POTENTIAL OF SYNTHETICALLY MODIFIED SURFACE EXUDATE FLAVONOIDS FROM *POLYGONUM SENEGALENSE* AS ANTI-PLASMODIAL PRINCIPLES

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A thesis submitted in partial fulfillment of the degree of Master of Science in Chemistry at the Department of Chemistry, University of Nairobi.

June **2010**

DECLARATION

I hereby declare that the material in this thesis is my original work and has not been presented for a degree in any university.

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This thesis has been submitted for examination with our consent as the University supervisors.

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To: My parents, Tetich and my friend Philipine.

ACKNOWLEDGEMENTS

I feel obliged to express my gratitude to various individuals and institutions, for their roles in the realization of this work. I particularly want to mention my supervisors; Professor Jacob O. Midiwo and Dr. Joseph M. Mwaniki, the success of this work could never have been possible without their close supervision and professional guidance.

I'm also indebted to Prof. Abiy Yenesew (University of Nairobi), Dr. Heydenreich (Potsdam University) and Hoseah Akala (KEMRI) for carrying out the NMR, MS spectroscopic analysis and anti-plasmodial tests, respectively.

I'm grateful to the Department of Chemistry, for having recommended me to the Board of Post-Graduate Studies for the award of University Scholarship. I extend my appreciation to all the teaching and technical staff of the Department of Chemistry for all the assistance they have accorded me during my studies here.

ABSTRACT

Polygonum senegalense produces surface exudates composed mainly of chalcones, flavanones and a homoisoflavanone. Synthetic transformation of some of the chalcones to new entities has been achieved via environmentally benign reactions ('green reactions'). Four chalcones were transformed into dihydrochalcones, flavanones and imines.

Reaction of the pinostrobin chalcone (**24**) with selected primary amines resulted into two flavanone imine derivatives. The reaction was carried out at room temperature in water. These derivatives were obtained in yields of 45% for 7-methoxy-4-(methyl imino)-2-phenylchroman-5-ol (**1**) and 78% for 4-(ethyl imino)-7-methoxy-2-phenylchroman-5-ol (**2**).

Four flavanones: 5-hydroxy-7,8-dimethoxyflavanone (**3**), alpinetin (**4**), 5-hydroxy-pinostrobin (**5**) and 5,7-dimethoxyflavanone (**6**) were prepared in aqueous media at the intervention of NaOH catalysis at room temperature. This is a single step reaction that does not require use of organic solvents since isolation of the products was achieved via simple filtration with low-high chalcone–flavanone conversion achieved.

Catalytic hydrogenation of the chalcones provided four dihydrochalcones: dihydropashanone (**7**), uvangolatin (**8**), 2',6'-dihydroxy-4'-methoxydihydrochalcone (**9**), lapathone (**10**). This was accomplished by bubbling hydrogen gas through solution of the respective chalcone with catalytic aid of palladium (5%) on charcoal to provide the dihydrochalcones in high yields (over 97% yields).

Kostanecki acetylation of the synthesized dihydrochalcones; **7**, **9** and **10** resulted to three compounds that include two acetylated enolates of the dihydrochalcones: 2-(1-acetoxy-3-phenylprop-1-enyl)-4,5-dimethoxy-1,3-phenylene diacetate (**12**), 2-(1-acetoxy-3-phenylprop-1-enyl)-5 methoxy 1,3-phenylenediacetate (**11**) and a homoisoflavone (3-benzyl-5, 7-dimethoxy-2-methyl-4*H*-chromen-4-one, **13**).

Nitro derivative of dihydrochalcone was prepared under mild nitrating conditions that include the use of nitric acid alone at temperatures below room temperature affording 2',6'-dihydroxy-4'-methoxy-3'-nitrodihydrochalcone (**14**).

Some of the products formed were tested to assess their *in vitro* anti-plasmodial activities. These compounds showed moderate anti-plasmodial activities of IC_{50} values: 9.32 and 11.6 μM (7-methoxy-4-(methyl imino)-2-phenylchroman-5-ol, **1**), 35.69 and 31.93 μM (4-(ethyl imino)-7-methoxy-2-phenylchroman-5-ol, **2**), 8.7 and 14.5 μM (7,8-dimethoxyflavanone, **3**), 12.4 and 8.6 μM (alpinetin,**4**), 8.0 and 6.1 μM (**6**) against chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains, respectively.

Characterization of these compounds was guided mainly by their ^{1}H, ^{13}C NMR and MS spectroscopic data.

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ABBREVIATIONS

MS: Mass Spectroscopy

$[M]^+$: Molecular ion

$[M]^+$: Molecular ion

m/z: Mass to charge ratio

EI-MS: Electron ionization mass spectroscopy

NMR: Nuclear magnetic resonance

^{13}C: Carbon -13 isotope

^1H: Proton

d: Doublet

m: Multiplet

δ: Chemical shift

t: Triplet

J: Coupling constant

ax: axial

eq: equatorial

Hz: Hertz

IC_{50}: Concentration causing 50% Inhibition

μM: micro molar

TLC.: Thin layer chromatography

THF: Tetrahydrofuran

EtOAc: Ethyl Acetate

Hex: hexane

CH_2Cl_2: dichloromethane

KEMRI: Kenya Medical Research Institute

CoA: coenzyme A

Nm: nano meter

ml: milliliter

mg: milligram

Mp: melting point

NaOH: Sodium hydroxide

CHAPTER 1
INTRODUCTION

1.0 General Introduction

Malaria is one of the most common infectious and life-threatening parasitic disease of the tropical and subtropical countries. Each year, up to three million deaths are due to malaria and close to five billion cases of clinical illness possibly meriting anti-malarial therapy occur throughout the world, with Africa having more than 90% of this burden [Breman *et al.*, **2004**]. Malaria is transmitted by mosquitoes and caused by *Plasmodium falciparum, P. malariae, P. ovale*, and *P. vivax*. *P. falciparum* is the most prevalent for the disease and it is responsible for about 80% of infections and 90% of deaths [Soon *et al.*, **2007**].

The life cycle, immunological defense mechanisms and clinical development of malaria in humans is a complex process. Clinical malaria is characterized by periodic high fever, chills, weak joints, and flu-like illness, which follow the lysis of infected erythrocytes, and caused mainly by the induction of cytokines interleukin-1 and tumor necrosis factor [Kaur *et al.*, **2009**]. *P. falciparum* infection can have serious effects, for example, anaemia, cerebral complications (from coma to convulsions), hypoglycemia, glomerulonephritis and death.

The disease is most serious among the non-immune individuals, including children and pregnant women. Most of the deaths, about 90%, are among young children in sub-Saharan Africa [Schwikkard and Heerde, **2002**].

1.1 Natural Products as source of Anti-malarial drugs

Traditionally, nature through plants has over the years offered medical solutions that form the basis of sophisticated traditional medicinal systems. Phytochemical studies have yielded clinical drugs used as natural product molecules, or as synthons for synthetic modifications or synthetic templates, particularly for chemotherapeutic treatment of cancer and malaria [Phillipson, **1994**]. Higher plants, many of which are threatened with extinction, are used as sources of pharmaceuticals and as ingredients of traditional medicines and are of value in new drug discovery. Several natural products which have been isolated from traditional medicinal plants have potent anti-plasmodial action *in vitro* and represent potential sources of new anti-malarial drugs [Phillipson, **2007**]. Therefore, plants have been a good source of lead compounds, especially against infectious. Example of naturally sourced lead compound against malaria is quinine (**15**). Quinine, a quinoline alkaloid was the first effective treatment against the *P. falciparum* parasite. Quinine was isolated in the 17^{th} century by the French scientists Caventou and Pelletier [Phillipson, **2001**] from the bark of cinchona tree and later used as a template for the synthesis of chloroquine (**16**), which became widely successful for several decades.

Anti-malarial drug resistance has emerged as one of the greatest challenges facing malaria control today. Multidrug resistance has rendered monotherapy for malaria useless in most parts of the world, and has also compromised the usefulness of many of the available combination chemotherapies [Kremsner, **2004**]. This calls for more active compounds with a new mode of action to replace the current ineffective drugs and therefore continuous search for new anti-malarial drugs is paramount.

Artemisinin (**17**), isolated from the Chinese plant *Artemisia annua*, has been used successfully against chloroquine resistant malaria. Artemisinin has had its structure modified to semi-synthetic derivatives that include arteether (**18**), artemether (**19**), which is a methyl ether of dihydroartemisinin, artesunate (**20**) and dihydroartemisinin (**21**). These derivatives have been formulated into drugs that are considered to be more bio-available with enhanced bio-activity. It is therefore evident that Natural Products Chemistry ranks high in this continuous war against malaria. The search for new anti-malarial compounds by combining natural sources and synthetic approaches is a continuous process.

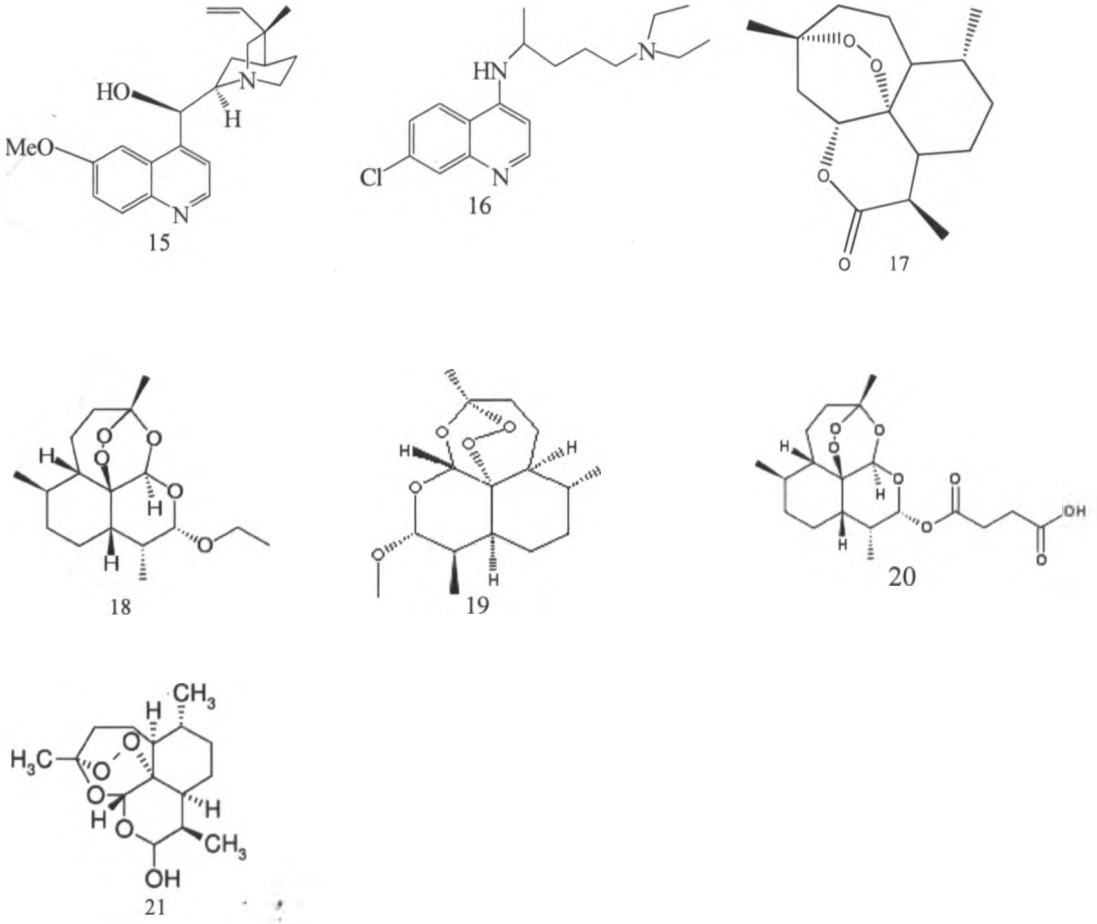
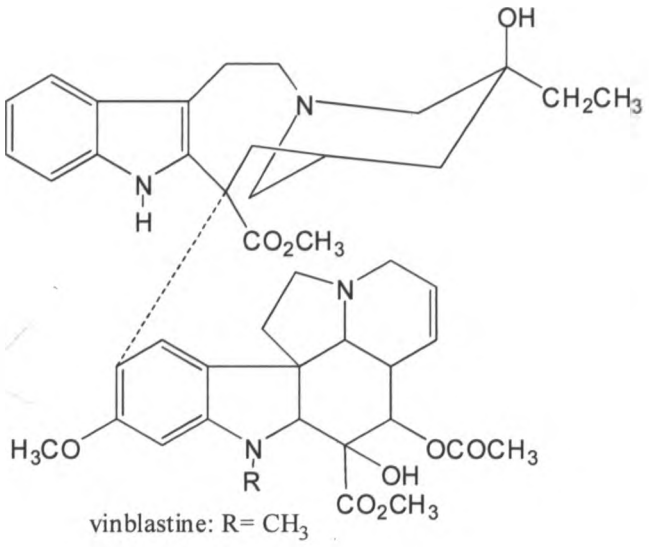


Figure 1: Examples of natural and synthetic compounds used to 'fight' malaria.

Other plant extracts that are famous for their use in medicine include vincristine, a drug that is administered for the treatment of acute leukemia, neuroblastoma, thyroid cancer, and lymphoma while vinblastine is a chemotherapy drug that is administered for the treatment of breast cancer, lung cancer, bladder cancer, and lymphoma. These drugs are from the plant *Catharanthus rosea* or the plant Madagascar periwinkle [Miura *et al.*, **1987**; Donoso *et al.*, **1977**].



vinblastine: R= CH_3

vincristine: R= CHO

Figure 2: Dimeric Indole Alkaloids of *Catharanthus roseus*

1.2 Bio-activity of Flavonoid Family of Natural Products

Secondary metabolites may be classified as polyketides, terpenoids and steroids, phenylpropanoid (C6-C3) compounds and alkaloids based on their biosynthetic building blocks. Flavonoids which are under the subclass phenylpropanoids are products from a cinnamoyl-CoA starter unit with chain extension using three molecules of malonyl-CoA. The enzyme chalcone synthase couples cinnamoyl-CoA unit with three malonyl-CoA units giving a chalcone [Dewick, **2002**].

Chalcone acts as a precursor for a vast range of flavonoid derivatives found throughout the plant kingdom. According to their chemical structure, flavanoids are subdivided into the following subgroups anthocyanidins, flavones, flavanones and flavanols [Ververidis *et al.*, **2007**]. The flavonoid family of natural products is widely distributed in nature and comprises a large group of polyphenolic secondary metabolites in plants. Flavonoids exhibit unrivalled bioactivity due to their diverse physiological functions. They possess a wide spectrum of therapeutic, enzyme inhibitory, anti-oxidant, anti-inflammatory [Pelzer *et al.*, **1998**], antiviral and antimicrobial [Alvarez *et al.*, **2008**]. Many natural and synthetic flavonoids possess antimalarial activity. Chalcones have a diverse array of substituents on the two aromatic rings of 1, 3-diphenyl-2-propen-1-one, which was derived by the cleavage of the C ring in flavonoids. Qualitative structure-activity analysis suggests that anti-plasmodial activity is governed more by substitution on ring B rather than on ring A of the chalcone template with most of the active compounds having methoxy or dimethoxy groups on ring B [Mei-Lin *et al.*, **2004**].

Interest in the anti-malarial activity of chalcones was prompted by the discovery of the anti-plasmodial activity of Licochalcone A (**Fig. 3**), an oxygenated chalcone isolated from the roots of the Chinese licorice [Chen *et al.*, **1994;** Frolich *et al*, **2005**].

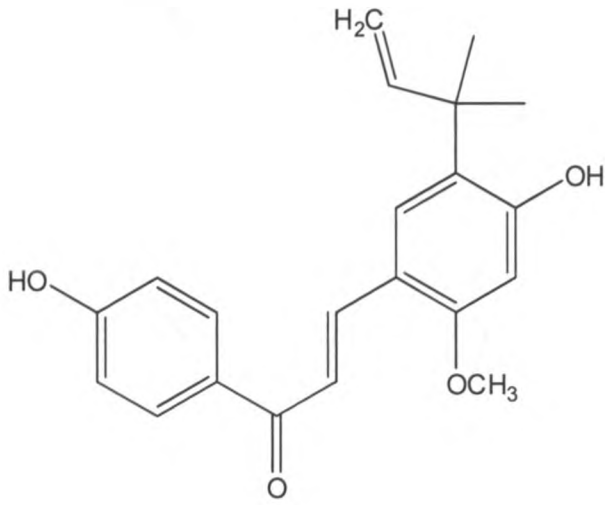


Figure 3: Structure of licochalcone A.

A large number of plants are famous for the presence of potentially malaria effective compounds. An example is *P. Senegalense* which is a rich source of flavonoids with moderate anti-plasmodial activities. Surface exudates extracted from *P. senegalense* are particularly rich in chalcones and flavanones that show anti-plasmodial activity [Midiwo *et al.*, **2007**].

New anti-plasmodial compounds can be obtained by testing natural product extracts and their structurally modified derivatives. The activity of these surface exudates may be enhanced by modifying the natural compounds to obtain more potent templates for malaria drugs through use of mild and environmentally benign chemical reactions.

1.2 Problem Statement

Malaria still remains a killer disease due to anti-malarial drug resistance that has become a major public health disaster in many areas of the tropical World [Fivelman et al., **2004**; White et al., **1999**]. Resistance by *P. falciparum* to anti-malarials makes chemotherapeutic choices increasingly limited [Kremsner *et al.*, **2004**]. Research effort to develop drugs that can fully cure malaria is steered by constant development of resistance by the malaria parasites. This continued resistance by malaria parasites to available commercial anti-malarial drugs such as chloroquine is the main reason driving search for new compounds that have potent activity against malaria parasites.

1.3 Justification

Several chalcones and flavanones which are surface exudate compounds of *Polygonum senegalense* have been isolated at the Department of Chemistry, University of Nairobi and have been reported to possess moderate anti-plasmodial activity [Midiwo *et al.*, **2007**].

Since most anti-malarial drugs that have been in use were developed from promising natural products as synthons or templates, these surface exudates may be modified by making their derivatives to produce potent anti-plasmodials. In this project, mild and environmentally benign chemical reactions were considered for modification. This was inspired by the fact that the feedstock for this work were from natural sources, therefore there was need to attempt green reactions to emulate the "green" processes in the natural system.

1.4 Objectives

The Overall Objective of this study is:

To prepare derivatives of bioactive flavanones, chalcones and dihydrochalcones synthetically, as possible lead compounds for future antimalarial drugs.

The Specific Objectives of the study are:

1. To transform chalcones to flavanones in water suspension media under base catalysis.
2. Transform the keto functionality of chalcones to imine by reaction with amines.
3. To hydrogenate chalcones to dihydrochalcones
4. Carry out chemical modification of dihydrochalcones by Kostanecki reactions.
5. To prepare nitro derivatives of dihydrochalcones.
6. To characterize products using physical and Spectroscopic methods of analysis.
7. Use green reactions where possible to carry out the synthetic transformations.
8. Evaluate *in-vitro* anti-plasmodial activities of the products and compare with the activities of the parent the natural products.

CHAPTER 2

LITERATURE REVIEW

2.0 The Chemistry and Histochemistry of *Polygonum senegalense*

Polygonum senegalense is a member of the Polygonaceae family, a medium sized, cosmopolitan family of herbs and shrubs. The stems are more or less covered with conspicuously slightly inflated sheaths. The leaves are lanceolate, acute, and hairless in the case of [Blundell, **1987**]. *Polygonum senegalense* is one of the 12 *Polygonum* species in Kenya. The others are: *P. baldschuanicum, P. convolvulus, P.amphibium, P.nepalense, P. capitatum, P. afromontanum, P. aviculare, P. strigosum, P. salicifolium, P. senegalense, P. pulchrum and P.setosulum* [Agnew and Agnew, **1994**]. There are two distinct forms of *Polygonum senegalense*: *P. senegalense* var. *senegalense* which is almost hairless except for small yellow gland and *P.senegalense* var. *albomentosum* which is densely white-hairy. *Polygonum senegalense* is unique amongst the *Polygonum* species because of the phenomenon of the surface exudates.

The leaf of *Polygonum senegalense* is up to 17% surface exudate and about thirteen non polar flavonoid derivatives (chalcones, dihydrochalcones, flavanones and a flavone) have been isolated and characterized from it [Midiwo *et al.*, **2007**]. The non polar aerial exudates of *Polygonum senegalense* have been reported to contain 12 flavonoids of the chalcone and flavanone types (**Fig. 4**) and they are distinctly different from internal tissue aglycones (**Fig. 5**) [Midiwo *et al.*, **2002**]. Reassessment of the aerial exudates of *P. senegalense* has given rise to the isolation of the first 9-hydroxyhomoisoflavanone (**26**) [Midiwo *et al.*, **2007**].

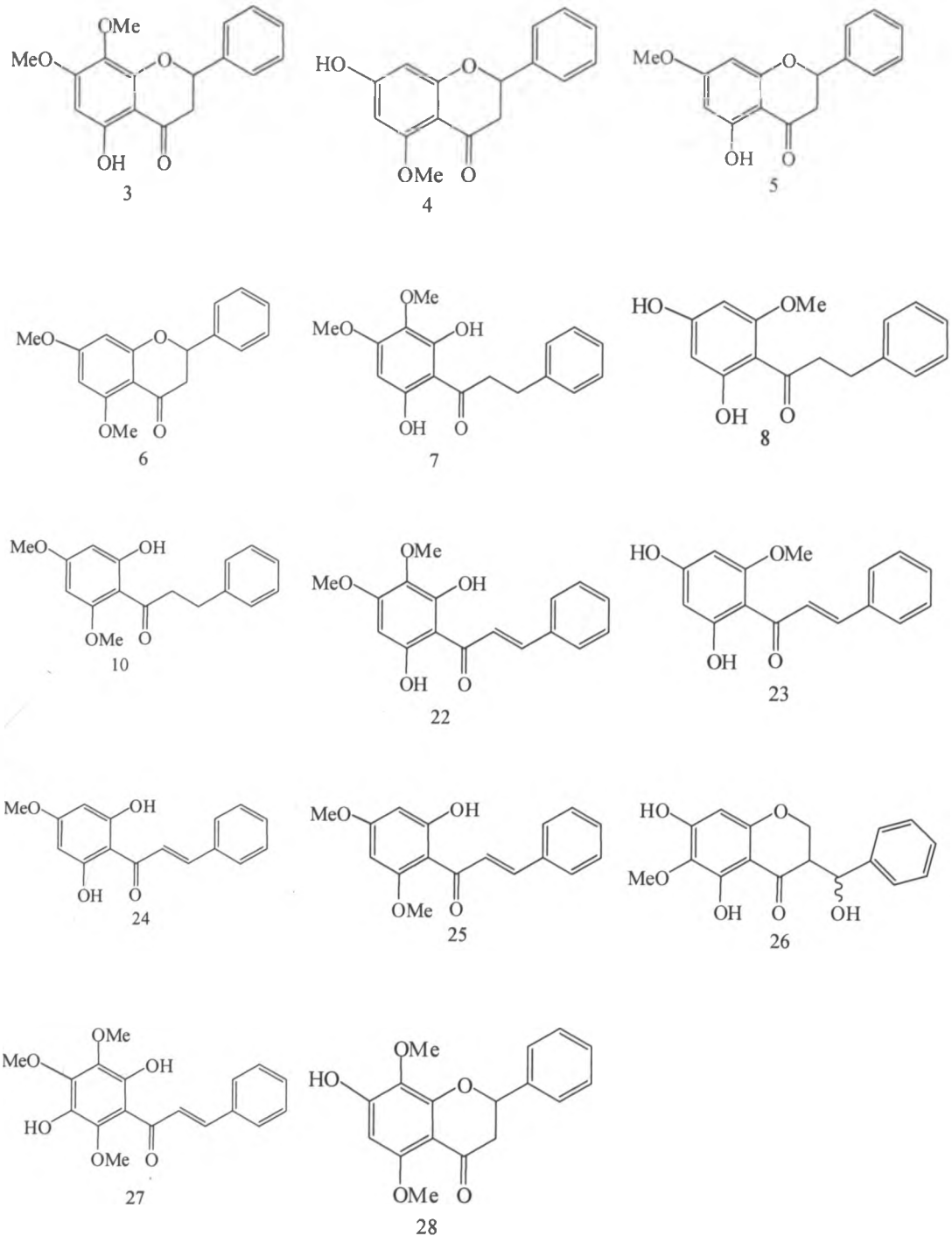
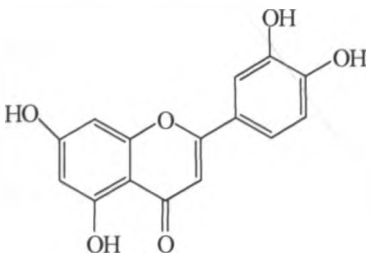


Figure 4: Non-polar Aerial surface exudate flavanoids isolated from *P. senegalense*.



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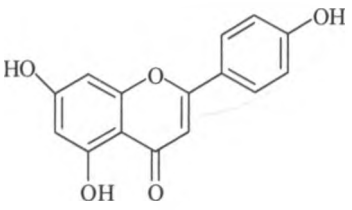

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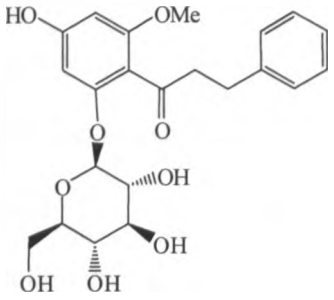

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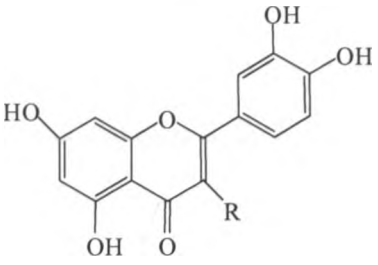


32: R=β-O-glucosyl
33: R=β-O-galactosyl
34: R=β-O-(2"-galloylglucosyl)

Figure 5: Internal tissue flavonoids isolated from *P.*senegalense.

2.1 Green Chemistry Consciousness

Green Chemistry, also known as sustainable chemistry, is the design of chemical processes and products that are more environmentally benign and reduce negative impacts to human health and the environment. It advocates for efficient utilization of raw materials (preferably renewable), waste minimization and avoids the use of toxic and/ or hazardous reagents and solvents in the manufacture and application of chemical products [Sheldon, **2007**]. The aim is the design of chemical processes and products that eliminate or reduce the use and generation of hazardous substances. Therefore, instead of limiting risk by controlling exposure to hazardous chemicals,

green chemistry attempts to reduce and preferentially eliminate the hazard thus negating the necessity to control exposure. The idea here is, if a process does not employ or produce hazardous substances then the risk is zero, and hence no need to worry about the treatment of hazardous substances or limiting our exposure to them.

Processes are not usually considered green or not green but wether a process is greener or less green. A process may be much greener than an old process but the process probably still has some negative environmental effects. To assess how green a chemical, a reaction or a process is, Anastas and Warner [**1998**] have developed the following twelve principles or tools of green chemistry:

1. **Prevention of waste:** Reactions should be designed so as to limit generation of waste rather than putting a measure for cleaning the waste after it is formed. It is very expensive to treat waste and contain it because the waste has to be monitored even after it has been contained.
2. **Atom Economy:** Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product. When creating materials it is important to maximize the incorporation of all the materials used into the final product meaning that waste as little material as possible.
3. **Less Hazardous Chemical Syntheses:** Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.

4. **Designing Safer Chemicals:** Chemical products should be designed to effect their desired function while minimizing their toxicity. Reducing toxicity of the products reduces hazards to people and to the environment.
5. **Safer Solvents and Auxiliaries:** Whenever possible, the use of additional substances such as solvents and separating agents should be discouraged. When required, these substances should be nontoxic substances. The use of auxiliary substances (e.g., solvents, separation agents, etc.) should be minimized where possible.
6. **Design for Energy Efficiency:** Less energy intensive processes need to be developed because energy requirements of chemical processes translate to their environmental and economic impacts that need to be minimized hence less energy intensive chemical processes are encouraged. Synthetic methods should be designed in a way that reactions are conducted at room temperature and pressure.
7. **Use of Renewable Feedstock:** When possible a raw material (feedstock) should come from a renewable resource. Renewable source of synthons rather than the depleting should be considered whenever possible.
8. **Reduction of Reaction Steps:** Unnecessary derivatization (use of blocking groups, protection or de-protection, temporary modification of physical and /or chemical processes) should be minimized or avoided if possible, because such steps require additional reagents which do not end up in the final product but instead lead to increase in waste and the number of reaction steps.

9. **Catalysis:** Catalytic reagents are superior to stoichiometric reagents. Use selective catalytic reagents over stoichiometric reagents because catalysts help a reaction occur at higher rate with less energy requirement.
10. **Design for Degradation:** Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment. The product that is made should break down into nontoxic substances after it has been used. This way, the product will not remain and build up in the environment
11. **Real-time analysis for Pollution Prevention:** Analytical methodologies need to be developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances. This includes monitoring while the process is happening, detection and control of the formation of hazardous substances, and monitoring after the substance has been disposed.
12. **Inherently Safer Chemistry for Accident Prevention:** The substance and the form of the substance (liquid, gas, etc) used in a chemical process should be carefully chosen to minimize the potential for chemical accidents, including releases, explosions, and fires during the chemical process.

Application of tools of green chemistry is beneficial in many ways. The new processes are less harmful to the environment. This means that the new processes are safer for workers, less expensive, more energy efficient, and require fewer materials.

2.2 Drug Discovery and Development.

The process of drug discovery is primarily based on the search and subsequent testing of drug candidates acting on a preselected therapeutic target. It involves the design and synthesis of new compounds, followed by evaluation of biological testing results and generation of a new hypothesis as the basis for further compound design and synthesis [Zheng *et al.*, **2006**]. This process is faced by hurdles that range from increasing complexity of current biological assays, which requires more extensive biological understanding hence facilitate incorporation of the available information into chemistry planning. This complexity applies to both the primary *in-vitro* assay for the biological target thought to be linked to clinical efficacy, as well as selectivity assays for undesired off-target *in-vitro* activities. The same considerations apply to the increasingly sophisticated assays for other aspects of drug discovery, absorption, distribution, metabolism, and elimination and safety [Johnson and Li, **2007**].

2.2.1 **Stages of drug development**

The process of getting a prescription drug after target identification starts with identification of a lead compound; a compound which shows the desired pharmaceutical activity. A lead compound might be discovered through a number of ways which include screening of natural compounds, synthetic banks, re-examining existing drugs, combinatorial synthesis, and designing lead compound by NMR [Baxter and Lockey, **2001**].

Drug development is a process with well-defined sequential phases. Each phase sets the stage for the next phase and a drug may be discarded at any of these phases for

causes such as toxicity, poor tolerability, and/ or lack of efficacy. The aim of this sequential approach in drug development is to reduce the uncertainty about the drug's effect [Preskorn, **2000**]. The following are the steps followed in the discovery and development of new drugs:

I. **Target identification:** Chemists and pharmacologists conduct a study to understand a particular disease area at a molecular level. They then search for a chemical, a 'hit', that can act on these molecular interactions and produce the desired pharmacological effect in the body, thus curing the disease or reversing its progress. Chemists or molecular biologists will then synthesize a number of different drugs with variations in their molecular structures.

II. **Lead identification:** Out of the several chemical or biological entities that are synthesized, a lead compound or substance is discovered that is believed to have the desired pharmacological effect in terms of curing the disease.

III. **Lead optimization:** The various lead compounds discovered in Step 2 are compared in order to select the most promising candidate that has the greatest potential to be developed into a safe and effective treatment of the disease condition.

IV. **Pre-clinical Testing:** The lead compound or substance that is believed to be most effective and safe is then tested extensively in laboratory animals to certify that it will be safe for use in human beings. It can take between one to five years to complete the evaluation of the compound at this stage. The various tests conducted during this phase will determine in what form the drug will be administered to human beings as oral tablets or intravenous injections.

Scientists will also ascertain the pharmaceutical composition of the drug, its safety and how it will be formulated and manufactured.

V. **Clinical Testing:** In this stage of drug development, the drug is administered to healthy human volunteers, as well as patients that suffer from the disease, to determine the safety of the drug in human beings. Clinical testing is conducted in three phases; Phase I, II & III. In all three phases increasing numbers of human volunteers are tested.

Phase I - Clinical Studies: These studies typically take six to nine months to complete and are designed to understand the safety and tolerability of the lead compound in humans. They involve administering the drug to a small number, between 50 and 100, of healthy volunteers for a short period of time. Scientists then note how the drug behaves in the body i.e. its absorption, distribution, metabolism and excretion.

Phase II - Clinical Studies: This phase usually takes anywhere between six months to three years to complete depending on the type of drug and the disease condition it treats. During this phase, scientists determine the effectiveness of the drug and further evaluate its safety in humans. The drug is administered to several hundreds of human volunteers who are suffering from the disease that the drug will be used to treat. The results of this phase will determine whether the drug is effective in treating the disease condition, the minimum and maximum effective doses of the drug and the safety and tolerability of the drug in patients.

Phase III - Clinical Studies: Depending on the type of drug and the disease condition, this phase usually requires one to four years to complete. During this phase several hundreds to thousands of patients will be tested. The study is designed to determine the safety and efficacy of the drug, at the desired dosages, in larger groups of patients.

IV. Application for Marketing the New Drug: The pharmaceutical companies that successfully complete all the stages of drug discovery and development, and have all the data gathered from these studies, are then required to approach the regulatory authorities of their respective countries to apply for permission to market the new drug. It may take six months to two years to obtain approval to market a new drug.

2.3 Synthetic Modifications on Chalcones

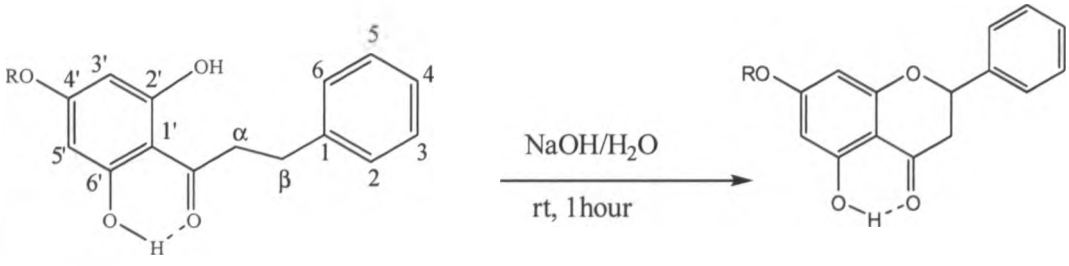
2.3.1 Conversion of Chalcones into Flavanones

Cyclization of chalcones to flavanones represents one of the most exhaustively studied reactions in heterocyclic chemistry, not only on account of its largely unexplained variability under oxidative conditions, but because its fluctuating course may represent parallels amongst those biogenetic sequences which lead to the range of natural flavonoids [Ferreira *et al.,* **1975]**. Ring closure to the β-position relative to the carbonyl proceeds with different types of chalcones under acidic or basic conditions. β -cyclization proceeds by 1, 4-Michael addition, and with conventional chalcones leads to the chalcone-flavanone equilibrium where the presence of a 6'-hydroxy group pushes the equilibrium in favour of the flavanones.

Preparation of flavanones has been carried out by intramolecular cyclization of 2'-hydroxychalcone under various catalytic conditions using acids, bases, electrolysis, photolysis and thermolysis. The yields of these reactions are often low-high (20–90% yield) and the reaction usually gives a mixture of the chalcone and flavanone, the separation of which requires a lot of organic solvent [Tanaka and Sugino, **2001**].

2.3.1.1 **Base catalyzed intramolecular cyclization of 2'-hydroxychalcone**

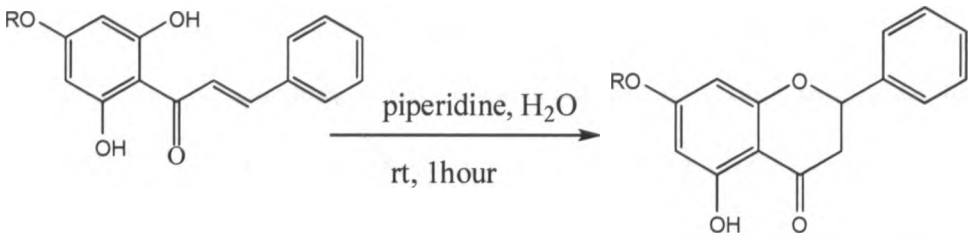
This is a single step reaction in which 2'-hydroxychalcones can be converted into flavanones very efficiently in water suspension medium and the products obtained simply by filtration. This intramolecular cyclization of 2'- hydroxychalcone is a base (NaOH) catalyzed reaction that is effected under environmentally benign aqueous medium with nearly 100% conversion [Tanaka and Sugino, **2001**]. The products (flavanones) are easily isolated by filtration followed by drying of the material in a desiccator.



Scheme 1: Sodium Hydroxide catalyzed cyclization of 2'- hydroxychalcone

2.3.1.2 **Piperidine catalyzed cyclization of 2'-hydroxychalcones to flavanones.**

Piperidine catalyzed cyclization reaction occurs in water suspension medium with higher degree of conversion [Tanaka and Sugino, **2001**]. The suspension of powdered 2'-hydroxychalcone in water containing piperidine is stirred at room temperature for one hour. The crude product is then collected by filtration, washed with water and dried in a desiccator to give flavanone.

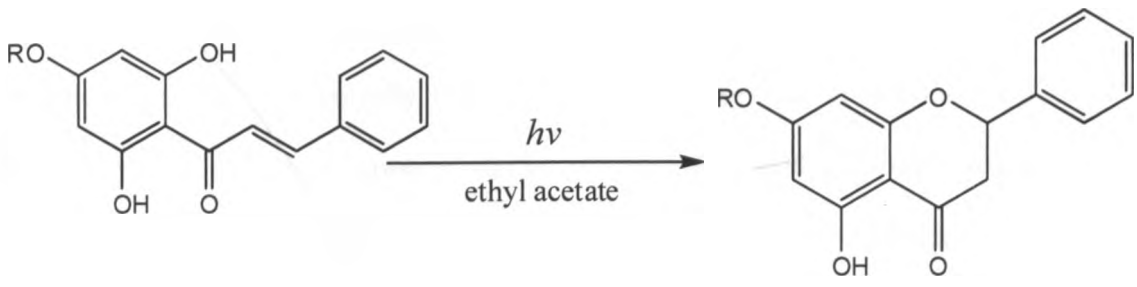


Scheme 2: Piperidine catalyzed reaction

2.3.1.3 **Photochemical cyclization of 2'-hydroxychalcones**

2'-Hydroxychalcones undergo facile photocyclization to give corresponding flavanones in ethyl acetate or dioxane when irradiated with light of wavelength ≥ 365 nm [Matsushima and Hirao, **1980**]. The lack of quenching by triplet quenchers and the lack of inhibition by free radical scavengers (2, 6-di t-butylphenol and dissolved oxygen) imply that the reaction does not proceed through mechanisms that involve triplet states or free radicals.

Photocyclization of 2'-hydroxychalcone proceeds via the zwitterionic mechanisms involving intramolecular proton transfer or electron transfer followed by proton transfer in the excited singlet states [Matsushima and Kageyama, **1985**]. Higher reactivity in polar solvents than in non-polar solvents suggest that photocyclization proceeds via ionic process.



Scheme 3: Photochemical cyclization of 2'-hydroxychalcones

2'- Hydroxychalcones undergo photocyclization to give the corresponding flavanones, in low quantum yields, when subjected to visible irradiation in polar aprotic solvents. The quantum yields are independent of the light intensity, implying a one-photon process, but strongly dependent on the wavelength of irradiation [Matsushima and Kageyama, **1985**].

2.3.2 Hydrogenation of Chalcones

Naringin chalcone is a flavonoid extracted from the peel of some citric fruits and responsible for the bitterness. The substance derived from hydrogenation of this flavonoid have a very intense sweet taste therefore their synthesis become interesting because of their industrial potential as sweeteners [Nazareno et al., **2000**].

Most carbon–carbon double bonds, whether substituted by electron-donating or electron-withdrawing substituent, can be catalytically hydrogenated; usually in quantitative or near-quantitative yields [March and Smith, **2007**].

An ideal catalyst produces an infinite amount of product (no deactivation, no poisoning) in 100% yield with no generation of byproducts and waste (complete selectivity). From a practical (and industrial) standpoint, the catalyst's availability,

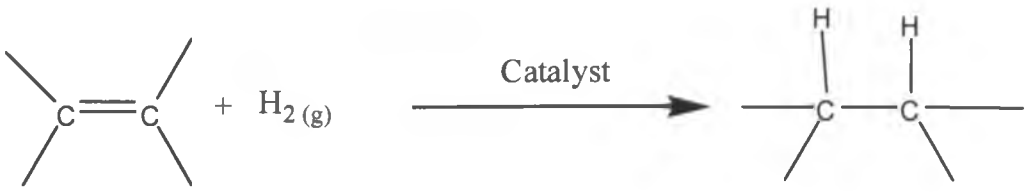
sensitivity, toxicity, and recovery are equally important. A recoverable catalyst is preferable to one that is eventually lost in a product stream. The relative merits of various recovering strategies, however, can be expected to depend upon the reaction under consideration, the reaction scale and engineering requirements. [Waldemar *et al.,* **2007]**

The catalysts used can be divided into two broad classes, both of which mainly consist of transition metals and their compounds:

(i) Catalysts insoluble in the reaction medium (heterogeneous catalysts); among the most effective are Raney nickel, palladium-on-charcoal, $NaBH_4$-reduced nickel (also called nickel boride), platinum metal or its oxide, rhodium, ruthenium, and zinc oxide.

(ii) Catalysts soluble in the reaction medium (homogeneous catalysts); An important example is chlorotris (triphenylphosphine)rhodium, $RhCl(Ph_3P)_3$ (Wilkinson's catalyst), which catalyzes the hydrogenation of many alkenyl compounds without disturbing functional groups such as ester (COOR), nitro (NO_2^+) and cyano (CN) present in the same molecule.

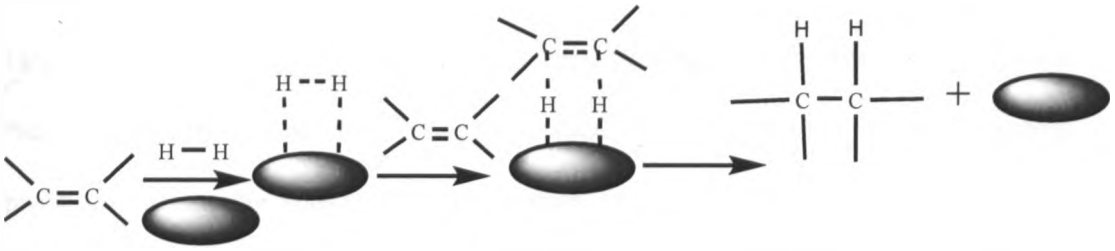
Recently the great importance of polymer supported metal catalysts have been recognized because of economic and safety aspects. An example is polymer incarcerated platinum catalyst (PI Pt), which has been prepared from Pt $(PPh_3)_4$ and styrene copolymers [Miyazaki *et al.,* **2006**].



Scheme 4: Catalytic hydrogenation of carbon-carbon double bond.

Catalytic hydrogenation reactions lead to addition of hydrogen atoms to carbon-carbon multiple bonds. These reactions depend on catalysts and are normally aided by hydrogenation catalysts such as platinum, palladium and nickel.

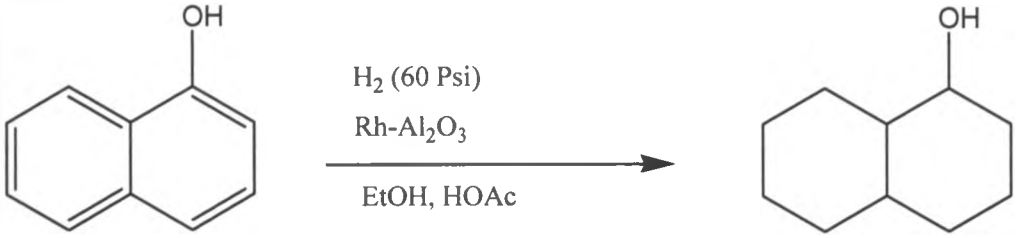
The catalyst adsorbs both the carbon molecules and the hydrogen gas on its surface, in such a way that the molecules are arranged in just the right position for addition to occur.



Scheme 5: Pictorial illustration of hydrogen syn addition to carbon-carbon double bond.

