PHYTOCHEMICAL INVESTIGATION OF *ZANTHOXYLUM HOLSTZIANUM* FOR ANTIPLSMODIAL, LARVICIDAL AND ANTINOCICEPTIVE PRINCIPLES

BY

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RESEARCH THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE REQUIREMENT OF THE AWARD OF THE DEGREE OF MASTERS OF SCIENCE IN CHEMISTRY OF UNIVERSITY OF NAIROBI

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DECLARATION

This research thesis is my original work and has never been presented for a degree in any higher institution of learning or University.

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THIS THESIS IS DEDICATED TO

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LIST OF ABBREVIATIONS AND SYMBOLS

brs	Broad singlet
CD	Circular dichorism
C C	Column chromatography
IC_{50}	Concentration of 50% inhibition
LC_{50}	Concentration of 50% lethality
COSY	Correlated spectroscopy
J	Coupling constant
CD_2Cl_2	Deutrated dichloromethane
$CDCl_3$	Deutrated trichloromethane
DMSO	Dimethylsulphoxide
DEPT	Distortionless enhanced polarization transfer
d	Doublet
dd	Doublet of doublet
EIMS	Electron ionization mass spectroscopy
GC/MS	Gas chromatography/Mass spectroscopy
HZ	Hertz
HMBC	Heteronuclear multiple bond correlation
HSQC	Heteronuclear single quantum coherence
m/z	Mass to charge ratio
MHz	Mega hertz
µg	Microgram
Mg	Milligram

$[M]^+$	Molecular ion
m	Multiplicity
NMR	Nuclear magnetic resonance
NOESY	Nuclear overhauser and exchange spectroscopy
PTLC	Preparative thin layer chromatography
s	Singlet
TLC	Thin layer chromatography
t	Tripplet
UV	Ultra violet
VLC	Vaccum liquid chromatography
WHO	World Health Organization

ABSTRACT

In this study, secondary metabolites from the root and stem bark of *Zanthoxylum holstzianum* were evaluated for antiplasmodial and mosquito larvicidal activities. In addition, the anti-nociceptive activities of the crude extract and some pure compounds were determined using tail flick method on animal model (mice).

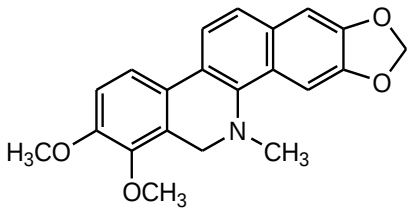
The air-dried and pulverized stem and root bark of *Z. holstzianum* was exhaustively extracted with CH_2Cl_2/MeOH (1:1) at room temperature. The stem bark extract was subjected to a combination of chromatographic techniques and resulted into isolation of eight compounds. These were five benzophenanthridine alkaloids; [8-acetonyldihydrochelerythrine **(3)**, nitidine **(6)**, dihydrochelerythine **(2)**, norchelerythrine **(2)**, arnottianamide **(7)]**; a 2-quinolone alkaloid [*N*-methylflindersine **(8)**]; a symmetrical lignan [4,4'-dihydroxy-3,3'-dimethoxylignan-9,9'-diyl diacetate **(10)**] and a *C-C* linked dimer of a benzophenanthridine and 2-quinoline [holstzianoquinoline **(9)]. ** Similarly, the root bark afforded eight compounds. These included five benzophenanthridine alkaloids [**1**, **2**, **3**, **7**, 9-demethyloxychelerythrine **(5)**]**,** a benzophenathridine and 2-quinoline dimer **(9)**, a symmetrical acetone bridged dimer of two benzophenanthridine alkaloids [chelerythridimerine **(4)**] and a 2-quinoline **(8).**

Overall, ten compounds were isolated from the stem and root bark of *Z. holstzianum*. Of these, compound **10** and **6** were only found in the stem bark while compounds **4** and **5** were only found in the root bark. Compounds **1**, **2**, **3**, **6**, **7**, **8** and **9** were found in both parts. Holstzianoquinoline (**9**) is a new compound and this is the second report of a *C-C* linked dimer of a benzophenanthridine and a 2-quinoline from nature. Compounds **4** and **10** are reported here for the first time from this genus. The identification of the isolated compounds was based on spectroscopic evidence, which included ^{1}H NMR, ^{13}C NMR, HMBC, HSQC, COSY, NOESY and MS.

The CH_2Cl_2/MeOH (1:1) stem bark extract (crude) and five isolates were tested for *in vitro* antiplasmodial activity against chloroquine resistant (W2) and chloroquine sensitive (D6), *Plasmodium falciparum* strains. The crude extract showed potent activity with IC_{50} values of 2.5 ± 0.3 and 2.6 ± 0.3 μgml^{-1} against W2 and D6 strains, respectively. Nitidine **(6)** showed potent activity with IC_{50} values of 0.11 ± 0.01 μgml^{-1} for both W2 and D6 while norchelerythrine **(2)** showed potent activity with IC_{50} value of 0.15 ± 0.01 μgml^{-1} against D6 strain but with moderate activity of 7.3 ±

0.3 μgml^{-1} against W2 strain. Dihydrochelerythrine **(1)** showed moderate activity of 3.8 ± 0.7 μgml^{-1} and 3.2 ± 0.8 μgml^{-1} and similarly 8-Acetonyldihydrochelerythrine with activity of 4.0 ± 0.2 and 3.8 ± 0.7 μgml^{-1} against W2 and D6 strains, respectively. *N*-methylflindersine (**8**) showed a weak activity against both W2 and D6.

Further, the crude extracts and some of the isolated compounds were tested for larvicidal activity against the early fourth instar mosquito larvae of *Aedes aegypti*. The root bark extract showed moderate activity (LC_{50} = 43.9 ± 5.89, LC_{90} = 61.0 ± 9.77 μgml^{-1} at 24 h and LC_{50} = 32.9 ± 3.66 and LC_{90} = 50.0 ± 9.12 μgml^{-1} at 48 h) while the stem bark extract showed weak activity. Two pure compounds showed high activity; *N*-methylflindersine (**8**) having a potent larvicidal activity (LC_{50} = 4.8 ± 0.3, LC_{90} = 6.9 ± 0.3 μgml^{-1} at 24 h and LC_{50} = 2.9 ± 0.3, LC_{90} = 5.13 ± 0.22 μgml^{-1} at 48 h) while 9-demethyloxychelerythrine (**5**) showed activity (LC_{50} = 12.6 ± 0.8, LC_{90} = 19.6 ± 1.5 μgml^{-1} at 24 h and LC_{50} = 6.5 ± 1.1, LC_{90} = 13.4 ± 1.87 μgml^{-1} at 48 h). Holstzianoquinoline (**9**) showed moderate activity while dihydrochelerythrine and 8-Acetonyldihydrochelerythrine had no activity at 20 μgml^{-1}. In addition, the crude extract (stem and root bark) showed statistically significant antinociceptive effect ($P<0.05$) at a dose of 100 and 200 mg kg^{-1} while *N*-methylflindersine, and dihydrochelerythrine showed extremely statistically significant antinociceptive effect ($p<0.0001$) at a dose of 100 and 50 mgkg^{-1}. Holstzianoquinoline showed extremely significant effect ($p<0.0001$) and highly significant effect ($p<0.001$) for 100 and 50 mgkg^{-1} dose.



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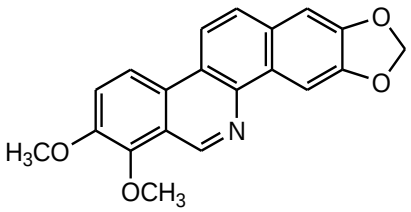

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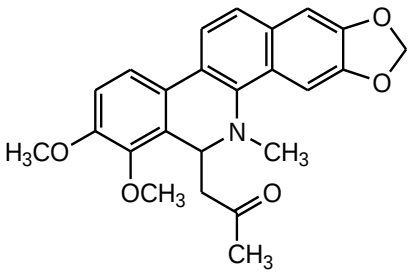

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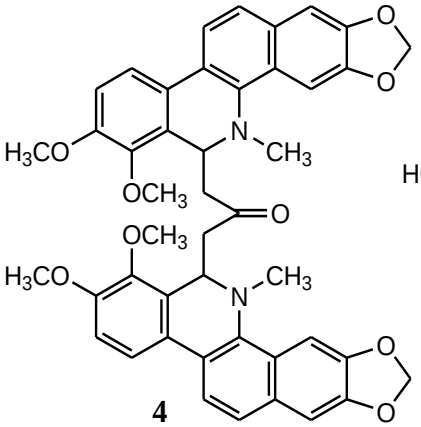

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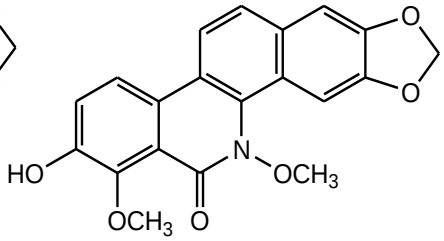

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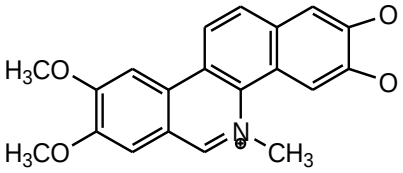

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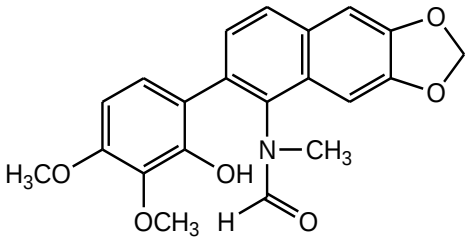

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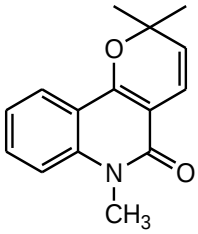

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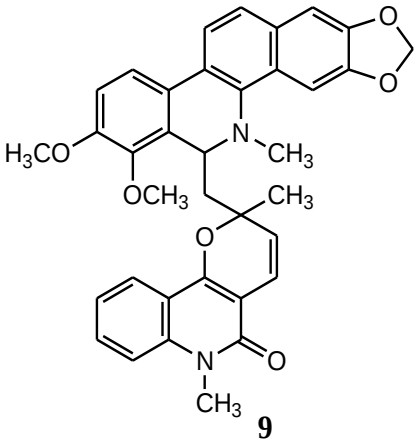

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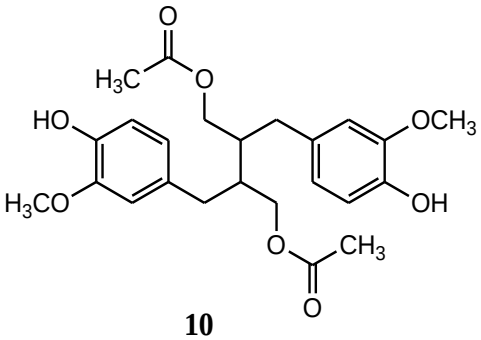

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CHAPTER ONE

INTRODUCTION

1.1 General

For thousands of years, natural products have played an important role throughout the world in treatement and prevention of diseases (Mouhseen, 2007). Plants, fungi, bacteria and animals produce natural products in attempt to defend themselves (Ji et al., 2009). To achieve this, organisms have for over millions of years evolved in synthesizing such protective chemicals that interact with specific proteins in their predator systems. Moreover, since the basic biochemistry of all living things is the same, these chemicals can interact with the same proteins in un intended targets including human beings, giving rise to the exploitation of such compounds for medicinal purposes (Ginsburg and Deharo, 2011). In fact, for over billions of years that bacteria have inhabited the earth, nature has been continually carrying out its own version of combinatorial chemistry (Zhang and Demian, 2005). This combinatorial chemistry practiced by nature proved to be more sophisticated than that in the laboratory, yielding interesting exotic structures rich in stereochemistry, concatenated rings and reactive functional groups (Zhang and Demain, 2005).

The direct role of nature in the primary health care is highly emphasized by remarkable use of herbal medicine by man (WHO, 2002). Historically the origin of herbal medicine is believed to be through observing which plants animals ate when they fell sick. Human beings through trial and error developed knowledge on useful plants, which became the world's indiginous folk medicine traditions. The fortunate chemists have frequently used this knowledge as a source of information for phytochemical investigation (Zhang and Demain, 2005).

Drug discovery and development has greatly benefited from sourcing compounds from nature. Infact between 1981 and 2002, 61% of the new chemical entities brought to the market can be traced

to, or were inspired by, natural sources (Newman et al., 2003); in this regard antimalarial drug discovery is a good example (Grimberg and Mehlotra, 2011). Molecules such as quinine, lapachol and artemisinin were originally isolated from herbal medicinal products. After improvement through medicinal chemistry and formulation technologies, as well as combination with other active ingredients, they now make the bulk of armametrium of medicines (Wells, 2011).

In the era where many mosquito vectors are increasingly developing resistance to insectcides and the lack of effective vaccine, chemoprophylaxis/chemotherapy remains the standard means of combating malaria (Grimberg and Mehlotra, 2011). Unfortunately, no new antimalarial chemical class has been introduced into clinical practice since 1996, despite the increasing trend of resistance of the malaria parasite strains to the newest drugs (Claudio and Lopes, 2011). This calls for an extra effort towards the discovery of new anti-malaria drugs as well as new larvicidal agents. In addition, since malaria has become a reality in the lives of human beings and is responsible for various forms of pain, it is also important to search for new anti-pain agents from nature.

The outstanding role of alkaloids in chemotherapy is well known. Quinine and its synthetic derivartives are the best examples (Achan et al., 2011). Some alkaloid producing plants such as *Zanthoxylum* have showed promising antiplasmodial activity in either crude form or as pure compounds (Jullian et al., 2006; Were et al., 2010). Further more, the crude and pure compounds from some *Zanthoxylum* species have showed interesting larvicidal activies (Ferdinand et al., 2011). Besides, the antiplasmodial and larvicical activities, *Zanthoxylum* species extracts and their pure alkaloid compounds show a range of biological activities including, antinociceptive, anticancer, anti-inflamatory, antioxdant and antimicrobial activities (Patino et al., 2012).

In Kenya, seven *Zanthoxylum* species have been recorded (Beentje, 1994). Despite the wide traditional uses of these plants, the phytochemical information on most of these species is scanty where available. Therefore, in this research, phytochemical investigation of *Zanthoxylum holstzianum* for secondary metabolites and for antiplasmodial, larvicidal and antinociceptive agents was conducted.

1.2 Problem statement

The continued emergence of anti-malarial drug resistance means that there will always be a need for new classes of lead structures to combat malaria (Wells, 2011). When the resistance to artemisinin drugs further develops and spreads, we will be caught un armed, without any effective weapon againsit this deadly parasite (Grimberg and Mehlotra, 2011). Mosquito vector resistance to the available insectcides especially in Africa has worsened the situation (Ranson et al., 2011). In addition, the limited number of insecticides available for malaria vector control has limited the options for effective insect resisitance management. Currently, WHO has approved only 4-classes of insectcides, which share two modes of action (Edi et al., 2012). Despite having these four classes, only one class (pyranthroids) is widely used. This adds fresh concerns about the problem of resistance to insecticides in malaria vectors. Notably, the use of pyrenthroids as larvicides is limited by their toxicity to non-target aquatic arganisms including fish (WHO, 2013). Generally, synthetic insectcides are not environmently friendly. This leaves a very big question to researchers as to which direction to follow.

1.3 Objectives of the study

The main objective of this study was to isolate and identify biologically active compounds from the stem and root bark of *Zanthoxylum holstzianum.*

Specific objectives;

i. To isolate secondary metabolites from the stem and root bark of *Zanthoxylum holstzianum* using a combination of chromatographic techniques.

ii. To elucidate chemical structures of the isolates using various spectroscopic methods

iii. To establish the *in vitro* antiplasmodial, larvicidal and anti-nociceptive activities of the crude and pure compounds.

1.4 Justification

There is a consensus among the scientific community that natural products have been playing a dorminant role in the discovery of leads for the development of drugs for the treatment of human diseases (Newman et al., 2003). Truly, the vast majority of the existing chemotherapeutic agents are based on natural products, which anticipates that new leads may certainly emerge from the tropical plant sources. This is because biological chemodiversity continues to be an important source of molecular templates in the search for new antimalarial drugs (Batesta, 2009).

Nature has not forgotten the discovery of insectcides and larvicides. By 1920's, the application of phytochemicals in mosquito control were in use. Furthermore, several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and other phenolics from different parts of plants have been reported for insecticidal activities (Anupam et al., 2012). Several *Zanthoxylum species* have not missed on the list of the plants with several important biological activities.

Some of the biological activities of the crude or pure compounds from *Zanthoxylum* genus include; antiparasitary, antinociceptive, larvicidal, antioxidant, anti-microbial, antihelmitic antiviral, ant-inflamatory, anti-cancer and antiplatelet (Patino et al., 2011). It is however, notable that there is no significant phytochemical and biological activity report on *Zanthoxylum holstzianum* for any of the

above actvivities. Therefore, the phytochemical investigation of the root and stem bark of *Zanthoxylum holstzianum* for antiplasmodial, larvicidal and antinociceptive activities was carried out.

CHAPTER TWO
LITERATURE REVIEW

2.1 The malaria burden

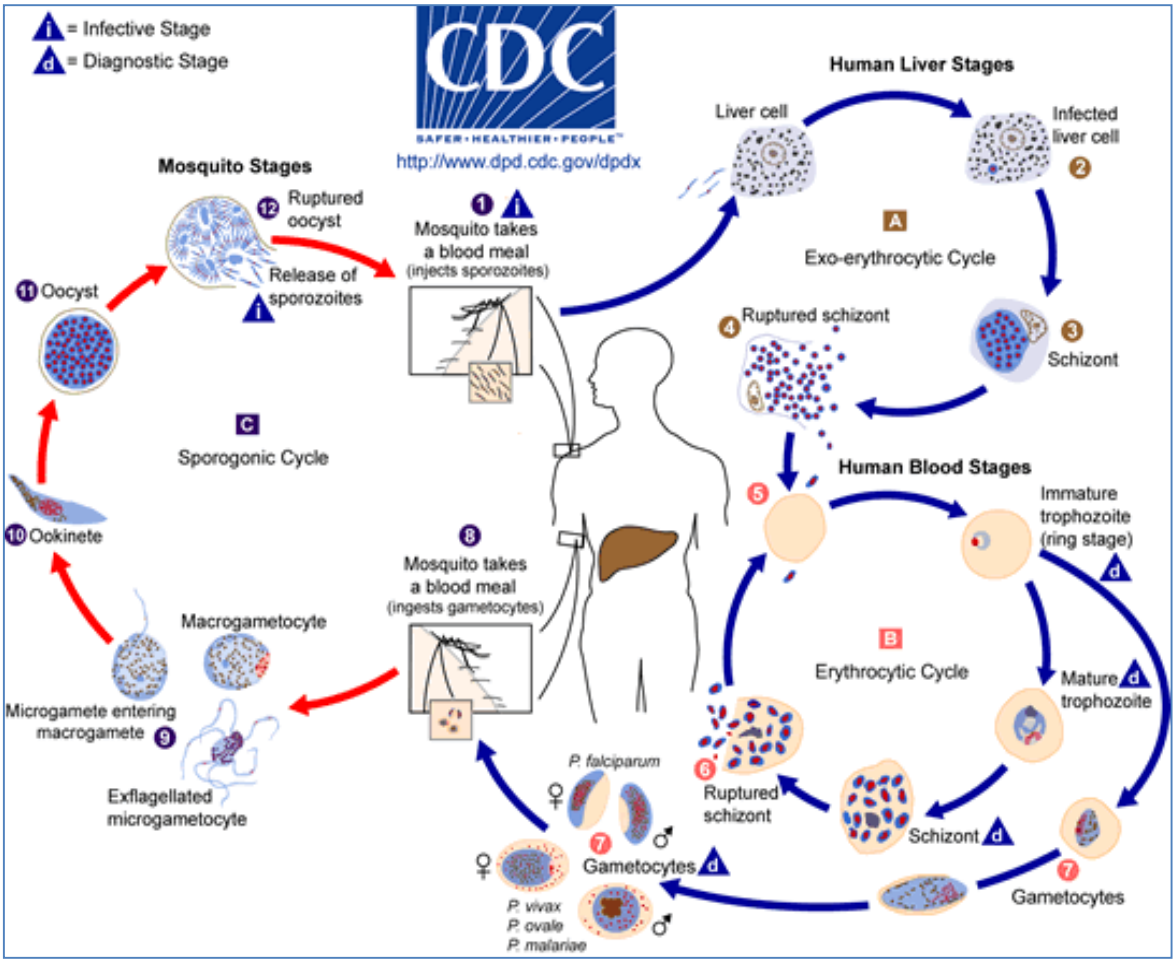
Malaria is a mosquito-borne, life threatening infectious disease caused by unicellular protozoan parasites of the genus *Plasmodium* (Mojab, 2012; Mehlotra, 2011). *Plasmodium* belongs to the Phylum Apicomplexa, a large group of eukaryotic microorganisms possessing a unique organelle called the apicoplast, and a complex structure termed as apical complex that is involved in host cell invasion (Sebisubi and Tan, 2010). A bite of infected female *Anopheles gambiae* mosquito, one of more than 70 *Anophelese* species is solely responsible for the transmission of malaria parasites from infected to healthy human beings. *Plasmodium falcipalum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* are the five *Plasmodium species* that are pathogenic to human beings (WHO, 2012).

It is important to note that malaria caused by *P. falciparum* is the most deadly and, unfortunately, it is the one that pre-dominates in Africa (WHO., 2012), a continent where the majority live in poverty. WHO (2012) reports that approximately 80% of malaria cases and 90% of death globally occur in sub-Saharan Africa. Less dangerous but still widely spread is malaria caused by *P. vivax*, while malaria from the other three species is less frequently encountered (WHO, 2012; Mojab, 2012). Despite malaria being associated with poverty, it is also a direct cause of poverty through loss of energy and work capacity. This ultimately contributes to the economic deterioration in the poorest countries more than any other human parasitic disease (Batista et al., 2009). The immunocompromised pregnant women and children under five years are always the victims of severe complications of malaria infection and disease (Rogerson, 2010; Schantz-Dunn and Nour, 2009).

2.2 The life cycle of malaria parasite

An infected female anopheles mosquito injects sporozoites into the human being. The sporozoites migrate to the liver, where they form merozoites. Merozoites are then released to invade red blood cells (RBC). In RBC, they become trophozoites. The trophozoites multiply, producing new merozoites and when the RBC raptures, the merozoites are released, infecting other RBCs. Some merozoites mature into gametocytes that are picked up by female mosquito from an infected human. The sexual cycle occurs in the mosquito, where sporozoites are formed (Batista et al., 2009)

Figure 2.1: The life cycle of malaria parasite



Adapted from centers for disease control and prevention. CDC 24/7: saving lives. Protecting people.

2.3 Antimalarial drugs

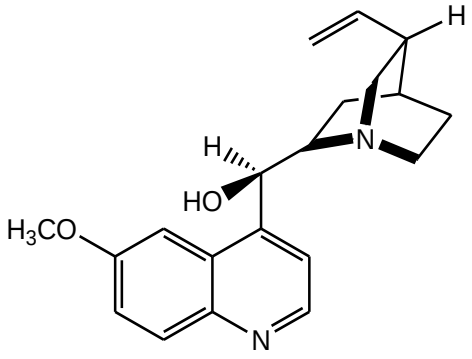
There are several classes of antimalarial drugs, which include quinoline containing compounds, antifoliates and artemisinins. In the next sections, they are discussed in detail.

2.3.1 Quinoline containing compounds

These contain the quinoline Skeleton. Some of the quinoline antimalarials are discussed below**.**

2.3.1.1 Quinine

Quinine (**11**) was isolated from the stem bark of Cinchona in 1820 by Caventuo and Pelletier (Razdan, 2010; Wells, 2011). It is the main alkaloid from Cinchona stem bark that contains about 20 alkaloids. *Cinchona* species are well known for their antimalarial properties, the active principle being quinine (**11)** which is still acknowledged as an effective drug. The less widely known stereoisomer quinidine, which also occur in this genus, is believed to be as potent if not more potent than quinine (White, 1985).



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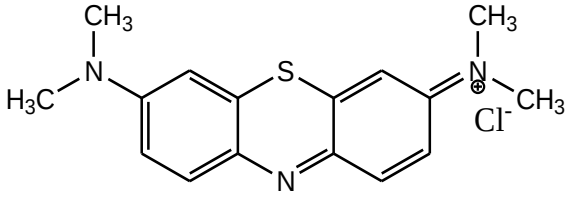
For a long time quinine was the only available drug ("single soldier") against malaria. Its economic importance rose substantially in the 19th century (Carmargo et al., 2009). Several attempts to synthesize quinine were un successful until 1944 when an efficient method was developed (Woodward and Doering., 1944). Despite quinine being used for many centuries, its high toxicity

and the fact that it has to be administered several times in a day, due to its short pharmacological half life (Camargo et al., 2009), triggered the need for suitable substitutes. Guttmann and Ehrlich (1891) brought hope to the human race by reporting the antimalarial properties of the synthetic compound methylene blue **(12)** (Camargo, 2005; Wells, 2011)



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2.3.1.2 Mode of action of quinine

Quinine is a rapidly acting, highly effective antimalarial drug against the blood schizonticide of *P. falciparum* parasite. It is also gametocidal against *P. vivax* and *P. ovale.* However, the drug is not effective against liver stage parasites, as it principally acts on mature trophozite stage of parasite development (Achan et al., 2011; Schlesinger et al., 1988). Although the mechanism of action of quinine and other quinolines has not yet been fully resolved, the widely held hypothesis involves the inhibition of the parasite heme detoxification (Sebisubi and Tan, 2010).

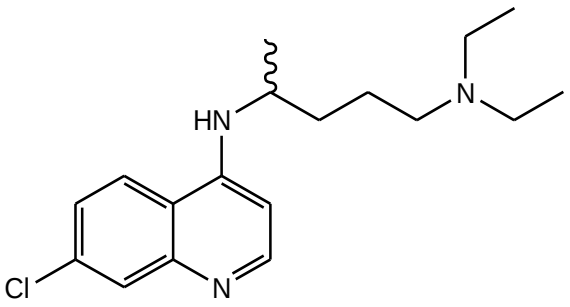
During the *intra* erythrocytic stage, *P. falciparum* proteolytically catabolises hemoglobin as a source of essential amino acid in an acidic and oxygen-rich lysosome-like digestive vacuole (Sullivan, 2002); from this process an enormous amount of free heme (ferroprotoporphyrin IX) is released (Bray et al., 2005). The free heme released is toxic to both the erythrocyte and the parasite by inducing the formation of the reactive oxygen species. Heme is oxidized to alpha-hematin (ferriprotoporphyrin IX) and subsequently polymerized to hemozoin, which is a non-toxic malaria pigment in the parasite food vacuole *via* the process called “bio-crystallisation”. Essentially

hemozoin is an ordered arrangement of dimeric heme units called beta- hematin (Sebisubi and Tan, 2010; Bohorquez, 2012).

The mono-protic basic nature of quinine facilitates its accumulation within the acidic vacuole by an ion trapping mechanism (Sebisubi and Tan, 2010). The protonable quinoline and terminal nitrogen atoms are important for the uptake and accumulation of the drug in the food vacuole (Sebisubi and Tan, 2010; Homewood et al., 1972). The protonated compound binds to heamatin and/or hemazon) within the food vacuole which inhibits the spontaneous process of crystal formation consequently leading to the death of the intraerythrocytic malaria parasite (Macomber and Sprinz, 1967; Chou et al., 1980). The death of the malaria parasite results from the quinine-heme complex formed which is toxic to it, just as free haeme is (Zhang et al., 1999). Trevor et al. (2007) hypothesized that the mechanism of action of quinine involves the binding of the drug to the double stranded DNA, resulting in the inhibition of DNA replication and the RNA transcription, consequently killing the malaria parasite.

2.3.1.3 Chloroquine

Chloroquine (CQ) (**14)** was first synthesized in 1934 and became the most widely used antimalarial drug by 1940's (Loeb, 1946). Its success is largely attributed to its excellent clinical efficacy, limited host toxicity, ease of use and the simple cost-effective synthesis. Most importantly, CQ treatment has always been affordable, a single dose costing as little as USD 0.10 in Africa (Wells and Polls, 2010). However, the value of quinoline-based antimalarials has seriously been eroded in recent years due to the development and spread of resistant parasite strains (Winstanley, 2002).



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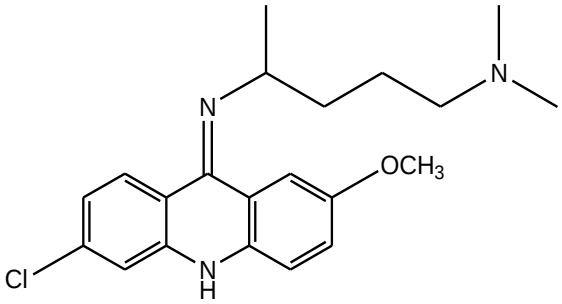
2.3.1.4 Mode of action of chloroquine (CQ)

CQ is active against the erythrocytic stages of malaria parasites but not against pre-erythrocytic or hypnozoite-stage parasites in the liver or mature gametocytes (Peters, 1970). Since CQ acts exclusively against those stages of the intra-erythrocytic cycle, during which the parasite is actively degrading haemoglobin, it was assumed that CQ somehow interferes with the parasite-feeding process. Like quinine, the 4-aminoquinoline chloroquine appears to act by blocking the formation of hemozoin from heme molecules once they are liberated from hemoglobin (Sullivan, 2002).

The ion-trapping or weak base mechanism and active transport explains how the drug finds itself in the acidic food vacuole of the parasite (Hawley et al., 1996; Geary et al., 1990; Walterkins et al., 2000). Since chloroquine is a diprotic weak base (P_{Ka1}=8.1, P_{Ka2}=10.2), it diffuses in its un protonated form through the membrane of the parasitized erythrocyte and accumulates in the acidic food vacuole (pH=5-5.2) (Hawley et al., 1996). It is then protonated making its membrane impermeable hence being trapped in the acidic compartment. CQ then forms a complex with Haeme (FP Fe (II) and / or the hyroxo or aqua complex of haematin (ferriprotoporphyrin IX, Fe III, FP), derived from parasite catabolism of the host haemoglobin (Adams et al., 1996). This blocks further sequestration of toxic heme, disrupting membrane functioning. Consequently, the parasite dies due to the accumulation of toxic heme in the membrane (Walterkins et al., 2000).

2.3.1.5 Other 4-aminoquinolines

The 4-aminoquinolines include quinacrine **(15)**, hydroxychloroquine **(16)**, sontoquine **(17)**, amodiaquine **(18)** and amopyroquine **(19)** (Razdan, 2010). Like in chloroquine, their activity is inclined to the asexual blood forms of all *Plasmodia* species that cause human malaria. They are not active against exoerythrocytic forms hence do not prevent the establishment of malaria (Razdan, 2010). Their mode of action is related to that of chloroquine as described above.



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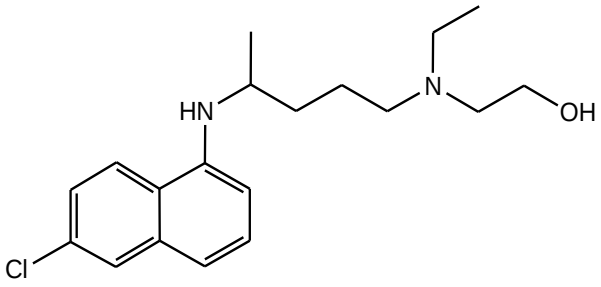

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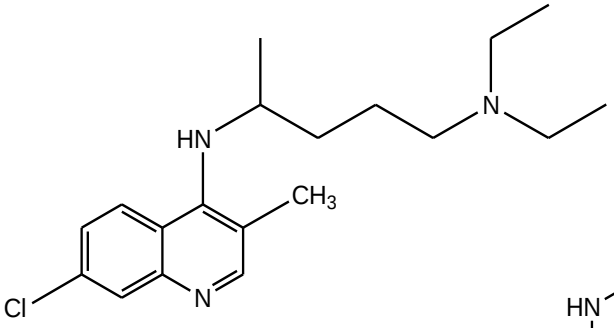

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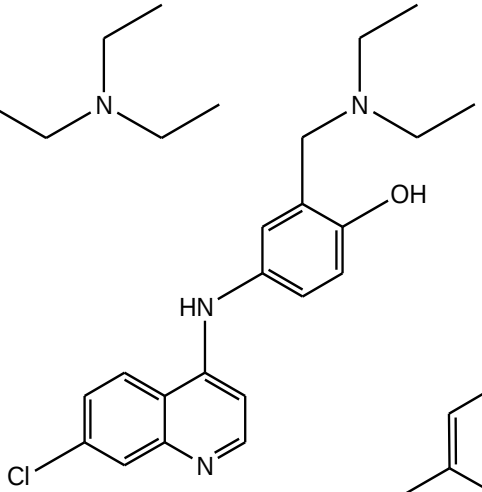

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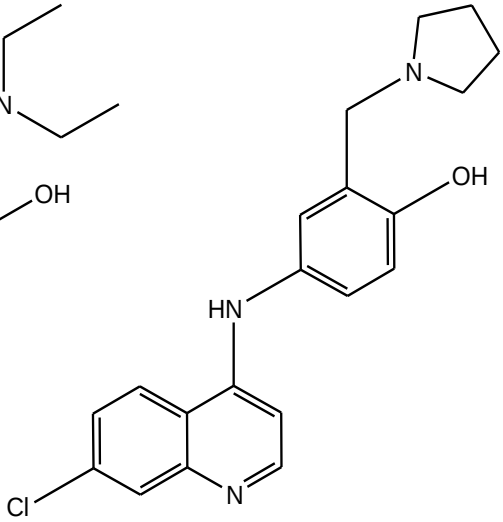

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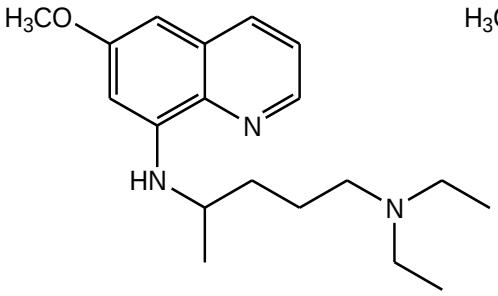
2.3.1.6 8-Aminoquinoline drugs

The 8-aminoquinolines include methylene blue **(12)**, pamaquine **(20)**, pentaquine **(21)**, isopentaquine (**22)**, and primaquine **(23)**. These are highly active against primary exo-erythrocytic forms of *P. vivax*, *P. falciparum* and gametocytes of all four species of malaria and tissue schizonts (Razdan, 2010). Among them primaquine is the most cherished. The mode of action is by generation of toxic metabolites and oxygen radicals in plasmodial mitochondria (Razdan, 2010).



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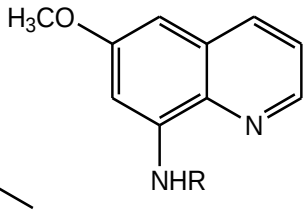
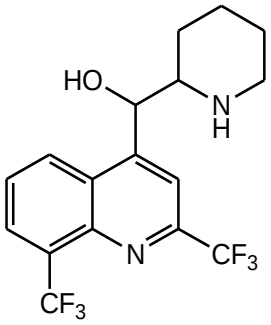


	R
21:	$-CH_2(CH_2)_4NHCH(CH_3)_2$
22:	$-CH(CH_2)_3NHCH(CH_3)_2$ $\vert$ CH_3
23:	$-CH(CH_2)_3NH_2$ $\vert$ CH_3

2.3.1.7 Quinoline methanols

The quinoline methanol antimalarials includes mefloquine (**26)** and halofantrine (**27)** (Foley and Tilley, 1998). Mefloquine was developed in 1970's by United State army in response to the increasing poor cure rates of chloroquine with its clinical trials starting in 1972 (Rieckmann et al., 1974). Nearly the same time, halofantrine was also developed (Saifi et al., 2013). The main target of mefaloquine is the blood stage schizonticide and its mode of action is through formation of toxic substance and swelling of the food vacuole resulting into the death of the parasite whereas halofantrine is an erythrocytic schizonticide that acts by inhibiting heme polymerase as well as vacuolar degredation (Razdan, 2010).



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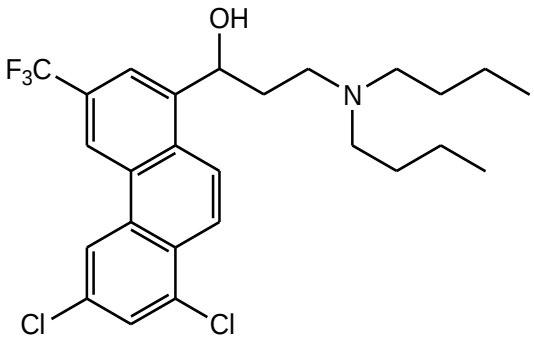

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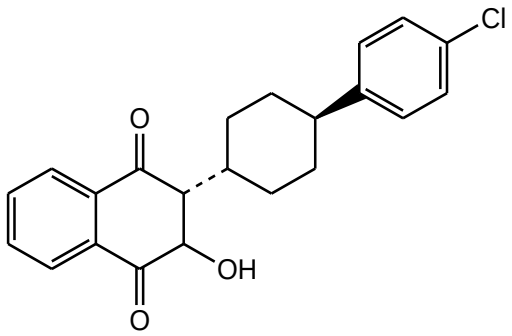
2.3.1.8 Hydroxynapthoquinones

Atovaquone **(24)** is the first effective antimalarial compound in this class and its antimalarial activity was discovered during World War II. Currently it is marketed under the trade name Malarone that contains a fixed combination of atovaquone and proguanil (Saifi et al., 2013).



24

2.3.1.9 Mode of action of atovaquone

Atovaquone {2-[trans-4-(4-chlorophenyl) cyclohexyl]-3-hydroxy 1,4-naphthoquinone}, hydroxyl naphthoquinone is used for both treatment and prevention of malaria in a fixed combination with proguanil. It is however not clear how it works in synergy with proguanil. Generally, it is agreed that atovaquone acts on the mitochondrial electron transfer chain inhibiting cytochrome C reductase activity in *P. falcparum* (Rudrapal, 2011; Fry and Pudney, 1992). The ubiquinone analogue, atovaquone binds on cytochrome BC1 complex of the parasite mitochondria electron transport

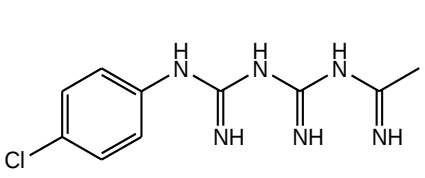
chain. The malaria mitochondria electron transport chain disposes off electrons generated by dihydroororate dihydrogenase during the synthesis of pyrimidines (Saifi et al., 2013). It has ben suggested that this inhibition process may kill the parasite (Hammond et al., 1985).

2.3.2 Antifolate drugs

These are antimalarial drugs that belong to the foliate antagonist class; however, their role in malaria control is hampered by rapid emergence of resistance under drug pressure (Plowe et al., 1998). They are blood stage schizonticide (Rudrapal, 2011) and their activity is exerted at all growing stages of sexual erythrocytic cycle and on young gametocytes (Saifi et al., 2013). Antifoliates can be classified into two;

i. Type I antifoliates which include sulfonamides and sulfones for example sulphadoxine (**29)** that mimic p-amino benzoic acid (PABA). They prevent the formation of dihydropteroate from hydroxymethyldihydropterin catalysed by dihydropteroate synthase (DHPS) by competing for the active site of DHPS which is a bifunctional enzyme in plasmodia coupled with 2-amino-4-hydroxy-6-hydroxy methyl-dihydropteridine pyrophosphokinase (PPPK) (Saifi et al., 2013)

ii. Type II antifoliates include pyrimethamine (**28)**, biguanides (proquinil **25**) and triazine metabolites as well as quinazolines. They inhibit dihydrofolate reductase (DHFR) which is a bifunctional enzyme in plasmodia coupled with thymidylate synthase (TS), thus preventing the NADPH-dependent reduction of H2 folate (DHF) to H4 folate (THF) by DHFR (Saifi et al., 2013).



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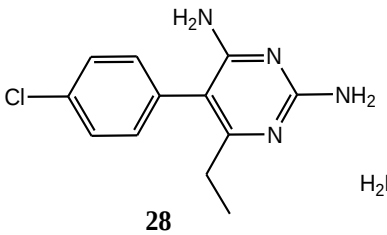

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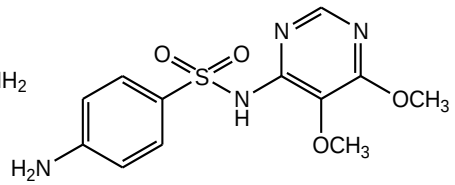


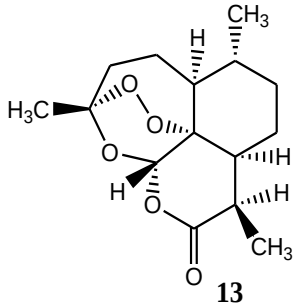
29

2.3.2.1 Mode of action of antifolates

The antifolates act by either inhibiting DHFR or DHPS, which are two key enzymes in the folate biosynthesis. Inhibition of this metabolic pathway leads to inhibition of biosynthesis of pyrimidines, purines and amino acids hence reduced DNA, serine and methionine formation that are key prayers in the malaria parasite survival (Seifa et al., 2013; Rudrapal, 2011)

2.3.3 Artemisinin

Artemisinin (**13)**, which originated from the Chinese herb Qing hao (*Artemisia annua*) is a sesquiterpine lactone that contains a 1,2,4-trioxane ring system (Taylor et al., 2004). The endopereoxide bond is the essential feature of the artemisinin and its derivertives. Its isolation as clourless crystals dates back as early as 1970's (Arrow et al., 2004). The clinical trials were launched by 1974 and it was found to have rapid action and low toxicity as well as being effective against chloroquine-resistant *P. falciprum* (Butler et al., 2010). Its structure was elucidated in 1975 with a molecular formula $C_{15}H_{23}O_5$, but it was not until 1979, that the structure was confirmed using X-ray crystallography (Liu et al., 1979).



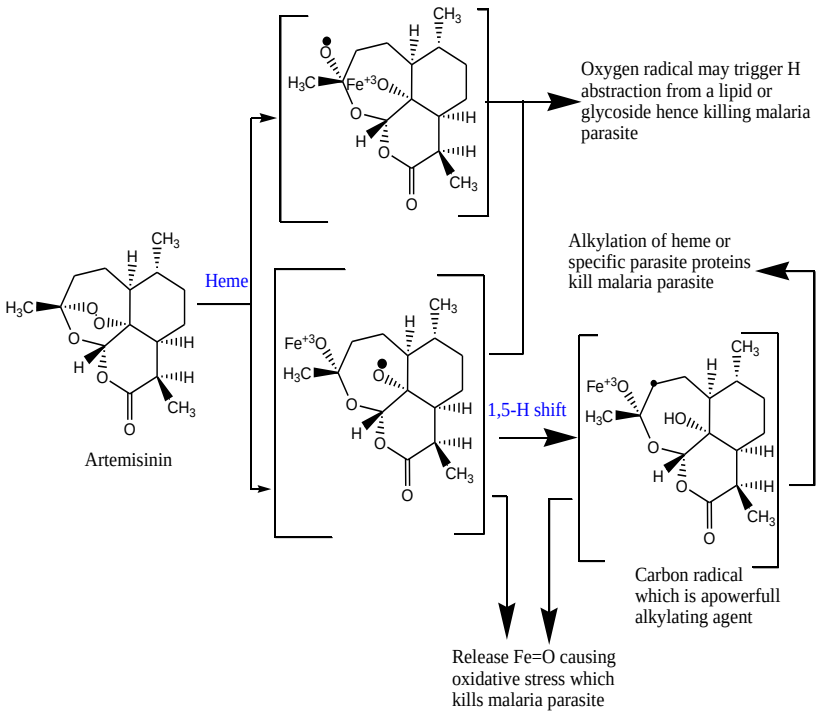
13

2.3.3.1 Artemisinin's mode of action

It has been proposed that, the active endoperoxide in artemisinin accumulate in the parasite cystosol and membranes where it interacts with the reduced heme (Taylor et al., 2004). In this reducing

environment the peroxide moiety is thought to react with FP-Fe II to form a cytotoxic carbon-centered radical intermediate that further react with susceptible groups within parasite enzymes and lipids. This emanates from reductive scission of the peroxide by low-valency transition metals (in this case Fe^{2+}) to generate oxygen-centred radicals (Meshnick, 2001). These radicals, due to affinity for hydrogen, might generate carbon-centred radicals and produce an FP=O, leading to an epoxide which is a highly active alkylating agent (Olliaro, 2001).

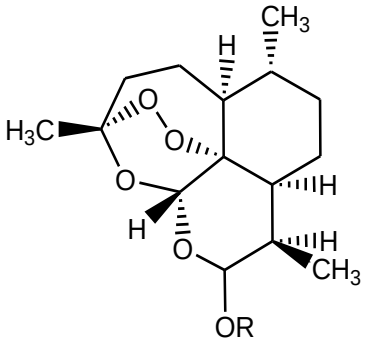
The carbon-centred radicals may alkylate to either heme itself or other proteins, such as Translationally Controlled Tumor Protein (TCTP) (Olliaro, 2001). According to Eckestein-indwig et al (2003), *P. falcipurum* homologue of the sarco/endoplasimic reticulum Ca^{2+}-ATpase (SARCA) is likely to be the major downstream target of the activated artemisinin in the parasite cytosol. This is in contrast with chloroquine and other quinoline drugs, which accumulate, in the acidic vacuole of the malaria parasite (Taylor et al., 2004). All the above, cause damage to the membrane of the parasite resulting into its death (Razdan, 2010). The scheme 2.1 shows a summary for the mechanism of action of artemisinin.



Scheme 2.1: Summary for the mechanism of action of artemisinin (Kerishna et al, 2004)

2.3.3.2 Derivatives of artemisinin

The artemisinin derivertives include artesunate (**30)**, artemether (**31)** and others. Their mode of action is related to that of the parent drug artemisinin that acts by formation of iron-catalysed free radical followed by alkylation of heme and consequently damaging the membrane of the parasite (Kerishna et al., 2004).



	R
30	-OCO$(CH_2)_2$COONa
31	Me

2.4 Antimalarial drug resistance

The antimalarial arsenal is severely compromised due to the parasite's remarkable ability to develop resistance to the known antimalarials, which results in the non-resolution of symptoms recrudescence and ultimately treatment failure (Price and Nosten, 2001). *P. falcipurum* and *P.vivax*, the most dominant malaria parasites have been reported to exhibit low to high level resistance to chloroquine, amodiaquine, mefaloquine, primaquine and sulphadoxine (Grimberg, 2011). The more recent heart breaking news is the resistance of *P. falciparum* to almost all the antimalarial drugs in current use (Warnsdorfer and Payne, 1991). According to WHO (2012), the parasite resistance to artemisinins has been detected in the four (4) countries of the Greater Mekong Sub-region in south East Asia: Cambodia, Myanmar, Thailand and Vietnam, the "historical hot spot" for multidrug resistance. In Cambodia particularly, resistance has been reported to both components of Multiple ACT's the only "double–edged sword" available against the life threatening malaria parasite. In response to this misfortune, special provisions for directly observed therapy using a non-artemisinin based combination (atovaquone-proguanil) have been put in place (WHO, 2012). Grimberg and Mehlotra (2011), states that drug resistant parasites are more likely to be selected if parasite populations are exposed to sub-therapeutic drug concentrations through;

i. Unregulated drug use
ii. The use of inadequate drug regimens, and/or
iii. The use of long half-life drugs singly or in non-artemisinin combination therapies

2.5 Antimalarial drug discovery

It is not debatable that the whole world is in a challenging situation of multidrug resistance of malaria parasites and therefore general approaches must be pursued towards the development of new antimalarials with novel modes of action (Rudrapal, 2011). The development of antimalarial drugs

can follow several strategies ranging from minor modifications of existing agents as well as combining the available agents to design novel antimalarial agents that act against new targets (Rosenthal, 2003). It is however regrettable, that there is small investment in antimalarial drug discovery and development since antimalarial markets are primarily in the developing countries which limits the market opportunities (Rosenthal, 2003).

The following approaches have been (or are being) pursued in an attempt to identify new antimalarials.

i. **Optimization of therapy with existing agents.** This is done by formulations of new dosing regimens, which optimizes the drug activity. The combination therapy including newer agents for example artemisinin derivertives/amodiaquine (Adjuik et al., 2002) and older agents for example amodiaquine/sulfadoxine/pyrimethamine (Dorsey et al., 2002) have been studied as first line therapies for Africa and other areas with widespread drug resistance. The combination is always aimed at improving antimalarial efficacy and providing additive or synergistic antiparasitic activity (Rosenthal, 2003).

ii. **Development of analogs of existing agents.** This approach focuses on improving on the existing antimalarials by chemical modification (Rosenthal, 2003). It is important to underline that, an attempt to improve on the activity of quinine resulted into the development of many existing antimalarials for example, chloroquine primaquine and mefaloquine (Stocks et al., 2001).

iii. **Natural products**. It is well documented that plant derived compounds have been the cornerstone for the malaria chemotherapy, which makes it the third prominent approach. This approach utilizes knowledge of medicinal plants among the native malarious regions, where there has been appreciable use of plant products to treat febrile illnesses (Rosenthal,

2003). The clear demonstration of the usefulness of this approach is the discovery of quinine and artemisinin, which have been and are still leading antimalarials from natural sources. In addition, simple modificacions of these compounds have led to a series of highly potent antimalarials that are playing an important role in the treatment of malaria (Meshnick, 2001; Rosenthal, 2003). There is much optimism that from various trials and evaluation of these natural products as potential new malaria therapies, may yield other important drugs for malaria chemotherapy (Rosenthal, 2003)

iv. **Compounds active against other diseases**. This involves identifying agents that are developed as treatment of other diseases and are tested for antimalarial activity. Folate antagonists, tetracyclines and other antibaotics were developed for their antibacterial properties and were later found to be active against malaria parasites (Clough and Wilson, 2001). Atovaquone that was initially identified as an antimalarial was later found to be active against pneumocystis and surprisingly its antimalarial potential was re-exploited in the combination drug malarone as well as its antimalarial synergy with proquanil (Canfield et al., 1995). These examples give a clear indication that screening new antimicrobial agents may result in discovery of inexpensive new antimalarials (Rosenthal, 2003).

v. **Drug resistance reversers**. Combining previously effective agents with compounds that reverse parasite resistance to the malaria agents is another best approach to chemotherapy (Rosenthal, 2003). Some drugs have shown the ability to reverse the resistance of *P. falciparum* to chloroquine *in vitro*, most notably the antihypertensive drug Verapamil (Martin et al., 1987). This combination with effective reversal of resistance keep alive the hope of resurrection of the failed first line, inexpensive, rapid acting and well-tolerated antimalarial drug (Rosenthal, 2003).

vi. **Compounds active against new targets.** The identification of new targets and subsequent discovery of compounds that act on these targets is arguably the most innovative approach to chemotherapy. The new targets for antimalarial chemotherapy are based on their locations within the malaria parasite (Rosenthal, 2003). These targets include; cytosolic targets where numerous metabolic pathways with hundreds of enzymes that are probably essential are inhibited for example the foliate metabolism will need the antifoliates that will treat effectively both bacteria and protozoan infections (Plowe, 2001).

Other exploitable targets include parasite membrane targets, food vacuole targets, mitochondria targets, epicoplast targets. In each case the biological and chemical composition of the parasite and in particular the target in question should be well studied and a drug is designed which can cause death to the parasite (Rosenthal, 2003). Important to note is that Liver stage drugs have the potential to hit new targets that are not present or essential during the blood stages of malaria, and these drugs would enjoy a tactical advantage over blood stage drugs because fewer parasites are involved, which should delay the development of resistance (Mazier et al., 2009).

2.6 Malaria prevention and control

It is notable that, the continued existence of malaria in an area requires a combination of high human population density, high mosquito population density and high rates of transmission from humans to mosquitoes and from mosquitoes to humans. If any of these is lowered sufficiently, the parasite will eventually disappear from that area, as it happened in North America, Europe and much of the Middle East (WHO, 2012). However, unless the parasite is eliminated from the whole world, it could become re-established if conditions revert to a combination that favours the parasite's reproduction. Many countries are seeing an increasing number of imported malaria cases due to

extensive travel and migration (WHO, 2011; Nilles and Arguin, 2012; Mavrogordato and Lever, 2012).

Strategies used to prevent the spread of disease, or to protect individuals in areas where malaria is endemic, include

i. **Vector control method**. This is achieved by indoor residual insecticide spraying using DDT, larval control, personal protection measures such as use of insecticide treated bed nets (ITNs), insect repellants and wearing appropriate clothing's (Ashleyet al., 2006, Gkrania-klotsas & Lever, 2007).

ii. **Use of Prophylactic drugs:** Several drugs, most of which are also used for treatment of malaria can be taken preventively. Generally, these drugs are taken daily or weekly, at a lower dose than would be used for treatment of a person who had actually contracted the disease. Use of prophylactic drugs is seldom practical for full-time residents of malaria-endemic areas, and their use is usually restricted to short-term visitors and travelers to malarial regions (Ashley et al., 2006). This is due to the cost of purchasing the drugs, negative side effects from long-term use, and because some effective antimalarial drugs are difficult to obtain outside of wealthy nations (Kimura et al., 2006)

iii. **Vaccination**. Vaccines for malaria are under development; however, there is no completely effective vaccine yet available (Gkrania-klotsas and Lever, 2007). Presently, there is a huge variety of vaccine candidates on the table. Pre-erythrocytic vaccines (vaccines that target the parasite before it reaches the blood), and in particular vaccines based on circumsporozoite protein (CSP), make up the largest group of research for the malaria vaccine (Schwartz et al., 2012). Other vaccine candidates include: those that seek to induce immunity to the blood stages of the infection; those that seek to avoid more

severe pathologies of malaria by preventing adherence of the parasite to blood venules and placenta as well as those that would stop the development of the parasite in the mosquito right after the mosquito has taken a blood meal from an infected person (All-Parliamentary Group on Malaria and Neglected Tropical Diseases Report, 2010–2011). It is anticipated that the sequencing of the *P. falciparum genome* will provide targets for new drugs or vaccines.

iv. **Education:** recognizing the symptoms of malaria has reduced the number of cases in some areas of the East Africa by as much as 20%. Early stage recognition of the disease can also stop the disease from becoming a killer. Education can also inform people to cover areas of stagnant and still water, for example Water Tanks, which are ideal breeding grounds for the parasite and mosquito. This cuts down the risk of the transmission among people (Toovey and Jamieson, 2003). This is best put in practice in urban areas where there are large centers of population in a confined space and transmission would be most likely.

2.7 The genus *Zanthoxylum*

2.7.1 An overview on the genus *Zanthoxylum*

The genus *Zanthoxylum* belongs to the Rutaceae family, subfamily Rutoideae in the tribe Zanthoxyleae (Fish and Waterman, 1973). The origin of *Zanthoxylum* can be traced from the word *Xanthoxylum* that derives from Greek: “Xanthon xylon” that means “yellow wood” (Patino et al., 2012). It is however notable that the inception of *Zanthoxylum* has been confused with the genus *Fagara*. In 1892, Eagler distinguished between the two genera by the following characteristics: species of the genus *Zanthoxylum* have simple perianth while in Fagara it is two-fold (Patino et al., 2012).

It was not until 1962, when Brizicky discovered that some species have intermediate perianth which suggested that simple perianth of *Zanthoxylum* was a result of a drift from *Fagara* due to failure of some sapels, which made him conclude that *Fagara* and *Zanthoxylum* are similar (Patino et al., 2012). In 1966, Hartley cemented Brizicky's argument by grouping *Zanthoxylum and Fagara* under one name *Zanthoxylum* (Fish and Waterman, 1973).

2.7.2 The distribution of the genus *Zathoxylum*

The genus *Zanthoxylum* comprises about 549 species distributed worldwide especially in tropical and warm temperate regions (Patino et al., 2012). In Kenya, there are seven *Zanthoxylum* species namely: *Z. usambarense* (Engl.) Kokwaro, *Z. holstzianum* (Engl.) Waterman, *Z. chalybeum* (Engl.var) Chalybeum, *Z. gillettii* (De Wild.) Waterman, *Z. mildbraedii* (Engl.) Waterman, *Z. paracanthum* (Mildbr.) Kokwaro and *Z. rubescens* Hooks. f (Beentje,1994).

2.7.3 Characteristics of genus *Zanthoxylum*

The genus include trees and shrubs, which are usually dioecious. Generally, the trees have leafy crown, with few branches and in most cases reach up to a height of 20 meters. The presence of re-curved spines on the trunk and branches is one of the conspicuous characteristics of the genus. The leaves are varied, may be alternate or opposite, simple or composed, imparipanadas or paripanadas, with up to 15 pairs of leaflets. The inflorescences are usually in form of panicles or umbel, compound, axillary or terminal of small flowers. The flowers are actinomorphic, hermaphrodite and unisexual, rarely bisexual, usually white or green. The fruits are follicles or esquizocarp contains from one to five carpels usually aromatic, and they are ordinarily bivalve with a single red or black shiny seeds (Patino et al., 2012; Beentje, 1994).

2.7.4 *Zanthoxylum holstzianum*

Zanthoxylum holstzianum (Engl.) Waterman, is only documented to be in the coastal region of Kenya in East Africa (Beentje, 1994). It is a shrub or tree which may grow to 4-15 m, which is some times described as scrabling; bark grey, with corky bosses. Branches have straight or recurved spines, which are 2-7 mm long. Its leaves have 7-9 leaflets that are 7-15 by 3-6.5 cm and glabrous. Its flowers are white, in terminal panicles 8-25 cm long; petals 1.5-3 mm long. The fruit is reddish to bright red, round and 4-6mm. It can easily be found in moist or dry forest or closed thicket near the sea (Beentje, 1994). Figure 2.2 below is a photo for the stem, leaves and the fruits of *Z. holstzianum*.

Figure 2.2: The stem, leaves and fruits of *Z. holistziaum* from Diani forest (Photo by Akampurira Denis)

2.7.5 Ethnomedicinal uses of *Zanthoxylum* species

The major ethnobotanical properties attributed to *Zanthoxylum* species are: relief of dental problems, treatment of malaria, gastrointestinal disorders, gonorrhea and lung diseases, antidiarrheal use in

animals and humans, emmenagogue action, effective for rheumatism, anthelmintic use in animals and humans, aphrodisiac, analgesic, action against various skin diseases, febrifuge, antihemorrhagic, effective for genito-urinary diseases, anticancer, diuretic, stomachic, anti-convulsive, tonic and stimulant. In addition to the medicinal properties, some species are used as pesticides (Patinoet al., 2012). Specific traditional uses of *Zanthoxylum* species are summarized in Table 2.1.

Due to the unlimited substantial ethnomedical properties of *Zanthoxylum* species, some have been used as components of natural medicines for example; Flora Medicinal. J. Monteiro da silva laboratory, has marketed *Z. tingoassuiba* since 1923, as part of herbal medicinal product called Uva do Mato® that is prescribed for muscle cramps and spasms (Da silva et al., 2008)

Table 2.1: Ethnomedicinal uses of *Zanthoxylum* species

Species	**Plant part used**	**Traditional use**	**Reference**
Z. usambarense	Stem and root back	Treat rheumatism and malaria	Matu and staden, 2003
	Young twigs	Tooth brushes	Kokwaro, 2009
	Leaves	Colds and flue	
Z. chalybeum	Stem back and root	Treat malaria, colds, coughs, toothache, sores, wounds and headache	Matu and Staden, 2003 and Kokwaro, 2009
	Fruits	Treat coughs	
	Leaves	Severe colds and pneumonia, kwashiorkor and snake bite	
Z. gilletti	Bark	Stomachache, toothache, joint pain, fever, washing wounds and venereal diseases	Dharani et al., 2010, Kokwaro, 2009
	Leaves	Antihypertensive, analgesic and to	Addae et al.,1989

Species	Plant part used	Traditional use	Reference
		treat gonorrhea	
Z. chiloperone	Root bark	As antimalaric,emmenagogue and Antirheumatic properties.	Ferreira et al., 2007
Z. macrophylla	Bark and seeds	Used for toothache, colds, fever, malaria, stomachache, rheumatism and urinogenital affections, as well as to prepare poisonous arrows.	Kuete et al., 2011 Tringali et al., 2001
Z. tingoassuiba	Stem bark	Antispasmodic, muscle relaxant, Analgesic, sudorific, antifungal, diuretic, antiplatelet, antiparasitic and antihypertensive.	Da Silva et al., 2008
Z. tetraspermum	Stem bark	Used for the treatment of dyspepsia, rheumatism and some forms of diarrhea	Nissanka et al., 2001
Z. rhoifolium	Bark	Antimalarial, tonic and febrifuge, toothache, venereal chancre, external parasites, antipyretic, digestive properties	Jullian et al., 2006
Z. ailanthoides	Leaves Stem and Root bark	Common cold Rheumatism, arthalgia, stasis, contusions, snake bite and stimulates blood circulation and anti-HIV activity	Cheng et al., 2005
Z. capense	Not specified	Parasiticide and anti-protozoal activity	Setzer, 2004

2.7.6 Biological activities of *Zanthoxylum* species

Zanthoxylum species have showed various biological activites, which include antiplasmodial, larvicidal, anticancer, antinociceptive activities and many others. Some of these are presented in detail in the following sections.

2.7.6.1 Antiplasmodial activity

It is on record that good antimalarial properties have been observed in alkaloids, sesquiterpene lactones, coumarins, triterpenoids and limonoids among natural products (Claudio and Lopes, 2011). *Zanthoxylum. chalybeum, Z. syncarpum, Z. zanthoxyloides, Z. gilletii, Z. limonella, Z. rhoifolium* and *Z. usambarense,* among others, are some of the species that have showed promising antimalarial properties (Patino et al., 2012). Nguta et al (2010), reports the IC_{50} of the crude extract from the root back of *Z. chalybeum* as 4.2 µg/ml and some quinoline alkaloids isolated from the same species exhibiting strong antiplasmodial activity against chloroquine root bark resistant *Plasmodium falciparum* strain. Muganga et al (2010), reports the methanol extract of the root bark to have an IC_{50} 1.9 ± 0.5 µg/ml against W2 of *P. falciparum.*

Decarine and syncarpamide, the two compounds isolated from *Z. syncarpum* have showed strong antiplasmodial activity againsit D6 (chloroquine sensitive clone) and W2 (chloroquine resistant clone) *P. falciprum* strains, having IC_{50} values lower than 6.1µM (Kaur et al., 2009; Ross et al., 2005). Fagaronine, a benzophenanthridine from *Fagara zanthoxyloides* inhibited *P. falciparum in vitro* at a low IC_{50} value of 0.018µg/ml (Adebayo and Krettli, 2011). The alkaloid fractions *of Z. rhoifolium* showed antiplasmodial activity of approximately 44% inhibition of *P. falciparum* at 10µg/ml and nitidine hydroxide (an isolate) at IC_{50} 1.8 µg/ml using LDH micro method (Jullian et al., 2006). Stem bark extracts from *Z. usambarense* were tested againsit *P. knowlesi* and *P. berghei*, which revealed that aqeous extracts had significant activity against the two parasites while all other organic solvent extracts were inactive. This suggested that polar substances in this species are responsible for the antiplasmodial activity (Were et al., 2010).

2.7.6.2 Larvicidal activity

The compounds 10-*O*-dimethyl-17-*O*-methylarnottianimide and 6-acetonyl-*N*-methyl-dihydrodecarine isolated from *Z. lemairei* showed larvicidal activity with mortality on *Anophlese gambiae* of 96.7 and 98.3% respectively, at a concentration of 250 mg/l (Ferdinand et al., 2011). At a concentration of 10 ppm, the ethanol extract of Z. *piperitum* fruit showed 85, 100 and 48% mortality in larvae for *Aedes aegyptti*, *Aedes tongoi* and *Culex pupiens,* respectively (Yang et al., 2004).

2.7.8 Phytochemistry of the genus *Zanthoxylum*

The most commonly reported secondary metabolites from the genus are alkaloids of various types, lignans, coumarins and amides. However, other metabolites such as flavonoids, terpenes, fatty acids, among others have not missed on the list (Patino et al., 2012).

2.7.8.1 Alkaloids of *Zanthoxylum*

Alkaloids are low molecular weight nitrogen-containing substances with characteristic toxicity and pharmacological activity, the properties that have been traditionally exploited by humans for hunting, execution and warfare (Vicenzo and Beniot, 2000). Infact, these properties have been the cornerstone for the treatment of diseases in humans (Vicenzo and Beniot, 2000). Alkaloids are mainly found in plants, but also to a lesser extent in microorganisms and animals. It is estimated that over 27,000 different alkaloids have been characterized and of these 21,000 (77.8%) are from plants. Alkaloids are mainly derived from amino acids (Dewick, 2009).

The fact that alkaloids are present in all parts and in particular abundant in the stem and root bark of most *Zanthoxylum* species makes *Zanthoxylum genus* very important (Patino et al., 2012). The alkaloids of *Zanthoxylum* are categorized mainly into two: isoquinoline and quinolines alkaloids

(Waterman and Grundon, 1983). Need to mention are some other types of alkaloids, which are found in some species for example bishoderninyl terpene, indolopyridoquinazoline, canthin-6-one, quinazoline and carbazole alkaloids (Patino et al., 2012). By 2011, 660 alkaloids had been reported from genus *Zanthoxylum* (DNP, 2011).

2.7.8.2 Isoquinoline alkaloids

The isoquinoline alkaloids from the genus *Zanthoxylum* are further classified into benzylisoquinoline, benzophenanthridine, protoberberine, berberine and aporphine (Waterman and Grundon, 1983).

2.7.8.3 Benzylisoquinoline alkaloids

The basic skeleton of benzylisoquinoline alkaloids is given in Figure 2.3 below.

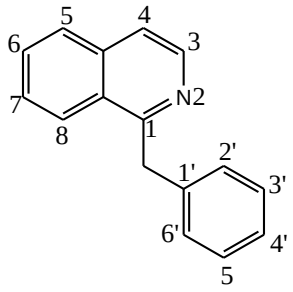
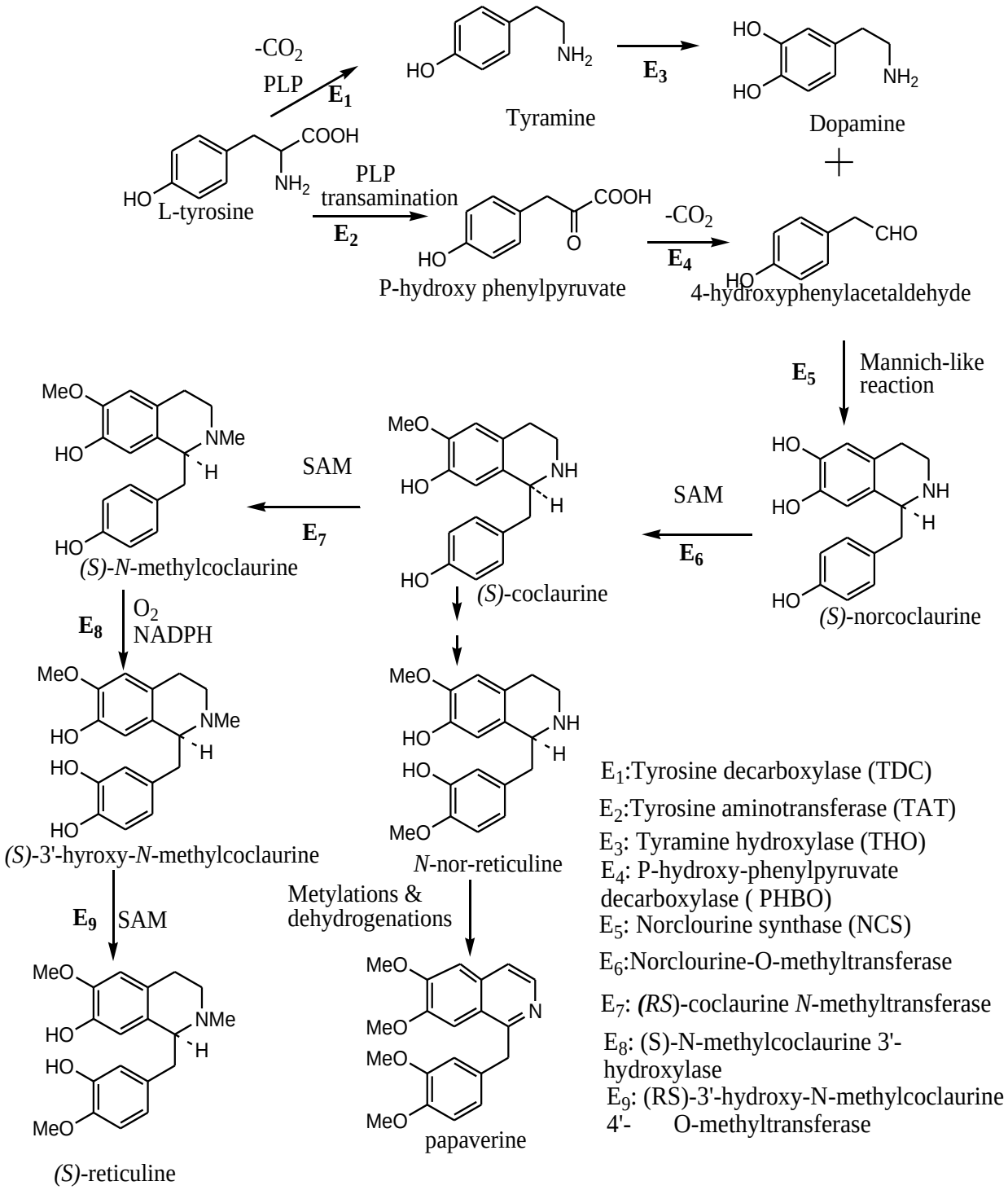


Figure 2.3: The basic skeleton of benzylisoquinoline alkaloids

Most benzylisoquinolines have methylated nitrogen atom as well as functional groups containing (OH, OCH_3, $-OCH_2O-$) at C-6, 7, 3' and 4'. It is, however, very important to note that benzylisoquinoline is the structural backbone of many alkaloids with a wide variety of structures, including benzophnanthridine, protoberberine and aporphine alkaloids among others.

2.7.8.4 Biosynthesis of benzylisoquinoline alkaloids

The biosynthetic pathway of benzylisoquinoline alkaloids utilizes two units of L-tyrosine in which one tyrosine molecule is metabolized to dopamine that constitutes the isoquinoline part while the origin of the benzylic part is from tyramine that arise from decarboxylation of tyrosine (Dewick, 2009). The scheme below shows the biosynthesis of benzylisoquinoline.



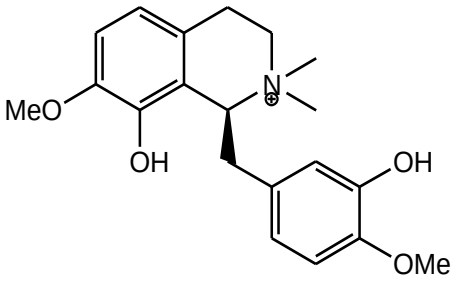
Adopted from Grycova et al., 2007; Facchini and Benoit, 2005 and Dewick, 2009

Scheme 2.2: Biosynthesis of benzylisoquinoline alkaloids form tyrosine

It is worth noting that from (*S*)-reticuline several compounds of *Zanthoxylum* are biosynthesized. For example benzophenanthridine, protoberberine and aporphine alkaloids as shown later.

2.7.8.5 Benzylisoquinoline alkaloids of *Zanthoxylum*

Although benzylisoquinoline alkaloids are not the most common alkaloids in the genus *Zanthoxylum,* they have been found in some species. For example the quaternary alkaloids (*R*)-(+)-isotembetarine (**60)** and (*S*)-(-)-xylopinidine (**61)**, have been isolated from the bark of *Z. quinduense* (Patino and Cuca, 2010). Generally, in the Rutaceae family, they are present in a group of five genera named proto-Rutaceae (*Phellodendron*, *Fagaropsis*, *Tetradium*, *Toddalia* and *Zanthoxylum*) (Ling et al., 2009; Waterman, 2007).



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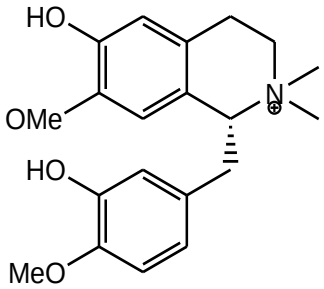

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2.7.8.6 Benzophenanthridine alkaloids

Benzophenanthridines are alkaloids with a basic skeleton as shown in the figure 2.4 below.

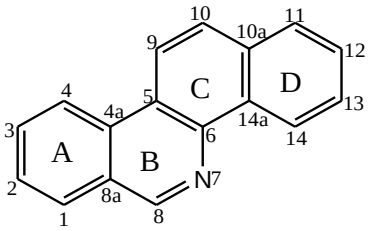
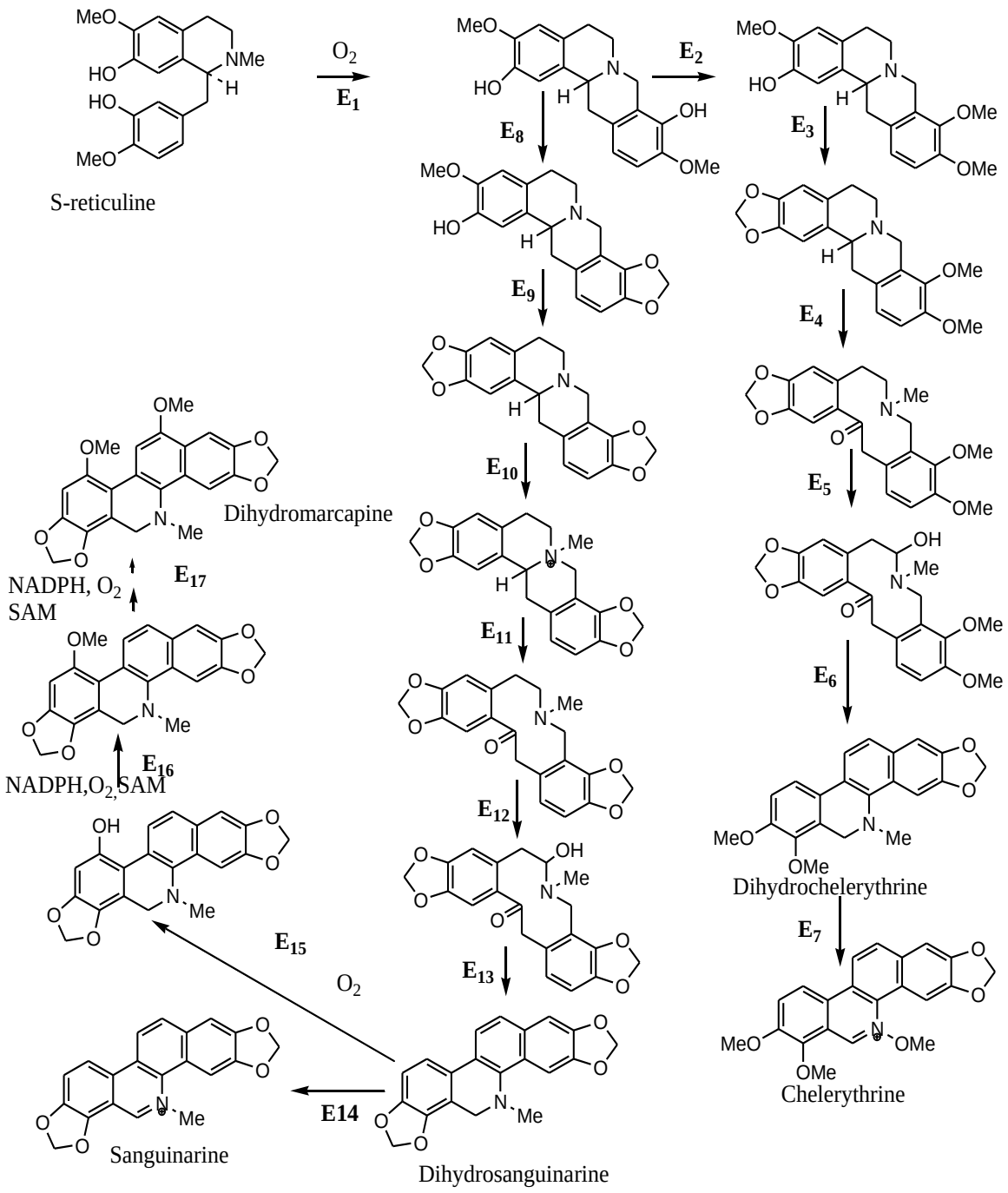


Figure 2.4: Basic Skeleton of benzophenanthridine alkaloids (Waterman, 1990).

