UNIVERSITY OF NAIROBI
COLLEGE OF BIOLOGICAL AND PHYSICAL SCIENCES
DEPARTMENT OF CHEMISTRY

PHYTOCHEMICAL INVESTIGATION OF THREE LEGUMINOSAE PLANTS FOR CANCER CHEMOPREVENTIVE AGENTS

BY
IVAN GUMULA

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)
IN CHEMISTRY OF THE UNIVERSITY OF NAIROBI

2014

DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.

Ivan Gumula
(I80/82102/2009)

This thesis has been submitted with our approval as University supervisors.

Date 23/7/2014

Prof. Abiy Yenesew
Department of Chemistry, University of Nairobi

Date 23-07-14

Dr. Solomon Derese
Department of Chemistry, University of Nairobi

Date 07.07.2014

Prof. Isaiah O. Ndiege
Department of Chemistry, Kyambogo University

DEDICATION

This work is dedicated to my family

ACKNOWLEDGEMENTS

First of all, I would like to thank the University of Nairobi for admitting me as a Doctoral student. I wish to extend my heartfelt gratitude to my supervisors; Prof. Abiy Yenesew, Dr. Solomon Derese and Prof. Isaiah O. Ndiege whose close supervision coupled with resourceful guidance/advice enriched me with the knowledge, skills and attitude resulting in the success of this research. I am grateful to the German Academic Exchange Services (DAAD) and the Natural Products Research Network for Eastern and Central Africa (NAPRECA) for financial support during my studies. I appreciate the help extended to me by Dr. Matthias Heydenreich of the University of Potsdam in spectroscopic/spectrometric analyses of some of the compounds reported in this thesis. Special thanks go to the Swedish Institute for sponsoring my research visit to the University of Gothenburg. I am indebted to my host supervisor, Prof. Máté Erdélyi, and the Halogen Bond Research Group of the Department of Chemistry and Molecular Biology, at the University of Gothenburg for his unwavering support and guidance in isolation and spectroscopic techniques and analysis. My sincere gratitude is extended to Dr. John P. Alao and Prof. Per Sunnerhagen of the Department of Chemistry and Molecular Biology at the University of Gothenburg for carrying out cytotoxicity assays. I acknowledge Mr Simon G. Mathenge of the East African Herbarium, Nairobi, for the identification of the plant species studied. I wish to thank my fellow postgraduate students in the research group at the Department of Chemistry, University of Nairobi: Dr. Milkyas Endale, Dr. Francis Machumi, Dr. Hannington Twinomuhwezi, Ms. Martha Induli, Mr. Denis Akampurira, Mr. Robert Masinde and Mr. Daniel Buyinza for their cooperation and moral support. Last but not least, I wish to appreciate my beloved wife Prossy and children Elizabeth, Nathan and Dorcus, for the support they accorded to me throughout the study period. Glory is to the Almighty God-the *alpha* and the *omega*.

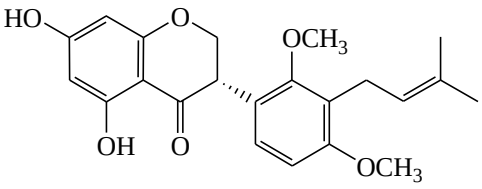
ABSTRACT

Cancer is one of the largest single and common causes of death in humans around the world. It is estimated to have claimed up to 8.2 million lives in the year 2012 and with an incidence of 14.1 million new cases. Plant metabolites such as flavonoids have been reported to display cancer chemopreventive properties such as anti-oxidant, anti-estrogenic effects and cytotoxicity. Three Leguminosae plants, *Platycelphium voënse, Millettia usaramensis* subsp. *usaramensis* and *Flemingia grahamiana,* were phytochemically investigated to give a range of flavonoid derivatives, two triterpenoids and an anthraquinone. Twenty three of the isolated compounds are new and their structures were established using a combination of techniques including NMR, UV, CD and MS.

From the stem bark of *Platycelphium voënse* twelve compounds, including seven isoflavanones, one isoflavone, two 3-methoxyflavones and two triterpenes, were isolated and identified. Five of the isoflavanones including platyisoflavanones A (**163**) and C (**166**) are new. Similar investigation of the root bark of *Millettia usaramensis* subsp. *usaramensis* resulted in the isolation of thirteen compounds including two new flavanone derivatives: (2*R*,3*R*)-4'-*O*-geranyl-7-hydroxyflavanonol (**179**) and (*S*)-4'-*O*-geranyl-7-hydroxyflavanone (**180**) in addition to seven known 12a-hydroxyrotenoids, two isoflavones, one chalcone and a cinnamoyl alcohol derivative. From the roots of *Flemingia grahamiana,* seven isoflavones and five prenylated flavanones, including a new flavanone derivative, 5,3',4'-trihydroxy-8-γ,γ-dimethylallyl-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**191**), along with a chromone and a gallocatechin derivative were obtained. Exposure of 8-(γ,γ-dimethylallyl)-2",2"-dimethylpyrano-[5",6":6,7]-flavanones to deuterated dimethylsulfoxide led to isomerization of the flavones yielding the corresponding 6-γ,γ-dimethylallyl-2"',2"'-dimethylpyrano-[5"',6"':8,7]-flavanone isomers. This is the first report of such rearrangement. From the leaves of *F. grahamiana,* a range of flavonoids were isolated and identified. These included the known chalcone, flemingin A (**196**) and eleven new chalcones [including flemingin G (**197**)], two new flavanones and two new *Z*-aurones [such as flemingiaurone A (**211**)].

Five rotenoids, one triterpene, one isoflavanone and two chalcones were active against the MCF-7 human breast cancer cell line. Compounds **163** and **196** were cytotoxic against Vero cells and both were active against *Mycobacterium tuberculosis*. Compound **196** and four other chalcones exhibited strong radical scavenging activity against DPPH.

Attempt to synthesize 2,5,2′-trihydroxy-4′,5′-dimethoxychalcone from 2-hydroxy-4,5-dimethoxyacetophenone and 2,5-diallyloxybenzaldehyde in the presence of KOH/methanol led to the formation of 2,5-diallyloxy-2′-hydroxy-4′,5′-dimethoxychalcone. Deprotection of the allyl groups using $Pd(PPh_3)_4$, prepared *in-situ*, in the presence of K_2CO_3/methanol, yielded a partially deprotected, 5-allyloxy-2,2′-dihydroxy-4′,5′-dimethoxychalcone.



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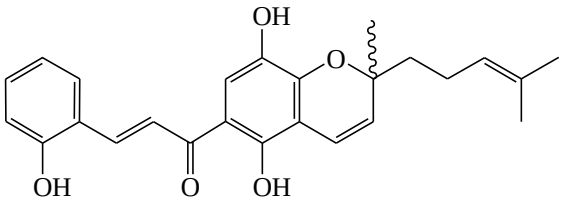

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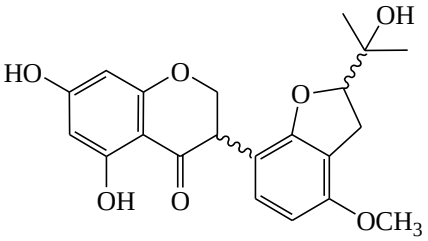

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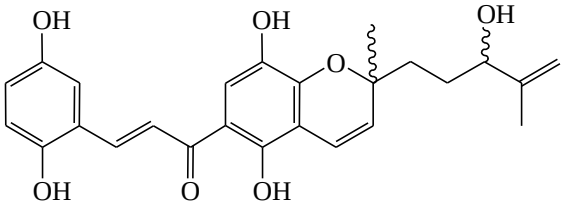

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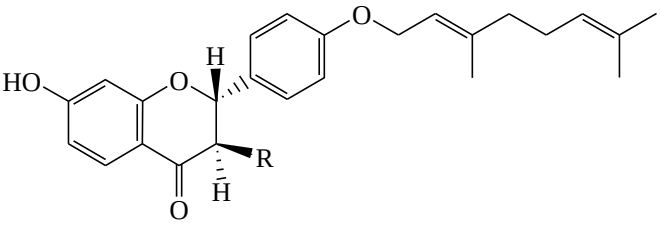
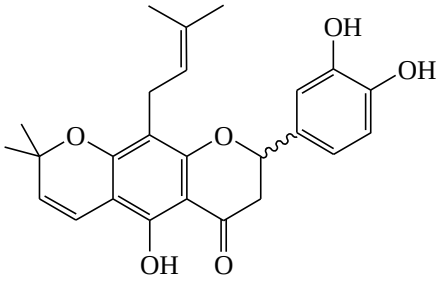


179: R = OH
180: R = H



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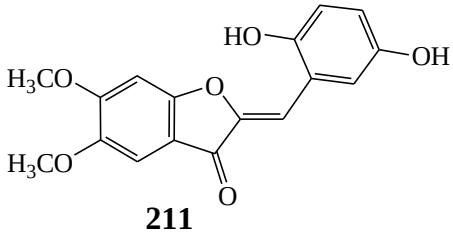

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LIST OF ABBREVIATIONS AND SYMBOLS

α_D^{24}	Specific rotation determined at the sodium *D*-line wavelength (589 nm) at 24 °C
BCR-ABL	Abelson gene-break point cluster gene fusion
br s	Broad singlet
CC	Column chromatography
CD	Circular dichroism
dd	Doublet of doublets
ddt	Doublets of doublets of triplets
DEPT	Distortionless enhancement by polarization transfer
δ	Chemical shift
DPPH	1,1-Diphenyl-2-picrylhydrazyl radical (α,α-Diphenyl-β-picrylhydrazyl)
dt	Double of triplets
EC_{50}	Concentration at 50% effectiveness
LC-ESI-MS	Liquid chromatography electro-spray ionization mass spectrometry
gCHSQC	Gradient carbon-proton heteronuclear single quantum coherence.
gCOSY	Gradient correlation spectroscopy
gHMBC	Gradient heteronuclear multiple bond correlation
gNOESY	Gradient nuclear overhauser effect spectroscopy
HPLC	High pressure liquid chromatography
HRESI-MS	High resolution electro-spray ionization mass spectroscrometry
Hz	Hertz
IARC	International agency of research on cancer
J	Coupling constant
λ_{max}	Wavelength at maximum absorption
MHz	Mega hertz
MIC	Minimum inhibition concentration
MPLC	Medium pressure liquid chromatography
$[M]^+$	Molecular ion peak
$[M+H]^+$	*Pseudo*-molecular ion peak
m	Multiplet
m/z	Mass to charge ratio
NMR	Nuclear magnetic resonance
NOE	Nuclear overhauser effect
NO	Not observed
prep-HPLC	Preparative high pressure liquid chromatography
prep-TLC	Preparative thin layer chromatography
Q-TOF-LC/MS	Quadrupole time-of-flight liquid chromatography-mass spectrometry
q	Quartet
[θ]	Degrees of ellipticity
t	Triplet
TLC	Thin layer chromatography
UV	Ultra violet

CHAPTER ONE
INTRODUCTION

1.1 General

Cancer refers to a group of diseases characterized by unregulated cell growth and division due to loss of balance between cell production and cell death within a tissue/organ of an individual [Nicolaou *et al.*, 1998; Stewart and Kleihues, 2003]. This uncontrolled growth usually leads to invasion of the surrounding parts of the body, causing interference in functioning of organs which, if not checked, may culminate into death [Boyle and Levin, 2008; Nicolaou *et al.*, 1998;]. It is estimated that cancer claimed up to 8.2 million lives in the year 2012 and with an incidence of 14.1 million new cases [Ferlay *et al*., 2013]. However, these figures may be underestimates because of unreliability of medical/deaths records especially in Africa, Asia and South America.

In the year 2012, prevalence of cancer stood at 32.6 million people (over 15 years of age) [Bray *et al*., 2013]. In the same year, lung cancer was the most commonly diagnosed form of cancer worldwide standing at 1.8 million cases, followed by cancer of the breast (1.7 million), and colorectum (1.4 million) [Ferlay *et al*., 2013]. The most common causes of cancer deaths in the same year, were lung (1.6 million), liver (0.8 million), and stomach cancer (0.7 million) [Ferlay *et al*., 2013].

In sub-Saharan Africa 34.8 new cases of cervical cancer are reported per 100, 000 women annually, and 22.5 lives lost compared to 6.6 and 2.5 per 100 000 women, respectively, in North America [Ferlay *et al*., 2013]. The differences in the figures have been attributed to lack of awereness, access to regular screening and medical facilities in Africa that would enable early diagnosis and treatment [Ferlay *et al*., 2013].

There are multiple treatment approaches available for cancer patients including surgery, radiotherapy, photodynamic therapy, chemotherapy and immunotherapy (biological therapy) [Thurston, 2007]. Each of these modalities is useful individually in particular situations and when combined they can offer effective treatment to cancer patients. An array of anticancer drugs has been developed, many of which are of plant origin. Although there has been tremendous success in use of chemotherapy in the treatment of childhood cancers such as

leukemia, Wilms and Ewing's sarcoma, testicular tumors, non-Hodgkin's lymphoma that were formerly fatal, there are still difficulties in the treatment of slow growing adult solid tumours such as lung, colon, breast, ovarian, prostatic, pancreatic and brain cancer [Suffness, 1987]. Furthermore, most drugs used in the treatment of cancer are highly toxic, not only to cancer cells, but also to normal host cells that have a high growth rate. Side effects such as alopecia (hair loss), gastro-intestinal ulceration, fertility impairment, immune suppression, and blood dyscrasis are unavoidable in cancer patients on chemotherapy [Thurston, 2007].

Since the 1960s, there is a growing interest in cancer chemoprevention which is focused at preventing, delaying or reversing cancer development using dietary (nutriceuticals) or pharmaceutical agents.

1.2 Problem Statement

Majority of the agents used in cancer chemotherapy are associated with several adverse side-effects. Furthermore, multiple drug resistance is a problem that has been experienced in attempts to treat cancer related diseases. It is, therefore, imperative to continue searching for compounds that may be safe and with novel modes of action.

1.3 Objectives

1.3.1 General Objective

The main objective of this study was to isolate compounds from three Leguminosae plants (*Platycelphium voënse, Millettia usaramensis* subsp *usaramensis* and *Flemingia grahamiana*) and determine their cancer chemopreventive activities.

1.3.2 Specific Objectives

The specific objectives of this study were to:

1. Isolate compounds from crude extracts of *Platycelyphium voënse, Millettia usaramensis* subsp. *usaramensis* and *Flemingia grahamiana*;
2. Elucidate the chemical structures of the isolated compounds;
3. Establish the cytotoxicity, antioxidant properties and anti-tuberculosis activities of the crude extracts and the isolated compounds;
4. Prepare analogues of compounds with significant biological activities.

1.4 Justification of the Study

Free radicals are some of the substances responsible for genotoxicity and initiation of carcinogenesis [Boik, 2001; Stewart and Kleihues, 2003; Thurston, 2007] therefore, compounds with radical scavenging activities may significantly prevent certain types of cancer. Flavonoids and isoflavonoids are among different natural products with potential cancer chemotherapy through radical scavenging.

Studies so far carried out on some Papilionoideae plants indicated that the sub-family furnishes a variety of compounds with diverse chemical structures [Harborne and Williams, 2000; Van Wyk, 2003; Veitch, 2007, 2009]. It was, therefore, deemed likey that this study would lead to identification of compounds with interesting biological activities, especially in regard to cancer prevention.

Plants from this sub-family are used in traditional medicine for the treatment of a wide range of diseases in tropical and sub-tropical regions [Bally, 1937; Pickard, and Cox, 1986; Kokwaro, 1993; Katende, 1995] and this was one of the motivating factors for the search of the active ingredients. The resinous oil from *Lonchocarpus*, *Millettia* and *Derris* species is a source of rotenoids that have found use as insecticidal agents [Fukami and Nakajima, 1971]. Some of the rotenoids (and other isoflavonoids) have shown anticancer properties [Ito, 2000; 2004a; Gerhäuser *et al.*, 1995; Fang et al., 1998].

Although the three Legumes: *Platycelphium voënse*, *Millettia usaramensis* and *Flemingia grahamiana* are known (or expected) to contain flavonoids and isoflavonoids, their chemical constituents have not been studied for cancer chemopreventive properties.

CHAPTER TWO
LITERATURE REVIEW

2.1 Causes of Cancer

Carcinogenesis is caused by modifications (mutation/damage) of the genome brought about by internal factors, external (environmental) factors or by hereditary factors [Thurston, 2007; Nicolaou *et al*, 1998].

2.1.1 Internal factors

Formation of a tumour may result from changes in the structure or sequence of the DNA double helix strand, brought about by malfunctions of the DNA processes within a given cell. The malfunctions may take the form of mutation, addition or deletion of genetic material, epigenetic changes (mechanisms to regulate gene expression independent of DNA sequence, but rather functional changes - usually methylation of cytosine), uncontrolled gene expression or gene amplification [Martinez *et al*., 2003; Thurston, 2007].

Free radicals (such as hydroxyl and superoxide) are among a range of agents that can cause DNA damage. Internally, free radicals may be side-products of cellular immune activity and respiration [Boik, 2001].

2.1.2 External (environmental) factors

External factors such as chronic infections from pathogens and parasites; contact or ingestion/inhalation of certain chemicals as well as exposure to hazardous radiations contribute to carcinogenesis [Stewart and Kleihues, 2003].

2.1.2.1 Chronic infections

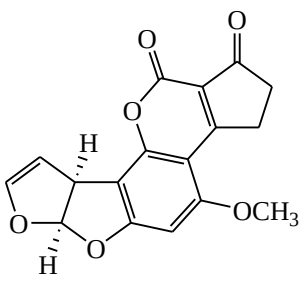
Chronic infections of certain bacteria, viruses and parasites may lead to cancer. Examples include *Helicobacter pylori* responsible for cancer of the stomach; hepatitis B and C, liver flukes (liver cancer); human papilloma virus (cervical cancer and cancer of the uterus); Epstein-Barr virus (lymphomas and nasopharyngeal carcinoma); HIV (Kaposi sarcoma); *Schistosoma haematobium* (bladder cancer); human T-cell lymphotropic virus (Adult T-cell leukaemia/lymphoma) [Pisani *et al.*, 1997; Walboomers *et al.*, 1999; Rolon *et al*., 2000].

2.1.2.2 Chemicals in the environment

Certain chemicals in the environment and some encountered at homesteads, as well as through diet, lifestyle, and at workplaces are carcinogenic or procarcinogenic. For example cigarette smoke contains carcinogenic agents: *N*-nitrosamines, volatile aldehydes, heavy metals such as polonium-210, polycyclic aromatic hydrocarbons such as benzo[a]pyrene [Rodgman *et al*., 2000; Brunnemann *et al*., 1996]. Thus, tobacco smoking has been associated with lung cancer, cancer of the bladder, oesophageal cancer, pancreatic cancer, kidney cancer and many others [Rodgman *et al*., 2000; Brunnemann *et al*., 1996].

Humans are also exposed to carcinogenic polycyclic aromatic hydrocarbons through overcooked meat (especially through frying and barbecuing) and this has been linked to cancer of the colon [O'Neill *et al.*, 1991; Layton *et al*., 1995]. Smoked fish are sources of potent carcinogenic nitroso compounds [O'Neill *et al.*, 1991; Layton *et al*., 1995]. These compounds may also be formed from reactions in foods containing added nitrites and nitrates as preservatives [Stewart and Kleihues, 2003].

Certain natural food contaminants including aflatoxins, such as aflatoxin B_1 (**1)** produced by *Aspergillus* fungi that grow on maize and peanut (groundnuts) in hot humid conditions, particularly during storage, have been categorized as carcinogenic harzards [Smela *et al.*, 2001; Wild and Hall, 2000]. Aflatoxins and hepatitis B virus (HBV) infection are the main risk factors for the high incidenc of liver cancer in sub-Saharan Africa, parts of Asia and South America [Wild and Hall, 2000]. Other carcinogenic food contaminants include: fumonisin B_1 (**2**) and fusarin C (**3**) both produced by *Fusarium verticillioides* (fungus) that grows on maize grain and is associated with oesophageal cancer; ochratoxin A (**4**, a fungal metabolite) and a contaminant of grain and pork products which is associated with nephropathy, uretherial urinary tract tumours [IARC, 1993; Castegnaro *et al*., 1991]; pyrrolizidine alkaloids (plant toxins), which are contaminants of cereals and some honey have been found to cause liver cancer in rodents [Prakash *et al.*, 1999]; certain industrial chemicals (biphenyls); and agrochemicals (pesticides, herbicides and fertilizers) pause health hazards to humans [Stewart and Kleihues, 2003].



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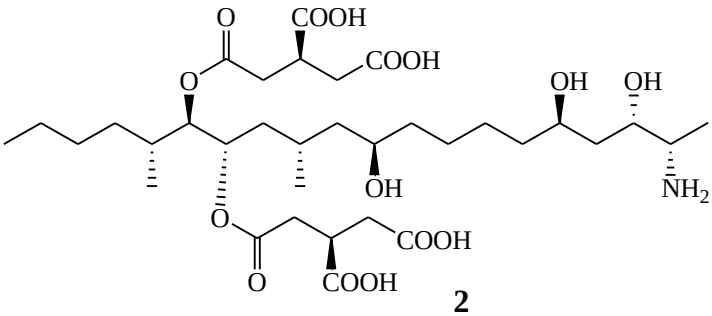

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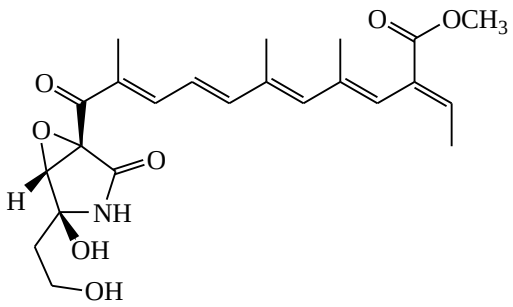

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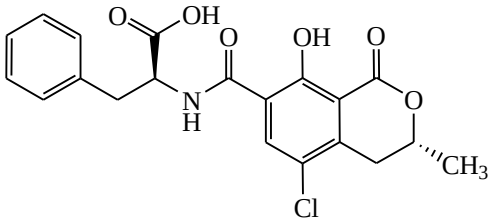

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The incidence of most cancers varies worldwide and this has been attributed, partly, to differences in diet. For instance high salt intake together with *H. pylori* infections are thought to contribute to gastric cancer [Stewart and Kleihues, 2003]. Consumption of Chinese-style salted fish has been associated with nasopharyngeal cancer in South-East Asia [IARC, 1993]. Consumption of both red and processed meat has been associated with colorectal cancer risk [Norat *et al.*, 2002]. However, consumption of vegetables and fruits is associated with reduced risk of cancers of pharynx, larynx, oesophagus, stomach and cervix/uterus. Vegetable consumption is associated with protection against colorectal cancers. Olive oil consumption is associated with reduction in cancer risk [Thurston, 2007]. Research on fat consumption gives moderately consistent indication that animal fat (except fish) is correlated to the risk of colorectal cancer [Stewart and Kleihues, 2003]. Low dietary intake of vitamin C is associated with increased risk of cancers of the stomach, mouth, pharynx and oesophagus whereas low vitamin E intake has been linked to cancers of the lung, cervix and colorectum [Stewart and Kleihues, 2003]. A diet rich in copper and cadmium and deficient of zinc is said to increase the risks of cancer development [Boik 2001]. Some studies also suggest that selenium deficiency may increase the risk of cancer [Clark *et al.*, 1998].

Heavy drinking of alcohol has been associated with a range of cancer types including cancers of the oral cavity, pharynx, larynx, oesophagus as well as liver; and may increase the risk of breast and colorectal cancer [Stewart and Kleihues, 2003].

Certain medical drugs including some anticancer ones and hormones as well as surgical implants have been found to cause cancer in some individuals [Stewart and Kleihues, 2003]. For example tamoxifen is an anti-breast cancer drug has been associated with increased risk of endometrial cancer (in this case a secondary cancer) [White, 2001]. Similarly diethylstilbestrol, a synthetic estrogen, originally used to prevent miscarriage, was found to cause malformation of reproductive organs and associated with vaginal adenocarcinoma in daughters exposed to the drug *in utero* [Laitman, 2002].

High risk occupations in relation to cancer are also known. Scrotal skin cancer was noted in workers of chimney sweeps due to deposits from coal fires as early as 1775 [Pott, 2008; Monson, 1996]. Some of the chemicals considered by WHO/IARC to be of carcinogenic hazard at workplaces include benzene from fuel and solvent industries (causing leukaemia); asbestos from insulation, filter materials and textile industries (lung, pleura, peritoneum cancer); benzidine from dye/pigment industries (bladder cancer); sulfuric acid mist from metal and battery industries (larynx and lung cancer); crystalline silica from stone cutting and mining (lung cancer); vinyl chloride from plastic industries (liver cancer); wood dust from furniture industry (nasal cavity cancer); chromium (VI) compounds from metal plating and dye/pigment industries (nasal and lung cancer) [Stewart and Kleihues, 2003], among many others.

2.1.2.3 Radiations and free radicals

Exposure to strong ionizing radiation such as γ- or X-rays that produce α and β particles causes damage to the DNA by disintegration through free radical formation. The free radicals not only destroy the DNA but also induce oxidative damage in cell membrane proteins as well as lipid peroxidation of membrane phospholipids and lipoproteins. Free radical damage has been implicated as a major contributor in degenerative diseases as well, including aging, cardiovascular disease, immune dysfunction, brain dysfunction and cataracts [Boik, 2001; Thurston, 2007].

It has been postulated that children living close to nuclear power stations are at risk of developing certain forms of leukaemia and brain tumours. This was supported by the incidence of various cancers in populations exposed to radiation as a result of an accident at the Chernobyl (Ukraine) Nuclear Power Station in 1986 [Stewart and Kleihues, 2003]. There are fears that several people may have been exposed to ionizing radiation when the Fukushima Nuclear Power station in Japan erupted on 11th March 2011 [Nebehay, 2013].

Radon is a naturally occurring radioactive gas found in high concentrations in certain types of granite rock and soils, and has been associated with increased risk of certain types of cancer including leukaemia [Forastiere *et al.*, 1998] and lung cancer [Darby, *et al.*, 2005].

Exposure to non-ionizing electromagnetic radiation particularly ultra violet (UV) rays is associated with skin cancer and melanoma. The UV light has been categorized into three sub-bands as UV-A, UV-B and UV-C. The UV-C (200-290 nm) band is considered to be the most carcinogenic and UV-B (290-320 nm) causes sun-burn. UV-A (320-400 nm) does not damage DNA directly like UV-B and UV-C, but it can generate reactive oxygen species such as hydroxyl and oxygen radicals, which in turn can damage the DNA [Boik, 2001; Thurston, 2007]. The ozone layer in the atmosphere absorbs the carcinogenic UV-C but there are concerns over the increasing depletion of the ozone layer by green house gases [Dobson, 2005; Toon and Turco, 1991]

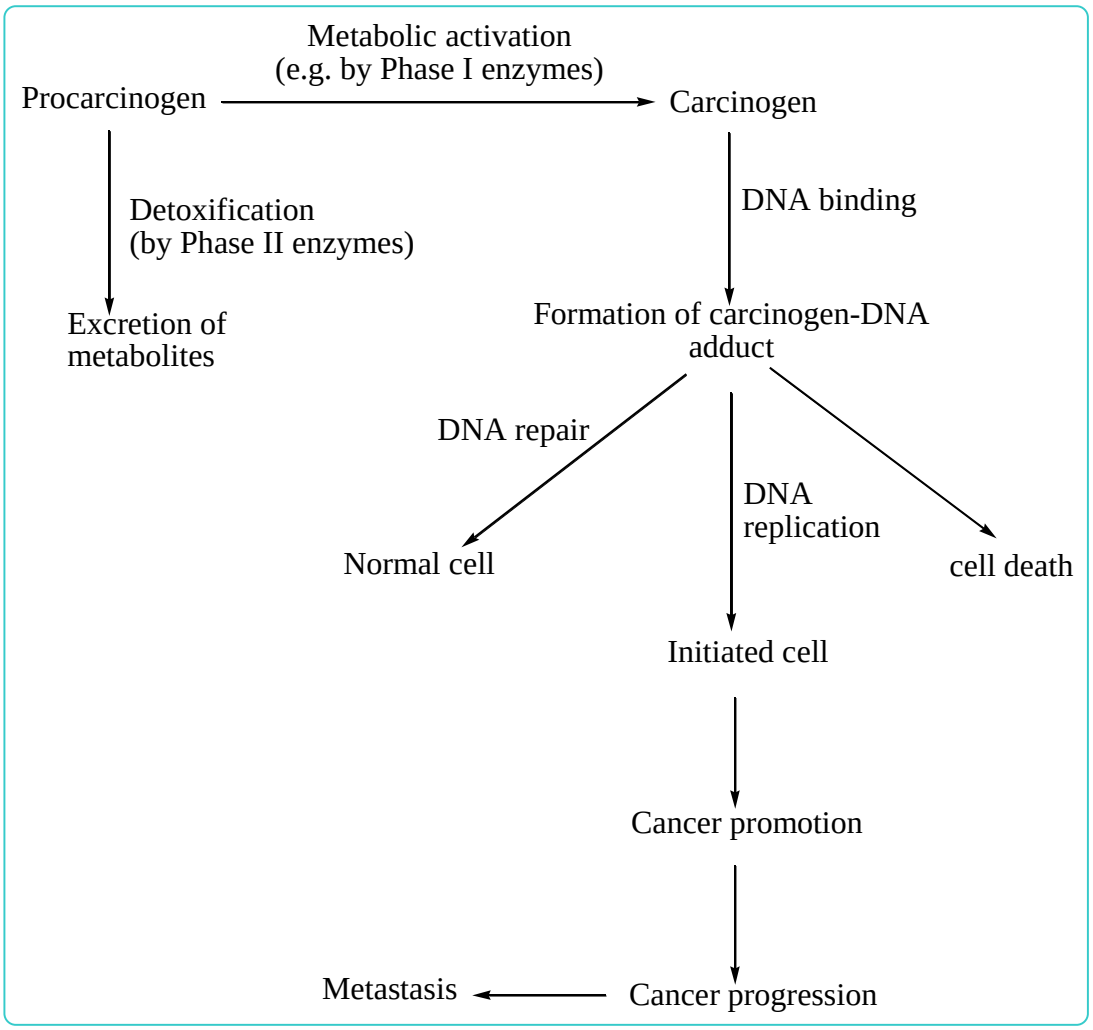
2.1.3 Hereditary factors

A number of genes have been identified that if inherited may increase the risk of certain cancers. For instance two genes, breast cancer gene 1 (BRCA1), and breast cancer gene 2 (BRCA2), have been associated with breast cancer. In some cases women who discover that they are carrying these genes have their breasts be removed at an early age as a preventive measure [Boik, 2001; Thurston, 2007]. Similarly genes responsible for colon tumours have been identified [Boik, 2001; Thurston, 2007].

2.2 Mechanism of Carcinogenesis

Carcinogenesis proceeds through a series of stages, many of which may overlap with one another. These stages include initiation (damage to cellular DNA by a carcinogen), promotion (initiated cells multiply to form a tumor), and progression (proliferation of the

tumor to a malignant state that can lead to metastasis (Scheme 1) [Stewart and Kleihues, 2003].



Scheme 1: Basic stages of carcinogenesis [Stewart and Kleihues, 2003; Martinez *et al.*, 2003]

Initiation is an irreversible event that occurs when a genotoxic chemical, or its reactive metabolite, causes a DNA mutation in a critical growth controlled gene [Martinez *et al.*, 2003]. The mutations that occur in the initiated cells can confer growth advantages, which allow them to evolve and/or grow faster by-passing normal cellular growth controls. Many chemical agents must be metabolically activated before they become carcinogenic. Most carcinogens and their active metabolites are strong electrophiles and bind to DNA to form

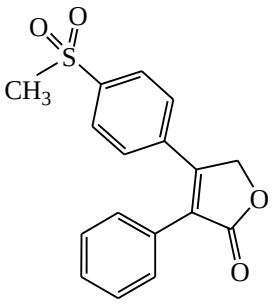
adducts that must be removed by DNA repair mechanisms. Failure to repair chemical adducts, followed by cell proliferation, results in permanent alterations or mutation(s) in the genome that lead to oncogene activation or inactivation of tumor suppressor genes [Martinez *et al.,* 2003].

Promotion is a reversible process in which chemical agents stimulate proliferation of initiated cells. Promoting agents are unable to form DNA adducts or cause DNA damage but are able to stimulate proliferation [Martinez *et al.,* 2003].

2.2.1 Role of enzymes in carcinogenesis

Phase I and II metabolizing enzymes play important roles in the metabolic activation and detoxification of carcinogenic agents. Phase I enzymes that include monooxygenases, cyclo-oxygenases, dehydrogenases, esterases, reductases and oxidases are responsible for activation of procarcinogens. Cyclo-oxygenase (COX) enzymes catalyze the rate-limiting step in prostaglandin synthesis, converting arachidonic acid to prostaglandins which play a role in biological processes including blood clotting, ovulation, bone metabolism, nerve growth and development, and immune response. There are two COX isoforms, COX-1 and COX-2. COX-1 is constitutively expressed in most cell types and is necessary in homeostasis of colonic epithelium and platelet aggregation. COX-2, on the other hand, is inducible by a variety of stimuli including growth factors, stress conditions and cytokines. COX-2 is commonly overexpressed in premalignant and malignant tissues. The enzyme upregulates the production of pro-inflammatory prostaglandins and stimulate tumor cell growth and suppress immune surveillance. In addition, COX-2 can activate carcinogens into forms that can damage the DNA [Martinez *et al.,* 2003; Cuendet and Pezzuto, 2000]. Selective inhibitors of COX-2 reduce the formation and proliferation of experimental tumours. A number of selective COX-2 inhibitors have been developed and being monitored for cancer chemoprevetion [Thurston, 2007; Dannenberg *et al*., 2001]. Among these agents include rofecoxib (Vioxx™) (**5**) and celecoxib (celebrex™) (**6**) [Thurston, 2007; Newman and Cragg, 2007]. Unfortunately, rofecoxib was withdrawn in 2004 from the market after it was discovered that patients on the drug showed increased risks of cardiovascular troubles including heart attack and stroke. This raised concern wether this was characteristic of all selective COX-2 inhibitors. However, the cardiovascular problems resulting from the use of

rofecoxib is thought to be compound-specific rather than class-specific because the safety of celecoxib has been ascertained [Thurston, 2007].



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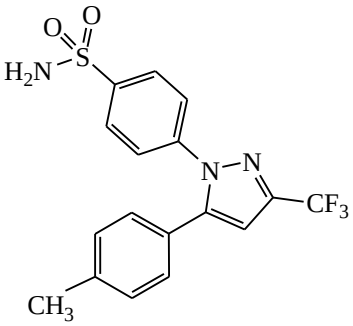

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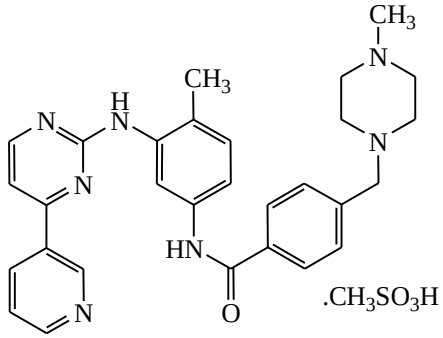
Phase II metabolizing enzymes are important for the detoxification and excretion of carcinogens. Some examples include epoxide hydrase, glutathione-*S*-transferase, uridine-5'-diphosphate (UDP) glucuronide transferase and quinone reductase (QR) [Martinez *et al.*, 2003]. There are also enzymes that exist only in cancer cells and not present in normal cells. Such enzymes include BCR-ABL tyrosine kinase enzyme. Differences of this kind could be exploited in cancer treatment with subsequent reduction in cytotoxicity to normal cells. The development of the drug imatinib (Gleevec™) (**7**), a BCR-ABL tyrosine kinase inhibitor, has been considered to be a break-through in targeted cancer therapy with significant selectivity [Matei *et al.*, 2004; Newman and Cragg, 2007; Thurston, 2007].



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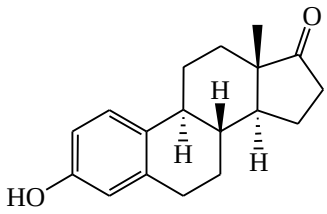
2.2.2 Estrogens, phytoestrogens and carcinogenesis

Estrogens are important sex steroidal hormones because of their role in the development of female sex organs and secondary sex characteristics and in regulation of menstrual cycle and pregnancy [Travis and Key, 2003]. However, these compounds have been implicated as carcinogens [Travis and Key, 2003]. In the female human body, there are three forms of estrogen in circulation, estrone (**8**), 17β-estradiol (**9**) and estriol (**10**). The two forms, **8** and **9**, are secreted by mature graafian follicles of the ovaries [Norman and Litwack, 1997]. Compound **10** is a metabolite of **9** with reduced activity and its concentration rises during pregnancy [Norman and Litwack, 1997].



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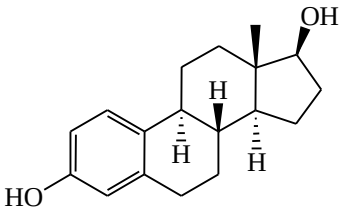

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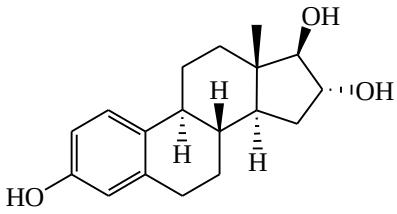

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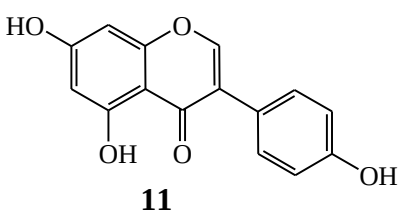
Another role of estrogen is to sustain the physiological balance in the maintenance of skeletal, cardiovascular, and central nervous system throughout the reproductive life of an individual [Parl, 2002]. The biological effects of estrogen are regulated by its concentration in serum and target tissues [Parl, 2002]. In premenopausal women the source of estrogen is mainly the ovary, but in postmenopausal women it is mainly secreted by peripheral adipose tissues. In the breast, the plasma/tissue ratio of 17β-estradiol is 1:1 in premenopausal women but tremendously changes to 1:10-50 in postmenopausal women [Parl, 2002]. Therefore, the risk of cancer in postmenopausal women is mainly associated with tissue-specific production of estrogen in the absence of an ovarian source [Travis and Key, 2003].

Natural or synthetic estrogens are the main components of oral contraceptives. However, the problem lies in the fact that these compounds have proved to be risk factors for the development of cancer in different estrogen responsive-organs including breast, uterine, endometrium, ovary, liver, lungs and pituitary as has been demonstrated in a number of animal experimental models [IARC, 1999; Tsutsui *et al*., 1997; Huseby, 1980]. Several observations suggest that estrogens exert their carcinogenic effects through two major

mechanisms; first these compounds accelerate cell proliferation by acting as initiators and promoters in carcinogenesis and secondly, estrogens induce angiogenesis (a pathobiological processing of sprouting and configuring new blood vessels from pre-existing blood vessels) [Browder *et al*., 2000]. Pregnancy appears to be important in breast cancer risk: women who have never been pregnant are at the greatest risk [Kelsey and Horn-Ross, 1993]. Pregnancy earlier in life is more protective than one later in life [Kelsey and Horn-Ross, 1993]. This has been attributed to the differentiation of breast epithelial cells into milk-producing cells that takes place in the breast during pregnancy and apoptosis of breast cells that happens during pregnancy and lactation periods [Hoffman *et al*., 1993].

Major attention has recently, been drawn towards those compounds that tend to mimic the actions of estrogen. Among these compounds include the plant constituents which are known to show complex biphasic modes of action with regards to carcinogenesis. These compounds are capable of potentiating and at the same time inhibiting estrogenic effects. Some of these compounds (phytoestrogens) include isoflavonoids (such as genistein (**11**)), lignans [(+)-sesamin (**12**)], coumestans [coumestrol (**13**)] and prenylated flavonoids [e.g. 8-prenylnarigenin (**14**)] [Wiseman, 2006]. Experiments have shown that at low concentrations these compounds impart estrogenic effects and promote tumour growth by competing with estrogen for estrogen-receptors (ERα and ERβ) in the nucleus of a given cell [Djigue *et al.*, 2009]. This is possible because the compounds are structurally related to 17β-estradiol [Thurston, 2007]. For example, the A-ring in isoflavones is analogous to the A-ring in 17β-estradiol. However, it has to be noted that the estrogenic activity of the phytoestrogens is much lower (~500 to 1000 times less) than estradiol [Thurston, 2007].

At relatively high concentrations, several phytoestrogens exhibit anti-estrogenic activities and thus inhibit tumour growth and proliferation. It has been suggested that the anti-estrogenic mechanism may not be *via* the estrogen receptor mechanism but rather through other ways such as induction of apoptosis, inhibition of angiogenesis, modulation of growth factor receptors, signal transduction, antioxidation, inhibition of DNA topoisomerase [Wiseman, 2006], and many others.



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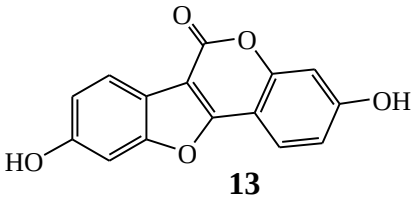

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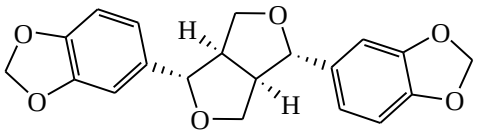

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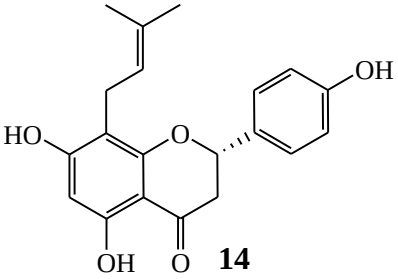

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2.3 Natural Product-Based Anticancer Agents

Natural products used in cancer chemotherapy include compounds from plants and micro-organisms. The following are some examples of natural-product derived drugs; vincristine (**15**), and vinblastine (**16**) extracted from *Catharanthus rosea.* Compounds **15** and **16** are used for the treatment of leukaemia in children and Hodgkin's disease, respectively [Thurston, 2007]. Vinblastine is also used in treatment of metastatic testicular tumours [Thurston, 2007]. Docetaxel (Taxotere™) (**17**) a semisynthetic derivative of Paclitaxel (taxol™) (**18**) obtained from the Pacific Yew tree is used for the treatment of ovarian and breast cancers [Thurston, 2007]. Compound **17** has better solubility properties compared to paclitaxel [Thurston, 2007; Pietra, 2002]. Table 1 shows some of the important anticancer drugs of plant origin. Etopside (**19**) and teniposide (**20**) are semisynthetic derivatives of the lignan podophyllotoxin (**21**) isolated from *Podophyllum peltatum* [Croteau *et al.,* 2000]. Ixabepilone (azaepothilone B) (**22**) is a derivative of the natural epothilone B (**23**) extracted from *Sorangium cellulosum.* Compound **22**, developed by Bristol-Myers Squibb, has been approved for the treatment of metastatic and locally advanced breast cancer that is either resistant or refractory to taxanes, anthracyclines and capecitabine [Kingston, 2009]. Flavopiridol (**24**), an inhibitor of cyclin-dependent kinases (CDKs), is a semisynthetic compound based on a chromonealkaloid rohitukine (**24a**) isolated from *Dysoxylum binectariferum* [Pietra, 2002]. Rotenone (**25**), which is used worldwide in pest management

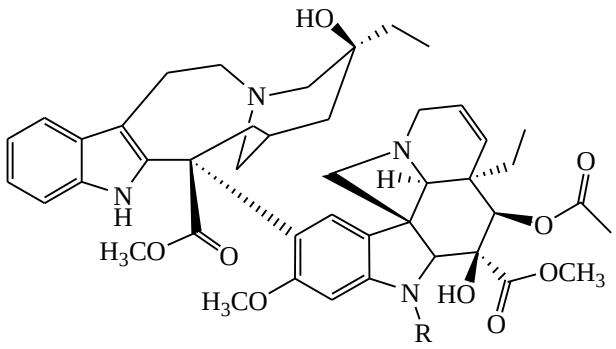
and skin disease control, is also a potent antitubulin agent, interacting with the colchicines binding site. It is used, clinically as an adjuvant, in combination with vinblastine [Jordan *et al.*, 1998].

Table 1: Natural product-based anticancer agents

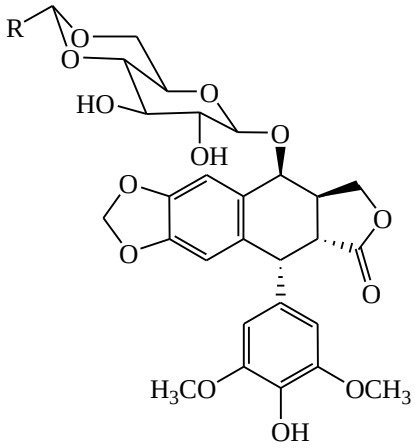
Anticancer Agent	**Source**	**Mechanism of action**
Vincristine (15)	*Catharanthus rosea*	**Interferes with microtubule assembly**
Vinblastine (16)	*Catharanthus rosea*	**Interferes with microtubule assembly**
Docetaxel (17)	Derivative of paclitaxel from Yew weed (*Taxus* sp.)	**Disrupts the depolymerisation of microtubule**
Etopside (19)	Semisynthetic from *Podophyllum peltatum*	**Topoisomerase II inhibitor**
Teniposide (20)	Semisynthetic from *Podophyllum peltatum*	**Topoisomerase II inhibitor**
Ixabepilone (22)	Derivative of epothilone B extracted from myxobacterium *Sorangium cellulosum*	**Disrupts the depolymerisation of microtubule**
Flavopiridol (24)	Semisynthetic based on rohitukine (**82a**), a chromonealkaloid obtained from *Dysoxylum binectariferum*	**Inhibits cyclin-dependent kinases**
Rotenone (25)	Several Papilionoideae plants	**Inhibitor of mitochondrial complex I and used in combination with vinblastine**
Englerin A (26)	*Phyllanthus engleri*	**Induction of necrotic cell death**
Combretastatin A-4 (27)	*Combretum caffrum*	**Inhibits tubulin polymerization**
Doxorubicin (29)	***Streptomyces peucetius***	**DNA intercalating agent**

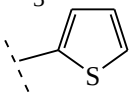
Englerin A (**26**) is guiane sesquiterpenoid obtained from the bark of *Phyllanthus engleri*, a plant native to Tanzania and Zimbabwe [Nicolaou *et al*., 2010; Ratnayake *et al.,* 2009]. Compound **26** exhibited very potent and highly selective cytotoxicity against renal cancer cells and it is a promising drug candidate [Sulzmaier *et al.,* 2012]. Combretastatin A-4 (**27**) is among the simpler *Z*-olefinic bridged biaryl compounds obtained from the South African willow tree, *Combretum caffrum* [Pettit *et al*., 1989]. Combretastatins have exhibited potent antitumour properties, through the inhibition of tubulin polymerization [Jordan *et al.*, 1998].

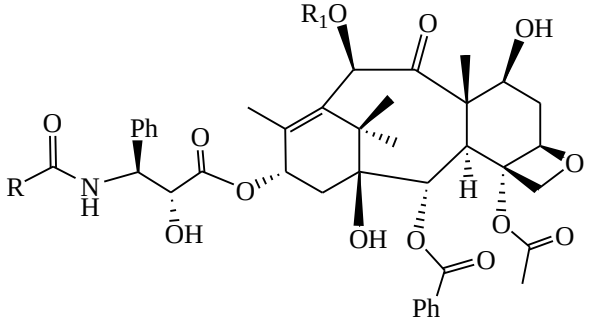
However, these compounds have a limitation of low water solubility, and so a range of analogues have been prepared, such as the phosphate prodrug CA4P (**28**) with improved solubility and high efficacy [Thurston, 2007]. It is currently used against cervical, lung, prostate, colorectal, thyroid and ovarian cancers [Kingston, 2009]. Doxorubicin (Caelyx™, Myocet™) (**29**) is an anthracycline (also known as anthraquinone) antibiotic first isolated from *Streptomyces peucetius,* and it is considered to be one of the most successful and widely used anticancer drugs due to its broad spectrum of activity [Thurston, 2007]. The drug is used in the treatment of acute leukaemia, lymphomas, solid tumours such as carcinomas of the breast, thyroid, lung, and ovary as well as soft tissue carcinomas [Thurston, 2007].



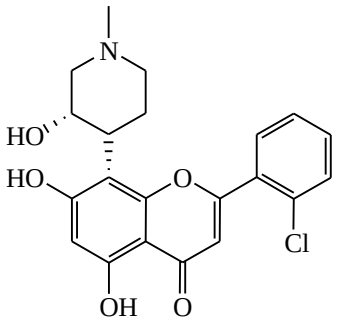
15: R = CHO
16: R = CH_3



19: R = CH_3
20: R = 



17: R = $OC(CH_3)_3$, R_1 = H
18: R = Ph, R_1 = Ac



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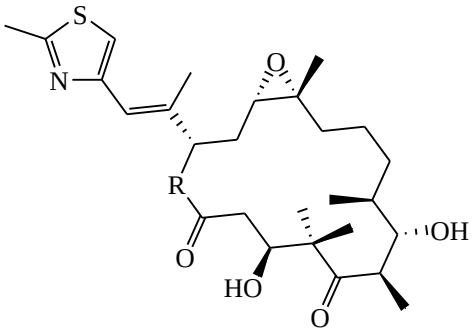
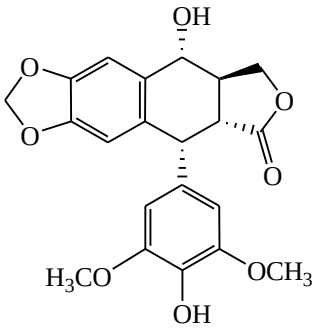


22: R = NH
23: R = O



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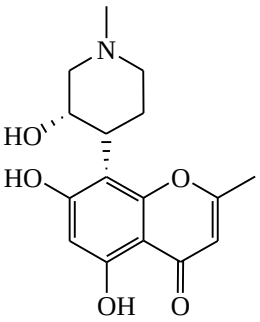

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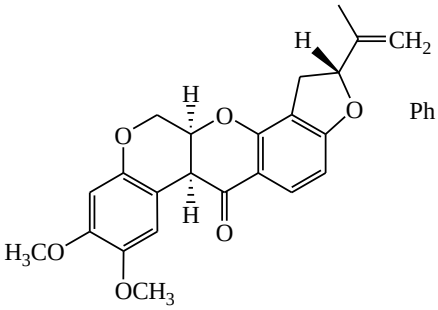

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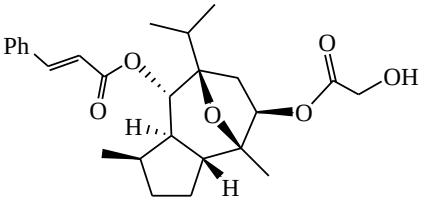

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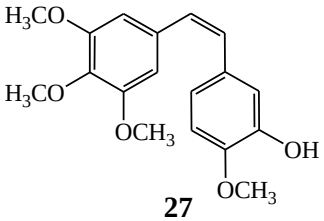

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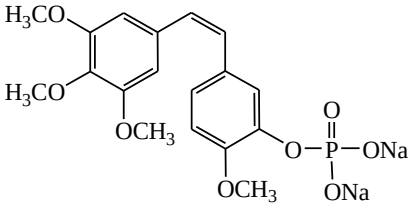

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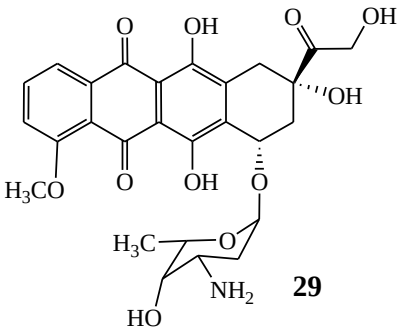

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A number of natural macromolecular anticancer agents are also known. These include bleomycins (DNA cleaving agent) [Thurston, 2007], bryostatin (inhibitor of protein kinase C) [Pettit, 1991], porfimer sodium (photodynamic agent) and Gemtuzumab ozogamicin (Mylotarg™) (an antibody) [Thurston, 20007].

2.4 Cancer Preventive Measures

Approaches to prevention of cancer have been in the form of reducing and minimizing human exposure to risk pre-disposing conditions. It has to be noted that exposure to certain carcinogenic hazards such as active smoking, drinking alcohol, sun exposure, are largely voluntary and based on individuals' lifestyle [Stewart and Kleihues, 2003]. Attempts to

prevent such risks have been through sensitization and public education. The control of passive tobacco smoking has been through legislations and enactment of laws that control, and in some countries prohibit, tobacco smoking in public places [WHO, 2013]. Reduction of exposure to potentially carcinogenic agents/conditions in the environment and at workplaces has been achieved by setting regulations that include replacement of carcinogens with alternative safe chemicals or processes, clean and well ventilated workplaces, re-engineered manufacturing processes and use of protective clothes/equipment [Stewart and Kleihues, 2003]. Prevention also requires regular medical check-up and screening for a suspected type of cancer. This is a prerequisite for early diagnosis and successful intervention (through chemotherapy or surgery) before a tumour becomes invasive and metastatic. Vaccination against human papillomavirus in teenage girls and hepatitis B virus in children are being carried out in several countries [WHO, 2014; Stewart and Kleihues, 2003; Coursaget and Muñoz, 1999; McNeil, 1997].

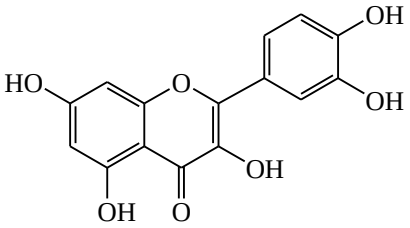
There is also a growing interest in cancer chemoprevention, a strategy aimed at reducing the risk of carcinogenesis through the use of pharmaceuticals or micro-nutrients. This approach focuses on interventions to prevent, or delay carcinogenesis or to enhance regression of abnormal cells or tissue with minimal or no side-effects [Crowell, 2005]. The activity of a variety of chemopreventive agents seems to be based on epigenetic changes that may take place during the latent period (20 or more years) before invasion and metastatic stages of tumourigenesis. Chemopreventive agents also include certain pharmaceuticals and hormonally active agents. For instance the breast cancer drug tamoxifen reduces the risk of developing a second cancer in the other breast [Stewart and Kleihues, 2003]. Similarly, reduction in the risk of colon cancer was observed after regular use of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin [IARC, 1997]. There is considerable evidence that some chemicals present in the diet at low concentrations (nutriceuticals) are important in the protection against carcinogenesis. Some of these agents include derivatives of folic acid, curcumin, genistein, selenium and tea catechins [National Cancer Institute, 2003].

2.5 Natural Products in Cancer Chemoprevention

Cancer chemoprevention can take a variety of approaches and several mechanisms have been proposed. Two major categories of cancer chemopreventive agents have been identified; the

anti-initiators and the anti-promotional (or suppressing) agents [Martinez *et al.*, 2003; Fischer *et al.*, 1986; Thurston, 2007]. Anti-initiators are thought to reduce body's exposure to carcinogens through inhibition of Phase I enzymes and enhancement of Phase II enzymes. Anti-initiators also act by promoting the expression of cytoprotective genes in cells including those that produce drug metabolizing enzymes such as glutathione-*S*-transeferase, uridine-5'-diphosphate (UDP) glucuronide transferase and NADPH/quinone oxido-reductase 1. Anti-promotional agents may act on molecular targets, such as hormone receptors, protein kinases, transcription factors such as NF-κB, tumour suppressor genes, and enzymes such as COX, ornithine decarboxylase, lipoxygenase [Fischer *et al.*, 1986; Thurston, 2007]. Different groups of compounds with diverse structures have been considered as lead compounds for cancer chemoprevention. Flavonoids like flavonols [quercetin (**30**)] and chalcones have antioxidant and radical scavenging activities, an important property in preventing carcinogenesis [Harborne and Williams, 2000]. Other natural products with strong anti-oxidant properties include ascorbic acid (**31**), caffeic acid (**32**), epigallocatechin gallate (**33**) [Boik, 2001; Thurston, 2007], and ergothioneine (**34**), a constituent of white button mushrooms and several exotic mushrooms, chicken liver and wheat germ [Thurston, 2007].

Curcumin (**35**), an ingredient of turmeric used as spice, is associated with reduction in cancer risks by inhibiting aminopeptidase N (APN), an enzyme that catalyses tumour invasiveness and angiogenesis [Thurston, 2007; Khan *et al.*,, 2008]. Isothiocyanates found in cruciferous vegetables, such as broccoli, cauliflower, kale and cabbage are linked to prevention of various cancer types. Examples of such isocyanates include sulforaphane (**36**) which is reported to induce Phase II detoxifying enzymes [Gerhäuser, *et al.*, 1997a]. Both oleic acid [(9Z)-9-octadecenoic acid (**37**)] and oleocanthal (**38**) found in virgin olive oil are considered to be cancer chemopreventive agents. Compound **37** is reported to inhibit the over-expression of the oncogene HER2/neu, a significant factor in breast cancer development. Compound **38** inhibits COX I and COX II enzymes just like the NSAID ibuprofen (**39**) [Thurston, 2007].



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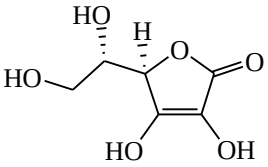

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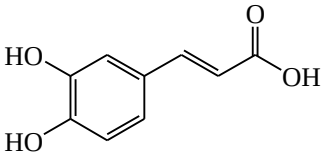

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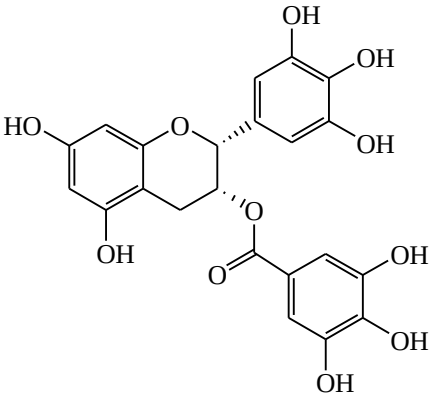

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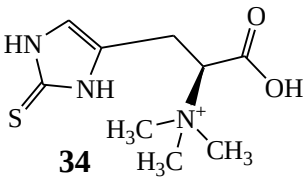

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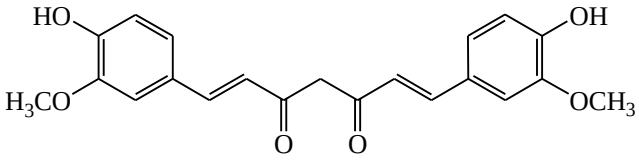

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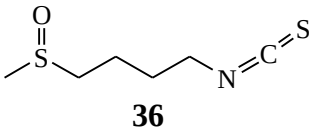

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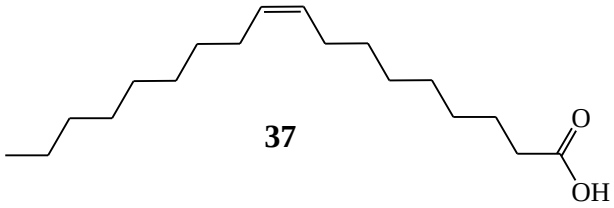

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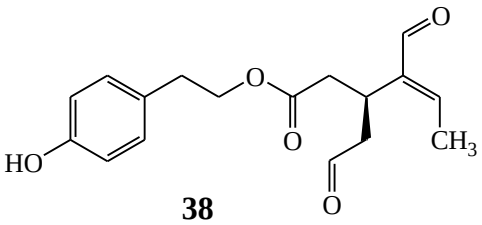

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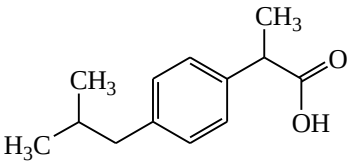

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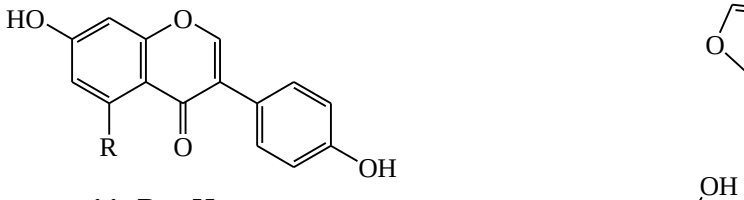
Phytoestrogens including isoflavonoids [such as genistein (**11**) and daidzein (**40**) (present in soybean and other Leguminosae plants)], lignans and coumestans have chemopreventive properties against cancers of the breast and the prostate [Wiseman, 2006; Khan *et al.*,, 2008]. It has been suggested that these compounds exert anti-estrogenic effects by binding to and

effectively blocking estrogen receptors while causing only weak estrogenic effects [Croteau *et al.*, 2000; Thurston, 2007; Khan *et al.*, 2008].

Recent investigations of the stems of *Derris trifoliata* (Leguminosae) resulted in the identification of 6a-α,12a-α-12a-hydroxyelliptone (**41**), deguelin (**42**) and α-toxicarol (**43**) as potential cancer chemopreventive rotenoids [Ito *et al.*, 2004a]. Lanceolatin B (**44**) obtained from *Millettia leucantha* also showed cancer chemopreventive properties [Phrutivorapongkul *et al.*, 2003].

Stilbenes have antitumour promotion properties, for example *trans*-resveratrol (**45**) present in grape (*Vitis vinifera* L., Vitaceae) and products manufactured from grapes suppress tumour formation [Croteau *et al.*, 2000; Jang *et al.*, 1997].



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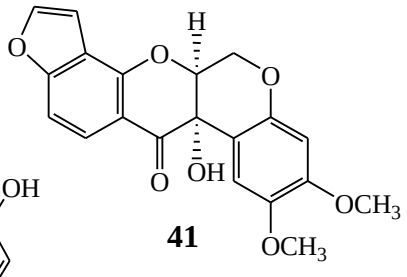
40: R = OH



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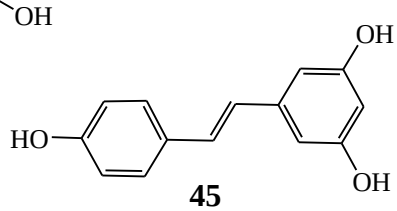

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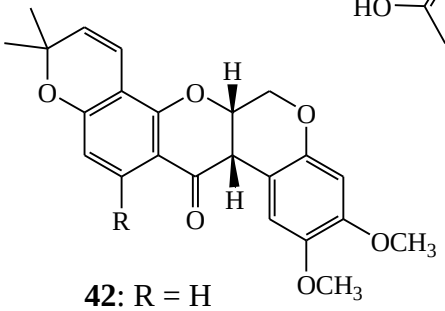
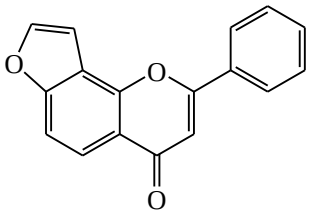


42: R = H
43: R = OH



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2.6 The Family Leguminosae

The three plant species (*Platycelphium voënse*, *Millettia usaramensis* subspecies *usaramensis* and *Flemingia grahamiana*) investigated in this study belong to the family Leguminosae in the Papilionoideae sub-family. The classification of the sub-family is summarized as shown below:

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Fabales
Family:	Leguminosae (Fabaceae)
Subfamily:	Papilionoideae (Faboideae)

The family Leguminosae (alternative name Fabaceae) comprises approximately 19,325 species in 727 genera [Lewis *et al.* 2005]. It is the third largest family after Asteraceae (Compositae) and Orchidaceae among the flowering plants. Three sub-families are currently recognized in the Leguminosae family: Caesalpinoideae, Mimosoideae and Papilionoideae. The three subfamilies are further divided into 36 tribes, 4 in the Caesalpinoideae, 4 in the Mimosoideae, and 28 in the Papilionoideae [Lewis, *et al.,* 2005].

2.6.1 The Sub-family Papilionoideae

Papilionoideae is the largest subfamily with about 28 tribes, 440 genera and 13,800 species which are trees, shrubs, lianas or most often herbs [Lewis, *et al.*, 2005]. The subfamily is distinguished from the other two by the presence of papilionoid flowers, a hilar valve in the seeds and their ability to synthesise quinolizidine alkaloids and isoflavonoids [Gillett *et al.,* 1971; Van Wyk, 2003; Veitch, 2007].

Although most of the genera are clearly defined, in some cases there is no clear delineation. This is especially the case among the three genera of *Millettia*, *Lonchocarpus* and *Derris.* More than one botanist has described a *Millettia* species in *Lonchocarpus* and *vice versa* [Gillett, *et al.,* 1971, Geesink, 1981]. *Lonchocarpus* shows a great vegetative and floral affinity with *Millettia, Pongamia* and *Piscidia* [Magalhães *et al*., 1996]. Such morphological complexity has resulted in the adoption of controversial systems by different botanists.

Gillett *et al.* (1971) placed the two genera *Derris* and *Lonchocarpus* in the tribe Dalbergieae and *Millettia* in Tephrosieae, whereas Geesink (1981) classified all the three genera as being under one tribe, Tephrosieae. In some cases two generic names are quoted for the same plant material, such as "*Derris* (*Lonchocarpus*) *urucu"* used to refer to a species found in Brazil [Gusmão *et al.,* 2002]. One of the common elements in these genera is that they all elaborate prenylated flavonoids/isoflavonoids with wide variety of biological activities. It is rewarding to study these genera not only for isolation of bioactive agents but also to examine the chemotaxonomic value of phytochemicals so as to establish the relationship among the genera.

2.6.2 Botanical information on the plants under study

The three plants investigated in this study are *Platycelphium voënse, Millettia usaramensis* subspecies *usaramensis and Flemingia grahamiana*. The botanical information on these plants is presented in this section.

2.6.2.1 Botanical information on *Platycelphium voënse*

Platycelphium is a monotypic genus classified under the tribe Sophoreae [Gillett *et al.,* 1971] within the Papilionoideae sub-family. According to Gillett *et al.* (1971), it is a small deciduous tree 3-7.5 m tall, sometimes several-stemed. Its bark is usually pale grey, brown or yellowish-green in colour which peels into paperly strips when old. The leaves are 3-7 foliolate and up to 10-27 cm long. Leaflets are lateral ovate-lanceolate to ovate-elliptic, 3.5-10 cm long and 2.5-6 cm wide. The flowers are in raceme forms with sweetly scented violet to deep blue corolla and purplish-green calyx. The flowers usually appear before leaves. The fruits are asymmetrically oblong-elliptic, narrowed at either end, 5-7.5 cm long 2-3.7 cm wide.

It occurs in the drier parts of Eastern Africa, particularly in Kenya, Ethiopia, Somalia and Tanzania [Gillett *et al.,* 1971]. Sophoreae has been described as "a tribe of convenience", comprising genera of least specialization and of diverse morphological features [Gillet *et al.,* 1971; Polhill, 1981]. The relationships within and between the mainly tropical Sophoreae genera are still poorly known [Van Wyk, 2003]. The tribe is further considered to be transitional between Papilionoideae and Caesalpinoideae sub-families [Bentham, 1841]. DNA sequencing studies have shown that the genera in Sophoreae are in need of taxonomic

realignment [Käss and Wink, 1995; Doyle *et al.*, 2000; Crisp *et al.*, 2000; Pennington *et al.*, 2001].

2.6.2.2 Botanical information on *Millettia*

The genus *Millettia*, represented by more than 200 species of climbers, shrubs and trees, is distributed in tropical Africa, Asia and Australia. This genus is represented by twenty four (24) and six (6) species in East Africa and Kenya, respectively. The species in Kenya include *M. lasiantha* Dunn, *M. leucantha* Vatke, *M. tanaensis* Gillett, *M. usaramensis* Taub., *M. oblata* Dunn and *M. dura* Dunn.

Figure 1: Leaves and fruits of *Millettia usaramensis* subspecies *usaramensis*

The genus is characterized by imparipinatte opposite leaves; stipels usually present and pulvinate base of rachis. Inflorescence is paniculate (often looking like a raceme). Their fruits are dehiscent and flat [Gillett *et al.*, 1971; Beentje, 1994].

2.6.2.3 Botanical information on *Flemingia grahamiana*

Flemingia is a genus consisting of 30-50 species, distributed in the tropical and subtropical regions. *Moghania*, the corrupted form of *Maughania*, is a synonym for *Flemingia*. In Africa the genus *Flemingia* is represented by two species, *Flemingia grahamiana* Wight & Arn and *Flemingia macrophylla* [Lewis *et al*., 2005].

Flemingia grahamiana Wight & Arn is an erect herb or sub-shrub up to 1.8 m tall, with tomentose young shoots and deep (sometimes tuberous) roots. Its leaves are digitately 3-foliate; stipules lanceolate or elliptic-lanceolate, about 1 cm long, deciduous. The plant has auxiliary inflorescence, densely fasciculate raceme and having a rachis 1-5 cm long, with imbricate bracts. Flowers are bisexual, pedicel *ca* 2 mm long, calyx tubular, *ca* 1.5 cm long, corolla yellow-white or pink, standard oblong, 7-8 mm long. Pods are inflated oblong, 2-seeded, 1 cm long, pubescent, and often covered with red viscous glands.

Figure 2: Leaves and flower buds of *Flemingia grahamiana* [Source: *Swaziland's Flora Database*. http://www.sntc.org.sz/flora/speciesinfo.asp?spid=1504]

It is distributed in tropical Africa and occurs in open and wooded savanna, sometimes near water in riverine vegetation, on hillside, termite mounds and along roadsides [Gillett *et al.*, 1971; Jansen, 2005]. The plant is also found in India, Laos, Myanmar, Vietnam and South Western Asia, particularly Yemen [Gillett *et al.*, 1971].

2.6.3 Ethnobotanical uses of Leguminosae

Plants from Leguminosae family are used in folklore medicines all around the world for treatment of a variety of diseases. They are used for the treatment of stomach problems, diarrhea, dysentery, syphilis, chest pain and coughs among others [Kokwaro, 1993]. They are

used as aphrodisiacs, purgatives and laxatives, anti-heliminthics as well as anti-dotes for snake bite [Kokwaro, 1993; Katende *et al.*, 1995; Pickard and Cox, 1986].

Many of the plants in the family are used as sources of food and vegetables, fodder for livestock, and are important in nitrogen fixation and soil conservation. Some, especially those with thorns and spines, are used in live and dry fencing [Katende *et al.*, 1995]. Others are used as pesticides and insecticides, molluscicides, and fish poison [Jain *et al.*, 1994; Joshi, 1986; Teesdale, 1954]. A range of species are cultivated as timber trees as well as shade and ornamental trees [Gillett *et al.*, 1971; Beentje, 1994]. There is no documented ethnobotanical use of *Platycelphium voënse.*

2.6.3.1 Ethnopharmacological uses of *Millettia*

Plants of the genus *Millettia* are used in traditional medicine across the tropical and sub-tropical regions. In East Africa, a decoction of the bark and leaves of *Millettia makondensis* is used to treat tooth-ache [Kokwaro, 1993]. The different plant parts of *M. oblata* are used for the treatment of stomachache, coughs, swellings, and bladder troubles [Kokwaro, 1993]. *M. usaramensis* is used as a snake bite antidote, whereas the leaves of *M. lasiantha* are used as part of an aphrodisiac preparation [Bally, 1937; Kokwaro, 1993]. The seeds of *M. dura* are used as a fish poison in Kenya [Teesdale, 1954]. In India, the leaves of *M. auriculata* are used to treat infertility in men [Choudhary *et al.*, 1990], as a vermicide, pesticide and fish poison [Jain *et al.*, 1994; Joshi, 1986]. The dried leaves and stem bark of *M. caerulea* are used as an antiseptic agent to reduce the chances of infection in cuts and wounds in Thailand [Anderson, 1986]. The roots of *M. extensa* are used as treatment for wounds and cuts in Nepal [Taylor *et al.*, 1996] as well as for stomach pain and prevention of conception in India [Singh and Maheshwari, 1994].

2.6.3.2 Ethnopharmacological uses of *Flemingia*

Plants from the genus *Flemingia* are widely used in traditional medicine in different parts of the world, as summarized in Table 2.

Table 2: Ethnopharmacological uses of selected *Flemingia* species

Plant Species	Disease Treated/Used as (plant part)	Place	Reference(s)
F. grahamiana **and** ***F. macrophylla***	'Warrus' - Cosmetic, dye, antihelmintic and anticough (pods)	Arabia and India	Ghalot, 2011
F. grahamiana	'Warrus' - Cosmetic and dye (fruits and flowers)	Uganda, Malawi and Zimbabwe	Jansen, 2005; Cardillo *et al*., 1968
F. grahamiana	Skin diseases and as a purgative (leaves and stems)	India	Jansen, 2005
F. grahamiana	Diarrhea and dysentery (roots)	Zimbabwe and Malawi	Jansen 2005
F. macrophylla	Rheumatism and inflammations (stems)	Taiwan	Yang and Lu, 1995a
F. chappar	Eye troubles and cataract (seeds) Insomnia, epilepsy, stomach troubles (roots)	India India	Ghalot *et al*., 2011 Oudhia 2003
F. chappar **and** ***F. strobilifera***	Hysteria, insomnia, epilepsy, pain reliever, stomach-ache and disorders (roots)	India	Ghalot, 2011
F. strobilifera	Diarrhea and dysentery (roots)	Nepal	Ghalot, 2011
	Vermifuge (leaves)	Java	
	Tuberculosis (leaves and flowers)	Philippine	
F. philippinensis	Rheumatism, arthropathy, leucorrhea, menalgia, menapausal syndrome, nephritis, improvement of bone-mineral density (roots)	China	Li *et al*., 2008 Su, 1977
F. tuberosa	Dysentery and vaginal discharge (leucorrhoea) (roots)	India	Ghalot, 2011

2.6.4 Phytochemistry of Leguminosae

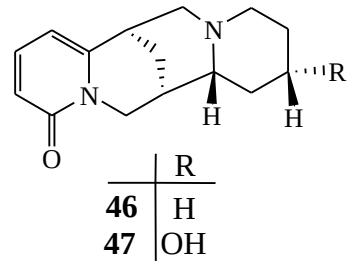
The plant family Leguminosae is a source of flavonoids, isoflavonoids (mostly occurring in Papilionoideae sub-family) [Veitch, 2007, 2009], cyanogenic glycosides [Bisby, 1994], anthocyanins [William and Grayer, 2004], quinolizidine alkaloids [Asres *et al.*, 2004; Van Wyk, 2003]; pyrolidone alkaloids and pyrrolizidine alkaloids [Van Wyk, 2003; Flores *et al.*, 2009]; pterocarpans [Jiménez-Gonzalez *et al.*, 2008], quinones, terpenoids and coumarins

[Bisby, 1994]. It is worth noting that isoflavonoids are almost exclusively restricted to the subfamily Papilionoideae. For instance it was reported that, in the year 2004, out of the 1,600 isoflavonoids reported, only 213 were from 48 non-leguminosae families [Veitch, 2007].

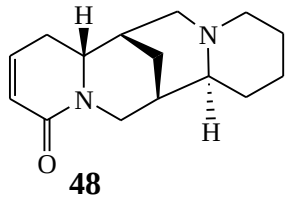
Many of the isolated compounds have a wide range of biological activities and are thus potential hit compounds in drug development. Among the compounds from the Leguminosae with economic importance are the rotenoids which are commercially used as insecticides [Fukami and Nakajima, 1971; Yenesew *et al.*, 2003a; Veitch, 2007].

2.6.4.1 Phytochemistry of *Platycelphium voënse*

Quinolizidine alkaloids are the major compounds identified from *Platycelphium voënse* including anagyrine (**46**), baptifoline (**47**), lupanine (**48**), cytisine (**49**), *N*-acetyl cytisine (**50**) and *N*-methyl cytisine (**51**) [Asres *et al.*, 1997; Van Wyk *et al.*, 1993].



	R
46	H
47	OH



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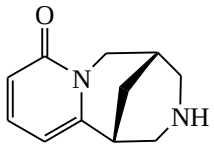

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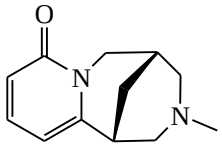

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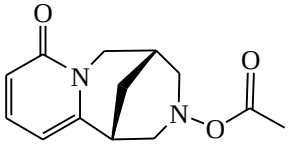

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2.6.4.2 Phytochemistry of the genus *Millettia*

Millettia is a rich source of flavonoids as the major constituents. Isoflavones, rotenoids, pterocarpans, isoflavanones, flavones and chalcones are the most common flavonoids from this genus. Other secondary metabolites like coumarins, benzoquinones, terpenes, sterols and guanidine alkaloids are some of the classes of minor constituents from the genus.

2.6.4.2.1 Isoflavones from *Millettia*

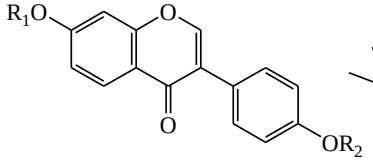
Table 3 gives examples of the typical isoflavones so far obtained from different *Millettia* species. The genus has been a source of a wide range of *C*- and *O*-prenylated as well as geranylated isoflavonoids with interesting ranges of biological activity.

Table 3: Isoflavones from *Millettia*

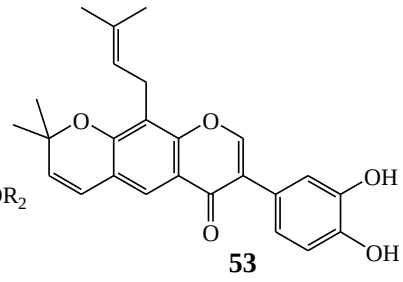
Isoflavone	**Source (plant part)**	**Reference**
Formononetin (52)	*Millettia atropurpurea* (seeds and stem bark)	**Ito *et al*., 2000**
Auriculasin (53)	*Millettia atropurpurea* (seeds and stem bark)	**Ito *et al*., 2000**
Griffonianone C (56)	,,	**,,**
Griffonianone D (55)	,,	**Yankep *et al*., 2003**
Conrauinone A (57)	*M. conraui* (stem bark)	**Fuendjiep *et al*., 1998a**
Conrauinone B (58)	,,	**,,**
Conrauinone C (59)	,,	**Fuendjiep *et al*., 1998b**
Conrauinone D (60)	,,	**,,**
Isoformonetin (61)	*M. atropurpurea* (seeds and Stem bark)	**Ito *et al*., 2000**
Calopogonium isoflavone A (62)	*M. dura* (stem bark)	**Yenesew, *et al*., 1996**
6-Methoxycalopogonium isoflavone A (63)	*M. dura* (seed pods)	**Yenesew, *et al*., 1997**
Barbigerone (64)	*M. ferruginea* ssp *ferruginea* (seeds) *M. usaramensis* (stem bark)	**Dagne and Bekele, 1990.** **Yenesew, *et al*., 1998**
Norisojamiacin (65)	*M. dura* (seed pods) *M. usaramensis* (stem bark)	**Yenesew, *et al*., 1997** **Yenesew, *et al*., 1998**
Calopogonium isoflavone B (66)	*M. ferruginea* ssp *ferruginea* and ssp *darassana* (stem bark and seeds)	**Dagne *et al*., 1989.**
Millepurone (54)	*M. atropurpurea* (seeds and stem bark)	**Ito *et al*., 2000**
Durmillone (67)	***M. dura* (seeds)**	**Dagne *et al*., 1991**

Some isoflavonoids from the genus have shown anti-neoplastic or cancer chemopreventive activities [Ito *et al.*, 2004b; 2000]. Thus, formononetin (**52**), auriculasin (**53**) and millepurone

(**54**) from *Millettia atropurpurea* showed inhibitory effects against Epstein-Barr virus early antigen activation induced by 12-*O*-tetradecanoylphorbol-13-acetate in Raji cells [Ito *et al.*, 2000]. Both griffonianone D (**55**) and the chloroform extract of *Millettia griffoniana* root bark exhibited anti-inflammatory activity [Yankep *et al.*, 2003].



52: R_1 = H, R_2 = CH_3
61: R_1 = CH_3, R_2 = H



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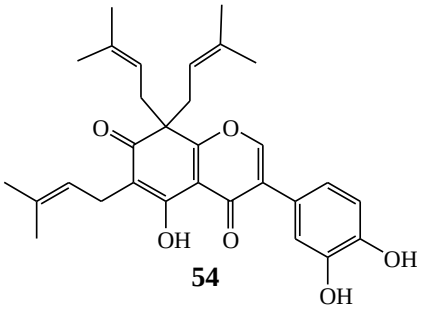

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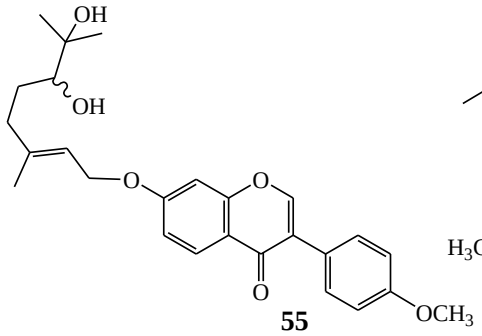

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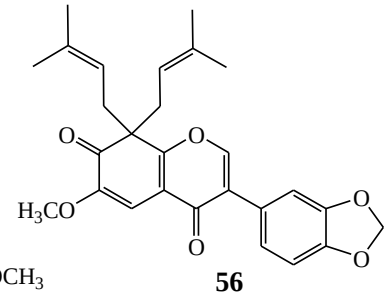

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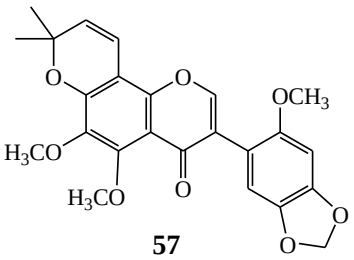

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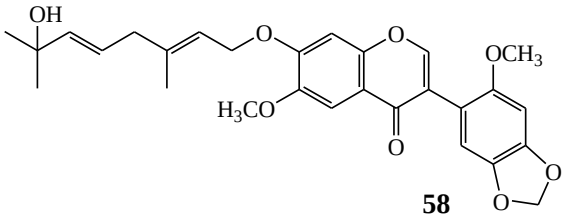

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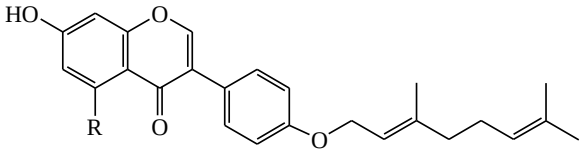
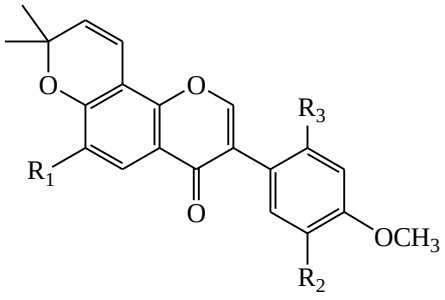
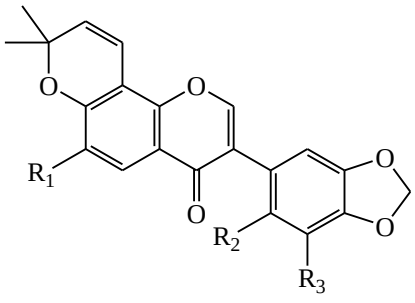


59: R = OCH_3
60: R = H



62: R_1 = R_2 = R_3 = H
63: R_1 = OCH_3, R_2 = R_3 = H
64: R_1 = H, R_2 = R_3 = OCH_3



65: R_1 = R_3 = H, R_2 = OH
66: R_1 = R_2 = R_3 = H
67: R_1 = OCH_3, R_2 = R_3 = H

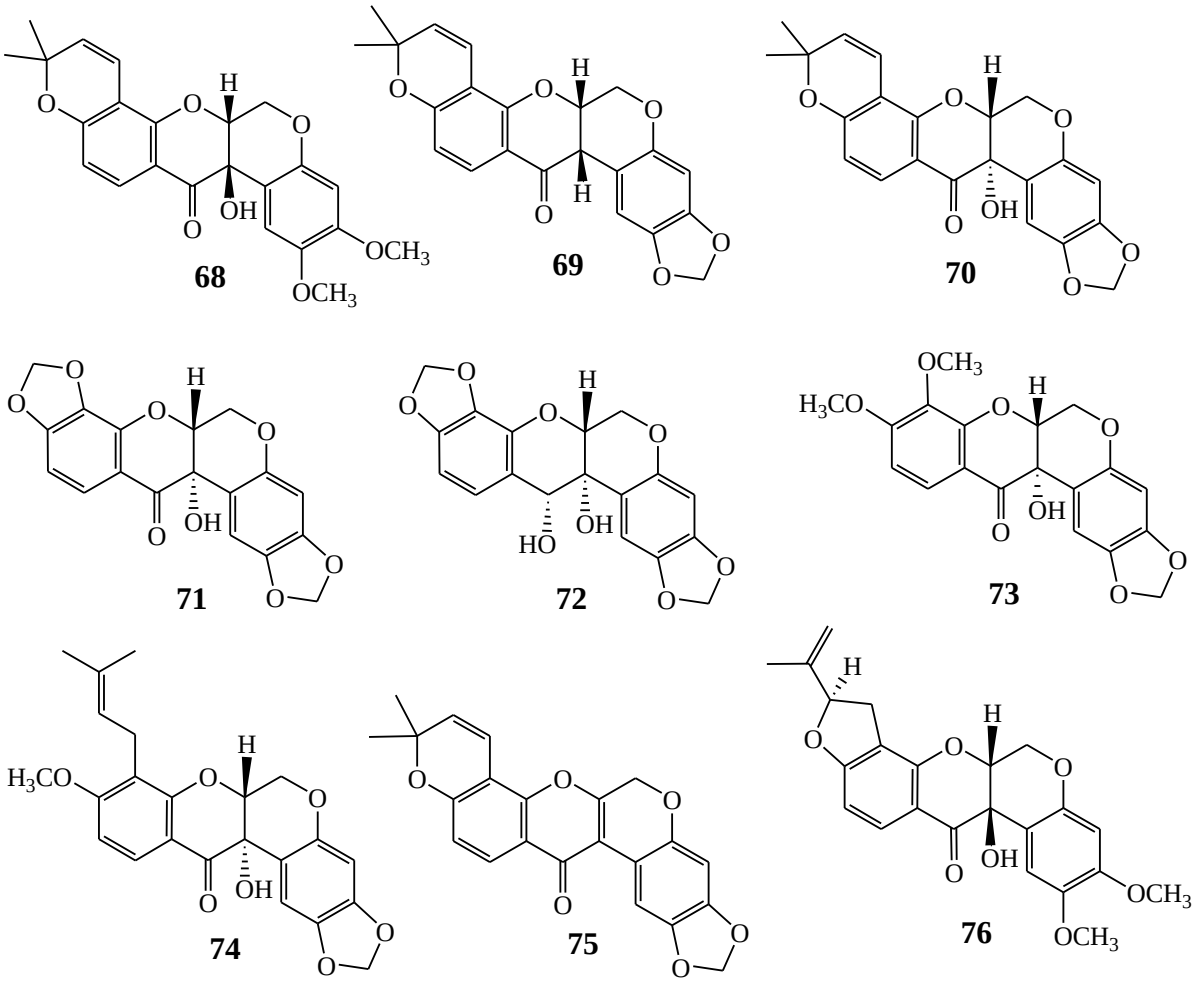
2.6.4.2.2 Rotenoids from *Millettia*

Rotenoids are among the major constituents of the genus *Millettia* [Veitch, 2007]. They contribute significantly to the insecticidal activities of the plant extracts [Allen and Allen, 1981; Fukami and Nakajima, 1971]. Some of the rotenoids furnished by the genus are represented in Table 4.

Rotenone (**25**) was found in a cytotoxic fraction of *M. pervilleana*, inhibited TPA-induced ornithine decarboxylase at the level of its mRNA expression and was therefore regarded as a promising cancer chemopreventive agent [Gerhäuser *et al.*, 1995].

Table 4: Rotenoids from *Millettia*

Rotenoid	**Source (Plant part)**	**Reference(s)**
Rotenone (25)	*M. pervilleana* (root bark) *M. dura* (seeds)	**Galeffi *et al*., 1997** **Yenesew *et al*., 2003a**
Tephrosin (68)	*M. dura* (seeds) *M. ferruginea* ssp *ferruginea* (seeds)	**Yenesew *et al*., 2003a** **Dagne and Bekele, 1990**
Deguelin (42)	*M. dura* (seeds)	**Dagne *et al*., 1991** **Yenesew *et al*., 2003a**
Millettone (69)	*M. dura* (seeds)	**Ollis *et al*., 1967** **Yenesew *et al*., 2003a**
12a-Epimilletttosin (70)	*M. usaramensis* ssp *usaramensis* (stem bark)	**Yenesew *et al*., 2003b**
Usararotenoid A (71)	*M. usaramensis* ssp *usaramensis* (stem bark)	**Yenesew *et al*., 2003b**
12-Dihydrousararotenoid A (72)	*M. usaramensis* ssp *usaramensis* (stem bark)	**Yenesew *et al*., 2003b**
(+)-Usararotenoid B (73)	*M. usaramensis* ssp *usaramensis* (stem bark)	**Yenesew *et al*., 2003b**
Usararotenoid C (74)	*M. usaramensis* ssp *usaramensis* (stem bark)	**Yenesew *et al*., 2003b**
6a,12a-Dehydromillettone (75)	*M. dura* (seeds) *M. usaramensis* ssp *usaramensis* (stem bark)	**Ollis *et al*., 1967** **Yenesew *et al*., 2003b**
***Cis*-12a-hydroxyrotenoid (76)**	***M. pachycarpa* (roots)**	**Singhal *et al*., 1982**

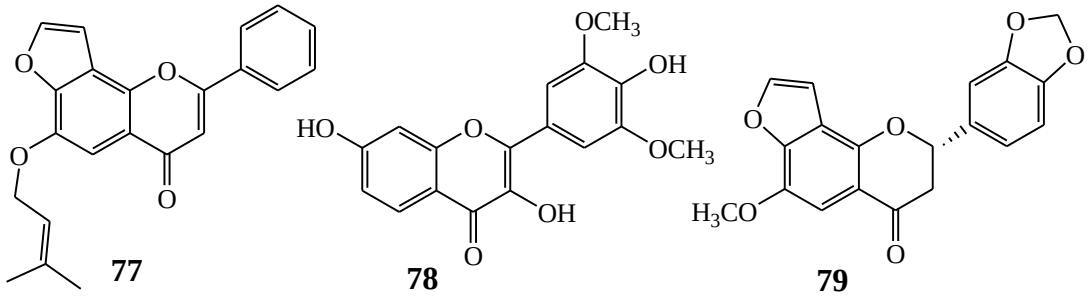


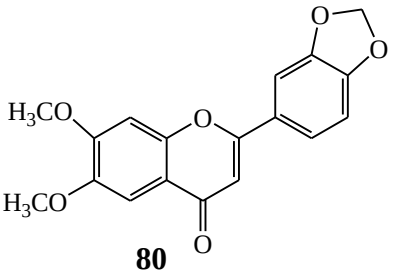
2.6.4.2.3 Flavones, flavanones and flavans from *Millettia*

Table 5 shows some of the flavones, flavanones and flavans isolated from the genus *Millettia*. A number of furano and prenylated flavonones have been isolated from this genus. Ovalifolin (**77**) was found to be moderately active against herpes simplex virus (HSV1 and HSV2) [Sritularak *et al.*, 2002].

Table 5: Flavones, flavanones and flavans from *Millettia*

Compound	Source (plant part)	Reference
Ovalifolin (77)	*M. ovalifolia* (Trunk bark) *M. erythrocalyx* (Stem bark)	**Khan and Zaman, 1974** **Sritularak *et al.*, 2002**
Laurentinol (78)	*M. laurentii* (Heart wood)	**Kamnaing *et al.*, 1999**
Lanceolatin B (44)	*M. ovalifolia* (Trunk bark) *M. hemsleyana* (Stem bark)	**Khan and Zaman, 1974** **Mahmoud and Waterman, 1985**
Milletenin B (79)	*M. ovalifolia* (Leaf)	**Khan and Zaman, 1974**
Milletenin C (80)	*M. ovalifolia*	**Khan and Zaman, 1974**
Karanjachromene (pongaflavone) (81)	*M. hemsleyana* (Stem bark)	**Mahmoud and Waterman, 1985**
(-)-Isolonchocarpin (82)	*M. ovalifolia* (Seeds)	**Krishnamurti and Islam, 1987**
Sanaganone (83)	*M. sanagana* (Roots)	**Mbafor *et al.*, 1995**
(-)-(*2S*)-6-Methoxy-[2″,3″:8,7]-furanoflavanone (84)	*M. erythrocalyx* (Roots)	**Sritularak *et al.*, 2002**
2,5-Dimethoxy-4-hydroxy-[2″,3″:8,7]-furanoflavan (85)	*M. erythrocalyx* (Roots)	**Sritularak *et al.*, 2002**
Pachycarin C (86)	*M. pachycarpa* (Roots)	**Shao *et al.*, 2001**
Pachycarin D (87)	*M. pachycarpa* (Roots)	**Shao *et al.*, 2001**
7-Methoxy-3′,4′-methylenedioxyflavone (88)	*M. erythrocalyx* (Stem bark) *M. hensleyana* (Stem bark) *M. leucantha* (Stem bark)	**Sritularak *et al.*, 2002** **Mahmoud and Waterman, 1985** **Phrutivorapongkul *et al.*, 2003**
Ovaliflavanone C (89)	*M. ovalifolia* (Seed)	**Islam *et al.*, 1980**
Ovaliflavanone D (90)	***M. ovalifolia* (Seed)**	**Islam *et al.*, 1980**





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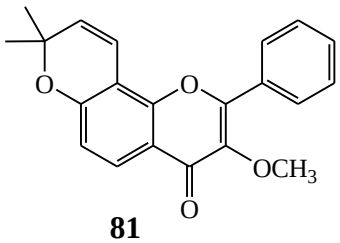

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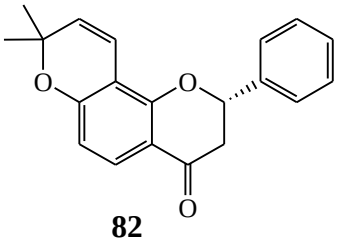

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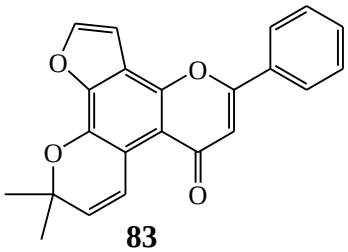

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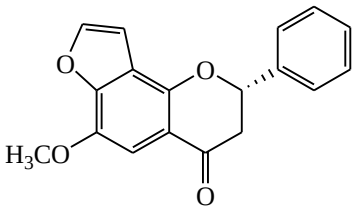

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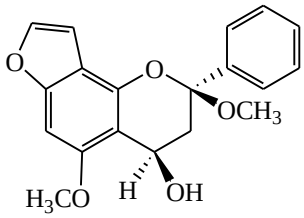

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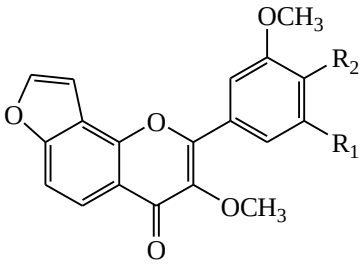
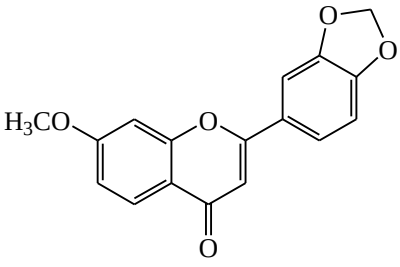


86: $R_1 = R_2 = OCH_3$
87: $R_1 = R_2 = H$



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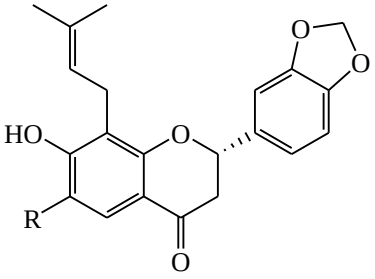


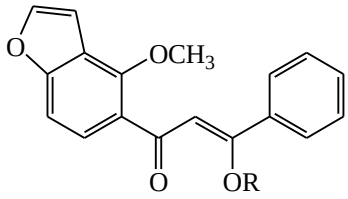
89: R = H
90: R = $CH_2CH=C(CH_3)_2$

2.6.4.2.4 Chalcones, coumarins, quinones and pterocarpans from *Millettia*

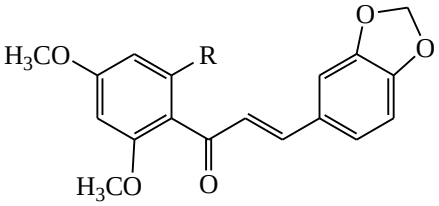
Furano and prenylated chalcones as well as oxymethylated chalcones have been reported from the genus *Millettia.* Coumarins have been reported mainly from the seeds and roots. Quinones have also been obtained from the heartwoods; and pterocarpans are minor constituents of the genus. Table 6a shows some examples of chalcones, coumarins and quinine derivatives. Examples of pterocarpans and alkaloids from *Millettia* are given Table 6b

Table 6a: Chalcones, coumarins and quiononoids from *Milletttia*

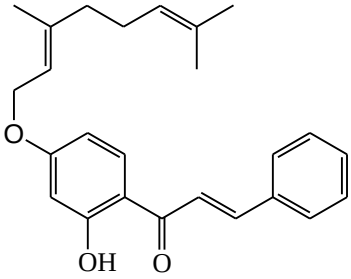
Compound	Source (plant part)	Reference
Chalcones		
Pongamol (91)	*M. erythrocalyx* (roots) *M. ovalifolia* (roots) *M. penguensis* (roots)	**Sritularak *et al.*, 2002** **Saxena *et al.*, 1987** **Ganapaty *et al.*, 1998**
***O*-Methylpongamol (92)**	*M. ovalifolia* (seeds)	**Gupta and Krishnamurti, 1977**
2′,4′,6′-Trimethoxy-3,4-methylenedioxychalcone (93)	*M. leucantha* (stem bark)	**Phrutivorapongkul *et al.*, 2003**
2′,4′-Dimethoxy-3,4-methylenedioxychalcone (94)	*M. erythrocalyx* (roots)	**Sritularak *et al.*, 2002**
Ovalitenone (95)	*M. erythrocalyx* (roots) *M. penguensis* (roots)	**Sritularak *et al.*, 2002** **Ganapaty *et al.*, 1998**
2′,4′,6′-Trimethoxy-3,4-methylenedioxydihydrochalcone (96)	*M. leucantha* (stem bark)	**Phrutivorapongkul *et al.*, 2003**
Derricidin (97)	*M. erythrocalyx* (roots)	**Sritularak *et al.*, 2002**
4′-*O*-Geranylisoliquiritigenin (98)	*M. ferruginea* (root bark) *M. usaramensis* ssp *usaramensis* (stem bark)	**Dagne *et al.*, 1990** **Yenesew *et al.*, 2003b**
Ovalitenone B (99)	*M. ovalifolia* (seeds)	**Gupta and Krishnamurti, 1977**
Coumarins		
Thonningine A (100)	*M. thonningii* (seeds)	**Khalid and Waterman, 1983**
Thonningine B (101)	,,	**Khalid and Waterman, 1983**
Thonningine C (102)	*M. thoningii* (seed pods)	**Asomaning *et al.*, 1995**
4-Hydroxy-5,6,7-trimethoxy-3-(3′,4′-methylenedioxy)phenyl coumarin (103)	*M. griffoniana* (roots)	**Yankep *et al.*, 1998**
Pervilleanine (104)	*M. pervilleana* (roots)	**Palazzino *et al.*, 2003**
Quinones		
Claussequinone (105)	*M. pendula* (heartwood)	**Hayashi *et al.*, 1978**
Pendulone (106)	*M. pendula* (heartwood)	**Hayashi *et al.*, 1978**
Laurentiquinone (107)	***M. lsurentii* (heartwood)**	**Kamnaing *et al.*, 1999**



91: R = H
92: R = CH_3



93: R = OCH_3
94: R = H



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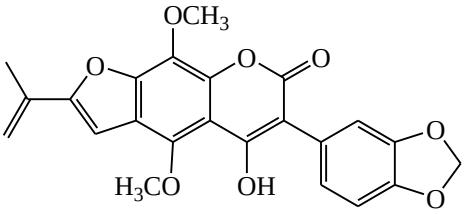

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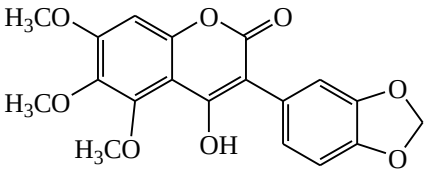

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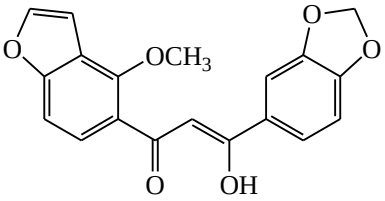

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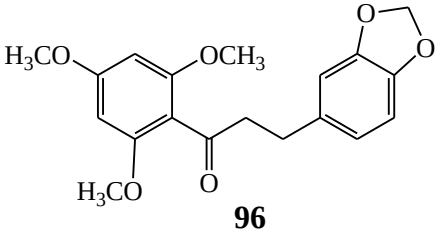

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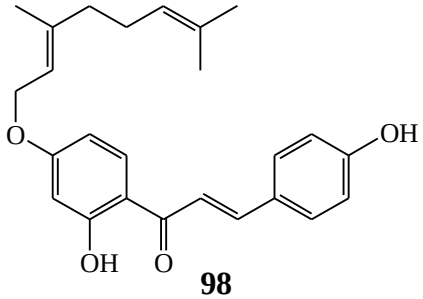

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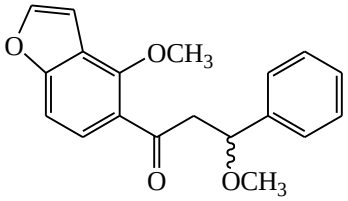

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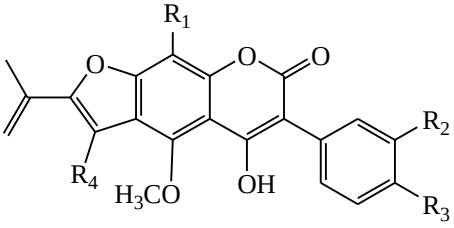
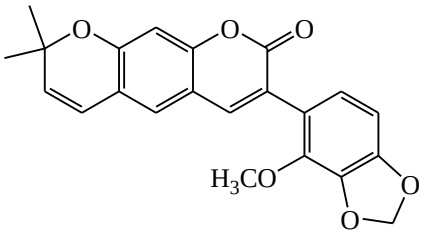


101: $R_1 = R_3 = OCH_3$, $R_2 = R_4 = H$

102: $R_1 = H$, $R_2 = R_3 = R_4 = OCH_3$



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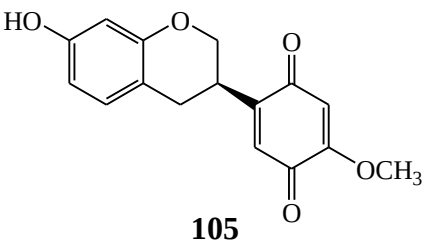

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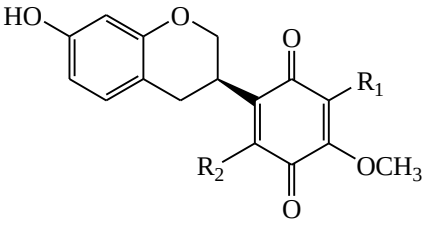
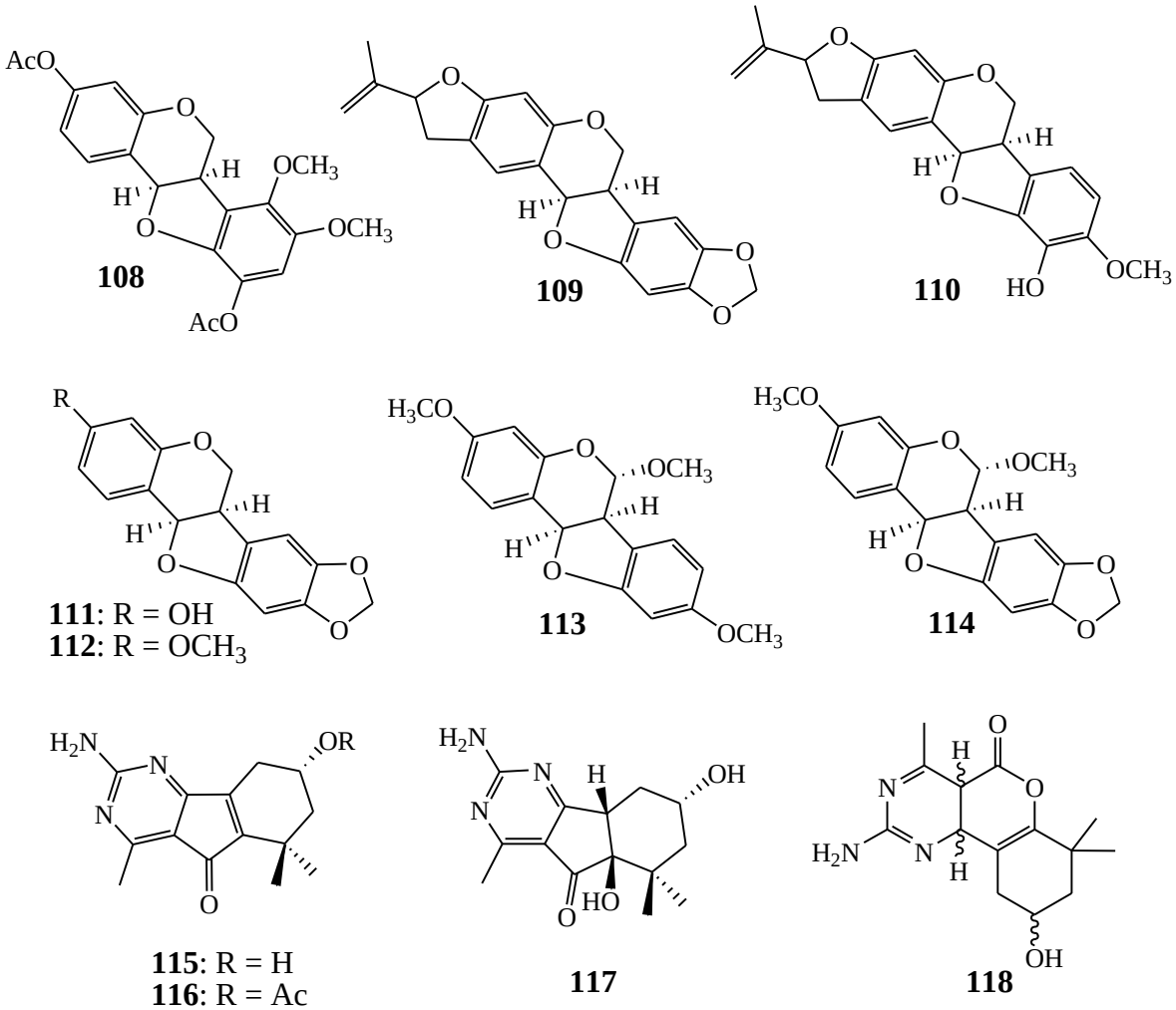


	R_1	R_2
106	OCH_3	H
107	H	OCH_3

Table 6b: Pterocarpans and alkaloids from *Milletttia*

Pterocarpans	**Source (plant part)**	**Reference(s)**
3,10-Diacetoxy-7,8-dimethoxypterocarpan (108)	*M.* species (heartwood)	**Mitsunaga *et al.*, 1987**
Emoroidocarpan (109)	*M. pervilleana* (root bark)	**Palazzino *et al.*, 2003**
Pervilline (110)	*M. pervilleana* (root bark)	**Palazzino *et al.*, 2003**
Maackiain (111)	*M. pulchra* (aerial parts) *M. speciosa* (roots) *M. pendula* (heartwood)	**Baruah *et al.*, 1984** **Uchiyama *et al.*, 2003** **Hayashi *et al.*, 1978**
Pterocarpin (112)	*M. pulchra* (aerial parts)	**Baruah *et al.*, 1984**
6α-Methoxyhomopterocarpin (113)	*M. pulchra* (aerial parts)	**Baruah *et al.*, 1984**
6α-Methoxypterocarpin (114)	*M. pulchra* (aerial parts)	**Baruah *et al.*, 1984**
Alkaloids		
Millaurine (115)	*M. laurentii* (seeds) *M. laurentii* (seeds)	**Ngamga *et al.*, 1993** **Ngamga *et al.*, 2007**
Acetylmillaurine (116)	*M. laurentii* (seeds)	**Ngamga *et al.*, 1993**
5a,9a-Dihydro-5a-hydroxymillaurine (117)	*M. laurentii* (seeds)	**Ngamga *et al.*, 1993**
Millettonine (118)	***M. laurentii* (stem bark)**	**Kamnaing *et al.*, 1994**



2.6.4.3 Phytochemistry of *Flemingia*

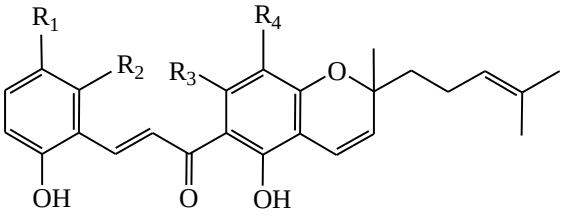
A range of compounds have been reported from the genus *Flemingia*. These include, isoflavones, chalcones, flavanones, and to a less extent flavonol glycosides, dihydrochalcones, quinones, coumestans and pterocarpans.

2.6.4.3.1 Chalcones from *Flemingia*

The genus *Flemingia* is known to contain chalcones, majority of which have a unique *para*-dioxygenation pattern in the B-ring together with a geranyl moiety in the form of an open chain or a chromene and an extended side chain [Cardillo *et al*., 1968, 1970; 1973; Rao *et al*., 1975]. A list of chalcones isolated from *Flemingia* species are represented in Table 7. Chalcones have been reported mainly from leaves.

Table 7: Chalcones from *Flemingia*

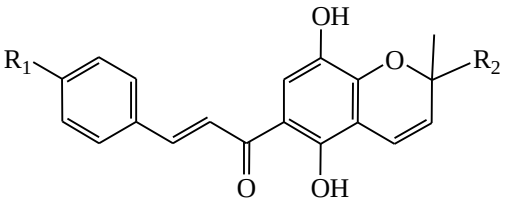
Compound	Source (plant part)	Reference(s)
Homoflemingin (119)	*F. rhodocarpa* (seeds pods) *F. grahamiana* (inflorescence)	Cardillo *et al.*, 1968
Flemiwallichin A (120)	*F. wallichii*	Rao *et al*., 1975
Flemiwallichin B (121)	*F. wallichii*	Rao et al., 1975
Flemiwallichin D (122)	*F. wallichii* (leaves)	Babu *et al.*, 1985
Flemichapparin A (123)	*F. chappar* (aerial part)	Cardillo *et al.*, 1970
Flemingin D (124)	*F. congesta* (leaves and flowers)	Cardillo *et al.*, 1973
Flemingin E (125)	*F. congesta* (leaves and flowers) *F. wallichii* (leaves)	Cardillo *et al.*, 1973 Babu *et al*., 1985
Flemingin F (126)	*F. congesta* (leaves and flowers) *F. wallichii* (leaves)	Cardillo *et al.*, 1973 Babu *et al*., 1985
Flemiwallichin F (127)	*F. wallichii* (leaves)	Babu *et al.*, 1985
Flemiculosin (128)	*F. fruticulose* (leaves)	Bhattacharyya *et al*., 1999
Flemistrictin C (129)	*F. stricta* (leaves)	Rao *et al*., 1976
Flemistrictin B (130)	*F. stricta* (leaves)	Rao *et al*., 1976
Flemistrictin A (131)	*F. stricta* (leaves)	Rao *et al*., 1976



120: R_1 = H; R_2 = OH; R_3 = OH; R_4 = H

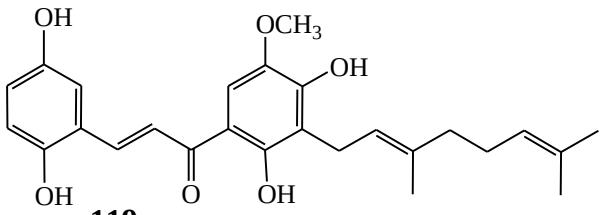
121: R_1 = OH; R_2 = H; R_3 = OH; R_4 = H

122: R_1 = OH; R_2 = H; R_3 = R_4 = H



123: R_1 = H; R_2 = Me

124: R_1 = OH; R_2 = 3-methylbut-3-enyl



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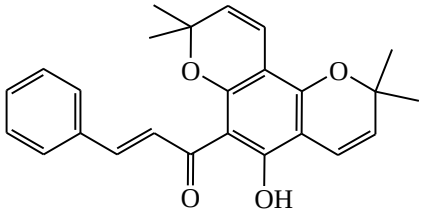

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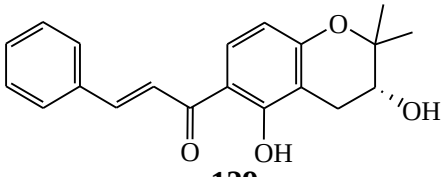

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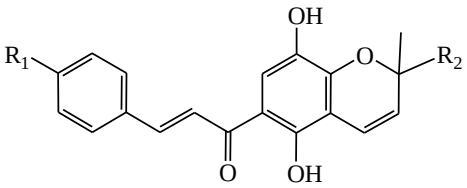
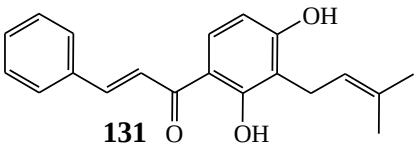


125: R_1 = OH; R_2 = (*E*)-4-hydroxy-4-methylpent-2-enyl

126: R_1 = OH; R_2 = 2-hydroxy-3-methylbut-3-enyl

127: R_1 = H; R_2 = (*E*)-4-hydroxy-4-methylpent-2-enyl



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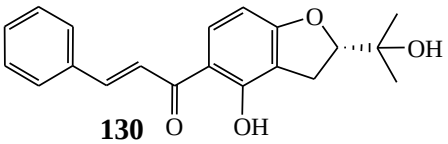


130

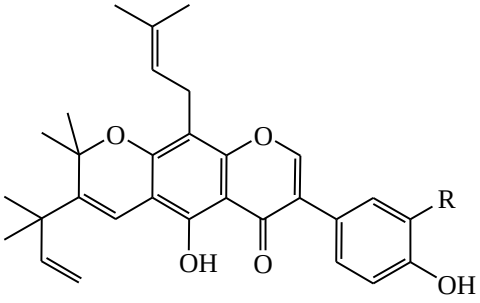
2.6.4.3.2 Isoflavones from *Flemingia*

Majority of the isoflavonoids from the genus *Flemingia* are prenylated and sometimes obtained as glycosides (Table 8a).

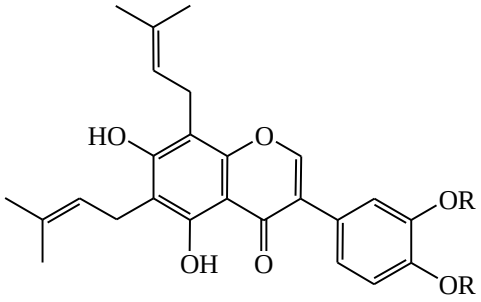
Table 8a: Isoflavones from *Flemingia*

Compound	Source (plant part)	Reference(s)
Flemiphilippinin A (132)	*F. philippinensis* (Roots)	Chen *et al*., 1991 Fu *et al*., 2012
Flemiphilippinin B (133)	*F. philippinensis* (roots)	Chen *et al*., 1991
Flemiphilippinin C (134)	*F. philippinensis* (roots)	Chen *et al*., 1991
Flemiphilippinin E (135)	*F. philippinensis* (roots)	Li *et al*., 2008
Erythrinin B (136)	*F. philippinensis* (roots)	Chen *et al*., 1991
Auriculasin (53)	*F. philippinensis* (roots)	Li *et al*., 2008 Fu *et al*., 2012
8-(1,1-Dimethylallyl)Genistein (137)	*F. philippinensis* (roots)	Li *et al*., 2008 Fu *et al*., 2012
Flemiphyllin (138)	*F. macrophylla* (stems)	Rao and Srimannarayana, 1984
5,7,4′-Trihydroxy-8,2′,5′-tri(3-methylbut-2-enyl)isoflavone (139)	*F. strobilifera* (roots)	Madan *et al.*, 2009
Flemiphilippinin G (140)	*F. philippinensis* (roots)	Fu *et al*., 2012
5,7,3′,4′-Tetrahydroxy-6,8-diprenylisoflavone (141)	*F. philippinensis* (roots)	Fu *et al*., 2012
5,7,4′-Trihydroxy-8-(1,1-dimethylprop-2-enyl)isoflavone (142)	*F. paniculata* (stem bark)	Rahman *et al*., 2004
5,7,2′,4′-Tetrahydroxy-8-(1,1-dimethylprop-2-enyl)isoflavone (143)	*F. paniculata* (stem bark) *F. philippinensis* (roots)	Rahman *et al*., 2004 Fu *et al*., 2012
5,2′,4′-Trihydroxy-2″,3″,3″(ξ)-trimethyl-2″,3″-dihydrofurano-[4″,5″:6,7]-isoflavone (144)	*F. paniculata* (stem bark)	Rahman *et al*., 2004
5,2′,4′-Trihydroxy-7-(3-methylbut-2-enyloxy)isoflavone (145)	*F. paniculata* (stem bark)	Rahman *et al*., 2004

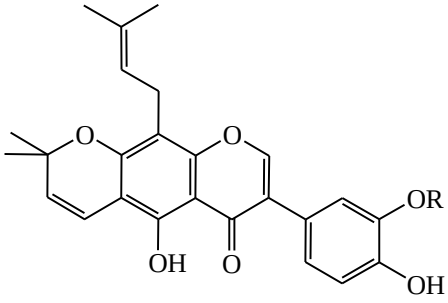
Flemiphilippinins A (**132**) and B (**133**) obtained from the roots of *F. philippinensis* exhibited significant activity against lymphocytic leukaemia [Chen *et al.*, 1991]. Flemiphilippinin G (**140**), also isolated from *F. philippinensis*, was strongly cytotoxic against human breast carcinoma cell line (MCF-7), human lung cancer cell line (A549) and human hepatoma cell line (Hep-G2) [Fu *et al.*, 2012].



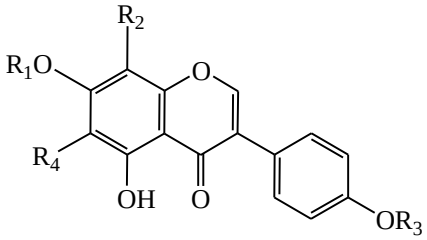
132: R = OH
135: R = H



133: R = Me
141: R = H

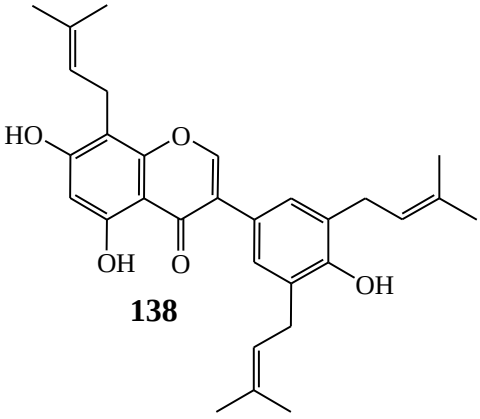


134: R = Me



136: $R_1 = R_2 = R_3 = H$; R_4 = 3,3-dimethylallyl

137: $R_1 = R_3 = R_4 = H$; R_2 = 1,1-dimethylallyl



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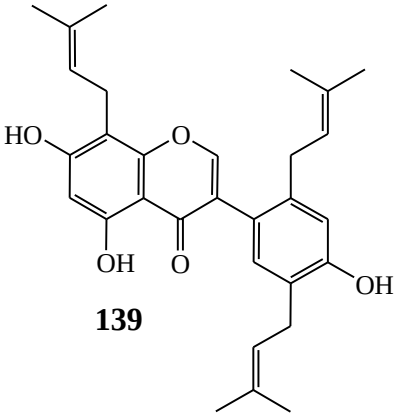

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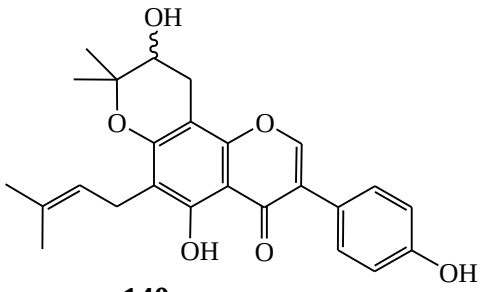

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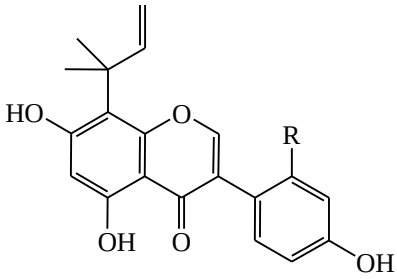
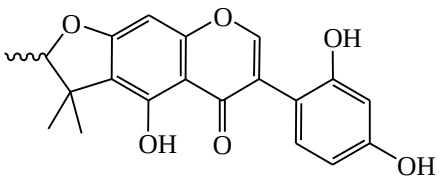
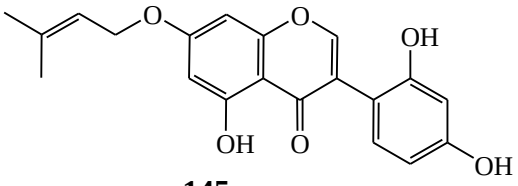


142: R = H
143: R = OH



144



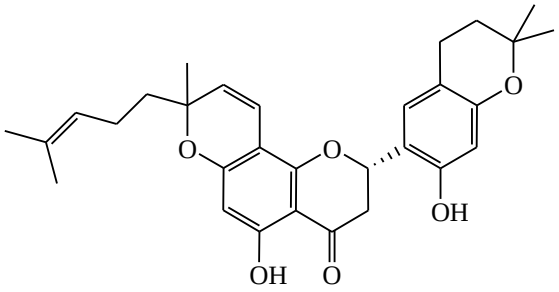
145

2.6.4.3.3 Flavanones from *Flemingia*

Several multiple prenylated flavanones have been reported from *Flemingia*. A summary of the flavanones which have been previously isolated from *Flemingia* is presented in Table 8b.

Table 8b: Flavanones from *Flemingia*

Compound	Source (plant part)	Reference(s)
Flemichin A (146)	*F. wallichii* (roots)	Rao *et al*., 1974
Flemichin C (147)	*F. wallichii* (roots)	Rao *et al*., 1975
Naringin (148)	*F. strobilifera* (leaves)	Saxena *et al.*, 1976
Fleminone A (149)	*F. macrophylla* (stems)	Rao and Srimannarayana, 1983
Flemichin D (150)	*F. wallichii* (roots) *F. philippinensis* (roots) *Moghania philippinensis* (roots)	Rao *et al*., 1975 Hua *et al*., 2009 Ahn *et al*., 2003
Lupinifolin (151)	*F. philippinensis* (roots)	Hua *et al*., 2009



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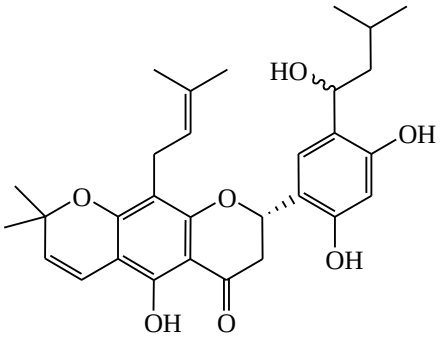

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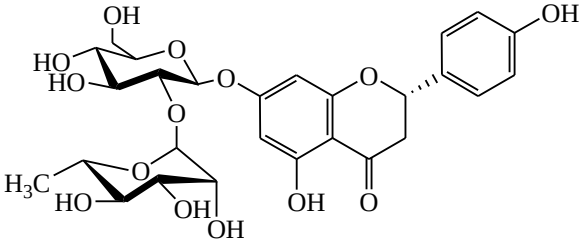

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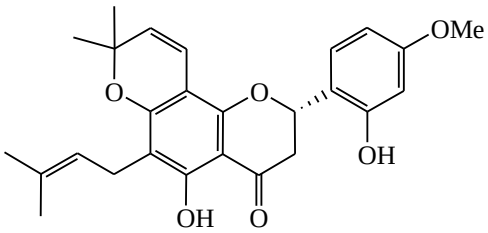

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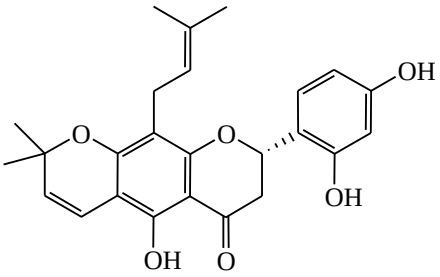

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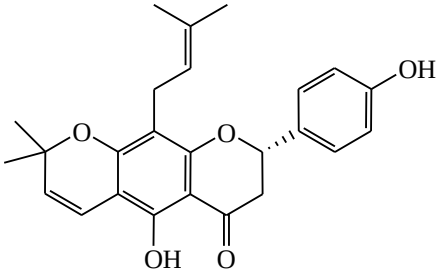
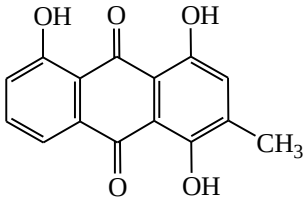


151

2.6.4.3.4 Anthraquinones from *Flemingia*

Anthraquinones have been reported from the roots of *F. philippinensis* and these include islandicin (**152**), chrysophanol (**153**) and physcion (**154**) [Hua *et al*., 2009].



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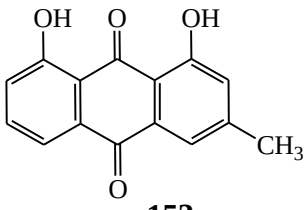

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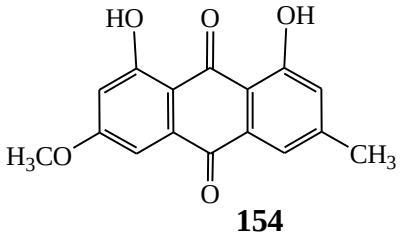
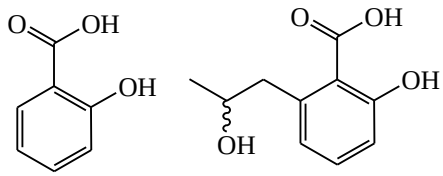


154

2.6.4.3.5 Salicylic acid derivatives from the Genus *Flemingia*

Salicylic acid (**155**) and 2-carboxy-3-(2-hydroxypropanyl)-phenol (**156**) have been reported from *F. philippinensis* [Hua *et al.*, 2009] and *F. paniculata* [Rahman *et al.*, 2004], respectively. These compounds could be responsible for the reported analgesic properties of *Flemingia* species [Ghalot, 2011].



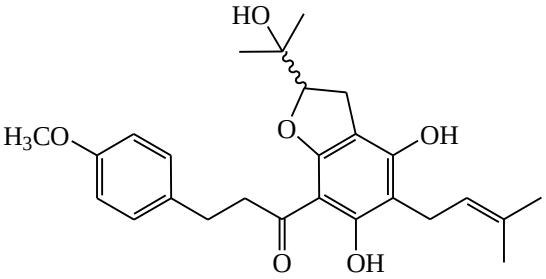
155 **156**

2.6.4.3.6 Other Minor Constituents from *Flemingia*

Other minor classes of compounds obtained from *Flemingia* species include dihydrochalcones, coumestans, pterocarpans, flavonol glycosides, some of which are represented in Table 8c. The aerial parts of *F. macrophylla* yielded flemingichalcone (**157**), medicagol (**159**) and aureole (**160**), among other natural products. Compound **162** significantly protected neurons of Harlan Sprague-Dawley rat pups from amyloid β protein-induced damage with an EC_{50} = 12.09 ± 2.55 μM [Shiao *et al*., 2005].

Table 8c: Other minor compounds from *Flemingia*

Compound	Source (Plant part)	Reference(s)
Dihydrochalcones		
Flemingichalcone (214)	*F. macrophylla* (aerial parts)	Shiao *et al*., 2005
Phlorizin (215)	*Flemingia strobilifera* (leaves)	Saxena *et al.*, 1976
Coumestans		
Medicagol (216)	*F. macrophylla* (aerial parts)	Shiao *et al*., 2005
Aureole (218)	*F. macrophylla* (aerial parts)	Shiao *et al*., 2005
Pterocarpan		
Flemichapparin B (219)	*F. chappar* (roots)	Adityachaudhury and Gupta, 1973
Flavonol glycoside		
Flemiphilippininside (220)	*F. philippinensis* (roots)	Fu *et al*., 2012



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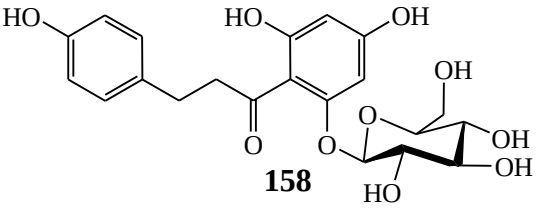

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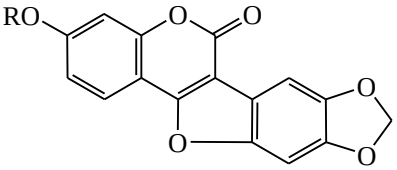

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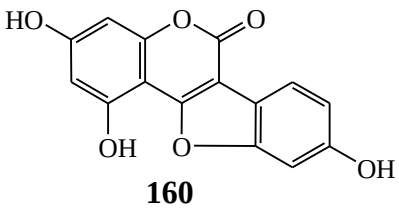

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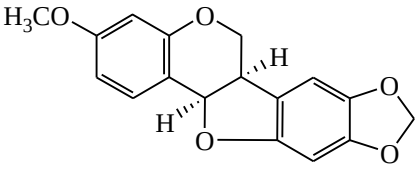

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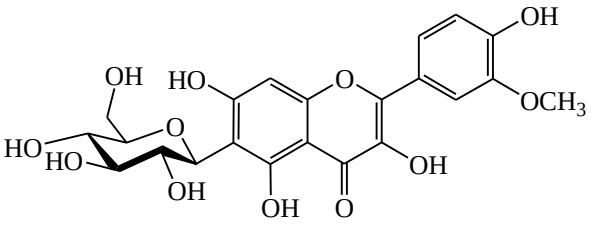

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CHAPTER THREE
MATERIALS AND METHODS

3.1 General

Column chromatography (CC) and medium pressure liquid chromatography (MPLC) were carried out on silica gel 60 (70-230 mesh or 230-400 mesh). Preparative thin layer chromatography (Prep-TLC) was done on glass plates (20 x 20 cm) coated with silica gel 60 PF_{254} (Merck) with a thickness of 0.25-1.0 mm. Analytical thin layer chromatography (TLC) was done on silica gel 60 F_{254} (Merck) pre-coated aluminium plates which, after development with an appropriate solvent system, were viewed under UV light (254 nm and 366 nm). Gel filtration was carried out over Sephadex® LH-20 (Pharmacia) suspended in CH_2Cl_2/MeOH (1:1). UV spectra were recored on a Specord S600 Analytik Jena AG, Germany; or Hewlett-Packard 8453 spectrophotometer. CD spectra were recorded on JASCO J-710 spectropolarimeter; EI-MS was determined by direct inlet, 70 eV on Micromass GC-TOF micro mass spectrometer (Micromass, Wythenshawe, Waters Inc., UK). LC-ESI-MS was run on a Perkin-Elmer PE SCIEX API 150EX spectrometer with a Turbolon spray ion source and a Gemini 5 mm RPC_{18} 110 Å column (H_2O/MeCN, 80:20-20:80 gradient solvent system).

High-resolution mass spectral data was obtained on a Q-TOF-LC/MS spectrometer (Stenhagen Analyslab AB, Gothenburg, Sweden) using a 2.1 x 30 mm 1.7 µm RPC_{18} column [H_2O/MeCN, 5:95-95:5 (with 0.2% formic acid) gradient solvent system]. Preparative HPLC was done on a Waters 600E machine using an RPC_8 Kromasil® (250 mm x 25 mm) column, solvent system; H_2O/MeOH or H_2O/MeCN (gradient, 90:10 to 5:95, in 30-80 min, flow rate of 8-15 ml/min) using Chromulan (Pikron Ltd) software. Evaporation of CC fractions was done by rotary evaporation under vacuum at 40-50 $^{\circ}C$ whereas HPLC fractions were evaporated using a V-10 evaporator (Biotage) or Genevac (Biotage).

NMR spectra were run on Varian spectrometers operating at 200, 400, 500, 600 or 800 MHz and, Bruker Avance spectrometers at either 300 or 600 MHz (for the ^1H nuclei) with test-samples dissolved in deuterated solvents. The spectra were processed using MestReNova-8.1.1 or Topspin softwares. Structural elucidation and NMR assignments were based on both one-dimensional (^1H, ^{13}C NMR) and two-dimensional (^1H-^1H gCOSY, gNOESY, gCHSQC, gHMBC) experiments using residual solvent peaks as references.

3.2 Plant Materials

The stem-bark of *Platycelphium voënse* was collected from Mwingi District, Eastern Province, Kenya, in January 2009. The leaves and roots of *Flemingia grahamiana* were collected from Kitale in western Kenya in October 2008. The roots of *Millettia usaramensis* subsp. *usaramensis* were collected from Coast Province, Kenya, in February 2008. The plant materials were authenticated at the National Museums of Kenya East African Herbarium, Nairobi, where voucher specimen are deposited (voucher Nos. Mathenge 2009/568 for *P. voënse*; Mathenge 2008/487 for *F. grahamiana*; and Mathenge 2008/374 for *M. usaramensis* subsp. *usaramensis*).

3.3 Extraction and Isolation

3.3.1 Extraction and isolation of the stem bark of *Platycelphium voënse*

The air-dried stem-bark of *P. voënse* (1.6 kg) was pulverized and extracted by soaking in CH_2Cl_2/MeOH (1:1) at room temperature twice, for 24 h for every soak. After evaporation of the solvent, 114 g of the crude extract (7.12 %) was obtained. A portion (110 g) of the crude extract was fractionated by column chromatography over silica gel using increasing amounts of EtOAc in *n*-hexane as the solvent, resulting in 10 fractions (each fraction *ca*. 1.5 L volume-elution). Fraction 2 that eluted with 1% EtOAc in *n*-hexane yielded *β*-amyrin (**172**) (21.0 mg). Fraction 3 (eluated with 4% EtOAc in *n*-hexane) consisted of four major compounds which were separated by further CC on silica gel (solvent: 0-15% acetone in *n*-hexane) to give compounds **163** (210.0 mg), **164** (37.2 mg), **165** (31.0 mg) and **169** (36.4 mg). Fraction 3 (eluted with 5% EtOAc in *n*-hexane) was subjected to CC on silica gel (0-30% EtOAc in *n*-hexane) followed by size exclusion chromatography over Sephadex LH-20 (eluent CH_2Cl_2/MeOH 1:1) giving betulin (**173**) (28.0 mg). Fraction 4 (eluted with 5-10% EtOAc in *n*-hexane) was further purified by medium pressure liquid chromatography (MPLC, solvent: 0-5% acetone in *n*-hexane; flow rate: 30 mL/min) to yield kumatakenin (**170**) (29.0 mg). Fraction 5 (15-20% EtOAc in *n*-hexane) was subjected to MPLC (solvent: 0-15% EtOAc in *n*-hexane; flow rate: 30 mL/min) to give formononetin (**52**) (10.1 mg), compounds **166** (40.0 mg), **168** (15.0 mg), **169** (25.3 mg), **167** (2.0 mg) and isokaempferide (**171**) (6.2 mg).

3.3.2 Extraction and isolation of the root-bark of *Millettia usaramensis* subsp. *usaramensis*

The air-dried root bark of *Millettia usaramensis* subsp. *usaramensis* (1.6 Kg) was pulverized and soaked in CH_2Cl_2/MeOH, 1:1 at room temperature twice with fresh solvent every 24 h giving 70 g of crude extract (4.3 % yield). The crude extract (68 g) was subjected to CC on silica gel (400 g) using gradient solvent elution (EtOAc in *n*-hexane) to give 12 fractions (*ca*. 3 L of elution per fraction). Fractions 1-2 (eluted with 0-1% EtOAc in *n*-hexane) contained non-UV active components (probably fatty acids) and were not followed. Fraction 3 (eluted with 2% EtOAc in *n*-hexane) was subjected to prep-TLC (eluted with 70 % CH_2Cl_2 in *n*-hexane) to give compound **175** (10.0 mg). Fraction 4 (eluted with 3% EtOAc in *n*-hexane) gave a precipitate which after washing with 2% EtOAc in *n*-hexane gave compound **70** (23.4 mg). Fraction 5 (eluted with 4% EtOAc in *n*-hexane) gave a white suspension which after filtration and washing with 3% EtOAc in n-hexane gave compound **74** (20.8 mg). The filtrate (mother liquor) was then subjected to size-exclusion chromatography over Sephadex LH-20 (CH_2Cl_2/MeOH, 1:1) giving compound **181** (470.2 mg). Fraction 6 (eluted with 5% EtOAc in *n*-hexane) was subjected to CC over silica gel (eluted with 0-60% CH_2Cl_2 in *n*-hexane) giving compound **71** (60.3 mg). Fraction 7 (eluted with 6-7% EtOAc in *n*-hexane) was subjected to CC over silica gel (eluted with 0-70% CH_2Cl_2 in *n*-hexane) giving nine sub-fractions. Sub-fraction six, "sfr6" (eluted with 50% CH_2Cl_2 in *n*-hexane) was prep-TLC (2% then 5% EtOAc in CH_2Cl_2) leading to compound **176** (7.6 mg). Sub-fraction seven, "sfr7" (eluted with 60% CH_2Cl_2 in *n*-hexane) was subjected to prep-TLC (12% acetone in *n*-hexane) giving compound **182** (8.2 mg) and more of compound **181** (5.0 mg). Sub-fraction eight, "sfr8", was subjected to CC over silica gel (1-10% acetone in *n*-hexane then methanol/acetone/hexane (1:3:6)) giving compounds **178** (4.5 mg), **179** (2.8 mg) and **180** (207.4 mg).

Fraction 8 (eluted with 10% EtOAc in *n*-hexane) was further fractionated by prep-TLC (eluted with 3% acetone in CH_2Cl_2) giving compound **174** (~ 1.0 mg). Fraction 9 (eluted with 12-15% EtOAc in *n*-hexane) gave a white precipitate which was washed with 8% EtOAc in hexane leading to compound **72** (48.6 mg). The mother liquor was subjected to prep-TLC (eluted with 4% acetone in CH_2Cl_2) yielding compound **177** (2.1 mg).

3.3.3 Extraction and isolation of the roots of *Flemingia grahamiana*

The air-dried and pulverized roots of *Flemingia grahamiana* (1.55 Kg) was extracted using CH_2Cl_2/MeOH (1:1) as previously described for *Platycelphium voënse* and *Millettia usaramensis* giving 93.4 g (6.0 %) of a brown coloured crude extract. A portion of the extract (62.0 g) was sonicated in CH_2Cl_2/EtOAc (1:1) for 30 min, left to stand for 12 h, and followed by decanting to give 15.0 g extract (after evaporation of solvent). The extract (13.0 g) was adsorbed on 20 g of silica gel 60 (230-400 mesh, Merck) then subjected to CC over silica gel eluting with *n*-hexane containing increasing amounts of EtOAc, followed by increasing amounts of MeOH in EtOAc, resulting in 13 fractions (*ca*. 4.0 L for each fraction). The fifth CC fraction (eluted at 30-40% EtOAc/*n*-hexane) was subjected to repeated prep-HPLC (RPC_8 250 mm x 25 mm, Kromasil® column; H_2O/MeCN eluate) to give flemichin D (**150**) (20.2 mg), eriosemaone (**189**) (2.0 mg), 5,3′,4′-trihydroxy-8-γ,γ-dimethylallyl-2″,2″-dimethylpyrano-[5″,6″:6,7]-flavanone (**191**) (3.0 mg), eriosematin (**192**) (1.5 mg), lupalbigenin (**188**) (2.5 mg), lupinifolin (**151**) (10.3 mg) and 5,2′,4′-trihydroxy-8,5′-di(γ,γ-dimethylallyl)-2″,2″-dimethylpyrano-[5″,6″:6,7]-flavanone (**190**) (10.4 mg). The sixth CC fraction (eluted at 50% EtOAc in *n*-hexane) was also subjected to prep-HPLC (H_2O/MeCN eluate) leading to isolation of genistein (**11**) (30.0 mg), biochanin A (**183**) (3.0 mg), caryolin (**187**) (1.5 mg) and flemiphilippinin F (**186**) (2.5 mg). The ninth CC fraction (eluted at 90% EtOAc in *n*-hexane) was further purified by prep-HPLC (H_2O/MeCN solvent system) giving 4′-*O*-methylgallocatechin (**193**) (4.0 mg). Preparative HPLC (H_2O/MeCN eluate) of the eleventh fraction (eluate: 20% MeOH/EtOAc) gave genistein 4′-*O*-β-glucopyranoside (also known as genistin, (**184**)) (10.0 mg) and genistein 7,4′-di-*O*-β-glucopyranoside (**185**) (5.0 mg).

3.3.4 Extraction and isolation of the leaves of *F. grahamiana*

The Dried and pulverized leaves (413.19 g) of *F. grahamiana* were soaked twice in CH_2Cl_2/MeOH (1:1) at room temperature, for 24 h in each case, to yield 29.56 g of crude extract after solvent evaporation. A portion of the extract (20 g) was adsorbed on silica gel 60 (70-230 mesh ASTM) (40 g) and loaded on a column of silica gel and eluted with increasing amounts of EtOAc in *n*-hexane to give 20 fractions (*ca*. 500 mL per fraction). Each of the fractions 7-20 were purified by gel filtration over Sephadex LH-20 [CH_2Cl_2/MeOH (1:1) eluate]. Fractions 7-9 (eluted during CC at 5-25% EtOAc in *n*-hexane) were combined and

further purified by CC (silica gel–deactivated with oxalic acid; gradient elution of EtOAc in *n*-hexane) leading to isolation of emodin (**213**) (2.1 mg) and flemingin A (**196**) (46.0 mg). The remaining sub-fractions from fraction 7-9 were recombined and subjected to prep-HPLC (RPC_8 250 mm x 25 mm, Kromasil® column; H_2O/MeCN eluate) leading to isolation of compounds **206** (2.5 mg) and **208** (3.0 mg). Fraction 15 (50% EtOAc in *n*-hexane) was subjected to prep-HPLC (H_2O/MeOH eluate) yielding compounds **197** (15.4 mg) and **198** (6.3 mg). Fractions 16 and 17 (50-70% EtOAc in *n*-hexane) were mixed and subjected to prep-HPLC (H_2O/MeOH eluate) to give compound **201** (13.2 mg). Fractions 18 and 19 (70% EtOAc in *n*-hexane-100% EtOAc) were combined and subjected to prep-HPLC (H_2O/MeOH eluate) giving crude mixtures "sfr4" (**203** plus **207**) and "sfr7" (**204** plus **205**). The sub-fraction sfr7 was further purified by prep-HPLC (H_2O/MeOH eluate) leading to separation of compounds **204** (1.5 mg) and **205** (2.3 mg). Sub-fraction sfr4 was purified by prep-HPLC (H_2O/MeCN eluate) to give the pure forms of **203** (4.2 mg) and **207** (5.0 mg). Fractions 12 and 13 (40% EtOAc in *n*-hexane) were subjected to prep-HPLC (H_2O/MeCN eluate) giving crude forms of **211** (0.8 mg), **202** (10.0 mg), **199** (3.0 mg) and **200** (2.6 mg) which were further purified by prep-HPLC (H_2O/MeOH eluate) to give the respective compounds. Combined fractions 10-12 (30% EtOAc in *n*-hexane) were subjected to prep-HPLC (H_2O/MeCN eluate) to give sub-fractions "sfrA-D". Sub-fraction sfrA was further purified by prep-HPLC (H_2O/MeOH eluate) to give compounds **209** (1.0 mg) and **210** (1.8 mg); and sub-fraction sfrC was subjected to prep-HPLC (H_2O/MeCN eluate) leading to isolation of **212** (4.0 mg), **194** (30.7 mg) and **195** (20.6 mg).

3.4 Cytotoxicity Test

Cytotoxicity assays of some of the isolated compounds against MCF-7 human breast cancer cells as described by Endale *et al*. (2012). The cells were cultured in Dulbecco's modified Eagle medium supplemented with fetal bovine serum (10% v/v), 2 mM *L*-glutamine, 100 units/mL penicillin and 100 μg/mL streptomycin. The medium was pre-incubated at 37 °C under humid conditions with 5% CO_2, 95% air. Cells were seeded in 96-well plates at optimal cell density to ensure exponential growth for the duration of the assay. The growth medium was replaced with the experimental medium containing an appropriate drug concentration or control (0.1% or 1.0% v/v, DMSO) after 24 h of pre-incubation. Cell viability was measured, after 48 h of incubation, using PrestoBlue cell viability reagent

(Inventrogen AB Lindingö, Sweden) in accordance with the manufacturer's instructions. Absorbance was measured at 570 nm with 600 nm as a reference wavelength. Results were expressed as mean ± SE for six replicates as a percentage of vehicle control (taken as 100%). Experiments were independently carried out at least three times. Statistical analyses were performed using a two-tailed student's *t*-test. $p < 0.05$ was considered statistically significant. 1-Isopropyl-3-(pyridine-4-ylethynyl)-1*H*-pyrazolo[3,4-d]pyrimidin-4-amine was used as the standard drug.

Cytotoxicity of some test compounds against Vero cells was performed as described by Falzari *et al.* (2005). The anti-TB agent rifampicin was used as the standard drug and showed no significant cytotoxicity (IC_{50} = 183 μM).

3.5 Radical Scavenging Activity Test

The method as described by Ohnishi *et al*. (1994) was used with minor modifications. Test sample solutions at 320, 160, 80, 40, 20, 10, 5 and 2.5 μg/mL were prepared in double-distilled methanol. From each concentration, 1.0 mL of the sample was added to 2.0 mL of 76 μM 1,1-diphenyl-2-picrylhydrazyl radical (α,α-diphenyl-β-picrylhydrazyl) (DPPH) that was dissolved in methanol. The mixture was allowed to stand at room temperature for 30 minutes and the absorbance of the remaining DPPH measured at 517 nm. The radical scavenging activity was measured as the decrease in absorbance due to DPPH radical expressed as a percentage of the control solution (1.0 mL of methanol and 2.0 mL of 76 μM DPPH solution). The activity was then expressed as EC_{50}, which is the concentration of the test compound required to give a 50% decrease of the absorbance from that of the control solution. Quercetin was used as positive control.

$$\% \text{ of scavenged DPPH} = \frac{A_{DPPH} - A_{Sample}}{A_{DPPH}} \times 100$$

Where A_{DPPH} is the absorbance of the solution containing DPPH but without a test sample; A_{Sample} is the absorbance of the mixture of test sample and DPPH solution.

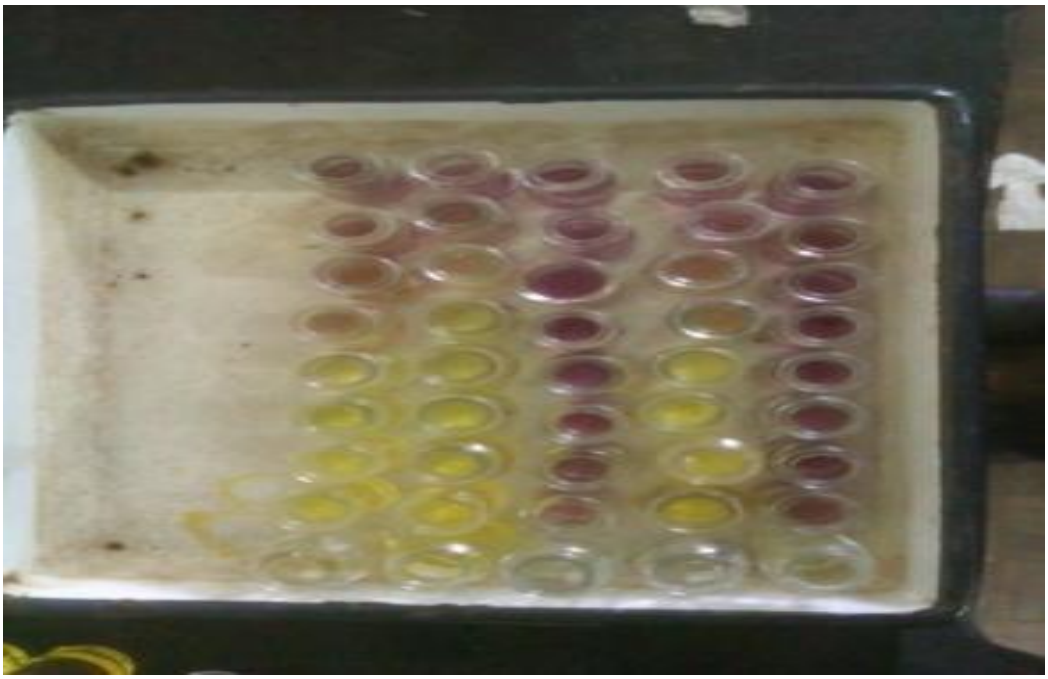


Figure 3: Vials containing varying concentrations of test samples in methanol with constant amount of DPPH

EC_{50} values were determined from graphs of percentage scavenged DPPH versus initial concentration of each sample. Activity index was calculated by comparing the EC_{50} value (in μM) for a given test compound with that of the standard.

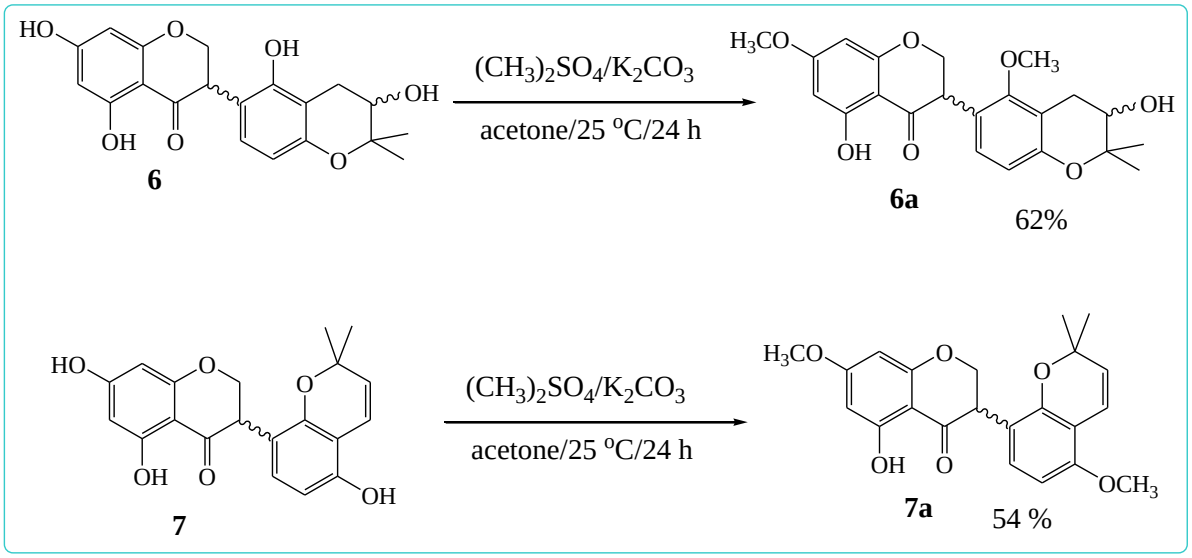
3.6 Anti-Tuberculosis Tests

Minimum inhibition concentrations (MICs) of some test samples against *Mycobacterium tuberculosis* were determined using the microplate alamar blue assay (MABA) as described by Falzari *et al.* (2005) as well as the low oxygen recovery assay (LORA) as described by Cho *et al.* (2007). Rifampicin was used the standard drug with MIC values of 0.06 and 1.8 μM in MABA and LORA, respectively.

3.7 Methylation of Platyisoflavanone D (168) and Glyasperin F (169)

Dimethyl sulphate (5 drops, 0.0263 mmol) and K_2CO_3 (300.0 mg, 2.17 mmol) were added to compound **168** (10.0 mg, 0.0269 mmol) in freshly distilled acetone (6 mL) and stirred at 25 $^{\circ}$C for 24 h. After filtration and concentration, the product was purified by prep-TLC (25% EtOAc in *n*-hexane) yielding compound **168a** (5.0 mg, 0.0125 mmol) in 62% yield.

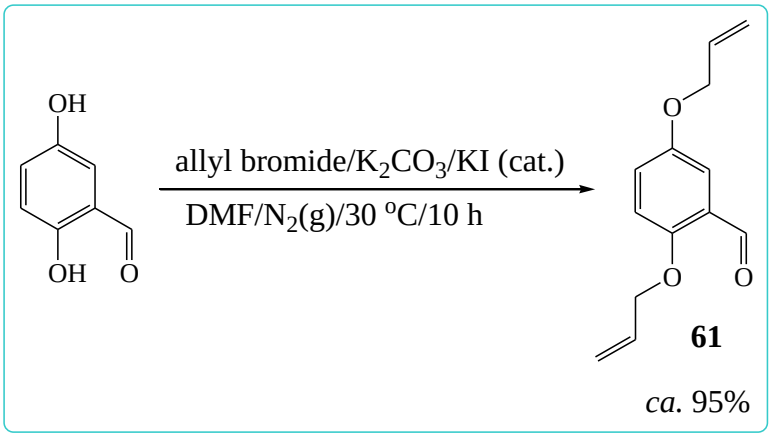
Similarly, compound **169** (10.2 mg, 0.0282 mmol) was methylated under the same conditions and the product purified by prep-TLC (mobile phase: EtOAc/*n*-hexane, 2:3) to give compound **169a** (5.6 mg, 0.0146 mmol) in 54% yield.



Scheme 2: Methylation of platyisoflavanone D (**168**) and glyasperin F (**169**)

3.8 2,5-Diallyloxybenzaldehyde (215)

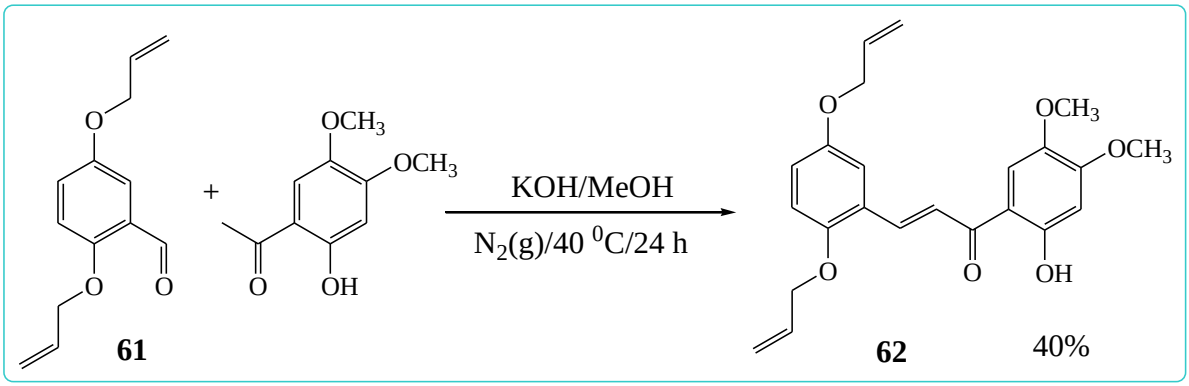
To a mixture of 2,5-dihydroxybenzaldehyde (300 mg, 2.17 mmol), K_2CO_3 (900.0 mg, 6.52 mmol) in dimethylformamide (2.0 mL) and allyl bromide (1.0 mL, 11.55 mmol), catalytic amount of KI (5 mole percent of 2,5-dihydroxybenzaldehyde) was added. The reaction mixture was then stirred at 30 °C and under nitrogen gas overpressure. The progress of the reaction was monitored by withdrawal of small quantities of the mixture followed by quenching with water and extracted with diethyl ether. After evaporation the product was analysed by ESI-LC/MS. The reaction was stopped (by quenching) after 10 h followed by extraction with diethyl ether. The crude product was then purified by CC over silica gel (70-230 mesh, Merck; gradient elution, EtOAc/*n*-hexane) to give 273.8 mg (57.7% recovery yield) of 2,5-diallyloxybenzaldehyde (eluate of 20% EtOAc in *n*-hexane).



Scheme 3: Preparation of 2,5-Diallyloxybenzaldehyde (**215**)

3.9 2,5-Diallyloxy-2′-hydroxy-4′,5′-dimethoxychalcone (216)

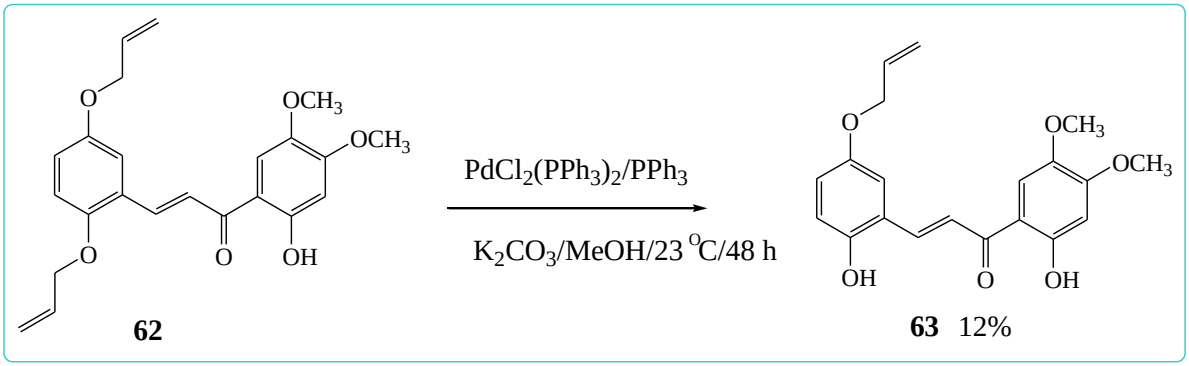
To a mixture of 2,5-diallyloxybenzaldehyde (273.8 mg, 1.25 mmol), 2-hydroxy-4,5-dimethoxyacetophenone (235.3 mg, 1.20 mmol) and potassium hydroxide (194.8 mg, 3.48 mmol), 6 mL of MeOH (solvent) was added and stirred at 40 °C on a water bath. After 48 h cold water was added to the reaction mixture and extracted with EtOAc. The organic layer was evaporated and redissolved in CH_3CN and left to stand, leading to formation of reddish-orange crystals of 2,5-diallyloxy-2′-hydroxy-4′,5′-dimethoxychalcone (**216**) (190.2 mg, 40% yield).



Scheme 4: Preparation of 2,5-Diallyloxy-2′-hydroxy-4′,5′-dimethoxychalcone (**216**)

3.10 Deprotection of 2,5-Diallyloxy-2′-hydroxy-4′,5′-dimethoxychalcone (216)

2,5-diallyloxy-2′-hydroxy-4′,5′-dimethoxychalcone (32 mg, 0.08 mmol) was mixed with triphenyl phosphine (0.84 mg, 0.0032 mmol), *bis*-triphenyl phosphine palladium (II) chloride (1.12 mg, 0.0016 mmol), K_2CO_3 (67.1 mg, 0.486 mmol) in 2 mL of MeOH (solvent) and stirred at 23 °C. After 48 h, the reaction was quenched with 0.1 M HCl and extracted with EtOAc. The concentrated sample was purified by CC on Sephadex® LH-20 (CH_2Cl_2/MeOH, 1:1 (v/v)) and then by prep-HPLC (H_2O/CH_3CN eluate) to yield 3.4 mg (12 % yield) of, 5-allyloxy-2,2′-dihydroxy-4′,5′-dimethoxychalcone (**217**) along with several unidentified side-products.



