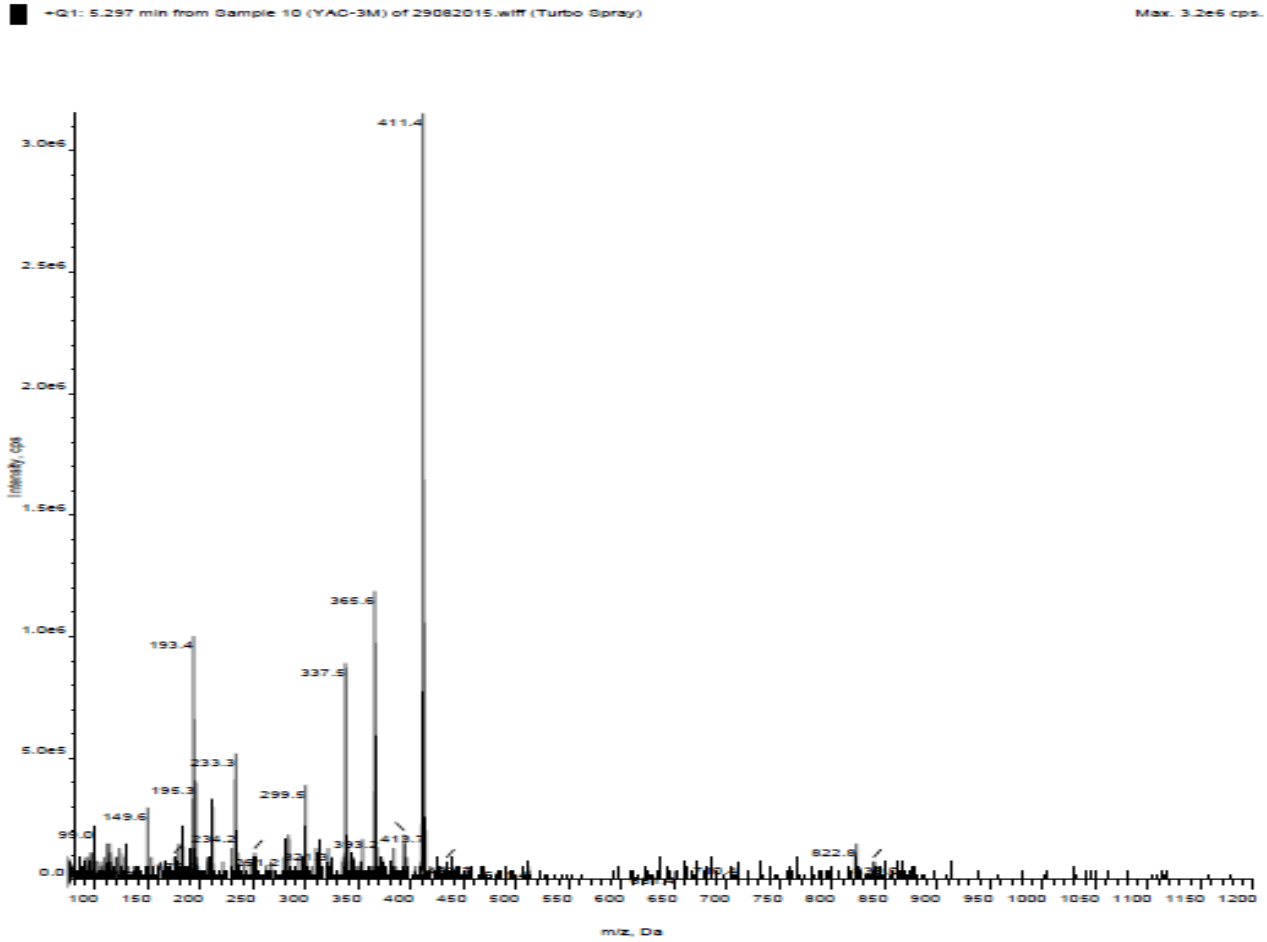
APPENDIX 37A: ^{1}H NMR Spectrum of 6-hydroxyrotenone (187) (800 MHz; $CDCl_3$)



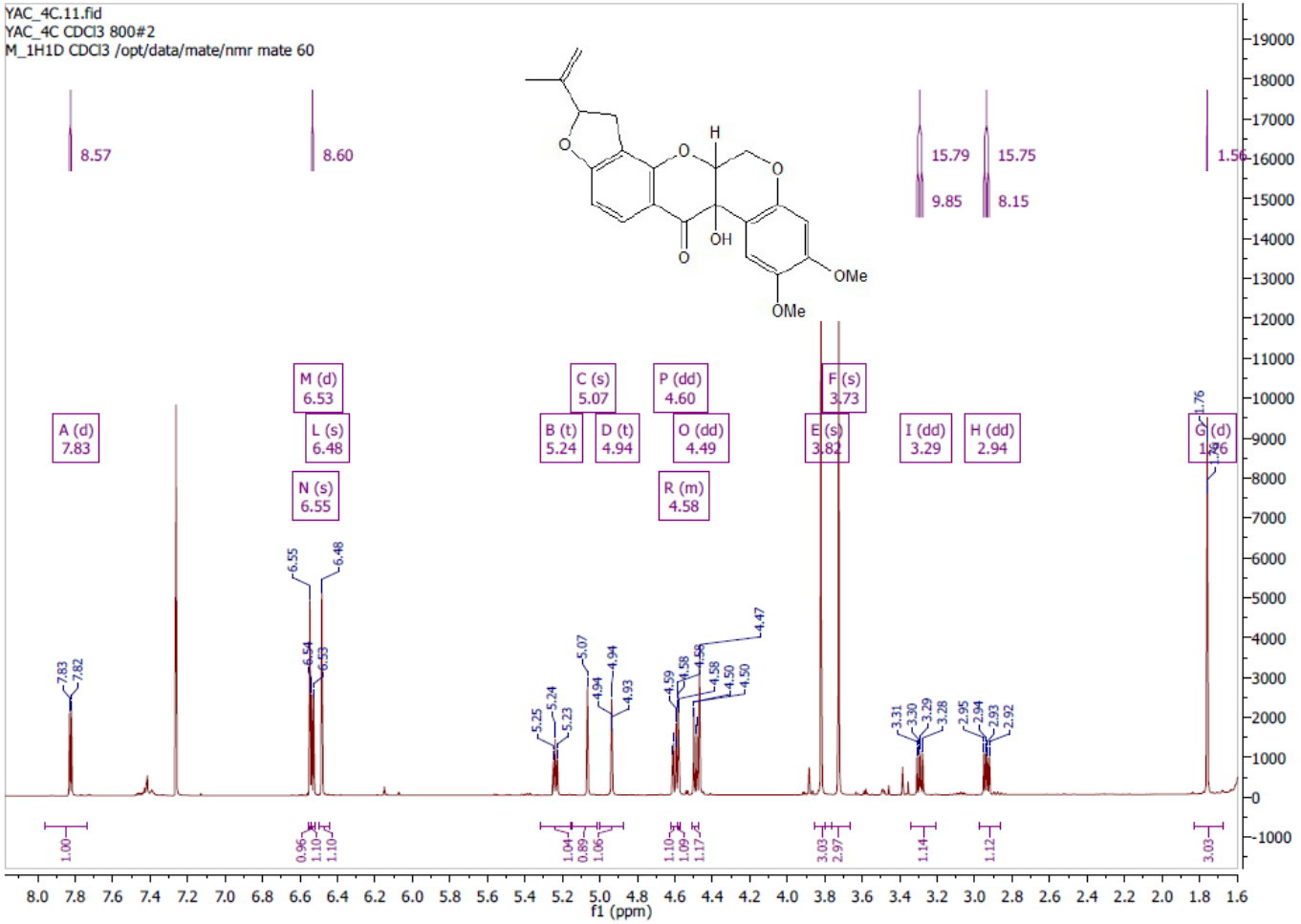
APPENDIX 37B: ^{13}C NMR Spectrum of 6-hydroxyrotenone (187) (200 MHz; $CDCl_3$)



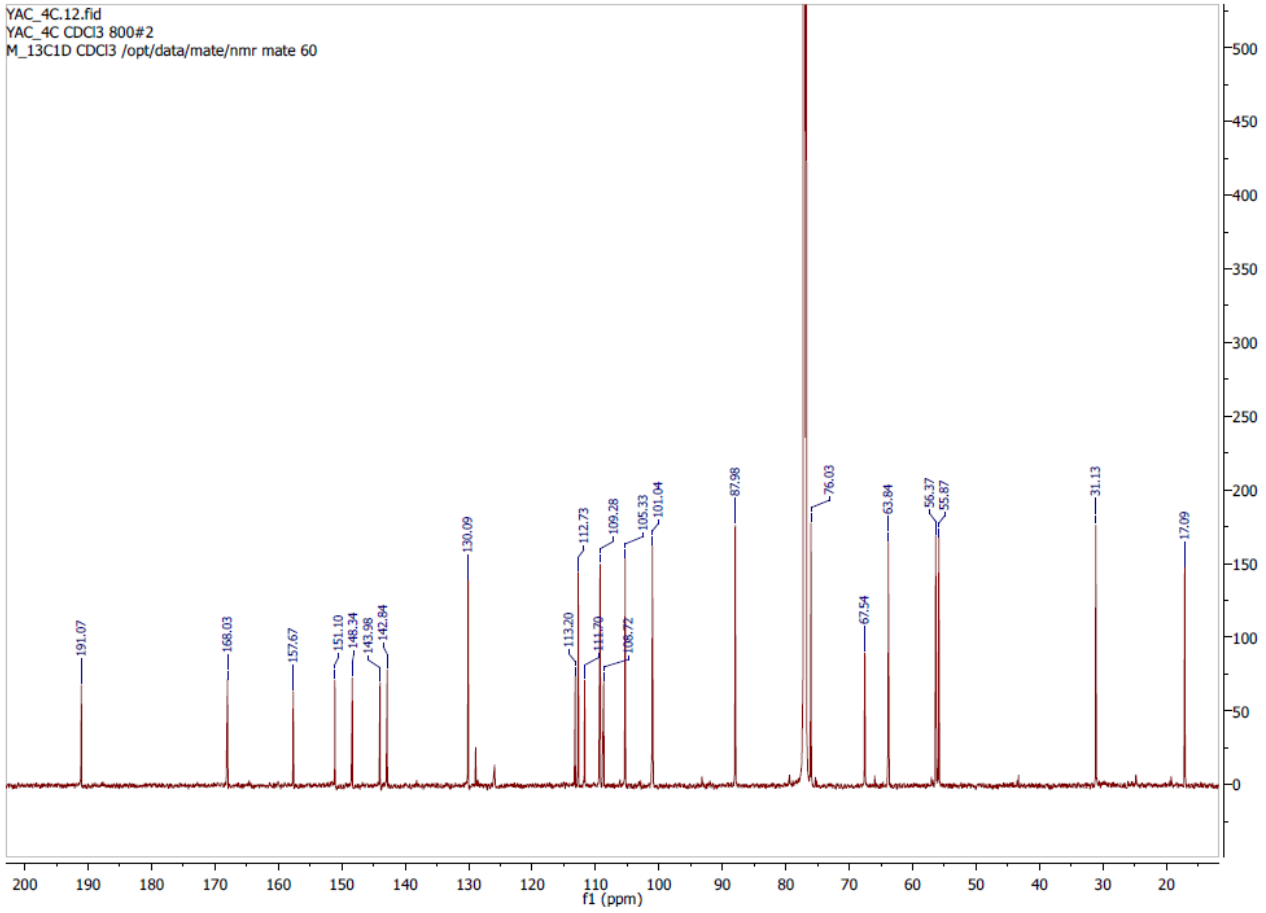
APPENDIX 37C: LCMS Spectrum of 6-hydroxyrotenone (187) ($CDCl_3$)



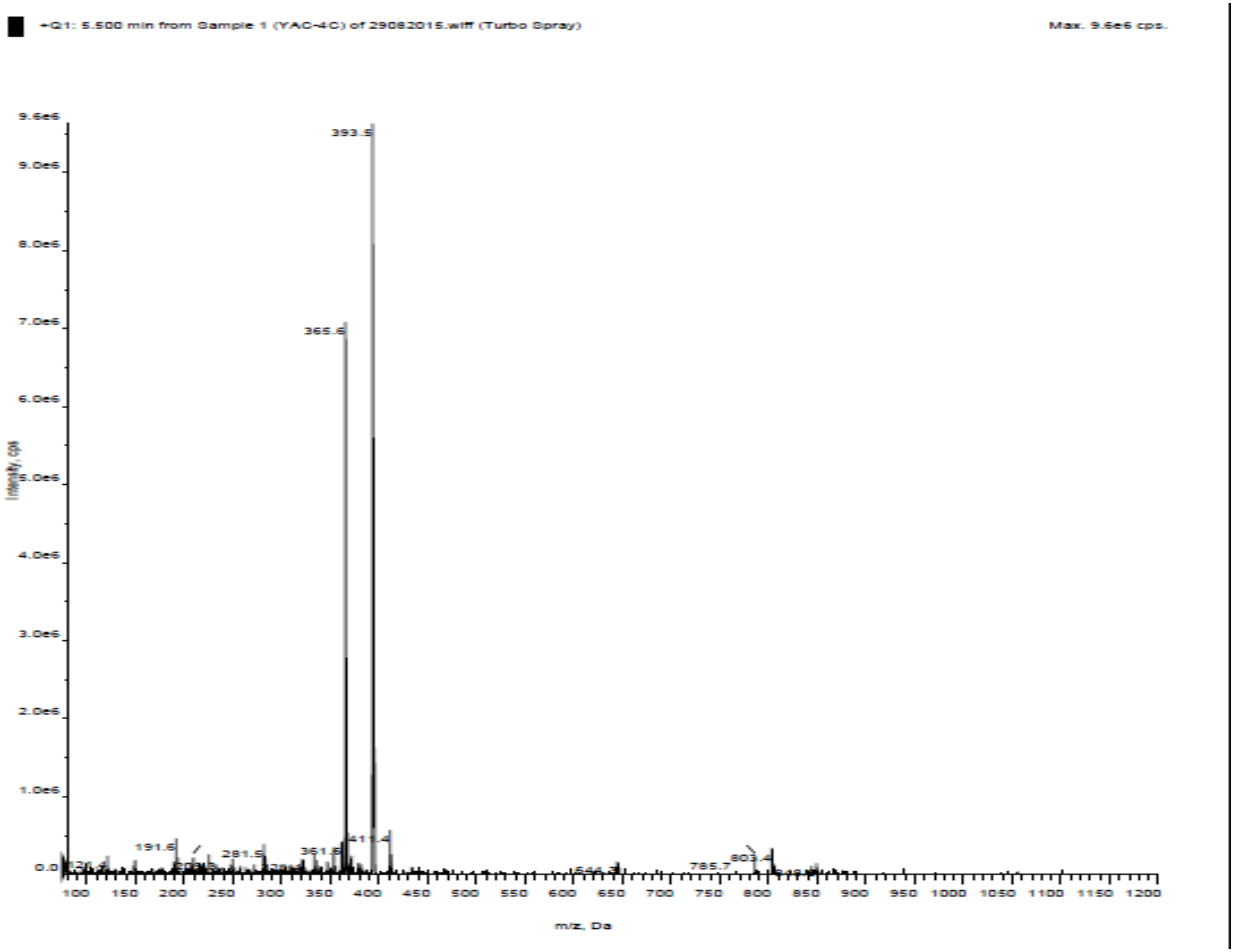
APPENDIX 38A: ^{1}H NMR Spectrum of 12a-hydroxyrotenone (129) (800 MHz; $CDCl_3$)



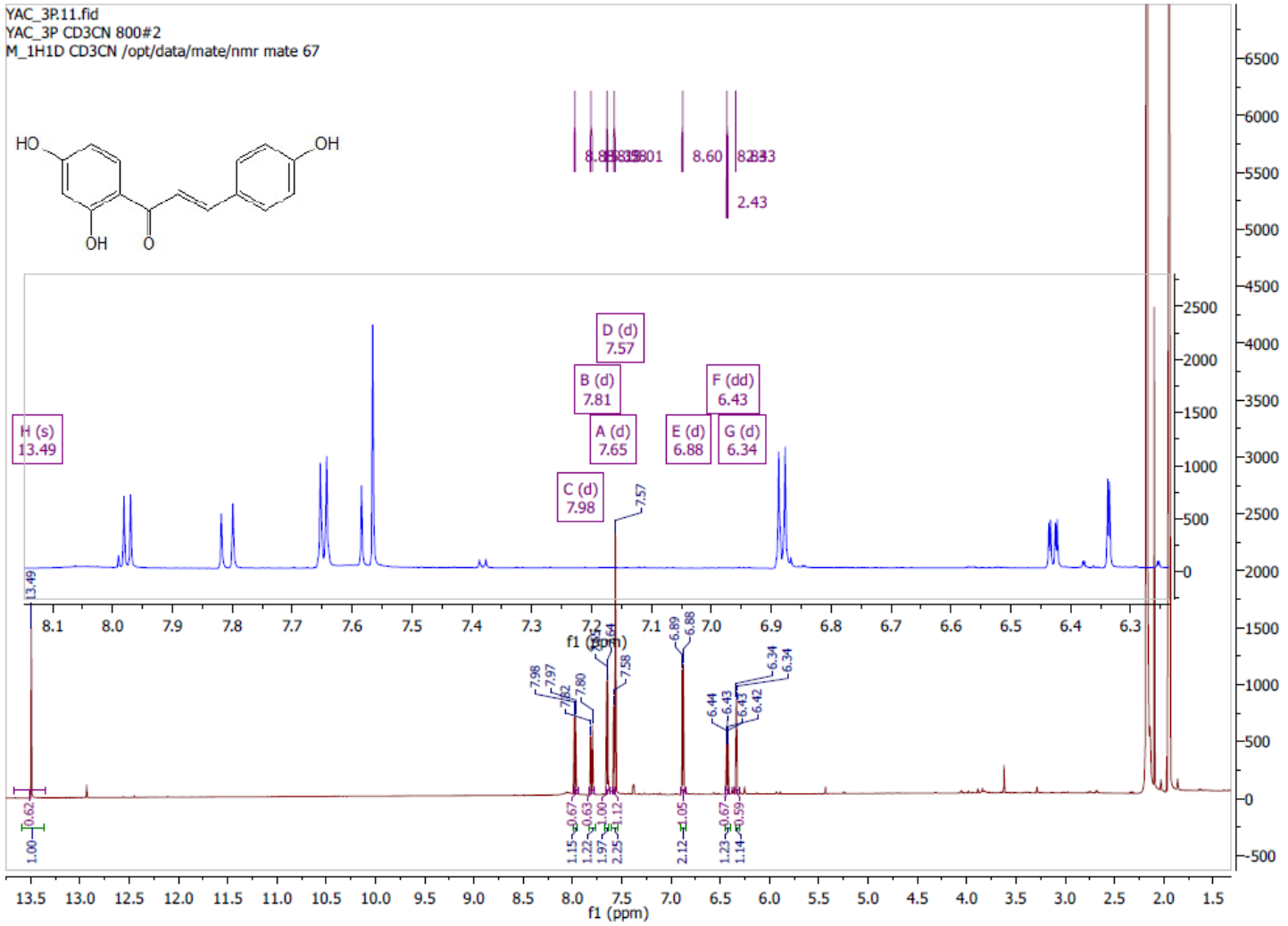
APPENDIX 38B: ^{13}C NMR Spectrum of 12a-hydroxyrotenone (129) (200 MHz; $CDCl_3$)



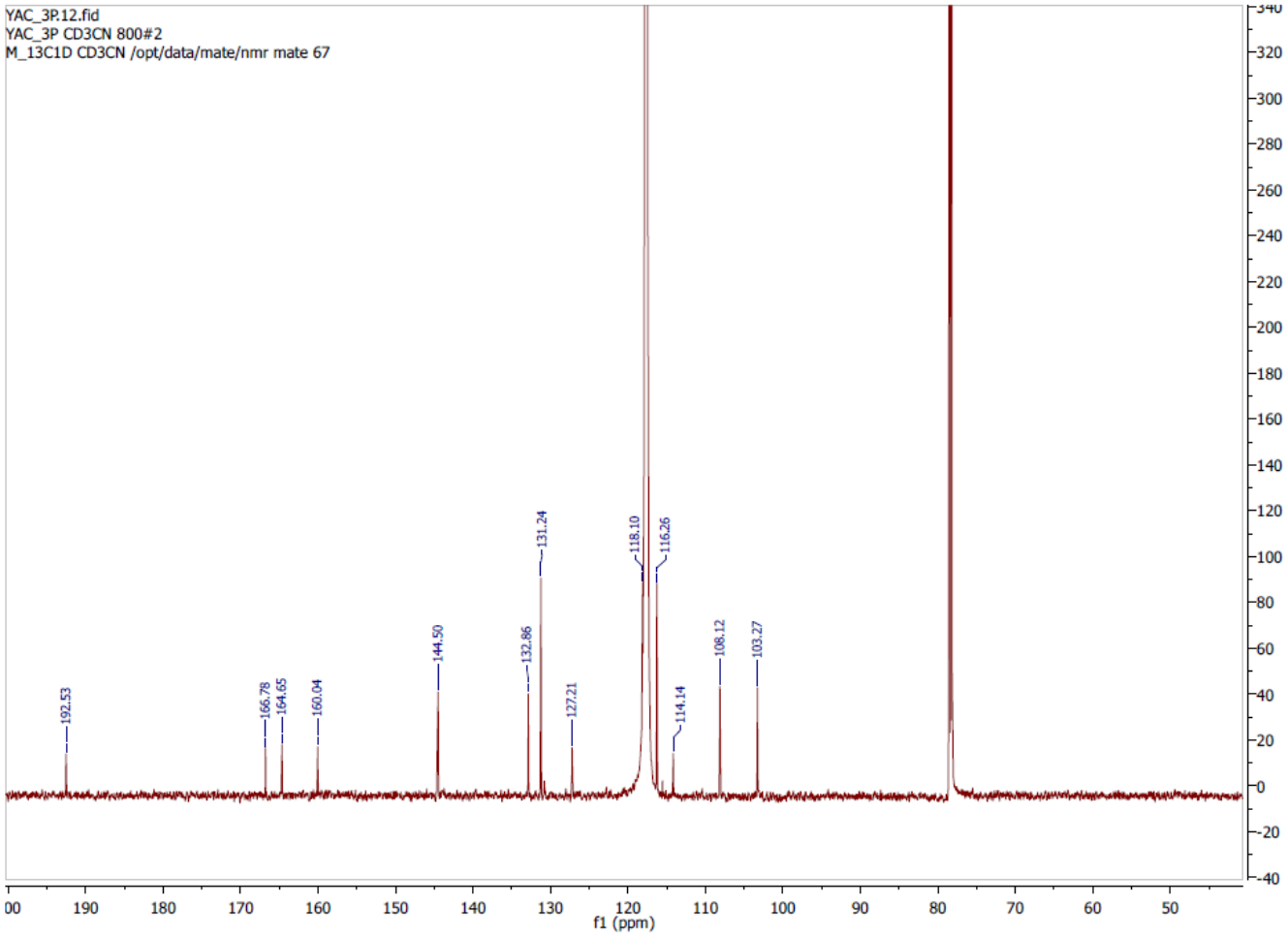
APPENDIX 38C: LCMS Spectrum of 12a-hydroxyrotenone (129)



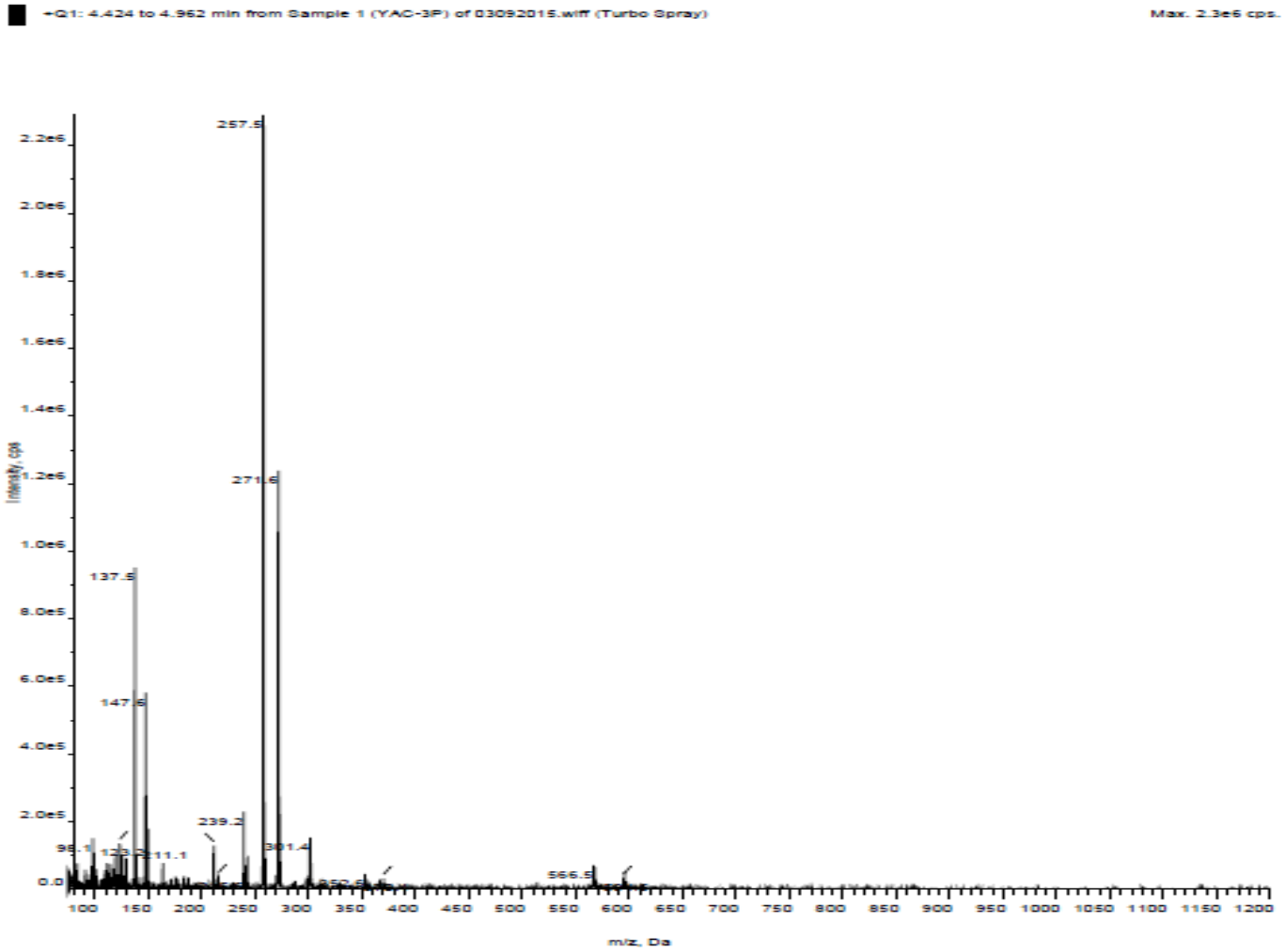
APPENDIX 39A: ^{1}H NMR Spectrum of Isoliquirtigenin (190) (800 MHz; $CDCl_3$)



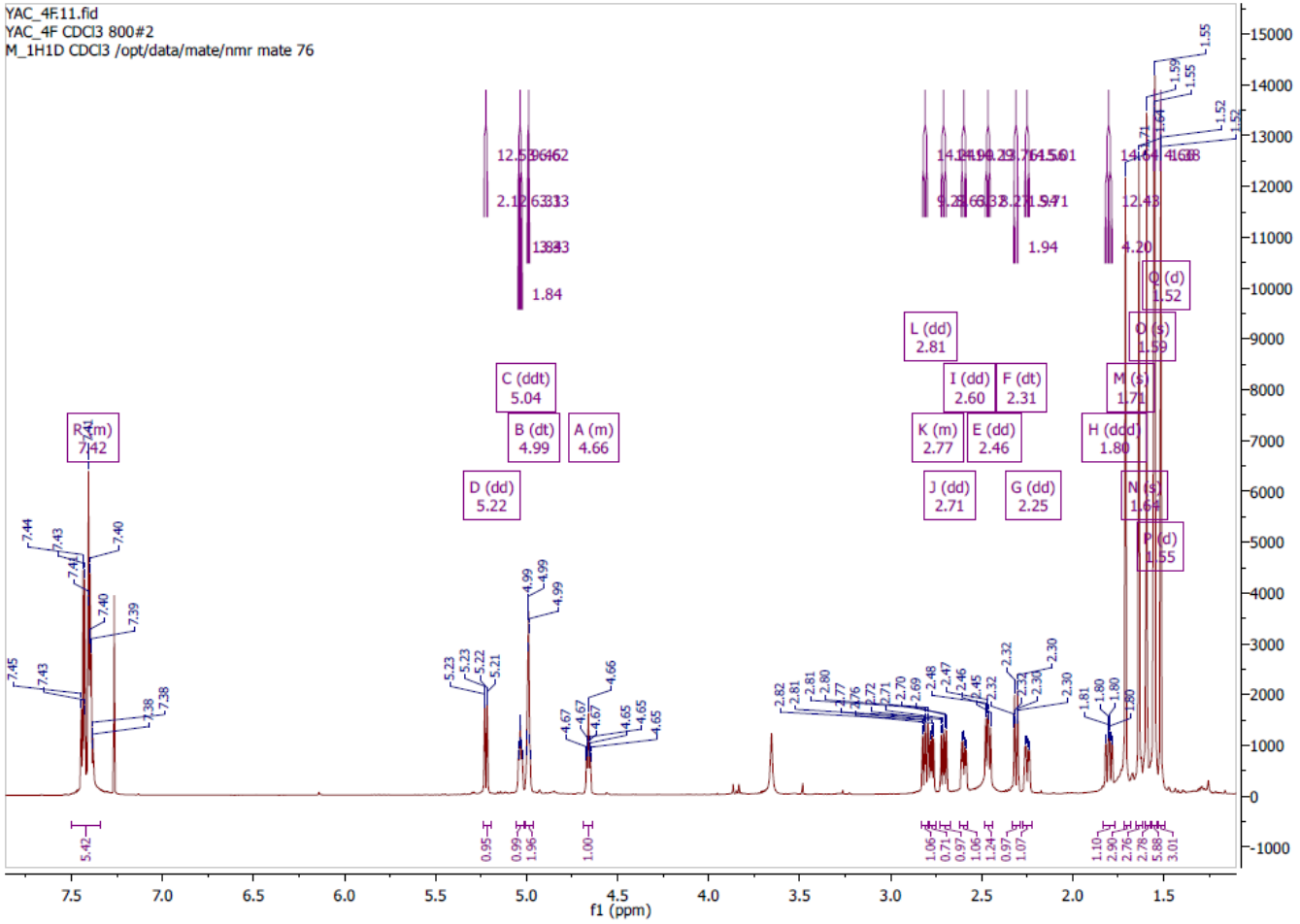
APPENDIX 39B: ^{13}C NMR Spectrum of Isoliquirtigenin (190) (800 MHz; $CDCl_3$)