Oxygenation in this type of alkaloids is mainly on rings A and D at C-1, 2, 3, 4, 12 and 13. Some compounds can be oxygenated at C-8 on ring B and though rare at C-10 on ring C. Both ring A and D can be oxygenated by methylenedioxy formation at C-1/2, 2/3 and 12/13. At position 7, *N*-methylation is very common. Apart from oxygenation, C-8 is the center for other attachments as well as dimerization. Some reperesentative examples of benzophenanthridine alkaloids of Zanthoxylum are given in the Table 2.2.

2.7.8.7 Biosynthesis of benzophenanthridine alkaloids

As earlier noted, benzophenanthridins are (*S*)-reticuline derived alkaloids. Therefore it is not wrong to mention that benzophenathridine alkaloids are derived from L-tyrosine which is the precursor for *(S)*-reticuline (Zenk, 1994). Scheme 2.3 illustrates how some benzophenanthridine alkaloids are biosynthesized.

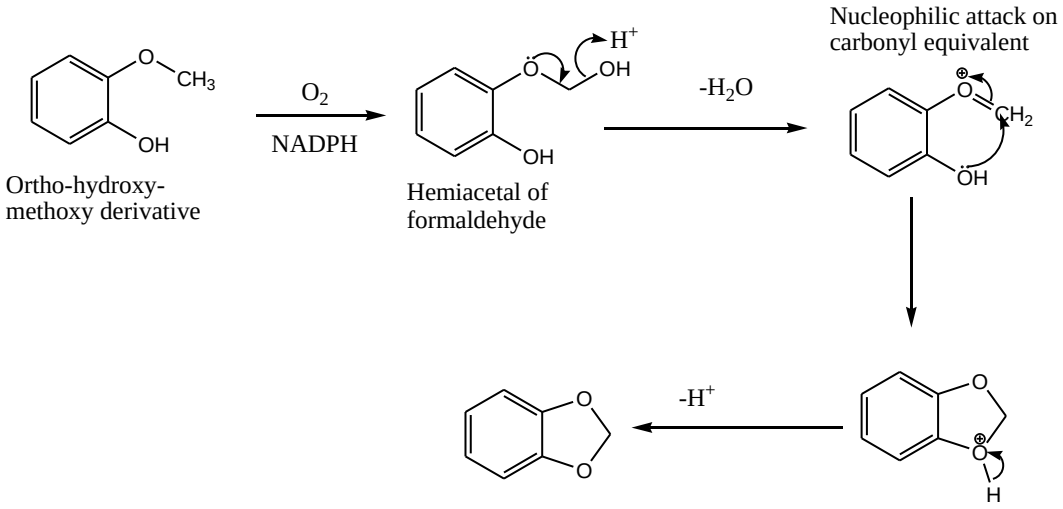


E1: Beberine bridge enzyme; E2: *O*-methyltransferase; E3: Stylopine synthase; E4: Tetrahydroberberine-*N*-methylsynthase & methylstypinehydroxylase; E5: Allocryptopine-6-hydroxylase; E6: spontaneous rearrangement; E7: Dihydrobenzophenanthridine oxidase; E8:Cheilanthifoline synthase; E9: Stylopine synthase; E10: Tetrahydroberberine-cis-N-methyltransferase; E11: *N*-methylstylopine-14-hydroxylase; E12: Protopine-6-hydroxylase; E13: Rearrangement, E14: Dihydrobenzophenanthridine oxidase, E15: Dihydrosaguinarine-4-hydroxylase; E16: 4-*O*-methyltransferase; E15:10-hydroxylase, O-methyltransferase.

Scheme 2.3: Biosynthesis of benzophenanthridine alkaloids

2.7.8.8 Formation of the methylenedioxy group

Oxidative cyclisation of *ortho*-hydoxy methoxy substituted aromatic system results into the formation of methylenedioxy. It involves cytochrome P-450-dependent mono oxygenase enzyme. The enzyme hydroxylates the methoxy methyl to yield an intermediate that is a hemiactetal of formaldehyde. This can cyclise to a methylenedioxy bridge (the acetal of formaldehyde) by an ionic mechanism (Dewick, 2009, Kutchuan et al., 1991). Scheme 2.4 shows the mechanism of formation of the methylenedioxy groups.



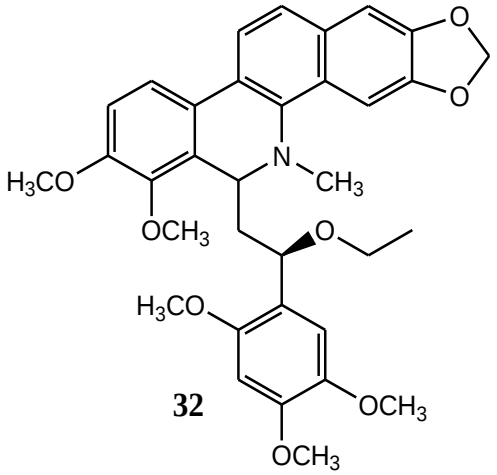
Scheme 2.4: Formation of methylenedioxy group

2.7.8.9 Benzophenanthridine alkaloids isolated from *Zanthoxylum*.

The genus *Zanthoxylum* is very well known to elaborate benzophenanthridine alkaloids (Waterman, 1990). In table, 2.2 various examples of benzophenanthridine alkaloids isolated from different species of *Zanthoxylum* genus are listed.

Table 2.2: Some examples of benzophenanthridine alkaloids of *Zanthoxylum*

Alkaloid	**Plant source**	**Reference**
Buesgeniine **(32)**	*Z. buesgenii*	Tane et al., 2005
8-Acetonyldihydronitidine **(33)**	*Z. tetraspernum* and *caudatum*	Nissanka et al, 2001
8-Acetonyldihydroavicine **(34)**		
Dihydrochelerythrine **(35)**	*Z. simulans*	Traus et al., 2006
Simulanoquinoline **(36)**	*Z. simulans*	Traus et al., 2006
Isoarnottianamide **(37)**	*Z. nitidum*	Hu et al., 2007
Arnottianamide **(38)**	*Z. nitidum*	Hu et al., 2007
Intergriam **(39)**	*Z. nitidum*	Hu et al., 2007
Chelerytherine **(40)**	*Z. nitidum*	Hu et al., 2007
Nitidine **(41)**	*Z. nitidum*	Hu et al., 2007
Sanguinarine **(42)**	*Z. nitidum*	Hu et al., 2007
Dihydrochelerythrine-8-acetaldehyde **(43)**	*Z. nitidum*	Hu et al., 2007
8-Hydroxydihydrochelerythrine **(44)**	*Z. nitidum*	Hu et al., 2007
8-Methoxydihydrochelerythrine **(45)**	*Z. nitidum*	Hu et al., 2007
Decarine **(46)**	*Z. nitidum*	Hu et al., 2007
Rhoifoline **(47)**	*Z. nitidum*	Hu et al., 2007
Oxyavicine **(48)**	*Z. nitidum*	Hu et al., 2007
4, 6-Hydroxydihydrochelerythrine **(49)**	*Z. davyi*	Tarus et al., 2006
5,6-Methoxy-7-dimethyldihydrochelerythrine **(50)**		
Bocconoline **(51)**		
6-Carboxymethyldihydrochelerythrine **(52)**	*Z.ssp*, root back	Kwok et al., 1987
6-(4-Methyl-2-oxopentanyldihydrochelerythrine **(53)**		
Chelelactam **(54)**		
Oxychelerythrine **(55)**		
Cayamandimerine **(56)**		
Norchelerythrine **(57)**		
Dimethoxyzanthoxyline **(58)**	*Z. rhoifoli*	Nausa et al., 1997
Zanthoxyline **(59)**		



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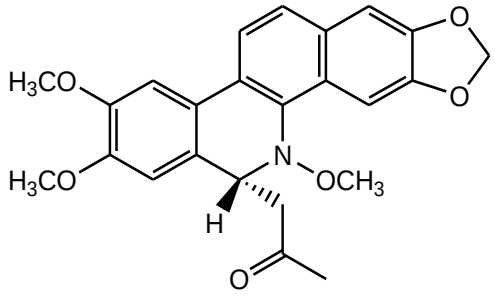

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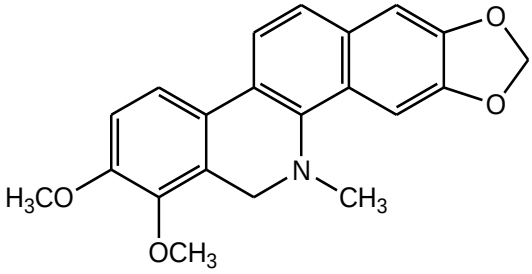

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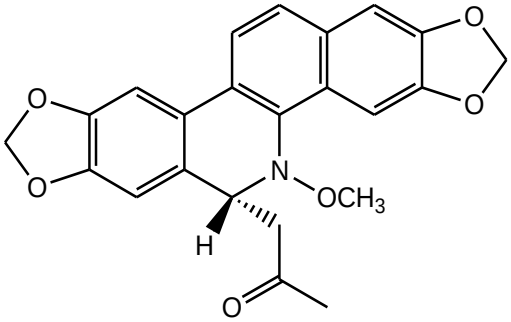

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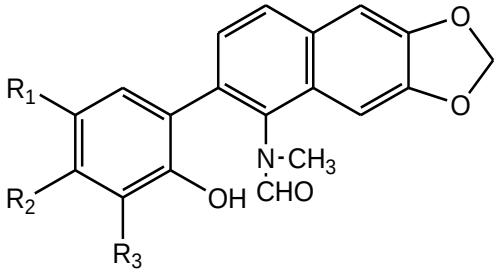
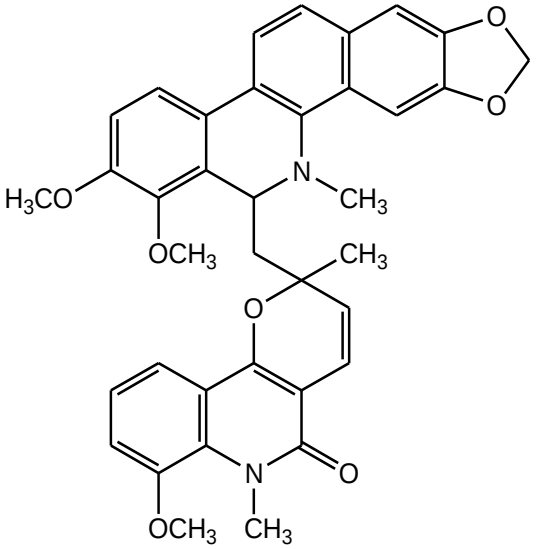


	R_1	R_2	R_3
37	OCH_3	OCH_3	H
38	H	OCH_3	OCH_3
39	OCH_2O		H



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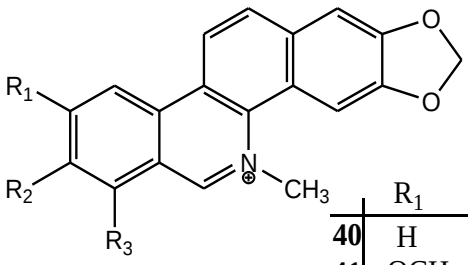
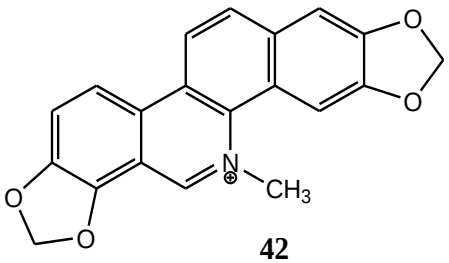


	R_1	R_2	R_3
40	H	OCH_3	OCH_3
41	OCH_3	OCH_3	H



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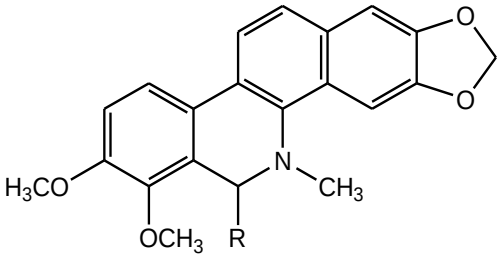
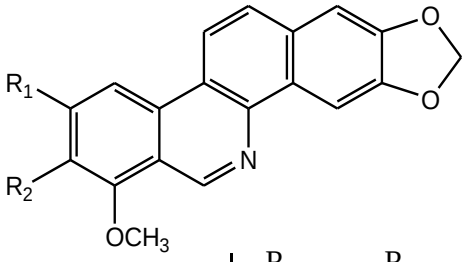
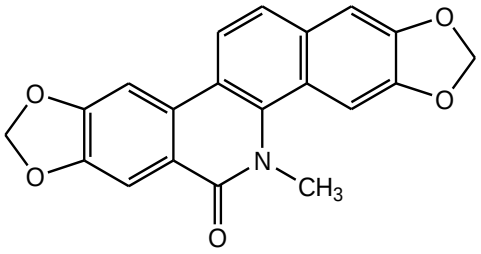


	43	**44**	**45**
R	CH_2CHO	OH	OCH_3



	R_1	R_2
46	H	OH
47	OCH_2O	



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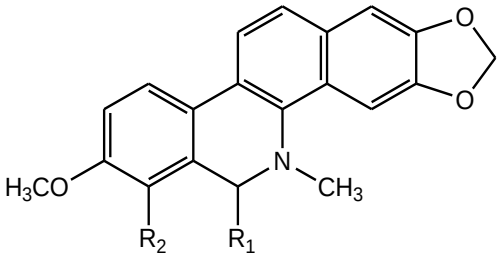

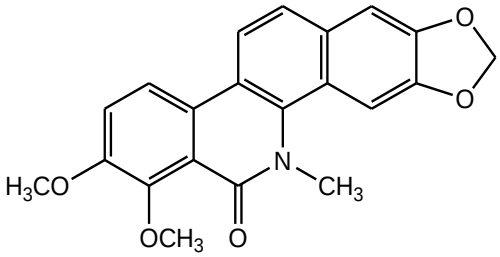

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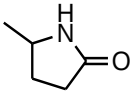
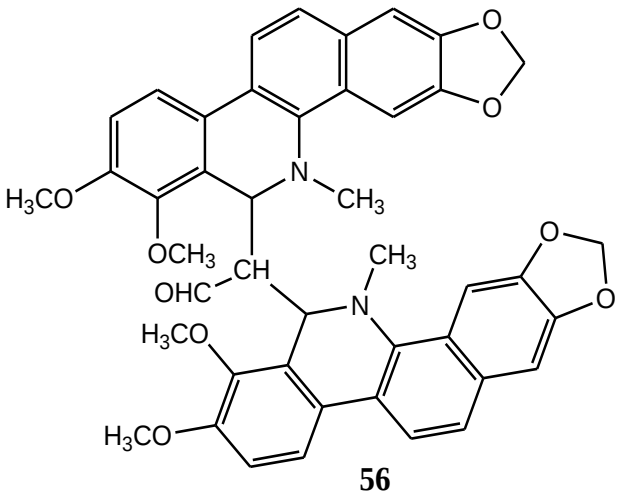
	R1	R2
49	OH	OCH_3
50	OCH_3	OH
51	CH_2OH	OCH_3
52	CH_2COOH	OCH_3
53	$CH_2COCH_2CH(CH3)_2$	OCH_3
54		OCH3



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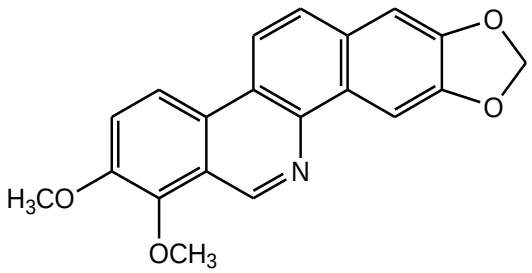

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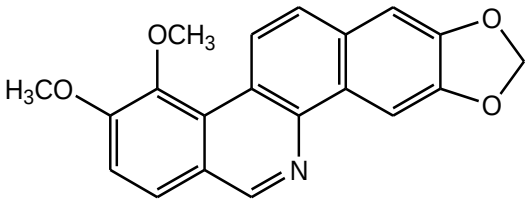

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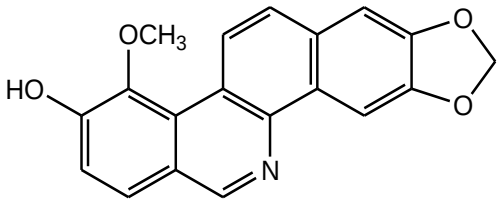

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2.7.8.10 Protoberberine alkaloids

Biogenetically protoberberine alkaloids are derived from tyrosine. Most protoberberines exist in plants either as tetrahydroprotoberberine or as quaternally protoberberine salts, though a few dihydroberberines do also exist. The basic skeleton of quaternary protoberberine alkaloids as shown in Figure 2.5, is 5,6-Dihydrodibenzo(a,g)quinolizinium ($C_{17}H_{14}N^{+}$) (Grycova et al., 2007)

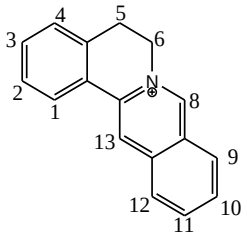


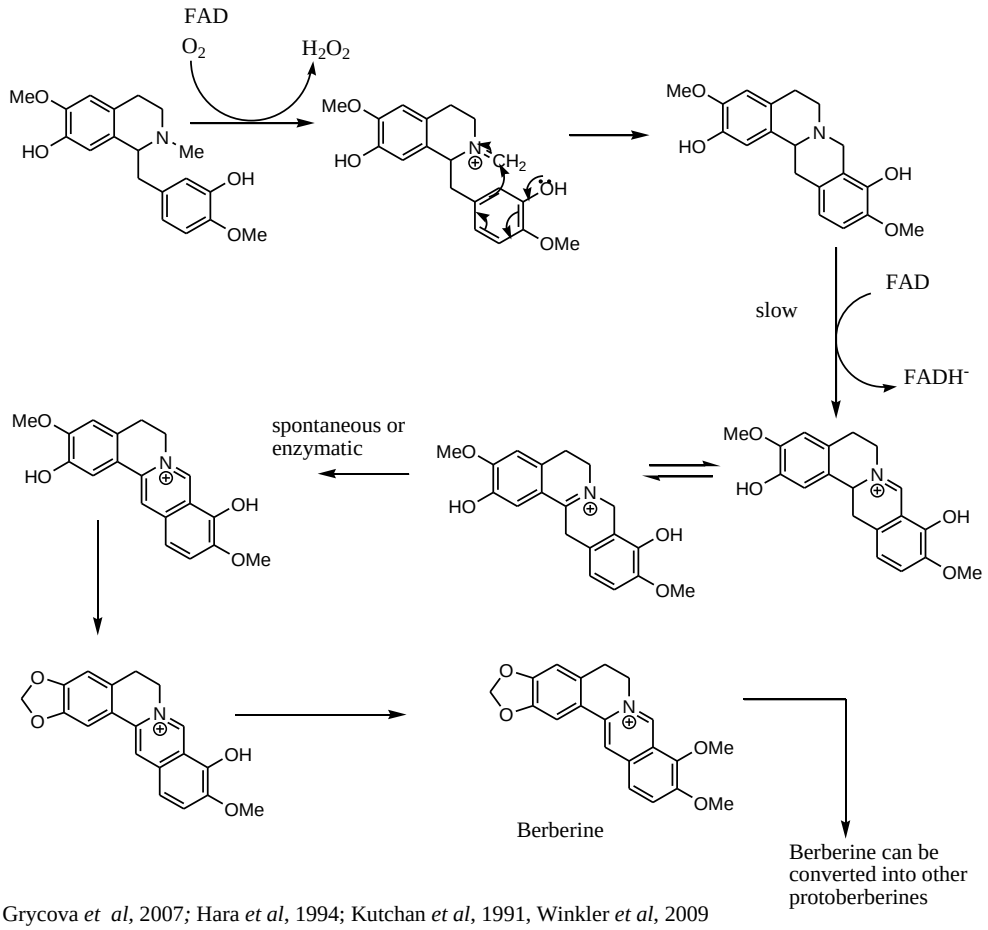
Figure 2.5: Basic structure of quaternary protoberberine alkaloids

Substituents, which are either hydroxyl, methoxy or methylenedioxy groups, are usually present at position 2, 3, 9, 10 or 2, 3, 10, 11. A prefix pseudo- is often used in the later substitution pattern. It

is however notable that compounds with substituents at position 1, 4, 5 and 13 have also been isolated from natural sources (Gryova et al., 2007).

2.7.8.11 Biosynthesis of protoberberine alkaloids

Biosynthesis of protoberberines can be traced from (*S*)-reticuline whose biosynthesis from tyrosine has been earlier discussed. The scheme 2.5, shows how (*S*)-reticuline can result into a protoberberine.

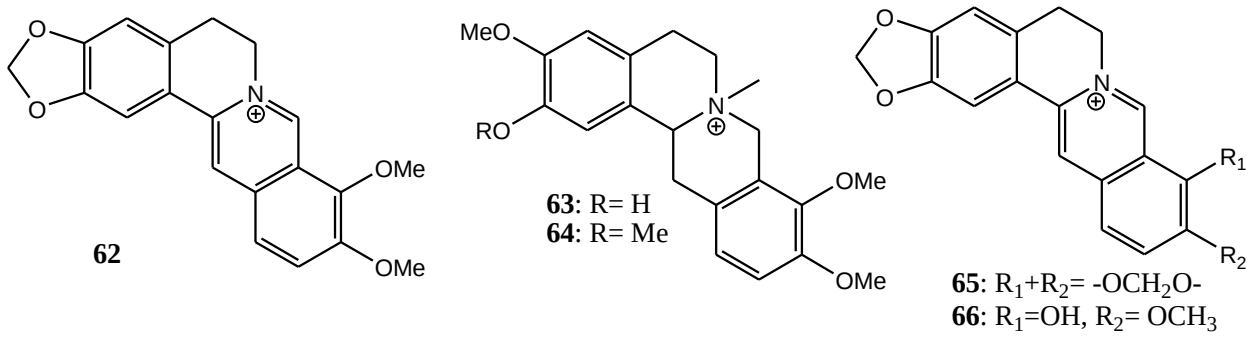


Grycova *et al*, 2007*;* Hara *et al*, 1994; Kutchan *et al*, 1991, Winkler *et al*, 2009

Scheme 2.5: Biosynthesis of protoberberines

2.7.8.12 Protoberberine alkaloids of *Zanthoxylum*

Examples of protoberberine alkaloids from *Zanthoxylum* genus are berberine (**62)** from *Z. monophyllum* (Patino and Cuca, 2011), N-methyltetrahydrocolumbamine **(63)** and *N*-methyl tetrahydropalmatine (**64)** from *Z. quidiense* (Patino and Cuca, 2010), berberrubine **(65)** and coptisine (**66)** from *Z. nitidin* (Jiang et al., 2007).



62

63: R= H
64: R= Me

65: R_1+R_2= -OCH_2O-
66: R_1=OH, R_2= OCH_3

2.7.8.13 Aporphine alkaloids

Aporphine alkaloids are a diverse family of isoquinoline alkaloids. They share a characteristic tetracyclic motif with different levels of oxidation on both aromatic rings (Lafrance et al., 2007). The figure below shows the basic skeleton of aporphine alkaloids.

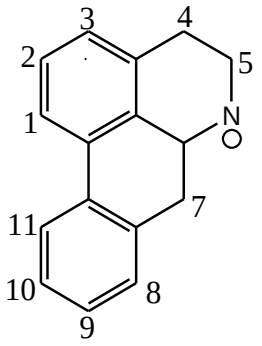
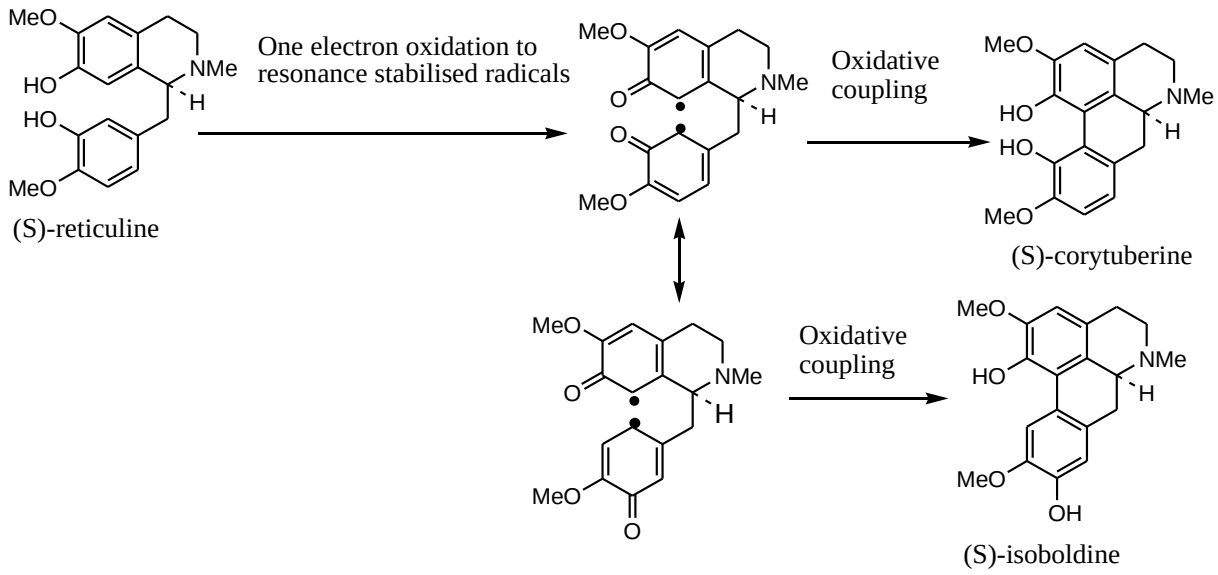


Figure 2.6: The basic skeleton of aporphine alkaloids

Aporphine alkaloids may be di, tri, tetra, penta, or hexa substituted to give different derivertives. The substituents are mainly hydroxyl, methoxy, methylenedioxy groups or sugar residues. These substituents may be located in all the four rings except methylenedioxy group, which can only be found on rings A and D. The most wide spread in nature are 1, 2, 9, 10 and 1, 2, 10, 11, tetra substituted bases, and however pent substituted aporphines in which functional groups occupy positions at various carbon atoms are found frequent (Israilov et al., 1980).

2.7.8.14 Biosynthesis of aporphine alkaloids

Amino acid tyrosine which through a series of reactions results into (*S*)-reticuline intermediate (Hara et al, 1994), is the genesis for the biosynthesis of aporphine alkaloids (Dewick, 2009). The scheme below illustrates how aporphine alkaloids are derived from (*S*)-reticuline (Dewick, 2009).



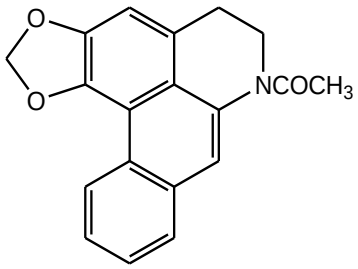
Scheme 2.6: Biosynthesis of aporphine alkaloids

2.7.8.15 Aporphine alkaloids of *Zanthoxylum*.

Reperesentatives of aporphine alkaloids of *Zanthoxylum* are summarized in the Table 2.3.

Table 2.3: An outline of some aporphine alkaloids isolated from genus *Zanthoxylum.*

Aporphine alkaloid	**Plant source**	**Reference**
N-acetyldehyderoanonaine **(67)**	*Z. simulans*	Chenet al., 1996
N-methylcorydine **(68)**	*Z. monophyllum*	Stermitz and Sharif, 1997
Magnoflorine **(69)**	*Z. williamsii/ Z.monophyllum*	Stermitz and Sharif, 1997; Stermitz et al., 1980
Laurifoline **(70)**	*Z. williamsii*	Stermitz et al., 1980
N- acetylanonaine **(71)**	*Z. bugeanum*	Chen et al., 1996
Liriodenine **(72)**	*Z. simulans*	Hufford et al., 1976
Zanthoxyphyllum **(73)**	*Z. oxyphyllum*	Ishii et al., 1961
Xanthoplanine **(74)**	*Z. planispium*	Ishii et al., 1961



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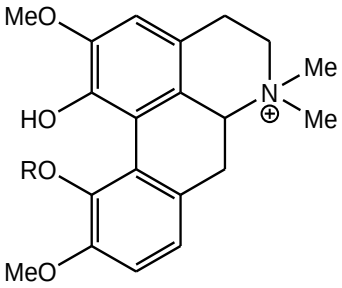
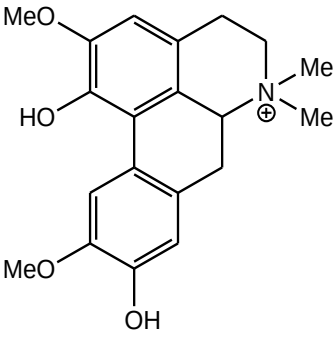


68: R= Me
69: R=H



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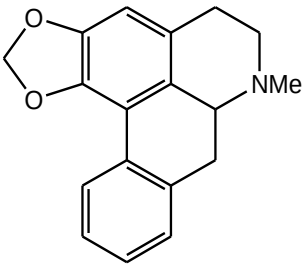

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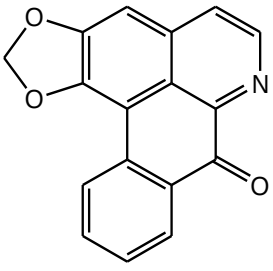

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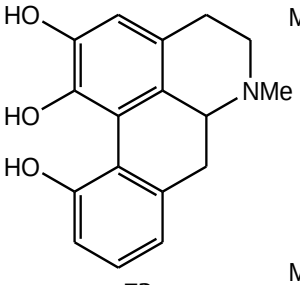

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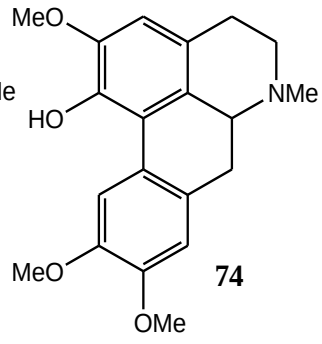


74

2.7.8.16 Quinoline alkaloids

Most quinoline alkaloids contain a carbonyl at C-2 of the simple quinolinic nucleus and hence called 2-quinolines (Waterman and Grundon, 1983). The Figure 2.7, shows is a basic skeleton of 2-quinoline alkaloids.

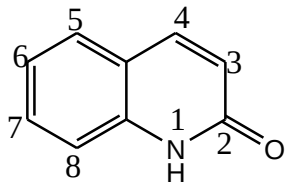
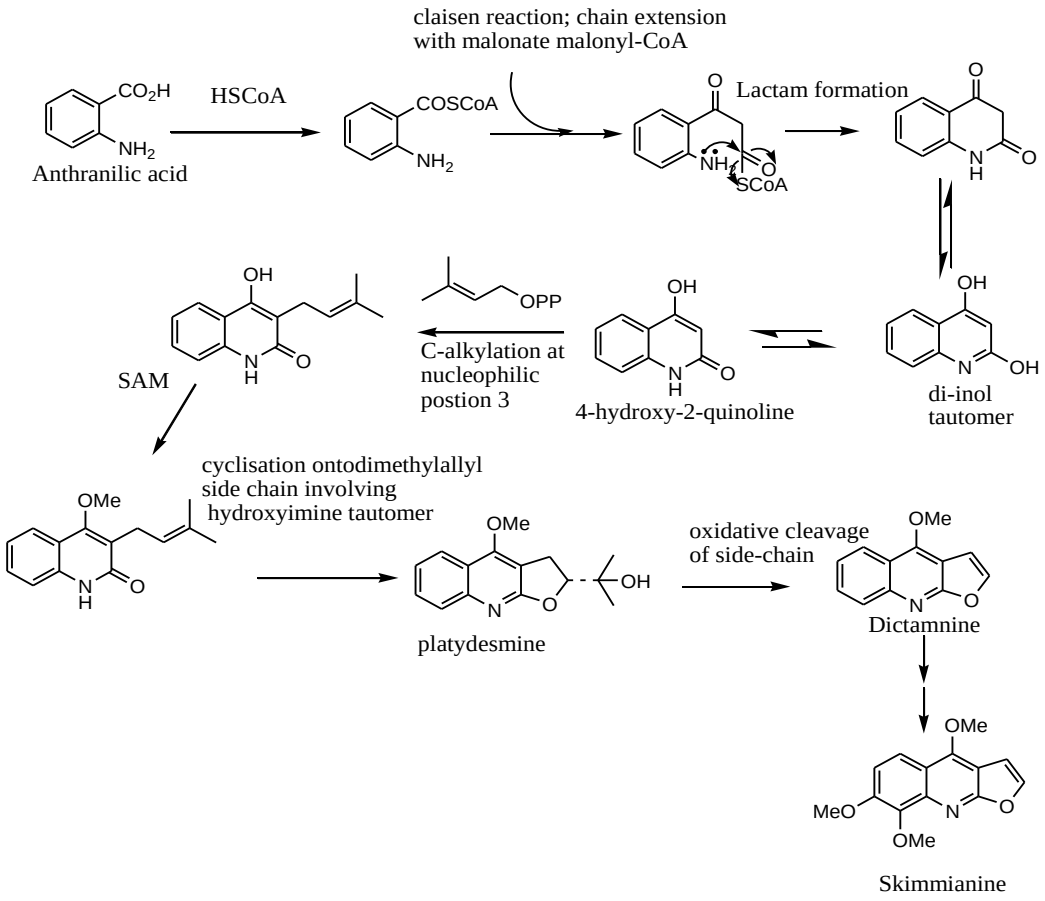


Figure 2.7: The basic structure of 2-quinoline alkaloids

2.7.8.17 Biosynthesis of quinoline alkaloids

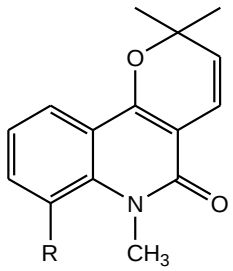
The precursor for the rutaceous quinoline alkaloids is anthranilic acid (Fish and Waterman, 1973). Quinazoline and acridine alkaloids are also derived from anthranilic acid. Below is a scheme showing how dictamnine (**77)** and skimmianine **(78)** are biosynthesized from anthranilic acid (Dewick, 2009). Scheme 2.7 illustarates how quinolone alkaloids are biosythesised.



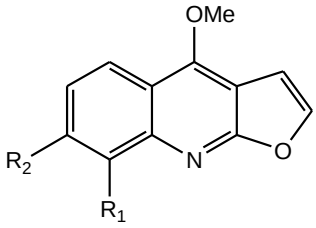
Scheme 2.7: Biosynthesis of quinolone alkaloids

2.7.8.18 2-quinoline alkaloids of *Zanthoxylum*

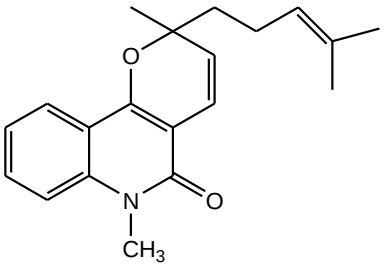
Pyranoquinolines and furanoquinolines are the two types of quinoline alkaloids found to be very common in genus *Zanthoxylum*. Rahman et al (2005) reports the isolation of two pyranoquinolines: *N*-methylflindersine (**75)** and Zanthobungeanine (**76)** together with two furanoquinolines, dictamine (**77)** and skimmianine (**78)** from *Z. bundruga*. Zhantosimulin (**79)** and haujiaosimulin (**80)** were also isolated from *Z. simulans* (Chen et al., 1996).



75: R=H
76: R=Me



77: R_1= R_2 = H
78: R_1= R_2= OMe



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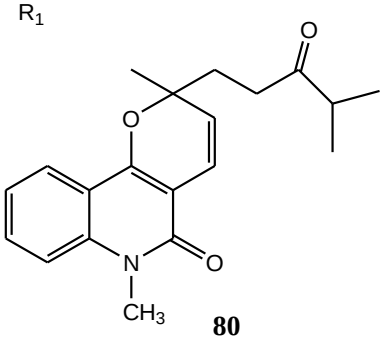


80

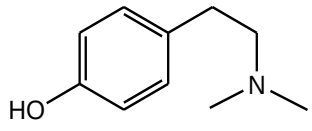
2.7.8.19 Other alkaloids from *Zanthoxylum*

Though not very common, some other types of alkaloids have been isolated from *Zanthoxylum* species. These include bishoderninyl terpene, indolopyridoquinazoline, canthin-6-one, quinazoline and carbazole alkaloids. Some of these alkaloids together with their sources are given in Table 2.4.

Table 2.4: Other alkaloids from *Zanthoxylum* genus

Alkaloid	**Type of alkaloid**	**Plant source**	**reference**
Hordenine (**81)**	Bishoderninyl alkaloid	*Z. culantrillo*	Swinehart and Stermitz, 1980
Candicine (**82)**	Bishoderninyl alkaloid	*Z. culantrillo*	,,
Synerphrine **(83)**	Bishoderninyl alkaloid	*Z. culantrillo*	,,
Hordenine (**84)**	Bishoderninyl alkaloid	*Z. culantrillo*	,,
Canthin-6-one (**85)**	Canthin-6-one	*Z. chiloperone*	Ferreira et al., 2002
5-Methoxy-canthin-6-one (**86)**	Canthin-6-one	*'Z. chiloperone*	Ferreira et al., 2002
Lunacridina (**87)**	quinazoline	*Z. budrunga*	Ahmad et al., 2003

Alkaloid	Type of alkaloid	Plant source	reference
3-Methoxy-9-methyl-9H-carbazol-2-ol (**88)**	carbazole	*Z. rhoifolium*	Taborda and Cuca, 2007
Rutaecarpine (**89)**	indolopyridoquinazoline	*Z. integrifoliolum*	Sheen et al., 1996



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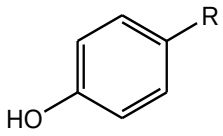
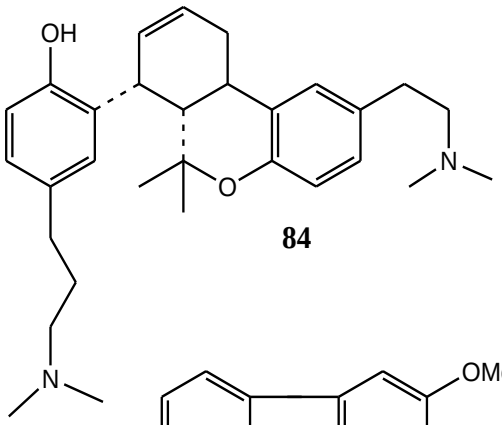


82: R= $CH_2CH_2NMe_2$
83: R= $CH(OH)CH_2NHMe$



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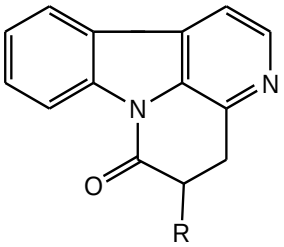
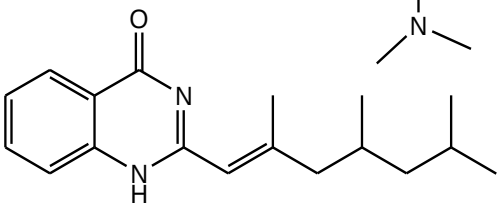


85: R = H
86: R = OCH_3



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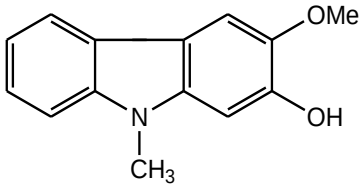

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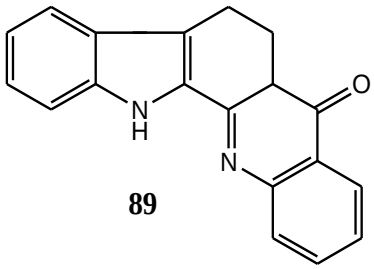


89

2.7.8.20 Coumarins

Coumarins are fragarant organic chemical compounds in the benzopyrone chemical class, which are found naturally in many plants. Coumarins appear as colourles crystalline substances in their standard states. The basic Skeleton of coumarin show a C_6-C_3 oxygenated heterocycle as shown in the Figure 2.8 (Dewick, 2009).

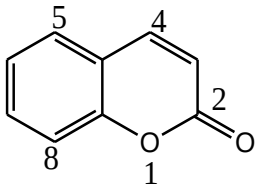
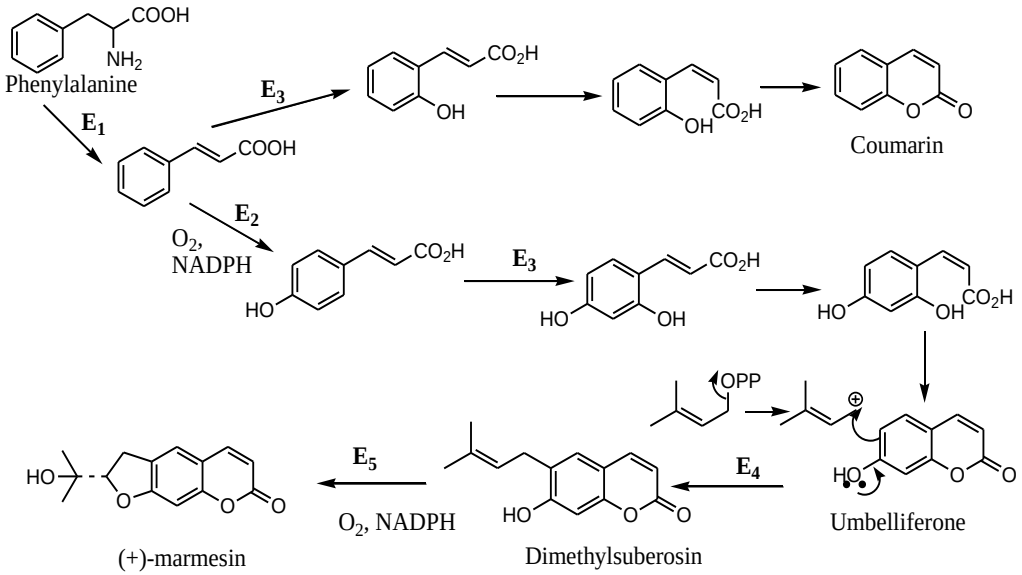


Figure 2.8: The basic Skelton of coumarins

2.7.8.20.1 Biosynthesis of coumarins

Shikimic acid pathway is responsible for the biosynthesis of coumarins in plants (Fish and Waterman, 1973). This accurs through hydroxylation, glycolysis and cyclisation of Cinnamic acid, which is an intermidiate from deamination of phenylalanine (Dewick, 2009). The scheme 2.8 shows how coumarins are biosynthesized from cinamic acid.



E_1: phenylalanine ammonia lyase (PAL), **E_2**: cinnamate-4-hydroxylase, **E_3**:cinnamate-2-hydroxylase, **E_4**: umbelliferone-6-prenyltransferase, **E_5**: marmesin synthase

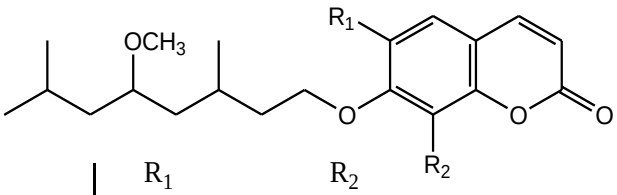
Scheme 2.8: Biosynthesis of coumarins

2.7.8.20.2 Coumarins of *Zanthoxylum*

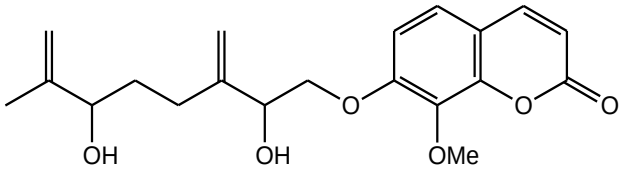
The genus *Zanthoxylum* is characterized by the presence of different types of coumarins, which include simple, linear, dihydrofuro-coumarins, furo-coumarins and pyroano-coumarins (Murray et al., 1989; Waterman and Grundon, 1983). The Table 2.5 lists some of the coumarins reported from the genus *Zanthoxylum.*

Table 2.5: Some of the coumarins from the genus *Zanthoxylum*

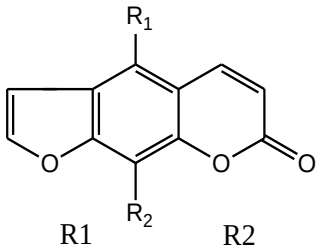
Coumarin	**Plant source**	**Reference**
5'-Methoxyauraptine (**90)**	*Z. avicennae*	Cho *et al*, 2012
6,5'-Dimethoxyaucollininraptene (**91)**	,,	,,
5'-Methoxycollinin (**92)**	,,	,,
7-(2',6'-Dihydroxy-7-methyl-3'-methyleneocta-7'-enioxy)-8-methoxy-coumarin **(93)**	*Z. schinofolium*	Chang *et al*, 1997
Isopimpinellin (**94)**	*Z. rhoifolium*	Arruda *et al*, 1992
Phellopterin (**95)**	,,	,,
Imperapterin (**96)**	,,	,,
Aurapten (**97)**	,,	,,
Umbelliferone (**98)**	,,	,,
5'-Hydroxyaurapten **(99)**	,,	,,



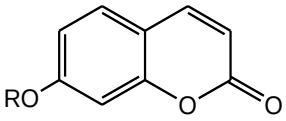
	R_1	R_2
90	H	H
91	OCH_3	H
92	H	OCH_3



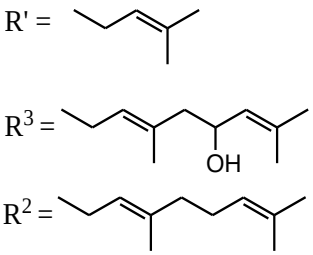
93



	R1	R2
94	OCH_3	CH_3
95	OCH3	R'
92	H	R'

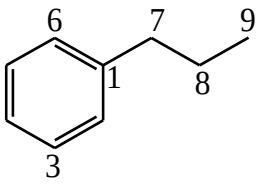


	R
97	R^2
98	H
99	R^3



2.7.8.21 Liginans and neoliginans

Liginans and neo-liginans are polyphenolic substances derived from phenylalanine via dimerization of substituted cinnamic alcohols (Dewick, 2009). The biogenetic synthesis of lignans and neolignans follows the shikimic acid pathway (Fish and Waterman, 1978). They are characterized by the coupling of the C_6-C_3 units. In nomenclature, the C_6-C_3 unit is treated as propylbenzene (a) and numbered from 1 to 6 in the ring, starting from the propyl group, and with the propyl group numbered from 7 to 9 starting from the benzene ring. Incase there is a second C_6-C_3 unit the numbers are primed (IUPAC commission, 1978).



(a)

When a bond between postions 8 and 8’ links the two C_6-C_3 units, the compound is reffered to and named as liginan **(b)**. In the absence of the C-8 to C-8’ bond, and where a carbon-carbon bond links the two C_6-C_3 units, it is reffered to and named as a neo-lignan **(c).** Where there is no direct carbon-carbon bond between the C_6-C_3 units and an ether oxygen atom links them, the compound is named as an oxy neo-ligna **(d)** (IUPAC commission, 1978)

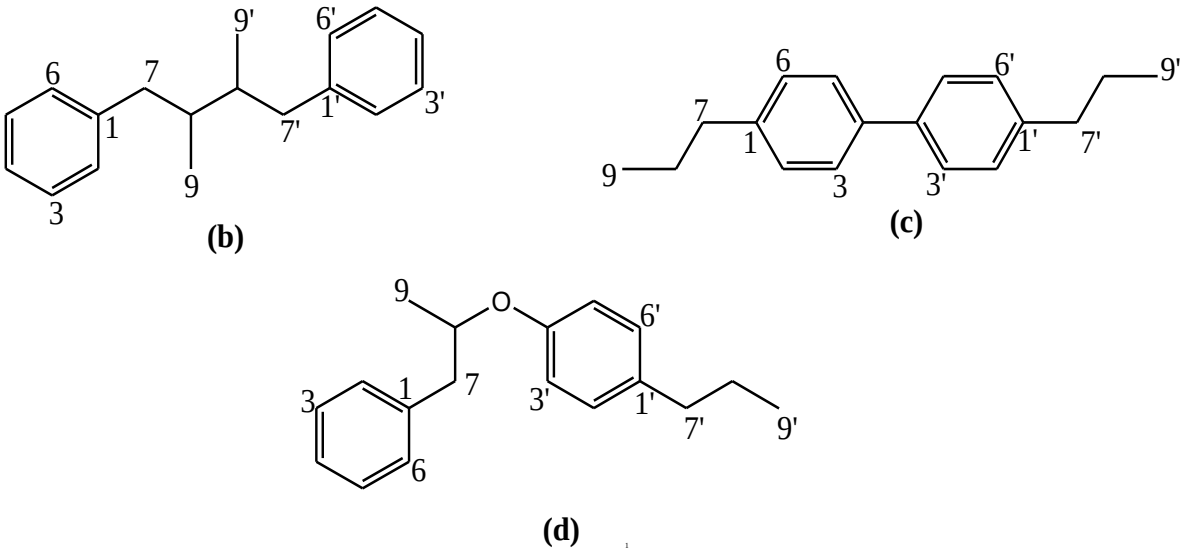


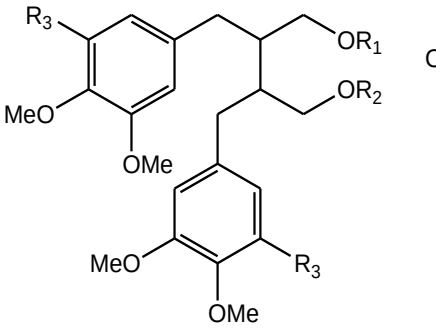
Figure 2.9: The basic skeleton of liginans and neo-lignan

2.7.8.21.1 Liginans and neoliginans of *Zanthoxylum*

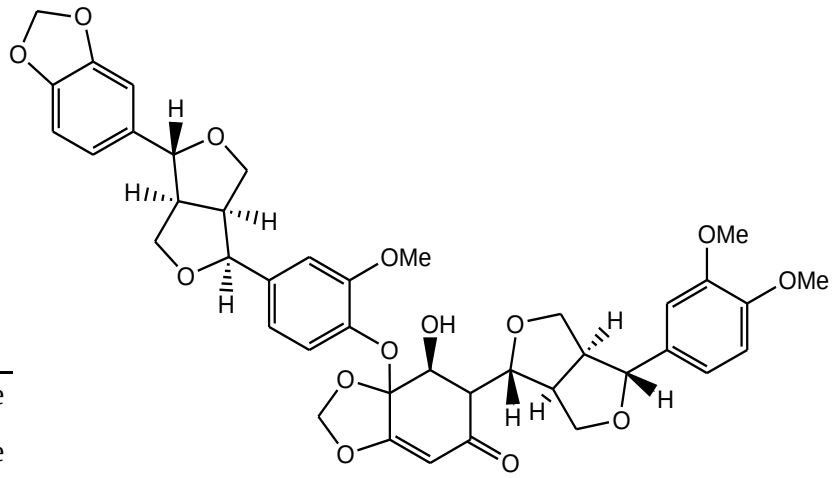
Table 2.6 below, shows examples of lignans and a neolignan that have been reported in the genus *Zanthoxylum*

Table 2.6: Examples of lignans of *Zanthoxylum* species

Lignan	**Plant source**	**References**
Meso-2,3-bis(3,4,5 trimethoxybenzyl)-1,4-butanediol (**100)**	*Z. heitzzi*	Ngou et al., 1994
4-Acetoxy-2,3-bis(3,4,5-trimethoxybenzyl)-1-butanol (**101)**	,,	,,
Zanthpodocarpin **(102)**	*Z. podocarpum*	Zhou et al., 2011
(-)-Seasamin (**103)**	*Z. bundruga*	Rahaman et al., 2005
(-)-Simulanol (**104)**	*Z. simulans*	Yang et al., 2002
7,9'-Epoxylignan (**105)**	*Z. culantrillo*	Luis et al., 1998
Eudesmin (**106)**	*Z. armatum*	Guo et al., 2011
Kobusin (**107)**	*Z. armatum*	Guo et al., 2011



	R_1	R_2	R_3
100	H	H	OMe
101	H	Ac	OMe



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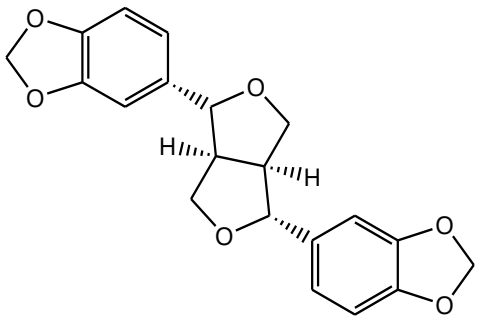

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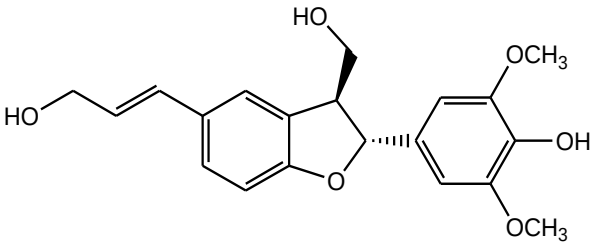

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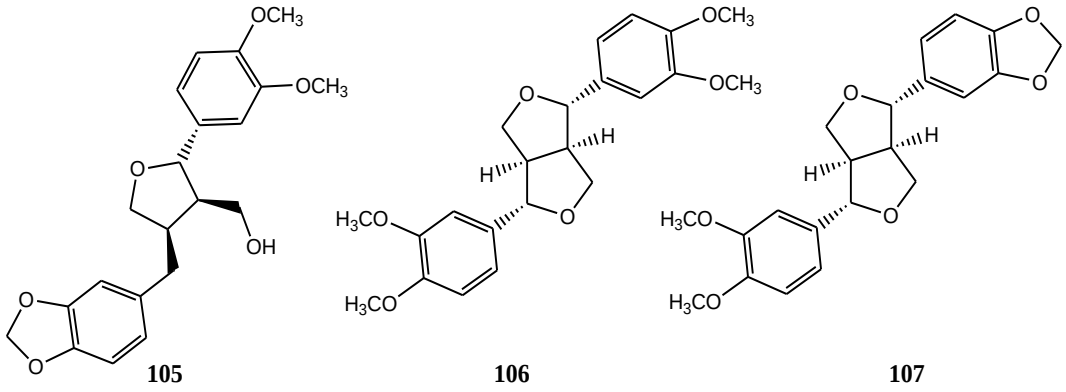


2.7.8.22 Flavonoids

Flavanoids are naturally occurring phenolic compounds with a characteristic unit of C_6-C_3-C_6 forming a 2-phenylbenzopyran-4-one structure (Federica et al., 2009). They are grouped in several subclasses such as anthocyanins, flavans, isoflavonoids, flavones, flavanones, flavanols and chalcones. Flavanoids are biosynthesized through the shikimate pathway utilizing cinnamoyl-CoA as a starter unit whose chain is extended by three molecules of malonyl-CoA (Dewick, 2009). Importantly flavanoids are known for their diverse biological properties, such as antioxidants, anti-inflammatory, antithrombotic, antibacterial, antihepatotoxic, antitumor, antihypertensive, antiviral, antiallergic and estrogenic (Andersson et al., 1996; Harborne and Williams, 2000). They are present in genus *Zanthoxylum* in almost all plant organs and play an important role in the antioxidant defense system.

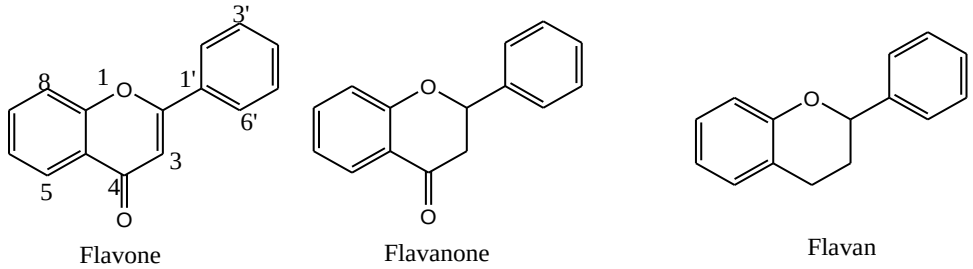
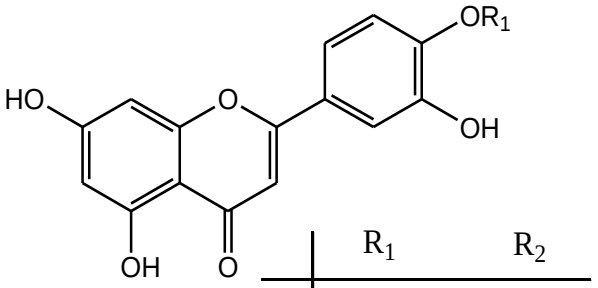


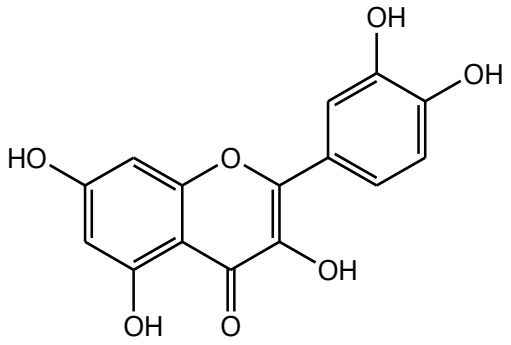
Figure 2.10: Basic Skelton of flavones, flavanone and flavan.

Table 2.7: Some of the flavanoids isolated from the species of genus *Zanthoxylum.*

Flavanoid	**Plant source**	**Reference**
Diosmetin (**108)**	*Z. avicennae/ leaves*	Cho et al., 2012
Apigenin (**109)**	,,	,,
Quercetin (**110)**	*Z. bungeanum*	Xiong et al., 1994
3,5-diacetyltambulin (**111)**	*Z. intergrifoliolum*	Chen et al., 1999
Isorhamnetin-7-O-glucoside(**112)**	*Z. armatum*	Xiong et al, 1994



	R_1	R_2
108	Me	OH
109	H	H



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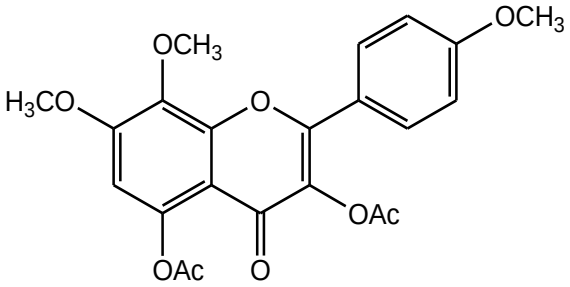

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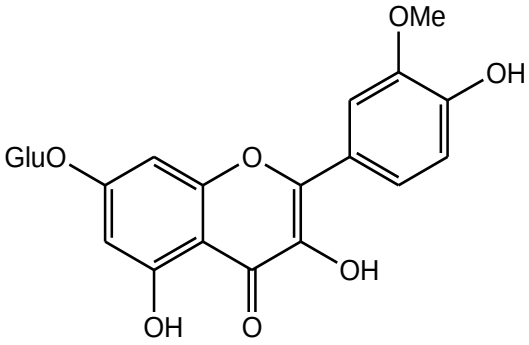

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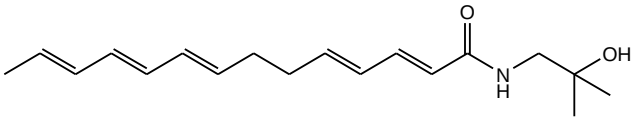
2.7.8.23 Amides of *Zanthoxylum*

The genus *Zanthoxylum* is characterized chemically by the frequent accumulation of olefinic alkamides (unsaturated aliphatic amides) whose biogenetic origin is the condensation of fatty acids with isobutyl amines. Notably isobutyl amides are biologically known to have insecticidal properties

where as alkamides have analgesic effects. Aromatic amides have also been isolated in several species of the same genus (Patino et al., 2012).

Table 2.8: Highlights some of the amides from *Zanthoxylum* genus

Amide	**Plant source**	**Reference**
(2E, 4E, 8E, 10E, 12E)-N-isobutyl-2, 4, 8,10, 12-tetra-decapentaenamide (**113)**	*Zanthoxylum spp* (fruits)	Kishiwanda et al., 1997
Rubesamide (**114)**	*Z. rubescens*	Adesina and Reisch, 1989
Zanthomamide (**115)**		
Rubemamin (**116)**		
Lunyuamide I (**117)**	*Z. intergrifoliolum*	Chen et al., 1999
Lunyuamide II (**118)**		



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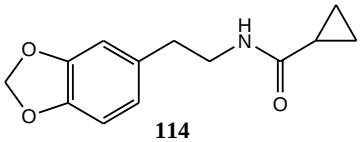

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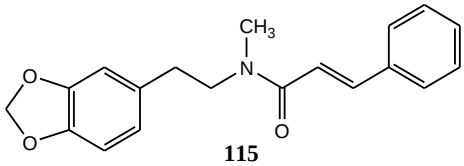

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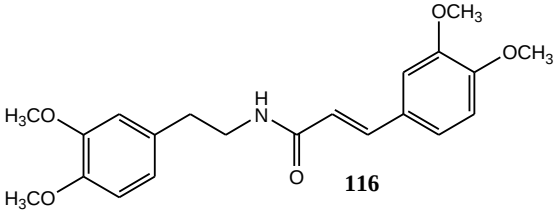

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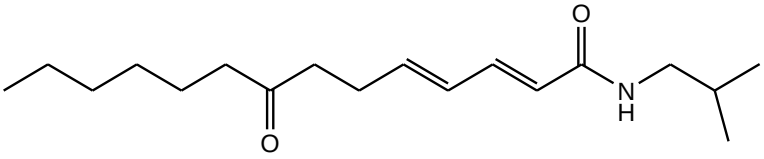

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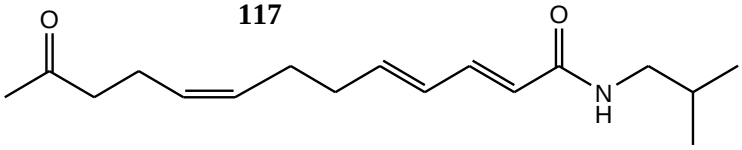

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CHAPTER THREE
MATERIALS AND METHODS

3.1 General

Merck silica gel 60 (70-230 mesh) and Sephadex LH-20 were used as stationary phases for column chromatography (CC). PTLC (1.0 mm, 20 x 20 cm) were prepared using Merck silica gel 60 ($PF_{254+366}$) for purification; factory made analytical aluminium TLC plates (silica gel 60 $F_{254,}$ Merck) were used to monitor the purity of the compounds by visualizing the spots under UV light at 254 or 366 nm, followed by spraying with iodine and Drangendorff's reagent.

The ^{1}H and ^{13}C NMR spectra were recorded using Varian-Mercury 200 MHz and Bruker Avance 500 and 600 MHz spectrometers. The Homonuclear Correlation Spectroscopy (COSY), Heteronuclear Single Quantum Coherence (HSQC) and Hetronuclear Multiple Bond Connectivity (HMBC) spectra were obtained using standard Bruker software. Chemical shifts were measured in ppm and are given in δ values relative to the internal standard tetramethyl silane (TMS). The EI-MS spectra were recorded at 70 eV on GC-TOF micromass spectrometer (micromass Wythenshawe, waters Inc. UK). The major solvents used for chromatography were acetone, dichloromethane, methanol, ethyl acetate and n-hexane, which in all cases were glass distilled.

3.2 Plant materials

Zanthoxylum holstzianum (stem bark) was collected from Diani Veminant Forest, Coastal Province of Kenya in February 2011, identified at the African Harbarium at the National Museum of Kenya where a voucher specimen is deposited. The root bark of the same plant was collected in June 2012 from the same Forest, identified by a botanist at the School of Biological Sciences, University of Nairobi where a voucher specimen (number 2012/AD-001) was deposited. In each case, the plant

material was air-dried and pulverized using a Willy mill available at the Department of Chemistry, University of Nairobi.

3.3 Extraction and isolation of compounds from stem bark of *Zanthoxylum holstzianum*

The shade, air-dried and ground (pulverized) stem back of *Zanthoxylum holstzianum* (3.4 kg) was defatted using (4 × 4 L) n-hexane for 24 hours, in each case by cold percolation. This yielded 170 g of oily extract. The marc was then dried and further extracted with (CH_2Cl_2/MeOH; 1:1 (6× 4 L) at room temperature, yielding 324 g (9.5%) of gummy oily extract. In each case, the extract was seperated by filtration and concentrated using an IKA-Werk 7813 Staufen (RV-05) rotary evaporator.

The gummy and oily extract (324 g) was acidified with 2 M HCl solution and extracted with distilled dichloromethane followed by ethyl acetate yielding 138 g of oily extract after concentration. The remaining aqueous layer was basified with 37% aqueous ammonia and extracted by CH_2Cl_2 followed by EtOAc two times, yielding 4 g of the extract after concentration.

The basic organic extract (4 g), was adsorbed on 5 g of silica gel and loaded over silica gel (200 g) n-hexane slurry of 200 g of silica gel in a column. The elution was started with 100% distilled n-hexane containing increasing amounts of CH_2Cl_2 (5, 10, 15, 20, 25, 30, 40, 60, and 100%) followed by CH_2Cl_2 containing increasing amounts of methanol (2, 4, 10, 20 and 50%) and finally the column was washed with 100% methanol. In total 86 fractions, each (250 ml) were collected. Fraction 15-17 (eluted at 30% of hexane in CH_2Cl_2), were combined, crystallized and purified using Sephadex LH-20 (CH_2Cl_2/MeOH; 1:1 v/v) yielding 11 mg of 8-acetonyldihydrochelerythrine (**3).** Fractions that were eluted at 20% of CH_2Cl_2/MeOH yielded 30 mg of nitidine (**6)**.

The acidic organic extract (138 g) was adsorbed on 160 g of normal silica gel and was defatted by VLC in a Buchner funnel packed with preparative TLC grade silica. The sample was then eluted with n-hexane until the eluent became colourless, yielding 120 g of oil after concentration. From this fraction, colourles crystals were obtained and re-crystallized to give 340 mg of dihydrochelerythrine (**1)**. Further fractionation was done using 100% CH_2Cl_2, CH_2Cl_2/MeOH (1:1 v/v) and 100% Methanol yielding 32.48 g, 40.75 g and 3.1 g, respectively, after concentration.

The CH_2Cl_2 fraction (32.4 g) was adsorbed on 60 g of normal silica and loaded over silica gel (400 g) n-hexane slurry of in a column. Elution began with 100% n-hexane increasing polarity with distilled acetone (1, 2, 3, 4, 6, 8, 10, 15, 20, 30 and 50%) and finally washing the column with 100% acetone. From the fractions eluted with acetone at 1%, colourles crystals were obtained, and recrystalisation yielded 3 g of *N*-methylflindersine (**8)**. Fractions eluted with acetone 2% yielded white crystals, which were purified by Sephadex LH-20 and recystalisation yielding 40 g of norchelerythrine **(2).** 62 mg of holstzianoquinoline (**9)** was obtained as white crystals at 6% and when dissolved in CH_2Cl_2 turned yellow, in addition it showed signs of decomposition on the TLC plates after multiple developments. Fractions eluted at 10% acetone gave colourles crystals, which were purified by crystalisation and Sephadex giving 30 mg of 4,4’-dihydroxy-3,3’-dimethoxylignan-9,9’-diyl diacetate (**10)**. 40 mg of arnottianamide (**7)** was obtained from fractions eluted at 15% acetone as white amorphous solid.

3.4 Extraction and isolation of compounds from the root bark of *Zanthoxylum holstzianum*

The shade air-dried and pulverized root back of *Zanthoxylum holstzianum* (1.6 g) was exhaustively extracted by cold percolation using CH_2Cl_2/MeOH; 1:1 v/v, (4L×5) for 24 hours in each case. Filtration was then done followed by concentration using IKA-Werk 7813 Staufen (RV-05) rotary evaporator yielding 292 g of highly gummy oily extract (18% yield). The extract was then

partitioned between water and ethyl acetate. The organic layer (ethyl acetate layer) yielded a very oily extract (230 g).

The organic layer (230 g) extract was adsorbed on 250g of silica gel and defatted by VLC in a Buchner funnel packed with preparative TLC grade silica. First eluting with sufficient n-hexane until a colourles eluent was obtained did the defatting process. After concentration, the hexane extract gave 120 g of oil. The fractionation process was continued by eluting with CH_2Cl_2 followed by EtOAc and finally with Methanol, yielding 47, 23 and 15 g respectively after concentration.

The CH_2Cl_2 (47 g) and EtOAc (22.6 g) fractions were combined resulting into 69.6 g of extract which was adsorbed on 100g of silica gel and loaded over n-hexane slurry of 450 g of silica gel in a column. The column was eluted with n-hexane containing increasing amounts of acetone (0.5, 1, 2, 3, 5, 8, 12, 16, 20, 30, 50 and finally 100% acetone). Fraction 2-5 (eluted with 100% hexane) yielded compound **(1) (**300 mg**)** that was purified by re-crystallization. Fractions 10-13 (eluted at 0.5% acetone in hexane) gave *N*-methylflindersine (**8)**) (4 g). Fractions 14-18 (eluted at 1% acetone in hexane) crystallized into white crystals, which showed two compounds on a TLC plate. Purification using sephadex and preparative TLC resulted into compounds (**5)** (15 mg) and (**3)** (13 mg). Crystallization of fractions 41-45 (eluted at 4% acetone in hexane) resulted into 24 mg of chelerythridimerine **(4).** Fraction 54-59 (eluted at 8% acetone in hexane) resulted into holstzianoquinoline (**9)** (250 mg). Fractions 77-82 (eluted at 12% of acetone in hexane) gave arnottianamide (**7)** (20 mg). Fraction 83-100 (eluted at 15% acetone in hexane) resulted into 9-Demethyloxychelerythrine (**5)** as white amorphous solid (300 mg).

3.5 Antiplasmodial assay test

The crude and the pure compounds were assayed using a non-radioctive assy technique (Smilkstein et al., 2004), with modifications according to Johnson et al. (2007) to determine 50% growth inhibition of the cultured parasites. Two *Plasmodium falciparum* parasite strains, chloroquine-sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2) were grown as described by Johnson et al (2007). Crude extract, pure compounds and the reference drug were dissolved in 99.5% DMSO and diluted in complete Rosewell Park Memorial Institute 1640 series of cell culture medium (RPMI 160) prepared as described by Akala et al (2007). The basic culture medium was prepared from RPMI 1640 powder and the complete RPMI 1640 media was stored at 4^{o}C and used within 2 weeks.

Two-fold serial dilutions of chloroquine and test samples were prepared on a 96-well plate, making sure that the amount of DMSO is equal or less than 0.0875%. The culture-adapted *P. falciparum* at 2% hematocrit and 1% parastemia, were added on the plate containing a range of drug doses and incubated in a gas mixture (5% CO_2, 5% O_2 and 90% N_2) at 37^{o}C. The termination of the assy was done 72 hours later by freezing at -80^{o}C. The parasite growth quantified as mean ± stardard deviation (Mean IC_{50} ± SD) as described by Johnson et al (2007).

3.5. Antinociceptive assay tests

3.5.1. Experimental animals and sample administration

Adult Swiss albino mice of both sexes weighing 22-27 g were used in the experiment. The breeds were obtained from the animal house, Medical Physiology Department, University of Nairobi. The animals were kept in cages with food and water and put in a room maintained at room temperature and controlled lighting (24 hour light / dark cycles). Twenty-four hours before the start of the experiments, habituation to the equipment was done. Laboratory animal care guidelines and

procedures were followed throughout the experiment. The tests were carried out during the daytime in a quite laboratory with ambient illumination and temperatures approximately equal to those in the animal house. The animals were allowed to acclimatize to the test laboratory conditions for one hour prior to the experiments. Five (5) animals (mice) were used in each set of experiment as well as in the control.

Each mouse was randomly and carefully picked by the tail and placed on a bench. Using the left hand, the loose skin of the dorsal side of the mouse's neck was placed on the left hand palm while holding the tail with the left hand little finger. This was followed by exposing the ventral side of the mouse and 0.2 ml of the sample or vehicle injected intraperitoneally (i. p.) using a 1 ml plastic syringe one hour prior to the test. The injection was done approximately 1 cm to the left of the midline. In order to ensure that the sample is not injected into the intestines, aspiration was done.

3.5.2 The tail flick test

A radiant heat, An IITC. Model 33 tail-flick analgesiometer was used to measure latency responses according to the method described in literature (D'Amour and Smith, 1941). Animals responded to a focused heat stimulus by removing or flicking their inflicted tail exposing a photocell in the apparatus immediately below the tail. The reaction time was recorded for animals pre-treated with vehicle (control), different doses of pure compounds and the stem bark crude extract approximately one hour before testing. To minimize tissue damage an automatic 15 seconds cut off was used.

3.5.3 Statistical analysis

The data was presented as mean ± S. E. M (Standard Error of the Mean) and was analysed using the unpaired t-test analysis (student t-test), one-way analysis of variance (ANOVA) followed by

Schaffe's *post hoc* test. The difference in the test versus control values was considered statistically significant at $P<0.05$ (95% confidence level).