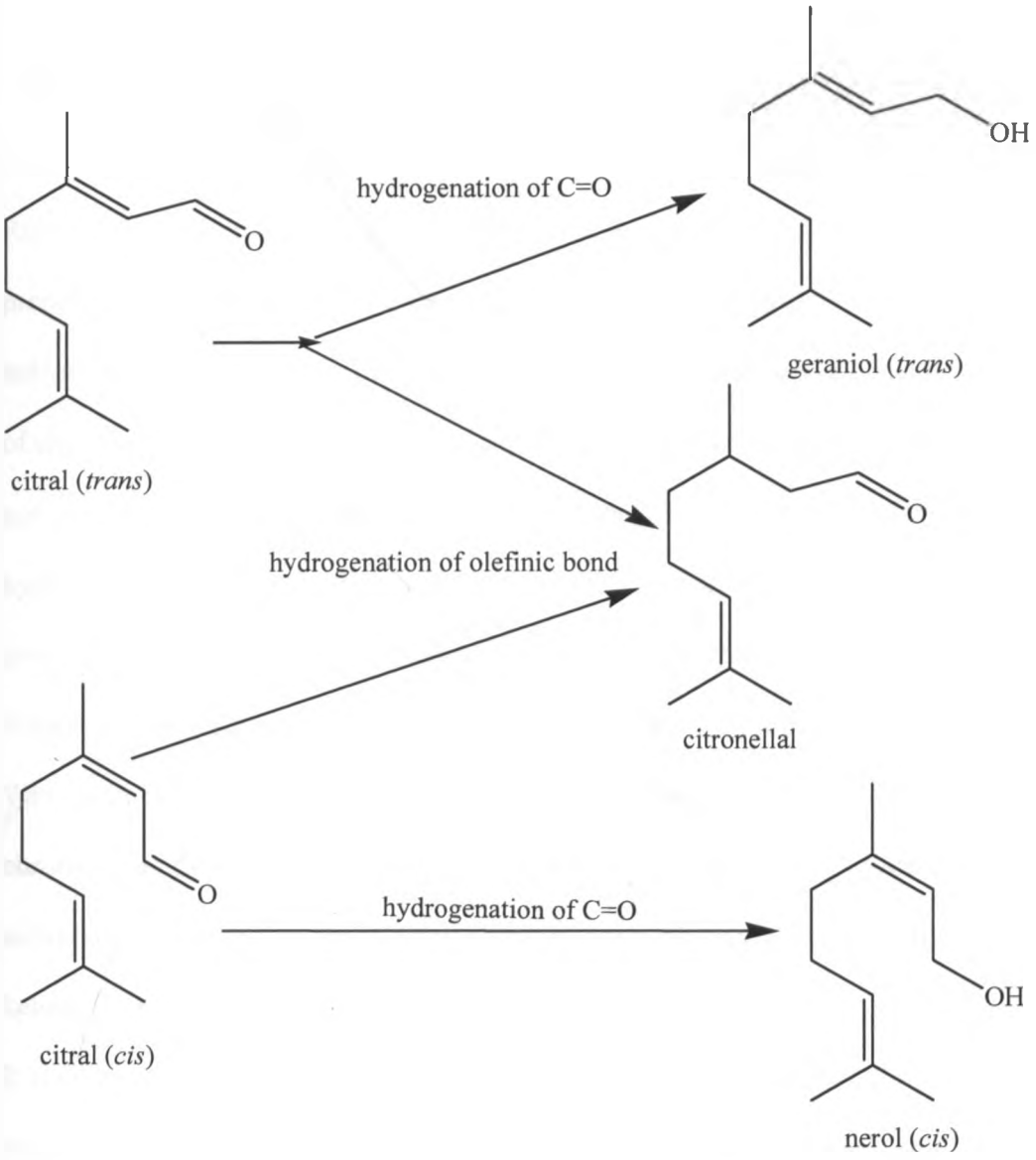
Most catalytic reductions of double or triple bonds, whether heterogeneous or homogeneous, have been shown to be *syn*, with the hydrogen atoms adding from the less-hindered side of the molecule [Meyers *et al.,* **1988**]. Even unsaturated aldehydes can be reduced to saturated aldehydes, although in this case decarbonylation may be a side reaction.

Since one of the reagents (hydrogen) is a gas, the reaction rate can be increased by pressurizing the reaction vessel, to a higher pressure than atmospheric pressure. In fact, hydrogenation under pressurized conditions leads to hydrogenation of aromatic nuclei [Meyers *et al.*, **1988**]. The Meyers group reported the reduction of 1-naphthol to 1-decalol using platinum, Raney nickel, and Raney copper. These reactions catalyzed by nickel and copper require elevated temperatures and pressure.



Scheme 6: Hydrogenation of 1-Naphtol

In chemical synthesis, heterogeneous hydrogenation catalysts are most useful for the reduction of various functional groups and, therefore, are widely applied in industrial processes where the selective hydrogenation of α, β-unsaturated olefinic bond is required over the carbonyl (C=O). For example, when the olefinic bond of citral is hydrogenated, citronellal is formed but hydrogenation of the C=O yields the unsaturated allyl-type alcohols geraniol and nerol in the *trans* and *cis* forms respectively.



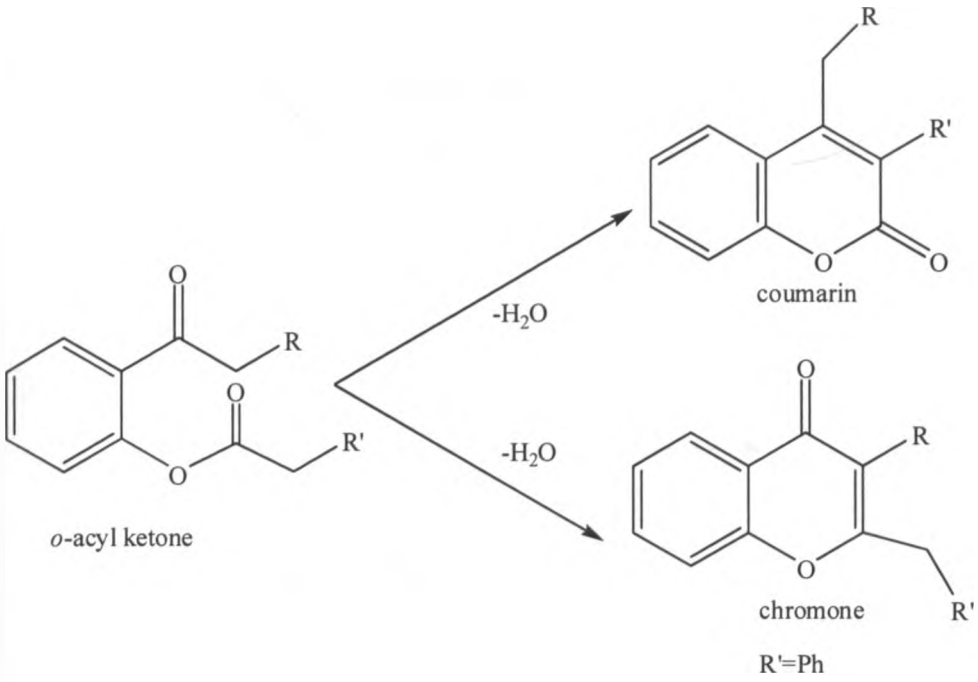
Scheme 7: Hydrogenation of C-C double bond versus carbonyl group

The selectivity (C=O vs. C=C group hydrogenation) can often be controlled by the nature of the individual metal, the presence of a second metal (bimetallic catalysts), metal particle size (dispersion), and electron-donating or electron-withdrawing ligand effects induced by the catalyst [Steffan *et al.*, **2008**].

2.3.3 The Kostanecki-Robinson Acetylation reactions

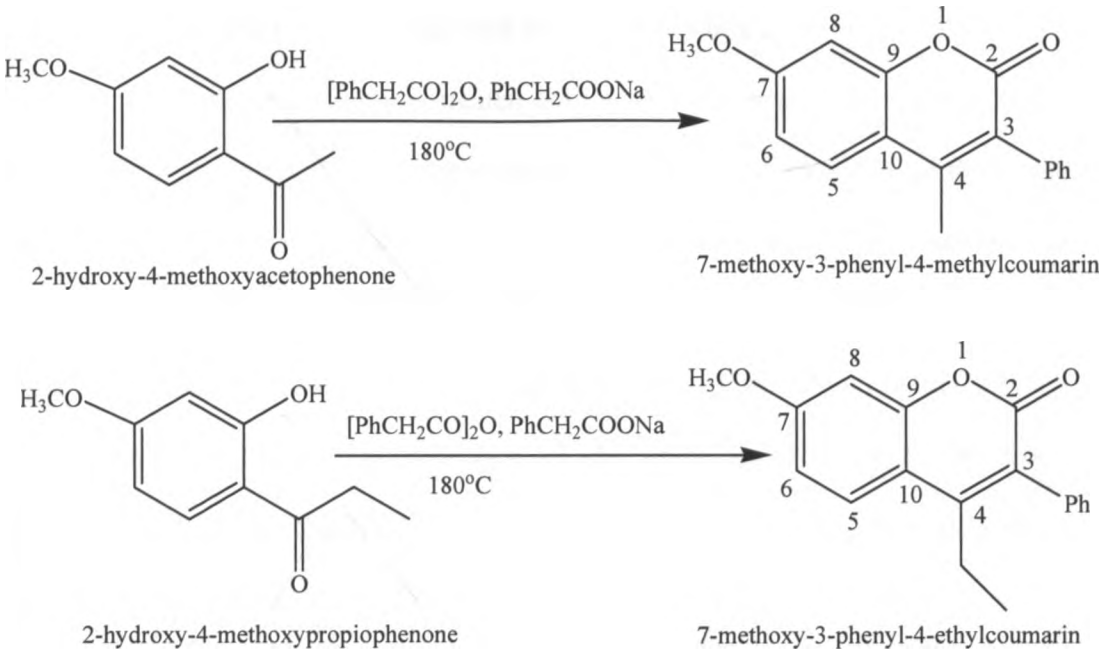
The reaction of *O*-hydroxy-acetophenones with sodium salt of fatty acids and their anhydrides to form coumarins and chromones is generally known as Kostanecki-Robinson reaction [Naik and Thakor, **1952**]. The Kostanecki reaction involves preparation of flavones or isoflavones by condensing *O*-hydroxyaryl ketones with anhydrides and sodium salts of fatty acids, though is not reliable as a general method of chromone synthesis, since course of the reaction has been shown to be dependent not only on the acid anhydride and the salt used, but also on the nature of the *O*-hydroxyphenyl ketone. Studies on the action of the anhydrides and sodium salt of propionic and butyric acids on 2-hydroxy-4-methoxyacetophenone and on 2-hydroxy-4-methoxypropiophenone show that more than one product is formed in each reaction. With the former ketone, the main product is the coumarin and with the latter it is the chromone. In these cases, therefore, varying the sodium salt and anhydride is of subsidiary importance to the effect of changing from the methyl ketone to the ethyl ketone [Heilbron *et al.*, **1934**].

It may be presumed that, in the reaction between an *o*-hydroxyphenyl ketone and a mixture of the anhydride and the sodium salt of a fatty or substituted fatty acid, the initial stage involves the formation of an *O*-acyl ketone which can lose water in one of two ways, to give either a chromone or a coumarin [Heilbron *et al.*, **1935**].



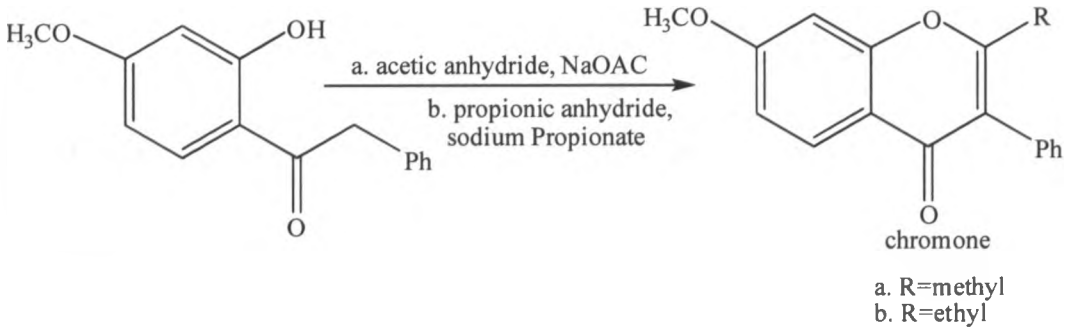
Scheme 8: Possible products of Kostanecki-Robinson reaction.

Experiments on the influence of a phenyl group in the sodium salt and the anhydride of the fatty acid was performed with regard to formation of chromone and coumarin. When either 2-hydroxy-4-methoxyacetophenone or 2-hydroxy-4-methoxypropiophenone is heated with sodium phenylacetate and phenylacetic anhydride the product has almost exclusively the coumarin structure, the compounds formed being 7-methoxy-3-phenyl-4-methylcoumarin and 7-methoxy-3-phenyl-4-ethylcoumarin respectively. In each case, the abstraction of the hydrogen at the reactive methylene group between phenyl and carbonyl is the precursor to ring closure. This influence is sufficiently powerful and outweigh the effects introduced either by replacing the methyl ketone by the ethyl ketone, or by using derivatives of propionic acid in place of those of acetic acid. The influence of the phenyl group on the course of the Kostanecki reaction, though more powerful, is in the same direction as that of the methyl group [Heilbron *et al.*, **1935**].



Scheme 9: Influence of a phenyl group on Kostanecki reaction product.

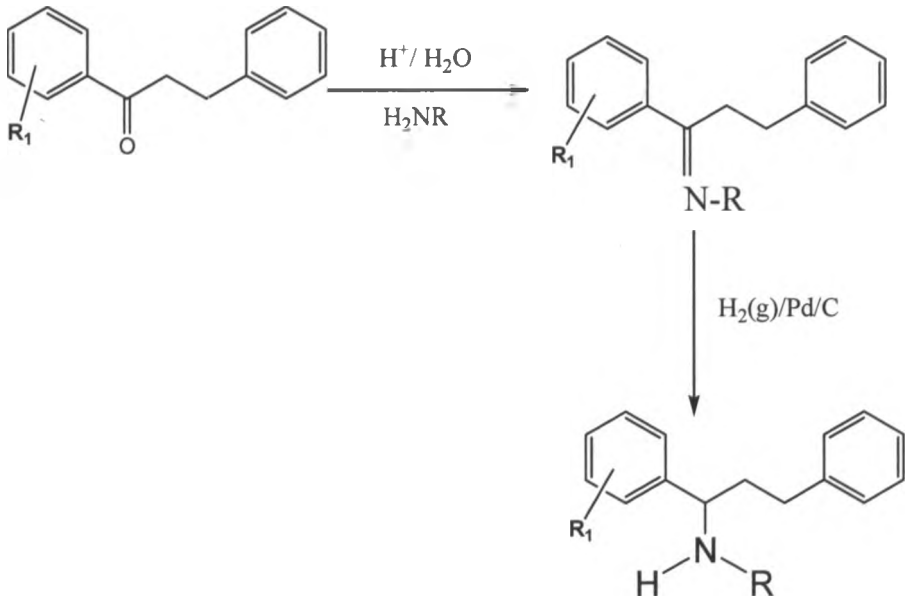
The action of the sodium salt and the anhydride of both acetic and propionic acid on 2-hydroxy-4-methoxyphenyl benzyl ketone gives rise to almost exclusive chromone formation, the products being 7-methoxy-3-phenyl-2-methylchromone (a) and 7-methoxy-3-phenyl-2-ethylchromone (b).



When the phenyl group is substituted in the sodium salt and the acid anhydride, both groups favour coumarin formation, but when substituted in the hydroxyacetophenone side chain both groups favour chromone formation [Heilbron *et al.*, **1935**].

2.3.4 Imine formation from ketone and reduction to amine.

When an aldehyde or a ketone is treated with a primary or secondary amine in the presence of hydrogen and a hydrogenation catalyst, reductive alkylation of the amine (or reductive amination of the carbonyl compound) takes place. The reaction can formally be regarded as occurring in the following manner (scheme 10). In this regard, the reaction of an aldehyde with an amine to give an imine (amine-carbonyl condensation) is followed by reduction of the imine functionality (C=N) using Sodium Borohydride ($NaBH_4$) or a variety of other reagents such as sodium cyanoborohydride ($NaBH_3CN$) [March and Smith, **2007**].



Scheme 10: Reductive Amination

The first step in this reaction involves alkylamino-de-oxo-bisubstitution. In this reaction, the nucleophile provides the electrons to form the new bond and the π- bond of the carbonyl group is broken as it "gets out of the way". The electrons move from this π- bond onto what was the carbonyl oxygen.

Strong nucleophiles (strong base) are capable of using one of its unshared pairs of electrons to make a new covalent bond. If a weak nucleophile is involved, like water, the reaction needs help in the form of acid catalysis. In this pattern, the H^+ initiates the reaction by making a bond with the carbonyl oxygen. The electrons which make this bond can be envisioned as coming from the carbonyl pi bond, which leaves a positive charge on the carbonyl carbon. Fewer acid molecules than there are amine molecules are required to initiate the reaction and to catalyze the removal of the water molecule. The reaction rate is higher if the pH is controlled so that half of the amine molecules are available to act as nucleophiles and the other half are present as the conjugate acid (ammonium salt).

The ammonium ion (RNH_3^+) actually serves as the acid catalyst since it is the strongest acid which can co-exist with the amine. Strong nucleophiles can attack directly, without help from an acid catalyst.

Amine-carbonyl condensation reactions that involve natural products have been shown by Kul'magambetova *et al.*, [**2002**] who synthesized pinostrobin hydrazone and pinostrobin oxime by treating pinostrobin with hydrazine hydrate (H_2N-$NH_2{\cdot}H_2O$) and hydroxylamine hydrochloride in pyridine [(NH_2OH) HCl] respectively.

2.3.5 Nitration Reactions

Aromatic compounds, whether of high or low reactivity, can be nitrated by use of a wide variety of nitrating agents. For benzene, the simple alkylbenzenes, and less reactive compounds, the most common reagent is a mixture of concentrated nitric and sulfuric acids, but for active substrates, the reaction can be carried out with nitric acid

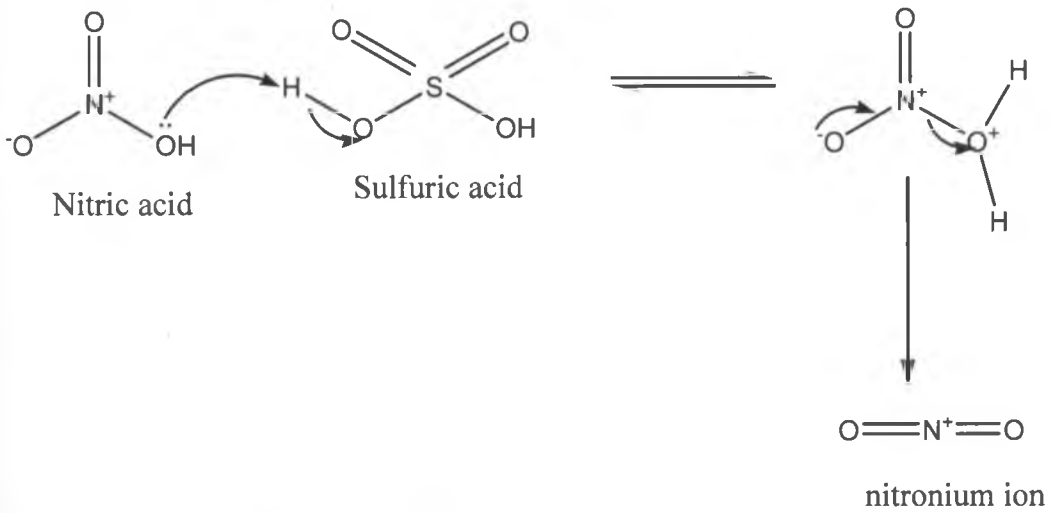
alone, or in water, acetic acid, acetic anhydride, or chloroform. In fact, milder conditions are necessary for active compounds, such as amines, phenols, and pyrroles, since reaction with mixed nitric and sulfuric acids would oxidize these substrates.

With reactive substrates, such as amines and phenols, nitration can be accomplished by nitrosation under oxidizing conditions with a mixture of dilute nitrous and nitric acids. The attacking species is the nitronium ion (NO_2^+) can be generated by the following ways [March and Smith, **2007**];

- **In concentrated sulfuric acid**, by an acid–base reaction in which nitric acid is the base:

  The first step is the formation of a very powerful electrophile, NO_2^+, by the interaction of the two strong acids. Sulfuric acid is the stronger and it protonates the nitric acid on the OH group so that a molecule of water can leave.

$$HNO_3 + 2H_2SO_4 \rightleftharpoons NO_2^+ + H_3O^+ + 2HSO_4^-$$



Scheme 11: Mechanism of generation of nitronium ion

- **In concentrated nitric acid alone**, a similar acid–base reaction as in the above mechanism occurs in which one molecule of nitric acid is the acid and another the base [Belson, **1989**].

$$2HNO_3 \rightleftharpoons NO_2^+ + NO_3^- + H_2O$$

This equilibrium lies to the left (~4% ionization), but enough NO_2^+ is formed for nitration to occur.

- **With N_2O_5 in CCl_4**, there is spontaneous dissociation:

$$N_2O_5 \rightleftharpoons NO_2^+ + NO_3^-$$

But in this case there is evidence that some nitration also takes place with undissociated N_2O_5 as the electrophile.

- **In the case of nitronium salts**, NO_2^+ is of course present to begin with. Esters and acyl halides of nitric acid ionize to form NO_2^+. Nitrocyclohexadienones are converted to NO_2^+ and the corresponding phenol [Fischer *et al.*, **1990**]. Nitrocyclohexadienones have been identified as intermediates formed in a range of nitration reactions. Some nitrocyclohexadienones containing electron-withdrawing substituents have been suggested as mild, selective and recyclable nitrating agents for phenols, naphthols and aromatic amines which do not involve problems of substrate oxidation [Coombes *et al.*, **1996**].

- **Some hydrated metal nitrates** (containing covalent nitro groups) and their dinitrogen tetroxide complex analogues have been used for the nitration of phenols under various conditions [Mohammad *et al.*, **2001**]. Heterogeneous reagent systems have many advantages such as simple experimental

procedures, mild reaction conditions and minimization of chemical wastes as compared to their liquid phase counterparts.

There is a great deal of evidence that NO_2^+ is present in most nitration reactions and that it is the attacking entity, for example, nitric acid has a peak in the Raman spectrum; when nitric acid is dissolved in concentrated sulfuric acid, the peak disappears and two new peaks appear, one at 1400 cm^{-1} attributable to $NO_2^+$and one at 1050 cm^{-1} due to HSO^-_4 [Ingold *et al.*, **1950**].

The rate-determining step is formation of nitronium ion and the substrate does not take part in the formation of the attacking entity. Therefore rate of the reaction with most reagents is proportional to the concentration of the nitronium ions, not to that of other species. When the reagent produces this ion in small amounts, the attack is slow and only activated (electron rich) substrates can be nitrated.

2.3.5.1 **Reduction of Nitro Compounds**

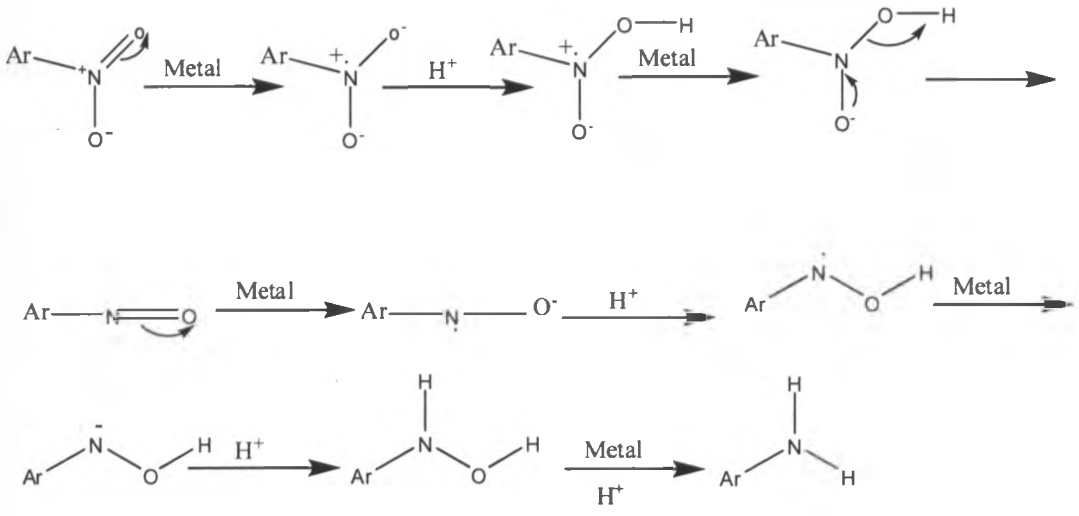
Both aliphatic and aromatic nitro compounds can be reduced to amines, although the reaction has been applied much more often to aromatic nitro compounds, owing to their greater availability. Many reducing agents have been used to reduce aromatic nitro compounds; the most common being Zinc, Tin, or Iron in an acid (commonly, hydrochloric acid), and catalytic hydrogenation. Indium metal in aqueous ethanol with ammonium chloride or with water in aq. THF also reduces aromatic nitro compounds to the corresponding aniline derivatives [March and Smith, **2007**].

Nitro-compounds have been reduced to amines by use of palladium, platinum, or rhodium metal catalyst with formic, phosphinic, or phosphorous acid. With formic

acid, nitro-compounds containing fluorine were reduced but not those containing chlorine, bromine, or iodine. With the other acids, nitro-compounds containing any of the halogens were reduced with retention of the halogen [Jackson *et al.*, **1977**].

The mechanisms of these reductions have not been studied much, although it is usually presumed that, at least with some reducing agents, nitroso compounds and hydroxyl amines are intermediates. Both these types of compounds give amines when exposed to most of these reducing agents, and hydroxylamines can be isolated.

With metals and acid the following path has been suggested: [March and Smith, 2007]



Scheme 12: Mechanism of reduction of Nitro group

CHAPTER THREE

RESULTS AND DISCUSSION

The compounds discussed here are of semi-synthetic origin obtained by transformation of natural chalcones (**22-25**) isolated from *P.senegalense* at the Department of Chemistry, University of Nairobi. These chalcones were tested and found to have anti-plasmodial activity [Midiwo *et al.*, **2007**].

The compounds formed include two imines (**1** and **2**), four flavanones (**3-6**), four dihydrochalcones (**7-10**), three products from Kostanecki reactions (**11-13**) and a nitro derivative of dihydrochalcone (**14**). The dihydrochalcones were obtained via catalytic hydrogenation, flavanones through sodium hydroxide catalyzed cyclization of 2'-hydroxychalcones, imines formed by reaction of chalcones with primary amines, nitration of dihydrochalcone in nitic acid and finally acetylated products and a flavone formed by subjecting the dihydrochalcones synthesized to Kostanecki conditions.

Some of the products formed were tested to assess their *in-vitro* anti-plasmodial activities. The tested compounds showed moderate anti-plasmodial activity with some having enhanced activities when compared to the parent chalcones against chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains respectively.

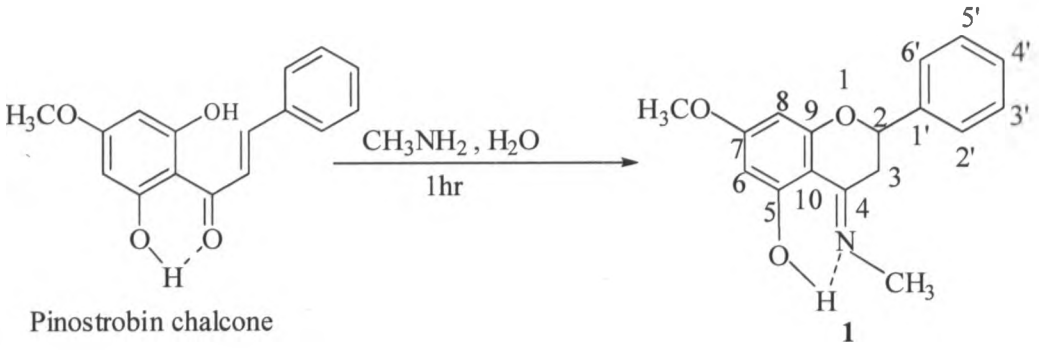
The structures of the synthesized compounds were determined using ^{1}H, ^{13}C NMR and MS techniques.

3.1 Synthesis and Characterization of Compounds

3.1.1 Imines from Pinostrobin chalcone

3.1.1.1 7-Methoxy-4-(methyl imino)-2-phenylchromn-5-ol (1)

Reaction of pinostrobin chalcone (**24**) with methyl amine in water produced 7-methoxy-4-(methyl imino)-2-phenylchroman-5-ol (Scheme 13). The product was filtered, washed with tap water and dried then recrystallised in CH_2Cl_2/hexane to furnish green-yellow needles (**1**), Mp 171-173°C , R_f = 0.35 (CH_2Cl_2: MeOH, 95:5 %) and yield 45%.

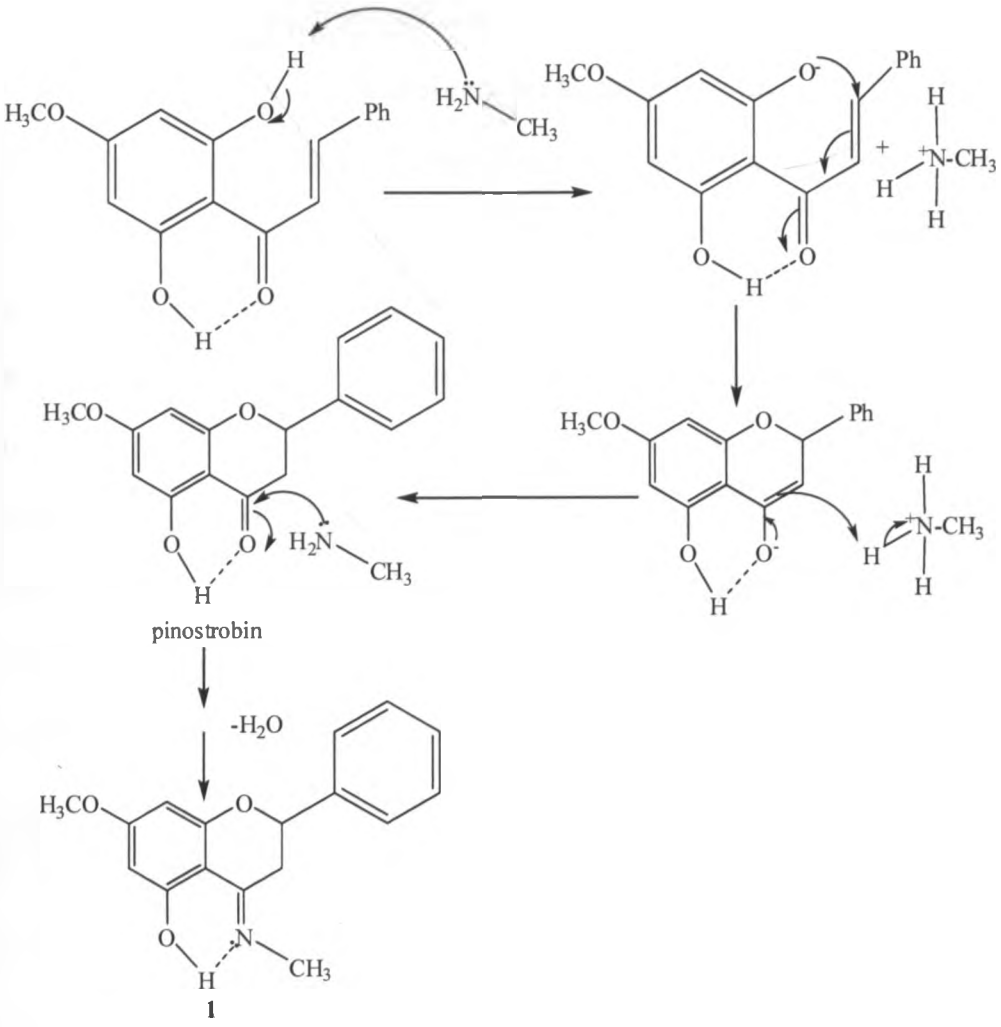


Pinostrobin chalcone

1

Scheme 13: Synthesis of 7-methoxy-4-(methylimino)-2-phenylchroman-5-ol

Compound **1** is formed following cyclization of the chalcone to a flavanone and subsequent attack of the carbonyl functionality by the excess methyl amine. Methyl amine being a base, deprotonates the *O*-hydroxyl generating a substituted phenoxide which then undergoes intramolecular 1,4 Michael addition like reaction with the 2,3 unsaturated enone to give pinostrobin as a result of ring closure at the β-position relative to the carbonyl. Reaction of the amine with the activated carbonyl carbon (via chelation) affords the flavanone imine (**1**) as explained by the following reaction mechanism (Scheme 14).



Scheme 14: Mechanism of formation of compound 1

Based on its ^{1}H, ^{13}C NMR (Table 1) and MS spectroscopic data, compound **1** was identified as a methyl imine flavanone. EI-MS analysis gave a molecular ion peak at *m/z* 283 corresponding to the molecular formula of C_{17} H_{17} N O_3. The M^+ is a odd number which is in agreement with the 'nitrogen rule' which states that an odd-numbered molecular weight requires an odd number of nitrogen atoms whereas a molecule of even-numbered molecular weight must contain either an even number of

nitrogen atoms or no nitrogen [Silverstein *et al.*, **2005**]. This confirmed methyl amine-carbonyl condensation and the above molecular formula.

The ^{1}H NMR data (Table 1) of compound **1** showed a *dd* at δ 5.15 ppm (indicating oxygenation), with *J* values of 12.5 and 3.0 Hz corresponding to splitting by two protons at an axial and equatorial positions respectively. Upfield signal at δ 3.02 ppm, a *dd* of *J* values 17.0 and 3.0 Hz due to one proton implied that this proton is coupled to two protons; geminal and axial, respectively and therefore this signal due to H-3_{eq}. Another *dd* at δ 2.83 ppm of *J* values 16.5 and 12.0 Hz indicated coupling with two protons which are geminal and axial, respectively therefore these peaks are due to H-3_{ax}. The two aromatic protons which were chemically equivalent (for the chalcone) appeared as *doublets*; H-6 (δ 5.98 ppm) and H-8 (δ 5.82 ppm) with coupling constants 2.34 Hz. This implied that H-6 and H-8 are chemically nonequivalent and cyclization to form γ-pyrone ring. Downfield at δ 7.37-7.48 ppm, a *multiplet* due to five phenyl protons was observed meaning ring B is not substituted. Unsubstitution at ring B is also supported by MS fragment at *m/z* 206 due to loss of a phenyl moiety. Also observed from ^{1}H NMR is a doublet at δ 3.18 ppm with J value of 3.5Hz due methyl protons of the methyl amine.

From ^{13}C NMR, the initially sp^2 hybridized α and β carbons are observed at δ 33.3 ppm and δ 79.6 ppm for the α-C and β-C, respectively. This indicates saturation and oxygenation at the α-C and β-C, respectively. Additional carbon from the methyl amine was observed at δ 32.2 ppm. Therefore, from ^{1}H, ^{13}C NMR and MS, **1** is an imine flavanone substituted at ring A (5-OH, δ 15.52 ppm (chelated H), 7-methoxy, ^{1}H; *singlet*, δ 3.76 ppm, ^{13}C; δ 55.5 ppm) with methyl amine moiety attached to it at C-4 forming an imine.

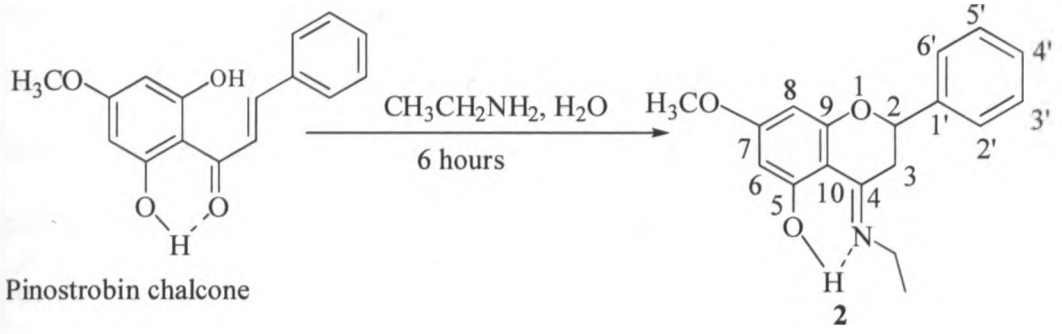
Table 1: Spectral assignments of ^{1}H, ^{13}C (500, 50 MHz) for compound **1**

Position	^{1}H δ ppm, *m*, (*J* in Hz) ($CDCl_3$)	^{13}C
2	5.15, *dd* (J_{3ax} 12.5, J_{3eq} 3.0)	76.9
3	Ha:2.83, *dd*,(J_2 16.5,J_{3ax} 12.0) He: 3.02,*dd*, (J_2 17.0, J_{3ax} 3.0)	33.3
4		173.5
5	-OH, 15.52, *s*,	166.8
6	5.98, *d* (2.0)	96.9
7		167.3
8	5.82, *d* (2.5)	92.2
9		159.5
10		100.6
1'		139.3
2'6'	} 7.37-7.48, *m*	126.4
3'5'		129.1
4'		126.4
11	3.19, *d*, (3.5)	32.2
OMe	3.76, *s*	55.5

3.1.1.2 **4-(Ethyl imino)-7-methoxy-2-phenylchroman-5-ol** (2).

Reaction of the pinostrobin chalcone with ethyl amine in water (Scheme 15) produced 4-(ethyl imino)-7-methoxy-2-phenylchroman-5-ol (**2**). The formation of this imine is via mechanism similar to that proposed for compound **1** (Scheme 14, on page 37).

The product was filtered, washed with tap water and dried then recrystallised in CH_2Cl_2/Hexane to give yellow crystals, Mp 118-120°C with R_f = 0.29 (Hex/EtOAc 1:4), yield 74%.



Scheme 15: Synthesis of 4-(ethyl imino)-7-methoxy-2-phenylchroman-5-ol

The structure of compound **2** was determined using ^1H, ^{13}C NMR and MS techniques. Its molecular formula was established based on EI-MS spectrum which gave a molecular ion peak at *m/z* 297 corresponding to the molecular formula of C_{18} H_{19} N O_3. The molecular ion peak is an odd number as predicted by the 'nitrogen rule' [Silverstein *et al.*, **2005**]. This confirms incorporation of nitrogen atom and by extension the above molecular formula.

The ^1H and ^{13}C NMR spectral data (Table 2) indicate that this compound is an imine flavanone. ^1H NMR spectrum of compound **2** displayed a *dd* (H-2) at δ 5.12 ppm, with coupling constants of 11.8 and 3.6 Hz corresponding to splitting by two protons one at axial and the other at equatorial orientation, respectively. The methylene

protons: H_{3e} at δ 3.03 ppm showed a *dd* of *J* values 17.2 and 3.6 Hz indicating coupling with two protons with one geminal and the other axial while H_{3a} at δ 2.8 ppm showed a *dd* of *J* values 17 and 11.8 Hz indicating coupling with two protons that are geminal and axial, respectively. H-6 (δ 5.98 ppm) and H-8 (δ 5.82 ppm) appeared as *doublets* with coupling constants 2.34 Hz implying that H-6 and H-8 which were chemically equivalent are now chemically nonequivalent and hence presence of a γ-pyrone ring. Downfield at δ 7.37-7.48 ppm, a *multiplet* due to five phenyl protons was observed meaning ring B is not substituted. Un-substitution at ring B is supported by MS fragment at *m/z* 220 showing loss of a phenyl group. ^{1}H NMR displayed a *triplet* at δ 1.34 ppm with *J* value of 7 Hz due to methyl protons split by methylene protons. A broad *multiplet* at δ 3.46 ppm is due to methylene protons coupling with methyl protons and the chelated proton at δ 15.65 ppm on C-5. Broadening is attributed to quadrupole effect of ^{14}N on the methylene protons.

^{13}C NMR spectrum displayed the initially sp^2 hybridized α and β carbons at δ 33.3 ppm and δ 79.6 ppm for the α-C and β-C, respectively. This indicated saturation and oxygenation at the α-C and β-C, respectively. Ethyl carbons were observed at δ 15.4 (C-12) and 40.1 ppm (C-11) confirming ethyl amine-carbonyl condensation to form **2**. Therefore, from ^{1}H and ^{13}C NMR, compound **2** is ethyl imine-flavanone substituted at ring A (5-OH, δ 15.52 ppm (chelated H), 7-methoxy, H; *singlet*, δ 3.76 ppm, ^{13}C; δ 55.5 ppm).

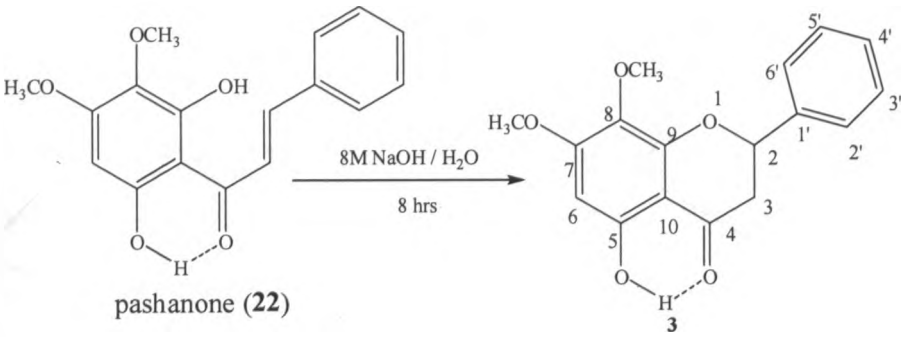
Table 2: Spectral assignments of ^{1}H, ^{13}C (500, 50 MHz) for compound **2**

Position	^{1}H δ ppm, *m*, (*J* in Hz) ($CDCl_3$)	^{13}C δ ppm
2	5.15, *d*, (J_{3ax} 12.0, J_{3eq} 3.0)	76.9
3	Hax: 2.85, *dd*, (J_2 17.0, J_{3ax} 12.0) Heq: 3.04, *dd*, (J_2 17.0, J_{3ax} 3.0)	33.2
4		174.0
5	-OH, 15.65, s	165.9
6	5.96, *d*, (2.0)	97.0
7		166.9
8	5.81,*d*,(2.5)	92.1
9		159.6
10		100.3
1'		139.3
2'6'	} 7.37-7.48, *m*	126.4
3'5'		129.1
4'		129.1
11	3.46, *m*	40.1
12	1.34, *t* , (7.0)	15.4
OMe	3.77, *s*	55.5

3.1.2 Synthesis and Characterization of Flavanones

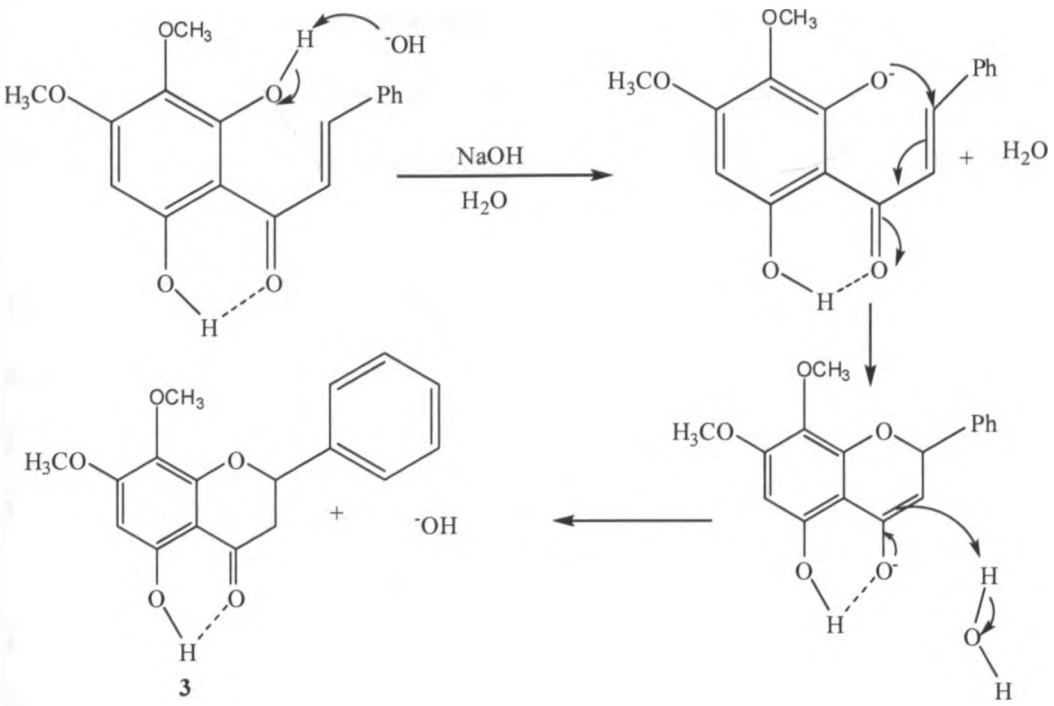
3.1.2.1 **5-Hydroxy, 7, 8-dimethoxyflavanone** (3)

Conversion of the pashanone (**22,** red crystals) to flavanone **3** (Scheme 16) was carried out in aqueous media under the catalytic aid of sodium hydroxide at room temperature for 8 hours. Compound 3 was then filtered out, washed with water, dried in a desiccator and recrystalised from CH_2Cl_2/Hexane to give colourless crystals, mp, 95-98 °C, R_f value 0.54 (Hex/ EtOAc, 7:3) and yield 19%.



Scheme 16: Synthesis of 5-hydroxy-7, 8-dimethoxyflavanone

Compound **3** was formed as a result of ring closure at the β-position relative to the carbonyl which proceeds with chalcones under basic conditions. In this case the base abstracts the *O*-hydroxyl proton forming a phenoxide and hence β -cyclization which proceeds by 1, 4-Michael addition leading to flavanone. The mechanism of this reaction is explained by (Scheme 17).



Scheme 17: Mechanism of cyclisation of 2'-chalcone

The structure of **3** was determined using ^{1}H and ^{13}C NMR data (Table 3) and comparison with reference data [Kamperdick *et al.,* **2002**] suggested that this compound is 5-hydroxy- 7, 8-dimethoxyflavanone. The ^{1}H NMR of compound **3** exhibit a *dd* at δ 5.48 ppm due to H-2, with coupling constants 12.11 and 3.52 Hz corresponding to splitting by two protons that are at axial and equatorial orientations respectively. Likewise, a signal observed at δ 3.10 ppm as a *dd* with *J* values 11.72 and 17.19 Hz indicating that this signal is due to $H\text{-}3_{ax}$ which is axially and geminally coupled. $H\text{-}3_{eq}$ is observed as a *dd* at δ 2.85ppm with *J* values of 17.19 and 3.52Hz indicating geminal and axial coupling, respectively. Ring B protons are responsible for unresolved *multiplet* at δ 7.37-7.46 ppm.

