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PHYTOCHEMICAL ANALYSIS OF *Murraya koenigii* (RUTACEAE) AND *IN VITRO* CYTOTOXIC ACTIVITY OF ITS ISOLATED COMPOUNDS AND THE SYNTHETIC ANALOGUES OF GIRINIMBINE AND MAHANIMBINE

TAN SIOW PING

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THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

FACULTY OF SCIENCE AND MATHEMATICS
UNIVERSITI PENDIDIKAN SULTAN IDRIS

2015

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ABSTRACT

The objectives of the study are to determine the phytochemical constituents from the bark and leaves of *Murraya koenigii*, to develop synthetic analogues from girinimbine and mahanimbine via Lewis acid-mediated reactions, and to evaluate cytotoxic activities. The isolation and purification of the chemical compounds were done by using various chromatography methodologies. These compounds were then structurally identified by various spectroscopic techniques such as NMR, IR, UV and mass spectrometry. *In vitro* cytotoxic activities were evaluated against human promyelocytic leukemia (HL-60), cervical cancer cell lines (HeLa) and the normal mouse embryonic fibroblasts (NIH/3T3) cell lines via MTT assay. As the findings, phytochemical analysis on bark and leaves of *M. koenigii* afforded a total of 36 chemical compounds included six new carbazoles, *viz.*, murrastanine-A, murrastinine-A, -B and -C, murrayatanine-A and bismahanimboline. Besides, two girinimbine analogues, *viz.*, murranimbine and epoxygirinimbine; and three mahanimbine analogues, *viz.*, bicyclomahanimbine, cyclomahanimbine and murrayazolinine, were successfully derived. Five carbazoles and two non-carbazoles have shown very strong to moderate *in vitro* cytotoxic activities (CD_{50} < 20 μg/mL) against both HL-60 and HeLa cell lines, *viz.*, murrayafoline-A, mahanine, murrayamine-J, murrastinine-C, murrayatanine-A, β-sitosterol and 2-hydroxy-4-methoxy-3,6-dimethylbenzoic acid. In conclusion, the isolated compounds from *M. koenigii* were found to possess potential *in vitro* cytotoxic activities against selected cancer cell lines. In fact, most of these results were first time reported. These findings have shown that *M. koenigii* is an important source for therapeutic discovery and may lead to the development of potential drugs.

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ANALISIS FITOKIMIA *Murraya koenigii* (RUTACEAE) DAN AKTIVITI SITOTOKSIK *IN VITRO* BAGI SEBATIAN KIMIA YANG DIPENCILKAN DAN ANALOG SINTETIK GIRINIMBINA DAN MAHANIMBINA

ABSTRAK

Kajian ini bertujuan menentukan kandungan fitokimia daripada kulit kayu dan daun *Murraya koenigii*, membina analog sintetik daripada girinimbina dan mahanimbina menggunakan asid Lewis sebagai pengantara, dan menguji aktiviti sitotoksik. Pemencilan dan penulenan sebatian kimia dijalankan dengan menggunakan pelbagai kaedah kromatografi. Struktur sebatian kemudiannya dikenalpasti melalui teknik spektroskopi iaitu NMR, IR, UV dan spektrometri jisim. Aktiviti sitotoksik *in vitro* diuji ke atas sel promielositik leukemia manusia (HL-60), sel kanser servik (HeLa) dan sel normal tikus fibroblas embrio (NIH/3T3) dengan rawatan MTT. Dapatan kajian menunjukan sebanyak 36 sebatian kimia termasuk enam karbazola baharu, iaitu murrastanina-A, murrastinina-A, -B dan -C, murrayatanina-A dan bismahanimbolina telah diperolehi. Selain itu, dua analog girinimbina, iaitu murranimbina dan epoksigirinimbina dan tiga analog mahanimbina, iaitu bisiklomahanimbina, siklomahanimbina dan murrayazolinina, telah berjaya disintesiskan. Lima karbazola dan dua sebatian bukan karbazola menunjukkan aktiviti sitotoksik yang sangat aktif ke sederhana aktif (CD_{50} < 20 μg/mL) terhadap kedua-dua sel HL-60 dan sel HeLa, iaitu murrayafolina-A, mahanina, murrayamina-J, murrastinina-C, murrayatanina-A, β-sitosterol dan asid 2-hidroksi-4-metoksi-3,6-dimetilbenzoik. Kesimpulannya, sebatian yang dipencilkan daripada *M. koenigii* didapati mempunyai potensi aktiviti sitotoksik *in vitro* ke atas sel kanser yang dipilih. Malah, sebahagian besar daripada keputusan ini dilaporkan pertama kali. Hasil kajian menunjukkan *M. koenigii* merupakan sumber penting bagi penemuan terapeutik dan membawa kepada pembangunan ubat-ubatan yang berpotensi.

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ABBREVIATIONS

α	Alpha
β	Beta
λ	Maximum Wave Length
δ	Chemical Shift
μg	Microgram
mg	Miligram
g	Gram
kg	Kilogram
M	Molar
mM	Milimolar
mL	Mililitre
μL	Microlitre
m	Meter
mm	Milimeter
μm	Micrometer
nm	Nanometer
cm^{-1}	Per centimeter
MHz	Mega Hertz
Hz	Hertz
UV	Ultraviolet
IR	Infrared
ppm	Part per Million
EA	Ethyl acetate

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Hex	*n*-Hexane
$CDCl_3$	Deuterated Chloroform
CD_3OD	Deuterated Methanol
DMSO	Dimethyl Sulfoxide
NH_4OH	Ammonia Solution
TMS	Tetramethylsilane
qC	Quaternary Carbon
CH	Methine Group
CH_2	Methylene Group
CH_3	Methyl Group
pH	Power of Hydrogen
TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
CC	Column Chromatography
NMR	Nuclear Magnetic Resonance
min	Minute
°C	Degree Celsuis
$[\alpha]_D^{25}$	Optical rotation at 25 °C using D-line sodium lamp
J	Coupling Constant
d	Doublet
dd	Doublet of doublets
t	Triplet
td	Triplet of doublets
s	Singlet
m	Multiplet

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1D NMR	One Dimension Nuclear Magnetic Resonance
2D NMR	Two Dimension Nuclear Magnetic Resonance
^{1}H	Proton NMR
^{13}C	13-Carbon NMR
^{1}H-^{1}H COSY	Correlation Spectroscopy
DEPT	Distortioness Enhancement by Polarization Transfer
HMQC	Heteronuclear Multiple Quantum Correlation
HMBC	Heteronuclear Multiple Bond Correlation
TOCSY	Total Correlated Spectroscopy
NOE	Nuclear Overhauser Effect
NOESY	Nuclear Overhauser Effect Spectroscopy
LC-MS	Liquid Chromatography-Mass Spectrometry
HRESIMS	High Resolution Electrospray Ionisation MS
MS	Mass Spectrometry
m/z	Mass per Charge
calcd. mass	Calculated Exact Mass
CGM	Complete Growth Medium
MTT	Microculture Tetrazolium Assay
v/v	Volume Concentration
CD_{50}	50% of Cytotoxic Dose
ED_{50}	50% of Effective Dose
HL-60	Human Promyelocytic Leukemia Cell
HeLa	Cervical Carcinoma Cancer Cell
NIH/3T3	Normal Mouse Embryonic Fibroblasts
MIC	Minimum Inhibitory Concentration

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CHAPTER 1

INTRODUCTION

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1.1 General Introduction

Plant-based drugs have been used and well documented for their medicinal uses since the dawn of medicine. The earliest written record on medicinal plants in the treatment of cough, cold and inflammation is well documented in the oldest medical text of ancient Mesopotamia in 2600 B.C. such as *Cupressus sempervirens* (cypress) and *Commiphora* species (myrrh). The Ebers Papyrus (1550 B.C.) is an Egyptian pharmaceutical record which documented over 700 plant-based drugs ranging from gargles, pills, infusions, to ointments. The Chinese Materia Medica (1100 B.C.) (Wu Shi Er Bing Fang, contains 52 prescriptions), the Shennong Herbal (~100 B.C., 365 drugs) and the Tang Herbal (659 A.D., 850 drugs) are documented records of the uses of natural products. The Greek physician, Dioscorides, (100 A.D.), recorded the

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collection, storage and the uses of medicinal herbs, while the Greek philosopher and natural scientist, Theophrastus (~300 B.C.), dealt with medicinal herbs (Cragg & Newman, 2005; Dias, Urban, & Roessner, 2012). According to Musselman (1999), in the Bible and the Quran, about 125 and 20 plants were mentioned, respectively, and being used as medicinal agents to treat various ailments.

Indeed, natural products derived from plants is an important source of potential drugs and continues to bring significant effects in today drugs discovery process. For examples, morphine **1** and codeine **2** from *Papaver somniferum*, digitoxin **3** from *Digitalis lanata*, quinine **4** and quinidine **5** from *Cinchona sp*., vincristine **6** and vinblastine **7** from *Catharanthus roseus* serve to explain the continuing value of plant-derived secondary metabolites as important entities for modern drug discovery and development (Croteau, Kutchen, & Lewis, 2000; Newman, Cragg, & Snader, 2000; Rates, 2001). Most importantly, natural products will continue to be an important source of medicinal agents. Therefore, natural products chemists have vital role in the early phase of drug discovery.

Malaysia, one of the world's biodiversity rich country, provides opportunities for the discovery of bioactive compounds with interesting biological activities. A Malaysian Rutaceae species, *Murraya koenigii* (Linn.) Spreng, which grows commonly in the gardens in Malaysia, was chosen for the investigation.

M. koenigii is known to be a rich source of carbazole alkaloids based on it chemotaxanomic classification. Although various kinds of monomeric and dimeric carbazole alkaloids have been isolated and investigated since 1965 (Chakraborty,

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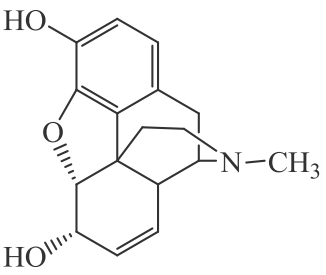
Barman, & Bose, 1965), but more than 95% of the studies on *M. koenigii* were only focused on Indian species. Most of these studies were depicted the fact that the crude organic extracts of leaves, fruits, roots, and bark of this plant are a rich source of biologically active carbazole alkaloids with promising biological properties such as cholesterol reducing property, antidiarrheal activity, cytotoxic activity, antioxidant property, antiulcer activity, antimicrobial, antibacterial potential (Bonde, Nemade, Patel, & Patel, 2011; Ramsewak, Nair, Strasburg, DeWitt, & Nitiss, 1999).

In the present study, Malaysian *Murraya koenigii* was chosen for investigation with emphasis on the discovery and structure characterization of new chemical compounds, the type of carbazole skeletons such as biscarbazole which is hardly isolated from Indian species, and the cytotoxic screening against selected cell lines in order to investigate the structure-activity relationship (SAR) among the isolated carbazole alkaloids with the aim to discover potential anticancer agents.

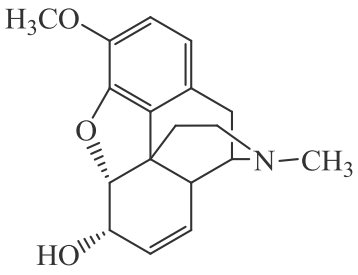
Carbazole alkaloids continue to provide strong interest because of their diverse structural varieties and promising biological activities. Further approach on the construction of dimeric and cyclic monoterpenoid pyrano[3,2-a]carbazole alkaloids via Lewis acid-mediated reaction and screening of their cytotoxic properties were also included in order to gain better understanding in the development of complex framework of carbazole alkaloids and their potential in anticancer activity.

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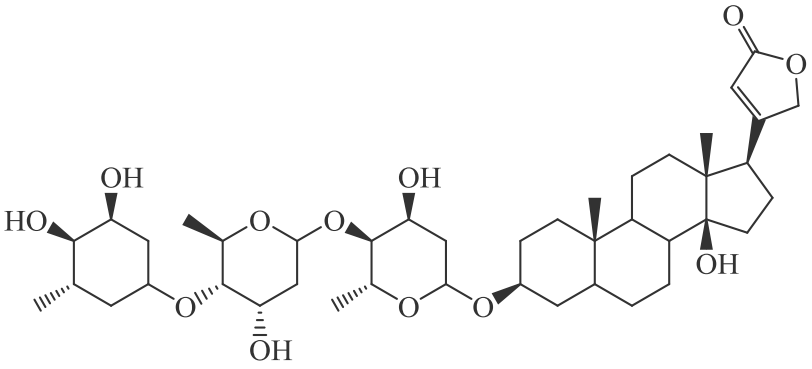
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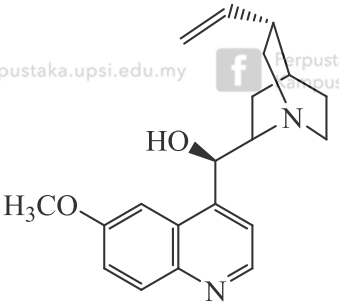
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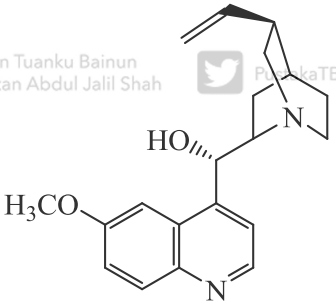
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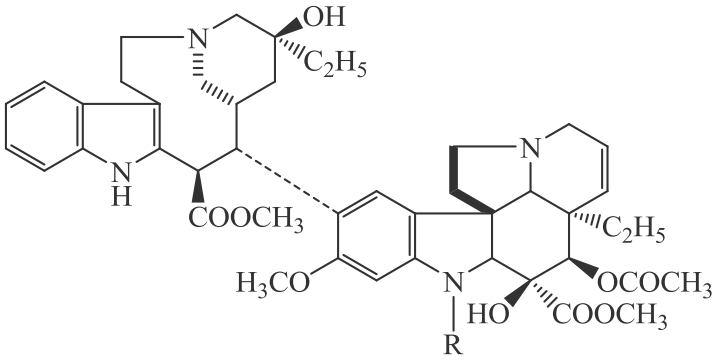
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4



5



6. R = CHO
7. R = CH_3

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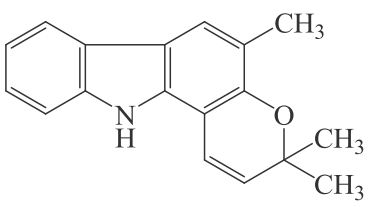
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1.2 Objectives of Study

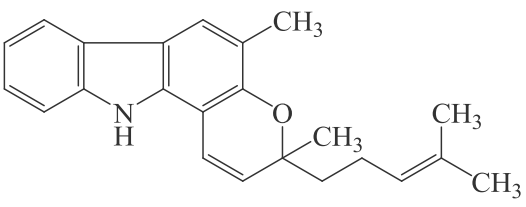
For the above reasons, the phytochemical study of *Murraya koenigii*, a Malaysian species from the Rutaceae family, has started. The main objective of the study was to isolate the secondary metabolites with potential cytotoxic properties from the selected plant species. *In vitro* cytotoxic activities were evaluated against selected cancer cell lines such as the human promyelocytic leukemia cell lines (HL-60) and cervical cancer cell lines (HeLa), as well as the normal mouse embryonic fibroblasts (NIH/3T3) cell lines via MTT assay. Their structure-activity relationship (SAR) analysis were also performed to investigate the relationship between the isolated chemical compounds and their cytotoxic properties.

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Further, development of synthetic analogues from the major compounds isolated from *M. koenigii*, namely girinimbine **8** and mahanimbine **9**, were also included. Anhydrous ferric (III) chloride ($FeCl_3$) and boron triflouride diethyletherate (BF_3-$O(C_2H_5)_2$) which promote oxidative coupling, rearrangement and cyclization reaction were used on the approach with the aim to gain better understanding on the development of complex carbazole framework with potential bioactivity properties. Attention will be paid on the structural characterization and the development of novel compounds of their natural and synthetic analogues. Structurally modified analogues were also examined for their cytotoxicity via MTT assay.



8



9

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1.3 Significance of Study

Natural products have played an integral and ongoing role in drug discovery, especially in the anticancer therapeutic areas. In view of the growing need for effective bioactive agents, discovery of natural products from plant source to serve as safe and effective bioactive agents has become significantly important.

In connection to this, Malaysian Rutaceae species, *Murraya koenigii* (Linn.) Spreng, was chosen for the investigation in order to investigate the differences between the Indian and Malaysian *M. koenigii*, the study in phytochemistry of *M. koenigii* with effective integration of information on chemical composition of its crude extracts would able to serve as part of the chemical characterization and documentation for future reference.

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In fact, there has been attracted strong interest of chemists and biologists due to the intriguing structural features and promising biological activities of carbazole alkaloids. The continuous interest in the study in cytotoxic screening on isolated carbazole alkaloids, that might possess certain health risks in human, would serve as part of the cytotoxic profile that vital importance during the early research phase of developing new pharmaceutical products to ensure the safety of the end-users.

Due to the limited information on the development or synthesis of complex molecule of carbazole alkaloids and their biological properties such as dimeric carbazole and cyclic monoterpenoid pyrano[3,2-a]carbazole alkaloids, the knowledge obtained from the development of synthetic analogues of carbazole alkaloids and its

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cytotoxic screening have served to provide an important source for therapeutic discovery and may lead to the development of potential drugs.

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CHAPTER 2

LITERATURE REVIEW

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2.1 The Plant - *Murraya koenigii* (Linn.) Spreng

Among the 14 species under the genus *Murraya*, *Murraya koenigii* (Linn.) Spreng and *Murraya paniculata* (Linn.) Jack, belonging to the Rutaceae family (also known as the citrus family), are commonly found in Malaysia (Kok et al., 2012; Sukari, Ahmad, Haron, & Muse, 2001).

Rutaceae, a large family of mostly trees, shrubs and woody vines, contains more than 160 genera and 1800 species of mostly tropical and subtropical distribution (Wei, Wang, & Li, 2012). The family is known for its economic importance: Edible fruits (e.g. *Citrus*), timber (e.g. *Flidersia sp.*) and aromatic oils (e.g. *Boronia sp.*) (Scott, McIntyre, & Playford, 2000).

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M. koenigii, an aromatic plant locally known as curry leaf tree, originates from Pakistan, Sri Lanka and India east to China. It has been widely distributed throughout Southeast Asia. In tropical Africa, it is cultivated in many countries, including Nigeria, Kenya, Tanzania and most of the Indian Ocean Islands, where Indian immigrants settled (Parul, Javed, Neha, Honey, & Anuj, 2012). It is widely cultivated for its aromatic leaves. Its leaves are used as traditional vegetable (*ulam* - salad), which help to promote appetite and digestion, and provide natural flavouring in southern Asian cuisine especially in Indian cuisine.

2.1.1 Morphological Characteristics

Murraya koenigii is a small spreading shrub, about 2.5 metres high; the main stem, dark green to brownish, with numerous dots on it; its bark can be peeled off longitudinally, exposing the white wood underneath; the girth of the main stem is 16 cm (Ajay et al., 2011) (Figure 2.1). Its roots are brown in colour and with lots of fine fibrous roots (Figure 2.2).

M. koenigii is an important leafy vegetable. Its leaves are widely used in Indian cookery for flavouring foodstuffs. Its leaves are exstipulate, bipinnately compound, 30 cm long, each bearing 24 leaflets, having reticulate venation; leaflets, lanceolate, 4.9 cm long, 1.8 cm broad, having 0.5-cm-long petiole (Ajay et al., 2011). The leaves have a slightly pungent, bitter and feebly acidic taste, and they retain their flavour and other qualities even after drying (Figure 2.3) (Joseph & Peter, 1985).

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Flowers of *M. koenigii* are bisexual, white, funnel-shaped, sweetly scented, stalked, complete, ebracteate, regular, actinomorphic, pentamerous, hypogynous, the average diameter of a fully opened flower being 1.12 cm; inflorescence, a terminal cyme, each bearing 60 to 90 flowers; calyx, 5-lobed, persistent, inferior, green; corolla, white, polypetalous, inferior, with five petals, lanceolate; length, 5 mm; androecium, polyandrous, inferior, with 10 stamens, dorsifixed, arranged into circles of five each; smaller stamens, 4 mm long whereas the longer ones reaches 5 to 6 mm; gynoecium, 5 to 6 mm long; stigma, bright, sticky; style, short; ovary, superior (Ajay et al., 2011) (Figure 2.3).

Its fruits are round to oblong, 1.4 to 1.6 cm long, 1 to 1.2 cm in diameter; weight, 880 mg; volume, 895 microlitres; fully ripe fruits, black with a very shining surface; pulp, Wistaria blue 640/2; the number of fruits per cluster varying from 32 to 80 (Figure 2.4) (Parmar & Kaushal, 1982).

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Figure 2.1. Bark of *M. koenigii*

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Figure 2.2. Roots of *M. koenigii*

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Figure 2.3. Leaves and Flowers of *M. koenigii*

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Figure 2.4. Fruits of *M. koenigii*

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2.1.2 Ethnobotanical Uses

Ethnobotanic medicine may be defined broadly as the use of plants by human as medicines. According to the World Health Organization (WHO), 80% of people still remain rely on plant-based traditional medicines for primary health care (Farnsworth, Akerele, Bingel, Soejarto, & Guo, 1985) and 80% of 122 plant derived drugs are related to their original ethnopharmacological purposes (Fabricant & Farnsworth, 2001).

Murraya koenigii, a medicinally important herb of Indian origin, has been mentioned in the Ayurveda and Siddha System of medicine (Gupta, Nahata, & Dixit, 2011; Nakamura et al., 2013). Traditionally, this plant was used as a tonic, stimulant, stomachic, febrifuge, analgesic (Birari, Javia, & Bhutani, 2010). It is also used in the treatment of diarrhoea, dysentery, insect bites, chronic fever, dropsy, nausea (Arulselvan & Subramanian, 2007; Murugan, Anandaraj, & Al-Sohaibani, 2013; Muthumani, Venkatraman, Ramseshu, Meera, & Devi, 2009; Nakamura et al., 2013; Rahman & Gray, 2005; Sathaye, Bagul, Gupta, Kaur, & Redkar, 2011; Sim & Teh, 2011), rheumatism and traumatic injury (Kong et al., 1986).

The bark and roots of the plant are used to relieve skin eruptions and bites by poisonous animals (Adebajo et al., 2004; Dastur, 1970; Kok et al., 2012) and also used as a stimulant by the physicians (Parul et al., 2012). The branches of the plants are used to strengthen gums, popularly used to clean teeth as datum (Prasan, 2013), while the roots are used externally to cure piles, inflammation and itching (Vinuthan, Girish-Kumar, Ravindra, Jayaprakash, & Narayana, 2004). Traditionally, the bark and leaves

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of the plant are used as folk medicine for analgesia and local anesthesia and for the treatment of eczema, rheumatism and dropsy (Wang, He, Shen, Hong, & Hao, 2003; Wu, Wang, Wu, Ito, & Furukawa, 1996).

Leaves of *M. koenigii* are widely used as important ingredient in the Indian cuisine to improve appetite and digestion. Leaves of the plant are used as flavouring agent in Indian dishes since ancient time (Pande & Pathak, 2009). According to Birari et al. (2010), the leaves are considered as a tonic, anthelmintic, analgesic, digestive and appetizer. In the folk, the green leaves are used to treat piles, inflammation, itching, fresh cuts, dysentery, vomiting, burse and dropsy (Abu Bakar et al., 2007; Birari et al., 2010; Ma et al., 2013). The leaves are also used for the treatment of headache, stomach ache, influenza, rheumatism, traumatic injury, insect bites, antivomiting, externally cure eruptions (Arulselvan & Subramanian, 2007; Pande & Pathak, 2009; Shah, Wakade, & Juvekar, 2008), diarrheal, dysentery, digestive disorders (Kok et al., 2012), hypertension, hepatitis, cough, hysteria inflammation (Birari et al., 2010) and constipation (Sathaye et al., 2011). Curry leaves are also used in calcium deficiency, it has Vitamin A, B, C, B2, calcium and iron in plenty. Its nutritional value benefits equally to both the young and the old. Women who suffer from calcium deficiency and osteoporosis can find an ideal natural calcium supplement in curry leaves (Parul et al., 2012).

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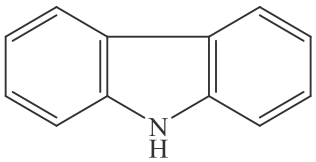
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2.2 Chemical Aspects

An intense search of the literature has revealed that the bark, leaves, fruits and roots of *Murraya koenigii* are rich sources of important natural products such as carbazole alkaloids and terpenes. The present section has included the classification of carbazole alkaloids and terpenes which could be isolated from *M. koenigii*.

2.2.1 Carbazole Alkaloids

Carbazoles **10** were initially isolated from anthraceae fraction of coal tar by Graebe & Glaser in 1872. The first simple carbazole **10** from plant sources was not discovered until the 1960s (Chakraborty & Roy, 1991; Cordell, 2008; Moody, 1994). This group of alkaloids has gradually become a huge class of alkaloids, and has proven to contain promising biological activities such as antifungal, antimicrobial, anti-tumour, anti-inflammatory and hypertensive effects (Kirsch, 2001). Besides, their intriguing structural features have also prompted strong interest and attracted the attention of chemists and biologists. Since then, these heteroaryl-condensed carbazoles have expanded enormously large due to their interesting biological properties (Kirsch, 2001).



10

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2.2.1.1 Occurrence and Structure Diversity

Over the past decades, the carbazole alkaloids with interesting structural features and wide spectrum of pharmacological properties have been isolated from diverse natural sources. They are a series of natural products which have been isolated from higher plants of the taxonomically related genera *Murraya*, *Glycosmis* and *Clausena* from the Rutaceae family (Krahl, Kataeva, Schmidt, & Knölker, 2013). The occurrence of carbazole alkaloids in these Rutaceae genera is known to be important to plant classification and chemotaxonomic.

The genus *Murraya* represents the richest source of carbazole alkaloids among all terrestrial plants. The first carbazole from biological source, the alkaloid murrayanine **11**, was isolated from the plant *Murraya koenigii* (Linn.) Spreng by Chakraborty and co-woker in 1962. The structure and antibiotic properties of murrayanine **11** were then reported in 1965 (Chakraborty et al., 1965). The natural sources of carbazole alkaloids can also be obtained from other sources such as microorganism, blue-green algae, coal tar, petroleum oil, soil humus, as well as the cigarette smoke (Cordell, 2008).

The *Murraya* trees that grow in southern Asia also able to provide great source of carbazole alkaloids (Furukawa, Wu, Ohta, & Kuoh, 1985; Wang et al., 2003). Although carbazole alkaloids are distributed throughout all parts of the plant, the occurrences of these compounds are influenced by the geographical variation, environmental conditions and seasonal changes. Various monomeric and biscarbazole alkaloids, which formed by the combination of two of the monomeric units, have been

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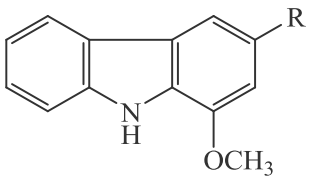
reported from the genus *Murraya* grown in Taiwan and Japan. According to Cordell (2008), Indian *Murraya* species hardly afford any biscarbazole. However, the first discovery of biscarbazole from Indian *M. koenigii* was reported from the fruits by Uvarani et al. (2014).

These alkaloids contain the basis carbazole nucleus derived from an indole nucleus and isoprene units in which one of the isoprene (dimethylallyl substituent) is annulated to the indole to form a benzene ring (Knölker, 2005) through biogenetic pathway. Based on their natural occurrences, oxygenation reaction at different positions of the carbazole framework, oxidation reaction of the methyl group, prenylation or geranylation, and subsequent annulation of additional rings at further downstream on the biogenetic pathway have been proven to provide diverse structural varieties of the carbazole alkaloids. To date, the carbazole alkaloids can be classified following two classification systems.

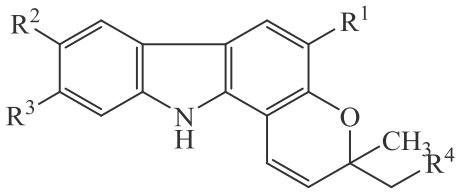
According to Chakraborty and Roy (2003), the carbazoles have classified into three basic groups according the number of isoprene unit attached to an indole. The C_{13} group contains an isoprene units attached to an indole nucleus forming the parent carbazole ring system such as koenoline **12** and murrayafoline-A **13**. The C_{18} group has an additional isoprene unit attached to the C-1 or C-4 of the carbazole nucleus such as girinimbine **8** and murrayacine **14**. The C_{23} group contains two isoprene units on the carbazole nucleus such as mahanimbine **9** and mahanine **15**. The biscarbazole, also known as dimeric carbazole alkaloids are formed when two monomeric carbazole alkaloids attached to each other by a biaryl bond such as bismurrayafoline-E **16**.

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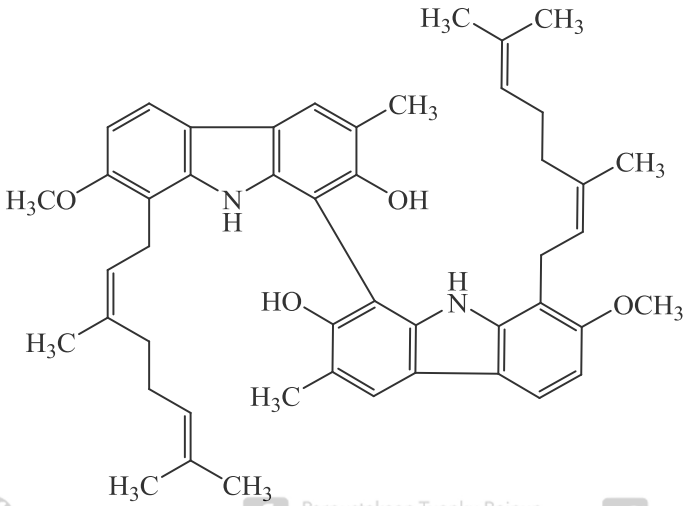
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11. Murrayanine, R = CHO
12. Koenoline, R = CH_2OH
13. Murrayafoline-A, R = CH_3



14. Murrayacine, R^1 = CHO, R^2 = H, R^3 = H, R^4 = H
15. Mahanine, R^1 = CH_3, R^2 = H, R^3 = OH, R^4 = prenyl



16. Bismurrayafoline-E

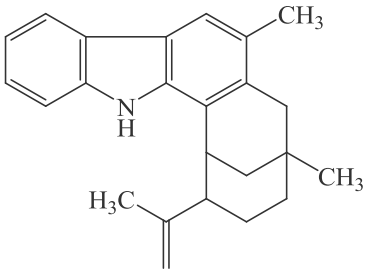
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Cordell (2008) and Nayak, Mandal, Banerji, and Banerji (2010) have classified carbazole alkaloids into four basic groups according to the number of cyclic present in the carbazole nucleus. The tricyclic carbazole-type of alkaloids consist only the parent carbazole ring system such as murrayanine **11** and koenoline **12**. The tetracyclic alkaloids, also known as pyrano[3,2-a]carbazole alkaloids, possessing the pyranocarbazole moiety where the carbazole nucleus has a fused pyran ring such as girinimbine **8** and mahanimbine **9**. The pentacyclic alkaloids, or cyclic monoterpenoid pyrano[3,2-a]carbazole-type of alkaloids, possessing the pyranocarbazole moiety with a five cyclic rings such as cyclomahanimbine **17** and murrayazolinine **18**. Hexacyclic alkaloids, the *N*-substituted hexacyclic pyranocarbazole type of alkaloids, also under the categories of cyclic monoterpenoid pyrano[3,2a]carbazoles such as

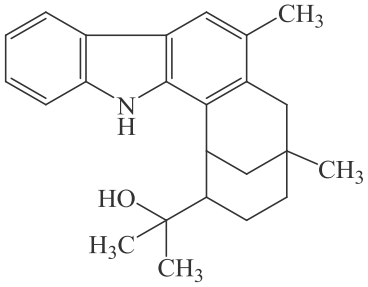
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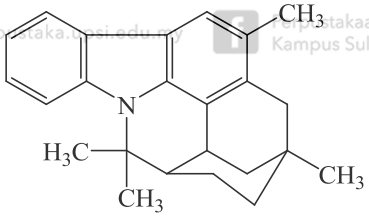
mahanimbidine **19** and murrayazolinol **20**. Biscarbazoles or binary carbazole alkaloids are normally the symmetrical dimers of the carbazole alkaloids such as bismurrayafoline-E **16**. Recently, another two types of carbazole alkaloids have been reported by Nayak et al. (2010), they are quinone type (e.g. koenigine-quinone A **21**) and *N*-substituted carbazole alkaloids (e.g. 9-carbethoxy-3-methylcarbazole **22**).



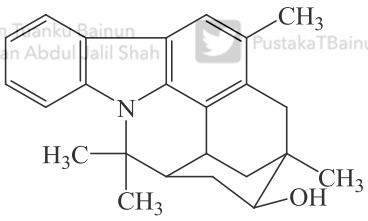
17. Cyclomahanimbine / Murrayazolidine / Curryanine



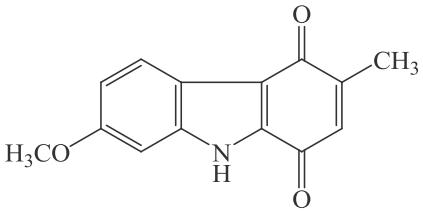
18. Murrayazolinine



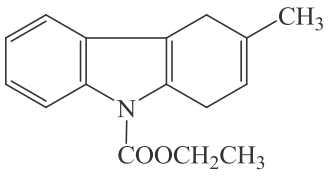
19. Murrayazoline / Mahanimbidine / Curryangin



20. Murrayazolinol



21. Koeniginequinone-A



22. 9-Carbethoxy-3-methylcarbazole

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2.2.1.2 Biosynthesis

The most important biogenetic features of all carbazole alkaloids isolated from the higher plants is the origin of the indole nucleus and the present of ring A, along with several interesting features, including an extra methyl group (or its equivalent) at the position 3 of the carbazole nucleus. However, the pathway formation of carbazole nucleus is still far from understood.

According to Cordell (2008), naturally occurring carbazole alkaloids are generally thought to be derived from 3-prenylquinolone **23** via a pathway involving shikimic acid **24** and mevalonic acid **25** (Scheme 2.1), anthranilic acid **26** and prephenic acid **27** via a pathway involving shikimic acid **24** (Scheme 2.2), and also tryptophan **28** involving the mevalonate pathway (Scheme 2.3). However, the pathway described in Scheme 2.1 is the most widely accepted among these biogenetic pathways to carbazole alkaloids in higher plants.

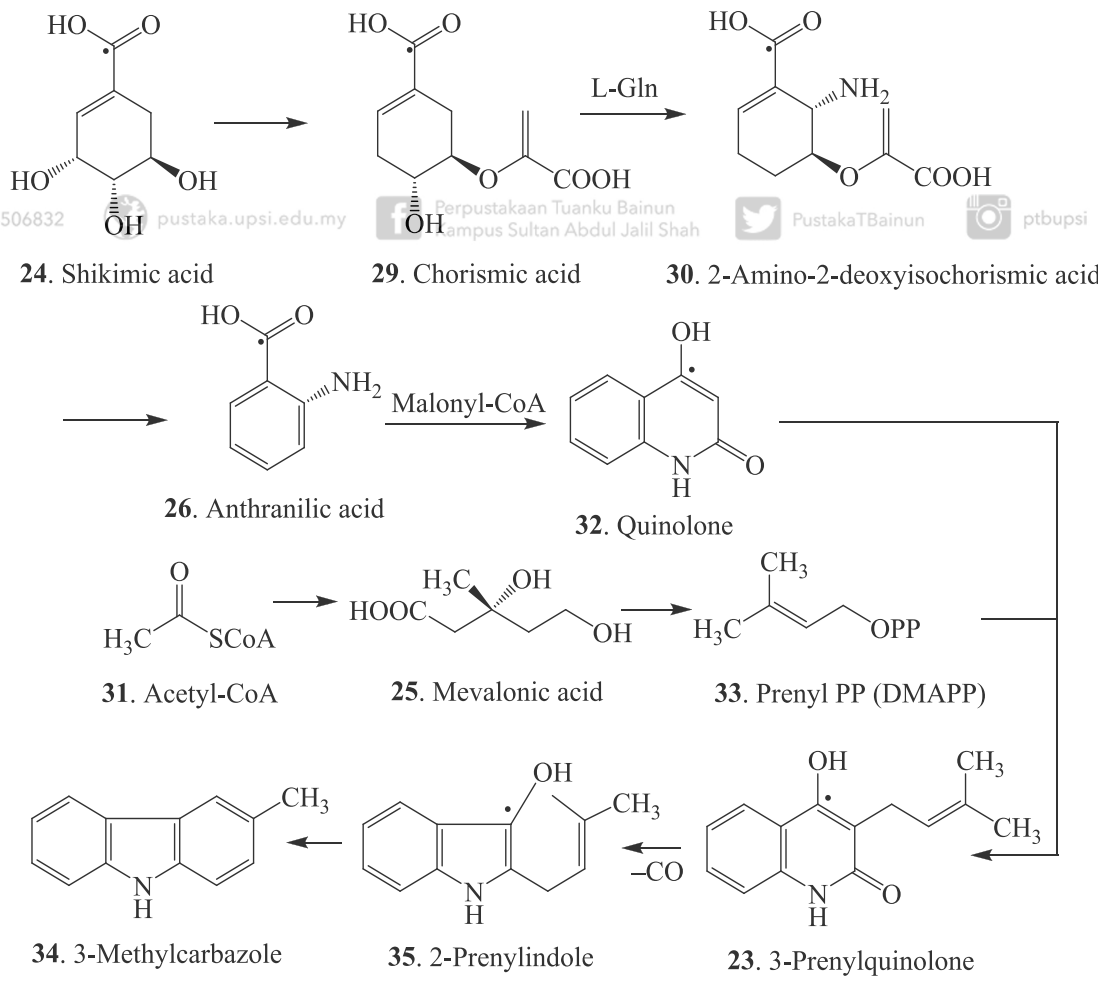
In the shikimate pathway (Scheme 2.1), shikimic acid **24** is derived from 3-dehydroquinic acid via 3-dehydroshikimic acid through a series of dehydration and reduction steps. While chorismic acid **29** is then derived from shikimic acid **24** via several addition – elimination reactions. Amination of chorismic acid **29** at C-2 from L-glutamine (L-Gln) with the elimination of water leads to 2-amino-2-deoxyisochorismic acid **30**.

Anthraniloyl-coenzyme A (CoA), obtained from anthranilic acid **26**, a very important branch point compound, and acetyl-coenzyme A (CoA) **31**, undergoes a

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chain extension to afford quinolone **32** via the addition of one molecule of malonyl-CoA through amide formation. Alkylation of quinolone **32** at the nucleophilic C-3 position with prenyl diphosphate (DMAPP) **33** affords 3-prenylquinolone **23**. The formation of 3-methylcarbazole **34** is then derived from 3-prenylquinolone **23** through 2-prenylindole **35** via elimination of carbon monoxide (CO) from 3-prenylquinolone **23** followed by oxidative cyclization of the 2-prenylindole **35**. From the findings, 3-methylcarbazole **34** is proved to be the key precursor for the carbazoles isolated from higher plants (Scheme 2.1) (Cordell, 2008).



Scheme 2.1. Shikimate Pathway

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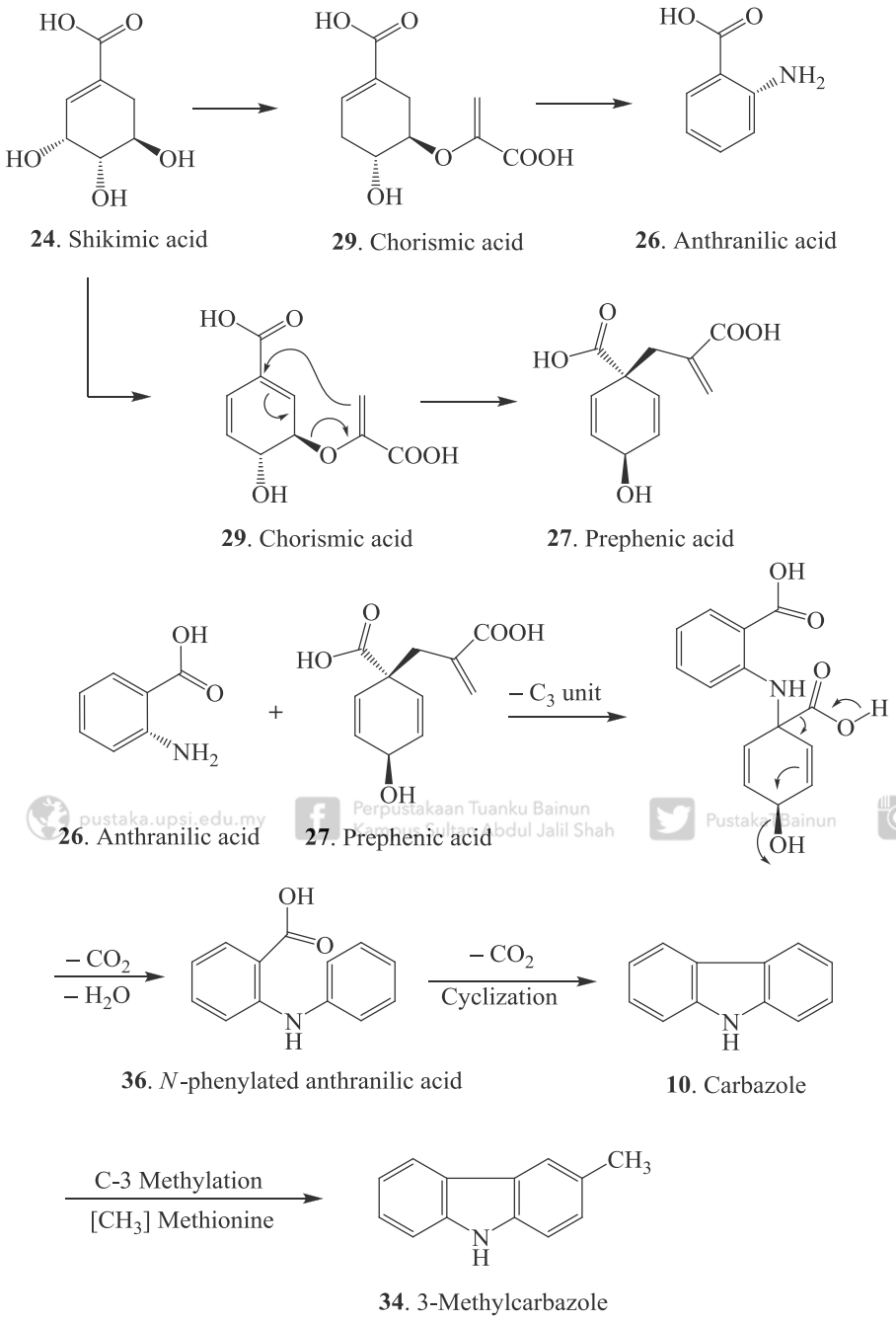
The biogenetic pathway in Scheme 2.2 suggested by Chakraborty (1969) for the formation of carbazole **10** and 3-methylcarbazole **34** through *N*-phenylated anthranilic acid **36**. Based on this biogenetic pathway, *N*-phenylated anthranilic acid **36** is derived from anthranilic acid **26** and prephenic acid **27** with the elimination of a C-3 unit, followed by CO_2 and H_2O. *N*-phenylated anthranilic acid **36** was then be transformed to carbazole **10** with the elimination of CO_2. The methyl group (or its equivalent) at the C-3 position, which could originate from C-methylation with methionine on carbazole **10**. This could also happen before the formation of the carbazole nucleus.

According to Narasimhan's approach (Scheme 2.3), an initial introduction of the isopentenyl chain at the 3-position of L-tryptophan **28**, followed by a favourable migration of the isopentenyl chain **37** to the 2-position, and subsequent cyclization to give the tetrahydrocarbazole derivative **38**. Loss of serine from tetrahydrocarbazole derivative **38** in the presence of pyridoxal co-enzyme, followed by dehydrogenation, would lead to 3-methylcarbazole **34** (Narasimhan, Paradkar, Chitguppi, & Kelkar, 1975).

Further from these, *in vivo* oxygenation of 3-methylcarbazole **34** to its oxidized functional variants such as CH_2OH, CHO, and COOH, which have been found in various alkaloids (Scheme 2.4). For examples, murrayanine **11**, the first member of the carbazole alkaloids, 2-hydroxy-3-methylcarbazole **39** and 3-formylcarbazole **40**.

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24. Shikimic acid **29**. Chorismic acid **26**. Anthranilic acid

29. Chorismic acid **27**. Prephenic acid

26. Anthranilic acid **27**. Prephenic acid

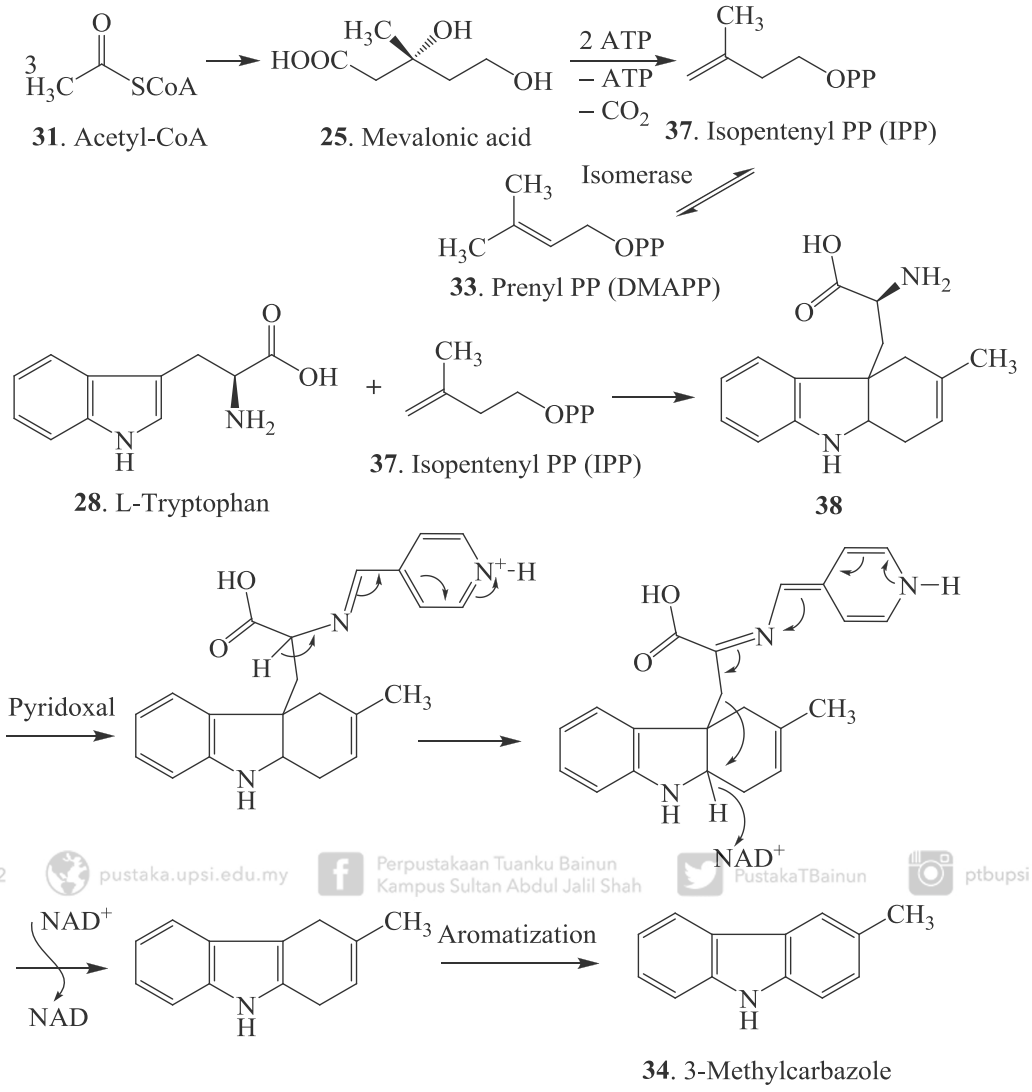
36. *N*-phenylated anthranilic acid **10**. Carbazole

34. 3-Methylcarbazole

Scheme 2.2. Biogenetic Pathway to Carbazole through *N*-phenylated Anthranilic Acid

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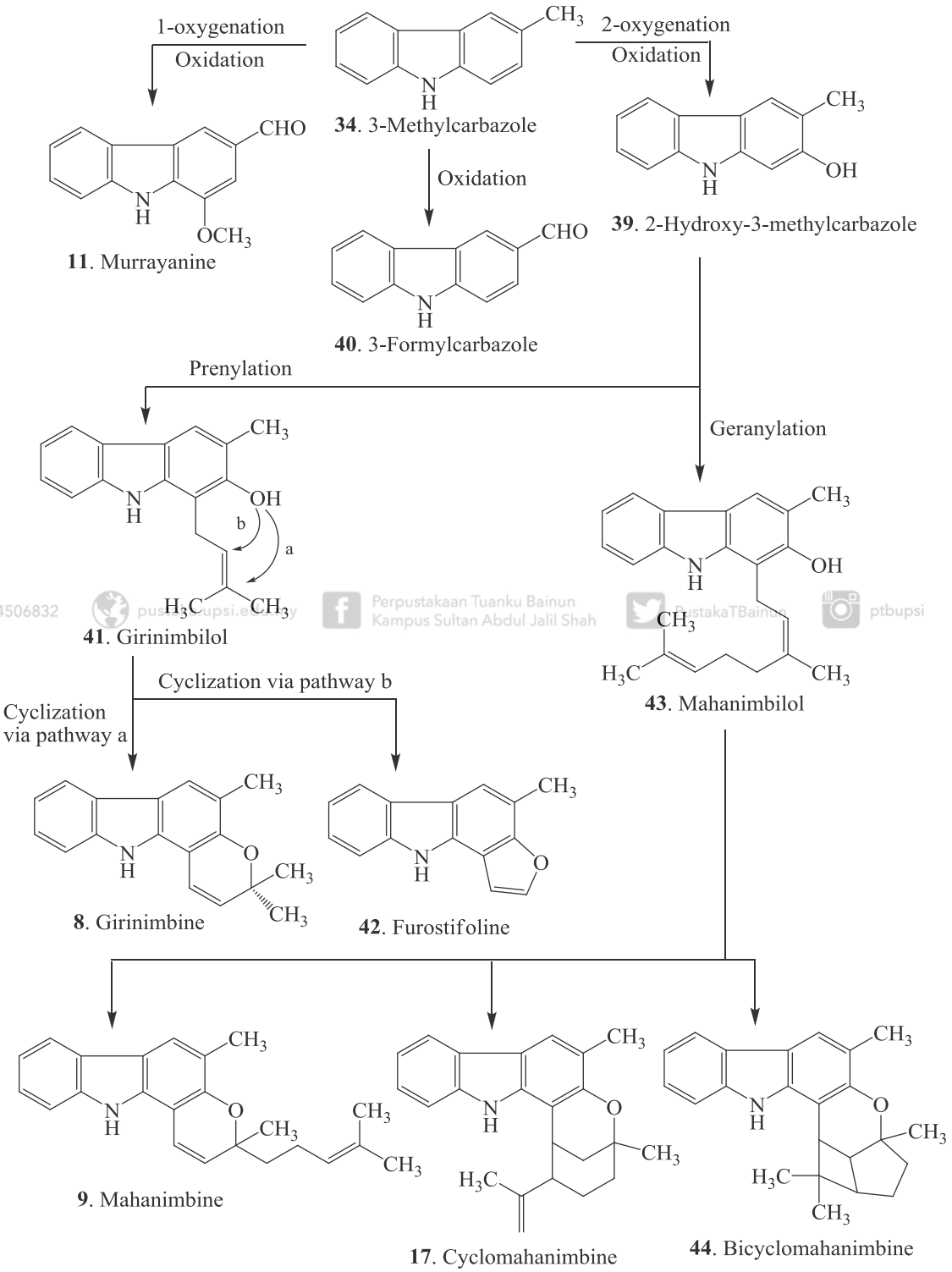
Scheme 2.3. Narasimhan's Approach Biogenetic Pathway

3-Methylcarbazole **34**, the origin of the carbazole alkaloids of higher plants, and the wider participation of 2-hydroxy-3-methylcarbazole **39** in the formation of pyranocarbazoles, as well as furocarbazoles, have been further proven by the isolation of several C_{18} derivatives of 3-methylcarbazole **34** such as girinimbilol **41**, girinimbine **8** and furostifoline **42**. Girinimbilol **41** could be considered as the precursor for furo- and pyrano[3,2-a]carbazole alkaloids when the furan and pyran

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ring, respectively, is formed via annulation of the prenylated congeners (Scheme 2.4) (Cordell, 2008).



Scheme 2.4. Biogenetic Pathway to Carbazole Alkaloid Derivatives

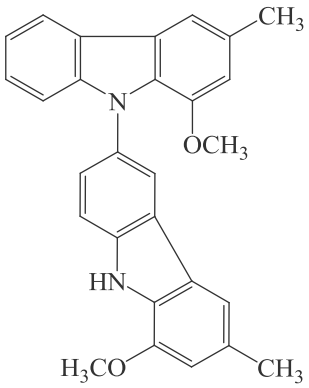
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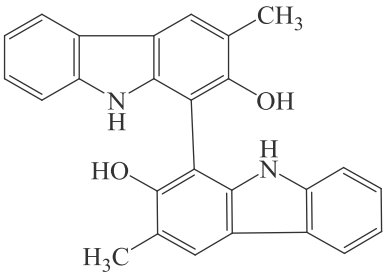
2-Hydroxy-3-methylcarbazole **39** could also act as a precursor for the formation of C_{23} carbazole alkaloids (Scheme 2.4). Insertion of a C_{10} unit, *viz.*, geraniol at C-1 of 2-hydroxy-3-methylcarbazole **39** would yield mahanimbinol (mahanimbilol) **43**. The geranyl monoterpene unit could undergo various transformations, thus giving rise to isomeric carbazole alkaloids with a C_{23} skeleton such as mahanimbine **9**, cyclomahanimbine **17** and bicyclomahanimbine **44**. Therefore, mahanimbinol (mahanimbilol) **43** is considered as the important intermediate of the carbazoles with a C_{23} skeleton (Cordell, 2008).

Further from these, oxidative dimerization or oxidative coupling would occur and yield various binary carbazole or biscarbazaole alkaloids such as murrastifoline-A **45** and bis-2-hydroxy-3-methylcarbazole **46**.

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45. Murrastifoline-A



46. Bis-2-hydroxy-3-methylcarbazole

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2.2.2 Terpenes

Terpenes are among the most widespread and chemically interesting known class of natural products produced primarily by a wide variety of plants, animals, insects and microorganisms. However, no report on the isolation and identification of terpene was found on species *Murraya koenigii* in the literature to date.

Terpenes are major biosynthetic building blocks within nearly every living organisms. These are thought to be the secondary metabolic products of plants. There are approximately 30,000 terpene structures were identified (Buckingham, 1998; Ramawat, Dass, & Mathur, 2009). Starting in 1970s, a number of terpenes were demonstrated to be toxins, repellents or attractants to other organisms, which led to the belief that they have ecological roles in antagonistic or mutualistic interactions among organisms (Gershenzon & Dudareva, 2007). Almost all terpenes have biological activities in animals including man and also play an important role in human medicine (Patocka, 2003).

2.2.2.1 Occurrence and Structure Diversity

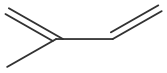
Scientists have studied terpenes for nearly two hundred years. According to Spurgeon and Proter (1981), several details about the discovery of terpenes were reported: A chemical compound with a molecular formula $C_{10}H_{16}$ was synthesized by heating the chemical compound with a formula of C_5H_8, which is also known as isoprene or 2-methylbutadiene **47**, and found that it was the same chemical compound that found in turpentine; Labillardière had published his first study of terpenes in 1818, in which he

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found that the turpentine oil, also known as "pine oleoresin", have a carbon to hydrogen ratio of 5:8; Faraday proved that the empirical formula of rubber, $(C_5H_8)_n$, in 1826.

In 1887, Otto Wallach has postulated the "isoprene rule", which states that chemical compounds can be considered as terpenes if they are having fundamental repeating five-carbon isoprene units (Croteau et al., 2000; Harry et al., 2006; Ramawat et al., 2009). This rule or C_5 rules was of great value also in the structural determination of new terpenes isolated from nature.



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Meanwhile, according to Ramawat et al. (2009), terpenes are literally defined as a unique group of hydrocarbon-based natural products that possess a structure that may be hypothetically derived from isoprene. The isoprene units usually arise from the head-to-tail condensation to form linear chain or ring compounds commonly containing a number of 10 (monoterpene), 15 (sesquiterpene) and 20 (diterpene) carbon atoms. While 30 (triterpene) and 40 (tetraterpene) carbon atoms which arise from head-to-head condensation of two C_{15} or C_{20} units. Terpenes with 25 carbon atoms are not commonly found.

Terpenes are technically only hydrocarbons, while terpenoids are modified terpenes, where methyl groups are moved or removed, or oxygen atoms are added. The oxygen-containing terpenoids are alcohol, aldehydes or ketones terpenoids. Generally, the term "terpene" refers to terpenes and terpenoids (Taiz & Zeiger, 2006).

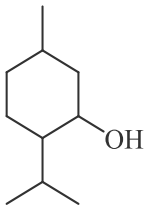
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Monoterpenes are C_{10} terpenes. Most of them are unsaturated hydrocarbons which commonly known as components of the volatile essences of flowers and the essential oils of herbs and spice (Croteau et al., 2000) such as pinene **48** and menthol **49**. They are widely used as insecticides and their pharmacological properties range from analgesic to anti-inflammatory (Taiz & Zeiger, 2006).

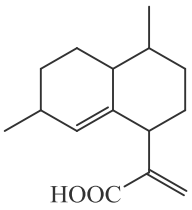


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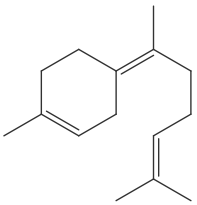


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Sesquiterpenes, C_{15} or compounds having 3-isoprene units, are found in essential oils such as arteannuic acid **50** and bisabolene **51**. They exhibit a variety of pharmacological activities such as antibacterials, antifungal, anthemistics, antimalarial and molluscidal (Taiz & Zeiger, 2006).



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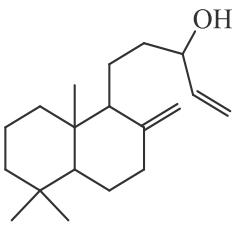


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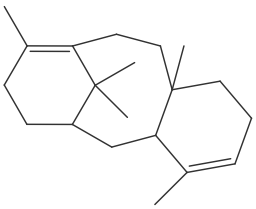
The diterpenes, C_{20} or compounds which contain 20 carbons, are present in animals and plants and some of them possess biological activities. There are about 2500 known diterpenes that belong to 20 major structural types. For examples, the famous Taxol® **3** and its derivatives are anticancer drugs (Heinig & Jennewein, 2009). Other examples are manool **52** and taxadiene **53**.

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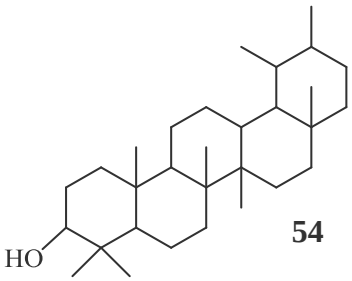
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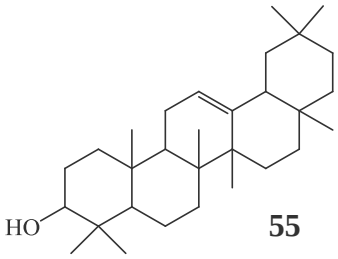
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The triterpenes (C_{30} compounds) are abundant in nature. They are composed of six isoprene units such as taraxasterol **54** and β-amyrin **55**, which are biosynthetically derived from squalene **56** (Ramawat et al., 2009). They are generated by the head-to-head joining of two C_{15} chains, each of which constitute three isoprene units joined head-to-tails (Croteau et al., 2000). They have a wide spectrum of biological activities such as antitumor, antiviral, bactericidal, fungicidal, analgesic, anti-inflammatory and cytotoxic activities (Patocka, 2003).

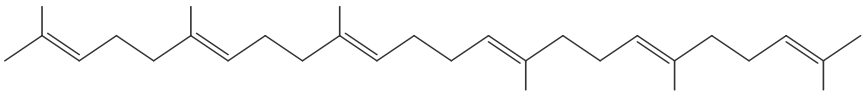
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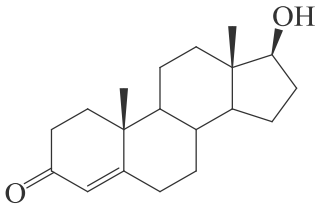
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Steroids are modified triterpenes. They are probably most familiar from their role as hormones such as testosterone **57** and progesterone **58**. Steroids are most often used as anti-inflammatory agents, for example cortisone **59**. They also been used in

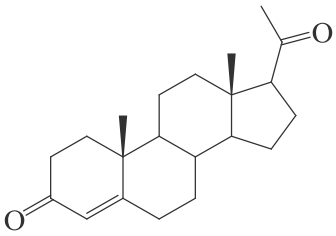
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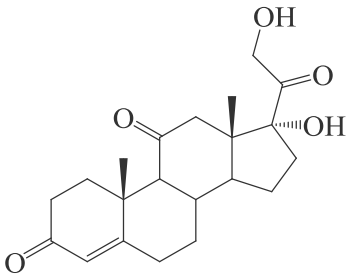
birth control pills. Prior to 1943, most steroids were obtained from natural sources (Taiz & Zeiger, 2006).



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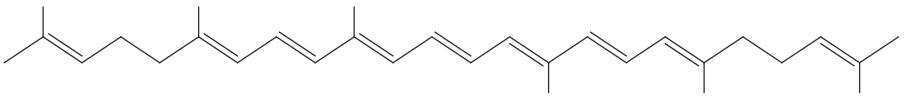
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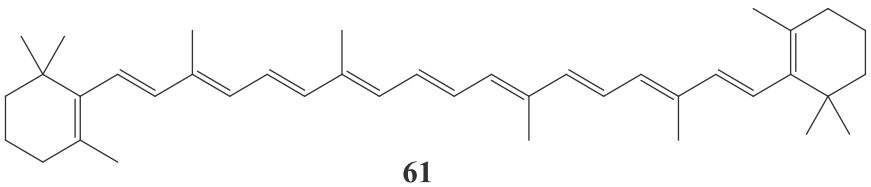
59

Tetraterpenes or carotenoids are terpenes consist of eight isoprene units with molecular formula $C_{40}H_{64}$. Tetraterpenes are found in all plants, bacteria and fungi. They are the world's most widely distributed pigments. Biologically important tetraterpenes include lycopene **60** and β-carotene **61** (Taiz & Zeiger, 2006). The classification of terpenes is summarized in Table 2.1.

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Table 2.1

The Classification of Terpenes

Group	Number of Carbon	Isoprene unit	Examples
Monoterpene	10	2	Pinane **48** , Methol **49**
Sesquiterpene	15	3	Arteannuic Acid **50**, Bisabolene **51**
Diterpene	20	4	Manool **52**, Taxadiene **53**
Triterpene	30	6	Taraxasterol **54**, β-amyrin 55
Tetraterpene	40	8	Lycopene **60**, β-carotene **61**
Polyisoprene	>40	>6	Rubber $(C_5H_8)_n$

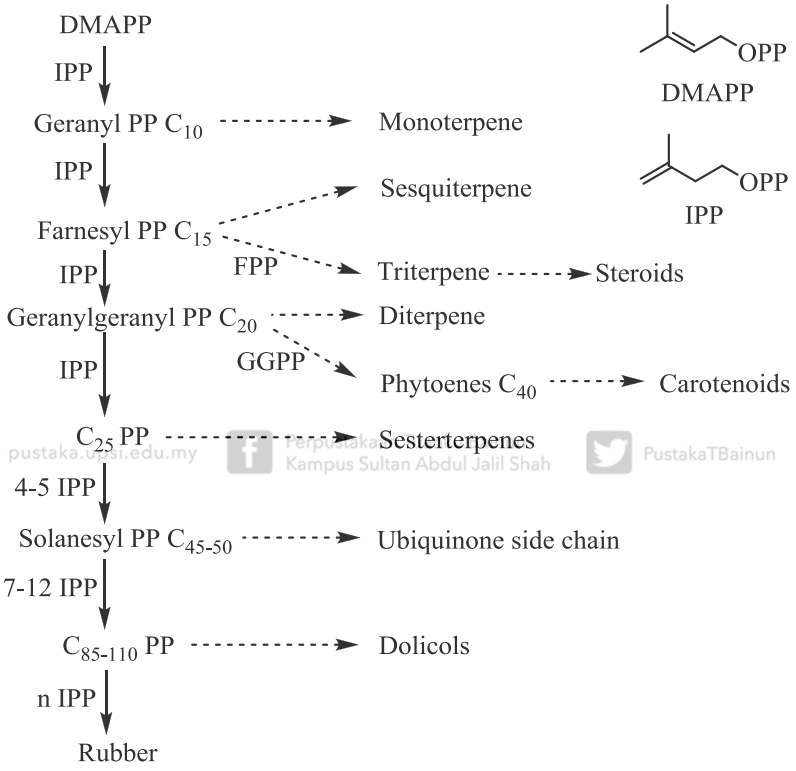
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2.2.2.2 Biosynthesis

Terpenes are a diverse family of secondary metabolites that are derived from the basic unit of isoprene. Members of this family include the monoterpenes, sesquiterpenes, diterpenes, steroids, carotenes, polyprenoids such as ubiquinone side chain, and the polymer rubber (Scheme 2.5).



Scheme 2.5. Terpene Family

Studies on the biosynthesis of terpene have led to the "biogenetic isoprene rule" which was originally proposed by Ruzicka (Spurgeon & Proter, 1981). This rule has successively improved and extended to cover the increasing number of terpenes isolated from natural sources. Its monomeric unit is isoprene, also known as 2-methylbut-1,3-diene **47**. The biosynthesis of terpene can be divided into three definite steps:

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1. The formation of an isopentene unit from acetate;
2. The condensation of isopentene unit to form acyclic terpenes; and
3. The conversion of acyclic terpenes into cyclic terpenes and the introduction of functional groups.

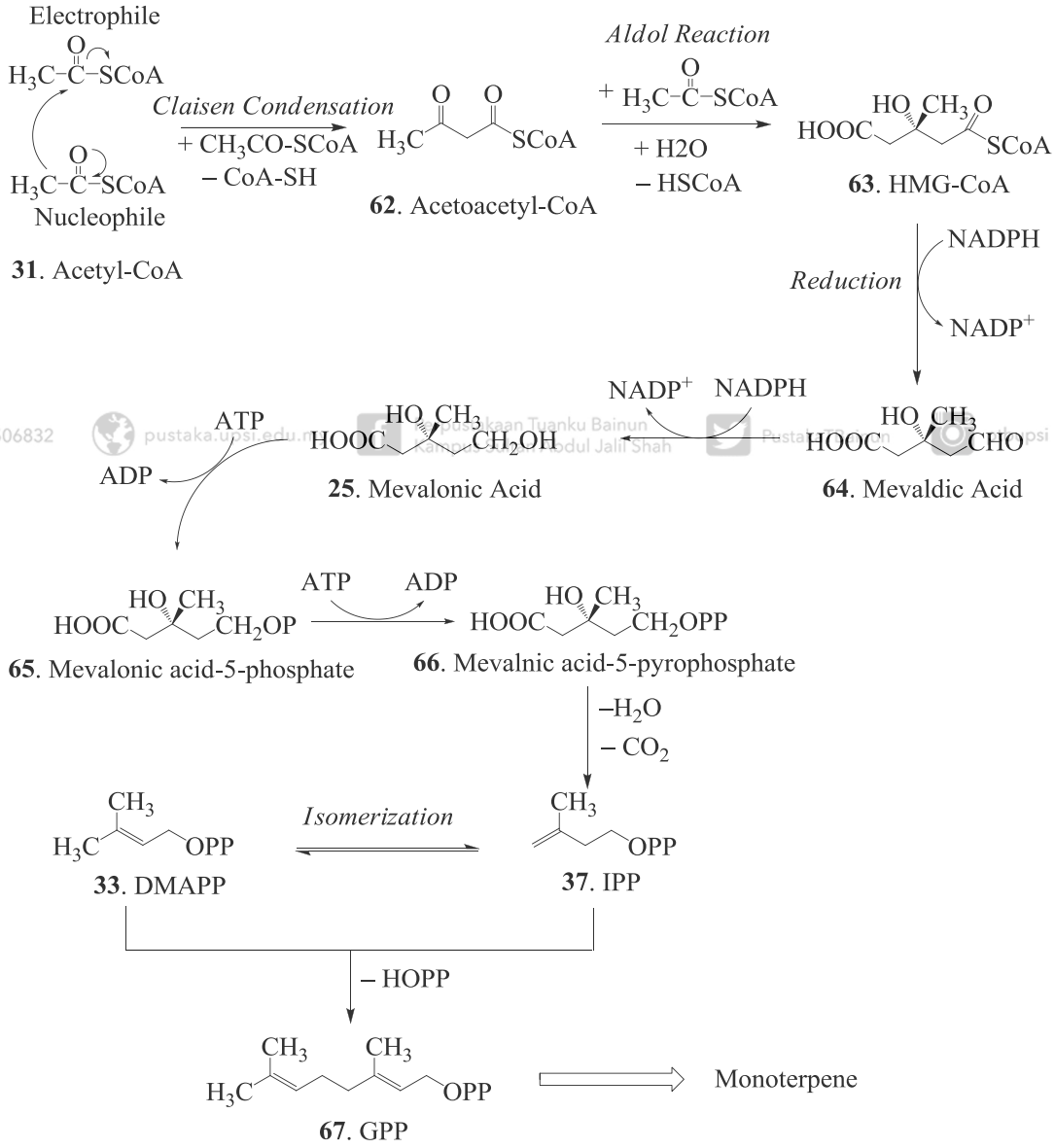
Acetyl-CoA **31** is the biosynthetic precursor for the formation of isopentene unit. Two molecules of acetyl-CoA **31**, derived mainly from carbohydrate or fat metabolism, condense to afford acetoacetyl-CoA **62**. Further condensation of acetoacetyl-CoA **62**, the aldol condensation, with another molecule of acetyl-CoA **31** gives α-hydroxy-β-methylglutaryl-CoA (HMG-CoA) **63**. Then, HMG-CoA **63** is reduced by the action of nicotinamide adenine dinucleotide phosphate (NADPH) to yield an intermediate compound, mevaldic acid **64**, which is responsible to the production of mevalonic acid (MVA) **25**, the building block of almost all isoprenoids (Scheme 2.6). The pathway from acetyl-CoA **31** to mevalonic acid **25**, and to various classes of terpenes is known as the mevalonate pathway (Spurgeon & Proter, 1981).

Phosphorylation of mevalonic acid **25** by adenosine triphosphate first produces mevalonic acid-5-phosphate ($P=H_2PO_3$) **65** which is followed by a second phosphorylation to give mevalnic acid-5-pyrophosphate ($PP=H_3P_2O_6$) **66**. Subsequently loss of a molecule of water and carbon dioxide yields 3-methylbut-3-enyl (isopentenyl) pyrophospate (IPP) **37**. The biogenetic isoprene unit is in the form of 3-methylbut-3-enyl pyrophosphate **37**, which in the presence of an appropriate enzyme isomerizes to 3-methylbut-2-enyl (β,β-dimethylallyl) pyrophosphate (DMAPP) **33** (Scheme 2.6) (Spurgeon & Proter, 1981).

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The next step in the biosynthesis of terpenes is the formation of geranyl pyrophosphate, a 10-carbon compound (GPP) **67** which arises by the combination of DMAPP **33** and IPP **37**. This IPP **37** acts as a nucleophile and DMAPP **33** as an electrophile to form a head-to-tail union as indicated in Scheme 2.6. The GPP **67** is the precursor for the formation of monoterpene (Scheme 2.6) (Spurgeon & Proter, 1981).

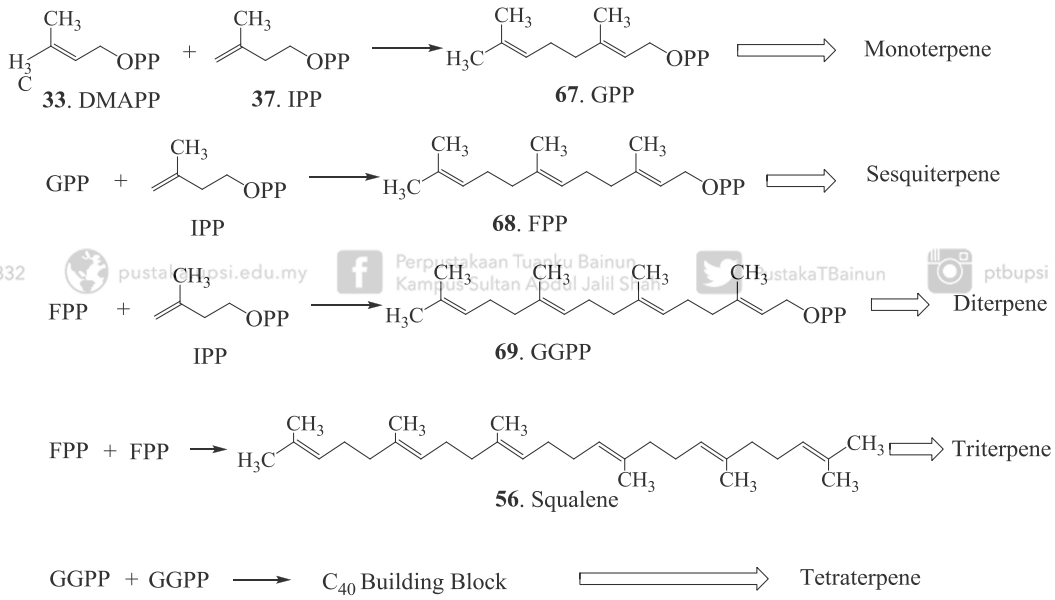


Scheme 2.6. The Biogenesis Pathway from Aceto-CoA to the Formation of Geranyl Pyrophosphate (GPP)

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Geranyl pyrophosphate (GPP) **67** coupling with 3-methylbut-3-enyl pyrophosphate to extend the chain by a five-carbon unit. This affords farnesyl pyrophosphate (FPP) **68**, a 15-carbon compound which is the biosynthetic precursor of sesquiterpene. The conversion of FPP **68** into the precursor of diterpene, geranyl-geranyl pyrophosphate (GGPP) **69** requires the addition of one more C5 unit. The coupling of two FPP units **68** in a head-to-head manner gives rise to squalene **56**. While, the coupling of two GGPP units **69** gives rise to biosynthetic precursor of tetraterpene (Scheme 2.7) (Spurgeon & Proter, 1981).



Scheme 2.7. Biosynthesis of Terpene Family

The remarkable process of cyclization of squalene **56** give rise to triterpene. Triterpene was then directly synthesized from isopentenyl pyrophosphate (IPP) **37** via squalene **56** which is the biological precursor of all triterpenes. Then, it was converted to (3*S*)-2,3-oxidosqualene **70** by oxidosqualene cyclase (Scheme 2.8) (Breitmaier, 2006).

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A significant number of triterpenes are believed to be the products synthesized via the intermediate oxidosqualene. The opening of the intermediate oxidosqualene initiates the cyclization process. This will determine the orientation of the biosynthesis towards either triterpenes or steriods (Breitmaier, 2006).

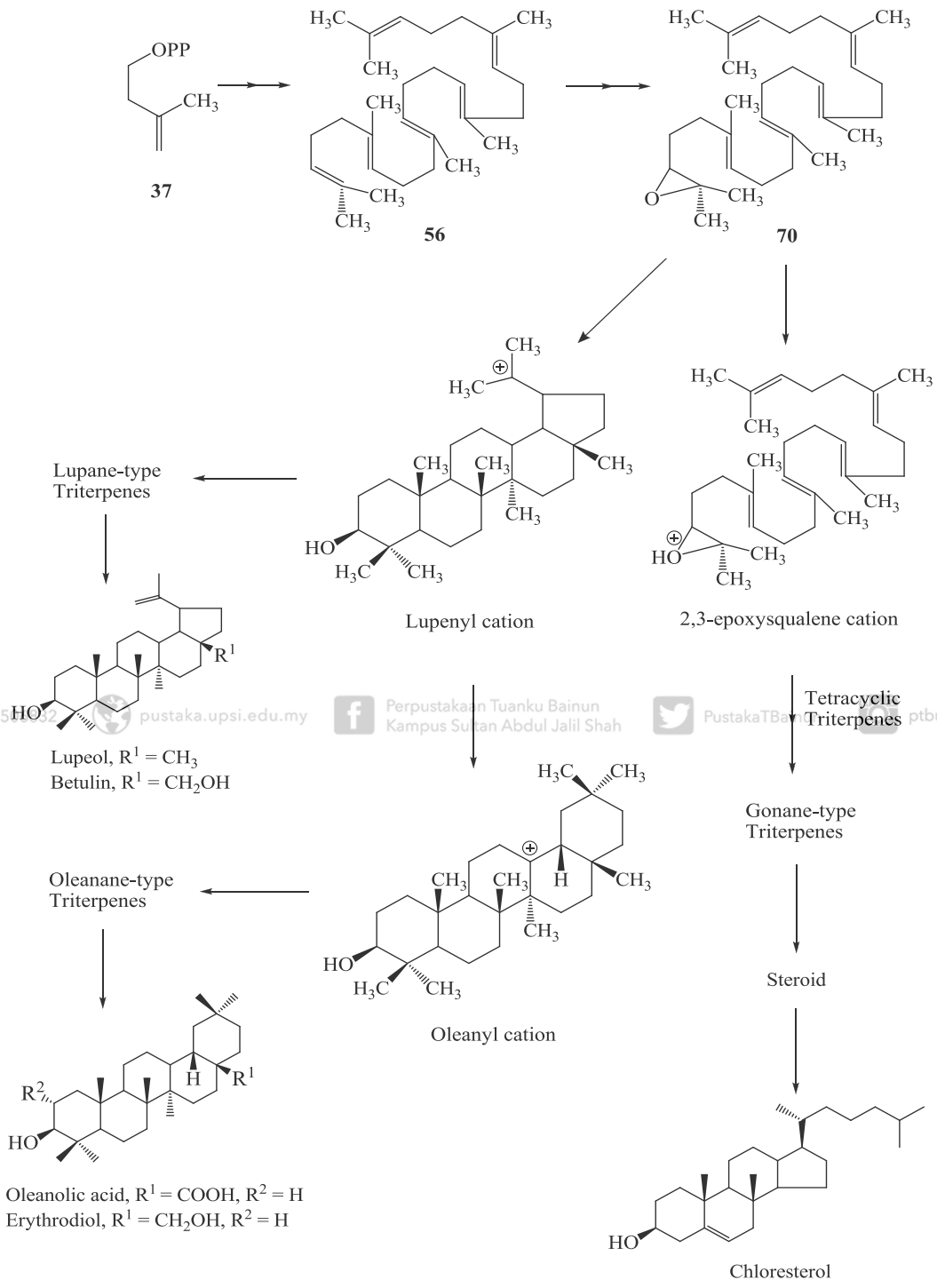
If oxidosqualene is at the suitably positioned and folded on the enzyme surface, a series of cyclization will occur. Then, the reaction followed by a sequence of concerted Wagner-Meerwein migrations of methyls and hydrides, which generate tetra- or pentacyclic triterpene skeletal diversity. The protonation of the epoxide group will then allow the opening of the ring (Breitmaier, 2006).

In typical triterpene cyclization, twelve to twenty covalent bonds are broken or formed, seven to nine chiral centers are established and four to five rings are built. (Fraga, Reina, Luis, & Rodriguez, 2003). Steroids are also derived from these tetracyclic triterpenes (Breitmaier, 2006). The possibilities of biosynthetic pathways of triterpenes are describe in Scheme 2.8.

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Scheme 2.8. Biosynthesis of Triterpenes

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2.3 Phytochemistry and Pharmacological Aspects of *M. koenigii*

This section has included a review on the work done by various researchers on phytochemical screening and pharmacological activities of crude extracts and chemical compounds from bark, leaves, fruits and roots of *Murraya koenigii*.

2.3.1 Phytochemistry

Phytochemistry screening on bark of the *Murraya koenigii* by Kaur, Gupta, Ahmad, and Alam (2011) showed the presence of alkaloids, carbohydrates, protein, amino acids, saponins, flavonoids and coumarins. Bark have also been reported by various researchers to contain carbazole alkaloids such as girinimbine **8**, murrayanine **11**, koenoline **12** and 3-methylcarbazole **34** (Table 2.2). The first carbazole alkaloids, murrayanine (3-formyl-1-methoxycarbazole) **11**, was isolated from the bark of *M. koenigii* by Chakraborty et al. (1965). Since then, there has been a strong interest in this area by the researchers due to its intriguing structural features and promising biological activities.

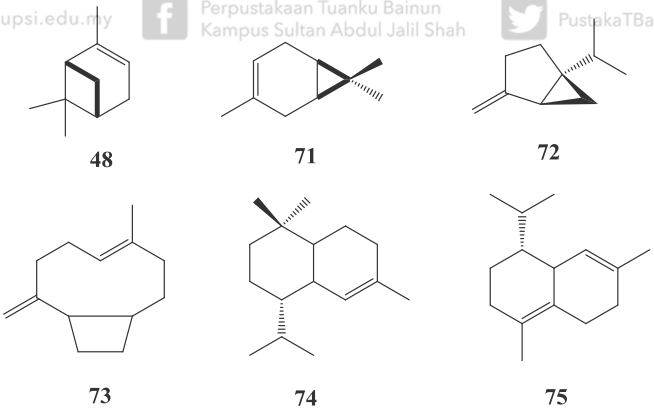
Phytochemical studies by Mishra, Yousof, Singh, and Aradhana (2009) showed the presence of phenols, tannins, flavonoids, terpenoids and alkaloids in the leaves extract of *M. koenigii*. The ethanol extract was observed to contain saponins and flavonoids (Pande, Ingale, & Gupta, 2009). The leaves have also been reported by Iyer and Uma Devi (2008) to contain amino acids, calcium, phosphorus, iron, thiamine, riboflavin, niacin, vitamin C, carotene and oxalic acid. Essential oil composition of the leaves of *M. koenigii* has been reported by several researchers

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(Chowdhury, Bhuiyan, & Yusuf, 2008; Onayade & Adebajo, 2000; Rana, Juyal, Rashmi, & Amparo Blazquez, 2004; Verma, Padalia, Chauhan, & Arya, 2012; Wong & Tie, 1993). The major essential oils responsible for the aroma and flavour of the plant have been reported as pinene **48**, carene **71**, sabinene **72**, caryophyllene **73**, cadinol **74** and cadiene **75** (Chowdhury et al., 2008; Malwal & Sarin, 2010; Nigam & Purohit, 1961; Onayade & Adebajo, 2000; Prakesh & Natarajan, 1974; Rana et al., 2004; WaBmuth-Wagner, Kalinowski, & Jork, 1995; Walde, Joythirmay, Rao, Shivaswamy, & Srinivas, 2005). The essential oils composition, mainly contributed by monoterpene hydrocarbons, but also possesses sesquiterpene hydrocarbons, were reported to depend considerably on seasonal climate changes (Verma et al., 2012). The carbazole alkaloids can also be obtained from leaves of the plant by extracting with various solvent (Table 2.2).

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Reisch, Goj, Wickramasinghe, Bandara Herath, and Henkel (1992) reported that the seeds of *M. koenigii* contained carbazole alkaloids (Table 2.2). However, the seeds have also been reported to have fatty oil by various researchers (Adebajo & Reisch, 2000; Bringmann et al., 2001; Charkrabarty et al., 1997; Hemavathy, 1991; Kureel, 1969a; Wassmuth-Wagner, Kalinowski, & Jork, 1995). The pulp of fruits were reported to contain protein, reducing sugar, vitamin C and minerals (Bonde et al., 2011; Prasan, 2013).

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Carbazole alkaloids either monomeric or binary, simple-, furo- and pyrano-coumarins (Adebajo, 1997; Adebajo, Olugbade, Elujoba, Aladesanmi, & Reisch, 1997; Atta-ur-Rahman, Zaidi, & Firdous, 1988; Bhattacharyya & Chakraborty, 1984; Bhattacharyya, Maiti, Basu, & Chowdhury, 1994; Chakraborty et al., 1997; Fiebig, Pezzuto, Soejarto, & Kinghorn, 1985; Hegnauer, 1990; Ito, Thoyama, Omura, Kajiura, & Furukawa, 1993; Nutan, Hasnat, & Rashid, 1998; Reisch et al., 1992, 1994a; Reisch, Adebajo, Kumar, & Aladesanmi, 1994b) have been reported from the various parts of the plant. Carbazole alkaloids isolated from *M. koenigii* are listed in Table 2.2.

Table 2.2

Carbazole Alkaloids Isolated from M. koenigii

No.	Isolated Compounds	Part of Plants	References
	Tricyclic Carbazole Alkaloids		
76	1-Formyl-3-methoxy-6-methylcarbzole	Leaves	(Chowdhury, Jha, Bhattacharyya, & Mukherjee, 2001)
77	1-Hydroxy-3-methylcarbazole	Bark	(Bhattacharyya et al., 1994)
39	2-Hydroxy-3-methylcarbazole	Roots	(Bhattacharyya, Jash, & Chowdhury, 1986)
78	2-Methoxy-3-methylcarbazole	Fruits/Seeds	(Bhattacharyya & Chowdhury, 1985)
79	2-Methoxycarbazole-3-carboxylate	Bark	(Bhattacharyya et al., 1994)
40	3-Formylcarbazole	Bark	(Rahman & Gray, 2005)
		Roots	(Ito et al., 1993)
80	3-Formyl-1,8-dimethoxycarbazole	Roots	(Intekhab & Aslam, 2008)

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Table 2.2 (Continued)

No.	Isolated Compounds	Part of Plants	References
34	3-Methylcarbazole	Bark	(Sukari et al., 2007)
		Roots	(Ahmad, Tan, Sukari, Ali, & Nafiah, 2014; Chakraborty et al., 1997; Sukari et al., 2001)
		Leaves	(Adesina, Olatunji, Bergenthal, & Reisch, 1988)
81	3,6-dimethyl-1-isopentenylcarbazole	Roots	(Begum, Chowdhury, Banerji, & Das, 2005)
82	6,7-Dimethoxy-1-hydroxy-3-methylcarbazole	Leaves	(Chowdhury et al., 2001)
83	Carbazole-3-carboxylic acid	Bark	(Rahman & Gray, 2005)
84	Clausazoline-K	Leaves	(Birari, Roy, Singh, & Bhutani, 2009)
85	Euchrestine-B	Leaves	(Ito et al., 2006; Nakamura et al., 2013; Tachibana, Kikuzaki, Lajis, & Nakatani, 2001; Yoshikawa, 2013)
86	Eustifoline-C	Roots	(Ito et al., 1993)
		Leaves	(Nakamura et al., 2013; Yoshikawa, 2013)
87	Glycozoline	Roots	(Ito et al., 1993)
		Leaves	(Adesina et al., 1988)
88	Isomurrayafoline-B	Bark	(Ito et al., 1993)
12	Koenoline	Bark	(Ito et al., 1993; Tantapakul, Phakhodee, Laphookhioe, Ritthiwigrom, & Cheenpracha, 2014)
		Roots	(Fiebig et al., 1985; Ito et al., 1993)
		Whole Plants	(Ma et al., 2013)

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Table 2.2 (Continued)

No.	Isolated Compounds	Part of Plants	References
43	Mahanimbilol/ Mahanimbinol	Bark	(Ito et al., 1993; Rama Roa, Bhide, & Mujumdar, 1980; Reich et al., 1994b; Tan, Nafiah, Ahmad, 2014a, b; Tantapakul et al., 2014)
		Roots	(Ito et al., 1993)
		Leaves	(Adebajo et al., 2006)
89	Mukoeic Acid	Bark	(Chowdhury & Chakraborty, 1969; Chowdhury & Chakraborty, 1971)
		Roots	(Ito et al., 1993)
41	Mukoenine-A/ Girinimbilol	Bark	(Ito et al., 1993; Reich et al., 1994b)
		Roots	(Ito et al., 1993)
		Leaves	(Adebajo et al., 2006)
90	Mukoenine-B	Aerial Parts	(Naz, Saied, Ahmed, & Shahid, 2014)
		Roots	(Ito et al., 1993)
91	Mukoenigatin	Aerial Parts	(Naz et al., 2014)
92	Mukolidine	Roots	(Roy, Bhattacharyya, & Chakraborty, 1982)
93	Mukoline	Roots	(Roy et al., 1982)
94	Mukonal	Bark	(Bhattacharyya & Chakraborty, 1984)
95	Mukonidine	Bark	(Chakraborty, Roy, & Guha, 1978)
96	Mukonine	Bark	(Chakraborty, Bhattacharyya, Roy, Bhattacharyya, & Biswas, 1978)
97	Murrayadinal	Aerial Parts	(Naz et al., 2014)

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Table 2.2 (Continued)

No.	Isolated Compounds	Part of Plants	References
13	Murrayafoline-A	Bark	(Ito et al., 1993; Sukari et al., 2007)
		Roots	(Ahmad et al., 2014; Haslizawati et al., 2007; Ito et al., 1993, Sukari et al., 2001)
11	Murrayanine	Bark	(Ahmad et al., 2014; Chakraborty et al. 1965; Das, Chakraborty, Bose, 1965; Ito et al., 1993; Rahman & Gray, 2005; Reisch et al., 1994b; Rao, Ramkrishna, Annamalai, Rajesh, & Mukhopadhyay, 2012; Sukari et al., 2007; Tantapakul et al., 2014)
		Roots	(Ahmad et al., 2014; Haslizawati et al., 2007; Ito et al., 1993)
		Leaves	(Gupta & Nigam, 1971; Wang et al., 2003)
		Whole Plants	(Ma et al., 2013)
98	Murrayanol	Leaves	(Ramsewak et al., 1999)
		Fruits/Seeds	(Reisch et al., 1992)
99	O-demethylmurrayanine	Whole plants	(Ma et al., 2013)
		***N*-Substituted Carbazole Alkaloids**	
100	3-Formyl-9-methoxycarbazole	Bark	(Rahman & Gray, 2005)
101	3-hydroxymethyl-9H-carbzole	Whole plants	(Ma et al., 2013)
22	9-carbethoxy-3-methylcarbazole	Roots	(Chakraborty et al., 1997)
102	9-formyl-3-methylcarbazole	Roots	(Chakraborty et al., 1997)

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Table 2.2 (Continued)

No.	Isolated Compounds	Part of Plants	References
103	Karapinamine-A/ Karapinchamines-A	Leaves	(Nakamura et al., 2013; Yoshikawa, 2013)
104	*N*-benzyl carbazole-A	Whole plants	(Ma et al., 2013)
105	*N*-benzyl carbazole-B	Whole plants	(Ma et al., 2013)
106	*N*-methoxy-3-hydroxymethyl-9H-carbazole	Whole plants	(Ma et al., 2013)
	Tricyclic Carbazole-1,4-quinone Alkaloids		
21	Koenigine-quinone-A	Bark	(Saha & Chowdhury, 1998)
107	Koenigine-quinone-B	Bark	(Saha & Chowdhury, 1998)
108	Murrayaquinone-A	Bark	(Ito et al., 1993; Tantapakul et al., 2014)
		Roots	(Ito et al., 1993)
109	Murrayaquinone-B	Bark	(Ito et al., 1993)
	Pyrano[3,2-a]carbazole Alkaloids		
8	Girinimbine	Bark	(Ahmad et al., 2014; Chakraborty, Barman, & Bose, 1964; Chakraborty, Saha, & Mukhapadhyay, 2009; Das et al., 1965; Dutta & Quasim, 1969; Ito et al., 1993; Joshi, Kamat, & Gwad, 1970; Kok et al., 2012; Rahman & Gray, 2005; Reich et al., 1994b; Sukari et al., 2007; Tantapakul et al., 2014)
		Roots	(Ahmad et al., 2014; Haslizawati et al., 2007; Ito et al., 1993; Mohan et al., 2013)
		Leaves	(Adebajo et al., 2006; Sim & Teh, 2011; Nakamura et al., 2013; Yoshikawa, 2013)

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Table 2.2 (Continued)

No.	Isolated Compounds	Part of Plants	References
8	Girinimbine	Fruits/Seeds	(Reisch et al., 1992; Reisch et al., 1994b)
		Whole plant	(Ma et al., 2013)
110	Isokoenidine	Whole plants	(Ma et al., 2013)
111	Isokoenigine	Whole plants	(Ma et al., 2013)
112	Isomahanine/ Pyrayafoline-D	Leaves	(Ito et al., 2006; Nakamura et al., 2013; Tachibana, Kikuzaki, Lajis, & Nakatani, 2003; Yoshikawa, 2013)
		Fruits/Seeds	(Reisch et al., 1992; Reisch et al., 1994b)
113	Koenigicine/ Koenimbidine/ Koenidine	Leaves	(Birari et al., 2009; Ito et al., 2006; Joshi et al., 1970; Kureel et al., 1969a; Narasimhan, Paradkar, & Kelkar, 1970; Narasimhan et al., 1975; Sim & Teh, 2011)
		Whole Plants	(Ma et al., 2013)
114	Koenigine	Leaves	(Ito et al., 2006; Narasimhan et al., 1970; Narasimhan et al., 1975; Rao, Ramalakshmi, Borse, & Raghavan, 2007; Wang, He, Shen, Hong, & Hao, 2002)
		Whole plants	(Ma et al., 2013)
115	Koenimbine / O-methylmukonal	Leaves	(Birari et al., 2009; Chakraborty et al., 2009; Ito et al., 2006; Joshi et al., 1970; Kureel et al., 1969a; Nakamura et al., 2013; Narasimhan et al., 1975; Srivastara, Ray, & Singh, 2007; Tachibana et al., 2003; Yoshikawa, 2013)
		Fruits/Seeds	(Mandal et al., 2010; Mandal, Nayak, Banerjee, Banerji, & Banerji, 2008; Narasimhan, Paradkar, & Chitguppi, 1968; Reisch et al., 1992; Reisch et al., 1994b; Uvarani et al., 2014)

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Table 2.2 (Continued)

No.	Isolated Compounds	Part of Plants	References
115	Koenimbine / O-methylmukonal	Whole Plants	(Ma et al., 2013)
116	Koenine (2',2',3-trimethyl-6-hydroxypyranocarbazole)	Leaves	(Ito et al., 2006; Narasimhan et al., 1970; Narasimhan et al., 1975)
		Fruits/Seeds	(Mandal et al., 2008; Mandal et al., 2010)
		Whole plants	(Ma et al., 2013)
117	Kurryam (4-hydrox-2',2',3-trimethyl-6,7-dimethoxypyrano carbazole)	Fruits/Seeds	(Mandal et al., 2010; Mandal et al., 2008)
118	Mahanimbicine/ Isomahanimbine	Leaves	(Joshi et al., 1970; Kureel, Kapil, Popli, & Satye, 1970; Nagappan et al., 2011; Nagappan et al., 2012; Nakamura et al., 2013; Narasimhan et al., 1975; Tachibana et al., 2001; Yoshikawa, 2013)
9	Mahanimbine	Bark	(Ahmad et al., 2014; Das et al., 1965; Dutta & Quasim, 1969; Haslizawati et al., 2007; Ito et al., 1993; Rahman & Gray, 2005; Reich et al., 1994b; Roy & Chakraborty, 1974; Sukari et al., 2007; Tan et al., 2014a; Tan, Nafiah, Ahmad, 2014b; Tantapakul et al., 2014)
		Roots	(Ito et al., 1993)

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Table 2.2 (Continued)

No.	Isolated Compounds	Part of Plants	References
9	Mahanimbine	Leaves	(Ahmad et al., 2014; Birari et al., 2010; Birari et al., 2009; Chakraborty et al., 2009; Dineshkumar, Mitra, & Mahadevappa, 2010; Ito et al., 2006; Joshi et al., 1970; Kumar, Mukherjee, Bhadra, Saha, & Pal, 2010; Kureel et al., 1969b; Nagappan et al., 2011; Nagappan et al., 2012; Nakamura et al., 2013; Narasimhan et al., 1975; Ramsewak et al., 1999; Rao et al., 2007; Tachibana et al., 2001; Sim & Teh, 2011; Yoshikawa, 2013)
		Fruits/Seeds	(Narasimhan et al., 1968; Reisch et al., 1992; Reisch et al., 1994b)
		Whole plant	(Ma et al., 2013)
119	Mahanimbinine	Leaves	(Kureel, Kapil, & Popli, 1970)
120	Mahanimboline	Roots	Roy, Gosh, & Chakraborty, 1979)
15	Mahanine	Bark	(Ito et al., 1993)
		Roots	(Ito et al., 1993)
		Leaves	(Ahmad et al., 2014; Atta-ur-Rahman et al., 1998; Ito et al., 2006; Nagappan et al., 2011; Nagappan et al., 2012; Narasimhan et al., 1970; Narasimhan et al., 1975; Ramsewak et al., 1999; Tachibana et al., 2001)
		Fruits/Seeds	(Reisch et al., 1992; Uvarani et al., 2014)
		Whole plants	(Ma et al., 2013)

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Table 2.2 (Continued)

No.	Isolated Compounds	Part of Plants	References
121	Mukoenine-C/ Murrayamine-A	Roots	(Ito et al., 1993)
		Whole Plants	(Ma et al., 2013)
122	Mukonicine	Leaves	(Mukherjee, Mukherjee, Shaw, & Ganguly, 1983)
14	Murrayacine	Bark	(Ahmad et al., 2014; Chakraborty & Das, 1968; Chakraborty, Das, & Chowdhury, 1971; Sukari et al., 2001)
		Leaves	(Joshi et al., 1970)
123	Murrayacinine	Bark	(Chakraborty, Bhattacharyya, Islam & Roy, 1974)
124	Murrayamine-B	Leaves	(Nakamura et al., 2013; Yoshikawa, 2013)
125	O-methylmahanine	Leaves	(Nakamura et al., 2013; Tachibana et al., 2003; Uvarani et al., 2014; Yoshikawa, 2013)
126	O-methylmurrayamine-A	Leaves	(Chakraborty et al., 2009; Tachibana et al., 2003)
	Cyclic Monoterpenoid Pyrano[3,2-a]carbazole Alkaloids		
127	7-Hydroxymurrayazolinine	Leaves	(Sim & Teh, 2011)
128	Bicyclomahanimbicine	Leaves	(Kureel et al., 1970; Nakamura et al., 2013)
129	Bicyclomahanimbiline	Bark	(Reich et al., 1994a, b)
44	Bicyclomahanimbine	Bark	(Tan et al., 2014a; Tan et al., 2014b)
		Leaves	(Kureel, Kapil, & Popli , 1969b; Nakamura et al., 2013; Sim & Teh, 2011)

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Table 2.2 (Continued)

No.	Isolated Compounds	Part of Plants	References
19	Curryangin/ Mahanimbidine/ Murrayazoline	Bark	(Ahmad et al., 2014; Dutta, Quasim, & Wadia, 1969; Sukari et al., 2001; Tantapakul et al., 2014)
		Leaves	(Kureel et al., 1969b)
130	Isomurrayazoline	Bark	(Bhattacharyya, Roy, & Chakraborty , 1982)
131	Karapinamine-B/ Karapinchamines-B	Leaves	(Nakamura et al., 2013; Yoshikawa, 2013)
132	Karapinamine-C	Leaves	(Yoshikawa, 2013)
133	Karapinamine-D	Leaves	(Yoshikawa, 2013)
134	Murrayakoeninol	Leaves	(Chakraborty et al., 2009; Tan et al., 2014a)
135	Murrayamine-D	Leaves	(Sim & Teh, 2011)
136	Murrayamine-E	Leaves	(Nakamura et al., 2013; Yoshikawa, 2013)
17	Murrayazolidine/ Cyclomahanimbine/ Curryanin	Bark	(Chakraborty, Islam, Basak, & Das, 1970; Tantapakul et al., 2014)
		Leaves	(Kureel et al., 1969a)
		Fruits/Seeds	(Reisch et al., 1992)
18	Murrayazolinine	Bark	(Chakraborty et al., 1973)
		Leaves	(Chakraborty et al., 2009)
20	Murrayazolinol	Bark	(Bhattacharyya, Chatterjee, Roy, & Chakraborty, 1989; Tan et al., 2014a; Tan et al., 2014b)
	<u>Biscarbazole Alkaloids</u>		
137	3,3'-[oxybis(methylene)]bis(9-methoxy-9H-carbazole)	Bark	(Rahman & Gray, 2005)

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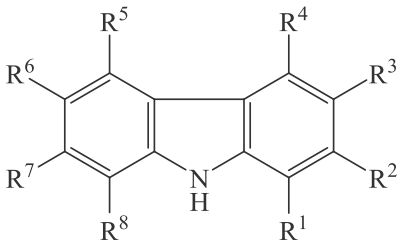
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Table 2.2 (Continued)

No.	Isolated Compounds	Part of Plants	References
138	8,8"-biskoenigine	Leaves	(Wang et al., 2002; Wang et al., 2003)
		Whole plants	(Ma et al., 2013)
139	8,10'-[3,3',11,11'-tetrahydro-9,9'-dihydroxy-3,3',5,8'-tetramethyl-3,3'-bis-(4-methyl-3-pentenyl)]bipyrano[3,2a]carbazole	Leaves	(Tachibana et al., 2003)
140	Bikoeniquinone-A	Bark	(Ito et al., 1993)
141	Bikoeniquinonine	Aerial Parts	(Naz et al., 2014)
46	Bis-2-hydroxy-3-methylcarbazole	Bark	(Ito et al., 1993)
142	Bisgerayafoline D	Fruits/Seeds	(Uvarani et al., 2014)
143	Bismahanimbinol	Fruits/Seeds	(Uvarani et al., 2014)
144	Bismahanine	Bark	(Ito et al., 1993)
		Leaves	(Tachibana et al., 2003)
16	Bismurrayafoline-E	Leaves	(Nutan, Hasan & Rashid, 1999; Tachibana et al., 2001)
145	Bismurrayaquinone-A	Bark	(Ito et al., 1993)
146	Bispyrayafoline	Leaves	(Tachibana et al., 2003; Uvarani et al., 2014)
147	Chrestifoline-A	Bark	(Ito et al., 1993)
148	Mahabinine-A	Leaves	(Ito et al., 2006)
149	Murrafoline-I	Leaves	(Ito et al., 2006)
45	Murrastifoline-A	Bark	(Ito et al., 1993)
150	Murrastifoline-F	Bark	(Ito et al., 1993)

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	Compound	R^1	R^2	R^3	R^5	R^6	R^7	R^8
77	1-Formyl-3-methoxy-6-methylcarbzole			CH_3		OCH_3		CHO
76	1-Hydroxy-3-methylcarbazole	OH		CH_3				
39	2-Hydroxy-3-methylcarbazole		OH	CH_3				
78	2-Methoxy-3-methylcarbazole		OCH_3	CH_3				
79	2-Methoxycarbazole-3-carboxylate		OCH_3	COOH				
80	3-Formyl-1,8-dimethoxycarbazole	OCH_3		CHO				OCH_3
40	3-Formylcarbazole			CHO				
34	3-Methylcarbazole			CH_3				
81	3,6-Dimethyl-1-isopentenylcarbazole	isopentenyl		CH_3		CH_3		
82	6,7-Dimethoxy-1-hydroxy-3-methylcarbazole	OH		CH_3		OCH_3	OCH_3	
83	Carbazole-3-carboxylic acid			COOH				
84	Clausazoline-K			CHO			OCH_3	
85	Euchrestine-B		OH	CH_3			OCH_3	geranyl
86	Eustifoline-C			CH_3	geranyl			

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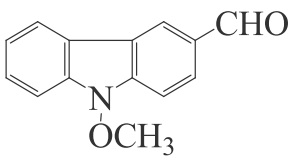
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	Compound	R^1	R^2	R^3	R^5	R^6	R^7	R^8
41	Girinimbilol / mukoenine A	prenyl	OH	CH_3				
87	Glycozoline			CH_3		OCH_3		
88	Isomurrayafoline B		OCH_3	CH_3			OCH_3	prenyl
12	Koenoline	OCH_3		CH_2OH				
43	Mahanimbinol / mahanimbilol	geranyl	OH	CH_3				
89	Mukoeic acid	OCH_3		COOH				
90	Mukoenine B	geranyl	OH	CHO				
91	Mukoenigatin	OCH_3		C19H35O3		OCH_3	OCH_2CH_3	OH
92	Mukolidine			CHO				OCH_3
93	Mukoline			CH_2OH				OCH_3
94	Mukonal		OH	CHO				
95	Mukonidine		OH	$COOCH_3$				
96	Mukonine	OCH_3		$COOCH_3$				
97	Murrayadinal	prenyl		CHO			OH	CHO
13	Murrayafoline-A	OCH_3		CH_3				
11	Murrayanine	OCH_3		CHO				
98	Murrayanol		OCH_3	CH_3			OH	geranyl
99	O-demethylmurrayanine	OH		CHO				

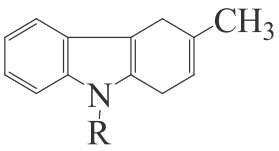
Note. Prenyl - $(CH_2)_2CH=C(CH_3)_2$; Gerenyl - $CH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)_2$

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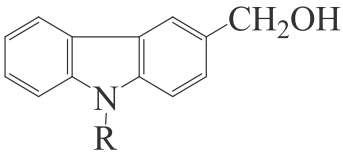
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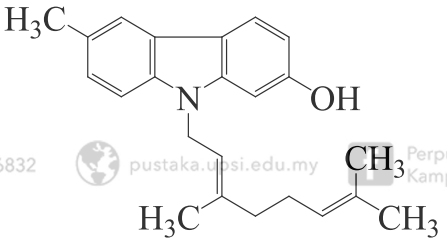
100. 3-Formyl-9-methoxycarbazole



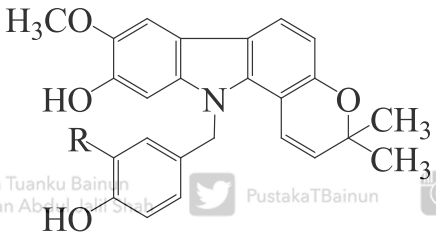
22. 9-Carbethoxy-3-methylcarbazole
R = $COOCH_2CH_3$
102. 9-Formyl-3-methylcarbazole
R = CHO



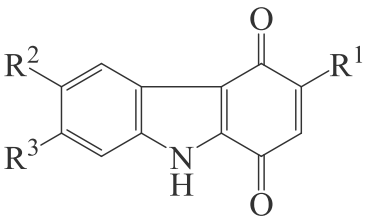
99. 3-Hydroxymethyl-9H-carbzole
R = OCH_3
106. *N*-methoxy-3-hydroxymethyl-9H-carbazole
R = H



103. Karapinchamines-A/
Karapinamine-A



104. *N*-benzyl carbazole-A
R = OCH_3
105. *N*-benzyl carbazole-B
R = H

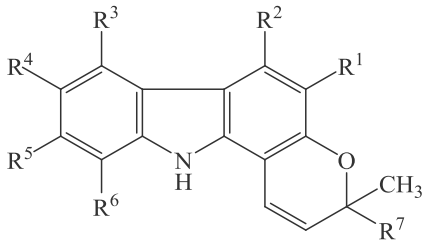


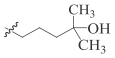
	Compound	**R^1**	**R^2**	**R^3**
21	Koeniginequinone-A	CH_3		OCH_3
107	Koeniginequinone-B	CH_3	OCH_3	OCH_3
108	Murrayaquinone-A	CH_3		
109	Murrayaquinone-B	CH_3	OCH_3	prenyl

Note. Prenyl - $(CH_2)_2CH=C(CH_3)_2$

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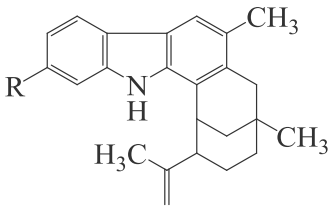


	Compound	R1	R2	R3	R4	R5	R6	R7
8	Girinimbine	CH_3						CH_3
110	Isokoenidine	CH_3		OCH_3			OCH_3	CH_3
111	Isokoenigine	CH_3		OH			OCH_3	CH_3
112	Isomahanine / pyrayafoline-D	prenyl			CH_3	OH		
113	Koenigicine / koenidine / koenimbidine	CH_3			OCH_3	OCH_3		CH_3
114	Koenigine	CH_3			OCH_3	OH		CH_3
115	Koenimbine	CH_3			OCH_3			CH_3
116	Koenine	CH_3			OH			CH_3
117	Kurryam	CH_3	OH		OCH_3	OCH_3		CH_3
118	Mahanimbicine / isomahanimbine				CH_3			prenyl
119	Mahanimbinine	CH_3						
120	Mahanimboline	CH_3						
9	Mahanimbine	CH_3						prenyl
15	Mahanine	CH_3				OH		prenyl
121	Mukoenine C / murrayamine A	CH_3				OH		CH_3
122	Mukonicine	CH_3			OCH_3		OCH_3	CH_3
14	Murrayacine	CHO						CH_3
123	Murrayacinine	CHO						prenyl
124	Murrayamine B	CH_3					OCH_3	prenyl
125	O-Methylmahanine	CH_3				OCH_3		prenyl
126	O-Methylmurrayamine A	CH_3				OCH_3		CH_3

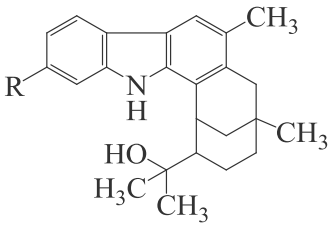
Note. Prenyl - $(CH_2)_2CH=C(CH_3)_2$

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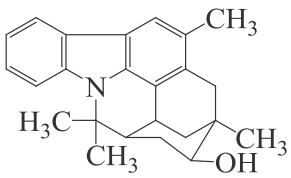
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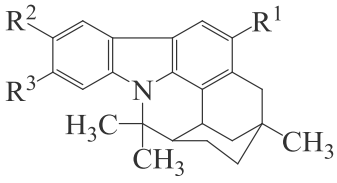
17. Cyclomahanimbine / Murrayazolidine / Curryanine
R = H
135. Murrayamine-D
R = OH



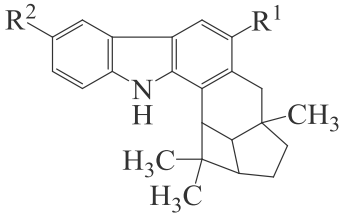
18. Murrayazolinine
R = H
127. 7-Hydroxymurrayazolinine
R= OH



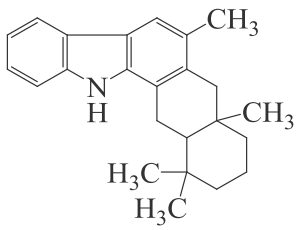
20. Murrayazolinol



19. Murrayazoline / Mahanimbidine / Curryangin
$R^1 = CH_3$; $R^2 = R^3 = H$
130. Isomurrayazoline
$R^2 = CH_3$; $R^1 = R^3 = H$
136. Murrayamine-E
$R^1 = CH_3$; R^2= H; R^3 = OH

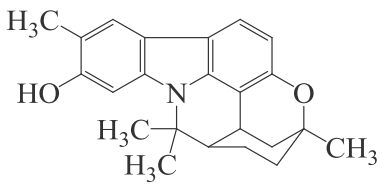


44. Bicyclomahanimbine
$R^1 = CH_3$, $R^2 = H$
128. Bicyclomahanimbicine
$R^1 = H$, $R^2 = CH_3$

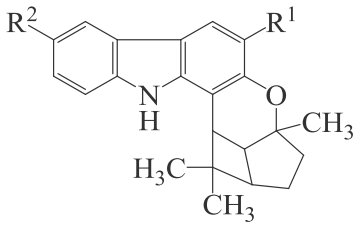


129. Bicyclomahanimbiline

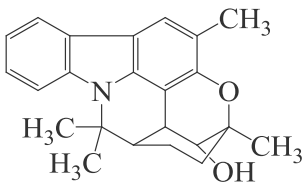
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131. Karapinchamines-B/ Karapinamine-B



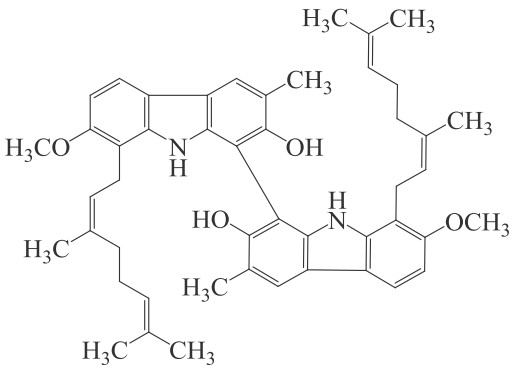
132. Karapinamine-C
$R^1 = H$; $R^2 = CH_3$
133. Karapinamine-D
$R^1 = CH_3$; $R^2 = H$



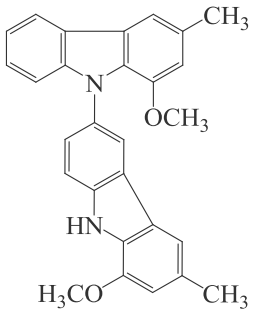
134. Murrayakoeninol

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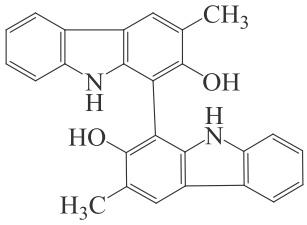
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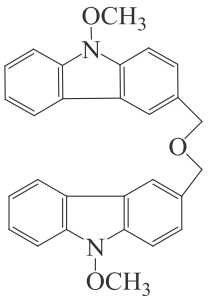
16. Bismurrayafoline-E



45. Murrastifoline-A

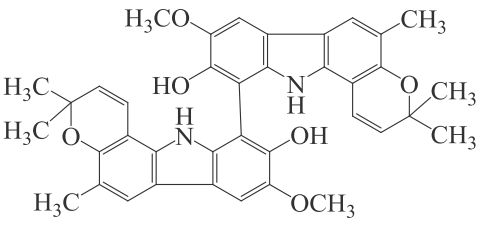


46. Bis-2-hydroxy-3-methylcarbazole

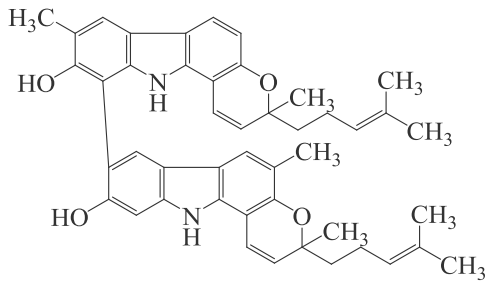


137. 3,3'-[Oxybis(methylene)]bis(9-methoxy-9H-carbazole)

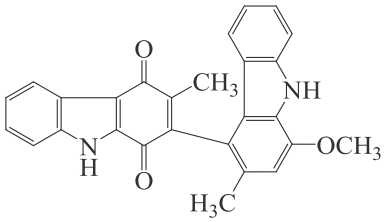
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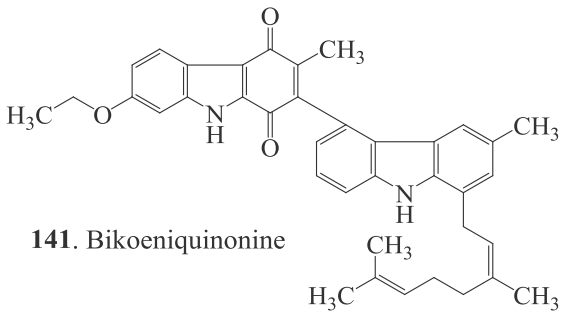
138. 8,8''-Biskoenigine



139. 8,10'-[3,3',11,11'-Tetrahydro-9,9'-dihydroxy-3,3',5,8'-tetramethyl-3,3-bis(4-methyl-3-pentenyl)]bipyrano[3,2-]carbazole



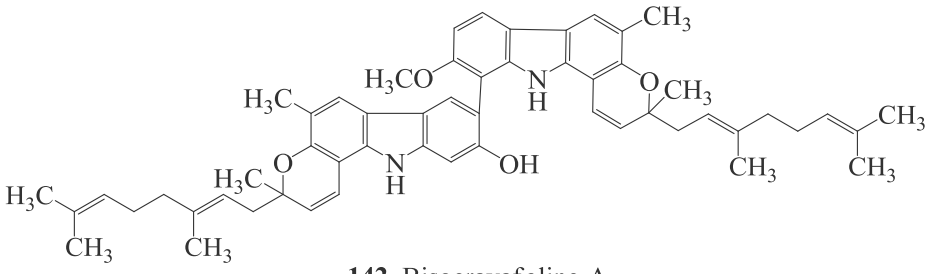
140. Bikoeniquinone-A



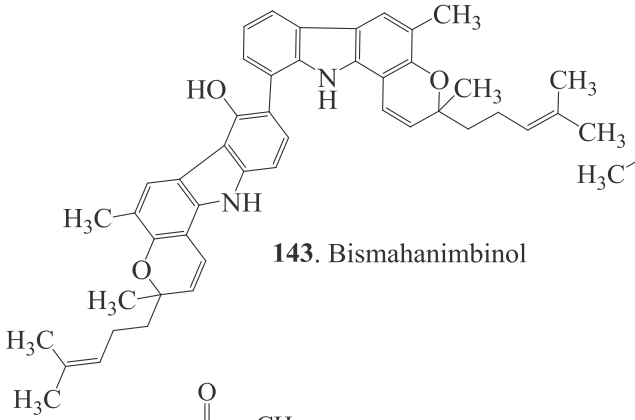
141. Bikoeniquinonine

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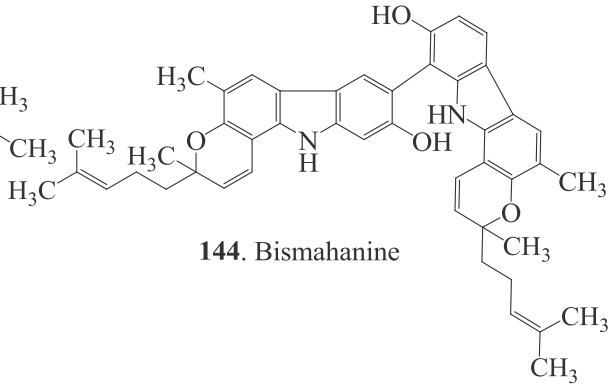
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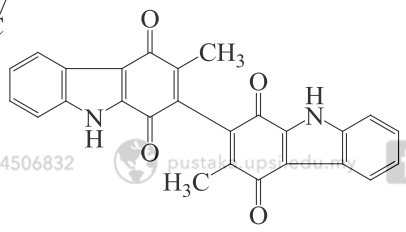
142. Bisgerayafoline-A



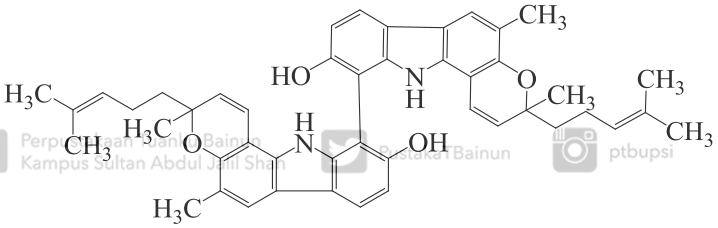
143. Bismahanimbinol



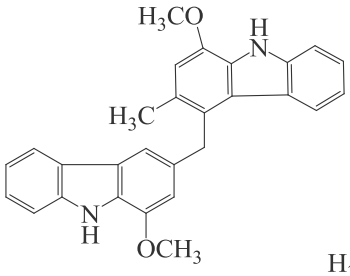
144. Bismahanine



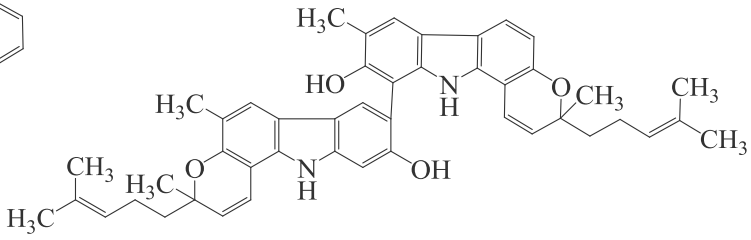
145. Bismurrayaquinone-A



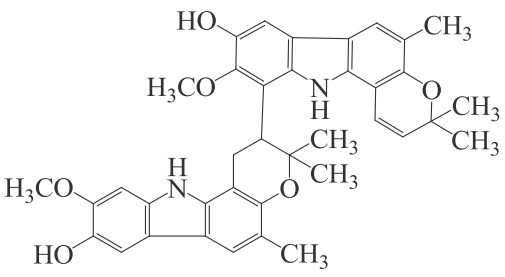
146. Bispyrayafoline



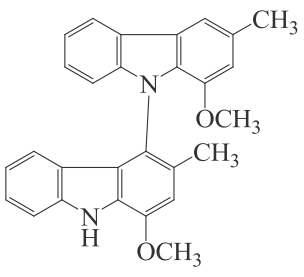
147. Chrestifoline-A



148. Mahabinine-A



149. Murrafoline-I



150. Murrastifoline-F

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2.3.2 Pharmacological Properties

Recently, the studies on plant-based research have increased all over the world and many evidences have been collected to show immerse potential of medicinal plants used in various traditional systems. *Murraya koenigii* is one of the plant species which has been recorded in the traditional medicinal system Ayurveda (Nayak et al., 2010). Many reports are available to validate scientifically the pharmacological properties on the crude extracts of various parts of this plant. Although many isolated compounds have been reported from various parts of *M. koenigii*, but only a few of them have been studied for their biological activities (Table 2.3).