3.6 Larvicidal assy test

The eggs of *Aedes aegypti L*. (Diptera: Culicidae) were obtained from Zoology Department, University of Nairobi. They were left to hatch at about 28^{o}C and develop into twenty-second (early fourth) instar larvae. The health looking larvae were then transferred in Petri-dishes containing the test extracts and pure compounds of different concentrations. The samples were prepared by dissolving twenty milligrams of each sample in 2 ml of dimethyl sulfoxide (DMSO). From the stock solution, different concentrations were prepared by serial dilution using distilled water. For the crude extract, the larvae were tested for mortality at 100, 50, 25, 12.5 and 6.25 µg/ml while pure compounds at 20, 10, 5, 2.5 and 1.25 µg/ml. The control in all cases received the amount of DMSO exactly as in the test larvae setup. Larvae mortality was checked after 24 and 48 hours. LC_{50} and LC_{90} mean values were determined from the three observations of each concentration using SPSS Ver.16.0, Regression probit analysis.

3.7 Physical and spectroscopic data of the isolated compounds

3.7.3 Dihydrochelerythrine (1)

Colourles crystals, CH_2Cl_2, Rf = 0.82 (25% ethylacetate in hexane) mp 150-160^{o}C. ^{1}H NMR (500 MHz, CD_2Cl_2): δ_H 6.96 (1H, *d*, *J* = 10 Hz, H-3), 7.51 (1H, *d*, *J* = 10 Hz, H-4), 4.27 (2H, *s*, H-8), 7.71 (1H, *d*, *J* =10 Hz, H-9), 7.48 (1H, *d*, *J* = 10 Hz, H-10), 7.12 (1H, *s*, H-11), 7.66 (1H, *s*, H-14), 6.14 (2H, *s*, O$C\underline{H}_2$O), 4.11 (3H, *s*, MeO-1), 4.04 (3H, *s*, MeO-2), 2.58 (3H, *s*, CH_3-7). ^{13}C NMR (125 MHz, CD_2Cl_2): δ_C 146.0 (C-1), 152.7 (C-2), 111.4 (C-3), 118.8 (C-4), 126.4 (C-4a), 126.3 (H-5), 145.0 (C-6), 48.9 (C-8), 124.6 (C-8a), 120.4 (C-9), 123.9 (C-10), 131.1 (C-10a) 104.4 (C-11),

148.4 (C-12), 148.7 (C-13), 100.8 (C-14), 126.7 (C-14a), 101.7 (O<u>C</u>H_2O), 61.7/56 (MeO-1/2), 41.4 (Me-7).

3.7.5 Norchelerythrine (2)

Colourles crystals, CH_2Cl_2. ^{1}H NMR (500 MHz, CD_2Cl_2): δ_H 7.63 (1H, *d*, *J* = 10 Hz, H-3), 8.36 (1H, *d*, *J* = 10 Hz, H-4), 9.70 (1H, *s*, H-8), 8.38 (1H, *d*, *J* = 10 Hz, H-9), 7.87 (1H, *d*, *J* =10 Hz, H-10), 7.28 (1H, s, H-11), 8.68 (1H, *s*, H-14), 6.14 (2H, *s*, OC<u>H</u>$_2$O), 4.11/4.04 (6H, *s*, 2×MeO-1/2). ^{13}C NMR (125 MHz, CD_2Cl_2): δ_C 145.6 (C-1), 159.6 (C-2), 118.9 (C-3), 118.2 (C-4), 121.9 (C-4a), 129.8 (H-5) 139.2 (C-6), 146.6 (C-8), 127.9 (C-8a), 118 (C-9), 126.9 (C-10), 120.0 (C-10a), 104.3 (C-11), 148.4 (C-12), 148.6 (C-13), 101.9 (C-14), 129.2 (C-14a), 101.7 (O<u>C</u>H_2O), 61.7/58.8 (MeO-1/2).

3.7.1 8-Acetonyldihydrochelerythrine (3)

Colourless crystals, CH_2Cl_2, Rf = 0.59 (25% ethylacetate in hexane), mp 165-170^{o}C, $[a]_D^{23.4}$ = +4.11^0 (c 0.035, CH_2Cl_2). ^{1}H NMR (600 MHz, CD_2Cl_2): δ_H 6.98 (1H, *d*, *J* = 9.0 Hz H-3), 7.55 (1H, *d*, *J* = 9.0 Hz, H-4), 5.04 (2H, *dd*, *J* = 3.6, 11 Hz, H-8), 7.73 (1H, *d*, *J* = 8.4 Hz, H-9), 7.50 (1H, *d*, *J* = 8.4 Hz, H-10), 7.12 (1H, *s*, H-11), 7.50 (1H, *s*, H-14), 2.57-2.21 (2H, *dd*, *J* = 3.6, 11, 3.2 Hz, H-1'), 2.09 (3H, *s*, CO<u>CH</u>$_3$), 6.05 (2H, *s*,–OC<u>H</u>$_2$O-), 3.93 (3H, *s*, OMe), 3.93 (3H, *s*, OMe), 2.61 (3H, *s*, N-CH_3).^{13}C NMR (150 MHz, CD_2Cl_2): δ_C 145.6 (C-1), 152.3 (C-2), 111.6 (C-3), 118.8 (C-4), 127.6 (C-4a), 131.3 (C-5), 139.5 (C-6), 55.0 (C-8), 128.5 (C-8a), 118.8 (C-9), 123.9 (C-10), 123.1 (C-10a), 104.2 (C-11), 147.7 (C-12), 148.2 (C-13), 100.4 (C-14), 127.6 (C-14a), 47 (C-1'), 206.9 (C-2'), 30.5 (H-3'), 101.4 (-O<u>C</u>H_2O-), 60.8/55.7 (MeO-1/2), 42.7 (N-<u>C</u>H_3). EIMS m/z: 405 $[M]^+$, 406, 349, 348, 333, 290.

3.7.9. Chelerythridimerine (4)

White crystals, MeOH+CH_2Cl_2, Rf = 0.55 (25% ethyl acetate in hexane). ^{1}H NMR (200 MHz, $CDCl_3$+MeOH): δ_H 6.84 (1H, *d*, *J* = 8.4 Hz, H-3), 7.40 (1H, *d*, *J* = 8.4 Hz, H-4), 5.04 (1H, *dd*, *J*= 4.4, 9.8 Hz, H-8), 7.51 (1H, *d*, *J* = 8.6 Hz, H-9), 7.30 (1H, *d*, *J*= 8.6 Hz, H-10), 6.92 (1H, s, H-11**),**7.28 (1H, *s*, H-14), 2.24 (2H, *m*, H-1'), 5.8 (1H, *d*, *J* = 12.4 Hz, OC<u>H</u>$_2$O), 3.88 (3H, *s*, MeO-1), 3.87 (3H, *s*, MeO-2), 2.50 (3H, *s*, Me-7).^{13}C NMR (50MHz, $CDCl_3$+MeOH): δ_C 145.6 (C-1), 152.2 (C-2), 111.6 (C-3), 118.9 (C-4), 127.4 (C-4a), 131.1 (C-5), 139.3 (C-6), 54.4 (C-8), 128.4 (C-8a), 119.7 (C-9), 123.9 (C-10), 123.3 (C-10a), 104.3 (C-11), 147.6 (C-12), 148.1 (C-13), 100.6 (C-14), 127.4 (C-14a), 47.1 (C-1'), 208.3 (C-2'), 101.0 (OC<u>H</u>$_2$O), 61.1 (MeO-1), 55.9 (MeO-2). EIMS *m/z*: 752 $[M]^+$, 753, 350, 359,348, 333, 290.

3.7.10 9-Demethyloxychelerythrine (5)

White armophous solid, DMSO, Rf = 0.55 (30% ethyl acetate in hexane), mp 125-130^{o}C. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 7.35 (1H, *d*, *J* = 8.0 Hz, H-3), 8.07 (1H, *d*, *J* = 8.0 Hz, H-4), 8.13 (1H, *d*, *J* = 8.0 Hz, H-9), 7.60 (1H, *d*, *J* = 8.0 Hz, H-10), 7.36 (1H, *s*, H-11), 7.60 (1H, *s*, H-14), 6.17 (2H, *s*, OC<u>H</u>$_2$O), 3.75 (3H, *s*, Me-7), 3.86 (3H, *s*, MeO-1), 9.68 (1H, *brs*, HO-2). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 147.2 (C-1), 150.4 (C-2), 122.1 (C-3), 118.6 (C-4), 127.2 (C-4a), 116.8 (C-5), 134.8 (C-6), 161.2 (C-8), 118.9 (C-8a), 118.6 (C-9), 123.2 (C-10), 131.2 (C-10a), 104.4 (C-11), 147.1 (C-12), 146.8 (C-13), 102.3 (C-14), 120.3 (C-14a), 101.6 (O<u>C</u>H$_2$O), 40.5 (Me-7), 61.0 (MeO-7).

3.7.2 Nitidine (6)

White armophous solid, Rf = 0.33 (1% methanol in dichloromethane), mp 280-290^{o}C. ^{1}H NMR (600 MHz, DMSO-d_6): δ_H7.95 (1H, *s*, H-1), 8.37 (1H, *s*, H-4), 9.96 (1H, *s*, H-8), 8.95 (1H, *d*, *J* = 8.8 Hz, H-9) 8.25 (1H, *d*, *J* = 8.8Hz, H-10), 7.78 (1H, *s*, H-11), 8.32 (1H, *s*, H-14), 6.36 (2H, *s*, -OC<u>H</u>$_2$O-), 4.91 (3H, *s*, NC<u>H</u>$_3$), 4.24 (3H, *s*, OC<u>H</u>$_3$), 4.05 (3H, *s*, OC<u>H</u>$_3$). ^{13}C NMR (150 MHz,

DMSO-d_6): δ_C 109.3 (C-1), 158.7 (C-2), 151.9 (C-3), 103.8 (C-4), 124.6 (C-4a), 120.4 (C-5), 132.9 (C-6), 151.8 (C-8), 132.9 (C-8a), 119.8 (C-9), 130.5 (C-10), 119.9 (C-10a), 106.2 (C-11), 149.3 (C-12), 148.9 (C-13), 105.1 (C-14), 132.5 (C-14a), 103.2 (O<u>C</u>H_2O), 51.9 (N-<u>C</u>H_3), 57.8 (O<u>C</u>H_3), 56.8 (O<u>C</u>H_3).

3.7.8. Arnottianamide (7)

White armophous solid, DMSO, Rf = 0.43 (30% ethyl acetate in hexane), mp 130-140^{o}C. ^{1}H NMR (600 MHz, DMSO-d_6): δ_H 6.56 (1H, *d*, *J* = 8.4 Hz, H-4), 6.76 (1H, *d*, *J* = 8.4 Hz, H-5), 7.96 (1H, *s*, H-9), 7.81 (1H, *d*, *J* = 8.4 Hz, H-10), 7.24 (1H, *d*, *J* = 8.4 Hz, H-11), 7.46 (1H, *s*, H-12), 7.01 (1H, *s*, H-15), 6.18 (2H, *d*, OC<u>H</u>$_2$O), 3.82 (1H, *s*, MeO-3), 3.69 (1H, *s*, MeO-2), 2.89 (1H, *s*, Me-7), 8.92 (1H, *s*, HO-1). ^{13}C NMR (150 MHz, DMSO-d_6): δ_C 147.7 (C-1), 136.2 (C-2), 152.5 (C-3), 103.1 (C-4), 125.5 (C-5), 119.8 (C-5a), 134.4 (C-6), 135.5 (C-7), 163.3 (C-9), 127.6 (C-10), 127.0 (C-11), 130.7 (C-11a), 104.3 (C-12), 148.9 (C-13), 147.9 (C-14), 98.7 (C-15), 125.5 (C-15a), 101.7 (-O<u>C</u>H_2O-), 60.4 (MeO-2), 55.7 (MeO-3), 32.8 (Me-8). EIMS *m/z*: 381 $[M]^+$, 383, 382, 353, 322, 307, 161, 152, 139.

3.7.4 *N*-methylflindersine (8)

Colourles crystals, CH_2Cl_2, Rf = 0.57 (25% ethylacetate in hexane), mp 75-80^{o}C. ^{1}H NMR (500 MHz, CD_2Cl_2): δ_H 7.97 (1H, *dd*, *J* = 8, 1.6 Hz, H-5), 7.25 (1H, *m*, *J* = 8, 7.3, 1.6, 1 Hz, H-6), 7.55 (1H, *m*, *J* = 7.3, 1.6 Hz, H-7), 7.34 (1H, *dd*, *J*= 8.5, 1 Hz, H-8), 6.73 (1H, *d*, *J* = 10 Hz, H-1'), 5.55 (1H, *d*, *J* = 10 Hz, H-2'), 1.5 (6H, *s*, 4'), 3.63 (3H, *s*, Me-1). ^{13}C NMR (125 MHz ,CD_2Cl_2): δ_C 160.5 (C-2), 105.5 (C-3), 154.9 (C-4), 115.9 (C-4a), 122.9 (C-5), 121.5 (C-6), 130.8 (C-7), 113.9 (C-8), 139.5 (C-8a), 117.8 (C-1'), 126.0 (C-2'), 78.7 (C-3'), 27.1 (C-4'').

3.7.6. Holstzianoquinoline (9)

White crystals, CH_2Cl_2, Rf = 0.47 (20% ethylacetate in hexane), mp 210-214ºC, $[a]_D^{24}$ = -1.56^0 (c 0.41, CH_2Cl_2), UV (CH_2Cl_2) (λ_{max} nm) absorption at 230 (4.8), 247 (4.52), 260 (4.42), 280 (4.52), 290 (4.34) 332 (4.15), 349 (3.99) and 369 (3.67). ^{1}H NMR (200 MHz, $CDCl_3$): δ_H 6.93 (1H, *d*, *J* = 8.8 Hz, H-3), 7.51 (1H, *d*, *J* = 8.8 Hz, H-4), 4.9 (1H, *dd*, *J*= 3.2, 8.8 Hz, H-8), 7.71 (1H, *d*, *J* = 8.8 Hz, H-9), 7.46 (1H, *d*, *J* = 8.8 Hz, H-10), 7.11 (1H, *s*, H-11), 7.55 (1H, *s*, H-14), 6.08, 6.09 (each 1H, *d*, *J* = 1.2 Hz, OC$\underline{H}_2$O), 3.91/3.71 (3H×2, *s*, MeO-1/2), 2.61 (3H, *s*, Me-7), 1.91,1.70 (2H, *m*, H-1'), 5.75 (1H, *d*, *J* = 10.2 Hz, H-3'), 6.91 (1H, *d*, *J* = 10.2 Hz, H-4'), 7.36 (1H, *d*, *J* = 8.4 Hz, H-8'), 7.57 (1H, *ddd*, *J* = 8.4, 7.2, 1.2 Hz, H-9'), 7.26 (1H, *t*, *J* = 8.0, 7.2 Hz, H-10'), 8.11 (1H, *dd*, *J* = 8.0, 1.2 Hz, H-11'), 1.35 (3H, *s*, Me-2'), 3.74 (3H, *s*, Me-7). ^{13}C NMR (50MHz, CD_2Cl_2): δ_C 145.5 (C-1), 152.5 (C-2), 111.3 (C-3), 118.9 (C-4), 127.4 (C-4a), 131.4 (C-5), 139.6 (C-6), 54.0 (C-8), 127.6 (C-8a), 120.0 (C-9), 123.9 (C-10), 123.6 (C-10a), 104.7 (C-11), 147.7 (C-12), 148.3 (C-13), 101.3 (C-14), 127.4 (C-14a), 101.5 (OCH_2O), 61.0/55.9 (MeO-1/2), 42.8 (Me-7), 45.6 (C-1'), 81.2 (C-2'), 125.1 (C-3'), 116.4 (C-4'), 105.7 (C-5'), 161.4 (C-6'), 140.0 (C-7a'), 118.9 (C-8'), 130.8 (C-9'), 121.9 (C-10'), 123.6 (C-11'), 115.6 (C-11a'), 155.3 (C-12'), 28.7 ($\underline{C}H_3$-2'), 29.5 ($\underline{C}H_3$-7'). EIMS *m/z*: 588 $[M]^+$, 589, 349, 348, 333, 241.

3.7.7 4,4'-dihydroxy-3,3'-dimethoxylignan-9,9'-diyl diacetate (10)

Colourles crystals, CH_2Cl_2, Rf = 0.29 (20 % ethyl acetate in hexane), mp 160-170ºC. ^{1}H NMR (500 MHz, CD_2Cl_2): δ_H 6.53 (1H, *d*, *J* = 1.9 Hz, H-2), 6.77 (1H, *d*, *J* = 8.0 Hz H-5), 6.58 (1H, *dd*, *J* = 8.0 Hz, 1.9 Hz, H-6), 2.76, 2.59 (each, *dd*, *J* = 13.9, 7.3 Hz, 14.0, 7.5 Hz, H-7), 2.09 (1H, *m*, H-8), 4.00, 4.15 (each *dd*, *J* = 11.3, 5.5, 11.3, 6.0 Hz, H-9), 2.03 (3H, *s*, C$\underline{H}_3$), 3.79 (3H, *s*, OC$\underline{H}_3$), 5.54 (1H, *s*, OH). ^{13}C NMR (125 MHz, CD_2Cl_2): δ_C 131.9 (C-1), 111.4 (C-2), 146.6 (C-3), 144.0 (C-4), 113.9

(C-5), 121.7 (C-6), 34.9 (C-7), 39.9 (C-8), 64.3 (C-9), 170.0 (C=O), 20.8 (<u>C</u>H_3), 55.8 (MeO-3). EIMS *m/z*: 446 $[M]^+$, 447, 189, 137, 122.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Compounds isolated from *Zanthoxylum holstzianum*

Both the stem and root bark of *Zanthoxylum holstzianum* were extracted using CH_2Cl_2/MeOH (1:1, v/v) by cold percolation method. The extracts were subjected to Column Chromatography and several other purification methods yielding ten compounds. Seven compounds had a benzophenanthridine alkaloid skeleton; [8-acetonyldihydrochelerythrine **(3),** nitidine **(6)** dihydrochelerythrine **(1)**, norchelerythrine **(2)**, arnottianamide **(7)**, chelerythridimerine **(4)** and 9-demethyloxychelerythrine **(5)**], one magnetically symmetrical lignan [4,4'-dihydroxy-3,3'-dimethoxylignan-9,9'-diyl diacetate **(10)**], one 2-quinoline alkaloid [*N*-methylflindersine **(8)**] and one dimer of a benzophenanthridine and 2-quinoline alkaloid [holstzianoquinoline **(9)**]. It is interesting to note that holstzianoquinoline (**9**) is a dimer of dihydrochelerythrine and *N*-methylflindersine, the two major compounds isolated from both the root and stem bark and it is a new compound. This is its first report from *Zanthoxylum* and the second report of a C-C benzophenanthridine 2-quinoline dimeric compound from nature.

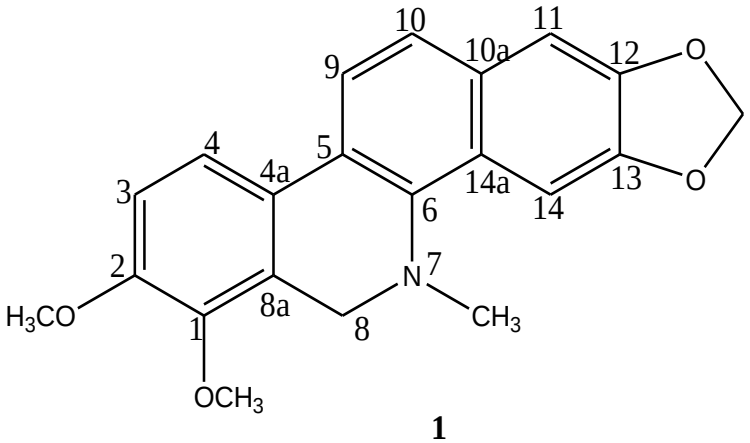
4.1.1 Dihydrochelerythrine (1)

Compound **3**, was obtained as colourles crystals. On TLC, it flouresced blue under UV (366 and 254 nm) and turned yellow on prolonged exposure to air and light. When sprayed with Drangendorff's reagent (alkaloid test), it changed to orange. These characteristics are typical of alkaloids with a benzophenanthridine skeleton (Kwok et al., 1987). Sixteen sp^2-hybridized carbons out of twenty-one carbon peaks were conspicuous on ^{13}C NMR spectrum, which is in agreement with a compound having a 5-ring benzophenanthridine skeleton (Nissanka et al., 2011). Further analysis of NMR

spectra data revealed the presence of two methoxyl (δ_H 4.11, 4.04; δ_C 61.7 and 56.8, respectively), methylenedioxy (δ_H 6.14, δ_C 101.7) and *N*-methyl (δ_H 2.58, δ_C 41.4) groups.

Biosynthetically, the common oxygenation in benzophenanthridine Skelton is at C-1, 2, 3 or 4 of ring A and at C-12 and C-13 of ring D. In few cases, oxygenation may occur in rings B and C (at C-8 and C-10). An ambiguous placement of methoxyl goups at C-1/C-2 and methylenedioxy at C-12/C-13 as well as *N*-methyl at C-7 was done, with the help of HMBC. Thus, ^3J correlation of the *N*-methyl with the -CH_2- (δ_H 4.27, δ_C 48.9) is consistent with the two groups being adjacent. Methylene protons (CH_2-8) showed a ^3J correlation with an oxygenated carbon δ_C 145.4 (C-1) and at the same time a methoxyl protons (δ_H 4.11, δ_C 61.7) showing a ^2J correlation with the same carbon which allows the placement of the two methoxyl groups at C-1 and C-2. The placement of the methoxyl groups at these positions was further confirmed from the chemical shift of one of the methoxyl at δ_C 61.7, which is typical for di-orthosubstituted methoxyls.

In addition, the ^{1}H NMR showed six aromatic protons; one set of *ortho*-coupled doublets at δ_H 6.96 and 7.51 (J =10 Hz) which showed a ^2J and ^3J HMBC correlation with C-2 leading to their assignment to H-3 and H-4, respectively. The second set of doublets at δ_H 7.71 and 7.45 (J = 10 Hz), were assigned to H-9 and H-10, respectively, based on the HMBC correlations (Table 1). Two singlets resonating at δ_H 7.12 and 7.66 showed ^2J and ^3J HMBC correlations with two oxygenated carbons δ_C 148.4 and 147.9, and at the same time the methylenedioxy protons showed ^2J HMBC correlation with the same two carbons. This is consistent with the placement of the methylenedioxy at C-12 and C-13 as well as the two singlet, para-protons at C-11 and C-14 (δ_H 7.12, H-11 and δ_H 7.66, H-14, respectively). All other carbon atoms were assigned using HMBC correlations as shown in the Table 1. Compound **(1)** is therefore, identified as dihydrochelerynthrine, an alkaloid that was previously reported from *Z. rubscens* (Waterman et al., 1976).



1

Table 4.1: ^{1}H NMR (CD_2Cl_2, 500MHz) and ^{13}C NMR (CD_2Cl_2, 125 MHz) data for dihydrochelerythrine (1)

Position	**δ_H (#H, *m*, *J*/Hz)**	**δ_C**	**HMBC (^2J, ^3J)**
1	-	146.0	-
2	-	152.7	-
3	6.96 (1H, *d*, *J* = 10 Hz)	111.4	C-4a, C-1, C-2
4	7.51 (1H, *d*, *J* = 10 Hz)	118.8	C-2, C-4a
4a	-	126.4	-
5	-	126.3	-
6	-	145.0	-
8	4.27 (2H, *s*)	48.9	C-4a, C-6, C-1
8a	-	124.6	-
9	7.71 (1H, *d*, *J* =10 Hz)	120.4	C-4a, C-10a, C-6
10	7.48 (1H, *d*, *J* = 10 Hz)	123.9	C-11, 124.6, , C-10a
10a	-	131.1	-
11	7.12 (1H, *s*)	104.4	C-10, C-12, C-13
12	-	148.4	-
13	-	148.7	-
14	7.66 (1H, *s*)	100.8	C-10a, C-6, C-12
14a	-	126.7	-
-OCH_2O-	6.14 (2H, *s*)	101.7	C-12, C-13
OCH_3	4.11 (3H, *s*)	61.7	C-1
OCH_3	4.04 (3H, *s*)	56.8	C-2
N-CH_3	2.58 (3H, *s*)	41.4	C-6, C-8

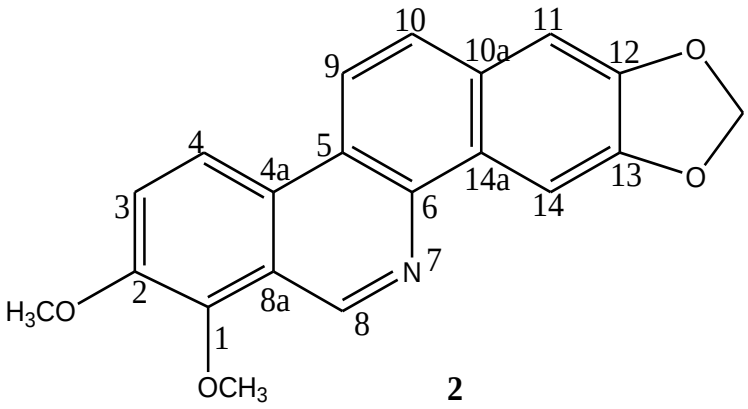
1.2 Norchelerythrine (2)

Compound **5** was isolated as colourless crystals. Like compound **1**, it showed a blue flourescence on TLC plate under UV (254 and 366 nm) and showed a positive test with the Drangendoff's reagent suggesting an alkaloid. The ^{13}C NMR spectra revealed presence of 17 sp^2 hybridised carbons, a methylenedioxy group (δ_C 101.6) and two methoxy (δ_C 61.7 and 56.8) peaks. Compound **5** is only different from compound **1** due to the absence of *N*-CH_3 peak and the presence a highly downfield shifted proton for H-8 (δ_H 9.70, δ_C 146.4).

The detailed ^1H NMR spectrum, in aromatic region, showed three one-proton singlets (δ_H 9.70 , 7.28, and 8.68), two one-proton doublets and one intense two-proton AB quartet indicating two doublets with close chemical shift values (δ_H 7.63, 8.36, 8.38, and 7.87). The proton at [δ_H 9.70, H-8, (δ_C 146.6, C-8)], was easy to identify and hence used as the starting point to assign other atoms. This is because of; its proximity to nitrogen atom on the pyridine ring, the ^1H and ^{13}C atoms resonates at low field. From the ^1H-^1H COSY, the H-8 proton showed long range coupling with H-4 (δ_H 8.36, 1H, *d*, *J* = 10 Hz) due to zig-zag (W) bonds between the two atoms. H-4 in turn showed a cross peak with H-3 (δ_H7.63, 1H, *d*, *J* = 10 Hz) completing the assignment of the A-ring protons and which otherwise is substituted with methoxyl groups at C-1 and C-2 as in compounds **1**.

^1H-^1H COSY spectrum also showed a pair of *ortho*-coupled protons (δ_H 8.36 and 7.87 *J* = 10 Hz). The two protons in addition to showing a cross peak with each other, the peak at δ_H 7.87 showed long range coupling with singlets at δ_H 7.28 and δ_H 8.68 allowing the assignment of the signal at δ_H 7.28 to H-10 and by extension, the signal at δ_H 8.36 to H-9. The two signals at δ_H 7.87 and δ_H 8.68 then belong to the two para-oriented protons (H-11 and H-14). With the help of HMBC and HSQC spectra (Table 5), the assignment of all atoms was established. Thus, compound **5** was elucidated to

be norchelerythrine, a benzophenanthridine alkaloid previously reported form *Z. bundrunga* (Balawant et al., 1991).



2

Table 4.2: ^{1}H NMR (CD_2Cl_2, 500 MHz) and ^{13}C NMR (CD_2Cl_2, 125 MHz) data for norchelerythrine (**2**)

Position	**δ_H (#H, *m*, *J*/Hz**	**δ_C**	**HMBC (^2J, ^3J)**
1	-	145.6	-
2	-	149.6	-
3	7.63 (1H, *d*, *J* = 10 Hz)	118.9	C-8a, C-1, C-2
4	8.36 (1H, *d*, *J* = 10 Hz)	118.2	C-8a, C-2, C-4a
4a	-	121.9	-
5	-	129.8	-
6	-	139.2	-
8	9.70 (1H, *s*)	146.6	C-8a, C-4a
8a	-	127.9	-
9	8.38 (1H, *d*, *J* = 10 Hz)	118.4	C-6, C-4a, C-5
10	7.87 (1H, *d*, *J* = 10 Hz)	126.9	C-11, C-10a
10a	-	120.0	-
11	7.28 (1H, *s*)	104.3	C-13, C-10, C-`14a
12	-	148.4	-
13	-	148.6	-
14	8.68 (1H, *s*)	101.9	C-6, C-12
14a	-	129.2	-
-OCH_2O-	6.14 (2H, *s*)	101.7	C-13, C-12
OCH_3	4.11 (3H, *s*)	61.7	C-1
OCH_3	4.11 (3H, *s*)	56.8	C-2

4.1.3 8- acetonyldihydrochelerythrine (3)

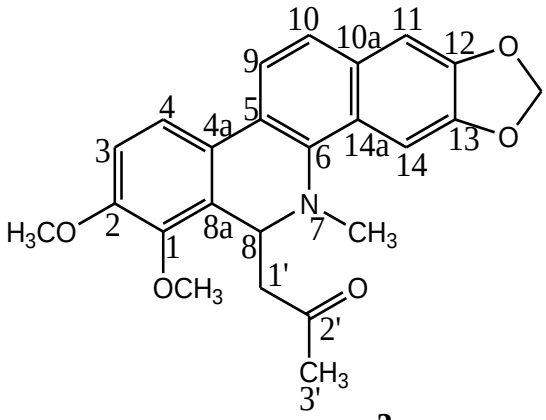
Compound (**1)** was isolated as colourless crystals. It showed strong flouresence under UV (254-366 nm) on silca gel plates and turned yellow on prolonged exposure to air and light. It showed a positive test with Drangendoff's reagent. Like compound **1** and **2**, these characteristics suggested an alkaloid with a benzophenanthridine skeleton (Kwok et al., 1987).

Out of twenty-four carbon peaks from ^{13}C NMR spectrum, sixteen were sp^2-hybridised carbons, one typical of methylenedioxy, two methoxy and *N*-methyl peaks. Biosynthetically, the oxygenation of benzophenanthridines is on ring A, B, D and in rare cases on ring C at postion 10. Therefore a tentative fixing of methoxy groups on C-1/C-2 on ring A and methylenedioxy on C-12/C-13 was done. From HMBC, the three proton singlet δ_H 2.61 (N-CH_3) showed a cross peak with the methine carbon [δ_C 55.0, (δ_H 5.04, *dd*, *J* = 3.6 Hz, 11 Hz)] which confirmed fixing of the benzylic proton at C-8.

The benzylic proton (H-8) showed a ^3J correlation with oxygyenated carbon C-1 (δ_C 145.4) and in addition, methoxy group (δ_H 3.91, δ_C 60.8) showed a ^2J correlation with the same carbon. This was a clear indication that the oxygenation on ring A is by methoxy groups particularly on C-1 and C-2. Therefore, methylenedioxy group is attached on ring D at C-12/C-13. The methyl singlet at δ_H 2.08 and the AMX system with *J* = 3.6, 7.2 and 11 Hz at δ_H 2.48, δ_H 2.25 and δ_H 5.04 due to H-8, indicated the presence of the acetonyl group (-CH_2COCH_3) with signals at δ_C 207.8 (C=O), δ_C 30.45 (-CO$\underline{C}H_3$) and δ_C 47.1 (-CO$\underline{C}H_2$). Using HMBC, ^1H-^1H-COSYand HSQC (Table 3) the attachement of the acetonyl group at C-8 was comfirmed.

The aromatic region of the ^1H NMR spectrum exhibited 6 protons; two sets of *ortho*-coupled protons and two one-proton singlets. A pair of one-proton doublets (δ_H 6.98 and δ_H 7.55), showed

^{1}H-^{1}H COSY correlation with each other and HMBC correlation with oxygenated carbons at C-1 and C-2. The two protons (δ_H 6.98 and δ_H 7.55) were thus assigned H-3 and H-4 respectively. The second pair of one-proton doublets (δ_H7.73 and 7.50) was assigned to H-9 and H-10 respectively using HMBC and HSQC spectra (Table 3). Futhermore, the two singlet proton peaks showed HMBC correlation with the two oxygenated carbons (C-12/C-13) of ring D and the methylenedioxy protons showed correlation with the same carbons. This indicated that the two protons were *para*-oriented to each other, hence placed at C-11/C-14. This confirmed the fixing of the methylenedioxy on C-12/C-13. The fixing of all other carbon atoms was confirmed using HMBC and HSQC (Table 3). The structure was further comfirmed by its molecular formular which was determined to be $C_{24}H_{25}NO_4$ from the EIMS ($[M]^+$ *m/z* 390). Basing on the above evidence, compound **3** was identified to be 8-acetonyldihydrochelerythrine; a known alkaloid from *Zanthoxylum* species (Neg et al., 2011).



3

Table 4.3: ^{1}H NMR (CD_2Cl_2, 600MHz) and ^{13}C NMR (CD_2Cl_2, 150MHz) data for 8-acetonyldihydrochelerythrine (**3**)

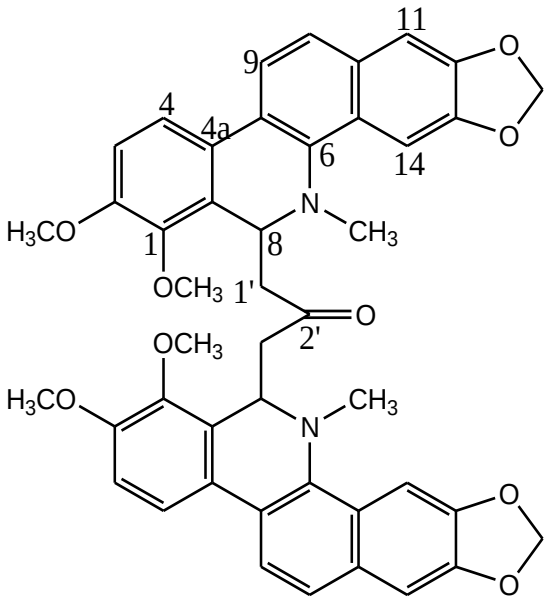
Position	**δ_H (#H, *m*, *J*/Hz)**	**δ_C**	**HMBC (^2J, ^3J)**
1	-	145.6	-
2	-	152.3	-
3	6.98 (1H, *d*, *J* = 9.0 Hz)	111.6	C-2, C-1, C-4a,
4	7.55 (1H, *d*, *J* = 9.0Hz)	118.8	C-3, C-8a, C-2
4a	-	127.6	-
5	-	131.3	-
6	-	139.5	-
8	5.04 (2H, *dd*, *J* = 3.6 Hz, 11 Hz)	55.0	C-8a C-1' C-4a C-6
8a	-	128.5	-
9	7.73 (1H, *d*, *J* = 8.4 Hz)	119.9	C-10a, C-10, C-5, C-6
10	7.50 (1H, *d*, *J* = 8.4 Hz)	123.9	C-5, C-10a, C-14a
10a	-	123.1	-
11	7.12 (1H, *s*)	104.2	C-12, C-13, C-10a, C-14a
12	-	147.7	-
13	-	148.2	-
14	7.50 (1H, *s*)	100.4	C-4a, C-12, C-13
14a	-	127.6	-
1'	2.57, 2.21 (2H, *dd*, *J* = 3.6, 11, 3.2 Hz)	47.1	C-8a, C-8, C-3' (C=O)
2'	-	206.9	-
3'	2.09 (3H, *s*)	30.5	C-1', C-2'(C=O)
-OCH_2O-	6.05 (2H, *s*)	101.4	C-12, C-13
OCH_3	3.93 (3H, *s*)	60.8	C-1
OCH_3	3.93 (3H, *s*)	55.7	C-2
***N*-CH_3**	2.61 (3H, *s*)	42.7	C-6, C-8

4.1.4 Chelerythridimerine (4)

Compound **9** was isolated as colourless crystals, which is only soluble in CH_2Cl_2/MeOH mixture. It had all the characteristics of benzophenantridine alkaloid, dihydrochelerythrine (**1**) which is substituted at C-8. The ^{1}H NMR spectrum had two sets of *ortho*-coupled protons: one at δ_H 6.84 and 7.40 (1H, *d*, *J* = 8.4 Hz) while the second set at δ_H 7.51 and 7.30 (1H, d, *J* = 8.6 Hz). Two singlets, at δ_H 6.92 and δ_H 7.28 (1H, *s*), a benzylic proton at δ_H 5.04 (1H, *dd*, *J* = 4.4, 9.8 Hz) and a methylene

at δ_H 2.24 (2H, *m*) were also visible. From ^{1}H-^{1}H COSY the benzylic proton (H-8) showed a cross peak with the methylene protons (H-1'). This data was comparable to that of compound **3** (8-acetonyl dihydrochelerythrine) except, the difference in the chemical shifts and the absence of the ketonic methyl group in compound **4.**

The ^{13}C NMR spectrum was similar to that of 8-acetonyl dihydrochelerythrine (compound **3**) except the absence of ketonic methyl peak in compound **4**. This suggested that compound **4** might be a symmetrical dimer of two dihydrobenzophenanthridine units linked by an acetone brigde. The molecular formular was determined to be $C_{45}H_{40}N_2O_9$ measured from EIMS ([M]$^+$ m/z 752). This and comparing with the literature data (Maclean et al., 1969), compound **9** was characterized as chelerythridimerine. Compound **4** was previously isolated from Canadian *Bocconia* species (Papaveraceae) by Maclean et al. (1969), however, this is the first isolation of this compound from genus *Zanthoxylum.*



4

Table 4.4: ^{1}H NMR ($CDCl_3$+MeO-d_4, 200 MHz) and ^{13}C NMR ($CDCl_3$+MeO-d_4, 50 MHz) data for chelerythridimerine (**4**)

Position	**δ_H (#H, *m*, *J*/Hz)**	**δ_C**
1	-	145.6
2	-	152.2
3	6.84 (1H, *d*, *J* = 8.4 Hz)	111.6
4	7.40 (1H, d, *J* = 8.4Hz)	118.9
4a	-	127.4
5	-	131.1
6	-	139.3
8	5.04 (1H, *dd*, *J* = 4.4, 9.8 Hz)	54.4
8a	-	128.4
9	7.51 (1H, *d*, *J* = 8.6 Hz)	119.7
10	7.30 (1H, *d*, *J* = 8.6Hz)	123.9
10a	-	123.3
11	6.92 (1H, *s***)**	104.3
12	-	147.6
13	-	148.1
14	7.28 (1H, *s*)	100.6
14a	-	127.4
1'	2.24 (2H, *m*)	47.1
2'	-	208.3
-OCH_2O-	5.8 (1H, *d*, *J* = 12.4 Hz)	101.0
OCH_3	3.88 (3H, *s*)	61.1
OCH_3	3.87 (3H, *s*)	55.9
N-CH_3	2.50 (3H, *s*)	42.7

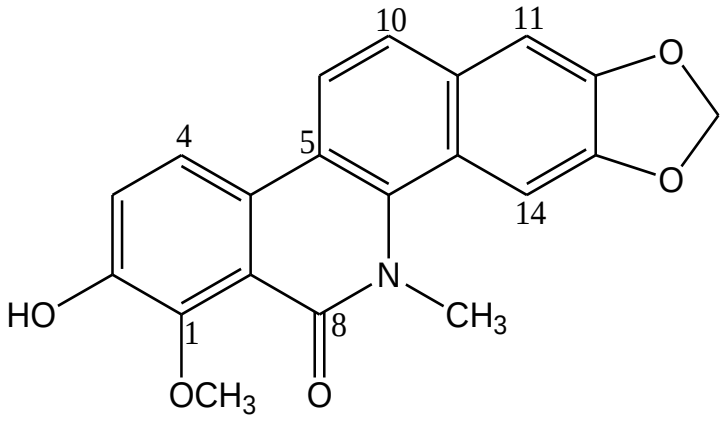
4.1.5 9-Demethyloxychelerythrine (5)

Compound **5** was isolated as white amorphous solid, which is only soluble in DMSO. On silca gel TLC and under UV (254 and 366 nm) light, it showed a strong blue fluorescence and was active with Drangendorff's reagent. The ^{1}H NMR spectrum showed in aromatic region, two sets of *ortho*-coupled protons; one set at δ_H 7.35 and δ_H 8.07 while the second set at δ_H 8.13 and 7.60 (1H, *d*, *J* = 8.0Hz). In addition two *para*-oriented one-proton singlets at δ_H 7.36 (1H, s) and δ_H 7.60 (1H, s)

were also observed. A broad singlet peak δ_H 9.67 (1H, s) suggested an exchangeable phenolic hydroxyl group.

The ^{13}C NMR spectrum (Table 4.5) had 16 sp^2-hybridised carbons, one shielded carbonyl, one methylenedioxy, one methoxy and N-methyl peaks. This pattern is typical of benzophenanthridine alkaloids. The upfield chemical shift (δ_C 161.2) of the carbonyl carbon suggested the presence of a conjugated amidic carbonyl. The fact that the peak at δ_H 3.75 (3H, *s*) had NOESY interaction with H-14 as well as HMBC correlation with C-6 and C-8 (C=O) allowed this downfield shifted proton signal to be assigned to the *N*-methyl group at position 7.

The HMBC and HSQC (Table 4.5) spectra are consistent with the placement of the methylenedioxy group at carbon C-12/C-13 as expected biogenetically on ring D. Further more one of the expected methoxy peaks on ring A was replaced by an OH group at δ_H 9.67 (1H, *brs*). The fixing of the OH and the methoxy groups at C-2 and C-1, respectively as well as the assignment of H-3 and H-4 was comfirmed using HMBC and HSQC (Table 10) spectra. The second set of the *ortho*-coupled protons could only be assigned to the ring C protons (H-9 and H-10), HMBC correlation of H-9 (δ_H 8.13) with C-6 (δ_C 134.8) confirmed their placement. In comparison with the matching literature data reported by Chen et al. (2005), compound **5** was identified as 9-demethyloxychelerythrine, a compound which was first reported from the root back of *Zanthoxylum integrifoliolum*, however, it had been previously synthesized by Ishikawa et al. (1995).



5

Table 4.5: ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) data for 9-demethyloxychelerythrine (**5**).

Position	**δ_H (#H, *m*, *J*/Hz)**	**δ_C**	**HMBC (^2J, ^3J)**
1	-	147.2	-
2	-	150.4	-
3	7.35 (1H, *d*, *J* = 8.0 Hz)	122.1	C-5, C-8a, C-2
4	8.07 (1H, *d*, *J* = 8.0 Hz)	118.6	C-4a,C-2, C-1
4a	-	127.2	-
5	-	116.8	-
6	-	134.8	-
8 (C=O)	-	161.2	-
8a	-	118.9	-
9	8.13 (1H, *d*, *J* = 8.0 Hz)	118.6	C-4a, C-10a, C-6
10	7.60 (1H, *d*, *J* = 8.0 Hz)	123.2	C-11, C-6, C-14a
10a	-	131.2	
11	7.36 (1H, *s*)	104.4	C-14a, C-10, C-13
12	-	147.1	-
13	-	146.8	-
14	7.60 (1H, *s*)	102.3	C-10a, C-6, C-12
14a	-	120.3	-
-OCH_2O-	6.17 (2H, *s*)	101.6	C-12, C-13
N-CH_3	3.75 (3H, s)	40.5	C-8, C-6
OCH_3	3.86 (3H, *s*)	61.0	C-1
OH	9.68 (1H, *brs*)	-	-

4.1.6 Nitidine (6)

Compound **6** was isolated as white armophous solid, which is only soluble in DMSO. It showed a positive reaction with Drangendorff's reagent suggesting that, the compound is an alkaloid. The ^{1}H NMR spectrum showed in aromatic region, five one proton singlet signals at (δ_H 7.78, 7.95, 8.32, 8.37, and 9.96), only one set of *ortho*-coupled protons at δ_H 8.95 and δ_H 8.25 (1H, *d, J* = 8.8 Hz), a singlet at δ_H 6.36 intergrating for two protons and three singlet signals at δ_H 4.05, 4.24, and 4.91 each intergrating for three protons.

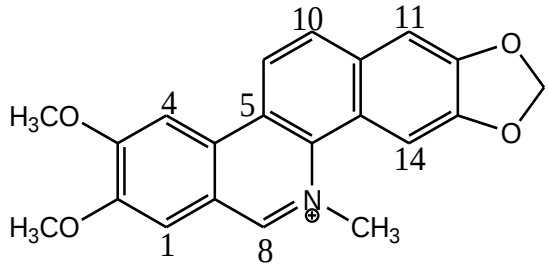
The singlet proton peaks at δ_H 7.78 and 8.38 showed a ^{2}J HMBC correlation with oxygenated carbons at δ_C 149.3, and 148.9. The methylenedioxy protons at δ_H 6.36 also showed a ^{2}J HMBC correlation with the two oxygenated carbons. In comparison with compounds, **1** and **2** an ambigous assignment of the methylenedioxy group at C-12/C-13 was done as well as assigning the protons at δ_H 7.78 and 8.38 at C-11 and C-14, respectively.

The higly disheilded proton at δ_H 9.96, showed a weak HSQC correlation with a carbon at δ_C 151.8. This suggested that this carbon is at postion 8 of the benzophenthridine skeleton and is attached to a nitrogen in a pyridine ring. The H-8 proton at (δ_H 9.96) showed a ^{2}J and ^{3}J HMBC correlation with, C-6 (132.9), C-8a (119.9), C-1 (109.3), C-4a (132.5) and *N*-CH_3 (51.9). In addition, the proton siginal of the *N*-CH_3 (δ_H 4.91) showed ^{3}J HMBC correlation with C-6 and C-8, as well as NOESY interaction with H-14 proton. This confimed the assignment of H-8 and the *N*-CH_3.

Futhermore the ^{3}J HMBC correlation of H-8 with C-1, H-1 with C-8, confirmed the assignment of the proton H-1 proton. The remaining one-singlet proton ($\delta_{H,}$ 8.37) could only be placed at postion 4 in para postion with H-1. This was confirmed by the ^{3}J HMBC correlation of proton H-4 with C-5

and C-2. The proton at δ_H 8.95 (1H, *d*) showed ^3J HMBC correlation with C-6 (δ_C 132.9) and C-10a (δ_C 132.9) while that at δ_H 8.25 (1H, *d*) showed ^3J HMBC correlation with C-11 (δ_C 106.2), and C-5 (δ_C 124.6). This was in agreement with the placement a pair of *ortho*-coupled protons (δ_H 8.95, 8.25) at C-9 and C-10 respectively as H-9 and H-10. The assignment of all carbons was done using HMBC and HSQC correlations.

The difference between this compound and other compounds with the same skeleton (for example compound **1**) was the substitution partern on ring A, which allowed the two protons to be *para*-oriented instead of the common *ortho*-coupling as seen in compound **1**. In addition, the high chemical shift values of the entire proton signals as compared to other compounds and its high porarity suggested an ammonium salt of a benzophenanthridine alkaloid. The data compared very well with that reported by Peilu et al. (2011), hence identifying the compound as nitidine.



6

Table 4.6: ^{1}H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) data for nitidine (**6**).

Position	**δ_H (#H, *m*, *J*/Hz)**	**δ_C**	**HMBC (^2J, ^3J)**	**NOESY**
1	7.95 (*s*, 1H)	109.3	C-4a, C-8, C-3	
2	-	158.7	-	
3	-	151.9	-	
4	8.37 (*s*, 1H)	103.8	C-8a, C-2, C-5	
4a	-	132.5	-	
5	-	124.6	-	
6	-	132.9	-	

Position	δ_H (#H, *m*, *J*/Hz)	δ_C	HMBC (^2J, ^3J)	NOESY
8	9.96 (*s*, 1H)	151.8	C-6, C-1, C-4a, C-8a, N-$\underline{C}H_3$	
8a	-	119.9	-	
9	8.95 (*d*, 1H, *J* = 8.8 Hz)	119.8	C-6, C-10a	
10	8.25 (*d*, 1H, *J* = 8.8 Hz)	130.5	C-11, C-5, C-14a,	
10a		132.9	-	
11	7.78 (*s*, 1H)	106.2	C-10, C-12, C-13, C-14a	
12	-	149.3	-	
13	-	148.9	-	
14	8.32 (*s*,1H)	105.1	C-12, C-13, C-6, C-14a	H-14 → N-Me
14a		120.4	-	
-OCH_2O-	6.36 (*s*, 2H)	103.2	C-12, C-13	
N-CH_3	4.91 (*s*, 3H)	51.9	C-8, C-6	N-Me → H-14
OCH_3	4.24 (*s*, 3H)	57.8	C-2	
OCH_3	4.05 (*s*, 3H)	56.8	C-3	

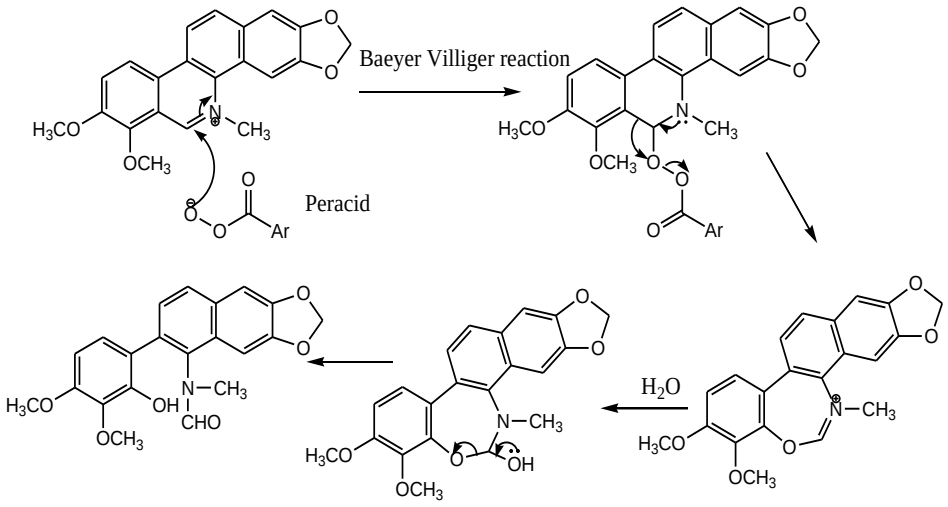
4.1.7 Arnottianamide (7)

Compound **7** was isolated as white amorphous powder, which is only soluble in DMSO. It showed a strong fluorescence under UV (366 and 254 nm) light on silca gel TLC plates. It also gave postive reactions with Drangendorff's reagent, which indicated that it is an alkaloid. The molecular formular of this compound was determined to be $C_{21}H_{19}NO_6$ from the EIMS ($[M]^+$*m/z* 381). The ^{1}H NMR spectrum displayed in the aromatic region, three singlet signals δ_H 7.96 (1H, *s*), 7.46 (1H, *s*) and 7.01 (1H, *s*) and a highly downfield shifted singlet at δ_H 8.92 (1H, *s*). Two sets of *ortho*-coupled doublets: one set at δ_H 7.81 and 7.24 (1H, *d*, *J* = 8.4 Hz), and a second set at δ_H 6.77 and 6.56 (1H, *d*, *J* = 8.4 Hz) were also clear on the ^{1}H NMR spectrum. A typical methylenedioxy peak at δ_H 6.19 (2H, *s*), *N*-methyl peak at δ_H 2.89 (3H, *s*) and two methoxy peaks at δ_H 3.82 (3H, *s*) and δ_H 3.69 (3H, s) were also evedent on the ^{1}H NMR spectrum.

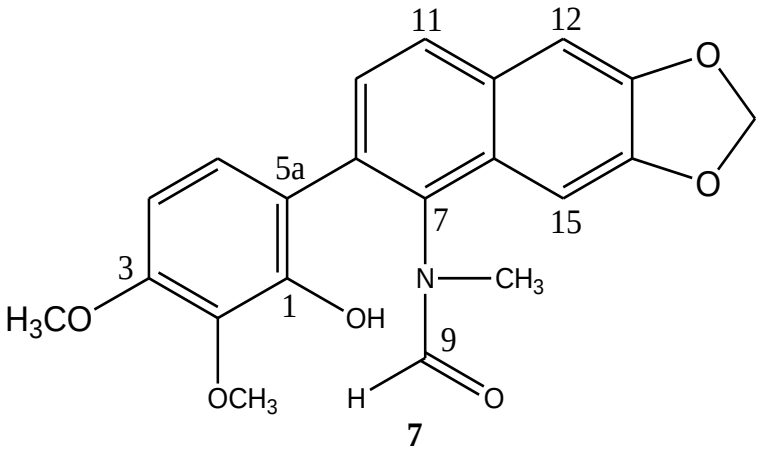
Based on HMBC, HSQC and COSY (Table 8), the proton signals at δ_H 2.89 (3H, *s*) and δ_H 7.96 (1H, s) were attributed to *N*-methyl formamide, of which the corresponding carbons signals appeared at δ_C 32.8 and δ_C 163.3 respectively in the ^{13}C NMR spectrum. The fact that, the highly

deshielded proton at δ_H 8.92 showed HMBC correlation with the quartenary carbon at δ_C 119.9 (C-5a) and an oxygenated carbon at δ_C 136.2 (C-2) suggested that, it is for an OH group at C-1 (δ_C 147.7). HMBC and HSQC further revealed that ring A was tetra-substituted allowing one set of *orth*-coupled protons (δ_H 6.56, 6.76, *d, J* = 8.4 Hz) to be fixed at C-4 and C-5 respectively with the two methoxyl groups being at carbon C-2 and C-3 while the OH group at C-1.

The methylenedoixy group was fixed on ring D at C-13/C-14, based on the HMBC correlation of the methylene protons with the two-para singlet protons, H-12 and H-15. The HMBC correlations (Table 8) allowed the assignment of another set of *ortho*-coupled protons, H-10 and H-11. Comparing the data with that reported by Hsiao and Chiang (1995), the compound was identified as arnottianamide. The substution pattern of this compound is similar to that of benzophenanthridine alkaloids, which suggests that, it is derived through some oxidative process involving Baeyer-Villiger like reaction on the ammonium groups of benzophenanthridine alkaloids, which results in the opening of ring B (Ishii and Ishikawa, 1976), as shown in Scheme 3.1.



Scheme 3.1: Proposed mechanism for the Baeyer villiger reaction for the formation of arnottianamide from nitidine.



7

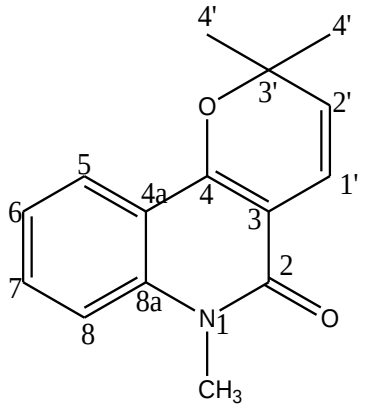
Table 4.7: ^{1}H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150MHz) data for arnottianamide (**7**)

Position	**δ_H (#H, *m*, *J*/Hz)**	**δ_C**	**HMBC (^{2}J, ^{3}J)**
1	-	147.7	-
2	-	136.2	-
3	-	152.5	-
4	6.56 (1H, *d*, *J* = 8.4 Hz)	103.1	C-5a, C-3, C-2
5	6.76 (1H, *d*, *J* = 8.4 Hz)	125.5	C-6, C-1, C-3
5a	-	119.8	-
6	-	134.4	-
7	-	135.5	-
9	7.96 (1H, *s*)	163.3	*N*-$\underline{C}H_3$
10	7.81 (1H, *d*, *J* = 8.4 Hz)	127.6	C-11a, C-7, C-5a
11	7.24 (1H, *d*, *J* = 8.4 Hz)	127.0	C-12, C-11a, C-6
11a	-	130.7	-
12	7.46 (1H, *s*)	104.3	C-11, C-14, C-15a
13	-	148.9	-
14	-	147.9	-
15	7.01 (1H, *s*)	98.7	C-11a, C-7, C-13
15a	-	125.5	-
-OCH_2O-	6.18 (2H, *d*)	101.7	C-14, C-13
OCH_3	3.82 (3H, *s*)	55.7	C-3
OCH_3	3.69 (3H, *s*)	60.4	C-2
***N*-CH_3**	2.89 (3H, *s*)	32.8	C-7, C-9
OH	8.92 (1H, *s*)	-	C-5a, C-1, C-2

4.1.8 *N*-methylflindersine (8)

Compound **8** was isolated as colourless crystals and the spot was clearly visible under UV (366 and 254 nm) on silica gel TLC plates. The ^{1}H NMR spectrum (Table 3) of the compound showed a *cis*-olefinic system with two clear doublets at δ_H 6.73 and 5.55 (*d*, *J* =10Hz) which together with the 6H singlet at δ_H 1.77 suggested the presence of a 2, 2-dimethylchromene (2, 2-dimethylpyrano substituent) group. In addition, the ^{1}H NMR displayed signals for four aromatic protons with chemical shifts in the range δ_H 7.34-7.97, *N*-Me [δ_H 3.63 (3H, *s*)] and particularly a signal for H-5 (δ_H 7.97) which are typical for a 2-quinoline skeleton (Claude et al., 1983; Adams et al., 1973). The presence of the downfield oxygenated quarternary carbon at δ_C 154.9 was an indication of probable cycllization of the prenyl group involving an adjacent hydroxyl group resulting in the formation of the pyran ring (Buyinza, 2012).

The HMBC revealed a ^{3}J correlation of the methyl protons $(CH_3)_2$-4' with C-3' as well as H-2' with C-3' and C-4' and H-1' with C-2, C-4 and C-2'. The *N*-Me group had ^{2}J correlation with the amidic carbonyl 160.5 (C-2) and the quarternally carbon at δ_C 139.5 (C-8a). As shown in the Table 4.8. The HSQC and HMBC correlation confirmed that the compound is *N*-methylflindersine, an alkaloid that has been reported from some *Zanthoxylum* species (Sheng et al., 1997; Stermitz et al., 1977).



8

Table 4.8: ^{1}H NMR (CD_2Cl_2, 500MHz) and ^{13}C NMR (CD_2Cl_2, 125 MHz) data for *N*-methyflindersine **(8)**

Position	**δ_H (#H, *m*, *J*/Hz)**	**δ_C**	**HMBC (^2J, ^3J)**
2	-	160.5	-
3	-	105.8	-
4	-	154.9	-
4a	-	115.9	-
5	7.97 (1H, *dd*, *J* = 8.0, 1.6 Hz)	122.9	C-7, C-8a, C-4
6	7.25 (1H, *m*, *J* = 8.0, 7.3, 1.6, 1 Hz)	121.5	C-5, C-7
7	7.55 (1H, *m*, *J* = 7.3, 1.6 Hz)	130.8	C-8a, C-5
8	7.34 (1H, *dd*, *J* = 8.5, 1 Hz)	113.9	C-4a, C-6
8a	-	139.5	-
1'	6.73 (1H, *d*, *J* = 10 Hz)	117.8	C-3', C-4
2'	5.55 (1H, *d*, *J* = 10 Hz)	126	C-4'', C-3', C-3
3'	-	78.7	-
4''	1.5 (6H, s)	27.9	-
***N*-CH_3**	3.63 (3H, s)	28.9	C-2, C-8a

4.1.9 Holstzianoquinoline (9)

Compound **9** was isolated as white crystals with a green fluorescence on silca gel TLC plates under UV (366 and 254 nm). On plolonged exposure to air and light, the spot turned yellow. It showed positive reaction with Drangendoff's reagent suggesting alkaloid nuclei. The ^{1}H NMR spectrum showed the presence of two sets of *ortho*-coupled protons [one set at δ_H 7.51 and 6.93 (1H, *d*, *J* = 8.8 Hz) and another set at δ_H 7.71 and 7.46 (1H, *d*, *J* = 8.8 Hz)]. In addition, two singlet one-proton peak signals (at δ_H 7.55 and 7.12), a benzylic proton at δ_H 4.90 (*dd*, *J* = 8.8, 3.2 Hz), three proton singlet signal at δ_H 2.61 (*N*-CH_3), two methoxyl peaks (at δ_H 3.92 and 3.79) and a double doublet intergrating for two protons at δ_H 6.07 (*J* = 1.2 Hz) which is typical of a methylenedioxy group were clearly seen in the NMR spectra. This data as in compound **3**, suggested the presence of a dihydrochelerythine moiety with a substituent at C-8.

Thus the substituent at C-8 was established to be *N*-methylflindersine (**8**), a co-metabolite. The ^{1}H NMR spectrum revealed the presence of *cis*-olefinic system with two coupled protons at δ_H 5.79 and δ_H 6.92 (1H, *d, J* = 10.2 Hz), one methyl at δ_H 1.35 (3H, *s*), methylene protons at δ_H 1.70-1.91. This corresponds to a 2, 2-dimethyl pyrano moiety (Wu and Chen, 1993). A down field *N*-methyl at δ_H 3.75 (3H, *s*) which had ^{3}J HMBC correlation with an amidic carbonyl, and d peaks at δ_H 8.12 (1H, *dd*, *J* = 8.0, 1.2 Hz), 7.57 (1H, *ddd*, *J* = 8.4, 7.2, 1.2 Hz), 7.36 (1H, *d*, *J* = 8.4 Hz) 7.26 (1H, *t*, *J* = 8.0, 7.2 Hz) were consistent with the 2-quinoline structure. In fact, a one-proton *dd* at δ_H 8.12 is typical of 2-quinolines at postion H-5. This moiety is connected to the other half through a 2, 2-dimethyl pyrano group where one of its methyl group was converted to a methylene during the attachement with C-8 of dihydrochelerythrine.

The C-8/C-1' linkage of the two moieties was established using HMBC. The methyl protons at δ_H 1.34 (3H, *s*) had ^{3}J HMBC correlation with C-1' (methylene carbon at δ_C 45.6), C-3 (δ_C 125.1) and ^{2}J with C-2' (δ_C 81.2).). In addition, the benzylic proton H-8 showed a ^{2}J HMBC correlation with C-1', ^{3}J with C-2' and N-$\underline{C}H_3$. This was further supported by COSY correlation cross peak between the benzylic proton (H-8) with the two methylene protons (H-1'and H-1'') at δ_H 1.70-1.91 (*m*, *J* = 8.8, 3.2 Hz). The *N*-methyl at δ_H 2.61 (3H, s) also showed ^{3}J HMBC correlation with C-8 and C-6 as in compound **3**. Complete assignment was done using clear features of HMBC, HSQC spectra as well as comparing with the spectra data with compounds **3** and **8**. The data was further compared with that of simulanoquinoline alkaloid, a related dimer isolated from *Zanthoxylum simulans* (Wu and Chen., 1993). The only difference was the absence of one methoxy group in compound **9**.

The mass pectrum of compound **6** showed $[M]^+$ and $[M+H]^+$ molecular peaks at *m/z* 588 and 589 respectively, ($C_{36}H_{32}N_2O_6$) and displayed important fragments at *m/z* 349 ($C_{21}H_{19}NO_4$), 348 ($C_{21}H_{18}NO_4$) and 226 ($C_{13}H_{12}NO_2$). This confirmed the structure of compound **9** as a dimer of two

compounds **1** (dihydrochelerythrine) and **8** (*N*-methylflindersine). Compound **9** is a new compound and therefore basing on the related compound simulanoquinoline alkaloid isolated from *Zanthoxylum simulans*, a trival name holstzianoquinoline was suggested. This is the second report of the C-C linked dihydrobenzophenanthridine and 2-quinoline dimeric alkaloid from natural sources.

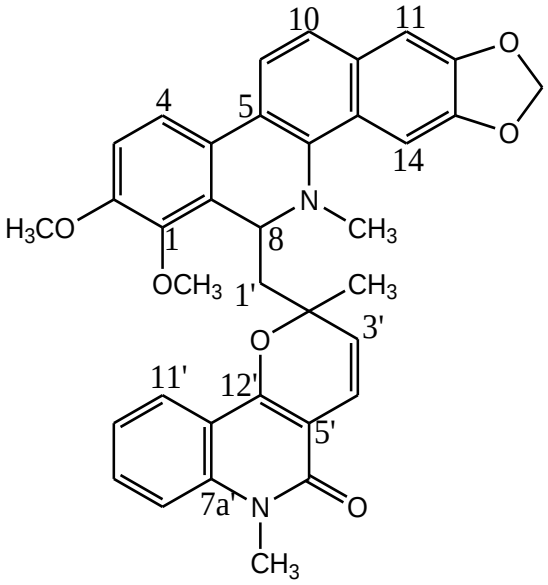


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Table 4.9: ^{1}H NMR ($CDCl_3$, 200 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz) data for holstzianoquinoline (**9**).

Position	**δ_H (#H, *m*, *J*/Hz)**	**δ_C**	**HMBC (*^2J*, *^3J*)**	**NOESY**
1	-	145.4	-	
2	-	152.5	-	
3	6.93 (1H, *d*, *J* = 8.8 Hz)	111.3	C-4a, C-1, C-2	
4	7.51 (1H, *d*, *J* = 8.8 Hz)	118.9	C-2, C-5, C-8a	
4a	-	127.4	-	
5	-	131.4	-	
6	-	139.6	-	
8	4.9 (1H, *dd*, *J* = 3.2, 8.8 Hz)	54.0	C-1', C-2', C-5, C-4a, C-1	H-8 → OMe, H-3'
8a	-	127.6		
9	7.71 (1H, *d*, *J* = 8.8 Hz)	120.0	C-6, C-5, C-10a	
10	7.46 (1H, *d*, *J* = 8.8 Hz)	123.9	C-11,	
10a	-	123.6	-	
11	7.11 (1H, *s*,)	104.7	C-13, C-14, C-14a,	
12	-	147.7	-	
13	-	148.3	-	
14	7.55 (1H, s)	101.3	C-12, C-13,	
14a	-	127.4	-	
-OCH_2O-	6.07 (2H, *d*, *J* = 1.2 Hz)	101.5	-	
OCH_3	3.91 (3H, *s*)	61.0	C-1	
OCH_3	3.71 (3H, *s*)	55.9	C-2	
N-CH_3	2.61 (3H, *s*)	42.8	C-6 ,C-8,	
1'	1.91 *m*, 1.70 *m*	45.6	C-8, C-2', C-8a, -CH_3	
2'		81.2		
3'	5.75 (1H, *d*, *J* = 10.2 Hz)	125.1	CH_3, C-2', C-1', C-5'	
4'	6.91 (1H, *d*, *J* = 10.2 Hz)	116.4	C-2', C-12', C-6'	
5'	-	105.7	-	
6'	-	161.4	-	
7'a	-	140.0	-	
8'	7.36 (1H, *d*, *J* = 8.4 Hz)	115.5	C-9', C-12', C-10'	
9'	7.57 (1H, *ddd*, *J* = 8.4, 7.2, 1.2 Hz)	130.8	C-7'a, C-11'	
10'	7.26 (1H, *t*, *J* = 8.0, 7.2 Hz)	121.9	C-11a,	
11'	8.11 (1H, *dd*, *J* = 8.0, 1.2 Hz)	123.4	C-12', C-7'a, C-9'	
11'a	-	115.6	-	
12'	-	155.3	-	
CH_3	1.35 (3H, *s*)	28.7	C-1', C-2', 3'	
N-CH_3	3.74 (3H, *s*)	29.5	C-6', C-7'a	

4.1.10 4,4'-Dihydroxy-3,3'-dimethoxylignan-9,9'-diyl diacetate (10)

Compound **7** was isolated as colourless crystals. It showed a green spot under UV (254 and 366 nm) on silca gel TLC plate. The ^{13}C NMR indicated the presence of twelve carbons, of these; six are sp^2-hybridized aromatic carbons, while three carbons were aliphatic. The ^{1}H NMR spectrum showed three aromatic protons with an ABX spin system at δ_H 6.53 (1H, *d*, *J* = 1.9Hz), 6.77 (1H, *d*, *J* = 8.0 Hz), and 6.58 (1H, *dd*, *J* = 8.0 Hz, 1.9 Hz), which were assigned to H-2, H-4 and H-5, respectively. This indicated that H-2 is meta-coupled with H-6 which in turn is *ortho*-coupled with H-5. In addition the NMR showed signal for one methoxyl group at δ_H 3.79 3H, *s*) and one phenolic hydroxyl group at δ_H 5.54 (1H, *s*). The ^{13}C NMR spectrum revealed the presense of six aromatic carbons of which three are quarternary while other three are methine carbons, which indicated that the aromatic ring is tetra substituted. Using HMBC, the methoxyl group and the phenolic hydroxyl group were fixed at C-3 and C-4, respectively. This confirmed the presence of the 4-hydroxy-3-methoxyphenyl moiety.

The aliphatic region in the ^{1}H NMR spectrum indicated the presence of an acetyl group 2.03 (3H, *s*) and a downfield signals at δ_H 4.00-4.15 (2H, *dd*) which suggested the presence of acetylated CH_2-OH group. This was further supported by ^{13}C NMR signals at δ_C 170.0 (C=O), 64.3 (-CH_2-O) and 20.8 (H_3C-C=O). The benzylic methylene group signals δ_H 2.76 (*dd*, *J* = 13.9 Hz, *J* = 7.3 Hz, H-7), 2.59 (1 H, *dd*, *J* = 14.0 Hz, *J* = 7.5 Hz, H-7) and the methine group signals δ_H 2.09 (1H, *m*) were assigned to postions C-7 and C-8 respectively with the help of HMBC, HSQC and COSY (Table 7). The side chain was therefore determined to have $-CH_2CHCH_2OCOCH_3$ structure.

From the mass spectra, compound **7** exhibited a molecular formular of $C_{24}H_{30}O_8$ calculated from EIMS ($[M]^+$ *m/z* 446). With this evidence and the fact that the ^{13}C NMR spectrum showed only 12 signals including those of acetyl, benzylic and methine groups suggested that compound **7** is a

dibenzyl butane that has a magnetically symmetrical structure. This data and assignment was in agreement with that reported by Martinez et al., (1999). It was then characterized as 4, 4'-dihydroxy-3, 3'-dimethoxylignan-9, 9'-diyl diacetate a compound that was first isolated from *Virola sebifera* leaves (Martinez et al., 1999). However, this is the first report of this compound from genus *Zathoxylum*.

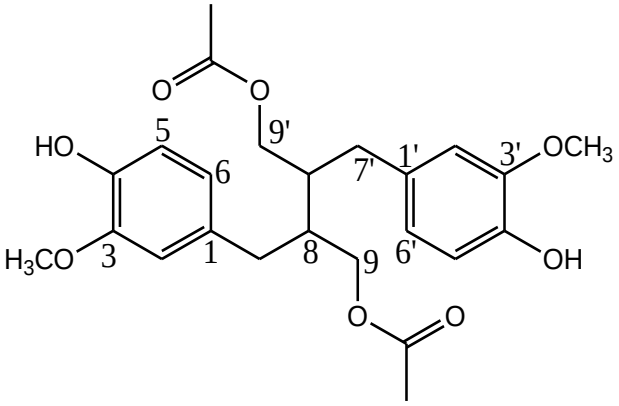

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Table 4.10: ^{1}H NMR (CD_2Cl_2, 500 MHz) and ^{13}C NMR (CD_2Cl_2, 125 MHz) data for 4, 4'-dihydroxy-3,3'-dimethoxylignan-9,9'-diyl diacetate (**10).**

Postion	**δ_H (#H, *m*, *J*/Hz)**	**δ_C**	**HMBC *(^2J, ^3J)***
1		131.9	
2	6.53 (1H, *d*, *J* =1.9 Hz)	111.4	C-6, C-1, C-3, C-4
3	-	146.6	-
4	-	144.0	-
5	6.77 (1H, *d*, *J* = 8.0 Hz)	113.9	C-1, C-3, C-4
6	6.58 (1H, *dd*, *J* = 8.0 Hz, 1.9 Hz)	121.7	C-7, C-2, C-4
7	2.76 (*dd*, *J* = 13.9, 7.3 Hz), 2.59 (*dd*, *J* = 14.0, 7.5 Hz)	34.97	C-8, C-9, C-2, C-6, C-1
8	2.09 (1H, *m*)	39.9	C-7, C-9
9	4.00 (*dd*, *J* = 11.3, 5.5 Hz), 4.15 (*dd*, *J* = 11.3, 6.0 Hz)	64.3	C-7, C-8, C=O
C=O	-	170	-
CH_3	2.03 (3H, *s*)	20.8	C=O
OCH_3	3.79 (3H, *s*)	55.8	C-3
OH	5.54 (1H, *s*)	-	C-5, C-4, C-3, C-6

4.2 Biological activity tests

The crudes and some pure compounds isolated from the stem and root bark of *Z. holstzianum* were tested for antiplasmodial, larvicidal and antinociceptive activities.

4.2.1 Antiplasmodial test results

The CH_2Cl_2/MeOH (1:1) extract of the stem bark of *Z. holstzianum* showed potent ant-plasmodial activity agansit W2 (chloroquine-resistant) and D6 (chloroquine sensitive) strains of *P. falciparum*, with IC_{50} values of 2.5 ± 0.3 and 2.6 ± 0.3 $\mu g ml^{-1}$, respectively. Among the compounds tested, nitidine **(6)** showed potent activity (0.11 ± 0.03 and 0.11 ± 0.01 µg/ml) agansit both W2 and D6. Gakunju et al (1995) reported the activity of nitidine ranging from 0.009-0.11 µg/ml, which is in agreement with the obtained activity. It is interesting to note that norchelerythrine **(2)** showed marginal activity of 7.3 ± 0.3 µg/ml against chloroquine-resistant (W2), but showed potent activity (0.15 ± 0.01 µg/ml) against chloroquine sensitive strain (D6). 8-acetonyldihydrochelerythrine **(3)** and dihydrochelerythine **(1)** showed good activity while *N*-methylflindersine (**8**) had the least activity against both W2 and D6 strains. The results are summarized in Table 4.11.

It is notable that the activity of nitidine against W2 and D6, and norchelerythrine agansit D6 was higher than that of the crude extract. The reduced activity of the crude extract could be attributed to the reduced concentration of the active compounds. This therefore suggests that the removal of the less active components can inprove the activity of the crude extract. The less activity of the 2-quinoline (*N*-methylflindersine) compared to all other benzophenanthridine alkaloids also suggests that, the benzophenathridine nucleus is responsible for the antiplasnodial activity.

Table 4.11: In-vitro IC_{50} values of the crude and alkaloids of *Z. holstzianum* against W2 and D6 strains of *P. falciparum*

Tested compounds	**IC50 in µg/ml (± SD)**	
	W2 (Chloroquine resistant strain)	**D6 (Chloroquine sensitive strain)**
Crude extract (stem bark)	2.5 ± 0.3	2.6 ± 0.3
8-Acetonyldihydrochelerythrine **(3)**	4.0 ± 0.2	3.8 ± 0.7
Nitidine **(6)**	0.11 ± 0.03	0.11 ± 0.01
Dihydrochelerythrine **(1)**	3.8 ± 0.7	3.2 ± 0.8
N-methylflindersine **(8)**	17.4 ± 2.0	13.4 ± 0.9
Norchelerythrine **(2)**	7.3 ± 0.3	0.15 ± 0.01
Chloroquine	0.1 ± 0.01	0.01 ± 0.001

4.2.1 Antinociceptive test results

The fact that many diseases including malaria result into painfull effects to human beings, the crude extract from the stem back of *Z. holstzianum* and three major pure compounds [dihydrochelerythrine **(1)**, *N*-methylflindersine **(8)** and the new compound holstzianoquinoline **(9)**] were tested for anti-nociceptive (anti-pain) activity using tail flick method on mice. The analysis was done using ANOVA (analysis of variance) followed by Schaffe's *post hoc* test. The difference in the test versus controll values was considered significant at $P < 0.05$.