From ^{13}C NMR, the initially sp^2 hybridized α and β carbons were observed at δ 43.6 ppm and δ 79.8 ppm for the α-C and β-C, respectively. This indicated saturation and oxygenation at the α-C and β-C, respectively. Therefore from ^{1}H and ^{13}C NMR

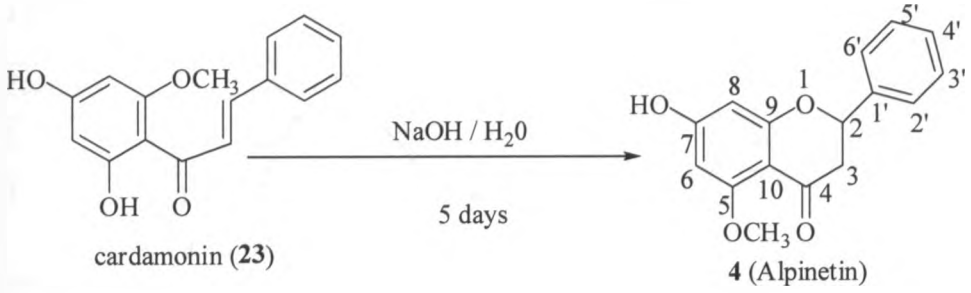
spectral data, a flavanone skeleton with substitutions at ring A (5-OH; *singlet*, δ 11.97 ppm, H-6; *singlet,* δ 6.12 ppm and 7, 8-dimethoxy; H, two *singlets* δ 3.90, 3.79 ppm, ^{13}C; δ 61.2 and 56.4 ppm) confirmed that **3** is a 5-hydroxy, 7, 8-dimethoxyflavanone.

Table 3: Spectral assignments of ^{1}H, ^{13}C (200, 50 MHz) for compound **3**

Position	^{1}H δ_{ppm}, *m*, (*J* in Hz)	^{13}C
2	5.48,*dd*, (J_{3ax}12.11, J_{3eq} 3.52)	79.8
3	Ha: 3.10, *dd*, ($J_2$17.19, J_{3ax} 11.72) He: 2.85, *dd*, ($J_2$17.19, J_{3ax} 3.52)	43.6
4		196.8
5	-OH, *s*, 11.97	158.9
6	6.12, *s*	93.4
7		161.2
8		130.8
9		155.3
10		103.1
7 –OMe	3.90, *s*	56.4
8-OMe	3.79, *s*	61.2
1'		138.51
2'6'	} *m*, 7.37-7.46	126.4
3'5'		129.2
4'		129.1

3.1.2.2 **Alpinetin** (4)

Alpinetin (**4**) was synthesized from cardamonin (**23**) in water under sodium hydroxide catalysis at room temperature after a period of five days (Scheme 18). Crude of compound **4** was obtained by filtration, washed with water followed by drying in a desiccator then recrystallized from CH_2Cl_2/Hexane giving white needles, Mp, 218-221°C , R_f value 0.23 (Hex. /EtOAc, 1:1) and yield 64%.



Scheme 18: Synthesis of Alpinetin

The mechanism of formation of this compound is similar to that of compound **3** (Scheme 17)

Basing on its ^1H, ^{13}C NMR spectroscopic data (Table 4*)* and reference to literature [Ching *et al.,* **2007**], tLhis compound was deduced to be 7-hydroxy-5-methoxyflavanone. The ^1H NMR of compound **4** (alpinetin) exhibied a *dd* at δ 5.46 ppm for H-2, with coupling constants of 12.11 and 3.13 Hz corresponding to splitting by two protons that are axial and equatorial, respectively. Likewise, H-3_{eq} at δ 2.62 ppm shows a *dd* of *J* values 16.4 and 3.12 Hz indicating coupling with two protons of which one is geminal and the other axial to it. Also H-3_{ax} was observed at δ 2.98 ppm as a *dd* with *J* values 12.11 and 16.02 Hz indicating coupling with two protons that are axial and geminal respectively. Ring A protons; H-6 (δ 6.15 ppm) and H-8(δ 6.09 ppm) appeared as *doublets* with *J* values of about 2 Hz indicating *meta* coupling.

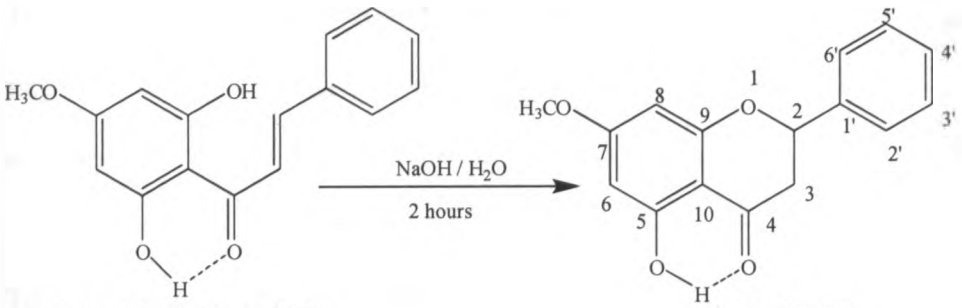
From ^{13}C NMR, the initially sp^2 hybridized α and β carbons were observed at δ 45.5ppm and δ 78.7 ppm for the α-C and β-C, respectively. This indicated saturation and oxygenation at the α-C and β-C, respectively. Therefore from ^{1}H NMR, a flavanone system with substitutions at ring A (7-OH, 5-methoxy, ^{1}H; *singlet*, δ 3.78 ppm, ^{13}C; δ 56.3 ppm) above confirms that **4** is a flavanone.

Table 4: Spectral assignments of ^{1}H, ^{13}C (200, 50 MHz) for compound **4**

Position	^{1}H δ ppm, *m*, (*J* in Hz)	^{13}C
2	5.46, *dd*, (J_{3ax} 12.11, J_{3eq} 3.13)	78.7
3	Ha: 2.98, *dd*, (J_2 16.02, J_{3ax} 12.11) He: 2.62, *dd*, (J_2 16.4, $J_{3\,ax}$ 3.12)	45.5
4		188.0
5		164.7
6	6.15, *d*, (1.99)	94.0
7		165.0
8	6.09, *d*, (1.56)	96.30
9		162.9
10		105.2
1'		139.8
2'6'	} 7.37-7.46, *m*	127.0
3'5'		129.2
4'		129.0
5-OMe	3.79, *s*	56.3

3.1.2.3 **Pinostrobin (5)**

A suspension of pinostrobin chalcone (red) in water was treated with sodium hydroxide solution and stirred for two hours (Scheme 19) to provide colourless plates, Mp 95-100°C , R_f value 0.67 (Hex. /EtOAc, 7:3) and yield 89 %.



pinostrobin chalcone (24) Pinostrobin (**5**)

Scheme 19: Synthesis of Pinostrobin (5)

The mechanism of formation of this compound is similar to that of compound **3** (Scheme 17, on page 44).

The structure of **5** was determined using the ^{1}H and ^{13}C spectra (Table 5), and confirmed by comparison of its spectroscopic data against literature data of pinostrobin obtained from natural sources [Ching *et al.,* **2007**; Kul'magambetova *et al.*, **2002**; Asakawa, **1970**]. ^{1}H NMR of compound **5** showed a *dd* at δ 5.42 ppm (H-2), with coupling constants of 12.5 and 3.12 Hz corresponding to splitting by two protons that are axial and equatorial, respectively. Likewise, $H\text{-}3_{eq}$ at δ 2.82 ppm shows a *dd* of *J* values 17.9 and 3.12 Hz indicating coupling with two protons which are geminal and axial, respectively. $H\text{-}3_{ax}$ at δ 3.12 ppm showed a *dd* of *J* values 17.9 and 12.89Hz indicating coupling with two protons that are geminal and axial respectively. H-6 (δ 6.08 ppm) and H-8 (δ 6.1 ppm) appear as *doublets* with coupling constants 2.34 Hz. This indicated that H-6 and H-8 are chemically nonequivalent

confirming cyclization to form ring C of the flavanone. From ^{13}C NMR, the initially sp^2 hybridized α and β carbons are observable at δ 43.6 and δ 79.5 ppm for the α-C and β-C respectively. This indicated saturation and oxygenation at the α-C and β-C, respectively.

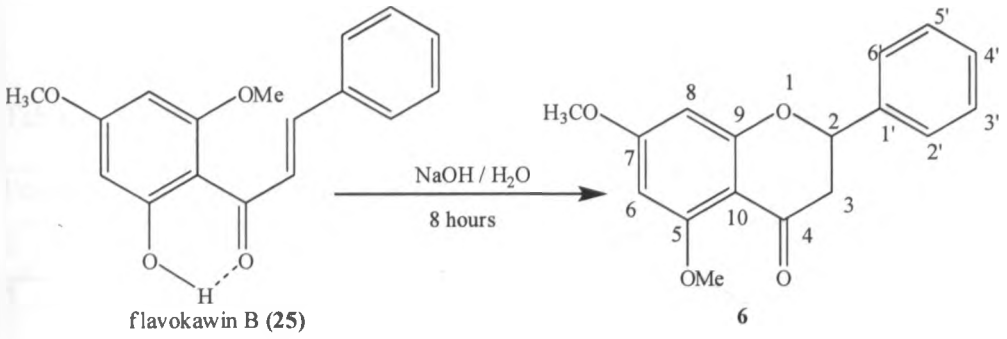
Therefore from ^{1}H, ^{13}C NMR and comparison to literature, a flavanone substituted at ring A (5-OH, δ 12.02 ppm, 7-methoxy, ^{1}H; *singlet*, δ 3.8 ppm, ^{13}C; δ 55.9 ppm) confirmed that indeed, **5** is a 5-hydroxy-7-methoxyflavanone.

Table 5: Spectral assignments of ^{1}H, ^{13}C (200, 50 MHz) for compound **5**

Position	^{1}H δ ppm, *m*, (*J* in Hz)	^{13}C
2	5.42, *dd*, (J_{3ax} 12.5, J_{3eq} 3.12)	79.5
3	H_{ax}: 3.12 *dd* (J_2 17.19, J_{3ax} 12.89) H_{eq}: 2.82, *dd*, (J_2 17.19, J_{3ax} 3.12)	43.6
4		196.0
5	-OH, 12.02, *s*	164.4
6	6.08, *d*, (2.34)	95.4
7		168.2
8	6.1, *d*, (2.34)	94.5
9		163.0
10		103.4
1'		138.4
2'6'	} 7.37-7.48, *m*	126.4
3'5'		129.1
4'		126.5
OMe	3.8, *s*	55.9

3.1.2.4 **5, 7-Dimethoxyflavanone** (6)

5, 7-Dimethoxyflavanone (**6**) was prepared by treating suspension of flavokawin B (**25**) in water with sodium hydroxide solution at room temperature, for eight hours (Scheme 20). The white material formed was filtered out, dried in a desiccator and recrystallised from methanol as colourless crystals, Mp 143-145°C, R_f value 0.36 (Hex/EtOAc, 1:1) and yield 86%.



Scheme 20: Synthesis of 5, 7-dimethoxyflavanone

The mechanism of formation of this compound is similar to that of compound **3** (Scheme 17, on page 44).

The ^1H and ^{13}C NMR spectra (Table 6) indicated that this compound is 5, 7-dimethoxyflavanone. The ^1H spectra of compound **6** showed a *dd* at δ 5.48 ppm (H-2), with coupling constants of 12.89 and 3.13Hz corresponding to splitting by two protons that are at axial and equatorial positions respectively. Likewise, H-3_{eq} at δ 2.82 ppm showed a *dd* of *J* values 16.41 and 3.13 Hz indicating coupling with two protons that are geminal and axial, respectively. Another signal at δ 3.06 ppm observed as a *dd* with *J* values 16.41 and 12.89 Hz indicated that the proton (H-3_{ax})

couples with two protons that are at geminal and axial positions relative to it. Protons H-6(δ 6.16 ppm) and H-8(δ 6.09 ppm) are *meta* coupled and therefore doublets with *J* value of 2.3Hz. From ^{13}C, the initially sp^2 hybridized α and β carbons are observable at δ 45.8 ppm and δ 79.5 ppm for the α-C and β-C, respectively. This indicates saturation and oxygenation at the α-C and β-C, respectively. From ^{13}C NMR data, a flavanone skeleton substituted at ring A (5, 7-dimethoxy, ^{1}H; two *singlets*, δ 3.82, 3.91 ppm, ^{13}C; δ 55.8, 56.4ppm) confirmed that indeed, **6** is a 5,7-dimethoxyflavanone.

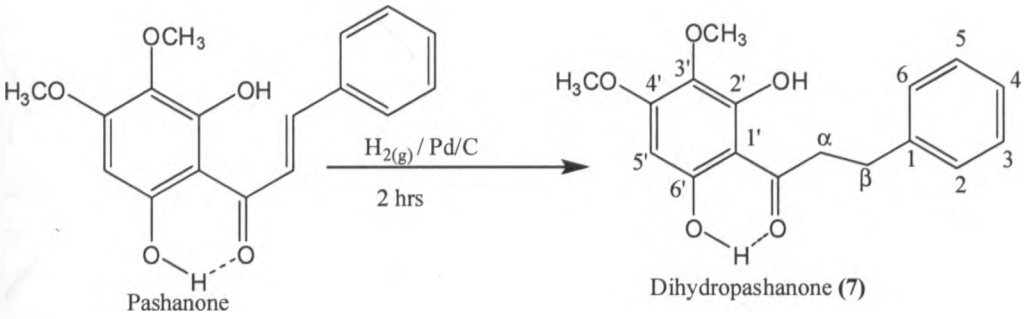
Table 6: Spectral assignments of ^{1}H, ^{13}C (200, 50 MHz) for compound **6**

Position	^{1}H δ ppm, *m*, (*J* in Hz)	^{13}C
2	5.39, *dd*, (J_{3ax} 12.89, J_{3eq} 3.13)	79.5
3	Ha: 3.06, *dd*, (J_2 16.41, J_{3ax} 12.89) He: 2.82, *dd*, (J_2 16.41, J_{3ax} 3.13)	45.8
4		189.5
5		165.2
6	6.16, *d*, (2.35)	93.4
7		166.2
8	6.09, *d* (2.34)	93.8
9		162.5
10		106.2
1'		139.0
2'6'	7.37-7.47, *m*	126.4
3'5'		129.0
4'		128.9
5-OMe	3.91, *s*	56.4
7-OMe	3.82, *s*	55.8

3.1.3 Synthesis of Dihydrochalcones

3.1.3.1 **Dihydropashanone (7)**

Dihydropashanone (7) was prepared by hydrogenating a solution of pashanone over 5% palladium catalyst on charcoal at room temperature and pressure for 2 hours (Scheme 21). White crystals were obtained following filtration, evaporation of the solvent then recrystallisation from CH_2Cl_2/ hexane, Mp 128-130°C, R_f =0.46 (Hex/ EtOAc, 7:3) and yield 96 %.



Scheme 21: Hydrogenation of pashanone

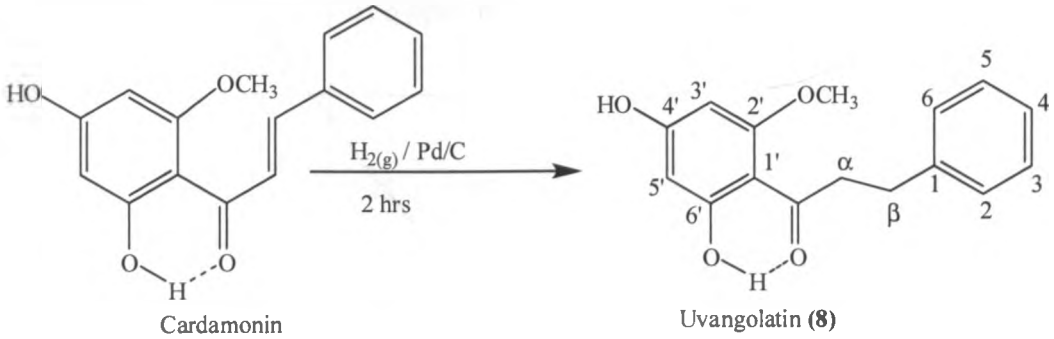
From ^{1}H NMR (Table 7) and reference to literature data of naturally sourced dihydropashanone isolated from *Lindera erythrocarpa* [Ichino *et al.*, **1988**], this compound is dihydropashanone. ^{1}H NMR displayed the characteristic A_2B_2 pattern for dihydrochalcones exhibited as *triplets* at δ 3.42 and 3.02 ppm, with coupling constant of 7.2 Hz corresponding to two α protons being split by two β protons respectively and vice versa. Furthermore, ^{1}H NMR showed two methoxy groups at δ 3.83 ppm (3H, *s*) and 3.89 ppm (3H, *s*), an aromatic proton at δ 6.63 (1H, *s*), a *multiplet* belonging to phenyl protons at δ 7.17-7.34 ppm (5H, *m*), and a chelated hydroxyl group at δ 13.42 ppm. These data suggested that this compound is dihydropashanone. ^{13}C NMR and DEPT spectra showed two methylene carbons at δ

45.4 and 30.6 ppm attributed to α and β carbons respectively. These set of *triplets* which is characteristic for dihydrochalcones and the chemical shift values of these carbons imply that these carbons are saturated confirming addition of hydrogen atoms at α and β positions, respectively.

Table 7: Spectral assignments of ^{1}H, ^{13}C (500, 125 MHz) for compound **7**

Position	^{1}H δ ppm, *m*, (*J* in Hz)	^{13}C
C=O		204.6
α	3.42, *t*, (7.2)	45.4
β	3.02, *t*, (7.2)	30.6
1		141.8
2/6	} 7.20-7.28, *m*	128.6
3/5		128.7
4		126.2
1'		103.9
2'		158.2
3'		128.0
4'		151.4
5'	6.75, *s*	92.8
6'-OH	13.42, *s*	162.2
3'-OMe	3.83, *s*	61.6
4'-OMe	3.89, *s*	56.2

3.1.3.2 **Uvangolatin (8)**



Scheme 22: Hydrogenation of cardamonin

Uvangolatin (**8**) was prepared by hydrogenation of a solution of cardamonin over 5% palladium catalyst on charcoal at room temperature and pressure for 2 hours (Scheme 22). This compound was obtained as colourless needles, Mp, 126-130°C, R_f = 0.46 (Hex/ EtOAc, 7:3) and yield 98%.

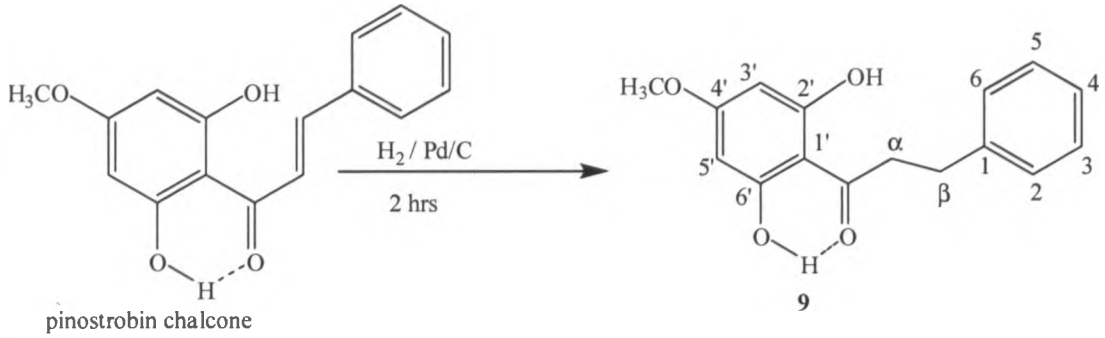
The structure of this compound was determined on the basis of its ^1H, ^{13}C NMR spectra (Table 8) and comparison to previous data of uvangolatin isolated from *Uvaria angolensis* [Hufford and Oguntimein, **1980**]. ^1H NMR showed the characteristic A_2B_2 pattern for dihydrochalcones exhibited as a *triplet* at δ 3.42 ppm, with coupling constant of 7.03 Hz corresponding to α protons being split by two β protons. Another set of a *triplet* at δ 3.02 ppm with coupling constant of 7.03 Hz is due to two protons at the β position being split by two α protons. Saturation at the initially sp^2 hybridized α and β carbons is also evident from ^{13}C NMR from which the two carbons (α and β) are now observed at δ 45.7 and δ 30.7 ppm respectively in the case of **8**. Therefore, from ^1H and ^{13}C NMR, the chalcone cardamonin was hydrogenated at α and β positions, respectively.

Table 8: Spectral assignments of ^{1}H, ^{13}C (200, 50 MHz) for compound **8**

Position	^{1}H δ ppm, *m*, (*J* in Hz)	^{13}C
C=O		-
α	3.42, *t*, (7.03)	45.7
β	3.02, *t*, (7.03)	30.7
1		142.1
2,6	} 7.14-7.30, *m*	128.5
3,5		128.6
4		126.0
1'		105.0
2'-OMe		163.9
3'	6.03, *d*, (2.2)	96.2
4'		167.7
5'	5.96,*d*,(2.2)	91.2
6'-OH	13.88, *s*	165.0
-OMe	3.89, *s*	55.5

3.1.3.3 **2', 6'-Dihydroxy-4'-methoxydihydrochalcone (9)**

Transformation of pinostrobin chalcone into the dihydrochalcone **9** was carried out by hydrogenation of solution of the chalcone over 5% palladium catalyst on charcoal at room temperature and pressure for 2 hours (Scheme 23). The catalyst was filtered off, the solvent evaporated using a rotary evaporator and finally recrystallization afforded colourless crystals, Mp 158-161°C, R_f =0.4 (Hex/EtOAc, 7:3), yield 99 %.



Scheme 23: Hydrogenation of pinostrobin chalcone

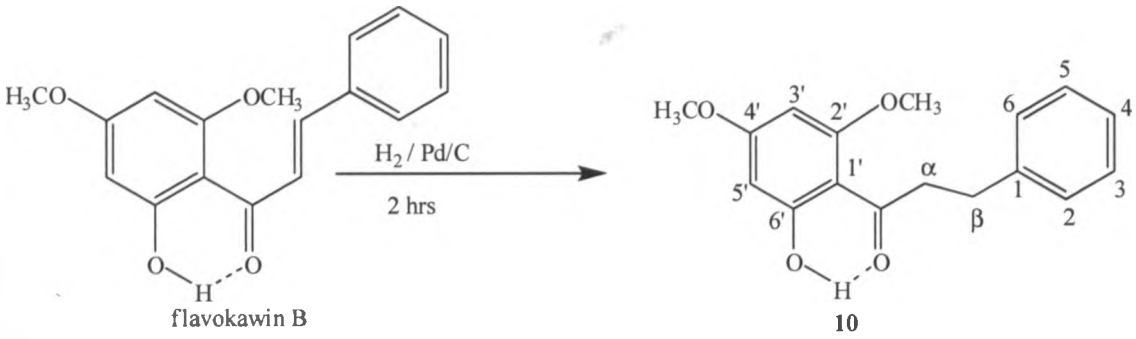
The ^1H, ^{13}C NMR spectra (Table 9) and reference data [Orjala *et al.*, **1994**] indicate that compound **9** is 2', 6'-dihydroxy-4'-methoxydihydrochalcone. The ^1H NMR of **9** exhibits a *triplet* at δ 3.42 ppm, with coupling constant of 7.03 Hz corresponding to α protons being split by two β protons. Likewise, the two β protons are responsible for a *triplet* at δ 3.02 ppm with coupling constant of 7.03 Hz. These set of *triplets* is a proof of addition of hydrogen atoms at α and β positions respectively. ^1H NMR also shows two aromatic protons on ring B at δ 5.99 (3'/5') ppm appearing as a *singlet* and the phenyl protons as a *multiplet* between δ 7.17-7.43. Hydrogenation at the α and β is supported by the chemical shift positions of the initially sp^2 hybridized carbons which for 9 are observed at δ 45.8 ppm and δ 30.6 ppm for the α-C and β-C respectively.

Table 9: Spectral assignments of ^1H, ^{13}C (200, 50 MHz) for compound **9**

Position	^{1}H δ ppm, *m*, (*J* in Hz)	^{13}C
C=O		204.9
α	3.42, *t*, (7.03)	45.8
β	3.02, *t*, (7.03)	30.6
1		142.2
2,6	} 7.173-7.341, *m*	126.0
3,5		128.6
4		128.5
1'		
2', 6'-OH	13.42, *s*	164.5
3', 5'	5.994, *s*	93.6
4'		166.2
-OMe	3.885, *s*	55.1

3.1.3.4 **Lapathone** (10)

Lapathone (**10**) was prepared by bubbling hydrogen gas into a solution of flavokawin B over 5% palladium catalyst on charcoal at room temperature and pressure for 2 hours (Scheme 24). Lapathone was obtained as colourless needles, Mp 102-105°C, R_f = 0.56 (Hex/ EtOAc, 4:1) and yield 99 %.



Scheme 24: Hydrogenation of flavokawin B

The ^1H and ^{13}C NMR spectra (Table 10) indicate that this compound is dihydrochalcone. The ^1H NMR of compound **10** exhibits a *triplet* at δ 3.27 ppm, with coupling constant of 7.03 Hz corresponding to α protons being split by two neighbouring protons (β). Likewise, two β protons are responsible for a *triplet* at δ 2.99 ppm with coupling constant of 7.03 Hz. This set of *triplets* confirmed addition of hydrogen atoms at α and β positions, respectively. Saturation is evident from the chemical shift positions of the initially sp^2 hybridized carbon atoms which are observed at δ 45.9 ppm and δ 30.9 ppm for the α-C and β-C, respectively.

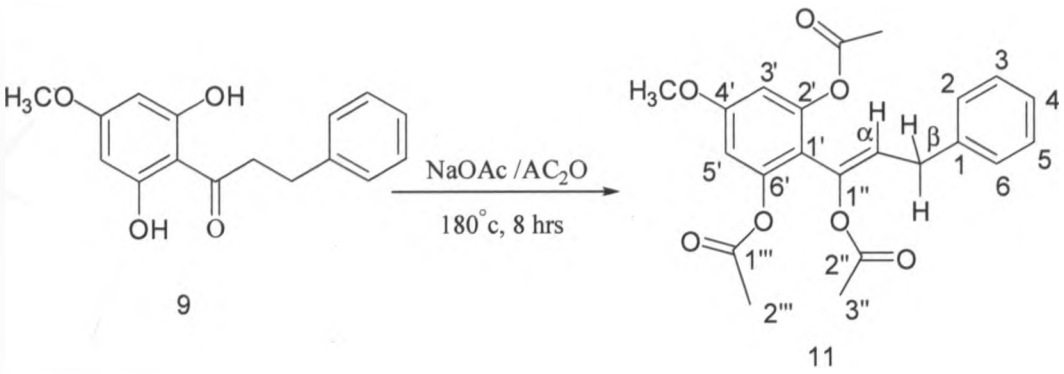
Table 10: Spectral assignments of ^{1}H, ^{13}C (200, 50 MHz) for compound **10**

Position	^{1}H δ ppm, *m*, (*J* in Hz)	^{13}C
C=O		204.7
α	3.27, *t*, (7.03)	45.9
β	2.99, *t*, (7.03)	30.9
1		141.9
2,6	} 7.20-7.35, *m*	126.2
3,5		128.68
4		128.65
1'		106.
2'		166.2
3'	6.08, *d*,(2.2)	91.1
4'		167.9
5'	5.92, *d*,(2.6)	93.9
6'- OH	14.01, *s*	162.9
2'- OMe	3.9, *s*	55.77
4'-OMe	3.83, *s*	55.81

3.1.4 Products from Kostanecki Reactions.

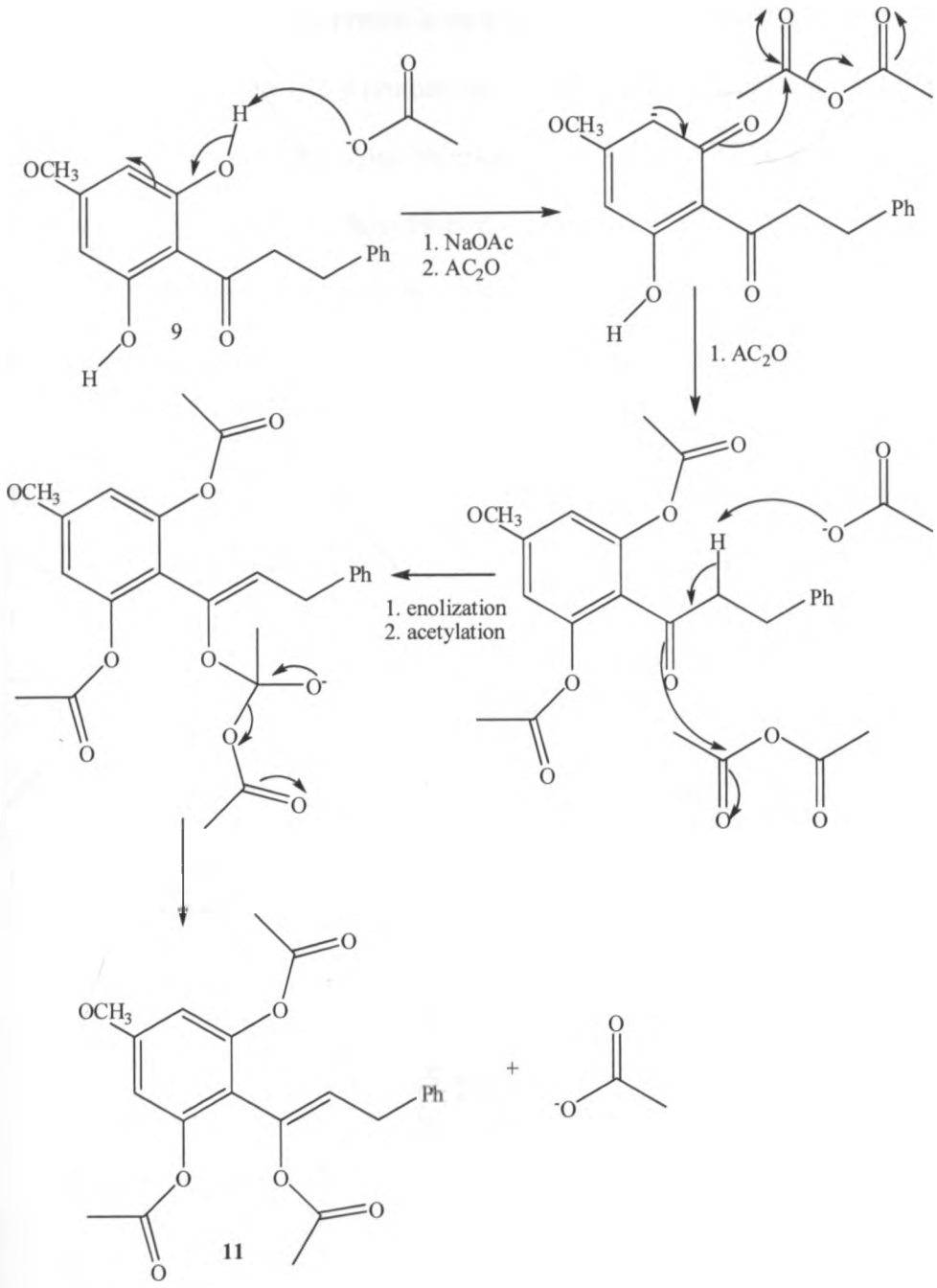
3.1.4.1 2-(1-Acetoxy-3-phenylprop-1-enyl)-5-methoxy-1,3-phenylene diacetate (11)

The dihydrochalcone **9** was heated with freshly fused sodium acetate and acetic anhydride for 8 hours (Scheme 25). The mixture was then added to ice cold water, giving a crude oily paste that was purified on silica gel column to give one pure fraction and two other products that were not purified since they were obtained in small amounts. Recrystallization of the pure fraction obtained from methanol gave white crystals, Mp 62-65°C, R_f =0.28 (Hex/ EtOAc, 7:3).



Scheme 25: Kostanecki acetylation of 9

Compound **11** is a side product formed due to acetylation of the enol tautomer of **9**. Acetylation of the hydroxyl groups at positions 2' and 6' were expected [Taylor and Weissberger, **1977**] but from the spectral data, there is also acetylation of what was the carbonyl oxygen. This is due to formation of an enol followed by acetylation due the reaction of the enol with acetic anhydride (Scheme 26).



Scheme 26: Mechanism of formation of compound 11

Assignment of ^{1}H and ^{13}C NMR spectral data (Table 11) indicated that compound **11** is an acetylated enolate of **9**. The ^{1}H NMR of compound **11** showed a *triplet* at δ 5.41 ppm, with coupling constant of 7.6 Hz corresponding to α proton being split by two

neighbouring protons. This proton is on a sp^2 carbon indicating that enolization occurred. Likewise, the two β protons are responsible for a *doublet* at δ 3.42 ppm with coupling constant of 7.6 Hz. Also observable is a singlet at δ 2.86 ppm due to methyl protons on C-2''' and C-3'' from the acetyl group.

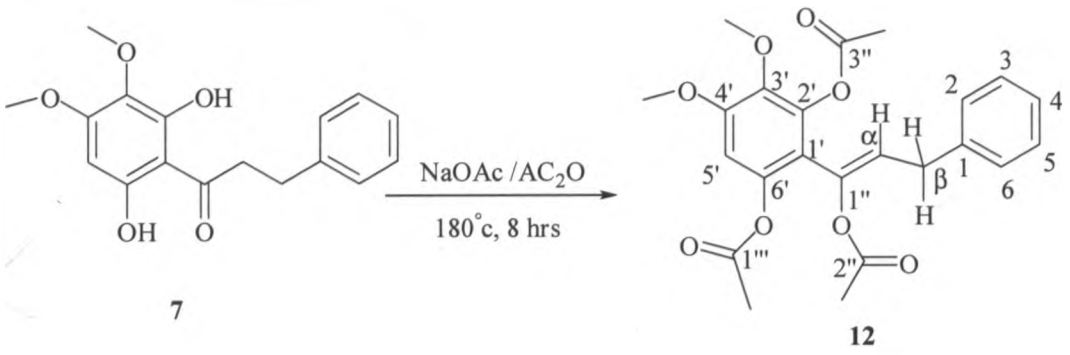
From ^{13}C NMR, methyl carbons are observed at δ 19.8 and 20.2 ppm for C-2''' and C-3'' which are the methyls of the acetyl groups.

Table 11: Spectral assignments of ^{1}H, ^{13}C (200, 50 MHz) for compound **11**

Position	^{1}H δ ppm, *m* (*J* in Hz)	^{13}C
1		137.8
2,6	} 7.24-7.33, *m*	126. 6
3,5		128.8
4		126.6
1'		116.0
2',6'		150.5
3',5'	6.33, *s*	107.2
4'		160.5
1''		139.6
2''		168.5
3''	2.86, *s*	20.2
1'''		169.6
2'''	2.86, *s*	19.8
4'-OCH_3	3.80, *s*	55.9
α	5.41, *t*, (7.6)	122.9
β	3.42, *d*, (7.6)	32.8

3.1.4.2 2-(1-Acetoxy-3-phenylprop-1-enyl) - 4, 5-dimethoxy -1,3-phenylene diacetate (**12**)

Dihydropashanone (**7**) was heated with freshly fused sodium acetate and acetic anhydride for 8 hours (Scheme 27). The mixture was then added to ice cold water, giving a crude oily paste that was purified on silica gel column to give compound **12**. Two other fractions with different R_f values from **12** could not purified further. Recrystallization of compound **12** from methanol gave white crystals, Mp 51-54°c, R_f = 0.54 (Hex/EtOAc, 7:3).



Scheme 27: Kostanecki acylation of dihydropashanone.

The mechanism of formation of compound **12** is similar to that of compound **11** (Scheme 26).

^1H and ^{13}C NMR spectra (Table 12) indicated that this compound is an acetylated enolate of dihydropashanone (**7**). The ^1H NMR of compound **12** showed a *triplet* at δ 5.46 ppm, with coupling constant of 7.5 Hz corresponding to α proton being split by two neighbouring protons. This proton is on a sp^2 carbon indicating that enolization occurred. Likewise, two β protons are responsible for a *doublet* at δ 3.41 ppm with coupling constant of 7.5 Hz. Also observable are singlets at δ 2.19, 2.09 and 2.11 ppm due to methyl protons on 2''-Me, 3''-Me and 1'''-Me from the acetyl group.

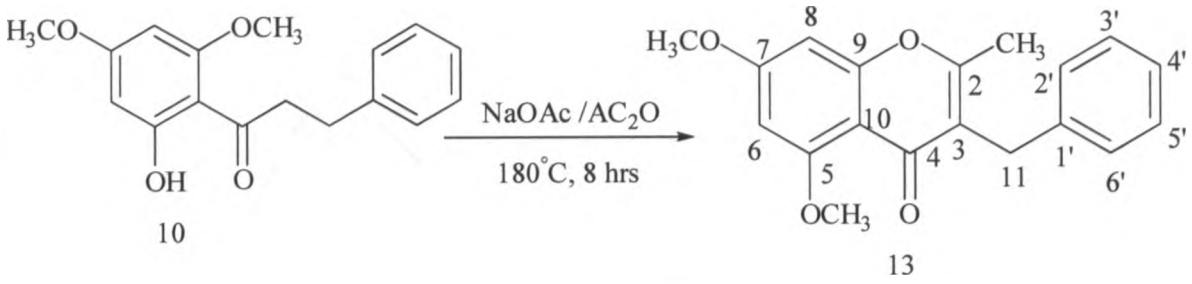
From ^{13}C NMR, methyl carbons are observed at δ 21.0, 20.8 and 20.6 ppm for 2''-Me, 3''-Me and 1'''-Me which are the methyls of the acetyl groups.

Table 12: Spectral assignments of ^{1}H, ^{13}C (300, 75 MHz) for compound **12**

Position	^{1}H δ ppm, *m* (*J* in Hz)	^{13}C
1		137.8
2,6	} 7.20-7.30, *m*	128. 8
3,5		128.6
4		126.5
1'		100.2
2'		139.5
3'		139.5
4'		153.8
5'	6.53, *s*	105.4
6'		144.7
1''		139.6
2''		168.4
2''-Me	2.19, *s*	21.0
3''		169.9
3''-Me	2.09, *s*	20.8
1'''		169.0
1'''-Me	2.11, *s*	20.6
3'-OCH_3	} 3.84, 3.80, *s*	}60.9, 56.2
4'-OCH_3		
α	5.46, *t*, (7.5)	123.2
β	3.41, *d*, (7.5)	32.8

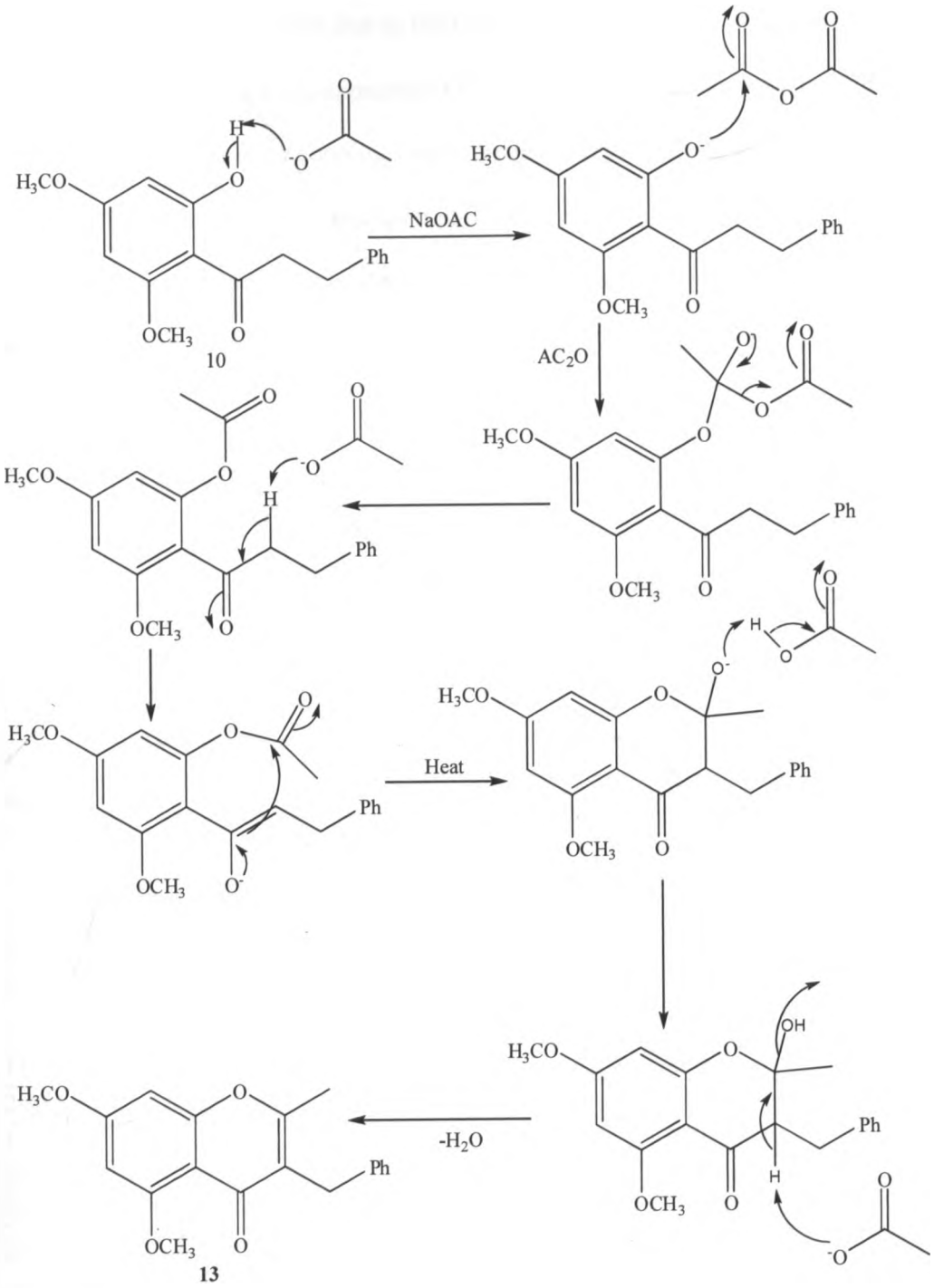
3.1.4.3 **3-Benzyl-5, 7-dimethoxy-2-methyl-4H-chromen-4-one (13)**

A mixture of dihydrochalcone **10**, freshly fused sodium acetate and acetic anhydride was heated for 8 hours (Scheme 28). The mixture was then added to cold water, giving a crude oily paste that was purified on silica gel column giving yellowish crystals, $R_f = 0.55$ (Hex/ EtOAc4:1).



*Scheme 28: Kostanecki acetylation of **10***

Scheme 29 below explains how compound **13** is formed from compound **10.**



Scheme 29: Mechanism of formation of 13

^{1}H NMR signal at δ 2.32 ppm due to the methyl protons was observed as a *singlet*. Two aromatic proton signals appeared at δ 6.32 and 6.38 ppm as *doublets* with *J* value of 2.2 Hz (indicating *meta* coupling) were assigned to H-6 and H-8, respectively. Ring B protons were observed as a *multiplet* at δ 7.13-7.30 ppm.

^{13}C NMR data (Table 13) showed additional methyl carbon at δ 18.5 ppm attributed to methyl attached to position 2 (2-Me). Shift in the δ position of the carbonyl (C-4) which in the case of dihydrochalcone was at δ 204.7 ppm to δ 179.0 ppm for **13** indicated α-β conjugation with the carbonyl of the homoisoflavone.

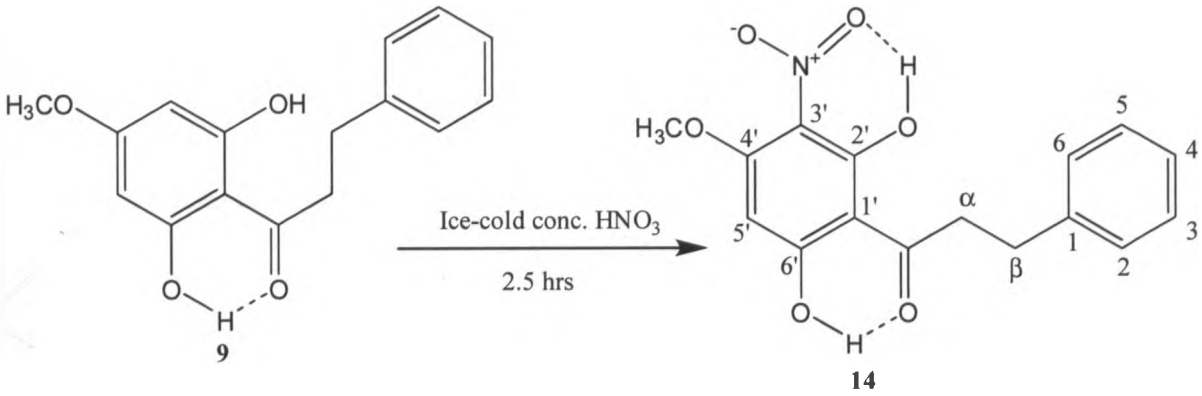
Table 13: Spectral assignments of ^{1}H, ^{13}C (200, 50 MHz) for compound **13**

Position	^{1}H δ ppm, *m*, (*J* in Hz)	^{13}C
2		159.7
3		121.7
4		179.0
5		161.2
6	6.32, *d*, (2.2)	92.5
7		163.8
8	6.38, *d*, (2.2)	95.9
9		160.8
10		119.9
11	3.8, *s*	30.1
2-Me	2.32, *s*	18. 5
1'		140.4
2'6'	7.13-7.30, *m*	128.7
3',5'		128.5
4'		126.1
5-OMe	3.93, *s*	56.5
7-OMe	3.86, *s*	55.9

3.1.5 Synthesis and Chacterisation of Nitro derivative of dihydrochalcone.

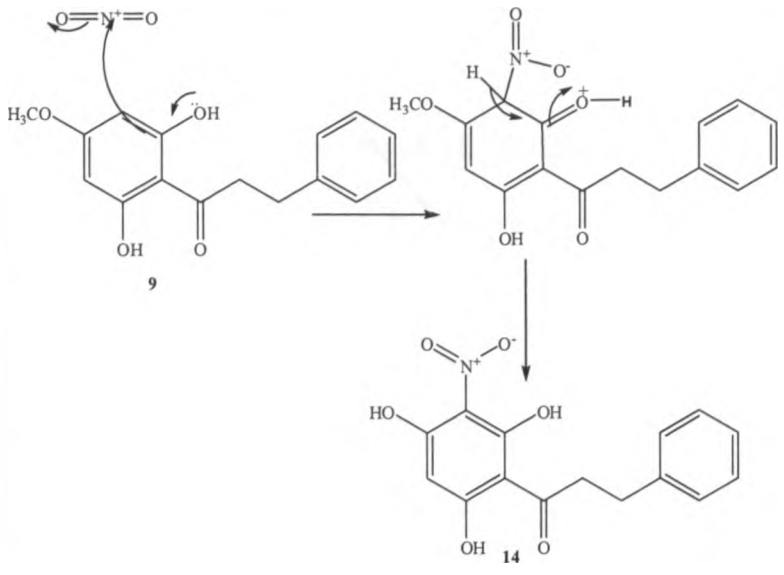
3.1.5.1 **2', 6'-Dihydroxy-4'-methoxy-3'-nitro dihydrochalcone** (14)

Compound **14** is a nitro derivative formed by nitration of compound **9** using an ice-cold concentrated nitric acid in ice-bath for 2.5 hours (Scheme 30). The product of this reaction was purified in a silica gel column to give 5.6 mg of **14,** R_f = 0.38 (hexane/EtOAc, 7:3).