Scheme 5: Deprotection of 2,5-Diallyloxy-2′-hydroxy-4′,5′-dimethoxychalcone (**216**)

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Isoflavanones, Formononetin and 3-Methoxyflavones from the Stem Bark of *Platycelphium voënse*

From the stem bark of *Platycelphium voënse* twelve compounds, including seven isoflavanones, one flavone, two 3-methoxyflavones and two triterpenes, were isolated and identified. Five of the isoflavanones are new. All the seven known compounds are reported from *Platycelphium voënse* for the first time.

4.1.1 Isoflavanones

The isoflavanones obtained from *Platycelphium voënse* included platyisoflavanone A (**163**), platyisoflavanone B (**164**), sophoraisoflavanone A (**165**), platyisoflavanone C (**166**), 5,7-dihydroxy-4'-methoxyfurano-[4",5":3',2']-isoflavanone (**167**), platyisoflavanone D (**168**), and glyasperin F (**169**).

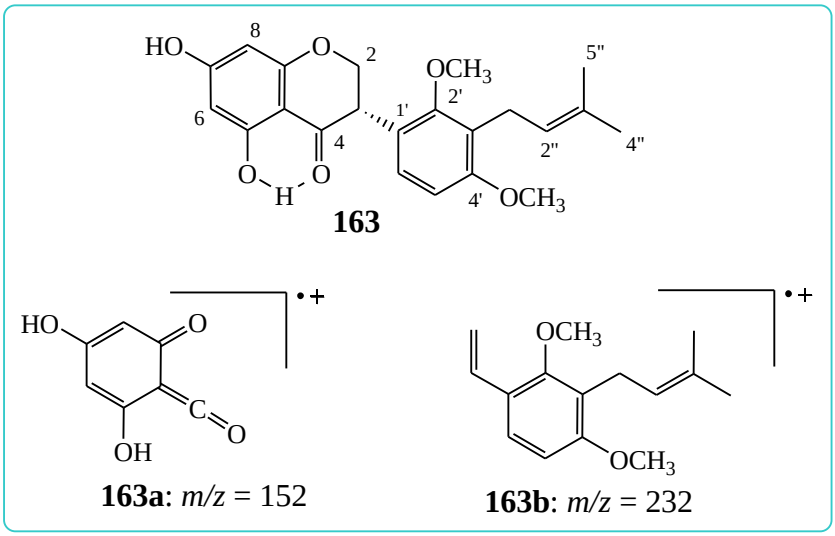
4.1.1.1 Platyisoflavanone A (163)

Compound **163**, obtained as a white amorphous solid, with a molecular ion peak at *m/z* 384.1597 in the HREI-MS corresponding to a molecular formula $C_{22}H_{24}O_6$. The presence of an isoflavanone skeleton was deduced from UV (λ_{max} 288 nm); ^{1}H NMR [δ_H 4.48 (dd, *J* = -11.1, 11.2 Hz, H-2ax); δ_H 4.66 (dd, *J* = -11.1, 5.6 Hz, H-2eq); δ_H 4.38 (dd, *J* = 11.2, 5.6 Hz, H-3ax)] (Table 9) and ^{13}C NMR [δ_C 71.6 for C-2; 45.9 for C-3 and 198.2 for C-4] data (Table 10). The ^{1}H NMR spectrum further revealed the presence of two methoxyl at δ_H 3.71 and 3.80 groups; a chelated hydroxyl (δ_H 12.18) at C-5 and a 3-methylbut-2-enyl moiety (Table 9).

The two *meta*-coupled proton doublets [δ 5.95 (*J* = 2.0 Hz) and 5.97 (*J* = 2.0 Hz)] were assigned to H-8 and H-6, respectively, implying that C-5 and C-7 in the A-ring are oxygenated as expected from biogenetic considerations. In the B-ring two *ortho*-coupled aromatic protons at δ_H 6.67 and 6.92 (*J* = 8.6 Hz) were assigned to H-5' and H-6', respectively, with C-2', C-3' and C-4' being substituted. The ^{13}C chemical shift values (Table

10) for the B-ring carbon are consistent with oxygenation at C-2' and C-4', and the 3-methylbut-2-enyl group being at C-3'. The fragment ions at *m/z* 152 (**163a**) and 232 (**163b**) in the EI-mass spectrum resulting from a typical *retro*-Diels-Alder (RDA) cleavage of the C-ring confirmed that the A-ring had two hydroxyl groups; and that the two methoxyl together with the 3-methylbut-2-enyl group are located on the B-ring.

In NOE experiments, the peak at δ_H 4.38 (H-3) was enhanced upon irradiation of the methoxyl group at δ_H 3.71; similarly, NOE interaction was observed between a signal at δ_H 6.67 (H-5') and the methoxyl group at δ_H 3.80 allowing the placement of the two methoxyl groups at C-2' and C-4', respectively. Indeed the down-field carbon shift of one of the methoxyl groups (δ 62.4) in the ^{13}C NMR spectrum, is typical of a di-*ortho* substituted methoxyl group [Park *et al.*, 2008], confirming its placement at C-2'. Furthermore, the HMBC experiment revealed a long range correlation of the CH_2-1" protons of the 3-methylbut-2-enyl moiety with C-2' and C-4', confirming that the 3-methylbut-2-enyl group is placed between the two methoxyl substituents (at C-3') as in structure **163** (Tables 9 and 10). Based on these data the compound was characterized as 5,7-dihydroxy-2',4'-dimethoxy-3'-(3-methylbut-2-enyl)-isoflavanone. The CD spectrum of compound **163** showed a negative Cotton effect at 344 nm (n → π* transition) which was consistent with 3*S* absolute configuration for this isoflavanone [Slade *et al.*, 2005] and thus the compound was identified as (*S*)-5,7-dihydroxy-2',4'-dimethoxy-3'-(3-methylbut-2-enyl)-isoflavanone. This is a new compound and is named platyisoflavanone A (**163**).



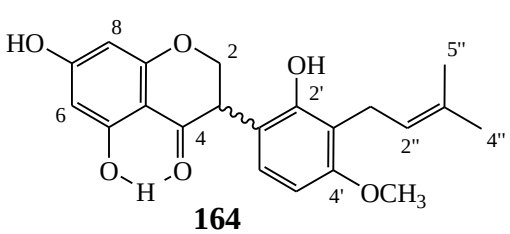
163

163a: *m/z* = 152

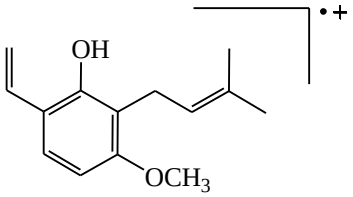
163b: *m/z* = 232

4.1.1.2 Platyisoflavanone B (164)

Compound **164** was obtained as a white amorphous solid and identified as an isoflavanone derivative from the UV (λ_{max} 294 nm); ^{1}H (Table 9) and ^{13}C (Table 10) NMR spectra. The HR-EI mass spectrum of the compound gave a molecular ion peak at *m/z* 370.1400 [M^{+}] corresponding to a molecular formula $C_{21}H_{22}O_6$. The ^{1}H and ^{13}C NMR data further revealed the presence of a chelated hydroxyl (5-OH), a methoxyl and a 3-methylbut-2-enyl group. Comparison of the ^{1}H (Table 9) and ^{13}C (Table 10) NMR spectral data of this compound with those of compound **163** revealed that the A-ring in the two compounds are identical, while the B-ring has similar substitution pattern to that in **163**. The only difference between these two compounds is that **164** has only one methoxyl group. The fragment ion, in the EI-MS, at *m/z* 218 (**164a**) was in agreement with the location of the methoxyl, the 3-methylbut-2-enyl and one hydroxyl group in the B-ring. The chemical shift of the methoxyl group was within the normal range (δ_C 56.0) in ^{13}C NMR spectrum, suggesting its location at C-4 rather than at C-2 [Park *et al*., 2008]. NOESY (which showed NOE interaction of the methoxyl protons with H-5') spectrum coupled with the HMBC observed between methoxy protons and C-5' confirmed the location of methoxyl group at C-4'. From the above spectral data this compound was characterized as, 5,7,2'-trihydroxy-4'-methoxy-3'-(3-methylbut-2-enyl)-isoflavanone (**164**). The near zero optical rotation together with insignificant Cotton effect in the CD spectrum revealed that the compound was isolated as a racemic mixture. Isoflavanones with free OH at C-4' and/or at C-2', are reported to undergo racemization through keto-enol tautomerism during extraction and isolation processes [Slade *et al.*, 2005]. This is a new compound and is named platyisoflavanone B (**164**).



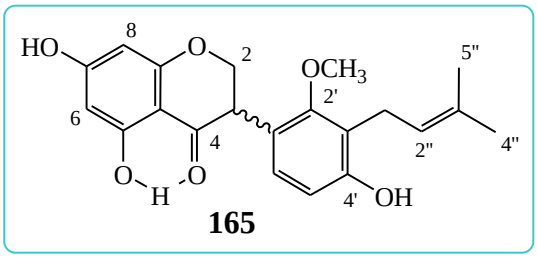
164



164a: *m/z* = 218

4.1.1.3 Sophoraisoflavanone A (165)

Compound **165** was isolated as a white amorphous solid and identified as an isoflavanone derivative due to ^{1}H and ^{13}C NMR data (Tables 9 and 10) that are similar to that of **165**, with oxygenation at C-5, C-7, C-2' and C-4', one methoxyl group and a 3-methylbut-2-enyl moiety. The high chemical shift value (δ_C 62.3) of the methoxyl group together with the signals due to two *meta*-coupled aromatic protons at δ_H 5.96 (1H, d, *J* = 2.0 Hz, H-8) and 6.00 (1H, d, *J* = 2.0 Hz, H-6) suggested that the methoxy is in the B-ring, specifically at C-2' with the 3-methylbut-2-enyl group at C-3'; and the hydroxyl groups placed at C-5, C-7 and C-4'. The electrospray ionization mass spectrum (ESI-MS) of the compound gave an $[M+H]^+$ peak at *m/z* 371.4 corresponding to the molecular formula $C_{21}H_{22}O_6$. Based on the above spectral data and comparison with published information, the compound was identified as 5,7,4'-trihydroxy-2'-methoxy-3'-(3-methylbut-2-enyl)-isoflavanone (**165**), with the trivial name sophoraisoflavanone A, a compound first obtained from the aerial parts of *Sophora tomentosa* [Komatsu *et al.*, 1978].



165

Table 9: ^{1}H NMR data for compounds **163**, **164** (CD_2Cl_2, 600 MHz,) and **165** ($CDCl_3$, 200 MHz)

Position	δ_H mult. (*J* in Hz) **163**		**164**		**165**	
2ax	4.48,	dd[a] (-11.1, 11.2)	4.62,	dd (-11.6, 5.0)	4.49,	m
eq	4.46,	dd[a] (-11.1, 5.6)	4.76,	dd (-11.6, 6.4)		
3	4.38,	dd (11.2, 5.6)	4.08,	t (5.7)	4.36,	dd (9.8, 6.4)
6	5.97,	d (2.3)	5.95,	m	6.00,	d (2.0)
8	5.95,	d (2.3)	5.95,	m	5.96,	d (2.0)
5'	6.67,	d (8.6)	6.48,	d (8.6)	6.63,	d (8.2)
6'	6.92,	d (8.5)	7.15,	d (8.6)	6.83,	d (8.2)
1"	3.32,	dd (-14.3, 6.6)	3.39,	m	3.43,	d (6.8)
	3.38,	dd (-14.4, 6.8)				
2"	5.25,	t (6.7)	5.17,	t (7.2)	5.25,	t (6.6)
4"	1.77,	s	1.79,	s	1.77,	s
5"	1.68,	s	1.70,	s	1.65,	s
2'-OCH_3	3.71,	s	-	-	3.74,	s
4'-OCH_3	3.80,	s	3.78,	s	-	-
5-OH	12.18,	s	11.87,	s	12.21,	s

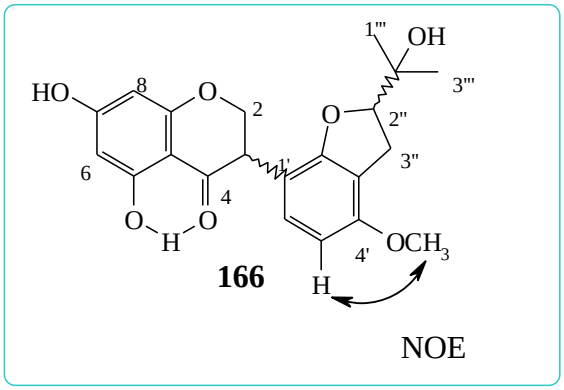
[a]Multiplicity was established after iteration according to Laatikainen *et al.* (1996a, 1996b).

Table 10: ^{13}C NMR spectral data for compounds **163**, **164** (CD_2Cl_2) and **165** ($CDCl_3$)

Position	δ_C **163** (150 MHz)	**164** (150 MHz)	**165** (50 MHz)
2	71.6	70.3	71.2
3	45.9	46.0	45.8
4	198.2	197.7	198.7
4a	103.8	102.6	103.6
5	164.9	165.3	164.3
6	96.7	96.8	96.7
7	164.9	165.4	164.7
8	95.5	95.4	95.2
8a	163.9	163.7	163.5
1'	120.5	115.5	119.8
2'	157.9	154.1	157.4
3'	124.2	117.4	120.8
4'	158.9	158.3	155.9
5'	107.2	103.7	112.5
6'	127.4	126.2	127.6
1"	23.7	22.7	23.7
2"	123.1	122.2	121.4
3"	131.8	134.0	135.6
4"	18.1	17.8	18.0
5"	25.9	25.7	25.8
2'-OCH_3	62.4	-	62.3
4'-OCH_3	56.0	56.0	-

4.1.1.4 Platyisoflavanone C (166)

Compound **166** also exhibited ^{1}H and ^{13}C NMR spectral features (Table 11) of an isoflavanone and having an A-ring that is identical to those in compounds **163-165**. The B-ring in compound **166** also showed a similar substitution pattern (a C_5 substituent at C-3' and oxygenation at C-2' and C-4'), but differed from the other compounds in that the five carbon unit constitutes a 2-(2-hydroxyisopyl)-dihydrofuranyl moiety fused to the B-ring. The 2-(2-hydroxyisopropyl)-dihydrofuranyl functionality was discernible from the ^{1}H NMR [an AXY spin system at δ_H 2.97 (1H, dd, *J* = -16.0 and 9.6 Hz), 3.01 (1H, dd, *J* = -16.0 and 7.0 Hz) attributed to C-3" methylene group, 4.52 (1H, dd, *J* = 9.6 and 7.1 Hz, H-2"), a pair of three-proton singlets at δ_H 1.03 (H-1'") and 1.04 (H-3'")] and ^{13}C NMR data: [δ_C 91.4 for an oxymethine carbon (C-2"); δ_C 29.5 (C-3"); δ_C 26.2 and 25.9 (C-1'" and C-3'") and δ_C 72.6 due to a quaternary carbon bonded to an oxygen (C-2'")] NMR spectra. Whereas one of the two oxygen groups in the B-ring is involved in cyclization, the second oxygen in this ring is methylated with the corresponding methoxyl group appearing at δ_H 3.68 and δ_C 56.4 in the NMR spectra. In the NOESY spectrum, this methoxyl signal showed NOE interaction with the aromatic proton resonating at δ_H 6.32 (H-5'), implying that it is located at C-4'; and hence the dihydrofuranyl ring should be between C-2' and C-3'. Furthermore, the 'normal' methoxyl resonance at δ_C 56.4 supports the placement of a methoxyl group at C-4' rather than at C-2' [Park *et al.,* 2008]. The high resolution EIMS for the compound gave an $[M^+]$ at *m/z* 386.1354 which was accounted for by a molecular formula $C_{21}H_{22}O_7$. Based on the spectral data discussed above, the compound was identified as 5,7-dihydroxy-4'-methoxy-2"-(2-hydroxyisopropyl)dihydrofurano-[4",5":3',2']-isoflavanone which is new and named platyisoflavanone C.



166

4.1.1.5 5,7-Dihydroxy-4'-methoxyfurano-[4'',5'':3',2']-isoflavanone (167)

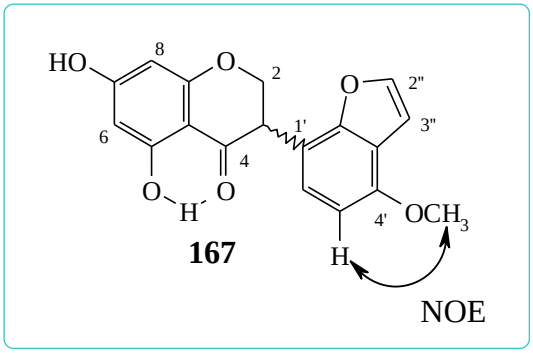
Compound **167** was identified as an isoflavanone with the A-ring identical to that in compound **166**. The oxygenation pattern in the B-ring is also similar to that of **166** with methoxyl at C-4'. Compound **167** differed from **166** due to the presence of a furan fused to the B-ring instead of five-carbon unit at C-3' in other compounds. The presence of the furan moiety was deduced from ^{1}H NMR spectral data [δ_H 6.87 (1H, d, *J* = 2.4 Hz, H-3") and 7.52 (1H, d, *J* = 2.0 Hz, H-2")] (Table 11).

Table 11: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data for compound **166** ($(CD_3)_2O$) and ^{1}H (200 MHz) NMR spectral data for compound **167** ($CDCl_3$)

Position	**166**		**167**
	δ_C	δ_H, mult. (*J* in Hz)	δ_H, mult. (*J* in Hz)
2	71.5	4.34, dd (-11.0, 5.7) 4.42, dd (-11.2, 11.2)	4.58, dd (-11.2, 5.4) 4.76, dd (-11.2, 7.4)
3	48.7	4.06, dd (11.4, 5.6)	4.46, dd (10.8, 5.0)
4	198.6	-	-
4a	104.2	-	-
5	166.4	-	-
6	97.6	5.84, d (2.2)	5.97, d (2.4)
7	168.0	-	-
8	96.3	5.82, d (2.2)	6.01, d (2.0)
8a	165.3	-	-
1'	110.6	-	-
2'	160	-	-
3'	115.7	-	-
4'	157.8	-	-
5'	104.7	6.32, d (8.4)	6.64, d (7.8)
6'	131.4	6.83, d (8.4)	7.06, d (8.2)
2"	91.4	4.52, dd (9.6, 7.1)	7.52, d (2.0)
3"	29.5	2.97, dd (-16.0, 9.6) 3.01, dd (-16.0, 7.0)	6.87, d (2.4)
1'"	26.2	1.03, s	-
2'"	72.6	-	-
3'"	25.9	1.04, s	-
5-OH	-	12.15, s	12.13, s
4'-OCH_3	56.0	3.68, s	3.93, s

The fact that the methoxyl protons exhibited NOE interaction with an aromatic proton resonating at δ_H 6.64 (H-5') implied that the methoxyl group was located at C-4' and

therefore the furan is fused to the B-ring at C-2' and C-3'. Based on the above spectral data and by comparison of the ^{1}H NMR data with that of **166** (Table 11), compound **167** was identified as 5,7-dihydroxy-4'-methoxyfurano-[4",5":3',2']-isoflavanone. It is also new, but due to paucity of the sample other experiments could not be carried out.



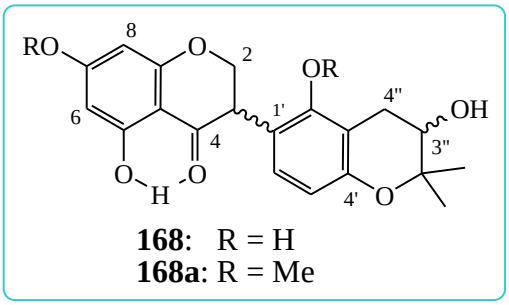
167

4.1.1.6 Platyisoflavanone D (168)

Compound **6** was identified as an isoflavanone with a 3-hydroxy-2,2-dimethyldihydropyrano moiety fused to the B-ring, as revealed from the ^{1}H and ^{13}C NMR spectra (Table 12). Two possible structures were considered for this compound: (i) one in which the dihydropyran ring is between C-2'/C-3', and (ii) the other with the dihydropyran moiety between C-3'/C-4'. In order to decide between two possible structures, compound **168** was methylated by treatment with dimethyl sulphate in the presence of potassium carbonate and acetone, at room temperature, to give a dimethylated product (**168a**) whose ^{13}C NMR spectrum (Table 12) displayed two methoxyl signals at δ_C 56.1 (7-OCH_3) and at δ_C 61.2; the latter deshielded signal, is typical of a *ortho*-disubstituted methoxyl carbon [Park *et al.*, 2008], and hence assigned to 2'-OCH_3. This implied that C-4' in **168a** and in the parent compound **168**, is part of the pyran ring. With one methoxy at C-2' in **168a**, the other methoxyl was assigned to C-7 based on the NOE observed between H-6/H-8 (δ_H 5.98/5.95) and methoxyl protons at δ 3.75. Therefore the isolated compound was characterized as 5,7,2',3"-tetrahydroxy-2",2"-dimethyldihydropyrano-[5",6":3',4']-isoflavanone which is also new and named platyisoflavanone D. Most of the ^{1}H and ^{13}C NMR signals appeared in duplicates indicating that the compound was isolated as a diastereomeric mixture.

Table 12: ^{1}H (600 MHz), and ^{13}C (150 MHz) NMR spectral data for compounds **168** ($(CD_3)_2CO$) and **168a** (CD_2Cl_2)

Position	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
	168		**168a**	
2	71.6/71.4	4.56/4.57, m 4.69/4.68, m	71.5/71.4	4.37, m 4.38, m
3	47.6	4.23/4.22, m	46.4/46.0	4.26, dd (-10.2, 7.6)/ 4.22, dd (-10.8, 5.4)
4	199.1	-	198.0	-
4a	103.8	-	103.8/103.7	-
5	166.5	-	164.8	-
6	97.6/97.7	5.96, m	95.1	5.98, d (1.8)
7	168.2	-	168.2	-
8	96.4/96.3	5.96, m	94.1	5.95, m
8a	165.2	-	163.6	-
1'	115.4	-	119.6	-
2'	155.3	-	158.14/158.12	-
3'	110.7	-	113.8/113.7	-
4'	155.0	-	154.2/154.1	-
5'	110.6	6.32, d (8.4)	113.4	6.51, d (8.4)
6'	129.0/128.9	6.97/6.96, d (8.4)	128.4/128.2	6.79, d (8.4)
2"	78.0/77.9	-	76.9	-
3"	70.3	3.78, m	69.3/69.2	3.73, m
4"-a 4"-b	28.3	2.62/2.58, dd (-16.2, 7.8) 2.98/2.96, m	27.3/27.2	2.66/2.70, dd (-17.1, 5.7) 2.92/2.94, dd (-17.9, 4.8)
5-OH	-	12.18/12.17, s	-	12.23, s
7-OCH_3	-	-	56.1	3.75, s
2'-OCH_3	-	-	61.2/61.1	3.65/3.66, s
2"-CH_3	21.2/21.0 26.7/26.6	1.22, s 1.33/1.32, s	21.8 25.0/24.9	1.24, s 1.25, s



168: R = H
168a: R = Me

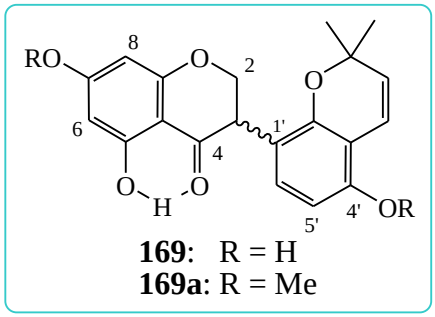
4.1.1.7 Glyasperin F (169)

Compound **7** was isolated as a white amorphous solid and identified as an isoflavanone derivative having an A-ring that is identical to that of compounds **163**-1**68**. The ^{13}C NMR spectrum (Table 13) revealed five peaks between δ_C 153 and 168. This was in agreement with the oxygenation at C-5,-7,-8a and suggesting that the other two oxygenated carbons are in the B-ring.

The ^{1}H NMR data revealed the presence of two *ortho*-coupled (d, J = 8.3 Hz) aromatic protons with signals centred at δ_H 6.40 and 6.86 (d, J = 8.3 Hz) attributed to H-5' and H-6', respectively, in the B-ring. Furthermore, in the gHMBC experiment, H-6' exhibited interactions with two oxygenated carbons resonating at δ_C 153.1 and 154.3, which is consistent with oxygenation at C-2' and C-4'.

The presence of a 2,2-dimethylpyrano ring was also evident from the ^{1}H and ^{13}C NMR spectral data (Table 13). With H-6, C-8, C-5', C-6' in place, there are two possible structures: (i) one in which the pyrano ring is fused to the B-ring at C-2'/3'; and (ii) where the pyran moiety is between C-3' and C-4'. The structure was confirmed by methylation (as in the case for compound **6**) giving an *O,O*-dimethylated product (**169a**) whereby one of the methoxyl groups, resonating at δ_H 3.72, gave a cross peak with H-5' (at δ_H 6.34) in the NOESY experiment, which was an indication that the OCH_3 was located at C-4'. This clearly implies that the pyran ring, in both **169a** and the parent compound **167**, must be between C-2'/C-3'.
From the spectral data above and comparison with published literature compound **167** was identified as the known 5,7,4'-trihydroxy-2",2"-dimethylpyrano-[5",6":3',2']-isoflavanone,

trivial name glyasperin F, that was reported for the first time from the roots of *Glyccrhiza aspera* [Zeng *et al.*, 1992].



169: R = H
169a: R = Me

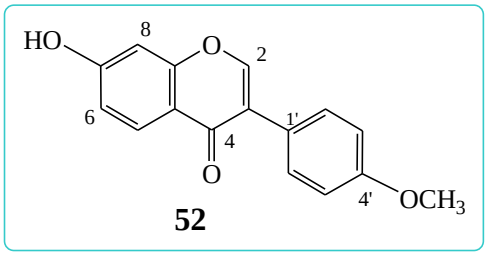
Table 13: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data for compounds **169** and **169a**

Position	**169** ($(CD_3)_2CO$)		**169a** (CD_2Cl_2)	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
2	71.8	4.41, dd (-10.9, 5.6)	70.8	4.32, dd (-10.8, 6.0)
		4.55, dd (-11.1, 11.1)		4.48, dd (-11.4, 11.4)
3	48.4	4.19, dd (11.1, 5.7)	47.4	4.10, dd (11.4, 6.0)
4	199.0	-	197.8	-
4a	104.4	-	103.8	-
5	166.3	-	164.8	-
6	97.6	5.98, d (2.2)	95.0	5.98, m
7	167.6	-	167.9	-
8	96.3	5.95, d (2.2)	94.1	5.92, m
8a	165.2	-	163.6	-
1'	115.5	-	115.1	-
2'	153.1	-	151.6	-
3'	111.0	-	110.9	-
4'	154.3	-	155.4	-
5'	109.1	6.40, d (8.3)	103.3	6.34, d (8.4)
6'	131.9	6.86, d (8.3)	130.4	6.82, d (8.4)
2"	77.8	-	76.8	-
3"	129.9	5.63, d (10.0)	129.3	5.48, d (9.6)
4"	118.4	6.68, d (9.9)	116.8	6.55, d (10.2)
5-OH	-	12.40, s	-	12.17, s
7-OCH_3	-	-	56.0	3.74, s
4'-OCH_3	-	-	55.9	3.72, s
2"-CH_3	28.4/28.9	1.33, s/1.35, s	27.5/28.0	1.24, s/1.25, s

It is worthy to note that the oxygenation pattern in six of the isoflavanones isolated from this plant (*P. voënse*) is identical (C-5, 7, 2' and 4'), and each with a five-carbon unit at C-3'. The other isoflavanone (compound **167**) has identical oxygenation pattern but differed from the rest by having furano moiety fused to the B-ring instead of five-carbon unit at C-3'. Isoflavanones with similar substitution pattern have also been isolated from related genera of *Bolusanthus* [Bojase *et al.,* 2001a, b] and *Sophora* [Iinuma *et al.,* 1993: Komatsu *et al.,* 1978] supporting the placement of the three genera in the same group (Sophora group); the latter two genera also elaborate isoflavanones with different oxygenation and prenylation patterns. It will be interesting to find out if another related genus *Dicraeopetalum* also elaborate isoflavanones with similar oxygenation as those obtained from *P. voënse*.

4.1.2 Formononetin (52)

The LC-ESI-MS of a white solid (**52**) gave an $[M+H]^+$ at *m/z* 269.5 corresponding to a molecular formula $C_{16}H_{12}O_4$. It was identified as an isoflavone derivative as deduced from ^{1}H NMR spectrum that exhibited a one proton singlet at δ_H 8.15 (H-2) with the corresponding relatively high intensity oxygenated carbon peak at δ_C 153.6 (C-2) in the ^{13}C NMR spectrum. Furthermore, a signal at δ_C 178.9 in the ^{13}C NMR spectrum is typical of α,β-unsaturated carbonyl (C-4) in an isoflavone skeleton. The presence of a low field doublet centred at δ_H 8.05 (1H, J = 8.8 Hz) in the ^{1}H NMR spectrum was attributed to H-5, experiencing a deshielding *peri*-effect from the carbonyl group, and hence (based on the multiplicity) indicating that C-6 is also unsubstituted. H-6 exhibited coupling with a proton resonating at δ_H 6.93 (1H, d, J = 2.2 Hz, H-8) showing that this ring is substituted at C-7. The ^{1}H NMR spectrum further revealed an AA'XX' spin system with a pair of multiplets centerd at δ_H 6.97 and 7.47, due to the B-ring protons. Methoxyl protons resonating at δ_H 3.82 exhibited NOE interaction with the two-proton multiplet at δ_H 6.97 (H-3', H-5') which is suggestive that the methoxy group is at C-4'. Careful analysis of the ^{1}H and ^{13}C NMR spectra (Table 14) in comparison with published data led to the identification of the isolated compound as formononetin (**52**) [Fuendjiep *et al*., 1998b].



52

Table 14: ^{1}H (CD_3OD, 200 MHz) and ^{13}C (50 MHz) NMR spectral data for compound **52**

Position	δ_C	δ_H, mult. (*J* in Hz)
2	153.6	8.15, s
3	124.5	-
4	176.9	-
4a	116.9	-
5	127.3	8.05, d (8.8)
6	115.4	6.93, dd (8.8, 2.2)
7	163.8	-
8	102.1	6.85, d (2.2)
8a	158.6	-
1'	124.3	-
2'/6'	130.2	7.47, m
3'/5'	113.6	6.97, m
4'	159.9	-
4'-OCH_3	54.5	3.82, s

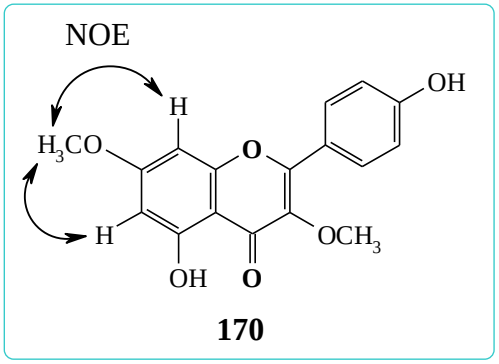
4.1.3 3-Methoxyflavones

Two flavones each with methoxyl group at C-3 were isolated from *P. voënse* and identified as kumatakenin (**170**) and isokaempferide (**171**).

4.1.3.1 Kumatakenin (170)

Compound **170** was obtained as a yellow solid. Its ^{1}H NMR spectra data (Table 15) displayed signals for a chelated hydroxyl group (δ_H 12.76, 1H, s, 5-OH); a pair of *meta*-coupled aromatic signals at δ_H 6.32 (1H, *J* = 2.2, H-8) and at δ_H δ 6.66 (1H, *J* = 2.2, H-6); an AA'XX' spin system (a pair of two-proton multiplets at δ_H 7.02 and 8.06) for aromatic protons implying a 4'-oxygenated B-ring of a flavonol; and two methoxyl peaks at δ_H 3.88 and 3.92. The absence of a signal for H-3 suggested a substitution at C-3, probably by an OH or an OCH_3 group. One of the methoxyl carbon resonated at δ_C 59.9, in the ^{13}C NMR spectrum

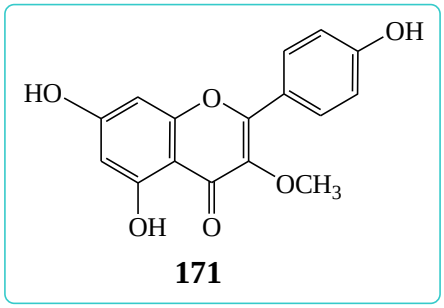
(Table 15), implying that it is di-*ortho*-substituted, and hence placed at C-3 rather than C-5, C-7 or C-4'. Upon irradiation of the protons resonating in the range δ_H 3.75-4.00 in an NOE-difference experiment, the H-6 and H-8 signals were enhanced, allowing the determination of the second methoxyl group at C-7. In view of the spectral data discussed above, the isolated compound was identified as 5,4'-dihydroxy-3,7-dimethoxyflavone also known as kumatakenin [Valesi *et al.*, 1972].



170

4.1.3.2 Isokaempferide (171)

Compound **171** was isolated as a yellow solid with ^{1}H and ^{13}C NMR spectra (Table 16) that are similar to those of **170**. Unlike compound **170** that has two methoxyl groups, compound **171** has only one methoxyl group (δ_H 3.87, δ_C 59.5) whose proton signal showed a weak NOE interaction with H-2'/6' at δ_H 8.03 indicating that the methoxyl is located at C-3. The down-field ^{13}C NMR chemical shift value for the methoxyl group (δ_C 59.5) is consistent with its placement at C-3. From these pieces of information, the compound was characterized as 5,7,4'-trihydroxy-3-methoxyflavone, trivial name isokaempferide [Yang *et al.*, 1995b].



171

Table 15: ^{1}H ($(CD_3)_2CO$, 200 MHz) and ^{13}C (50 MHz) NMR spectral data for compounds **170** and **171**

	170		**171**	
Position	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
2	NO	-	NO	-
3	NO	-	NO	-
4	NO	-	180.4	-
4a	100.1	-	NO	-
5	NO	-	NO	-
6	98.1	6.66, d (2.2)	98.7	6.49, d (2.0)
7	166.2	-	164.2	-
8	92.5	6.32, d (2.2)	93.8	6.25, d (2.0)
8a	162.5	-	162.6	-
1'	NO	-	122.0	-
2'/6'	130.9	8.05, m	130.5	8.02, m
3'/5'	116.1	7.02, m	115.7	7.01, m
4'	160.7	-	160.2	-
5-OH	-	12.76, s	-	12.80, s
3-OCH_3	59.9	3.88, s	59.5	3.87, s
7-OCH_3	56.1	3.92, s	-	-

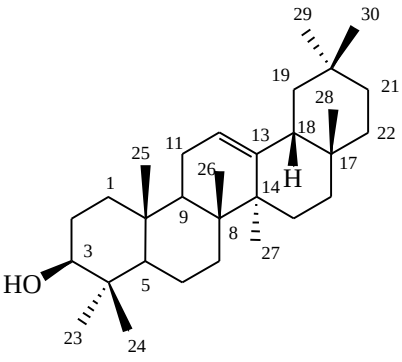
NO = Not observed

4.1.4 Triterpenes from the Stem Bark of *Platycelphium voënse*

Two triterpenes were isolated and identified from the stem bark of *P. voënse*, and identified as *β*-amyrin (**172**) and betulin (**173**).

4.1.4.1 *β*-Amyrin (172)

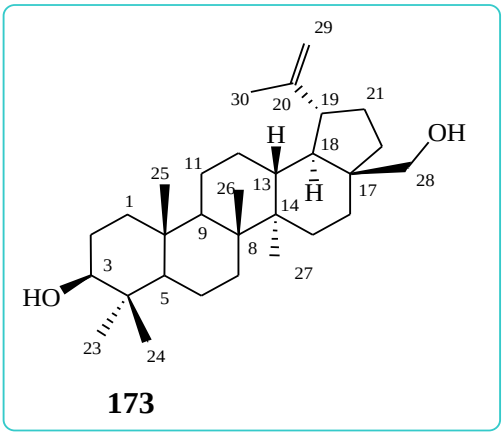
Compound **172** was obtained as a white amorphous solid and identified as a terpenoid derivative based on its ^{1}H NMR spectrum (that had most of the peaks in the high field region). The ^{13}C NMR spectrum (Table 16) showed thirty peaks typical of a triterpene. The ^{1}H NMR signals [δ_H 0.77 (3H, s); 0.83 (3H, s); 0.87 (6H, s); 0.93 (3H, s); 0.97 (3H, s); 1.00 (3H, s); 1.13 (3H, s); 3.19 (1H, m) and 5.18 (1H, m)] together with the ^{13}C NMR/DEPT experiment [(CH_3 groups at δ_C 15.7, 15.8, 17.0, 23.9, 26.2, 28.3, 28.6 and 33.6); an oxygenated methine at δ_C 79.2 and two sp^2 carbons at δ_C 121.9 (CH) and 145.4 (quaternary)] revealed the presence of eight methyl groups, a hydroxymethine group and an olefinic functionality. Comparison of ^{1}H and ^{13}C NMR spectral data (Table 16) of the compound with published information led to the identification of the compound as *β*-amyrin [Mahato and Kundu, 1994].



172

4.1.4.2 Betulin (173)

Compound **173** was also obtained as a white amorphous solid with NMR spectral features (Table 16) typical of a highly saturated compound. The ^{1}H NMR spectrum displayed six methyl singlets (δ_H 0.81, 0.91, 1.01, 1.07, 1.11, and 1.75) one of which (δ_H 1.75) is attributable to a vinylic methyl group. The ^{1}H NMR spectrum further exhibited two broad singlets at δ_H 4.61 and 4.74 (due to geminal methylene protons of a terminal double bond), a multiplet at δ_H 3.80 (due to CH-O) and a typical lupenol H_β-19 multiplet at δ_H 2.50 [Kuo *et al.*, 1997]. The ^{13}C NMR and DEPT spectra revealed a total of thirty carbons including six methyls, twelve methylenes, six methines and six quaternary carbons, which were suggestive of a triterpnoid derivative. The presence, in the ^{13}C NMR spectrum, of two sp^2 hybridized carbons at δ_C 109.4 (C-29) and 151.1 (C-20); an sp^3 oxymethine carbon at δ_C 78.0 (C-3), a hydroxymethylene carbon resonating at δ_C 59.2 (C-28) and the six methyl carbons (at δ_C 14.5, 15.5, 15.8, 16.0, 18.5 and 28.0) are consistent with the structure of this compound being the triterpenoid betulin [Siddiqui *et al*., 1988].



173

Table 16: ^{13}C NMR (50 MHz) spectral data for compound **172** ($CDCl_3$) and **173** ($(CD_3)_2CO$)

172			**173**	
Position	δ_C	DEPT	δ_C	DEPT
1	38.8	CH_2	38.9	CH_2
2	27.4	CH_2	27.7	CH_2
3	79.2	CH	78.0	CH
4	39.0	q-C	38.9	q-C
5	55.4	CH	55.7	CH
6	18.6	CH_2	18.5	CH_2
7	32.9	CH_2	34.5	CH_2
8	40.0	q-C	41.1	q-C
9	47.8	CH	50.7	CH
10	37.4	q-C	37.3	q-C
11	23.7	CH_2	21.0	CH_2
12	121.9	CH	25.6	CH_2
13	145.4	q-C	37.6	CH
14	41.9	q-C	42.8	q-C
15	26.4	CH_2	27.3	CH_2
16	27.1	CH_2	29.6	CH_2
17	32.7	q-C	48.2	q-C
18	47.4	CH	48.9	CH
19	47.0	CH_2	48.2	CH
20	31.3	q-C	151.1	q-C
21	34.9	CH_2	30.0	CH_2
22	37.2	CH_2	34.2	CH_2
23	28.3	CH_3	28.0	CH_3
24	15.7	CH_3	15.5	CH_3
25	15.8	CH_3	16.0	CH_3
26	17.0	CH_3	15.8	CH_3
27	26.2	CH_3	14.5	CH_3
28	28.6	CH_3	59.2	CH_2
29	33.6	CH_3	109.4	CH_2
30	23.9	CH_3	18.7	CH_3

4.2 Rotenoids, Isoflavones, Flavanones and a Chalcone from the Roots of *Millettia usaramensis* subsp. *usaramensis*

A total of thirteen secondary metabolites were isolated from the roots of *Millettia usaramensis* subsp. *usaramensis* and these include seven 12a-hydroxyrotenoids, two isoflavones, two new flavanones, a chalcone 4-*O*-geranylisoliquiritigenin (**181**) and a cinnamoyl alcohol derivative colenemol (**182**).

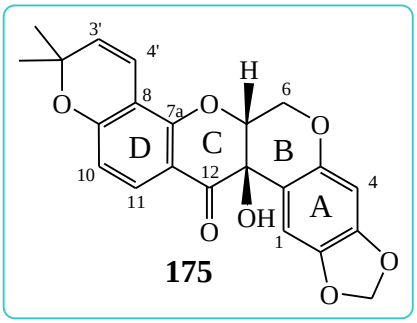
4.2.1 12a-Hydroxyrotenoids

The seven 12a-Hydroxyrotenoids obtained and identified from the root bark of *Millettia usaramensis* subsp. *usaramensis* included usararotenoid-A (**71**), 12-Dihydrousararotenoid-A (**72**), 12-Dihydrousararotenoid-B (**174**), millettosin (**175**), 12a-epimillettosin (**70**), usararotenoid-C (**74**) and 12-Dihydrousararotenoid-C (**176**). All these compounds, except millettosin (**175**), have been earlier reported from the stem bark [Yenesew *et al.,* 1998] and another portion of the root bark extract [Musyoki, 2011] from *Millettia usaramensis* subsp. *usaramensis*.

4.2.1.1. Millettosin (175)

Compound **175** was isolated as a white solid with NMR spectral data (Table 17) characteristic of rotenoid derivative [Agrawal, 1989; Yenesew *et al.,* 1998]. The gCHSQC spectrum of the compound revealed an oxymethylene carbon at δ_C 63.6 which correlated with two diastereotopic protons at δ_H 4.37 (d, *J* = -12.0 Hz, H-6α) and 4.55 (dd, *J* = -12.0, 2.4 Hz, H-6β); an oxymethine carbon at δ_C 75.6 (C-6a) with a corresponding proton signal at δ_H 4.70, (dd, *J* = 2.4, ~0.8 Hz, H-6a). The ^{13}C NMR spectra displayed a typical C-12a quaternary carbon signal at δ_C 67.4 characteristic of 12a-hydroxyrotenoids [Yenesew *et al.,* 1998]. This deduction was supported by a ^{3}J correlation, in the gHMBC, from H-1 (δ_H 6.49) to C-12a. Another noticeable feature of the ^{1}H NMR spectrum was the presence of two *ortho*-coupled aromatic proton signals at δ_H 6.49 (1H, d, *J* = 8.8 Hz, H-10) and 7.61 (1H, d, *J* = 8.8 Hz, H-11) due to H-10 and H-11, respectively. This assignment was supported by gHMBC correlation between H-11 and C-12 (at δ 190.8). The chemical shift value for H-1 (δ_H 6.49) is consistent with a *cis*-B/C ring junction [Yenesew *et al.,* 1998].

The ^{1}H NMR spectrum revealed the presence of a 2,2-dimethylpyrano ring and a methylenedioxy group which, based on both gHMBC and ^{13}C NMR spectral analyses, were placed between C-8/C-9 (δ_C 108.3/159.1) and C-2/C-3 (δ_C 141.2/148.7), respectively. With the spectral data above and by comparison with literature information the isolated compound was identified as millettosin (**175**) [Ollis *et al*., 1967]. This is the first time millettosin is being reported from *M. usaramensis.*



175

Table 17: ^{13}C (150 MHz) and ^{1}H (800 MHz) NMR data for compound **175** ($(CD_3)_2SO$)

Position	δ_C	δ_H mult. (*J* in Hz)	HMBC (H → C)
1	106.2	6.49, s	C-2, 3, 4a, 12a
2	141.2	-	-
3	148.7	-	-
4	98.5	6.55, s	C-2, 3, 12a, 12b
4a	149.1	-	-
6α	63.6	4.37, d (-12.0)	C-6a, 7a
6β		4.55, dd (-12.0, 2.4)	C-4a, 7a, 12a
6a	75.6	4.70, dd (2.4, ~0.8)	C-6, 12, 12a, 12b
7a	155.5	-	-
8	108.3	-	-
9	159.1	-	-
10	111.1	6.49, d (8.8)	C-8, 9, 11, 11a
11	128.1	7.61, d (8.8)	C-7a, 8, 9, 12
11a	112.1	-	-
12	190.8	-	-
12a	67.4	-	-
12b	109.5	-	-
2'	77.8	-	-
3'	129.6	5.75, d (9.6)	C-8, 2', 2'-CH_3
4'	114.6	6.52, d (10.2)	C-7a, 8, 9, 2'
2-OCH_2O-3	101.3	5.87, *br* s/5.94, *br* s	C-2, 3
2'-CH_3	27.8/28.1	1.33, s/1.40, s	C-2', 3', (2'-CH_3)'/C-2', 3', 2'-CH_3
12a-OH	-	6.58, *br* s	NO

NO = Not Observed

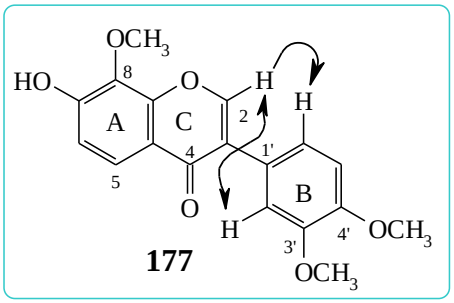
4.2.2. Isoflavones

Two isoflavones were obtained from the roots of *Millettia usaramensis*, and these include 7-hydroxy-8,3',4'-trimethoxyisoflavone (**177**) and Jamaicin (**178**).

4.2.2.1. 7-Hydroxy-8,3',4'-trimethoxyisoflavone (177)

Compound **177** was isolated as a white solid. That this compound is an isoflavone was based on gCHSQC experiment that gave a correlation between a one-proton singlet at δ_H 8.46 and the characteristic C-2 of an isoflavone at δ_C 153.3, and the presence, in the ^{13}C NMR spectrum, of a typical α,β-unsaturated isoflavone carbonyl peak at δ 174.7 (C-4). The presence of a hydroxyl and three methoxyl substituents was evident from the ^{1}H and ^{13}C NMR data (Table 18). Two *ortho*-coupled protons as displayed by both the ^{1}H NMR and ^{1}H-^{1}H gCOSY spectra [δ_H 7.02 (1H, d, J = 8.8 Hz) and 7.72 (1H, d, J = 8.8 Hz)] were assigned to H-6 coupled to H-5, the latter of which is deshielded by the *peri*-effect from the C-4 carbonyl group. A C-7/C-8 oxygenation in the A-ring was established from HMBC between H-5 and C-7/C-8a (δ 154.8/150.6); and ^{3}J correlations from H-6 to C-4a/C-8 (δ 117.4/134.7). The methoxyl protons at δ_H 3.87 exhibited HMBC with a carbon at δ_C 134.7, hence assignable to C-8. This was supported by the high chemical shift value of the methoxyl (δ 60.8), typical of *ortho*-disubstituted methoxyl groups for 8-OCH_3, in the ^{13}C NMR spectrum.

The ^{1}H NMR spectrum also displayed an AMX spin system [δ_H 7.19 (d, J = 1.6 Hz); 7.13 dd, J = 8.0, 1.6 Hz) and 7.00 (d, J = 8.8 Hz)] which, together with carbon signals at δ_C 148.3 and 148.6, are consistent with oxygenation at C-3' and C-4' in the B-ring. The location of the two remaining methoxyl groups were ascertained to be C-3' and C-4' based on the observation that three of the six methoxyl protons with overlapping signals at δ_H 3.78 exhibited HMBC with the carbon resonating at δ_C 148.3 and the other three protons with the carbon at δ_C 148.6. In agreement with the proposed structure, the NOESY spectrum showed a cross peak between H-2 and H-2'/6'. From the above observations it was concluded that compound **177** is the known 7-hydroxy-8,3',4'-trimethoxyisoflavone previously isolated from *Dipteryx alata* (Leguminosae) [Puebla *et al*., 2010], but hereby reported for the first time from *Millettia* species.



177

Table 18: ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data for compound **177** $(CD_3)_2SO$)

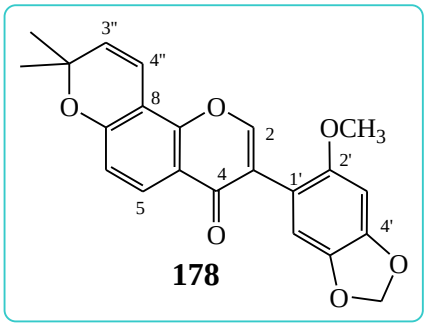
Position	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
2	153.3	8.46, s	C-3, 4, 8a, 1'
3	124.4	-	-
4	174.7	-	-
4a	117.4	-	-
5	120.8	7.72, d (8.8)	C-7, 8a
6	115.2	7.02, d (8.8)	C-4a, 8
7	154.8	-	-
8	134.7	-	-
8a	150.6	-	-
1'	123.0	-	-
2'	112.8	7.19, d (1.6)	C-1', 4', 6'
3'	148.3	-	-
4'	148.6	-	-
5'	111.7	7.00, d (8.8)	C-3, 3'
6'	121.3	7.13, dd (8.0, 1.6)	C-2', 4'
8-OCH_3	60.8	3.87, s	C-8
3'-OCH_3	55.6	3.78, s	C-3'
4'-OCH_3	55.5	3.78, s	C-4'
7-OH	-	10.62, s	NO

4.2.2.2. Jamaicin (178)

Compound **178**, a white solid, exhibited NMR spectral features that are characteristic of an isoflavone [δ_H 7.90 (1H, s, H-2); δ_C 153.8 (C-2); 122.0 (C-3)]. Furthermore, the ^{1}H NMR spectrum displayed an AX spin system due to two *ortho*-coupled aromatic protons [δ_H 8.04 (1H, d, *J* = 8.8 Hz, and 6.79, 1H, d, *J* = 8.8 Hz)] which were assigned to H-5 and H-6, respectively. The low-field δ_H value for H-5 was attributed to the deshielding *peri*-effect of C-4 carbonyl group. Oxygenation at C-2', C-4', C-5' in B-ring was deduced from the two

singlets at δ_H 6.62 and 6.82, in the aromatic region; and the chemical shift positions of three oxygenated carbons at δ_C 141.1, 148.4, 152.4 (Table 19).

Furthermore the presence of a methylenedioxy substituent [δ_H 5.95 (2H, s); δ_C 101.4 (C-4'/5'), with C-7 and C-8 being substituted with a 2,2-dimethylpyrano ring [δ_H 1.25 (3H, s); 1.49 (3H, s); 5.71 (1H, d, *J* = 10.4 Hz); 6.84 (1H, d, *J* = 10.0 Hz)] and one methoxyl group [δ_H 3.72 (3H, s); δ_C 56.9 (C-2')] in this ring were discernible from both ^{1}H and ^{13}C NMR spectra (Table 19). Upon comparing the data with what is reported in literature, the compound was identified as jamaicin, a known isoflavone [Dagne *et al.*, 1989].



178

Table 19: ^{1}H ($CDCl_3$, 200 MHz) and ^{13}C (50 MHz) NMR data for compound **178**

Position	δ_C	δ_H, mult. (*J* in Hz)
2	153.8	7.90, s
3	122.0	-
4	NO	-
4a	118.3	-
5	126.7	8.04, d (8.8)
6	115.1	6.79, d (8.8)
7	152.9	-
8	109.2	-
8a	157.2	-
1'	112.8	-
2'	152.4	-
3'	95.4	6.62, s
4'	148.4	-
5'	141.1	-
6'	111.2	6.82, s
2"	77.6	-
3"	130.2	5.71, d (10.4)
4"	115.1	6.84, m
2'-OCH_3	56.9	3.72, s
4'-OCH_2O-5'	101.4	5.95, s
2"-$(CH_3)_2$	28.8/29.7	1.49, s/1.25, s

NO = Not observed

4.2.3. Flavanone derivatives

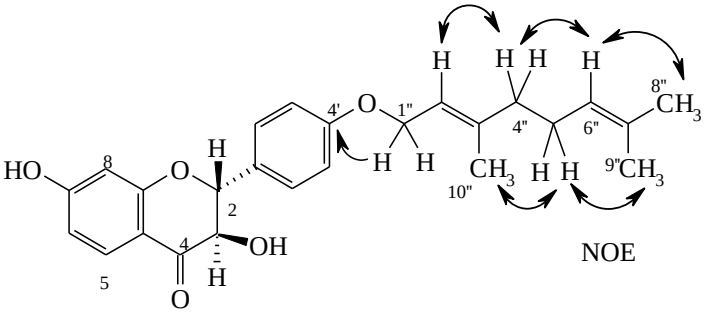
The roots of *Millettia usaramensis* also yielded two new flavanone derivatives that were characterized as (2*R*,3*R*)-4'-*O*-geranyl-7-hydroxyflavanonol (**179**) and (*S*)-4'-*O*-geranyl-7-hydroxyflavanone (**180**).

4.2.3.1. (2*R*,3*R*)-4'-*O*-Geranyl-7-hydroxyflavanonol (179)

Compound **179**, obtained as a white amorphous solid, UV: λ_{max} 279, 305 nm, showed for an $[M+H]^+$ at *m/z* 409.2020 which corresponded with the molecular formula $C_{25}H_{28}O_5$ in the HRESI-MS (calcd. 409.2015). The compound exhibited NMR spectral features typical of a flavanonol derivative [δ_H 5.09 (1H, d, *J* = 11.6 Hz, H-2); 4.50 (1H, dd, *J* = 11.6, 2.5 Hz, H-3) and 5.52 (1H, d, *J* = 3.1 Hz, 3-OH) with the corresponding carbons resonating at δ_C 83.1 (C-2) and 72.5 (C-3); and a carbonyl peak at δ_C 192.3 (C-4)]. The large coupling constant (*J* = 11.6 Hz) between H-2 and H-3 is in agreement with a 1,2-diaxial relative configuration for the two protons. Furthermore, the ^{1}H NMR spectrum revealed an AMX system at δ_H 7.63 [d, *J* = 8.7 Hz, H-5), which is deshielded by the *peri*-effect of the C-4 carbonyl]; 6.52 (dd, *J* = 8.7, 2.2 Hz, H-6) and 6.28 (d, *J* = 2.2 Hz, H-8). Long range ^3J correlations observed between the proton at δ_H 7.63 (H-5) and two oxygenated aromatic carbons at δ_C 162.8 and 165.2 in the HMBC experiment allowed the assignment of these to C-7 and C-8a. Analysis of the ^{1}H NMR and gCHSQC spectra also revealed an AA'XX' spin system at δ_H 6.95 (H-3'/H-5') and 7.42 (H-2'/H-6') for the B-ring protons with corresponding carbon peaks being at δ_C 114.2 and 129.3, respectively. The above data corroborated by HMBC that exhibited interactions between H-2'/H-6' and an sp^2 carbon at δ_C 158.6 indicated that the B-ring is oxygenated at C-4'.

The ^{1}H NMR spectrum gave features of a geranyloxy (or neryloxy) side chain [three methyl singlets (δ_H 1.57, 1.64, 1.71), two methylene multiplets (δ_H 2.06, 2.08), an oxymethylene doublet (δ_H 4.56) and two vinylic proton signals (δ_H 5.08, 5.43)]. This group was deduced to be linked to C-4' on the basis of HMBC correlation observed between an oxymethylene proton at δ_H 4.56 (H-1") and an oxygenated carbon at δ_C 158.6 (C-4'). The chemical shift values at δ_C 16.3 for C-10" carbon and δ_C 39.0 for C-4" methylene carbon are in agreement with a geranyl rather than a neryl side chain (*ca*. δ_C 25 and 32, respectively) (Blanc *et al.*, 2005). Complete assignment (Table 20) was achieved using a combination of one

dimensional (^{1}H and ^{13}C) and two dimensional (^{1}H-^{1}H gCOSY, NOESY, gCHSQC, HMBC) NMR spectroscopy.



179

Table 20: ^{1}H ((CD$_3$)$_2$SO, 800 MHz) and ^{13}C (150 MHz) NMR data for compound **179**

Position	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
2	83.1	5.09, d (11.6)	C-3, 4, 8a, 2'/6'
3	72.5	4.50, dd (11.6, 2.5)	C-2, 4, 1'
3-OH	-	5.52, d (3.1)	NO
4	192.3	-	-
4a	111.9	-	-
5	128.6	7.63, d (8.7)	C-4, 7, 8a
6	111.0	6.52, dd (8.7, 2.2)	C-4a, 8
7	165.2	-	-
8	102.4	6.28, d (2.1)	C-4a, 6, 7, 8a
8a	162.8	-	-
1'	129.5	-	-
2'/6'	129.3	7.42, d (8.7)	C-2, 1', 4'
3'/5'	114.2	6.95, d (8.6)	C-1', 4', 3'/5'
4'	158.6	-	-
1"	64.4	4.56, d (6.6)	C-4', 2", 3"
2"	119.7	5.43, t (6.6)	C-4", 10"
3"	140.2	-	-
4"	39.0	2.06, m	C-2", 3", 5", 10"
5"	25.8	2.08, m	C-3", 4", 6", 7"
6"	123.8	5.08, m	C-8", 9"
7"	131.0	-	-
8"/9"	25.5/17.6	1.64, s/1.57, s	C-6", 7", 9"/C-6", 7", 8"
10"	16.3	1.71, s	C-2", 3", 4"

NO = Not observed

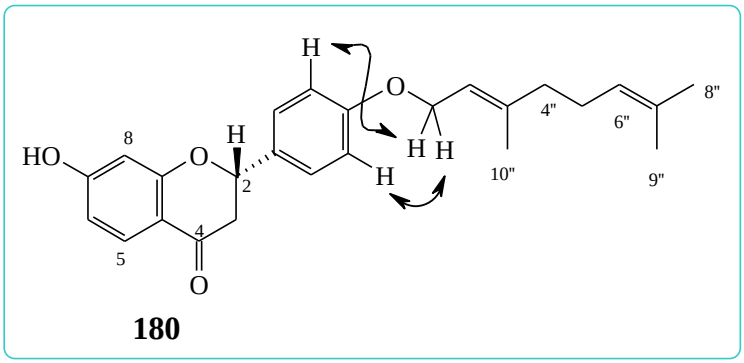
From the value of coupling constant between H-2 and H-3 (*J* = 11.6 Hz) it was deduced that the two vicinal protons are in diaxial *trans* orientation and therefore implied two possible

absolute configurations; either (2*R*,3*R*) or (2*S*,3*S*) [Slade *et al.*, 2005]. The CD spectrum of compound **179** gave a positive cotton effect (CE) at 335 nm (n → π* transition) which is consistent with 2*R* absolute configuration for flavanonols (dihydroflavonols) [Slade *et al.*, 2005] and, hence a 3*R* configuration. Based on the above data, the isolated compound was characterized as (2*R*,3*R*)-4'-*O*-geranyl-7-hydroxyflavanonol (**179**) which is a new compound.

4.2.3.2. (*S*)-4'-*O*-Geranyl-7-hydroxyflavanone (180)

Compound **180** was obtained as a white solid showing a $[M+H]^+$ at *m/z* 393.2068 in the HRESI-MS which corresponding to a molecular formula $C_{25}H_{28}O_4$ (calcd. 393.2066). It exhibited NMR spectral features that are similar to those of compound **179**: an AA'XX' spin system [δ_H 6.95 (H-3'/H-5') and 7.38 (H-2'/H-6')]; an AMX system [δ_H 7.85 (d, *J* = 8.8 Hz, H-5), 6.55 (dd, *J* = 8.8, 2.1 Hz, H-6) and 6.45 (d, *J* = 2.4 Hz, H-8)]; and characteristic features of an *O*-geranyl moiety (Table 21)]. The ^{1}H NMR spectrum displayed another AMX spin system for C-ring protons [δ_H 5.40 (dd, *J* = 13.2, 3.2 Hz, H-2), 3.06 (dd, *J* = -17.0, 13.2 Hz, H-3a) and 2.80 (dd, *J* = -17.0, 3.2 Hz, H-3b) which was suggestive of a flavanone skeleton instead of flavanonol as in **179**. In the NOESY spectrum of **180**, the doublet at δ_H 4.56 (H-1'') showed an NOE cross peak with a multiplet centred at δ_H 6.95 (H-3'/H-5') implying that the geranyloxy moiety is attached to C-4 on ring B as in compound **179**. Thus, the two compounds have the same substitution pattern, except that **180** is a flavanone and **179** is a flavanonol (or dihydroflavonol). Complete NMR assignment was achieved by comparison of the observed spectral data (^{1}H, ^{13}C NMR, DEPT and NOESY) with that for **179**.

The CD spectrum of compound **180** showed a negative cotton effect (CE) at 275 nm (π → π* transition) and a positive CE at 330 nm (n → π* transition) which is characteristic of 2*S* absolute configuration for flavanones (Slade *et al.*, 2005). The compound was therefore characterized as (*S*)-4'-*O*-geranyl-7-hydroxyflavanone, which is also new.



180

Table 21: ^{1}H ($CDCl_3$, 200 MHz) and ^{13}C (50 MHz) NMR data for compound **180**

Position	δ_C	$\delta_{H,}$ mult. (*J* in Hz)	NOESY
2	79.9	5.40, dd (13.2, 3.2)	3, 2'/6'
3	44.3	3.06, dd (-17.0, 13.2)	2
		2.80, dd (-17.0, 3.2)	2
4	191.6	-	
4a	115.2	-	
5	129.6	7.85, d (8.8)	6
6	110.8	6.55, dd (8.8, 2.1)	5
7	164.0	-	
8	103.6	6.45, d (2.4)	-
8a	163.3	-	
1'	130.8	-	
2'/6'	127.9	7.38, m	2
3'/5'	115.2	6.95, m	1"
4'	159.5	-	
1"	65.2	4.56, d (6.8)	3'/5'
2"	119.4	5.49, t (7.8)	1", 4", 10"
3"	141.8	-	
4"	39.8	2.10, m	2"
5"	26.5	2.10, m	6"
6"	124.0	5.09, m	5", 8"
7"	132.1	-	
8"/9"	25.9/17.9	1.68, s/1.60, s	6"/NO
10"	16.9	1.74, s	2"

4.3. Isoflavones and Prenylated Flavanones from the Roots of *Flemingia grahamiana*

Seven isoflavones and five prenylated flavanones together with a chromone as well as a gallocatechin derivative were obtained from the roots of *Flemingia grahamiana*. The flavanones exhibited signs of rearrangement in deuterated dimethylsulfoxide. One of the flavanones is new.

4.3.1 Isoflavones

The isoflavone derivatives reported here include genistein (**11**), biochanin A (**183**), genistin (genistein 7-*O*-β-glucopyranoside) (**184**), genistein 7,4'-di-*O*-β-glucopyranoside (**185**), flemiphilippinin-F (**186**), corylin (**187**) and lupalbigenin (**188**).

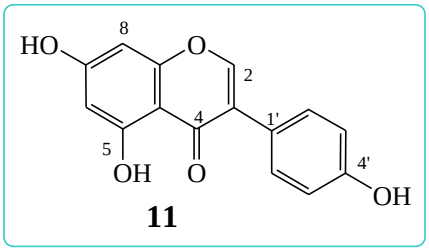
3.3.1.1 Genistein (11)

Compound **11** was obtained as a white solid. It was established to be an isoflavone derivative based on the presence of a carbonyl carbon peak at δ_C 189.3 (C-4) in the ^{13}C NMR spectrum and the gCHSQC experiment that gave a correlation between a carbon at δ_C 154.4 (C-2) and a one-proton singlet at δ_H 8.0 corresponding to H-2 (Table 22).

The ^1H NMR spectrum revealed the presence of a chelated 5-OH (δ_H 12.94, 1H, s) and two *meta*-coupled aromatic protons at δ 6.38 (*J* = 2.2 Hz, H-8) and 6.25 (*J* = 2.2 Hz, H-6) with carbon cross peaks in the gCHSQC at δ 94.7 and 99.8, respectively. Furthermore, the proton at δ 6.38 (H-8) correlated with two quaternary carbons at 159.1/164.7 while the proton at δ 6.25 (H-6) correlated with 163.8/164.7 in the HMBC. This implied a 5,7,8a-trioxygenation in the A-ring.

In the B-ring the ^1H NMR spectral data (Table 22), revealed an AA'XX' spin system centred at δ_H 7.38 (H-2'/6') and 6.88 (H-3'/5') with corresponding carbon peaks at δ_C 131.4 and 116.0, respectively, suggesting symmetry in the B-ring with a substituent at C-4'. The three bond HMBC correlation observed between H-2'/6' and an oxygenated carbon at δ 158.0 is consistent with C-4' oxygenation. The LC-ESI-MS of **11** gave an $[M+H]^+$ peak at *m/z* 271.7 which is in agreement with a molecular formula $C_{15}H_{10}O_5$ Based on these features it was concluded that compound **11** is genistein a known isoflavone which is common in

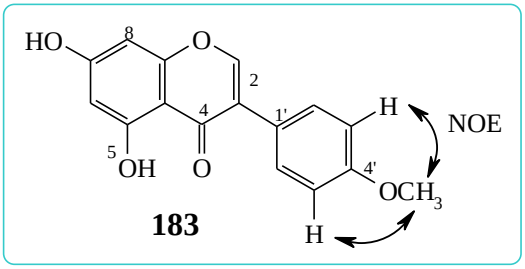
Leguminosae [Veitch, 2007; Stobiecki *et al*., 2010; Chen *et al*., 1991; Fu *et al*., 2012; Madan *et al*., 2009; Rahman *et al*., 2004; Das *et al*., 2007].



11

4.3.1.2 Biochanin A (183)

Compound **183** was isolated as a white solid with NMR (Table 22) spectral features that are similar to those of genistein (**11**) except for the presence of signals attributable to a methoxyl group (δ_C 56.0; δ_H 3.82, 3H, s) in the ^{1}H and ^{13}C NMR spectra of compound **183**. The UV spectrum with a λ_{max} at 260 nm is characteristic of an isoflavone derivative (Sekine *et al*., 1999). The LC-ESI-MS of **183** gave an $[M+H]^+$ peak at *m/z* 285.5 which is in agreement with a molecular formula $C_{16}H_{12}O_5$. The methoxyl protons exhibited NOE interaction with H-3'/5' (δ 7.00, 2H, m) as well as HMBC correlation with C-4' (δ_C 160.7), hence enabling its placement at C-4'. The compound was therefore identified as 5,7-dihydroxy-4'-methoxyisoflavone with a trivial name biochanin A, which is among the common isoflavones in Leguminosae [Veitch, 2007; Ahn, *et al.*, 2003; Stobiecki *et al*., 2010].



183

Table 22: ^{1}H (CD_3CN, 800 MHz) and ^{13}C (150 MHz) NMR data for compounds **11** and **183**

Position	**11**		**183**	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
2	154.4	8.00, s	154.7	8.02, s
3	124.1	-	123.6	-
4	189.3	-	181.9	-
4a	106.4	-	106.1	-
5	163.8	-	163.8	-
6	99.8	6.25, d (2.2)	99.9	6.26, d (2.1)
7	164.7	-	164.7	-
8	94.7	6.38, d (2.2)	94.7	6.39, d (2.1)
8a	159.1	-	158.9	-
1'	123.5	-	124.0	-
2'/6'	131.4	7.38, m	131.3	7.48, m
3'/5'	116.0	6.88, m	114.7	7.00, m
4'	158.0	-	160.7	-
5-OH	-	12.94, s	-	12.92, s
4'-OCH_3	-	-	56.0	3.82, s

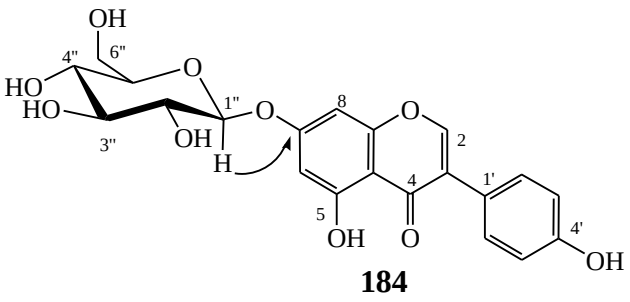
4.3.1.3 Genistin (184)

Compound **184** was isolated as a white powder, UV: λ_{max} 255 nm, and identified as an *O*-glucoside of genistein (**11**). The ^{1}H and ^{13}C NMR spectral data (Table 23) revealed features of an isoflavone skeleton similar to those of compound **11** but with relatively deshielded H-6 (δ 6.47) and H-8 (δ 6.72) protons. This was suggestive of an etheric bond at C-7 probably resulting from 7-*O*-glucosylation. The presence of a glucopyranose group was deduced from typical saturated oxymethine/oxymethylene proton signals in the ^{1}H NMR spectrum [δ 5.06 (1H, d, *J* = 7.5 Hz, H-1"); 3.70 (1H, m, H-6"b); 3.46 (1H, m, H-6"a); 3.44 (1H, m, H-5"); 3.29 (1H, m, H-3"); 3.26 (1H, m, H-2") and 3.16 (1H, m, H-4")] in combination with gCOSY. This was supported by both ^{13}C NMR spectrum [δ_C 99.8, (C-1')'; 77.2 (C-5"); 76.4 (C-3"); 73.1 (C-2"); 69.6 (C-4"); 60.6 (C-6")] and gCHSQC spectra. The ^{1}H NMR spectrum further exhibited signals at δ 5.40 (1H, d, *J* = 4.2 Hz), 4.13 (1H, d, *J* = 4.1 Hz), 5.07 (1H, m), 4.60 (1H, t, *J* = 5.4 Hz) which, based on gCOSY, TOCSY (and with no cross peaks in gCHSQC), were assigned to 2"-OH, 3"-OH, 4"-OH and 6"-OH, respectively.

The configuration at C-1" was determined by running an H-C undecoupled (or H-C coupled) gCHSQC experiment so as to determine the coupling constant between ^{13}C-1" and ^{1}H-1", ($^{1}J[^{13}CH(1")]$). The coupling constant (J_{CH} = 163.2 Hz) obtained was consistent with an axial

oriented H-1", hence a β-glucoside form, rather than an α-glucoside substituent (with the expected value $^{1}J[^{13}CH(1")] \approx 170$ Hz) (Bock and Pedersen, 1974). This is in agreement with a large coupling constant observed between H-1" and H-2" (J = 7.2 Hz) thus implying a 1-2 *trans*-diaxial relationship.

In the gHMBC experiment, an interraction was observed between H-1" and C-7 (δ 163.0) which is in agreement with the placement of the glucosyl moiety at C-7 of the isoflavone unit. From the above discussed data and by comparison with published data, it was concluded that the isolated compound is genistein 7-*O*-β-glucopyranoside also known as genistin [Lewis *et al.*, 1998].



184

4.3.1.4 Genistein 7,4'-di-*O*-β-glucopyranoside (185)

Compound **185** was obtained as a white solid with UV λ_{max} at 260 nm. The ^{1}H NMR data of **185** (Table 23) was, to a great extent, similar to that of genistin (**184**) but with the signals for the glucosyl moiety duplicated. Two anomeric-proton signals were observed at δ_H 5.05 and 4.96 with respective carbon peaks at δ_C 101.6 and 102.3. The observation of an NOE correlation between H-3'/5' (δ 7.17, 2H, *m*) and the anomeric proton resonating at δ 4.96 (H-1''') enabled the assignment of one of the glycosyl residues to C-4' of the B-ring of an isoflavone skeleton.

The chemical shift values for the *meta*-coupled protons H-6 (δ_H 6.53, 1H, d, J = 2.2 Hz) and H-8 (δ_H 6.72, 1H, d, J = 2.2 Hz) in compound **185** were identical to those in **184** (in CD_3OD) and therefore implying a 7-*O*-glucosyl substituent in both compounds.

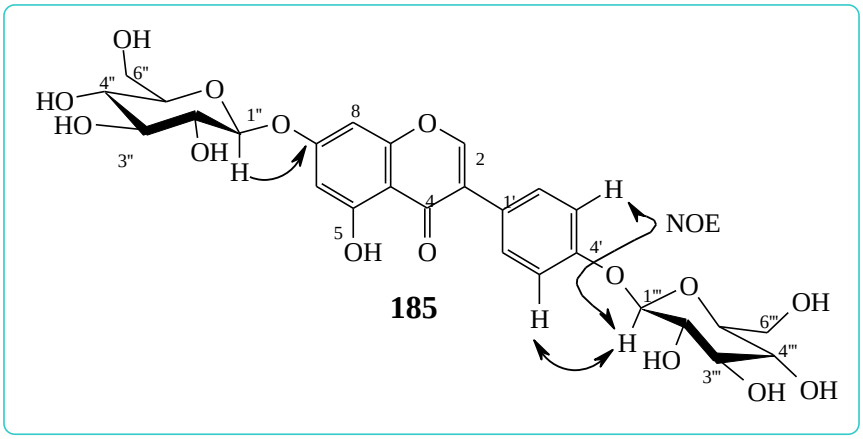
Table 23: ^{1}H (800 MHz) and ^{13}C (150 MHz) NMR data for compounds **184** and **185**

Position	**184** ($(CD_3)_2SO$)		**185** (CD_3OD)	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
2	154.6	8.43, s	155.7	8.19, s
3	122.5	-	126.1	-
4	180.5	-	182.3	-
4a	106.1	-	108.0	-
5	161.6	-	163.6	-
6	99.6	6.47, d (2.2)	101.2	6.53, d (2.2)
7	163.0	-	164.9	-
8	94.5	6.72, d (2.2)	95.9	6.72, d (2.2)
8a	157.4	-	159.2	-
1'	121.0	-	124.7	-
2'/6'	130.1	7.40, m	131.3	7.51, m
3'/5'	115.1	6.83, m	117.7	7.17, m
4'	157.5	-	159.2	-
1"	99.8	5.06, d (7.2)	101.6	5.06, m
2"	73.1	3.26, m	[a]74.7	3.44-3.55, m
3"	76.4	3.29, m	77.8	3.44-3.55, m
4"	69.6	3.16, m	71.4	3.42, m
5"	77.2	3.44, m	78.2	3.44-3.55, m
6"a	60.6	3.46, m	62.4	3.71, m
b		3.70, m		3.91, m
1'"	-	-	102.2	4.96, m
2'"	-	-	[a]74.9	3.44-3.55, m
3'"	-	-	78.0	3.44-3.55, m
4'"	-	-	71.2	3.42, m
5'"	-	-	78.4	3.44-3.55, m
6'"a	-	-	62.5	3.71, m
b				3.91, m
5-OH	-	12.95, s	-	NO
4'-OH	-	NO	-	-
2"-OH	-	5.40, d (4.2)	-	NO
3"-OH	-	4.13, d (4.1)	-	NO
4"-OH	-	5.07, m	-	NO
6"-OH	-	4.60, t, (5.4)	-	NO

NO = Not observed; [a] ^{13}C NMR respective assignments may be interchanged.

The ten peaks displayed in the range δ_C 60-79 of the ^{13}C NMR spectrum further supports the presence of two glucosyl residues. The $^{1}J[^{13}CH(1")]$ and $^{1}J[^{13}CH(1'")]$ in the H-C coupled gCHSQC, of 161.6 and 164.8 Hz, respectively, were consistent with β-glucoside moieties with axial H-1" and H-1'" [Bock and Pedersen, 1974]. Based on these data the compound was characterised as genistein 7,4'-di-*O*-β-glucopyranoside (or genistin 4'-*O*-β-glucopyranoside) [Watanabe *et al.*, 1993]. The rest of the assignments were done by comparison of the data

with those of genistin (**184**). The compound is reported here for the first time from the genus *Flemingia*



185

4.3.1.5 Flemiphilippinin F (186)

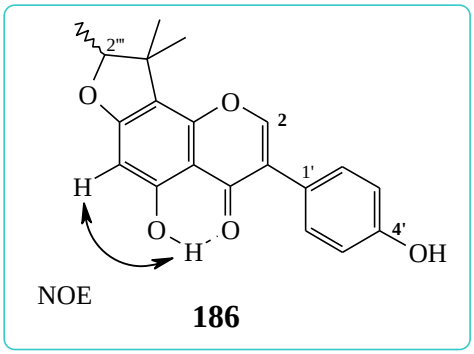
Compound **186** was obtained as a white solid, the presence of an isoflavone skeleton was deduced based on both ^{1}H and ^{13}C NMR spectra (Table 24) and supported by gCHSQC experiment that gave a peak with co-ordinates for C-2/H-2 at δ_C/δ_H 154.1/8.01. Furthermore, the HMBC correlation observed between H-2 and a ketonic carbon at δ_C 181.9 (C-4) confirmed the isoflavone skeleton.

The presence of two relatively intense peaks at δ_C 131.1 (C-2'/6') and 116.1 (C-3'/5'), in the ^{13}C NMR spectrum, with corresponding proton peaks at δ 7.38 (m, 2H) and 6.88 (m, 2H), respectively, is consistent with a 4'-substituted B-ring. This, supported by HMBC correlation between H-2'/6' (at δ_H 7.38) with a quaternary carbon resonating at δ_C 158.1, indicated an oxygenation at C-4' similar to compounds **11** and **183**.

The ^{1}H NMR spectrum, further exhibited a one-proton singlet at δ_H 6.24 (with a corresponding carbon peak at δ_C 95.0) assignable to H-6 on the basis of NOE interaction between this proton and the chelated 5-OH as well as HMBC correlation between 5-OH and C-6 (δ 95.0). This implied that C-7 and C-8 of the A-ring are substituted.

The presence of a 2,3,3-trimethyldihydrofuranyl moiety fused to C-7/C-8 of the A-ring was deduced from the ^{1}H NMR spectrum that revealed a quartet at δ 4.56 (1H, J = 6.5 Hz, for H-2") coupled to methyl protons at δ 1.38 (d, J = 6.5 Hz, 2"-CH_3), as well as two methyl

singlets at δ 1.49 and 1.24. This was supported by the ^{13}C NMR spectrum (and gCHSQC experiment) with peaks at δ 91.8 (C-2"), 44.6 (C-3"), 25.8/21.6 (3"-*gem*-CH_3), 14.5 (2"-CH_3). With H-6 in place, the placement of the dihydrofuranyl moiety must be fused to A-ring at C-7/8, was supported by HMBC correlations of 3"-dimethyl protons and C-8 (δ 113.9) together with a 3-bond correlation between H-6 and C-8.



186

Table 24: ^{1}H (800 MHz) and ^{13}C (150 MHz) NMR data for compound **186** (CD_3CN)

Position	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
2	154.1	8.01, s	C-2, 3, 4, 1'
3	124.0	-	
4	181.9	-	
4a	106.8	-	
5	164.2	-	
6	95.0	6.24, s	C-4a, 5, 8
7	164.2	-	
8	114.0	-	
8a	154.3	-	
1'	123.5	-	
2'/6'	131.1	7.38, m	C-3, 4', 6'/2'
3'/5'	116.1	6.88, m	C-1'
4'	158.1	-	
2"	91.8	4.56, q (6.5)	
3"	44.6	-	
2"-CH_3	14.5	1.38, d (6.5)	C-2", 3"
3"-CH_3	21.6	1.24, s	C-8, 2", 3", 3"-CH_3
	25.8	1.49, s	C-8, 2", 3", 3"-CH_3
5-OH	-	13.22, s	C-4a, 6, 7

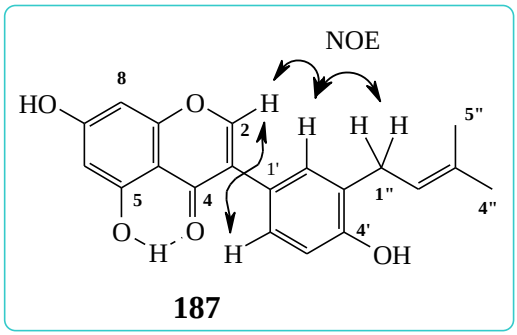
Compound **186** was therefore characterized as 5,4'-dihydroxy-2",3",3"-trimethyl-2",3"-dihydrofurano-[4",5":8,7]-isoflavone, trivial name flemiphilippinin F, previously isolated

from the roots of *Flemingia philippinensis* [Li *et al.,* 2008]. The configuration at C-2" is not established here but the sample isolated from the roots of *F. philippinensis* was obtained as a racemic mixture [Li *et al.,* 2008].

4.3.1.6 Corylin (187)

Compound **187**, UV: λ_{max} 255 nm, was isolated as a white amorphous solid and identified as the known prenylated isoflavone corylin [Nkengfack *et al.,* 1994]. The ^{1}H NMR data (Table 25) gave a characteristic H-2 singlet at δ_H 7.97 (1H) with a corresponding carbon peak (C-2) at δ_C 154.1 in the gCHSQC. The ^{1}H NMR spectrum also revealed the presence of a chelated 5-OH (δ_H 12.96, 1H, s) and two *meta*-coupled aromatic protons [δ_H 6.37(*J* = 2.0 Hz) and 6.24 (*J* = 2.0 Hz)]. These features are similar to those observed for compound **11**, and is typical of a C-5 and C-7-oxygenation A-ring. This was supported by gCHSQC spectrum which gave the corresponding carbon signals at δ_C 94.3 (C-8) and 99.6 (C-6).

An ABX spin system [δ 7.26 (1H, d, *J* = 2.0 Hz, H-2'); 7.21 (1H, dd, *J* = 8.2, 2.0 Hz, H-6'); 6.85 (1H, d, *J* = 8.2 Hz, H-5') also observed in the ^{1}H NMR spectrum was indicative of a C-3', -4' substituted B-ring of the isoflavone. The presence of a γ,γ-dimethylallyl group was deduced from both ^{1}H NMR and gCHSQC spectra [(δ_H 5.32, 1H, t, *J* = 7.4 Hz, H-2"/δ_C 122.9, C-2"); (δ_H 3.30, 2H, d, *J* = 7.2 Hz, H-1"/ δ_C 28.9, C-1"); (δ_H 1.74, 3H, s, H-5"/δ_C 17.0, C-5"); (δ_H 1.74, 3H, s, H-4"/δ_C 25.1, C-4")])] and was placed at C-3' based on the observation of a NOE interaction between H-1" (δ 3.30) and H-2' (δ 7.25). Other NOE interactions were between H-2 and H-2'/H-6', which further support the proposed structure. ^{13}C NMR and HMBC spectra could not be fully generated due to paucity of the sample (only protonated carbons were detected, Table 25) but based on the above data this compound was established as corylin (**187**), previously reported from *Erythrina sigmoidea* [Nkengfack *et al.,* 1994] and the root bark of *Erythrina addisoniae* [Nguyen *et al.,* 2010]. However this is the first report of corylin (**187**) from the genus *Flemingia*.



187

4.3.1.7 Lupalbigenin (188)

Compound **188** was also identified as an isoflavone derivative with H-2 resonating at δ_H 7.96 in the ^{1}H NMR spectrum; C-2 at δ_C 154.3 and C-4 at δ 182.1 in the ^{13}C NMR spectrum (Table 25). The presence of two γ,γ-dimethylallyl groups was obvious from both ^{1}H and ^{13}C NMR spectra (Table 25). An isolated aromatic proton signal at δ 6.43 (1H, s), in the ^{1}H NMR spectrum, corresponding to a carbon with a signal at δ_C 94.0 (deduced from gCHSQC) and showing long range HMBC correlations to two oxygenated aromatic carbons at δ_C 159.6 and 162.3 is suggestive of the aromatic proton being at either C-6 or C-8 in the A-ring with a substituent (γ,γ-dimethylallyl group) on the other carbon. NOE interaction between a chelated hydroxyl proton [δ 13.25 (s, 5-OH) and the methylene protons [δ 3.31 (m, CH_2-1")] of one of the γ,γ-dimethylallyl groups and thus implying the placement of the isoprenyl group at C-6 and therefore an aromatic proton is located at C-8. Furthermore, the observation of HMBC of 5-OH with three quaternary carbons at δ_C 106.3 (C-4a), δ_C 160.5 (C-5) and δ_C 112.4 (C-6) is indeed a confirmation of the proposition.

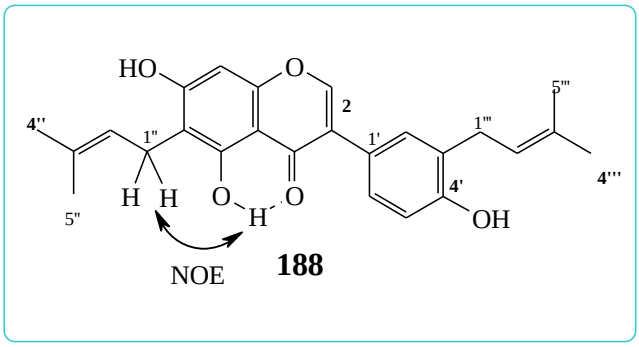


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Table 25: ^{1}H NMR for compound **187** and **188** (CD_3CN, 800 MHz); gCHSQC data for compound **187** and ^{13}C NMR data for compound **188**

	187		**188**		
Position	δ_C	δ_H, mult. (*J* in Hz)	Position	δ_C	δ_H, mult. (*J* in Hz)
2	-	7.97, s	2	154.6	7.96, s
3	-	-	3	124.2	-
4	-	-	4	182.1	-
4a	-	-	4a	106.3	-
5	-	-	5	160.5	-
6	99.6	6.24, d (2.0)	6	112.4	-
7	-	-	7	162.3	-
8	94.3	6.36, d (2.0)	8	94.0	6.43, s
8a	-	-	8a	156.9	-
1'	-	-	1'	123.8	-
2'	131.2	7.26, d (2.0)	2'	131.5	7.25, d (2.2)
3'	-	-	3'	128.9	-
4'	-	-	4'	155.6	-
5'	115.4	6.85, d (8.2)	5'	115.8	6.85, d (8.2)
6'	128.6	7.21, dd (8.2, 2.0)	6'	128.8	7.21, dd (8.2, 2.2)
-	-	-	1"	22.0	3.29, m
-	-	-	2"	123.0	5.20, t (7.0)
-	-	-	3"	132.7	-
-	-	-	4"	25.79	1.66, s
-	-	-	5"	17.92	1.77, s
1"	28.9	3.30, d (7.2)	1'"	29.2	3.29, m
2"	122.9	5.32, t (7.4)	2'"	123.3	5.31, t (7.2)
3"	-	-	3'"	133.4	-
4"	25.1	1.74, s	4'"	25.84	1.73, s
5"	17.0	1.74, s	5"	17.90	1.71, s
5-OH	-	12.96, s	5-OH	-	13.25, s

The presence of an ABX spin system in B-ring was evident from ^{1}H NMR data [δ 7.25 (1H, d, *J* = 2.2 Hz, H-2'); 7.21 (1H, dd, *J* = 8.2, 2.2 Hz, H-6') and 6.85 (1H, d, *J* = 8.2 Hz, H-5')]. The biogenetically expected oxygenation at C-4 (4'-OH) was supported from the HMBC correlations from both H-2' and H-6' to the carbon resonating at δ_C 155.6. The placement of the second isoprenyl group at C-3' is consistent with a 1',3',4' substitution pattern and an ABX system of the B-ring. From the information above it was concluded that compound **188** is lupalbigenin, previously obtained from *Derris scandens* [Sekine *et al.,* 1999].

4.3.2 Flavanones and a chromone derivative

Five flavanones, together with one prenylated chromone derivative were identified from the roots of *Flemingia grahamiana*. The flavanones include flemichin D (**150**), eriosemaone A (**189**), lupinifolin (**151**), 5,2',4'-trihydroxy-8,5'-di(γ,γ-dimethylallyl)-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**190**), 5,3',4'-trihydroxy-8-γ,γ-dimethylallyl-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**191**). In addition to flavanones, a prenylated chromone derivative with trivial name eriosematin was also obtained.

The 8-γ,γ-dimethylallyl-2",2"-dimethylpyrano-[5",6":6,7]-flavanones in dimethyl sulfoxide were noted to undergo partial rearrangement into the respective 6-γ,γ-dimethylallyl-2"',2"'-dimethylpyrano-[5"',6"':8,7]-flavanone regioisomers.

4.3.2.1 Flemichin D (150)

Compound **150** was isolated as a yellow solid and identified as a flavanone derivative. The UV [λ_{max} 270 and 300 nm]; ^{1}H NMR [(AMX spin pattern at δ_H 5.61 (dd, *J* = 13.1, 3.0 Hz, H-2); 3.13 (dd, *J* = -17.2, 13.0 Hz, H-3ax) and 2.72 (dd, *J* = -17.2, 3.0 Hz, H-3eq)] and ^{13}C NMR [δ_C 42.3 (C-3); 75.5 (C-2) and 198.8 (C-4)] spectra (Table 26) suggested a flavanone skeleton.

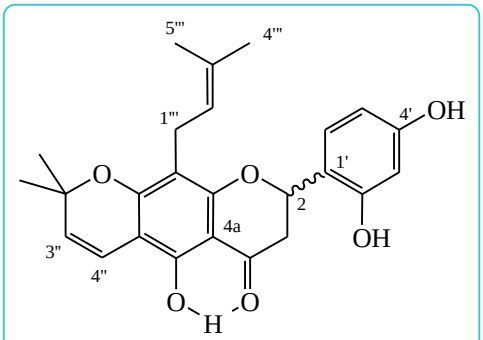
Furthermore, the ^{1}H NMR spectrum showed an AXY spin pattern [δ_H 6.36 (d, *J* = 2.0 Hz, H-3'), 6.40 (dd, *J* = 8.4 and 2.0 Hz, H-5') and 7.25 (d, *J* = 8.4 Hz, H-6') in the B-ring. This assignment was based on observed ^{3}J HMBC correlation of H-6' with C-2 (δ_C 75.5) and two oxygenated quaternary carbons at δ_C 156.4 (C-2') and 159.2 (C-4').

The ^{1}H and ^{13}C NMR spectra (Table 26) also revealed the presence of chelated hydroxyl group [δ_H 12.41 (s, 5-OH)], a 2,2-dimethylpyrano ring and a γ,γ-dimethylallyl moiety on A-ring. In the presence of 5-OH, there are two possible arrangements: (i) the pyrano ring between C-6/C-7 and the prenyl unit at C-8 and (ii) vice versa. The 5-OH and one of the pyran protons [δ_H 6.57 (d, *J* = 10.0 Hz, H-3")] exhibited HMBC correlation with the signal at δ_C 157.3 (C-5) which implied that the pyran ring is between C-6/C-7 (with C-7 oxygenated), and hence the isoprenyl unit is located at C-8. Indeed, H-1"' of the γ,γ-dimethylallyl unit correlated with both C-7 (δ_C 160.1) and C-8a (δ_C 161.0), in gHMBC experiment, thus

confirming the structure. This compound was therefore characterized as the known 5,2',4'-trihydroxy-8-γ,γ-dimethylallyl-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**150**) (trivial name flemichin D) [Ma *et al.,* 1995]. The absolute configuration at C-2 is not yet established but the coupling constant between H-2 and one of the C-3 protons (J = 13.0 Hz) indicates that H-2 is in an axial orientation and, thus, the B-ring assuming the equatorial position.

Table 26: ^{1}H (800 MHz) and ^{13}C (150 MHz) NMR data for compound **150** (CD_3CN)

Position	δ_C	δ_H, mult. (J in Hz)	HMBC (H → C)
2	75.5	5.61, dd (13.0, 3.0)	C-1', 2', 6'
3ax	42.3	3.13, dd (-17.2, 13.0)	C-2, 4, 1'
eq		2.72, dd (-17.2, 3.0)	C-4, 6
4	198.8	-	
4a	103.5	-	
5	157.3	-	
6	103.4	-	
7	160.1	-	
8	109.2	-	
8a	161.0	-	
1'	117.7	-	
2'	156.4	-	
3'	103.5	6.36, d (2.0)	C-1', 2', 4', 5'
4'	159.2	-	
5'	108.0	6.40, dd (8.4, 2.0)	C-1', 3', 4'
6'	129.2	7.25, d (8.4)	C-2, 2', 4'
2"	79.0	-	
3"	127.5	5.59, d (10.0)	C-6, 2", 2"-CH_3
4"	116.0	6.57, d (10.0)	C-5, 7, 2"
2"-CH_3	28.4	1.41, s	C-2", 3", 4", 2"-CH_3
	28.4	1.42, s	C-2", 3", 4", 2"-CH_3
1'"	22.1	3.15, d (7.4)	C-8, 8a, 2'", 3'"
2'"	123.3	5.12, t (7.3)	C-1'", 4'", 5'"
3'"	132.0	-	
4'"	25.8	1.62, d (1.1)	C-8, 2'", 3'", 5'"
5'"	18.0	1.64, d (1.0)	C-2'", 3'", 4'"
5-OH	-	12.41, s	C-4a, 5, 6



150

4.3.2.2 Eriosemaone A (189)

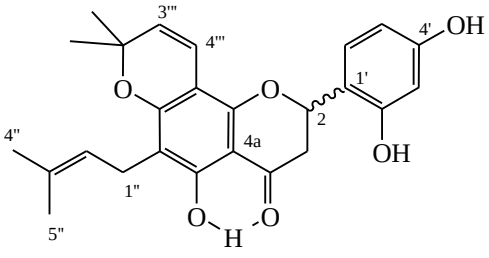
Compound **189**, a yellow solid whose UV spectrum gave λ_{max} at 260 and 300 nm, was isolated as a minor constituent from the roots of *Flemingia grahamiana*. The ^{1}H and ^{13}C NMR spectra (Table 27) of **189** were similar to those of compound **150** with an apparent difference being that the chemical shift value for 5-OH is shifted down field (δ_H 12.54) compared to that of **150** (δ_H 12.41). Another noticeable difference was that one of the methyl signals of the γ,γ-dimethylallyl moiety shifted down field (δ_H 1.77, H-5") compared to the value in **150** (δ_H 1.64, H-5'''). Furthermore, the olefinic proton H-4''' of the pyrano-ring in **189** resonated at a higher field (δ_H 6.47) than that of H-4" (δ_H 6.57) in **150**. This suggested that compounds **150** and **189** are regioisomers having interchanged locations of the 2,2-dimethylpyrano and γ,γ-dimethylallyl systems. This deduction was supported by HMBC correlations from both 5-OH and CH_2-1" (protons of γ,γ-dimethylallyl group) to C-5 (δ_C 161.8) consistent with the placement of the γ,γ-dimethylallyl substituent at C-6 in compound **189**.

The compound is therefore 5,2',4'-trihydroxy-6-γ,γ-dimethylallyl-2''',2'''-dimethylpyrano-[5''',6''':8,7]-flavanone (**189**), trivial name eriosemaone A, previously reported from the roots of *Eriosema tuberosum* [Ma *et al.*, 1995]. However, this is the first report of the compound from the genus *Flemingia*.

The absolute configuration at C-2 is not yet established but it was deduced that H-2 is in an axial position and the B-ring in the equatorial orientation based on the coupling constant between H-2 and one of the C-3 protons (J = 13.1 Hz).

Table 27: ^{1}H (800 MHz) and ^{13}C (150 MHz) NMR data for compound **189** (CD_3CN)

Position	δ_C	δ_H, mult. (J in Hz)	HMBC (H → C)
2	75.5	5.64, dd (13.1, 3.0)	NO
3ax	42.3	3.16, dd (-17.1, 13.1)	C-2, 4
eq		2.72, dd (-17.1, 3.0)	C-4
4	198.6	-	
4a	103.4	-	
5	161.8	-	
6	109.9	-	
7	160.2	-	
8	102.6	-	
8a	156.6	-	
1'	117.5	-	
2'	159.3	-	
3'	103.6	6.37, d (2.3)	C-1', 2', 4', 5'
4'	156.4	-	
5'	108.1	6.40, dd (8.4, 2.2)	C-1', 3'
6'	129.2	7.25, d (8.4)	C-2, 2', 4'
1"	21.6	3.21, d (7.0)	C-5, 6, 7, 2", 3"
2"	123.3	5.16, t (7.4)	
3"	132.1	-	
4"	25.8	1.65, d (1.0)	C-2", 3", 5"
5"	18.0	1.77, s	C-2", 3", 4"
2"'	79.0	-	
3"'	127.6	5.56, d (10.0)	C-8, 2"'
4"'	116.4	6.47, d (10.0)	C-7, 2"'
2"'-CH_3	28.2	1.39, s	C-2"', 3"', 2"'-CH_3
	28.5	1.43, s	C-2"', 3"', 2"'-CH_3
5-OH	-	12.55, s	C-4a, 5, 6



189

In the presence of dimethylsulfoxide, compound **150,** underwent rearrangement into its regioisomer **189**. This was also observed for compounds **151** and **190** which, similarly, isomerised into **150a** and **190a**, respectively.

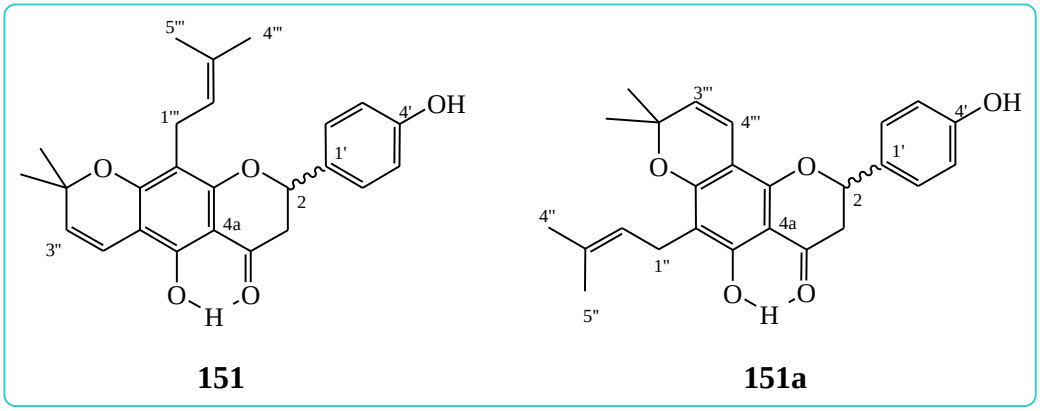
3.3.2.3 Lupinifolin (151)

Compound **151** was obtained as a yellow powder with UV spectrum at λ_{max} 260 and 304 nm. In the ^{1}H NMR spectrum (Table 28), three typical flavanone signals [δ_H 5.40 (1H dd, *J* = 12.9, 3.0 Hz, H-2); 3.12 (1H, dd, *J* = -17.2, 12.9 Hz, H-3a) and 2.78 (1H, dd, *J* = -17.0, 3.0 Hz)] were observed. The ^{13}C NMR showed a C-4 carbonyl signal (δ_C 198.3), an oxymethine peak [δ_C 79.8 (C-2)] and a methylene signal [δ_C 43.3 (C-3)]. The ^{1}H NMR also displayed a hydrogen bonded OH [δ_H 12.39 (s, 5-OH)] implying a fully substituted A-ring as in **150** and **189**.

A C-4' oxygenated (δ_C 158.3) B-ring was evident from an AA'XX' pattern observed in ^{1}H NMR spectrum (Table 28) and supported by the two relatively intense peaks at δ_C 129.1 (C-2'/6') and 116.2 (C-3'/5') in the ^{13}C NMR spectrum.

The ^{1}H,^{13}C NMR and gCHSQC spectra revealed the presence of a 2,2-dimethylpyrano-ring and γ,γ-dimethylallyl group in A-ring. Analysis of gHMBC spectrum showed interactions between H-4" (δ_H 6.58) and 5-OH with C-5 (δ_C 157.3). This allowed the placement of the dimethylpyrano ring between C-6/7, and therefore the γ,γ-dimethylallyl moiety is attached at C-8. Furthermore, the HMBCs correlations of H-1'" to C-7 (δ_C 160.2), C-8 (δ_C 109.2) and C-8a (δ_C 160.6) were in agreement with the placement of the dimethylallyl group at C-8. Compound **151** was therefore characterized as 5,4'-dihydroxy-8-γ,γ-dimethylallyl-2",2"-dimethylpyrano-[5",6":6,7]-flavanone, trivial name lupinifolin [Smalberger *et al.*, 1974; Mahidol *et al.*, 1997].

In the presence of dimethylsulfoxide, compound **151** underwent rearrangement into its regioisomer **151a**, whose ^{1}H NMR spectral data are given in Section 4.7.



151 **151a**

Table 28: ^{1}H (CD_3CN, 800 MHz) NMR and ^{13}C (150 MHz) data for compound **151**

Position	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
2	79.8	5.40, dd (12.9, 3.0)	
3ax	43.3	3.12, dd (-17.2, 12.9)	C-2, 4, 1'
eq		2.78, dd (-17.0, 3.0)	C-4
4	198.3	-	
4a	103.4	-	
5	157.3	-	
6	103.5	-	
7	160.2	-	
8	109.2	-	
8a	160.6	-	
1'	131.2	-	
2'/6'	129.1	7.34, d (8.5)	C-2, 1', 6'/2'
3'/5'	116.2	6.86, d (8.5)	C-1', 4', 5'/3'
4'	158.3	-	
2''	79.1	-	
3''	127.5	5.60, d (9.9)	C-6, 2''
4''	116.0	6.58, d (10.0)	C-5, 7, 2''
2''-CH_3	28.4	1.41, s	C-2'', 3'', 2''-CH_3
	28.5	1.42, s	C-2'', 3'', 2''-CH_3
1'''	22.1	3.16, d (7.2)	C-7, 8, 8a, 2''', 3'''
2'''	123.3	5.12, t (7.4)	C-4''', 5'''
3'''	132.1	-	
4'''	25.8	1.63, s	C-2''', 3''', 5'''
5'''	18.0	1.63, s	C-2''', 3''', 4'''
5-OH	-	12.39, s	C-4a, 5, 6

4.3.2.4 5,2',4'-Trihydroxy-8,5'-di(γ,γ-dimethylallyl)-2'',2''-dimethylpyrano-[5'',6'':6,7]-flavanone (190)

Compound **190** was isolated as a yellow powdery solid and identified as a flavanone. This was deduced from its UV spectrum [λ_{max}: 260 and 305 nm] and three sets of double doublets for C-ring protons in the ^{1}H NMR spectrum [δ_H 5.61 (1H, *J* = 12.2, 2.5 Hz, H-2); 3.11 (1H, *J* = -17.4, 12.7 Hz, H-3ax) and 2.73 (1H, *J* = -17.2, 2.7 Hz, H-3eq)] (Table 29). A broad singlet at δ_H 12.42 (1H) was attributed to 5-OH hydrogen-bonded to C-4 carbonyl. The ^{13}C NMR spectrum of compound **190** showed characteristic signals for C-2 (δ_C 75.5), C-3 (δ_C 42.3) and C-4 (δ_C 198.8) of the C-ring of a flavanone skeleton.

The A-ring had a substitution pattern similar to that of flemichin D (**150**) and lupinifolin (**151**) as deduced from both ^{1}H and ^{13}C NMR spectral data (Table 29) and a combination of gCHSQC, ^{1}H-^{1}H gCOSY, gHMBC and NOESY spectral analyses. However, compound **190**, was different from **150** and **151** in that the it has two γ,γ-dimethylallyl groups instead of one as in **150** and **151**. Furthermore, the B-ring signals in the ^{1}H NMR spectrum consisted of two singlets [δ_H 7.10 (1H, H-6') and 6.38 (1H, H-3')] due to two *para*-oriented protons of a trisubstituted ring. With C-2' and C-4' oxygenated (Table 29), as in compound **150**, the second γ,γ-dimethylallyl group could be located at C-5' based on HMBC spectrum that revealed ^{3}J correlations of H-1'''' (δ_H 3.18) to C-4' and C-6' (δ_C 127.4)].

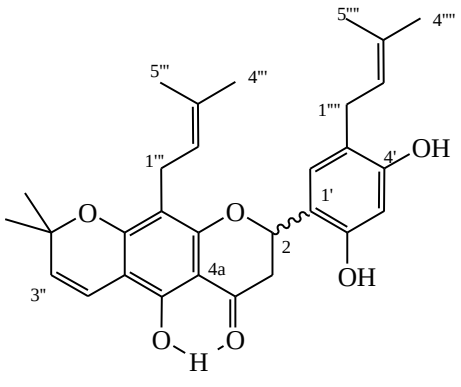
This compound was therefore identified as 5,2',4'-trihydroxy-8, 5'-di(γ,γ-dimethylallyl)-2'',2''-dimethylpyrano-[5'',6'':6,7]-flavanone (**190**) which was previously isolated from the roots of *Moghania philippinensis* (Leguminosae) [Ahn *et al.*, 2003].

Just like compounds **150** and **151** when dissolved in dimethyl sulfoxide, compound **190** exists in equilibrium with its regioisomer **190a**. The ^{1}H NMR spectrum (Table 30) of compound **190a** was similar to that of **190**, deduced as a flavanone derivative, with one dimethylpyrano, two γ,γ-dimethylallyl groups and with identical B-ring. However, it was noted that 5-OH in compound **190a** is more deshielded (δ_H 12.56) compared to the case in compound **190** (δ_H 12.42). Similar differences were observed for 5-OH chemical shifts in isomeric pairs; **150** and **189**; as well as **151** and **151a** (Table 31).

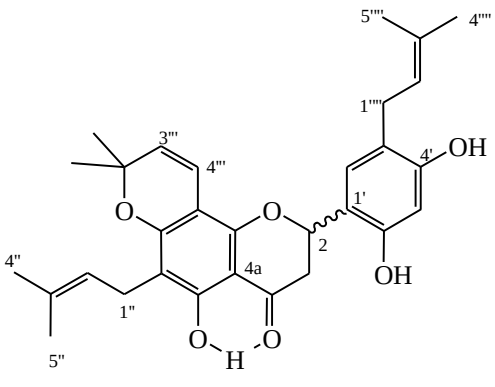
From the NOESY experiment, an interaction was observed between H-1" (δ_H 3.20) of a γ,γ-dimethylallyl moiety and the chelated hydroxyl proton at C-5. This together with gHMBC correlations of H-1" to the three carbons C-5 (δ_C 161.7, also correlated to 5-OH), C-6 (δ_C 109.8) and C-7 (δ_C 160.1) was a clear indication that one of the γ,γ-dimethylallyl groups is located at C-6.

The dimethylpyrano ring was assigned between C-7 and C-8 on the basis of gHMBC correlations exhibited by H-3‴ of the pyrano group with C-8 (δ_C 102.5) as well as similar correlations from H-4‴ to C-7 (δ_C 160.1) and C-8a (δ_C 156.3).

Other noticeable differences between compounds **190** and **190a** include the down-field shift of the methyl-proton signal for CH_3-5" (δ_H 1.77) of the γ,γ-dimethylallyl group when located at C-6 as in isomer **190a** compared to the signal for CH_3-5‴ (δ_H 1.64) when the isoprenyl is at C-8 as in structure **190**. Such differences were also observed between compounds **151** and **151a**; as well as between **150** and its isomer **189**. The reason for the difference could be that when the isoprenyl unit is at C-8, the methyl groups of the prenyl group experience a shielding anisotropic effect [Pavia *et al.*, 1995] due to the π-bond electrons resonating in the B-ring. When in position C-6 the prenyl group is far away from the shielding effect of the B-ring.



190



190a

Table 29: ^{1}H (800 MHz) and ^{13}C (150 MHz) NMR data for compound **190** (CD_3CN)

Position	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
2	75.5	5.61, dd (12.2, 2.5)	
3ax	42.3	3.11, dd (-17.4, 12.7)	C-2, 4, 1'
eq		2.73, dd (-17.2, 2.7)	C-4
4	198.8	-	
4a	103.5	-	
5	157.3	-	
6	103.3	-	
7	160.1	-	
8	109.2	-	
8a	160.9	-	
1'	117.4	-	
2'	154.0	-	
3'	103.5	6.38, s	C-2, 1', 2', 4'
4'	156.3	-	
5'	120.5	-	
6'	129.1	7.10, s	C-2, 2', 4', 2"-CH_3
2"	79.1	-	
3"	127.4	5.59, d (9.7)	C-6, 2"
4"	116.0	6.57, d (10.0)	C-5, 7, 2"
2"-CH_3	28.4/28.5	1.41, s/1.42, s	C-2", 3", 2"-CH_3/C-2", 3", 2"-CH_3
1"'	22.1	3.15, d (7.2)	C-7, 8a, 2"', 3"'
2"'	123.3	5.13, t (7.0)	C-1"', 4"', 5"'
3"'	132.0	-	
4"'	25.9	1.62, s	C-2"', 3"', 5"'
5"'	18.0	1.64, s	C-2"', 3"', 4"'
1""	28.4	3.18, d (7.2)	C-4', 5', 6', 2"", 3""
2""	123.7	5.25, t (6.8)	C-1"", 4"", 5""
3""	133.1	-	
4""	25.8	1.70, s	C-2"", 3"", 5""
5""	17.8	1.67, s	C-2"", 3"", 4""
5-OH	-	12.42, s	C-4a, 5, 6, 7

Table 30: ^{1}H (800 MHz) and ^{13}C (150 MHz) NMR data for compound **190a** (CD_3CN)

Position	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
2	75.6	5.64, dd (12.4, 3.0)	
3ax	42.1	3.16, m	C-2, 4, 1'
eq		2.74, dd (-17.2, 3.3)	C-4
4	198.5	-	
4a	103.3	-	
5	161.7	-	
6	109.8	-	
7	160.1	-	
8	102.5	-	
8a	156.3	-	
1'	117.1	-	
2'	154.1	-	
3'	103.5	6.37, s	C-2, 1', 2', 4', 5'
4'	156.2	-	
5'	120.5	-	
6'	129.1	7.08, s	C-2, 2', 4', 1""
1"	21.5	3.20, m	C-5, 6, 7, 2", 3"
2"	123.2	5.16, t (7.2)	
3"	132.0	-	
4"	25.8	1.65, s	C-2", 3", 5"
5"	18.8	1.77, s	C-2", 3", 4"
2"'	78.9	-	
3"'	127.5	5.56, d (10.0)	C-8, 2"'
4"'	116.4	6.46, d (10.0)	C-8, 8a, 2"'
2"'-CH_3	28.3	1.40, s	C-2"', 3"', 2"'-CH_3
	28.5	1.42, s	C-2"', 3"', 2"'-CH_3
1""	28.4	3.18, m	C-4', 5', 6', 2"", 3""
2""	123.6	5.25, t (7.2)	
3""	133.2	-	
4""	25.8	1.70, s	C-2"", 3"", 5""
5""	17.8	1.67, s	C-2"", 3"", 4""
5-OH	-	12.56, s	C-4a, 5, 6

NO = Not observed

Table 31: δ_H values of 5-OH in CD_3CN for compounds **150**, **189**, **151**, **151a**, **190** and **190a**

Isomeric pair	δ_H for the 1st isomer	δ_H for the 2nd isomer	$\Delta\delta_H$
150, **189**	12.41	12.55	0.14
151, **151a**	12.39	12.54	0.15
190, **190a**	12.42	12.56	0.14

4.3.2.5 5,3',4'-Trihydroxy-8-γ,γ-dimethylallyl-2'',2''-dimethylpyrano-[5'',6'':6,7]-flavanone (191)

Compound **191** was obtained as a yellow solid and identified to be a flavanone derivative as deduced from its UV (λ_{max}: 265 and 310 nm) and NMR (Table 32) spectra. The molecular formula the compound was established to be $C_{25}H_{26}O_6$ by HRESI-MS giving a *quasi*-molecular ion peak, $[M+H]^+$ at *m/z* 423.1809 (calcd. 423.1763). Both the ^1H and ^{13}C NMR spectra (Table 32) of the compound revealed characteristic features of a flavanone skeleton having an A-ring substitution pattern identical to that in compounds **150**, **151** and **190**. However, the B-ring substitution pattern was quite different from these compounds. Thus, the ^1H NMR spectrum signals in the B-ring included a one-proton broad singlet (δ_H 6.96) and two overlapping singlets (δ_H 6.85). The signals at δ_C 145.7 and 145.8 in the ^{13}C NMR spectrum are characteristic of two oxygenated aromatic carbons that are either *ortho* or *para* relative to each other [Agrawal, 1989]. From these pieces of information, two substitution patterns in the B-ring were considered: 3,4-disubstitution (partial-structure **D**) and 2,5-disubstitution (partial-structure **E**). The proton resonating at δ_H 6.96 (H-2') and one of the protons at δ_H 6.85 (H-6') exhibited NOE correlations with H-2 and H-3_{ax} (in the C-ring of the flavanone derivative). This would not be consistent with the partial-structure **E**, but rather consistent with partial-structure **D** [Agrawal, 1989]. Thus, the signals at δ_H 6.96 and δ_H 6.85 in the ^1H NMR spectrum, were respectively assigned to H-1' and H-5'/6', respectively, in partial-structure **D**. In gHMBC experiment, interactions from H-6' to C-2 (δ_C 79.7) further supported the proposition.

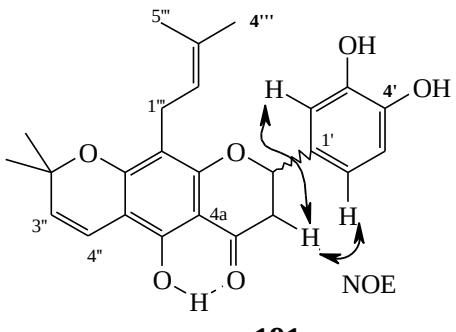
The presence of 2,2-dimethylpyrano ring [δ_H 6.57 (1H, d, *J* = 10.0 Hz, H-4"); 5.60 (1H, d, *J* = 10.0 Hz, H-3"); 1.42 (3H, s, 2"-CH_3) and 1.41 (3H, s, 2"-CH_3)]; and a γ,γ-dimethylallyl moiety [δ_H 5.13 (1H, t, *J* = 7.3 Hz, H-2'"); 3.17 (2H, d, *J* = 7.4 Hz, H-1'"); 1.65 (3H, d, *J* = 1.3 Hz, H-5'") and 1.64 (3H, d, *J* = 1.4 Hz, H-4'")] were evident from ^1H NMR spectrum.

The location of the 2,2-dimethylpyrano ring at C-6/7 was established on the basis of HMBC correlation from the olefinic proton at δ_H 6.57 (H-4") of the pyrano ring to C-5 (δ_C 157.1) and C-7 (δ_C 160.2) in conjunction with a correlation between the 5-OH and C-5. Thereby implying that the γ,γ-dimethylallyl chain is located at C-8. This compound was therefore

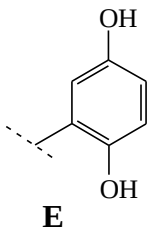
characterized as 5,3',4'-trihydroxy-8-γ,γ-dimethylallyl-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**191**) and is new.

Table 32: ^{1}H (800 MHz) and ^{13}C (150 MHz) NMR data for compound **191** (CD_3CN)

Position	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
2	79.7	5.35, dd (12.6, 3.2)	
3ax	43.4	3.09, dd (-17.1, 12.5)	C-2, 4, 1'
eq		2.77, dd (-17.1, 3.1)	
4	198.3	-	
4a	103.4	-	
5	157.3	-	
6	103.5	-	
7	160.2	-	
8	109.2	-	
8a	160.5	-	
1'	132.1	-	-
2'	114.7	6.96, s	C-2, 4', 5'
3'	145.7	-	-
4'	145.8	-	-
5'	116.1	6.85, s	C-1', 3'
6'	119.6	6.85, s	C-2, 2'
2"	79.1	-	-
3"	127.5	5.60, d (10.0)	C-6, 2"
4"	116.0	6.57, d (10.0)	C-5, 7, 2"
2"-CH_3	28.4/28.5	1.41, s/1.42, s	C-2", 3", 2"-CH_3/C-2", 3", 2"-CH_3
1'"	22.1,	3.17, d (7.4)	C-8, 2'", 3'"
2'"	123.3	5.13, t (7.3)	
3'"	132.1	-	
4'"	25.8	1.64, d (1.3)	C-2'", 3'", 5'"
5'"	18.0	1.65, d (1.4)	C-2'", 3'", 4'"
5-OH	-	12.39, s	C-4a, 5



191



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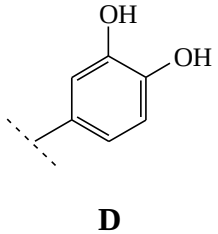
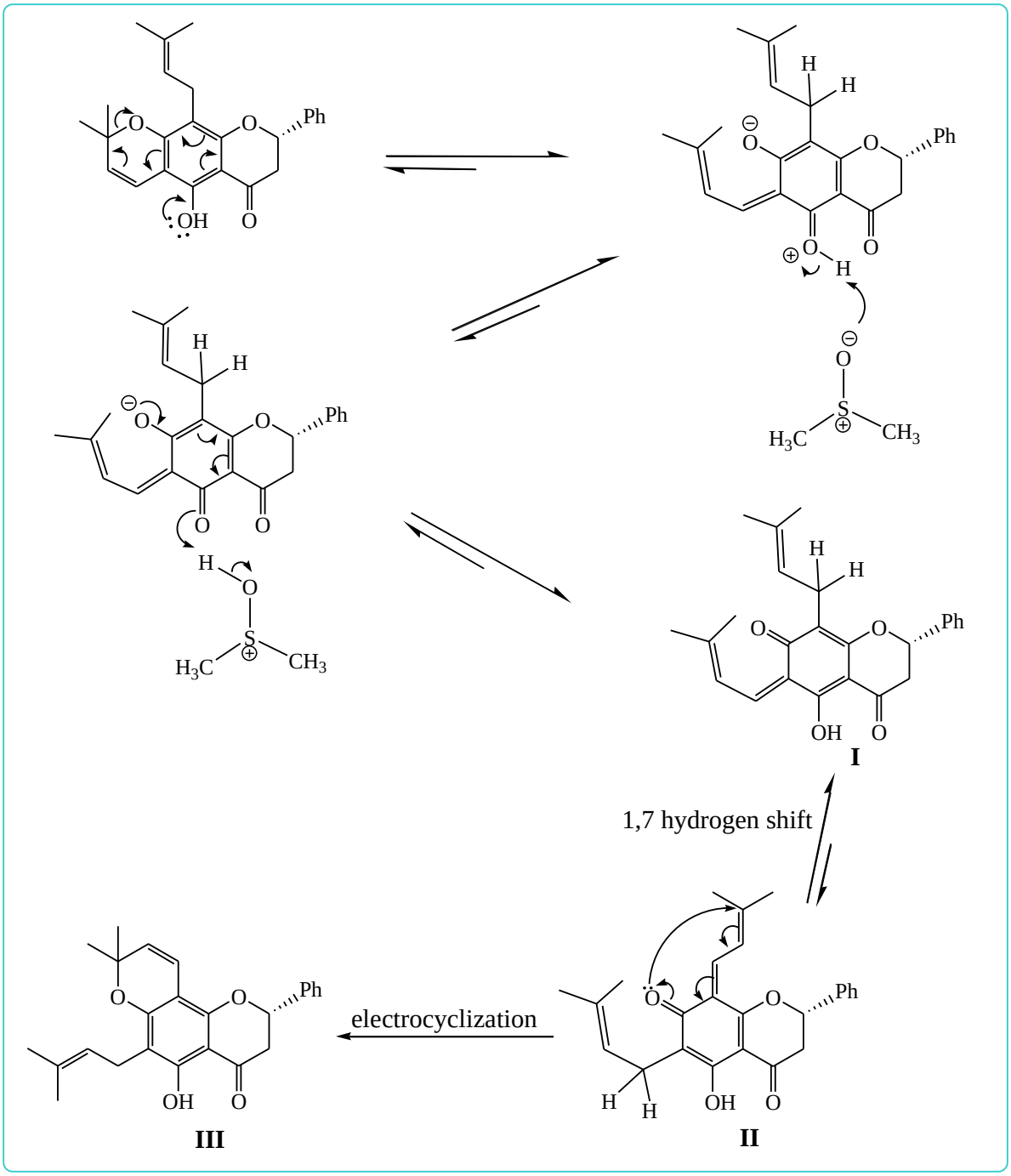


D

4.3.2.6 Proposed mechanisms for the rearrangement of 8-γ,γ-dimethylallyl-2'',2''-dimethylpyrano-[5'',6'':6,7]-flavanones

The flavanones **150**, **151** and **190** were found to rearrange to the isomeric products **189**, **151a** and **190a**, respectively, in deuterated DMSO solutions. This rearrangement is reported for the first time and proposed mechanisms are as shown in Schemes 6a, 6b and 6c.

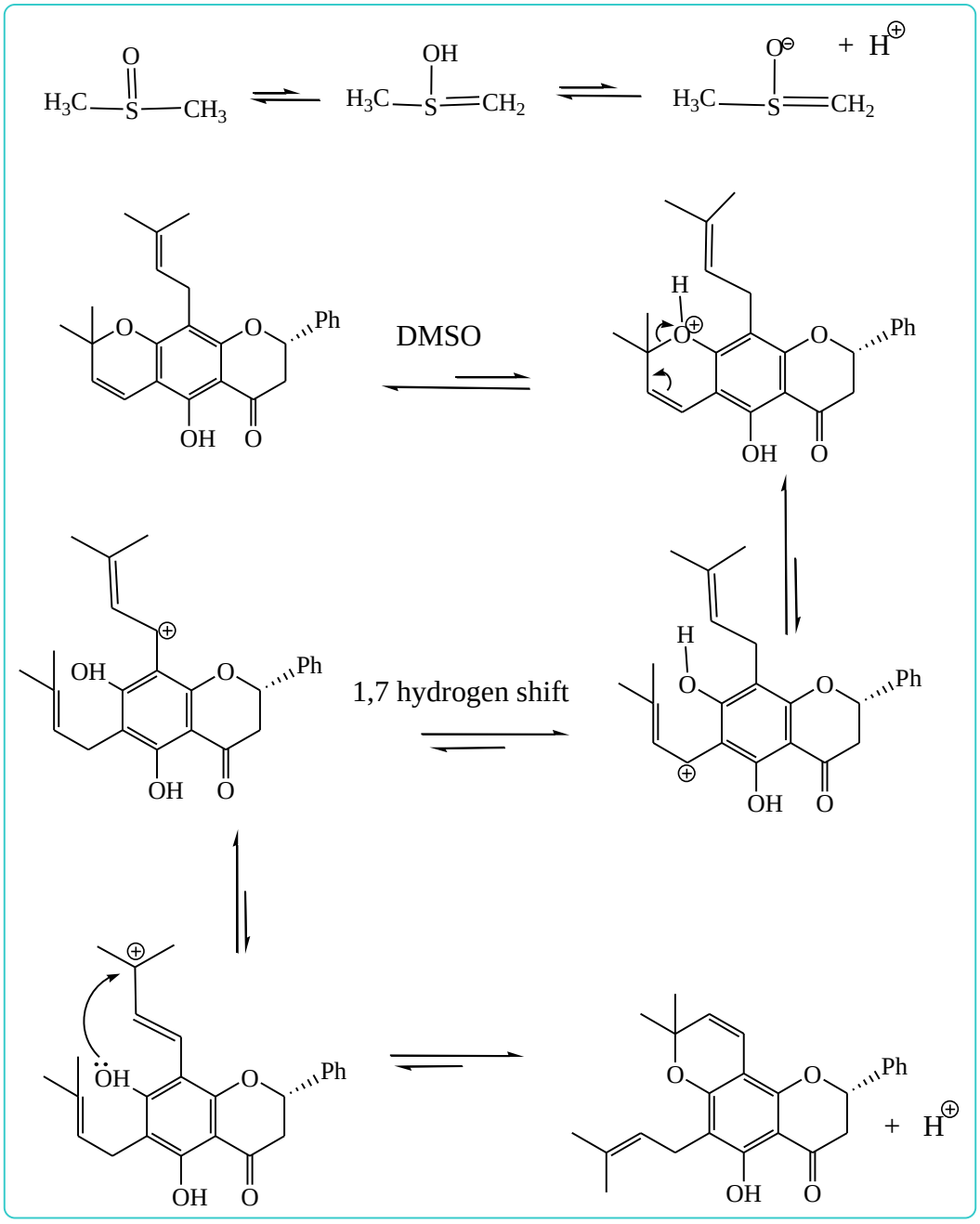
As shown in Scheme 6a, it appears that one of the lone pairs of electrons on the 5-OH oxygen is involved in the electron-resonance of the A-ring conjugated with the pyrano ring. This results in the opening of the pyrano ring and creation of a negative charge on the C-7 oxygen atom and a positive charge on the C-5 oxygen. This is, apparently, followed by the abstraction of the 5-OH proton by negatively charged oxygen of DMSO. This leads to formation of a 4,7-dienone derivative (**I**) which, after 1,7 hydrogen shift [Jiao and Schleyer, 1993; Hoeger and Okamura, 1985], is transformed into compound **II**. This is followed by electrocyclization [Wyatt and Warren, 2007] involving the C-8 prenyl group and the C=O at C-7, with the corresponding formation of 6-γ,γ-dimethylallyl-2''',2'''-dimethylpyrano-[5''',6''':8,7]-flavanone derivative (**III**). The process seems to be reversible.



Scheme 6a: Proposed mechanism for the rearrangement of compounds **150**, **151**, **190** in DMSO.

Alternatively, dimethyl sulfoxide would be considered to undergo “enolization” leading to release of protons which attack the oxygen of the pyrano ring (Scheme 6b), resulting in the opening of the ring and creation of a cation at C-1". This would be followed by a reversible 1,7 hydrogen shift from C-1'" to C-1" as in **I** which rearranges to give a relatively stable

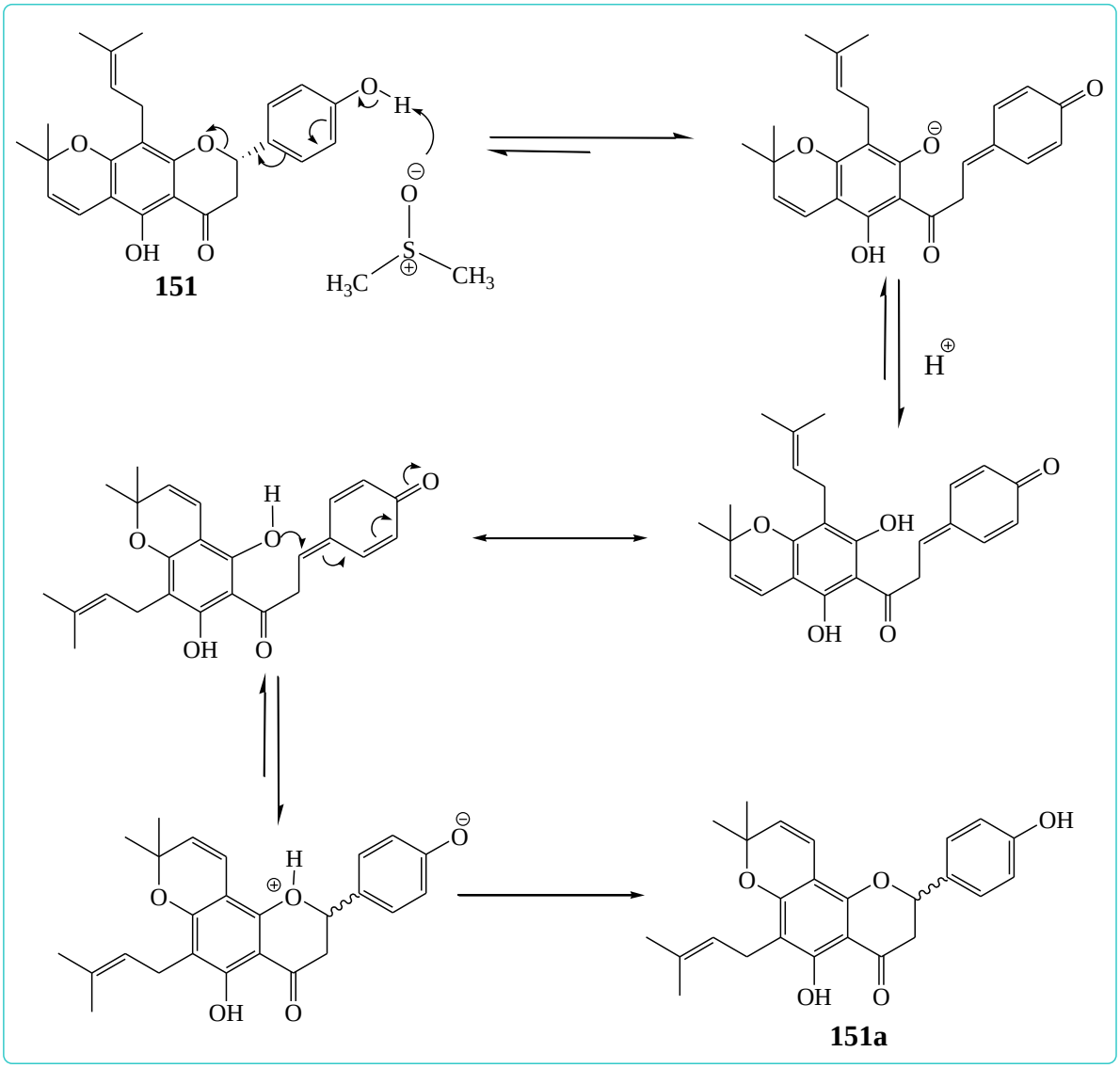
cation **II**. The OH at C-6 in **II** then neutralizes the cation leading to pyrano formation and release of a proton.



Scheme 6b: Alternative proposed mechanism for the rearrangement of compounds **150**, **151**, **190** in DMSO.

A third proposal for the isomerization is as illustrated in Scheme 6c. Thus, considering the fact that flavanones with 4'-OH and 2'-OH (or 4'-OH) groups undergo racemization [Yáñez *et*

al., 2007], it appears that the negatively charged oxygen of DMSO catalyses the racemization process by the abstracting the 4'/2'-OH proton with the resultant opening of the C-ring. This allows free rotation for the bond between C-4 and C-4a and hence the possibility for the interchange of the parental C-5 with C-8a oxygenated positions. This would be followed by cyclization to form the flavanone C-ring and the stable aromatic B-ring as in isomer **151a**.



Scheme 6c: A third proposed mechanism for the rearrangement of compounds **150**, **151**, **190** in DMSO.

4.3.2.7 Eriosematin (192)

Compound **192** was isolated as a yellow solid (UV: λ_{max} 275 nm). It was considered to be a chromone derivative on the basis of ^{1}H NMR spectrum (Table 33) with signals at δ_H 7.95 (1H, d, *J* = 5.8 Hz, H-2) and 6.19 (1H, d, *J* = 5.8 Hz, H-3). Due to paucity of the sample, the carbon signals could only be indirectly determined, from both gCHSQC and gHMBC spectra without ^{13}C-trace. Thus, that the compound is a chromone derivative was supported by HMBC correlation between H-2 and a characteristic *α,β*-unsaturated carbonyl carbon resonating at δ_C 183.3.

The presence of 5-OH (δ_H 13.01) group and a γ,γ-dimethylallyl moiety [δ_H 5.16 (1H, t, *J* = 7.1 Hz, H-2"); 3.37 (1H, d, *J* = 7.1 Hz, H-1"); 1.80 (3H, *s*, H-5"); 1.66 (3H, s, H-4")] were evident from the ^{1}H NMR spectrum (Table 33). Noteworthy is that H-1" of the dimethylallyl group exhibited HMBC correlations with C-7 (δ_C 157.5) and C-8a (δ_C 155.7). This was an indication that the γ,γ-dimethylallyl moiety is located at C-8 and, therefore, allowing the placement of the dimethylpyrano ring at C-6/C-7. Thus, the compound was characterized as 5-hydroxy-8-γ,γ-dimethylallyl-2',2'-dimethylpyrano-[5',6':6,7]-chromone (**192**) with the trivial name, eriosematin, a compound previously obtained from the roots of *Eriosema tuberosum* [Ma *et al.,* 1996]. This is, however, the first report of the compound from the genus *Flemingia.*

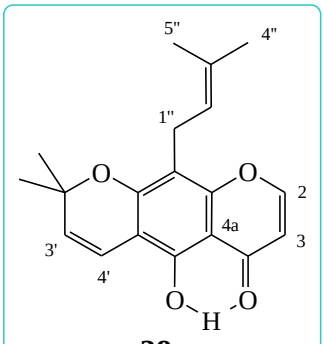


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Table 33: ^{13}C (200[a] MHz) and ^{1}H (800 MHz) NMR data for compound **192** (CD_3CN)

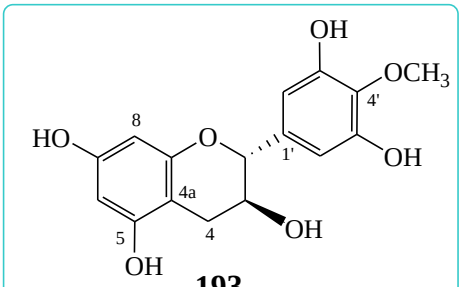
Position	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
2	157.6	7.95, d (5.8)	C-4, 8a
3	111.1	6.19, d (5.8)	C-4a
4	183.3	-	-
4a	107.0	-	-
5	155.3	-	-
6	106.3	-	-
7	157.5	-	-
8	108.5	-	-
8a	155.7	-	-
2'	78.9	-	-
3'	129.6	5.73, d (10.0)	NO
4'	115.7	6.65, d (10.0)	NO
2'-CH_3	28.2	1.44, s	C-2', 3', 2'-CH_3
	28.2	1.44, s	C-2', 3', 2'-CH_3
1"	21.4	3.37, d (7.1)	C-7, 8, 8a, 2", 3"
2"	122.6	5.16, t (7.1)	NO
3"	132.7	-	-
4"	25.6	1.66, s	C-2", 3", 5"
5"	18.0	1.80, s	C-2", 3", 4"
5-OH	-	13.03, s	C-5, 6

[a]^{13}C NMR signals was determined indirectly, from gCHSQC and gHMBC spectra.

4.3.3 4'-*O*-Methylgallocatechin (193)

Compound **193** was obtained as a white amorphous solid. The ^{1}H NMR spectrum (Table 34) gave characteristic flavan-3-ol derivative signals at δ_H 4.55 (1H, d, *J* = 7.2 Hz, H-2), δ_H 3.96 (1H, ddd, *J* = 7.3, 7.3, 5.4 Hz, H-3), 2.79 (1H, dd, *J* = -16.0, 5.2 Hz, H-4eq) and 2.51 (1H, dd, *J* = -16.0, 7.6 Hz, H-4ax) supported by ^{13}C NMR spectrum [δ_C 82.6 (C-2), 68.7 (C-3), 28.1 (C-4), 157.6 (C-5), 157.9 (C-7), 156.7 (C-8a)]. Two *meta*-coupled aromatic protons at δ_H 5.92 (*J* = 2.0 Hz) and δ_H 5.87 (*J* = 2.0 Hz) were, respectively, assigned to H-6 and H-8 of the A-ring which is oxygenated at C-5 and C-7 as expected biogenetically. A two-proton singlet [δ_H 6.41 (H-2'/6')], in ^{1}H NMR spectrum, that correlated with a high intensity carbon peak at δ_C 107.4, in the gCHSQC, and the chemical shift values for the oxygenated aromatic carbons at δ_C 151.6 (C-3/5) and 136.4 (C-4'), in the ^{13}C NMR spectrum, was consistent with a 3',4',5'-trioxygenated B-ring. The coupling constant (*J* = 7.2 Hz) between H-2 and H-3

implied a 2,3-*trans* relative orientation [Lee *et al.*, 1992]. Complete assignments were achieved with the help of gHMBCs. A methoxyl group was evident [^{1}H δ_H 3.79 (3H, s) and δ_C 60.7] in the NMR spectra. The 4'-OCH_3 substitution was deduced on the basis of the observed HMBC between the methoxyl protons with C-4' and supported by the high methoxyl carbon chemical shift value (δ_C 60.7), typical of an *ortho*-disubstituted methoxyl group [Park *et al.*, 2008]. From the above data and comparison with published information [Lee *et al.*, 1992], compound **193** was identified as 4'-*O*-methylgallocatechin which is reported for the first time from the genus *Flemingia*.



193

Table 34: ^{1}H (800 MHz) and ^{13}C (150 MHz) NMR data for compound **193** (CD_3OD)

Position	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
2	82.6	4.55, d (7.2)	C-3, 4, 8a, 1', 2'/6'
3	68.7	3.96, ddd (7.3, 7.3, 5.4)	C-4a
4ax	28.1	2.51, dd (-16.0, 7.6)	C-2, 3, 4a, 5, 8a
4eq		2.79, dd (-16.0, 5.2)	
4a	100.6	-	-
5	157.6	-	-
6	96.5	5.92, d (2.0)	C-4a, 5, 7, 8
7	157.9	-	-
8	95.7	5.87, d (2.0)	C-4a, 5, 6, 7
8a	156.7	-	-
1'	137.7	-	-
2'/6'	107.4	6.41, s	C-2, 3'/5', 4', 6'/2'
3'/5'	151.6	-	-
4'	136.4	-	-
4'-OCH_3	60.7	3.79, s	C-4'

4.4 Geranylated Chalcones, Flavanones, Aurones and an Anthraquinone from the Leaves of *Flemingia grahamiana*

The leaves of *Flemingia grahamiana* yielded fifteen chalcones including four known and eleven new ones, named as flemingins G-Q (**197**-**207**). Two *Z*-aurones were also obtained. Other compounds identified from the leaves included two new diastereomeric flavanones as well as the known anthraquinone emodin (**213**). This is the first report of emodin from *Flemingia grahamiana*, but previously reported from the roots of *F. philippinensis* [Hua *et al.*, 2009].

4.4.1 Geranylated Chalcones

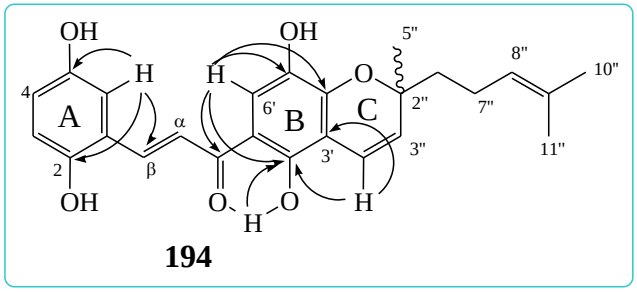
From the leaves of *Flemingia grahamiana* the following new chalcones were isolated and identified; flemingin G (**197**), H (**198**), I (**199**), J (**200**), K (**201**), L (**202**), M (**203**), N (**204**), O (**205**), P (**206**), Q (**207**) in addition to the known chalcones, flemingin C (**194**), B (**195**), A (**196**), each having a geranyl substituent modified into a chromene derivative with a residual chain or a cyclohexene derivative as in **206**, and deoxyhomoflemingin(**208**) with an open geranyl group.

4.4.1.1 Flemingin C (194)

Compound **194** was isolated as a yellow powder and identified as the known chalcone, flemingin C [Cardillo *et al.*, 1968]. The presence of the chalcone moiety was deduced from UV (λ_{max} 290, 375 400 nm) and from ^{1}H NMR data (Table 35) which exhibited a pair of doublets centred at δ 8.05 (1H, 15.6 Hz, H-β) and 7.66 (1H, 15.6 Hz, H-α) with both protons showing HMBCs with a carbonyl resonating at δ_C 193.5. The ^{1}H NMR spectrum further showed a peak at δ_H 13.51 (1H, s) which is characteristic of 2′-OH chelated to the oxygen of the chalconyl ketone.

The ^{1}H NMR and ^{1}H-^{1}H gCOSY spectra exhibited a proton multiplet at δ 7.17 correlating with a two-proton signal at δ 6.78 (m, H-3 overlapped with H-4). These spectral features were indicative of a trisubstituted aromatic ring. With the help of gHMBC spectrum the signal centred at δ 7.17 was assigned to H-6 in A-ring of the chalcone structure with C-2 (δ_C 151.2) and C-5 (δ_C 151.2) being oxygenated according to the biogenetic expectations. The presence of a geranyl moiety modified into a chromene ring with an extended side chain was

deduced from ^{1}H NMR data [δ 6.72 (1H, d, *J* = 10.0 Hz, H-4″); 5.66 (1H, d, *J* = 10.0 Hz, H-3″); δ 5.12 (1H, distorted triplet, *J* = 7.2 Hz, H-8″); δ 2.11 (2H, *br* q, *J* = 8.0 Hz, H-7″); 1.67-1.86 (2H, m, H-6″); 1.64(3H, s, H-11″); 1.55 (3H, s, H-10″) and 1.45 (3H, s, H-5″)] with the proton at δ_H 6.72 showing HMBC correlation with two quaternary oxygenated aromatic carbons (δ_C 155.7 and 149.2), one of which (δ_C 155.7, C-2′) is attached to the chelated OH as revealed from HMBC spectrum. From this piece of information it was inferred that the chromene group is fused with the B-ring *via* C-3′ and C-4′. The presence of the chromene group was further supported by the ^{13}C NMR spectrum (Table 35). A proton singlet at δ_H 7.40 that showed long range HMBC correlation with the chalconyl carbon (δ_C 193.5) implied that C-6′ was un-substituted. The structure was confirmed by gCOSY, gCHSQC and gHMBC and upon comparison with published ^{1}H NMR spectral data, the compound was identified as the known chalcone flemingin C (**194**) [Cardillo *et al.*, 1968].



194

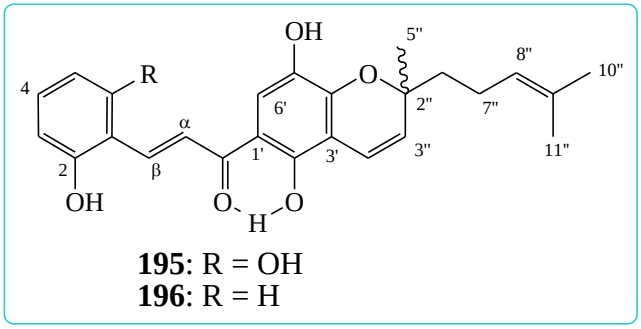
4.4.1.2 Flemingin B (195)

Compound **195** (UV λ_{max}: 350 nm) had NMR spectral features (Tables 35 and 36) similar to those of **194** (chalcone moiety, chelated OH, chromene and side chain), but differed in that the signals of the three aromatic protons in the A-ring appeared in form of an AX_2 system: δ_H 7.02 (1H, dd, *J* = 8.2, 8.2 Hz, H-4) and δ_H 6.37 (2H, d, *J* = 8.4 Hz, H-3 and H-5). The compound was identified as the known chalcone flemingin B (**195**) [Cardillo *et al.*, 1968]

4.4.1.3 Flemingin A (196)

Compound **196** was obtained as an orange powder. The ESI mass spectrum gave a *pseudo* molecular ion peak at *m/z* 407.7 $[M+H]^+$ corresponding to the formula $C_{25}H_{26}O_5$. Both the ^{1}H (Table 35) and ^{13}C (Table 36) NMR spectra of **196** gave characteristic chalcone signals

similar to those for compounds **194** and **195** except for the peaks due to a 2-hydroxylated A-ring [δ_H 7.60 (1H, dd, *J* = 7.8, 1.8 Hz, H-6); 7.32 (1H, ddd, *J* = 7.6, 7.6, 1.7 Hz, H-4); 7.00 (1H, ddd, *J* = 7.5, 7.5, 0.9 Hz, H-5); 6.86 (1H, dd, *J* = 8.4, 1.2 Hz, H-3); δ_C 155.6 (C-2), 131.7 (C-4), 130.1 (C-6)] 122.2 (C-1), 121.1 (C-5) and 116.5 (C-3. From both the above spectral data and the literature, compound **196** was identified as the known chalcone flemingin A [Cardillo *et al.*, 1968].



195: R = OH
196: R = H

Table 35: ^{1}H NMR data for compounds **194** (CD_3CN, 800 MHz), **195** (CD_3OD, 800 MHz) and **196** ($CDCl_3$, 300 MHz)

	δ_H, mult. (*J* in Hz)		
Position	**194**	**195**	**196**
3	6.78, m	6.37, d (8.4)	6.86, dd (8.4, 1.2)
4	6.78, m	7.02, dd (8.2, 8.2)	7.32, ddd (7.6, 7.6, 1.7)
5	-	6.37, d (8.4)	7.00, ddd (7.5, 7.5, 0.9)
6	7.17, m	-	7.60, dd (7.8, 1.8)
β	8.05, d (15.6)	8.37, d (15.6)	8.15, d (15.6)
α	7.66, d (15.6)	8.15, d (15.6)	7.73, d (15.6)
6′	7.40, s	7.28, s	7.40, s
3″	5.66, d (10.0)	5.62, d (10.0)	5.58, d (10.2)
4″	6.73, d (10.0)	6.75, d (10.0)	6.83, d (10.5)
5″	1.45, s	1.46, s	1.50, s
6″a	1.73, m	1.69, m	1.75, m
b	1.82, m	1.83, m	1.82, m
7″	2.11, q (8.0)	2.14, q (8.0)	2.14, q (7.8)
8″	5.12, t (7.2)	5.12, t (7.2)	5.11, t (7.2)
10″	1.64, s	1.64, d (1.2)	1.68, d (0.9)
11″	1.55, s	1.56, *br* s	1.59, *br* s
2′-OH	13.51, s	-	13.46, s

Table 36: ^{13}C NMR data for compounds **194**, **195** (CD_3CN, 150 MHz) and **196** ($CDCl_3$, 75 MHz)

Position	δ_C		
	194	**195**	**196**
1	123.3	111.2	122.2
2	151.2	159.3	155.6
3	118.1	108.4	116.5
4	120.1	132.9	131.7
5	151.2	108.4	121.1
6	115.1	159.3	130.1
β	139.9	136.5	140.0
α	121.5	123.0	121.5
C=O	193.5	194.4	192.7
1′	113.5	113.7	112.9
2′	155.7	155.6	155.2
3′	110.1	110.2	109.3
4′	149.2	148.9	147.3
5′	138.4	138.4	136.6
6′	115.6	115.2	113.8
2″	82.1	82.0	81.7
3″	128.6	128.6	126.9
4″	116.9	117.0	116.7
5″	27.2	27.3	27.2
6″	41.9	42.0	41.5
7″	23.4	23.5	22.7
8″	124.8	124.8	123.6
9″	132.7	132.7	132.2
10″	25.6	25.7	25.6
11″	17.5	17.7	17.7

4.4.1.4 Flemingin G (197)

Compound **197**, a yellow solid, was obtained as diastereomeric mixure. HRESI mass spectrum for the compound gave a pseudo-molecular ion peak at *m/z* 439.18 $[M+H]^+$, which corresponds to the molecular formula $C_{25}H_{27}O_7$ ($[M+H]^+$, calcd. 439.1757). Its NMR spectra resembled those of compounds **194**-**196** (Tables 37 and 38), and were thus indicative of a chalcone substituted with a geranyl group modified into chromene ring possessing a side chain. Its ^1H NMR spectrum exhibited a pair of doublets at δ_H 8.06 (1H, H-β) and δ_H 7.72 (1H, H-α) with the vicinal coupling constant $^3J_{HH}$ = 15.2 Hz indicative of their *trans*-relationship. The corresponding carbons C-β (δ_C 141.7) and C-α (δ_C 121.3) were identified using HSQC (Table 38). Both H-α (δ_H 7.72) and H-β (δ_H 8.06) showed HMBC correlations to the carbonyl carbon at δ_C 194.0, suggesting that they are part of an *α,β*-unsaturated ketone

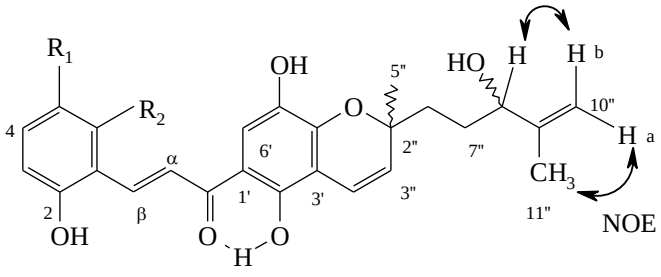
moiety of a chalcone. The ^{1}H NMR signals of the A-ring protons of **197** gave a two proton multiplet at δ_H 6.74 arising from H-3 and H-4, and a doublet of a doublets ($^{4}J_{HH}$; $^{5}J_{HH}$ =1.8, 1.8 Hz) at δ_H 7.03 (1H, H-6), the spin system of which was confirmed by COSY. The assignment was supported by the HMBC cross peaks of H-6 to C-β ($^{3}J_{CH}$ to δ_C 141.7), C-2 ($^{3}J_{CH}$ to δ_C 152.3) and C-4 ($^{3}J_{CH}$ to δ_C 120.5). The HMBC cross peaks H-β to C-2 ($^{3}J_{CH}$ to δ_C 152.3) and C-6 ($^{3}J_{CH}$ to δ_C 115.6) provided further confirmation of the nature of the A-ring. The singlet at δ_H 7.33 (1H) was assigned to H-6′, in the B-ring, based on NOE between this signal and H-α (δ_H 7.72).

The substitution pattern of the B-ring, with a chromene at C-3′ and C-4′ formed by cyclization of a geranyl group, and two hydroxyl groups at C-2′ and C-5′, is similar to that of compounds **194-196** [Cardillo *et al.,* 1968]; the only exception being the nature of the C-2″ substituent. The assignment of the one-proton singlet at δ_H 7.33 to H-6′ was confirmed by its HMBC correlations to three quaternary aromatic carbons, C-2′ ($^{3}J_{CH}$ to δ_C 155.7), C-4′ ($^{3}J_{CH}$ to δ_C 150.1), and C-5′ ($^{2}J_{CH}$ to δ_C 138.9), and the carbonyl carbon, C=O ($^{3}J_{CH}$ to δ_C 194.0). The low chemical shift of C-5′ is attributed to the shielding effect of oxygenation at C-2′ and C-4′. Duplicate signals of two *ortho* olefinic protons at δ_H 5.63/5.63 (1H, d, J = 10.1/10.4 Hz) and δ_H 6.76/6.76 (1H, d, J = 10.1/10.4 Hz) were attributed to H-3″ and H-4″, respectively, of the chromene ring, whose placement at C-3′/C-4′ was defined by the HMBC cross peaks H-3″ to C-3′ ($^{3}J_{CH}$ to δ_C 110.7) , and H-4″ to C-2′ ($^{2}J_{CH}$ to δ_C 155.7) and C-4′ ($^{3}J_{CH}$ to δ_C 150.1). The deduction that the side chain of compound **197** has a terminal double bond at C-9″ (δ_C 148.6) and a hydroxyl group at C-8″ (δ_C 76.5/76.6) was based on the multiplet at δ_H 3.99-4.01 (1H, m) attributed to H-8″, the signals at δ_H 4.80 (1H) and δ_H 4.91 (1H) due to the H-10″a (1H, dd, J = 5.4 and 1.0 Hz) and 10″b (1H, dd, J = 5.4 and 1.4 Hz) methylene protons of the terminal double bond, and the allylic methyl signal at δ_H 1.68 (3H, dd, J = 1.4 and 1.0 Hz, H-11″). The latter proton signal showed HMBC correlations to C-8″ ($^{3}J_{CH}$ to δ_C 76.6/76.5), C-10″ ($^{3}J_{CH}$ to δ_C 111.4/111.7) and C-9″ ($^{2}J_{CH}$ to δ_C 148.6). Moreover, H-6″a/b (δ_H 1.63/1.78, 1H and 1.78/1.89, 1H) exhibited $^{3}J_{CH}$ correlations to C-3″ (δ_C 128.2) and C-5″ (δ_C 27.4/27.6), indicating that C-2″ is the position of attachment of the substituent in the chromene ring. Assignment of the 10″a and 10″b protons (δ_H 4.80, 1H and 4.91, 1H) was based on the NOE observed between H-10″a and CH_3-11″ (δ_H 1.68, 3H), as well as

between H-10″b (δ_H 4.91, 1H) and H-8″ (δ_H 3.99/4.01, 1H). The signal duplications, observed in the ^1H and ^{13}C NMR spectra, for positions 3″, 4″, 6″ and 8″ indicate that compound **197** was isolated as a diastereomeric mixture, with its stereocenters being at C-2″ and C-8″. This new compound was therefore characterized as 2,5,2′,5′-tetrahydroxy-2″-(3-hydroxy-4-methylpent-4-enyl)-2-methylpyrano-[5″,6″:3′,4′]-chalcone and was assigned the trivial name flemingin G.

4.4.1.5 Flemingin H (198)

Compound **198**, whose UV absorption spectrum gave λ_{max} (MeOH) at 170 and 365 nm, was also identified as a diastereomeric mixture. It exhibited NMR spectral properties (Tables 37 and 38) that were very similar to those of **197** except for the presence of an AX_2 spin pattern [δ_H 7.02 (1H, dd, *J* = 8.2, 8.2 Hz, H-4), 6.37 (2H, d, *J* = 8.2 Hz, H-3/5)], in the ^1H NMR spectrum, due to an A-ring that is oxygenated at C-2 and C-6. The molecular ion peak at *m/z* 439.1866 $[M+H]^+$ in the HRESI-MS of the compound, was consistent with a molecular formula $C_{25}H_{26}O_7$. Based on the above spectral data this compound was characterized as 2,6,2′,5′-tetrahydroxy-2″-(3-hydroxy-4-methylpent-4-enyl)-2-methylpyrano-[5″,6″:3′,4′]-chalcone which is new and was given the trivial name flemingin H.



197: R1 = OH; R_2 = H

198: R_1 = H; R_2 = OH

Table 37: ^{1}H (400 MHz) and ^{13}C (150 MHz) NMR data for compounds **197** and **198** (CD_3OD)

Position	**197**		**198**	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
1	123.5	-	111.5	-
2	152.3	-	160.6	-
3	117.7	6.74, m	107.8	6.37, d (8.4)
4	120.5	6.74, m	132.9	7.02, dd (8.2, 8.2)
5	151.3	-	107.8	6.37, d (8.4)
6	115.6	7.03, dd (1.8, 1.8)	160.6	-
β	141.7	8.06, d (15.2)	138.0	8.37, d (15.6)
α	121.3	7.72, d (15.2)	122.4	8.14, d (15.6)
C=O	194.0	-	195.2	-
1′	113.8	-	114.1	-
2′	155.7	-	155.6	-
3′	110.7	-	110.7	-
4′	150.1	-	149.7	-
5′	138.9	-	138.8	-
6′	116.0	7.33, s	116.0	7.28, s
2″	81.7/81.8	-	81.5/81.7	-
3″	128.3	5.63/ 5.63, d (10.0/10.4)	128.2	5.62/5.62, d (10.0/10.0)
4″	118.0	6.76/6.76, d (10.4/10.0)	117.8	6.76/6.76, d (10.0/10.0)
5″	27.4/27.6	1.46, s	27.4/27.5	1.46, s
6″a	38.5/38.5	1.63/1.78, m	38.5/38.5	1.63/1.77, m
b		1.78/1.89, m		1.78/1.89, m
7″a	30.2/30.3	1.66/1.71, m	30.2/30.4	1.65/1.67, m
b		1.71/1.78, m		1.72/1.74, m
8″	76.5/76.6	3.99/4.01, m	76.5/76.6	3.99/4.01, m
9″	148.6	-	148.6	-
10″a	111.4/111.7	4.80, dd (5.4, 1.0)	111.7	4.80, ddd (5.3, 1.8, 1.8)
b		4.91, dd (5.4, 1.4)		4.91, m
11″	17.5/17.7	1.68, dd (1.4, 1.0)	17.5/17.7	1.69, m

Table 38: HMBC (CD_3OD, 800 MHz) data for compounds **197** and **198**

	197	**198**
Position	HMBC (H → C)	HMBC (H → C)
3	C-1, 5	C-1, 2, 5
4	C-2, 6	C-2, 6
5	-	C-1, 2, 3
6	C-β, 2, 4	-
β	C-α, 2, 6, C=O	C-α, 2, 6, C=O
α	C-β, 1, C=O	C-1, C=O
6′	C-2′, 4′, 5′, C=O	2′, 4′, 5′, C=O
3″	C-3′, 2″	C-3′, 2″
4″	C-2′, 4′, 2″	C-2′, 4′, 2″
5″	C-2″, 3″, 6″	C-2″, 3″, 6″
6″a	C-3″, 5″	C-2″, 5″, 8″
b	C-3″, 5″	C-2″, 5″, 8″
7″a	C-6″, 8″	C-6″, 8″
b	C-6″, 8″	C-6″, 8″
8″	C-6″, 7″, 9″,10″, 11″	C-6″, 7″, 9″,10″, 11″
10″a	C-8″, 9″, 11″	C-8″, 9″, 11″
b	C-8″, 9″, 11″	C-8″, 9″, 11″
11″	C-8″, 9″, 10″	C-8″, 9″, 10″

4.4.1.6 Flemingin I (199) and Flemingin J (200)

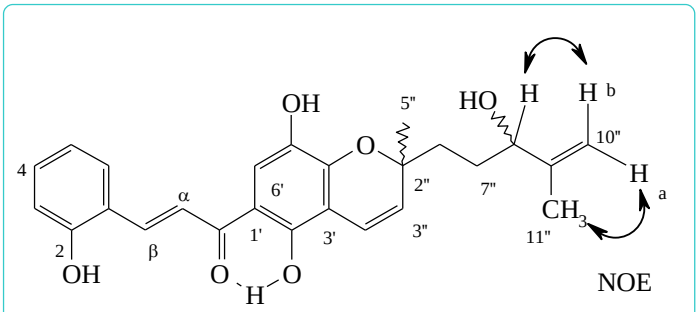
Compounds **199** (UV λ_{max}: 275 and 365 nm), and **200** were isolated as separate diastereomers, eluting as distinctive fractions in the prep-HPLC. The NMR spectra (Tables 39 and 40) for both compounds are closely related and indicated the presence of a chalcone derivative with a B-ring substitution and a chromene with a side chain identical to that in compounds **197** and **198**. However, in **199** and **200**, the A-ring is hydroxylated at C-2 just like in flemingin A (**196**).

A significant difference was noticed in the chemical shift values of H-6′ for the two diastereomers. The proton at C-6′ (in the B-ring) resonated at δ_H 7.43 and 7.49 in the ^1H NMR spectra for **199** and **200**, respectively, yet in both cases HMBC interactions were observed between C=O and H-6′. Furthermore, H-6a and H-6b resonated at δ_H 1.63 and 1.68, respectively, for **199** as compared to δ_H 1.53 and 1.76 in compound **200**. These observations are a clear revelation that **199** and **200** are diastereomers of the same compound and not regioisomers.

Table 39: ^{1}H ($(CD_3)_2SO$, 800 MHz) and ^{13}C (150 MHz) NMR spectral data for diastereomers **199** and **200**

	199		**200**	
Position	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
1	121.3	-	121.3	-
2	157.4	-	157.4	-
3	116.3	6.93, d (8.0)	116.3	6.95, d (8.0)
4	132.2	7.27, dd (8.0, 7.2)	132.2	7.28, dd (8.0, 7.2)
5	119.5	6.87, dd (8.0, 7.2)	119.5	6.88, dd (8.0, 7.2)
6	129.3	7.83, d (8.0)	129.3	7.84, d (8.0)
β	139.6	8.06, d (15.2)	139.6	8.07, d (15.2)
α	119.8	7.79, d (15.2)	119.8	7.80, d (15.2)
C=O	192.0	-	192.0	-
1′	112.1	-	112.1	-
2′	153.8	-	153.8	-
3′	108.9	-	109.0	-
4′	149.0	-	148.9	-
5′	137.9	-	137.9	-
6′	115.4	7.43, s	115.4	7.49, s
2″	80.2	-	80.1	-
3″	127.8	5.68, d (9.6)	127.9	5.70, d (9.6)
4″	115.9	6.63, d (9.6)	115.9	6.65, d (9.6)
5″	26.7	1.38, s	26.6	1.39, s
6″a/b	36.9	1.63, m/1.68, m	36.9	1.53, m/1.76, m
7″a/b	29.0	1.22, m/1.50, m	29.0	1.24, m/1.53, m
8″	73.7	3.85, m	73.8	3.85, m
9″	147.9	-	147.8	-
10″a/b	110.1	4.70, m/4.83, m	110.2	4.72, m/4.85, m
11″	17.6	1.59, s	17.5	1.60, m
2-OH	-	10.34, s	-	10.35, s
2′-OH	-	13.54, s	-	13.55, s
5′-OH	-	8.70, s	-	8.72, s
8″-OH	-	10.19, s	-	10.2, s

The HR-ESI mass spectra of **199** and **200** gave a molecular ion peak at *m/z* 423.1821 and 423.1924, $[M+H]^+$ respectively, both being in conformity with the molecular formula $C_{25}H_{26}O_6$. Complete assignments were achieved with the help of gCOSY, gCHSQC, gHMBC and NOESY. The CD spectrum for the two compounds did not show significant cotton effect which was an indication that each was a racemate. From the above data **199** and **200** were characterized as different diastereomers of 2,2′,5′-trihydroxy-2″-(3-hydroxy-4-methylpent-4-enyl)-2″-methylpyrano-[5″,6″:3′,4′]-chalcone being new and the trivial names flemingin I and flemigin J are respectively given.