The results obtained after treating the mice with 100 and 50 mg/kg of *N*-Methylflindersine (**8)**, showed an extremely statistically significant antinociceptive effect ($p<0.0001$) when compared to the vehicle treated animals. The effects of 50 mg/kg compared to 100 mg/kg of *N*- methylflindersine (**8)** also showed extremely statistically significant effect ($p < 0.0001$). This indicated that increasing the dose increases the anti-pain effect hence dose dependent. 50 mg/kg of dihydrochelerythrine and

holstzianoquinoline showed extremely statstically significant effect ($p < 0.0001$) and highly significant effect ($p < 0.001$), respectively. It is however, notable that the effect of dihydrochelerythrine and *N*-methylflindersine is higher than that of holstzianoquinoline, which is a dimer of the two. The reduced activity could be attributed to the loss of the methylene group on postion 8 of dihyrochelerythrine and the methyl group of *N*-methylflindersine during dimerization.

Despite the crude extract having a highly significant effect ($p<0.001$) for 100 mg/kg and significant effect ($p<0.05$) for 200 mg/kg, its effect in all cases is lower than that of the pure compounds tested. More interesting to note is that, the effect of a higher dose (200 mg/kg) for the crude extract is lower than that of 100 mg/kg of the same. This could probably be due to antagonistic effect of other compounds, which stimulate the stimulus transmission within the animal. Therefore increasing the concentration of the crude increases percentage of such compounds, hence reducing the concentration of the compounds with ant-pain activity.

When compared with the effect of the crude extract, *N*-methylflindersine (100 mg/kg) had a higher effect than both 200 mg/kg and 100 mg/kg doses of the crude extract. This could be attributed to the antagonistic effect due to other compounds in the crude extract. It is however notable, that there was no significant difference between the effect of 50 mg/kg of *N*-methylflindersine and 200 mg/kg of the crude extract.

The activity of both dihydrochelerythrine and *N*-methlyflindersine was found to be higher than that of the the mild anti-pain killer drug (Asprin). In addition, there was no significant difference between the activity of holstzianoquinoline and asprin. This therefore, suggests that the three pure compounds (dihydrochelerythrine, *N*-methylflindersine and holstzianoquinoline) are potential leads for the anti-pain agents.

Table 4.12: Antinociceptive test results for the crude and some alkaloids of *Z. holstzianum.*

Sample	Dose (mg/kg)	Tail flick latency (Seconds) ± SD
***N*-Methylflindersine (8)**	100	5.81 ± 0.21^{c}
	50	4.10 ± 0.12^{c}
Holstzianoquinoline (9)	100	4.64 ± 0.17^{c}
	50	3.89 ± 0.10^{b}
Dihydrochelerythrine (1)	50	4.37 ± 0.18^{c}
Crude (stem bark)	200	3.15 ± 0.05^{a}
	100	3.69 ± 0.07^{b}
Asprin	100	5.07 ± 0.22^{c}
Vehicle (control)		2.80 ± 0.09

The data is represented as the mean ± SEM of 5 animals. $^{a}p<0.05$, $^{b}p<0.001$ and $^{c}p< 0.0001$ when compared with the control value subsequent to ANOVA and Scheffe's *post hoc* test for latency.

4.2.2 Larvicidal test results

In search for new active larvicidal compounds, the root and stem bark (CH_2Cl_2/MeOH, 1:1) extracts of *Z. holstzianum* and some of its pure alkaloids were tested againsit *Aedes aegypti* larvae. The mortality was observed at 24 hours and 48 hours and the LC_{50} and LC_{90} was calculated. The results are summarized in Table 4.13.

The root bark extract showed moderate and dose dependant larvicidal activity (LC_{50} = 43.9 and LC_{90} = 61.0 µg/ml), at 24 hours; confidence interval 34.8-50.8 and 39.7-71.2, respectively. At 48 hours the activity was LC_{50} = 32.9 and LC_{90} = 50 µg/ml, confidence interval 25.5-41.6 and 41.5-65.3, respectively. It is important to note that with in 48 hours (2 days) both 100 and 50 µg/ml of the root bark caused 100% mortality. Compared to the root bark, the stem bark showed a less activity [(LC_{50} = 83.9 and LC_{90} = 118.4 µg/ml), at 24 hours; (LC_{50} =61.1 and LC_{90} = 95.6 µg/ml) at 48 hours.

Among the five pure alkaloid compounds tested, *N*-methylflindersine (**8**) showed potent and dose dependant larvicidal activity (LC_{50} = 4.8 and LC_{90} = 6.9 µg/ml, at 24 hours; confidence interval 3.76-6.11 and 5.72-9.42 respectively), while at 48 hours the activity improved to LC_{50} = 2.9 and LC_{90} = 5.13 µg/ml, confidence interval 2.05-3.93 and 4.07-7.17, respectively. Interestingly, *N*-methylflindersine caused 100% mortality at 5 µg/ml in 72 hours (3 days). 9-Demethyloxychelerythrine (**5**) showed good larvicidal activity (LC_{50} = 12.6 and LC_{90} = 19.5 µg/ml, at 24 hours, confidence interval 10.0-15.9 and 16.3-25.2, respectively, while at 48 hours, it showed activity (LC_{50} = 6.5 and LC_{90} = 13.4 µg/ml, confidence interval of 4.3-9.1 and 10.6-18.4, respectively). Holstzianoquinoline (**9**) showed a moderate activity [(LC_{50} = 26.5 and LC_{90} = 37.9 µg/ml, confidence interval of 20.1-36.6 and 29.8-53.6, respectively, at 24 hours); whereas at 48 hours the activity improved (LC_{50} =20.0 and LC_{90} = 31.5 µg/ml, confidence interval of 15.4-27.4 and 24.9-44.5 respectively)]. Other two-benzophenanthridine alkaloids tested (dihydrochelerythrine and 8-acetonyldihydrochelerythrine) did not show any larvicidal activity at 20 µg/ml.

It is important to note that, the two most active compounds (*N*-methylflindersine and 9-demethyloxychelerythrine) were isolated from the root bark. However, 9-Demethyloxychelerythrine was not isolated in the stem bark. This could account for the less activity of the stem bark extract compared to the activity of root bark extract. Despite holstzianoquinoline **(9)** being a dimer of the most active compound *N*-methylflindersine (**8**) and none active (at 20 µg/ml) dihydrochelerythrine, its activity was lower than that of *N*-methylflindersine (**8**). This suggests that, the methyl groups could be responsible for the potent activity of *N*-methylflindersine. This is because; one of the two-methyl groups is converted into the methylene during attachement to the dihydrochelerythrine moiety. It can also be suggested that, the reduced activity is due to the less percentage of the active compound in the dimer compared to when they are separate.

The antagonistic effect and less concentration of the active compounds compared to the active ones could be the reasons behind the reduced activity the crude extracts compared to some of the active compounds found in *Z. holstzianum*. It is worth noting that since *N*-methylflindersine is the major compound in this plant and has a potent larvicidal activity; it can be a good lead for larvicidal agents.

Table 4.13: Lavicidal activities of crudes and pure alkaloid compounds of *Z. holstzianum*

Sample	**LC_{50} (µg/ml)**		**LC_{90} (µg/ml)**	
	24 hours	**48 hours**	**24 hours**	**48 hours**
Crude (root bark)	43.93 ± 5.89	32.93 ± 3.66	61.02 ± 9.77	50.01 ± 9.12
Crude (stem bark)	83.95 ± 4.53	61.12 ± 5.91	118.39 ± 6.75	95.56 ± 9.03
***N*-methylflindersine (4)**	4.76 ± 0.32	2.90 ± 0.27	6.95 ± 0.34	5.10 ± 0.22
9-demethyloxychelerythrine (10)	12.25 ± 0.84	6.51 ± 1.09	19.54 ± 1.57	13.43 ± 1.87
Holstzianoquinoline (6)	26.53 ± 1.37	20.04 ± 1.32	37.97 ± 1.57	31.48 ± 2.76
Dihydrochelerythrine (3)	Not active at 20 µg/ml			
8-acetonyldihydrochelerythrine (1)	Not active at 20 µg/ml			

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This research study involved isolation, characterization and biological activity tests of the crude and pure compounds from the stem and root bark of *Zanthoxylum holstzianum.* Ten compounds were isolated. Except for one lignan (**10),** all compounds isolated were alkaloids, eight of which had a benzophenanthridine Skelton. The crude extracts and some of the pure compounds were tested for antiplasmodial, larvicidal and antinociceptive activities. The conclusions drawn from the study are outlined below;

i. Eight compounds were isolated from the stem bark. These included five benzophenanthridine alkaloids [8-acetonyldihydrochelerythrine (**3**), nitidine (**6**)**,** dihydrochelerythrine (**1**), norchelerythrine (**2**), and arnottianamide (**7**)], a 2-quinoline alkaloid [*N*-methylflindersine (**8**)], a symmetrical diameric lignan [4,4'-Dihydroxy-3,3'-dimethoxylignan-9,9'-diyl diacetate, **(10)]** and unsymmetrical dimeric alkaloid [holstzianoquinoline (**9**)]**.**

ii. Eight compounds, six of which having a benzophenathridine skeleton (**3**, **1**, **2**, **7**, chelerythridimerine **4**, and 9-Demethyloxychelerythrine, (**5**), one 2-quinoline (**8**) and the dimer (**9**) were isolated from the root bark**.**

iii. Nitidine (**6**), and 4,4'-Dihydroxy-3,3'-dimethoxylignan-9,9'-diyl diacetate (**10**) were isolated from the stem bark but not from the root bark, while 9-demethyloxychelerythrine (**5**) and chelerythridimerine (**4**) were isolated from the root bark but not from the stem bark.

iv. Holstzianoquinoline (**9)** is a new compound isolated from both the stem and the root bark of this plant. It is a dimer of two major co-metabolites (Dihydrochelerythrine and *N*-methylflindersine). This is the second report of a *C-C* linked unsymmetrical dimer with a benzophenathridine and 2-quinoline moieties from natural sources. In addition, this is the first report of chelerythridimerine (**4**) and 4,4'-dihydroxy-3,3'-dimethoxylignan-9,9'-diyl diacetate (**10**) from the genus *Zanthoxylum.*

v. The stem and the root bark of this plant was found to have high content of oil.

vi. Most *Zanthoxylum* species elaborate benzophenanthridine alkaloids. It was found out from this study that 80% of the metabolites isolated had benzophenanthridine Skeltons.

vii. The crude extract and some of the pure compounds isolated from stem bark were tested for *in vitro* antiplasmodial activity. The crude extract showed high activity. Among the pure compounds, nitidine showed potent activity agansit both W2 and D6 *Plasmodium falciparum* strains. Norchelerythrine showed potent activity against D6 but with marginal activity with W2. Others showed moderate activity with *N*-methylflindersine showing the least activity. This revealed that the benzophenathridine nucleus is responsible for the good activity of the benzophenanthridines.

viii. The crude extracts of the stem and root bark and isolated alkaloids were tested for larvicidal activities. The crude extract showed moderate to weak activity; among the pure compounds tested, *N*-methylflindersine **(8)** had the highest larvicidal activity, followed by 9-Demethyloxychelerythrine **(5)** and holstzianoquinoline **(9).** Dihydrochelerythrine **(1)** and 8-acetonyldihydrochelerythrine **(3),** showed no activity at 20 µg/ml. This study therefore indicated that *N*-methylflindersine could be used to control the population of mosquito.

ix. The stem bark crude extract and three pure alkaloids were tested for anti-nociceptive (anti-pain) activity. *N*-methylflindersine (**8**) and dihydrochelerythrine (**1**) had extremely significant antinociceptive effect. In fact, these two compounds have a higher activity than asprin, the commonly used mild anti-pain agent; while the new compound holstzianoquinoline (**9**) showed comparable effect to that of asprin.

5.2 Recommendations

i. ***N***-methyflindersine should be tested against other species of mosquito larvae including the malaria vector.

ii. More reseach on the antinociceptive effect of *N*-methylflindersine, dihydrochelerythrine and holstzianoquinoline is recommended in an attempt to develop them into antinociceptive agents. The cytotocicity of these alkaloids should also be done.

iii. Due to the high oil content the plant, GC-MS analysis as well as the biological activity test should be done on oil.

iv. More *in vitro* antiplasmodial and in vivo antimalarial studies along with and cytotoxicity tests should be conducted on crude extracts and pure compounds in order to explore the potential of these extractives in malaria control.

v. The absolute configuration of holstzianoquinoline should be determined.

REFERENCES

Achan, J., Talisuna, A. O., Erhart, Y A., Tibenderana, J. K., Baliraine, F. N., Rosethal, P. J, & D'Alessandro. (2011). Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. *Malaria Journal*, **10**,144-147

Adams, C. D., Taylor, D. R., & Warner J. M. (1973). *N*-methylflindersine from spathelia sorbifolia. *Phytochemistry*, **12**, 1359-1360.

Adams, P. A., Berman, P. A., Egan, T. J., Marsh, P. J, & Silver, J. (1996). The iron environment in heme and heme-antimalarial complexes of pharmacological interest. *J Inorg Biochem*, **63**, 69–77.

Addae-Mensah, I., Munenge, R., Guantai, A. N. (1989). Comparative Examination of two *Zanthoxylum* Benzophenanthridine Alkaloids for Cardiovascular Effects in Rabbits. *Phytotherapy Research*, **3**, 165-169.

Adesina, S. K., & Reisch, J. (1989). Amides from ***Z****anthoxylum rubescens***.** *Phytochemistry*, **28**(3). 839-842**.**

Adjuik, M., Agnamey, P., Babiker, A., Borrmann, S., Brasseur, P; Cisse, M; Cobelens, F; Diallo, S., Faucher, J. F., & Garner (2002). Amodiaquine-artesunate versus amodiaquine for un complicated plasmodium falciparum malaria in african children: a randomized multicenter trial. *Lancet*, **359**, 1365-1372.

Ahmad, M. U., Rahman, M. A., Hug, E., & Chowdhury, R. (2003). Alkaloids of *Zanthoxylum* with Antiplatelet Aggregation Activity from *Zanthoxylum integrifoliolum*. *Planta Medica*, **62**, 175-176.

Akala, M. H., Eyase, F. L., Cheruiyot, A. C., Omondi, A. A., Ogutu, B. R., Waters, N. C, Johnson, J. D., Mark, E. P., Schnabel, D. C. & Walsh, D. S. (2011). Antimalarial Drug sensitivity profile of western Kenya *Plasmodium falciparum* field determined by a SYBR Green I *in vitro* assay and molecular analysis. *Am. J.prop.Md.Hyg*, **81**(1), 34-41.

All-Parliamentary Group on Malaria and Neglected Tropical Diseases Report 2010 – 2011 targeting Zero: Sustaining Success in Malaria Control.

Andersson, C. M., Halberg, A., Hogberg, T. (1996). Advances in the Development of Pharmaceutical Antioxidants. *Advances in Drug Research*, **28**, 65-180.

Anupam, G., Nandita, C., Goutam, C. (2012). Plant extracts as potential mosquito Larvicides. *Indian J Med Res***. 135,** 581-598.

Arrow, K. J., Panosian, C. B., & Gelband, H. (2004). Saving lives, buying time: Economics of malaria drugs in an age of resistance, 126-128.

Arruda, M. S. P., Fernandes, J. B., Vieira, P. C., Da-silva, M. F. D., & Pirani, J. R. (1992). Chemistry of *Zanthoxylum rhoifolium*: a new sacofuroquinoline alkaloid. *Biochemical systematics and ecology*, **20**(2), 173-178.

Ashley, E., McGready, R., Proux, S., & Nosten, F. (2006). Malaria. A review. *Travel Medicine and Infectious Disease*, **4**, 159–173.

Balawant, S. J., Kristi, M. M., & Pelletier, S. W. (1991). Alkaloids of *Zanthoxylum budrunga* Wall. NMR assignments of Dihydrochelerythrine, (A)-Evodiamine and Zanthobungeanine: *Phytochemical analysis*, **2**, 20-25.

Batista, R., Junior, A. D. S., & Olivera, A. B., (2009). Plant- Derived antimalarial agents: new leads and efficient phytomedicines. Part II. None, alkaloidal natural products. *Molecules*, **14**, 3037-3072.

Beentje, H. J. (**1994)**: Kenya trees, shrubs and lianas: National Museum of Kenya, 372-374.

Bohorquez, B. E., Chua, M., & Meshnick, S. R., (2012). Quinine localizes to a non-acidic compartment within the food vacuole of the malaria parasite: *Malaria Journal*, **11**, 350.

Bray, P. G., Ward, S. A., & O'Neill, P. M. (2005). Quinolines and artemisinin: Chemistry, biology and history. *CTMI*, **295**, 3-38.

Butler, A. R., Khan, E., and Ferguson, *J. R. (2010)* .A brief history of malaria chemotherapy. *Physicians Edinb*; **40**, 172–177.

Buyinza, D. (2012)**.** Phytochemical investigation of *Zanthoxylum holstzianum* for antimicrobial principles. Thesis, University of Nairobi repository

Canfield, C. J., Pudney, M., & Gutteridge, W. E. (1995). Interactions of atovaquone with other antimalarial drugs against *Plasmodium falciparum* in vitro. *Exp. Parasitol,* **80**, 373-381.

Carmargo, L. M., Olivera, S. D., Basano, S., Garcia, C. (2009). Antimalarials and the fight against malaria in Brazil: *Therapeutics and Clinical Risk Management,* **5**, 311–317.

Centers for disease control and prevention. CDC 24/7: saving lives. Protecting people.

Chang, C-T., Doong, S-L., Tsai, I-L., and Chen, I-S.(1997). Coumarins and anti-HIV constituents from *Zanthoxylum schinifolium. Phyrochemistry,* **45**(7), 3419-1422.

Chen, I.; Shwu, W., Yann, L.; Inn, T., & Tian, W. (**1996**): Alkaloids from root bark of *Zanthoxylum simulans*: *Phytochemistry*, **42**, (1), 2157-219.

Chen, J-J., Fang, H-Y., Duh, C-Y., Chen, I-S. (2005). New indolopyridinoquinazoline, benzophenanthridines and cytotoxic constituents form *Zanthoxylum integrifoliolum*. *Plant med,* **71**, 470-475.

Chen, T-L., Lin, W-Y., Tsai, I-L., Chen, Y-C., & Chen, I-S. (1999). Isobutylamides from the fruit of *Zanthoxylum integrifoliolum*. *Phytochemistry*, **52**. 357-360.

Cheng, M.; Kuo, L., Ian, T., and I. H. C. (**2005**): Two new sesquiterpenoids and ant-HIV principles from the root bark of *Zanthoxylum ailanthoides*: *Bioorganic and Medicinal Chemistry*, **13**, 5915-5920.

Cho, J-Y., Hwang, T-L., Chang, T-H., Lim, Y-P., Sung, P-J., Lee, T-H., Chen, J-J. (2012). New coumarins and anti-inflammatory constituents from *Zanthoxylum avicennae*. *Food chemistry*, **135**, 17-23.

Chou, A.C, Chevli, R., & Fitch, C. D.(1980): Ferriprotoporphyrin IX fulfils the criteria for identification as the chloroquine receptor of malaria parasites. *Biochemistry,* **19,** 1543-1549.

Claude, M., Komar, R. W., Loiseau, J. G. P., Stanislas, E., & Moretti, C. (1983). 2-quinolone alkaloid from *Almeidea guyanenszs*. *Phytochemistry,* **22** (9), 2095-20961.

Claudio, R. N., & Lopes L. M. X. (2011). Antiplasmodial natural products (review). *Molecules,* **16**, 2146-2190.

Clough, B., and Wilson, R. J. M. (2001). Antibiotics and the plasmodial plastid organelle. In *Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery* (ed. P. J. Rosenthal) *Totawa, NJ: Humana Press,*. 265-286.

Constant, V.A., Koudou, B. G., Jones, C. M. J., David Weetman. D., & Ranson, H. (2012). Multiple-Insecticide Resistance in *Anopheles gambiae* Mosquitoes, Southern Côted'Ivoire**.** Emerging Infectious Diseases. *www.cdc.gov/eid,* **18**(9), 2012.

Cuca, L., Juan **S.,** Martinez, C., and Monache, F. D. (1998). 7,9'-epoxylignan and other constituents of *Zanthoxylum culantrillo***.** *Phytochemistry,* **47**(7), 1439.

D'Amour, F. E., & Smith, D. L. (1941). A method for determining loss of pain sensation. *J Comp neurol*, **72**, 74-79.

Da-Silva, C. V., Detoni, C. B., Da-Silva, E., Da-Silva, M. L. (2008). Alcalóides e Outros Metabólitos do Caule e Frutos de *Zanthoxylum tingoassuiba*. *Quimica Nova,* **31**(8), 2052-2055.

Dewick, P. M., (2009). Medicinal natural products. *Abiosynthetic approach 3rd Edition*. John Willy and sons limited. ISBN: 978-0-470-74168-9.

Dharani, N., Rukunga, G., Yenesew, A., Mbora, A., Mwaura, L., Dawson, I., & Jamnadass, R. (**2010**). Common Antimalarial Trees and Shrubs of East Africa: Description of Species and Guide to Cultivation and Conservation through Use. Dawson I ed. *World Agroforestry Centre* (ICRAF), Nairobi Kenya, 73-78.

DNP, (**2011**): Dictionary of Natural Product, CRC Press.

Dorsey, G., Njama, D., Kamya, M. R., Cattamanchi, A.; Kyabayinze, D., Staedke, S. G., Gasasira, A., & Rosenthal, P.J. (2002). Sulufadoxine/pyrimethamine alone with amodiaquine or artesunate for treatement of uncomplicated malaria: a longitudinal randomized trial. *Lancet*, **360**, 2031-2038.

Eckstein-ludwig, U., Webb, R. T., Van Guethen, I. D., East, J. M., Lee, A. G., Kimura, M., O'Neil, P. M., Bary, P. G., Ward, S. A, Krishna, S. Artemisinin target the SARCA of plasmodium falciprum. *Nature*, **424**, 957-961.

Edi, P. J., Kaudou, B. G., Jones, C. M., Weetman, D & Hillary, R. (2012). Multiple insecticide resistance in anophelese gambiae mosquitoes, Southern cote D'Ivoire. Emerging infectious diseases, **18**(9), 2012.

Facchini, P. J., & Benoit, S-P., (2005). Synthesis and trafficking of alkaloid biosynthetic enzymes. *Current Opinion in Plant Biology*, **8**, 657–666

Federica, M; Cornelis, E; Frank, V.D.K; Young, H.C and Verpoorte, R. (**2009**): Identification of Natural Epimeric Flavanone Glycosides by NMR Spectroscopy: *Food Chemistry*, **116**, 575–579.

Ferdinand, M., Talontsi, J. C., Matasyoh, R. M., Ngoumfo, R C. (2011). Mosquito larvicidal activity of alkaloids from *Zanthoxylum lemairei* against the malaria vector Anopheles gambiae. *Pesticide Biochemistry and Physiology*, **99**(2011), 82-85.

Ferreira, M.E., Rojas, D-A. A., Torres, D-O. S., Inchausti, A., Nakayama, H., Thouvenel, C., Hocquemiller, R., & Fournet, .A (2002). Leishmanicidal activity of two canthin-6-one alkaloids, two major constituents of *Zanthoxylum chiloperone*. *Journal of Ethnopharmacology*, **80**, 199-2002

Fish, F. and Waterman P. G. (1973). Chemosystematics in the Rutaceae II. The Chemosystematics of the *Zanthoxylum/Fagara* complex: *Taxon*, **22**, (2/3) 177-203.

Ferreira, M. H., Nakayama, H., Rojas, A., Schinini, A., Vera, N., Serna, E., Lagoutte, D., Soriano-Agatón, F., Poupon, E., Hocquemiller, R., &Fournet, A. (2007). Effects of canthin-6-one alkaloids from

Zanthoxylum chiloperone on *Trypanosoma cruzi*-infected mice. *Journal of Ethnopharmacology*, **109**, 258-263.

Foley, M., & Tilley, L. (1998). Quinolone antimalarials. Mechanisms of action and resistance and prospects for the new agents. Pharmacol. Ther, **79** (1), 55-89

Fry, M., & Pudney, M. (1992). Site of action of the antimalarial hydroxynaphthoquinone, 2-[trans-4-(49-chlorophenyl) cyclohexyl]-3- hydroxy-1,4-naphthoquinone (566C80). *Biochem. Pharmacol*, **43**, 1545–1553.

Gkrania-Klotsas, E. & Lever, A. M. L. (2007). An update on malaria prevention, diagnosis and treatment for the returning traveller Blood Reviews, **21**, 73–87

Geary, T. G., Divo, A. D., Jensen, J. B., Zangwill, M., & Ginsburg, H. (1990). Kinetic modelling of the response of *Plasmodium falciparum* to chloroquine and its experimental testing in vitro. Implications for mechanism of action of and resistance to the drug. *Biochem Pharmacol*, **40**, 685–691.

Ginsburg, H., & Deharo, E. (2011). A call for using natural product compounds in development of new antimalarial treatments-an introduction. *Malaria journal*, **10**(1) S1

Grimberg, B. T., & Mehlotra, R. K., (2011). Expanding the Antimalarial Drug Arsenal-Now, But How?. Review: *Pharmaceuticals*, **4**, 681-712.

Grycova, L., Jırı, D, & Marek, R. (2007). Quaternary protoberberine alkaloids: Review. *Phytochemistry,* **68**, 150–175.

Guo, T., Deng, Y-X., Hui X., Yao C-Y., Cai, C-C., Pan, S-l., & Yang-Lin W. **(2011)**. Antinociceptive and anti-inflammatory activities of ethylacetate fraction from *Zanthoxylum armatum* in mice. *Fitoterapia*, **82**, 347-351.

Hammond, D. J., Burchell, J. R., & Pudney, M. (1985). Inhibition of pyrimidine biosynthesis *de novo* in *Plasmodium falciparum* by 2-(4-tbutylcyclohexyl)-3-hydroxy-1-naphthoquinone *in vitro*. *Mol. Biochem. Parasitol*, **14**, 97-109.

Harbone, J. B., Williams, C. A. (2000). Advances in Flavonoid research since 1992. *Phytochemistry*, **55**, 481-504.

Hawley, S. R., Bray, P. G., Park, B. K., &Ward, S. A. (1996). Amodiaquine accumulation in *Plasmodium falciparum* as a possible explanation for its superior antimalarial activity over chloroquine. *Mol Biochem Parasitol*, **80**, 15–25.

Hisashi, I., & Ishikawa, T. (1976). Arnottianamide and isoarnottianamide: the structural establishment due to chemical conversion from the known benzo[c]phenanthridine alkaloids by the novel: Baeyer-villiger like oxidation of an immonium group. *Tetrabsdron Letter*, **15**, 1203–1206.

Homewood, C. A., Warhurst, D. C., Peters, W., & Baggaley, V. C (1972): Lysosomes, pH and the anti-malarial action of chloroquine**.** *Nature*, **235,** 50-54.

Hsiao, J-J., and Chiang, H-C. (1995). Lignans from the wood of Aralia bipinnate. *Phytochemistry*, **39**(4) 899-902.

Hu, J., Zhang, W-D., Shen, Y-H., Zhang, C., Xu, L., Liu, R-H., Wang, B., & Xu, X-K. (2007). Alkaloids from *Zanthoxylum nitidum* (Roxb.) DC. *Biochemical Systematics and Ecology*, **35**, 114-117.

Hufford, C.D. (**1976**): Aporphine Alkaloids of *Z.simulans* and *Z. nigrescens*: *Phytochemistry*, **15**, 1169-1172.

Ishii, H. (**1961**). Alkaloids of rutaceous plants XVI. Alkaloids of *Zanthoxylum schinifolium*. *Journal of Pharmacology*. *Soc. Jap*, **81**, 1633-1635.

Ishikawa, T., Takami, A., Abe, M., Chen, I S., Harayama, T., Ishii, H. (1995). Concise synthesis of the oxo-derivatives of benzophenanthridine bases by *N*-deformylated cyclisation based on Vilsmecer-Haack reaction. *Chem. Pharm bull*, **43**, 766-770.

Israilov, I. A., Karimova, S.U., Yunusov, M. S., & Yunusov, S (1980). Aporphine alkaloids: Institute of the chemistry of plant substances. *Academy of sciences of the Uzbek USSR*, **3**, 279-312.

IUPAC. (1978). Commission on the Nomenclature of Organic Chemistry (CNOC), Nomenclature of Organic Chemistry, Section F: Natural Products and Related Compounds, Recommendations 1976. *Eur. J. Biochem*, **86**, 1-8.

Ji, **H-F**., Li X-J., & Zhang, H-Y. (2009). Natural products and drug discovery. Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia? *European Molecular Biology Organization* EMBO *reports*, **10**(3), 194-200.

Jiang, H; Wei, Z; Yun, S; Chuan, Z; Lei, X; Run, L; Bin, W and Xi, X. (**2007**). Alkaloids from *Zanthoxylum nitidum* (Roxb.) DC: *Biochemical Systematics and Ecology*, **35**, 114-117.

Johnson, J. D; Dennull, R. A; Gerena, L; Lopez-Sanchez, M; Roncal, N. E & Waters, N. C. (2007). Assessment and continued validation of the malaria SYBR Green I-based fluorescence assay for use in malaria drug screening. *Antimicrobial Agents Chemotherapy*, 1, 1926-1933

Jullian, V., Bourdy, G, Georges, S., Maurel, S., & Sauvain, M. (**2006**): Validation of use of a traditional antimalarial remedy from French Guiana, *Zanthoxylum rhoifolium* Lam: *Journal of Ethnopharmacology*, **106,** 348–352.

Kammerer, L., Wanchai, D. E-E., & Meinhart H. Z., (1994**).** Enzymic 12-hydroxylation and 12-*O*-methylation of dihydrochelirubine in dihydromacarpine formation by *thalictrum bulgaricum. Hycoc-y*, **36**(6), 1409-1416.

Kashiwanda, Y., Chikashi, I., Hitoshi, K., Izumi, M., Katsuko, K., Suneo, N., & Yasumasa, I. (1997): Alkylamides of the Fruit of *Zanthoxylum SSP*: *Phytochemistry*, **44**(6), 1125-1127.

Kaur, K., Jain, M., Kaur, T., Jain, R. (2009). Antimalarials from nature. *Bioorganic & Medicinal Chemistry*, **17**, 3229-3256.

Kimura, M., Kawakami, K., Hashimoto, M., & Hamada, M. (2006). Malaria prevention and stand-by emergency treatment among Japanese traveler's. *Travel Medicine and Infectious Disease*, **4**, 81–85.

Kokwaro J.O. (**2009**). Medicinal Plants of East Africa, Third Edition: *University of Nairobi Press*, 256-257.

Kerishna, S., Uhlemann, A. C., & Haynes, R. K. (2004). Artemisinins: mechanisms of action and potential for resistance.*Drug Resistance Updates*, **7**, 233–244.

Kuetea, V., Krusche, B., Youns, M., Voukeng, I., Fankama, A. G., Tankeo, S., Lacmata, S., Efferth, T. (2011). Cytotoxicity of some Cameroonian spices and selected medicinal plant extracts. *Journal of Ethnopharmacology*, **134**, 803-812.

Kutchan, T. M., Dittrich, H., Bracher, D., & Zenk M.H (**1991)**. Enzymology and molecular biology of alkaloid biosynthesis. *Tetrahc & on*, **41**(31) 5945-5954.

Kwok, M.N. G., Alexander, I.G and Waterman, P G. (**1987**): Benzophenanthridine Alkaloids from the Stem Bark of a *Zanthoxylum* Species: *Phytochemistry*, **26**,(12) 3251-3254.

Lafrance, M., Blaquiere, N., & Fagnou, K (2007). Aporphine Alkaloid Synthesis and Diversification via Direct Arylation**.** *Eur. J. Org. Chem.* **2007**, 811–825.

Li, Y, (2007) editor. *Discovery and development of new antimalarial drug (artemisinin). Shanghai scientific & Technical Publishers*; 2007.

Ling, K.-H., Wang, Y., Poon, W.-S., Shaw, P.-S., But, P. P.-H. (2009). The Relationship of *Fagaropsis* and *Luvunga* in Rutaceae. *Taiwania*, 54, 338-342.

Liu, J-M., Ni, M-Y., & Fan, Y-F. (1979). Structure and reactions of arteannuin. *Acta Chimica Sinica*, **37**, 129–141.

Luis, E., Cuca, S., Juan, C., Martinez, V., and Franco, D. M. (1998). 7,9'-Epoxylignan and other constituents of zanthoxylum culantrillo. *Phytochemistry*, **47**(7), 1437-1439.

Loeb, L. F., Clarke, W. M., Coatney, G. R., Coggeshall, L. T., Dieuaide, F. R., & Dochez, A. R. (1946). Activity of a new antimalarial agent, Chloroquine (SN 7618). *JAMA*, **130**, 1069–1070.

MacLean, D. B., Gracey, D. E. F., Saunders, J. K., Rodrigo, R. & R.H.F. Manske, R. H. F. (1969). *Can. J . Chem.*, **47**, 195.

Macomber, P. B., & Sprinz, H. (1967): Morphological effects of chloroquine on *Plasmodium berghei* in mice**.** *Nature*, **214**, 937.

Martin, S. K., Oduola, A. M., & Milhous, W. K. (1987). Reversal of chloroquine resistance in *Plasmodium falciparum* by verapamil. *Science*, **235**, 899-901.

Martinez, J C., Aldan J., Martha I., Cuca S. & Luis E. (1999). Dibenzylbutane lignans from *Virola Sebifera* leaves. *Phytochemistry*, **50**(5), 883-886

Matu, E. N., & Staden, J. 2003). Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. *Journal of Ethno pharmacology*, **87**(2003) 35–41

Mavrogordato, A., & Lever, A. M. L. (2012). A cluster of *Plasmodium vivax* malaria in an expedition group to Ethiopia: Prophylactic efficacy of atovaquone/proguanil on liver stages of *P. vivax*. *Journal of infection*, **65**, 269-274.

Mazier, D., Renia, L., & Snounou, G. (2009) A pre-emptive strike against malaria's stealthy hepatic forms. *Nat Rev Drug Discovery*, **8**, 854–864.

Meshnick, S. R. (2001). Artemisinin and its derivatives. In *Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery* (ed. P. J. Rosenthal). *Totowa, NJ: HumanaPress*, 191-201..

Mojab, F. (2012). Antimalarial natural products: A review: *Avicenna Journal of Phytomedicine*, **2**, 52-62.

Mouhseen, L. (2007). Secreening of natural products for drug discovery: expert opin. *Drug discovery*, **2**(5): 697-705.

Muganga, R., Angenot, L., Tits, M., Frédérich, M, (2010). Antiplasmodial and cytotoxic activities of Rwandan medicinal plants used in the treatment of malaria. *Journal of Ethnopharmacology*, **128**, 52–57.

Murray, R.D. H., Méndez, J., & Brown, S. A. (1982). The Natural Coumarins. *Ocurrence, Chemistry and Biochemistry*. *John Wiley & Sons LTD.* pp. 343-345. ISBN: 0471280577, Chichester.

Negi, J. S., Bisht, V. K., Bhandari, A. K., Shingh, P., & Sundriyal, R. C. (**2011**): Chemical constituents and biological activity of the genus *Zanthoxylum:* A review: *African Journal of Pure and Applied Chemistry*, **5(**12**)**, 412-416.

Neusa, F. D; Heraldo, B. R; Emilia, C. S. M., Eduardo, M. E.; Nilo, Z., & Ademir, F. M., (**1997**): Benzophenanthridine Alkaloids from *Zannthoxylum rhoifolium*: *Phytochemistry*, **46**(8), 1443-1446.

Newman, D. J., Cragg, G. M., Snader, K. M. (2000). The influence of natural products upon drug discovery. *Nat Prod Rep*, **17**(3), 215–234.

Newman, D. J., Cragg, G. M., Snader, K. M. (2003). Natural products as sources of new drugs over the period 1981–2002. *J Nat Prod*, **66**(7), 1022–1037.

Ngou, S., Tsamo, E., &Joseph, D. (1994**).** Lignans and other constituents of *Zanthoxylum heztzzi*. *PtWaknlmy***,** **37**(3), 867-869.

Nguta, J. M., Mbaira, J. M., Gakuya, D. W., Gathumbi, P. K., Kiama, S. G. (2010). Antimalarial herbal remedies of Msambweni, Kenya. *Journal of Ethnopharmacology*, **128**, 424-432.

Nilles, E. J., & Arguin, P. M., (2012). Imported malaria: an update.*American Journal of Emergency Medicine*, **30**, 972–980.

Nissanka, A.P.K., Karanaratne, V., Bandara, B. M. R., Kumar, V., Nakanisha, T., Nishi, M., Inada, A., Tellekeratne, L. M. V., Wijesundara, D. S. A., & Gunatilaka, A. A. (2001). Antimicrobial alkaloids from *Zanthoxylum tetraspernum* and *caudatum*. *Phytochemistry*, **56**, 857-861.

Olliaro, P. (2001). Mode of action and mechanisms of resistance for antimalarial drugs. *Pharmacology and Therapeutics*, **89**(2), 207-219.

Patino, L.O.J., Prieto, R.J.A., & Cuca, S. L. E. (2012). *Zanthoxylum* Genus as Potential Source of Bioactive Compounds in Phytomedicine. *Prof. Iraj Rasooli (Ed.)*, ISBN: 978-953-307-805-2, (186-218).

Patino, O. J., & Cuca, L. E. (2010). Isoquinoline Alkaloids of *Zanthoxylum quinduense* (Rutaceae). *Biochemical Systematic and Ecology*, **38**, 853-856.

Patino, O. J., Cuca, L. E. (2011). Monophyllidin, a New Alkaloid *L*-Proline Derivative from *Zanthoxylum monophyllum* (Rutaceae). *Phytochemistry Letters*, **4**, 22-25.

Pei, L., Kanglun, H., Longguan, X., & Xiaohua, X. (2011). Supplementary Material (ESI) for Organic & Biomolecular Chemistry. Palladium-Catalyzed Tandem Reaction to Construct Benzo[c]phenanthridine: application to the total synthesis of Benzo[c]phenanthridine alkaloids. Electronic supporting information for: *Org. Biomol. Chem.The Royal Society of Chemistry*, 2011.

Peters, W. (1970). Chemotherapy and drug resistance in malaria. *Academic, London.*

Plowe, C. V. (2001). Folate antagonists and mechanisms of resistance. In Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery (ed. P. J. Rosenthal). ***Totowa, NJ: Humana Press*, 46**, 173-190**.**

Plowe, C., Kublin, J. G., & Doumbo, O. K. (1998). *P. falciparum* dihydrofolate reductase and dihydropteroate synthase mutations: Epidemiology and role of clinical resistance to antifolates. *Drug Resistance Updates,* **1**, 389-396.

Price, R. N., & Nonsten, F., (2001). Drug resistant falcipurum malaria: Clinical consequences and strategies for prevention. Drug resistance update, **4**, 187-196.

Rahman, M. M., Islam, M. A., Khondkar, P., Gray, A. I. (2005). Alkaloids and lignans from *Zanthoxylum budrunga (Rutaceae). Biochemical Systematics and Ecology* **33**, 91–96.

Rahman, M. M., Islam, M. A., Khondkar, P., Gray, A. I. (2005). Alkaloids and Lignans from *Zanthoxylum budrunga* (Rutaceae). *Biochemical Systematic and Ecology*, **33**, 91-96.

Ranson, H., N'Guessan, R., Lines, J., Moiroux, N., Nkuni, Z., Corbel, V., (2011). Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol.27, 91–98. *Indian J Med Res*. **135(**5), 581–598.

Razadam, B. (2010). Medicinal chemistry: CBS publishers & distributers Pvt Ltd prahland street India. ISBN: 978-239-1790-0.

Rieckman, K. H., Trenholme, G. M., Williams, R. L., Carson, P. E., Frischer, H., & Desjardins, R. E. (1974). Prophylactic activity of mefloquine hydrochloride (WR 142490) in drug resistant malaria. *Bull. WHO,* **51**, 375-377.

Rogerson, S. J., (2010). Malaria in pregnancy and the newborn. *Adv.Exp. Med. Biol*, **659**, 139-152.

Rosenthal, P. J. (2001a). Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery. *Totowa, NJ: HumanaPress*.

Rosenthal, P. J. (2003). Antimalarial drug discovery: old and new approaches. *Journal of experimental Biology*, **206**, 3735-3744.

Ross, S. A., Al-Azeib, M. A., Krishnavei, K. S., Fronczek, F. R., Burandt, C. L. (2005). Alkamides from the Leaves of *Zanthoxylum syncarpum*. *Journal of Natural Products*, **68**, 1297-1299.

Rudrapal, M. (2011). A brief review on malaria and current antimalarial drugs. *Current Phamma Research CPR*, **1**(3), 286-290.

Saifi, M. A., Beg, T., Harrath, A. H., Fahad, S. H., Altayalan, F. H. S., & Saleh, S. A: Antimalarial drugs: Mode of action and status of resistance**.** *African Journal of Pharmacy and Pharmacology*, 7(5), 148-156.

Sanjib, B. M., & Kamaruz, Z. (2009). Pharmacognostical evaluation of *Zanthoxylum nitidum* bark International Journal of PharmTech Research, **1**(2), 292-298.

Schantz-Dunn, J., & Nour, N. M, (2009). Malaria and pregnancy: A global health perspective. *Rev. Obstet. Gynecol*, **2**, 186-192.

Schneider, B**.,** & Zenk, M. H. (1993). Unusual methyl transfer in the biosynthesis of aporphine and protoberberine alkaloids.*Phytochemistry*, **32**(4), 897-903.

Schwartz, L., Brown, G., Genton, B., & Moorthy, V. S. (2012). A review of malaria vaccine clinical projects based on the WHO rainbow table. *Malaria Journal*, **11**, 11.

Schlesinger, P. H., Krogstad, D. J., & Herwald, B. L. (1988). Antimalarial Agents: Mechanisms of Action. *American Society for Microbiology, antimicrobial agents and chemotherapy*, **1988**, 793-798

Sebisubi, M.F. &Tan, G.T (2012). Natural products with antimalarial activity. Phytochemistry and phamacogonosy. *UNESCO-Encyclopedia of life support systems (EOLSS) sample chapters* 2012.

Setzer, W. N., Noletto, J. A., Lawton, R.O., & Haber, W.A. (2004). Leaf essential oil composition of five *Zanthoxylum* species Monteverde, Costa Rica: *MolecularDiversity*, **9**, 3-13.

Sheen, W. S., Tsai, I. L., Teng, C. M., Ko, F. N. Chen, I. S. (1996). Indolopyridoquinazoline alkaloids with antiplatelet aggregation activity from *Zathoxylum intergrifoliolum*. *Plant medica***, 62**(2), 175-176.

Sheng, C; Inn, T; Che, T; Jih, C; Ya, C; Feng, K; Matthias, C. L and Pezzuto, J. M. (**1997**). Pyranoquinoline Alkaloids from *Zanthoxylum simulans*: *Phytochemistry*, **46**(3), 525-529.

Stermitz, F. R., &. Sharif, I. A. (**1997)**. Alkaloids of *Zanthoxylum monophyllum* and Z. *punctatum* .ckmlsny, **16**, 2000-2006.

Stermitz, F.R., Caolo, M. A, & Swinehart, J. A. (1980). Alkaloids and other constituents of *Zanthoxylum williamsii, Z. monophyllum* and Z. *fagara. Phym & mirtry*, **19**(180), 1469-1472.

Stocks, P. A., Raynes, K. J. and Ward, S. A**.** (2001). Novel quinoline antimalarials in Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery (ed. P. J. Rosenthal). *Totowa, NJ: Humana Press*, 235-253.

Sullivan, D. J. (2002). Theories on malarial pigment formation and quinoline action. *Int. J. Parasitol*, **32**, 1645–1653.

Sullivan, D. J., Gluzman, I. Y., Russell, D. G., & Goldberg, D. E (1996). On the molecular mechanism of chloroquine's antimalarial action. *Proc Natl Acad Sci USA*, **93**, 11865–11870.

Swinehart, J. A., & Stermitz, F. R. (**1980)**. Bishordeninyl terpene alkaloids and other constituents of *zanthoxylum culantrillo* and z. *coriaceum"*. *Phytochemistry*, **19**, 1219-1223.

Taborda, M. E., & Cuca, L. E. (2007). Un Nuevo Alcaloide Carbazolico de *Zanthoxylum rhoifolium*. *Scientia et Technica*, **13**, 191-192.

Tane, P., Hippolyte, K., Wabo, J. D. (2005). A new benzophenanthridine alkaloid from *Zanthoxylum buesgenii*. *Connolly Fitoterapia*, **76**, 656–660.

Tarus, P.K., Coombes, P.H., Crouch, N. R., & Mulholland, D. A. (2006). Benzo[c]phenanthridine alkaloids from stem bark of the Forest Knobwood, *Zanthoxylum davyi* (Rutaceae). *South African Journal of Botany*, **72,** 555–558.

Taylor, D. K., Avery, T. D., Greafrex, B. W., Tenkink, E. R. T., Macreadie, I. G., & Hump A. D **(2004)**. Novel endoperoxide antimalarials: synthesis, heme binding and antimalarial activity. *J. Med.Chem*, **47**, 1833-1839.

Timothy, N. C., & Wells. (2011). Natural products as starting points for future anti-malarial therapies: going back to our roots? *Malaria Journal*, **10**(1):S3.

Toovey, S., & Jamieson, A., (2003). Rolling back malaria: how well is Europe doing? *Travel Medicine and Infectious Disease*, **1**, 167–175.

Trevor, A., Katzung, B., Masters, S., Katzung & Trevor's. (2007) Pharmacology Examination and Board Review, *Eight Edition*. *USA: McGraw-Hill Medical*, 2007.

Tringali, C., Spatafora, C., Calì, V., & Simmonds, M. S. J. (2001). Antifeedant constituents from *Fagara macrophylla*. *Fitoterapia*, **72**, 538-543.

Vincenzo D. L., & Benoit, S-P. (2000). The cell and developmental biology of alkaloid biosynthesis. *Trends in plant science*, **5**(4), 1360-1385.

Walmir, S. G., Fernanda R. G., Lilliam, M. G. E. & Da-Silva, L. H. (2009). Larvicidal activity against Aedes aegypti of some plants native to the West-Central region of Brazil. *Bioresource Technology*, **100**, 6647–6650.

Wanchai, D-E., Takao, T., Meinhart, H. Z (1992). Enzymic, l0-hydroxylation and l0-0-methylation of dihydrosanguinarine in dihydrochelirubine formation by ***eschscholtzia.*** Phyrochemistry, **1**(8), 2713-2717.

Waterman, P. G.; Alexander, G., & Elizabeth, G. C. (**1976**): Acomparative Study on the alkaloids of *Zanthoxylum leprieurii*, *Z. lemailei* and *Z. rubescens* from Ghana: *Biochemical Systematics and Ecology*, **4**, 259-262.

Waterman, P. G. (1990). Chemosystematics of the Rutaceae: comments on the interpretation of

Da-Silva et al. *Pl. syst. Evol*, **173**, 39-48.

Waterman, P. G. (2007). The Status of Chemical Systematics. *Phytochemistry*, **68**, 2896-2903.

Waterman, P. G., & Grundon, M. F. (1983). *Chemistry and Chemical Taxonomy of the Rutales*.*Academic press*, ISBN: 0127376801 London.

Watkins, E. R., & Meshnick, S. R. (2002). Drugs for Malaria**.** *Seminars in Pediatric Infectious Diseases*, **11**(3), (202-212).

Wells T. N., & Poll, E. M. (2010). When is enough enough? The need for a robust pipeline of high quality antimalarials. *Discov Med*, **9**, 389–398.

Wells, T. N.C. (2011). Natural products as starting points for future anti-malarial therapies: going Back to our roots?, *Malaria Journal*, **10**(1), S3

Warnsdorfer, W. H., & Payne, D. (1991). The Dynamics of drug resistance in *Plasmodium falciparum*. *Pharmacol. Ther*, **50**, 95-121.

Were, P, S., Kinyanjuia, P, Gicheru, M. M., Mwangi, E, Ozwara, H. S. (2010). Prophylactic and curative activities of extracts from *Warburgia ugandensis* and *Zanthoxylum usambarense* against *Plasmodium knowlesi* and *Plasmodium berghei. Journal of ethnophamacology*, **130**, 158-162.

White, N. J. (1985). Clinical Pharmacokinetics of antimalrial drugs. *Clinical pharmacokinet*, **10**, 187-215.

Winkler, A., Puhl, M., Weber, H., Kutchan, T. M., Gruber. K., & Macheroux, P. (2009). Berberine bridge enzyme catalyzes the six electron oxidation of (S)-reticuline to Dehydroscoulerine. *Phytochemistry*, **70**, 1092–1097.

Winstanley, P. A., Ward, S. A., & Snow, R. W. (2002). Clinical status and implications of antimalarial drug resistance. *Microb Infect*, **4**, 157–164.

Woodward, R. B., & Doering, W. E., (1944). The total synthesis of quinine. J. *AM. Chem. Soc*, **66**(5), 849-849.

World health organization, (2012). Summary of world malaria report 2012, ISBN 9759241564533.

World health organization. (2002). The World healthy report 2002. Reducing risks and promoting healthy life.

World health organization. (2013). World healthy statistics 2013. ISBN 9789241564588.

Wu, S-J. & Chen, I-S. (1993). Alkaloids from *Zanthoxylum simulans*. Phytochemistry, **34**(6) 1659-1661.

Xiong, Q., Dawen, S., & Mizuno. (1994): Flavanol glucosides in Pericarps of *Zanthoxylum bungeanum*: *Phytochemistry*, **39**(3), 723-729.

Yang, Y. C., Park, K., Kim, E. H., Lee, H. S., & Ahn, Y. J. (2004) . Larvicidal Activity of Medicinal Plant Extracts against *Aedes aegypti, Ochlerotatus togoi,* and *Culex pipiens pal/ens* (Diptera: Culicidae)**.** *Asia-Pacific Entomol*, **7**(2): 227 -232.

Yang, Y-P., Cheng, M-J., Teng, C-M., Chang, Y-L., Tsai, I-L., Chen, I-S. (2002). Chemical and anti-platelet constituents from Formosan *Zanthoxylum simulans. Phytochemistry,* **61**(2002), 567–572

Zenk, M. H. (1994). The formation of benzophenanthridine Alkaloids. *Pure* & *Appl. Chem*, **66**, 10/11, 2023-2028.

Zhang, J., Krugliak, M., & Ginsburg, H. (1999). The fate of ferriprotorphyrin IX in malaria infected erythrocytes in conjonction with the mode of action of antimalarial drugs. *Mol. Biochem. Parasitol*, **99**, 129-141.

Zhang, L., & Demain, A. L. (2005). Natural products: Drug discovery and therapic medicine. *Humana press, tokowa, New Jersey*. ISBN: 978-1-59259-976-9.

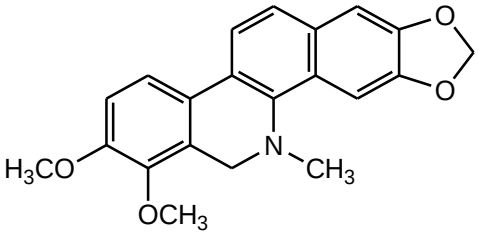
Zhou, X-J., Chen, X-L., Li, X.S., Su, J., He, J-B., Wang, Y-H., Li, Y., Cheng, Y-X. (2011). Two dimeric lignans with an unusual a, b-unsaturated ketone motif from Zanthoxylum podocarpum and their

inhibitory effects on nitric oxide production. *Bioorganic and medicinal chemistry letters*, **21**, 373-376.

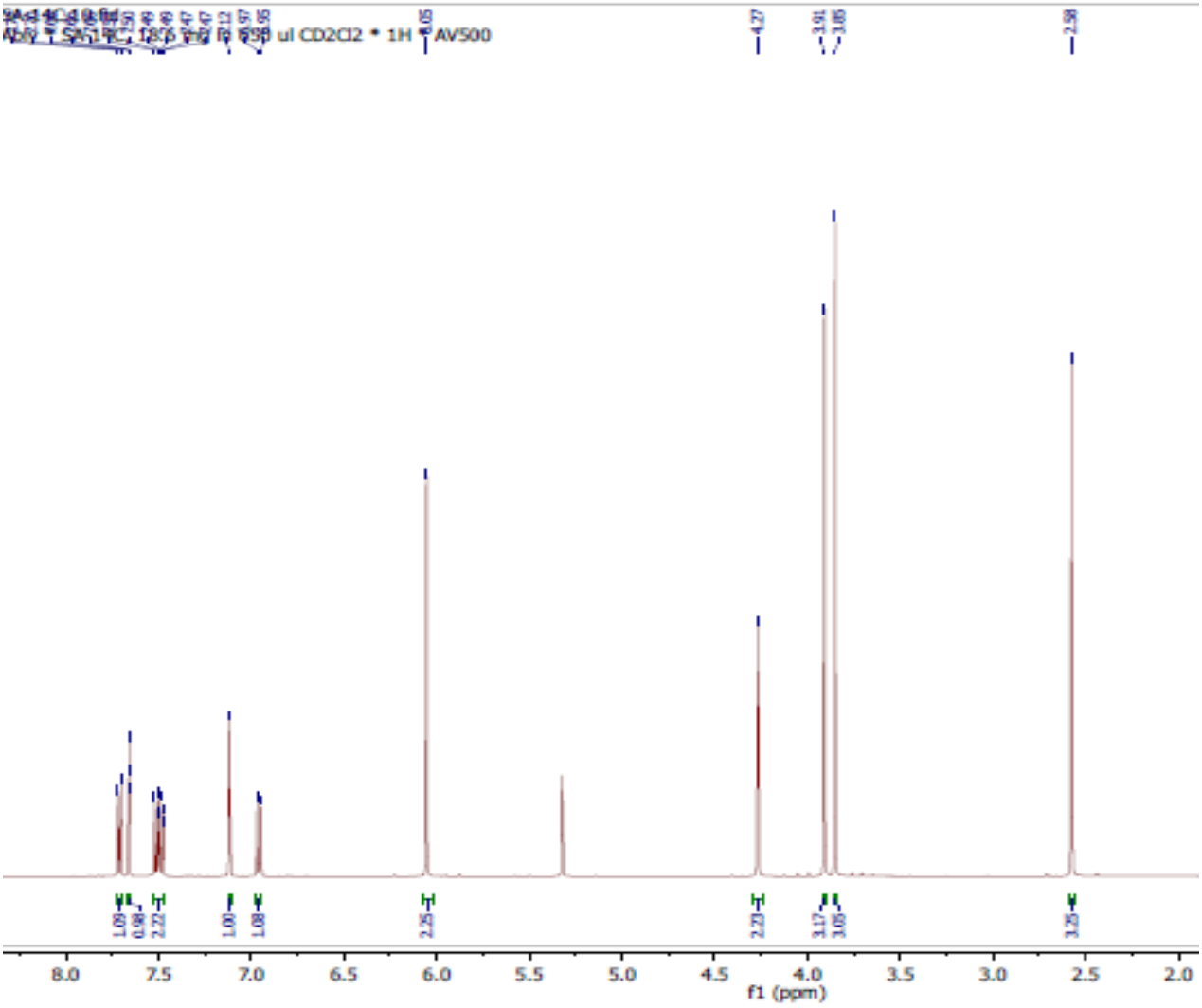
Zirihi, G. N., N'Guessan, Koffi, E. D. T., & Serikouassi. (**2009)**. Evaluation in vitro of antiplasmodial activity of ethanolic extracts of *Funtumia elastica* , *Rauvolfiavomitoria* and *Zanthoxylum gilletii* on *Plasmodium falciparum* isolates from Côted'Ivoire .Badama Ph. *Journal of Animal & Plant Sciences*, **5**(1), 406 - 413.

APPENDICES

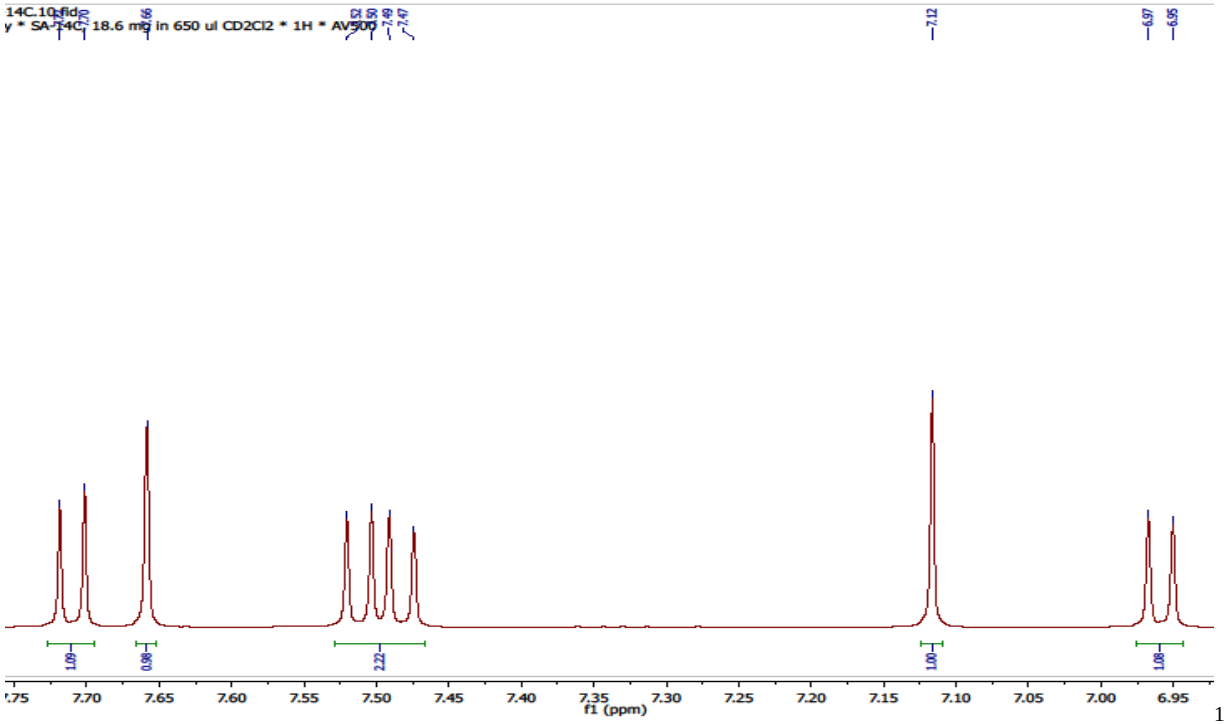
Appendix 1: The spectra of dihydrichelerythrine (1)



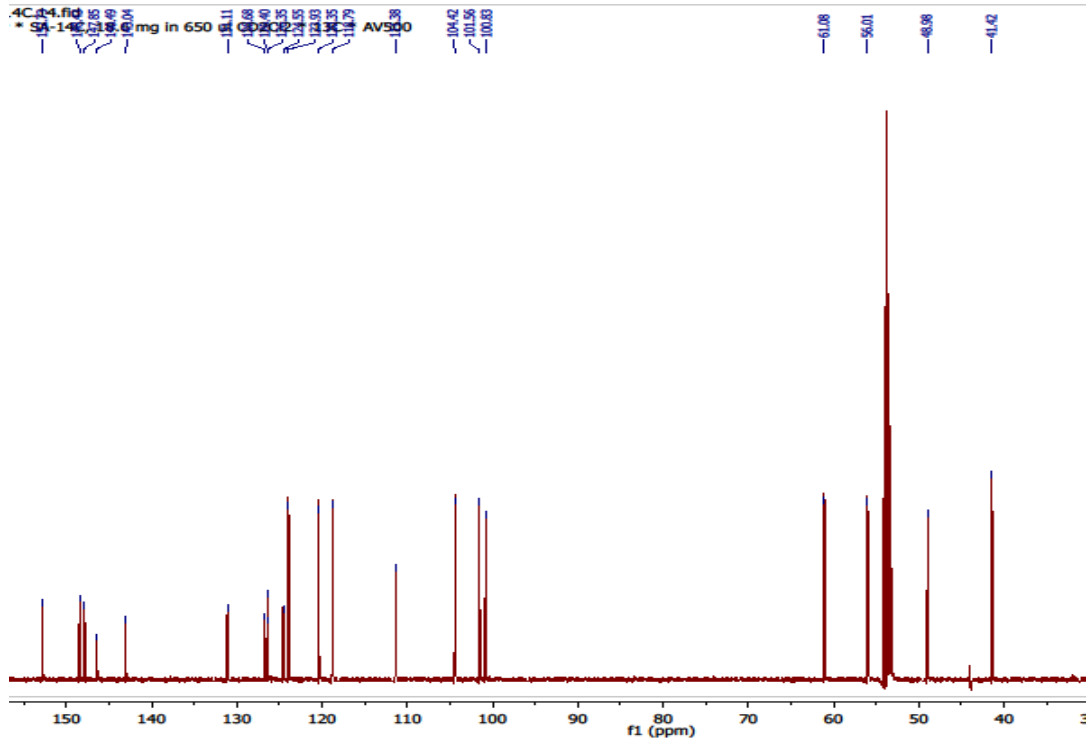
^{1}H NMR spectrum for dihydrochelerythrine **(1)**



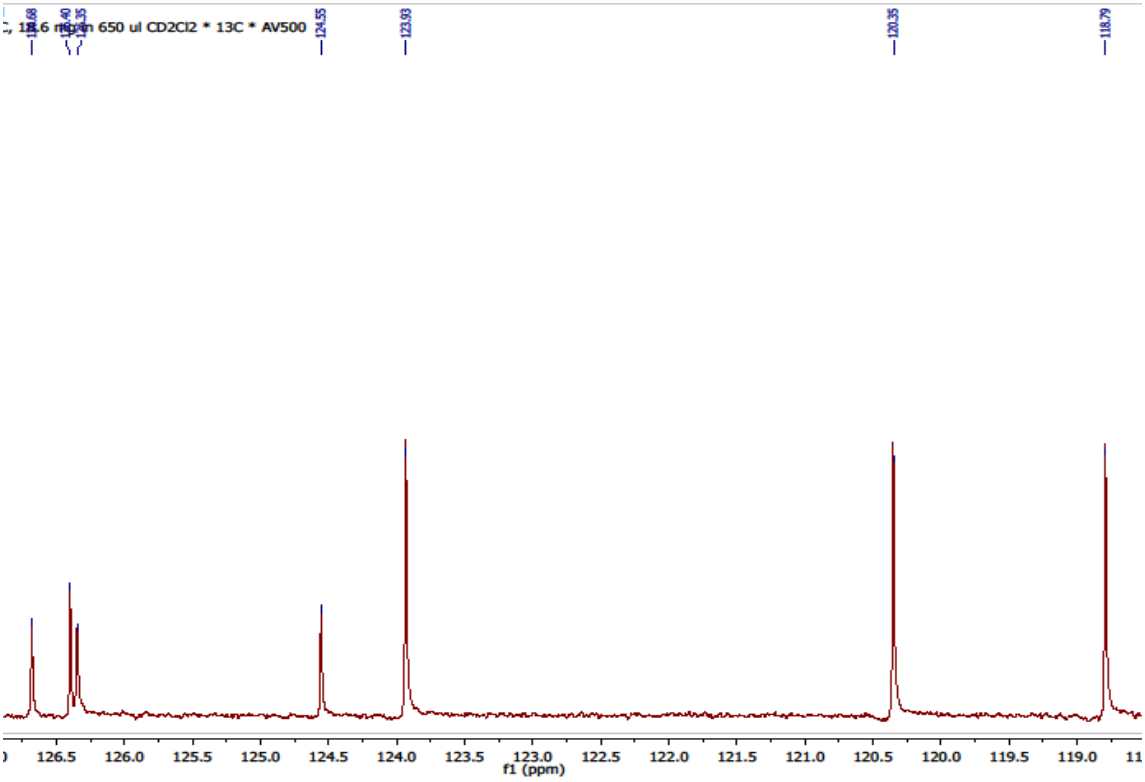
^{1}H NMR spectra for dihydrochelerythrine (**1**) (aromatic region expanded)



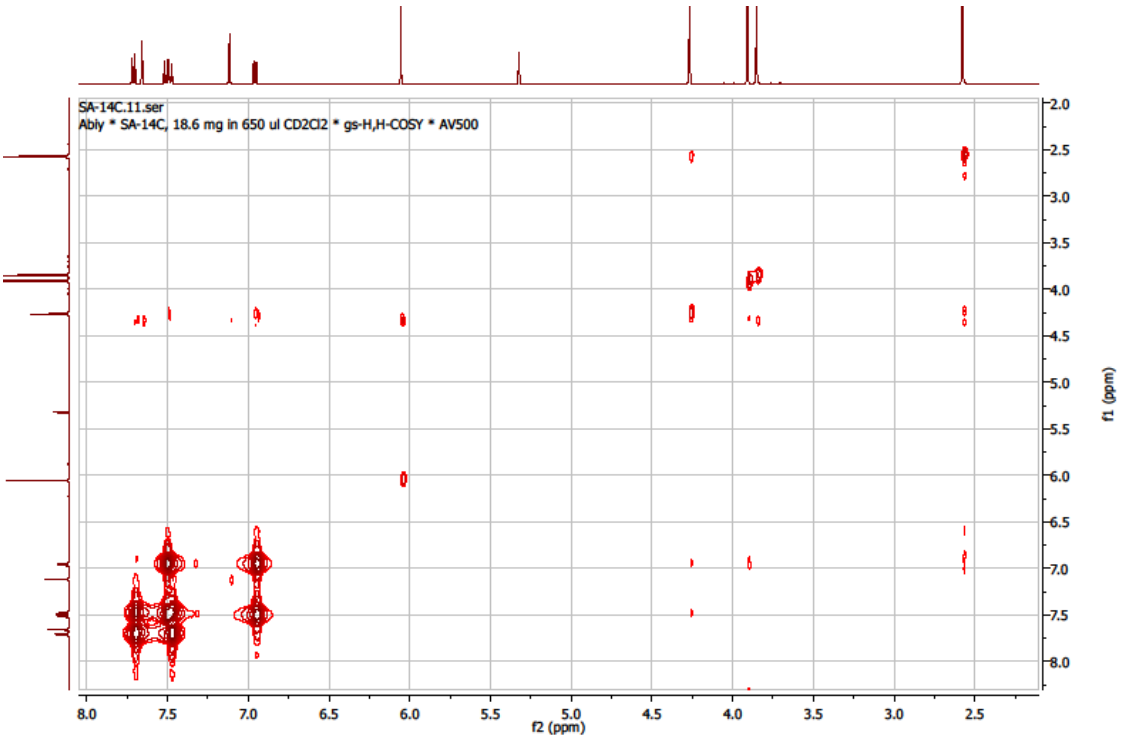
^{13}C NMR spectrum for dihydrochelerythrine **(1)**



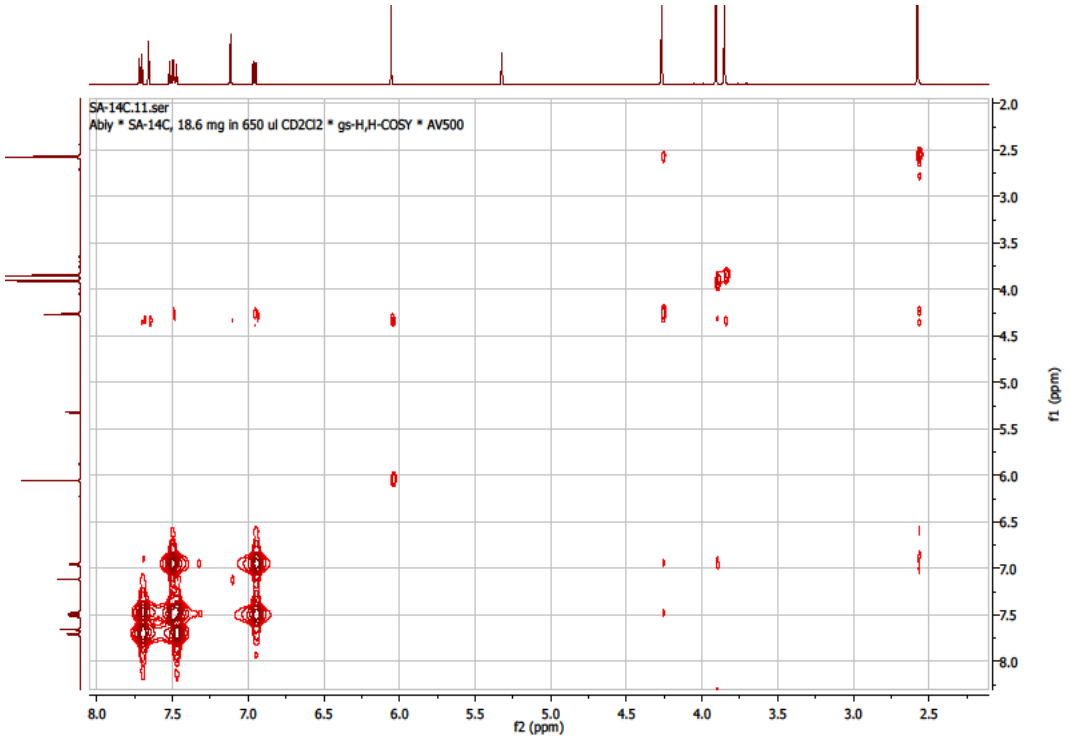
^{13}C NMR for dihydrochelerythrine **(1)** (expansion)



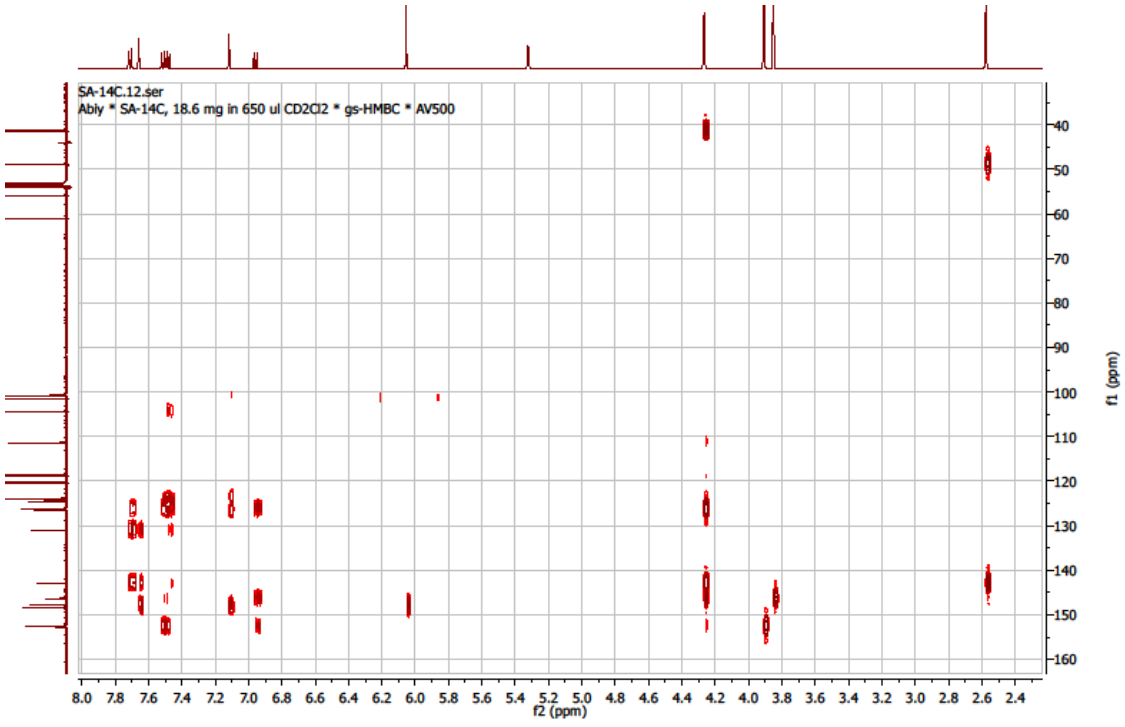
^{1}H-^{1}H-COSY for dihydrochelerythrine **(1)**



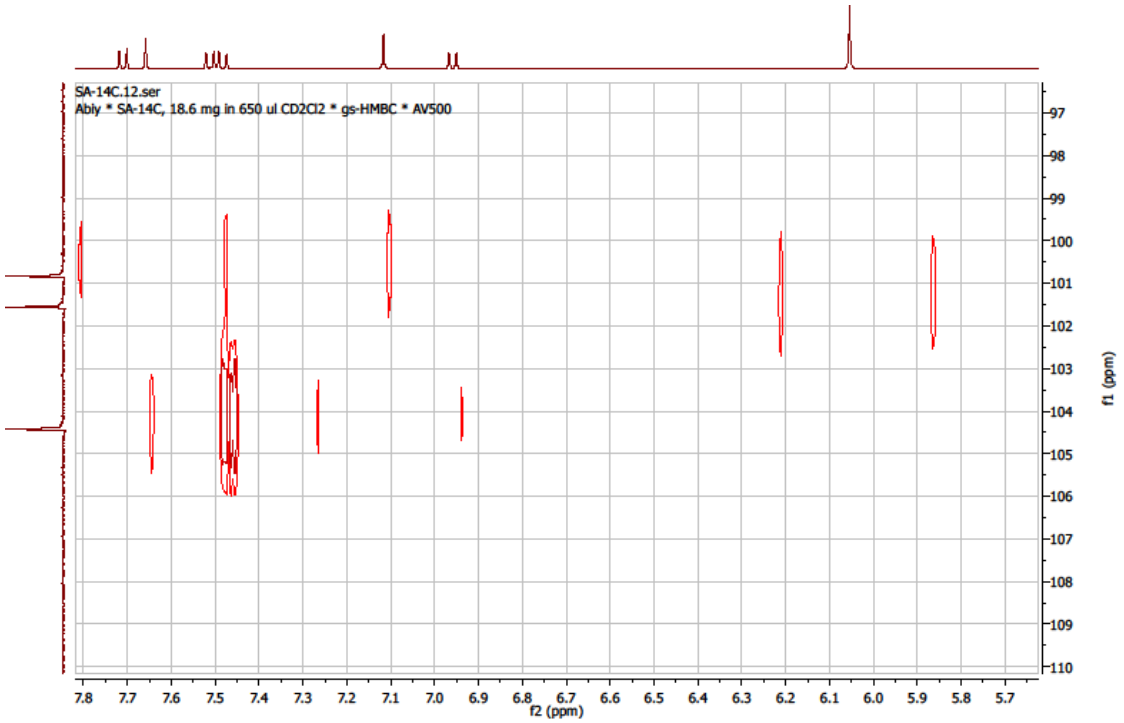
^{1}H-^{1}H-COSY for dihydrochelerythrine **(1)** (expansion)