Scheme 30: Synthesis of 2', 6'-dihydroxy-4'-methoxy-3'-nitrodihydrochalcone

Scheme 31 explains the nitration of **9.**



Scheme 31: Mechanism of nitration of ***9***

^{1}H and ^{13}C NMR data (Table 14) showed that this compound is a 2', 6'-dihydroxy-4'-methoxy-3'-nitro dihydrochalcone. Nitration at C-3' is exhibited by chelation of the hydroxyl hydrogen at C-2' observed as a *singlet* at δ 14.78 ppm with the nitro functionality being an additional chelating centre to the carbonyl. ^{1}H NMR of **14** exhibited A_2B_2 spin system, a characteristic of a dihydrochalcone. This is shown as a *triplet* at δ 3.02 ppm, with coupling constant of 7.03 Hz corresponding to two α protons being split by two neighbouring β protons. Likewise, two β protons are responsible for a *triplet* at δ 3.47ppm with coupling constant of 7.03 Hz. The *singlet* at δ 6.05 ppm due to the proton at C-5' confirms mononitration at C-3'. Nitration at this carbon was expected due to the fact that this position is *ortho* to two activating groups (methoxy and hydroxyl functions). The *multiplet* observed between δ 7.19-7.28 ppm is due to the phenyl protons of ring A implying that only ring B was nitrated

because it is activated (has two hydroxy and a methoxy which are electron donating groups).

Table 14: Spectral assignments of ^{1}H, ^{13}C (500, 125 MHz) for compound **14**

Position	^{1}H δ ppm, *m*, (*J* in Hz)	^{13}C
C=O		205.6
α	3.02, *t*, (7.03)	46.2
β	3.47, *t*, (7.03)	30.2
1		141.1
2,6	} 7.19-7.28, *m*	128.6
3,5		
4		126.3
1'		102.0
2'-OH	14.78, *s*	160.0
3'		
4'		163.0
5'	6.05,s	93.8
6'-OH	13.58, *s*	171.6
4-OMe	3.95,*s*	57.3

3.2 Anti-plasmodial Activities

Some of the compounds synthesized were screened for anti-plasmodial activities against chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains of *Plasmodium falciparum* using the hypoxanthine incorporation technique [Desjardins *et al.*, 1979].

The synthesized compounds showed good to poor activities (IC_{50} values ranging from 8.0 to 34.8 μM) against the *Plasmodium* parasite with some the flavanones having enhanced activity when compared to the parent chalcones. Chloroquine and mefloquine were used as the reference drugs in this experiment.

IC_{50} is the concentration of an inhibitor that is required for 50% inhibition of its target.

3.2.1 **Anti-plasmodial Activities of the Flavanones against W2 and D6 strains**

Flavanones **3**, **4** and **6** showed good anti-plasmodial activity when tested on W2 and D6 strains of the *Plasmodium* parasite with the following IC_{50} values: 12.4 and 8.6 μM (**4**), 8.0 and 6.1 μM (**6**) against W2 and D6 strains, respectively. Compound **4** and **6** have higher activities compared to their parent chalcones; cardamonin (IC_{50} =14.0 ± 1.7and 9.5 ± 1.1μM) and flavokawin B (IC_{50} =11.3 ± 0.2 and 15.7 ± 1.2 μM), respectively. Pashanone (IC_{50} = 2.4 ± 0.3and 3.1 ± 0.8 μM) had its activity diminished on conversion to flavanone **3**, (IC_{50} = 8.7 and 14.5 μM).

3.2.2 **Anti-plasmodial Activities of the Imines against W2 and D6 strains**

The methyl and ethyl imine flavanones showed moderate antiplasmodial activities with the methyl amine derivative, **1** (IC_{50} = 9.32 and 11.6 μM) being more active as compared to the ethyl amine derivative, **2** (IC_{50}= 35.69 and 31.93 μM). The methyl derivative is more active compared to the parent chalcone, pinostrobin chalcone **24**

(IC_{50} =17.09 and 13.6 μM) and pinostrobin (**5**) (IC_{50} =11.6 and 16.3 μM)) whereas the ethyl amine derivative is less active compared to both the parent chalcone and pinostrobin.

3.2.3 **Anti-plasmodial Activities of dihydrochalcones**

Three dihydrochalcones **8** (IC_{50} = 21.80 and 13.49 μM), **9** (IC_{50} =26.18 and 70.40 μM) and **10** (IC_{50} =14.31 and 34.38 μM) tested on W2 and D6 strains of *P. falciparum* showed moderate anti-plasmodial activity. All the dihydrochalcones had lower activities compared to the parent chalcones cardamonin (IC_{50} =14.0 ± 1.7 and 9.5 ± 1.1μM), pinostrobin chalcone (IC_{50} =17.09 and 13.49 μM) and flavokawin B (IC_{50} =11.3 ± 0.2 and 15.7 ± 1.2 μM) respectively. The low activity of the dihydrochalcones compared to the parent chalcones is attributed to loss of α-β conjugation.

Table 15 below summarizes the anti-plasmodial activities (in **μM**) of the formed compounds against the activities of the starting materials (parent chalcones).

Table 15: In-vitro anti-plasmodial activities of the synthesized compounds

Compound	IC_{50} in µM of compound Vs parent chalcones against W2 and D6 strains			
	W2		**D6**	
Flavanones	compound	parent chalcone	compound	parent chalcone
3	8.7	2.4 ± 0.3	14.5	3.1 ± 0.8
4	12.4	14.0 ± 1.7	8.6	9.5 ± 1.1
6	8.0	11.3 ± 0.2	6.1	15.7 ± 1.2
Imines				
1	9.32	17.09	11.6	13.6
2	35.69	17.09	31.93	13.6
Reference drugs				
Chloroquine	0.35		0.35	
Mefloquine	0.01		0.08	
Kostanecki product (IC_{50} in µg/ml)				
12	2.23	0.72	1.31	0.93

Anti-plasmodial activities continued…

Compound	IC_{50} in µM of compound Vs parent chalcones against W2 and D6 strains			
	W2		**D6**	
Dihydrochalcones	compound	parent chalcone	compound	parent chalcone
8	21.80	14.0 ± 1.7	13.49	9.5 ± 1.1
9	26.18	17.09	70.40	13.6
10	14.31	11.3 ± 0.2	34.38	15.7 ± 1.2
Pinostrobin chalcone	17.09		13.6	

CHAPTER FOUR
CONCLUSIONS AND RECOMMENDATIONS

4.1 CONCLUSIONS

In this research, four chalcones were derivatized into flavanones, imines, dihydrochalcones, enol acetates and a nitro derivative of dihydrochalcones. A total of fourteen compounds were prepared and their *in-vitro* anti-plasmodial activities evaluated. Specific conclusions drawn in this study include;

1. Reaction of amines (ethyl and methyl amine) with pinostrobin chalcone gave two new imines (compound **1** and **2**).
2. Flavanones (**3-6**) and dihydrochalcones (**7-10**) that are naturally obtained in small quantities have been synthesized in large quantities by performing synthetic transformation of chalcones.
3. Kostanecki-Robinson acetylation provided a homoisoflavone (**13**) and two side products which are acetylated enolates of the dihydrochalcones (**11** and **12**).
4. Nitration of **9** provided a nitro compound (**14**)
5. The simplicity of a chemical process often correlates well with its 'greenness'. Relatively simple one-step reactions that did not require use of organic solvents either in the reaction or in the work-up have been employed in flavanone and imine formation. In these reactions, the products were formed as a suspension in water and the organic product separated simply by filtration. Simple basic catalysts were used to ensure fast reactions under 'green' conditions.

6. The *in-vitro* anti-plasmodial activities of some the compounds formed are higher as compared to the activities of the parent compounds. Flavanones are more active compared to the parent chalcones except for the pashanone which is more active compared to its flavanone. Methyl imine of pinostrobin (**1**) is more active compared to the parent chalcone whereas the ethyl imine of pinostrobin (**2**) is less active when compared to parent chalcone. The dihydrochalcones are less active compared to the parent chalcones.

4.2 RECOMMENDATIONS

Based on this study the following recommendations are suggested:-

1. The compounds synthesized above to be screened for other biological activities such as antimicrobial, antibacterial, anti-tumor, anti-HIV and anti-cancer.

2. Larvicidal activity tests to be carried out on the compounds in order to determine their potential in larval control.

3. The imines synthesized above to be reduced to transform them to amines and their bioactivity determined.

4. The use of natural products as synthons to be considered when designing synthetic reactions.

CHAPTER FIVE
EXPERIMENTAL

5.1 General

The four chalcones (**22-25**) transformed were obtained from Natural Products Library, Department of Chemistry, University of Nairobi where they were previously isolated and their structures determined. Melting points were determined using an oil bath. Analytical TLC. was performed on Merck pre-coated silica gel 60 F_{254} plates. Column chromatography was carried out using Merck silica gel 40 (720-230 mesh). Solvents: laboratory grade, purified by distillation to constant boiling temperature range. The NMR spectra were recorded on a Varian-Mercury 200 or Bruker 500 MHz instruments. The chemical shifts (δ) were measured in ppm relative to tetramethylsilane (TMS) as the internal standard. EIMS spectra were recorded on SSQ 710 Finnigan MAT spectrometer at 70 eV.

5.2 Synthesis of Imines

Two imines were prepared by modifying the general procedure of amine-carbonyl condensation in which water was used as the reaction medium.

5.2.1 **Synthesis of 7-methoxyflavanone methyl imine (1) from pinostrobin chalcone**

A quantity of 50 mg of pinostrobin chalcone was introduced into a 25 ml conical flask. Methyl amine (1ml) was added then tightly corked. The reaction was allowed to proceed at room temperature while shaking at intervals of 15 minutes for one hour.

Greenish-yellow powder formed which was then filtered, washed with tap water and then dried in a desiccator.

28.7 mg of crude was obtained. The crude was recrystallized from CH_2Cl_2/Hexane to give greenish-yellow needles of **1** (24.6 mg).Yield = 44.7%

TLC. profile (CH_2Cl_2: MeOH (95:5 %) showed a single spot for **1** (Mp. 171-173°c) which is different from that of the pinostrobin chalcone.

Physical and spectroscopic data for compound **1**

Greenish-yellow needles, Mp. 171-173°C, R_f 0.35 (CH_2Cl_2: MeOH (95:5 %), *m/z* 283 and yield 45%

^{13}C ($CDCl_3$, 50 MHz): 76.9 (**C-2**), 33.3 (**C-3**), 173.5 (**C-4**), 166.8 (**C-5**), 97.0 (**C-6**), 167.4 (**C-7**), 92.2 (**C-8**), 159.5 (**C-9**), 100.6 (**C-10**), 32. 2 (**C-11**), 139.3 (**C-1'**), 126.4 (**C-2'/6'**), 129.1 (**C-3'/5'**), 126.4 (**C-4'**), 55.5 (**7-OMe**).

^{1}H ($CDCl_3$, 500 MHz): 5.15 (1H, *dd*, J_{3ax} 12.5 and J_{3eq} 3.0Hz, **H-2**), 2.83 (1H, *dd*, J_2 16.5 and J_{3ax} 12.0Hz, **H-3$_{ax}$**), 3.02 (1H, *dd*, J_2 17.0 and J_{3ax} 3.0Hz, **H-3 $_{eq}$**), 15.52 (1^{1}H, *s*, **5-OH**), 5.98 (1H, *d*, 2.0Hz, **H-6**), 5.82 (1H, *d*, 2.5Hz, **H-8**), 3.76, (3H, *s*, **H-11**), 7.37-7.47 (5H, *m*, **H-2'/3'/4'/5'/6'**), 3.76 (3H, *s*, **7-OMe**).

5.2.2 **Synthesis of 7-methoxyflavanone ethyl imine (2) from pinostrobin chalcone**

A quantity of 50 mg of pinostrobin chalcone was introduced into a 25 ml conical flask. A volume of 1ml 70% ethyl amine was added then tightly corked. The reaction was allowed to proceed at room temperature while shaking for two hours. Ten milliliters of tap water was added while swirling gently upon which yellow powder

was formed after four hours. The crude was then filtered, washed with tap water and then dried in a desiccator giving 44.59 mg of **2**. The crude was recrystalized from CH_2Cl_2/Hexane to obtain 43 mg yellow needles of **2**, Mp 118-120°C, and Yield = 74%.

Physical and spectroscopic data for compound **2**

Yellow needles, Mp 118-120°C, R_f 0.29 (Hex/EtOAc 1:4), *m/z* 297 and yield 74%..

^{13}C ($CDCl_3$, 50 MHz): 76. 9 (**C-2**), 33.2 (**C-3**), 174.0 (**C-4**), 165.9 (**C-5**), 97.0 (**C-6**), 166.9 (**C-7**), 92.1 (**C-8**), 159.6 (**C-9**), 100.3 (**C-10**), 40.1 (**C-11**), 15.4 (**C-12**), 139.3 (**C-1'**), 126.4 (**C-2'/6'**), 129.1 (**C-3'/5'**), 129.1 (**C-4'**), 55.5 (**7-OMe**).

^{1}H ($CDCl_3$, 500 MHz): 5.15 (1H, *dd*, J_{3ax} 12.0 , J_{3eq} 3.0 Hz, **H-2**), 2.85 (1H, *dd*, J_2 17.0 and J_{3ax} 12.0 Hz, **H-3$_{ax}$**), 3.04 (1H, *dd*, J_2 17.0 and J_{3ax} 3.0 Hz, **H-3$_{eq}$**), 15.65 (1H, *s*, **5-OH**), 5.96 (H, *d*, 2.34 Hz, **H-6**), 5.81 (1H, *d*, 2.34 Hz, **H-8**), 3.46, (2H, *m*, **H-11**), 1.34,(3H, *t*, 7.0 Hz,**H-12**), 7.37-7.48 (5H, *m*, **H-2'/3'/4'/5'/6'**), 3.77 (3H, *s*, **7-OMe**).

5.3 Conversion of Chalcones into Flavanones

Preparation of flavanones was carried out using the procedure previously used by Tanaka and Sugino [**2001**] in which intramolecular cyclization of 2'-hydroxychalcones was effected under environmentally benign sodium hydroxide catalyzed aqueous medium to give respective flavanones.

5.3.1 **Synthesis of 5-hydroxy-7, 8-dimethoxyflavanone** (3) **from pashanone**

A sample of 100 mg,of pashanone was introduced into a 100 ml conical flask. 10 milliliters of tap water was added forming a suspension. A volume of 0.3 ml of 8M

NaOH was then added upon which the suspended particles dissolved into the aqueous media. The reaction was allowed to proceed with continuous shaking for 3 hours at room temperature.

After 8 hours, colourless crystalline product formed which was then filtered, washed with tap water and then dried in a desiccator giving 21 mg of material. The crude crystals obtained were recrystallized from CH_2Cl_2/Hexane system to give 19 mg (19%) of 5-hydroxy, 7, 8-dimethoxyflavanone (Mp 95-98 °C) as colourless needles.

Physical and spectroscopic data for compound **3**

Colourless crystals, Mp 95-98 °C, R_f 0.54 (Hex/EtOAc 7:3).

^{13}C ($CDCl_3$, 50 MHz): 79.8 (**C-2**), 43.6 (**C-3**), 196.6 (**C-4**), 158.9 (**C-5**), 93.4 (**C-6**), 161.2 (**C-7**), 130.8 (**C-8**), 155.3 (**C-9**), 103.1 (**C-10**), 138.5 (**C-1'**), 126.4 (**C-2'/6'**), 129.1 (**C-3'/5'**), 129.1 (**C-4'**), 56.4 (**7-OMe**), 61.1 (**8-OMe**).

^{1}H ($CDCl_3$, 200 MHz): 5.48 (1H, *dd*, J_{3ax} 12.11 and J_{3eq} 3.52 Hz, **H-2**), 3.10 (1H, *dd*, J_2 17.2 and J_{3ax} 11.7 Hz, **H-3** $_{ax}$), 2.85 (1H, *dd*, J_2 17.2 and $J_{3\,ax}$ 3.52 Hz, **H-3** $_{eq}$), 12.0 (1H, *s*, **5-OH**), 6.12 (1H, *s*, **H-6**), 7.37-7.46 (5H, *m*, **H-2'/3'/4'/5'/6'**), 3.90 (3H, *s*, **7-OMe**), 3.79 (3H, *s*, **8-OMe**).

5.3.2 **Synthesis of Alpinetin (4) from cardamonin**

A 100 mg of cardamonin was weighed and put in a 100 ml conical flask. Ten milliliters of tap water was added forming a suspension. A quantity of 0.3 ml of 8M NaOH was then added upon which the suspended particles dissolved into the aqueous

media. The reaction was allowed to proceed with continuous shaking for one hour at room temperature.

After 5 days, crystalline material was formed which was then filtered, washed with tap water and then dried in a desiccator giving 68.4 mg of crude material.

The crude crystals obtained were recrystallized from CH_2Cl_2/Hexane system to give 64.3 mg (64%) of 7-hydroxy, 5-methoxyflavanone (Mp 218-220°c) as colourless needles.

Analysis by TLC. (Hex. /EtOAc 1:1) showed a single spot for **4** which is more polar than cardamonin.

Physical and spectroscopic data for compound **4**

Colourless needles, Mpt 218-220°C, R_f 0.23 (Hex. /EtOAc 1:1).

^{13}C (DMSO, 50 MHz): 78.7 (**C-2**), 45.5 (**C-3**), 188.032 (**C-4**), 164.7 (**C-5**), 94.0 (**C-6**), 165.0 (**C-7**), 96.303 (**C-8**), 162.9 (**C-9**), 105.2 (**C-10**), 139.8 (**C-1'**), 127.0 (**C-2'/6'**), 129.2 (**C-3'/5'**), 129.0 (**C-4'**), 56.3 (**7-OMe**).

^{1}H (DMSO, 200 MHz): 5.46 (1H, *dd*, J_{3ax} 12.11 and J_{3eq} 3.13 Hz, **H-2**), 2.98 (1H, *dd*, J_2 16.02 and J_{3ax} 12.11 Hz, **H-3$_{ax}$**), 2.62 (1H, *dd*, J_2 16.4 and J_{3ax} 3.12 Hz, **H$_{eq}$-3**), 6.15 (1H, *s*, 1.99 Hz, **H-6**), 6.09 (1H, *s*, 1.56Hz, **H-8**), 7.37-7.46 (5H, *m*, **H-2'/3'/4'/5'/6'**), 3.79 (3H, *s*, **5-OMe**).

5.3.3 **Synthesis of Pinostrobin (5) from pinostrobin chalcone**

Pinostrobin chalcone ,(100 mg) was weighed into a 100 ml conical flask and suspended in 10 ml of tap water. 0.3 ml of 8M NaOH was then added upon which the

suspended particles dissolved into the aqueous media. The reaction was allowed to proceed with continuous shaking for one hour at room temperature.

After 2 hrs, colourless powder was formed which were then filtered, washed with tap water and then dried in a desiccator giving 91 mg of compound **5**.

The crude crystals obtained were recrystallized from methanol to give 89 mg (89 %) of pinostrobin (Mp 95-100°c) as colourless plates. Analysis by TLC. (Hex./EtOAc 7:3) showed a single spot for pinostrobin which is less polar than the pinostrobin chalcone.

Physical and spectroscopic data for compound **5**

Colourless plates, Mp 95-100°c, R_f 0.67 (Hex. /EtOAc 7:3).

^{13}C ($CDCl_3$, 50 MHz): 79.5 (**C-2**), 43.6 (**C-3**), 196.0 (C-4), 164.4 (**C-5**), 95.4(**C-6**), 168.2 (**C-7**), 94.5 (**C-8**), 163.0 (**C-9**), 103.4 (**C-10**), 138.4 (**C-1'**), 126.4 (**C-2'/6'**), 129.1 (**C-3'/5'**), 126.5 (**C-4'**), 55.9 (**7-OMe**).

^{1}H ($CDCl_3$, 200 MHz): 5.42 (1H, *dd*, J_{3ax} 12.5 and J_{3eq} 3.12 Hz, **H-2**), 3.12 (1H, *dd*, J_2 17.19 and J_{3ax} 12.89 Hz, **H-3$_{ax}$**), 2.82 (1H, *dd*, J_2 17.19, and J_{3ax} 3.12 Hz, **H-3$_{eq}$**), 12.02 (H, *s*, **5-OH**), 6.08 (1H, *d*, 2.34 Hz, **H-6**), 6.1 (1H, *d*, 2.34 Hz, **H-8**), 7.4-7.47 (5H, *m*, **H-2'/3'/4'/5'/6'**), 3.90 (3H, *s*, **7-OMe**).

5.3.4 **Synthesis of 5, 7-dimethoxyflavanone (6) from flavokawin B**

100 mg of flavokawin B was suspended in 10 ml of tap water in a 100 ml conical flask. A volume of 0.3 ml of 8M NaOH was then added upon which the suspended

particles dissolved into the aqueous media. The reaction was allowed to proceed with continuous shaking for 2 hours at room temperature.

After 8 hrs, colourless powder formed which were then filtered, washed with tap water and then dried in a desiccator giving 89 mg of **6**.

The crude crystals obtained were recrystallized from methanol to give 86 mg (86%) of pure 5, 7- dimethoxyflavanone, **6** (Mp 143-145°c) as colourless needles.

Analysis by TLC. (Hex. /EtOAc 1:1) showed a single spot for **6** which is more polar than flavokawin B.

Physical and spectroscopic data for compound **6**

Colourless needles, Mp 143-145°C, R_f 0.36 (Hex. /EtOAc 1:1).

^{13}C ($CDCl_3$, 50 MHz): 79.5 (**C-2**), 45.8 (**C-3**), 189.5 (**C-4**), 165.2 (**C-5**), 93.4 (**C-6**), 166.2 (**C-7**), 93.8 (**C-8**), 162.5 (**C-9**), 106.2 (**C-10**), 139.0 (**C-1'**), 126.3 (**C-2'/6'**), 129.0 (**C-3'/5'**), 128.9 (**C-4'**), 56.4 (**5-OMe**), 55.8 (**7-OMe**).

^{1}H ($CDCl_3$, 200 MHz): 5.39 (1H, *dd*, J_{3ax} 12.89 and J_{3eq} 3.13Hz, **H-2**), 3.06 (1H, *dd*, J_2 16.41 and J_{3ax} 12.89Hz, **H-3$_{ax}$**), 2.82 (1H, *dd*, J_2 16.41 and J_{3ax} 3.13Hz, **H-3$_{eq}$**), 6.16 (1H, *d*, 2.34Hz, **H-6**), 6.09,(1H, *d*, 2.34 Hz, **H-8**), 7.37-7.47 (5H, *m*, **H-2'/3'/4'/5'/6'**), 3.91 (3H, *s*, **5-OMe**), 3.82 (3H, *s*, **7-OMe**).

5.4 Synthesis of Dihydrochalcones (Hydrogenation of chalcones)

5.4.1 **Dihydropashanone (7) from pashanone**

1.2 grams of the pashanone was dissolved in dichloromethane in 100 ml conical flask. 100 milligrams of 5% palladium on charcoal was added to the solution and then hydrogen gas was bubbled into the solution. The hydrogenation process was allowed to proceed at room temperature and pressure for 2 hours after which the coloured solution turned clear. At the end of the reaction the catalyst was removed by filtration and the filtrate evaporated in a rotary evaporator, upon which colourless crystalline material was deposited on the walls of the flask. This material was scratched off then recrystallized from CH_2Cl_2/Hexane to give **7** (1.154 g, 96 %, Mp. 126-130°c) as colourless needles.

Physical and spectroscopic data for compound 7

Colourless needles, Mp 126-130°C, R_f =0.46 (Hex/ EtOAc7:3) and yield 96 %.

^{13}C ($CDCl_3$, 125 MHz): 204.6 (**C=O**), 45.4 (C-**α**), 30.6 (C-**β**), 141.8(C-1), 128.6 (C-2/6), 128.7 (**C-3/5**), 126.2 (**C-4),** 103.9 (**C-1'**), 158.2 (**C-2'**), 128.0 (**C-3'**), 151.4 (**C-4'**), 91.8 (**C-5'**), 162.2 (**C-6'**), 61.6 (**3'-OMe**), 56.2(**4'-OMe**).

^{1}H ($CDCl_3$, 500 MHz): 3.02 (2H, *t*, 7.2Hz, **H-α**), 3.42 (2H, *t*, 7.2 Hz, **H -β**), 7.20-7.28 (5H, *m*, **H-2/3/4/5/6**), 6.75 (1H, *s*, **H-5'**), 13.42 (1H, *s*, **6'-OH**), 3.83 (3H, *s*, **3'-OMe**), 3.89 (3H, *s*, **4'-OMe**).

5.4.2 **Synthesis of uvangolatin (8) from 2', 4'-dihydroxy, 6'-methoxychalcone**

Compound **8** was prepared from cardamonin under the same hydrogenation conditions applied to pashanone (section 5.4.1, above).

Physical and spectroscopic data for compound **8**

Colourless needles, Mp148-151°C, R_f =0.4(Hex/EtOAc,7:3) and yield 98%

^{13}C (Acetone-d_6, 50 MHz) : (**C=O**) 45.7 (**C-α**), 30.7 (**C-β**), 126.0 (**C-1**), 128.5 (**C-2, 6**), 128.6 (**C-3, 5**), 126.0 (**C-4**), 105 (**C-1'**), 165.0 (**C-2'**), 96.2 (**C-3'**), 167.7 (**C-4'**), 91.2 (**C-5'**), 165.0 (**C-6'**), 55.5 (**2'-OMe**).

^{1}H (Acetone-d_6, 200 MHz): 3.02 (2H, *t*, 7.03 Hz, **H-α**), 3.42 (2H, *t*, 7.03 Hz, **H-β**), 7.14-7.30, (5H,*m*, **H-2/3/4/5/6**), 13.88,(1H,*s*, **6'-OH**), 6.03 (1H, *d*,2.2 Hz, **H-3'**), 5.96 (1H, *d*, 2.2 Hz, **H-5'**), 3.89 (3H, *s*, 2'- **OMe**).

5.4.3 **Synthesis of 2', 6'-dihydroxy-4'-methoxydihydrochalcone** (9) **from pinostrobin chalcone**

Compound **9** was obtained by subjecting pinostrobin chalcone to the same hydrogenation conditions as in hydrogenation of pashanone (section 5.4.1, on page 84).

Physical and spectroscopic data for compound **9**

Colourless needles, Mp157-161°c, R_f =0.4 (Hex/EtOAc, 7:3) yield 99 %.

^{13}C ($CDCl_3$, 50 MHz): 204.9 (**C=O**), 45.8 (**C-α**), 30.6 (**C-β**), 142.2 (**C-1**), 126.0 (**C-2, 6**), 128.6 (**C-3, 5**), 128.5 (**C-4**), (**C-1'**), 164.5 (**C-2', 6'**), 93.7 (**C-3', 5'**), 166.2 (**C-4'**), 55.1 (**4'-OMe**).

^{1}H ($CDCl_3$, 200 MHz): 3.02 (2H, *t*, 7.03 Hz, **H-α**), 3.42 (2H, *t*, 7.03 Hz, **H-β**), 7.17-7.34, (5H, *m*, H-**2/3/4/5/6**), 13.42,(2H, *s*, **2'/6'-OH**), 5.99 (2H, *s*, **H-3'/5'**), 3.89(3H, *s*, **4'-OMe**).

5.4.4 **Synthesis of lapathone (10) from flavokawin B.**

Compound **10** was prepared from flavokawin B under the same hydrogenation conditions applied to pashanone (section 5.4.1, on page 84).

Physical and spectroscopic data for compound **10**

Colourless needles, Mp 102-105°c, R_f =0.56 (Hex/EtOAc, 4:1) and yield 99 %

^{13}C ($CDCl_3$, 50 MHz): 204.7 (**C=O**) 30.9 (**C-α**), 45.9 (**C-β**), 141.9 (**C-1**), 126.2 (**C-2, 6**), 128.7 (**C-3, 5**), 128.7 (**C-4**), 106.0 (**C-1'**), 166.2 (**C-2'**), 93.9 (**C-3'**), 167.9 (**C-4'**), 91.1 (**C-5'**), 162.9 (**C- 6'**), 55.8 (**2'-OMe**), 55.8 (**4'-OMe**).

^{1}H ($CDCl_3$, 200 MHz): 2.99 (2H, *t*, 7.03 Hz, **H- α**), 3.27 (2H, *t*, 7.03 Hz, **H -β**), 7.20-7.35, (5H, *m*, H-**2/3/4/5/6**), 6.08 (1H, *d*, 2.2 Hz, **H-3'**), 5.92 (1H, *d*, 2.6 Hz, **H-5'**), 14.01 (1H,*s*, **6'-OH**), 3.89 (3H, *s*, **2'-OMe**), 3.83 (3H, *s*, **4'-OMe**).

5.5 Kostanecki-Robinson Acetylation of Dihydrochalcones.

The Chalcone was first hydrogenated to obtain dihydrochalcone which was then subjected to the Kostanecki-Robinson reaction conditions.

Freshly fused sodium acetate was prepared by heating sodium acetate in a porcelain evaporating basin over a free flame until the salt liquefied and a steam evolved. This was followed by solidification indicating removal of the water of crystallization. Residual water was removed by heating the salt in a larger flame, then the fused salt was allowed to solidify then removed while still warm.

5.5.1 **Synthesis of 2-(1-acetoxy-3-phenylprop-1-enyl)-5-methoxy-1,3phenylene diacetate) (11)**

A mixture of 200 mg of the dihydrochalcone **9**, freshly fused sodium acetate (300 mg) and acetic anhydride (5 ml) were heated at 160-180°c for 8 hours. The reaction was monitored by TLC. (Solvent system: Hexane / EtOAc, 7:3). After a period of 8 hours, the reaction mixture was treated with 10 milliliters of hot water to give a brown oily paste (142.6 mg). The crude oily paste was purified by passing through silica gel (15g) column using hexane/ ethyl acetate (4:1 then 7:3) as the eluent. One pure fraction, 11 was obtained then recrystallized from methanol to give 58 mg of 11.

Physical and spectroscopic data of compound **11**

Colourless crystals, Mp 62-65°C, R_f =0.28 (Hex/EtOAc, 7:3).

^{13}C (Acetone-d_6, 50 MHz): 122.9 (**C-α**), 32.8 (**C-β**), 137.8 (**C-1**), 126.6(**C-2, 6**), 128.8 (**C-3, 5**), 126.6 (**C-4**), 116.1(**C-1'**), 150.5 (**C-2'/6'**), 107.2 (**C-3'/5'**), 160.5 (**C-4'**), 139.6 (**C-1''**), 168.5 (**C-2''**), 20.2 (**2''-Me**), 169.69 (**C-1'''**), 19.8 (**1'''-Me**), 55. 9 (**4'-OMe**).

^{1}H (Acetone-d_6, 200 MHz): 5.41 (1H, *t*, 7.6 Hz, **H-α**) ,3.42 (2H, *d*,7.6 Hz **H-β**),7.21-7.33, (5H, *m*, **H-2/3/4/5/6**), 6.33 (2H, *s*, **H–3'/5'**), 2.86 (9H, *s*, **H-2'''/3''**), 3.80 (3H, *s*, **4'-OMe**).

5.5.2 **Synthesis of 2-(1-acetoxy-3-phenylprop-1-enyl)-4,5-dimethoxy-1,3-phenylenediacetate** (12)

A mixture of 150 mg of the dihydropashanone (7), freshly fused sodium acetate (300 mg) and acetic anhydride (5 ml) were heated at 160-180°c for 8 hours. The reaction was monitored by TLC. (Solvent system: Hexane / EtOAc, 7:3). After a period of 8 hours, the reaction mixture on treatment with 10 milliliters of hot water gave a brown oily paste (135.6 mg). The crude oily paste was purified by passing through silica gel (10 g) column using hexane/ ethyl acetate (9:1, 4:1, 7:3 and 1:1). One pure fraction was obtained then recrystallized from methanol to give 58 mg of 12.

Physical and spectroscopic data of compound **12**

Colourless crystals, Mp 51-54°C, R_f =0.54 (Hex/EtOAc, 7:3).

^{13}C ($CDCl_3$, 75 MHz): 123.2 (**C-α**), 32.8 (**C-β**), 137.8 (**C-1**), 128. 8 (**C-2, 6**), 128.6 (**C-3**), 126. 6 (**C-4**), 100.2 (**C-1'**), 139.5 (**C-2'**), 139.5 (**C-3'**), 153.8 (**C-4'**), 105.4 (C-5'), 144.7 (**C-6'**), 139.6 (**C-1''**), 168.4 (**C-2''**), 21.0 (**2''-Me**), 169.9 (**C-3''**), 20.8 (**3''-Me**), 169.0 (**C-1'''**), 20.6 (**1'''-Me**), 56.2, 60.9 (**3,4'-OMe**).

^{1}H ($CDCl_3$, 300 MHz): 5.46 (1H, *t*,7.5 Hz **H-α**) ,3.41 (2H, *d*,7.5 Hz, **H-β**), 7.20-7.30, (5H, *m*, **H-2/3/4/5/6**), 6.53 (1H, *s*, **H–5'**), 2.09, 2.11, 2.19 (9H, 3*s*, **H-2'',3'',1'''-Me**), 3.84, 3.80 (6H, 2*s*, **3',4'-OMe**).

5.5.3 **Synthesis of 3-benzyl-5, 7-dimethoxy-2-methyl-4H-chromen-4-one** (13)

A mixture of **10** (150 mg), freshly fused sodium acetate (200 mg) and acetic anhydride (5 ml) was heated at 160-180°c for 8 hours. The reaction was monitored by

TLC. (Solvent system: hexane / EtOAc, 7:3).The reaction mixture on treatment with hot water and sodium hydrogen carbonate gave a brown oily paste. The crude paste (98mg) was purified by passing through silica gel (10 g) column over hexane/ ethyl acetate (7:3, 3:1) which gave 8.6 mg of a yellowish compound named **13**.

Physical and spectroscopic data of **13**

Yellowish crystals, R_f = 0.55 (Hex/EtOAc,7:3)

^{13}C ($CDCl_3$, 50 MHz): 159.7 (**C-2**), 121.7 (**C-3**), 179.0 (**C-4**), 161.2(**C-5**), 92.4(**C-6**), 163.8(**C-7**), 95.9 (**C-8**), 160.8(**C-9**), 119.9(**C-10**), 30.1(**C-11**), 18.5(**2-Me**), 140.4(**C-1'**), 128.7 (C-2'/6'), 128.6 (C-3'/5'), 126.1 (C-4'), 56.5 (5-OMe), 55.9 (7-OMe).

^{1}H ($CDCl_3$, 200 MHz) 6.32 (1H,*d*,2.2 Hz, **H-6**), 6.38(1H, *d*,2.2 Hz, **H-8**),71-7.30 (5H, m, **H-2'/3'/4'/5'/6'**), 2.32 (3H, *s*, **H–2Me**), 3.92(3H, *s*, **5-OMe**), 3.86 (3H, *s*, **7-OMe**).

5.6 Nitration of 2', 6'-dihydroxy-4'-methoxy-3'-dihydrochalcone

5.6.1 **Synthesis of 2', 6'-dihydroxy-4'-methoxy-3'-nitrodihydrochalcone** (14)

70 mg of **9** was added into 1 ml of ice-cold concentrated nitric acid, stirred and left in the ice-bath for 2.5 hours after which ice -cold water was added into the reaction vessel. The crude product formed was filtered out, washed with tap water then dried in a dessicator giving 68.2 mg crude product.

On TLC. (Hex/EtOAc, 1:1), this product showed two spots. The product was then treated on silica gel column. 15 g of silica gel was packed on a column, the product was loaded into the column and then the product was eluted sequentially using 150

milliliters of Hex/EtOAc (9:1, 7:3, and 1:1) and finally 100% ethyl acetate upon which 9.6 mg of compound 14 R_f =0.38 (Hex/EtOAc (7:3) and 5 mg of another compound that was not analyzed R_f =0.33 (Hex/EtOAc (3:2) was obtained.

Physical and spectroscopic data for **14**

Yellow powder, R_f =0.38 (Hex/EtOAc, (7:3)

^{13}C ($CDCl_3$, 125 MHz): 205.6 (**C=O**), 46.2 (**C-α**), 30.2 (**C-β**), 141.1 (**C-1**), 128.6 (**C-2, 3, 5,6**), 126.3 (**C-4**), 102.0 (**C-1'**), 160.0 (**C-2'**), 171.6 (**6'**), (**C-3'**), 93.7 (**5'**), 163.0 (**C-4'**), 57.3 (**4'-OMe**).

^{1}H ($CDCl_3$, 500 MHz): 3.47 (2H, *t*, 7.03 Hz, **H-α**), 3.02 (2H, *t*, 7.03 Hz, **H-β**), 7.19-7.28 (5H, *m*, H-**2/3/4/5/6**), 14.78 (1H, *s*, **2'-OH**), 6.05 (1H, *s*, **H-5'**), 13.58 (1H ,*s*, **6'-OH**), 3.95 (3H, *s*, **4'-OMe**).

5.7 Biological Activity Studies

5.7.1 **Anti-plasmodial Activity Assay.**

The anti-plasmodial tests were done at the KEMRI (Kisumu). The compounds were assayed using an automated micro-dilution technique to determine 50% growth inhibition of cultured parasites [Chulay *et al.*, **1983**; Desjardins *et al.*, **1979**].

Two different strains of *Plasmodium falciparum* parasite cultures were used in this study. The chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains were grown in a continuous culture supplemented with mixed gas (90% nitrogen, 5% oxygen, and 5% carbon dioxide), 10% human serum, and 6% hematocrit of A+ red blood cells. Once cultures reach a parasitemia of 3% with at least a 70% ring

developmental stage present, parasites were transferred to a 96 well microtiter plate with wells pre-coated with compound. The samples were serially diluted across the plate to provide a range of concentration used to accurately determine IC_{50} values. Plates were incubated in a mixed gas incubator for 24 hours.

Following the specified incubation time, ^{3}H-hypoxanthine was added and parasites allowed growing for an additional 18 hours. Cells were processed with a plate harvester (Tom Tec) onto filter paper and washed to eliminate unincorporated isotope. Filters were measured for activity in a microtiter plate scintillation counter (Wallac). Data from the counter was imported into a Microsoft Excel spreadsheet, which is then imported into an Oracle database/ program to determine IC_{50} values.

The anti-plasmodial assay results are summarised in (Table 15, on page 73).