199 & **200**

Table 40: HMBC ($(CD_3)_2SO$, 800 MHz) data for diastereomers **199** and **200**

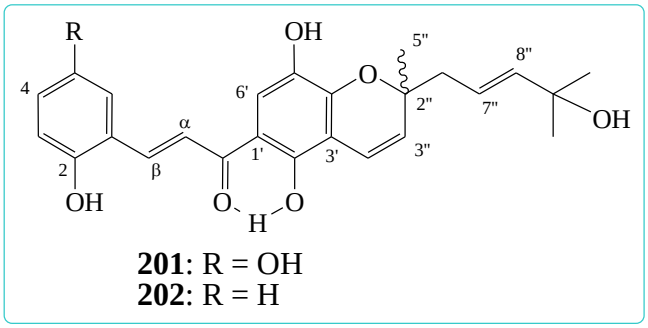
	199	**200**
Position	HMBC (H → C)	HMBC (H → C)
3	C-1, 5	C-1, 5
4	C-2, 6	C-2, 6
5	C-1, 3	C-1, 3
6	C-β, 2, 4	C-β, 2, 4
β	C-α, 2, 6, C=O	C-α, 2, 6, C=O
α	C-1, C=O	C-1, C=O
6′	C-2′, 4′, 5′, C=O	C-2′, 4′, 5′, C=O
3″	C-3′, 2″	C-3′, 2″
4″	C-2′, 4′, 2″	C-2′, 4′, 2″
5″	C-2″, 3″, 6″	C-2″, 3″, 6″
6″a	C-2″, 3″, 7″, 8″	NO
b	C-2″, 3″, 7″, 8″	NO
7″a	NO	NO
b	NO	NO
8″	C-10″	NO
10″a	C-8″, 11″	C-8″, 11″
b	C-8″, 11″	C-8″, 11″
11″	C-8″, 9″, 10″	C-8″, 9″, 10″
2-OH	C-1, 2	C-1, 2
2′-OH	C-1′, 2′, 3′	C-1′, 2′, 3′
5′-OH	C-4′, 5′, 6′	C-4′, 5′, 6′
8″-OH	NO	NO

4.4.1.7 Flemingin K (201)

Compound **201** was obtained as a yellow solid and was assigned the molecular formula $C_{25}H_{26}O_7$ based on HRMS (ESI) analysis ($C_{25}H_{27}O_7$, $[M+H]^+$ *m/z* obs. 439.1733, calcd. 439.1757). It was identified as being a chalcone derivative with NMR spectroscopic features (Table 41) highly similar to those of compound **197**, but with an aliphatic chain attached to its C-2″ position, isomeric to that of **197**, and thus carrying a hydroxyl group at C-9″ and a double bond between its C-7″ and C-8″ carbons. This double bond was identified by the COSY correlations of the diastereotopic protons δ_H 2.42 (1H, m, H-6″a), and 2.49 (1H, m, H-6″b) with the olefinic protons resonating at δ_H 5.68 (2H, m, H-7″ and H-8″, Table 41). The assignment was further supported by the HMBC correlation of H-6″ (δ_H 2.42 and 2.49) and the olefinic carbon at δ_C 121.8 (C-7″), and of the two methyl groups CH_3-10″ and CH_3-11″ (δ_H 1.16, 3H and δ_H 1.17, 3H) to the sp^2 methine carbon at δ_C 143.4 ($^3J_{CH}$ to C-8″) as well as to an sp^3 oxygenated quaternary carbon at δ_C 71.2 ($^2J_{CH}$ to C-9″,). As the $^3J_{HH}$ was not readable due to the identical chemical shift of H-7″ and H-8″ in CD_3OD, the proposed *E*-configuration of the C-7″ - C-8″ double bond was based on the chemical shift of its carbons (predicted values for *E*: $\delta_{C\text{-}8''}$ 142 and $\delta_{C\text{-}7''}$ 124, and for *Z*: $\delta_{C\text{-}8''}$ 137 and $\delta_{C\text{-}7''}$ 120; observed $\delta_{C\text{-}8''}$ 143.4 and $\delta_{C\text{-}7''}$ 121.8) [Prokofev *et al.*, 1980]. The configuration is in good agreement with biogenetic considerations [Dewick, 2002]. The compound was therefore characterized as 2,5,2′,5′-tetrahydroxy-2″-((*E*)-4-hydroxy-4-methylpent-2-enyl)-2″-methylpyrano-[5″,6″:3′,4′]-chalcone (**201**), with the suggested trivial name flemingin K.

4.4.1.8 Flemingin L (202)

Compound **202** was obtained as a yellow solid whose HRESI-MS gave an $[M+H]^+$ peak at *m/z* 423.1881 corresponding to the molecular formula $C_{25}H_{26}O_6$. The ^1H NMR spectral data (Table 41) for the compound reflected the presence of a chalcone derivative.



201: R = OH
202: R = H

Table 41: ^{1}H and ^{13}C (150 MHz) NMR data for compounds **201** (CD_3OD) and **202**

	201		**202**		
Position	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	
		(800 MHz)	($(CD_3)_2SO$)	($(CD_3)_2SO$, 800 MHz)	(CD_3OD, 400 MHz)
1	123.5	-	121.3	-	-
2	152.3	-	157.0[a]	-	-
3	118.0	6.74, m	116.1	6.94, dd (8.0, 1.6)	6.88, m
4	120.5	6.74, m	132.2	7.28, ddd (8.0,.8.0, 1.6)	7.25, ddd (7.8, 7.8, 1.6)
5	151.4	-	119.5	6.88, ddd (8.0, 8.0, 1.6)	6.90, dd (8.0, 2.0)
6	115.7	7.03, *br* s	129.3	7.85, dd (8.0, 1.6)	7.63, dd (8.0, 2.0)
β	141.7	8.05, d (15.2)	139.6	8.07, d (15.6)	8.11, d (15.6)
α	121.3	7.72, d (15.2)	119.4	7.80, d (15.6)	7.82, d (15.6)
C=O	194.0	-	191.2	-	-
1′	113.9	-	112.1	-	-
2′	155.7	-	153.8	-	-
3′	111.0	-	110.0	-	-
4′	150.2	-	148.9	-	-
5′	138.9	-	137.9	-	-
6′	115.9	7.32, m	115.5	7.49, s	7.34, s
2″	81.5	-	79.9	-	-
3″	128.1	5.64, d (9.6)	127.4	5.68, d (10.1)	5.64, d (10.2)
4″	117.8	6.76, d (9.6)	116.3	6.65, d (10.1)	6.76, d (10.0)
5″	27.2	1.49, s	26.3	1.40, s	1.49, s
6″a	45.3	2.42, m	43.4	2.33, m	2.44, m
b		2.49, m		2.38, m	2.47, m
7″	121.8	5.68, m	119.8	5.53, ddd (15.6, 7.0, 7.0)	5.68, m
8″	143.4	5.68, m	143.3	5.62, d (15.6)	5.68, m
9″	71.2	-	68.8	-	-
9″-CH_3	29.6	1.16, s	29.8	1.07	1.16, s
	29.7	1.17, s	29.8	1.07	1.17, s
2-OH*	-	NO	-	8.69, s	NO
2′-OH	-	NO	-	13.55, s	NO
5′-OH*	-	NO	-	10.37, s	NO

[a]The chemical shift value for C-2 was determined indirectly from gHMBC.
*Assignements may be interchanged

The spectroscopic features of the B- and C-rings, as well as for the C-2″ substituent, were virtually identical to those of compound **201**, however, **202** possesses a 2-hydroxylated A-ring. As for compound **201**, the scalar coupling constant between H-7″ and H-8″ was not measurable in methanol solution of **202** due to their highly similar chemical shifts, but its magnitude determined from dimethyl sulfoxide solution (J = 15.6 Hz) was indicative of a *trans*-double bond between C-7″ and C-8″. This new compound was therefore identified as 2,2′,5′-trihydroxy-2″-((*E*)-4-hydroxy-4-methylpent-2-enyl)-2″-methylpyrano-[5″,6″:3′,4′]-chalcone, and was given the trivial name flemingin L.

Table 42: HMBC correlations for compounds **201** and **202** (800 MHz, CD_3OD)

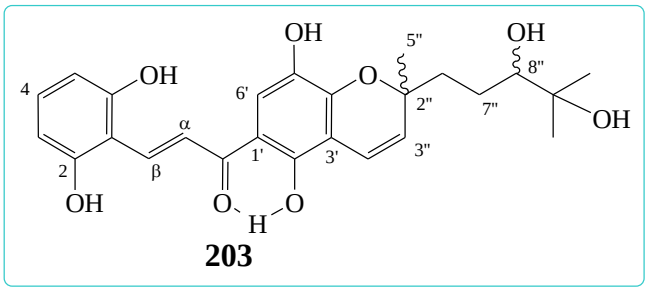
	201	**202**
Position (or H)	HMBC (H → C)	HMBC (H → C)
3	C-1, 5	C-1, 5
4	NO	C-2, 6
5	-	C-1, 3
6	C- β, 2, 4	C-β, 2, 4
β	C-α, 2, 6, C=O	C-2, 6, C=O
α	C-1, C=O, C-β	C-1, C=O
6′	C-2′, 4′, 5′, C=O	C-2′, 4′, 5′, C=O
3″	C-3′, 2″	C-3′, 2″
4″	C-2′, 4′, 2″	C-4′, 2″
5″	C-2″, 3″, 6″	C-2″, 3″, 6″
6″a	C-2″, 7″, 8″	C-7″, 8″
b	C-2″, 7″, 8″	C-7″, 8″
7″	C-6″, 9″	C-6″, 9″
8″	C-6″, 9″	C-6″, 9″
9″-CH_3	C-8″, 9″, 9″-CH_3′	C-8″, 9″, 9-CH_3′
9″-CH_3′	C-8″, 9″, 9″-CH_3	C-8″, 9″, 9″-CH_3
2′-OH	NO	NO

NO = Not Observed

4.4.1.9 Flemingin M (203)

Compound **203**, also a yellow solid, was isolated as a mixture of diastereomers, as indicated by the duplication of some of its ^{1}H NMR signals (e.g. CH_3-10″ and CH_3-11′, Table 43). The AX_2 spin pattern of its A-ring (δ_H 7.02, 1H, dd, H-4, and δ_H 6.37, 2H, d, H-3/5, in CD_3OD) is consistent with dioxygenation at C-2 and C-6, similar to compounds **195** and **198**. The three singlets, each integrating for three protons, at δ_H 1.11/1.12, 1.14/1.15 and 1.46/1.47 ppm of the methanol solution of **203**, correspond well to the signals of CH_3-10″, -11″and -5″of compound **201**. The ^{1}H NMR signals of the CH_2-6″ and CH_2-7″ of compound **203** at δ_H 1.24-

2.13 showed chemical shifts very similar to the corresponding protons of **197**-**200** than of **201**, suggesting a lack of unsaturation of its C-2″ substituent. The chemical shift of its H-8″ proton (δ_H 3.85) was comparable to that of **197**-**200**, indicating hydroxylation of C-8″ adjacent to a saturated, quaternary C-9″. Hence, the ^{1}H NMR of **203** indicated vicinal dihydroxy-substitution at C-8″ and C-9″ and A-C rings identical to compounds **195** and **198**.



203

Table 43: ^{1}H (400 MHz) and ^{13}C (150 MHz) NMR spectral data for compound **203** (CD_3OD)

Position	δ_C	δ_H, mult. (*J* in Hz)
1	111.3	-
2	159.3	-
3	108.2	6.37, d (8.0)
4	122.3	7.02, dd (8.2, 8.2)
5	108.2	6.37, d (8.0)
6	159.3	-
β	136.5	8.37, d (15.6)
α	122.8	8.14, d (15.6)
C=O	194.1	-
1′	N.O.	-
2′	155.5	-
3′	111.3	-
4′	149.1	-
5′	138.4	-
6′	115.3	7.28, s
2″	79.1	-
3″	128.7	5.64/5.66, d (10.4/10.0)
4″	117.1	6.76/6.77, d (10.0/10.0)
5″	27.2	1.46/1.47, s
6″a	39.5	1.67-1.90, m
b		2.01-2013, m
7″a	26.9	1.24-1.43, m
b		1.78-1.89, m
8″	79.1	3.85, m
9″	73.0	-
10″	24.7	1.11/1.12, s
11″	26.0	1.14/1.15, s

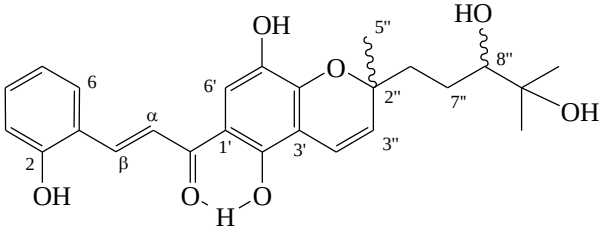
The *m/z* 457.6 $[M+H]^+$ observed by LC-ESI-MS was consistent with the molecular formula $C_{25}H_{29}O_8$, and the new compound was accordingly identified as a diastereomeric mixture of 2,6,2′,5′-tetrahydroxy-2″-(3,4-dihydroxy-4-methylpentyl)-2″-methylpyrano-[5″,6″:3′,4′]-chalcone (**203**), for which the trivial name flemingin M is proposed.

4.4.1.10 Flemingin N (204) and Flemingin O (205)

Compounds **204** and **205** (UV λ_{max}: 268 and 345 nm), were obtained as yellow solids and identified to be diastereomers of a chalcone with a chromene ring side-chain identical with that in compound **203** but with a C-2 oxygenated A-ring as in flemingin A (**196**) [Cardillo *et al*., 1968], compounds **198**, **201** and **202**.

Compounds **204** and **205** had almost superimposable ^{1}H NMR spectra (Table 44) except for the chemical shift values for H-6 and H-7 protons. Furthermore, during prep-HPLC fractionation the two fractions were eluted successively showing distinct peaks on the chromatogram.

The HRESI-MS for the two compounds **204** and **205** gave $[M+H]^+$ ion peaks at *m/z* 441.1779 and 441.1912, respectively, both of which corresponded to the molecular formula $C_{25}H_{28}O_7$. The possibility of **204** and **205** being constitutional isomers was ruled-out based on the fact that H-6′, resonating as a singlet in ^{1}H NMR spectra for both **204** and **205**, exhibited HMBC correlations with C=O, C-2′, 4′ and 5′. Furthermore when ^{1}H NMR analyses were carried out in $(CD_3)_2SO$ each spectrum gave a signal due to a chelated hydroxyl group. From the above data it was concluded that the two compounds flemingin N and flemingin O are diastereomers of 2,2′,5′-trihydroxy-2″-(3,4-dihydroxy-4-methylpentyl)-2″-methylpyrano-[5″,6″:3′,4′]-chalcone and both are new. The CD spectrum for the two compounds showed no significant cotton effect and thus indicating that each was a racemate probably at C-2″.



204 & **205**

Table 44: ^{1}H (CD_3OD, 400 MHz) for compounds **204** and **205**; ^{13}C (150 MHz) NMR and HMBC spectral data for **205** (800 MHz)

	204		205		
Position	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
1	121.3	-	123.2		-
2	157.4	-	159.0		-
3	115.9	6.94, d (8.3)	117.1	6.88, m	C-1, 5
4	132.2	7.28, ddd (8.3, 7.3, 1.6)	132.9	7.24, ddd (8.0, 8.0, 1.6)	C-2, 6
5	119.5	6.89, dd (8.0, 7.3)	120.9	6.90, m	C-1, 3
6	129.3	7.84, dd (8.0, 1.6)	131.1	7.63, dd (8.0, 2.0)	C-2, 4, β
β	139.6	8.08, d (15.6)	141.8	8.11, d (15.6)	C-2, 6, C=O
α	119.8	7.81, d (15.6)	121.5	7.82, d (15.6)	C-1, C=O
C=O	191.9	-	194.2	-	-
1′	115.3	-	113.9	-	-
2′	153.8	-	155.8	-	-
3′	109.0	-	110.8	-	-
4′	149.0	-	150.1	-	-
5′	138.0	-	139.0	-	-
6′	112.0	7.49, s	116.0	7.35, s	C-2′, 4′, 5′, C=O
2″	80.5	-	81.8	-	-
3″	128.0	5.69, d (10.1)	128.7	5.67, d (10.0)	C-3′, 2″
4″	116.3	6.65, d (10.1)	117.7	6.76, d (10.0)	C-4′, 2″
5″	29.0	1.39, s	27.2	1.47, s	C-2″, 3″, 6″
6″	38.6	1.68, m 1.95, m	39.8	1.70, m 2.12, m	NO
7″	26.7	1.23, m 1.68, m	26.7	1.42, m 1.79, m	NO
8″	77.6	3.04, dd (10.1, 6.1)	79.7	3.25, dd (10.8, 2.0)	NO
9″	71.6	-	73.9	-	-
9″-CH_3	24.4,	0.96, s	24.9	1.12, s	C-8″, 9″, 9″-CH'_3
9″-CH'_3	25.4	1.03, s	25.8	1.15, s	C-8″, 9″, 9″-CH_3

4.4.1.11 Flemingin P (206)

Compound **206** was also obtained as a yellow solid, and was identified as a chalcone derivative possessing a monooxygenated A-ring, which was revealed by the similar chemical shifts and COSY spectrum to **196**, **199**, **200**, **204** and **205**. The nature of the A-ring was further confirmed by the presence of only one carbon, C-2, in this ring with a chemical shift above 150 ppm in ^{13}C NMR (Table 45), and also by the NOE correlation of H-β (δ_H 8.06, 1H) and H-6 (δ_H 7.81, 1H).

Unlike chalcones **194**-**205**, where the C-ring has a residual side chain at C-2″, the side chain of compound **206** is fused with a methylcyclohexene ring. Thus, the ^{13}C NMR spectrum of

206 showed seven aliphatic carbons, two of them tertiary CHs at δ_C 31.0 (C-1″) and 38.7 (C-6″), two methylenes at δ_C 20.3 (C-5″) and 28.9 (C-4″), and three methyl carbons at δ_C 23.5 (C-10″), 24.9 (C-8″) and 25.2 (C-9″). This could be attributed either to a highly saturated geranyl chain or a cyclohexene ring system, the latter having been deduced from the COSY correlation of the benzylic/allylic proton δ_H 3.56 (1H, H-1″) to the olefinic proton at δ_H 6.26 (1H, H-2″), and to the aliphatic proton at δ_H 1.83 (1H, H-6″).

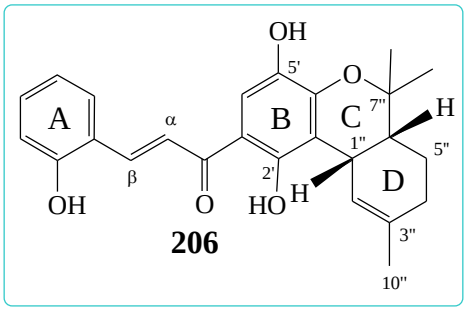
Table 45: ^{1}H ($(CD_3)_2SO$, 400 MHz), ^{13}C (150 MHz) NMR and gHMBC (800 MHz) spectral data for compound **206**

Position	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
1	121.4	-	-
2	157.3	-	-
3	116.3	6.94, dd (8.2, 1.2)	C-1, 5
4	132.1	7.28, ddd (8.2, 7.5, 1.6)	C-2, 6
5	119.5	6.88, ddd (7.9, 7.5, 1.6)	C-1, 3
6	129.3	7.84, dd (7.9, 1.6)	C-2, 4
β	139.2	8.07, d (15.6)	C-2, 6, C=O
α	120.1	7.81, d (15.6)	C-1, C=O
C=O	192.0	-	-
1′	112.7	-	-
2′	158.6	-	-
3′	111.3	-	-
4′	149.7	-	-
5′	138.8	-	-
6′	111.9	7.40, s	C-2′, 4′, 5′, C=O
1″	31.0	3.56, m	NO
2″	121.6	6.26, m	NO
3″	133.6	-	-
4″a	28.9	1.91, m	NO
b		1.98, m	NO
5″a	20.3	1.27, m	NO
b		1.92, m	NO
6″	38.7	1.83, m	NO
7″	78.1	-	-
8″	24.9	1.25, s	C-6″, 7″, 9″
9″	25.2	1.41, s	C-6″, 7″, 8″
10″	23.4	1.63, s	C-2″, 3″, 4″
2-OH	-	10.35, s	NO
2′-OH	-	14.08, s	C-1′, 2′, 3′
5-OH	-	8.47, s	NO

NO = Not observed

The presence of a cyclohexene ring in **206** was further supported by the HMBC correlations of H-10″ (δ_H 1.63, 3H) to the sp^2 carbons at δ_C 121.6 (C-2″), and 133.6 (C-3″) and to the sp^3 methylene carbon at δ_C 28.9 (C-4″), and the HMBC correlations of CH_3-8″/9″ (δ_H 1.25, 3H and δ_H 1.41, 3H) and C-6″ (δ_C 38.7).

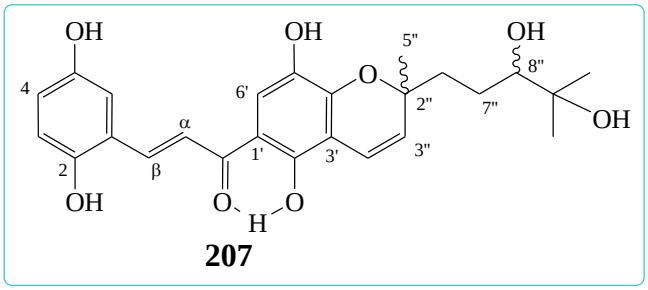
The *cis* configuration of the dihydropyran - cyclohexene ring junction was established from the NOE interaction between H-1″ (δ_H 3.56, 1H) and H-6″ (δ_H 1.83, 1H). Based on the above data, **206** was identified as (*E*)-1-((6a,10a)-1,4-dihydroxy-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6*H*-benzo[*c*]chromen-2-yl)-3-(2-hydroxyphe-nyl)prop-2-en-1-one, and was given the trivial name flemingin P.



206

4.4.1.12 Flemingin Q (207)

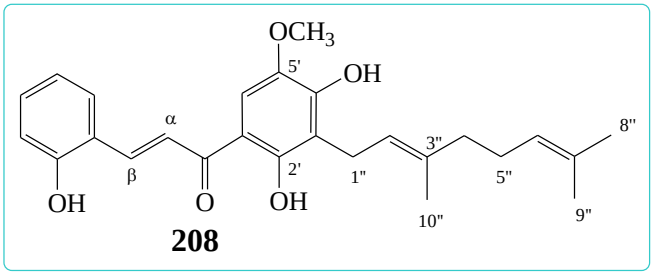
Compound **207**, obtained as a yellow solid, was also identified as a chalcone having a 2,5-dihydroxylated A-ring and a chromene ring fused to the B-ring as in compounds **194**, **197** and **201**. The ^{1}H NMR spectral data (Section 4.7) for compound **207** reflected the presence of a saturated side chain, with two vicinal dihydroxy groups at C-8″ and C-9″ [δ_H 3.25 (1H, dd, J = 10.8, 2.0 Hz, H-8″); 1.12/1.15 (6H s, 9-CH_3)], identical to compounds **203**-**205**. The compound gave a molecular ion peak at *m/z* 457.6 $[M+H]^+$ in the LC-ESI-MS correspoding to the molecular formula $C_{25}H_{28}O_8$. Based on the above data the compound was characterized as 2,5,2′,5′-tetrahydroxy-2″-(3,4-dihydroxy-4-methylpentyl)-2″-methylpyrano-[5″,6″:3′,4′]-chalcone and is reported for the first time and a trivial name flemingin Q is hereby suggested. The compound decomposed before other experiments could be carried out.



207

4.4.1.13 Deoxyhomoflemingin (208)

Compound **208** was obtained as a yellow solid with characteristic NMR features (Table 46) of a chalcone derivative having an oxygenation pattern similar to that of flemingin A (**196**) but with an open geranyl chain at C-3′. The ^{1}H NMR displayed three methyl singlets at δ_H 1.52, 1.58, 1.72; two distorted triplets at δ_H 5.02 (J = 6.8 Hz) and 5.17 (J = 7.2 Hz) which is characteristic of an open geranyl/neryl side chain. This was supported by the presence of six sp^3 carbon peaks, in the ^{13}C NMR spectrum [δ_C 16.0 (C-10″), 17.5 (C-9″), 21.6 (C-1″), 25.4 (C-8″), 26.2 (C-5″) and 39.2 (C-4″); two sp^2 methine carbon signals [δ_C 122.2 (C-2″) and 124.1 (C-6″)]; and two quaternary carbon resonances [δ_C 130.6 (C-7″) and 131.9 (C-3″)]. The chemical shift values for C-10″ (δ_C 16.0) and C-4″ (δ_C 39.2) are consistent with a geranyl rather than a neryl chain (*ca*. δ_C 25 and 32, respectively) [Blanc *et al.*, 2005]. Signals for the methoxyl group were observed in both ^{1}H (δ_H 3.85, 3H, s) and ^{13}C (δ_C 56.6) NMR spectra. It was placed at C-5′ on the basis of HMBC correlations between the methoxyl protons and C-5′. This compound was identified as the known 2,2',3'-trihydroxy-5'-methoxy-3'-geranylchalcone (**208**), trivial name deoxyhomoflemingin [Cardillo *et al.*, 1968].



208

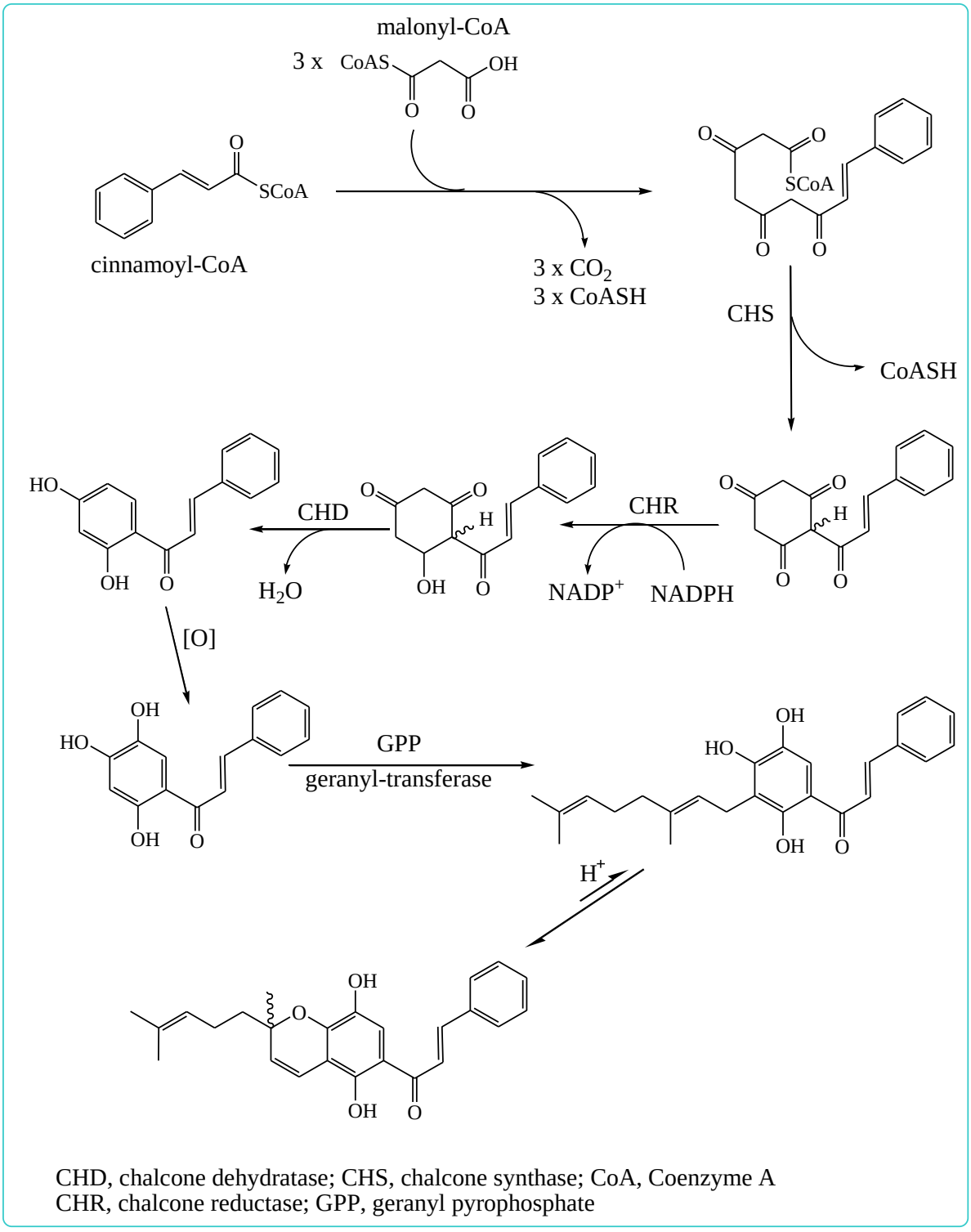
Table 46: ^{1}H (400 MHz), ^{13}C (150 MHz) NMR and gHMBC (800 MHz) spectral data for compound **208** ($(CD_3)_2SO$)

Position	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
1	121.2	-	-
2	157.0	-	-
3	116.2	6.93, d (8.0)	C-1, 5
4	131.8	7.27, ddd (8.2, 8.2, 1.6)	C-2, 6
5	119.2	6.88, dd (8.4, 8.4)	C-1, 5
6	128.5	7.95, dd (8.2, 1.6)	C-2, 4
β	138.2	8.13, d (15.2)	NO
α	119.9	7.87, d (15.6)	NO
C=O	NO	-	-
1′	118.1	-	-
2′	159.3		-
3′	115.1	-	-
4′	149.8	-	
5′	140.8	-	-
6′	109.2	7.45, s	NO
1″	21.6	3.24*	C-2′, 3′, 2″, 3″
2″	122.2	5.17, t (7.2)	C-1″, 4″, 10″
3″	131.9	-	-
4″	39.2	1.90, m	C-2″, 3″, 5″, 6″, 10″
5″	26.2	1.99, m	C-3″, 4″, 6″, 7″
6″	124.1	5.02, t (6.8)	C-8″, 9″
7″	130.6	-	-
8″	25.4	1.58, s	C-6″, 7″, 9″
9″	17.5	1.52, s	C-6″, 7″, 8″
10″	16.0	1.72, s	C-2″, 3″, 4″
5′-OCH_3	56.6	3.85, s	C-5′

*Masked by solvent; NO – Not observed

4.4.2.1 Proposed biogenesis for the flemingins

The chalcone skeleton in the flemingins appear to have been biogenetically formed from the condensation of three molecules of malonyl Coenzyme A per one molecule of cinnamoyl Coenzyme A in the presence of chalcone synthase giving a tetraketide derivative [Dewick, 2002; Veitch, 2009]. This may be followed by reduction of one of the ketone groups of the tetraketide in the presence of chalcone reductase and then loss of water in the presence of dehydratase giving 6′-deoxychalcones [Veitch, 2009]. The next step may involve an oxidation process giving a 2′,4′,5′-trihydroxychalcone derivative (Scheme 3). Geranylated chalcones are then formed in the presence of geranyl transferase and geranyl pyrophosphate. The geranyl chain can undergo further modifications such as cyclization and epoxidation (Scheme 4) [Dewick, 2002].

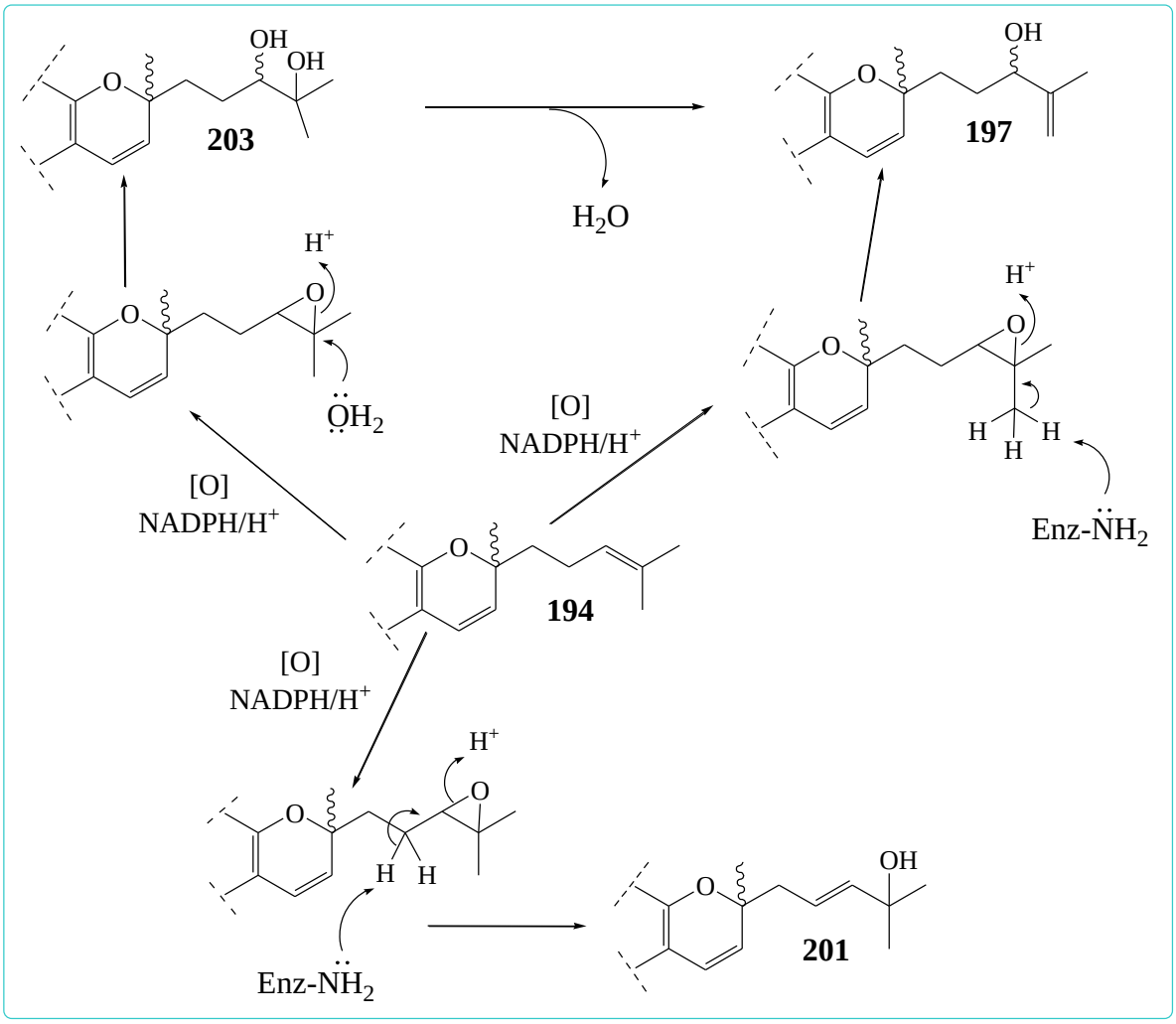


CHD, chalcone dehydratase; CHS, chalcone synthase; CoA, Coenzyme A
CHR, chalcone reductase; GPP, geranyl pyrophosphate

Scheme 7: Proposed biogenesis of flemingins

4.4.2.2 Biogenetic modifications of 2-methyl-2-(4-methylpent-3-enyl) chromene ring in flemingins

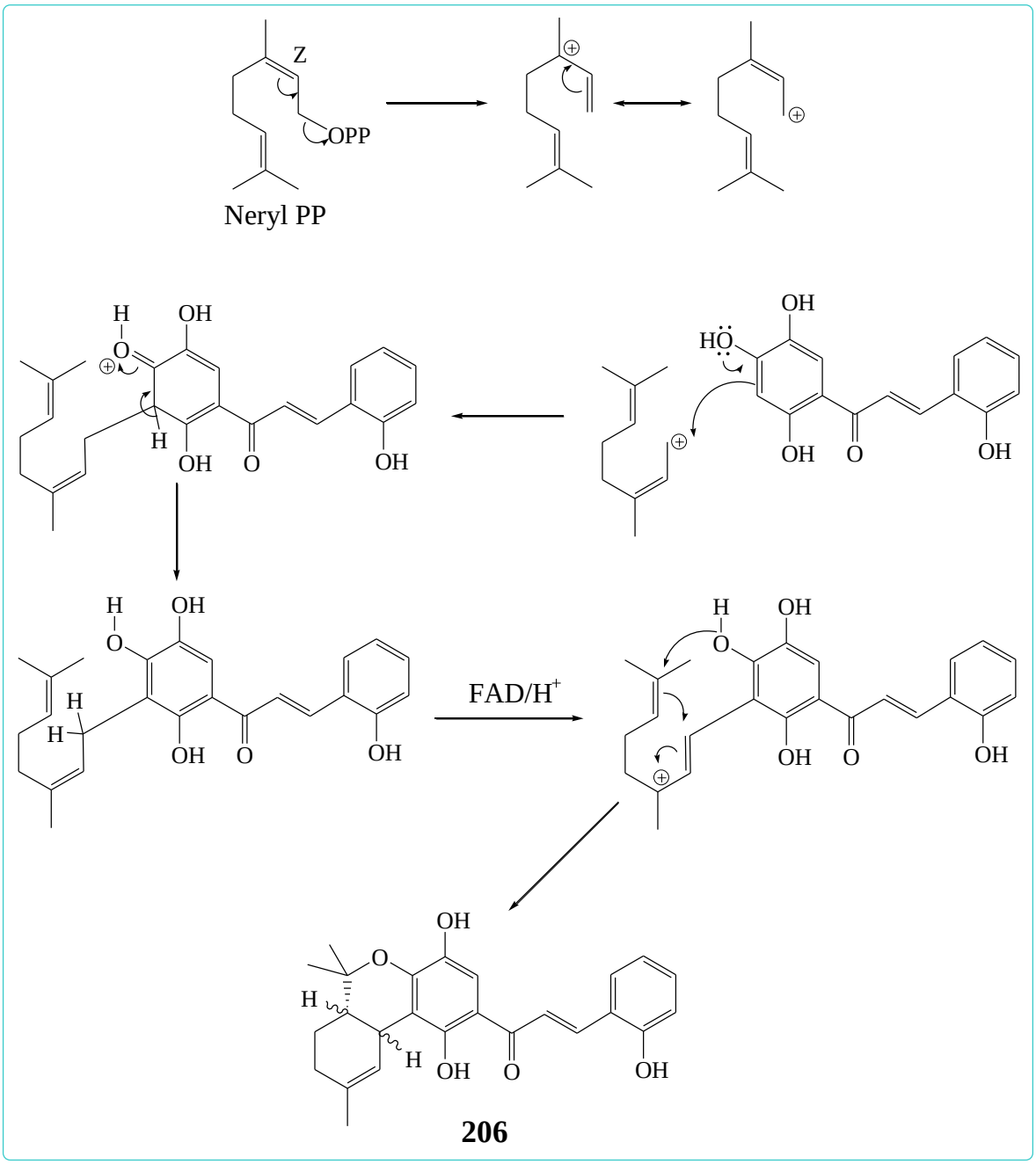
The 2-methyl-2-(4-methylpent-3-enyl) chromene ring appears to undergo epoxidation in the presence of mono-oxidase enzyme. The strained epoxide ring is then opened by either water to give vicinal dihydroxyl moiety or by the basic end of the enzyme residue leading to allylic alcohols as shown in Scheme 4 below.



Scheme 8: Biogenetic modification of 2-methyl-2-(4-methylpent-3-enyl) chromene ring in flemingins [Dewick, 2002].

4.4.2.3 Proposed biogenetic modifications of the geranyl chain resulting in flemingin P (206)

Flemingin R is thought to be resulting from oxido-cyclization of the geranyl chain initiated by FAD in the presence of an acid; similar to the biogenetic formation reported for cannabinoids in *Cannabis sativa* [Taura, *et al*., 2007].



Scheme 9: Proposed biogenesis of flemingin P (**206**)

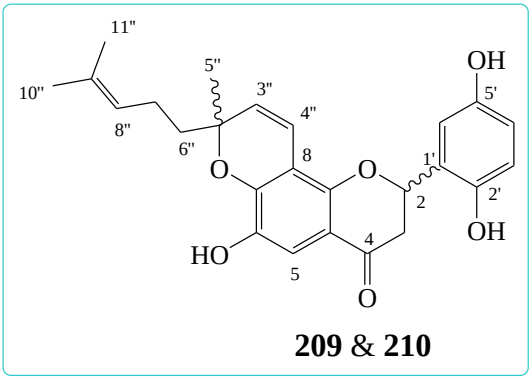
4.4.3 Flavanones

Two new diasteriomeric flavanones were isolated and identified from the leaf extract of *Flemingia grahamiana*. They have been given the trivial names fleminginflavanone A and fleminginflavanone B.

4.4.3.1 Fleminginflavanone A (209) and fleminginflavanone B (210)

Compounds **209** and **210** were identified to be diastereomers having the same flavanone skeleton. The HRESI-mass spectral analysis for compound **209** gave an $[M+H]^+$ peak at *m/z* 423.1854 corresponding to a molecular formula $C_{25}H_{26}O_6$. The ^1H NMR spectrum (Table 47) of each sample displayed an AMX spin pattern characteristic of flavanones as well as an ABX spin system of the B-ring. Furthermore, an aromatic singlet at δ_H 7.11, in the ^1H NMR spectrum for each diastereomer, was attributed to H-5 in the A-ring based on the gHMBC correlations, observed in NMR spectra for diastereomer **210**, between H-5 and C-4/6/7/8a. The presence of a 2-methyl-2-(4-methylpent-3-enyl)-pyranyl ring was evident from the NMR spectra and its placement at C-7/8 was based on HMBC experiment (Table 47).

From these pieces of data, it was concluded that samples **209** and **210** are diastereomers of 6,2′,5′-trihydroxy-2″-methyl-2″-(4-methylpent-3-enyl)-pyrano-[5″,6″:8,7]-flavanone which are new compounds and the trivial names fleminginflavanone A and fleminginflavanone B, respectively, are suggested. However, the configurations at C-2 and C-2″ have not been determined.



209 & **210**

Table 47: ^{1}H NMR for compounds **209** (CD_3CN, 400 MHz) and **210** (CD_3CN, 800 MHz); ^{13}C (150 MHz) NMR and HMBC (800 MHz) spectral data for **210** (CD_3CN).

	209	**210**		
Position		δ_C		HMBC (H → C)
2	5.65, dd (12.8, 3.2)	76.3	5.64, dd (12.5, 2.7)	NO
3ax	2.90, dd (-17.2, 13.2)	43.5	2.90, dd (-16.8, 12.8)	C-2, 4
eq	2.74, dd (-16.8, 2.8)		2.75, dd (-16.8, 2.4)	C-4
4	-	191.6	-	-
4a	-	106.9	-	-
5	7.11, s	111.4	7.11, s	C-4, 6, 7, 8a
6	-	140.7	-	-
7	-	147.9	-	-
8	-	110.5	-	-
8a	-	152.5	-	-
1′	-	127.3	-	-
2′	-	147.6	-	-
3′	6.72, d (8.8)	117.1	6.72, d (8.8)	C-1′, 2′, 5′
4′	6.66, dd (8.8, 2.8)	116.6	6.66, dd (8.8, 2.4)	C-2′
5′	-	150.9	-	-
6′	6.95, d (2.8)	114.2	6.95, d (2.4)	C-2′, 4′, 5′
2″	-	81.8	-	-
3″	5.67, d (10.4)	129.4	5.68, d (9.6)	C-8, 2″
4″	6.64, d (10.4)	116.7	6.64, d (10.4)	C-7, 2″
5″	1.45, s	27.1	1.43, s	C-2″, 3″, 6″
6″	1.71, m	41.9	1.72, m	NO
	1.80, m		1.82, m	
7″	2.11, m	23.8	2.10, m	NO
8″	5.11, t (7.0)	124.8	5.12, t (7.2)	C-10″, 11″
9″	-	132.7	-	-
10″	1.64, s	25.7	1.64, s	C-8″, 9″, 11″
11″	1.55, s	17.7	1.56, s	C-8″, 9″, 10″

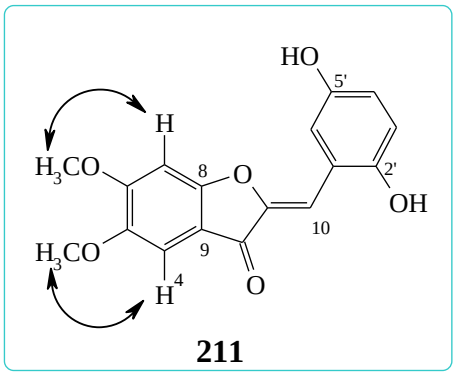
NO = Not observed

4.4.4 Aurones

Two hitherto unreported aurones were obtained from the leaves of *Flemingia grahamiana*. They include flemingiaurone A (**211**) and 5,2′,5′-trihydroxy-2″-methyl-2″-(4-methylpent-3-enyl)pyrano-[5″,6″:7,6]-aurone (**212**).

4.4.4.1 Flemingiaurone A (211)

Compound **57** was obtained as a yellow solid and identified to be an aurone derivative based on NMR spectral data (Table 48) which revealed the presence of a carbonyl at δ_C 184.5, an exocyclic olefinic methine group (CH-10) [δ_C 108.6/δ_H 7.33 (s)], together with HMBCs from H-10 to C-2 (148.2) and C-1′ (117.4). The flavone moiety was ruled out because H-10 (almost corresponding to H-3 in flavone derivatives) resonates at a lower field (δ_H 7.33) than the range observed for H-3 in flavones (δ_H 6.28-7.05) [Nascimento *et al.*, 1976; Yoon *et al*., 2011]. The chemical shift value for C-10 (δ_C 108.6) is an indication of a 2′-oxy-*Z*-aurone since the exocyclic olefinic methine carbon in *E*-aurones resonates in the range δ_H 114.7-122.2 compared to δ_H 104-112.8 in *Z*-aurones [Agrawal, 1989]. Analysis of the HRESI-MS gave a molecular ion peak at *m/z* 315.0865 $[M+H]^+$ corresponding to the molecular formula $C_{17}H_{14}O_6$. The UV spectrum showed an absorption maximum at 320 nm, supporting the presence of an aurone [Nascimento *et al.*, 1976]. The ^{1}H NMR spectrum also displayed two other singlets at δ_H 7.05 and 7.20 assigned to H-7 and H-4, respectively, on the basis of HMBC data (Table 48). Furthermore, NOESY experiment showed an interaction between H-4 and a methoxyl-group protons resonating at δ_H 3.88, allowing the placement of the CH_3O-group at C-5. Similarly, the second methoxy group was assigned to C-6 based on NOE interaction between H-7 and the methoxyl protons at δ_H 4.00. The ^{1}H NMR revealed a B-ring with an AXY spin pattern [δ_H 7.70, (1H, dd, *J* = 2.4, 0.8 Hz, H-6′); 6.74 (2H, m, H-3′ and H-4′)]. The compound was therefore identified as 2′,5′-dihydroxy-6,7-dimethoxyaurone which is a new compound named flemingiaurone A. This is the first report of an aurone from *Flemingia grahamiana* but aurones have been previously reported from *F. strobilifera* [Nigam and Saxena, 1975].



211

Table 48: ^{1}H (400 MHz) and ^{13}C (200 MHz) NMR spectral data for compound **211** (CD_3OD)

Position	$^{\#}\delta_C$	δ_H, mult. (*J* in Hz)	HMBC (H → C)
2	148.2	-	-
3	184.5	-	-
4	104.7	7.20, s	C-5, 6, 8
5	148.1	-	-
6	159.8	-	-
7	96.5	7.05, s	C-5, 6, 8, 9
8	164.7	-	-
9	113.5	-	-
10	108.6	7.33, s	C-2, 1′
1′	117.4	-	-
2′	152.3	-	-
3′	116.3	6.74, m	NO
4′	120.2	6.74, m	NO
5′	151.2	-	-
6′	117.8	7.70, dd (2.4, 0.8)	C-2′
5-OCH_3	56.6	3.88, s	C-5
6-OCH_3	56.9	4.00, s	C-6

$^{\#}$The ^{13}C NMR peaks were determined from gHMBC and gCHSQC but with no carbon traces; NO = Not observed.

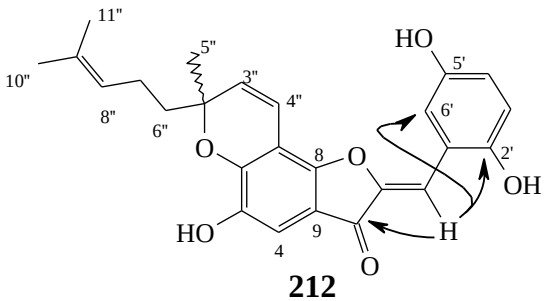
4.4.4.2 5,2′,5′-Trihydroxy-2″-methyl-2″-(4-methylpent-3-enyl)pyrano-[5″,6″:7,6]-aurone (212)

Compound **212**, was isolated as a yellow solid whose HRESI-MS gave a *pseudo*-molecular ion peak at *m/z* 421.1651 $[M+H]^+$ corresponding to the molecular formula $C_{25}H_{24}O_6$. It was identified to be an aurone based on its UV spectrum [λ_{max} (CH_3OH): 410 nm]. This was supported by the ^{1}H NMR (Table 49) spectral singlet at δ_H 7.05 which was attributed to the

characteristic exocyclic olefinic methine proton (H-10) and the ^{13}C NMR (Table 49) spectrum that showed an *α,β*-unsaturated carbonyl peak [δ_C 183.6 (C-3)], an oxygenated olefinic carbon [δ_C 148.6 (C-2)] and a methine carbon [δ_C 105.6 (C-10)] characteristic of 2′-oxy-Z-aurones [Agrawal, 1989] similar to compound **211**. That the compound is an auronoid derivative was confirmed by ^3J HMBCs from H-10 to C-3 (in the C-ring), to C-2′ (151.1) and C-6′ (117.5) (in the B-ring). The ^1H NMR spectrum furthermore displayed an AXY spin system [δ_H 7.63 (1H, d, *J* = 2.8 Hz, H-6′); 6.79 (1H, d, *J* = 8.8 Hz, H-3′) and 6.74 (1H, dd, *J* = 9.0, 3.0 Hz, H-4′)] while HMBC (Table 49) analysis revealed oxygenation at C-2′ and C-5′ in the B-ring.

Aromatic proton resonating as a singlet at δ_H 6.98, in the ^1H NMR spectrum, was assigned to H-4 of a trisubstituted A-ring due to the fact that it exhibited HMBCs to C-3 (carbonyl carbon) and three oxygenated aromatic carbons at δ_C 143.3 (C-5), 149.5 (C-6), 157.8 (C-8). From the data it was confirmed that C-5 and C-6, in addition to C-8, were oxygenated. The substitution pattern is similar to that observed in the B-ring of the flemingins and homoflemingins which were earlier described.

A chromene ring with an extended carbon chain, formed through cyclization of a geranyl group as in flemingin C (**194**), was also evident from both ^1H and ^{13}C NMR spectral data (Table 49) and was placed between C-6 and C-7 as confirmed by HMBCs from H-4″ to C-6/C-8, together with long range interactions between H-3″ and C-7. This compound was therefore elucidated as 5,2′,5′-trihydroxy-2″-methyl-2″(4-methylpent-3-enyl)pyrano-[5″,6″:7,6]-aurone (**212**) and is most likely to be an artefact formed after flemingin C (**194**) underwent oxidative-cyclization involving the H-α proton (Scheme 10), probably during fractionation.

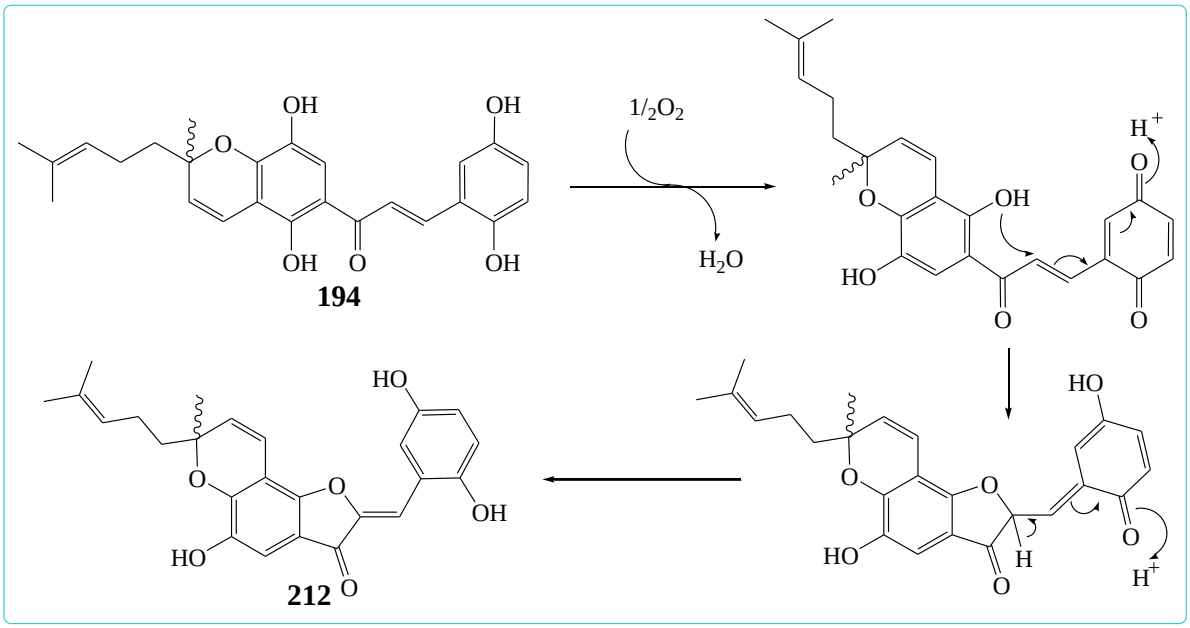


212

This is based on the fact that the peak for compound **212** in the prep-HPLC chromatogram could only appear, at a very short retention time, during purification of flemingin B whose crude NMR spectral data had been obtained prior to the purification process. It is not clear if compound **211** could also be an artifact or natural metabolite.

Table 49: ^{1}H (400 MHz) and ^{13}C (150 MHz) NMR spectral data for compound **212** (CD_3CN)

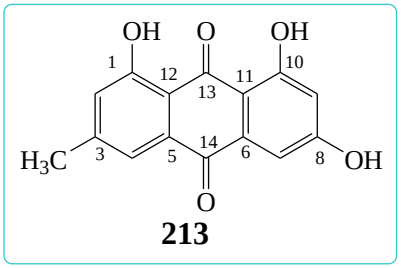
Position	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
2	148.6	-	-
3	183.6	-	-
4	108.5	6.98, s	C-3, 5, 6, 8
5	143.3	-	-
6	149.5	-	-
7	107.2	-	-
8	157.8	-	-
9	114.3	-	-
10	105.6	7.05, s	C-2, 3, 2′, 6′
1′	120.9	-	-
2′	151.1	-	-
3′	117.5	6.79, d (8.8)	C-1′, 5′
4′	119.6	6.74, dd (9.0, 3.0)	C-2′, 6′
5′	151.2	-	-
6′	117.4	7.63, d (2.8)	C-2, 2′, 4′
2″	83.0	-	-
3″	130.8	5.86, d (10.4)	C-7, 2″
4″	115.6	6.84, d (10.4)	C-6, 8, 2″
5″	23.5	1.50, s	C-2″, 3″, 6″
6″a	41.9	1.78, m	C-7″, 8″
b		1.87, m	C-2″, 3″, 7″, 8″
7″	23.5	2.16, m	
8″	124.7	5.13, t (7.0)	C-7″, 10″, 11″
9″	132.8	-	-
10″	25.7	1.63, s	C-8″, 9″, 11″
11″	17.7	1.55, s	C-8″, 9″, 10″



Scheme 10: Proposed mechanism for the formation of compound **212** from **194**.

4.4.5. Emodin (213)

Compound **213** was obtained as an orange solid. The ^{1}H NMR spectrum (Table 50) displayed two signals of hydroxyls each intra-molecularly hydrogen-bonded to a carbonyl functionality. The TLC spot of the compound turned red-brown on exposure to ammonia which was suggestive of a quinone. The ^{1}H NMR spectrum, further contained four aromatic proton signals including two doublets at δ_H 7.34 (J = 2.4 Hz) and 6.75 (J = 2.4 Hz) suggesting a *meta* interaction as shown in the coupling patterns. The rest of the signals were singlets at δ_H 7.66 and 7.23 which implied the absence of any vicinal protons. Another salient feature of the ^{1}H NMR spectrum was the presence of a three-proton singlet at δ_H 2.54 whose chemical shift suggests a methyl group attached to an aromatic system. After comparison of the spectral data with published work, it was concluded that compound **213** is, emodin [Danielsen *et al.*, 1992] the known anthraquinone having a methyl group at C-3, and three hydroxyl groups at C-1, 8 and 10 as shown in structure **213**. This is the first time an emodin is being reported from *Flemingia grahamiana* but previously obtained, together with other anthraquinones, from the roots of *F. philippinensis* [Hua *et al.*, 2009].



213

Table 50: ^{1}H ($CDCl_3$ 200 MHz) NMR spectral data for compound **213**

Position	δ_H, mult. (J in Hz)
2	6.75, d (2.4)
3-CH_3	2.54, s
4	7.66, *br* s
7	7.34, d (2.4)
9	7.23, *br* s
1-OH#	12.27, *br* s
10-OH#	12.15, *br* s

#Assignments may be interchanged.

4.5 SYNTHESIS OF CHALCONES

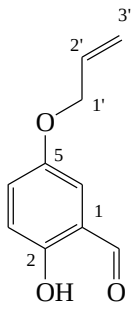
Attempts were made to synthesize flemingin analogues such as 2,5,2′-Trihydroxy-4′,5′-dimethoxychalcone from 2-Hydroxy-4,5-dimethoxyacetophenone and 2,5-Diallyloxybenzaldehyde in the presence of KOH/methanol. 2,5-Diallyloxy-2′-hydroxy-4′,5′-dimethoxychalcone was prepared which and the allyl groups deprotected using an *in-situ*-prepared $Pd(PPh_3)_4$ in the presence of K_2CO_3/methanol to yield a partially deprotected, 5-Allyloxy-2,2′-dihydroxy-4′,5′-dimethoxychalcone.

4.5.1 5-Allyloxy-2-hydroxybenzaldehyde (214) and 2,5-Diallyloxybenzaldehyde (215)

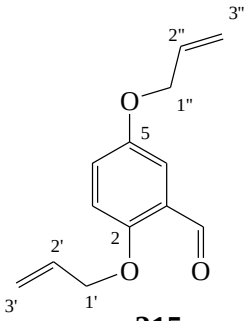
Treatment of 2,5-Dihydroxybenzaldehyde with Allyl bromide in the presence of potassium carbonate suspended in dimethylformamide at room temperature gave a mixture of 5-Allyloxy-2-hydroxybenzaldehyde (**214**) and 2,5-Diallyloxybenzaldehyde (**215**) in a ratio of 3:2 after 24 h. When the the reaction was repeated in the presence of catalytic amount of potassium iodide at 30 $^{\circ}$C, full conversion to **215** was achieved within 10 h (Scheme 3, section 3.8).

The ^{1}H NMR spectral data (Table 51) of **214** revealed a chelated OH at δ_H 9.42 together with an *O*-allyl functionality [δ_H 4.62, (2H, ddd, *J* = 5.1, 1.6, 1.6 Hz, H-1′); 6.06 (1H, ddt, *J* = 17.2, 10.5, 5.2 Hz, H-2′); 5.27 (1H, ddt, *J* = 10.5, 1.6, 1.6 Hz, H-3a′) and 5.42 (1H, ddt, *J* = 17.2, 1.7, 1.7 Hz, H-3b′)] which was placed at C-5 based on HMBCs between H-1′ and an oxygenated aromatic carbon at δ_C 154 (C-5). The 2-OH is not readily available to the protecting group because of the intramolecular hydrogen bonding the carbonyl group. The ^{1}H NMR further showed an ABC spin pattern between δ_H 7.00 and 7.10 attributed to the aromatic protons H-3, H-4 and H-6.

The ^{1}H NMR spectrum of **215** indicated the presence of two allyloxy groups with the peaks slightly overlapping (Table 52). One allyloxy group was located at C-2 and the other at C-5 as supported by gHMBC experiment (Table 52).



214



215

Table 51: ^{1}H (400 MHz) and ^{13}C (150 MHz) NMR spectral data for compound **214** **(**$(CD_3)_2SO$)

Position	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
1	125.0	-	-
2	151.2	-	-
3	115.7	7.07, m	C-1, 2
4	123.4	7.050, m	C-6
5	154.0	-	-
6	112.1	7.055, m	C=O, C-5
C=O	189.0	10.34, s	C-2
1′	69.5	4.62, ddd (5.1, 1.6, 1.6)	C-5
2′	133.4	6.06, ddt (17.2, 10.5, 5.2)	C-1′
3′a	117.5	5.27, ddt (10.5, 1.6, 1.6)	C-1′
b		5.42, ddt (17.2, 1.7, 1.7)	C-1′, 2′
2-OH	-	9.42, *br* s	NO

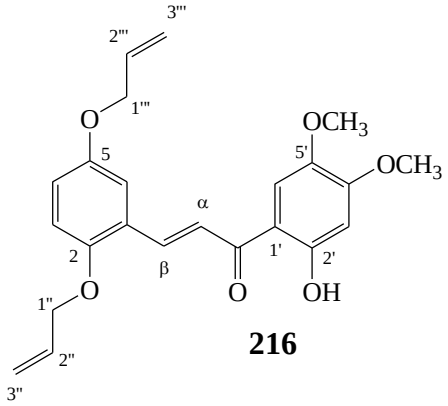
Table 52: ^{1}H ($(CD_3)_2SO$, 400 MHz) and ^{13}C (150 MHz) NMR spectral data for compound **215**

Position	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
1	124.7	-	-
2	152.1	-	-
3	115.8	7.18, d (9.1)	C-1, 5
4	123.7	7.27, dd (9.1, 3.2)	C-2, 5
5	155.2	-	-
6	111.4	7.19, d (3.2)	C=O, C-2
C=O	188.7	10.4, s	C-1, 2, 6
1′	68.7	4.56, ddd (5.2, 1.6, 1.6)	C-2, 2′, 3′
2′	133.6	6.02, ddt (17.2, 10.5, 5.2)	C-1′
3′a	117.4	5.25, ddt (10.5, 1.8, 1.8)	C-1′
b		5.38, ddt (17.3, 1.8, 1.8)	C-1′
1″	69.4	4.68, ddd (5.2, 1.6, 1.6)	C-5, 2″, 3″
2″	133.3	6.07, ddt (17.2, 10.5, 5.2)	C-1″
3″a	117.7	5.28, ddt (10.5, 1.8, 1.8)	C-1″
b		5.44, ddt (17.2, 1.8, 1.8)	C-1″

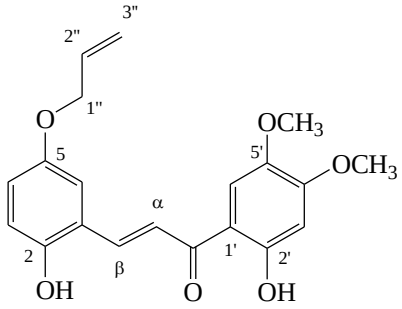
4.5.2 2,5-Diallyloxy-2′-hydroxy-4′,5′-dimethoxychalcone (216) and 5-Allyloxy-2,2′-dihydroxy-4′,5′-dimethoxychalcone (217)

2,5-Diallyloxy-2′-hydroxy-4′,5′-dimethoxychalcone (**216**) was prepared by mixing compound **215** with 2-Hydroxy-4,5-dimethoxyacetophenone, potassium hydroxide in methanol. The reaction mixture was stirred at 40 $^{\circ}$C. Attempts to deprotect **216** with the aim of obtaining 2,5,2′-Trihydroxy-4′,5′-dimethoxychalcone were not successful, instead giving 5-Allyloxy-2,2′-dihydroxy-4′,5′-dimethoxychalcone (**217**) along with several un-identified side products.

Compounds **216** and **217** were deduced to be chalcone derivatives having identical B-ring substitution pattern with a 2′-hydroxyl group chelated to the carbonyl. The ^{1}H and ^{13}C NMR spectra (Tables 53 and 54), in each case, revealed the presence of a *trans*-double bond and a carbonyl carbon characteristic of a chalcone. Unlike compound **216** with two allyloxy groups in the A-ring, compound **217** has only one allyloxy substituent that was placed at C-5, on the basis of the HMBC observed between H-1″ protons (of the allyloxy substituent) and C-5. The HRESI-MS for **216** and **217** gave molecular ion peaks at *m/z* 397.1668 $[M+H]^{+}$ and 357.1375 $[M+H]^{+}$ corresponding to molecular formulae $C_{23}H_{24}O_6$ and $C_{20}H_{20}O_6$, respectively. A noticeable difference is that in the ^{1}H NMR spectra for compound **63**, the aromatic proton signals of the A-ring appeared as a well resolved AMX spin pattern [δ_H 6.85 (1H, d, *J* = 8.9 Hz, H-3); 6.95 (1H, dd, *J* = 8.7, 3.0 Hz, H-4) and 7.53 (1H, d, *J* = 3.0 Hz, H-6)] unlike in compound **216** where the A-ring signals appeared as an ABX system [δ_H 7.06 (2H, *br* s, H-3 and H-4); 7.61 (1H, *br* s, H-6)]. The two allyloxy substituents at C-2 and C-5 in compound **216** are responsible for the almost chemical equivalence of H-3 and H-4 as opposed to the 2-OH and 5-allyloxy substituents in **217** and hence significant differences in chemical shift values for H-3, and H-4. In each case, the presence of two methoxyl groups at C-4′ and C-5′ in the B-ring were evident from ^{1}H and ^{13}C NMR spectra (Tables 53 and 54).



216



217

Table 53: ^{1}H (400 MHz) and ^{13}C (200 MHz) NMR spectral data for compound **216** $(CD_3)_2SO$)

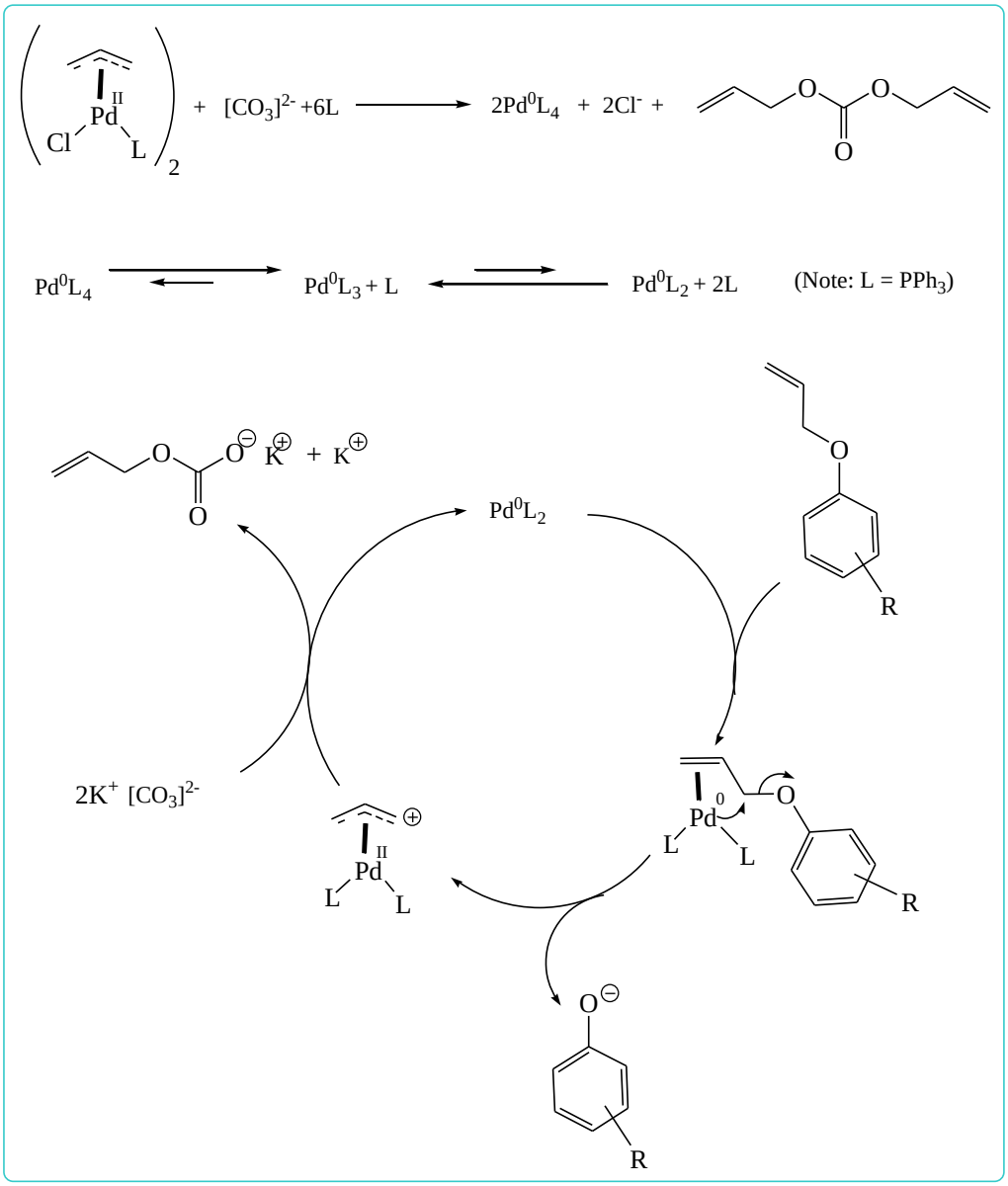
Position	δ_C	δ_H, mult. (*J* in Hz)
1	123.8	-
2	152.2	-
3	117.6	7.06, m
4	118.5	7.06, m
5	151.8	-
6	114.3	7.61, *br* s
β	138.3	8.15, d (15.8)
α	121.5	7.97, d (15.7)
C=O	191.5	-
1′	111.7	-
2′	160.8	-
3′	100.6	6.59, s
4′	157.7	-
5′	141.8	-
6′	112.2	7.69, s
1″	68.8	4.61, ddt (5.1, 1.5, 1.5)
2″	133.8	6.95, ddt (17.2, 10.5, 5.2)
3″a	117.6	5.43, ddt (11.0, 1.5, 1.5)
b		5.27, ddt (17.2, 1.5, 1.5)
1‴a	69.3	4.62, ddt (5.2, 1.5, 1.5)
2‴	133.6	6.14, ddt (17.2, 10.5, 5.2)
3‴a	117.7	5.44, ddt (17.2, 1.9, 1.9)
b		5.30, ddt (11.0, 1.7, 1.7)
2′-OH	-	13.47, *br* s
4′-OCH_3	56.0	3.85, s
5′-OCH_3	56.7	3.83, s

The electron withdrawing effect of the chalconyl group causes electron deficiency at C-2, in compound **216**, and therefore weakening the carbon-oxygen bond. This explains the preferential deprotection at C-2. The deprotection reaction could not, however, go to completion (at C-5) possibly because of the reversibility of the oxidative addition or/and the

the reductive elimination precesses (Scheme 11). Therefore a stronger base such as an amine which readily forms *N*-allyl compounds [Guibe, 1998] is required instead of the carbonate used in this case. Alternatively, other protecting groups such as methoxymethyl ether (from methoxymethyl chloride) could be used as protecting groups for the hydroxyl groups of 2,5-dihydroxybenzaldehyde which could then be cleaved by use of dimethylboron bromide.

Table 54: ^{1}H (400 MHz) and ^{13}C (200 MHz) NMR spectral data for compound **217** $(CD_3)_2SO$)

Position	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
1	121.6	-	-
2	151.9	-	-
3	117.1	6.85, d (8.9)	C-1, 5
4	119.7	6.95, dd (8.7, 3.0)	C-2, 6
5	151.1	-	-
6	113.5	7.53, d (3.0)	C-β, C-2, 4
β	139.3	8.15, d (15.5)	C=O, C-2, 6
α	120.1	7.89, d (15.6)	C=O, C-1′
C=O	191.7	-	-
1′	111.8	-	-
2′	160.1	-	-
3′	100.7	6.59, s	C-1′, 2′, 4′, 5′
4′	157.0	-	-
5′	141.8	-	-
6′	112.4	7.62, s	C=O, C-2′, 4′
1″	69.0	4.56, ddd (5.4, 1.5, 1.5)	C-5, 2″, 3″
2″	134.0	6.05, ddt (17.3, 10.3, 5.3)	NO
3″a	117.5	5.26, ddt (10.3, 1.5, 1.5)	C-1″
b		5.42, ddt (17.2, 1.5, 1.5)	C-1″, 2″
2′-OH	-	13.52, s	NO
4′-OCH_3	56.0	3.85, s	C-4′
5′-OCH_3	56.8	3.83, s	C-5′



Scheme 11: Mechanism for the deprotection of allyl phenyl ethers (**216**) [Guibé, 1998]

4.6 BIOLOGICAL ACTIVITY

The crude extracts and a selection of the isolated compounds were subjected to biological tests that included cytotoxicity against MCF-7 human breast cancer cells and Vero cells, radical scavenging activity against DPPH and anti-TB test.

4.6.1 CYTOTOXICITY

Twenty compounds were tested against MCF-7 human breast cancer cells (Table 55). The 12-Dihydro-12a-hydroxyrotenoid **176** with an open prenyl group and flemingins C (**194**) and A (**196**) exhibited potent activity against the cells. Compounds **70**, **71**, **171**, **173** and **175** were moderately active. Colenemol (**182**), genistein (**11**) and genistin (**184**) were inactive in this test. The cytotoxicity of the rotenoids has been associated with their ability to inhibit the induction of ornithine decarboxylase [Gerhäuser *et al.,* 1997a] as well as inhibition of NADH/ubiquinone oxidoreductase [Cunningham *et al.,* 1995; Fang and Casida, 1998].

Table: 55: Cytotoxicity results of selected compounds

Compound	**IC_{50} (μg/mL)**	**IC_{50} (μM)**
Isokaempferide (**171**)	21.7	72.3
Betulin (**173**)	30.0	67.9
Usararotenoid-A (**71**)	34.0	95.5
12-Dihydrousararotenoid-A (**72**)	> 100	-
Millettosin (**175**)	18.0	45.6
12a-Epimillettosin (**70**)	24.0	60.9
12-Dihydrousararotenoid-C (**176**)	3.7	9.0
4'-*O*-Geranyl-7-hydroxyflavanone (**180**)	55	140.3
4-*O*-Geranylisoliquiritigenin (**181**)	39.9	101.8
Colenemol (**182**)	> 100	
Genistein (**11**)	> 100	
Genistin (**184**)	> 100	
Flemingin C (**194**)	3.2	7.6
Flemingin B (**195**)	> 100	
Flemingin A (**196**)	3.6	8.9
Flemingin G (**197**)	> 100	
Flemingin K (**201**)	> 100	
Flemingin L (**202**)	> 100	
5,2′,5′-Trihydroxy-2″-methyl-2″-(4-methylpent-3-enyl)pyrano-[5″,6″:7,6]-aurone (**212**)	> 100	
Emodin (**213**)	85.7	317.4
1-Isopropyl-3-(pyridine-4-ylethynyl)-1*H*-pyrazolo[3,4-d]pyrimidin-4-amine*		5.0 nM

*Standard positive control.

Anticancer activity of chalcones has been associated with, among a range of mechanisms, their ability to inhibit tubulin polymerization and thus affect cellular mitosis [Dyrager *et al.,*

2011]. It seems the modified geranyl group also contributes to the cytotoxicity of the flemingins.

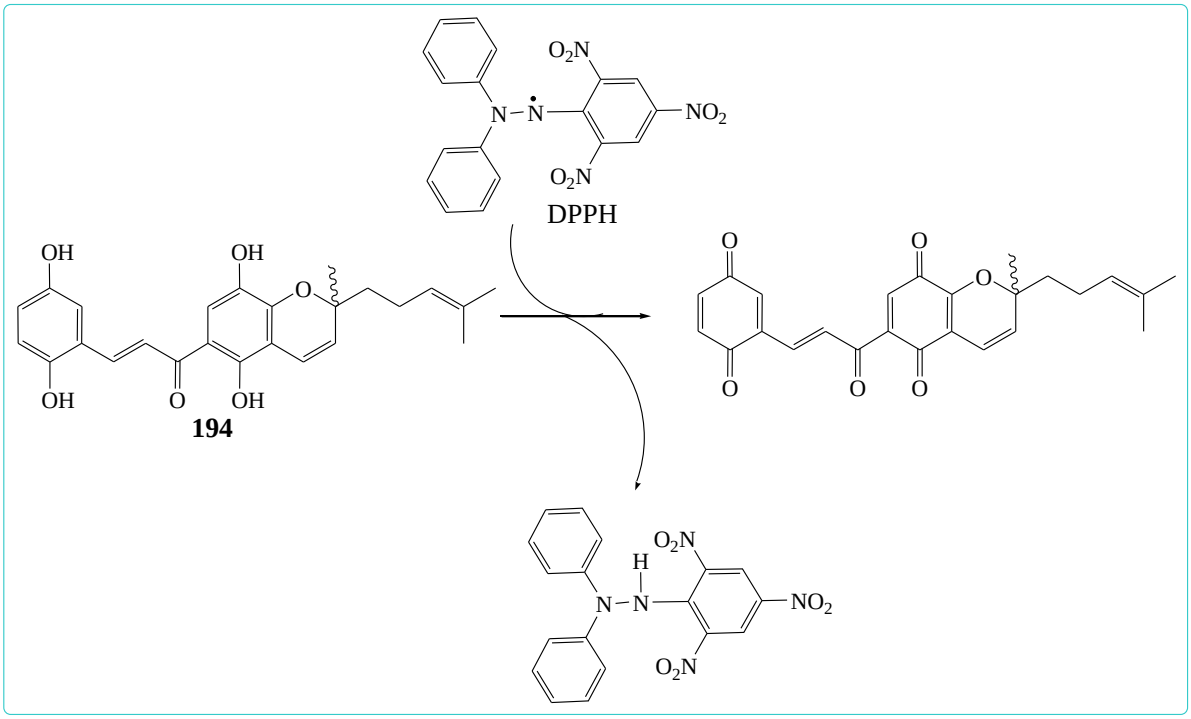
4.6.2 RADICAL SCAVENGING ACTIVITY

The crude extracts of the roots and leaves of *Flemingia grahamiana* and nine compounds were tested separately against DPPH (Table 56).

From the results it was noted that the leaf extract and the isolated chalcones have strong radical scavenging properties. It is envisaged that the 2′, 5′-*para* dihydroxylation in the B-ring is responsible for the strong radical scavenging activity of the flemingins because of a stable quinone that results after radical scavenging (Scheme 12). It is not clear why compound **196** is the most active of all the test compounds.

Table 56: Radical scavenging activity of selected compounds and crude extracts of leaves and roots of *F. grahamiana*

Sample	**ED_{50} (μg/mL)**	**ED_{50} (μM)**	**Activity Index**
4'-*O*-Geranyl-7-hydroxyflavanone (**180**)	> 106		
4-*O*-Geranylisoliquiritigenin (**181**)	> 106		
Genistein (**11**)	52.0	192.59	76.42
Genistin (**184**)	> 106		
Flemingin C (**194**)	2.50	5.92	2.35
Flemingin B (**195**)	3.28	7.77	3.08
Flemingin A (**196**)	1.80	4.43	1.76
Flemingin G (**197**)	3.65	8.33	3.30
Flemingin H (**198**)	3.90	8.90	3.53
F. grahamiana (leaves, minus chlorophyll)	5.9	-	-
F. grahamiana (roots)	42.0	-	-
Quercetin (**30**) (standard)	0.76	2.52	1.00



Scheme 12: Conversion of *para*-dihydroxychalcones into quinones by DPPH

Chalcone **181** was only mildly active because of the absence of a *para*-dihydroxylation (or an *ortho*-dihydroxylation) pattern in the B-ring and the fact that the oxygen atom at C-4 is etheric and therefore not available for radical scavenging. Similarly genistein (**11**) was weakly active and glycosylation at C-7 as in genistin (**184**) resulted in significant inactivation. This explains the role of free phenolic hydroxyls in radical scavenging activity.

4.6.3 ANTI-TB ACTIVITY

Compound **196** exhibited strong activity in the MABA test, moderate activity in the LORA test against *Mycobacterium tuberculosis* and strong cytotoxicity against Vero cells (Table 57). This reveals the potential of flemingins in the fight agaist TB as well as considering them for renal and kidney related cancers. Compound **163** was moderately active in the MABA test, weakly active in the LORA test and moderately cytotoxic against Vero cells. Compound **165** was inactive in all the tests probably because it was isolated in a racemic form and therefore the test sample also included the totally inactive enantiomer and the other enantiomer that may be active against the TB pathogen.

Table 57: Anti-TB test results of selected compounds

	MIC (%Inh at highConc)				**IC_{50}**	
	MABA		**LORA**		**Cytotoxicity (Vero Cell)**	
Compound	(μg/mL)	μM	(μg/mL)	μM	(μg/mL)	μM
163	9.1	23.7	35.4	92.2	8.1	21.1
165	> 20 (52%)	> 54.0 (52%)	> 20 (33%)	> 54.0 (33%)	18.6	50.3
169	> 50 (84%)	>136.7 (84%)	> 50 (74%)	> 136.7 (74%)	32.3	88.2
196	5.9	14.5	10.5	25.8	4.7	11.6
Rifampicin		0.06		1.8		183.8
Isoniazid		0.17		> 128(63%)		NT

NT = Not tested

4.7 Physical and Spectroscopic Properties of Compounds Isolated/Discussed

*Platyisoflavanone A (**163**)*

Amorphous powder. $[\alpha]_D^{24}$ = +14.1 (CH_2Cl_2, *c* = 1% w/v). UV λ_{max} (CH_2Cl_2): 288 nm. CD (CH_2Cl_2, *c* = 0.0047): $[\theta]_{350}$ -100, $[\theta]_{344}$ -120, $[\theta]_{332}$ 0, $[\theta]_{315}$ +490, $[\theta]_{294}$ 0, $[\theta]_{285}$ -340, $[\theta]_{255}$ 0, $[\theta]_{245}$ +50. ^{1}H NMR: (Table 9) and ^{13}C NMR: (Table 10). EIMS *m/z* (rel. int.): 384 (80, $[M]^+$), 232 (100), 217 (69), 205 (83), 189 (47), 177 (54), 152 (65),124 (95), 115 (30), 91 (32),69 (32), 51 (53), 45 (54). HREI-MS $[M]^+$: found *m/z* 384.1597 for $C_{22}H_{24}O_6$ (calcd. 384.1567)

*Platyisoflavanone B (**164**)*

Amorphous white powder. $[\alpha]_D^{24}$ = 0 (CH_2Cl_2, 1% w/v). UV λ_{max} (CH_2Cl_2): 294 nm. CD (CH_2Cl_2, *c* = 1% w/v): no Cotton effect. ^{1}H NMR: (Table 9) and ^{13}C NMR: (Table 10). EIMS *m/z* (rel. int.): 370 (92, $[M]^+$), 314 (40), 218 (100), 163 (65), 162 (70), 153 (43), 51 (32). HREI-MS $[M]^+$: found *m/z* 370.1400 for $C_{21}H_{22}O_6$ (calcd. 370.1411)

*Sophoraisoflavanone A (**165**)*

Amorphous white solid. ^{1}H and ^{13}C NMR (Tables 9 and 10), in agreement with literature [Komatsu *et al*, 1978].

*Platyisoflavanone C (**166**)*

Amorphous white solid. UV λ_{max} (CH_2Cl_2): 290 nm. $[\alpha]_D^{24}$ = 0 (CH_2Cl_2, *c* = 1% w/v). ^{1}H and ^{13}C NMR: (Table 11). EI-MS *m/z* (rel. int.): 386 (10, $[M]^+$), 328 (100), 234 (8), 201 (14), 180 (30), 176 (60), 161 (25), 83 (26), 59 (28). HREI-MS $[M]^+$: found *m/z* 386.1354 for $C_{21}H_{22}O_7$ (calcd. 386.1366).

*5,7-Dihydroxy-4'-methoxyfurano-[4",5":3',2']-isoflavanone (**167**)*

Yellowish Brown gum. ^{1}H NMR (Table 11).

*Platyisoflavanone D (**168**)*

Amorphous white solid. UV λ_{max} (CH_2Cl_2): 289 nm. ^{1}H and ^{13}C NMR: (Table 12). EI-MS *m/z* (rel. int.): 372 (60, $[M]^+$), 220 (48), 187 (45), 153 (86), 149 (46), 124 (28), 109 (21), 91 (30), 69 (50), 46 (100). HREI-MS $[M]^+$: found *m/z* 372.1202 for $C_{20}H_{20}O_7$ (calcd. 372.1209).

*7,2′-O,O-Dimethylplatyisoflavanone D (**168a**)*

Amorphous white solid. UV λ_{max} (CH_2Cl_2): 295 nm. ^1H and ^{13}C NMR (Table 12). EI-MS *m/z* (rel. int.): 400 (35, $[M]^+$), 235 (15), 234 (100), 201 (15), 163 (25), 149 (13), 91 (10). HREI-MS $[M]^+$: found *m/z* 400.1510 for $C_{22}H_{24}O_7$ (calcd. 400.1522).

*Glyasperin F (**169**)*

White amorphous solid. ^1H and ^{13}C NMR (Table 13) in agreement with literature [Zeng *et al.*, 1992].

*7,4′-O,O-Dimethylglyasperin F (**169a**)*

Amorphous white solid. ^1H and ^{13}C NMR (Table 13). EI-MS *m/z* (rel. int.): 382 (27, $[M]^+$), 368 (35), 367 (100), 201 (68), 186 (24), 45 (25), 43 (37). HREI-MS $[M]^+$: found *m/z* 382.1400 for $C_{22}H_{22}O_6$ (calcd. 382.1416).

*Formononetin (**52**)*

White amorphous solid. ^1H and ^{13}C NMR (Table 14) in agreement with literature, [Fuendjiep *et al*., 1998b]. LC-ESI-MS: $[M+H]^+$ at *m/z* 269.5.

*Kumatakenin (**170**)*

Yellow amorphous solid. ^1H and ^{13}C NMR (Table 15).

*Isokaempferide (**171**)*

Yellow amorphous solid. ^1H and ^{13}C NMR: (Table 15).

*β-Amyrin (**172**)*

White amorphous solid. ^1H and ^{13}C NMR (Table 16).

*Betulin (**173**)*

White amorphous solid. ^1H and ^{13}C NMR (Table 16).

*12a-Epimillettosin (**70**)*

White solid. ^1H NMR (800 MHz, $(CD_3)_2SO$): δ 7.64 (1H, d, *J* = 8.6 Hz, H-11), 7.52 (1H, s, H-1), 6.66 (1H, *br* s, 12a-OH), 6.60 (1H, d, *J* = 10 Hz, H-4′), 6.56 (1H, d, *J* = 8.6 Hz, H-10),

6.51 (1H, s, H-4), 5.99 (2H, d, *J* = 1.6 Hz, 2-OCH_2O-3), 5.81 (1H, d, *J* = 10.0 HZ, H-3′), 4.76 (1H, dd, *J* = 11.0 and 5.0 Hz, H-6a), 4.36 (1H, dd, *J* = 9.7 and 5.0 Hz, H-6α), 4.34 (1H, dd, *J* = 9.7 and 9.7 Hz, H-6β). ^{13}C NMR (150 MHz, $(CD_3)_2SO$): δ 187.7 (C-12), 158.3 (C-9), 155.4 (C-7a), 149.8 (C-4a), 148.2 (C-3), 141.0 (C-2), 129.8 (C-3′), 128.8 (C-11), 114.8 (C-4′), 114.0 (C-11a), 111.9 (C-12b), 110.7 (C-10), 109.8 (C-1), 108.2 (C-8), 101.3 (2-OCH_2O-3), 97.8 (C-4), 77.5 (C-2′), 75.8 (C-6a), 65.2 (C-12a), 61.0 (C-6).

*Usararotenoid-A (**71**)*

White amorphous solid. ^{1}H NMR (800 MHz, $(CD_3)_2SO$): δ 7.48 (1H, d, *J* = 8.3 Hz, H-11), 7.46 (1H, s, H-1), 6.80 (1H, d, *J* =8.3 Hz, H-10), 6.76 (1H, *br* s, 12a-OH), 6.51, (1H, s, H-4), [6.19 (1H, *br* s) and 6.14 (1H, *br* s), 8-OCH_2O-9], 5.99 (2H, d, *J* = 3.3 Hz, 2-OCH_2O-3), 4.83 (1H, dd, *J* = 11.3 and 4.3 Hz, H-6a), 4.35 (1H, dd, *J* = 9.6 and 4.6 Hz, H-6α), 4.31 (1H, dd, *J* = 10.5 and 9.7 Hz, H-6β). ^{13}C NMR (150 MHz, $(CD_3)_2SO$): δ 187.3 (C-12), 153.4 (C-9), 149.8 (C-4a), 148.3 (C-3), 143.4 (C-7a), 141.1 (C-2), 133.6 (C-8), 123.3 (C-11), 116.7 (C-11a), 111.7 (C-12b), 109.9 (C-1), 103.3 (C-10), 102.8 (8-OCH_2O-9), 101.3 (2-OCH_2O-3), 97.8 (C-4), 75.9 (C-6a), 65.9 (C-12a), 60.9 (C-6).

*12-Dihydrousararotenoid-A (**72**)*

White amorphous solid. ^{1}H NMR (800 MHz, $(CD_3)_2SO$): δ 7.85 (1H, s, H-1), 6.98 (1H, d, *J* = 8.2 Hz, H-11), 6.59 (1H, d, *J* = 8.2 Hz, H-10), 6.45 (1H, s, H-4), [5.97 (1H *br* s) and 5.96 (1H, *br* s), 8-OCH_2O-9], 5.95 (2H, *br* s, 2-OCH_2O-3), 4.73 (1H, *br* s, H-12), 4.36 (1H, dd, *J* = 10.3 and 5.7 Hz, H-6a), 4.24 (1H, m, H-6β), 4.22 (1H, m, H-6α). ^{13}C NMR (150 MHz, $(CD_3)_2SO$): δ 148.7 (C-4a), 147.7 (C-3), 147.2 (C-9), 141.2 (C-2), 137.8 (C-7a), 132.7 (C-8), 122.9 (C-11a), 120.9 (C-11), 117.4 (C-12b), 107.7 (C-1), 101.2 (8-OCH_2O-9), 101.1 (2-OCH_2O-3), 97.6 (C-4), 73.1 (C-6a), 70.1 (C-12), 63.2 (C-12a), 61.5 (C-6).

*Usararotenoid-C (**74**)*

White solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.84 (1H, d, *J* = 8.8 Hz, H-11), 7.66 (1H, s, H-1), 6.67 (1H, d, *J* = 8.8 Hz, H-10), 6.38 (1H, s, H-4), 5.91 (2H, s, 2-OCH_2O-3), 5.16 (1H, t, *J* = 7.4 Hz, H-2′), 4.57 (1H, dd, *J* = 10.8 and 4.8 Hz, H-6a), 4.44 (1H, dd, *J* = 10.0 and 10.0 Hz, H-6β), 4.34 (1H, dd, *J* = 9.2 and 4.4 Hz, H-6α), 3.89 (3H, s, 9-OCH_3), 1.76 (3H, s, H-4′), 1.66 (3H, s, H-5′). ^{13}C NMR (50 MHz, $CDCl_3$): δ 188.0 (C-12), 163.5 (C-9), 158.3 (C-

7a), 150.9 (C-4a), 149.5 (C-3), 142.5 (C-2), 132.3 (C-3′), 128.5 (C-11), 121.8 (C-2′), 117.5 (C-8), 114.2 (C-11a), 110.8 (C-12b), 109.7 (C-1), 106.1 (C-10), 101.7 (2-OCH_2O-3), 98.7 (C-4), 76.6 (C-6a), 66.6 (C-12a), 61.5 (C-6).

*12-Dihydrousararotenoid-B **(174)***

White amorphous solid. ^{1}H NMR: similar to what is in literature [Musyoki, 2011].

*Millettosin **(175)***

White solid. ^{1}H and ^{13}C NMR (Table 17).

*12-Dihydrousararotenoid-C **(176)***

White solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.80 (1H, s, H-1), 7.41 (1H, d, *J* = 9.2 Hz, H-11), 6.64 (1H, d, *J* = 8.8 Hz, H-10), 6.39 (1H, s, H-4), 5.92 (2H, s, 2-OCH_2O-3), 5.18 (1H, t, *J* = 7.4, H-2′), 4.90 (1H, s, H-12), 4.34 (1H, m, H-6a), 4.28 (1H, m, H-6α and H-6β). ^{13}C NMR (50 MHz, $CDCl_3$): δ 158.0 (C-9), 151.4 (C-7a), 149.8 (C-4a), 149.3 (C-3), 142.7 (C-2), 131.7 (C-3′), 127.1 (C-11), 122.4 (C-2′), 118.2 (C-8), 117.1 (C-11a), 115.3 (12b), 107.4 (C-1), 105.6 (C-10), 101.6 (2-OCH_2O-3), 98.6 (C-4), 73.2 (C-12), 70.9 (C-6a), 64.7 (C-12a), 62.5 (C-6), 56.0 (9-OCH_3), 26.0 (C-4′), 22.5 (C-1′), 18.0 (C-5′).

*7-Hydroxy-8,3',4'-trimethoxyisoflavone **(177)***

White solid. ^{1}H and ^{13}C NMR (Table 18), in agreement with literature [Puebla *et al*., 2010].

*Jamaicin **(178)***

White solid. ^{1}H and ^{13}C NMR (Table 19), in agreement with literature [Dagne *et al.*, 1989].

*(2R,3R)-4'-O-Geranyl-7-hydroxyflavanonol **(179)***

White amorphous solid. UV λ_{max} (CH_3OH): 279, 305 nm. ^{1}H and ^{13}C NMR (Table 20). HRESI-MS $[M+H]^+$: found *m/z* 409.2020 for $C_{25}H_{28}O_5$ (calcd. 409.2015).

*(S)-4'-O-Geranyl-7-hydroxyflavanone **(180)***

White amorphous solid. ^{1}H and ^{13}C NMR (Table 21). LC-ESI-MS $[M+H]^+$ at *m/z* 393.5.

*4-O-Geranylisoliquiritigenin **(181)***

Yellow gum. ^{1}H and ^{13}C NMR: similar to what is in literature [Musyoki, 2011].

*Colenemol (**182**)*

White solid. ^{1}H (200 MHz, $CDCl_3$) NMR: δ 7.32 (2H, m, H-2/6), 6.86 (2H, m, H-3/5), 6.55 (1H, d, *J* = 16.2 Hz, H-7), 6.30 (1H, dt, *J* = 16.2 and 6.0 Hz), 5.44 (1H, t, *J* = 6.4 Hz), 5.15 (1H, m, H-6′), 4.53 (2H, d, *J* = 6.4 Hz, H-1′), 4.29 (2H, dd, *J* = 5.8 and 0.8 Hz, H-9), 2.09 (4H, m, H-4′ and H-5′), 1.73 (3H, s, H-10′), 1.67 (3H, s, H-8′), 1.60 (3H, s, H-9′). ^{13}C NMR (50 MHz, $CDCl_3$): δ 158.9 (C-4), 141.5 (C-3′), 131.9 (C-7′), 131.3 (C-7), 129.5 (C-1), 127.8 (C-2/6), 126.3 (C-8), 124.0 (C-6′), 119.6 (C-2′), 115.0 (C-3/5), 65.1 (C-1′), 64.2 (C-9), 39.8 (C-4′), 26.5 (C-5′), 25.9 (C-8′), 17.9 (C-10′), 16.9 (C-9′).

*Genistein (**11**)*

White solid. ^{1}H and ^{13}C NMR (Table 22). LC-ESI-MS $[M+H]^+$ at *m/z* 271.7.

*Biochanin A (**183**)*

White solid. UV λ_{max} (CH_3OH): 260 nm. ^{1}H and ^{13}C NMR (Table 22). LC-ESI-MS $[M+H]^+$ at *m/z* 285.5.

*Genistin (**184**)*

White solid. UV λ_{max} (CH_3OH): 255 nm. ^{1}H and ^{13}C NMR (Table 23).

*Genistein 7,4'-di-O-β-glucopyranoside (**185**)*

White solid, UV λ_{max} (CH_3OH): 260 nm. ^{1}H and ^{13}C NMR (Table 23).

*Flemiphilippinin F (**186**)*

White solid. ^{1}H and ^{13}C NMR (Table 24).

*Corylin (**187**)*

White amorphous solid. UV λ_{max} (CH_3OH): 255 nm. ^{1}H NMR and gCHSQC spectra (Table 25)

*Lupalbigenin (**188**)*

White amorphous solid. ^{1}H and ^{13}C NMR (Table 25).

*Flemichin D (**150**)*

Yellow solid. UV λ_{max} (CH_3OH): 270 nm. ^{1}H and ^{13}C NMR (Table 26).

Eriosemaone A ***(189)***

Yellow solid. UV λ_{max} (CH_3OH): 260 nm. ^{1}H and ^{13}C NMR (Table 27).

Lupinifolin ***(151)***

Yellow powder. UV λ_{max} (CH_3OH): 260 nm. ^{1}H and ^{13}C NMR (Table 28).

5,4'-Dihydroxy-6-γ,γ-dimethylallyl-2''',2'''-dimethylpyrano-[5''',6''':8,7]-flavanone ***(151a)***

Yellow solid. ^{1}H (400 MHz, CD_3OD) NMR: δ 7.33 (2H, d, *J* = 8.3 Hz, H-2'/6'), 6.82 (2H, d, *J* = 8.3 Hz, H-3'/5'), 6.50 (1H, d, *J* = 10.0 Hz, H-4'''), 5.53 (1H, d, *J* = 10.0 Hz, H-3'''), 5.36 (1H, dd, *J* = 13.2 and 2.7 Hz, H-2), 5.14 (1H, t, *J* = 7.0 Hz, H-2''), 3.20 (2H, m, H-1''), 3.19 (1H, m, H-3ax), 2.74 (1H, dd, *J* = -17.2 and 2.8 Hz, H-3eq), 1.76 (3H, s, H-5''), 1.65 (3H, s, H-4''), 1.42 (3H, s, 2'''-CH_3) and 1.39 (3H, s, 2'''-CH_3).

5,2',4'-Trihydroxy-8,5'-di(γ,γ-dimethylallyl)-2'',2''-dimethylpyrano-[5'',6'':6,7]-flavanone ***(190)***

Yellow powder. UV λ_{max} (CH_3OH): 260 nm. ^{1}H and ^{13}C NMR (Table 29).

5,2',4'-Trihydroxy-6,5'-di(γ,γ-dimethylallyl)-2''',2'''-dimethylpyrano-[5''',6''':8,7]-flavanone ***(190a)***

Yellow solid. ^{1}H and ^{13}C NMR (Table 30).

5,3',4'-Trihydroxy-8-γ,γ-dimethylallyl-2'',2''-dimethylpyrano-[5'',6'':6,7]-flavanone ***(191)***

Yellow solid. UV λ_{max} (CH_3OH): 265 nm. ^{1}H and ^{13}C NMR (Table 32). HRESI-MS: $[M+H]^+$: found *m/z* 423.1809 for $C_{25}H_{26}O_6$ (calcd. 423.1763).

Eriosematin ***(192)***

Yellow solid. UV λ_{max} (CH_3OH): 275 nm. ^{1}H NMR and gCHSQC (Table 33).

4'-O-Methylgallocatechin ***(193)***

White amorphous solid. ^{1}H and ^{13}C NMR (Table 34).

Flemingin C ***(194)***

Yellow powder. UV λ_{max} (CH_3OH): 290, 375 400 nm. ^{1}H NMR (Table 35) and ^{13}C NMR (Table 36).

Flemingin B ***(195)***

Yellow powder. UV λ_{max} (CH_3OH): 350 nm. ^{1}H NMR (Table 35) and ^{13}C NMR (Table 36).

Flemingin A ***(196)***

Orange powder. ^{1}H NMR (Table 35) and ^{13}C NMR (Table 36). The ESI-MS $[M+H]^+$ at *m/z* 407.7.

Flemingin G ***(197)***

Yellow powder. ^{1}H and ^{13}C NMR (Tables 37 and 38). HRESI-MS $[M+H]^+$: found, *m/z* 439.1875 for $C_{25}H_{26}O_7$ (calcd. 439.1757).

Flemingin H ***(198)***

Yellow powder. UV λ_{max} (CH_3OH): 170 and 365 nm. ^{1}H and ^{13}C NMR (Tables 37 and 38). HRESI-MS $[M+H]^+$: found, *m/z* 439.1866 for $C_{25}H_{26}O_7$ (calcd. 439.1757).

Flemingin I ***(199)***

Yellow powder. UV λ_{max} (CH_3OH): 275, 365 nm. ^{1}H and ^{13}C NMR (Tables 39 and 40). HRESI-MS $[M+H]^+$: found *m/z* 423.1821 for $C_{25}H_{26}O_6$ (calcd. 423.1808).

Flemingin J ***(200)***

Yellow powder. ^{1}H and ^{13}C NMR (Tables 39 and 40). HRESI-MS $[M+H]^+$: found *m/z* 423.1924 for $C_{25}H_{26}O_6$ (calcd. 423.1808)

Flemingin K ***(201)***

Yellow powder. ^{1}H and ^{13}C NMR (Tables 41 and 42). HRESI-MS $[M+H]^+$: found *m/z* 439.1733 for $C_{25}H_{26}O_7$ (calcd. 439.1757).

Flemingin L ***(202)***

Yellow powder. ^{1}H and ^{13}C NMR (Tables 41 and 42). HRESI-MS $[M+H]^+$: found *m/z* 423.1881 for $C_{25}H_{26}O_6$ (calcd. 423.1808).

Flemingin M ***(203)***

Yellow solid. ^{1}H NMR (Table 43).

*Flemingin N (**204**)*

Yellow solid. ^{1}H NMR (Table 44). HRESI-MS $[M+H]^{+}$: found *m/z* 441.1779 for $C_{25}H_{28}O_7$ (calcd. 441.1813)

*Flemingin O (**205**)*

Yellow solid. ^{1}H NMR (Table 44) and ^{13}C NMR: (Table 44). HRESI-MS $[M+H]^{+}$: found *m/z* 441.1912 for $C_{25}H_{28}O_7$ (calcd. calcd. 441.1813).

*Flemingin P (**206**)*

Yellow solid. UV λ_{max} (CH_3OH): 300, 360 nm. ^{1}H and ^{13}C NMR (Table 45).

*Flemingin Q (**207**)*

Yellow solid. ^{1}H NMR (CD_3OD): δ_H 8.06 (1H, d, *J* = 15.4 Hz, H-β), 7.73 (1H, d, *J* = 15.4 Hz, H-α), 7.34 (1H, s, H-6′), 7.04 (1H, m, H-6), 6.76 (1H, d, *J* =10.2 Hz, H-4″), 6.75 (2H, m, H-3 and H-4), 5.67 (1H, d, *J* = 10.2 Hz, H-3″), 3.22 (1H, dd, *J* = 10.8 and 2.0 Hz, H-8″), 2.01 (1H, m, H-6″b), 1.85 (1H, m, H-7″b), 1.72 (1H, m, H-6″a), 1.47 (3H, s, H-5″), 1.40 (1H, m, H-7″a), 1.15 (3H, s, H-11″), 1.12 (3H, s, H-10″). LC-ESI-MS $[M+H]^{+}$: *m/z* 457.6.

*Deoxyhomoflemingin (**208**)*

Yellow solid. ^{1}H and ^{13}C NMR (Table 46).

*Fleminginflavanone A (**209**)*

Yellow solid. ^{1}H NMR (Table 47). HRESI-MS $[M+H]^{+}$: found *m/z* 423.1854 for $C_{25}H_{26}O_6$ (calcd. 423.1808).

*Fleminginflavanone B (**210**)*

Yellow solid. ^{1}H (Table 47) and ^{13}C NMR (Table 47)

*Flemingiaurone A (**211**)*

Yellow solid. UV λ_{max} (CH_3OH): 320 nm. ^{1}H and gCHSQC (Table 48). HRESI-MS $[M+H]^{+}$: found *m/z* 315.0865 for $C_{17}H_{14}O_6$ (calcd. 315.0869).

*5,2′,5′-Trihydroxy-2″-methyl-2″-(4-methylpent-3-enyl)pyrano-[5″,6″:7,6]-aurone (**212**)*

Yellow solid. UV λ_{max} (CH_3OH): 410 nm. ^{1}H and ^{13}C NMR (Table 49). HRESI-MS $[M+H]^{+}$: found *m/z* 421.1651 for $C_{25}H_{24}O_6$ (calcd. 421.1606).

*Emodin (**213**)*

Orange solid. ^{1}H NMR (Table 50).

*5-Allyloxy-2-hydroxybenzaldehyde (**214**)*

A brown gum. ^{1}H and ^{13}C NMR (Table 51).

*2,5-Diallyloxybenzaldehyde (**215**)*

Brownish oil. ^{1}H and ^{13}C NMR (Table 52).

*2,5-Diallyloxy-2'-hydroxy-4',5'-dimethoxychalcone (**216**)*

Reddish orange crystals (CH_3CN). Mp 129-130 ºC. ^{1}H and ^{13}C NMR (Table 53). HRESI-MS $[M+H]^+$: found *m/z* 397.1668 for $C_{23}H_{24}O_6$ (calcd. 397.1651).

*5-Allyloxy-2,2'-dihydroxy-4',5'-dimethoxychalcone (**217**)*

Brown gum. ^{1}H and ^{13}C NMR (Table 54). HRESI-MS $[M+H]^+$: found *m/z* 357.1375 for $C_{20}H_{20}O_6$ (calcd. 357.1293).

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Phytochemical and cancer chemopreventive activity studies were carried out on three Papilionoidea plant species (Leguminosae): *Platycelphium voënse*, *Millettia usaramensis* subsp. *usaramensis*, and *Flemingia grahamiana*. A total of fifty nine compounds were isolated and identified including fifty six flavonoids and two terpenoids and one quinone. Five derivatives were also prepared. Twenty three of the isolated compounds were new.

From the stem bark extract of *Platycelphium voënse*, twelve compounds were obtained including seven isoflavanone derivatives (compounds **163-169**), one isoflavone (**52**), two 3-methoxyflavonols (**170** and **171**), and two triterpenes (**172** and **173**). This was the first report of such compounds to be identified from the plant. Five of the isoflavanones (**163**, **164**, **166-168**) were new compounds. The results support the taxonomic placement of *Platycelphium voënse* in the Sophora group, within the tribe Sophoreae. One isoflavanone (**163**) was active against *Mycobacterium tuberculosis* and cytotoxic against African green monkey kidney Vero cells. Compounds (**171**) and (**173**) were active against MCF-7 human breast cancer cells.

Phytochemical investigations of the root extract of *Millettia usaramensis* subsp. *usaramensis* led to characterization of thirteen compounds which included seven 12a-hydroxyrotenoids (**70-72, 74, 174-176**), two isoflavones (**177** and **178**), two flavanone derivatives (**179** and **180**), one chalcone (**181**), and one cinnamoyl derivative (**182**). This was the first time for compound **175** to be obtained from the roots of *Millettia usaramensis* and the first time for compound **177** to be reported from the genus *Millettia*. Compounds **179-181** are new compounds. Eight compounds from this plant were tested aginst MCF-7 human breast cancer cells, and compound **176** exhibited potent cytotoxicity. Compounds **70** and 1**75** were moderately active in the same assay. Chalcone **181** was mildly active against DPPH in the radical scavenging test.

From the root extract of *Flemingia grahamiana*, chromatographic fractionation gave fourteen compounds that included seven isoflavones (compounds **11, 183-188**), five multiprenylated

flavanones (**150, 151, 189-191**), one chromone derivative (**192**) and one gallocatechin derivative (**193**). Compounds **150**, **151** and **190** underwent rearrangement into the respective 6-γ,γ-dimethylallyl-2''',2'''-dimethylpyrano-[5''',6''':8,7]-flavanones regioisomers (**189**, **151a**, **190a**). The flavanone **191** is a new compound. In the antioxidant test the crude root extract of *Flemingia grahamiana* was weekly active, compound **11** was also weakly active and the 7-glucoside derivative of compound **11** (i.e. compound **184**) was inactive against DPPH.

Fractionation and phytochemical analysis of the leaf extract of *Flemingia grahamiana* led to the isolation and identification of nineteen compounds, including fourteen chalcones with a B-ring having a *para*-oxygenation and a geranyl group in form of an open chain, modified in into a chromene with a residual side chain or in form of a cyclohexene derivative (**194-208**); two diastereomeric flavanones (**209** and **210**); two aurones (**211** and **212**) and an anthraquinone (**213**). Compounds **197-212** are new and reported for the first time. In biological activity testing, both chalcones **194** and **196** exhibited potent activity against MCF-7 human breast cancer cells. Compound **196** was strongly cytotoxic against Vero cells and strongly active against *Mycobacterium tuberculosis*. The crude extract of the leaves (without chlorophyll) and the five chalcones **194-198** were strongly active against DPPH.

A chalcone derivative, 2,5-diallyloxy-2′-hydroxy-4′,5′-dimethoxychalcone was successfully prepared, but the removal of the allyl groups from positions 2 and 5 using $Pd^0(PPh_3)_4$ as the catalyst was not effectively achieved.

5.2 RECOMMENDATIONS

Another portion of the stem bark of *Platycelphium voënse* should be investigated wih the aim of carrying out exhaustive isolation, and if facilities allow, by using a prep-HPLC. Furthermore, the leaves and roots of the same plant should also be investigated for phytochemical markers as this may be important in chemotaxonomy and biogenesis within the plant. Plant species of the botanically related genus *Dicraepetalum* should be investigated in order to establish a chemotaxonomic relationship within the Sophoreae tribe.

More compounds from *Platycelphium voënse* should be tested against different micro-organisms and cancer cell lines. This is because compound **163** exhibited strong activity against Vero cells and TB.

The rotenoids from *Millettia usaramensis* subsp. *usaramensis* should be investigated against other cell lines in addition to MCF-7 human breast cancer cell. Another portion of the root extract should be investigated in order to scale up the newly isolated flavanonol. The leaves of the plant should also be investigated as well.

The rearrangement of multiprenylated flavanones, such as those obtained from the roots of *Flemingia grahamiana*, should be studied further using isotopic labeling to ascertain the mechanism. Furthermore, the effects of other solvent on these compounds should be comprehensively studied.

With regards to isolation of compounds from the leaves of *Flemingia grahamiana*, the hydroxyl groups in the diastereomeric mixtures should be acetylated so as to increase the retention time of the compounds on the HPLC column and thus facilitate the separation of the diasteomers. Derivatives of compound **196**, which was active in all tests carried out, should be prepared and tested against DPPH, different cancer cell lines and pathogenic micro-organisms. In the process of synthesising flemingin analoges, other protecting groups such as methoxymethyl ethers (other than allyl groups) should be used for the hydroxyls of the benzaldehyde derivatives. Alternative deprotecting methods should be tried in the removal of the allyl groups from the *O*-allyl chalcone derivatives.

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APPENDICES

PART I: ARTICLES PUBLISHED

A: First Paper

Phytochemistry Letters 5 (2012) 150–154

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Four isoflavanones from the stem bark of *Platycelphium voënse*

Ivan Gumula [a,b], Matthias Heydenreich [c], Solomon Derese [a], Isaiah O. Ndiege [b], Abiy Yenesew [a,*]

[a] Department of Chemistry, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya
[b] Department of Chemistry, Kyambogo University, P.O. Box 1, Kyambogo, Kampala, Uganda
[c] Institut für Chemie, Universität Potsdam, P.O. Box 60 15 53, D-14415, Potsdam, Germany

ARTICLE INFO

Article history:
Received 29 October 2011
Received in revised form 26 November 2011
Accepted 28 November 2011
Available online 9 December 2011

Keywords:
Platycelphium voënse
Stem bark
Leguminosae
Isoflavanones
Platyisoflavanone
Mycobacterium tuberculosis

ABSTRACT

From the stem bark of *Platycelphium voënse* (Leguminosae) four new isoflavanones were isolated and characterized as (*S*)-5,7-dihydroxy-2′,4′-dimethoxy-3′-(3″-methylbut-2″-enyl)-isoflavanone (trivial name platyisoflavanone A), (±)-5,7,2′-trihydroxy-4′-methoxy-3′-(3″-methylbut-2″-enyl)-isoflavanone (platyisoflavanone B), 5,7-dihydroxy-4′-methoxy-2″-(2‴-hydroxyisopropyl)-dihydrofurano-[4″,5″:3′,2′]-isoflavanone (platyisoflavanone C) and 5,7,2′,3″-tetrahydroxy-2″,2″-dimethyldihydropyrano-[5″,6″:3′,4′]-isoflavanone (platyisoflavanone D). In addition, the known isoflavanones, sophoraisoflavanone A and glyasperin F; the isoflavone, formononetin; two flavones, kumatakenin and isokaempferide; as well as two triterpenes, betulin and β-amyrin were identified. The structures were elucidated on the basis of spectroscopic evidence. Platyisoflavanone A showed antibacterial activity against *Mycobacterium tuberculosis* in the microplate alamar blue assay (MABA) with MIC = 23.7 μM, but also showed cytotoxicity (IC_{50} = 21.1 μM) in the vero cell test.

1. Introduction

Platycelphium (family Leguminosae, sub-family Papilionoideae, tribe Sophoreae) is a monotypic genus that occurs in the drier parts of Eastern Africa, particularly in Kenya, Ethiopia, Somalia and Tanzania (Gillett et al., 1971). Sophoreae, containing genera of least specialization and diverse morphological features, has been described as "a tribe of convenience" (Gillett et al., 1971; Polhill, 1981). The tribe is considered to be transitional between the subfamilies Papilionoideae and Caesalpinoideae (Bentham, 1841) and DNA sequencing studies have shown that Sophoreae needs taxonomic realignment (Crisp et al., 2000; Doyle et al., 2000; Käss and Wink, 1995; Pennington et al., 2001). From morphological point of view the genus *Platycelphium* is closely related to the genera *Dicraeopetalum* and *Bolusanthus* all belonging to the Sophora group within the Sophoreae tribe (Polhill, 1994.). Whereas, there is some phytochemical information on the genus *Bolusanthus* (Asres et al., 1985; Bojase et al., 2001a,b), the information available on *Platycelphium voënse* (Asres et al., 1997b; Van Wyk et al., 1993) and *Dicraeopetalum* (Asres et al., 1997a; Van Wyk et al., 1993) is limited to the identification of quinolizidine alkaloids through GC–MS analysis of the leaves and twigs of plants from the two genera. Quinolizidine alkaloids have also been reported from *Bolusanthus* (Asres et al., 1986). With interest to see if phytochemical information supports the close association among these genera in the Sophora group within the Sophoreae tribe, the stem bark of *P. voënse* was investigated. This paper describes the isolation and characterization of four new prenylated isoflavanones along with seven known compounds (two isoflavanones, an isoflavone, two 3-methoxyflavones and two triterpenes).

2. Results and discussion

Column chromatography of the CH_2Cl_2–MeOH (1:1) extract of the stem bark of *P. voënse*, using n-hexane containing increasing amounts of ethyl acetate as the eluent and subsequent purification of the fractions, resulted in the isolation of eleven compounds including four new isoflavanones, **1–4** (Fig. 1).

Compound **1**, obtained as a white amorphous solid, showed a $[M]^+$ at *m/z* 384.1597 in the HREI-mass spectrum suggesting a molecular formula of $C_{22}H_{24}O_6$. The presence of an isoflavanone skeleton was deduced from UV (λ_{max} 288 nm), ^1H (δ 4.48, *dd*, *J* = −11.1, 11.2 Hz, H-2_{ax}; δ 4.66, *dd*, *J* = −11.1, 5.6 Hz, H-2_{eq}; δ 4.38, *dd*, *J* = 11.2, 5.6 Hz, H-3_{ax}) and ^{13}C (δ 71.6 for C-2; 45.9 for C-3 and 198.2 for C-4) NMR spectra. The ^1H NMR spectrum further revealed the presence of two methoxyl (δ 3.71 and 3.80), a chelated hydroxyl (δ 12.18) at C-5 as well as a 3-methylbut-2-enyl moiety (Table 1).

Two *meta*-coupled doublets at δ 5.95 and 5.97 (*J* = 2.0 Hz) were attributable to H-8 and H-6 implying that C-5 and C-7 of A-ring are oxygenated as expected from biogenetic point of view. In the

* Corresponding author. Tel.: +254 733 832576; fax: +254 204 446138.
E-mail address: ayenesew@uonbi.ac.ke (A. Yenesew).

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doi:10.1016/j.phytol.2011.11.012

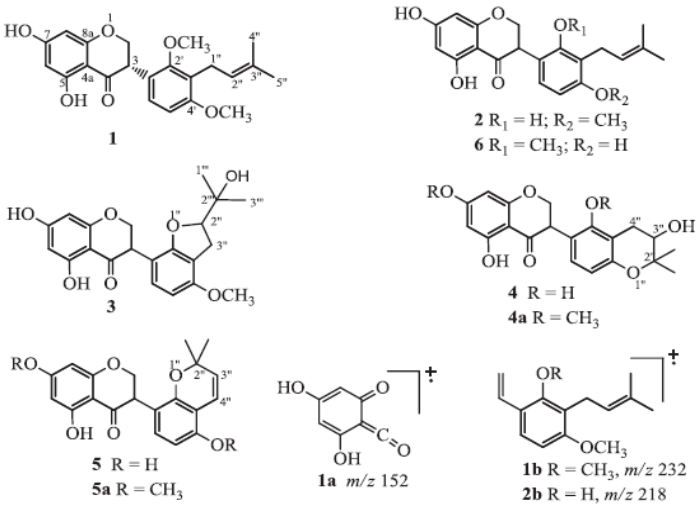

Fig. 1. Structures of compounds **1**–**6** and RDA fragment ions in the EI-mass spectra of compounds **1** and **2**.

Table 1
^{1}H NMR (600 MHz) spectral data for compounds **1**–**5**.

Position	δ_H *J* (in Hz)				
	[a]**1**	[a]**2**	[b]**3**	[b]**4**	[b]**5**
2	4.48[c] *dd* (−11.1, 11.2) 4.46[c] *dd* (−11.1, 5.6)	4.62 *dd* (11.6, 5.0) 4.76 *dd* (11.6, 6.4)	4.34 *dd* (11.0, 5.7) 4.42 *dd* (11.2, 11.2)	4.56 *m*/4.57 *m* 4.69 *m*/4.68 *m*	4.41 *dd* (10.9, 5.6) 4.55 *dd* (11.1, 11.1)
3	4.38 *dd* (11.2, 5.6)	4.08 *t* (5.7)	4.06 *dd* (11.4, 5.6)	4.23 *m*/4.22 *m*	4.19 *dd* (11.1, 5.7)
5-OH	12.18 *s*	11.87 *s*	12.15 *s*	12.18 *s*/12.17 *s*	12.40 *s*
6	5.97 *d* (2.3)	5.95 *m*	5.84 *d* (2.2)	5.96 *m*	5.98 *d* (2.2)
7	–	–	–	–	–
8	5.95 *d* (2.3)	5.95 *m*	5.82 *d* (2.2)	5.96 *m*	5.95 *d* (2.2)
5′	6.67 *d* (8.6)	6.48 *d* (8.6)	6.32 *d* (8.4)	6.32 *d* (8.4)	6.40 *d* (8.3)
6′	6.92 *d* (8.5)	7.15 *d* (8.6)	6.83 *d* (8.4)	6.97 *d* (8.4)/6.96 *d* (8.4)	6.86 *d* (8.3)
1″	3.32 *dd* (14.3, 6.6) 3.38 *dd* (14.4, 6.8)	3.39 *m*	–	–	–
2″	5.25 *t* (6.7)	5.17 *t* (7.2)	4.52 *dd* (9.6, 7.1)	–	–
3″	–	–	2.97 *dd* (16.0, 9.6) 3.01 *dd* (16.0, 7.0)	3.78 *m*	5.63 *d* (10.0)
4″	1.77 *s*	1.79 *s*	–	2.62 *dd* (10.2, 10.2)/2.58 *dd* (10.2, 10.2) 2.98 *dd* (8.4, 6.0)/2.96 *dd* (8.4, 6.0)	6.68 *d* (9.9)
5″	1.68 *s*	1.70 *s*	–	–	–
1‴	–	–	1.03 *s*	–	–
2‴	–	–	–	–	–
3‴	–	–	1.04 *s*	–	–
2′-OCH_3	3.71 *s*	–	–	–	–
4′-OCH_3	3.80 *s*	3.78 *s*	3.68 *s*	–	–
2″-CH_3	–	–	–	1.22 *s* 1.33 *s*/1.32 *s*	1.33 *s* 1.35 *s*

[a] Spectra recorded in CD_2Cl_2.
[b] Spectra recorded in CD_3OCD_3.
[c] Multiplicity was clear after iteration according to Laatikainen et al. (1996a, 1996b).

B-ring, two *ortho*-coupled aromatic protons at δ 6.67 and 6.92 (J = 8.6 Hz) were assigned to H-5′ and H-6′, respectively, with C-2′, C-3′ and C-4′ being substituted. The ^{13}C chemical shift values (Table 2) for the B-ring carbon atoms are consistent with oxygenation at C-2′ and C-4′, with the 3-methylbut-2-enyl group being at C-3′. The fragment ions at *m*/*z* 152 (**1a**) and 232 (**1b**) (Fig. 1) in the EI-mass spectrum resulting from a typical *retro*-Diels–Alder (RDA) cleavage confirmed that the A-ring had two hydroxyl groups; and that the two methoxyl together with the 3-methylbut-2-enyl group are located on the B-ring.

In NOE experiments, the peak at δ_H 4.38 (H-3) was enhanced upon irradiation of the methoxyl group at δ_H 3.71; similarly, NOE interaction was exhibited between a signal at δ_H 6.67 (H-5′) and the methoxyl group at δ_H 3.80 allowing the placement of the two methoxyl groups at C-2′ and C-4′. Indeed, the down-field shift of one of the methoxyl group (δ_C 62.4) in the ^{13}C NMR spectrum, is typical of di-*ortho* substituted methoxyl group (Park et al., 2008), confirming its placement at C-2′. The HMBC experiment revealed a long range correlation of the CH_2-1″ protons of the 3-methylbut-2-enyl moiety with C-2′ and C-4′, implying that the 3-methylbut-2-enyl group is

Table 2
^{13}C NMR (150 MHz) spectral data of compounds **1**–**5a**.

Position	δ_C						
	[a]**1**	[a]**2**	[b]**3**	[b]**4**	[a]**4a**	[b]**5**	[a]**5a**
2	71.6	70.3	71.5	71.6/71.7	71.5/71.4	71.8	70.8
3	45.9	46.0	48.7	47.6	46.4/46.0	48.4	47.4
4	198.2	197.7	198.6	199.1	198.0	199.0	197.8
4a	103.8	102.6	104.2	103.8	103.8/103.7	104.4	103.8
5	164.9	165.3	166.4	166.5	164.8	166.3	164.8
6	96.7	96.8	97.6	97.6/97.7	95.1	97.6	95.0
7	164.9	165.4	168.0	168.2	168.2	167.6	167.9
8	95.5	95.4	96.3	96.4/96.3	94.1	96.3	94.1
8a	163.9	163.7	165.3	165.2	163.6	165.2	163.6
1′	120.5	115.5	110.6	115.4	119.6	115.5	115.1
2′	157.9	154.1	160.6	155.3	158.14/158.12	153.1	151.6
3′	124.2	117.4	115.7	110.7	113.8/113.7	111.0	110.9
4′	158.9	158.3	157.8	155.0	154.2/154.1	154.3	155.4
5′	107.2	103.7	104.7	110.6	113.4	109.1	103.3
6′	127.4	126.2	131.4	129.0/128.9	128.4/128.2	131.9	130.4
1″	23.7	22.7	–	–	–	–	–
2″	123.1	122.2	91.4	78.0/77.9	76.9	77.8	76.8
3″	131.8	134.0	29.5	70.3	69.3/69.2	129.9	129.3
4″	18.1	17.8	–	28.3	27.3/27.2	118.4	116.8
5″	25.9	25.7	–	–	–	–	–
1‴	–	–	26.2	–	–	–	–
2‴	–	–	72.6	–	–	–	–
3‴	–	–	25.9	–	–	–	–
7-OCH_3	–	–	–	–	56.1		56.0
2′-OCH_3	62.4	–	–	–	61.2/61.1	–	–
4′-OCH_3	56.0	56.0	56.4	–	–	–	55.9
2″-CH_3	–	–	–	21.2/21.0	21.8	28.4	27.5
				26.7/26.6	25.0/24.9	28.9	28.0

[a] Spectra recorded in CD_2Cl_2.
[b] Spectra recorded in CD_3OCD_3.

placed between the two methoxyl substituents (at C-3′) as in structure **1** (Tables 1 and 2). Based on these data the new compound (**1**) was characterized as 5,7-dihydroxy-2′,4′-dimethoxy-3′-(3″-methylbut-2″-enyl)-isoflavanone, hereby named platyisoflavanone A. The CD spectrum of compound **1** showed a negative Cotton effect at 344 nm ($n \rightarrow \pi^*$ transition) which was consistent with 3*S* absolute configuration for this isoflavanone (Slade et al., 2005).

Compound **2**, obtained as a white amorphous solid, was also identified as an isoflavanone derivative from the UV (λ_{max} 294 nm), ^{1}H (Table 1) and ^{13}C (Table 2) NMR spectra. The HREI-mass spectrum of the compound gave $[M]^+$ at *m*/*z* 370.1400 corresponding to molecular formula of $C_{21}H_{22}O_6$. The ^{1}H and ^{13}C NMR spectra further revealed the presence of a chelated hydroxyl (5-OH), a methoxyl and a 3-methylbut-2-enyl group. Comparison of the ^{1}H (Table 1) and ^{13}C (Table 2) NMR data of this compound with those of **1** showed identical A-ring, while the B-ring has similar substitution pattern. In fact, the only difference between these two compounds is that **2** has only one methoxyl group. The fragment ion, in EI-MS, at *m*/*z* 218 (**2b**) (Fig. 1) was in agreement with the placement of the methoxyl, the 3-methylbut-2-enyl unit and one hydroxyl groups in the B-ring. The methoxyl group was within the normal range (δ_C 56.0) suggesting its placement at C-4′ rather than at C-2′ (Park et al., 2008). NOESY (which showed NOE interaction of the methoxyl protons with H-5′) as well as HMBC (correlation of methoxy protons with C-4′) spectra confirmed the placement of the methoxyl group at C-4′. Therefore this new compound was characterized as 2′,5,7-trihydroxy-4′-methoxy-3′-(3″-methylbut-2″-enyl)-isoflavanone, named platyisoflavanone B. The nearly zero optical rotation together with insignificant Cotton effect in the CD spectrum revealed that the compound was isolated as a racemic mixture. Isoflavanones with free OH at C-4′ and/or at C-2′, are reported to undergo racemization during extraction and isolation processes (Slade et al., 2005).

Compounds **3**–**5**, also exhibited ^{1}H (Table 1) and ^{13}C (Table 2) NMR spectral features of isoflavanones with identical A-ring as in **1** and **2**. The B-ring in these compounds also showed similar substitution pattern (a C_5 substituent at C-3′ and oxygenation at C-2′ and C-4′), except for the cyclization of the 3-methylbut-2-enyl group at C-3′ involving one of the adjacent hydroxyl groups giving rise to three different metabolites (**3**–**5**).

In the case of compound **3** ($[M]^+$ at *m*/*z* 386.1354, $C_{21}H_{22}O_7$), the cyclization has resulted in the formation of a 2-(2-hydroxyisopropyl)-dihydrofuranyl moiety in B-ring (Kijjoa et al., 1998) as shown from the ^{1}H [an ABX system at δ 2.97, *dd*, *J* = -16.0, 9.6 Hz and 3.01, *dd*, *J* = −16.0, 7.0 Hz were attributed to CH_2-3″; δ 4.52, *dd*, *J* = 9.6, 7.1 Hz (H-2″) and a pair of three-proton singlets at 1.03 (H-1‴) and 1.04 (H-3‴)] and ^{13}C [δ_C 91.4 an oxymethine carbon (C-2″); δ_C 29.5 (C-3″); δ_C 26.2 and 25.9 (C-1‴ and C-3‴) and δ_C 72.6 a quaternary carbon bonded to an oxygen (C-2‴)] NMR spectra. Whereas one of the two oxygen groups in B-ring is involved in cyclization, the second oxygen in this ring is methylated with the corresponding methoxyl group appearing at δ_H 3.68 and δ_C 56.4 in the NMR spectra. In the NOESY spectrum, this methoxyl signal showed NOE interaction with the aromatic proton resonating at δ_H 6.32 (H-5′), allowing its placement at C-4′, and hence the dihydrofuranyl ring should be between C-2′ and C-3′. Furthermore, the 'normal' methoxyl resonance at δ_C 56.4 supports the placement of a methoxyl group at C-4′ rather than at C-2′ (Park et al., 2008). This compound was therefore characterized as 5,7-dihydroxy-4′-methoxy-2″-(2‴-hydroxyisopropyl)-dihydrofurano-[4″,5″:3′,2′]-isoflavanone with a trivial name platyisoflavanone C. The configuration at C-3 and C-2″ has not been established.

The fourth new isoflavanone (compound **4**), $[M]^+$ at *m*/*z* 372.1202, $C_{20}H_{20}O_7$, has a 3-hydroxy-2,2-dimethyldihydropyrano moiety fused to the B-ring, as shown from ^{1}H (Table 1) and ^{13}C (Table 2) NMR spectra. Two possible structures were considered for this compound – one in which the dihydropyran ring is between C-2′/C-3′ and the other with the dihydropyrano moiety between C-3′/C-4′. In order to decide between the two structures, compound **4** was methylated with dimethyl sulphate in the presence of potassium carbonate and acetone, at room temperature, to give a dimethylated product (**4a**) whose ^{13}C NMR spectrum

(Table 2) displayed two methoxyl signals at δ_C 56.1 (7-OCH_3) and at δ_C 61.2; the latter deshielded signal, is typical of a di-*ortho* substituted methoxyl carbon (Park et al., 2008), and hence assigned to 2′-OCH_3. This implied that C-4′, in **4a** and the parent compound **4**, is part of the pyran ring. Therefore, the isolated compound was characterized as 5,7,2′,3″-tetrahydroxy-2″,2″-dimethyldihydropyrano-[5″,6″:3′,4′]-isoflavanone which is also new, with trivial name platyisoflavanone D. Most of the ^{1}H (Table 1) and ^{13}C (Table 2) NMR signals appeared in duplicates indicating that this compound was isolated as a diasteriomeric mixture.

Compound **5** was identified as the pyranoisoflavanone, glyasperin F, which had already been reported from the roots of *Glycerrhiza aspera*, by comparison of its spectral features (Tables 1 and 2) with published data (Zeng et al., 1992) and also by conversion to 7,4′-*O*,*O*-dimethylglyasperin F (**5a**). The ^{1}H NMR spectrum (Section 3.9) of compound **5a** revealed a methoxyl signal at 3.72 ppm that exhibited NOE interaction with a one-proton doublet at δ 6.34 (J = 8.4 Hz, H-5′), showing that the pyran ring (in both **5** and **5a**) is between C-2′/C-3′ rather than between C-3′/C-4′. The presence of two methoxyl signals below δ_C 59 [δ 55.9 (C-2′) and 56.0 (C-7)] in the ^{13}C NMR of compound **5a** (Table 2) confirmed the placement of the pyran ring between C-2′/C-3′. The sixth isoflavanone was identified as sophoraisoflavanone A (**6**) (Komatsu et al., 1978).

It is worthy to note that the oxygenation pattern in all the six isoflavanones isolated from this plant (*P. voënse*) is identical (at C-5, -7, -2′ and -4′), and each with a five-carbon unit at C-3′. Isoflavanones with this substitution pattern have also been isolated from the related genera *Bolusanthus* (Bojase et al., 2001a,b) and *Sophora* (Iinuma et al., 1993; Komatsu et al., 1978) supporting the placement of the three genera in the same group (Sophora group); the latter two genera also elaborate isoflavanones with different oxygenation and prenylation patterns. It will be interesting to find out if the related genus *Dicraeopetalum* also elaborate isoflavanones, possibly with the same oxygenation pattern as the isoflavanones obtained from *P. voënse*.

Other known compounds identified included the 3-methoxyflavones, kumatakenin (Valesi et al., 1972) and isokaempferide (Yang et al., 1995); the isoflavone, formononetin (Balasubramanian and Nair, 2000); and triterpenes, betulin (Siddiqui et al., 1988) and β-amyrin (Bahato and Kundu, 1994). Neither flavonoids nor terpenoids had, prior to this paper, been reported from this plant.

Compound **1** exhibited moderate in vitro anti-TB activity against *Mycobacterium tuberculosis* in the microplate alamar blue assay (MABA, MIC value of 23.7 μM) and weak activity in the low-oxygen-recovery assay (LORA, MIC = 92.2 μM). However, this compound also showed cytotoxicity (IC_{50} = 21.1 μM) in the vero cell test. Compounds **5** and **6** were inactive against TB in the two tests but showed moderate cytotoxicity in the vero cell test, IC_{50} = 88.3 μM for **5** and IC_{50} = 50.3 μM for **6**. The rest of the compounds were not tested.

3. Experimental

3.1. General

Analytical TLC: Merck pre-coated silica gel 60 F_{254} plates. CC and MPLC were carried out on silica gel 60 (70–230 mesh). Gel filtration on Sephadex LH-20. UV spectra were recorded on a Specord S600, Analytik Jena AG, Germany. CD spectra were recorded on JASCO J-710 Spectropolarimeter. EI-MS: direct inlet, 70 eV on Micromass GC-TOFmicro mass spectrometer (Micromass, Wythenshawe, Waters Inc., UK). ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) were recorded on a Bruker Avance 600 Spectrometer using the residual solvent peaks as reference. For compound **2** ^{13}C NMR (50 MHz) was recorded on a Varian 200 Spectrometer. HSQC and HMBC spectra were acquired using the standard Bruker software. The PERCH Program (PERCH solutions Ltd., Kuopio, Finland; Laatikainen et al., 1996a,b) was used for iteration of the ABX spin system of compound **1**.

3.2. Plant material

The stem of *P. voënse* was collected from Mwingi District, Eastern Province, Kenya, in January 2009. The plant was identified at the National Museums of Kenya East African Herbarium, Nairobi, where a voucher specimen is deposited (voucher No. Mathenge-2009/568).

3.3. Extraction and isolation

The air-dried stem bark (1.6 kg) of *P. voënse* was pulverized and extracted twice with CH_2Cl_2–MeOH (1:1) at room temperature for 48 h. Evaporation of the solvent afforded brown gummy extract (114 g). A 110 g portion of the extract was subjected to CC on silica gel, using increasing amounts of EtOAc in n-hexane as the eluate, resulting in 10 fractions, each *ca.* 5.0 L volume-elution.

Fraction 2, eluted with 1% EtOAc in n-hexane, afforded β-amyrin (21 mg). Fraction 3, eluted with 4% EtOAc in n-hexane, contained a mixture of four major compounds which was separated by CC on silica gel (solvent, 0–15% acetone in n-hexane) to give **1** (210 mg), **2** (37 mg), **6** (31 mg) and **5** (36 mg), while fraction 3 eluted with 5% EtOAc was subjected to CC on silica gel (0–30% EtOAc in n-hexane) followed by Sephadex LH-20 (CH_2Cl_2:MeOH, 1:1) yielded betulin (28 mg). Fraction 4 (5% EtOAc in n-hexane) was subjected to Medium Pressure Liquid Chromatography (MPLC, solvent: 0–5% acetone in n-hexane; flow rate: 30 ml/min) to give kumatakenin (29 mg). Fraction 5, obtained after elution with 15–20% EtOAc in n-hexane, was subjected to MPLC (solvent: 0–15% EtOAc in n-hexane; press: 30 ml/min) to give formononetin (10 mg), **3** (40 mg), **4** (15 mg), **5** (25 mg) and isokaempferide (6 mg).

3.4. (*S*)-Platyisoflavanone A (**1**)

Amorphous powder, m.p. 160–164 °C, $[\alpha]_D^{24.0}$ = +14.1 (CH_2Cl_2, c = 1%, w/v). UV: λ_{max} (CH_2Cl_2) 288 nm. CD (CH_2Cl_2, *c* 0.0047): $[\theta]_{350}$ −100, $[\theta]_{344}$ −120, $[\theta]_{332}$ 0, $[\theta]_{315}$ +490, $[\theta]_{294}$ 0, $[\theta]_{285}$ −340, $[\theta]_{255}$ 0, $[\theta]_{245}$ +50. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2). EI-MS *m*/*z* (rel. int.): 384 (80, $[M]^+$), 232 (100), 217 (69), 205 (83), 189 (47), 177 (54), 152 (65),124 (95), 115 (30), 91 (32),69 (32), 51 (53), 45 (54). HREI-MS $[M]^+$: found *m*/*z* 384.1597 for $C_{22}H_{24}O_6$ (calcd. 384.1567).

3.5. (±)-Platyisoflavanone B (**2**)

Amorphous white powder, m.p. 133–138 °C. UV: λ_{max} (CH_2Cl_2) 288 nm. $[\alpha]_D^{24.0}$ = 0 (CH_2Cl_2, c = 1%, w/v), UV: λ_{max} (CH_2Cl_2) 294 nm: CD (CH_2Cl_2, *c* 0.0046): no Cotton effect. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2). EI-MS *m*/*z* (rel. int.): 370 (92, $[M]^+$), 314 (40), 218 (100), 163 (65), 162 (70), 153 (43), 51 (32). HREI-MS $[M]^+$: found *m*/*z* 370.1400 $C_{21}H_{22}O_6$ (calcd. 370.1411).

3.6. Platyisoflavanone C (**3**)

Amorphous white solid. UV: λ_{max} (CH_2Cl_2) 290 nm. $[\alpha]_D^{24.0}$ = 0 (CH_2Cl_2, c = 1%, w/v). ^{1}H NMR (Table 1). ^{13}C NMR (Table 2). EI-MS *m*/*z* (rel. int.): 386 (10, $[M]^+$), 328 (100), 234 (8), 201 (14), 180 (30), 176 (60), 161 (25), 83 (26), 59 (28). HREI-MS $[M]^+$: found *m*/*z* 386.1354 $C_{21}H_{22}O_7$ (calcd. mass 386.1366).

3.7. Platyisoflavanone D (**4**)

Amorphous white solid. UV: λ_{max} (CH_2Cl_2) 289 nm. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2). EI-MS *m*/*z* (rel. int.): 372 (60, $[M]^+$),

220 (48), 187 (45), 153 (86), 149 (46), 124 (28), 109 (21), 91 (30), 69 (50), 46 (100). HREI-MS [M]$^+$: found m/z 372.1202 for $C_{20}H_{20}O_7$ (calcd. 372.1209).

3.8. 7,2′-O,O-Dimethylplatyisoflavanone D (4a)

Amorphous white solid. UV: λ_{max} (CH_2Cl_2) 295 nm. ^{1}H NMR (600 MHz, CD_2Cl_2): δ 12.23 (1H, s, 5-OH), 6.79 (1H, d, J = 8.4 Hz, H-6′), 6.51 (1H, d, J = 8.4 Hz, H-5′), 5.98 (1H, d, J = 1.8 Hz, H-6), 5.95 (1H, m, H-8), 4.38 (1H, m, H-2b), 4.37 (1H, m, H-2a), 4.24 (1H, dd, J = 10.8 and 6.0 Hz, H-3), 3.75 (3H, s, 7-OCH_3), 3.73 (1H, m, H-3″), 3.65 (3H, s, 2′-OCH_3), 2.93 (1H, dd, J = 11.4 and 11.4 Hz, H-4″a), 2.68 (1H, m, H-4″b), 1.25 (3H, s, 2″-CH_3), 1.24 (3H, s, 2″-CH_3). ^{13}C NMR (Table 2). EI-MS m/z (rel. int.): 400 (35, [M]$^+$), 235 (15), 234 (100), 201 (15), 163 (25), 149 (13), 91 (10). HREI-MS [M]$^+$:found m/z 400.1510 for $C_{22}H_{24}O_7$ (calcd. 400.1522).

3.9. 7,4′-O,O-Dimethylglyasperin F (5a)

Amorphous white solid. ^{1}H NMR (600 MHz, CD_2Cl_2): δ 12.17 (1H, s, 5-OH), 6.79/6.78 (1H, d, J = 8.4 Hz, H-6′), 6.55 (1H, d, J = 10.2 Hz, H-4″), 6.34 (1H, d, J = 8.4 Hz, H-5′), 5.98 (1H, m, H-6), 5.92 (1H, m, H-8), 4.37 (2H, m, H-2), 4.26/4.23 (1H, dd, J = 10.7 and 6.3 Hz, H-3), 3.74 (3H, s, 7-OCH_3), 3.72 (1H, dd, J = 10.8 and 5.4 Hz, H-3″), 3.66/3.65 (3H, s, 2′-OCH_3), 2.94/2.92 (1H, dd, J = 17.4 and 4.8 Hz, H-4″a), 2.69/2.66 (1H, dd, J = 17.4 and 5.7 Hz, H-4″b), 1.25 (3H, s, 2″-CH_3), 1.24 (3H, s, 2″-CH_3). ^{13}C NMR (Table 2). EI-MS m/z (rel. int.): 382 (27, [M]$^+$), 368 (35), 367 (100), 201 (68), 186 (24), 45 (25), 43 (37). HREI-MS [M]$^+$: found m/z 382.1400 for $C_{22}H_{22}O_6$ (calcd. 382.1416).

3.10. Methylation of platyisoflavanone D (4) and glyasperin F (5)

Five drops of dimethyl sulphate and K_2CO_3 (300 mg) were added to compound **4** (10 mg) in acetone (6 ml) and stirred at room temperature for 24 h. The product was filtered, concentrated and purified by prep. TLC (solvent: 25% EtOAc in n-hexane) to afford compound **4a** (5 mg, 62%). Similarly, compound **5** (10 mg) was methylated using the same conditions and the product purified on prep. TLC (solvent: 40% EtOAc in n-hexane) to give compound **5a** (5.6 mg, 52%).

3.11. Anti-TB assays

The MICs of test samples against *M. tuberculosis* were determined by the microplate alamar blue assay (MABA) as described by Falzari *et al.* (2005) and by the low-oxygen-recovery assay (LORA) as described by Cho *et al.* (2007). Rifampicin was used as a standard drug with MIC values of 0.06 and 1.8 μM in the MABA and LORA tests, respectively.

3.12. Cytotoxicity test

The cytotoxicity test was carried on the vero cells as described by Falzari et al. (2005). Rifampicin did not show significant cytotoxicity (IC_{50} value 183 μM).

Acknowledgements

One of the authors (I.G.) is grateful to the German Academic Exchange Services (DAAD) for a Scholarship that was awarded through the Natural Products Research Network for Eastern and Central Africa (NAPRECA). Mr. S.G. Mathenge is acknowledged for identification of the plant. We thank Mr. Klaus Gast for recording the CD spectra. Dr. Joshua Odingo, of the Infectious Disease Institute, Seattle, Washington, USA, is acknowledged for facilitating the anti-TB and cytotoxicity tests.

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B: Abstract of Second Paper Resubmitted to *Journal of Natural Products*

Flemingins G-P, Cytotoxic and Antioxidant Constituents of the Leaves of *Flemingia grahamiana*

Ivan Gumula,[†,‡] John Patrick Alao,[‡] Isaiah Omolo Ndiege,[⊥] Per Sunnerhagen,[‡] Abiy Yenesew[,†] and Máté Erdélyi[*,‡,§]*

[†]Department of Chemistry, University of Nairobi, POB 30197-00100, Nairobi, Kenya

[‡]Department of Chemistry and Molecular Biology, University of Gothenburg, SE-412 96 Gothenburg, Sweden

[⊥]Office of the Vice Chancellor, Kyambogo University, POB 1, Kyambogo-Kampala, Uganda

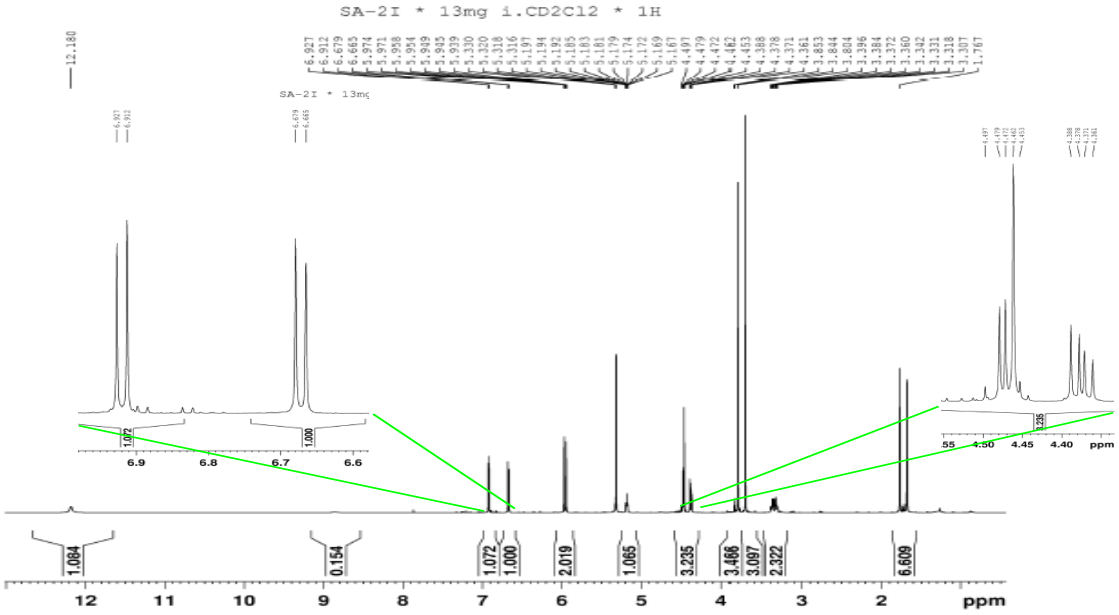
[§]Swedish NMR Centre, University of Gothenburg, SE-40530 Gothenburg, Sweden

ABSTRACT: Ten new chalcones, named flemingin G, H, I, J, K, L, M, N, O and P along with the known flemingins A, B, and C, deoxyflemingin and emodin were isolated from the leaf extract of *Flemingia grahamiana*. The isolated chalcones have a geranyl substituent modified into a chromene ring possessing a residual chain, as shown by spectroscopic methods. The leaf extract showed IC_{50}= 5.9 μg/mL DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity. The chalcones flemingin A, B, C, G, and H were very active in the DPPH radical scavenging assay (ED_{50}= 4.4-8.9 μM) whilst flemingins A and C showed strong cytotoxicity against MCF-7 human breast cancer cells (IC_{50}= 8.9 and 7.6 μM, respectively).