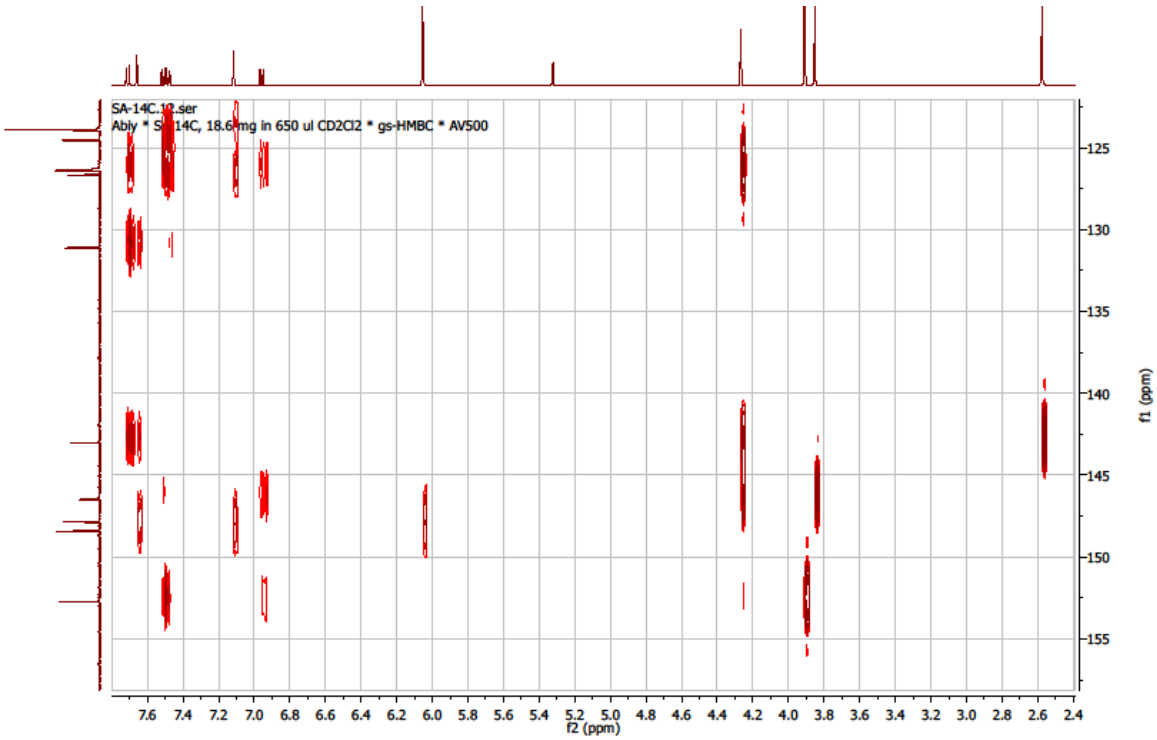
HMBC for dihydrochelerythrine **(1)**



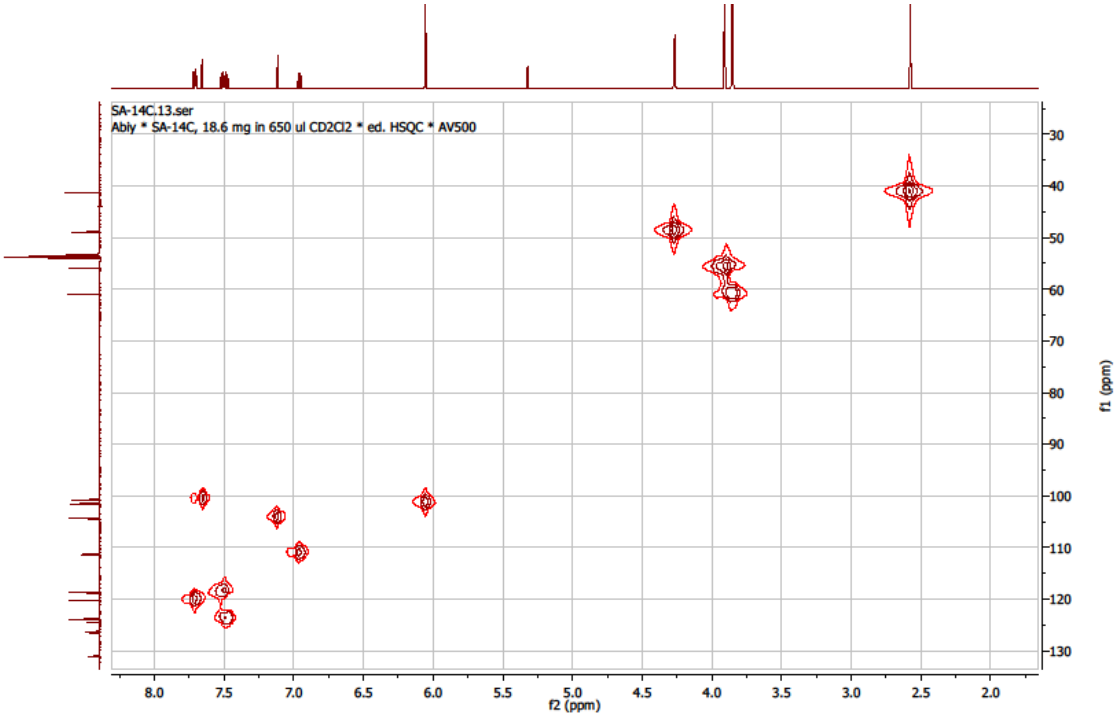
HMBC for dihydrochelerythrine **(1)** (expansion)



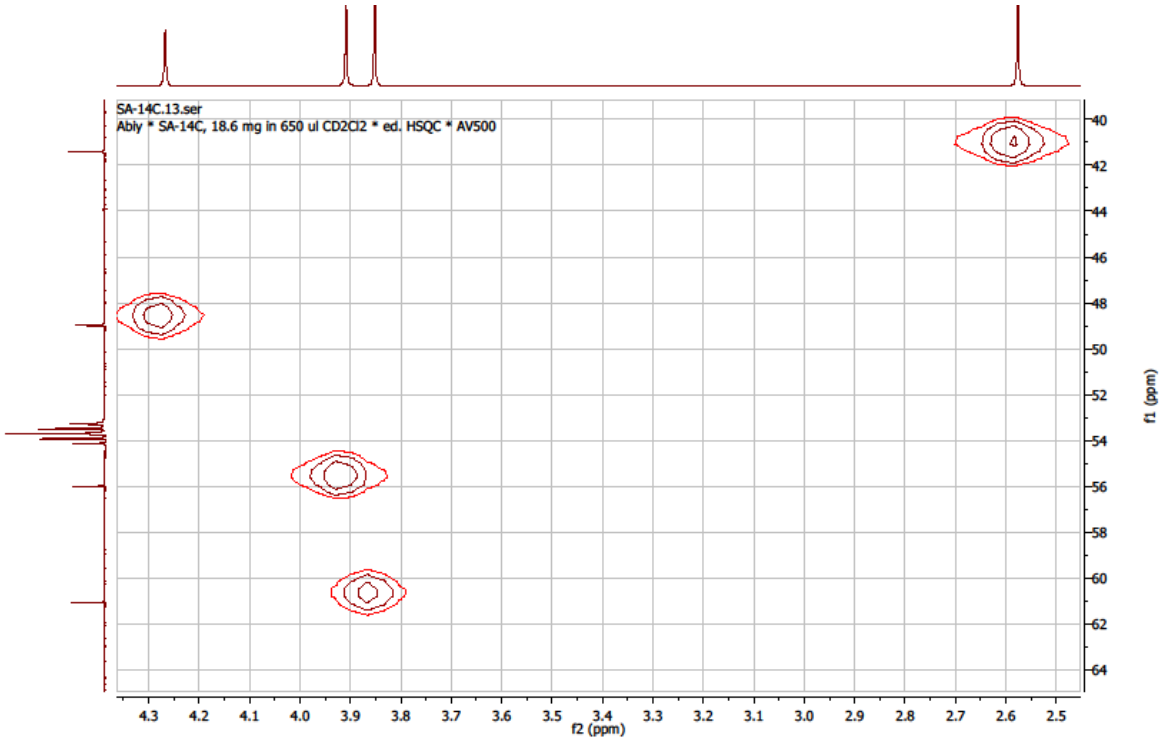
HMBC for dihydrochelerythrine (**1**) (expansion)



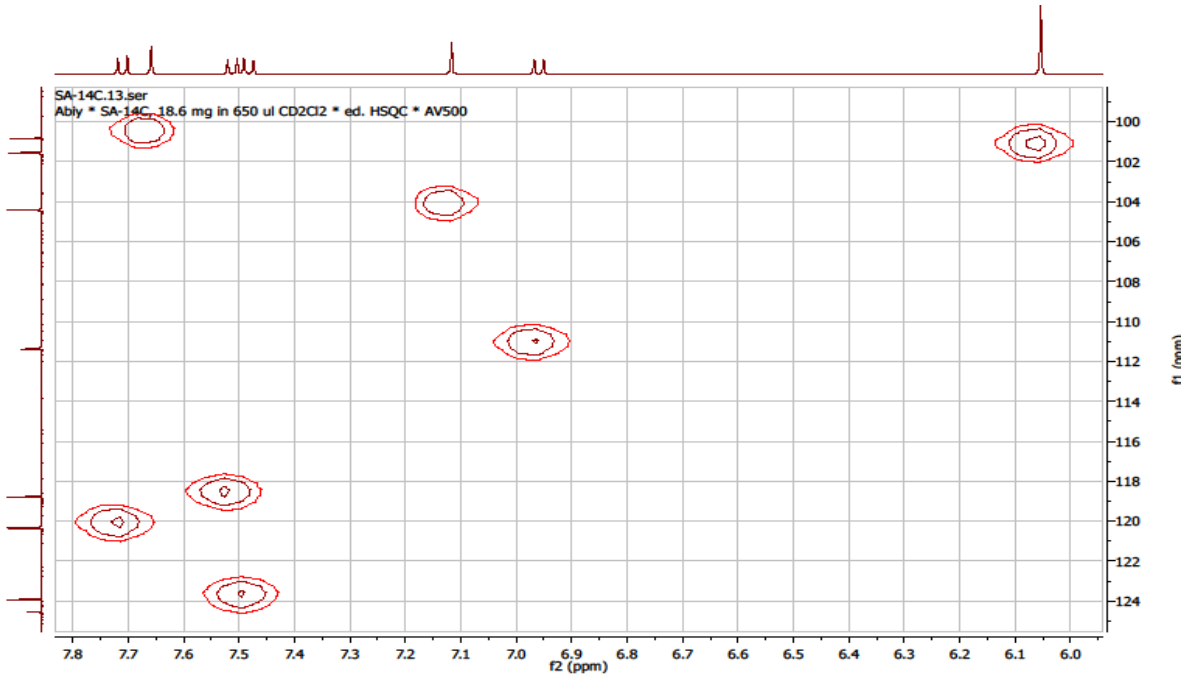
HSQC for dihydrochelerythrine **(1)**



HSQC for dihydrochelerythrine **(1)** (expansion)



HSQC for dihydrochelerythrine **(1)** (expansion)



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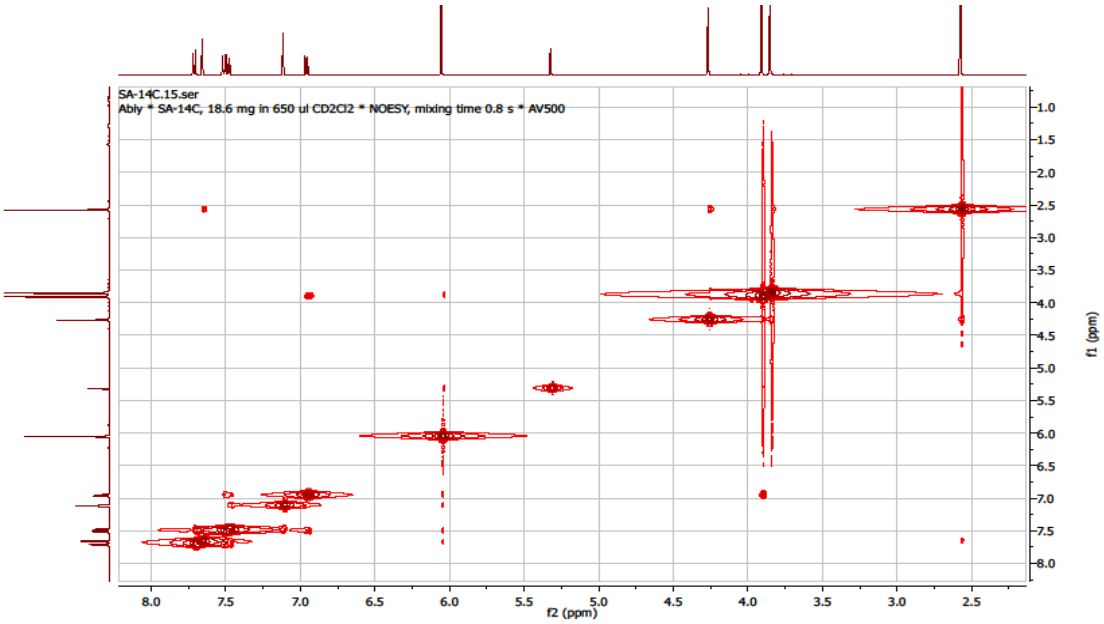
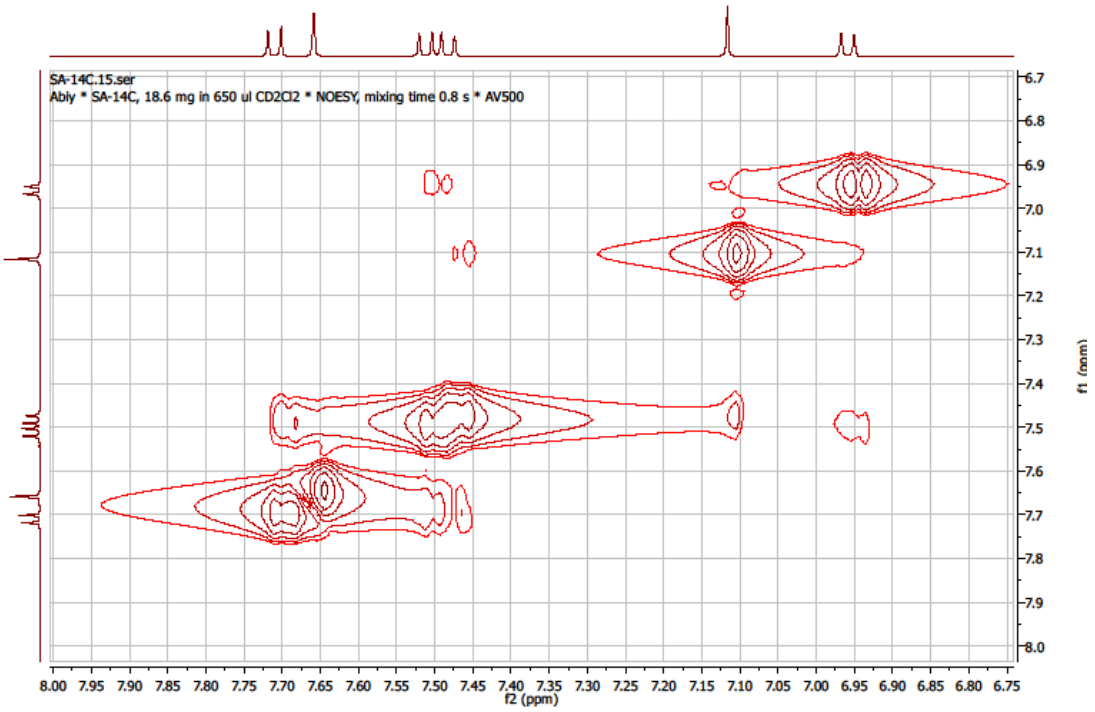
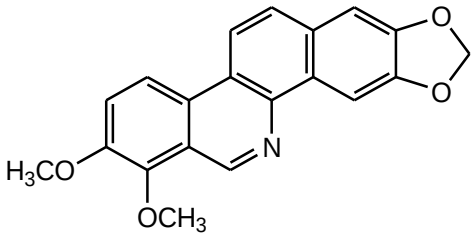


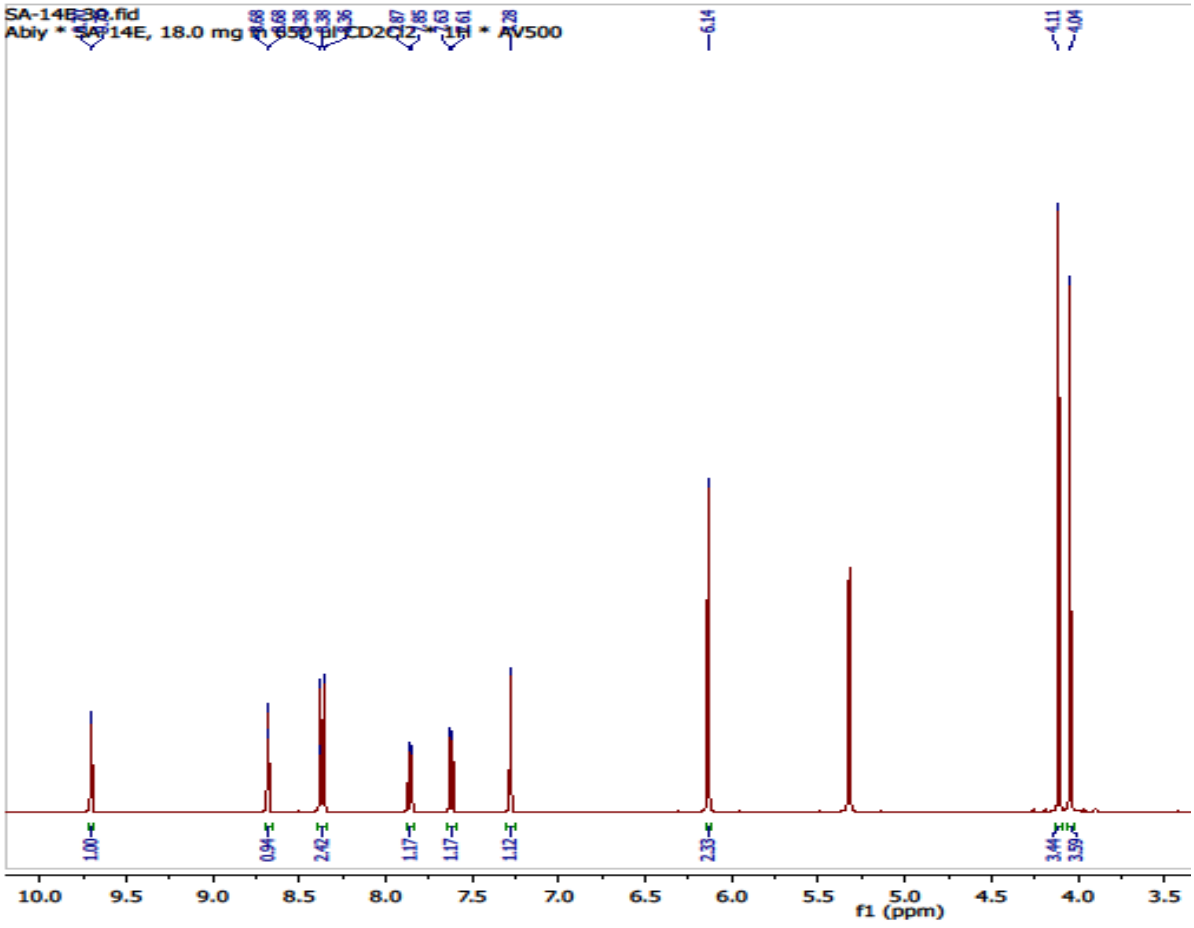
NOESY for dihydrochelerythrine (**1**) (expansion)



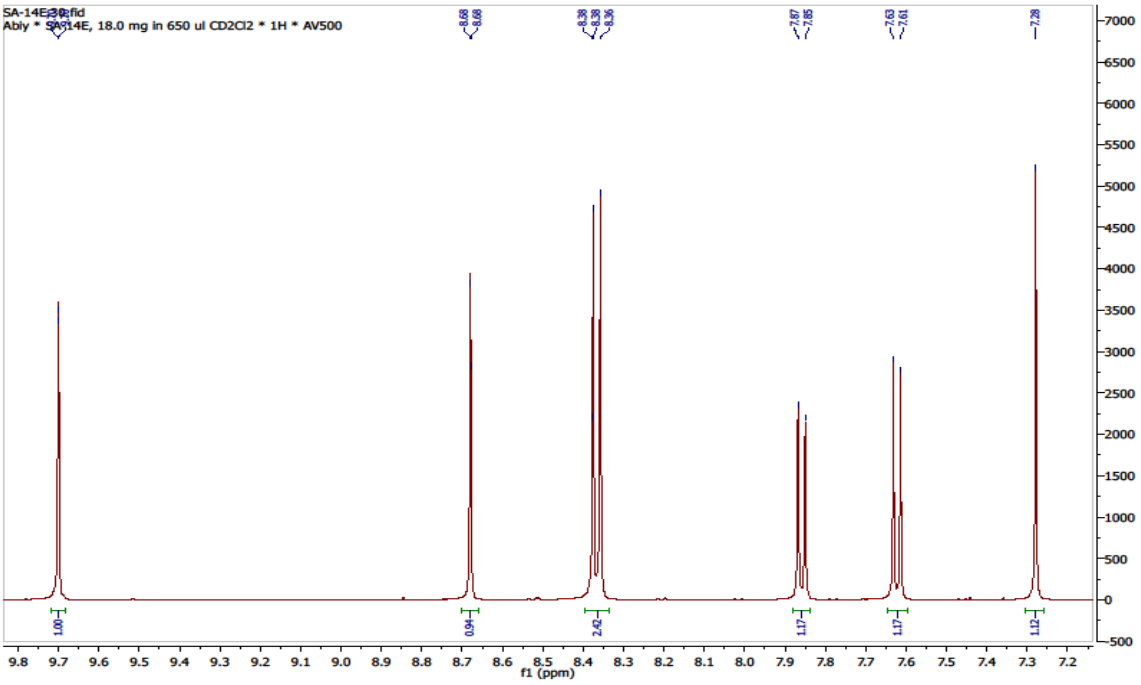
Appendix 2: The spectra for norchelerythrine (2)



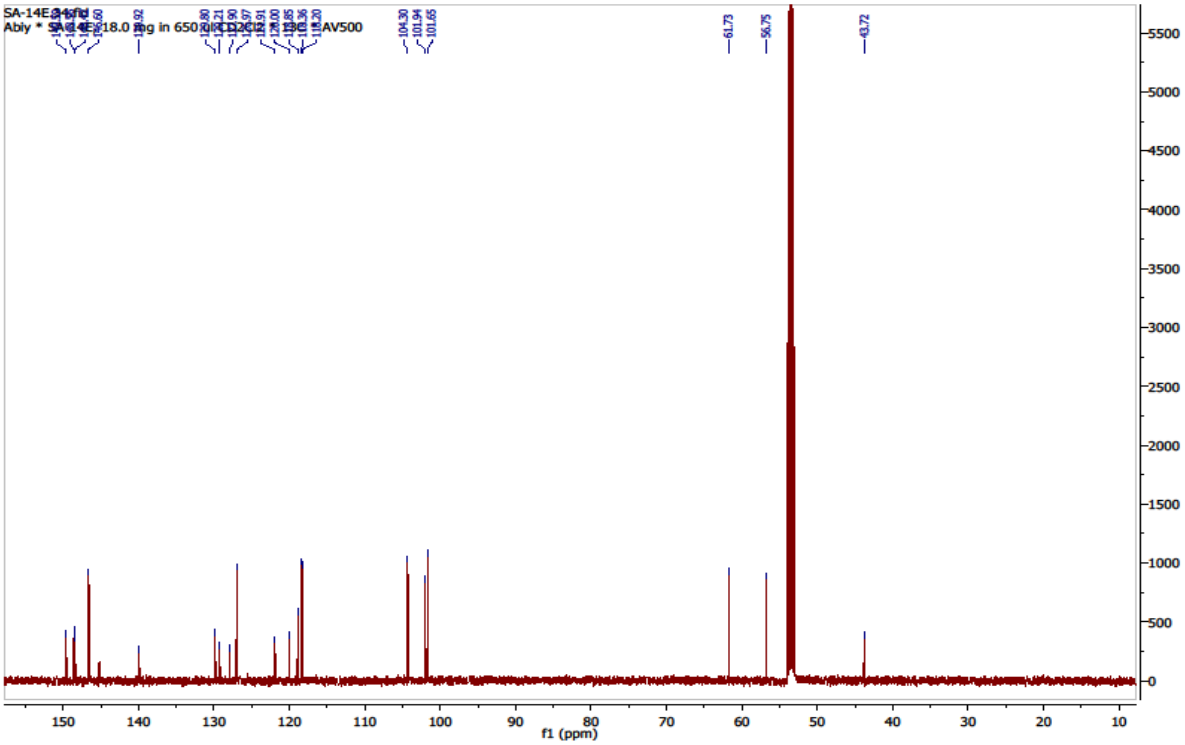
^{1}H NMR spectrum for norchelerythrine (**2**)



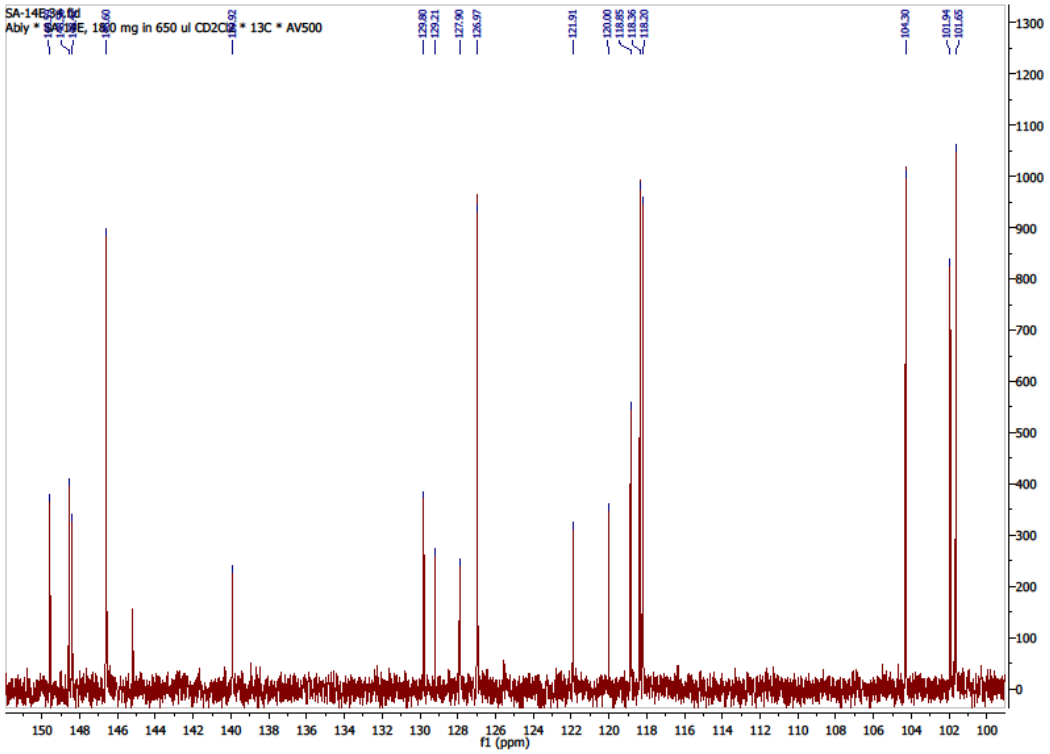
^{1}H NMR spectrum for norchelerythrine **(2)** (expansion)



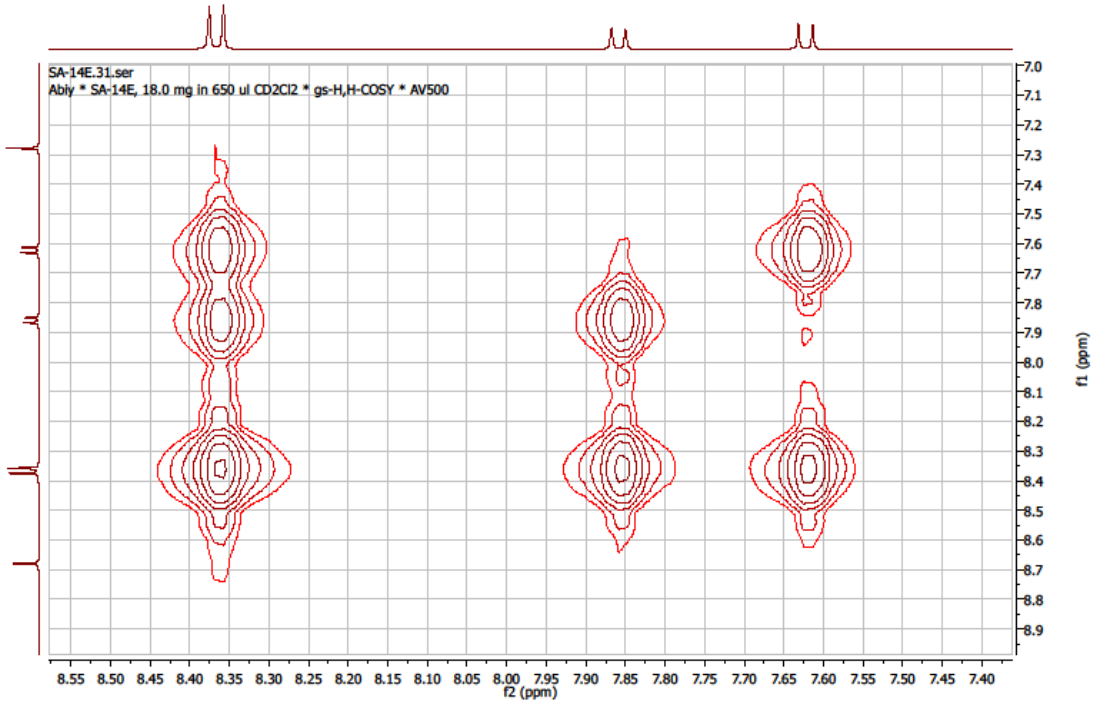
^{13}C NMR for norchelerythrine (**2**)



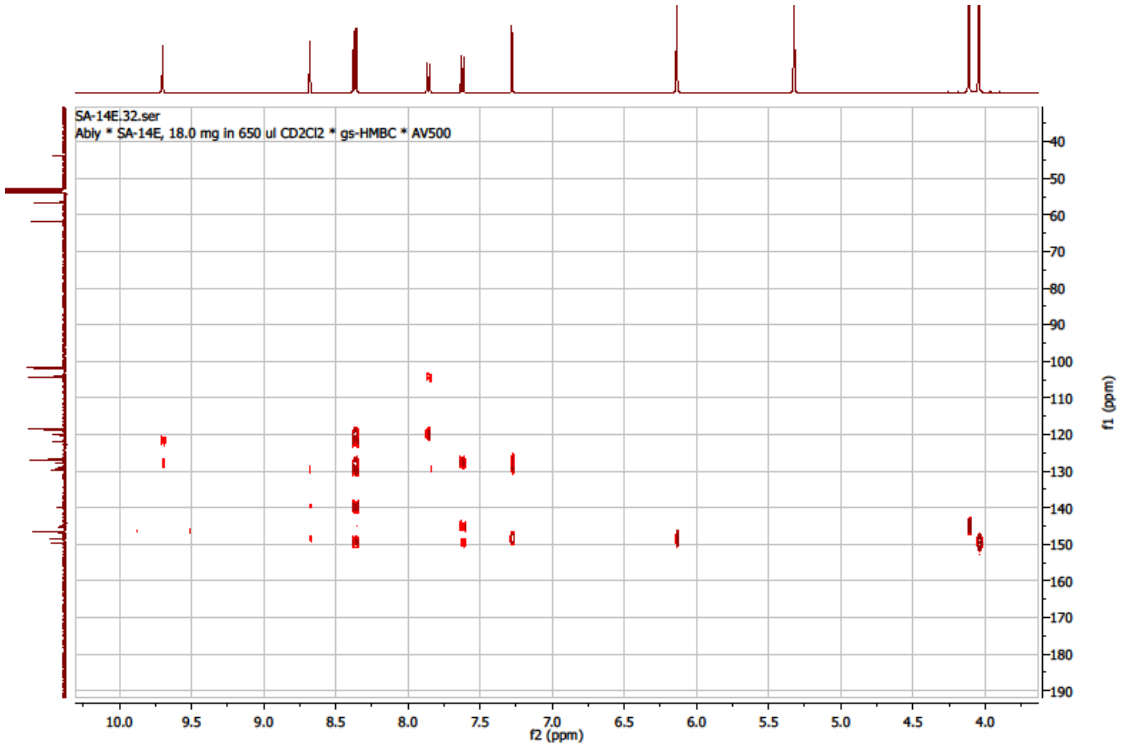
^{13}C NMR for norchelerythrine **(2)** (expansion)



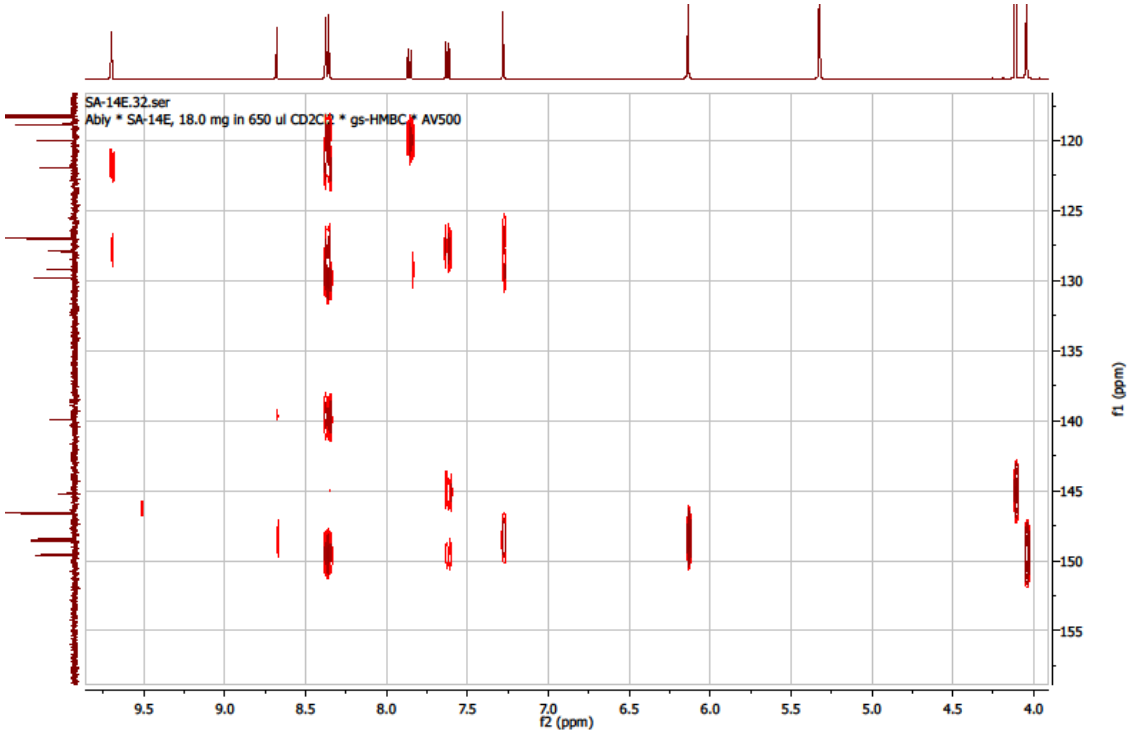
^{1}H-^{1}H-COSY for norchelerythrine (**2**)



HMBC for norchelerythrine (**2**)



HMBC for norchelerythrine (**2**) (expansion)



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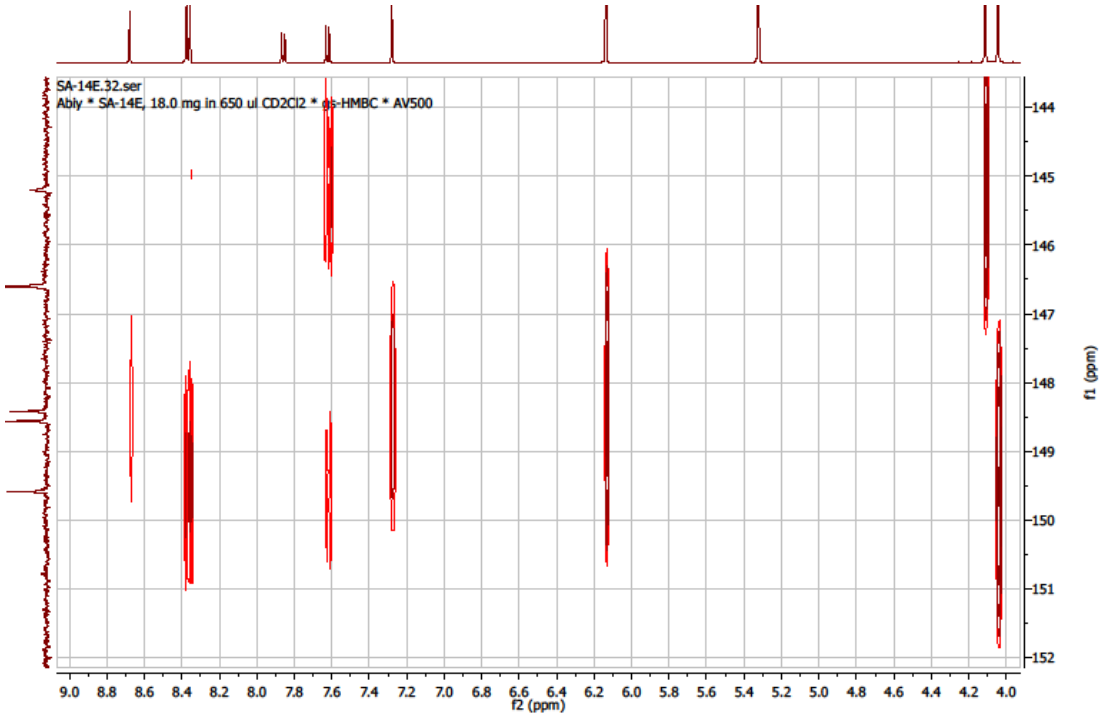
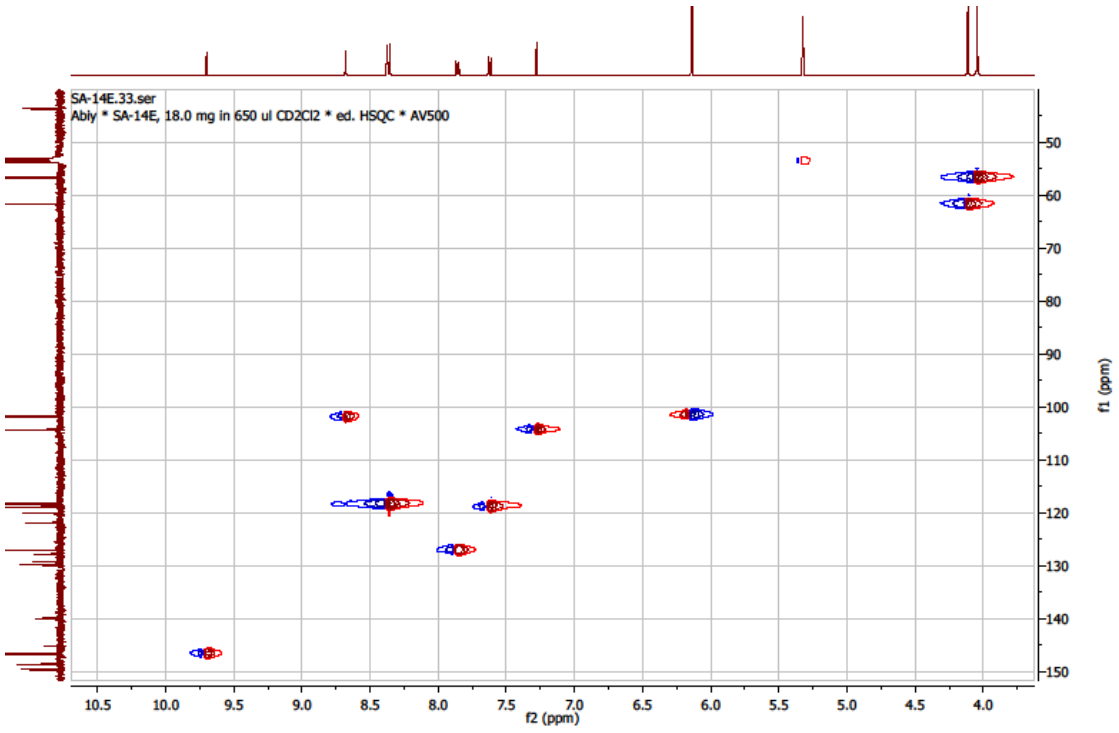
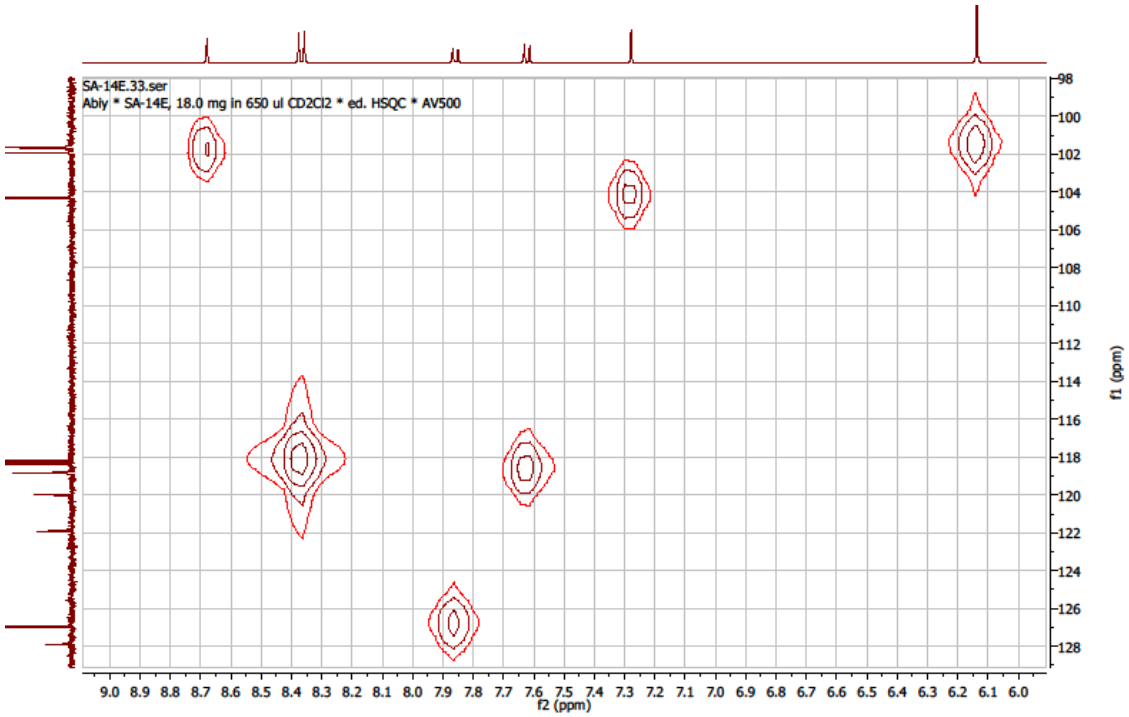


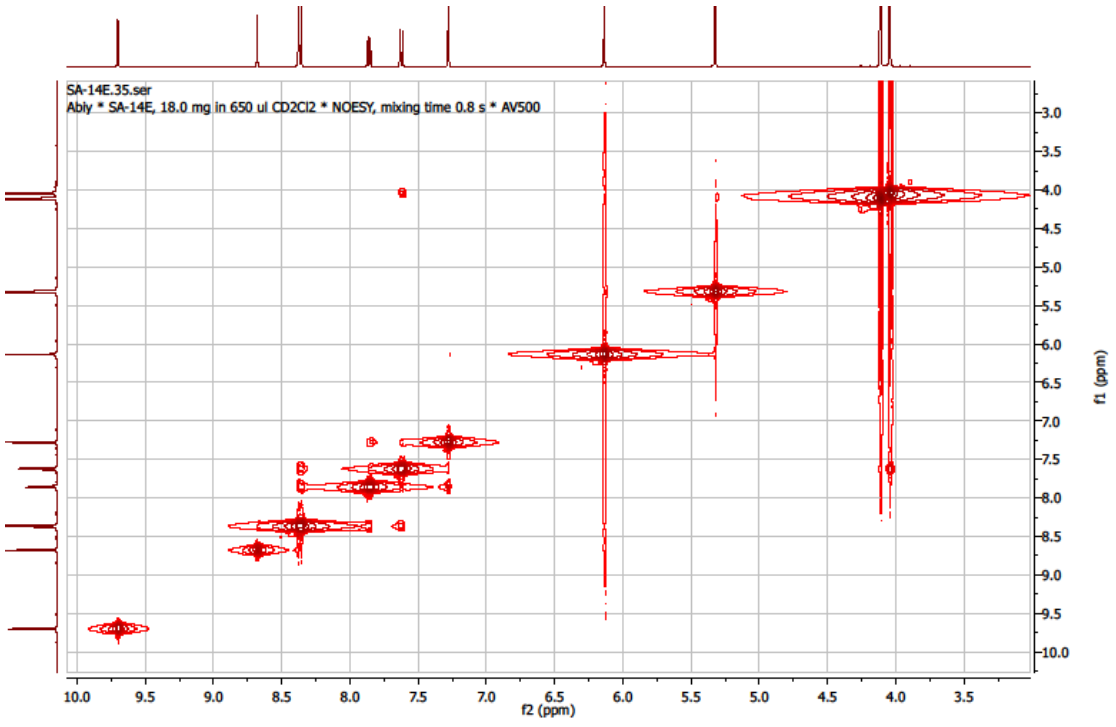
HSQC for norchelerythrine (**2**)



HSQC for norchelerythrine (**5**) (expansion)



NOESY for norchelerythrine



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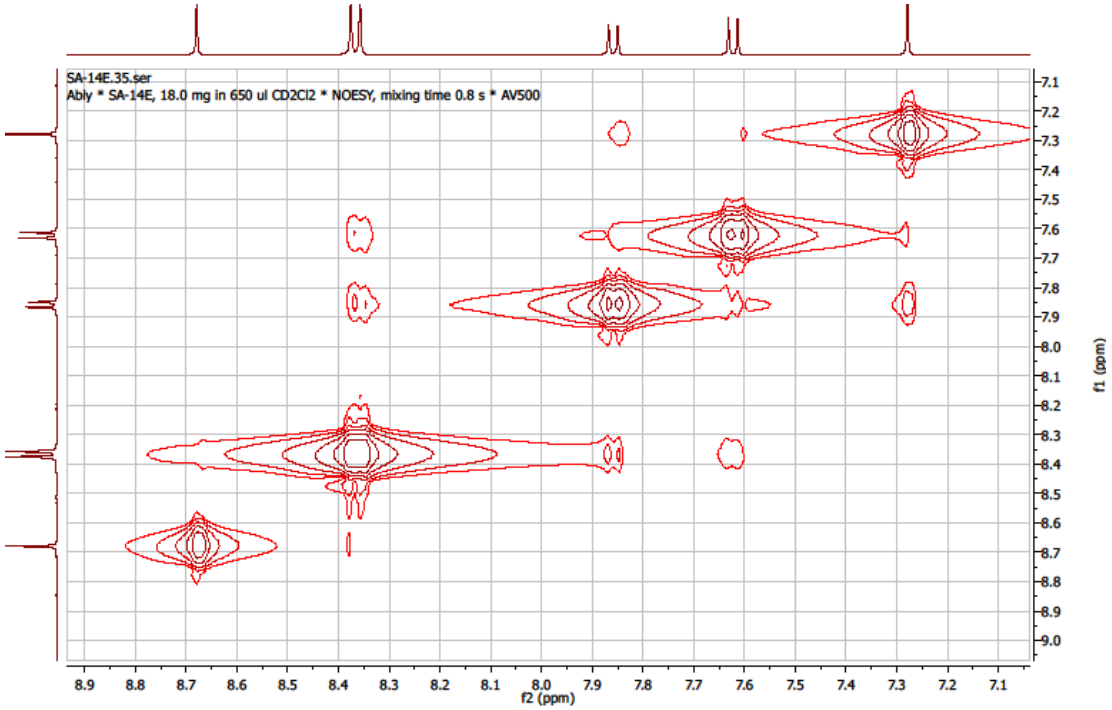
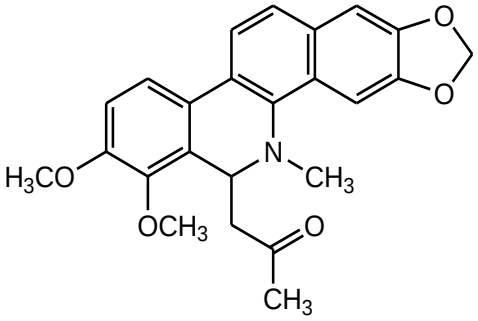
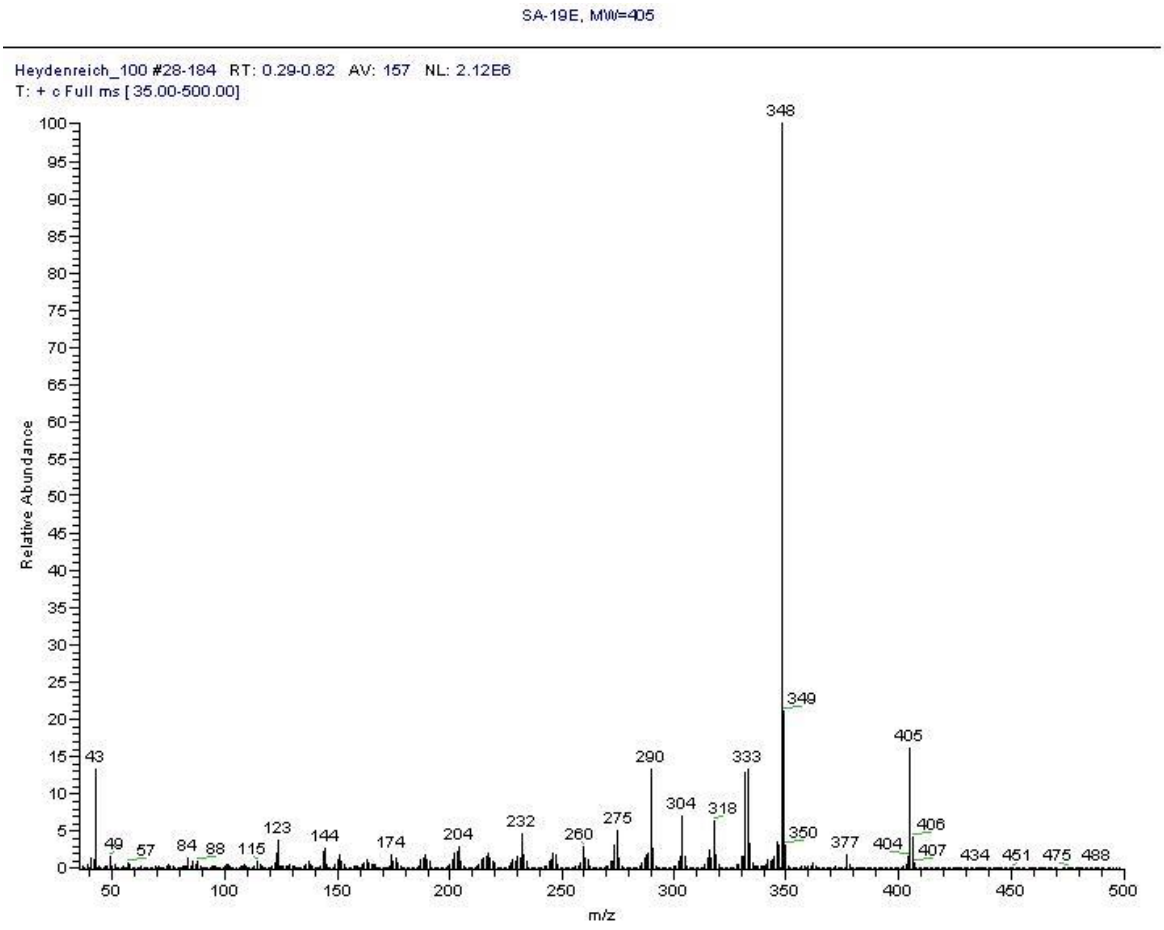


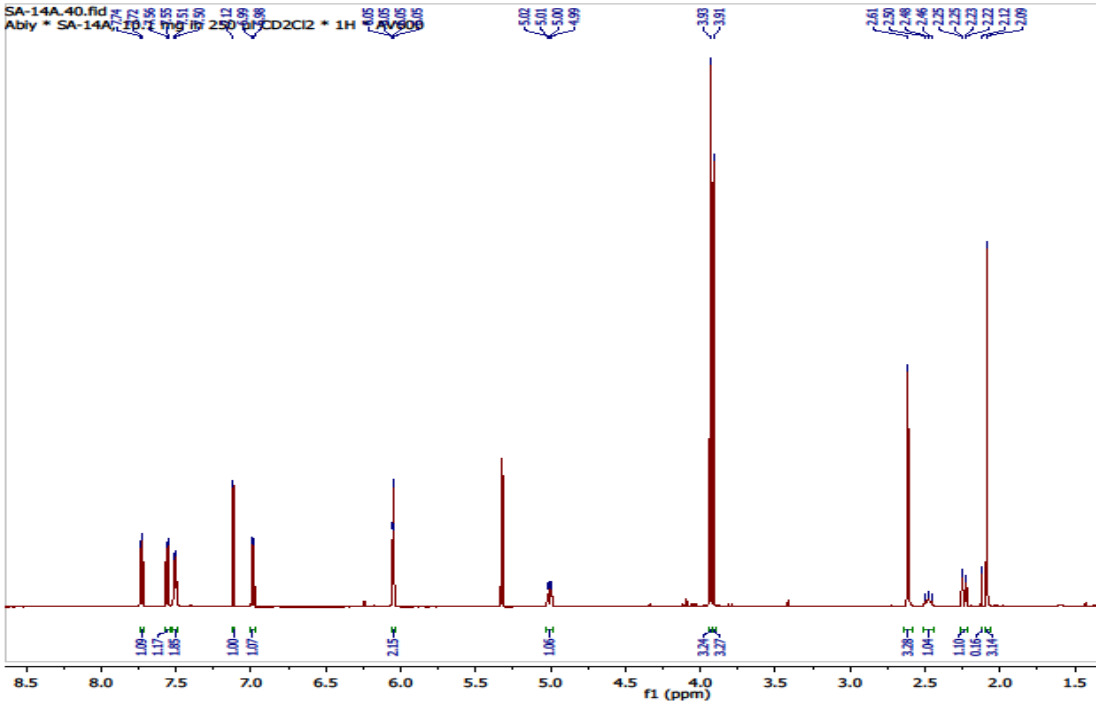
Appendix 3: The spectra for 8-Acetonyldihydrochelerythrine (3)



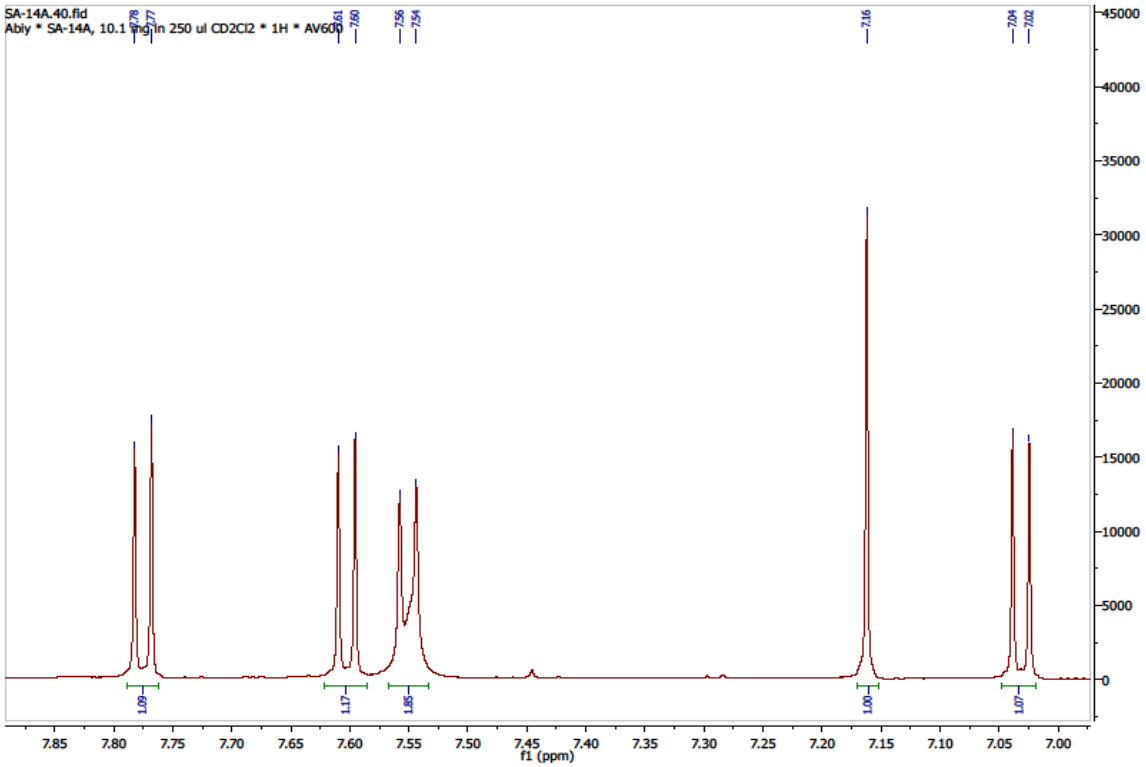
Mass spectrum for 8-Acetonyldihydrochelerythrine (**3**)



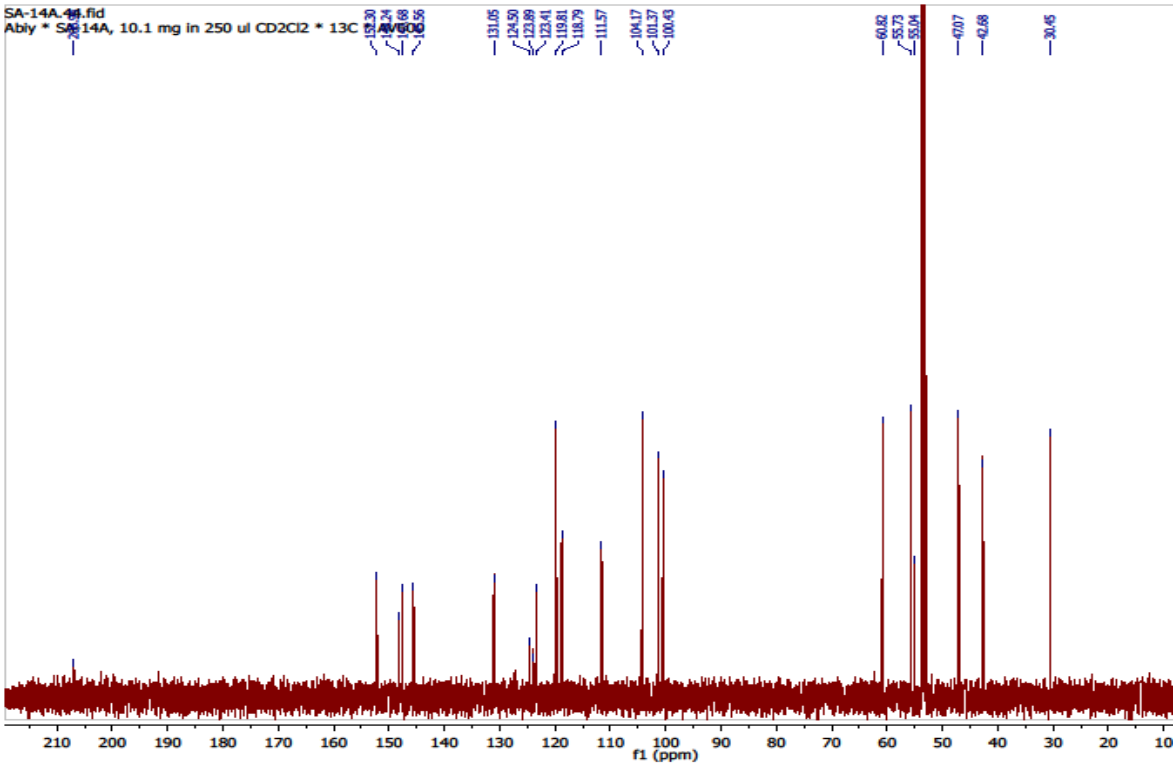
^{1}H NMR spectrum for 8-acetonyldihydrochelerythrine **(3)**



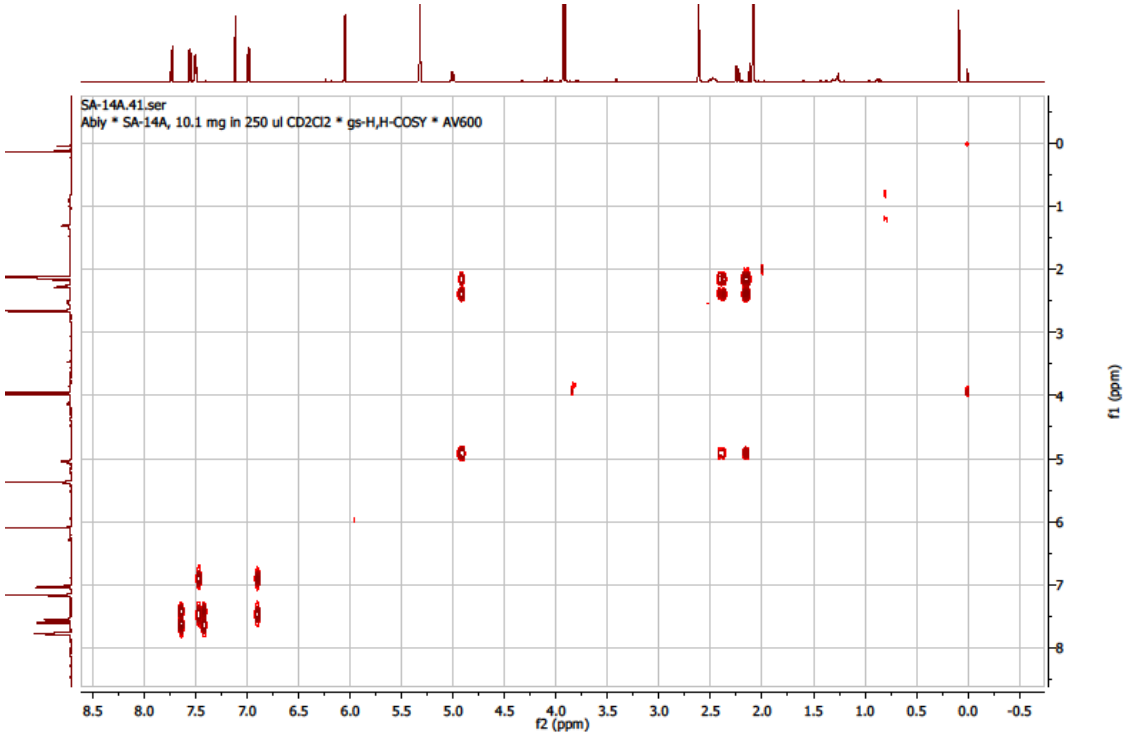
^{1}H NMR spectrum for 8-Acetonyldihydrochelerythrine **(3)** (expansion)



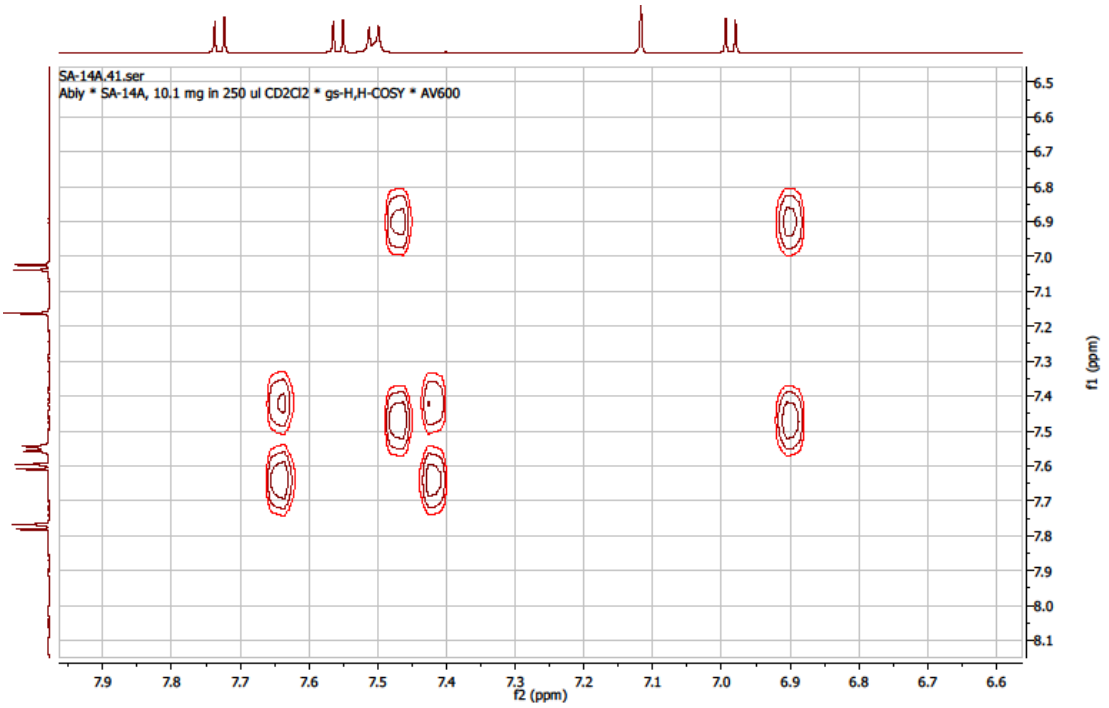
^{13}C NMR spectrum for 8-Acetonyldihydrochelerythrine (**3**)



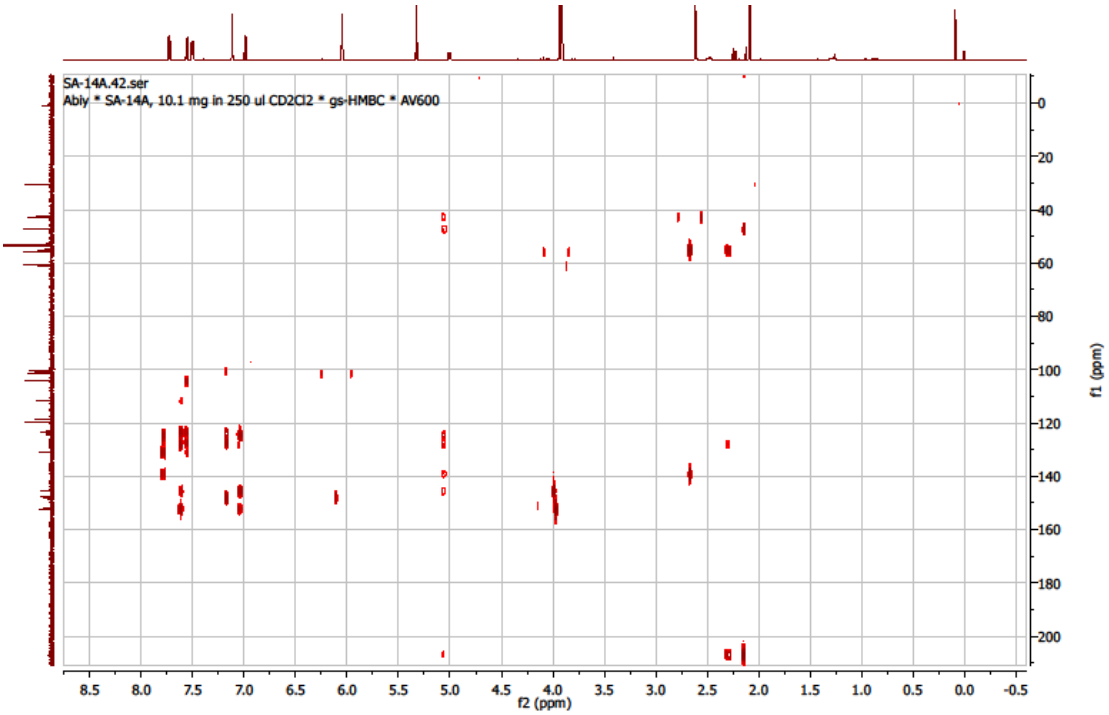
^{1}H,-^{1}H-COSY for 8-Acetonyldihydrochelerythrine (**3**)



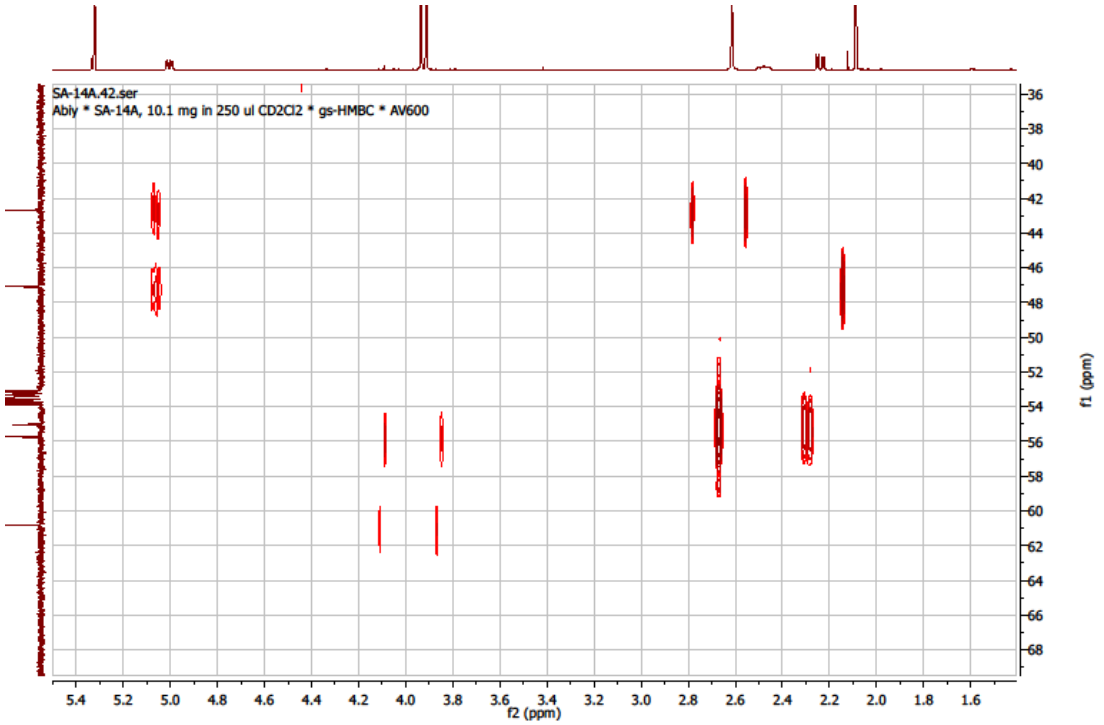
^{1}H-^{1}H-COSY for 8-Acetonyldihydrochelerythrine **(3)** (expansion)



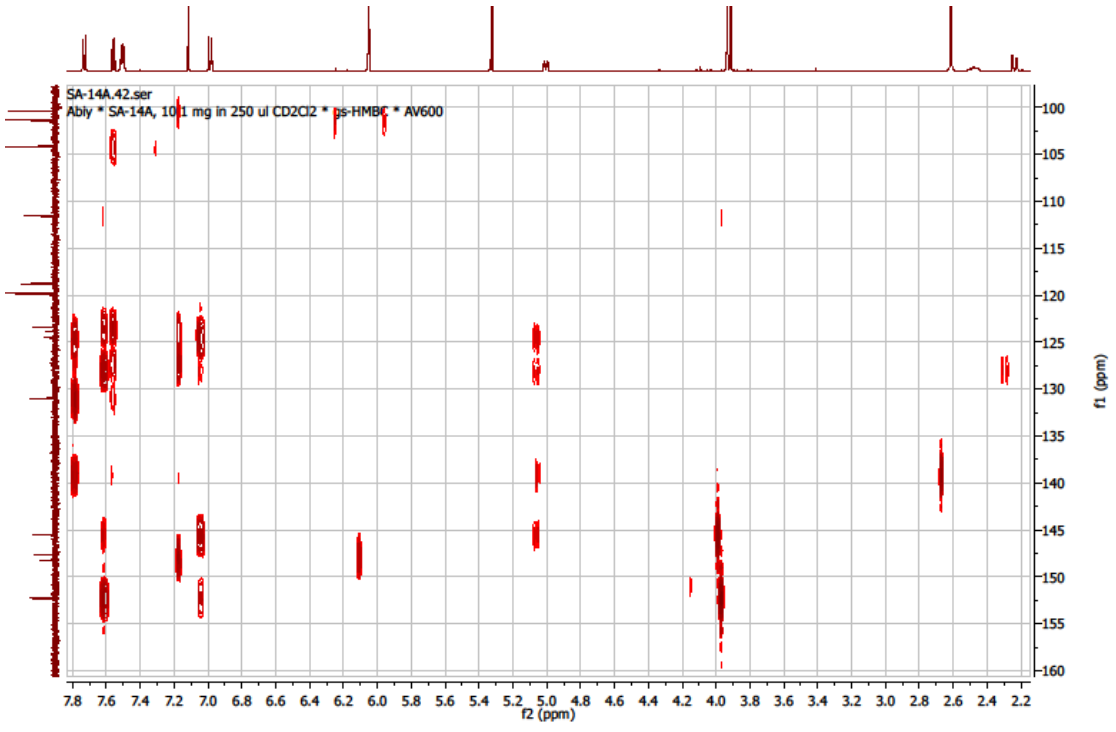
HMBC for 8-Acetonyldihydrochelerythrine **(3)**



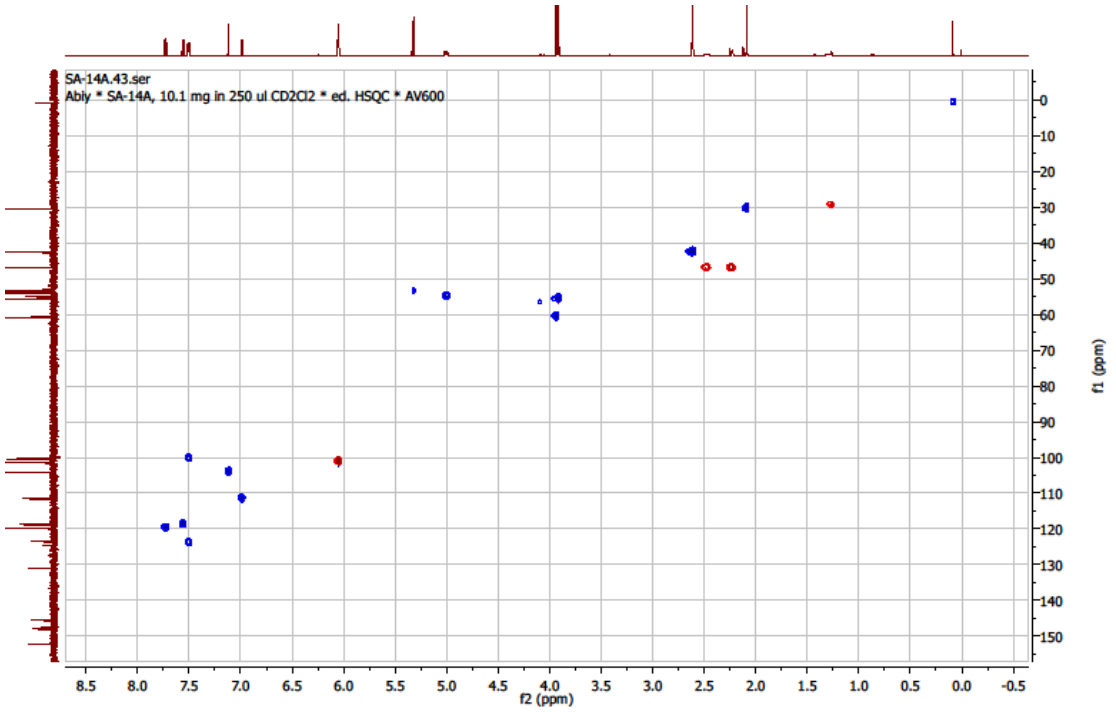
HMBC for 8-Acetonyldihydrochelerythrine **(3)**