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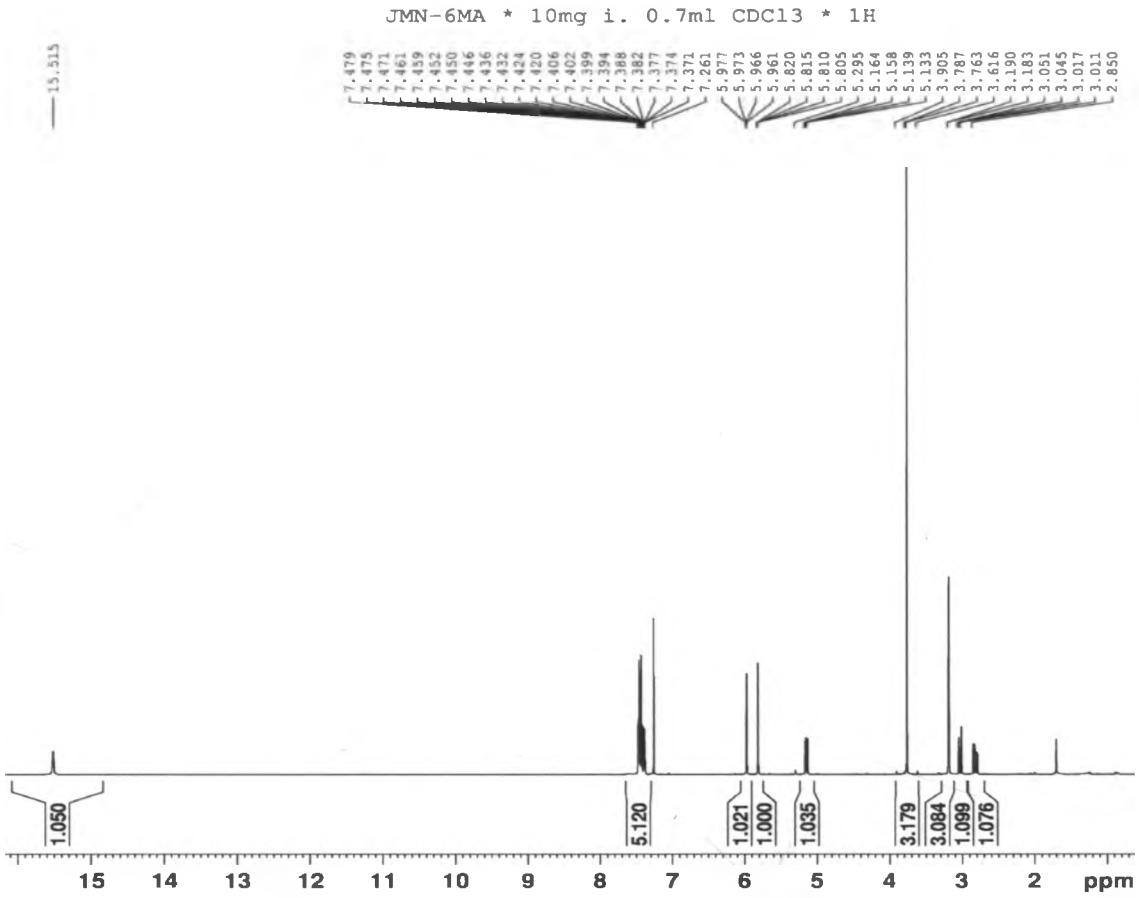
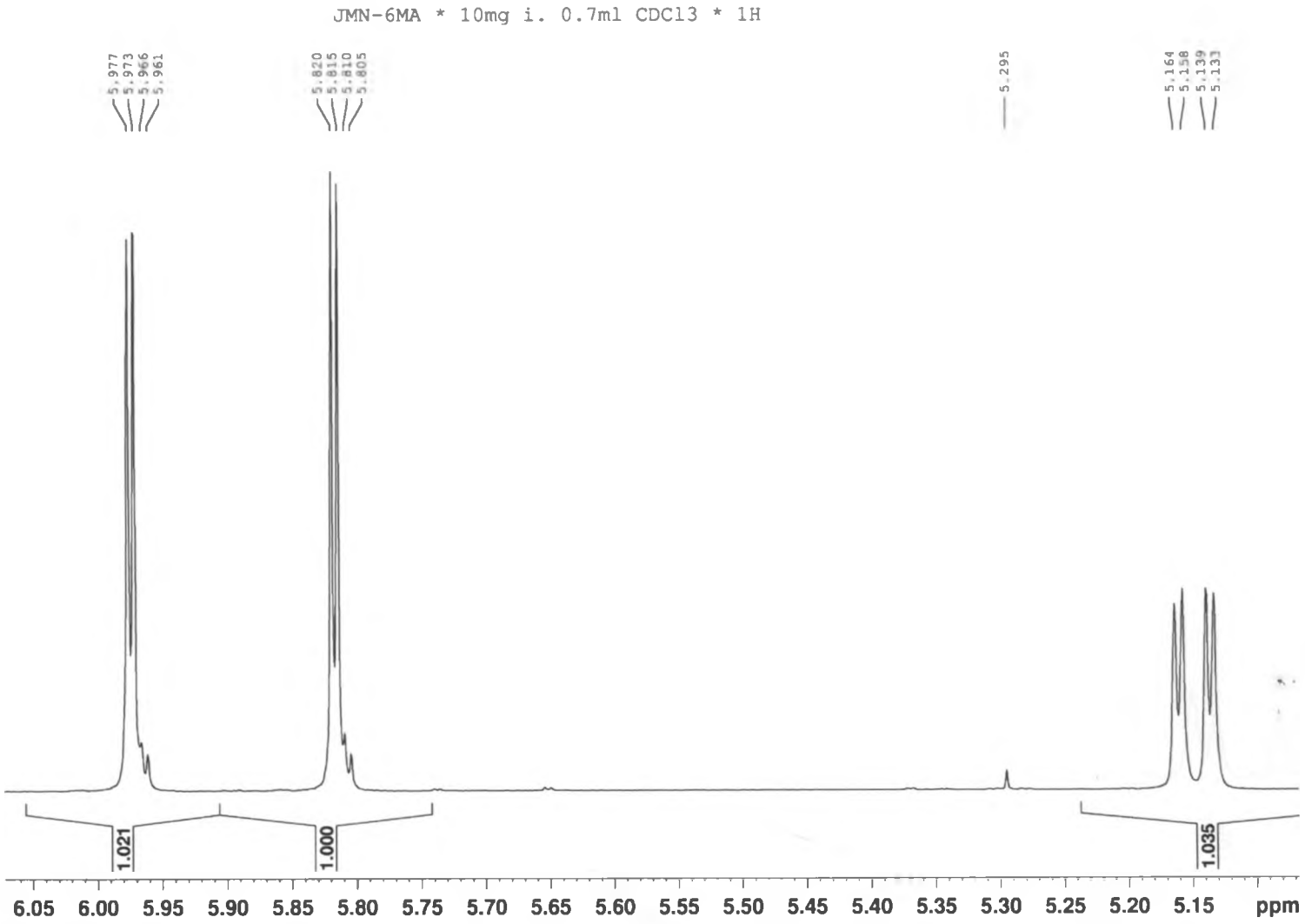
APPENDICES

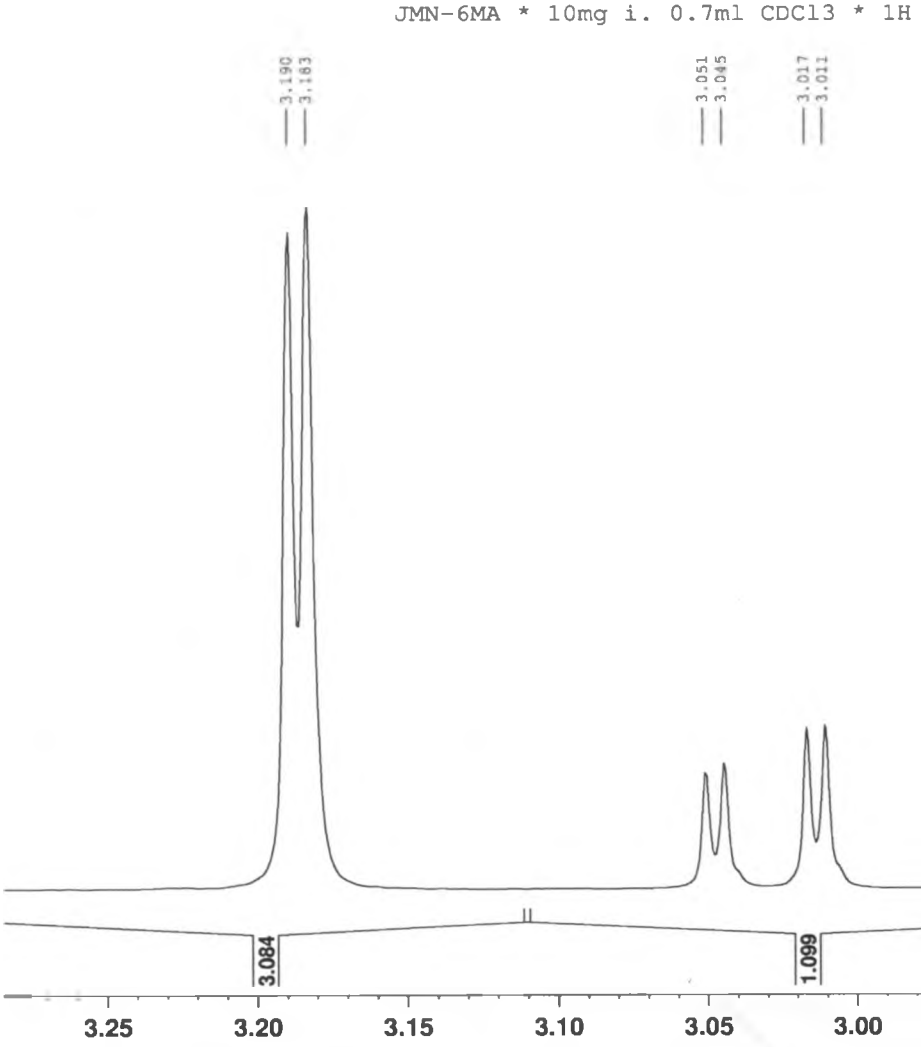
Appendix 1: Spectra for compound **1**

^{1}H NMR SPECTRUM (500 MHz, CDCl3)



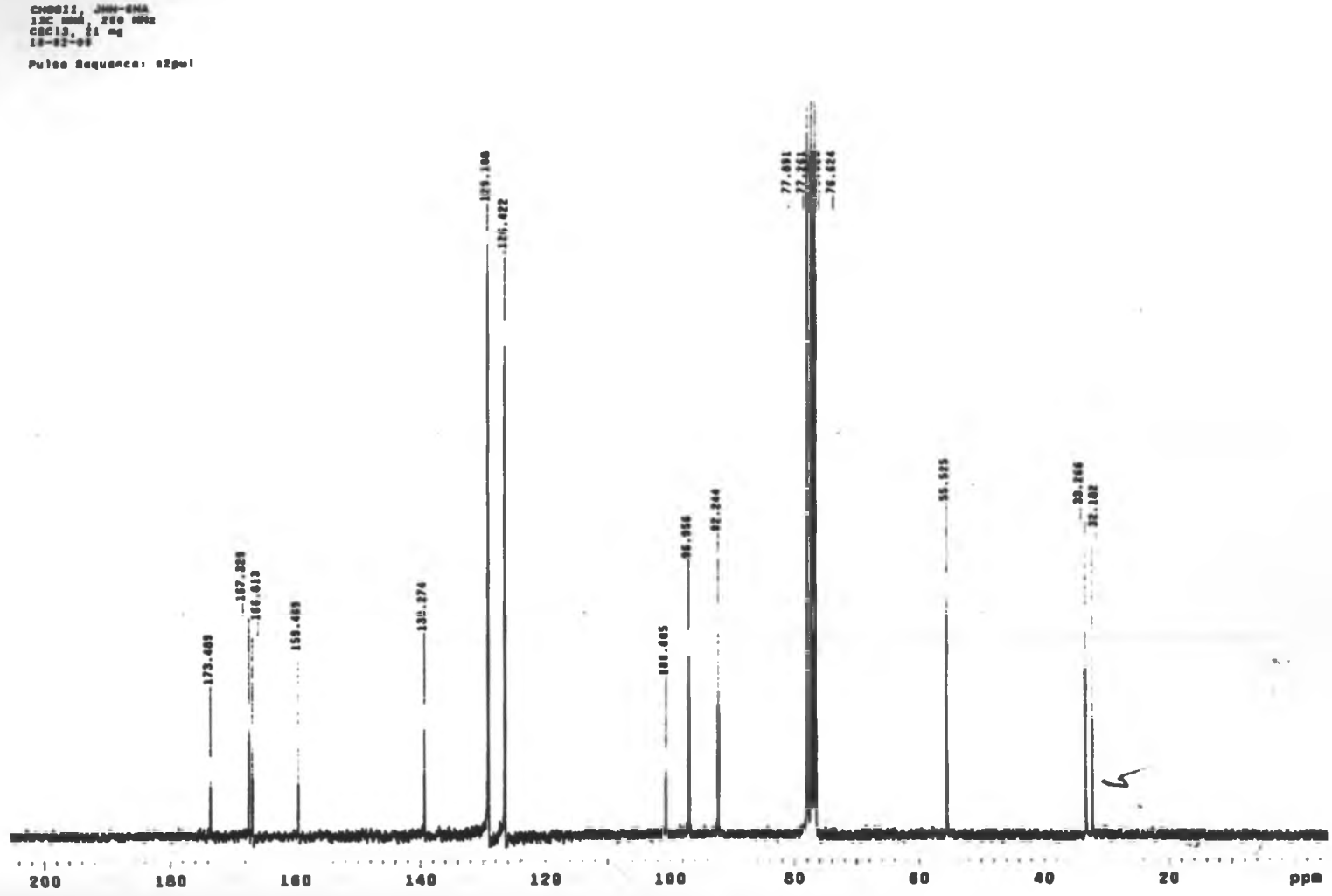
NAME	AV500_Mar23-2009-mh
EXPNO	30
PROCNO	1
Date_	20090323
Time	11.58
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDC13
NS	64
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	228.1
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
========	CHANNEL f1 ========
NUC1	1H
P1	9.10 usec
PL1	-3.00 dB
SFO1	500.1730888 MHz
SI	32768
SF	500.1700119 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00


JMN-6MA * 10mg i. 0.7ml CDCl3 * 1H
5.977
5.973
5.966
5.961
5.820
5.815
5.810
5.805
5.295
5.164
5.158
5.139
5.133
1.021
1.000
1.035
6.05 6.00 5.95 5.90 5.85 5.80 5.75 5.70 5.65 5.60 5.55 5.50 5.45 5.40 5.35 5.30 5.25 5.20 5.15 ppm


JMN-6MA * 10mg i. 0.7ml CDCl3 * 1H
3.190
3.183
3.051
3.045
3.017
3.011
3.084
1.099
3.25
3.20
3.15
3.10
3.05
3.00

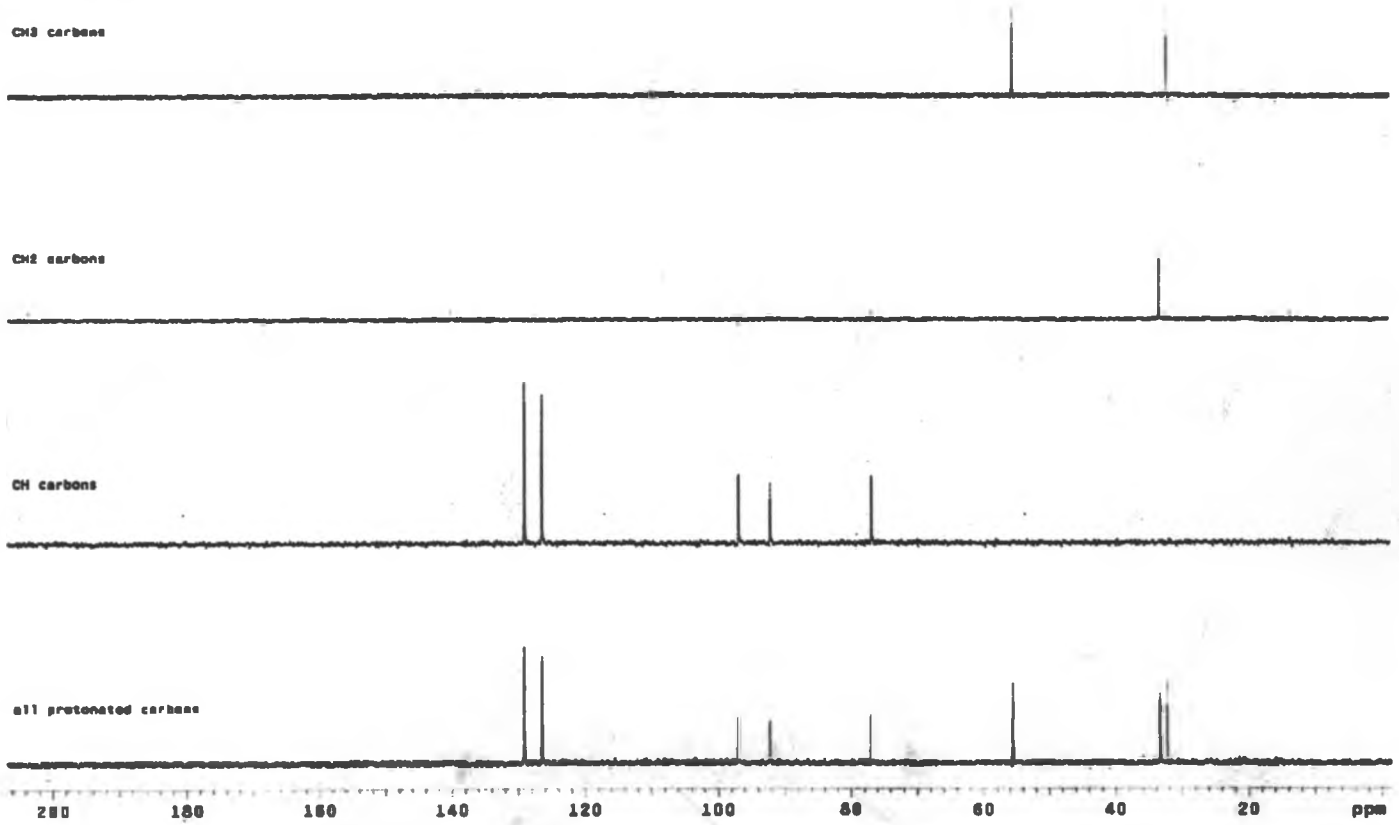
2.850
2.826
2.817
2.802
2.792
1.076
2.95
2.90
2.85
2.80
ppm

^{13}C NMR SPECTRUM (50 MHz, $CDCl_3$)



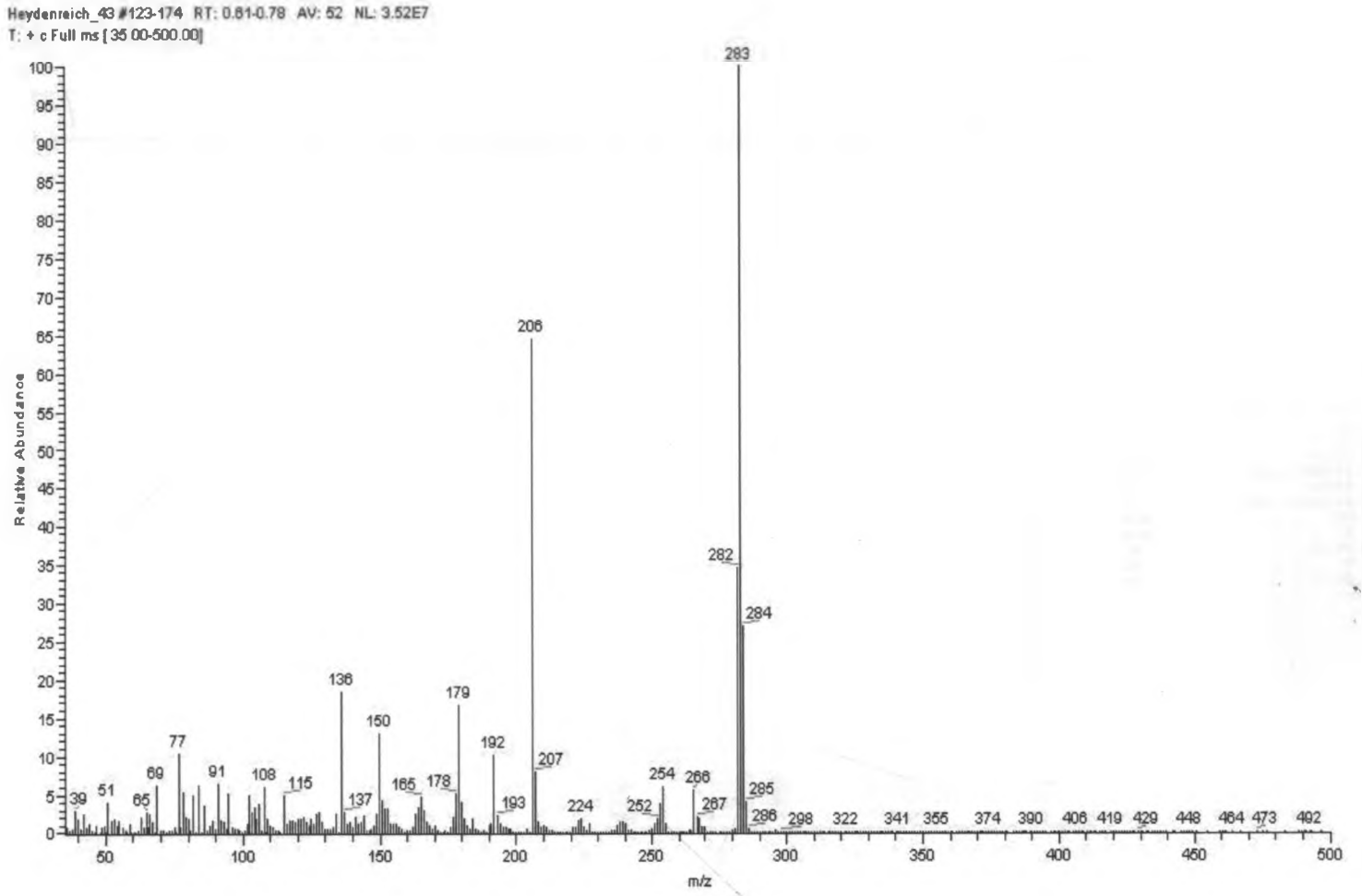
DEPT SPECTRUM ($CDCl_3$)

Clogii
JMN-6MA
$CDCl_3$
DEPT
10/02/09



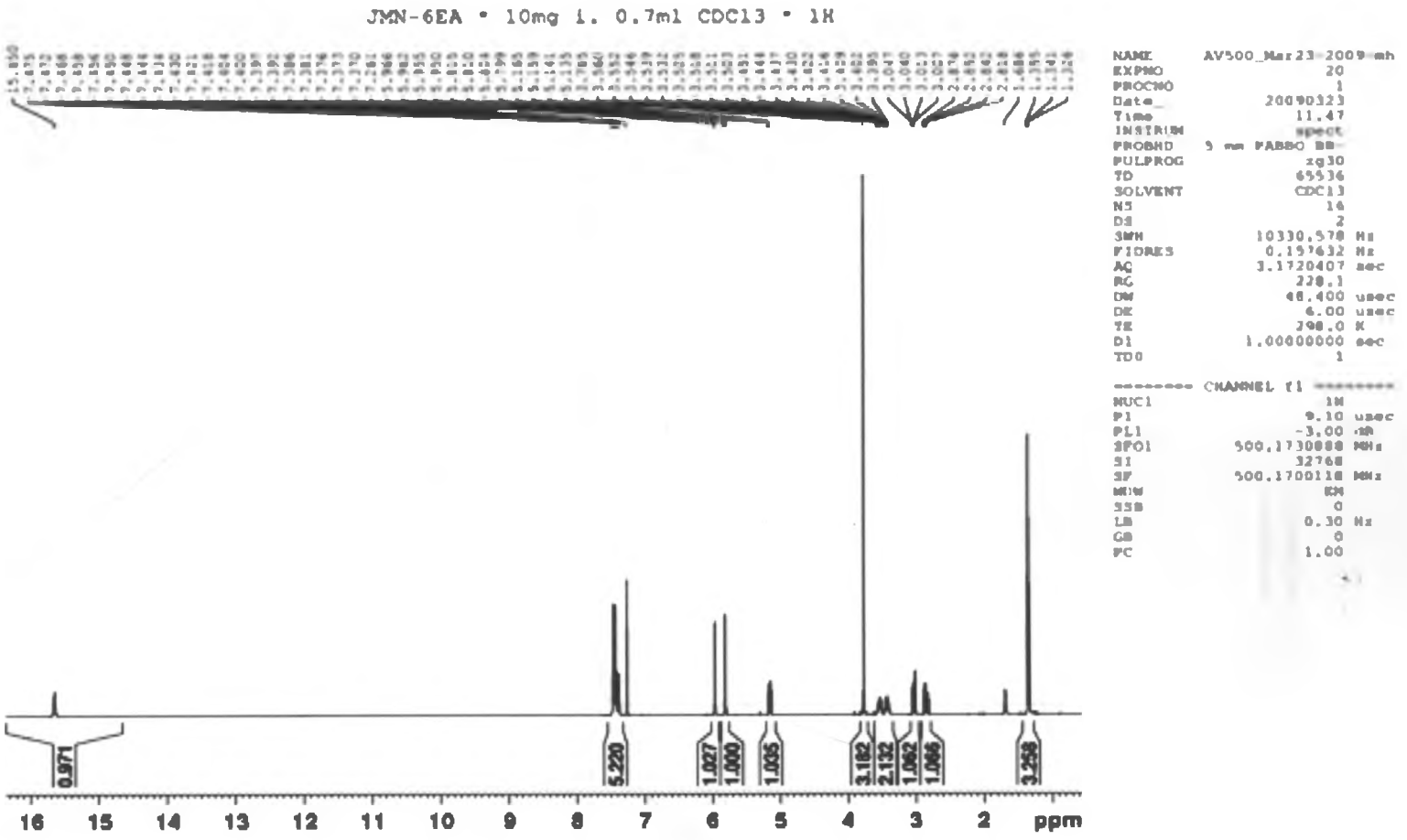
MS SPECTRUM (compound 1)

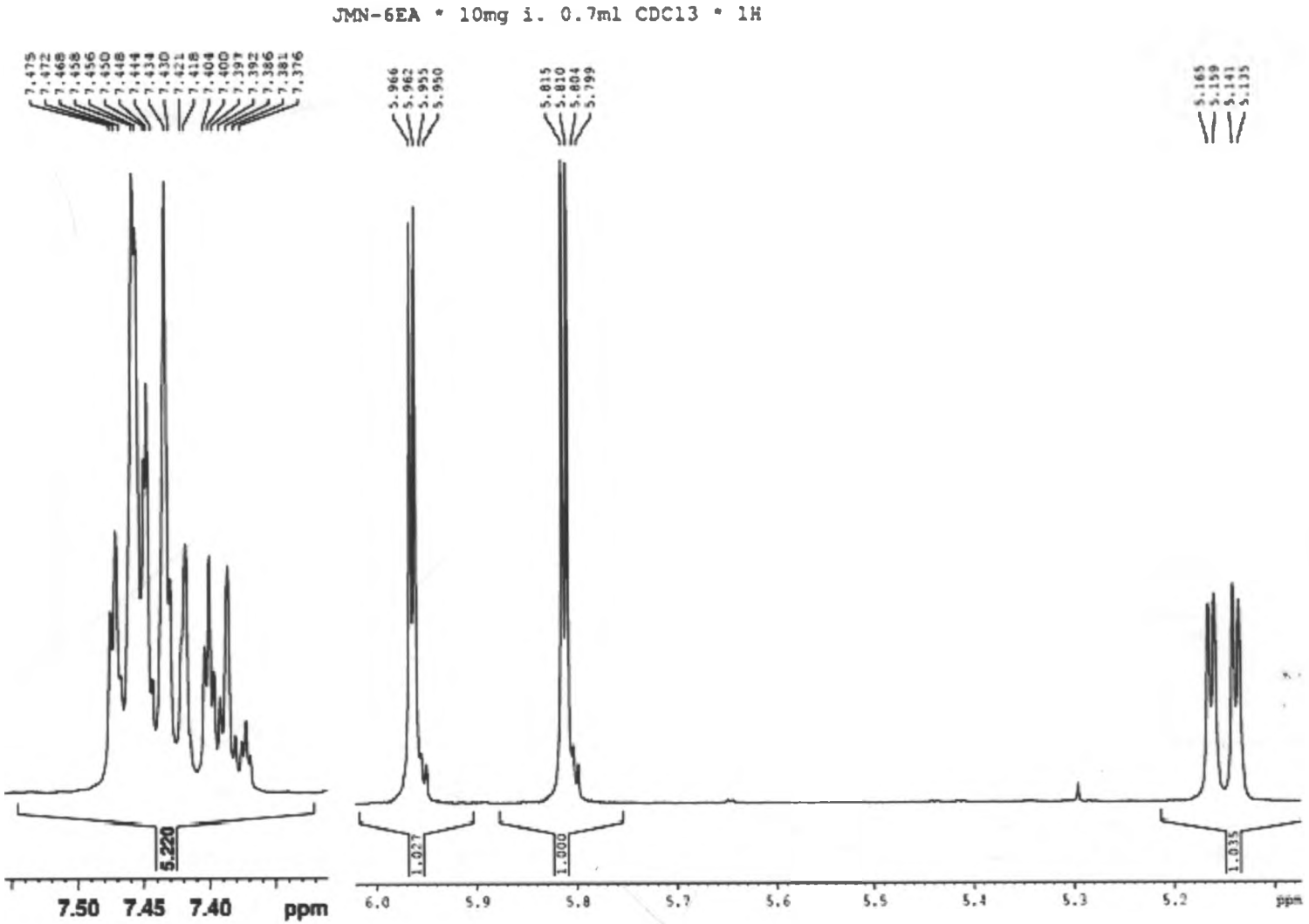
C:\Xcalibur\data\Projekte\Heydenreich_43 JMN-6MA, MW=283 DSQ

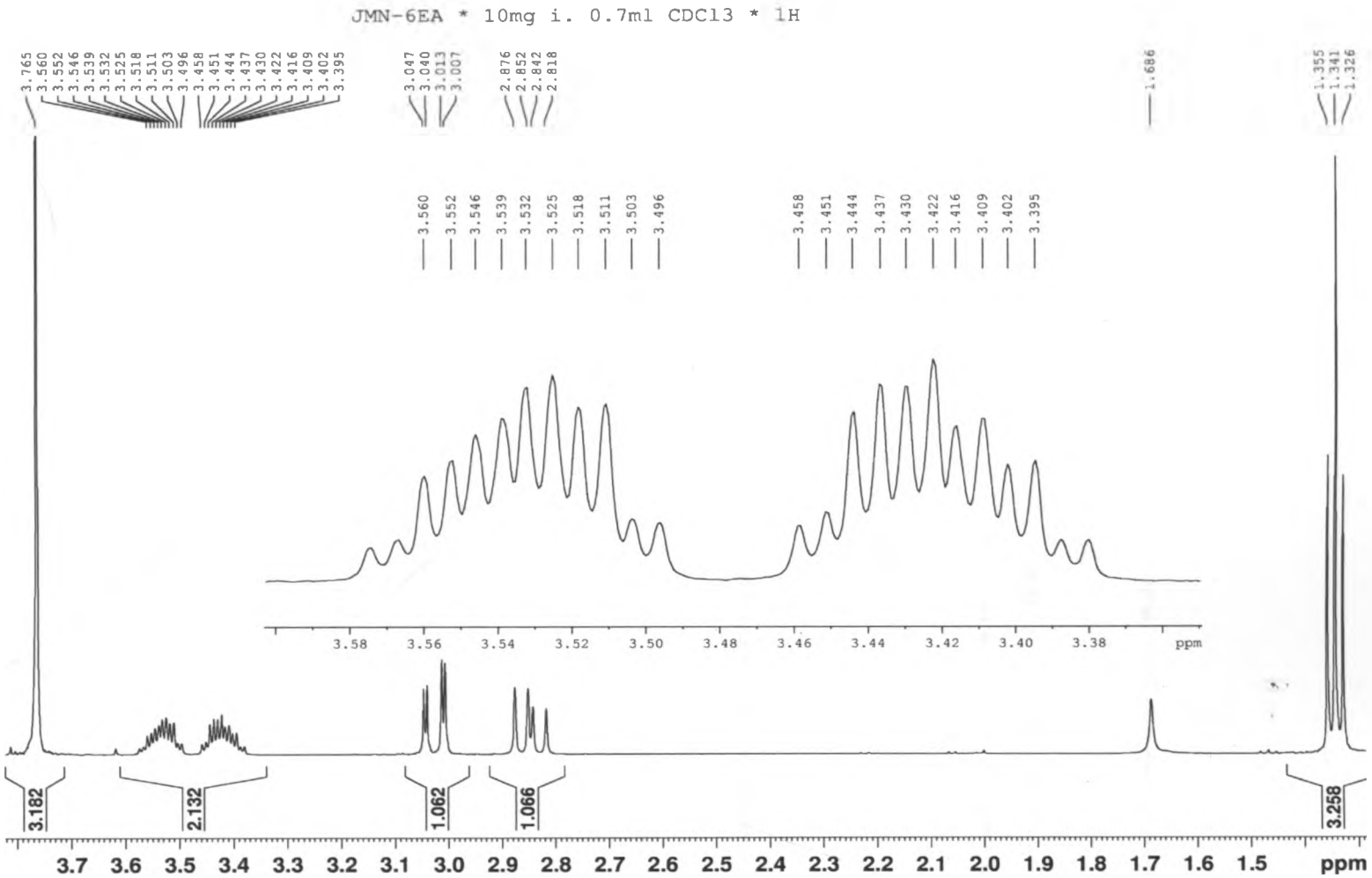


Appendix 2: Spectra for compound **2**

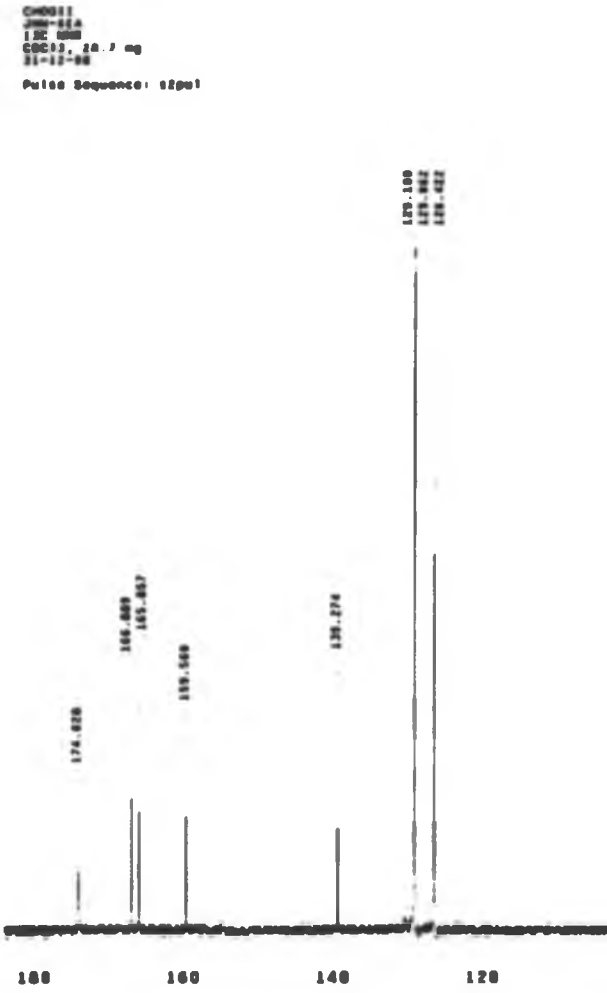
^{1}H NMR SPECTRUM (500 MHz, $CDCl_3$)




JMN-6EA * 10mg i. 0.7ml CDCl3 * 1H
7.475
7.472
7.468
7.458
7.456
7.450
7.448
7.444
7.434
7.430
7.421
7.418
7.404
7.400
7.397
7.392
7.386
7.381
7.376
5.966
5.962
5.955
5.950
5.815
5.810
5.804
5.799
5.165
5.159
5.141
5.135
5.220
1.027
1.000
1.035
7.50
7.45
7.40
ppm
6.0
5.9
5.8
5.7
5.6
5.5
5.4
5.3
5.2
ppm


JMN-6EA * 10mg i. 0.7ml CDCl3 * 1H
3.765
3.560
3.552
3.546
3.539
3.532
3.525
3.518
3.511
3.503
3.496
3.458
3.451
3.444
3.437
3.430
3.422
3.416
3.409
3.402
3.395
3.047
3.040
3.013
3.007
2.876
2.852
2.842
2.818
1.686
1.355
1.341
1.326
3.58
3.56
3.54
3.52
3.50
3.48
3.46
3.44
3.42
3.40
3.38
ppm
3.182
2.132
1.062
1.066
3.258
3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 ppm

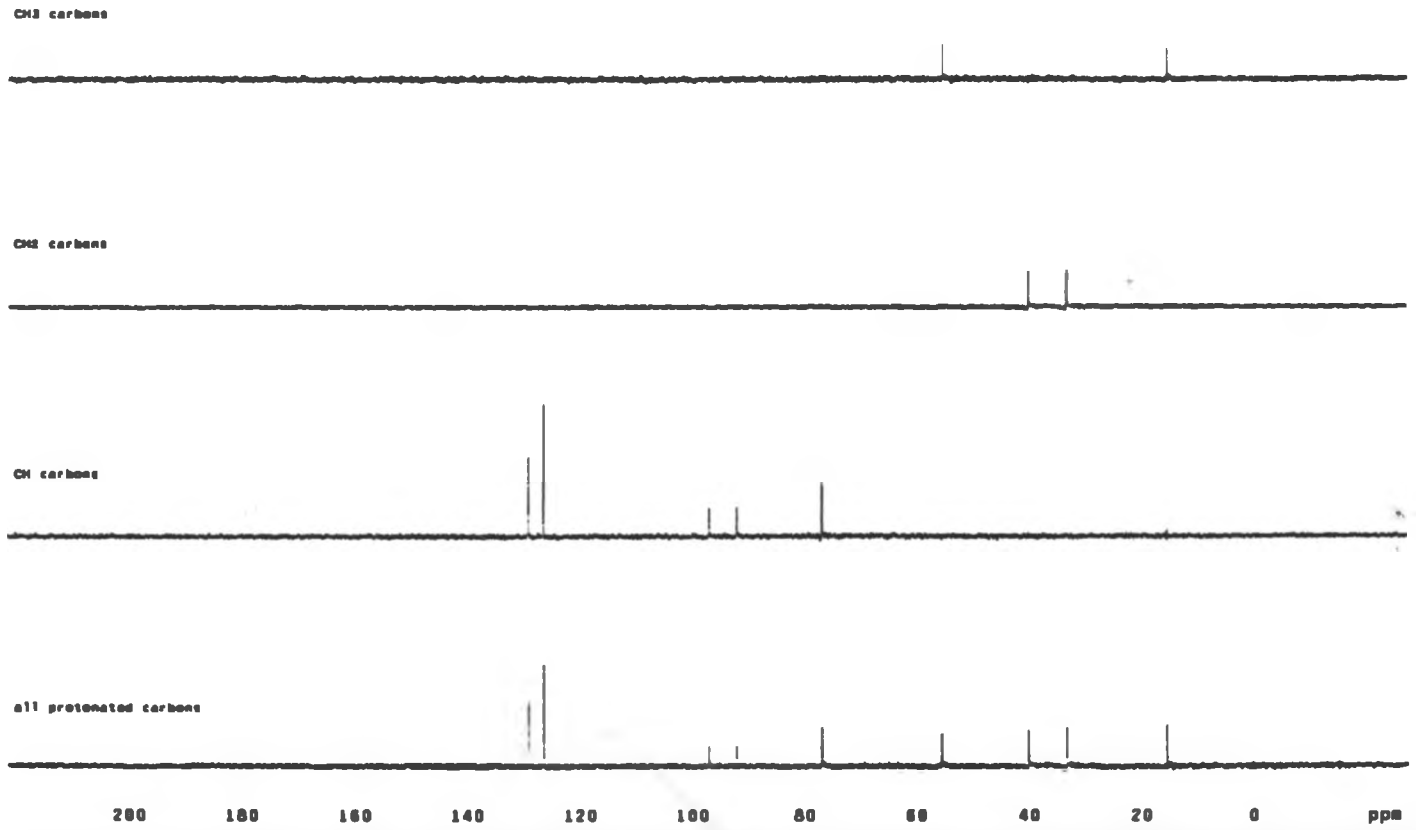
^{13}C NMR SPECTRUM ($CDCl_3$)



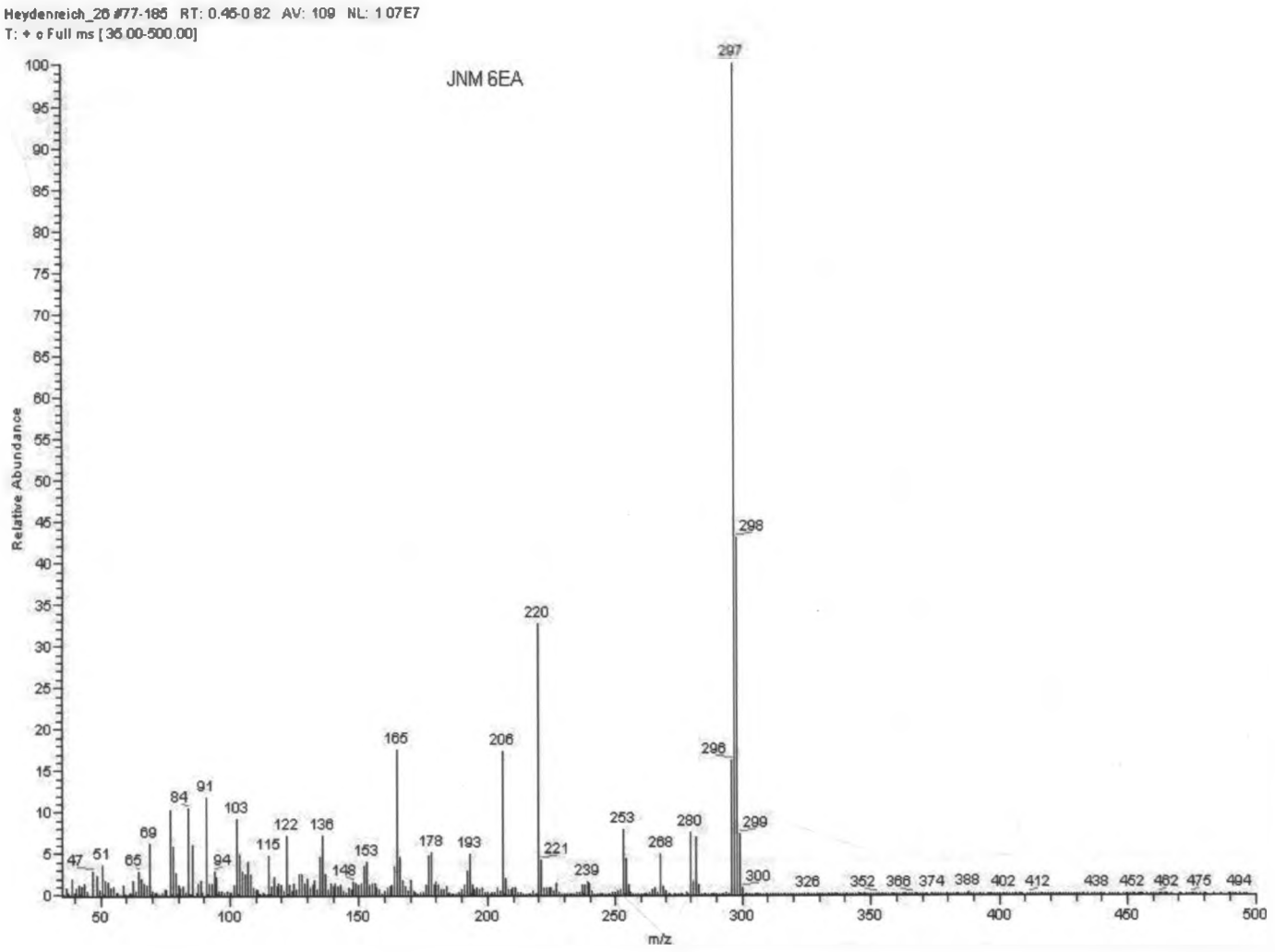
164.332
97.000
92.133
77.913
77.276
76.639
76.446
55.525
40.125
33.329
15.302
100
80
60
40
20
ppm

DEPT SPECTRUM

JMN-6cA
CDCl3
DEPT
02-01-09

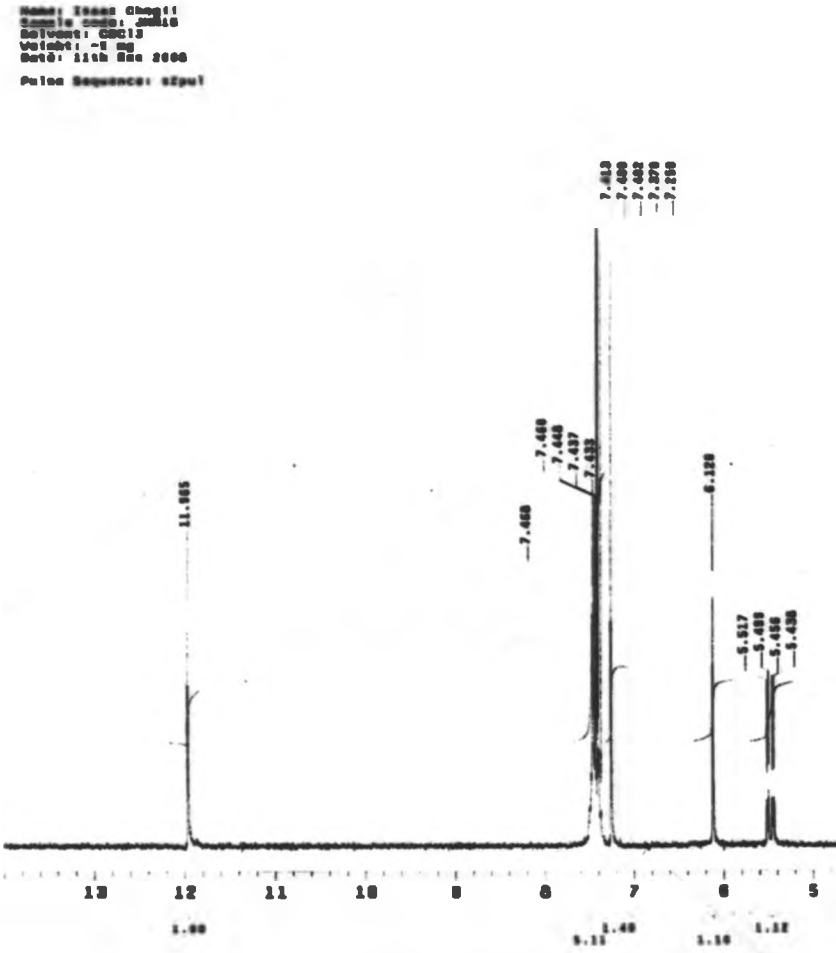


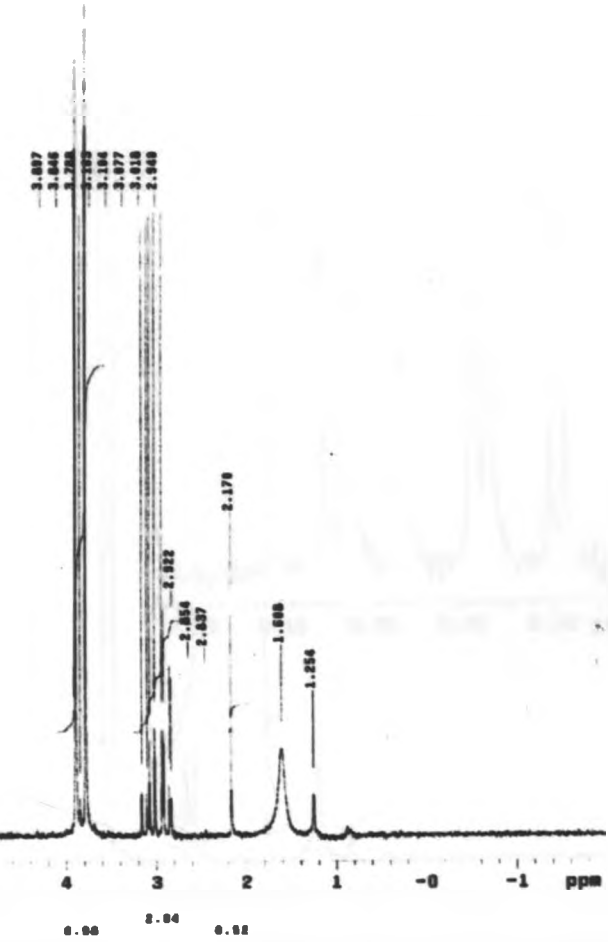
MS SPECTRUM (compound 2)