Several reports have shown that *M. koenigii* were found to possess antidiabetic activity (hypoglycaemic activity). In the study carried out by Narayana and Sastry (1975), the aqueous extract of the leaves of *M. koenigii* showed significant hypoglycaemic action in normal and alloxan diabetic dogs. Oral administration of ethanolic extract of *M. koenigii* in Streptomycin induced diabetic rats for a period of 30 days, the blood glucose level, glycosylated haemoglobin, urea, uric acid and creatinine of the diabetic treated group of animals were decreased significantly (Arulselvan, Senthilkumar, Sathish Kumar, & Subramanian, 2006). Several others studies have also shown that the extract of this species significantly decreased the blood glucose in different animal models (Arulselvan et al., 2006; Khan, Abraham, & Leelamma, 1995; Kesari, Gupta, & Watal, 2005; Lawal, Atiku, Khelpai, & Wannang, 2008; Tembhurne & Sakarkar, 2009 and 2010; Vinuthan et al., 2004; Yadav, Vats, Dhunnoo, & Grover, 2002). However, Dineshkumar et al. (2010) has shown that a carbazole alkaloid, mahanimbine **9**, which was isolated from the petroleum ether

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extract of the plant exhibited significant antidiabetic activity at a dose of 50 mg/kg and 100 mg/kg in streptozotocin induced Wister rats. Another study was carried out by Biswas et al. (2010) to determine the hypoglycaemic activity of another isolated carbazole, mahanine **15**. The finding showed that mahanine **15** showed encouraging possibility to deal with lipid induced insulin resistance after the authors administered the compound on the nutritionally induced type 2 diabetic golden hamsters. Mahanine **15** has significantly improved hyperglycemia in all the treated animals (Biswas et al., 2010).

The studies on antioxidative activities of various parts of *M. koenigii* were also reported by several researchers (Table 2.3). This property was determined based on their oil stability index (OSI) as well as their radical scavenging ability against 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Arulselvan & Subramanium, 2007; Kureel et al., 1969a; Ma et al., 2013; Tachibana et al., 2001 and 2003). In the study conducted by Mitra et al. (2012), the aqueous extract of *M. koenigii* leaves showed significant protection on rat cardiac tissue against cadmium-induced oxidative stress probably due to its antioxidative activity. Several researchers also reported the antioxidant activities on the isolated carbazole alkaloids. For example, Kureel et al. (1970) reported mahanimbine **9** displayed antioxidant activity at 33.1 μg/mL which was isolated from the acetone extract of the fresh leaves of *M. koenigii*. According to Rao et al. (2007), mahanimbine **9** and koenigine **114** from the leaves of *M. koenigii* showed significant antioxidant activities of 75% and 84%, respectively, when compared to butylated hydroxyanisole (BHA) (92%). However, koenigine **114** also showed a high degree of radical-scavenging properties (91.6%) when compared with BHA (95.6%). Another report for carbazole alkaloids, namely, mahanine **15**,

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bismurrayafoline-E **16** and euchrestine-B **85** from the CH_2Cl_2 extract showed strong antioxidant activity by measuring its OSI values and DPPH radical-scavenging activity (Tachibana et al., 2003).

Chemical constituents of *M. koenigii* were also found to have antimicrobial and antifungal activities (Table 2.3). Nagappan et al. (2011) reported that three carbazole alkaloids, *viz.*, mahanimbine **9,** mahanine **15** and mahanimbicine **118** from the leaves of the plant were evaluated for the effects on the growth of five antibiotic-resistant pathogenic bacteria such as *Staphylococcus aureas*, *Pseudomonas arruginosa*, *Klebsiella pneumonia*, *Escherchia coli* and *Streptococcus pneumoniae* with significant minimum inhibitory concentration (MIC) values (25.0-175.0 mg/mL). However, antimicrobial activities of the plants were also reported by other researchers. According to Das et al. (1965), three carbazole alkaloids, namely, girinimbine **8**, mahanimbine **9** and murrayanine **11**, isolated from stem bark showed antifungal activity against human pathogenic fungi. 1-Formyl-3-methoxy-6-methylcarbazole **76** and 6,7-dimethoxy-1-hydroxy-3-methylcarbazole **82** were also reported to possess antibacterial and antifungal properties by Chowdhury et al. (2001). Ramsewak et al. (1999) found that mahanimbine **9**, mahanine **15** and murrayanol **98** from fresh leaves showed antimicrobial and topoisomerase I and II inhibitory activity. Essential oil and aqueous extract of leaves were found active against *Staphylococcus epidermidis*, *S. aureus* and *Streptococcus* species. Crude extract and chloroform soluble fraction and petroleum ether soluble fraction showed a promising antibacterial activity against all the tested bacteria (Akerel & Ayinde, 1998; Srivastava & Singh, 2001)**.**

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Carbazoles alkaloids represent an important heterogeneous class of anticancer agents, which have grown considerably over the last two decades. Many carbazole derivatives have been tested for the cyctotoxic activities (Table 2.3), and some of them have entered clinical trials (Asche & Demeunynck, 2007). Nagappan et al. (2011) reported that three carbazole alkaloids, namely, mahanimbine **9**, mahanine **15** and mahanimbicine **118** have shown significant antitumor activity against three cancer cell lines (MCF-7, P388 and HeLa). Ito et al. (2006) have shown that three carbazole alkaloids, *viz.*, mahanine **15**, pyrayafoline-D **112** and murrafoline-I **147**, which were isolated from *M. koenigii* leaves, were found to induce apoptosis effects on human promyelocytic leukemia cell line (HL-60) and could be used for the treatment of cancer as chemopreventive agents. Koenoline **12** isolated from root bark were found exhibited cytotoxic activity against the KB cell culture test system (Bandyopadhyaya et al., 2002). Chakraborty et al. (1997) reported that 9-formyl-3-methylcarbazole **102** isolated from the roots of *M. koenigii* displayed weak cytotoxic activity against both mouse melanoma B16 and adriamycin-resistant P388 mouse leukemia cell lines. The *in vitro* anti-tumour promoting activity and antioxidant properties of girinimbine **8** isolated from the stem bark of *Murraya koenigii* was studied by Kok et al. (2012). The *in vitro* anti-tumour promoting activity of girinimbine **8** was determined by measuring the percentage inhibition of induced early antigen (EA) of EBV on the surface of Raji cells. This compound was found able to suppress restricted early antigen (EA-R), but diffuse early antigen (EA-D) was partially suppressed when used at 32.0 μg/mL (Kok et al., 2012). Syam et al. (2011) reported that girinimbine **8** inihibited cell proliferation, produced cytotoxic and induced apoptosis on liver hepatocellular cells (HepG2). Another report by Wang, Cai, Cui, Yan, and Wu (2007) and Wang, Cai, Cui, Yan, and Wu (2008), girinimbine **8** induced apoptosis on another two type of cancer cells such

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as human, colon, colorectal adenocarcinoma cell lines (HCT-15) and human acute myelocytic leukemia cell line (K562). Work also done on another carbazole alkaloid, mahanine **15**, isolated from *M. koenigii* and has found to induce apoptosis in human promyelocytic leukemia cell line (HL-60) (Roy, Thalang, Trakoontivakorn, & Nakahara, 2004). Another study reported on mahanine **15**, which was purified from the leaves of *M. koenigii*, has a dose- and time-dependent antiproliferative activity in acute lymphoid (MOLT-3) and chronic myeloid (K562) leukemic cell lines and in the primary cells of leukemic and myeloid patients, with minimal effect on normal immune cells including CD34(+) cells (Bhattacharya et al. 2010). Itoigawa et al. (2000) reported that murrayaquinone-A **108** demonstrated significant cytotoxicity against SK-MEL-5 and Colo-205 cells, with ED_{50} values of 2.58 and 3.85 μg/mL, respectively. In contrast, the report also showed that murrayafoline-A **13** exhibited marginal or weak cytotoxicity against SK-MEL-5, Colo-205, HCT-8, KB, and A-549 tumor cell lines, with ED_{50} values ranging from 5.31 to 7.52 μg/mL (Itoigawa et al., 2000). Study of cytotoxic activity on the isolated carbazole alkaloid, *viz.*, girinimbine **8**, mahanimbine **9**, murrayanine **11**, koenoline **12**, murrayazolidine **17**, murrayazoline **19**, mahanimbinol **43** and murrayaquinone-A **108**, against KB, MCF-7 and NCI-H187 were also reported (Tantapakul et al., 2014). Another report on cytotoxic and anti-tumour promoting activity on the crude extract and isolated compounds from the roots of *M. koenigii* were also reported. All crude extracts of the roots including the isolated compounds, mahanimbine **9**, mahanine **15** and murrayafoline-A **13** exhibited significant cytotoxic activity against CEM-SS cell line with IC_{50} 3 μg/mL. While, girinimbine **8** were found that inhibited EBV-activation in the antitumor promoting assay (Ahmad et al., 2014).

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There are other several important studies on the pharmacological properties of *M. koenigii* such as anti-inflammatory activity (Darvekar, Patil, & Choudhari, 2011; Gupta, George, Singhal, Sharma, & Grag, 2010; Mathur, Prasad, & Dua, 2011; Mathur, Verma, Singh, Prasad, & Dua, 2011; Mohammed Usman & Barhate, 2012; Parmar, Gangwal, & Sheth, 2010; Ramsewak et al., 1999), hypolipidemic effects (Birari et al., 2010; Dineshkumar et al., 2010; Kumar et al., 2010; and Lawal et al., 2008,), anti-amnesic and wound healing (Patidar, Yadav, Nakra, Sharma, & Bagherwal, 2010; Vasudevan & Parle, 2009), anti-diarrhoeal (Mandel et al., 2008; Mandal et al., 2010; Sharma et al., 2012), hepatoprotective (Ghosh et al., 2013; Ma et al., 2013; Sathaye et al., 2011; Sathaye et al., 2012), analgesic (Gupta et al., 2010), antinociceptive activities (Patil, Langeda, Dighade, & Hiray, 2012), anti-trichromonal (Adebajo et al., 2004; Adebajo et al., 2006) and promoting hair growth (Hamada, Okui, Sugimoto, & Komaki, 2005, Rao et al., 2012), were also reported recently by various researchers. Several pharmacological properties on the chemical constituents isolated from *M. koenigii* are listed in the Table 2.3.

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Table 2.3

Pharmacological Properties of Carbazole Alkaloids Isolated from M. koenigii

No.	Carbazole Alkaloids	Pharmacological Properties
76	1-formyl-3-methoxy-6-methylcarbazole	1. Antimicrobial (Chowdhury et al., 2001)
40	3-formylcarbazole	1. Anti-trichomonal (Adebajo et al., 2009)
34	3-methylcarbazole	1. Anti-inflammatory (Taechowisan, Chanaphat, Ruensamran, & Phutdhawong, 2012)
82	6,7-dimethoxy-1-hydroxy-3-methylcarbazole	1. Antimicrobial (Chowdhury et al., 2001)
102	9-Formyl-3-methylcarbazole	1. Cytotoxic (B16, P338) (Chakraborty et al., 1997)
16	Bismurrayafoline-E	1. Antioxidant (Tachibana et al., 2003)
85	Euchrestine-B	1. Antioxidant (Tachibana et al., 2003)
8	Girinimbine	1. Antimicrobial (Das et al., 1965) 2. Anti-tumour promoting (Ahmad et al., 2014; Kok et al., 2012) 3. Antioxidant (Kok et al., 2012) 4. Cytotoxic and Induce apoptosis (HepG2) (Syam et al., 2011) 5. Induce apoptosis (HCT-15, K562) (Wang et al., 2007; Wang et al., 2008) 6. Induce apoptosis (A549) (Mohan et al., 2013) 7. Anti-platelet (Ko, Lee, Wu, & Teng, 1994) 8. Cytotoxic (KB, MCF-7, NCI-H187) (Tantapakul et al., 2014)
114	Koenigine	1. Antioxidant (Rao et al., 2007)
115	Koenimbine	1. Anti-diarrhoeal (Mandal et al., 2010)
12	Koenoline	1. Cytotoxic (KB cell) (Bandyopadhyaya et al., 2002) 2. Cytotoxic (KB, MCF-7, NCI-H187) (Tantapakul et al., 2014)
117	Kurryam	1. Anti-diarrhoeal (Mandal et al., 2010)

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Table 2.3 (Continued)

No.	Carbazole Alkaloids	Pharmacological Properties
118	Mahanimbicine	1. Antimicrobial (Nagappan et al., 2011) 2. Antitumour (MCF-7, P338, HeLa) (Nagappan et al., 2011)
9	Mahanimbine	1. Antidiabetic (Dineshkumar et al., 2010) 2. Antioxidant (Kureel et al., 1970; Ramsewak et al., 1999; Rao et al., 2007) 3. Antimicrobial (Das et al., 1965; Nagappan et al., 2011; Ramsewak et al., 1999) 4. Antitumour (MCF-7, P338, HeLa) (Nagappan et al., 2011) 5. Anti-mosquitocidal (Ramsewak et al., 1999) 6. Hypolipidemic Effects (Dineshkumar et al., 2010) 7. Cytotoxic (KB, MCF-7, NCI-H187) (Tantapakul et al., 2014) 8. Cytotoxic (CEM-SS) (Ahmad et al., 2014)
43	Mahanimbinol	1. Cytotoxic (KB, MCF-7, NCI-H187) (Tantapakul et al., 2014)
15	Mahanine	1. Antidiabetic (Biswas et al., 2010) 2. Antioxidant (Tachibana et al., 2003) 3. Antimicrobial (Nagappan et al., 2011; Ramsewak et al., 1999) 4. Antitumour (MCF-7, P338, HeLa) (Nagappan et al., 2011) 5. Induced apoptosis effect (HL-60) (Ito et al., 2006; Roy et al., 2004) 6. Antiproliferation (MOLT-3, K562) (Bhattacharya et al., 2010) 7. Anti-mosquitocidal (Ramsewak et al., 1999) 8. Cytotoxic (CEM-SS) (Ahmad et al., 2014)
13	Murrayafoline-A	1. Cytotoxic (SK-MEL-5, Colo-205) (Itoigawa et al., 2000) 2. Cytotoxic (CEM-SS) (Ahmad et al., 2014)
147	Murrafoline-I	1. Induced apoptosis effect (HL-60) (Ito et al., 2006)
11	Murrayanine	1. Antimicrobial (Das et al., 1965) 2. Cytotoxic (KB, MCF-7, NCI-H187) (Tantapakul et al., 2014)
96	Murrayanol	1. Antimicrobial (Ramsewak et al., 1999) 2. Anti-inflammatory (Ramsewak et al., 1999) 3. Anti-mosquitocidal (Ramsewak et al., 1999)

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Table 2.3 (Continued)

No.	Carbazole Alkaloids	Pharmacological Properties
108	Murrayaquinone-A	1. Cytotoxic (SK-MEL-5, Colo-205) (Itoigawa et al., 2000) 2. Cytotoxic (KB, MCF-7, NCI-H187) (Tantapakul et al., 2014)
17	Murrayazolidine	1. Cytotoxic (KB, MCF-7, NCI-H187) (Tantapakul et al., 2014)
19	Murrayazoline	1. Cytotoxic (KB, MCF-7, NCI-H187) (Tantapakul et al., 2014)
112	Pyrayafoline-D	1. Induced apoptosis effect (HL-60) (Ito et al., 2006)

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CHAPTER 3

MATERIALS AND METHODS

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3.1 Phytochemical Analysis on *Murraya koenigii*

The following described the details analytical methodologies involved in the phytochemical analysis of *Murraya koenigii* (Linn.) Spreng.

3.1.1 General Methods

1D (^{1}H, ^{13}C, DEPT) and 2D (^{1}H-^{1}H COSY, NOESY, TOCSY, HMQC, HMBC) Nuclear Magnetic Resonance spectra (NMR) were carried out using JEOL ECX (500 MHz). Deuterated chloroform ($CDCl_3$) and deuterated methanol (CD_3OD) were used as NMR solvent. Tetramethylsilane (TMS) was used as the internal standard. Chemical shifts (δ) was reported in ppm and coupling constants (*J*) in Hz and

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internally referred to the solvent signals in $CDCl_3$ [^{1}H: δ 7.26 (*s*); ^{13}C: δ 77.1] or CD_3OD [^{1}H: δ 4.80 (*s*) and δ 3.35 (*m*); ^{13}C: δ 49.0].

The mass spectra (HRESIMS) were obtained on an Agilent Technologies 6530 Accurate-Mass-Q-TOF LC/MS with a ZORBAX Agilent Eclipse XDB-C18 rapid resolution HT 4.6 mm × 50 mm × 1.8 μm column (P.N.927975.902). The column temperature was at 40 °C. Auto sampler temperature was ambient. Solvents used as mobile phase with ratio A : B (10% water : 90% methanol). Sample flow rate at 0.25 mL/min. The injection volume was 1 μL. The mass range between *m/z* 100 to 1000.

Optical rotation ($[\alpha]_D^{25}$) were measured on an Autopol III Automatic Polarimeter (Rudolph Research Analtytical). Rotation of degrees were measured using 0.01 – 0.02 g/100 mL of sample which dissolve in methanol for 100 mm path length of the solution at 25 °C by using D-line sodium lamp (589 nm).

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The ultraviolet spectra (UV) were obtained in CH_3OH on a Perkin Elmer UV-visible spectrophotometer and the wavelength of the spectrum was determined in the range of 200 to 400 nm. The infrared spectra (IR) were recorded on a Nicolet 6700 FTIR spectrophotometer, with CH_3OH as dilution solvent of the sample.

Column chromatography was prepared by using Silica Gel 60F 70 – 230 mesh ASTM (Merck 1.07734.1000), Silica Gel 60F 230 – 400 mesh ASTM (Merck 1.09385.1000) or Sephadex® LH-20 silica (Sigma Aldrich LH20100) as stationary phase. The ratio of silica gel to the sample was approximately 30:1 for crude samples

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with flow rate 1 second per drop and 100:1 for fractions that need further purification with flow rate 3 second per drop. The normal silica gel was made into slurry with hexane before it was packed onto the column and was allowed to equilibrate for at least an hour before use. The Sephadex® LH-20 silica was made into slurry in CH_2Cl_2: MeOH (1:1).

Analytical thin layer chromatography (TLC) was used to separate and detect various chemical entities present in the crude extracts, fractions from chromatography, and isolated pure compounds. TLC sheets used were commercially pre-coated aluminium plates, silica gel $60F_{254}$ (Merck 1.05554.0001) with 0.25 mm in thickness. The TLC plates were spotted with a piece of fine glass capillary tube and then developed in saturated chromatographic tanks with various solvent systems at room temperature. The dark spots were visualized by examination of the TLC plates under UV light (254 nm), followed by spraying with 5.0% solution of sulphuric acid.

Preparative TLC was prepared by using Silica Gel 60 containing Gypsum F_{254} (Merck 1.07749.1000) on 20 cm × 20 cm glass plate equally. It was applied on the glass plate as slurry with the aid of Brickmann apparatus. The plate was dried in an oven at 100 ^{o}C for two hour to activate the plate. After being cooled in room temperature, it is ready to be used for isolation and separation process.

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3.1.2 Chemical Reagents

Preliminary screening of the alkaloids found in the crude extract was done by using Mayer's reagent (potassium mercuric iodide). A positive result was indicated when white precipitate was formed under acidic condition. The Mayer's reagent was prepared by mixing a solution of 1.4 g of mercuric iodide in 60 mL distilled water together with a solution of 5.0 g of potassium iodide in 10 mL of distilled water.

A 5.0% solution of sulphuric acid was used as spraying reagents to detect the presence of terpenes and carbazole alkaloids spotting on the TLC plates. A positive result is indicated by the formation of reddish purple spot for terpene and blue to purple spot for carbazole alkaloids on the TLC plates once the plate is heated to 110 °C for 5 minutes.

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The industrial grade solvents, such as *n*-hexane (Hex), dichloromethane (CH_2Cl_2), ethyl acetate (EA) and methanol (CH_3OH), were used for the extraction, isolation and purification processes. These solvents were distilled before used in the chromatographic separation of the compounds.

Spectroscopic grade solvents such as LiChrosolv® Reag. Ph Eur methanol, deuterated chloroform ($CDCl_3$) and deuterated methanol (CD_3OD) were used for the spectroscopic analysis.

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3.1.3 Plant Materials

The species selected for the current study is *Murraya koenigii* (Linn.) Spreng which was collected from Jerantut, Pahang, Malaysia in February 2012. This species was collected and identified by the Phytochemistry Group, Chemistry Department, Faculty Science and Mathematics, Universiti Pendidikan Sultan Idris. The voucher specimen (TM1006) was deposited at the Herbarium, Chemistry Department, Faculty Science and Mathematics, Universiti Pendidikan Sultan Idris, Tanjung Malim, Perak, Malaysia.

3.1.4 Extraction Methods

The dried bark and leaves of the plant were powdered and separately defatted in *n*-hexane for 72 hours. The extracts were dried under reduced pressure. After dried, the residue of various parts of the plant materials were then separately immersed with 25% ammonia solution and left for 2 hours at room temperature. This is to extract out the nitrogen-containing compounds from the plant materials. Then, the plant materials were reextracted sequentially by using dichloromethane (CH_2Cl_2) and methanol (CH_3OH), each 72 hours, respectively. The crude extracts were then concentrated under reduced pressure using rotary-evaporator. Subsequently, the CH_3OH crude extracts for both the plant materials were separately undergone solvent-solvent partitioning by using ethyl acetate (EA) and dichloromethane (DCM) to obtain EA and DCM crude extracts. The details extraction process were shown in the Scheme 3.1 to Scheme 3.2. The yield of the dried crude extracts obtained were recorded in Table 3.1.

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Table 3.1

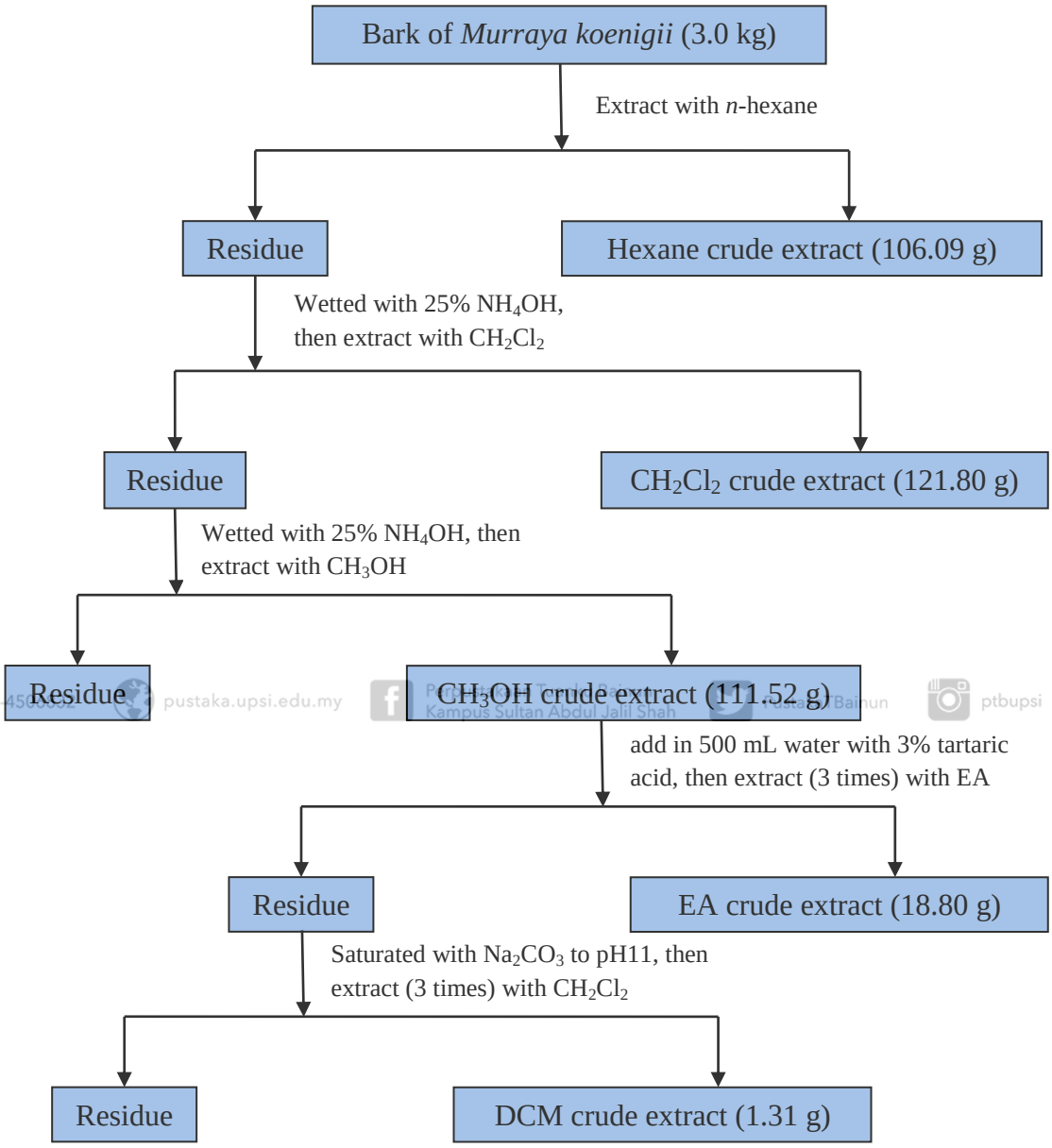
Yields of Plant Materials and Crude Extracts

	Bark	Leaves
Plant Materials	3.00 kg	0.62 kg
Hexane Crude	106.09 g	21.78 g
CH_2Cl_2 Crude	121.80 g	36.14 g
CH_3OH Crude	111.52 g	75.50 g

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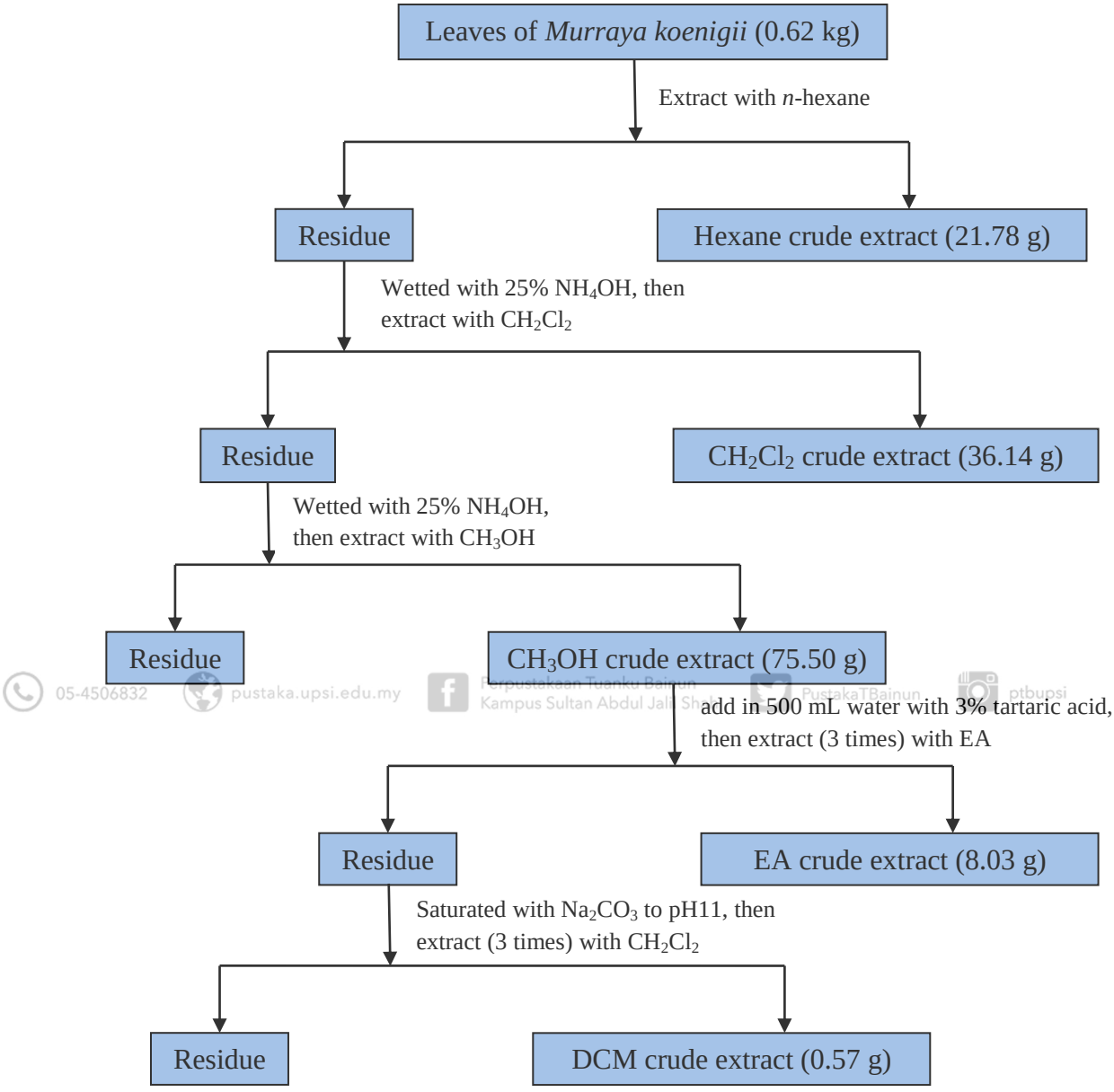
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Scheme 3.1. Extraction Process of *Murraya koenigii* (Bark)

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Scheme 3.2. Extraction Process of *Murraya koenigii* (Leaves)

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3.1.5 Isolation and Purification

For preliminary fractionation, the crude extracts from bark and leaves of *Murraya koenigii* were separately loaded to column chromatography over silica gel and eluted with a gradient mixture of *n*-hexane, dichloromethane and methanol by increasing its polarity. The amount of silica gel used was on the ratio of 1 g of crude extract to 30 g of silica gel.

Eluents were collected in fractions and were concentrated using rotary-evaporator. Then, each concentrated fraction was monitored by the development of TLC in a closed tank with a suitable solvent system, which the atmosphere in the tank was saturated with the vapour of the eluent. The developed TLC was then visualized under visible and ultra-violet light (254 nm). Fractions which having spots with same retention factor (R_f) value on the TLC were grouped together. Each grouped fraction was treated separately by extensive column chromatography by using normal silica, Sephadex® LH-20 silica or preparative TLC for further purification process. The purified constituent was determined by a single spot on the TLC which was visualized under visible and ultra-violet light (254 nm). Initial characterization of different compounds was facilitated by measuring the R_f values of each TLC system.

The isolation of chemical entities from the bark and leaves of the plants were summarized in Scheme 3.3 to Scheme 3.7, respectively.

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3.1.5.1 Chemical Constituents from Bark of *M. koenigii*

Extraction of the bark of *M. koenigii* (3.0 kg) with *n*-hexane yielded a brown syrup of hexane crude extract (106.09 g). 30.0 g of hexane crude extract was then subjected to column chromatography over normal silica gel and eluted with a gradient mixture of *n*-hexane, dichloromethane and methanol by increasing its polarity to give four fractions labelled as BH1 to BH4. Fractions BH1 and BH2 were combined and rechromatographed over a silica gel column eluted with Hex-CH_2Cl_2 (1:1) yielded three carbazole alkaloids; murrayafoline-A (**Mk2**, 27 mg), girinimbine (**Mk12**, 2.7 g) and mahanimbine (**Mk15**, 1.8 g). Fraction BH4 was also rechromatographed over silica gel column eluting successively with gradient mixtures of Hex, CH_2Cl_2 and CH_3OH yielded seven known carbazoles; murrayanine (**Mk1**, 142 mg), mukoenine-B (**Mk8**, 8 mg), murrayaquinone-A (**Mk10**, 9 mg), girinimbine (**Mk12**, 126 mg), mahanimbine (**Mk15**, 55 mg), murrayamine-J (**Mk19**, 5 mg) and murrayazolinol (**Mk23**, 12 mg), along with four non-carbazoles; β-sitosterol (**Mk31**, 11 mg), stigmast-4-ene-3-one (**Mk32**, 13 mg), squalene (**Mk33**, 11 mg) and 2-hydroxy-4-methoxy-3,6-dimethylbenzoic acid (**Mk35**, 5 mg). A total of 12 chemical entities were isolated from the hexane crude extract of the bark. The details of isolation process was summarized in Scheme 3.3.

The residues of the bark were then reextracted with dichloromethane at room temperature to give dark brown syrup (121.8 g) of CH_2Cl_2 crude extract. 50 g of the concentrated CH_2Cl_2 extract was subjected to silica gel column chromatography and eluted with *n*-hexane, Hex-CH_2Cl_2 (9:1, 3:2, 1:1, 2:3), CH_2Cl_2, CH_2Cl_2-CH_3OH (99:1, 9:1) and CH_3OH, successively to give six fractions labelled as BD1 to BD6. Fraction

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BD2 was subjected to preparative TLC developed with Hex-CH_2Cl_2 (1:1) to afford girinimbine (**Mk12**, 12 mg), mahanimbine (**Mk15**, 11 mg) and bicyclomahanimbine (**Mk25**, 8 mg). Fraction BD3 was rechromatographed over a silica gel column, eluting successively with gradient mixtures of *n*-hexane, CH_2Cl_2 and methanol afforded four known carbazole alkaloids; murrayafoline-A (**Mk2**, 12 mg), mahanimbilol (**Mk6**, 14 mg), girinimbine (**Mk12**, 24 mg) and murrayakoeninol (**Mk24**, 8 mg), along with a new biscarbazole alkaloid, bismahanimboline (**Mk30**, 5 mg). Fraction BD5 was also subjected to a silica gel column afforded four fractions labelled as BD5-1 to BD5-4. Fraction BD5-2 was subjected to preparative TLC developed with CH_2Cl_2 yielded two known and a new carbazole alkaloids; murrayaquinone-B (**Mk11**, 5 mg), murrayacine (**Mk14**, 8 mg) and murrastinine-A (**Mk20**, 8 mg), respectively. Fraction BD5-3 was also subjected to preparative TLC developed with CH_2Cl_2-CH_3OH (98:2) to afford a known carbazole; koenoline (**Mk3**, 12 mg), and a new carbazole alkaloid; murrastinine-B (**Mk21**, 6 mg). Fraction BD5-4 was also subjected to preparative TLC developed with CH_2Cl_2-CH_3OH (95:5) afforded another two carbazole alkaloids; 2-hydroxy-3-methylcarbazole (**Mk5**, 9 mg) and a new carbazole; murrastinine-C (**Mk22**, 8 mg). A total of 14 carbazole alkaloids consists of 10 known and four new carbazoles were successfully isolated from the CH_2Cl_2 crude extract from the bark of *M. koenigii*. The isolation of chemical entities from the CH_2Cl_2 extract of *M. koenigii* was summarized in Scheme 3.4.

Further, the residues from the extraction process were then reextracted with methanol to give 111.52 g of methanol crude extract. Solvent-solvent partitioning (Scheme 3.5) were done by using ethyl acetate (EA) and dichloromethane (DCM) to obtain EA crude extract (18.80 g) and DCM crude extract (2.17 g). EA crude extract

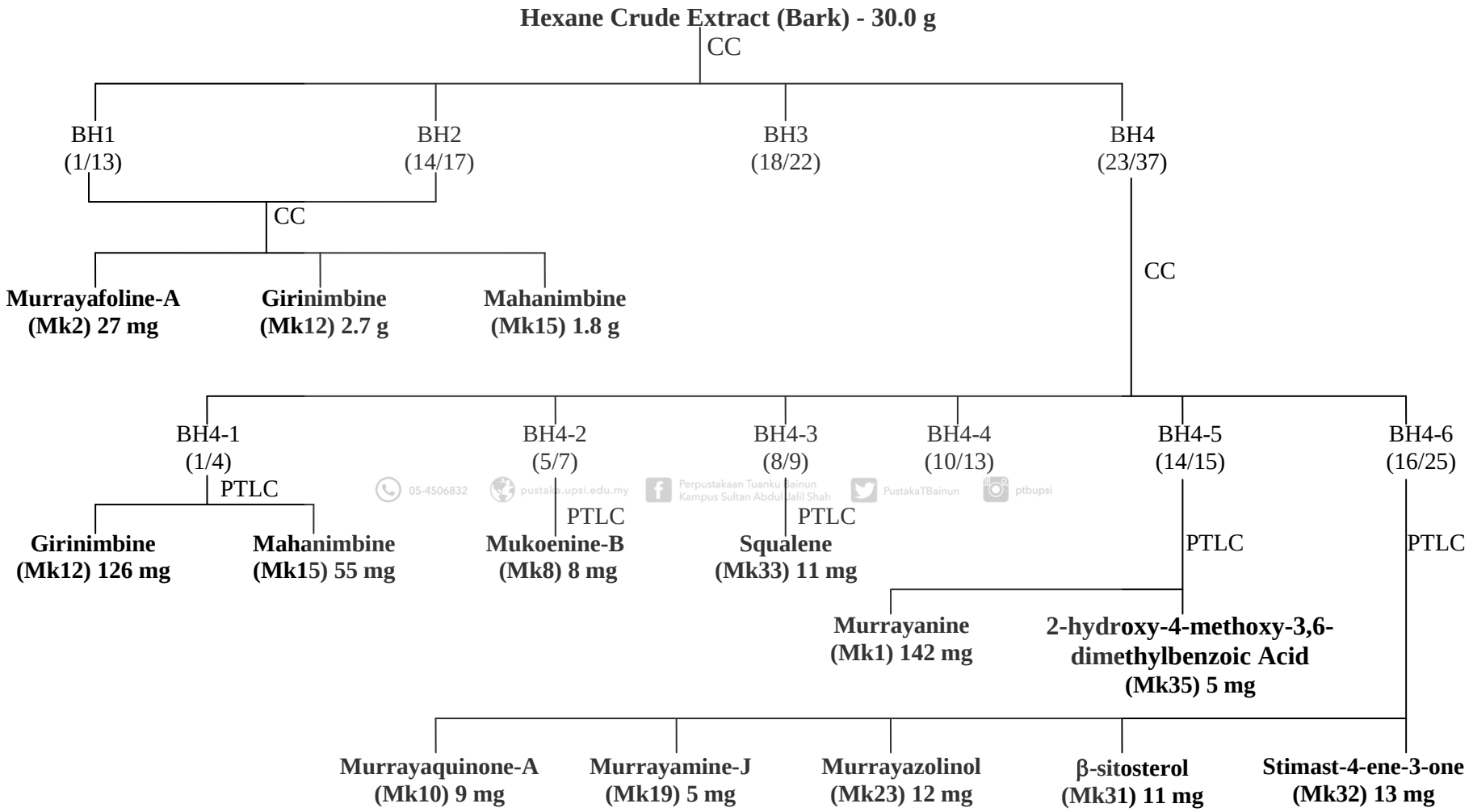
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was then subjected to a silica gel column afforded four fractions labelled as BEA1 to BEA4. Fraction BEA2 was subjected to preparative TLC developed with CH_2Cl_2 yielded two carbazole alkaloids; murrayafoline-A (**Mk2**, 8 mg) and mahanimbine (**Mk15**, 12 mg). Fraction BEA4 was also subjected preparative TLC developed with CH_2Cl_2 yielded a known and a new carbazole alkaloids; koenoline (**Mk3**, 16 mg) and murrastanine-A (**Mk9**, 9 mg), respectively. The DCM crude extract was also subjected to silica gel column chromatograph afforded four fractions (BDCM1 to BDCM4). Fraction BDCM2 was subjected to preparative TLC developed with CH_2Cl_2 yielded two alkaloids; murrayafoline-A (**Mk2**, 6 mg) and mahanimbine (**Mk15**, 12 mg). Fraction BDCM3 was also subjected to preparative TLC developed with CH_2Cl_2-CH_3OH (99:1) yielded another two carbazoles; murrayanine (**Mk1**, 6 mg) and koenoline (**Mk3**, 14 mg). While fraction BDCM4 was also subjected to column chromatography eluted with CH_2Cl_2-CH_3OH (1:1) using Sephadex® LH-20 silica afforded two known carbazole alkaloids; murrayanine (**Mk1**, 6 mg) and 3-formylcarbazole (**Mk4**, 6 mg). A total of seven carbazoles were obtained, including a new carbazole from the methanol crude extract (Scheme 3.5).

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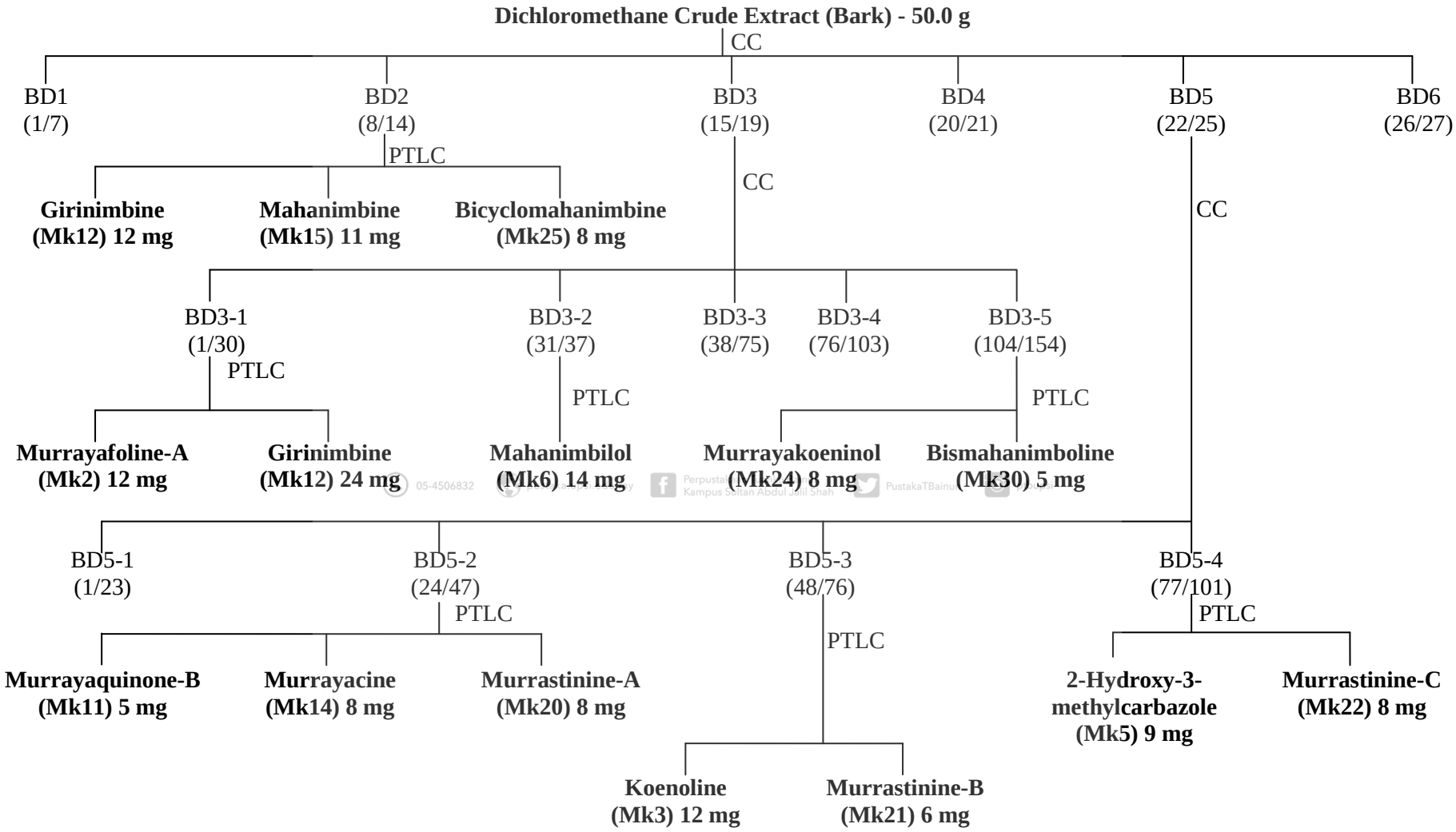
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Scheme 3.3. Isolation of Chemical Constituents of Hexane Crude Extract (Bark)

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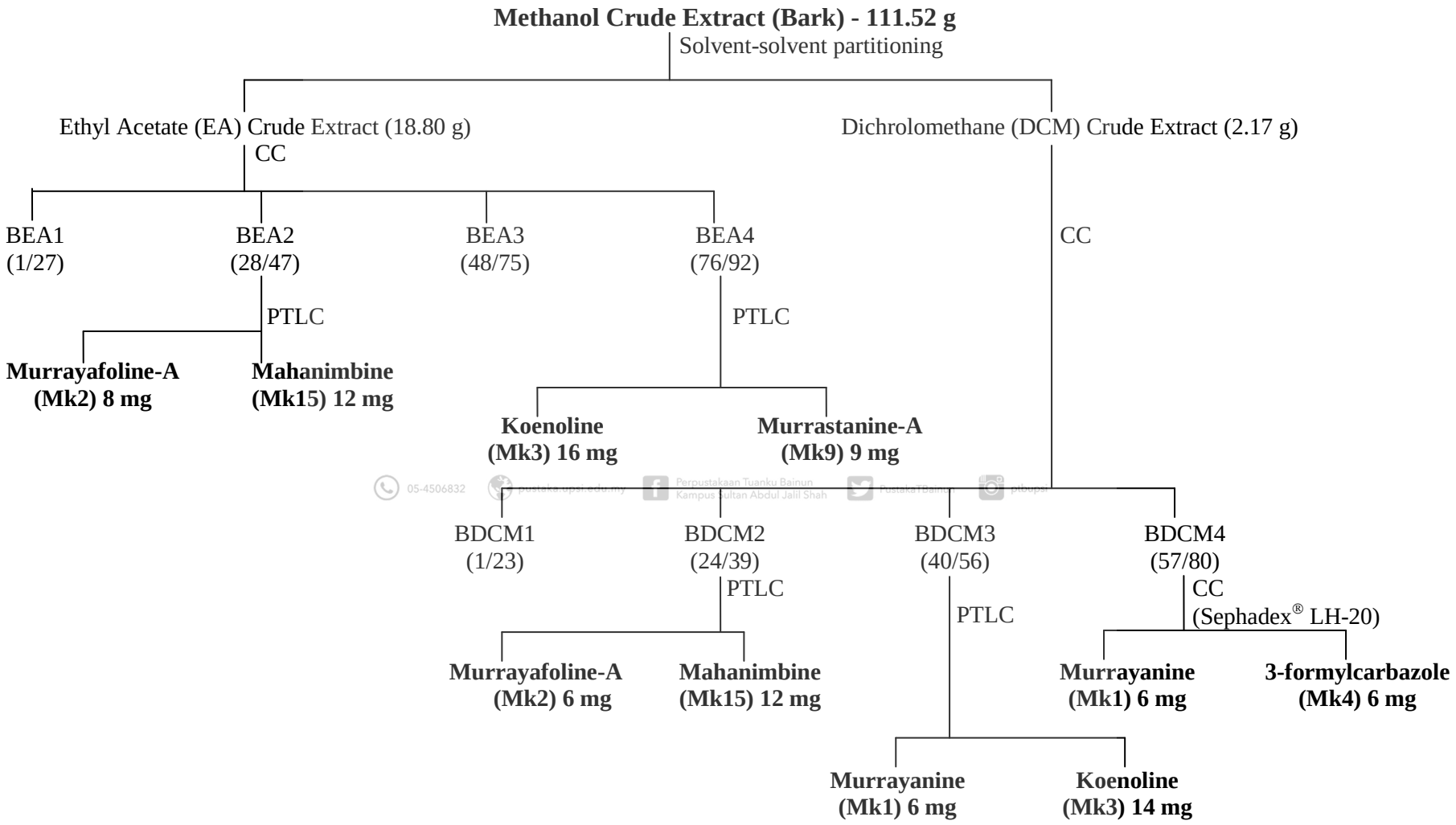
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Scheme 3.4. Isolation of Chemical Constituents of Dichloromethane Crude Extract (Bark)

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Scheme 3.5. Isolation of Chemical Constituents of Methanol Crude Extract (Bark)

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3.1.5.2 Chemical Constituents from Leaves of *M. koenigii*

The dried leaves (0.62 kg) of *M. koenigii* were extracted with *n*-hexane at room temperature yielded light brown (21.78 g) of hexane crude extract. The hexane crude extract (20.0 g) was subjected to silica gel column chromatography eluted with Hex, Hex-CH_2Cl_2 (9:1, 8:2, 1:1, 2:8), CH_2Cl_2, CH_2Cl_2-CH_3OH (99:1, 95:5, 1:1), CH_3OH, successively, gave seven fractions and labelled as LH1 to LH7. Fraction LH2 and LH3 were combined and subjected to preparative TLC developed with Hex-CH_2Cl_2 (1:1) to yield murrayafoline-A (**Mk2**, 12 mg) and mahanimbine (**Mk15**, 23 mg). Fraction LH5 was rechromatographed over silica gel column and eluted with a gradient mixture of *n*-hexane, dichloromethane and methanol by increasing its polarity to give six fractions (LH5-1 to LH5-6). Fraction LH5-2 subjected to preparative TLC developed with Hex-CH_2Cl_2 (3:2) yielded two carbazoles; murrayafoline-A (**Mk2**, 6 mg) and mahanimbine (**Mk15**, 9 mg). Fraction LH5-4 subjected to preparative TLC developed with Hex-CH_2Cl_2 (1:1) yielded mahanimbine (**Mk15**, 8 mg) and a compound with terpene side chain; α-tocopherol (**Mk34**, 15 mg). Fraction LH5-5 subjected to preparative TLC developed with Hex-CH_2Cl_2 (1:4) yielded another non-carbazole entities; squalene (**Mk33**, 11 mg). Fraction LH6 was further purified by silica gel column and eluted with a gradient mixture of *n*-hexane, dichloromethane and methanol by increasing its polarity to afford six fractions (LH6-1 to LH6-6). Fraction LH6-2 subjected to preparative TLC developed with Hex-CH_2Cl_2 (1:1) yielded murrayafoline-A (**Mk2**, 5 mg), mahanimbine (**Mk15**, 8 mg) and murrayamine-B (**Mk18**, 9 mg). Fraction LH6-4 was also subjected to preparative TLC developed with Hex-CH_2Cl_2 (1:4) yielded a known carbazole, koenimbine (**Mk13**, 11 mg). Fraction LH6-6 subjected to another preparative TLC developed with CH_2Cl_2

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yielded a biscarbazole alkaloid; bismurrayafoline-E (**Mk27**, 8 mg). There are five carbazoles and two non-carbazole compounds were afforded from the hexane extract from the leaves of *M. koenigii* (Scheme 3.6).

The residues of the leaves were then reextracted with CH_2Cl_2 at room temperature to give dark brown syrup (36.14 g) of CH_2Cl_2 crude extract. 30 g of the concentrated extract was subjected to silica gel column chromatography and eluted with a gradient mixture of *n*-hexane, dichloromethane and methanol by increasing its polarity gave nine fractions and labelled as LD1 to LD9 (Scheme 3.7). Fractions LD1 and LD2 were combined and subjected to preparative TLC afforded mahanimbine (**Mk15**, 11 mg). Fraction LD4 was also subjected to preparative TLC and afforded a known and a new carbazole alkaloid; mahanimbine (**Mk15**, 12 mg) and murrayatanine-A (**Mk26**, 6 mg), respectively. Fraction LD6 was subjected to preparative TLC and developed with CH_2Cl_2 afforded three carbazole alkaloids; euchrestine-B (**Mk7**, 9 mg), bismurrayafoline-E (**Mk27**, 11 mg) and bispyrayafoline (**Mk28**, 8 mg). While LD7 was rechromatographed over silica gel column to give five fractions (LD7-1 to LD7-5). There are two carbazoles and one sesquiterpene were afforded from this fraction; bismurrayamine-E (**Mk27**, 5 mg), bisisomahanine (**Mk29**, 9 mg) and selin-11-en-4α-ol (**Mk36**, 9 mg), respectively. Fraction LD8 was subjected to preparative TLC and developed with CH_2Cl_2 afforded three carbazoles; mahanine (**Mk16**, 12 mg), isomahanine (**Mk17**, 5 mg) and bismurrayafoline-E (**Mk27**, 11 mg). There are a total of nine chemical compounds were afforded; seven known and a new carbazole alkaloids, along with a sesquiterpene were isolated from this crude extracts (Scheme 3.7).

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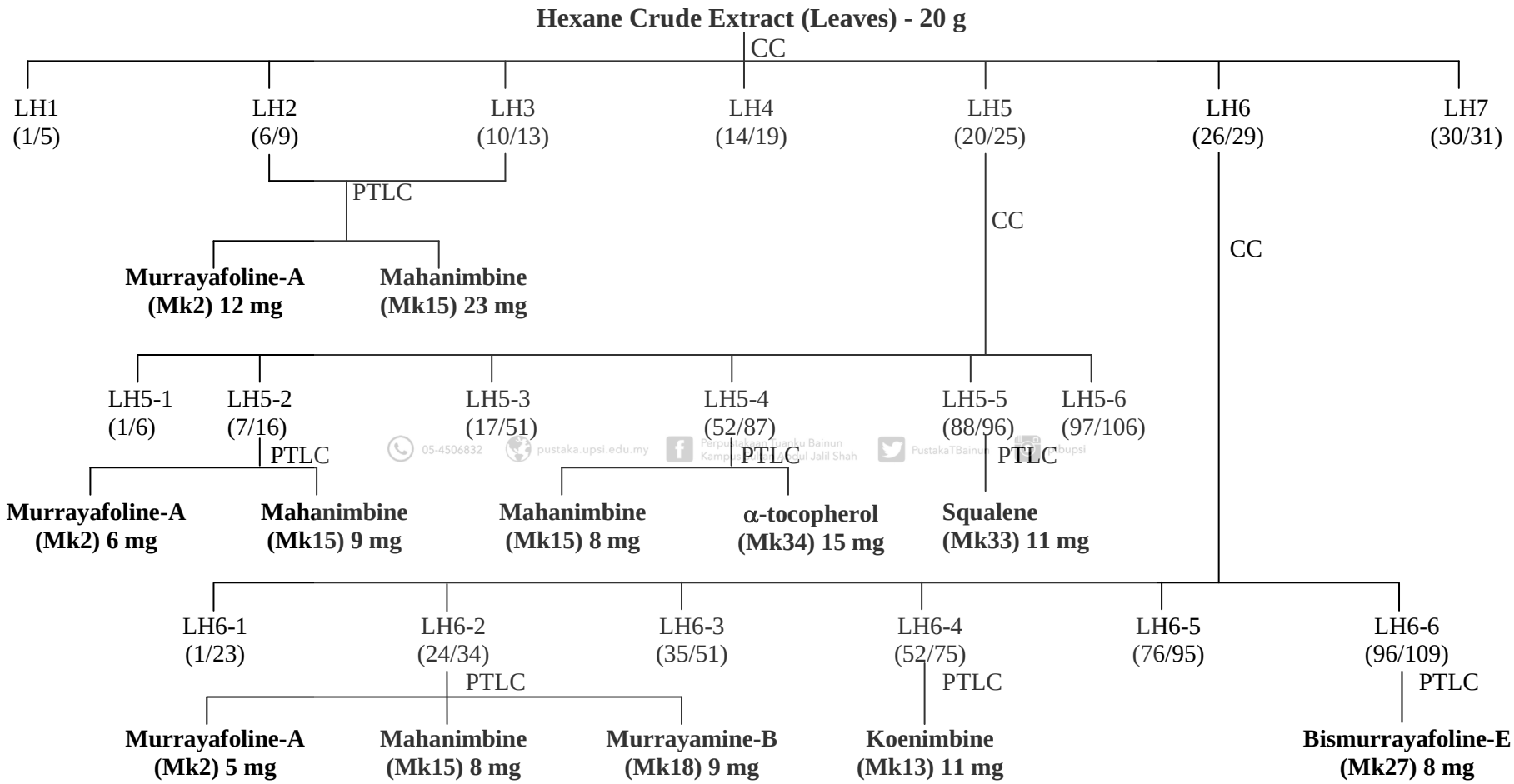
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Solvent-solvent partitioning (Scheme 3.2) were also done by using EA and DCM to obtain LEA crude extract (0.87 g) and LDCM crude extract (0.12 g), respectively. Unfortunately, no alkaloid was afforded from these crude extract. Thus, only 11 carbazoles and three non-carbazoles were successfully isolated the leaves of *M. koenigii* crude extracts.

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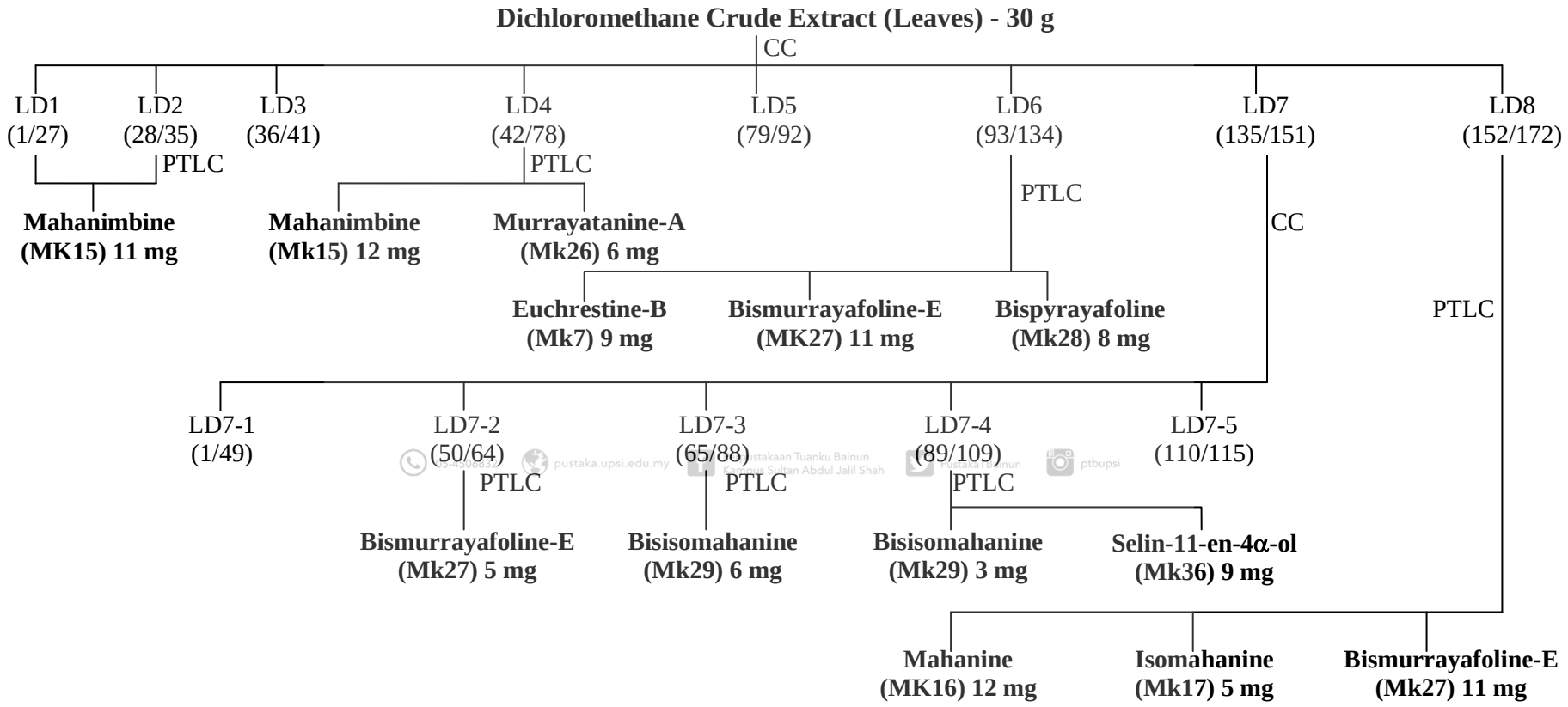
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Scheme 3.6. Isolation of Chemical Constituents of Hexane Crude Extract (Leaves)

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Scheme 3.7. Isolation of Chemical Constituents of Dichloromethane Crude Extract (Leaves)

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3.1.6 Physical and Spectral Data of Isolated Chemical Constituents from *M. koenigii*

Murrayanine (Mk1) : Silver plate solid

Molecular Formula : $C_{14}H_{11}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 238 (4.48), 249 (4.39), 273 (4.38), 288 (4.24), 329 (4.26)

IR (CH_3OH) ν_{max}, cm^{-1} : 3369, 1637, 1019

HRESIMS, *m/z* : 226.0856 $[M+H]^+$

Melting point, °C : 155–156

^1H NMR ($CDCl_3$), δ ppm :

7.47 (*s*, H-2, 1H), 8.20 (*s*, H-4, 1H), 8.12 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.33 (*td*, *J* = 8.0, 1.1 Hz, H-6, 1H), 7.49 (*td*, *J* = 8.0, 1.1 Hz, H-7, 1H), 7.52 (*d*, *J* = 8.0 Hz, H-8, 1H), 8.48 (*br s*, NH, 1H), 4.08 (*s*, 1-OCH_3, 3H), 10.06 (*s*, 3-CHO, 1H).

^{13}C NMR ($CDCl_3$), δ ppm :

146.2 (C-1), 134.1 (C-1a), 103.6 (C-2), 130.3 (C-3), 120.5 (C-4), 123.7 (C-4a), 120.8 (C-5), 123.7 (C-5a), 120.8 (C-6), 126.7 (C-7), 111.7 (C-8), 139.5 (C-8a), 55.9 (1-OCH_3), 192.0 (3-CHO).

Murrayafoline-A (Mk2) : Brownish oil

Molecular Formula : $C_{14}H_{13}NO$

UV (CH_3OH) λ_{max} (log ε), nm : 237 (4.36), 249 (4.25), 268 (4.31), 290 (4.24)

IR (CH_3OH) ν_{max}, cm^{-1} : 3366, 2257, 1374, 1019

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HRESIMS, *m/z* : 212.1063 $[M+H]^+$

^{1}H NMR ($CDCl_3$), δ ppm :

6.75 (*s*, H-2, 1H), 7.49 (*s*, H-4, 1H), 8.03 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.21 (*td*, *J* = 8.0, 1.2 Hz, H-6, 1H), 7.40 (*td*, *J* = 8.0, 1.2 Hz, H-7, 1H), 7.43 (*d*, *J* = 8.0 Hz, H-8, 1H), 8.17 (*br s*, NH, 1H), 4.00 (*s*, 1-OCH_3, 3H), 2.55 (*s*, 3-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

145.4 (C-1),129.5 (C-1a), 107.7 (C-2), 128.0 (C-3), 112.6 (C-4), 125.7 (C-4a), 120.5 (C-5), 123.8 (C-5a), 119.2 (C-6), 125.6 (C-7), 111.0 (C-8), 139.5 (C-8a), 55.5 (1-OCH_3), 22.0 (3-CH_3).

Koenoline (Mk3) : Colourless needles

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Molecular Formula : $C_{14}H_{13}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 232 (4.24), 251 (4.01), 290 (4.23), 324 (4.16)

IR (CH_3OH) ν_{max}, cm^{-1} : 3367, 3235, 2258, 1019

HRESIMS, *m/z* : 227.1531 $[M]^+$

Melting point, °C : 129–130

^{1}H NMR ($CDCl_3$), δ ppm :

6.94 (*s*, H-2, 1H), 7.65 (*s*, H-4, 1H), 8.03 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.22 (*td*, *J* = 8.0, 1.5 Hz, H-6, 1H), 7.41 (*td*, *J* = 8.0, 1.5 Hz, H-7, 1H), 7.45 (*d*, *J* = 8.0 Hz, H-8, 1H), 8.32 (*br s*, NH, 1H), 4.00 (*s*, 1-OCH_3, 3H) 4.83 (*s*, 3-CH_2OH, 2H), 1.66 (*br s*, 3-CH_2OH, 1H).

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^{13}C NMR ($CDCl_3$), δ ppm :

145.8 (C-1), 132.9 (C-1a), 105.7 (C-2), 129.5 (C-3), 111.8 (C-4), 124.1 (C-4a), 120.6 (C-5), 123.6 (C-5a), 119.5 (C-6), 125.9 (C-7), 111.1 (C-8), 139.5 (C-8a), 55.6 (1-OCH_3), 66.5 (3-CH_2OH).

3-Formylcarbazole (Mk4) : White solid

Molecular Formula : $C_{13}H_9NO$

UV (CH_3OH) λ_{max} (log ε), nm : 238 (4.24), 269 (4.23), 292 (4.23), 324 (4.13)

IR (CH_3OH) ν_{max}, cm^{-1} : 3331, 1680

HRESIMS, *m/z* : 196.0747 $[M+H]^+$

Melting point, °C : 158–160

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^{1}H NMR ($CDCl_3$), δ ppm :

7.51 (*d*, *J* = 8.1 Hz, H-1, 1H), 7.95 (*dd*, *J* = 8.1, 1.7 Hz, H-2, 1H), 8.65 (*d*, *J* = 1.7 Hz, H-4, 1H), 8.17 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.26 (*td*, *J* = 8.1, 1.2 Hz, H-6, 1H), 7.46 (*td*, *J* = 8.0, 1.2 Hz, H-7, 1H), 7.56 (*d*, *J* = 8.0 Hz, H-8, 1H), 8.54 (*br s*, NH, 1H), 10.01 (*s*, 3-CHO, 1H).

^{13}C NMR ($CDCl_3$), δ ppm :

111.1 (C-1), 128.4 (C-1a), 126.4 (C-2), 144.2 (C-3), 124.2 (C-4), 125.2 (C-4a), 120.1 (C-5), 123.2 (C-5a), 119.9 (C-6), 126.3 (C-7), 110.9 (C-8), 140.9 (C-8a), 192.7 (3-CHO).

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2-Hydroxy-3-methylcarbazole (Mk5) : Pale yellow oil

Molecular Formula : $C_{13}H_{11}NO$

UV (CH_3OH) λ_{max} (log ε), nm : 237 (4.19), 261 (4.08), 301 (4.12), 330 (4.11)

IR (CH_3OH) ν_{max}, cm^{-1} : 3369, 1375, 1019

HRESIMS, *m/z* : 197.0812 $[M]^+$

^1H NMR ($CDCl_3$), δ ppm :

6.84 (*s*, H-1, 1H), 7.78 (*s*, H-4, 1H), 7.94 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.18 (*td*, *J* = 8.0, 1.0 Hz, H-6, 1H), 7.31 (*td*, *J* = 8.0, 1.0 Hz, H-7, 1H), 7.35 (*d*, *J* = 8.0 Hz, H-8, 1H), 7.88 (*br s*, NH, 1H), 4.86 (*br s*, 2-OH, 1H), 2.40 (*s*, 3-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

96.5 (C-1), 139.4 (C-1a), 153.1 (C-2), 123.5 (C-3), 121.9 (C-4), 116.3 (C-4a), 119.4 (C-5), 124.7 (C-5a), 119.3 (C-6), 124.5 (C-7), 110.3 (C-8), 139.5 (C-8a), 16.3 (3-CH_3).

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Mahanimbilol (Mk6) : Pale yellow oil

Molecular Formula : $C_{23}H_{27}NO$

UV (CH_3OH) λ_{max} (log ε), nm : 239 (4.07), 269 (4.14), 287 (4.04), 304 (4.26)

IR (CH_3OH) ν_{max}, cm^{-1} : 3455, 3371, 1639, 1375, 1019

HRESIMS, *m/z* : 334.2151 $[M+H]^+$

^1H NMR ($CDCl_3$), δ ppm :

7.68 (*s*, H-4, 1H), 7.93 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.17 (*td*, *J* = 8.0, 1.2 Hz, H-6, 1H), 7.30 (*td*, *J* = 8.0, 1.2 Hz, H-7, 1H), 7.37 (*d*, *J* = 8.0 Hz, H-8, 1H), 3.62 (*d*, *J* = 6.9 Hz, H-9, 2H), 5.38 (*t*, *J* = 6.9 Hz, H-10, 1H), 7.85 (*br s*, NH, 1H), 5.21 (*br s*, 2-OH, 1H),

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2.40 (*s*, 3-CH_3, 3H), 2.09 (*m*, H-1', 2H), 2.11 (*m*, H-2', 2H), 5.06 (*t*, *J* = 7.0 Hz, H-3', 1H), 1.90 (*s*, 11-CH_3, 3H), 1.66 (*s*, 4'-CH_3, 3H), 1.59 (*s*, 4'-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

107.9 (C-1), 138.5 (C-1a), 151.3 (C-2), 124.0 (C-3), 119.4 (C-4), 117.2 (C-4a), 119.5 (C-5), 116.8 (C-5a), 119.5 (C-6), 124.3 (C-7), 110.4 (C-8), 139.5 (C-8a), 24.8 (C-9), 121.4 (C-10), 139.2 (C-11), 16.7 (3-CH_3), 39.7 (C-1'), 26.5 (C-2'), 123.7 (C-3'), 132.2 (C-4'), 16.5 (11-CH_3), 25.8 (4'-CH_3), 17.8 (4'-CH_3).

Euchrestine-B (Mk7) : Pale yellow oil

Molecular Formula : $C_{24}H_{29}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 237 (4.44), 264 (4.39), 308 (4.26), 325 (4.35)

IR (CH_3OH) ν_{max}, cm^{-1} : 3323, 1025

HRESIMS, *m/z* : 363.1845 $[M]^+$

^{1}H NMR ($CDCl_3$), δ ppm :

6.80 (*s*, H-1, 1H), 7.67 (*s*, H-4, 1H), 7.71 (*d*, *J* = 8.6 Hz, H-5, 1H), 6.83 (*d*, *J* = 8.6 Hz, H-6, 1H), 3.62 (*d*, *J* = 6.8 Hz, H-9, 2H), 5.33 (*t*, *J* = 6.9 Hz, H-10, 1H), 7.76 (*br s*, NH, 1H), 4.80 (*br s*, 2-OH, 1H), 2.42 (*s*, 3-CH_3, 3H), 3.91 (*s*, 7-OCH_3, 3H), 2.05 (*m*, H-1', 2H), 2.10 (*m*, H-2', 2H), 5.06 (*t*, *J* = 6.9 Hz, H-3', 1H), 1.88 (*s*, 11-CH_3, 3H), 1.63 (*s*, 4'-CH_3, 3H), 1.57 (*s*, 4'-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

96.7 (C-1), 139.7 (C-1a), 152.2 (C-2), 117.9 (C-3), 121.2 (C-4), 118.0 (C-4a), 117.2 (C-5), 115.9 (C-5a), 104.6 (C-6), 154.8 (C-7), 111.2 (C-8), 140.3 (C-8a), 23.9 (C-9), 122.5 (C-10), 136.6 (C-11), 16.2 (3-CH_3), 56.8 (7-OCH_3), 39.7 (C-1'), 26.7 (C-2'), 124.1 (C-3'), 131.7 (C-4'), 16.5 (11-CH_3), 25.8 (4'-CH_3), 17.8 (4'-CH_3).

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Mukoenine-B (Mk8) : Pale yellow oil

Molecular Formula : $C_{23}H_{25}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 239 (4.22), 269 (4.26)

IR (CH_3OH) v_{max}, cm^{-1} : 3371, 1637, 1019

HRESIMS, *m/z* : 348.1963 $[M+H]^+$

^1H NMR ($CDCl_3$), δ ppm :

8.07 (*s*, H-4, 1H), 7.91 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.17 (*td*, *J* = 8.0, 1.2 Hz, H-6, 1H), 7.40 (*td*, *J* = 8.0, 1.2 Hz, H-7, 1H), 7.39 (*d*, *J* = 8.0 Hz, H-8, 1H), 3.67 (*d*, *J* = 6.9 Hz, H-9, 2H), 5.34 (*t*, *J* = 6.9 Hz, H-10, 1H), 8.24 (*br s*, NH, 1H), 11.67 (*s*, 2-OH, 1H), 9.93 (*s*, 3-CHO, 1H), 2.11 (*m*, H-1', 2H), 2.10 (*m*, H-2', 2H), 5.08 (*t*, *J* = 6.9 Hz, H-3', 1H), 1.90 (*s*, 11-CH_3, 3H), 1.61 (*s*, 4'-CH_3, 3H), 1.57 (*s*, 4'-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

109.1 (C-1), 138.2 (C-1a), 157.9 (C-2), 123.7 (C-3), 125.5 (C-4), 117.4 (C-4a), 119.4 (C-5), 115.5 (C-5a), 119.4 (C-6), 126.0 (C-7), 110.9 (C-8), 140.2 (C-8a), 24.0 (C-9), 121.3 (C-10), 138.0 (C-11), 195.5 (3-CHO), 39.7 (C-1'), 26.6 (C-2'), 123.9 (C-3'), 132.0 (C-4'), 16.6 (11-CH_3), 25.8 (4'-CH_3), 17.8 (4'-CH_3).

Murrastanine-A (Mk9) : Brownish oil

Molecular Formula : $C_{13}H_{11}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 239 (4.32), 250 (4.42), 269 (4.37), 276 (4.27)

IR (CH_3OH) v_{max}, cm^{-1} : 3371, 1637, 1375, 1019

HRESIMS, *m/z* : 214.0890 $[M+H]^+$

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^{1}H NMR ($CDCl_3$), δ ppm :

7.62 (*s*, H-2, 1H), 8.44 (*s*, H-4, 1H), 8.05 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.18 (*td*, *J* = 8.0, 1.2 Hz, H-6, 1H), 7.39 (*td*, *J* = 8.0, 1.2 Hz, H-7, 1H), 7.50 (*d*, *J* = 8.0 Hz, H-8, 1H), 8.44 (*br s*, NH, 1H), 4.06 (*s*, 1-OCH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

145.3 (C-1), 132.9 (C-1a), 106.4 (C-2), 145.4 (C-3), 115.5 (C-4), 123.5 (C-4a), 119.9 (C-5), 123.4 (C-5a), 119.2 (C-6), 125.6 (C-7), 111.3 (C-8), 140.5 (C-8a), 56.1 (1-OCH_3).

Murrayaquinone-A (Mk10) : Brown solid

Molecular Formula : $C_{13}H_9NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 225 (4.32), 258 (4.30), 293 (4.34), 398 (4.35)

IR (CH_3OH) ν_{max}, cm^{-1} : 3367, 1638, 1374, 1019

HRESIMS, *m/z* : 212.0705 $[M+H]^+$

Melting point, °C : 245–247

^{1}H NMR ($CDCl_3$), δ ppm :

6.54 (*q*, *J* = 1.7 Hz, H-2, 1H), 8.25 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.35 (*td*, *J* = 8.0, 1.1 Hz, H-6, 1H), 7.41 (*td*, *J* = 8.0, 1.1 Hz, H-7, 1H), 7.49 (*d*, *J* = 8.0 Hz, H-8, 1H), 9.14 (*br s*, NH, 1H), 2.18 (*d*, *J* = 1.7 Hz, 3-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

183.7 (C-1), 135.5 (C-1a), 131.6 (C-2), 149.0 (C-3), 180.2 (C-4), 117.9 (C-4a), 123.1 (C-5), 119.1 (C-5a), 124.2 (C-6), 127.1 (C-7), 112.8 (C-8), 136.9 (C-8a), 16.3 (3-CH_3).

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Murrayaquinone-B (Mk11) : Dark brown solid

Molecular Formula : $C_{19}H_{19}NO_3$

UV (CH_3OH) λ_{max} (log ε), nm : 230 (4.38), 233 (4.03), 264 (4.15), 288 (4.25), 310 (4.13)

IR (CH_3OH) ν_{max}, cm^{-1} : 3366, 1638, 1375, 1019

HRESIMS, *m/z* : 310.1425 $[M+H]^+$

Melting point, °C : 221–222

^1H NMR ($CDCl_3$), δ ppm :

6.43 (*q*, *J* = 1.7 Hz, H-2, 1H), 8.01 (*d*, *J* = 9.2 Hz, H-5, 1H), 7.03 (*d*, *J* = 9.2 Hz, H-6, 1H), 8.91 (*br s*, NH, 1H), 2.13 (*d*, *J* = 1.8 Hz, 3-CH_3, 3H), 3.91 (*s*, 7-OCH_3, 3H), 3.57 (*d*, *J* = 6.9 Hz, H-1', 2H), 5.27 (*t*, *J* = 7.0 Hz, H-2', 1H), 1.75 (*s*, 3'-CH_3, 3H), 1.85 (*s*, 3'-CH_3, 3H).

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^{13}C NMR ($CDCl_3$), δ ppm :

183.9 (C-1), 135.2 (C-1a), 131.6 (C-2), 148.3 (C-3), 179.9 (C-4), 117.3 (C-4a), 110.8 (C-5), 119.0 (C-5a), 121.2 (C-6), 156.1 (C-7), 112.6 (C-8), 138.0 (C-8a), 16.2 (3-CH_3), 56.8 (7-OCH_3), 23.8 (C-1'), 121.6 (C-2'), 134.2 (C-3'), 25.8 (3'-CH_3), 18.1 (3'-CH_3).

Girinimbine (Mk12) : Colourless crystal

Molecular Formula : $C_{18}H_{17}NO$

UV (CH_3OH) λ_{max} (log ε), nm : 237 (4.12), 287 (4.08), 327 (4.16)

IR (CH_3OH) ν_{max}, cm^{-1} : 3363, 1638, 1375, 1018

HRESIMS, *m/z* : 264.1364 $[M+H]^+$

Melting point, °C : 176–178

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^{1}H NMR ($CDCl_3$), δ ppm :

7.69 (*s*, H-4, 1H), 7.94 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.19 (*td*, *J* = 8.0, 1.1 Hz, H-6, 1H), 7.32 (*td*, *J* = 8.0, 1.1 Hz, H-7, 1H), 7.36 (*d*, *J* = 8.0 Hz, H-8, 1H), 6.60 (*d*, *J* = 9.8 Hz, H-9, 1H), 5.69 (*d*, *J* = 9.8 Hz, H-10, 1H), 7.87 (*br s*, NH, 1H), 2.35 (*s*, 3-CH_3, 3H), 1.50 (*s*, 11-CH_3, 3H), 1.50 (*s*, 11-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

104.5 (C-1), 134.9 (C-1a), 149.9 (C-2), 118.7 (C-3), 121.3 (C-4), 116.8 (C-4a), 119.4 (C-5), 124.0 (C-5a), 119.6 (C-6), 124.4 (C-7), 110.5 (C-8), 139.6 (C-8a), 117.3 (C-9), 129.5 (C-10), 76.0 (C-11), 16.2 (3-CH_3), 27.7 (11-CH_3), 27.7 (11-CH_3).

Koenimbine (Mk13) : Brown solid

Molecular Formula : $C_{19}H_{19}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 238 (4.32), 262 (4.34), 268 (4.37), 295 (4.36)

IR (CH_3OH) ν_{max}, cm^{-1} : 3334, 1637, 1025

HRESIMS, *m/z* : 294.2071 $[M+H]^+$

Melting point, °C : 193–194

^{1}H NMR ($CDCl_3$), δ ppm :

7.63 (*s*, H-4, 1H), 7.41 (*d*, *J* = 2.9 Hz, H-5, 1H), 6.95 (*dd*, *J* = 8.6, 2.9 Hz, H-7, 1H), 7.28 (*d*, *J* = 8.6 Hz, H-8, 1H), 6.61 (*d*, *J* = 9.8 Hz, H-9, 1H), 5.68 (*d*, *J* = 9.8 Hz, H-10, 1H), 7.72 (*br s*, NH, 1H), 2.33 (*s*, 3-CH_3, 3H), 3.90 (*s*, 6-OCH_3, 3H), 1.48 (*s*, 11-CH_3, 3H), 1.48 (*s*, 11-CH_3, 3H).

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^{13}C NMR ($CDCl_3$), δ ppm :

104.6 (C-1), 135.8 (C-1a), 149.9 (C-2), 118.4 (C-3), 121.1 (C-4), 116.9 (C-4a), 102.6 (C-5), 124.5 (C-5a), 154.0 (C-6), 113.1 (C-7), 111.1 (C-8), 134.4 (C-8a), 117.3 (C-9), 129.4 (C-10), 76.8 (C-11), 16.2 (3-CH_3), 56.1 (6-OCH_3), 27.7 (11-CH_3), 27.7 (11-CH_3).

Murrayacine (Mk14) : Brown solid

Molecular Formula : $C_{18}H_{15}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 238 (4.22), 273, (4.26), 285 (4.18), 338 (4.06)

IR (CH_3OH) ν_{max}, cm^{-1} : 3365, 1637, 1019

HRESIMS, *m/z* : 278.1168 $[M+H]^+$

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Melting point, °C : 242–244

^{1}H NMR ($CDCl_3$), δ ppm :

8.42 (*s*, H-4, 1H), 7.98 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.25 (*td*, *J* = 8.0, 1.1 Hz, H-6, 1H), 7.39 (*td*, *J* = 8.0, 1.1 Hz, H-7, 1H), 7.40 (*d*, *J* = 8.0 Hz, H-8, 1H), 6.63 (*d*, *J* = 9.7 Hz, H-9, 1H), 5.80 (*d*, *J* = 9.7 Hz, H-10, 1H), 8.18 (*br s*, NH, 1H), 10.50 (*s*, 3-CHO, 1H), 1.57 (*s*, 11-CH_3, 3H), 1.57 (*s*, 11-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

104.2 (C-1), 140.3 (C-1a), 154.9 (C-2), 118.8 (C-3), 119.9 (C-4), 118.3 (C-4a), 120.4 (C-5), 124.2 (C-5a), 121.0 (C-6), 126.1 (C-7), 110.8 (C-8), 140.1 (C-8a), 116.3 (C-9), 130.2 (C-10), 77.2 (C-11), 189.4 (3-CHO), 27.8 (11-CH_3), 27.8 (11-CH_3).

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Mahanimbine (Mk15) : White Solid

Optical Rotation, $[\alpha]_D^{25}$: +25.5 (CH_3OH; c 0.02)

Molecular Formula : $C_{23}H_{25}NO$

UV (CH_3OH) λ_{max} (log ε), nm : 235 (4.48), 288 (4.28), 302 (4.14), 357 (4.18)

IR (CH_3OH) ν_{max}, cm^{-1} : 3367, 1637, 1374, 1121, 1019

HRESIMS, *m/z* : 332.2002 $[M+H]^+$

Melting point, °C : 93–95

^1H NMR ($CDCl_3$), δ ppm :

7.69 (*s*, H-4, 1H), 7.94 (*d*, *J* = 8.1 Hz, H-5, 1H), 7.21 (*td*, *J* = 8.1, 1.2 Hz, H-6, 1H), 7.33 (*td*, *J* = 8.1, 1.2 Hz, H-7, 1H), 7.35 (*d*, *J* = 8.1 Hz, H-8, 1H), 6.60 (*d*, *J* = 9.8 Hz, H-9, 1H), 5.65 (*d*, *J* = 9.8 Hz, H-10, 1H), 7.81 (*br s*, NH, 1H), 2.37 (*s*, 3-CH_3, 3H), 1.79 (*t*, *J* = 8.9 Hz, H-1', 2H), 2.20 (*m*, H-2', 2H), 5.15 (*t*, *J* = 7.5 Hz, H-3', 1H), 1.48 (*s*, 11-CH_3, 3H), 1.62 (*s*, 4'-CH_3, 3H), 1.72 (*s*, 4'-CH_3, 3H).

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^{13}C NMR ($CDCl_3$), δ ppm :

104.2 (C-1), 134.8 (C-1a), 149.9 (C-2), 118.4 (C-3), 121.2 (C-4), 116.6 (C-4a), 119.3 (C-5), 123.9 (C-5a), 119.4 (C-6), 124.2 (C-7), 110.4 (C-8), 139.4 (C-8a), 117.5 (C-9), 128.4 (C-10), 78.1 (C-11), 16.1 (3-CH_3), 40.7 (C-1'), 22.7 (C-2'), 124.2 (C-3'), 131.6 (C-4'), 25.8 (11-CH_3), 17.6 (4'-CH_3), 25.7 (4'-CH_3).

Mahanine (Mk16) : Yellowish solid

Optical Rotation, $[\alpha]_D^{25}$: +4.0 (CH_3OH; c 0.02)

Molecular Formula : $C_{23}H_{25}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 240 (4.01), 262 (4.02), 287 (4.18), 303 (4.16)

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IR (CH_3OH) v_{max}, cm^{-1} : 3367, 1640, 1375, 1020

HRESIMS, *m/z* : 347.1365 $[M]^+$

Melting point, °C : 94–96

^1H NMR ($CDCl_3$), δ ppm :

7.54 (*s*, H-4, 1H), 7.70 (*d*, *J* = 8.6 Hz, H-5, 1H), 6.68 (*dd*, *J* = 8.6, 1.7 Hz, H-6, 1H), 6.79 (*d*, *J* = 1.7 Hz, H-8, 1H), 6.58 (*d*, *J* = 9.7 Hz, H-9, 1H), 5.63 (*d*, *J* = 9.7 Hz, H-10, 1H), 7.77 (*br s*, NH, 1H), 2.32 (*s*, 3-CH_3, 3H), 1.75 (*t*, *J* = 8.8 Hz, H-1', 2H), 2.16 (*m*, H-2', 2H), 5.11 (*t*, *J* = 7.5 Hz, H-3', 1H), 1.43 (*s*, 11-CH_3, 3H), 1.58 (*s*, 4'-CH_3, 3H), 1.66 (*s*, 4'-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

104.4 (C-1), 134.8 (C-1a), 149.0 (C-2), 118.3 (C-3), 120.4 (C-4), 116.7 (C-4a), 120.1 (C-5), 118.1 (C-5a), 108.4 (C-6), 153.6 (C-7), 97.0 (C-8), 140.8 (C-8a), 117.6 (C-9), 128.8 (C-10), 78.1 (C-11), 16.2 (3-CH_3), 40.7 (C-1'), 22.8 (C-2'), 124.3 (C-3'), 131.8 (C-4'), 25.8 (11-CH_3), 17.7 (4'-CH_3), 25.7 (4'-CH_3).

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Isomahanine (Mk17) : White solid

Optical Rotation, $[\alpha]_D^{25}$: +6.0 (CH_3OH; c 0.02)

Molecular Formula : $C_{23}H_{25}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 237 (4.11), 262 (4.13), 286 (4.13), 303 (4.16)

IR (CH_3OH) v_{max}, cm^{-1} : 3500, 3420, 1450, 1025

HRESIMS, *m/z* : 347.3143 $[M]^+$

Melting point, °C : 183–184

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^{1}H NMR ($CDCl_3$), δ ppm :

6.68 (*d*, *J* = 8.6 Hz, H-3, 1H), 7.63 (*d*, *J* = 8.6 Hz, H-4, 1H), 7.63 (*s*, H-5, 1H), 6.82 (*s*, H-8, 1H), 6.62 (*d*, *J* = 9.8 Hz, H-9, 1H), 5.65 (*d*, *J* = 9.8 Hz, H-10, 1H), 7.79 (*br s*, NH, 1H), 2.38 (*s*, 6-CH_3, 3H), 4.89 (*br s*, 7-OH, 1H), 1.71 (*m*, H-1', 2H), 2.15 (*m*, H-2', 2H), 5.10 (*t*, *J* = 7.4 Hz, H-3', 1H), 1.44 (*s*, 11-CH_3, 3H), 1.57 (*s*, 4'-CH_3, 3H), 1.65 (*s*, 4'-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

104.7 (C-1), 136.2 (C-1a), 150.2 (C-2), 109.4 (C-3), 119.5 (C-4), 117.6 (C-4a), 121.0 (C-5), 117.9 (C-5a), 116.3 (C-6), 152.1 (C-7), 96.8 (C-8), 139.3 (C-8a), 117.4 (C-9), 128.9 (C-10), 78.2 (C-11), 16.2 (6-CH_3), 40.8 (C-1'), 22.8 (C-2'), 124.2 (C-3'), 131.8 (C-4'), 26.0 (11-CH_3), 17.7 (4'-CH_3), 25.8 (4'-CH_3).

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Murrayamine-B (Mk18) : Brownish oil

Optical Rotation, $[\alpha]_D^{25}$: +5.0 (CH_3OH; c 0.015)

Molecular Formula : $C_{24}H_{27}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 236 (4.03), 262 (3.83), 288 (4.09), 302 (4.02)

IR (CH_3OH) ν_{max}, cm^{-1} : 3378, 2257, 1640, 1375, 1019

HRESIMS, *m/z* : 361.1364 $[M]^+$

^{1}H NMR ($CDCl_3$), δ ppm :

7.63 (*s*, H-4, 1H), 7.52 (*d*, *J* = 8.1 Hz, H-5, 1H), 7.10 (*t*, *J* = 8.1 Hz, H-6, 1H), 6.81 (*d*, *J* = 7.5 Hz, H-7, 1H), 6.68 (*d*, *J* = 9.8 Hz, H-9, 1H), 5.65 (*d*, *J* = 9.8 Hz, H-10, 1H), 8.07 (*br s*, NH, 1H), 2.33 (*s*, 3-CH_3, 3H), 4.00 (*s*, 8-OCH_3, 3H), 1.75 (*t*, *J* = 8.8 Hz, H-

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1', 2H), 2.17 (*m*, H-2', 2H), 5.11 (*t*, *J* = 7.3 Hz, H-3', 1H), 1.44 (*s*, 11-CH_3, 3H), 1.58 (*s*, 4'-CH_3, 3H), 1.66 (*s*, 4'-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

104.5 (C-1), 134.7 (C-1a), 149.9 (C-2), 118.6 (C-3), 121.4 (C-4), 117.1 (C-4a), 112.2 (C-5), 125.0 (C-5a), 119.8 (C-6), 104.8 (C-7), 145.6 (C-8), 143.4 (C-8a), 117.7 (C-9), 128.5 (C-10), 78.2 (C-11), 16.2 (3-CH_3), 55.6 (8-OCH_3), 40.8 (C-1'), 22.8 (C-2'), 124.3 (C-3'), 131.8 (C-4'), 25.9 (11-CH_3), 17.7 (4'-CH_3), 25.8 (4'-CH_3).

Murrayamine-J (Mk19) : Pale yellow oil

Optical Rotation, $[\alpha]_D^{25}$: +35.5 (CH_3OH; c 0.01)

Molecular Formula : $C_{23}H_{23}NO_2$

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UV (CH_3OH) λ_{max} (log ε), nm : 215 (4.09), 248 (4.05), 265 (4.31), 310 (3.99)

IR (CH_3OH) ν_{max}, cm^{-1} : 3367, 1639, 1375, 1018

HRESIMS, *m/z* : 345.1364 $[M]^+$

^{1}H NMR ($CDCl_3$), δ ppm :

6.81 (*d*, *J* = 8.6 Hz, H-3, 1H), 7.83 (*d*, *J* = 8.6 Hz, H-4, 1H), 8.46 (*d*, *J* = 1.7 Hz, H-5, 1H), 7.89 (*dd*, *J* = 8.6, 1.7 Hz, H-7, 1H), 7.47 (*d*, *J* = 8.6 Hz, H-8, 1H), 6.66 (*d*, *J* = 9.7 Hz, H-9, 1H), 5.71 (*d*, *J* = 9.7 Hz, H-10, 1H), 8.35 (*br s*, NH, 1H), 10.07 (*s*, 6-CHO, 1H), 1.74 (*m*, H-1', 2H), 2.16 (*m*, H-2', 2H), 5.10 (*t*, *J* = 7.3 Hz, C-3', 1H), 1.47 (*s*, 11-CH_3, 3H), 1.58 (*s*, 4'-CH_3, 3H), 1.66 (*s*, 4'-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

105.1 (C-1), 136.8 (C-1a), 152.7 (C-2), 111.0 (C-3), 121.0 (C-4), 117.2 (C-4a), 122.9 (C-5), 124.3 (C-5a), 129.5 (C-6), 126.5 (C-7), 110.8 (C-8), 143.4 (C-8a), 116.8 (C-9),

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129.6 (C-10), 78.8 (C-11), 192.1 (6-CHO), 41.0 (C-1'), 22.8 (C-2'), 124.0 (C-3'), 132.0 (C-4'), 26.2 (11-CH_3), 17.7 (4'-CH_3), 25.8 (4'-CH_3).

Murrastinine-A (Mk20) : Pale yellow oil

Optical Rotation, $[\alpha]_D^{25}$: +11.5 (CH_3OH; c 0.02)

Molecular Formula : $C_{23}H_{23}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 238 (4.36), 249 (4.48), 273 (4.44), 286 (4.38), 329 (4.16)

IR (CH_3OH) ν_{max}, cm^{-1} : 3371, 1638, 1019

HRESIMS, *m/z* : 346.1789 $[M+H]^+$

^1H NMR ($CDCl_3$), δ ppm :

7.67 (*s*, H-4, 1H), 7.91 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.17 (*td*, *J* = 8.0, 1.2 Hz, H-6, 1H), 7.31 (*td*, *J* = 8.0, 1.2 Hz, H-7, 1H), 7.38 (*d*, *J* = 8.0 Hz, H-8, 1H), 6.68 (*d*, *J* = 9.8 Hz, H-9, 1H), 5.61 (*d*, *J* = 9.8 Hz, H-10, 1H), 7.92 (*br s*, NH, 1H), 2.32 (*s*, 3-CH_3, 3H), 1.45 (*s*, 11-CH_3, 3H), 2.09 (*m*, H-1'α, 1H), 2.11 (*m*, H-1'β, 1H), 2.86 (*m*, H-2'α, 1H), 2.98 (*m*, H-2'β, 1H), 5.71 and 5.93 (*d*, *J* = 1.7 Hz each, 4'-CH_2, 2×2H), 1.86 (*s*, 4'-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

104.0 (C-1), 134.9 (C-1a), 149.7 (C-2), 118.2 (C-3), 121.5 (C-4), 116.9 (C-4a), 119.4 (C-5), 123.9 (C-5a), 119.6 (C-6), 124.4 (C-7), 110.5 (C-8), 139.5 (C-8a), 118.3 (C-9), 127.9 (C-10), 78.0 (C-11), 16.2 (3-CH_3), 27.8 (11-CH_3), 35.6 (C-1'), 32.7 (C-2'), 202.0 (C-3'), 144.4 (C-4'), 124.9 (4'-CH_2), 17.8 (4'-CH_3).

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Murrastinine-B (Mk21) : Brown amorphous

Molecular Formula : $C_{18}H_{17}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 238 (4.11), 273 (4.08), 285 (4.08), 338 (4.19)

IR (CH_3OH) ν_{max}, cm^{-1} : 3372, 1637, 1020

HRESIMS, *m/z* : 279.1583 $[M]^+$

^1H NMR ($CDCl_3$), δ ppm :

7.82 (*s*, H-4, 1H), 7.89 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.15 (*td*, *J* = 8.0, 1.1 Hz, H-6, 1H), 7.29 (*td*, *J* = 8.0, 1.1 Hz, H-7, 1H), 7.37 (*d*, *J* = 8.0 Hz, H-8, 1H), 6.62 (*d*, *J* = 9.8 Hz, H-9, 1H), 5.66 (*d*, *J* = 9.8 Hz, H-10, 1H), 7.90 (*br s*, NH, 1H), 4.16 (*s*, 3-CH_2OH, 2H), 1.37 (*s*, 11-CH_3, 3H), 1.37 (*s*, 11-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

104.6 (C-1), 135.0 (C-1a), 150.1 (C-2), 124.4 (C-3), 122.3 (C-4), 116.7 (C-4a), 119.4 (C-5), 122.4 (C-5a), 119.5 (C-6), 124.2 (C-7), 110.4 (C-8), 139.6 (C-8a), 117.4 (C-9), 129.5 (C-10), 76.0 (C-11), 65.1 (3-CH_2OH), 27.5 (11-CH_3), 27.5 (11-CH_3).

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Murrastinine-C (Mk22) : Pale yellow oil

Molecular Formula : $C_{18}H_{15}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 240 (4.21), 262 (4.10), 287 (3.98), 309 (4.14)

IR (CH_3OH) ν_{max}, cm^{-1} : 3366, 1638, 1020

HRESIMS, *m/z* : 278.1174 $[M+H]^+$

^1H NMR ($CDCl_3$), δ ppm :

6.81 (*d*, *J* = 8.6 Hz, H-3, 1H), 7.83 (*d*, *J* = 8.6 Hz, H-4, 1H), 8.45 (*d*, *J* = 1.2 Hz, H-5, 1H), 7.85 (*dd*, *J* = 8.6, 1.2 Hz, H-7, 1H), 7.45 (*d*, *J* = 8.6 Hz, H-8, 1H), 6.63 (*d*, *J* = 9.7

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Hz, H-9, 1H), 5.73 (*d*, *J* = 9.7 Hz, H-10, 1H), 8.50 (*br s*, NH, 1H), 10.06 (*s*, 6-CHO, 1H), 1.50 (*s*, 11-CH_3, 3H), 1.50 (*s*, 11-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

105.3 (C-1), 136.8 (C-1a), 152.5 (C-2), 111.1 (C-3), 120.9 (C-4), 117.3 (C-4a), 122.9 (C-5), 124.3 (C-5a), 129.4 (C-6), 126.5 (C-7), 110.8 (C-8), 143.5 (C-8a), 116.5 (C-9), 130.4 (C-10), 76.4 (C-11), 192.2 (6-CHO), 27.7 (11-CH_3), 27.7 (11-CH_3).

Murrayazolinol (Mk23) : Brown solid

Optical Rotation, $[\alpha]_D^{25}$: +31.0 (CH_3OH; c 0.02)

Molecular Formula : $C_{23}H_{25}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 245 (4.08), 265 (4.08), 305 (4.12)

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IR (CH_3OH) ν_{max}, cm^{-1} : 3360, 1639, 1018

HRESIMS, *m/z* : 348.1957 $[M+H]^+$

Melting point, °C : 289–290

^{1}H NMR ($CDCl_3$), δ ppm :

7.49 (*s*, H-4, 1H), 7.90 (*d*, *J* = 7.5 Hz, H-5, 1H), 7.16 (*td*, *J* = 8.0, 1.2 Hz, H-6, 1H), 7.25 (*td*, *J* = 8.0, 1.2 Hz, H-7, 1H), 7.47 (*d*, *J* = 8.0 Hz, H-8, 1H), 3.32 (*m*, H-9, 1H), 1.22 (*m*, H-10, 2H), 2.36 (*s*, 3-CH_3, 3H), 1.76 (*dd*, *J* = 16.1, 6.9 Hz, H-1', 2H), 3.83 (*m*, H-2', 1H), 2.17 (*m*, H-3', 1H), 1.50 (*s*, 11-CH_3, 3H), 1.93 (*s*, 4'-CH_3, 3H), 1.29 (*s*, 4'-CH_3, 3H).

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^{13}C NMR ($CDCl_3$), δ ppm :

105.3 (C-1), 140.7 (C-1a), 153.3 (C-2), 118.5 (C-3), 119.7 (C-4), 115.1 (C-4a), 120.1 (C-5), 127.2 (C-5a), 119.5 (C-6), 123.1 (C-7), 113.6 (C-8), 142.4 (C-8a), 21.5 (C-9), 36.9 (C-10), 79.5 (C-11), 15.5 (3-CH_3), 36.0 (C-1'), 72.2 (C-2'), 49.5 (C-3'), 60.6 (C-4'), 25.1 (11-CH_3), 23.1 (4'-CH_3), 30.2 (4'-CH_3).

Murrayakoeninol (Mk24) : Pale yellow oil

Optical Rotation, $[\alpha]_D^{25}$: +11.0 (CH_3OH; c 0.015)

Molecular Formula : $C_{23}H_{25}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 242 (4.09), 275 (4.04), 308 (4.10)

IR (CH_3OH) ν_{max}, cm^{-1} : 3372, 1640, 1375, 1020

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HRESIMS, *m/z* : 348.1943 $[M+H]^+$

^{1}H NMR ($CDCl_3$), δ ppm :

7.50 (*s*, H-4, 1H), 7.89 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.16 (*td*, *J* = 8.1, 1.2 Hz, H-6, 1H), 7.26 (*td*, *J* = 8.1, 1.2 Hz, H-7, 1H), 7.47 (*d*, *J* = 8.1 Hz, H-8, 1H), 3.31 (*m*, H-9, 1H), 3.82 (*d*, *J* = 8.3 Hz, H-10, 1H), 4.43 (*br s*, 2-OH, 1H), 2.23 (*s*, 3-CH_3, 3H), 1.53 (*m*, H-1', 2H), 1.74 (*m*, H-2', 2H), 2.18 (*m*, H-3', 1H), 1.49 (*s*, 11-CH_3, 3H), 1.92 (*s*, 4'-CH_3, 3H), 1.30 (*s*, 4'-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

105.4 (C-1), 140.7 (C-1a), 153.3 (C-2), 118.5 (C-3), 119.7 (C-4), 115.0 (C-4a), 120.1 (C-5), 127.2 (C-5a), 119.5 (C-6), 123.1 (C-7), 113.7 (C-8), 142.4 (C-8a), 36.9 (C-9), 72.2 (C-10), 79.9 (C-11), 15.5 (3-CH_3), 36.0 (C-1'), 21.5 (C-2'), 49.5 (C-3'), 60.6 (C-4'), 25.2 (11-CH_3), 23.1 (4'-CH_3), 30.2 (4'-CH_3).

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Bicyclomahanimbine (Mk25) : Brown solid

Optical Rotation, $[\alpha]_D^{25}$: +26.0 (CH_3OH; c 0.02)

Molecular Formula : $C_{23}H_{25}NO$

UV (CH_3OH) λ_{max} (log ε), nm : 242 (4.09), 255 (4.18), 260 (4.03), 303 (4.10)

IR (CH_3OH) ν_{max}, cm^{-1} : 3379, 1639, 1109, 1019

HRESIMS, *m/z* : 332.2008 $[M+H]^+$

Melting point, °C : 145–147

^1H NMR ($CDCl_3$), δ ppm :

7.67 (*s*, H-4, 1H), 7.92 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.16 (*td*, *J* = 8.0, 1.2 Hz, H-6, 1H), 7.28 (*td*, *J* = 8.0, 1.2 Hz, H-7, 1H), 7.37 (*d*, *J* = 7.5 Hz, H-8, 1H), 3.29 (*d*, *J* = 9.2 Hz, H-9, 1H), 2.06 (*m*, H-10a, 1H), 1.55 (*m*, H-10b, 1H), 7.43 (*br s*, NH, 1H), 2.34 (*s*, 3-CH_3, 3H), 2.50 (*t*, *J* = 7.5 Hz, C-1', 1H), 1.65 (*m*, H-2'a, 1H), 1.56 (*m*, H-2'b, 1H), 2.70 (*t*, *J* = 7.4 Hz, H-3', 1H), 1.44 (*s*, 11-CH_3, 3H), 0.74 (*s*, 4'-CH_3, 3H), 1.57 (*s*, 4'-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

106.4 (C-1), 137.8 (C-1a), 150.3 (C-2), 120.1 (C-3), 119.4 (C-4), 115.8 (C-4a), 119.3 (C-5), 124.2 (C-5a), 119.3 (C-6), 124.0 (C-7), 110.4 (C-8), 139.3 (C-8a), 37.5 (C-9), 38.4 (C-10), 83.6 (C-11), 16.8 (3-CH_3), 46.5 (C-1'), 25.7 (C-2'), 37.9 (C-3'), 39.5 (C-4'), 27.5 (11-CH_3), 18.7 (4'-CH_3), 35.2 (4'-CH_3).

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Murrayatanine-A (Mk26) : Pale yellow oil

Optical Rotation, $[\alpha]_D^{25}$: +4.5 (CH_3OH; c 0.015)

Molecular Formula : $C_{24}H_{27}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 241 (4.82), 255 (4.67), 268 (5.03), 325 (5.11)

IR (CH_3OH) ν_{max}, cm^{-1} : 3329, 1025

HRESIMS, *m/z* : 362.2106 $[M+H]^+$

^1H NMR ($CDCl_3$), δ ppm :

7.54 (*s*, H-4, 1H), 7.74 (*d*, *J* = 8.6 Hz, H-5, 1H), 6.77 (*dd*, *J* = 8.6, 2.3 Hz, H-6, 1H), 6.83 (*d*, *J* = 2.3 Hz, H-8, 1H), 3.38 (*m*, H-9, 1H), 2.00 (*m*, H-10β, 1H), 2.06 (*m*, H-10α, 1H), 2.32 (*s*, 3-CH_3, 3H), 3.89 (*s*, 7-OCH_3, 3H), 1.42 (*s*, 11-CH_3, 3H), 7.64 (*br s*, NH, 1H), 1.61 (*m*, H-1'β, 1H), 1.99 (*m*, H-1'α, 1H), 1.64 (*m*, H-2'β, 1H), 1.68 (*m*, H-2'α, 1H), 2.56 (*m*, H-3', 1H), 1.50 (*s*, 4'-CH_3, 3H), 4.75 (*d*, *J* = 1.9 Hz, 4'β-CH_2), 4.83 (*d*, *J* = 1.9 Hz, 4'α-CH_2, 1H).