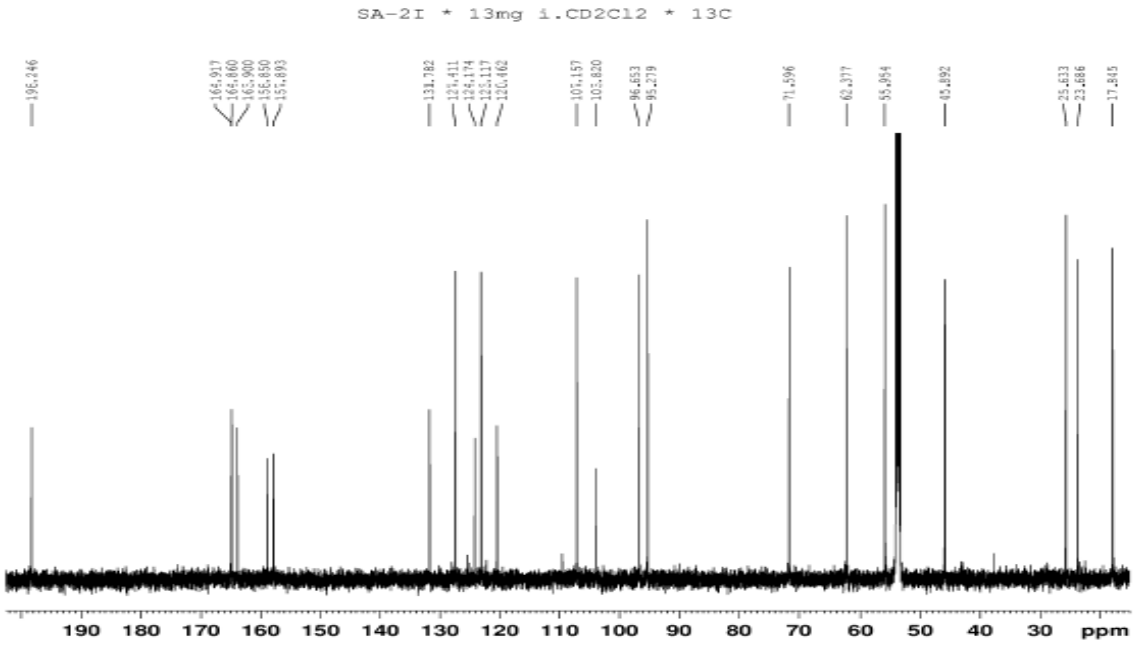
APPENDICES

PART II: SPECTRA FOR THE COMPOUNDS DISCUSSED

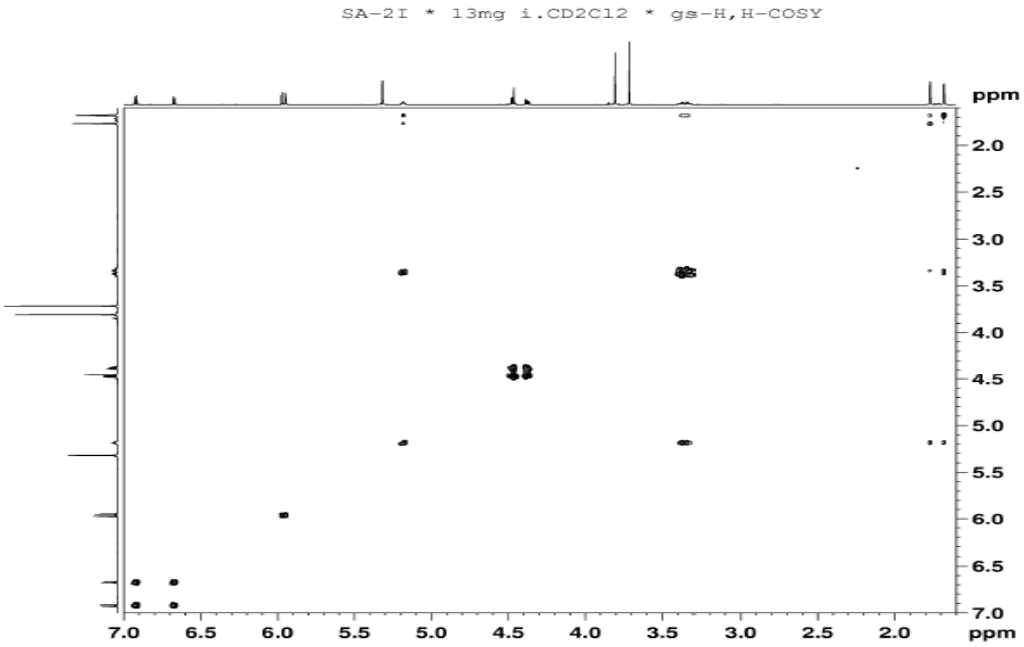
Appendix 1A: ^{1}H (CD_2Cl_2, 600 MHz) NMR spectrum of platyisoflavanone A (**163**)



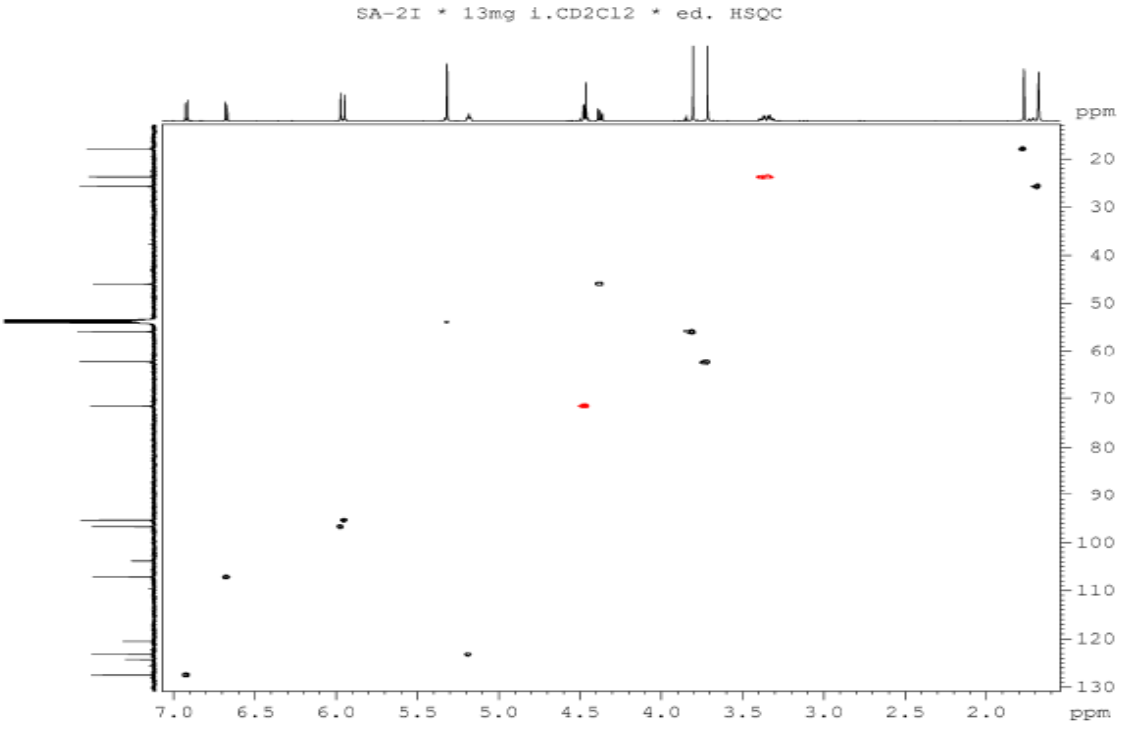
Appendix 1B: ^{13}C (CD_2Cl_2, 150 MHz) NMR spectrum of platyisoflavanone A (**163**)



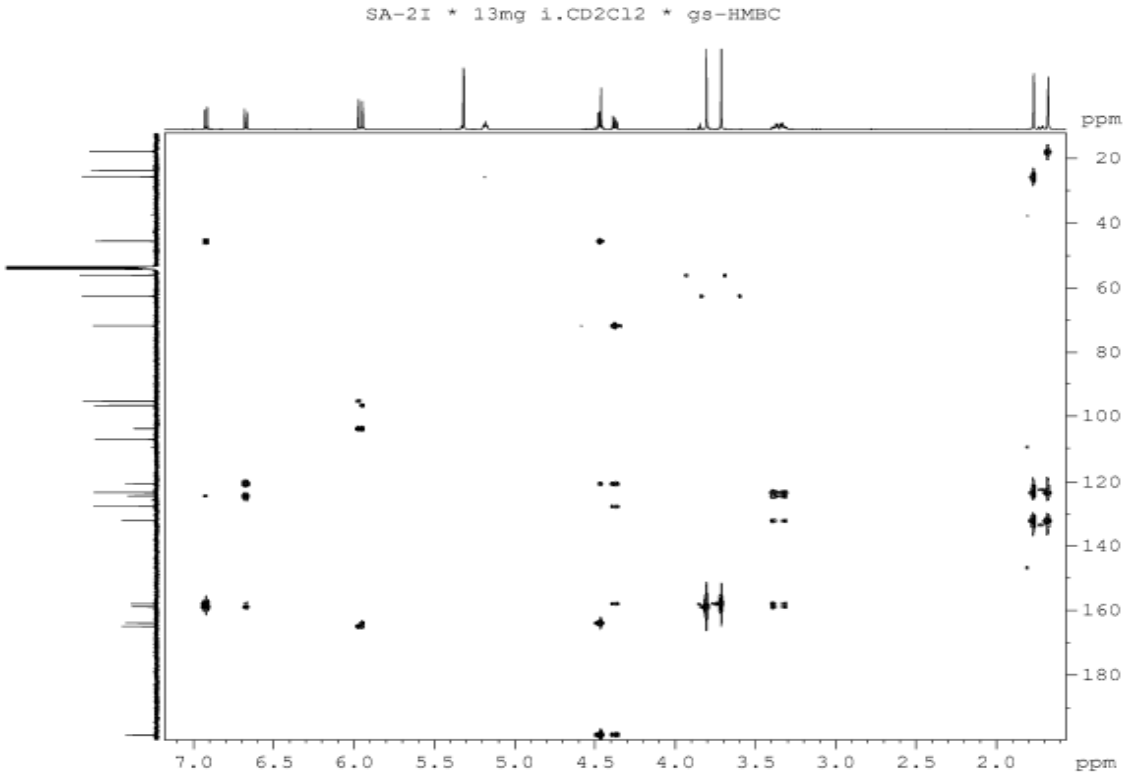
Appendix 1C: ^{1}H-^{1}H gCOSY (CD_2Cl_2, 600 MHz) spectrum of platyisoflavanone A (**163**)



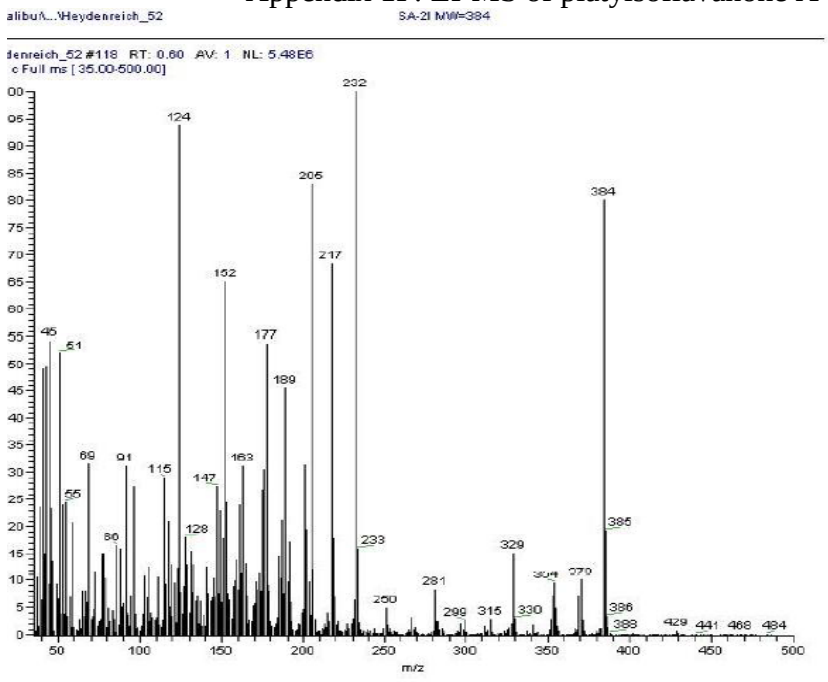
Appendix 1D: gHSQC (CD_2Cl_2, 600 MHz) spectrum of platyisoflavanone A (**163)**



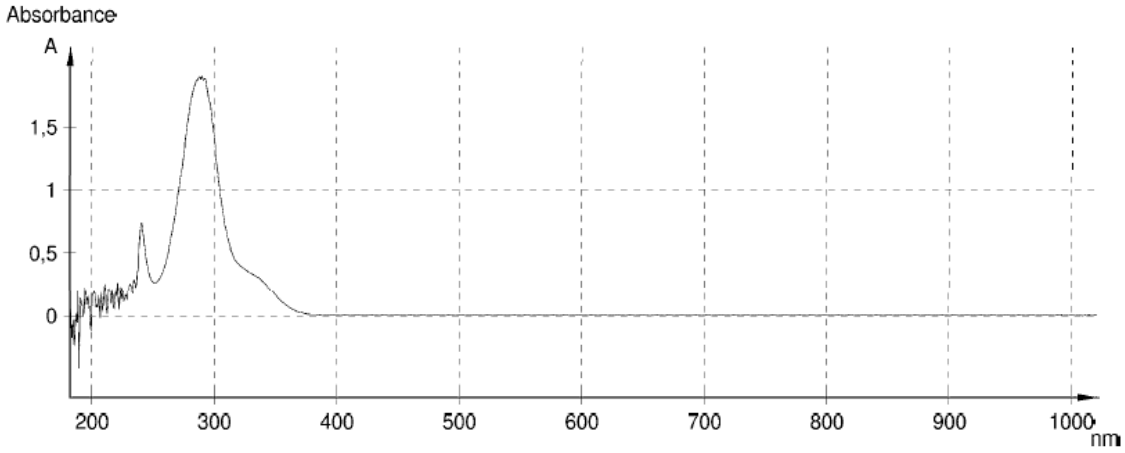
Appendix 1E: gHMBC (CD_2Cl_2, 600 MHz) spectrum of platyisoflavanone A (**163)**



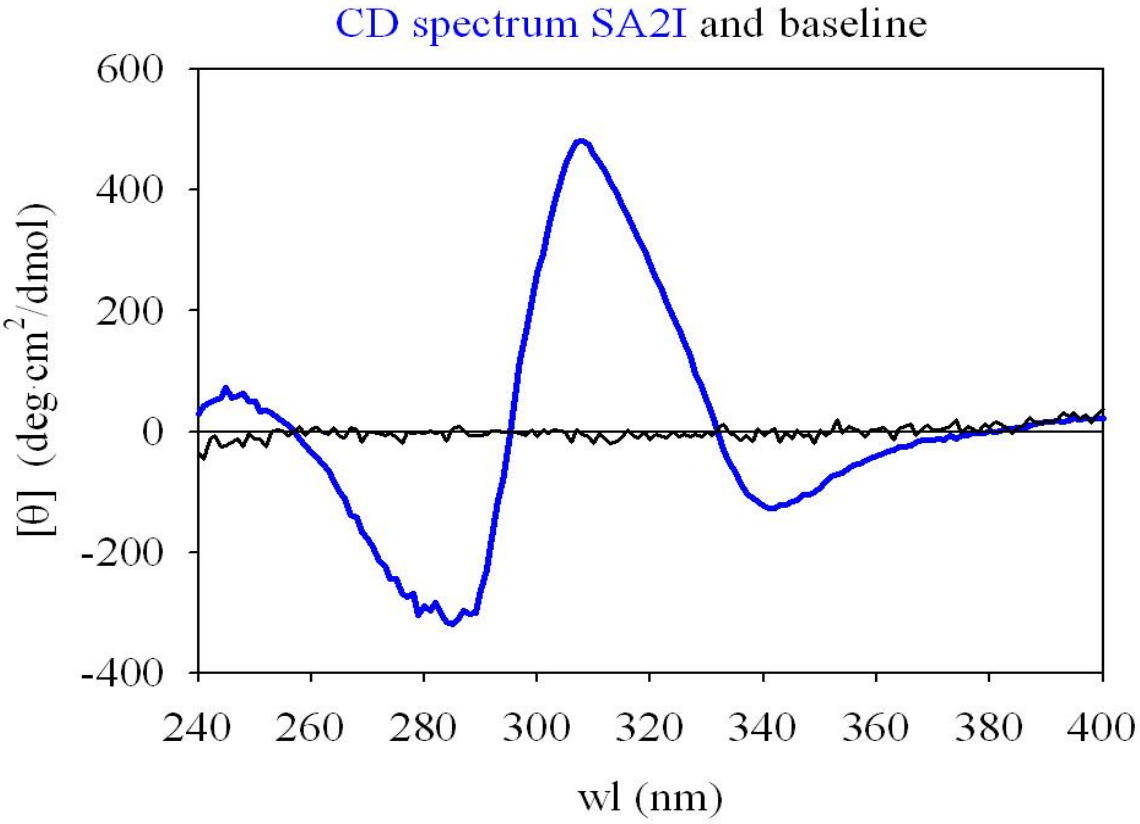
Appendix 1F: EI-MS of platyisoflavanone A (**163**)



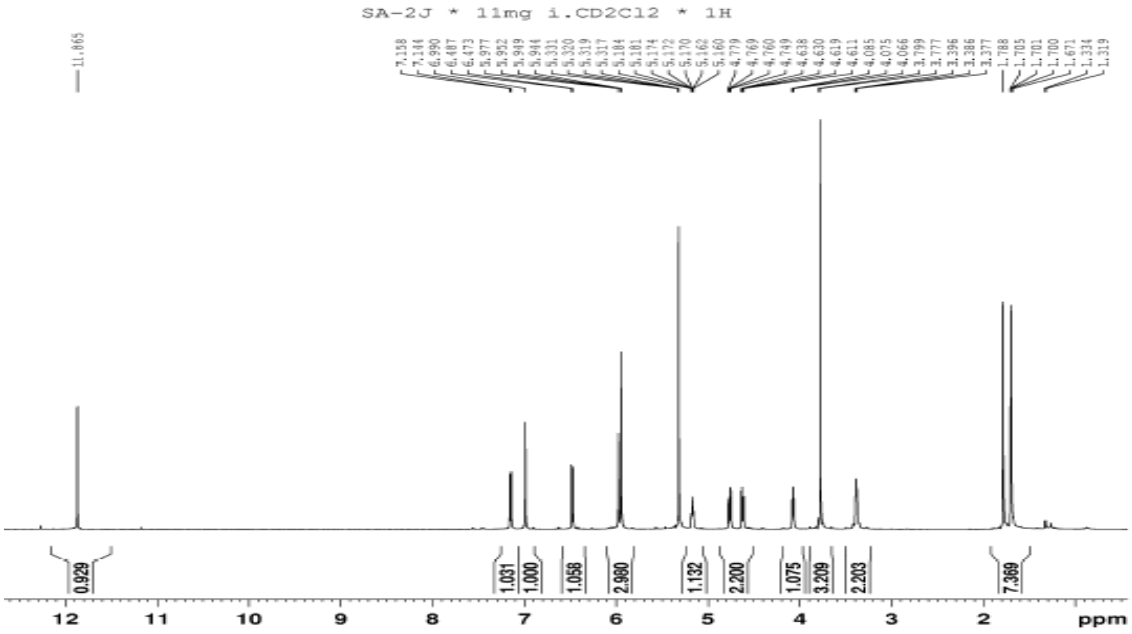
Appendix 1G: UV (CH_2Cl_2) spectrum of platyisoflavanone A (**163**)



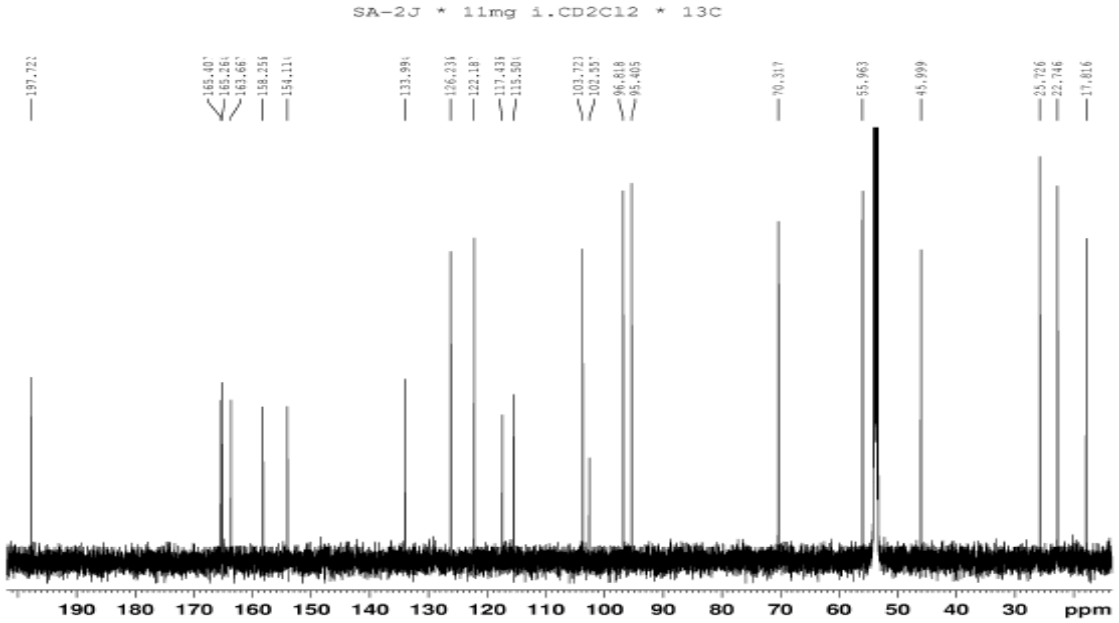
Appendix 1H: CD spectrum of platyisoflavanone A (**163**)



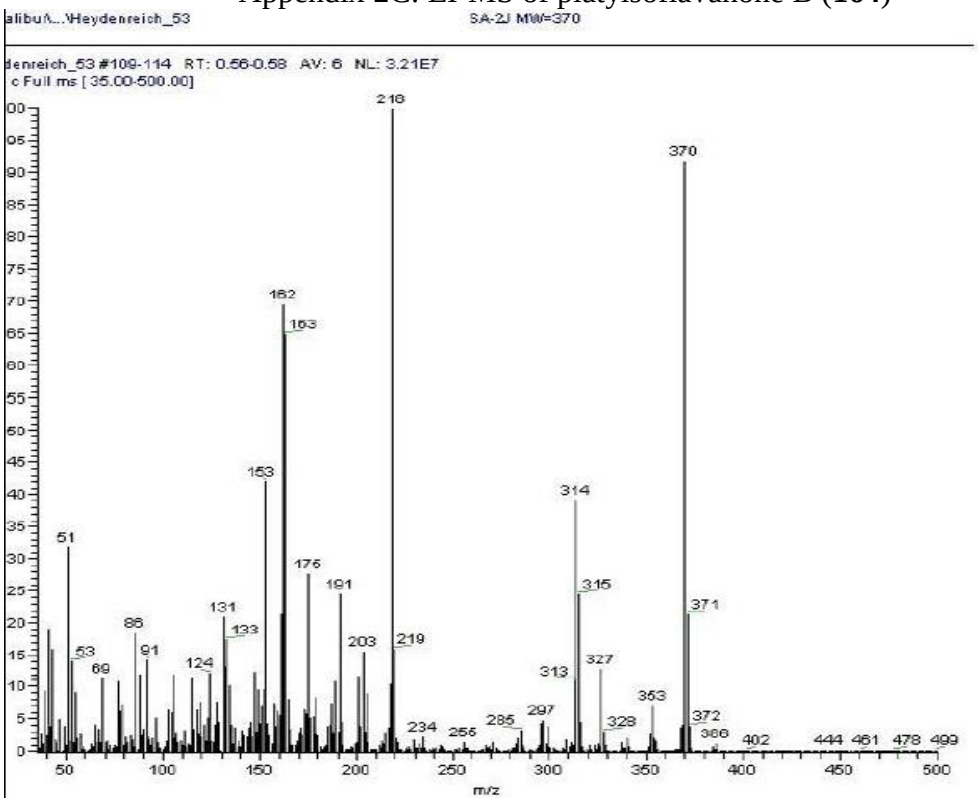
Appendix 2A: ^{1}H (CD_2Cl_2, 600 MHz) NMR spectrum of platyisoflavanone B (**164**)



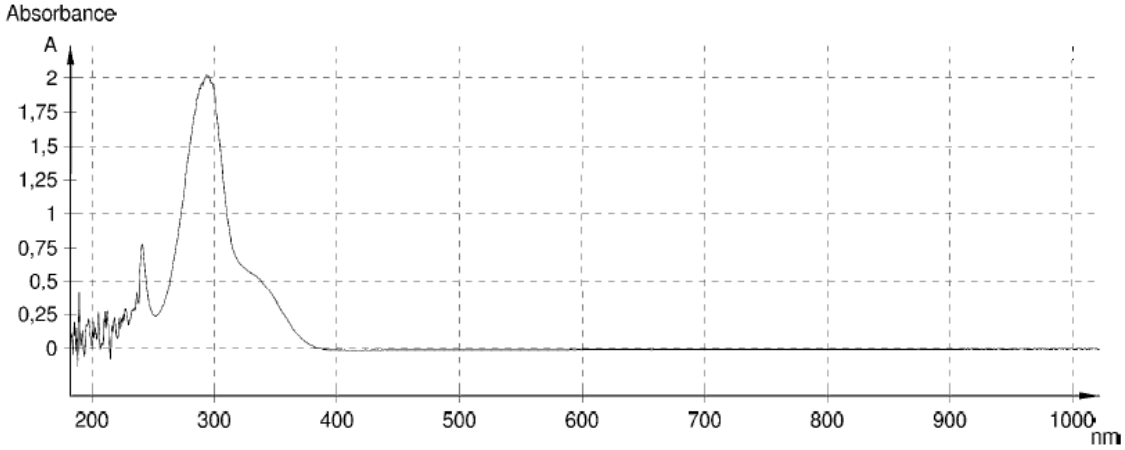
Appendix 2B: ^{13}C (CD_2Cl_2, 150 MHz) NMR spectrum of platyisoflavanone B (**164**)



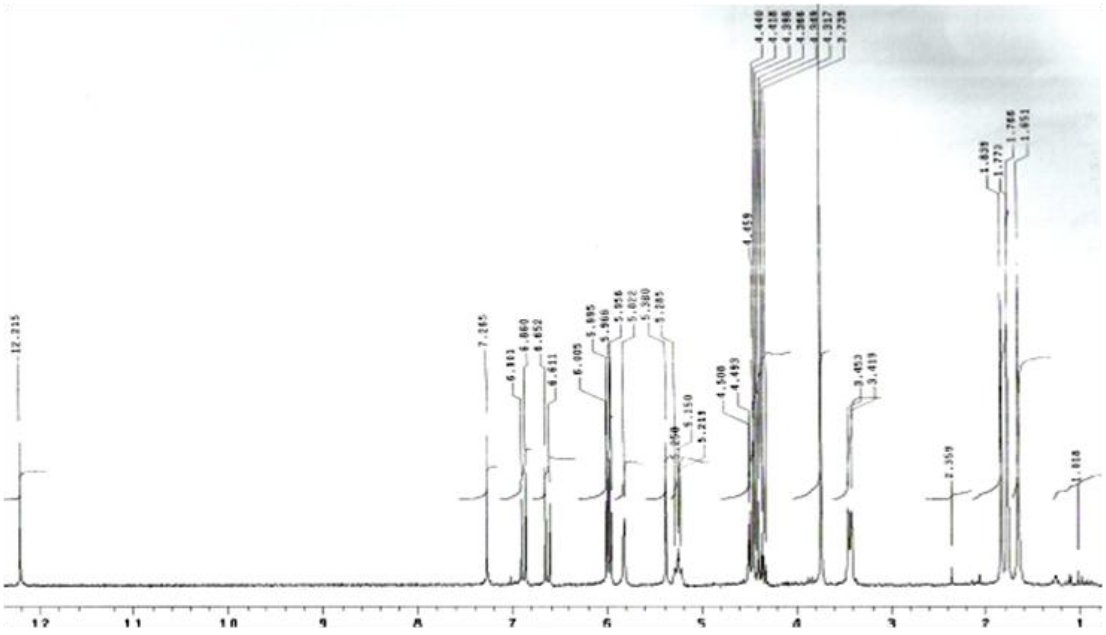
Appendix 2C: EI-MS of platyisoflavanone B (**164**)



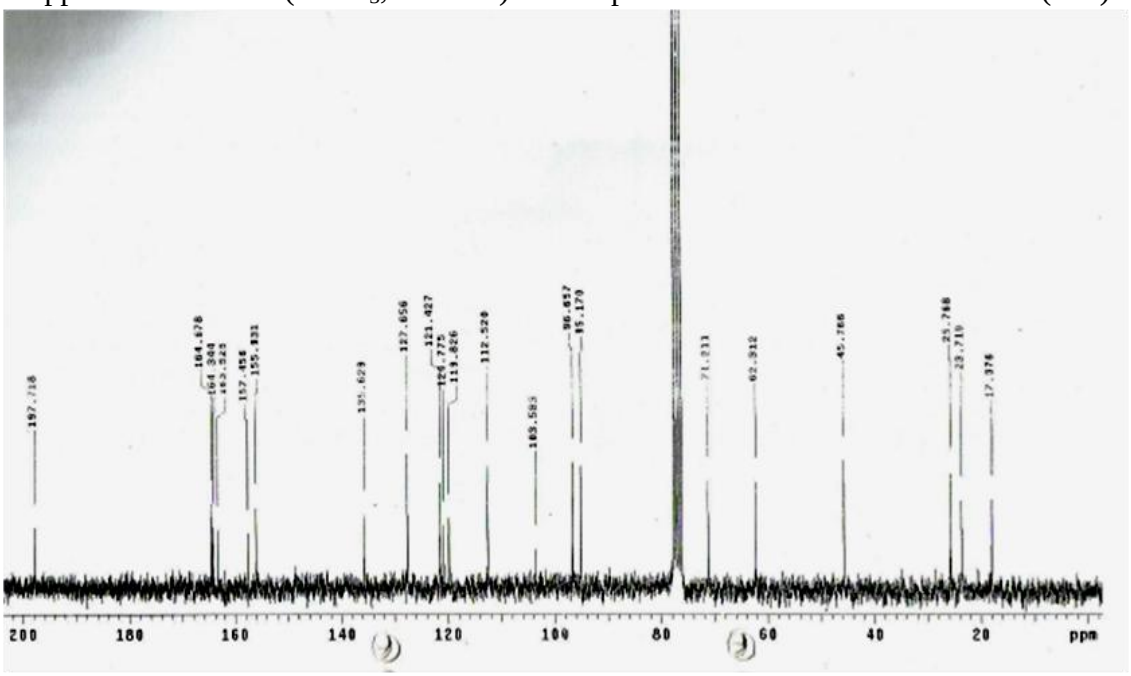
Appendix 2D: UV (CH_2Cl_2) spectrum of platyisoflavanone B (**164**)



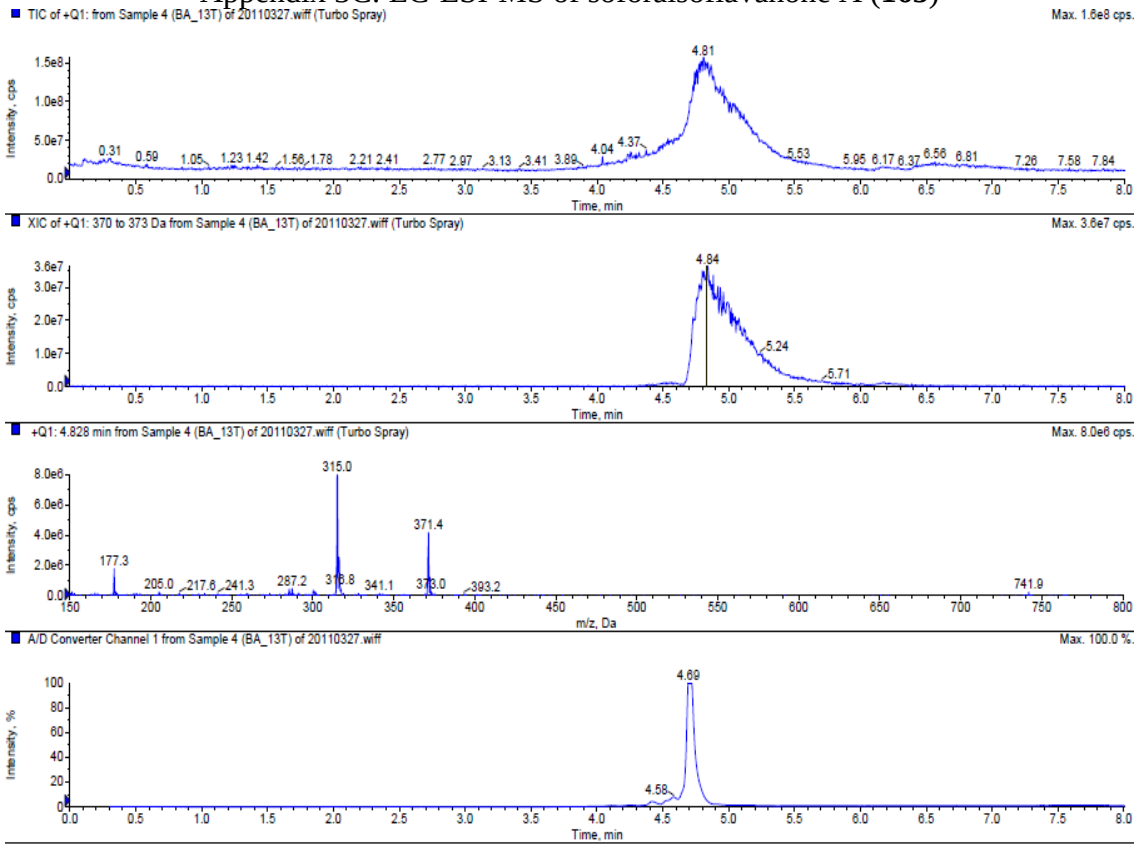
Appendix 3A: ^{1}H ($CDCl_3$, 200 MHz) NMR spectrum of soforaisoflavanone A (**165**)



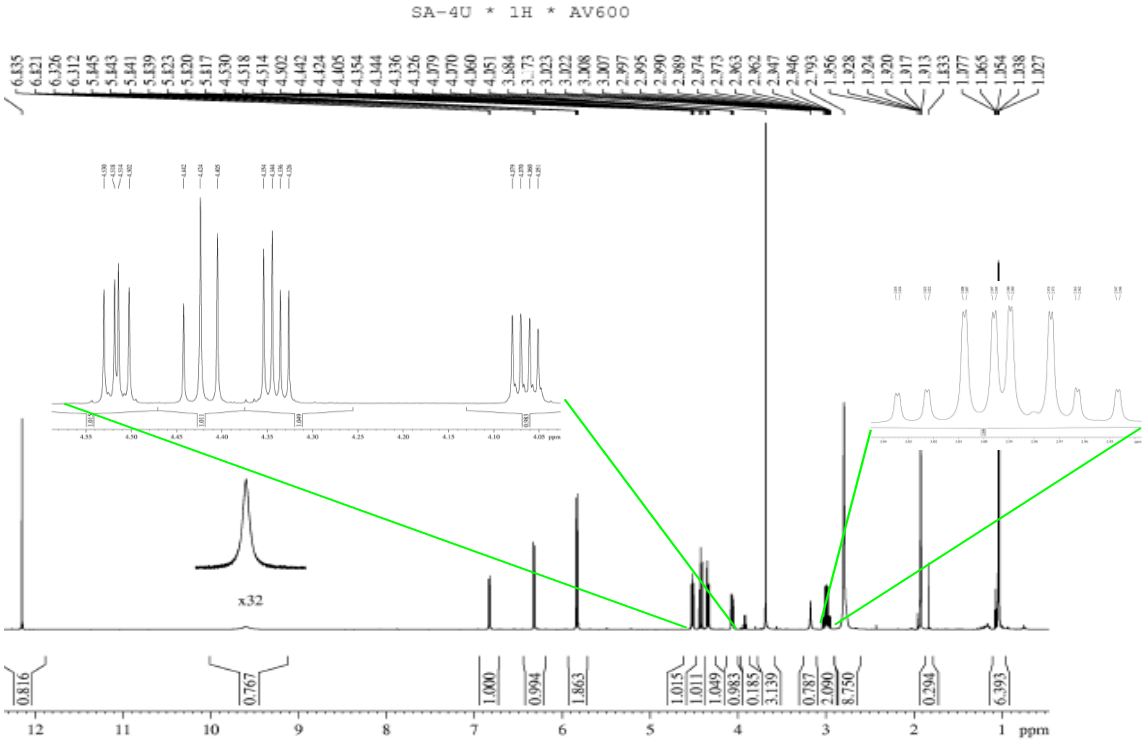
Appendix 3BA: ^{13}C ($CDCl_3$, 50 MHz) NMR spectrum of soforaisoflavanone A (**165**)



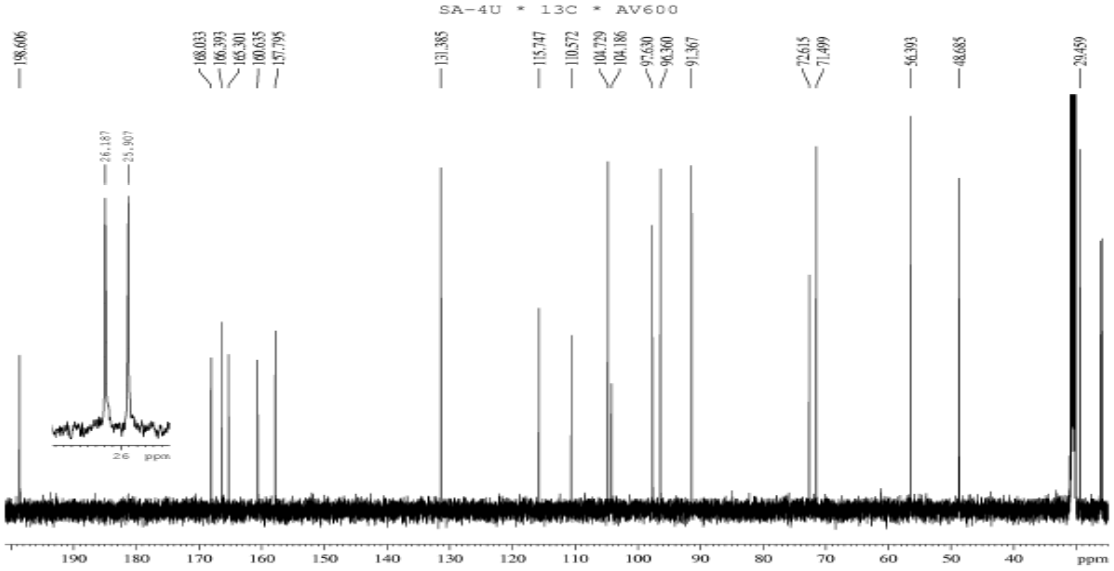
Appendix 3C: LC-ESI-MS of soforaisoflavanone A (**165**)



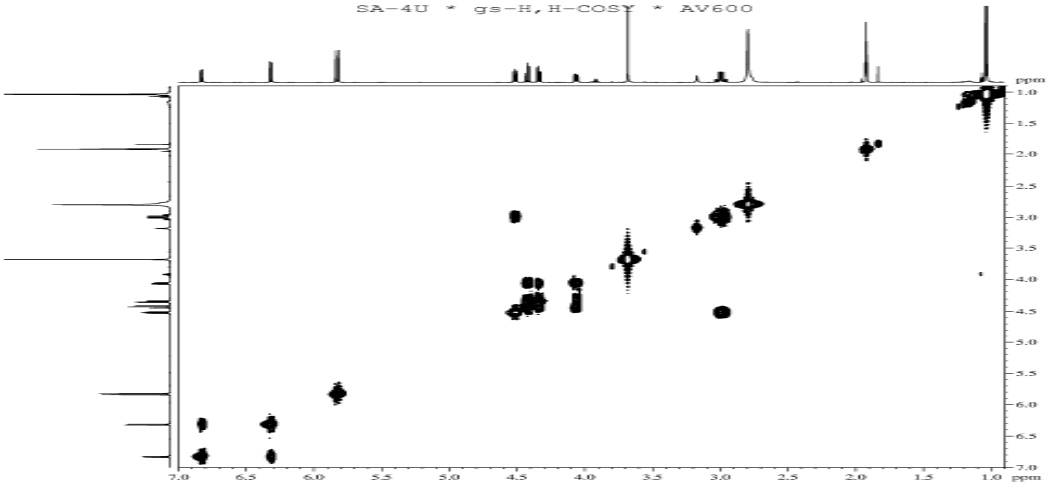
Appendix 4A: ^{1}H (CD_2Cl_2, 600 MHz) NMR spectrum for platyisoflavanone C (**166**)



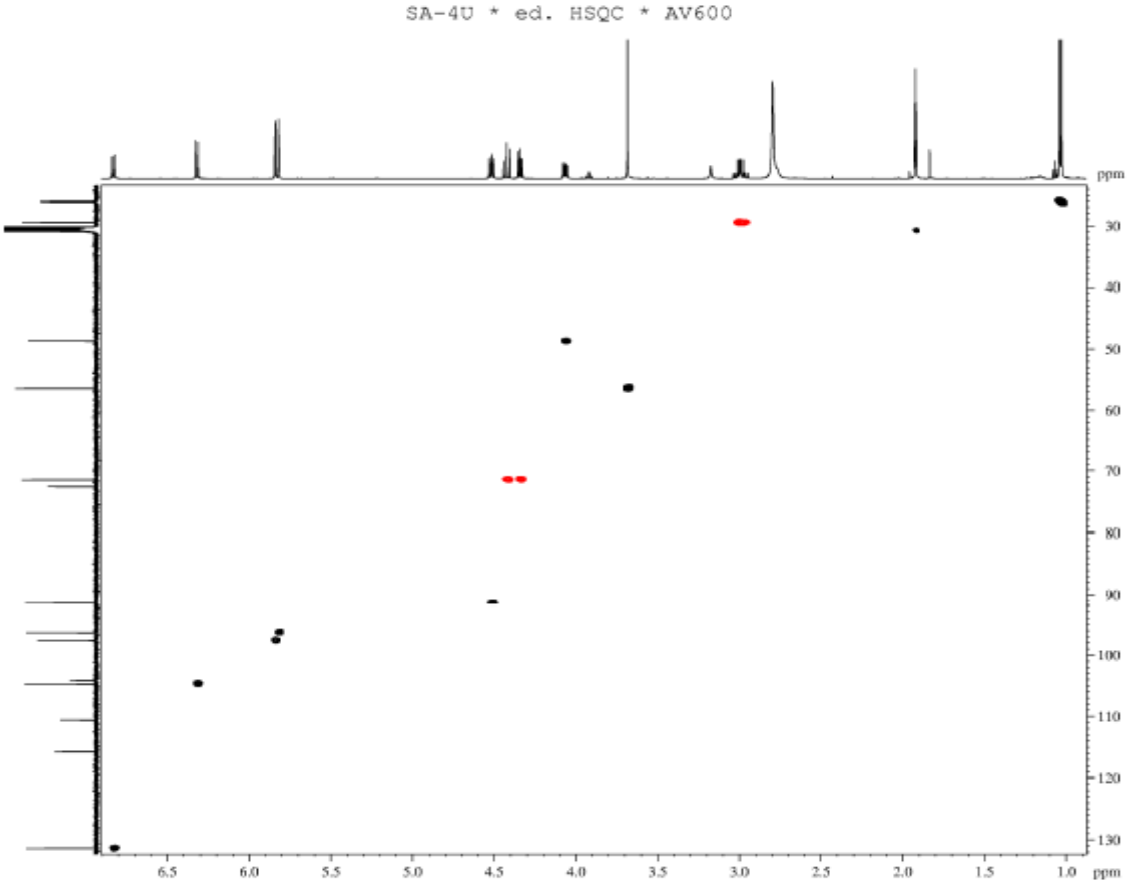
Appendix 4B: ^{13}C (CD_2Cl_2, 150 MHz) NMR spectrum for platyisoflavanone C (**166**)



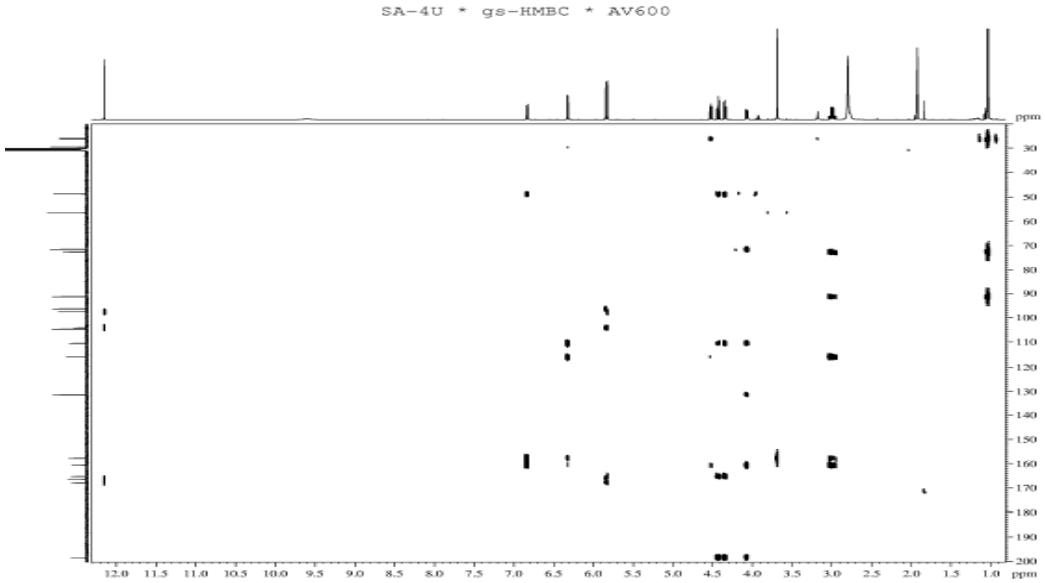
Appendix 4C: ^{1}H-^{1}H gCOSY (CD_2Cl_2, 600 MHz) spectrum of platyisoflavanone C (**166)**



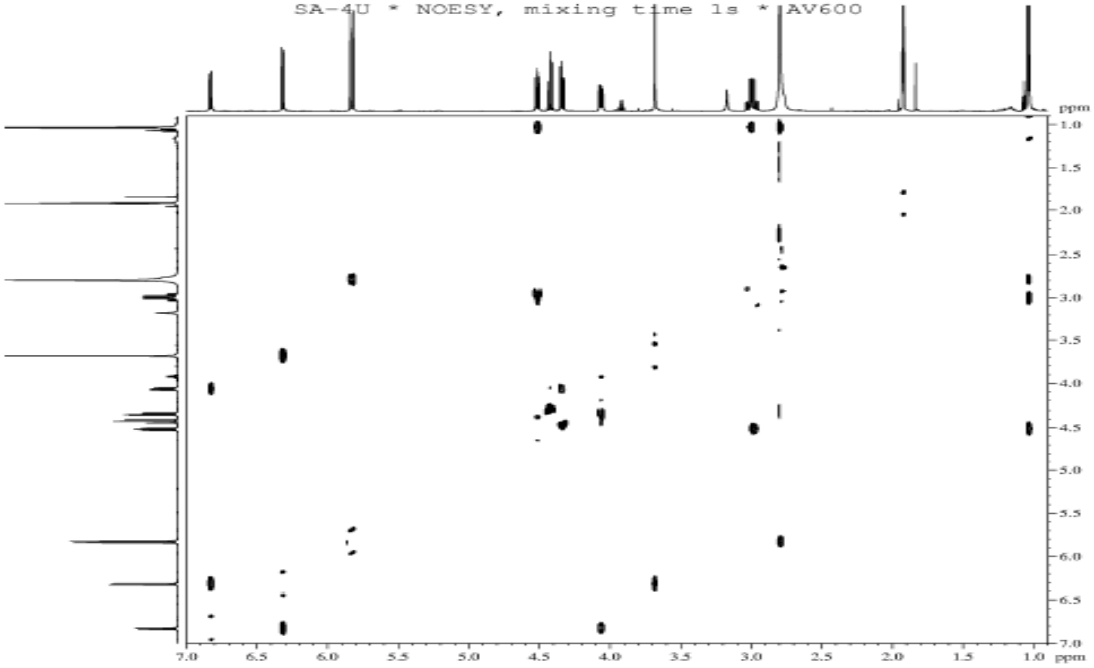
Appendix 4D: gHSQC (CD_2Cl_2, 600 MHz) spectrum of platyisoflavanone C (**166)**



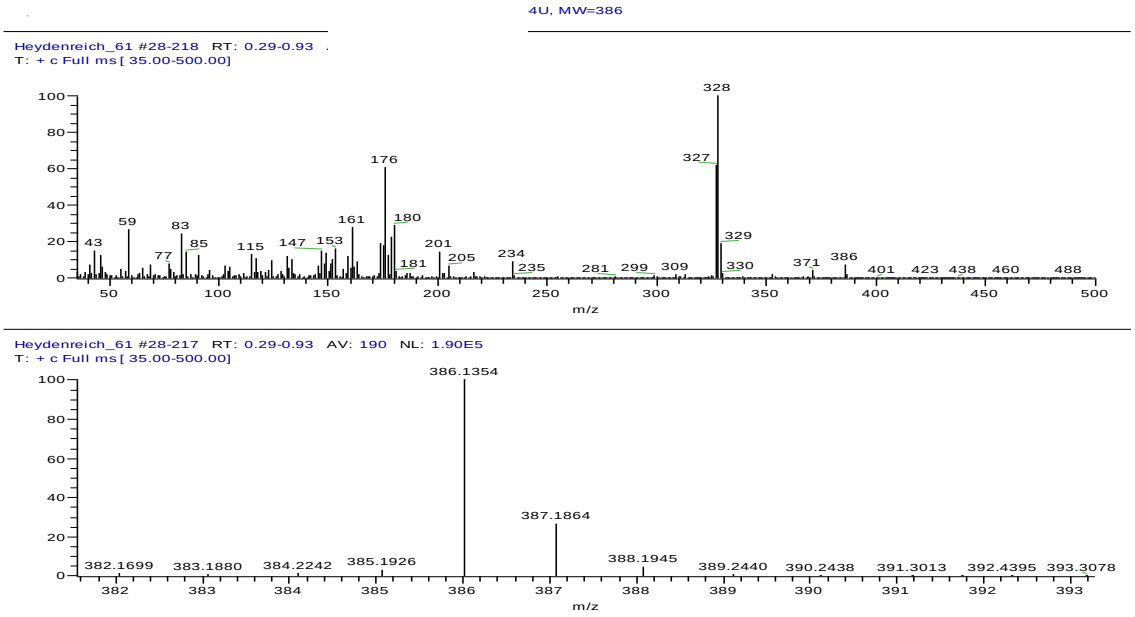
Appendix 4E: gHMBC (CD_2Cl_2, 600 MHz) spectrum of platyisoflavanone C (**166)**



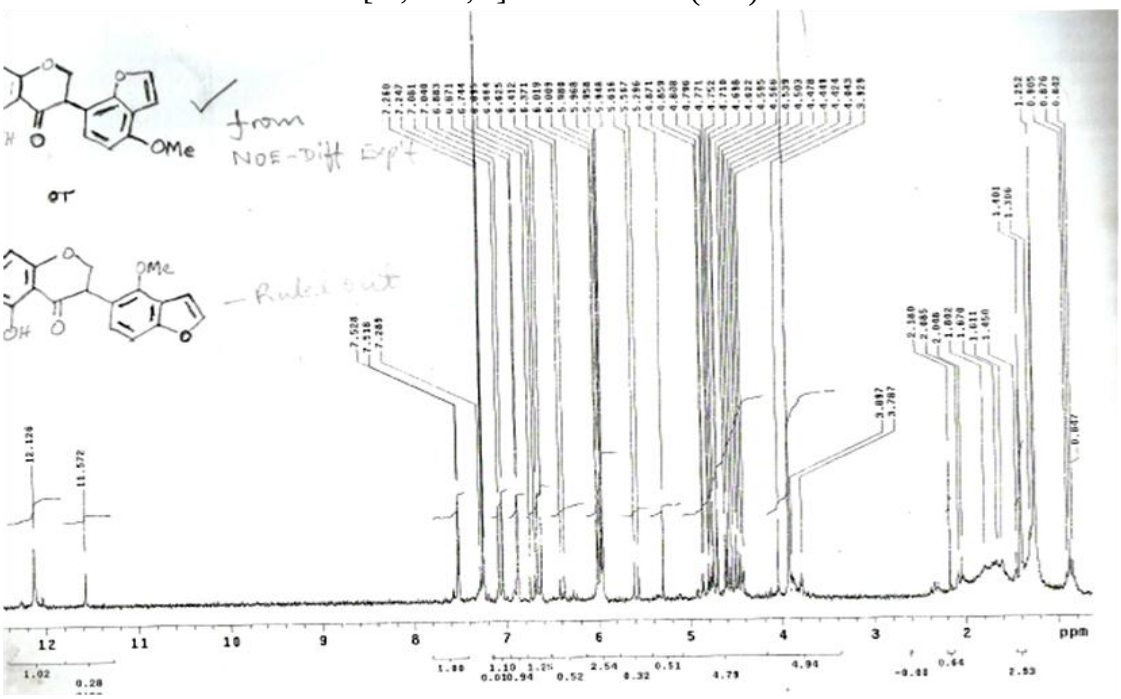
Appendix 4F: NOESY (CD_2Cl_2, 600 MHz) spectrum of platyisoflavanone C (**166)**



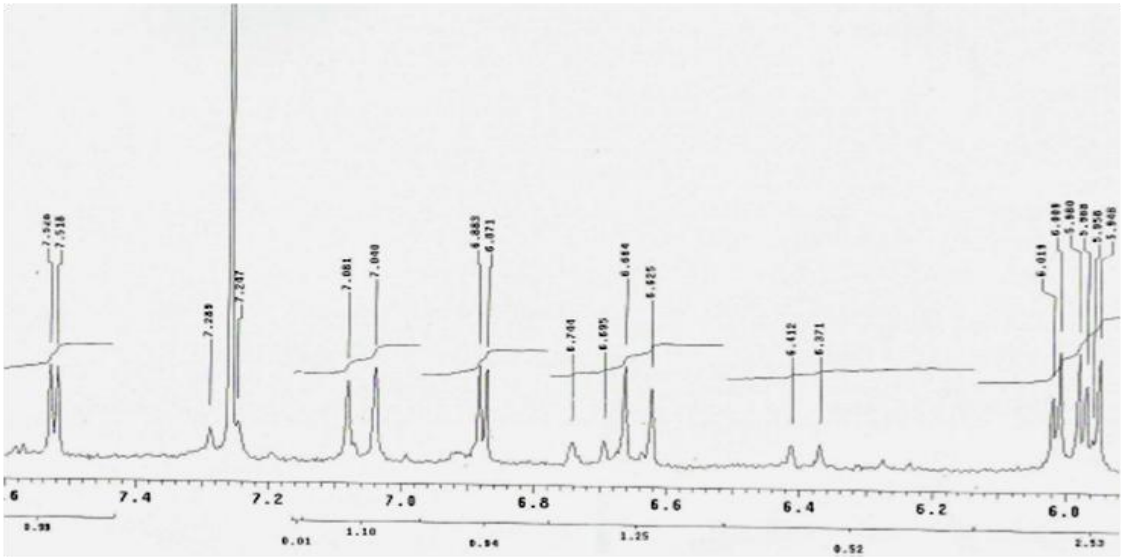
Appendix 4G: EI and HREI-MS of platyisoflavanone C (**166)**



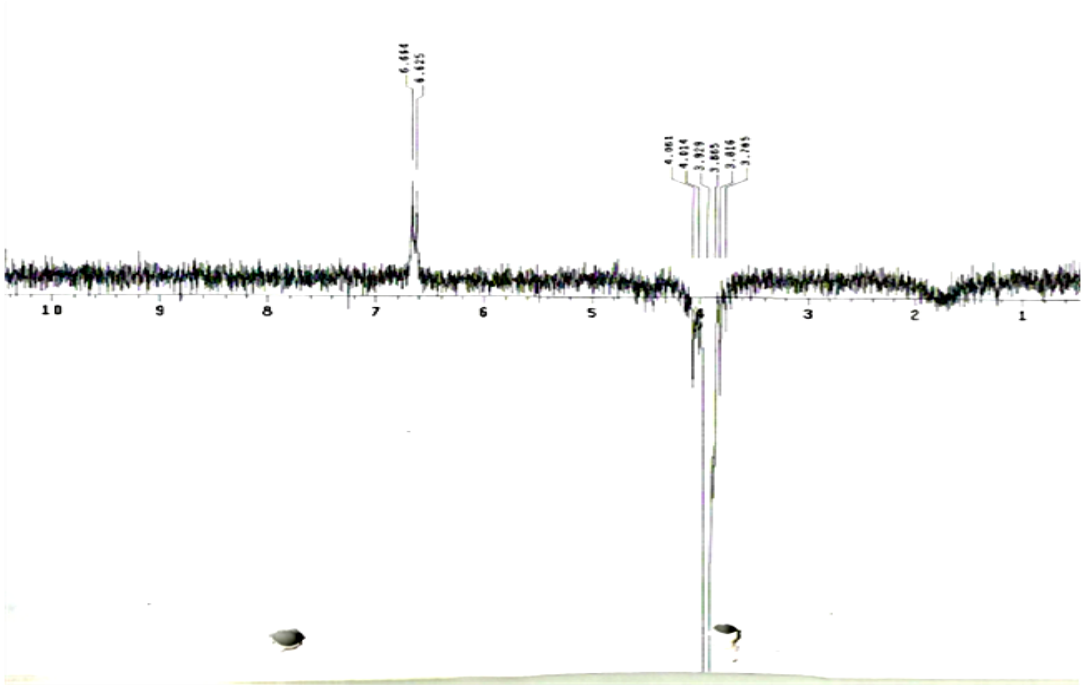
Appendix 5A: ^{1}H ($CDCl_3$, 200 MHz) NMR spectrum of 5,7-dihydroxy-4'-methoxyfurano-[4",5":3',2']-isoflavanone (**167**)



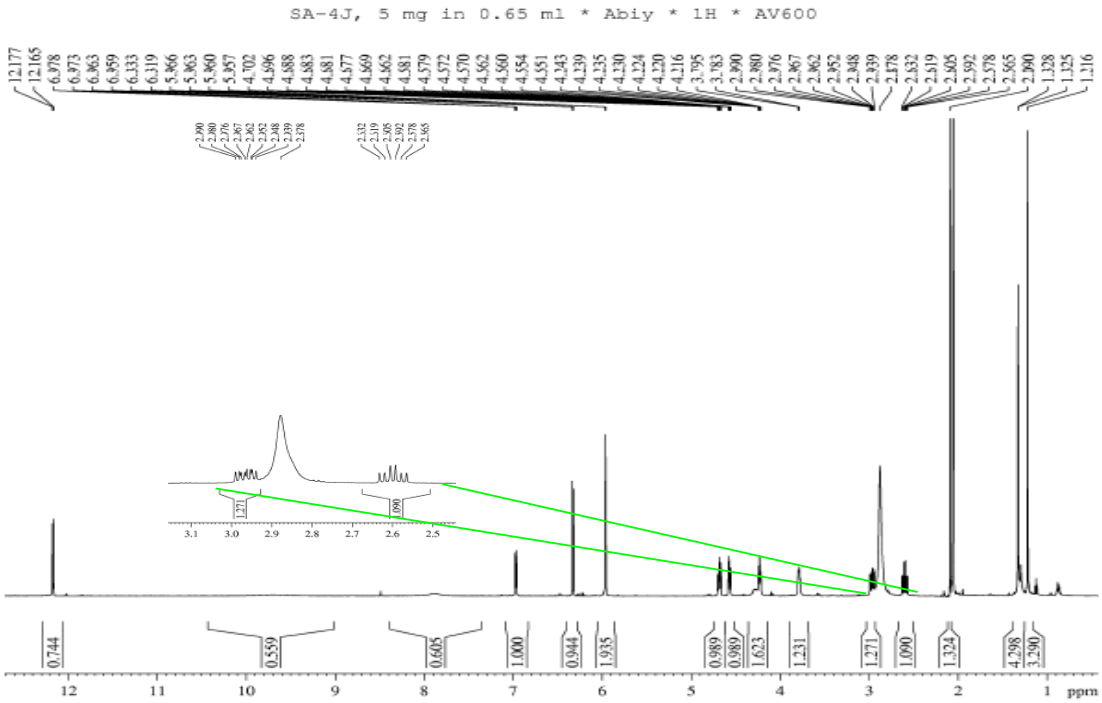
Appendix 5B: expanded ^{1}H ($CDCl_3$, 200 MHz) NMR spectrum of 5,7-dihydroxy-4'-methoxyfurano-[4",5":3',2']-isoflavanone (**167**)



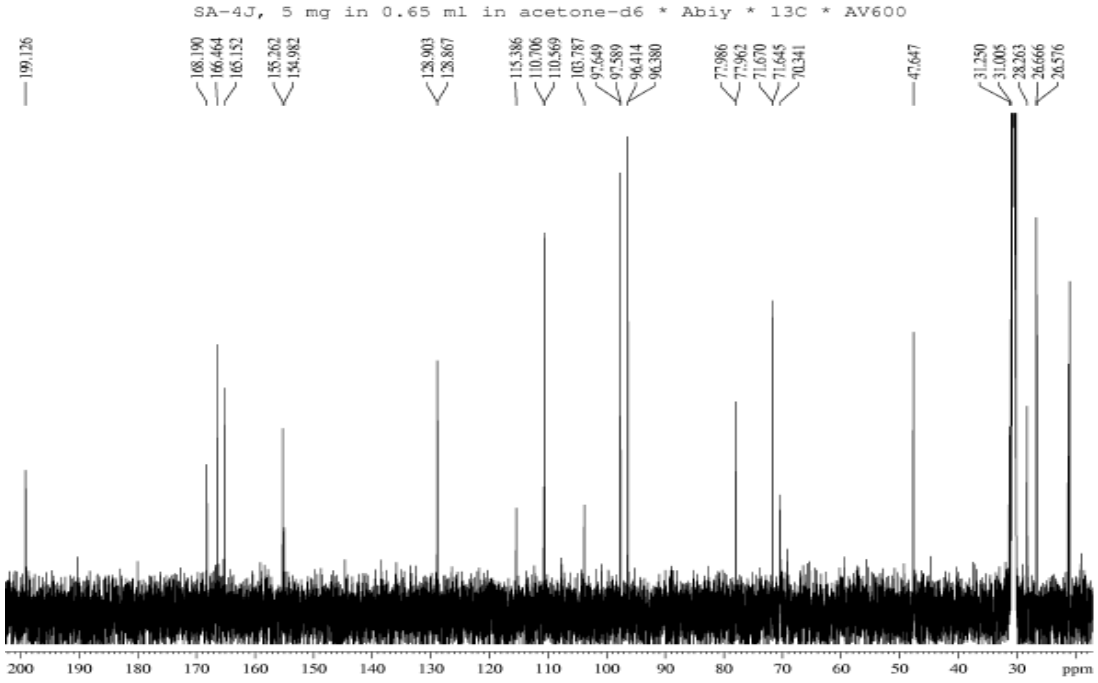
Appendix 5C: NOE-DIFF ($CDCl_3$, 200 MHz) spectrum of 5,7-dihydroxy-4'-methoxyfurano-[4",5":3',2']-isoflavanone (**167**)



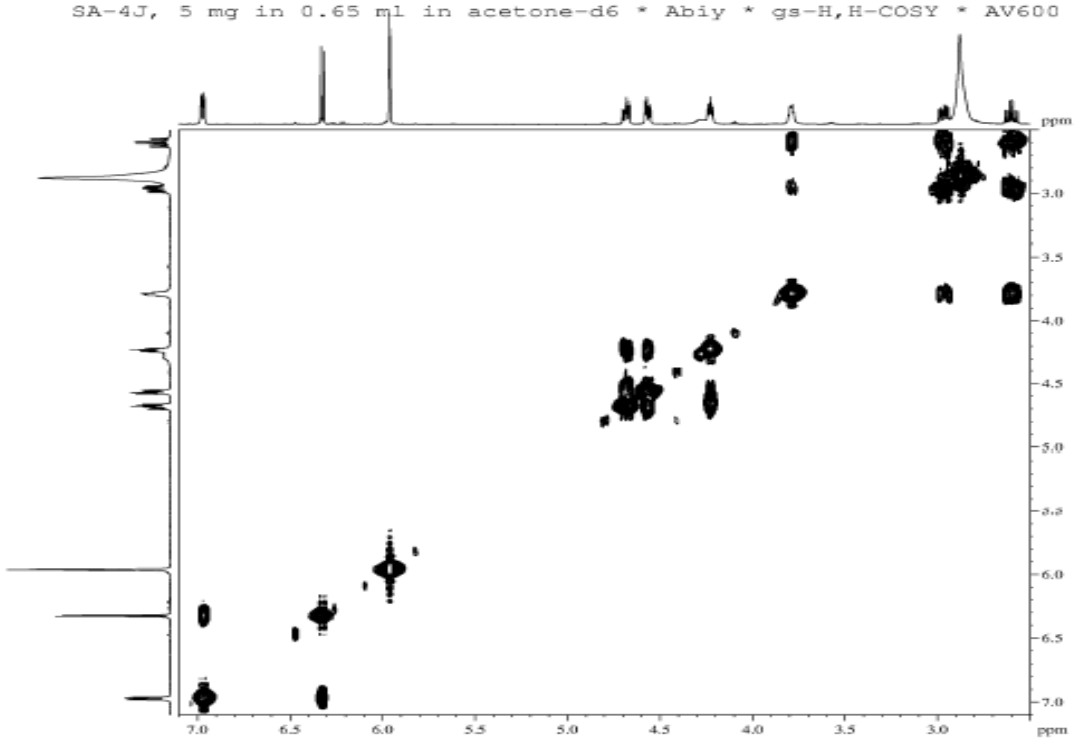
Appendix 6A: ^1H ($(CH_3)_2CO$, 600 MHz) NMR spectrum for platyisoflavanone D (**168**)



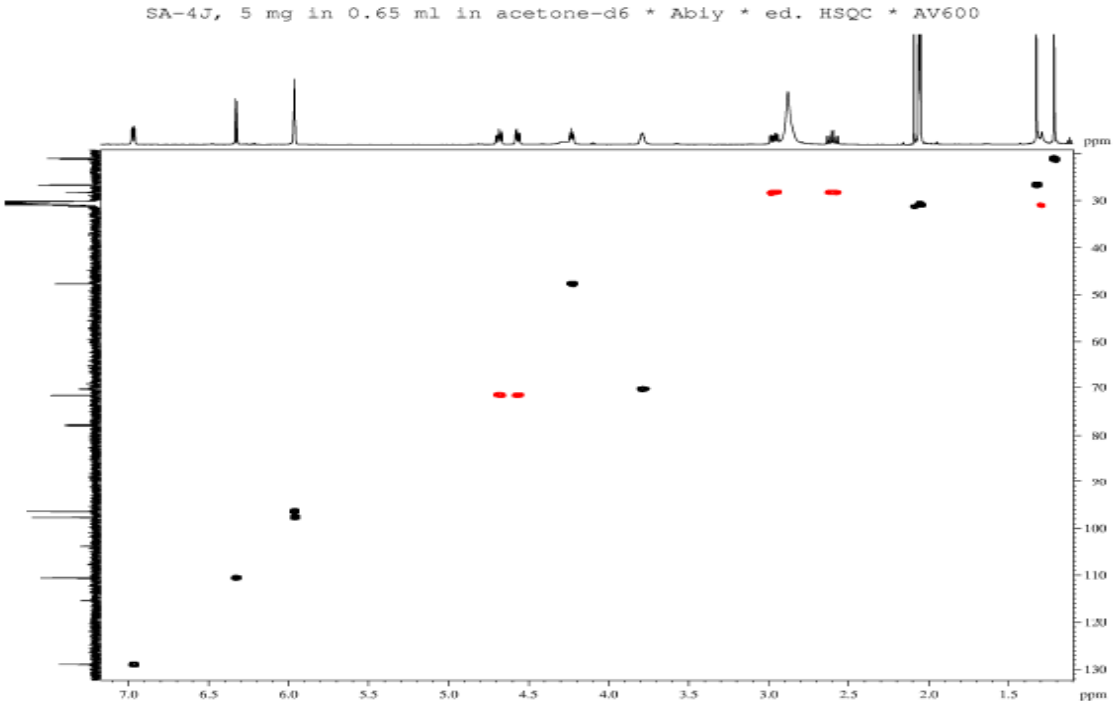
Appendix 6B: ^{13}C ($(CH_3)_2CO$, 150 MHz) NMR spectrum for platyisoflavanone D (**168**)



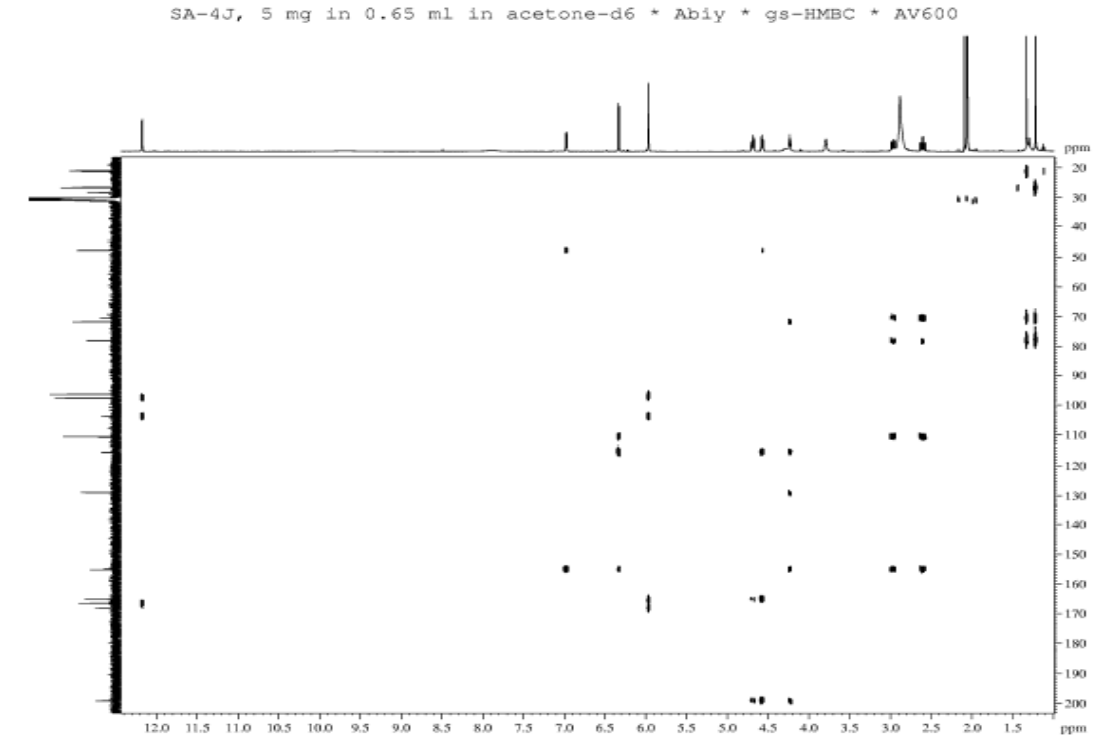
Appendix 6C: gCOSY ($(CH_3)_2CO$, 600 MHz) spectrum for platyisoflavanone D (**168**)



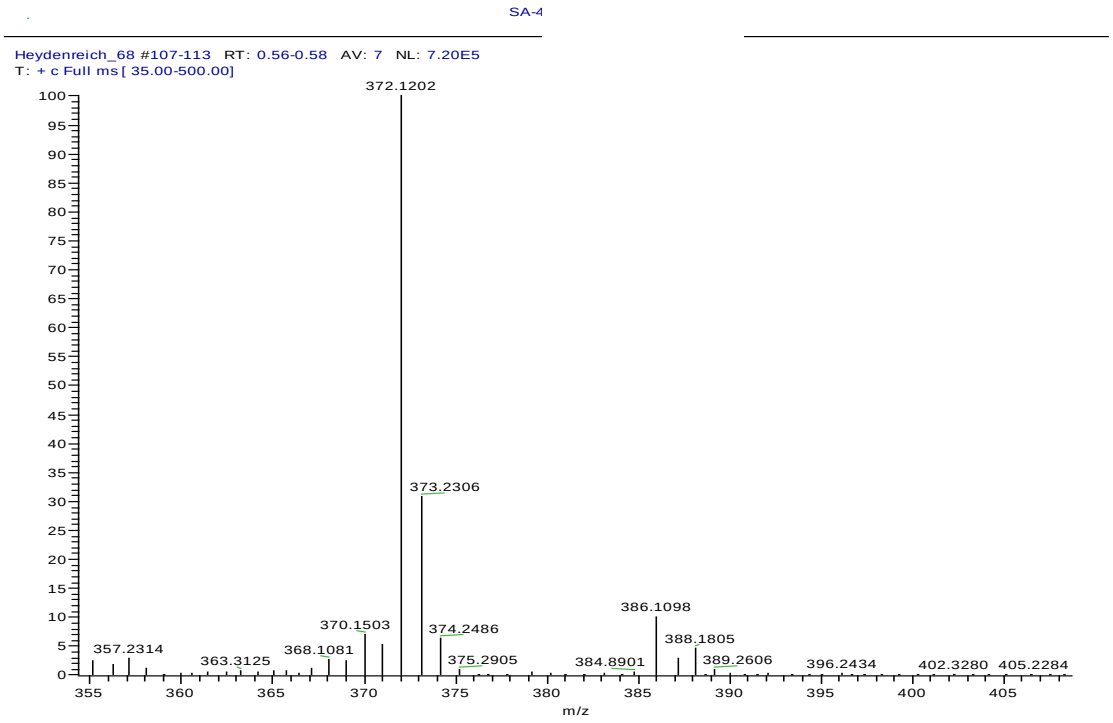
Appendix 6D: gHSQC ($(CH_3)_2CO$, 600 MHz) spectrum for platyisoflavanone D (**168**)



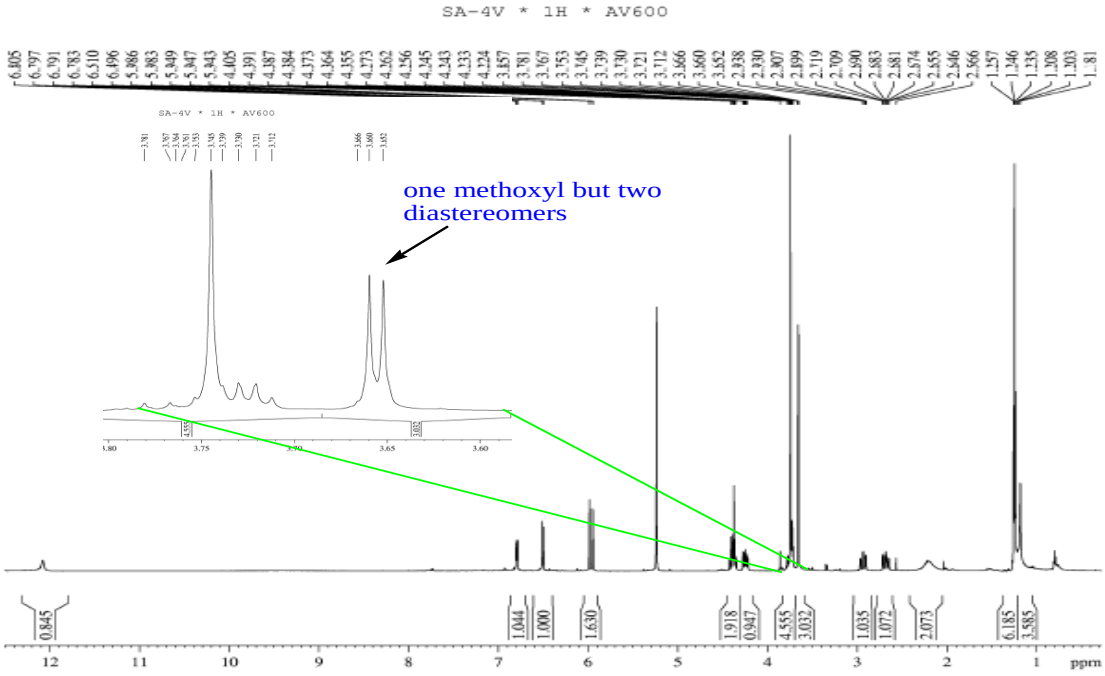
Appendix 6E: gHMBC ($(CH_3)_2CO$, 600 MHz) spectrum for platyisoflavanone D (**168**)



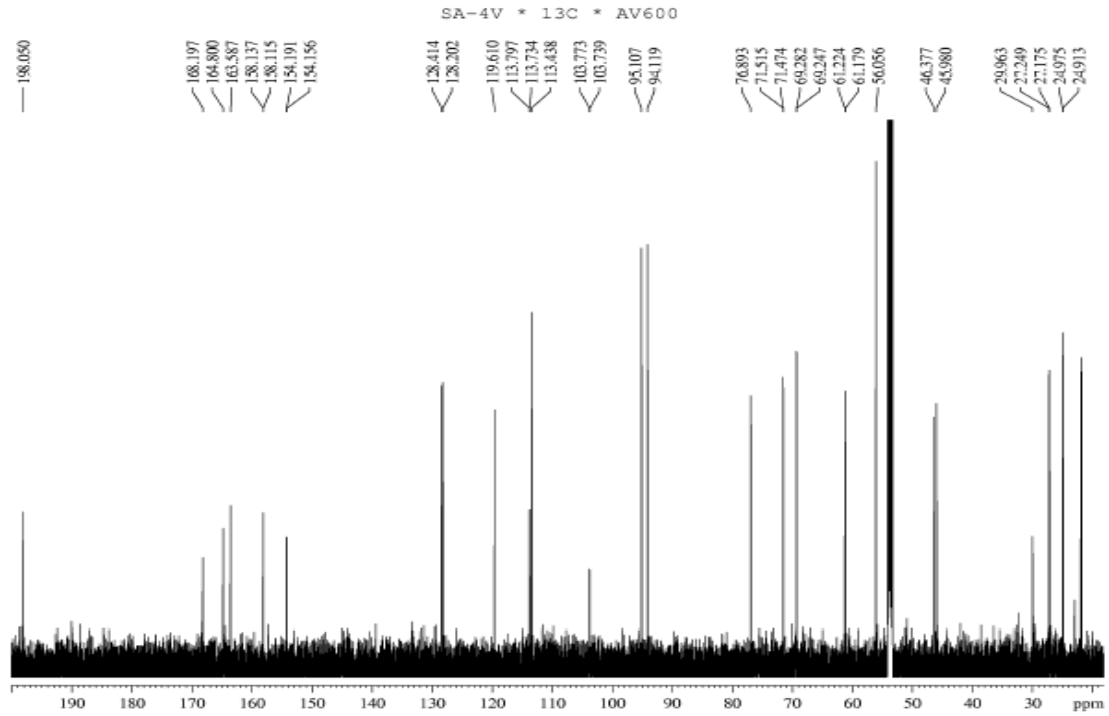
Appendix 6F: HREI-MS for platyisoflavanone D (**168**)



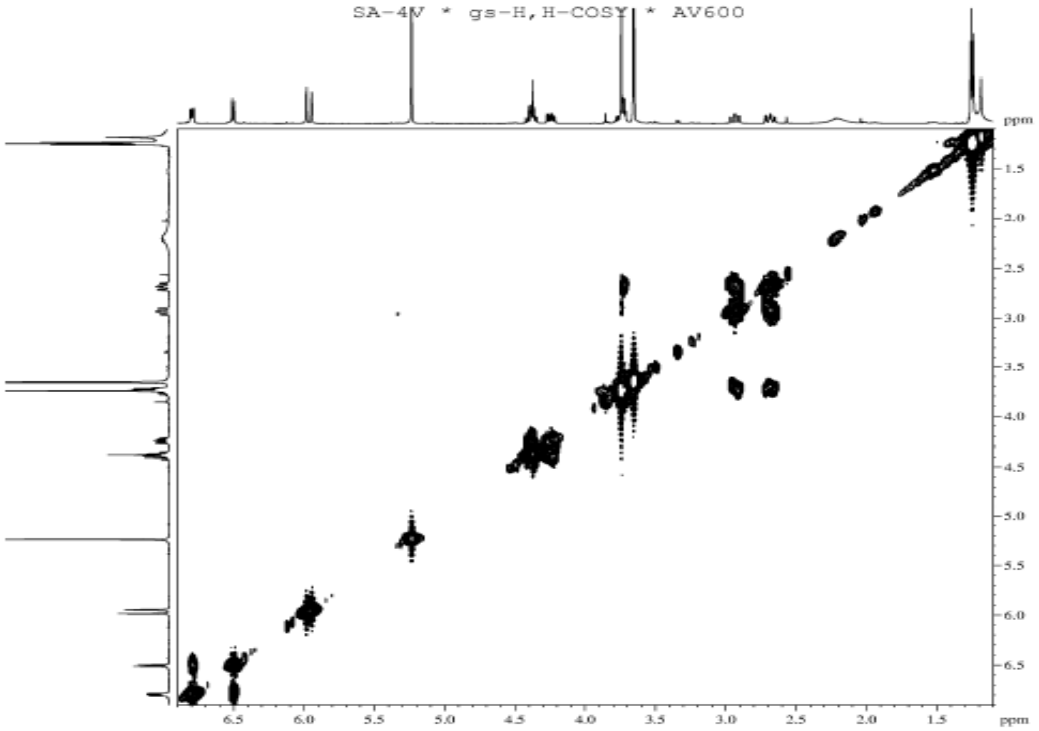
Appendix 6G: ^{1}H (CD_2Cl_2, 600 MHz) NMR spectrum for compound **168a**



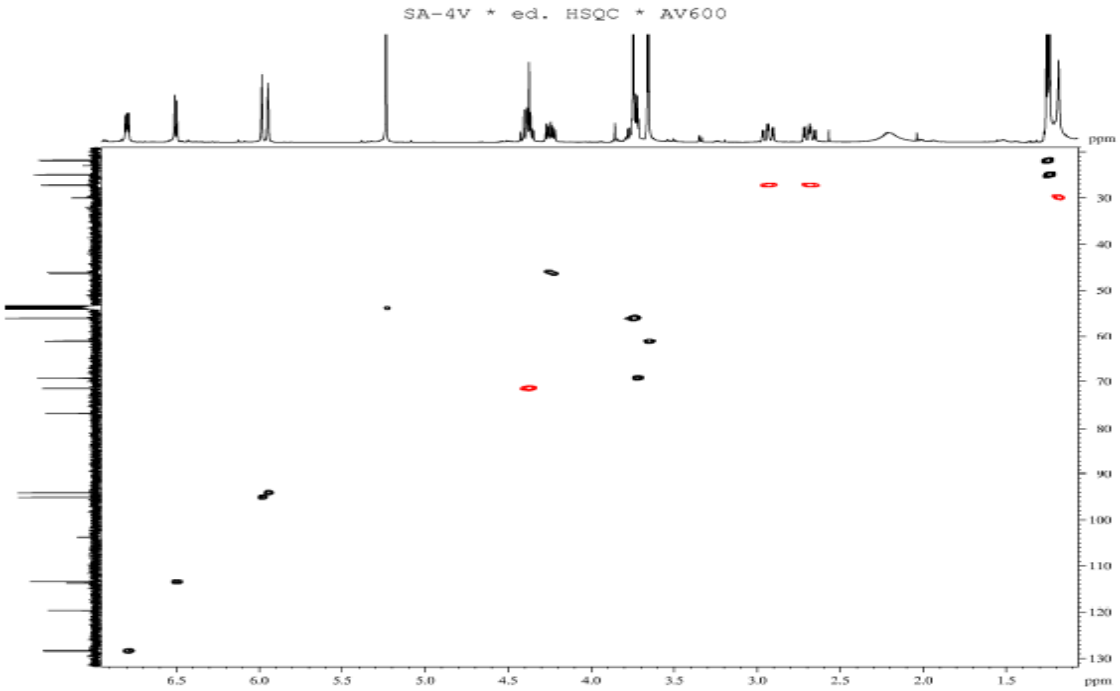
Appendix 6H: ^{13}C (CD_2Cl_2, 150 MHz) NMR spectrum for compound **168a**



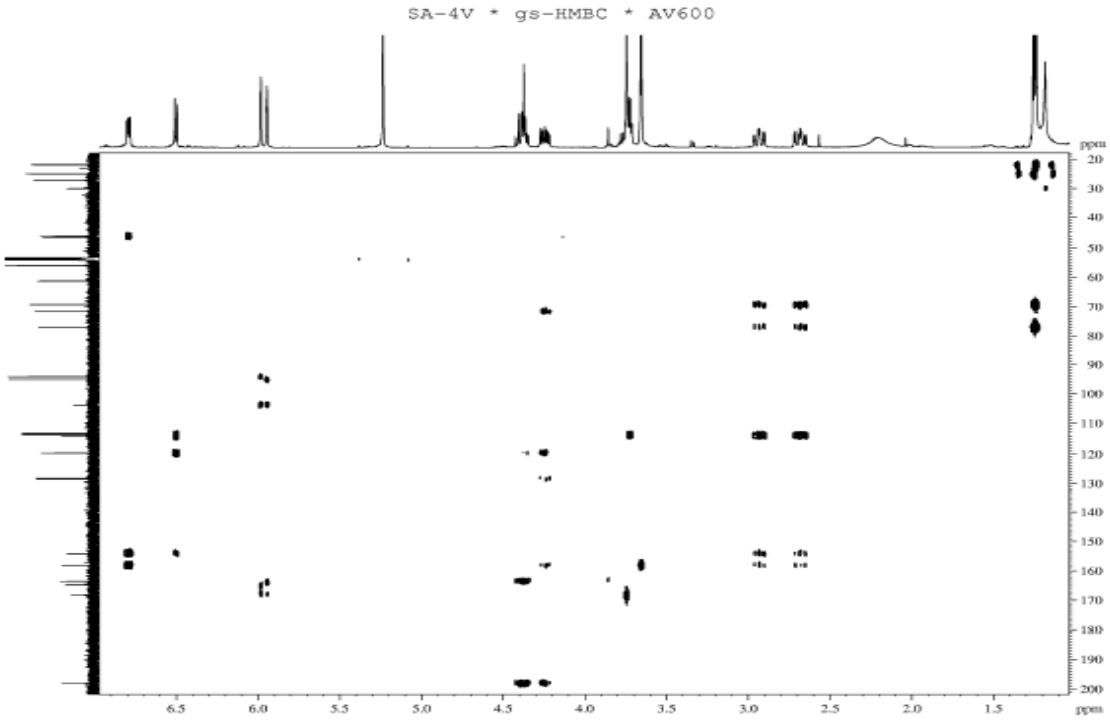
Appendix 6J: ^{1}H-^{1}H Gcosy (CD_2Cl_2, 600 MHz) spectrum for compound **168a**



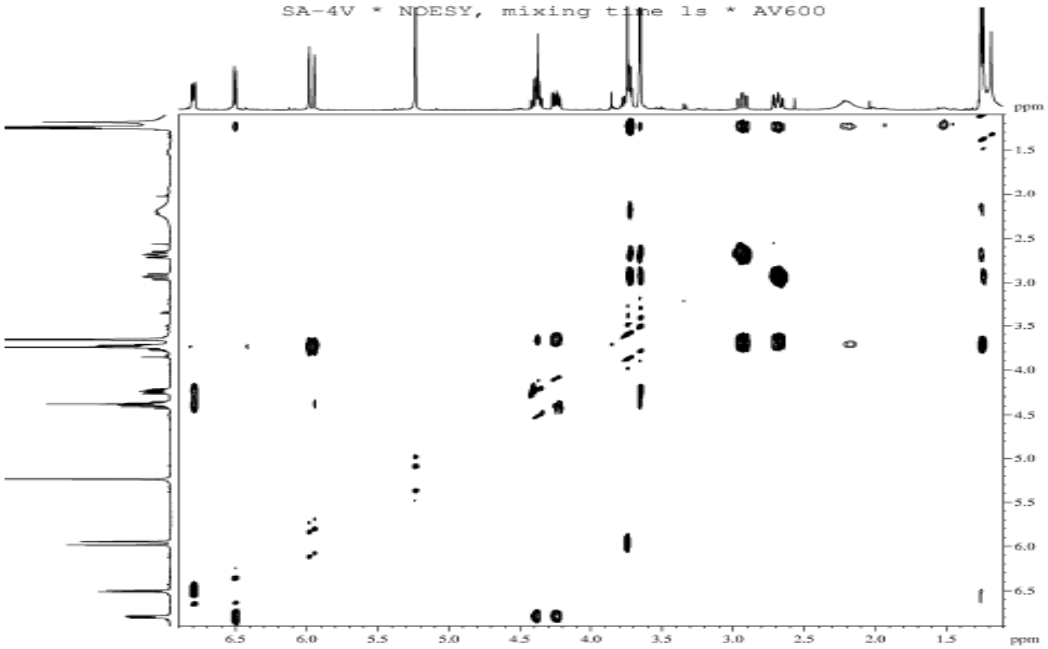
Appendix 6K: gHSQC (CD_2Cl_2, 600 MHz) spectrum for compound **168a**



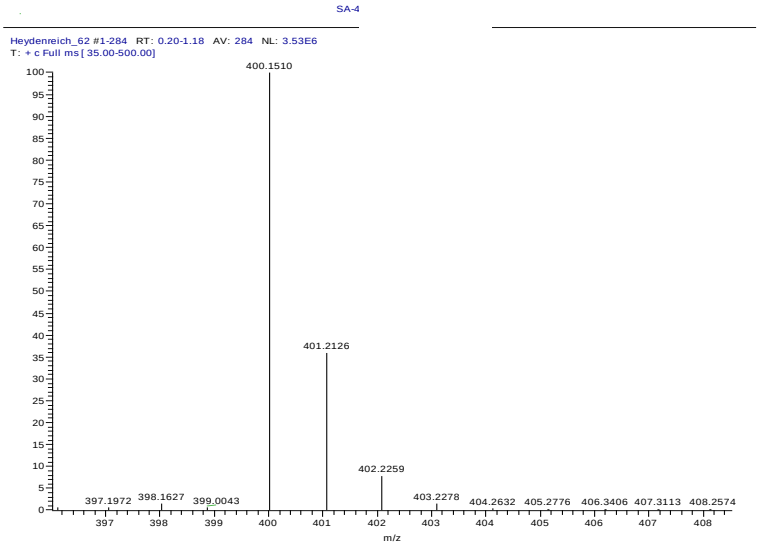
Appendix 6L: gHMBC (CD_2Cl_2, 600 MHz) spectrum for compound **168a**



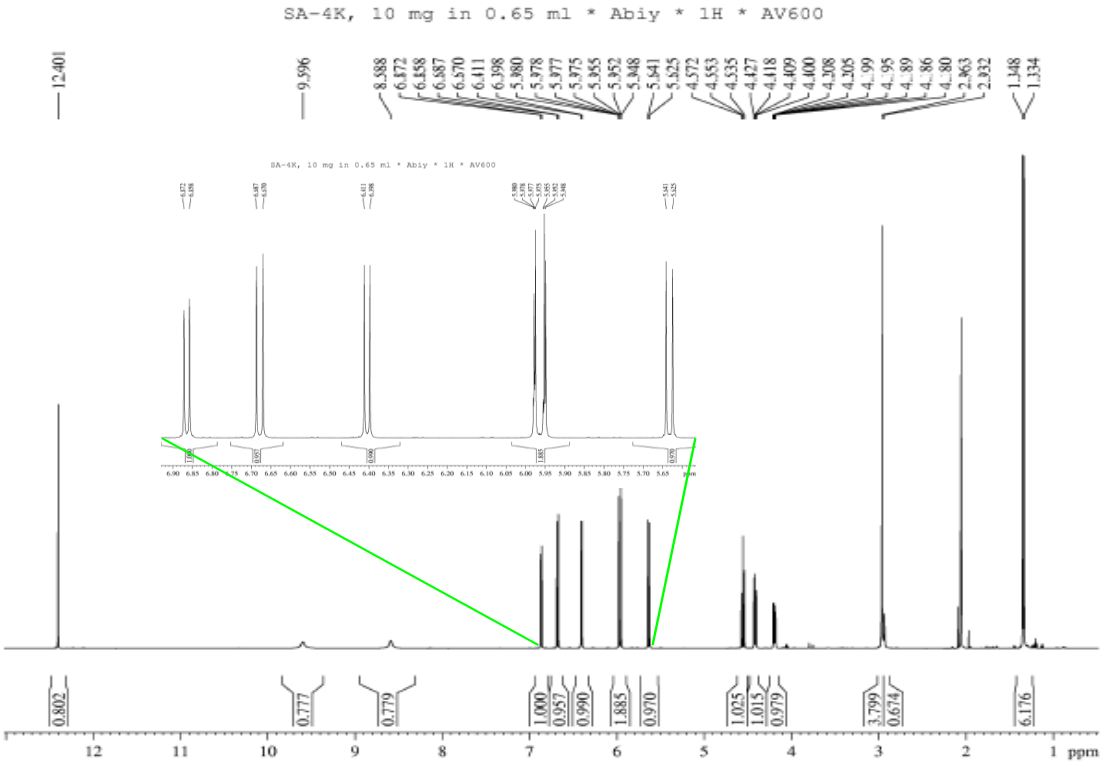
Appendix 6M: NOESY (CD_2Cl_2, 600 MHz) spectrum for compound **168a**



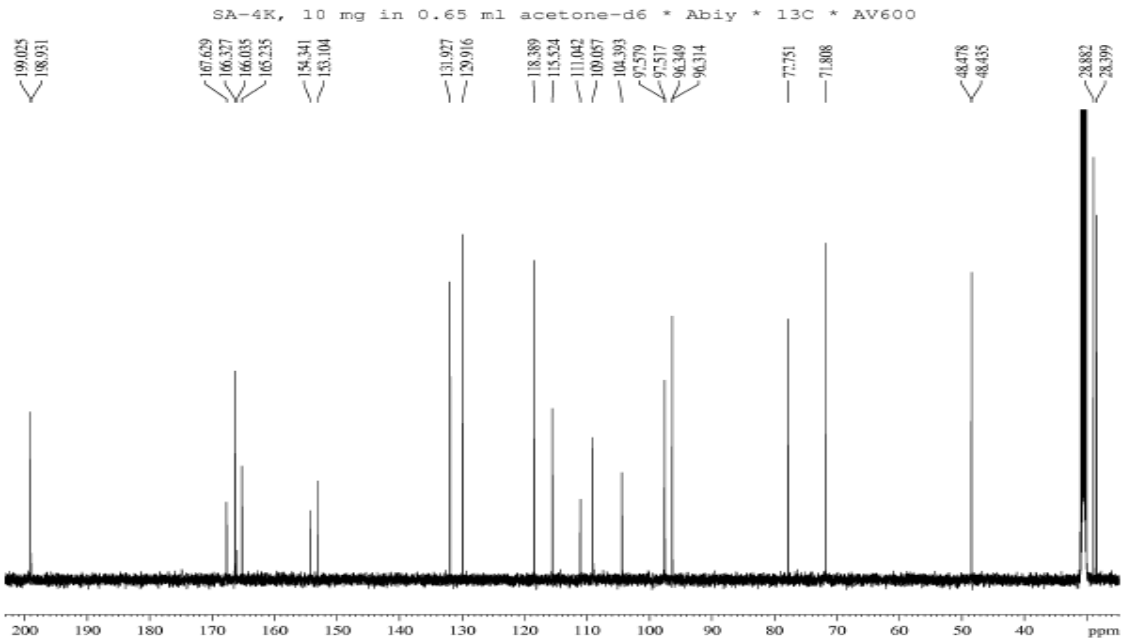
Appendix 6N: HREI-MS for compound **168a**



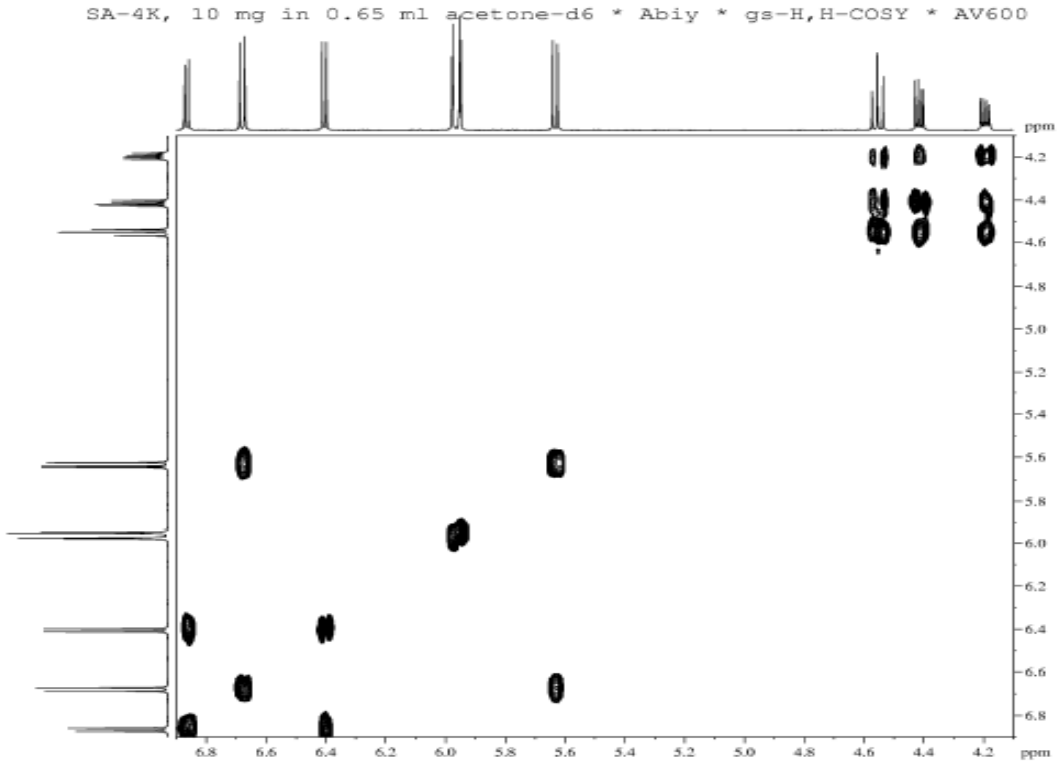
Appendix 7A: ^{1}H ($(CD_3)_2CO$, 600 MHz) NMR spectrum for glyasperin F (**169**)



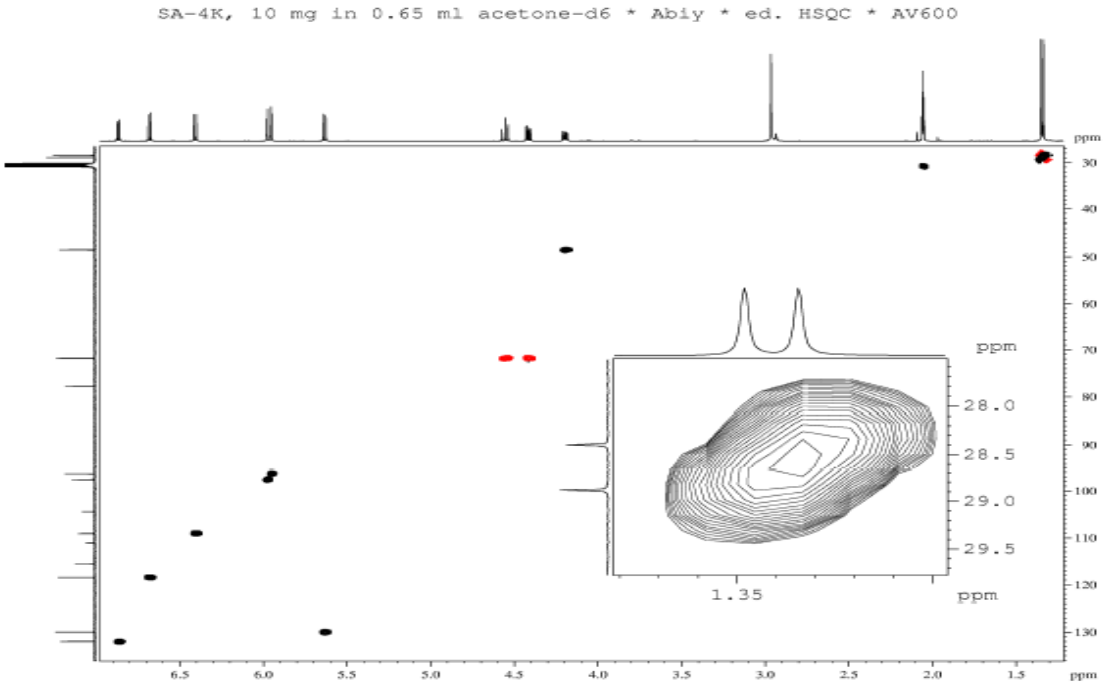
Appendix 7B: ^{13}C ($(CD_3)_2CO$, 150 MHz) NMR spectrum for glyasperin F (**169**)



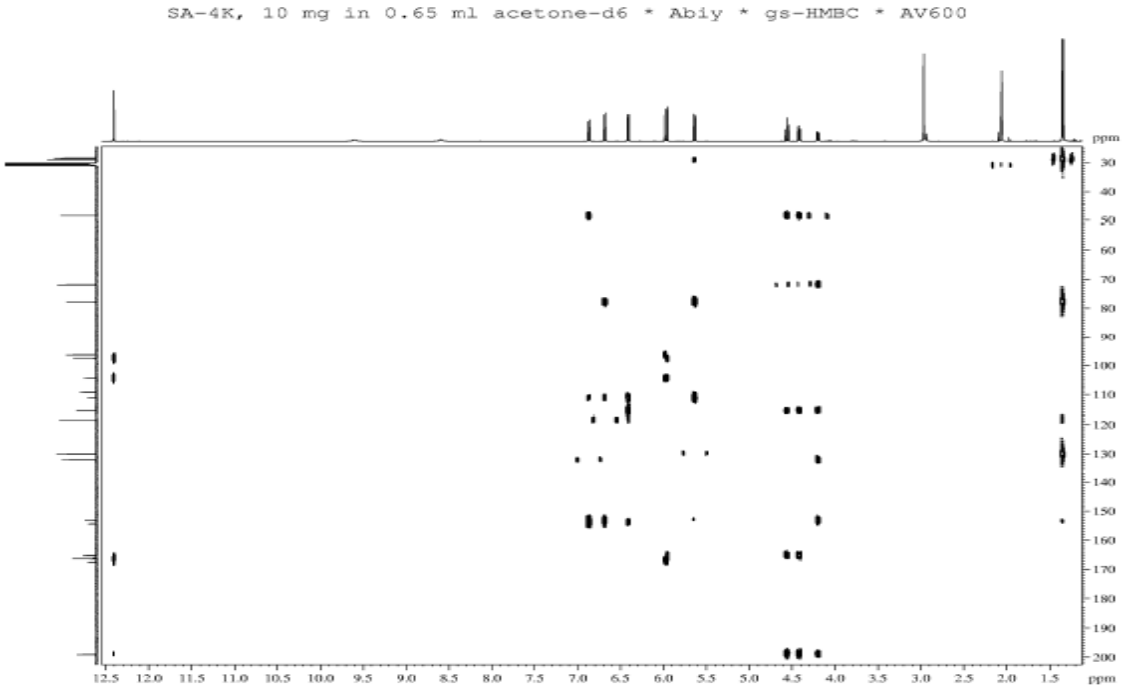
Appendix 7C: ^{1}H-^{1}H gCOSY ($(CD_3)_2CO$, 600 MHz) spectrum for glyasperin F (**169)**



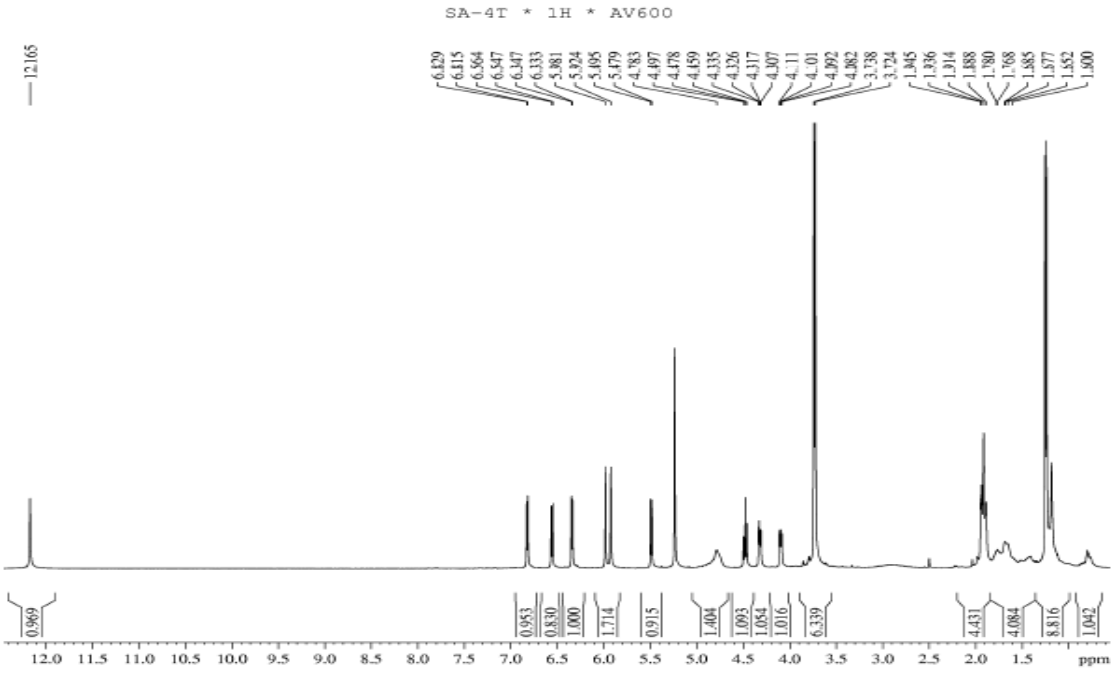
Appendix 7D: gHSQC ($(CD_3)_2CO$, 600 MHz) spectrum for glyasperin F (**169**)



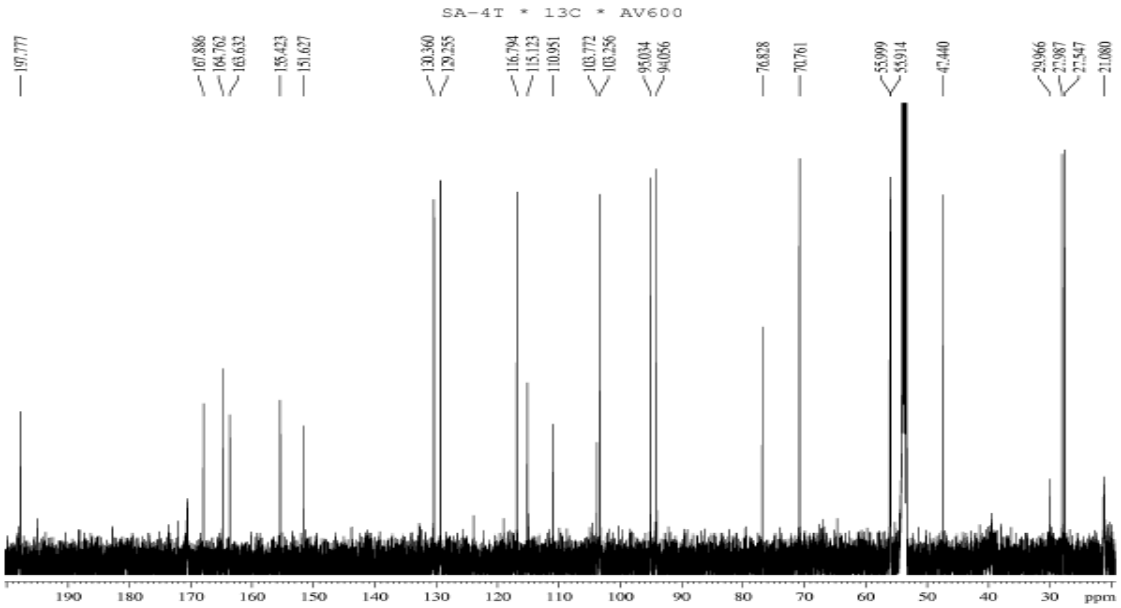
Appendix 7E: gHMBC ($(CD_3)_2CO$, 600 MHz) spectrum for glyasperin F (**169**)



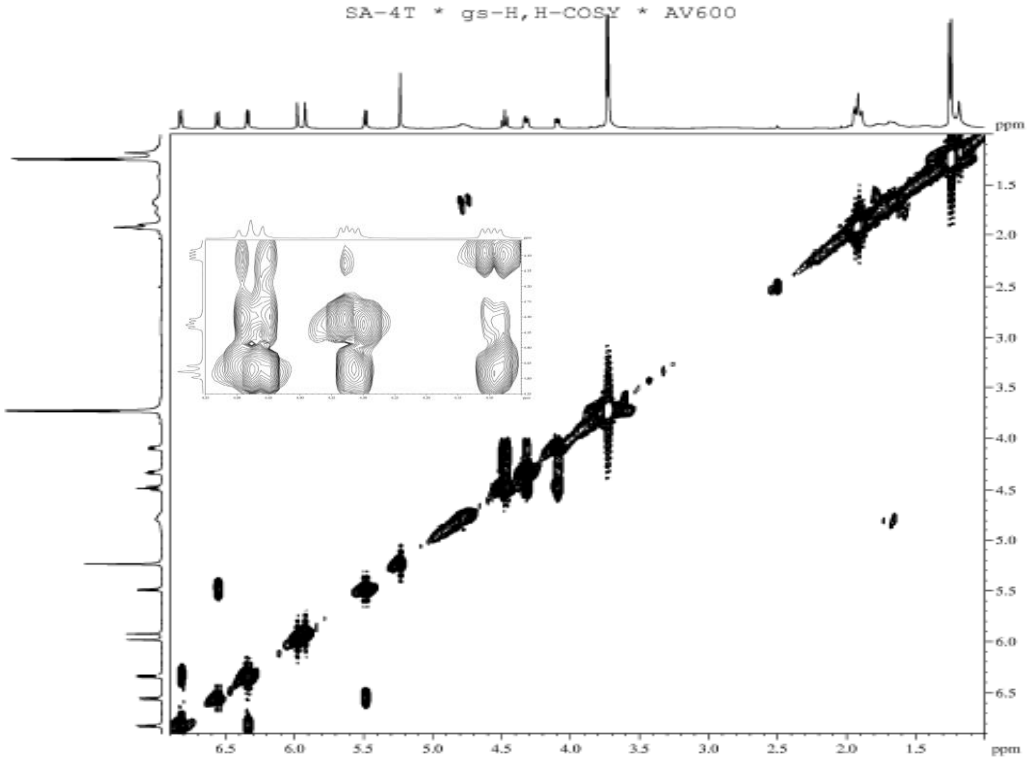
Appendix 7F: ^1H (CD_2Cl_2, 600 MHz) NMR spectrum for compound **169a**



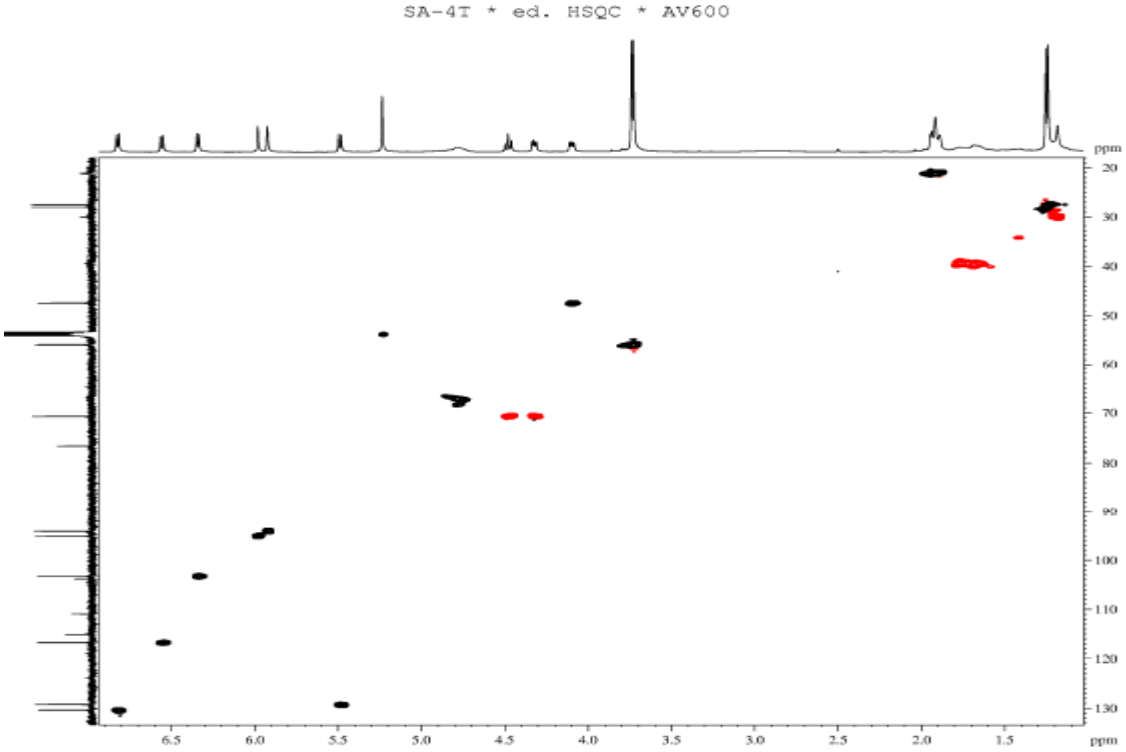
Appendix 7G: ^{13}C (CD_2Cl_2, 150 MHz) NMR spectrum for compound **169a**



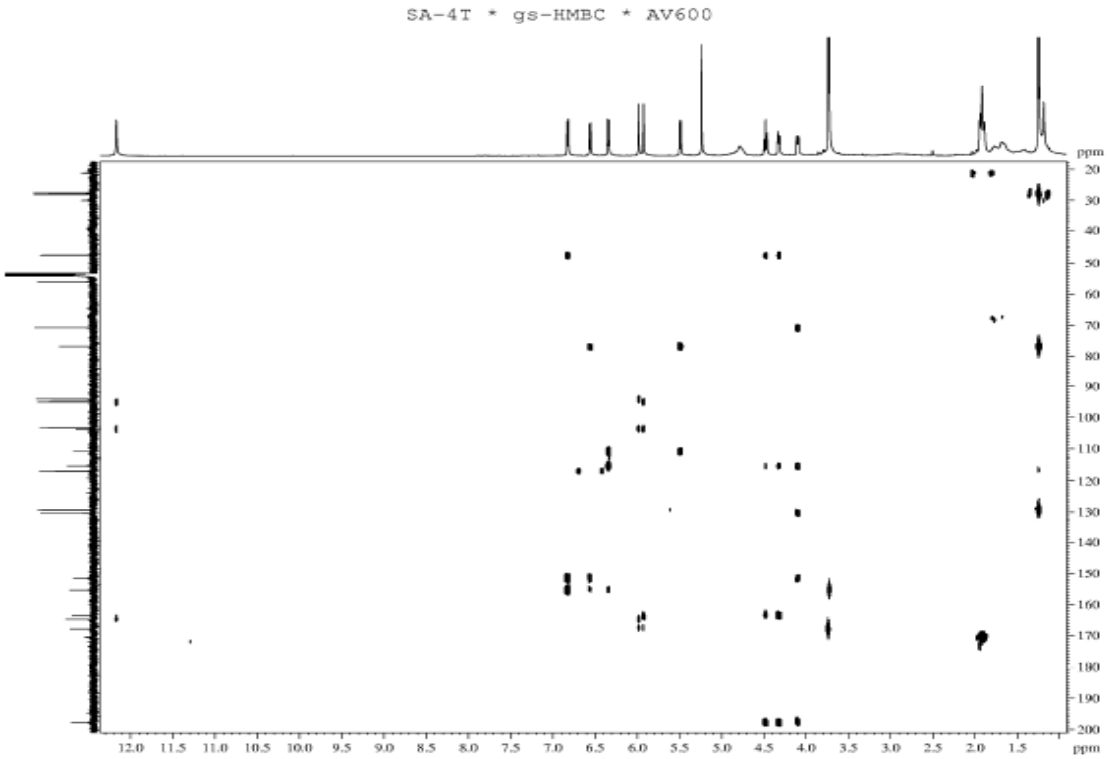
Appendix 7H: ^{1}H-^{1}H gCOSY (CD_2Cl_2, 600 MHz) spectrum for compound **169A**



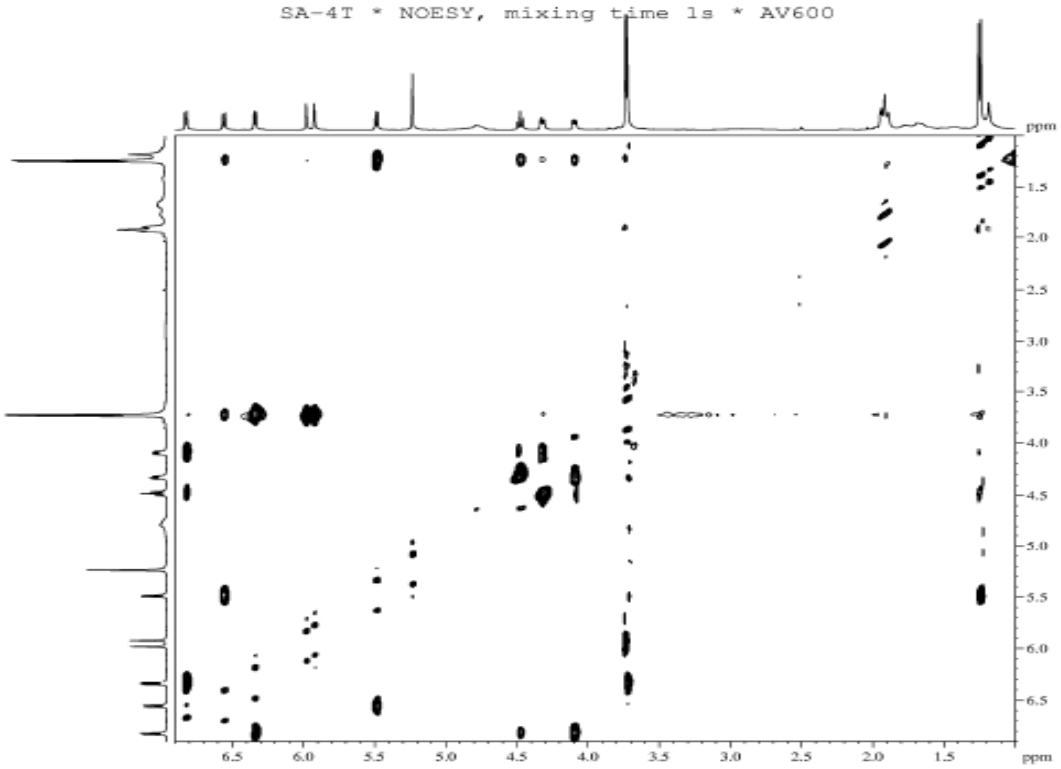
Appendix 7J: gHSQC (CD_2Cl_2, 600 MHz) spectrum for compound **169a**



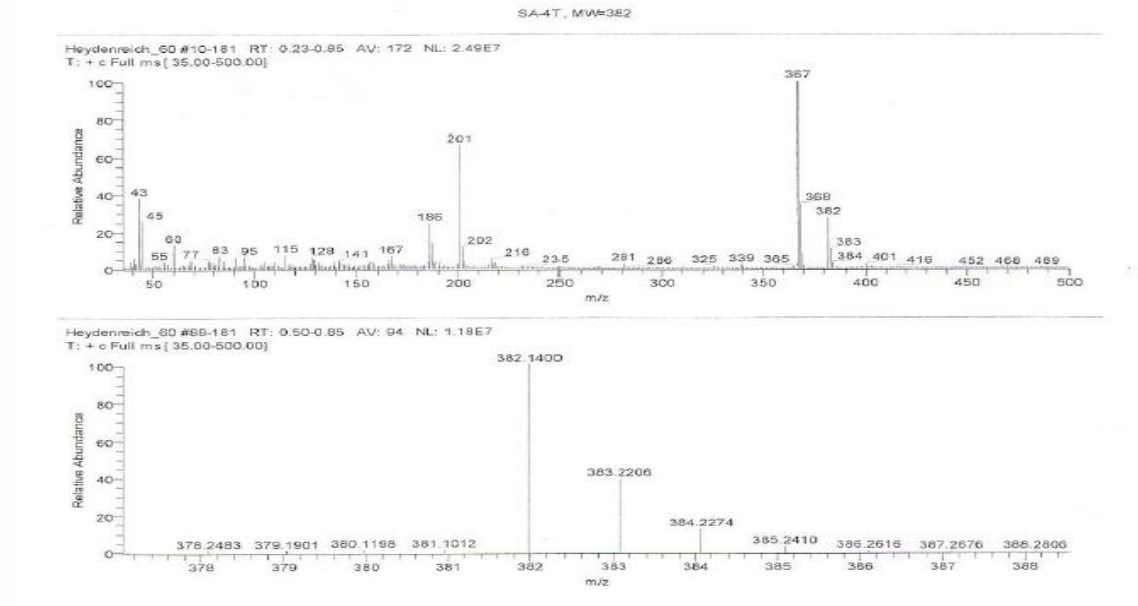
Appendix 7K: gHMBC (CD_2Cl_2, 600 MHz) spectrum for compound **169a**



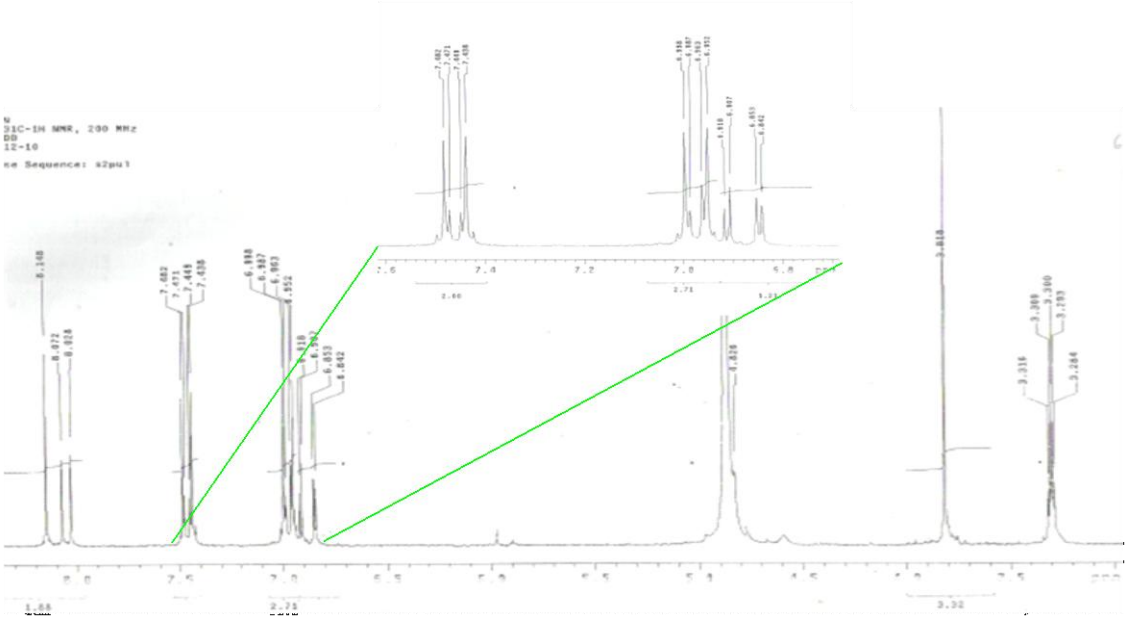
Appendix 7L: NOESY (CD_2Cl_2, 600 MHz) spectrum for compound **169a**



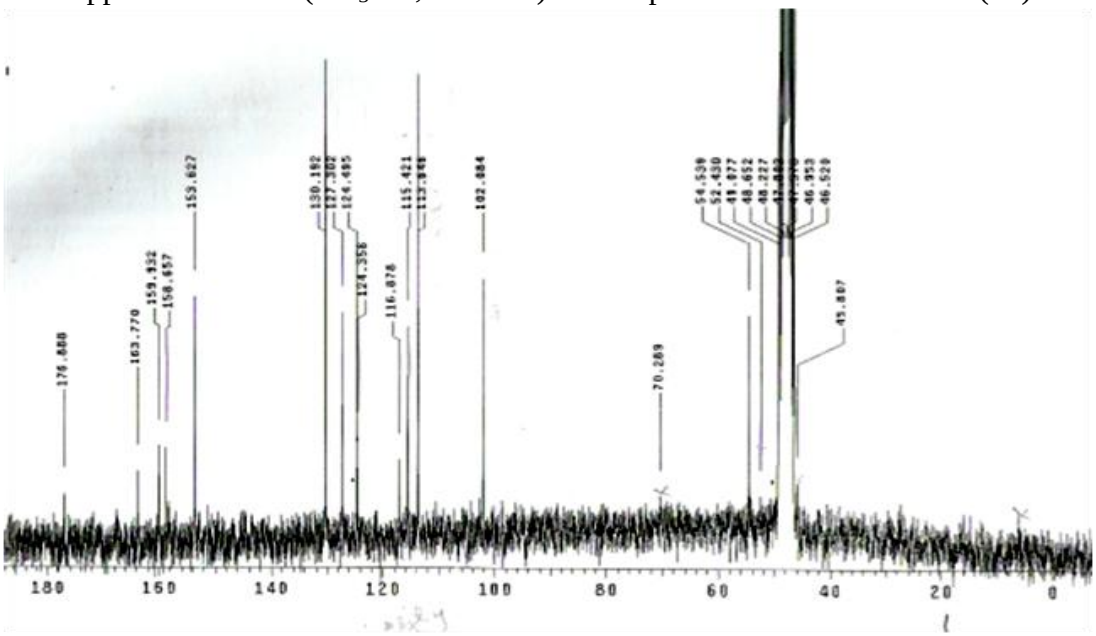
Appendix 7M: HREI-MS for compound **169a**



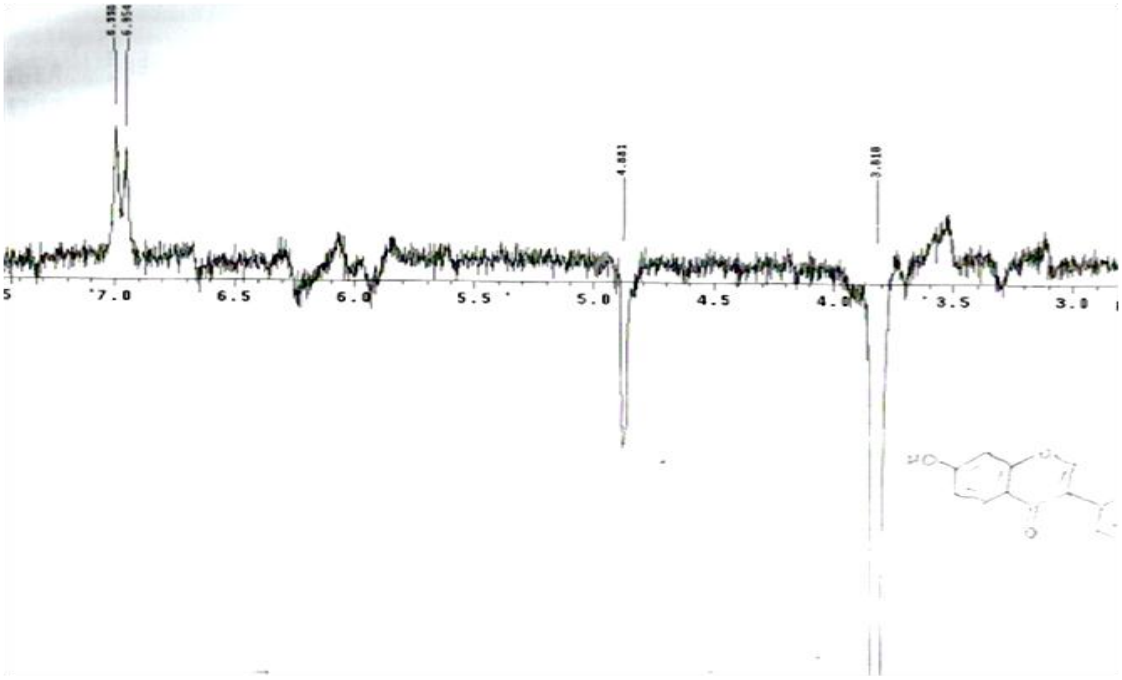
Appendix 8A: ^{1}H (CD_3OD, 200 MHz) NMR spectrum of formononetin (**52**)



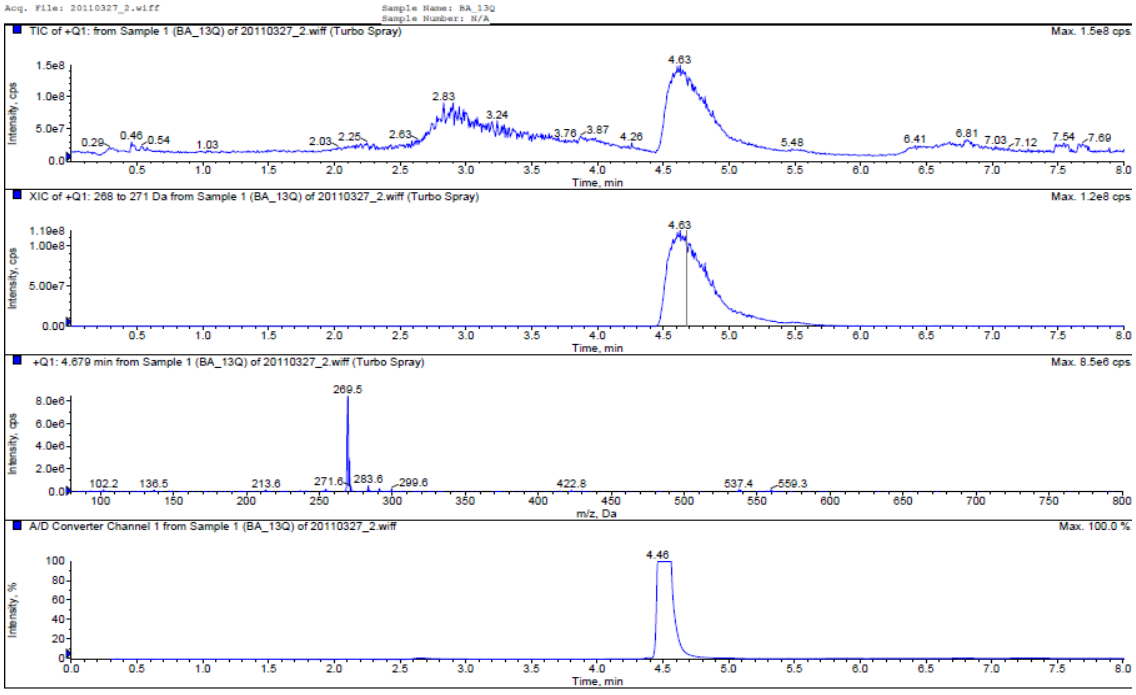
Appendix 8B: ^{13}C (CD_3OD, 50 MHz) NMR spectrum of formononetin (**52**)



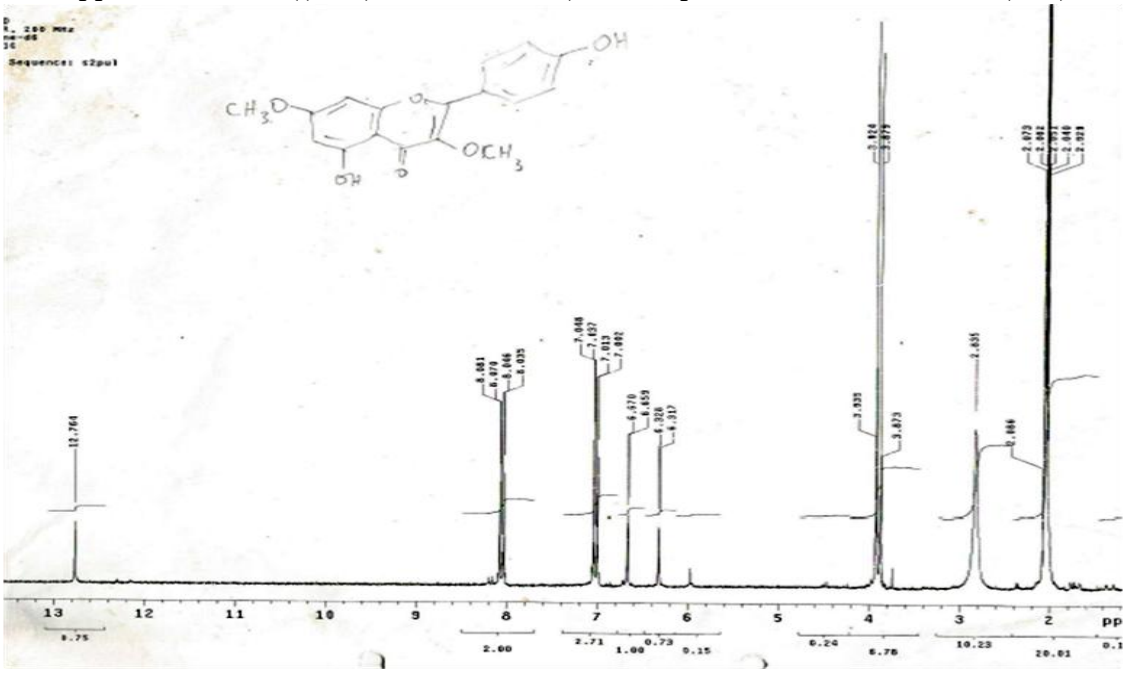
Appendix 8C: NOE-DIFF (CD_3OD, 200 MHz) spectrum of formononetin (**52**)



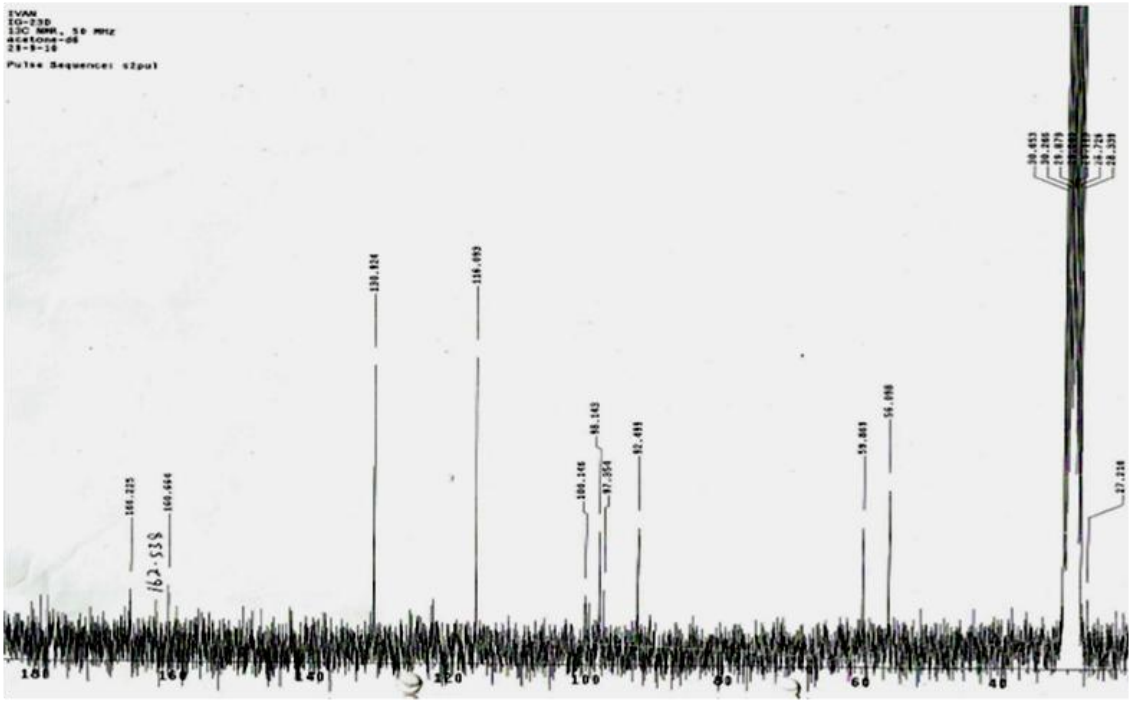
Appendix 8D: LC-ESI-MS for formononetin (**52**)



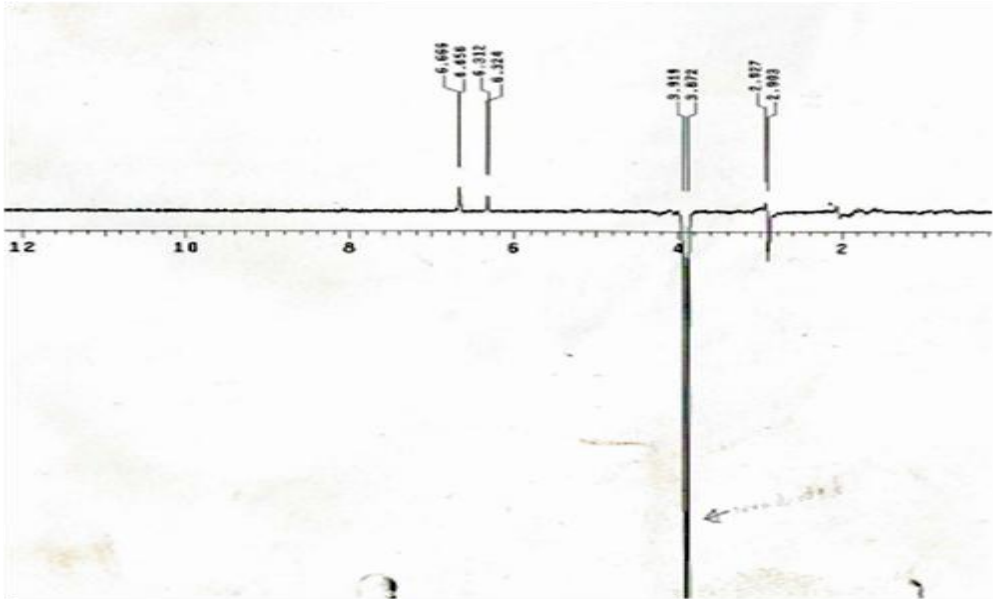
Appendix 9A: ^{1}H ($(CD_3)_2CO$, 200 MHz) NMR spectrum of kumatakenin (**170**)



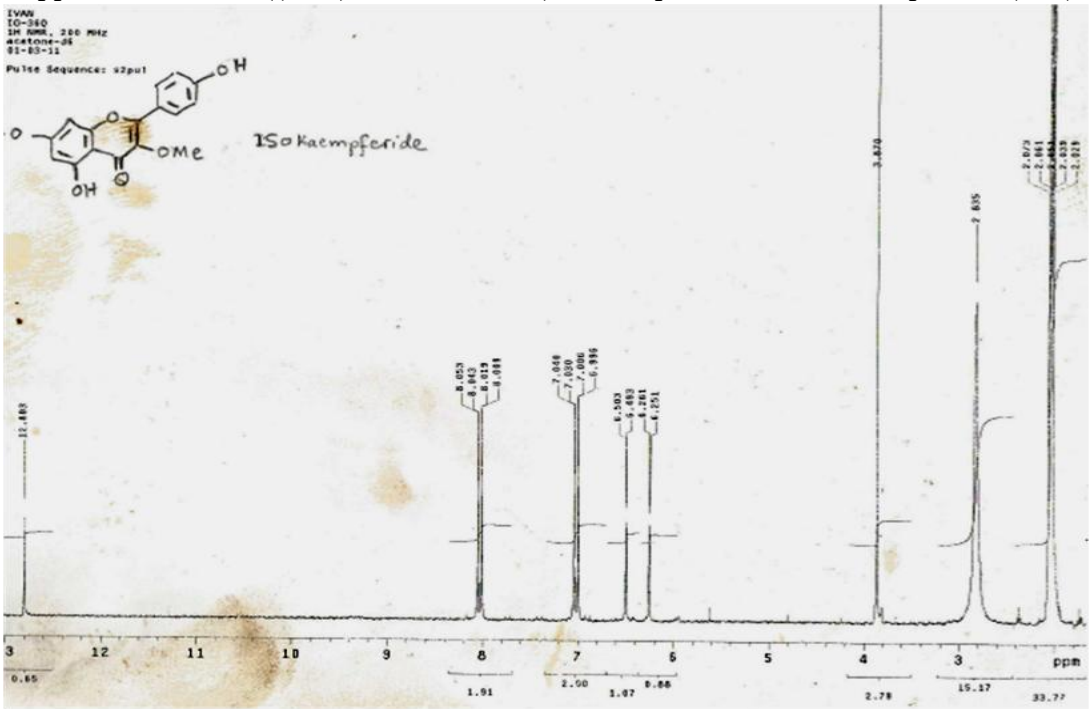
Appendix 9B: ^{13}C ($(CD_3)_2CO$, 50 MHz) NMR spectrum of kumatakenin (**170**)



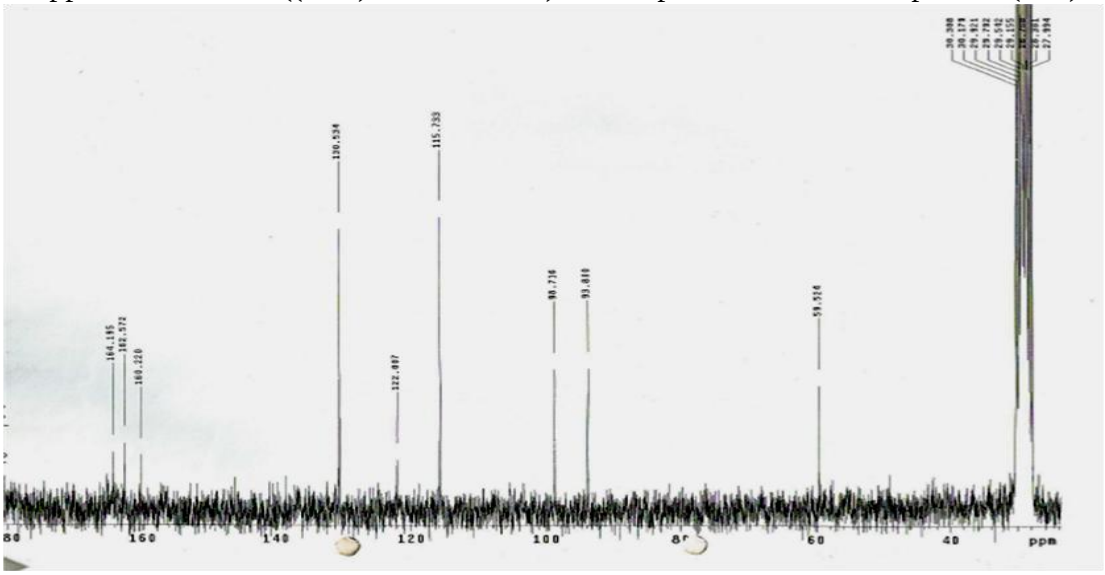
Appendix 9C: NOE-DIFF ($(CD_3)_2CO$, 200 MHz) spectrum of kumatakenin (**170**)



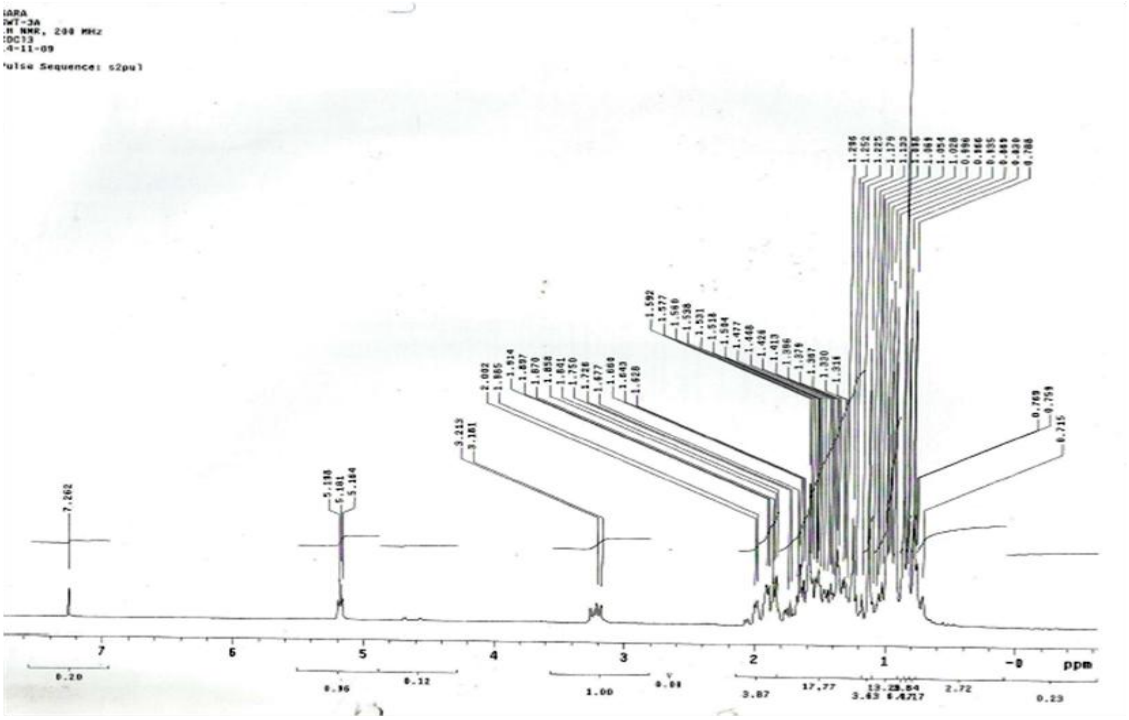
Appendix 10A: ^{1}H ($(CD_3)_2CO$, 200 MHz) NMR spectrum of isokaempferide (**171**)



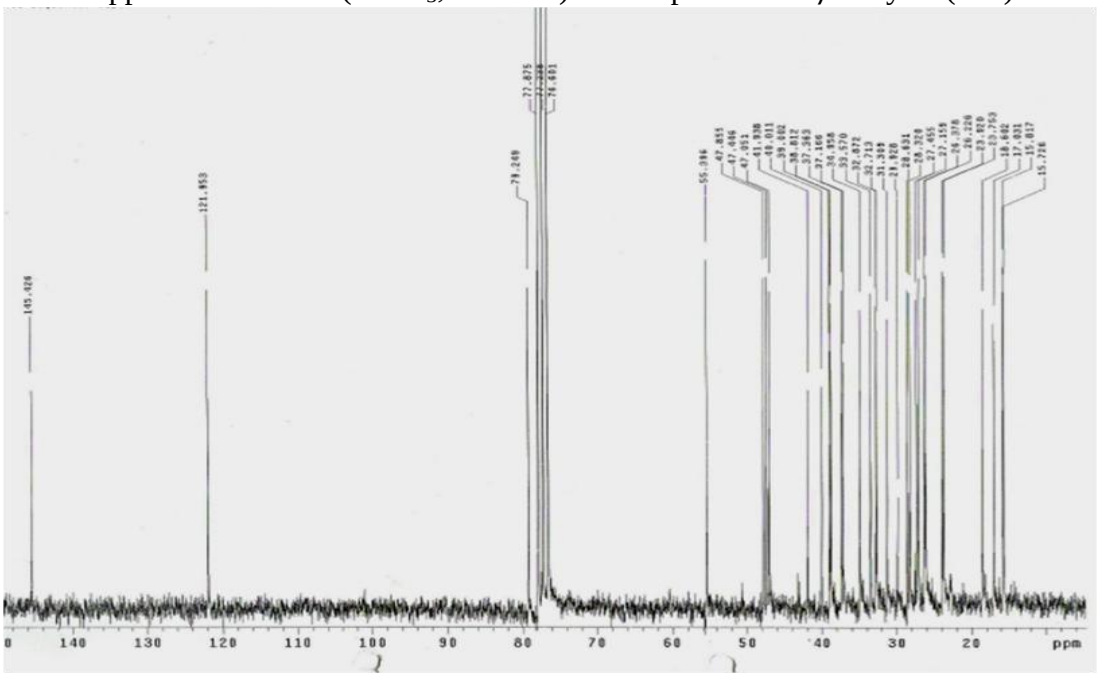
Appendix 10B: ^{13}C ($(CD_3)_2CO$, 50 MHz) NMR spectrum of isokaempferide (**171**)



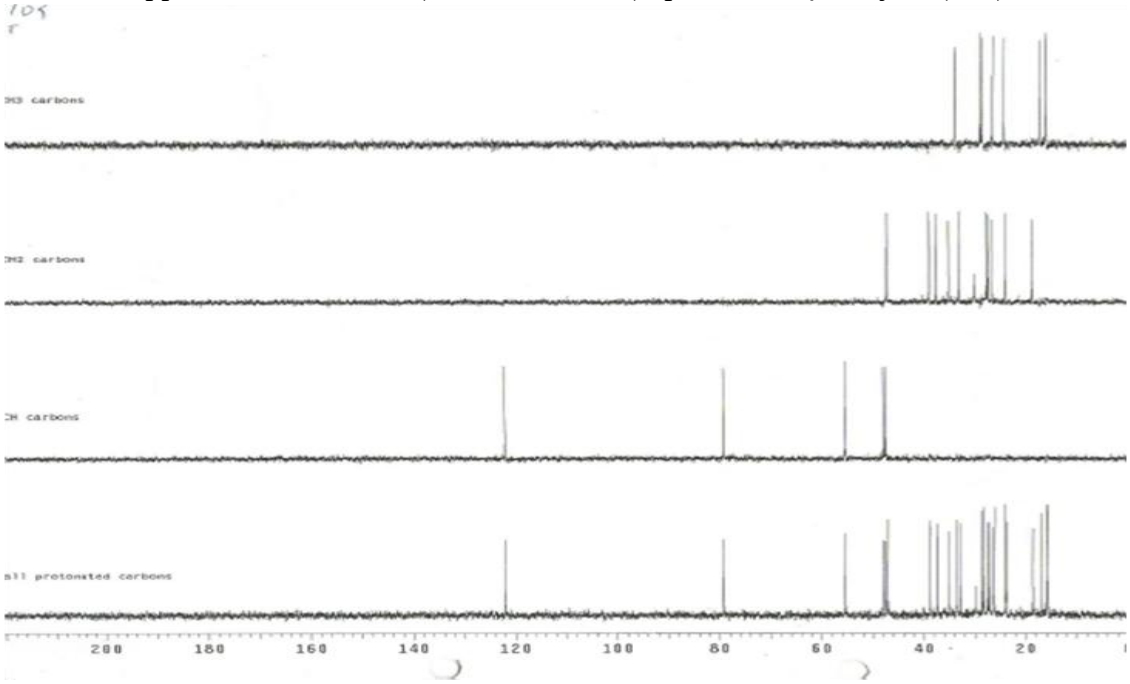
Appendix 11A: ^{1}H ($CDCl_3$, 200 MHz) NMR spectrum of β-amyrin (**172**)



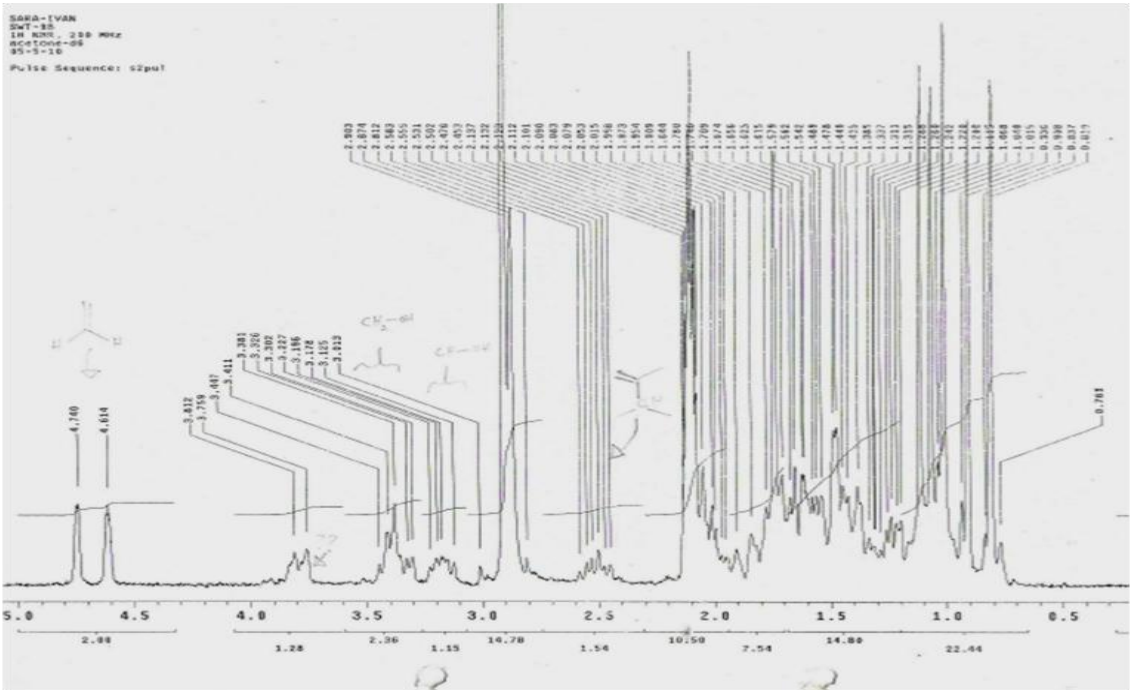
Appendix 11B: ^{13}C ($CDCl_3$, 50 MHz) NMR spectrum of β-amyrin (**172**)



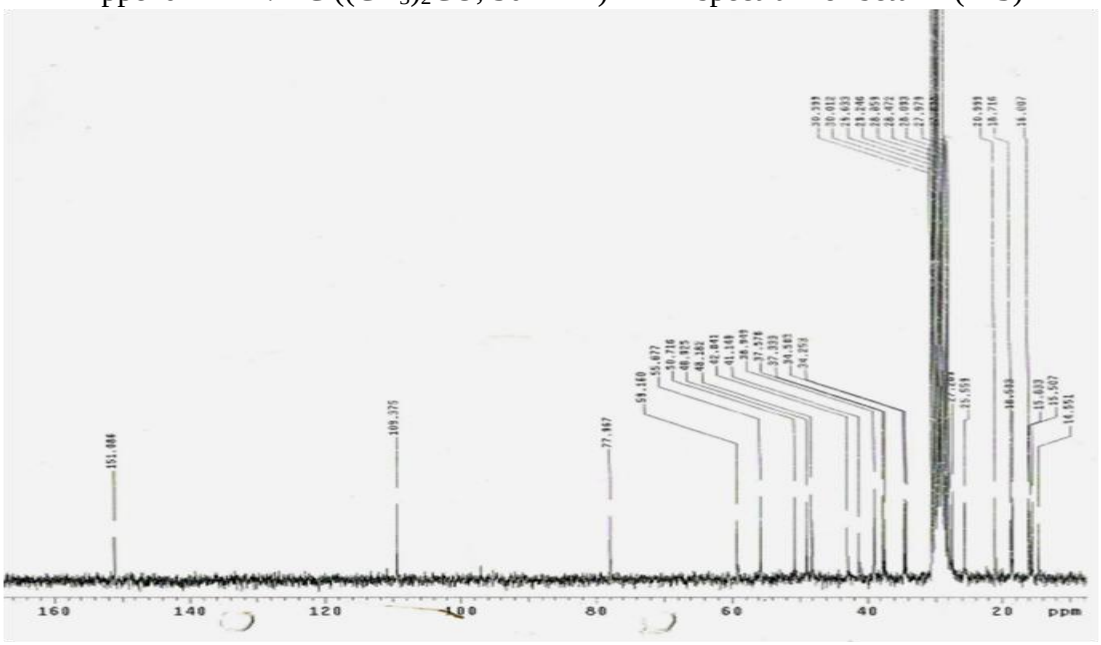
Appendix 11C: DEPT ($CDCl_3$, 50 MHz) spectrum of β-amyrin (**172**)



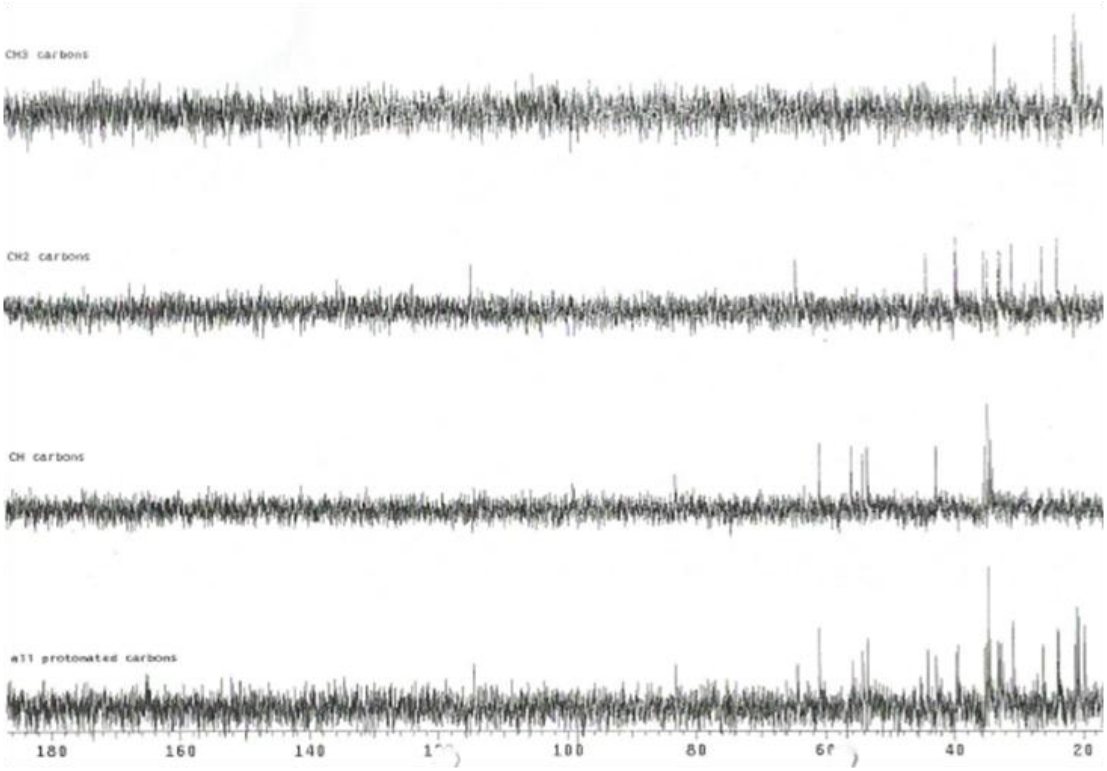
Appendix 12A: ^1H ($(CD_3)_2CO$, 200 MHz) NMR spectrum of betulin (**173**)



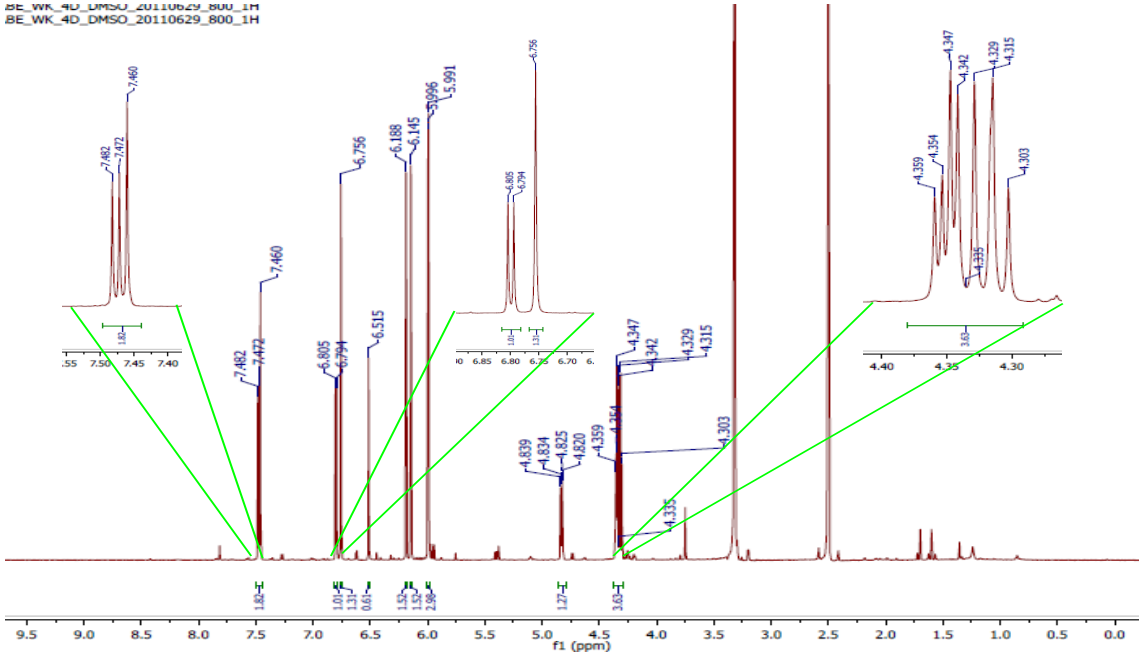
Appendix 12B: ^{13}C ($(CD_3)_2CO$, 50 MHz) NMR spectrum of betulin (**173**)



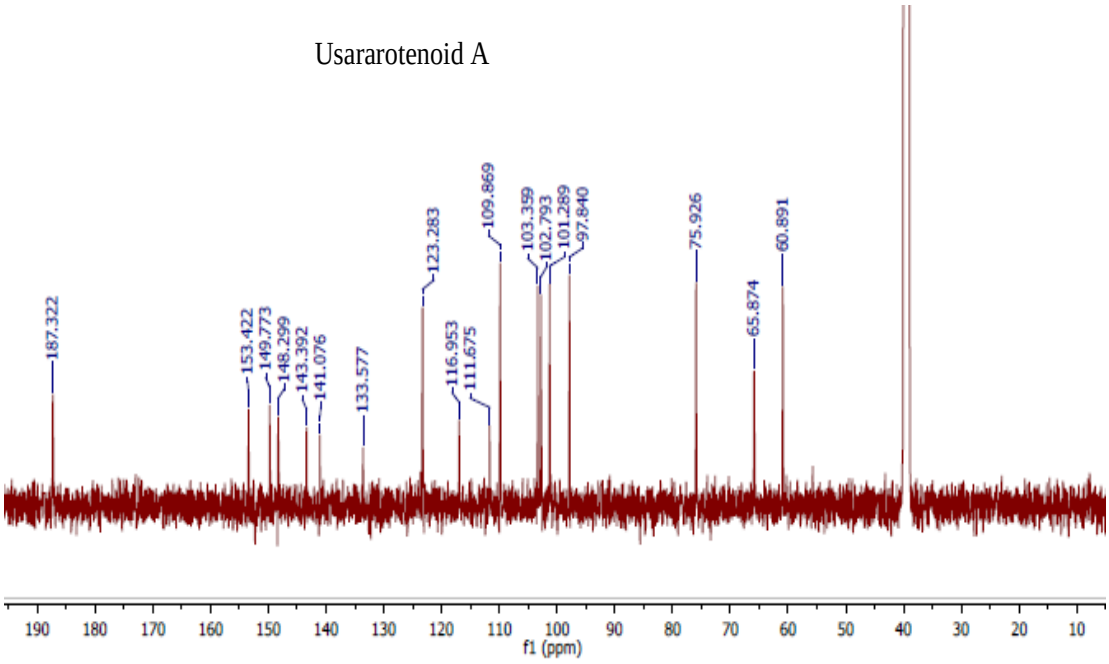
Appendix 12C: DEPT ($(CD_3)_2CO$, 50 MHz) spectrum of betulin (**173**)



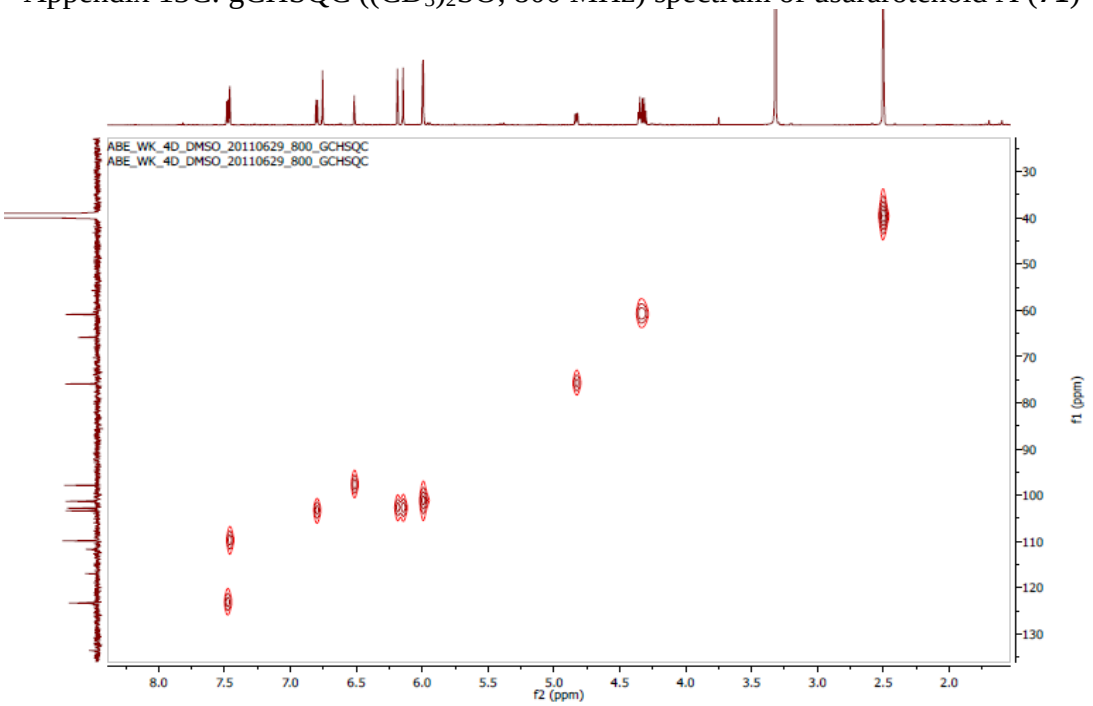
Appendix 13A: ^{1}H ($(CD_3)_2SO$, 800 MHz) NMR spectrum of usararotenoid A (**71**)



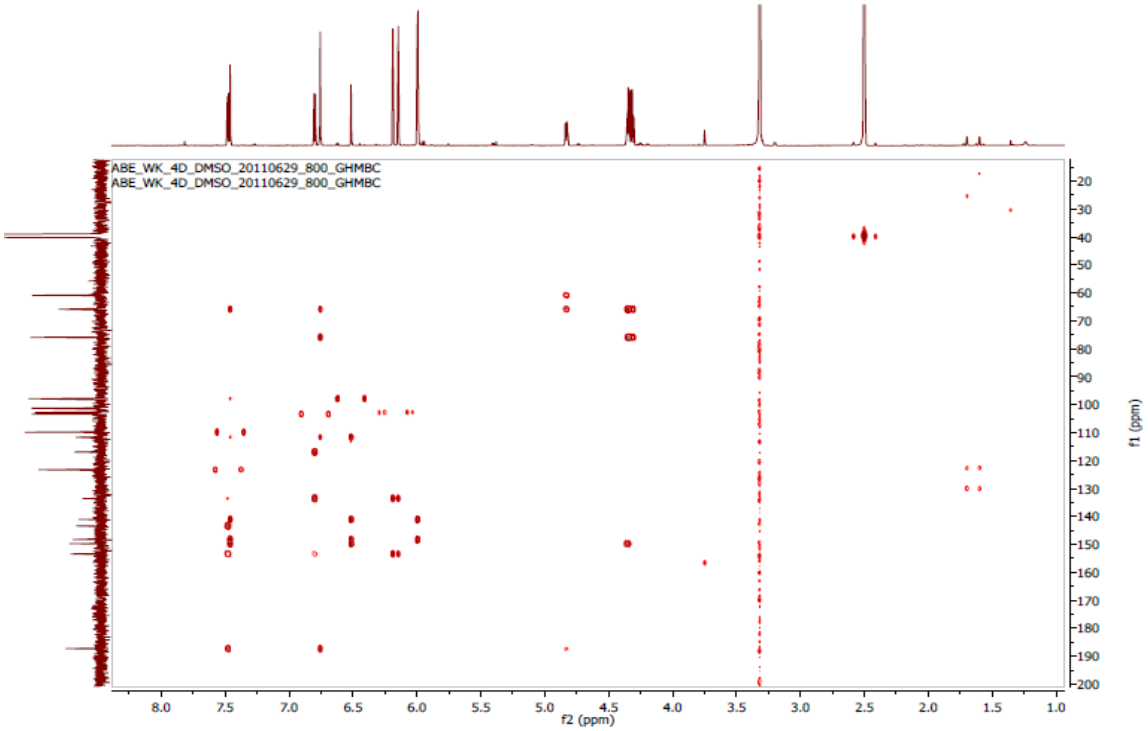
Appendix 13B: ^{13}C ($(CD_3)_2SO$, 150 MHz) NMR spectrum of usararotenoid A (**71**)



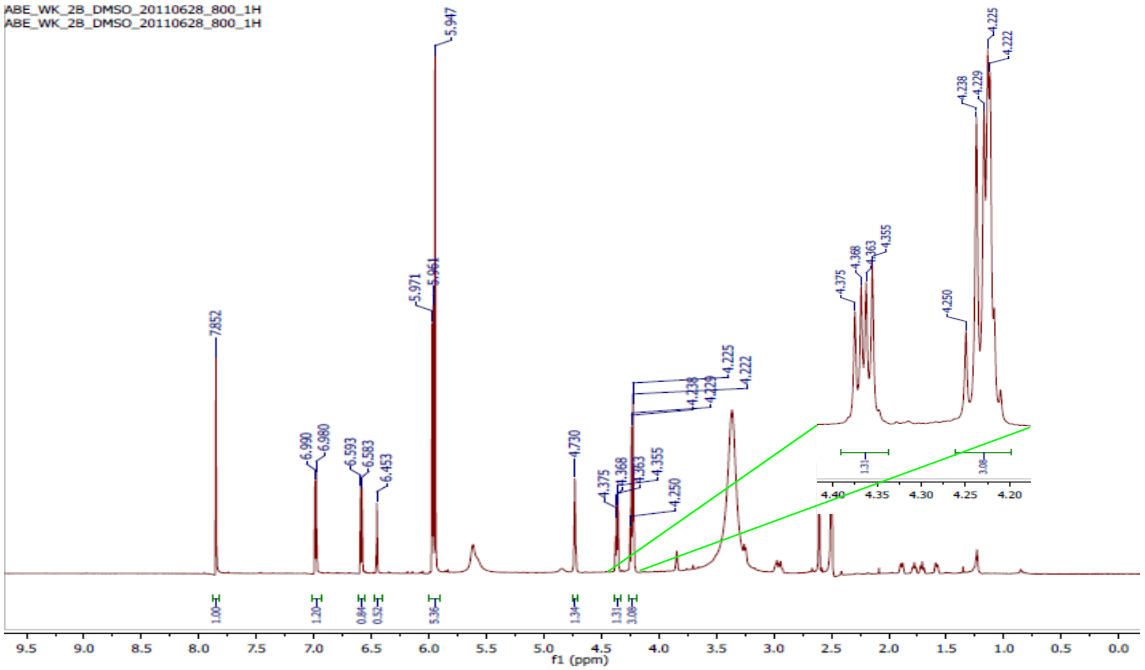
Appendix 13C: gCHSQC ($(CD_3)_2SO$, 800 MHz) spectrum of usararotenoid A (**71**)



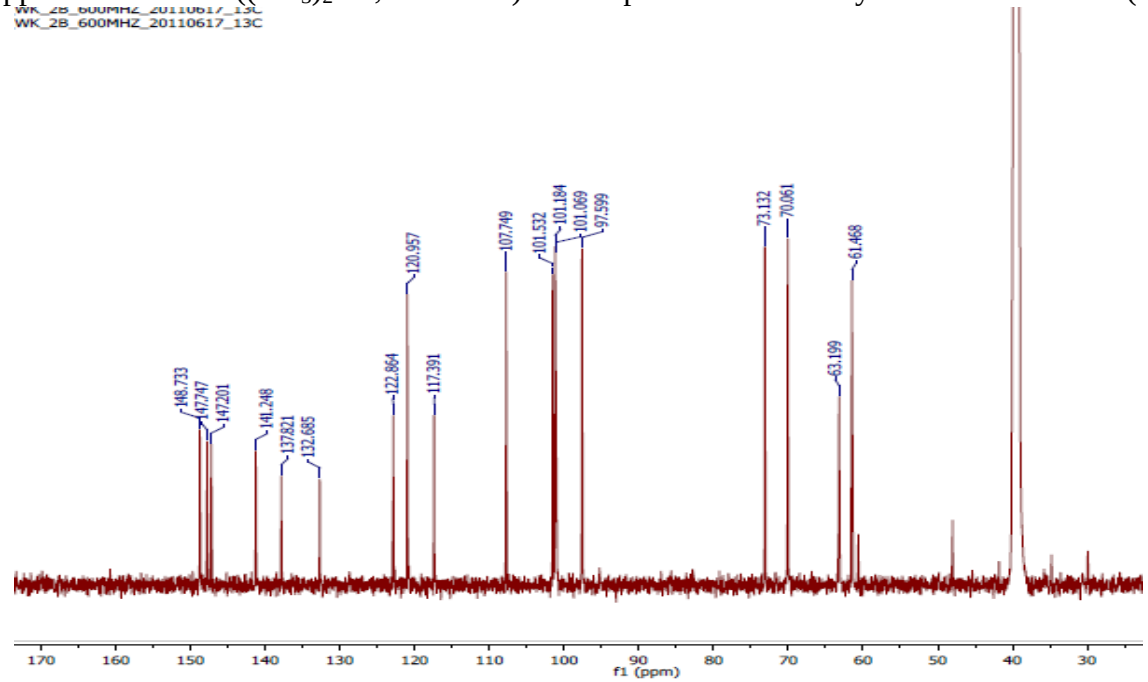
Appendix 13D: gHMBC ($(CD_3)_2SO$, 800 MHz) spectrum of usararotenoid A (**71**)