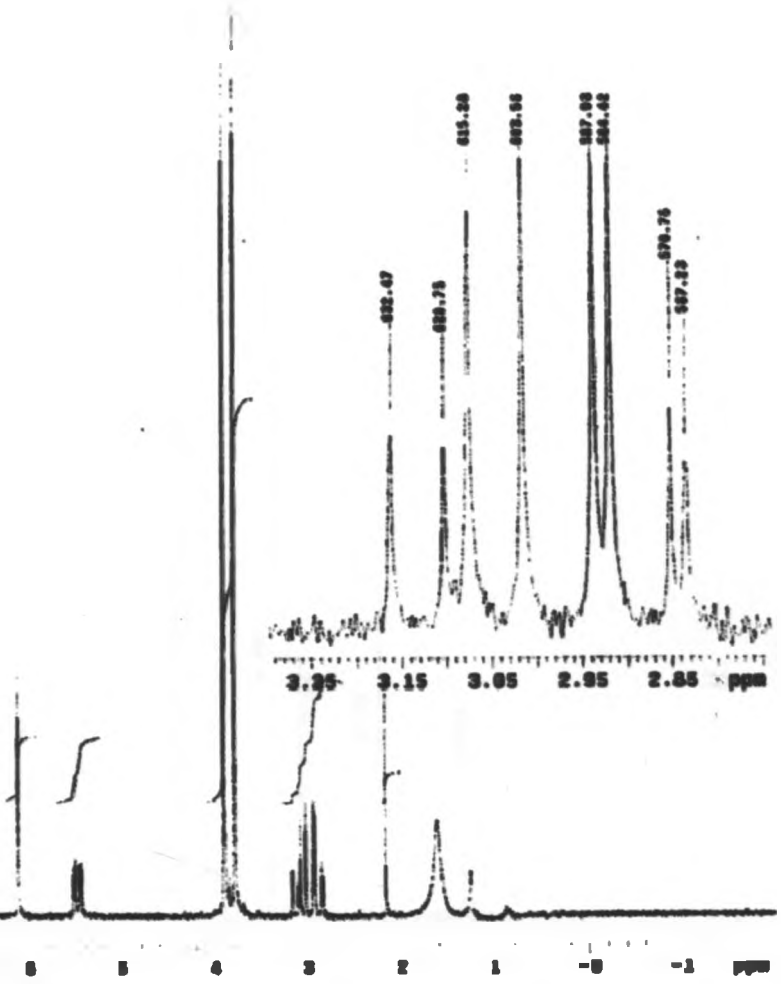


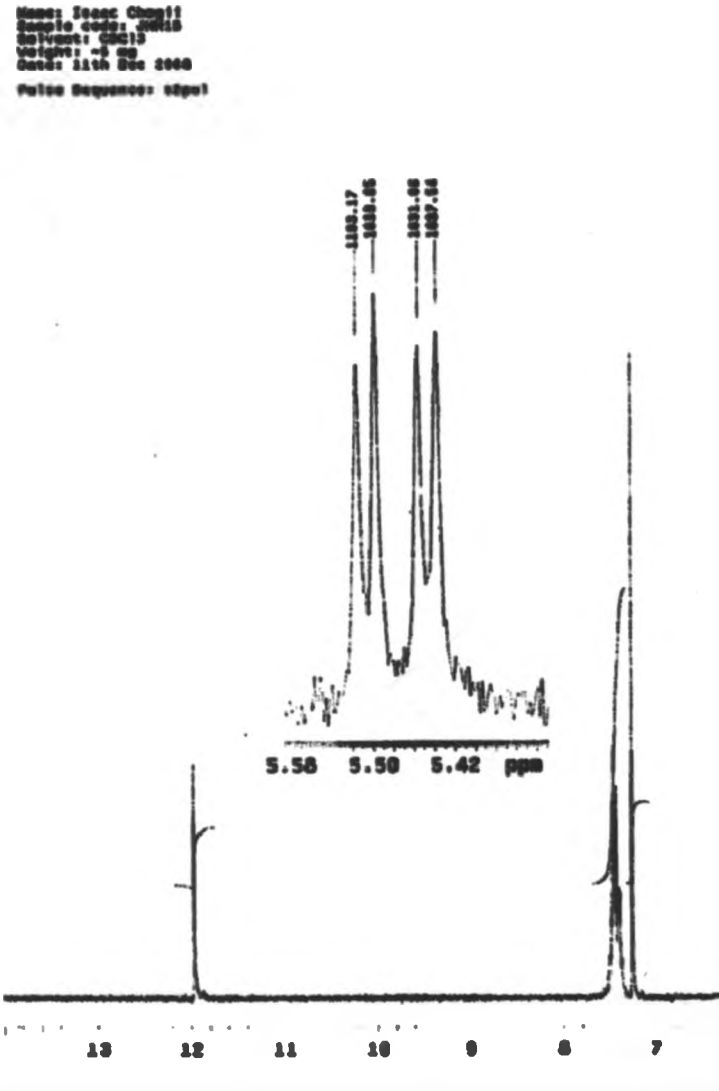
Appendix 3: Spectra for compound **3**

^{1}H NMR SPECTRUM (200 MHz, $CDCl_3$)

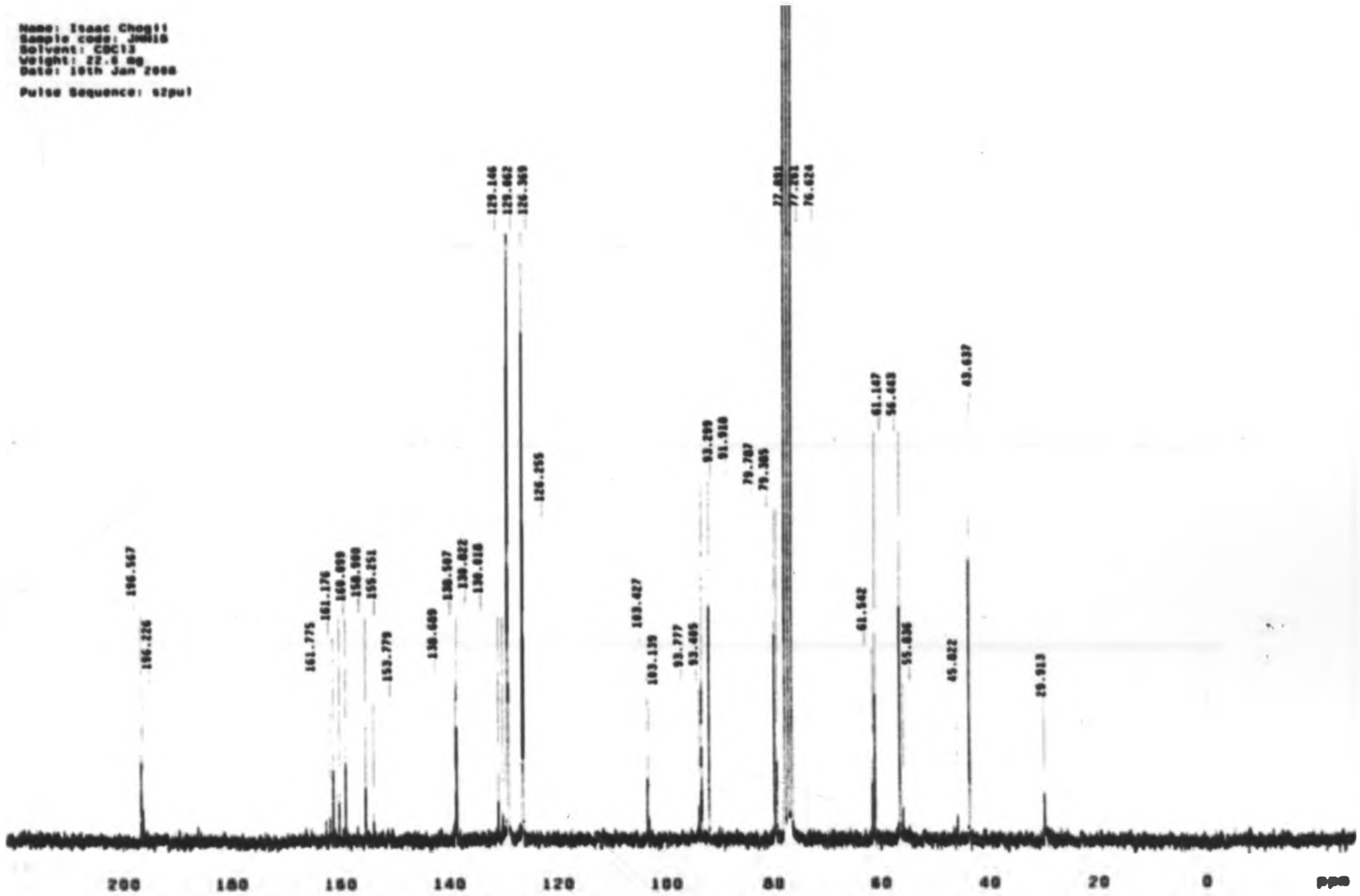



3.007
3.046
3.084
3.184
3.077
3.018
2.949
2.922
2.854
2.837
2.170
1.686
1.254
4
3
2
1
-0
-1
ppm
6.00
2.04
0.82

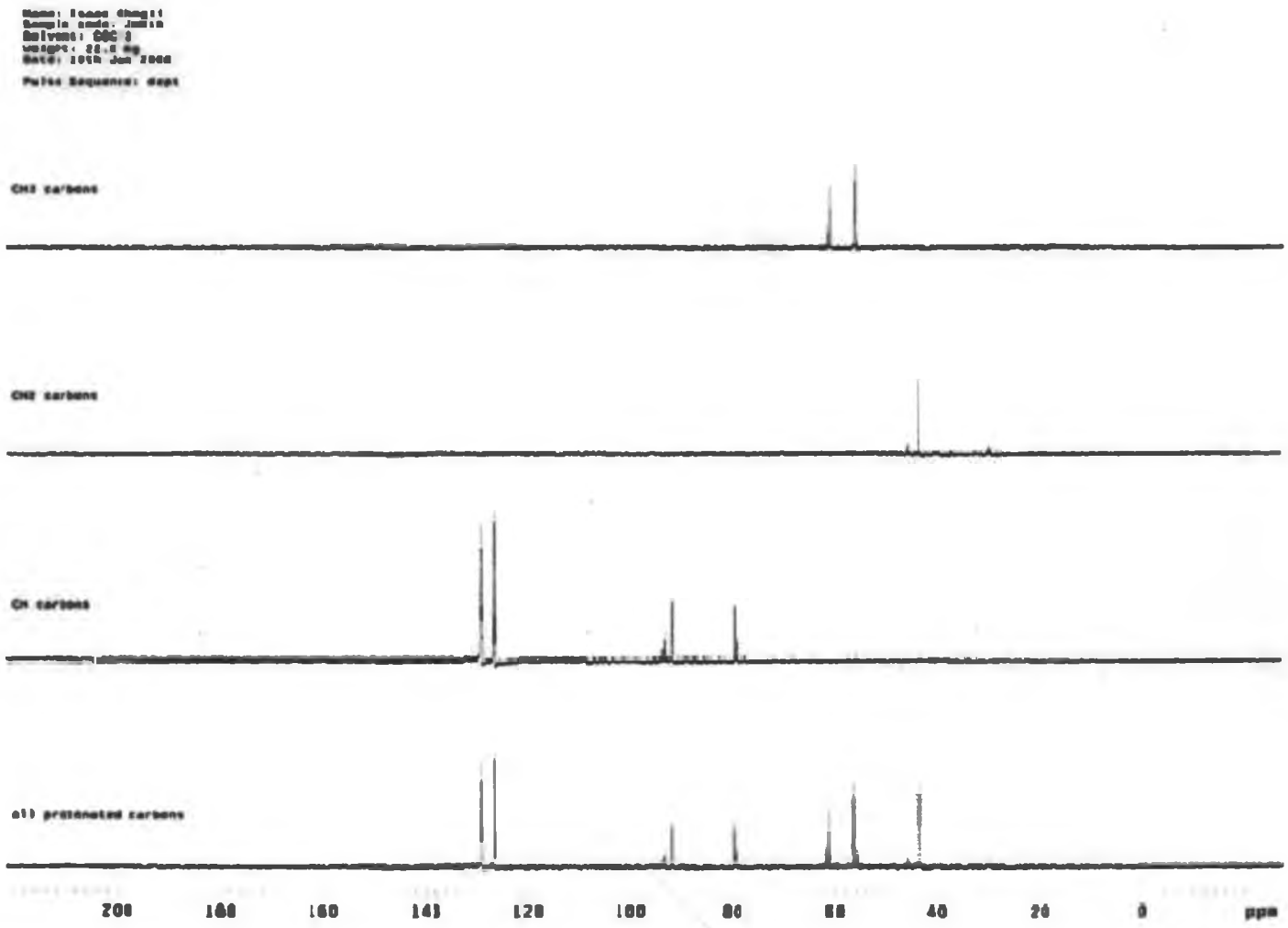

3.25
3.15
3.05
2.95
2.85
ppm
8
5
4
3
2
1
-0
-1
ppm


5.58
5.50
5.42
ppm
13
12
11
10
9
8
7

^{13}C NMR SPECTRUM (50 MHz, $CDCl_3$)

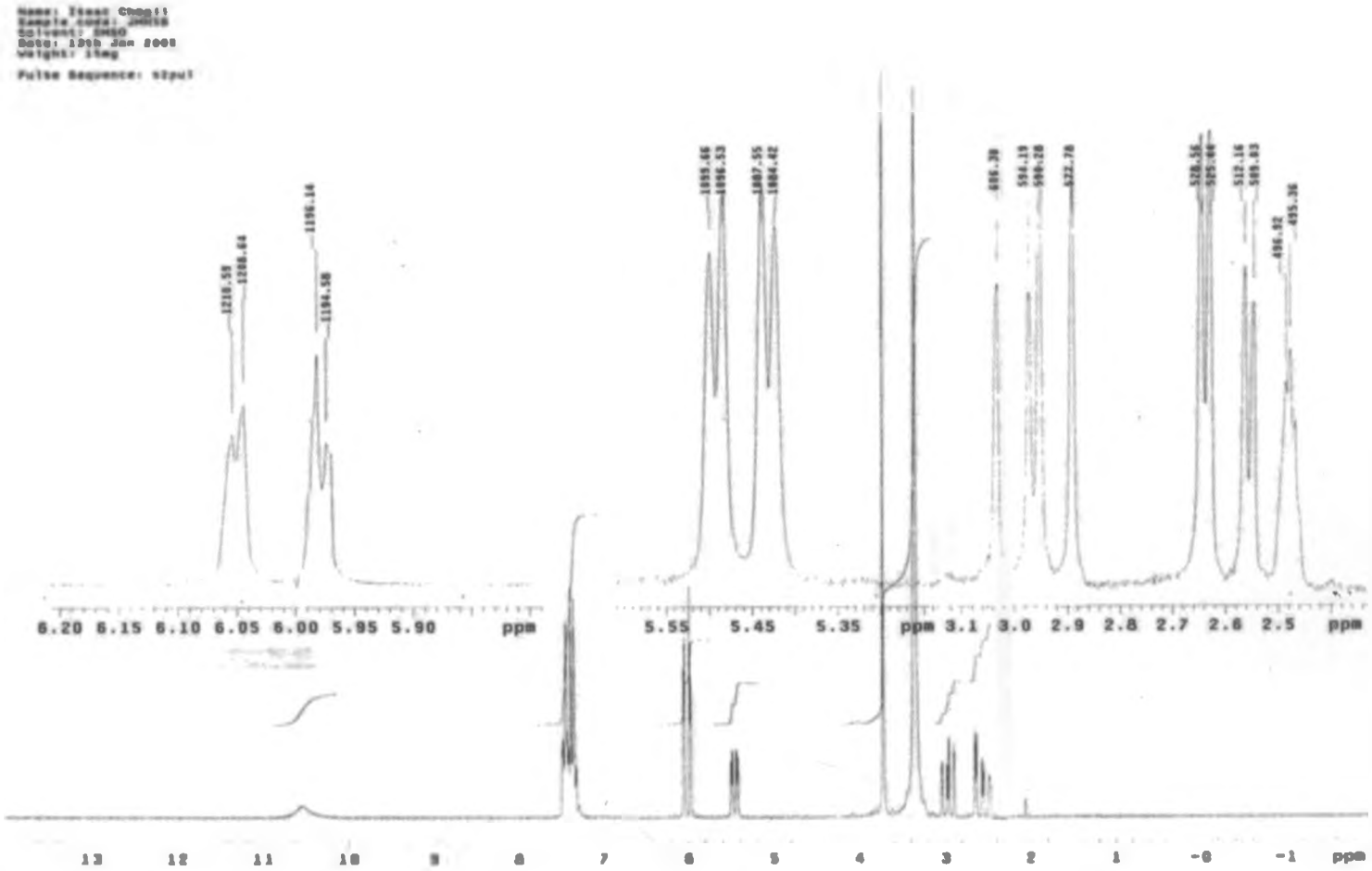


DEPT SPECTRUM

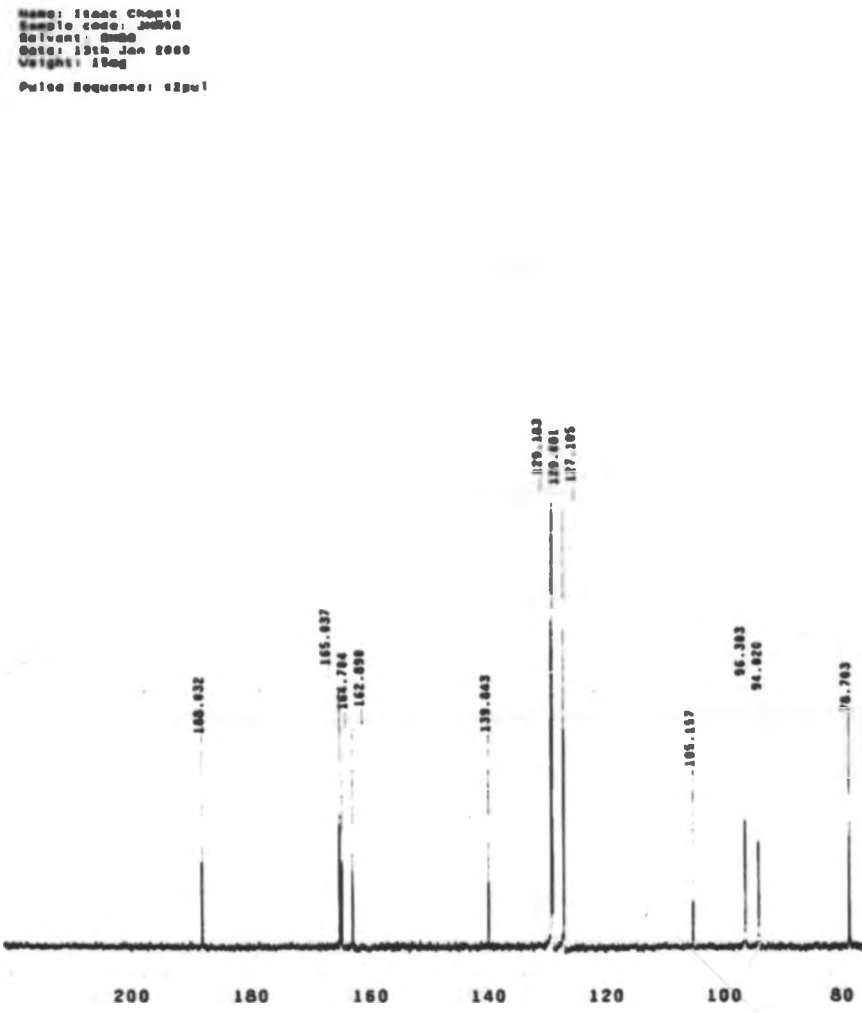


Appendix 4: Spectra for compound **4**

^{1}H NMR SPECTRUM (200 MHZ, DMSO)

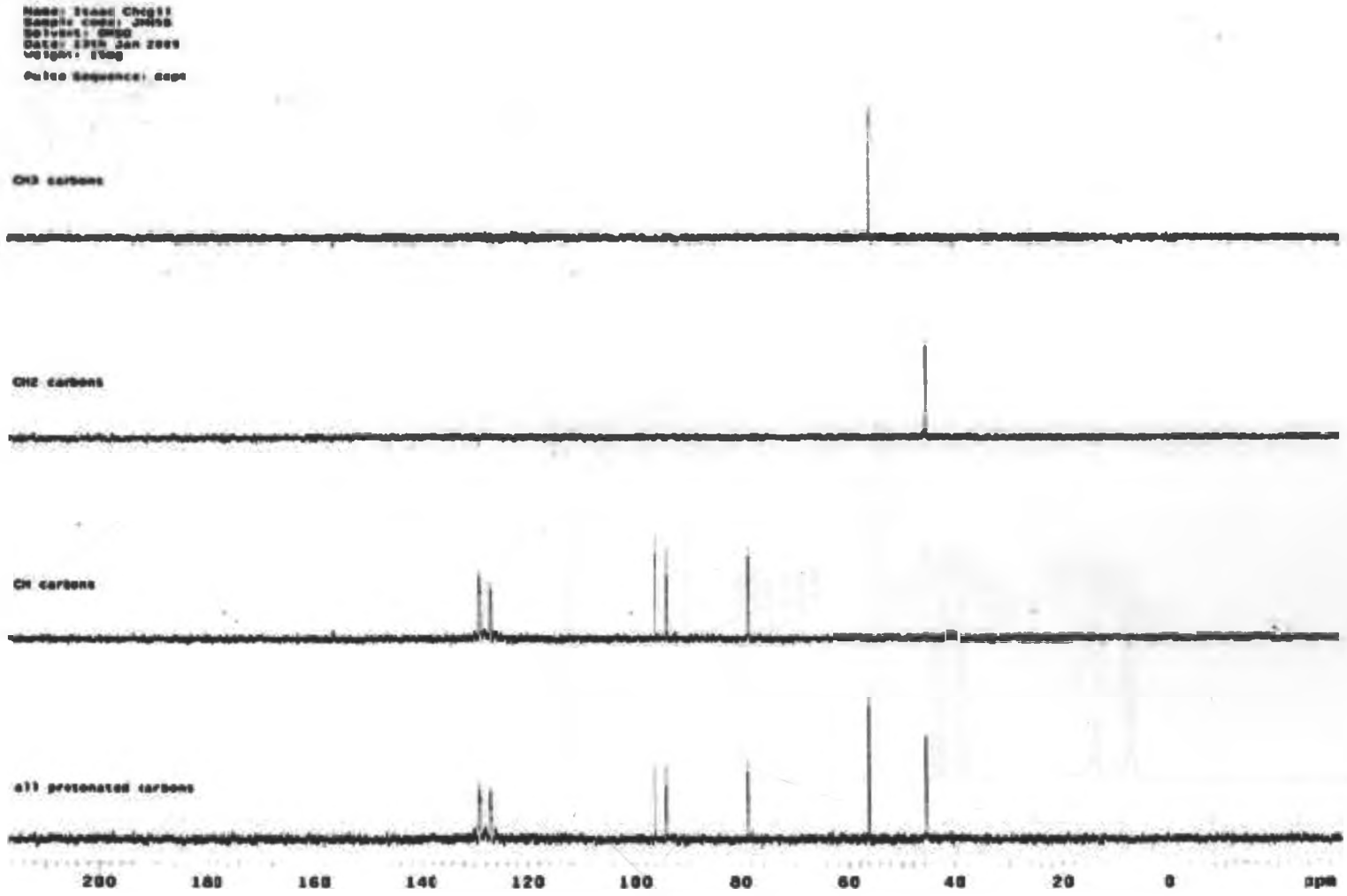


^{13}C NMR SPECTRUM (50 MHZ, DMSO)



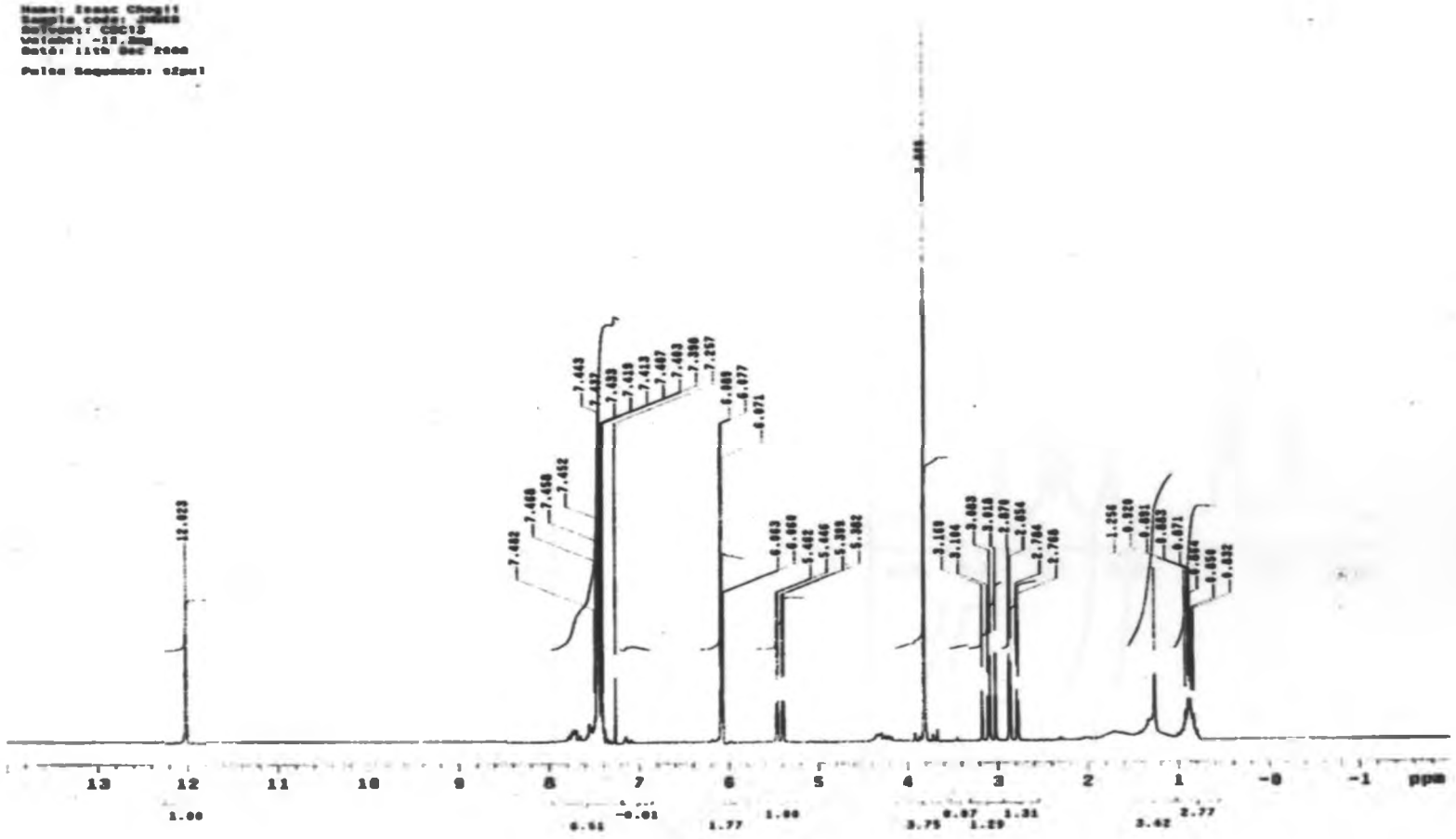
56.300
45.504
41.422
41.405
40.908
40.171
39.753
39.336
38.919
60
40
20
0
ppm

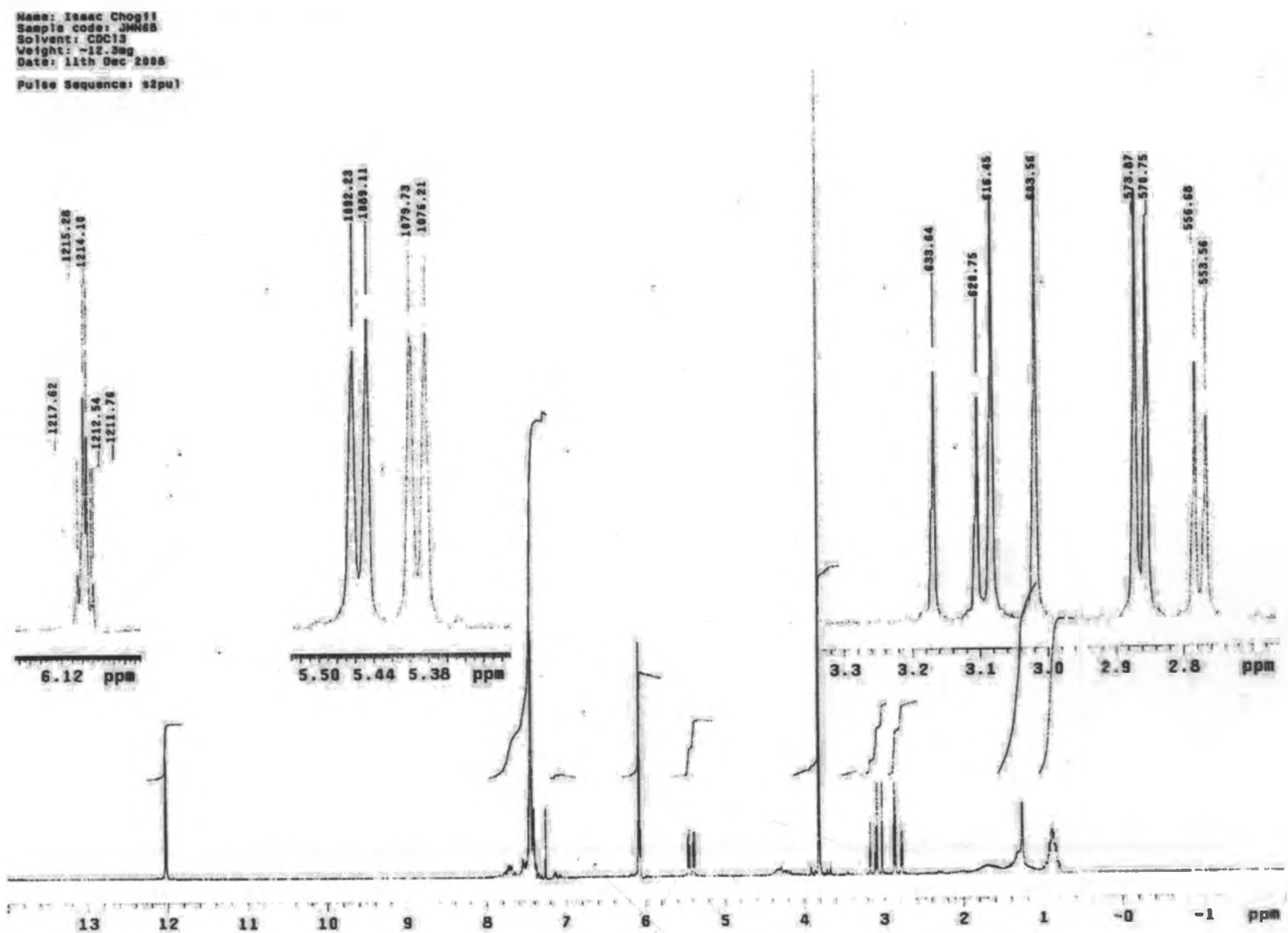
DEPT SPECTRUM



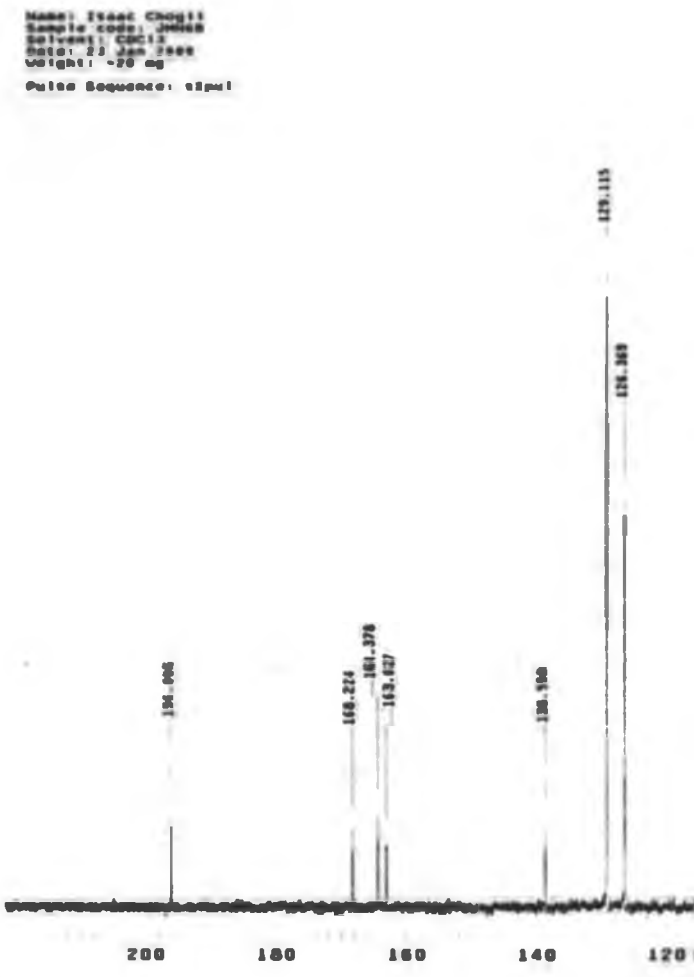
Appendix 5: Spectra for compound **5**

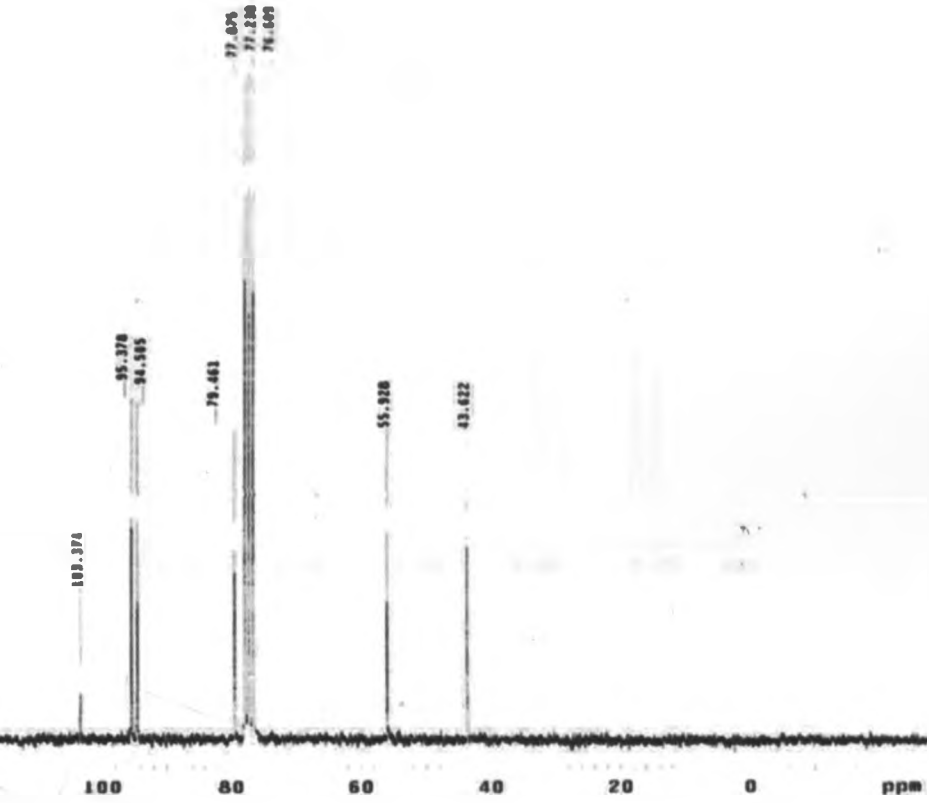
^{1}H NMR SPECTRUM (200 MHz, $CDCl_3$)




Name: Isaac Chogii
Sample code: JMN68
Solvent: CDCl3
Weight: ~12.3mg
Date: 11th Dec 2008
Pulse Sequence: s2pul
1217.62
1215.28
1214.18
1212.54
1211.76
1892.23
1889.11
1879.73
1876.21
633.64
620.75
616.45
583.58
573.87
570.75
556.68
553.58
6.12 ppm
5.50 5.44 5.38 ppm
3.3 3.2 3.1 3.0 2.9 2.8 ppm
13 12 11 10 9 8 7 6 5 4 3 2 1 -0 -1 ppm

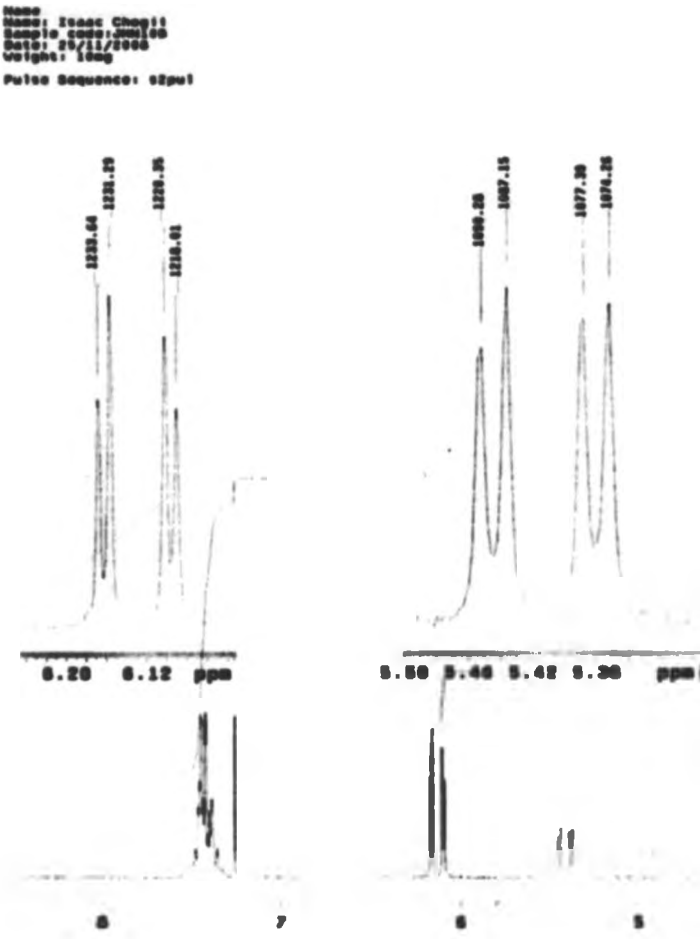
^{13}C NMR SPECTRUM (50 MHz, $CDCl_3$)

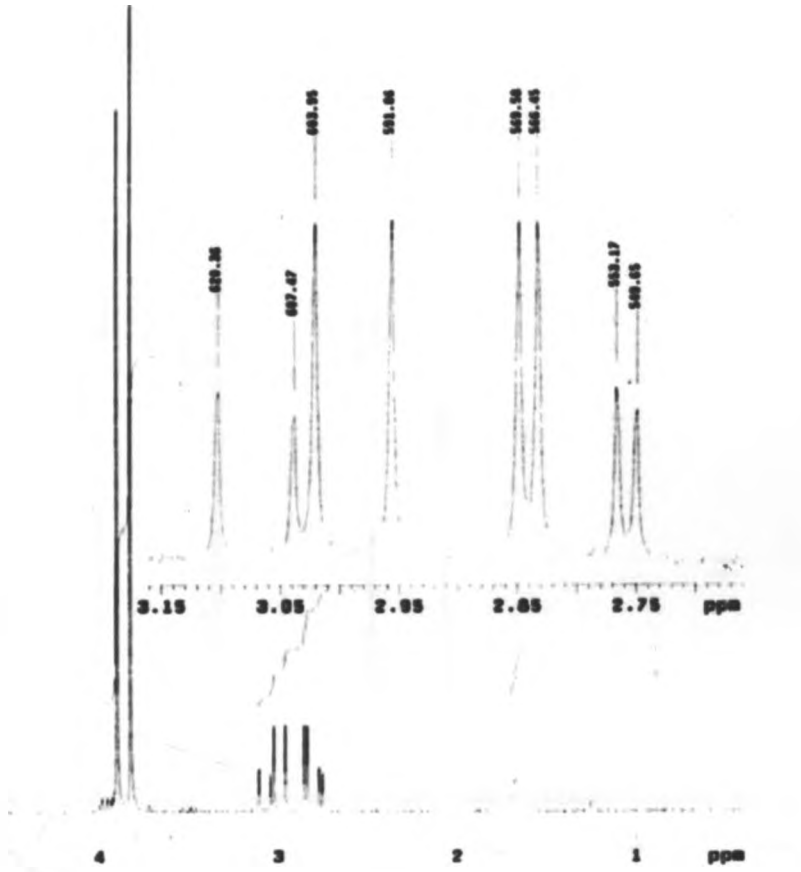



95.378
94.585
79.461
55.928
43.622
100
80
60
40
20
0
ppm

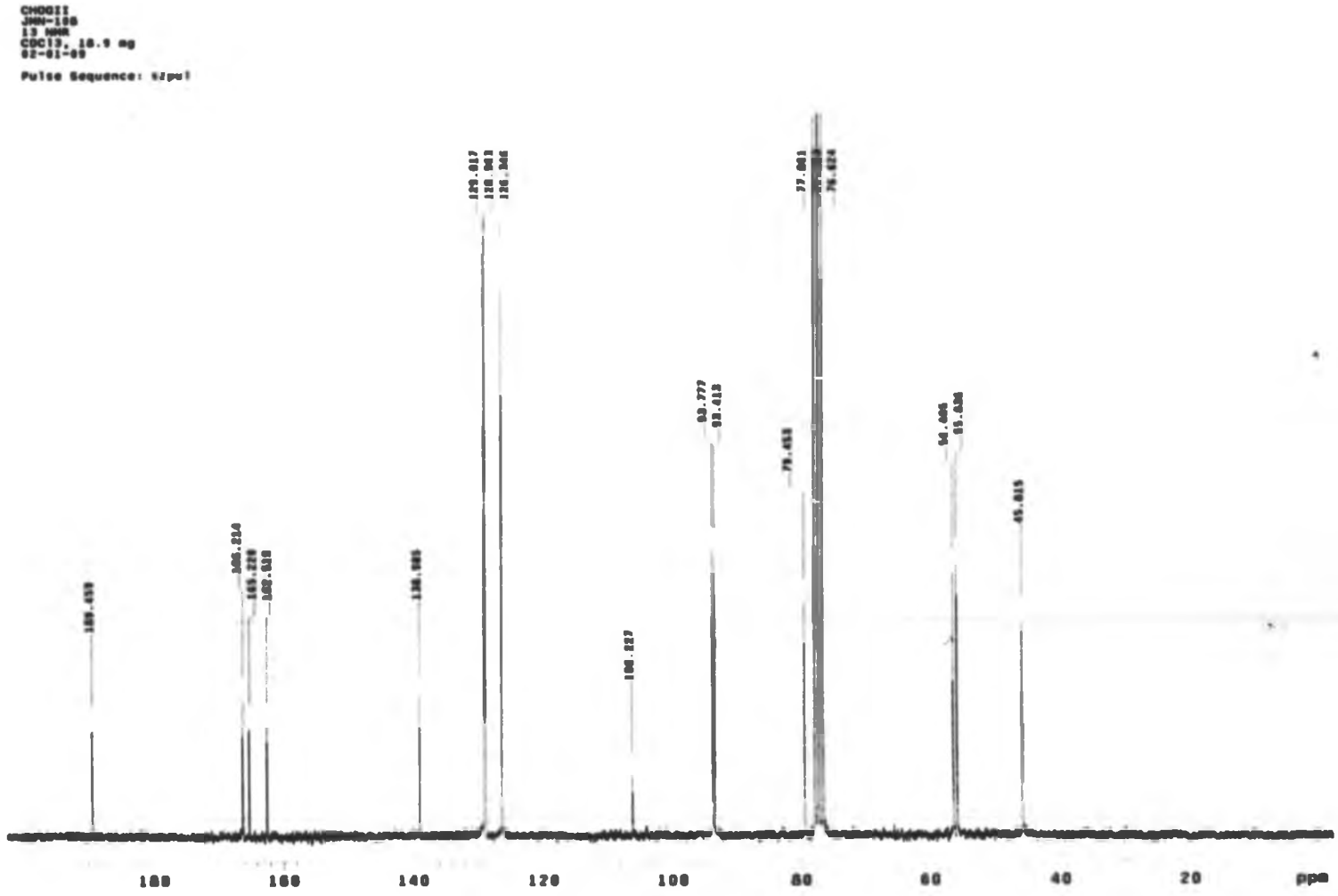
Appendix 6: Spectra for compound **6**

^{1}H NMR SPECTRUM (200 MHz, $CDCl_3$)

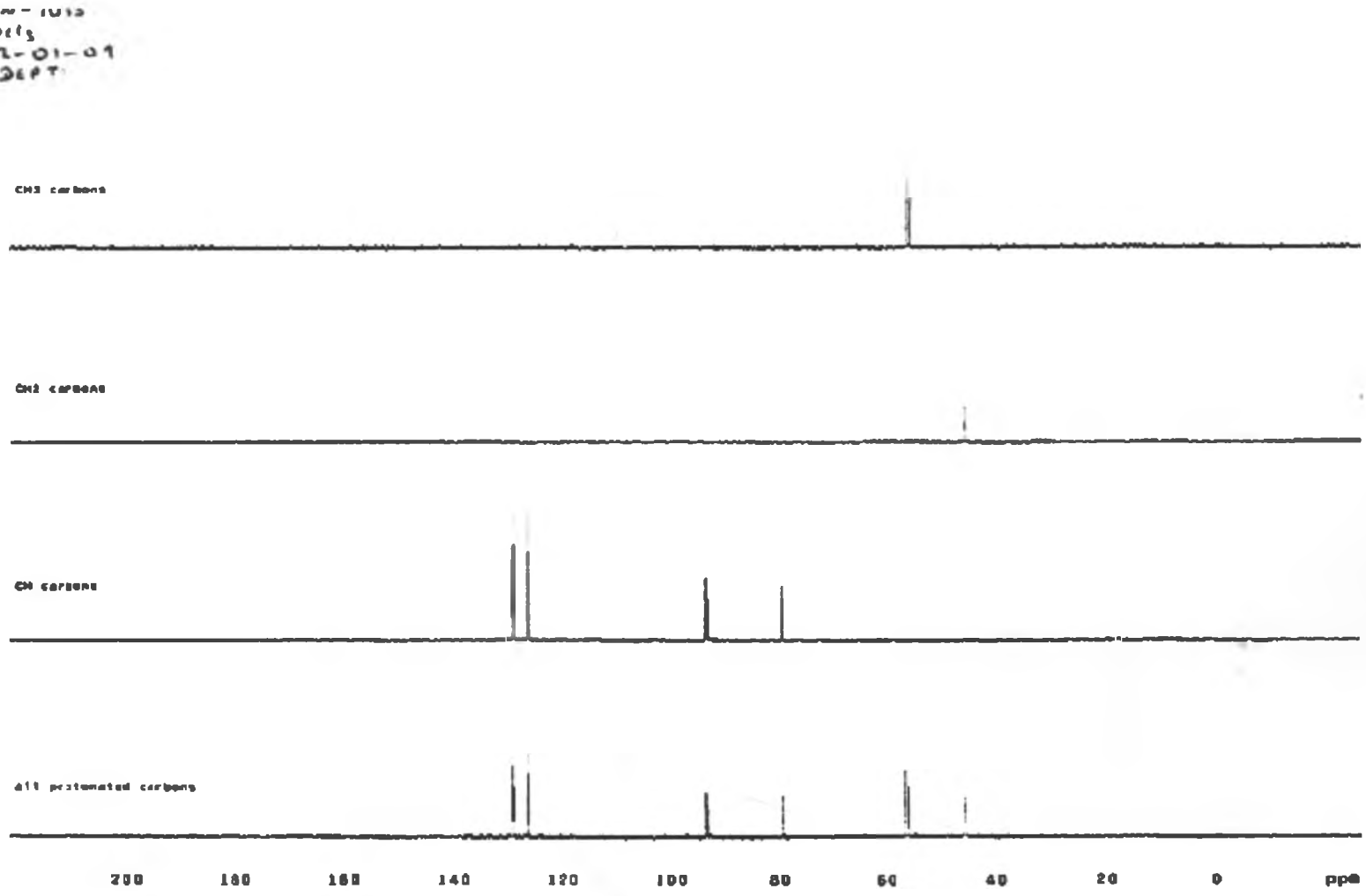



3.15
3.05
2.95
2.85
2.75
ppm
4
3
2
1
ppm

^{13}C NMR SPECTRUM (50 MHz, $CDCl_3$)