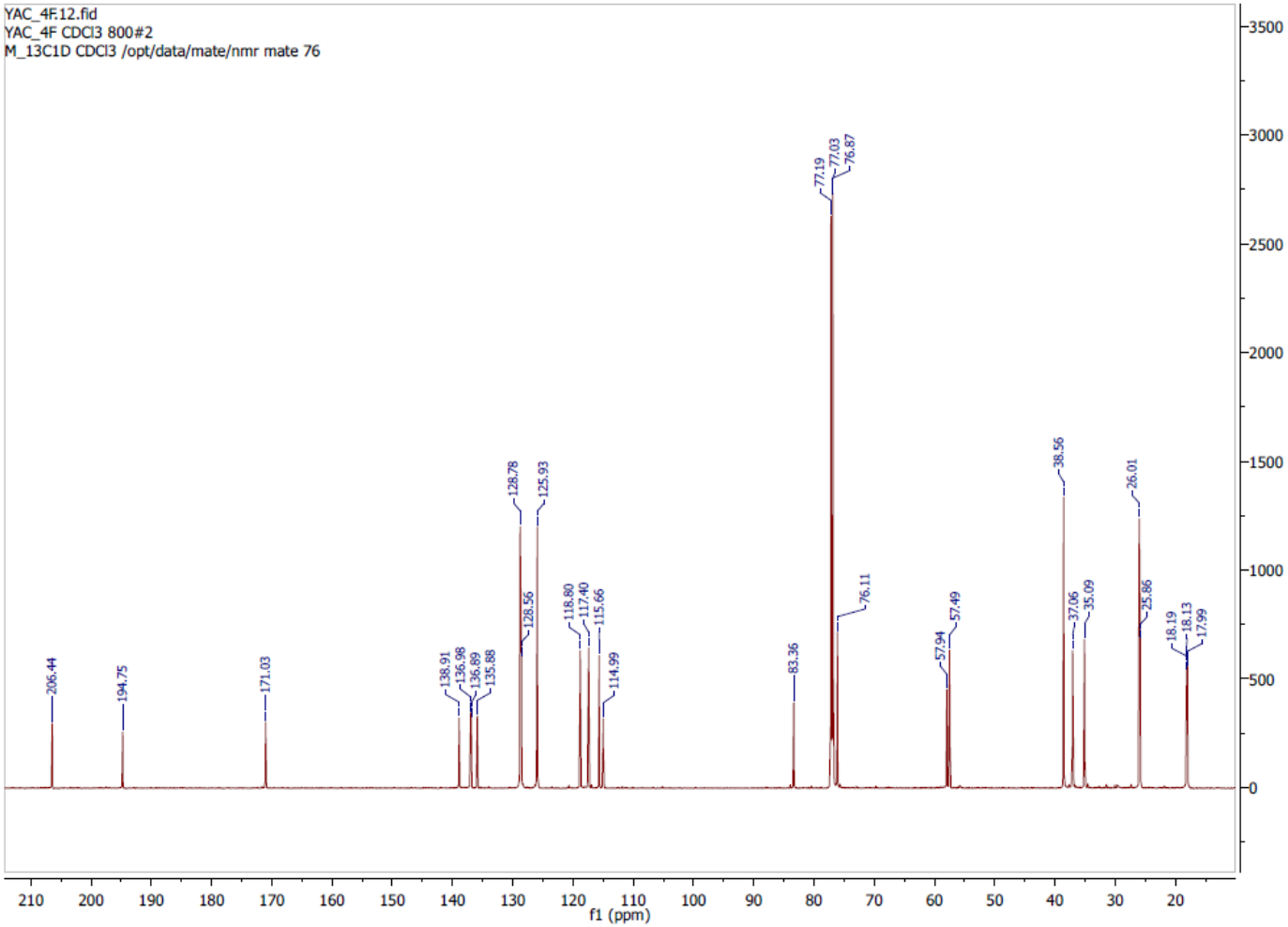
APPENDIX 39CE: LCMS Spectrum of Isoliquirtigenin (190) ($CDCl_3$)



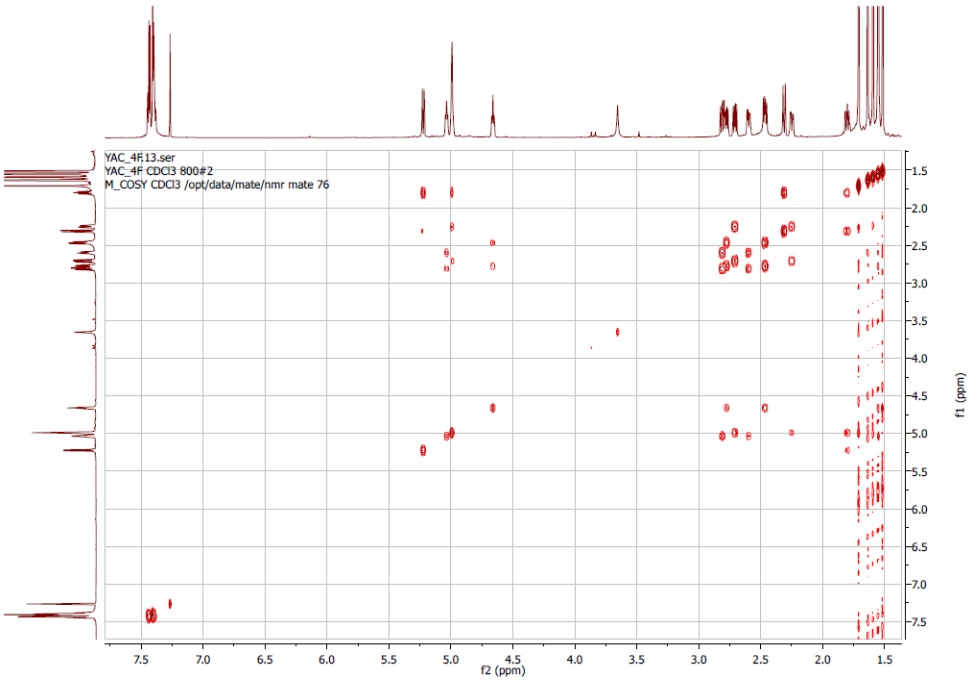
APPENDIX 40A: ^{1}H NMR Spectrum of Rhodiflavan A (191) (800 MHz; $CDCl_3$)



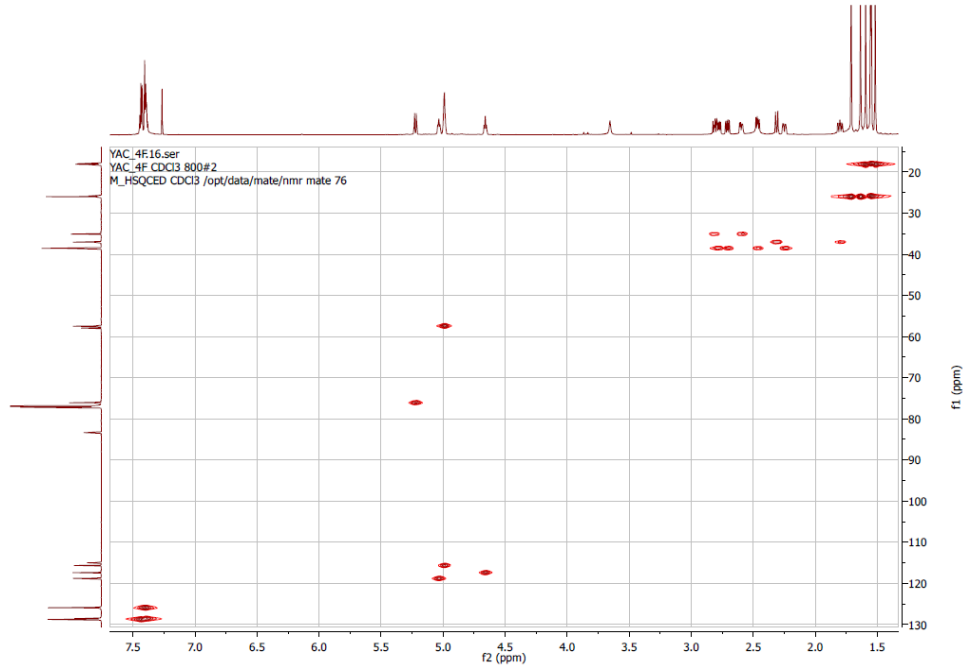
APPENDIX 40B: ^{13}C NMR Spectrum of Rhodiflavan A (191) (200 MHz; $CDCl_3$)



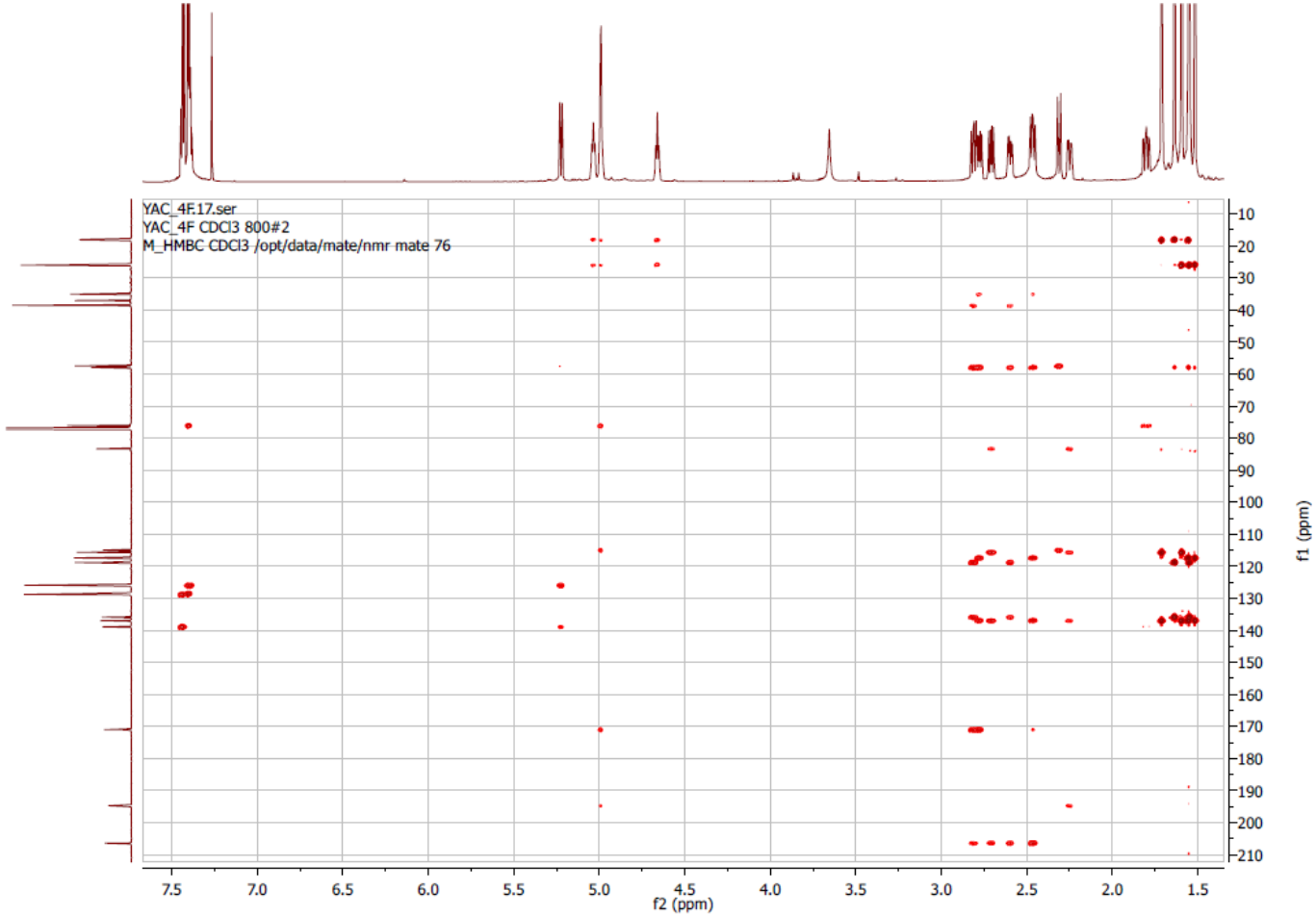
APPENDIX 40C: HH-COSY Spectrum of Rhodiflavan A (191) ($CDCl_3$)



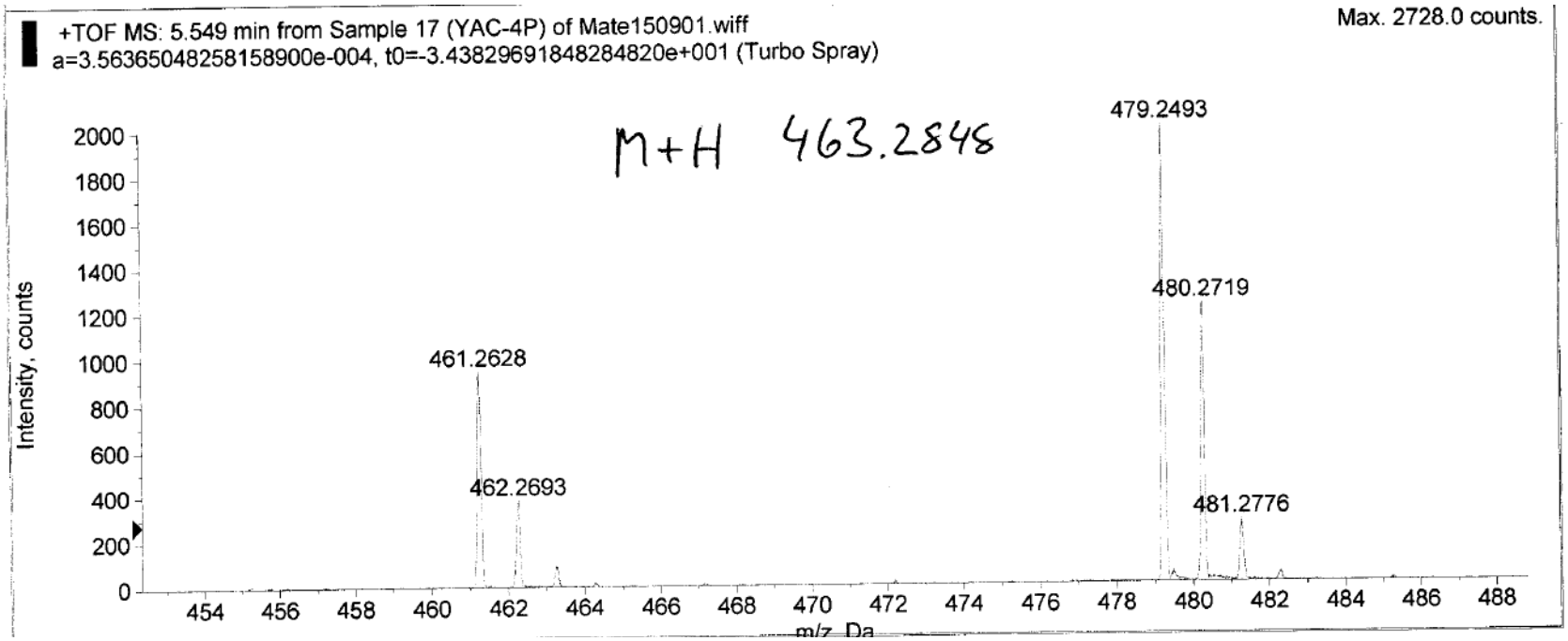
APPENDIX 40D: HSQC Spectrum of Rhodiflavan A (191) ($CDCl_3$)



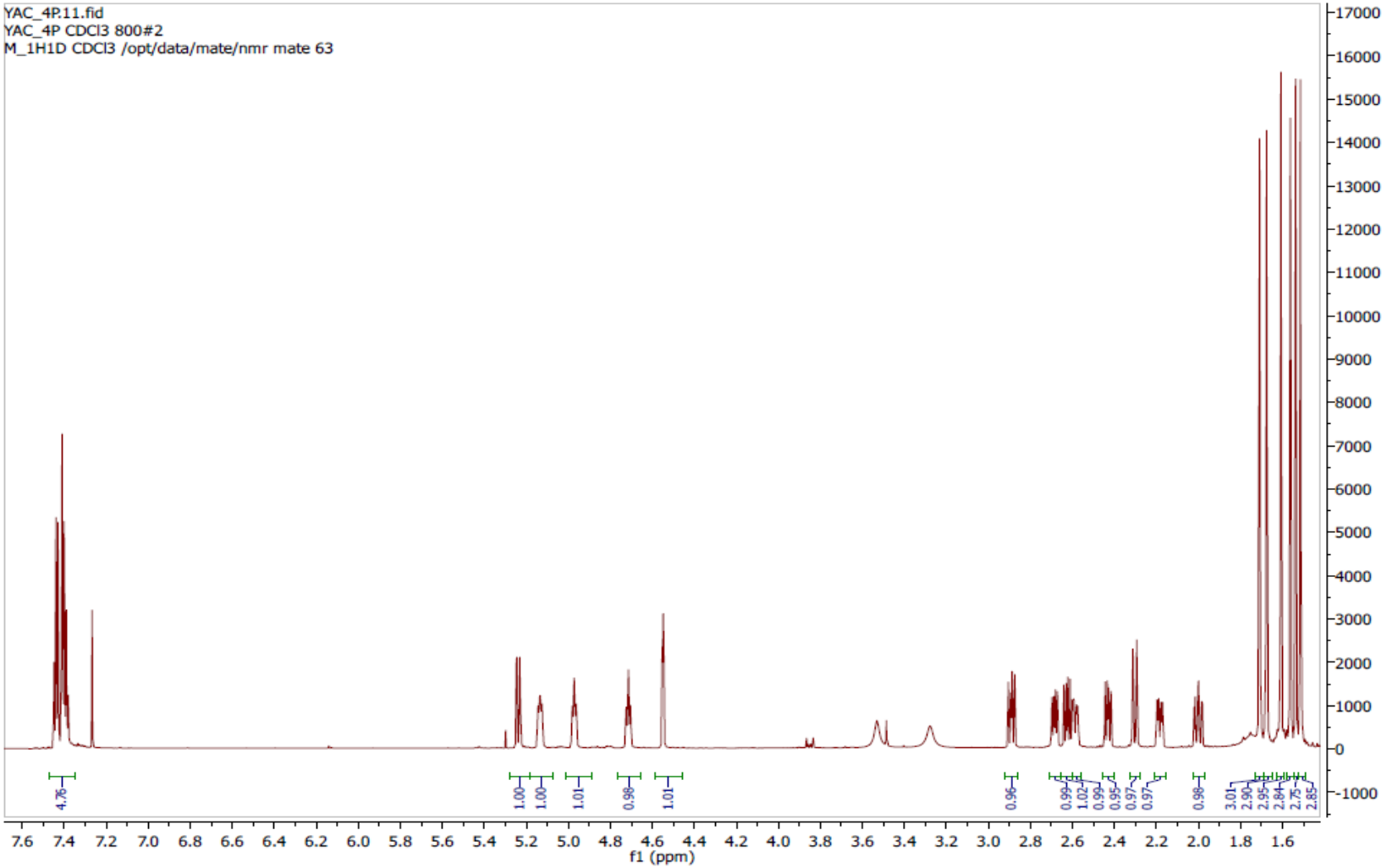
APPENDIX 40E: HMBC Spectrum of Rhodiflavan A (191) ($CDCl_3$)



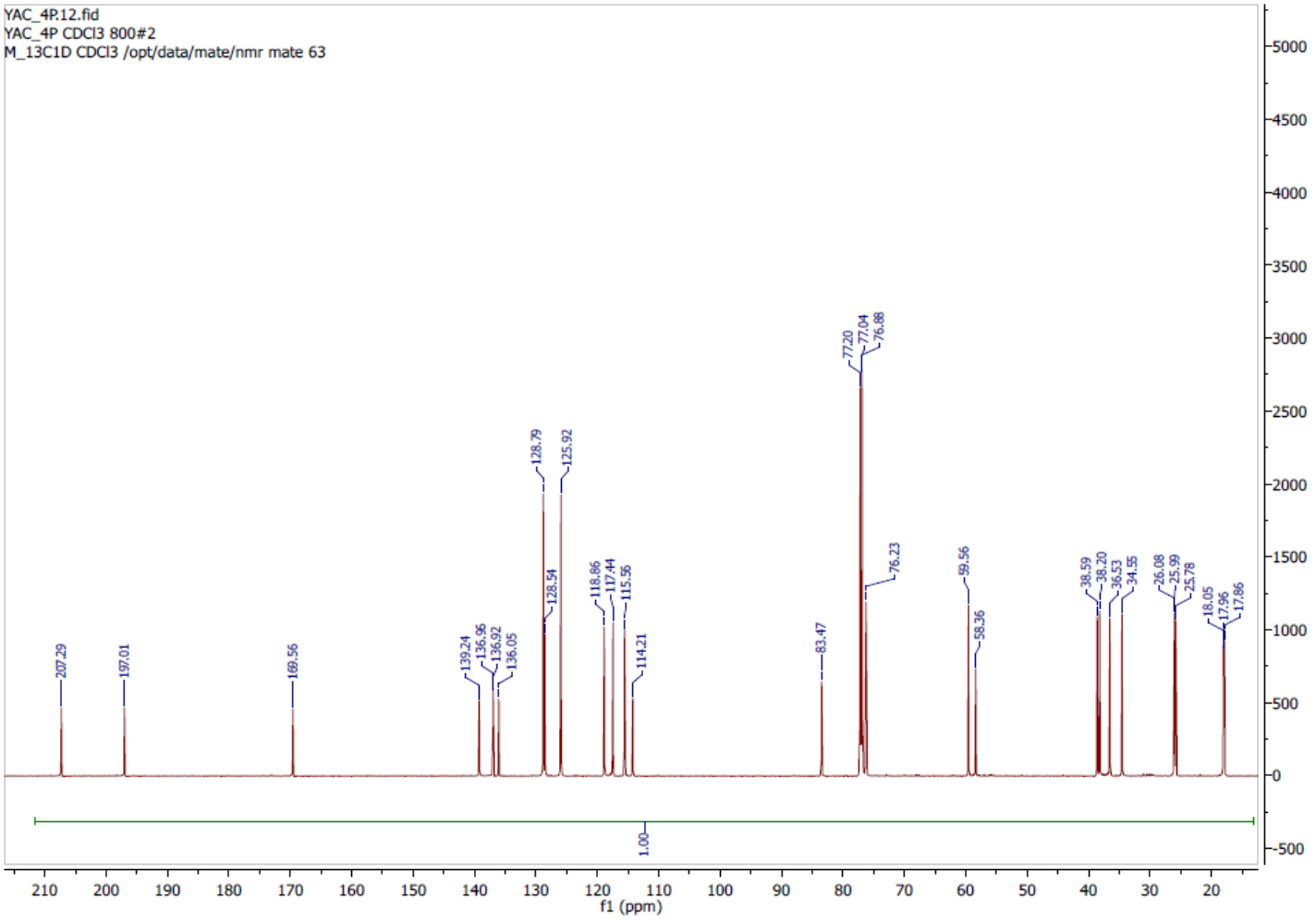
APPENDIX 40E: HRMS Spectrum of Rhodiflavan A (191)



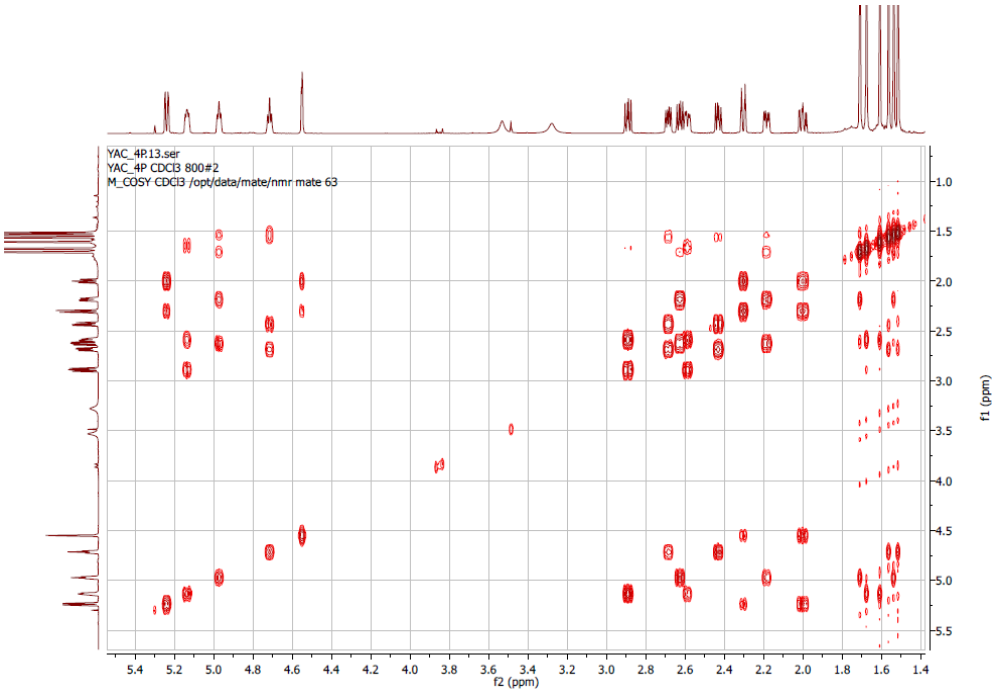
APPENDIX 41A: ^{1}H NMR Spectrum of Rhodiflavan B (192) (800 MHz; $CDCl_3$)



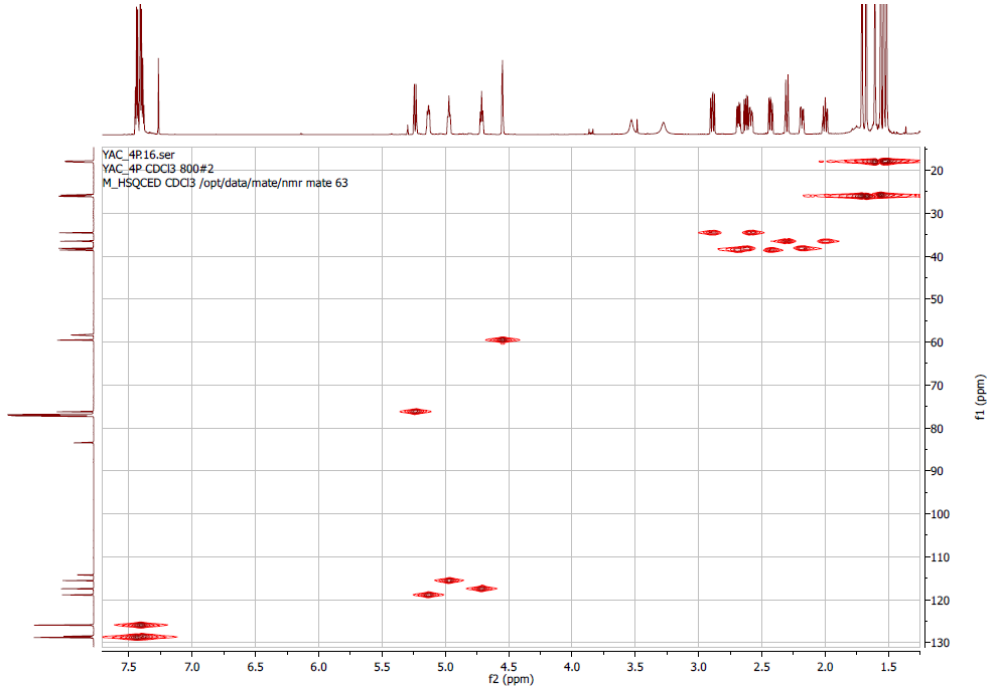
APPENDIX 41B: ^{13}C NMR Spectrum of Rhodiflavan B (192) (800 MHz; $CDCl_3$)



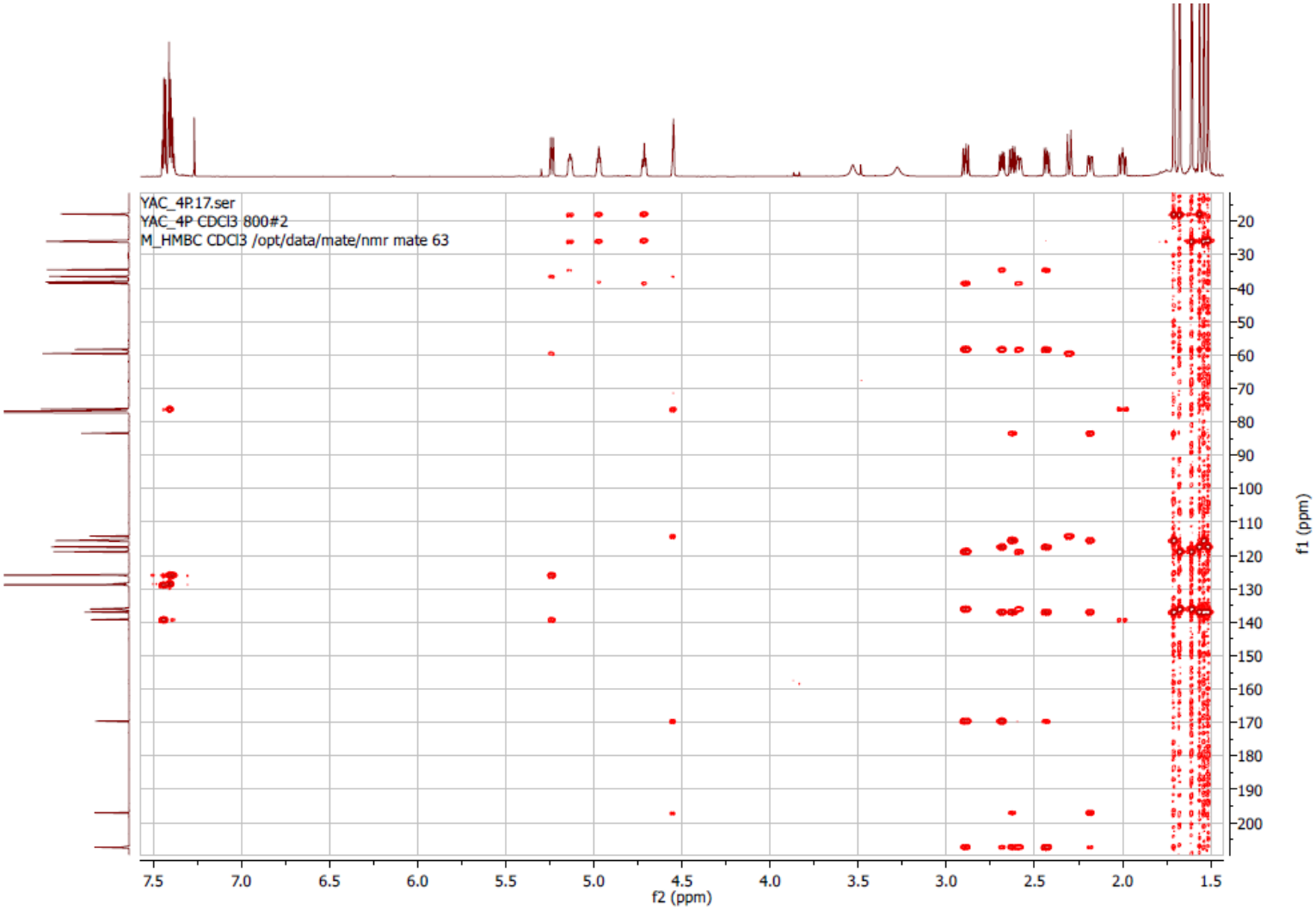
APPENDIX 41C: HH-COSY Spectrum of Rhodiflavan B (192) ($CDCl_3$)



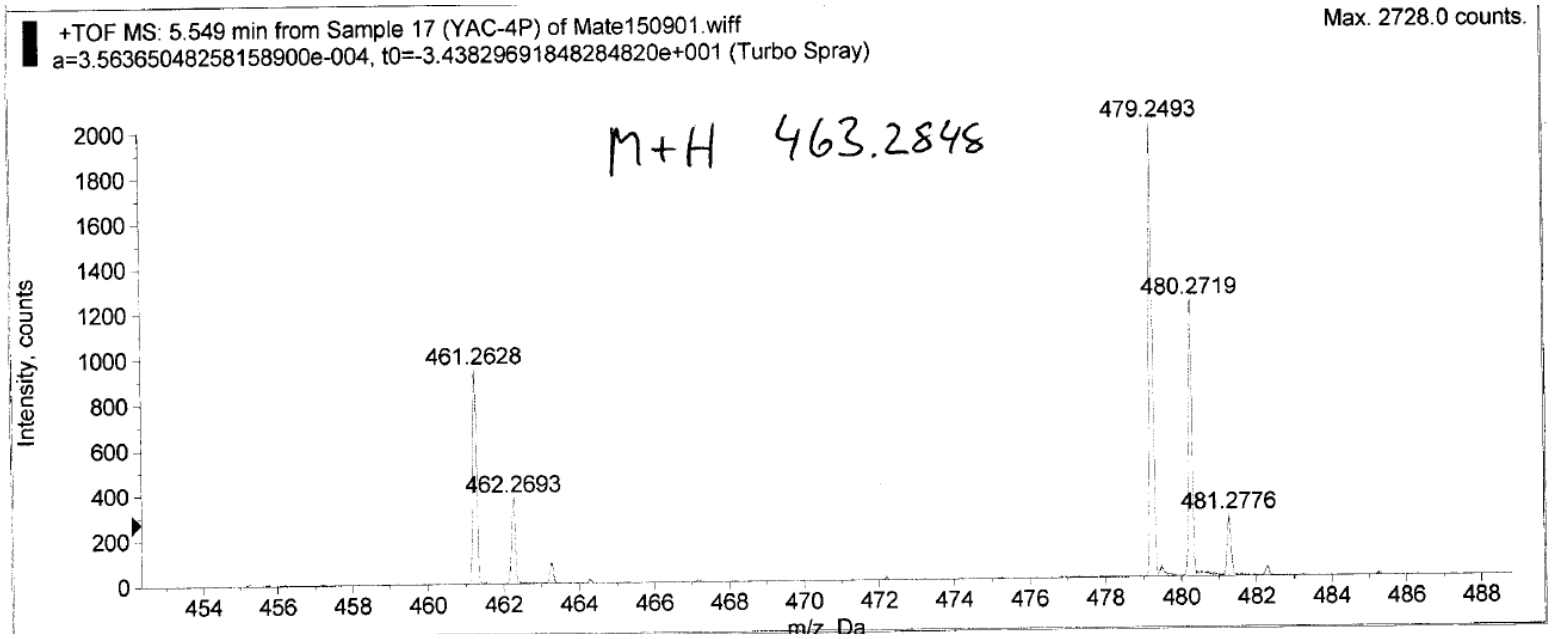
APPENDIX 41D: HSQC Spectrum of Rhodiflavan B (192) ($CDCl_3$)