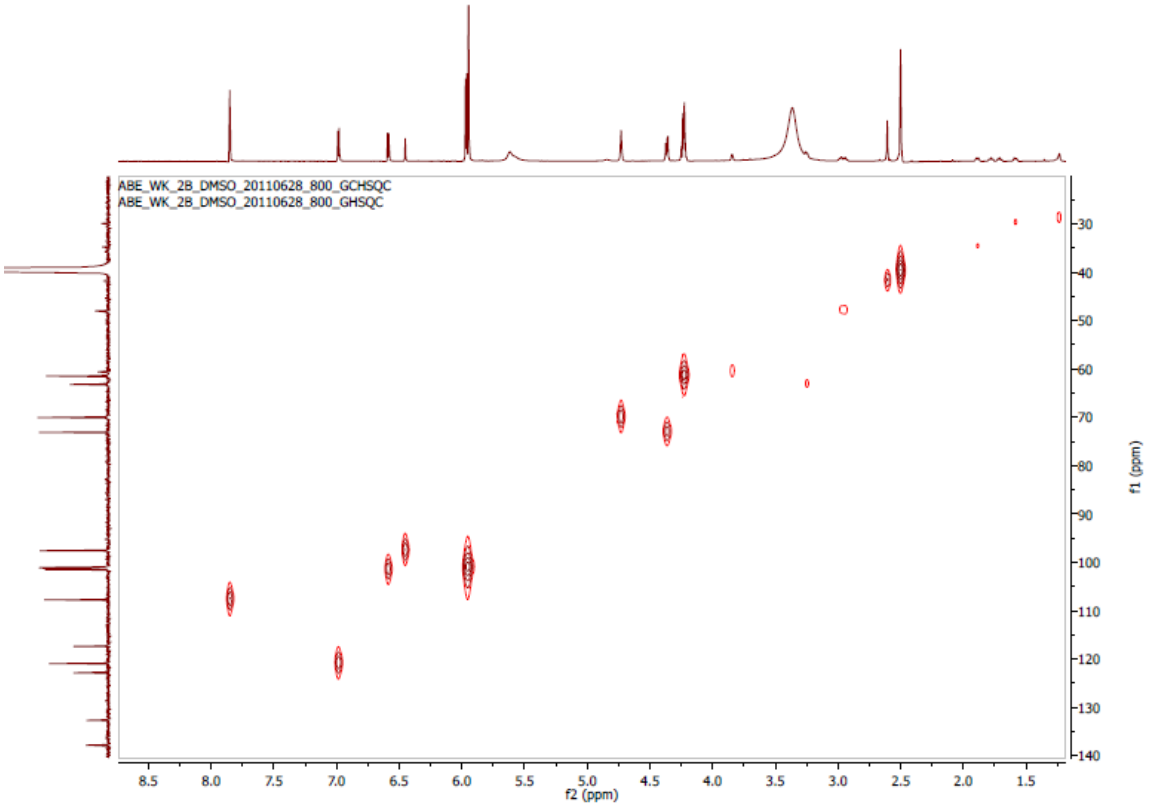
Appendix 14A: ^{1}H ($(CD_3)_2SO$, 800 MHz) NMR spectrum of 12-dihydrousararotenoid A (**72**)



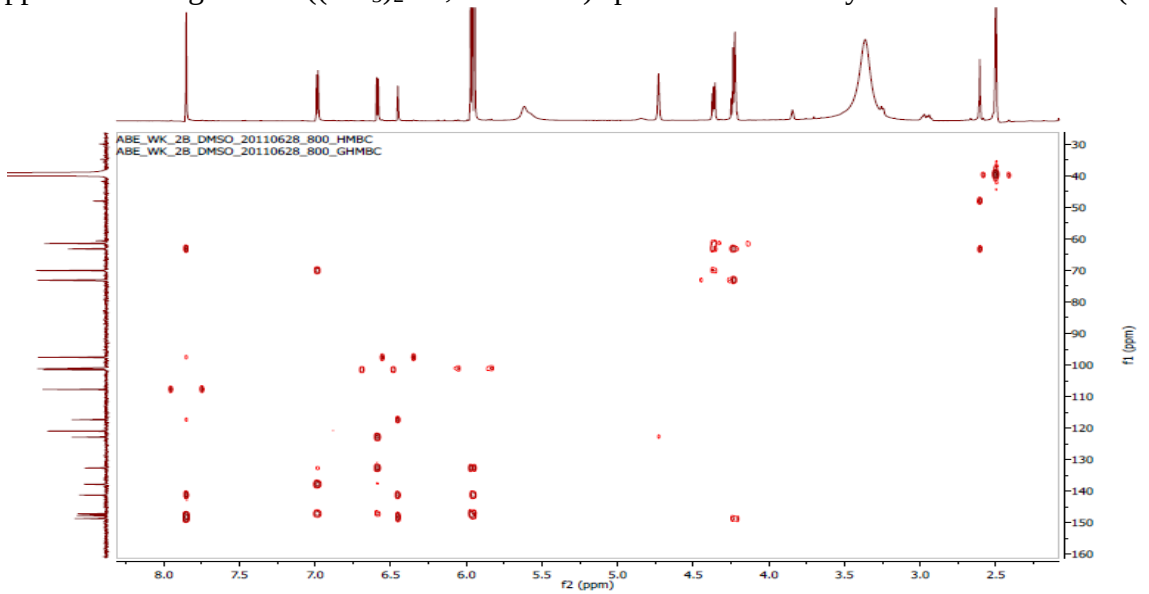
Appendix 14B: ^{13}C ($(CD_3)_2SO$, 150 MHz) NMR spectrum of 12-dihydrousararotenoid A (**72**)



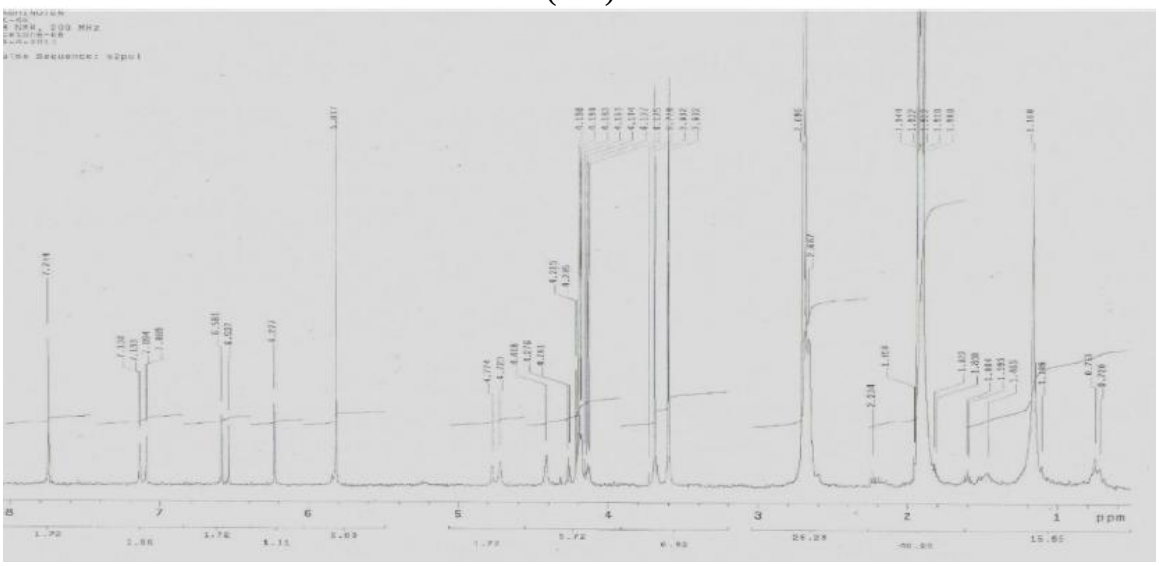
Appendix 14C: gCHSQC ($(CD_3)_2SO$, 800 MHz) spectrum of 12-dihydrousararotenoid A (**72**)



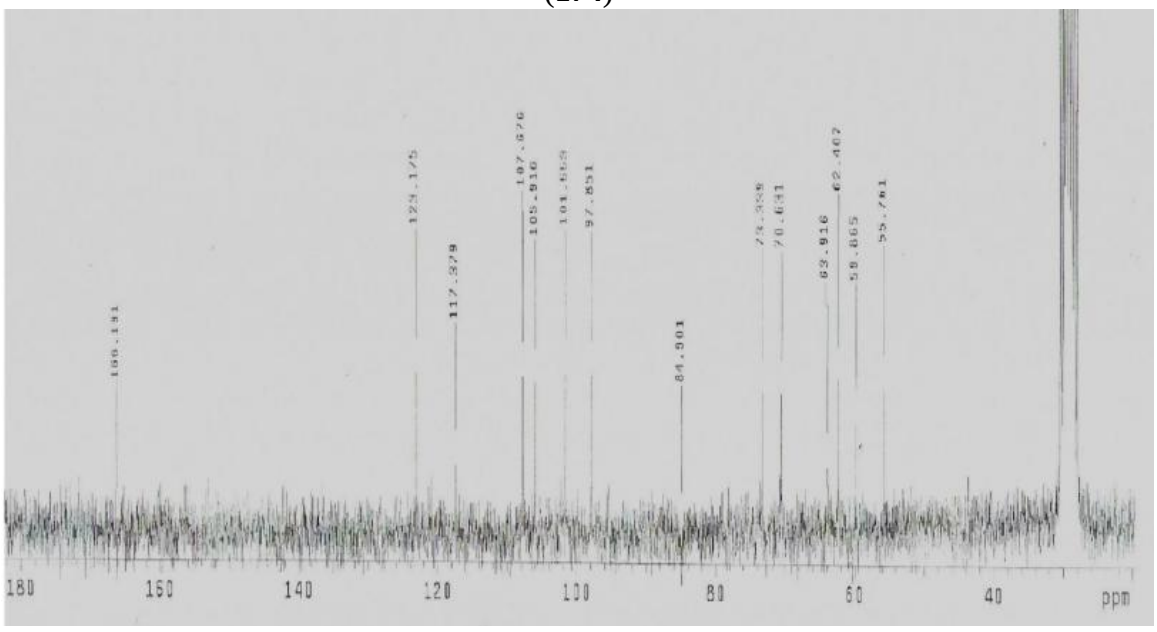
Appendix 14D: gHMBC ($(CD_3)_2SO$, 800 MHz) spectrum of 12-dihydrousararotenoid A (**72**)



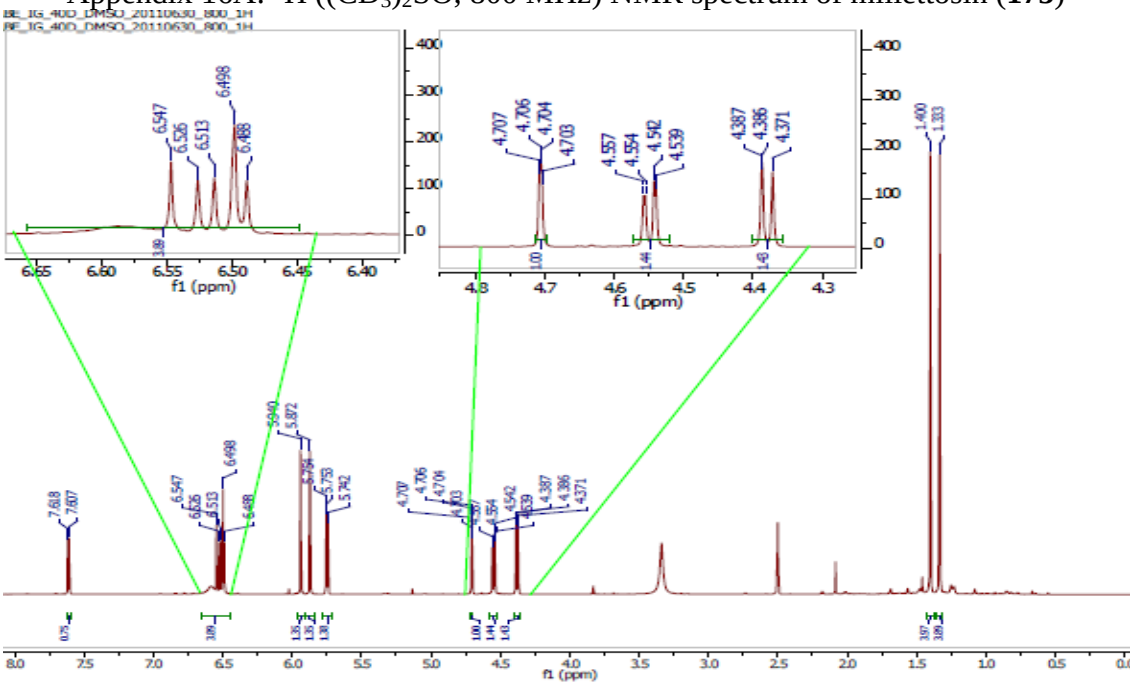
Appendix 15A: ^{1}H NMR ($(CD_3)_2CO$, 200 MHz) spectrum of 12-dihydrousararotenoid B (**174**)



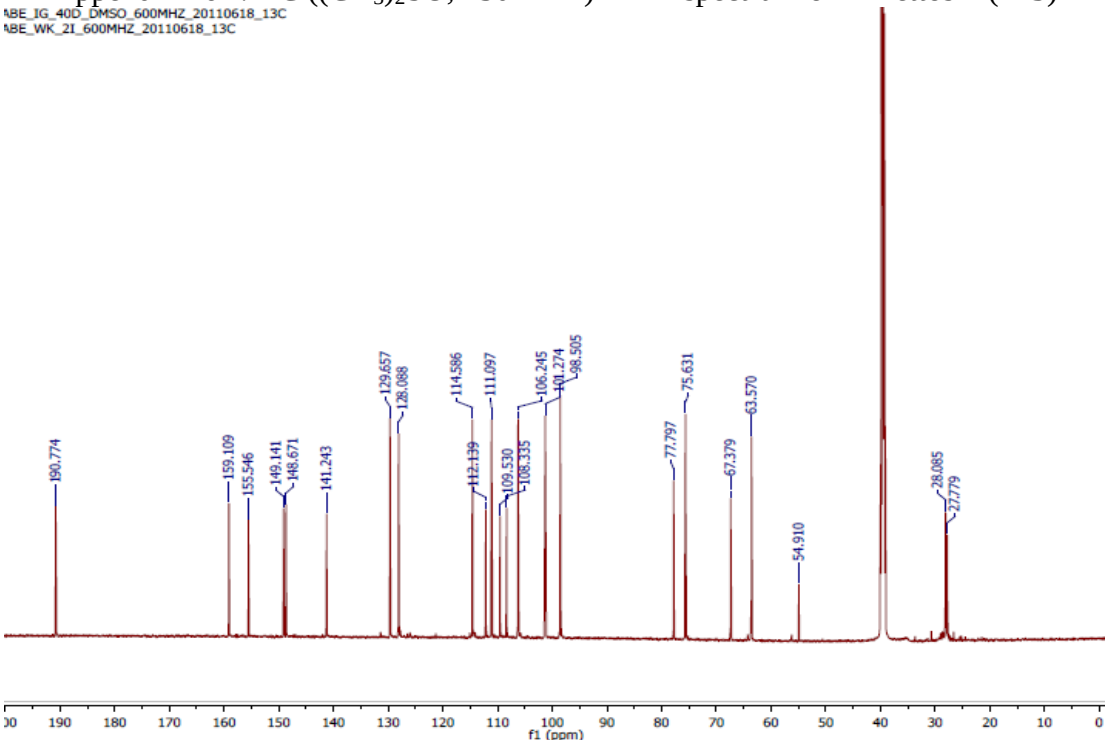
Appendix 15B: ^{13}C NMR ($(CD_3)_2CO$, 50 MHz) spectrum of 12-dihydrousararotenoid B (**174**)



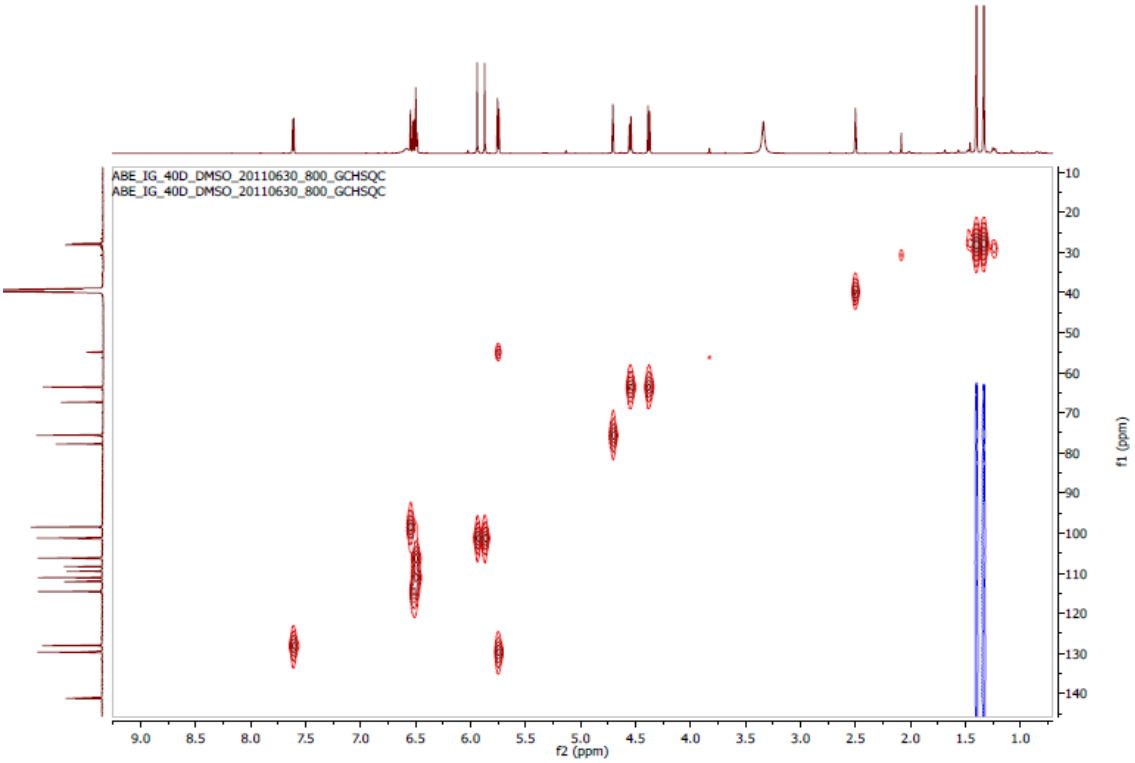
Appendix 16A: ^{1}H ($(CD_3)_2SO$, 800 MHz) NMR spectrum of millettosin (**175**)



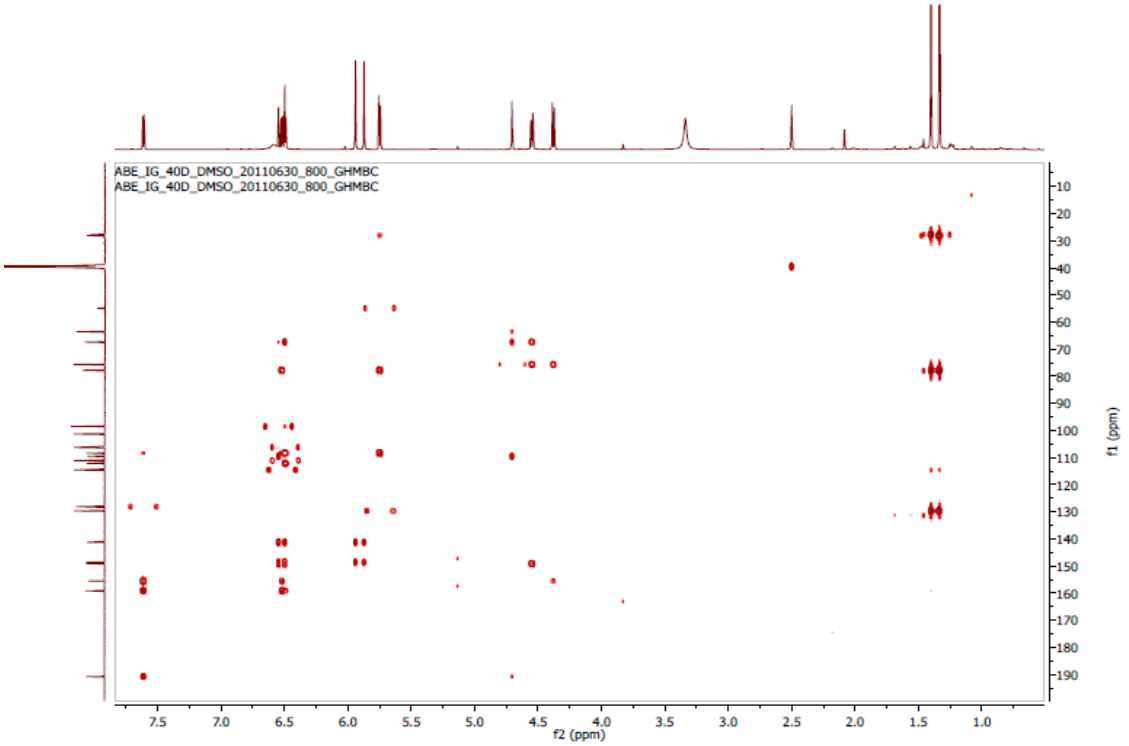
Appendix 16B: ^{13}C ($(CD_3)_2SO$, 150 MHz) NMR spectrum of millettosin (**175**)



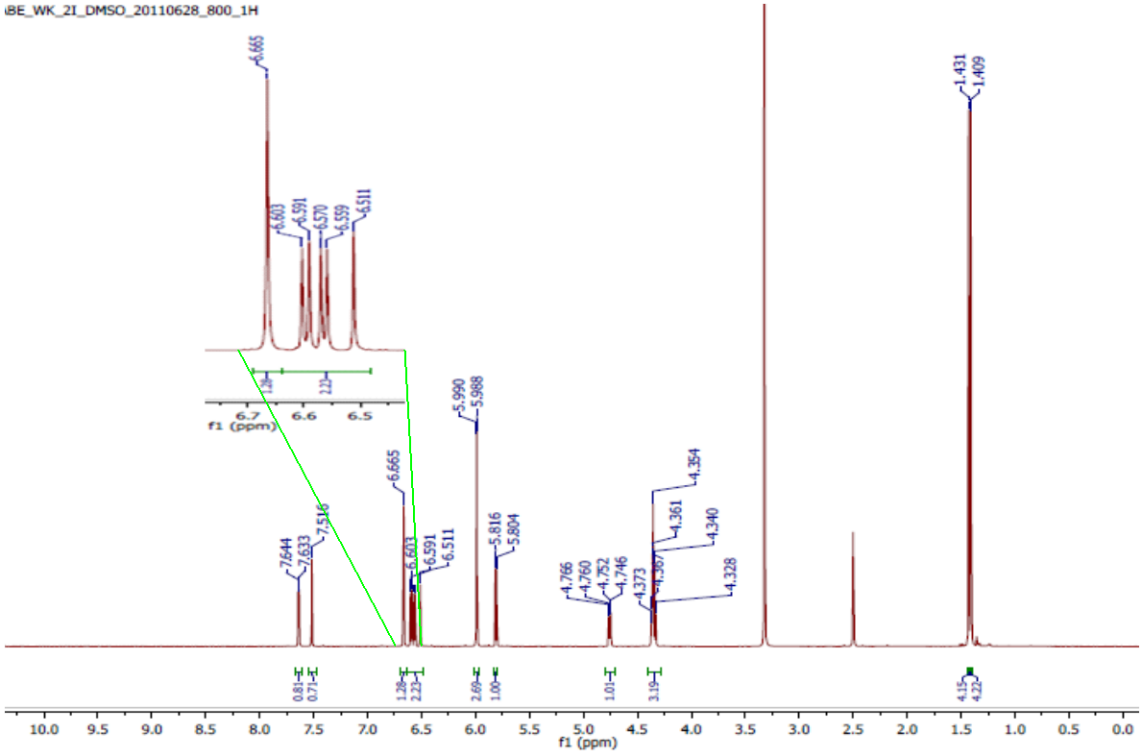
Appendix 16C: gCHSQC ($(CD_3)_2SO$, 800 MHz) spectrum of millettosin (**175**)



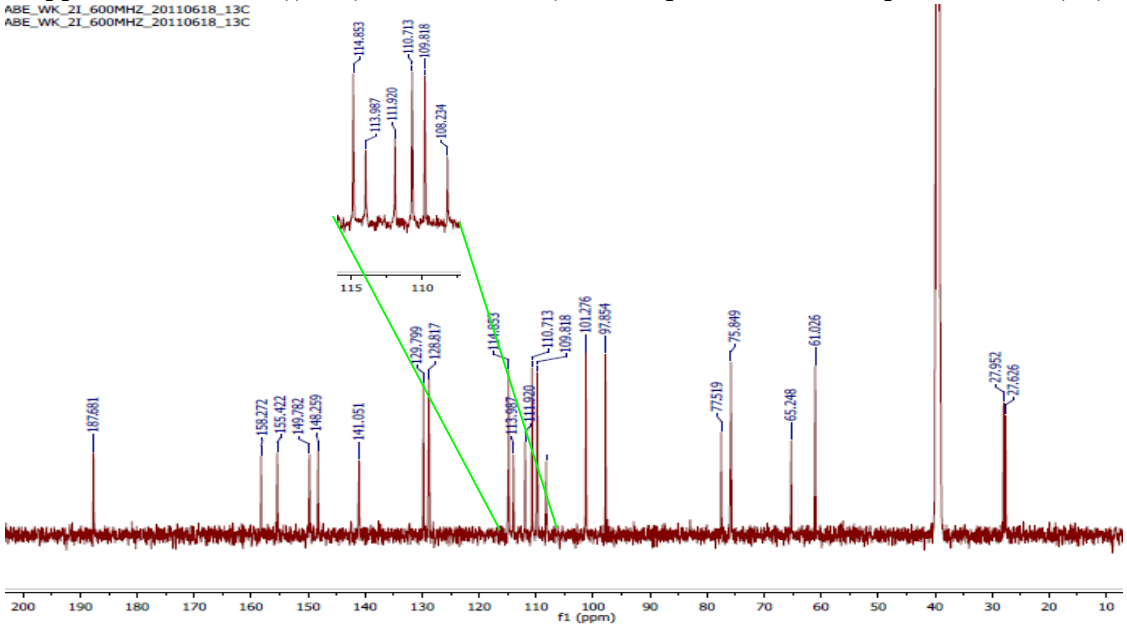
Appendix 16D: gHMBC ($(CD_3)_2SO$, 800 MHz) spectrum of millettosin (**175**)



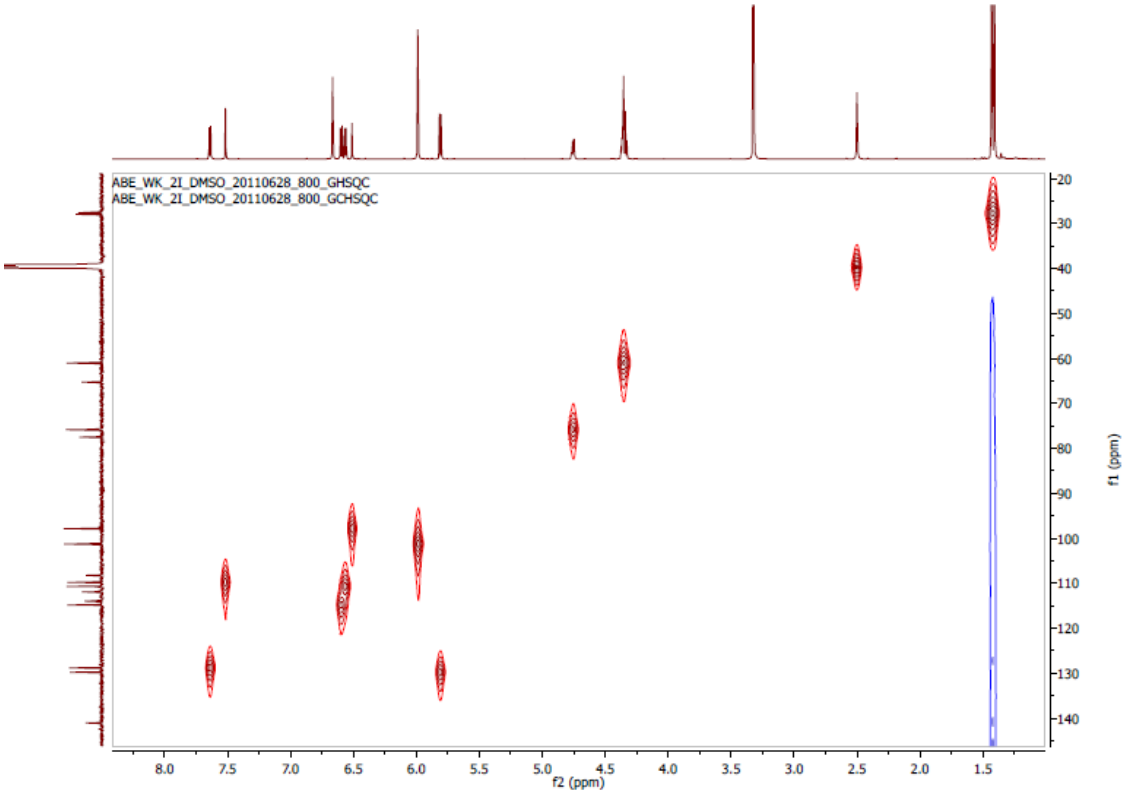
Appendix 17A: ^{1}H ($(CD_3)_2SO$, 800 MHz) NMR spectrum of 12a-epimillettosin (**70**)



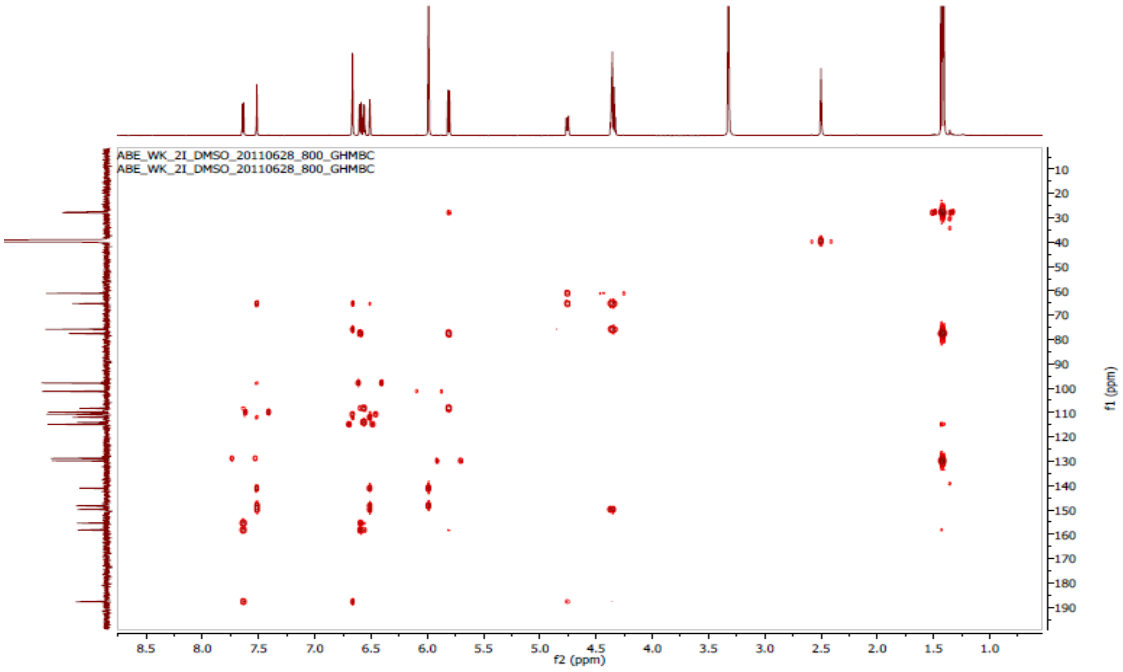
Appendix 17B: ^{13}C ($(CD_3)_2SO$, 150 MHz) NMR spectrum of 12a-epimillettosin (**70**)



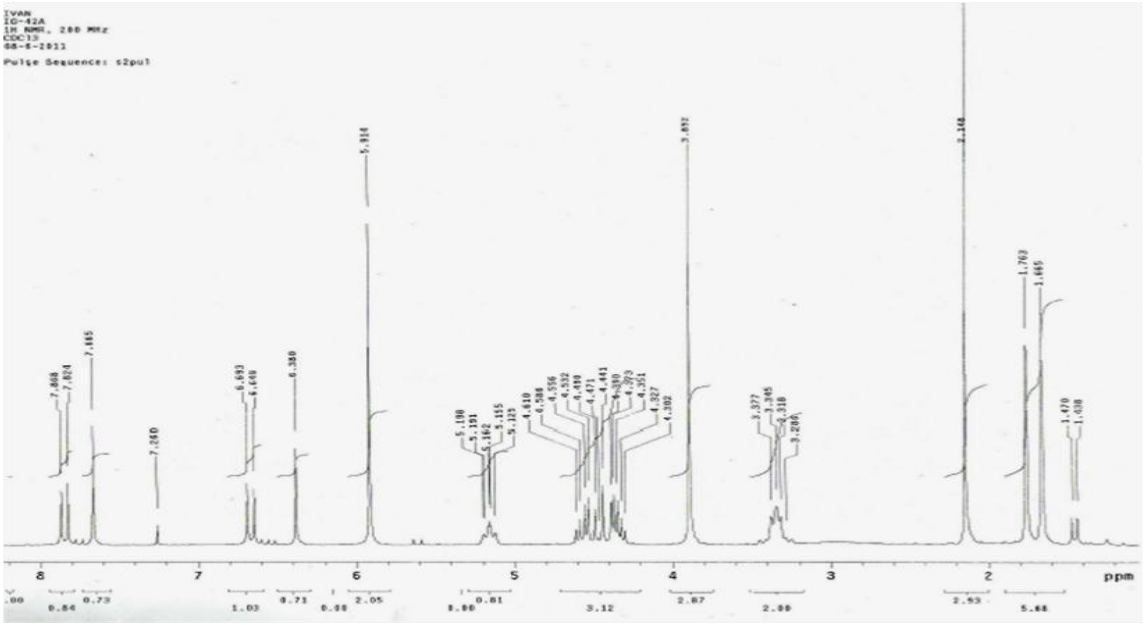
Appendix 17C: gCHSQC ($(CD_3)_2SO$, 800 MHz) spectrum of 12a-epimillettosin (**70**)



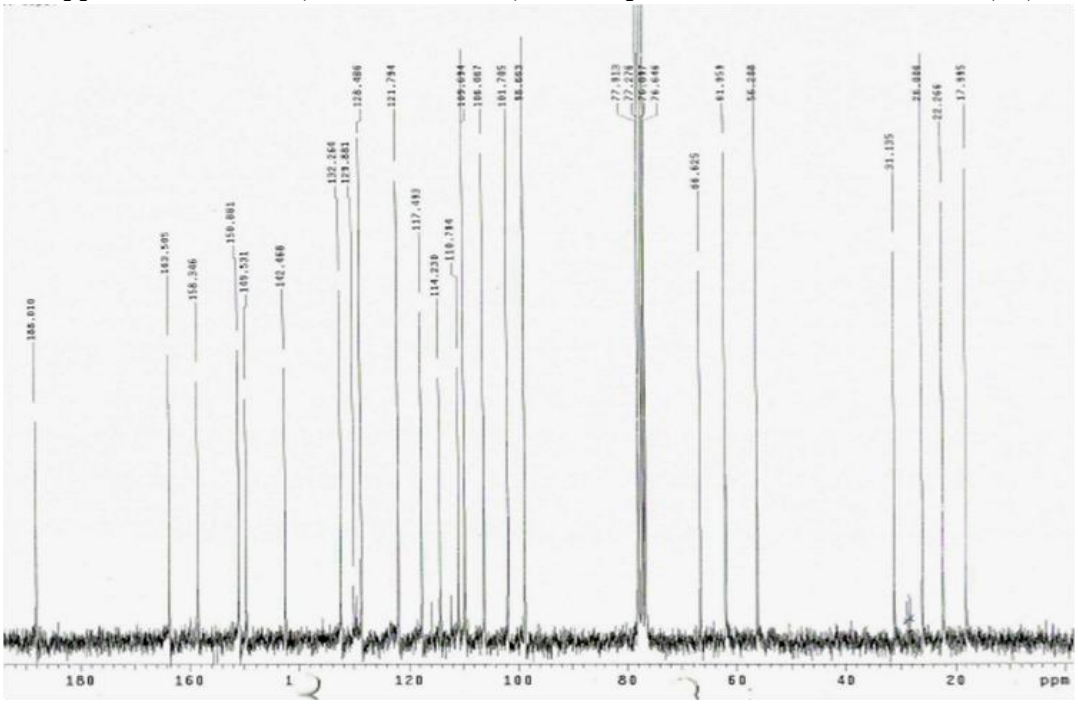
Appendix 17D: gHMBC ($(CD_3)_2SO$, 800 MHz) spectrum of 12a-epimillettosin (**70**)



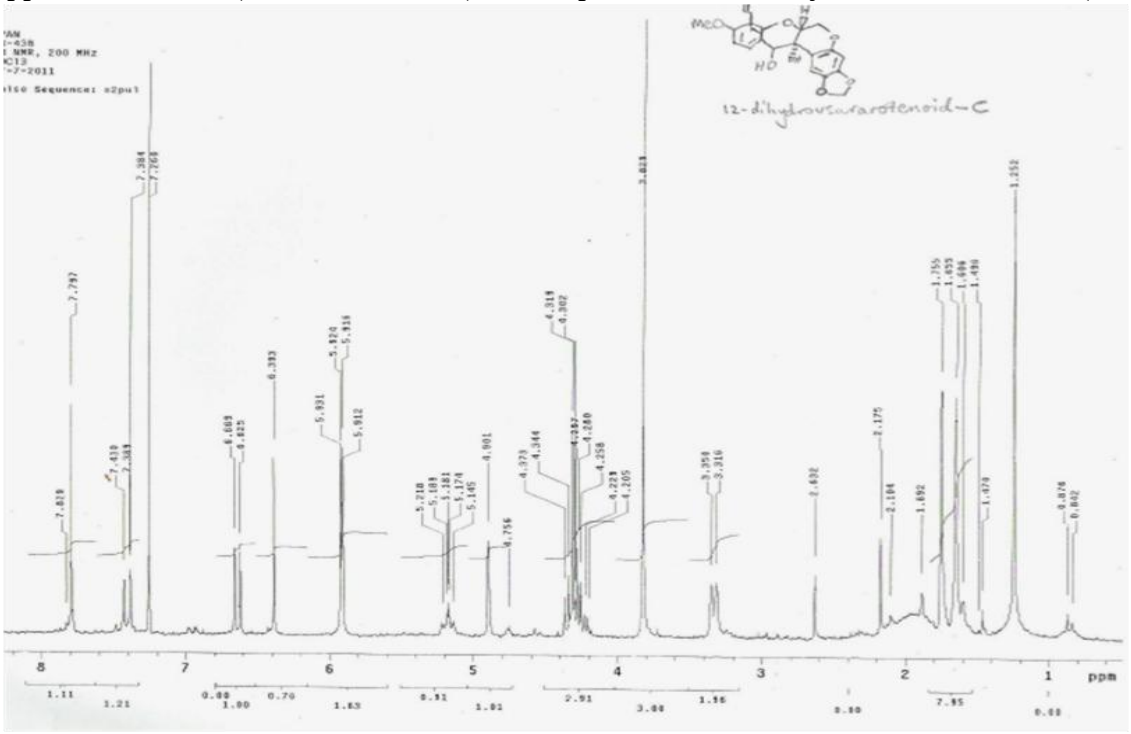
Appendix 18A: ^{1}H ($CDCl_3$, 200 MHz) NMR spectrum of usararotenoid C (**74**)



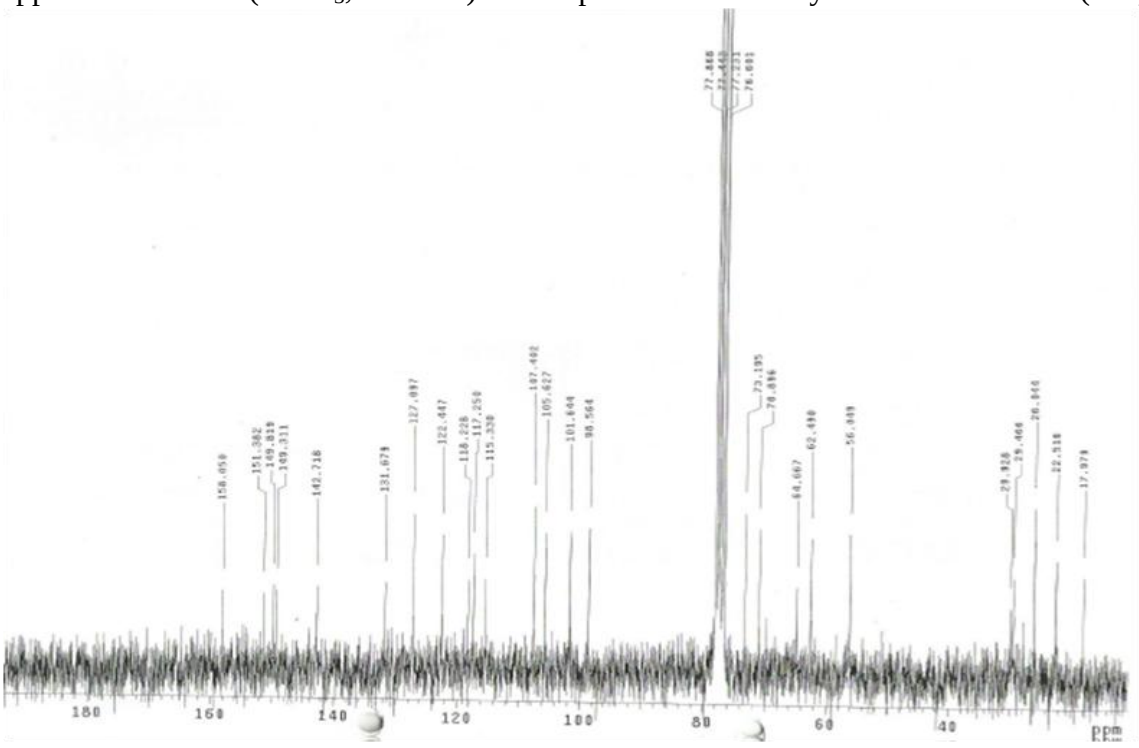
Appendix 18B: ^{13}C ($CDCl_3$, 50 MHz) NMR spectrum of usararotenoid C (**74**)



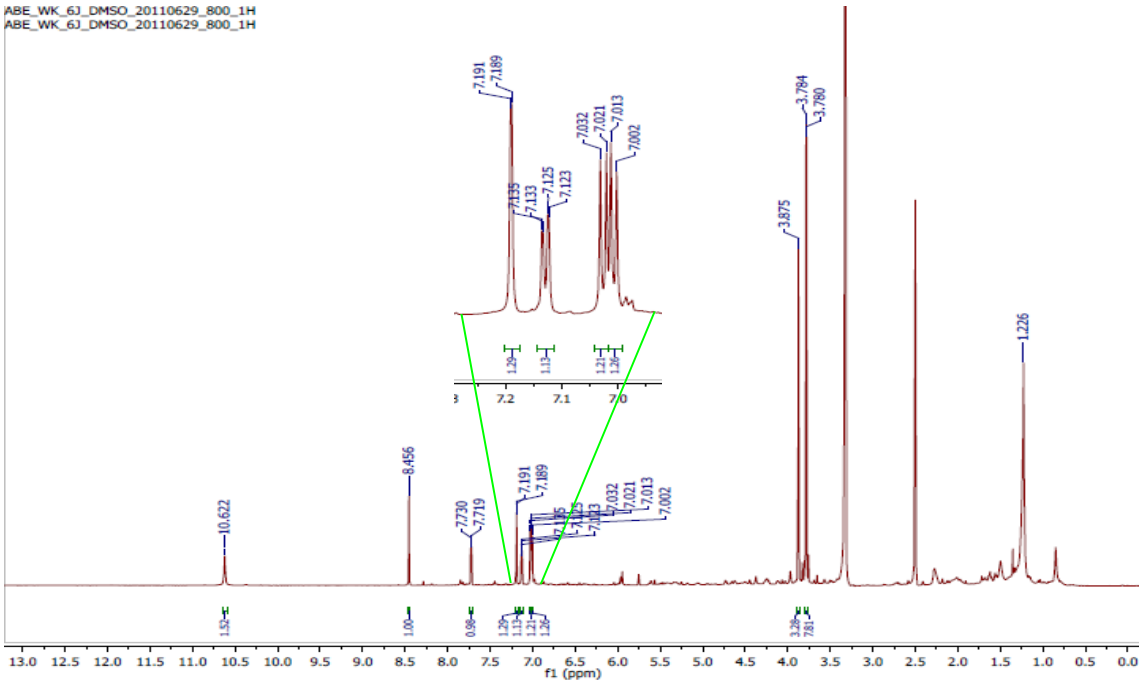
Appendix 19A: ^{1}H ($CDCl_3$, 200 MHz) NMR spectrum of 12-dihydrousararotenoid C (**176**)



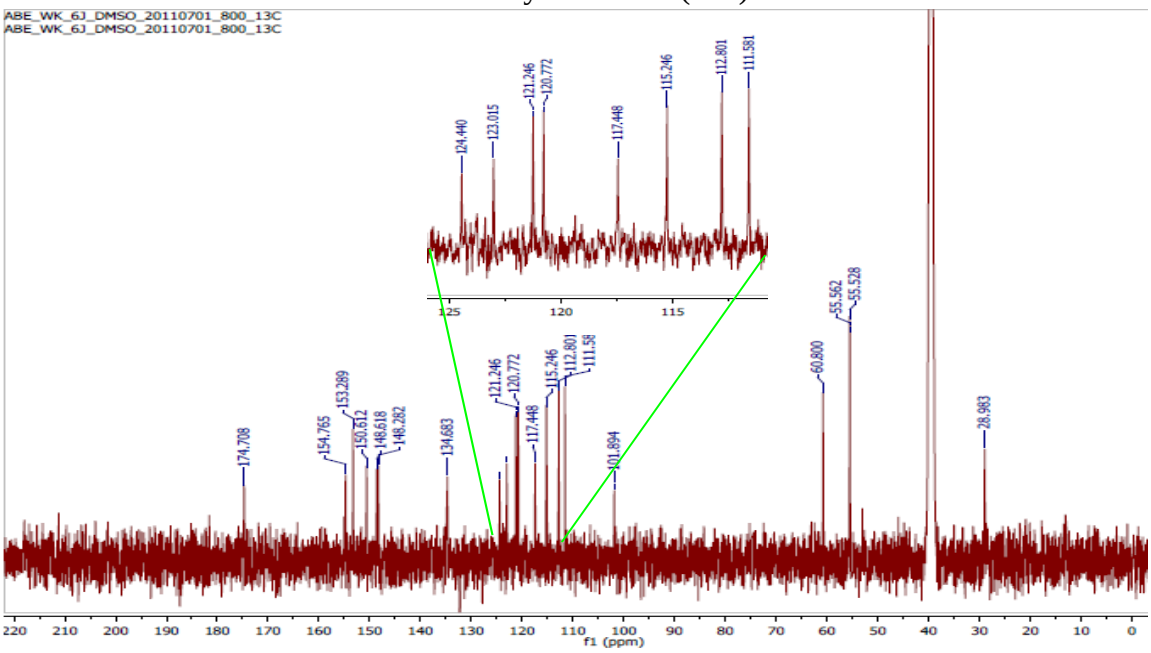
Appendix 19B: ^{13}C ($CDCl_3$, 50 MHz) NMR spectrum of 12-dihydrousararotenoid C (**176**)



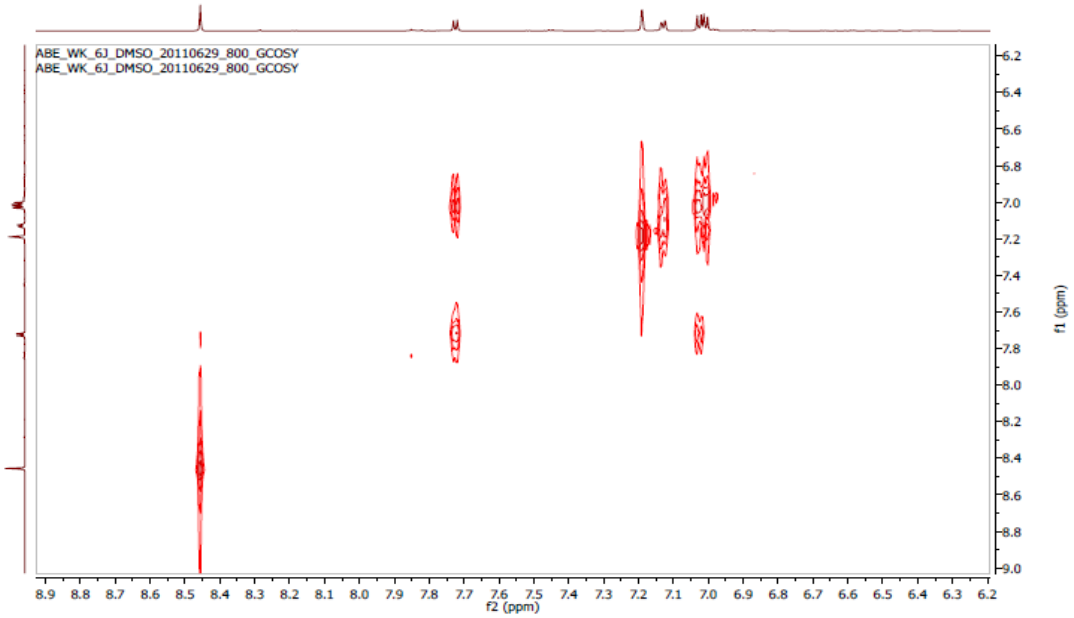
Appendix 20A: ^{1}H ($(CD_3)_2SO$, 800 MHz) NMR spectrum of 7-hydro-8,3',4'-trimethoxyisoflavone (**177**)



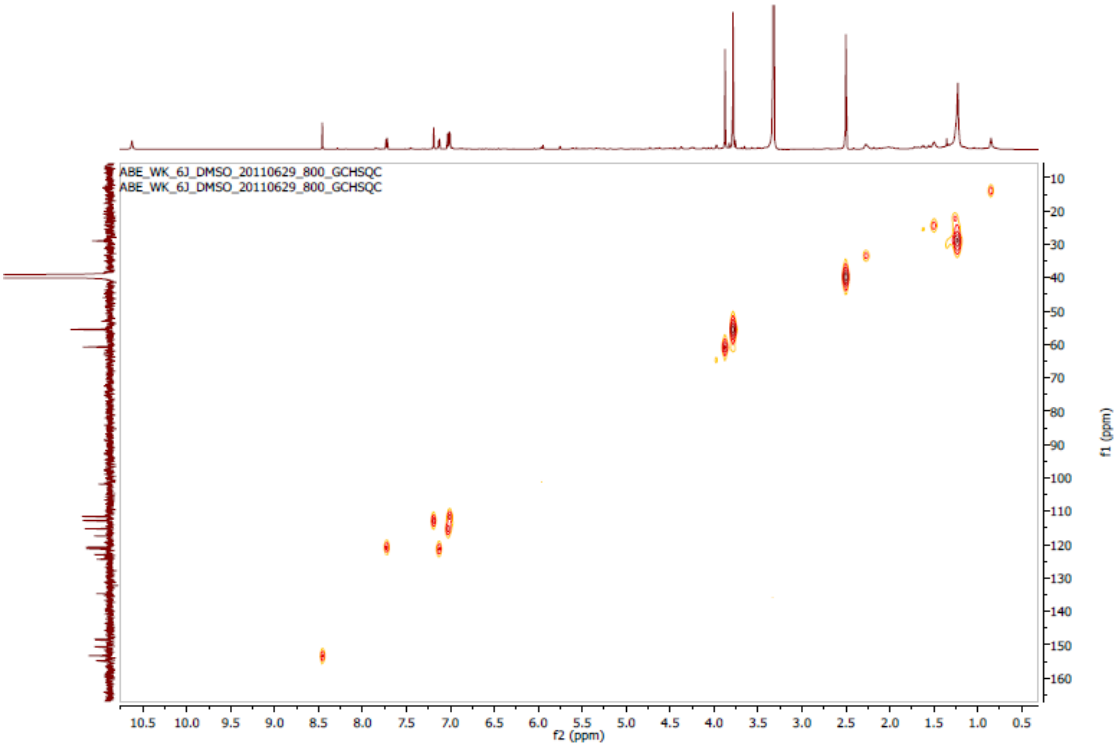
Appendix 20B: ^{13}C ($(CD_3)_2SO$, 150 MHz) NMR spectrum of 7-hydro-8,3',4'-trimethoxyisoflavone (**177**)



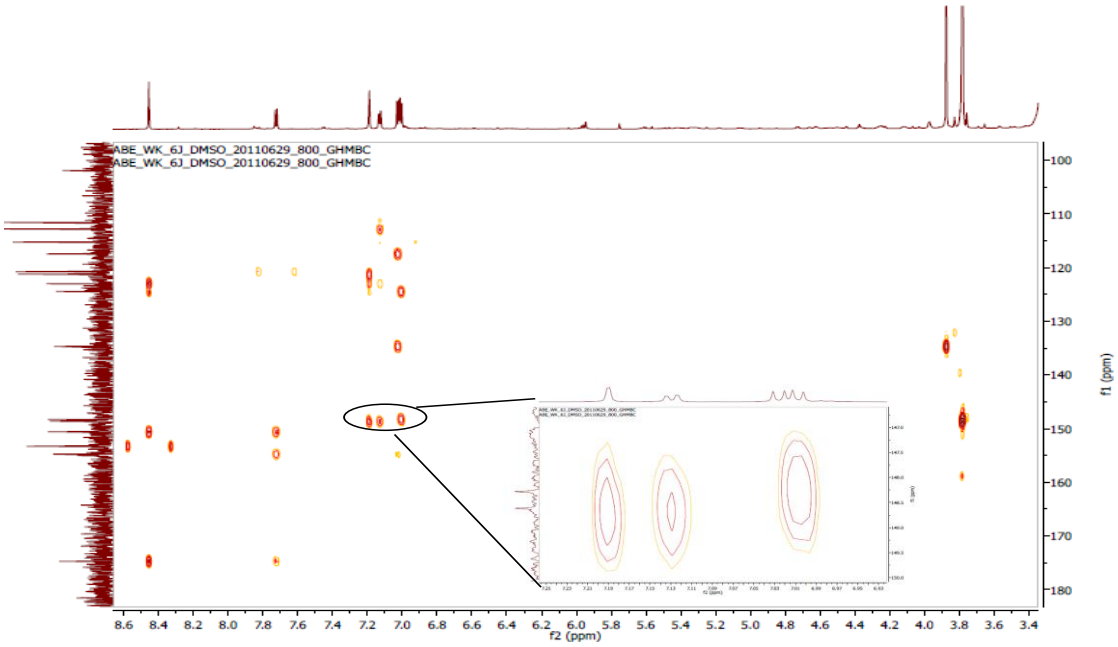
Appendix 20C: ^{1}H-^{1}H gCOSY ($(CD_3)_2SO$, 800 MHz) spectrum of 7-hydro-8,3',4'-trimethoxyisoflavone (**177**)



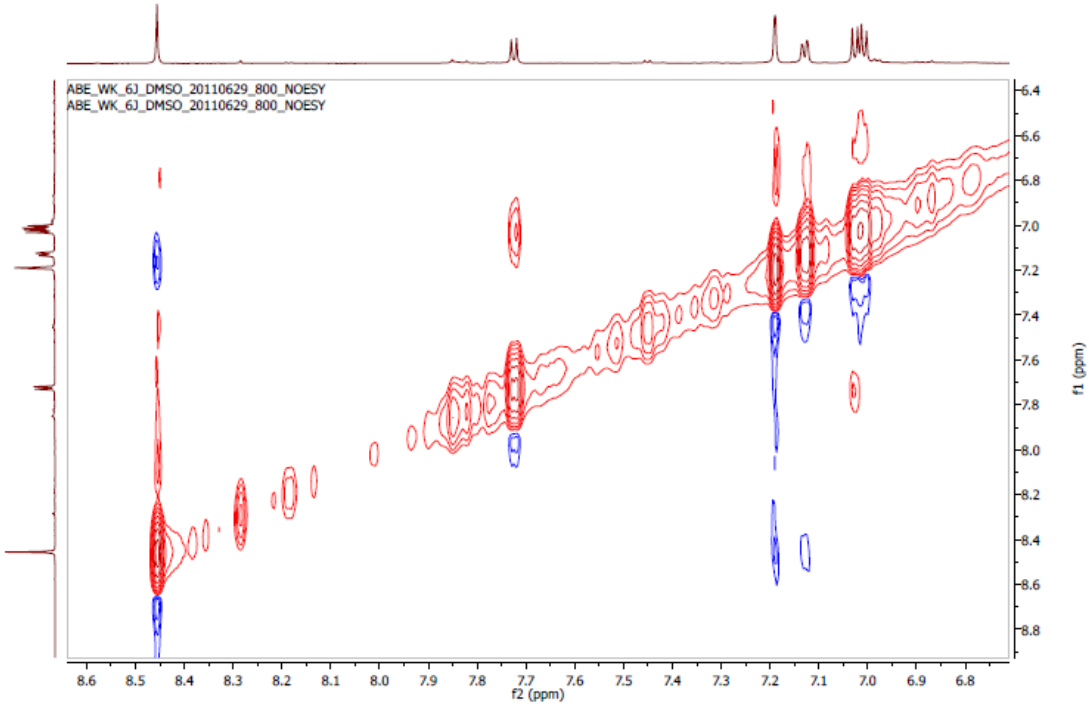
Appendix 20D: gCHSQC ($(CD_3)_2SO$, 800 MHz) spectrum of 7-hydro-8,3',4'-trimethoxyisoflavone (**177**)



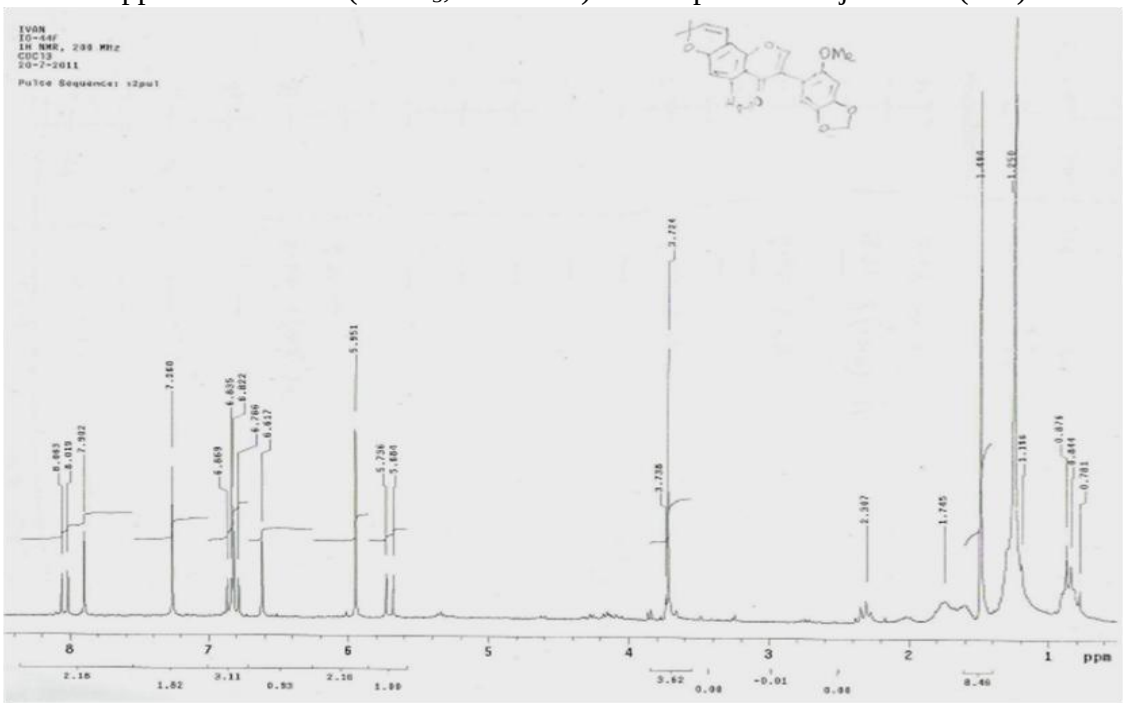
Appendix 20E: gHMBC ($(CD_3)_2SO$, 800 MHz) spectrum of 7-hydro-8,3',4'-trimethoxyisoflavone (**177**)



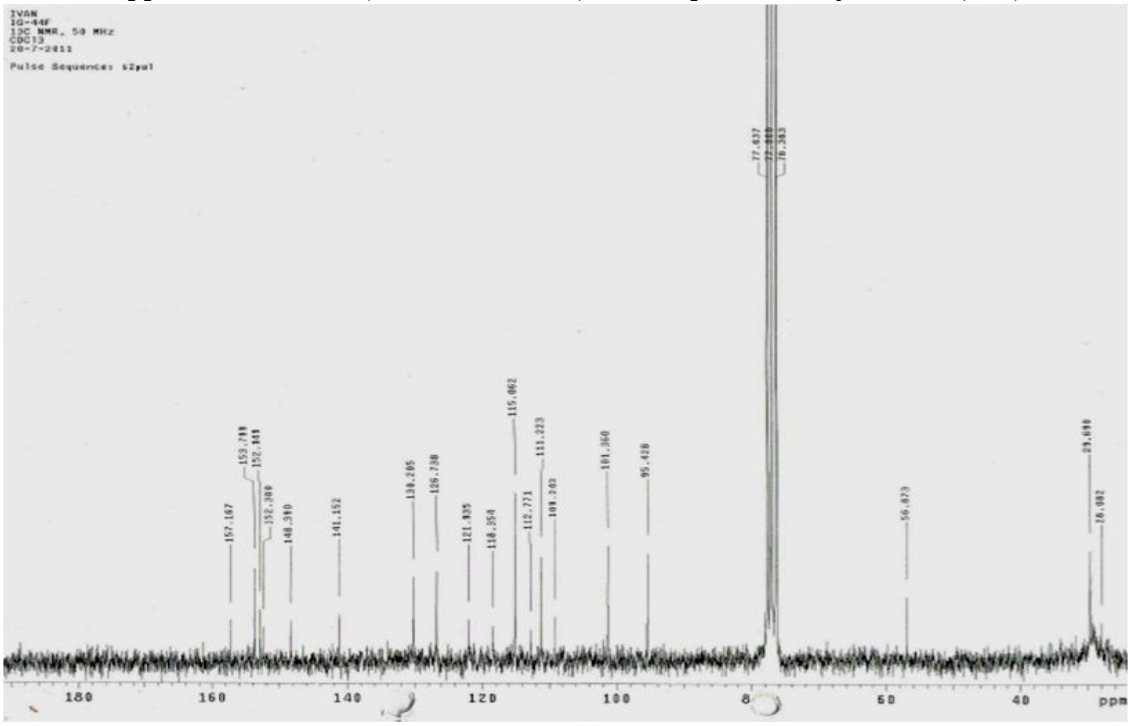
Appendix 20F: gNOESY ($(CD_3)_2SO$, 800 MHz) spectrum of 7-hydro-8,3',4'-trimethoxyisoflavone (**277**)



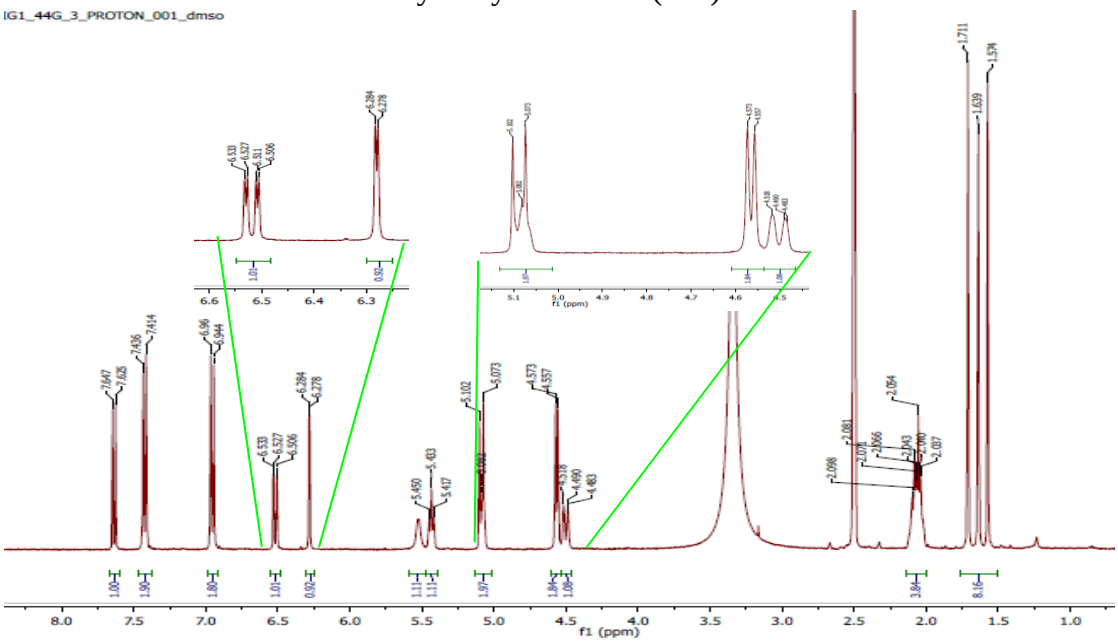
Appendix 21A: ^{1}H ($CDCl_3$, 200 MHz) NMR spectrum of jamaicin (**178**)



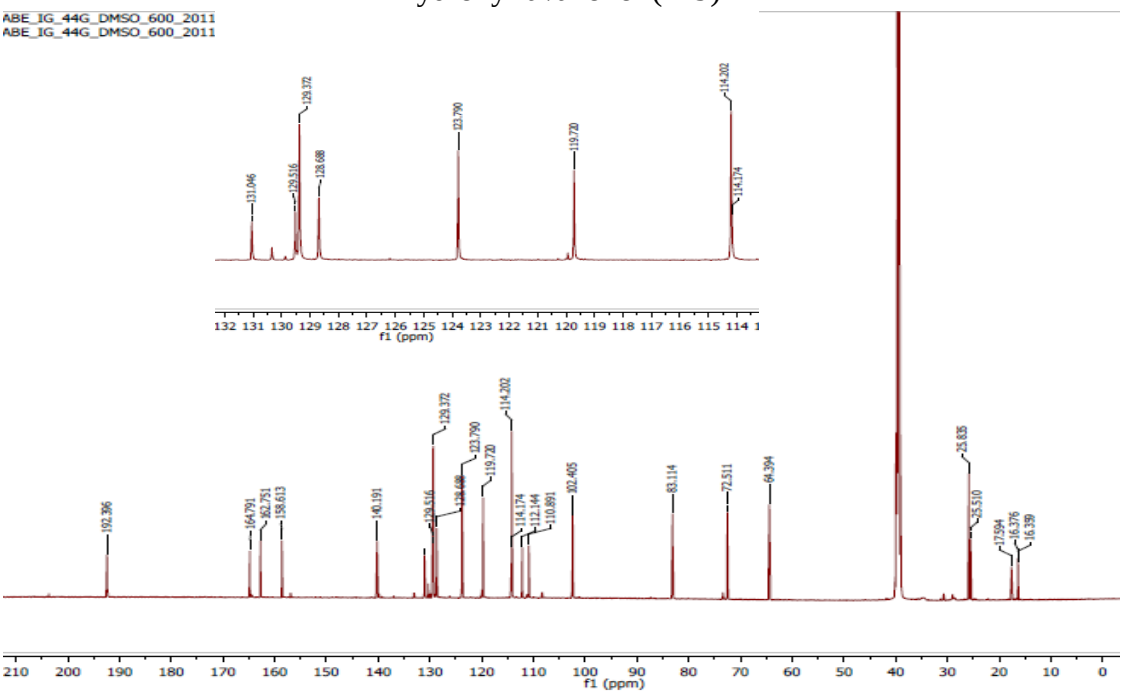
Appendix 21B: ^{13}C ($CDCl_3$, 50 MHz) NMR spectrum of jamaicin (**178**)



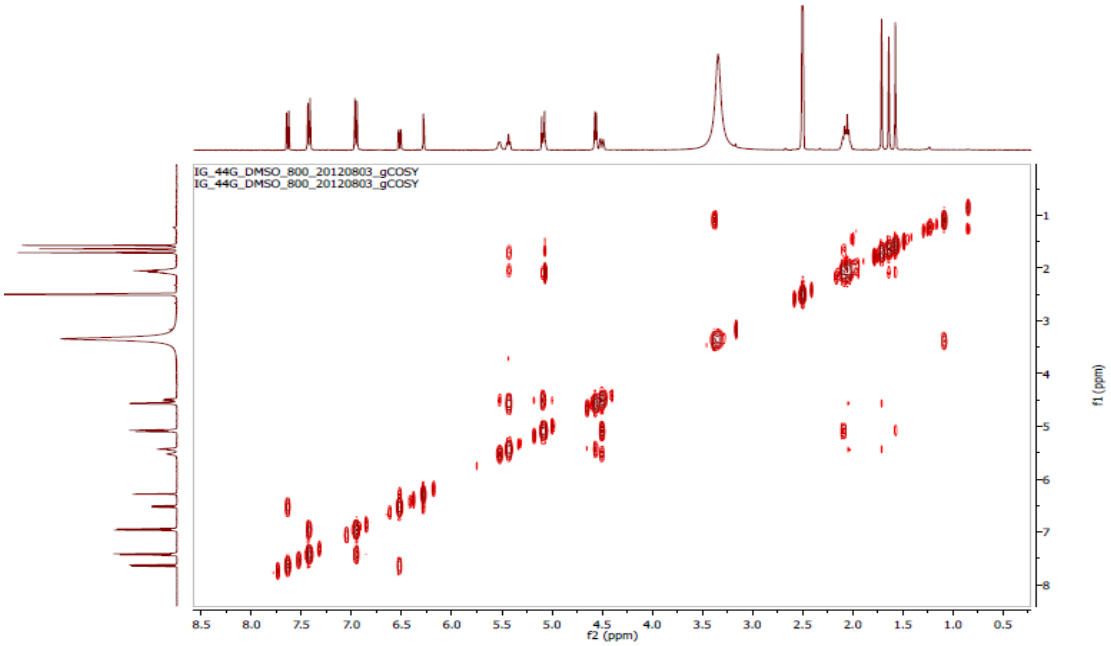
Appendix 22A: ^{1}H ($(CD_3)_2SO$, 400 MHz) NMR spectrum of (2*R*,3*R*)-4'-*O*-geranyl-7-hydroxyflavanonol (**179**)



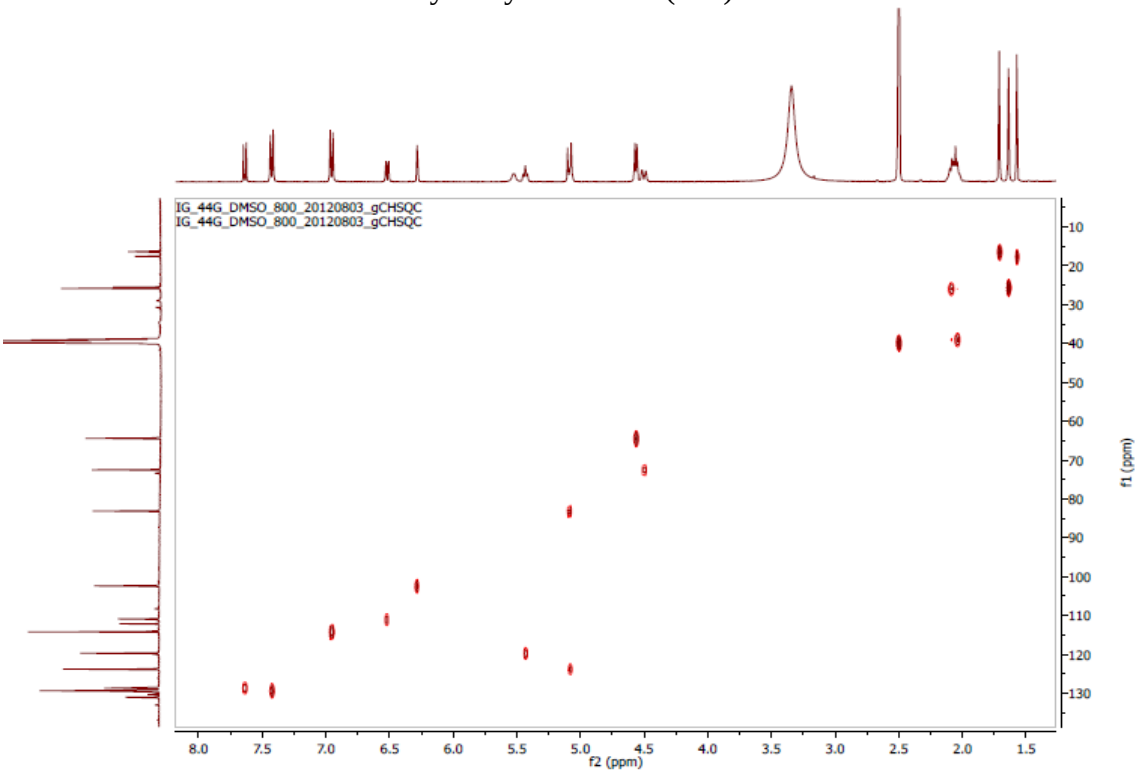
Appendix 22B: ^{13}C ($(CD_3)_2SO$, 150 MHz) NMR spectrum of (2*R*,3*R*)-4'-*O*-geranyl-7-hydroxyflavanonol (**179**)



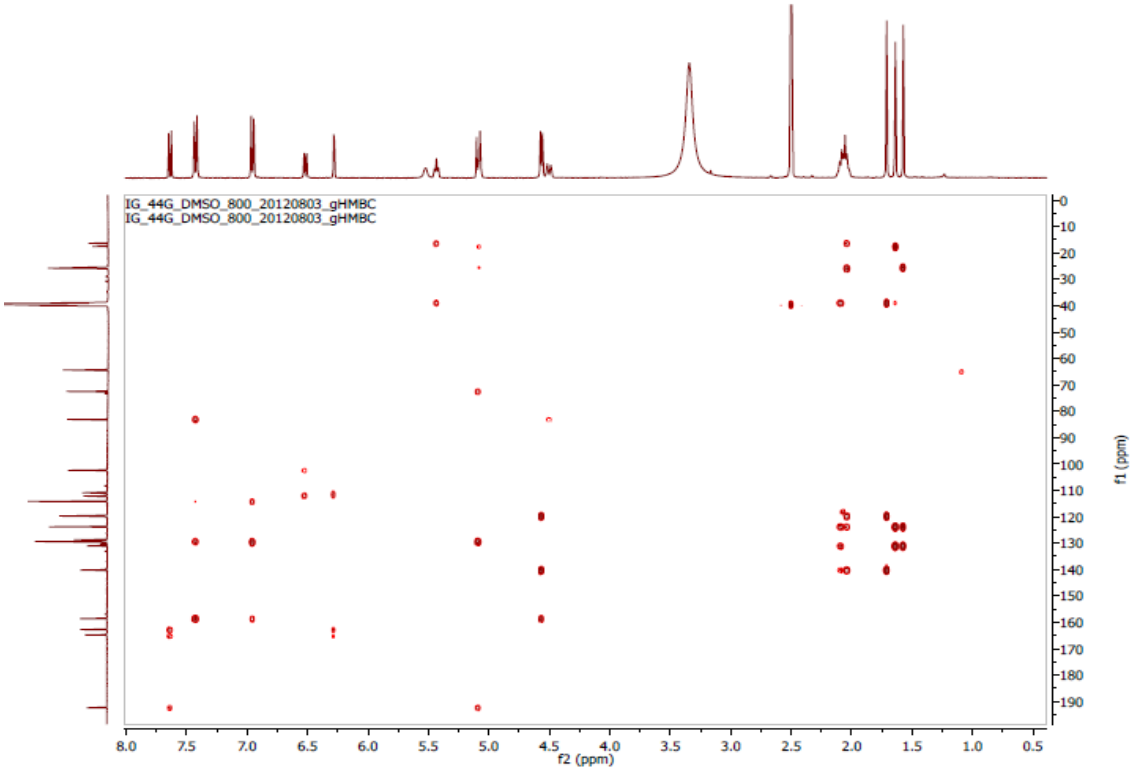
Appendix 22C: ^{1}H-^{1}H gCOSY ($(CD_3)_2SO$, 800 MHz) spectrum of (2*R*,3*R*)-4'-*O*-geranyl-7-hydroxyflavanonol (**179**)



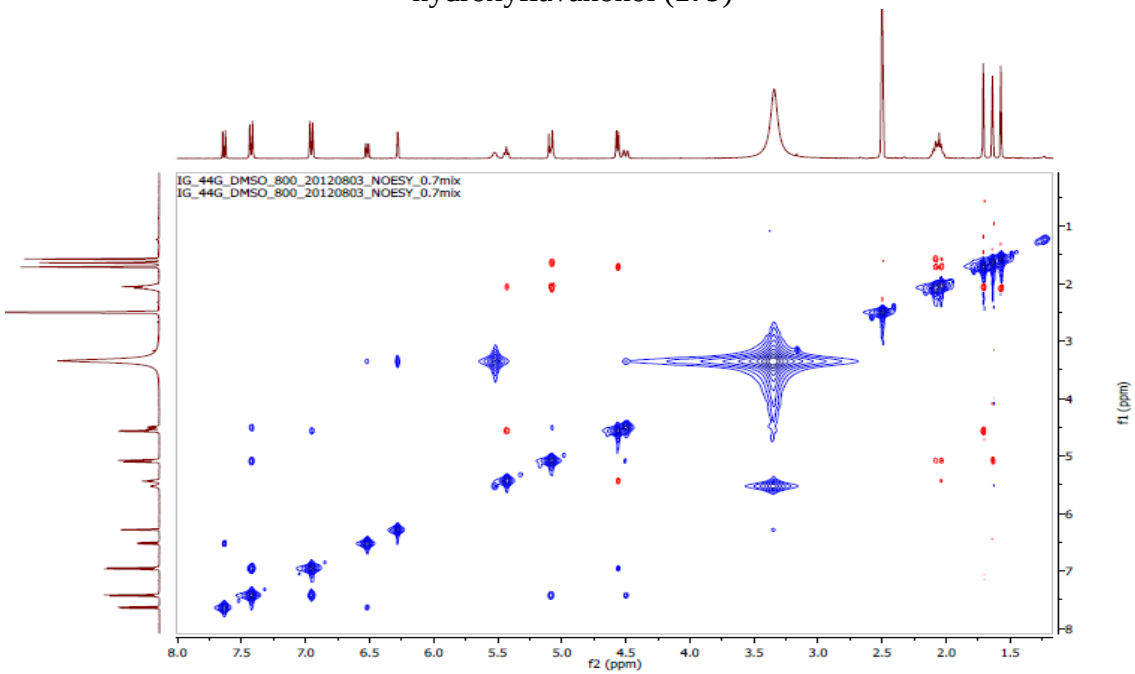
Appendix 22D: gCHSQC ($(CD_3)_2SO$, 800 MHz) spectrum of (2*R*,3*R*)-4'-*O*-geranyl-7-hydroxyflavanonol (**179**)



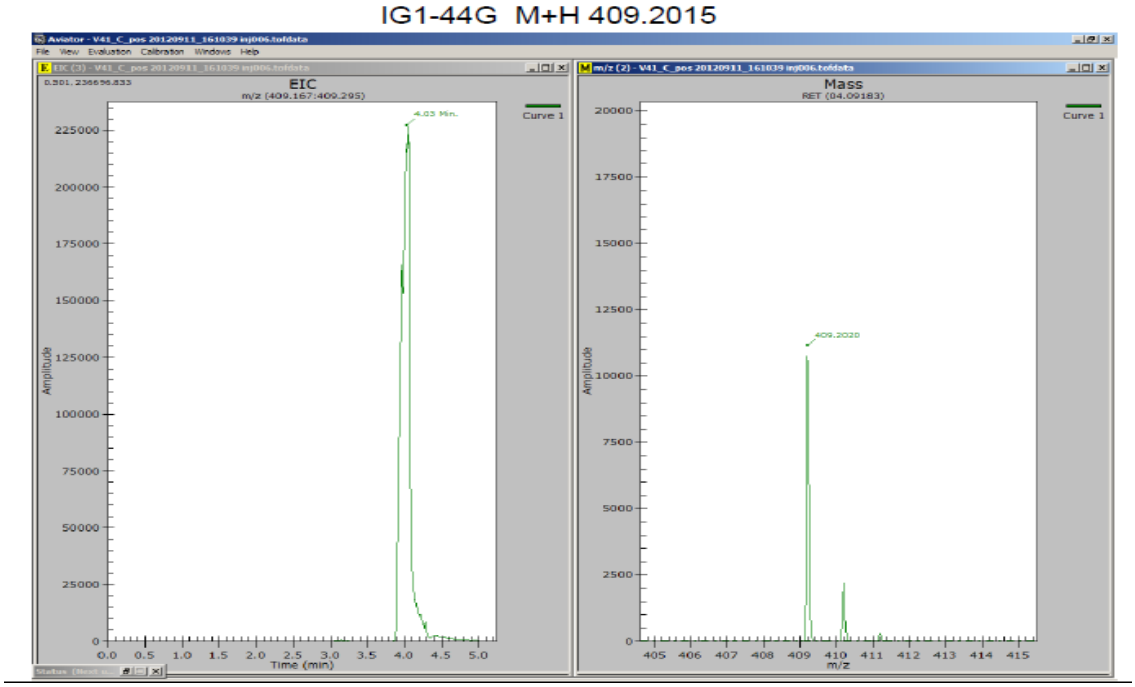
Appendix 22E: gHMBC ($(CD_3)_2SO$, 800 MHz) spectrum of (2*R*,3*R*)-4'-*O*-geranyl-7-hydroxyflavanonol (**179**)



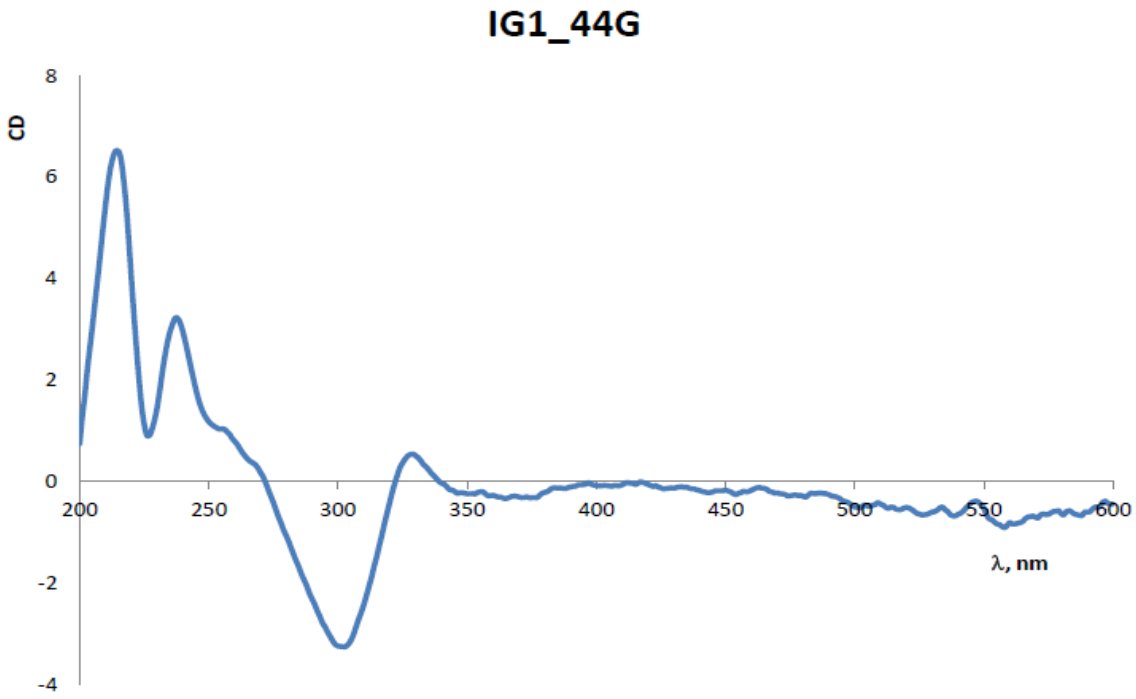
Appendix 22F: gNOESY ($(CD_3)_2SO$, 800 MHz) spectrum of (2*R*,3*R*)-4'-*O*-geranyl-7-hydroxyflavanonol (**179**)



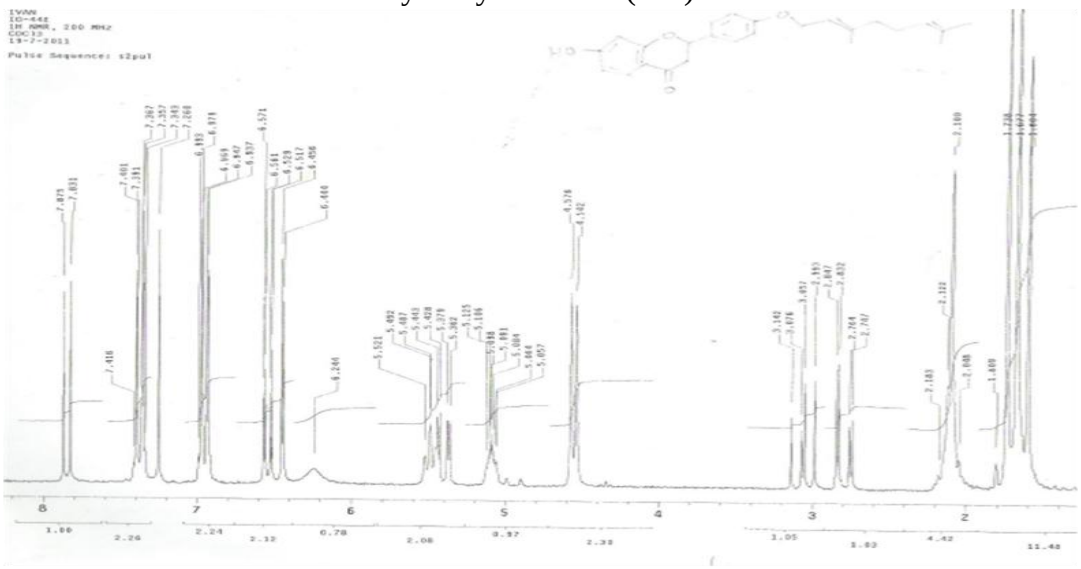
Appendix 22G: HRESI-MS of (2*R*,3*R*)-4'-*O*-geranyl-7-hydroxyflavanonol (**179**)



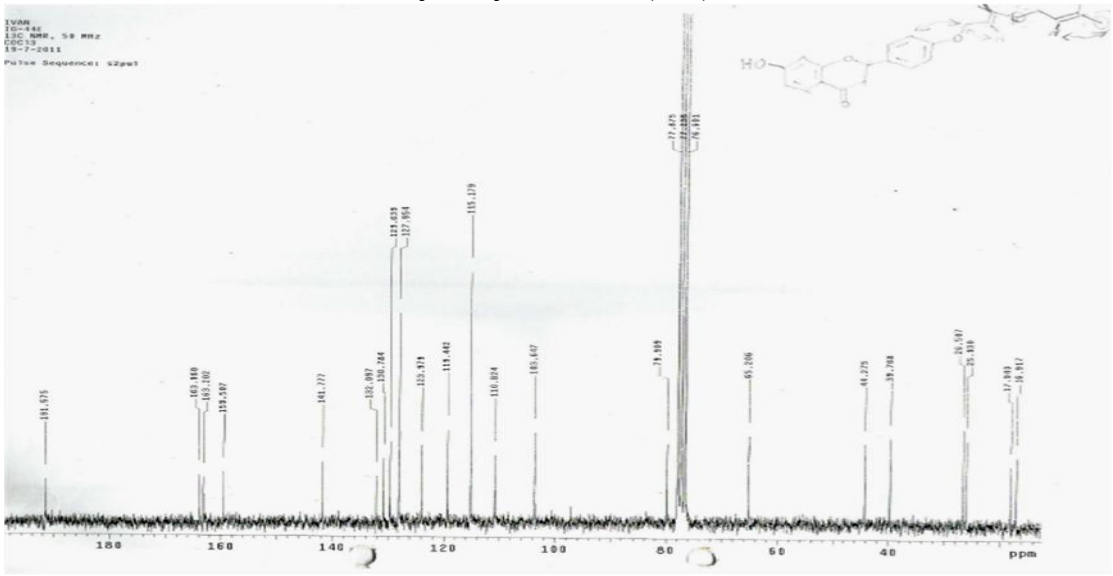
Appendix 22G: CD spectrum of (2*R*,3*R*)-4'-*O*-geranyl-7-hydroxyflavanonol (**179**)



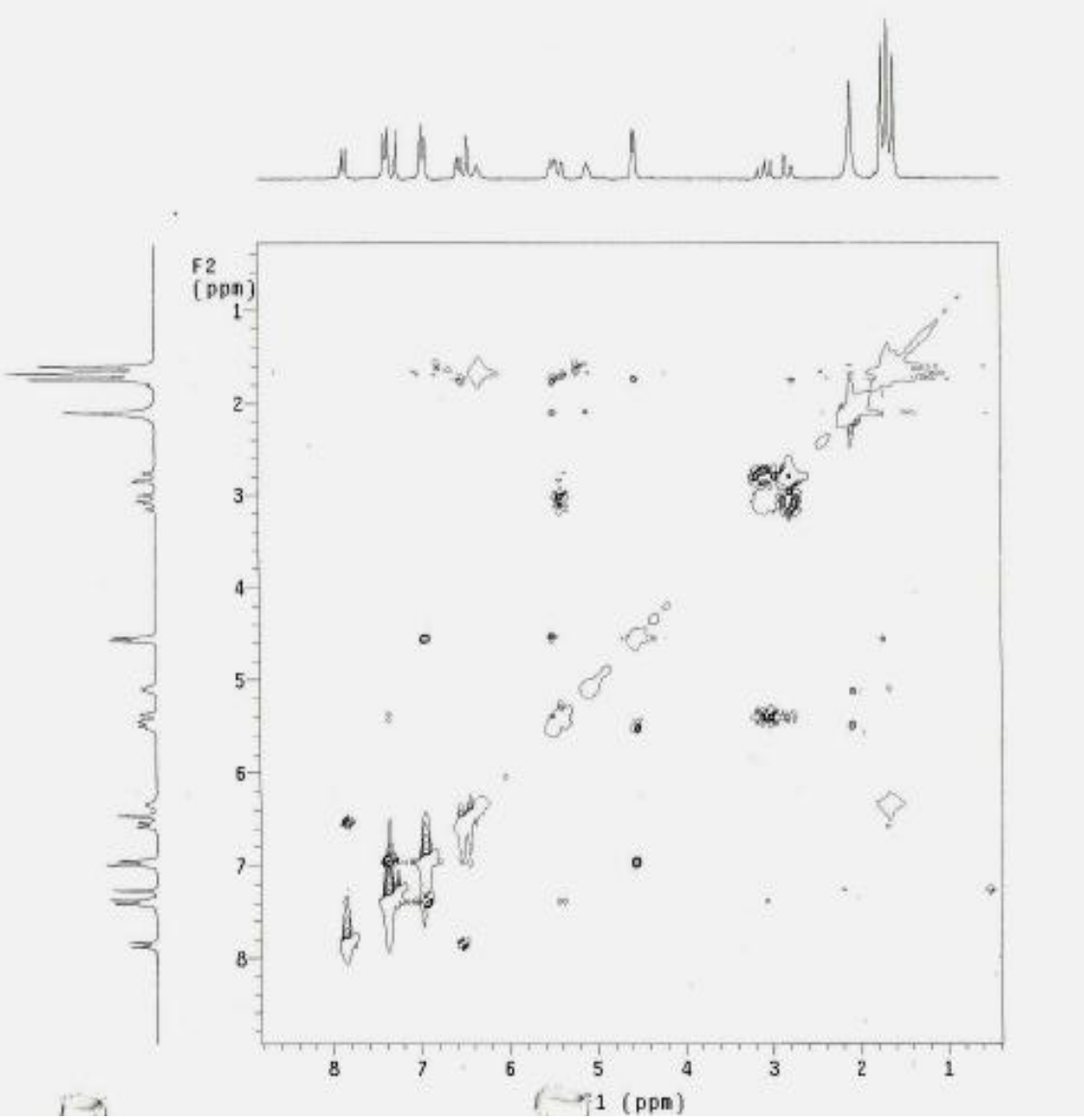
Appendix 23A: ^{1}H ($CDCl_3$, 200 MHz) NMR spectrum of (*S*)-4'-*O*-geranyl-7-hydroxyflavanone (**180**)



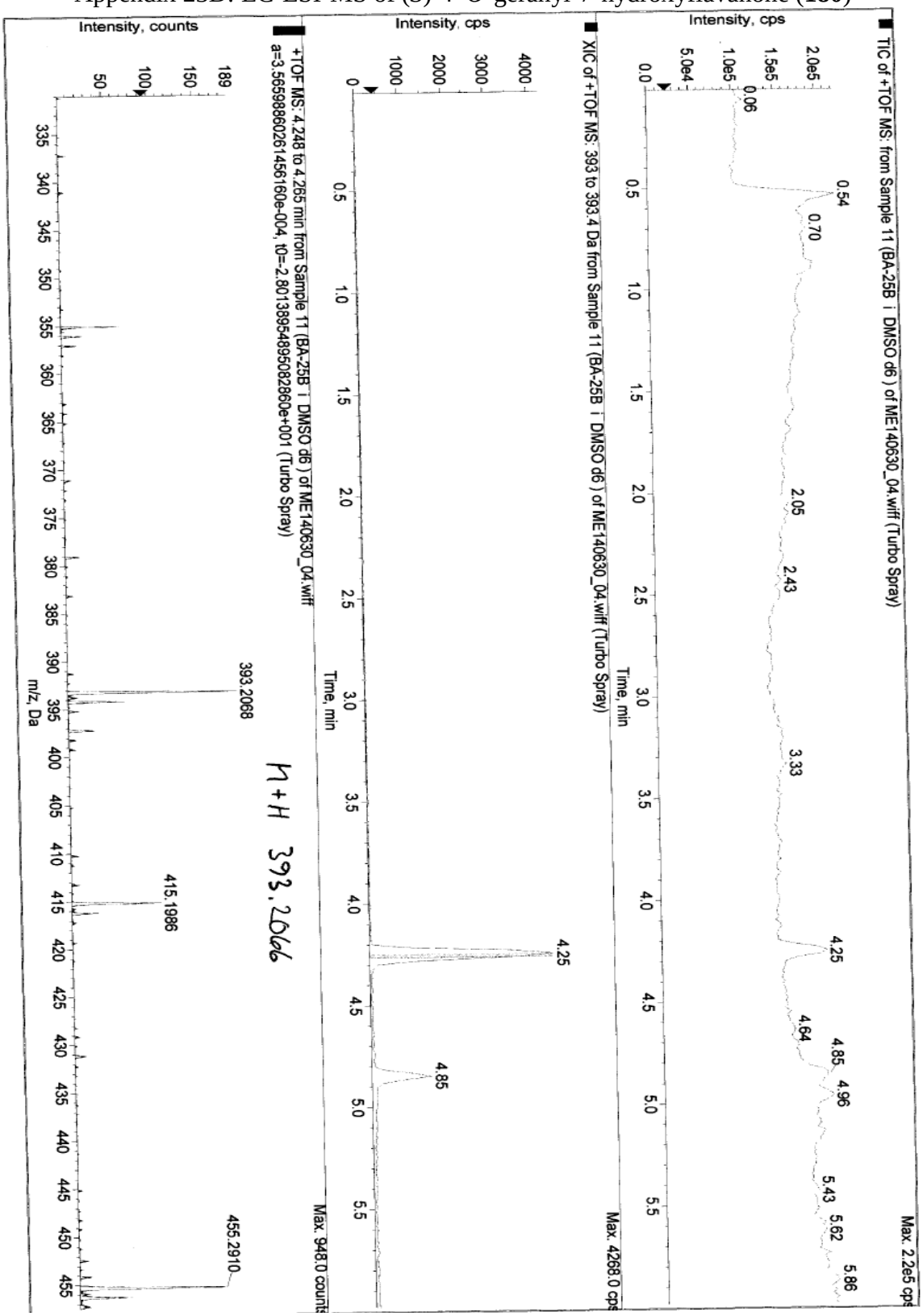
Appendix 23B: ^{13}C ($CDCl_3$, 50 MHz) NMR spectrum of (*S*)-4'-*O*-geranyl-7-hydroxyflavanone (**180**)



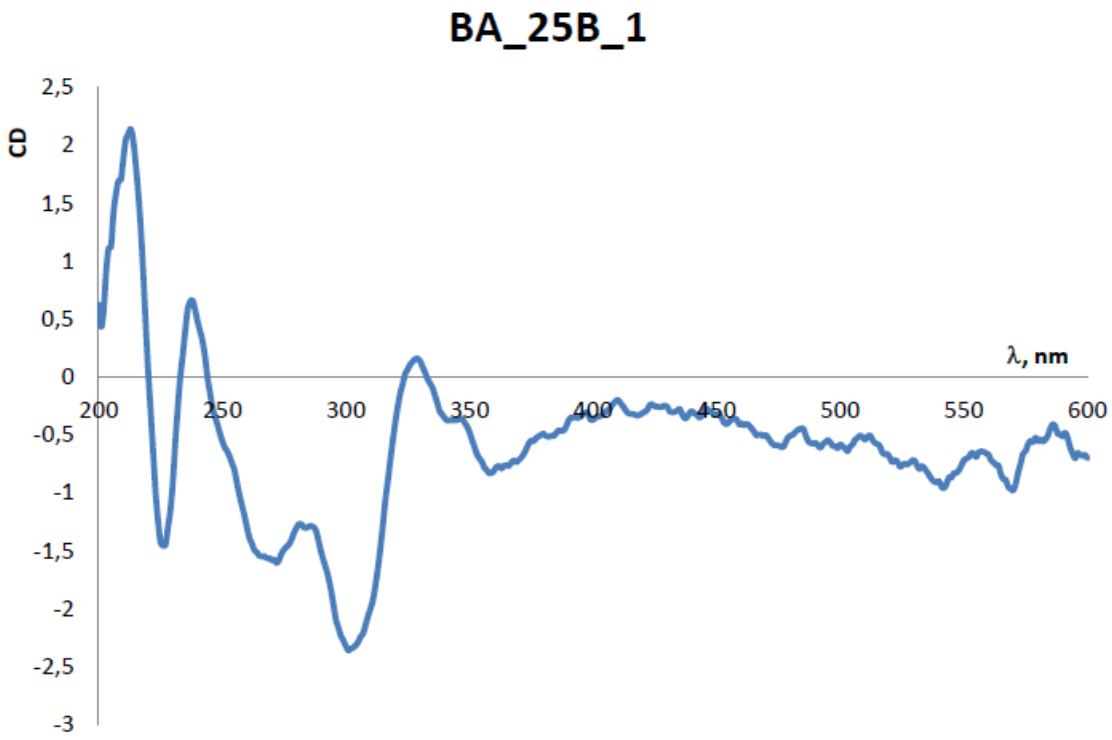
Appendix 23C: NOESY ($CDCl_3$, 200 MHz) spectrum of (*S*)-4'-*O*-geranyl-7-hydroxyflavanone (**180**)



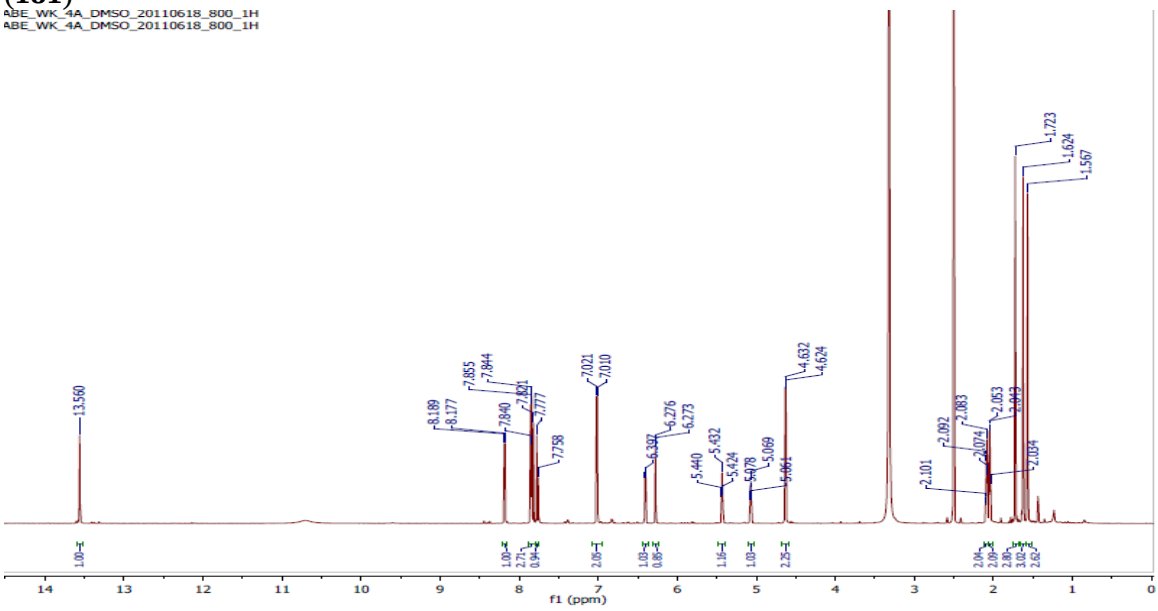
Appendix 23D: LC-ESI-MS of (*S*)-4'-*O*-geranyl-7-hydroxyflavanone (**180**)



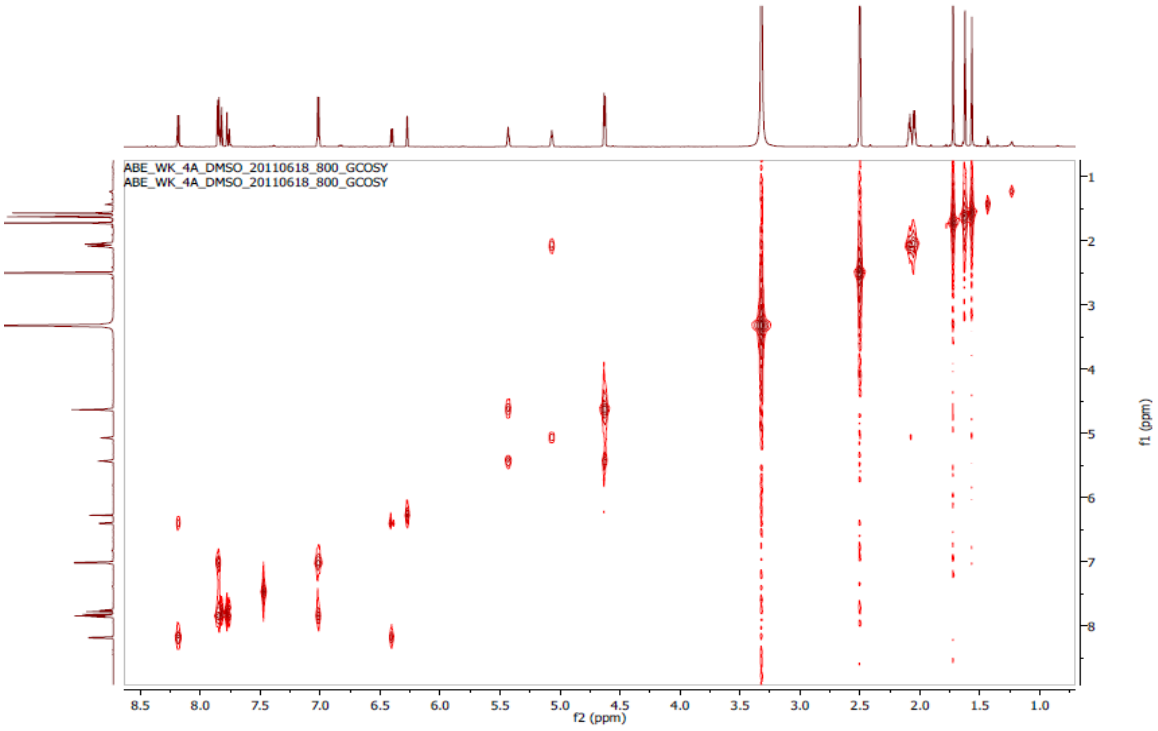
Appendix 23E: CD spectrum of (*S*)-4'-*O*-geranyl-7-hydroxyflavanone (**180**)



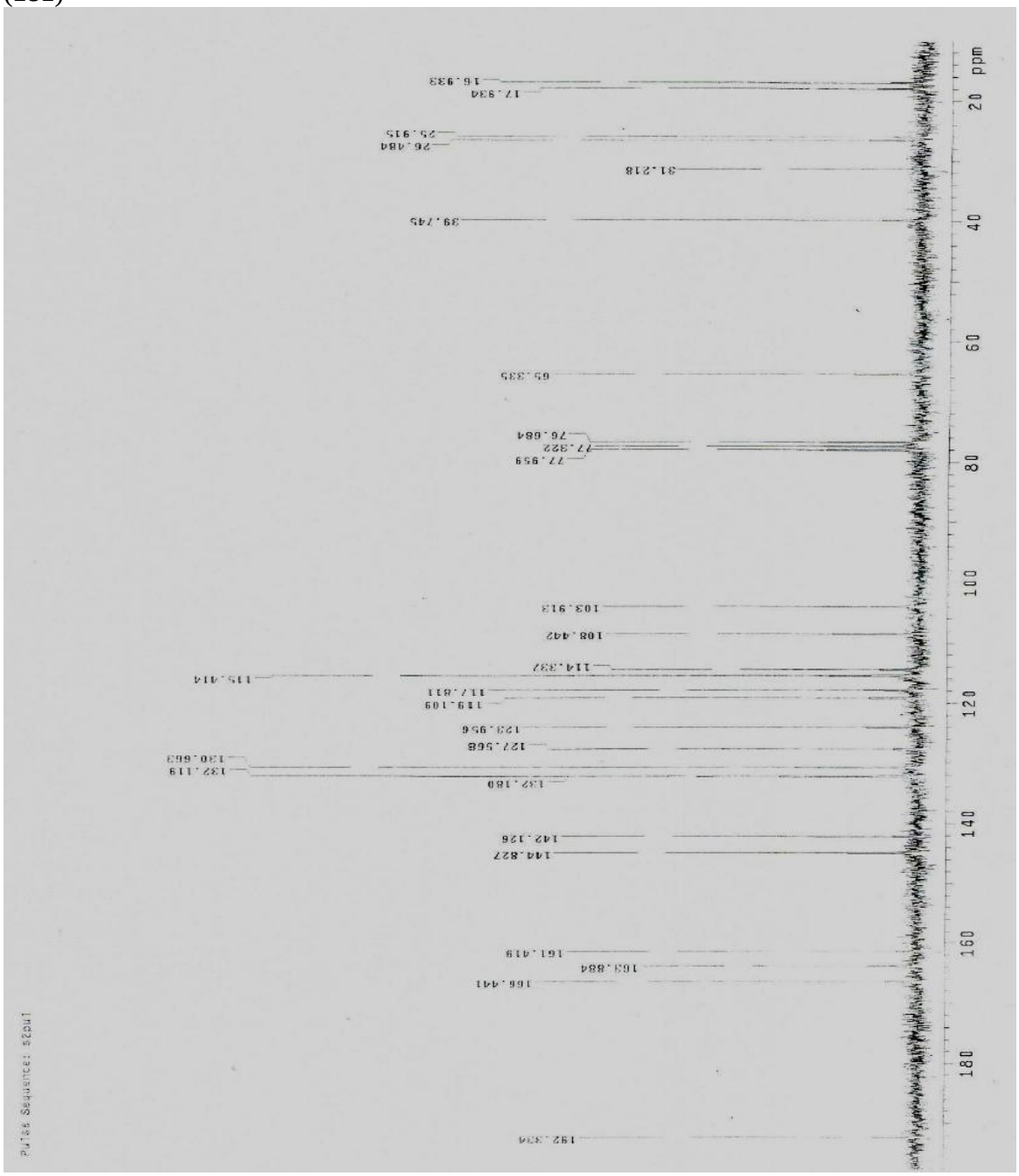
Appendix 24A: ^{1}H NMR ($(CD_3)_2SO$, 800 MHz) spectrum of 4-*O*-geranylisoliquiritigenin (**181**)



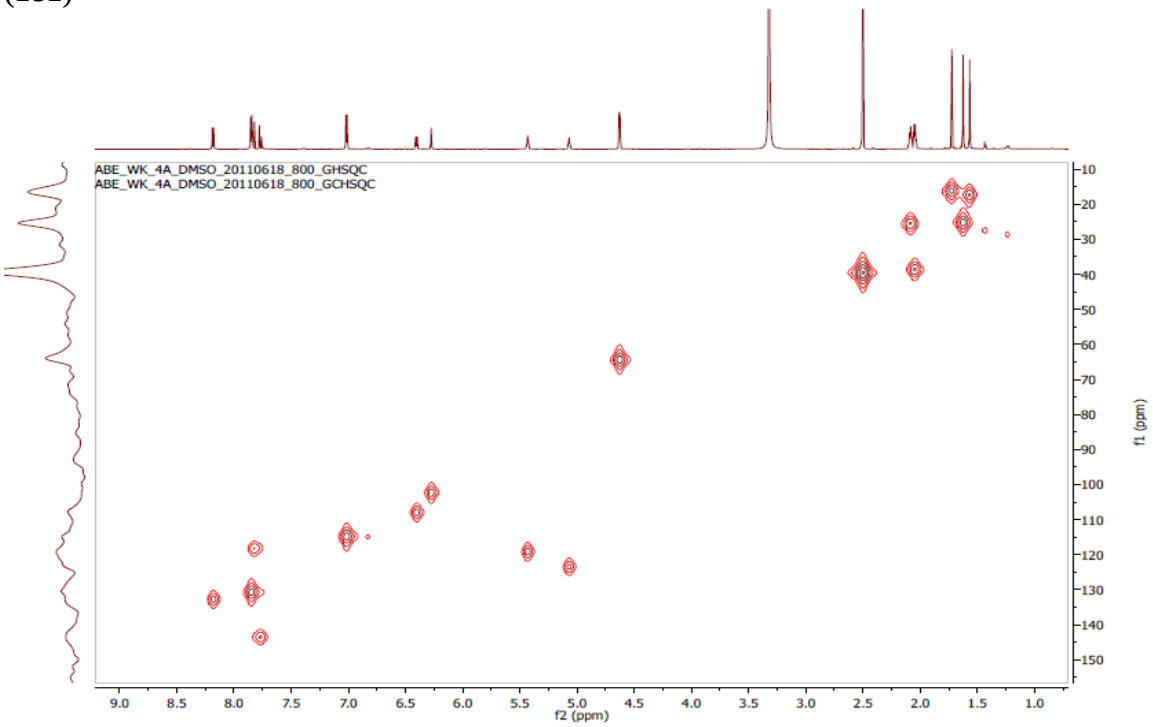
Appendix 24B: gCOSY ($(CD_3)_2SO$, 800 MHz) spectrum of 4-*O*-geranylisoliquiritigenin (**181**)



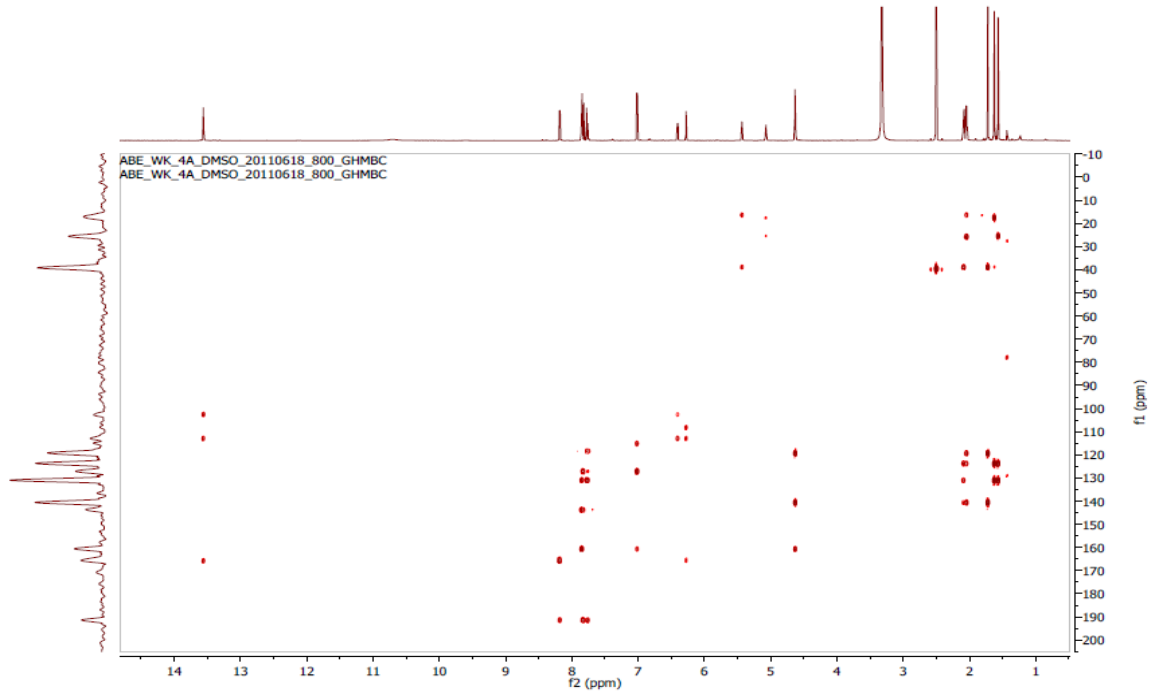
Appendix 24C: gCOSY ($(CD_3)_2SO$, 800 MHz) spectrum of 4-*O*-geranylisoliquiritigenin (**181**)



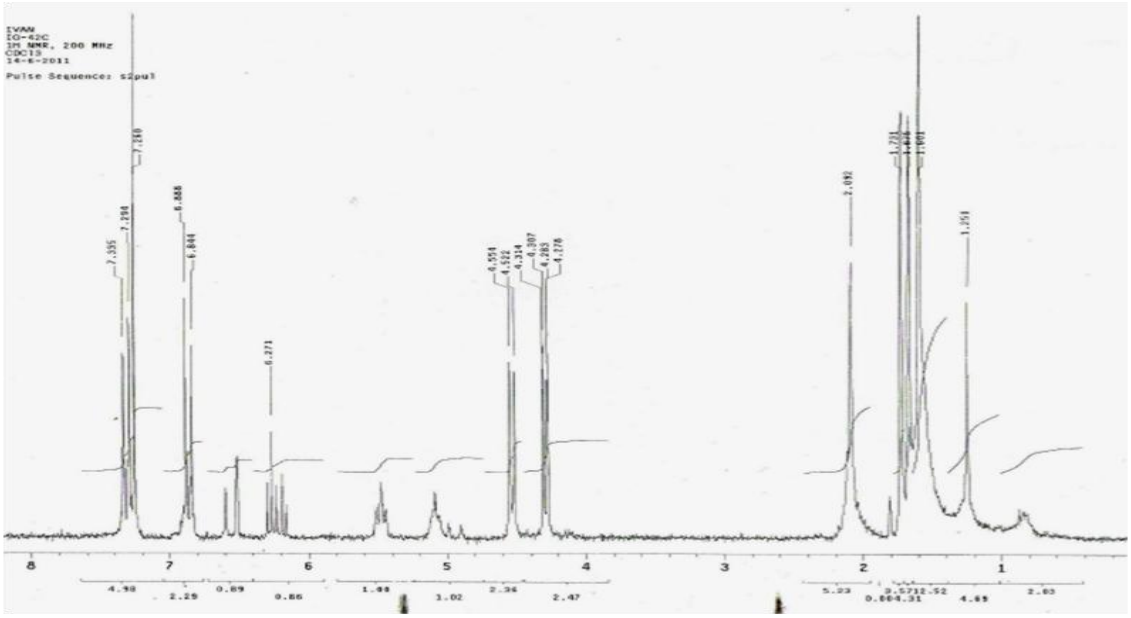
Appendix 24D: gCHSQC ($(CD_3)_2SO$, 800 MHz) spectrum of 4-*O*-geranylisoliquiritigenin (**181**)



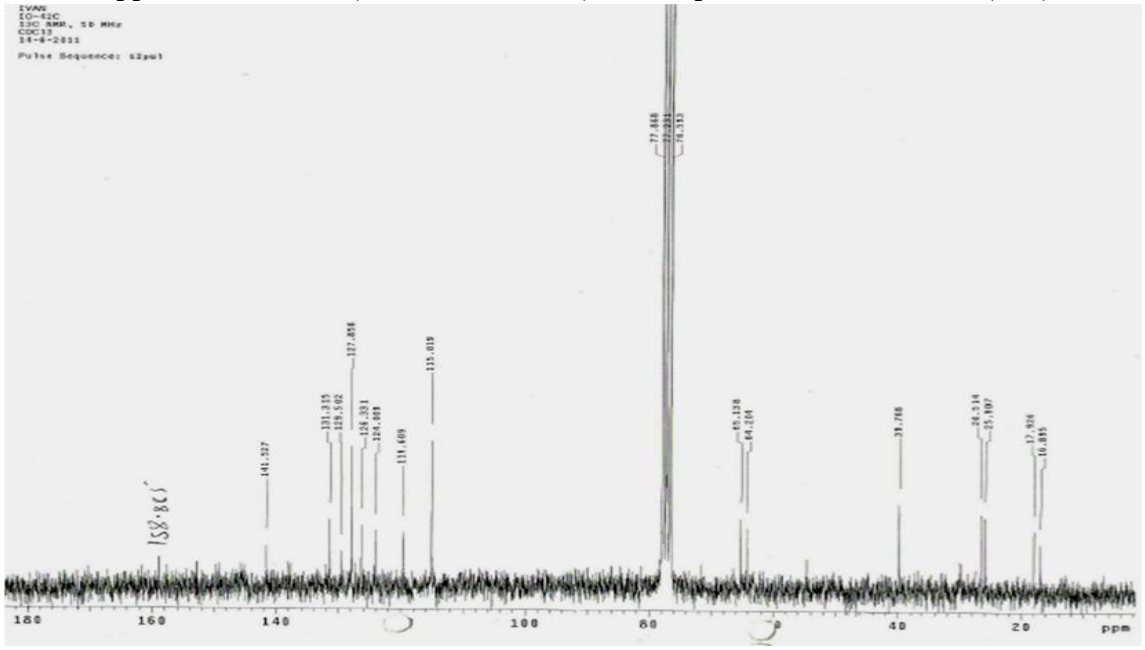
Appendix 24E: gHMBC ($(CD_3)_2SO$, 800 MHz) spectrum of 4-*O*-geranylisoliquiritigenin (**181**)



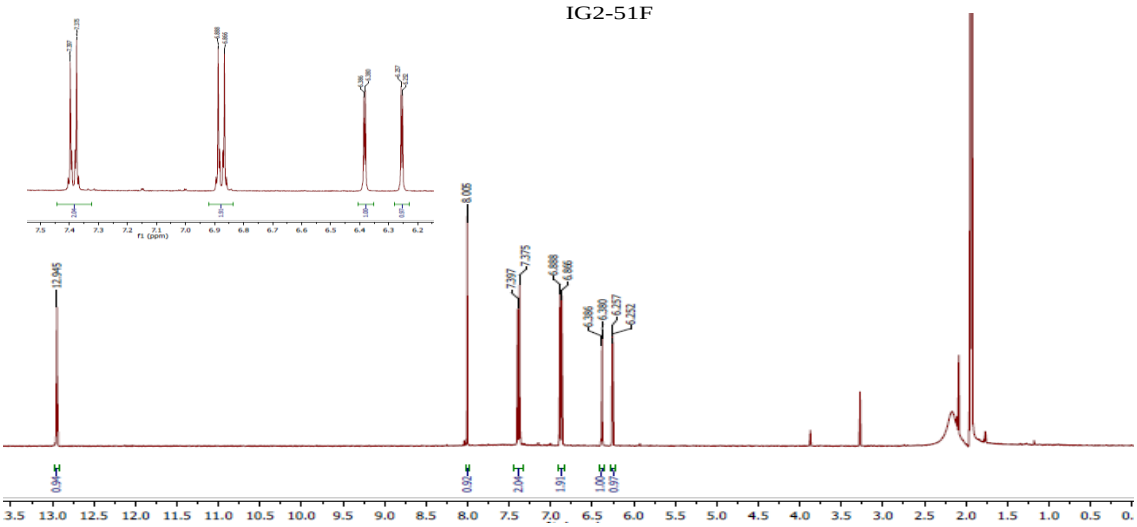
Appendix 25A: ^{1}H ($CDCl_3$, 200 MHz) NMR spectrum of colenemol (**182**)



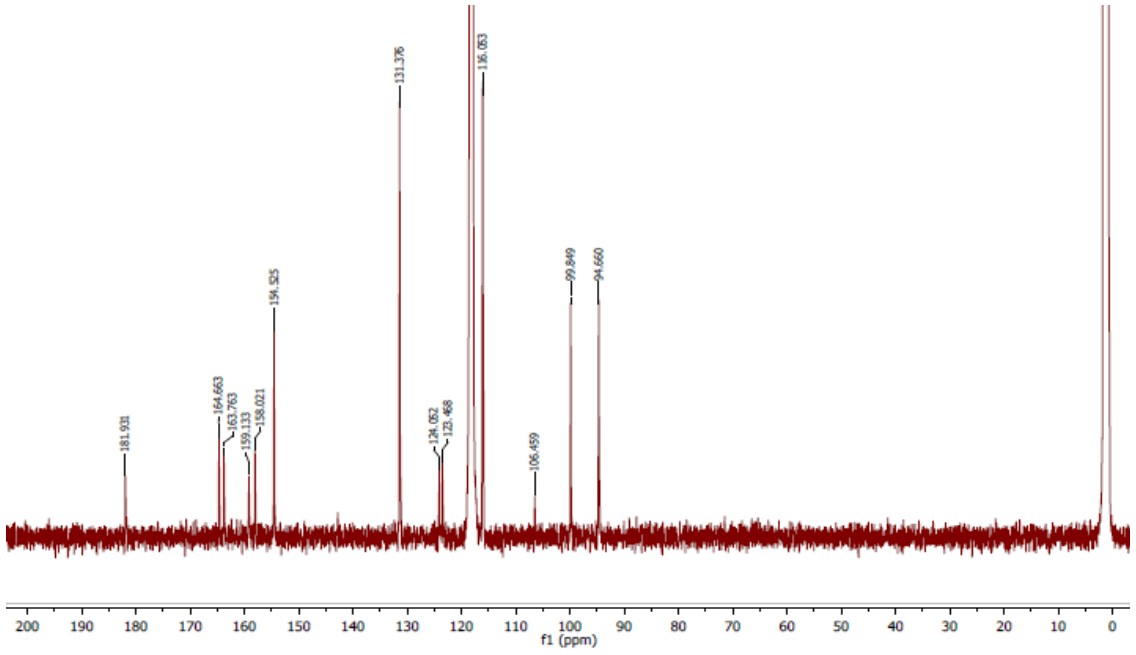
Appendix 25B: ^{13}C ($CDCl_3$, 200 MHz) NMR spectrum of colenemol (**182**)



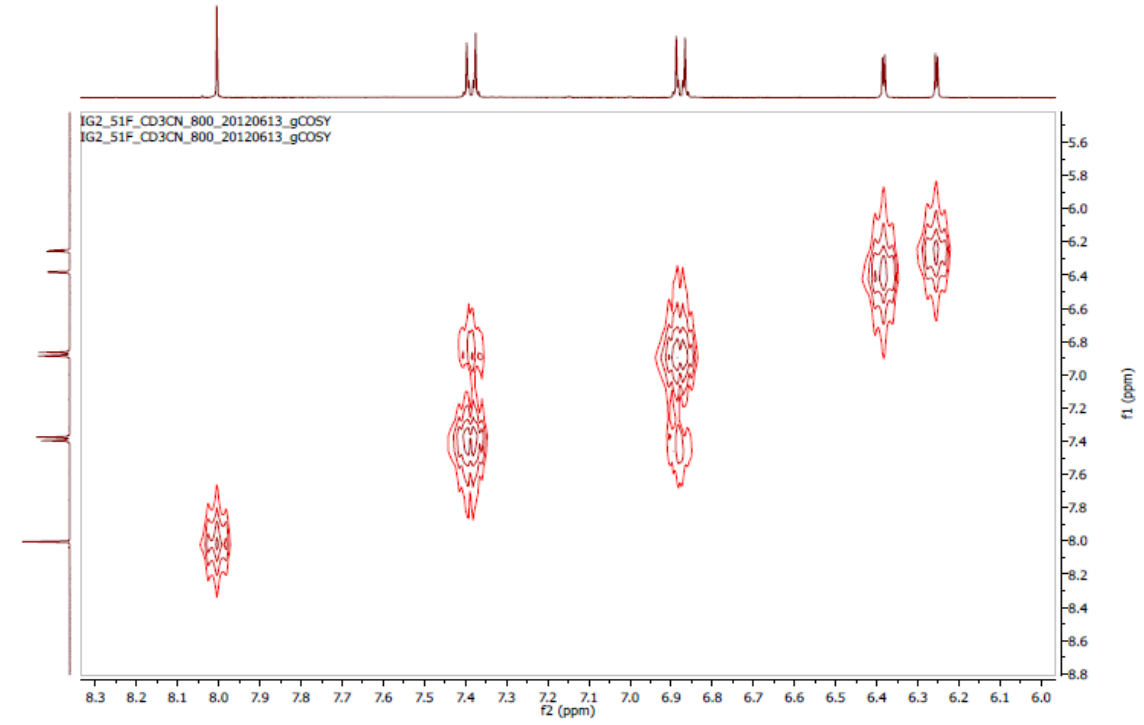
Appendix 26A: ^{1}H (CD_3CN, 400 MHz) NMR spectrum of genistein (**11**)



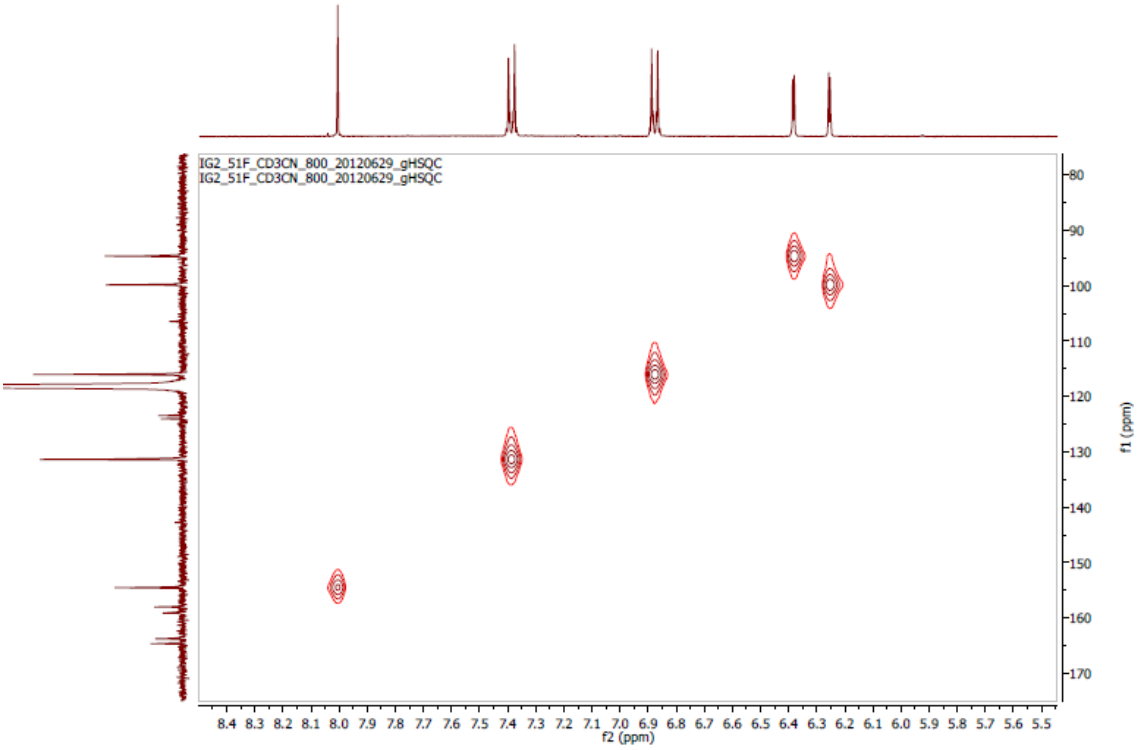
Appendix 26B: ^{13}C (CD_3CN, 150 MHz) NMR spectrum of genistein (**11**)



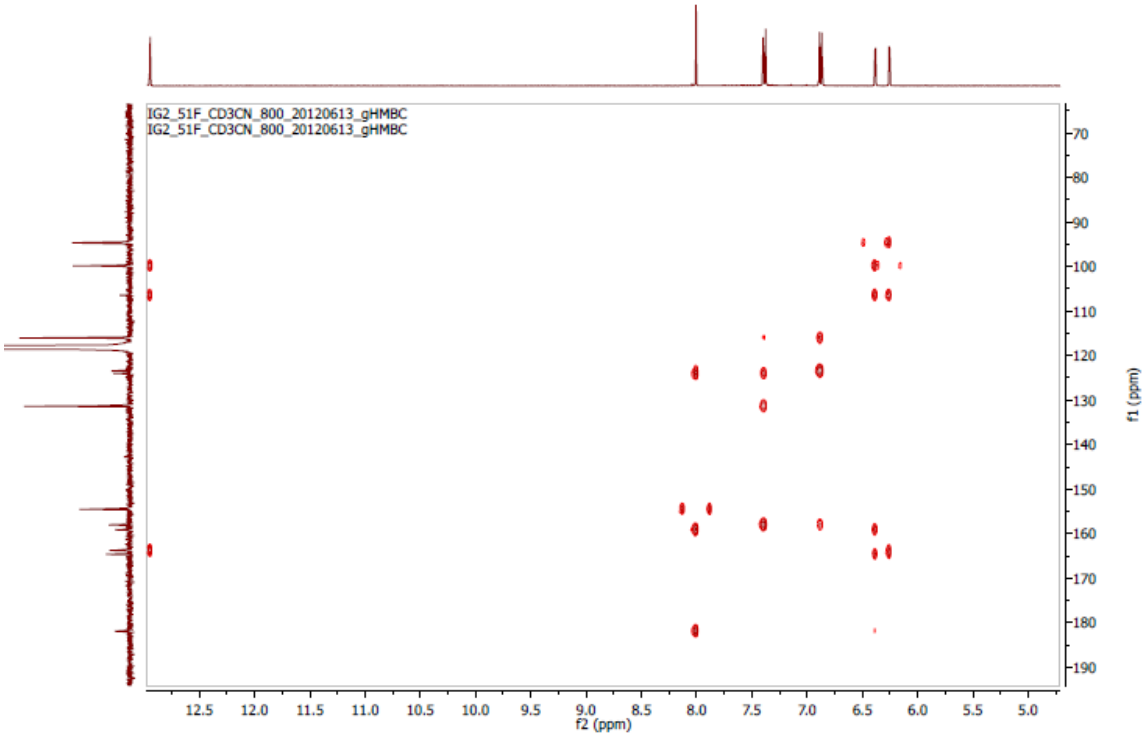
Appendix 26C: ^{1}H-^{1}H gCOSY (CD_3CN, 800 MHz) spectrum of genistein (**11**)



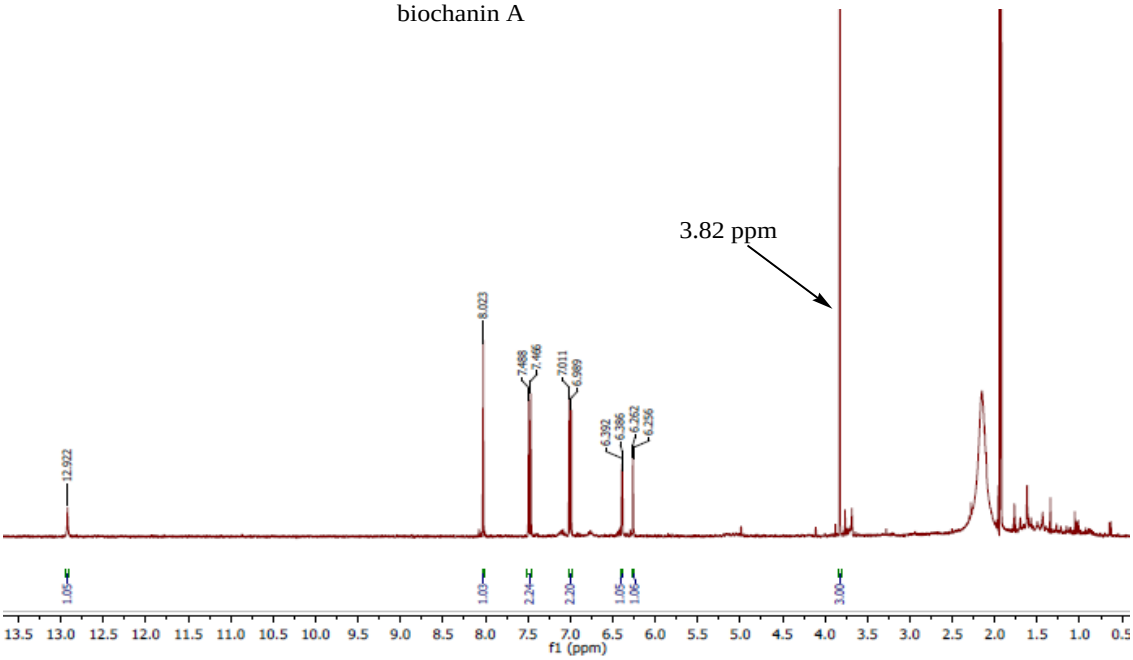
Appendix 26D: gCHSQC (CD_3CN, 800 MHz) spectrum of genistein (**11**)



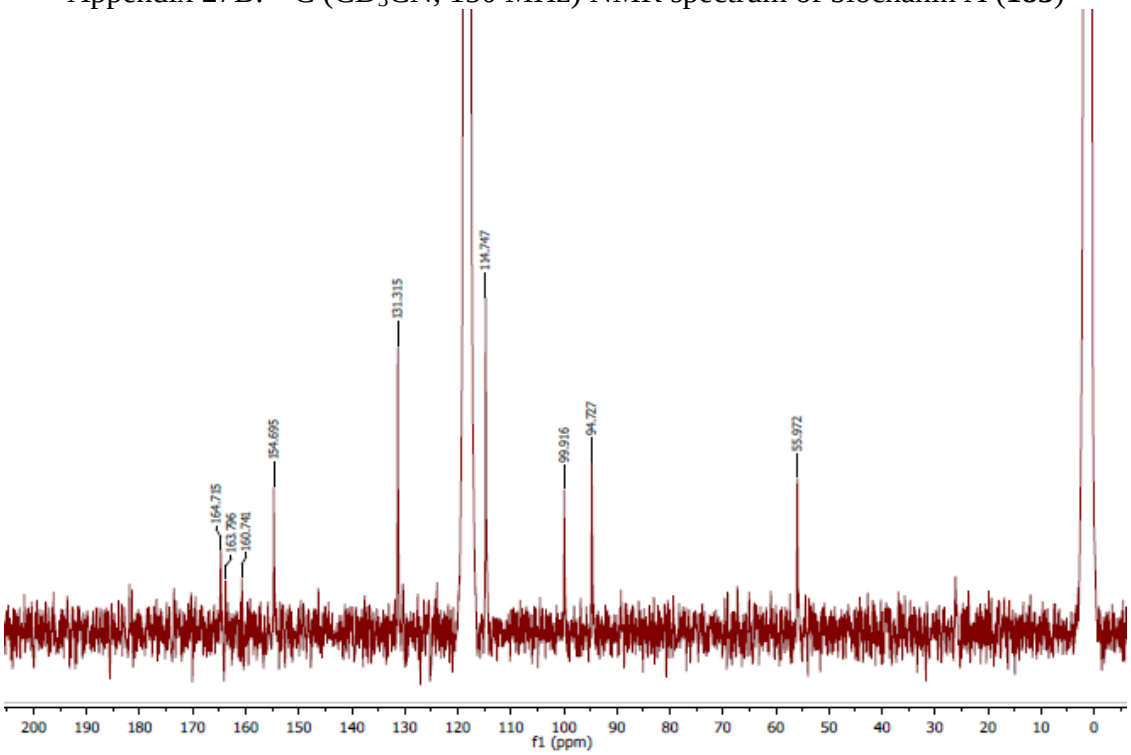
Appendix 26E: gHMBC (CD_3CN, 800 MHz) spectrum of genistein (**11**)



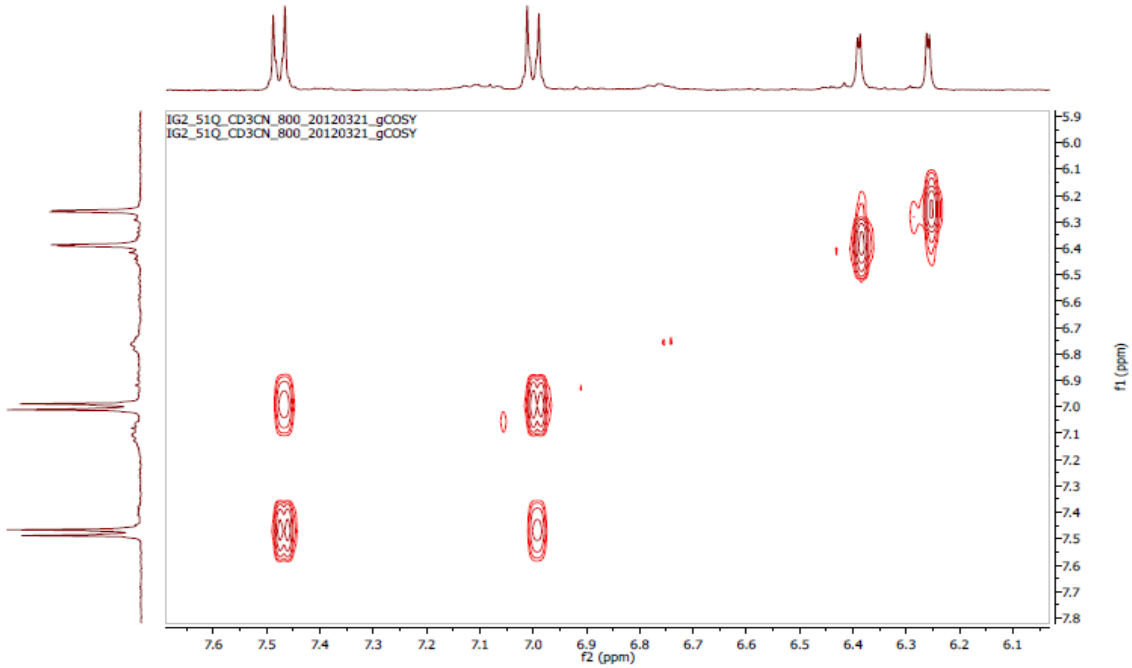
Appendix 27A: ^1H (CD_3CN, 400 MHz) NMR spectrum of biochanin A (**183**)



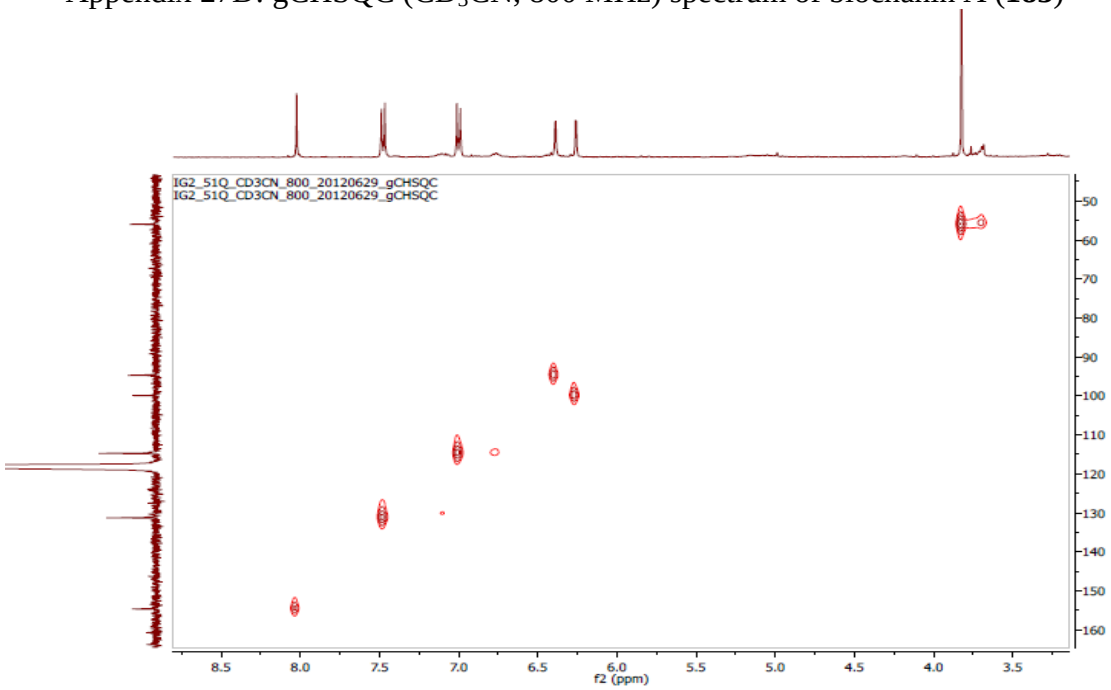
Appendix 27B: ^{13}C (CD_3CN, 150 MHz) NMR spectrum of biochanin A (**183**)



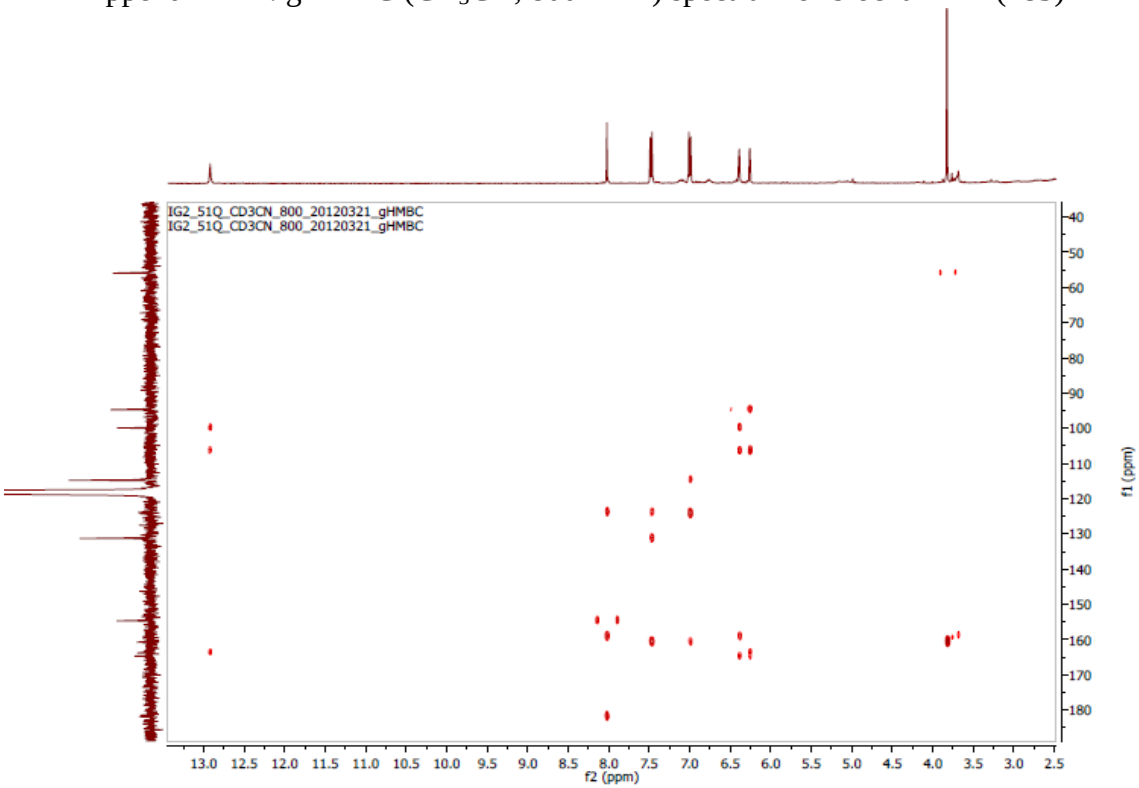
Appendix 27C: ^{1}H-^{1}H gCOSY (CD_3CN, 800 MHz)spectrum of biochanin A (**183**)



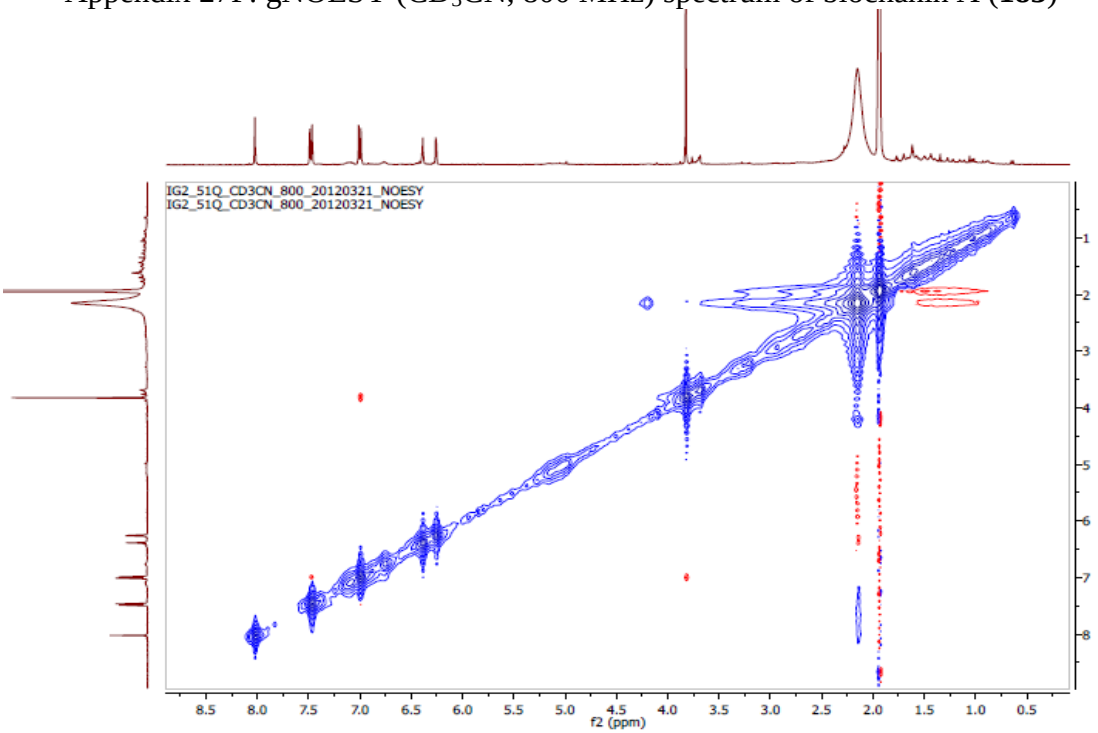
Appendix 27D: gCHSQC (CD_3CN, 800 MHz) spectrum of biochanin A (**183**)



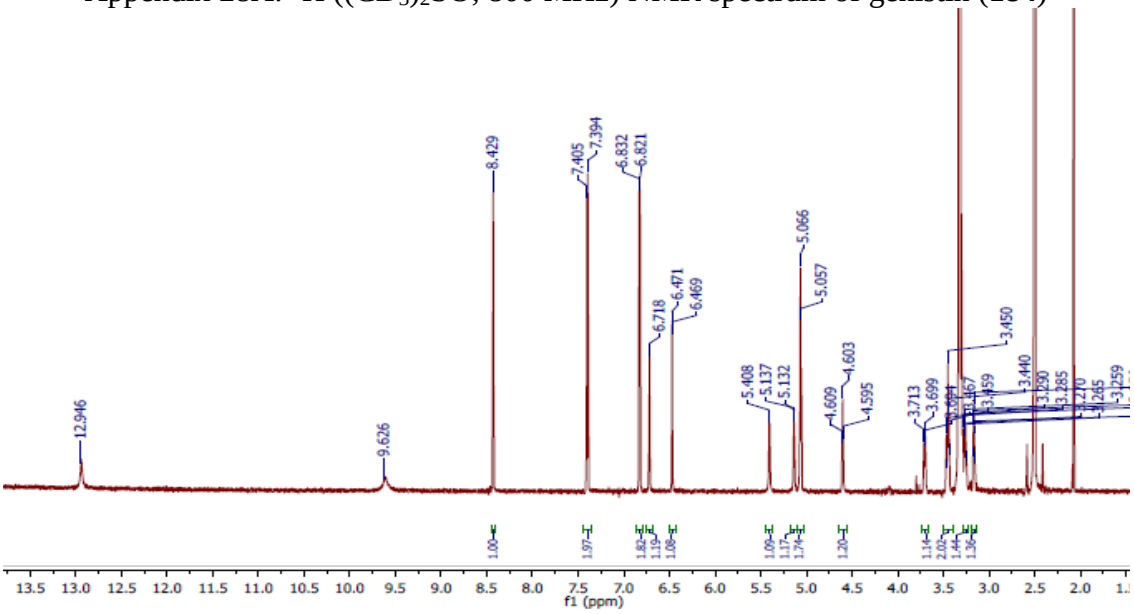
Appendix 27E: gHMBC (CD_3CN, 800 MHz) spectrum of biochanin A (**183**)



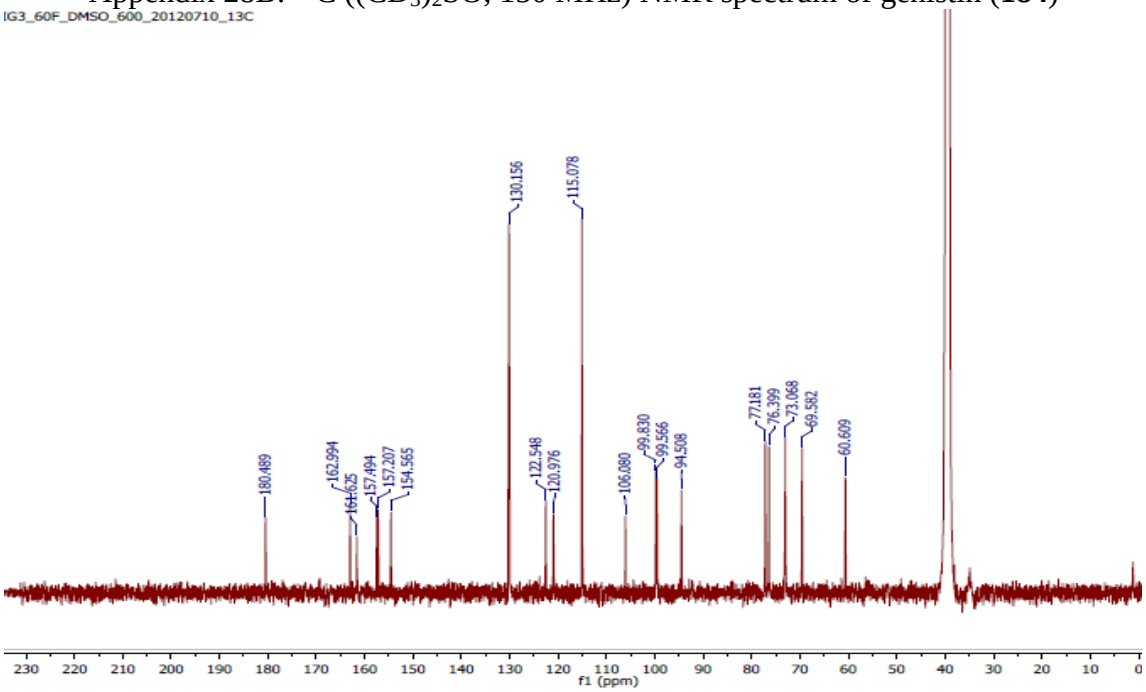
Appendix 27F: gNOESY (CD_3CN, 800 MHz) spectrum of biochanin A (**183**)



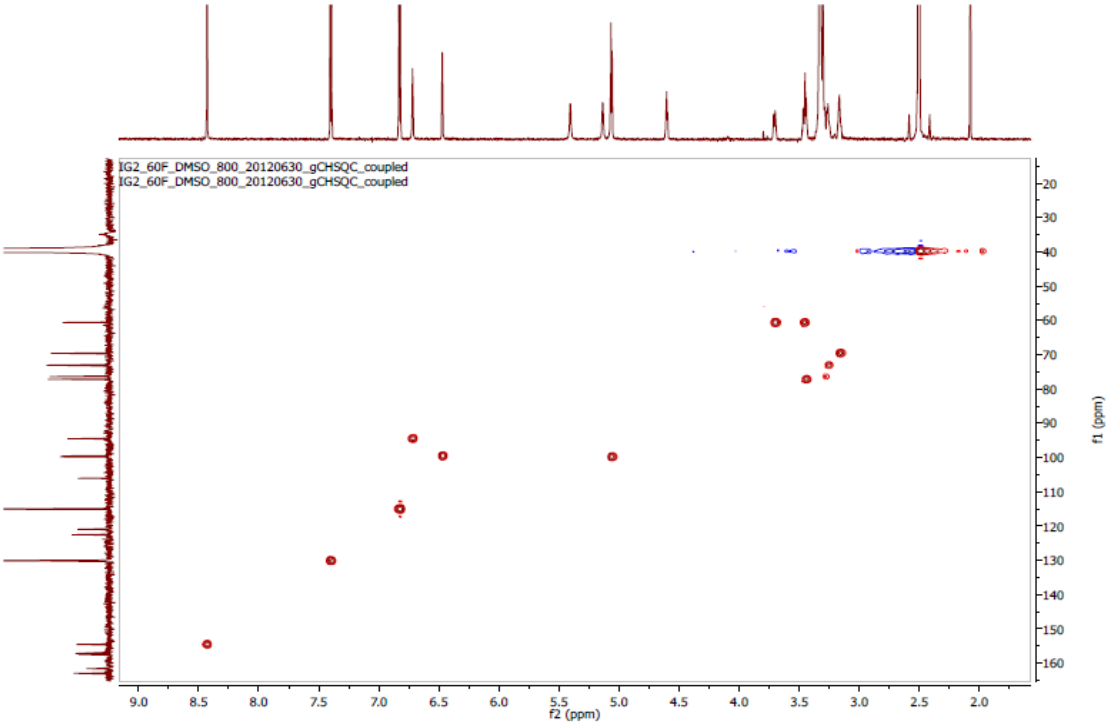
Appendix 28A: ^1H ($(CD_3)_2SO$, 800 MHz) NMR spectrum of genistin (**184**)



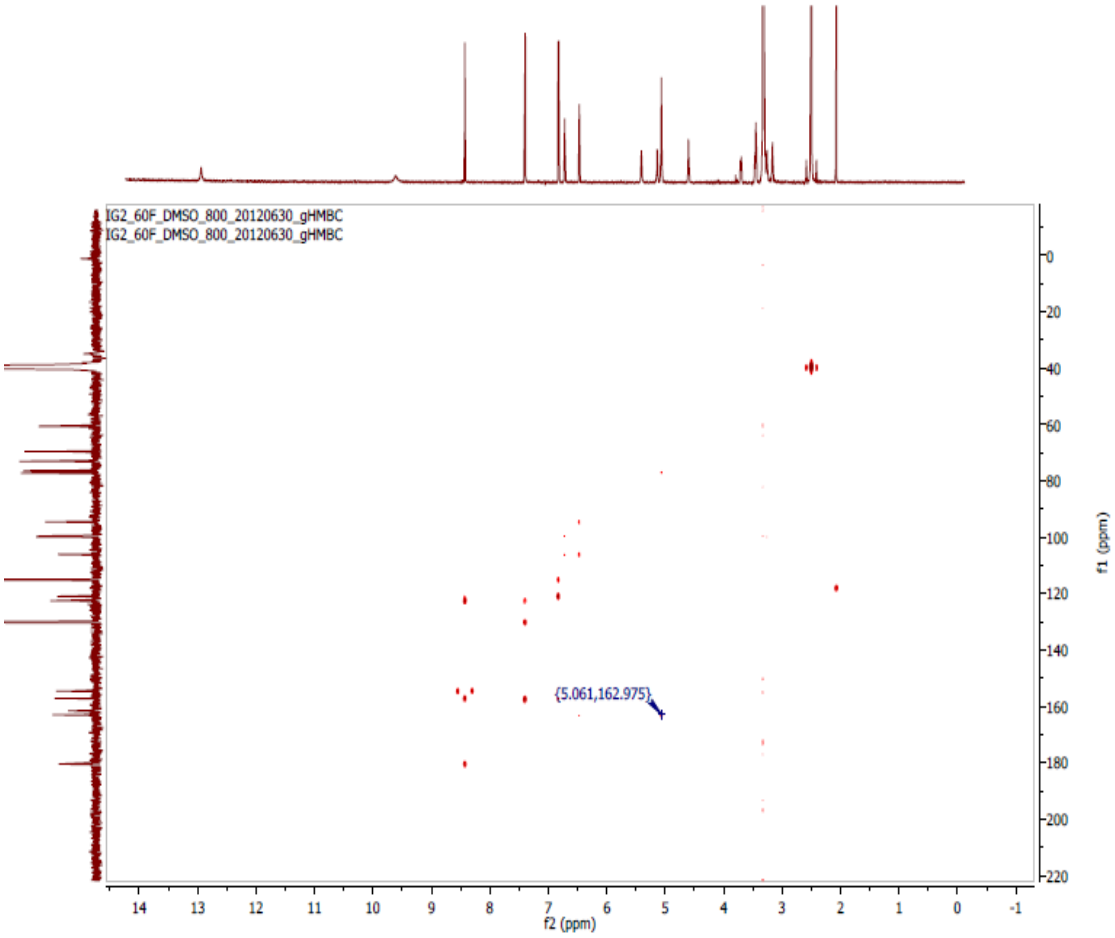
Appendix 28B: ^{13}C ($(CD_3)_2SO$, 150 MHz) NMR spectrum of genistin (**184**)