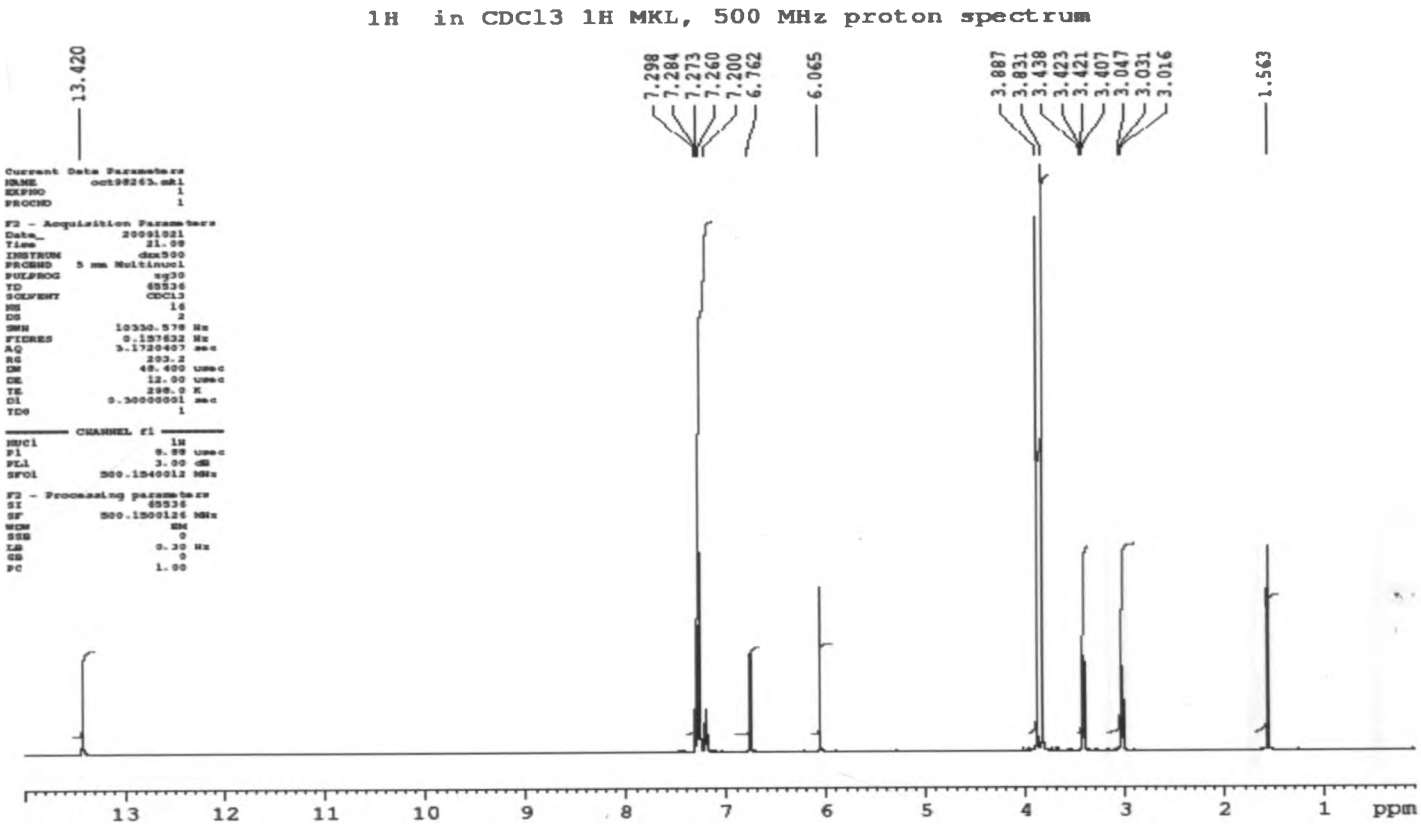


DEPT SPECTRUM

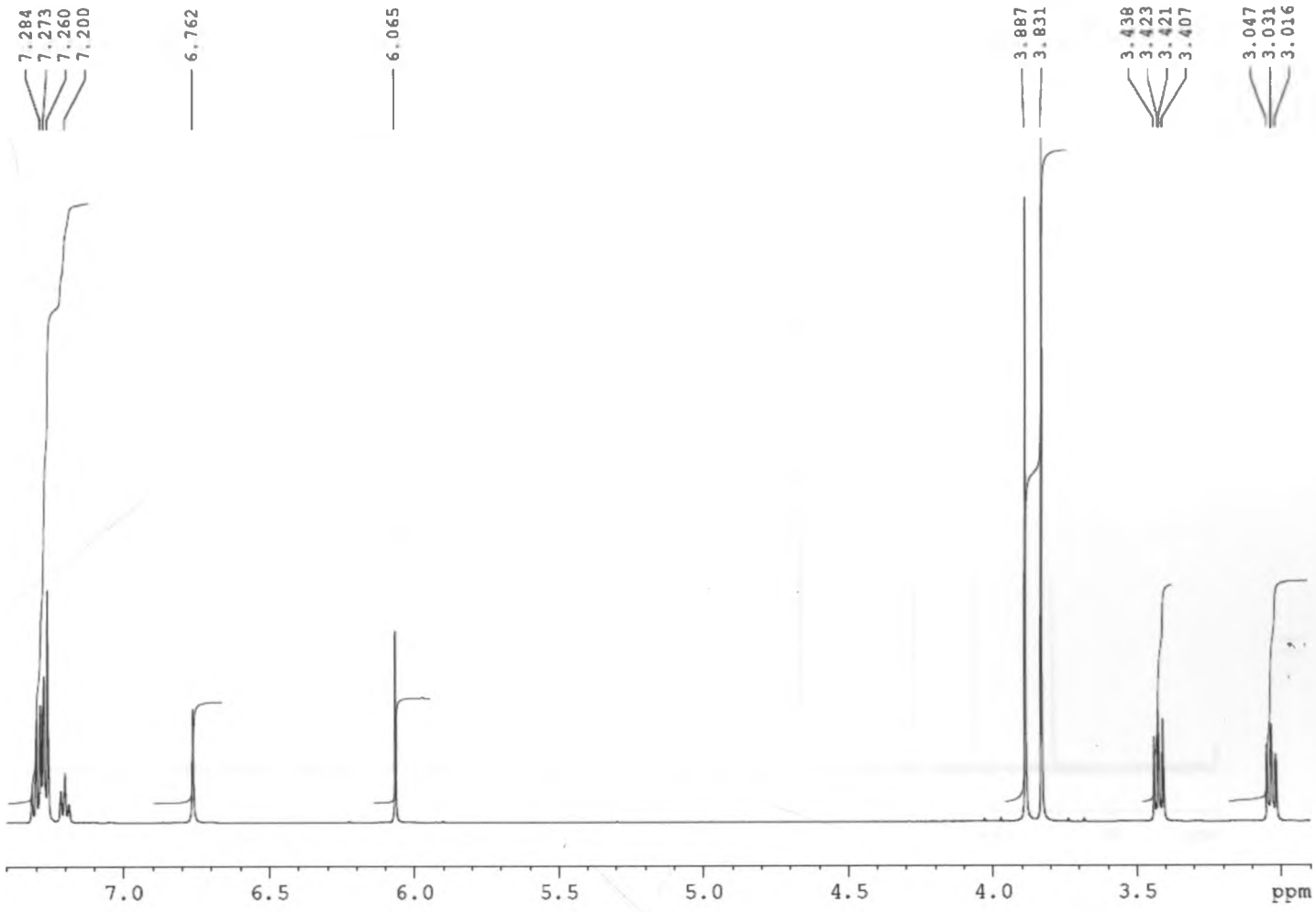


Appendix 7: Spectra for compound 7

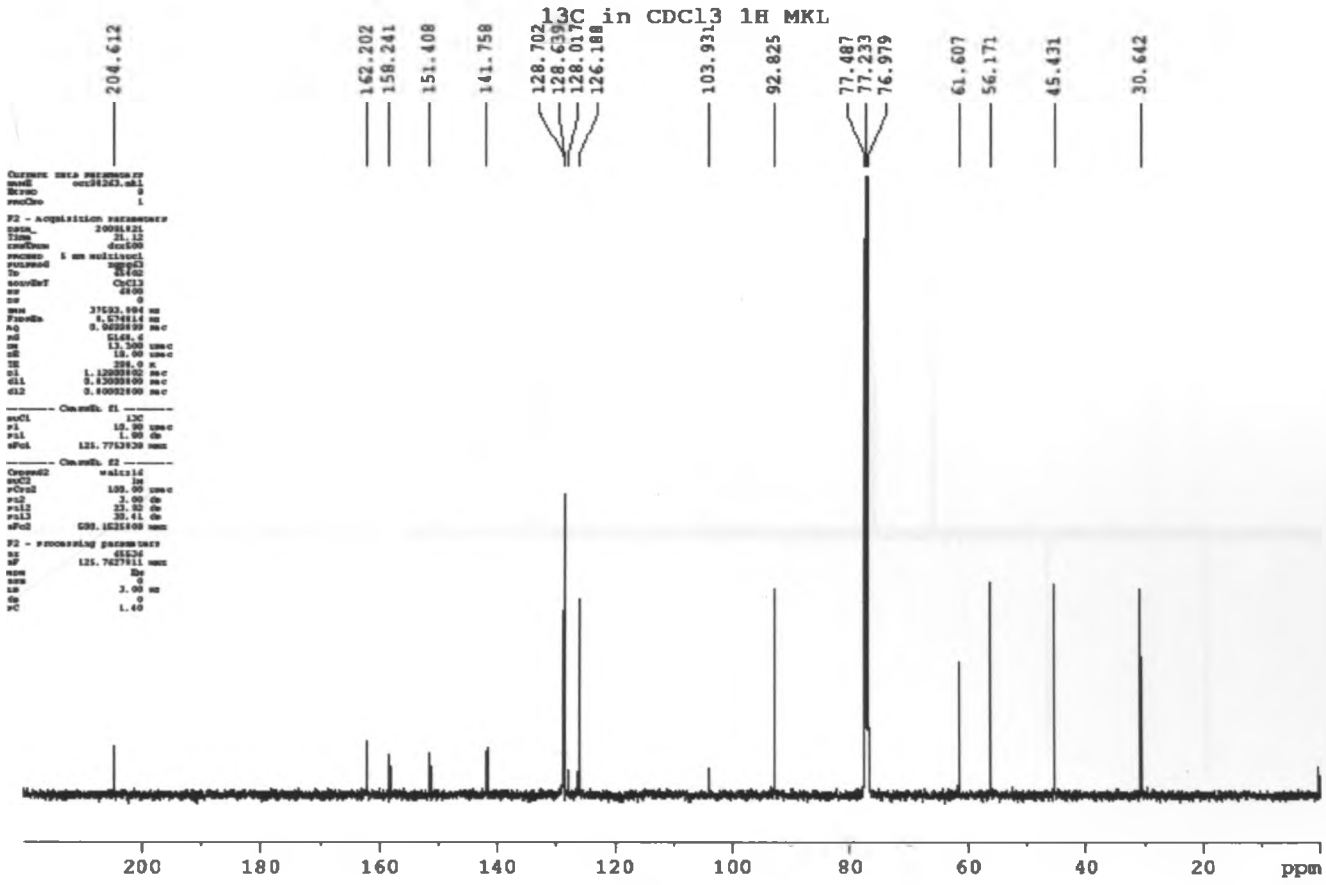
^{1}H NMR SPECTRUM (500 MHz, $CDCL_3$)



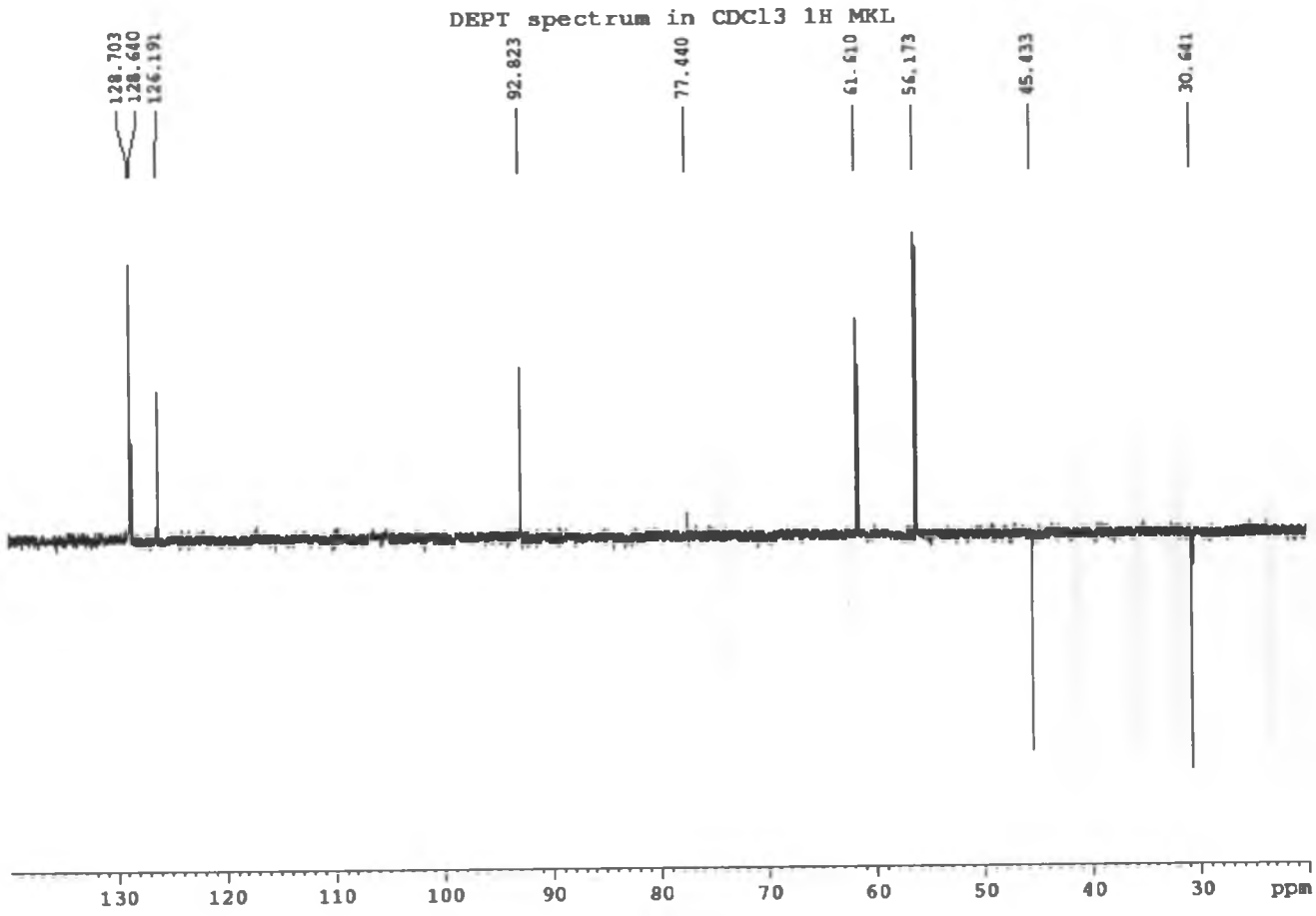
1H in CDCl3 1H MKL, 500 MHz proton spectrum



^{13}C SPECTRUM (125 MHZ, $CDCL_3$)

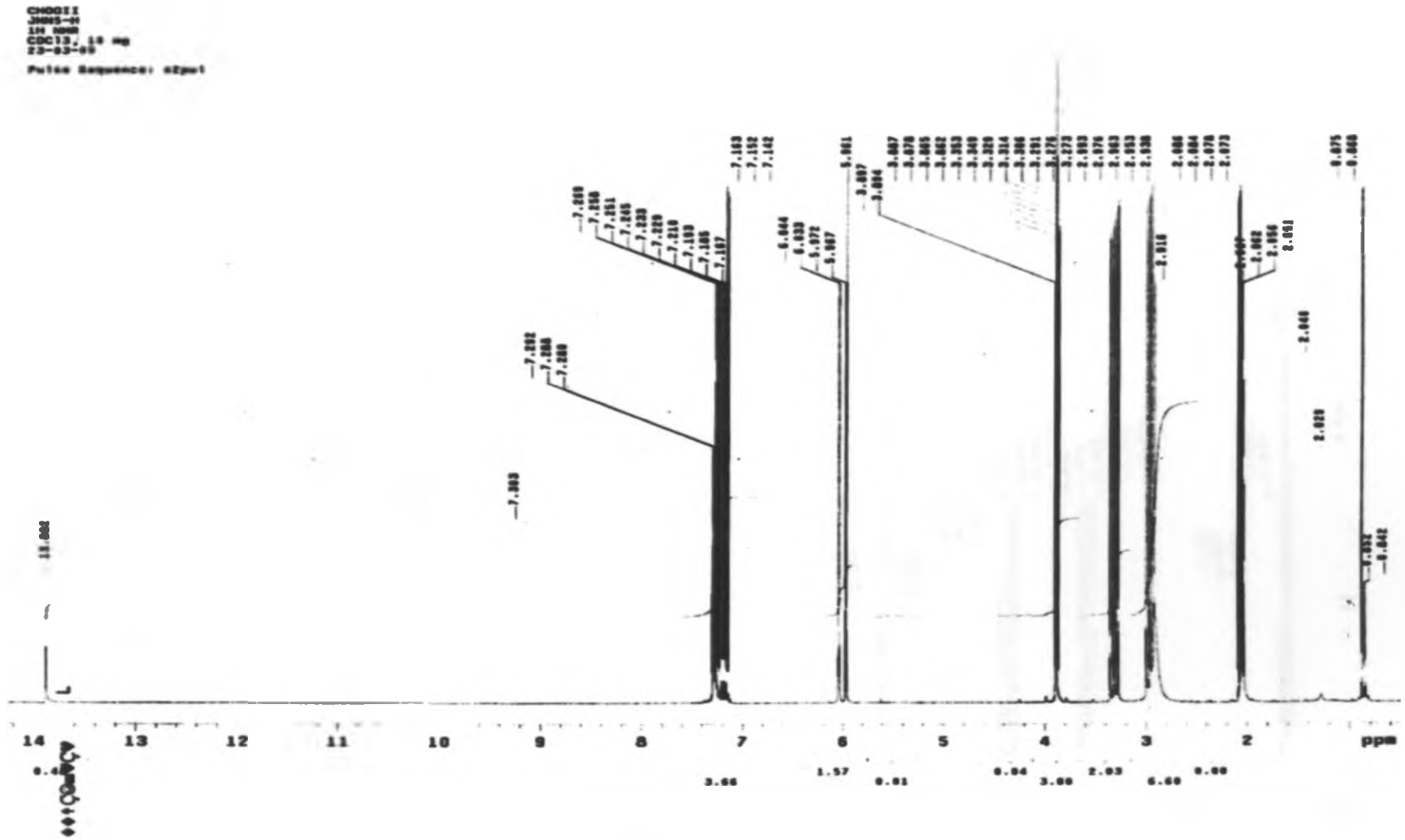


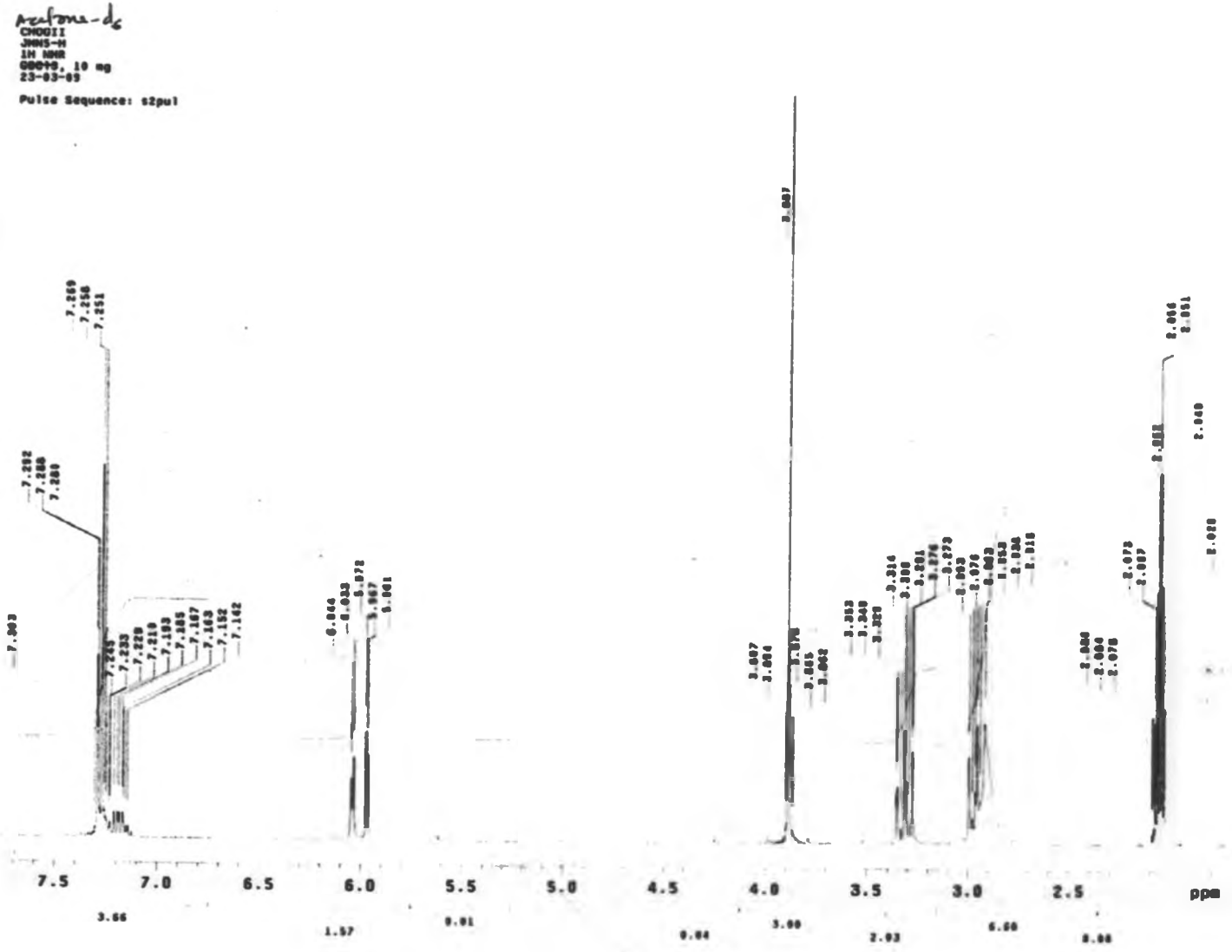
DEPT SPECTRUM



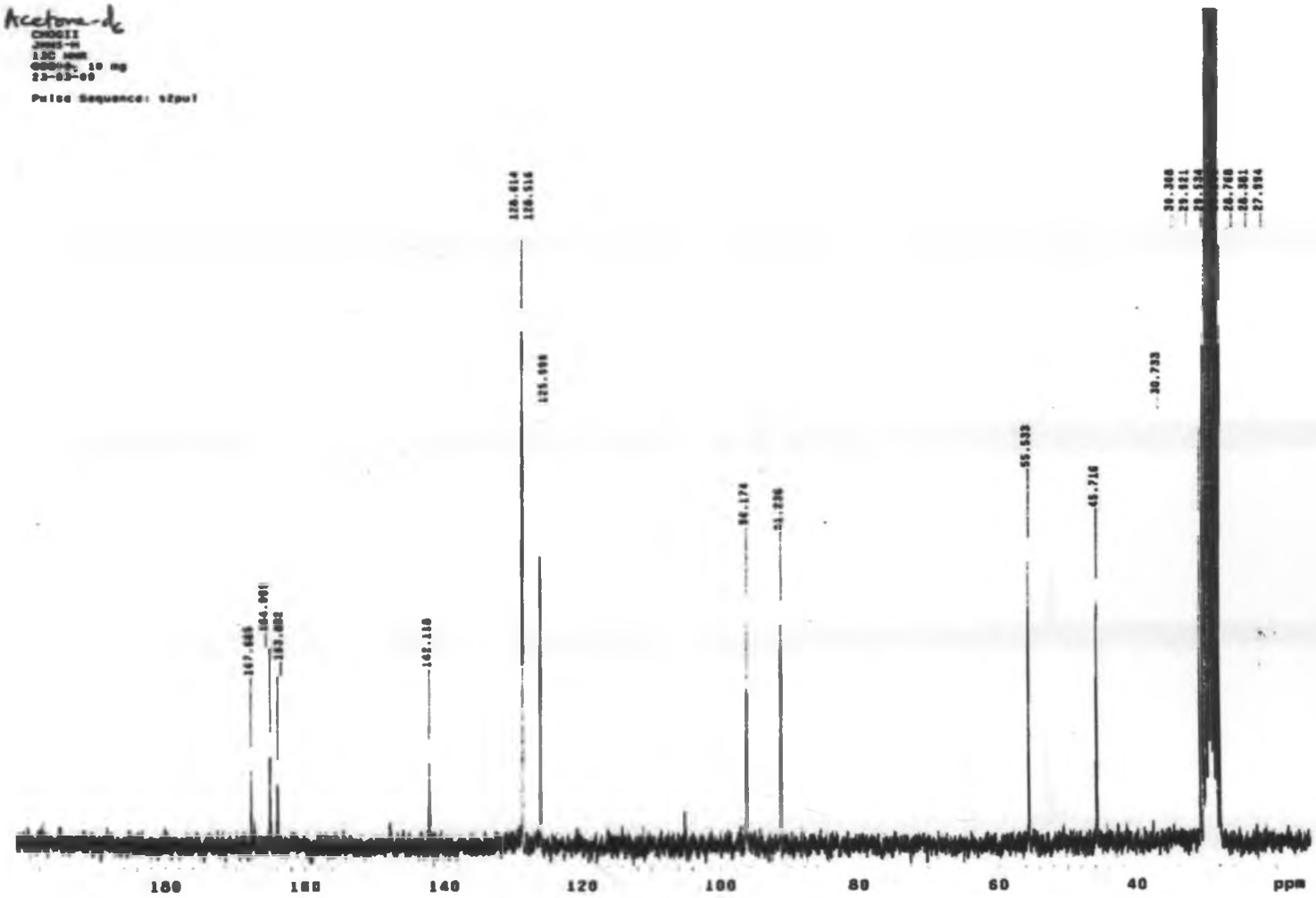
Appendix 8: Spectra for compound **8**

^{1}H SPECTRUM (200 MHz, Acetone-d6)

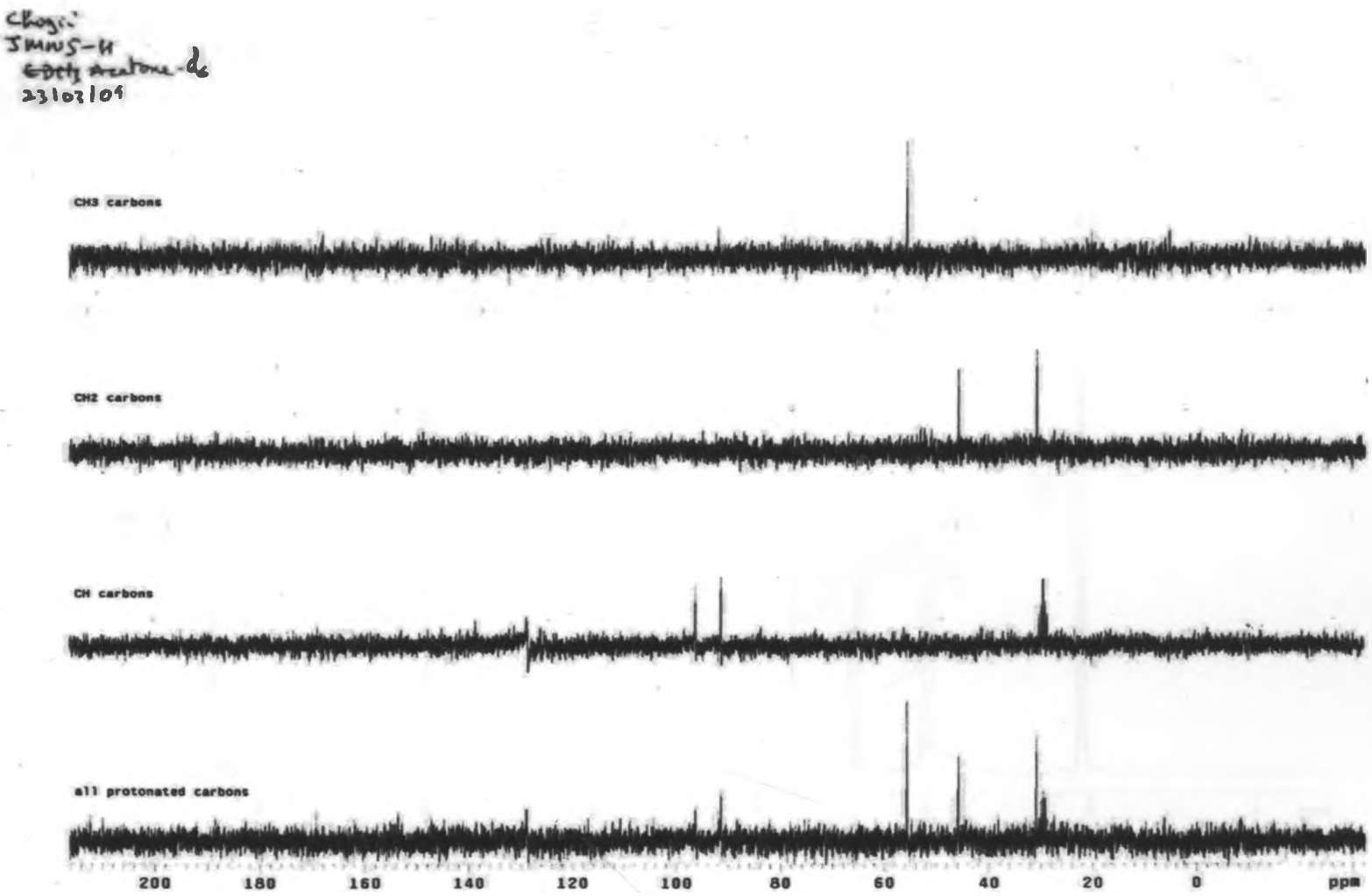



Acetone-d6
CH0021
JHNS-H
1H NMR
CD010, 10 mg
23-03-05
Pulse Sequence: s2pul
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm
3.66
1.57
0.91
0.84
3.00
2.03
6.00
0.98

^{13}C NMR SPECTRUM (50 MHz, Acetone-d6)

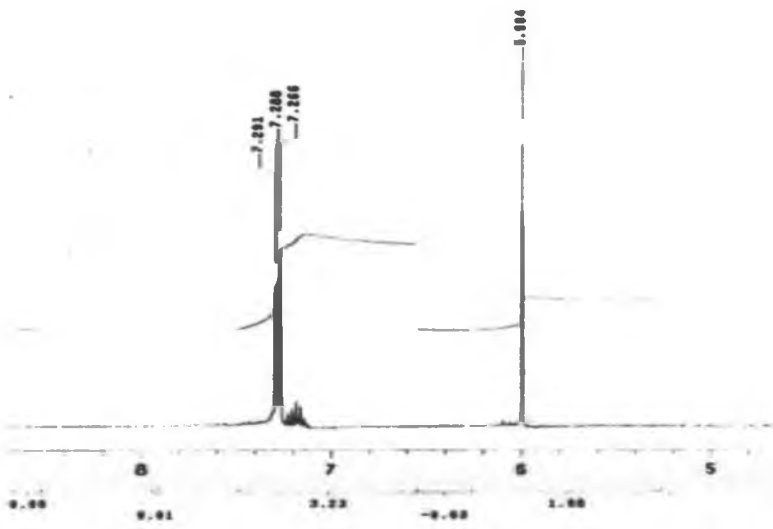


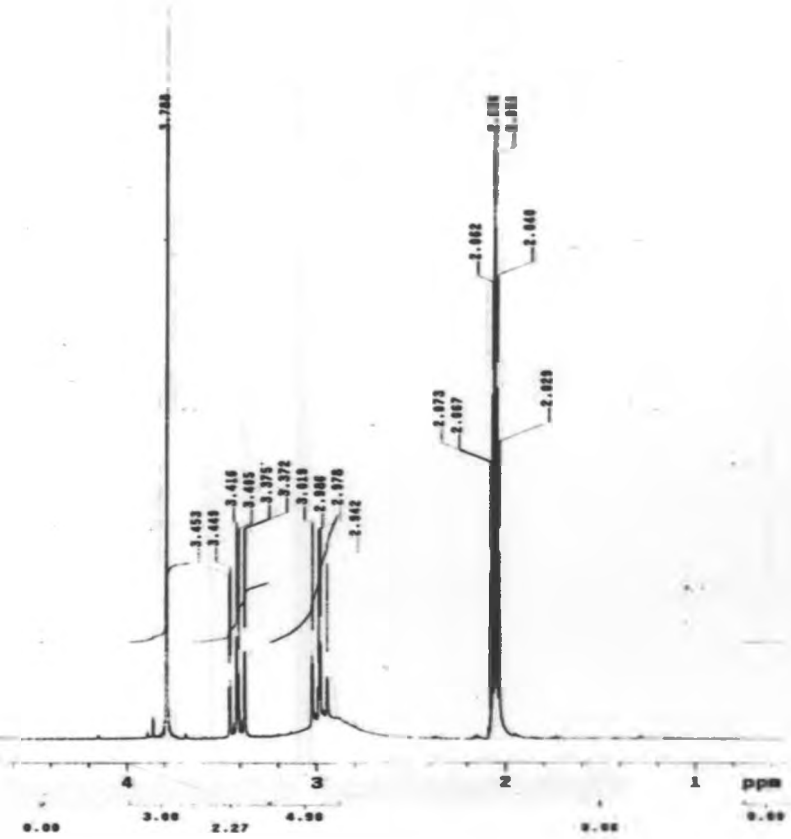
DEPT SPECTRUM



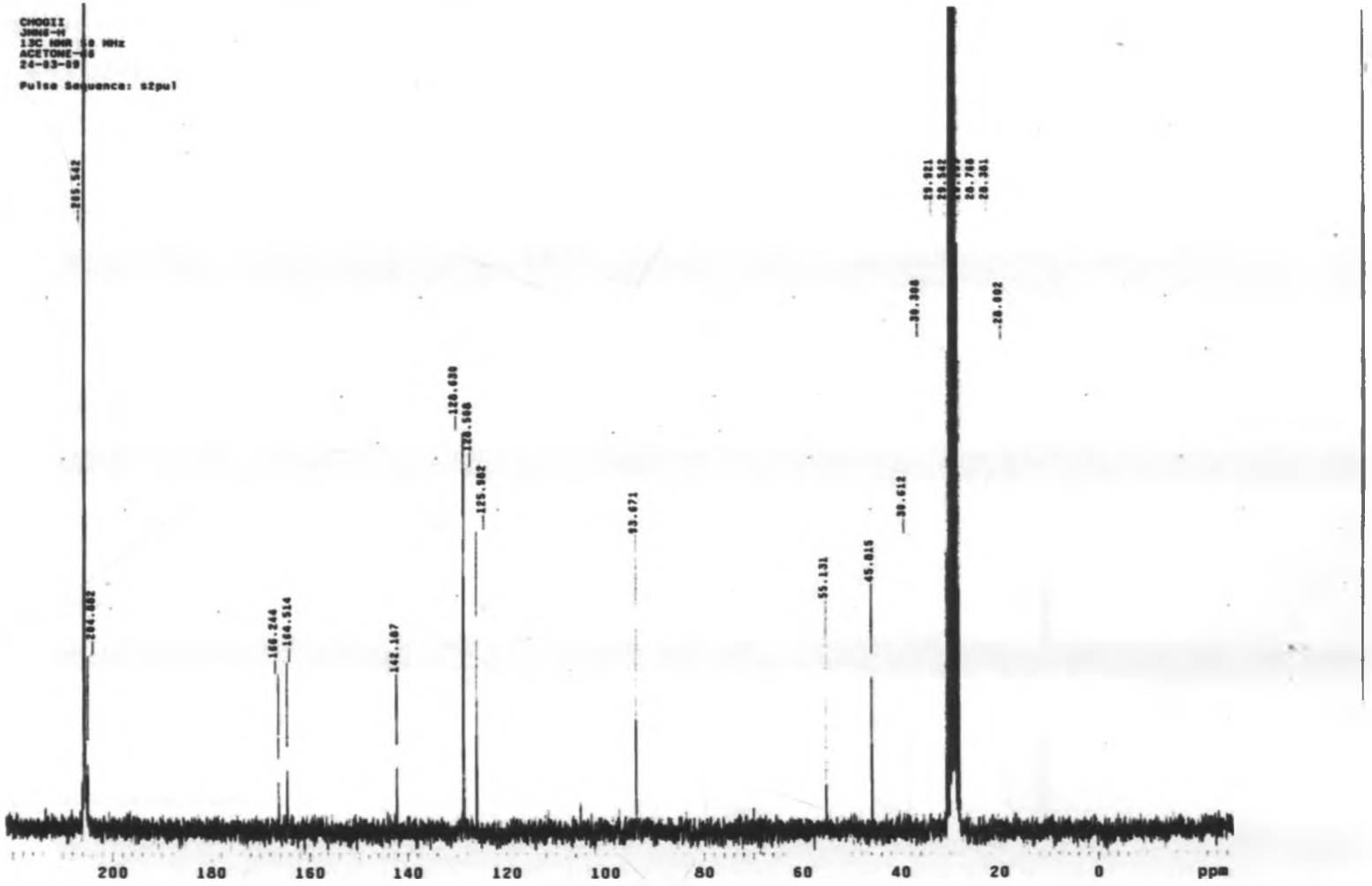
Appendix 9: Spectra for compound **9**

^{1}H NMR SPECTRUM (200 MHz, Acetone-d6)



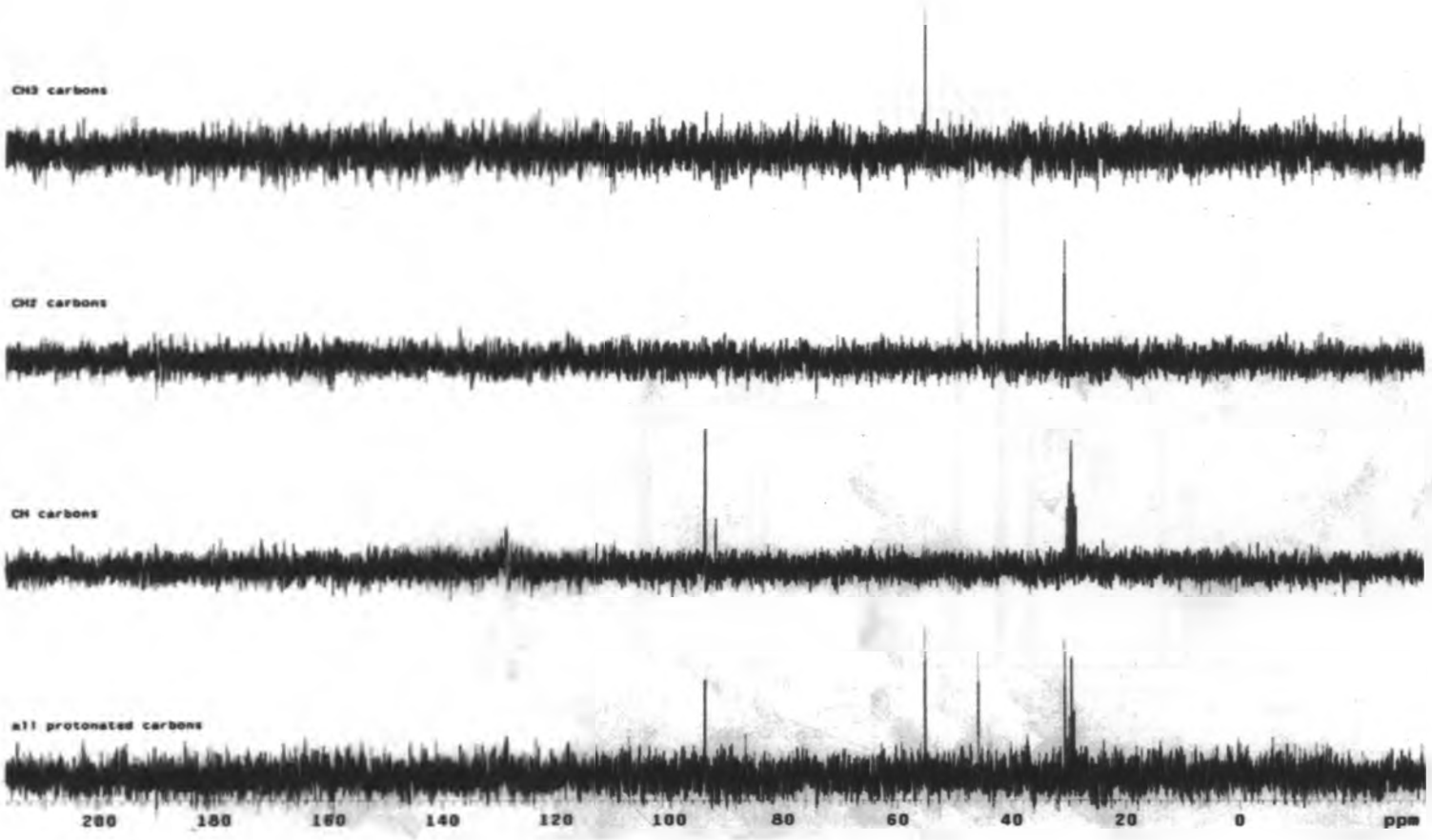

4
3
2
1
ppm
3.00
2.27
4.00

^{13}C NMR SPECTRUM (50 MHz, Acetone-d6)