^{13}C NMR ($CDCl_3$), δ ppm :

105.4 (C-1), 138.3 (C-1a), 152.8 (C-2), 117.4 (C-3), 118.9 (C-4), 114.3 (C-4a), 119.7 (C-5), 118.3 (C-5a), 107.4 (C-6), 157.5 (C-7), 95.0 (C-8), 140.6 (C-8a), 36.4 (C-9), 37.4 (C-10), 73.8 (C-11), 16.8 (3-CH_3), 55.7 (7-OCH_3), 29.1 (11-CH_3), 40.0 (C-1'), 23.2 (C-2'), 48.8 (C-3'), 150.3 (C-4'), 21.7 (4'-CH_3), 112.1 (4'-CH_2).

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Bismurrayafoline-E (Mk27) : Colourless oil

Molecular Formula : $C_{48}H_{56}N_2O_4$

UV (CH_3OH) λ_{max} (log ε), nm : 232 (4.13), 240 (4.08), 262 (3.89), 308 (4.11), 325 (4.15)

IR (CH_3OH) ν_{max}, cm^{-1} : 3367, 1638, 1019

HRESIMS, *m/z* : 724.3515 $[M]^+$

^1H NMR ($CDCl_3$), δ ppm :

7.85 (*s*, H-4/H-4", 2H), 7.77 (*d*, *J* = 8.6 Hz, H-5/H-5", 2H), 6.85 (*d*, *J* = 8.6 Hz, H-6/H-6", 2H), 3.43 (*ddd*, *J* = 40.7, 16.0, 6. 9 Hz, H-9/H-9", 4H), 5.09 (*m*, H-10/H-10", 2H), 7.57 (*br s*, NH/NH", 2H), 5.20 (*br s*, 2-OH/2"-OH, 2H), 2.48 (*s*, 3-CH_3/3"-CH_3, 6H), 3.89 (*s*,7-OCH_3/7"-OCH_3, 6H), 1.25 (*d*, *J* = 1.1 Hz, 11-CH_3/11"-CH_3, 6H), 1.48 (*m*, H-1'/H-1'", 4H), 2.06 (*m*, H-2'/H-2'", 4H), 4.77 (*t*, *J* = 6.6 Hz, H-3'/H-3'", 2H), 1.56 (*s*, 4'-CH_3/ 4'"-CH_3, 6H), 1.42 (*s*, 4'-CH3/4'"-CH_3, 6H).

^{13}C NMR ($CDCl_3$), δ ppm :

99.5 (C-1/C-1"), 137.8 (C-1a/C-1a"), 150.1 (C-2/C-2"), 117.5 (C-3/C-3"), 122.3 (C-4/C-4"), 117.3 (C-4a/C-4a"), 117.4 (C-5/C-5"), 118.3 (C-5a/C-5a"), 104.9 (C-6/C-6"), 154.9 (C-7/C-7"), 111.0 (C-8/C-8"), 140.5 (C-8a/C-8a"), 23.8 (C-9/C-9"), 121.8 (C-10/C-10"), 136.5 (C-11/C-11"), 16.8 (3-CH_3/3"-CH_3), 55.8 (7-OCH_3/7"-OCH_3), 15.3 (11-CH3/11"-CH_3), 39.1 (C-1'/C-1'"), 26.4 (C-2'/C-2'"), 124.1 (C-3'/C-3'"), 131.1 (C-4'/ C-4'"), 25.7 (4'-CH_3/4'"-CH_3), 17.5 (4'-CH_3/4'"-CH_3).

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Bispyrayafoline (Mk28) : Brownish oil

Optical Rotation, $[\alpha]_D^{25}$: +6.0 (CH_3OH; c 0.02)

Molecular Formula : $C_{46}H_{48}N_2O_4$

UV (CH_3OH) λ_{max} (log ε), nm : 217 (4.11), 262 (4.08), 285 (4.13), 335 (4.17)

IR (CH_3OH) ν_{max}, cm^{-1} : 3680, 3329, 1454, 1031

HRESIMS, *m/z* : 692.3610 $[M]^+$

^1H NMR ($CDCl_3$), δ ppm :

7.85 (*s*, H-4/H-4", 2H), 7.71 (*d*, *J* = 8.6 Hz, H-5/H-5", 2H), 6.73 (*d*, *J* = 8.6 Hz, H-6/H-6", 2H), 6.44 (*d*, *J* = 10.3 Hz, H-9/H-9", 2H), 5.54 (*d*, *J* = 10.3 Hz, H-10/H-10", 2H), 7.55 (*br s*, NH/NH", 2H), 2.49 (*s*, 3-CH_3/3"-CH_3, 6H), 5.16 (*br s*, 7-OH/7"-OH, 2H), 1.39 (*s*, 11-CH_3/11"-CH_3, 6H), 1.73 (*m*, H-1', 2H), 1.67 (*m*, H-1'", 2H), 2.11 (*m*, H-2'/H-2'", 4H), 5.11 (*t*, *J* = 6.7 Hz, H-3'/H-3'", 2H), 1.63 (*s*, 4'-CH_3, 3H), 1.62 (*s*, 4'"-CH_3, 3H), 1.54 (*s*, 4'-CH_3, 3H), 1.53 (*s*, 4'"-CH_3, 3H).

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^{13}C NMR ($CDCl_3$), δ ppm :

105.0 (C-1/C-1"), 136.1 (C-1a/C-1a"), 150.4 (C-2/C-2"), 117.8 (C-3), 117.9 (C-3"), 122.4 (C-4/C-4"), 118.0 (C-4a/C-4a"), 119.5 (C-5/C-5"), 117.7 (C-5a/C-5a"), 110.0 (C-6/C-6"), 151.2 (C-7/C-7"), 99.1 (C-8/C-8"), 137.9 (C-8a/C-8a"), 117.5 (C-9/C-9"), 128.8 (C-10), 128.9 (C-10"), 78.3 (C-11/C-11"), 16.8 (3-CH_3/3"-CH_3), 16.8 (11-CH_3/11"-CH_3), 40.8 (C-1'), 40.7 (C-1'"), 22.8 (C-2'), 22.7 (C-2'"), 124.2 (C-3'), 124.1 (C-3'"), 131.7 (C-4'), 131.8 (C-4'"), 25.7 (4'-CH_3/4'"-CH_3), 17.7 (4'-CH_3/4'"-CH_3).

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Bisisomahanine (Mk29) : Pale yellow solid

Optical Rotation, $[\alpha]_D^{25}$: +3.5 (CH_3OH; c 0.02)

Molecular Formula : $C_{46}H_{48}N_2O_4$

UV (CH_3OH) λ_{max} (log ε), nm : 211 (4.18), 241 (4.02), 268 (4.25), 335 (4.14)

IR (CH_3OH) ν_{max}, cm^{-1} : 3720, 3339, 1454, 1058, 1025

HRESIMS, *m/z* : 692.3601 $[M]^+$

Melting point, °C : 135–137

^1H NMR ($CDCl_3$), δ ppm :

6.73 (*d*, *J* = 8.6 Hz, H-3/H-3", 2H), 7.71 (*d*, *J* = 8.6 Hz, H-4/H-4", 2H), 7.85 (*s*, H-5/H-5", 2H), 6.44 (*d*, *J* = 10.3 Hz, H-9/H-9", 2H), 5.65 (*d*, *J* = 10.3 Hz, H-10/H-10", 2H), 7.54 (*br s*, NH/NH", 2H), 2.49 (*s*, 6-CH_3/6"-CH_3, 6H), 5.15 (*br s*, 7-OH/7"-OH, 2H), 1.39 (*s*, 11-CH_3/11"-CH_3, 6H), 1.66 (*m*, H-1', 2H), 1.75 (*m*, H-1'", 2H), 2.10 (*m*, H-2'/H-2'", 4H), 5.06 (*t*, *J* = 7.1 Hz, H-3'/H-3'", 2H), 1.64 (*s*, 4'-CH_3/4'"-CH_3, 6H), 1.55 (*s*, 4'-CH_3/4'"-CH_3, 6H).

^{13}C NMR ($CDCl_3$), δ ppm :

105.0 (C-1/C-1"), 136.1 (C-1a/C-1a"), 151.1 (C-2/C-2"), 110.0 (C-3/C-3"), 119.5 (C-4/C-4"), 117.7 (C-4a), 117.8 (C-4a"), 122.4 (C-5/C-5"), 118.0 (C-5a/C-5a"), 117.5 (C-6/C-6"), 150.4 (C-7/C-7"), 99.0 (C-8/C-8"), 137.9 (C-8a/C-8a"), 117.5 (C-9/C-9"), 128.8 (C-10), 128.9 (C-10"), 78.3 (C-11/C-11"), 16.8 (6-CH_3/6"-CH_3), 26.0 (2 × 11-CH_3), 40.7 (C-1'), 40.8 (C-1'"), 22.7 (C-2'), 22.8 (C-2'"), 124.1 (C-3'/C-3'"), 131.7 (C-4'), 131.8 (C-4'"), 25.7 (4'-CH_3/4'"-CH_3), 17.7 (4'-CH_3/4'"-CH_3).

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Bismahanimboline (Mk30) : Brownish oil

Optical Rotation, $[\alpha]_D^{25}$: –5.5 (CH_3OH; c 0.015)

Molecular Formula : $C_{46}H_{52}N_2O_4$

UV (CH_3OH) λ_{max} (log ε), nm : 239 (4.08), 262 (4.09), 298 (4.15), 338 (4.12)

IR (CH_3OH) ν_{max}, cm^{-1} : 3377, 1639, 1375, 1019

HRESIMS, *m/z* : 696.3125 $[M]^+$

^1H NMR ($CDCl_3$), δ ppm :

7.66 (*s*, H-4/H-4", 2H), 7.91 (*d*, *J* = 8.0 Hz, H-5/H-5", 2H), 7.18 (*td*, *J* = 8.0, 1.0 Hz, H-6/H-6", 2H), 7.31 (*td*, *J* = 8.0, 1.0 Hz, H-7/H-7", 2H), 7.38 (*d*, *J* = 8.0 Hz, H-8/H-8", 2H), 6.69 (*d*, *J* = 9.8 Hz, H-9/H-9", 2H), 5.63/5.66 (*d*, *J* = 9.8 Hz, H-10/H-10", 2H), 7.90 (*s*, NH/NH", 2H), 2.31 (*s*, 3-CH_3/3"-CH_3, 6H), 1.46 (*s*, 11-CH_3/11"-CH_3, 6H), 1.90/1.91 (*m*, H-1'/H-1'", 4H), 1.73/1.74 (*m*, H-2'/H-2'", 4H), 2.72/2.74 (*t*, *J* = 7.2 Hz, H-3'/H-3'", 2H), 1.27/1.28 (*s*, 4'-CH_3/4'"-CH_3, 6H), 1.23 (*s*, 4'-CH_3/4'"-CH_3, 6H).

^{13}C NMR ($CDCl_3$), δ ppm :

104.0 (C-1), 104.2 (C-1"), 134.9 (C-1a/C-1a"), 149.8 (C-2), 149.7 (C-2"), 118.4 (C-3/C-3"), 121.5 (C-4/C-4"), 116.8 (C-4a/C-4a"), 119.4 (C-5/C-5"), 123.9 (C-5a/C-5a"), 119.6 (C-6/C-6"), 124.4 (C-7/C-7"), 110.5 (C-8/C-8"), 139.5 (C-8a/C-8a"), 118.2 (C-9), 118.0 (C-9"), 127.9 (C-10), 128.4 (C-10"), 78.2 (C-11), 77.8 (C-11"), 16.2 (3-CH_3/3"-CH_3), 25.7 (11-CH_3), 26.4 (11"-CH_3), 37.7 (C-1'), 37.2 (C-1'"), 24.1 (C-2'), 23.8 (C-2'"), 64.6 (C-3'), 64.3 (C-3'"), 58.7 (C-4'), 58.6 (C-4'"), 24.9 (4'-CH_3/4'"-CH_3), 18.6 (4'-CH_3), 18.7 (4'"-CH_3).

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β-sitosterol (Mk31) : White solid

Optical Rotation, $[\alpha]_D^{25}$: –11.0 (CH_3OH; c 0.02)

Molecular Formula : $C_{29}H_{50}O$

UV (CH_3OH) λ_{max} (log ε), nm : 262 (4.31), 268 (4.52), 277 (4.28)

IR (CH_3OH) ν_{max}, cm^{-1} : 3346, 1024

HRESIMS, *m/z* : 415.3010 $[M+H]^+$

Melting point, °C : 134–136

^1H NMR ($CDCl_3$), δ ppm :

1.10 (*m*, H-1a, 1H), 1.85 (*m*, H-1b, 1H), 1.53 (*m*, H-2a, 1H), 1.98 (*m*, H-2b, 1H), 3.52 (*m*, H-3, 1H), 2.30 (*m*, H-4, 2H), 5.35 (*dd*, *J* = 5.2, 1.0 Hz, H-6, 1H), 1.86 (*m*, H-7, 2H), 1.49 (*m*, H-8, 1H), 0.93 (*m*, H-9, 1H), 1.50 (*m*, H-11, 2H), 1.15 (*m*, H-12a, 1H), 2.01 (*m*, H-12b, 1H), 1.00 (*m*, H-14, 1H), 1.58 (*m*, H-15, 2H), 1.26 (*m*, H-16a, 1H), 1.82 (*m*, H-16b, 1H), 1.10 (*m*, H-17, 1H), 1.36 (*m*, H-18, 1H), 0.92 (*d*, *J* = 6.5 Hz, H-19, 3H), 1.01 (*m*, H-20a, 1H), 1.33 (*m*, H-20b, 1H), 1.16 (*m*, H-21, 2H), 0.99 (*m*, H-22, 1H), 1.26 (*m*, H-23, 2H), 0.85 (*t*, *J* = 7.2 Hz, H-24, 3H), 1.68 (*m*, H-25, 1H), 0.84 (*d*, *J* = 6.4 Hz, H-26, 3H), 0.81 (*d*, *J* = 6.4 Hz, H-27, 3H), 0.68 (*s*, H-28, 3H), 1.01 (*s*, H-29, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

37.3 (C-1), 31.7 (C-2), 71.9 (C-3), 42.4 (C-4), 140.8 (C-5), 121.8 (C-6), 32.0 (C-7), 32.1 (C-8), 50.2 (C-9), 36.6 (C-10), 21.2 (C-11), 39.9 (C-12), 42.4 (C-13), 56.8 (C-14), 24.4 (C-15), 28.3 (C-16), 56.1 (C-17), 36.2 (C-18), 19.5 (C-19), 34.0 (C-20), 26.1 (C-21), 45.9 (C-22), 23.1 (C-23), 12.0 (C-24), 29.2 (C-25), 19.9 (C-26), 19.0 (C-27), 18.9 (C-28), 12.1 (C-29).

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Stigmast-4-ene-3-one (Mk32) : White amorphous

Optical Rotation, $[\alpha]_D^{25}$: +33.0 (CH_3OH; c 0.02)

Molecular Formula : $C_{29}H_{48}O$

UV (CH_3OH) λ_{max} (log ε), nm : 242 (4.12)

IR (CH_3OH) ν_{max}, cm^{-1} : 1678, 1454, 1025

HRESIMS, *m/z* : 413.2655 $[M+H]^+$

^1H NMR ($CDCl_3$), δ ppm :

1.69 (*m*, H-1a, 1H), 2.00 (*m*, H-1b, 1H), 1.30 (*m*, H-2a, 1H), 2.39 (*m*, H-2b, 1H), 5.72 (*s*, H-4, 1H), 2.24 (*m*, H-6a, 1H), 2.28 (*m*, H-6b,1H), 1.00 (*m*, H-7a, 1H), 1.84 (*m*, H-7b, 1H), 1.44 (*m*, H-8, 1H), 0.91 (*m*, H-9, 1H), 1.44 (*m*, H-11a, 1H), 1.53 (*m*, H-11b, 1H), 1.15 (*m*, H-12a, 1H), 2.02 (*m*, H-12b, 1H), 1.02 (*m*, H-14, 1H), 1.00 (*m*, H-15a, 1H), 1.61 (*m*, H-15b, 1H), 1.64 (*m*, H-16a, 1H), 1.84 (*m*, H-16b, 1H), 1.11 (*m*, H-17, 1H), 1.38 (*m*, H-18, 1H), 0.91 (*d*, *J* = 6.0 Hz, H-19, 3H), 2.32 (*m*, H-20, 2H), 1.16 (*m*, H-21, 2H), 0.92 (*m*, H-22, 1H), 1.28 (*m*, H-23, 2H), 0.86 (*t*, *J* = 6.8 Hz, H-24, 3H), 1.25 (*m*, H-25, 1H), 0.83 (*d*, *J* = 7.6 Hz, H-26, 3H), 0.81 (*d*, *J* = 7.6 Hz, H-27, 3H), 1.18 (*s*, H-28, 3H), 0.70 (*s*, H-29, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

35.8 (C-1), 33.9 (C-2), 199.8 (C-3), 123.8 (C-4), 171.9 (C-5), 33.1 (C-6), 32.1 (C-7), 35.7 (C-8), 53.9 (C-9), 38.7 (C-10), 21.1 (C-11), 39.7 (C-12), 42.5 (C-13), 55.9 (C-14), 24.3 (C-15), 28.3 (C-16), 56.1 (C-17), 36.2 (C-18), 18.8 (C-19), 34.1 (C-20), 26.1 (C-21), 45.9 (C-22), 23.1 (C-23), 12.1 (C-24), 29.2 (C-25), 19.9 (C-26), 19.1 (C-27), 17.5 (C-28), 12.0 (C-29).

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Squalene (Mk33) : Colourless oil

Molecular Formula : $C_{30}H_{50}$

UV (CH_3OH) λ_{max} (log ε), nm : 238 (4.12), 277 (4.08), 289 (4.14)

IR (CH_3OH) ν_{max}, cm^{-1} : 1400, 1510, 1025

HRESIMS, *m/z* : 410.3476 $[M]^+$

^1H NMR ($CDCl_3$), δ ppm :

1.69 (*s*, H-1/H-24, 6H), 5.13 (*m*, H-3/H-22, 2H), 2.08 (*m*, H-4/H-21, 4H), 1.99 (*m*, H-5/H-20, 4H), 5.13 (*m*, H-7/H-18, 2H), 2.00 (*m*, H-8/H-17, 4H), 2.10 (*m*, H-9/H-16, 4H), 5.14 (*m*, H-11/H-14, 2H), 2.06 (*m*, H-12/H-13,4H), 1.75 (*s*, H-25/H-30, 6H), 1.61 (*s*, H-26/H-27/H-28/H-29,12H).

^{13}C NMR ($CDCl_3$), δ ppm :

25.8 (C-1/C-24), 131.3 (C-2/C-23), 124.3 (C-3/C-22), 26.5 (C-4/C-21), 39.9 (C-5/C-20), 135.0 (C-6/C-19), 124.6 (C-7/C-18), 26.5 (C-8/C-17), 32.3 (C-9/C-16), 135.4 (C-10/H-15), 125.1 (C-11/C-14), 26.9 (C-12/C-13), 17.9 (C-25/C-30), 16.1 (C-26/C-27/C-28/C-29).

α-Tocopherol (Mk34) : Colourless oil

Optical Rotation, $[\alpha]_D^{25}$: +16.0 (CH_3OH; c 0.02)

Molecular Formula : $C_{29}H_{50}O_2$

UV (CH_3OH) λ_{max} (log ε), nm : 225 (4.28), 254 (4.22), 268 (4.26), 288 (4.16)

IR (CH_3OH) ν_{max}, cm^{-1} : 3327, 1452

HRESIMS, *m/z* : 430.2458 $[M]^+$

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^{1}H NMR ($CDCl_3$), δ ppm :

1.78 (*m*, H-3, 2H), 2.60 (*t*, *J* = 13.8 Hz, H-4, 2H), 1.23 (*s*, 2-CH_3, 3H), 2.11 (*s*, 5-CH_3, 3H), 4.20 (*s*, 6-OH, 1H), 2.16 (*s*, 7-CH_3, 3H), 2.11 (*s*, 8-CH_3, 3H), 1.48 (*m*, H-1'a, 1H), 1.53 (*m*, H-1'b, 1H), 1.38 (*m*, H-2', 2H), 1.06 (*m*, H-3'a, 1H), 1.25 (*m*, H-3'b, 1H), 1.38 (*m*, H-4', 1H), 1.30 (*m*, H-5', 2H), 1.15 (*m*, H-6', 2H), 1.30 (*m*, H-7', 2H), 1.38 (*m*, H-8', 1H), 1.30 (*m*, H-9', 2H), 1.05 (*m*, H-10', 2H), 1.14 (*m*, H-11', 2H), 1.51 (*m*, H-12', 1H), 0.87 (*d*, *J* = 6.9 Hz, H-13', 3H), 0.84 (*d*, *J* = 6.9 Hz, 4'-CH_3, 3H), 0.85 (*d*, *J* = 6.9 Hz, 8'-CH_3, 3H), 0.86 (*d*, *J* = 6.9 Hz, 12'-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

74.6 (C-2), 31.6 (C-3), 20.8 (C-4), 117.4 (C-4a), 118.5 (C-5), 144.6 (C-6), 121.1 (C-7), 122.7 (C-8), 145.6 (C-8a), 23.9 (2-CH_3), 11.4 (5-CH_3), 12.3 (7-CH_3), 11.9 (8-CH_3), 39.9 (C-1'), 21.1 (C-2'), 37.5 (C-3'), 32.9 (C-4'), 37.4 (C-5'), 24.5 (C-6'), 37.4 (C-7'), 32.8 (C-8'), 37.5 (C-9'), 24.9 (C-10'), 39.5 (C-11'), 28.1 (C-12'), 22.8 (C-13'), 19.8 (C-4'-CH_3), 19.7 (8'-CH_3), 22.7 (12'-CH_3).

2-Hydroxy-4-methoxy-3,6-dimethylbenzoic acid (MK35) : Pale yellow crystal

Molecular Formula : $C_{10}H_{12}O_4$

UV (CH_3OH) λ_{max} (log ε), nm : 268 (5.12), 289 (5.16), 316 (5.02)

IR (CH_3OH) ν_{max}, cm^{-1} : 3332, 1025

HRESIMS, *m/z* : 197.0799 $[M+H]^+$

Melting point, °C : 187–188

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^{1}H NMR ($CDCl_3$), δ ppm :

6.21 (*s*, H-5, 1H), 12.04 (*s*, 1-COOH, 1H), 5.19 (*br s*, 2-OH, 1H), 2.10 (s, 3-CH_3, 3H), 3.92 (*s*, 4-OCH_3, 3H), 2.46 (*s*, 6-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

105.4 (C-1), 163.3 (C-2), 108.7 (C-3), 158.2 (C-4), 110.7 (C-5), 140.3 (C-6), 172.8 (1-COOH), 7.8 (3-CH_3), 52.0 (4-OCH_3), 24.3 (6-CH_3).

Selin-11-en-4α-ol (Mk36) : White solid

Optical Rotation, $[\alpha]_D^{25}$: –7.0 (CH_3OH; c 0.02)

Molecular Formula : $C_{15}H_{26}O$

UV (CH_3OH) λ_{max} (log ε), nm : 262 (4.78), 268 (4.67)

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IR (CH_3OH) ν_{max}, cm^{-1} : 3327, 1114, 1025

HRESIMS, *m/z* : 222.2021 $[M]^+$

Melting point, °C : 60–62

^{1}H NMR ($CDCl_3$), δ ppm :

1.38 (*m*, H-2, 1H), 1.81 (*m*, H-2, 1H), 1.55 (*m*, H-3, 2H), 1.10 (*m*, H-4, 1H), 1.40 (*m*, H-4, 1H), 1.20 (*m*, H-5a, 1H), 1.44 (*m*, H-5b, 1H), 1.41 (*m*, H-6a, 1H), 1.58 (*m*, H-6b, 1H), 1.95 (*m*, H-7, 1H), 1.28 (*m*, H-8, 1H), 1.86 (*m*, H-8', 1H), 1.25 (*m*, H-8a, 1H), 1.11 (*s*,1-CH_3, 3H), 0.88 (*s*, 4a-CH_3, 3H), 1.74 (*s*, 9-CH_3, 3H), 4.71 (*d*, *J* = 1.2 Hz, 9a-CH_2, 1H), 4.69 (*d*, *J* = 1.2 Hz, 9a-CH_2, 1H).

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^{13}C NMR ($CDCl_3$), δ ppm :

72.3 (C-1), 43.4 (C-2), 20.2 (C-3), 41.1 (C-4), 34.7 (C-4a), 44.7 (C-5), 26.9 (C-6), 46.4 (C-7), 26.1 (C-8), 54.9 (C-8a), 150.8 (C-9), 22.8 (1-CH_3), 18.8 (4a-CH_3), 21.2 (9-CH_3), 108.2 (9-CH_2).

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3.2 Development of Synthetic Analogues of Girinimbine and Mahanimbine

3.2.1 General Introduction

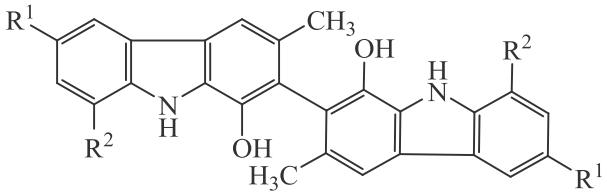
A broad range of carbazole alkaloids with promising biological activities have been isolated by several research groups from the family Rutaceae (Furukawa et al., 1985; Ito et al, 1993). Therefore, a variety of classical synthetic approaches and new methods using transition metals have been developed for the synthesis of carbazole alkaloids (Bauer & Knölker, 2012; Chakraborty & Roy, 1991; Chakraborty & Roy, 2003; Gassner et al., 2014; Hesse, Kataeva, Schmidt, & Knölker, 2014; Hesse, Jäger, Schmidt, & Knölker, 2014; Julich-Gruner, Schmidt, & Knölker, 2014; Knölker, 2009; Knölker & Reddy, 2002; Roy, Jama, & Mal, 2012; Schmidt, Reddy, & Knölker, 2012).

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Although several isolation of bioactive biscarbazoles have been described. For examples, clausenamine-A **151** was isolated from the stem and root bark of *Clausena excavata* (Wu, Huang, & Wu, 1996), which was used as folk medicine for detoxication caused by the poisonous snakebike in China, and a dimeric *O*-demethylmurrayafoline-A **152** was found to exhibit *in vitro* antiplasmodial activity against *P. falciparum* (Bringman et al., 1998). In fact, only a few synthetic approaches have been reported for the development of biscarbazole with limited information about their biological properties (Bauer & Knölker, 2012; Börger, Schmidt, & Knölker, 2014; Chakraborty & Roy, 1991; Chakraborty & Roy, 2003; Cordell, 2008; Knölker, 2005; Knölker & Reddy, 2002; Moody, 1994; Schmidt et al., 2012).

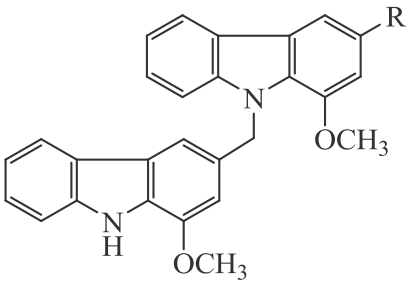
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151. Clausenamine-A
$R^1 = R^2 = OCH_3$
152. *O*-demethylmurrayafoline-A
$R^1 = R^2 = H$

After the first isolation of methylene-bridged bioactive biscarbazole alkaloid, bismurrayafoline-A **153** from the root of *M. euchrestifolia* Hayata by Furukawa, Wu, and Ohta (1983), it were then reported to exhibit a weak activity against several cancer cell lines by Itoigawa et al. (2000). In 2001, Bringmann and Tasler reported the synthesis of bismurrayafoline-A **153** with yield up to 19% as a by-product of the reduction of mukonine **96** (7 steps, 9% overall yield of **153**). In 2012, Börger, Kataeva, & Knölker have described a total synthesis of bismurrayafoline-A **153** via Ullmann coupling (6 steps, 28% overall yield of **153**).



153. Bismurrayafoline-A, $R = CH_3$
154. Bismurrayafolinol, $R = CH_2OH$

Another report from Ito and co-workers (1987), a dimeric carbazole, bismurrayafolinol **154**, was isolated from the root bark of *M. euchrestifolia* Hayata, was synthesized as a by-product during the synthesis of bismurrayafoline-A **153** with 6% yield (7 steps, 3% overall yield of **154**) was reported by Bringmann et al. (2001).

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Among the groups of carbazole alkaloids, the pyrano[3,2-a]carbazoles such as girinimbine **(Mk12)** and mahanimbine **(Mk15)**, is an important group of carbazole alkaloids which exhibit diverse structural features. Both of these compounds derive from 2-hydroxy-3-methylcarbazole **39** biogenetically by fusion with either prenyl or geranyl diphosphate follow by cyclization/annulation of the corresponding prenyl- or geranyl-substituent (Narasimhan et al., 1975).

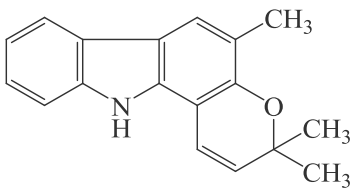
Herein, two major compounds, girinimbine **(Mk12)** and mahanimbine **(Mk15)** which isolated from *M. koenigii* in the previous section, have been chosen for the investigation. At the end, the approach not only able to provide us complex molecule of natural products, but also led us to gain better understanding on the development of biomimetic synthetic pathway routes of dimeric and cyclic monoterpenoid pyrano[3,2-a]carbazole alkaloids. The development of synthesis routes to girinimbine and mahanimbine analogues via oxidative coupling- and cyclization-mediated synthetic approaches will be discussed.

In general, anhydrous ferric (III) chloride ($FeCl_3$) has been of important for the development of the oxidative coupling reaction for the formation of carbon-carbon bonds in an organic molecule (Ahmad et al., 2009). Meanwhile, boron trifluoride diethyletherate (BF_3-$O(C_2H_5)_2$), a Lewis acid, was found to be an efficient acid at the cyclization/cleavage reaction. Commercially available anhydrous $FeCl_3$ and BF_3-$O(C_2H_5)_2$ were used as effective reagents for the oxidative coupling of electron-rich polysubstituted alkaloids to induce oxidative dimerization and/or cyclization. These

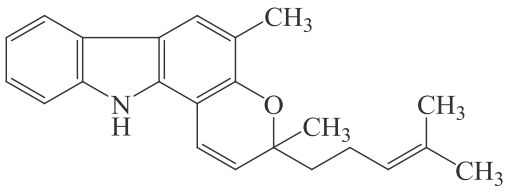
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reactions give interest on producing both complex molecules of natural products and to generate compounds for suitable biological investigations.



Girinimbine **(Mk12)**



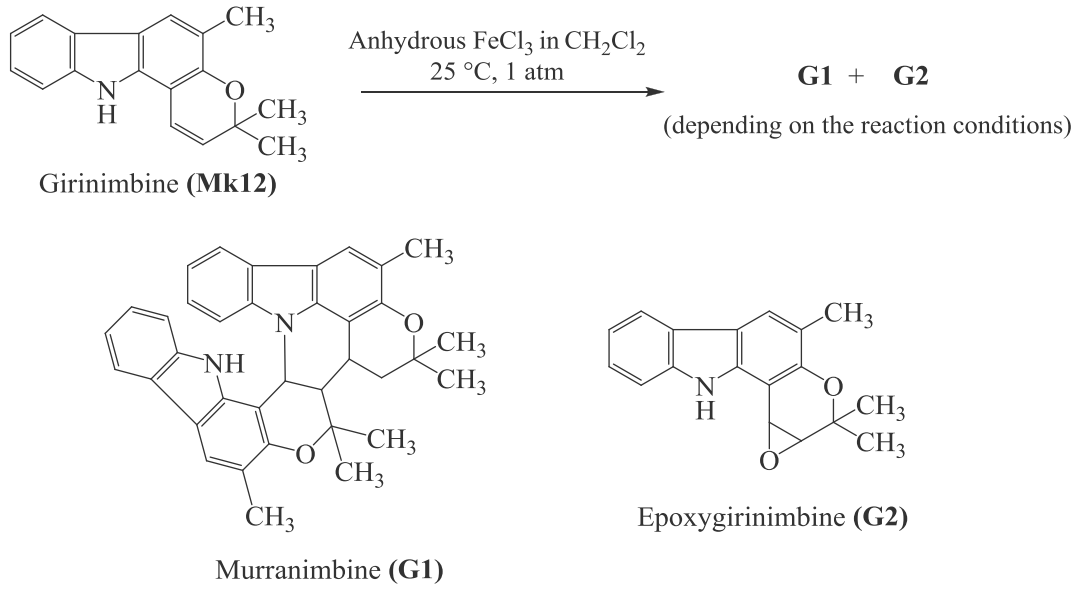
Mahanimbine **(Mk15)**

3.2.2 Oxidative Coupling/Cyclization Reaction Using Anhydrous Ferric (III) Chloride

Girinimbine (**Mk12**, 30 mg, 1.1×10^{-4} mmol) was dissolved in 10 ml CH_2Cl_2. Anhydrous $FeCl_3$ (18.5 mg, 1.1×10^{-4} mmol) was then added to the solution. The mixture was stirred for 10 minutes at room temperature and monitored by TLC. After the consumption of the starting material, the reaction mixture was diluted with ammonium chloride solution and extracted with ethyl acetate (EA) (three times). The combined EA extracts were dried over anhydrous magnesium sulphate. The crude products were finally purified by column chromatography and preparative TLC. Different attempts have been carried out to develop possible girinimbine analogues via oxidative coupling using anhydrous $FeCl_3$ (Table 3.2). The outcome of this reaction was the formation of murranimbine **(G1)** and epoxygirinimbine **(G2)** depending on the reaction conditions (Scheme 3.11). Structural assignments of **G1** and **G2** were ambiguously verified by spectroscopic analysis such as 1D and 2D NMR, IR, UV and MS.

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Scheme 3.8. Synthesis of Girinimbine Analogues with Anhydrous $FeCl_3$

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Table 3.2

Synthesis of Girinimbine Analogues with Anhydrous $FeCl_3$

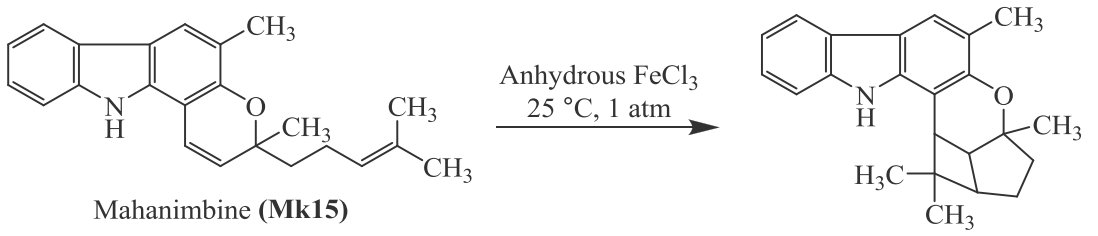
Entry	Anhydrous $FeCl_3$ (mmol)	Solvent used, mL	Time (min)	Yield (%)	
				G1	**G2**
1	1.1×10^{-4}	CH_2Cl_2, 10 mL	10	26.7	18.7
2	1.1×10^{-4}	CH_2Cl_2, 10 mL	30	23.3	19.3
3	1.1×10^{-4}	CH_2Cl_2, 10 mL	60	24.7	18.0
4	As catalyst	CH_2Cl_2, 10 mL	10	19.3	18.7
5	As catalyst	CH_2Cl_2, 10 mL	30	16.3	19.0
6	As catalyst	CH_2Cl_2, 10 mL	60	17.0	19.6
7	1.1×10^{-4}	Acetonitrile, 10 mL	60	27.3	14.0
8	1.1×10^{-4}	Acetonitrile, 10 mL	120	26.7	13.7
9	1.1×10^{-4}	Acetone, 10 mL	60	26.2	14.0
10	1.1×10^{-4}	Acetone, 10 mL	120	27.1	14.1

Note. No reaction takes place when using methanol as solvent.

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The reaction described above was repeated on mahanimbine (**Mk15**, 30 mg, 9.0×10^{-5} mmol) and was dissolved in 10 mL CH_2Cl_2, anhydrous $FeCl_3$ (14.7 mg, 9.0×10^{-5} mmol) was added (Scheme 3.9). Different attempts have been done to develop possible mahanimbine analogues via cyclization reaction using anhydrous $FeCl_3$ (Table 3.3). In this one step anhydrous $FeCl_3$-mediated approach, a hexacyclic compound, bicyclomahanimbine (**M1**), was successfully derived from mahanimbine (**Mk15**).



Mahanimbine **(Mk15)**

Bicyclomahanimbine **(M1)**

Scheme 3.9. Synthesis of Mahanimbine Analogues with Anhydrous $FeCl_3$

Table 3.3

Synthesis of Mahanimbine Analogues with Anhydrous $FeCl_3$

Entry	Anhydrous $FeCl_3$ (mmol)	Solvent used, mL	Time (min)	Yield (%)
				M1
1	9.0×10^{-5}	CH_2Cl_2, 10 mL	30	40.3
2	9.0×10^{-5}	CH_2Cl_2, 10 mL	60	41.0
3	As catalyst	CH_2Cl_2, 10 mL	30	39.3
4	As catalyst	CH_2Cl_2, 10 mL	60	39.7
5	9.0×10^{-5}	Acetonitrile, 10 mL	60	39.2
6	9.0×10^{-5}	Acetonitrile, 10 mL	120	39.5
7	9.0×10^{-5}	Acetone, 10 mL	60	41.7
8	9.0×10^{-5}	Acetone, 10 mL	120	39.7

Note. No reaction takes place when using methanol as solvent.

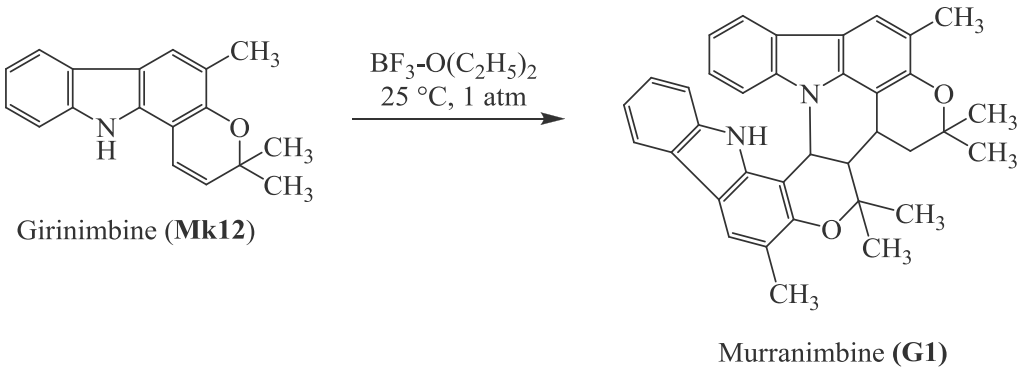
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3.2.3 Oxidative Coupling/Cyclization Reaction Using Boron Trifluoride Diethyletherate

A solution of girinimbine (**Mk12**, 30 mg, 1.1 × 10^{-4} mmol) in 10 ml CH_2Cl_2. Boron trifluoride diethyletherate (BF_3-$O(C_2H_5)_2$) (0.5 mL) was then added to the solution. The mixture was stirred for 60 minutes at room temperature and monitored by TLC. This reaction was performed according to Chakraborty and co-worker (2010) with slight modification. After the consumption of the starting material, the reaction mixture was diluted with ammonium chloride solution and extracted with ethyl acetate (3 × 20 mL). The combined EA extracts were dried over anhydrous magnesium sulphate (Scheme 3.10). The crude products were finally purified by chromatographic methods. Several attempts have been done to develop possible girinimbine analogues using BF_3-$O(C_2H_5)_2$ (Table 3.4). Murranimbine **(G1)** is the only compound obtained in this approach. The structural elucidation of **G1** is verified by complete spectroscopic analysis.

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Girinimbine (**Mk12**)

Murranimbine **(G1)**

Scheme 3.10. Synthesis of Girinimbine Analogues with BF_3-$O(C_2H_5)_2$

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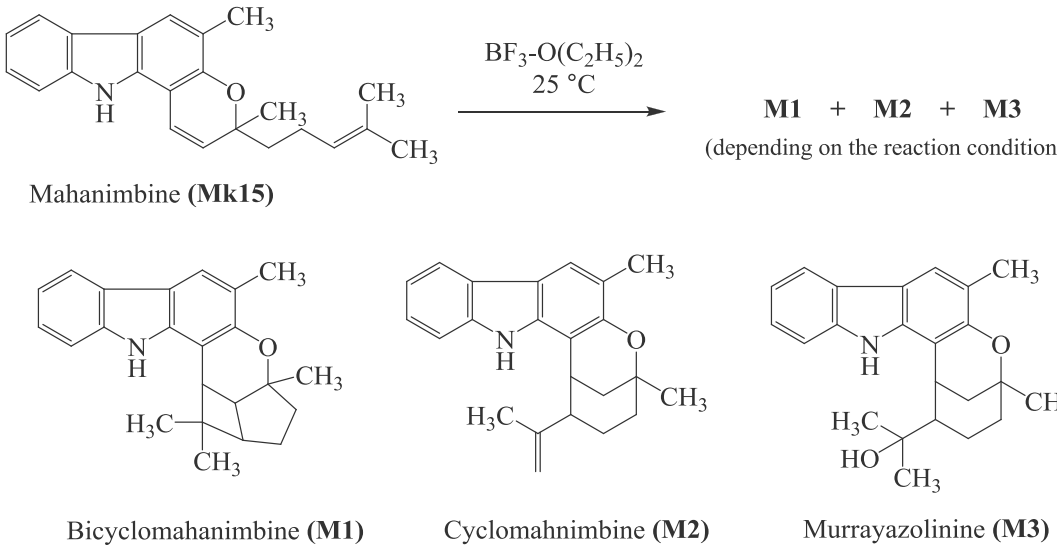
Table 3.4

Synthesis of Girinimbine Analogues with BF_3-$O(C_2H_5)_2$

Entry	BF_3-$O(C_2H_5)_2$, mL	Solvent used, mL	Time (min)	Yield (%) of **G1**
1	0.5 mL	CH_2Cl_2, 10 mL	60	-
2	0.5 mL	CH_2Cl_2, 10 mL	120	-
3	0.5 mL	Acetonitrile, 10 mL	30	-
4	0.5 mL	Acetonitrile, 10 mL	60	-
5	0.5 mL	Acetone, 10 mL	30	40.0
6	0.5 mL	Acetone, 10 mL	60	41.7

Note. No reaction takes place when using methanol as solvent.

The reaction described above was repeated on mahanimbine (**Mk15**, 30 mg, 9.0×10^{-5} mmol) (Scheme 3.11). Several attempts have been done to develop possible mahanimbine analogues via this new Lewis acid-mediated cyclization reaction using BF_3-$O(C_2H_5)_2$ (Table 3.5). In the approach, not only bicyclomahanimbine **(M1)**, but along with another two pentacyclic carbazole alkaloids, cyclomahanimbine **(M2)** and murrayazolinine **(M3)**, were also afforded.



Scheme 3.11. Synthesis of Mahanimbine Analogues with BF_3-$O(C_2H_5)_2$

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Table 3.5

Synthesis of Mahanimbine Analogues with BF_3-$O(C_2H_5)_2$

Entry	BF_3-$O(C_2H_5)_2$, mL	Solvent used, mL	Time (min)	Yield (%)		
				M1	**M2**	**M3**
1	0.5 mL	CH_2Cl_2, 10 mL	10	10.9	19.7	-
2	0.5 mL	CH_2Cl_2, 10 mL	30	11.3	19.7	-
3	0.5 mL	Acetonitrile, 10 mL	10	9.7	14.3	-
4	0.5 mL	Acetonitrile, 10 mL	30	9.7	16.3	-
5	0.5 mL	Acetone, 10 mL	10	10.0	19.3	42.7
6	0.5 mL	Acetone, 10 mL	30	10.3	20.0	44.0

Note. No reaction takes place when using methanol as solvent.

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3.2.4 Physical and Spectral Data of the Synthetic Analogues of Girinimbine and Mahanimbine

Murranimbine (G1) : Dark purple powder

Optical Rotation, $[\alpha]_D^{25}$: –30.5 (CH_3OH; c 0.02)

Molecular Formula : $C_{36}H_{34}N_2O_2$

UV (CH_3OH) λ_{max} (log ε), nm : 240 (4.12), 262 (4.15), 304 (4.03), 340 (4.02)

IR (CH_3OH) ν_{max}, cm^{-1} : 3325, 1032

HRESIMS, *m/z* : 526.2682 $[M]^+$

^1H NMR ($CDCl_3$), δ ppm :

7.71 (*s*, H-4, 1H), 8.11 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.34 (*td*, *J* = 8.0, 1.0 Hz, H-6, 1H), 7.42 (*td*, *J* = 8.0, 1.0 Hz, H-7, 1H), 7.52 (*d*, *J* = 8.0 Hz, H-8, 1H), 3.13 (*td*, *J* = 11.5, 5.2 Hz, H-9, 1H), 2.37 (*dd*, *J* = 11.6, 5.2 Hz, H-10α, 1H), 1.63 (*dd*, *J* = 11.5, 5.2 Hz, H-10β, 1H), 2.30 (*s*, 3-CH_3, 3H), 1.71 (*s*, 11-CH_3, 3H), 1.48 (*s*, 11-CH_3, 3H), 7.76 (*s*, H-4', 1H), 7.82 (*d*, *J* = 8.0 Hz, H-5', 1H), 7.02 (*td*, *J* = 8.0, 1.0 Hz, H-6', 1H), 7.06 (*td*, *J* = 8.0, 1.0 Hz, H-7', 1H), 6.66 (*d*, *J* = 8.0 Hz, H-8', 1H), 6.12 (*d*, *J* = 5.2 Hz, H-9', 1H), 2.16 (*dd*, *J* = 10.9, 5.3 Hz, H-10', 1H), 7.18 (*br s*, NH', 1H), 2.42 (*s*, 3'-CH_3, 3H), 1.71 (*s*, 11'-CH_3, 3H), 1.22 (*s*, 11'-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

103.2 (C-1), 136.3 (C-1a), 150.4 (C-2), 119.1 (C-3), 120.1 (C-4), 112.6 (C-4a), 120.7 (C-5), 124.6 (C-5a), 120.1 (C-6), 124.0 (C-7), 108.8 (C-8), 139.6 (C-8a), 27.2 (C-9), 39.8 (C-10), 75.1 (C-11), 16.6 (3-CH_3), 30.9 (11-CH_3), 30.7 (C11-CH_3), 106.7 (C-1'), 136.1 (C-1a'), 150.4 (C-2'), 118.9 (C-3'), 120.8 (C-4'), 117.7 (C-4a'), 118.8 (C-5'),

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122.4 (C-5a'), 119.0 (C-6'), 124.1 (C-7'), 110.3 (C-8'), 139.1 (C-8a'), 50.5 (C-9'), 46.5 (C-10'), 79.0 (C-11'), 16.8 (3'-CH_3), 28.8 (11'-CH_3), 25.2 (11'-CH_3).

Epoxygirinimbine (G2) : Brown amorphous

Optical Rotation, $[\alpha]_D^{25}$: +4.5 (CH_3OH; c 0.02)

Molecular Formula : $C_{18}H_{17}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 238 (4.26), 262 (4.38), 270 (4.26), 344 (4.16)

IR (CH_3OH) ν_{max}, cm^{-1} : 3347, 1025

HRESIMS, *m/z* : 279.1591 $[M]^+$

^{1}H NMR ($CDCl_3$), δ ppm :

7.76 (*s*, H-4, 1H), 7.94 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.20 (*td*, *J* = 8.0, 1.0 Hz, H-6, 1H), 7.32 (*td*, *J* = 8.0, 1.0 Hz, H-7, 1H), 7.22 (*d*, *J* = 8.0 Hz, H-8, 1H), 5.22 (*d*, *J* = 6.3 Hz, H-9, 1H), 2.65 (*d*, *J* = 6.9 Hz, H-10, 1H), 8.17 (*br s*, NH, 1H), 2.33 (*s*, 3-CH_3, 3H), 1.61 (*s*, 11-CH_3, 3H), 1.46 (*s*, 11-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

104.3 (C-1), 137.3 (C-1a), 150.5 (C-2), 118.9 (C-3), 121.5 (C-4), 116.6 (C-4a), 119.5 (C-5), 123.7 (C-5a), 119.6 (C-6), 124.5 (C-7), 110.5 (C-8), 139.3 (C-8a), 71.2 (C-9), 48.6 (C-10), 76.4 (C-11), 16.4 (3-CH_3), 28.4 (11-CH_3), 24.2 (11-CH_3).

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Bicyclomahanimbine (M1) : Brown Solid

Optical Rotation, $[\alpha]_D^{25}$: –33.0 (CH_3OH; c 0.02)

Molecular Formula : $C_{23}H_{25}NO$

UV (CH_3OH) λ_{max} (log ε), nm : 242 (4.13), 261 (4.16), 304 (4.20), 342 (4.02)

IR (CH_3OH) ν_{max}, cm^{-1} : 3340, 1025

HRESIMS, *m/z* : 332.2012 $[M+H]^+$

Melting Point, °C : 146–147

^1H NMR ($CDCl_3$), δ ppm :

7.66 (*s*, H-4, 1H), 7.93 (*d*, *J* = 8.1 Hz, H-5, 1H), 7.15 (*td*, *J* = 8.1, 1.0 Hz, H-6, 1H), 7.28 (*td*, *J* = 8.1, 1.0 Hz, H-7, 1H), 7.37 (*d*, *J* = 8.1 Hz, H-8, 1H), 3.30 (*d*, *J* = 9.2 Hz, H-9, 1H), 2.04 (*m*, H-10a, 1H), 1.55 (*m*, H-10b, 1H), 7.44 (*br s*, NH, 1H), 2.33 (*s*, 3-CH_3, 3H), 2.50 (*t*, *J* = 7.4 Hz, H-1', 1H), 1.66 (*m*, H-2'a, 1H), 1.56 (*m*, H-2'b, 1H), 2.71 (*t*, *J* = 7.5 Hz, H-3', 1H), 1.43 (*s*, 11-CH_3, 3H), 0.74 (*s*, 4'-CH_3, 3H), 1.57 (*s*, 4'-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

106.5 (C-1), 137.8 (C-1a), 150.2 (C-2), 120.1 (C-3), 119.2 (C-4), 115.8 (C-4a), 119.4 (C-5), 124.2 (C-5a), 119.4 (C-6), 124.0 (C-7), 110.4 (C-8), 139.3 (C-8a), 37.5 (C-9), 38.4 (C-10), 83.5 (C-11), 16.8 (3-CH_3), 46.5 (C-1'), 25.7 (C-2'), 37.9 (C-3'), 39.5 (C-4'), 27.5 (11-CH_3), 18.6 (4'-CH_3), 35.2 (4'-CH_3).

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Cyclomahanimbine (M2) : Brown solid

Optical Rotation, $[\alpha]_D^{25}$: –1.0 (CH_3OH; c 0.02)

Molecular Formula : $C_{23}H_{25}NO$

UV (CH_3OH) λ_{max} (log ε), nm : 248 (4.10), 263 (4.16), 270 (4.22)

IR (CH_3OH) ν_{max}, cm^{-1} : 3326, 1025

HRESIMS, *m/z* : 331.1928 $[M]^+$

Melting Point, °C : 146–147

^1H NMR ($CDCl_3$), δ ppm :

7.64 (*s*, H-4, 1H), 7.89 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.14 (*td*, *J* = 8.0, 1.1 Hz, H-6, 1H), 7.27 (*td*, *J* = 8.0, 1.1 Hz, H-7, 1H), 7.32 (*d*, *J* = 8.0 Hz, H-8, 1H), 3.41 (*d*, *J* = 2.9 Hz, H-9, 1H), 2.02 (*dd*, *J* = 12.6, 2.9 Hz, H-10a, 1H), 1.91 (*m*, H-10b, 1H), 7.72 (*br s*, NH, 1H), 2.33 (*s*, 3-CH_3, 3H), 1.44 (*s*, 11-CH_3, 3H), 2.08 (*m*, H-1'a, 1H), 1.62 (*m*, H-1'b, 1H), 2.04 (*m*, H-2'a, 1H), 1.62 (*m*, H-2'b, 1H), 2.58 (*m*, H-3', 1H), 4.74 (*d*, *J* = 1.7 Hz, H-5'a, 1H), 4.81 (*d*, *J* = 1.7 Hz, H-5'b, 1H), 1.50 (*s*, 4'-CH_3, 3H).

^{13}C NMR ($CDCl_3$), δ ppm :

105.3 (C-1), 138.4 (C-1a), 153.7 (C-2), 117.7 (C-3), 119.6 (C-4), 114.7 (C-4a), 119.1 (C-5), 124.3 (C-5a), 119.2 (C-6), 123.6 (C-7), 110.3 (C-8), 139.4 (C-8a), 36.3 (C-9), 37.4 (C-10), 74.0 (C-11), 16.9 (3-CH_3), 29.1 (11-CH_3), 40.0 (C-1'), 23.2 (C-2'), 48.8 (C-3'), 150.2 (C-4'), 112.1 (C-5'), 21.7 (4'-CH_3).

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Murrayazolinine (M3) : Brown amorphous

Optical Rotation, $[\alpha]_D^{25}$: –39.5 (CH_3OH; c 0.02)

Molecular Formula : $C_{23}H_{27}NO_2$

UV (CH_3OH) λ_{max} (log ε), nm : 240 (4.23), 250 (4.16), 302 (4.42), 338 (4.26)

IR (CH_3OH) ν_{max}, cm^{-1} : 3331, 1025

HRESIMS, *m/z* : 350.1750 $[M+H]^+$

^1H NMR ($CDCl_3$), δ ppm :

7.66 (*s*, H-4, 1H), 7.91 (*d*, *J* = 8.0 Hz, H-5, 1H), 7.14 (*td*, *J* = 8.0, 1.2 Hz, H-6, 1H), 7.27 (*td*, *J* = 8.0, 1.2 Hz, H-7, 1H), 7.37 (*d*, *J* = 8.0 Hz, H-8, 1H), 3.80 (*m*, H-9, 1H), 1.85 (*q*, *J* = 2.9 Hz, H-10, 2H), 9.59 (*br s*, NH, 1H), 2.34 (*s*, 3-CH_3, 3H), 1.43 (*s*, 11-CH_3, 3H), 2.09 (*m*, H-1'a, 1H), 1.62 (*m*, 1'b, 1H), 1.45 (*m*, H-2', 2H), 1.90 (*m*, H-3', 1H), 1.32 (*s*, 4'-CH_3, 3H), 0.55 (*s*, 4'-CH_3, 3H), 1.72 (*br s*, 4'-OH, 1H).

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^{13}C NMR ($CDCl_3$), δ ppm :

105.9 (C-1), 139.0 (C-1a), 153.7 (C-2), 117.5 (C-3), 119.5 (C-4), 114.8 (C-4a), 119.1 (C-5), 124.4 (C-5a), 118.7 (C-6), 123.4 (C-7), 110.6 (C-8), 139.8 (C-8a), 29.3 (C-9), 38.3 (C-10), 74.4 (C-11), 16.9 (3-CH_3), 29.0 (11-CH_3), 40.5 (C-1'), 22.9 (C-2'), 52.8 (C-3'), 74.4 (C-4'), 33.4 (4'-CH_3), 23.3 (4'-CH_3).

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3.3 Biological Activity

3.3.1 General Introduction

A large number of plant-derived biologically active carbazole alkaloids have been isolated and being reported by several researchers. Many of these chemical entities have shown biological properties such as antifungal, antimicrobial, anti-tumour, anti-inflammatory, cytotoxic and hypertensive effects (Chowdhury et al., 2001; Das et al., 1965; Ito et al., 2006; Kok et al., 2012; Nagappan et al, 2011; Taechowisan et al., 2012; Tantapakul et al., 2014). As synthetic organic compounds, many carbazole derivatives such as tetrahydrocarbazoles were also reported to exhibit anticancer activities against different cell lines such as kidney adenocarcinoma (ACHN), pancreas carcinoma (Panc-1), lung carcinoma (GIII) (Calu1), non-small cell lung carcinoma (H460), colon cancer cell (HCT116) and normal breast epithelium (MCF10A) cell lines (Shrungesh Kumar, Mahadevan, & Kumara, 2014). Their useful bioactivities and interesting structural features have attracted the attention of synthetic chemists and led to the development of many different synthetic strategies. Since then, the field has expanded enormously large due to the promising biological activities of many of the carbazole alkaloids.

Since *M. koenigii* being rich in bioactive carbazole alkaloids and only a few studies have been involved in cytotoxic analysis (Asche & Demeunynck, 2007; Bandyopadhyaya et al, 2002; Chakraborty et al., 1997; Itoigawa et al., 2000; Kok et al., 2012; Wang, Cai, & Cui, 2007; Wang, Cai, Cui, Yan, & Wu, 2008). In our continuing interest in biological studies, this section describes the methodology for a

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further examination of cytotoxic potential of the isolated carbazole alkaloids and the synthetic analogues of girinimbine **(Mk12)** and mahanimbine **(Mk15)**.

3.3.2 Materials and Chemical Reagents

The human promyelocytic leukemia cell lines (HL-60), cervical cancer cell lines (HeLa) and the normal mouse embryonic fibroblasts (NIH/3T3) cell lines were obtained from the American Type Culture Collection (ATCC), USA.

Commercially available RPMI-1640 (Sigma-Aldrich, USA), a culture medium, with 10% v/v foetal calf serum 10270 (Gibco, South America) and a Penicillin-Streptomycin Mixed Solution (5000 units/mL penicillin and 5 mg/mL streptomycin in 0.85% NaCl, Kyota, Japan) as a complete growth medium (CGM).

Freeze medium with 10% dimethyl sulfoxide (DMSO) and 90% foetal calf serum for cryopreservation of cell lines. Phosphate Buffer Solution (PBS) used as buffer solution which can be dispensed into aliquots. Trypsin/EDTA (TrypLE^{TM} Express, Gibco, Denmark) were used to bring adherent and semi-adherent cells into suspension. 0.4% Trypan Blue Solution (dilution factor = 2) (Sigma Aldrich, USA) is routinely used as cell stain during cell counting.

Dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), a yellow tetrazole, added to cells in culture, usually at a final concentration of 0.2 – 0.5 mg/mL, and incubated for 1 to 4 hours. The quantity of purple formazan crystal which is insoluble precipitate inside cells (presumably directly proportional to the number of

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viable cells) is measured by recording changes in absorbance at 570 nm using a plate reading spectrophotometer. A wavelength of 630 nm is used as reference. DMSO will be used as solubilisation solution to dissolve the formazan prior to recording absorbance readings.

3.3.3 Culture of Cells

The human promyelocytic leukemia cell lines (HL-60), cervical cancer cell lines (HeLa) and the normal mouse embryonic fibroblasts (NIH/3T3) cell lines were cultured in a CGM. For HL-60 cell lines (a suspension cell line), the cells were cultured in a flask and incubated at 37 °C in 5% (v/v) CO_2 for a few days until it has grown confluently in the flask. Cells were counted and prepared to a need concentration at 1×10^5 cells/mL for MTT assay before seeded into the 96-well microplate. For HeLa and NIH/3T3 cell lines (the adherent cell lines), the cells were seeded and preculture in a 96-well microplate at 1×10^5 cells/mL and incubated at 37 °C in 5% v/v CO_2 for 24 hours (Mackeen et al., 2000).

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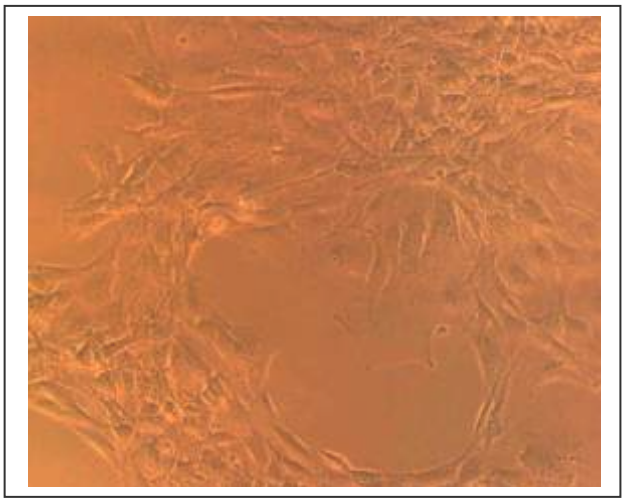


Figure 3.1. NIH/3T3 Cell Lines

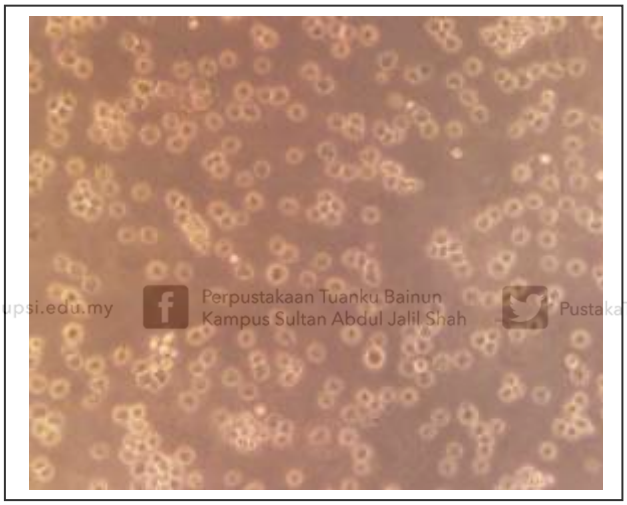


Figure 3.2. HL-60 Cell Lines

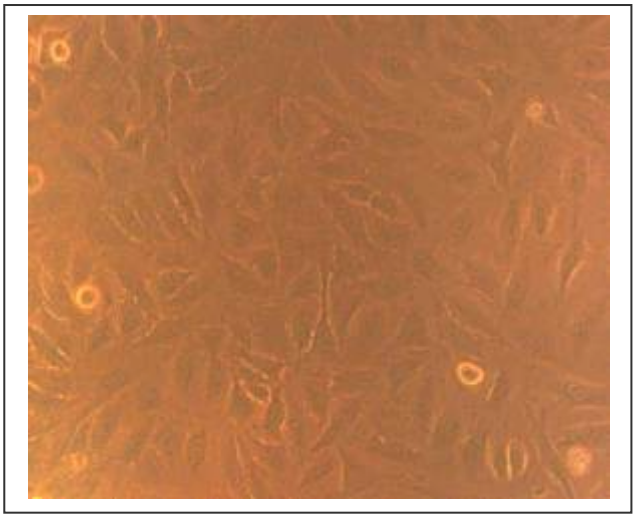


Figure 3.3. HeLa Cell Lines

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3.3.4 Cytotoxic Assay

Cytotoxic assay was determined using MTT assay developed by Mosmann (1983). For suspension cells MTT assay, varying concentrations 0.5 - 60.0 μg/mL of the isolated compounds were prepared from the stock samples by serial dilution in RPMI-1640 to give a fresh medium containing of test compounds (Ali et al., 1996) in a 96-well microplate. Each well was then filled with 100 μL of the selected cell suspension in CGM at 1.0×10^5 cells/mL. For adherent cells MTT assay, varying concentrations of the isolated compounds, started with 60 μg/mL, will be added to the 96-well microplate after the seeding of the selected cell lines for 24 hours. Controls that contained only cells lines and medium as negative control were included for each sample. Anticancer chemotherapy drug vincristine (VCR) (started with 60 μg/mL each) were used as positive control.

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The assay for each concentration of test compound was performed in triplicate and the culture plates were kept at 37 ℃ with 5% v/v CO_2 for 72 hours. After 3 days of incubation, 20 μL of MTT (5 mg/mL) in phosphate buffer solution (PBS) was added to each well and the plate was then further incubated for 4 hours. After the incubation, all remaining supernatant were removed and 100 μL of DMSO was then added to each well and mixed thoroughly to dissolve the resultant formazan crystal. After 20 minutes of incubation to ensure all crystals were dissolved, the cytotoxic effect of compounds on the selected cell lines were detected by measuring the absorbance of each well at 570 nm and 630 nm as reference wavelength by using ELISA microplate reader. Mean absorbance for each compound concentration was expressed as a percentage of control untreated well absorbance and plotted verses

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compound dose. Results were expressed as a percentage of control giving a certain percentage of cell viability after 72 hours exposure to the test compounds.

The percentage of cytotoxicity compared to the untreated cells was determined with the equation given as follow (Prayong, Barusrux, & Weerapreeyakul, 2008).

$$\% \text{ of Cytotoxicity} = \frac{(\text{Abs of cell without treatment} - \text{Abs of cell with treatment}) \times 100\%}{\text{Abs of cell without treatment}}$$

Finally, the concentrations of dose sample that can inhibit concentration of 50% of cell viability (CD_{50}) were determined from plotting of graph cell viability percentage versus concentration of test compounds.

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CHAPTER 4

RESULTS AND DISCUSSION

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4.1 General Introduction

The first part of the study was about the isolation and structural elucidation of the chemical constituents from Malaysian Rutaceae plant species, *Murraya koenigii* (Linn.) Spreng (TM1006). Various chromatographic techniques such as column chromatography (CC), thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) have been used for the isolation and purification process. A total of 39 chemical compounds were successfully isolated from the bark and leaves of this species, while three of them were repeated compounds obtained from both bark and leaves of the plants. The details were listed in Table 4.2.

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These structures were then elucidated by a combination of spectroscopic methods such as 1D NMR (^{1}H, ^{13}C, DEPT), 2D NMR (^{1}H-^{1}H COSY, NOESY, TOCSY, HMBC and HMQC), UV, IR, optical rotation, and mass spectrometry.

Then, naturally isolated chemical compounds were examined for their cytotoxicity via MTT assay against human promyelocytic leukemia cell lines (HL-60) and cervical cancer cell lines (HeLa) along with the normal mouse embryonic fibroblasts (NIH/3T3) in order to search for potential bioactive compounds from *M. koenigii* by determine their cytotoxic properties by comparison with a reputed cytotoxic agents vincristine (VCR). Their structure-activity relationship (SAR) analysis was also performed to investigate the relationship between the isolated carbazole alkaloids and their cytotoxic properties.

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Further study from this approach, development of synthetic analogues from two major carbazole alkaloids, which were isolated from the selected plant, *viz*., girinimbine **(Mk12)** and mahanimbine **(Mk15)**, by using anhydrous ferric (III) chloride ($FeCl_3$) and boron trifluoride diethyletherate (BF_3-$O(C_2H_5)_2$). These are the effective reagents to induce oxidative dimerization and cyclization reaction in electron-rich polysubstituted alkaloids. Two synthetic derivatives were successfully derived from girinimbine **(Mk12)**, *viz.*, murranimbine **(G1)**, a known carbazole alkaloids isolated previously from *M. euchrestifolia* (Ito & Furukawa, 1991), and a novel carbazole alkaloid with an epoxide functionality, namely epoxygirinimbine **(G2)**. Another three known carbazole deriviatives were also successfully synthesized from mahanimbine **(Mk15)**, *viz.*, bicyclomahanimbine **(M1)**, cyclomahanimbine **(M2)** and murrayazolinine **(M3)**. Attention will be paid on their structural characterization

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and their possible mechanistic pathways. The structurally modified analogues of girinimbine and mahanimbine were also examined for their cytotoxicity via MTT assay against HL-60, HeLa and NIH/3T3 cell lines. The findings showed that one of these molecules has been effectively increased the potency and ability to inhibit the selected cell lines when compared to girinimbine **(Mk12)** and mahanimbine **(Mk15)** and also comparison with VCR which served as a positive control in the cytotoxic analysis.

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4.2 Chemical Constituents Isolated from *M. koenigii*

Phytochemical investigation on the bark of *M. koenigii* afforded 25 chemical compounds, included 21 carbazole alkaloids and four non-carbazole entities, of which six are new carbazoles. These chemical entities are made up of eight tricyclic carbazoles, two carbazolequinones, seven pyrano[3,2-a]carbazole alkaloids, three cyclic monoterpenoid pyrano[3,2-a]carbazole alkaloids, one biscarbazole alkaloids and four non-carbazole compounds (Table 4.2).

Isolations and purifications of chemical constituents from the leaves of *M. koenigii* yielded 14 chemical compounds, included three non-carbazole entities and 11 carbazole alkaloids, of which two are tricyclic carbazoles, five pyrano[3,2-a]carbazole alkaloids, three biscarbazole alkaloids, along with a new cyclic monoterpenoid pyrano[3,2-a]carbazole alkaloids (Table 4.2). The list of chemical constituents isolated from the bark and leaves of *M. koenigii* and its composition were summarised in Table 4.2.

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Table 4.2

The Chemical Constituents Isolated from M. koenigii

Alkaloids	Part of the plants	R_f value	Yield (g/kg)	Chapter
Tricyclic Carbazole Alkaloids				
Murrayanine **(Mk1)**	Bark	0.53 [CH_2Cl_2]	0.0493	4.2.1.1
Murrayafoline-A **(Mk2)**	Bark, Leaves	0.50 [Hex: CH_2Cl_2 (1:1)]	0.0177 (bark)	4.2.1.1
Koenoline **(Mk3)**	Bark	0.52 [CH_2Cl_2:CH_3OH (99:1)]	0.0140	4.2.1.1
3-Formylcarbazole **(Mk4)**	Bark	0.49 [CH_2Cl_2:CH_3OH (99:1)]	0.00017	4.2.1.1
2-Hydroxy-3-methylcarbazole **(Mk5)**	Bark	0.42 [CH_2Cl_2:CH_3OH (99:1)]	0.0030	4.2.1.2
Mahanimbilol **(Mk6)**	Bark	0.69 [CH_2Cl_2]	0.0047	4.2.1.3
Euchrestine-B **(Mk7)**	Leaves	0.38 [CH_2Cl_2]	0.0145	4.2.1.3
Mukoenine-B **(Mk8)**	Bark	0.48 [Hex:EA (9:1)]	0.0027	4.2.1.3
Murrastanine-A **(Mk9)***	Bark	0.31 [CH_2Cl_2:CH_3OH(95:5)]	0.0030	4.2.1.4
Carbazolequinone Alkaloid				
Murrayaquinone-A **(Mk10)**	Bark	0.43 [Hex: EA (3:2)]	0.0030	4.2.2.1
Murrayaquinone-B **(Mk 11)**	Bark	0.54 [CH_2Cl_2]	0.0017	4.2.2.1

Note. ( )* new compound; ( )# known compound first time isolated and reported from *M. koenigii*.

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Table 4.2 (Continued)

Alkaloids	Part of the plants	R_f value	Yield (g/kg)	Chapter
Pyrano[3,2-a]carbazole Alkaloids				
Girinimbine **(Mk12)**	Bark	0.35 [Hex:CH_2Cl_2 (1:1)]	0.9540 (bark)	4.2.3.1
Koenimbine **(Mk13)**	Leaves	0.53 [Hex:CH_2Cl_2 (1:1)]	0.0177	4.2.3.1
Murrayacine **(Mk14)**	Bark	0.46 [CH_2Cl_2]	0.0037	4.2.3.1
Mahanimbine **(Mk15)**	Bark, Leaves	0.47 [Hex:CH_2Cl_2 (1:1)]	0.6300 (bark)	4.2.3.2
Mahanine **(Mk16)**	Leaves	0.32 [CH_2Cl_2:CH_3OH (99:1)]	0.0194	4.2.3.2
Isomahanine **(Mk17)**	Leaves	0.38 [CH_2Cl_2:CH_3OH (98:2)]	0.0806	4.2.3.2
Murrayamine-B **(Mk18)**	Leaves	0.84 [[Hex:CH_2Cl_2 (3:7)]	0.0145	4.2.3.2
Murrayamine-J **(Mk19)**#	Bark	0.41 [CH_2Cl_2:CH_3OH (99:1)]	0.0017	4.2.3.2
Murrastinine-A **(Mk20)***	Bark	0.48 [CH_2Cl_2]	0.0027	4.2.3.3
Murrastinine-B **(Mk21)***	Bark	0.73 [CH_2Cl_2]	0.0020	4.2.3.4
Murrastinine-C **(Mk22)***	Bark	0.30 [CH_2Cl_2]	0.0027	4.2.3.5
Cyclic Monoterpenoid Pyrano[3,2-a]carbazole Alkaloids				
Murrayazolinol **(Mk23)**	Bark	0.38 [CH_2Cl_2:CH_3OH(99:1)]	0.0040	4.2.4.1
Murrayakoeninol **(Mk24)**	Bark	0.33 [CH_2Cl_2:CH_3OH(99:1)]	0.0027	4.2.4.1
Bicyclomahanimbine **(Mk25)**	Bark	0.51 [Hex:CH_2Cl_2 (1:1)]	0.0027	4.2.4.2
Murrayatanine-A **(Mk26)***	Leaves	0.71 [Hex:CH_2Cl_2(3:7)]	0.0017	4.2.4.3

Note. ( )* new compound; ( )# known compound first time isolated and reported from *M. koenigii*.

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Table 4.2 (Continued)

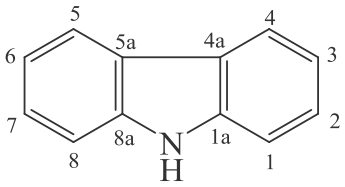
Alkaloids	Part of the plants	R_f value	Yield (g/kg)	Chapter
Biscarbazole Alkaloids				
Bismurrayafoline-E **(Mk27)**	Leaves	0.61 [CH_2Cl_2]	0.0565	4.2.5.1
Bispyrayafoline **(Mk28)**	Leaves	0.49 [CH_2Cl_2]	0.0129	4.2.5.2
Bisisomahanine **(Mk29)**#	Leaves	0.40 [CH_2Cl_2]	0.0097	4.2.5.3
Bismahanimboline **(Mk30)***	Bark	0.45 [Hex:CH_2Cl_2 (2:8)]	0.0017	4.2.5.4
Terpenoids				
β-Sitosterol **(Mk31)**#	Bark	0.36 [Hex:EA (8:2)]	0.0037	4.2.6.1
Stigmast-4-ene-3-one **(Mk32)**#	Bark	0.47 [Hex:EA (8:2)]	0.0043	4.2.6.2
Squalene **(Mk33)**#	Bark, Leaves	0.68 [Hex:CH_2Cl_2 (2:8)]	0.0037 (bark)	4.2.6.3
α-Tocopherol **(Mk34)**#	Leaves	0.35 [Hex:CH_2Cl_2 (1:1)]	0.0242	4.2.6.4
2-Hydroxy-4-methoxy-3,6-dimethylbenzoic acid **(Mk35)**#	Bark	0.25 [CH_2Cl_2]	0.0040	4.2.6.5
Selin-11-en-4α-ol **(Mk36)**#	Leaves	0.32 [CH_2Cl_2]	0.0017	4.2.6.6

Note. ( )* new compound; ( )# known compound first time isolated and reported from *M. koenigii*.

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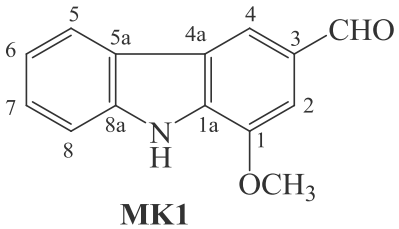
4.2.1 Tricyclic Carbazole Alkaloids



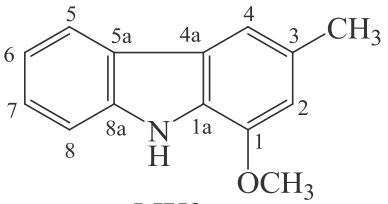
10

This group of carbazole alkaloids consists only the basic ring system of carbazole **10**. A total of nine tricylic carbazole alkaloids, included a new tricyclic carbazole, were isolated from bark and leaves of *M. koenigii*. Structural elucidation and identification of this type of carbazoles were based on NMR, UV, IR, and mass spectral data, as well as direct comparison with an authentic sample.

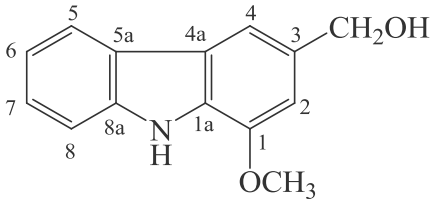
4.2.1.1 Murrayanine (Mk1), Murrayafoline-A (Mk2), Koenoline (Mk3) and 3-Formylcarbazole (Mk4)



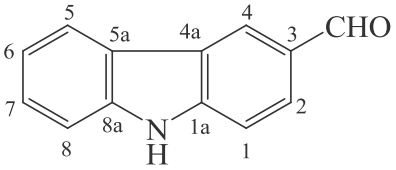
MK1



MK2



MK3



MK4

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Murrayanine **(Mk1)** was isolated as silver plate solid with melting point 155–156 °C (m.p.: 155 °C; Cuong, Hung, Sung, & Taylor, 2004) from the bark of the plant. The mass spectrum indicated a molecular ion peak at *m/z* 226.0856 $[M+H]^+$ which is consistent with the molecular formula $C_{14}H_{11}NO_2$. The IR spectrum of **Mk1** indicated the presence of a broad band at 3369 cm^{-1} due to a NH group and a strong carbonyl band (C=O) for an aromatic aldehyde at 1637 cm^{-1}. This was further confirmed by the ^{13}C NMR which indicated the presence of C=O peak at δ 192.0. Its UV spectrum showed characteristic absorbance for tricyclic moiety with λ_{max} 238, 249, 273, 288 and 329 nm, which was depicted of a 3-formyl-l-methoxycarbazole derivative. It was readily recognized as 1-oxygenated tricyclic carbazole derivative from its spectral data (Cordell, 2008).

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In the ^1H NMR spectrum ($CDCl_3$, 500 MHz) of **Mk1** (Table 4.2.1.1a and Figure 4.2.1.1a), a 3H singlet was seen at δ 4.08 and a lower-field 1H singlet at δ 10.06, which were assignable to a methoxyl and a formyl group, respectively. A broad downfield signal at δ 8.48 assignable to the presence of NH in the carbazole nucleus. In the aromatic region, two signals at δ 7.49 (*td*, *J* = 8.0, 1.1 Hz, H-7) and δ 7.33 (*td*, *J* = 8.0, 1.1 Hz, H-6) exhibiting one *meta*- and two *ortho*-couplings. However, the resonances at δ 8.12 (*d*, *J* = 8.0 Hz, H-5) and δ 7.52 (*d*, *J* = 8.0 Hz, H-8) showed only *ortho*-coupling, suggested that the aromatic ring-C was unsubstituted. The remaining aromatic protons, H-2 and H-4 proton, appeared as singlet at δ 7.47 and δ 8.20 at ring-A of the carbazole nucleus. The ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) indicated the presence of 14 carbons resonances (Table 4.2.1.1b and Figure 4.2.1.1b) with six quaternary carbons at δ 146.2 (C-1), δ 134.1 (C-1a), δ 130.3 (C-3), δ 123.7 (C-4a), δ

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123.7 (C-5a), δ 139.5 (C-8a); six methine carbons at δ 103.6 (C-2), δ 120.5 (C-4), δ 120.8 (C-5), δ 120.8 (C-6), δ 126.7 (C-7), δ 111.7 (C-8); an aldehydic carbon at δ 192.0 (3-CHO); and a methoxy carbon at δ 55.9 (1-OCH_3). The complete assignments of proton and carbon signals and location of substituents on the tricyclic skeleton of **Mk1** were achieved on the basis of DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectra. The structure of **Mk1** was readily assigned as murrayanine as its NMR data were identical with those reported in the literature (Cuong et al., 2004; Sukari et al., 2007).

Mk2 was isolated as brownish oil from both the hexane crude extract of bark and leaves of *M. koenigii*. The IR spectrum exhibited absorption essentially similar to those for **Mk1**, of which NH group at 3366 cm^{-1} and C–O stretching at 1019 cm^{-1}. The IR and UV (λ_{max} 237, 249, 268 and 290 nm) spectra (Cordell, 2008) also suggested the presence of a 1-oxygenated carbazole framework. The molecular formula of this compound was established by its HRESIMS as $C_{14}H_{13}NO$ with molecular ion peak at *m/z* 212.1063 $[M+H]^+$.

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The ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of **Mk2** was in a similar manner to those of **Mk1** (Table 4.2.1.1a and Figure 4.2.1.1c) except for the presence of a methyl signals at δ 2.55 instead of a formyl group at position 3 of ring-A. Similar to **Mk1**, the ^{1}H NMR spectrum exhibited the presence of NH group with a broad singlet signal at δ 8.17 and one phenolic methoxyl group at δ 4.00. The aromatic region of ^{1}H NMR spectrum showed the characteristic features appropriate of carbazole skeleton. Two singlet signals occurred at δ 6.75 and δ 7.49 were attributed to H-2 and H-4, respectively, of ring-A which were observed a higher field shift due to the presence of

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a methyl group (δ 2.55, *s*) at the position 3. In the ring-C of carbazole nucleus of **Mk2**, the signals at δ 8.03 (*d*, *J* = 8.0 Hz, H-5), δ 7.21 (*td*, *J* = 8.0, 1.2 Hz, H-6), δ 7.40 (*td*, *J* = 8.0, 1.2 Hz, H-7) and δ 7.43 (*d*, *J* = 8.0 Hz, H-8), were deduced for an unsubstituted carbazole ring that is similar to **Mk1**. Meanwhile in the ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) (Table 4.2.1.1b and Figure 4.2.1.1d), this compound gave 14 signals for each of 14 carbons presence in the molecule. The complete assignment of **Mk2** was achieved on the basis of ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectra. The structure of this compound was finally assigned as murrayafoline-A (Abu Bakar et al., 2007).

Mk3 was obtained from the CH_2Cl_2 crude extract from the bark of *M. koenigii* as colourless needles with melting point 129–130 °C (m.p.: 130 °C; Fiebig et al., 1985). Its molecular formula of $C_{14}H_{13}NO_2$ was supported by its HERSIMS with a molecular ion peak at *m/z* 227.1531 $[M]^+$. The UV spectrum of **Mk3**, with the absorption maxima recorded at 232, 251, 290, 324 nm, is identical to those in **Mk1**. In its IR spectrum, absorptions occurred characteristic of NH (3367 cm^{-1}), O–H (3235 cm^{-1}) and C–O stretching (1019 cm^{-1}) functionalities.

The ^{1}H NMR of **Mk3** (Table 4.2.1.1a and Figure 4.2.1.1e) was also identical to those of **Mk1**, except for a methylene signal at δ 4.83 and a methoxy signal at δ 4.00. Two broad singlets that occurred at δ 8.32 and δ 1.66 were assignable to a NH and an OH group, respectively. The position of the substituents in the carbazole ring system of **Mk3** could be deduced by analysing the coupling constants of its aromatic protons. Two signals at δ 7.22 (*td*, *J* = 8.0, 1.5 Hz, H-6) and δ 7.41 (*td*, *J* = 8.0, 1.5 Hz,

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H-7), both exhibiting one *meta*- and two *ortho*-couplings, suggested that one aromatic ring was unsubstituted which is also similar to **Mk1** and **Mk2**. However, the resonances at δ 7.45 (*d, J* = 8.0 Hz, H-8) and δ 8.03 (*d, J* = 8.0 Hz, H-5) showed only *ortho*-coupling. The two singlets at δ 7.65 and δ 6.94, considered to represent protons affixed to the 1-methoxy-3-hydroxymethyl-substituted carbazole aromatic ring-A, were therefore assigned to H-4 and H-2, respectively. These were further confirmed by the ^{13}C NMR spectrum of **Mk3**, obtained in $CDCl_3$, at 125 MHz (Table 4.2.1.1b and Figure 4.2.1.1f), which showed the signals at δ 66.5 (CH_2OH) and δ 55.6 (OCH_3) in addition to twelve carbon signals between δ 105.7 and δ 145.8. According to DEPT experiment, six quaternary carbons (C-l, C-1a, C-3, C-4a, C-5a, C-8a) could easily be differentiated from the unsubstituted carbons (C-2, C-4, C-5, C-6, C-7, C-8) of the carbazole nucleus. The complete assignments of carbon signals and location of substituents on tricyclic skeleton of **Mk3** were achieved on the basis of DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectra. The structure of **Mk3** was identified as koenoline (Fiebig et al., 1985).

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Mk4 was obtained as white solid with melting point at 158–160 °C (m.p.: 158–159 °C; Li, McChesney, & El-Feraly, 1990) from the methanol crude extract of the bark of *M. koenigii*. It has IR and UV spectral characteristics resembling those reported for **Mk1**, **Mk2** and **Mk3**, further confirmed that it is also a tricyclic carbazole alkaloid. The IR spectrum of **Mk4** showed the presence of NH (3331 cm^{-1}) and conjugated carbonyl (1680 cm^{-1}) functionalities. The mass spectrum indicated a prominent ion peak at *m/z* 196.0747 $[M+H]^+$ which is consistent with the molecular formula $C_{13}H_9NO$.

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The complete assignments of carbon signals and location of substituent on the **Mk4** were achieved on the basis of ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectra. In the ^{1}H NMR spectrum (CD_3OD, 500 MHz) (Table 4.2.1.1a and Figure 4.2.1.1g), a 1H singlet appeared at δ 10.01 was assigned to an aldehydic proton which was further confirmed by a typical low field signal at δ 192.7 in the ^{13}C NMR spectrum (CD_3OD, 125 MHz) (Table 4.2.1.1b and Figure 4.2.1.1h). The position of CHO group was further confirmed by the presence of a set of ABX mutually-coupled proton system at δ 7.51 (*d*, *J* = 8.1 Hz, H-1), δ 7.95 (*dd*, *J* = 8.1, 1.7 Hz, H-2), and δ 8.65 (*d*, *J* = 1.7 Hz, H-4), which were deshielded by an aldehyde substituent at C-3. Meanwhile, the signals at the aromatic region of the ^{1}H NMR spectrum were strikingly similar for those in the unsubstituted ring-C of **Mk1**, **Mk2** and **Mk3** [δ 8.17 (*d*, *J* = 8.0 Hz, H-5), δ 7.26 (*td*, *J* = 8.1, 1.2 Hz, H-6), δ 7.46 (*td*, *J* = 8.0, 1.2 Hz, H-7) and δ 7.56 (*d*, *J* = 8.0 Hz, H-8)]. Based on the coupling behaviour of the aromatic protons, as well as the characteristic of all the ^{13}C NMR shift, the structure of **Mk4** was then assigned as 3-formylcarbazole (Li et al., 1990)

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Table 4.2.1.1a

*^{1}H NMR [500 MHz, δ_H (J Hz)] of Murrayanine **(Mk1)**, Murrayafoline-A **(Mk2)**, Koenoline **(Mk3)** and 3-Formylcarbazole **(Mk4)***

Position	**Mk1**	**Mk2**	**Mk3**	**Mk4**
1				7.51 (*d*, 8.1 Hz)
1a				
2	7.47 (*s*)	6.75 (*s*)	6.94 (*s*)	7.95 (*dd*, 8.1, 1.7 Hz)
3				
4	8.20 (*s*)	7.49 (*s*)	7.65 (*s*)	8.65 (*d*, 1.7 Hz)
4a				
5	8.12 (*d*, 8.0 Hz)	8.03 (*d*, 8.0 Hz)	8.03 (*d*, 8.0 Hz)	8.17 (*d*, 8.0 Hz)
5a				
6	7.33 (*td*, 8.0, 1.1 Hz)	7.21 (*td*, 8.0, 1.2 Hz)	7.22 (*td*, 8.0, 1.5 Hz)	7.26 (*td*, 8.1, 1.2 Hz)
7	7.49 (*td*, 8.0, 1.1 Hz)	7.40 (*td*, 8.0, 1.2 Hz)	7.41 (*td*, 8.0, 1.5 Hz)	7.46 (*td*, 8.0, 1.2 Hz)
8	7.52 (*d*, 8.0 Hz)	7.43 (*d*, 8.0 Hz)	7.45 (*d*, 8.0 Hz)	7.56 (*d*, 8.0 Hz)
8a				
N-H	8.48 (*br s*)	8.17 (*br s*)	8.32 (*br s*)	8.54 (*br s*)
1-OCH_3	4.08 (*s*)	4.00 (*s*)	4.00 (*s*)	
3-CHO	10.06 (*s*)			10.01 (*s*)
3-CH_3		2.55 (*s*)		
3-$\mathbf{CH_2}$OH			4.83 (*s*)	
3-CH_2**OH**			1.66 (*br s*)	

Note. *Solvent used was $CDCl_3$ for Mk1, Mk2 and Mk3 and CD_3OD was solvent used for Mk4; assignments based on COSY, HMQC and HMBC.

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Table 4.2.1.1b

*^{13}C NMR [125 MHz, δ_C] of Murrayanine **(Mk1)**, Murrayafoline-A **(Mk2)**, Koenoline **(Mk3)** and 3-Formylcarbazole **(Mk4)***

Position	**Mk1**	**Mk2**	**Mk3**	**Mk4**
1	146.2	145.4	145.8	111.1
1a	134.1	129.5	132.9	128.4
2	103.6	107.7	105.7	126.4
3	130.3	128.0	129.5	144.2
4	120.5	112.6	111.8	124.2
4a	123.7	125.7	124.1	125.2
5	120.8	120.5	120.6	120.1
5a	123.7	123.8	123.6	123.2
6	120.8	119.2	119.5	119.9
7	126.7	125.6	125.9	126.3
8	111.7	111.0	111.1	110.9
8a	139.5	139.5	139.5	140.9
1-OCH_3	55.9	55.5	55.6	
3-CHO	192.0			192.7
3-CH_3		22.0		
3-CH_2OH			66.5	

Note. *Solvent used for Mk1, Mk2 and Mk3 was $CDCl_3$ and for Mk4 was CD_3OD; assignments based on DEPT, COSY, HMQC and HMBC.

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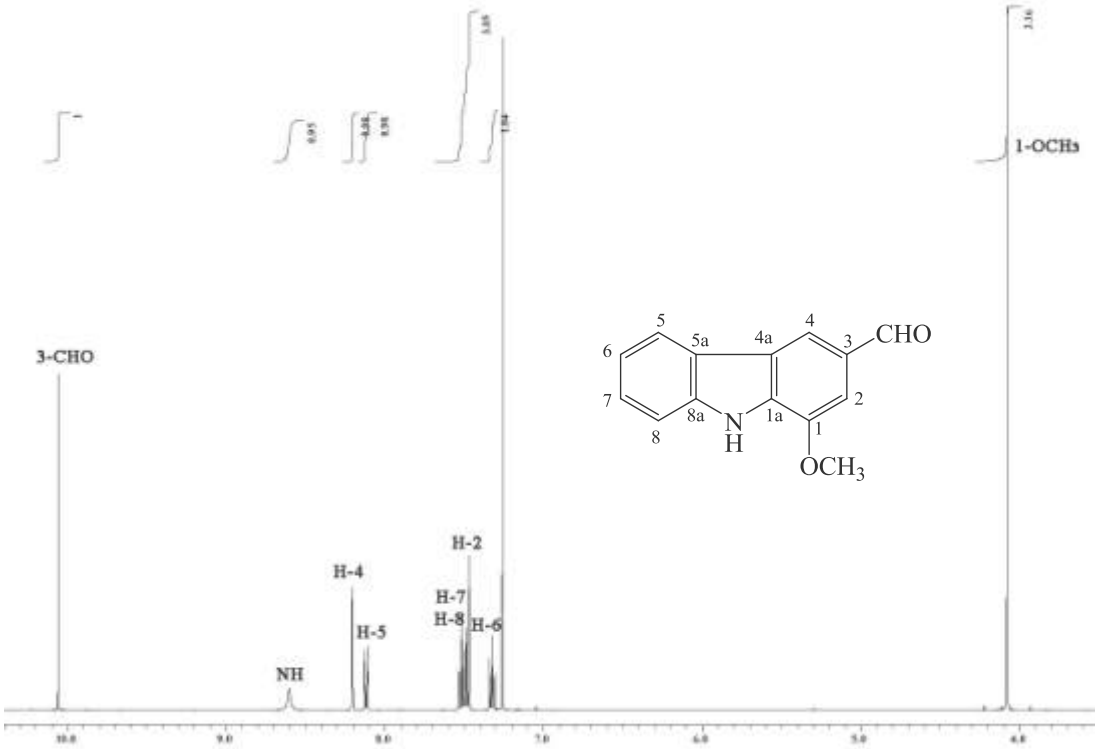


Figure 4.2.1.1a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Murrayanine **(Mk1)**

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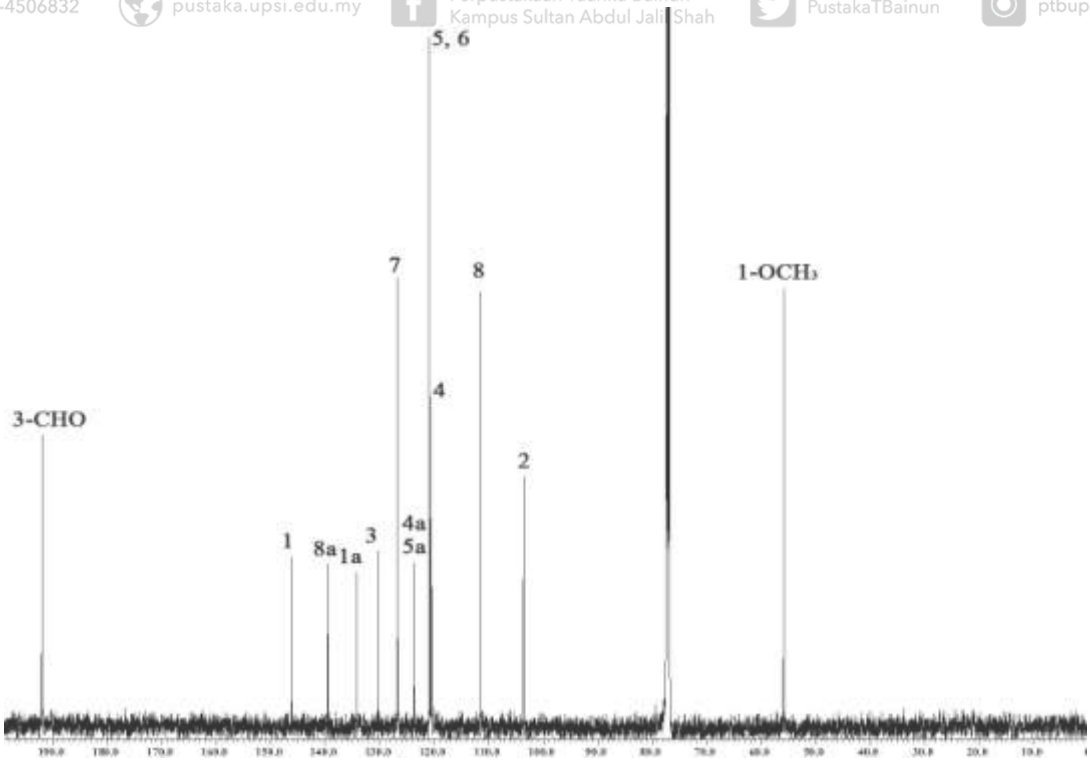


Figure 4.2.1.1b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Murrayanine **(Mk1)**

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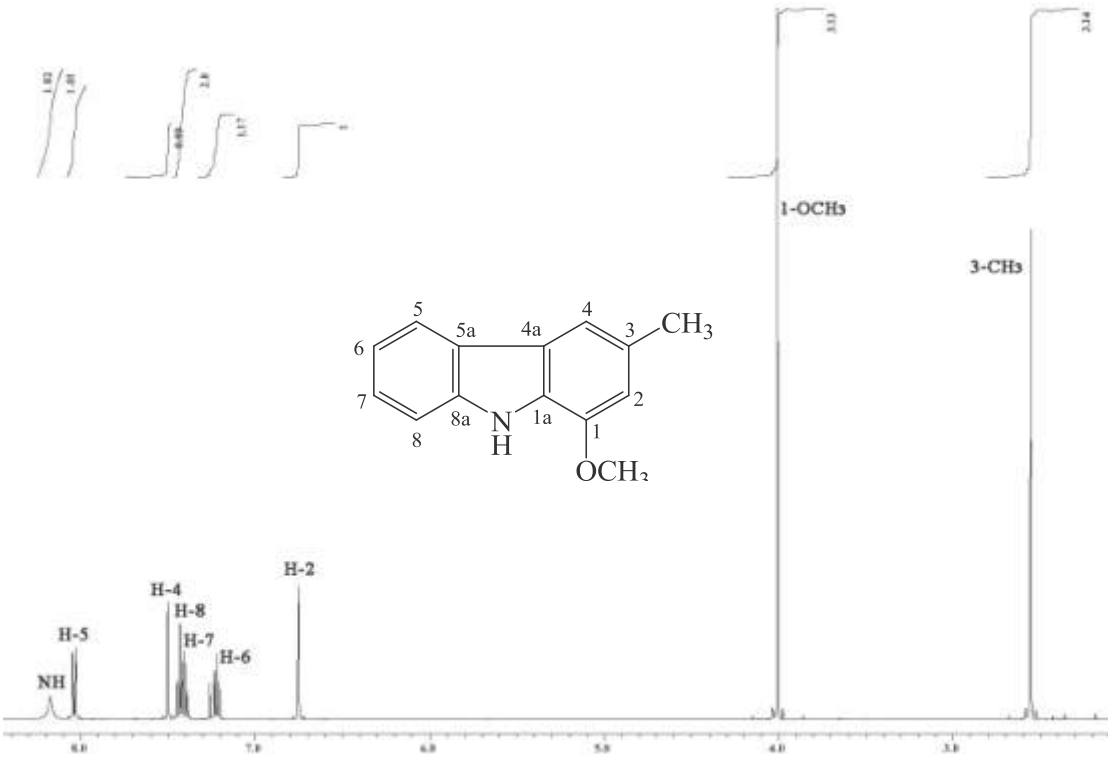


Figure 4.2.1.1c. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Murrayafoline-A **(Mk2)**

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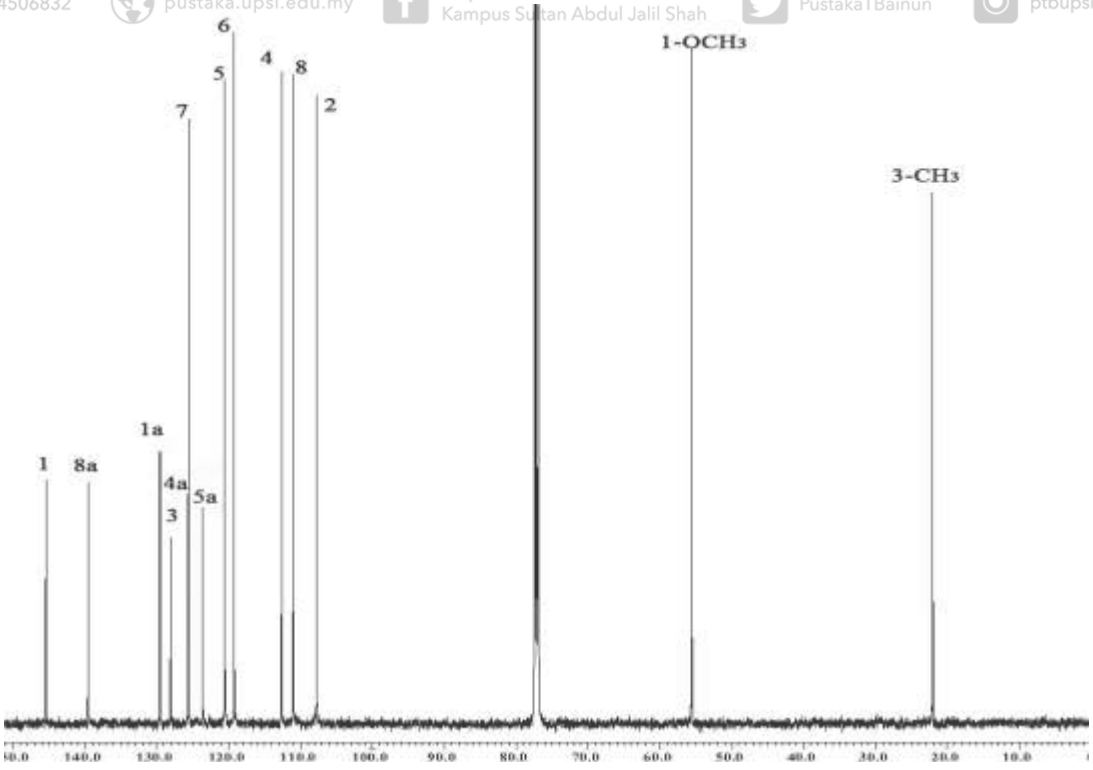


Figure 4.2.1.1d. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Murrayafoline-A **(Mk2)**

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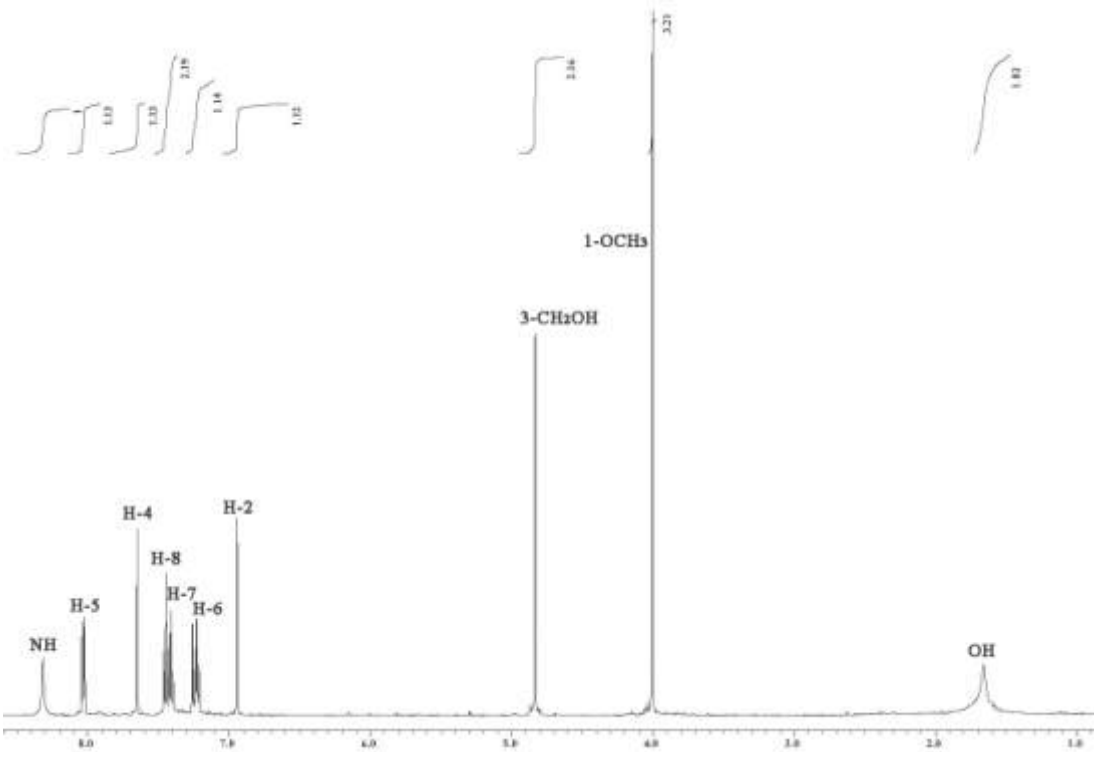


Figure 4.2.1.1e. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Koenoline **(Mk3)**

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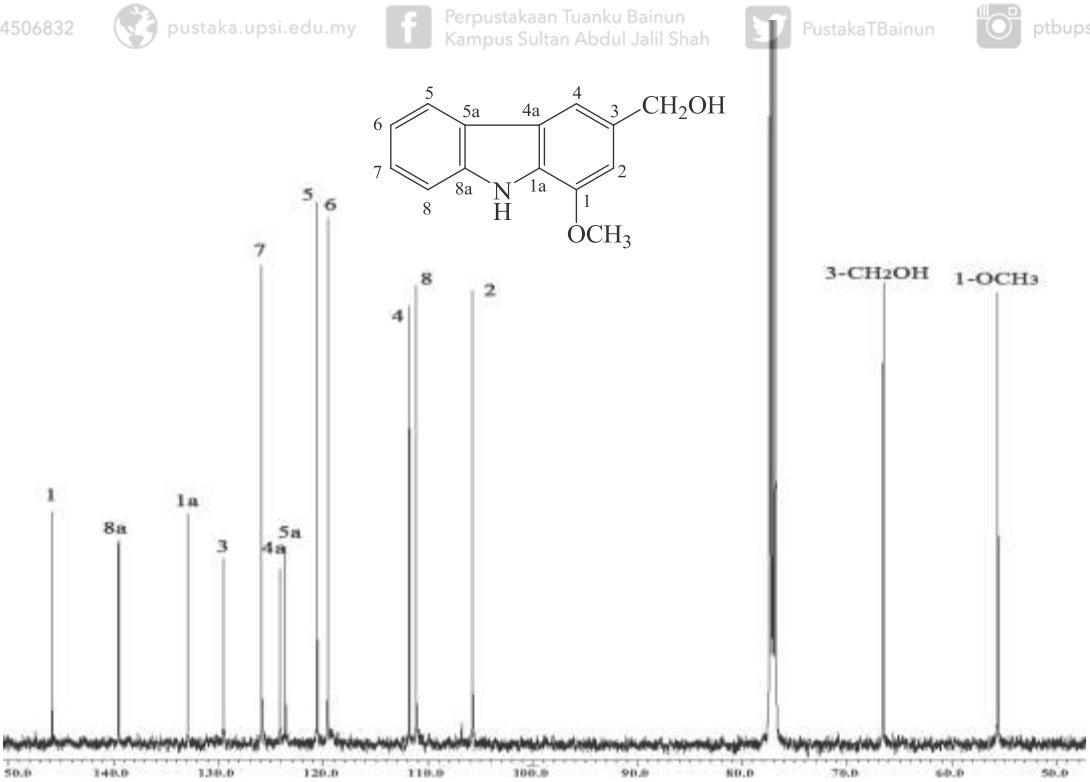


Figure 4.2.1.1f. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Koenoline **(Mk3)**

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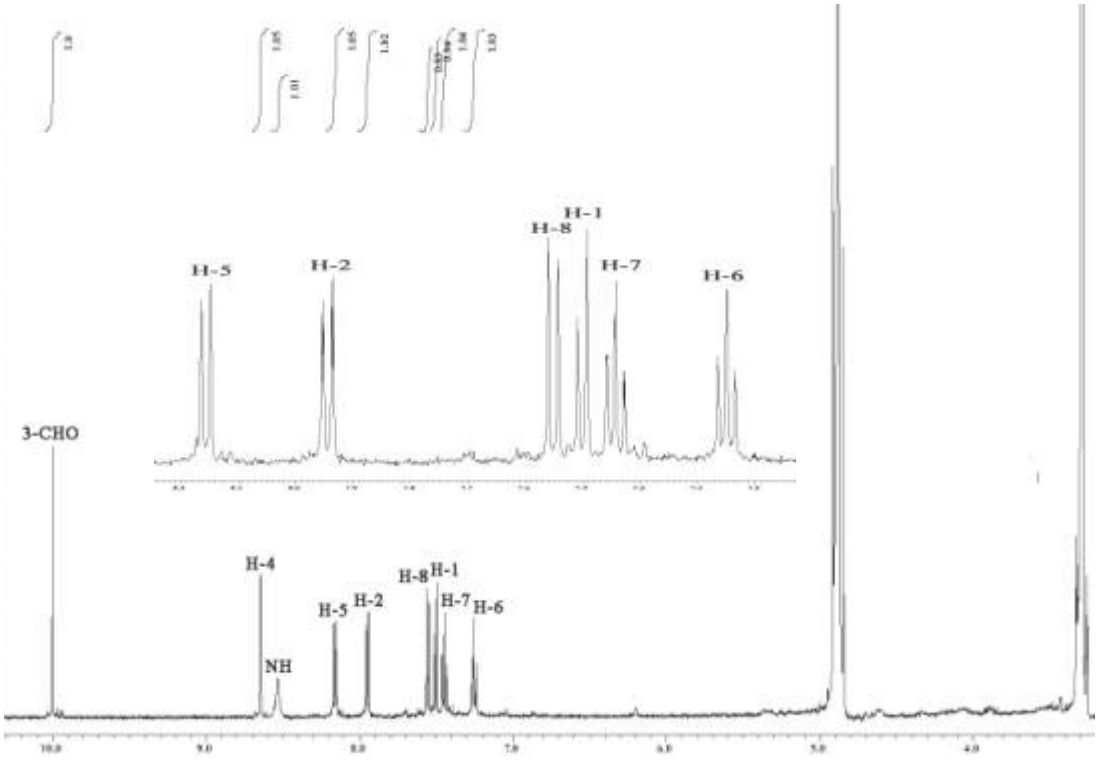


Figure 4.2.1.1g. ^{1}H NMR Spectrum (CD_3OD, 500 MHz) of 3-Formylcarbazole **(Mk4)**

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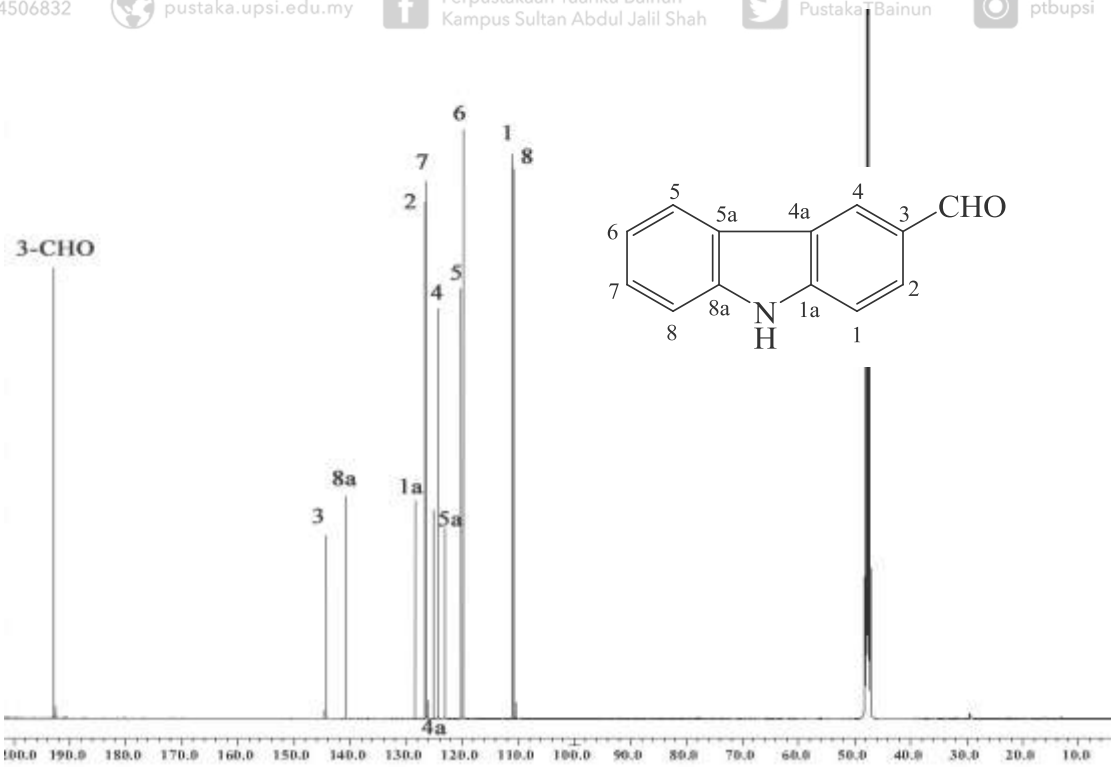
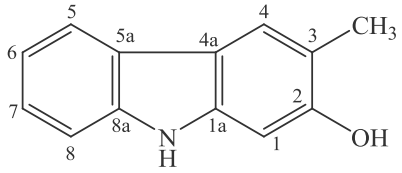


Figure 4.2.1.1h. ^{13}C NMR Spectrum (CD_3OD, 125 MHz) of 3-Formylcarbazole **(Mk4)**

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4.2.1.2 2-Hydroxy-3-methylcarbazole (Mk5)



Mk5

Mk5, with molecular formula $C_{13}H_{11}NO$, was isolated from the bark CH_2Cl_2 crude extract of *M. koenigii* as pale yellow oil. Its molecular formula was determined by HRESIMS with a molecular ion peak at *m/z* 197.0812 $[M]^+$. The UV spectrum exhibited absorption bands at λ_{max} 237, 261, 301, 330 nm indicates the characteristics of a tricyclic carbazole skeleton (Cordell, 2008). The IR spectrum showed typical bands due to NH and/or OH group at ν_{max} 3369 cm^{-1}.

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In the ^1H NMR spectrum of **Mk5**, four mutually coupled protons for an unsubstituted ring C of the carbazole nucleus [δ 7.94 (*d*, *J* = 8.0 Hz, H-5), δ 7.18 (*td*, *J* = 8.0, 1.0 Hz, H-6), δ 7.31 (*td*, *J* = 8.0, 1.0 Hz, H-7), δ 7.35 (*d*, *J* = 8.0 Hz, H-8)], a hydroxy signals at δ 4.86 (*br s*) and a singlet for an aryl methyl signal (δ 2.40) were observed. Two singlets were also observed at δ 6.84 and δ 7.78 for the position 1 and 4, respectively (Table 4.2.1.2 and Figure 4.2.1.2a).

The assignment of carbon signals (Table 4.2.1.2 and Figure 4.2.1.2b) and the positions of its substituents were achieved on the basis of ^{13}C NMR, ^1H-^1H COSY, HMQC and HMBC experiments. **Mk5** was confirmed to be 2-hydroxy-3-methylcarbazole (Bhattacharyya et al., 1986).