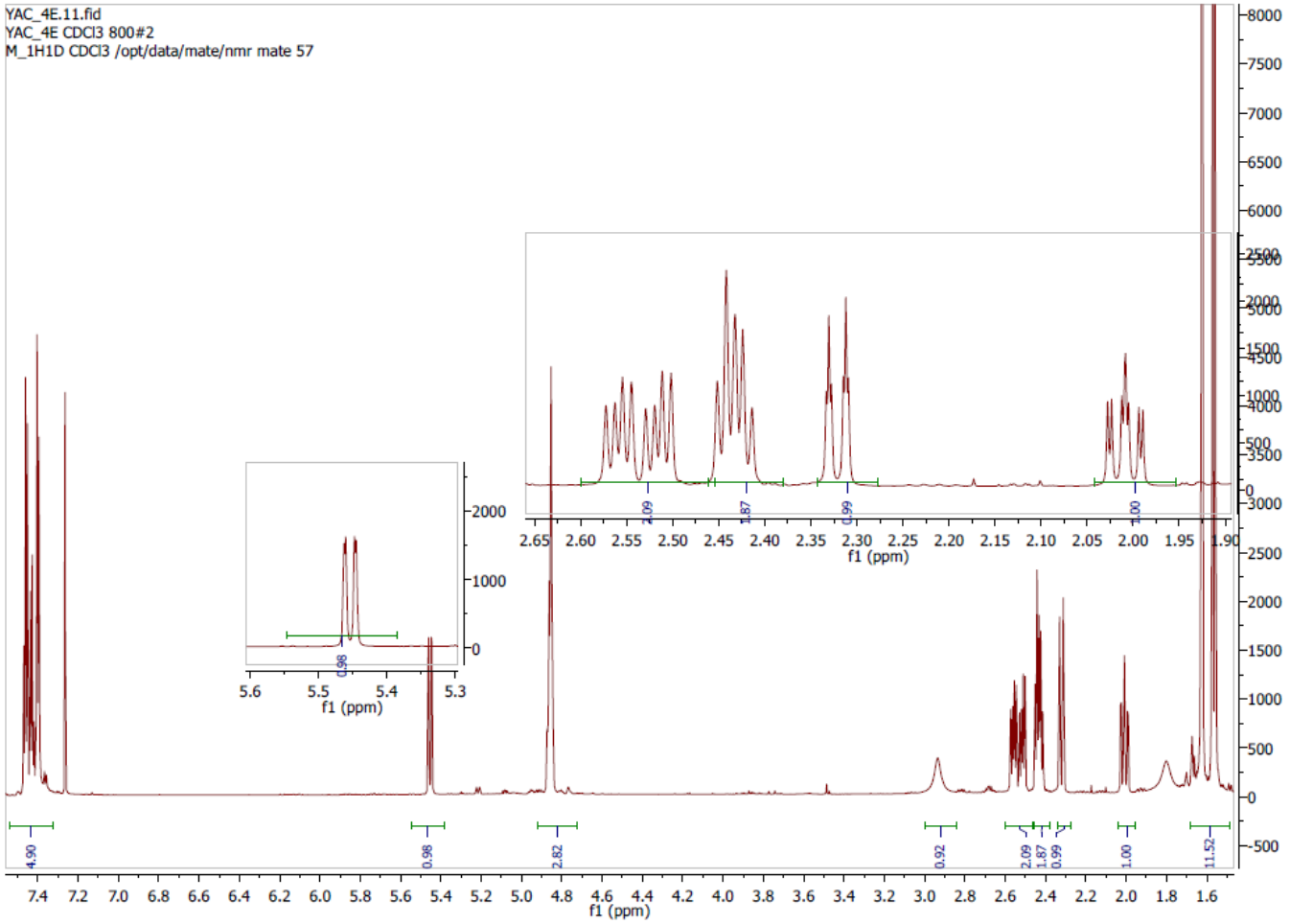
APPENDIX 41E: HMBC Spectrum of Rhodiflavan B (192) ($CDCl_3$)



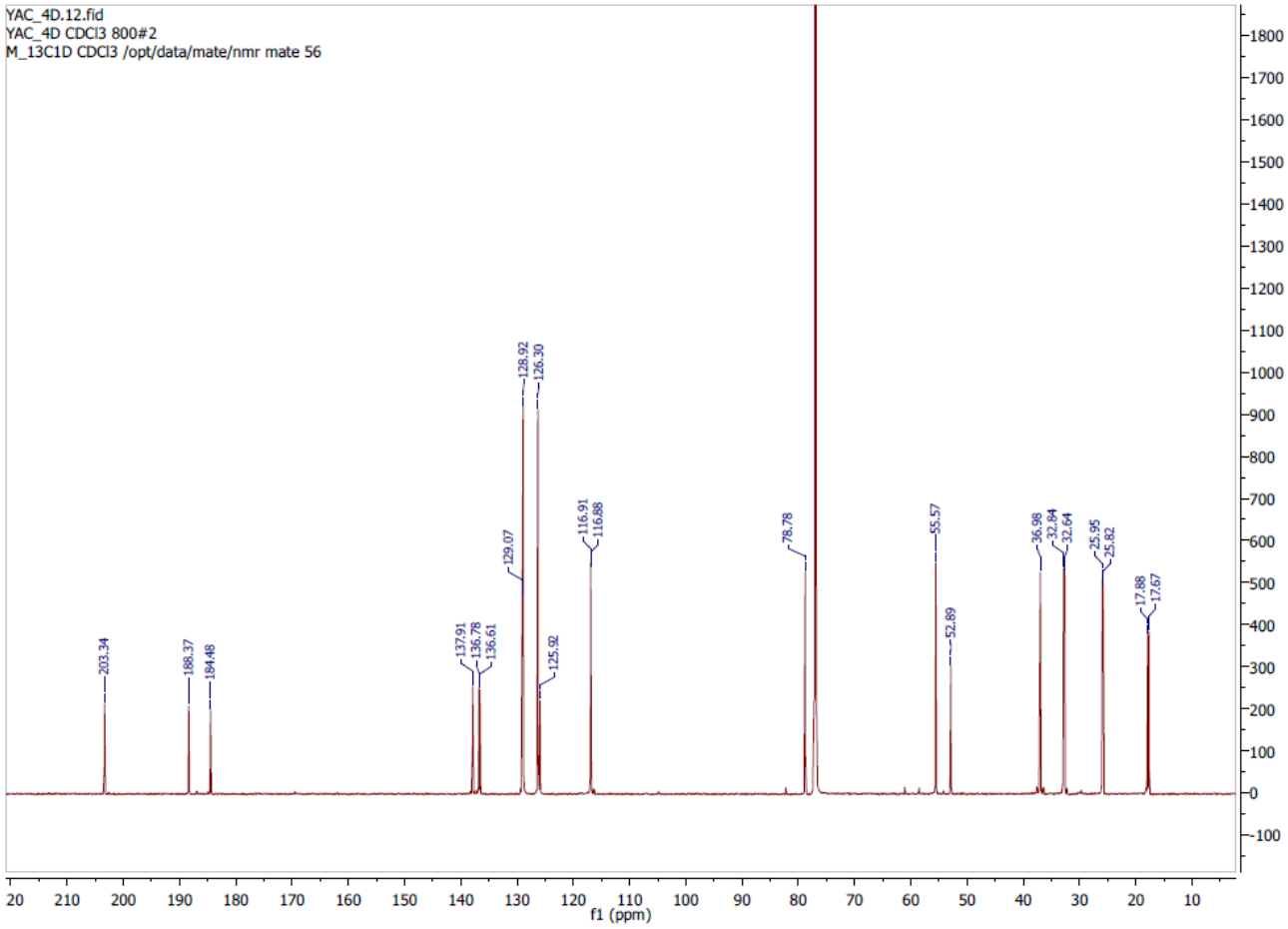
APPENDIX 41F: HMBC Spectrum of Rhodiflavan B (192) ($CDCl_3$)



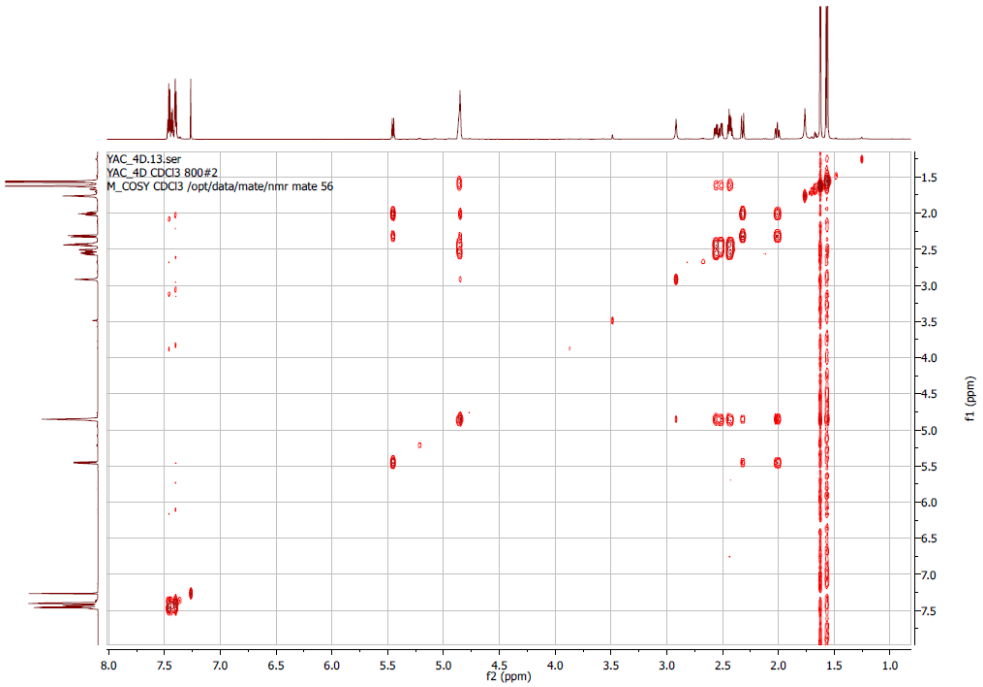
APPENDIX 42A: ^{1}H NMR Spectrum of Rhodiflavan C (193) (800 MHz; $CDCl_3$)



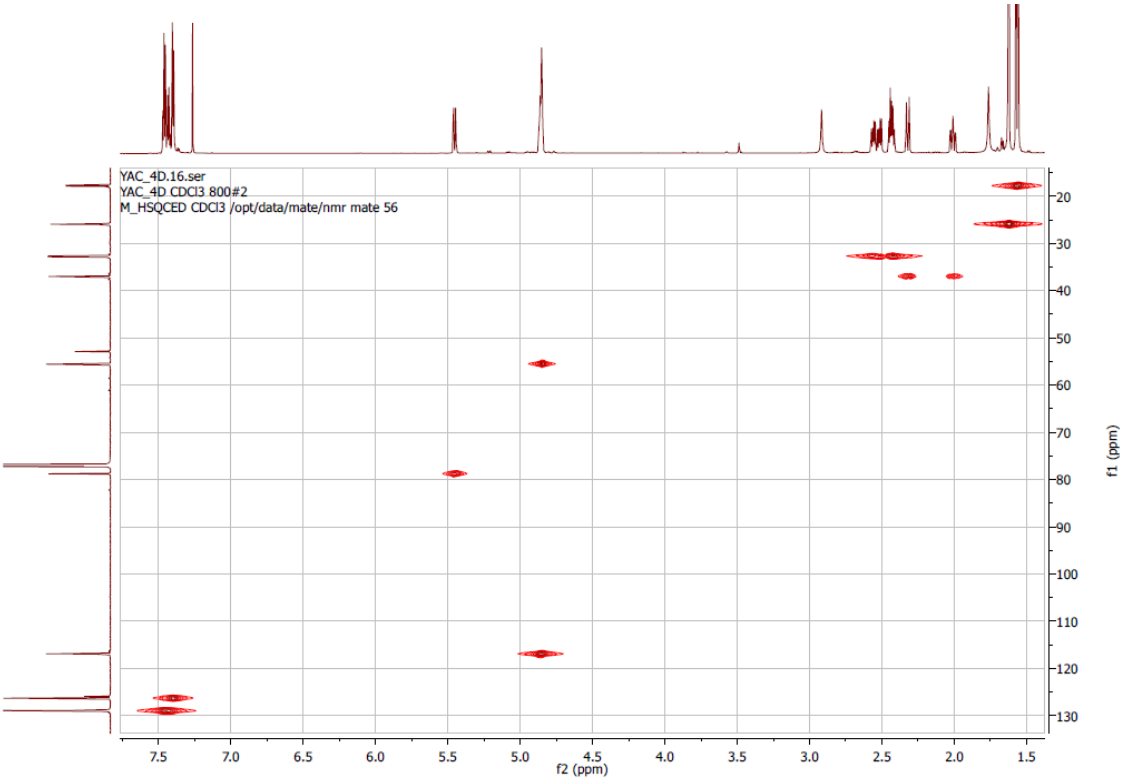
APPENDIX 42B: ^{13}C NMR Spectrum of Rhodiflavan C (193) (200 MHz; $CDCl_3$)



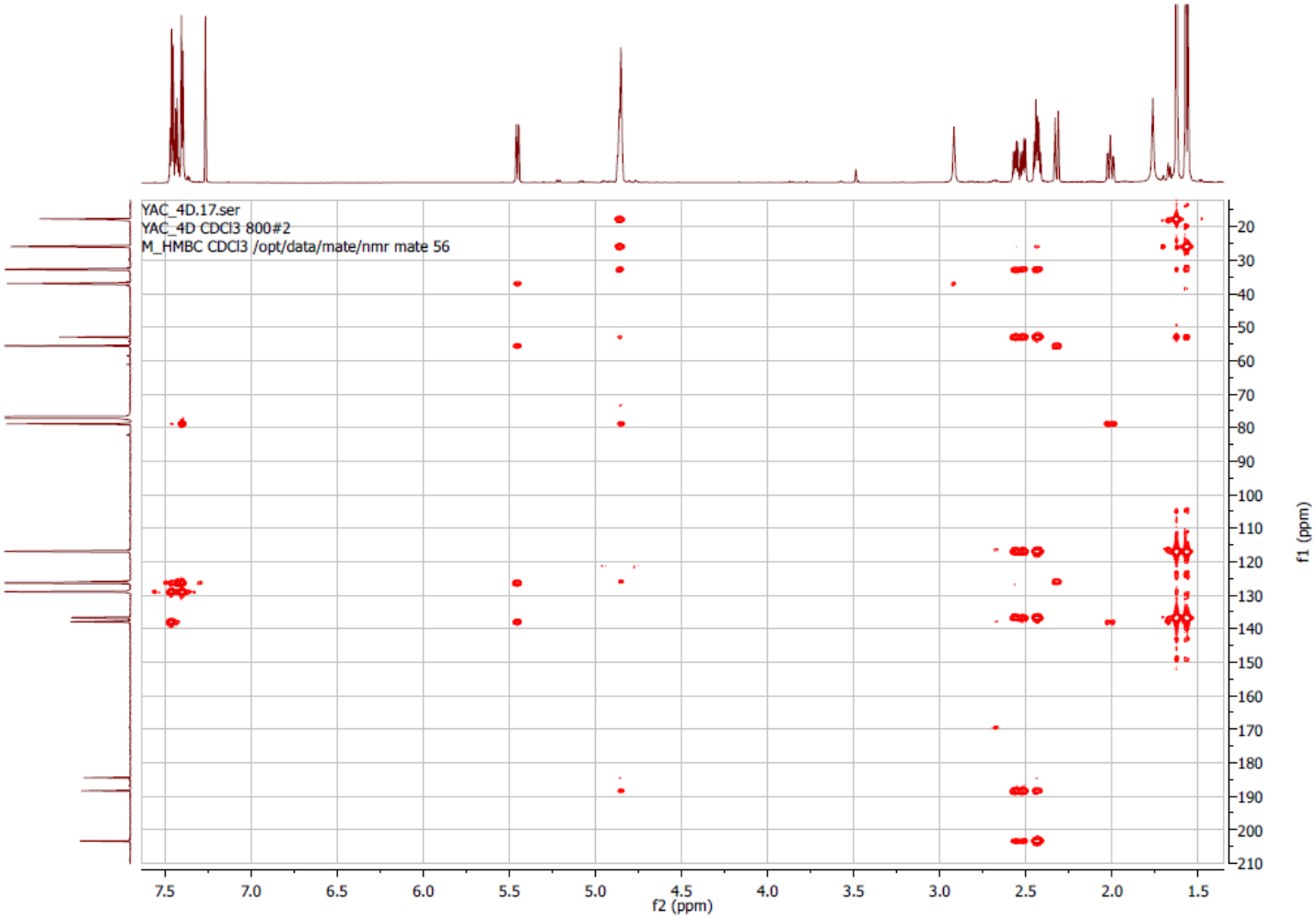
APPENDIX 42C: HH-COSY Spectrum of Rhodiflavan C (193) ($CDCl_3$)



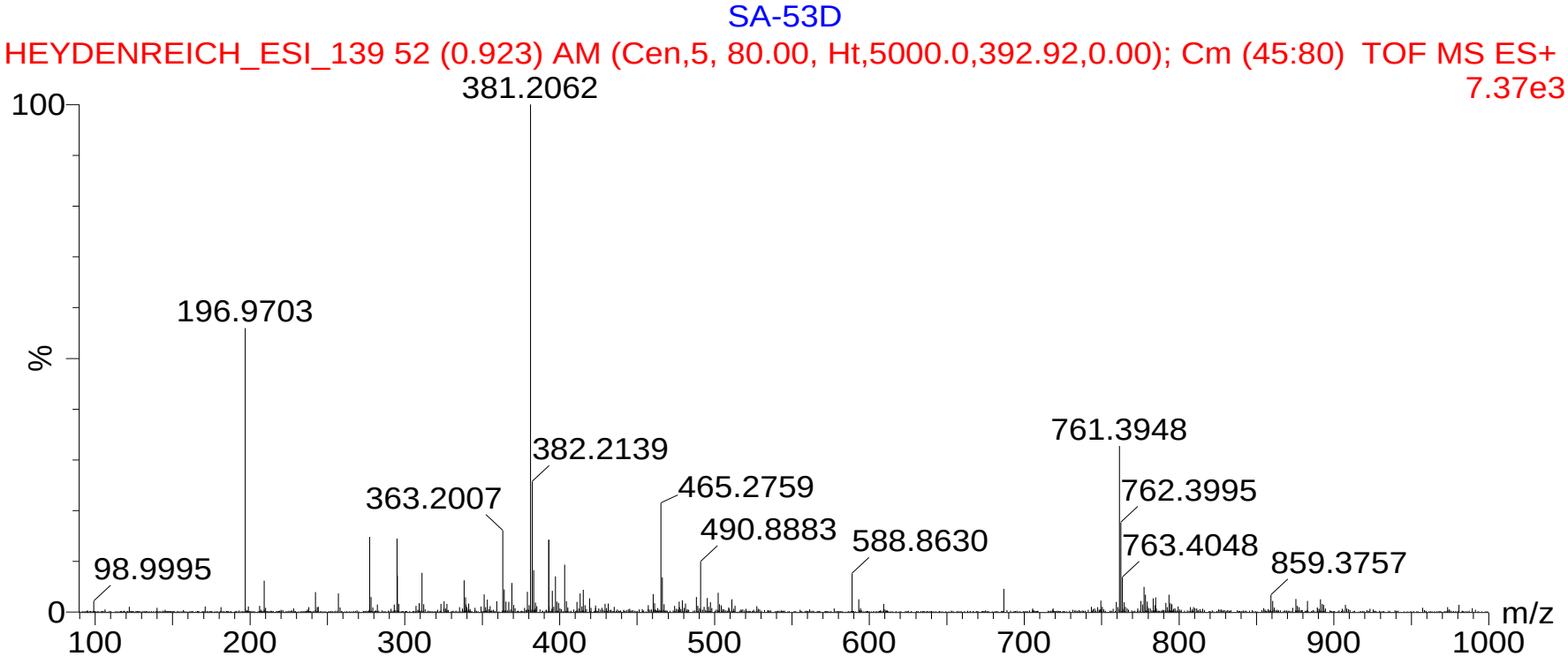
APPENDIX 42D: HSQC Spectrum of Rhodiflavan C (193) ($CDCl_3$)



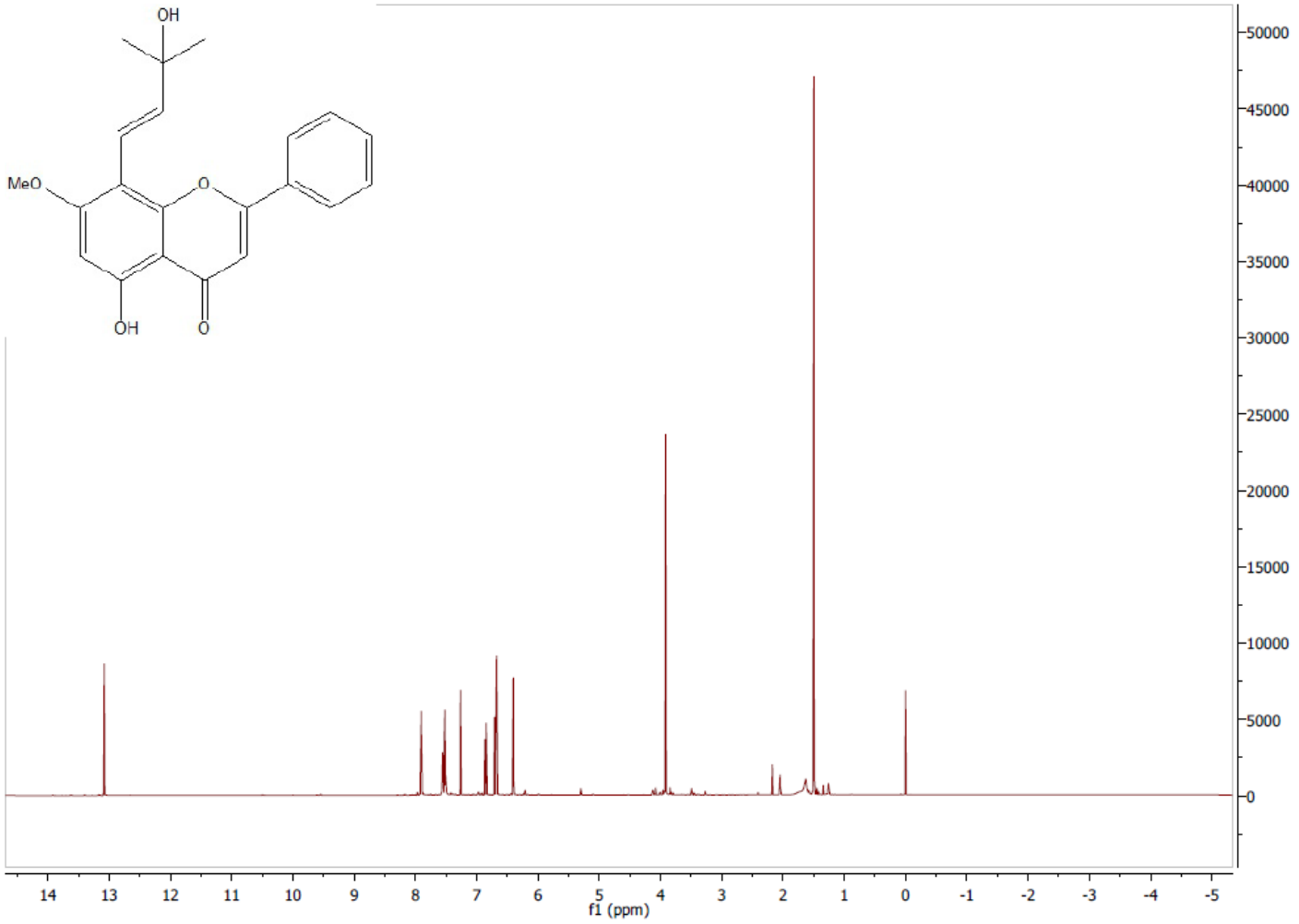
APPENDIX 42E: HMBC Spectrum of Rhodiflavan C (193) ($CDCl_3$)



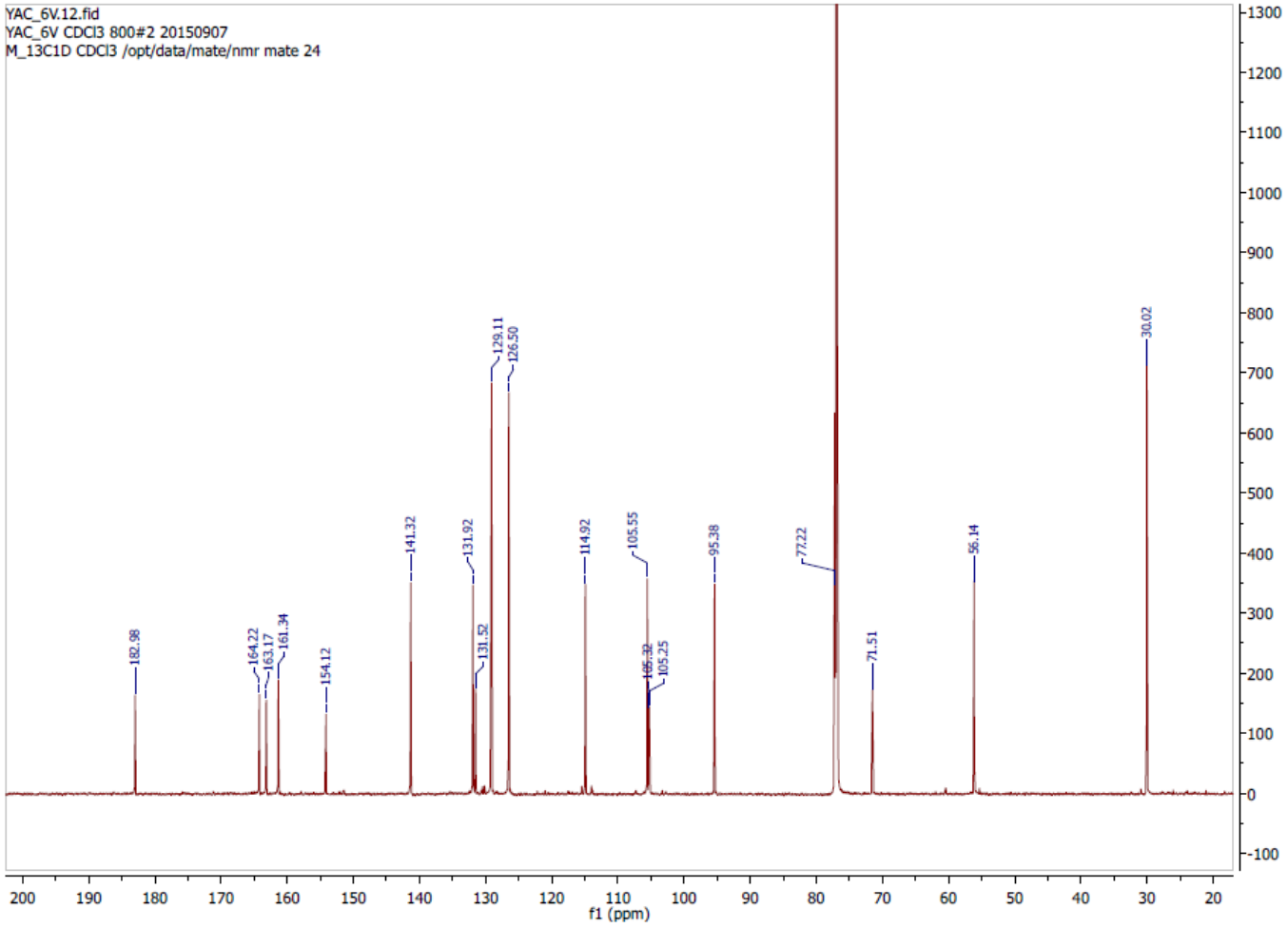
APPENDIX 42F: HRMS Spectrum of Rhodiflavan C (193) ($CDCl_3$)



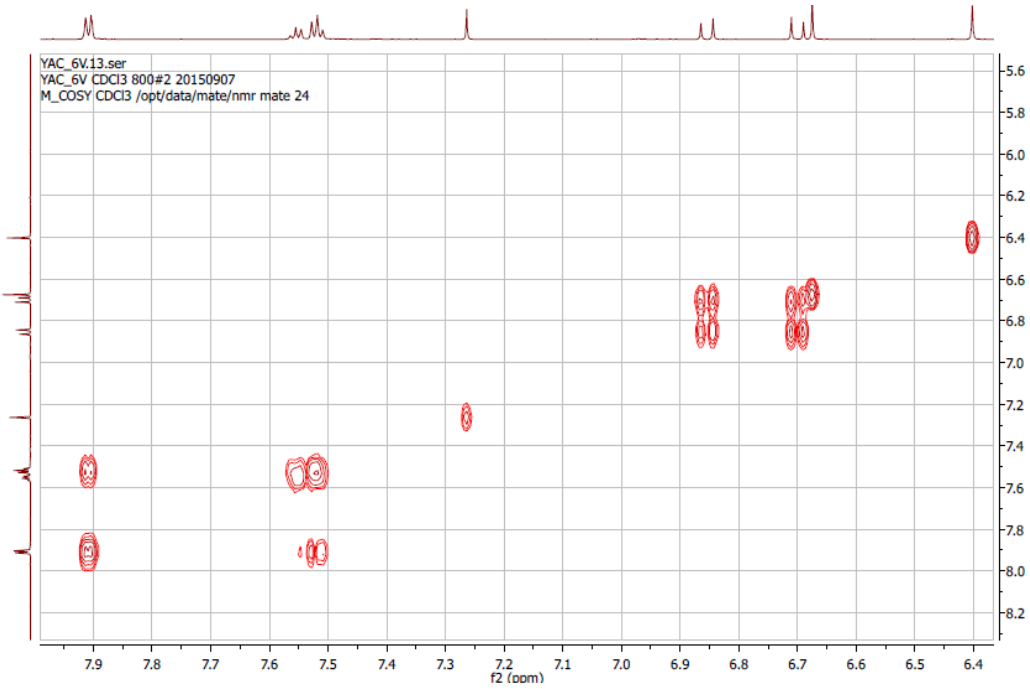
APPENDIX 43A: ^{1}H NMR Spectrum of (*E*)-5-Hydroxy-tephrostachin (198) (800 MHz; $CDCl_3$)