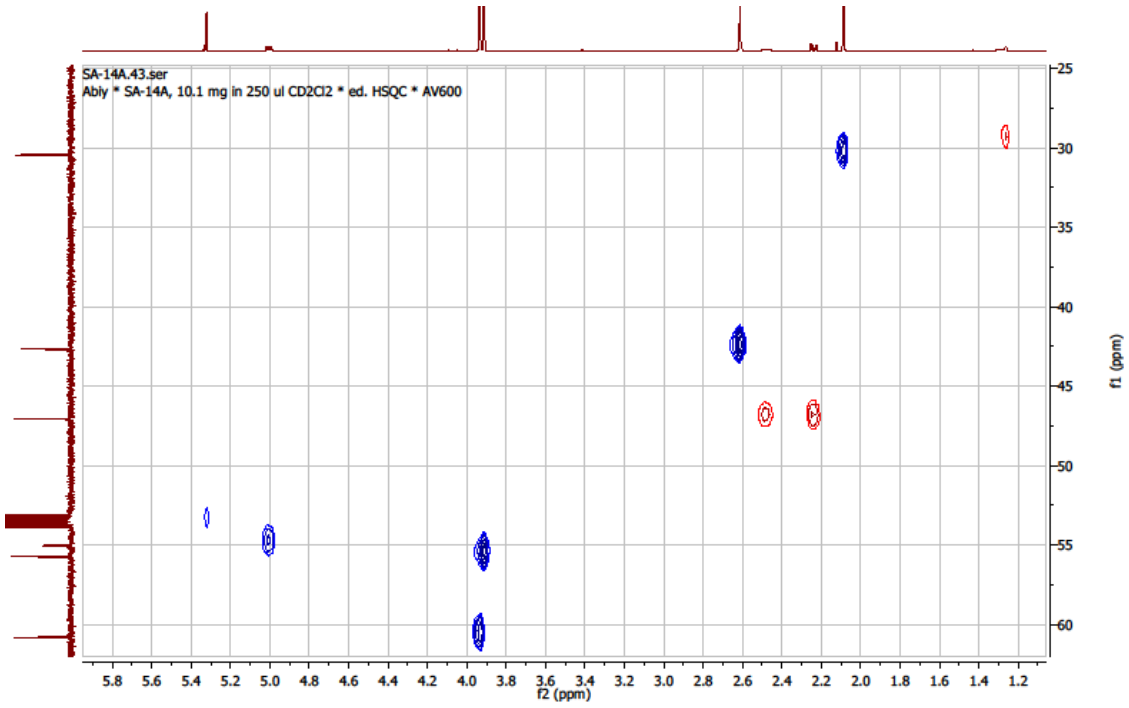
HMBC for 8-Acetonyldihydrochelerythrine **(3)** (expansion)



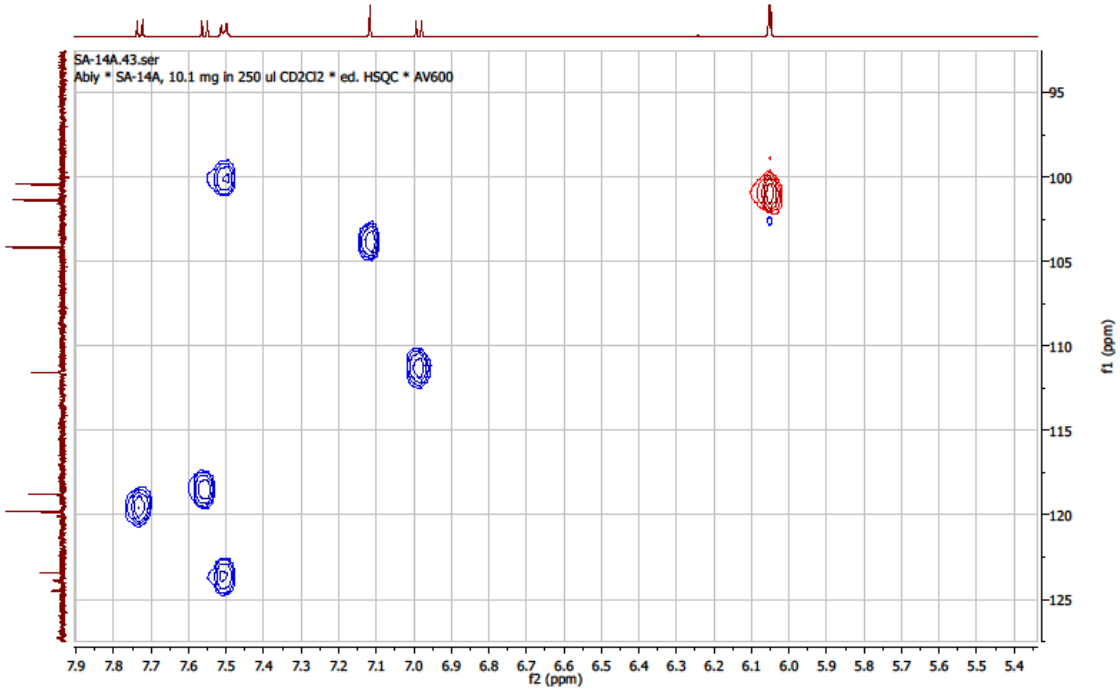
HSQC for 8-Acetonyldihydrochelerythrine **(3)**



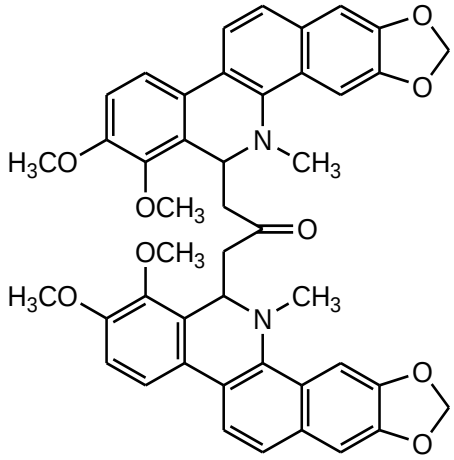
HSQC for 8-Acetonyldihydrochelerythrine **(3)**



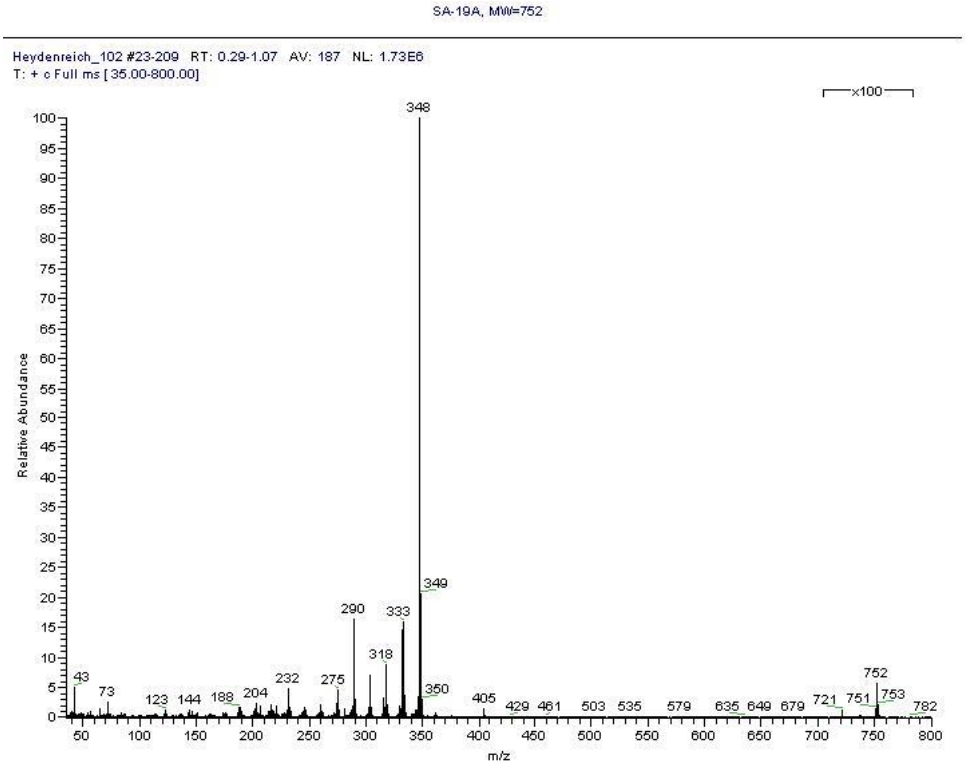
HSQC for 8-Acetonyldihydrochelerythrine **(3)**



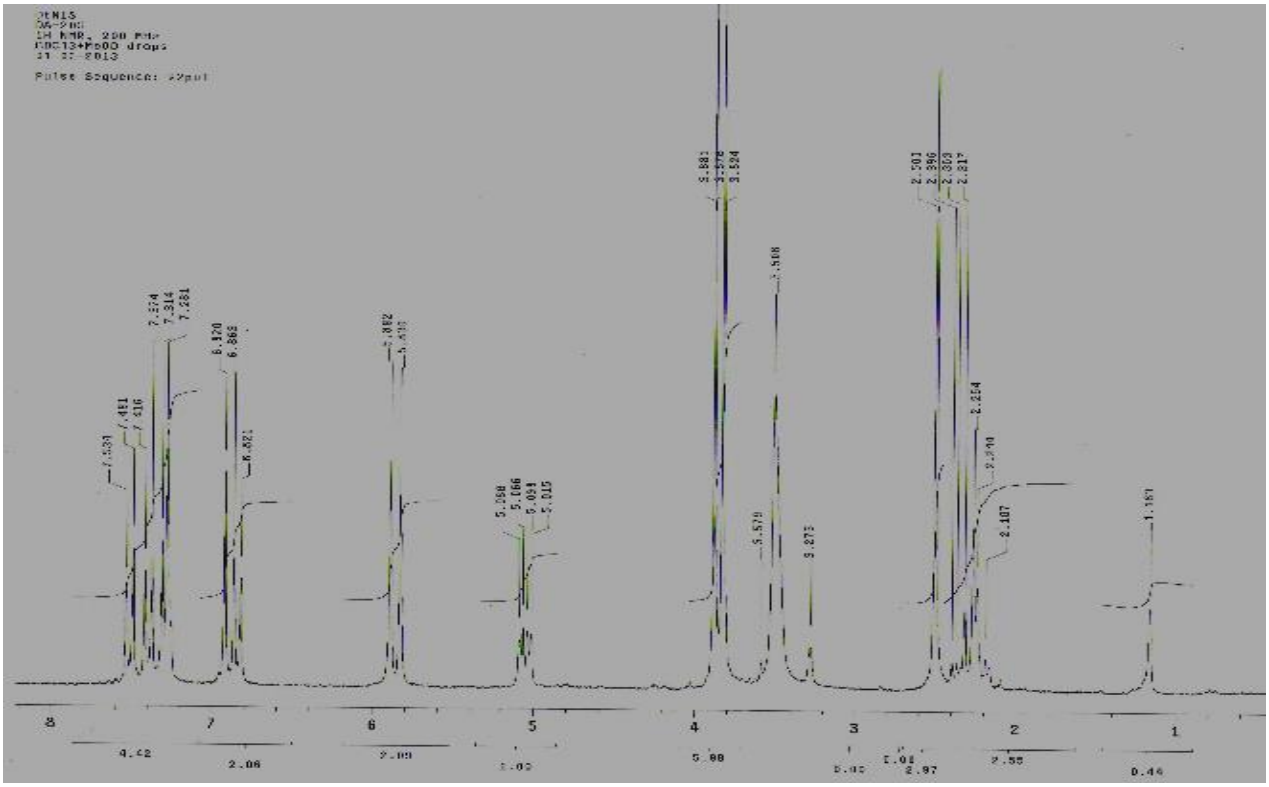
Appendix 4: Spectra for chelerythrindimerine (4)



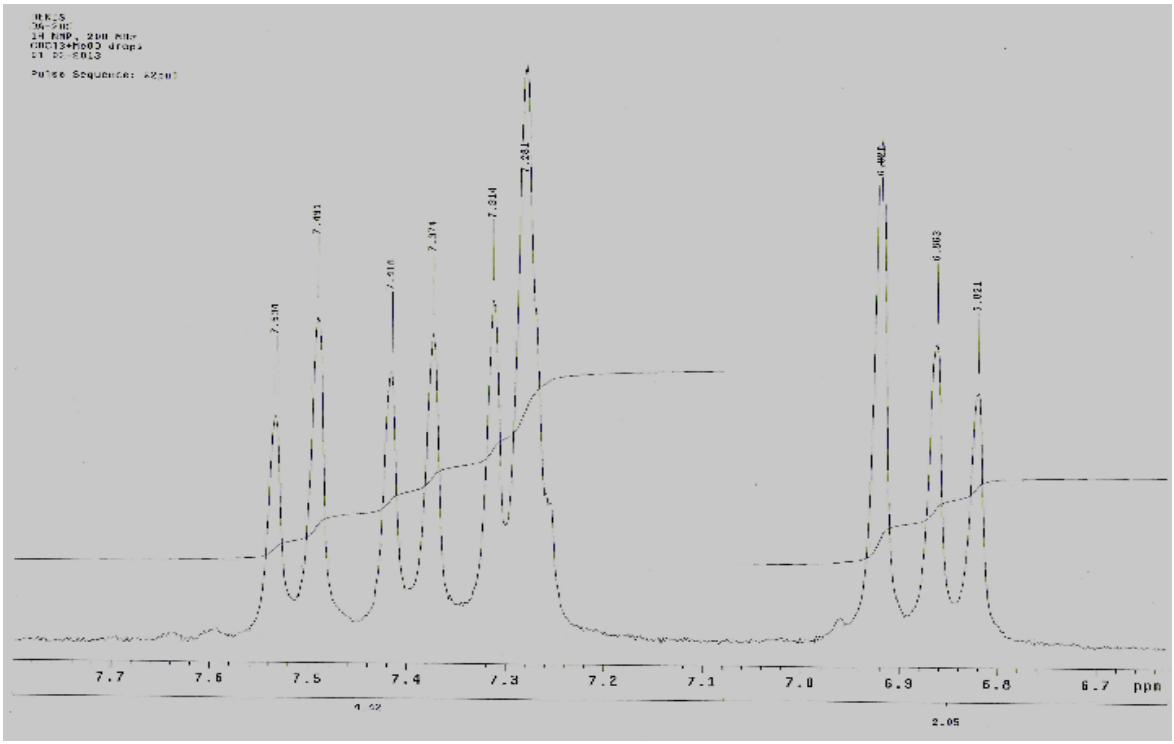
Mass spectrum for chelerythrindimerine (**4**)



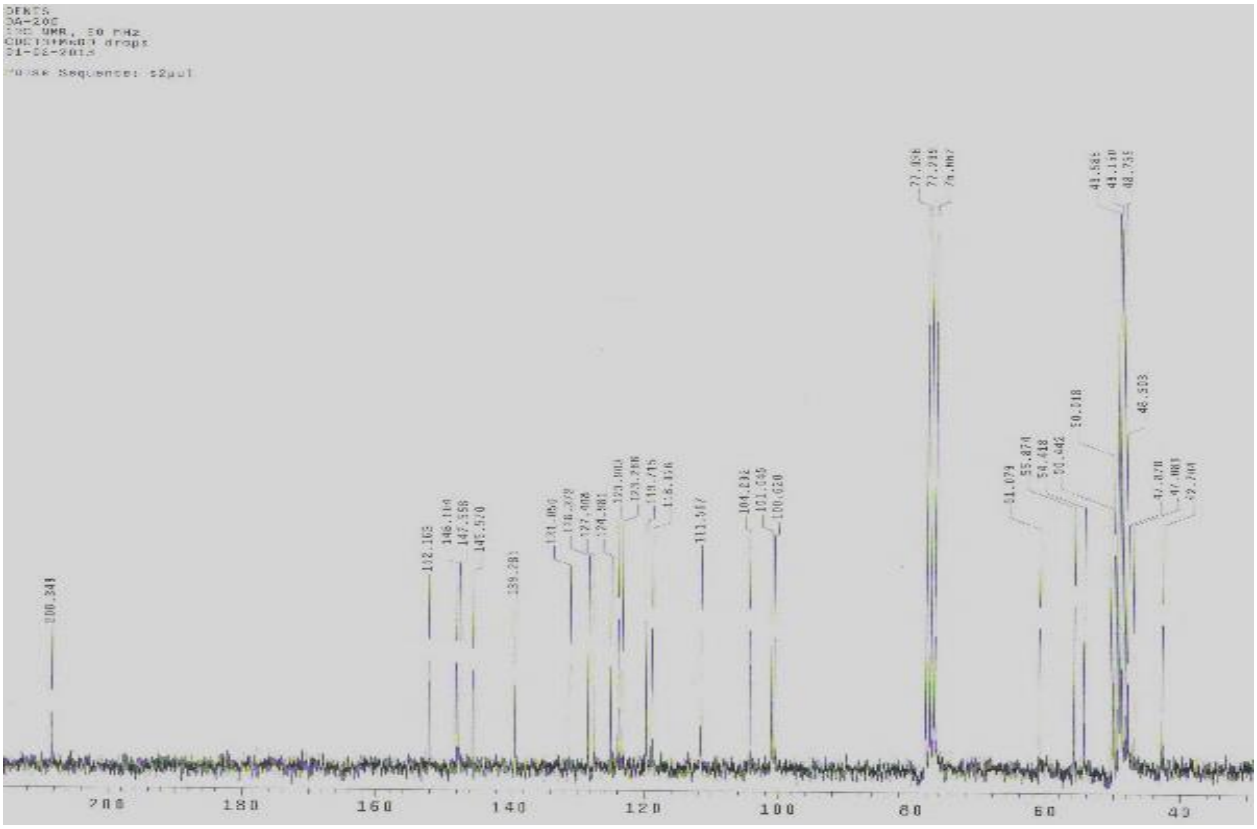
^{1}H-^{1}HNMR spectra chelerythrindimerine (**4**)



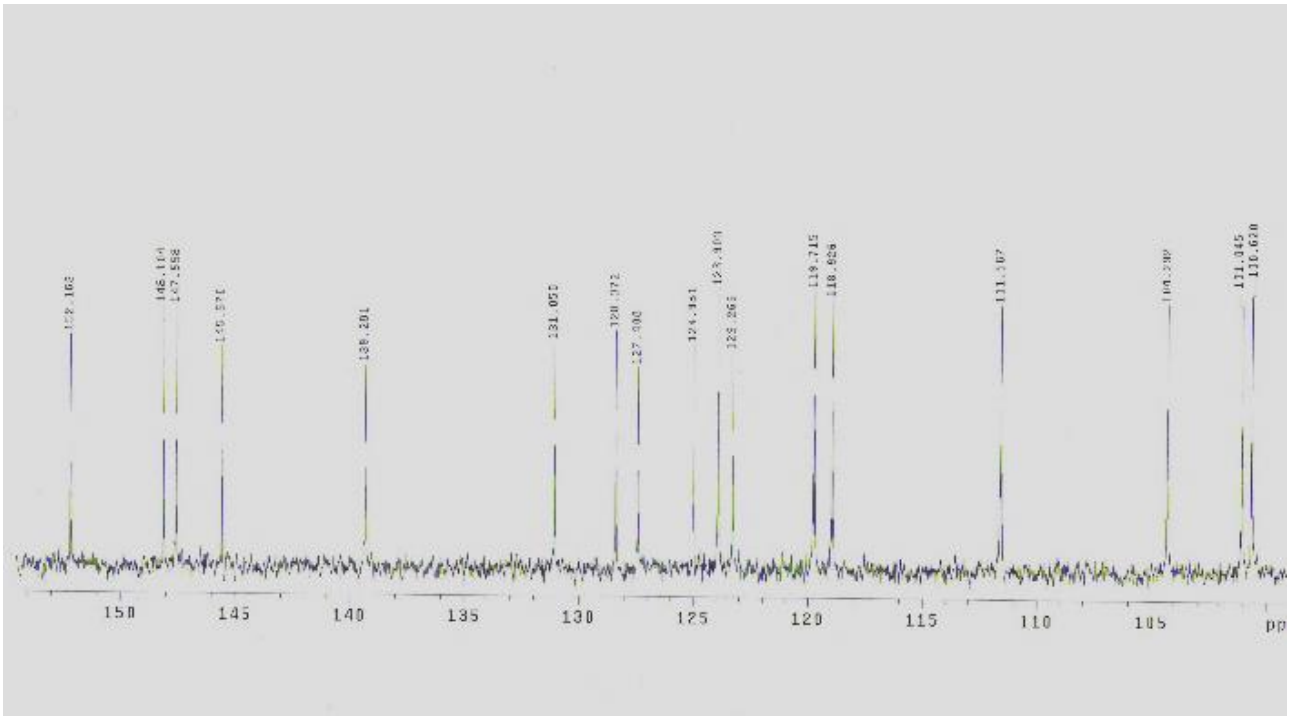
^{1}H NMR spectra chelerythrindimerine **(9)** (expansion)



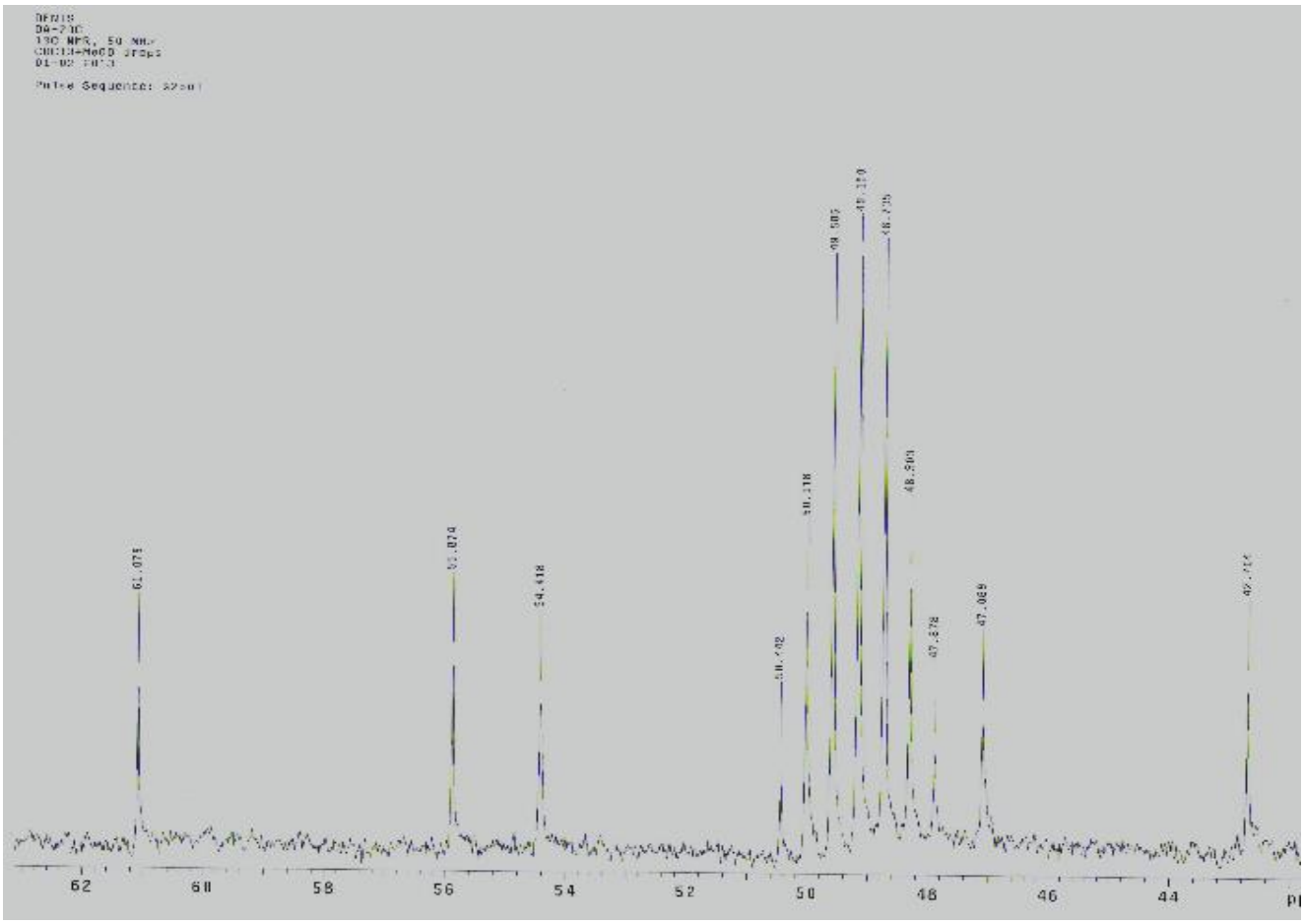
^{13}C NMR spectra for chelerythrindimerine **(4)**



^{13}C NMR spectra for Chelerythrindimerine **(4)** (expanded)



^{13}C NMR spectrum for chelerythrindimerine **(4)** (expansion)


62
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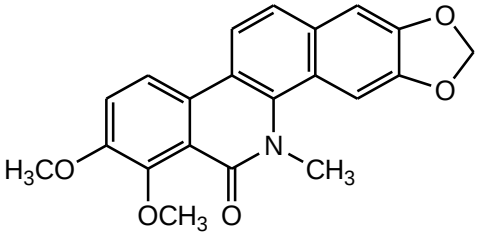
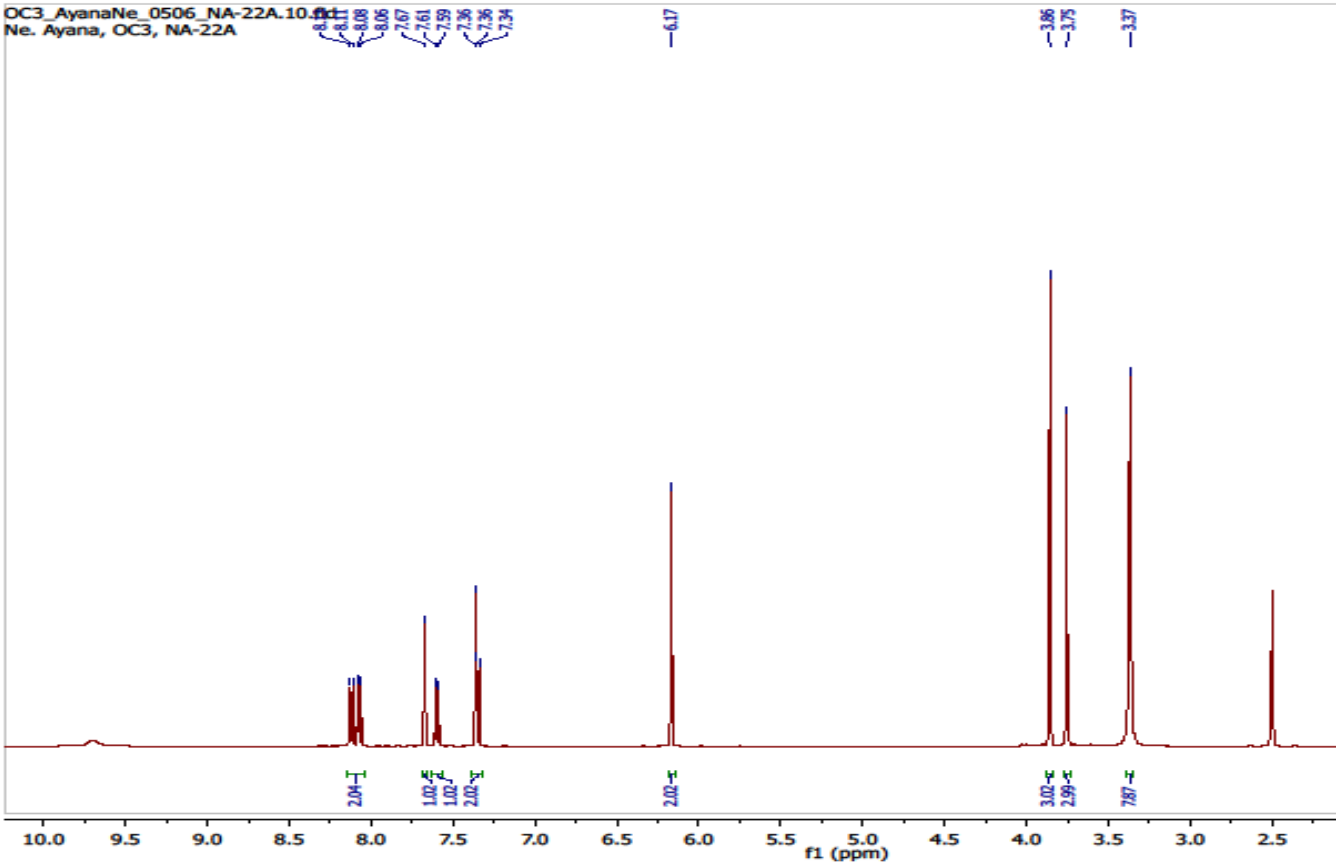
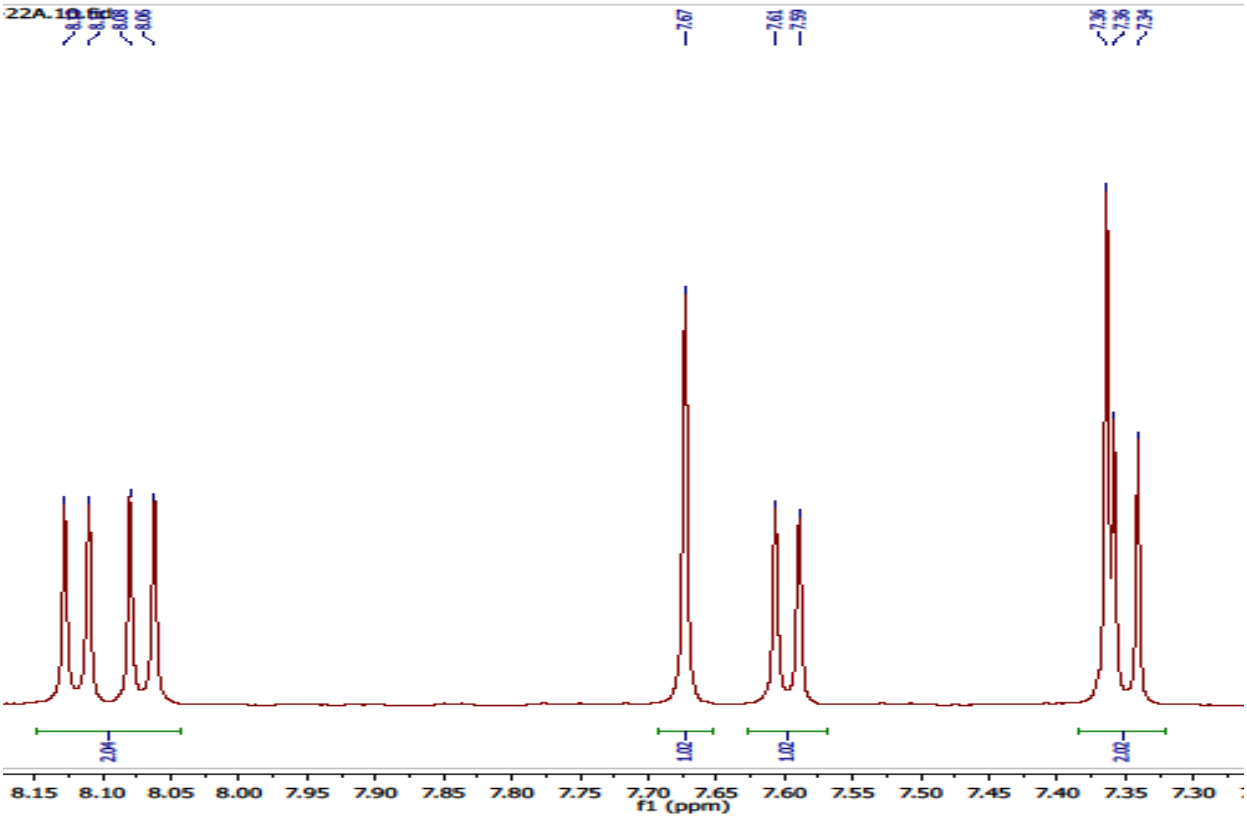
Appendix 5: Spectra for 9-Demethyloxychelerythrine (5)



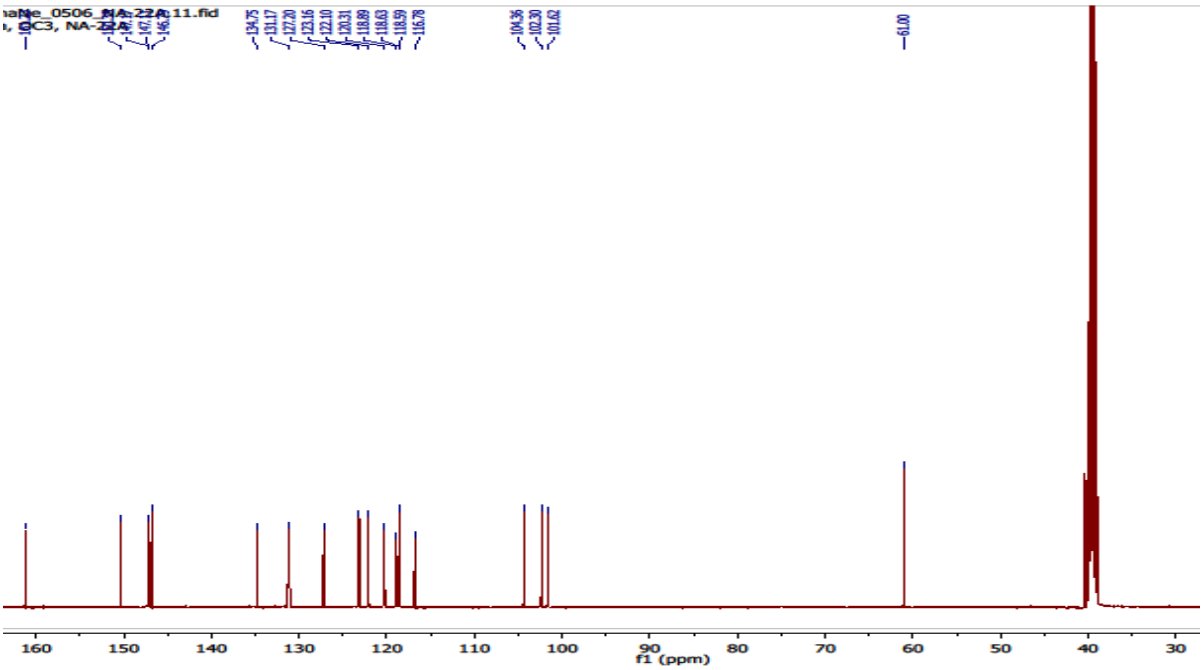
^1H-^1H NMR spectrum for 9-Demethyloxychelerythrine (**5**)



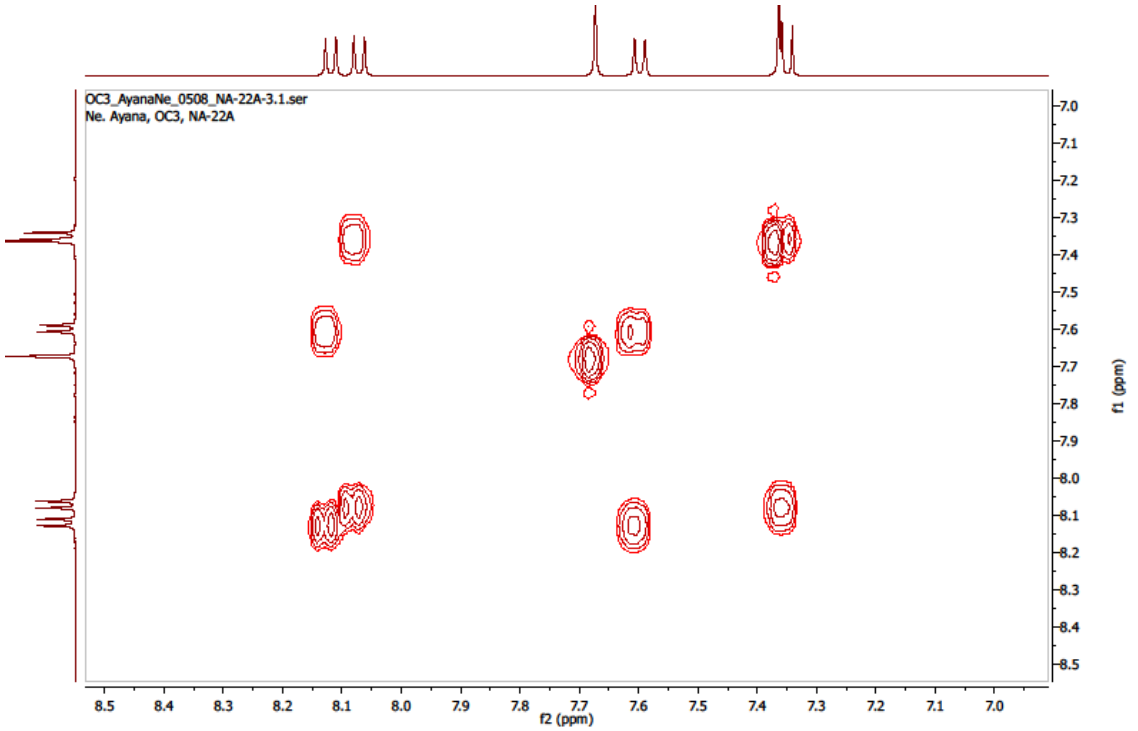
^{1}H-^{1}H NMR for 9-Demethyloxychelerythrine (**5**)



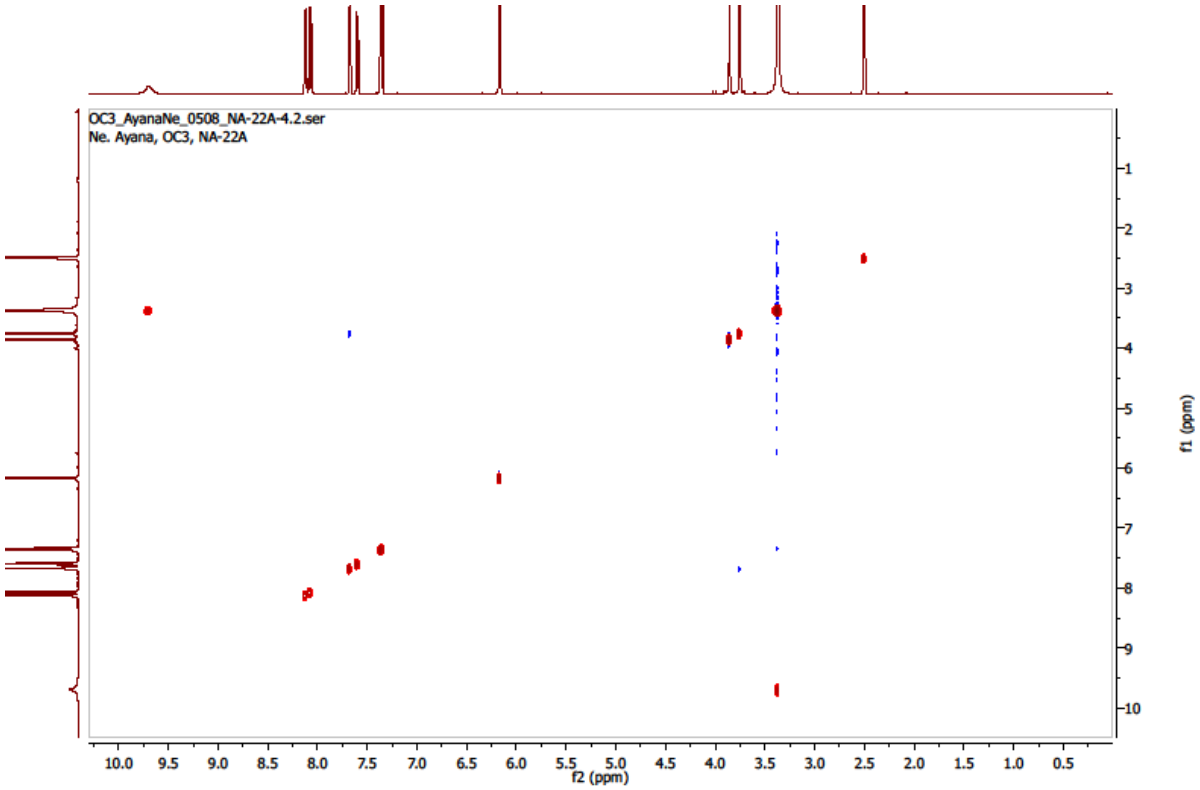
^{13}C NMR for compound (**5**)



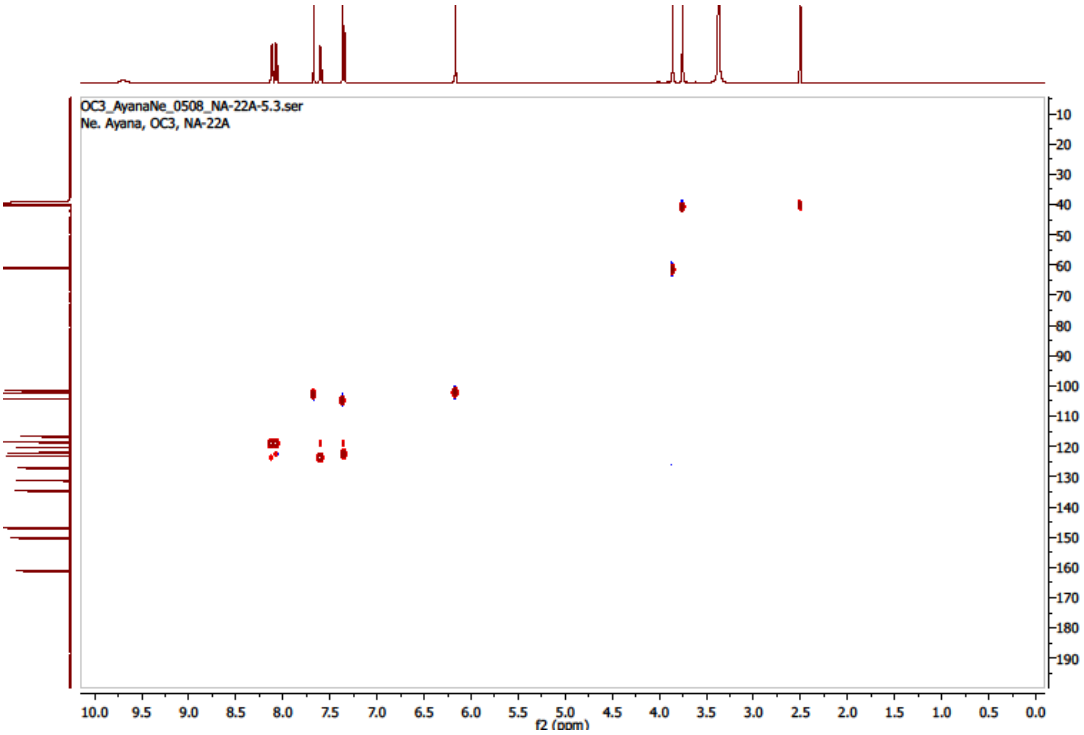
^{1}H-^{1}H-COSY for 9-Demethyloxychelerythrine (**5**)



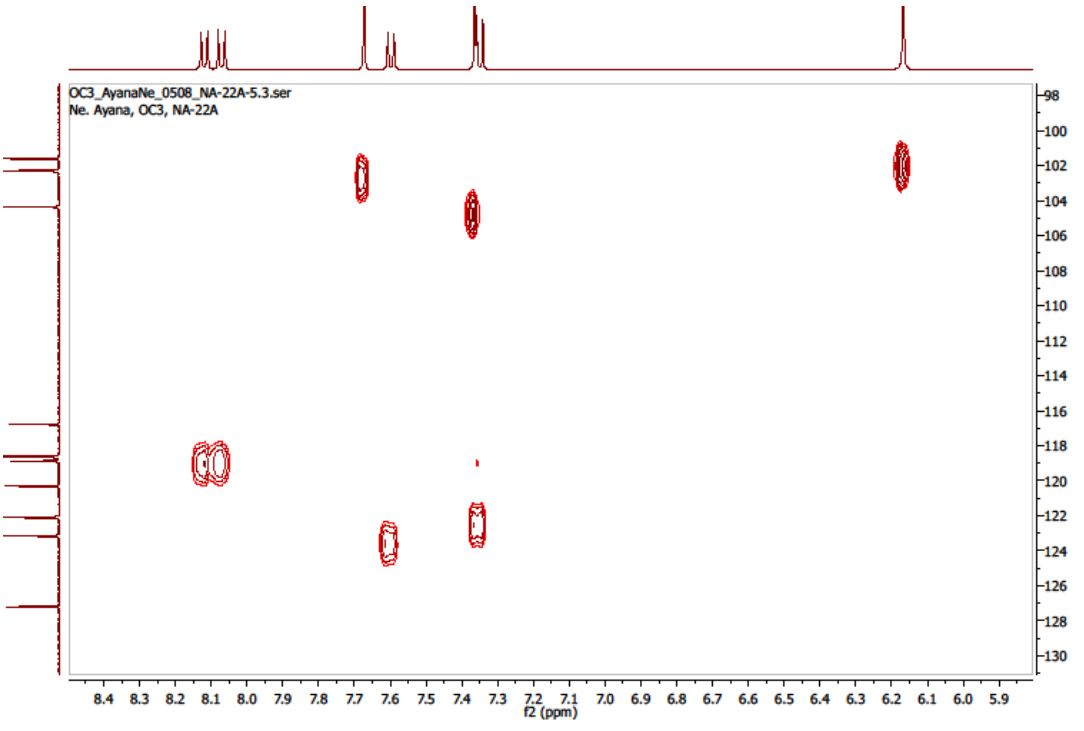
NOESY for 9-Demethyloxychelerythrine (**5**)



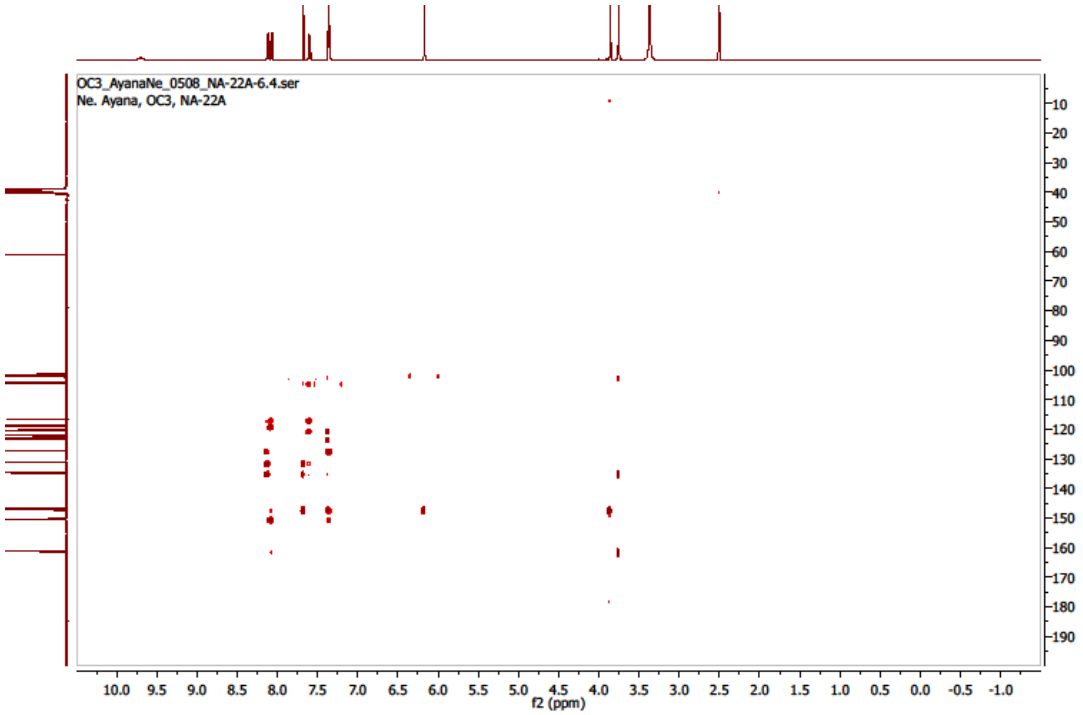
HSQC for 9-Demethyloxychelerythrine (**5**)



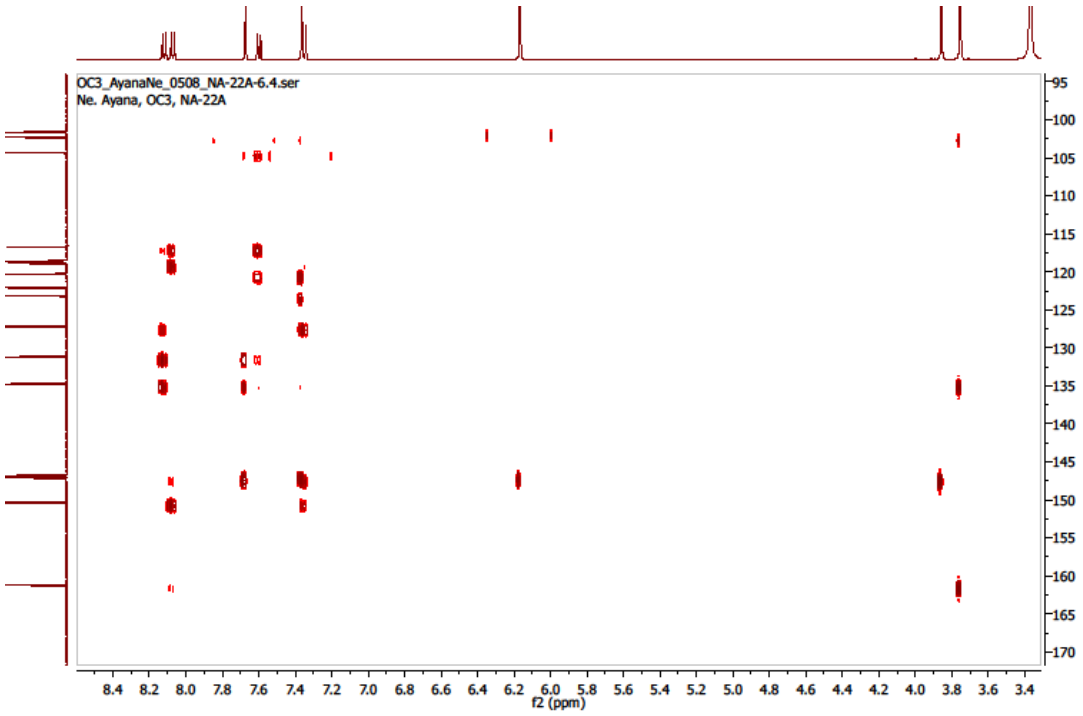
HSQC for 9-Demethyloxychelerythrine (**5**) (expansion)



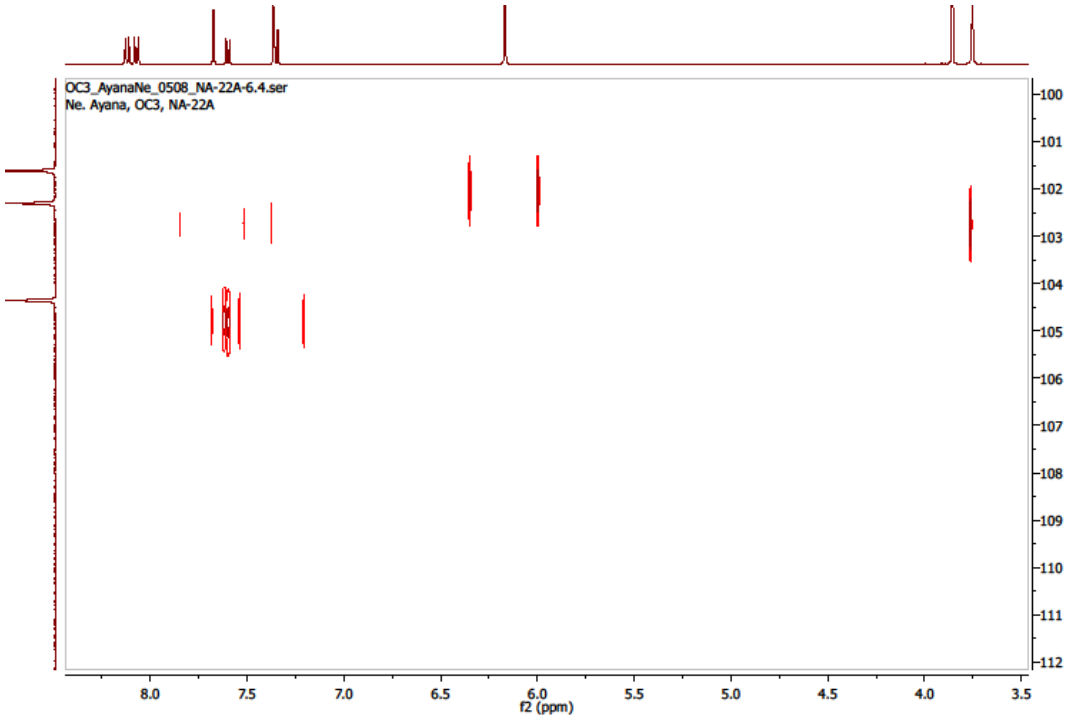
HMBC for 9-Demethyloxychelerythrine (**5**)



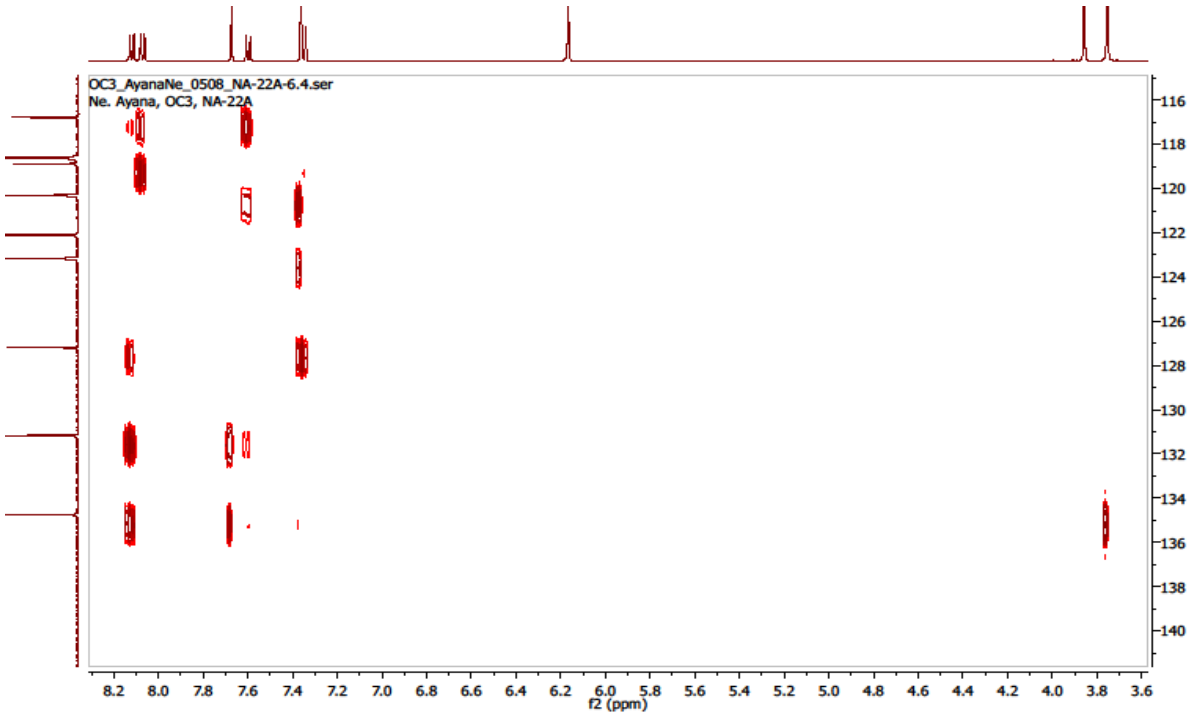
HMBC for 9-Demethyloxychelerythrine (**5**) (expansion)



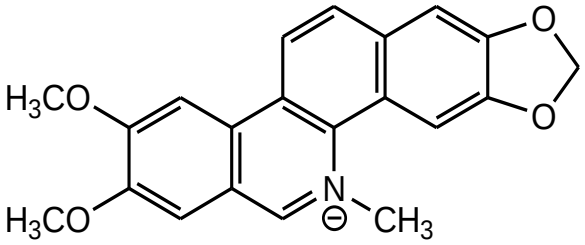
HMBC for 9-Demethyloxychelerythrine **(5)** (expansion)



HMBC for 9-Demethyloxychelerythrine (**5**) (expansion)



Appendix 6: Spectra for nitidine (6)



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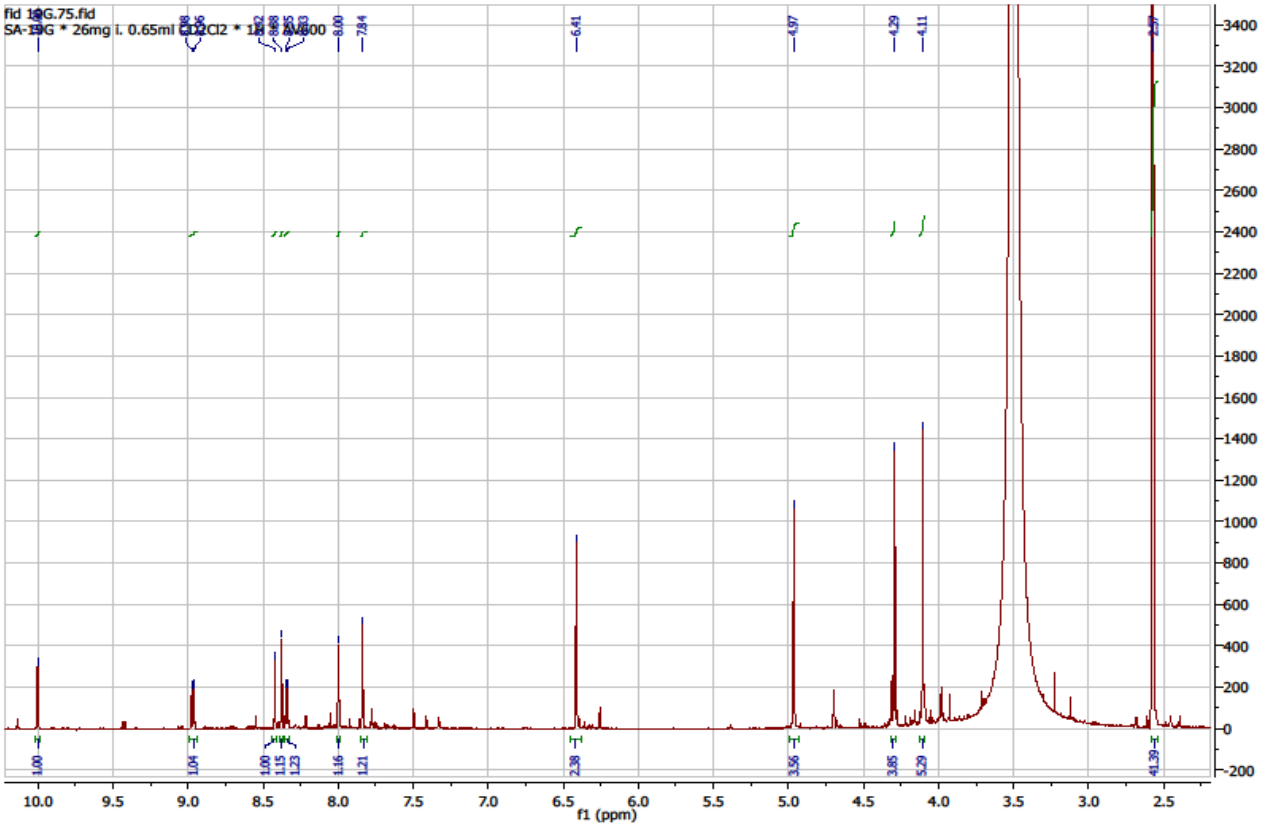
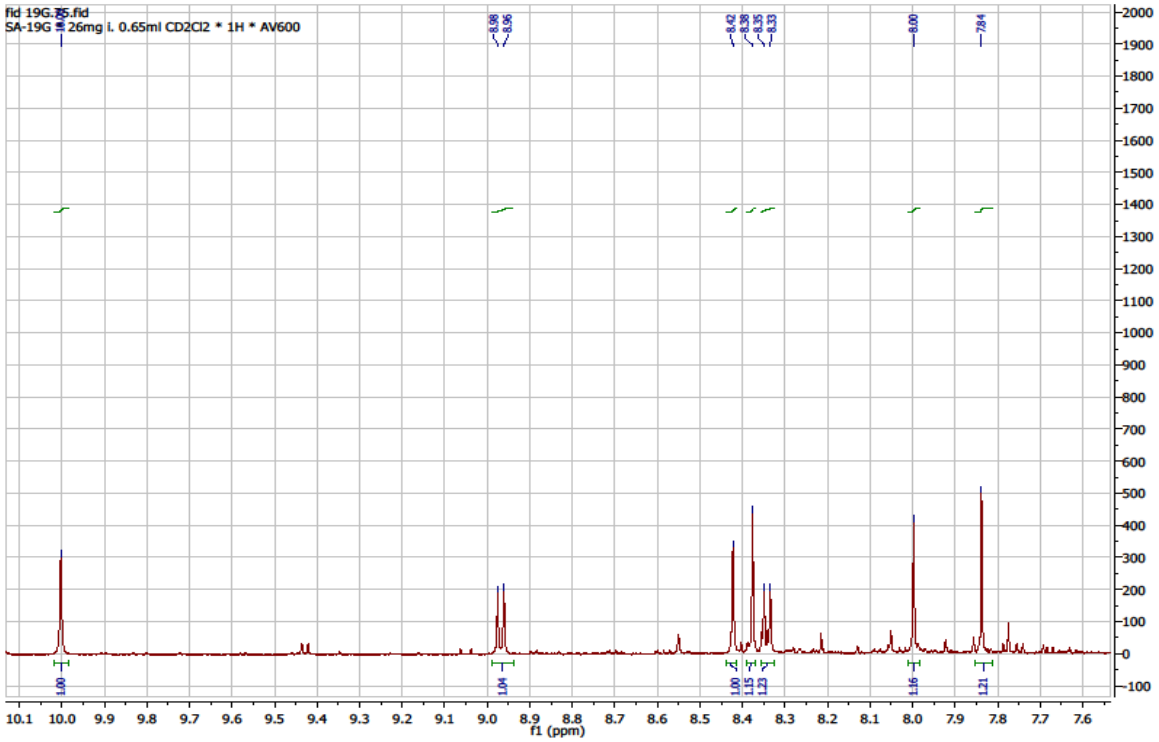
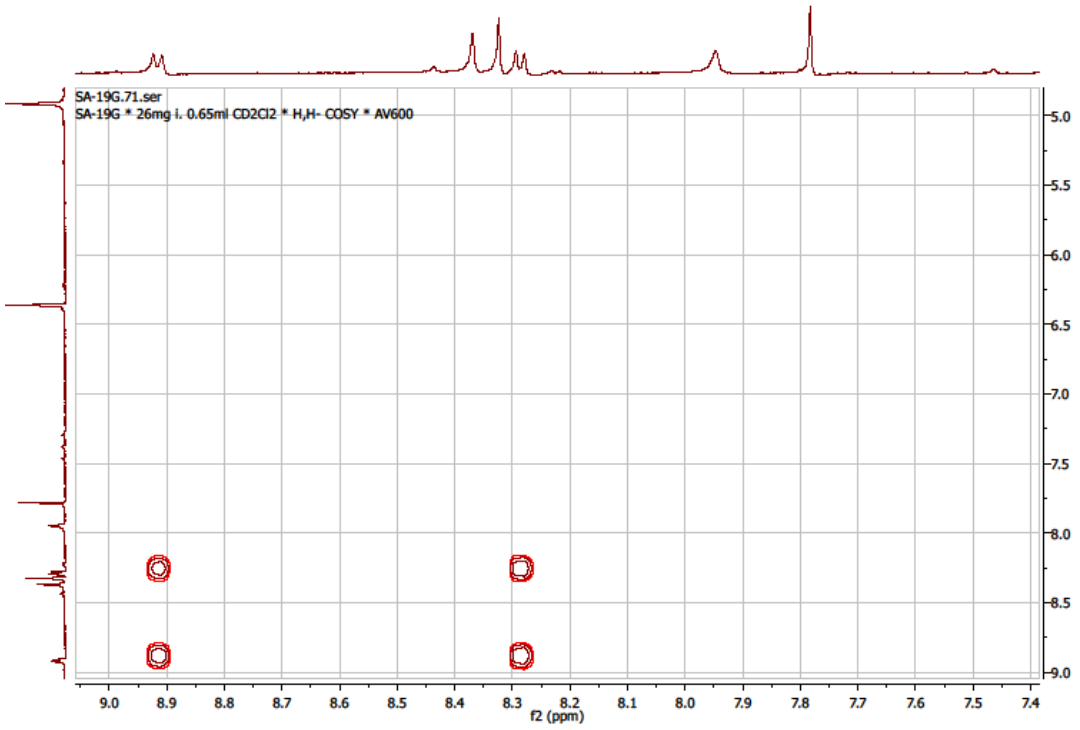
^{1}H NMR spectrum for nitidine **(6)**



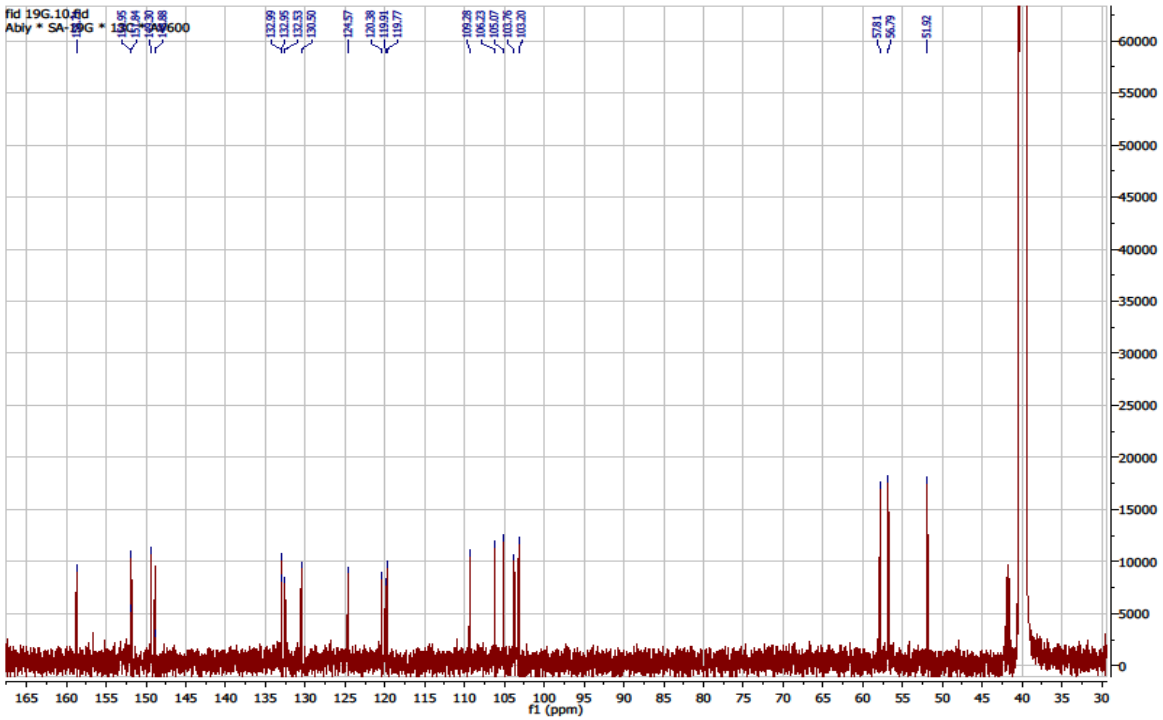
^{1}H-^{1}H NMR spectrum for compound **(6)**



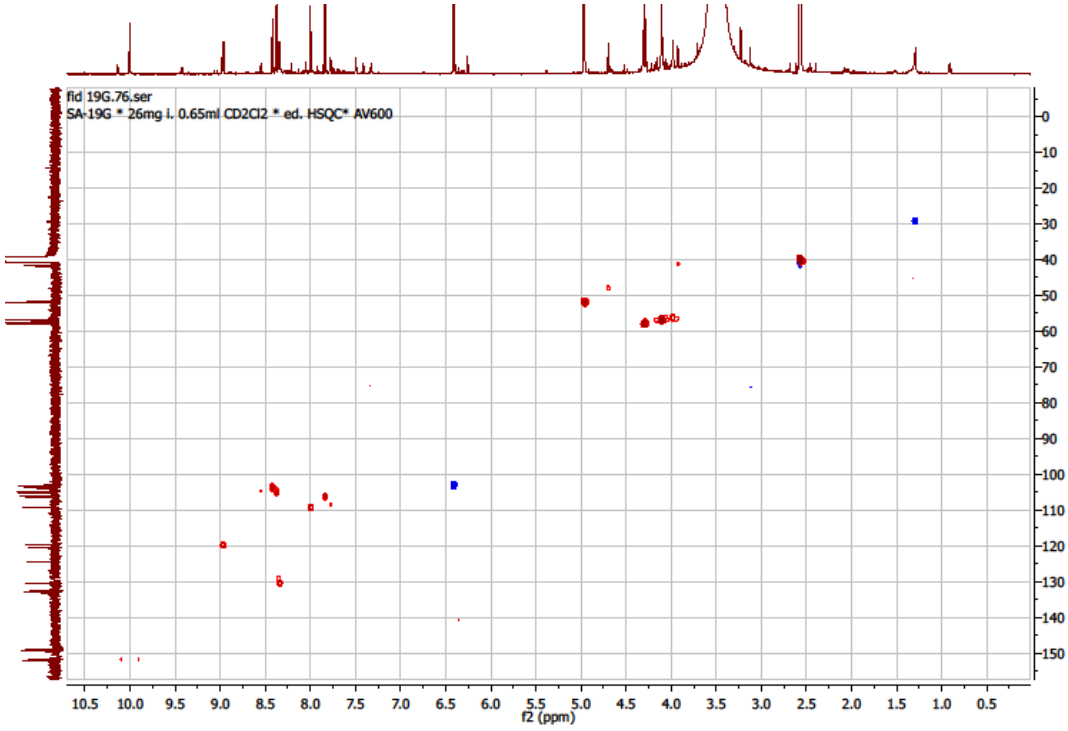
^{1}H-^{1}H-COSY for nitidine (**6**)



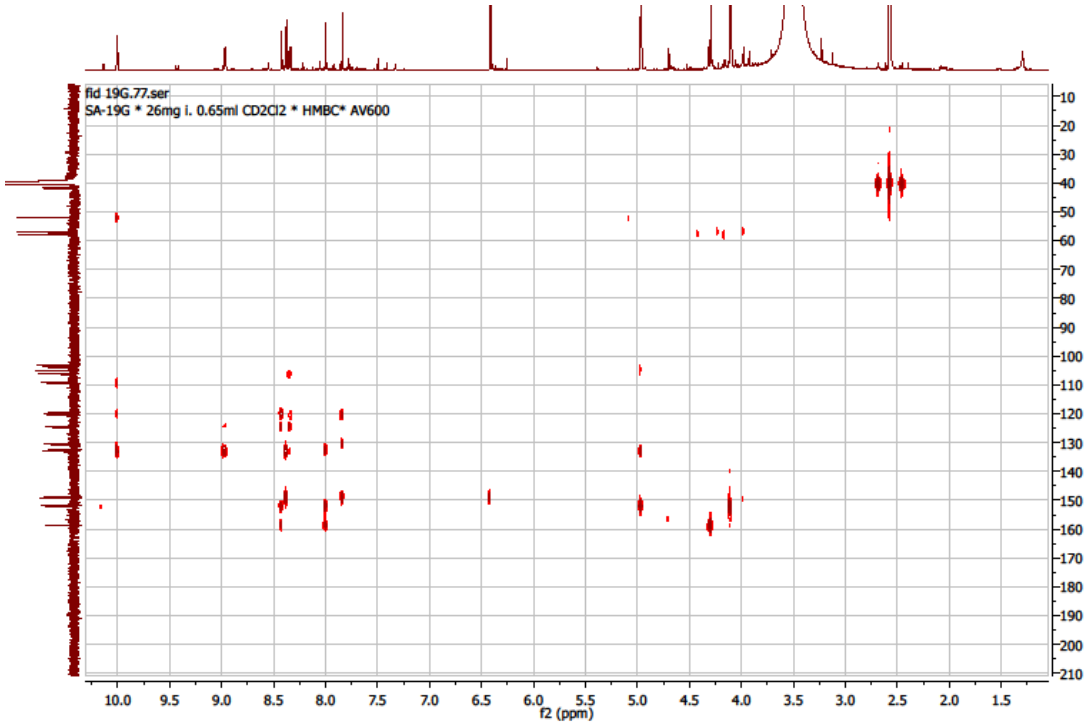
^{13}C NMR for nitidine (**6**)