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Table 4.2.1.2

^{1}H NMR [500 MHz, δ_H (J Hz)] and ^{13}C NMR [125 MHz, δ_C] of 2-Hydroxy-3-methylcarbazole ***(Mk5)***

Position	$\delta\ ^{13}C$	DEPT	$\delta\ ^{1}H$
1	96.5	CH	6.84 (*s*)
1a	139.4	qC	
2	153.1	qC	
3	123.5	qC	
4	121.9	CH	7.78 (*s*)
4a	116.3	qC	
5	119.4	CH	7.94 (*d*, 8.0 Hz)
5a	124.7	qC	
6	119.3	CH	7.18 (*td*, 8.0, 1.0 Hz)
7	124.5	CH	7.31 (*td*, 8.0, 1.0 Hz)
8	110.3	CH	7.35 (*d*, 8.0 Hz)
8a	139.5	qC	
NH			7.88 (*br s*)
2-OH			4.86 (*br s*)
3-CH_3	16.3	CH_3	2.40 (*s*)

Note. *Solvent used was $CDCl_3$; assignments based on DEPT, COSY, HMQC and HMBC.

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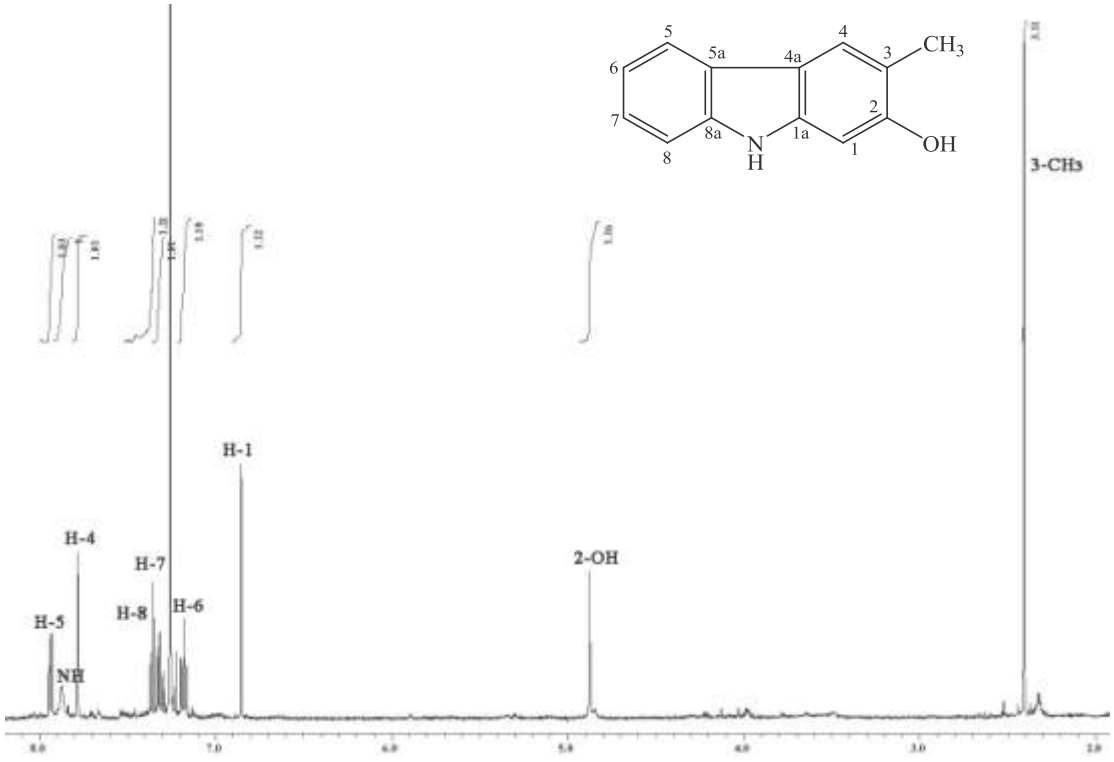


Figure 4.2.1.2a. ^{1}H NMR Spectrum (CD_3OD, 500 MHz) of 2-Hydroxy-3-methylcarbazole **(Mk5)**

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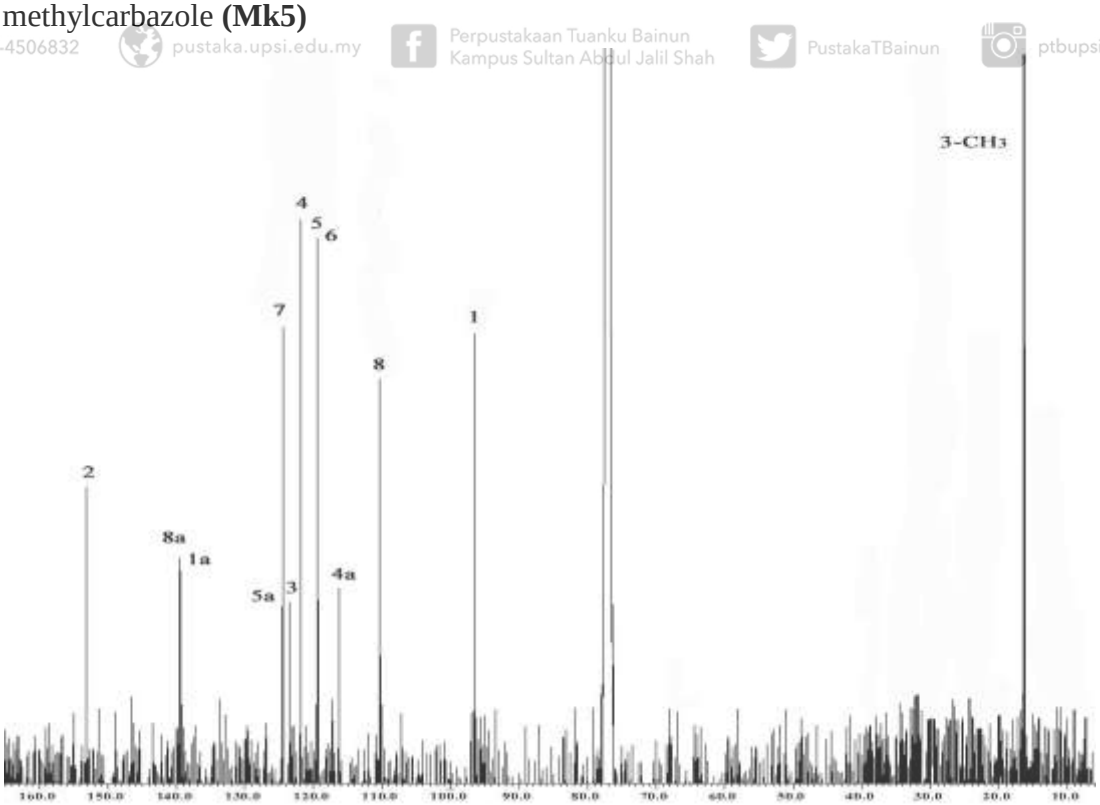
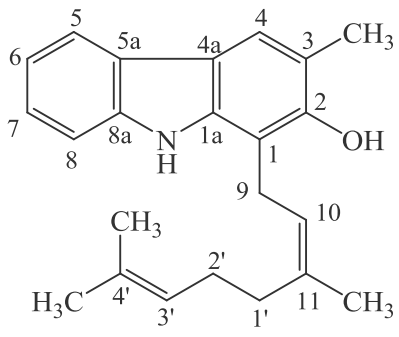


Figure 4.2.1.2b. ^{13}C NMR Spectrum (CD_3OD, 125 MHz) of 2-Hydroxy-3-methylcarbazole **(Mk5)**

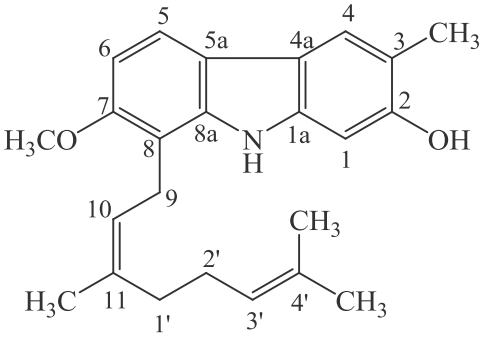
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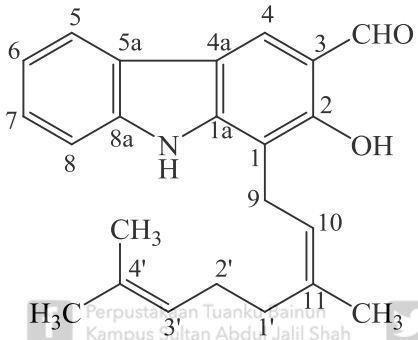
4.2.1.3 Mahanimbilol (Mk6), Euchrestine-B (Mk7) and Mukoenine-B (Mk8)



Mk6



Mk7



Mk8

Mk6 was obtained as pale yellow oil isolated from the CH_2Cl_2 crude extract of the bark. This compound was corroborated by HRESIMS with a molecular ion peak at *m/z* 334.2151 $[M+H]^+$ and analysed to be $C_{23}H_{27}NO$. The IR spectrum showed absorptions at 3455 and 3371 cm^{-1} revealed the presence of a phenolic OH group and an indolic NH group, of which can be confirmed by ^1H NMR spectrum with signals at δ 5.21 and δ 7.85, respectively. The UV spectrum (λ_{max} 239, 269, 287, 304 nm), ^1H and ^{13}C NMR spectra of **Mk6** indicated them to be a trisubstituted carbazole alkaloid with the same tricyclic carbazole skeleton to **Mk1** with the presence of a methyl group at position 3, an OH group at position 2 and a geranyl moiety at the position 1 in ring-A of the carbazole nucleus.

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In the ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of **Mk6** (Table 4.2.1.3a and Figure 4.2.1.3a), signals for three methyls at δ 1.59 (4'-CH_3), 1.66 (4'-CH_3) and 1.90 (11-CH_3), a four proton multiplets at δ 2.09 – 2.11 for H-1' and H-2', a benzylic CH_2 doublet at δ 3.62 (*d*, *J* = 6.9 Hz, H-9) and two vinyl protons at δ 5.06 (*t*, *J* = 7.0 Hz, H-3') and δ 5.38 (*t*, *J* = 6.9 Hz, H-10) indicated the presence of a geranyl substituent. The presence of a singlet at H-4 (δ 7.68, *s*) and a typical unsubstituted carbazole ring (H-5, H-6, H-7 and H-8) further confirmed that all three substituents were on the same ring of the carbazole nucleus. The OH substituent was proven to be at C-2 when the chemical shift of OH signal was not chelated to the carbazole nitrogen, which carbon signal (C–OH) shifted to a lower field at δ 151.3 when compared to those C–OH at C-1 which would appear at a higher field at about δ 145.0 (Table 4.2.1.3b and Figure 4.2.1.3b). The long range coupling between the aromatic methyl protons and H-4 indicated the methyl group to be at C-3, suggesting that **Mk6** was 2-hydroxyl-3-methyl-1-(3,7-dimethyl-2,6-octadienyl)carbazole, also known as mahanimbilol (Reisch et al., 1994b). Further correlating information were confirmed by the experiment of DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectra.

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Compound **Mk7** was purified as pale yellow oil from the dichloromethane crude extract of the leaves of *M. koenigii*. The UV spectrum of **Mk7** (237, 264, 308 and 325 nm) revealed a typical absorption of a tricyclic carbazole skeleton (Cordell, 2008). IR spectrum characteristics resembling those reported for **Mk6** and confirmed that it is also a tricyclic carbazole alkaloid. The molecular formula of **Mk7** was calculated as $C_{24}H_{29}NO_2$ based on a combination of mass spectral data (*m/z* 363.1845 $[M]^{+}$) and its NMR spectra.

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The ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) (Table 4.2.1.3a and Figure 4.2.1.3c) showed signals for one NH proton at δ 7.76, two AB-type doublets at δ 6.83 (*d*, *J* = 8.6 Hz, H-6) and 7.71 (*d*, *J* = 8.6 Hz, H-5) due to *ortho*-located aromatic protons of ring-C, and two 1H singlets of aromatic protons at δ 6.80 (H-1) and 7.67 (H-4) of ring-A of the carbazole nucleus. The ^{1}H NMR spectrum of **Mk7** also exhibited signals for a geranyl moiety [three methyls at δ 1.57 (4'-CH_3), 1.63 (4'-CH_3) and 1.88 (11-CH_3); two 2H multiplets at δ 2.05 (H-1') and 2.10 (H-2'); a benzylic CH_2 doublet at δ 3.62 (H-9); and two vinyl protons at δ 5.06 (*t*, *J* = 6.9 Hz, H-3') and 5.33 (*t*, *J* = 6.9 Hz, H-10)]. The presence of a phenolic hydroxy at δ 4.80, a methoxy at δ 3.91 and a methyl substituents at δ 2.42 were also observed in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of **Mk7** contained signals of 24 carbons (Table 4.2.1.3b and Figure 4.2.1.3d). According to DEPT experiment, ten quaternary carbons, six methine carbons, three methylene carbons and five methyl carbons were easily be identified. In agreement with all the experiments of ^{1}H-^{1}H COSY, HMQC and HMBC, and also comparison with its literature data, **Mk7** was readily assigned as euchrestine-B (Ito, Nakagawa, & Wu, 1991; Tachibana et al., 2001).

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Mk8 was also isolated as pale yellow oil isolated from the hexane crude extract of the bark of the plant. The HRESIMS showed a quasi-molecular ion peak at *m/z* 348.1963 $[M+H]^+$ and its molecular formula is determined to be $C_{23}H_{25}NO_2$. The IR spectrum showed absorptions at 3371 cm^{-1} revealed the presence of an OH group in addition to the NH group. This was confirmed by the presence of a broad signal at δ 8.24 for the NH group and δ 11.67 for the OH group in the ^{1}H NMR spectrum. The

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UV spectrum, ^{1}H and ^{13}C NMR spectra of **Mk8** resembled similar carbazole skeleton of **Mk7**.

In the ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of **Mk8** (Table 4.2.1.3a and Figure 4.2.1.3e), the presence of a 1H singlet of the formyl group (δ 9.93) at C-3 (δ 195.5) was suggested by a lower chemical shift value at δ 8.07 (*s*) of H-4 when compared to **Mk6** and **Mk7**, which was affected by the deshielding effect of the formyl group at ring-A. Whereas, signals for three methyls at δ 1.57 (4'-CH_3), 1.61 (4'-CH_3) and 1.90 (11-CH_3), two multiplets at δ 2.10 – 2.11 for H-1' and H-2', a 2H doublet at δ 3.67 (*d*, *J* = 6.9 Hz, H-9) and two vinyl protons at δ 5.08 (*t*, *J* = 6.9 Hz, H-3') and 5.34 (*t*, *J* = 6.9 Hz, H-10) indicated the presence of a geranyl moiety, of which identical to those in **Mk6** and **Mk7**. Four mutually coupled protons at ring C of the carbazole nucleus [δ 7.91 (*d*, *J* = 8.0 Hz, H-5), δ 7.17 (*td*, *J* = 8.0, 1.2 Hz, H-6), δ 7.40 (*td*, *J* = 8.0, 1.2 Hz, H-7), δ 7.39 (*d*, *J* = 8.0 Hz, H-8)] were deduced for the presence of an unsubstituted ring-C of the carbazole nucleus of **Mk8**. Meanwhile, the presence of a singlet at H-4 and a typical unsubstituted carbazole ring-C indicated that all three substituents were on the same ring of the carbazole (Ring-A). The long range coupling between the aromatic aldehyde proton and H-4 also indicated the formyl group to be at C-3 and suggesting that **Mk8** was a 2-hydroxy-3-formyl-1-(3,7-dimethyl-2,6-octadienyl)carbazole, of which also known as mukoenine-B (Ito et al., 1993). Other correlated data were further confirmed by the experiments of DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectra.

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Table 4.2.1.3a

^{1}H NMR [500 MHz, δ_H (J Hz)] of Mahanimbilol ***(Mk6)****, Euchrestine-B* ***(Mk7)*** *and Mukoenine-B* ***(Mk8)****

Position	**Mk6**	**Mk7**	**Mk8**
1		6.80 (*s*)	
1a			
2			
3			
4	7.68 (*s*)	7.67 (*s*)	8.07 (*s*)
4a			
5	7.93 (*d*, 8.0 Hz)	7.71 (*d*, 8.6 Hz)	7.91 (*d*, 8.0 Hz)
5a			
6	7.17 (*td*, 8.0, 1.2 Hz)	6.83 (*d*, 8.6Hz)	7.17 (*td*, 8.0, 1.2 Hz)
7	7.30 (*td*, 8.0, 1.2 Hz)		7.40 (*td*, 8.0, 1.2 Hz)
8	7.37 (*d*, 8.0 Hz)		7.39 (*d*, 8.0 Hz)
8a			
9	3.62 (*d*, 6.9 Hz)	3.62 (*d*, 6.8 Hz)	3.67 (*d*, 6.9 Hz)
10	5.38 (*m*)	5.33 (*m*)	5.34 (*m*)
11			
N-H	7.85 (*br s*)	7.76 (*br s*)	8.24 (*br s*)
2-OH	5.21 (*br s*)	4.80 (*br s*)	11.67 (*s*)
3-CH_3	2.40 (*s*)	2.42 (*s*)	
3-CHO			9.93 (*s*)
7-OCH_3		3.91 (*s*)	
1'	2.09 (*m*)	2.05 (*m*)	2.11 (*m*)
2'	2.11 (*m*)	2.10 (*m*)	2.10 (*m*)
3'	5.06 (*t*, 7.0 Hz)	5.06 (*t*, 6.9 Hz)	5.08 (*t*, 6.9 Hz)
4'			
11-CH_3	1.90 (*s*)	1.88 (*s*)	1.90 (*s*)
4'-CH_3	1.66 (*s*)	1.63 (*s*)	1.61 (*s*)
4'-CH_3	1.59 (*s*)	1.57 (*s*)	1.57 (*s*)

Note. *Solvent used was $CDCl_3$; assignments based on COSY and HMQC.

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Table 4.2.1.3b

*^{13}C NMR [125 MHz, δ_C] of Mahanimbilol **(Mk6)**, Euchrestine-B **(Mk7)** and Mukoenine-B **(Mk8)***

Position	**Mk6**	**Mk7**	**Mk8**
1	107.9	96.7	109.1
1a	138.5	139.7	138.2
2	151.3	152.2	157.9
3	124.0	117.9	123.7
4	119.4	121.2	125.5
4a	117.2	118.0	117.4
5	119.5	117.2	119.4
5a	116.8	115.9	115.5
6	119.5	104.6	119.4
7	124.3	154.8	126.0
8	110.4	111.2	110.9
8a	139.5	140.3	140.2
9	24.8	23.9	24.0
10	121.4	122.5	121.3
11	139.2	136.6	138.0
2-OH			
3-CH_3	16.7	16.2	
3-CHO			195.5
7-OCH_3		56.8	
1'	39.7	39.7	39.7
2'	26.5	26.7	26.6
3'	123.7	124.1	123.9
4'	132.2	131.7	132.0
11-CH_3	16.5	16.5	16.6
4'-CH_3	25.8	25.8	25.8
4'-CH_3	17.8	17.8	17.8

Note. *Solvent used was $CDCl_3$; assignments based on HMQC and HMBC.

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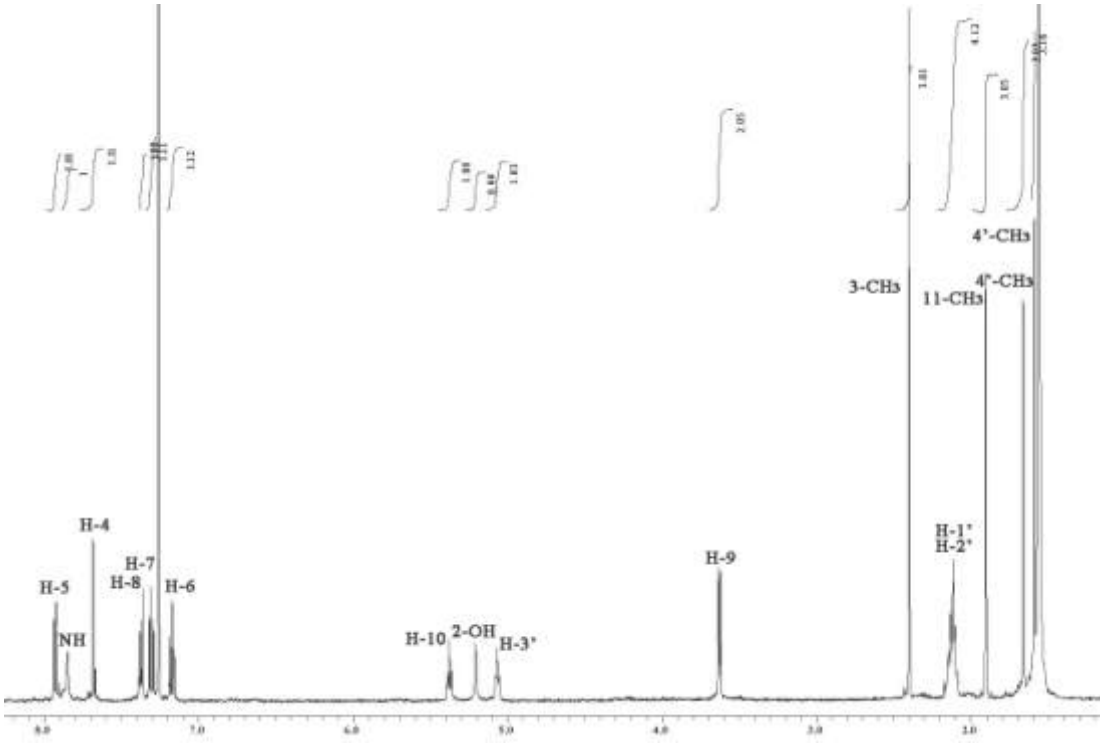


Figure 4.2.1.3a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Mahanimbilol **(Mk6)**

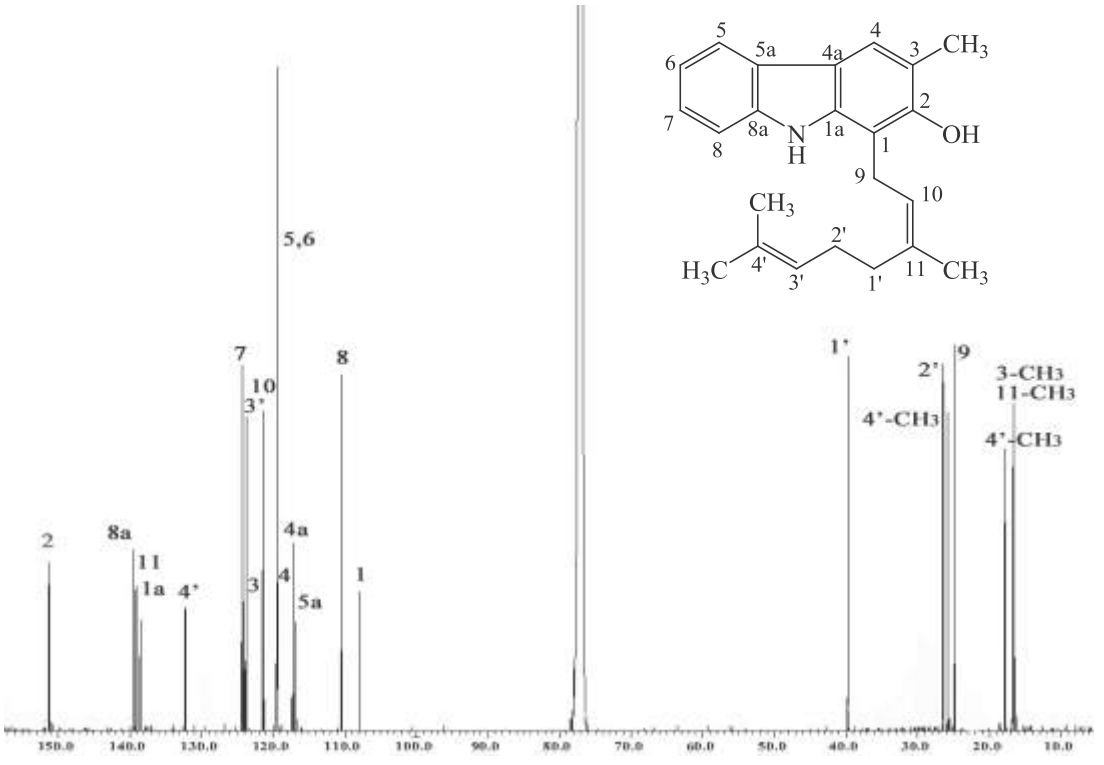


Figure 4.2.1.3b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Mahanimbilol **(Mk6)**

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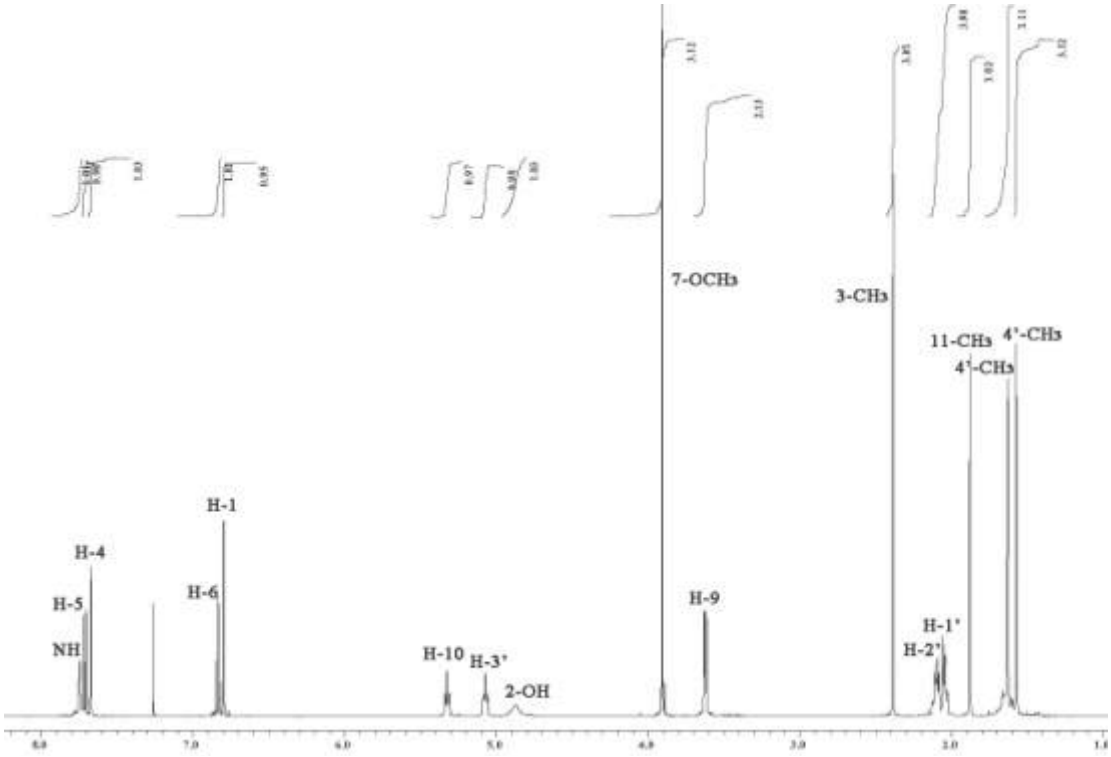


Figure 4.2.1.3c. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Euchrestine-B **(Mk7)**

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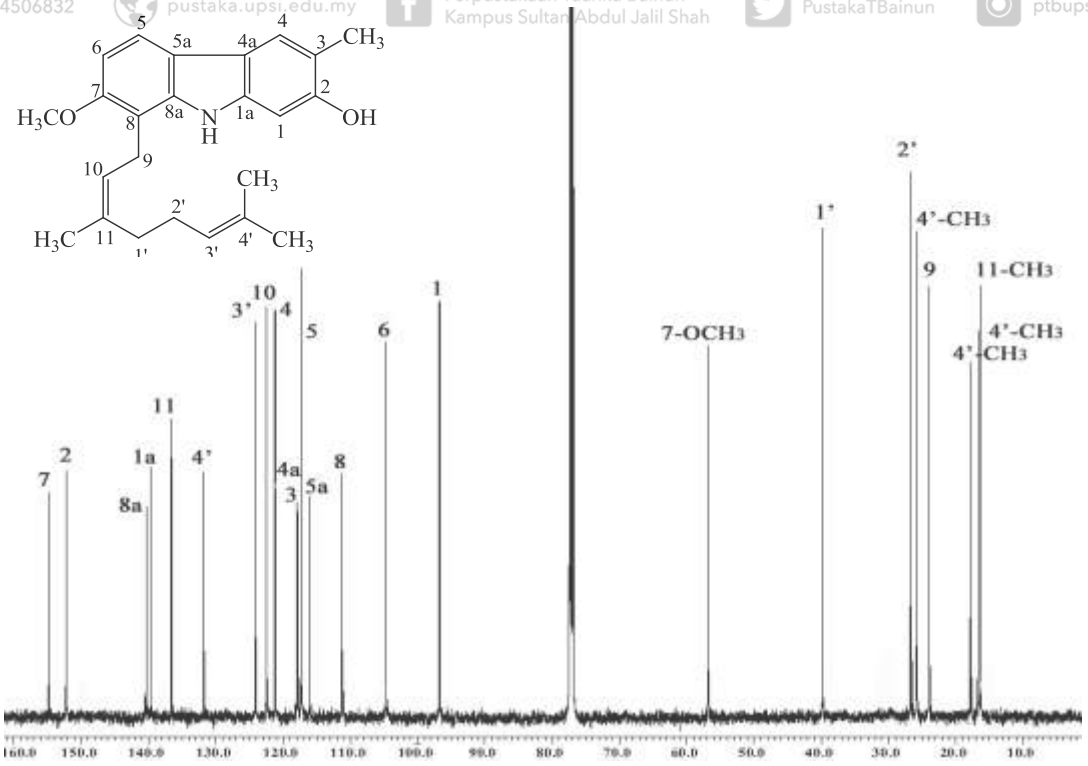


Figure 4.2.1.3d. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Euchrestine-B **(Mk7)**

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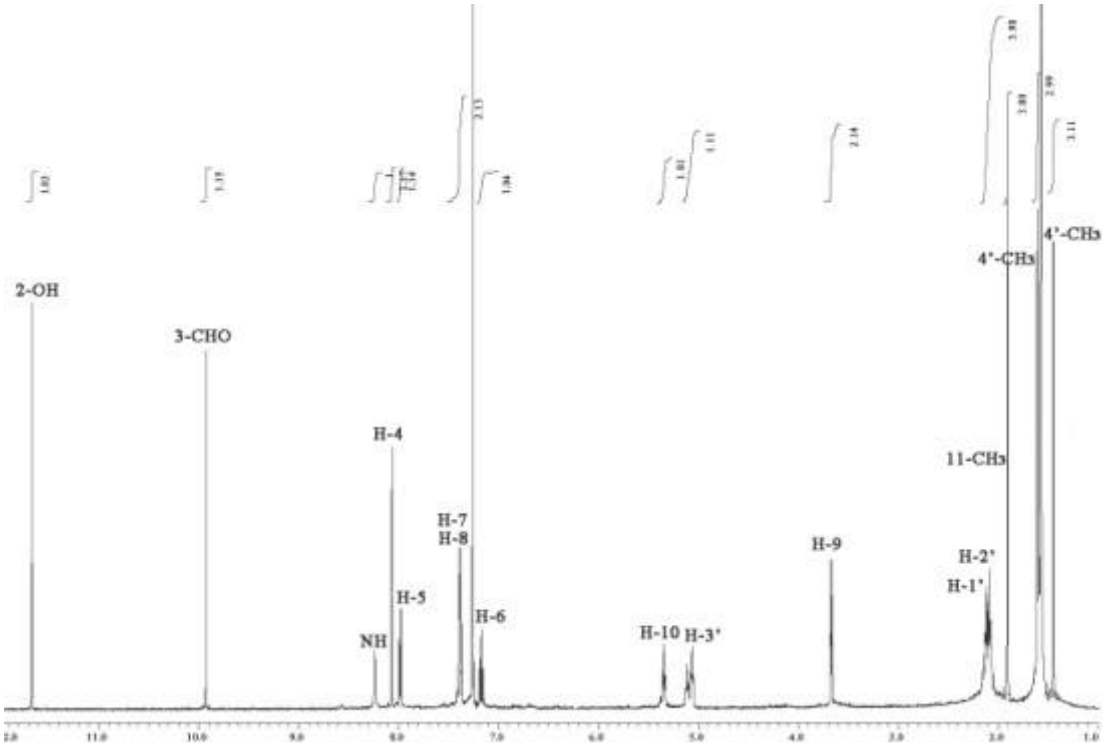


Figure 4.2.1.3e. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Mukoenine-B **(Mk8)**

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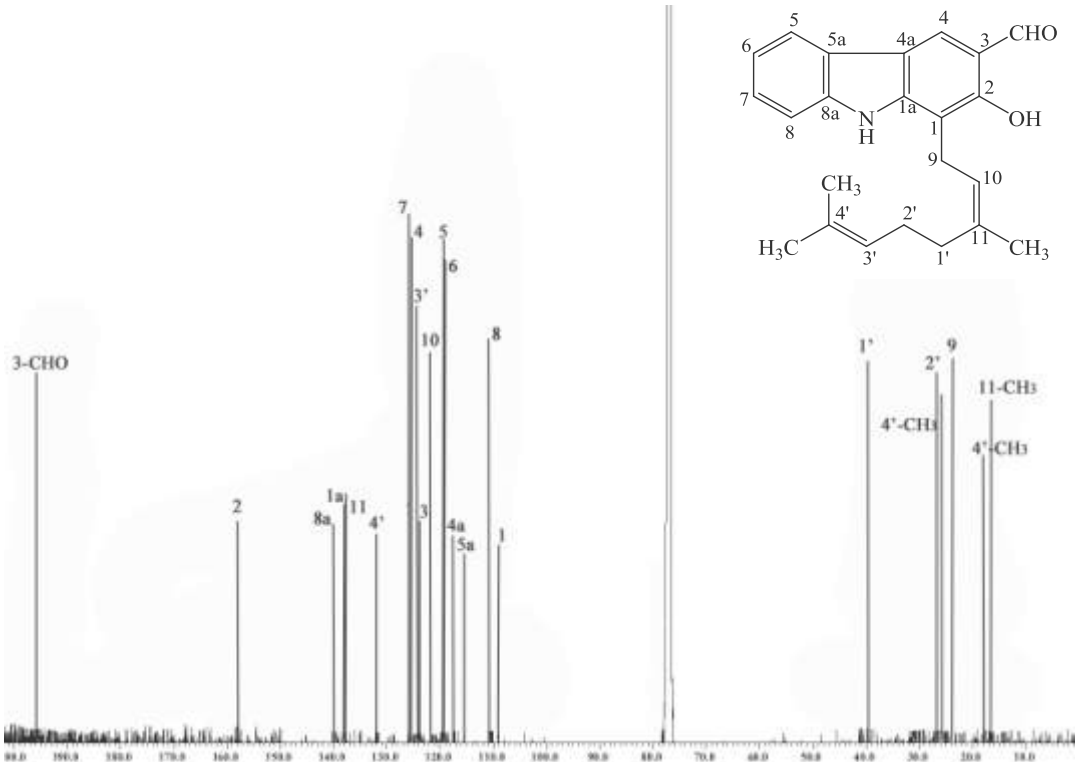
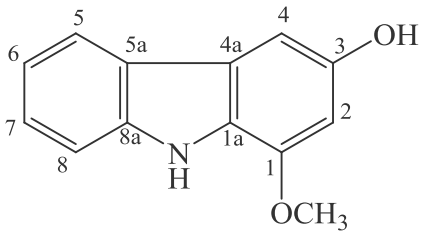


Figure 4.2.1.3f. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Mukoenine-B **(Mk8)**

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4.2.1.4 Murrastanine-A (Mk9)



Mk9

Mk9 was isolated from the methanol crude extract of the bark of *Murraya koenigii* as brownish oil. Its 1D and 2D NMR spectra were shown in Figure 4.2.1.4a to 4.2.1.4h). The IR spectrum of **Mk9** (Figure 4.2.1.4i) indicated the presence of a broad band at 3371 cm^{-1} due to the presence of an amine and/or a hydroxyl group. The absorption observed at 1019 cm^{-1} was assigned to the C–O group. Its UV spectrum resembled the characteristic for 1,3-oxygenated tricyclic moiety with λ_{max} 239, 250, 269 and 276 nm (Cordell, 2008). This was further agreed with the ^{13}C NMR which was readily recognized as tricylic carbazole derivative from its preliminary spectral data.

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In the ^{1}H NMR spectrum (CD_3OD, 500 MHz) of **Mk9** (Table 4.2.1.4 and Figure 4.2.1.4a), a 3H singlet was seen at δ 4.06 assignable to a methoxyl group at position 1 (ring-A) of the carbazole nucleus, which was further substantiated with the aid of HMBC experiment by showing long-range coupling between 1-OCH_3 and C-1. The NOE experiment (Figure 4.2.1.4b) enhancement of 7.14% for H-2 (δ 7.53) was also observed upon irradiation of the methoxy signal at δ 3.97. Therefore, the methoxyl group must be located *ortho* to H-2 and there is no other proton *ortho* to this substituent. Furthermore, the methoxyl group was proven to be at C-1 instead of C-3 due to the chemical shift of C-1, which has indicated it was chelated to the carbazole

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nitrogen with a higher field carbon peak at δ 145.3, but not lower field as if the methoxy is attached at C-3 and would be expected appear at more or less 155 ppm. The HRESIMS of **Mk9**, which is consistent with the molecular formula $C_{13}H_{11}NO_2$, gave evidence for the presence of the phenolic group by indicated a molecular ion peak at *m/z* 214.0890 ($[M+H]^+$, calcd. mass: 214.0868) (Figure 4.2.1.4j). Another broad downfield signal at δ 8.44 assignable for the presence of NH proton in the carbazole nucleus. In a similar manner in the aromatic region, two doublets at δ 8.05 (*J* = 8.0 Hz, H-5) and δ 7.50 (*J* = 8.0 Hz, H-8) and two triplet of doublets at δ 7.39 (*J* = 8.0, 1.2 Hz, H-7) and δ 7.18 (*J* = 8.0, 1.2 Hz, H-6) were deduced for an unsubstituted carbazole ring. The remaining aromatic protons, H-2 and H-4, appeared as singlet at δ 7.62 and δ 8.44, respectively, at ring-A of the carbazole nucleus.

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The ^{13}C NMR spectrum (CD_3OD, 125 MHz) indicated the presence of 13 carbons resonances (Table 4.2.1.4 and Figure 4.2.1.4c). Among the 13 carbons, the ^{13}C data indicated the presence of six quaternary carbons: δ 145.3 (C-1), δ 132.9 (C-1a), δ 145.4 (C-3), δ 123.5 (C-4a), δ 123.4 (C-5a), δ 140.5 (C-8a); six methine carbons: δ 106.4 (C-2), δ 115.5 (C-4), δ 119.9 (C-5), δ 119.2 (C-6), δ 125.6 (C-7), δ 111.3 (C-8); and a methoxy signal at δ 56.1. The complete assignments of proton and carbon signals and location of substituents on the tricyclic skeleton of **Mk9** were also agreed with all the assignments in DEPT, NOE, HMQC, ^1H-^1H COSY and HMBC (Figure 4.2.1.4d to Figure 4.2.1.4h). The ^1H-^1H COSY (Figure 4.2.1.4f) provided a significant fragment of C-5 – C-6 – C-7 – C-8 gave further evidence for the presence of an unsubstituted ring in its carbazole nucleus. The HMBC experiments (Figure 4.2.1.4g) confirmed the presence of long-range correlations included: H-2 to C-1, C-

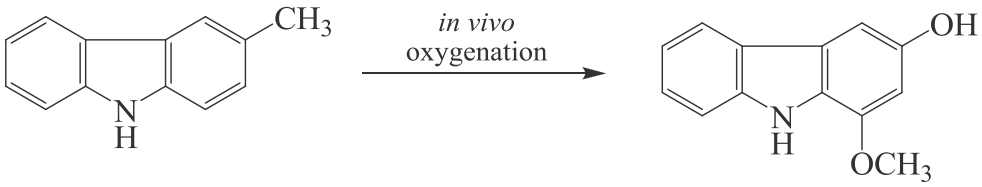
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1a, C-3 and C-4; H-4 to C-1a, C-2, C-3, C-4a and C-5a; H-5 to C-5a, C-6, C-7 and C-8a; H-6 to C-5, C-5a, C-7 and C-8; H-7 to C-5, C-6, C-8 and C-8a; and H-8 to C-5a, C-6, C-7 and C-8a. These correlations confirmed the attribution of all the ^{13}C signals of **Mk9**, especially gave evidence to show that a methoxyl group was connected to C-1 and the phenolic group attached to C-3. Based on the spectra analysis, the structure of **Mk9** was suggested to be 1-methoxy-9*H*-carbazol-3-ol, also assigned as murrastanine-A. This compound was found to be a new carbazole alkaloid isolated from *M. koenigii*.

The possible biogenetic pathway was suggested in Scheme 4.2.1.4, by which the 3-methylcarbazole is the key precursor for the formation of **Mk9** when *in vivo* oxygenation occurs naturally at C-1 and C-3 of the carbazole nucleus (Cordell, 2008).

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34. 3-Methylcarbazole

Mk9

Scheme 4.2.1.4. Proposed Biogenetic Pathway to **Mk9**

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Table 4.2.1.4

*1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of Murrastanine-A **(Mk9)****

Position	δ ^{13}C	DEPT	δ ^{1}H	HMBC correlations	COSY
1	145.3	qC		H-2	
1a	132.9	qC		H-2, H-4	
2	106.4	CH	7.62 (*s*)	H-4	
3	145.4	qC		H-2, H-4	
4	115.5	CH	8.44 (*s*)	H-2	
4a	123.5	qC		H-2, H-5	
5	119.9	CH	8.05 (*d*, 8.0 Hz)	H-6, H-7	H-6
5a	123.4	qC		H-5, H-6, H-8	
6	119.2	CH	7.18 (*td*, 8.0, 1.2 Hz)	H-5, H-7, H-8	H-5, H-7
7	125.6	CH	7.39 (*td*, 8.0, 1.2 Hz)	H-5, H-6, H-8	H-6, H-8
8	111.3	CH	7.50 (*d*, 8.0 Hz)	H-6, H-7	H-7
8a	140.5	qC		H-5, H-7, H-8	
N-H			8.44 (*br s*)		
1-OCH_3	56.1	CH_3	4.06 (*s*)	H-2	
3-OH			#		

Note. *Solvent used was CD_3OD; assignments based on DEPT, COSY, HMQC and HMBC; # peak not available

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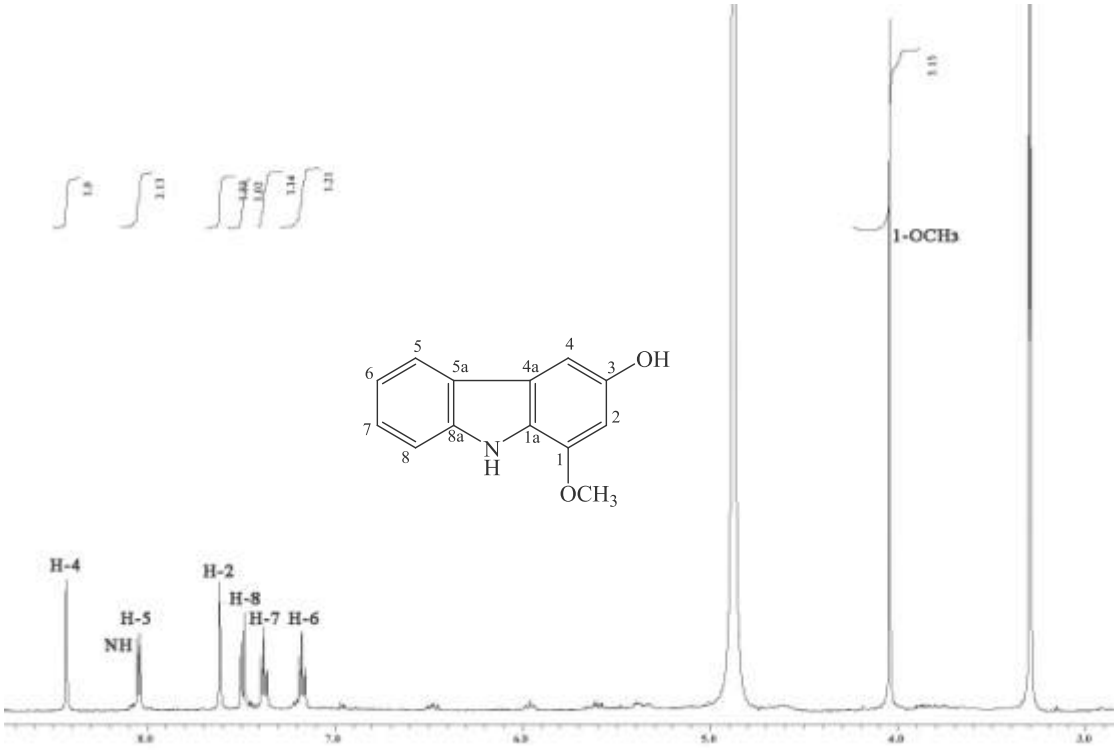


Figure 4.2.1.4a. ^{1}H NMR Spectrum (CD_3OD, 500 MHz) of Murrastanine-A **(Mk9)**

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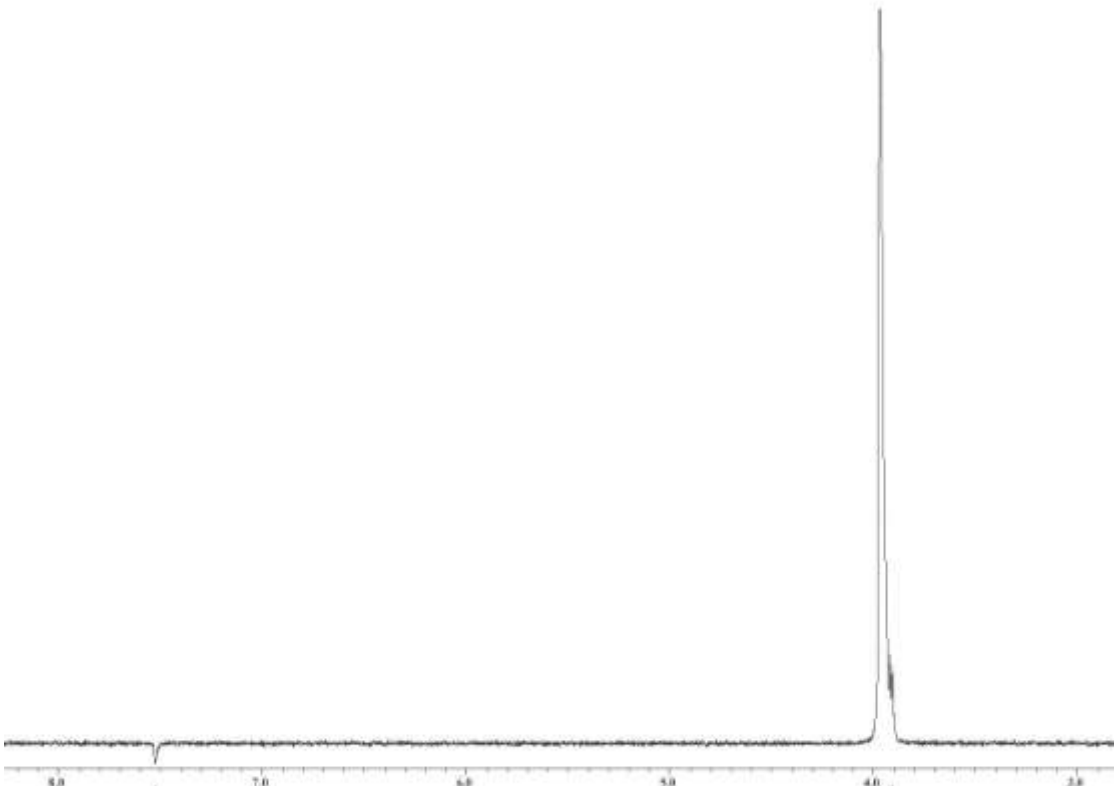


Figure 4.2.1.4b. NOE NMR Spectrum (CD_3OD, 500 MHz) of Murrastanine-A **(Mk9)**

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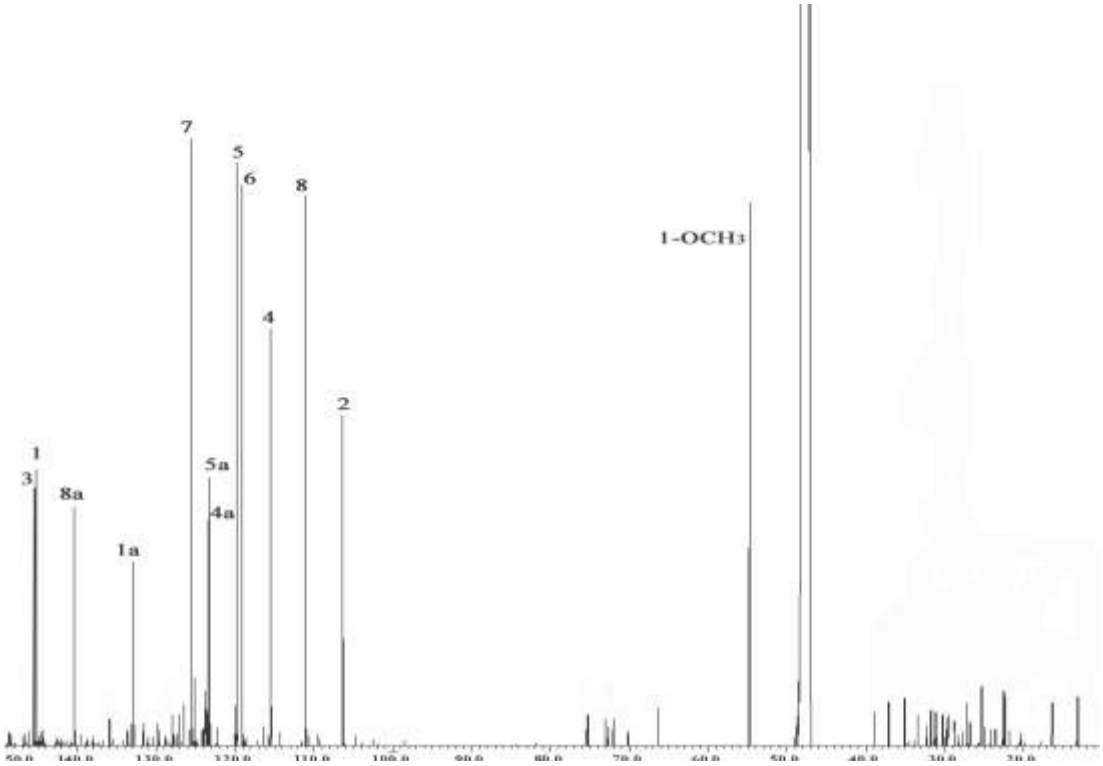


Figure 4.2.1.4c. ^{13}C NMR Spectrum (CD_3OD, 125 MHz) of Murrastanine-A **(Mk9)**

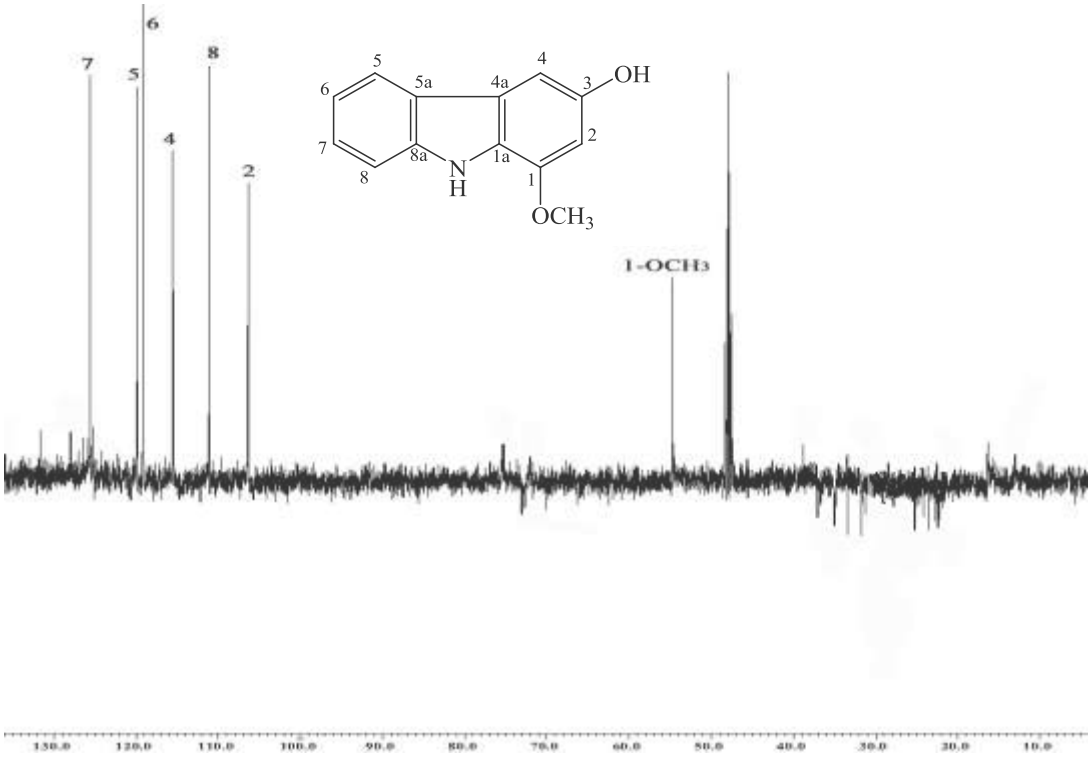


Figure 4.2.1.4d. DEPT-135 Spectrum (CD_3OD, 125 MHz) of Murrastanine-A **(Mk9)**

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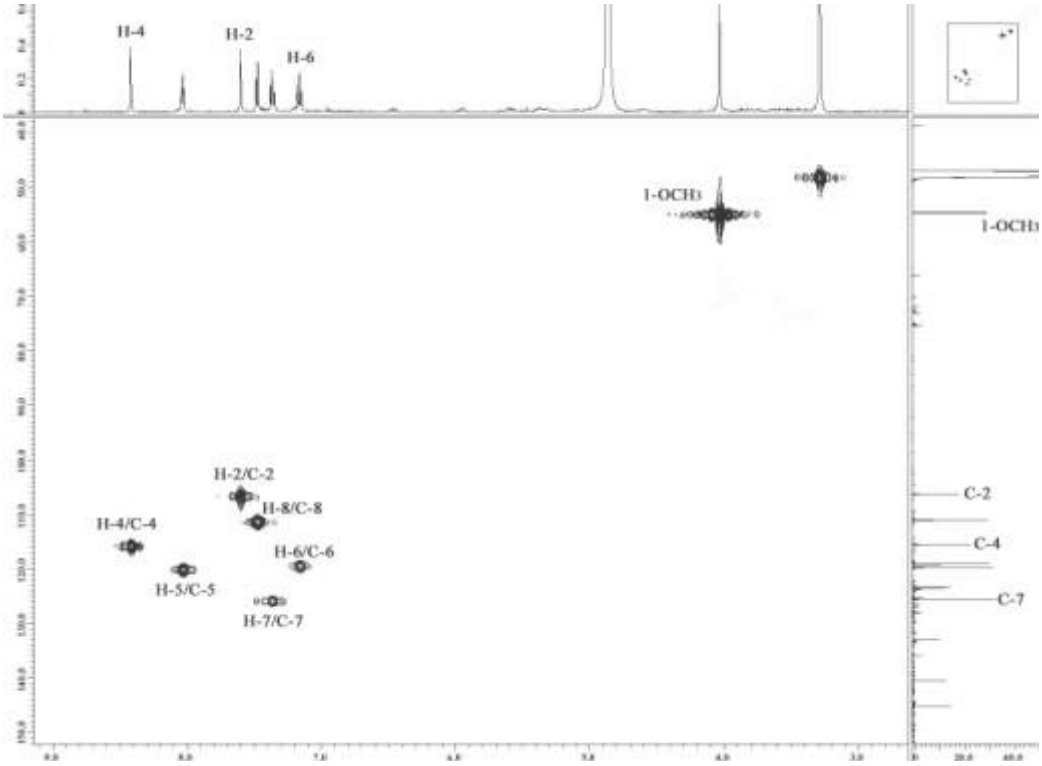


Figure 4.2.1.4e. HMQC Spectrum (CD_3OD, 500 MHz) of Murrastanine-A **(Mk9)**

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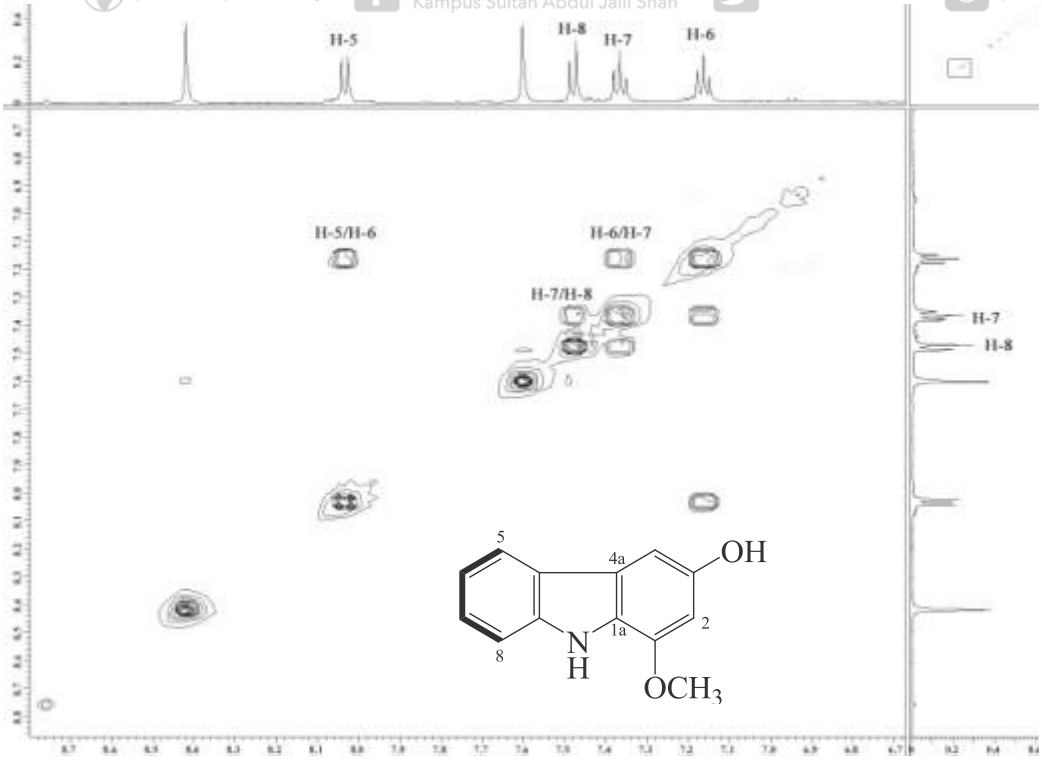


Figure 4.2.1.4f. ^{1}H-^{1}H COSY Spectrum (CD_3OD, 500 MHz) of Murrastanine-A**(Mk9)**

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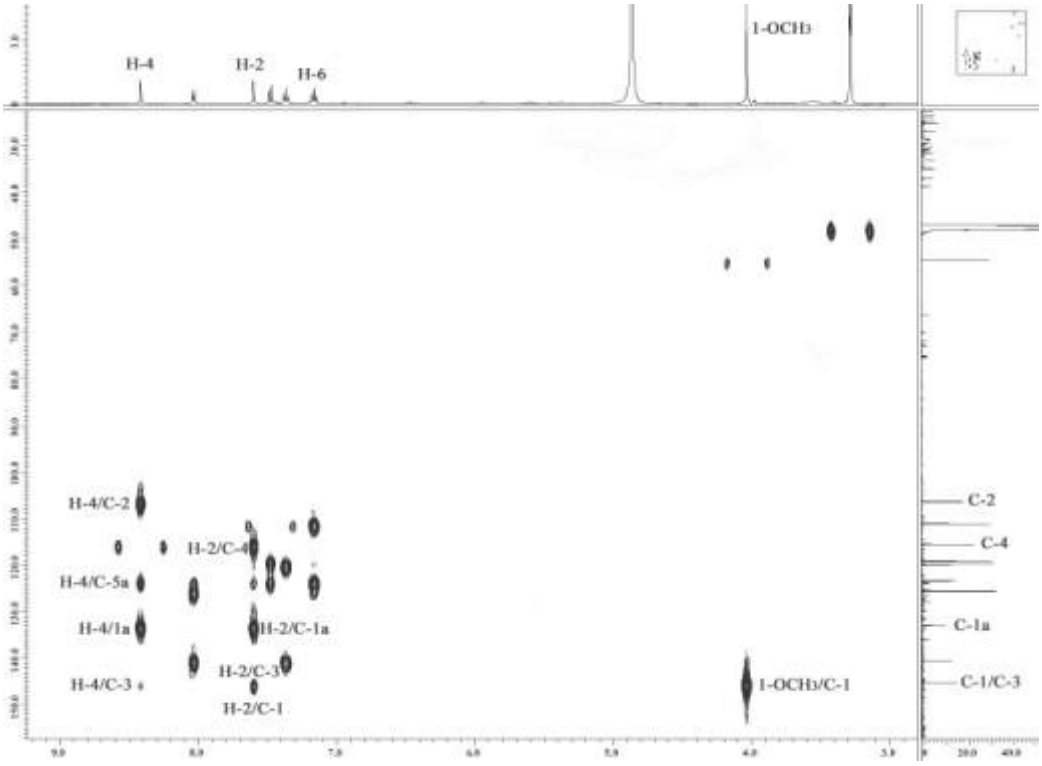


Figure 4.2.1.4g. HMBC Spectrum (CD_3OD, 500 MHz) of Murrastanine-A **(Mk9)**

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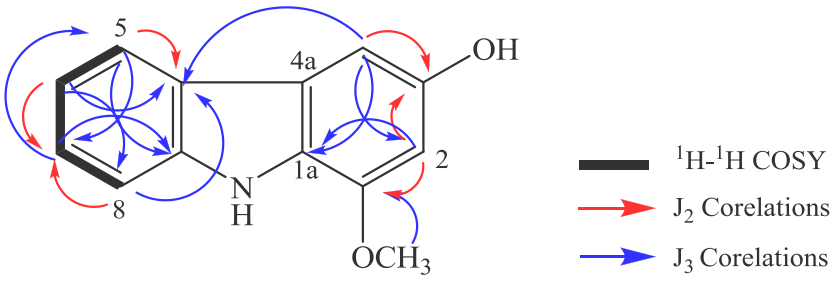


Figure 4.2.1.4h. Selected ^{1}H-^{1}H COSY and HMBCs of **Mk9**

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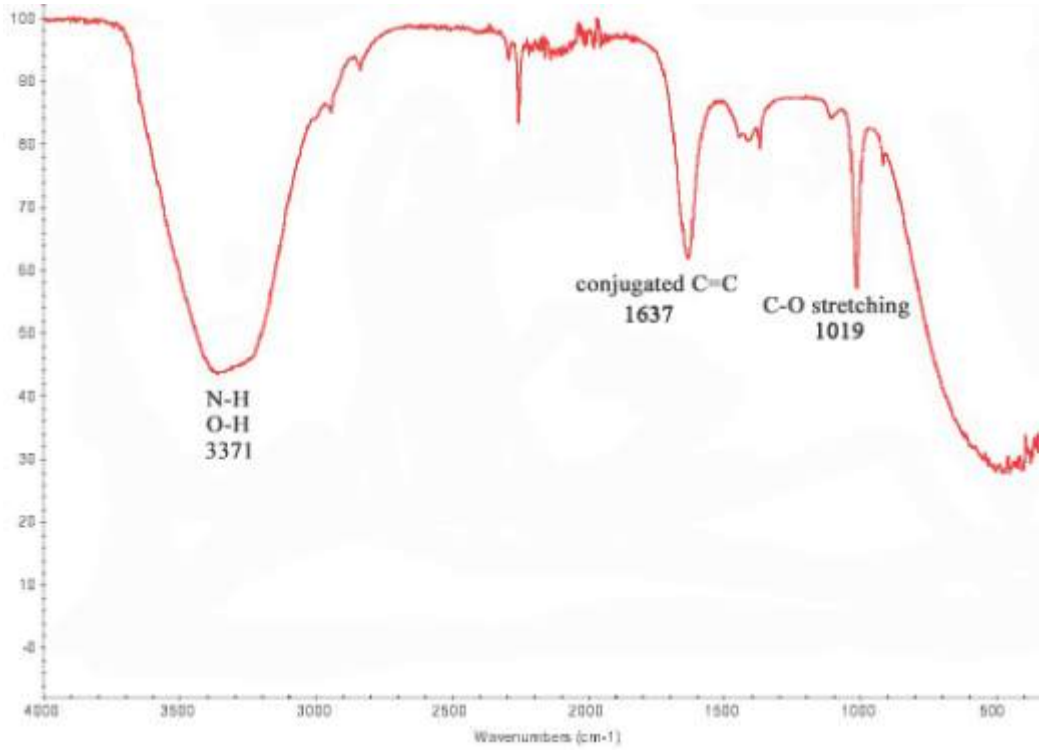


Figure 4.2.1.4i. IR Spectrum of Murrastanine-A **(Mk9)**

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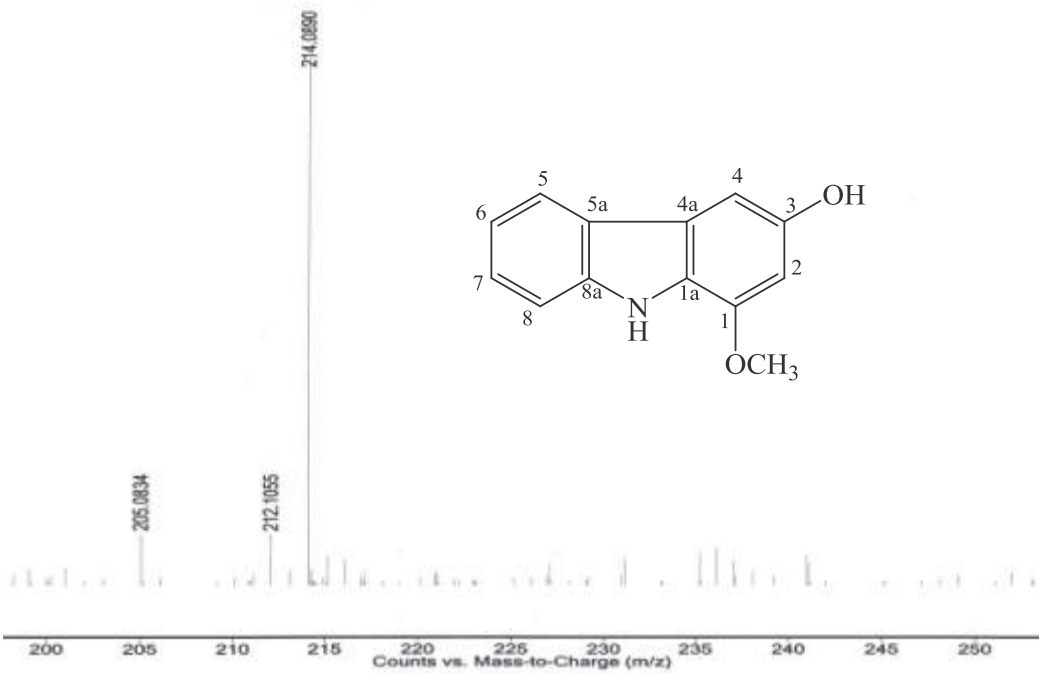


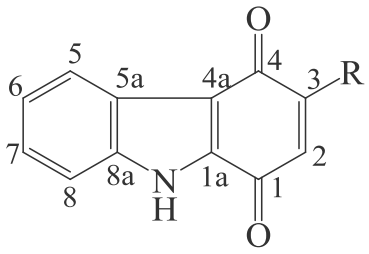
Figure 4.2.1.4j. Mass Spectrum of Murrastanine-A**(Mk9)**

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4.2.2 Carbazolequinone Alkaloid

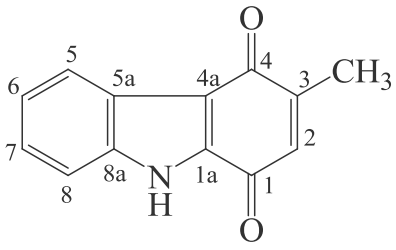
An important type of carbazole alkaloid, the carbazole-1,4-quinone **155,** which can be isolated as minor compound from the Rutaceae species, especially from the genus of *Murraya*. In the study, two known carbazolequinone alkaloids were successfully isolated from the bark of *M. koenigii*. The structural elucidation and identification of these alkaloids were also based on various spectroscopic analysis such as NMR, UV, IR and mass spectrometry.



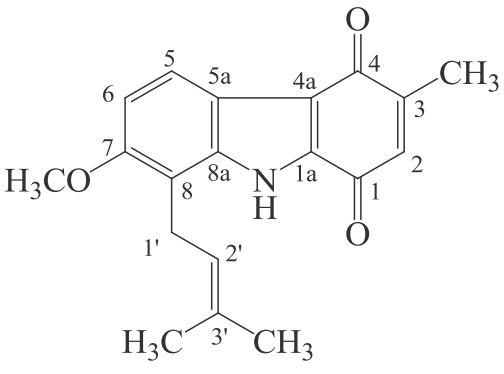
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4.2.2.1 Murrayaquinone-A (Mk10) and Murrayaquinone-B (Mk11)



Mk10



Mk11

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Mk10 was isolated as brown solid with melting point at 245–247 °C (m.p.: 246–247 °C; Furukawa et al.,1985) and showed molecular ion peak at *m/z* 212.0705 $[M+H]^+$ by HRESIMS in agreement with the molecular formula $C_{13}H_9NO_2$. The UV spectrum showed absorption at ν_{max} 225, 258, 293 and 398 nm (Cordell, 2008) and the IR spectrum showed absorption bands at 1638 (C=O) and 3367 (NH) cm^{-1} of **MK10** suggesting a carbazole-1,4-quinone type of molecule. This was supported by the ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) with two typical downfield signals for the carbonyl carbons at C-1 (δ 183.7) and C-4 (δ 180.4) in the molecule (Table 4.2.2.1b and Figure 4.2.2.1b). The ^1H NMR spectrum ($CDCl_3$, 500 MHz) (Table 4.2.2.1a and Figure 4.2.2.1a) exhibited signals for an allylic methyl group as a doublet at δ 2.18 (*d*, *J* = 1.7 Hz, 3-CH_3) and a quinonoid proton as a quartet at δ 6.54 (*q*, *J* = 1.7 Hz, H-2) due to the presence of the magnetic non-equivalence of neighbouring protons which attributed to the occurrence of an asymmetric center in the molecule. Both of these signals showed long-range coupling in its HMBC experiment and gave further evidence for the presence of a quinone ring in the carbazole nucleus. In the aromatic region, signal for a deshielded proton at δ 8.25 (*d*, *J* = 8.0 Hz) could be assigned to H-5 due to the deshielding effect of the C-4 carbonyl moiety. The remaining signals in the aromatic region of **Mk10** at δ 7.35 (*td*, *J* = 8.0, 1.1 Hz, H-6), δ 7.41 (*td*, *J* = 8.0, 1.1 Hz, H-7) and δ 7.49 (*d*, *J* = 8.0 Hz, H-8) were deduced for an unsubstituted carbazole ring-C. The complete assignments of carbon signals and location of substituents on the carbazolequinone skeleton of **Mk10** were achieved on the basis of DEPT, ^1H-^1H COSY, HMQC and HMBC spectra, as well as comparison with those reported in literature (Furukawa et al.,1985). The structure of **Mk10** was finally identified as murrayaquinone-A.

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Another carbazolequinone, **Mk11**, was obtained as dark brown solid with melting point 221–222 °C (m.p.: 221–223 °C; Furukawa et al.,1985). The molecular formula $C_{19}H_{19}NO_3$ was established for **Mk11** by HRESIMS at a quasi-molecular ion peak at *m/z* 310.1425 $[M+H]^+$. The UV spectrum showed absorption at ν_{max} 230, 233, 264, 288 and 310 nm (Cordell, 2008) and the IR spectrum revealed absorption peaks at 1019 (C–O), 1638 (C=O) and 3366 (NH) cm^{-1}, which are similar to those of **Mk10**, suggesting a carbazole-1,4-quinone nucleus in the carbazole molecule. These could be further supported by the presence of two signals for carbonyl carbons at δ 183.9 and 179.9 for C-1 and C-4, respectively, in the ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) (Table 4.2.2.1b and Figure 4.2.2.1d). The ^1H NMR spectrum of **Mk11** (Table 4.2.2.1a and Figure 4.2.2.1c) showed similar signals pattern to **Mk10**, except for the additional of a methoxy signal at δ 3.91 (*s*, 7-OCH_3) and a prenyl group which attached to C-8 with signals at δ 3.57 (*d*, *J* = 6.9 Hz, H-1'), δ 5.27 (*t*, *J* = 7.0 Hz, H-2'), δ 1.75 (*s*, 3'-CH_3) and δ 1.85 (*s*, 3'-CH_3). In addition, long range coupling between a 3H proton signal at δ 2.13 (*d*, *J* = 1.8 Hz, 3-CH_3) and a 1H quartet signal at δ 6.43 (*q*, *J* = 1.7 Hz, H-2) were also observed in its HMBC experiment. The complete assignments of all the proton and carbon signals for the carbazolequinone skeleton of **Mk11** were identical to those in the literature. The structure of **Mk11** was determined as murrayaquinone-B (Furukawa et al.,1985).

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Table 4.2.2.1a

*^{1}H NMR [500 MHz, δ_H (J Hz)] of Murrayaquinone-A **(Mk10)** and Murrayaquinone-B **(Mk11)***

Position	**Mk10**	**Mk11**
1		
1a		
2	6.54 (*q*, 1.7 Hz)	6.43 (*q*, 1.7 Hz)
3		
4		
4a		
5	8.25 (*d*, 8.0 Hz)	8.01 (*d*, 9.2 Hz)
5a		
6	7.35 (*td*, 8.0, 1.1 Hz)	7.03 (*d*, 9.2 Hz)
7	7.41 (*td*, 8.0, 1.1 Hz)	
8	7.49 (*d*, 8.0 Hz)	
8a		
N-H	9.14 (*br s*)	8.91 (*br s*)
3-CH_3	2.18 (*d*, 1.7 Hz)	2.13 (*d*, 1.8 Hz)
7-OCH_3		3.91 (*s*)
1'		3.57 (*d*, 6.9 Hz)
2'		5.27 (*t*, 7.0 Hz)
3'		
3'-CH_3		1.75 (*s*)
3'-CH_3		1.85 (s)

Note. *Solvent used was $CDCl_3$; assignments based on COSY and HMQC.

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Table 4.2.2.1b

*^{13}C NMR [125 MHz, δ_C] of Murrayaquinone-A **(Mk10)** and Murrayaquinone-B **(Mk11)****

Position	**Mk10**	**Mk11**
1	183.7	183.9
1a	135.5	135.2
2	131.6	131.6
3	149.0	148.3
4	180.2	179.9
4a	117.9	117.3
5	123.1	110.8
5a	119.1	119.0
6	124.2	121.2
7	127.1	156.1
8	112.8	112.6
8a	136.9	138.0
3-CH_3	16.3	16.2
7-OCH_3		56.8
1'		23.8
2'		121.6
3'		134.2
3'-CH_3		25.8
3'-CH_3		18.1

Note. *Solvent used was $CDCl_3$; assignments based on HMQC and HMBC.

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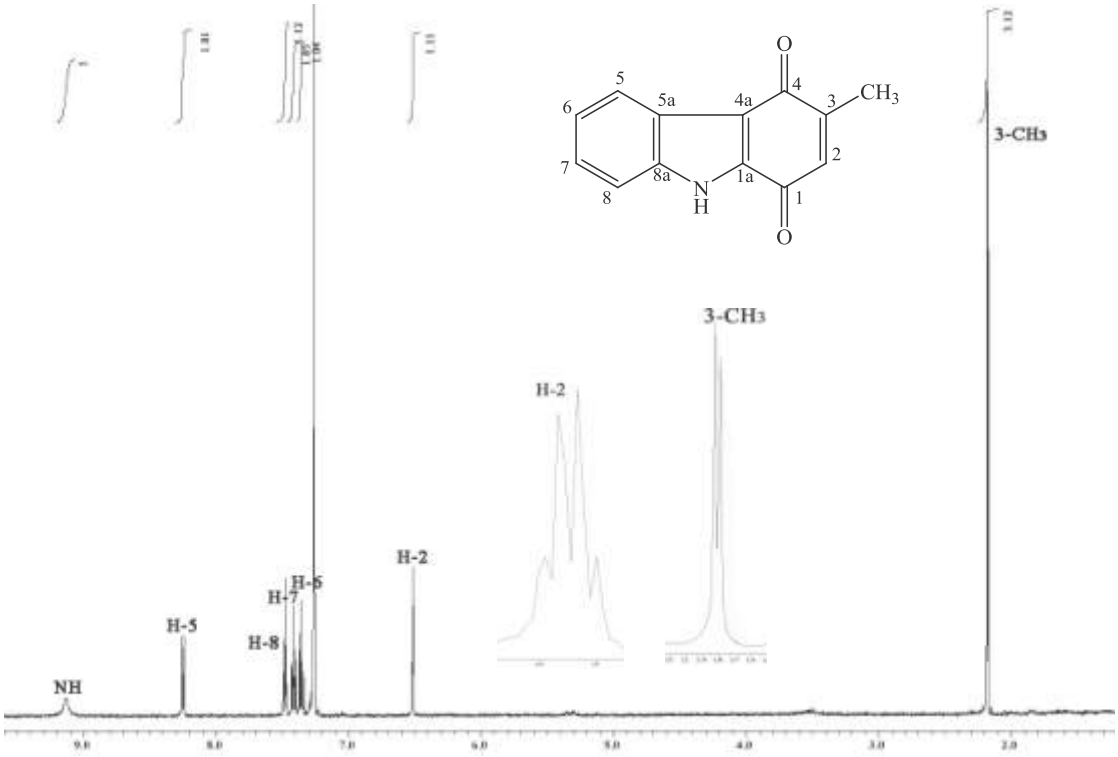


Figure 4.2.2.1a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Murrayaquinone-A **(Mk10)**

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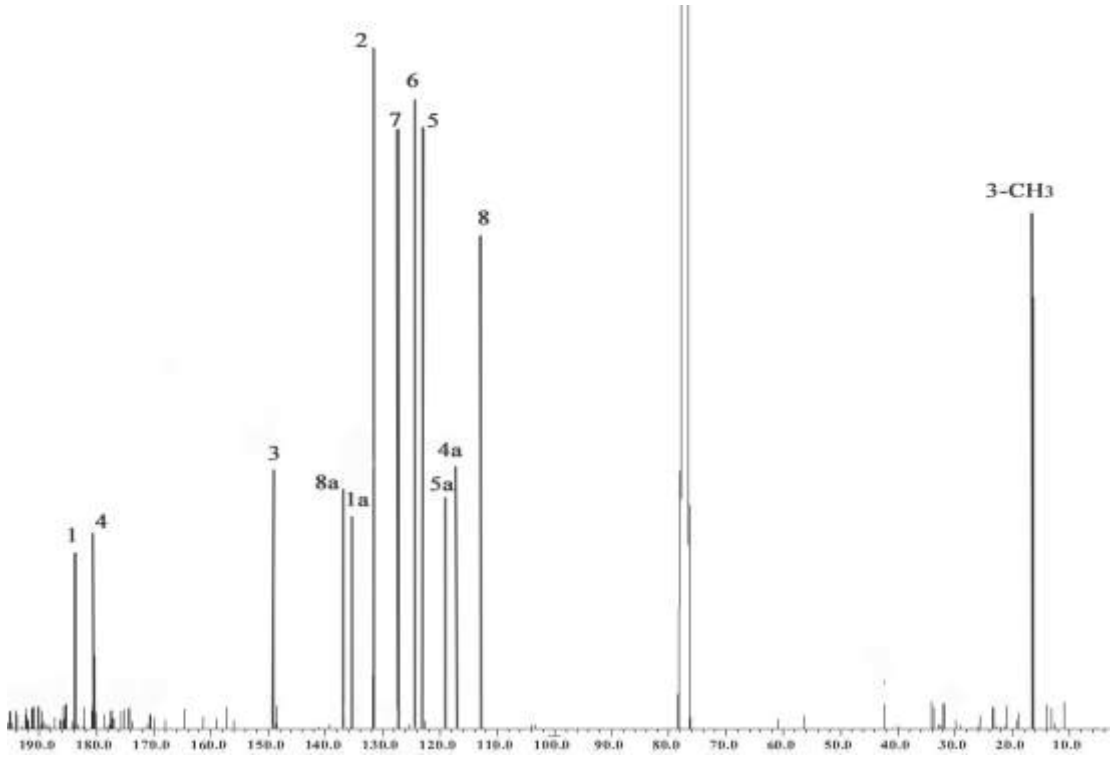


Figure 4.2.2.1b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Murrayaquinone-A **(Mk10)**

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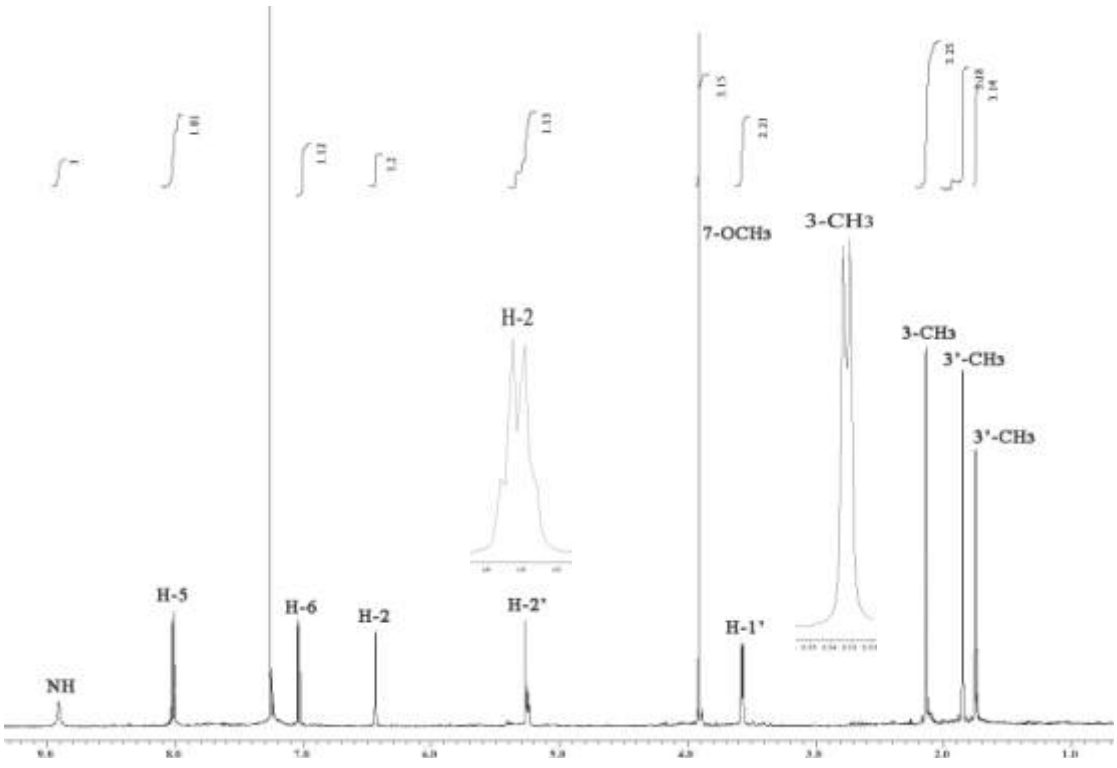


Figure 4.2.2.1c. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Murrayaquinone-B **(Mk11)**

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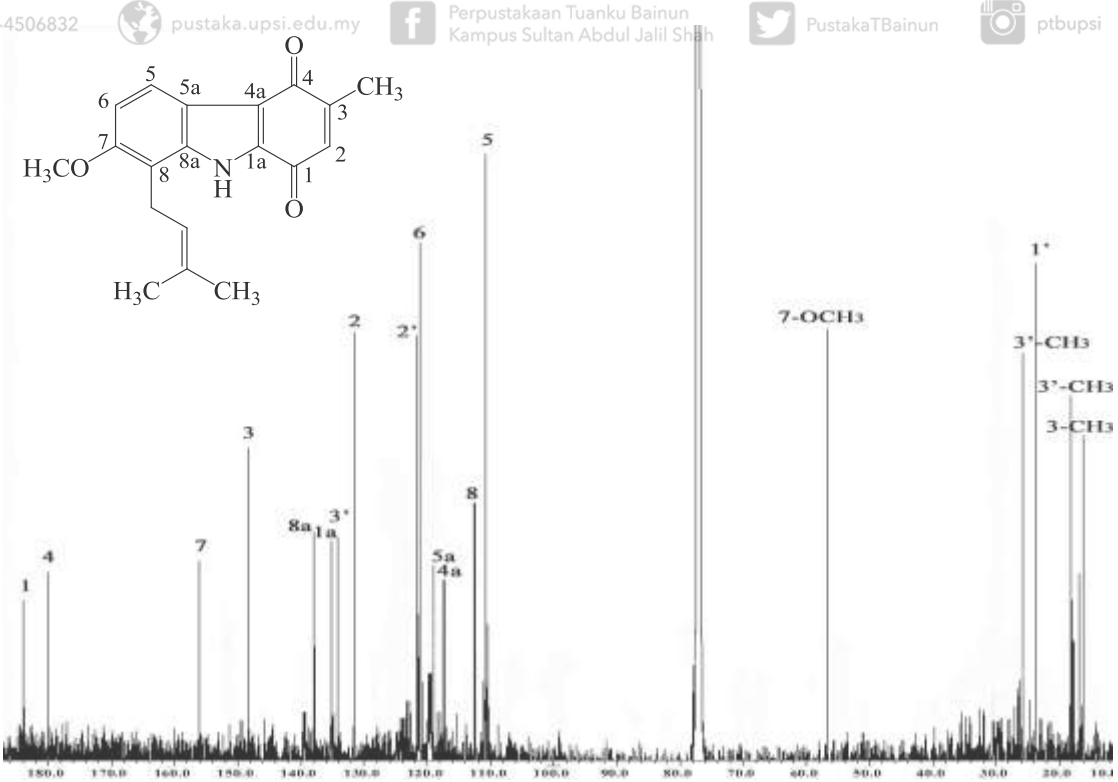
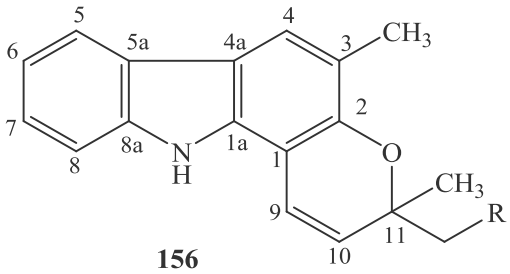


Figure 4.2.2.1d. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Murrayaquinone-B **(Mk11)**

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4.2.3 Pyrano[3,2-a]carbazole Alkaloids

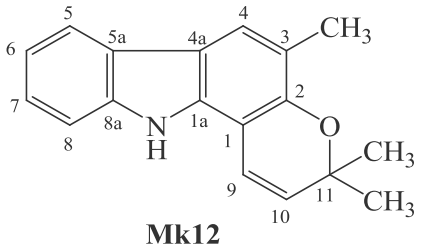


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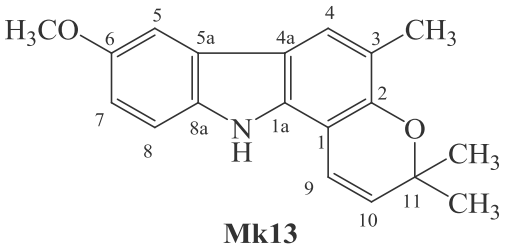
R = prenyl or geranyl

Eight known and three new pyrano[3,2-a]carbazoles **156** were afforded from the bark and leaves of *M. koenigii*. Biogenetically, these compounds were derived from 2-hydroxy-3-methylcarbazole **39** by fusion with either prenyl [-$CH_2CH=C(CH_3)_2$] or geranyl [-$CH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)_2$] substituents. The structural assignments of this type of carbazole alkaloids could be agreed and supported by NMR, UV, IR and mass spectral data, as well as direct comparison with the spectral data from the literature.

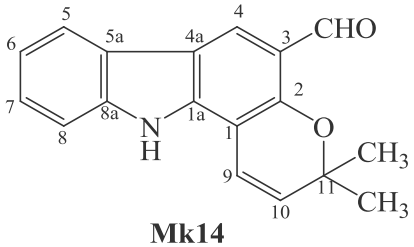
4.2.3.1 Girinimbine (Mk12), Koenimbine (Mk13) and Murrayacine (Mk14)



Mk12



Mk13



Mk14

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Mk12 was obtained as a major compound from the hexane extract of bark of *M. koenigii* as colourless crystal with melting point at 176–178 °C (m.p.: 175–177 °C; Abu Bakar et al., 2007). The IR spectrum of **Mk12** indicated the presence of NH group (3363 cm^{-1}) and C–O (1018 cm^{-1}). The UV spectrum of this compound with λ_{max} 237, 287 and 327 nm showed characteristic of an angular pyranocarbazole skeleton. The mass spectrum of this compound gave a prominent base peak at *m/z* 264.1364 $[M+H]^+$, which was consistent with a molecular formula of $C_{18}H_{17}NO$.

The ^1H NMR spectrum ($CDCl_3$, 500 MHz) (Table 4.2.3.1a and Figure 4.2.3.1a) of **Mk12** showed a broad downfield signal at δ 7.87 (*br s*) was assigned to NH group in the carbazole nucleus. Along with an aromatic methyl group (δ 2.35) at C-3, the ^1H NMR spectrum of this compound clearly showed that the molecule contained a singlet proton for H-4 at δ 7.69. Another four mutually coupled aromatic protons for the unsubstituted ring-C of the carbazole nucleus at δ 7.94 (*d*, *J* = 8.0 Hz, H-5), δ 7.36 (*d*, *J* = 8.0 Hz, H-8), δ 7.32 (*td*, *J* = 8.0, 1.1 Hz, H-7) and δ 7.19 (*td*, *J* = 8.0, 1.1 Hz, H-6) were also observed. Two doublets at δ 6.60 (*d*, *J* = 9.8 Hz, H-9) and δ 5.69 (*d*, *J* = 9.8 Hz, H-10) of the pyran ring double bond were depicted for the presence of pyranocarbazole framework. The remaining two methyl signals at δ 1.50 (6H, *s*, 2 × 11-CH_3) adjacent to a oxygen functionality represented a dimethyl pyranone ring fused to the ring-A of carbazole. The ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) (Table 4.2.3.1b and Figure 4.2.3.1b) indicated the presence of 17 carbon resonances with one overlapping signal at δ 27.7 for the *gem*-dimethyl group at C-11. The position of the carbon resonances were also further supported by 2D NMR and mass spectral data.

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On the basis of these data, the structure of **Mk12** was confirmed to be girinimbine (Abu Bakar et al., 2007).

Mk13 was obtained from the leaves extract as brown solid with melting point at 193–194 °C (m.p.: 194–196 °C; Narasimhan et al., 1968). The ^{1}H and ^{13}C NMR spectra of **Mk13** were identical to **Mk12** except for the presence of a methoxyl group at the C-6 [δ_H: 3.90 (*s*), δ_C: 56.1] of the carbazole nucleus. The IR spectrum indicated the presence of NH group (3334 cm^{-1}) and C–O stretching (1025 cm^{-1}). The UV spectrum of this compound with λ_{max} 238, 262, 268 and 295 nm was suggesting the presence of an angular pyranocarbazole skeleton (Cordell, 2008). The HRESIMS of **Mk13** gave a molecular ion peak at *m/z* 294.2071 $[M+H]^+$, which was consistent with a molecular formula of $C_{19}H_{19}NO_2$.

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The ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) (Table 4.2.3.1a and Figure 4.2.3.1c) of **Mk13** showed a broad downfield signal at δ 7.72 (*br s*) assignable to the presence of NH group in the carbazole nucleus. An aromatic methyl group (δ 2.33) at C-3, as well as a singlet proton for H-4 at δ 7.63 were observed in the ^{1}H NMR spectrum of **Mk13**. An ABX spin pattern at δ 7.41 (*d*, *J* = 2.9 Hz), δ 6.95 (*dd*, *J* = 8.6, 2.9 Hz) and δ 7.28 (*d*, *J* = 8.6 Hz) were observed for H-5, H-7 and H-8, respectively. Two doublets with coupling constant 9.8 Hz at δ 6.61 (H-9) and δ 5.68 (H-10) in its ^{1}H NMR spectrum, of which identical with those signals in **Mk12**, gave further evidence for the presence of pyranocarbazole moiety. Meanwhile, a 6H proton signal at δ 1.48 (*s*) was deduced to a *gem*-dimethyl group which adjacent to an oxygen functionality represented a dimethyl pyranone ring fused to the ring-A as in the **Mk12**. The ^{13}C

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NMR spectrum ($CDCl_3$, 125 MHz) (Table 4.2.3.1b and Figure 4.2.3.1d) indicated the presence of 19 carbons resonances with one overlapping signal at δ 27.7 for the *gem*-dimethyl group at C-11. Additional of one carbon signal, the methoxy carbon at C-6 (δ 56.1) was presence when compared to **Mk12**. The position of all the carbon resonances were further supported by ^{1}H-^{1}H COSY, HMQC and HMBC spectra and also comparison with the spectral data reported by Tachibana et al. (2003). On the basis of these data, the structure of **Mk13** was revealed to be koenimbine.

Mk14 was obtained from the bark of the plant as brown solid with melting point at 242–244 °C (m.p.: 242–244 °C; Chakraborty et al., 1971). The ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) (Table 4.2.3.1a and Figure 4.2.3.1e) of **Mk14** showed a typical downfield signal at δ 10.50 (1H, *s*, 3-CHO) indicated the presence of a formyl group (CHO), of which further confirmed by the presence C=O (1637 cm^{-1}) in its IR spectrum. The UV spectrum of **Mk14** with λ_{max} 238, 273, 285 nm was also suggesting the presence of a pyranocarbazole framework (Cordell, 2008). The mass spectrum of this compound gave a molecular ion peak at *m/z* 278.1168 $[M+H]^+$ and supported with the assigned molecular formula of $C_{18}H_{15}NO_2$.

In ^{1}H NMR, a broad singlet at δ 8.18 (*br s*) assignable to the presence of NH group in the carbazole nucleus. Besides, the ^{1}H NMR also showed signal for the presence of a singlet proton for H-4 at δ 8.42 which was deshielded and shifted to a lower field due to the presence of the aldehyde group at C-3 as compare to those in **Mk12**. Four mutually coupled aromatic protons at δ 7.98 (*d*, *J* = 8.0 Hz, H-5), δ 7.25 (*td*, *J* = 8.0, 1.1 Hz, H-6), δ 7.39 (*td*, *J* = 8.0, 1.1 Hz, H-7) and δ 7.40 (*d*, *J* = 8.0 Hz,

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H-8) were depicted for an unsubstituted ring-C carbazole. In a similar manner with **Mk12** and **Mk13**, the presence of the pyranocarbazole moiety were substantially confirmed by the presence of two doublets at δ 6.63 (*J* = 9.7 Hz, H-9) and δ 5.80 (*J* = 9.7 Hz, H-10), along with two overlapped methyl signal at δ 1.57 (*s*, 2 × 11-CH_3) in the ^{1}H NMR spectrum of **Mk14** which corresponding to a signal at δ 27.8 for a *gem*-dimethyl group at C-11 in its ^{13}C NMR spectrum (Table 4.2.3.1b and Figure 4.2.3.1f). The position of all the 18 carbon resonances and their corresponding protons were further confirmed by 1D and 2D NMR spectra. After the consideration of these spectral data, the structure of **Mk14** was determined as murrayacine (Chakraborty et al., 1971).

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Table 4.2.3.1a

*^{1}H NMR [500 MHz, δ_H (J Hz)] of Girinimbine **(Mk12)**, Koenimbine **(Mk13)** and Murrayacine **(Mk14)***

Position	**Mk12**	**Mk13**	**Mk14**
1			
1a			
2			
3			
4	7.69 (*s*)	7.63 (*s*)	8.42 (*s*)
4a			
5	7.94 (*d*, 8.0 Hz)	7.41 (*d*, 2.9 Hz)	7.98 (*d*, 8.0 Hz)
5a			
6	7.19 (*td*, 8.0, 1.1 Hz)		7.25 (*td*, 8.0, 1.1 Hz)
7	7.32 (*td*, 8.0,1.1 Hz)	6.95 (*dd*, 8.6, 2.9 Hz)	7.39 (*td*, 8.0, 1.1 Hz)
8	7.36 (*d*, 8.0 Hz)	7.28 (*d*, 8.6 Hz)	7.40 (*d*, 8.0 Hz)
8a			
9	6.60 (*d*, 9.8 Hz)	6.61 (*d*, 9.8 Hz)	6.63 (*d*, 9.7 Hz)
10	5.69 (*d*, 9.8 Hz)	5.68 (*d*, 9.8 Hz)	5.80 (*d*, 9.7 Hz)
11			
N-H	7.87 (*br s*)	7.72 (*br s*)	8.18 (*br s*)
3-CH_3	2.35 (*s*)	2.33 (*s*)	
3-CHO			10.50 (*s*)
6-OCH_3		3.90 (*s*)	
11-CH_3	1.50 (*s*)	1.48 (*s*)	1.57 (*s*)
11-CH_3	1.50 (*s*)	1.48 (*s*)	1.57 (*s*)

Note. *Solvent used was $CDCl_3$; assignments based on COSY and HMQC.

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Table 4.2.3.1b

*^{13}C NMR [125 MHz, δ_C] of Girinimbine **(Mk12)**, Koenimbine **(Mk13)** and Murrayacine **(Mk14)***

Position	**Mk12**	**Mk13**	**Mk14**
1	104.5	104.6	104.2
1a	134.9	135.8	140.3
2	149.9	149.9	154.9
3	118.7	118.4	118.8
4	121.3	121.1	119.9
4a	116.8	116.9	118.3
5	119.4	102.6	120.4
5a	124.0	124.5	124.2
6	119.6	154.0	121.0
7	124.4	113.1	126.1
8	110.5	111.1	110.8
8a	139.6	134.4	140.1
9	117.3	117.3	116.3
10	129.5	129.4	130.2
11	76.0	76.8	77.2
3-CH_3	16.2	16.2	
3-CHO			189.4
6-OCH_3		56.1	
11-CH_3	27.7	27.7	27.8
11-CH_3	27.7	27.7	27.8

Note. *Solvent used was $CDCl_3$; assignments based on HMQC and HMBC.

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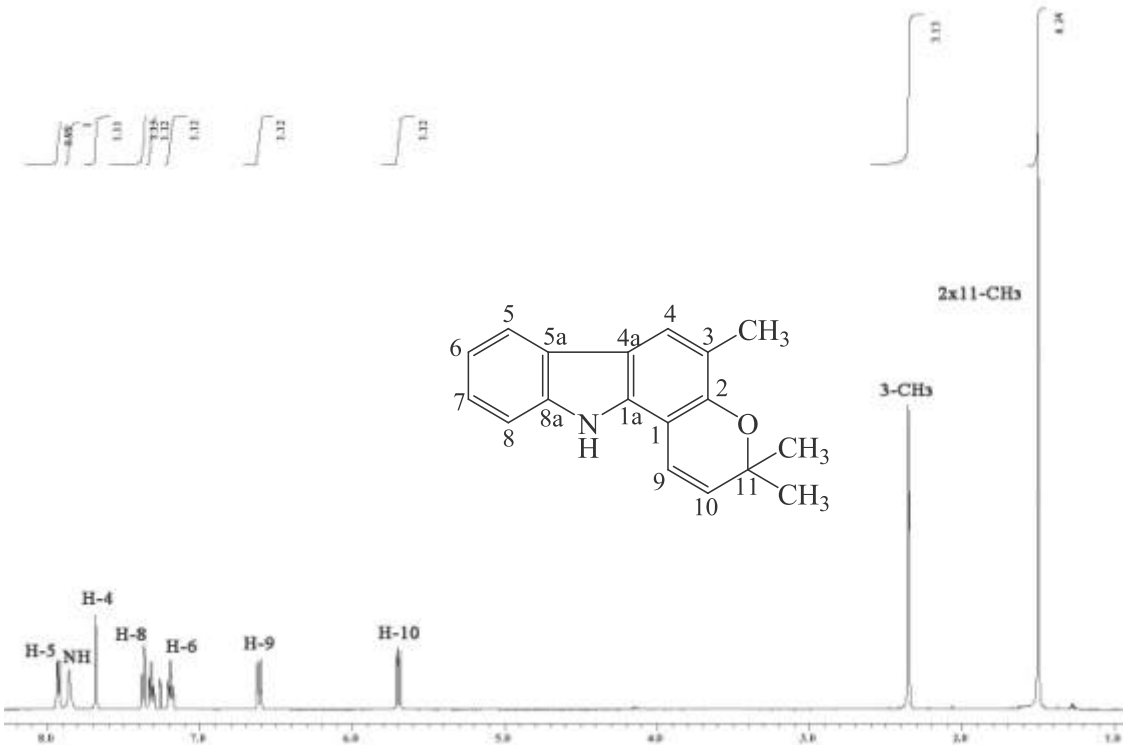


Figure 4.2.3.1a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Girinimbine **(Mk12)**

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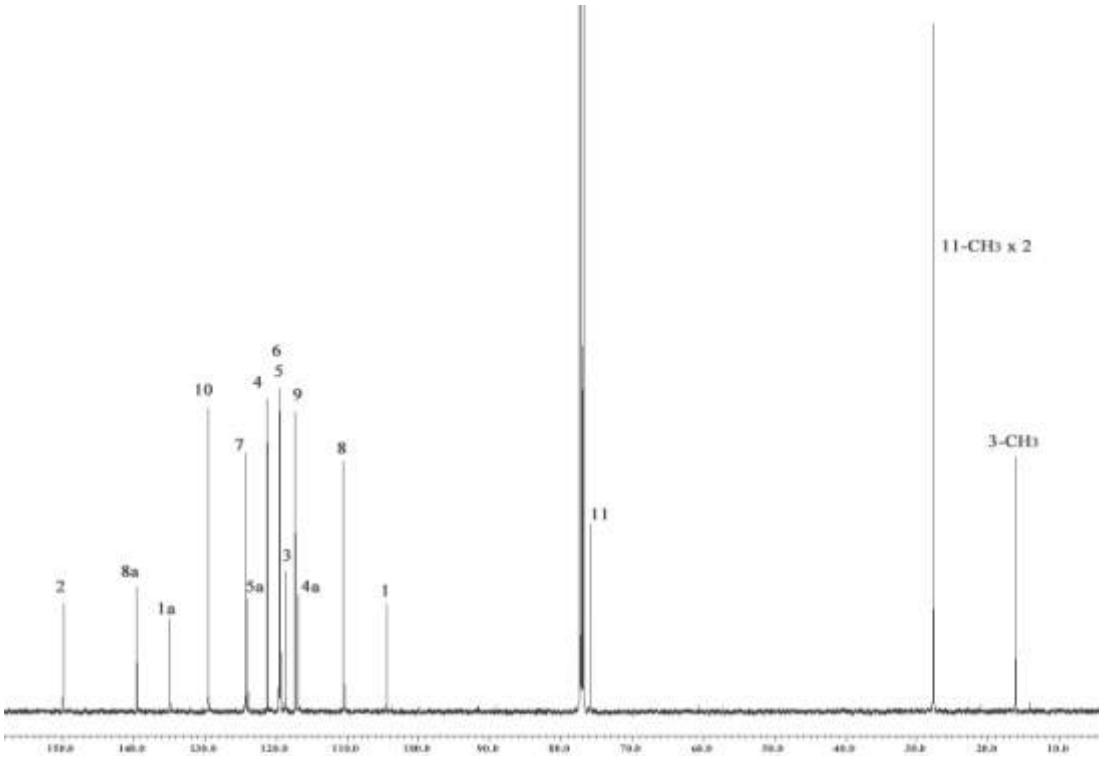


Figure 4.2.3.1b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Girinimbine **(Mk12)**

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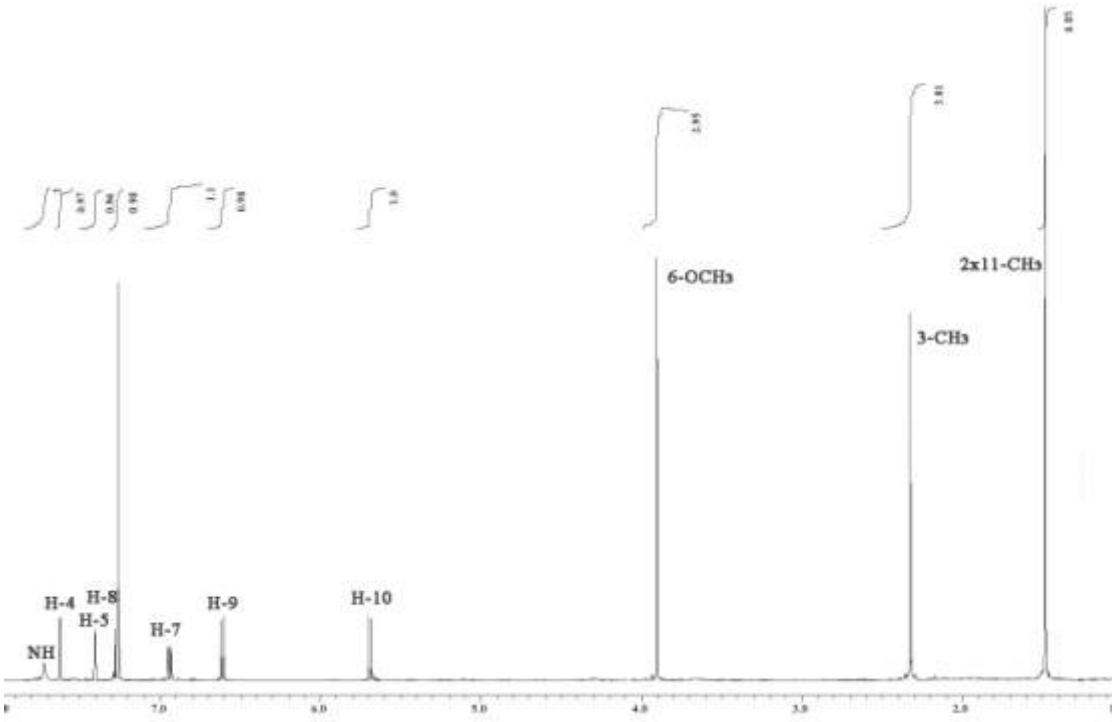


Figure 4.2.3.1c. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Koenimbine **(Mk13)**

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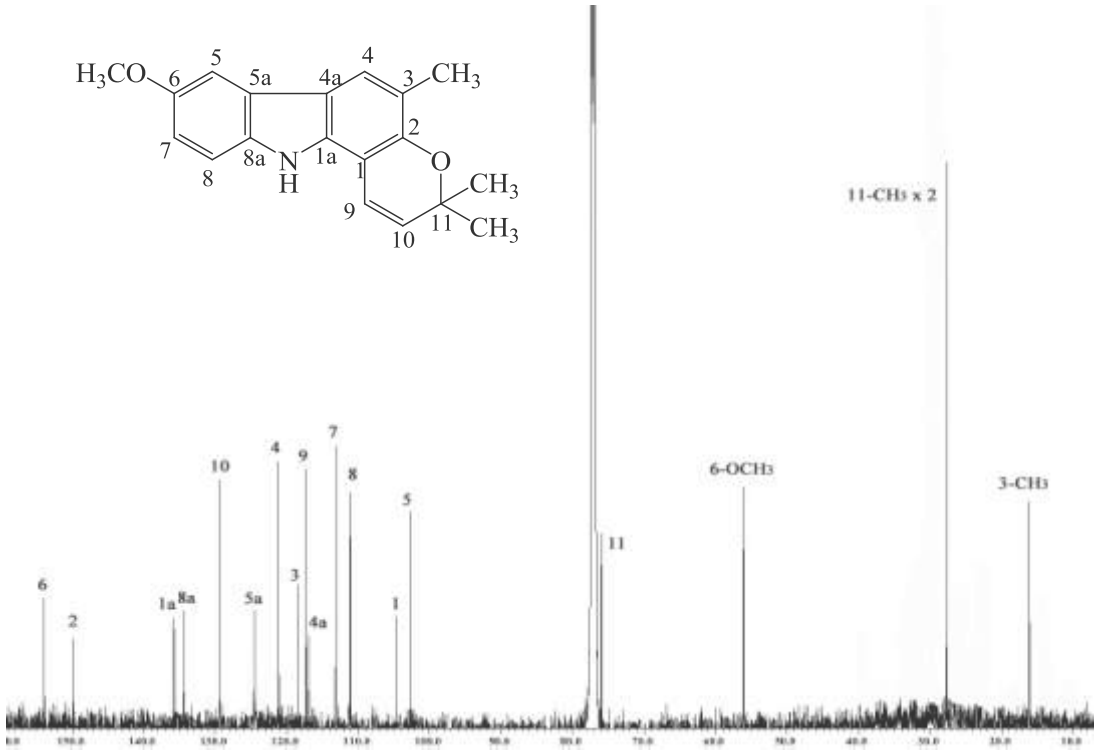


Figure 4.2.3.1d. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Koenimbine **(Mk13)**

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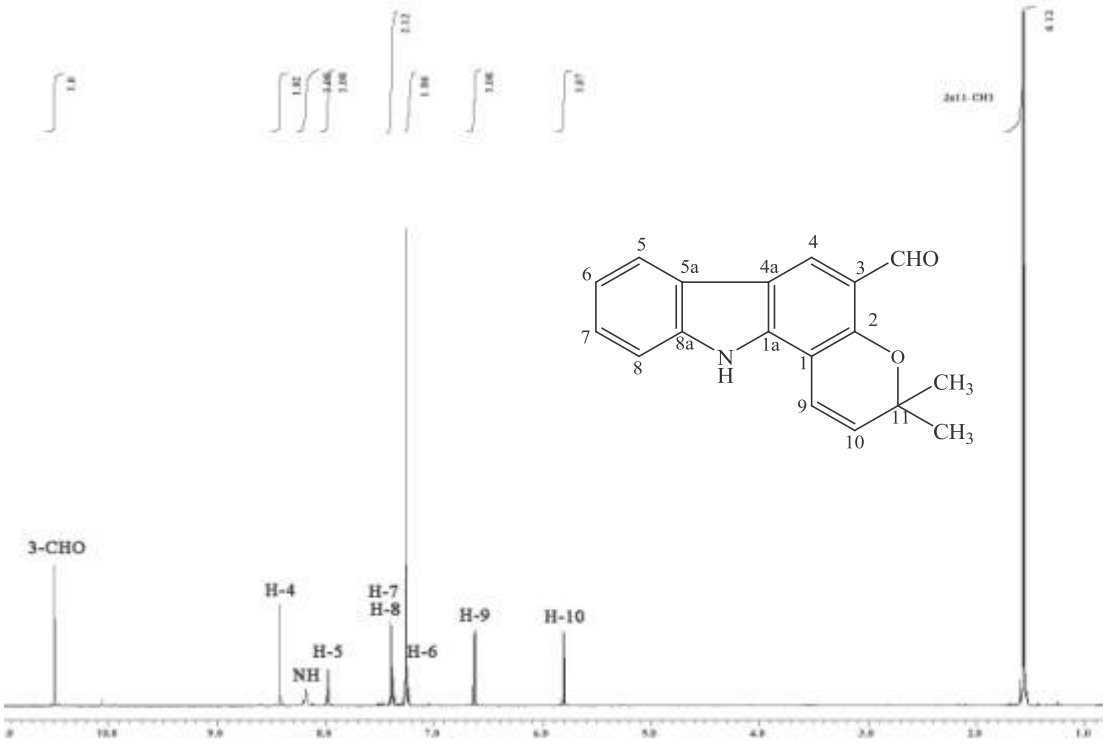


Figure 4.2.3.1e. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Murrayacine **(Mk14)**

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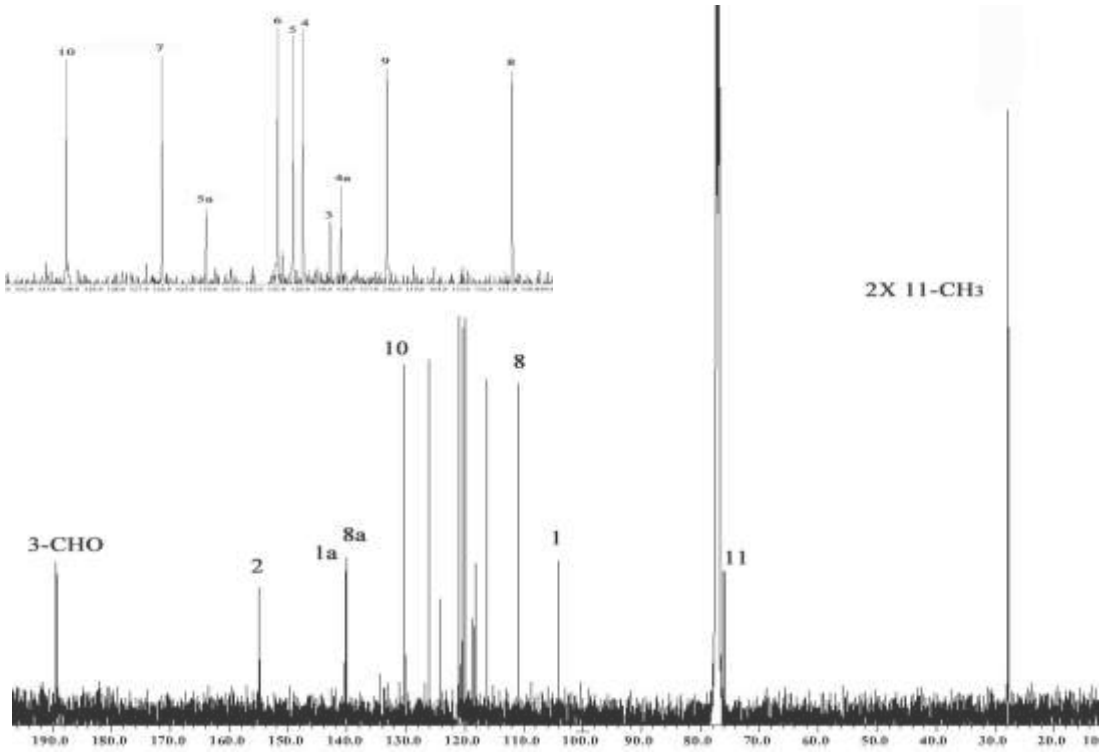
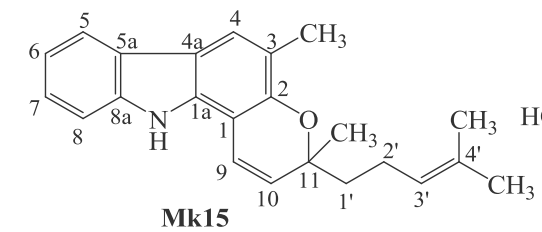


Figure 4.2.3.1f. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Murrayacine **(Mk14)**

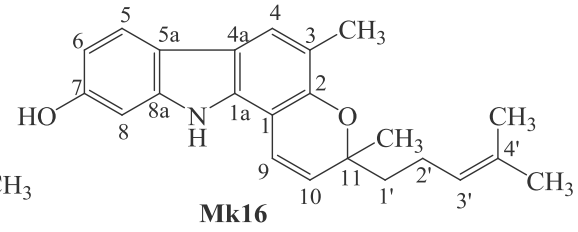
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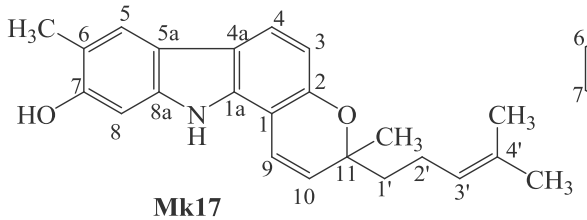
4.2.3.2 Mahanimbine (Mk15), Mahanine (Mk16), Isomahanine (Mk17), Murrayamine-B (Mk18) and Murrayamine-J (Mk19)



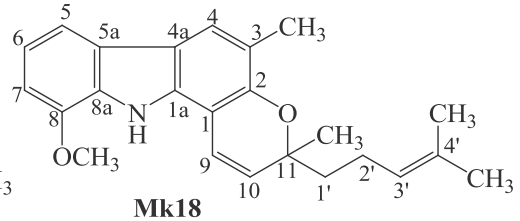
Mk15



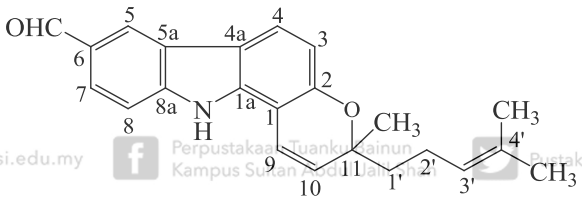
Mk16



Mk17



Mk18



Mk19

Mk15 was obtained as white solid [m.p.: 93–95 °C; m.p.: 94–95 °C (Narasimhan et al., 1968)] and optically active with $[\alpha]_D^{25}$ +25.5 (CH_3OH; c 0.02). Its UV spectrum resembled to those of pyranocarbazole moiety with λ_{max} 235 and 288 nm (Cordell, 2008). The IR spectrum of **Mk15** indicated the presence of NH (3367 cm^{-1}) and C–O (1121 cm^{-1}). It was readily recognized as a pyranocarbazole derivative from its preliminary spectral data. Its molecular formula was deduced as $C_{23}H_{25}NO$ at *m/z* 332.2002 $[M+H]^+$ by its HERSIMS.