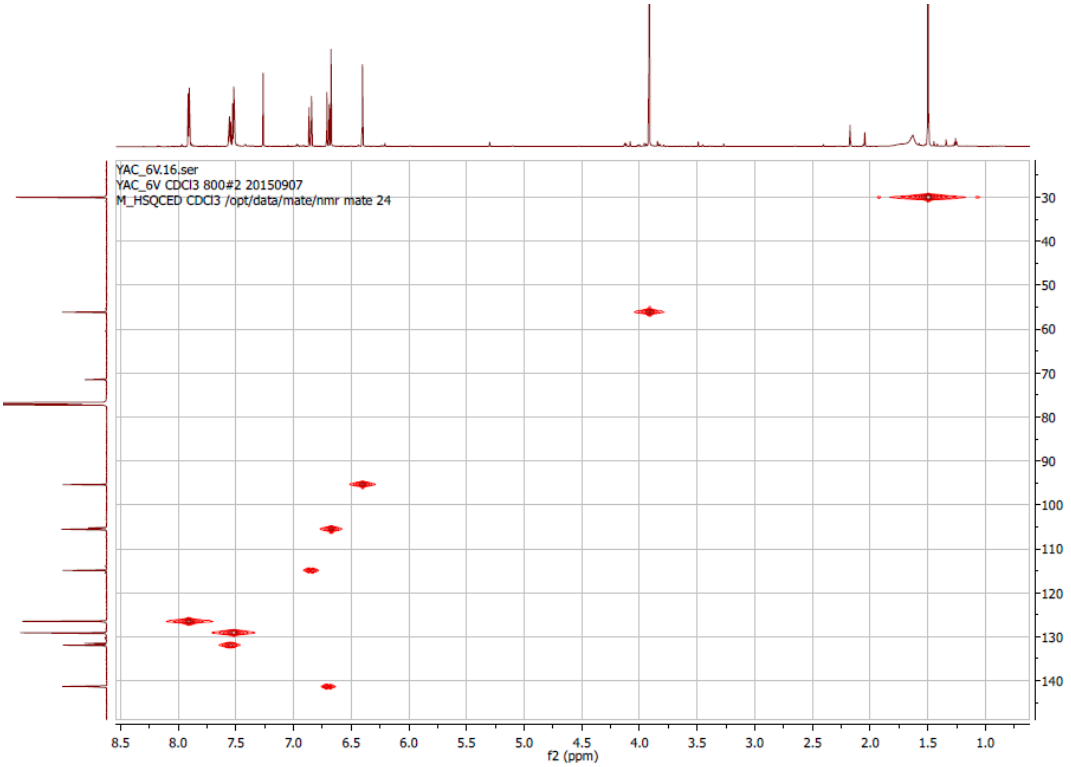
APPENDIX 43B: ^{13}C NMR Spectrum of (*E*)-5-Hydroxy-tephrostachin (198) (200 MHz; $CDCl_3$)



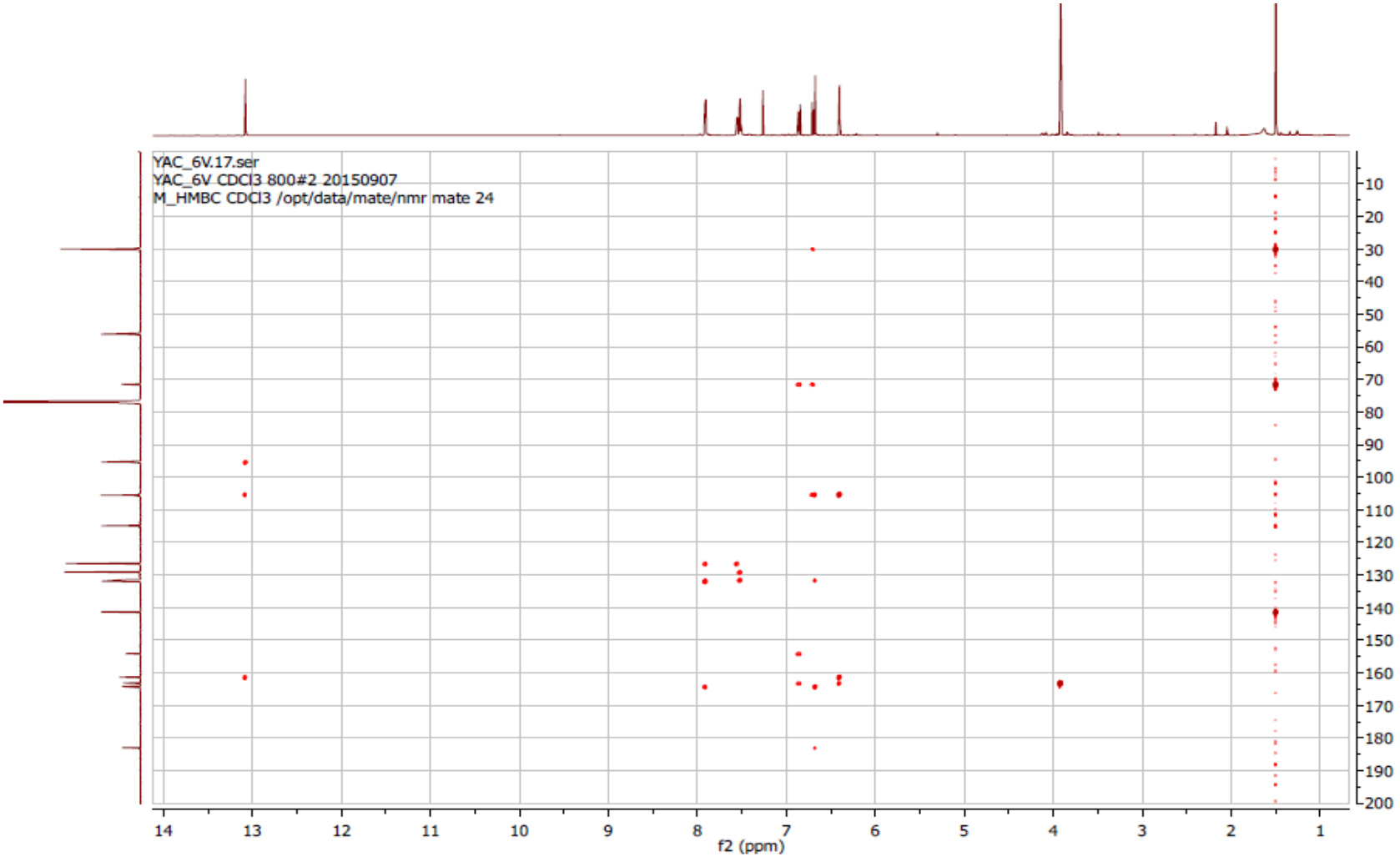
APPENDIX 43C: HH-COSY Spectrum of (*E*)-5-Hydroxy-tephrostachin (198) ($CDCl_3$)



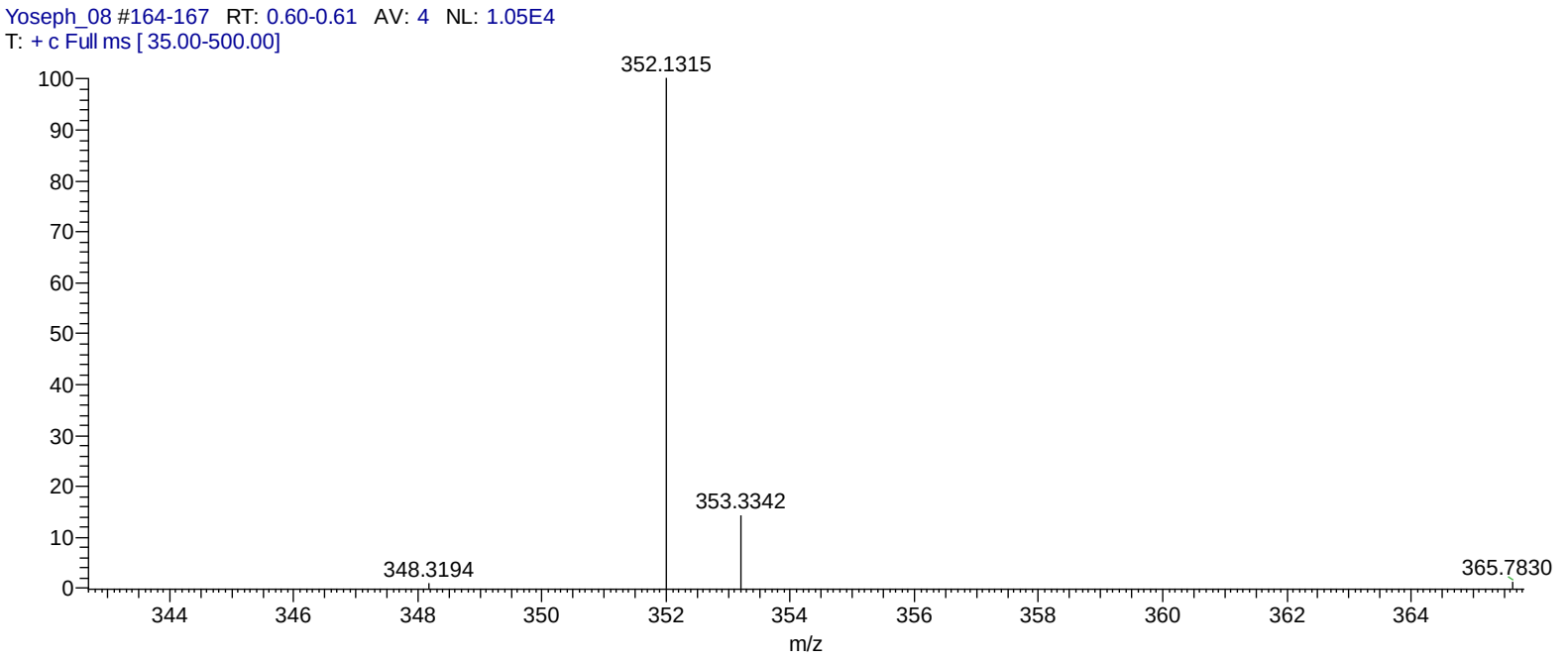
APPENDIX 43D: HSQC Spectrum of (*E*)-5-Hydroxy-tephrostachin (198) ($CDCl_3$)



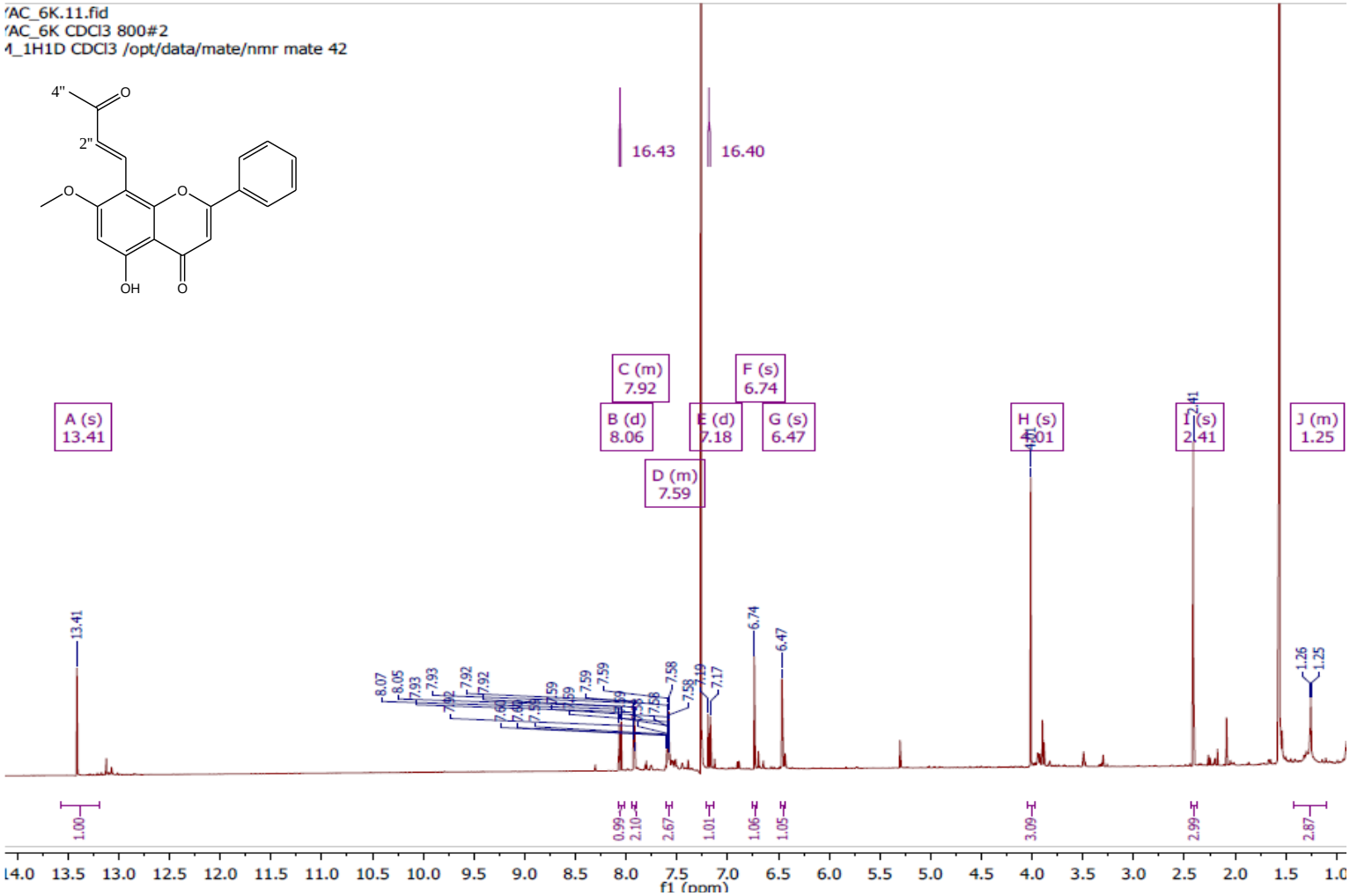
APPENDIX 43E: HMBC Spectrum of (*E*)-5-Hydroxy-tephrostachin (198) ($CDCl_3$)



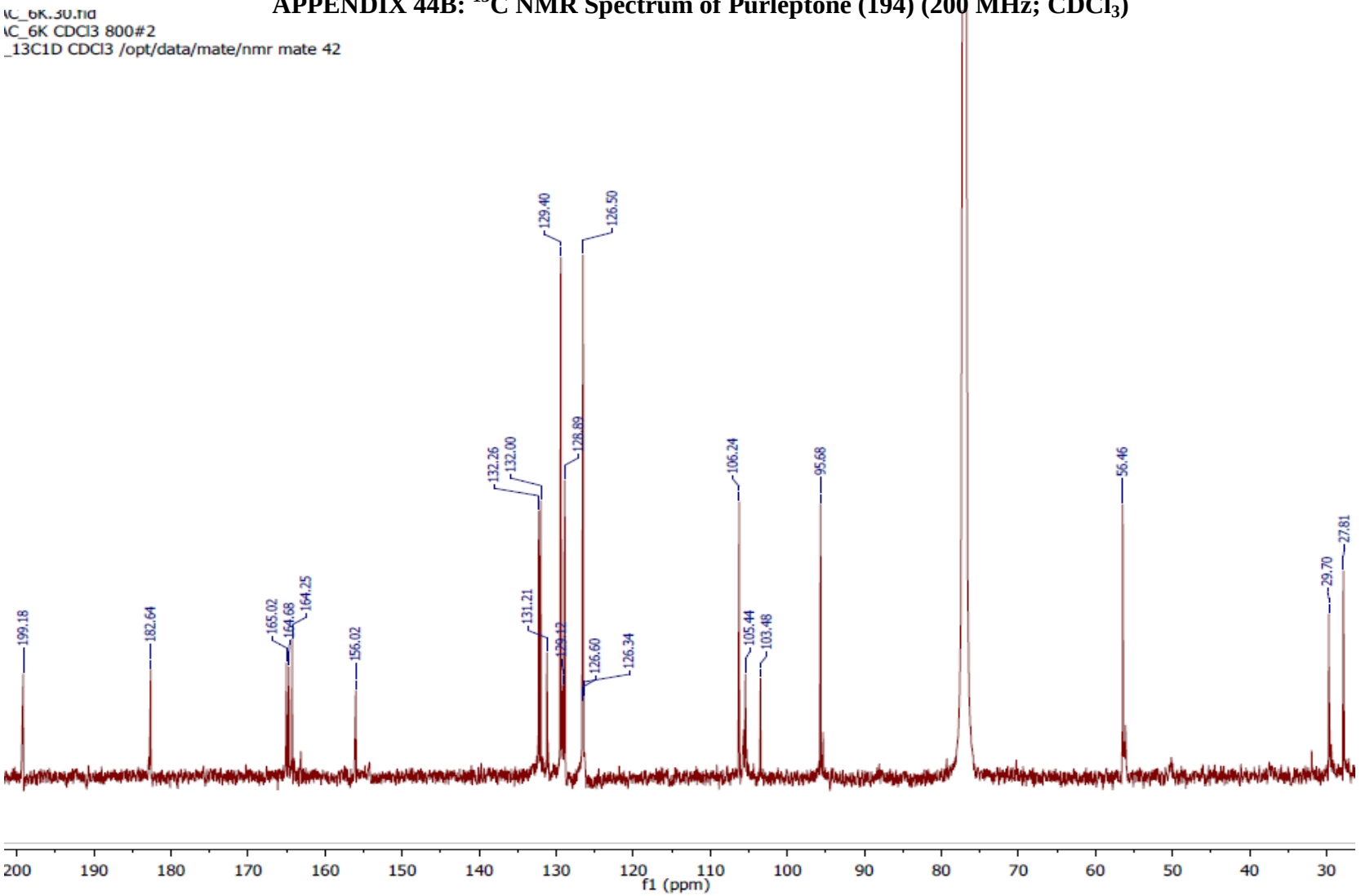
APPENDIX 43F: HRMS Spectrum of (*E*)-5-Hydroxy-tephrostachin (198)



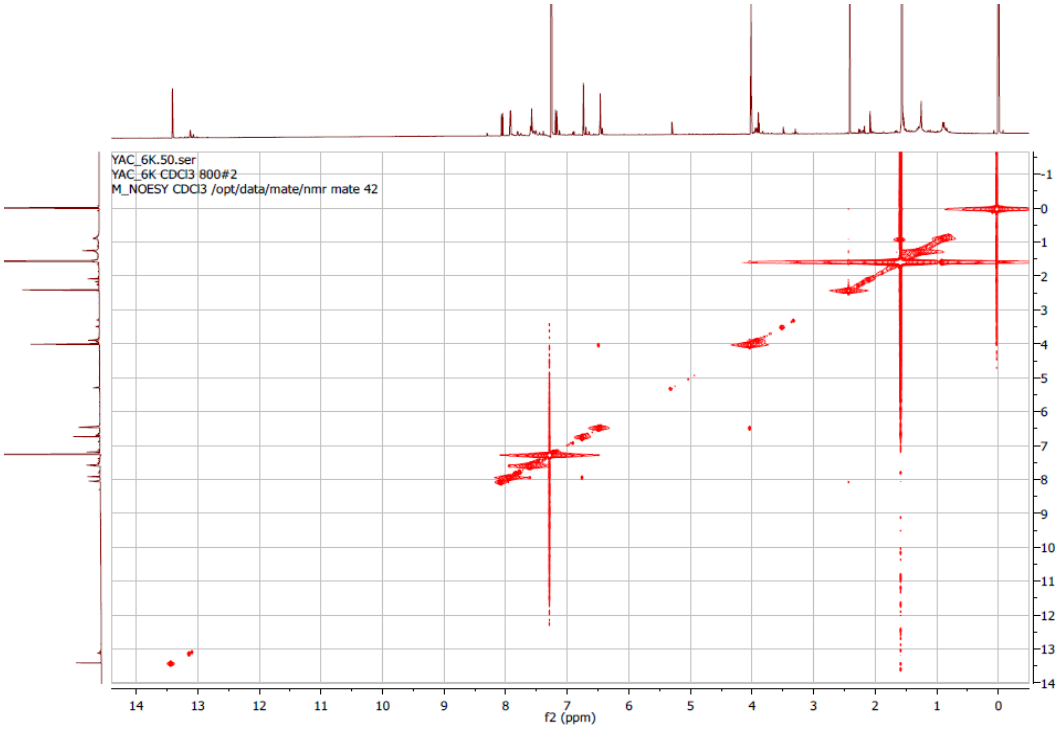
APPENDIX 44A: ^{1}H NMR Spectrum of Purleptone (194) (800 MHz; $CDCl_3$)



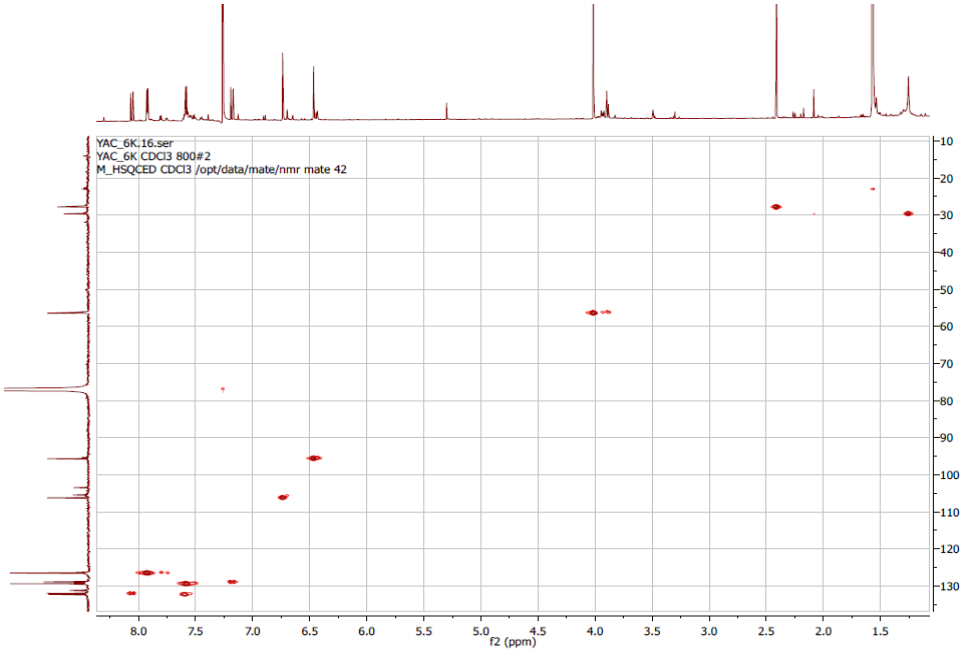
APPENDIX 44B: ^{13}C NMR Spectrum of Purleptone (194) (200 MHz; $CDCl_3$)



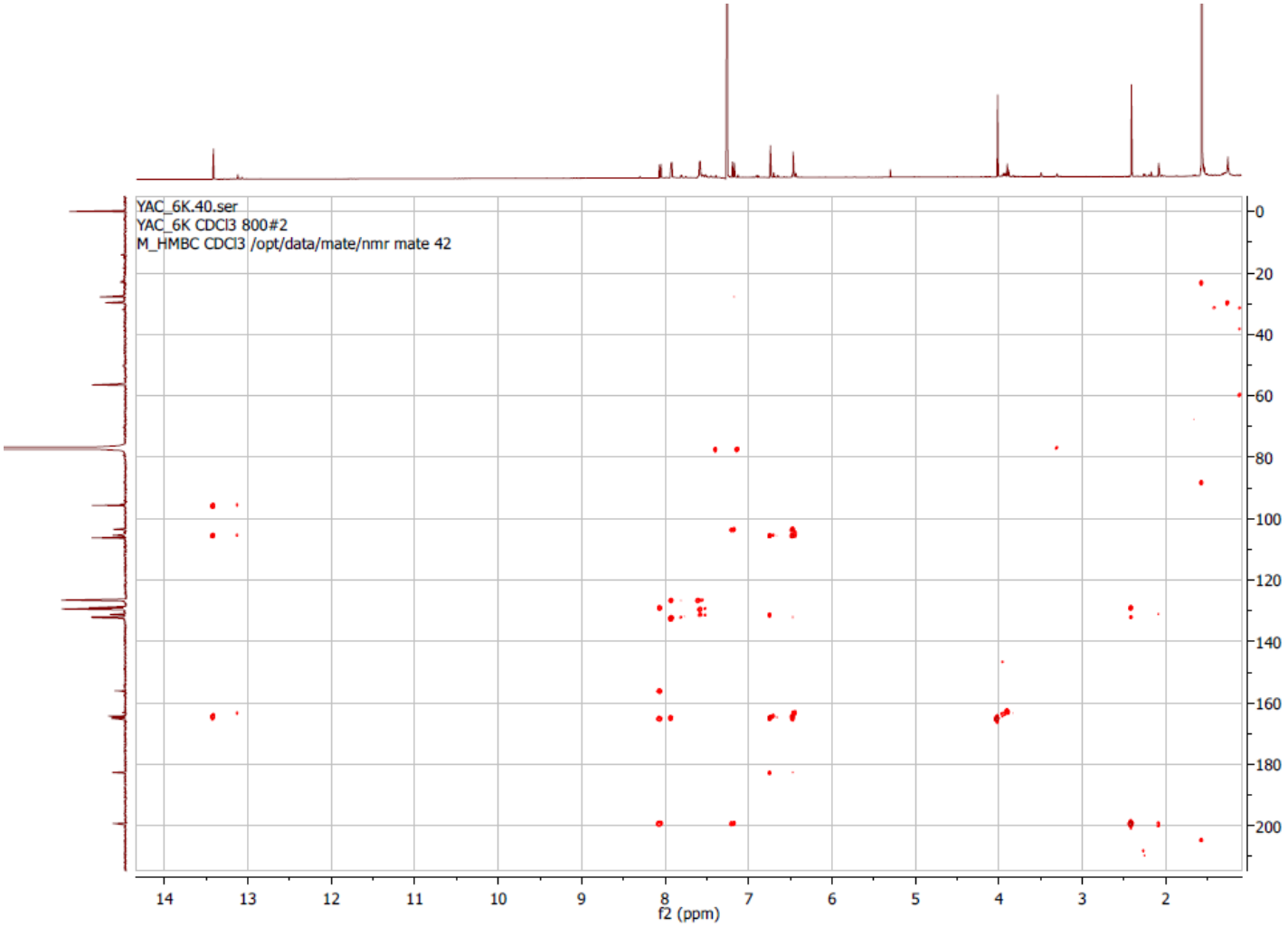
APPENDIX 44C: NOESY Spectrum of Purleptone (194) ($CDCl_3$)



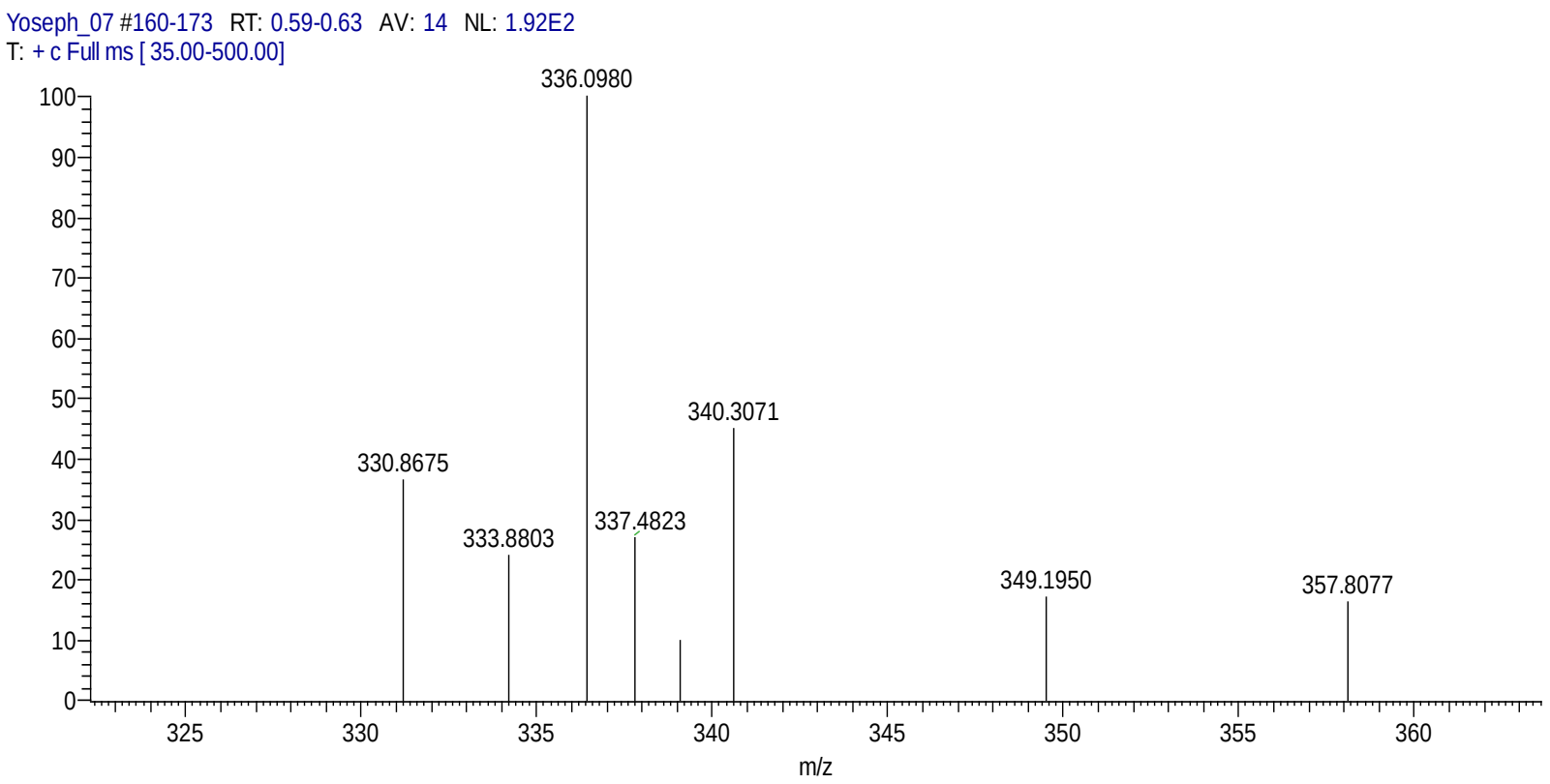
APPENDIX 44D: HSQC Spectrum of Purleptone (194) ($CDCl_3$)

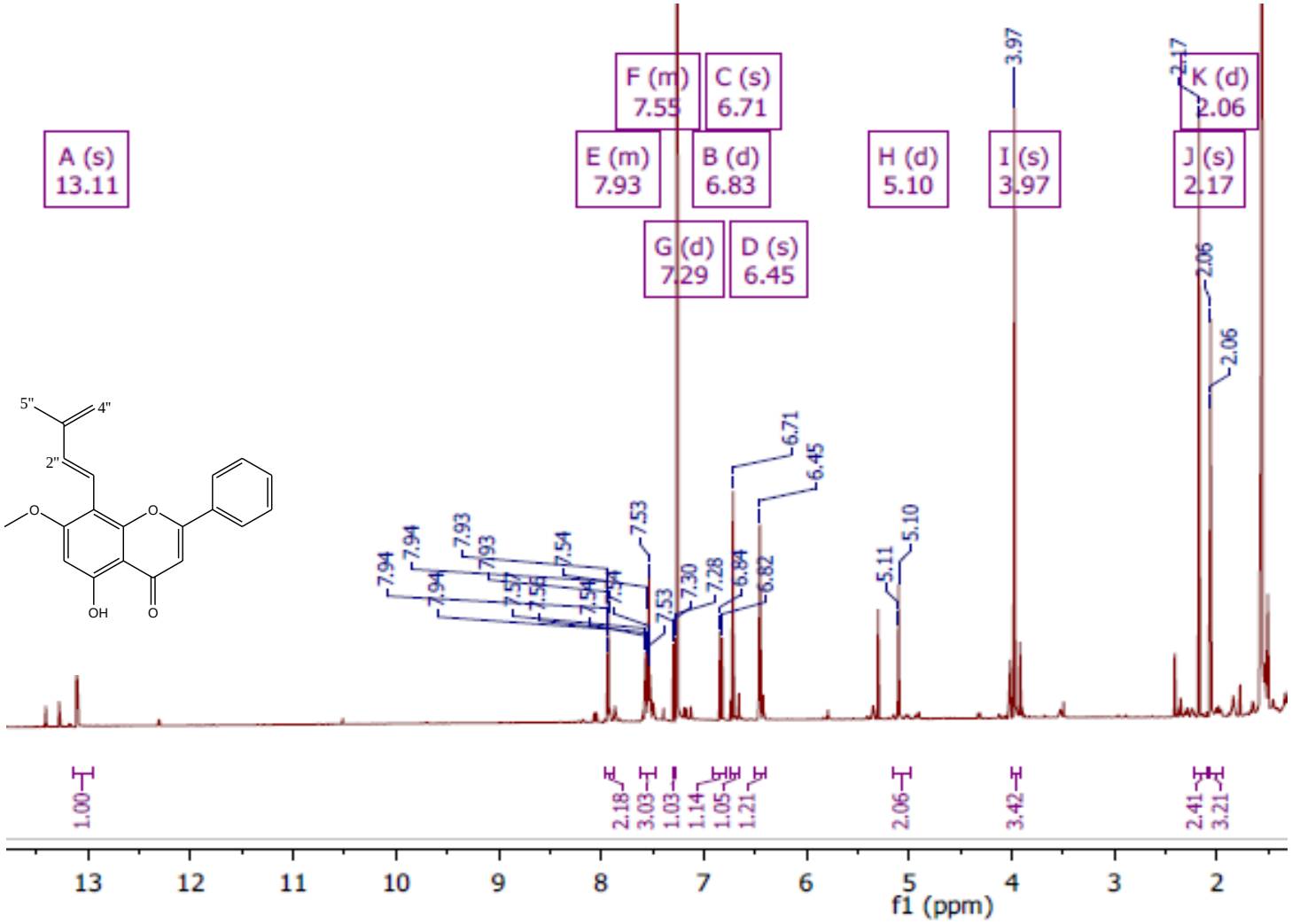


APPENDIX 44E: HMBC Spectrum of Purleptone (194) ($CDCl_3$)