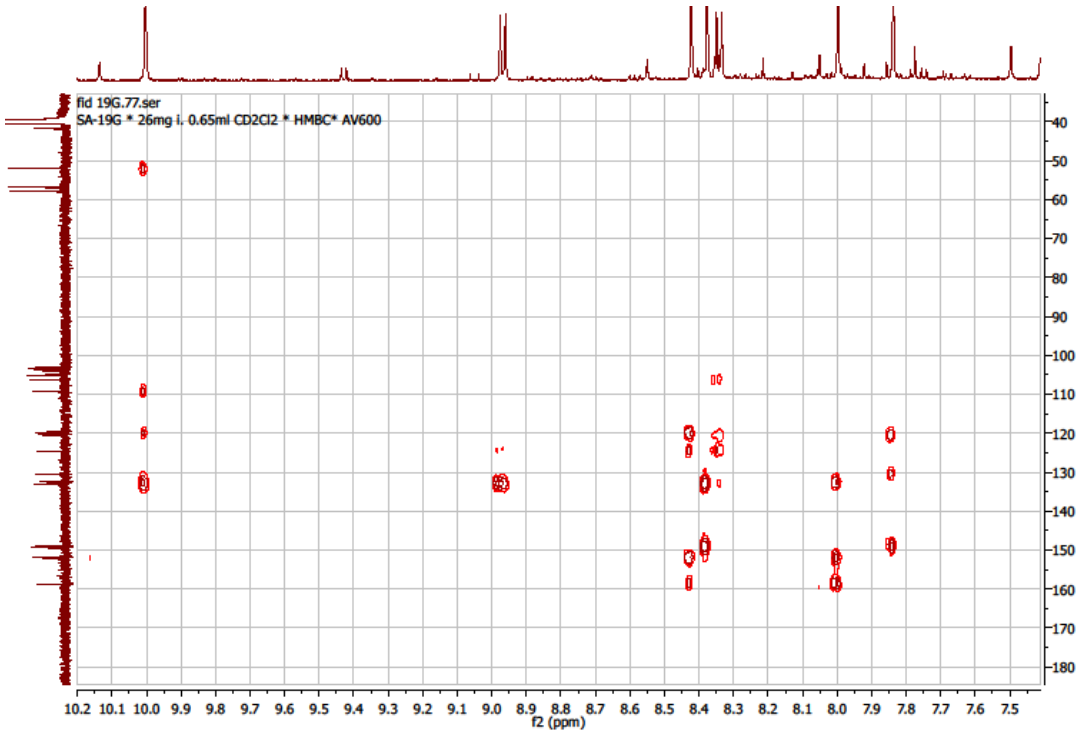
HSQC for nitidine (**6**)



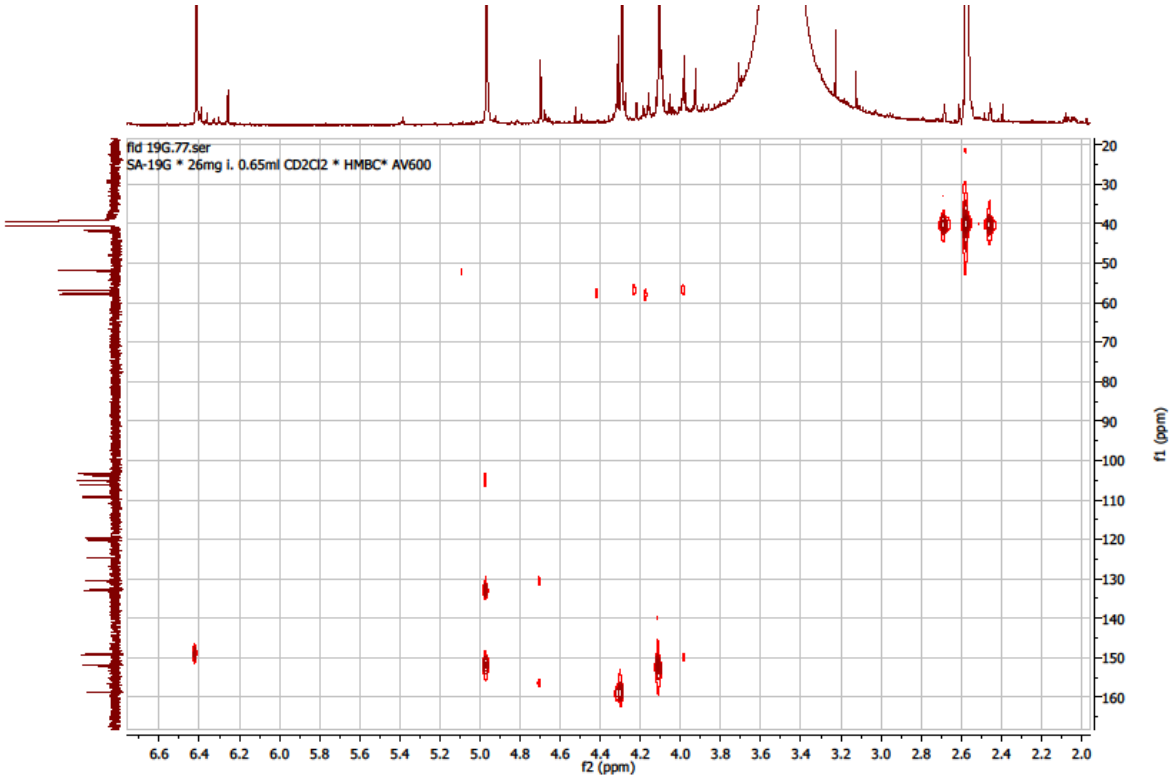
HMBC for nitidine (**6**)



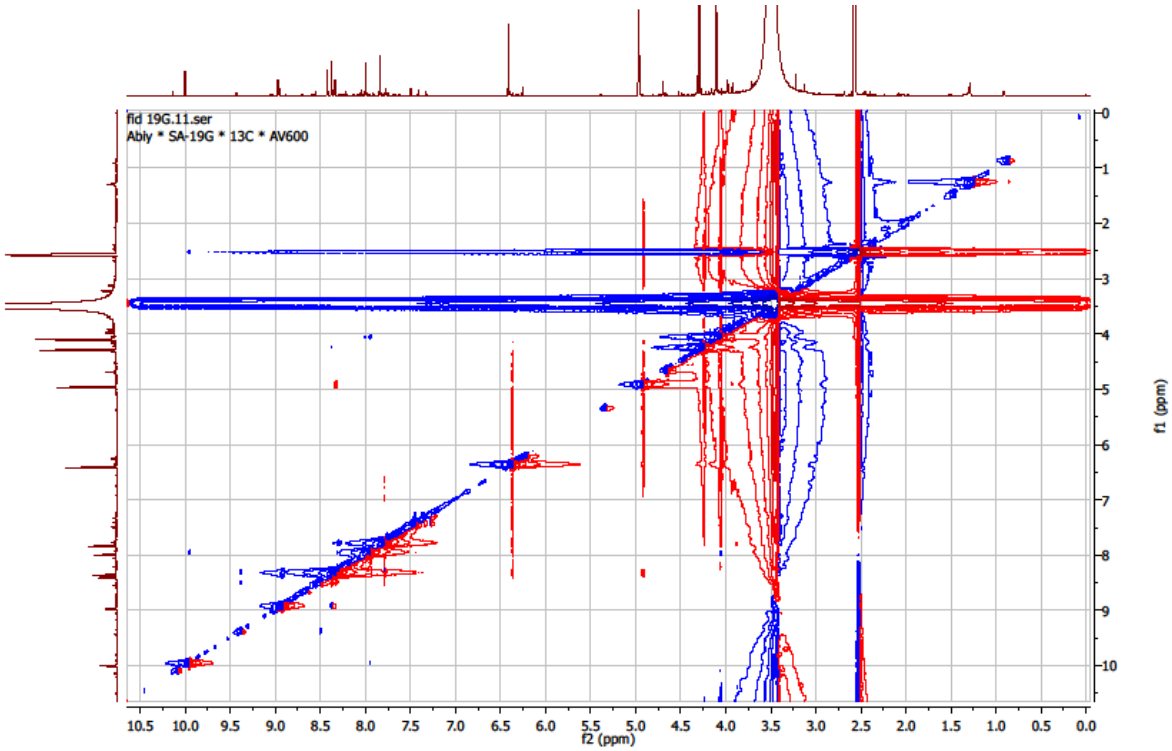
HMBC for nitidine (**6**) (expansion)



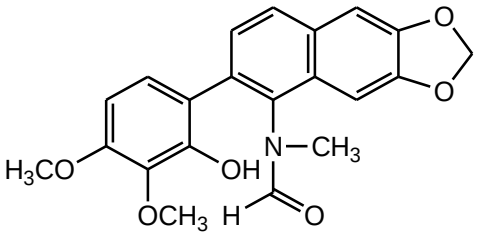
HMBC for nitidine (**6**) (expansion)



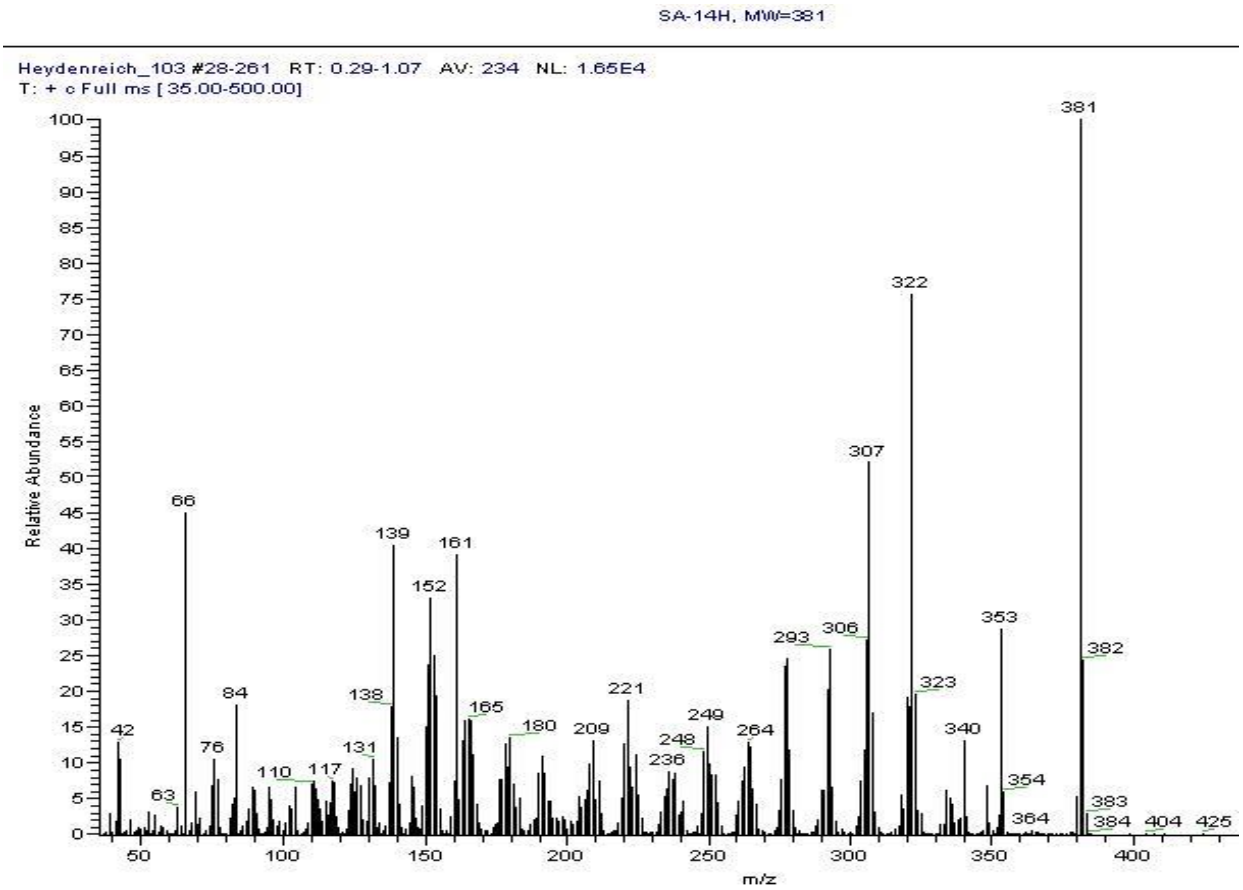
NOESY for nitidine (**6**)



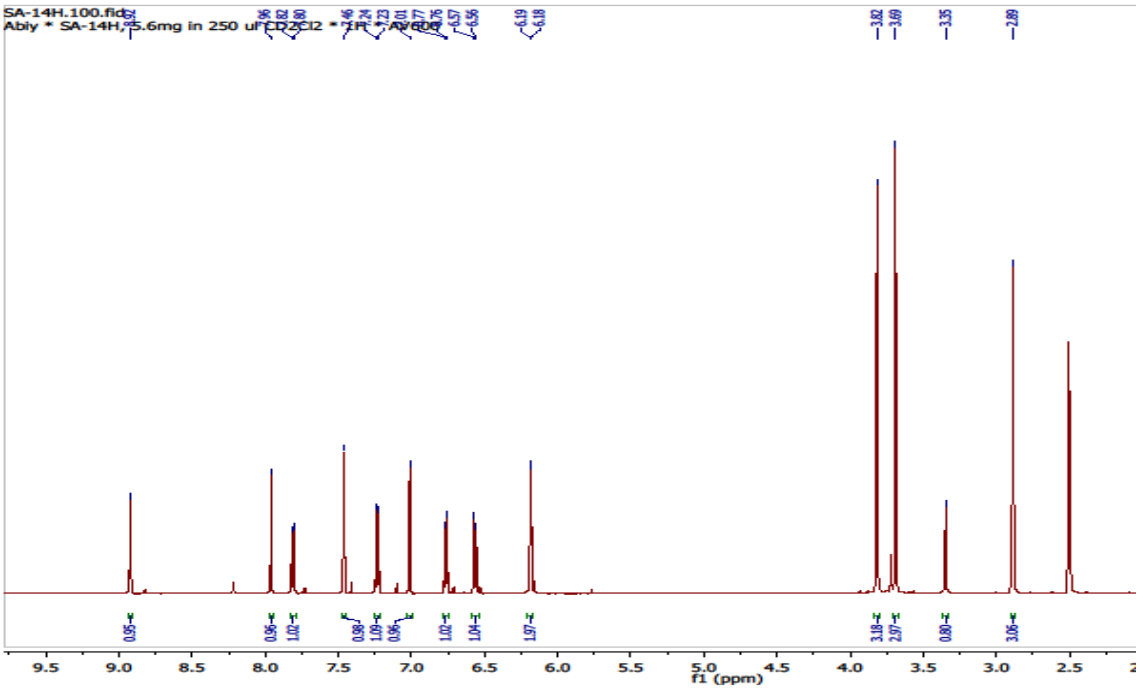
Appendix 7: Spectra for arnottianamide (7)



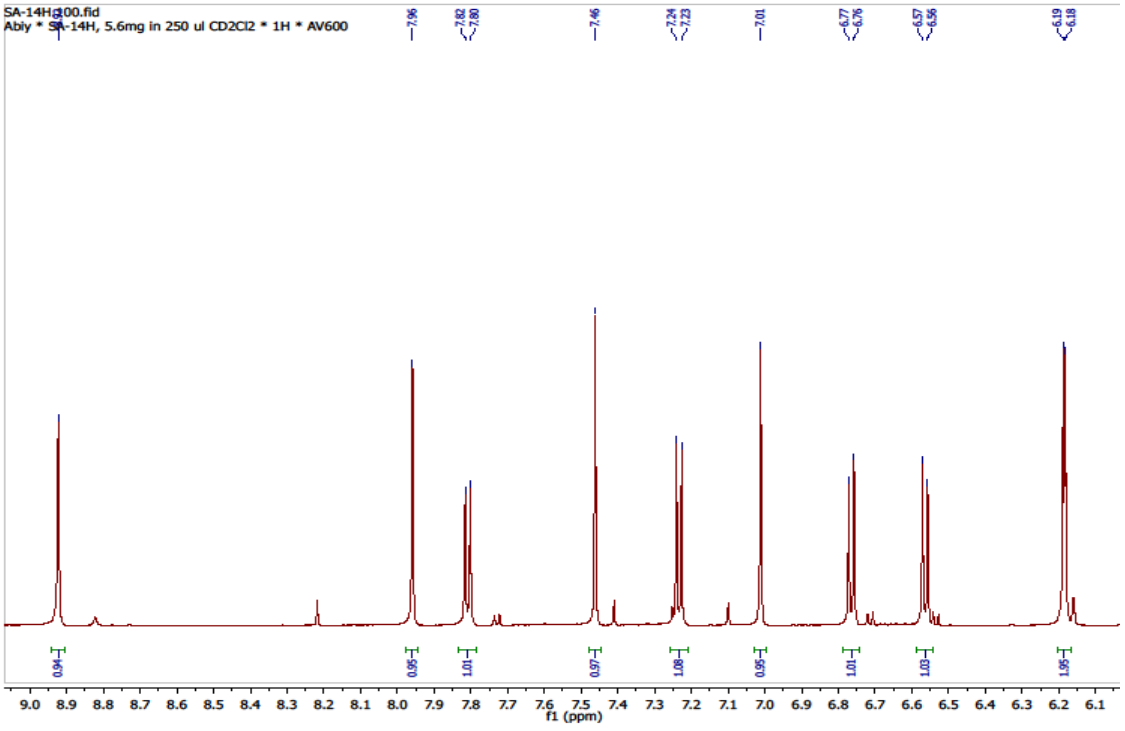
Mass spectrum forarnottianamide (**7**)



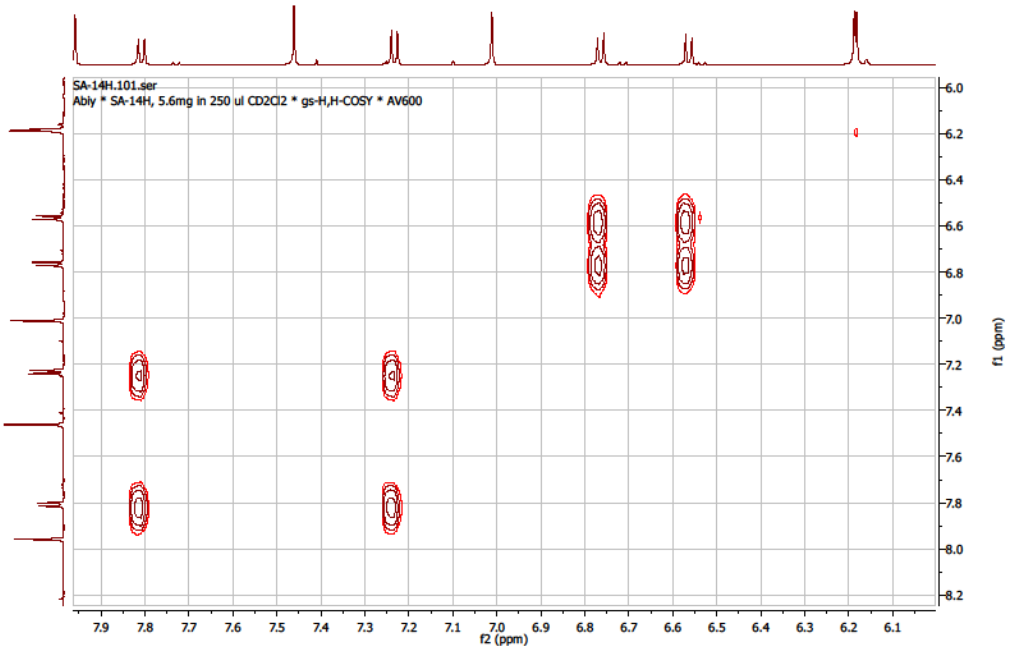
^{1}H-^{1}H NMR for arnottianamide (**7**)



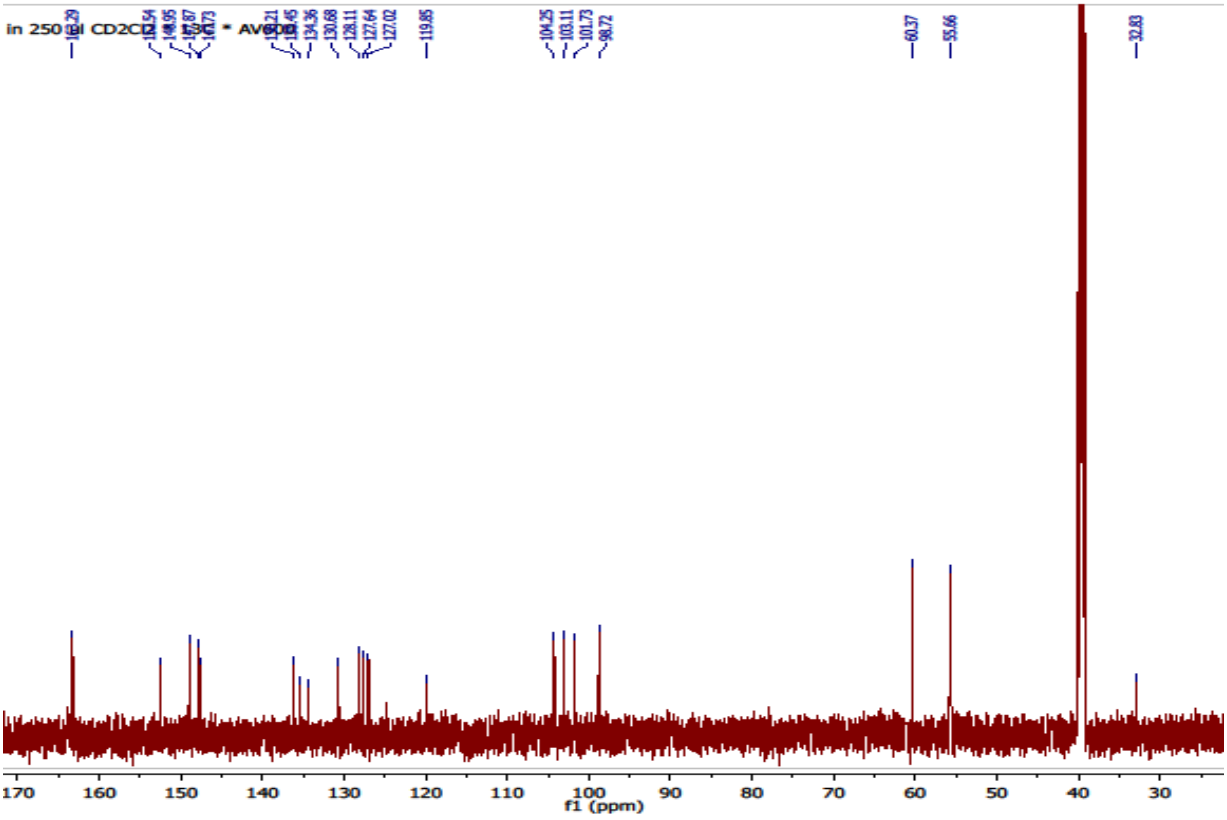
^{1}H-^{1}HNMR for arnottianamide (**7**) (expansion)



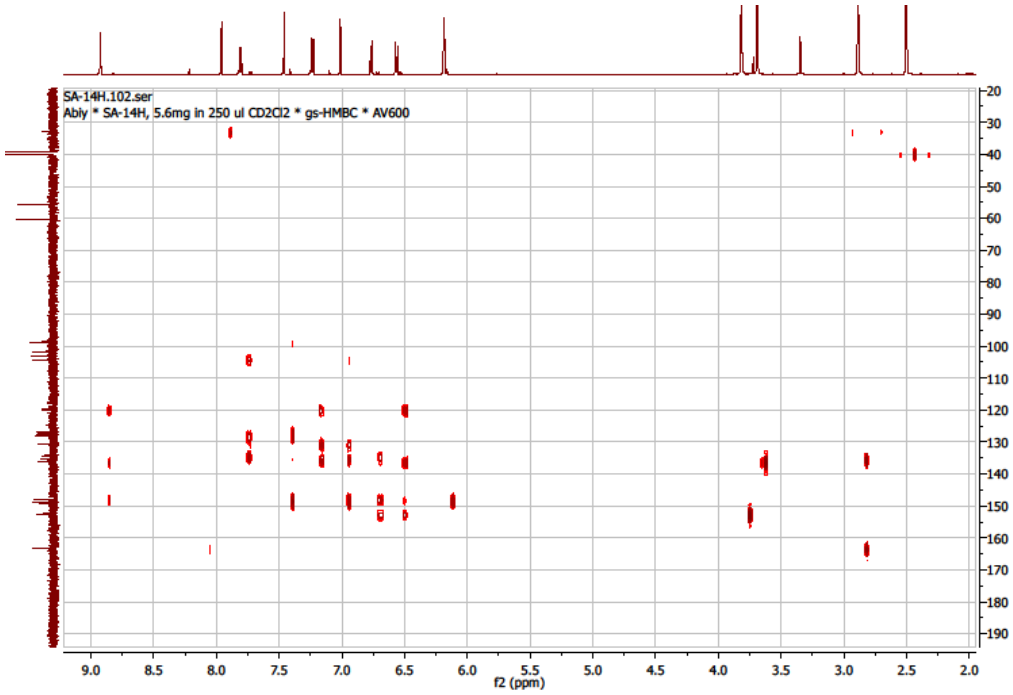
^{1}H-^{1}H-COSY for arnottianamide (**7**)



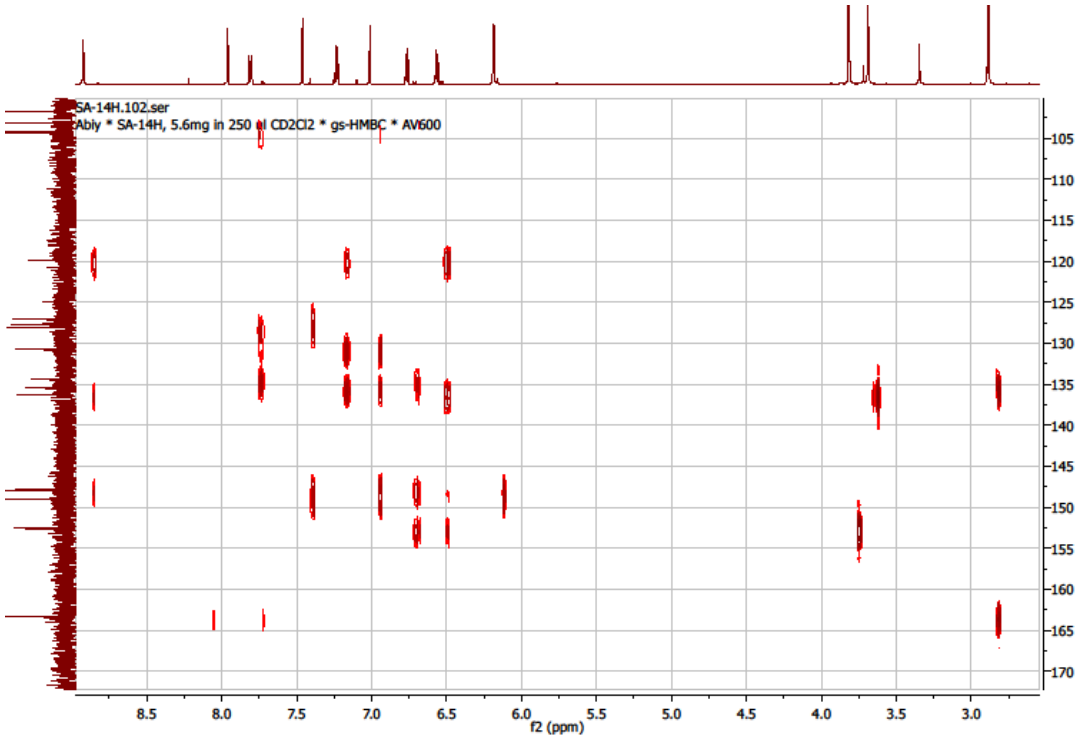
^{13}C NMR for arnottianamide (**7**)



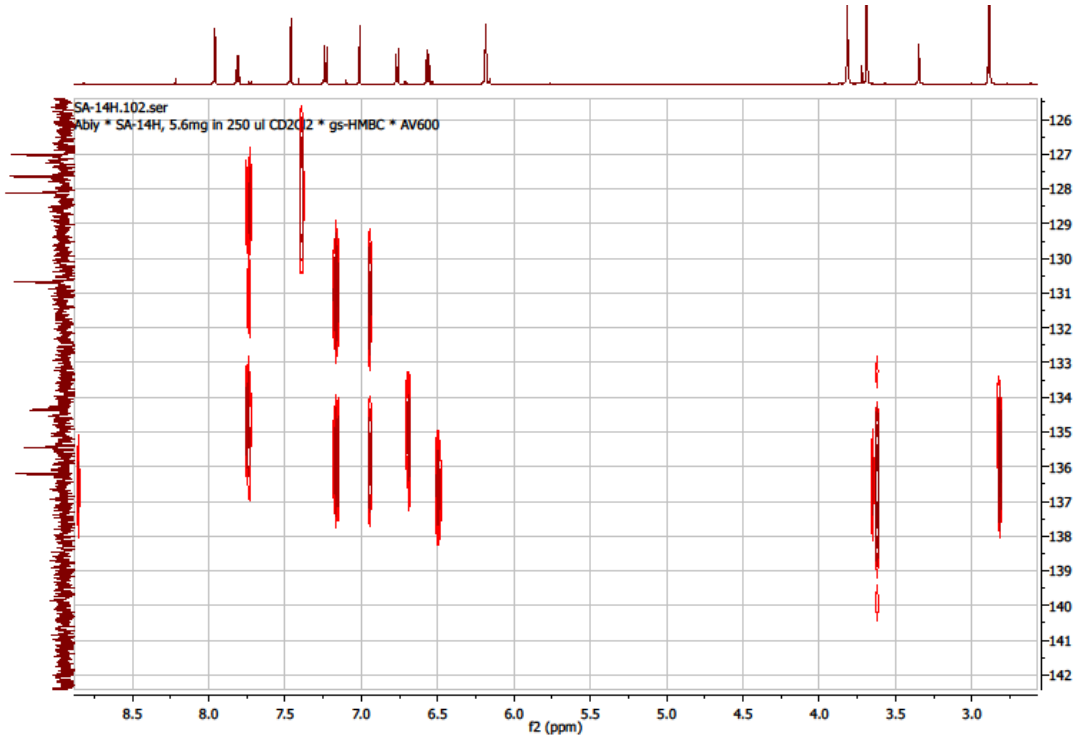
HMBC for arnottianamide (**7**)



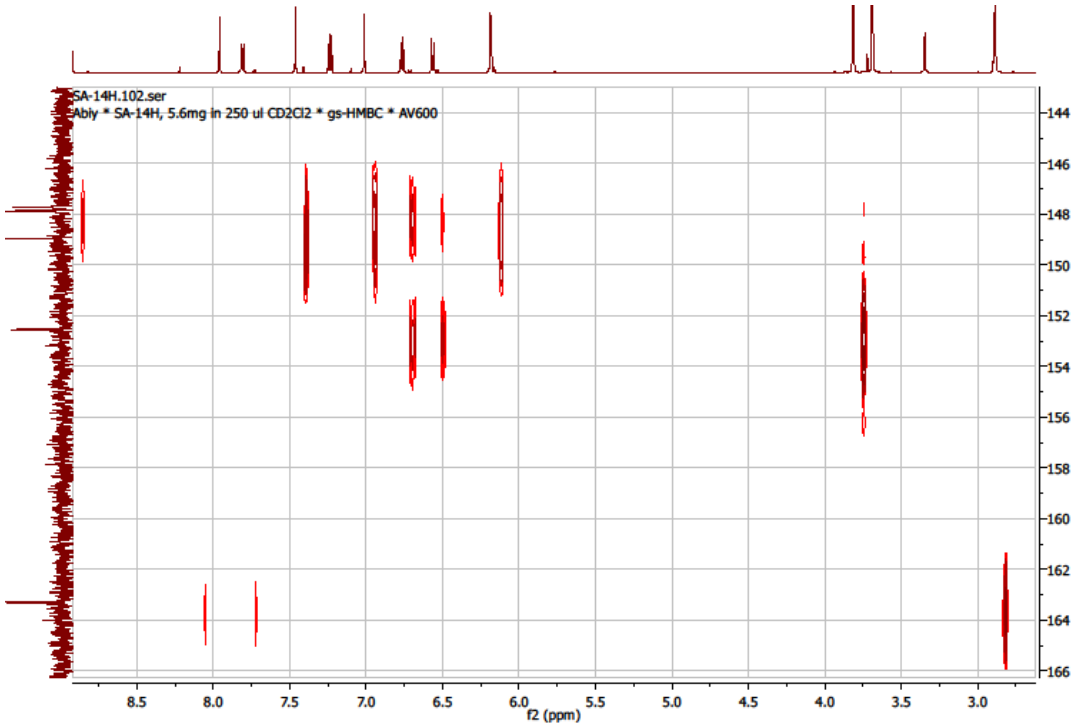
HMBC for arnottianamide (**7**) (expansion)



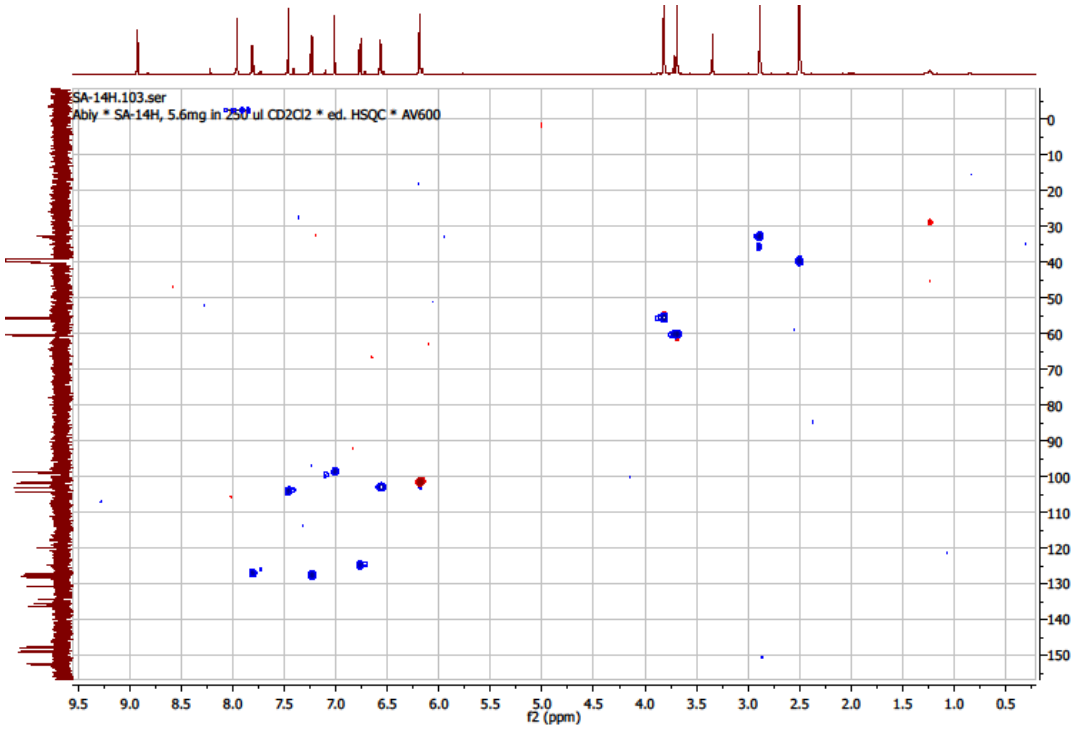
HMBC for arnottianamide (**7**) (expansion)



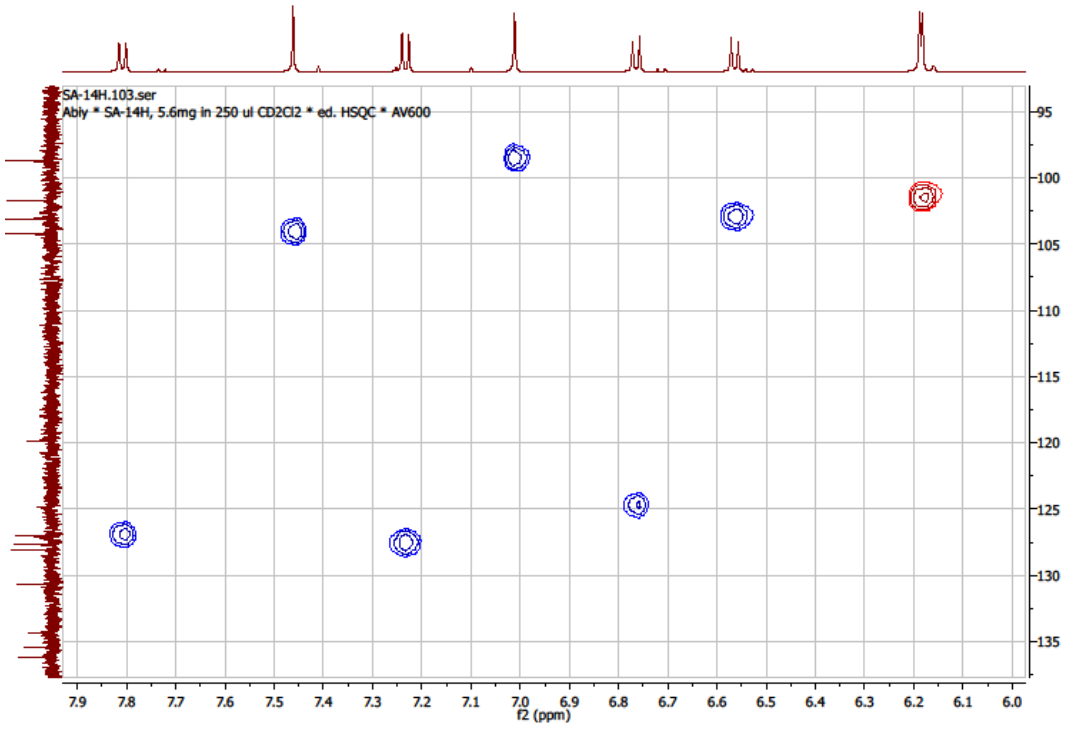
HMBC for arnottianamide (**7**) (expansion)



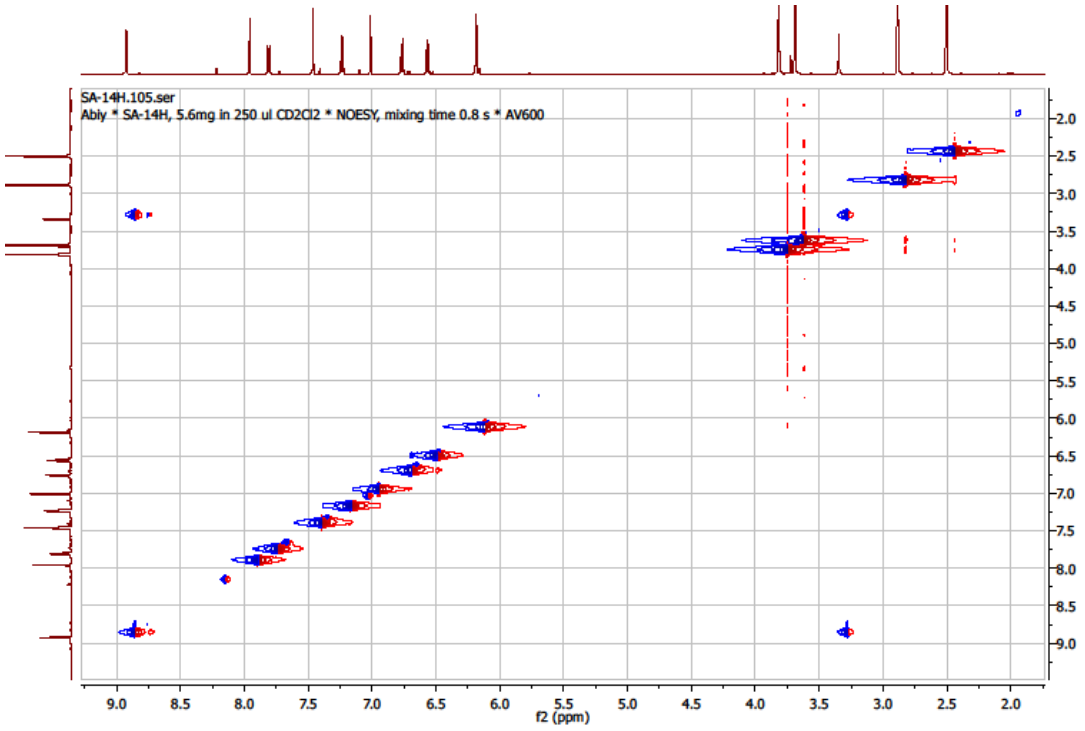
HSQC for arnottianamide (**7**)



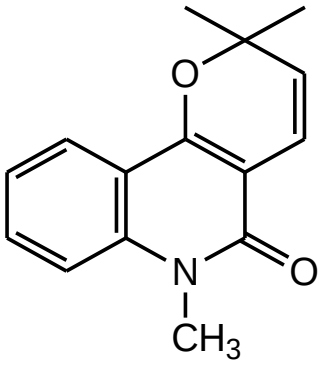
HSQC for arnottianamide (**7**) (expansion)



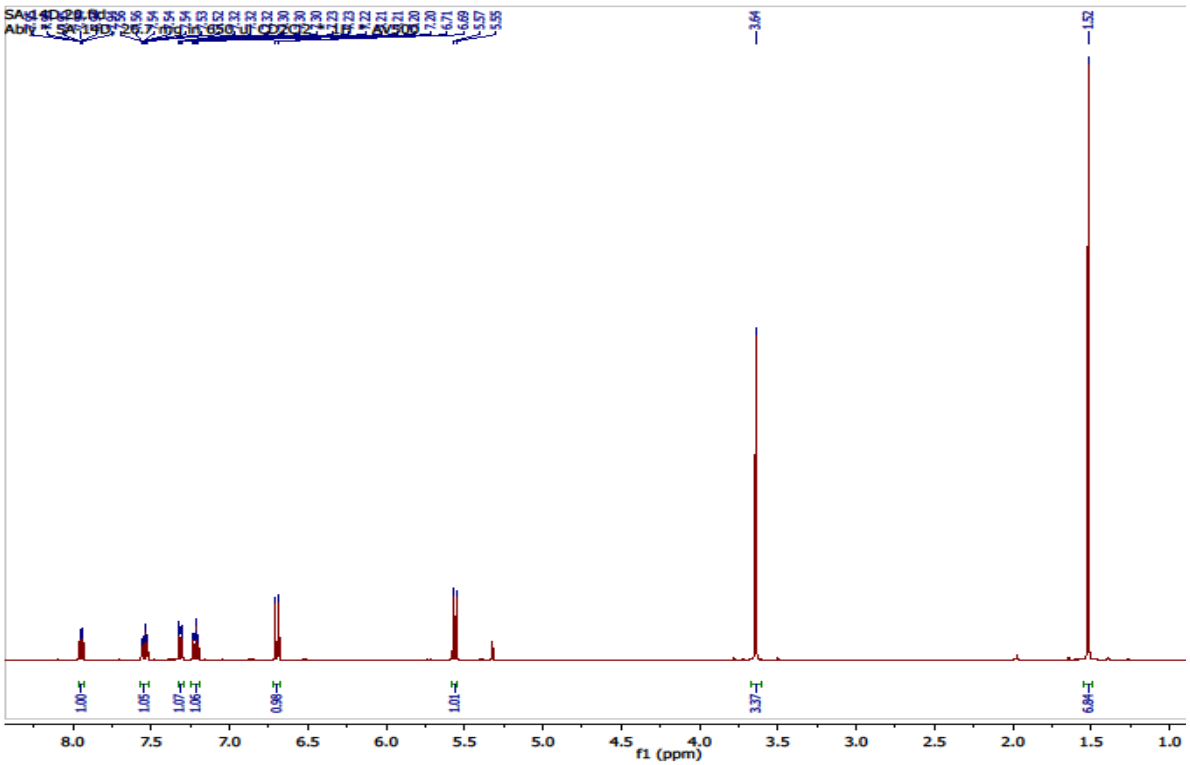
NOESY for arnottianamide (**7**) (expansion)



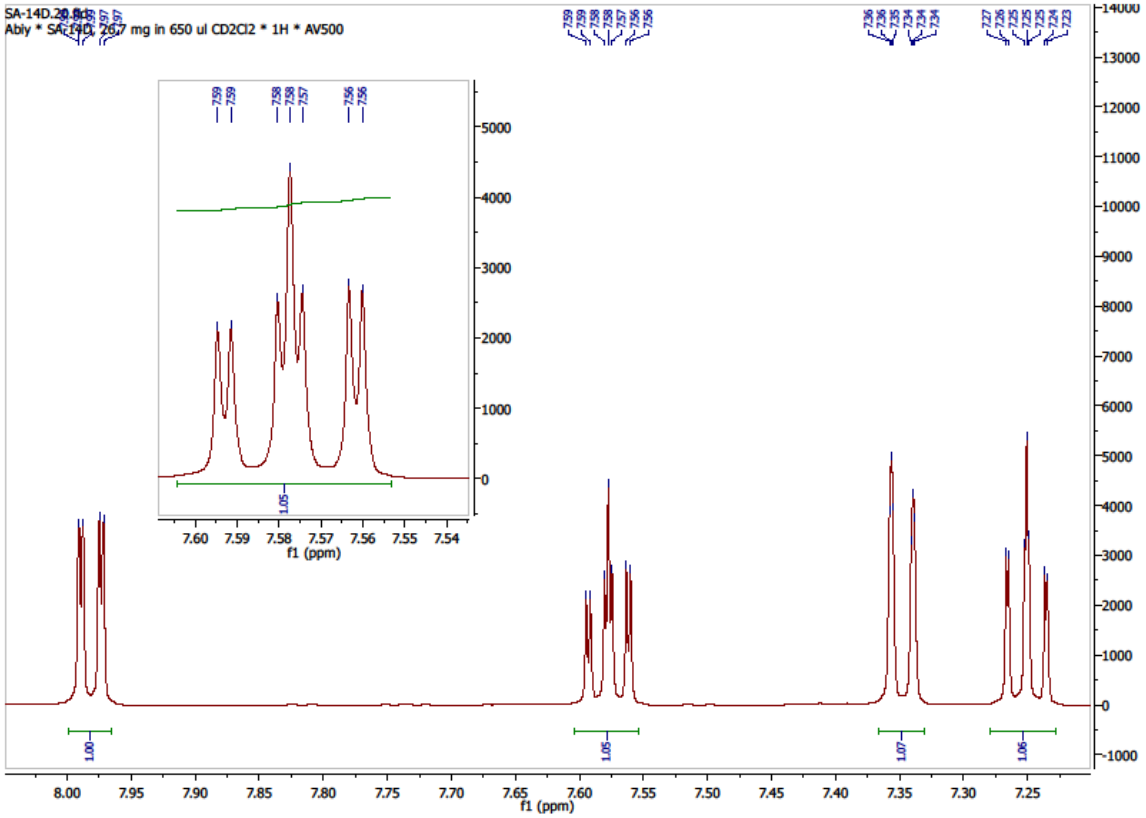
Appendix 8: Spectra for *N*-methylflindersine (8)



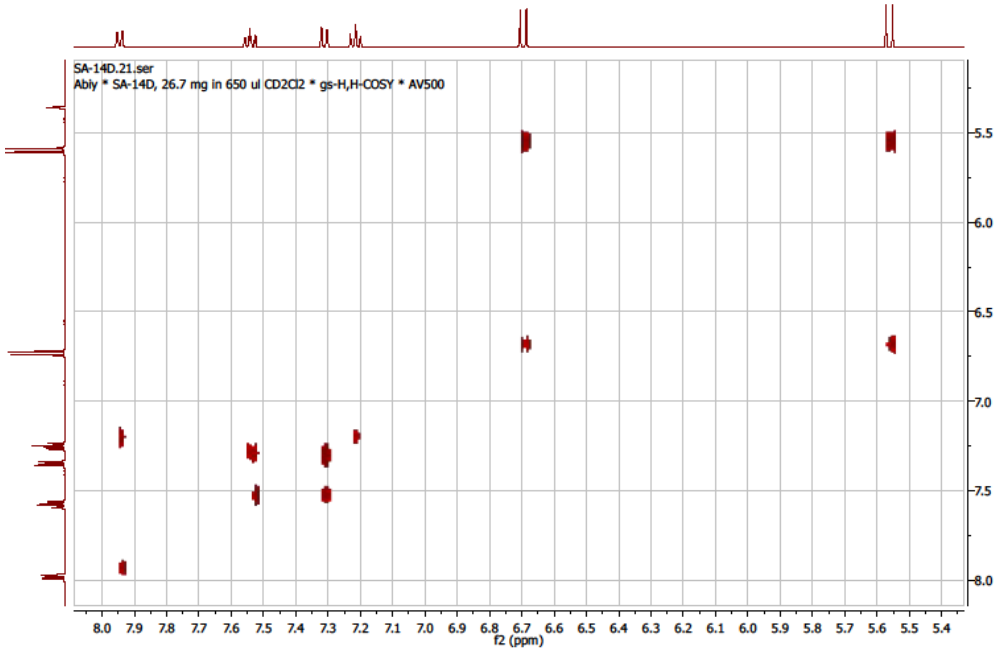
^{1}H-^{1}H NMR for *N*-methylflindersine (**8**)



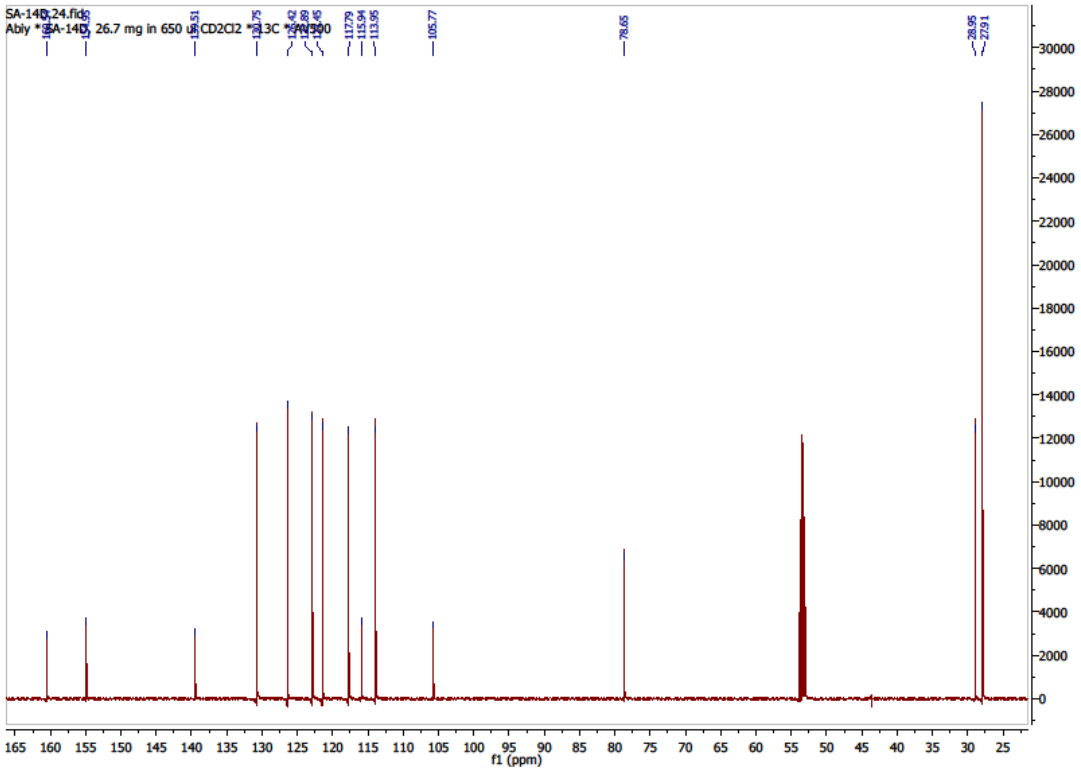
^{1}H-^{1}H NMR for *N*-methylflindersine (**4**) (expansion)



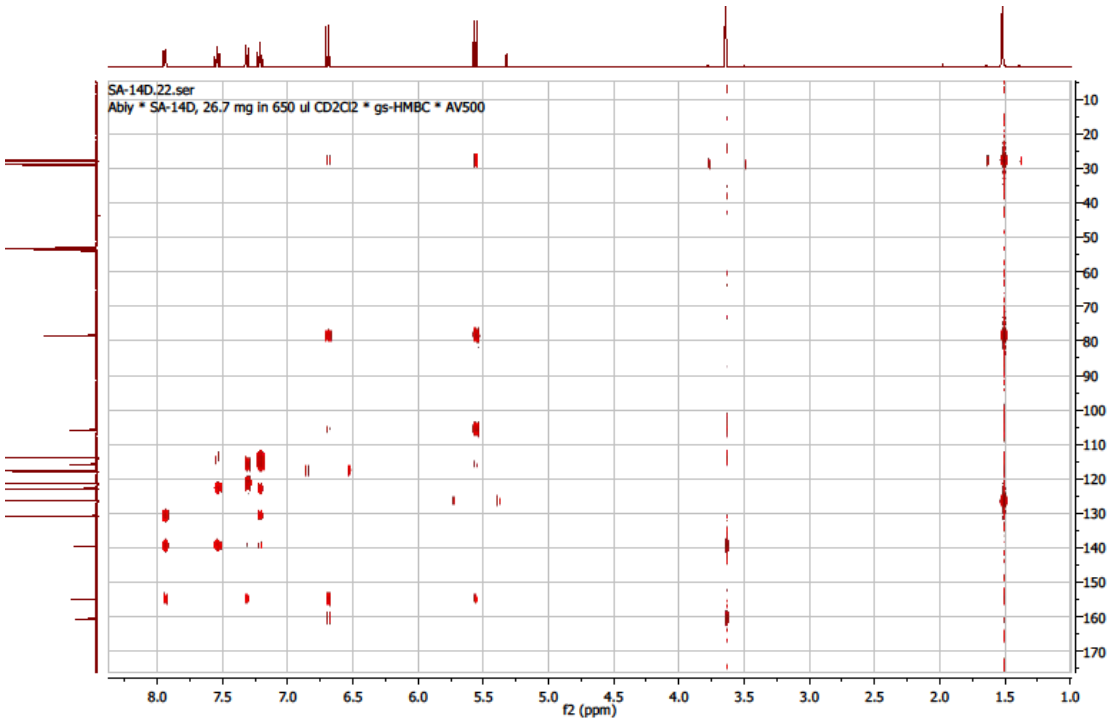
^{1}H, ^{1}H-COSY for compound (**8**)



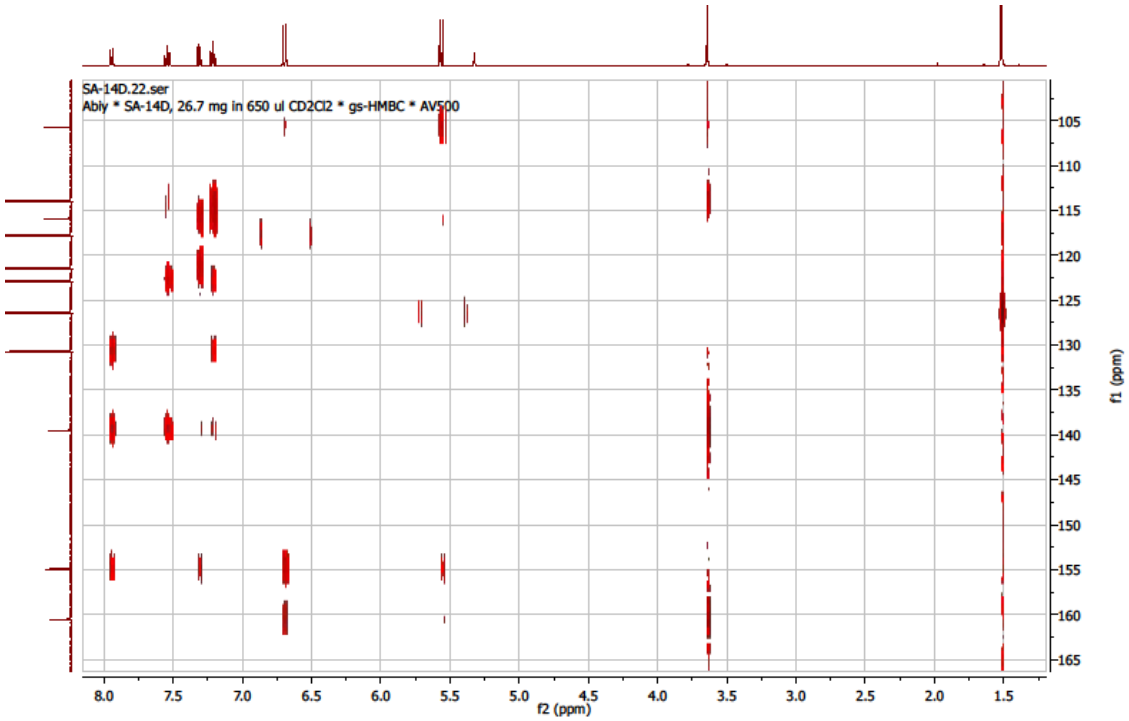
^{13}C NMR for *N*-methylflindersine (**8**)



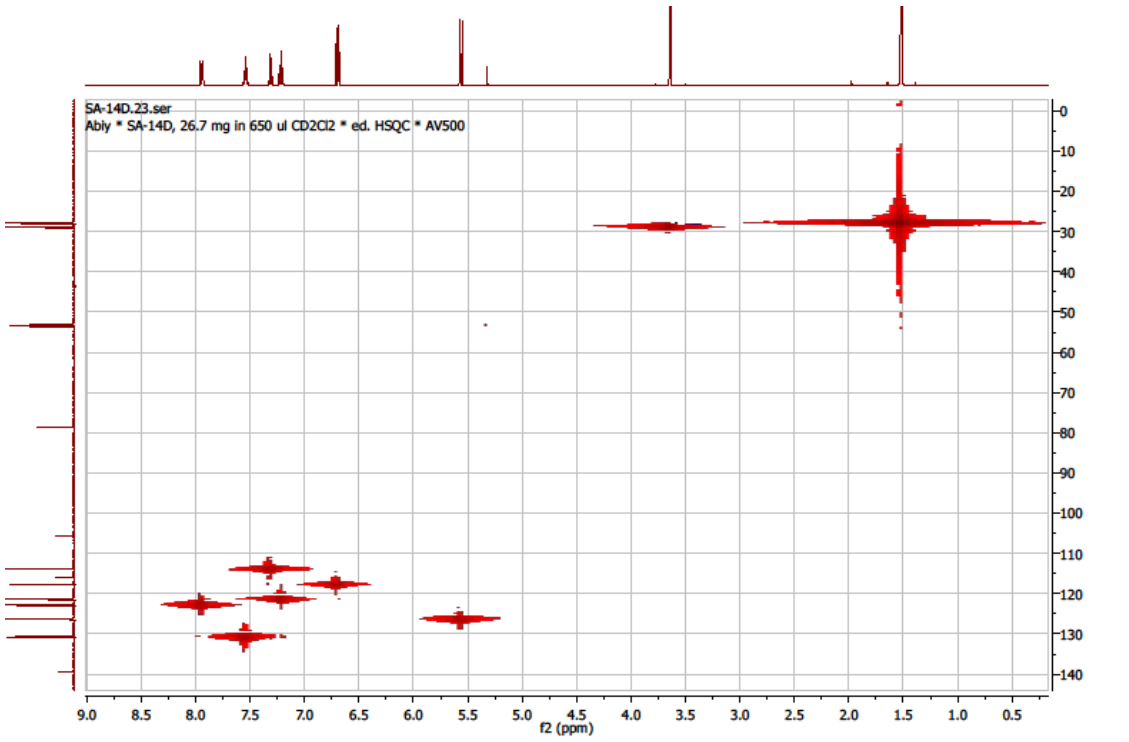
HMBC for *N*-methylflindersine (**8**)



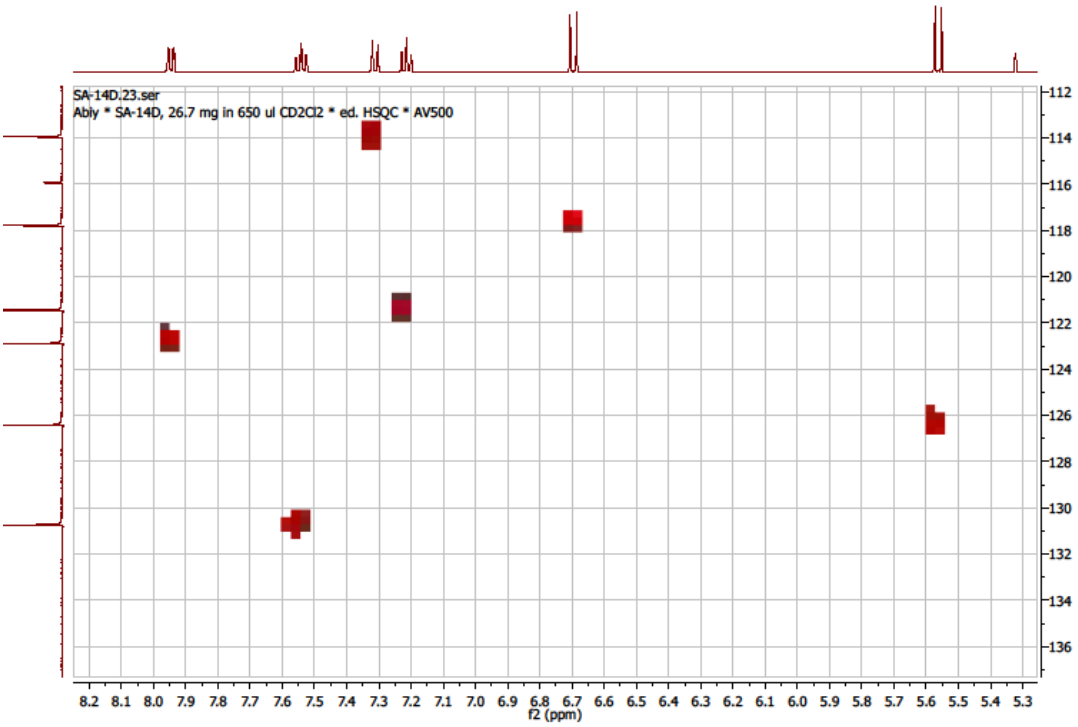
HMBC for *N*-methylflindersine (**8**) (expansion)



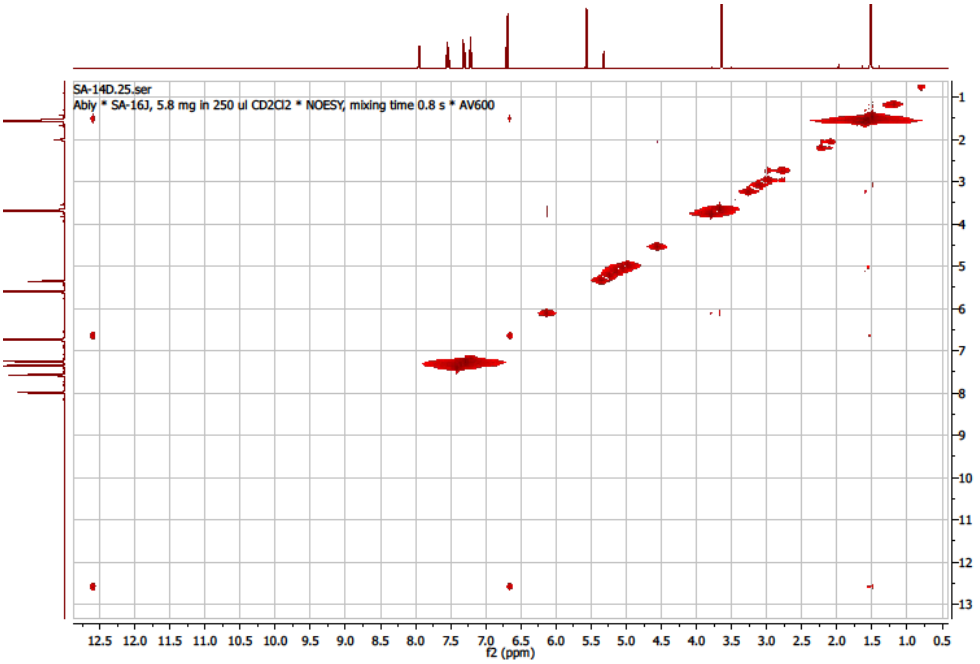
HMBC for *N*-methylflindersine (**8**) (expansion)



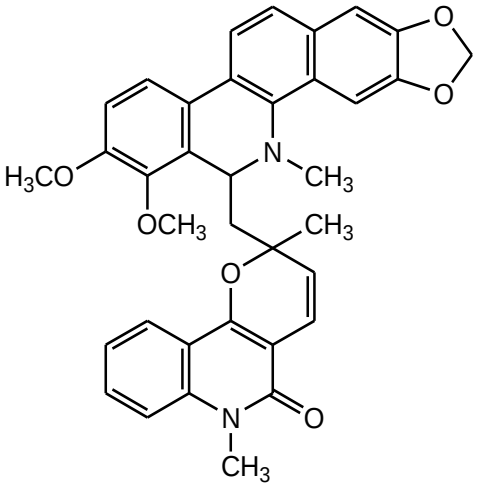
HMBC for *N*-methylflindersine (**8**) (expansion)



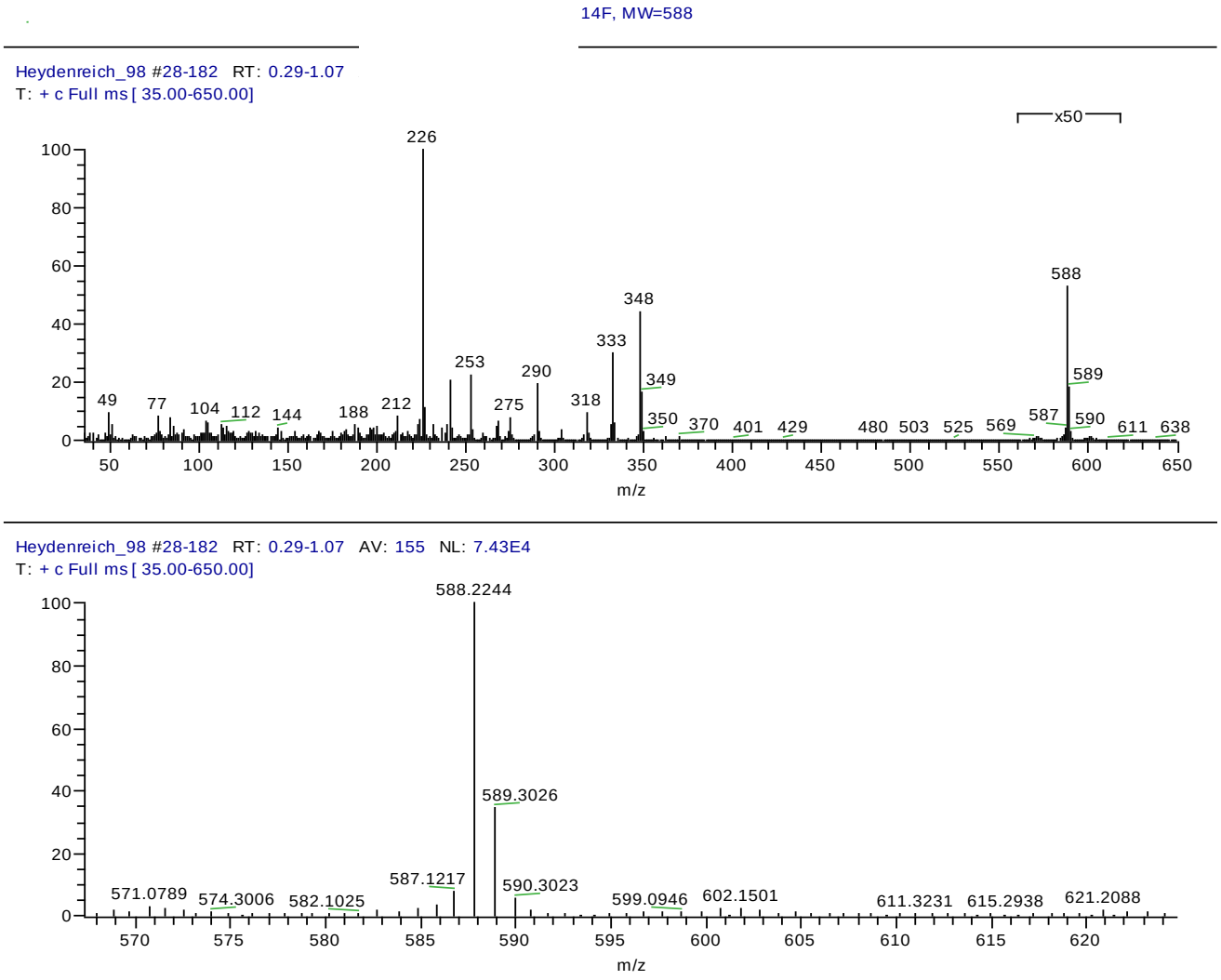
NOESY for *N*-methylflindersine **(8)**



Appendix 9: Spectra for holstzianoquinoline (9)



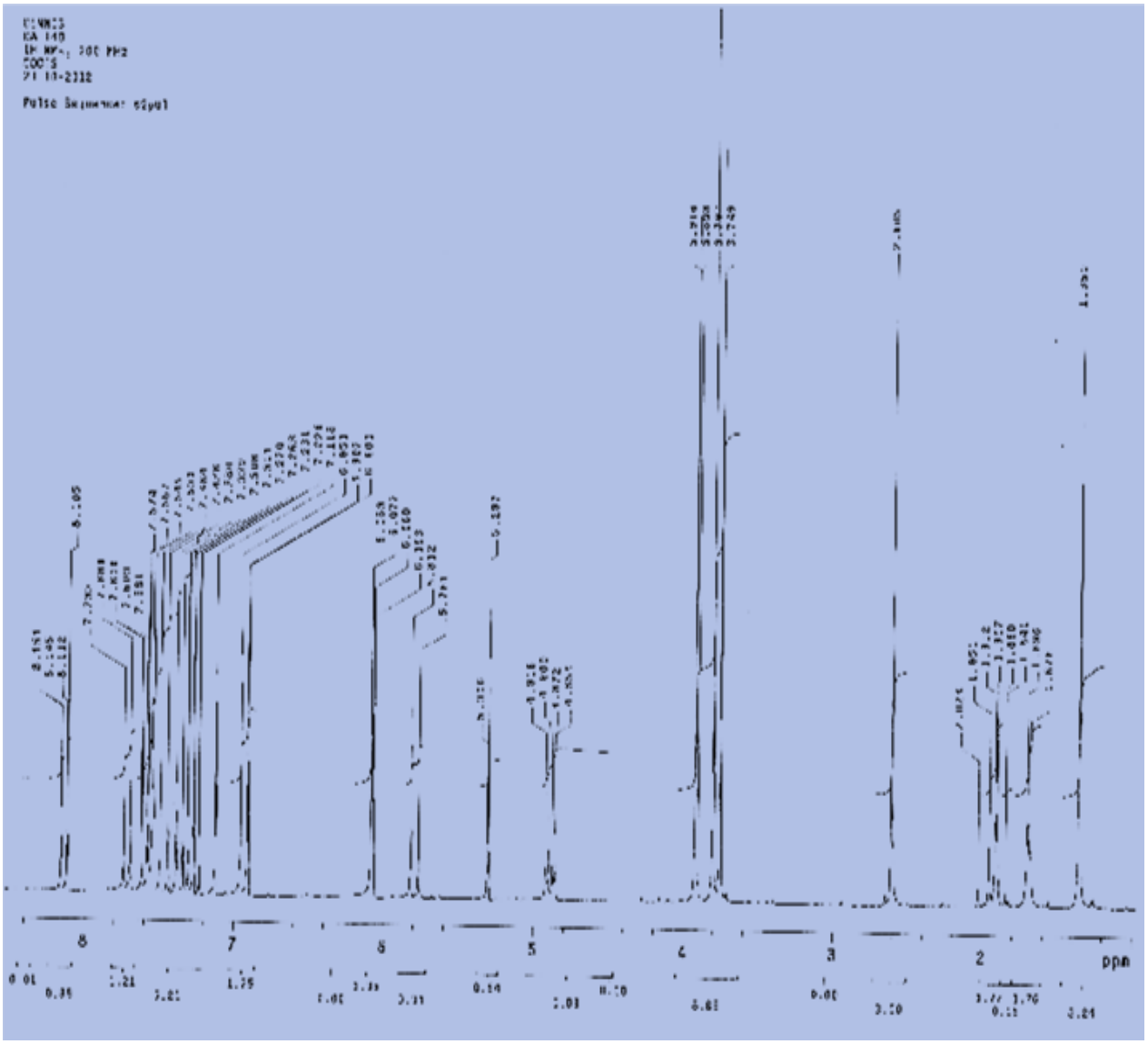
Mass spectrum for holstzianoquinoline (**9**)



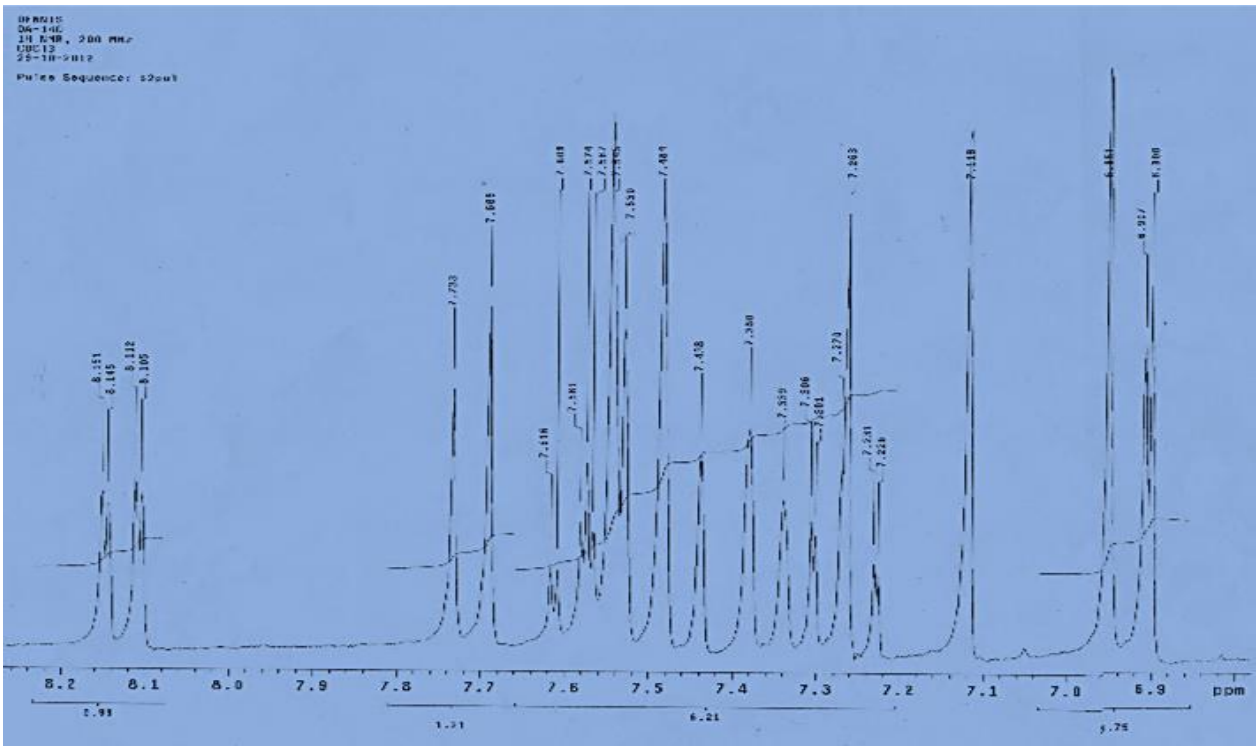
Elemental Composition Report

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
588.2244	588.2234	1.8	17.5	$C_{33}\,H_{34}\,O_9\,N_1$
	588.2260	-2.8	22.0	$C_{36}\,H_{32}\,O_6\,N_2$
	588.2207	6.3	13.0	$C_{30}\,H_{36}\,O_{12}$
	588.2292	-8.2	8.5	$C_{26}\,H_{38}\,O_{14}\,N_1$