Several features in ^1H NMR spectrum ($CDCl_3$, 500 MHz) of **Mk15** were similar to those of **Mk12** (Table 4.2.3.2a and Figure 4.2.3.2a), except for the presence

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of a prenyl group at C-11 (δ 78.1) with signals at δ 5.15 (*t*, *J* = 7.5 Hz, H-3'), 2.20 (*m*, H-2'), 1.79 (*t*, *J* = 8.9 Hz, H-1'), 1.72 (*s*, 4'-Me) and 1.62 (*s*, 4'-Me). The remaining signals such as a broad downfield signal at δ 7.81 assignable to the presence of a NH group in the carbazole nucleus, four mutually coupled signals at δ 7.94 (*d*, *J* = 8.1 Hz, H-5), δ 7.35 (*d*, *J* = 8.1 Hz, H-8), δ 7.33 (*td*, *J* = 8.1, 1.2 Hz, H-7) and δ 7.21 (*td*, *J* = 8.1, 1.2 Hz, H-6) were deduced for an unsubstituted carbazole ring-C, two olefinic protons at δ 6.60 (*d*, *J* = 9.8 Hz, H-9) and δ 5.65 (*d*, *J* = 9.8 Hz, H-10) due to a pyran ring annulated to a carbazole moiety, a signal for an aromatic methyl signal at δ 2.37 (*s*), and a methyl singlet signal at 11-CH_3 (δ 1.48). The ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of **Mk15** indicated the presence of 23 carbons resonances (Table 4.2.3.2b and Figure 4.2.3.2b). The complete assignments of all the proton and carbon signals and location of substituents of **Mk15** were achieved on the basis of DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectra. The structure of **MK15** was finally identified as mahanimbine (Tachibana et al., 2001).

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Mk16, yellowish solid with melting point at 94–96 °C (m.p.: 95–96 °C; Wu, 1991), was isolated as optically active compound, $[\alpha]_D^{25}$ +4.0 (CH_3OH; c 0.02) from the leaves of *M. koenigii*. This compound was identified as mahanine, of which was supported by various spectroscopic data and comparison with the literature data reported by Tachibana et al. (2001) and Wu (1991). The molecular formula $C_{23}H_{25}NO_2$ was based on its HRESIMS (*m/z* 347.1365 $[M]^+$) and NMR spectra. This structure was nearly identical to mahanimbine **(Mk15)**, except for the presence of a phenolic group (OH) at C-7 with a quaternary carbon signal at δ 153.6 in the ^{13}C and DEPT NMR spectra of **Mk16** (Table 4.2.3.2b and Figure 4.2.3.2d).

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In the aromatic region in ^{1}H NMR spectrum of **Mk16** (Table 4.2.3.2a and Figure 4.2.3.2c), an ABX spin pattern at δ 7.70 (*d*, *J* = 8.6 Hz, H-5), δ 6.68 (*dd*, *J* = 8.6, 1.7 Hz, H-6) and δ 6.79 (*d*, *J* = 1.7 Hz, H-8) were observed. The signals of two *cis*-olefinic protons at δ 5.63 (*d*, *J* = 9.7 Hz, H-10) and δ 6.58 (*d*, *J* = 9.7 Hz, H-9) due to a pyran ring annulated to a carbazole moiety at C-1 and C-2 of the pyrancarbazoles were also detected. The remaining signals included a NH proton at δ 7.77 (*br s*), a phenolic methyl at δ 2.32 (*s*, 3-CH_3), a methyl signal at δ 1.43 (*s*, 11-CH_3) and a prenyl substituents at δ 1.58 (*s*, 4'-CH_3), δ 1.66 (*s*, 4'-CH_3), δ 1.75 (*t*, *J* = 8.8 Hz, H-1'), δ 2.16 (*m*, H-2') and δ 5.11 (*t*, *J* = 7.5 Hz, H-3'). The ^{13}C NMR spectrum of **Mk16** ($CDCl_3$, 125 MHz) indicated the presence of 23 carbon resonances which is identical to those in **Mk15**. Furthermore, the ^{1}H-^{1}H COSY, HMQC and HMBC measurements allowed complete assignments of protons and carbons and determination of location of its substituents on the carbazole pyran skeleton.

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Mk17 was isolated as white solid [m.p. 183–184 °C; m.p.: 184 °C (Reisch et al., 1992)] with $[\alpha]_D^{25}$ +6.0 (CH_3OH; c 0.02). The IR spectrum of **Mk17** showed absorptions at ν_{max} 3500 and 3420 cm^{-1} indicated the presence of a phenolic hydroxyl group and a NH group. There were further confirmed by the appearance of proton signals at δ 4.89 (*br s*) and δ 7.79 (*br s*), respectively, in its ^{1}H NMR spectrum (Table 4.2.3.2a and Figure 4.2.3.2e). The UV spectrum of this compound, with λ_{max} 237, 262, 286, and 303, was similar to those of mahanine **Mk16** and suggesting the presence of a pyranocarbazole moiety (Cordell, 2008). The HRESIMS of **Mk16** revealed the molecular formula $C_{23}H_{25}NO_2$ with a molecular ion peak at *m/z* 347.3143 $[M]^+$.

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Two doublets at δ 6.62 and δ 5.65 (each *d, J* = 9.8 Hz) at H-9 and H-10, respectively, in its ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) gave further evidence for the presence of a pyranocarbazole skeleton. The signals of a methyl and a prenyl substituents, of which identical to mahanine **Mk16**, were indicated by comparison of the ^{1}H NMR spectra of these two compounds. In addition, the presence of an AB doublet of doublets at δ 6.68 and δ 7.63 (each *J* = 8.6 Hz) for H-3 and H-4, respectively, indicated two unsubstituted *ortho*-positions at ring-A, along with two singlets at δ 6.82 (H-8) and δ 7.63 (H-5), suggested that the methyl and hydroxyl group should be at the positions 6 and 7, respectively, in the ring-C. These were also confirmed by the long-range coupling in the HMBC experiment. Complete assignments of all the proton and carbon signals by 1D and 2D NMR and also comparison with literature data reported by Reisch and coworkers in 1992, **Mk17** was then confirmed to be isomahanine.

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Mk18 was isolated as brownish oil and optically active with $[\alpha]_D^{25}$ +5.0 (CH_3OH; c 0.015). Its molecular formula was established as $C_{24}H_{27}NO_2$ with its HRESIMS at *m/z* 361.1364 $[M]^+$. It showed UV spectrum similar to those of **Mk15**, indicated the presence of same carbazole skeleton.

In ^{1}H NMR spectrum (Table 4.2.3.2a and Figure 4.2.3.2g), the signals at δ 7.52 (*d, J* = 8.1 Hz) and 7.63 (*s*) were assigned to the mutually deshielded protons at H-5 and H-4, respectively, of carbazole nucleus. A high field singlet for an aromatic methyl group at δ 2.33 (*s*, 3-CH_3), two olefinic *gem*-dimethyl at δ 1.58 and δ 1.66 at C-4' (δ 131.8), and a tertiary methyl adjacent to an oxygen as a singlet at δ 1.44 (11-

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CH_3) were observed. Two methylene groups at δ 1.75 (*t*, *J* = 8.8 Hz, H-1') and δ 2.17 (*m*, H-2') along with an olefinic proton at δ 5.11 (*t*, *J* = 7.3 Hz, H-3') were depicted for the presence of a prenyl moiety. An AB type spin system at δ 6.68 and δ 5.65 (each *d*, *J* = 9.8 Hz) were assigned to H-9 and H-10, respectively, for the pyran ring double bond were also detected. Furthermore, the structure was also supported by the ^{13}C (Table 4.2.3.2b and Figure 4.2.3.2h), DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC experiments. Consequently, the structure of **Mk18** was concluded to be murrayamine-B (Wu, 1991).

Compound **Mk19** was obtained as pale yellow oil with $[\alpha]_D^{25}$ +35.5 (CH_3OH; c 0.01). This compound was determined to have molecular formula $C_{23}H_{23}NO_2$ by HRESIMS with a molecular ion peak at *m/z* 345.1364 $[M]^+$. The UV spectrum, at 215, 248, 265, 310 nm, suggested that this compound was thought to be a pyranocarbazole derivatives (Cordell, 2008). Examination of the aromatic region of the ^{1}H NMR spectrum suggested that **Mk19** was also identical to those of **Mk15**.

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The ^{1}H NMR spectrum of **Mk 19** (Table 4.2.3.2a and Figure 4.2.3.2i) showed a broad downfield signal at δ 8.35 deduced the presence of a NH group in the carbazole nucleus. A characteristic aldehyde singlet at δ 10.07 in its ^{1}H NMR along with a low field signal at δ 192.1 in its ^{13}C NMR spectrum (Table 4.2.3.2b and Figure 4.2.3.2j) corroborated with a strong carbonyl band at 1639 cm^{-1}, revealed the presence of a formyl group attached to C-6. The position of this formyl group was confirmed with its HMBC experiment by showing cross peak between the -CHO and C-6 of the carbazole nucleus. In addition, one set of ABX mutually-coupled proton system at δ

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8.46 (*d*, *J* = 1.7 Hz, H-5), δ 7.89 (*dd*, J = 8.6, 1.7 Hz, H-7) and δ 7.47 (*d*, *J* = 8.6 Hz, H-8), of which were deshielded by an aldehyde substituent at C-6 in ring-C, further confirmed the position of this CHO group substituent. An AB type spin system at δ 6.66 (*d*, *J* = 9.7 Hz) and δ 5.71 (*d*, *J* = 9.7 Hz) for H-9 and H-10, respectively, gave further evidence for the characteristic of pyranocarbazole skeleton. Conversely, the proton signals in the high field region consist of a methyl signal at δ 1.47 (*s*), a prenyl group adjacent to an oxygen in pyran ring system at C-11 with signals at δ 5.10 (*t*, *J* = 7.3 Hz, H-3'), δ 2.16 (*m*, H-2'), δ 1.74 (*m*, H-1'), and two *gem*-dimethyl signals at δ 1.68 (*s*, 4'-Me) and δ 1.58 (*s*, 4'-Me) were also observed. The complete assignments of proton and carbon signals and location of substituents on the pyranocarbazole skeleton of **Mk19** were achieved by the combination of NMR experiments. The structure of **Mk19** was readily identified as murrayamine-J (Wu et al., 1996).

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Table 4.2.3.2a

*^{1}H NMR [500 MHz, δ_H (J Hz)] of Mahanimbine **(Mk15)**, Mahanine **(Mk16)**, Isomahanine **(Mk17)**, Murrayamine-B **(Mk18)** and Murrayamine-J **(Mk19)****

Position	**Mk15**	**Mk16**	**Mk17**	**Mk18**	**Mk19**
1					
1a					
2					
3			6.68 (*d*, 8.6 Hz)		6.81 (*d*, 8.6 Hz)
4	7.69 (*s*)	7.54 (*s*)	7.63 (*d*, 8.6 Hz)	7.63 (*s*)	7.83 (*d*, 8.6 Hz)
4a					
5	7.94 (*d*, 8.1 Hz)	7.70 (*d*, 8.6 Hz)	7.63 (*s*)	7.52 (*d*, 8.1 Hz)	8.46 (*d*, 1.7 Hz)
5a					
6	7.21 (*td*, 8.1, 1.2 Hz)	6.68 (*dd*, 8.6, 1.7Hz)		7.10 (*t*, 8.1 Hz)	
7	7.33 (*td*, 8.1, 1.2 Hz)			6.81 (*d*, 7.5 Hz)	7.89 (*dd*, 8.6,1.7 Hz)
8	7.35 (*d*, 8.1 Hz)	6.79 (*d*, 1.7 Hz)	6.82 (*s*)		7.47 (*d*, 8.6 Hz)
8a					
9	6.60 (*d*, 9.8 Hz)	6.58 (*d*, 9.7 Hz)	6.62 (*d*, 9.8 Hz)	6.68 (*d*, 9.8 Hz)	6.66 (*d*, 9.7 Hz)
10	5.65 (*d*, 9.8 Hz)	5.63 (*d*, 9.7 Hz)	5.65 (*d*, 9.8 Hz)	5.65 (*d*, 9.8 Hz)	5.71 (*d*, 9.7 Hz)
11					
N-H	7.81 (*br s*)	7.77 (*br s*)	7.79 (*br s*)	8.07 (*br s*)	8.35 (*br s*)
3-CH_3	2.37 (*s*)	2.32 (*s*)		2.33 (*s*)	
6-CH_3			2.38 (*s*)		
6-CHO					10.07 (*s*)
7-OH		#	4.89 (*br s*)		
8-OCH_3				4.00 (*s*)	
1'	1.79 (*t*, 8.9 Hz)	1.75 (*t*, 8.8 Hz)	1.71 (*m*)	1.75 (*t*, 8.8 Hz)	1.74 (*m*)
2'	2.20 (*m*)	2.16 (*m*)	2.15 (*m*)	2.17 (*m*)	2.16 (*m*)
3'	5.15 (*t*, 7.5 Hz)	5.11 (*t*, 7.5 Hz)	5.10 (*t*, 7.4 Hz)	5.11 (*t*, 7.3 Hz)	5.10 (*t*, 7.3 Hz)
4'					
11-CH_3	1.48 (*s*)	1.43 (*s*)	1.44 (*s*)	1.44 (*s*)	1.47 (*s*)
4'-CH_3	1.62 (*s*)	1.58 (*s*)	1.57 (*s*)	1.58 (*s*)	1.58 (*s*)
4'-CH_3	1.72 (*s*)	1.66 (*s*)	1.65 (*s*)	1.66 (*s*)	1.66 (*s*)

Note. *Solvent used was $CDCl_3$; assignments based on COSY and HMQC. # peak not available.

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Table 4.2.3.2b

*^{13}C NMR [125 MHz, δ_C] of Mahanimbine **(Mk15)**, Mahanine **(Mk16)**, Isomahanine **(Mk17)**, Murrayamine-B **(Mk18)** and Murrayamine-J **(Mk19)***

Position	**Mk15**	**Mk16**	**Mk17**	**Mk18**	**Mk19**
1	104.2	104.4	104.7	104.5	105.1
1a	134.8	134.8	136.2	134.7	136.8
2	149.9	149.0	150.2	149.9	152.7
3	118.4	118.3	109.4	118.6	111.0
4	121.2	120.4	119.5	121.4	121.0
4a	116.6	116.7	117.6	117.1	117.2
5	119.3	120.1	121.0	112.2	122.9
5a	123.9	118.1	117.9	125.0	124.3
6	119.4	108.4	116.3	119.8	129.5
7	124.2	153.6	152.1	104.8	126.5
8	110.4	97.0	96.8	145.6	110.8
8a	139.4	140.8	139.3	143.4	143.4
9	117.5	117.6	117.4	117.7	116.8
10	128.4	128.8	128.9	128.5	129.6
11	78.1	78.1	78.2	78.2	78.8
3-CH_3	16.1	16.2		16.2	
6-CH_3			16.2		
6-CHO					192.1
8-OCH_3				55.6	
1'	40.7	40.7	40.8	40.8	41.0
2'	22.7	22.8	22.8	22.8	22.8
3'	124.2	124.3	124.2	124.3	124.0
4'	131.6	131.8	131.8	131.8	132.0
11-CH_3	25.8	25.8	26.0	25.9	26.2
4'-CH_3	17.6	17.7	17.7	17.7	17.7
4'-CH_3	25.7	25.7	25.8	25.8	25.8

Note. *Solvent used was $CDCl_3$; assignments based on COSY and HMQC.

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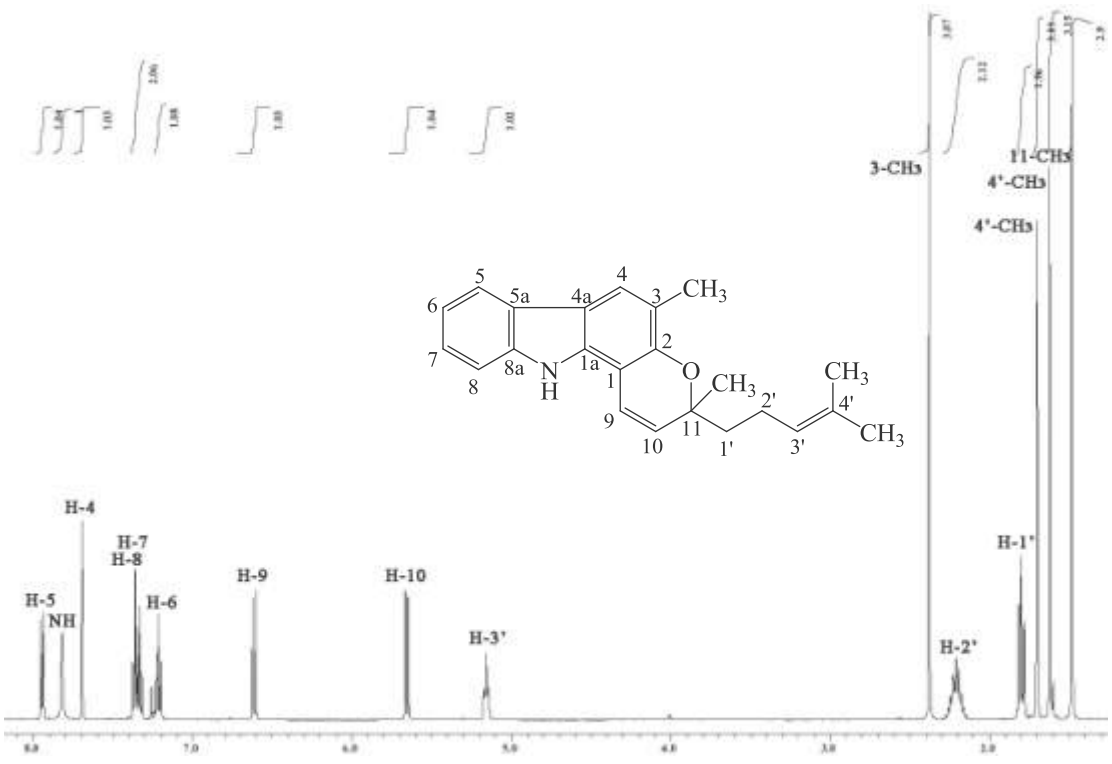


Figure 4.2.3.2a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Mahanimbine **(Mk15)**

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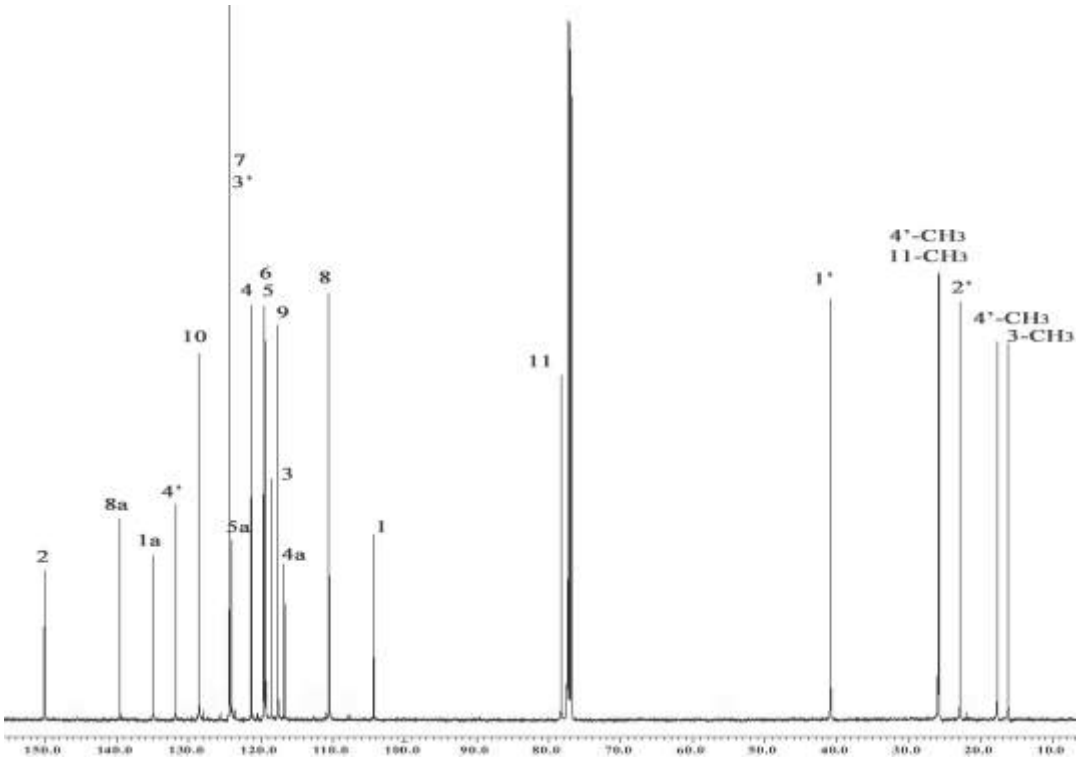


Figure 4.2.3.2b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Mahanimbine **(Mk15)**

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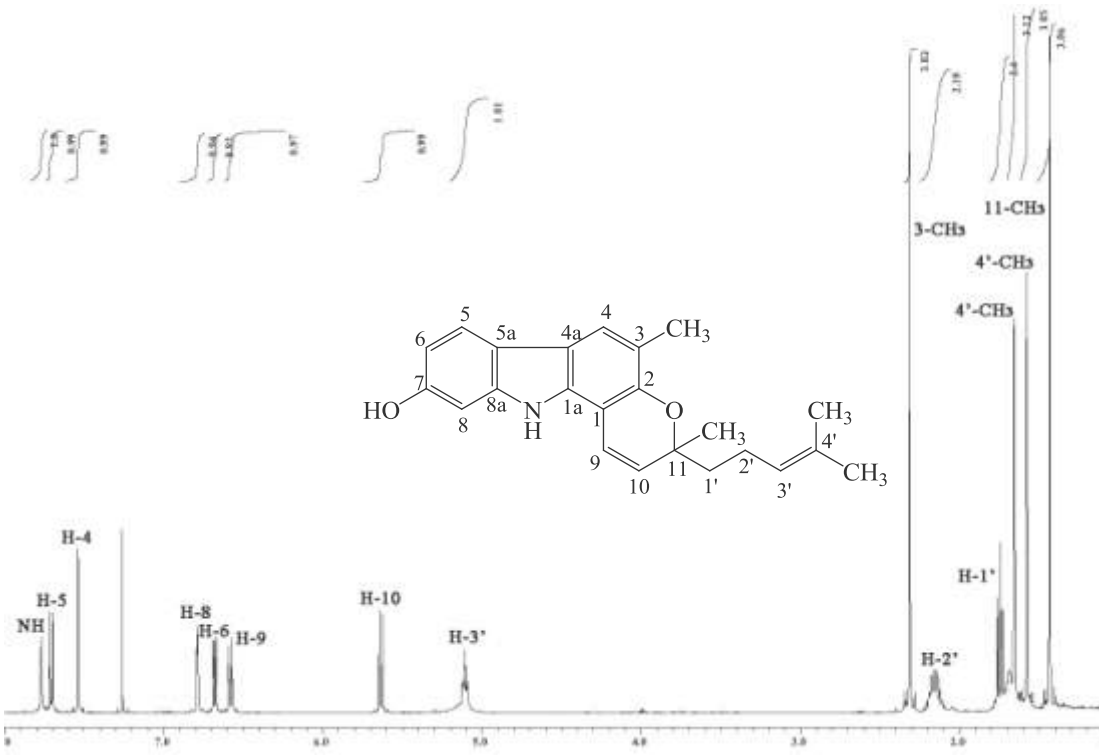


Figure 4.2.3.2c. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Mahanine **(Mk16)**

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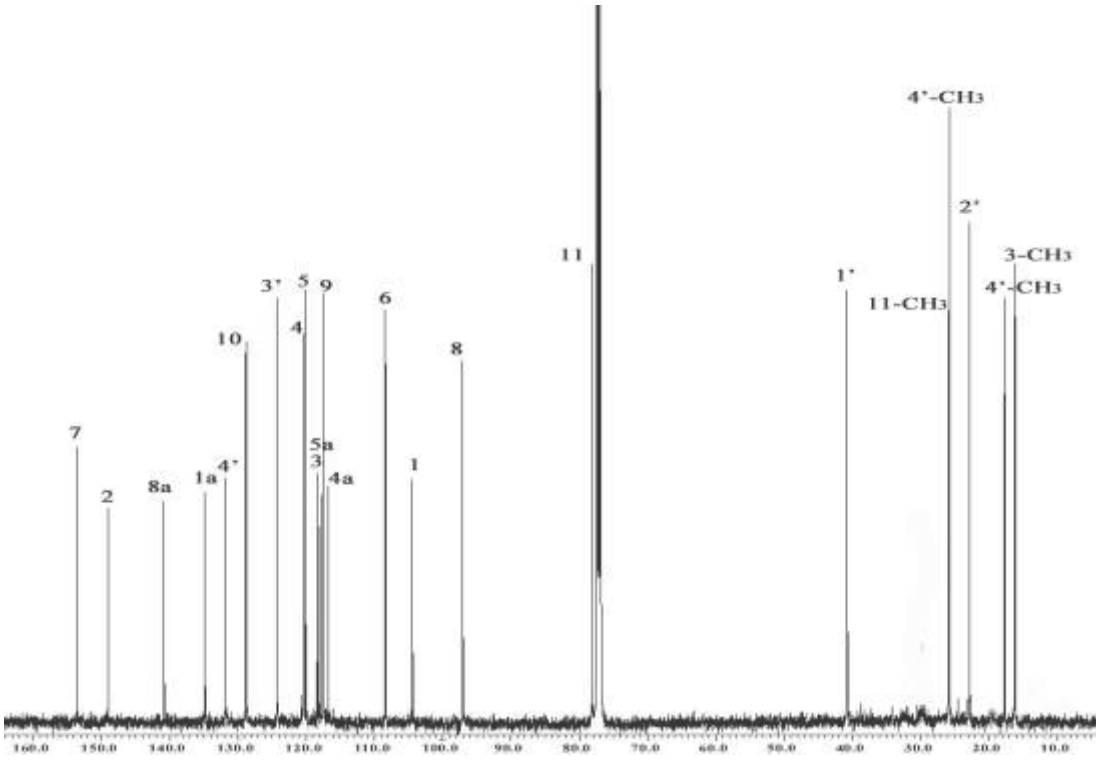


Figure 4.2.3.2d. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Mahanine **(Mk16)**

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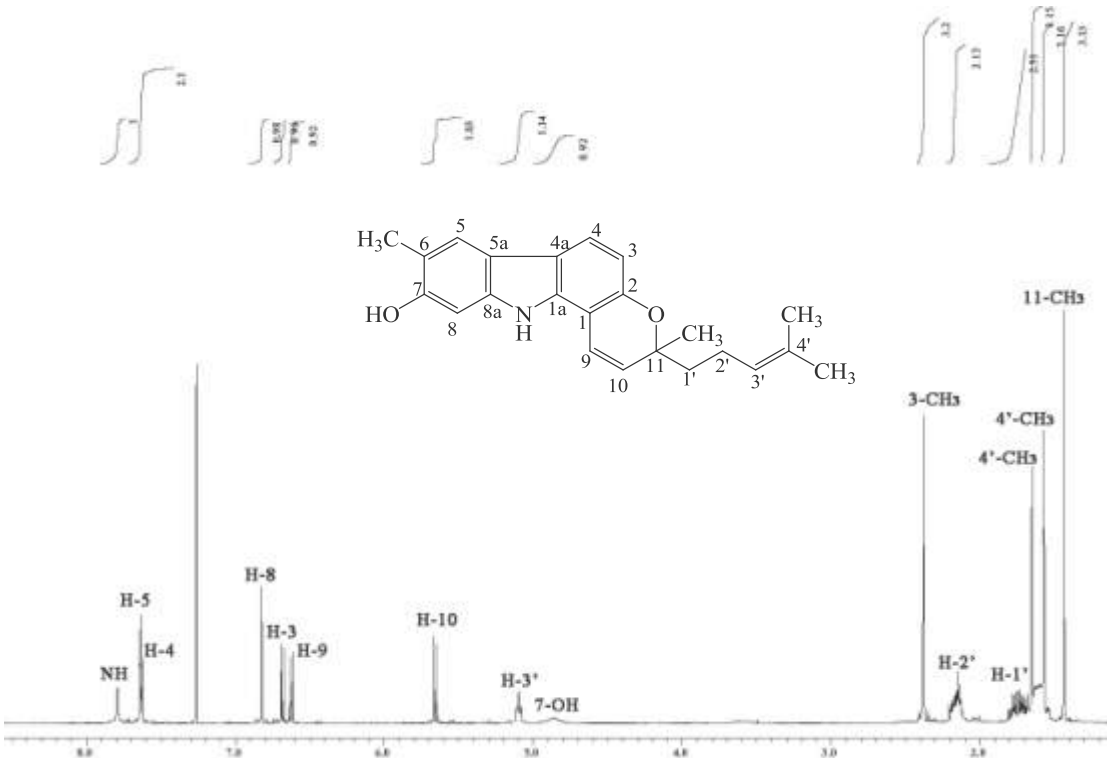


Figure 4.2.3.2e. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Isomahanine **(Mk17)**

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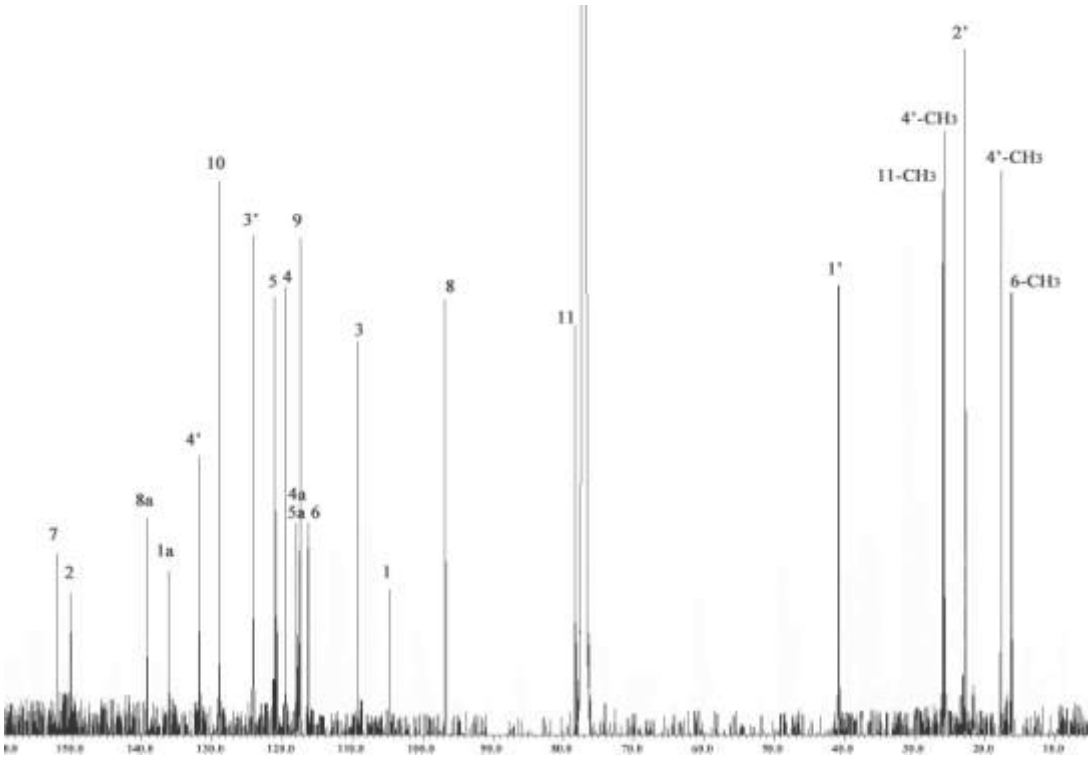


Figure 4.2.3.2f. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Isomahanine **(Mk17)**

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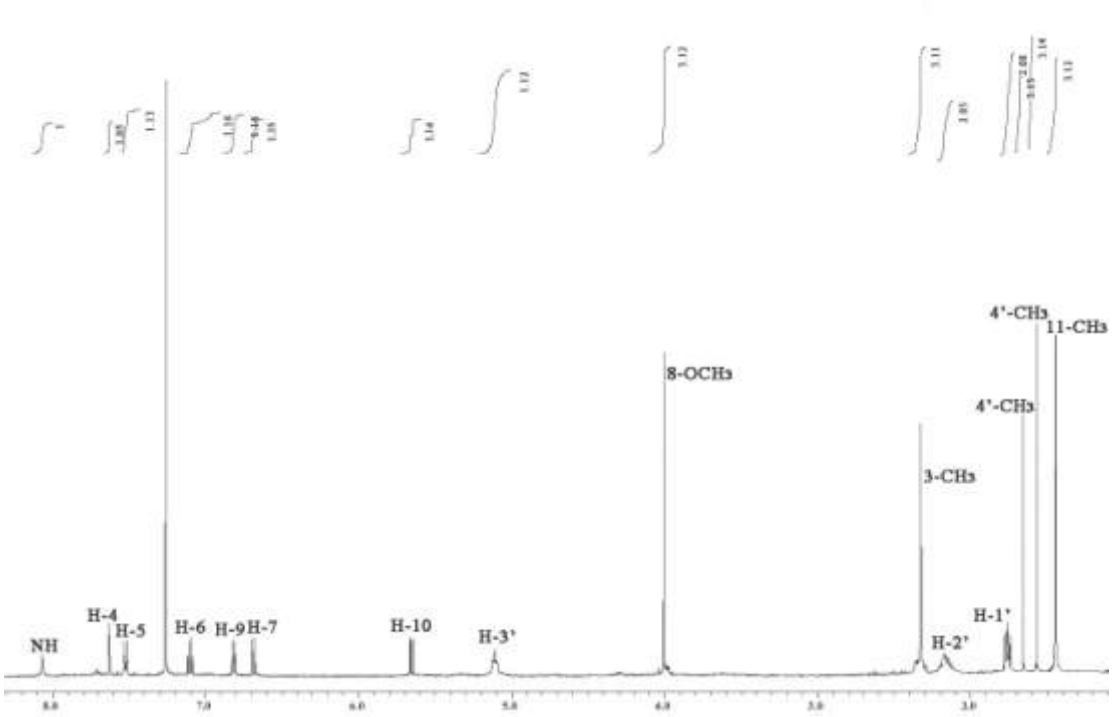


Figure 4.2.3.2g. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Murrayamine-B **(Mk18)**

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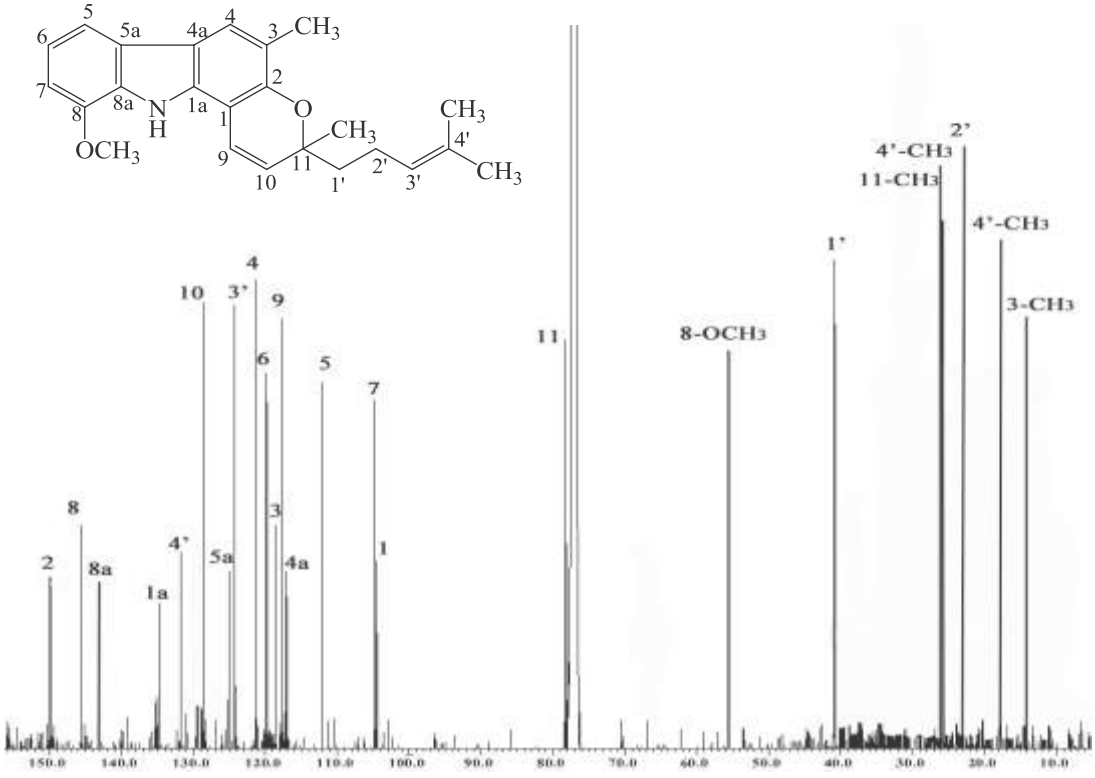


Figure 4.2.3.2h. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Murrayamine-B **(Mk18)**

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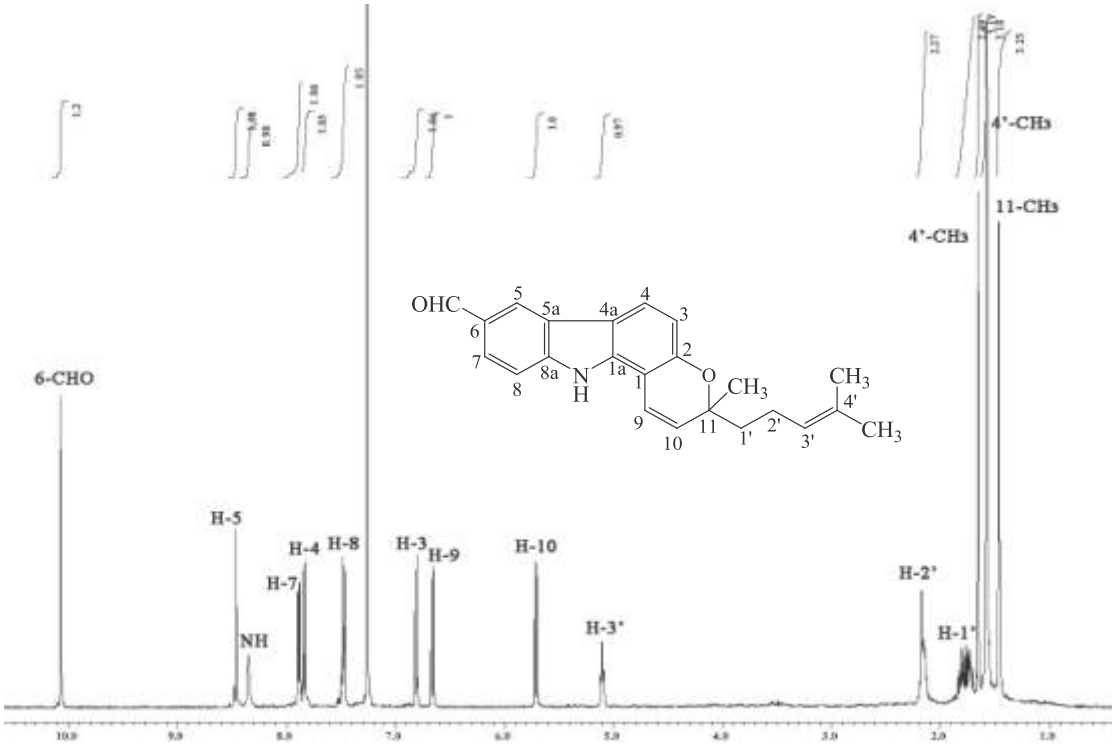


Figure 4.2.3.2i. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Murrayamine-J **(Mk19)**

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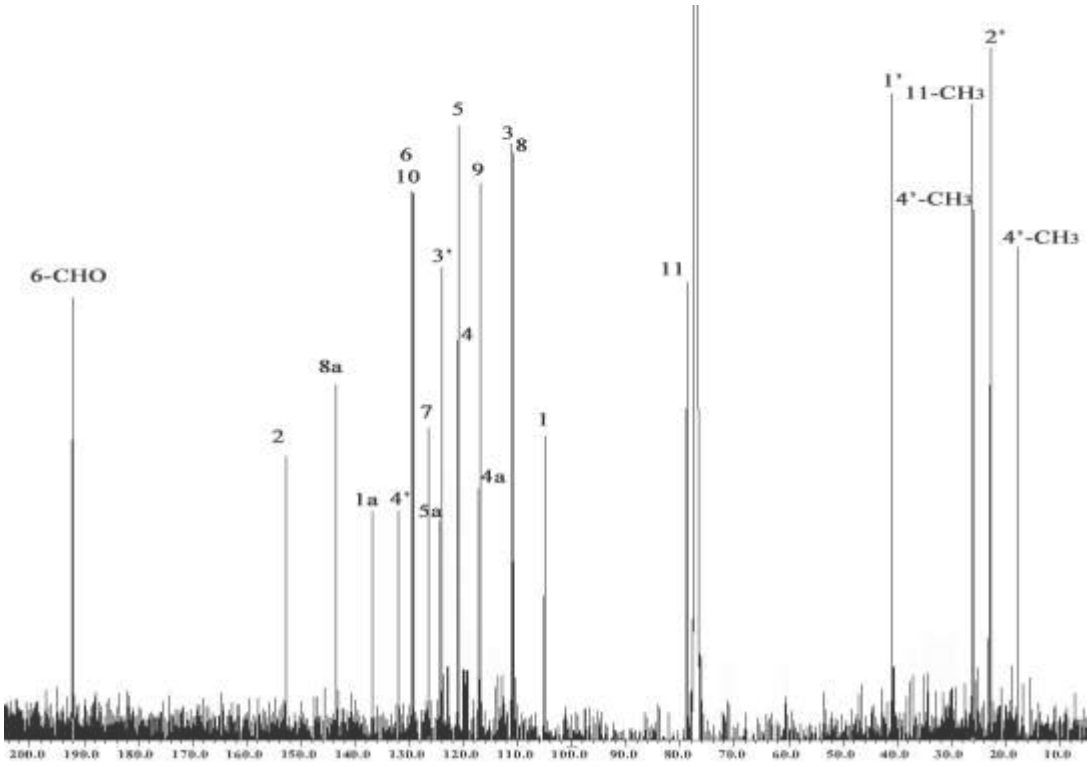
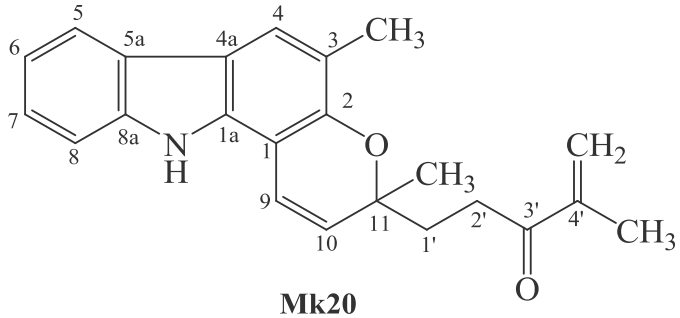


Figure 4.2.3.2j. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Murrayamine-J **(Mk19)**

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4.2.3.3 Murrastinine-A (Mk20)



Mk20

Mk20 was a new compound isolated as pale yellow oil with $[\alpha]_D^{25}$ +11.5 (CH_3OH; c 0.02). Figure 4.2.3.3a to 4.2.3.3l showed the preliminary spectral data of **Mk20**. The IR spectrum of **Mk20** (Figure 4.2.3.3k) indicated the presence of NH (3371 cm^{-1}), C=O (1638 cm^{-1}) and C–O stretching (1019 cm^{-1}). Its molecular formula was deduced as $C_{23}H_{23}NO_2$ with a molecular ion peak at *m/z* 346.1789 ($[M+H]^+$, calcd. mass: 346.1807) (Figure 4.2.3.3l). Its UV spectrum showed characteristic absorbance for pyranocarbazole moiety with λ_{max} 238, 249, 273, 286 and 329 nm which was similar to those of mahanimbine **(Mk15)**. It was readily recognized as pyranocarbazole derivative from its preliminary spectral data (Cordell, 2008).

The ^1H NMR spectrum ($CDCl_3$, 500 MHz) of **Mk20** was similar to those of **Mk15** (Table 4.2.3.3 and Figure 4.2.3.3a), except for the presence of a ketone (C=O) with a ethylenic proton (-CH_2) at C-3' and C-4', respectively, assignable to –$CH_2CH_2COC(CH_2)CH_3$ side chain instead of a prenyl group (–$CH_2CH_2CH=C(CH_3)_2$) side chain at the pyran ring. These were confirmed by the presence of long range coupling between a pair of doublets of 4'-CH_2 [δ 5.71 (*d*, *J* = 1.7 Hz) and δ 5.93 (*d*, *J* = 1.7 Hz)] and a carbonyl carbon signal at C-3' (δ 202.0). The ^1H NMR spectrum of

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Mk20 showed a general similarity with those of **Mk15** such as a broad downfield signal at δ 7.92 was assignable to the presence of NH in the carbazole nucleus, signals at δ 7.91 (*d*, *J* = 8.0 Hz, H-5), δ 7.17 (*td*, *J* = 8.0, 1.2 Hz, H-6), δ 7.31 (*td*, *J* = 8.0, 1.2 Hz, H-7) and δ 7.38 (*d*, *J* = 8.0 Hz, H-8) were deduced for an unsubstituted carbazole ring-C of **Mk20**, and two olefinic protons at δ 6.68 (*d*, *J* = 9.8, H-9) and δ 5.61 (*d*, *J* = 9.8, H-10) due to a pyran ring annulated to a carbazole moiety gave further evidence for the characteristic of pyranocarbazole skeleton. Finally, the remaining ^{1}H NMR spectrum revealed signals for an aromatic methyl at δ 2.32 (*s*, 3-CH_3), a methyl group at δ 1.45 (*s*, 11-CH_3) and a oxidized prenyl group adjacent to an oxygen at C-11 with signals at δ 2.09 (*m*, H-1'α), δ 2.11 (*m*, H-1'β), δ 2.86 (*m*, H-2'α) and δ 2.98 (*m*, H-2'β), a pair of doublets of 4'-CH_2 [δ 5.71 (*d*, *J* = 1.7 Hz) and δ 5.93 (*d*, *J* = 1.7 Hz)] and δ 1.86 (*s*, 4'-CH_3).

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The ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) indicated the presence of 23 carbons resonances (Table 4.2.3.3 and Figure 4.2.3.3b). There are nine quaternary carbons: δ 104.0 (C-1), δ 134.9 (C-1a), δ 149.7 (C-2), δ 118.2 (C-3), δ 116.9 (C-4a), δ 123.9 (C-5a), δ 139.5 (C-8a), δ 78.0 (C-11), δ 144.4 (C-4'); seven methine carbons: δ 121.5 (C-4), δ 119.4 (C-5), δ 119.6 (C-6), δ 124.4 (C-7), δ 110.5 (C-8), δ 118.3 (C-9), δ 127.9 (C-10); three methylene carbons: δ 35.6 (C-1'), δ 32.7 (C-2'), δ 124.9 (4'-CH_2); three methyl carbons: δ 16.2 (3-CH_3), δ 27.8 (11-CH_3), δ 17.8 (4'-CH_3); and a carbonyl signal at δ 202.0 (C-3'). Two ethylenic carbon signals at δ 144.4 and δ 124.9 were assigned to C-4' and 4'-CH_2, respectively.

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The above assignments were further substantiated with the help of DEPT, ^{1}H-^{1}H COSY, HMQC, TOCSY, NOESY and long-range coupling in HMBC. The ^{1}H-^{1}H COSY provided the following correlation: H-5 – H-6 – H-7 – H-8, H-9 – H-10, and H-1' – H-2'. The HMBC experiment confirmed the presence of the carbonyl group at C-3' showed cross peaks with H-2', 4'-CH_2 and 4'-CH_3. Other correlations included: H-9 to C-1, C-1a, C-2, C-10 and C-11; H-10 to C-1, C-11 and 11-CH_3; H-1' to C-11, C-2' and 11-CH_3; and H-2' to C-11, C-1' and C-3'. Several other correlations in aromatic region were similar to those in **Mk15**, which confirmed the attribution of all the ^{13}C signals of **Mk20**, especially gave evidence to confirm that a carbonyl group at C-3' was connected to C-2' and C-4'. In the NOESY experiment, cross peaks at H-10/11-CH_3, H-10/H-1' and H-1'/H-2' at the β-orientation were observed, of which in turn allowed the more deshielded protons at position 1' and 2' to be assigned as H-1'α and H-2'α. By then, further confirmed the relative stereochemistry of **Mk20**.

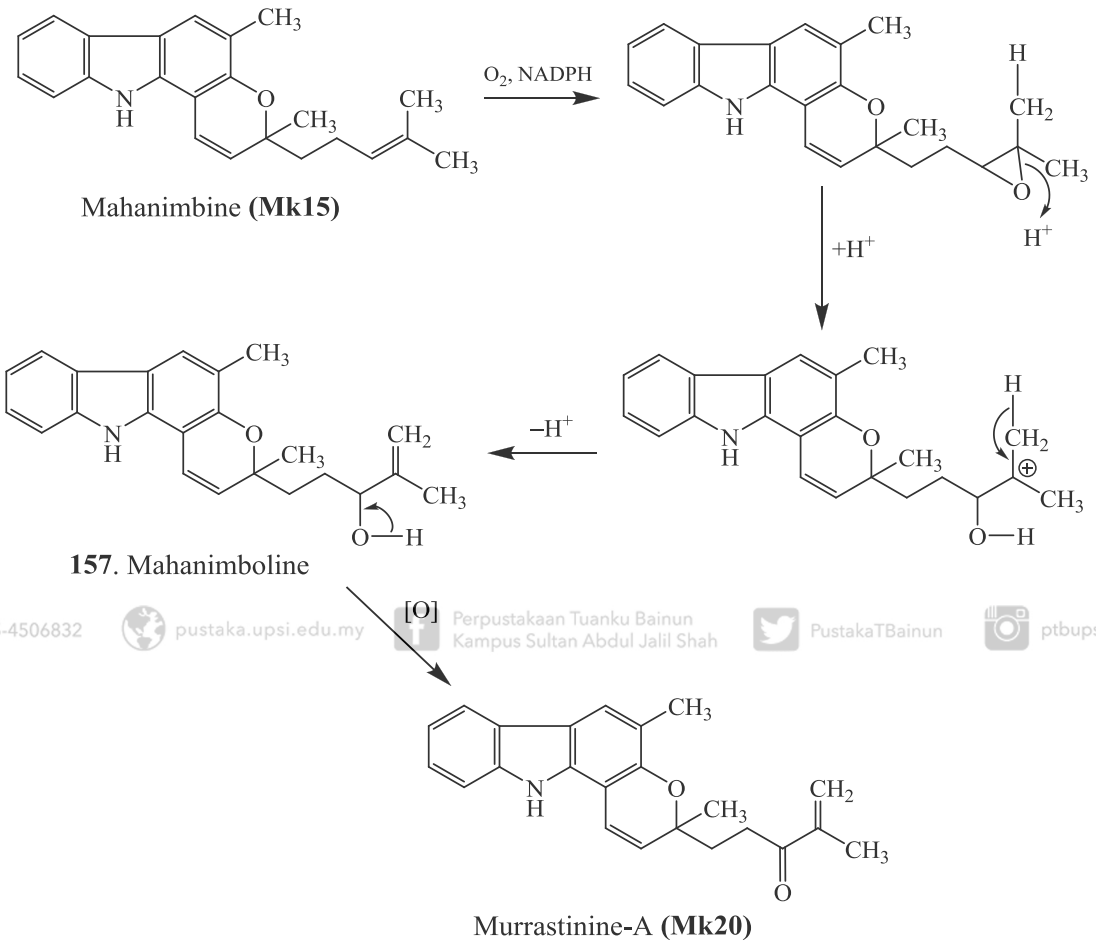
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A possible biogenetic route to **Mk20** is shown in Scheme 4.2.3.3, forming an unsaturated ketone type of precursor, which was detected as a minor carbazole alkaloid in the bark of *M. koenigii*. This was suggested to be a NADPH-induced epoxidation reaction onto an electron-deficient electrophilic double bond at C-3', led to the formation of an epoxide moiety and the disappearance of the double bond at C-3'. A new bond is formed after that at C-4' when protonation of epoxide oxygen took place. This was then followed by oxidation reaction on the hydroxyl group (the protonated epoxide) at C-3' for the formation of carbonyl group which showed with the presence of deshielded carbon signal at δ 202.0 in the ^{13}C NMR.

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This compound was determined as 5-(3,5-dimethyl-3,11-dihydropyrano[3,2-a]carbazol-3-yl)-2-methylpent-1-en-3-one, and also named as murrastinine-A **(Mk20)**. This is first time obtained naturally from the bark of *M. koenigii*.



Scheme 4.2.3.3. Proposed Biogenetic Pathway to **Mk20**

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Table 4.2.3.3

1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of Murrastinine-A ***(Mk20)****

Position	δ ^{13}C	DEPT	δ ^{1}H	HMBC correlations	COSY
1	104.0	qC		H-9, H-10	
1a	134.9	qC		H-4, H-9	
2	149.7	qC		H-4, H-9, 3-CH_3	
3	118.2	qC		3-CH_3	
4	121.5	CH	7.67 (*s*)	3-CH_3	
4a	116.9	qC			
5	119.4	CH	7.91 (*d*, 8.0 Hz)	H-7	H-6
5a	123.9	qC		H-6, H-8	
6	119.6	CH	7.17 (*td*, 8.0, 1.2 Hz)	H-5, H-7, H-8	H-5, H-7
7	124.4	CH	7.31 (*td*, 8.0, 1.2 Hz)	H-5, H-6, H-8	H-6, H-8
8	110.5	CH	7.38 (*d*, 8.0 Hz)	H-6, H-7	H-7
8a	139.5	qC		H-5, H-7, H-8	
9	118.3	CH	6.68 (*d*, 9.8 Hz)	H-10	H-10
10	127.9	CH	5.61 (*d*, 9.8 Hz)	H-9	H-9
11	78.0	qC		H-9, H-10, H-1', H-2', 11-CH_3	
N-H			7.92 (*br s*)		
3-CH_3	16.2	CH_3	2.32 (*s*)	H-4	
11-CH_3	27.8	CH_3	1.45 (*s*)	H-10, H-1'	
1'α	35.6	CH_2	2.09 (*m*)	H-2', 11-CH_3	H-2'
1'β			2.11 (*m*)		
2'α	32.7	CH_2	2.86 (*m*)	H-1'	H-1'
2'β			2.98 (*m*)		
3'	202.0	qC		H-2', 4'-CH_2, 4'-CH_3	
4'	144.4	qC		4'-CH_2, 4'-CH_3	
4'-CH_2	124.9	CH_2	5.71 (*d*, 1.7 Hz) 5.93 (*d*, 1.7 Hz)	4'-CH_3	4'-CH_2
4'-CH_3	17.8	CH_3	1.86 (*s*)	4'-CH_2	

Note. *Solvent used was $CDCl_3$; assignments based on DEPT, COSY, NOESY, TOCSY, HMQC and HMBC.

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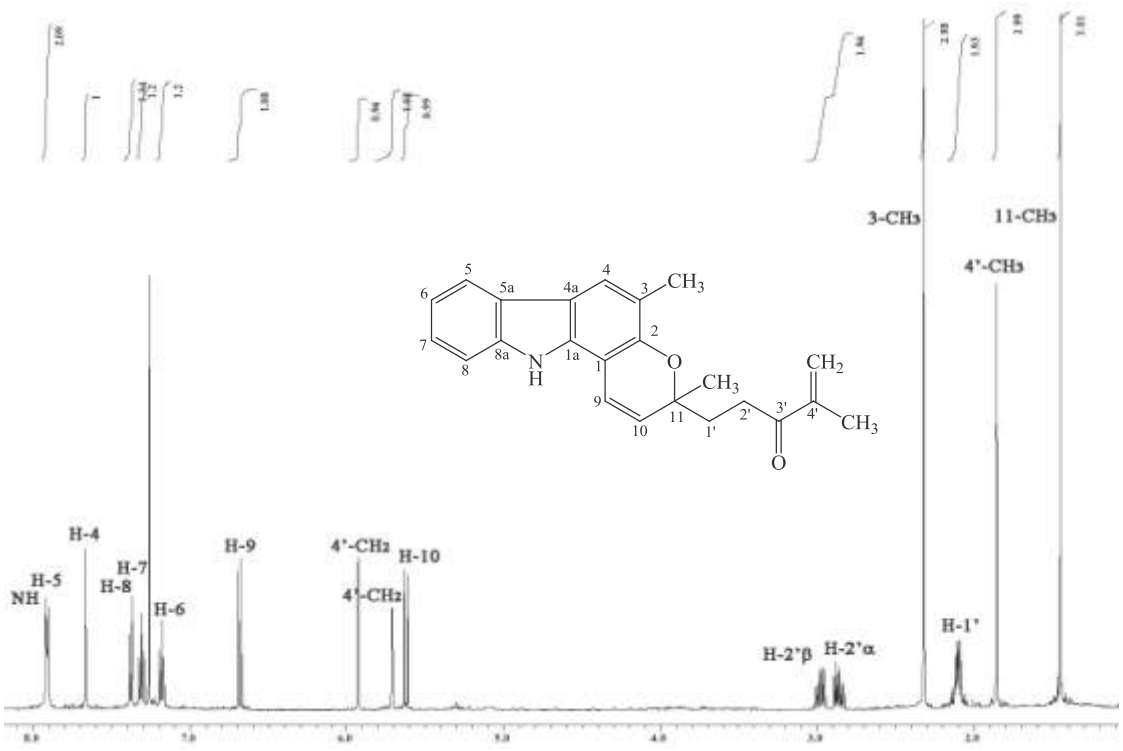


Figure 4.2.3.3a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Murrastinine-A **(Mk20)**

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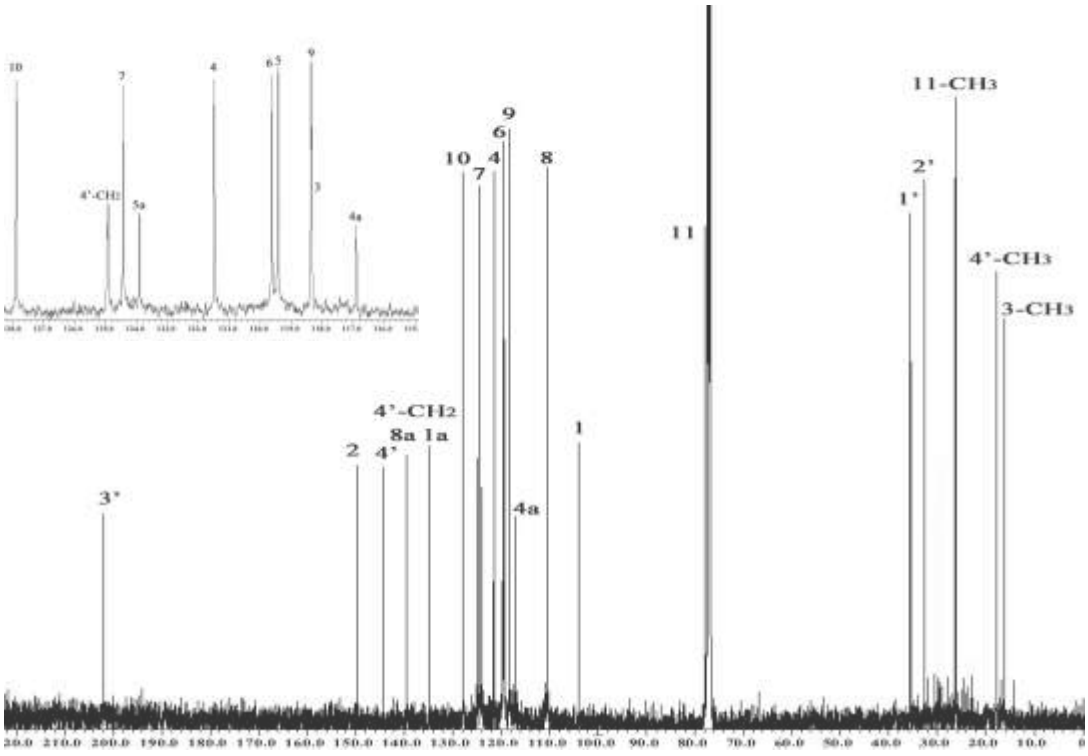


Figure 4.2.3.3b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Murrastinine-A **(Mk20)**

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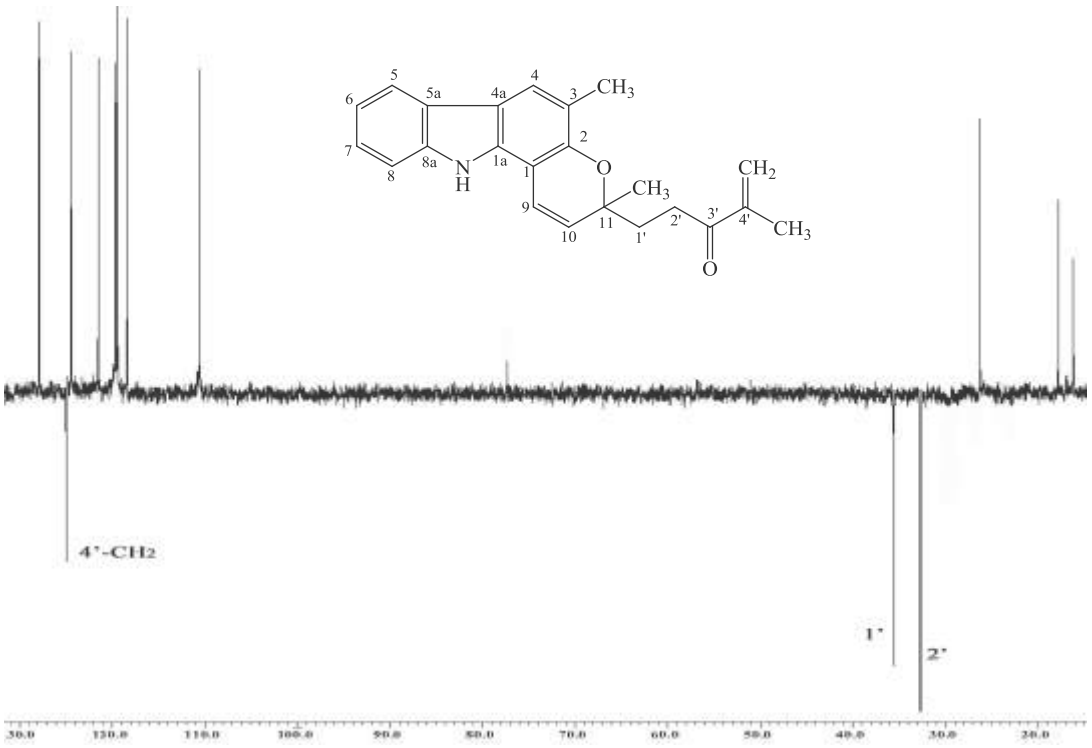


Figure 4.2.3.3c. DEPT-135 Spectrum ($CDCl_3$, 125 MHz) of Murrastinine-A **(Mk20)**

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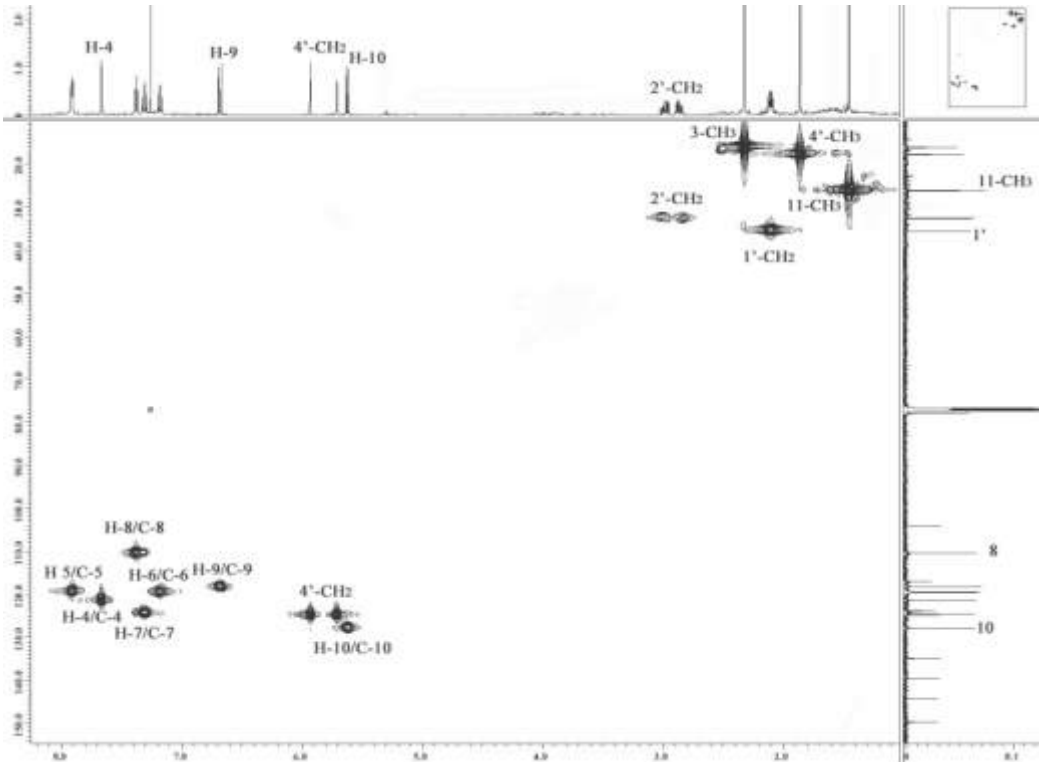


Figure 4.2.3.3d. HMQC Spectrum ($CDCl_3$, 500 MHz) of Murrastinine-A **(Mk20)**

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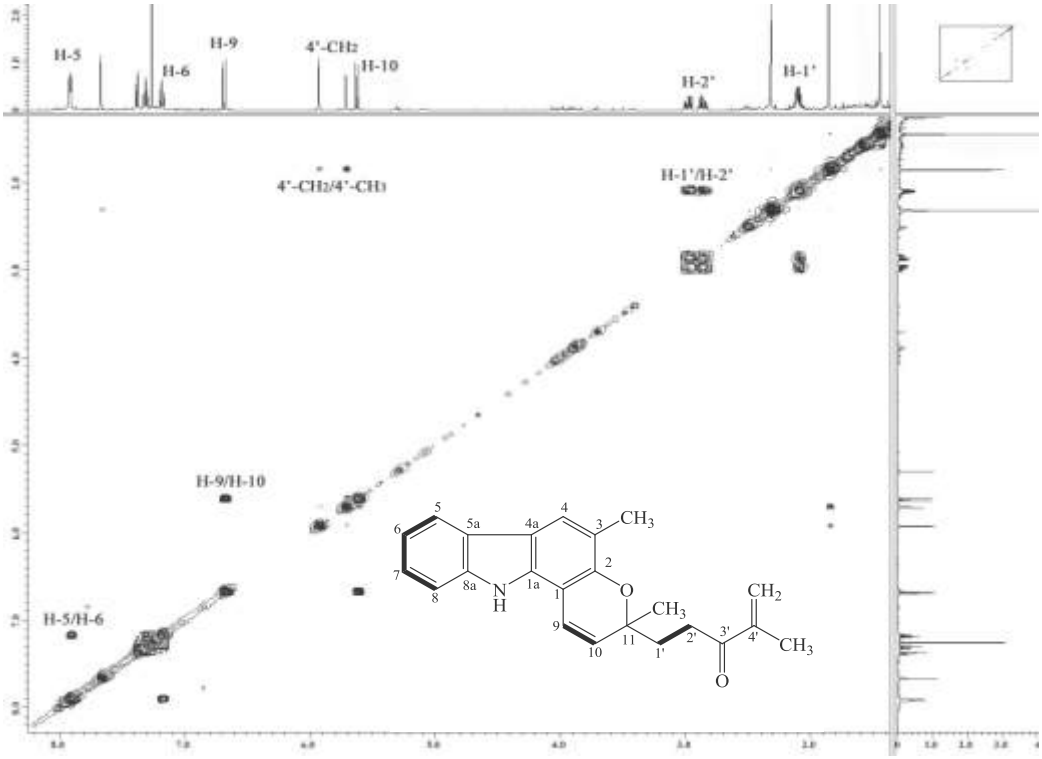


Figure 4.2.3.3e. ^{1}H-^{1}H COSY Spectrum ($CDCl_3$, 500 MHz) of Murrastinine-A **(Mk20)**

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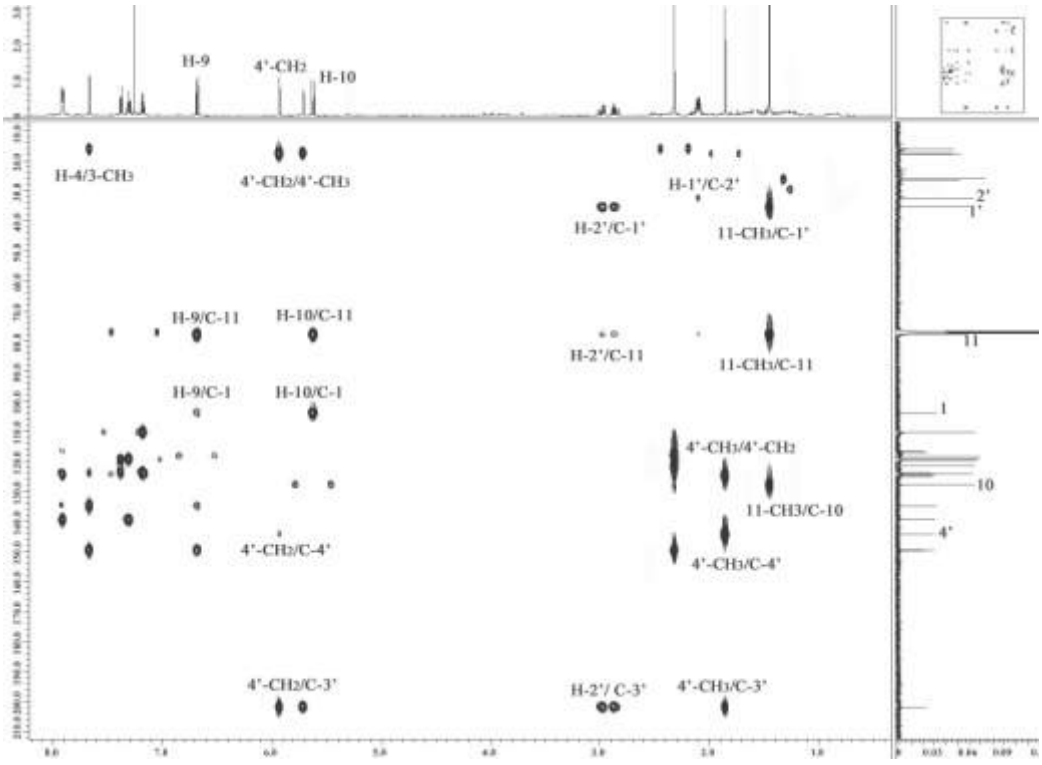


Figure 4.2.3.3f. HMBC Spectrum ($CDCl_3$, 500 MHz) of Murrastinine-A **(Mk20)**

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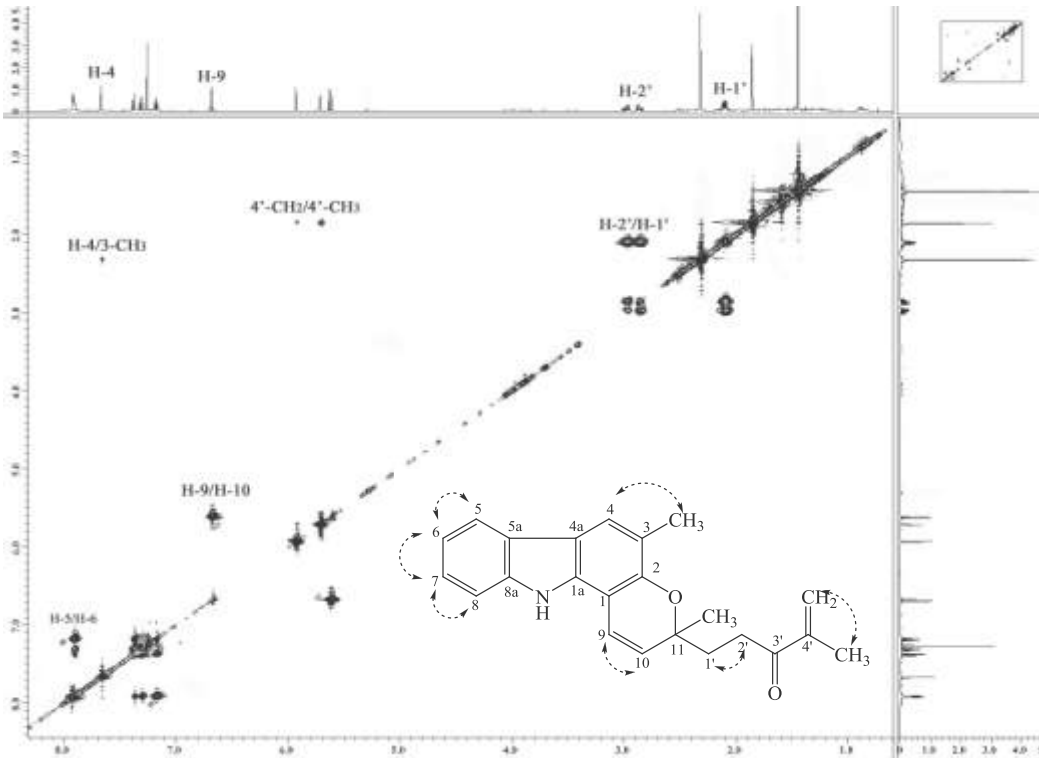


Figure 4.2.3.3g. TOCSY Spectrum ($CDCl_3$, 500 MHz) of Murrastinine-A **(Mk20)**

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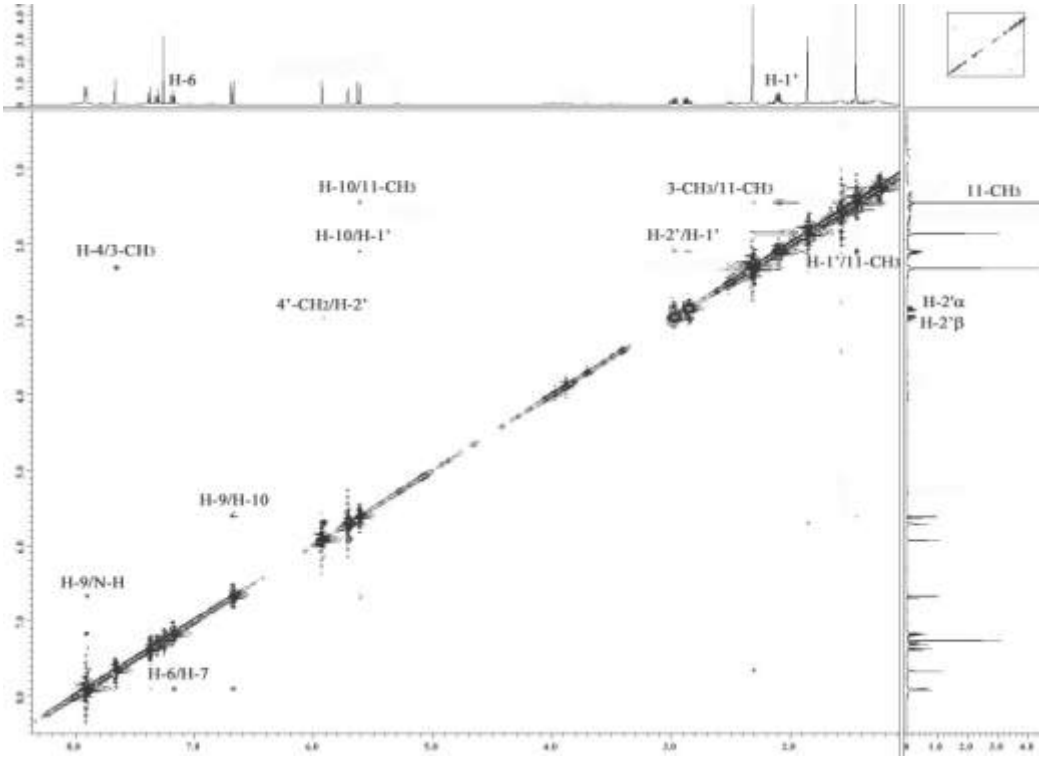


Figure 4.2.3.3h. NOESY Spectrum ($CDCl_3$, 500 MHz) of Murrastinine-A **(Mk20)**

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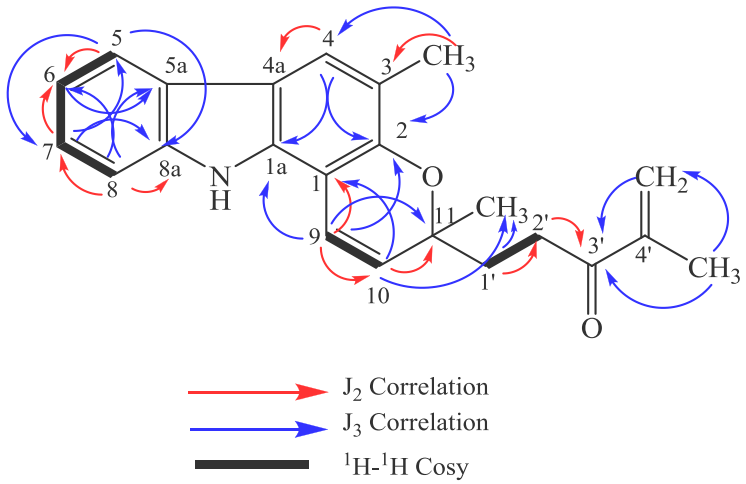


Figure 4.2.3.3i. Selected ^{1}H-^{1}H COSY and HMBCs of **Mk20**

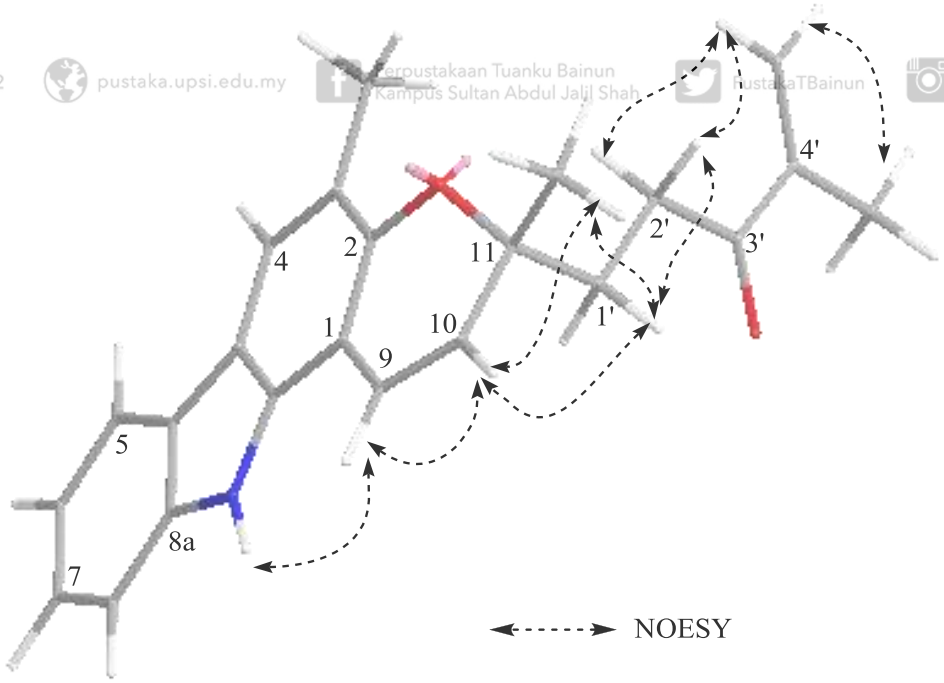


Figure 4.2.3.3j. Selected NOESY Correlations and Relative Stereochemistry for **Mk20**

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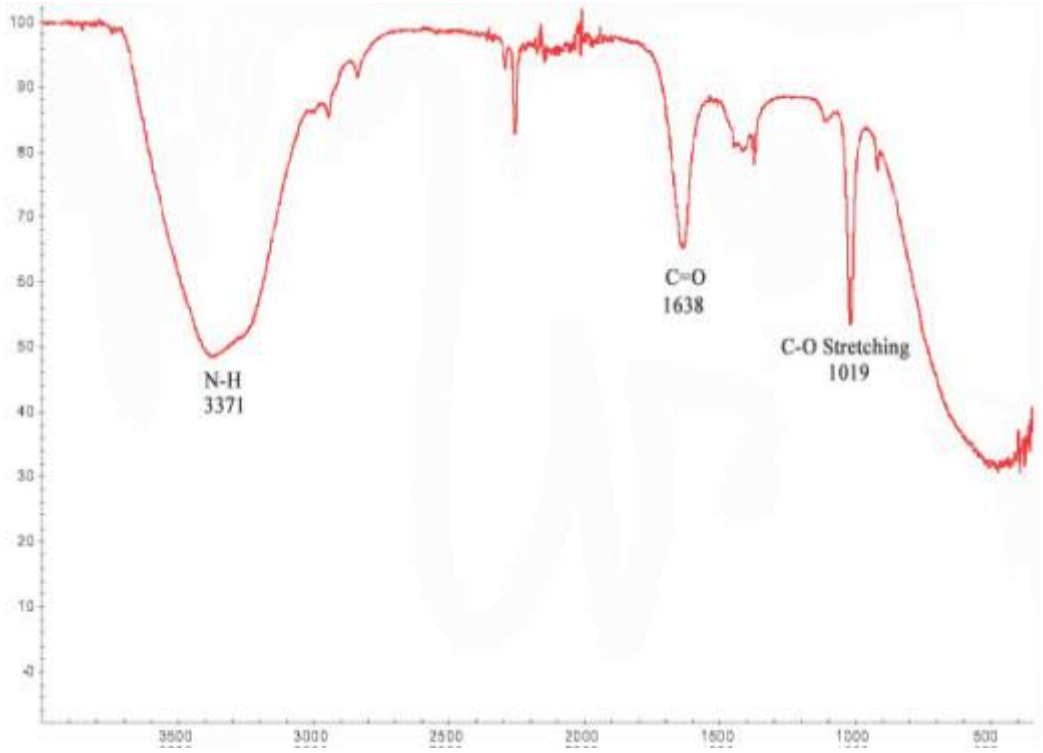


Figure 4.2.3.3k. IR Spectrum of Murrastinine-A **(Mk20)**

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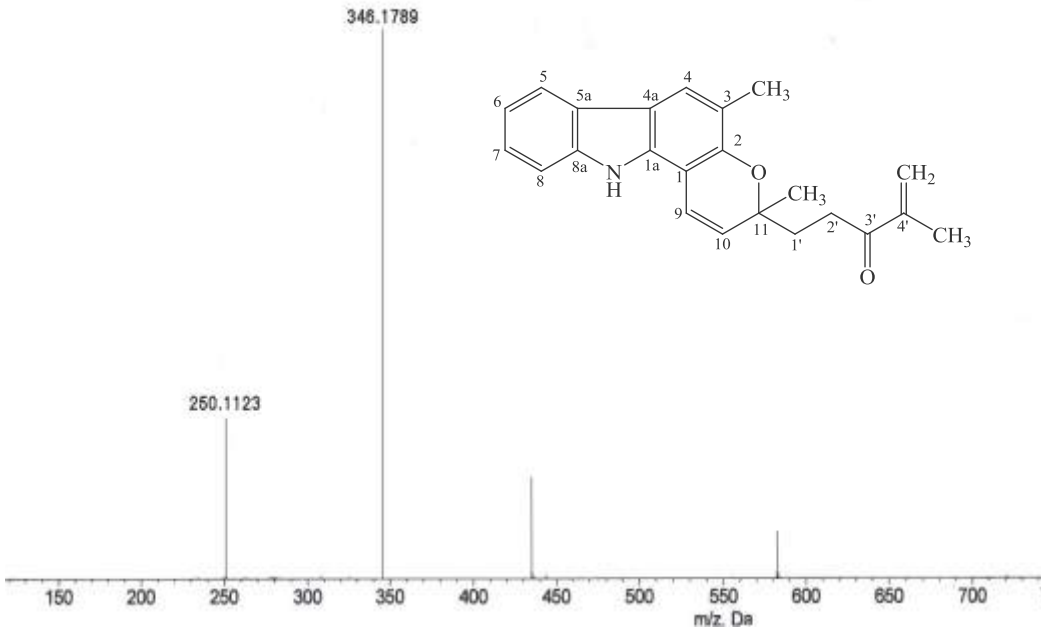
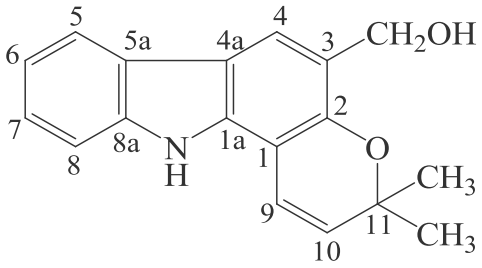


Figure 4.2.3.3l. Mass Spectrum of Murrastinine-A **(Mk20)**

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4.2.3.4 Murrastinine-B (Mk21)



Mk21

Another new compound, **Mk21**, was obtained from the bark of *M. koenigii* as brown amorphous. The preliminary spectral data of **Mk21** (Figure 4.2.3.4a to Figure 4.2.3.4i) was identical to those in **Mk12**, except for the presence of a hydroxymethyl group instead of methyl group at position 3 of the carbazole nucleus. The IR spectrum (Figure 4.2.3.4h) indicated the presence of NH and OH group (3372 cm^{-1}) as well as a C–O group (1020 cm^{-1}). The UV spectrum of **Mk21**, with λ_{max} 238, 273, 285 and 338 nm, resembled to those of pentacyclic pyranocarbazole framework (Cordell, 2008). Its HRESIMS (Figure 4.2.3.4i) with a molecular ion peak at *m/z* 279.1583 ($[M]^+$, calcd. mass: 279.1259) was consistent with molecular formula of $C_{18}H_{17}NO_2$. Despite some similarities, there are however significant differences in the mass with 16 molecular mass units more than **Mk12** (refer section 4.2.3.1) further revealed that **Mk21** contained an oxygen atom.

The ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of **Mk21** (Table 4.2.3.4 and Figure 4.2.3.4a) showed a typical downfield singlet signal at δ 4.16 (*s*) further indicated the presence of a CH_2OH group and a broad singlet at δ 7.90 (*br s*) assignable to the presence of a NH group in the carbazole nucleus. Similarly to **Mk12**, the ^{1}H NMR spectrum of **Mk21** also showed that the molecule contained a singlet signal for H-4 at

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δ 7.82, four mutually coupled aromatic protons with long-range coupling at δ 7.89 (*d*, *J* = 8.0 Hz, H-5), δ 7.15 (*td*, *J* = 8.0, 1.1 Hz, H-6), δ 7.29 (*td*, *J* = 8.0, 1.1 Hz, H-7) and δ 7.37 (*d*, *J* = 8.0 Hz, H-8) were assigned for the unsubstituted ring-C of the carbazole, and two doublets at δ 6.62 (*J* = 9.8 Hz, H-9) and δ 5.66 (*J* = 9.8 Hz, H-10) for the presence of the pyran ring double bond. The remaining signals, included one overlapping methyl signal at δ 1.37 (6H, *s*, 2 × 11-CH_3) adjacent to a oxygen functionality represented a *gem*-dimethyl pyranone ring fused to the ring-A of carbazole.

The ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) showed the presence of 18 carbons resonances (Table 4.2.3.4 and Figure 4.2.3.4b). Among the 18 carbons, the ^{13}C data indicated the presence of eight quaternary carbons: δ 104.6 (C-1), δ 135.0 (C-1a), δ 150.1 (C-2), δ 124.2 (C-3), δ 116.7 (C-4a), δ 122.4 (C-5a), δ 139.6 (C-8a) and δ 76.0 (C-11); seven methines: δ 122.3 (C-4), δ 119.4 (C-5), δ 119.5 (C-6), δ 124.2 (C-7), δ 110.4 (C-8), δ 117.4 (C-9) and δ 129.5 (C-10); a methylene signal at δ 65.1 (3-CH_2OH); and one overlapping signal at δ 27.5 for the *gem*-dimethyl group attached at C-11.

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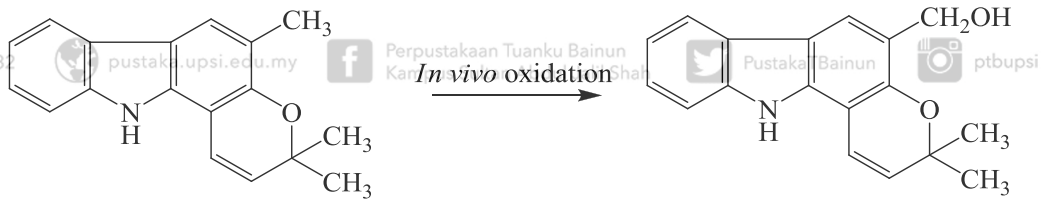
The complete assignments of proton and carbon signals and location of substituent on **Mk21** were also supported by the experiments of DEPT, ^1H-^1H COSY , HMQC and HMBC (Figure 4.2.3.4c to Figure 4.2.3.4g). The ^1H-^1H COSY (Figure 4.2.3.4e) showed correlation for the following fragments: C-5 – C-6 – C-7 – C-8 and C-9 – C-10. The HMBC experiments (Figure 4.2.3.4f) confirmed the presence of cross peaks for H-4 to C-1a, C-2 and C-3; H-5 to C-4a and C-8a; H-6 to C-7 and C-8;

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H-7 to C-6 and C-8a; H-8 to C-5; H-9 to C-1, C-1a, C-2 and C-11; H-10 to C-1, C-11 and 11-CH_3; 11-CH_3 to C-10 and C-11; and 3-CH_2OH to C-2, C-3 and C-4. These correlations were observed to confirm the attribution of all proton and carbon signals of **Mk21**, especially gave evidence to confirm the CH_2OH group was connected to C-3 of carbazole nucleus. The possible biogenetic pathway was suggested to be an *in vivo* oxidation reaction on the methyl group of an girinimbine **(Mk12)**.

Based on the above analysis, the structure of **Mk21** was suggested to be (3,3-dimethyl-3,11-dihydropyrano[3,2-a]carbazol-5-yl)methanol and assigned as murrastinine-B, which is first time isolated from the bark of *M. koenigii*.



Girinimbine **(Mk12)** Murrastinine-B **(Mk21)**

Scheme 4.2.3.4. Proposed Biogenetic Pathway to **Mk21**

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Table 4.2.3.4

1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of Murrastinine-B ***(Mk21)****

Position	δ ^{13}C	DEPT	δ ^{1}H	HMBC correlations	COSY
1	104.6	qC		H-9. H-10	
1a	135.0	qC		H-4, H-9	
2	150.1	qC		H-4, H-9. 3-CH_2OH	
3	124.4	qC			
4	122.3	CH	7.82 (*s*)	3-CH_2OH	
4a	116.7	qC		H-4, H-5	
5	119.4	CH	7.89 (*d*, 8.0 Hz)		H-6
5a	122.4	qC			
6	119.5	CH	7.15 (*td*, 8.0, 1.1 Hz)	H-7, H-8	H-5, H-7
7	124.2	CH	7.29 (*td*, 8.0, 1.1 Hz)	H-6. H-8	H-6, H-8
8	110.4	CH	7.37 (*d*, 8.0 Hz)	H-6	H-7
8a	139.6	qC		H-5, H-7	
9	117.4	CH	6.62 (*d*, 9.8 Hz)		H-10
10	129.5	CH	5.66 (*d*, 9.8 Hz)	11-CH_3	H-9
11	76.0	qC		H-9, H-10, 11-CH_3	
N-H			7.90 (*br s*)		
3-CH_2OH	65.1	CH_2	4.16 (*s*)		
11-CH_3	27.5	CH_3	1.37 (*s*)	H-10, 11-CH_3	
11-CH_3	27.5	CH_3	1.37 (*s*)	H-10, 11-CH_3	

Note. *Solvent used was $CDCl_3$; assignments based on DEPT, COSY, HMQC and HMBC.

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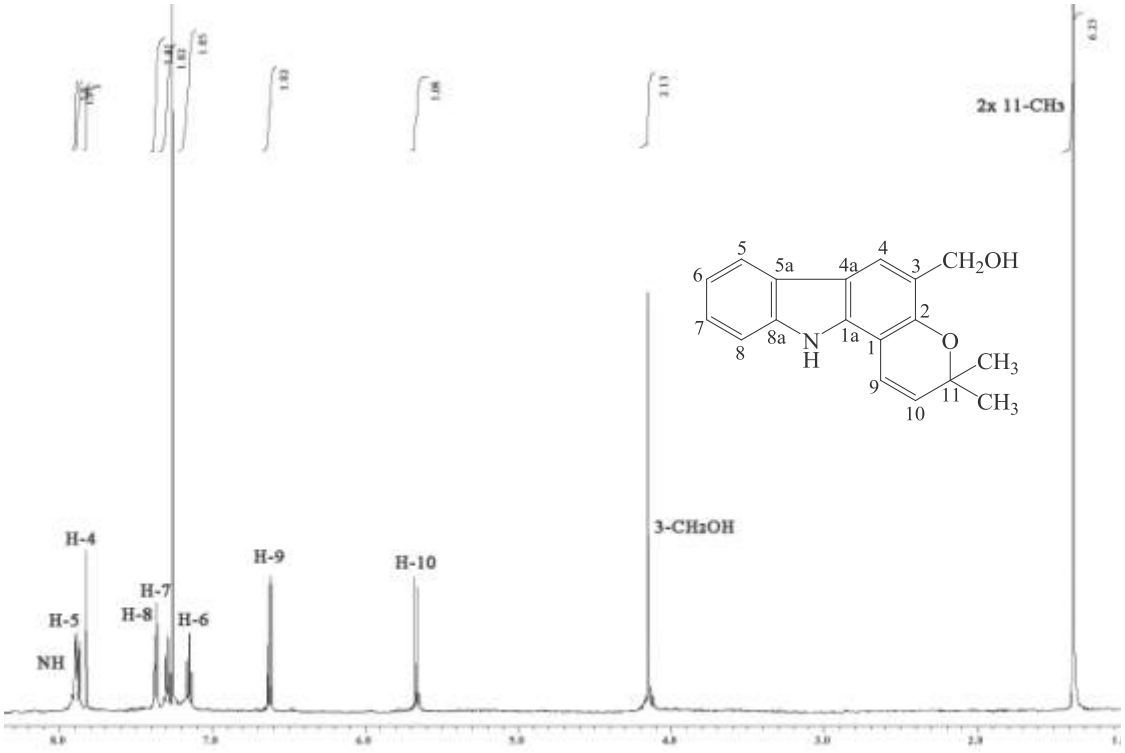


Figure 4.2.3.4a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Murrastinine-B **(Mk21)**

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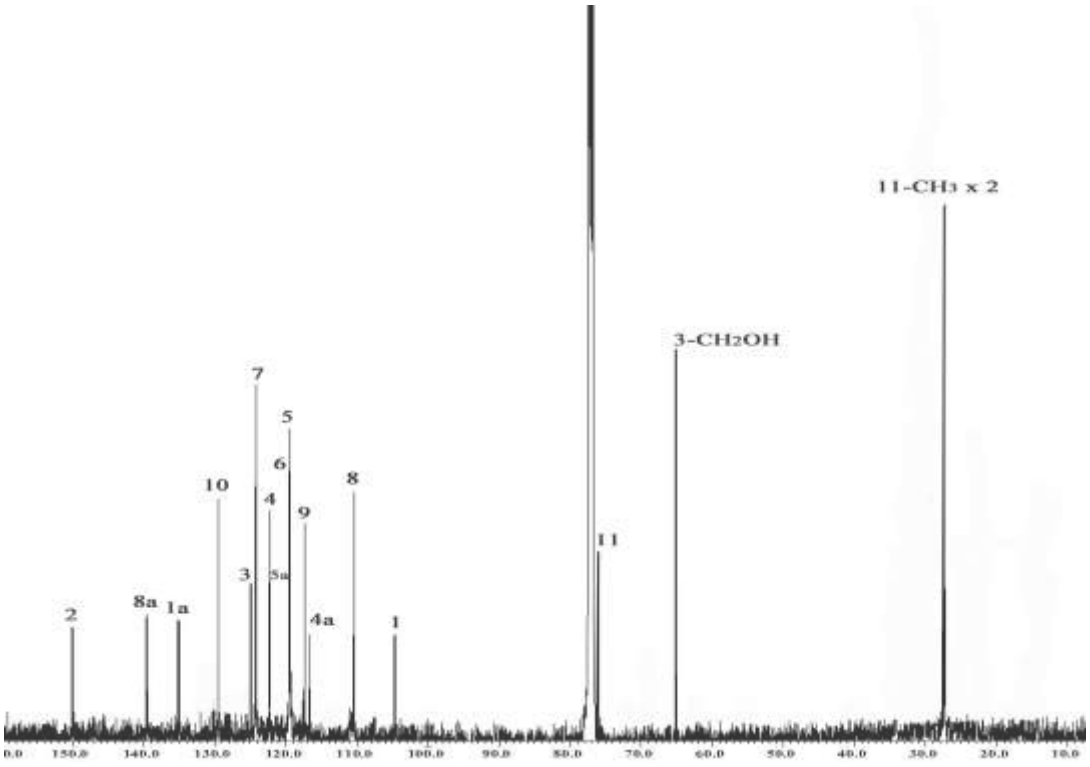


Figure 4.2.3.4b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Murrastinine-B **(Mk21)**

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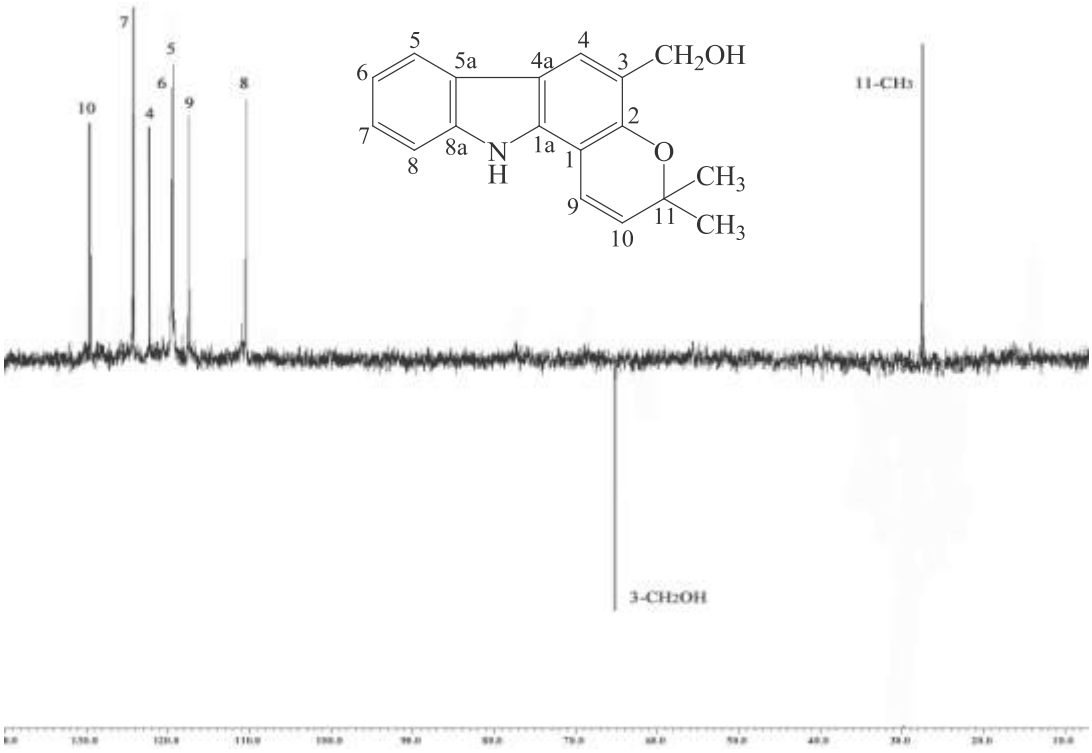


Figure 4.2.3.4c. DEPT-135 Spectrum ($CDCl_3$, 125 MHz) of Murrastinine-B **(Mk21)**

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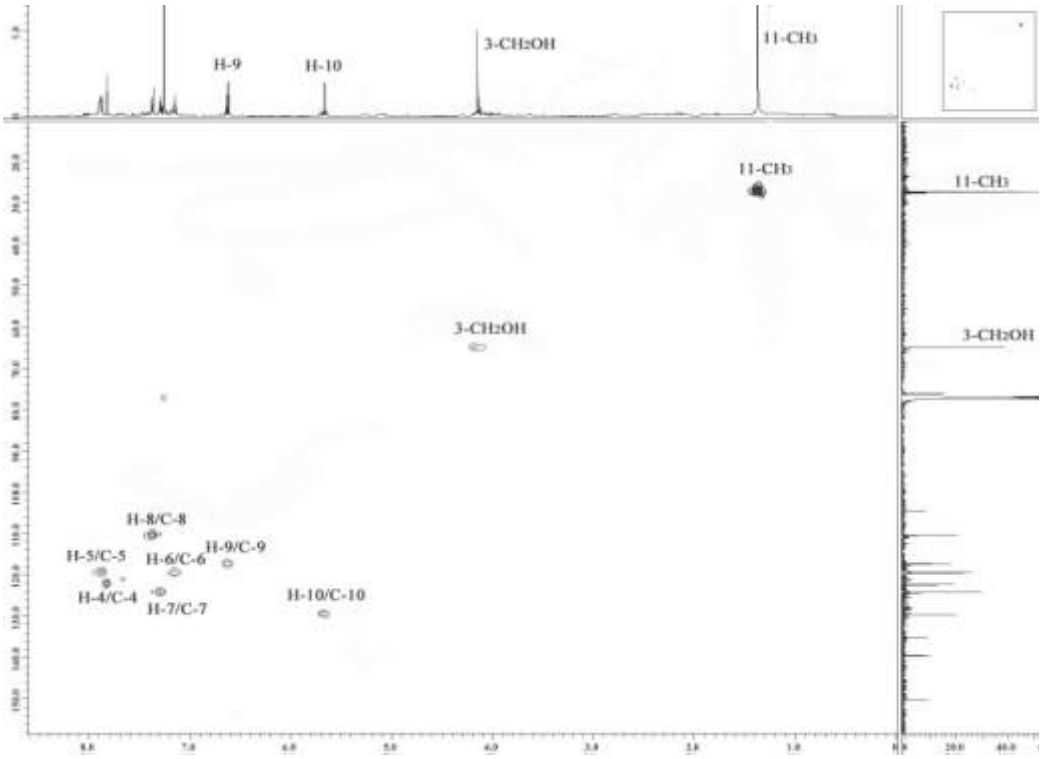


Figure 4.2.3.4d. HMQC Spectrum ($CDCl_3$, 500 MHz) of Murrastinine-B **(Mk21)**

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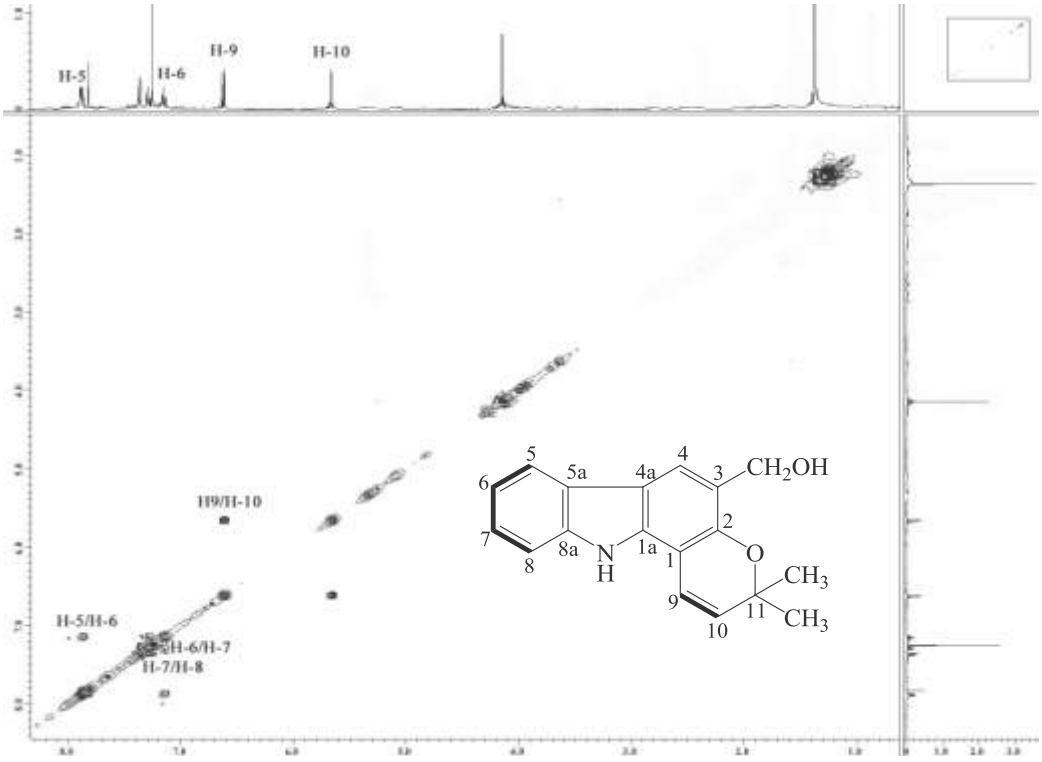


Figure 4.2.3.4e. ^{1}H-^{1}H COSY Spectrum ($CDCl_3$, 500 MHz) of Murrastinine-B **(Mk21)**

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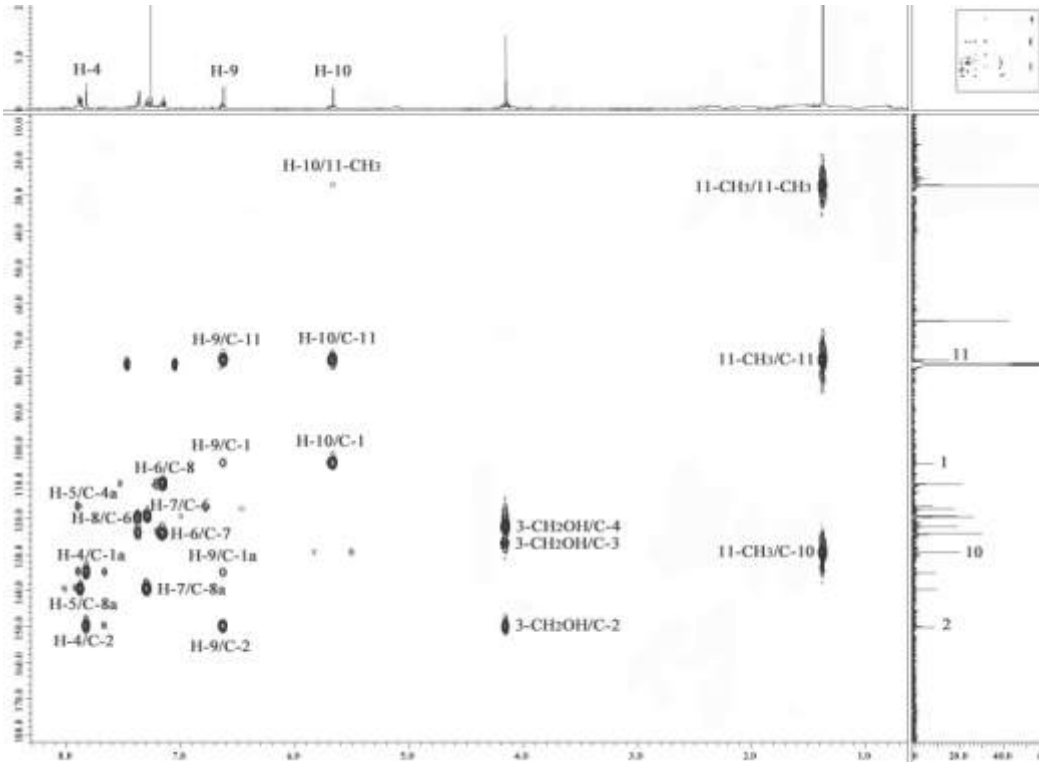


Figure 4.2.3.4f. HMBC Spectrum ($CDCl_3$, 500 MHz) of Murrastinine-B **(Mk21)**

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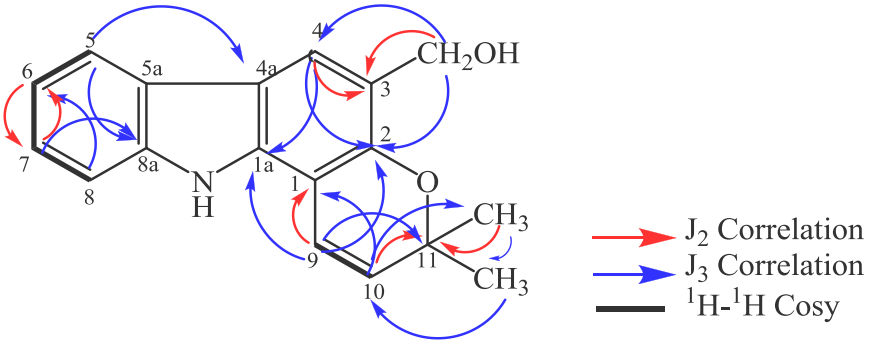


Figure 4.2.3.4g. Selected ^{1}H-^{1}H COSY and HMBCs of **Mk21**

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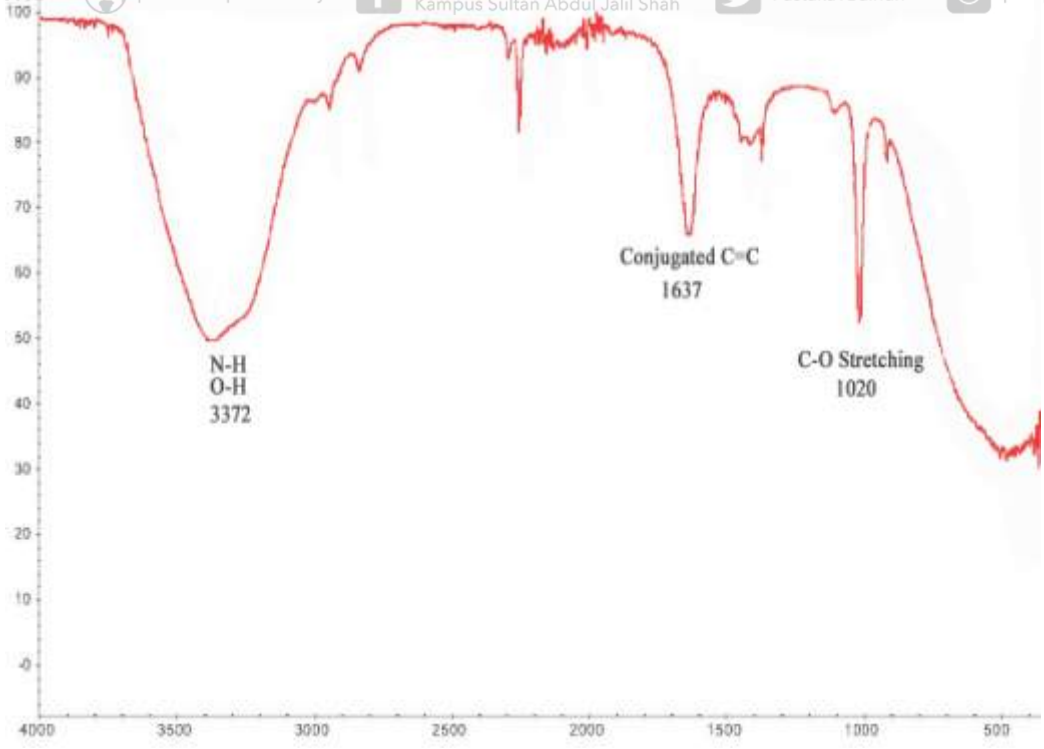


Figure 4.2.3.4h. IR Spectrum of Murrastinine-B **(Mk21)**

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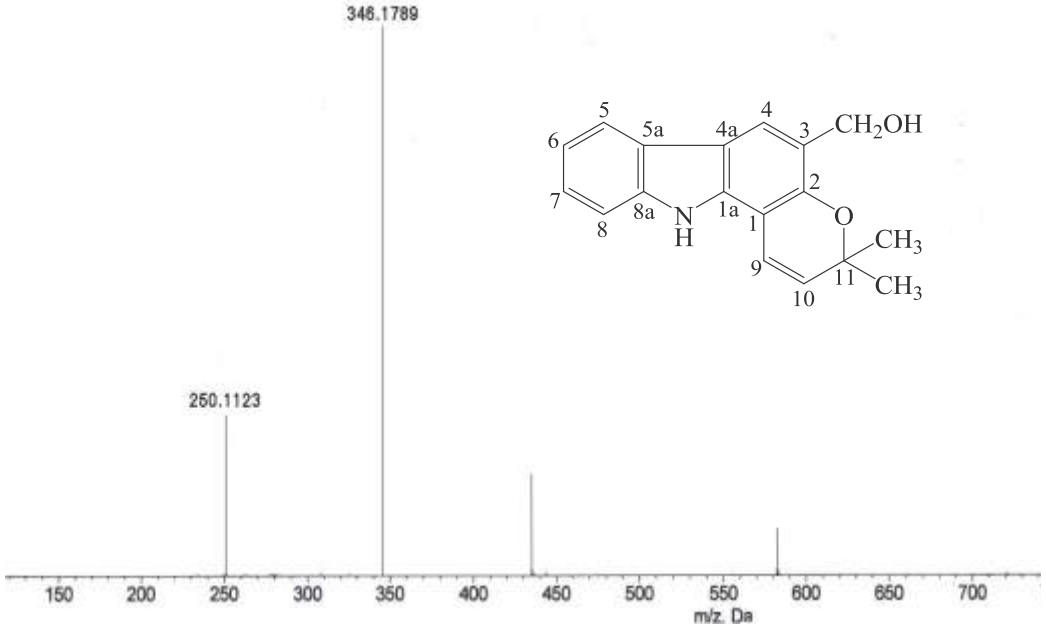


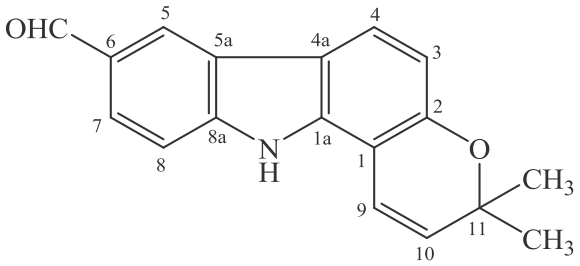
Figure 4.2.3.4i. Mass Spectrum of Murrastinine-B **(Mk21)**

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4.2.3.5 Murrastinine-C (Mk22)



Mk22

Mk22 was obtained as pale yellow oil. The preliminary spectroscopic data of **Mk22** (Figure 4.2.3.5a to 4.2.3.5i) was identical to those in murrayamine-J **(Mk19)**, except for the absence of prenyl group at C-11. The IR spectrum (Figure 4.2.3.5h) of **Mk22** indicated the presence of NH (3366 cm^{-1}), C–O (1020 cm^{-1}) as well as a C=O group (1638 cm^{-1}) in its molecule. **Mk22** was determined to have molecular formula of $C_{18}H_{15}NO_2$ by its HRESIMS (Figure 4.2.3.5i) with a molecular ion peak at *m/z* 278.1174 ($[M+H]^+$, calcd. mass: 278.1181). The UV spectrum of this compound, with λ_{max} 240, 262, 287, 309 nm, has revealed the characteristic absorption of the presence of pyranocarbazole skeleton (Cordell, 2008).