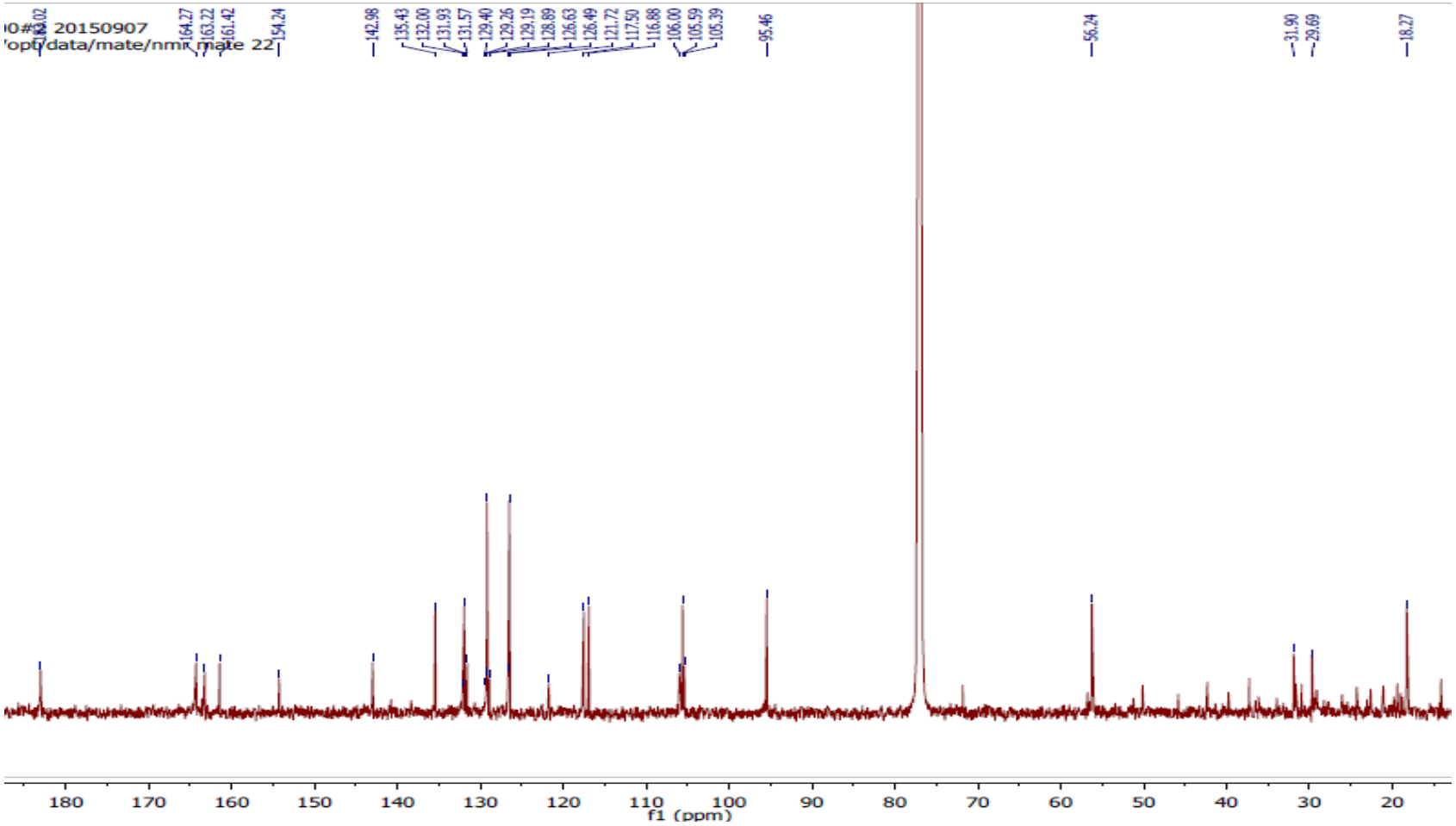
APPENDIX 44F: HRMS Spectrum of Purleptone (194) ($CDCl_3$)



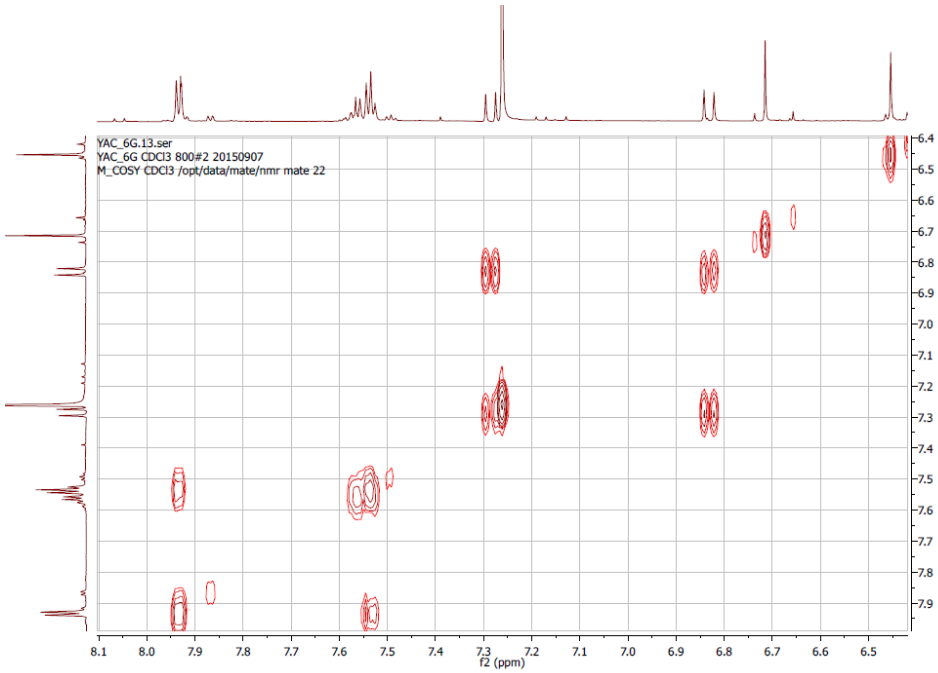


APPENDIX 45A: ^{1}H NMR Spectrum of 5-oxo-anhydrotephrostachin (195) (800 MHz; $CDCl_3$)

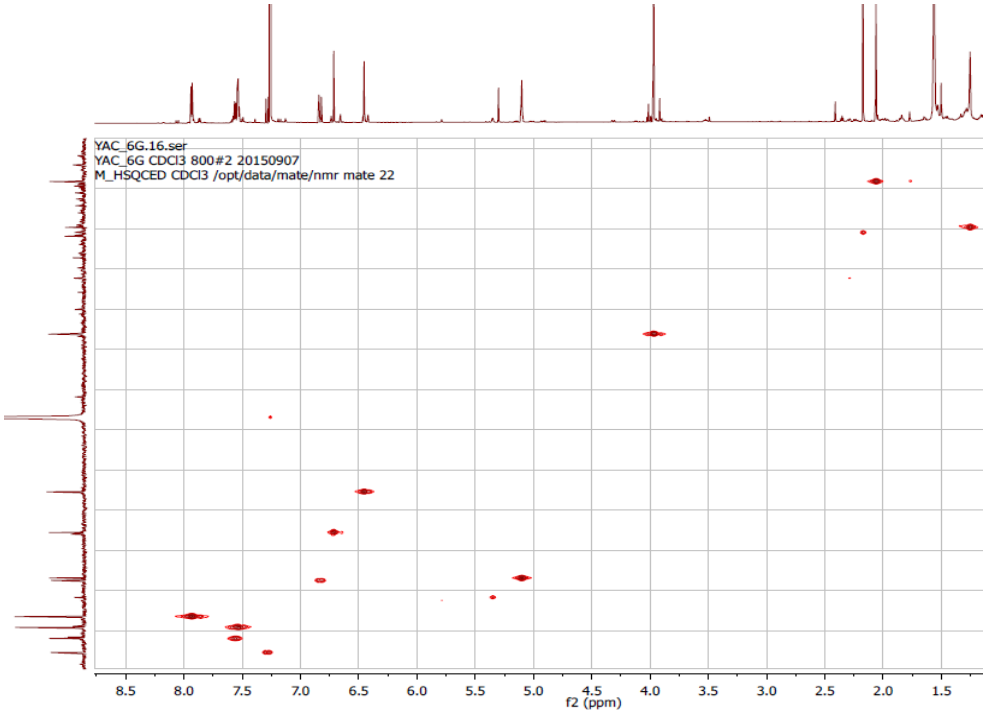
APPENDIX 45B: ^{13}C NMR Spectrum of 5-oxo-anhydrotephrostachin (195) (200 MHz; $CDCl_3$)



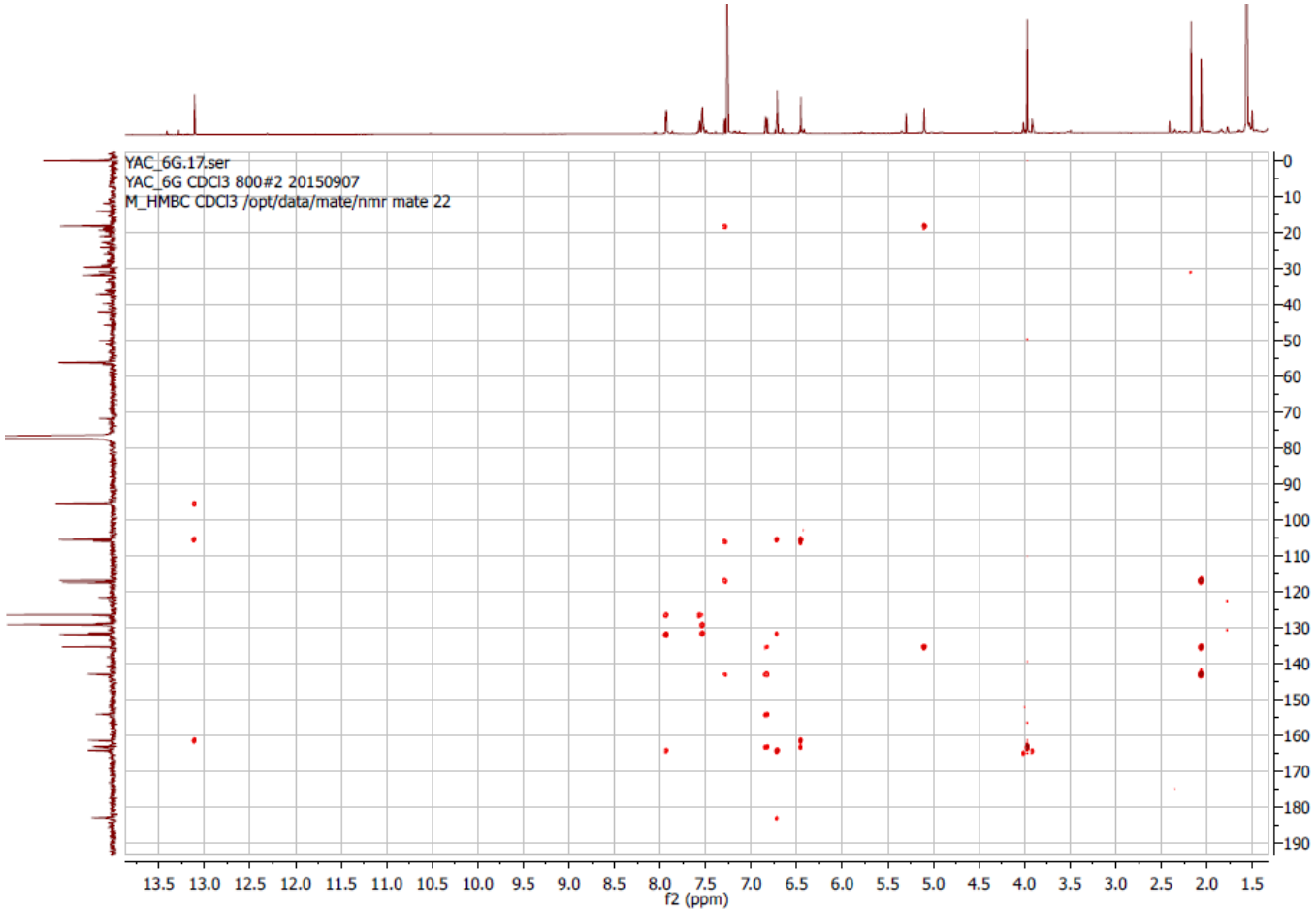
APPENDIX 45C: HH-COSY Spectrum of 5-oxo-anhydrotephrostachin (195) ($CDCl_3$)



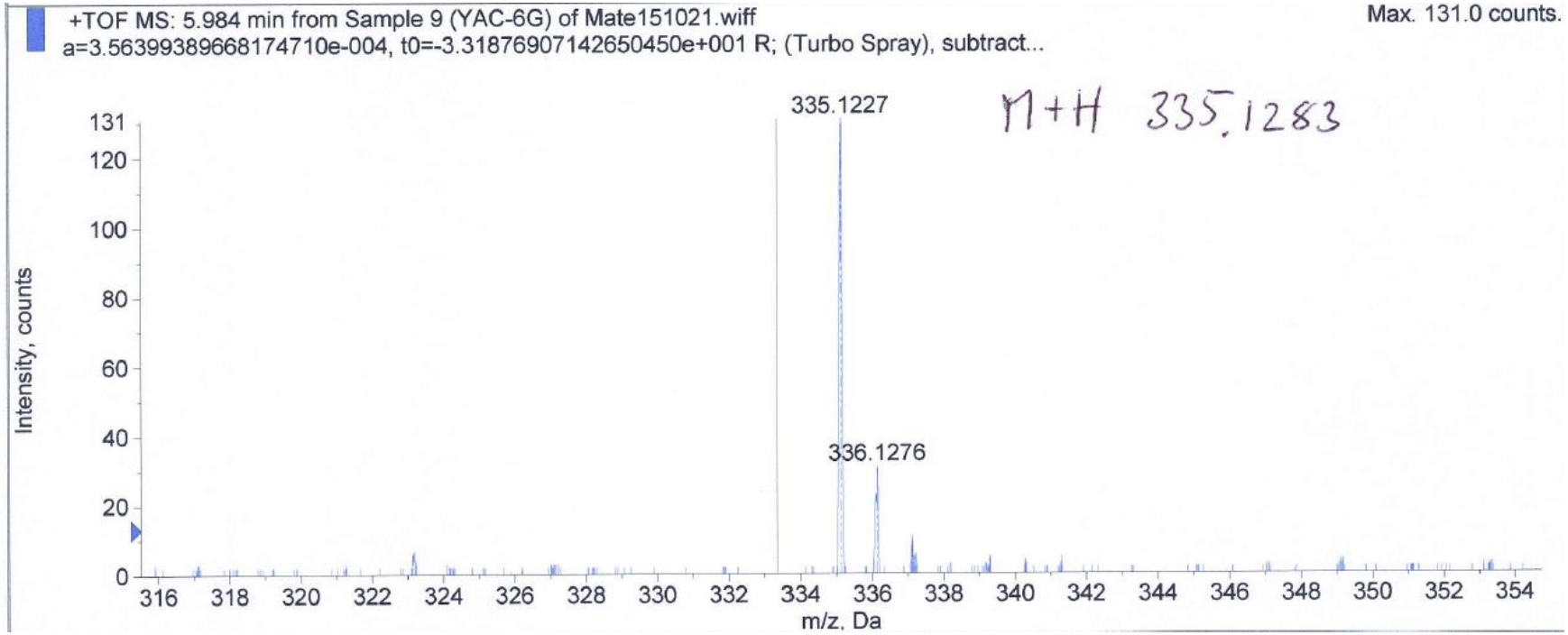
APPENDIX 45D: HSQC Spectrum of 5-oxo-anhydrotephrostachin (195) ($CDCl_3$)



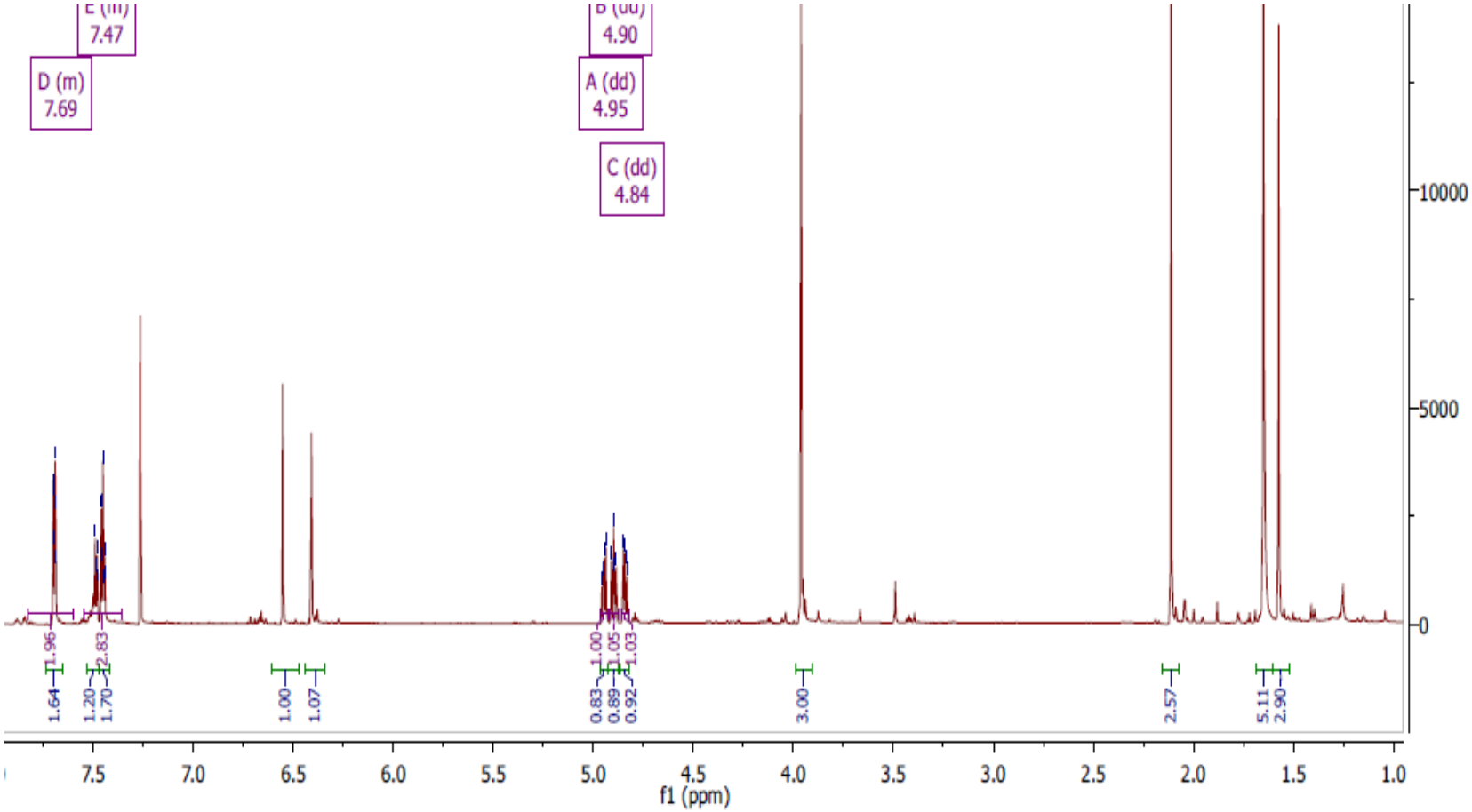
APPENDIX 45E: HMBC Spectrum of 5-oxo-anhydrotephrostachin (195) ($CDCl_3$)



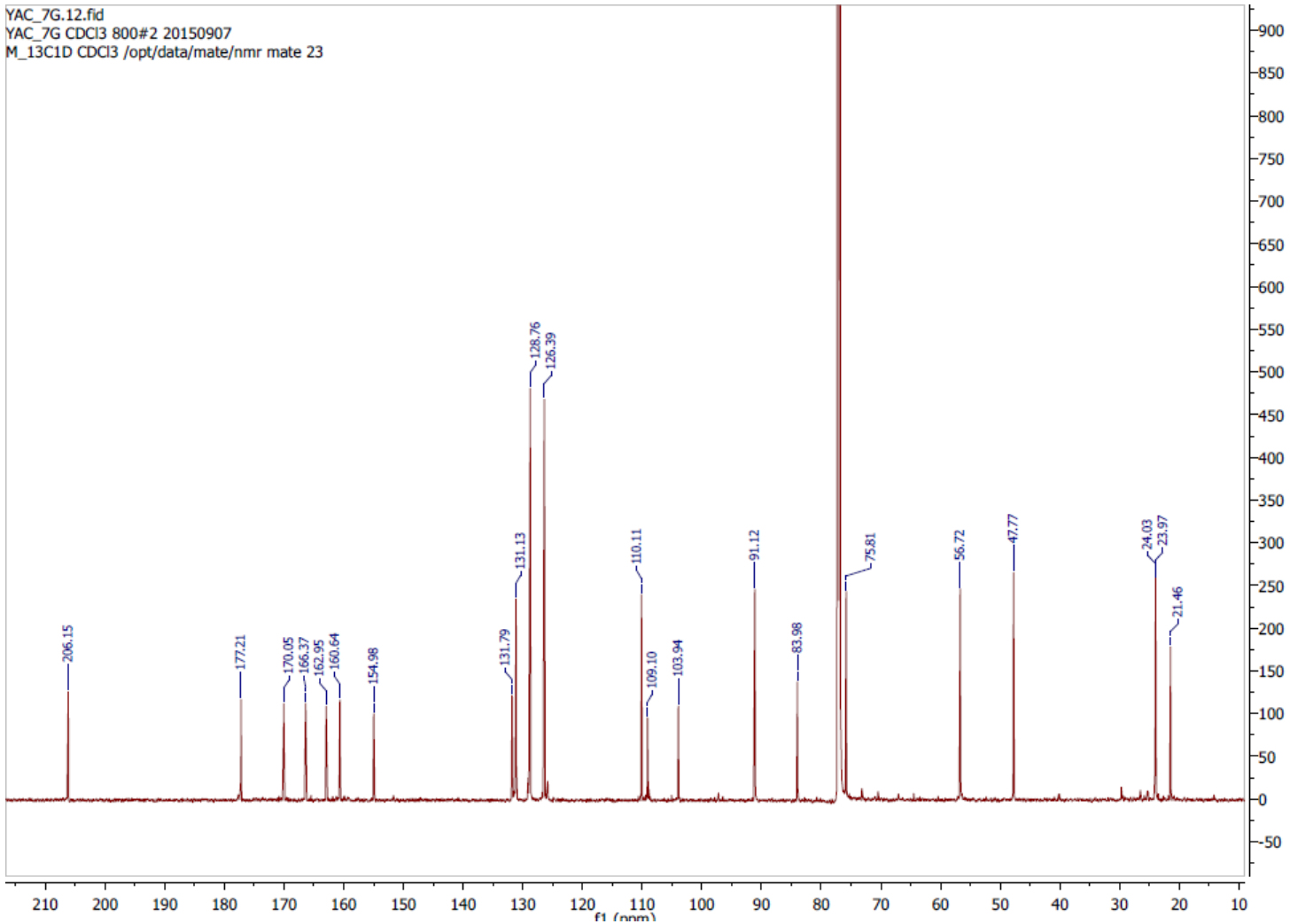
APPENDIX 45F: HRMS Spectrum of 5-oxo-anhydrotephrostachin (195)



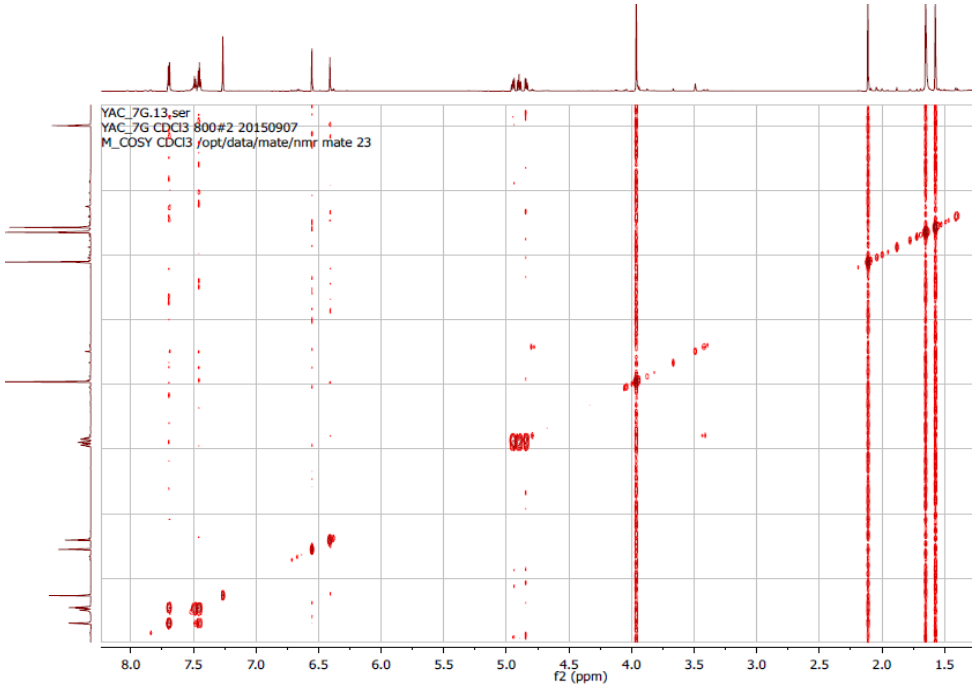
APPENDIX 46A: ^{1}H NMR Spectrum of Terpurlepflavone (199) (800 MHz; $CDCl_3$)



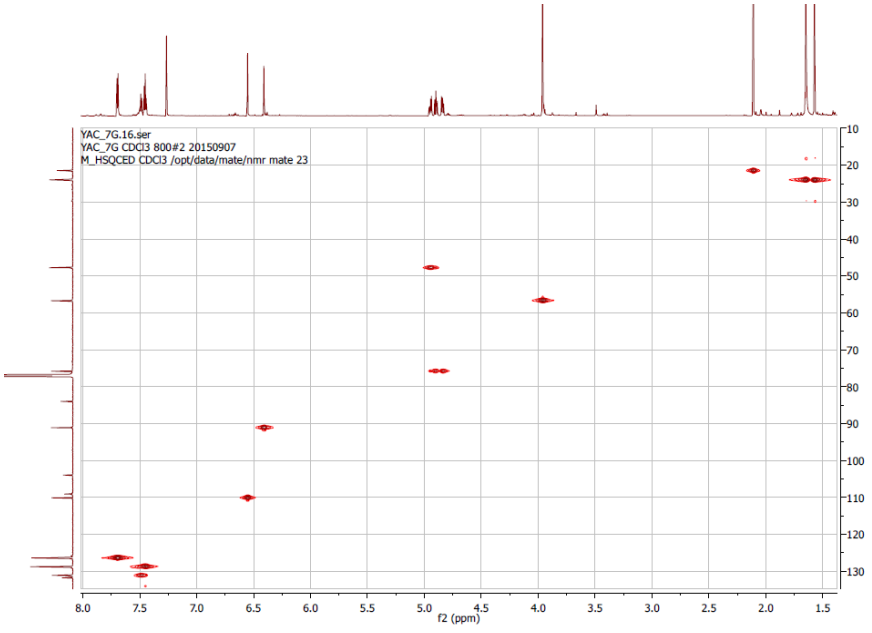
APPENDIX 46B: ^{13}C NMR Spectrum of Terpurlepflavone (199) (200 MHz; $CDCl_3$)



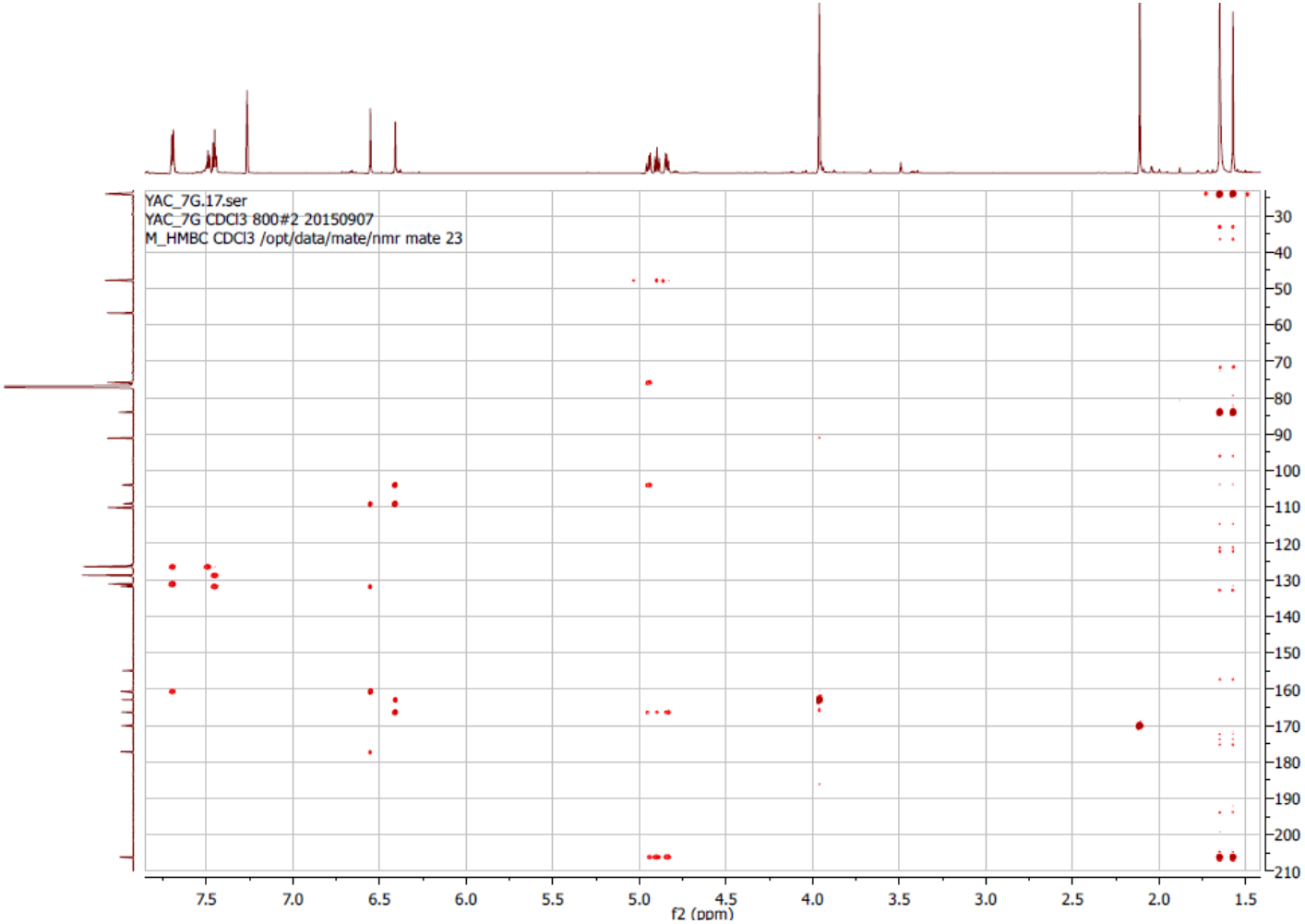
APPENDIX 46C: HH-COSY Spectrum of Terpurlepflavone (199) (CDCl3)