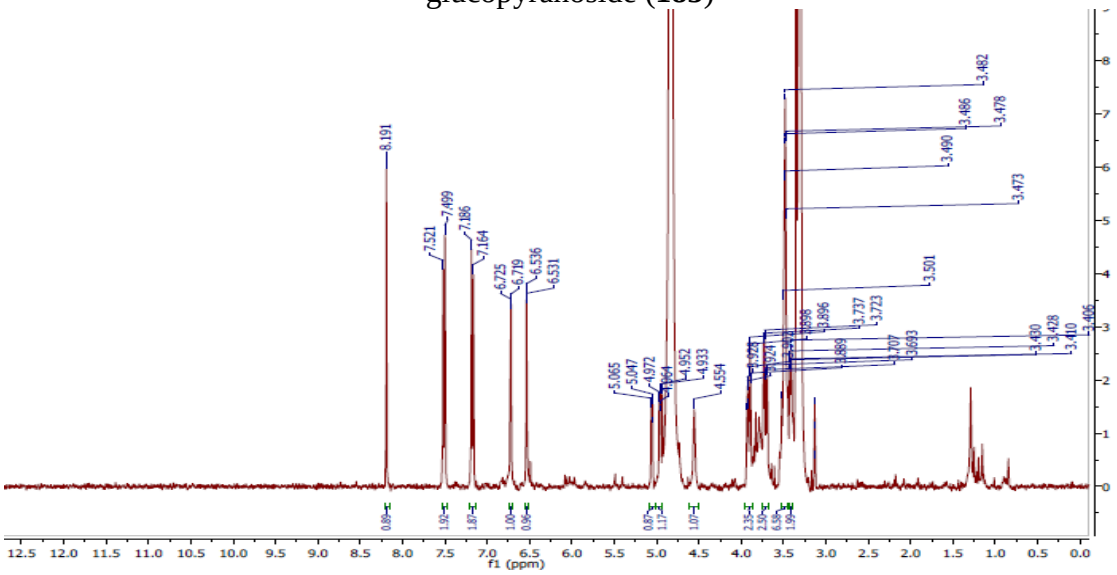
Appendix 28C: gCHSQC ($(CD_3)_2SO$, 800 MHz) spectrum of genistin (**184**)



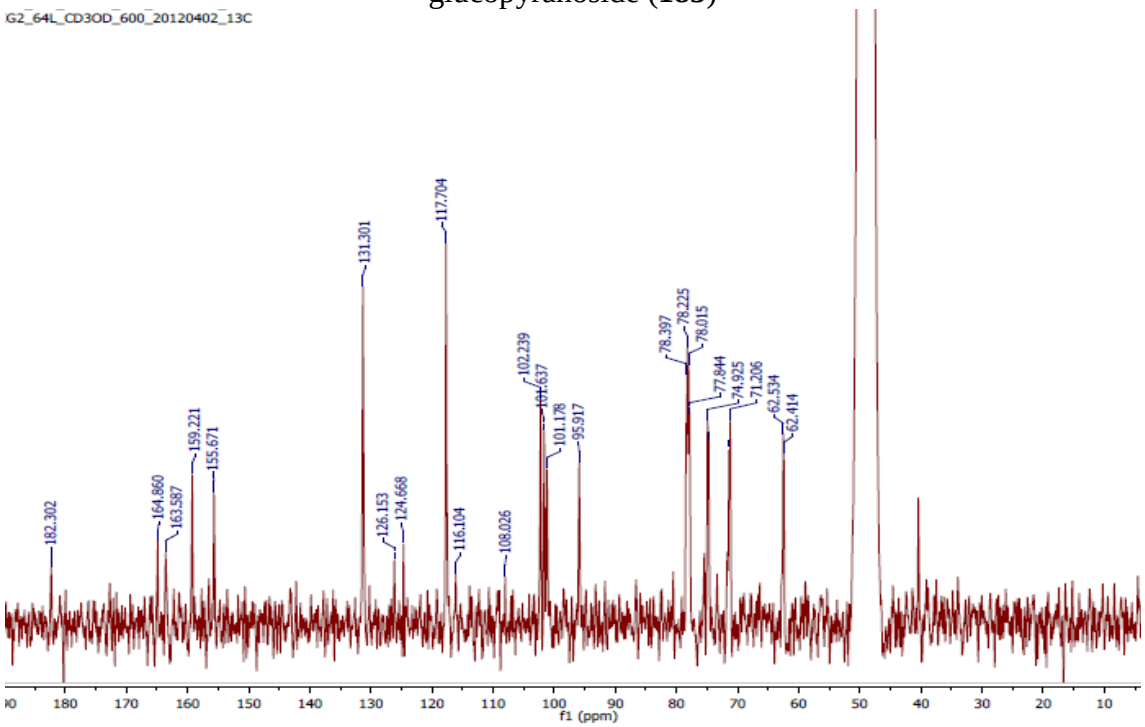
Appendix 28D: gHMBC ($(CD_3)_2SO$, 800 MHz) spectrum of genistin (**184**)



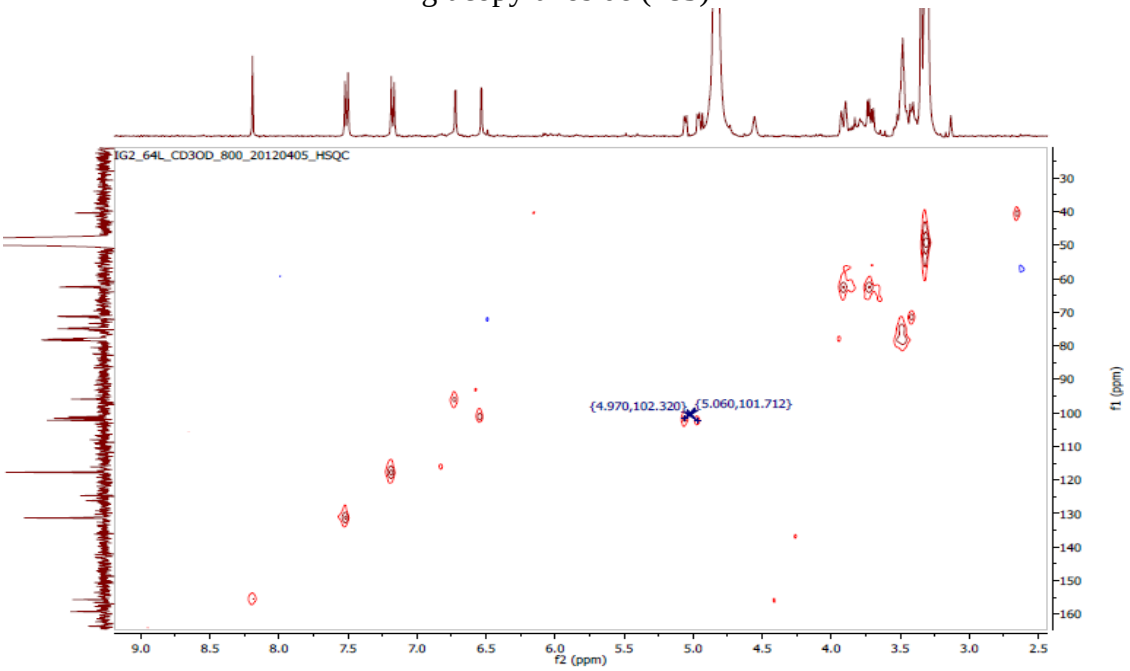
Appendix 29A: ^{1}H (CD_3OD, 800 MHz) NMR spectrum of genistein 7,4-di-*O*-β-glucopyranoside (**185**)



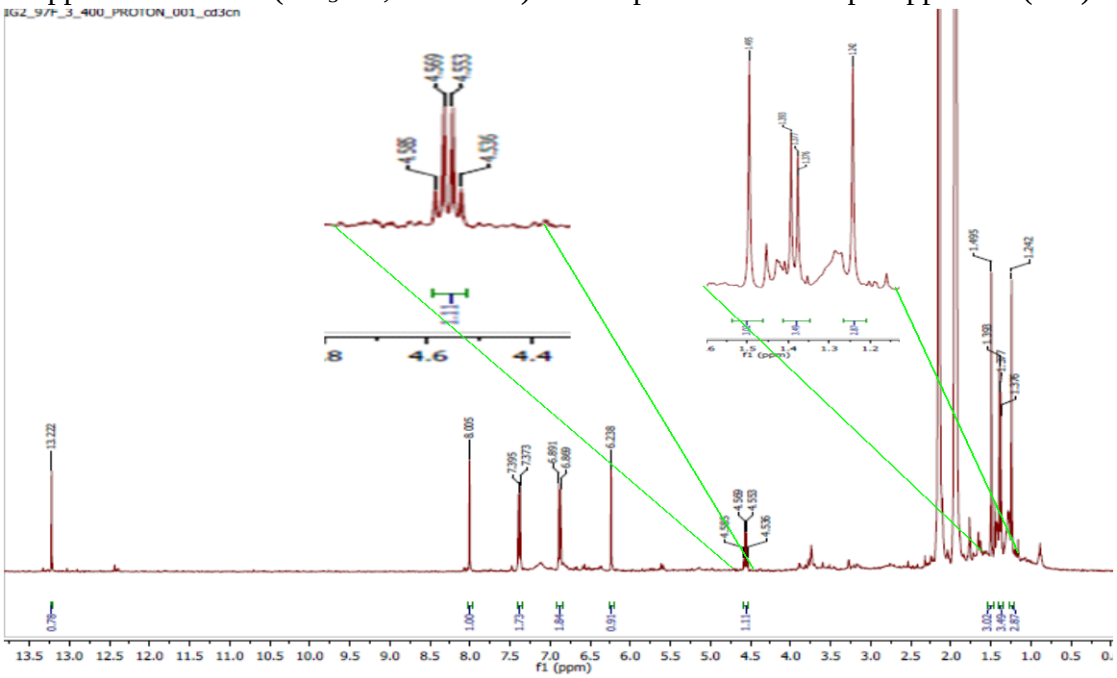
Appendix 29B: ^{13}C (CD_3OD, 150 MHz) NMR spectrum of genistein 7,4-di-*O*-β-glucopyranoside (**185**)



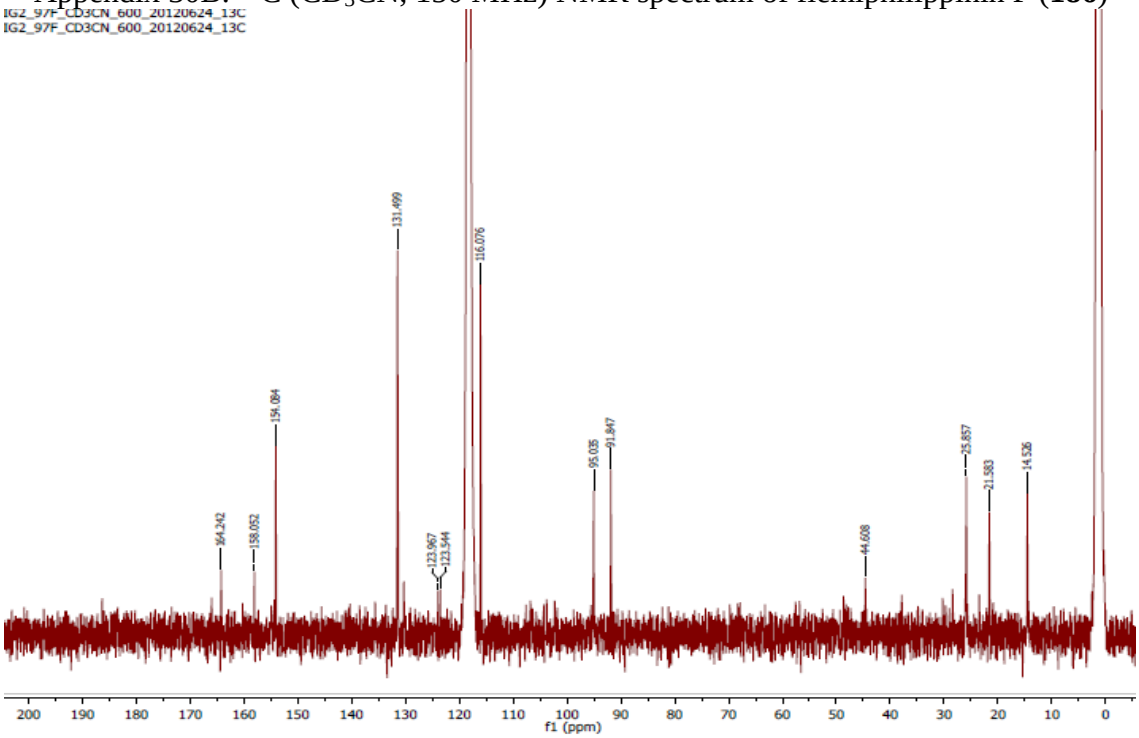
Appendix 29C: gCHSQC (CD_3OD, 800 MHz) spectrum of genistein 7,4-di-*O*-β-glucopyranoside (**185**)



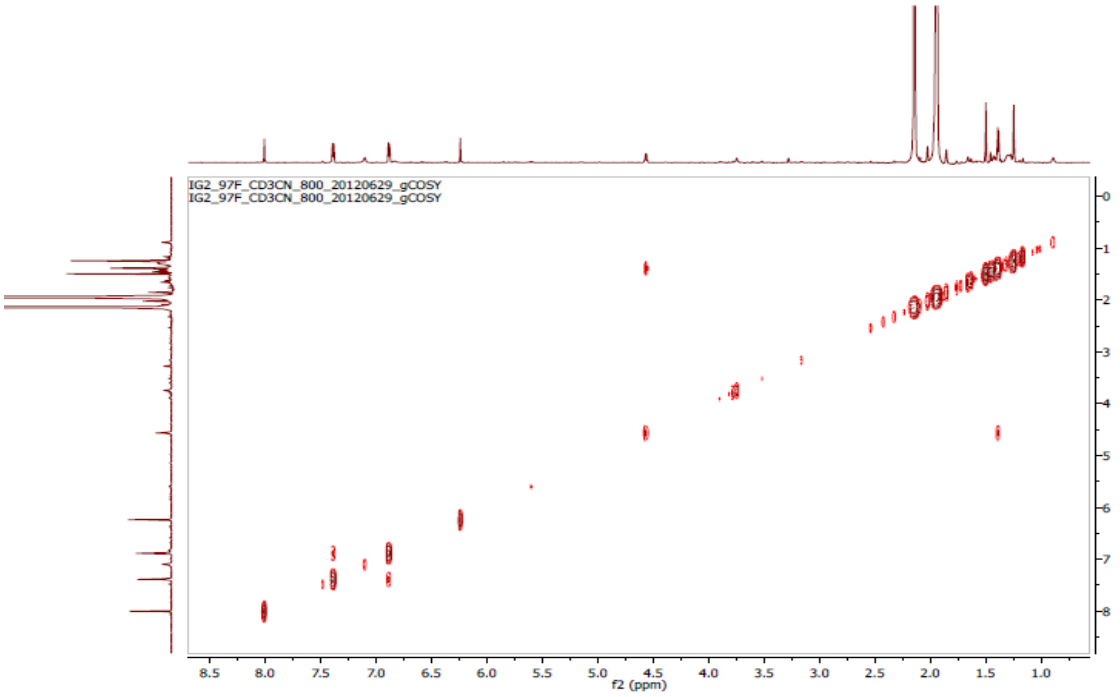
Appendix 30A: ^{1}H (CD_3CN, 400 MHz) NMR spectrum of flemiphilippinin F (**186**)



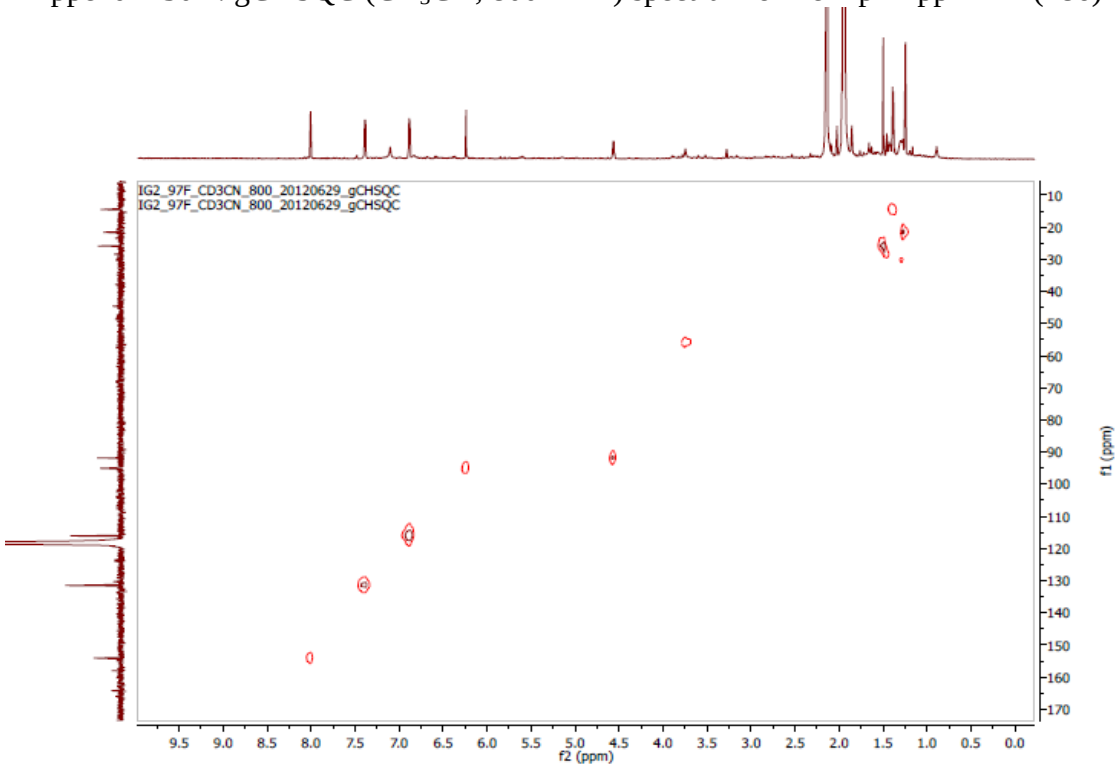
Appendix 30B: ^{13}C (CD_3CN, 150 MHz) NMR spectrum of flemiphilippinin F (**186**)



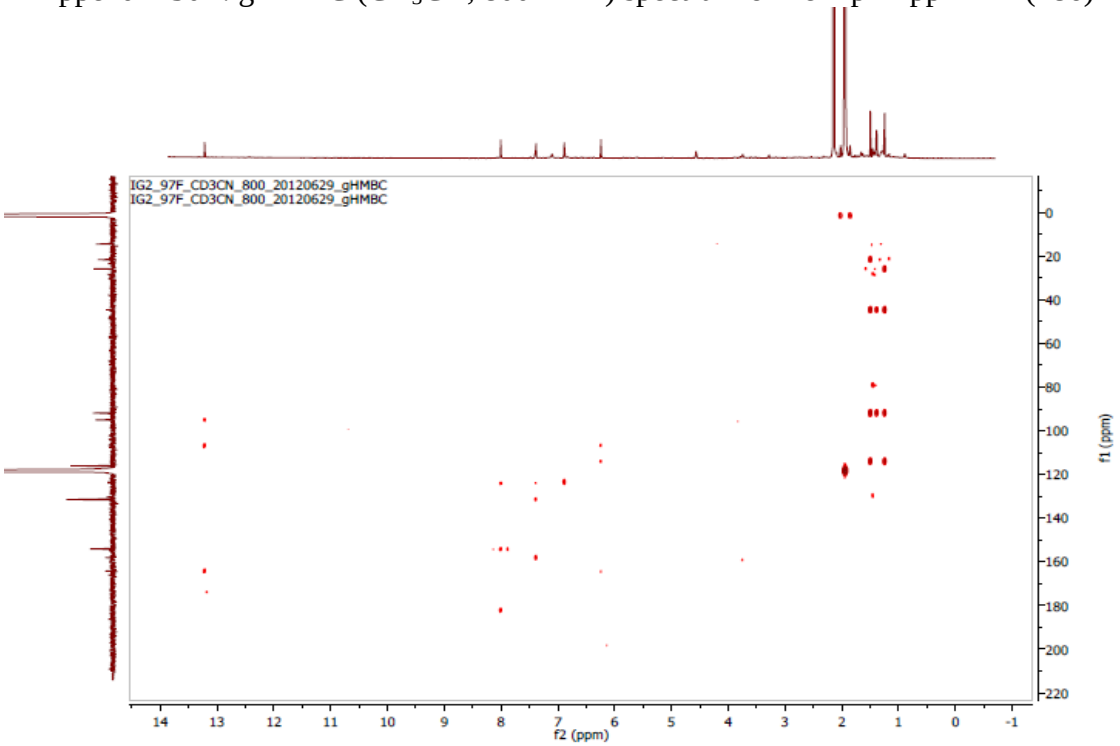
Appendix 30C: ^{1}H-^{1}H gCOSY (CD_3CN, 800 MHz) spectrum of flemiphilippinin F (**186**)



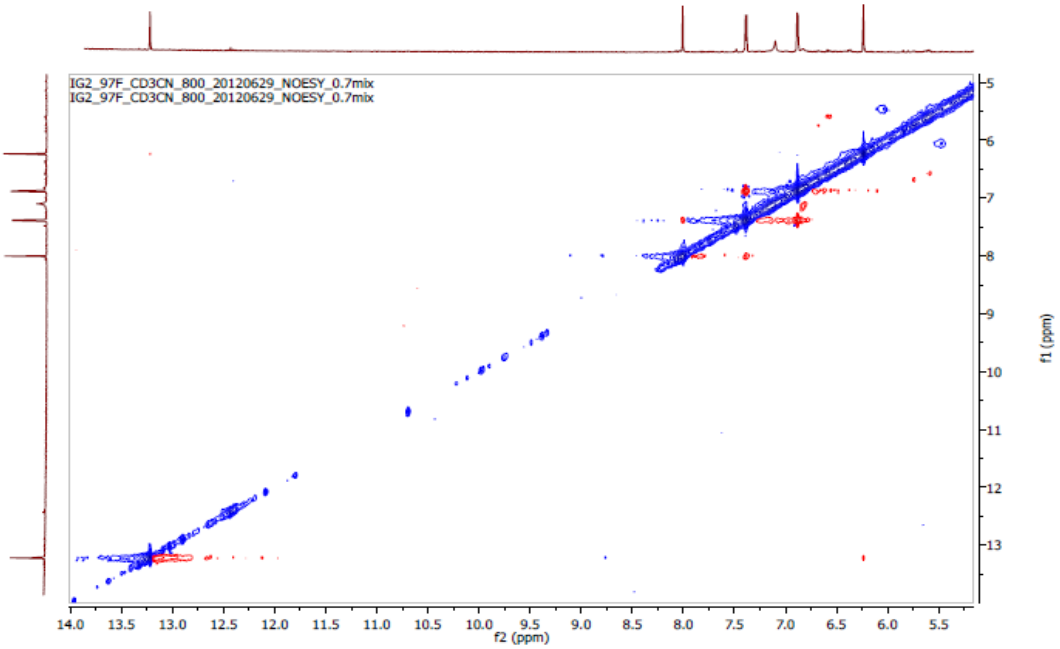
Appendix 30D: gCHSQC (CD_3CN, 800 MHz) spectrum of flemiphilippinin F (**186**)



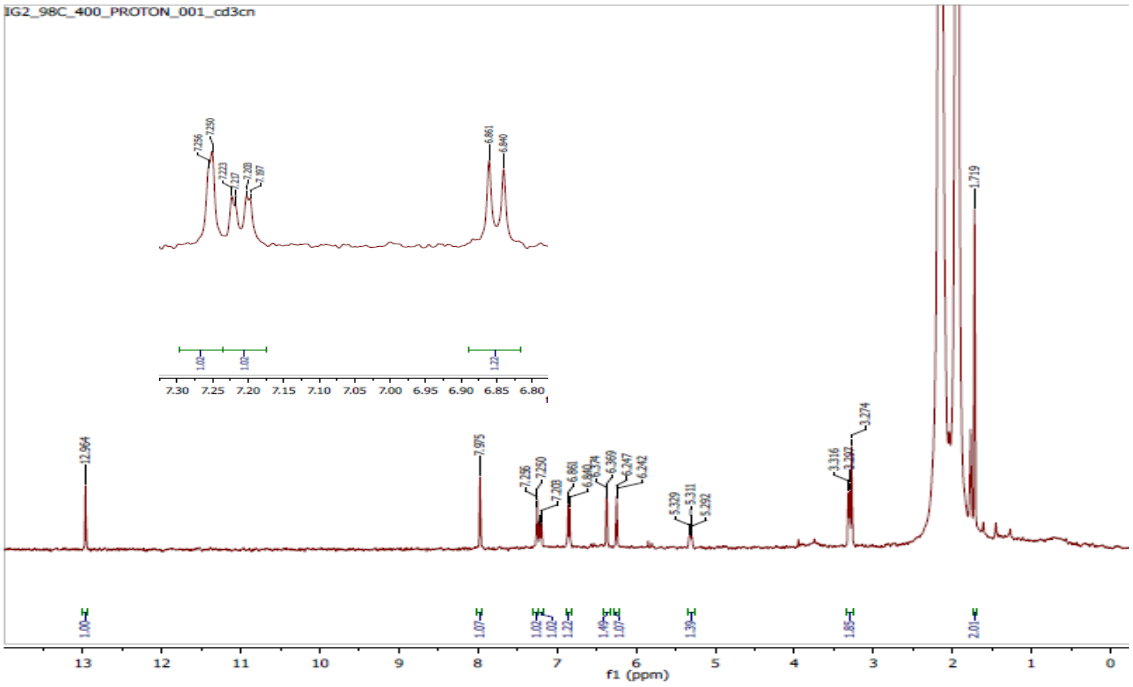
Appendix 30E: gHMBC (CD_3CN, 800 MHz) spectrum of flemiphilippinin F (**186**)



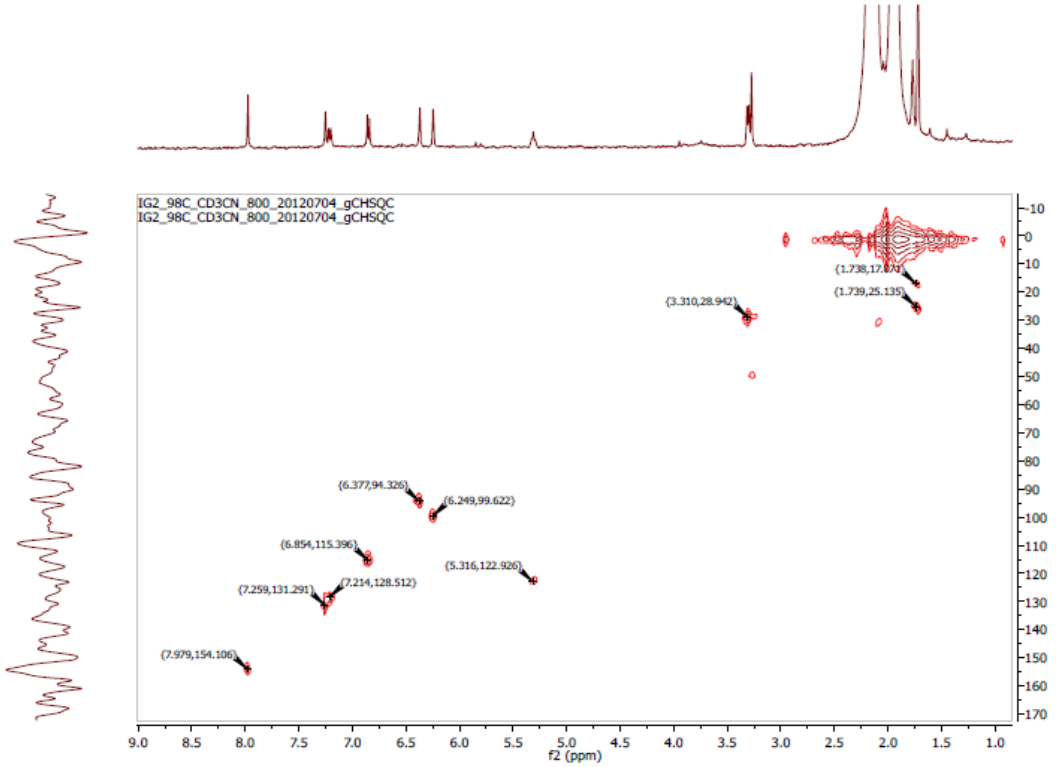
Appendix 30F: gNOESY (CD_3CN, 800 MHz) spectrum of flemiphilippinin F (**186**)



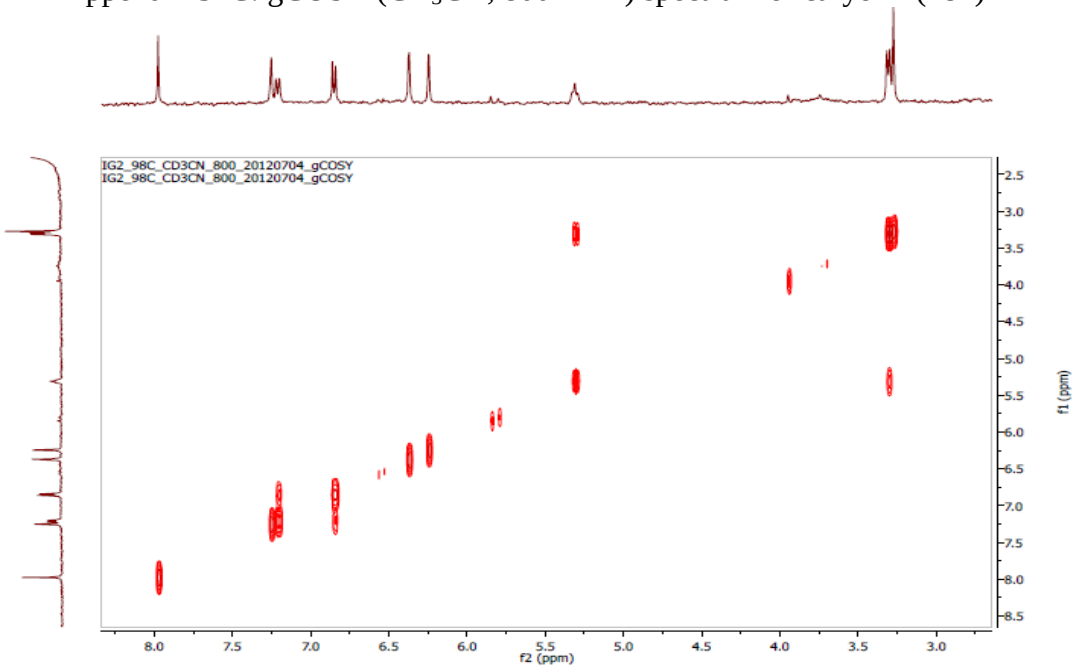
Appendix 31A: ^{1}H (CD_3CN, 400 MHz) NMR spectrum of caryolin (**187**)



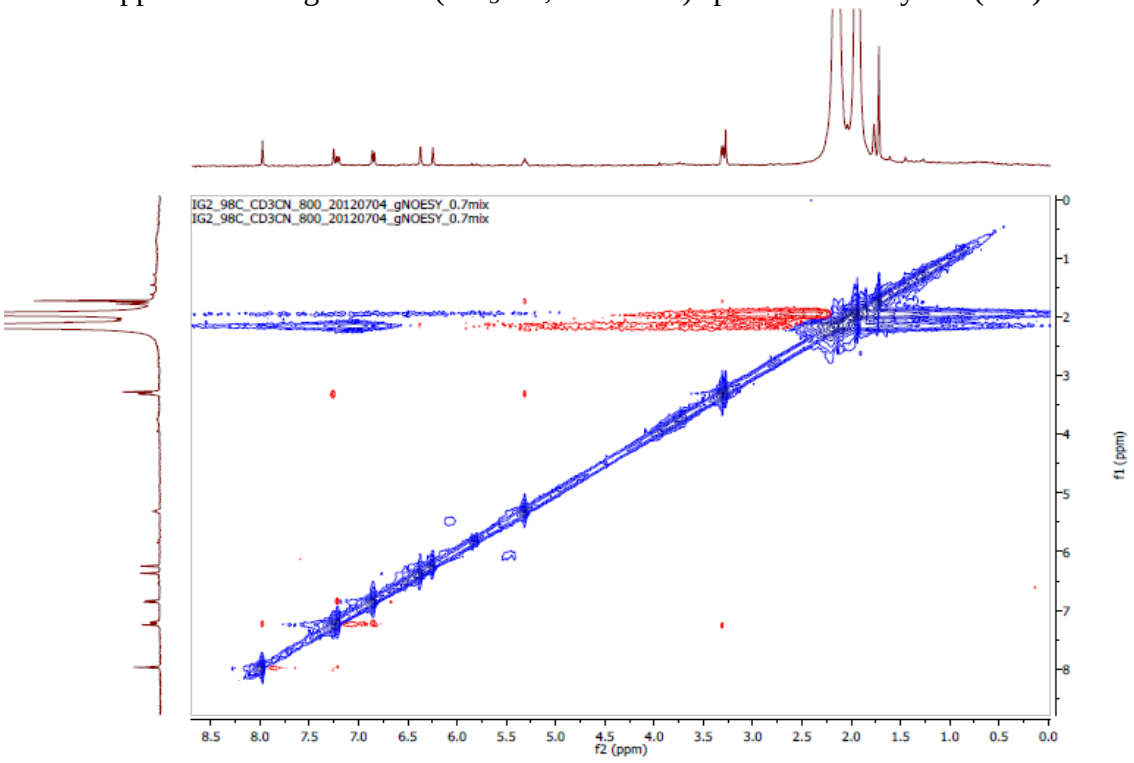
Appendix 31B: gCHSQC (CD_3CN, 800 MHz) spectrum of caryolin (**187**)



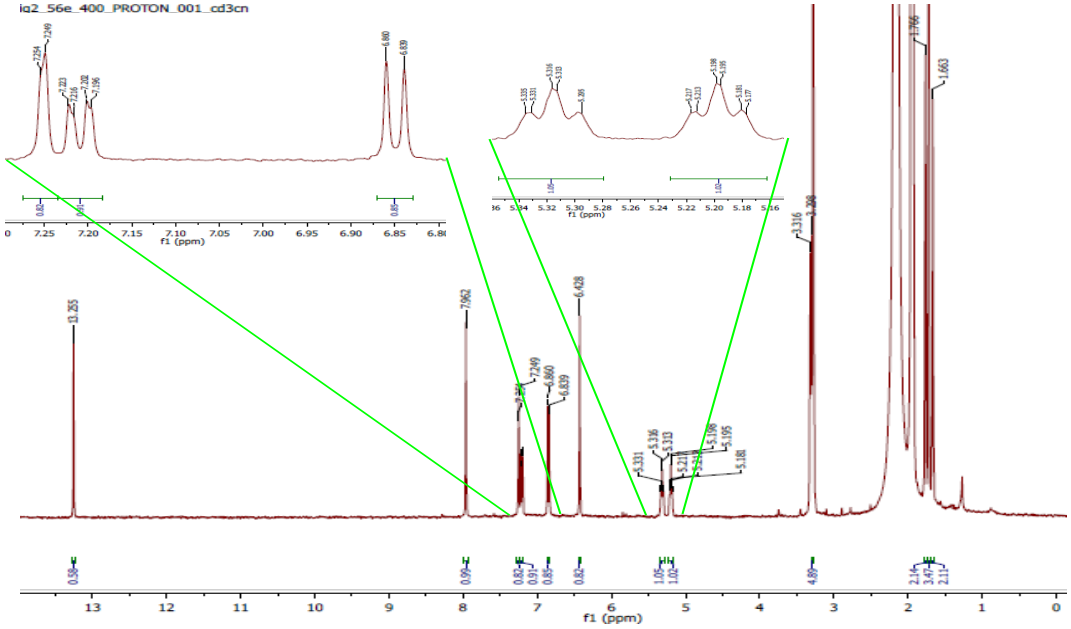
Appendix 31C: gCOSY (CD_3CN, 800 MHz) spectrum of caryolin (**187**)



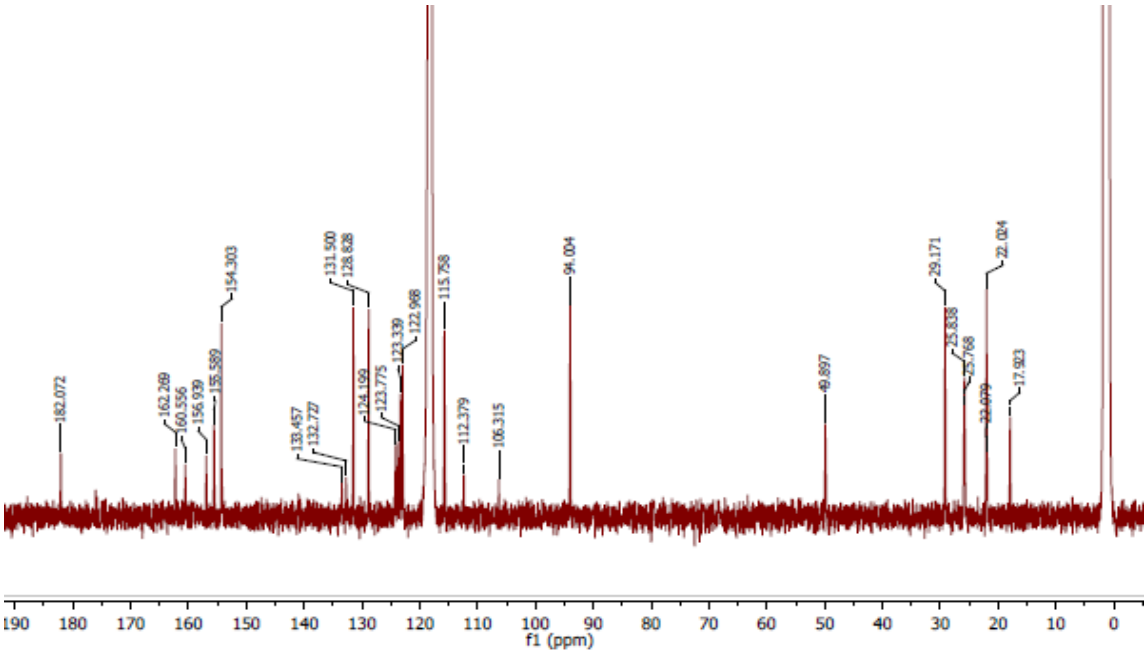
Appendix 31D: gNOESY (CD_3CN, 800 MHz) spectrum of caryolin (**187**)



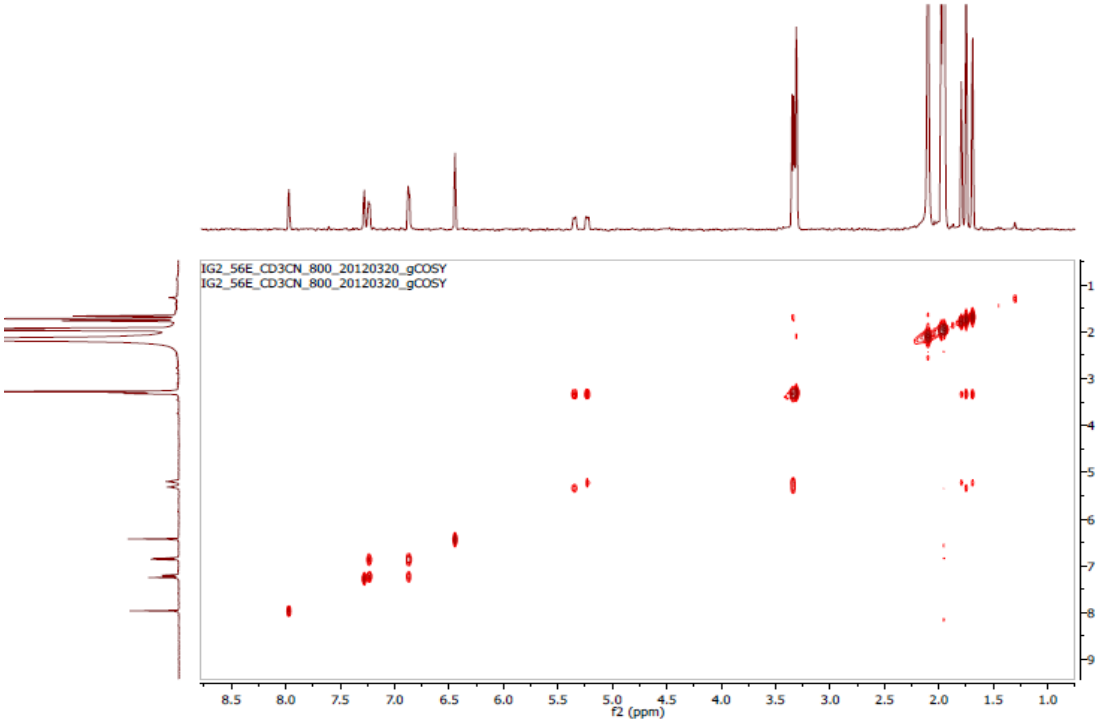
Appendix 32A: ^{1}H (CD_3CN, 400 MHz) NMR spectrum of lupalbigenin (**188**)



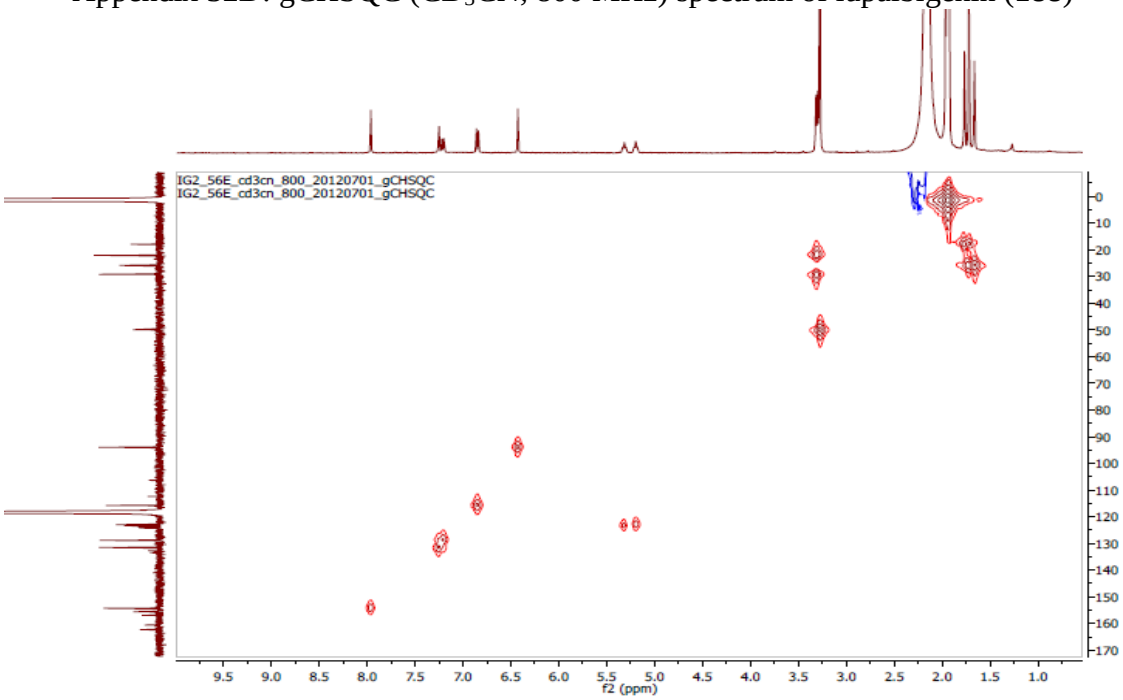
Appendix 32B: ^{13}C (CD_3CN, 150 MHz) NMR spectrum of lupalbigenin (**188**)



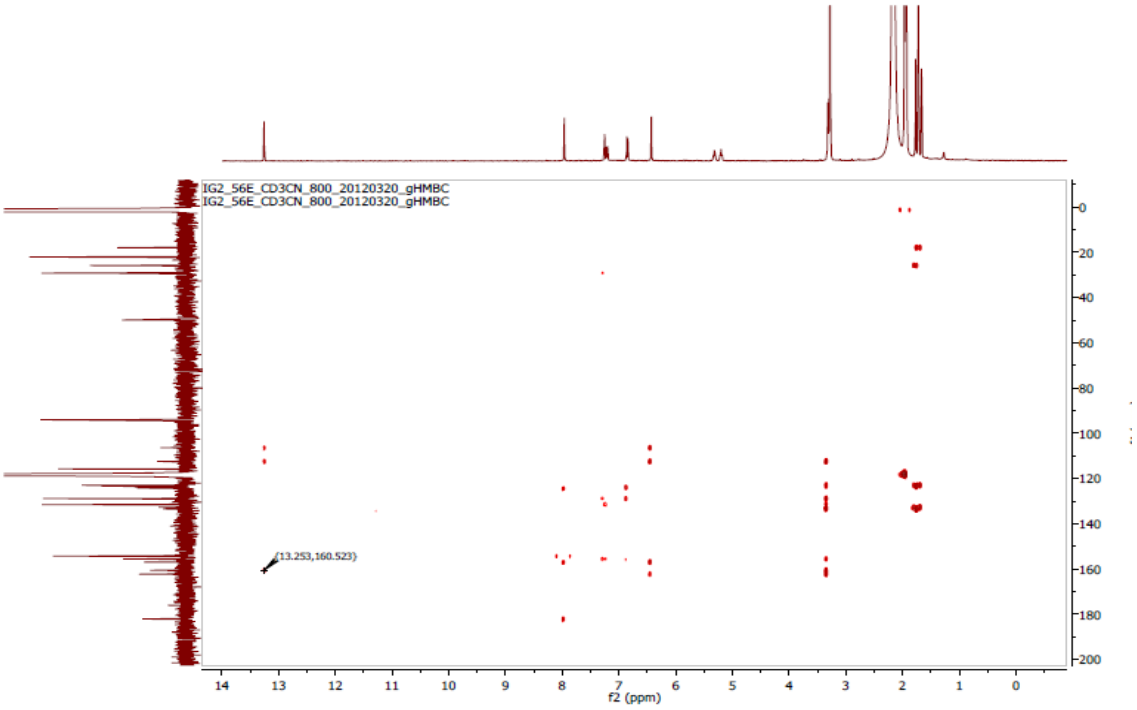
Appendix 32C: ^{1}H-^{1}H gCOSY (CD_3CN, 800 MHz) spectrum of lupalbigenin (**188**)



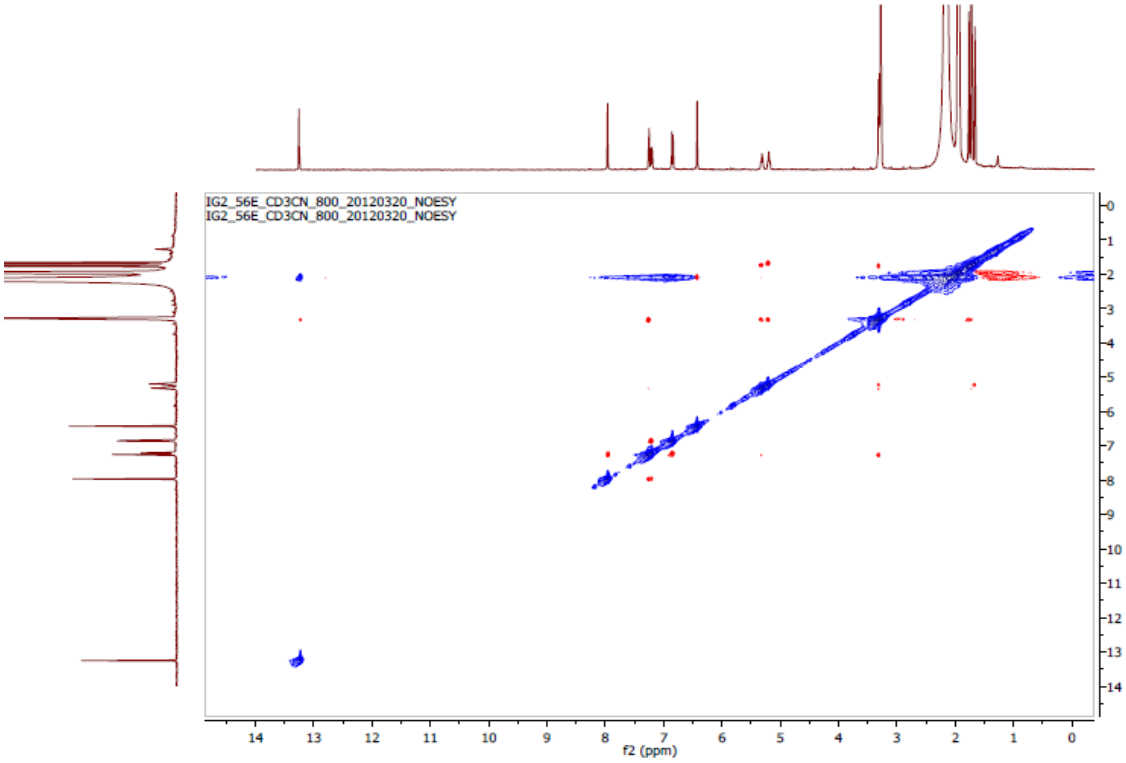
Appendix 32D: gCHSQC (CD_3CN, 800 MHz) spectrum of lupalbigenin (**188**)



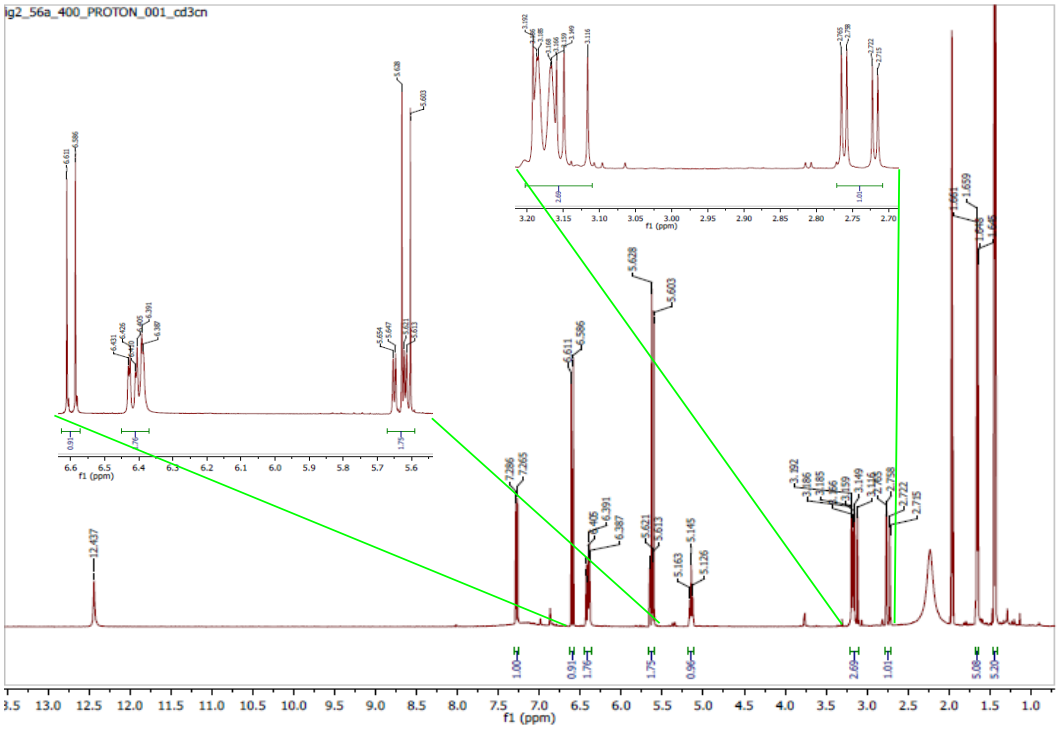
Appendix 32E: gHMBC (CD_3CN, 150 MHz) spectrum of lupalbigenin (**188**)



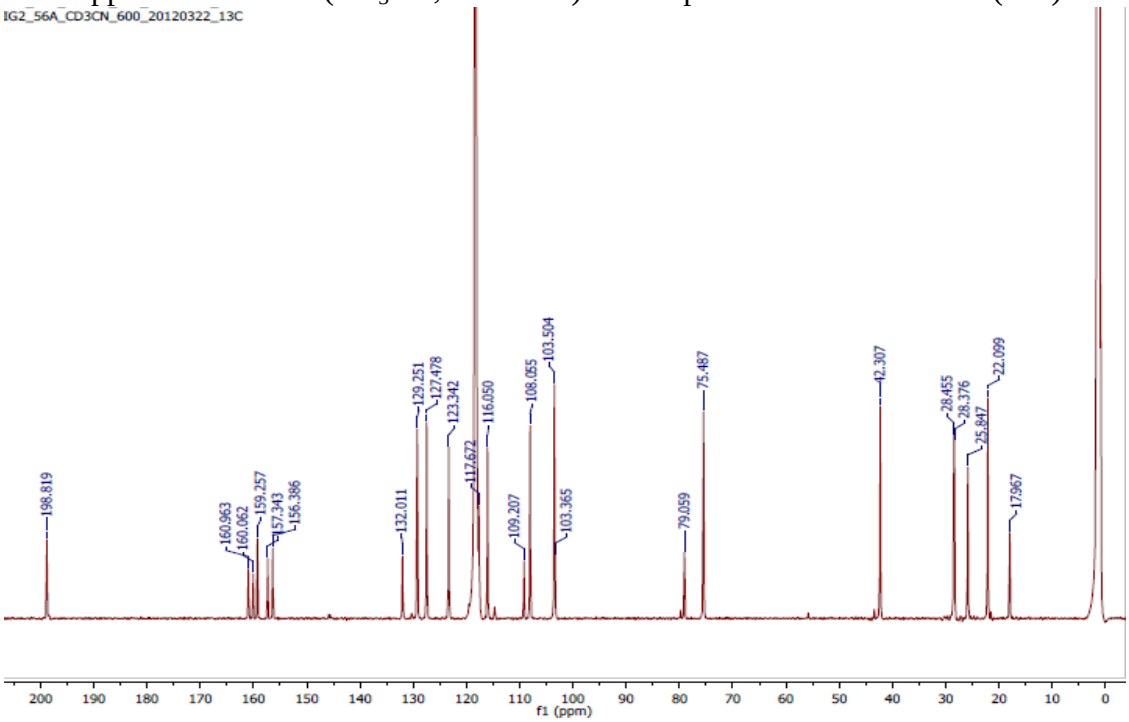
Appendix 32F: gNOESY (CD_3CN, 800 MHz) spectrum of lupalbigenin (**188**)



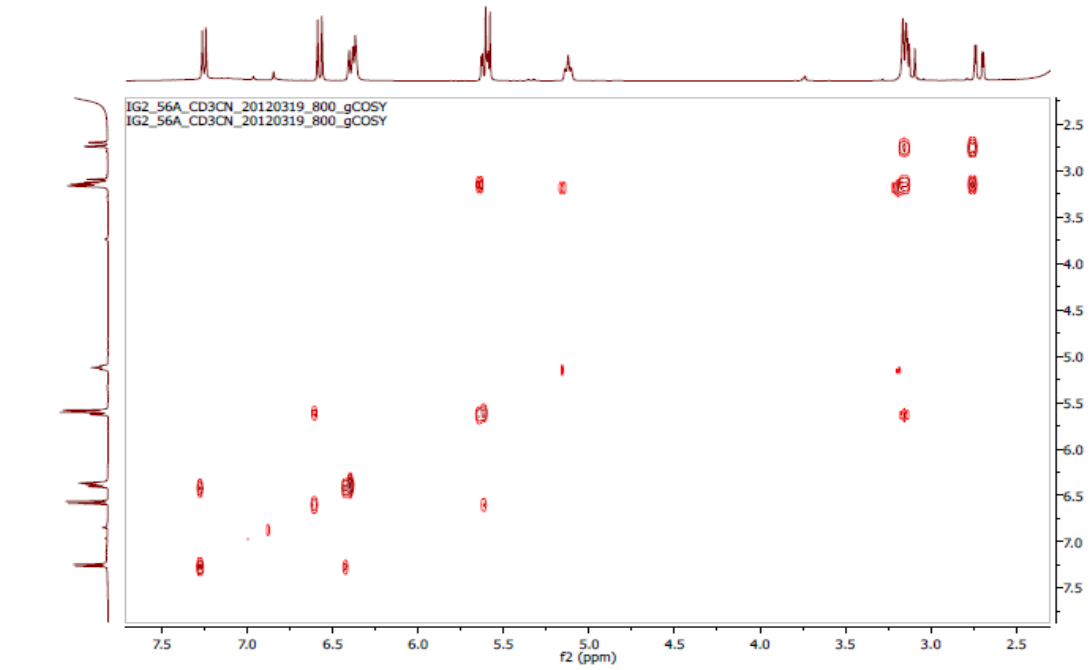
Appendix 33A: ^{1}H (CD_3CN, 400 MHz)NMR spectrum of flemichin D (**150**)



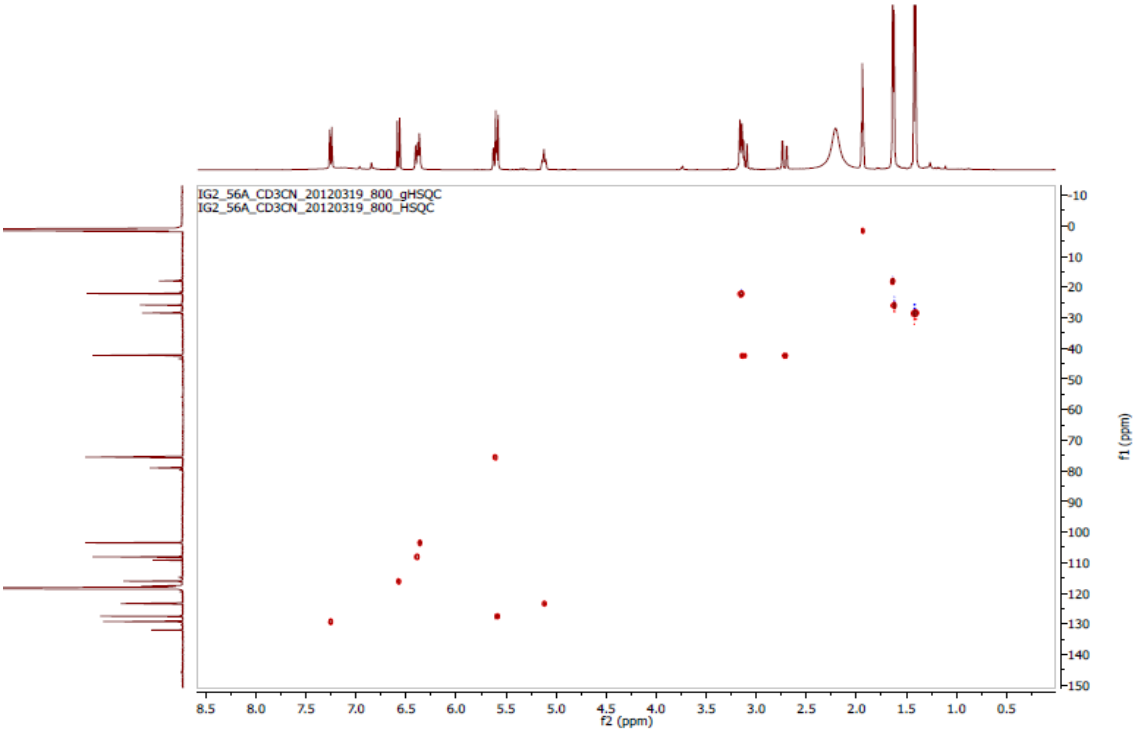
Appendix 33B: ^{13}C (CD_3CN, 800 MHz) NMR spectrum of flemichin D (**150**)



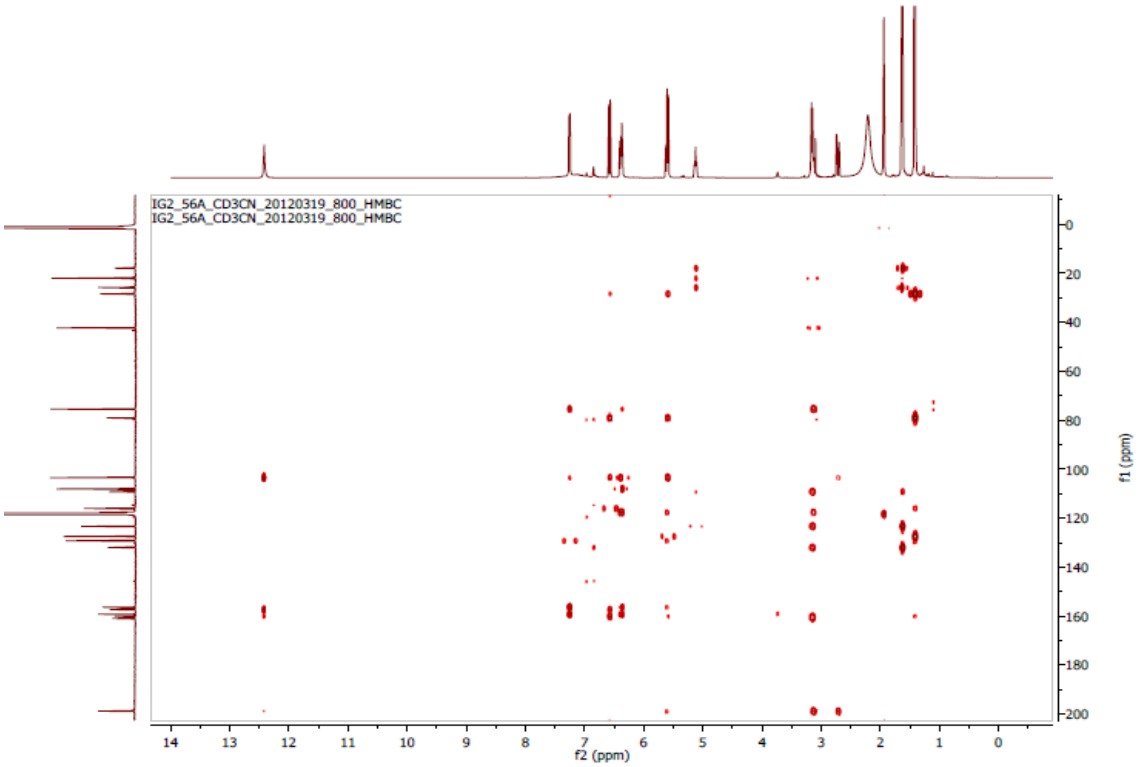
Appendix 33C: ^{1}H-^{1}H gCOSY (CD_3CN, 800 MHz) spectrum of flemichin D (**150**)



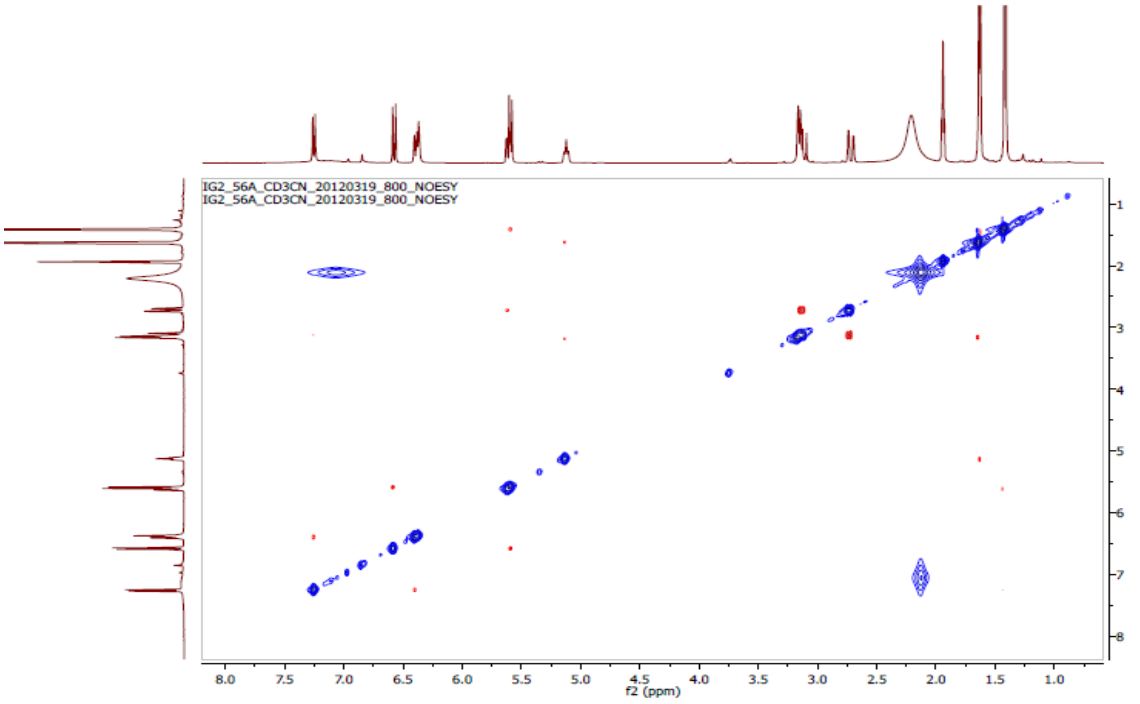
Appendix 33D: gCHSQC (CD_3CN, 800 MHz) spectrum of flemichin D (**150**)



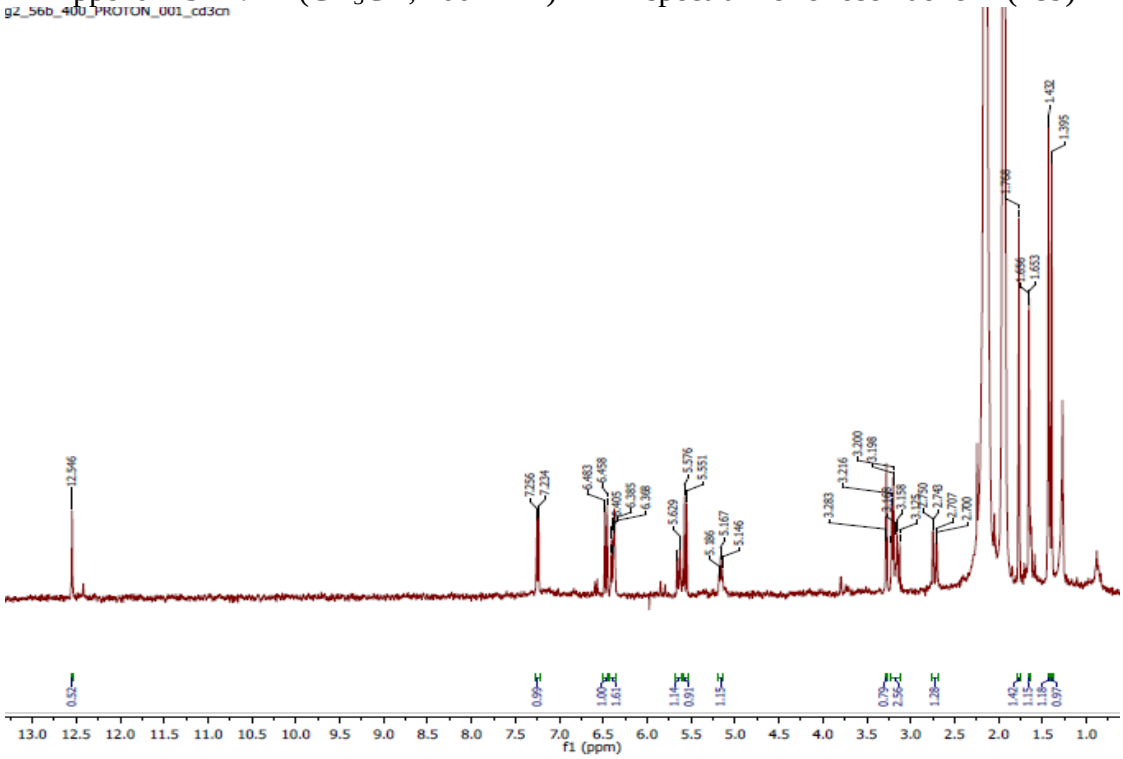
Appendix 33E: gHMBC (CD_3CN, 800 MHz) spectrum of flemichin D (**150**)



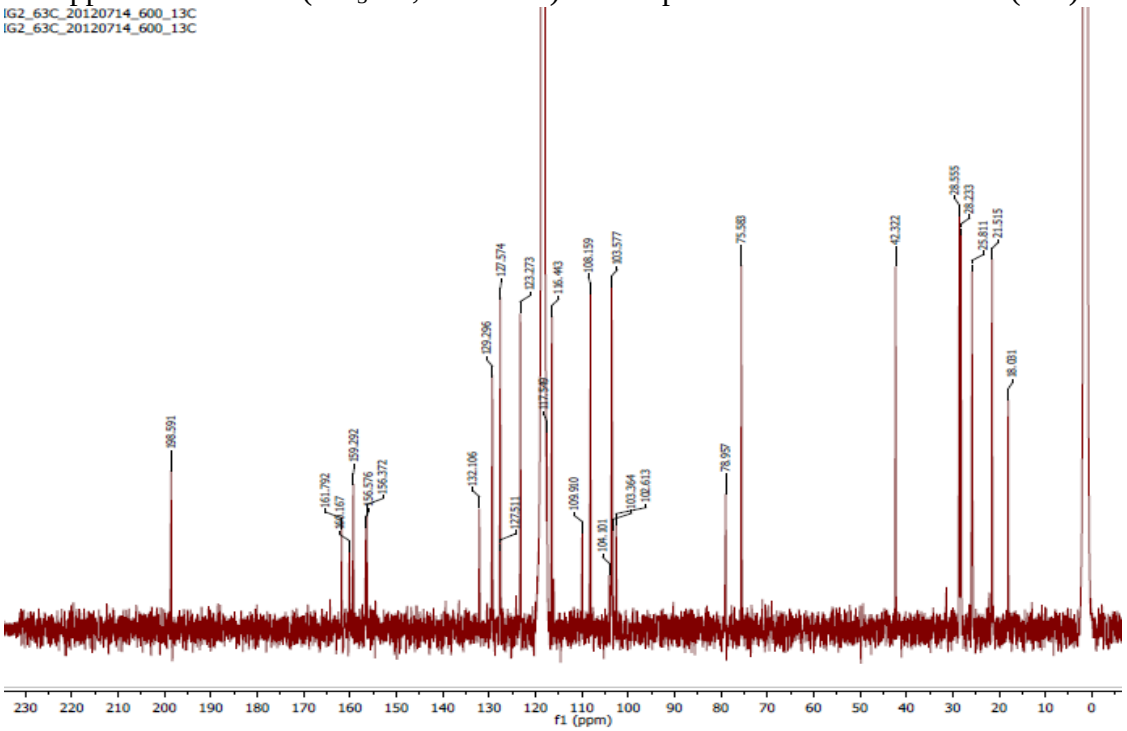
Appendix 33F: gNOESY (CD_3CN, 800 MHz) spectrum of flemichin D (**150**)



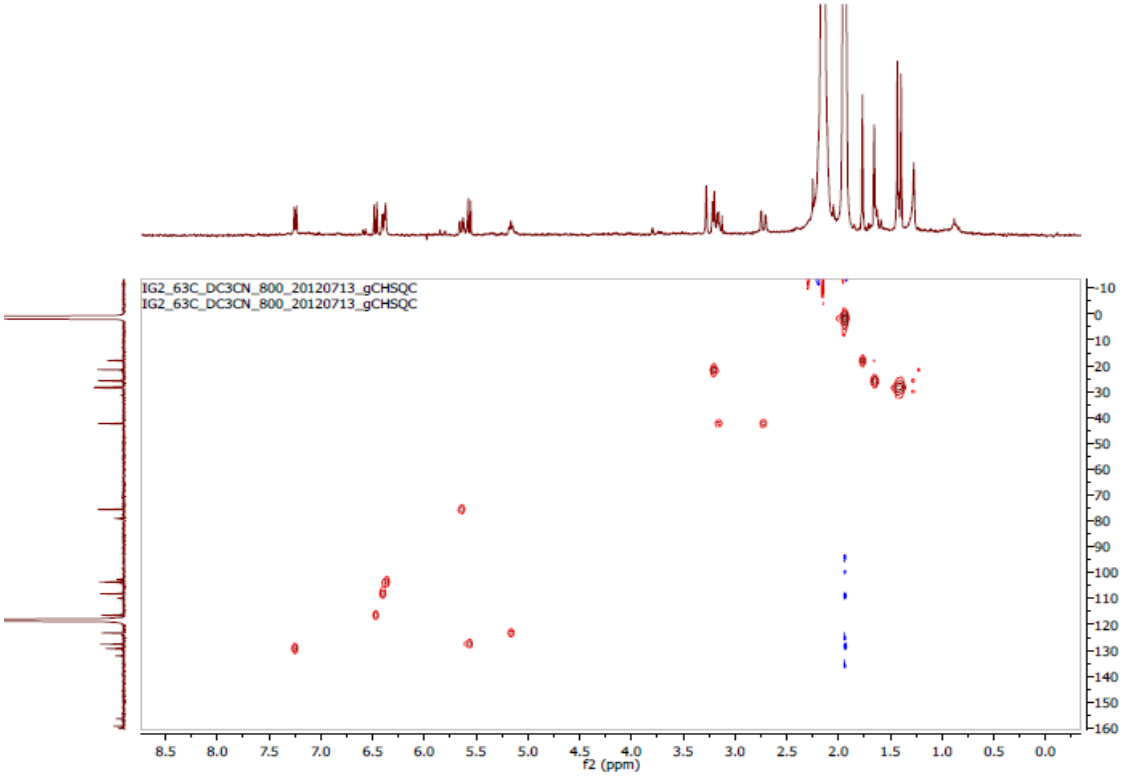
Appendix 34A: ^{1}H (CD_3CN, 400 MHz) NMR spectrum of eriosemaone A (**189**)



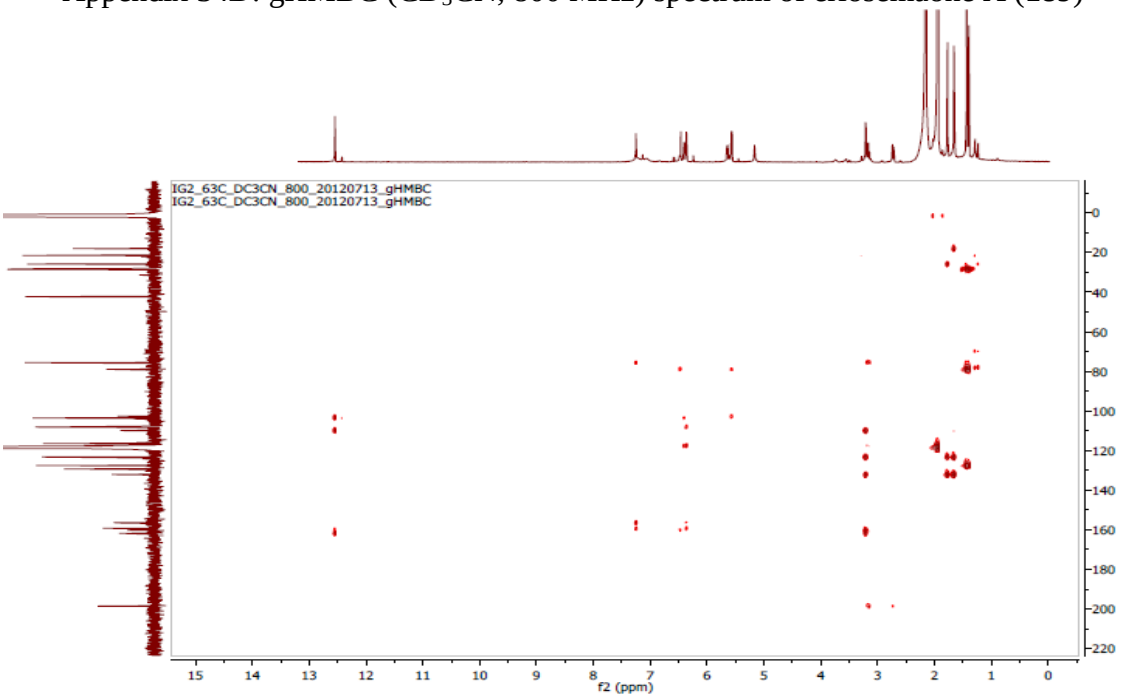
Appendix 34B: ^{13}C (CD_3CN, 150 MHz) NMR spectrum of eriosemaone A (**189**)



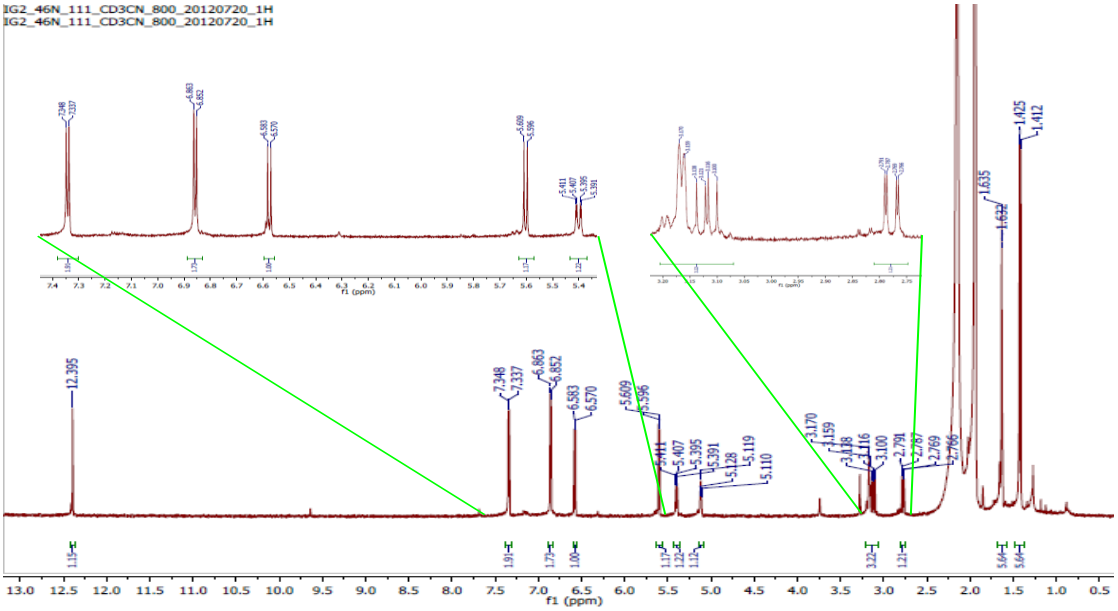
Appendix 34C: gCHSQC (CD_3CN, 800 MHz) spectrum of eriosemaone A (**189**)



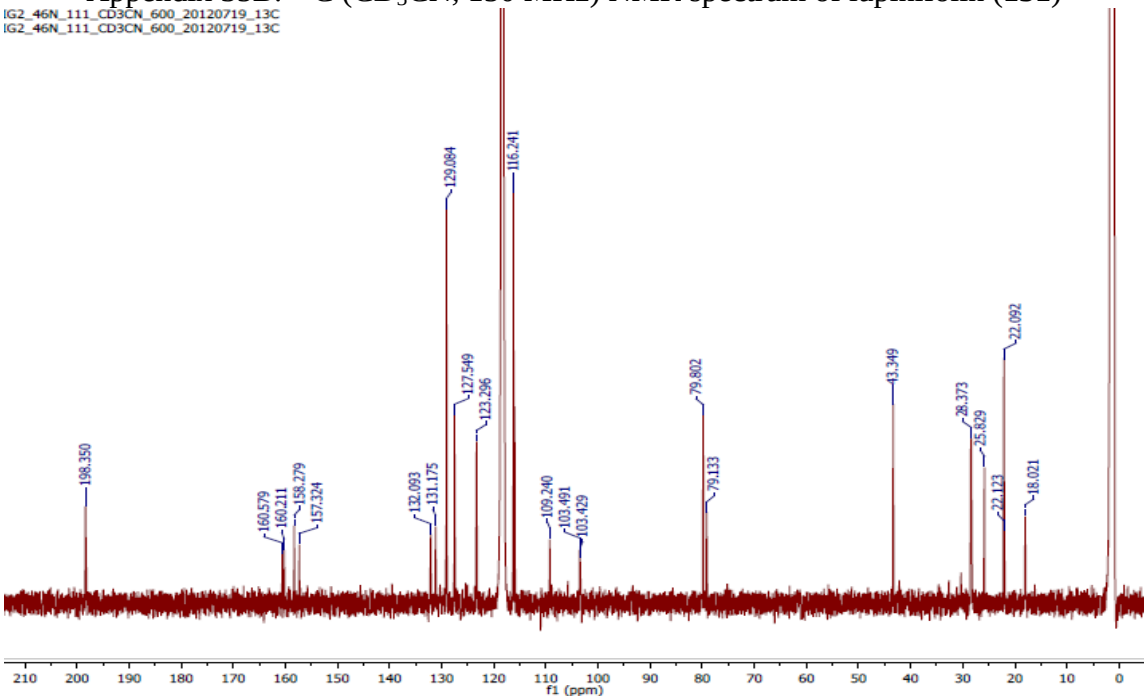
Appendix 34D: gHMBC (CD_3CN, 800 MHz) spectrum of eriosemaone A (**189**)



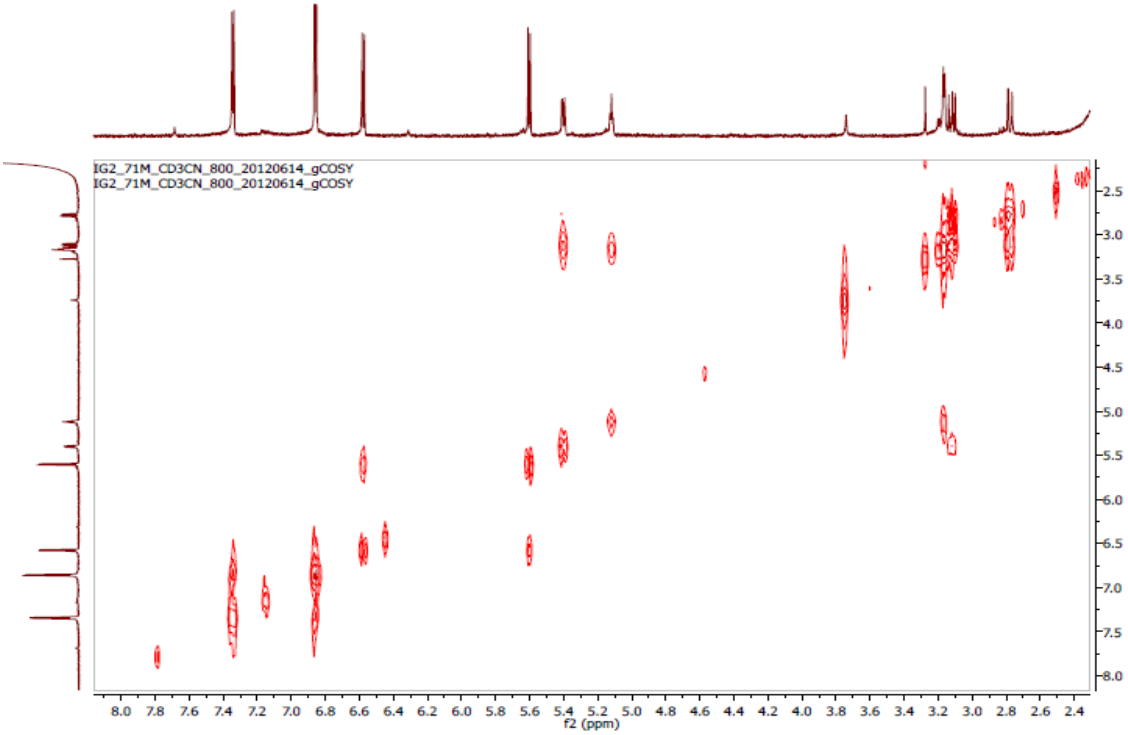
Appendix 35A: ^{1}H (CD_3CN, 800 MHz) NMR spectrum of lupinifolin (**151**)



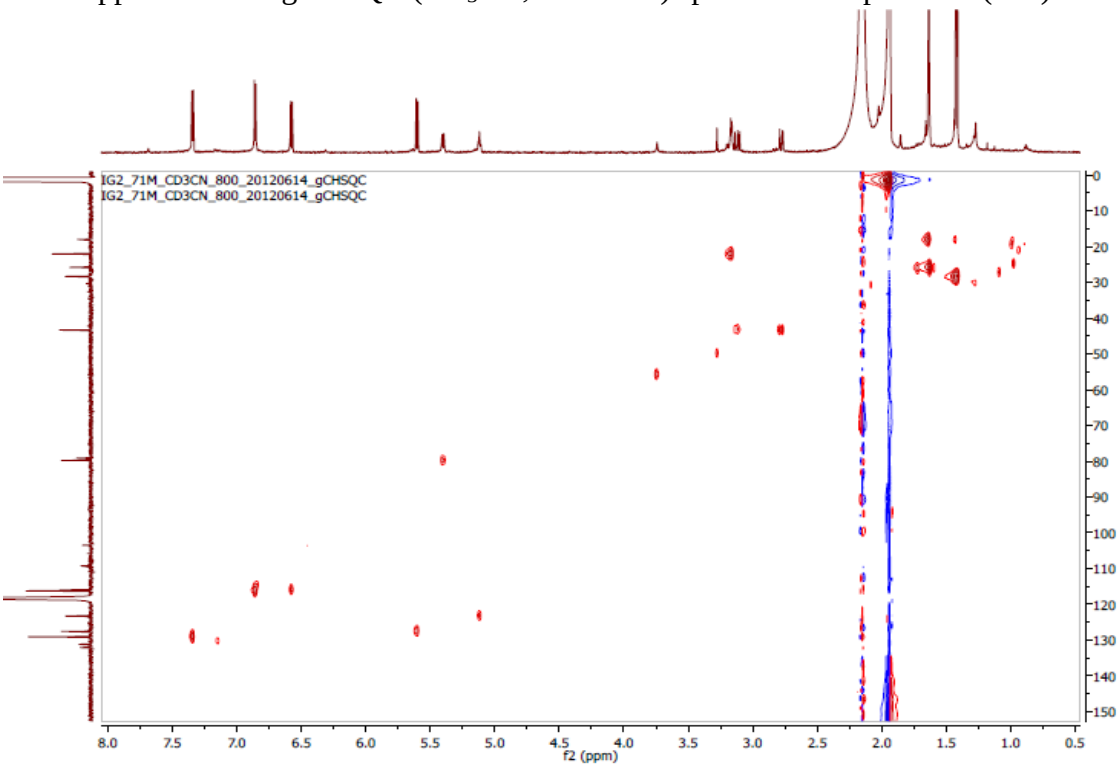
Appendix 35B: ^{13}C (CD_3CN, 150 MHz) NMR spectrum of lupinifolin (**151**)



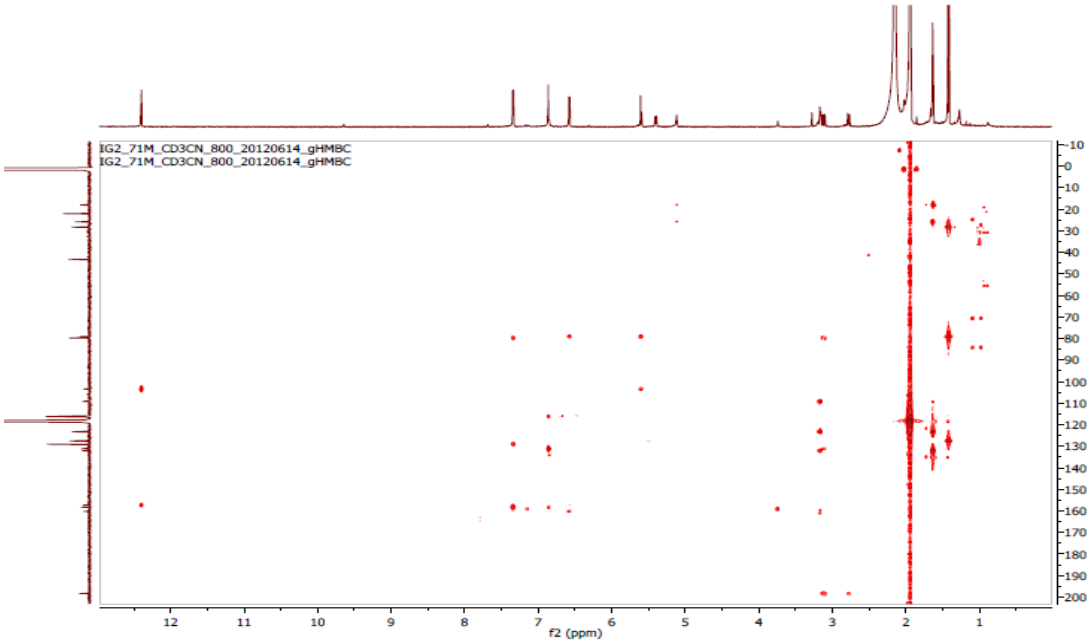
Appendix 35C: gCOSY (CD_3CN, 800 MHz) spectrum of lupinifolin (**151**)



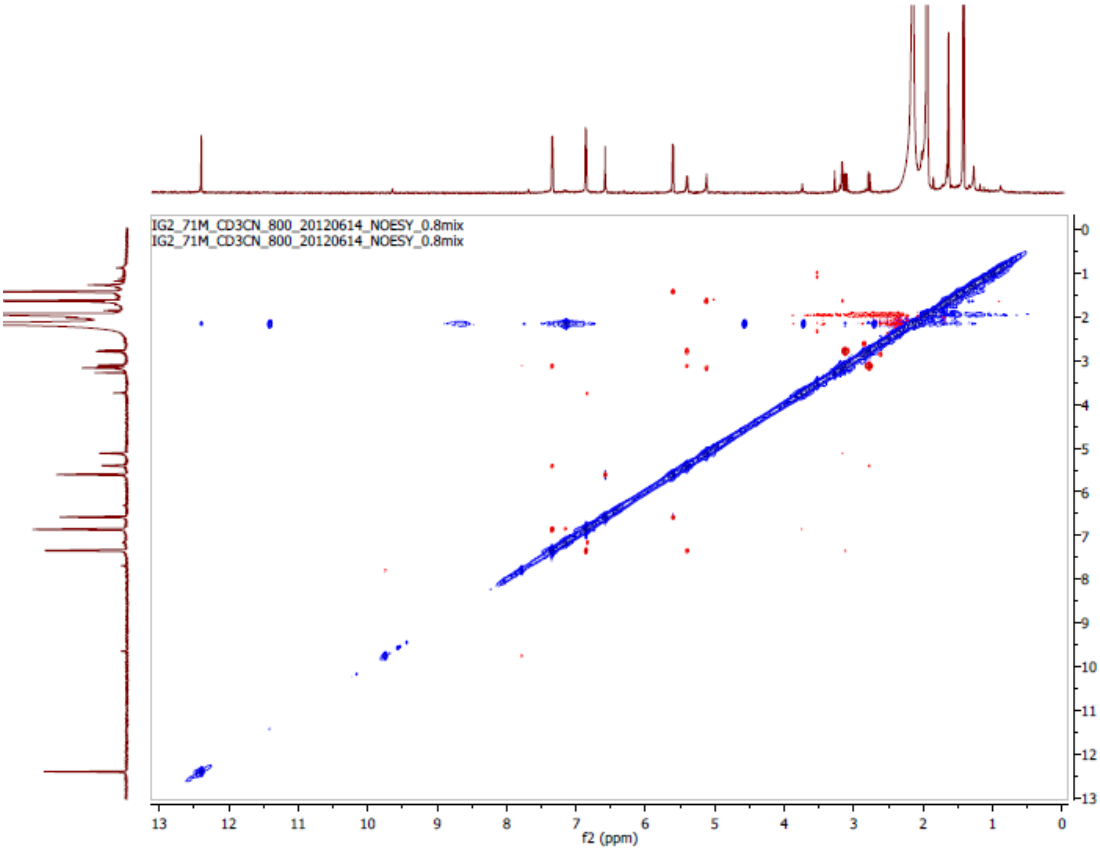
Appendix 35D: gCHSQC (CD_3CN, 800 MHz) spectrum of lupinifolin (**151**)



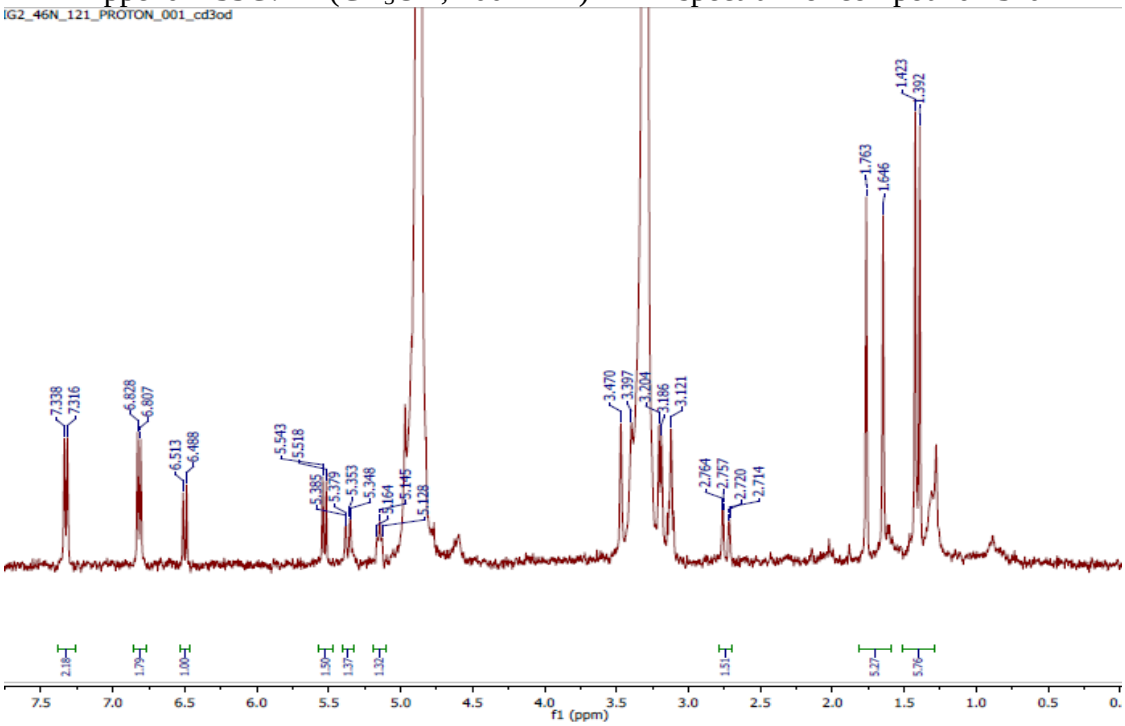
Appendix 35E: gHMBC (CD_3CN, 800 MHz) spectrum of lupinifolin (**151**)



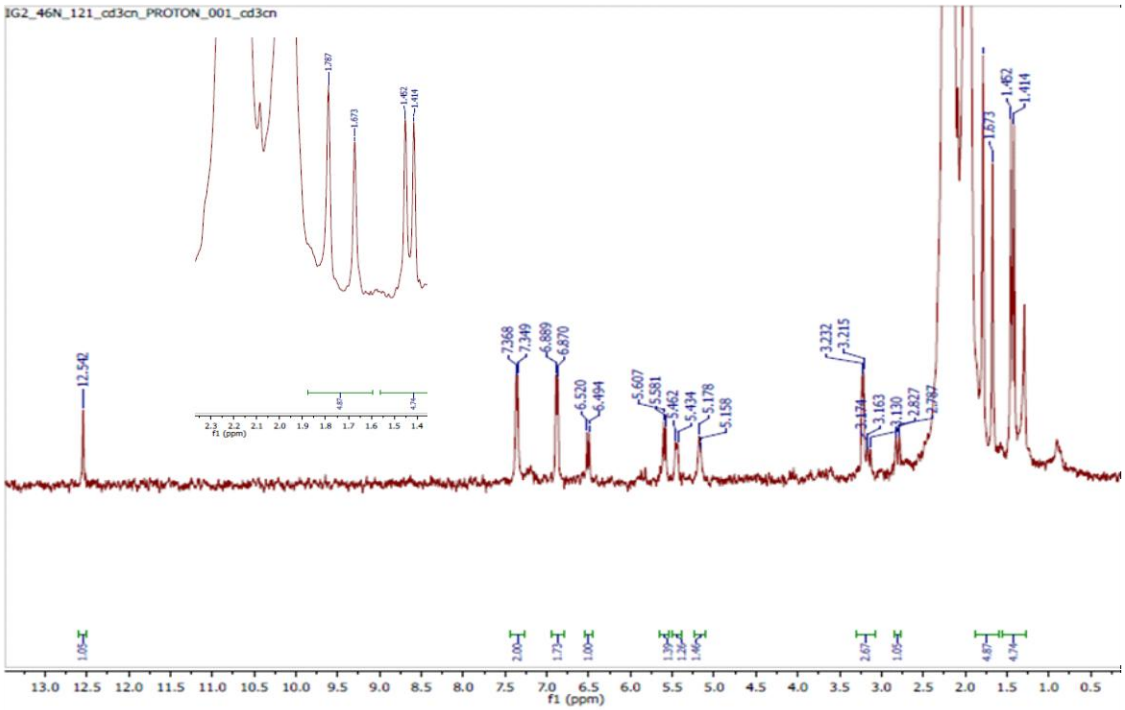
Appendix 35F: gNOESY (CD_3CN, 800 MHz) spectrum of lupinifolin (**151**)



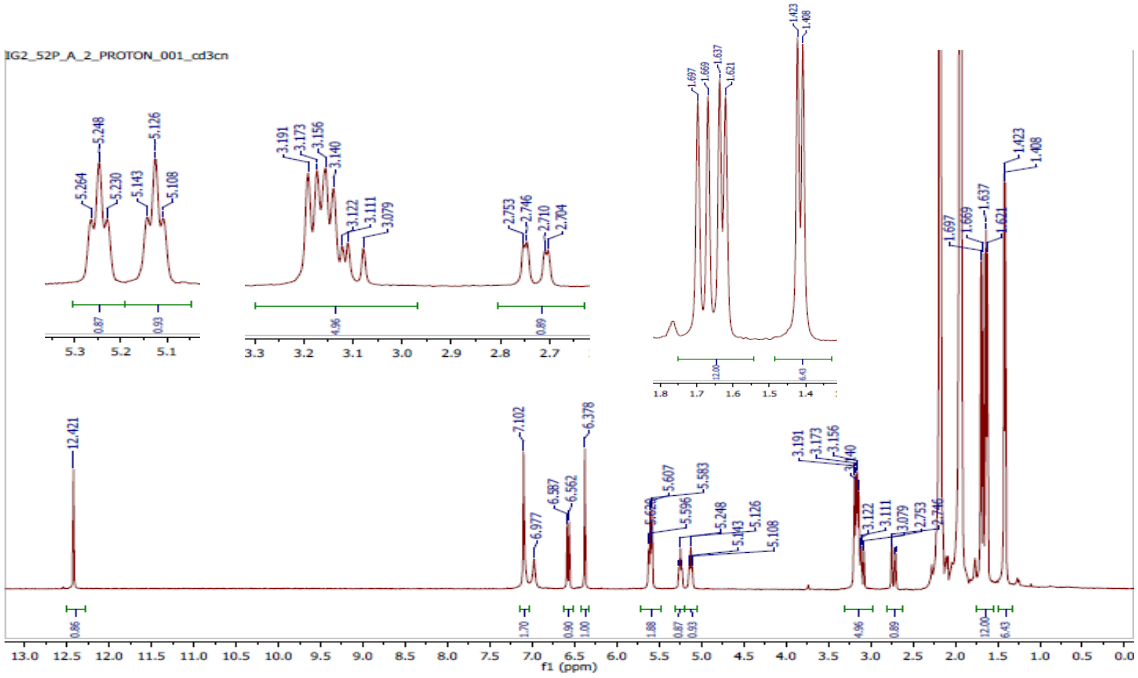
Appendix 35G: ^{1}H (CD_3OD, 400 MHz) NMR spectrum of compound **151a**



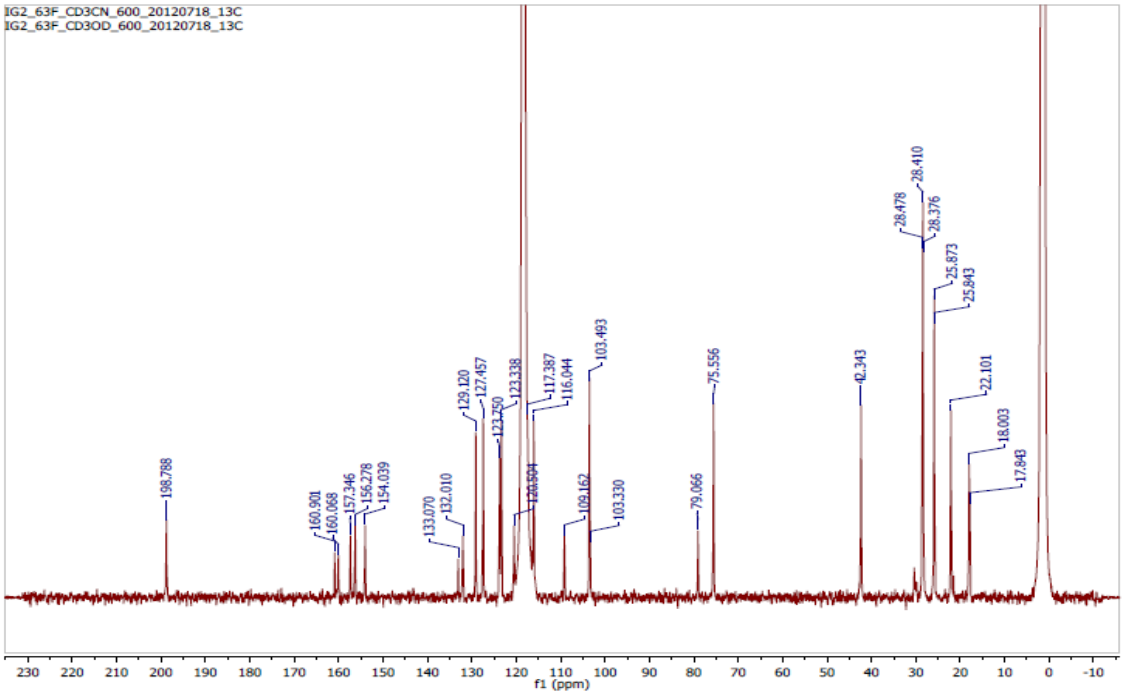
Appendix 35H: ^{1}H (CD_3CN, 400 MHz) NMR spectrum of compound **151a**



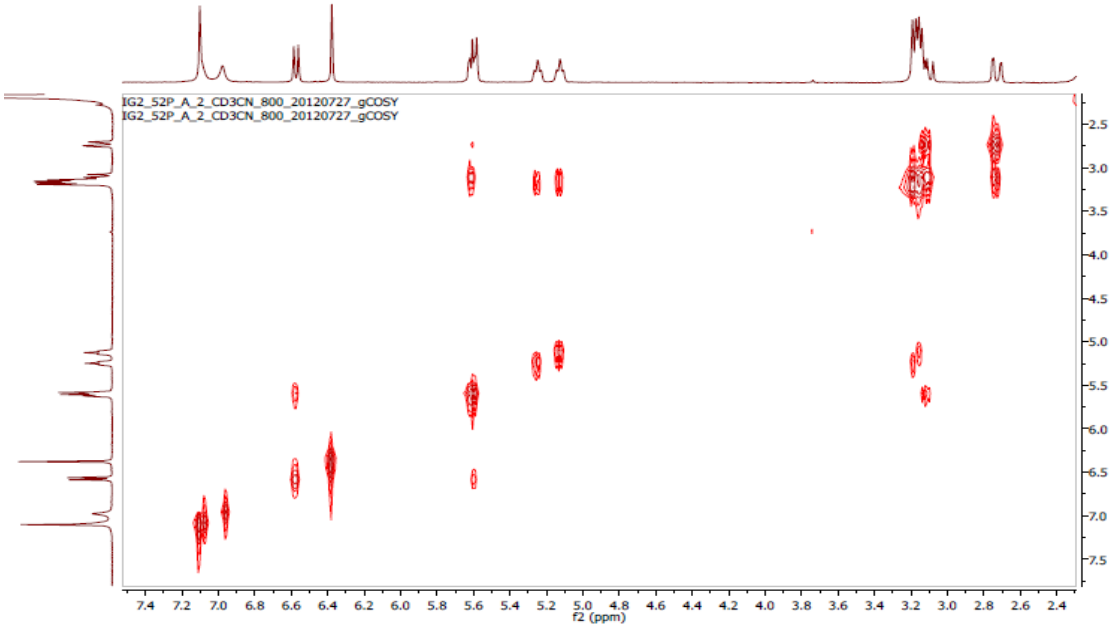
Appendix 36A: ^{1}H (CD_3CN, 400 MHz) NMR spectrum of 5,2',4'-trihydroxy-8, 5'-di(γ,γ-dimethylallyl)-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**190**)



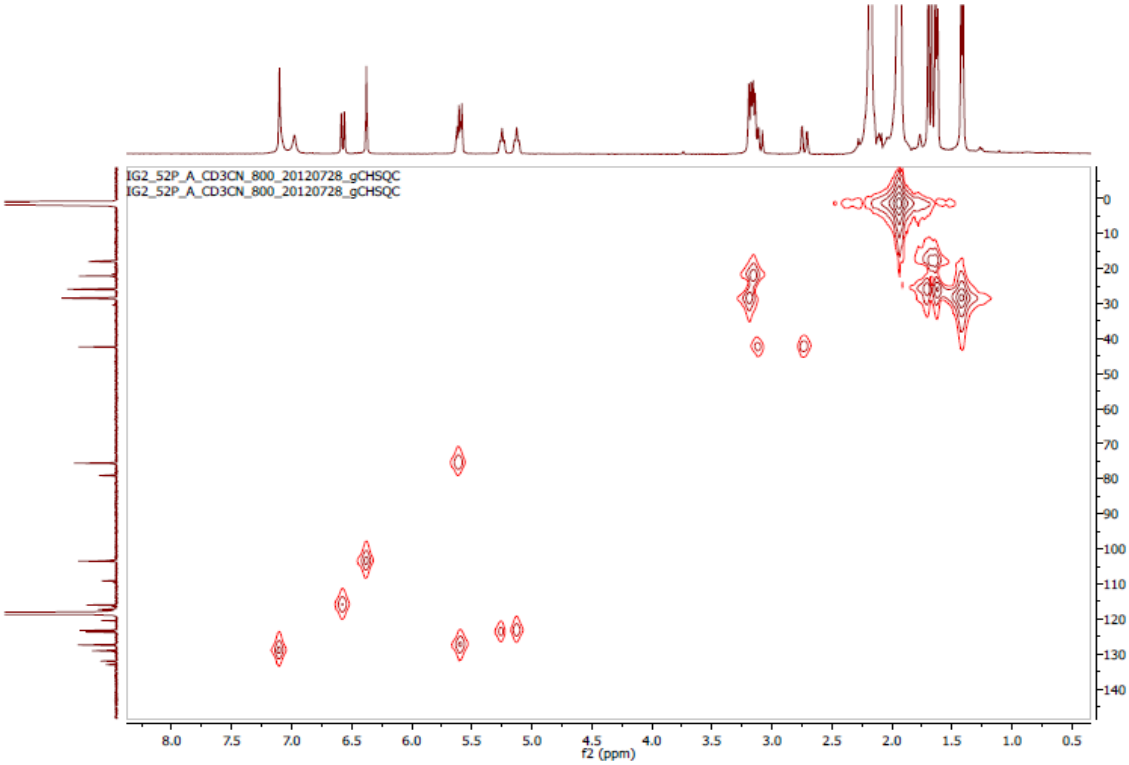
Appendix 36B: ^{13}C (CD_3CN, 150 MHz) NMR spectrum of 5,2',4'-trihydroxy-8, 5'-di(γ,γ-dimethylallyl)-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**190**)



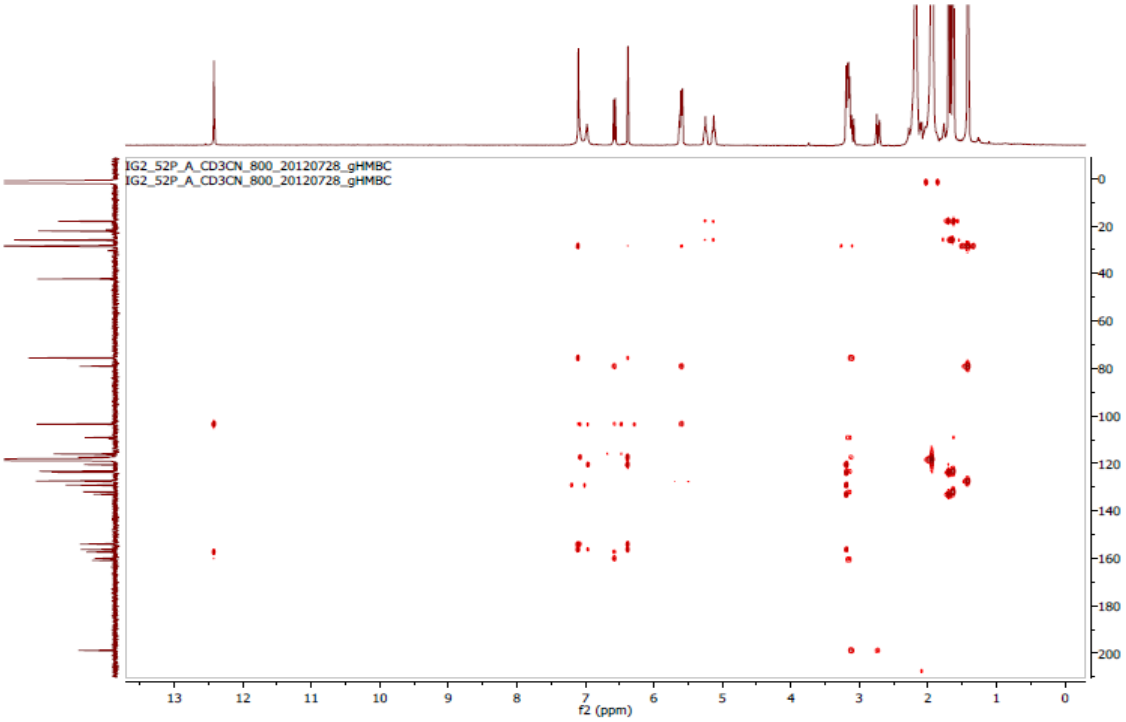
Appendix 36C: ^{1}H-^{1}H gCOSY (CD_3CN, 800 MHz) spectrum of 5,2',4'-trihydroxy-8, 5'-di(γ,γ-dimethylallyl)-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**190)**



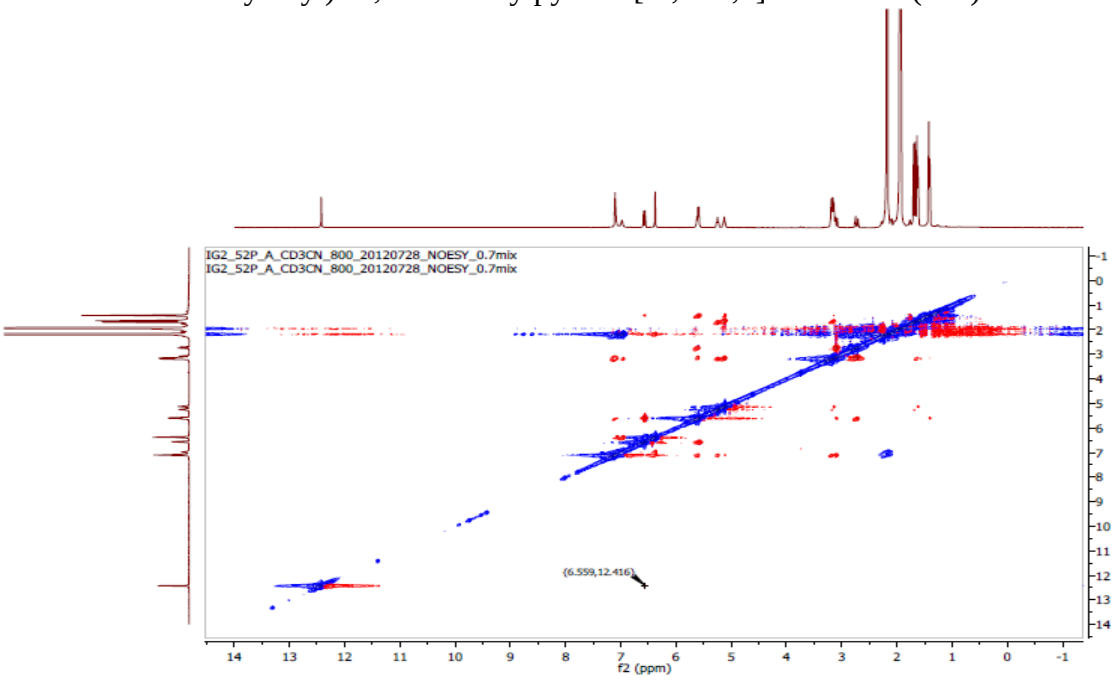
Appendix 36D: gCHSQC (CD_3CN, 800 MHz) spectrum of 5,2',4'-trihydroxy-8, 5'-di(γ,γ-dimethylallyl)-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**190**)



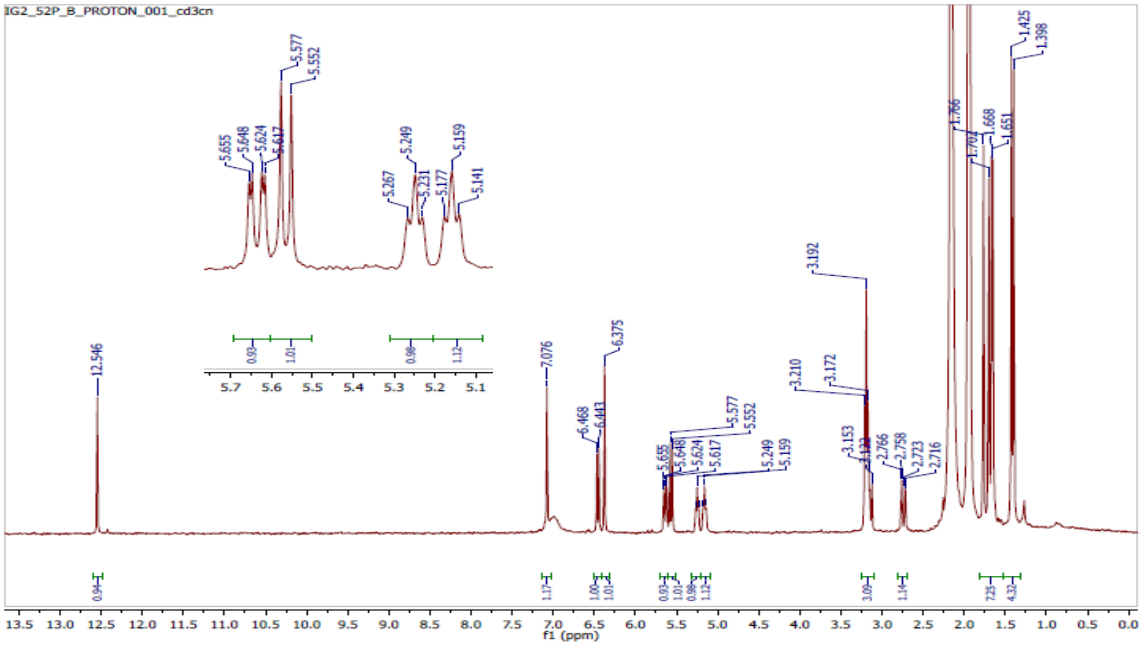
Appendix 36E: gHMBC (CD_3CN, 800 MHz) spectrum of 5,2',4'-trihydroxy-8, 5'-di(γ,γ-dimethylallyl)-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**190**)



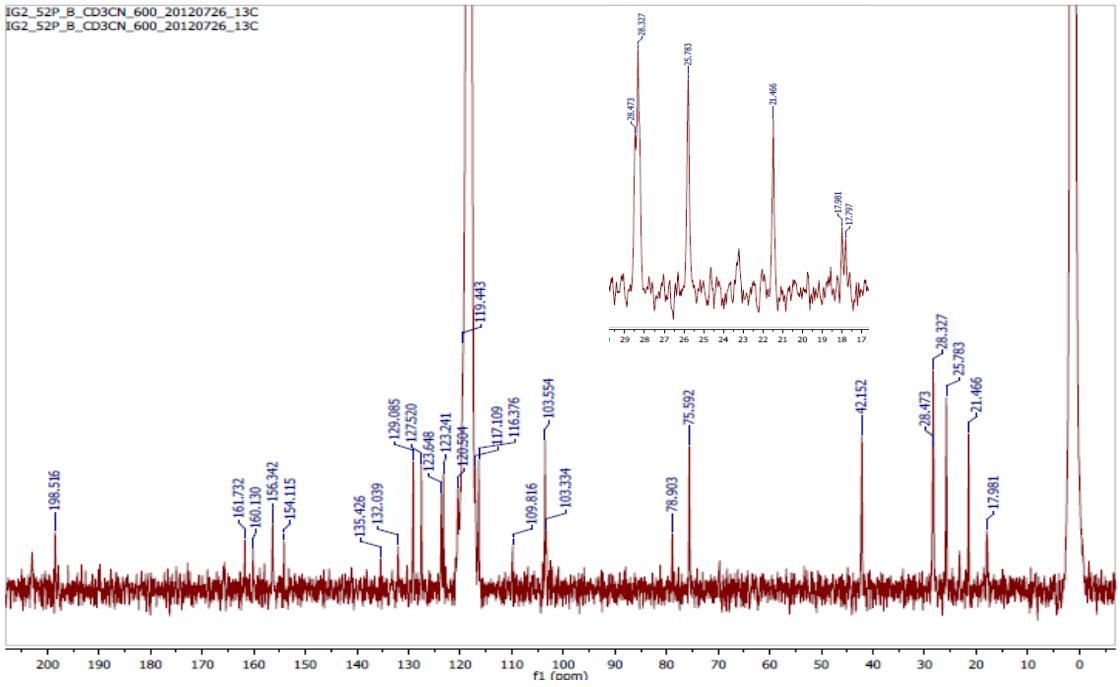
Appendix 36F: gNOESY (CD_3CN, 800 MHz) spectrum of 5,2',4'-trihydroxy-8, 5'-di(γ,γ-dimethylallyl)-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**190**)



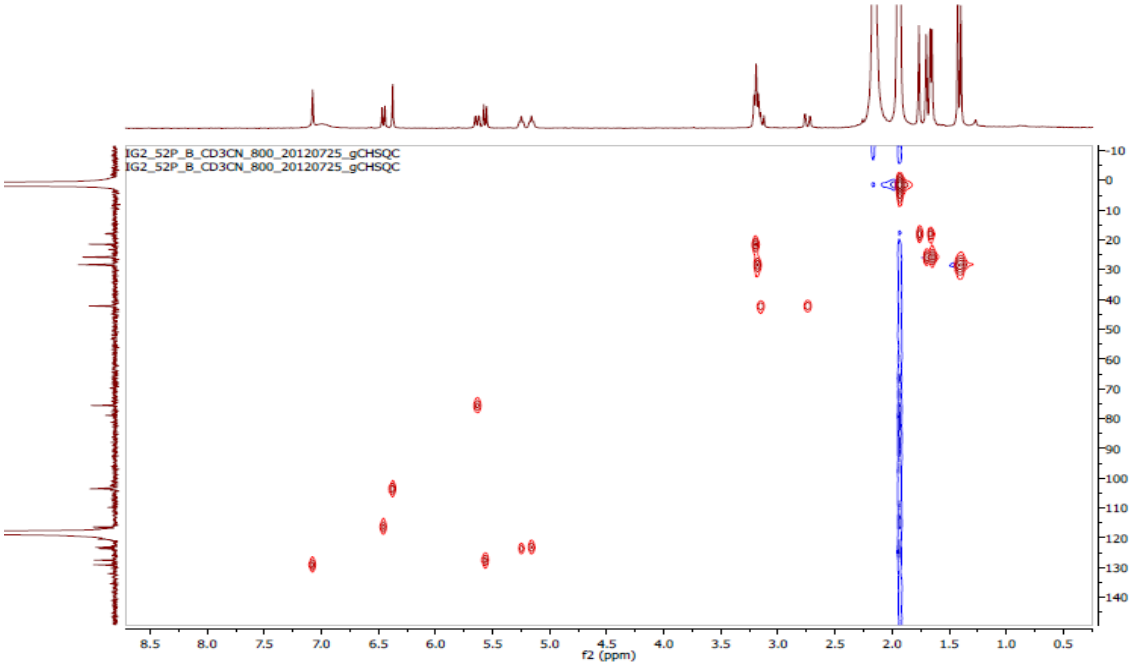
Appendix 36G: ^{1}H (CD_3CN, 400 MHz) NMR spectrum of compound **190a**



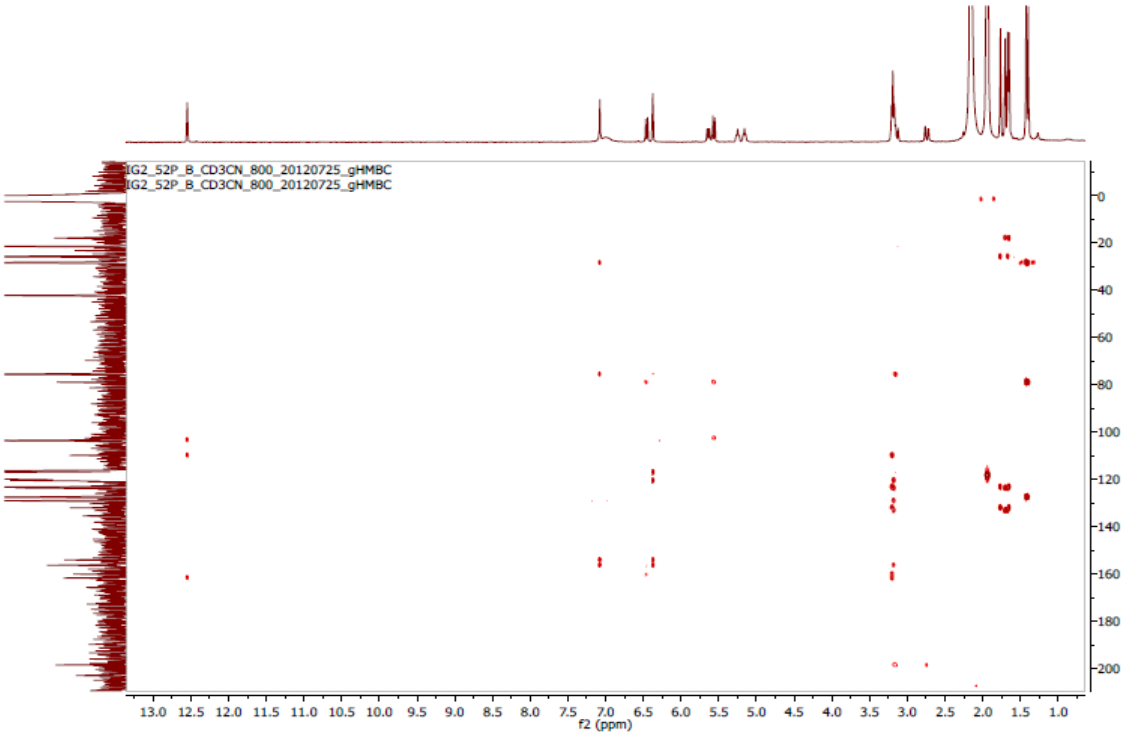
Appendix 36H: ^{13}C (CD_3CN, 150 MHz) NMR spectrum of compound **190a**



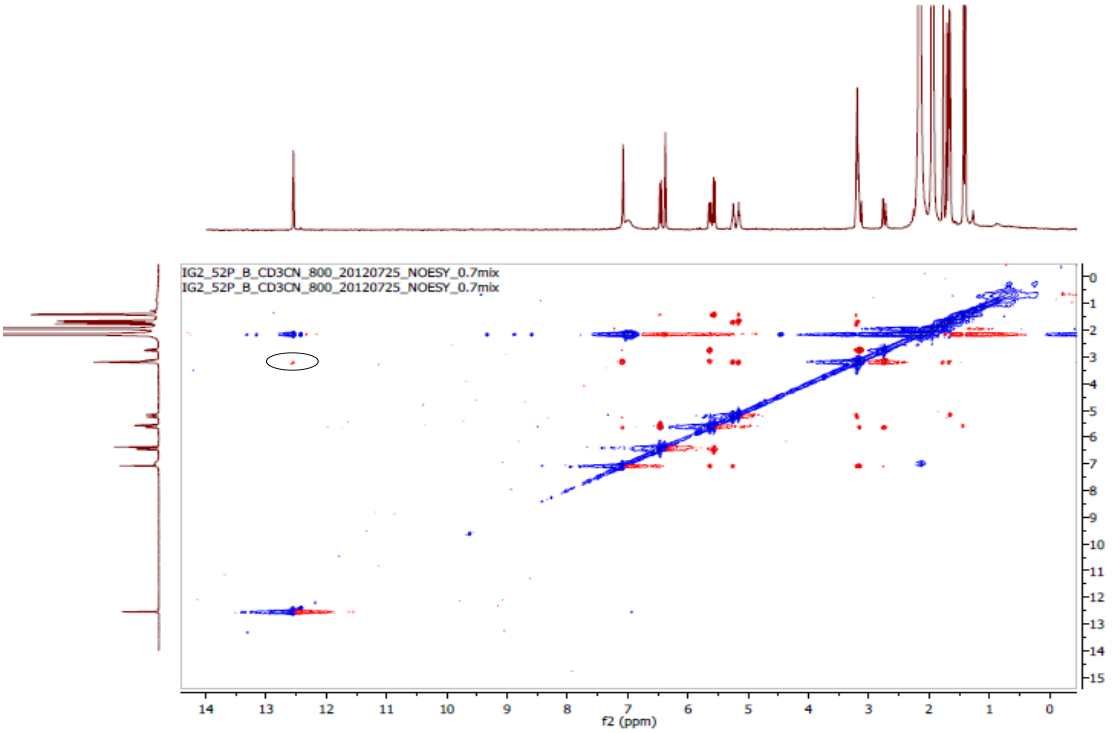
Appendix 36I: gCHSQC (CD_3CN, 800 MHz) spectrum of compound **190a**



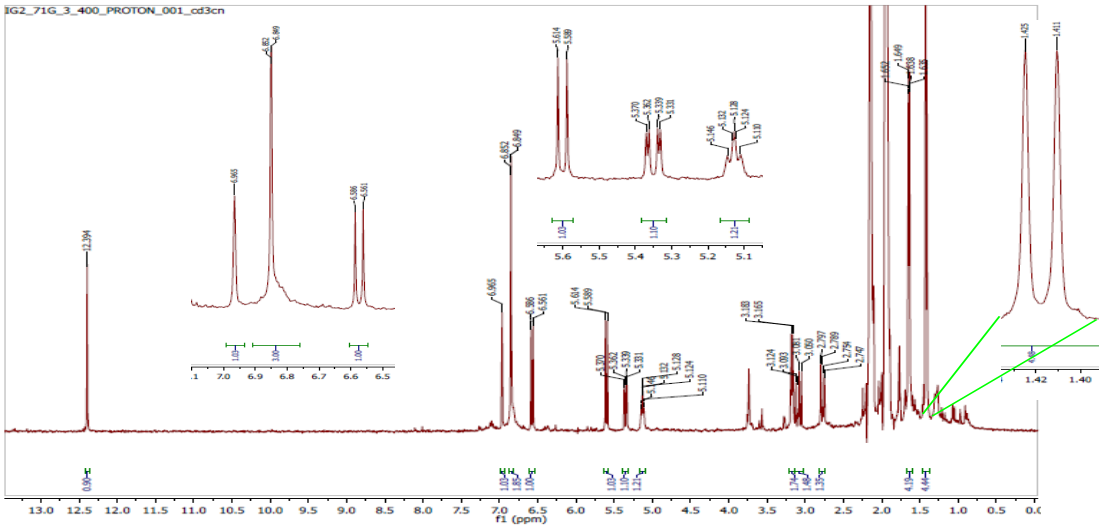
Appendix 36J: gHMBC (CD_3CN, 800 MHz) spectrum of compound **190a**



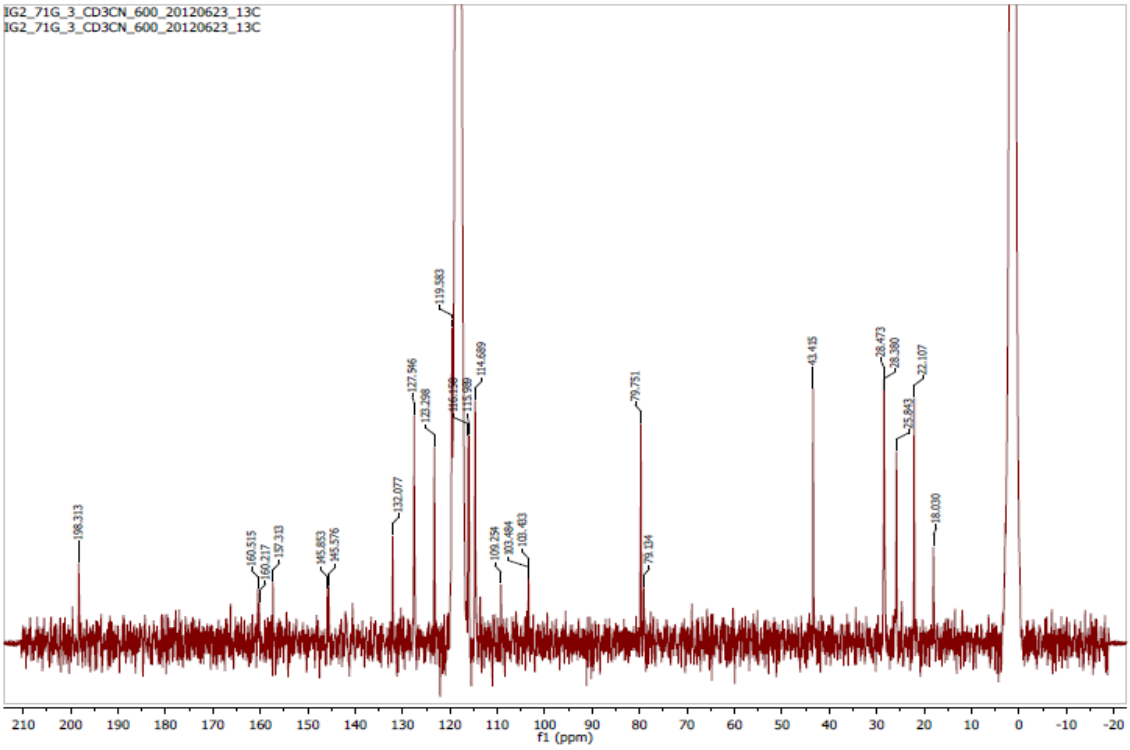
Appendix 36K: gNOESY (CD_3CN, 800 MHz) spectrum of compound **190a**, CD_3CN



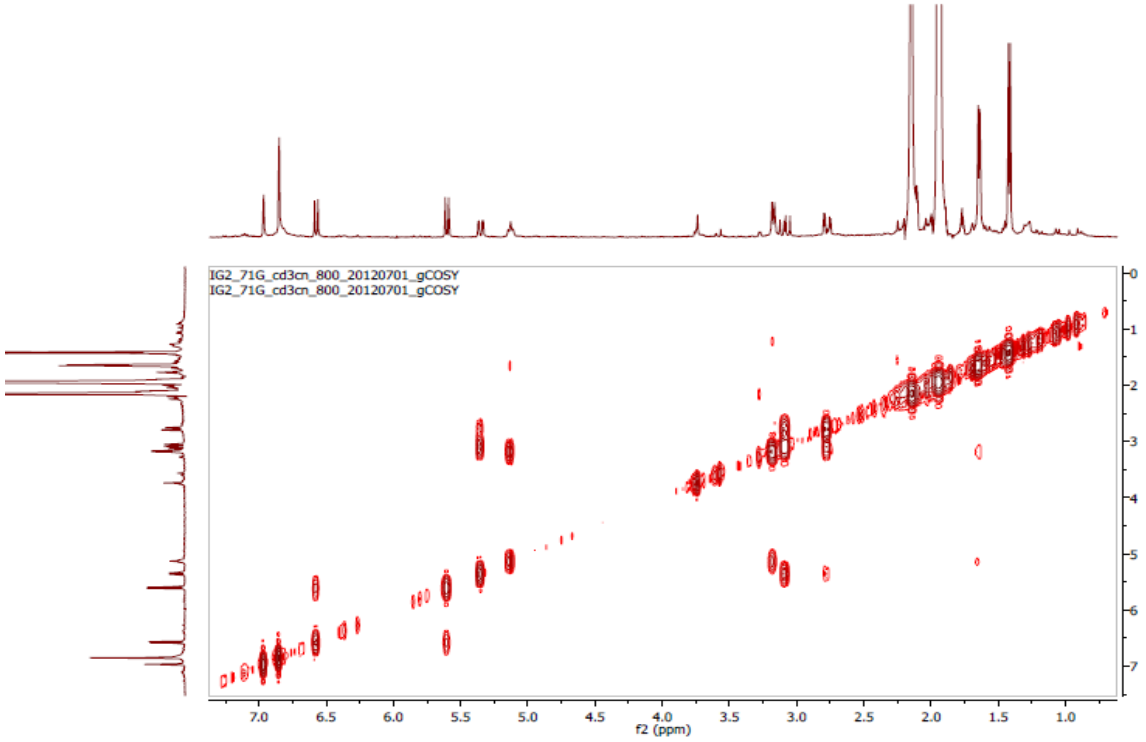
Appendix 37A: ^{1}H (CD_3CN, 400 MHz) NMR spectrum of 5,3',4'-trihydroxy-8-γ,γ-dimethylallyl-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**191**)



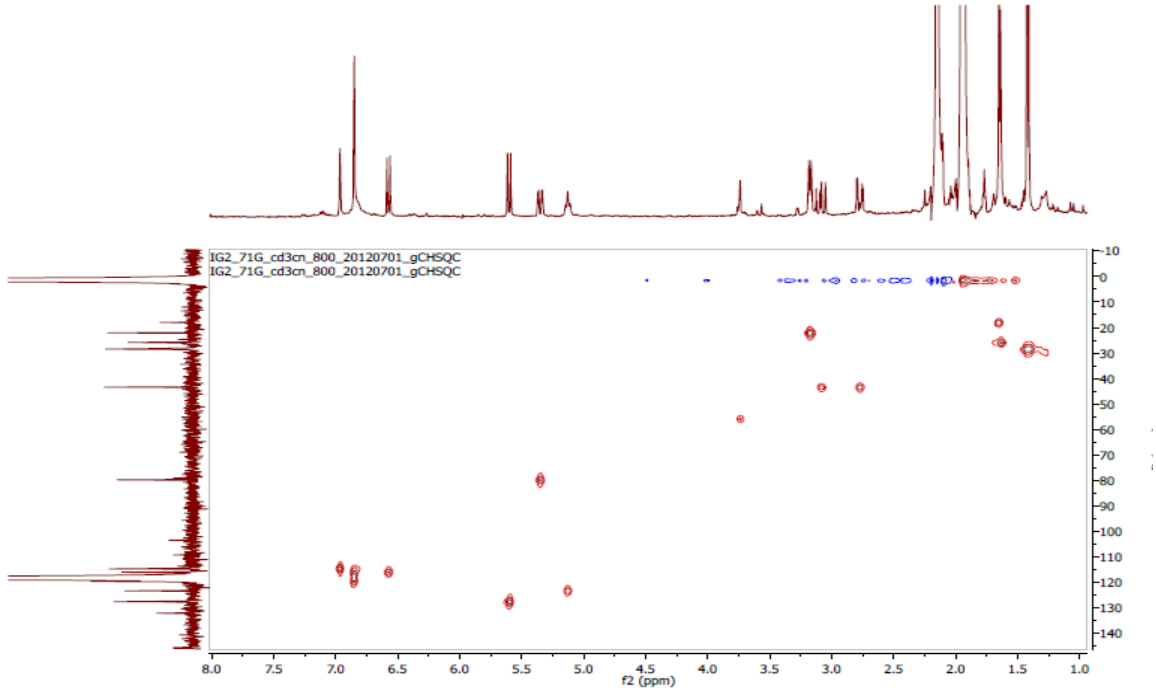
Appendix 37B: ^{13}C (CD_3CN, 150 MHz) NMR spectrum of 5,3',4'-trihydroxy-8-γ,γ-dimethylallyl-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**191**)



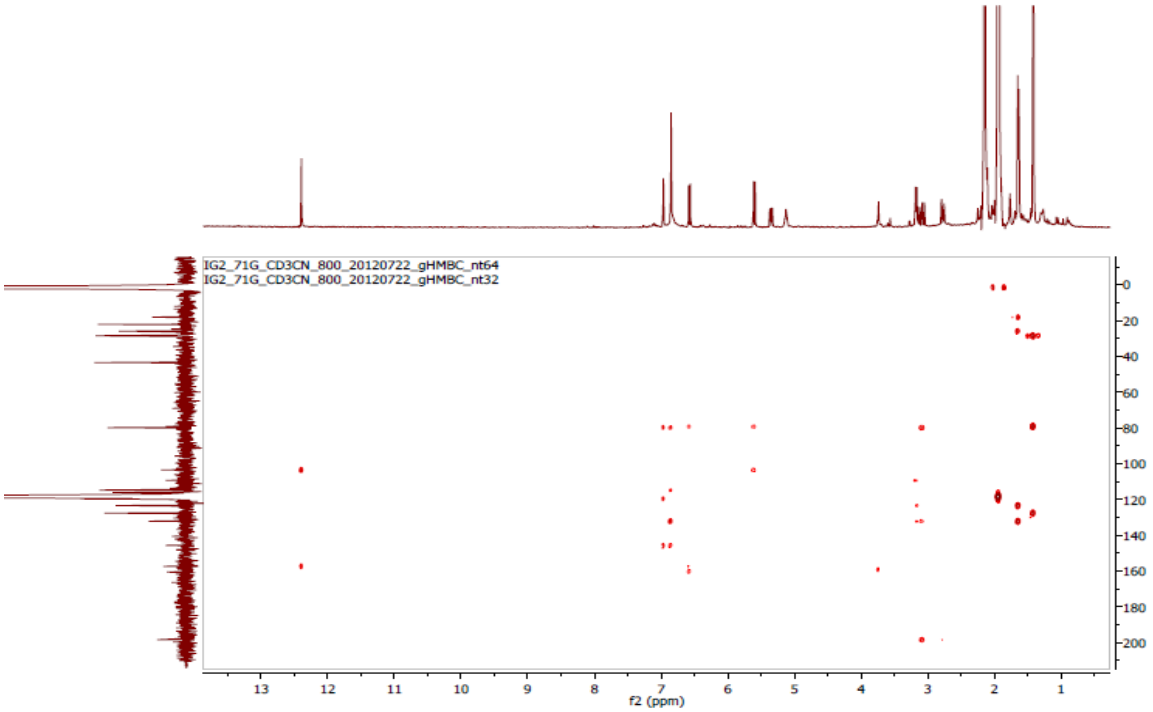
Appendix 37C: ^{1}H-^{1}H gCOSY (CD_3CN, 800 MHz) spectrum of 5,3',4'-trihydroxy-8-γ,γ-dimethylallyl-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**191**)



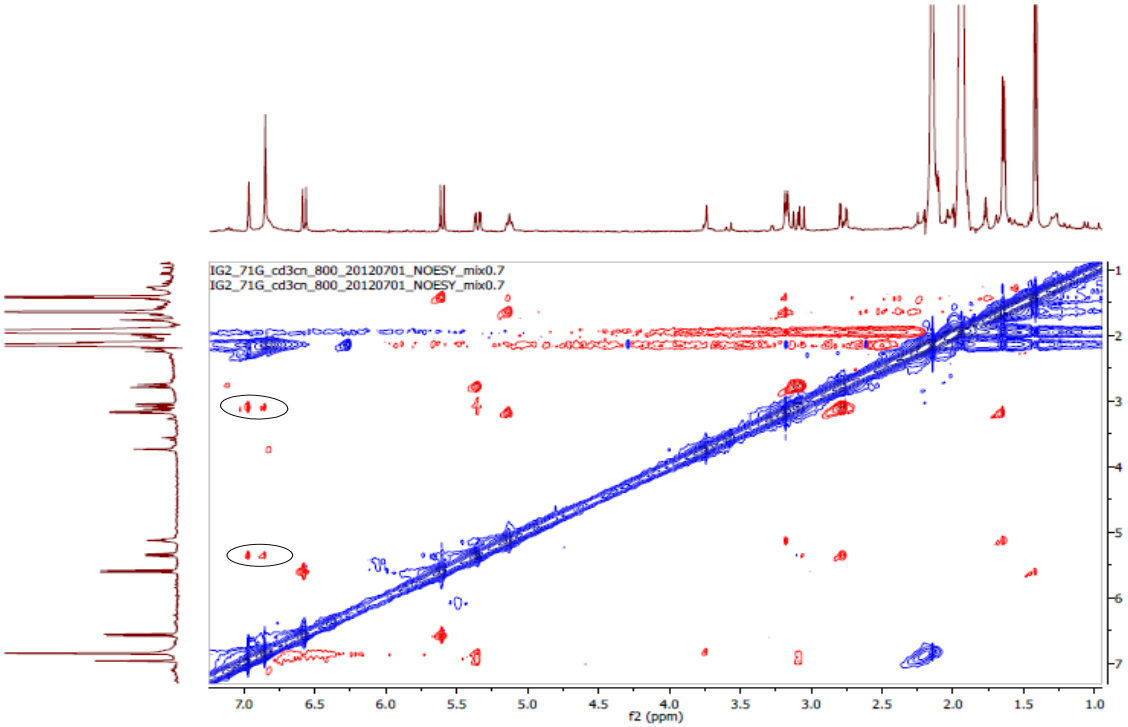
Appendix 37D: gCHSQC (CD_3CN, 800 MHz) spectrum of 5,3',4'-trihydroxy-8-γ,γ-dimethylallyl-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**191**)



Appendix 37E: gHMBC (CD_3CN, 800 MHz) spectrum of 5,3',4'-trihydroxy-8-γ,γ-dimethylallyl-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**191**)

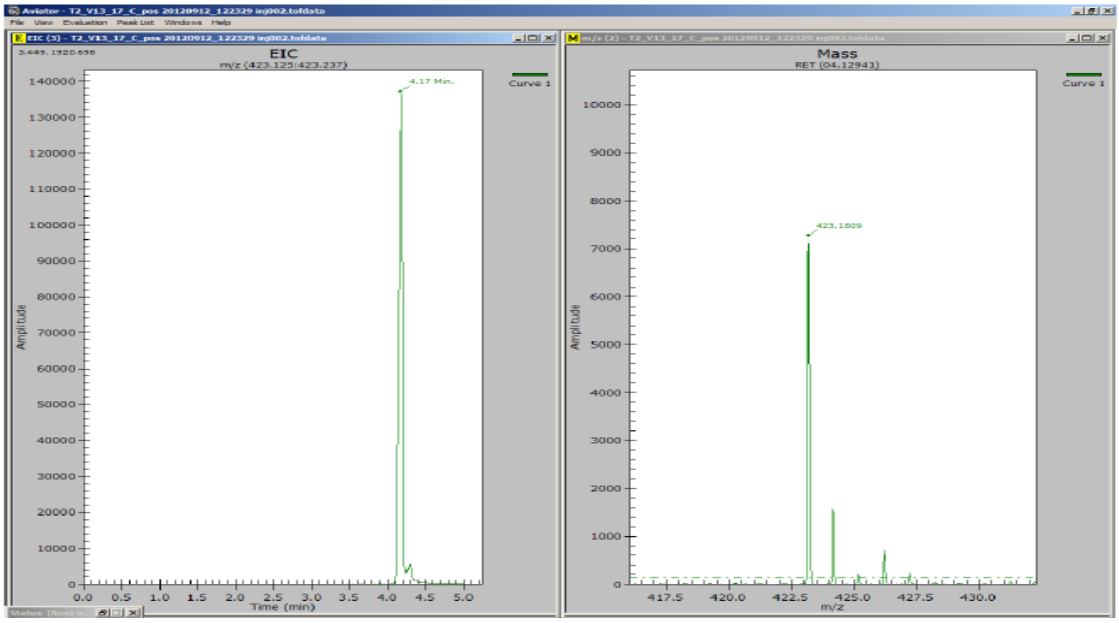


Appendix 37F: gNOESY (CD_3CN, 800 MHz) spectrum of 5,3',4'-trihydroxy-8-γ,γ-dimethylallyl-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**191**)

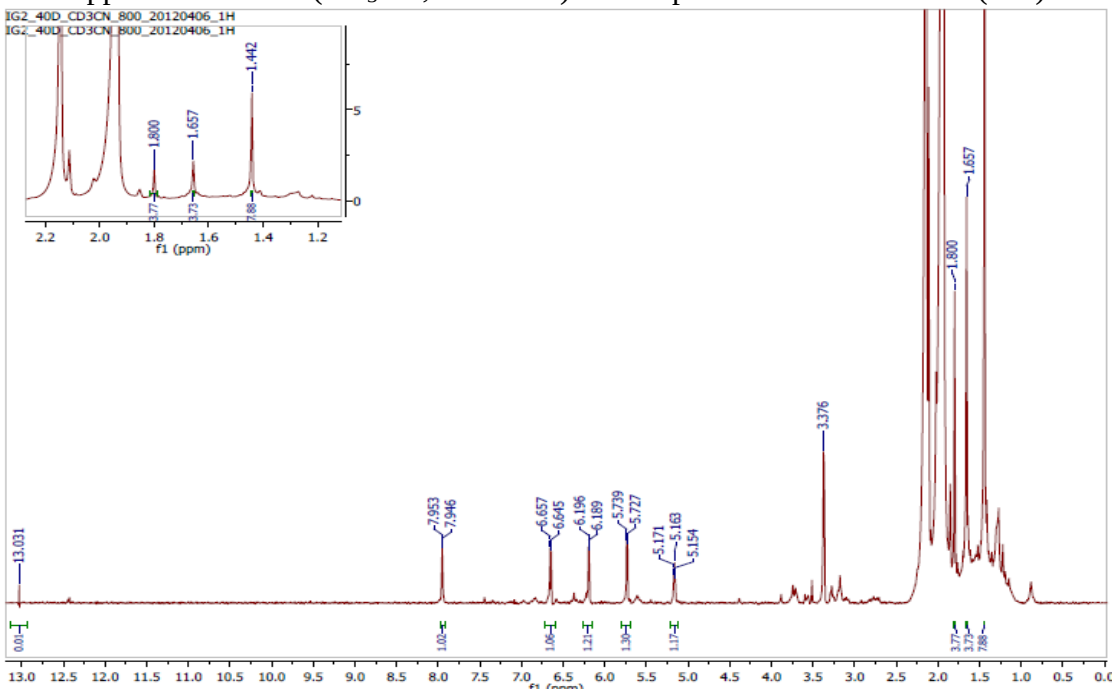


Appendix 37G: HRESIMS of 5,3',4'-trihydroxy-8-γ,γ-dimethylallyl-2",2"-dimethylpyrano-[5",6":6,7]-flavanone (**191**)

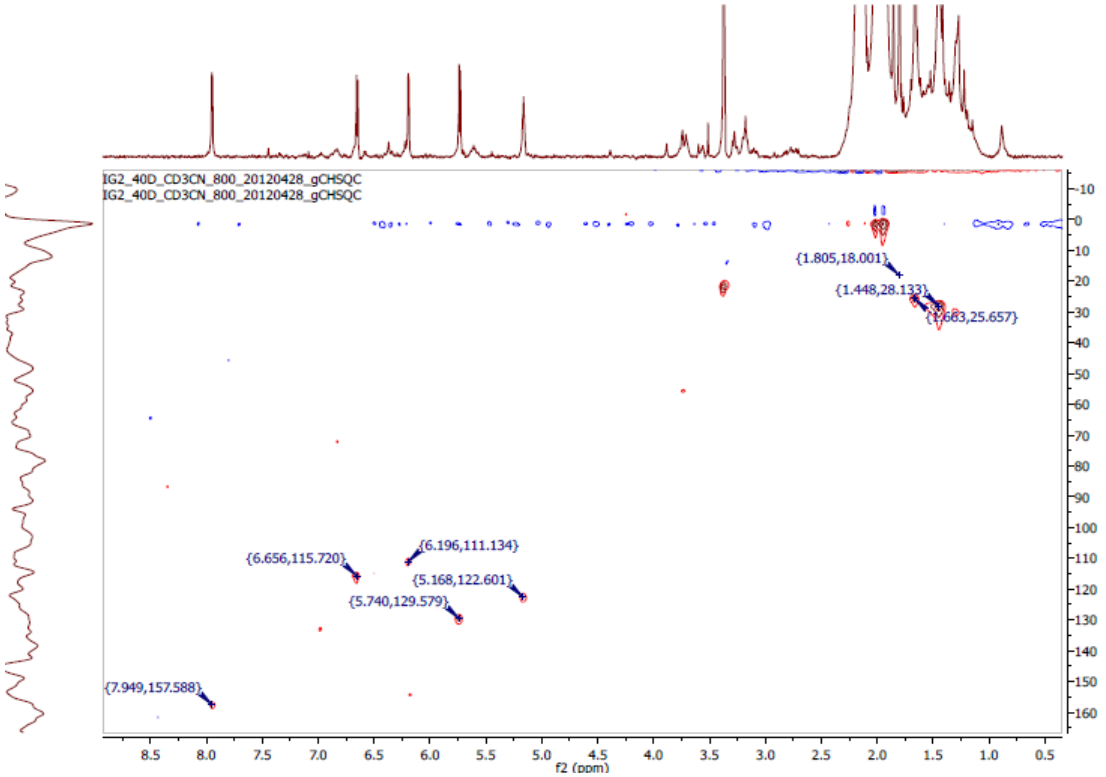
IG2-71G-3 M+H 423.1808



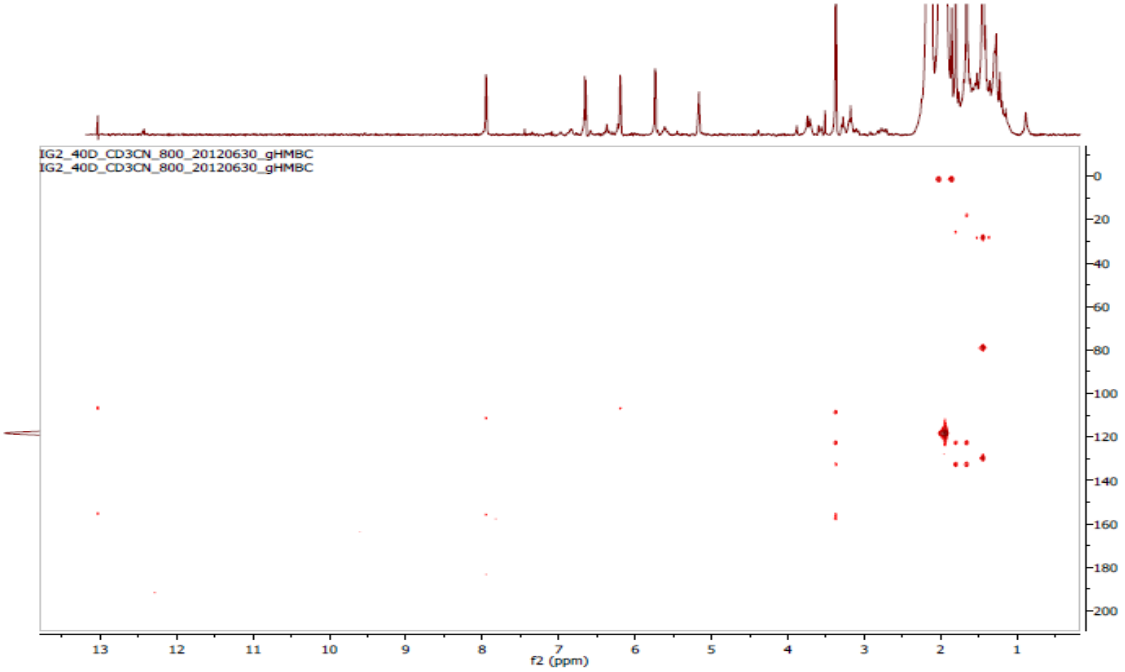
Appendix 38A: ^{1}H (CD_3CN, 800 MHz) NMR spectrum of eriosematin (**192**)



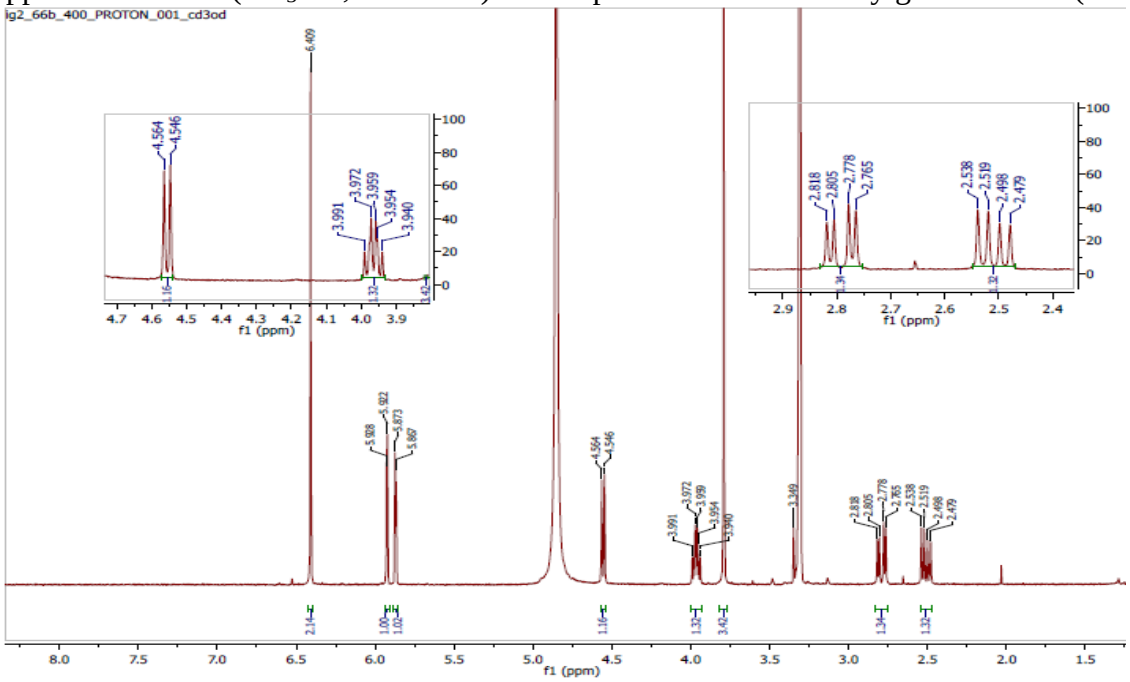
Appendix 38B: gCHSQC (CD_3CN, 800 MHz) spectrum of eriosematin (**192**)



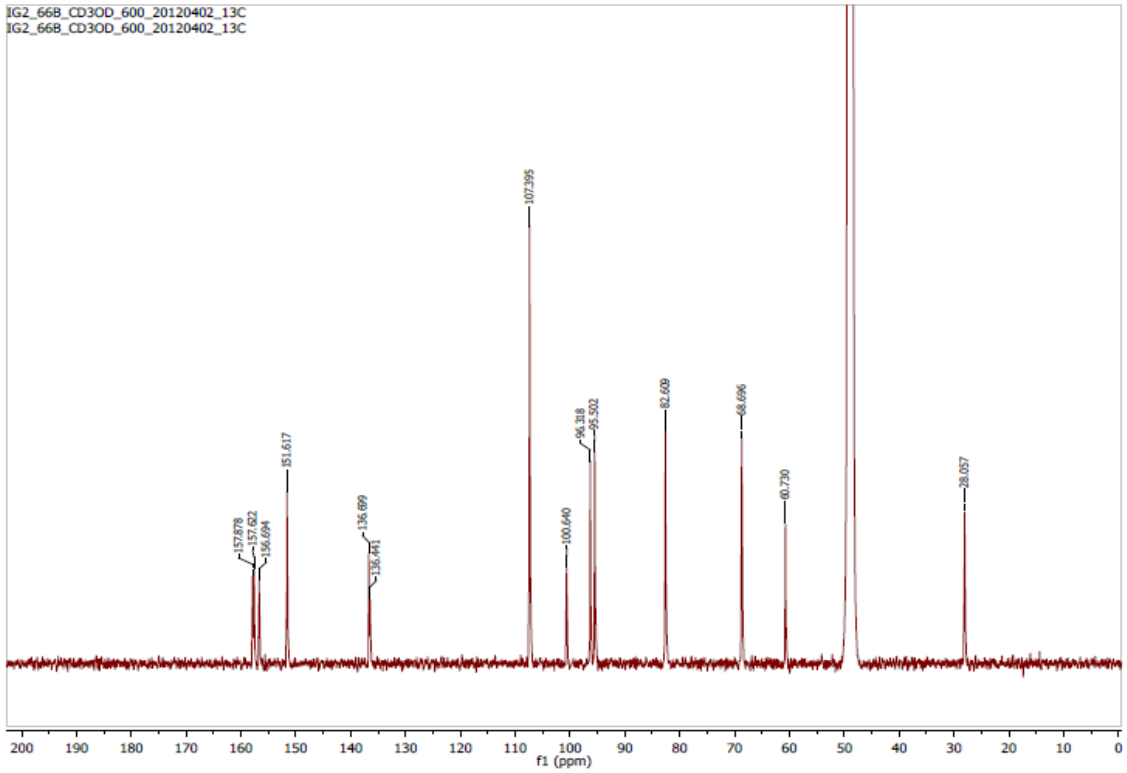
Appendix 38C: gHMBC (CD_3CN, 800 MHz) spectrum of eriosematin (**192**)



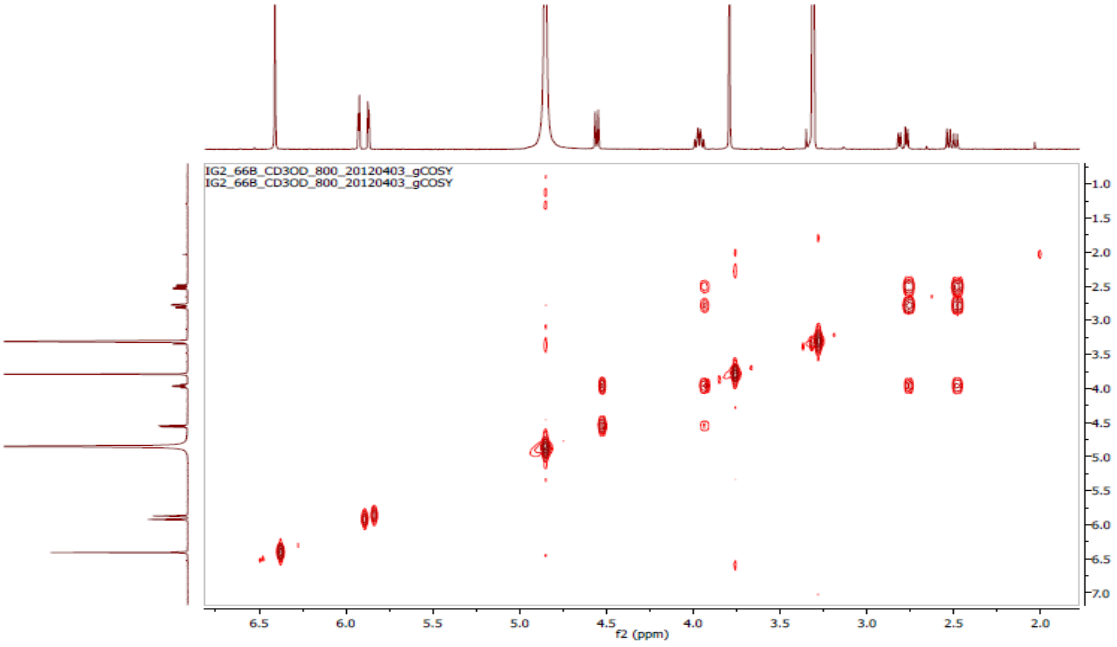
Appendix 39A: ^{1}H (CD_3OD, 400 MHz) NMR spectrum of 4'-*O*-methylgallocatechin (**193**)