The ^1H NMR spectrum ($CDCl_3$, 500 MHz) of **Mk22** (Table 4.2.3.5 and Figure 4.2.3.5a) allowed several signals could be readily assigned such as a typical downfield signal at δ 10.06 (*s*) further indicated for the presence of a CHO group, a broad singlets at δ 8.50 (*br s*) assignable to the presence of a NH group, an ABX spin pattern was observed at position 5, 7 and 8 with δ 8.45 (*d*, *J* = 1.2 Hz, H-5), δ 7.85 (*dd*, *J* = 8.6, 1.2 Hz, H-7) and δ 7.45 (*d*, *J* = 8.6 Hz, H-8), respectively, and a AB spin system at position 3 and 4 with δ 6.81 (*d*, *J* = 8.6 Hz, H-3) and δ 7.83 (*d*, *J* = 8.6 Hz, H-4),

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respectively. Another AB spin pattern of two doublets at δ 6.63 (*d*, *J* = 9.7 Hz, H-9) and δ 5.73 (*d*, *J* = 9.7 Hz, H-10) in its ^{1}H NMR spectrum gave further evidence for the presence of pyrancarbazole moiety.

The ^{13}C NMR spectrum ($CHCl_3$, 125 MHz) indicated the presence of 18 carbons resonances (Table 4.2.3.5 and Figure 4.2.3.5b) included: eight quaternary carbons at δ 105.3 (C-1), δ 136.8 (C-1a), δ 152.5 (C-2), δ 117.3 (C-4a), δ 124.3 (C-5a), δ 129.4 (C-6), δ 143.5 (C-8a) and δ 76.4 (C-11); seven methine carbons at δ 111.1 (C-3), δ 120.9 (C-4), δ 122.9 (C-5), δ 126.5 (C-7), δ 110.8 (C-8), δ 116.5 (C-9) and δ 130.4 (C-10); one overlapping carbon signal at δ 27.7 for the *gem*-dimethyl group at C-11; and a carbonyl signal at δ 192.2 (6-CHO).

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The above assignments were further substantiated with the help of ^{1}H-^{1}H COSY, HMQC and long-range coupling in HMBC (Figure 4.2.3.5c to Figure 4.2.3.5g). The ^{1}H-^{1}H COSY showed correlation between H-3 – H-4, H-7 – H-8, as well as H-9 – H-10. The HMBC experiment confirmed the presence of the formyl (CHO) group at C-6 with long-range coupling correlations between H-5 and H-7 to 6-CHO. Other cross peaks included H-3, H-4 and H-9 to C-2, and H-3, H-9 and H-10 to C-1. All correlations depicted in its aromatic region were identical to those in murrayamine-J **(Mk19)**, which further confirmed the attribution of all the ^{1}H and ^{13}C signals of **Mk22**.

Mk22 is an angular pyrano-type of carbazole framework and detected as a minor carbazole alkaloid in the bark of *M. koenigii*. Two possible biogenetic route to

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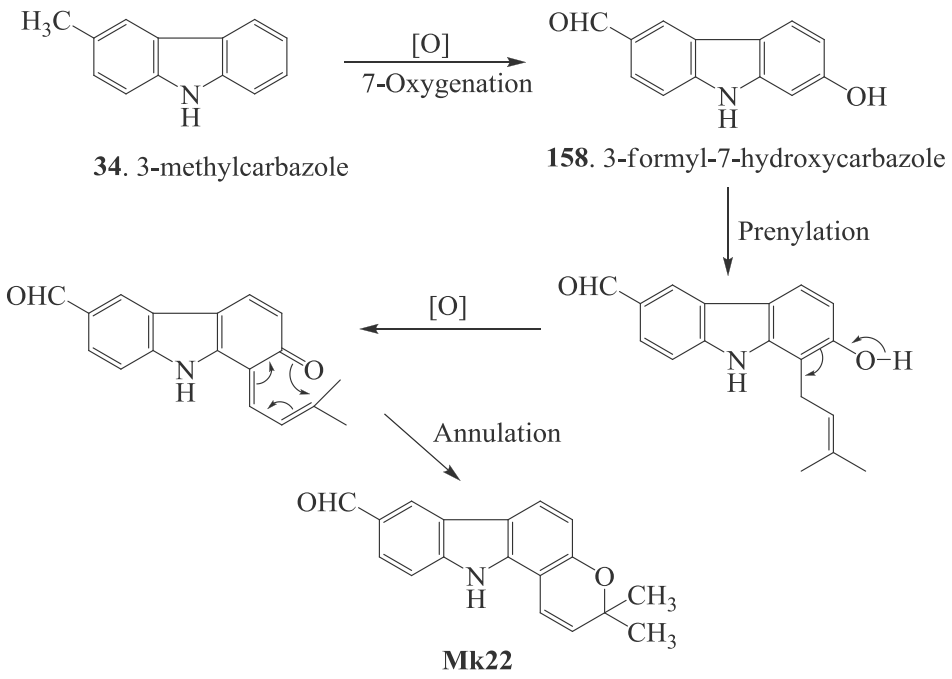
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Mk22 were proposed and showed in Scheme 4.2.3.5. 3-Methylcarbazole **34** is the key precursor for the formation of **Mk22**. An oxidation and 7-oxygenation reaction leads to the formation of 3-formyl-7-hydroxycarbozle **158**. This is then followed by prenylation and cyclization reaction at C-8 and C-9 for the formation of a pyran ring to the carbazole nucleus, or vice versa. Finally, thorough analysis of its spectral data suggested **Mk22** is a new compound and determined as 3,3-dimethyl-3,11-dihydropyrano[3,2-a]carbazole-8-carbaldehyde, and named as murrastinine-C **(Mk22)**.

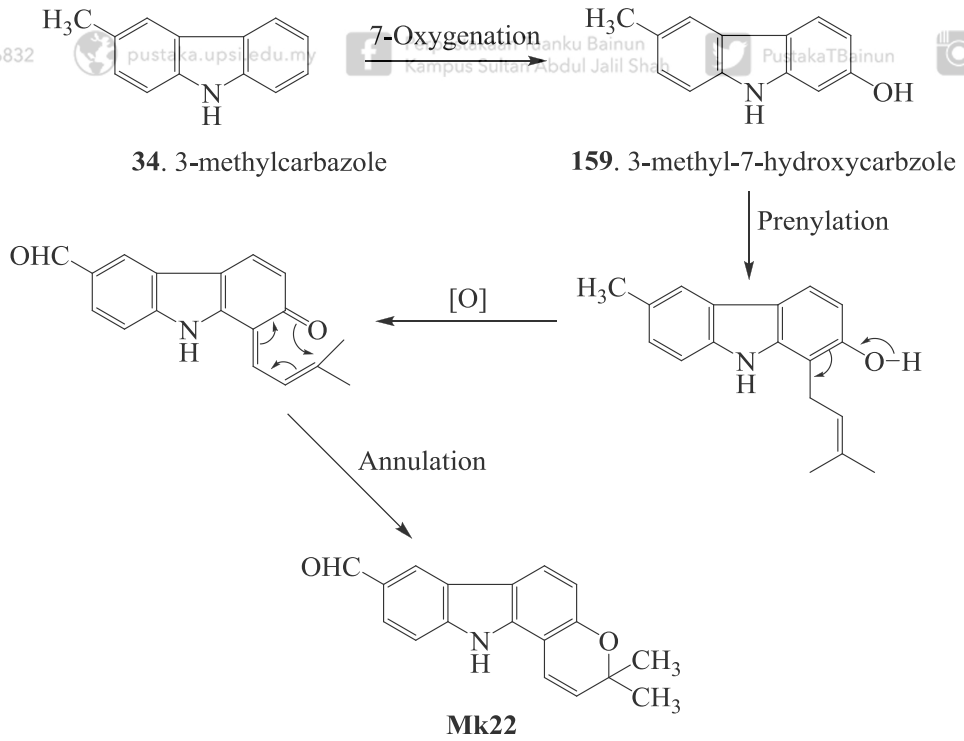
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(a) Possible pathway A



(b) Possible pathway B

Scheme 4.2.3.5. Proposed Biogenetic Pathway to **Mk22**

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Table 4.2.3.5

1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of Murrastinine-C ***(Mk22)****

Position	δ ^{13}C	DEPT	δ ^{1}H	HMBC correlations	COSY
1	105.3	qC		H-3, H-9, H-10	
1a	136.8	qC		H-4, H-9	
2	152.5	qC		H-3, H-4	
3	111.1	CH	6.81 (*d*, 8.6 Hz)	H-4	H-4
4	120.9	CH	7.83 (*d*, 8.6 Hz)	H-3	H-3
4a	117.3	qC		H-3, H-4	
5	122.9	CH	8.45 (*d*, 1.2 Hz)	H-7, 6-CHO	
5a	124.3	qC		H-4, H-5	
6	129.4	qC		H-5, H-8, 6-CHO	
7	126.5	CH	7.85 (*dd*, 8.6, 1.2 Hz)	H-8, 6-CHO	H-8
8	110.8	CH	7.45 (*d*, 8.6 Hz)		H-7
8a	143.5	qC		H-5, H-7	
9	116.5	CH	6.63 (*d*, 9.7 Hz)	H-10	H-10
10	130.4	CH	5.73 (*d*, 9.7 Hz)	H-9, 11-CH_3	H-9
11	76.4	qC		H-9, H-10, 11-CH_3	
N-H			8.50 (*br s*)		
6-CHO	192.2	CHO	10.06 (*s*)	H-5, H-7	
11-CH_3	27.7	CH_3	1.50 (*s*)	H-10	
11-CH_3	27.7	CH_3	1.50 (*s*)	H-10	

Note. *Solvent used was $CDCl_3$; assignments based on DEPT, COSY, HMQC and HMBC.

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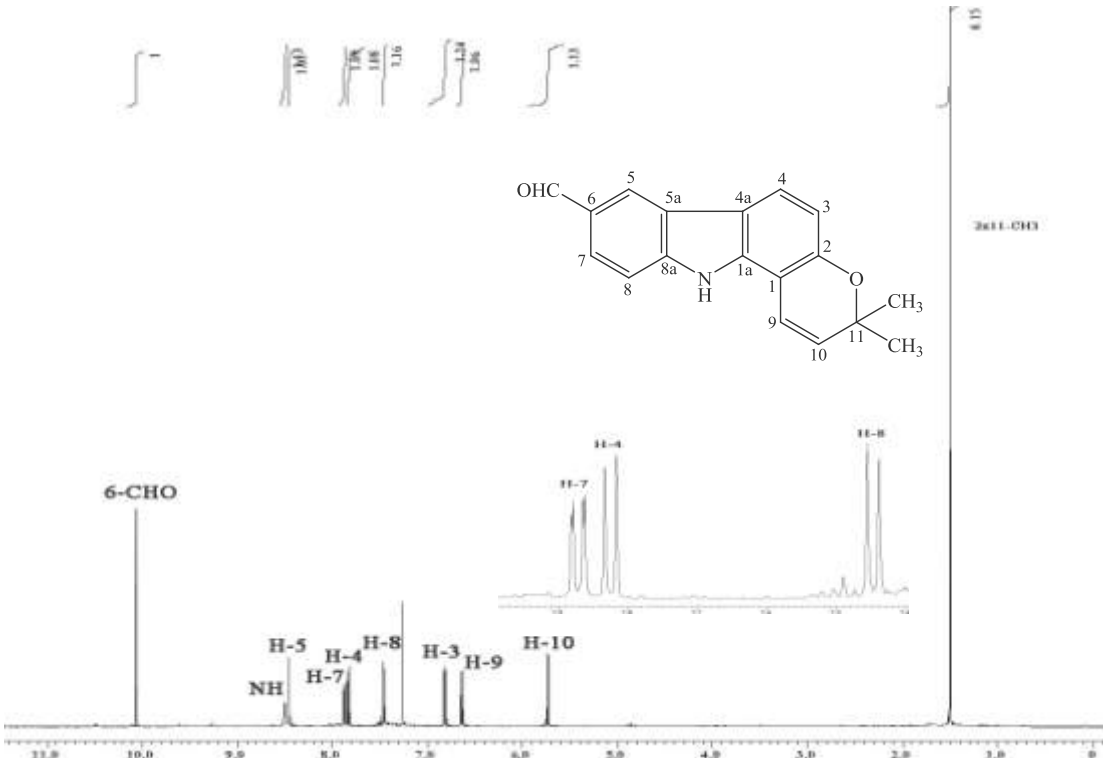


Figure 4.2.3.5a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Murrastinine-C **(Mk22)**

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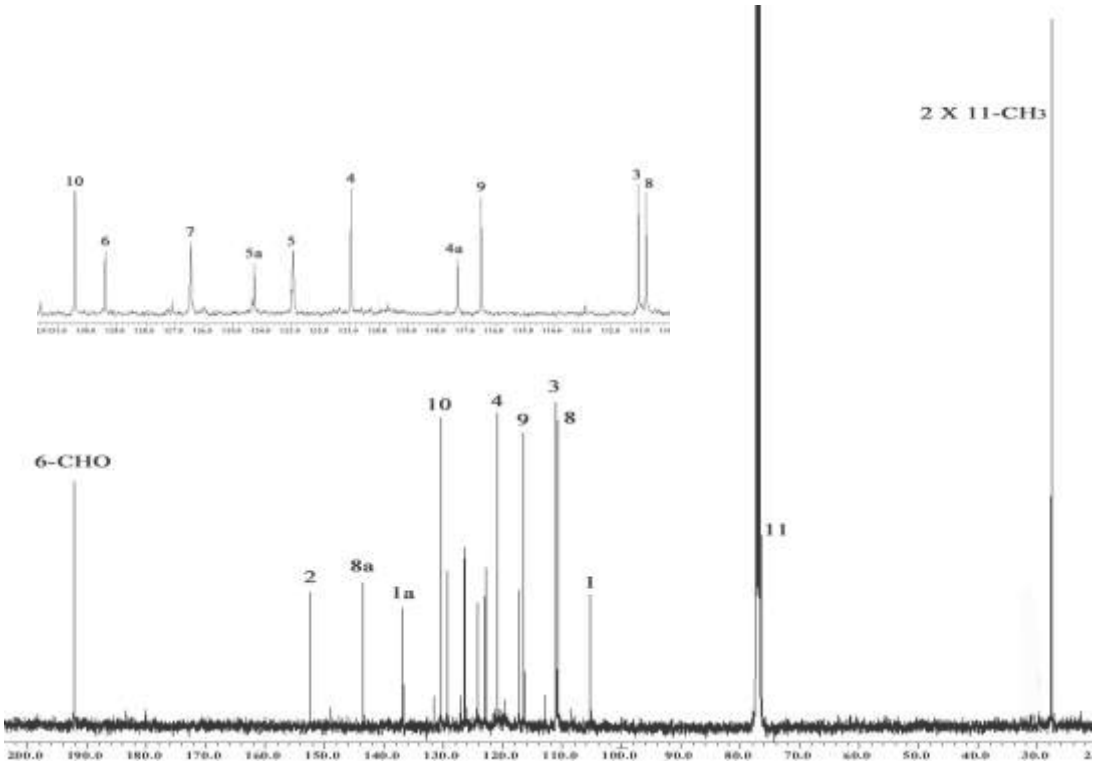


Figure 4.2.3.5b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Murrastinine-C **(Mk22)**

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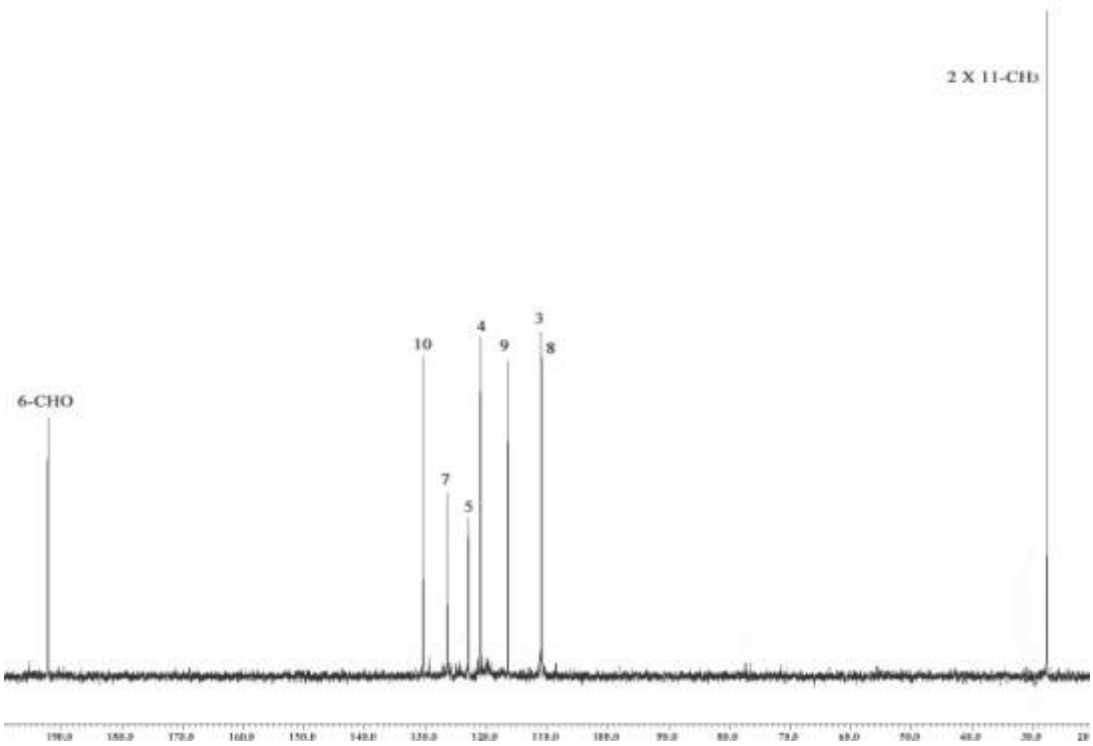


Figure 4.2.3.5c. DEPT-135 Spectrum ($CDCl_3$, 125 MHz) of Murrastinine-C **(Mk22)**

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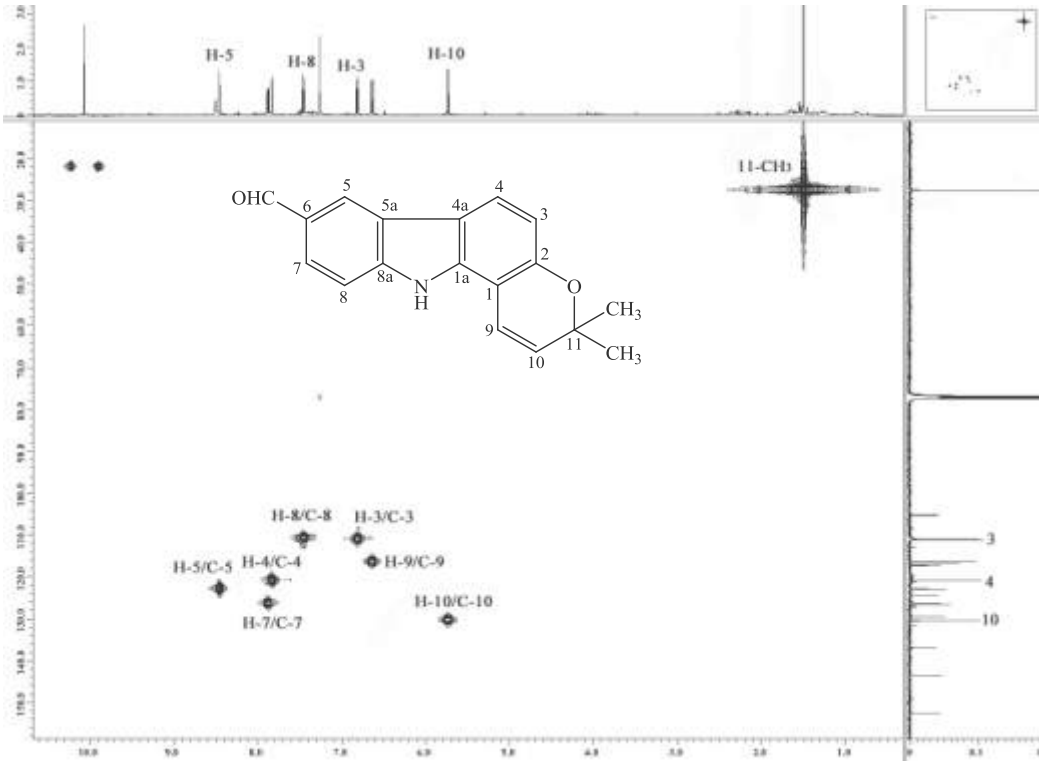


Figure 4.2.3.5d. HMQC Spectrum ($CDCl_3$, 500 MHz) of Murrastinine-C **(Mk22)**

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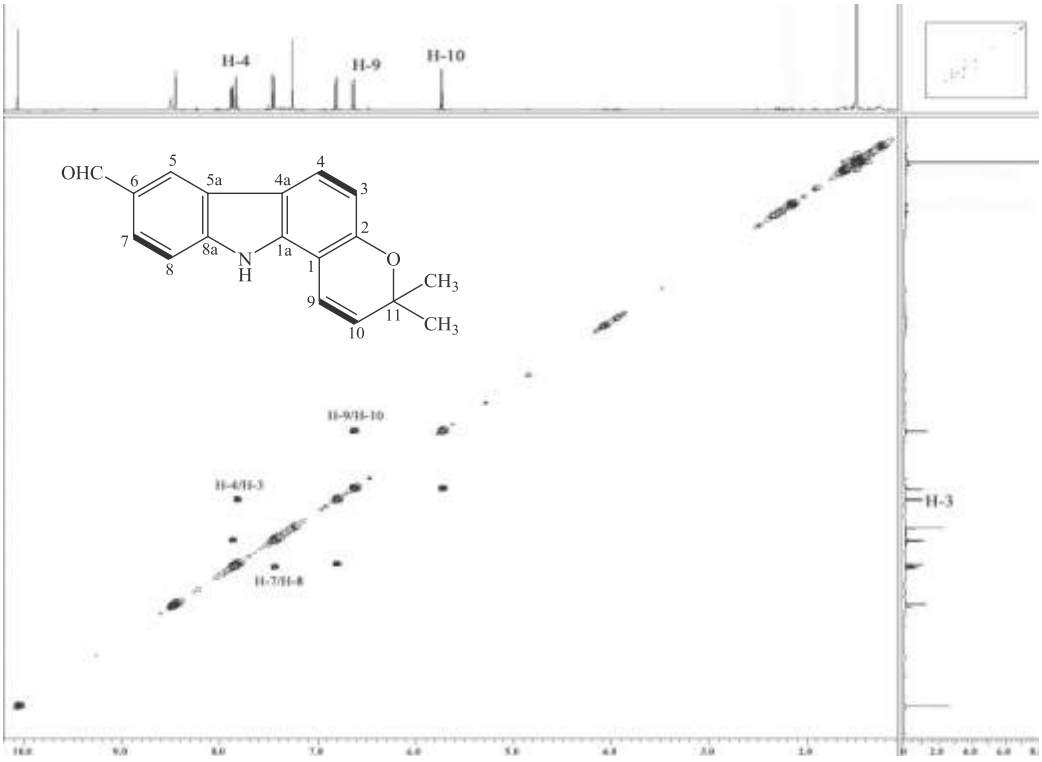


Figure 4.2.3.5e. ^{1}H-^{1}H COSY Spectrum ($CDCl_3$, 500 MHz) of Murrastinine-C **(Mk22)**

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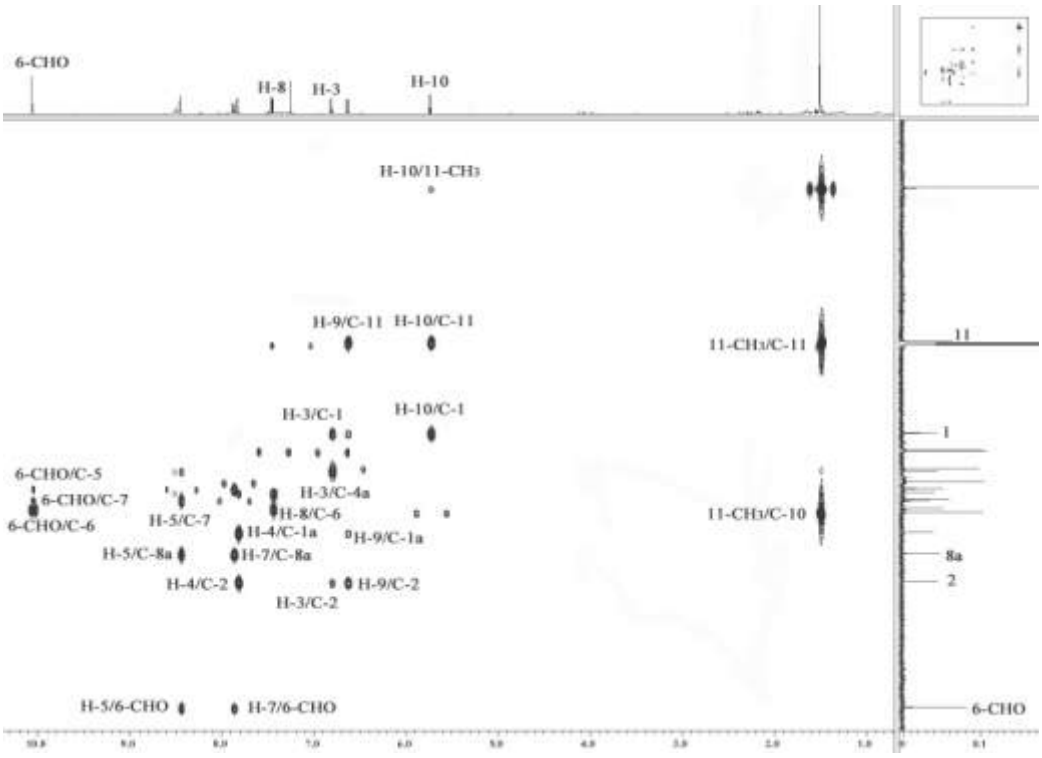


Figure 4.2.3.5f. HMBC Spectrum ($CDCl_3$, 500 MHz) of Murrastinine-B **(Mk22)**

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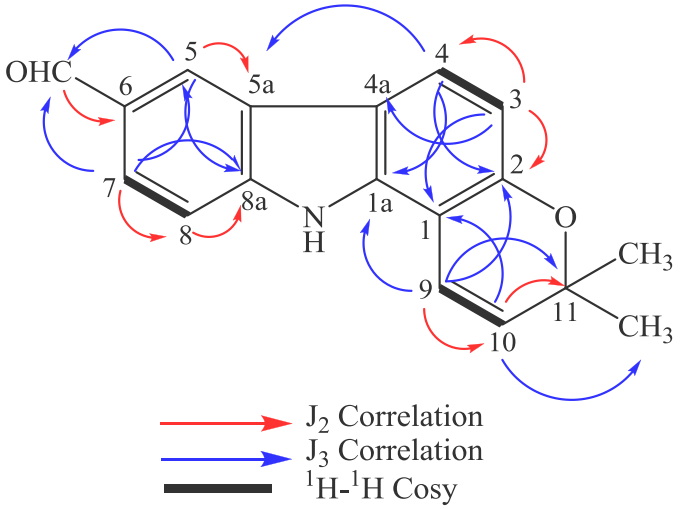


Figure 4.2.3.5g. Selected ^{1}H-^{1}H COSY and HMBCs of **Mk22**

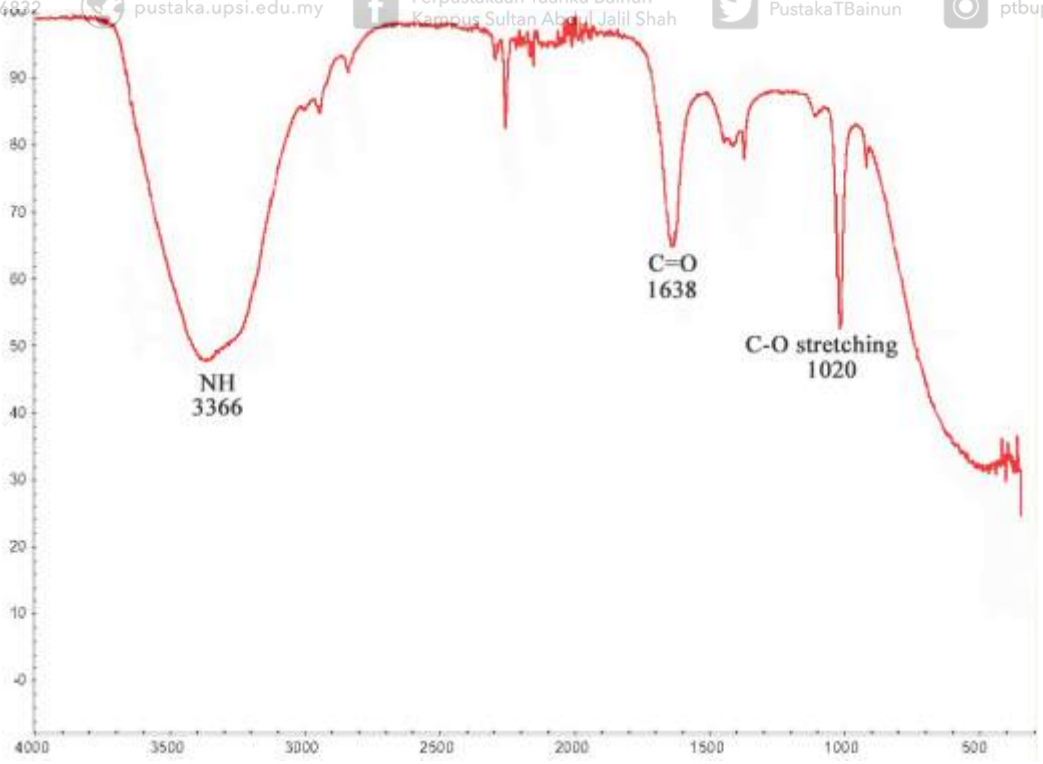


Figure 4.2.3.5h. IR Spectrum of Murrastinine-C **(Mk22)**

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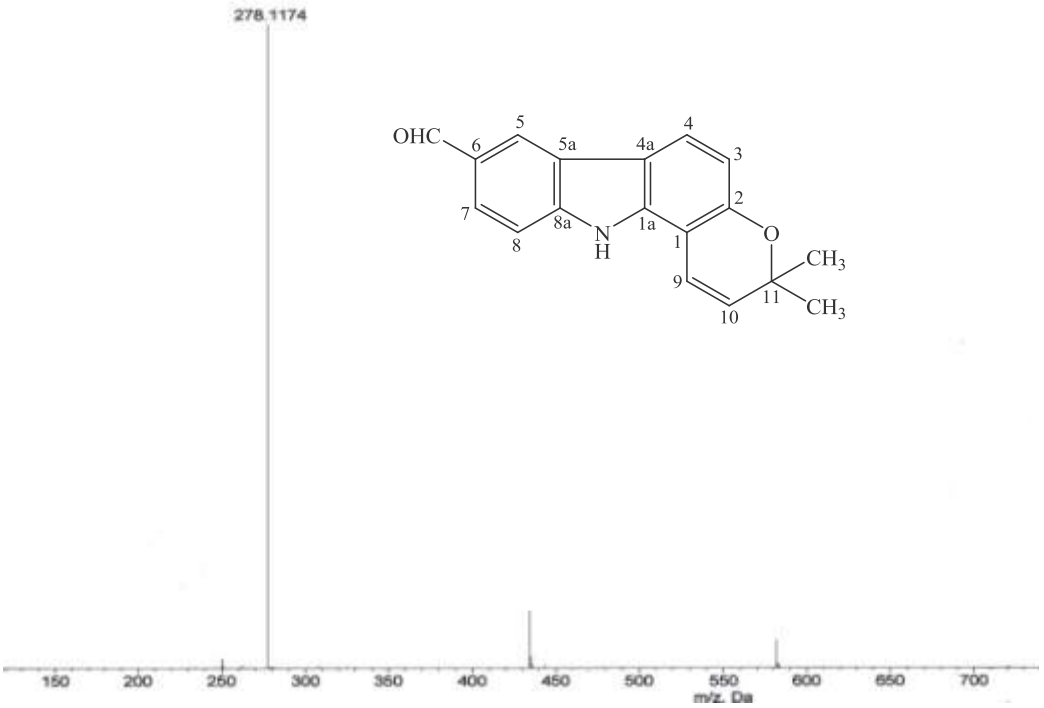


Figure 4.2.3.5i. Mass Spectrum of Murrastinine-C **(Mk22)**

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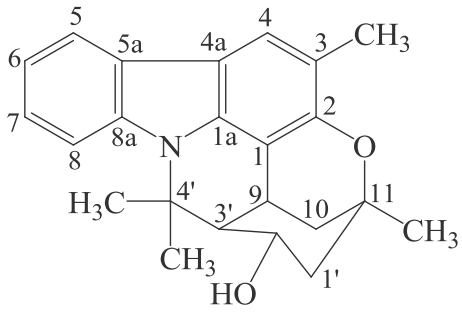
4.2.4 Cyclic Monoterpenoid Pyrano[3,2-a]carbazole Alkaloids

The cyclic monoterpenoid pyrano[3,2-a]carbazoles is another subclass of pyranocarbazole alkaloids. Biogenetically, these compounds are also derived from 2-hydroxy-3-methylcarbazole **39** by fusion with a geranyl (C_{10} building block) substituents. Subsequently, annulation of additional rings occur at further downstream on its biogenetic pathway (Cordell, 2008).

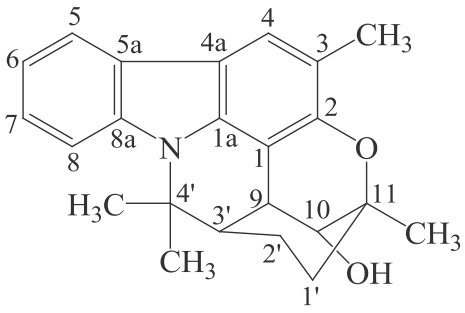
In the study, four of them were afforded from the bark and leaves of *Murraya koenigii* included a new cyclic monoterpenoid pyrano[3,2-a]carbazole. The structural assignments of these carbazoles were agreed and supported by combinations of NMR experiments, UV, IR and mass spectral data.

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4.2.4.1 Murrayazolinol (Mk23) and Murrayakoeninol (Mk24)



Mk23



Mk24

Mk23 was isolated as brown solid with melting point at 289–290 °C (m.p.: 290 °C; Bhattacharyya et al., 1989). It is an optically active compound with $[\alpha]_D^{25}$ +31.0 (CH_3OH; c 0.02) possesses a molecular formula $C_{23}H_{25}NO_2$ by its HERSIMS at *m/z* 348.1957 $[M+H]^+$. The IR spectrum showed absorption peaks at 3360 cm^{-1} confirmed

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the presence of an OH group in the molecule of **Mk23**. The UV spectrum, at 245, 265 and 305 nm, was readily recognized as a pyranocarbazole derivative from its preliminary spectral data (Cordell, 2008).

The ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of **Mk23** (Table 4.2.4.1a and Figure 4.2.4.1a) exhibited signal for an aromatic methyl group at δ 2.36 (3-CH_3) and confirmed an aromatic substitution pattern similar to girinimbine **(Mk12)**. The differences between **Mk12** and **Mk23** are the additional substitution of a monoterpenoid moiety [δ 1.76 (*dd*, *J* = 16.1, 6.9 Hz, H-1'), δ 3.83 (*m*, H-2') and δ 2.17 (*m*, H-3')], the absence of olefinic protons at H-9 and H-10, as well as the missing of NH signal in ^{1}H NMR spectrum of **Mk23**. Its ^{1}H NMR spectrum also showed signal for carbinyl hydrogen of a secondary alcohol, which appeared as a multiplet at δ 3.83 (*m*, H-2').

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The ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) indicated the presence of 23 carbons resonances and confirmed to be a C_{23}-carbazole (Table 4.2.4.1b and Figure 4.2.4.1b). The complete assignments of proton and carbon signals and location of substituents on the skeleton of **Mk23** were also supported on the basis of DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectra. Based on the spectral data, structure **Mk23** was determined as murrayazolinol, which was previously reported as minor compound from *M. koenigii* by Bhattacharyya et al. (1989).

The molecular formula of **Mk24** was also determined as $C_{23}H_{25}NO_2$ with a molecular ion peak at *m/z* 348.1943 $[M+H]^+$ by its HRESIMS, which displayed absorption bands at λ_{max} 242, 275 and 308 nm in the IR spectrum indicated the

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presence of an oxygenated carbazole chromophore (Cordell, 2008). The ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) (Table 4.2.4.1b and Figure 4.2.4.1d) of **Mk24** displayed signals for 23 carbons which has been proven to be a C_{23}-skeleton of carbazole alkaloid and identical to those of **Mk23**. It was also isolated as optically active pale yellow oil with $[\alpha]_D^{25}$ +11.0 (CH_3OH; c 0.015).

The ^1H NMR spectrum ($CDCl_3$, 500 MHz) of **Mk24** (Table 4.2.4.1a and Figure 4.2.4.1c) exhibited signals for five aromatic methine protons, of which one appeared as singlet at δ 7.50 (H-4), two as doublets at δ 7.89 (*J* = 8.0 Hz, H-5) and δ 7.47 (*J* = 8.1 Hz, H-8) and two as triplet of doublets at δ 7.16 (*J* = 8.1, 1.2 Hz, H-6) and δ 7.26 (*J* = 8.1, 1.2 Hz, H-7). The HMBC spectrum of **Mk24** suggested the presence of an unsubstituted carbazole ring by showing correlation of these four aromatic methine protons with neighbouring carbons resembled those in girinimbine **(Mk12)**. Other proton positions were also confirmed by ^1H-^1H COSY and HMBC spectra. The ^1H NMR spectrum of **Mk24** also showed signals for an aromatic methyl at δ 2.23 (*s*, 3-CH_3), a methyl group attached to an oxygen bearing group at δ 1.49 (*s*, 11-CH_3) and one *gem*-dimethyl group at C-4' with δ 1.30 and δ 1.92. The position of OH substituent was further confirmed by the HMBC spectrum with correlations of aliphatic methyl protons (at 11-CH_3) with mutually coupled methylene carbon (at H-1') and the OH bearing carbon (H-10). It also showed cross peak between H-10 at δ 3.82 (*d*, *J* = 8.3) and C-1 (δ 105.4) to give further evidence to confirmed the exact position of OH group. **Mk24** was closely resembling to **Mk23** except for the position of OH substituent. Based on the spectroscopic data, **Mk24** was assigned as murrayakoeninol (Chakraborty et al., 2009).

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Table 4.2.4.1a

^{1}H NMR [500 MHz, δ_H (J Hz)] of Murrayazolinol ***(Mk23)*** *and Murrayakoeninol* ***(Mk24)****

Position	**Mk23**	**Mk24**
1		
1a		
2		
3		
4	7.49 (*s*)	7.50 (*s*)
4a		
5	7.90 (*d*, 7.5 Hz)	7.89 (*d*, 8.0 Hz)
5a		
6	7.16 (*td*, 8.0, 1.2 Hz)	7.16 (*td*, 8.1, 1.2 Hz)
7	7.25 (*td*, 8.0, 1.2 Hz)	7.26 (*td*, 8.1, 1.2 Hz)
8	7.47 (*d*, 8.0 Hz)	7.47 (*d*, 8.1 Hz)
8a		
9	3.32 (*m*)	3.31 (*m*)
10	1.22 (*m*)	3.82 (*d*, 8.3 Hz)
11		
N-H		
2-OH	#	4.43 (*br s*)
3-CH_3	2.36 (*s*)	2.23 (*s*)
1'	1.76 (*dd*, 16.1, 6.9 Hz)	1.53 (*m*)
2'	3.83 (*m*)	1.74 (*m*)
3'	2.17 (*m*)	2.18 (*m*)
4'		
11-CH_3	1.50 (*s*)	1.49 (*s*)
4'-CH_3	1.93 (*s*)	1.92 (*s*)
4'-CH_3	1.29 (*s*)	1.30 (*s*)

Note. *Solvent used was $CDCl_3$; assignments based on COSY and HMQC. # peak not available.

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Table 4.2.4.1b

*^{13}C NMR [125 MHz, δ_H (J Hz)] of Murrayazolinol **(Mk23)** and Murrayakoeninol **(Mk24)****

Position	**Mk23**	**Mk24**
1	105.3	105.4
1a	140.7	140.7
2	153.3	153.3
3	118.5	118.5
4	119.7	119.7
4a	115.1	115.0
5	120.1	120.1
5a	127.2	127.2
6	119.5	119.5
7	123.1	123.1
8	113.6	113.7
8a	142.4	142.4
9	21.5	36.9
10	36.9	72.2
11	79.5	79.9
3-CH_3	15.5	15.5
1'	36.0	36.0
2'	72.2	21.5
3'	49.5	49.5
4'	60.6	60.6
11-CH_3	25.1	25.2
4'-CH_3	23.1	23.1
4'-CH_3	30.2	30.2

Note. *Solvent used was $CDCl_3$; assignments based on HMQC and HMBC.

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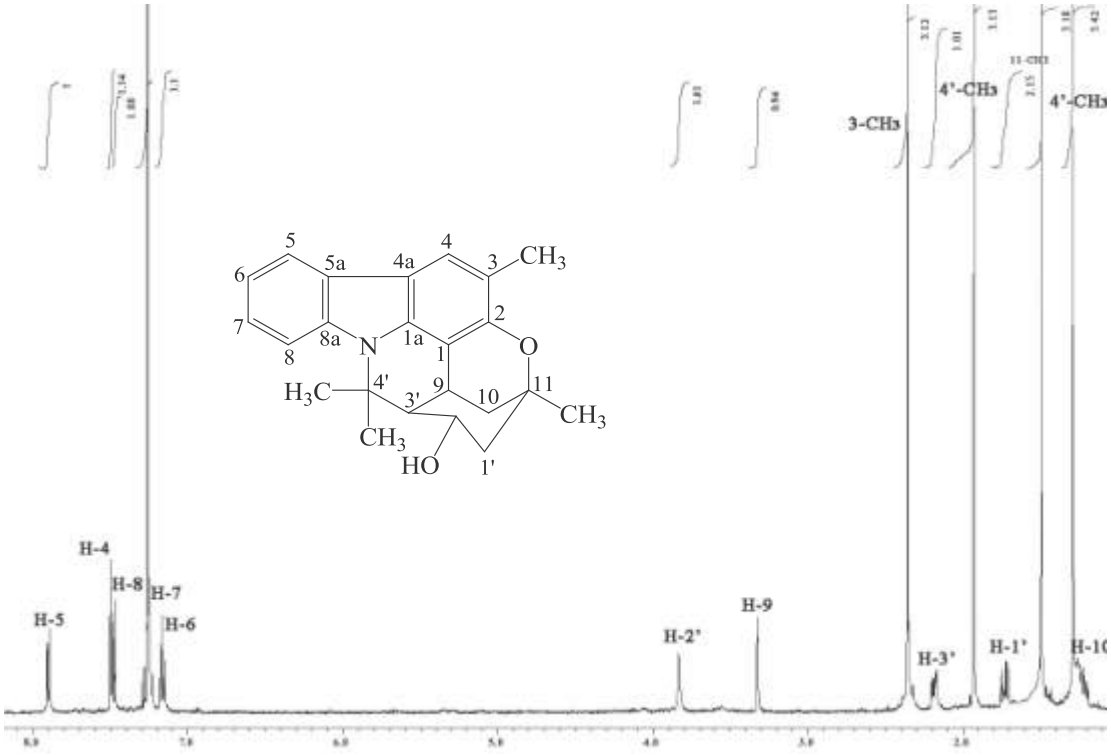


Figure 4.2.4.1a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Murrayazolinol **(Mk23)**

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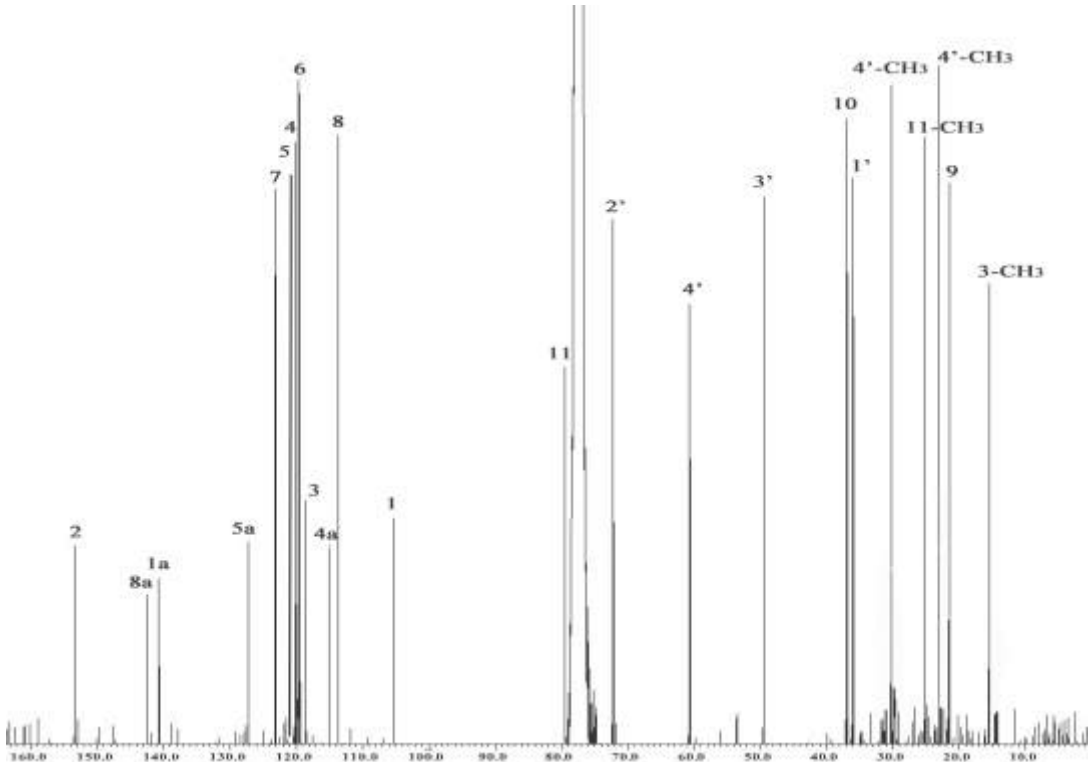


Figure 4.2.4.1b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Murrayazolinol **(Mk23)**

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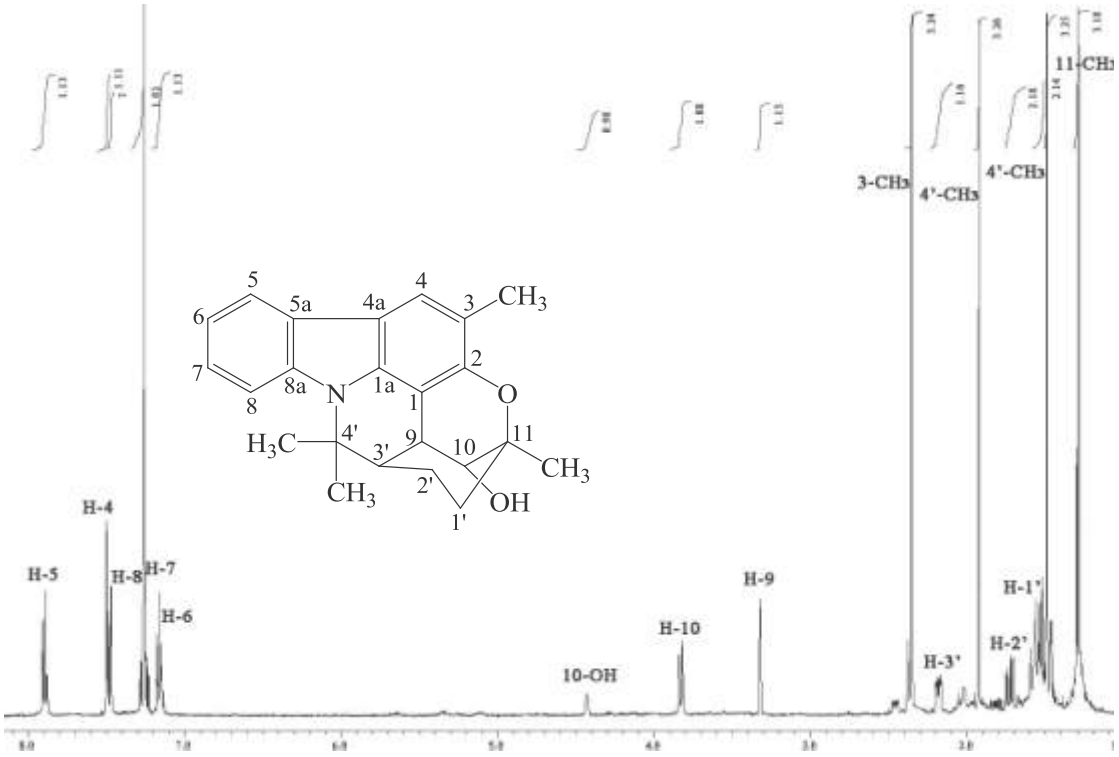


Figure 4.2.4.1c. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Murrayakoeninol **(Mk24)**

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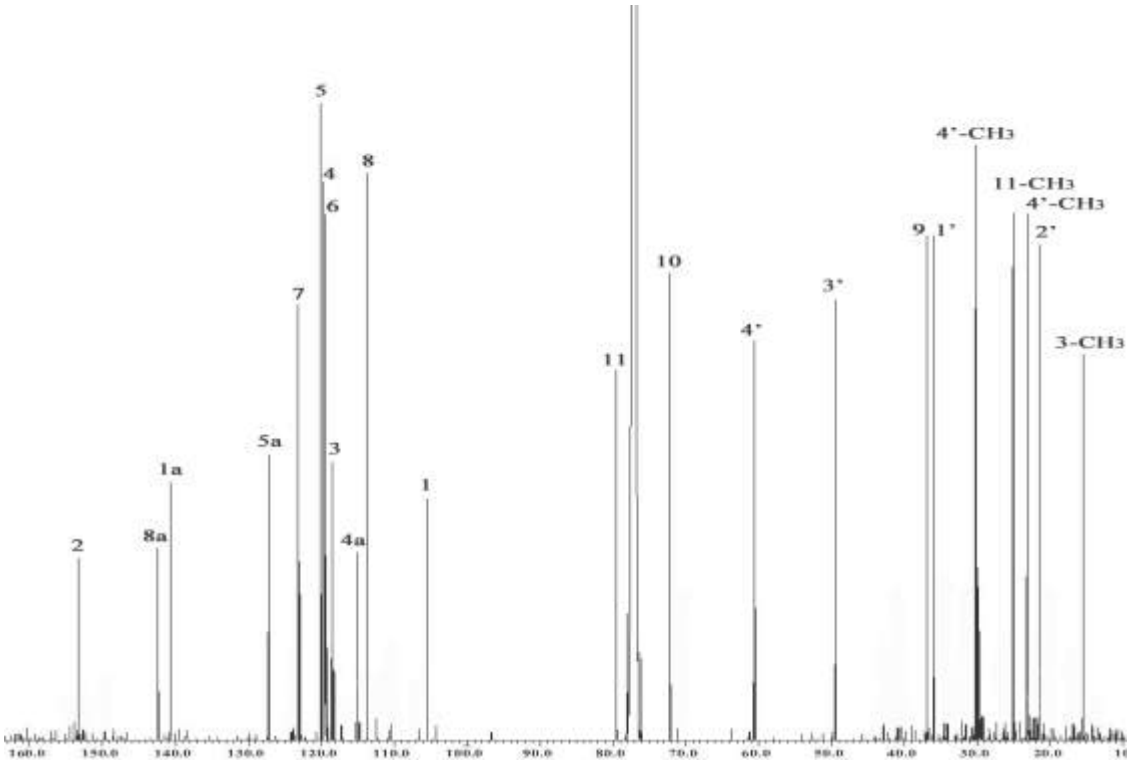
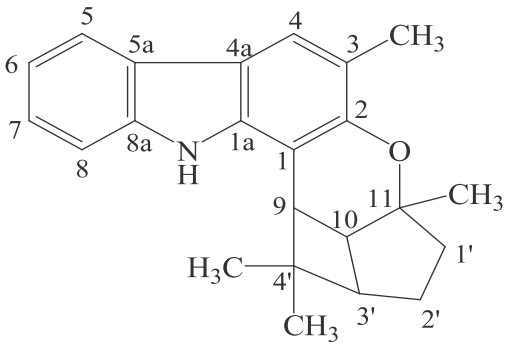


Figure 4.2.4.1d. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Murrayakoeninol **(Mk24)**

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4.2.4.2 Bicyclomahanimbine (Mk25)



Mk25

Mk25, as brown solid with melting point at 145–147 °C (m.p.: 145 °C; Kureel et al., 1969b), was an optically active compound with $[\alpha]_D^{25}$ +26.0 (CH_3OH; c 0.02). It HERSIMS showed molecular ion peak at *m/z* 332.2008 $[M+H]^+$ which consistent with molecular formula $C_{23}H_{25}NO$. The UV spectrum of **Mk25** showed characteristic absorbance at 242, 255, 260 and 303 nm for the typical absorption of cyclic monoterpenoid pyrano[3,2-a]carbazole chromophore (Cordell, 2008). The strong IR absorption at 3379 cm^{-1} and 1019 cm^{-1} indicated the presence of NH and C–O group, respectively.

The aromatic region of ^1H NMR spectrum ($CDCl_3$, 500 MHz) resembled to those of mahanimbine **(Mk15)**, indicated a similar aromatic substitution pattern on the carbazole skeleton (Table 4.2.4.2 and Figure 4.2.4.2a). However, the monoterpenoid moiety fused at C-1 and C-2 was different. In consideration of the molecular formula and the absence of olefinic protons, bicyclomahanimbine **(Mk25)** was suggested to be a hexacyclic base with a cyclobutane system. The presence of the cyclobutane ring was further confirmed by a downfield shift of its methyl group at C-4' to δ 0.74 in bicyclomahanimbine when compared to those of mahanimbine **(Mk15)** at δ 1.62. The

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^{13}C NMR spectrum ($CDCl_3$, 125 MHz) also showed the presence of 23 carbon resonances (Table 4.2.4.2 and Figure 4.2.4.2b) which was also showed either in **Mk15**, **Mk23** or **Mk24**. The complete assignments of proton and carbon signals and location of substituents on the skeleton of **Mk25** were confirmed by the combinations of NMR experiments. Based on these spectroscopic data, the structure of **Mk25** was revealed to be bicyclomahanimbine (Kureel et al., 1969b).

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Table 4.2.4.2

^{1}H NMR [500 MHz, δ_H (J Hz)] and ^{13}C NMR [125 MHz, δ_C] of Bicyclomahanimbine ***(Mk25)****

Position	δ ^{13}C	δ ^{1}H
1	106.4	
1a	137.8	
2	150.3	
3	120.1	
4	119.4	7.67 (*s*)
4a	115.8	
5	119.3	7.92 (*d*, 8.0 Hz)
5a	124.2	
6	119.3	7.16 (*td*, 8.0, 1.2 Hz)
7	124.0	7.28 (*td*, 8.0, 1.2 Hz)
8	110.4	7.37 (*d*, 7.5 Hz)
8a	139.3	
9	37.5	3.29 (*d*, 9.2 Hz)
10	46.5	2.50 (*t*, 7.5 Hz)
11	83.6	
N-H		7.43 (*br s*)
3-CH_3	16.8	2.34 (*s*)
1'	38.4	2.06 (*m*)
		1.55 (*m*)
2'	25.7	1.65 (*m*)
		1.56 (*m*)
3'	37.9	2.70 (*t*, 7.4 Hz)
4'	39.5	
11-CH_3	27.5	1.44 (*s*)
4'-CH_3	18.7	0.74 (*s*)
4'-CH_3	35.2	1.57 (*s*)

Note. *Solvent used was $CDCl_3$; assignments based on COSY, HMQC and HMBC.

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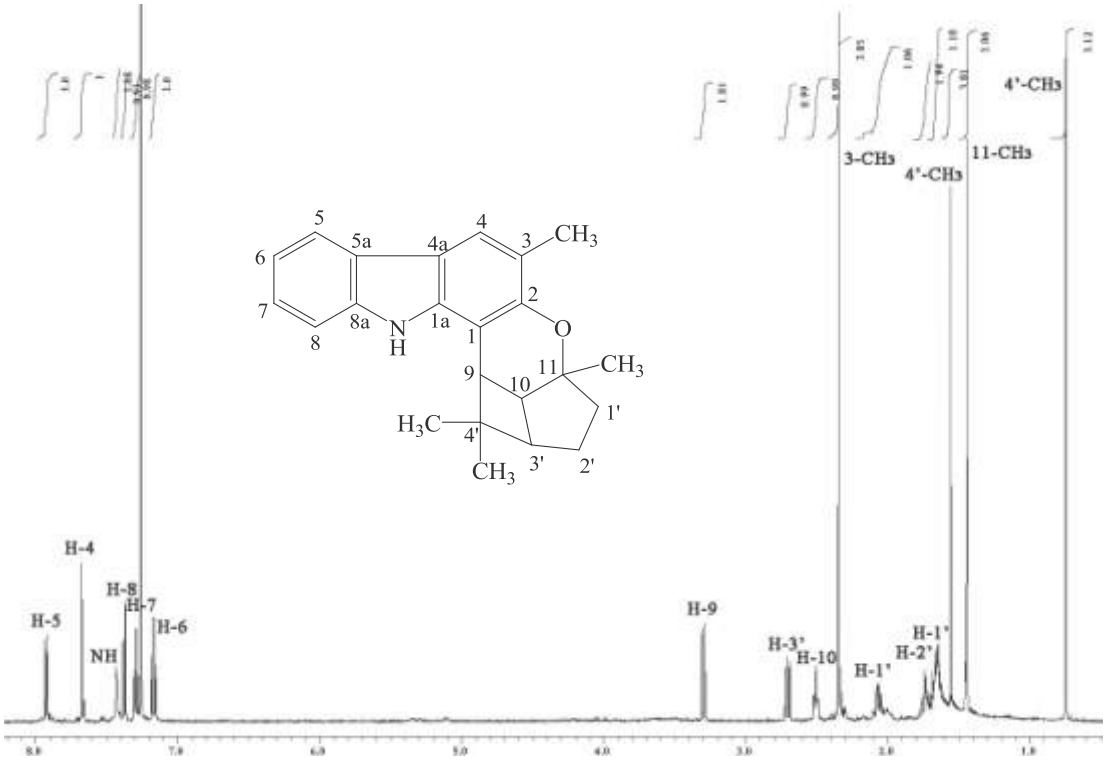


Figure 4.2.4.2a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Bicyclomahanimbine **(Mk25)**

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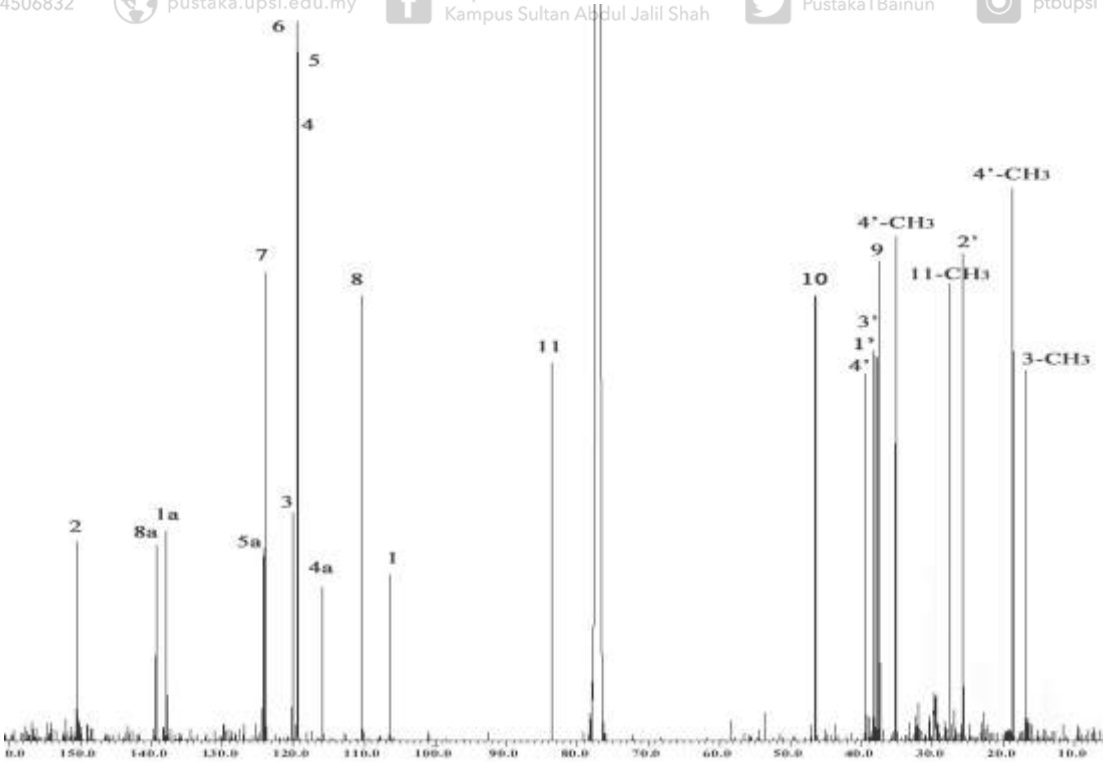
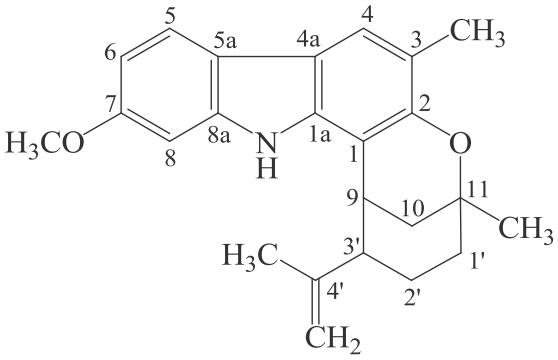


Figure 4.2.4.2b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Bicyclomahanimbine **(Mk25)**

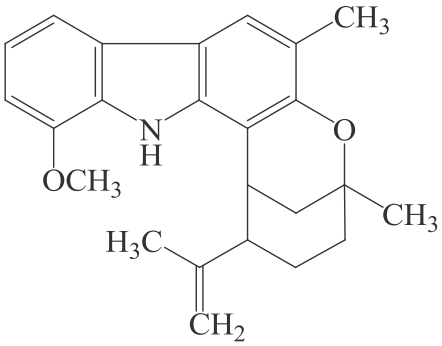
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4.2.4.3 Murrayatanine-A **(Mk26)**



Mk26



160. Murrayamine-H

Another new carbazole, murrayatanine-A **(Mk26)**, was successfully isolated as minor carbazole from *M. koenigii* as pale yellow oil with $[\alpha]_D^{25}$ +4.5 (CH_3OH; c 0.015) and found to be a structural isomer of murrayamine-H **160** which was isolated previously from *M. euchrestifolia* by Wu et al. (1996). All the substituents, included an aromatic methyl at δ 2.32 (3H, *s*), an aromatic methoxyl group at δ 3.89 (3H, *s*), a bicyclic ring with isopropenyl group and its methyl substituents in ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of **Mk26** (Table 4.2.4.3 and Figure 4.2.4.3a), were identical to those in murrayamine-H **160** (Wu et al., 1996). The only difference between them was the location of their methoxyl group. The methoxyl group of **Mk26** was attached to a quaternary carbon at position 7 with signal at δ 157.5 of the carbazole nucleus as indicated by the mutual HMBC correlations (Figure 4.2.4.3f). Other cross peaks were also found to support the location of methoxyl group such as the cross peaks of H-8 (δ 6.83, *d*, *J* = 2.3 Hz) and H-6 (δ 6.77, *dd*, *J* = 8.6, 2.3 Hz) to C-7. In NOESY experiment (Figure 4.2.4.3g), correlation from proton of 7-OCH_3 to H-6 and H-8, again confirmed the position of its methoxyl group. According to the proton signals in the aromatic region, a mutually coupled ABX pattern at δ 7.74 (*d*, *J* = 8.6 Hz, H-5), δ

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6.77 (*dd*, *J* = 8.6, 2.3 Hz, H-6) and δ 6.83 (*d*, *J* = 2.3 Hz, H-8) were attributed to H-5, H-6 and H-8, respectively, gave further evidence to support the position of methoxyl group (7-OCH_3).

The ^{13}C NMR spectrum ($CHCl_3$, 125 MHz) indicated the presence of 24 carbon resonances (Table 4.2.4.3 and Figure 4.2.4.3b) included ten quaternary carbons at δ 105.4 (C-1), δ 138.3 (C-1a), δ 152.8 (C-2), δ 117.4 (C-3), δ 114.3 (C-4a), δ 118.3 (C-5a), δ 157.5 (C-7), δ 140.6 (C-8a), δ 73.8 (C-11) and δ 150.3 (C-4'); six methine carbons at δ 118.9 (C-4), δ 119.7 (C-5), δ 117.4 (C-6), δ 95.0 (C-8), δ 36.4 (C-9) and δ 48.8 (C-3'); four methylene carbons at δ 37.4 (C-10), δ 40.0 (C-1'), δ 23.2 (C-2') and δ 112.1 (4'-CH_3); three methyl carbons at δ 16.8 (3-CH_3), δ 29.1 (11-CH_3), δ 21.7 (4'-CH_3); and a methoxy carbon at δ 55.7 for 7-OCH_3.

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The above assignments were further confirmed by DEPT, ^1H-^1H COSY, HMQC, NOESY and long-range coupling in HMBC experiment (Figure 4.2.4.3c to Figure 4.2.4.3j). Two fragments at C-5 – C-6 and C-10 – C-9 – C-3' – C-2' – C-1' were observed in its ^1H-^1H COSY (Figure 4.2.4.3e) experiment. Other correlations in the HMBC measurement (Figure 4.2.4.3f) were also observed such as cross peaks of H-4 to C-1a, C-2 and 3-CH_3; H-10 to C-1, C-1' and C-11; and 4'-CH_3 to C-3', C-4' and 4'-CH_2. The correlations were observed between H-3'/H-1'β, H-3'/4'β-CH_2, but no correlations between H-3' and H-9 in the NOESY system, established the orientation of H-3' at β-position and H-9 at the α-orientation. This further confirmed the relative stereochemistry of **Mk26**.

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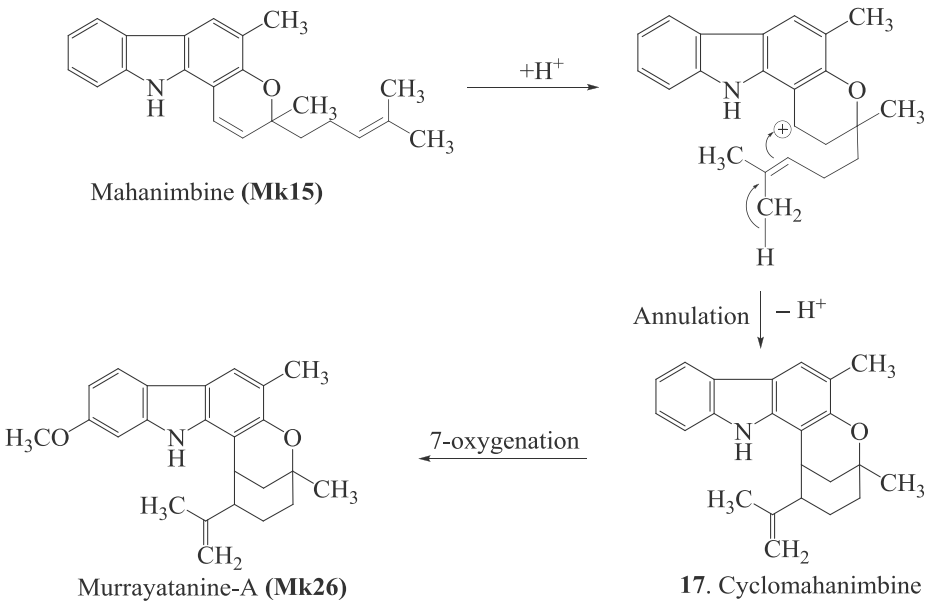
The IR spectrum of **Mk26** (Figure 4.2.4.3k) also indicated the presence of NH (3329 cm^{-1}) and C–O stretching (1025 cm^{-1}). Its molecular formula was deduced as $C_{24}H_{27}NO_2$ with a molecular ion at *m/z* 362.2106 ($[M+H]^+$, calcd. mass: 362.2120) by its HRESIMS (Figure 4.2.4.3l). Its UV spectrum showed characteristic absorbance for monoterpenoid pyranocarbazole moiety with λ_{max} 241, 255, 268 and 325 nm (Cordell, 2008) which were similar to those of murrayamine-H **160** (Wu et al., 1996). It was readily recognized as monoterpenoid pyranocarbazole derivative from its spectral data.

Two possible biogenetic routes to **Mk26** were proposed (Scheme 4.2.4.3). Mahanimbine (**Mk15**) was suggested to be the key precursor for the formation of **Mk26**. A combination of protonation, cyclization and 7-oxygenation reactions on mahanimbine led to the formation of **Mk26**, or vice versa. **Mk26** was then determined as murrayatanine-A in agreement with all the data.

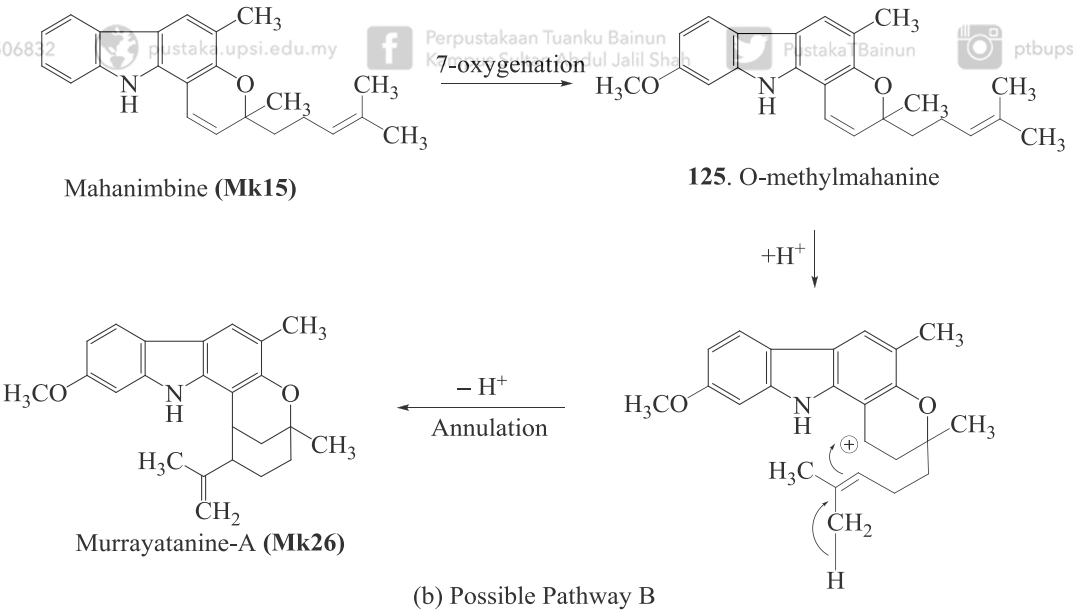
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(a) Possible Pathway A



(b) Possible Pathway B

Scheme 4.2.4.3. Proposed Biogenetic Pathway to **Mk26**

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Table 4.2.4.3

1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of Murrayatanine-A ***(Mk26)****

Position	δ ^{13}C	DEPT	δ ^{1}H	HMBC correlations	COSY
1	105.4	qC		H-10	
1a	138.3	qC		H-4	
2	152.8	qC		H-4, 3-CH_3	
3	117.4	qC		H-4	
4	118.9	CH	7.54 (*s*)	3-CH_3	
4a	114.3	qC		H-4	
5	119.7	CH	7.74 (*d*, 8.6 Hz)	H-6	H-6
5a	118.3	qC			
6	107.4	CH	6.77 (*dd*, 8.6, 2.3 Hz)	H-8	H-5
7	157.5	qC		H-5, H-6, 7-OCH_3	
8	95.0	CH	6.83 (*d*, 2.3 Hz)	H-6	
8a	140.6	qC		H-5	
9	36.4	CH	3.38 (*m*)		H-10, H-3'
10β	37.4	CH_2	2.00 (*m*)		H-9
10α			2.06 (*m*)		
11	73.8	qC		H-10, 11-CH_3	
3-CH_3	16.8	CH_3	2.32 (*s*)	H-4	
7-OCH_3	55.7	CH_3	3.89 (*s*)		
11-CH_3	29.1	CH_3	1.42 (*s*)	H-10, H-1'	
N-H			7.64 (*br s*)		
1'β	40.0	CH_2	1.61 (*m*)	H-10, H-2'	H-2'
1'α			1.99 (*m*)		
2'β	23.2	CH_2	1.64 (*m*)	H-1', H-3'	H-1', H-3'
2'α			1.68 (*m*)		
3'	48.8	CH	2.56 (*m*)	4'-CH_2, 4'-CH_3	H-9, H-2'
4'	150.3	qC		4'-CH_2, 4'-CH_3	
4'-CH_3	21.7	CH_3	1.50 (*s*)	H-3', 4'-CH_2	
4'β-CH_2	112.1	CH_2	4.75 (*d*, 1.9 Hz)	H-3', 4-CH_3	
4'α-CH_2			4.83 (*d*, 1.9 Hz)		

Note. *Solvent used was $CDCl_3$; assignments based on DEPT, COSY, NOESY, HMQC and HMBC.

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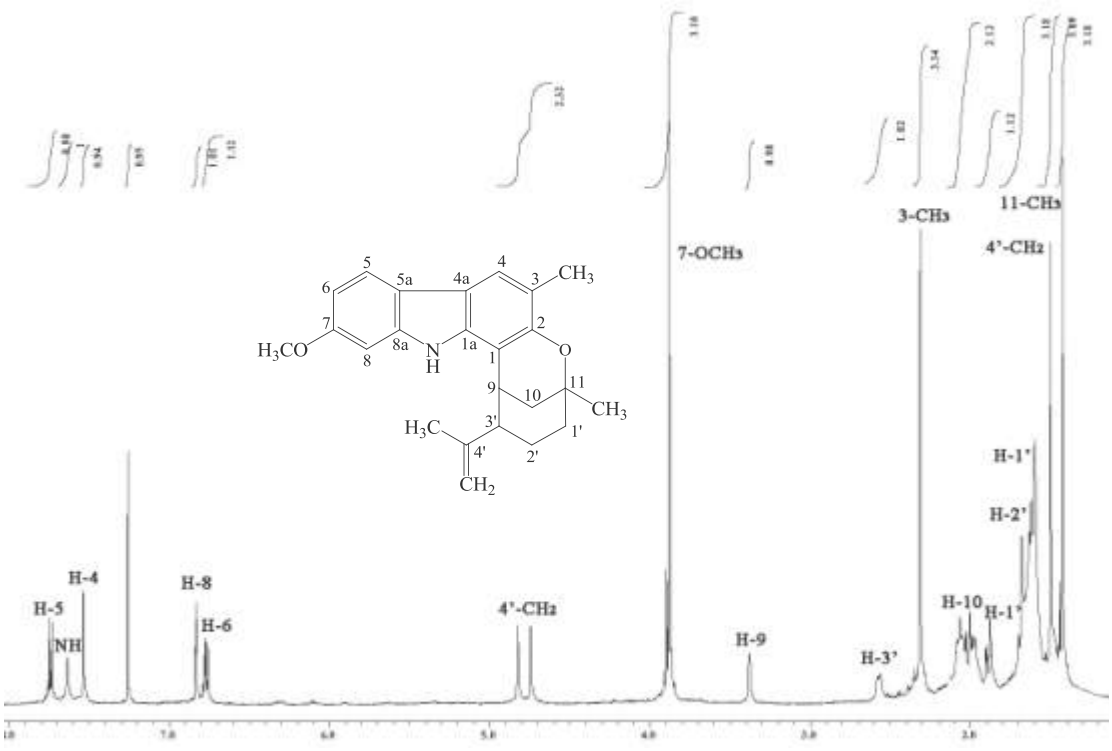


Figure 4.2.4.3a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Murrayatanine-A **(Mk26)**

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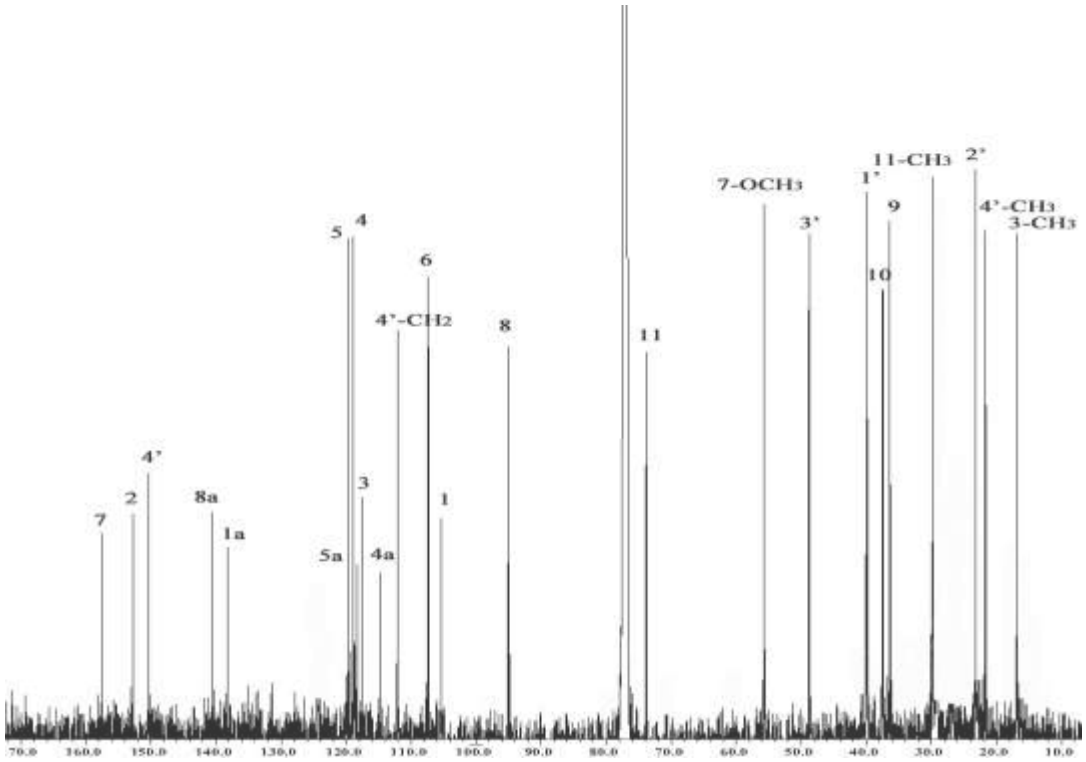


Figure 4.2.4.3b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Murrayatanine-A **(Mk26)**

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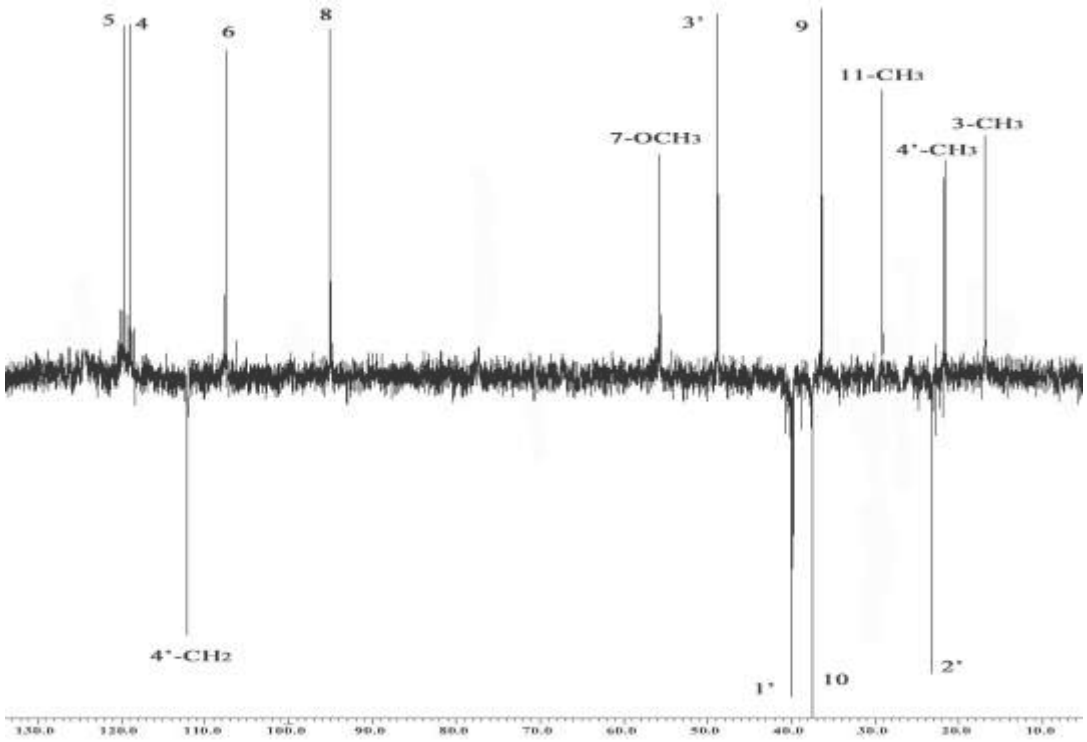


Figure 4.2.4.3c. DEPT-135 Spectrum ($CDCl_3$, 125 MHz) of Murrayatanine-A **(Mk26)**

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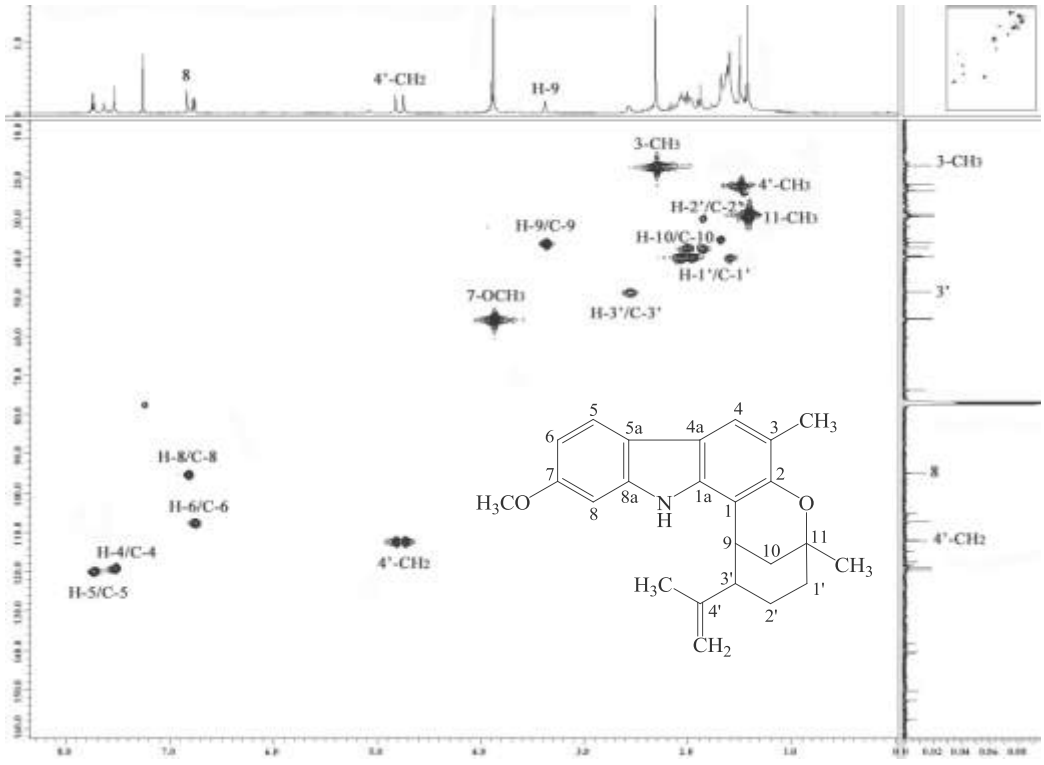


Figure 4.2.4.3d. HMQC Spectrum ($CDCl_3$, 500 MHz) of Murrayatanine-A **(Mk26)**

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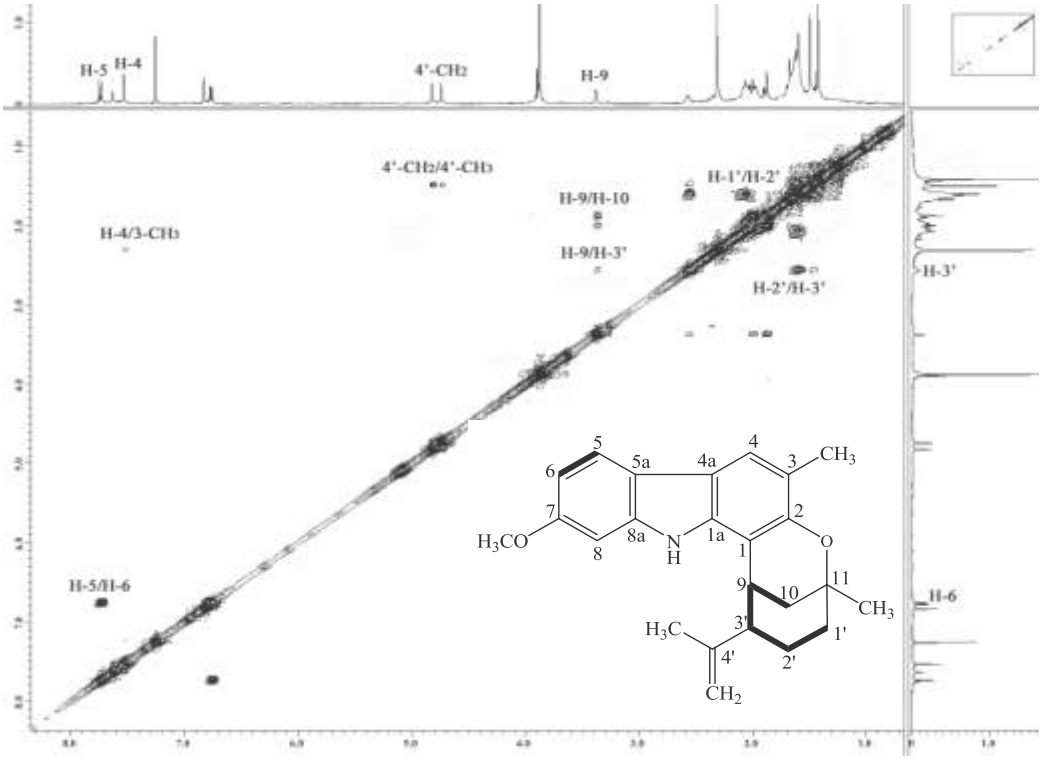


Figure 4.2.4.3e. ^{1}H-^{1}H COSY Spectrum ($CDCl_3$, 500 MHz) of Murrayatanine-A **(Mk26)**

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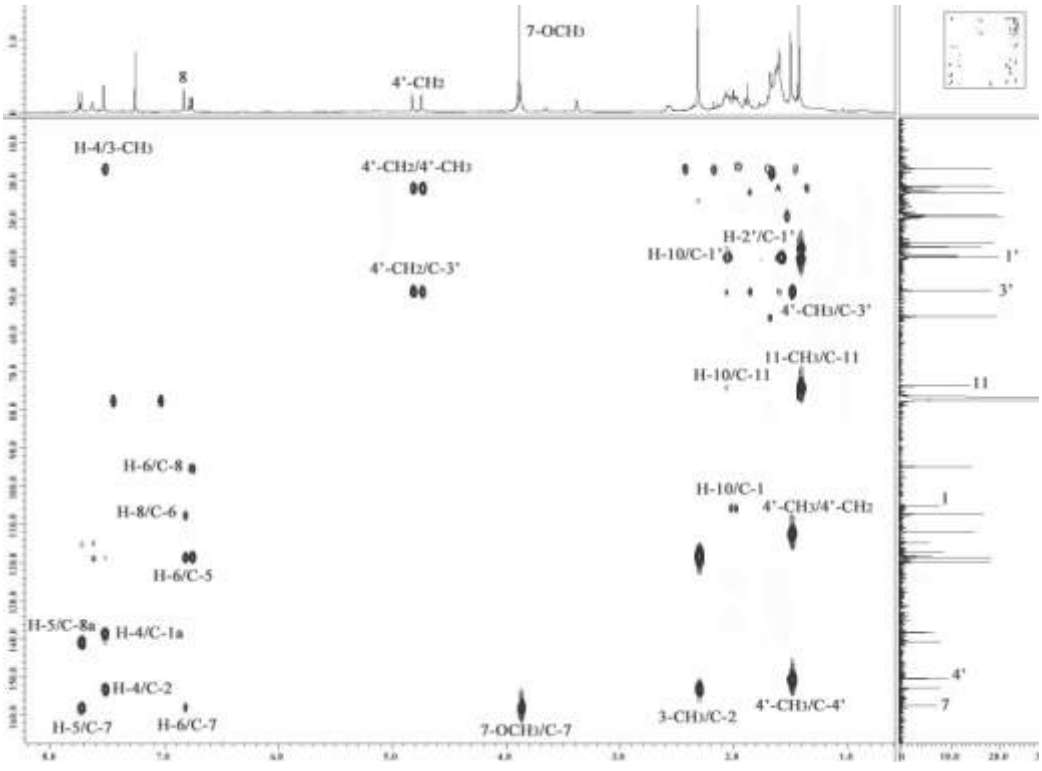


Figure 4.2.4.3f. HMBC Spectrum ($CDCl_3$, 500 MHz) of Murrayatanine-A **(Mk26)**

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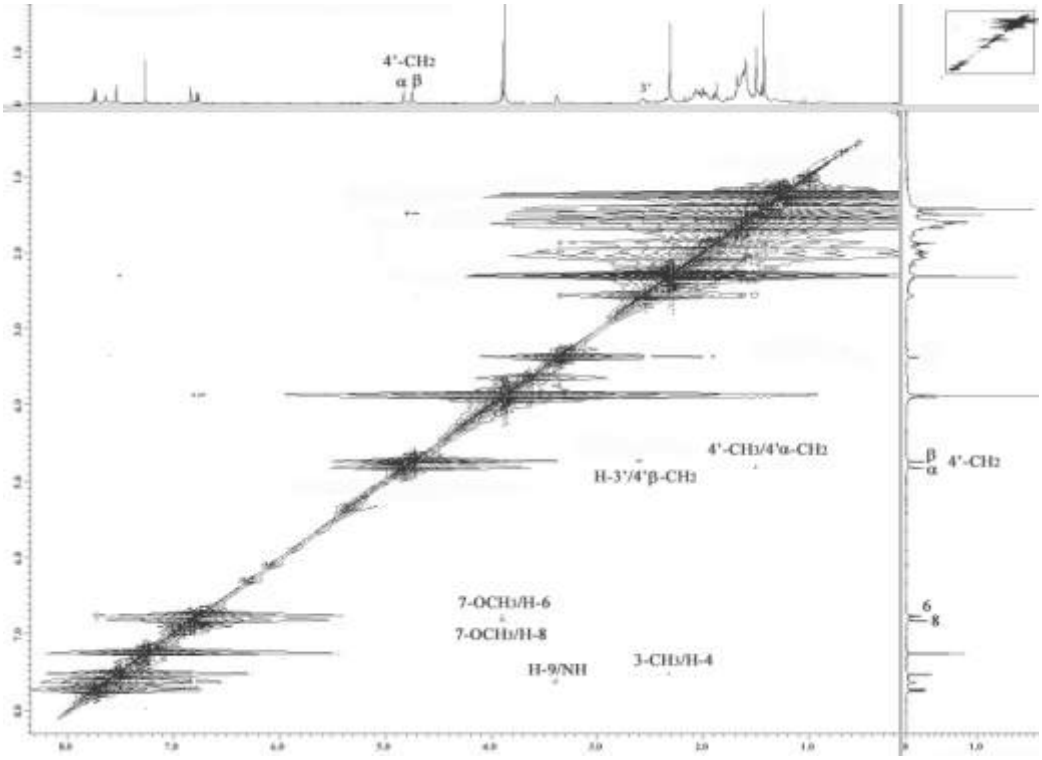


Figure 4.2.4.3g. NOESY Spectrum ($CDCl_3$, 500 MHz) of Murrayatanine-A **(Mk26)**

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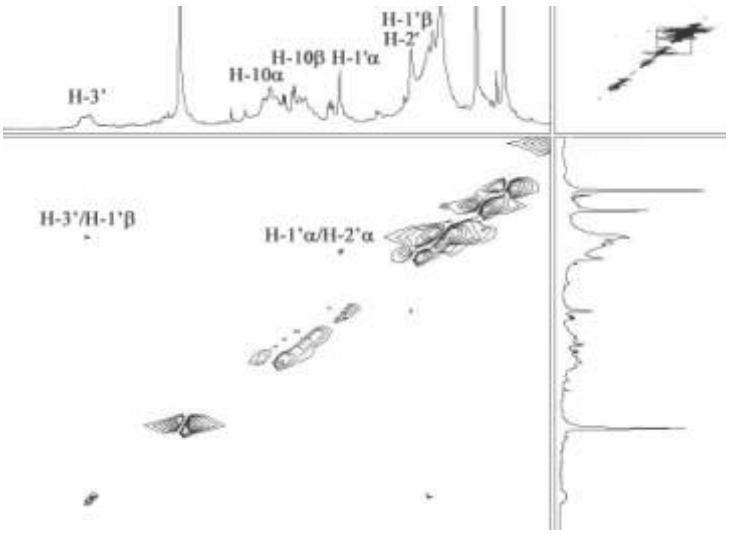


Figure 4.2.4.3h. Expanded NOESY Spectrum ($CDCl_3$, 500 MHz) of Murrayatanine-A **(Mk26)**

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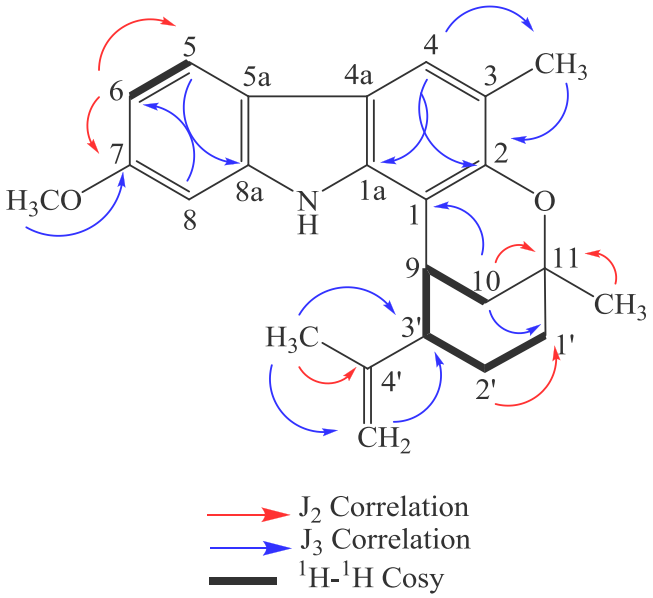


Figure 4.2.4.3i. Selected ^{1}H-^{1}H COSY and HMBCs of **Mk26**

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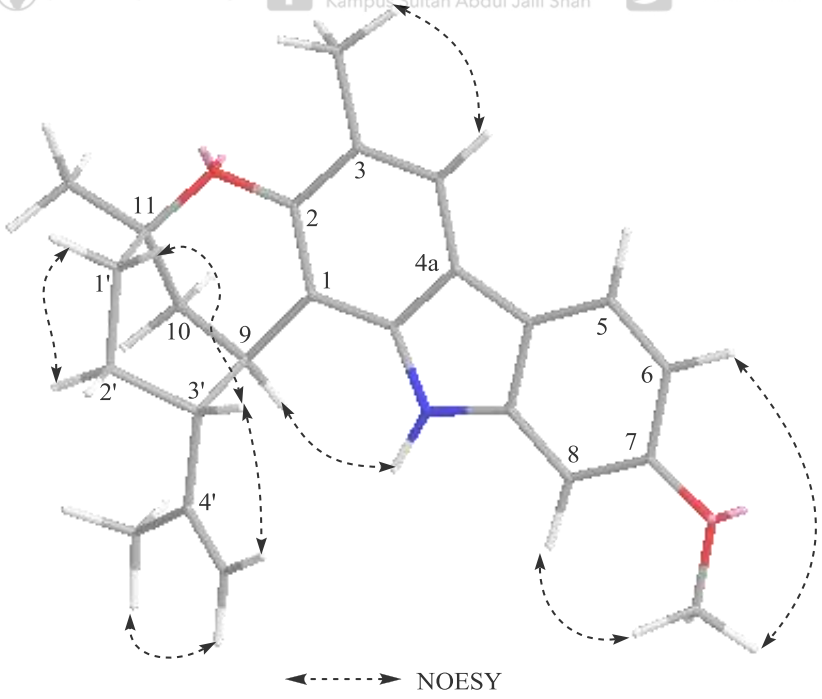


Figure 4.2.4.3j. Selected NOESY Correlations and Relative Stereochemistry for **Mk26**

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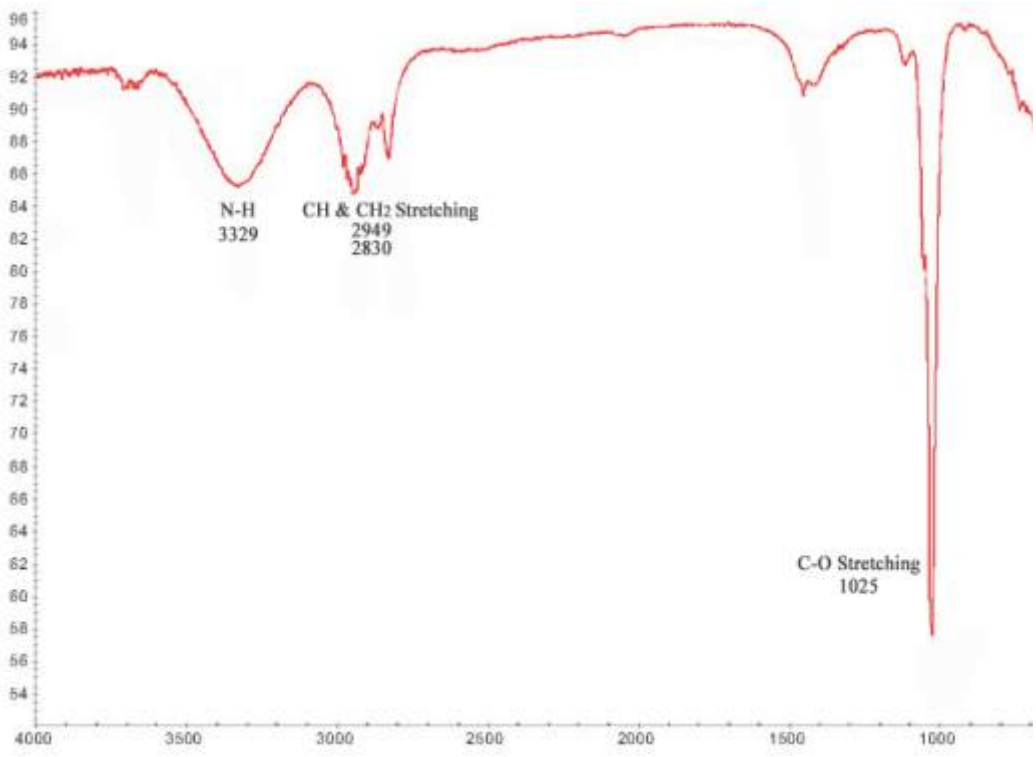


Figure 4.2.4.3k. IR Spectrum of Murrayatanine-A **(Mk26)**

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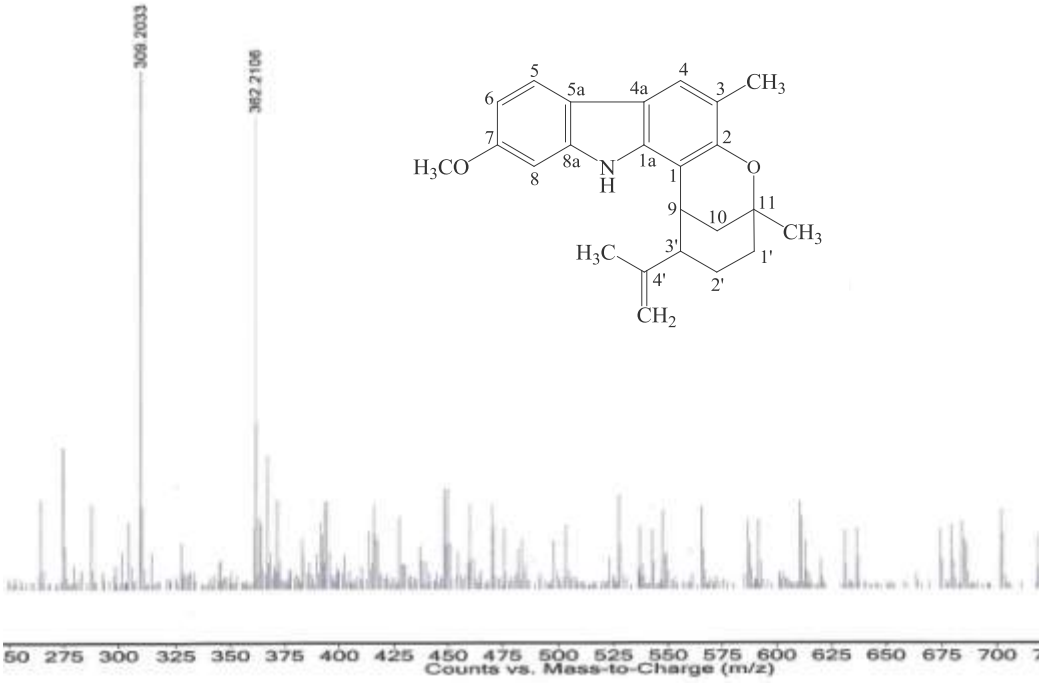


Figure 4.2.4.3l. Mass Spectrum of Murrayatanine-A **(Mk26)**

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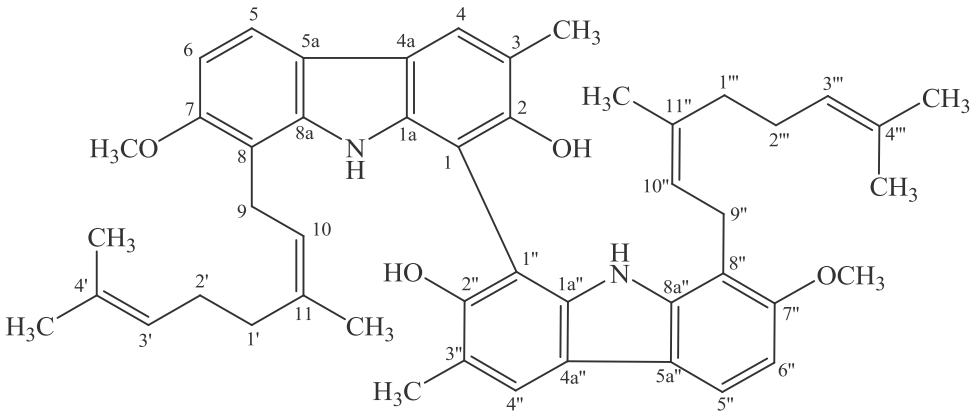
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4.2.5 Biscarbazole Alkaloids

Carbazole alkaloids attract strong interest because of their structural varieties and promising pharmacological activities. Biscarbazoles, also known as dimeric carbazole alkaloids, biogenetically forming linkage between one or two carbazole frameworks with either benzylic linkage, aryl-pyran linkage, or *N*-aryl linkage at further downstream on the biogenetic pathway (Cordell, 2008). Four biscarbazole alkaloids were mostly isolated from the leaves of *M. koenigii*, included a new biscarbazole. The structural assignments of these carbazoles will be supported by NMR, UV, IR and mass spectral data.

4.2.5.1 Bismurrayafoline-E (Mk27)

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Mk27

Mk27 was obtained as colourless oil from hexane crude extract of the leaves of *M. koenigii*. The molecular formula was determined as $C_{48}H_{56}N_2O_4$ by HRESIMS at *m/z* 724.3515 $[M]^+$. The ^1H NMR spectrum (Table 4.2.5.1 and Figure 4.2.5.1a) coupled with the result of ^{13}C NMR spectrum (Table 4.2.5.1 and Figure 4.2.5.1b) of **MK27**

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showed that only half the number of its proton and carbon signals of that expected from the molecular formula, suggested that **Mk27** has a completely symmetrical structure. The similarity of UV spectrum (λ_{max} at 232, 240, 262, 308, 325 nm) of this compound to those of euchrestine-B **(Mk7)** coupled with the ^{1}H NMR spectrum confirmed that euchrestine-B is the structural unit of **Mk27**.

The ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of **Mk27** was similar to those of euchrestine-B **(Mk7)** (refer Section 4.2.1.3), except for the lack of one proton signal for C-1. These data suggested the structure of **Mk27** corresponding to a symmetrical dimer of **Mk7** with a carbon-carbon linkage at C-1 and C-1".

The assignment of carbon signals and location of substituents of **Mk27** were achieved on the basis of ^{13}C NMR, DEPT, COSY, HMQC and HMBC measurements. On the basis of these data, **Mk27** was identified as bismurrayafoline-E (Nutan et al., 1999).

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Table 4.2.5.1

^{1}H NMR [500 MHz, δ_H (J Hz)] and ^{13}C NMR [125 MHz, δ_C] of Bismurrayafoline-E ***(Mk27)****

Position	δ ^{13}C	DEPT	δ ^{1}H
1, 1"	99.5	qC	
1a, 1a"	137.8	qC	
2, 2"	150.1	qC	
3, 3"	117.5	qC	
4, 4"	122.3	CH	7.85 (*s*)
4a, 4a"	117.3	qC	
5, 5"	117.4	CH	7.77 (*d*, 8.6 Hz)
5a, 5a"	118.3	qC	
6, 6"	104.9	CH	6.85 (*d*, 8.6 Hz)
7, 7"	154.9	qC	
8, 8"	111.0	qC	
8a, 8a"	140.5	qC	
9, 9"	23.8	CH	3.43 (*ddd*, 40.7, 16.0, 6.9 Hz)
10, 10"	121.8	CH	5.09 (*m*)
11, 11"	136.5	qC	
NH, NH"			7.57 (*br s*)
2-OH, 2"-OH			5.20 (*br s*)
3-CH_3, 3"-CH_3	16.8	CH_3	2.48 (*s*)
7-OCH_3, 7"-OCH_3	55.8	CH_3	3.89 (*s*)
11-CH_3,11"-CH_3	15.3	CH_3	1.25 (*d*, 1.1 Hz)
1', 1"'	39.1	CH_2	1.48 (*m*)
2', 2"'	26.4	CH_2	2.06 (*m*)
3', 3"'	124.1	CH	4.77 (*t*, 6.6 Hz)
4', 4"'	131.1	qC	
4'-CH_3, 4"'-CH_3	25.7	CH_3	1.56 (*s*)
4'-CH_3, 4"'-CH_3	17.5	CH_3	1.42 (*s*)

Note. *Solvent used was $CDCl_3$; assignments based on COSY, HMQC and HMBC.