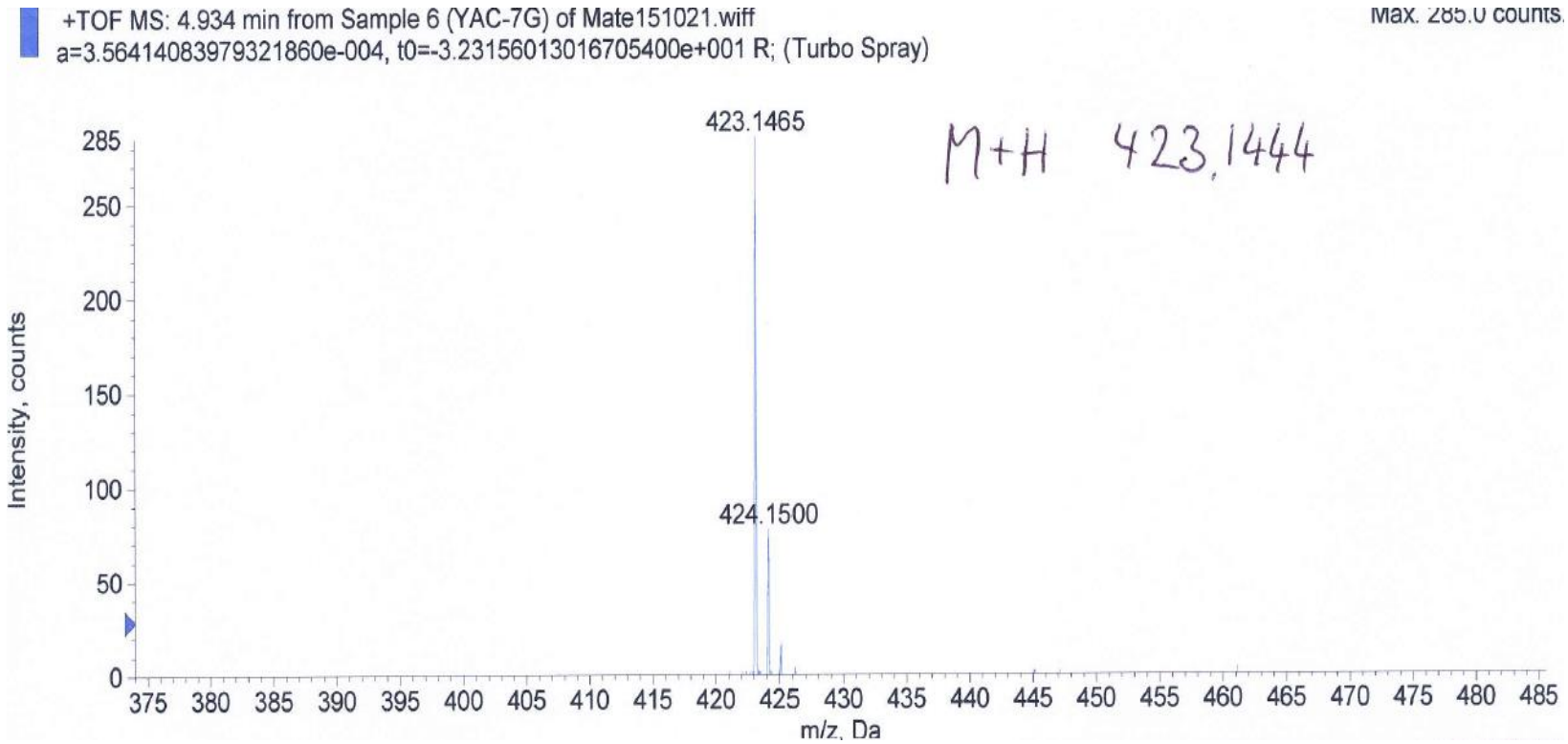
APPENDIX 46D: HSQC Spectrum of Terpurlepflavone (199) ($CDCl_3$)



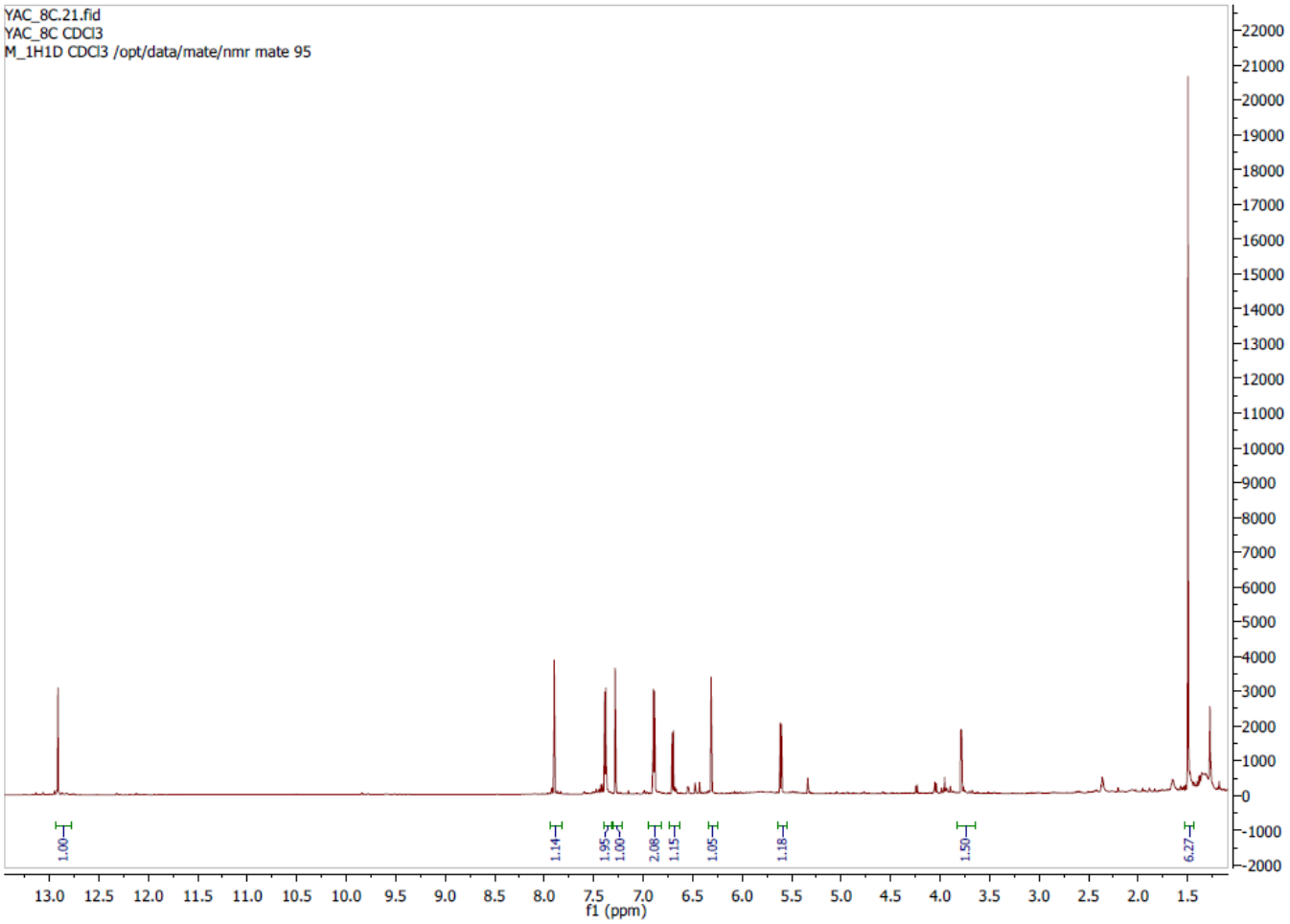
APPENDIX 46E: HMBC Spectrum of Terpurlepflavone (199) ($CDCl_3$)



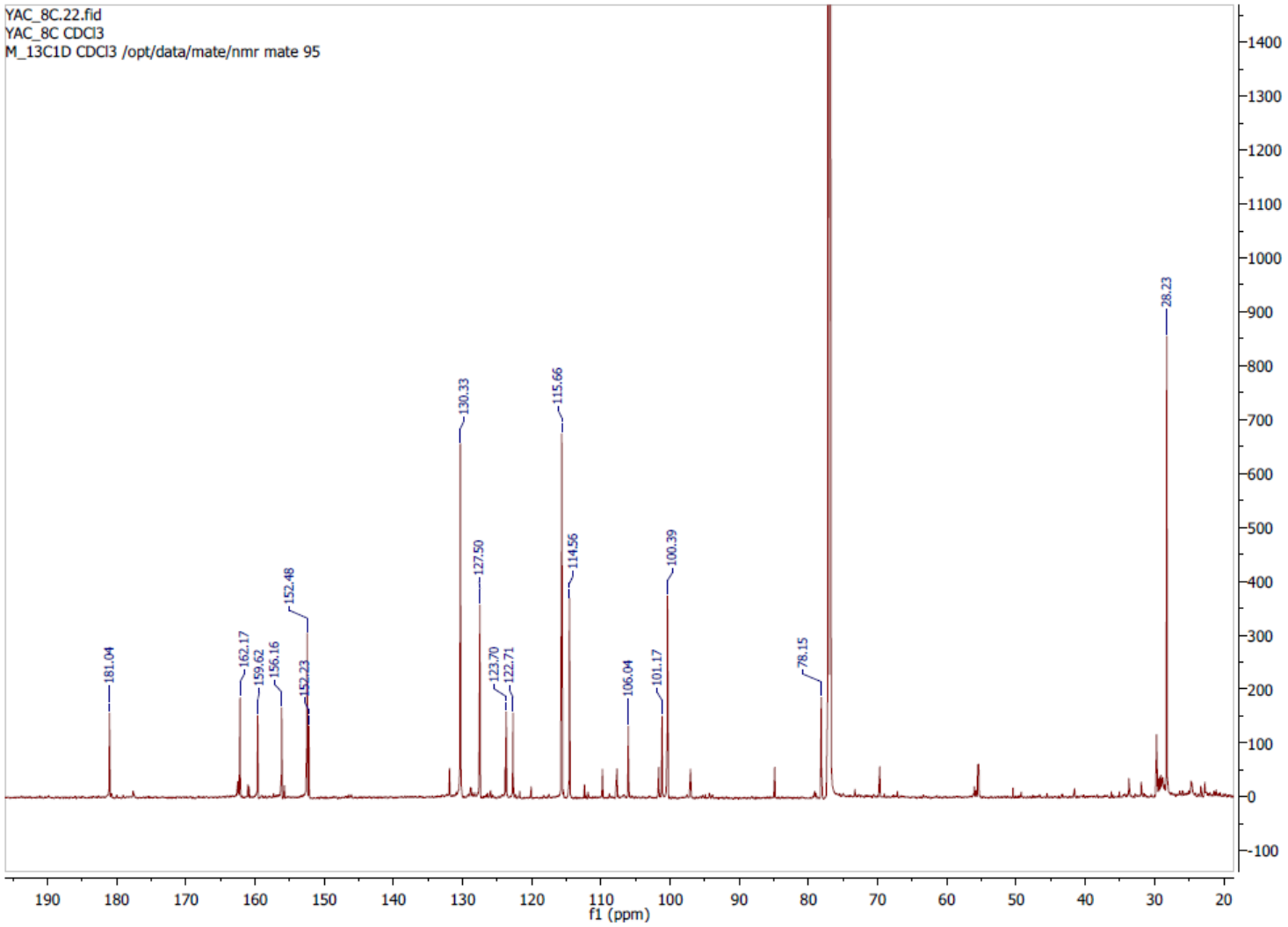
APPENDIX 46F: HMBC Spectrum of Terpurlepflavone (199)



APPENDIX 47A: ^{1}H NMR Spectrum of Derrone (196) (800 MHz; $CDCl_3$)

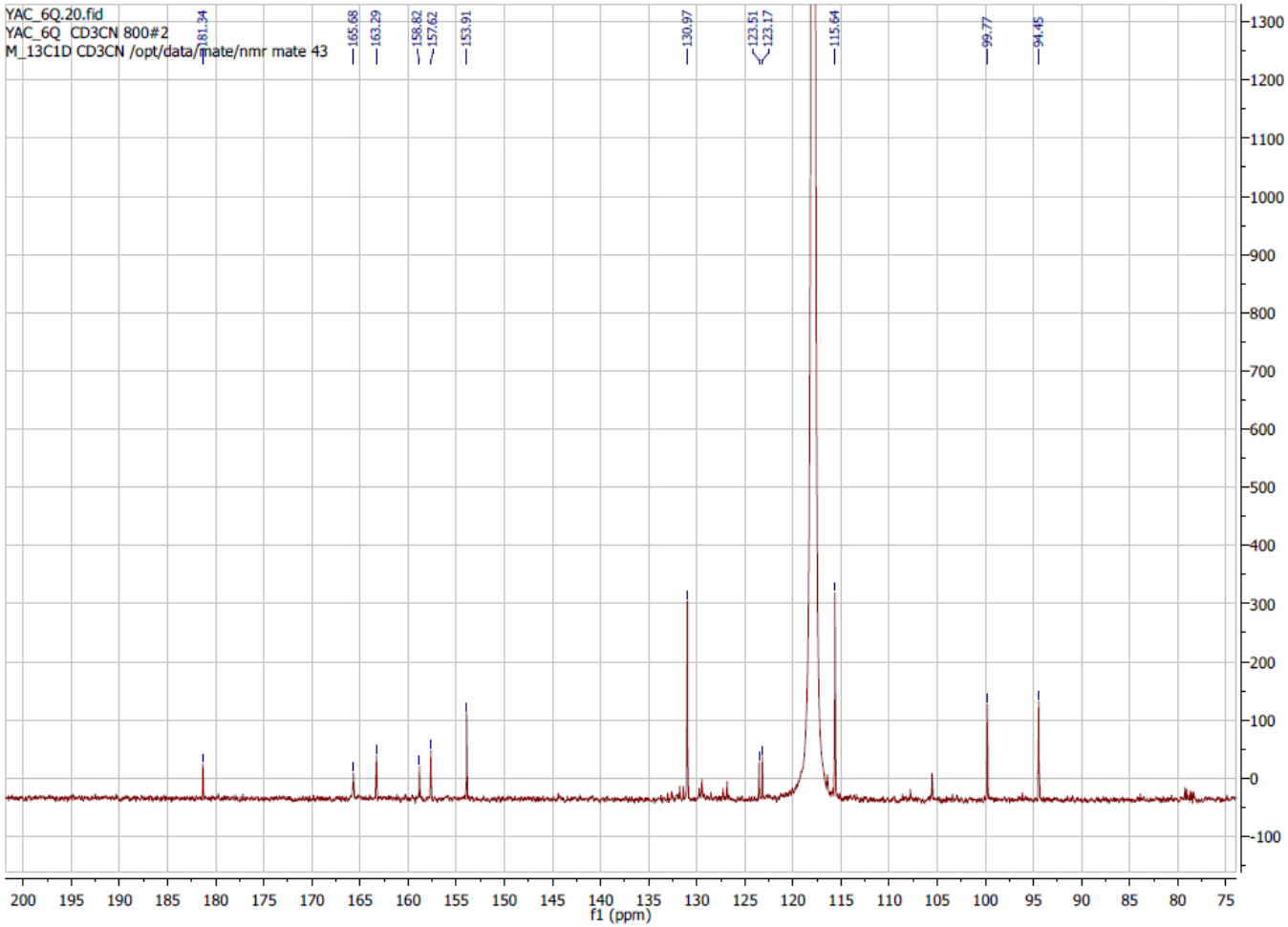


APPENDIX 47B: ^{13}C NMR Spectrum of Derrone (196) (200 MHz; $CDCl_3$)

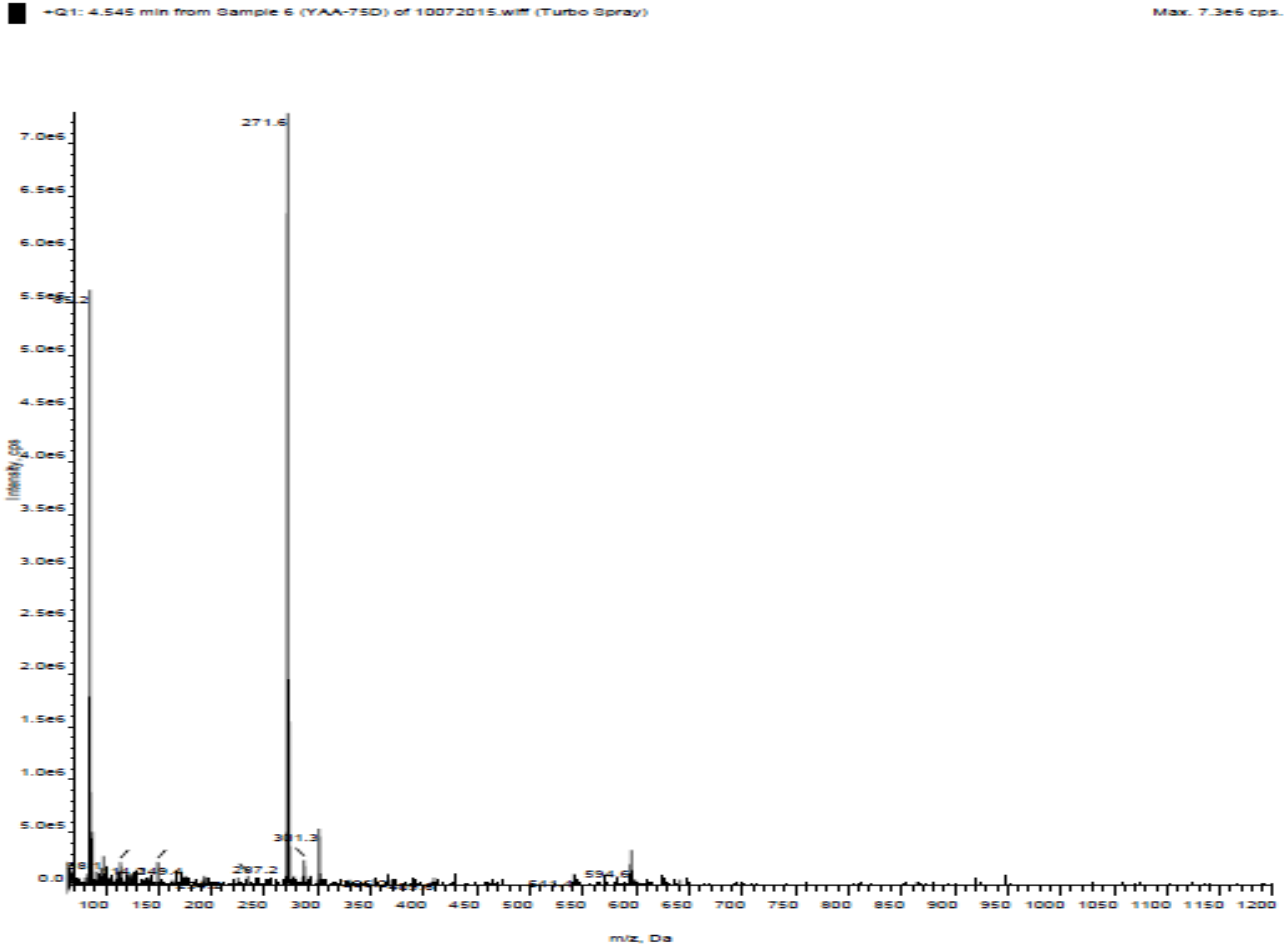


APPENDIX 48A: ^{1}H NMR Spectrum of Genistein (197) (800 MHz; $CDCl_3$)

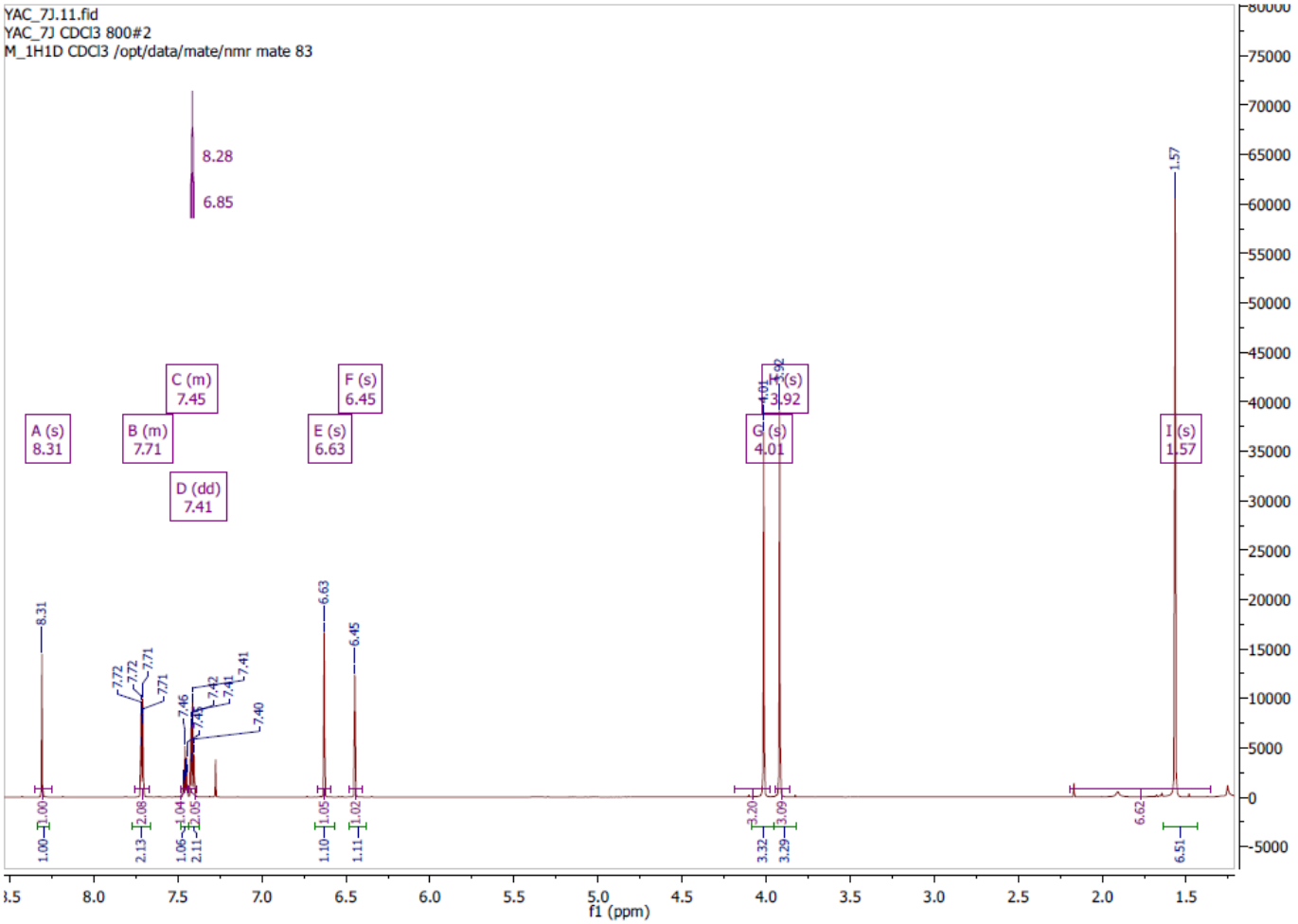
APPENDIX 48B: ^{13}C NMR Spectrum of Genistein (197) (200 MHz; $CDCl_3$)



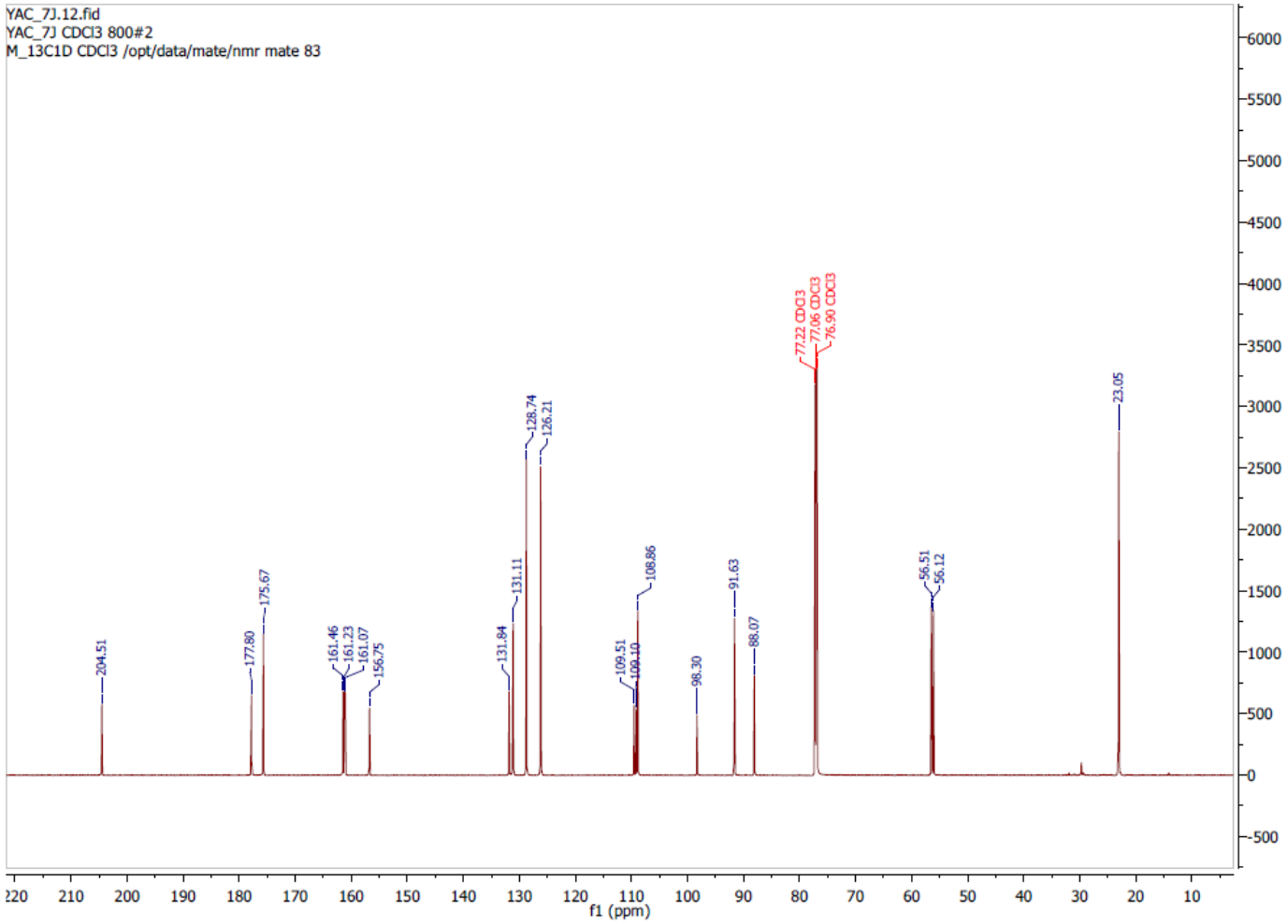
APPENDIX 48C: LCMS Spectrum of Genistein (197) ($CDCl_3$)



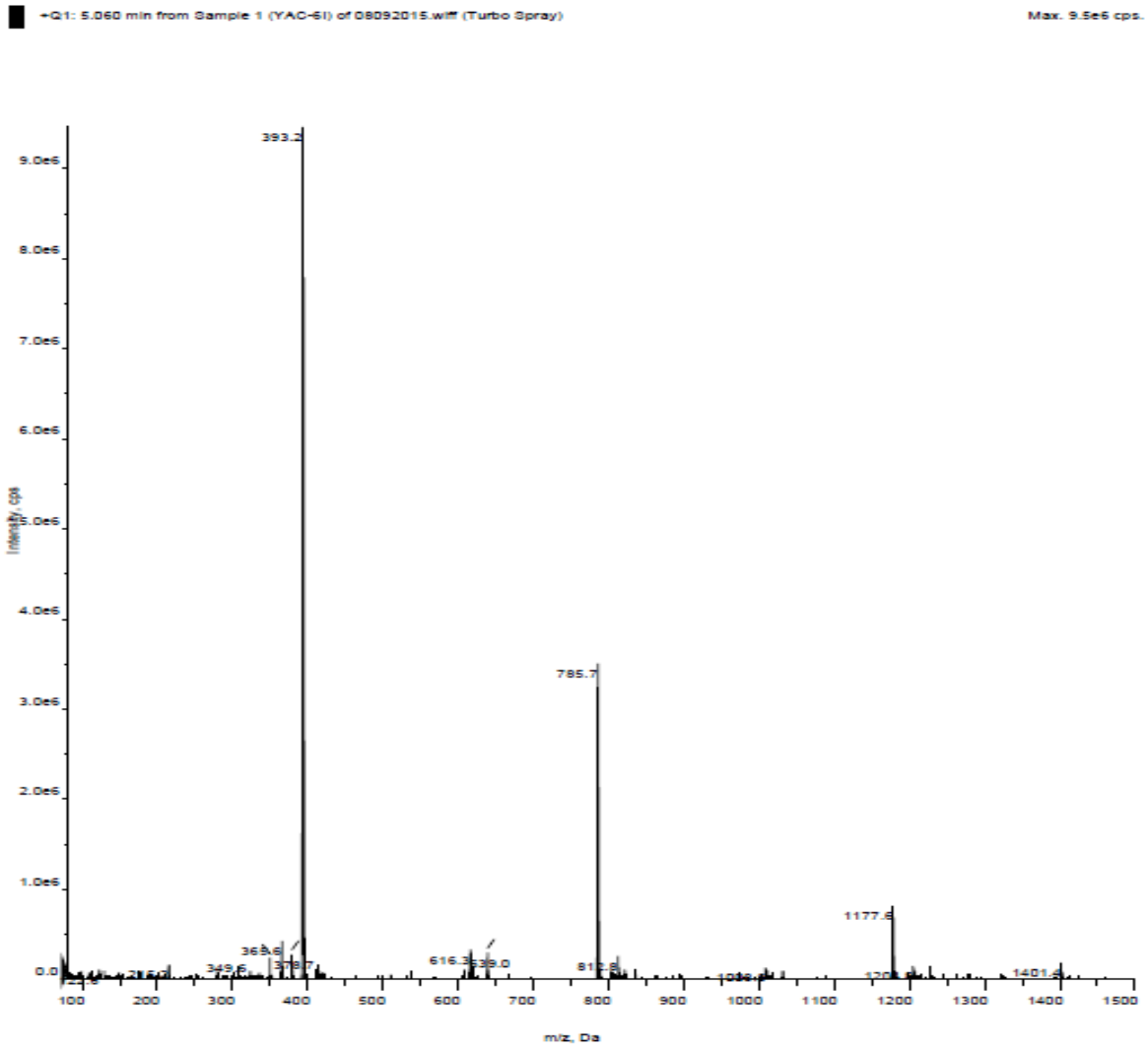
APPENDIX 49A: ^{1}H NMR Spectrum of Tachrosin (41) (800 MHz; $CDCl_3$)