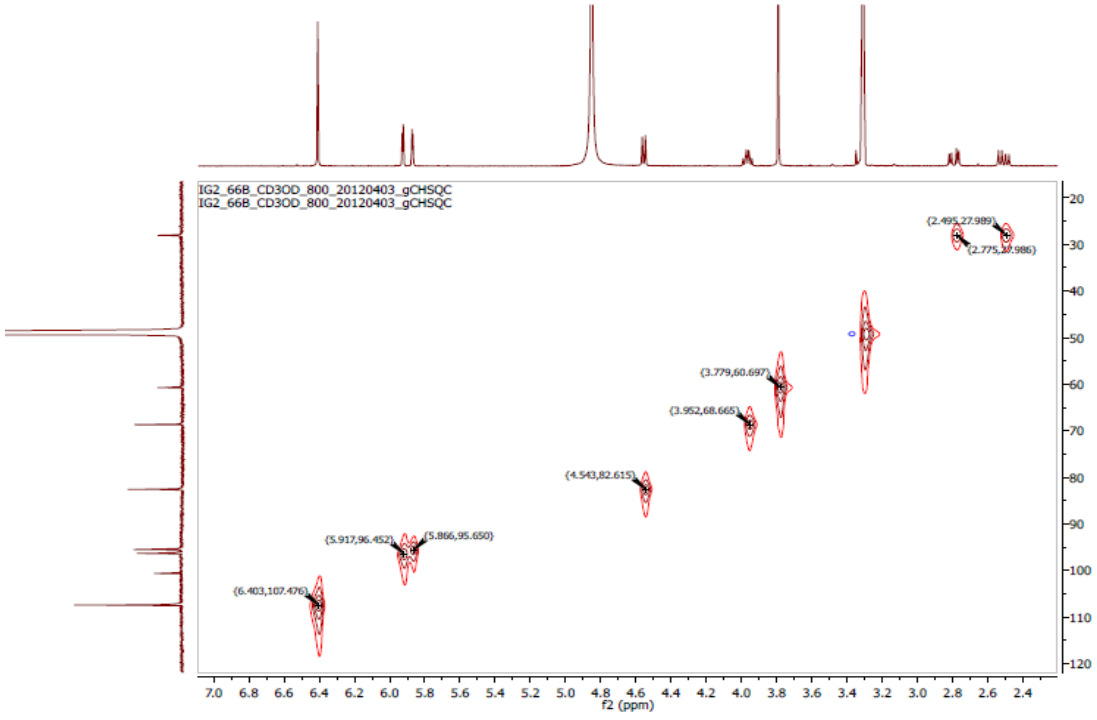
Appendix 39B: ^{13}C (CD_3OD, 150 MHz) NMR spectrum of 4'-*O*-methylgallocatechin (**193**)



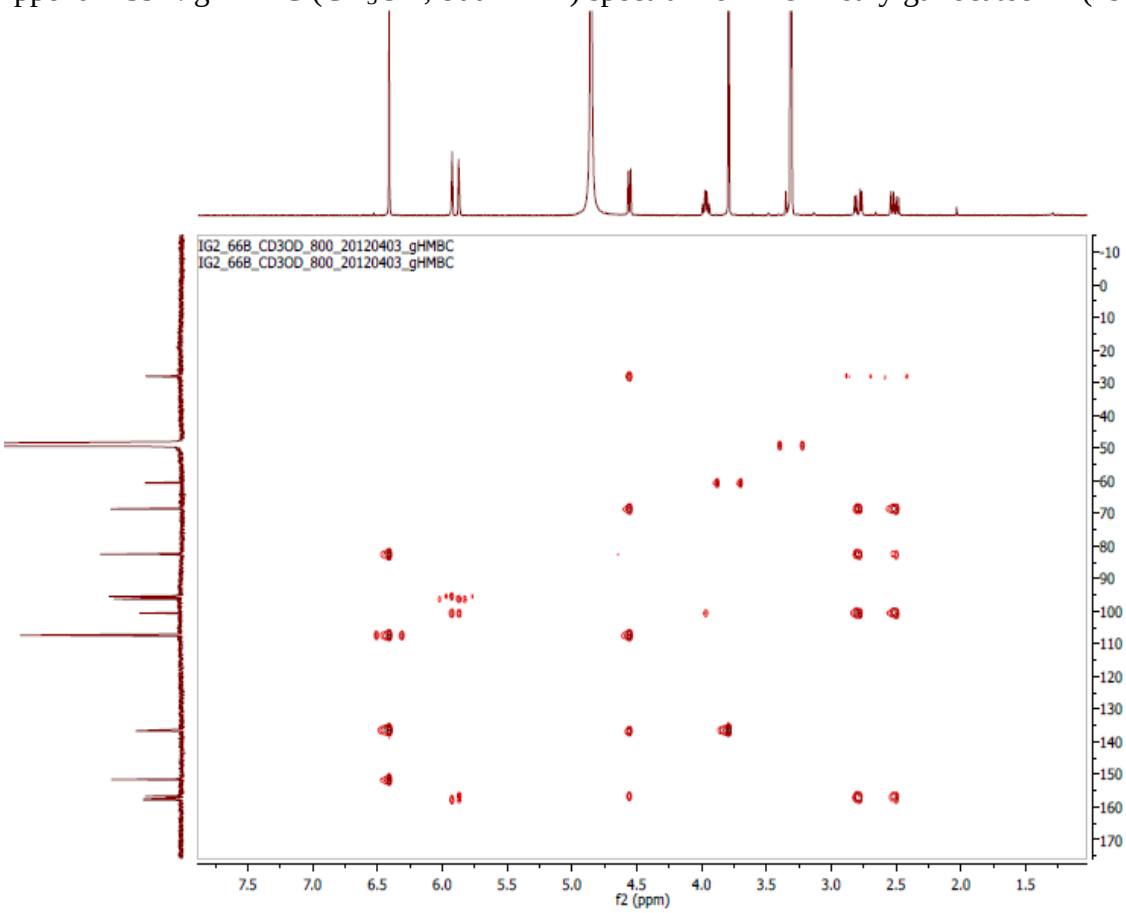
Appendix 39C: gCOSY (CD_3OD, 800 MHz) spectrum of 4'-*O*-methylgallocatechin (**193**)



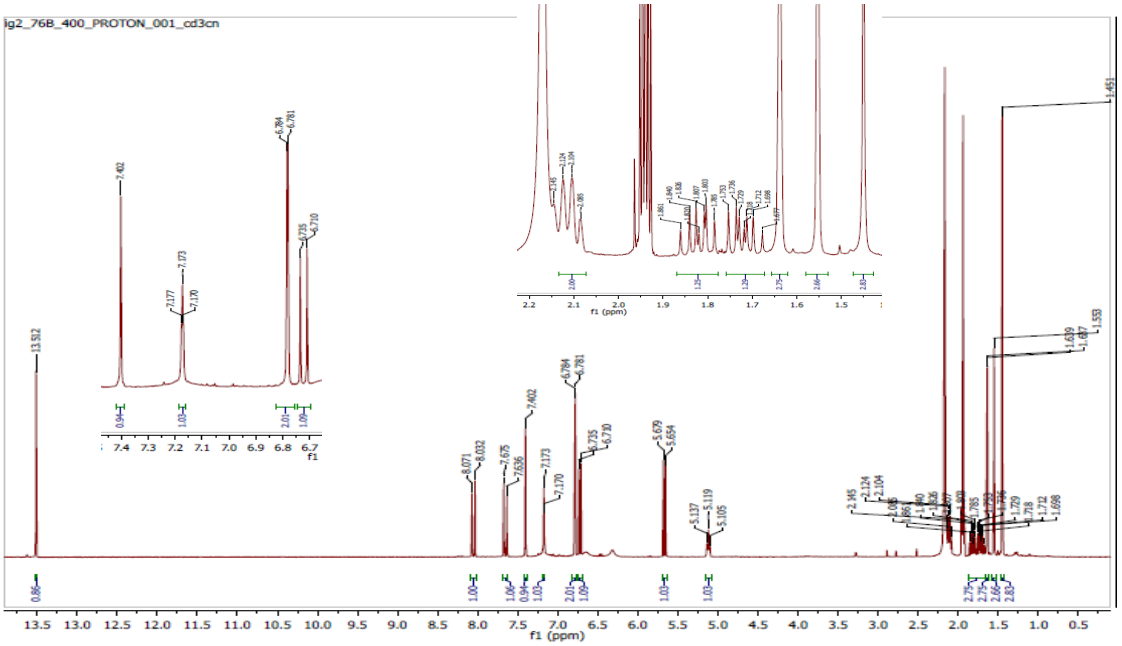
Appendix 39D: gCHSQC (CD_3OD, 800 MHz) NMR spectrum of 4'-*O*-methylgallocatechin (**193**)



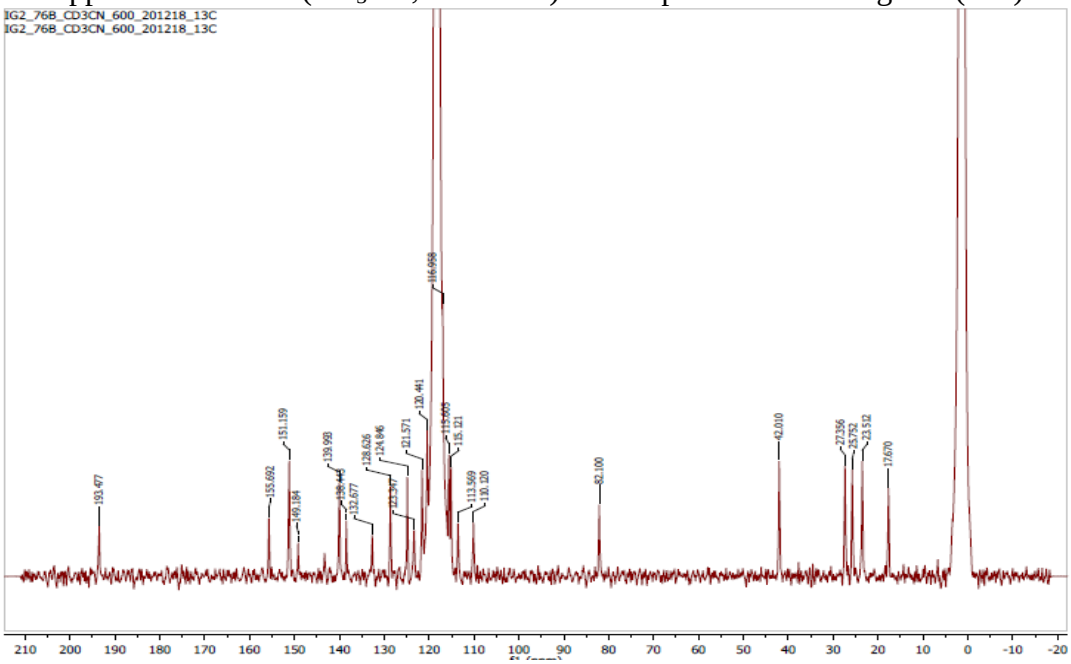
Appendix 39E: gHMBC (CD_3OD, 800 MHz) spectrum of 4'-*O*-methylgallocatechin (**193**)



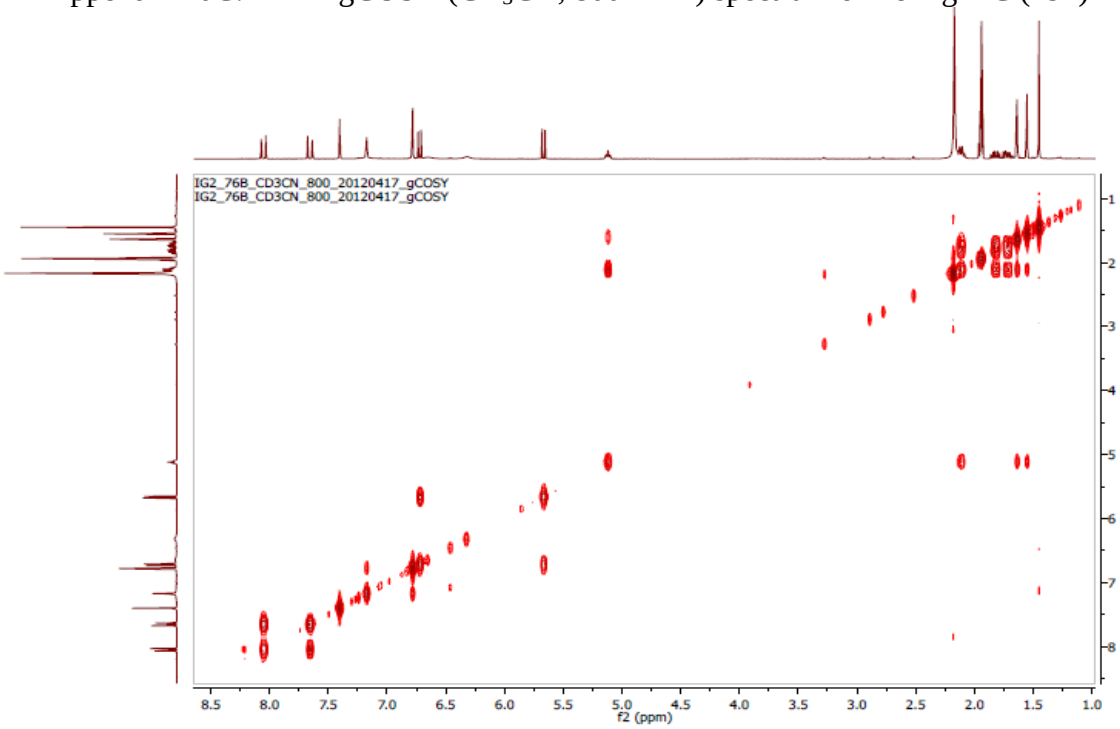
Appendix 40A: ^{1}H (CD_3CN, 400 MHz) NMR spectrum of flemigin C (**194**)



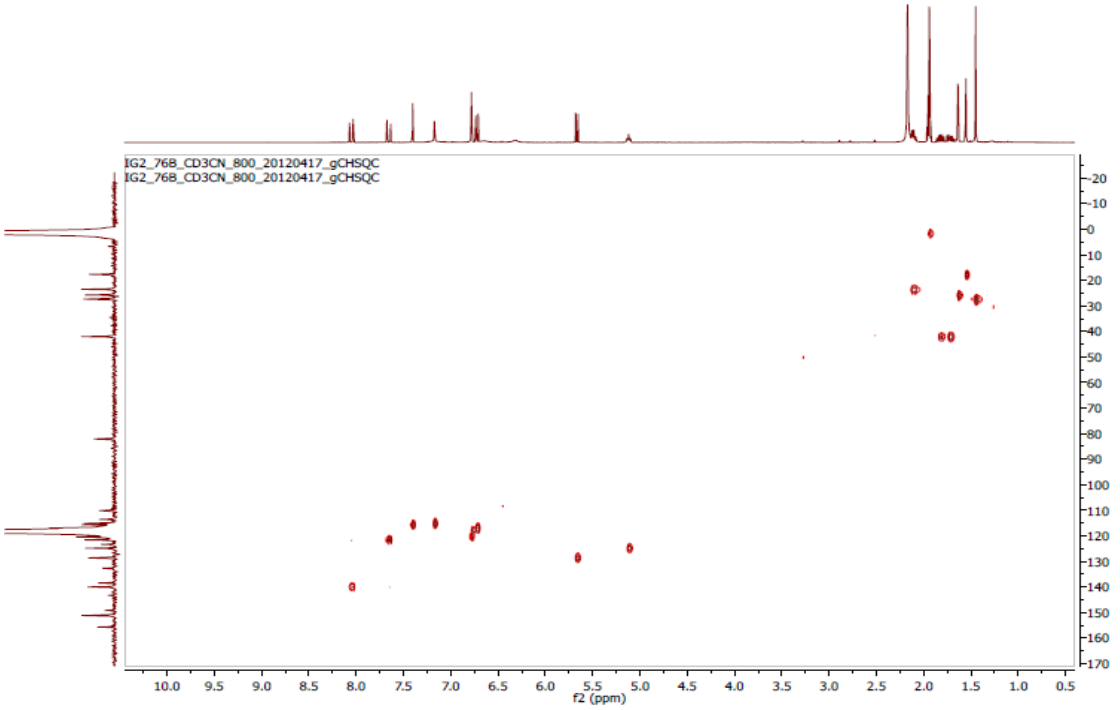
Appendix 40B: ^{13}C (CD_3CN, 150 MHz) NMR spectrum of flemigin C (**194**)



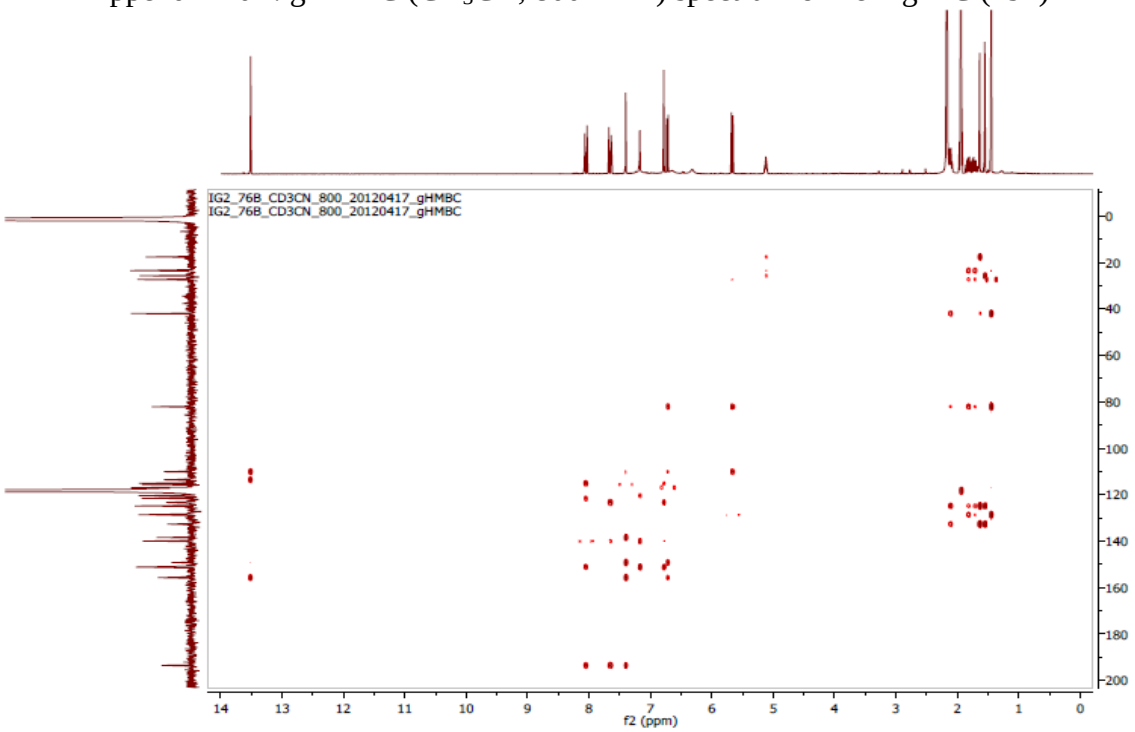
Appendix 40C: ^{1}H-^{1}H gCOSY (CD_3CN, 800 MHz) spectrum of flemigin C (**194**)



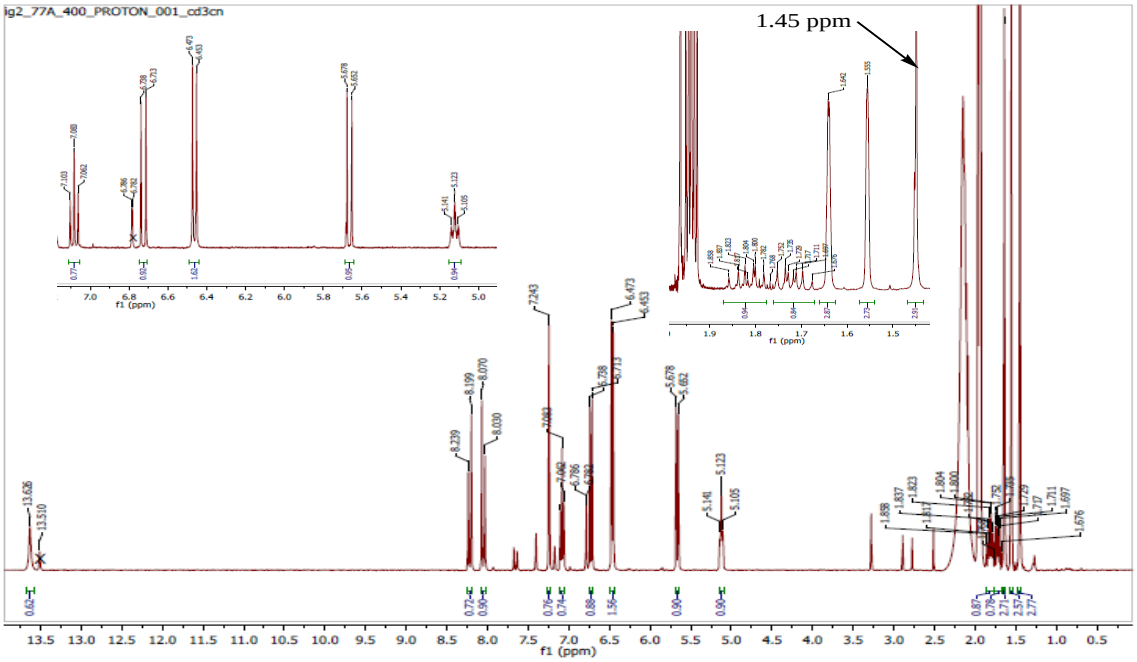
Appendix 40D: gCHSQC (CD_3CN, 800 MHz) spectrum of flemigin C (**194**)



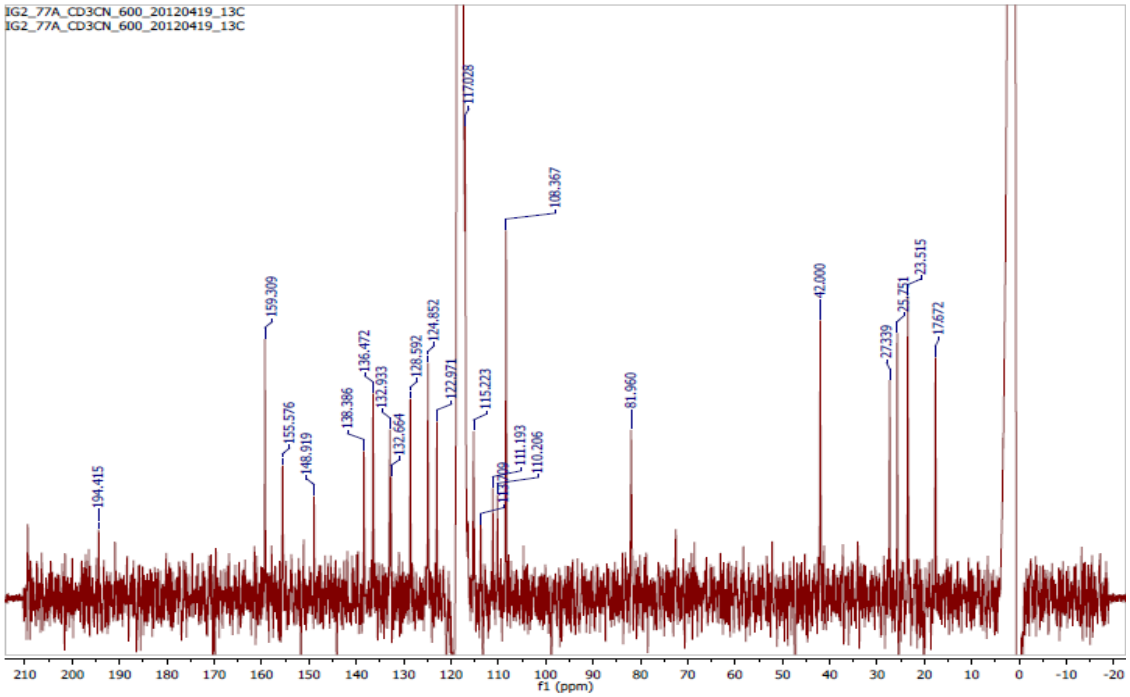
Appendix 40E: gHMBC (CD_3CN, 800 MHz) spectrum of flemigin C (**194**)



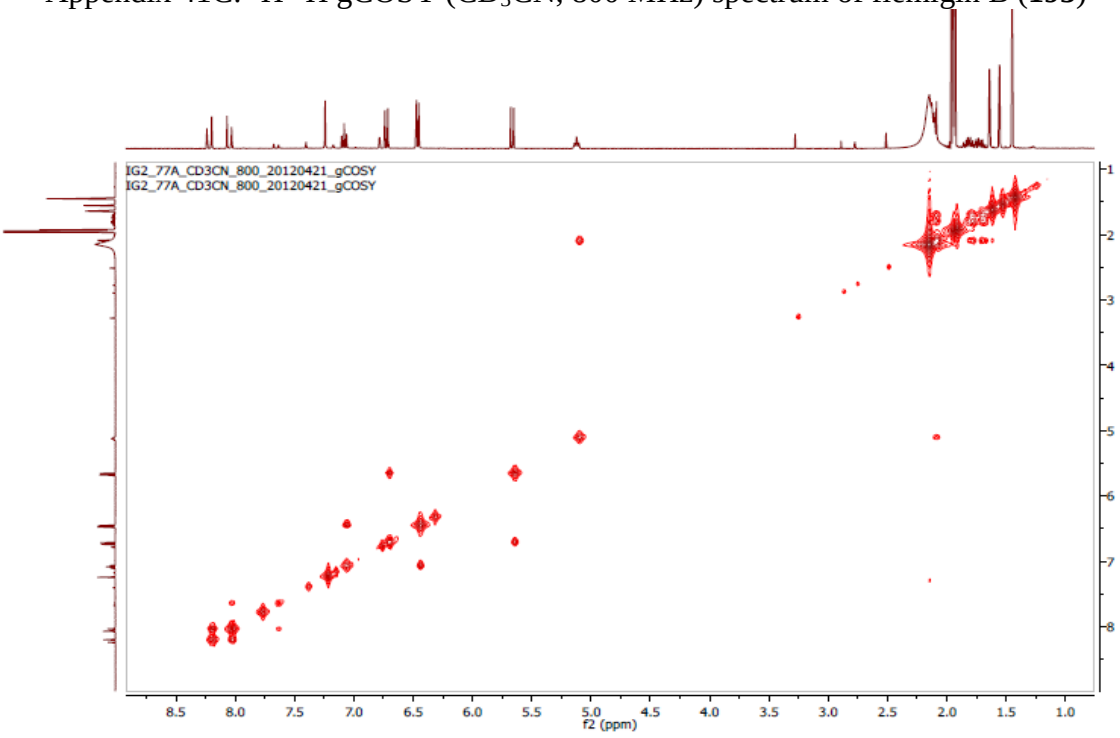
Appendix 41A: ^{1}H (CD_3CN, 400 MHz) NMR spectrum of flemigin B (**195**)



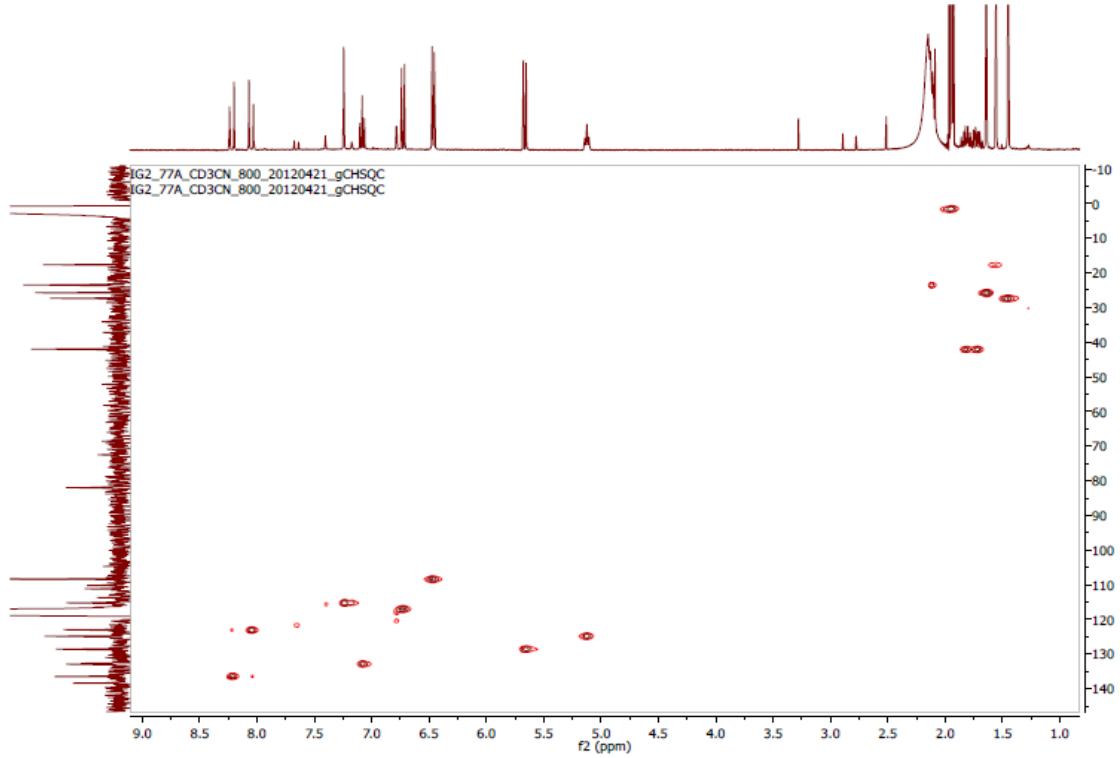
Appendix 41B: ^{13}C (CD_3CN, 150 MHz) NMR spectrum of flemigin B (**195**)



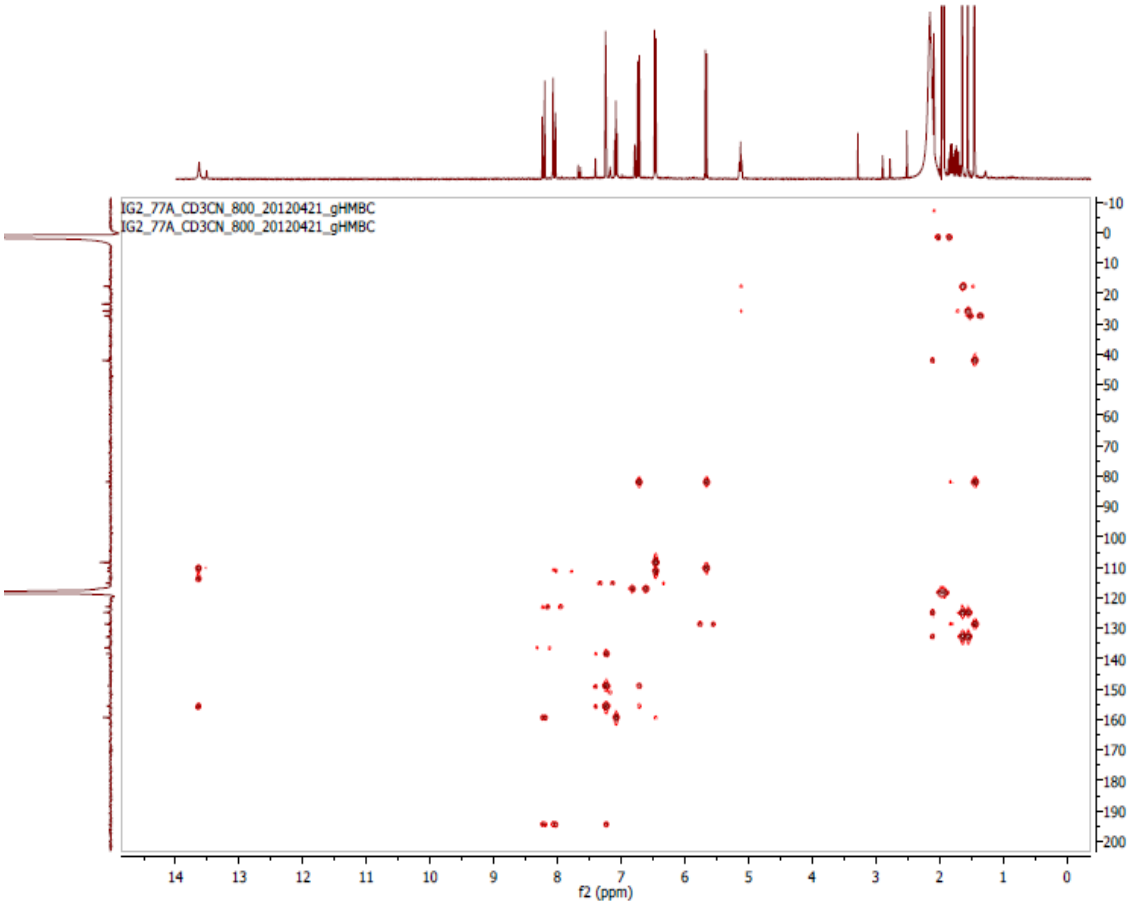
Appendix 41C: ^{1}H-^{1}H gCOSY (CD_3CN, 800 MHz) spectrum of flemigin B (**195**)



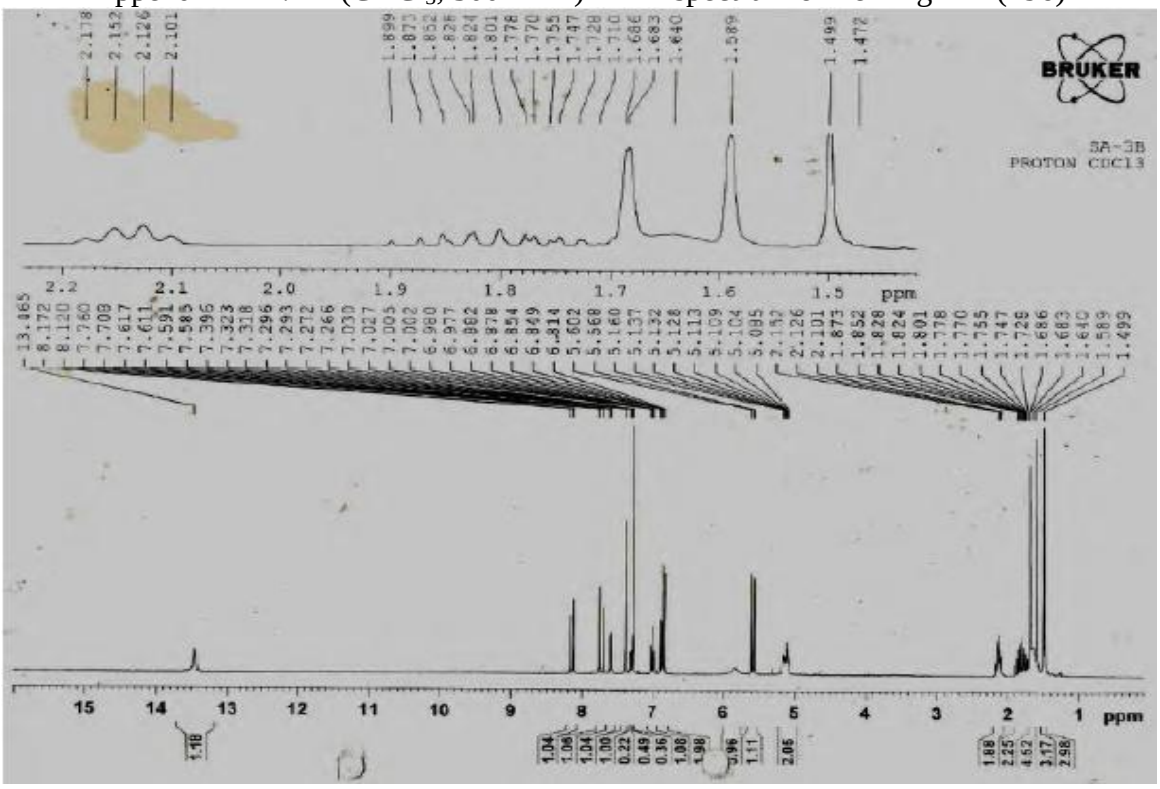
Appendix 41D: gCHSQC (CD_3CN, 800 MHz) spectrum of flemigin B (**195**)



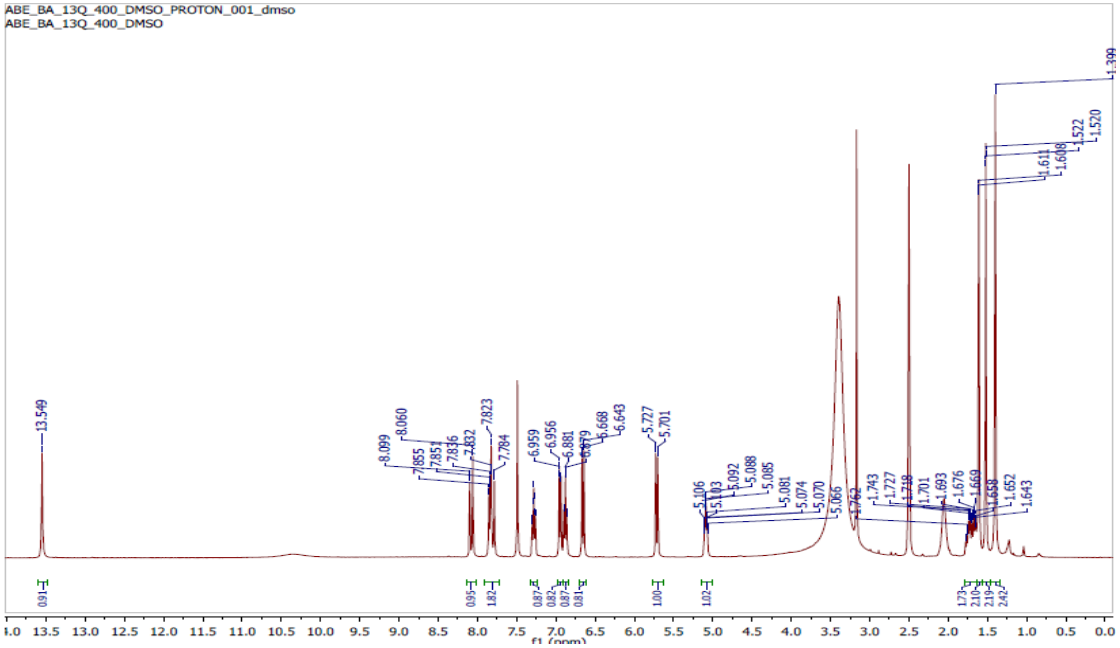
Appendix 41E: gHMBC (CD_3CN, 800 MHz) spectrum of flemigin B (**195**)



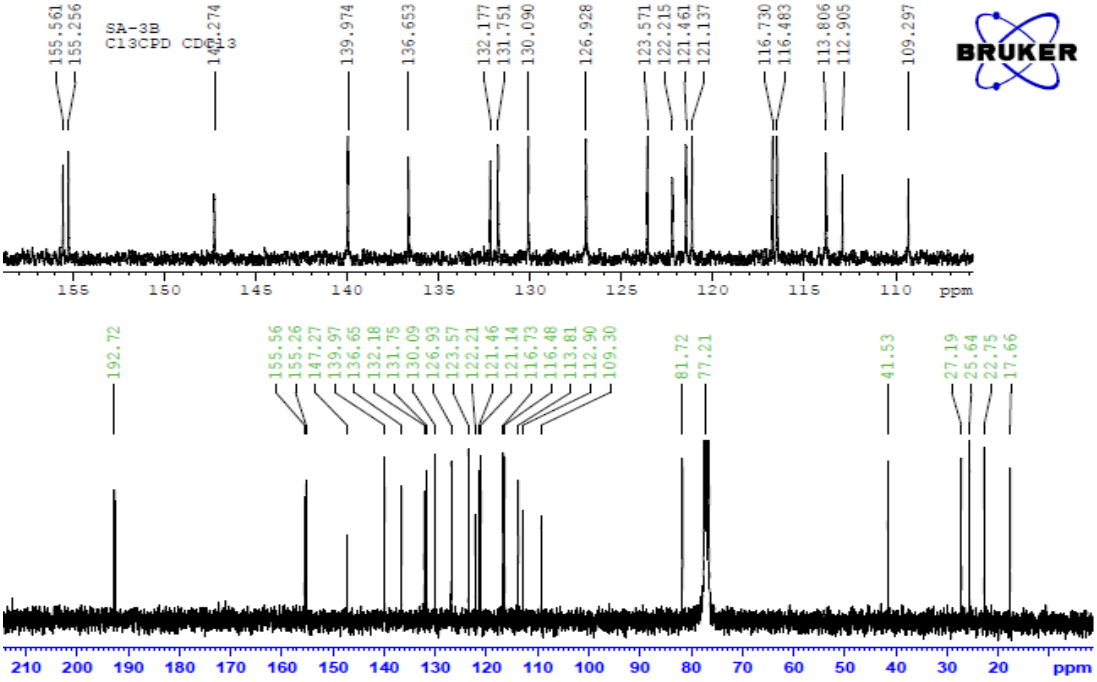
Appendix 42A: ^{1}H ($CDCl_3$, 300 MHz) NMR spectrum of flemingin A (**196**)



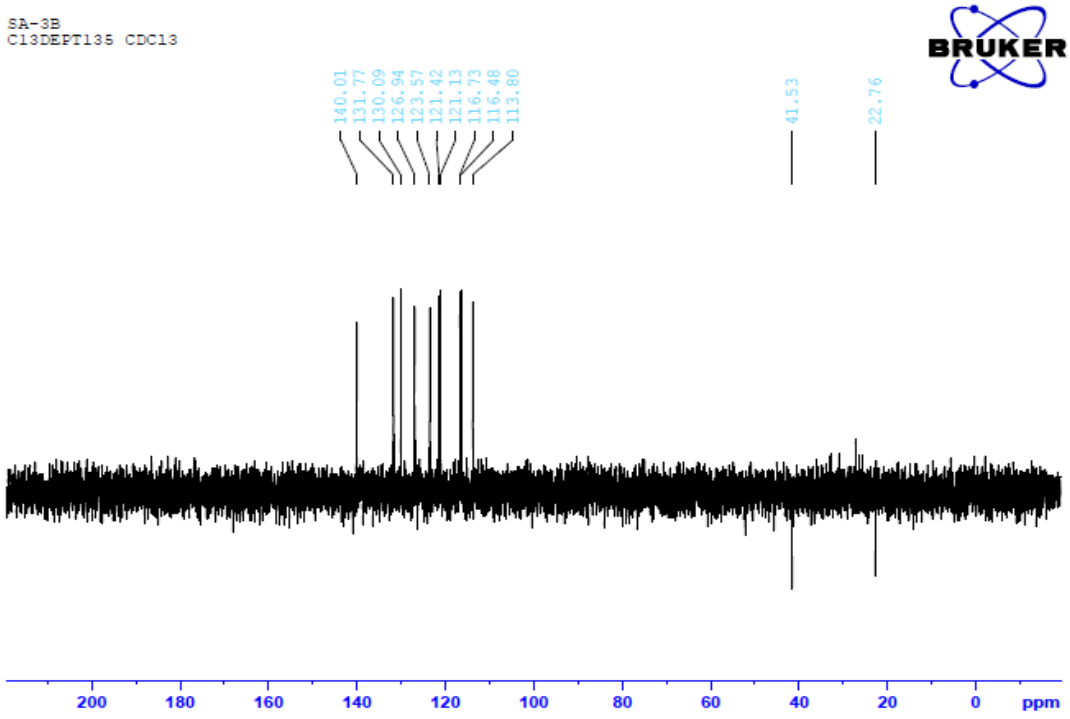
Appendix 42B: ^{1}H ($(CD_3)_2SO$, 400 MHz) NMR spectrum of flemingin A (**196**)



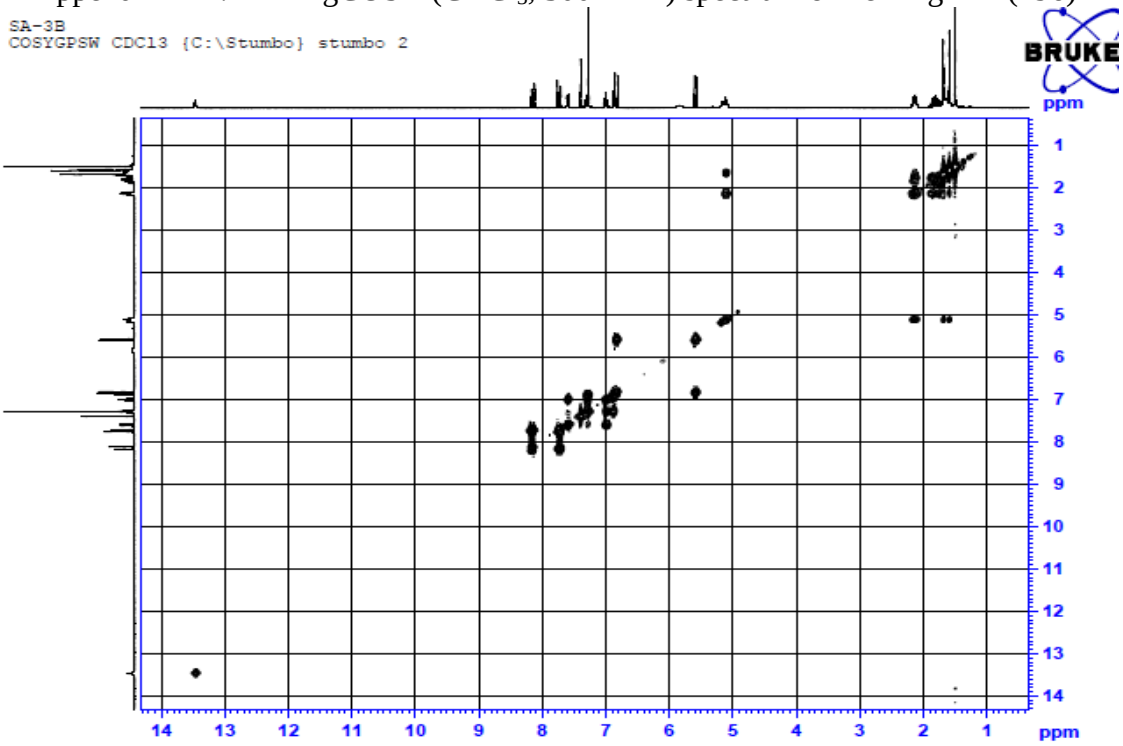
Appendix 42C: ^{13}C ($CDCl_3$, 75 MHz) NMR spectrum of flemingin A (**196**)



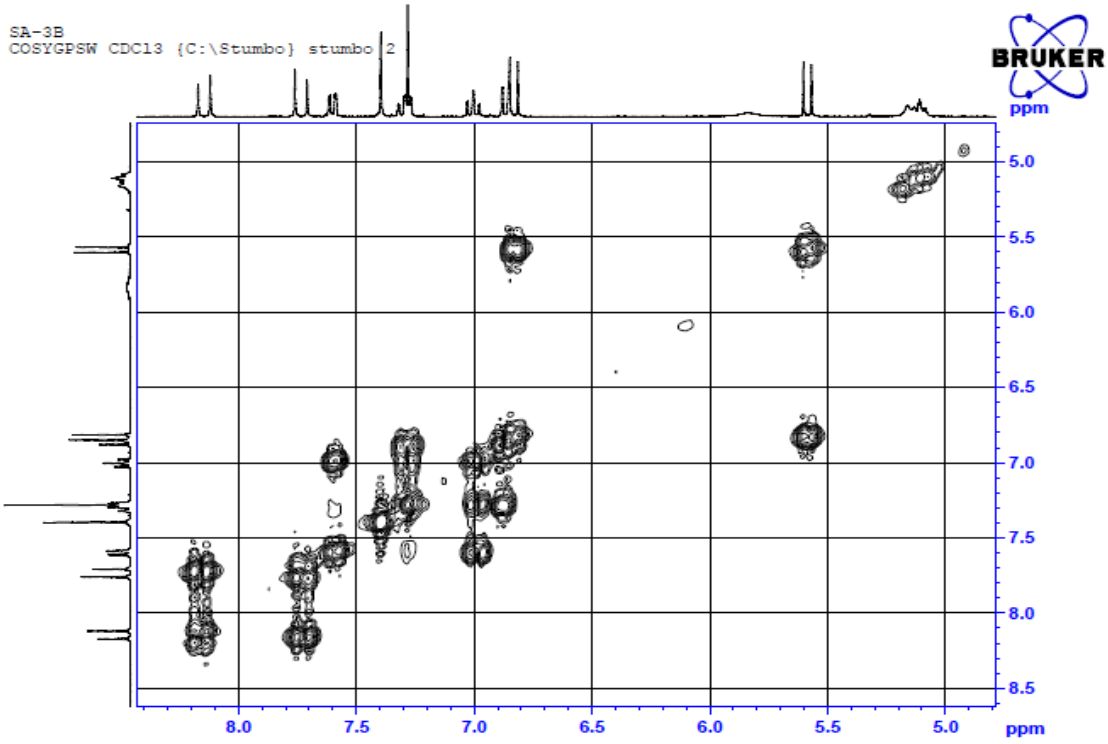
Appendix 42D: DEPT 135 ($CDCl_3$, 75 MHz) spectrum of flemingin A (**196**)



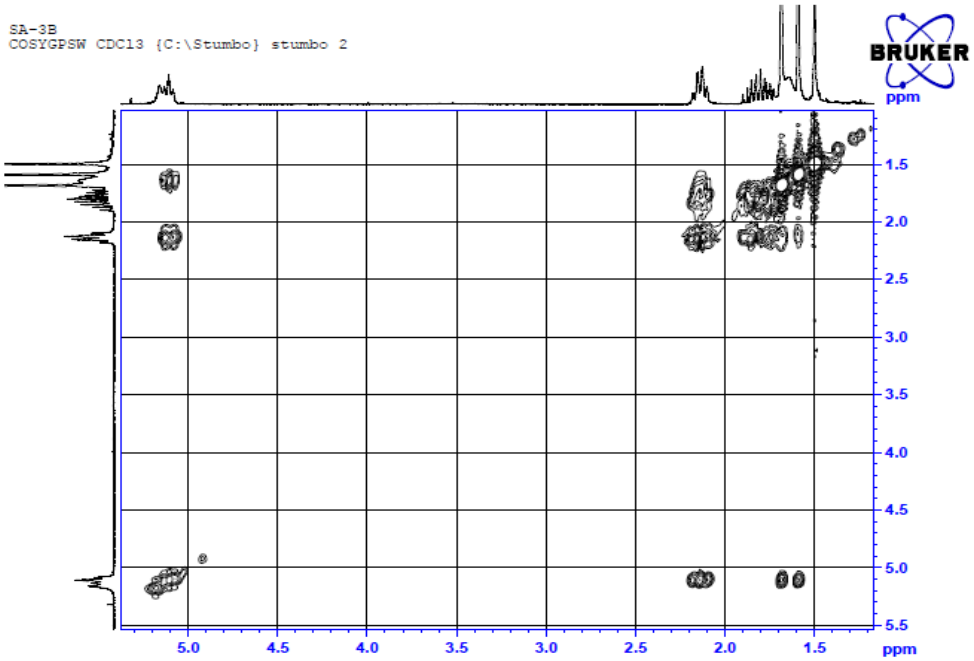
Appendix 42E: ^{1}H-^{1}H gCOSY ($CDCl_3$, 300 MHz) spectrum of flemingin A (**196**)



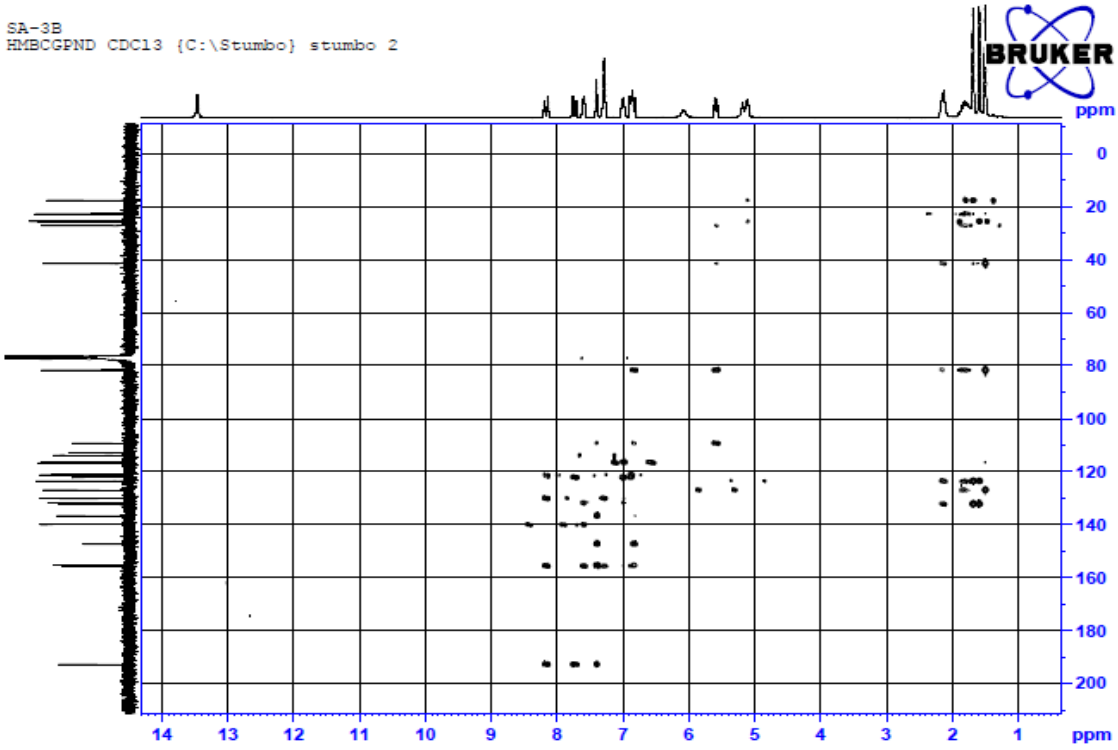
Appendix 42F: expanded gCOSY ($CDCl_3$, 300 MHz) spectrum of flemingin A (**196**)



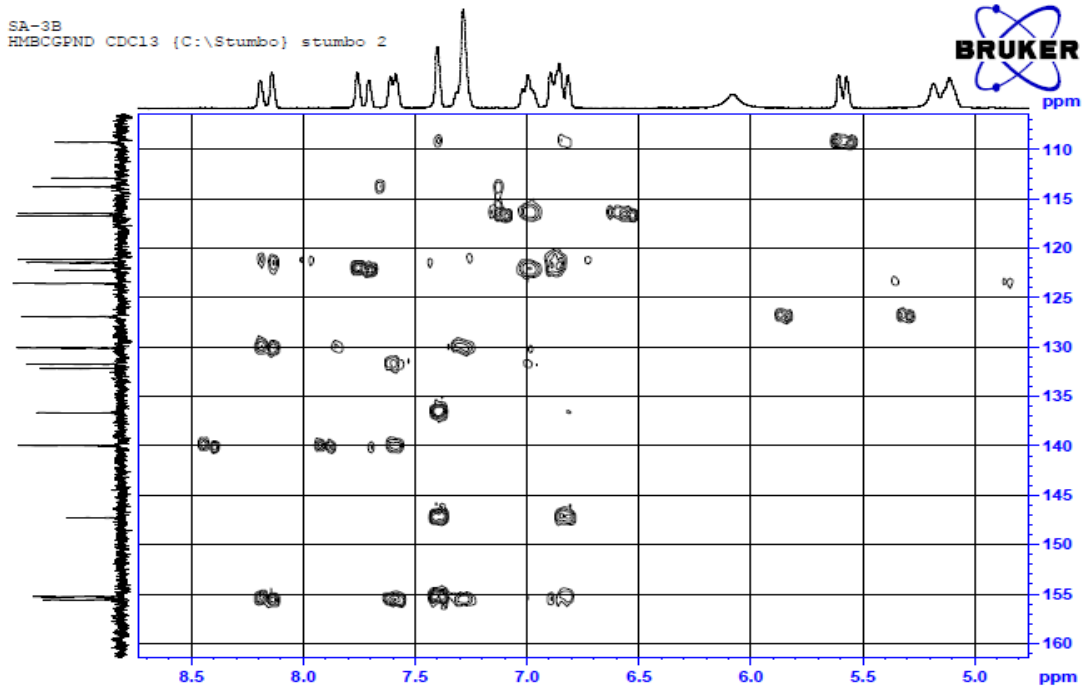
Appendix 42G: expanded gCOSY ($CDCl_3$, 300 MHz) spectrum of flemingin A (**196**)



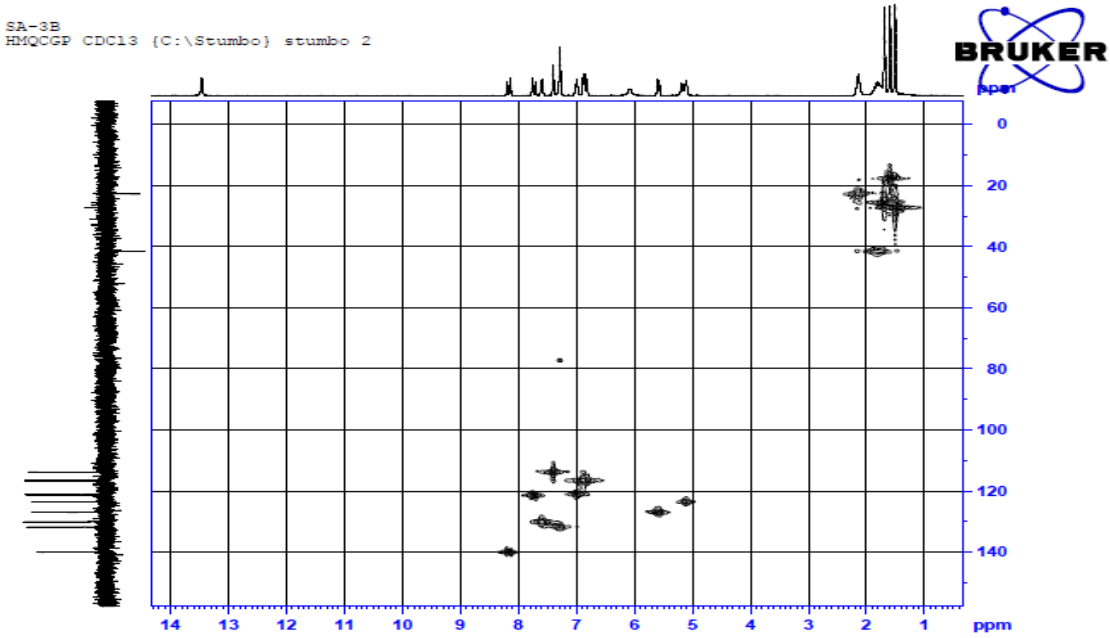
Appendix 42H: gHMBC ($CDCl_3$, 300 MHz)spectrum of flemingin A (**196**)



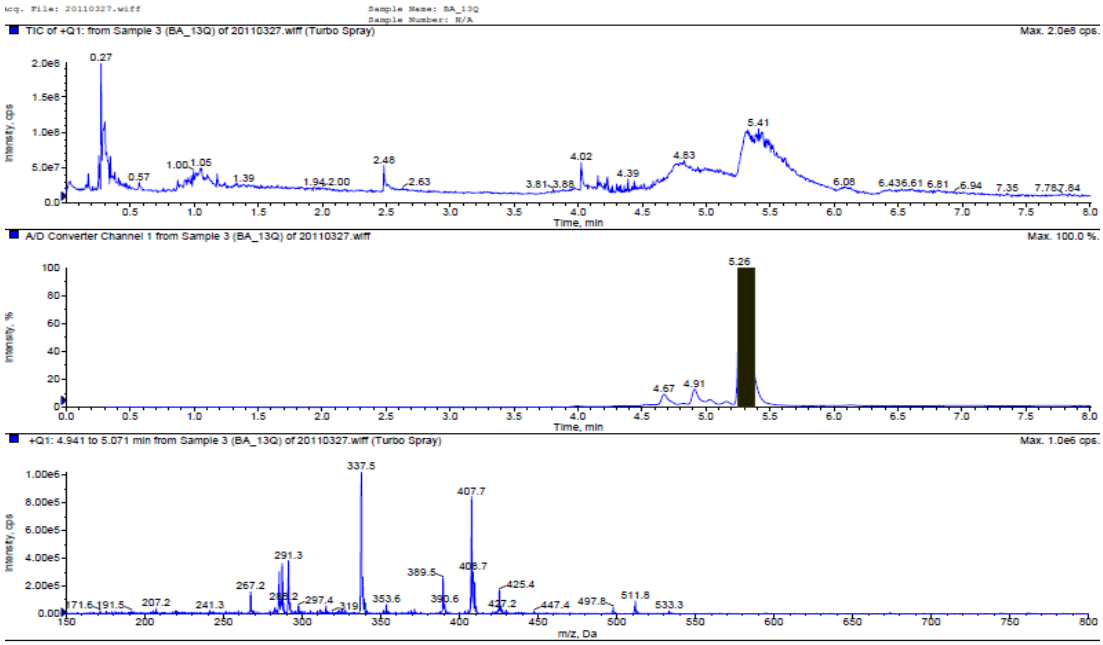
Appendix 42I: expanded gHMBC ($CDCl_3$, 300 MHz) spectrum of flemingin A (**196**)



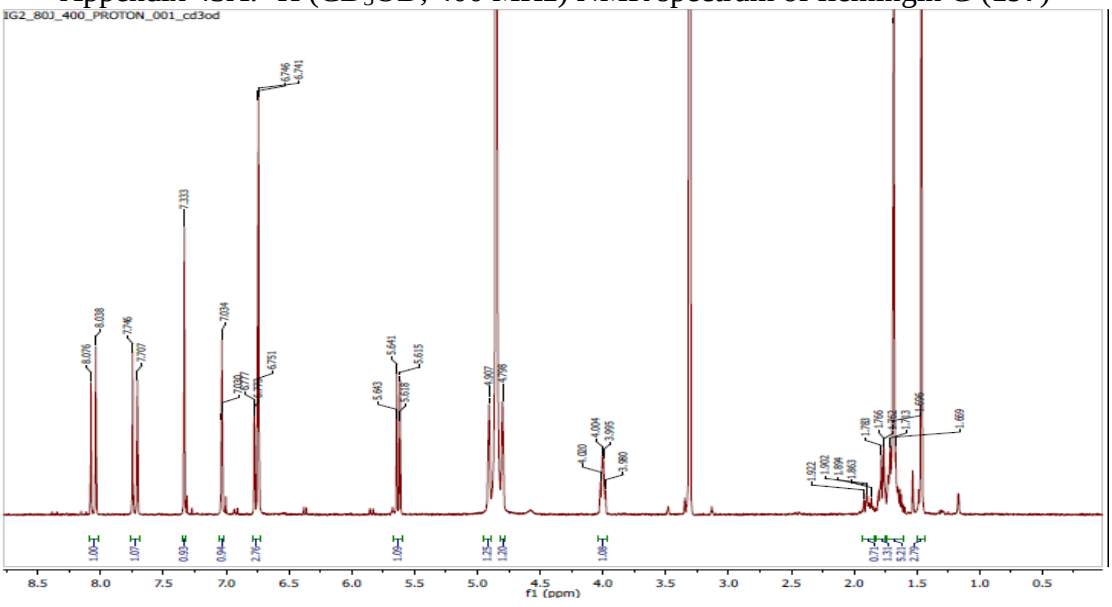
Appendix 42J: gHMQC ($CDCl_3$, 300 MHz) spectrum of flemingin A (**196**)



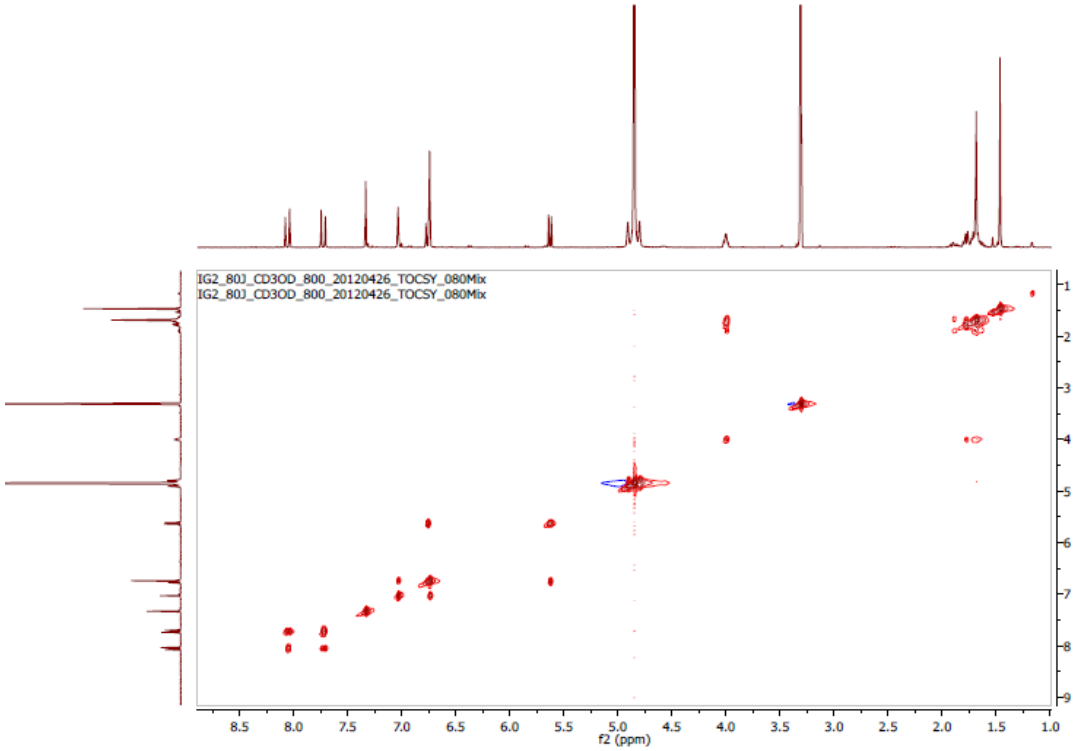
Appendix 42K: LC-ESI-MS of flemingin A (**196**)



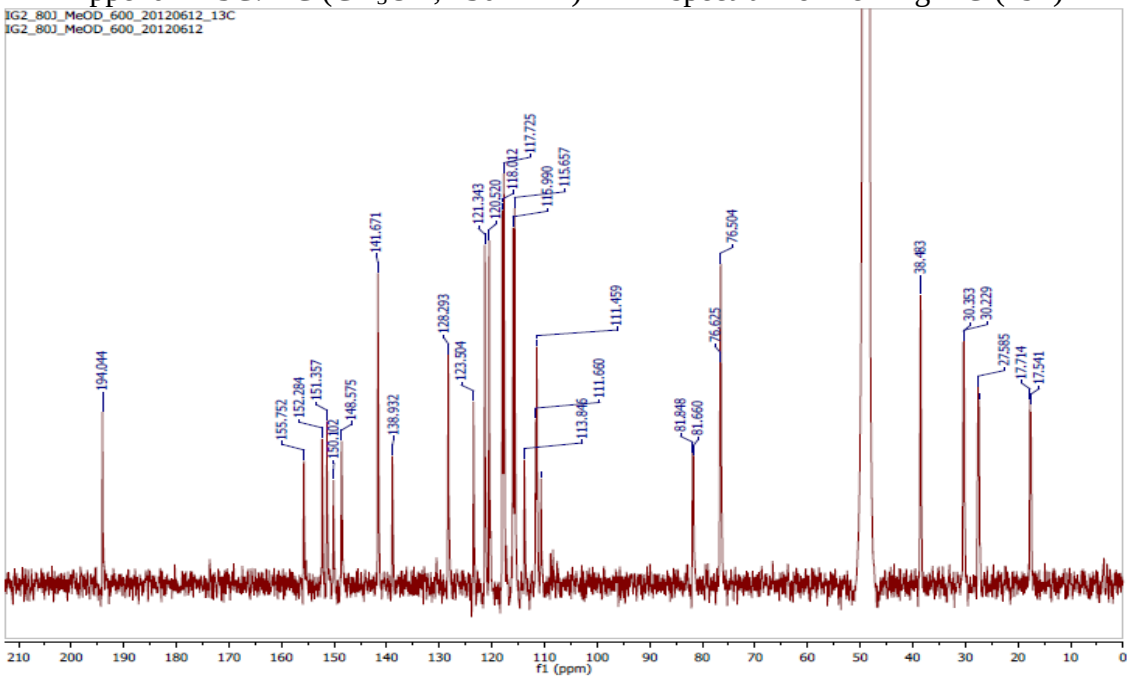
Appendix 43A: ^{1}H (CD_3OD, 400 MHz) NMR spectrum of flemingin G (**197**)



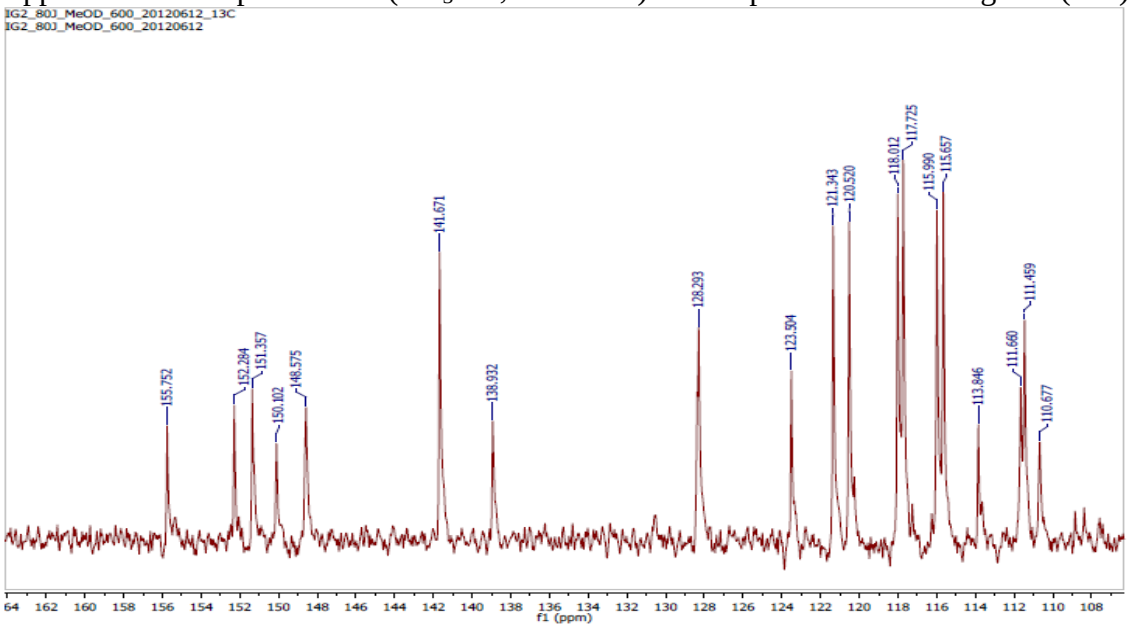
Appendix 43B: ^{1}H-^{1}H gCOSY (CD_3OD, 800 MHz) spectrum of flemingin G (**197**)



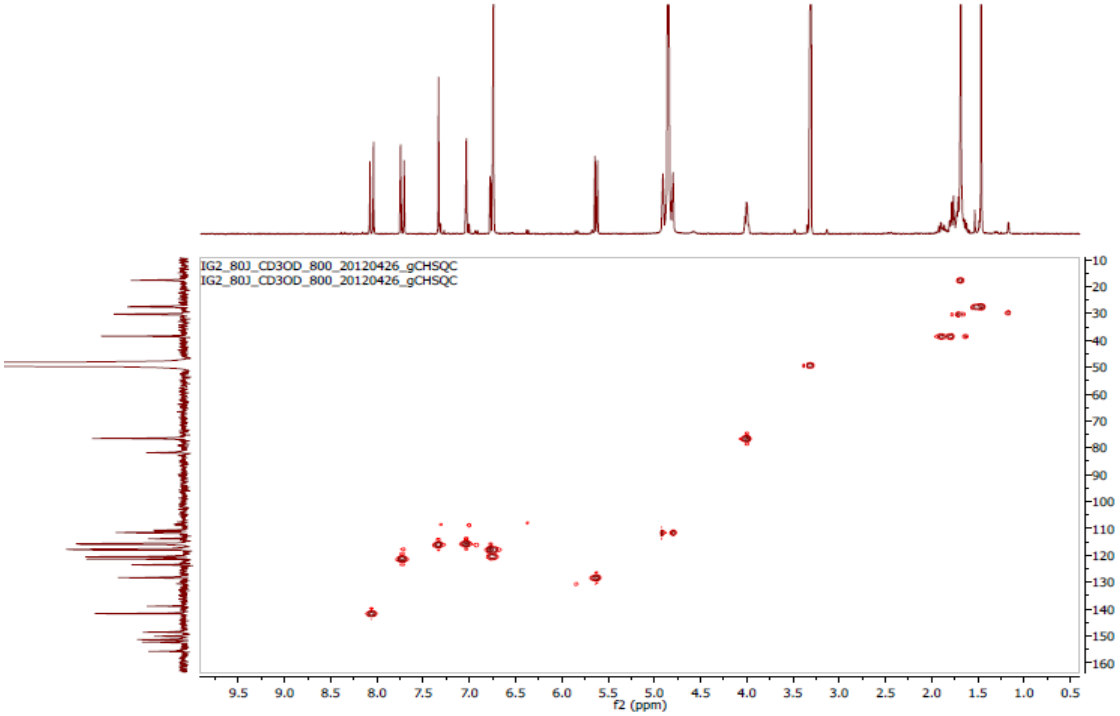
Appendix 43C: ^{13}C (CD_3OD, 150 MHz) NMR spectrum of flemingin G (**197**)



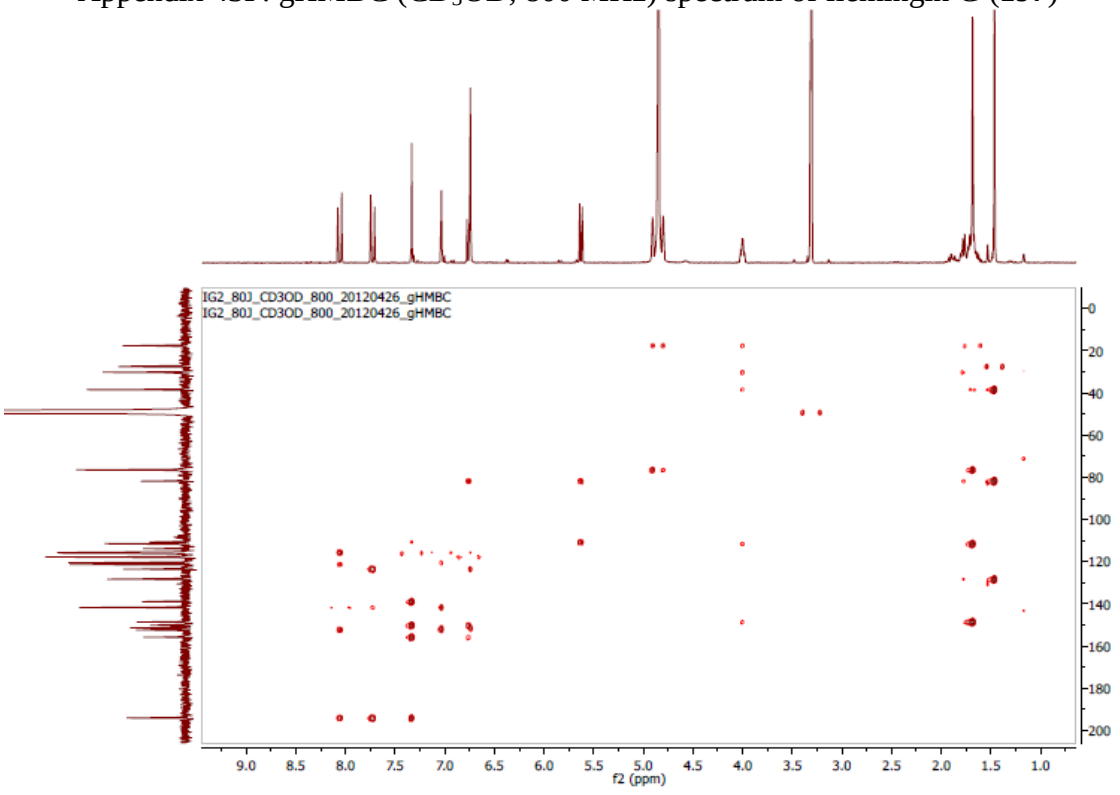
Appendix 43D: expanded ^{13}C (CD_3OD, 150 MHz) NMR spectrum of flemingin G (**197**)



Appendix 43E: gCHSQC (CD_3OD, 800 MHz) spectrum of flemingin G (**197**)

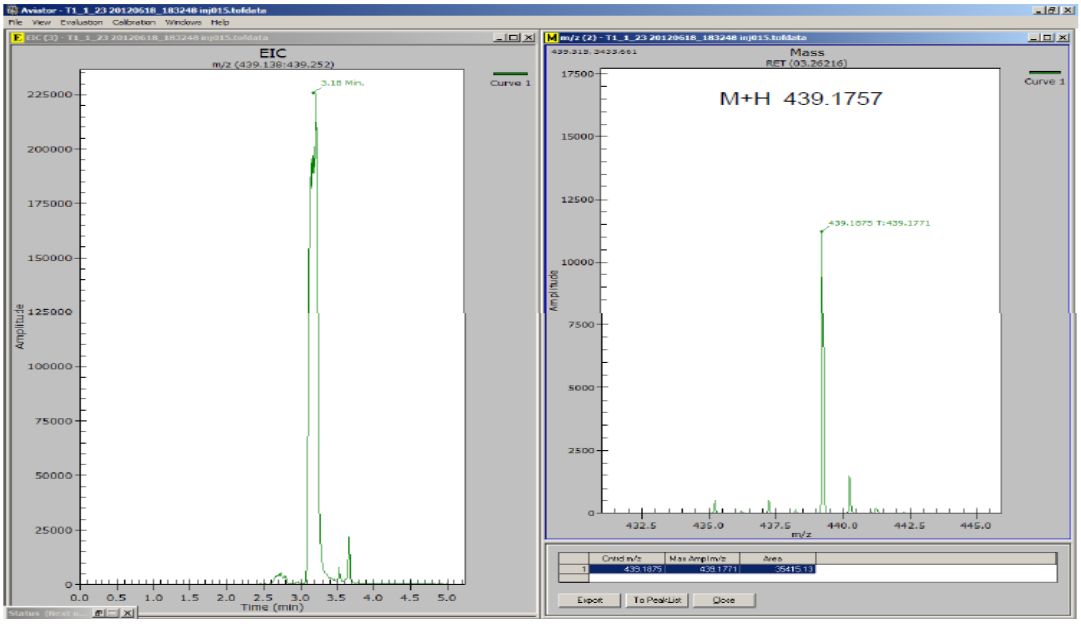


Appendix 43F: gHMBC (CD_3OD, 800 MHz) spectrum of flemingin G (**197**)

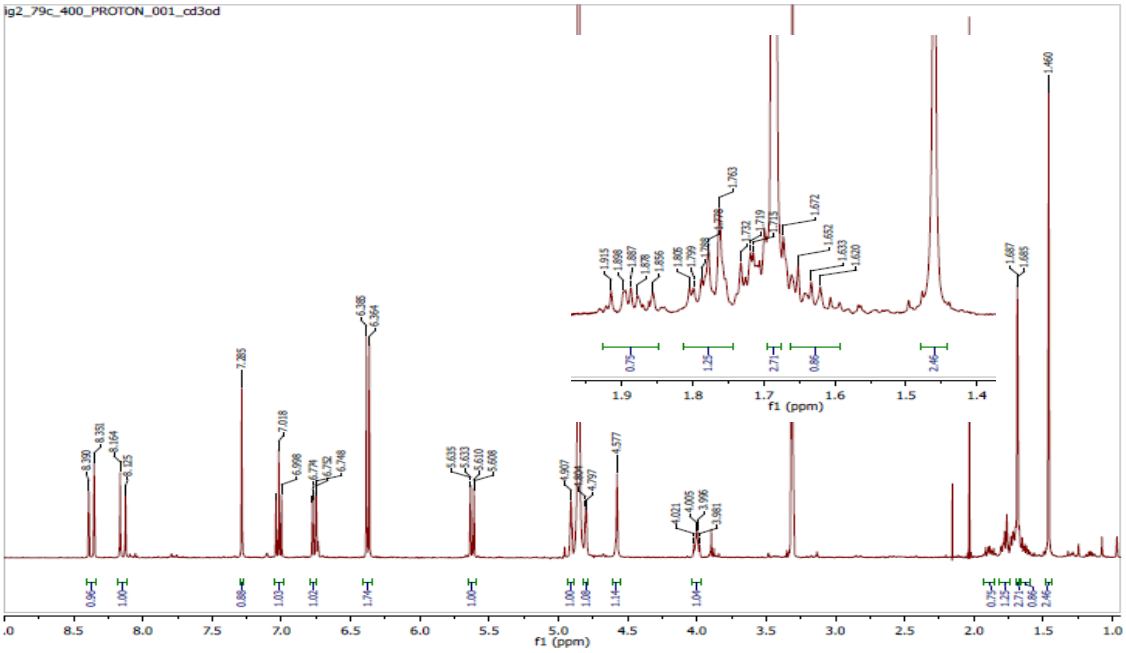


Appendix 43N: HRESI-MS of flemingin G (**197**)

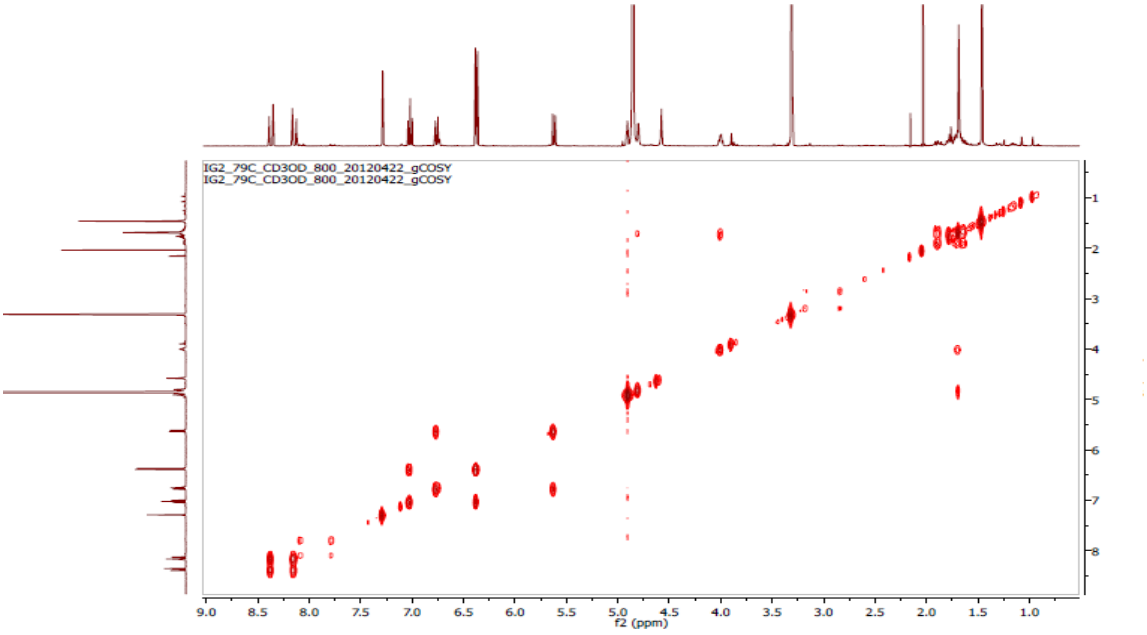
15 IG2-80J



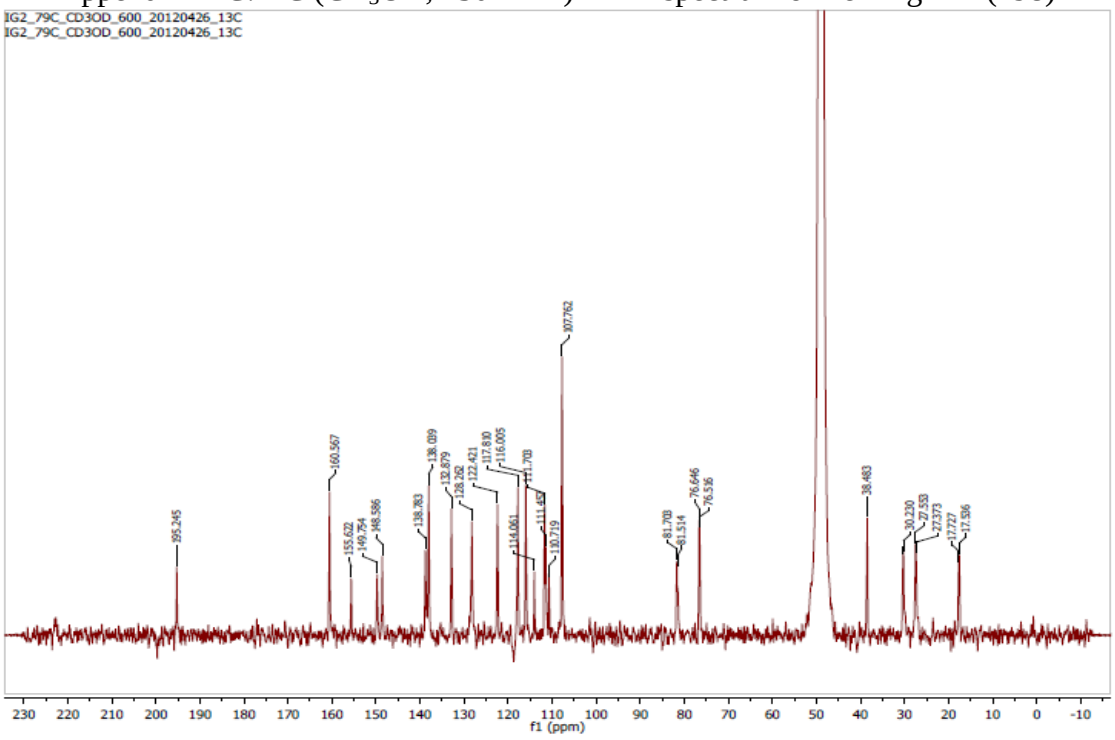
Appendix 44A: ^{1}H NMR (CD_3OD, 400 MHz) spectrum of flemingin H (**198**)



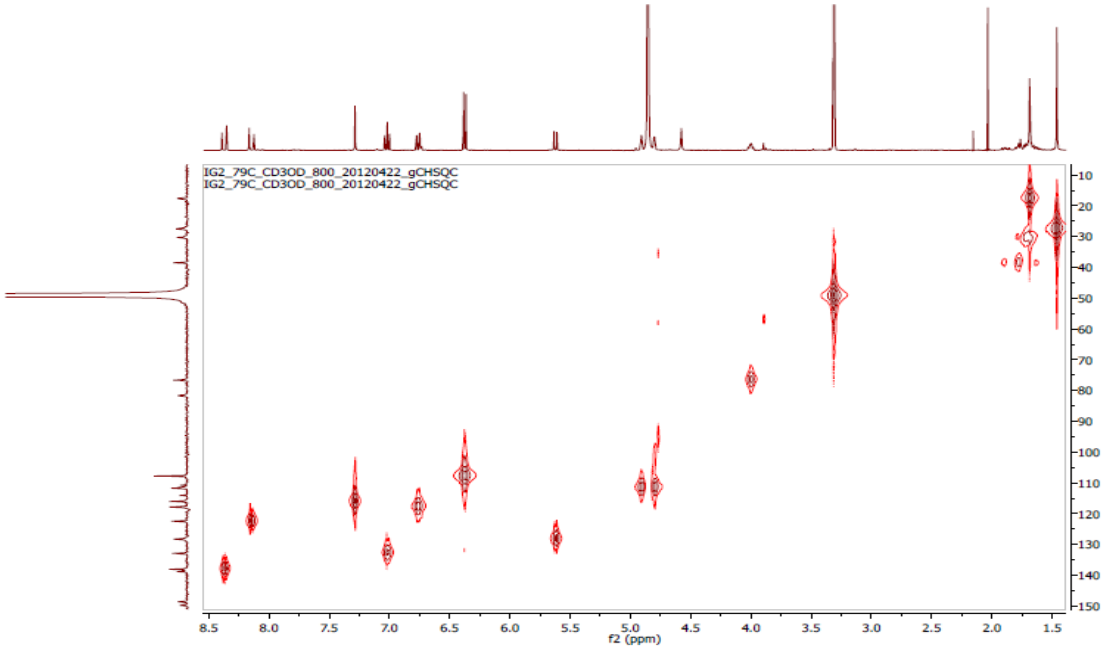
Appendix 44B: ^{1}H-^{1}H gCOSY (CD_3OD, 800 MHz)spectrum of flemingin H (**198**)



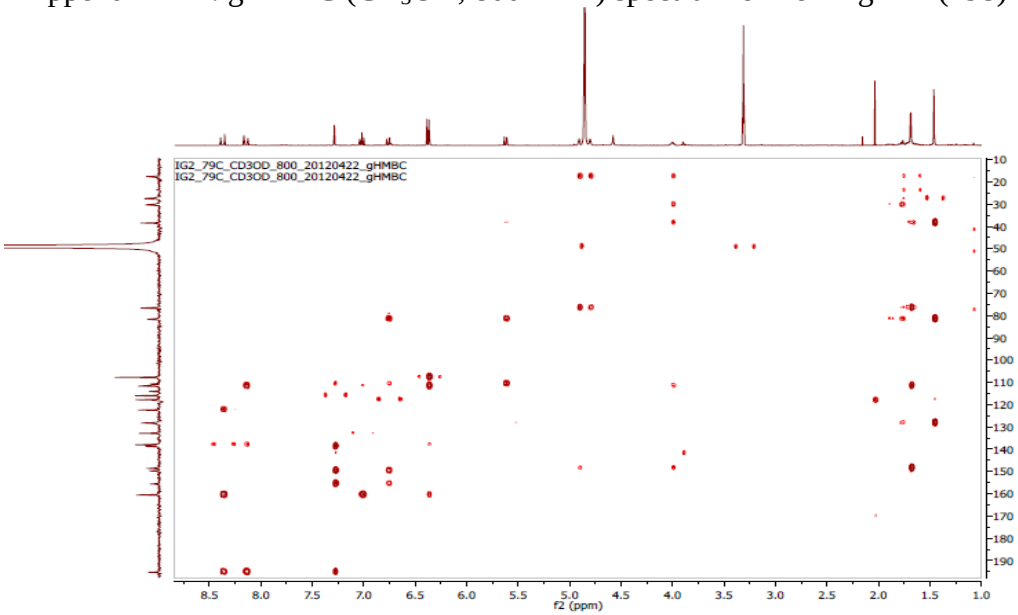
Appendix 44C: ^{13}C (CD_3OD, 150 MHz) NMR spectrum of flemingin H (**198**)



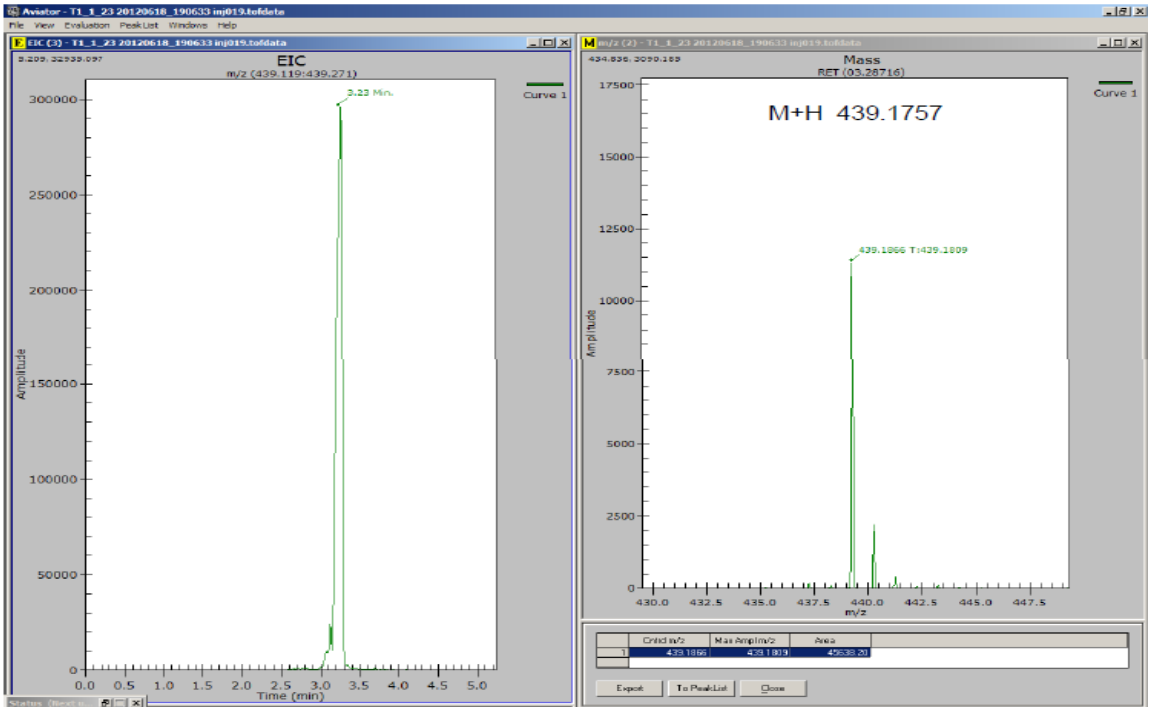
Appendix 44D: gCHSQC (CD_3OD, 800 MHz)spectrum of flemingin H (**198**)



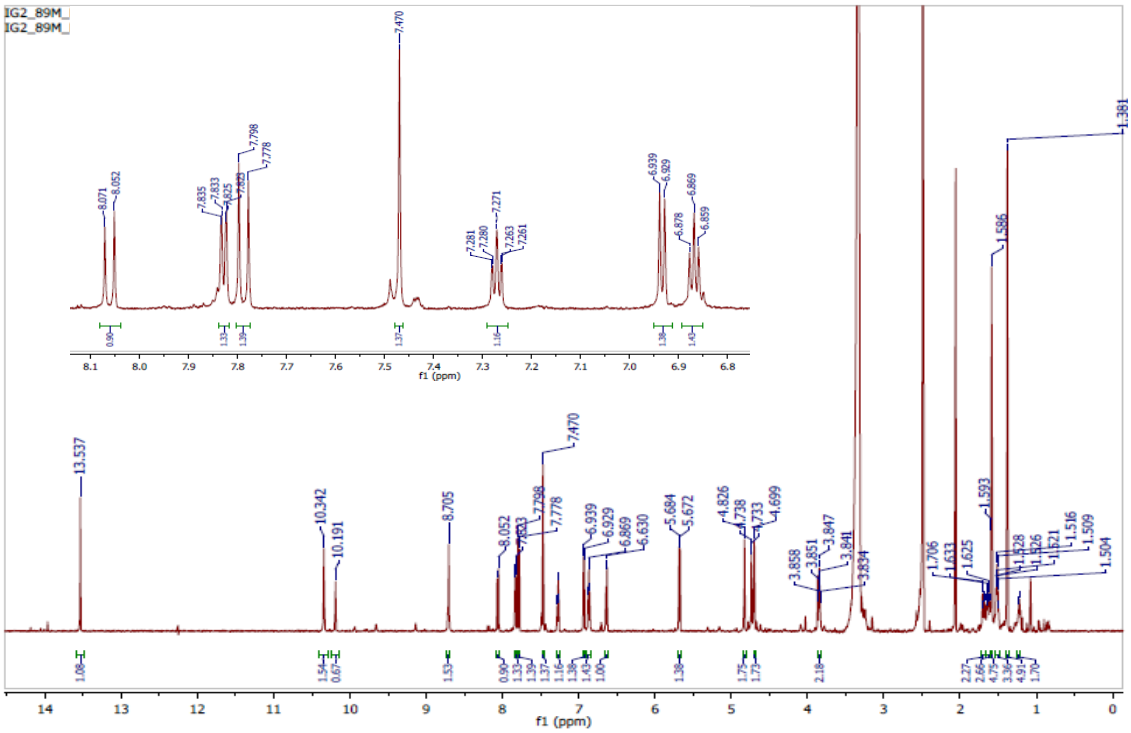
Appendix 44E: gHMBC (CD_3OD, 800 MHz) spectrum of flemingin H (**198**)



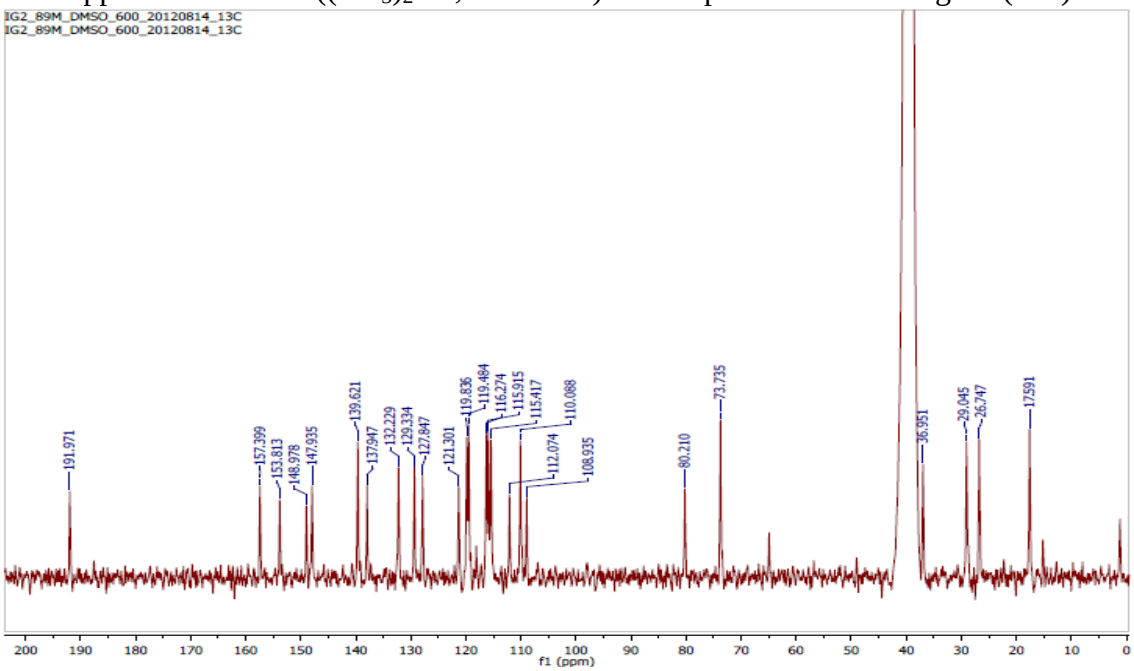
Appendix 44F: HRESI-MS of flemingin H (**198**)



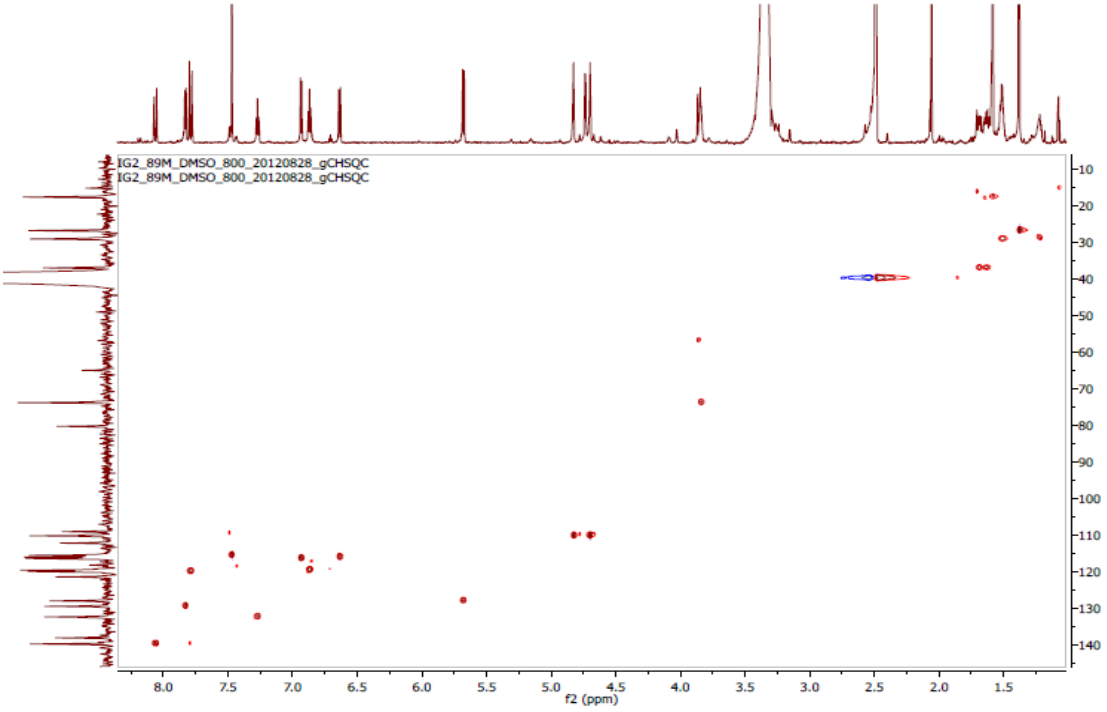
Appendix 45A: ^{1}H ($(CD_3)_2SO$, 800 MHz) NMR spectrum of flemingin I (**199**)



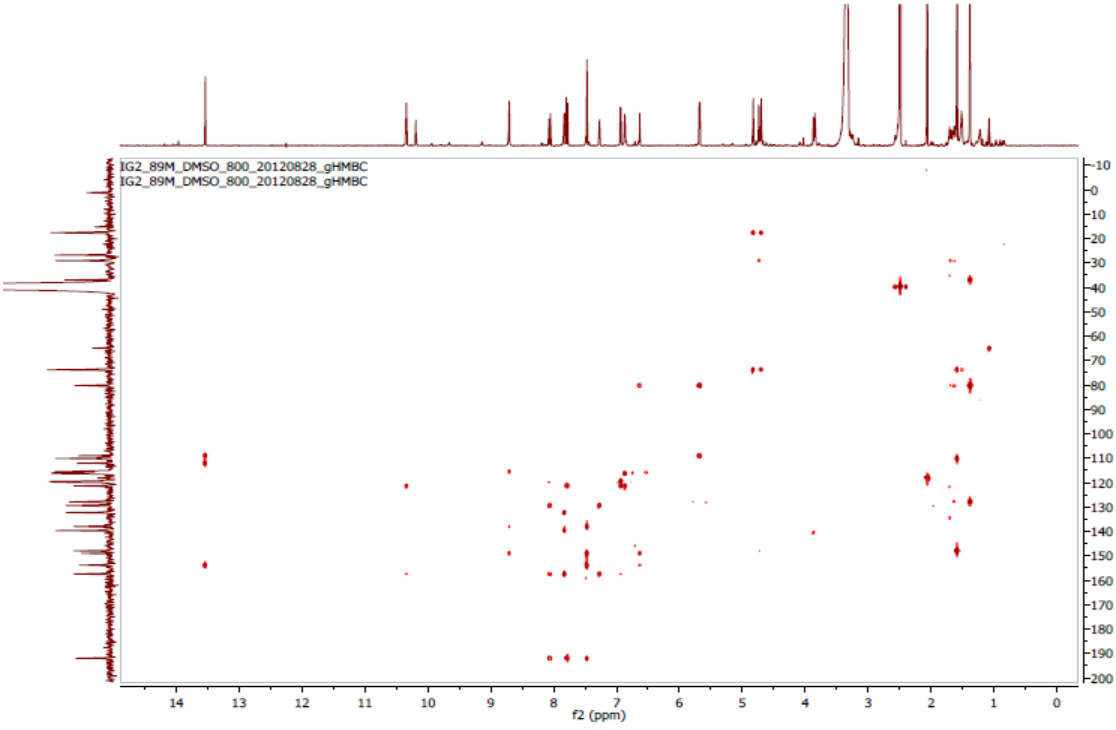
Appendix 45B: ^{13}C ($(CD_3)_2SO$, 150 MHz) NMR spectrum of flemingin I (**199**)



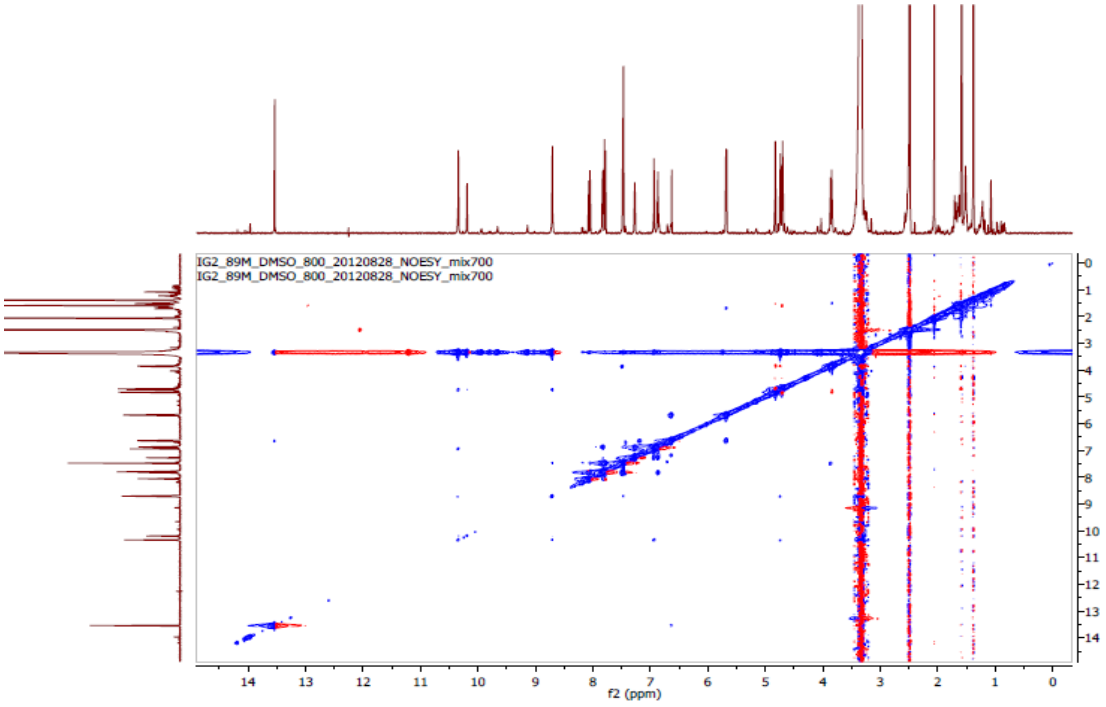
Appendix 45C: gCHSQC ($(CD_3)_2SO$, 800 MHz) spectrum of flemingin I (**199**)



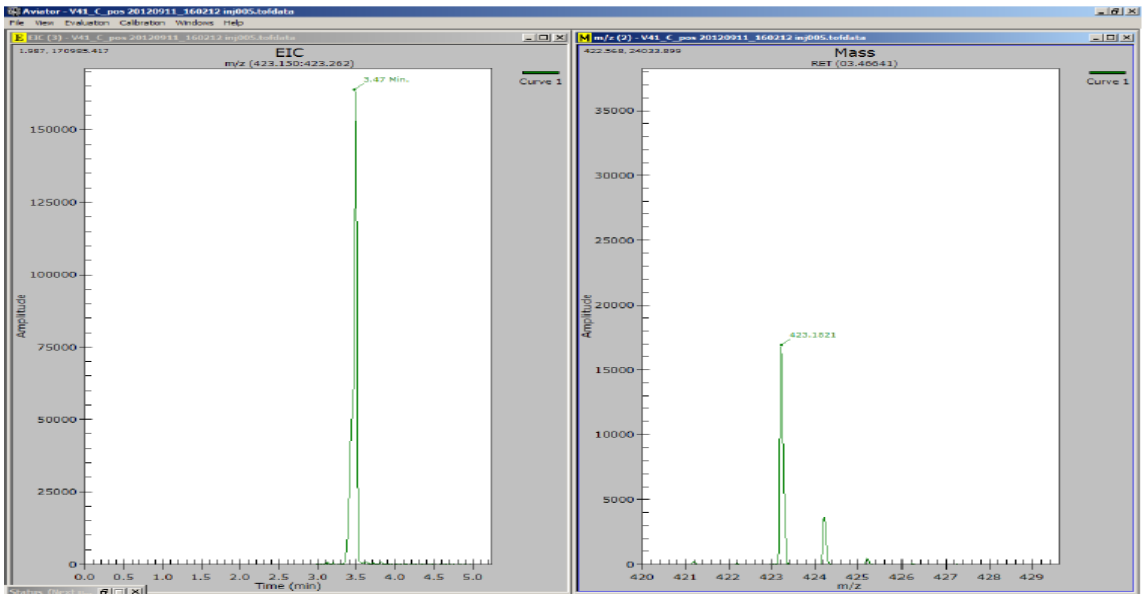
Appendix 45D: gHMBC ($(CD_3)_2SO$, 800 MHz) spectrum of flemingin I (**199**)



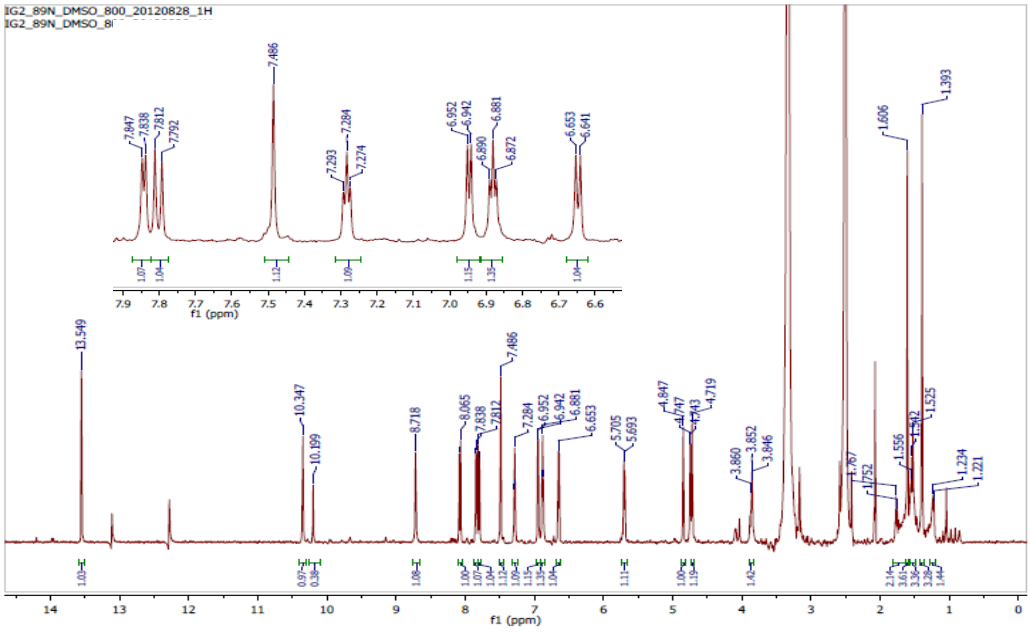
Appendix 45E: gNOESY ($(CD_3)_2SO$, 800 MHz) spectrum of flemingin I (**199**)



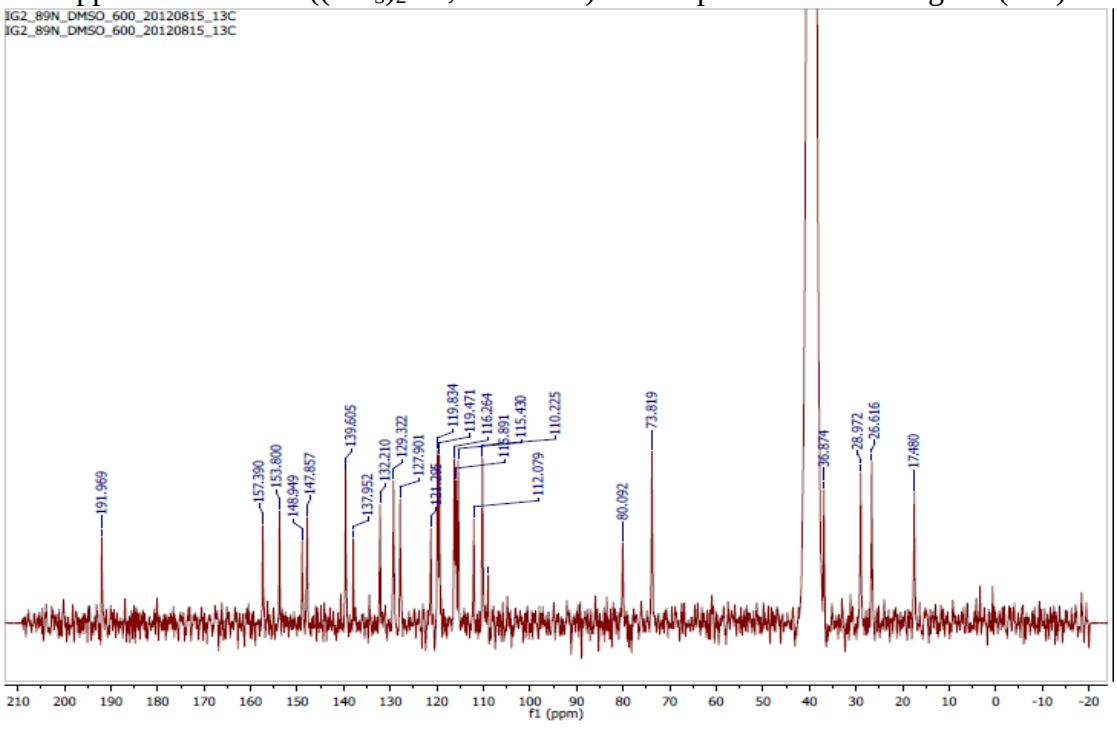
Appendix 45F: HRESI-MS of flemingin I (**199**)



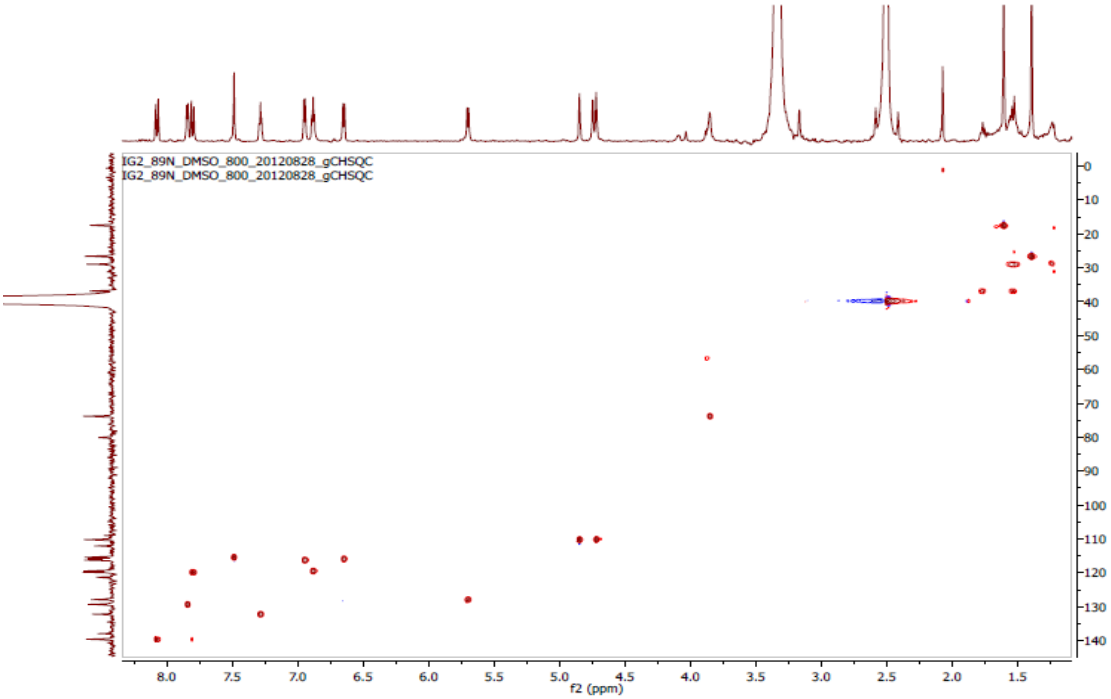
Appendix 46A: ^{1}H ($(CD_3)_2SO$, 800 MHz) NMR spectrum of flemingin J (**200**)



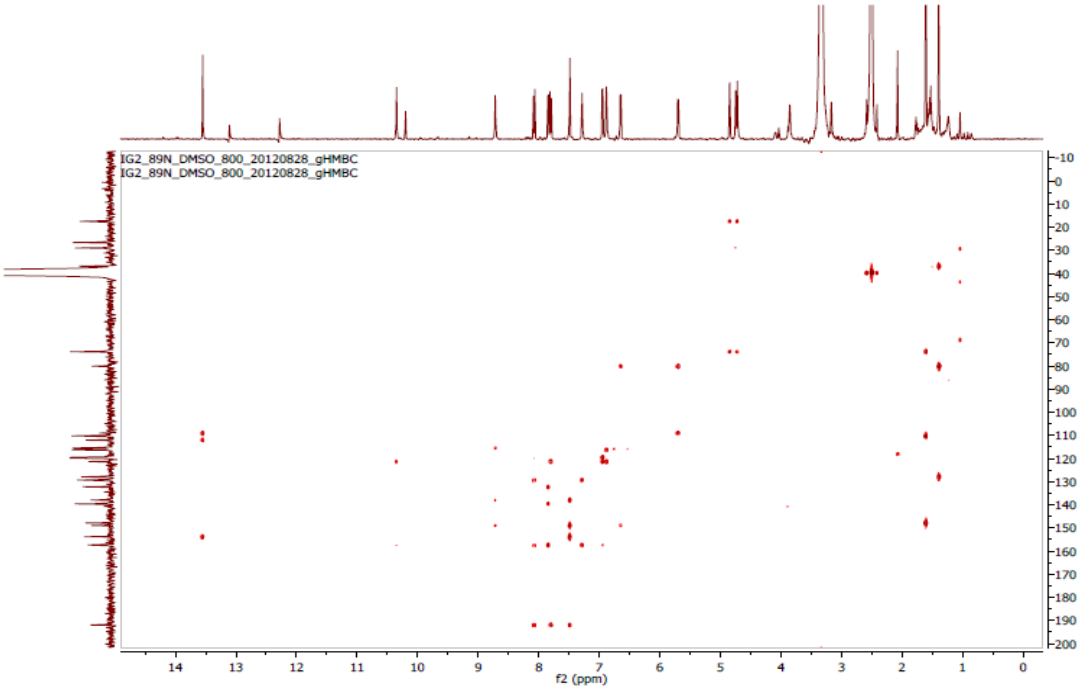
Appendix 46B: ^{13}C ($(CD_3)_2SO$, 150 MHz) NMR spectrum of flemingin J (**200**)



Appendix 46C: gCHSQC ($(CD_3)_2SO$, 800 MHz) spectrum of flemingin J (**200**)

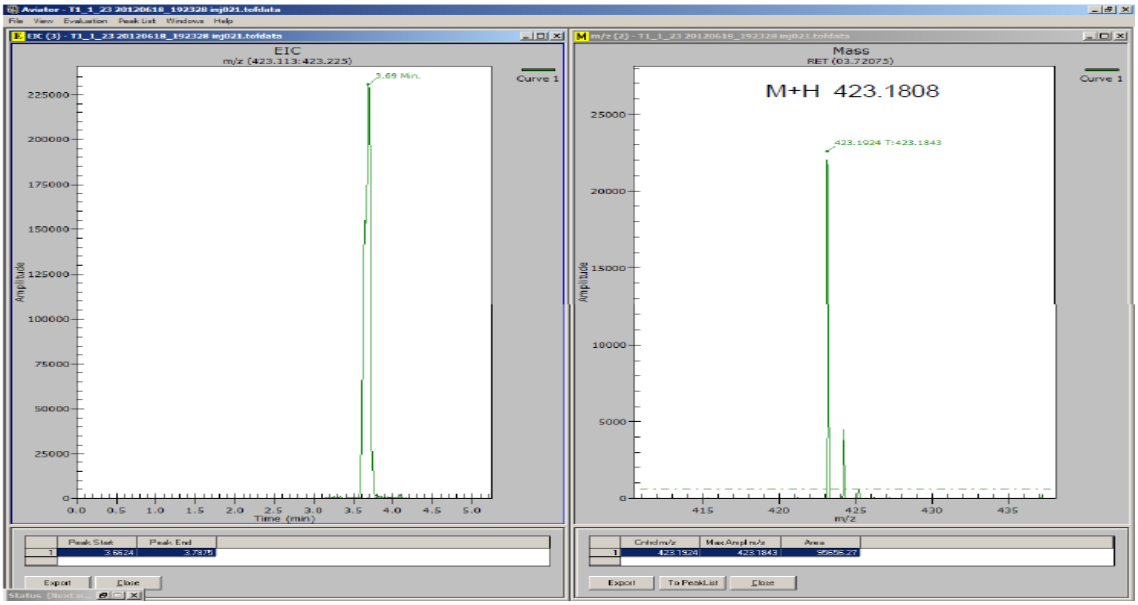


Appendix 46D: gHMBC ($(CD_3)_2SO$, 800 MHz) spectrum of flemingin J (**200**)

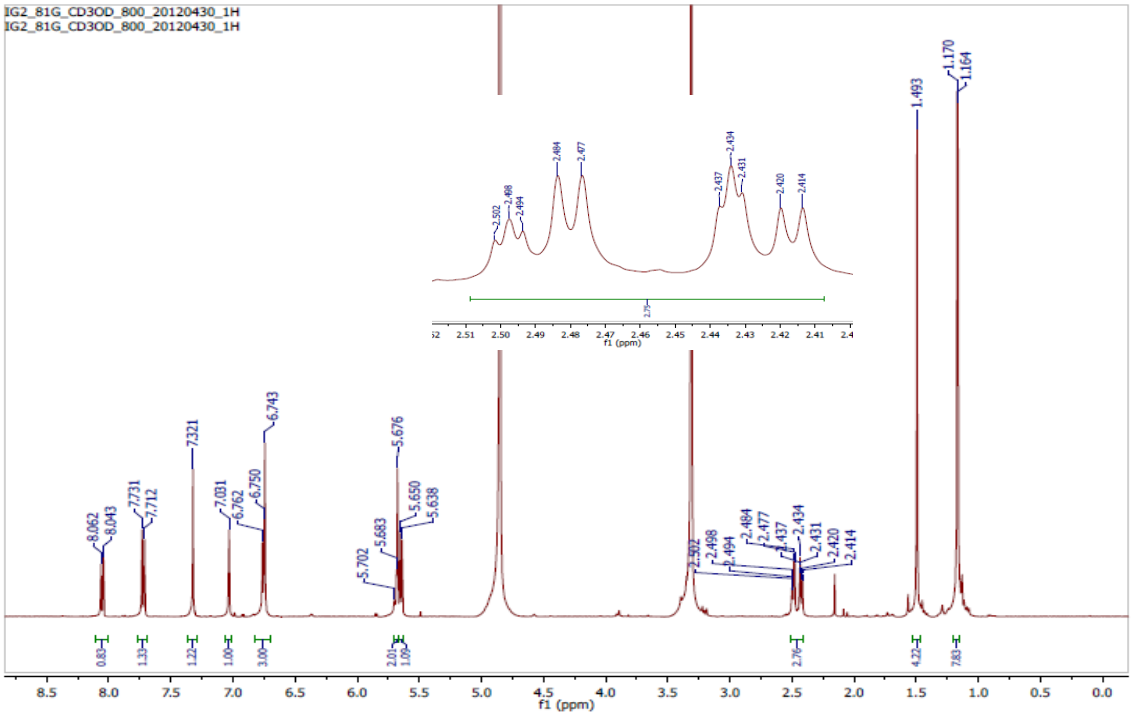


Appendix 46E: HRESI-MS of flemingin J (**200**)

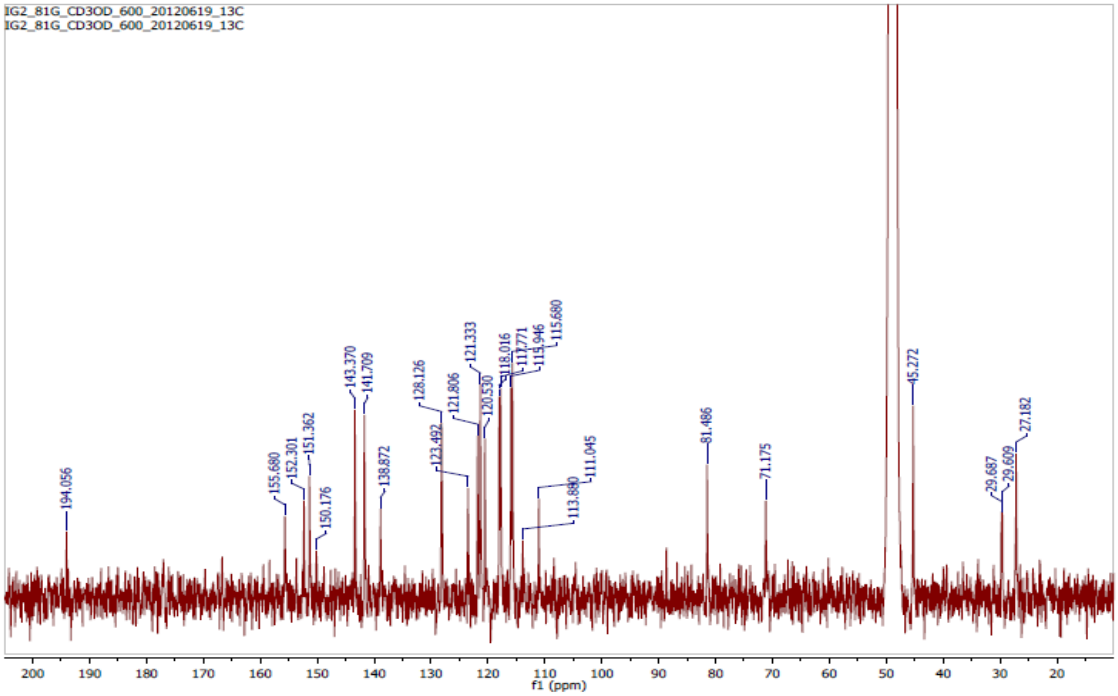
21 IG2-89N



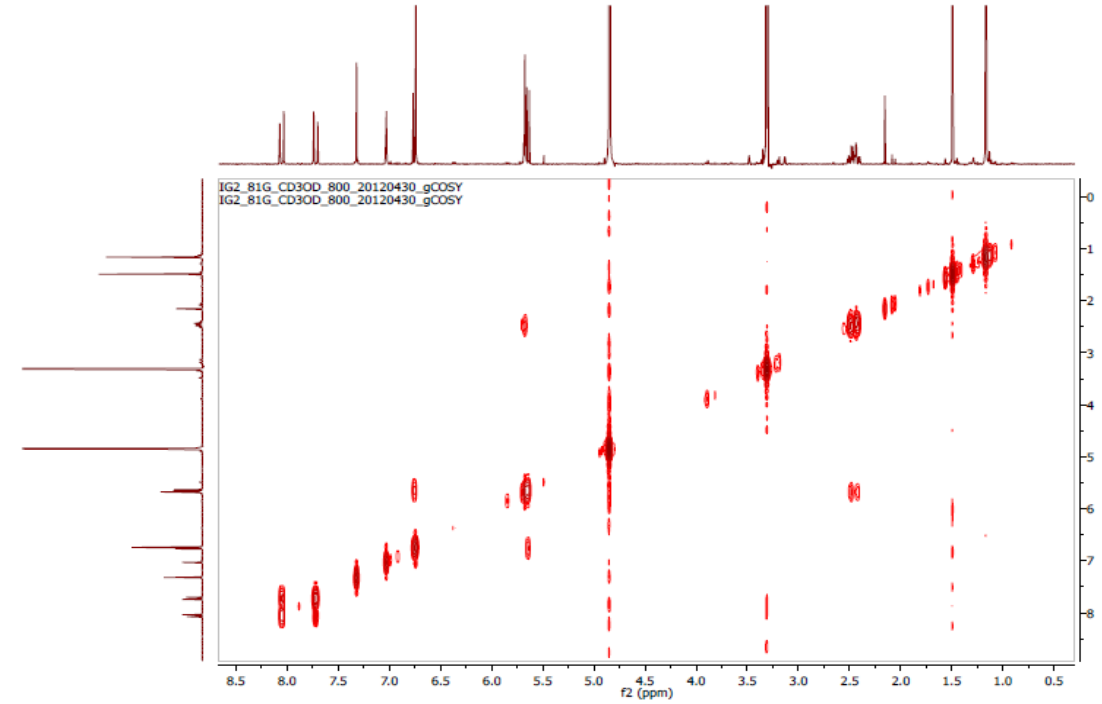
Appendix 47A: ^{1}H (CD_3OD, 800 MHz) NMR spectrum of flemingin K (**201**)



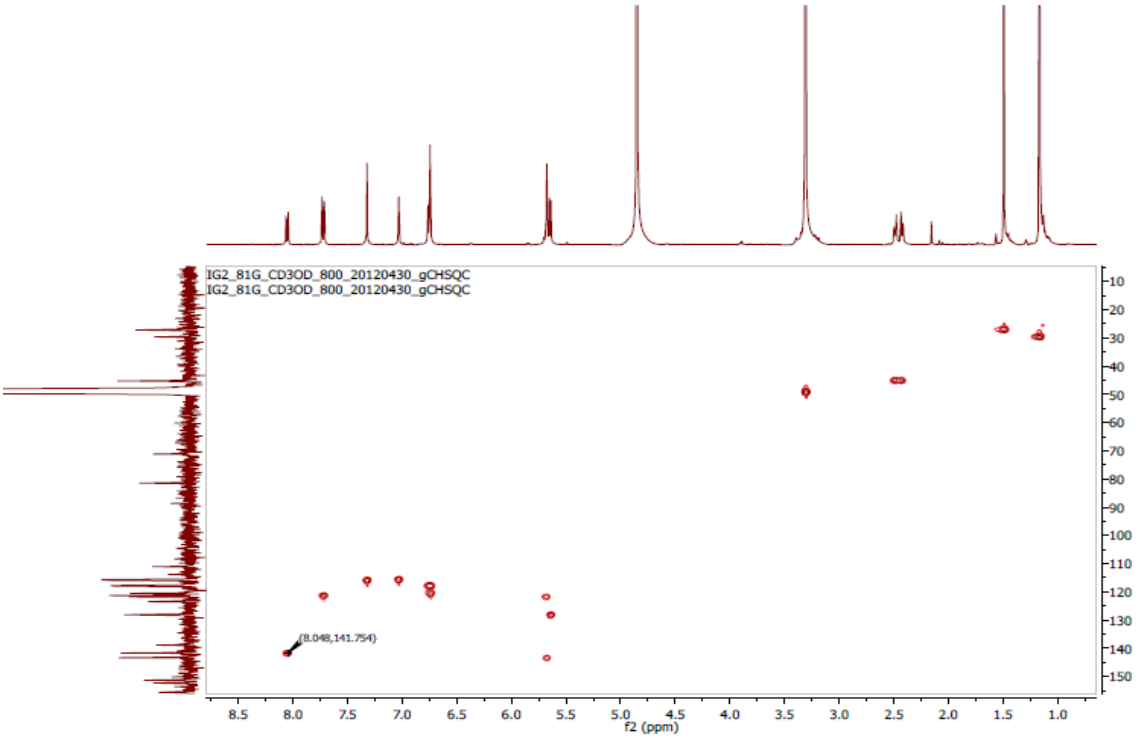
Appendix 47B: ^{13}C (CD_3OD, 150 MHz) NMR spectrum of flemingin K (**201**)



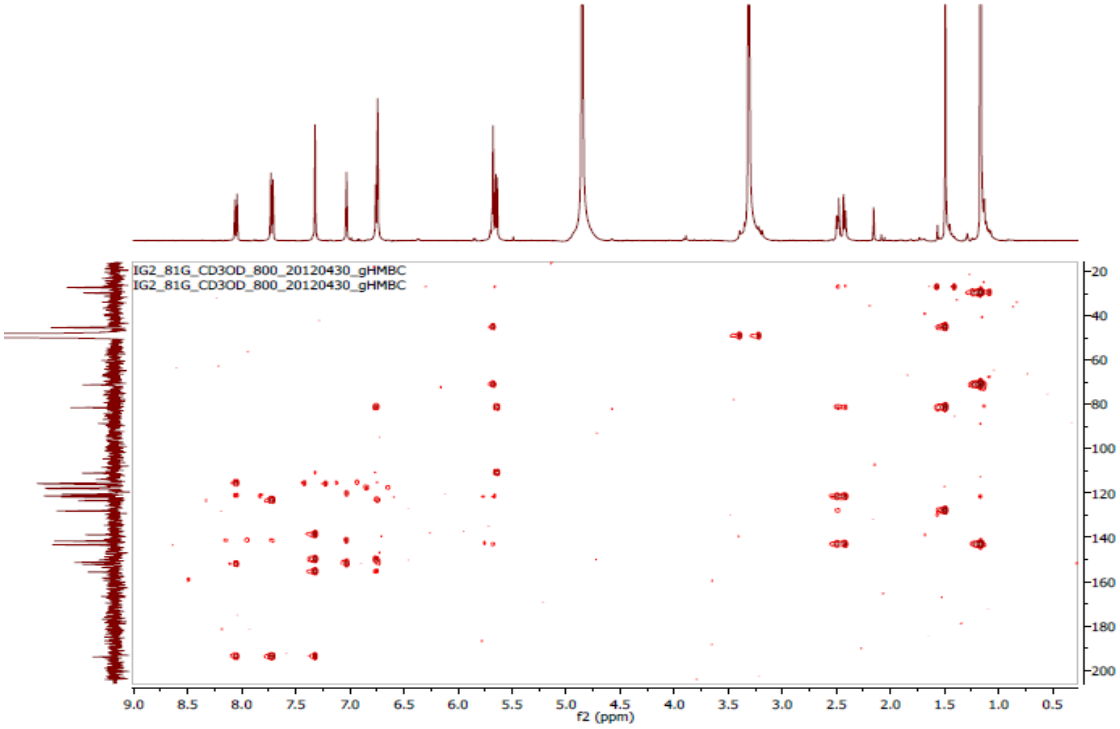
Appendix 47C: ^{1}H-^{1}H gCOSY (CD_3OD, 800 MHz) spectrum of flemingin K (**201**)



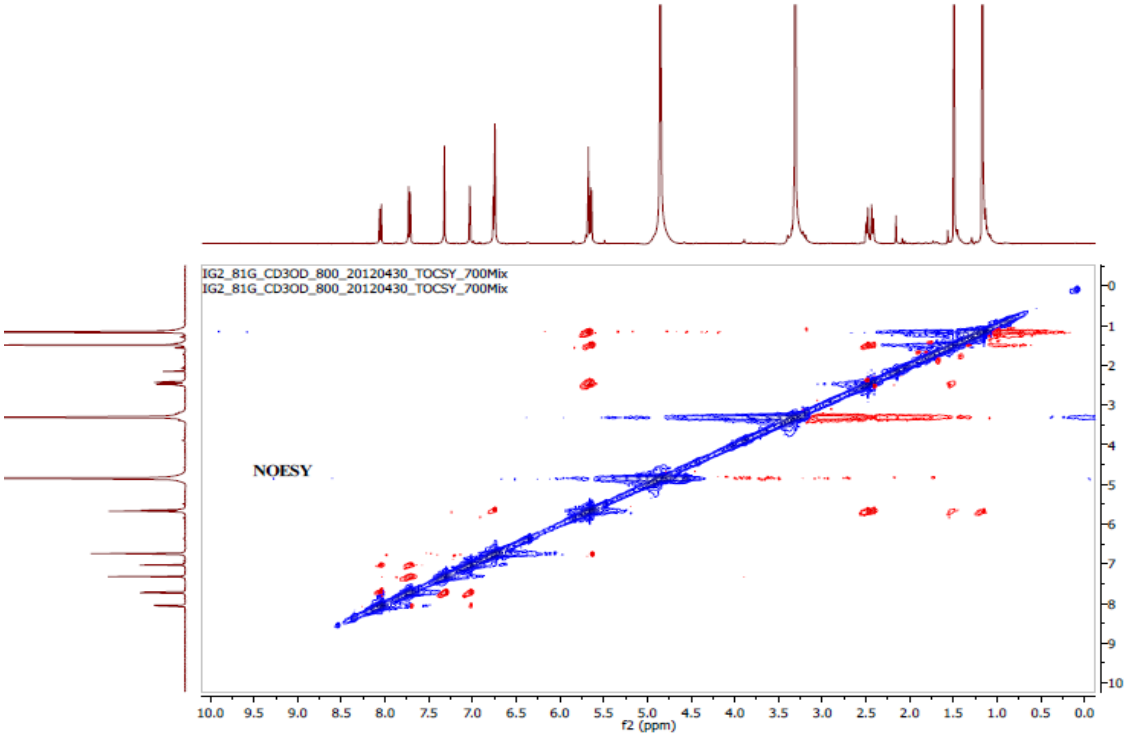
Appendix 47D: gCHSQC (CD_3OD, 800 MHz) spectrum of flemingin K (**201**)



Appendix 47E: gHMBC (CD_3OD, 800 MHz) spectrum of flemingin K (**201**)

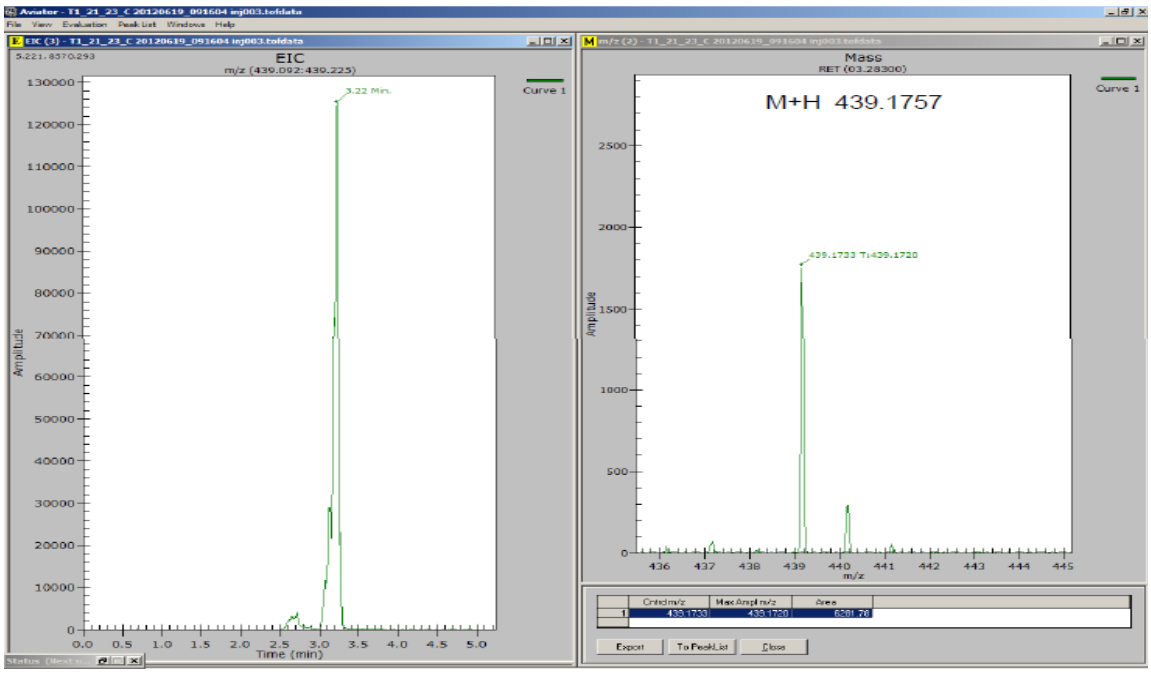


Appendix 47F: gNOESY (CD_3OD, 800 MHz) spectrum of flemingin K (**201**)

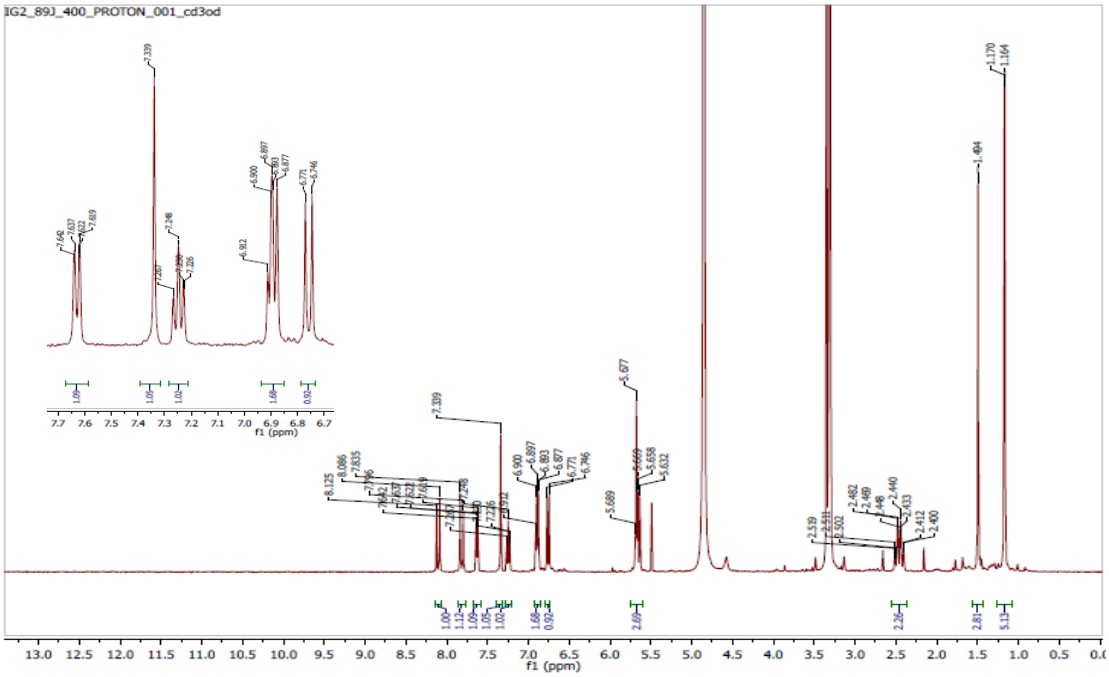


Appendix 47G: HRESI-MS of flemingin K (**201**)

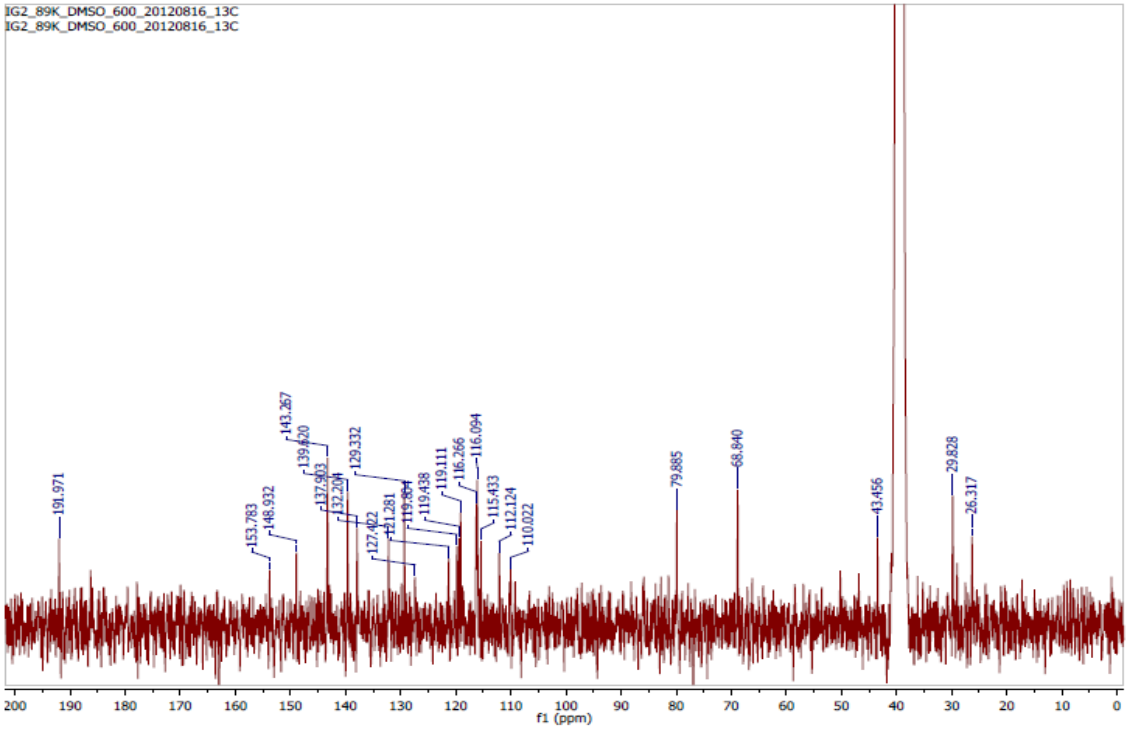
23 IG2-91E



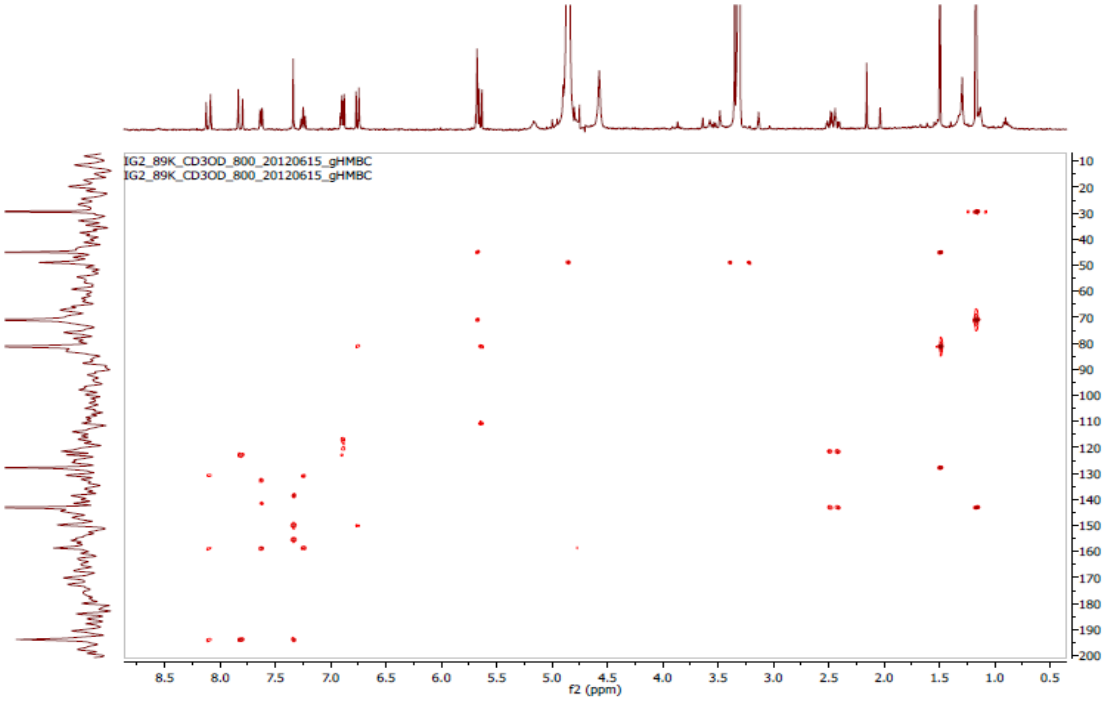
Appendix 48A: ^{1}H (CD_3OD, 400 MHz) NMR spectrum of flemingin L (**202**)



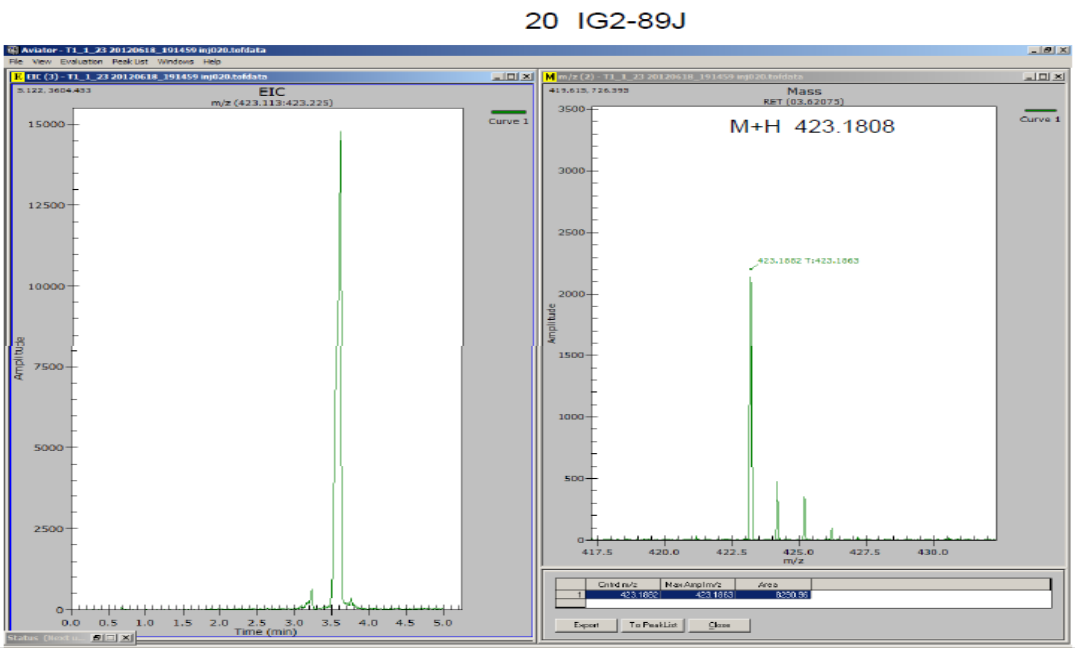
Appendix 48B: ^{13}C ($(CD_3)_2SO$, 150 MHz) NMR spectrum of flemingin L (**202**)



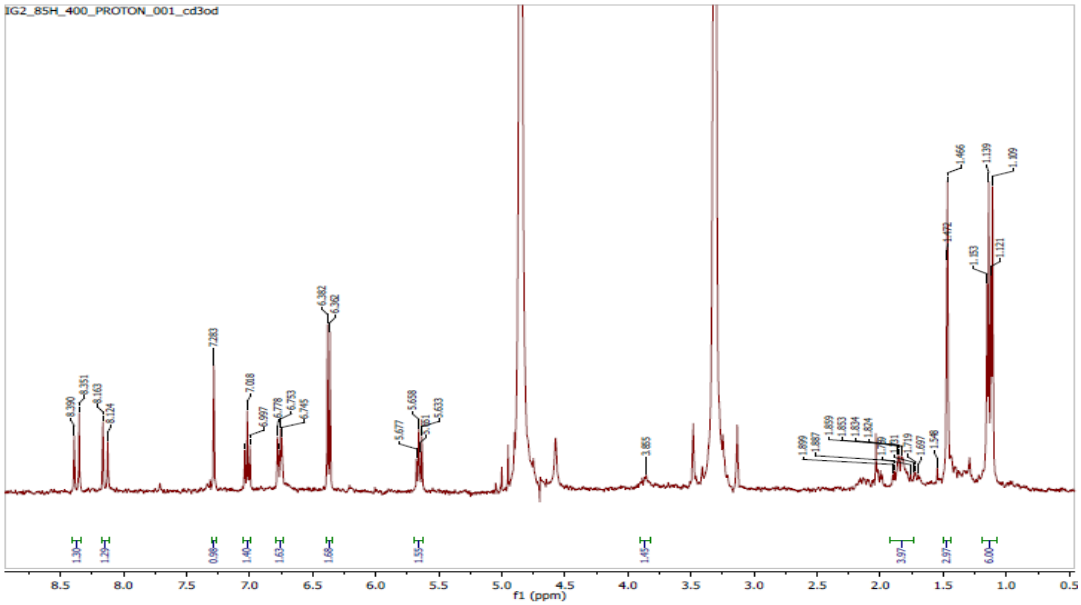
Appendix 48C: gHMBC (CD_3OD, 800 MHz) spectrum of flemingin L (**202**)



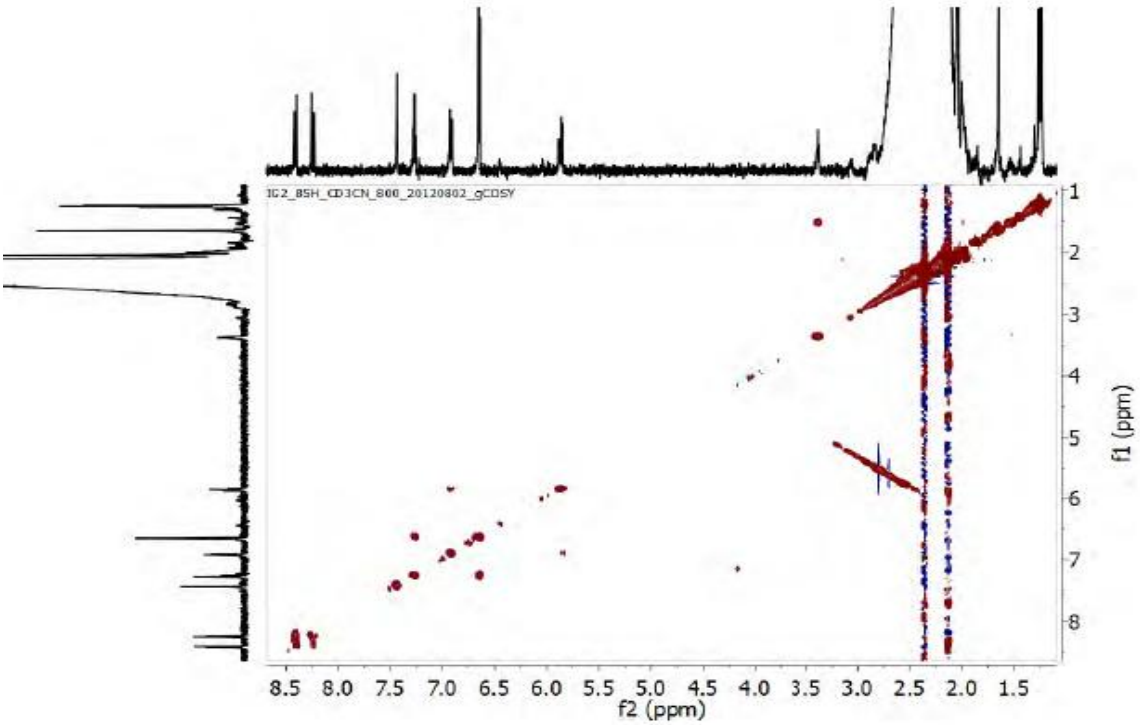
Appendix 48D: HRESI-MS of flemingin L (**202**)



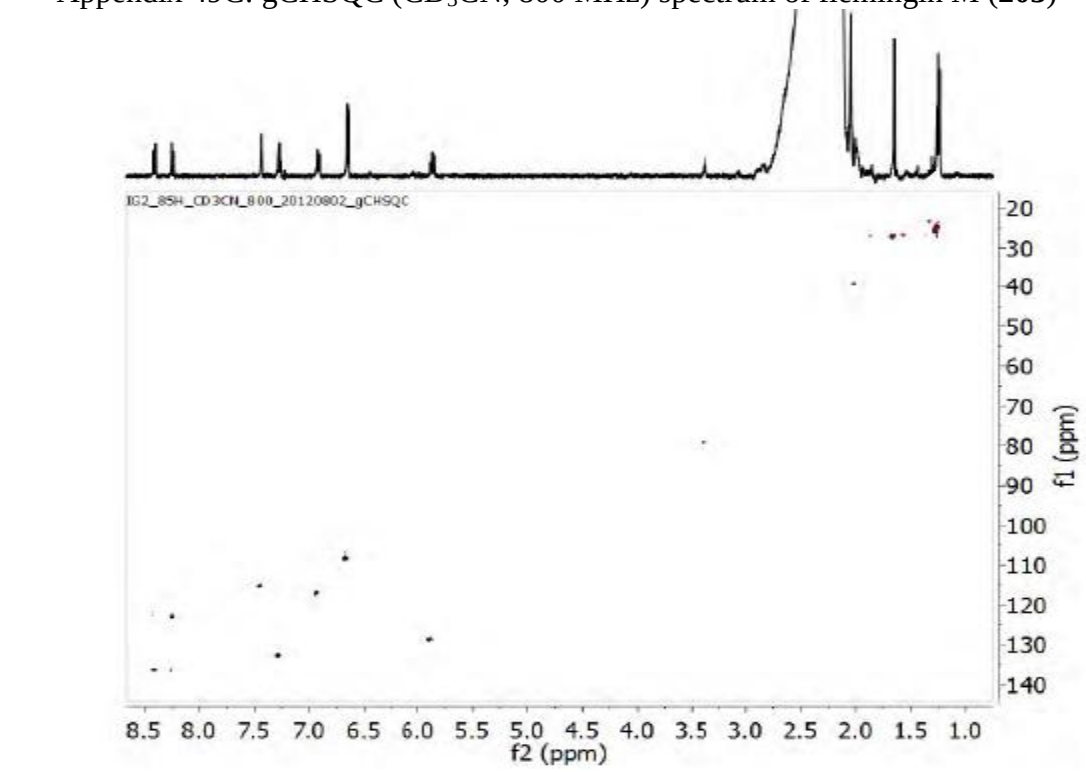
Appendix 49A: ^{1}H NMR (CD_3OD, 400 MHz) spectrum of flemingin M (**203**)



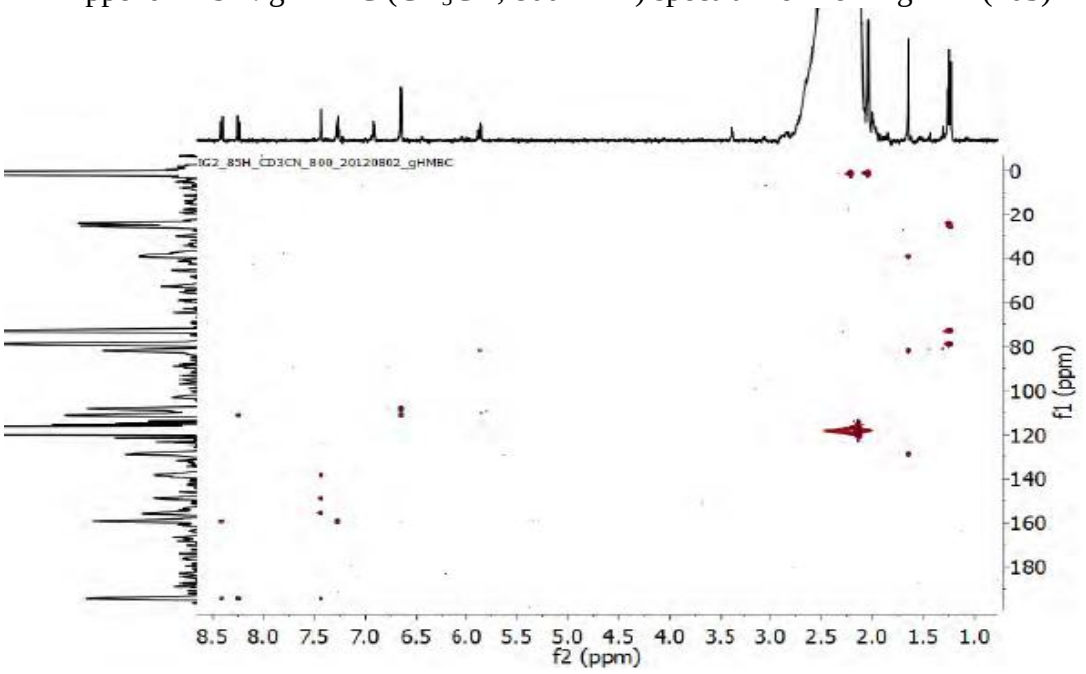
Appendix 49B: gCOSY (CD_3CN, 800 MHz) spectrum of flemingin M (**203**)