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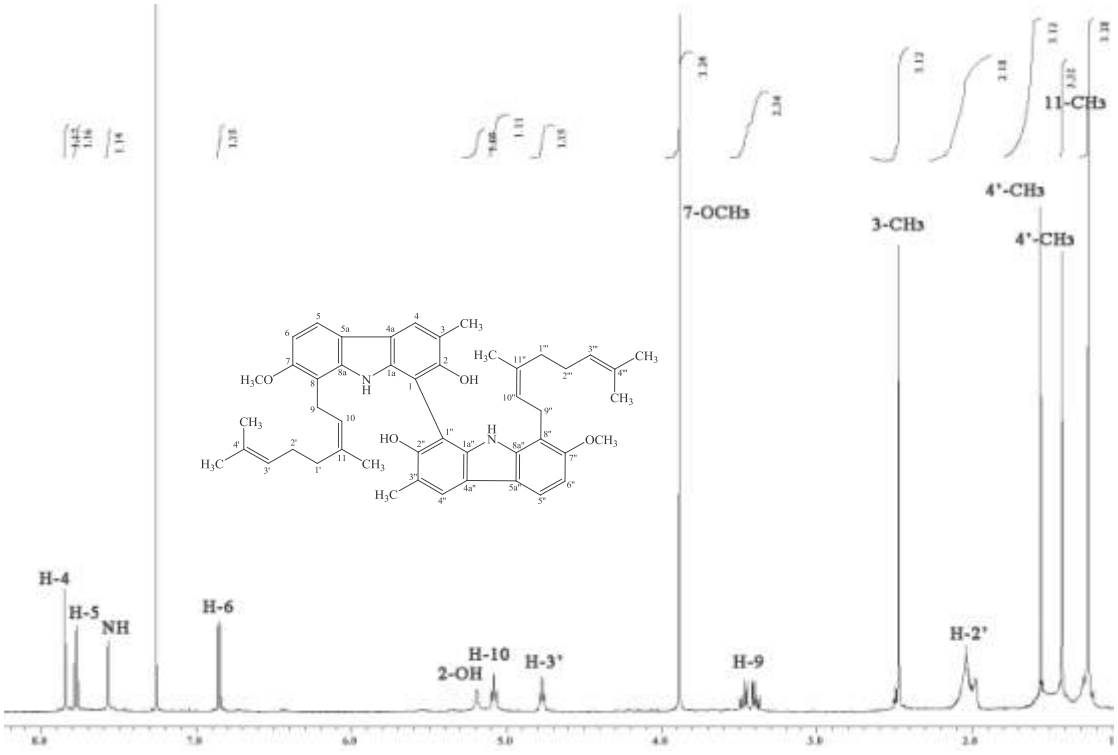


Figure 4.2.5.1a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Bismurrayafoline-E **(Mk27)**

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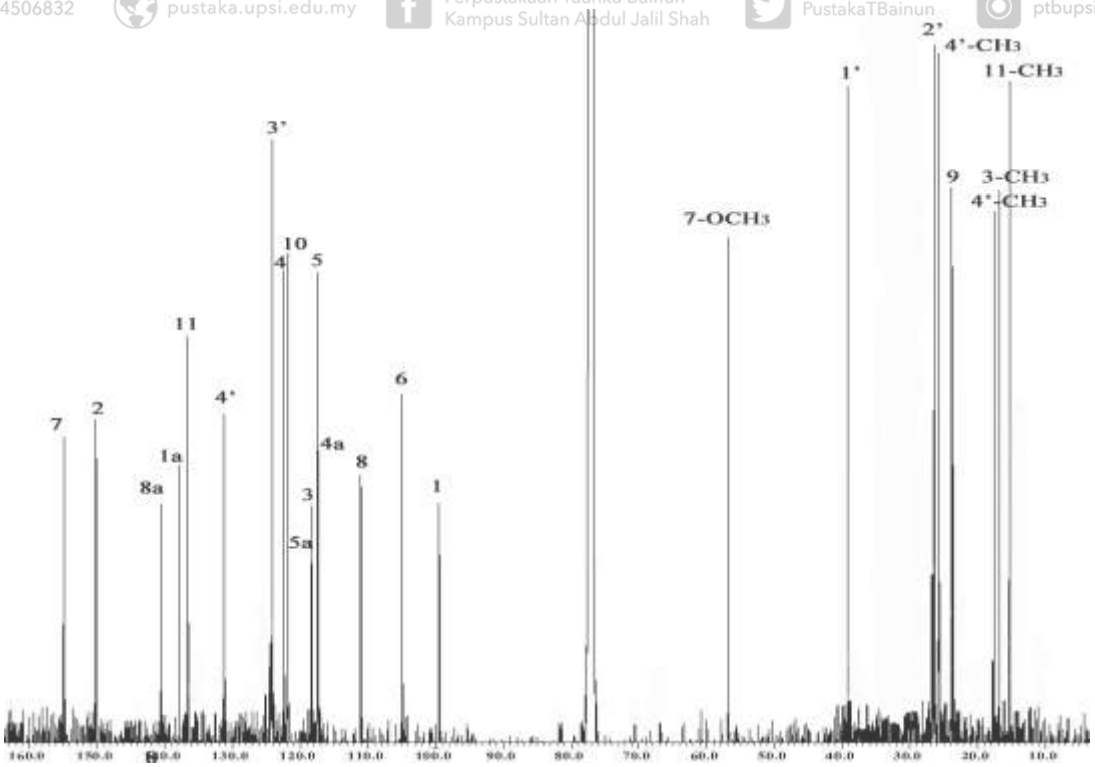
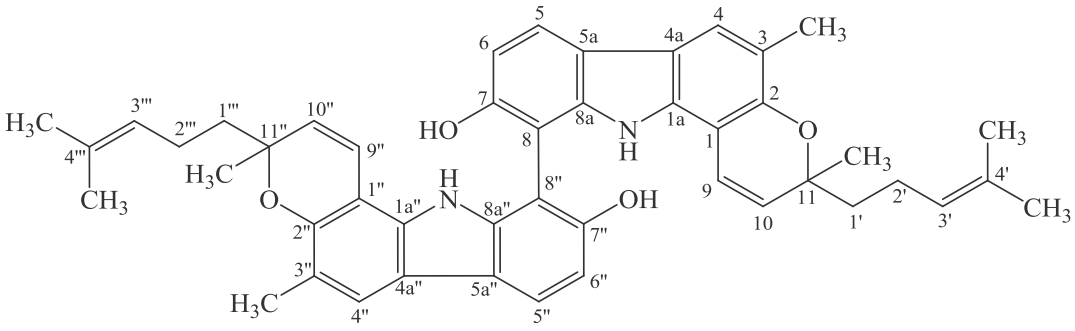


Figure 4.2.5.1b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Bismurrayafoline-E **(Mk27)**

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4.2.5.2 Bispyrayafoline (Mk28)



Mk28

Bispyrayafoline **(Mk28)** was isolated from the leaves of *M. koenigii* as brownish oil with $[\alpha]_D^{25}$ +6.0 (CH_3OH; c 0.02). The UV spectrum of **Mk28** showed absorptions at 217, 262, 285 and 335 nm which were closely similar to those of mahanine **(Mk16)** (refer Section 4.2.3.2) and thus indicated the presence of typical features of monomeric pyranocarbazole skeleton in **Mk28** (Cordell, 2008). The IR spectrum displayed intense absorption bands at 3680 and 3329 cm^{-1} indicated the presence of OH and NH functionalities. This was further confirmed by the signals at δ 5.16 and δ 7.55 which denoted to the presence of OH and NH group, respectively, in the ^1H NMR spectrum (Table 4.2.5.2 and Figure 4.2.5.2a) of **Mk28**. The mass spectral of **Mk28** showed the $[M]^+$ peak at *m/z* 692.3610 by HRESIMS. A combination of mass spectral, ^1H and ^{13}C NMR spectroscopic data provided a molecular formula of $C_{46}H_{48}N_2O_4$.

Dimeric carbazole alkaloid of **Mk28** was determined from the ^{13}C NMR signals which showed six pairs of unoverlapped carbon signals (Table 4.2.5.2 and Figure 4.2.5.2b) at δ 117.9 and δ 117.8 (C-3 and C-3"), δ 128.9 and δ 128.8 (C-10 and C-10"), δ 40.8 and δ 40.7 (C-1' and C-1'"), δ 22.8 and δ 22.7 (C-2' and C-2'"), δ 124.2

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and δ 124.1 (C-3' and C-3'"), and δ 131.8 and δ 131.7 (C-4' and C-4'"). The remaining carbon signals in its ^{13}C NMR spectrum were overlapped. The DEPT spectrum also helped to differentiate its carbons as eight methyls, four methines, 12 methylenes and 22 quaternary carbons.

The ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of **Mk28** indicated the presence of two prenyl units, inferred from signals at δ 5.11 (*t*, *J* = 6.7 Hz, H-3'), 2.11 (*m*, H-2'), 1.73, 1.67 (*m*, H-1'), 1.63, 1.62 (*s*, 4'-CH_3, 3H each), 1.54, 1.53 (*s*, 4'-CH_3, 3H each) and a methyl signal at δ 2.49 (*s*, 3-CH_3). A set of AB-type doublets, integrated for the presence of two protons each in the aromatic region (ring-C) at δ 7.71 (*d*, *J* = 8.6 Hz, H-5) and δ 6.73 (*d*, *J* = 8.6 Hz, H-6) of the carbazole nucleus. The positions of these signals were also supported by long range HMBC interactions. Based on these spectroscopic studies, structure **Mk28** was determined as bispyrayafoline (Tachibana et al., 2001).

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Table 4.2.5.2

^{1}H NMR [500 MHz, δ_H (J Hz)] and ^{13}C NMR [125 MHz, δ_C] of Bispyrayafoline ***(Mk28)****

Position	δ ^{13}C	DEPT	δ ^{1}H
1, 1"	105.0	qC	
1a, 1a"	136.1	qC	
2, 2"	150.4	qC	
3, 3"	117.8, 117.9	qC	
4, 4"	122.4	CH	7.85 (*s*)
4a, 4a"	118.0	qC	
5, 5"	119.5	CH	7.71 (*d*, 8.6 Hz)
5a, 5a"	117.7	qC	
6, 6"	110.0	CH	6.73 (*d*, 8.6 Hz)
7, 7"	151.2	qC	
8, 8"	99.1	qC	
8a, 8a"	137.9	qC	
9, 9"	117.5	CH	6.44 (*d*, 10.3 Hz)
10, 10"	128.8, 128.9	CH	5.54 (*d*, 10.3 Hz)
11, 11"	78.3	qC	
NH, NH"			7.55 (*br s*)
3-CH_3, 3"-CH_3	16.8	CH_3	2.49 (*s*)
7-OH, 7"-OH			5.16 (*br s*)
11-CH_3,11"-CH_3	16.8	CH_3	1.39 (*s*)
1', 1"'	40.8, 40.7	CH_2	1.73 (*m*), 1.67 (*m*)
2', 2"'	22.8, 22.7	CH_2	2.11 (*m*)
3', 3"'	124.2, 124.1	CH	5.11 (*t*, 6.7 Hz)
4', 4"'	131.7, 131.8	qC	
4'-CH_3, 4"'-CH_3	25.7	CH_3	1.63 (*s*), 1.62 (*s*)
4'-CH_3, 4"'-CH_3	17.7	CH_3	1.54 (*s*), 1.53 (*s*)

Note. *Solvent used was $CDCl_3$; assignments based on COSY, HMQC and HMBC.

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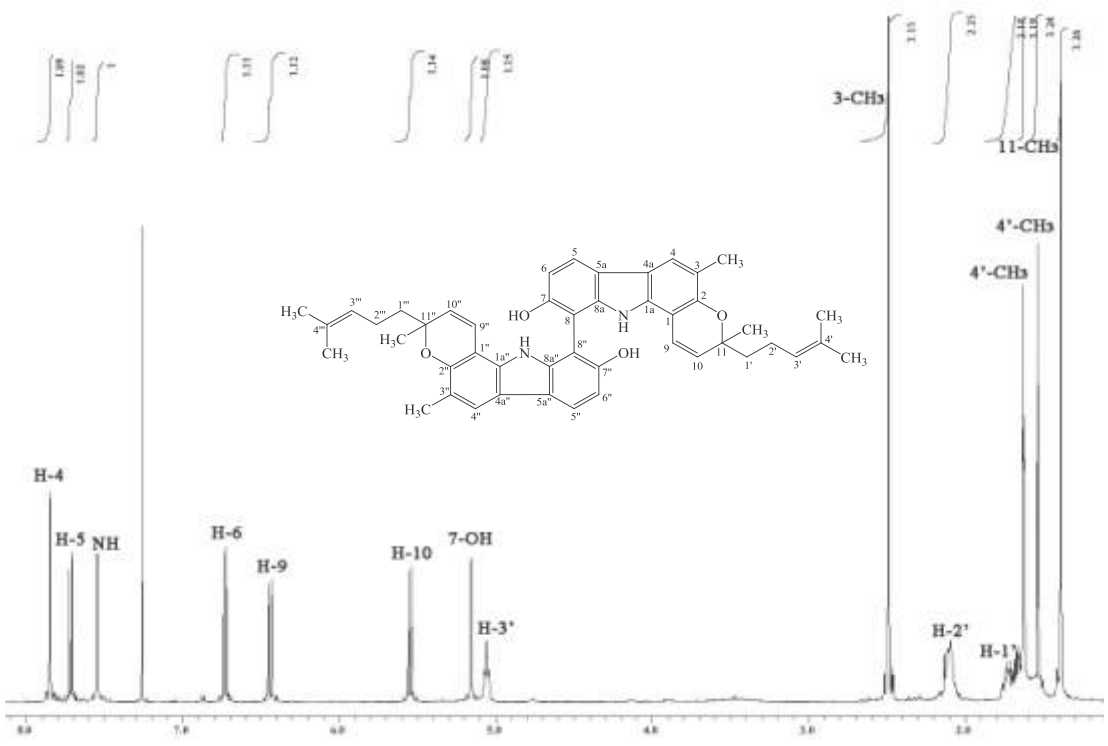


Figure 4.2.5.2a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Bispyrayafoline **(Mk28)**

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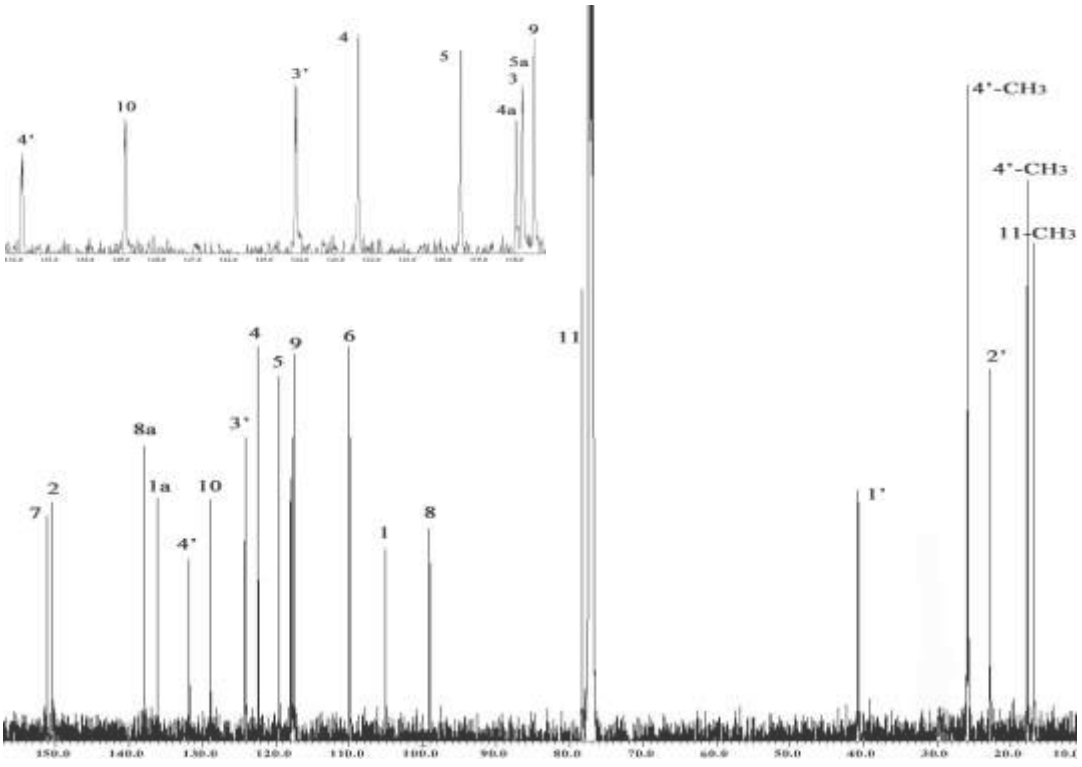
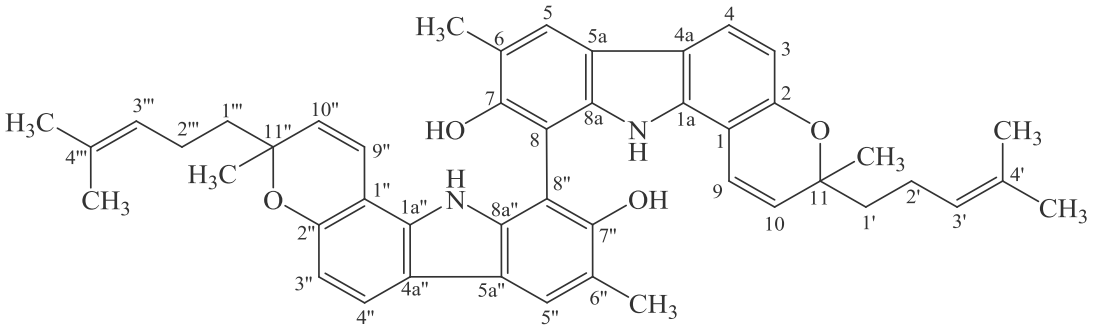


Figure 4.2.5.2b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Bispyrayafoline **(Mk28)**

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4.2.5.3 Bisisomahanine (Mk29)



Mk29

Mk29 was an optically active compound first time isolated from *M. koenigii* with $[\alpha]_D^{25}$ +3.5 (CH_3OH; c 0.02) as pale yellow solid [m.p.: 135–137 °C; m.p.: 130–140 °C (Cuong et al., 2004)]. A molecular ion peak at *m/z* 692.3601 in its mass spectral data revealed a molecular formula of $C_{46}H_{48}N_2O_4$. The IR spectrum of **Mk29** was identical to **Mk17** showed absorptions at ν_{max} 3720 and 3339 cm^{-1} indicated the presence of a NH group and a phenolic hydroxyl group. This was further confirmed by the appearance of proton signals at δ 7.54 and δ 5.15, respectively, in its ^{1}H NMR spectrum (Table 4.2.5.3 and Figure 4.2.5.3a). The UV spectrum of this compound with λ_{max} 211, 241, 268 and 335 was closely similar to those of isomahanine **(Mk17)** and thus further suggesting for the presence of an angular pyranocarbazole skeleton of carbazole alkaloids (Cordell, 2008).

In the ^{1}H NMR spectrum ($CDCl_3$, 500 MHz), the presence of a prenyl substituent [δ 5.06 (*t*, *J* = 7.1 Hz, H-3'), 2.10 (*m*, H-2'), 1.75, 1.66 (*m*, H-1'), 1.64 (*s*, 4'-CH_3) and 1.55 (*s*, 4'-CH_3)] and the methyl substituent (δ 2.49) at C-6, respectively, were also found to be identical to those in isomahanine **(Mk17)**. The presence of a

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pair of *othro*-coupled proton at δ 6.73 (*d*, *J* = 8.6 Hz, H-3) and δ 7.71 (*d*, *J* = 8.6 Hz, H-4), and a singlet at δ 7.85 (*s*, H-5), suggested that the methyl group and the hydroxyl group should be at the positions 6 and 7, respectively. The number of these signals were only half of that expected, suggested that **Mk29** has a completely symmetrical structure.

Most of the 1D and 2D NMR spectra of **Mk29** were similar to those in **Mk17**, with the exception of an additional nonprotonated aromatic carbon signal at C-8 (δ 99.0) and lack of one methine aromatic proton of the carbazole nucleus. Dimeric carbazole alkaloid of **Mk29** was further supported by the ^{13}C NMR signals of seven unoverlapped pair of carbon signals (Table 4.2.5.3 and Figure 4.2.5.3b) at δ 117.8 and δ 117.7 (C-4a and C-4a"), δ 128.9 and δ 128.8 (C-10 and C-10"), δ 40.8 and δ 40.7 (C-1' and C-1'"), δ 22.8 and δ 22.7 (C-2' and C-2'"), δ 124.1 (C-3' and C-3'"), δ 26.0 (11-CH_3' and 11-CH_3'"), and δ 131.8 and δ 131.7 (C-4' and C-4'"), and the remaining were overlapping.

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Based on these data, the basic structural unit of **Mk29** was concluded to be isomahanine **(Mk17)**. The position of the linkage of two molecules of **Mk29** is between the quaternary carbon C-8 and C-8" for both the carbazole nucleus. These data confirmed the structure **Mk29** for bisisomahanine, a dimeric carbazole alkaloid of isomahanine **(Mk17)**, which was previously reported from *Glycosmis stenocarpa* by Cuong et al. (2004).

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Table 4.2.5.3

^{1}H NMR [500 MHz, δ_H (J Hz)] and ^{13}C NMR [125 MHz, δ_C] of Bisisomahanine ***(Mk29)****

Position	δ ^{13}C	DEPT	δ ^{1}H
1, 1"	105.0	qC	
1a, 1a"	136.1	qC	
2, 2"	151.1	qC	
3, 3"	110.0	CH	6.73 (*d*, 8.6 Hz)
4, 4"	119.5	CH	7.71 (*d*, 8.6 Hz)
4a, 4a"	117.7, 117.8	qC	
5, 5"	122.4	CH	7.85 (*s*)
5a, 5a"	118.0	qC	
6, 6"	117.5	qC	
7, 7"	150.4	qC	
8, 8"	99.0	qC	
8a, 8a"	137.9	qC	
9, 9"	117.5	CH	6.44 (*d*, 10.3 Hz)
10, 10"	128.8, 128.9	CH	5.65 (*d*, 10.3 Hz)
11, 11"	78.3	qC	
NH, NH"			7.54 (*br s*)
6-CH_3, 6"-CH_3	16.8	CH_3	2.49 (*s*)
7-OH, 7"-OH			5.15 (*br s*)
11-CH_3, 11"-CH_3	26.0, 26.0	CH_3	1.39 (*s*)
1', 1"'	40.7, 40.8	CH_2	1.66, 1.75 (*m*)
2', 2"'	22.7, 22.8	CH_2	2.10 (*m*)
3', 3"'	124.1, 124.1	CH	5.06 (*t*, 7.1 Hz)
4', 4"'	131.7, 131.8	qC	
4'-CH_3, 4"'-CH_3	25.7	CH_3	1.64 (*s*)
4'-CH_3, 4"'-CH_3	17.7	CH_3	1.55 (*s*)

Note. *Solvent used was $CDCl_3$; assignments based on COSY, HMQC and HMBC.

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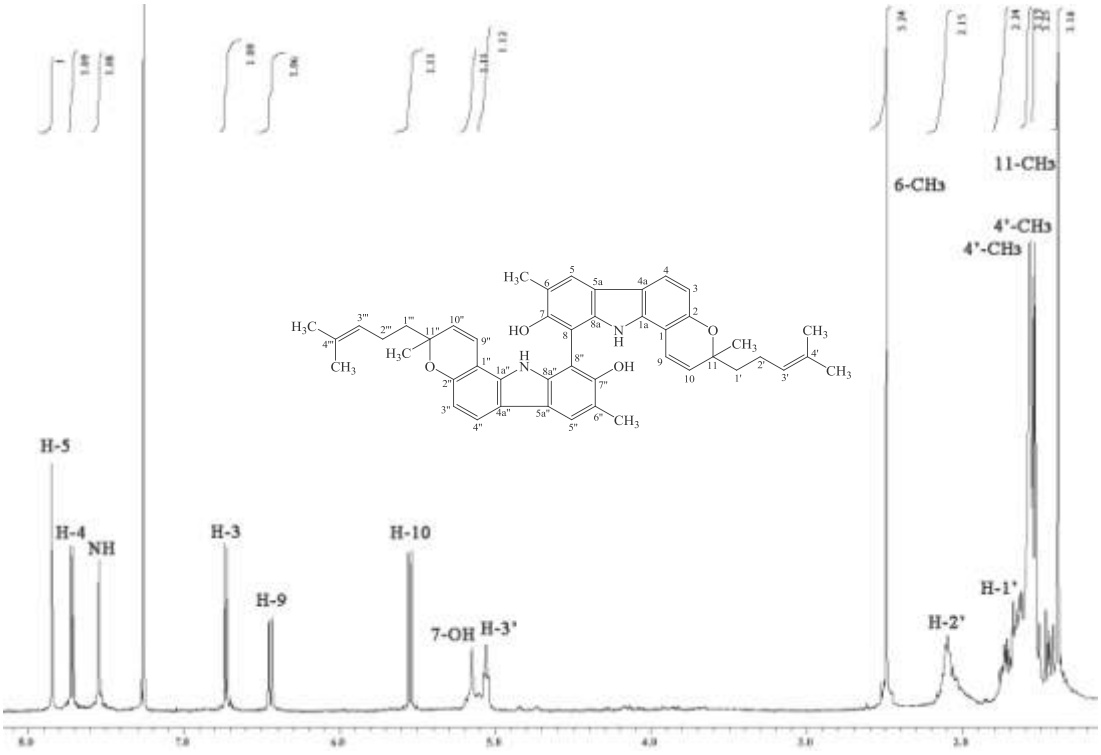


Figure 4.2.5.3a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Bisisomahanine **(Mk29)**

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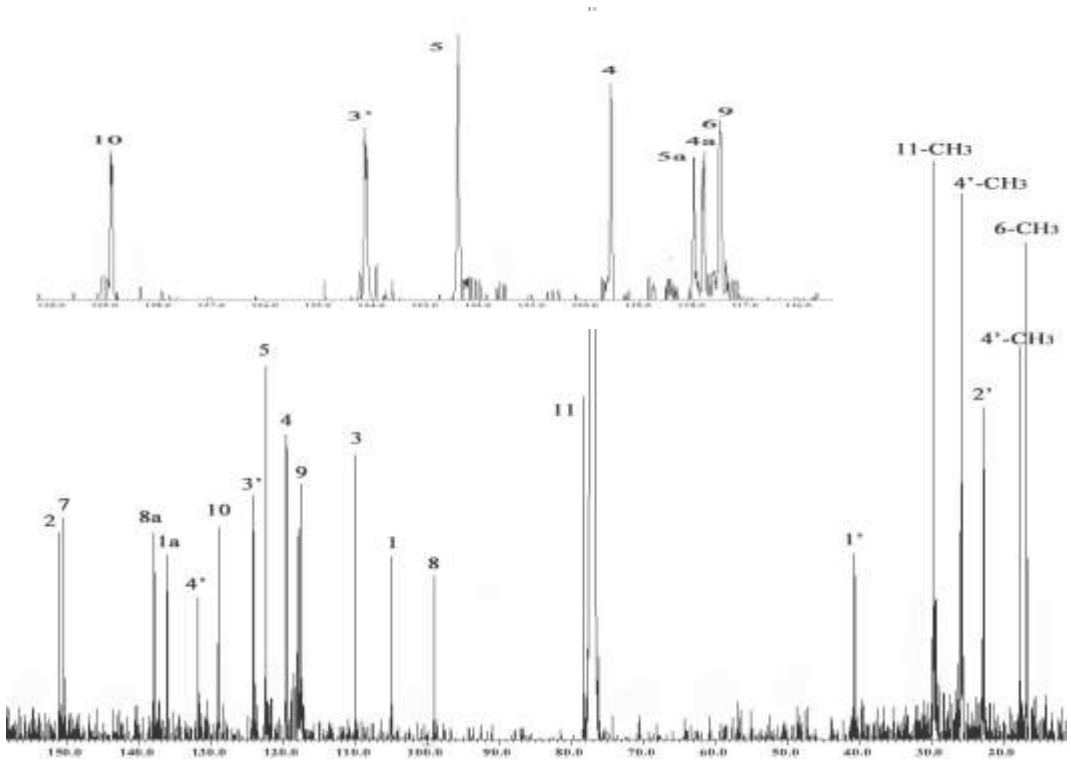
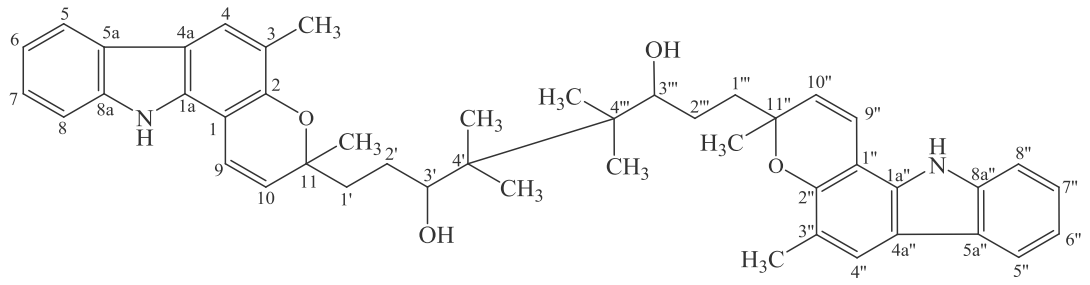


Figure 4.2.5.3b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Bisisomahanine **(Mk29)**

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4.2.5.4 Bismahanimboline (Mk30)



Mk30

Mk30 was isolated as brownish oil with $[\alpha]_D^{25}$ –5.5 (CH_3OH; c 0.015). The IR spectrum of **Mk30** (Figure 4.2.5.4j) showed absorptions at ν_{max} 3377 cm^{-1} indicated the presence of a phenolic hydroxyl and/or NH group. The UV spectrum of this compound (λ_{max} 239, 262, 298 and 338) was identical to those of mahanimbine **(Mk15)** and suggested the presence of an angular pyranocarbazole skeleton (Cordell, 2008).

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The spectroscopic data was listed in Table 4.2.5.4. The NMR spectra are in the Figure 4.2.5.4a to Figure 4.2.5.4i. The ^{13}C NMR and DEPT spectra of **Mk30** (Figure 4.2.5.4b and Figure 4.2.5.4c) showed the presence of five olefinic, overlapped pair of aromatic sp^2 methine carbon signals at δ 110.5 (C-8, C-8"), 119.4 (C-5, C-5"), 119.6 (C-6, C-6"), 121.5 (C-4, C-4") and 124.4 (C-7, C-7"); two sp^3 aliphatic methine carbon signals at δ 64.3 (C-3'), 64.6 (C-3'"); 14 sp^2 quaternary carbon signals 104.0 (C-1), 104.2 (C-1"), 116.8 (C-4a, C-4a"), 118.4 (C-3, C-3"), 123.9 (C-5a, C-5a"), 134.9 (C-1a, C-1a"), 139.5 (C-8a, C-8a"), 149.7 (C-2), 149.8 (C-2"); four sp^3 quaternary carbon signals at δ 58.6 (C-4'"), 58.7 (C-4'), 78.2 (C-11) and 77.8 (C-11"); eight sp^3 methyl carbon signals at δ 16.2 (3-CH_3, 3"-CH_3), 18.6 (4'-CH_3), 18.7 (4'"-CH_3), 24.9 (4'-CH_3,

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4'''-CH_3), 25.7 (11-CH_3), 26.4 (11''-CH_3); and four sp^3 methylene carbon signals at δ 24.1 (C-2'), 23.8 (C-2'''), 37.2 (C-1''') and 37.7 (C-1'). Two pair of olefinic carbon signals [δ 118.0 (C-9''), 118.2 (C-9), 127.9 (C-10), 128.4 (C-10'')] were also found for the pyrancarbazole. A pair of aliphatic methine carbons at C-3' (δ 64.3, 64.6) was detected, which has been shifted to a higher field when compared to those pyranocarbazole with a prenyl group at C-3' of the pyran ring. A likely pathway proposed in Scheme 4.2.5.4, a NADPH-induced epoxidation reaction onto an electron-deficient electrophilic double bond at C-3', led to the formation of an epoxide moiety and the disappearance of the double bond at C-3'. A new ethylenic double bond was formed after that at C-4' when protonation of epoxide oxygen took place led to the formation of a hydroxyl group, followed by the creation of new C-C linkage between C-4' and C-4''' among the two monomers via radical-radical dimerization reaction.

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In the ^{1}H NMR spectrum (Figure 4.2.5.4a), the number of proton signals were half of that expected suggested that this compound is with a dimeric configuration. A pair of AB doublets at H-9 (δ 6.69) and H-10 (δ 5.63, δ 5.66) with coupling constant 9.8 Hz each, gave further evidence for the presence of two sets of pyran ring double bond in **Mk30**. The ^{1}H-^{1}H COSY, NOESY, HMQC and HMBC data (Figure 4.2.5.4d to Figure 4.2.5.4g) also disclosed the spatial structure which corresponding to the monomer of **Mk30**. This can be explained when two identical monomeric units are linked via carbon-carbon linkages from C-4' of one half to C-4''' of the other, which can be distinguished by ^{13}C NMR signals. This was further confirmed by its mass spectrometry revealed the molecular formula $C_{46}H_{52}N_2O_4$ with a molecular ion peak at *m/z* 696.3125 ($[M]^+$, calcd. mass: 696.3927) along with a base signal at *m/z* 348.1956 by its HRESIMS (Figure 4.2.5.4k).

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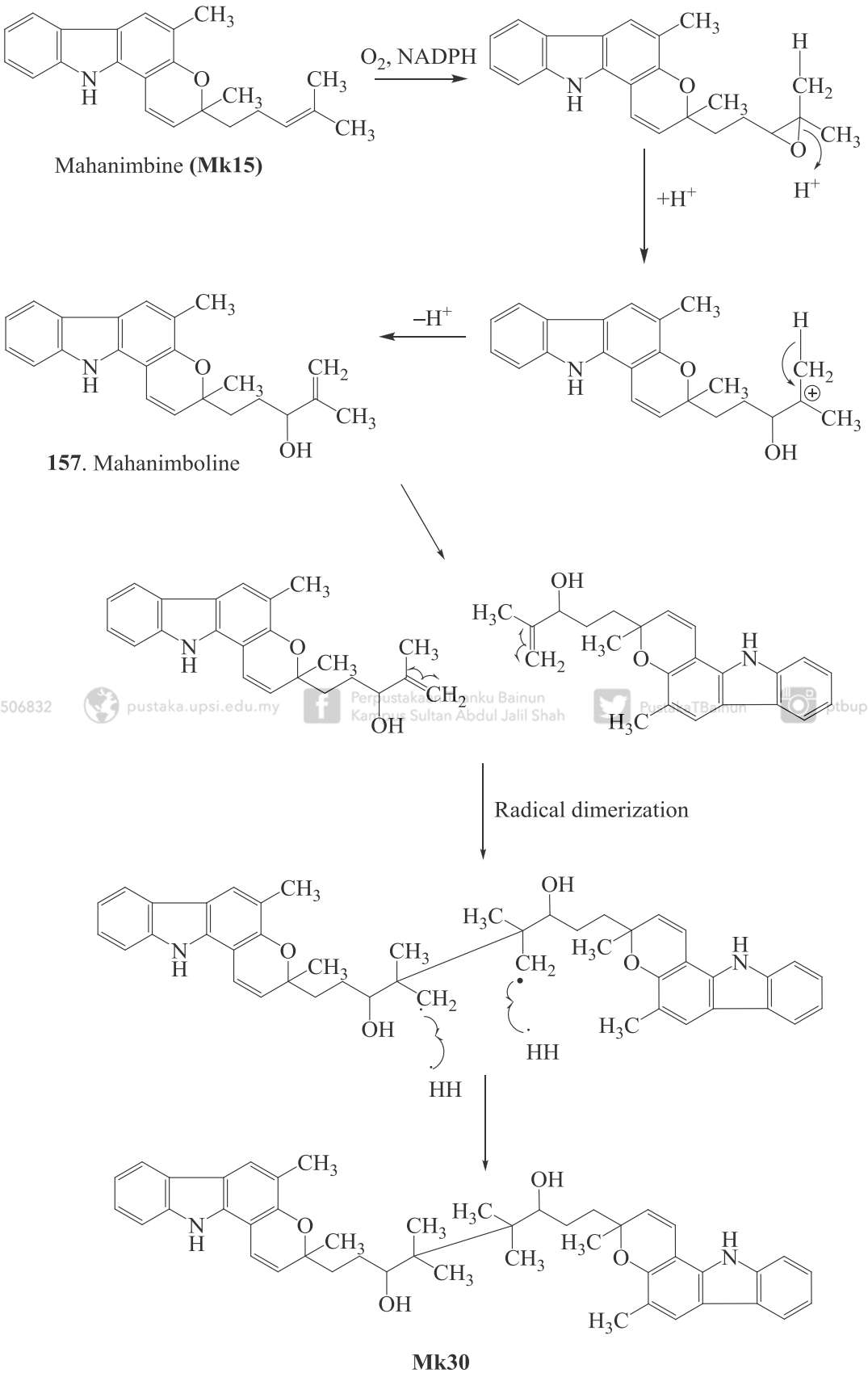
The stereochemistry at C-3' and C-3''' can be determined by NOESY experiment (Figure 4.2.5.4g). Since in the alternative arrangement in which the relative stereochemistry of H-1'α has correlation with 11-CH_3 and H-3'. Meanwhile, H-1'''β showed correlation with 11-CH_3 and H-2''' at the β orientation. H-3''' was observed to be correlated to the H-1''' at the α orientation. These observations required both H-3' and H-3''' to be disposed *anti* with respect to each other. A key finding obtained from NOESY is the correlation observed between H-3' and H-3''' which indicated that the hydroxyl moiety and the proton at H-3' and H-3''' must be on the same side. These observations therefore allowed the configurations at C-3' and C-3''' to be assigned as either 3'(*S*), 3'''(*S*) or 3'(*R*), 3'''(*R*), and the 3'(*S*), 3'''(*R*) and 3'(*R*), 3'''(*S*) diastereomers are ruled out by the observed H-3'/H-1'α and H-3'''/H-1'''α in NOESY.

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Based on these data, the basic structural unit of **Mk30** was concluded to be that of mahanimboline **157** (Roy et al., 1979). These data suggested the structure **Mk30** for bismahanimboline, a new dimeric carbazole alkaloid of mahanimboline and was a new compound that first time isolated from *M. koenigii*.

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Scheme 4.2.5.4. Proposed Biogenetic Pathway to **Mk30**

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Table 4.2.5.4

1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of Bismahanimboline ***(Mk30)****

Position	δ ^{13}C	DEPT	δ ^{1}H	HMBC correlations	COSY
1, 1"	104.0, 104.2	qC		H-9, H-10	
1a, 1a"	134.9	qC		H-4, H-9	
2, 2"	149.8, 149.7	qC		H-4, H-9, 3-CH_3	
3, 3"	118.4	qC		3-CH_3	
4, 4"	121.5	CH	7.66 (*s*)	3-CH_3	
4a, 4a"	116.8	qC			
5, 5"	119.4	CH	7.91 (*d*, 8.0 Hz)	H-7	H-6
5a, 5a"	123.9	qC		H-4, H-5, H-6	
6, 6"	119.6	CH	7.18 (*td*, 8.0, 1.0 Hz)	H-5, H-7	H-5, H-7
7, 7"	124.4	CH	7.31 (*td*, 8.0, 1.0 Hz)		H-6, H-8
8, 8"	110.5	CH	7.38 (*d*, 8.0 Hz)	H-7	H-7
8a, 8a"	139.5	qC		H-5, H-7	
9, 9"	118.2, 118.0	CH	6.69 (*d*, 9.8 Hz)		H-10
10, 10"	127.9, 128.4	CH	5.63, 5.66 (*d*, 9.8 Hz each)	11-CH_3	H-9
11, 11"	78.2, 77.8	qC		H-9, H-10, 11-CH_3	
NH, NH"			7.90 (*s*)		
3-CH_3, 3"-CH_3	16.2	CH_3	2.31 (*s*)	H-4	
11-CH_3, 11"-CH_3	25.7, 26.4	CH_3	1.46 (*s*)		
1α', 1α"'	37.7, 37.2	CH_2	1.91 (*m*)	11-CH_3	H-2'
1β', 1β"'			1.90 (*m*)		
2α', 2α"'	24.1, 23.8	CH_2	1.74 (*m*)	H-1', H-3'	H-1', H-3'
2β', 2β"'			1.73 (m)		
3', 3"'	64.6, 64.3	CH	2.72, 2.74 (*t*, 7.2 Hz each)	4'-CH_3	H-2'
4', 4"'	58.7, 58.6	qC		4'-CH_3	
3'-OH, 3"'-OH			#		
4'-CH_3, 4"'-CH_3	24.9	CH_3	1.27 (*s*), 1.28 (*s*)	4'-CH_3	
4'-CH_3, 4"'-CH_3	18.6, 18.7	CH_3	1.23 (*s*)	4'-CH_3	

Note. *Solvent used was $CDCl_3$; assignments based on COSY, NOESY, HMQC and HMBC. # Peak not available

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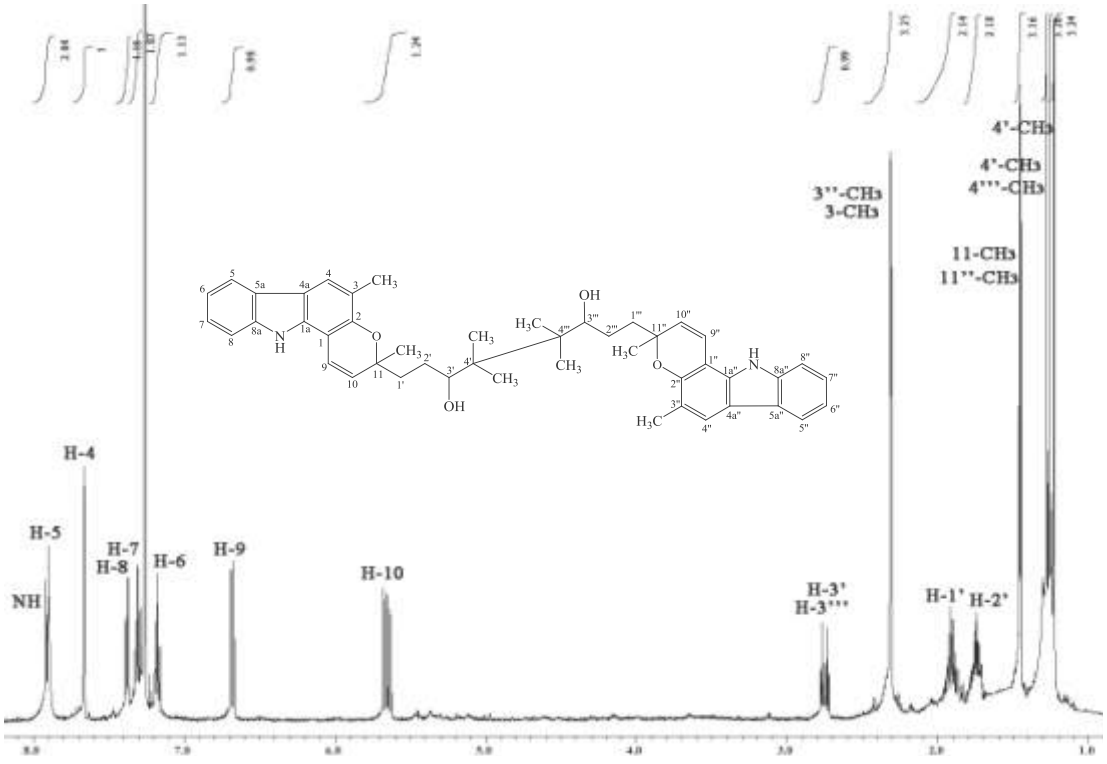


Figure 4.2.5.4a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Bismahanimboline **(Mk30)**

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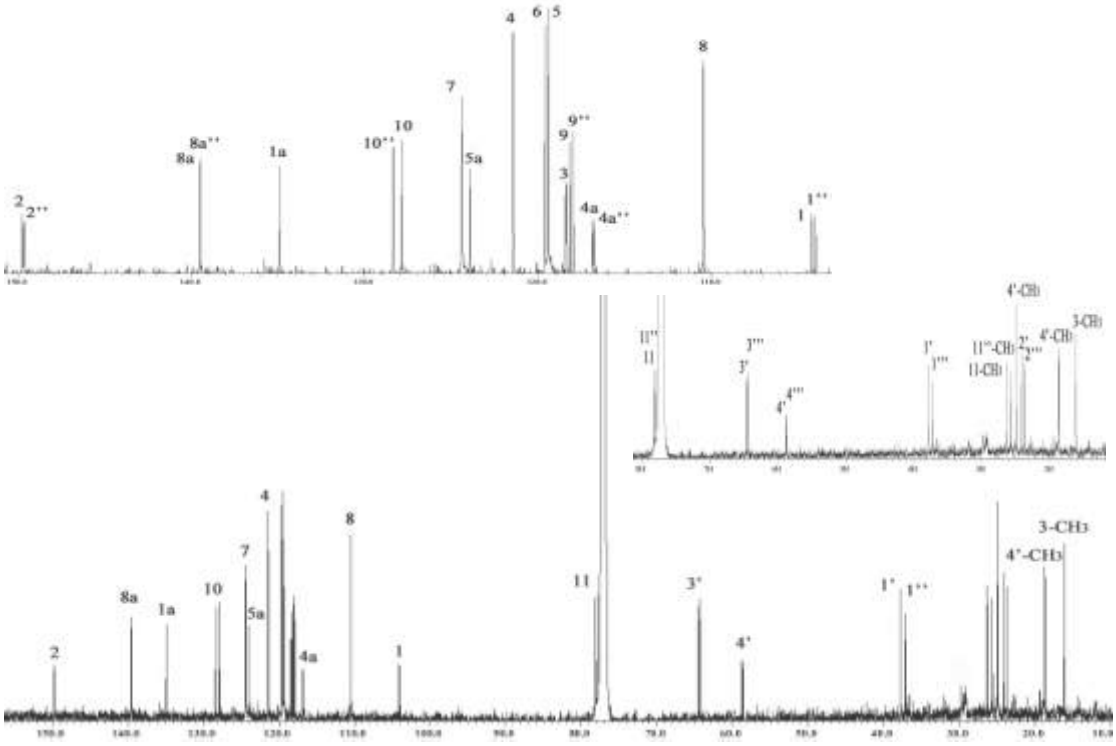


Figure 4.2.5.4b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Bismahanimboline **(Mk30)**

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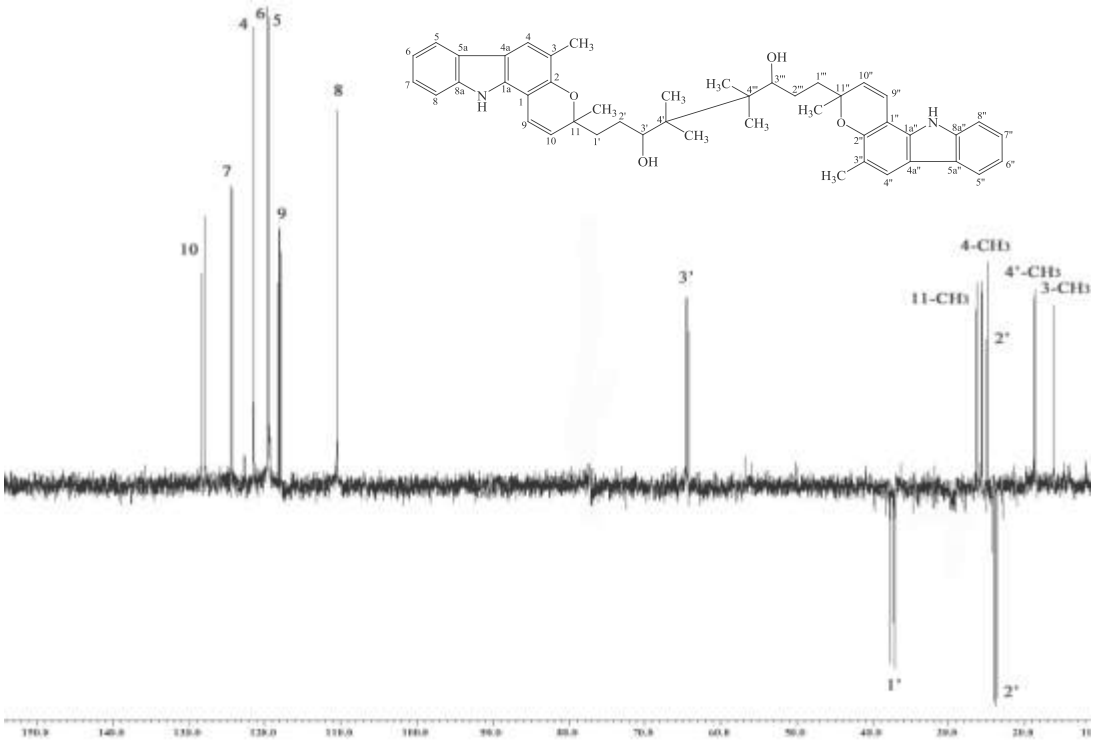


Figure 4.2.5.4c. DEPT-135 Spectrum ($CDCl_3$, 125 MHz) of Bismahanimboline **(Mk30)**

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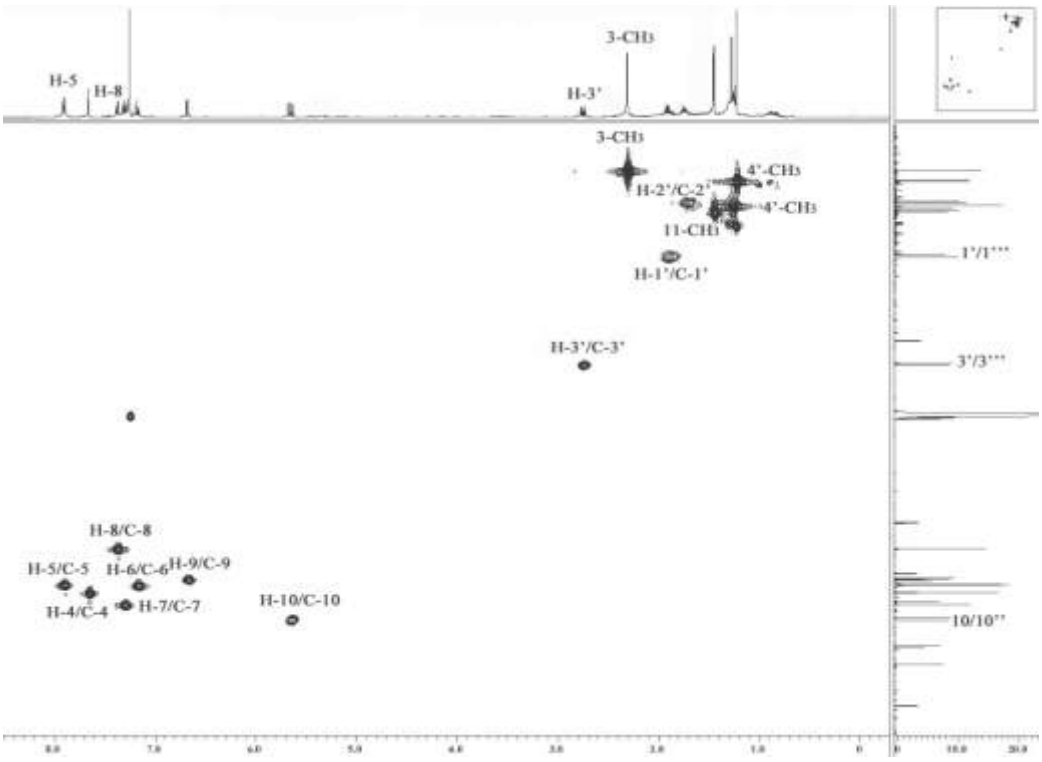


Figure 4.2.5.4d. HMQC Spectrum ($CDCl_3$, 500 MHz) of Bismahanimboline **(Mk30)**

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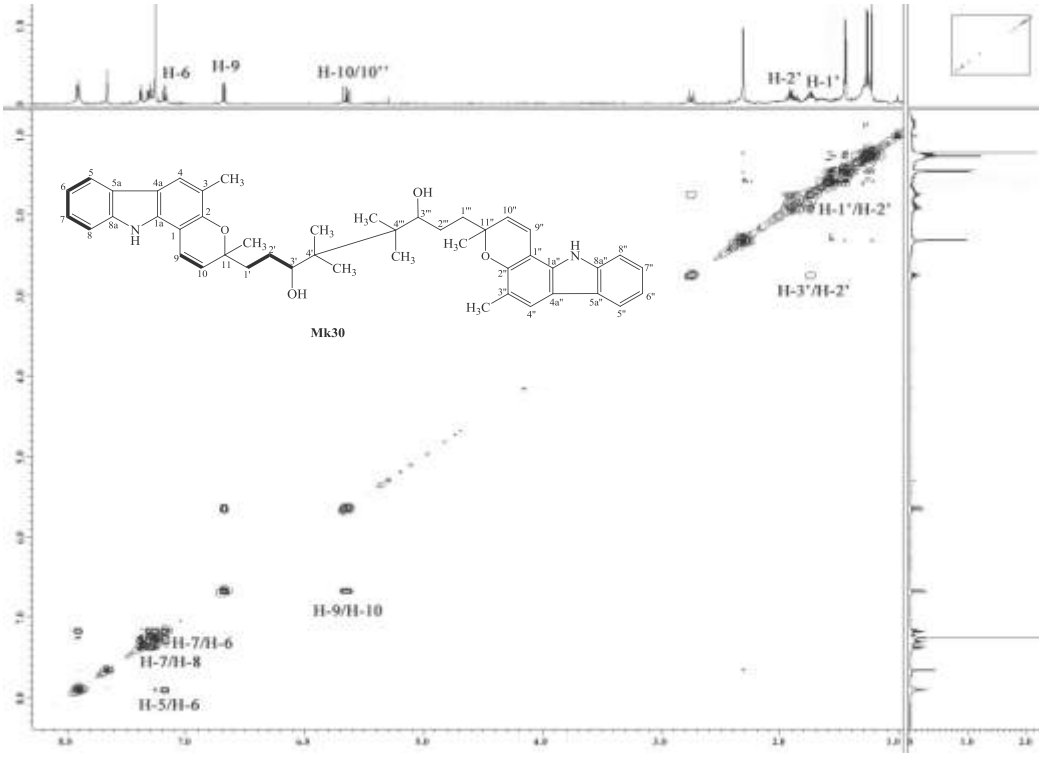


Figure 4.2.5.4e. ^{1}H-^{1}H COSY Spectrum ($CDCl_3$, 500 MHz) of Bismahanimboline **(Mk30)**

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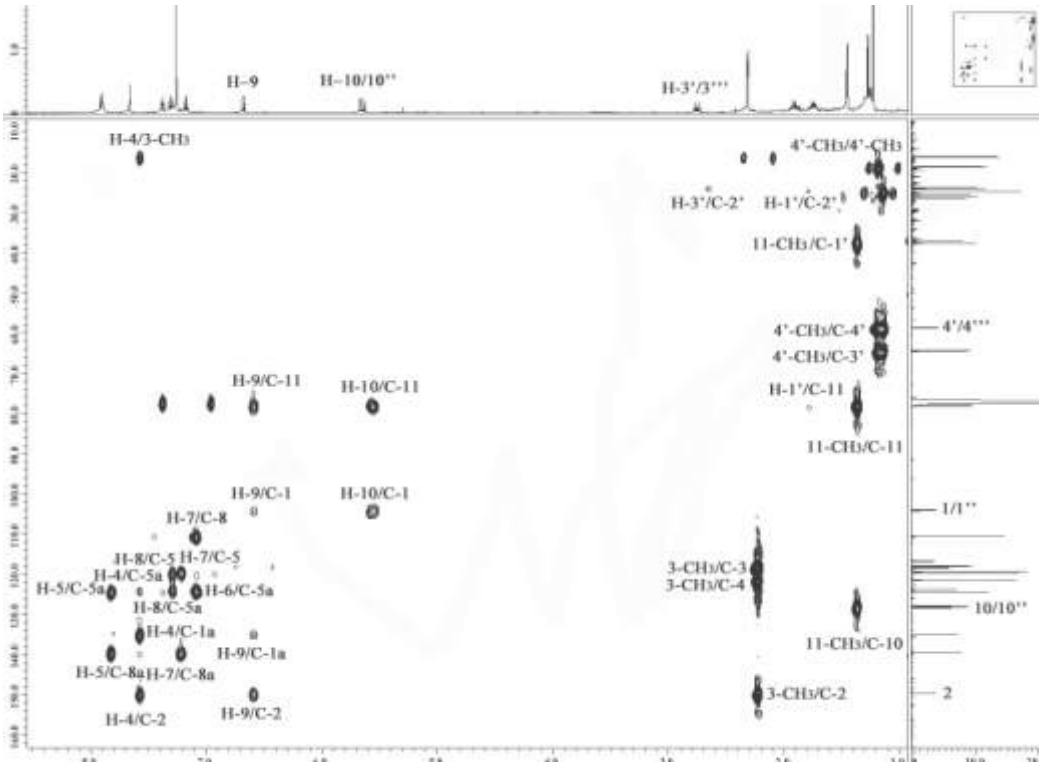


Figure 4.2.5.4f. HMBC Spectrum ($CDCl_3$, 500 MHz) of Bismahanimboline **(Mk30)**

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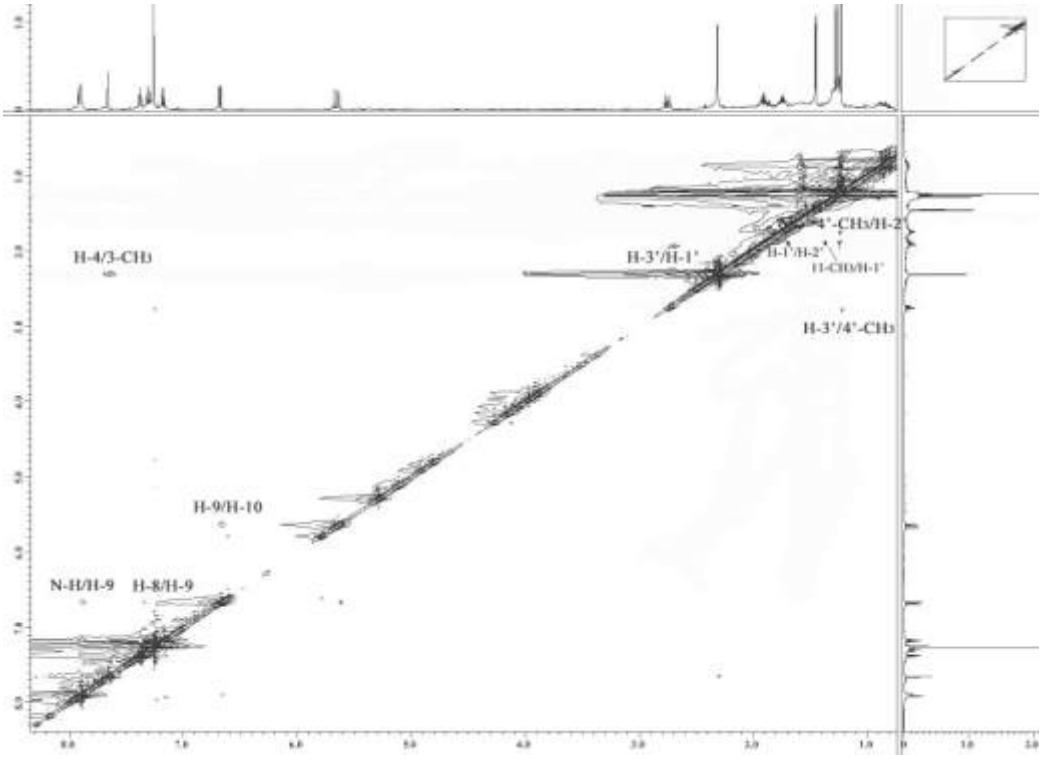


Figure 4.2.5.4g. NOESY Spectrum ($CDCl_3$, 500 MHz) of Bismahanimboline **(Mk30)**

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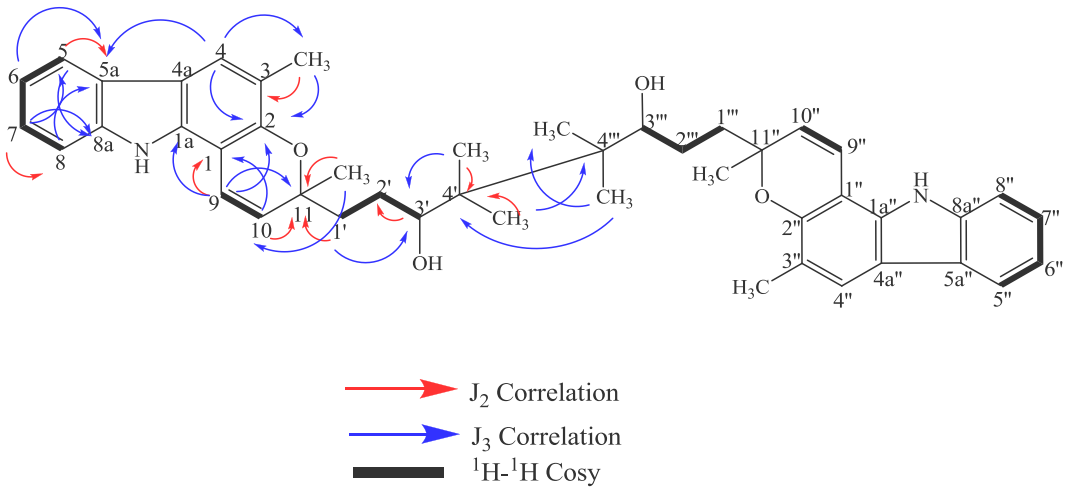


Figure 4.2.5.4h. Selected ^1H-^1H COSY, HMBCs and NOESY of **Mk30**

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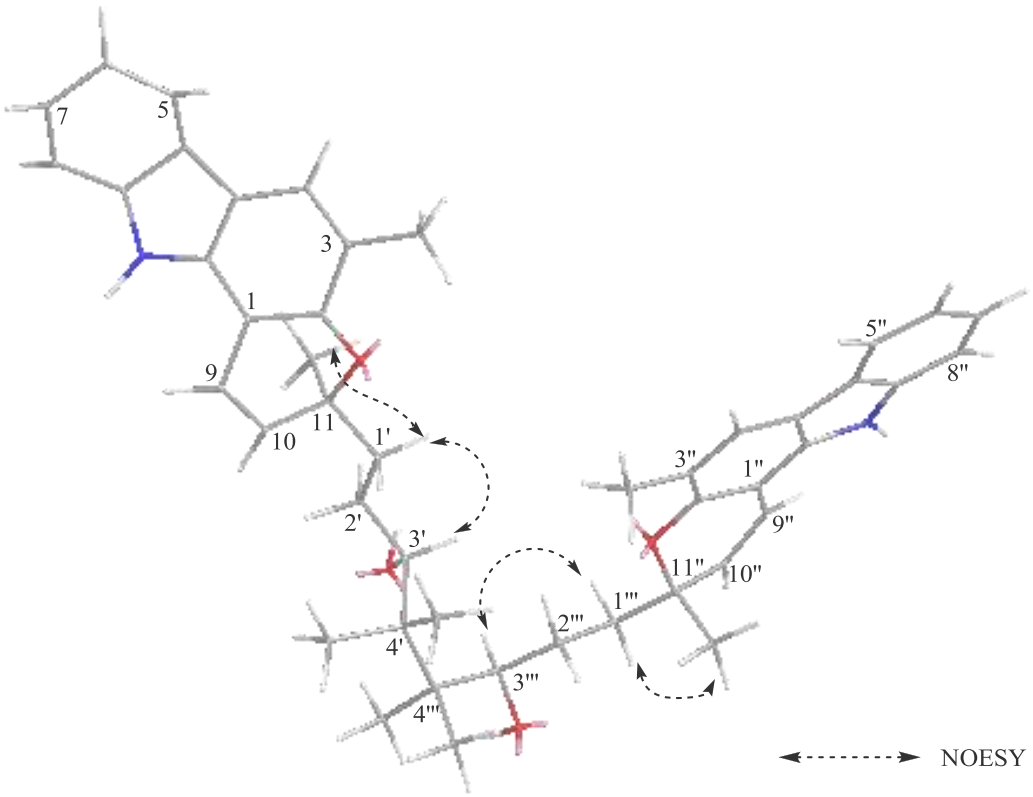


Figure 4.2.5.4i. Selected NOESY Correlations and Relative Stereochemistry for **Mk30**

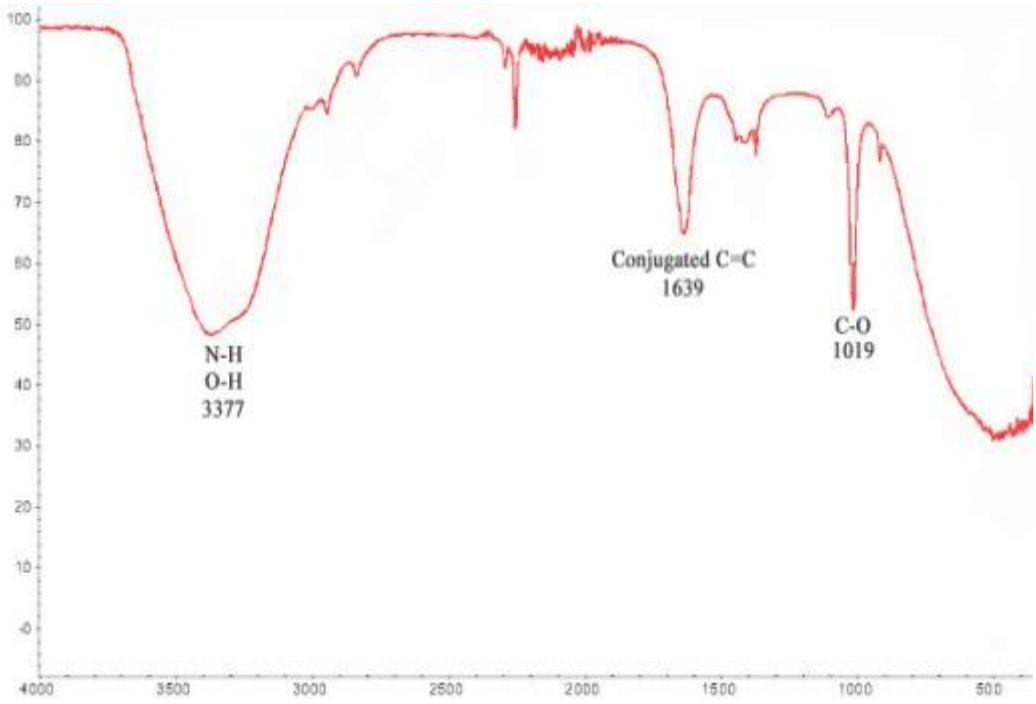


Figure 4.2.4.3j. IR Spectrum of Bismahanimboline **(Mk30)**

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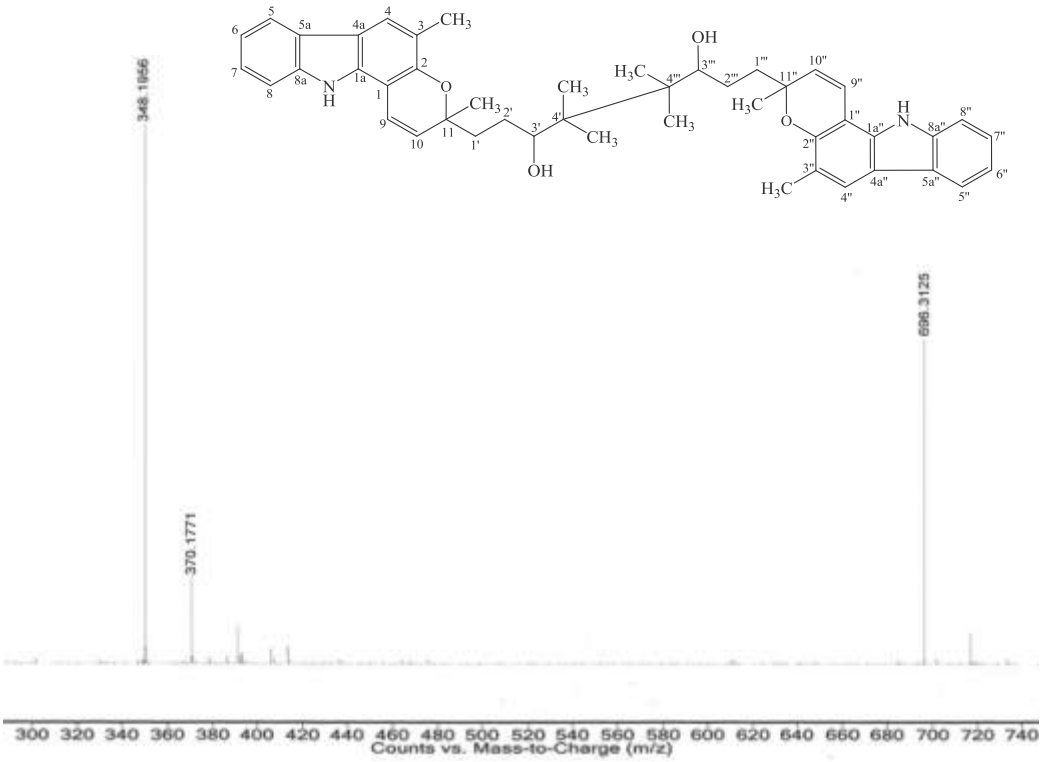


Figure 4.2.4.3k. Mass Spectrum of Bismahanimboline **(Mk30)**

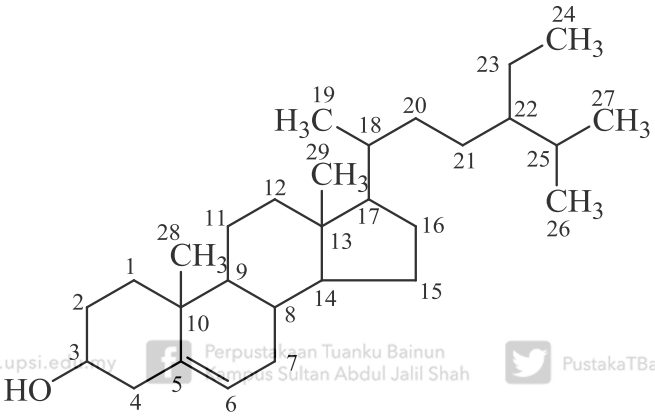
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4.2.6 Others

A wide range of bioactive compounds such as steroids, flavonoids and terpenoids may also be isolated from *M. koenigii*. The details of isolated compounds which are not under the categories carbazole alkaloids were discussed in the following section.

4.2.6.1 β-Sitosterol (Mk31)



Mk31

Mk31 was isolated as white solid [m.p.: 134–136 °C; m.p.: 134–135 °C (Chaturvedula & Prakasu, 2012)] with $[\alpha]_D^{25}$ –11.0 (CH_3OH; c 0.02). The mass spectral data of **Mk31** with molecular ion peak at 415.3010 $[M+H]^+$ was consistent with its molecular formula $C_{29}H_{50}O$, which was also supported by NMR spectra. **Mk31** was found to be an unsaturated sterol, which showed only 29 carbons instead of 30 carbons for tetracyclic triterpenes. This was further supported by the UV spectrum which showed maxima absorption at 262, 268 and 277 nm. The IR spectrum displayed stretching band for O–H (3346 cm^{-1}), C=C (1645 cm–1) and C–O (1024 cm^{-1}).

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The ^{1}H NMR spectrum of **Mk31** ($CDCl_3$, 500 MHz) (Table 4.2.6.1 and Figure 4.2.6.1a) showed the presence of six methyl signals that appeared as two methyl singlets at δ 0.68 (H-28) and δ 1.01 (H-29); three methyl doublets that appeared at δ 0.81 (*J* = 6.4 Hz, H-27), δ 0.84 (*J* = 6.4 Hz, H-26), and δ 0.92 (*J* = 6.5 Hz, H-19); and a methyl triplet at δ 0.85 (*J* = 7.2 Hz, H-24). The ^{1}H NMR spectrum of **Mk31** also showed signal for one olefinic proton at δ 5.35 (*dd*, *J* = 5.2, 1.0 Hz, H-6) and a proton signal at δ 3.52 corresponding to C-3 hydroxyl group which appeared as a multiplet.

The ^{13}C NMR (Table 4.2.6.1 and Figure 4.2.6.1b) along with ^{1}H-^{1}H COSY, HMQC and HMBC showed twenty nine carbon signals included six methyls, eleven methylenes, nine methines and three quaternary carbons. Thus, the structure of **Mk31** was determined to be β-sitosterol that was consistent to the literature values reported by Chaturvedula and Prakasu (2012).

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Table 4.2.6.1

^{1}H NMR [500 MHz, δ_H (J Hz)] and ^{13}C NMR [125 MHz, δ_C] of β-Sitosterol ***(Mk31)****

Position	δ ^{13}C	DEPT	δ ^{1}H
1	37.3	CH_2	1.10 (*m*)
			1.85 (*m*)
2	31.7	CH_2	1.53 (*m*)
			1.98 (*m*)
3	71.9	CH	3.52 (*m*)
4	42.4	CH_2	2.30 (*m*)
5	140.8	qC	
6	121.8	CH	5.35 (*dd*, 5.2, 1.0 Hz)
7	32.0	CH_2	1.86 (*m*)
8	32.1	CH	1.49 (*m*)
9	50.2	CH	0.93 (*m*)
10	36.6	qC	
11	21.2	CH_2	1.50 (*m*)
12	39.9	CH_2	1.15 (*m*)
			2.01 (*m*)
13	42.4	qC	
14	56.8	CH	1.00 (*m*)
15	24.4	CH_2	1.58 (*m*)
16	28.3	CH_2	1.26 (*m*)
			1.82 (*m*)
17	56.1	CH	1.10 (*m*)
18	36.2	CH	1.36 (*m*)
19	19.5	CH_3	0.92 (*d*, 6.5 Hz)
20	34.0	CH_2	1.01 (*m*)
			1.33 (*m*)
21	26.1	CH_2	1.16 (*m*)
22	45.9	CH	0.99 (*m*)
23	23.1	CH_2	1.26 (*m*)
24	12.0	CH_3	0.85 (*t*, 7.2 Hz)
25	29.2	CH	1.68 (*m*)
26	19.9	CH_3	0.84 (*d*, 6.4 Hz)
27	19.0	CH_3	0.81 (*d*, 6.4 Hz)
28	18.9	CH_3	0.68 (*s*)
29	12.1	CH_3	1.01 (*s*)

Note. *Solvent used was $CDCl_3$; assignments based on DEPT, COSY, HMQC and HMBC.

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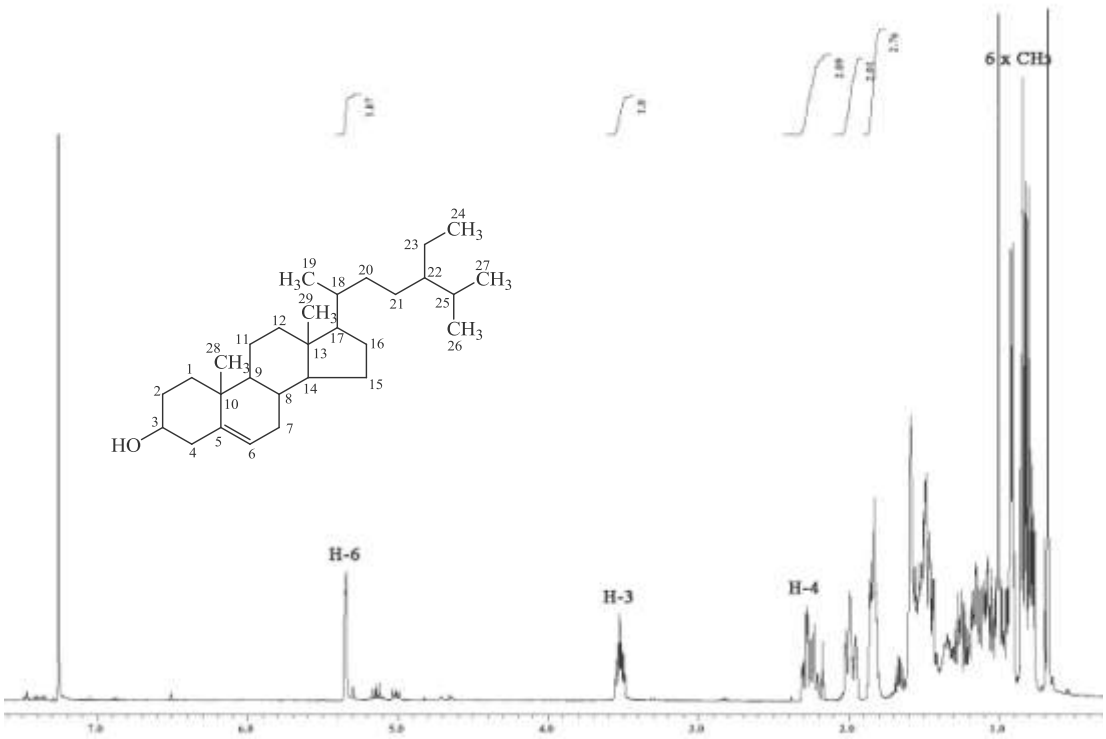


Figure 4.2.6.1a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of β-Sitosterol **(Mk31)**

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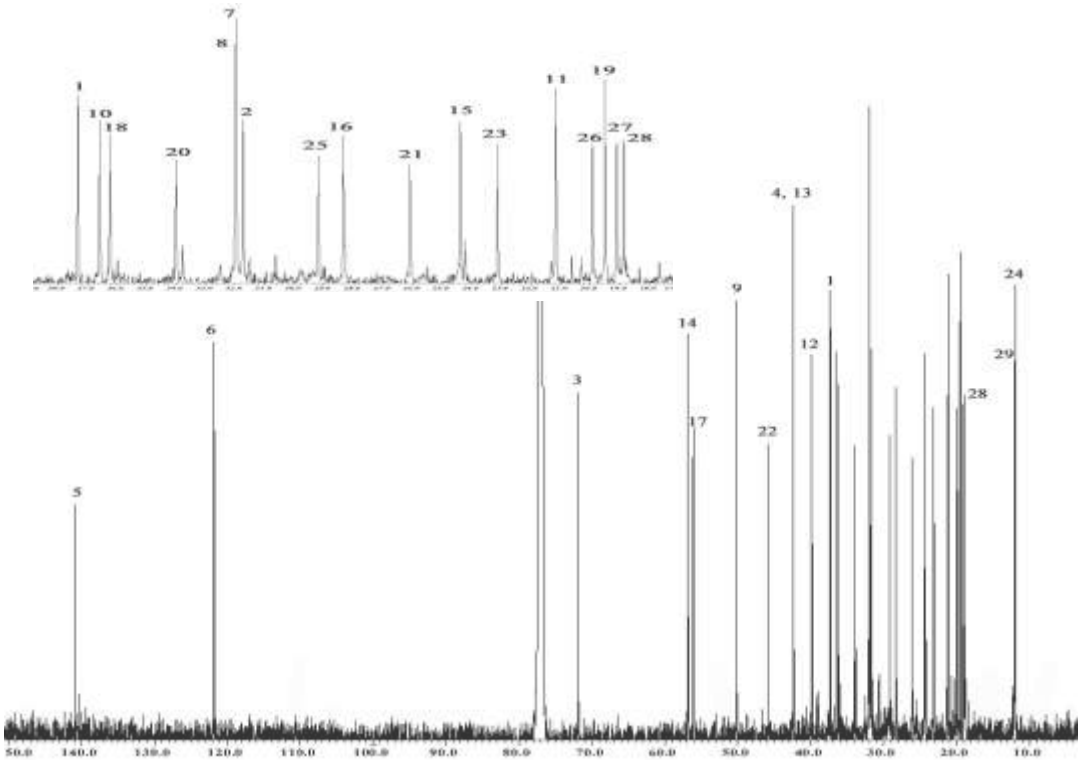
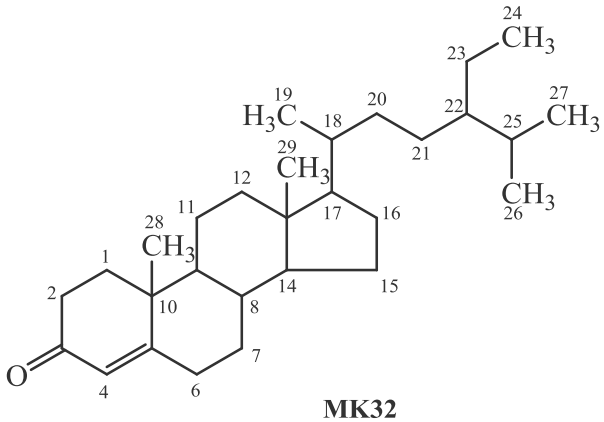


Figure 4.2.6.1b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of β-Sitosterol **(Mk31)**

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4.2.6.2 Stigmast-4-ene-3-one (Mk32)



MK32

Mk32 was optically active $[\alpha]_D^{25}$ +33.0 (CH_3OH; c 0.02) and isolated as white amorphous from the bark of *M. koenigii*. The HRESIMS of **Mk32** showed molecular ion peak $[M+H]^+$ at *m/z* 413.2655 which was compatible with a molecular composition of $C_{29}H_{48}O$. The IR spectrum of **Mk32** showed absorption bands at 1678 cm^{-1}, suggesting the presence of an α,β-unsaturated carbonyl system. The ^1H NMR spectrum (Table 4.2.6.2 and Figure 4.2.6.2a) presented a singlet at δ 5.72 (*s*, H-4), consistent with the presence of a vinylic proton. Other signals included the presence of six methyl signals that appeared as two methyl singlets at δ 0.70 (H-29) and δ 1.18 (H-28); three methyl doublets that appeared at δ 0.81 (*J* = 7.6 Hz, H-27), δ 0.83 (*J* = 7.6 Hz, H-26), and δ 0.91 (*J* = 6.0 Hz, H-19); and a methyl triplet at δ 0.84 (*J* = 6.8 Hz, H-24) were also observed and suggested a sterol skeleton for **Mk32**.

In the ^{13}C NMR spectrum (Table 4.2.6.2 and Figure 4.2.6.2b), the resonances of the α,β-unsaturated carbonyl system were referred to the signals at δ 123.8 (C-4), δ 171.9 (C-5), and δ 199.8 (C-3). The structure of **Mk32** was further confirmed by the

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analysis of its ^{13}C NMR spectrum, which showed six methyl groups, 11 methylene groups, eight methine groups and four quaternary carbons. The detailed analysis of the ^{1}H-^{1}H COSY, HMQC and HMBC spectra of **Mk32** allowed the unequivocal assignments of all proton and carbon resonances and confirmed its structure as stigmast-4-ene-3-one, which is also known as stigmasterone (Barla, Birman, Kültür, & Oksüz, 2006).

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Table 4.2.6.2

^{1}H NMR [500 MHz, δ_H (J Hz)] and ^{13}C NMR [125 MHz, δ_C] of Stigmast-4-ene-3-one ***(Mk32)****

Position	δ ^{13}C	DEPT	δ ^{1}H
1	35.8	CH_2	1.69 (*m*)
			2.00 (*m*)
2	33.9	CH_2	1.30 (*m*)
			2.39 (*m*)
3	199.8	qC	
4	123.8	CH	5.72 (*s*)
5	171.9	qC	
6	33.1	CH_2	2.24 (*m*)
			2.28 (*m*)
7	32.1	CH_2	1.00 (*m*)
			1.84 (*m*)
8	35.7	CH	1.44 (*m*)
9	53.9	CH	0.91 (*m*)
10	38.7	qC	
11	21.1	CH_2	1.44 (*m*)
			1.53 (*m*)
12	39.7	CH_2	1.15 (*m*)
			2.02 (*m*)
13	42.5	qC	
14	55.9	CH	1.02 (*m*)
15	24.3	CH_2	1.00 (*m*)
			1.61 (*m*)
16	28.3	CH_2	1.64 (*m*)
			1.84 (*m*)
17	56.1	CH	1.11 (*m*)
18	36.2	CH	1.38 (*m*)
19	18.8	CH_3	0.91 (*d*, 6.0 Hz)
20	34.1	CH_2	2.32 (*m*)
21	26.1	CH_2	1.16 (*m*)
22	45.9	CH	0.92 (*m*)
23	23.1	CH_2	1.28 (*m*)
24	12.1	CH_3	0.86 (*t*, 6.8 Hz)
25	29.2	CH	1.25 (*m*)
26	19.9	CH_3	0.83 (*d*, 7.6 Hz)
27	19.1	CH_3	0.81 (*d*, 7.6 Hz)
28	17.5	CH_3	1.18 (*s*)
29	12.0	CH_3	0.70 (*s*)

Note. *Solvent used was $CDCl_3$; assignments based on DEPT, COSY, HMQC and HMBC.

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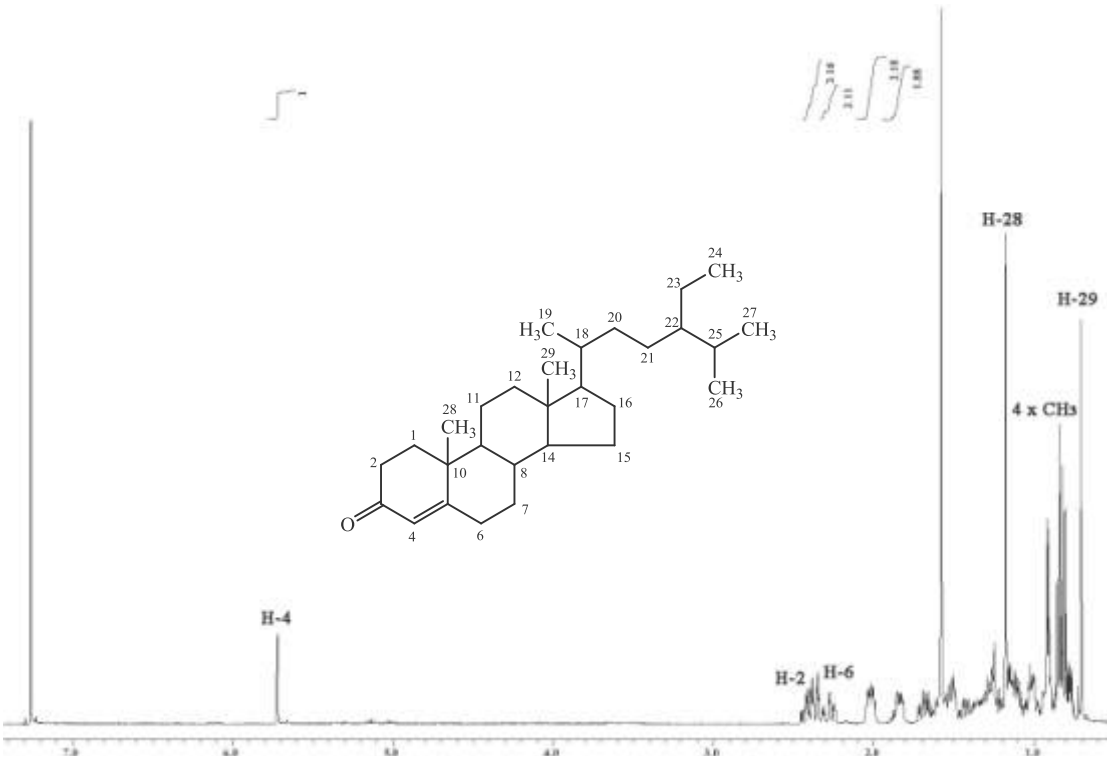


Figure 4.2.6.2a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Stigmast-4-ene-3-one **(Mk32)**

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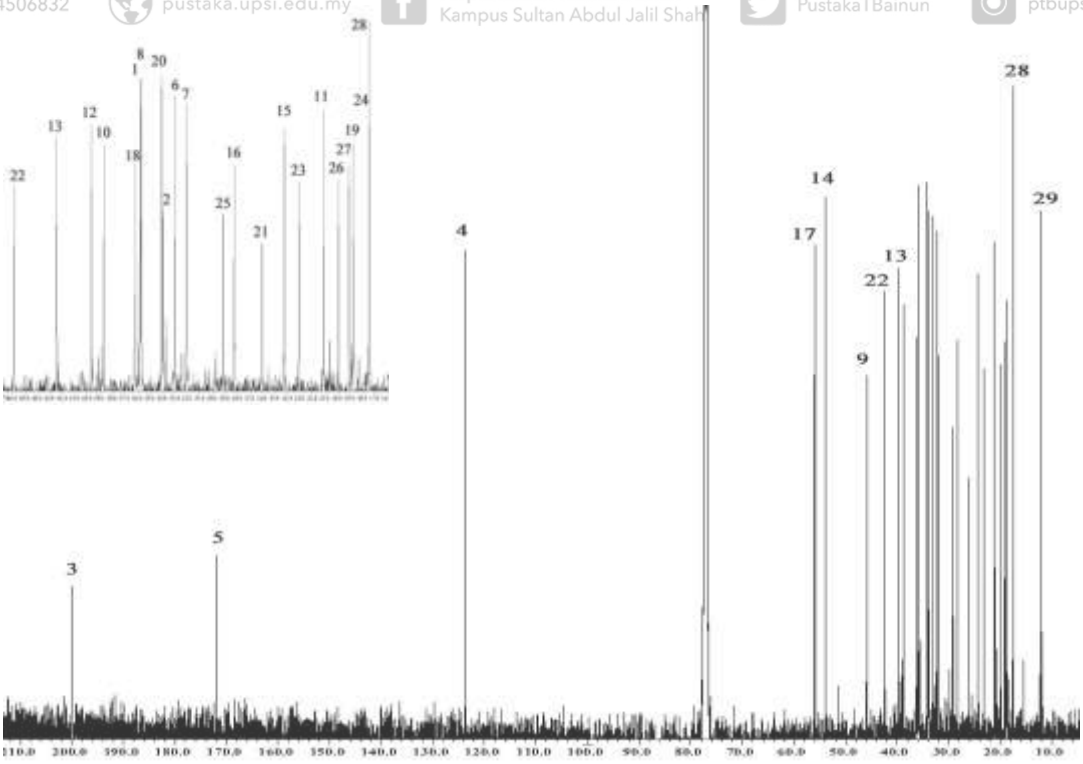
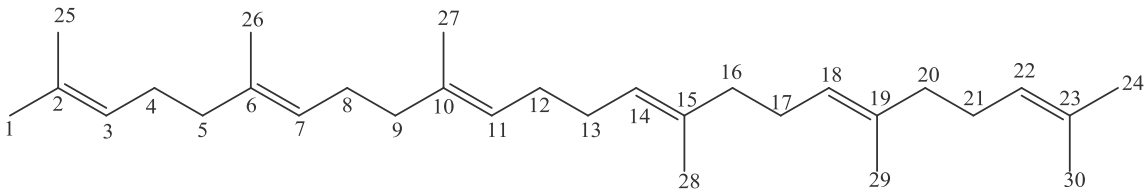


Figure 4.2.6.2b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Stigmast-4-ene-3-one **(Mk32)**

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4.2.6.3 Squalene (Mk33)



MK33

Mk33 was isolated as colourless oil. Its molecular formula confirmed by HRESIMS at *m/z* 410.3476 and determined to be $C_{30}H_{50}$ $[M]^+$. The ^1H NMR (500 MHz, $CDCl_3$) of **Mk33** (Table 4.2.6.3 and Figure 4.2.6.3a) showed signals at δ 1.61 (12H, *s*, CH_3 × 4), δ 1.69 (6H, *s*, CH_3 × 2), δ 1.75 (6H, *s*, CH_3 × 2), δ 1.99 – 2.10 (20H, *m*, CH_2 × 10), olefinic protons at δ 5.13 – 5.14 (6H, *m*, *J* = 6.1 Hz, C=C-H × 6). These signals indicated that the compound was squalene, an acyclic triterpenoid.

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In the ^{13}C NMR of squalene (**Mk33**) (Table 4.2.6.3 and Figure 4.2.6.3b), displayed distinct resonances represents 30 carbons, while DEPT experiment showed the presence of six trisubstituted quaternary carbons; six methine carbons; 10 methylene carbons; and eight methyl carbons. The ^{13}C NMR spectrum of **Mk33** also showed that the out-chain methyl groups resonating at δ 17.9 and δ 16.1 × 2 indicated the geometry of the six trisubstituted double bonds, while the signals appearing at δ 25.8, confirmed its in-chain position. The detailed analysis of the ^1H-^1H COSY, HMQC and HMBC spectra features of **Mk33** was identified to be squalene (Borchman, Yappert, Milliner, Smith, & Bhola, 2013).

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Table 4.2.6.3

^{1}H NMR [500 MHz, δ_H (J Hz)] and ^{13}C NMR [125 MHz, δ_C] of Squalene ***(Mk33)****

Position	$\delta^{13}C$	DEPT	$\delta^{1}H$
1, 24	25.8	CH_3	1.69 (*s*)
2, 23	131.3	qC	
3, 22	124.3	CH	5.13 (*m*)
4, 21	26.5	CH_2	2.08 (*m*)
5, 20	39.9	CH_2	1.99 (*m*)
6, 19	135.0	qC	
7, 18	124.6	CH	5.13 (*m*)
8, 17	26.5	CH_2	2.00 (*m*)
9, 16	32.3	CH_2	2.10 (*m*)
10, 15	135.4	qC	
11, 14	125.1	CH	5.14 (*m*)
12, 13	26.9	CH_2	2.06 (*m*)
25, 30	17.9	CH_3	1.75 (*s*)
26, 27, 28 29	16.1	CH_3	1.61 (*s*)

Note. *Solvent used was $CDCl_3$; assignments based on DEPT, COSY, HMQC and HMBC.

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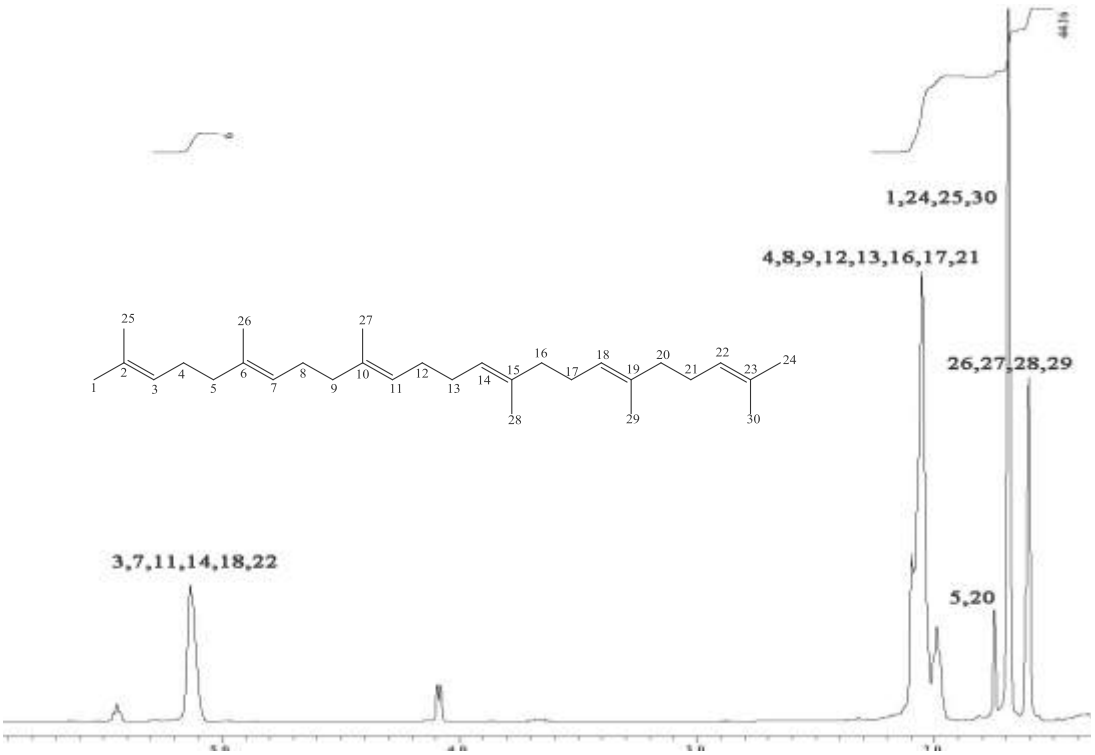


Figure 4.2.6.3a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Squalene **(Mk33)**

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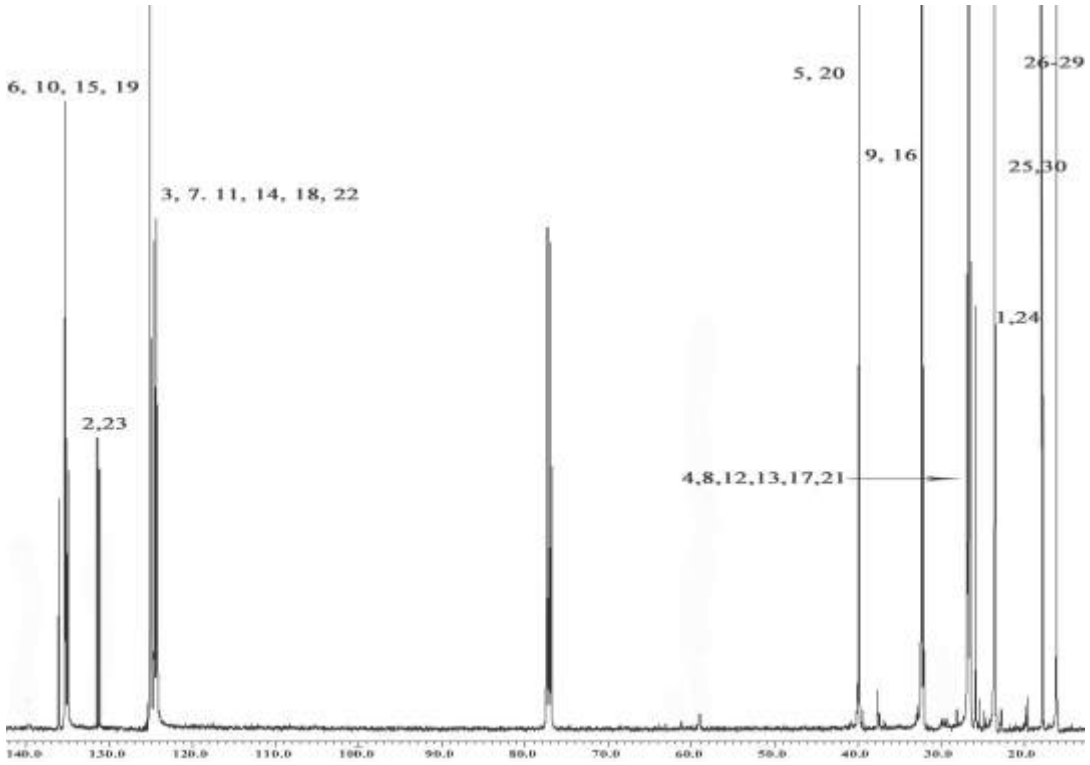
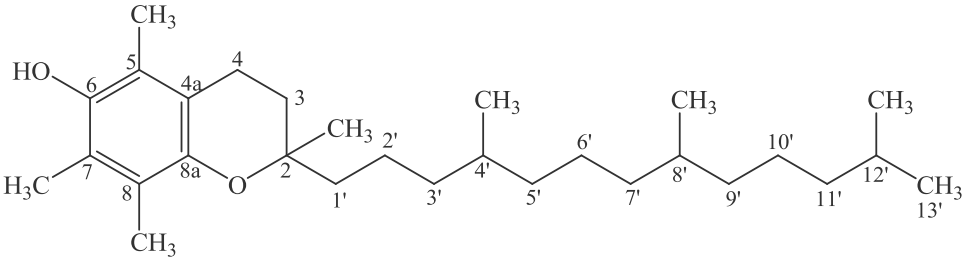


Figure 4.2.6.3b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Squalene **(Mk33)**

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4.2.6.4 α-Tocopherol (Mk34)



Mk34

Mk34 was obtained as colourless oil from the leaves of *M. koenigii* with $[\alpha]_D^{25}$ +16.0 (CH_3OH; c 0.02). Its mass spectral displayed a molecular ion peaks at *m/z* 430.2458 $[M]^+$ which consistent with a molecular formula of $C_{29}H_{50}O_2$. The IR spectrum showed bands attributable to a hydroxyl group (3327 cm^{-1}) and an aromatic group (1452 cm^{-1}). UV absorptions at λ_{max} 225, 254, 268 and 288 nm suggested that **Mk34** contained a phenol moiety in its molecule.

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The ^1H NMR spectrum of **Mk34** (Table 4.2.6.4 and Figure 4.2.6.4a) showed signals assignable to four doublet methyls at δ 0.84-0.87 (3H × 4), four high field singlet methyls at δ 1.23, δ 2.11 × 2 and δ 2.16 (3H each, s), a triplet methylene at δ 2.60 (*t*, *J* = 6.9 Hz, H-4) and a hydroxyl proton at δ 4.20 (*s*, 6-OH).

The ^{13}C NMR (Table 4.2.6.4 and Figure 4.2.6.4b) and DEPT spectra of **Mk34** exhibited only 29 carbon signals, which were sorted into eight methyls, eleven methylenes, three methines, one sp^3 quaternary carbons with an oxygen substituent at δ 74.6 (C-2) and six sp^2 quaternary carbons contributed to a benzene ring. The position of hydroxyl group (6-OH) was also confirmed from the cross peaks between

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6-OH (δ 4.20) with C-5 (δ 118.5), C-6 (δ 144.6) and C-7 (δ 121.1). Further, the long range correlations between 6-OH (δ 7.44) with 5-CH_3 (δ 2.11) were also observed in the HMBC experiment. From the above evidence, **Mk34** was established and determined as α-tocopherol (Matsuo & Urano, 1976). Complete ^{1}H and ^{13}C chemical shifts were further supported by DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectra.

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Table 4.2.6.4

^{1}H NMR [500 MHz, δ_H (J Hz)] and ^{13}C NMR [125 MHz, δ_C] of α-Tocopherol ***(Mk34)****

Position	δ ^{13}C	DEPT	δ ^{1}H
1			
2	74.6	qC	
3	31.6	CH_2	1.78 (*m*)
4	20.8	CH_2	2.60 (*t*, 13.8 Hz)
4a	117.4	qC	
5	118.5	qC	
6	144.6	qC	
7	121.1	qC	
8	122.7	qC	
8a	145.6	qC	
2-CH_3	23.9	CH_3	1.23 (*s*)
5-CH_3	11.4	CH_3	2.11 (*s*)
6-OH			4.20 (*s*)
7-CH_3	12.3	CH_3	2.16 (*s*)
8-CH_3	11.9	CH_3	2.11 (*s*)
1'	39.9	CH_2	1.48 (*m*)
			1.53 (*m*)
2'	21.1	CH_2	1.38 (*m*)
3'	37.5	CH_2	1.06 (*m*)
			1.25 (*m*)
4'	32.9	CH	1.38 (*m*)
5'	37.4	CH_2	1.30 (*m*)
6'	24.5	CH_2	1.15 (*m*)
7'	37.4	CH_2	1.30 (*m*)
8'	32.8	CH	1.38 (*m*)
9'	37.5	CH_2	1.30 (*m*)
10'	24.9	CH_2	1.05 (*m*)
11'	39.5	CH_2	1.14 (*m*)
12'	28.1	CH	1.51 (*m*)
13'	22.8	CH_3	0.87 (*d*, 6.9 Hz)
4'-CH_3	19.8	CH_3	0.84 (*d*, 6.9 Hz)
8'-CH_3	19.7	CH_3	0.85 (*d*, 6.9 Hz)
12'-CH_3	22.7	CH_3	0.86 (*d*, 6.9 Hz)

Note. *Solvent used was $CDCl_3$; assignments based on DEPT, COSY, HMQC and HMBC.

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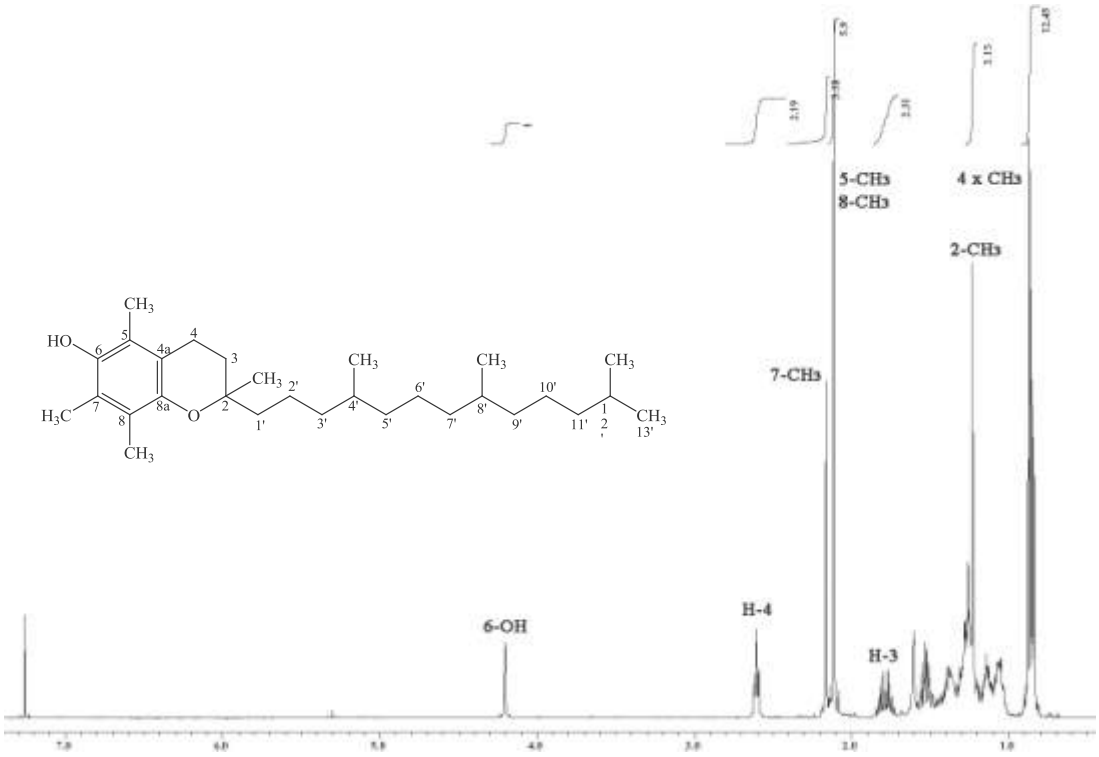


Figure 4.2.6.4a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of α-Tocopherol **(Mk34)**

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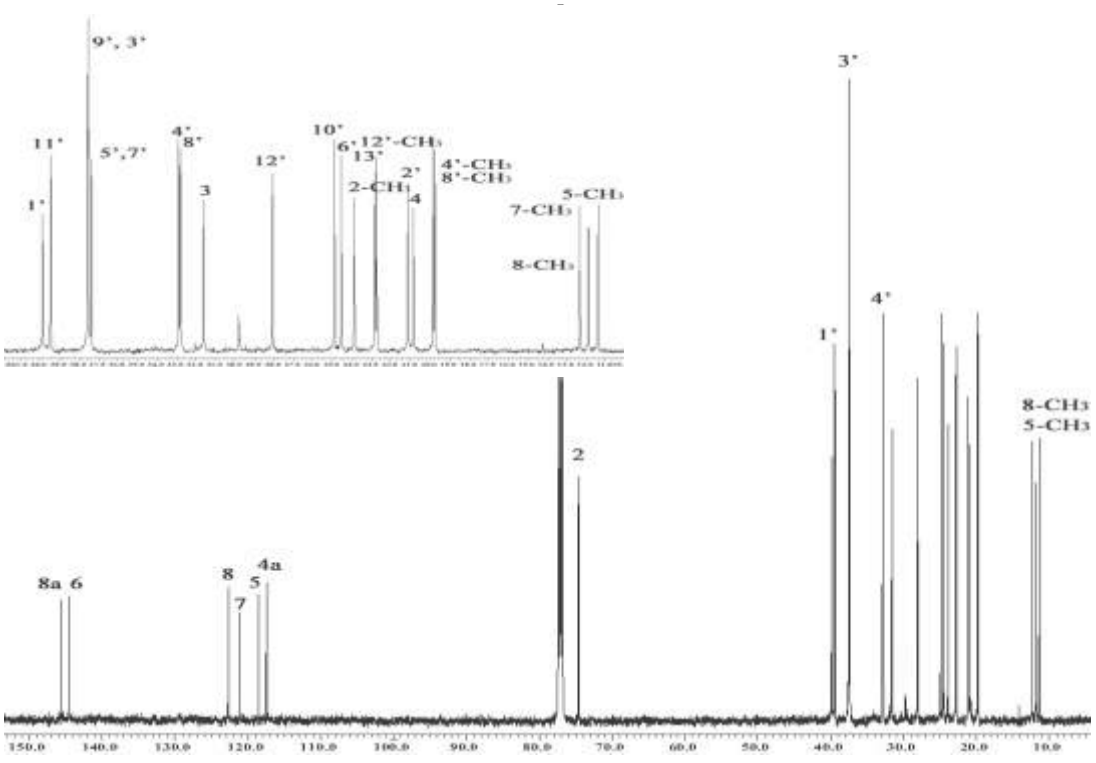
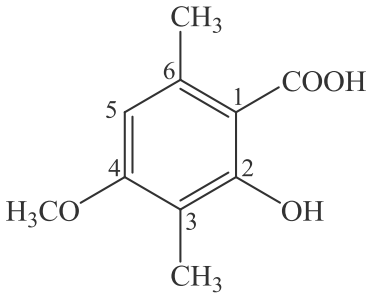


Figure 4.2.6.4b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of α-Tocopherol **(Mk34)**

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4.2.6.5 2-Hydroxy-4-methoxy-3, 6-dimethylbenzoic Acid (Mk35)



MK35

The NMR spectroscopic data of **Mk35**, which isolated from the bark of *M. koenigii*, is listed in Table 4.2.6.5. All the proton and carbon NMR peak assignments (Figure 4.2.6.5a and Figure 4.2.6.5b) were completed with the help of ^{1}H-^{1}H COSY, DEPT, HMQC and long-range coupling in HMBC.

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In the ^{1}H NMR ($CDCl_3$, 500 MHz), an aromatic singlet (δ 6.21, H-5), a methoxy singlet (δ 3.92, 3H, 4-OCH_3), two methyl proton signals at 3-CH_3 (δ 2.46, *s*) and 6-CH_3 (δ 2.10, *s*), along with a significant hydroxy signal of the carboxylic acid at δ 12.04 (*s*, 1-COOH) were also observed.

In ^{13}C and DEPT experiments, six quaternary carbons (δ 105.4, 108.7, 140.3, 158.2, 163.3 and 172.8), one methine carbon (δ 110.7) and two methyl carbons (δ 24.3 and 7.8) and a methoxy carbon (δ 52.0) were observed. The position of all proton and carbon signals were achieved by the combination of the NMR experiments. In the HMBC experiment, the presence of the hydroxyl group was confirmed to be attached at C-2, which showed long-range coupling from 3-CH_3 and 1-COOH to C-2 (δ 163.3). Other cross peaks included the following: 6-CH_3 to C-1 (δ 105.4), C-5 (δ 110.7) and

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C-6 (δ 140.3); 3-CH_3 to C-2 (δ 163.3), C-3 (δ 108.7) and C-4 (δ 158.2); 4-OCH_3 to C-4 (δ 158.2), which confirmed the attribution of all the ^{13}C signals of **Mk35** especially gave evidence to confirm the hydroxyl group of the carboxylic acid was connected to C-1 with a very down field region at δ 172.8.

Mk35 was obtained as pale yellow crystal with pleasant smell [m.p.: 187–188 °C; 186–188 °C (Rawat, Shukla, Negi, & Pant, 2006)]. The molecular formula of **Mk35** was determined as $C_{10}H_{12}O_4$ which corresponding to a molecular ion peak at *m/z* 197.0799 $[M+H]^+$. The IR (ν_{max} at 3332, 1025 cm^{-1}), UV spectrum (λ_{max} at 268, 289 and 316 nm) of **Mk35** coupled with the its NMR spectrum, suggested that this compound was deduced as 2-hydroxy-4-methoxy-3,6-dimethylbenzoic acid (Rawat et al., 2006), which was first time reported from *M. koenigii*.