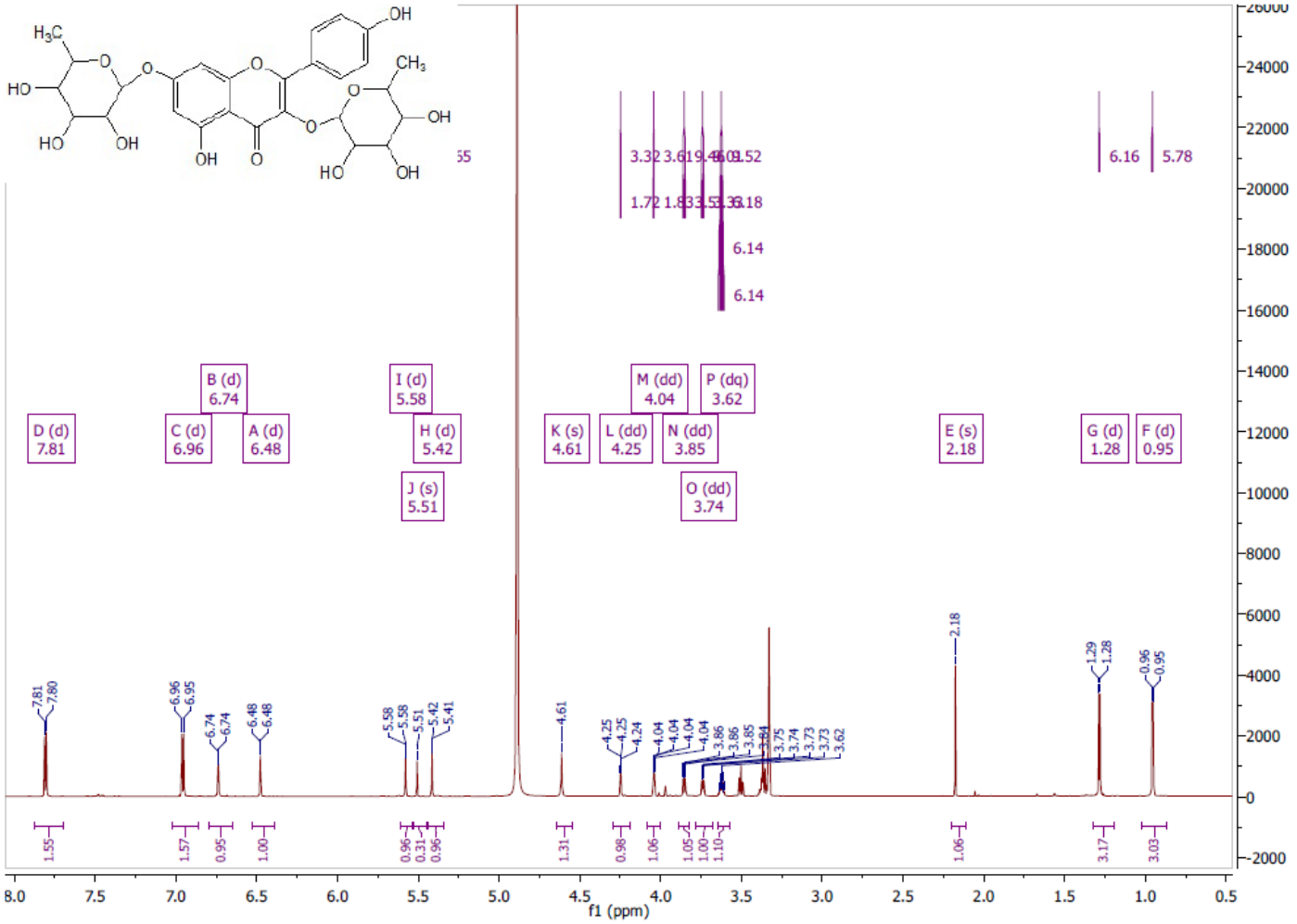
APPENDIX 49B: ^{13}C NMR Spectrum of Tachrosin (41) (200 MHz; $CDCl_3$)



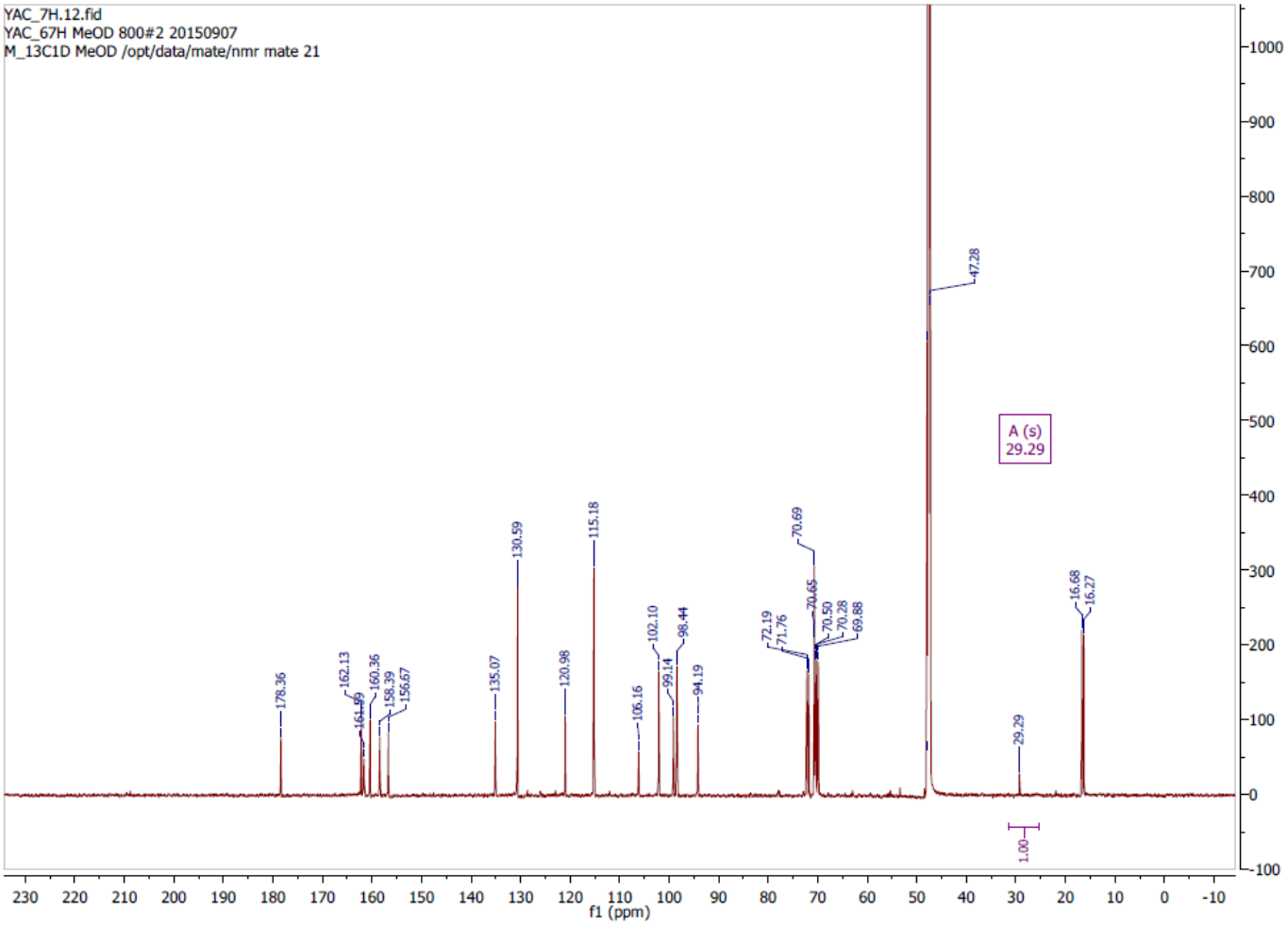
APPENDIX 49C: LCMS Spectrum of Tachrosin (41)



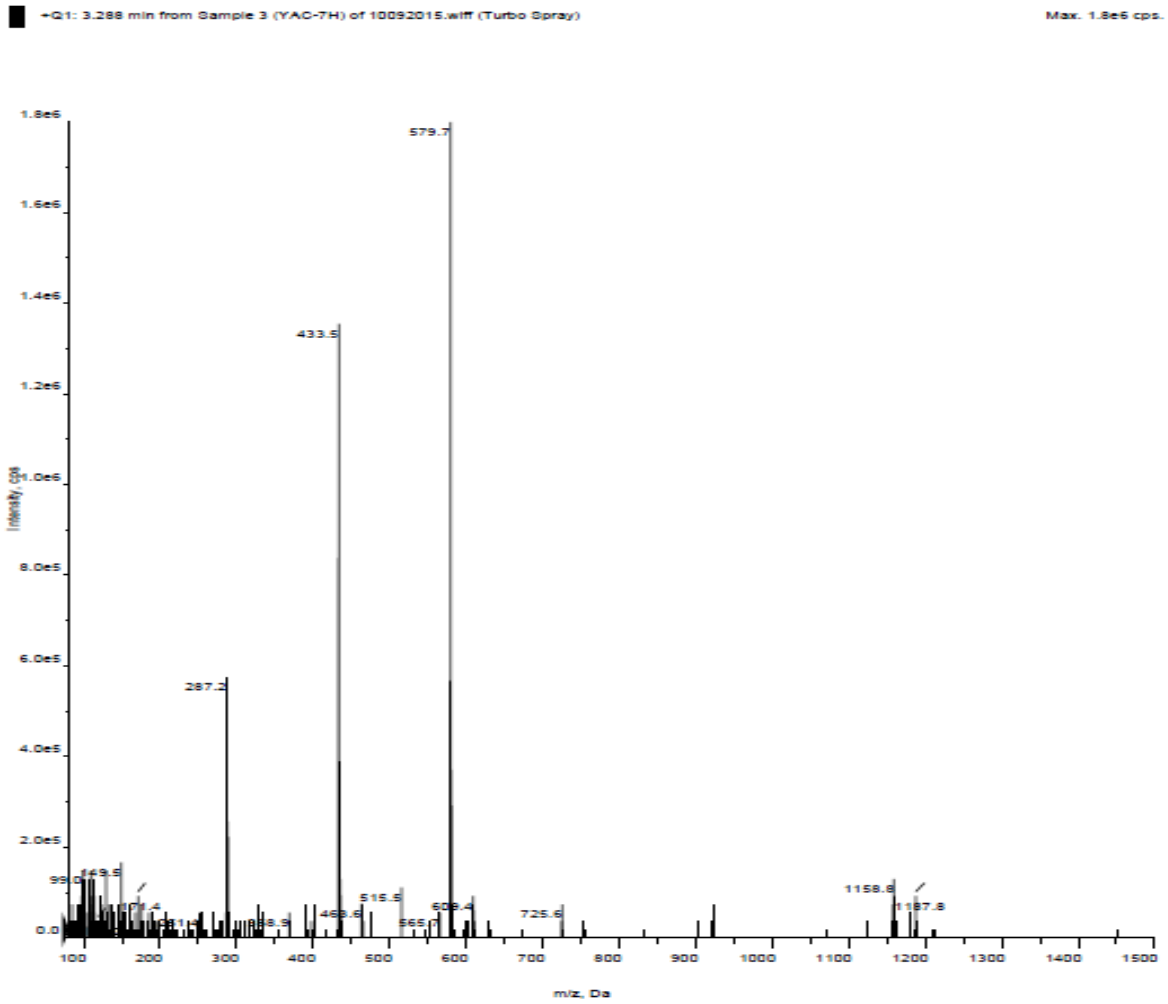
APPENDIX 50A: ^{1}H NMR Spectrum of Kaempferitrin (200) (800 MHz; MeOD)



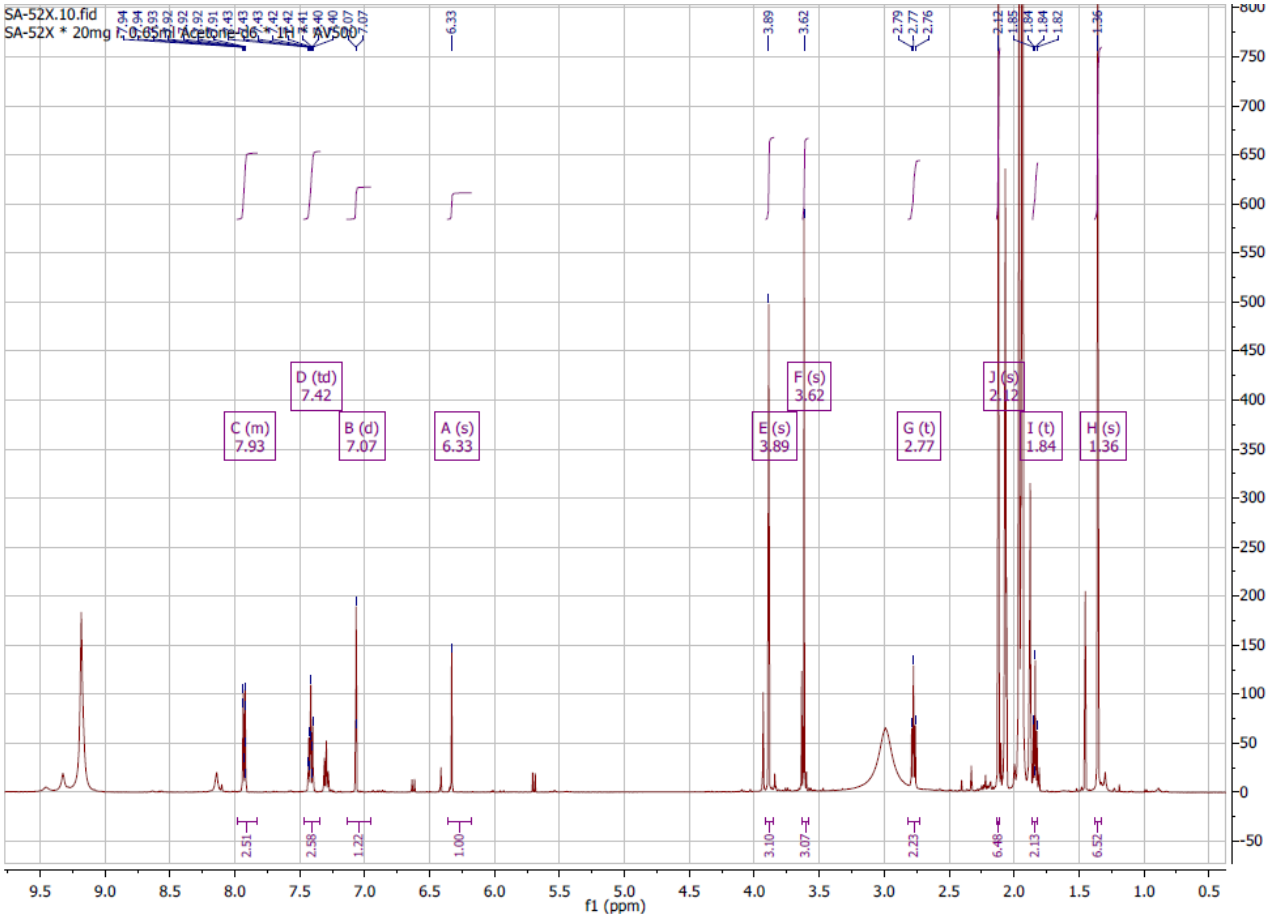
APPENDIX 50B: ^{13}C NMR Spectrum of Kaempferitrin (200) (200 MHz; MeOD)



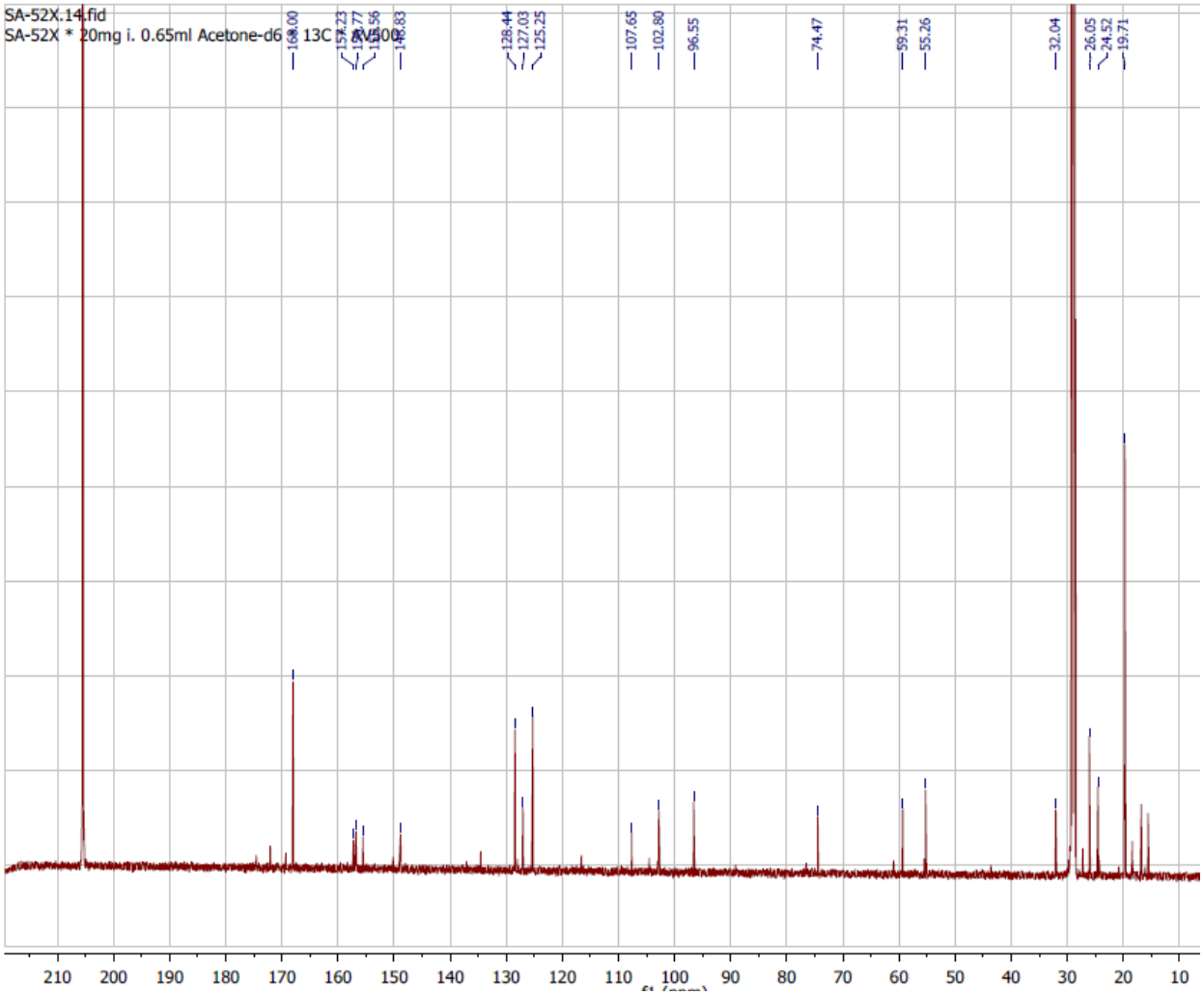
APPENDIX 50C: LCMS Spectrum of Kaempferitrin (200) (MeOD)



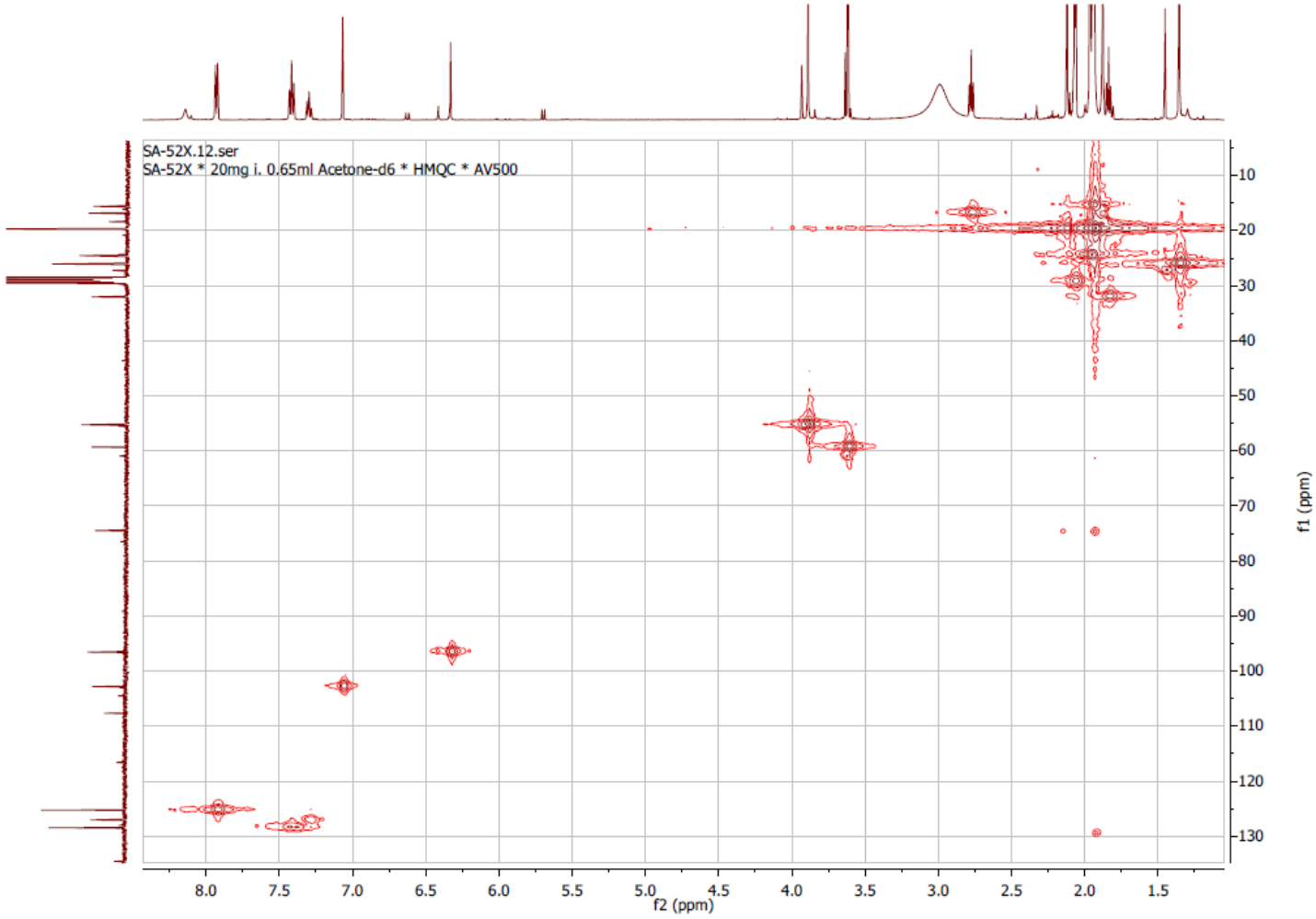
APPENDIX 51A: ^{1}H NMR Spectrum of Pyrazopraecansone B (201) (500 MHz; Acetone)



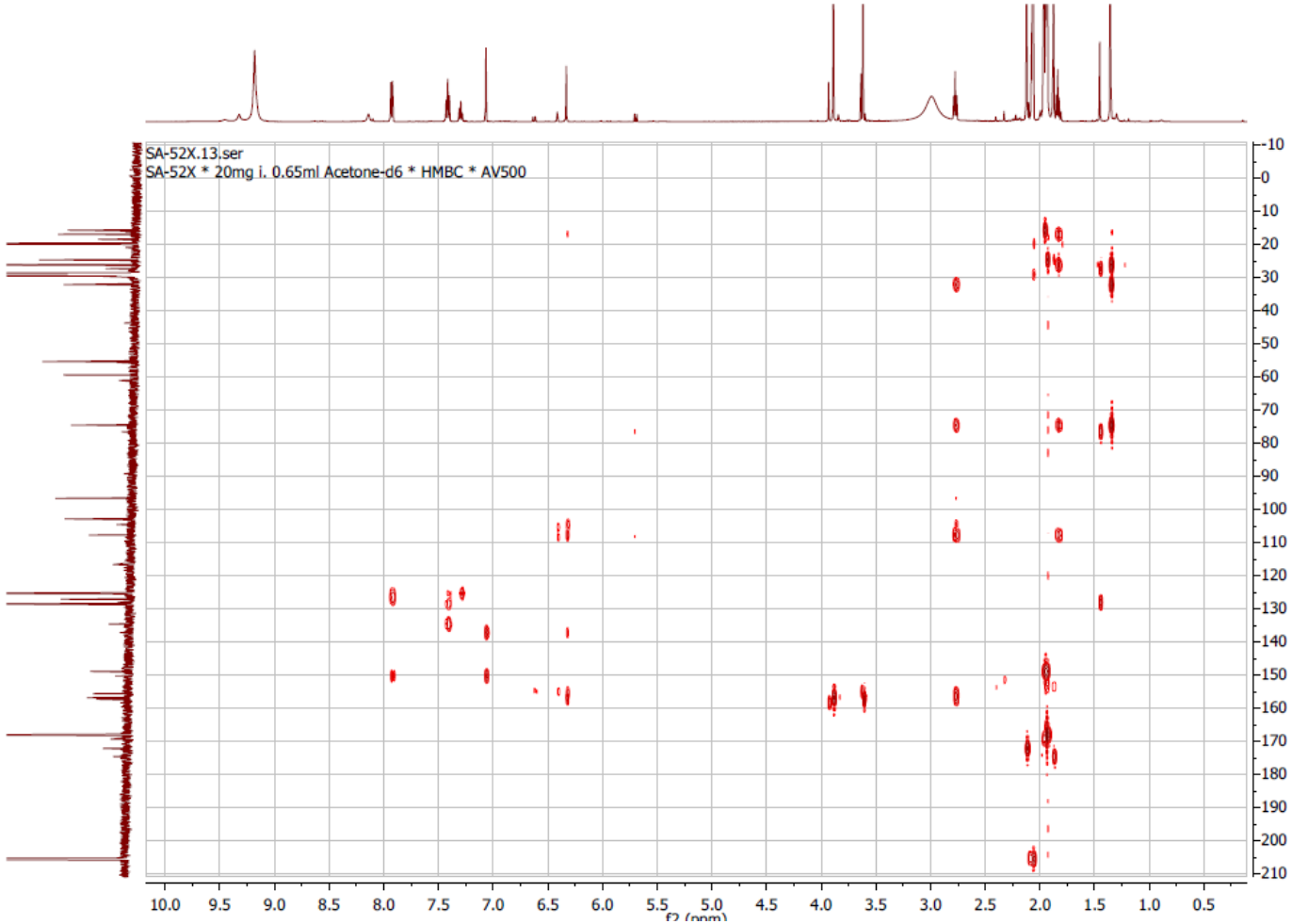
APPENDIX 51B: ^{13}C NMR Spectrum of Pyrazopraecansone B (201) (125 MHz; Acetone)



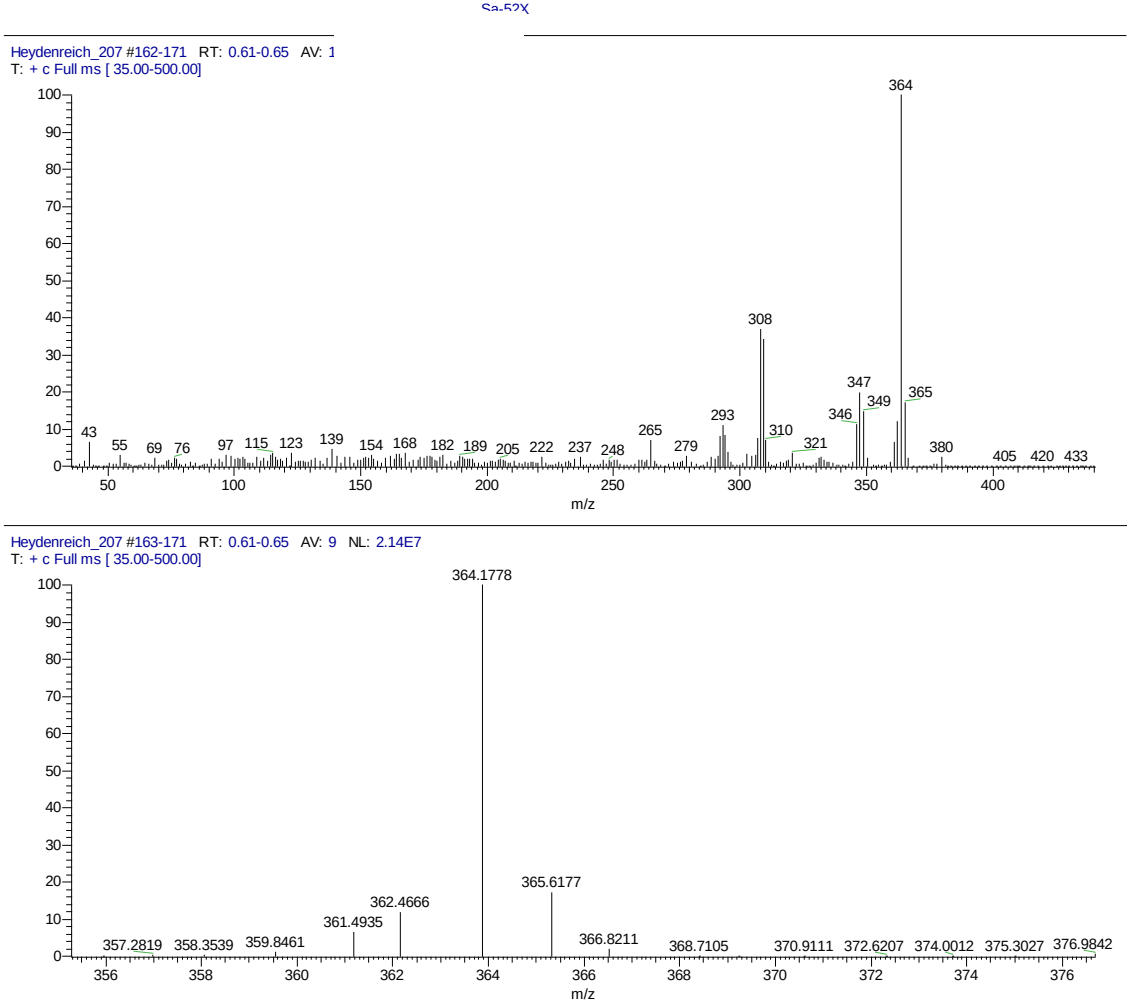
APPENDIX 51C: HSQC Spectrum of Pyrazopraecansone B (201) (Acetone)



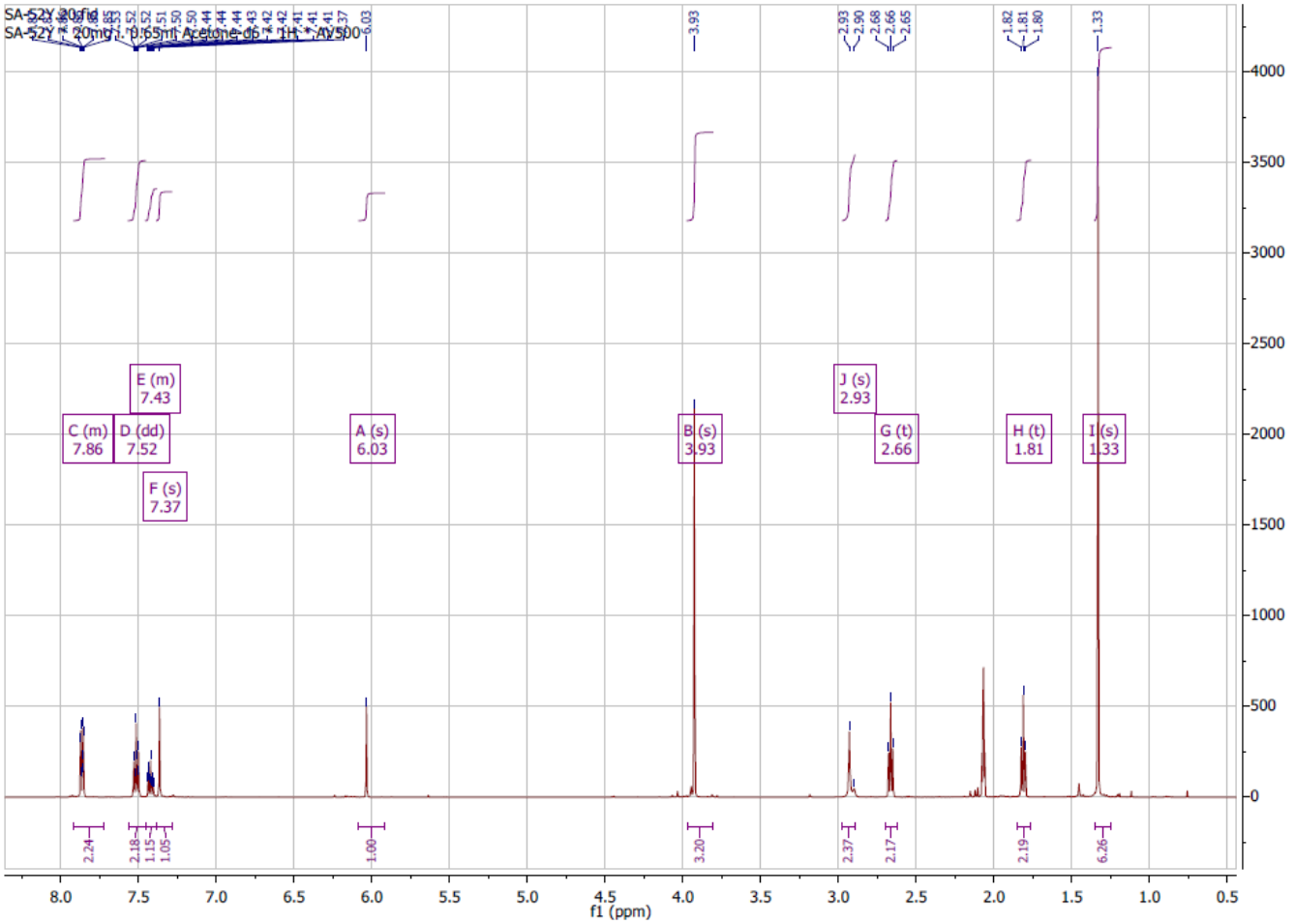
APPENDIX 51D: HMBC Spectrum of Pyrazopraecansone B (201) (Acetone)