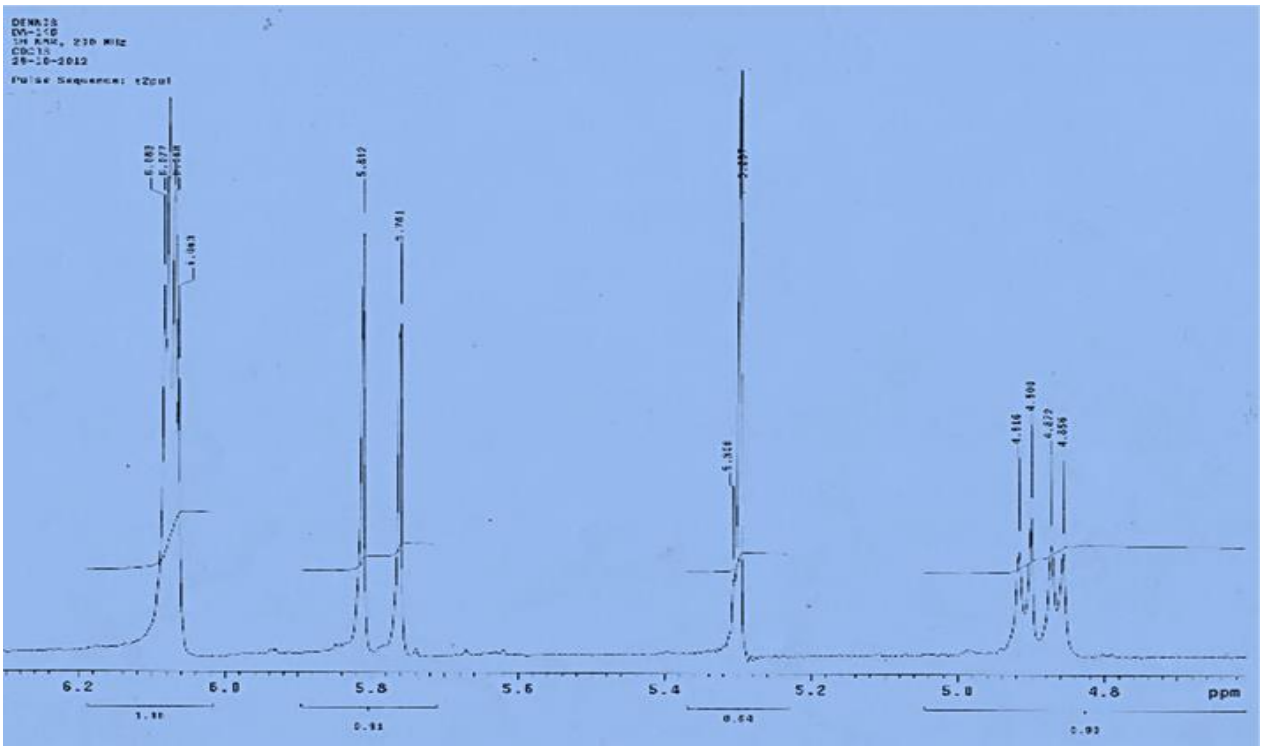
^{1}H, ^{1}H NMR for holstzianoquinoline (**9**)



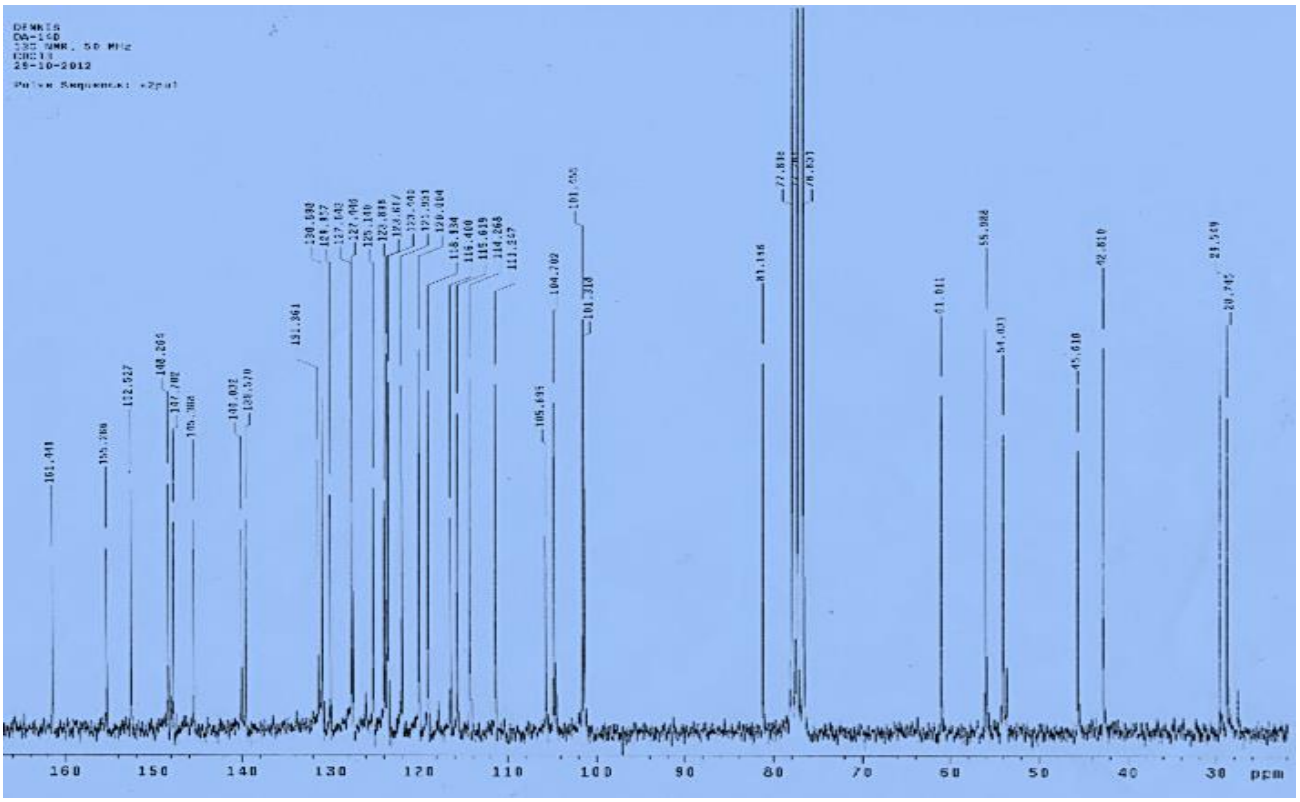
^{1}H-^{1}H NMR for holstzianoquinoline **(9)** (expansion)



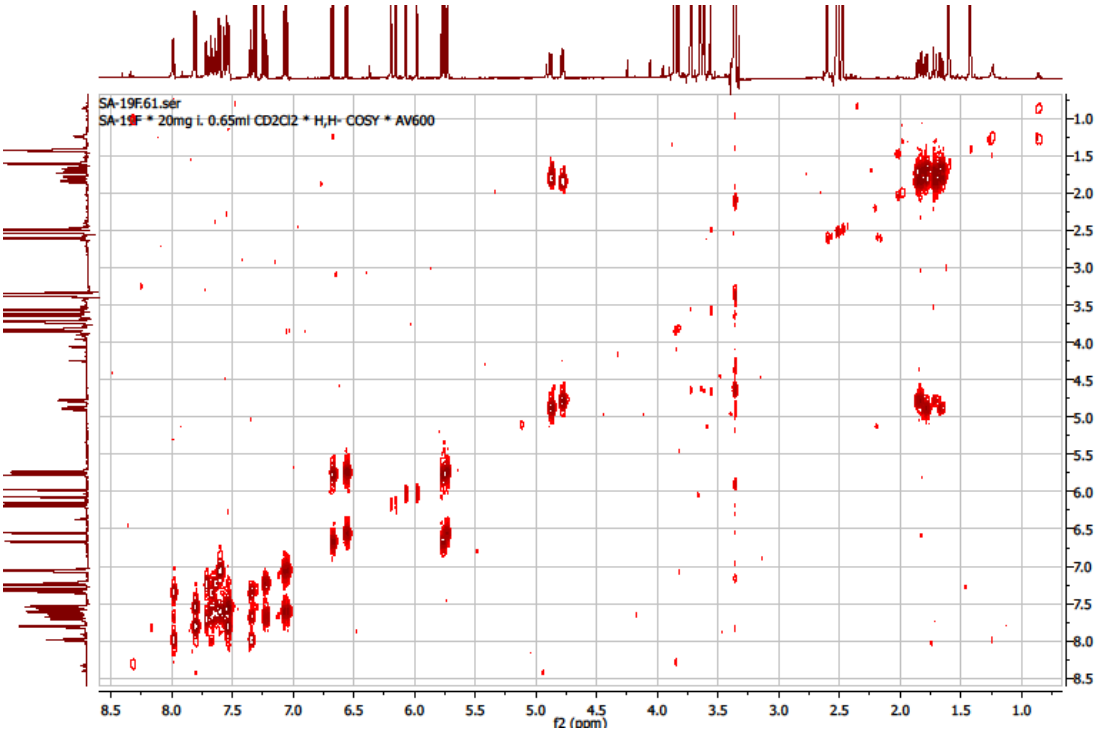
^{1}H-^{1}H NMR for holstzianoquinoline **(9)** (expansion)



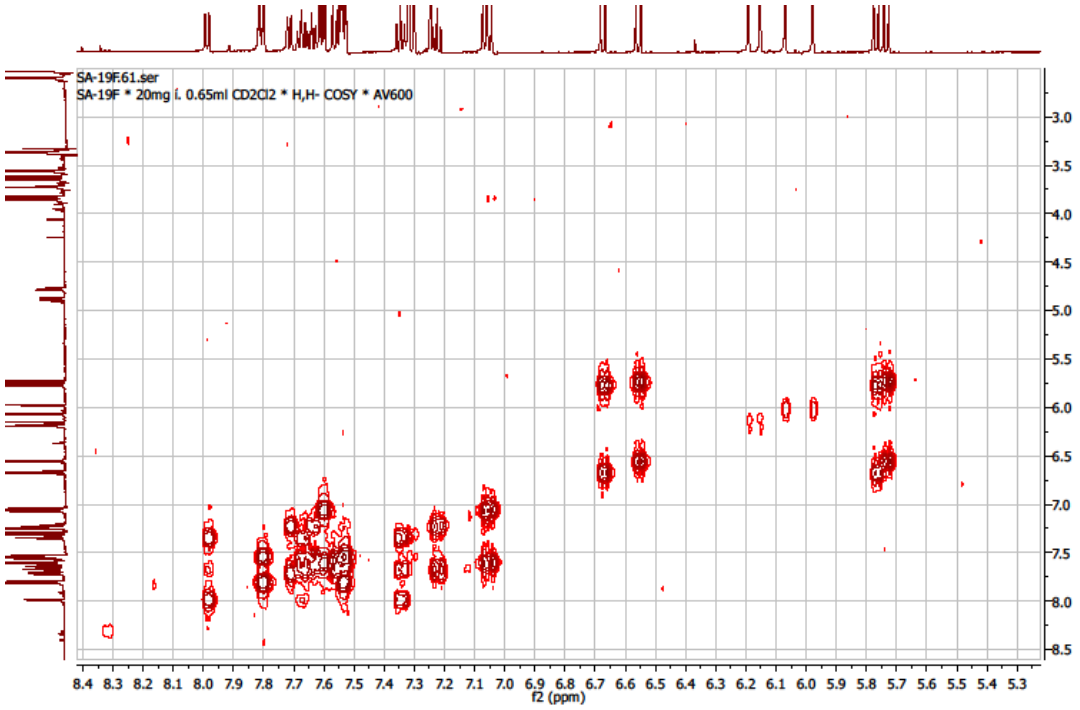
^{13}C NMR for holstzianoquinoline **(9)**



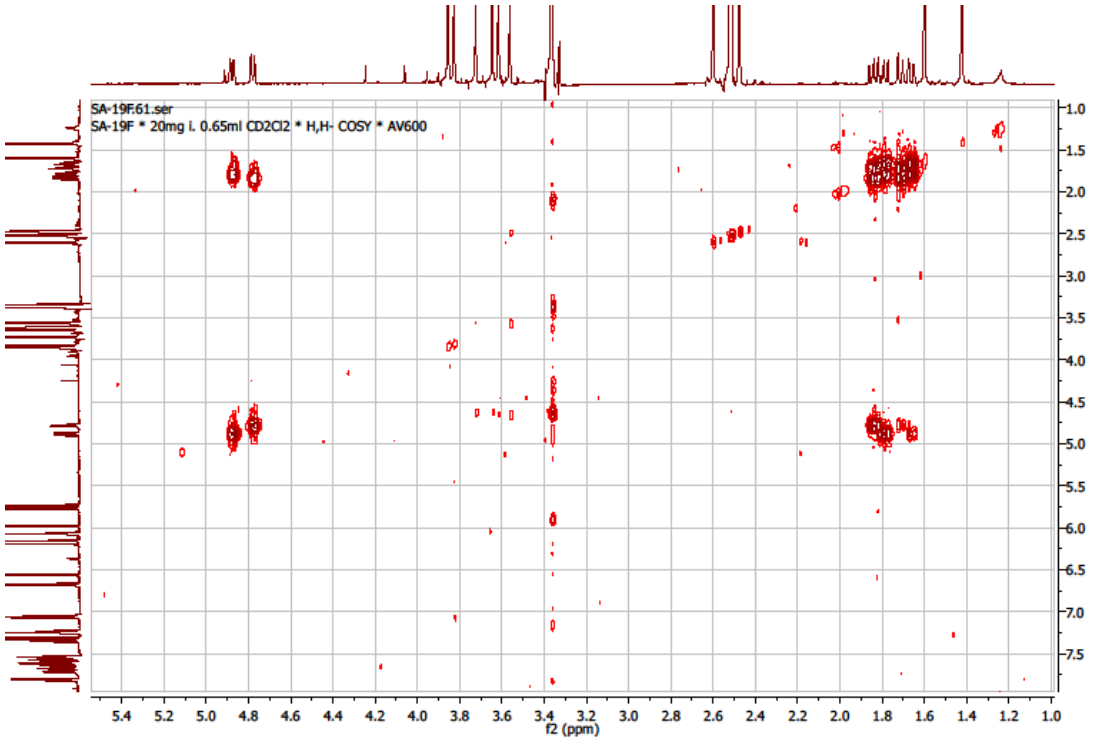
^{1}H-^{1}H-COSY for holstzianoquinoline (**9**)



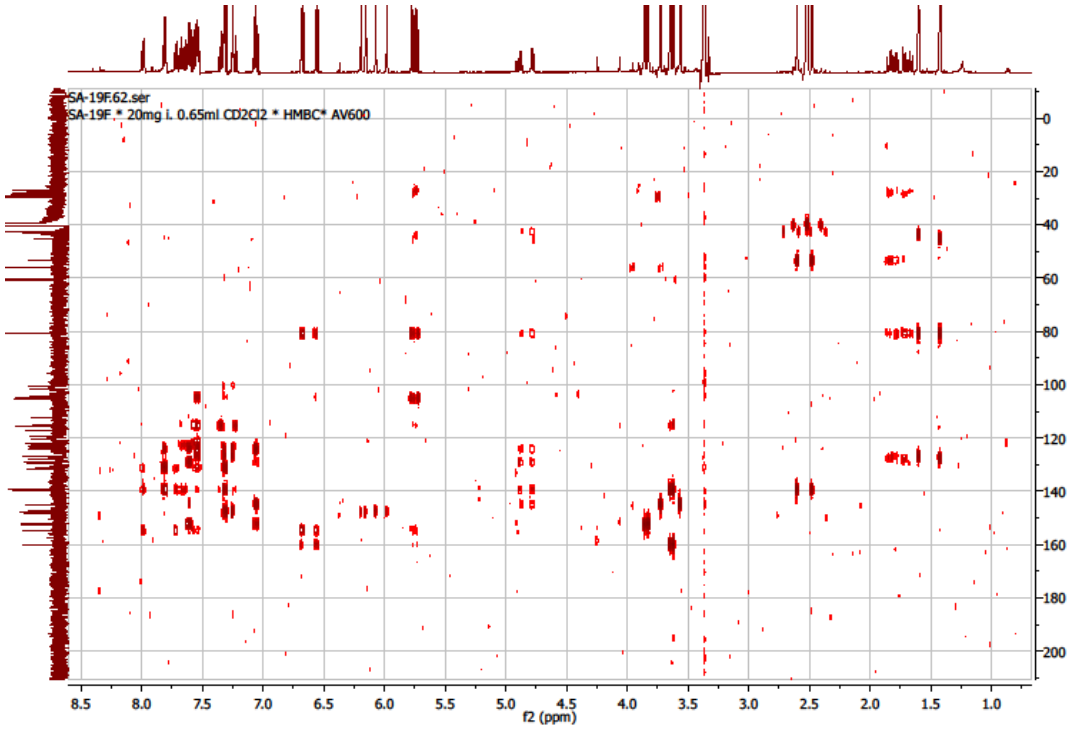
^{1}H-^{1}H-COSY for holstzianoquinoline **(9)** (expansion)



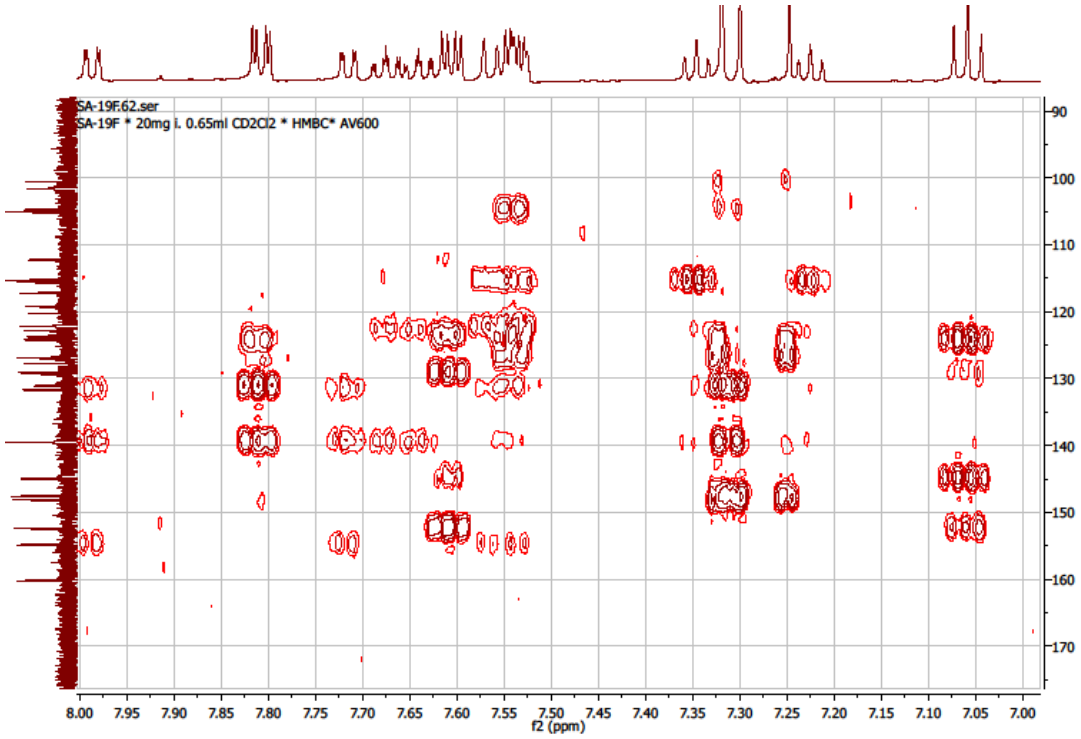
^{1}H-^{1}H COSY for holstzianoquinoline **(9)** (expansion)



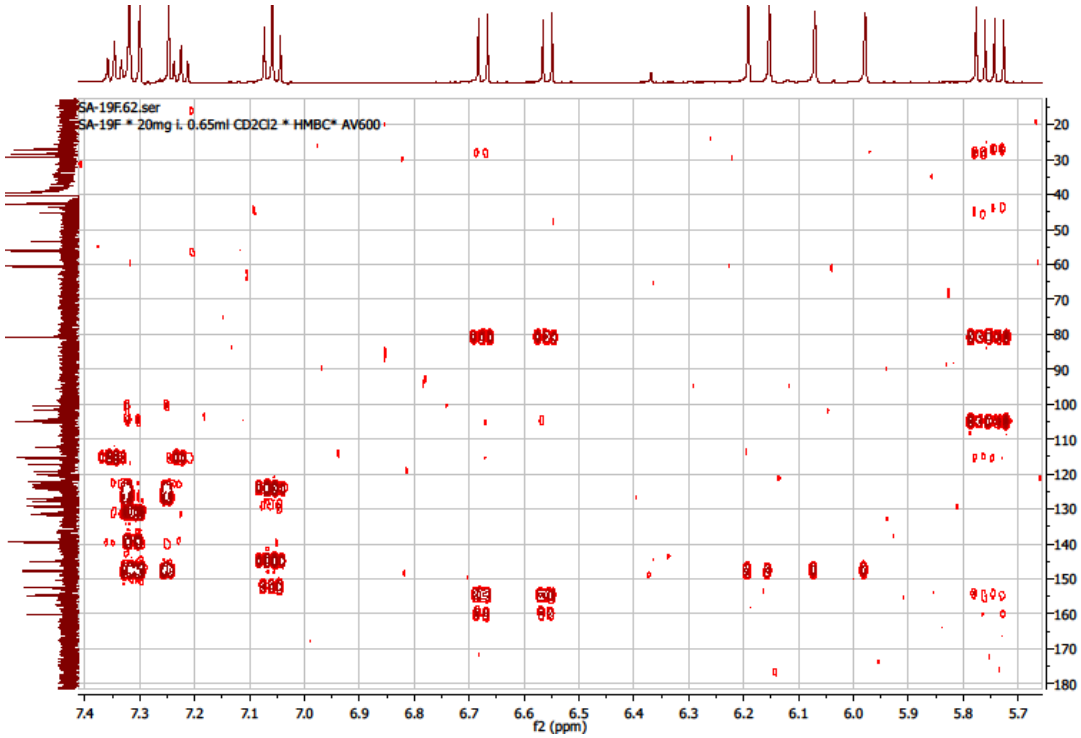
^{1}H-^{1}H COSY for holstzianoquinoline **(9)**



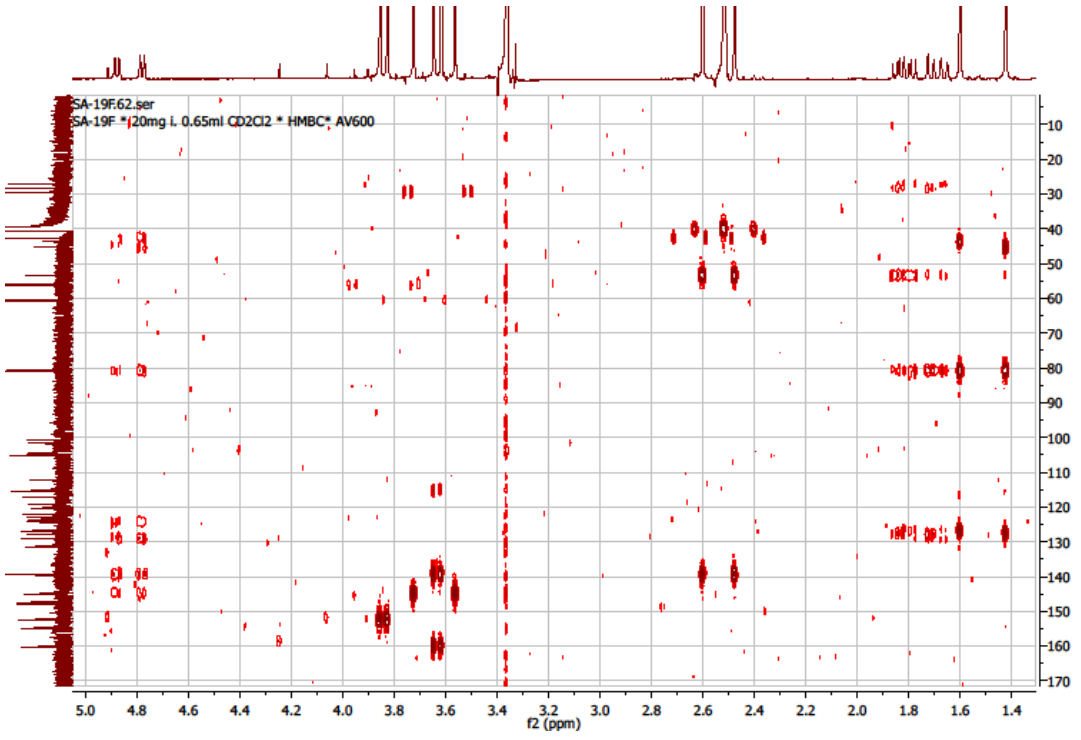
HMBC for holstzianoquinoline **(9)** (expansion)



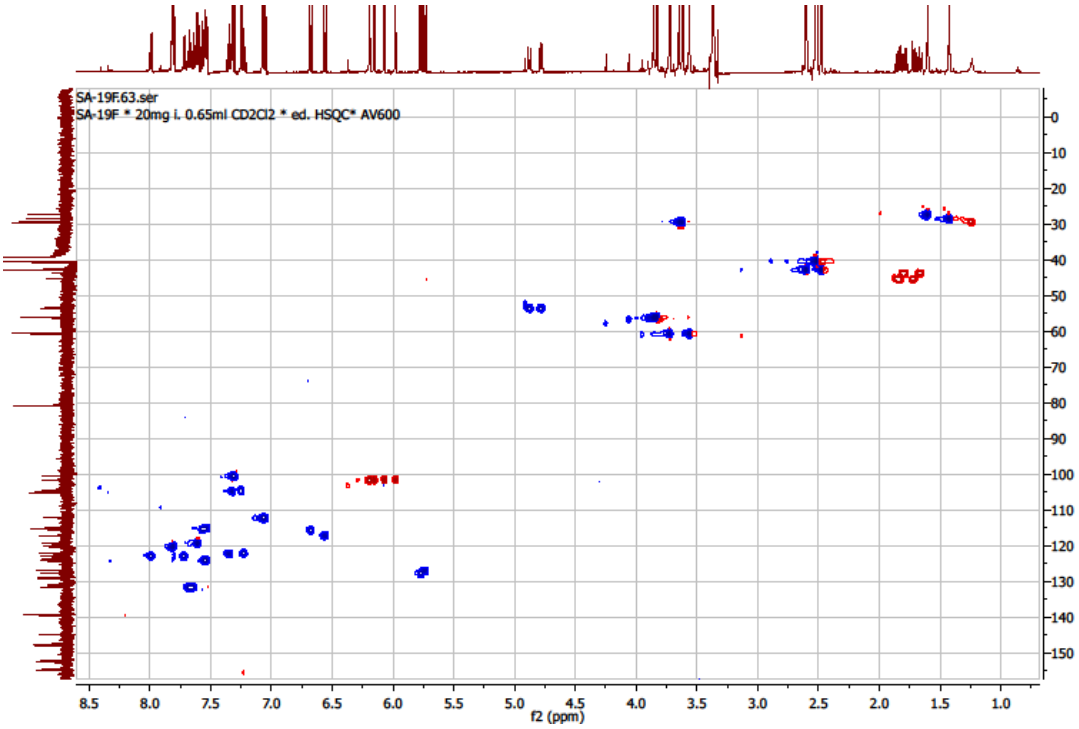
HMBC for holstzianoquinoline **(9)**



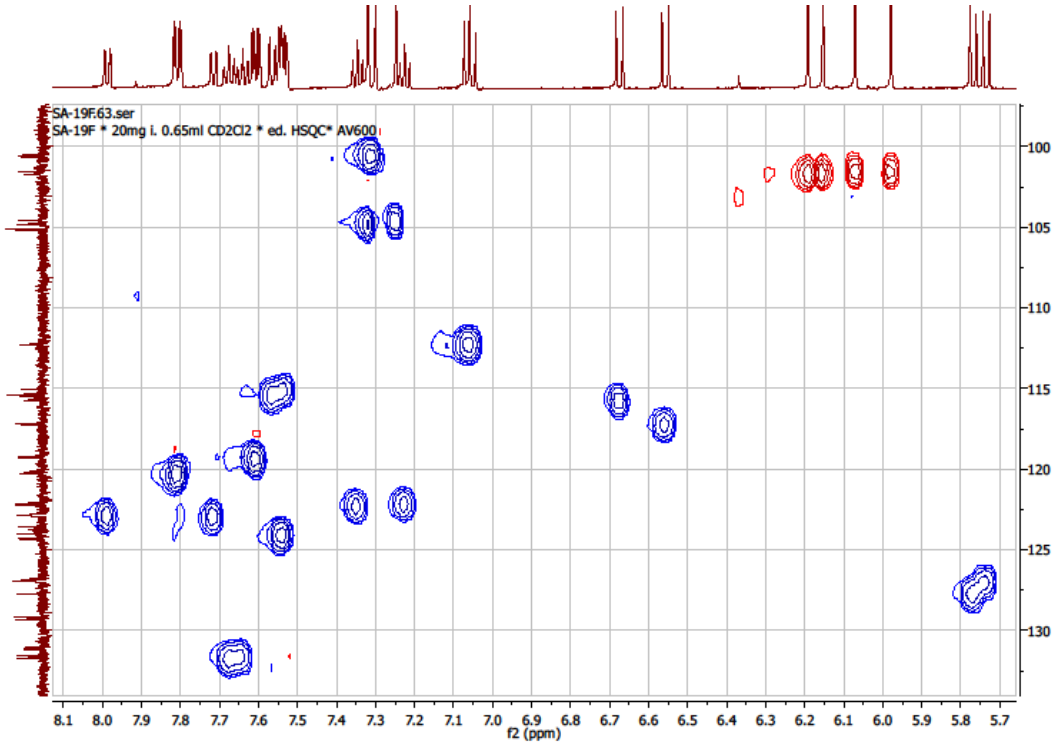
HMBC for holstzianoquinoline **(9)**

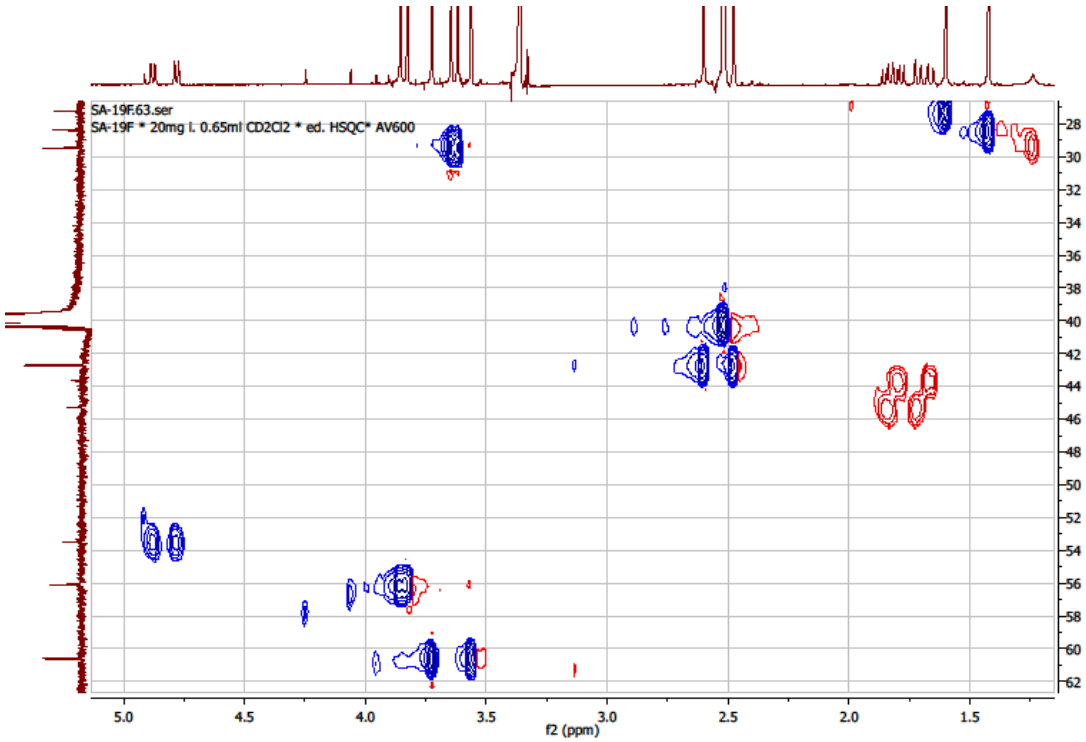


^{1}H-^{1}H COSY for holstzianoquinoline **(9)**

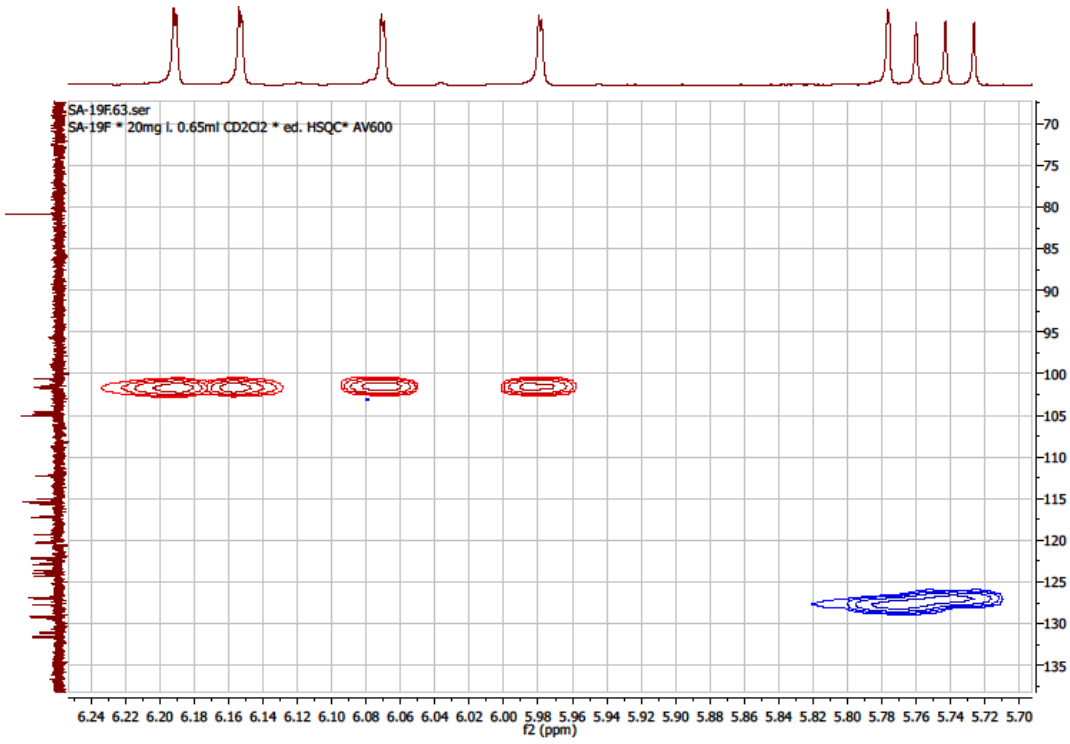


HSQC for holstzianoquinoline **(9)**

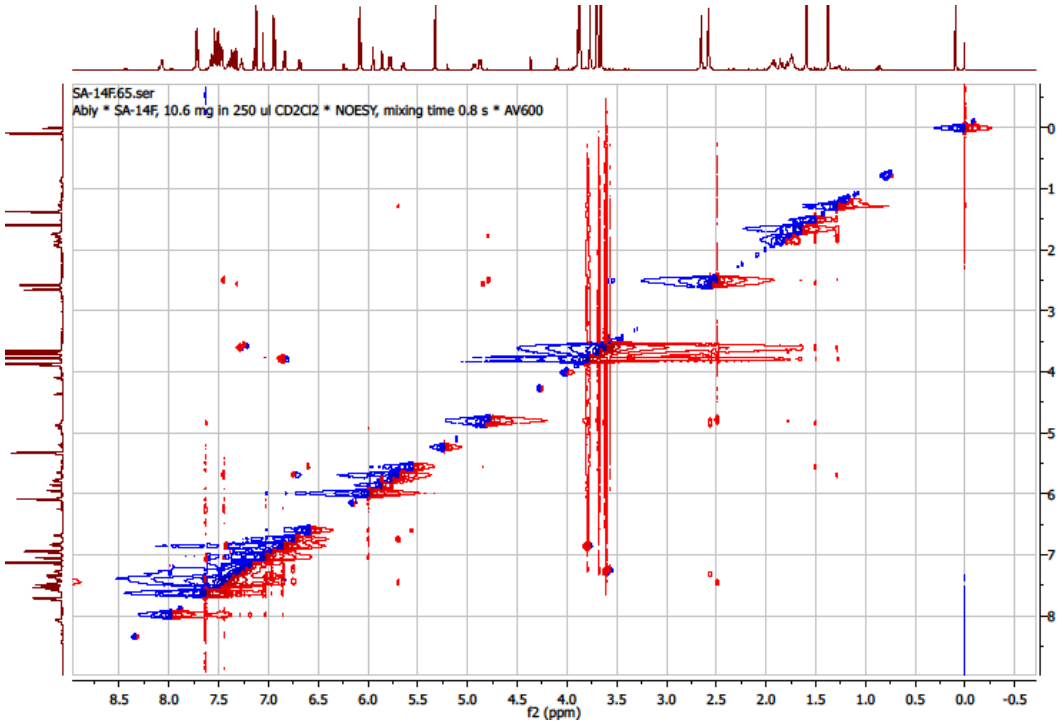




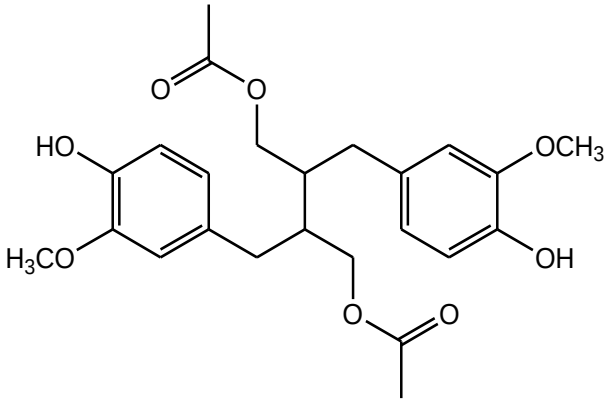
HSQC for holstzianoquinoline **(9)**



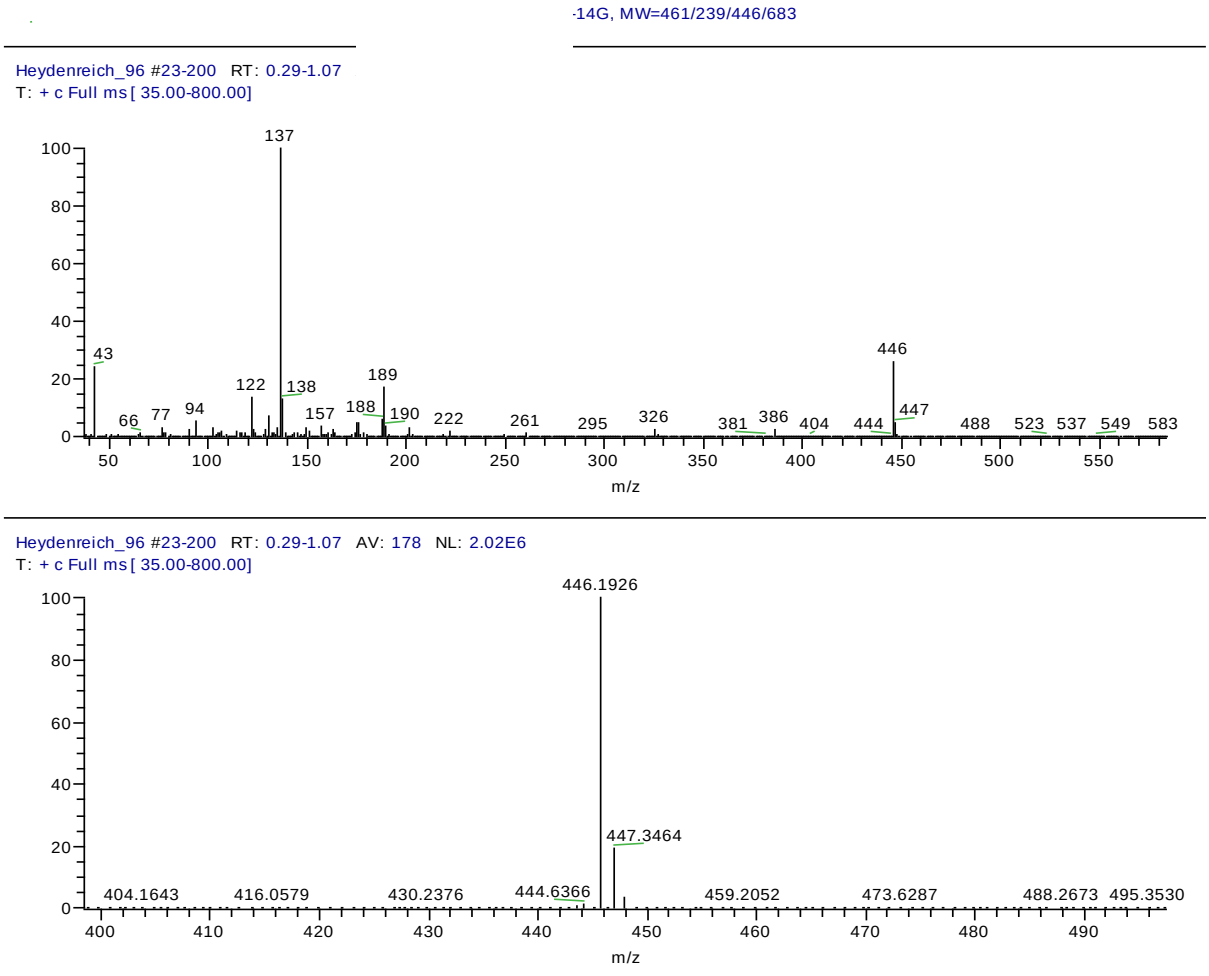
NOESY for holstzianoquinoline **(9)**



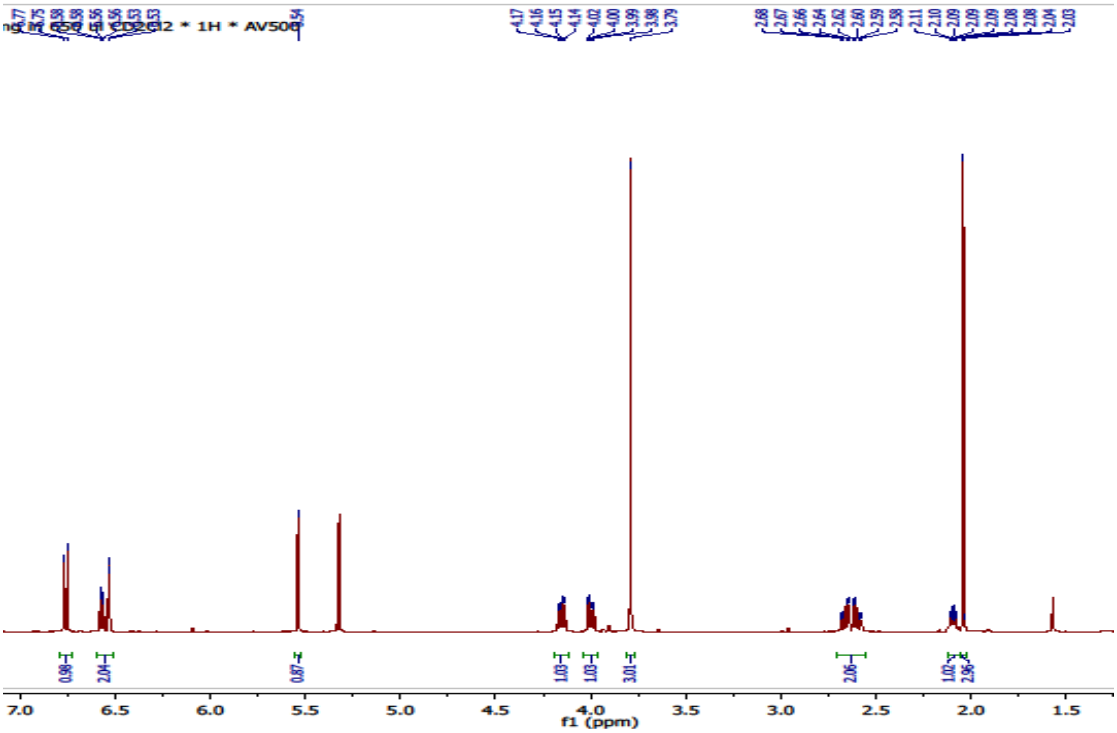
Appendix 10: Spectra for 4,4’-Dihydroxy-3,3’-dimethoxylignan-9,9’-diyldiacetate (10)



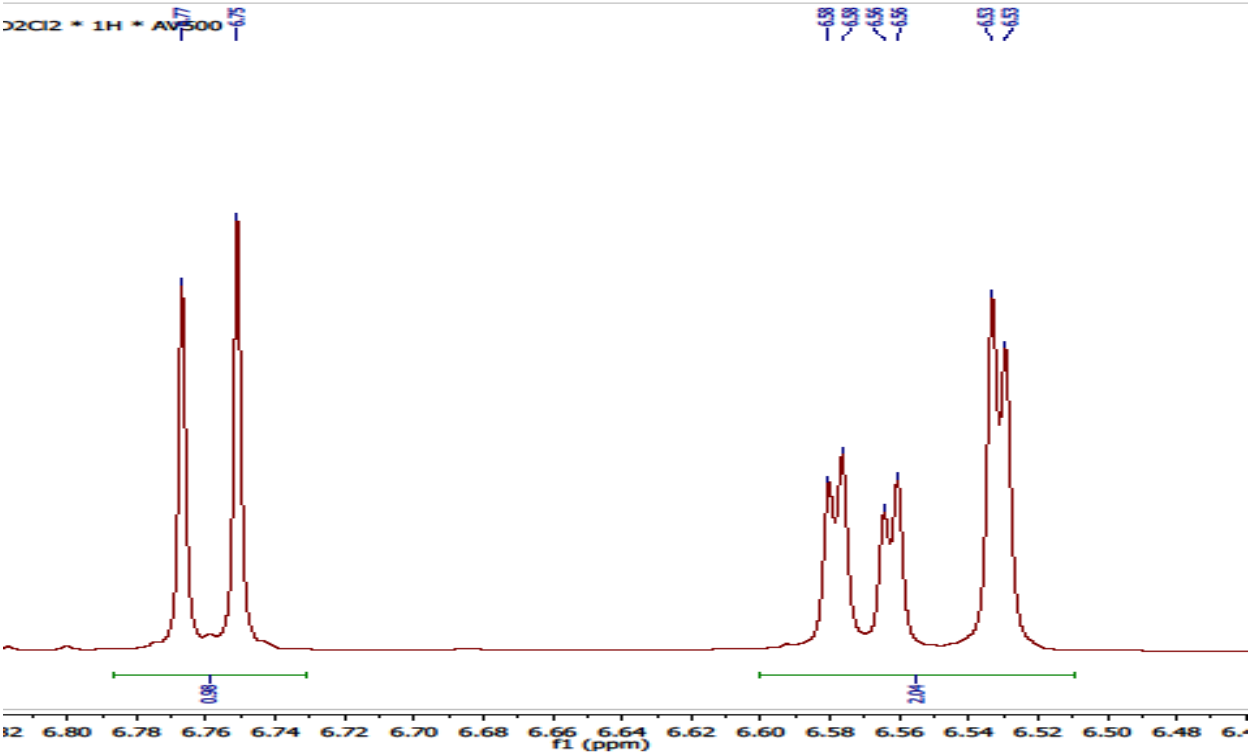
Mass spectrum for compound **(10)**



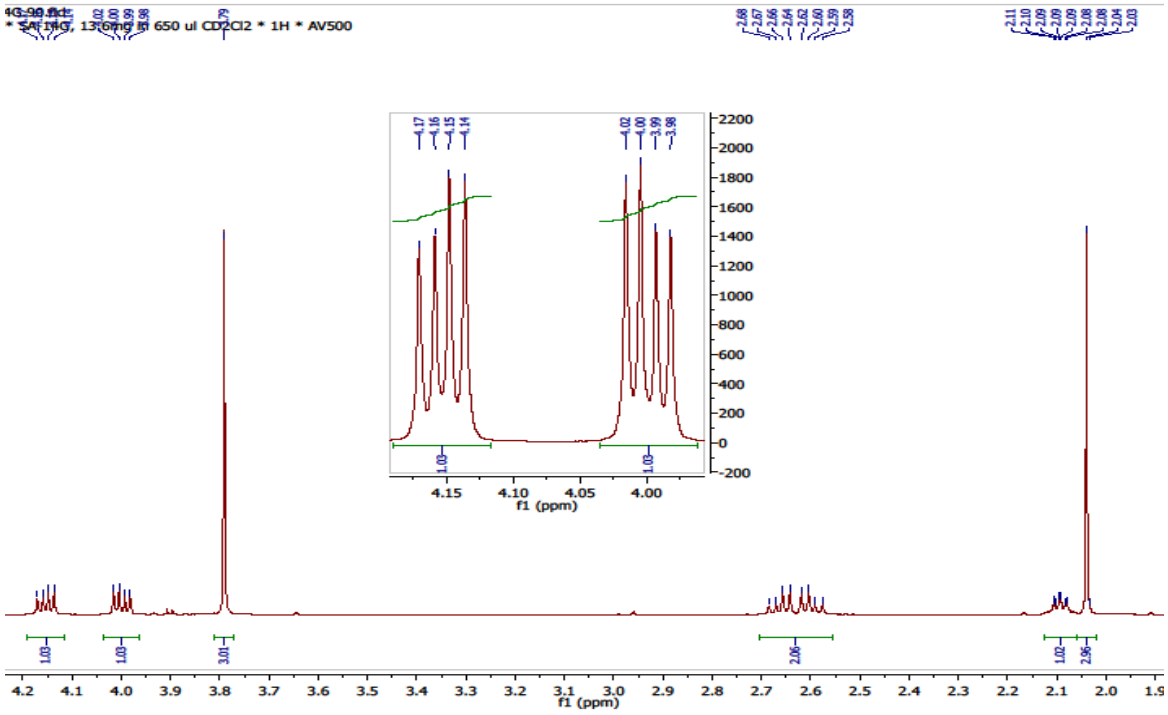
^{1}H-^{1}H NMR spectrum for compound **(10)**



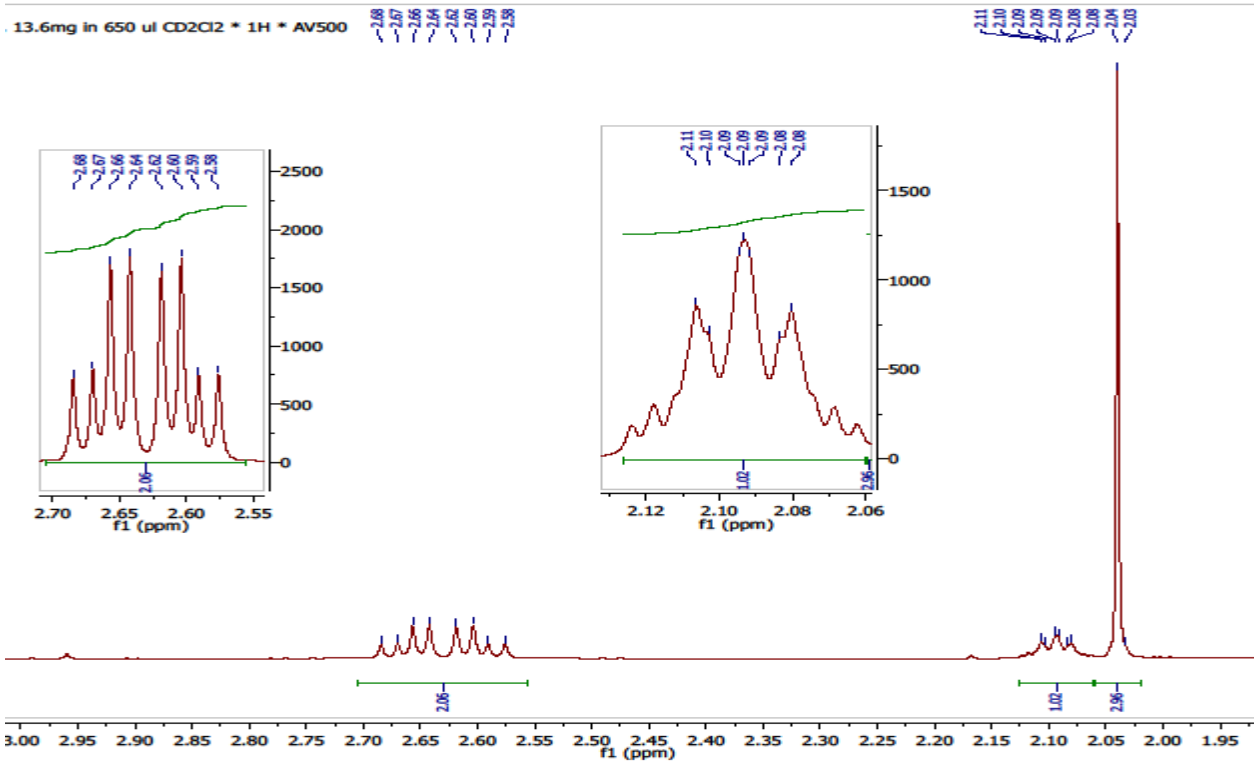
^{1}H-^{1}H NMR spectrum for compound **(10)** (expansion)



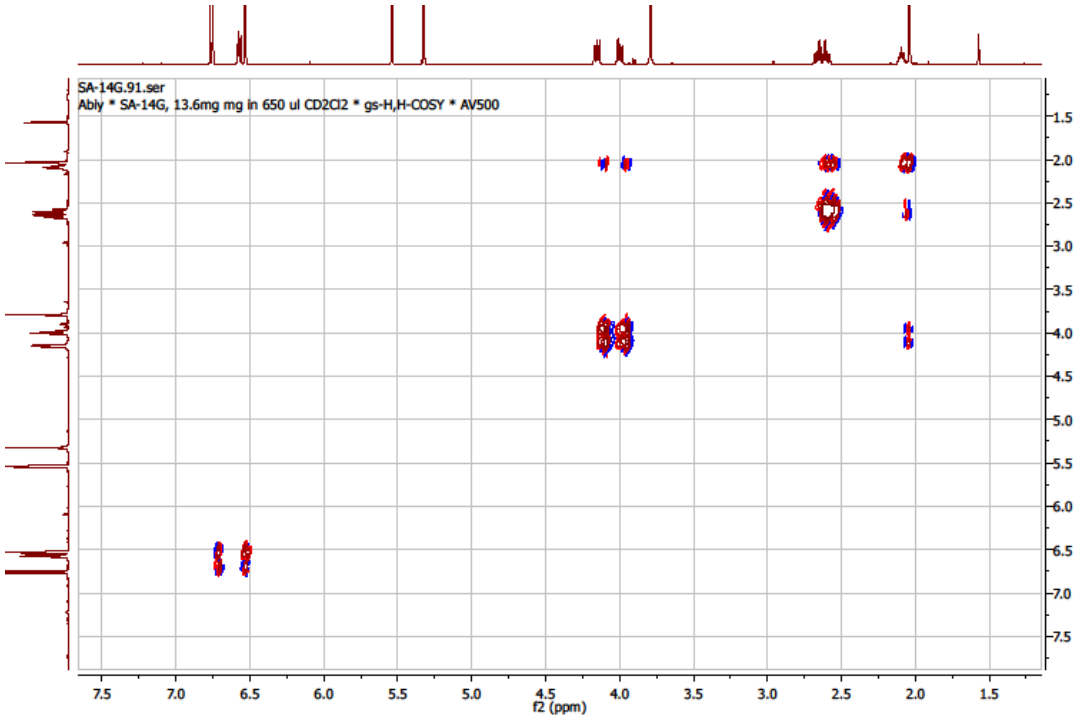
^{1}H-^{1}H NMR spectrum for compound (**10**) (expansion)



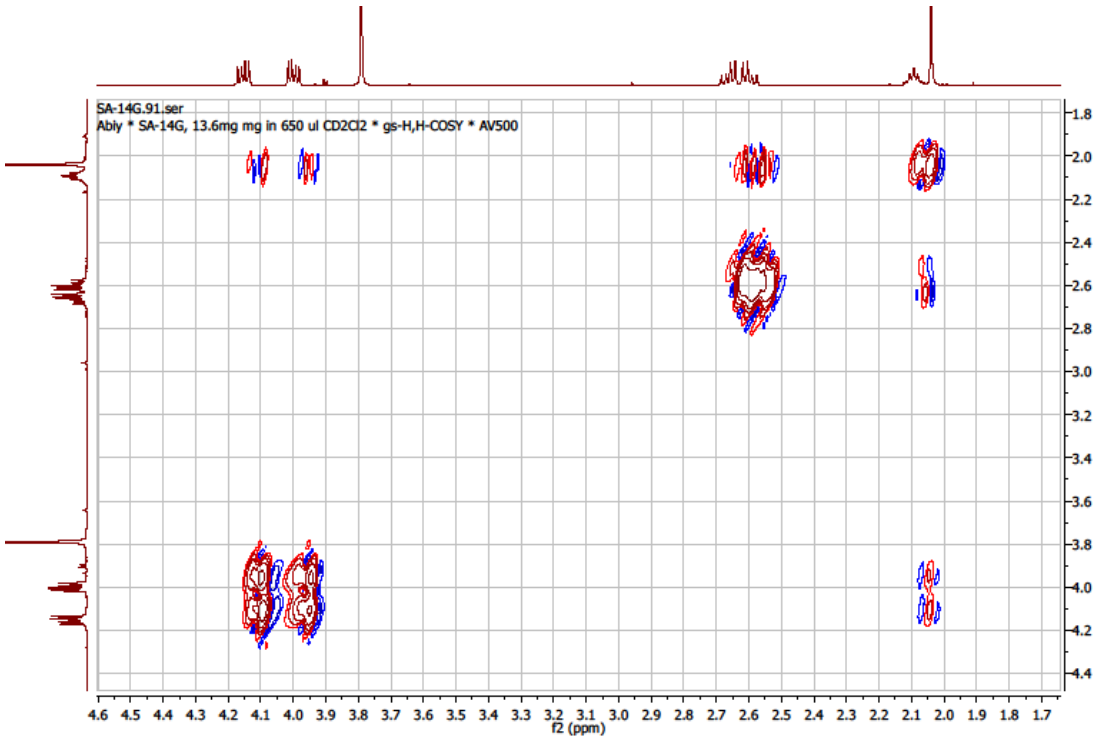
^{1}H-^{1}H NMR spectrum for compound **(10)** (expansion)



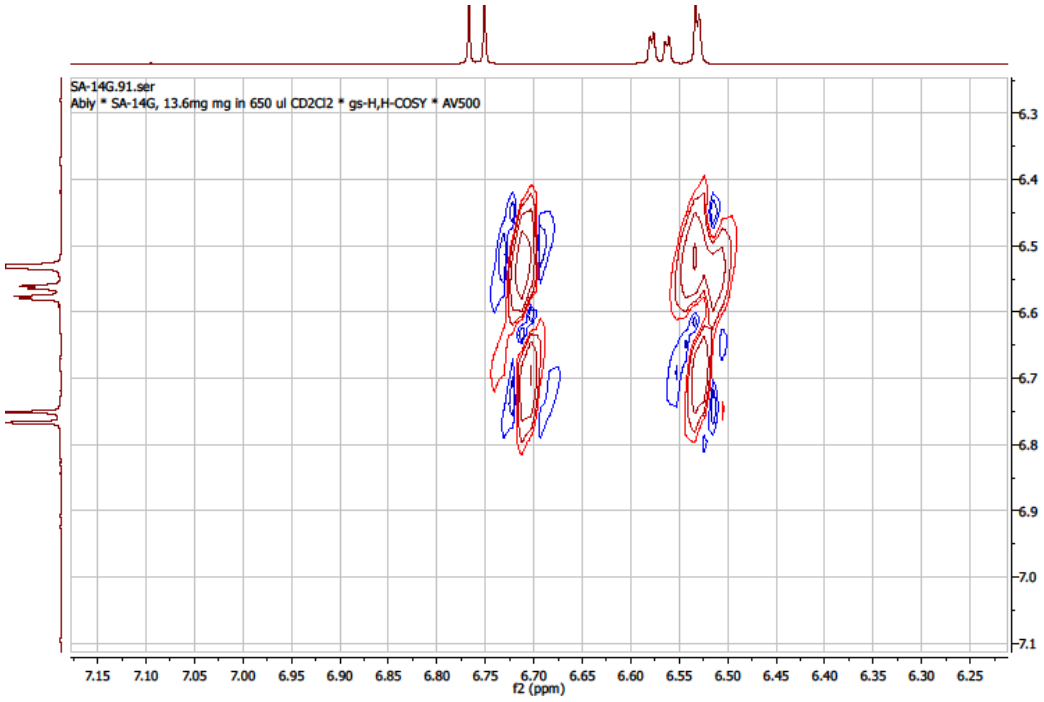
^{1}H-^{1}H COSY for compound **(10)**



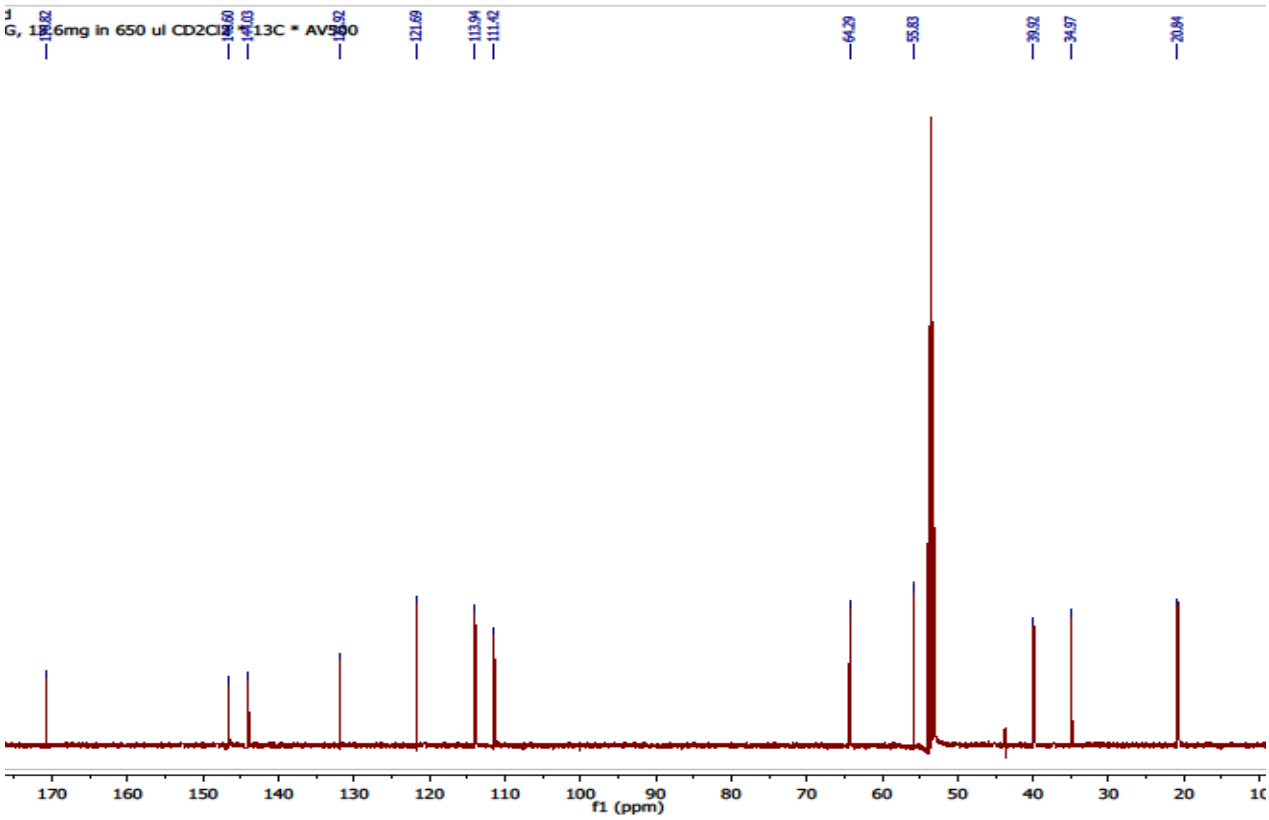
^{1}H-^{1}H COSY for compound **(10)**



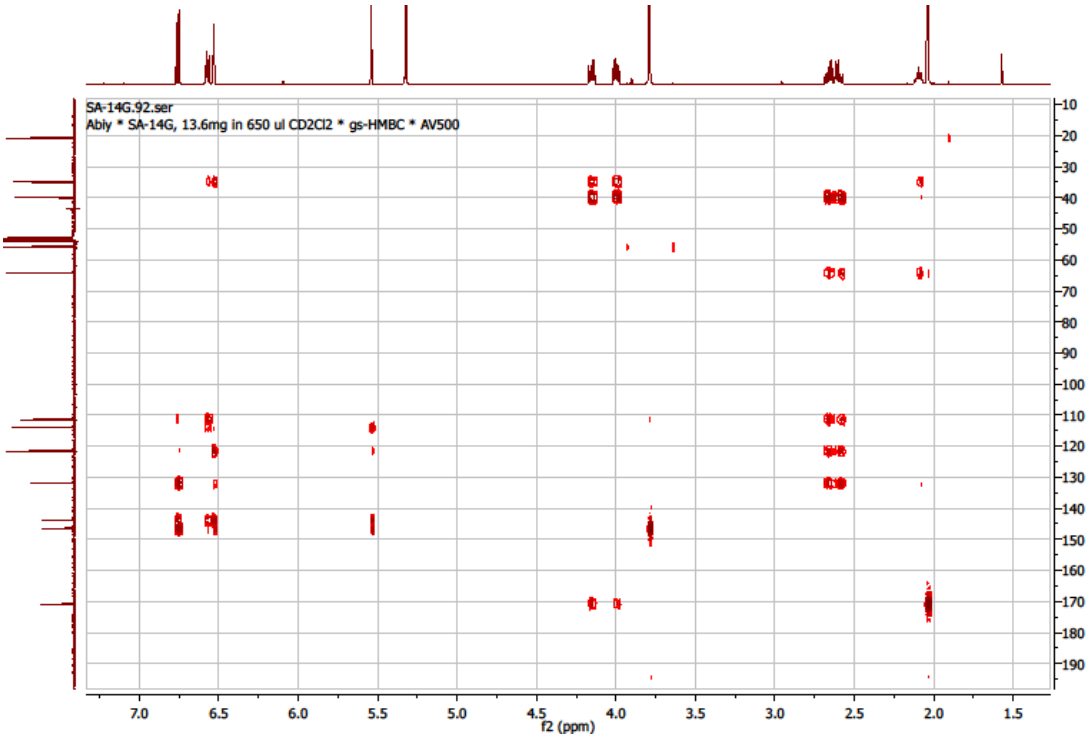
^{1}H-^{1}HCOSY for compound **(10)**



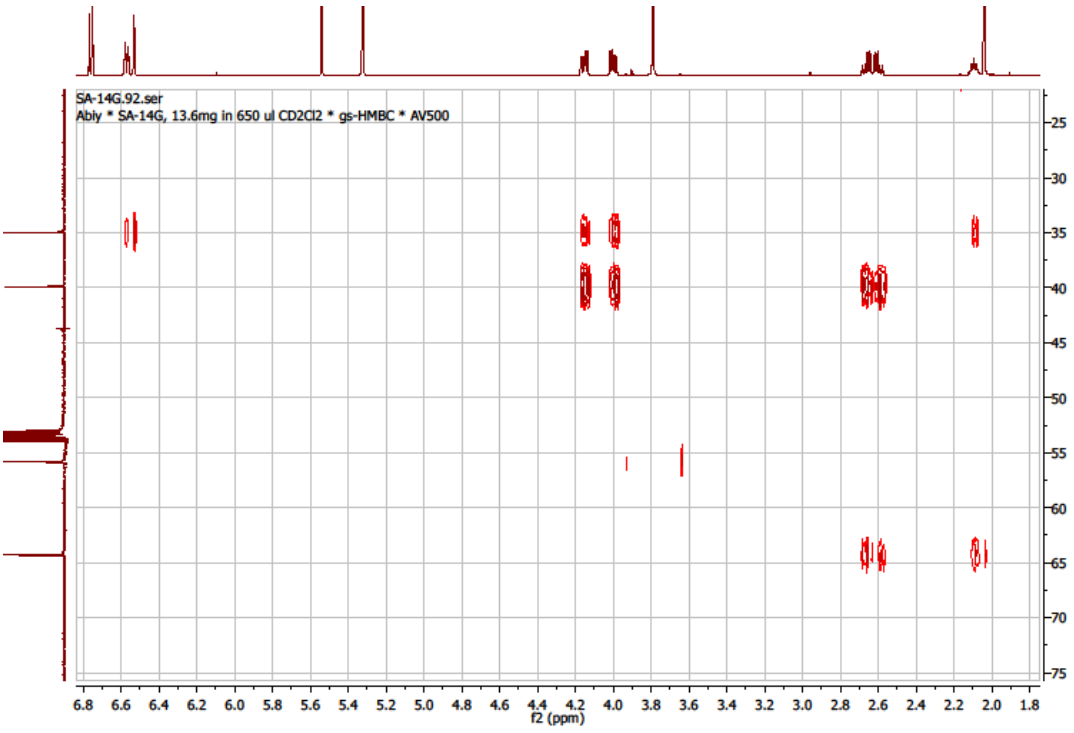
^{13}C NMR spectrum of compound **(10)**



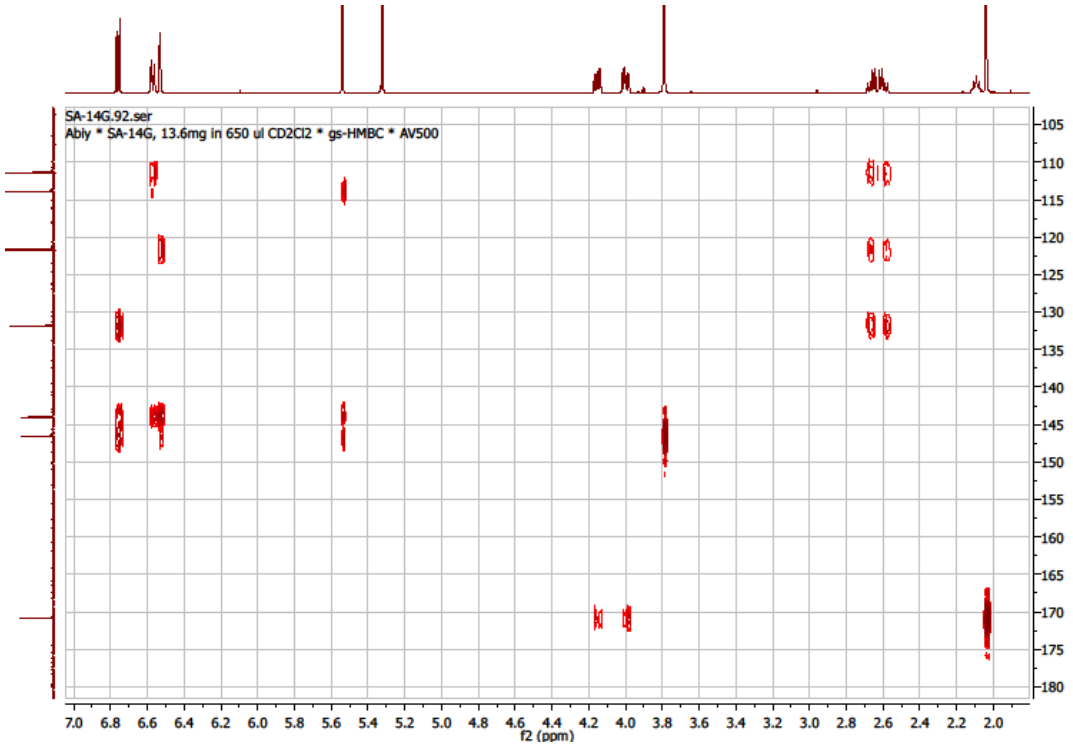
HMBC spectrum for compound **(10)**



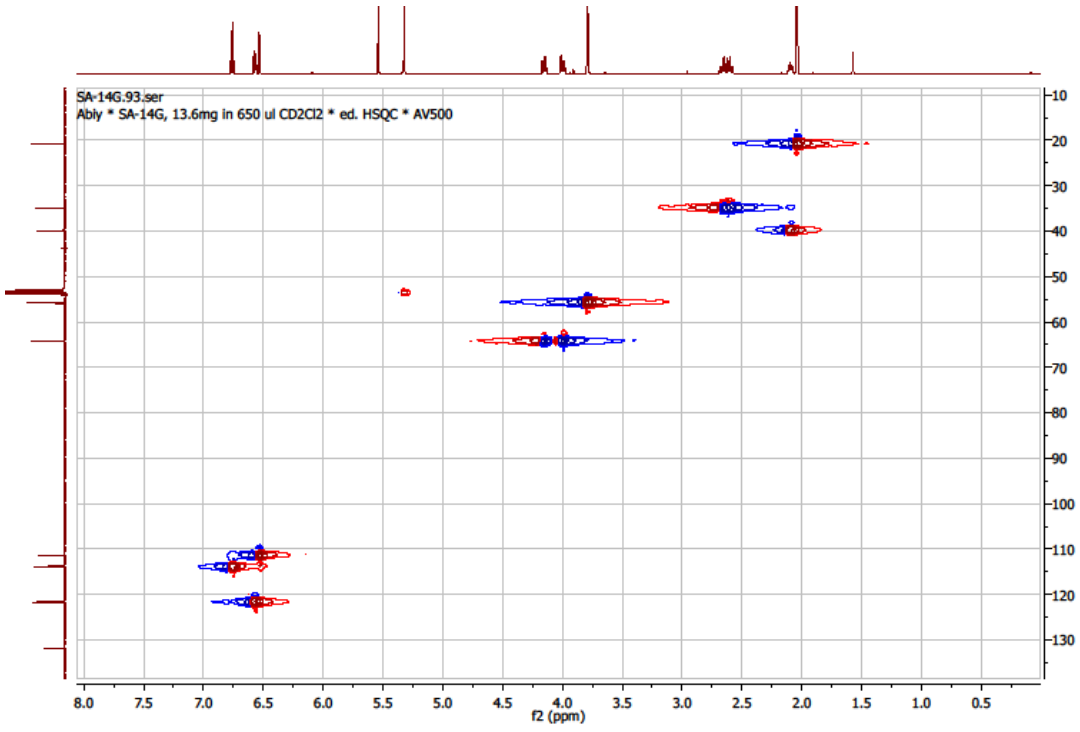
HMBC spectrum for compound **(10)**



HMBC spectrum for compound **(10)**



HSQC for compound **(10)**



NOESY for compound **(10)**