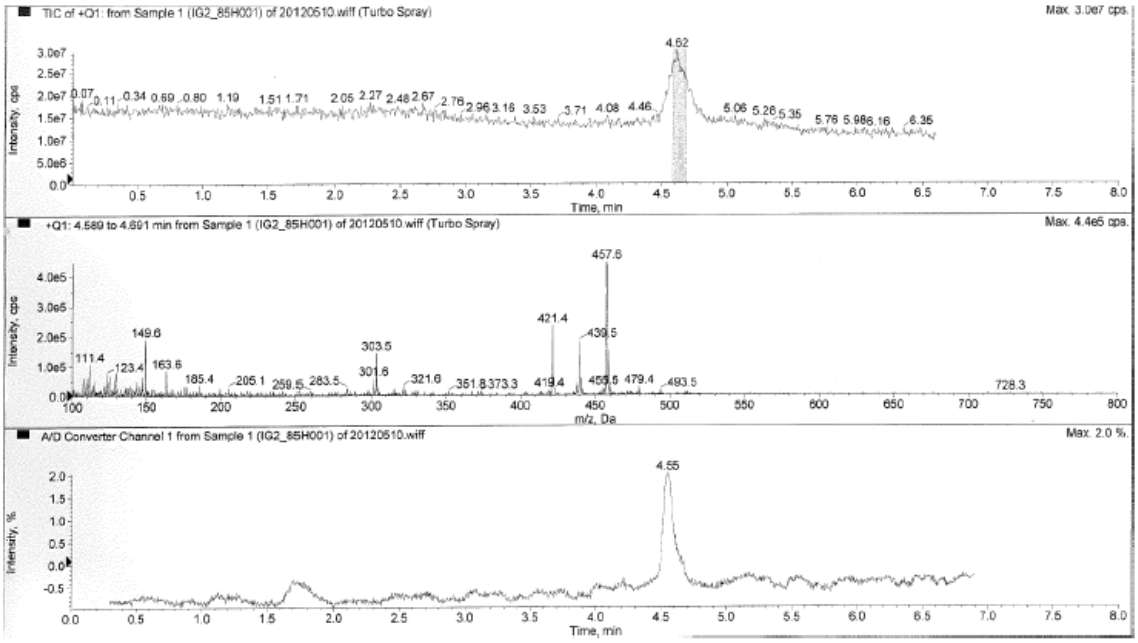
Appendix 49C: gCHSQC (CD_3CN, 800 MHz) spectrum of flemingin M (**203**)



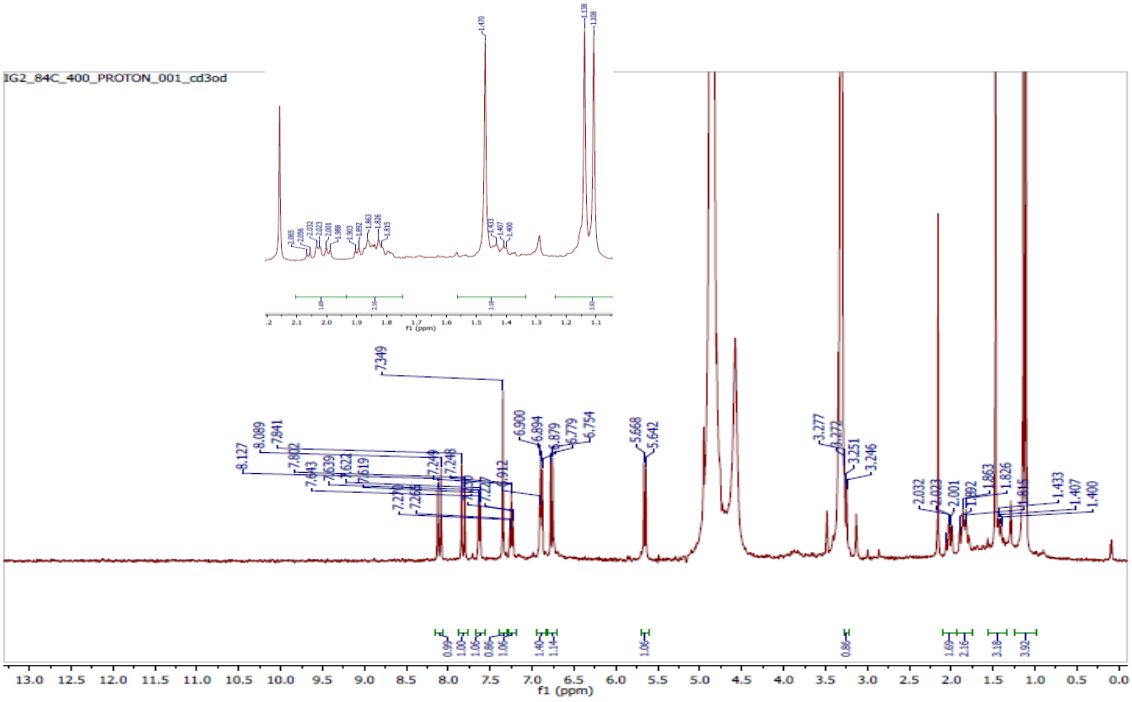
Appendix 49D: gHMBC (CD_3CN, 800 MHz) spectrum of flemingin M (**203**)



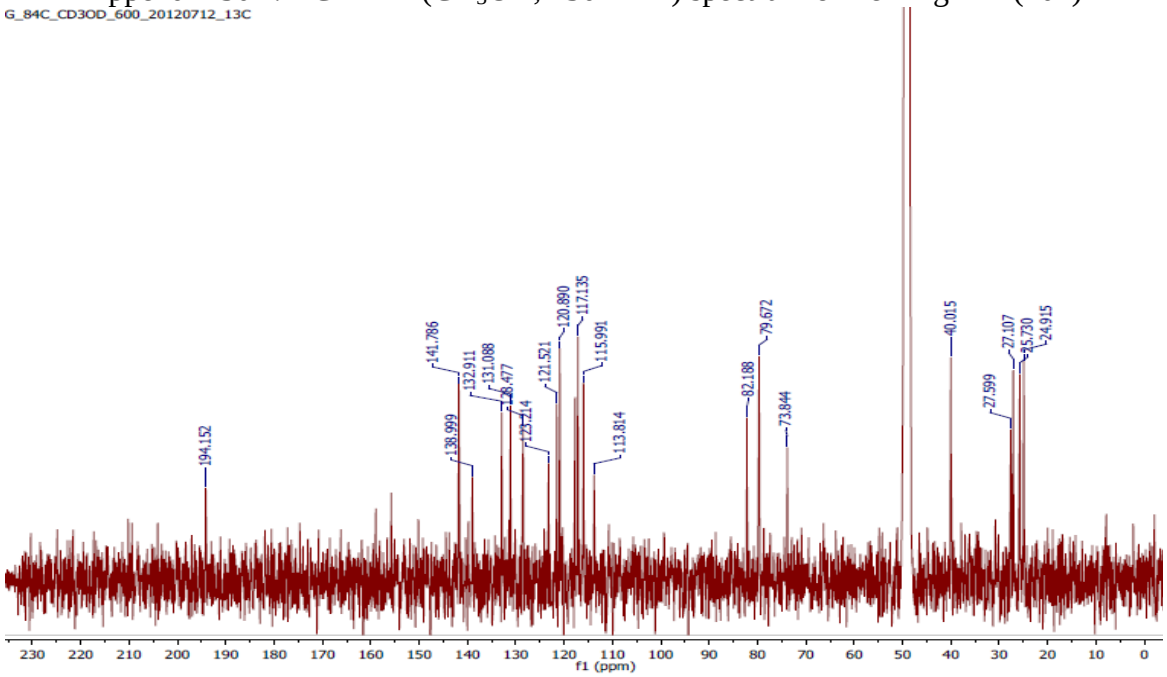
Appendix 49E: LC-ESI-MS of flemingin M (**203**)



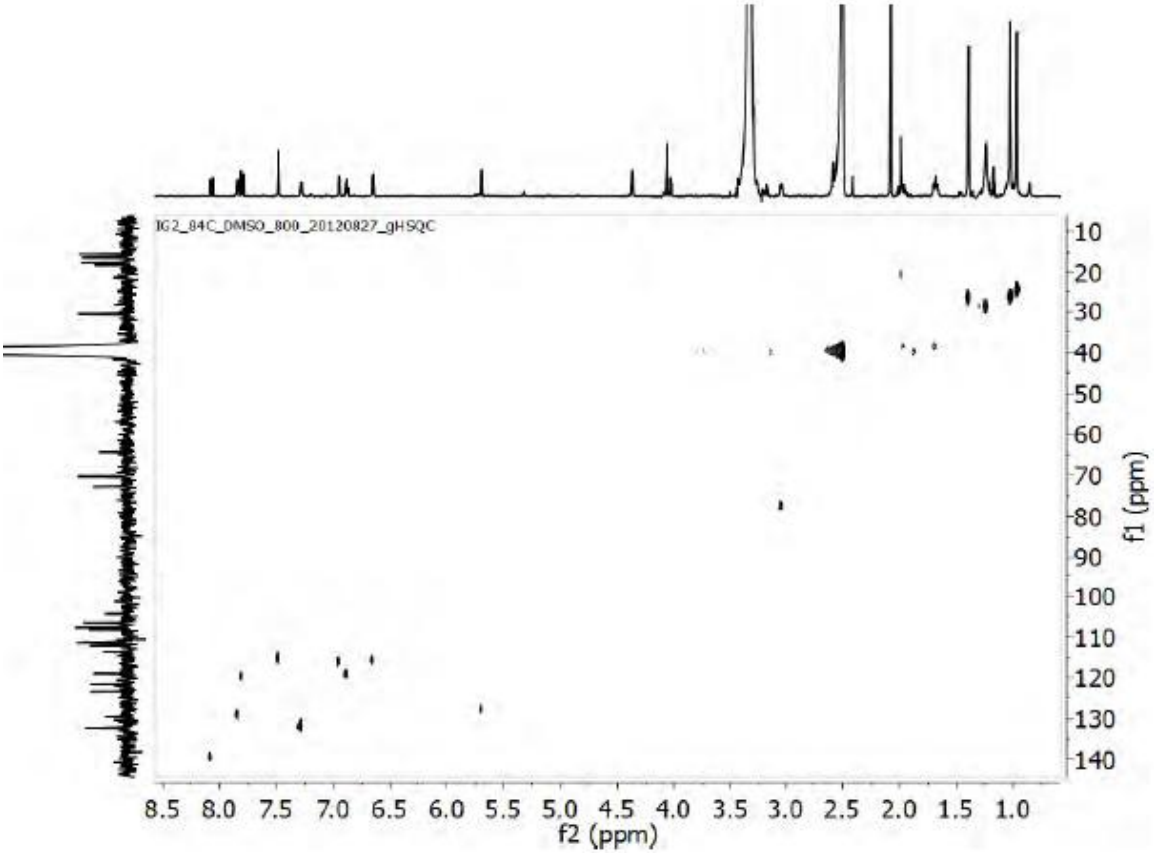
Appendix 50A: ^{1}H NMR (CD_3OD, 400 MHz) spectrum of flemingin N (**204**)



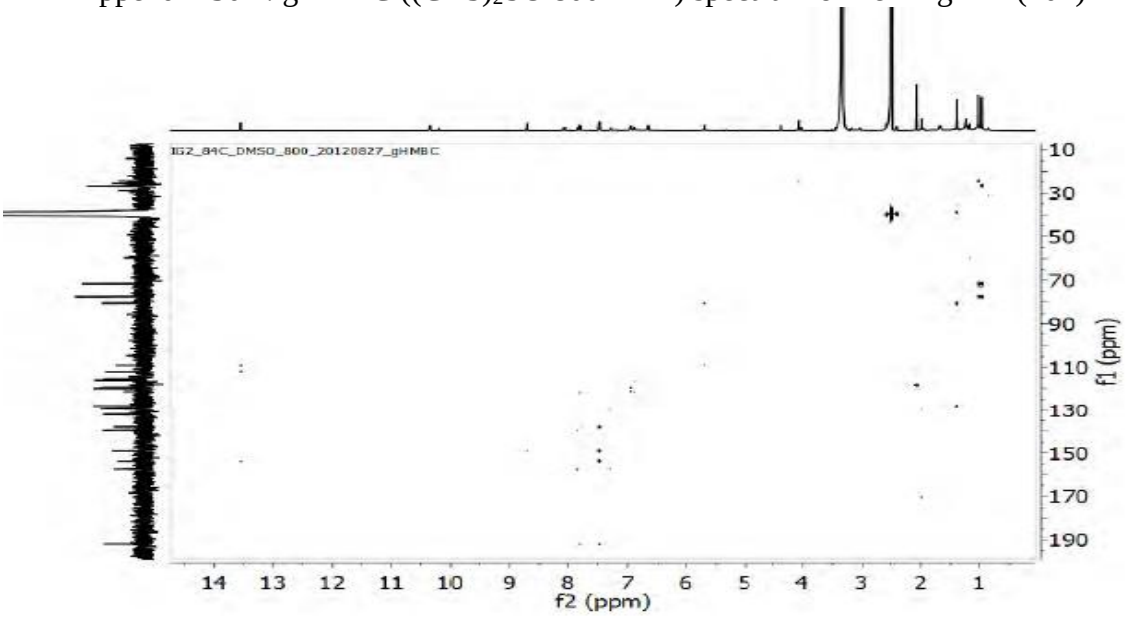
Appendix 50B: ^{13}C NMR (CD_3OD, 150 MHz) spectrum of flemingin N (**204**)



Appendix 50C: gCHSQC ($(CD3)_2SO$ 800 MHz) spectrum of flemingin N (**204**)



Appendix 50D: gHMBC ($(CD3)_2SO$ 800 MHz) spectrum of flemingin N (**204**)



Appendix 50E: HRESI-MS of flemingin N (**204**)

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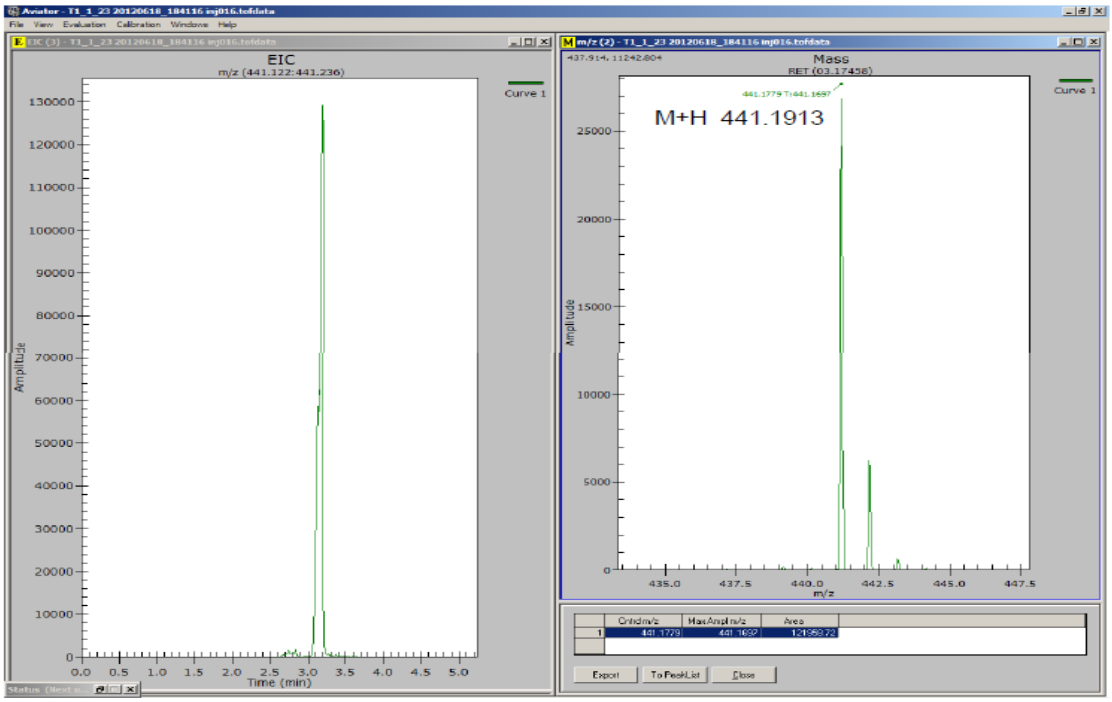
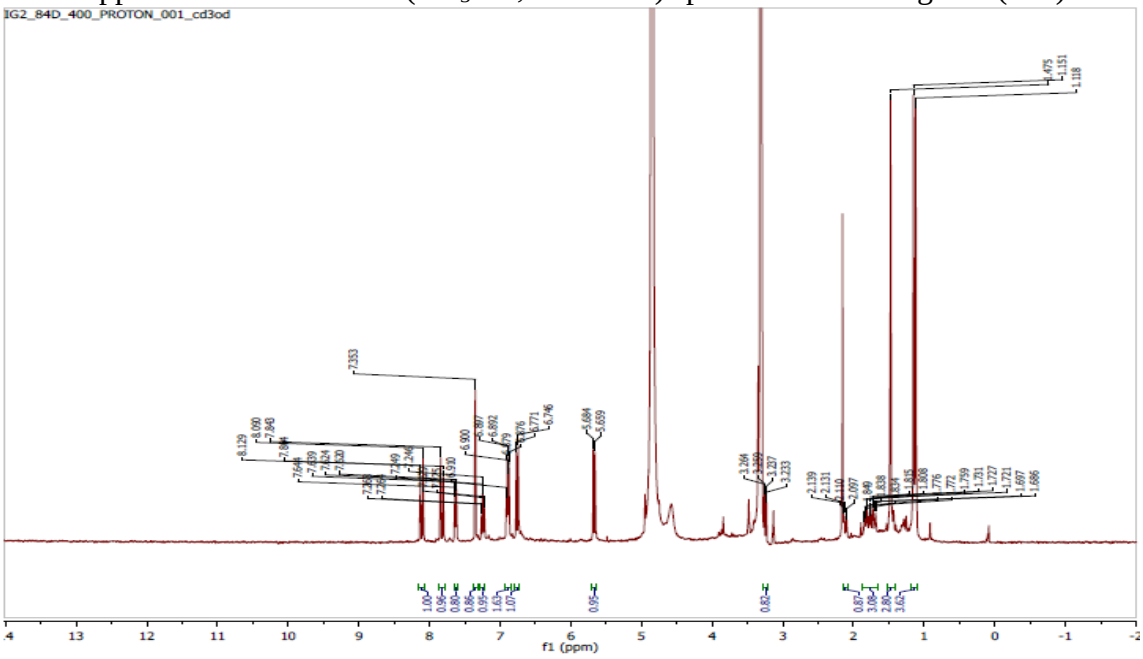
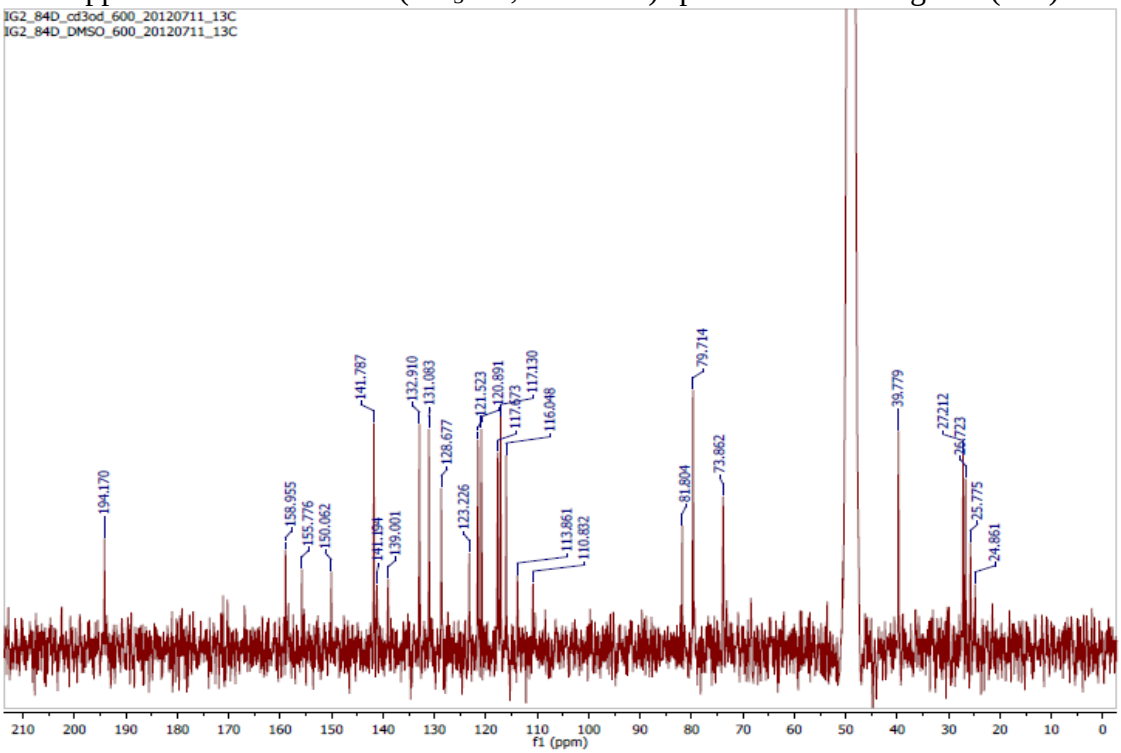


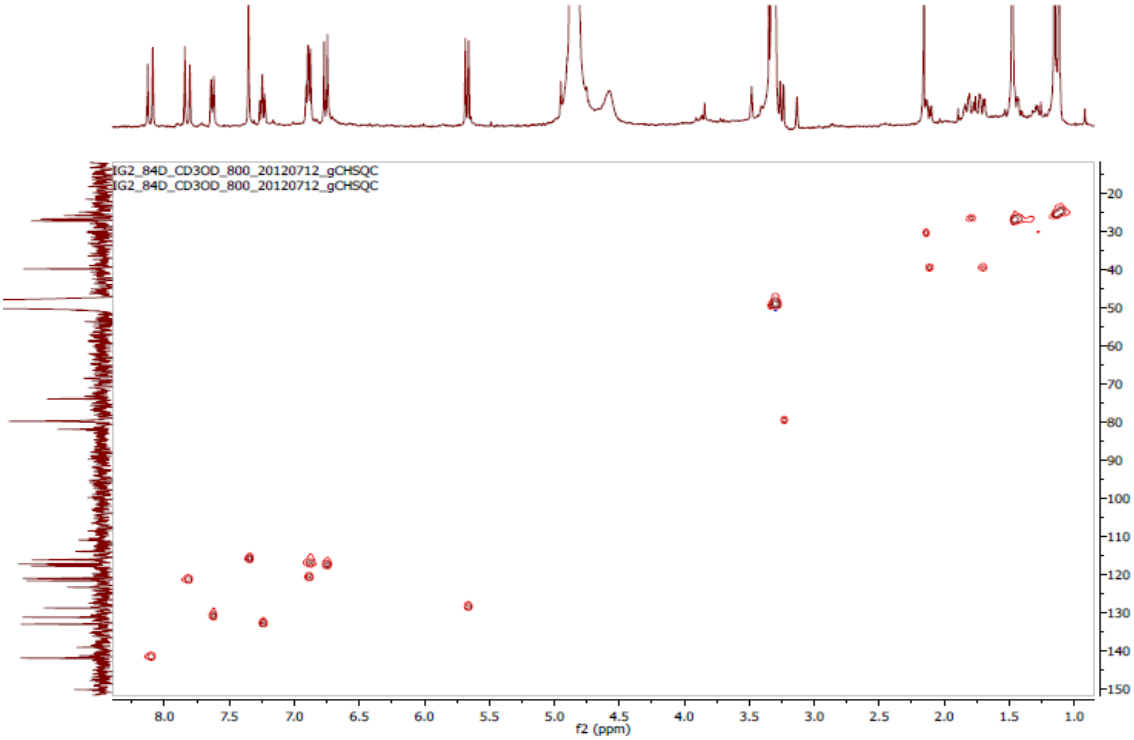
Appendix 51A: ^{1}H NMR (CD_3OD, 400 MHz) spectrum of flemingin O (**205**)



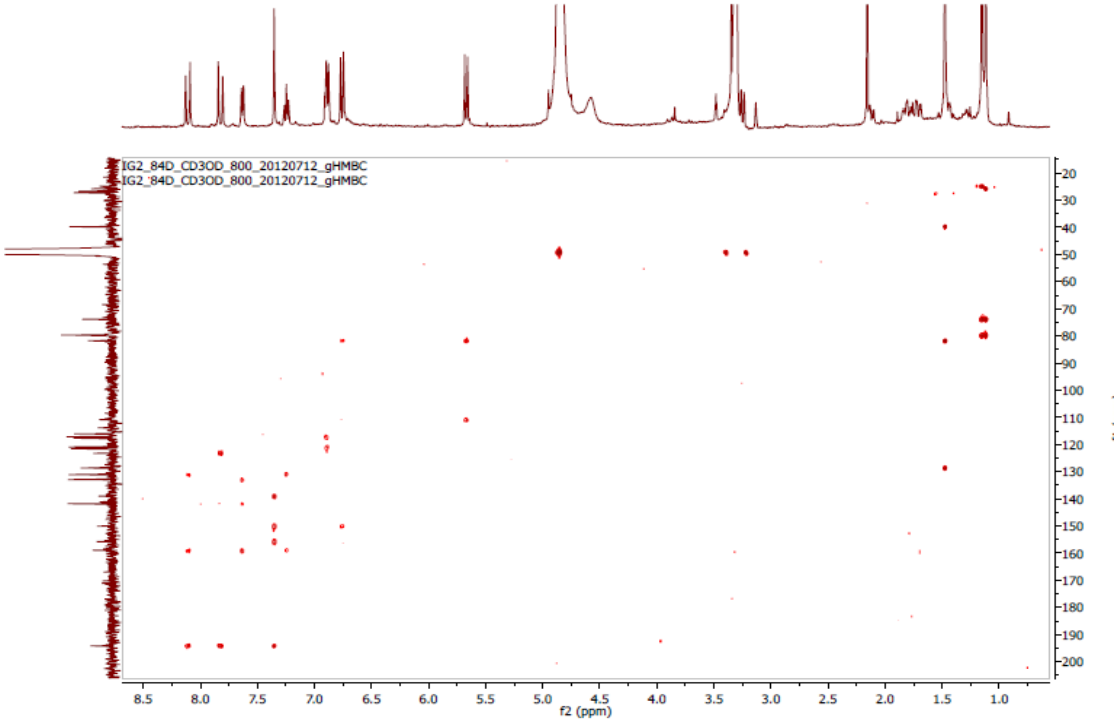
Appendix 51B: ^{13}C NMR (CD_3OD, 150 MHz) spectrum of flemingin O (**205**)



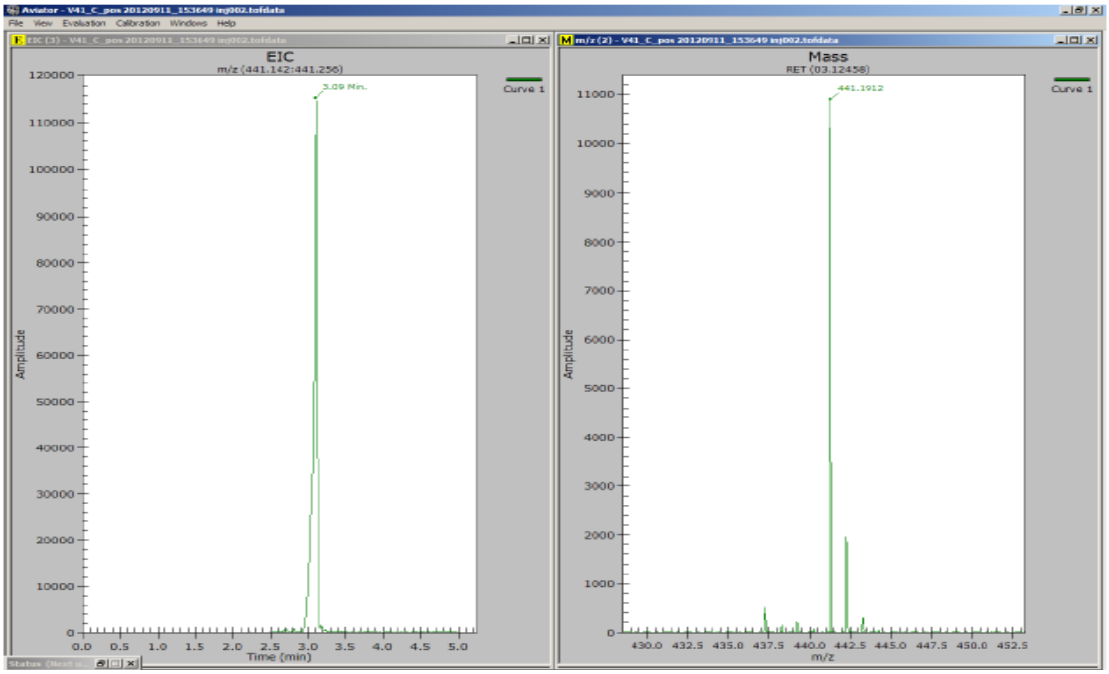
Appendix 51C: gCHSQC (CD_3OD, 800 MHz) spectrum of flemingin O (**205**)



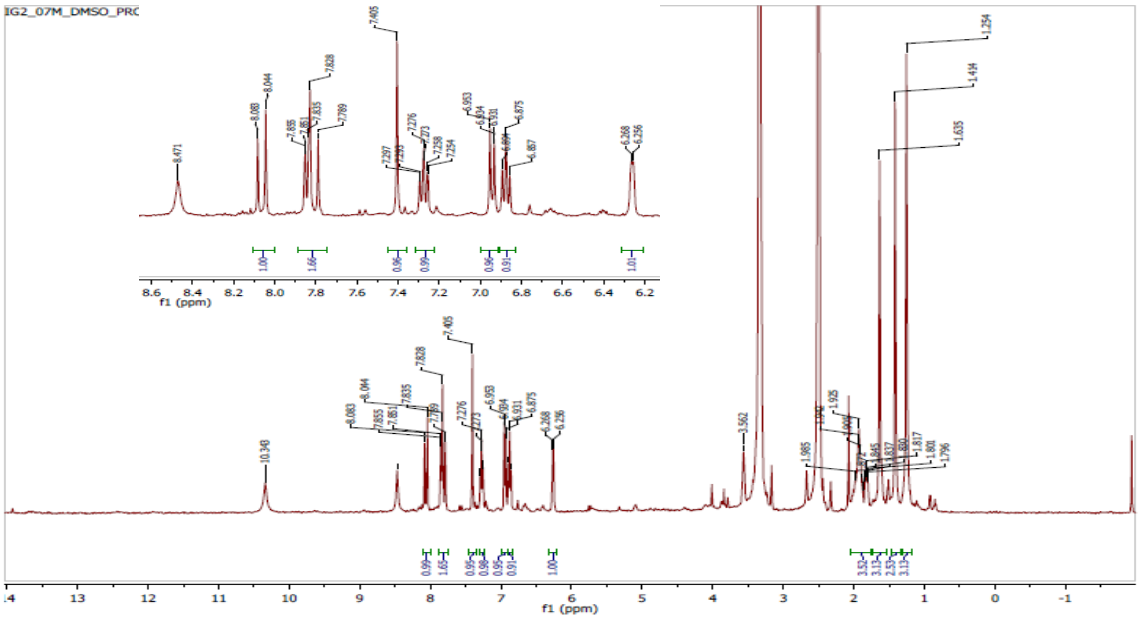
Appendix 51D: gHMBC (CD_3OD, 800 MHz) spectrum of flemingin O (**205**)



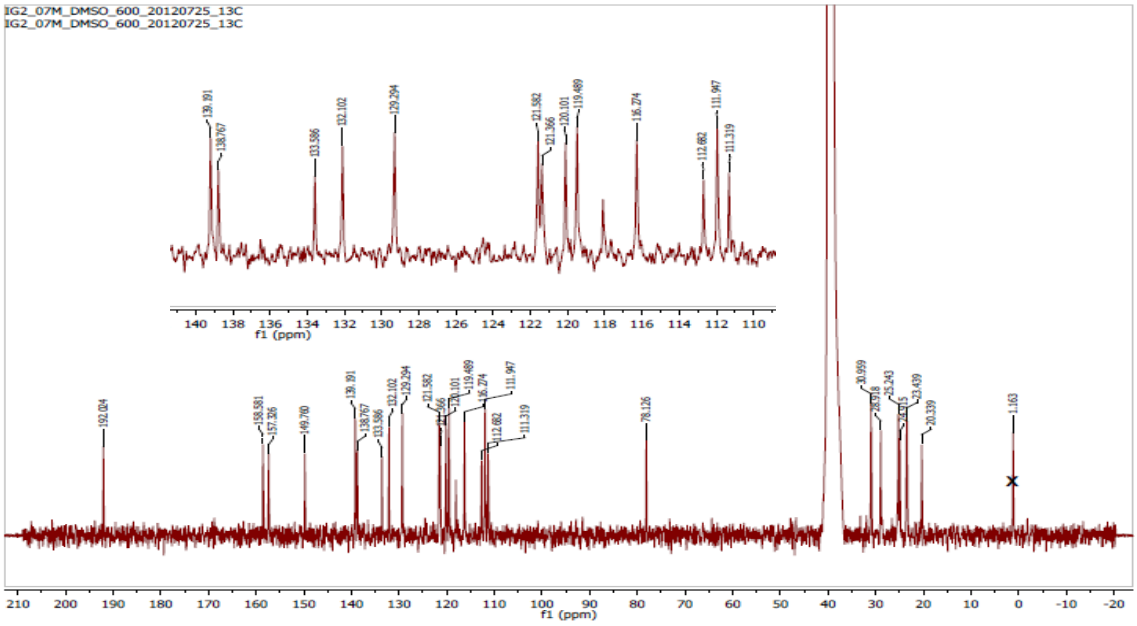
Appendix 51E: HRESI-MS of flemingin O (**205**)
IG2-84D M+H 441.1913



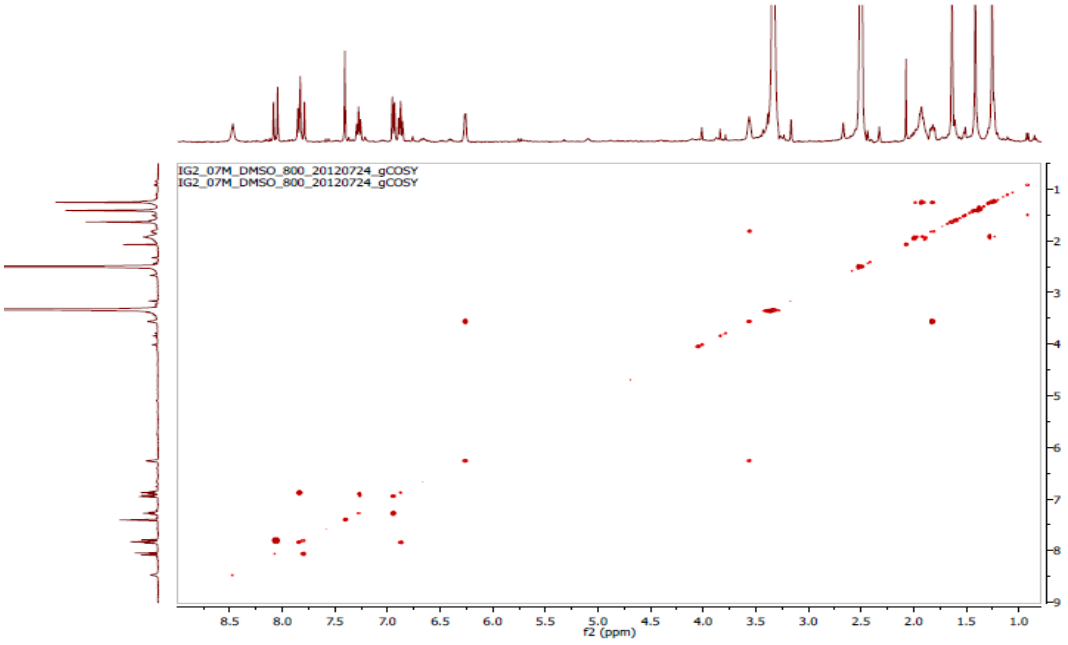
Appendix 52A: ^{1}H ($(CD_3)_2SO$, 400 MHz) NMR spectrum of flemingin P (**206**)



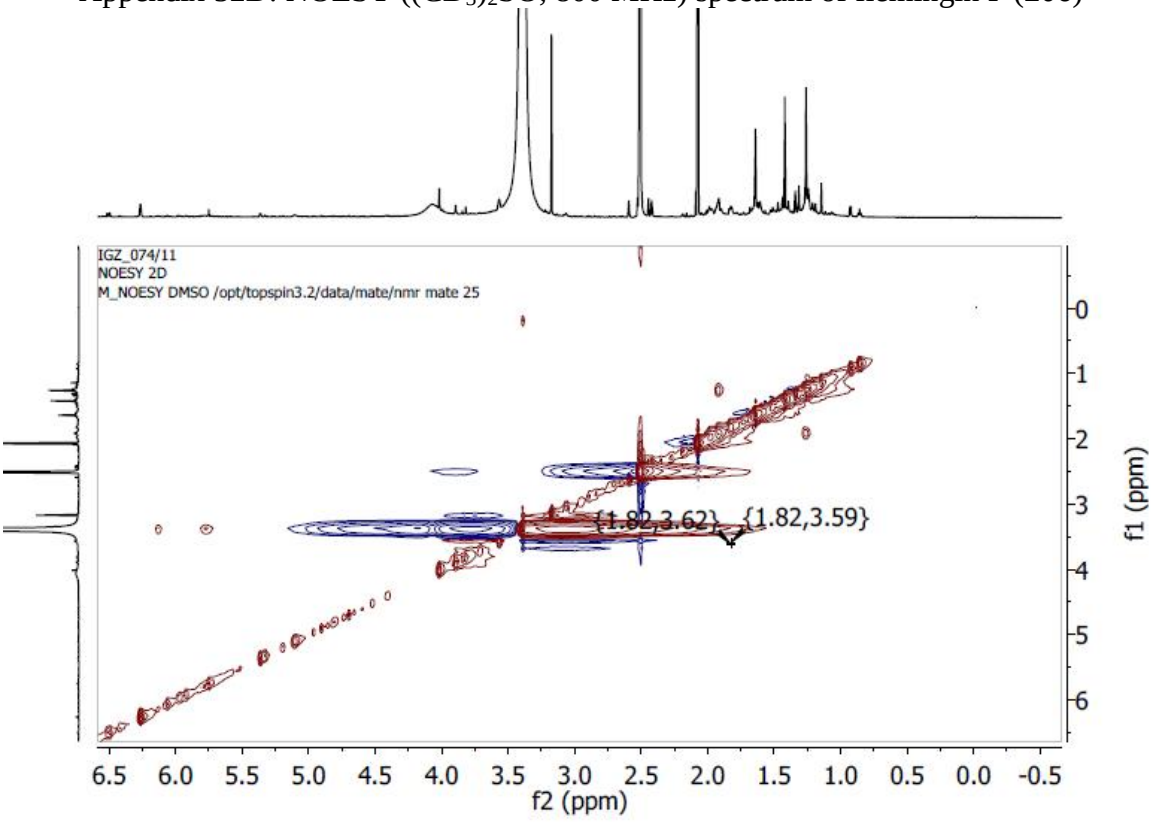
Appendix 52B: ^{13}C ($(CD_3)_2SO$, 150 MHz) NMR spectrum of flemingin P (**206**)



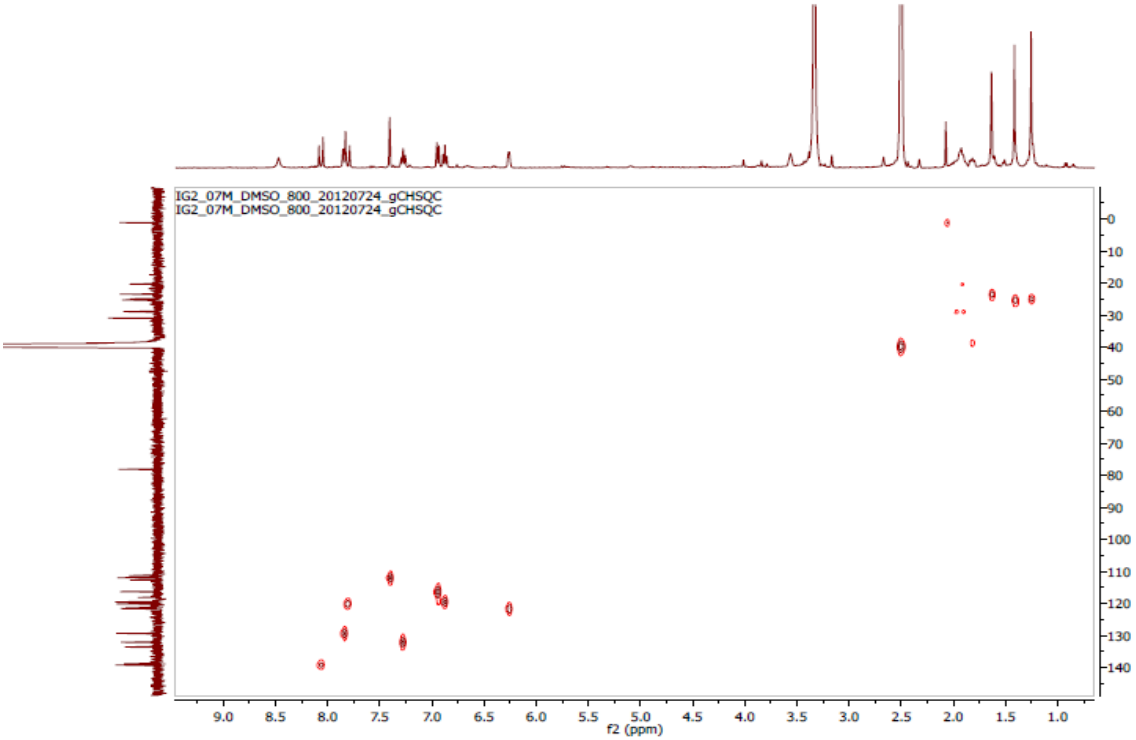
Appendix 52C: ^{1}H-^{1}H gCOSY ($(CD_3)_2SO$, 800 MHz) spectrum of flemingin P (**206**)



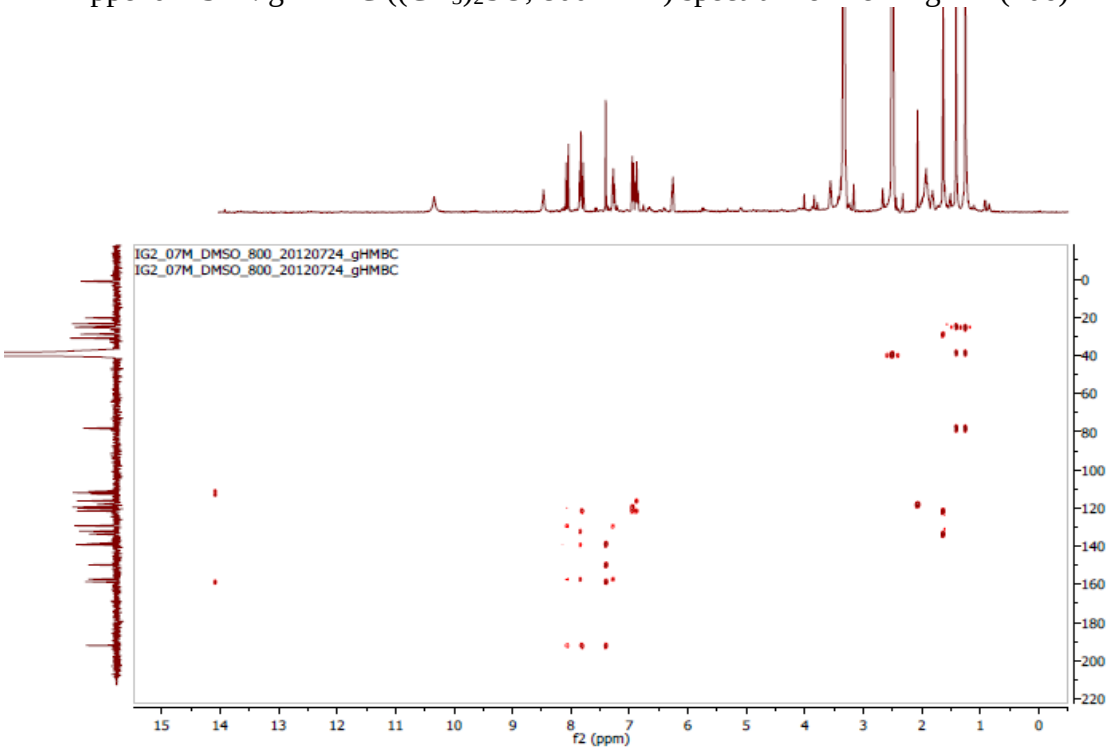
Appendix 52D: NOESY ($(CD_3)_2SO$, 800 MHz) spectrum of flemingin P (**206**)



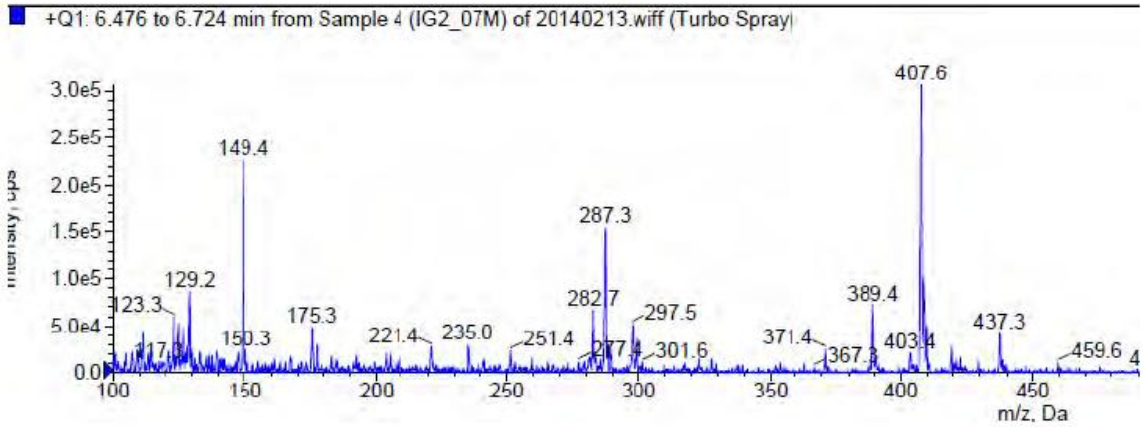
Appendix 52E: gCHSQC ($(CD_3)_2SO$, 800 MHz) spectrum of flemingin P (**206**)



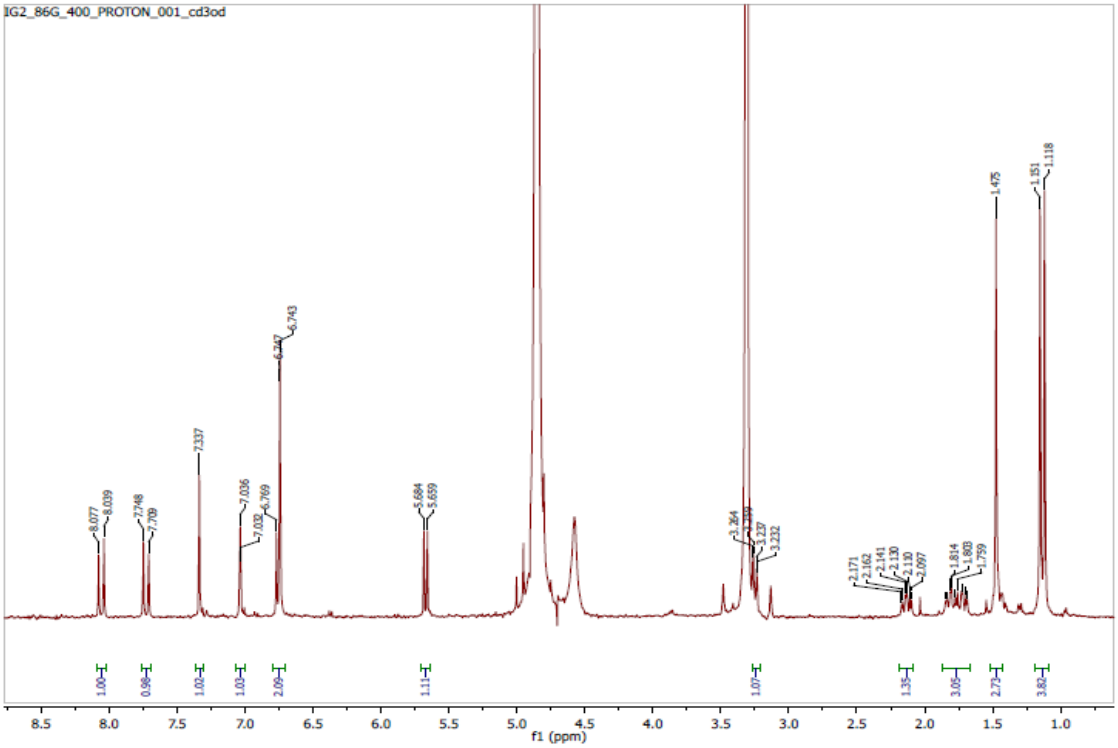
Appendix 52F: gHMBC ($(CD_3)_2SO$, 800 MHz) spectrum of flemingin P (**206**)



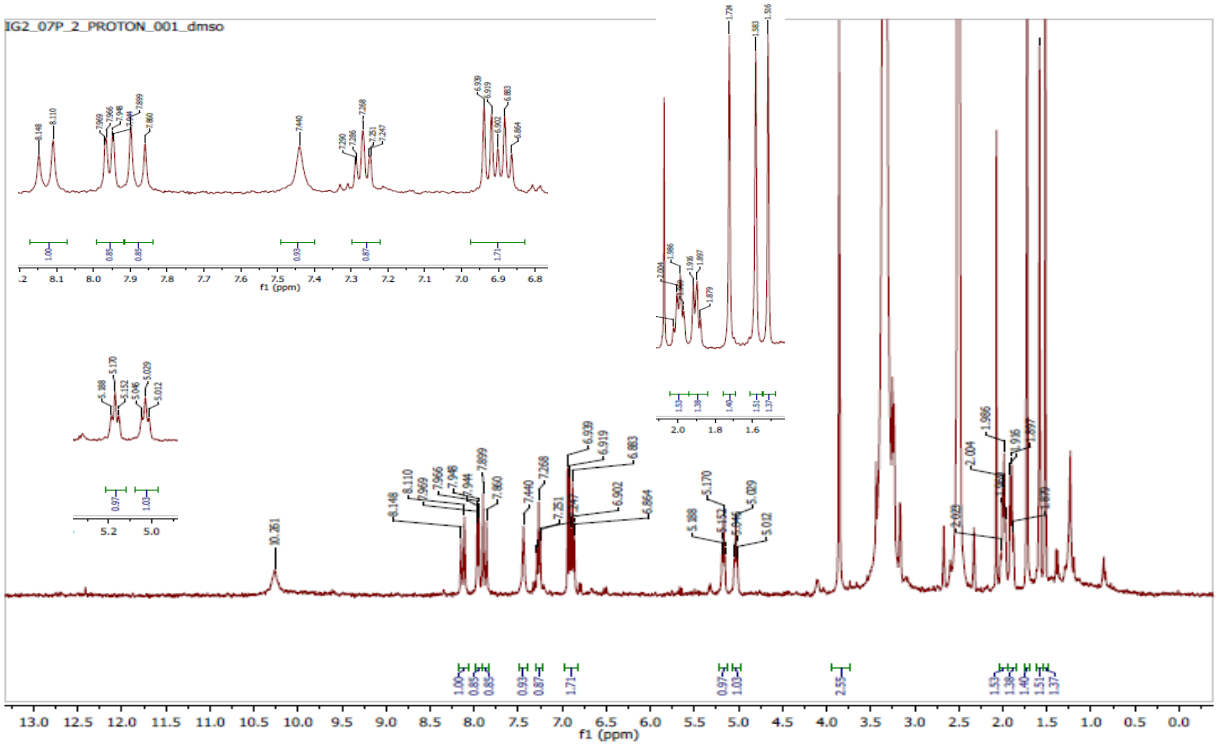
Appendix 52G: LC-ESI-MS of flemingin P (**206**)



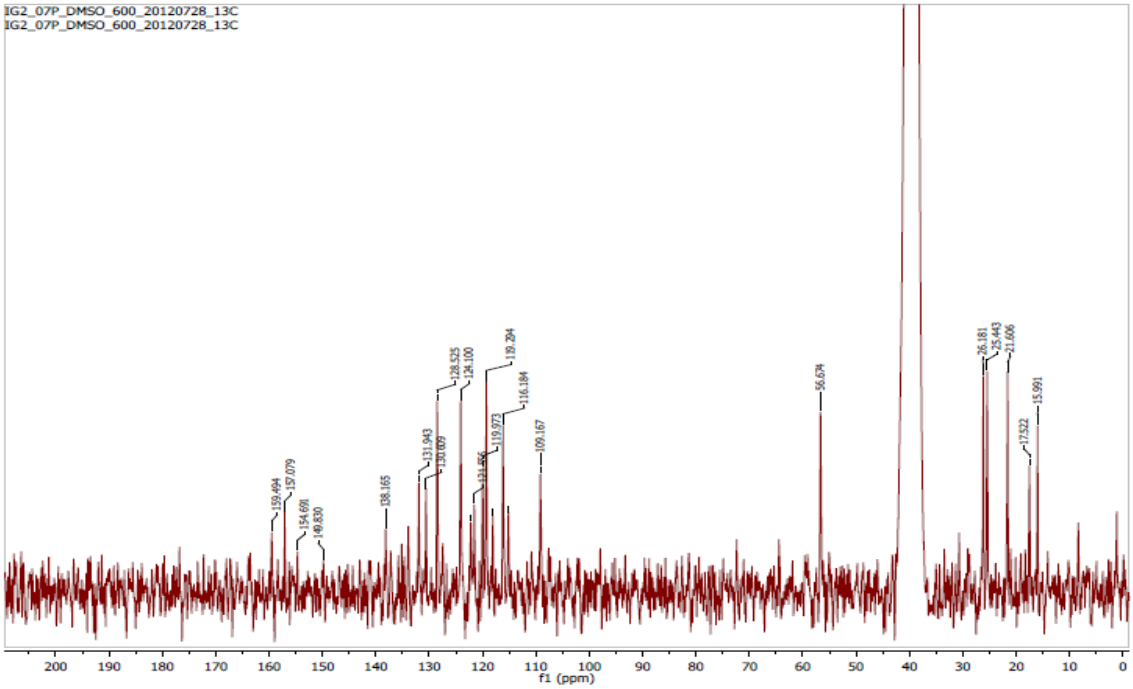
Appendix 53A: ^{1}H (CD_3OD, 400 MHz) NMR spectrum of flemingin Q (**207**)



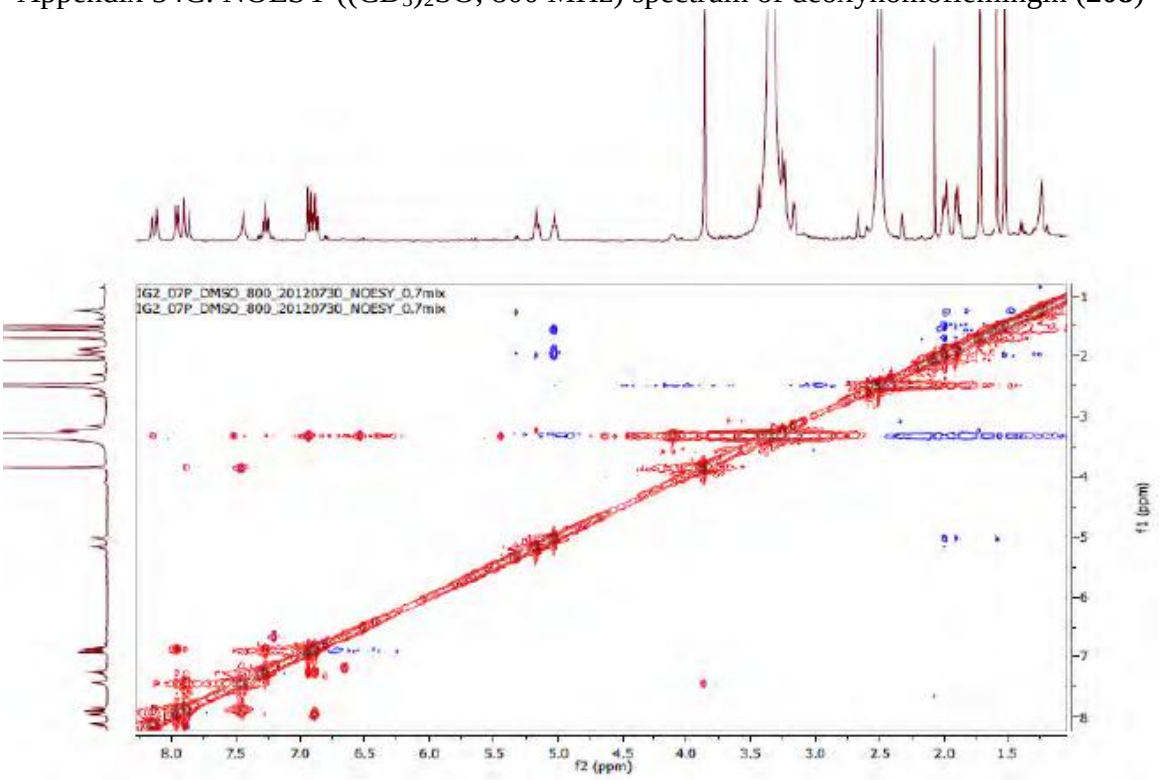
Appendix 54A: ^{1}H ($(CD_3)_2SO$, 400 MHz) NMR spectrum of deoxyhomoflemingin (**208**)



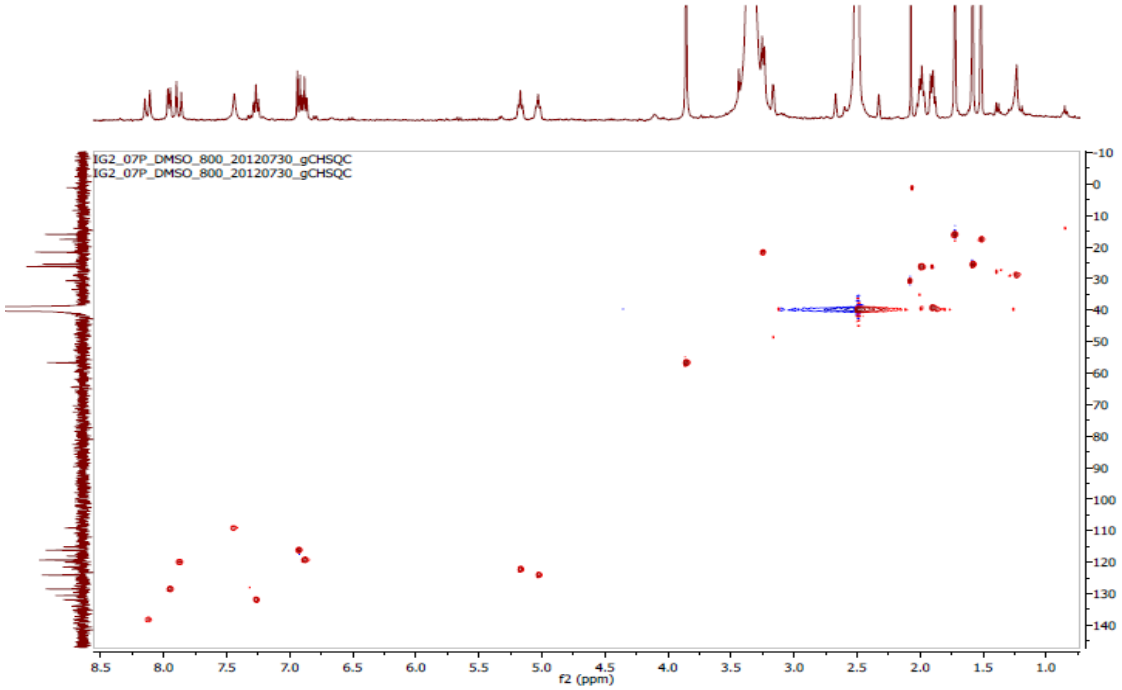
Appendix 54B: ^{13}C ($(CD_3)_2SO$, 150 MHz) NMR spectrum of deoxyhomoflemingin (**208**)



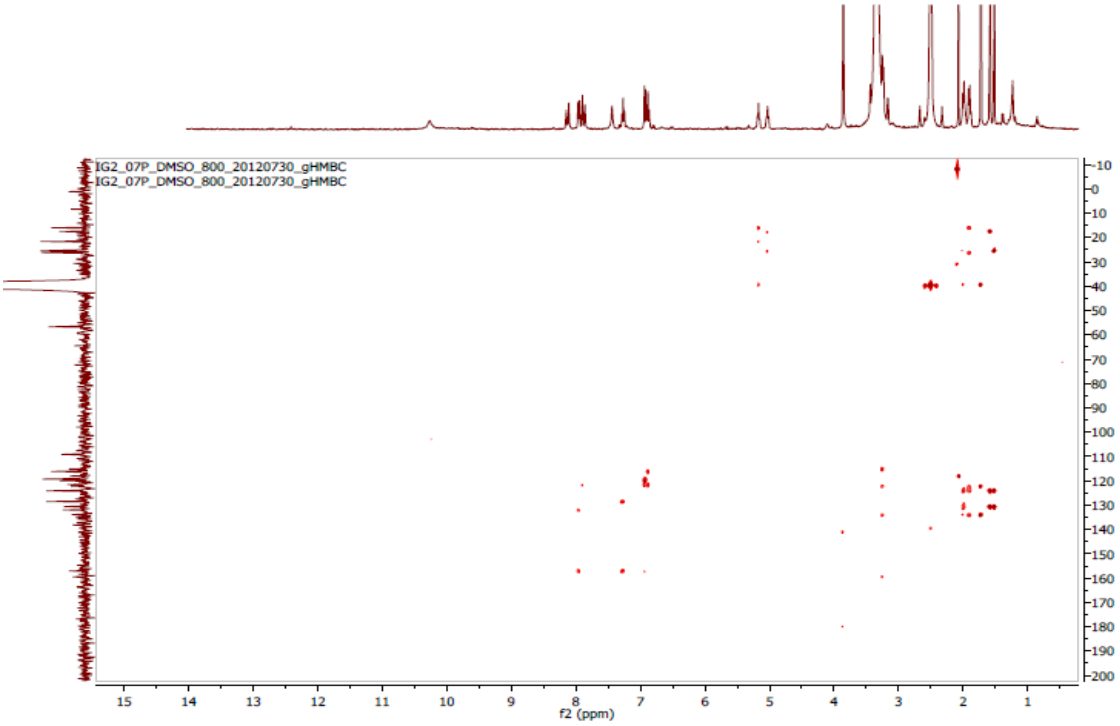
Appendix 54C: NOESY ($(CD_3)_2SO$, 800 MHz) spectrum of deoxyhomoflemingin (**208**)



Appendix 54D: gCHSQC ($(CD_3)_2SO$, 800 MHz) spectrum of deoxyhomoflemingin (**208**)



Appendix 54E: gHMBC ($(CD_3)_2SO$, 800 MHz) spectrum of deoxyhomoflemingin (**208**)



Appendix 54F: LC-ESI-MS spectrum of deoxyhomoflemingin (**208**)

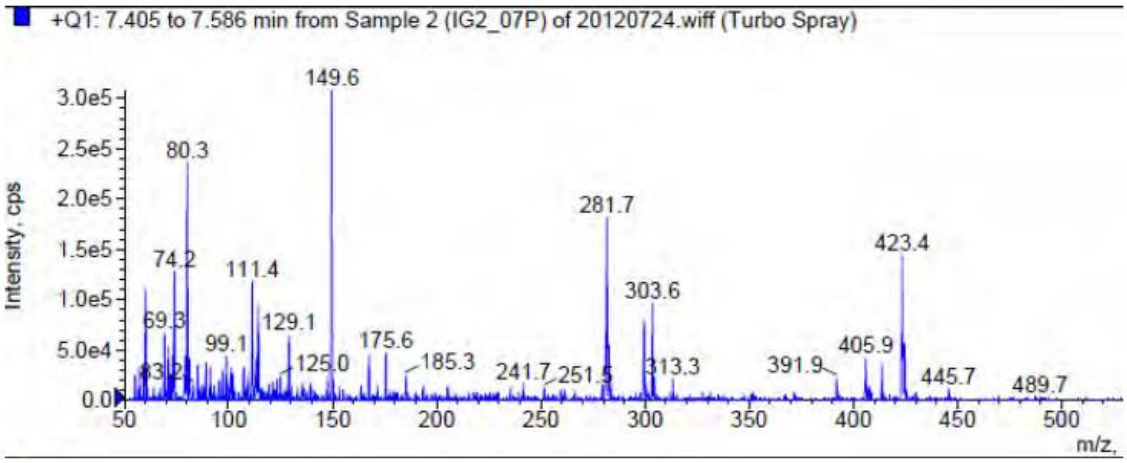
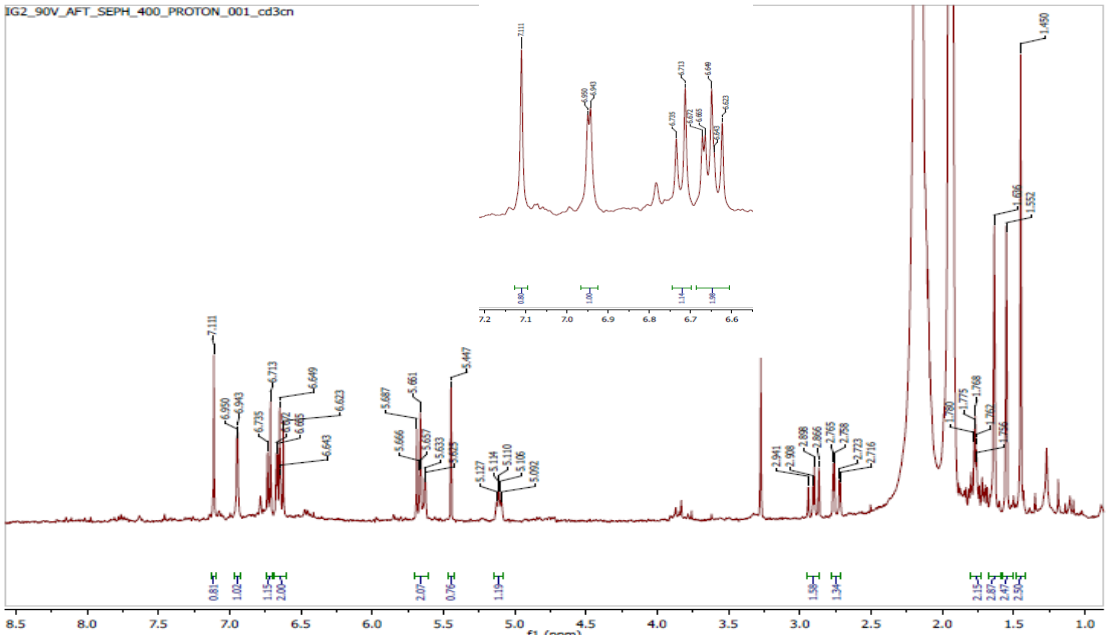
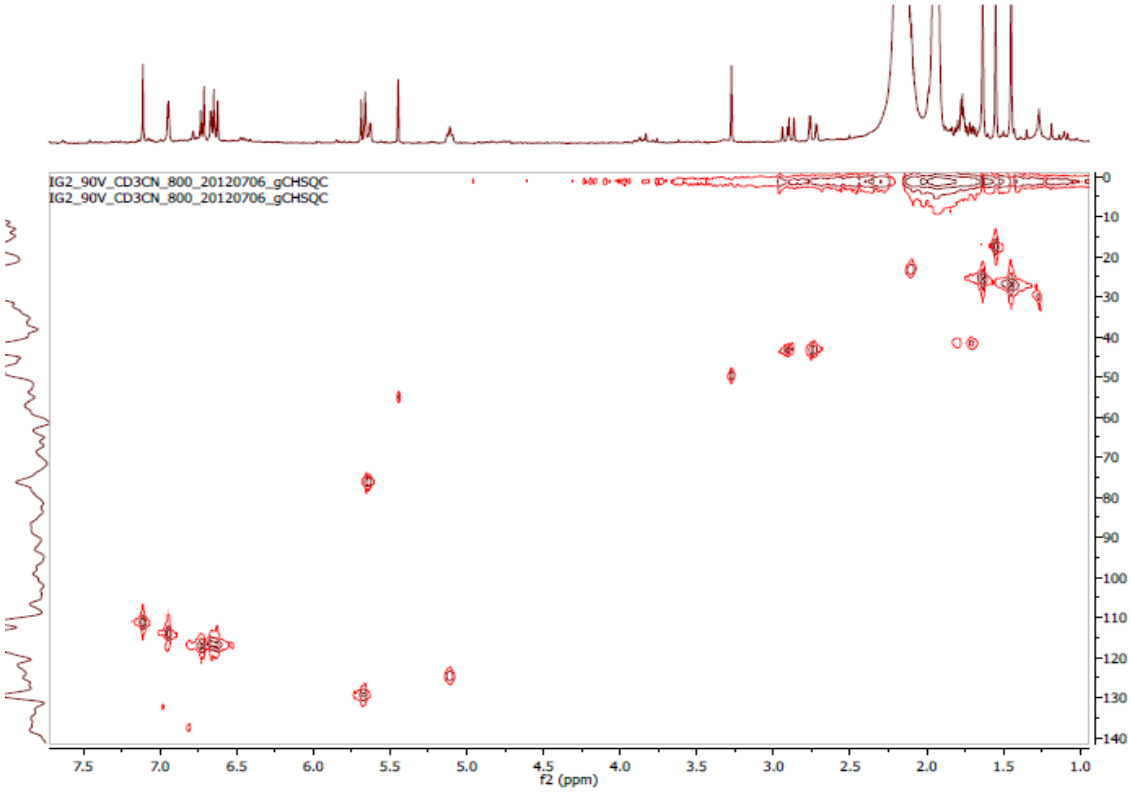


Figure S111. The ESI MS of deoxyhomoflemingin (**14**).

Appendix 55A: ^{1}H (CD_3CN, 400 MHz) NMR spectrum of fleminginflavanone A (**209**)



Appendix 55B: gCHSQC (CD_3CN, 800 MHz) spectrum of fleminginflavanone A (**209**)



Appendix 55C: HRESI-MS of fleminginflavanone A (**209**)

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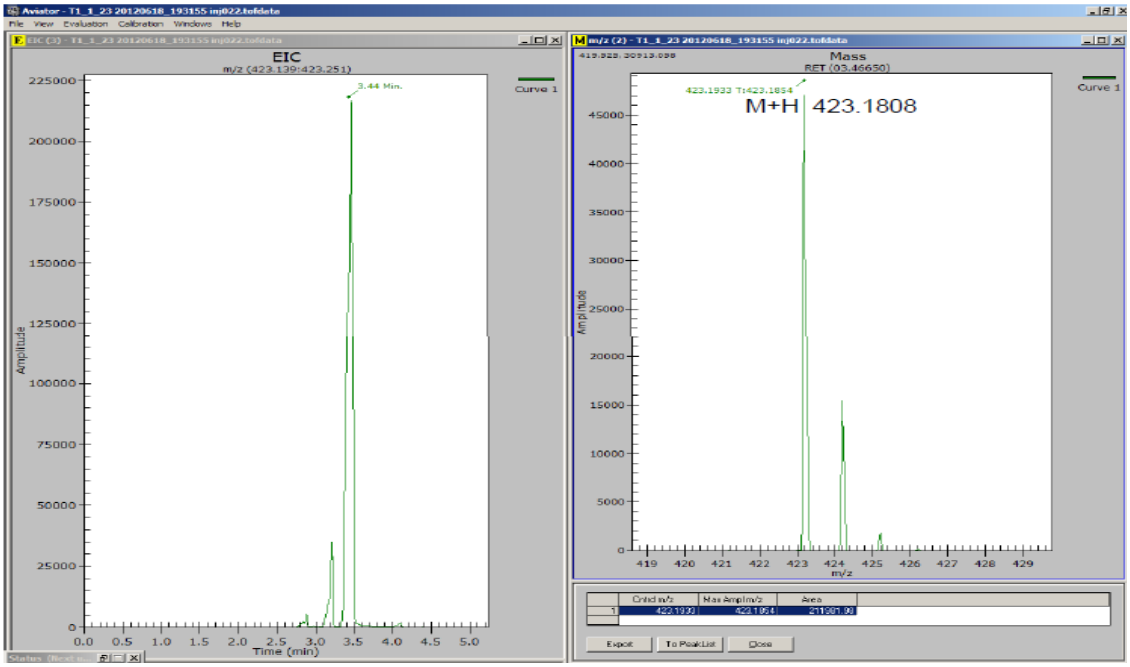
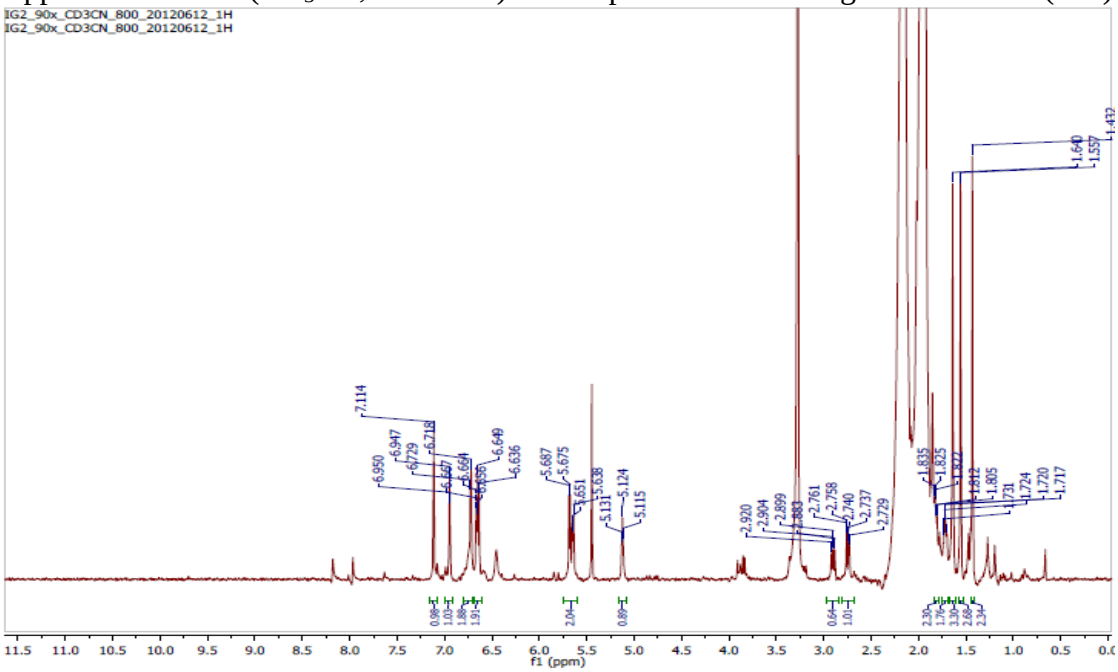
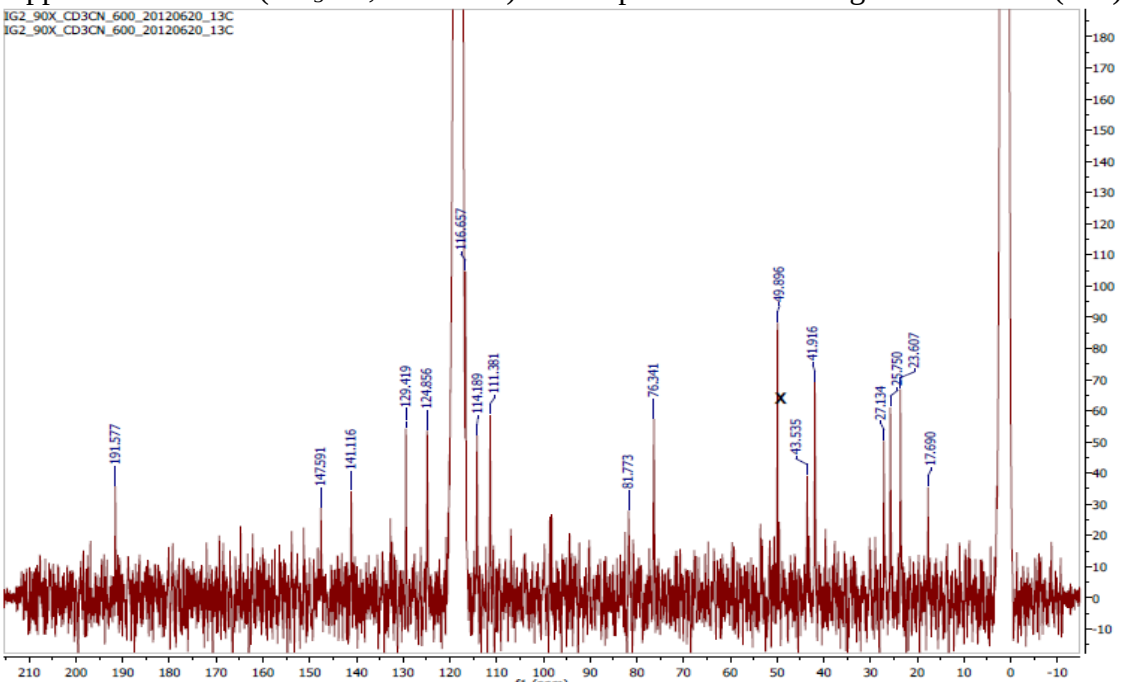


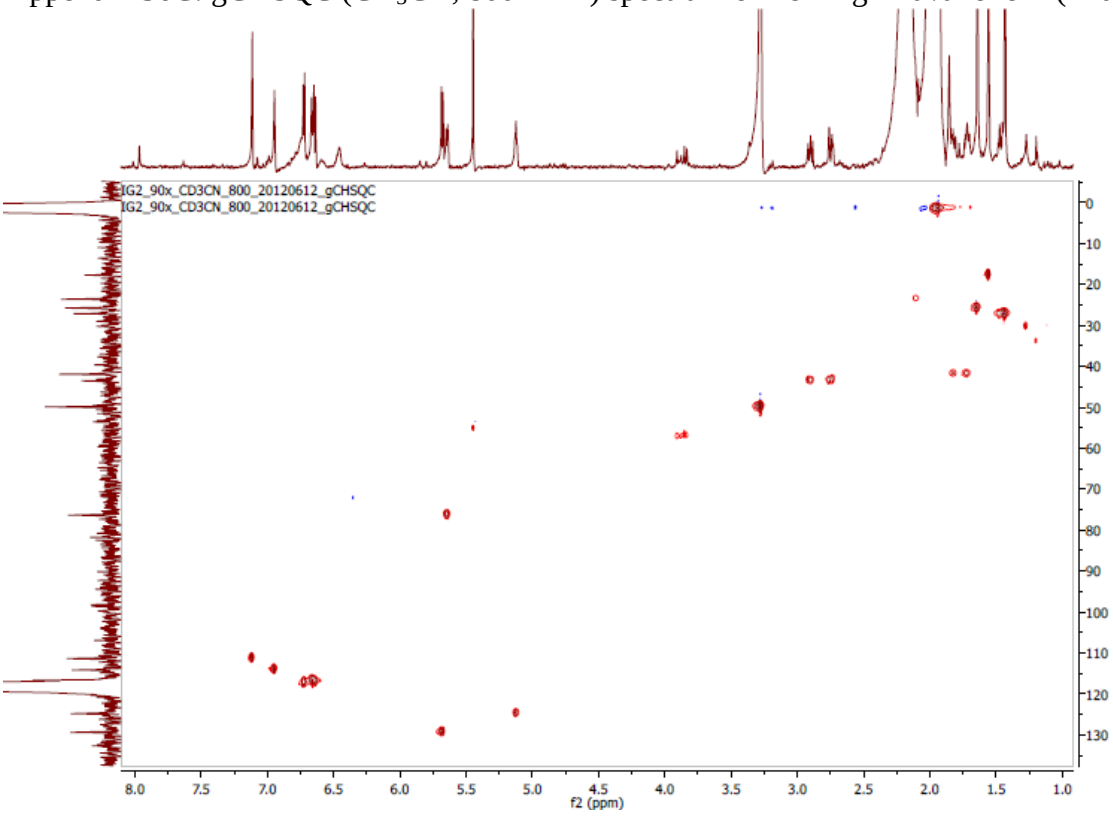
Appendix 56A: ^{1}H (CD_3CN, 800 MHz) NMR spectrum of fleminginflavanone B (**210**)



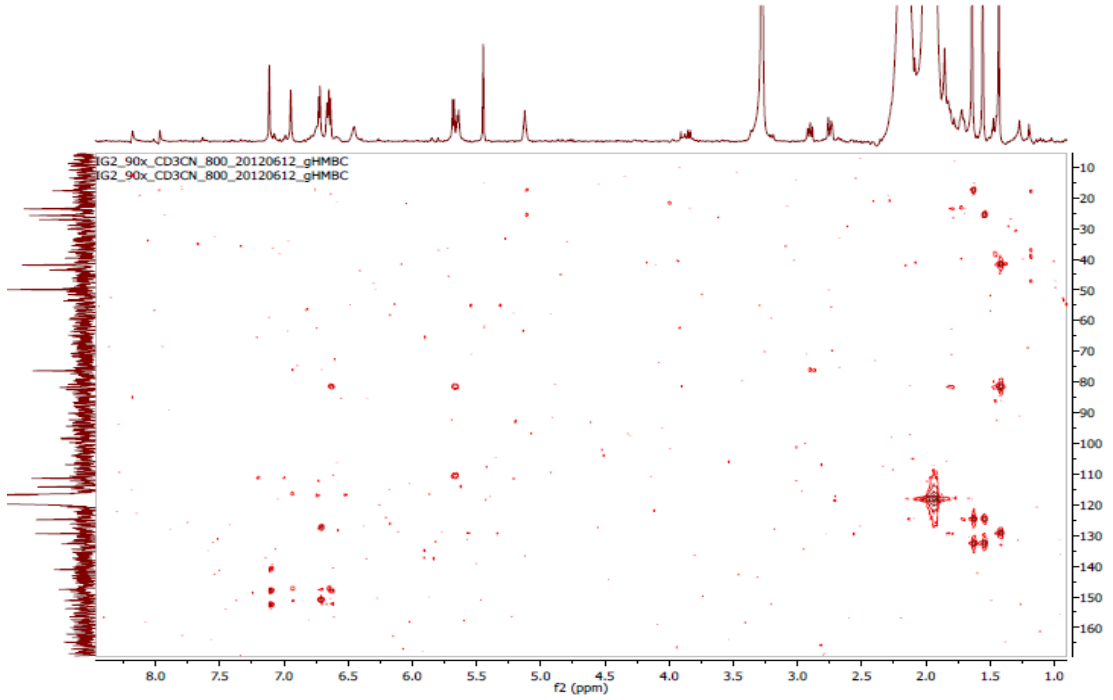
Appendix 56B: ^{13}C (CD_3CN, 150 MHz) NMR spectrum of fleminginflavanone B (**210**)



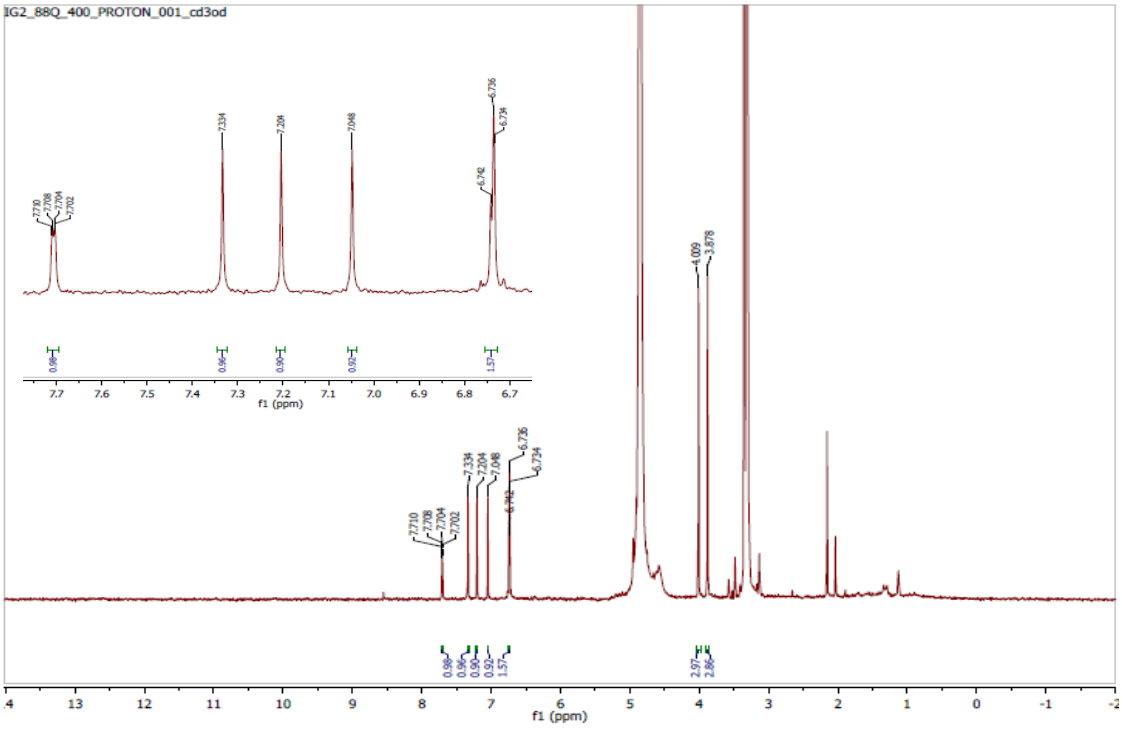
Appendix 56C: gCHSQC (CD_3CN, 800 MHz) spectrum of fleminginflavanone B (**210**)



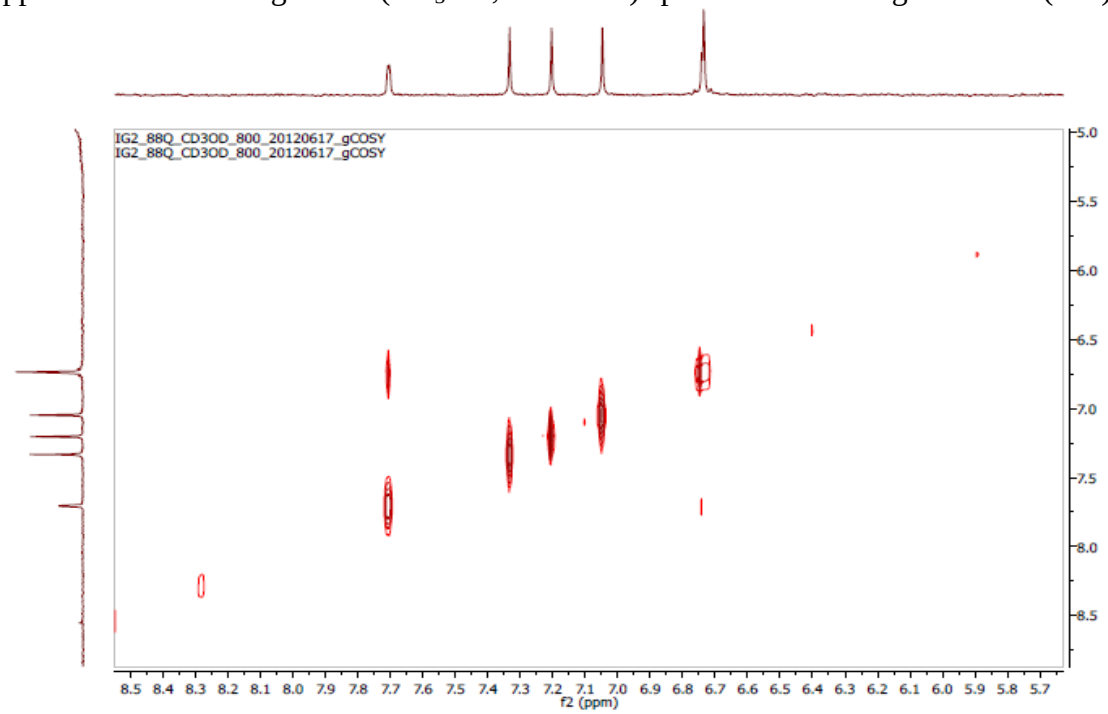
Appendix 56D: gHMBC (CD_3CN, 800 MHz) spectrum of fleminginflavanone B (**210**)



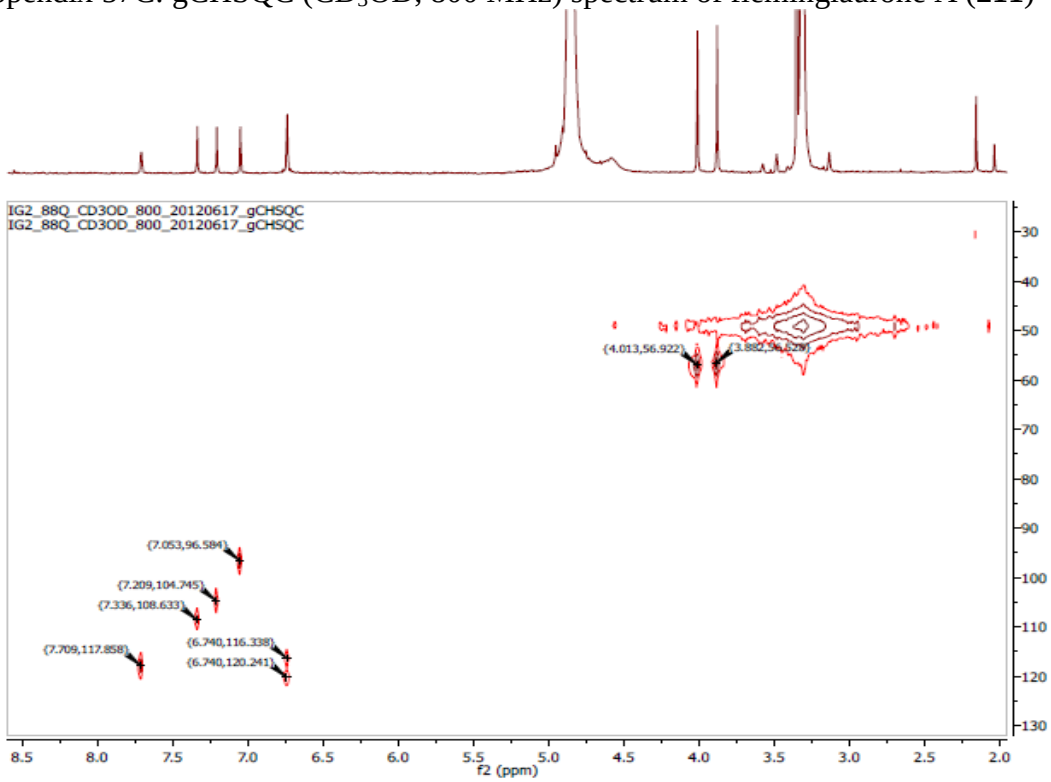
Appendix 57A: ^{1}H (CD_3OD, 400 MHz) NMR spectrum of flemingiaurone A (**211**)



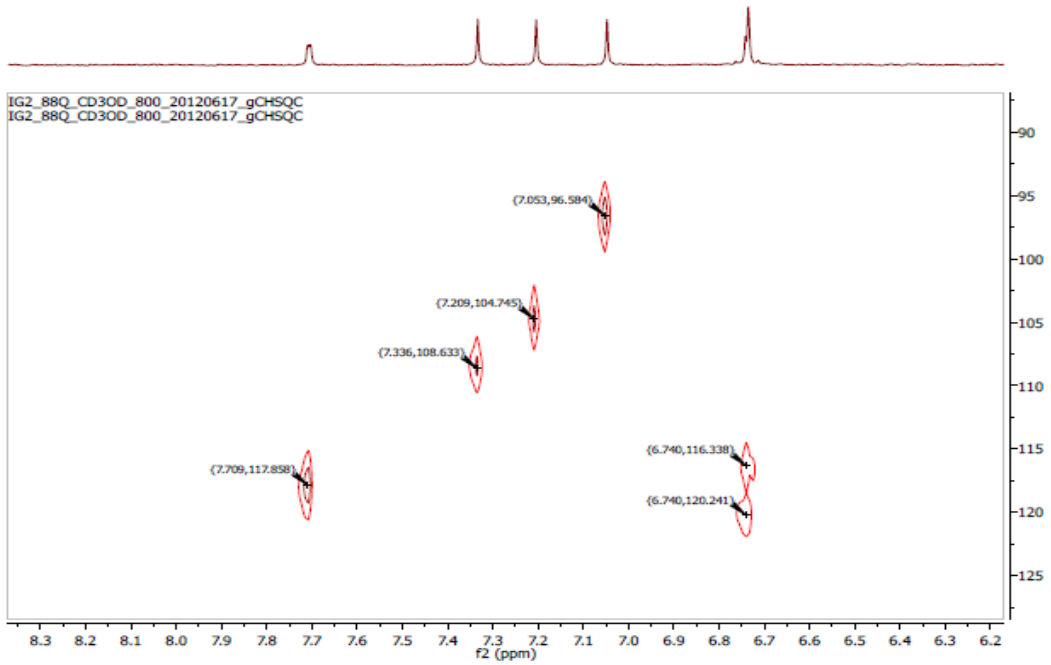
Appendix 57B: ^{1}H-^{1}H gCOSY (CD_3OD, 800 MHz) spectrum of flemingiaurone A (**211**)



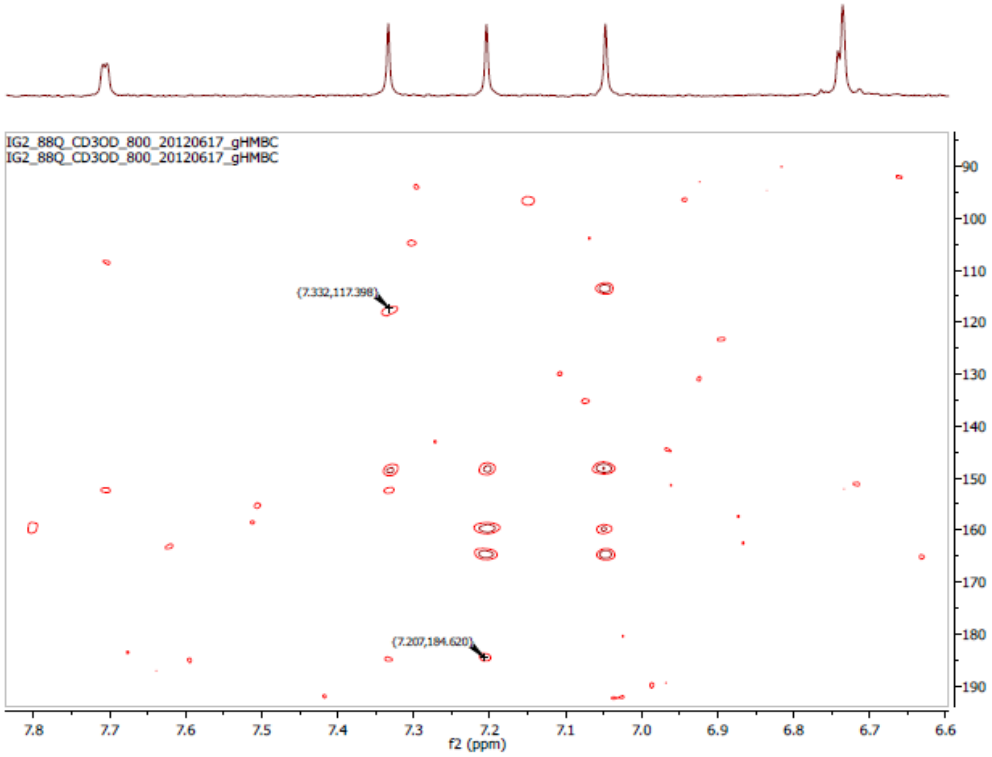
Appendix 57C: gCHSQC (CD_3OD, 800 MHz) spectrum of flemingiaurone A (**211**)



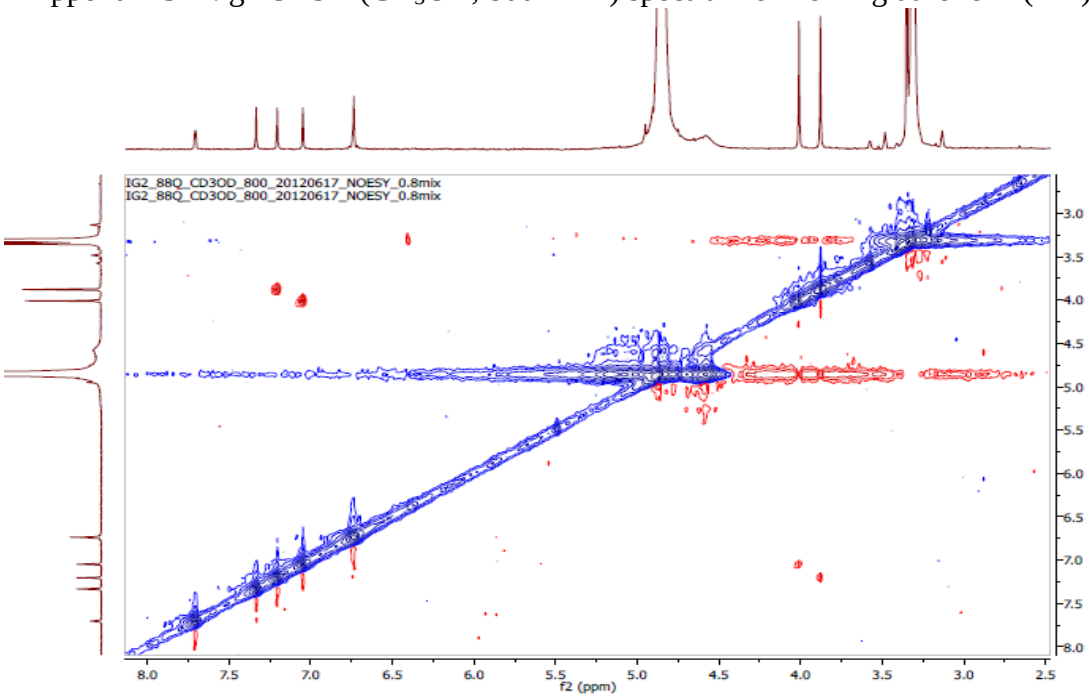
Appendix 57D: expanded gCHSQC (CD_3OD, 800 MHz) spectrum of flemingiaurone A (**211**)



Appendix 57E: gHMBC (CD_3OD, 800 MHz) spectrum of flemingiaurone A (**211**)

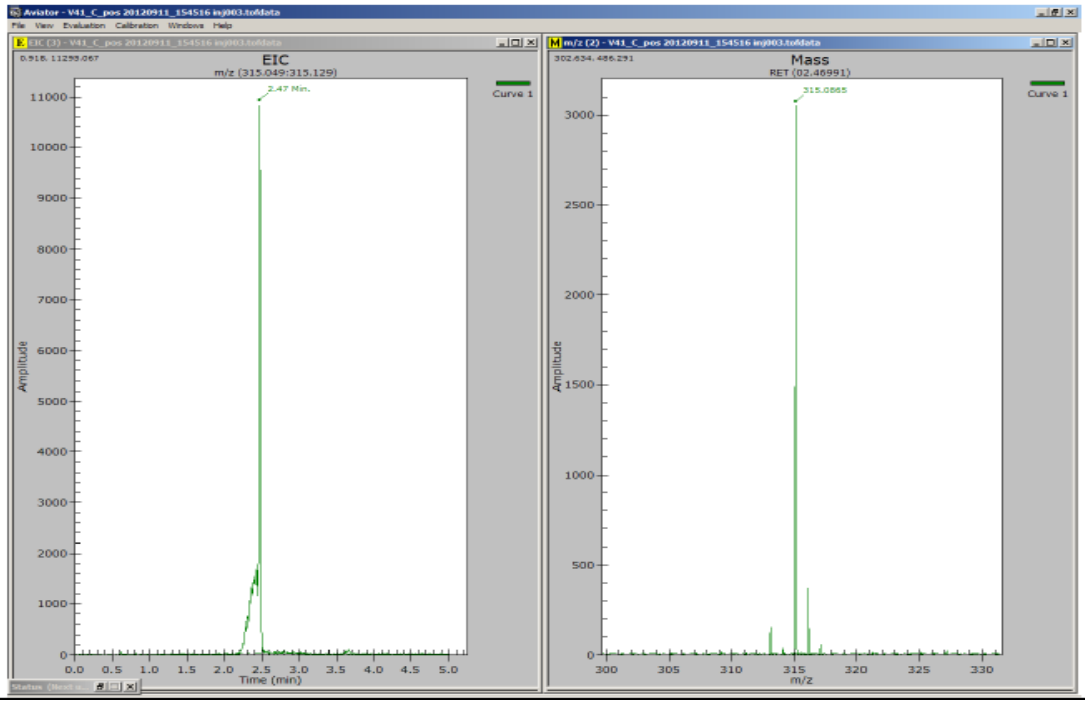


Appendix 57F: gNOESY (CD_3OD, 800 MHz) spectrum of flemingiaurone A (**211**)

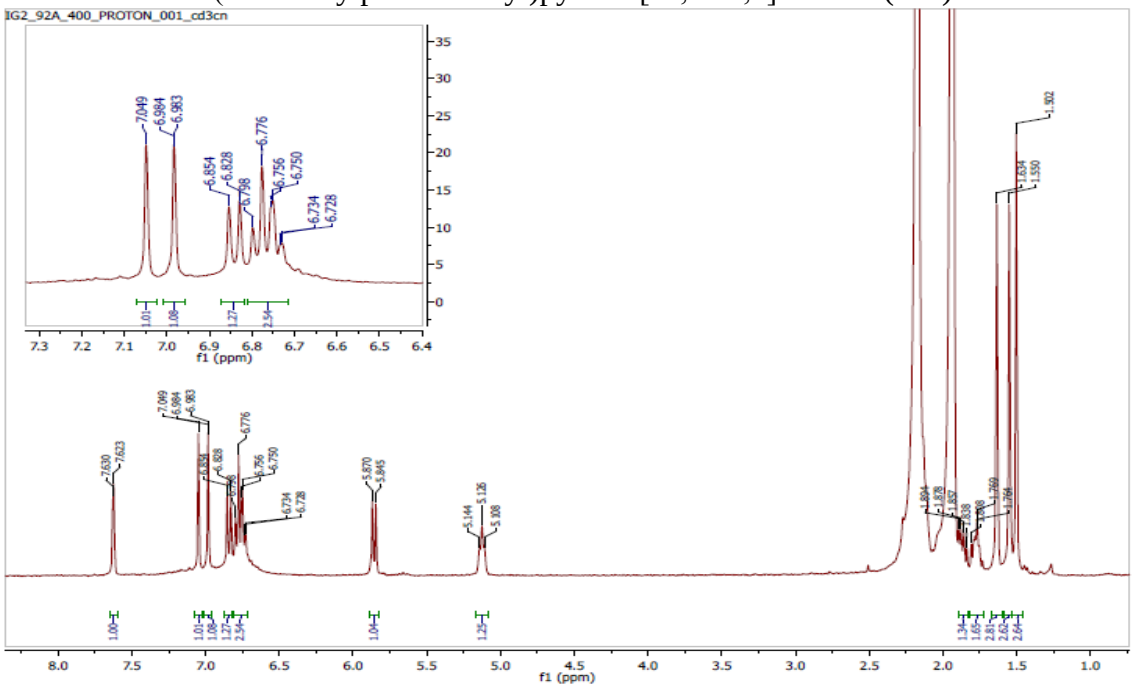


Appendix 57G: HRESI-MS of flemingiaurone A (**211**)

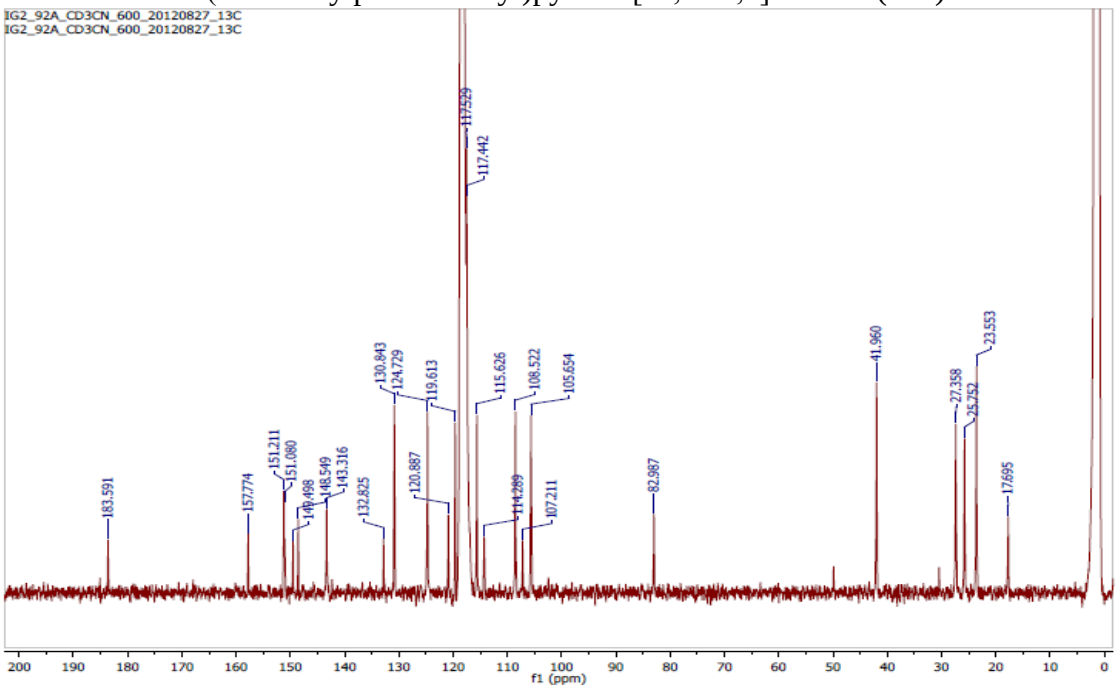
IG2-88Q M+H 315.0869



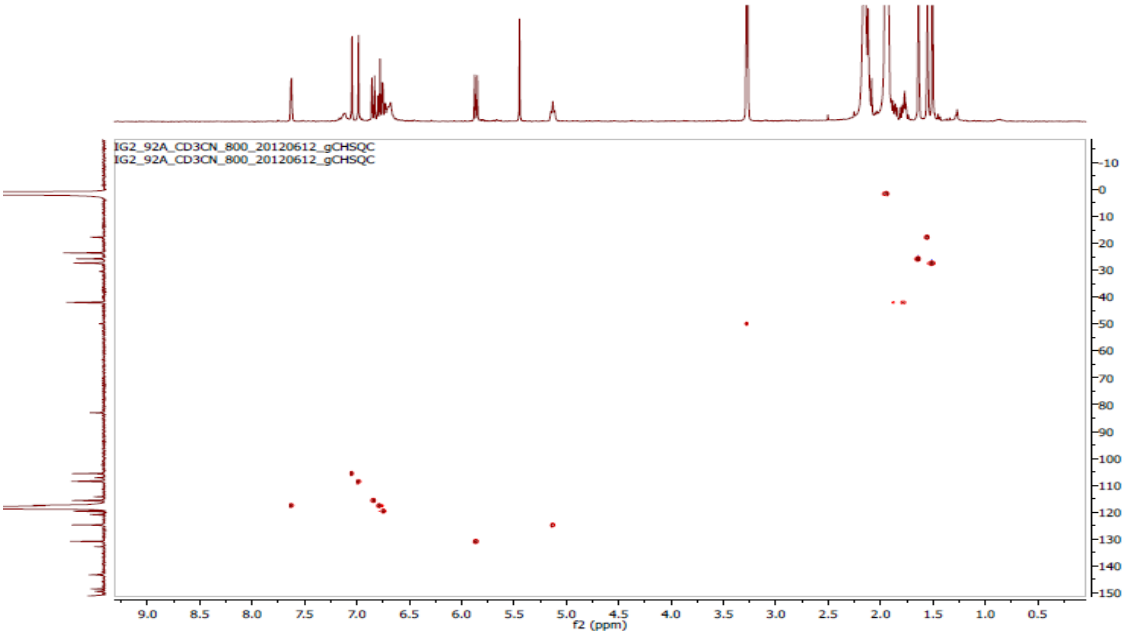
Appendix 58A: ^{1}H (CD_3CN, 400 MHz) NMR spectrum of 5,2′,5′-trihydroxy-2″-methyl-2″(4‴-methylpent-3‴-enyl)pyrano-[5″,6″:7,6]-aurone (**212**)



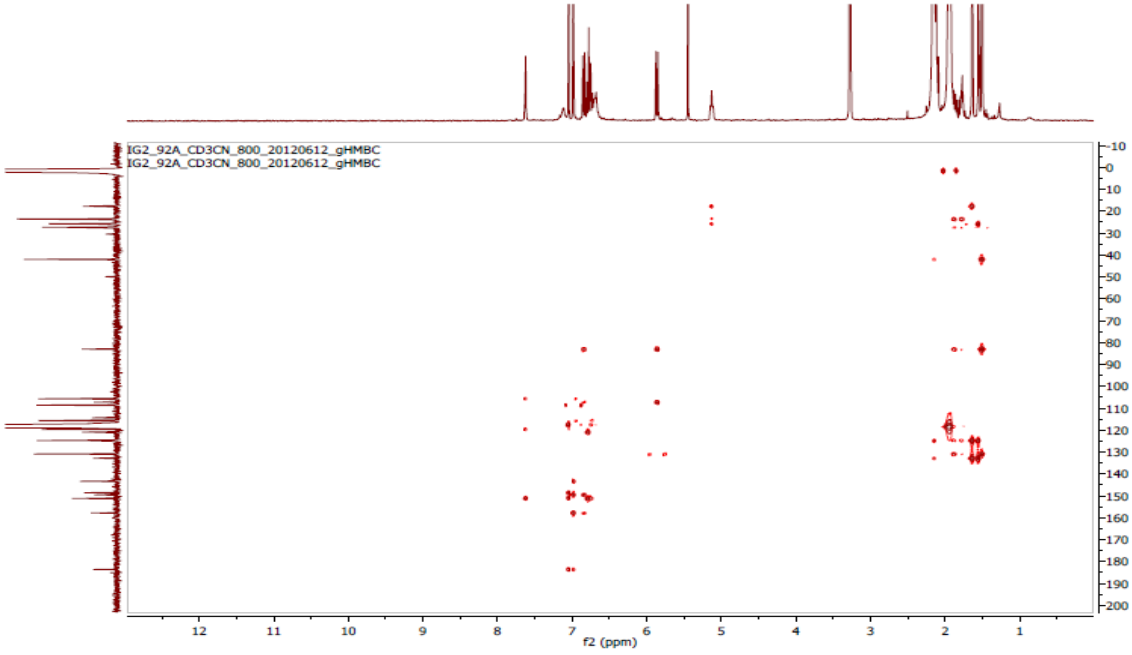
Appendix 58B: ^{13}C (CD_3CN, 150 MHz) NMR spectrum of 5,2′,5′-trihydroxy-2″-methyl-2″(4‴-methylpent-3‴-enyl)pyrano-[5″,6″:7,6]-aurone (**212**)



Appendix 58C: gCHSQC (CD_3CN, 800 MHz) spectrum of 5,2′,5′-trihydroxy-2″-methyl-2″(4‴-methylpent-3‴-enyl)pyrano-[5″,6″:7,6]-aurone (**212**)

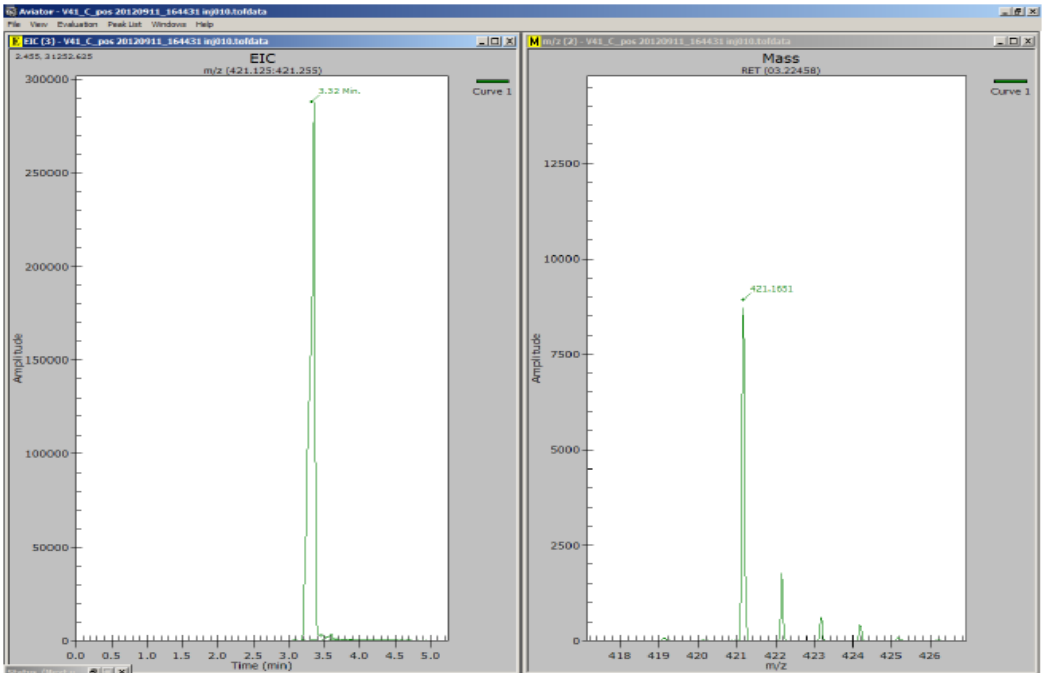


Appendix 58D: gHMBC (CD_3CN, 800 MHz) spectrum of 5,2′,5′-trihydroxy-2″-methyl-2″(4‴-methylpent-3‴-enyl)pyrano-[5″,6″:7,6]-aurone (**212**)

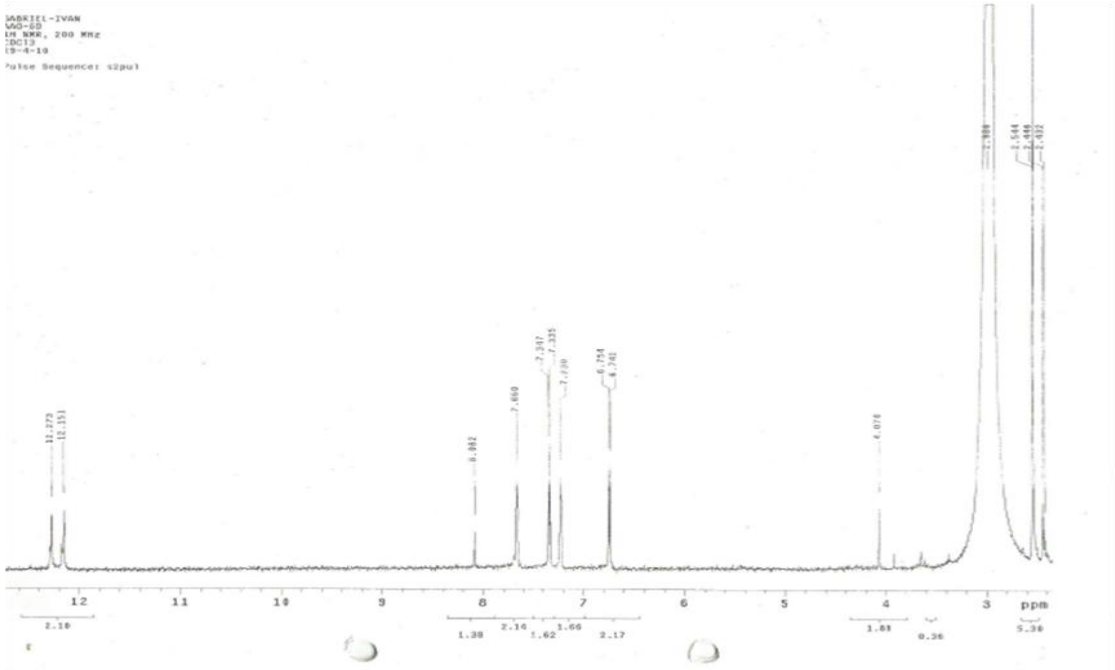


Appendix 58E: HRESI-MS of 5,2′,5′-trihydroxy-2″-methyl-2″(4‴-methylpent-3‴-enyl)pyrano-[5″,6″:7,6]-aurone (**212**)

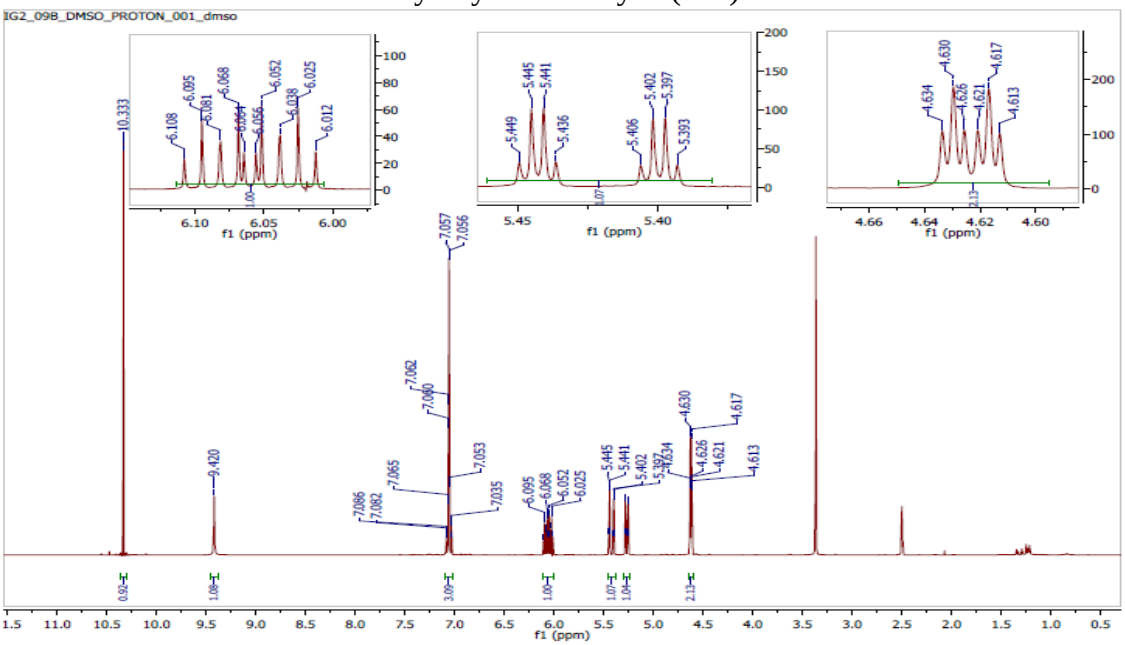
IG2-91A M+H ?



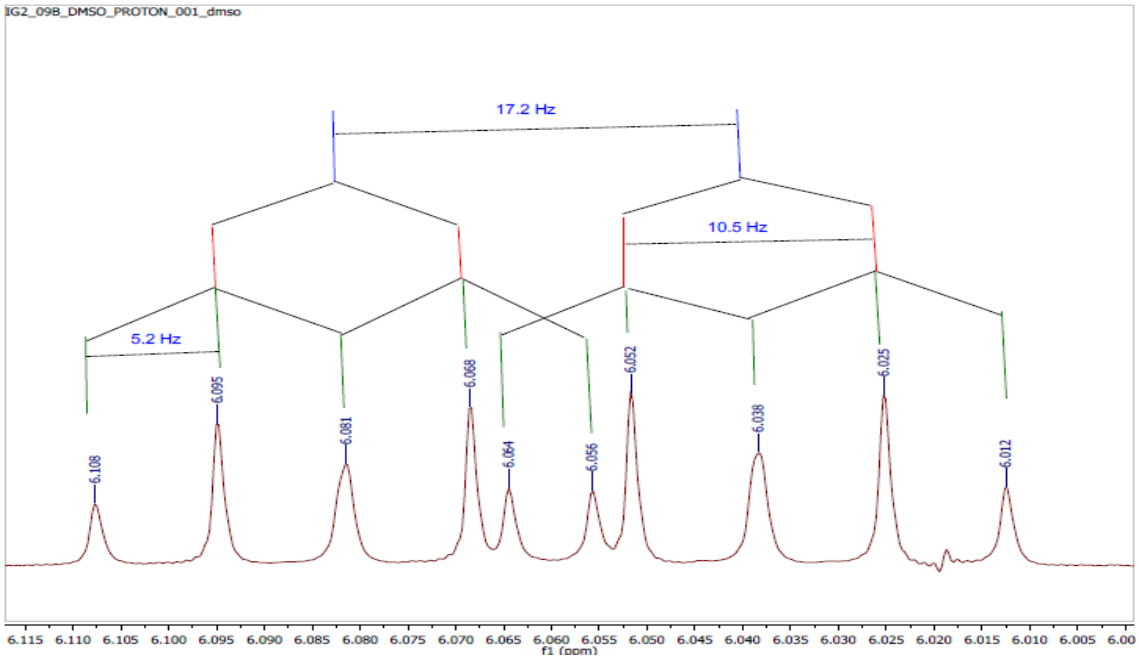
Appendix 59A: ^{1}H ($CDCl_3$, 200 MHz) NMR spectrum of emodin (**213**)



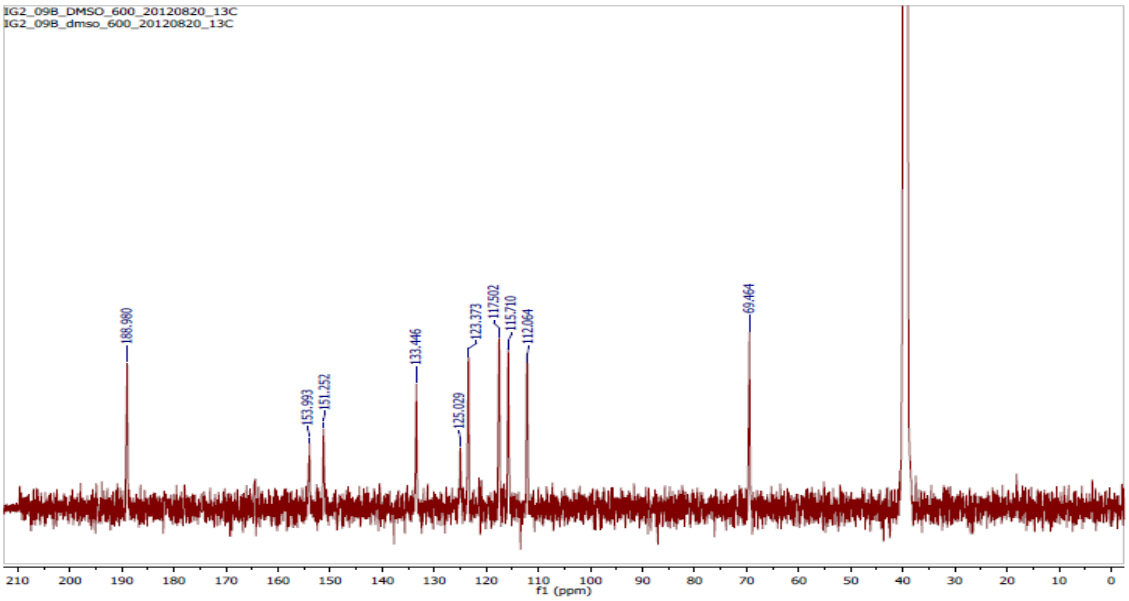
Appendix 60A: ^{1}H ($(CD_3)_2SO$, 400 MHz) NMR spectrum of 2-hydroxy-5-allyloxybenzaldehyde (**214**)



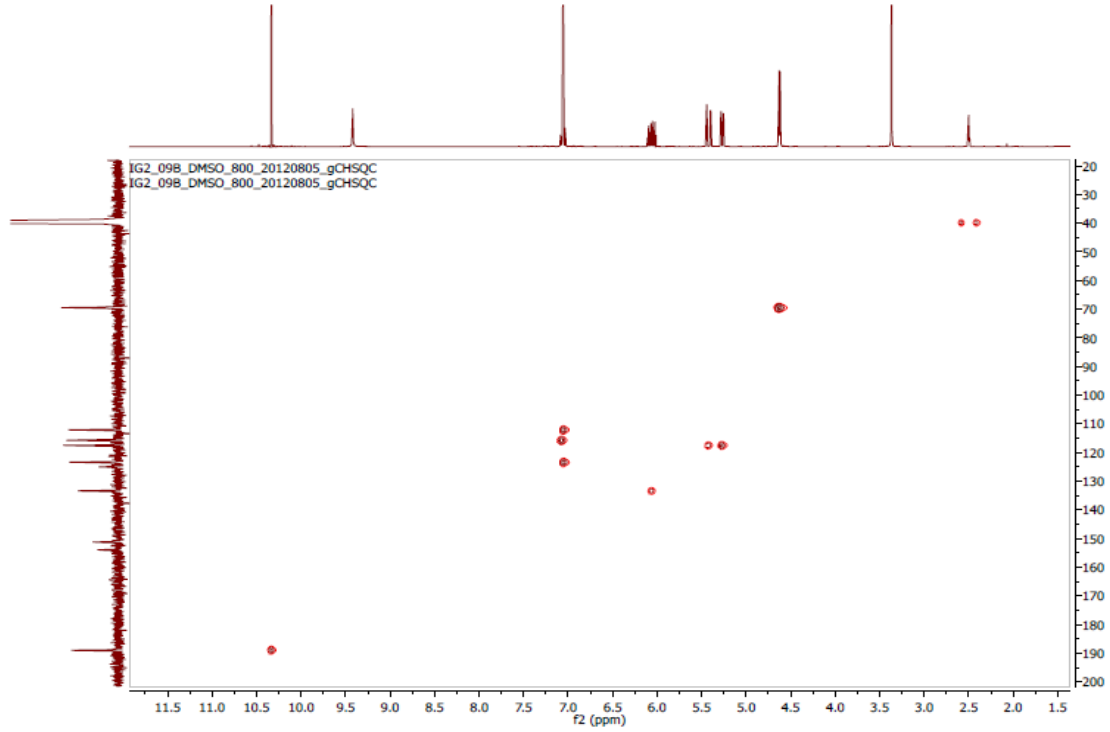
Appendix 60B: Graphical analysis of the multiplicity of H-2′ in ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) spectrum of 2-hydroxy-5-allyloxybenzaldehyde (**214**)



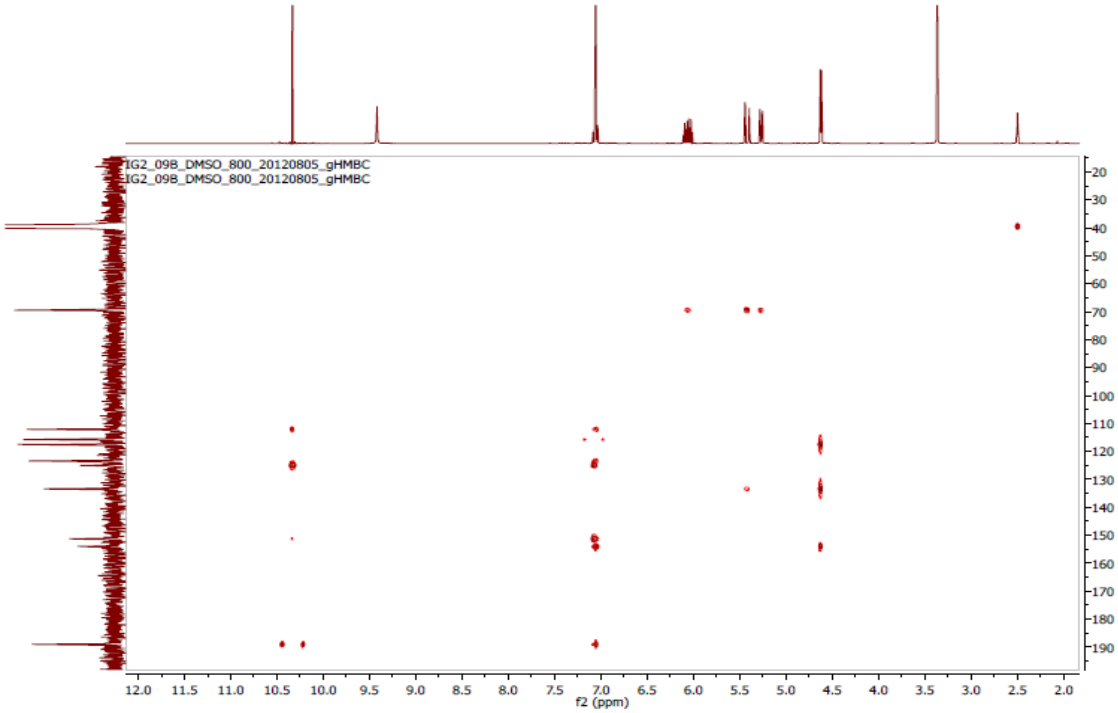
Appendix 60C: ^{13}C ($(CD_3)_2SO$, 150 MHz) NMR spectrum of 2-hydroxy-5-allyloxybenzaldehyde (**214**)



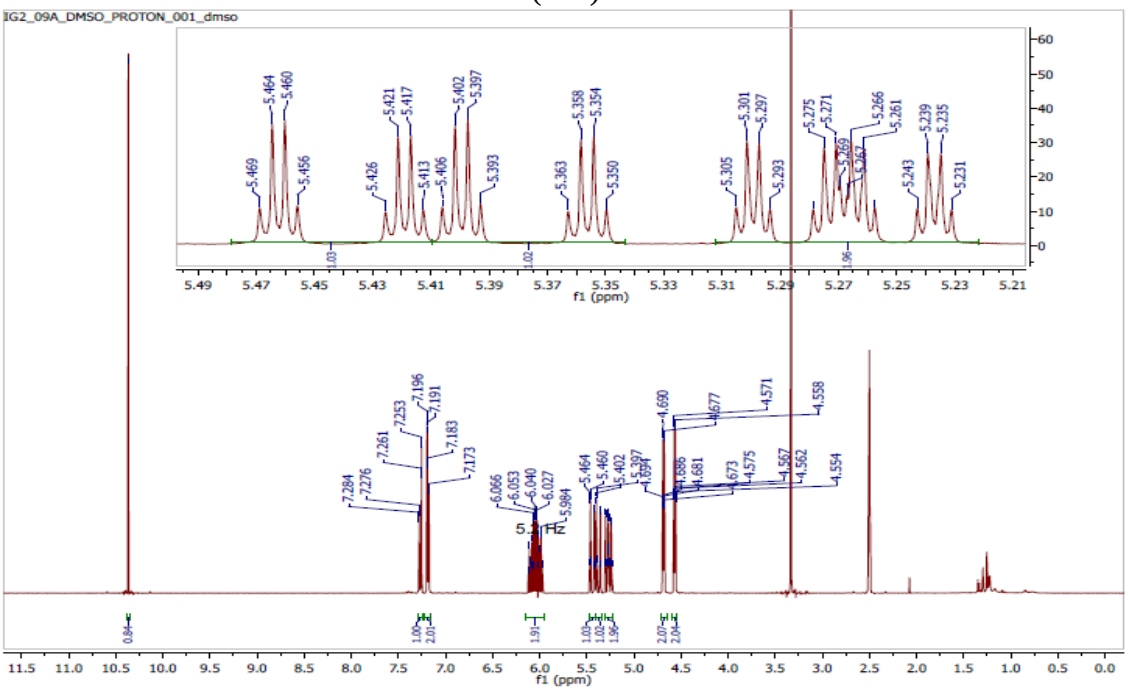
Appendix 60D: gCHSQC ($(CD_3)_2SO$, 800 MHz) spectrum of 2-hydroxy-5-allyloxybenzaldehyde (**214**)



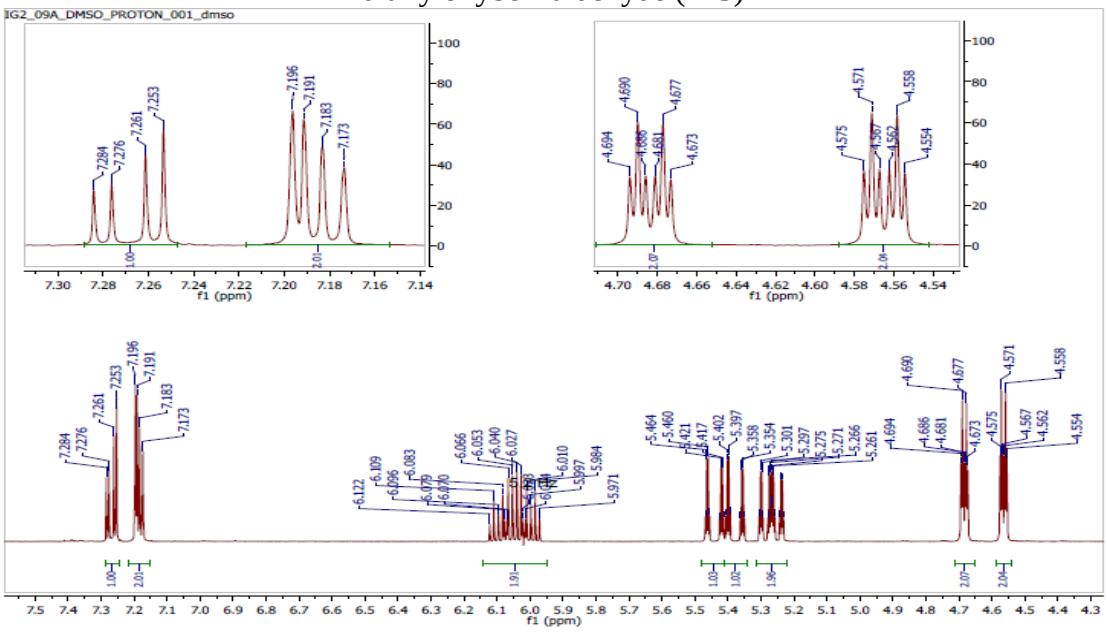
Appendix 60E: gHMBC ($(CD_3)_2SO$, 800 MHz) spectrum of 2-hydroxy-5-allyloxybenzaldehyde (**214**)



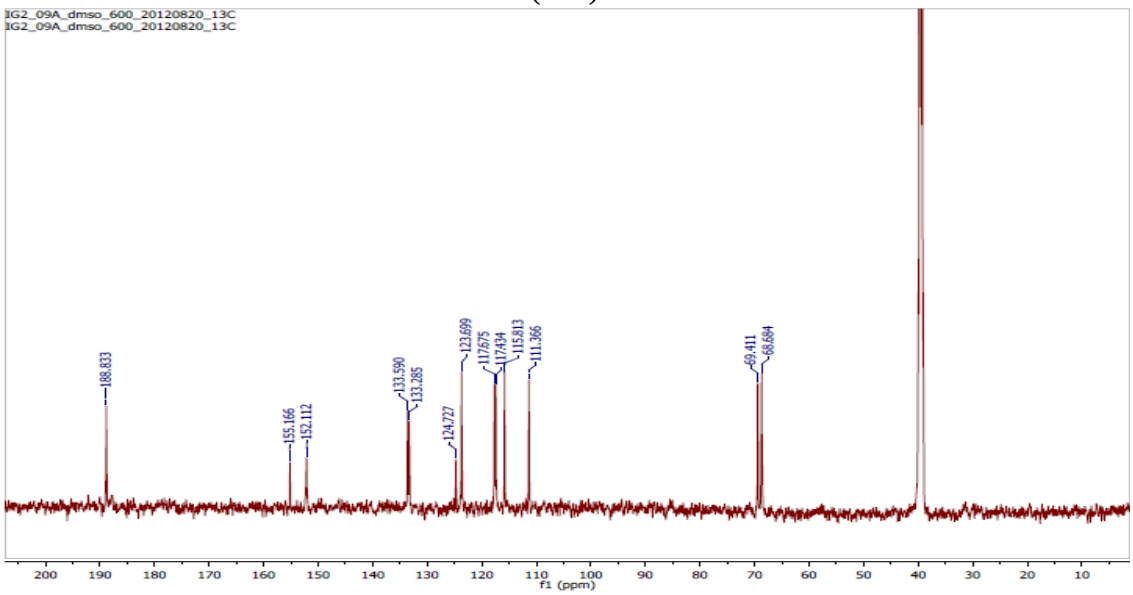
Appendix 61A: ^{1}H ($(CD_3)_2SO$, 400 MHz) NMR spectrum of 2,5-diallyloxybenzaldehyde (**215**)



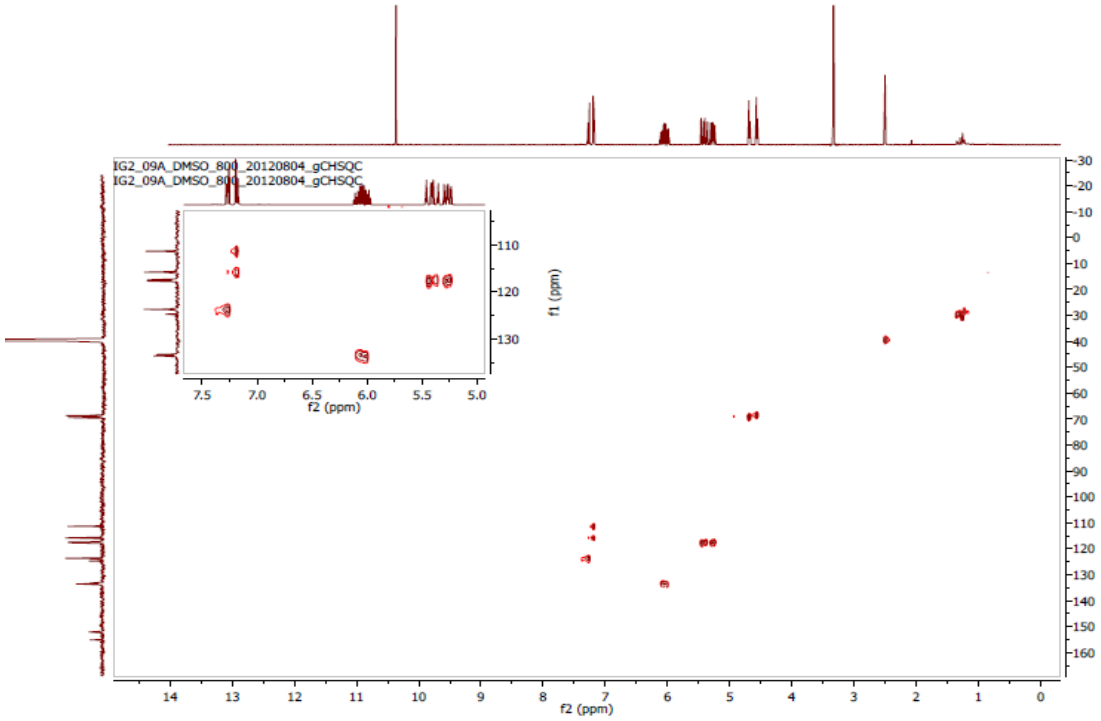
Appendix 61B: expanded ^{1}H ($(CD_3)_2SO$, 400 MHz) NMR spectrum of 2,5-diallyloxybenzaldehyde (**215**)



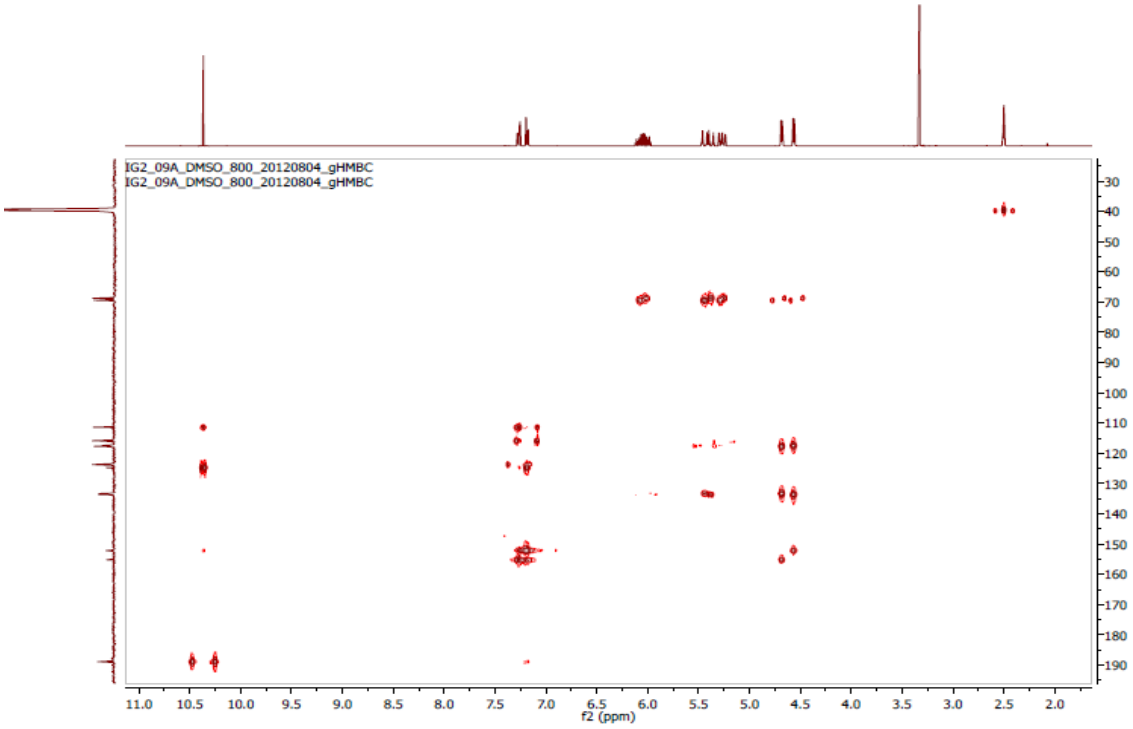
Appendix 61C: ^{13}C ($(CD_3)_2SO$, 150 MHz) NMR spectrum of 2,5-diallyloxybenzaldehyde (**215**)



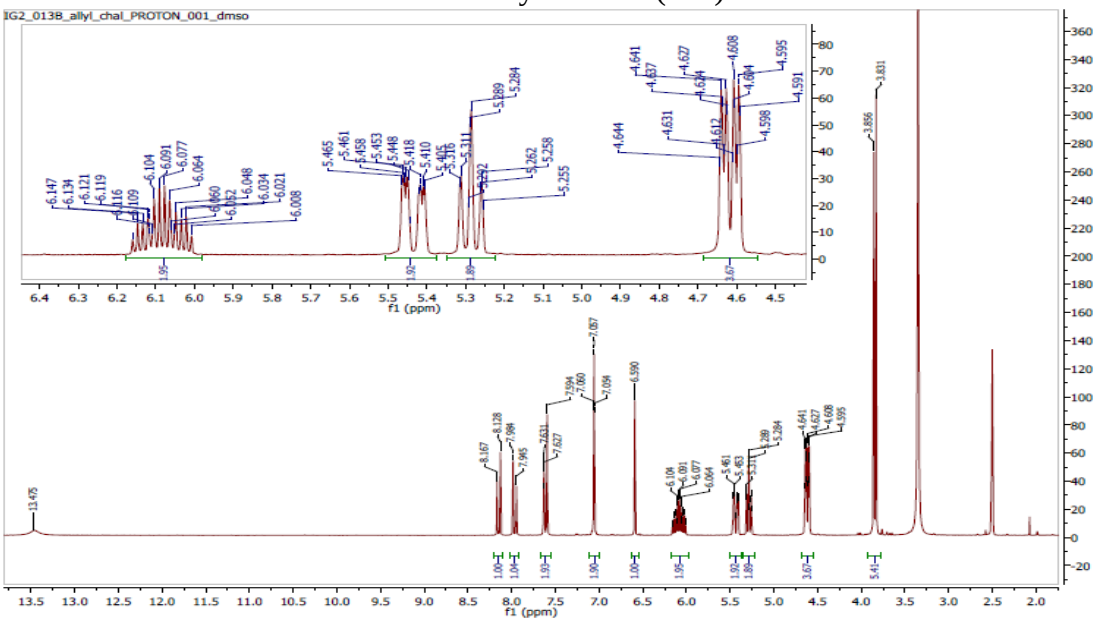
Appendix 61D: gCHSQC ($(CD_3)_2SO$, 800 MHz) spectrum of 2,5-diallyloxybenzaldehyde (**215**)



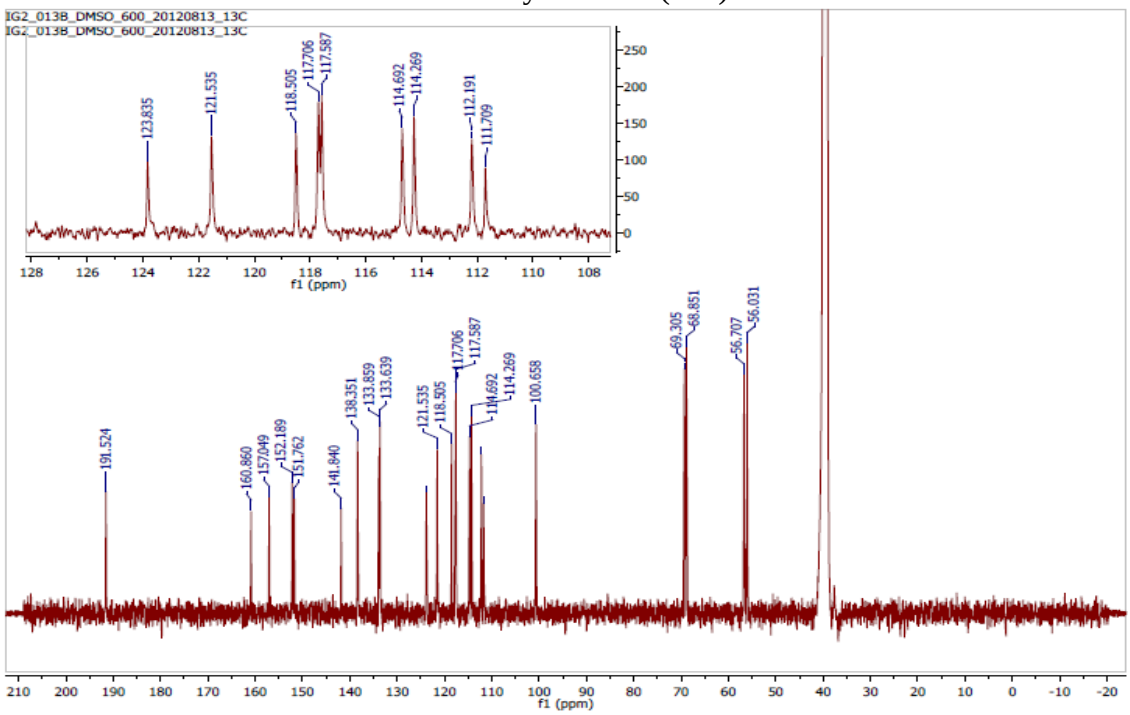
Appendix 61E: gHMBC ($(CD_3)_2SO$, 800 MHz) spectrum of 2,5-diallyloxybenzaldehyde (**215**)



Appendix 62A: ^{1}H ($(CD_3)_2SO$, 400 MHz) NMR spectrum of 2,5-diallyloxy-2′-hydroxy-4′,5′-dimethoxychalcone (**216**)

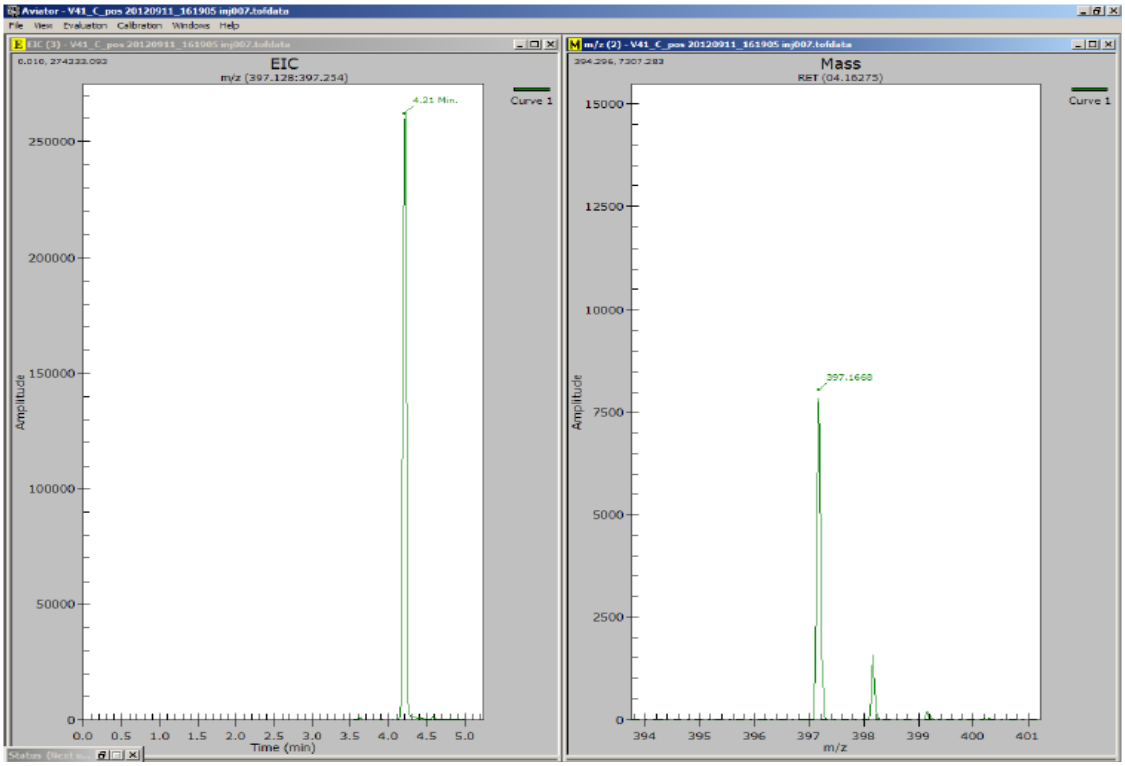


Appendix 62B: ^{13}C ($(CD_3)_2SO$, 150 MHz) NMR spectrum of 2,5-diallyloxy-2′-hydroxy-4′,5′-dimethoxychalcone (**216**)

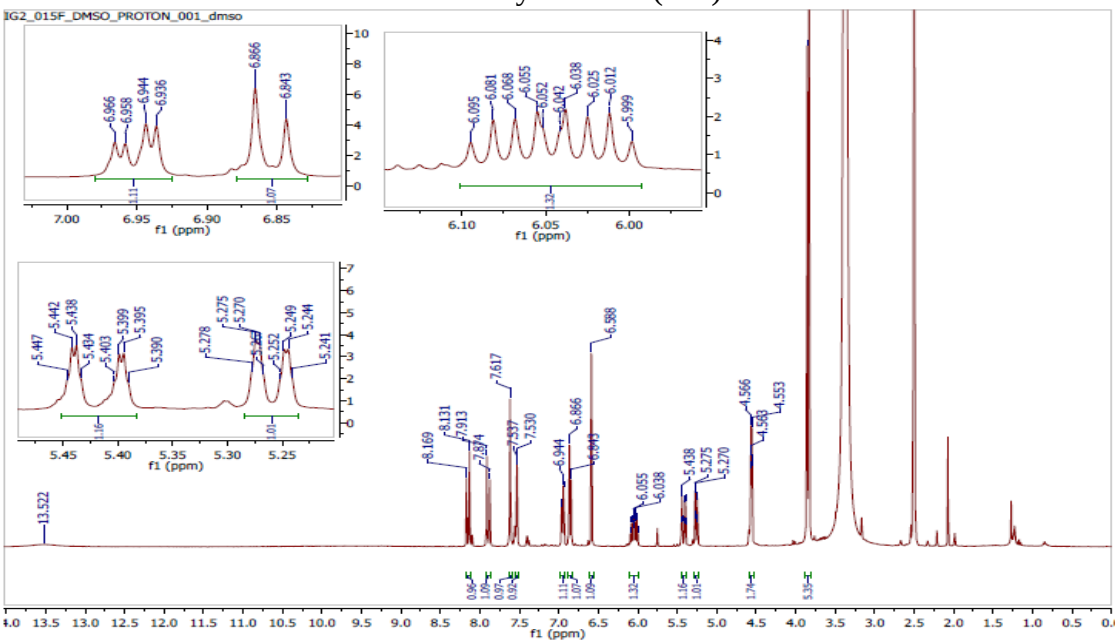


Appendix 62C: HRESI-MS of 2,5-diallyloxy-2′-hydroxy-4′,5′-dimethoxychalcone (**216**)

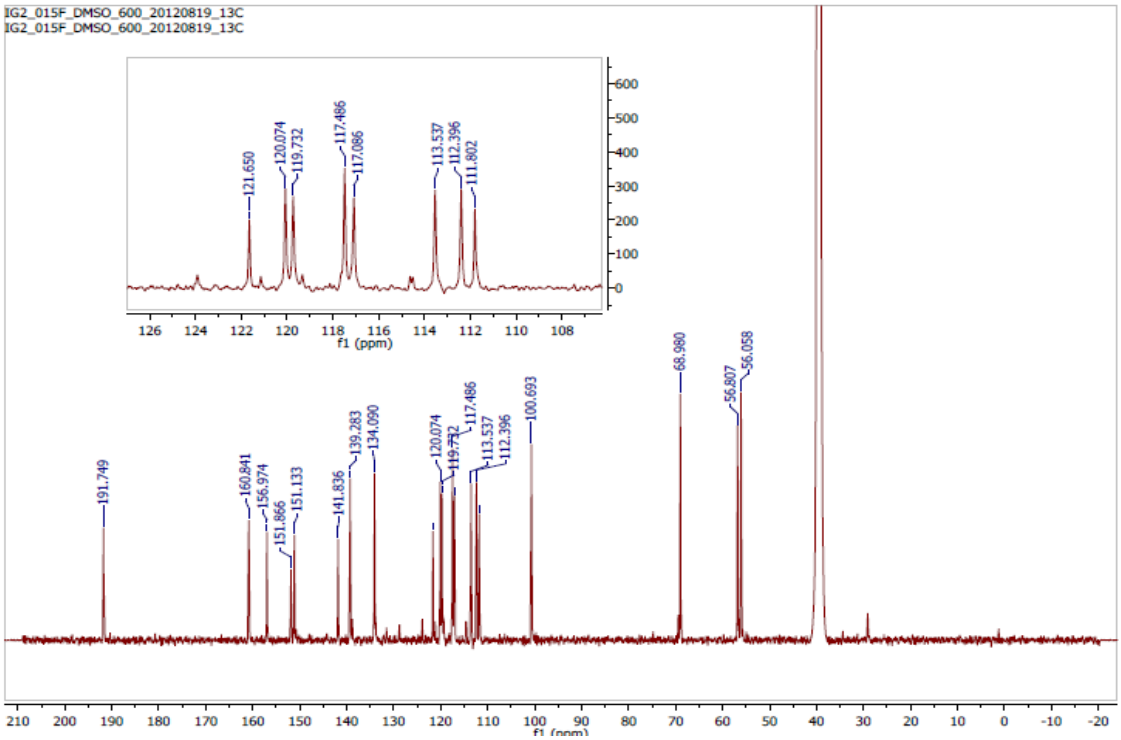
IG2-13B M+H 397.1651



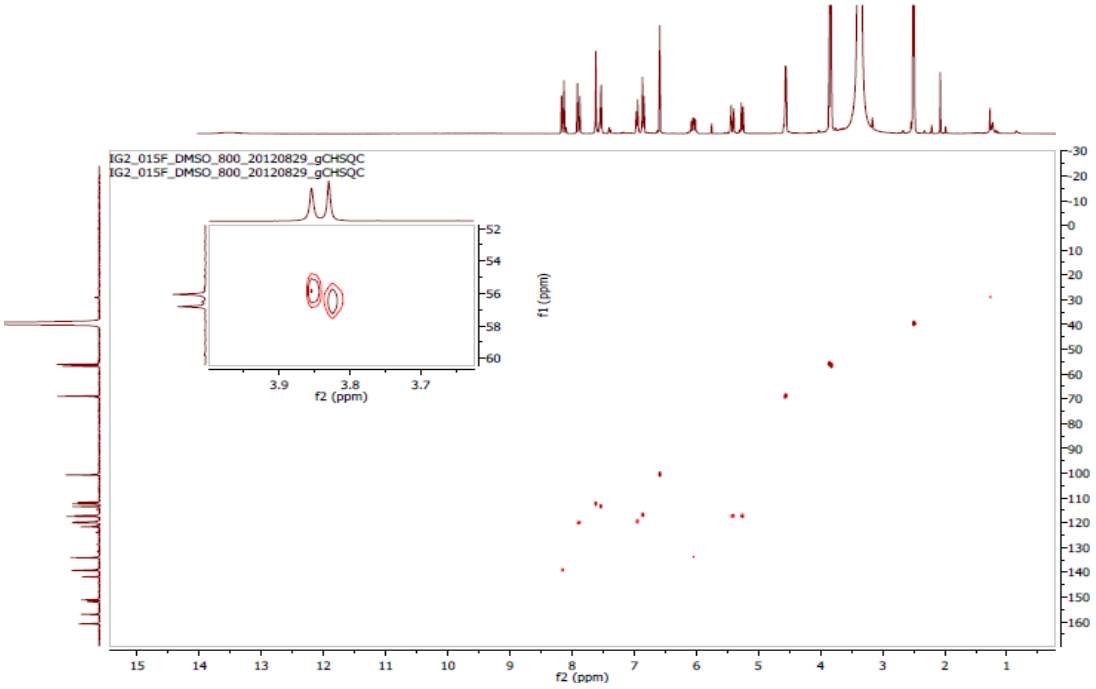
Appendix 63A: ^{1}H ($(CD_3)_2SO$, 400 MHz) NMR spectrum of 5-allyloxy-2,2′-dihydroxy-4′,5′-dimethoxychalcone (**217**)



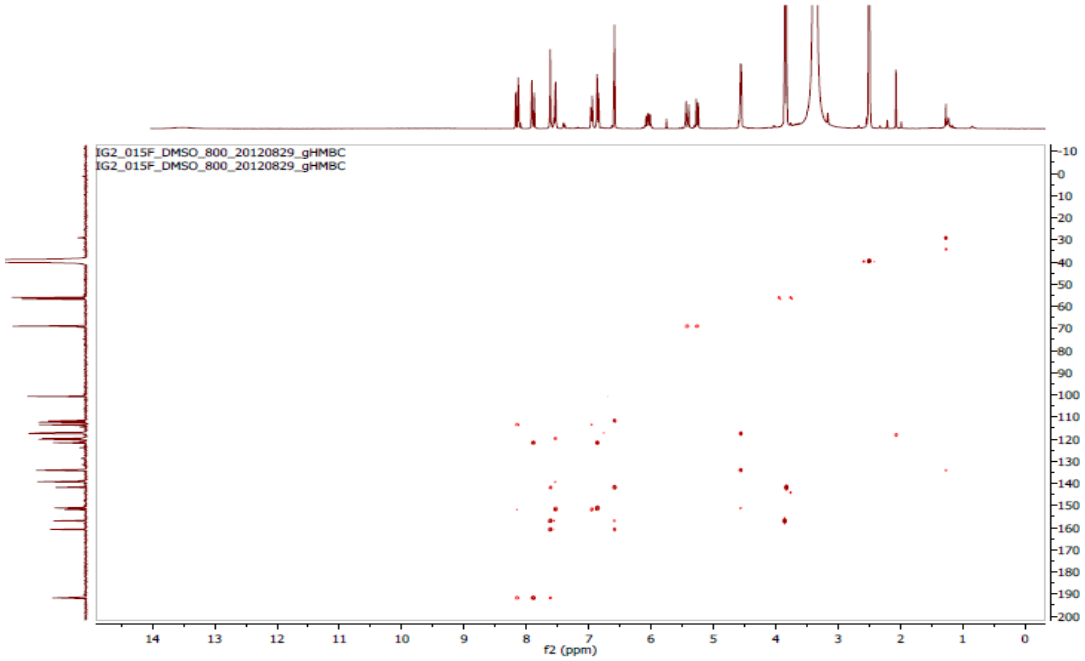
Appendix 63B: ^{13}C ($(CD_3)_2SO$, 150 MHz) NMR spectrum of 5-allyloxy-2,2′-dihydroxy-4′,5′-dimethoxychalcone (**217**)



Appendix 63C: gCHSQC ($(CD_3)_2SO$, 800 MHz) spectrum of 5-allyloxy-2,2′-dihydroxy-4′,5′-dimethoxychalcone (**217**)



Appendix 63E: gHMBC ($(CD_3)_2SO$, 800 MHz) spectrum of 5-allyloxy-2,2′-dihydroxy -4′,5′-dimethoxychalcone (**217**)



Appendix 63H: HRESI-MS of 5-allyloxy-2,2′-dihydroxy-4′,5′-dimethoxychalcone (**217**)