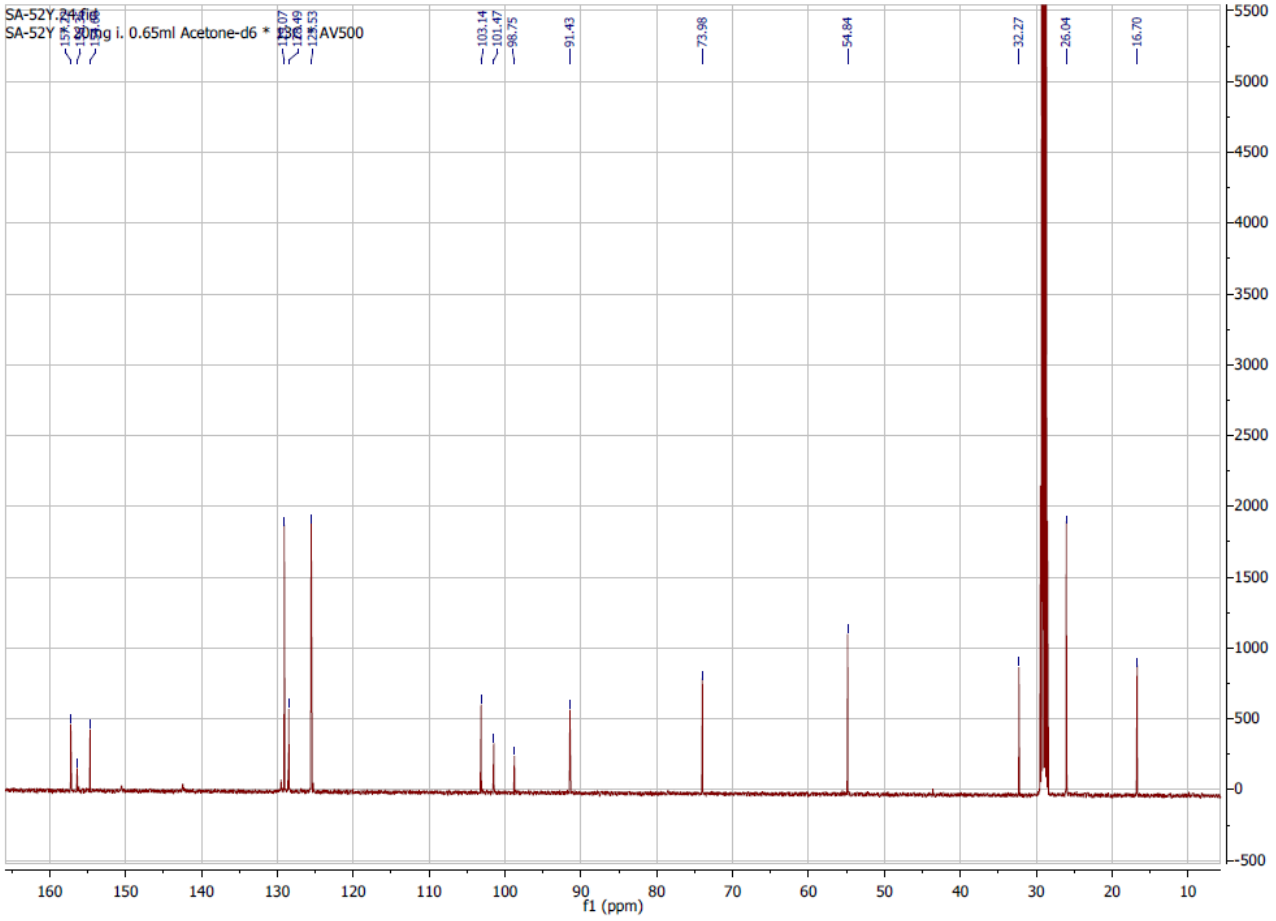
APPENDIX 51E: HRMS Spectrum of Pyrazopraecansone B (201)



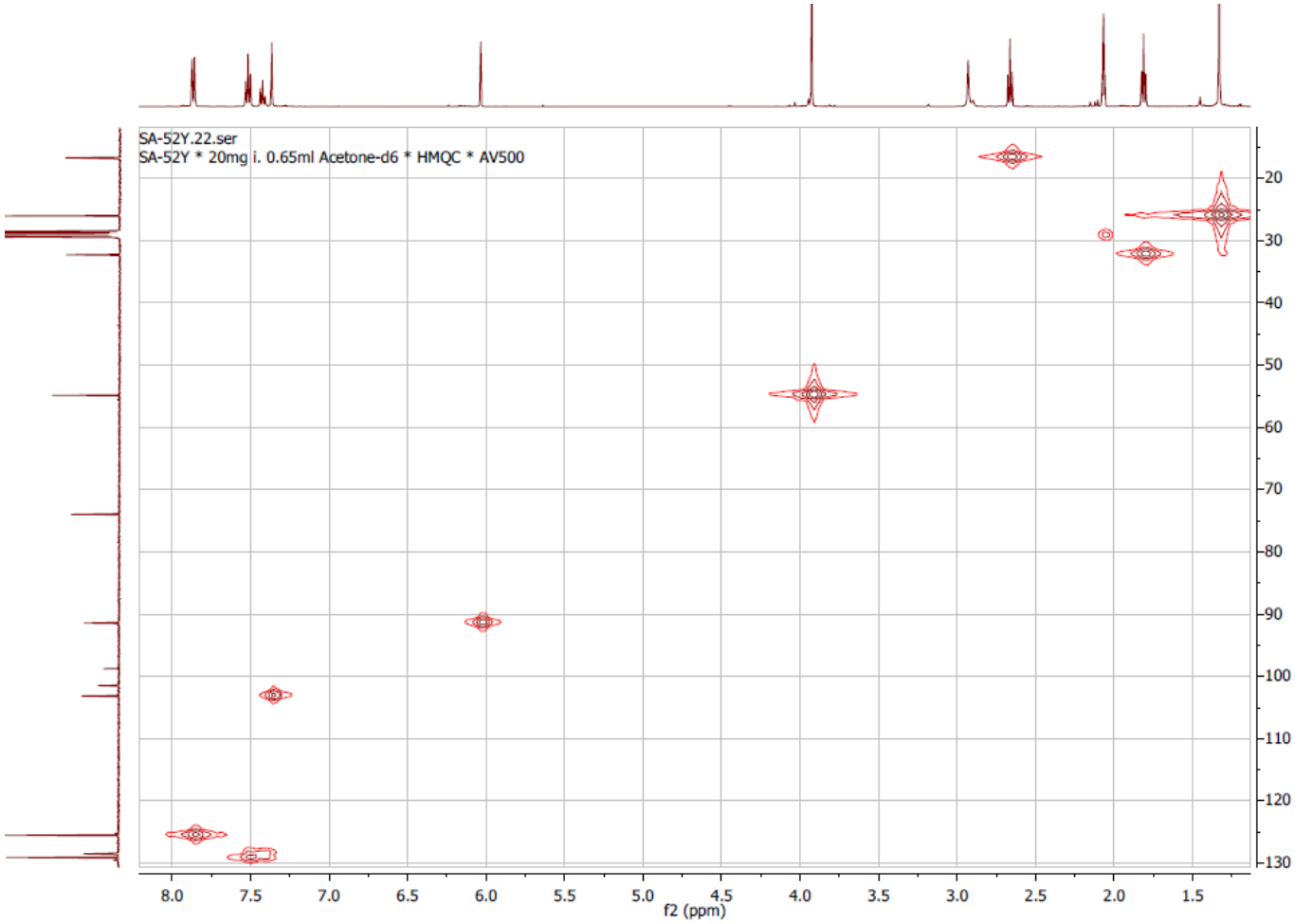
APPENDIX 52A: ^{1}H NMR Spectrum of Pyrazoisopongaflavone (202) (500 MHz; Acetone)



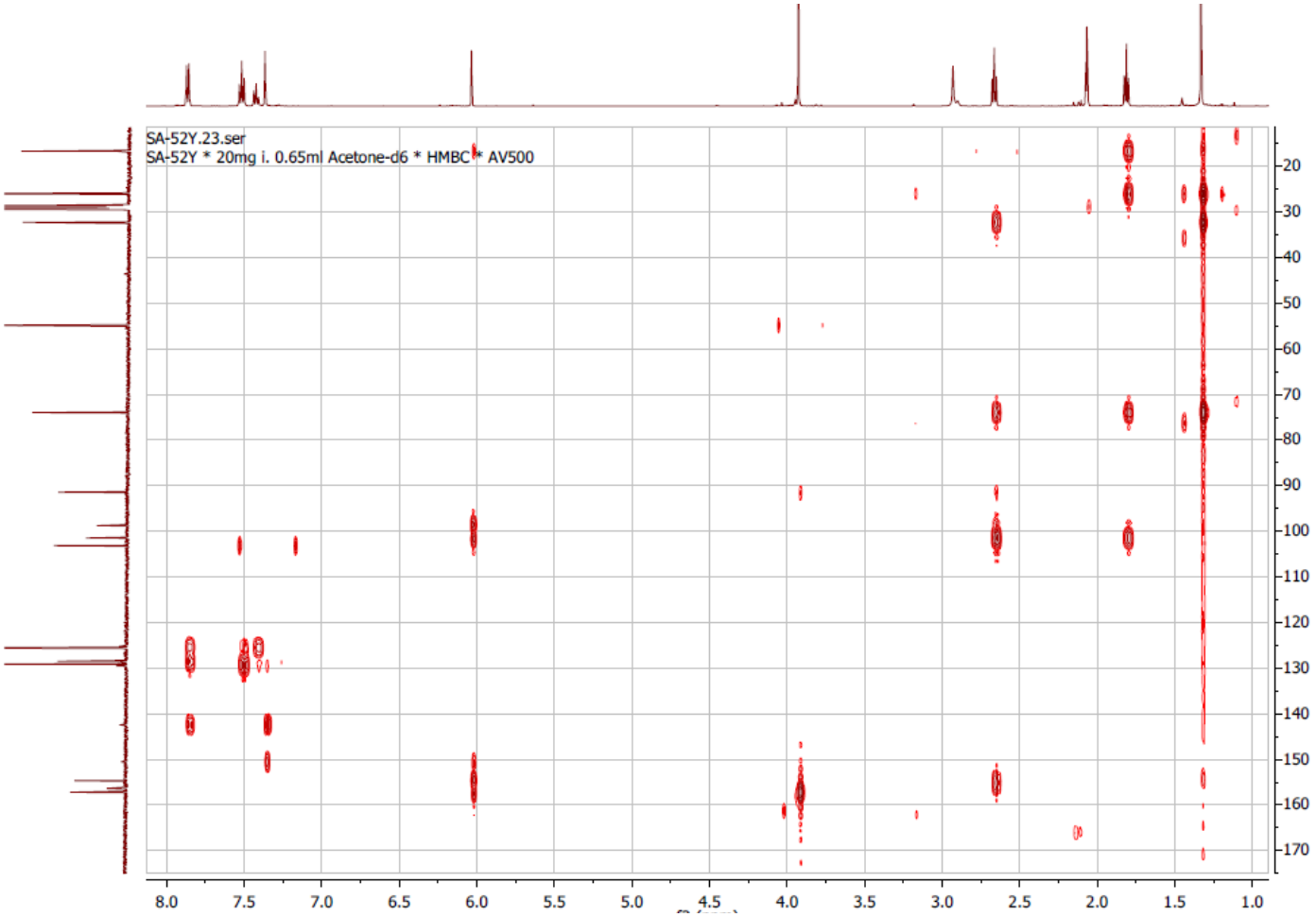
APPENDIX 52C: ^{13}C NMR Spectrum of Pyrazoisopongaflavone (202) (125 MHz; Acetone)



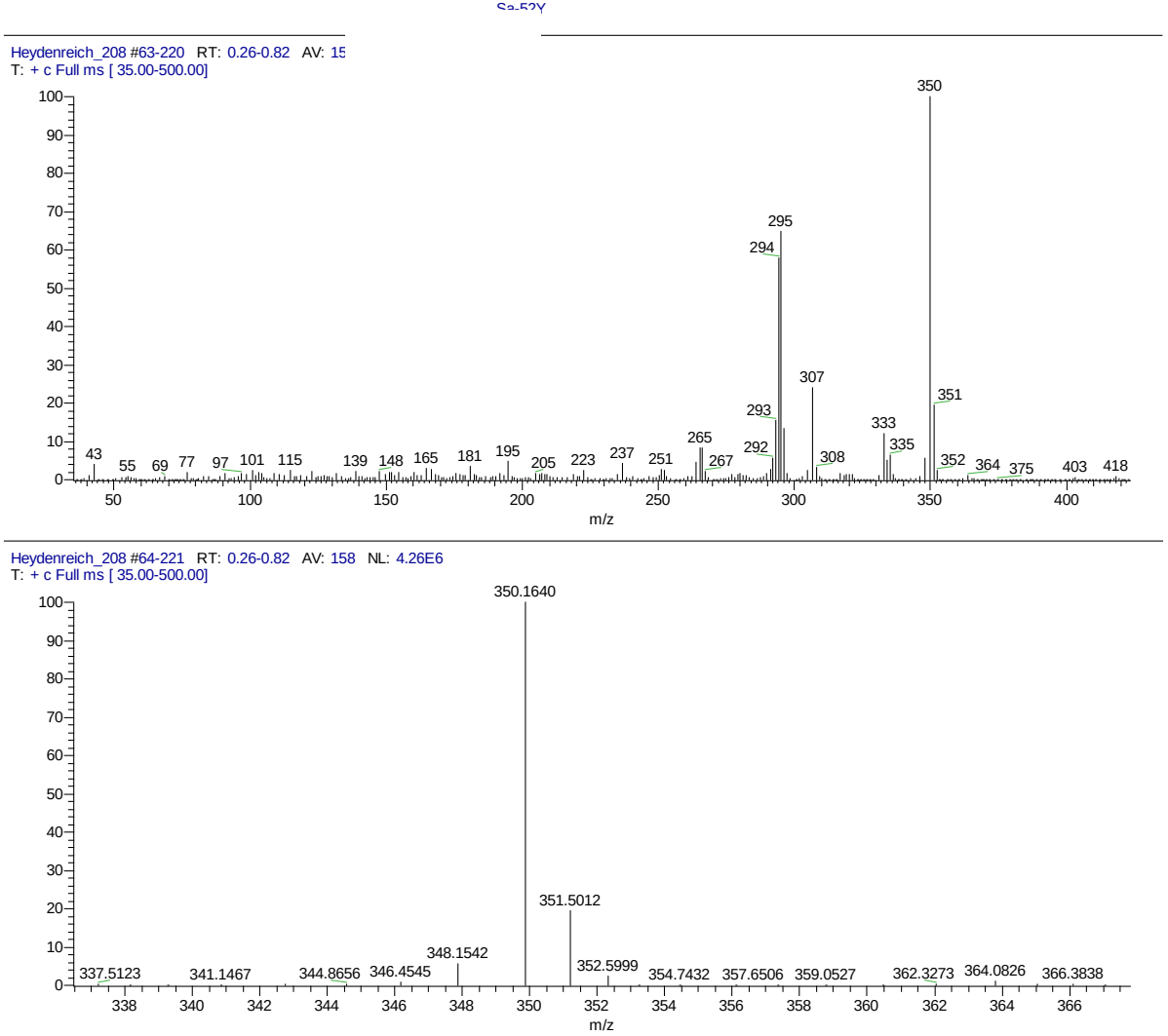
APPENDIX 52D: HSQC Spectrum of Pyrazoisopongaflavone (202) (Acetone)



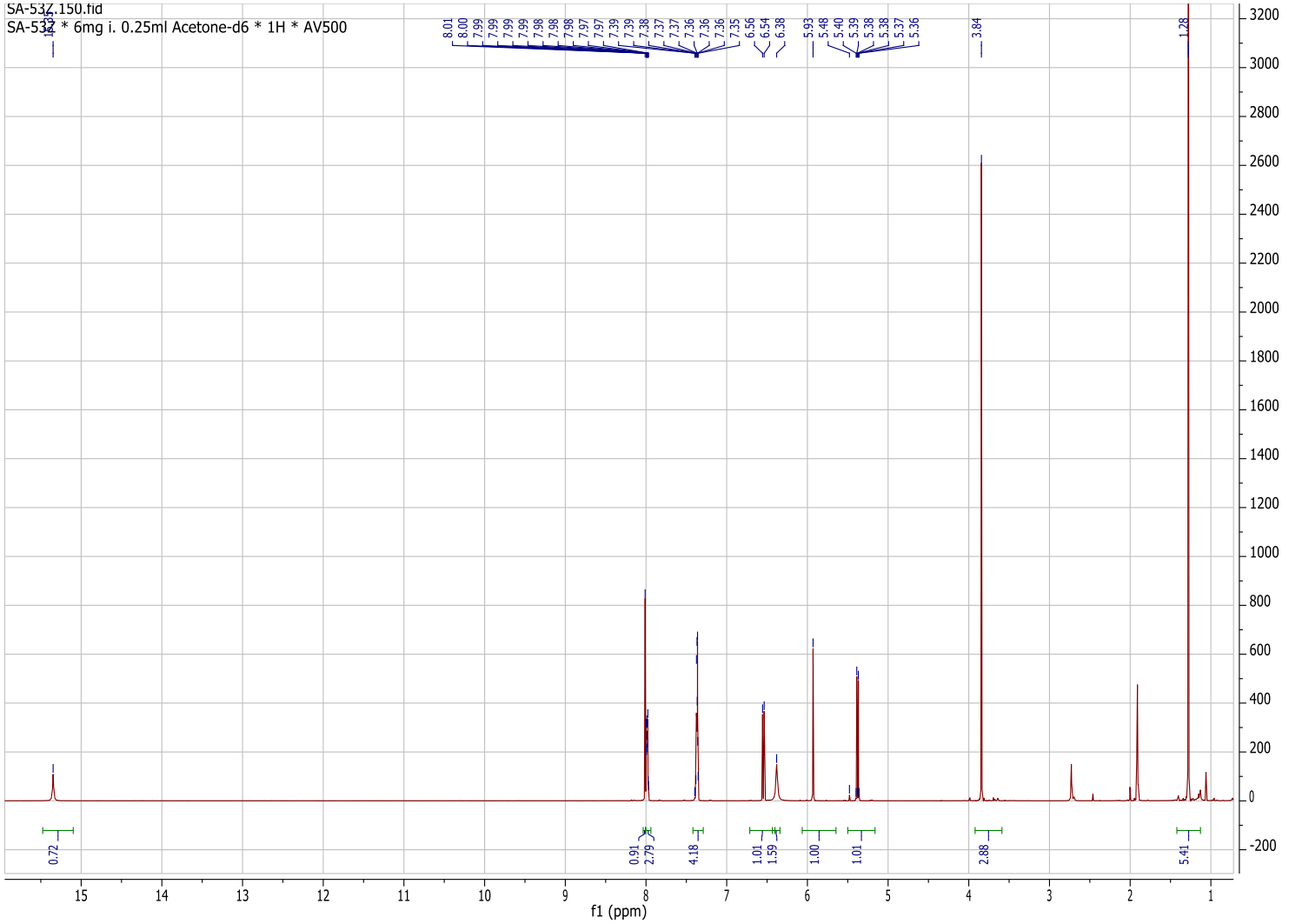
APPENDIX 52D: HMBC Spectrum of Pyrazoisopongaflavone (202) (Acetone)



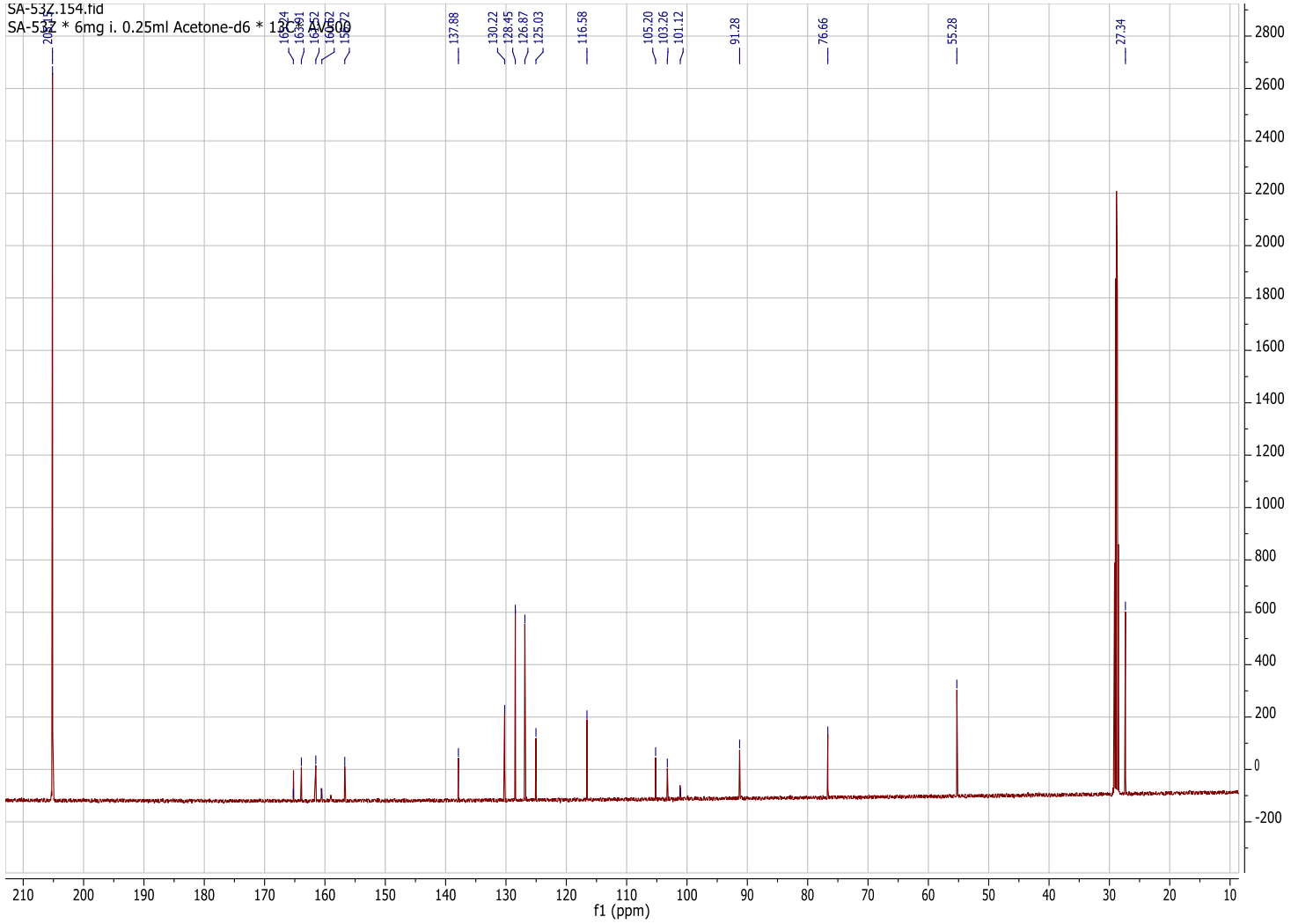
APPENDIX 52E: HRMS Spectrum of Pyrazoisopongaflavone (202)



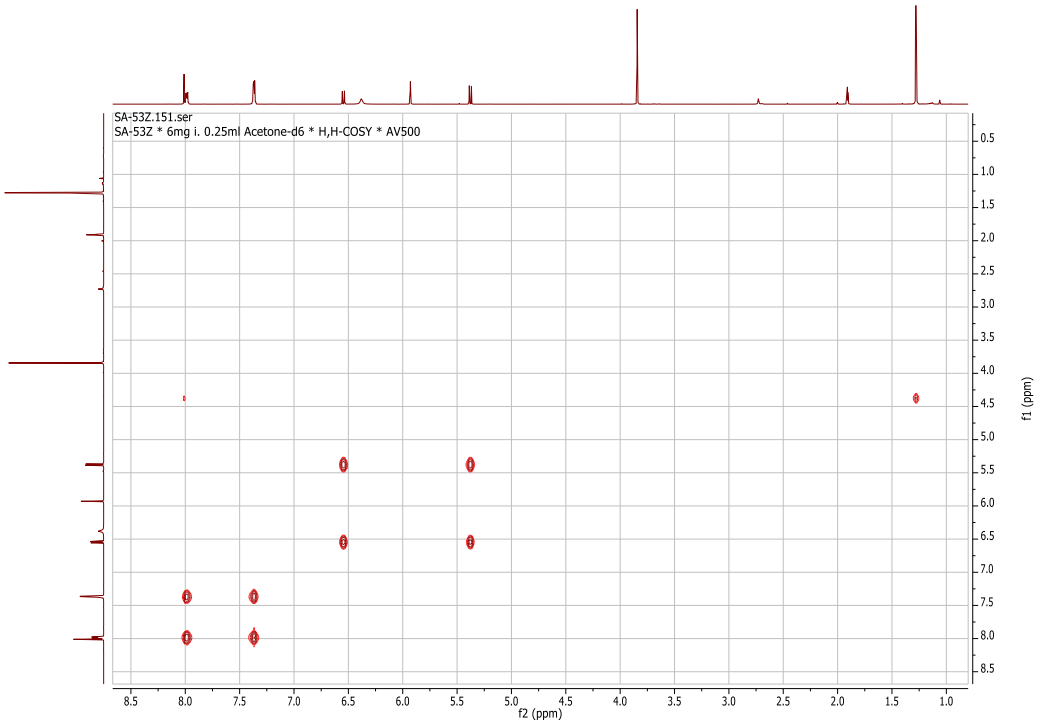
APPENDIX 53A: ^{1}H NMR Spectrum of Guanidinoisopongaflavone (203) (500 MHz; Acetone)



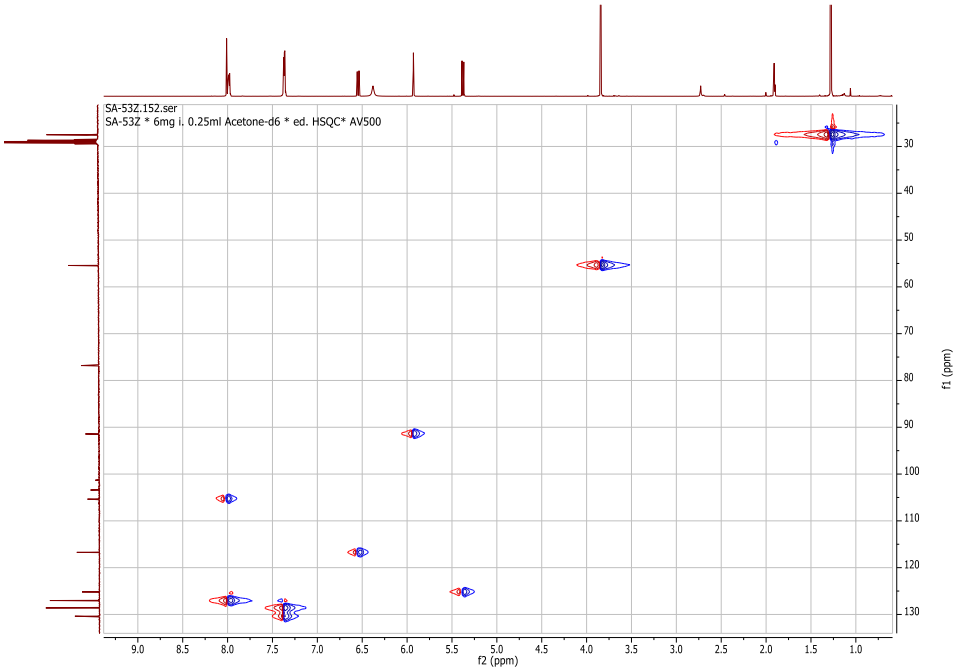
APPENDIX 53B: ^{13}C NMR Spectrum of Guanidinoisopongaflavone (203) (125 MHz; Acetone)



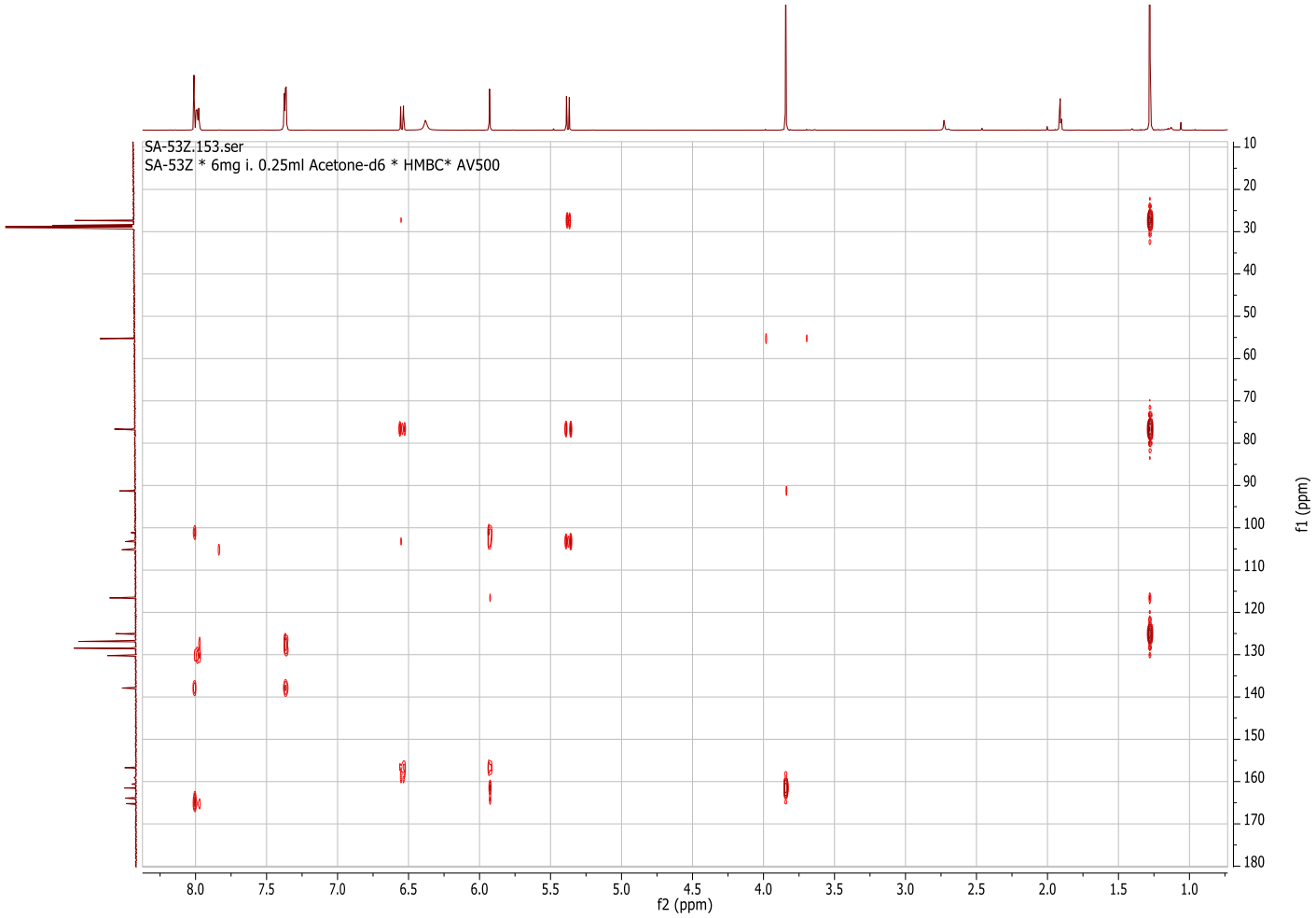
APPENDIX 53C: HH-COSY Spectrum of Guanidinoisopongaflavone (203) (Acetone)



APPENDIX 53D: HSQC Spectrum of Guanidinoisopongaflavone (203) (Acetone)



APPENDIX 53E: HMBC Spectrum of Guanidinoisopongaflavone (203) (Acetone)



APPENDIX 53F: HRMS Spectrum of Guanidinoisopongaflavone (203)