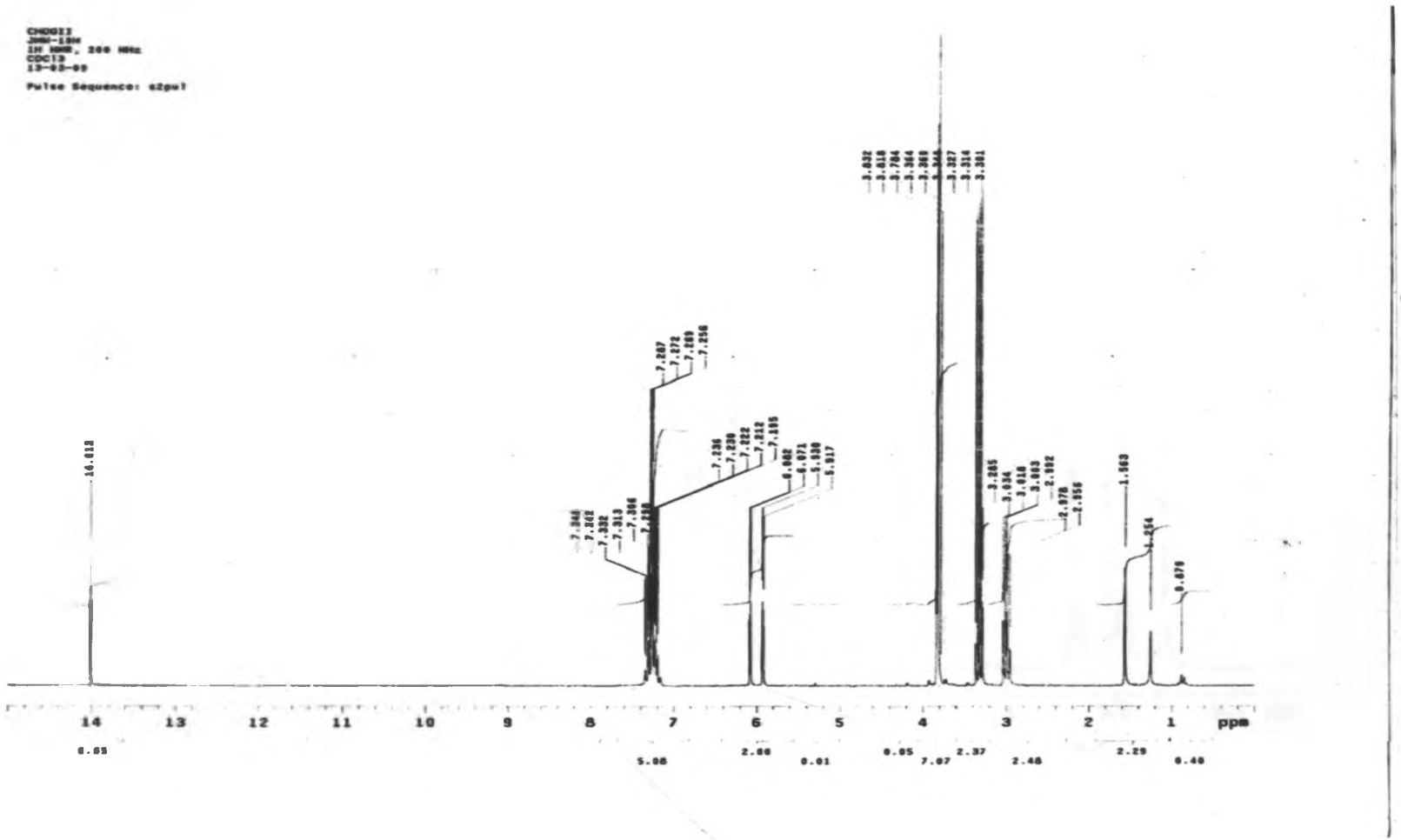
DEPT SPECTRUM

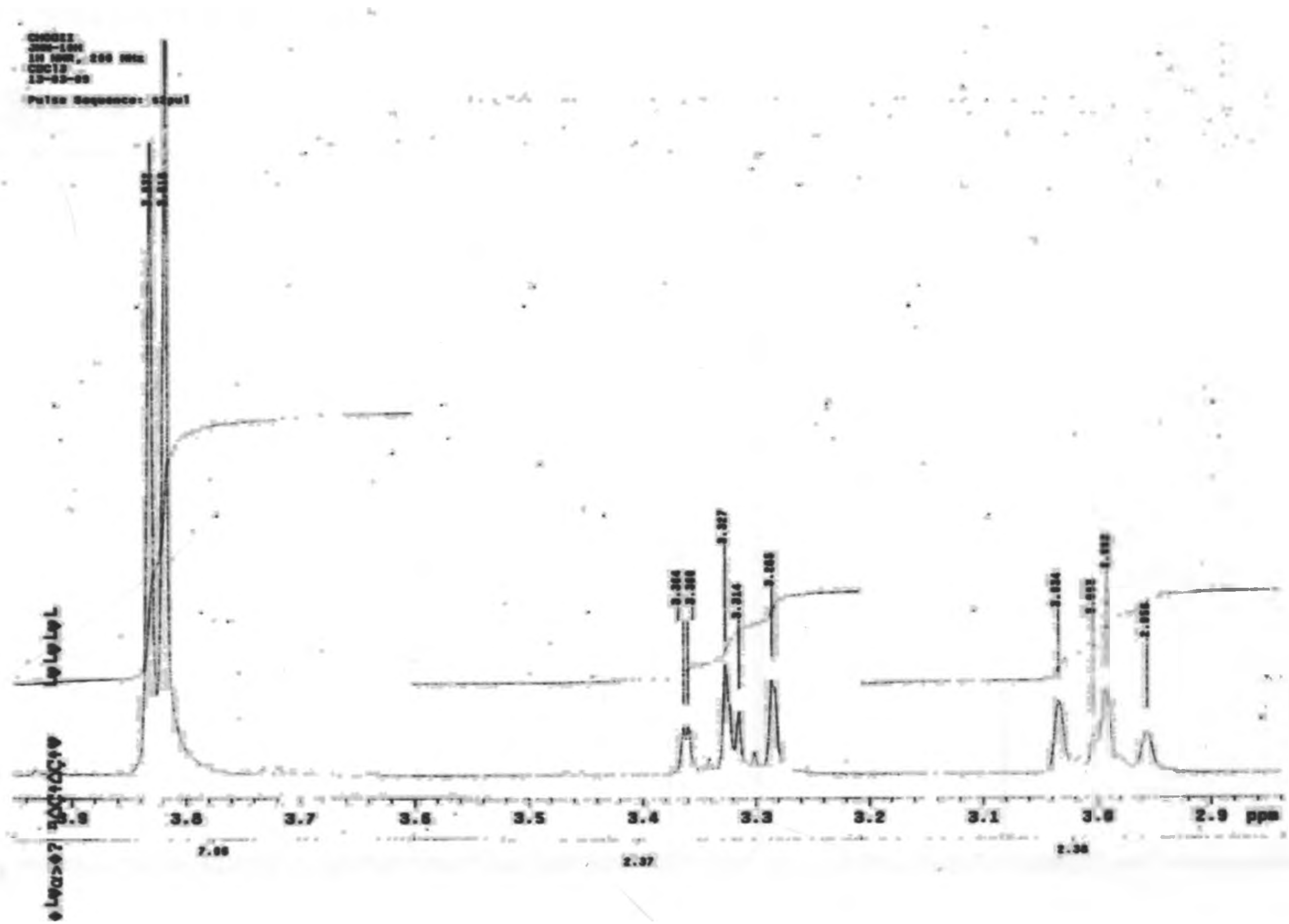
JMN6-H
DEPT
24/03/09



Appendix 10: Spectra for compound **10**

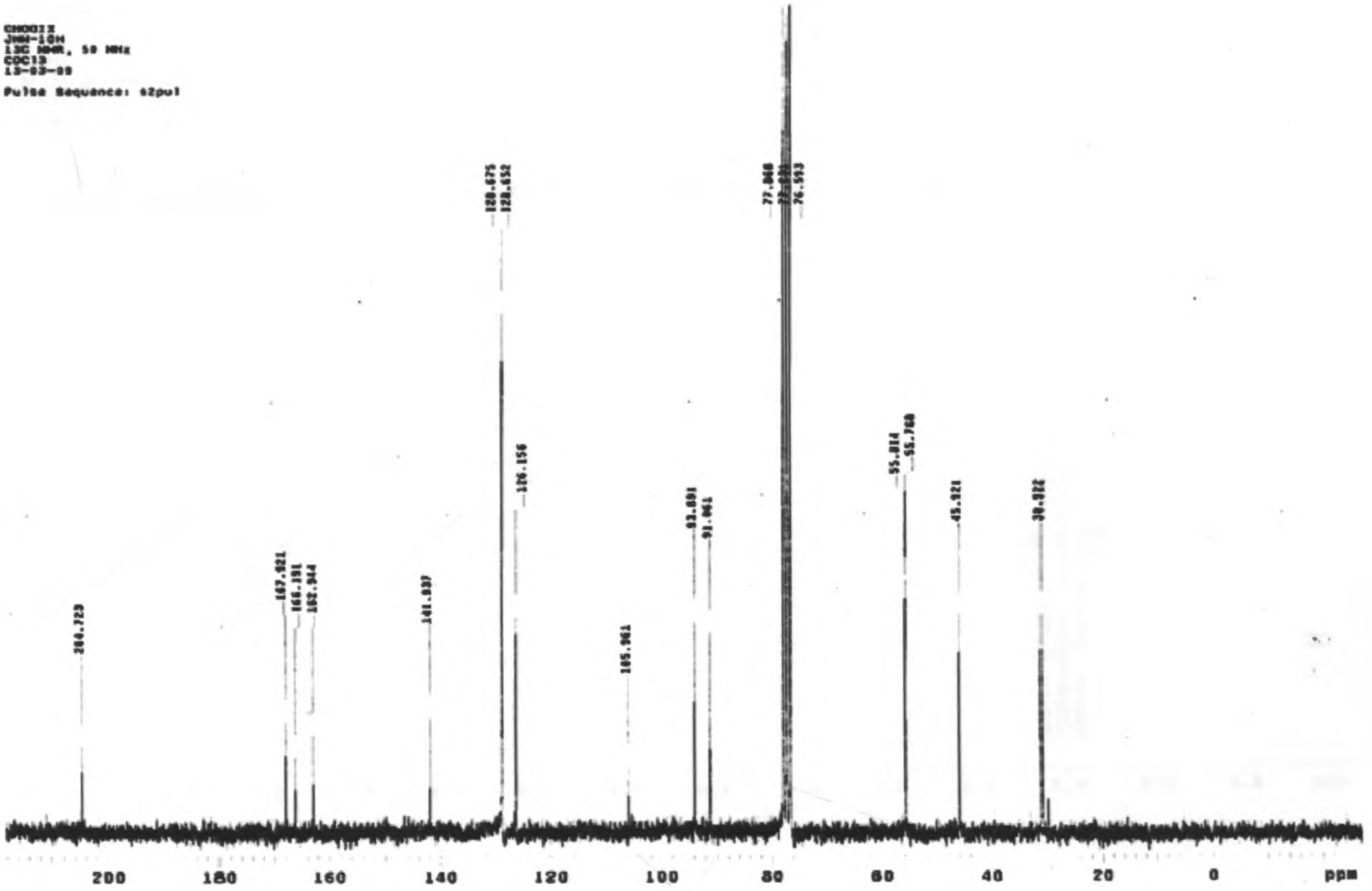
^{1}H SPECTRUM (200 MHz, $CDCl_3$)





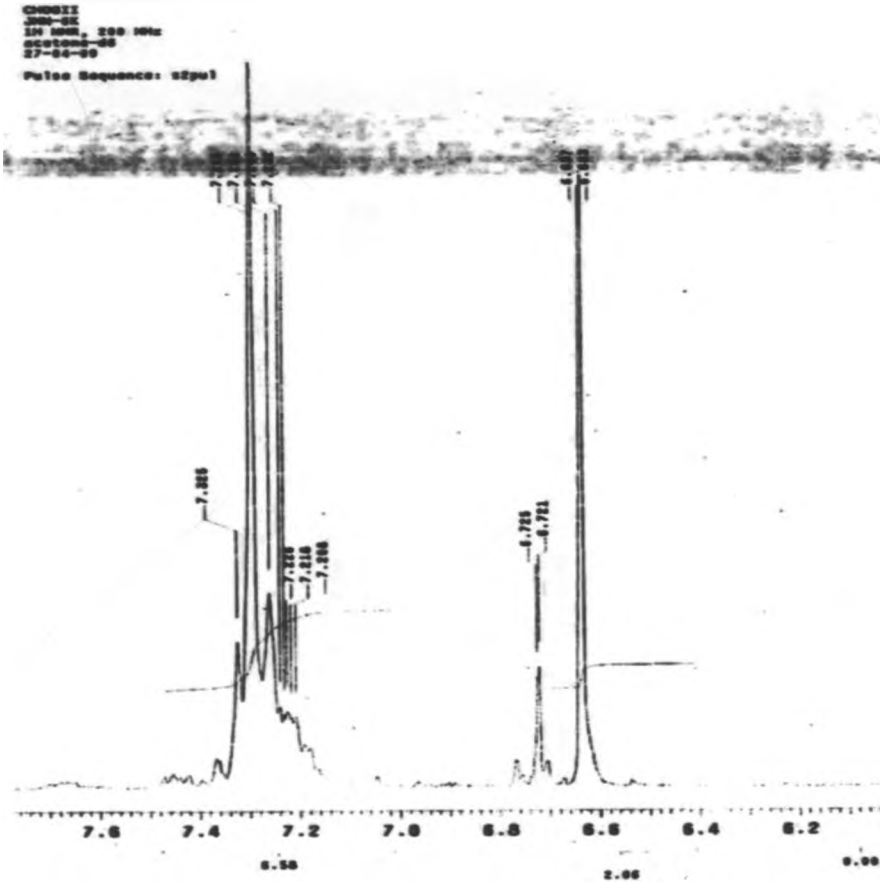
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3.0
2.9
ppm

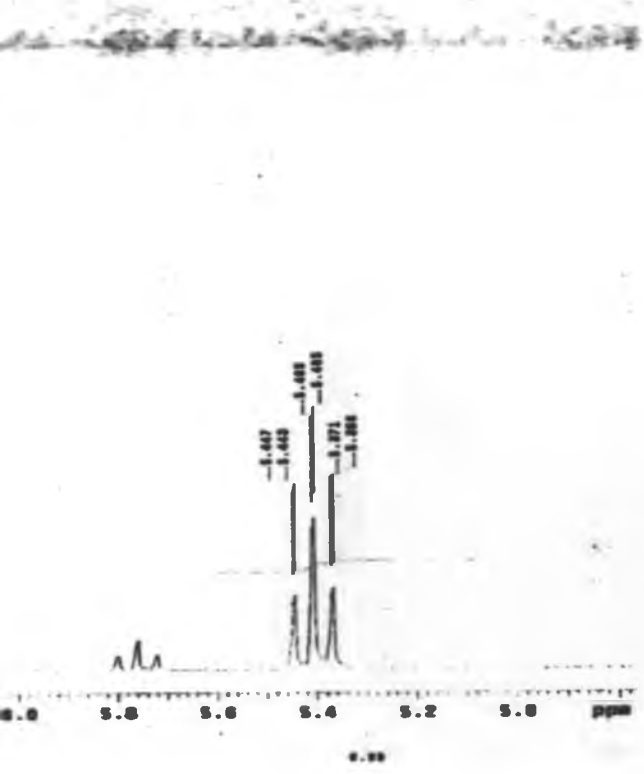
^{13}C NMR SPECTRUM (50 MHz, CDCl3)

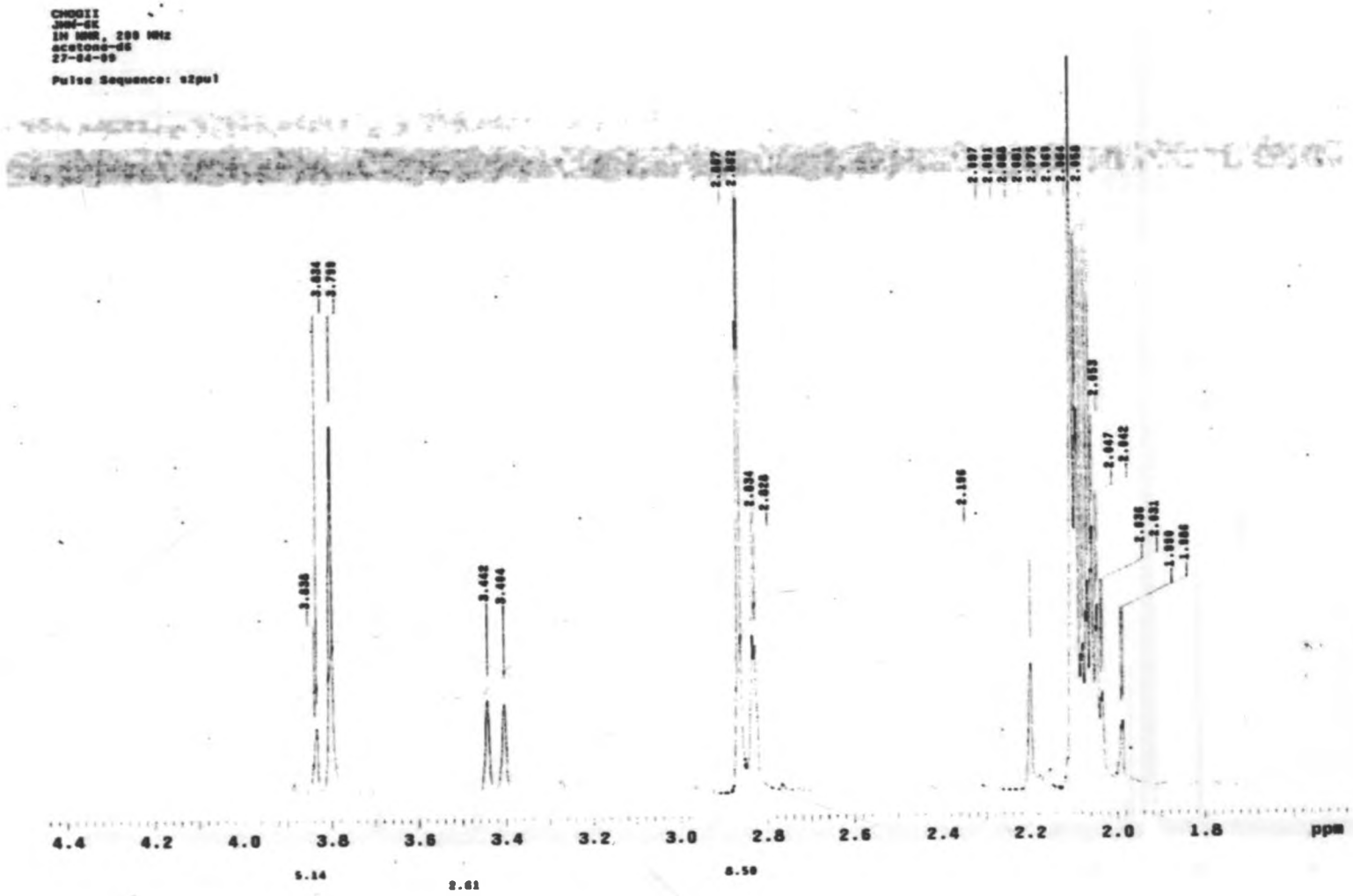


Appendix 11: Spectra for compound **11**

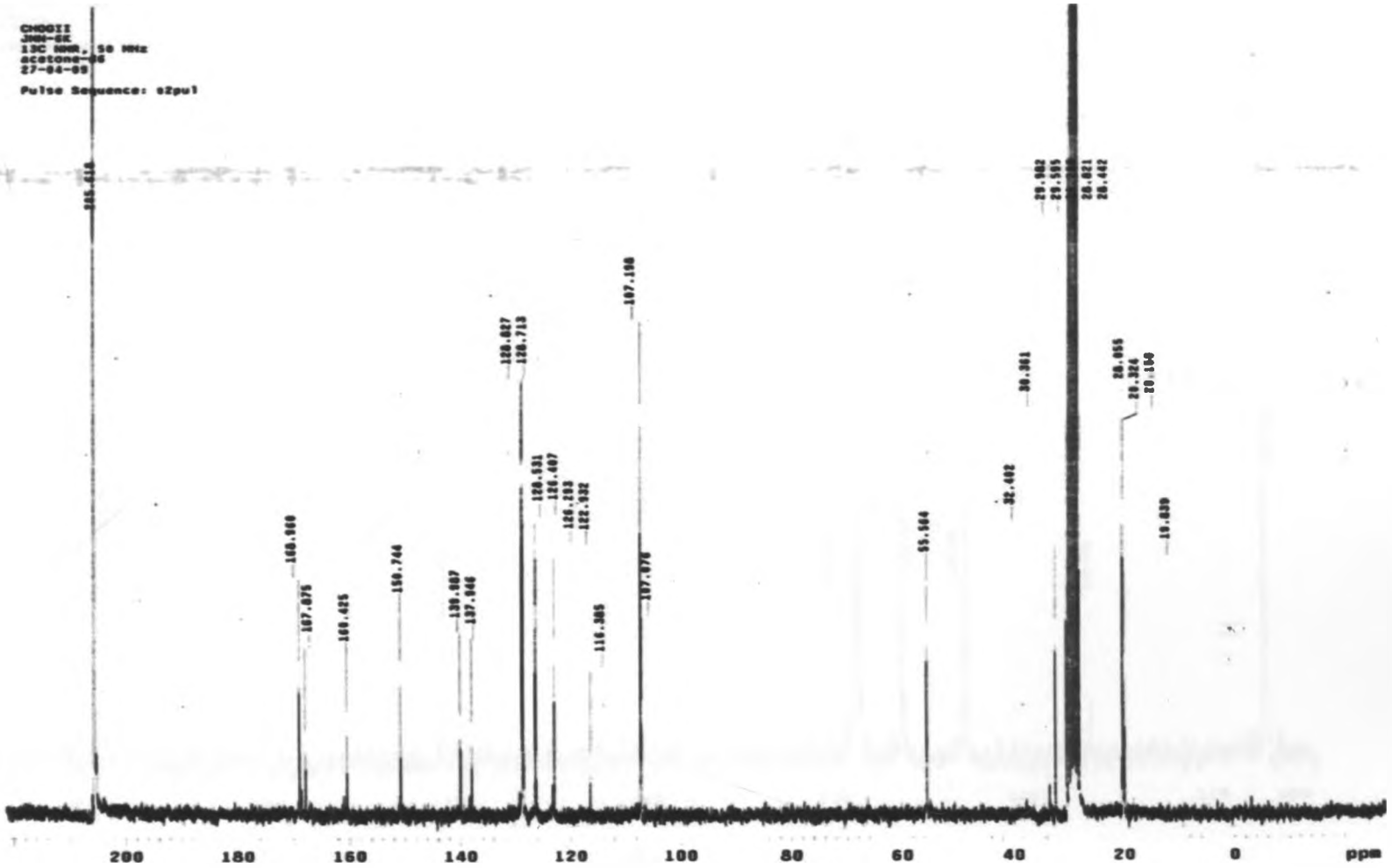
^{1}H NMR SPECTRUM (200 MHz, CDCl3)

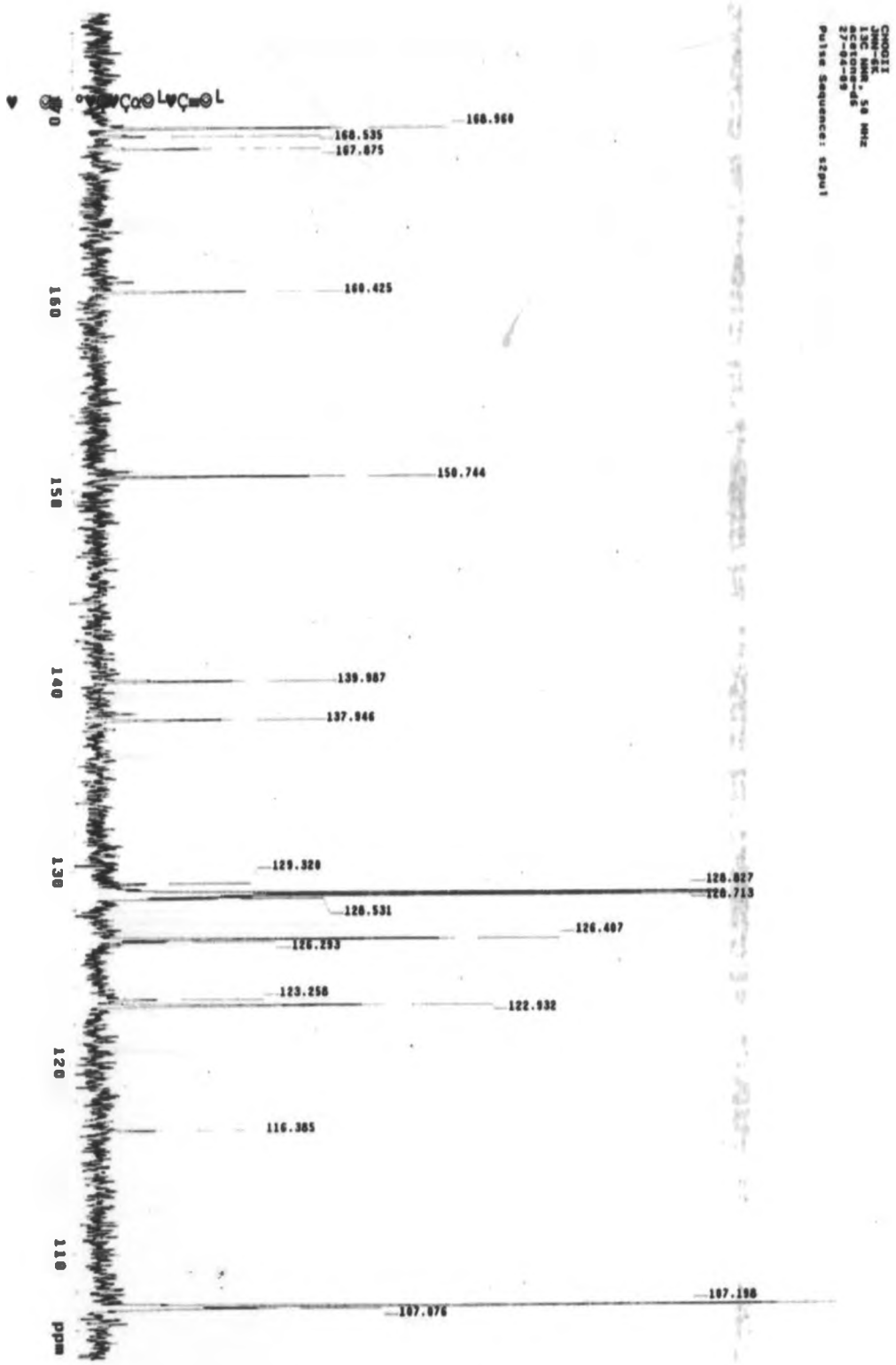



5.8
5.6
5.4
5.2
5.0
ppm


CH0DII
JNM-GX
1H NMR, 200 MHz
acetone-d6
27-04-99
Pulse Sequence: s2pul
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
ppm
5.14
2.61
6.50

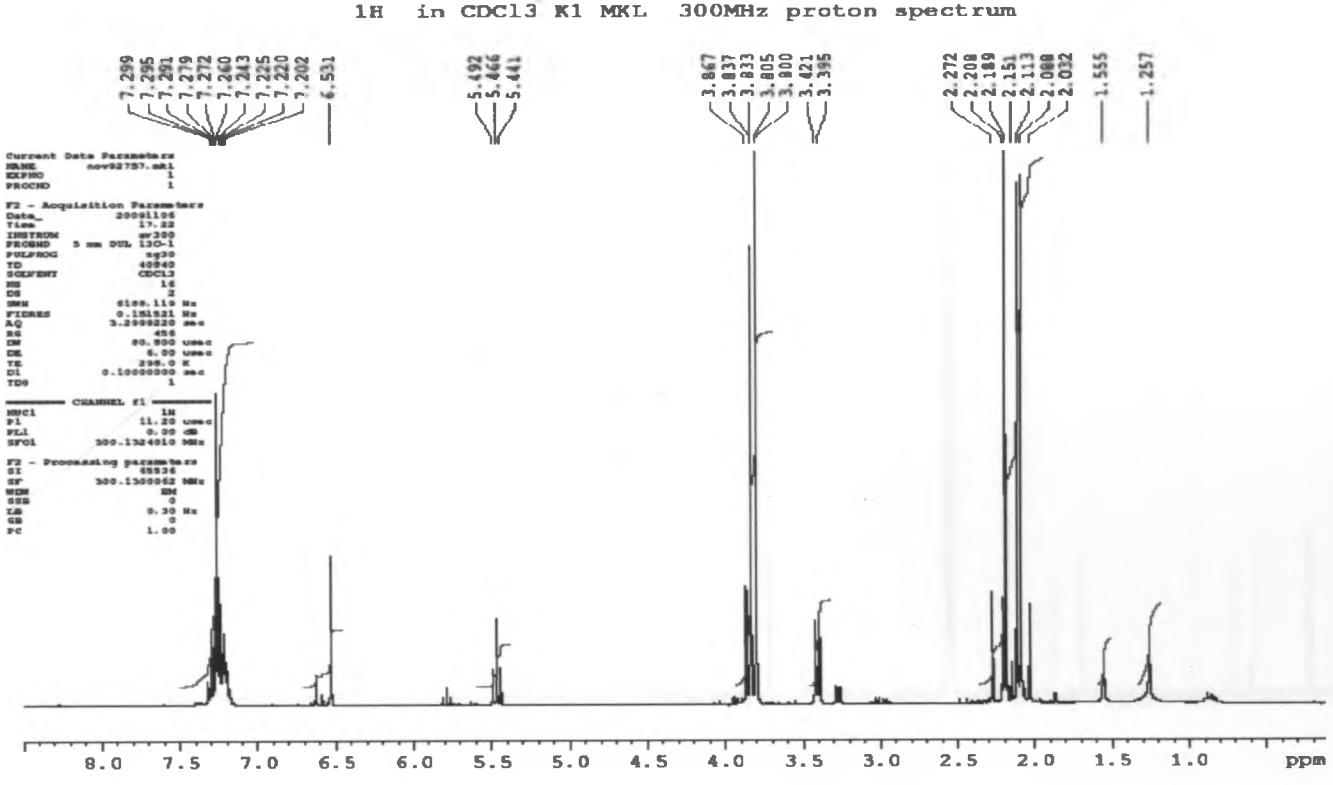
^{13}C NMR SPECTRUM (50 MHz, $CDCl_3$)



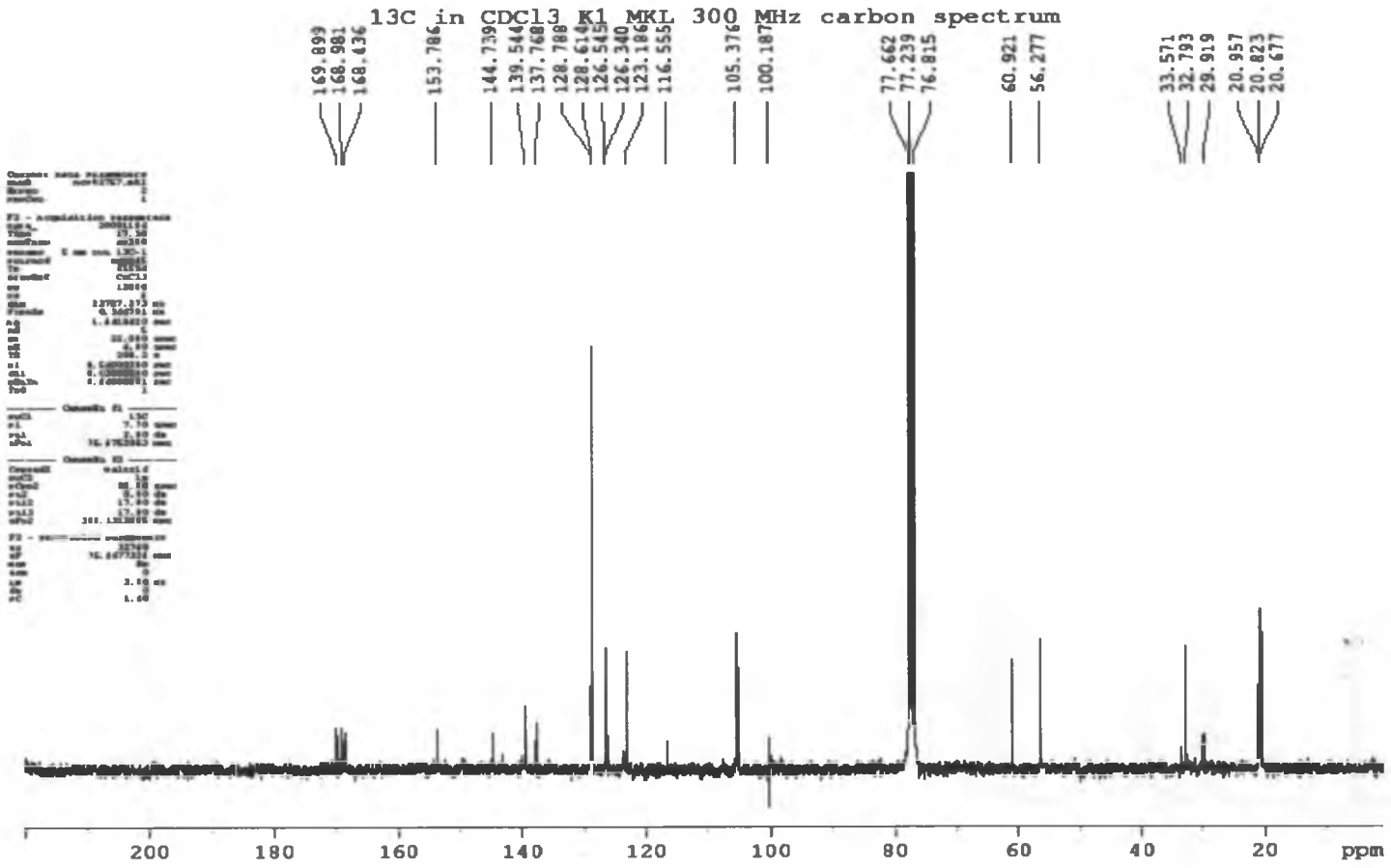

CHOOII
JNM-GSX
13C NMR, 50 MHz
acetone-d6
27-04-06
Pulse Sequence: s2pul
168.960
168.535
167.875
160.425
150.744
139.987
137.946
129.320
128.827
128.713
128.531
126.407
126.293
123.258
122.932
116.385
107.196
107.076
ppm

Appendix 12: Spectra for compound **12**

^{1}H NMR SPECTRUM (300 MHz, $CDCl_3$)

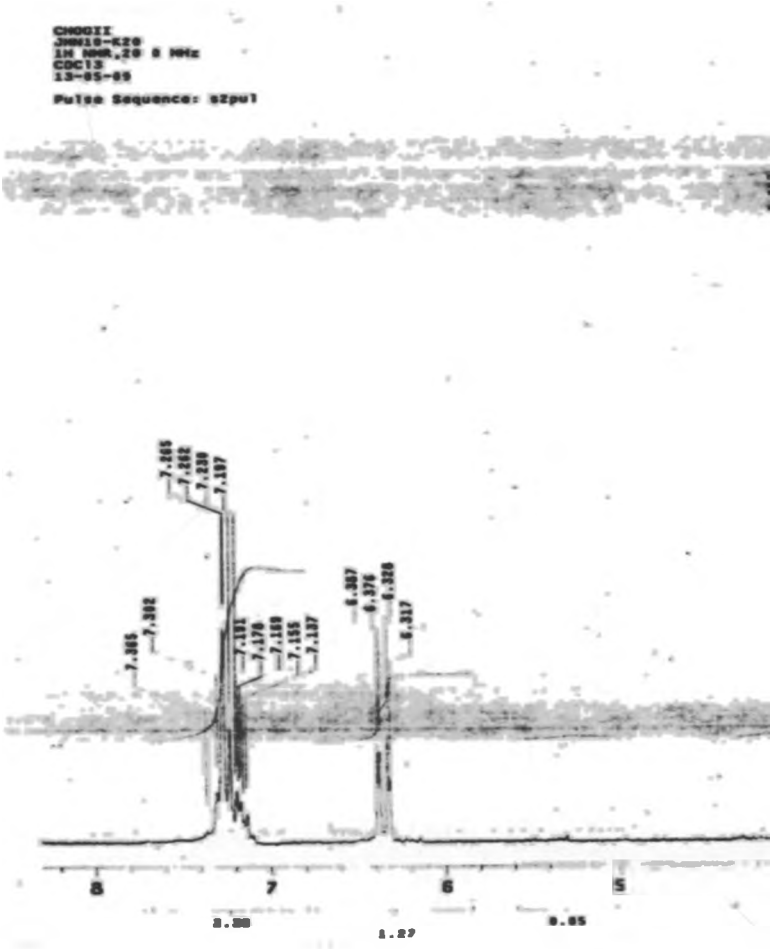


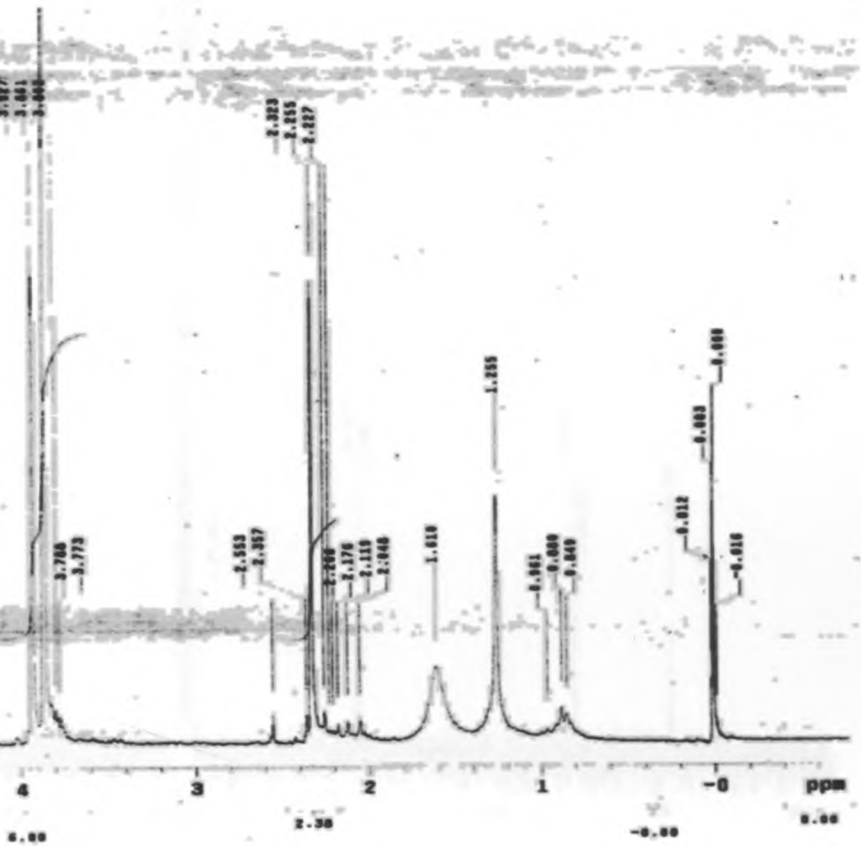
^{13}C NMR SPECTRUM (75 MHz, $CDCl_3$)



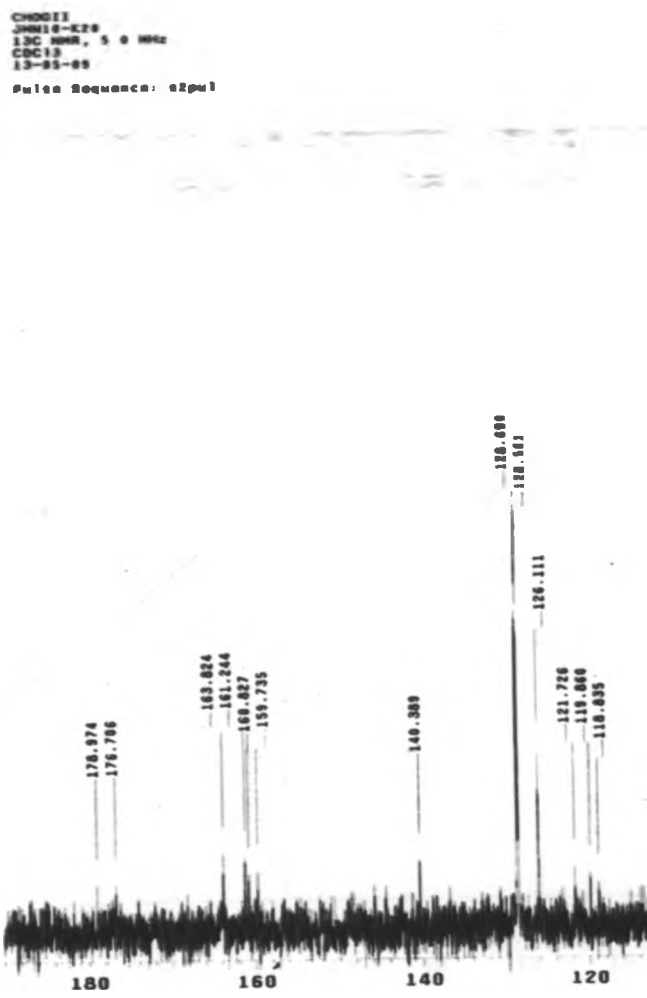
Appendix 13: Spectra for compound **13**

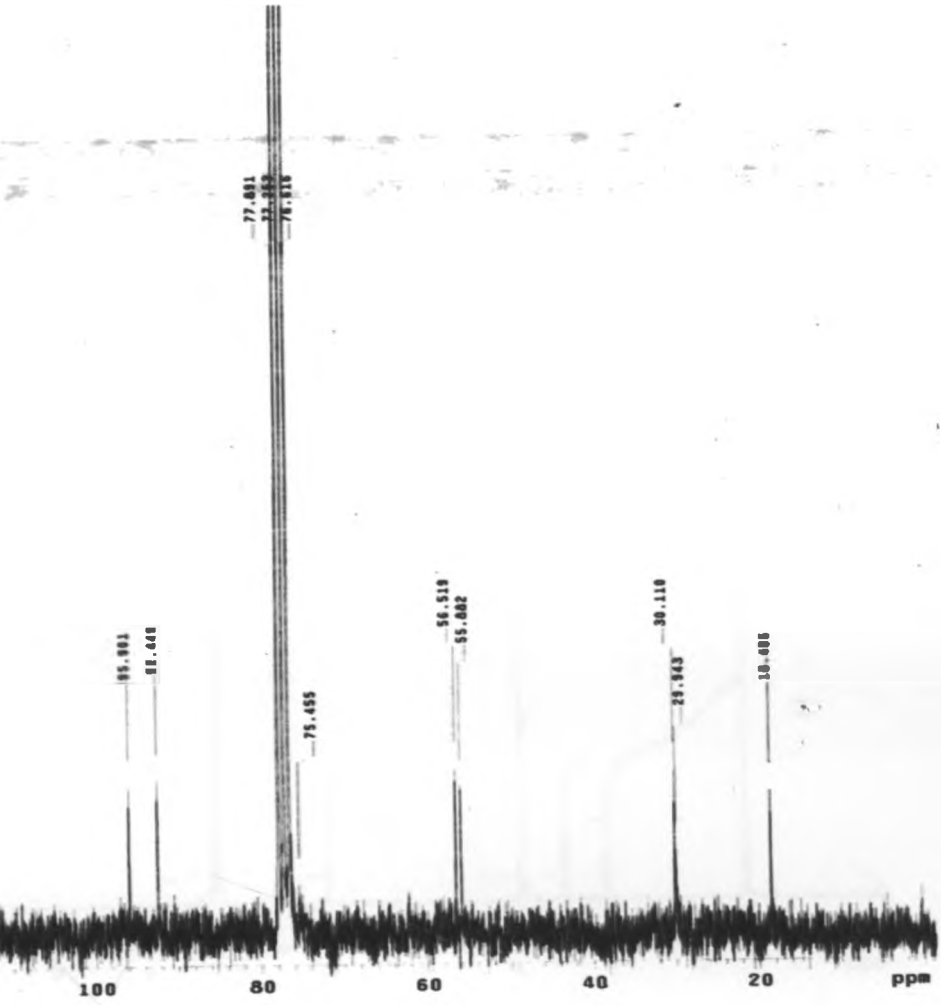
^{1}H NMR SPECTRUM (200 MHz, $CDCl_3$)




4
3
2
1
-0
ppm

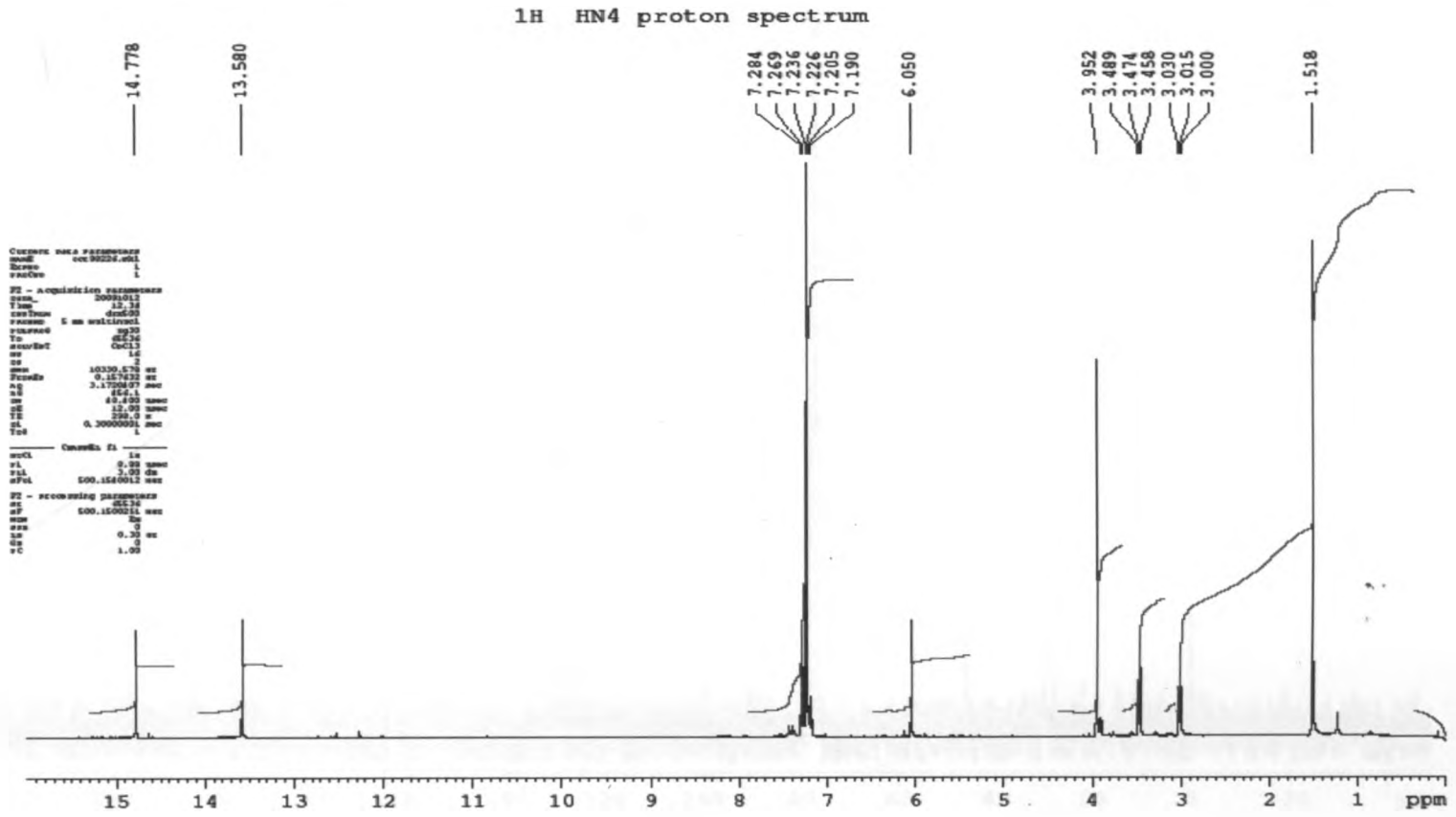
^{13}C NMR SPECTRUM (50 MHz, CDCl3)




77.401
75.455
56.519
55.882
30.110
29.943
18.405
100
80
60
40
20
ppm

Appendix 14: Spectra for compound **14**

^{1}H NMR SPECTRUM (500 MHz, $CDCl_3$)



^{13}C NMR SPECTRUM (125 MHz, $CDCl_3$)