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Table 4.2.6.5

*^1H NMR [500 MHz, δ_H (J Hz)] and ^{13}C NMR [125 MHz, δ_C] of 2-Hydroxy-4-methoxy-3,6-dimethylbenzoic acid **(Mk35)***

Position	$\delta^{13}C$	DEPT	δ^1H
1	105.4	qC	
2	163.3	qC	
3	108.7	qC	
4	158.2	qC	
5	110.7	CH	6.21 (*s*)
6	140.3	qC	
1-**C**OOH	172.8	qC	
1-CO**OH**			12.04 (*s*)
2-OH			5.19 (*br s*)
3-CH_3	7.8	CH_3	2.10 (*s*)
4-OCH_3	52.0	CH_3	3.92 (*s*)
6-CH_3	24.3	CH_3	2.46 (*s*)

Note. *Solvent used was $CDCl_3$; assignments based on DEPT, COSY, HMQC and HMBC.

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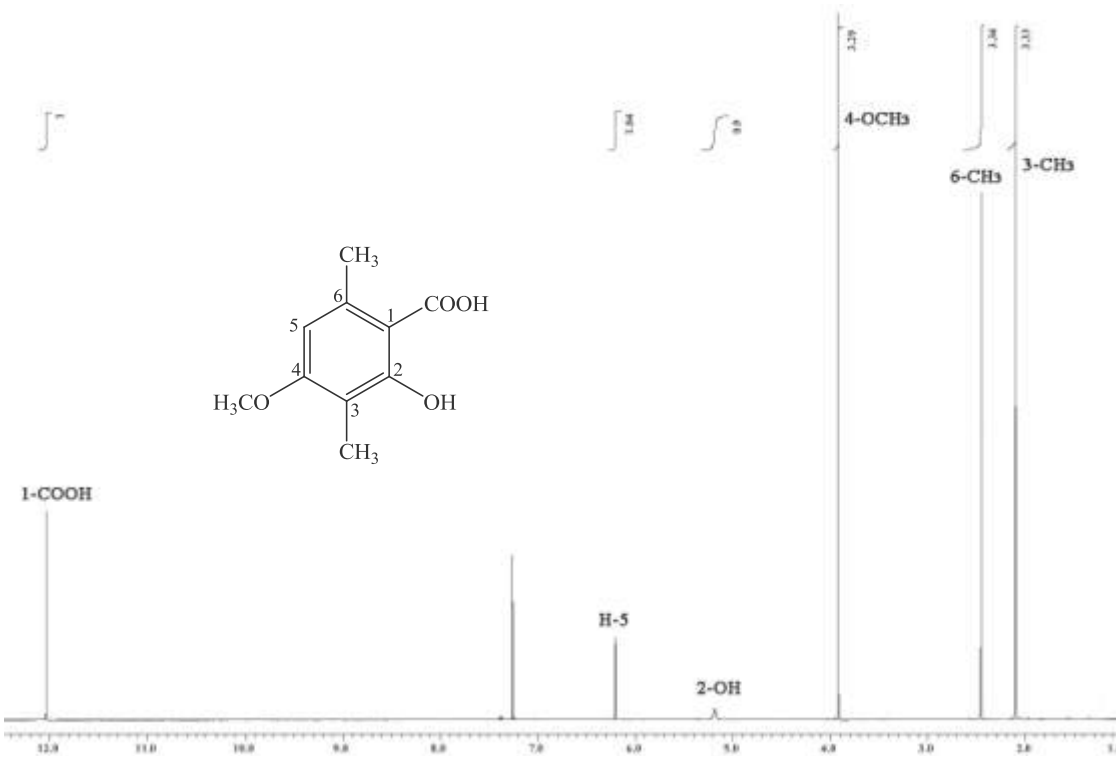


Figure 4.2.6.5a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of 2-Hydroxy-4-methoxy-3,6-dimethylbenzoic acid **(Mk35)**

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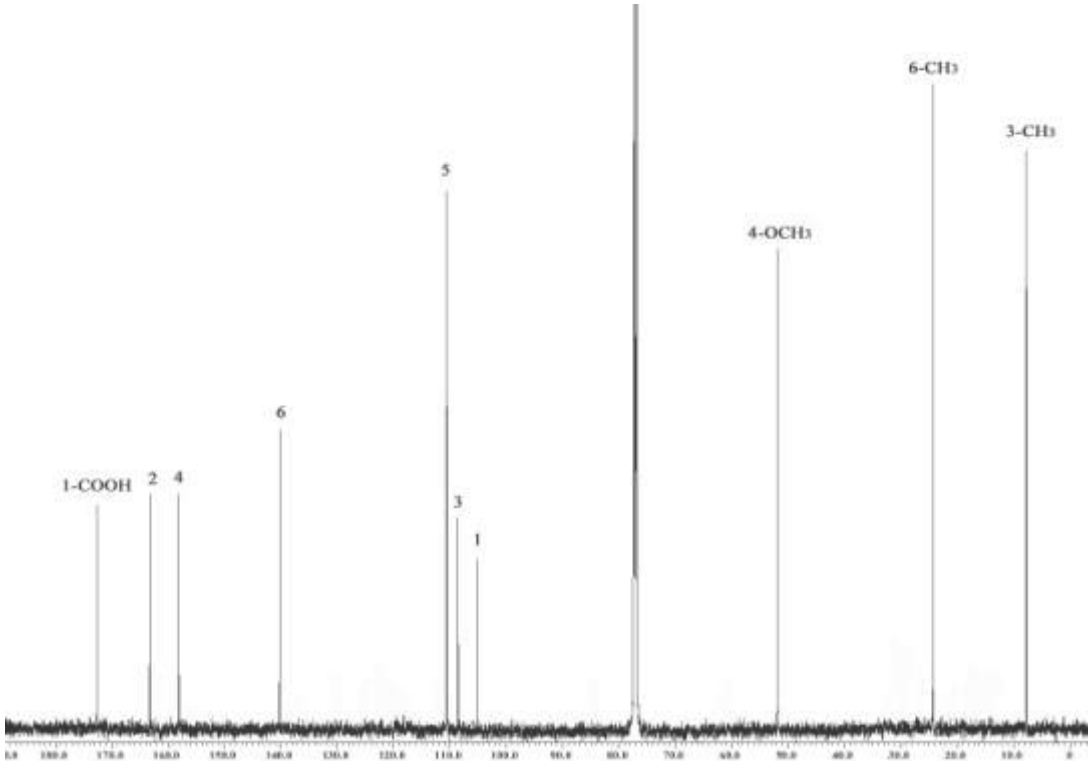
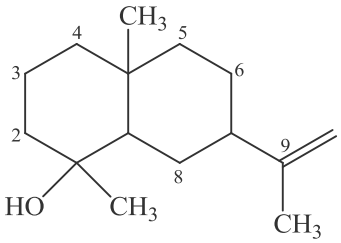


Figure 4.2.6.5b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of 2-Hydroxy-4-methoxy-3,6-dimethylbenzoic acid **(Mk35)**

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4.2.6.6 Selin-11-en-4α-ol (Mk36)



Mk36

An eudesmane sesquiterpene, selin-11-en-4α-ol **(Mk36)** was afforded from the leaves of *M. koenigii*. It appears as white solid with melting point 60–62 °C (m.p.: 61–62 °C; Kesselmans, Wijnberg, & Minnaard, 1991) and displayed a molecular ion peaks at *m/z* 222.2021 $[M]^+$ by its HERSIMS which consistent with its molecular formula $C_{15}H_{26}O$. The IR showed absorption at 3327 cm^{-1}(O–H), 1644 cm^{-1} (C=C) and 1025 cm^{-1} (C–O) revealed the presence of a hydroxyl group in the molecule.

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In the ^1H NMR spectrum ($CDCl_3$, 500 MHz) (Table 4.2.6.6 and Figure 4.2.6.6a), a total of three methyl signals at: δ 0.88 (*s*, 4a-CH_3) for an angular methyl, δ 1.11 (*s*, 1-CH_3) for the methyl attached with the carbon bearing hydroxyl group, and δ 1.74 (*s*, 9-CH_3) for methyl attached to C-9 with an exocyclic double bond. A pair of doublets of 9-CH_2 at δ 4.71 (*d*, *J* = 1.2 Hz) and δ 4.69 (*d*, *J* = 1.2 Hz), due to the exocyclic methylene protons, was also observed.

The ^{13}C and DEPT NMR spectra of **Mk36** showed a total of 15 carbon signals and their assignments marked the presence of three methyls, seven methylenes, three methines and two quaternary carbons. The above assignments were further confirmed

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by the combination of NMR experiments. **Mk36** was thus identified as selin-11-en-4α-ol as all its NMR data were identical with those reported in the literature (Chanotiya, Sammal, & Mathela, 2005; Kesselmans et al., 1991).

Table 4.2.6.6

^{1}H NMR [500 MHz, δ_H (J Hz)] and ^{13}C NMR [125 MHz, δ_C] of Selin-11-en-4α-ol ***(Mk36)****

Position	δ ^{13}C	DEPT	δ ^{1}H
1	72.3	qC	
2	43.4	CH_2	1.38 (*m*)
			1.81 (*m*)
3	20.2	CH_2	1.55 (*m*)
4	41.1	CH_2	1.10 (*m*)
			1.40 (*m*)
4a	34.7	qC	
5	44.7	CH_2	1.20 (*m*)
			1.44 (*m*)
6	26.9	CH_2	1.41 (*m*)
			1.58 (*m*)
7	46.4	CH	1.95 (*m*)
8	26.1	CH_2	1.28 (*m*)
			1.86 (*m*)
8a	54.9	CH	1.25 (*m*)
9	150.8	qC	
1-CH_3	22.8	CH_3	1.11 (*s*)
1-OH		#	
4a-CH_3	18.8	CH_3	0.88 (*s*)
9-CH_3	21.2	CH_3	1.74 (*s*)
9-CH_2	108.2	CH_2	4.71 (*d*, 1.2 Hz)
			4.69 (*d*, 1.2 Hz)

Note. *Solvent used was $CDCl_3$; assignments based on DEPT, COSY, HMQC and HMBC. # peak not available.

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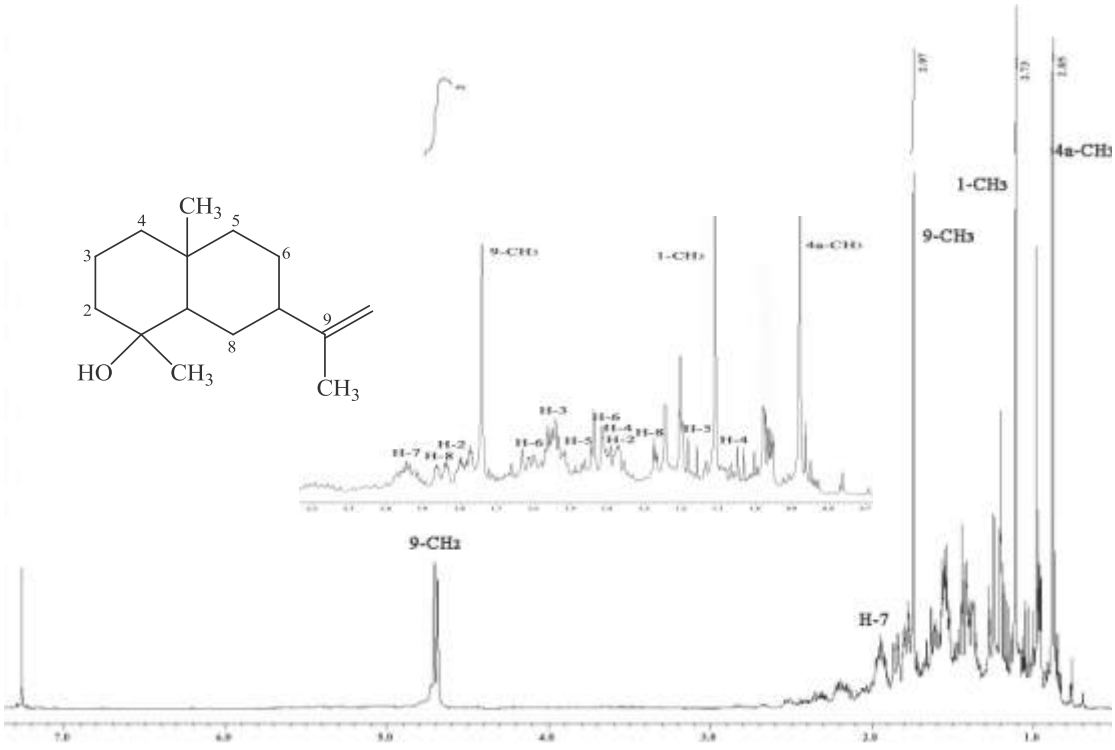


Figure 4.2.6.6a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of Selin-11-en-4α-ol **(Mk36)**

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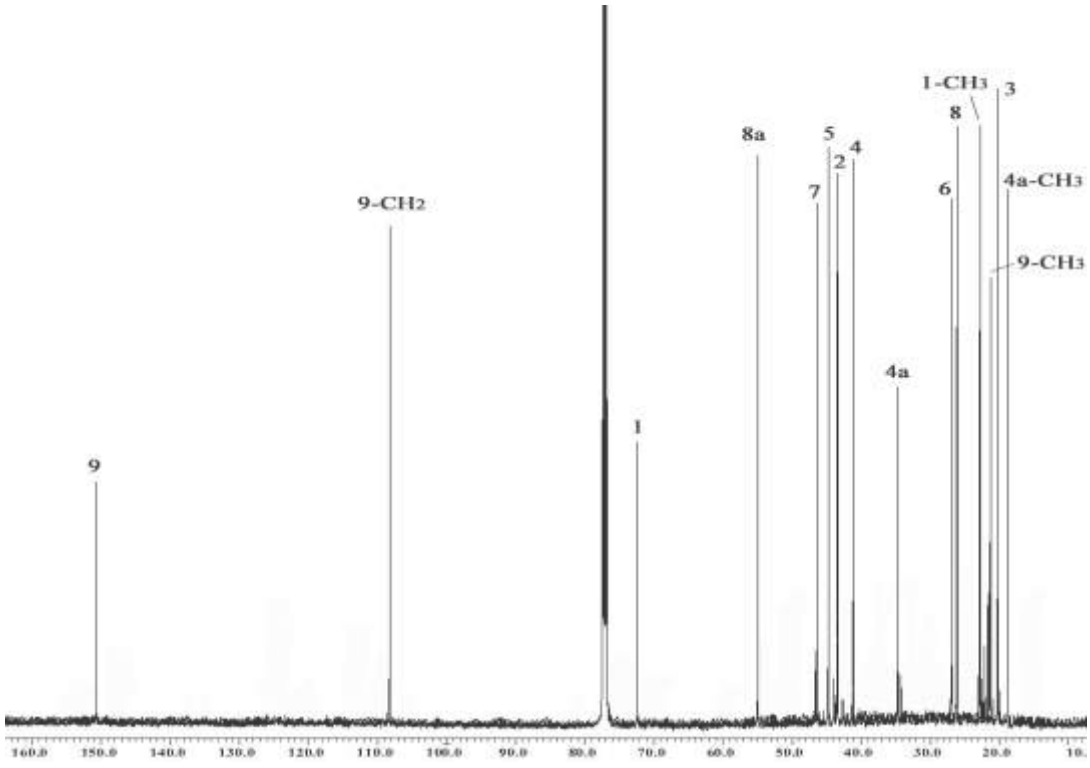


Figure 4.2.6.6b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of Selin-11-en-4α-ol **(Mk36)**

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4.3 Synthesis of Girinimbine Analogues

Girinimbine **(Mk12)**, the carbazole alkaloid isolated as major compound from the bark of *Murraya koenigii*, was chosen for further investigation. Chemical synthesis or modification of girinimbine **(Mk12)** by using two effective oxidative coupling and/or dimerization reagents, *viz.*, anhydrous ferric (III) chloride ($FeCl_3$) (Scheme 3.8) and boron trifluoride diethyletherate (BF_3-$O(C_2H_5)_2$) (Scheme 3.10), successfully derived two analogues, namely murranimbine **(G1)** and epoxygirinimbine **(G2)**. The rationale behind the development of synthetic analogues of these compounds were expected to be able to derive new synthetic analogues and to increase in potency and selectivity in biological activity.

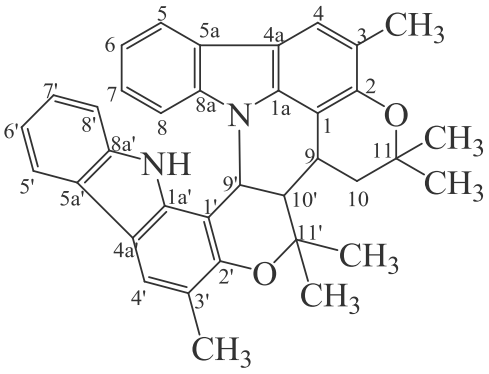
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The elucidation and identification of the synthetic analogues by various spectroscopic methods such as 1D NMR (^1H, ^{13}C, DEPT), 2D NMR (^1H-^1H COSY, NOESY, HMBC and HMQC), UV, IR, specific rotation, and mass spectrometry will be discussed in this section.

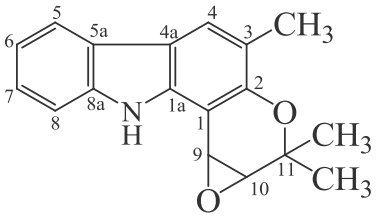
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4.3.1 Spectroscopic Analysis of Murranimbine (G1) and Epoxygirinimbine (G2)



G1



G2

In the synthetic approach showed in Scheme 3.8, treatment of girinimbine **(Mk12)** with anhydrous $FeCl_3$ afforded a dimeric compound **G1**, also known as murranimbine, in up to 27.3% yield (Table 3.2, entry 7), which was previously isolated as natural products from *Murraya euchrestifolia* (Ito & Furukawa, 1991). Interestingly, a new oxygenated compound assigned as epoxygirinimbine **(G2)** was also afforded from this reaction (up to 19.6% yield, entry 6) (Table 3.2). For the synthesis using BF_3-$O(C_2H_5)_2$, only **G1** was obtained with a higher yield (entry 5: 40.0% and entry 6: 41.7%) (Table 3.4) when compared to the previous reaction using anhydrous $FeCl_3$. Therefore, optimization towards the formation of **G1** can be achieved by using BF_3-$O(C_2H_5)_2$ with suitable solvent instead of anhydrous $FeCl_3$.

The positions of proton and carbon resonances were confirmed by the combination of 1D and 2D NMR experiments and the data from the literature. Table 4.3.1 and Table 4.3.2 showed the NMR spectral data of **G1** and **G2**, respectively.

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Figure 4.3.1a to 4.3.1j and Figure 4.3.2a to 4.3.2h showed the ^{1}H, ^{13}C, COSY, HMQC, HMBC and NOESY NMR spectra of **G1** and **G2**, respectively.

The molecular formula of murranimbine **(G1)** was established to be $C_{36}H_{34}N_2O_2$ with a molecular ion peak at *m/z* 526.2682 ($[M]^+$, calcd. mass: 526.2620), coupled with its ^{1}H and ^{13}C NMR spectral data (Table 4.3.1). In the ^{1}H NMR of **G1** (Figure 4.3.1a), characteristic of two set of unsubstituted ring-C [δ 8.11 (*d*, *J* = 8.0 Hz, H-5), δ 7.34 (*td*, *J* = 8.0, 1.0 Hz, H-6, δ 7.42 (*td*, *J* = 8.0, 1.0 Hz, H-7), δ 7.52 (*d*, *J* = 8.0 Hz, H-8), δ 7.82 (*d*, *J* = 8.0 Hz, H-5'), δ 7.02 (*td*, *J* = 8.0, 1.0 Hz, H-6'), δ 7.06 (*td*, *J* = 8.0, 1.0 Hz, H-7') and δ 6.66 (*d*, *J* = 8.0 Hz, H-8')], two deshielded proton signals for H-4 [δ 7.71 (*s*)] and H-4' [δ 7.76 (*s*)] which showed long range coupling with two aryl methyls at δ 2.30 (*s*) and δ 2.42 (*s*), respectively, along with four geminal methyls at δ 1.48, 1.74 attached to C-11 and δ 1.22, 1.71 attached to C-11', each in the carbazole nucleus, were observed. These data suggested that molecule **G1** contained two units of girinimbine. In ^{1}H-^{1}H COSY (Figure 4.3.1f), correlations between the proton at C-9 [δ_C: 27.2; δ_H: 3.13 (*td*, *J* = 11.5, 5.2 Hz)] and C-10' [δ_C: 46.5; δ_H: 2.16 (*dd*, *J* = 10.9, 5.3 Hz)], indicated the two girinimbine units are linked with a C–C linkage between C-9 and C-10'. This was further supported by the long-range couplings in HMBC experiment (Figure 4.3.1g). In addition, correlation between H-9' (δ 6.12, *d*, *J* = 5.2 Hz) and C-8a (δ 139.6) confirmed that the presence of a C–N linkage (C-9' to N atom) as well. This was further confirmed by a lower chemical shift value of the doublet at H-9' (δ 6.12, *d*, *J* = 5.2 Hz) is considered to be attached to N atom of the first units of girinimbine. In NOESY experiment (Figure 4.3.1i), correlations between H-10β/H-10', H-10'/H-9' and H-9'/H-8 at the β-orientation were

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observed. Meanwhile, H-9 was correlated with 11-CH_3 at the α position and unambiguously determined the relative stereochemistry of **G1**. The position of all carbon resonances and their corresponding protons were further confirmed by DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectra (Figure 4.3.1d to Figure 4.3.1j). On the basis of these data, the structure of compound **G1** was confirmed to be murranimbine (Ito & Furukawa, 1991).

G2 was first time afforded from the reaction using anhydrous $FeCl_3$ and its NMR spectra further confirmed that it is an epoxide, a new synthetic analogues of girinimbine. The ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of **G2** (Table 4.3.2) showed similar resonances with those observed in girinimbine **(Mk12)**. These signals included: A broad downfield signal at δ 8.17 (*br s*) for the NH group in the carbazole nucleus; an aromatic methyl group (δ 2.33) attached to C-3; a singlet proton signal for H-4 at δ 7.76; an unsubstituted ring-C with four mutually coupled aromatic protons at δ 7.94 (*d*, *J* = 8.0 Hz, H-5), δ 7.20 (*td*, *J* = 8.0, 1.0 Hz, H-6), δ 7.32 (*td*, *J* = 8.0, 1.0 Hz, H-7) and δ 7.22 (*d*, *J* = 8.0 Hz, H-8); and two methyls at δ 1.50 (*s*, 2 × 11-CH_3) adjacent to a oxygen functionality.

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The signal of two doublets at H-9 and H-10 have been shifted to a shielded region were observed in its ^{1}H NMR spectrum (Figure 4.3.2a) and the missing of two olefinic carbon signals for C-9 and C-10 in its ^{13}C NMR spectrum (Figure 4.3.2b), gave further evidence for the missing of the double bond at the pyranocarbazole skeleton. However, the COSY fragment (Figure 4.3.2e), H-10 [δ 2.65 (*d*, *J* = 6.9 Hz)] with H-9 [δ 5.22 (*d*, *J* = 6.3 Hz)] still can be observed. The HMBC was also found to

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be used to connect this fragment: δ 2.65 (H-10) to δ 71.2 (C-9) and δ 5.22 (H-9) to δ 48.6 (C-10). The position of H-9 and H-10 were determined to be at the same side by NOESY cross peak at NH/H-9 and H-9/H-10, and confirmed that the oxygen of this *cis*-epoxide is at the α-orientation (Figure 4.3.2g). Therefore, the relative stereochemistry of H-9 and H-10 is at β-position. A total of 18 carbon resonances presence in the ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) and the position of the proton and carbon resonances were also further supported by ^{1}H-^{1}H COSY, HMQC and HMBC spectra (Figure 4.3.2c to Figure 4.3.2h).

The molecular formula of **G2** was established to be $C_{18}H_{17}NO_2$ by the mass spectrometry (Figure 4.3.2k) with a molecular ion peaks at *m/z* 279.1591 ($[M]^+$, calcd. mass: 279.1259). An extra of 16 units of mass when compared with girinimbine (**Mk12**, calcd. mass: 263.1310), which further proved that the presence of an epoxide functionalities for **G2**. On the basis of these data, the structure of compound **G2** was proposed to be a new compound and suggested to be named as epoxygirinimbine.

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Table 4.3.1

1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of ***G1****

Position	δ ^{13}C	DEPT	δ ^{1}H	HMBC correlations	COSY
1	103.2	qC		H-9, H-10	
1a	136.3	qC		H-4, H-9	
2	150.4	qC		H-4, H-9	
3	119.1	qC		3-CH_3	
4	120.1	CH	7.71 (*s*)		
4a	112.6	qC		H-5	
5	120.7	CH	8.11 (*d*, 8.0 Hz)	H-7	H-6
5a	124.6	qC		H-4, H-8	
6	120.1	CH	7.34 (*td*, 8.0, 1.0 Hz)	H-8	H-5, H-7
7	124.0	CH	7.42 (*td*, 8.0,1.0 Hz)	H-5	H-6, H-8
8	108.8	CH	7.52 (*d*, 8.0 Hz)	H-9	H-7
8a	139.6	qC		H-5, H-7, H-9'	
9	27.2	CH	3.13 (*td*, 11.5, 5.2 Hz)	H-10, H-10'	H-10, H-10'
10α	39.8	CH_2	2.37 (*dd*, 11.6, 5.2 Hz)	H-9, 11-CH_3	H-9
10β			1.63 (*dd*, 11.5, 5.2 Hz)		
11	75.1	qC		11-CH_3	
3-CH_3	16.6	CH_3	2.30 (*s*)	H-4	
11-CH_3	30.9	CH_3	1.71 (*s*)	H-10	
11-CH_3	30.7	CH_3	1.48 (*s*)	H-10	
1'	106.7	qC		H-9', H-10'	
1a'	136.1	qC		H-4', H-9'	
2'	150.4	qC		H-4', H-9', 3'-CH_3	
3'	118.9	qC			
4'	120.8	CH	7.76 (*s*)		
4a'	117.7	qC		H-5'	
5'	118.8	CH	7.82 (*d*, 8.0 Hz)	H-7'	H-6'
5a'	122.4	qC		H-4', H-8'	
6'	119.0	CH	7.02 (*td*, 8.0, 1.0 Hz)	H-8'	H-5', H-7'
7'	124.1	CH	7.06 (*td*, 8.0, 1.0 Hz)	H-5'	H-6'
8'	110.3	CH	6.66 (*d*, 8.0 Hz)	H-6'	H-7'
8a'	139.1	qC		H-5', H-7'	
9'	50.5	CH	6.12 (*d*, 5.2 Hz)		H-10'
10'	46.5	CH_q	2.16 (*dd*, 10.9, 5.3 Hz)	H-9'	H-9', H-10
11'	79.0	qC			
N-H'			7.18 (*br s*)		
3'-CH_3	16.8	CH_3	2.42 (*s*)	H-4'	
11'-CH_3	28.8	CH_3	1.71 (*s*)	H-10'	
11'-CH_3	25.2	CH_3	1.22 (*s*)	H-10'	

Note. *Solvent used was $CDCl_3$; assignments based on COSY, NOESY, TOCSY, HMQC and HMBC.

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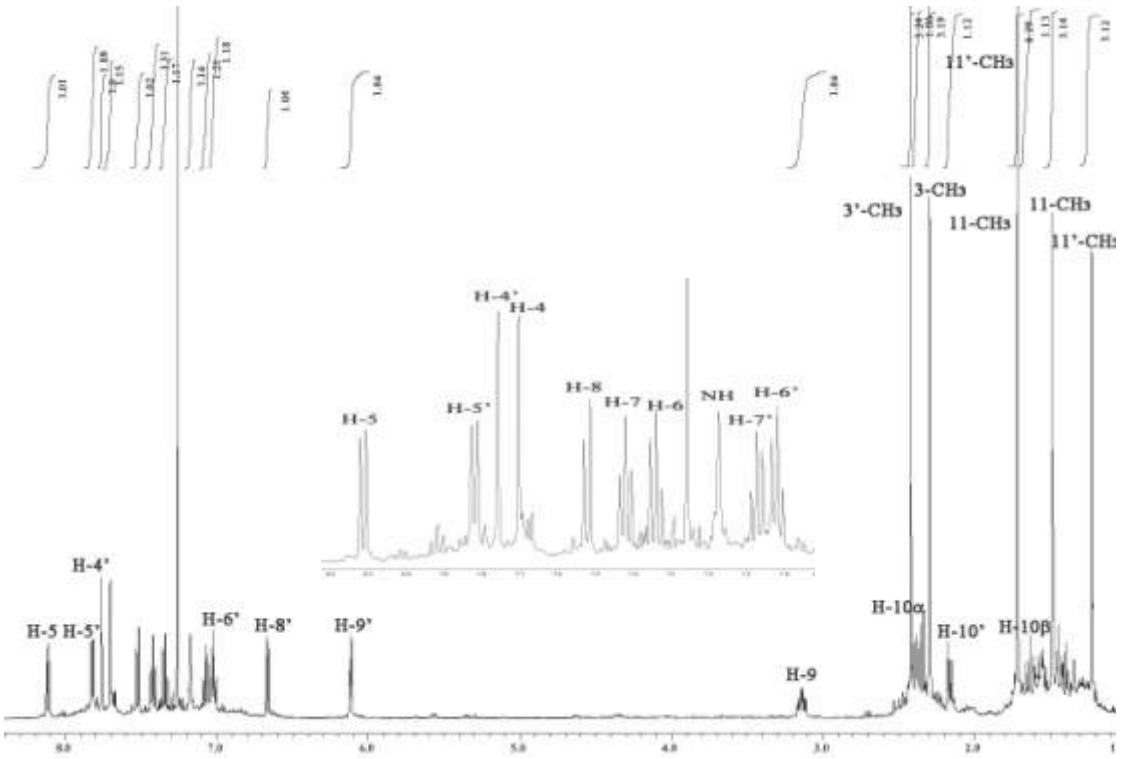


Figure 4.3.1a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of **G1**

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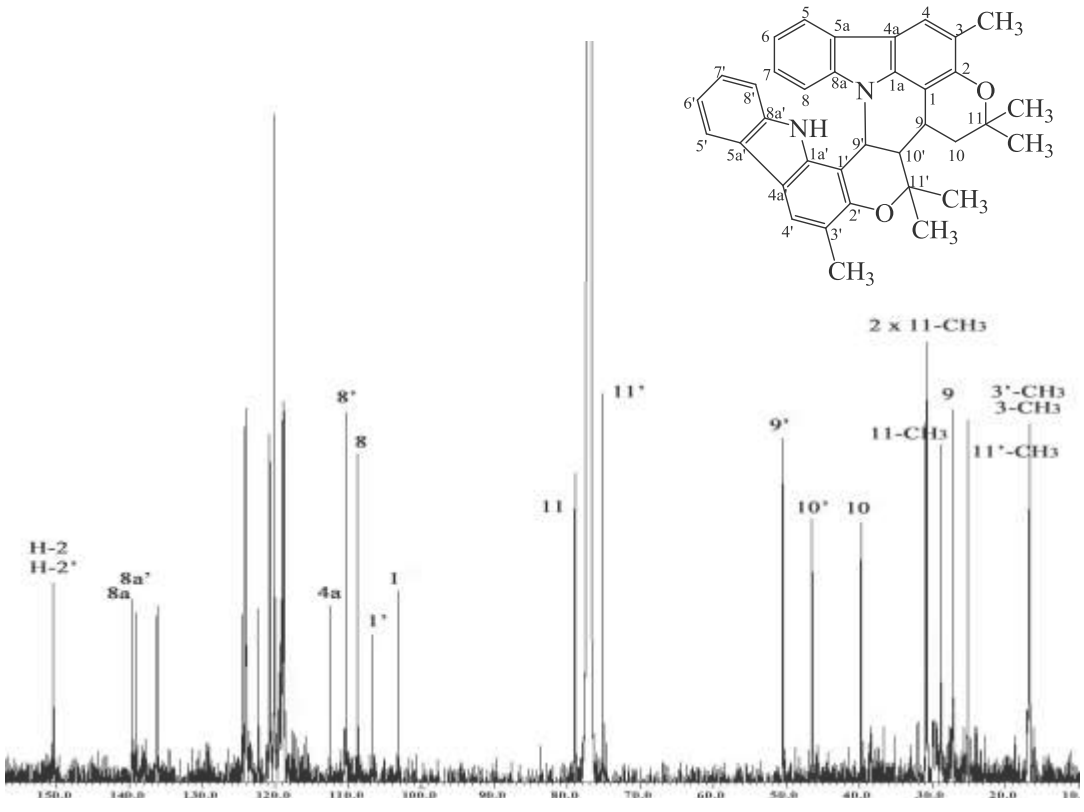


Figure 4.3.1b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of **G1**

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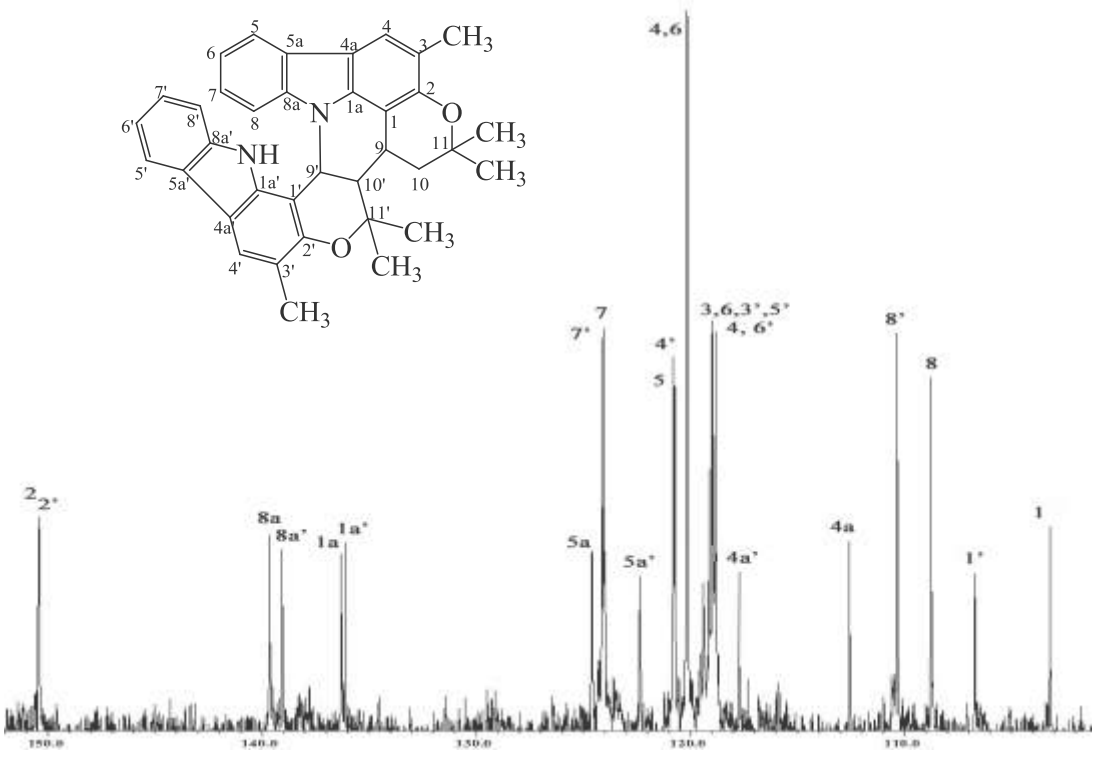


Figure 4.3.1c. Expanded ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of **G1**

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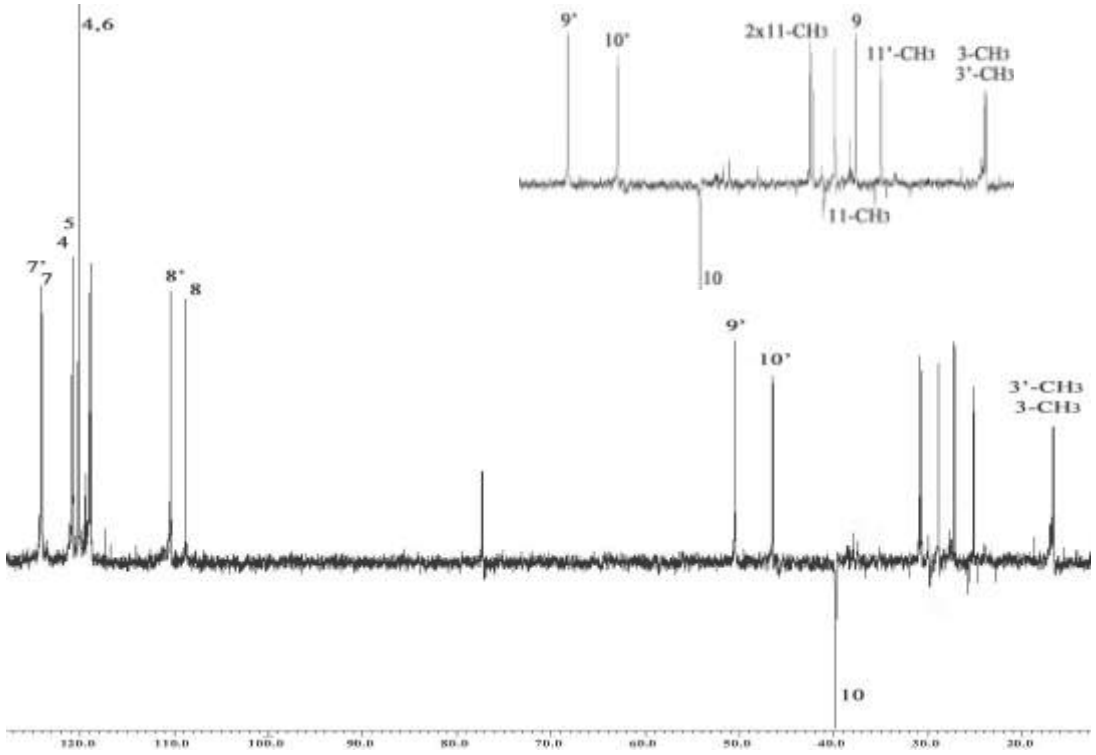


Figure 4.3.1d. DEPT-135 Spectrum ($CDCl_3$, 125 MHz) of **G1**

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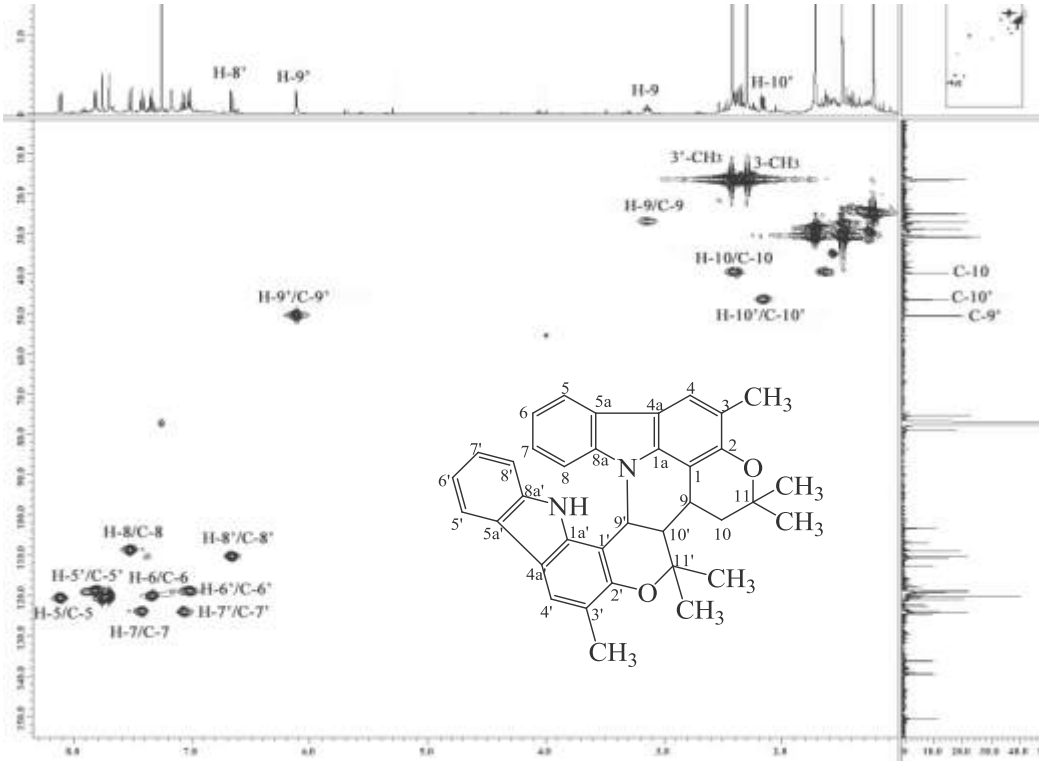


Figure 4.3.1e. HMQC Spectrum ($CDCl_3$, 500 MHz) of **G1**

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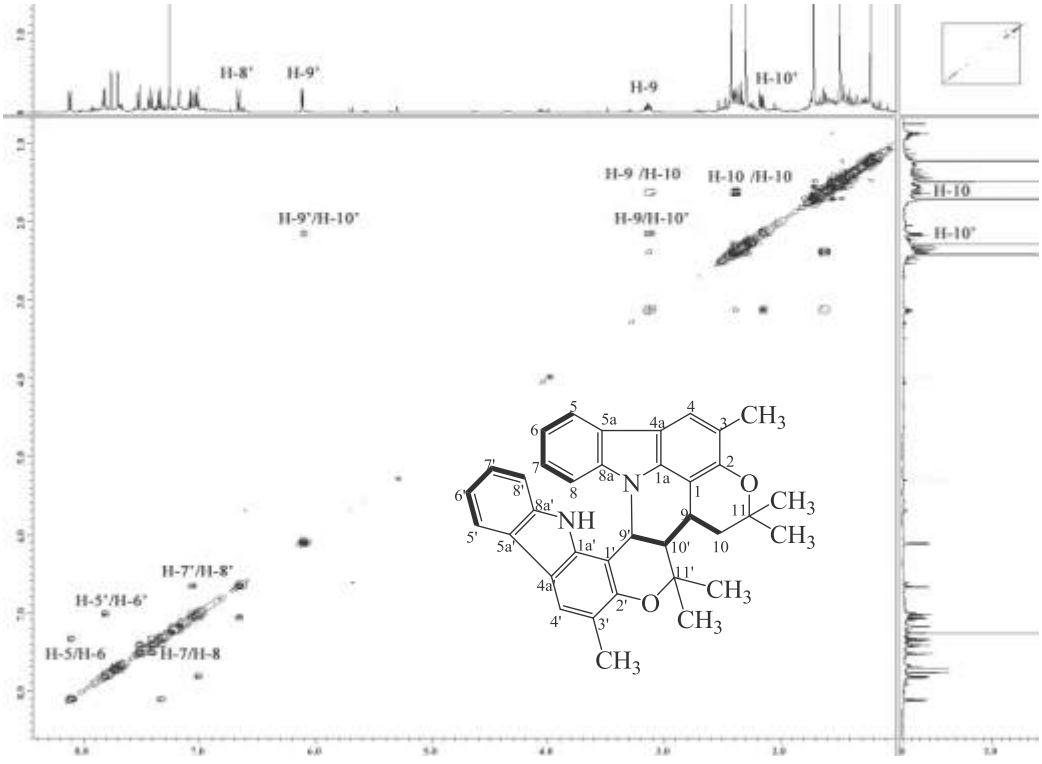


Figure 4.3.1f. ^{1}H-^{1}H COSY Spectrum ($CDCl_3$, 500 MHz) of **G1**

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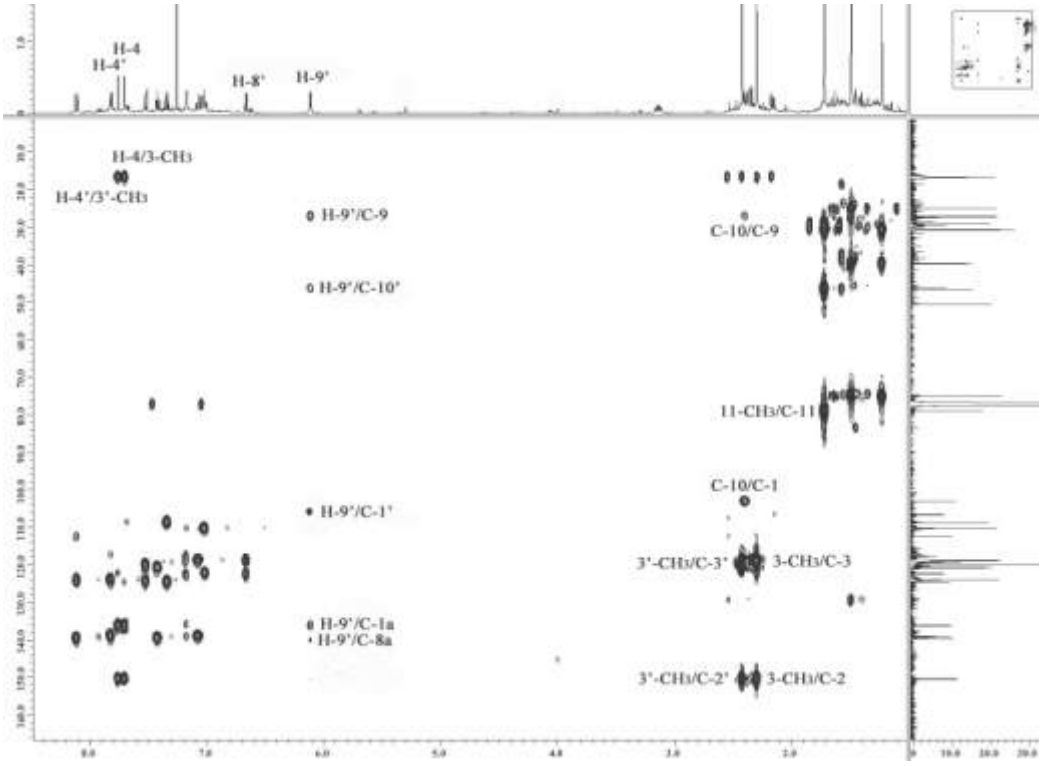


Figure 4.3.1g. HMBC Spectrum ($CDCl_3$, 500 MHz) of **G1**

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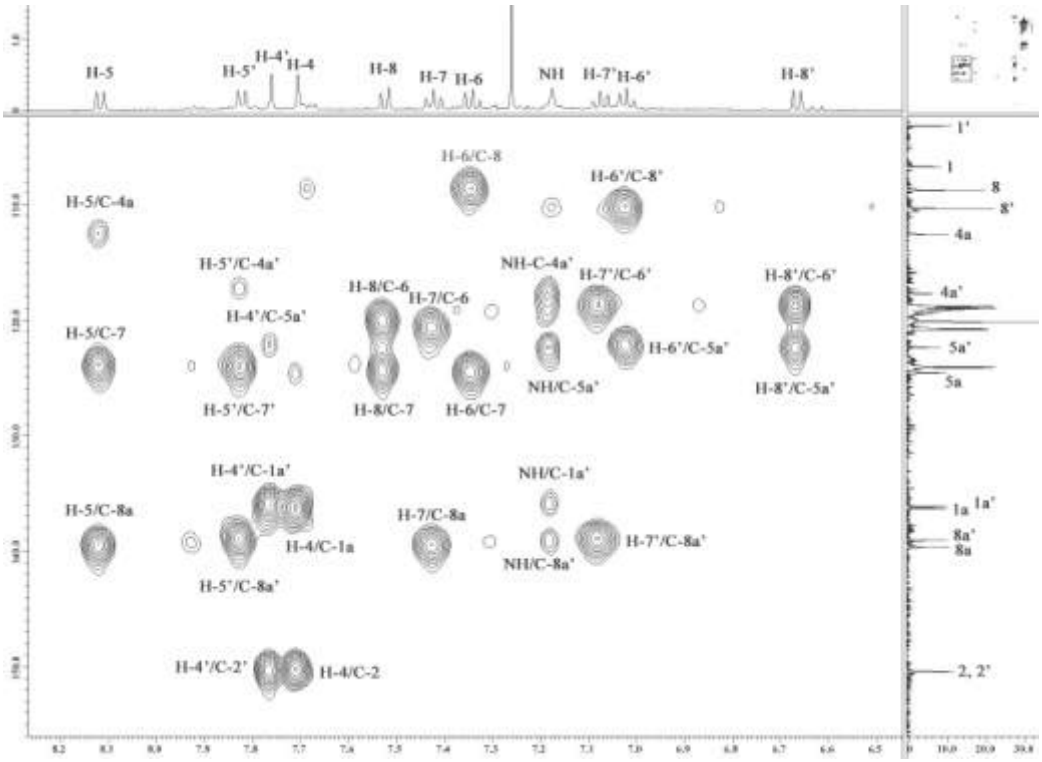


Figure 4.3.1h. Expanded HMBC Spectrum ($CDCl_3$, 500 MHz) of **G1**

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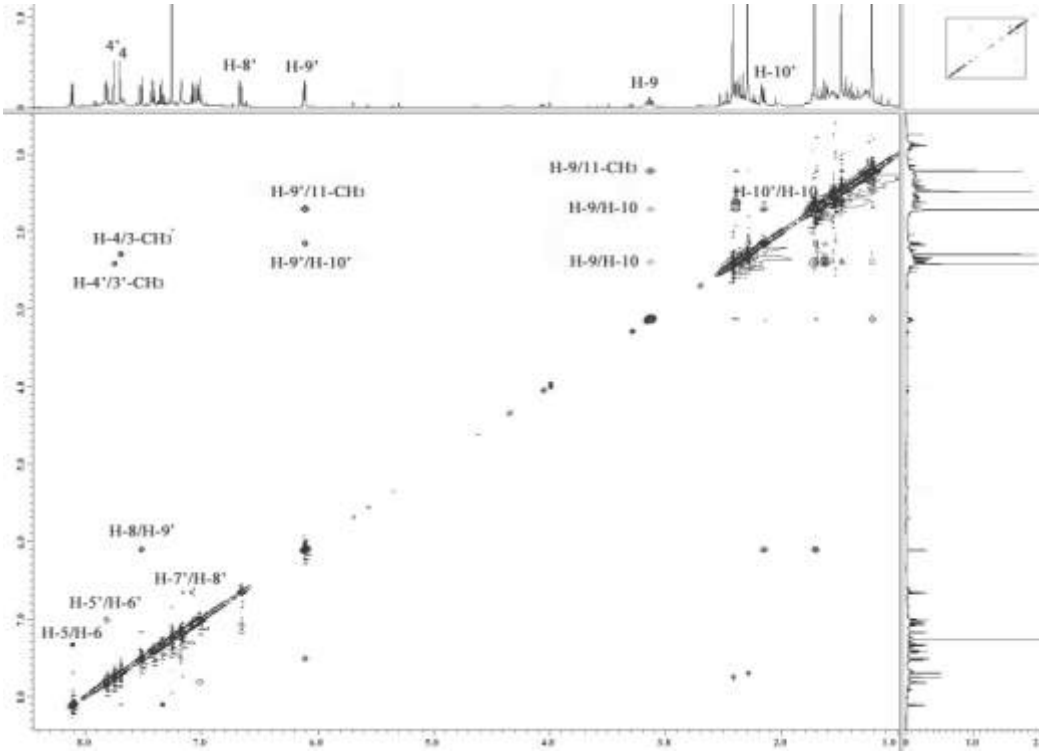


Figure 4.3.1i. NOESY Spectrum ($CDCl_3$, 500 MHz) of **G1**

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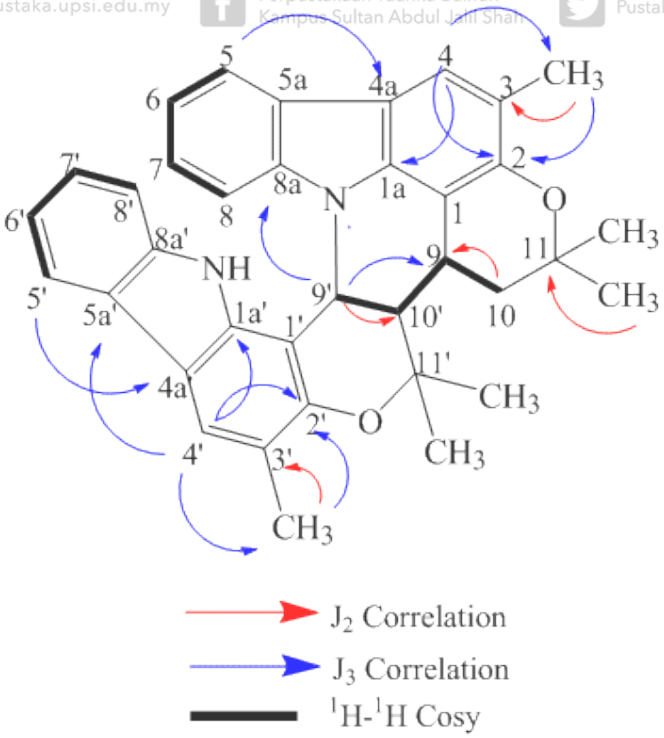


Figure 4.3.1j. Selected ^1H-^1H COSY and HMBCs of **G1**

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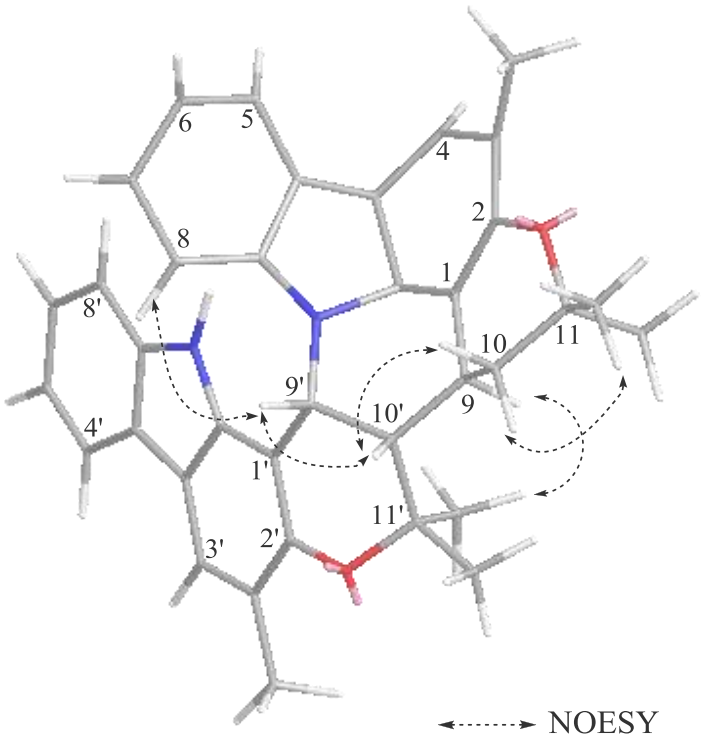


Figure 4.3.1k. Selected NOESY Correlations and Relative Stereochemistry for **G1**

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Table 4.3.2

1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of ***G2****

Position	δ ^{13}C	DEPT	δ ^{1}H	HMBC correlations	COSY
1	104.3	qC		H-9	
1a	137.3	qC		H-4, H-9	
2	150.5	qC		H-4, H-9, 3-CH_3	
3	118.9	qC		3-CH_3	
4	121.5	CH	7.76 (*s*)	3-CH_3	
4a	116.6	qC		H-5	
5	119.5	CH	7.94 (*d*, 8.0 Hz)	H-6	H-6
5a	123.7	qC		H-6	
6	119.6	CH	7.20 (*td*, 8.0, 1.0 Hz)	H-5, H-8	H-5, H-7
7	124.5	CH	7.32 (*td*, 8.0,1.0 Hz)	H-5, H-6	H-6, H-8
8	110.5	CH	7.22 (*d*, 8.0 Hz)	H-6	H-7
8a	139.3	qC		H-5, H-8	
9	71.2	CH	5.22 (*d*, 6.3 Hz)	H-10	H-10
10	48.6	CH	2.65 (*d*, 6.9 Hz)	H-9, 11-CH_3	H-9
11	76.4	qC		H-9, H-10, 11-CH_3	
N-H			8.17 (*br s*)		
3-CH_3	16.4	CH_3	2.33 (*s*)		
11-CH_3	28.4	CH_3	1.61 (*s*)	H-10, 11-CH_3	
11-CH_3	24.2	CH_3	1.46 (*s*)	H-10, 11-CH_3	

Note. *Solvent used was $CDCl_3$; assignments based on COSY, NOESY, HMQC and HMBC.

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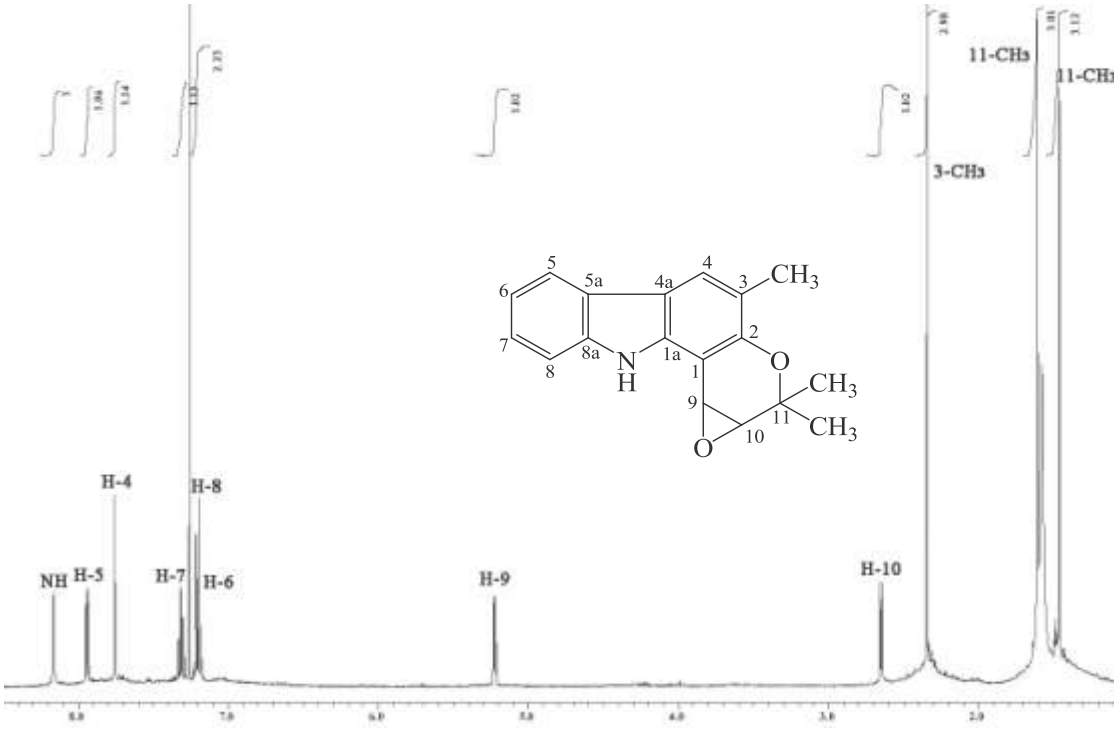


Figure 4.3.2a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of **G2**

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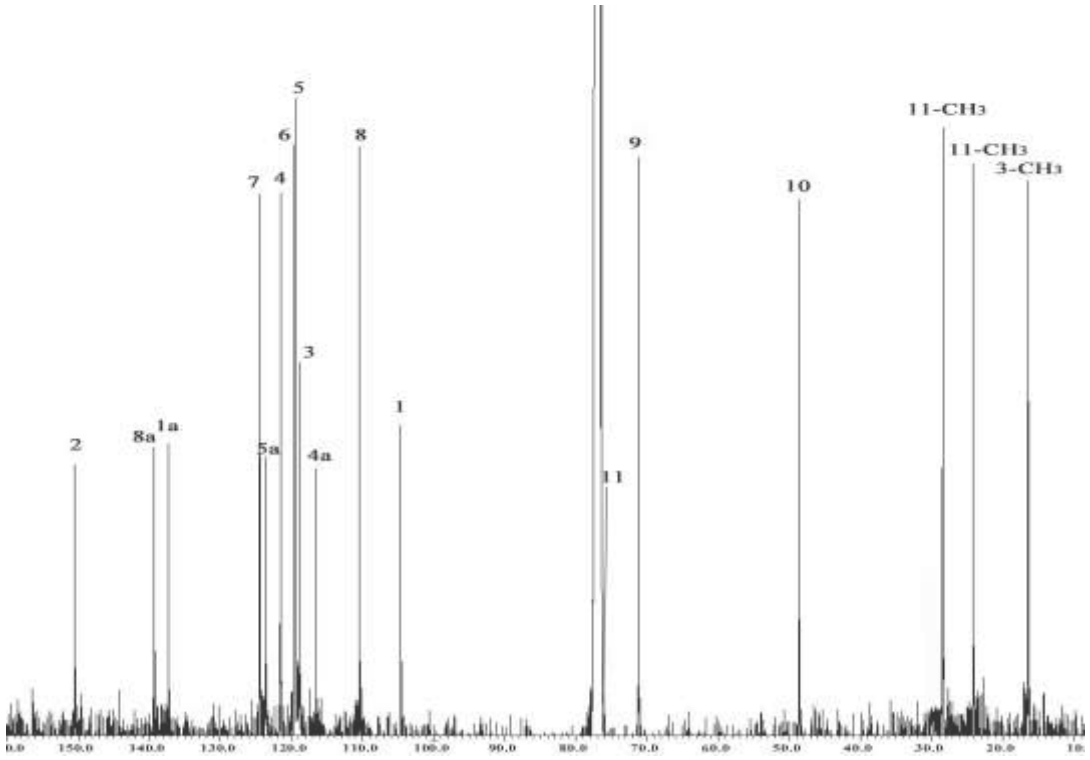


Figure 4.3.2b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of **G2**

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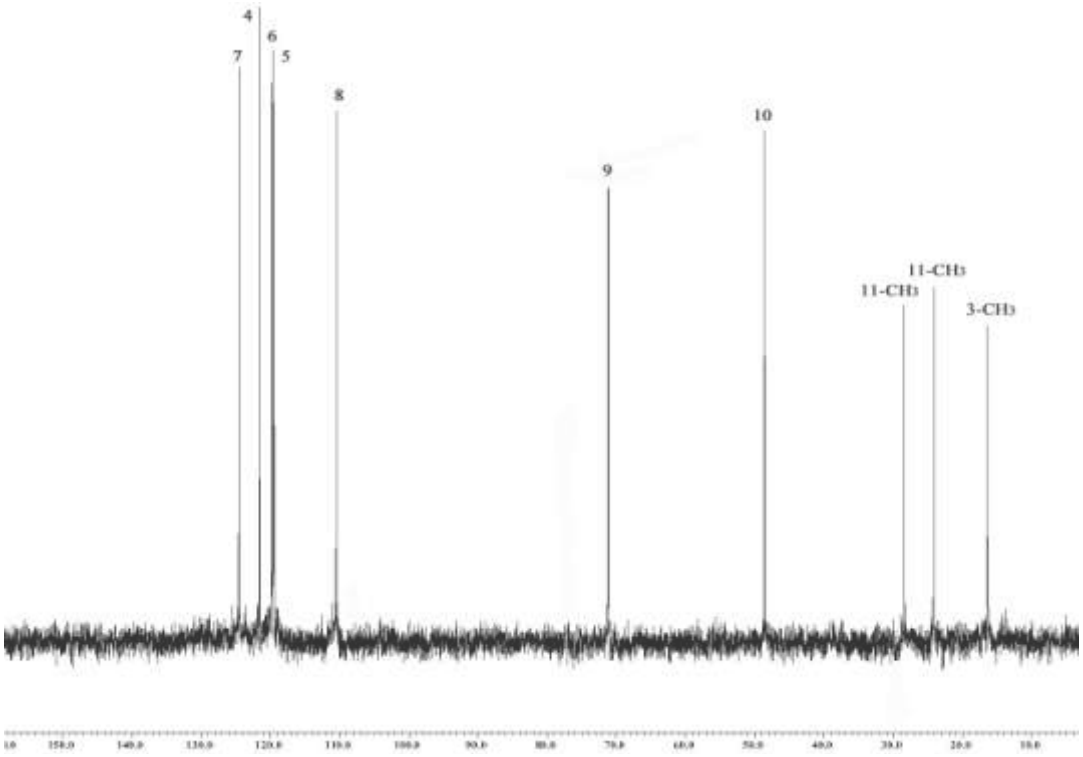


Figure 4.3.2c. DEPT-135 Spectrum ($CDCl_3$, 125 MHz) of **G2**

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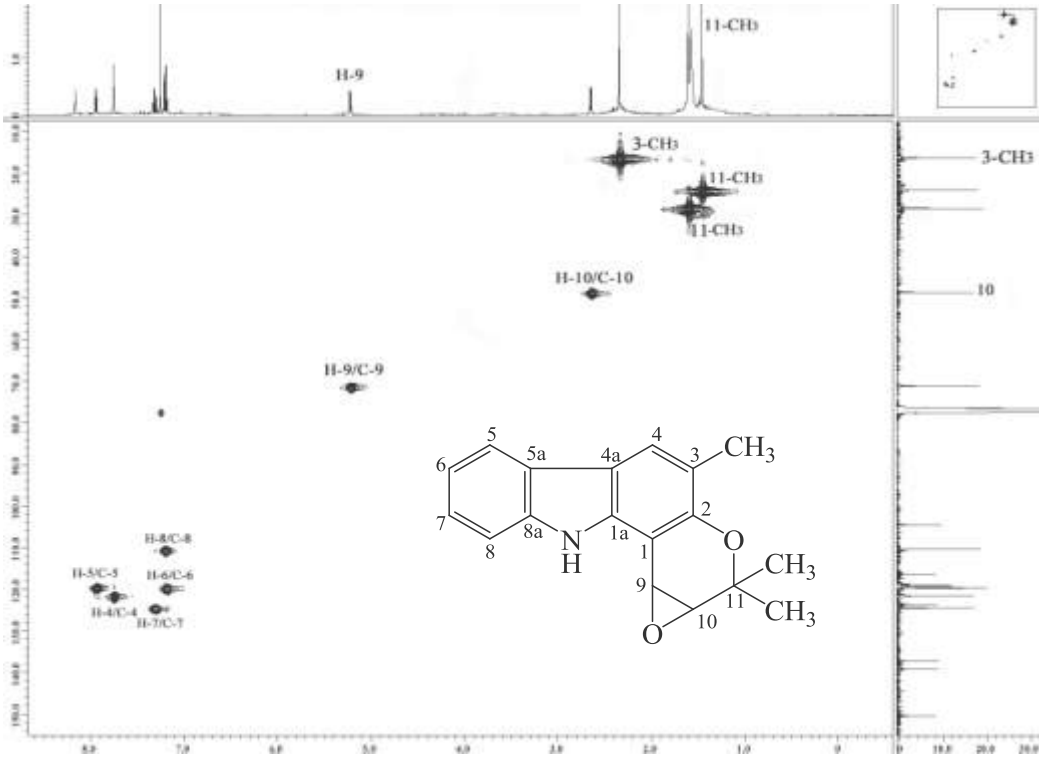


Figure 4.3.2d. HMQC Spectrum ($CDCl_3$, 500 MHz) of **G2**

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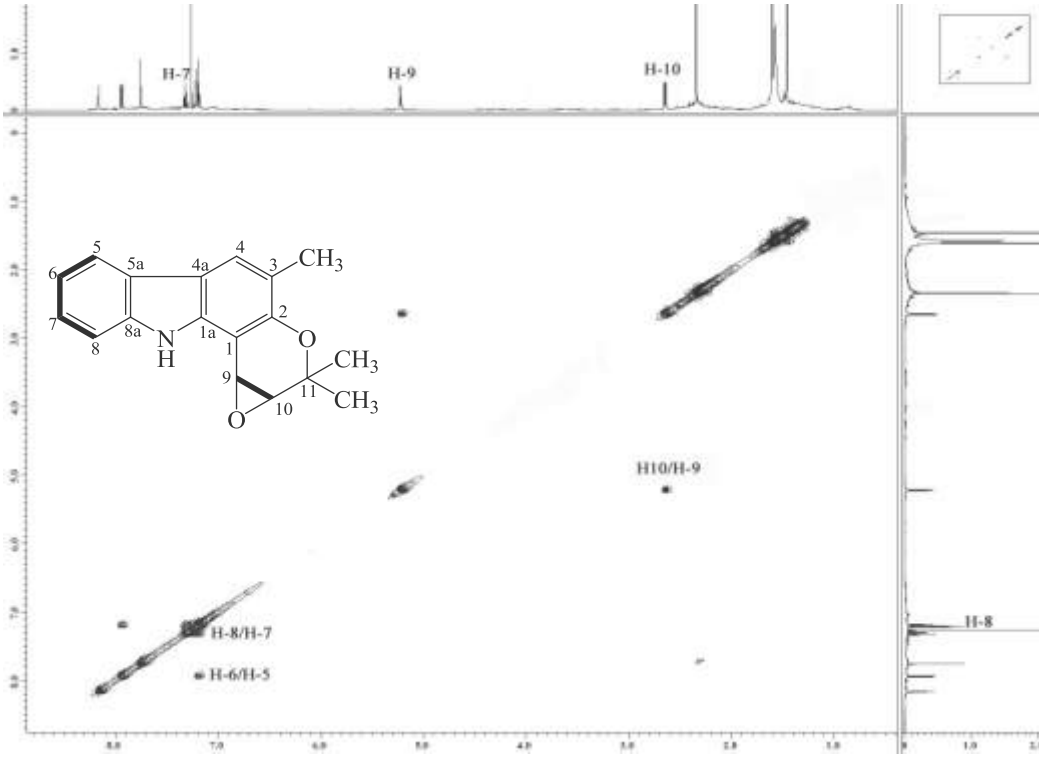


Figure 4.3.2e. ^{1}H-^{1}H COSY Spectrum ($CDCl_3$, 500 MHz) of **G2**

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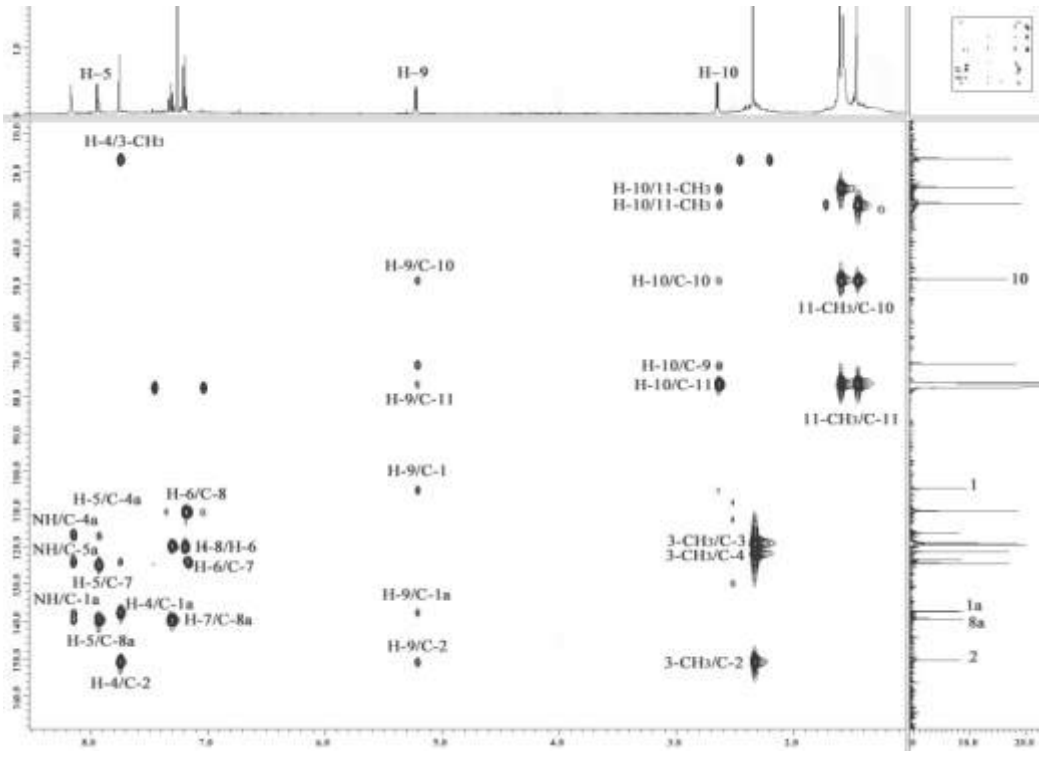


Figure 4.3.2f. HMBC Spectrum ($CDCl_3$, 500 MHz) of **G2**

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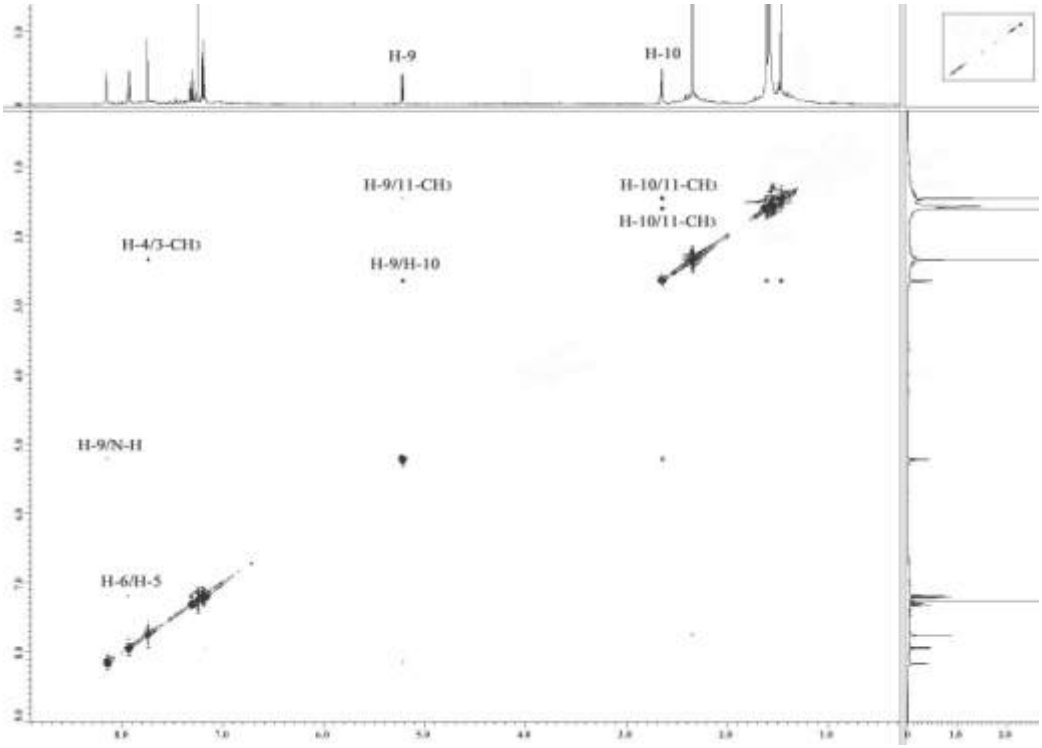


Figure 4.3.2g. NOESY Spectrum ($CDCl_3$, 500 MHz) of **G2**

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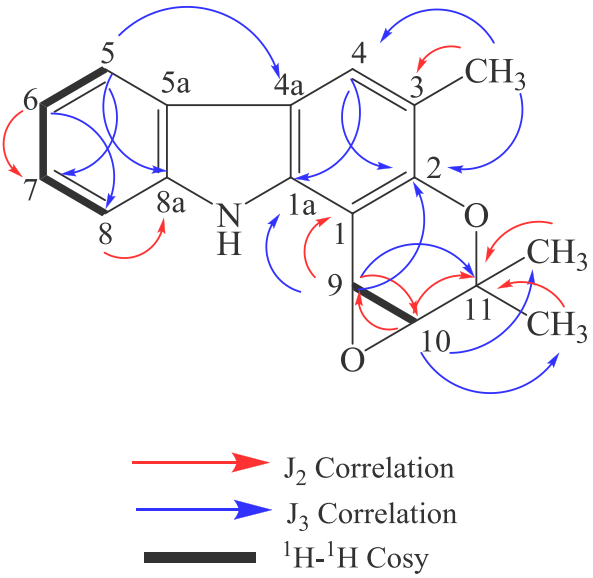


Figure 4.3.2h. Selected ^1H-^1H COSY and HMBCs of **G2**

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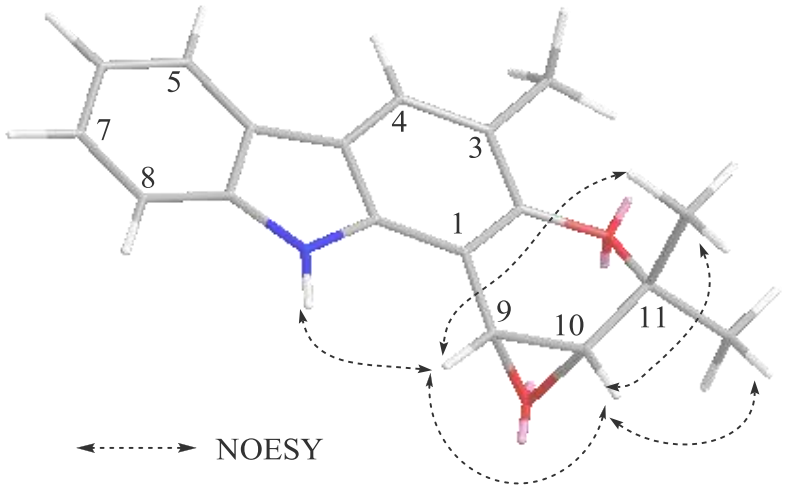


Figure 4.3.2i. Selected NOESY Correlations and Relative Stereochemistry for **G2**

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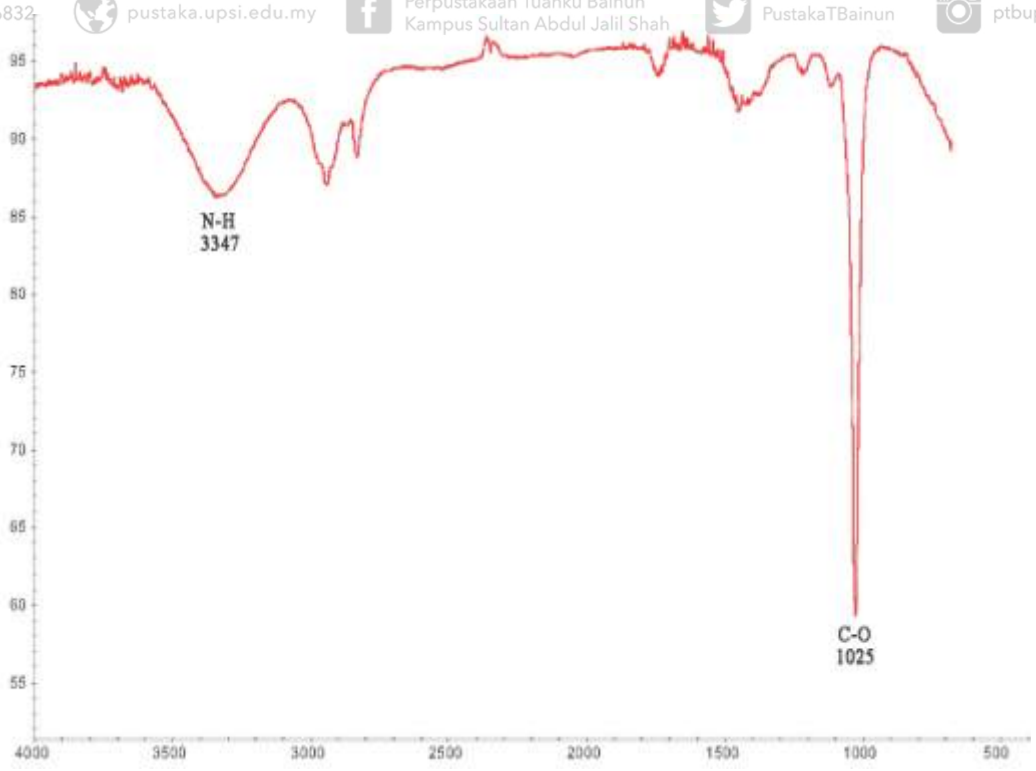


Figure 4.3.2j. IR Spectrum of Epoxygirinimbine **(G2)**

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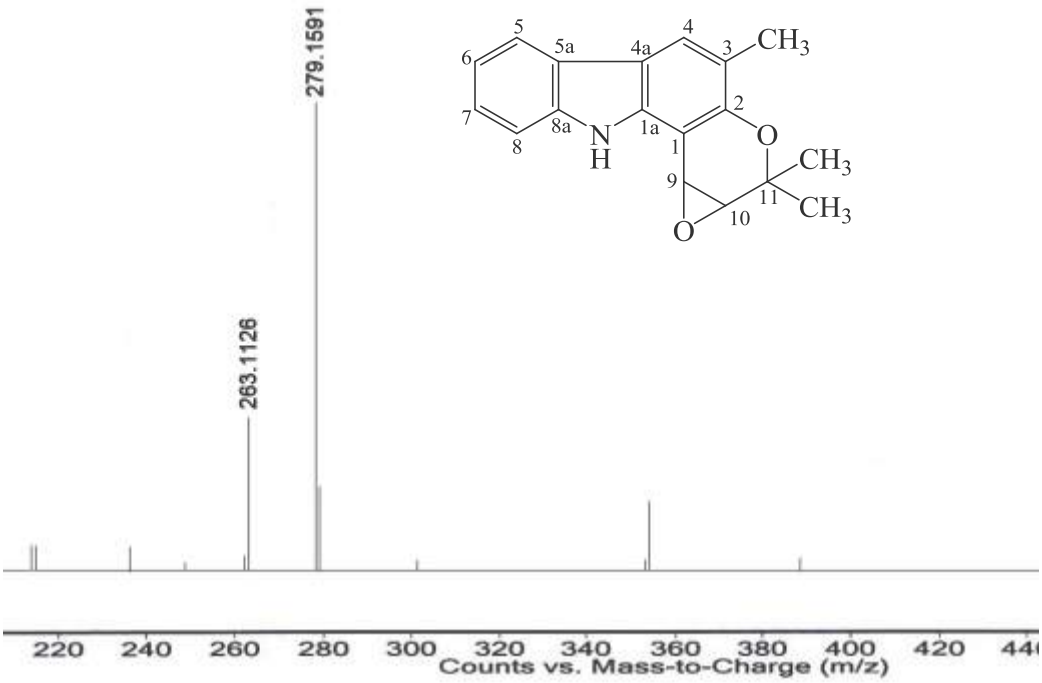


Figure 4.3.2k. Mass Spectrum of Epoxygirinimbine **(G2)**

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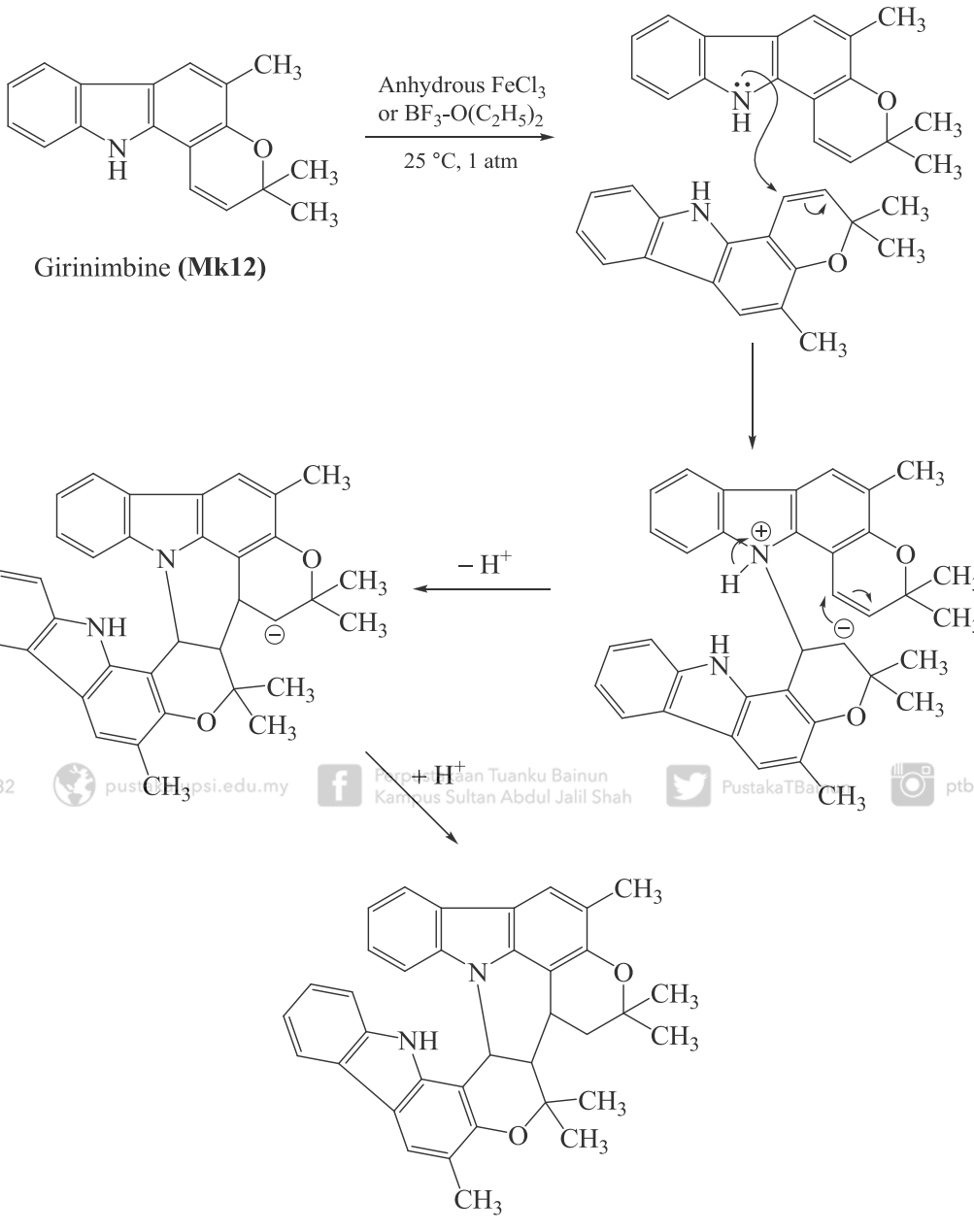
4.3.2 Mechanistic Studies

One-pot synthesis of murranimbine **(G1)** was known to be an oxidative coupling reaction using selected Lewis acid. The mechanism proposed for the formation of **G1** was initiated by the bimolecular nucleophilic substitution by the lone pair of NH group of the first molecule to the electron deficient chromene double bond at C-9' of the second molecule of girinimbine **(Mk12)**, followed by the ring closure resulting by the formation of a C–N and C–C linkage between two of molecules of girinimbine **(Mk12)** (Scheme 4.3.1).

A new epoxygirinimbine **(G2)** was derived as a *cis*-epoxide by using anhydrous $FeCl_3$, which was proposed to be a simple and efficient approach towards metal-catalyzed enantioselective epoxidation of girinimbine **(Mk12)** (Colonna, Manfredi, Annunziata, Gaggero, & Casella, 1990). In this approach, formation of the oxygen evolving complex, the iron(IV)-oxo moiety [Cl_3–Fe(III)=O] in the form of radical, acted as the stoichiometric oxidant (Nam, 2007).

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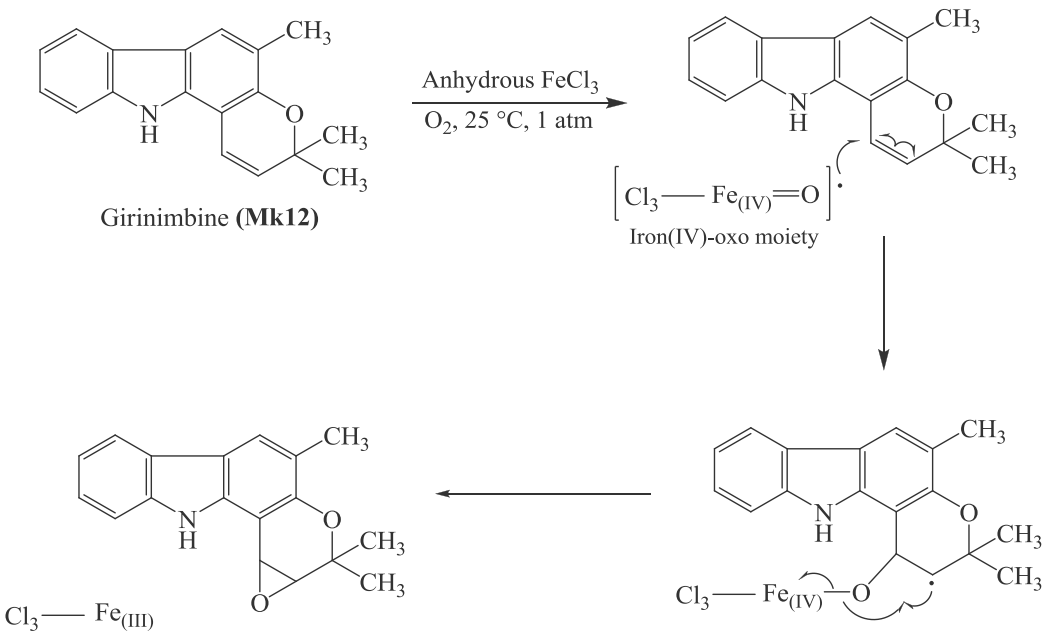
Girinimbine **(Mk12)**

Murranimbine **(G1)**

Scheme 4.3.1. A Possible Mechanistic Pathway to **G1**

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Girinimbine **(Mk12)**

Epoxygirinimbine **(G2)**

Scheme 4.3.2. A Possible Mechanistic Pathway to **G2**

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4.4 Synthesis of Mahanimbine Analogues

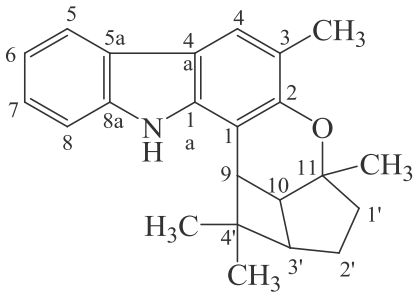
Another major compound isolated from *M. koenigii*, namely mahanimbine **(Mk15)**, was the second compound chosen for the synthesis reaction by using the same reagents, *viz.*, anhydrous ferric (III) chloride ($FeCl_3$) and boron trifluoride diethyletherate (BF_3-$O(C_2H_5)_2$). As a result, three known analogues, namely bicyclomahanimbine **(M1)**, cyclomahanimbine **(M2)** and murrayazolinine **(M3)**, were successfully derived.

The structural assignments of **M1**, **M2** and **M3** were based on their spectroscopic evidences from 1D NMR (^1H, ^{13}C, DEPT), 2D NMR (^1H-^1H COSY, NOESY, HMBC and HMQC) and mass spectrometry which were in full agreement with those reported for their corresponding natural products.

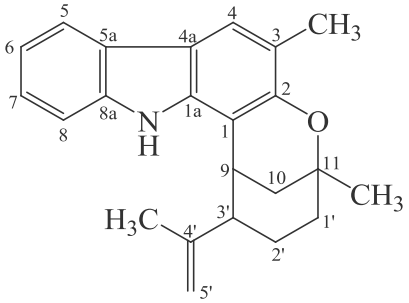
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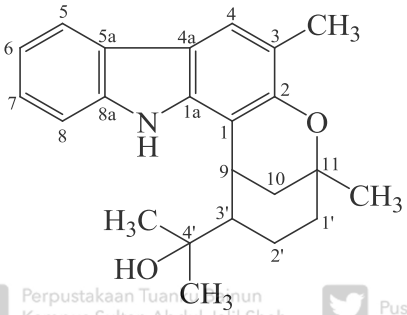
4.4.1 Spectroscopic Analysis of Bicylcomahanimbine (M1), Cyclomahanimbine (M2) and Murrayazolinine (M3)



M1



M2



M3

One step anhydrous $FeCl_3$-mediated approach, a hexacyclic compound, bicyclomahanimbine **(M1)**, was successfully derived from mahanimbine **(Mk15)** with 41.7% yield (Table 3.3, entry 5). The synthetic approach is shown in Scheme 3.9. This compound was also naturally isolated from the bark of *M. koenigii* during the phytochemical analysis of the selected plant. Several attempts have been tried to improve the turnover by modification of the reaction conditions (time, amount of anhydrous $FeCl_3$, solvent) gave no improvement with yield ranging from 39.2% to 41.7%. Meanwhile, for the synthesis using BF_3-$O(C_2H_5)_2$, not only **M1** (overall yield up to only 11.3%, entry 2) but along with another two pentacyclic carbazole, cyclomahanimbine **(M2)** (yield up to 20.0%, entry 6) and murrayazolinine **(M3)**

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(yield up to 44.0%, entry 6), were also obtained (Table 3.5). Therefore, optimization towards the formation of **M1** can be achieved by using anhydrous $FeCl_3$. However, to obtain more cyclised compounds in one-pot step can be achieved by using BF_3-$O(C_2H_5)_2$ as the catalyst for the reaction.

The structure of these cyclised compounds were determined with the combination of 1D and 2D NMR measurements and by comparing the data from the literature. Table 4.4.1, Table 4.4.2 and Table 4.4.3 showed the NMR spectral data of **M1**, **M2** and **M3**, respectively. Figure 4.4.1a to 4.4.1g, Figure 4.4.2a to 4.4.2g and Figure 4.3.2a to 4.3.2g showed the ^1H, ^{13}C, COSY, HMQC and HMBC NMR spectra of **M1**, **M2** and **M3**, respectively.

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M1 with a quasi-molecular ion peak at *m/z* 332.2012 ($[M+H]^+$, calcd. mass: 332.2014) in its mass spectral data consistent with the molecular formula $C_{23}H_{25}NO$ which has been discussed previously in section 4.2.4.2. The aromatic region of the ^1H NMR spectrum ($CDCl_3$, 500 MHz) (Figure 4.4.1a), indicated a similar aromatic substitution pattern of mahanimbine skeleton. The presence of a hexacylic base cyclobutane ring system was supported by the downfield shift of the methyl group at 4'-CH_3 to δ 0.74 in bicyclomahanimbine when compared to the methyl group of mahanimbine **(Mk15)** at δ 1.62. The complete assignments for **M1** is based on its spectroscopic data and also with those reported for the natural products. The structure of **Mk25** was assigned as bicyclomahanimbine (Kureel et al., 1969b).

M2, a cyclic monoterpenoid pyrano[3,2a]carbazole alkaloid, namely cyclomahanimbine which was derived from mahanimbine **(Mk15)** via one-pot BF_3-

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$O(C_2H_5)_2$-mediated approach. Its molecular formula was established to be $C_{23}H_{25}NO$ by HRESIMS at *m/z* 331.1928 ($[M]^+$, calcd. mass: 331.1936). The ^1H NMR ($CDCl_3$, 500 MHz) of **M2** (Figure 4.4.2a) exhibited signals for five aromatic methine protons, of which one appeared as singlet (H-4, δ 7.64), two as doublets (H-5 and H-8) and two as triplet of doublets (H-6 and H-7). The HMBC spectrum of **M2** suggested the presence of an unsubstituted carbazole ring by showing correlation of these four mutually coupled aromatic methine protons with neighbouring carbons which were resembled to those in mahanimbine **(Mk15)**. Their proton positions were further confirmed by ^1H-^1H COSY (Figure 4.4.2e) and HMBC spectra (Figure 4.4.2f). The ^1H NMR spectrum also showed three methyl signals. For examples, an aromatic methyl at δ 2.23, a methyl group attached to a tetrasubstituted oxygen bearing group at δ 1.44 and a methyl group at C-4' with δ 1.50. The position of a pair of methylene signals at H-5' [δ 4.74 (*d*, *J* = 1.7 Hz), δ 4.81 (*d*, *J* = 1.7 Hz)] was further confirmed by the HMBC experiment by showing correlation between the aliphatic methyl protons (4'-CH_3, δ 1.50) with the mutually coupled methylene protons bearing carbon (C-5', δ 112.1). It also showed correlations from H-5' to C-3' (δ 48.8) and 4'-CH_3 (δ 21.7) to gave further evidence to confirm the exact position of the methylene proton of C-5'. Based on the spectroscopic data, **M2** was assigned to cyclomahanimbine (Kureel et al., 1969b).

The molecular formula of **M3** was established to be $C_{23}H_{27}NO_2$ by its HRESIMS at molecular ion peak at *m/z* 350.1750 ($[M+H]^+$, calcd. mass: 350.2120) coupled with ^1H and ^{13}C NMR spectral data (Table 4.4.3). The ^1H NMR spectrum of **M3** ($CDCl_3$, 500 MHz) (Figure 4.4.3a) showed signals for five aromatic protons [δ

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7.66 (*s*, H-4), δ 7.91 (*d*, *J* = 8.0 Hz, H-5), δ 7.14 (*td*, *J* = 8.0, 1.2 Hz, H-6), δ 7.27 (*td*, *J* = 8.0, 1.2 Hz, H-7), δ 7.37 (*d*, *J* = 8.0 Hz, H-8)] similar to those in mahanimbine **(Mk15)**. Two board signals indicated the appearance of a NH signal at δ 9.59 (*br s*) and an OH signal at δ 1.72 (*s*). Other signals were also observed for one benzylic methyl protons at δ 2.34 (*s*, 3-CH_3), six methylene protons [δ 1.85 (*q*, *J* = 2.9 Hz, H-10, 2H), δ 2.09 (*m*, H-1'a), δ 1.62 (*m*, H-1'b), δ 1.45 (*m*, H-2', 2H)], the cyclohexane ring methine proton [δ 3.80 (*m*, H-9), 1.90 (*m*, H-3')] and one aliphatic methyl group [δ 1.43 (*s*, 11-CH_3)]. A 3H singlets at δ 1.32 (*s*, 4'a-CH_3) was attributed to one of the methyl group of the *gem*-dimethyl function, while a highly shielded signal at δ 0.55 (*s*, 4'b-CH_3), which due to its aromatic ring currect effect (held rigidly above the plane of the benzene ring), was attributed to another methyl group of the *gem*-dimethyl functionality. Furthermore, the structure was also supported by the DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC measurements (Figure 4.4.3c to Figure 4.4.3g). Based on the spectroscopic data and the data from the literature, **M2** was assigned to murrayazolinine, which was previously isolated as natural products by Chakraborty and coworkers (1973).

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Table 4.4.1

1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of ***M1****

Position	δ ^{13}C	DEPT	δ ^{1}H	HMBC correlations	COSY
1	106.5	qC		H-9	
1a	137.8	qC		H-4, H-9	
2	150.2	qC		H-4, H-9, 3-CH_3	
3	120.1	qC		3-CH_3	
4	119.2	CH	7.66 (*s*)	3-CH_3	
4a	115.8	qC			
5	119.4	CH	7.93 (*d*, 8.1 Hz)	H-7	H-6
5a	124.2	qC		H-5	
6	119.4	CH	7.15 (*td*, 8.1, 1.0 Hz)	H-8	H-5, H-7
7	124.0	CH	7.28 (*td*, 8.1, 1.0 Hz)	H-6, H-8	H-6, H-8
8	110.4	CH	7.37 (*d*, 8.1 Hz)	H-6	H-7
8a	139.3	qC		H-5, H-7	
9	37.5	CH	3.30 (*d*, 9.2 Hz)		H-10, H-3'
10	46.5	CH_2	2.50 (*t*, 7.4 Hz)		H-9
11	83.5	qC		H-10, 11-CH_3	
N-H			7.44 (*br s*)		
3-CH_3	16.8	CH_3	2.33 (*s*)	H-4	
1'	38.4	CH	2.04 (*m*)	H-2', 4'-CH_3	H-2'
			1.55 (*m*)		
2'	25.7	CH_2	1.66 (*m*)		H-1', H-3'
			1.56 (*m*)		
3'	37.9	CH	2.71 (*t*, 7.5 Hz)	H-9, 4'-CH_3	H-9, H-2'
4'	39.5	qC		H-9, H-3', 4'-CH_3	
11-CH_3	27.5	CH_3	1.43 (*s*)	H-9, H-10, 11-CH_3	
4'-CH_3	18.6	CH_3	0.74 (*s*)		
4'-CH_3	35.2	CH_3	1.57 (*s*)		

Note. *Solvent used was $CDCl_3$; assignments based on COSY, HMQC and HMBC.

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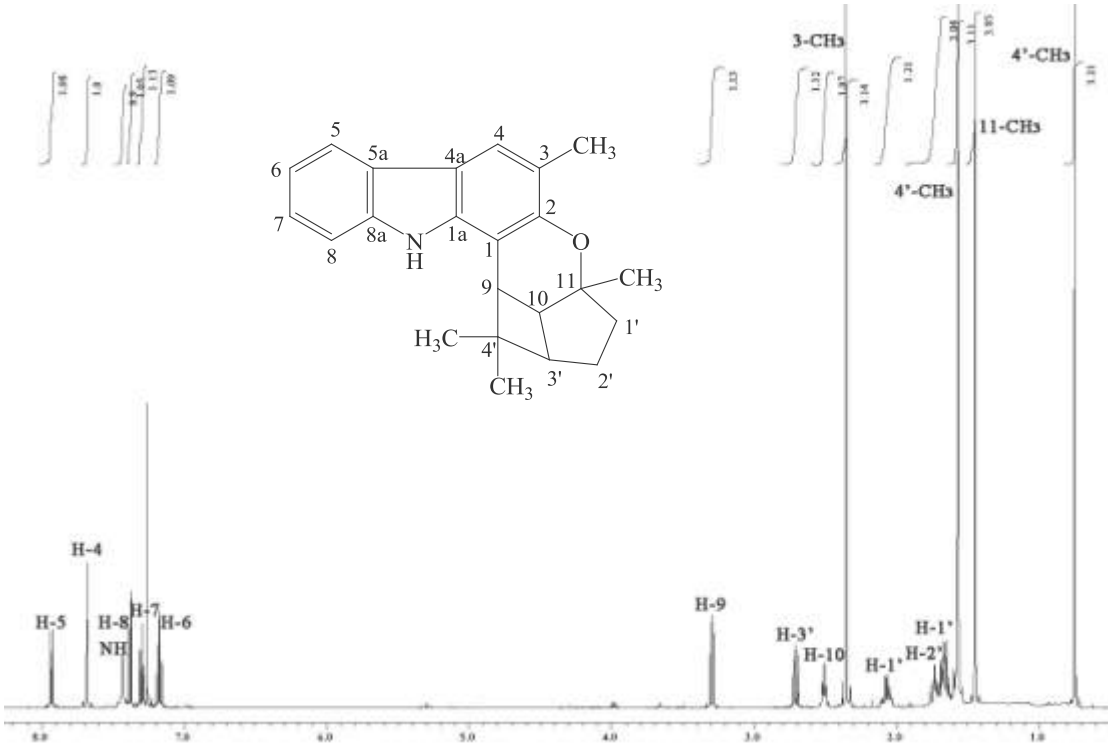


Figure 4.4.1a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of **M1**

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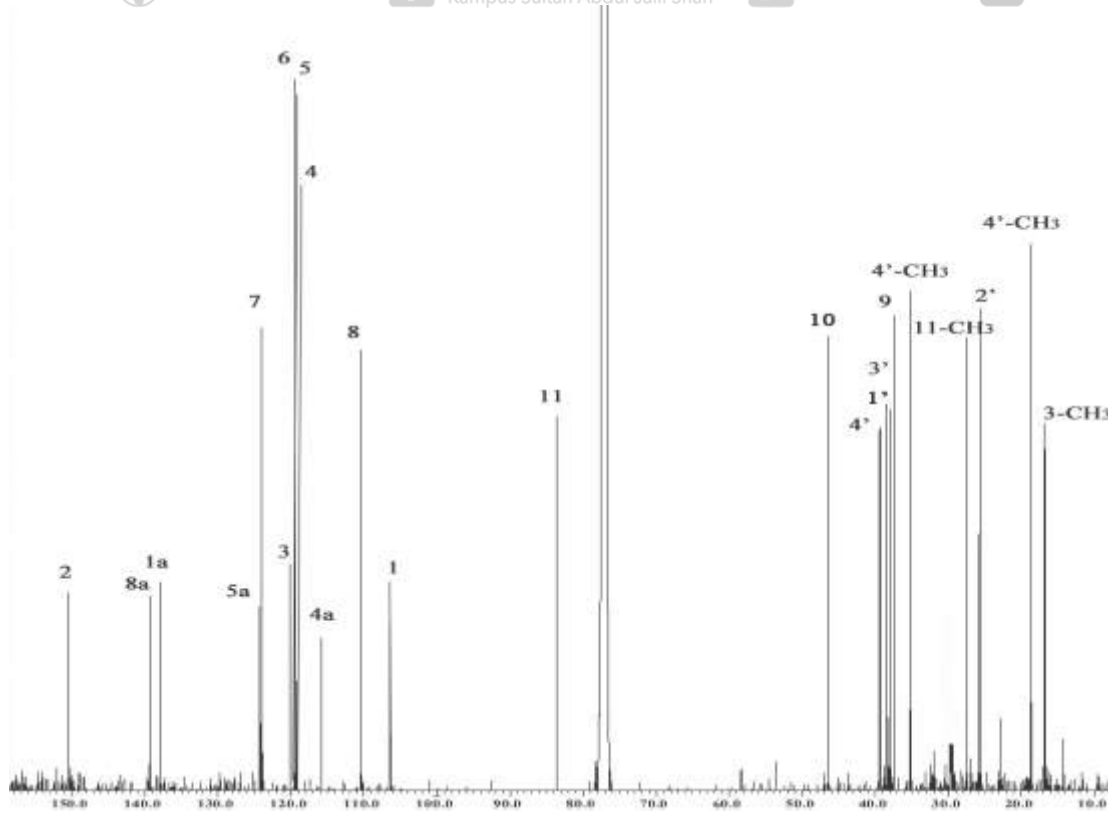


Figure 4.4.1b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of **M1**

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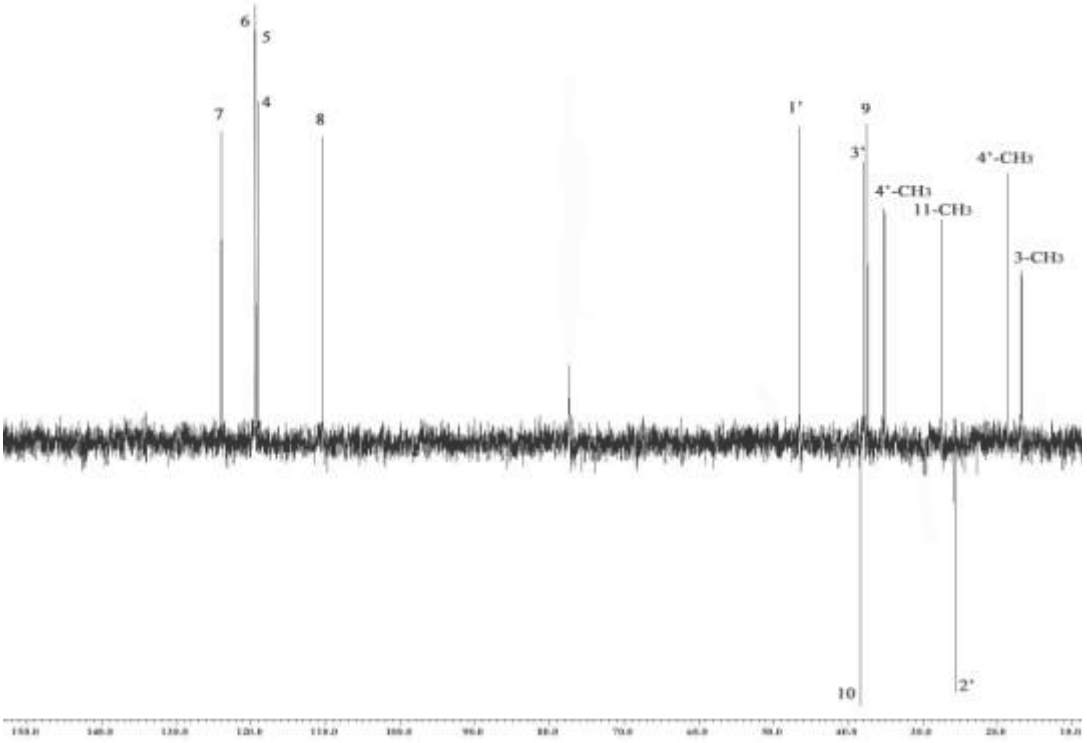


Figure 4.4.1c. DEPT-135 Spectrum ($CDCl_3$, 125 MHz) of **M1**

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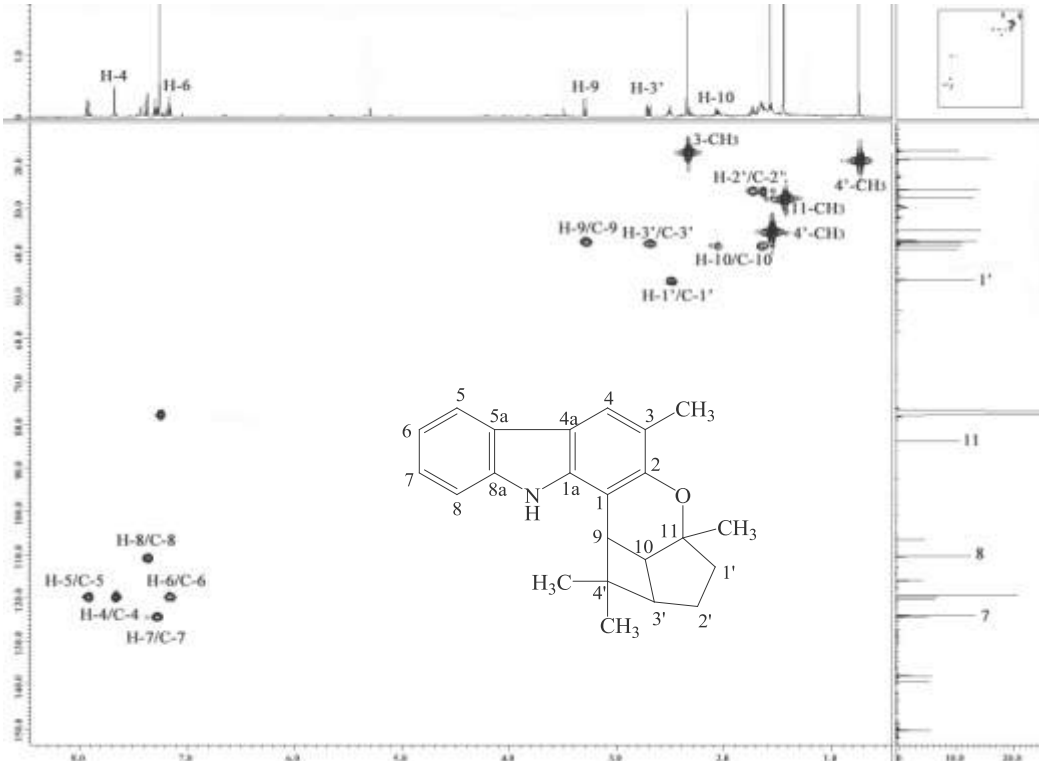


Figure 4.4.1d. HMQC Spectrum ($CDCl_3$, 500 MHz) of **M1**

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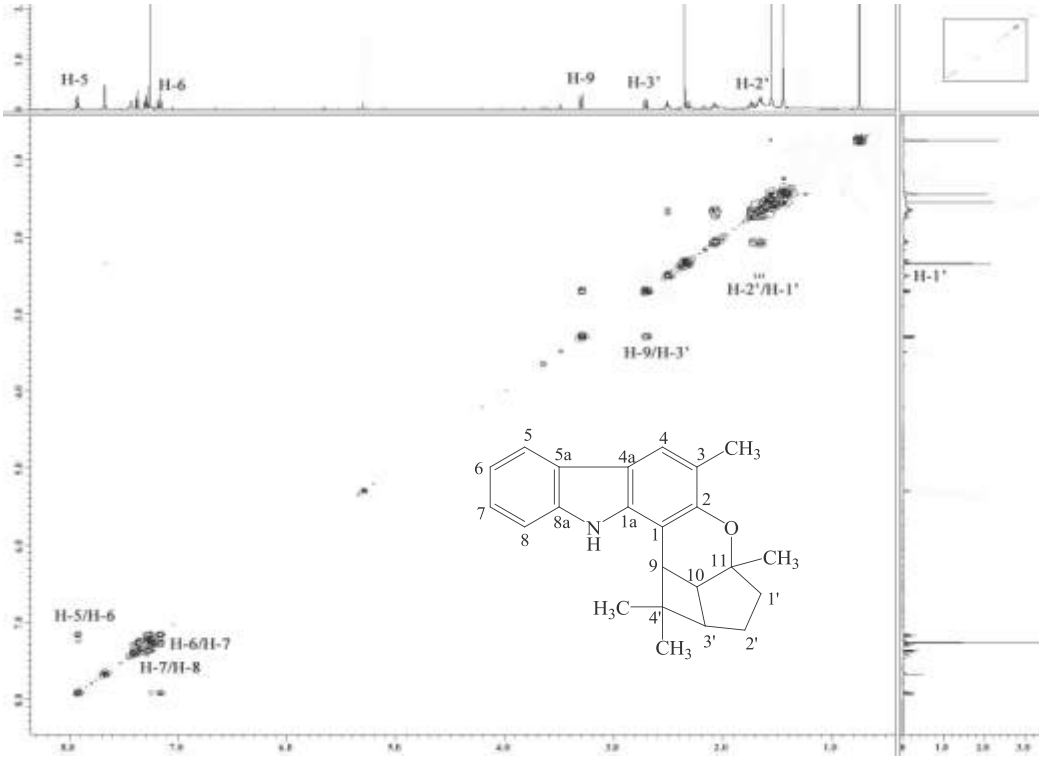


Figure 4.4.1e. ^{1}H-^{1}H COSY Spectrum ($CDCl_3$, 500 MHz) of **M1**

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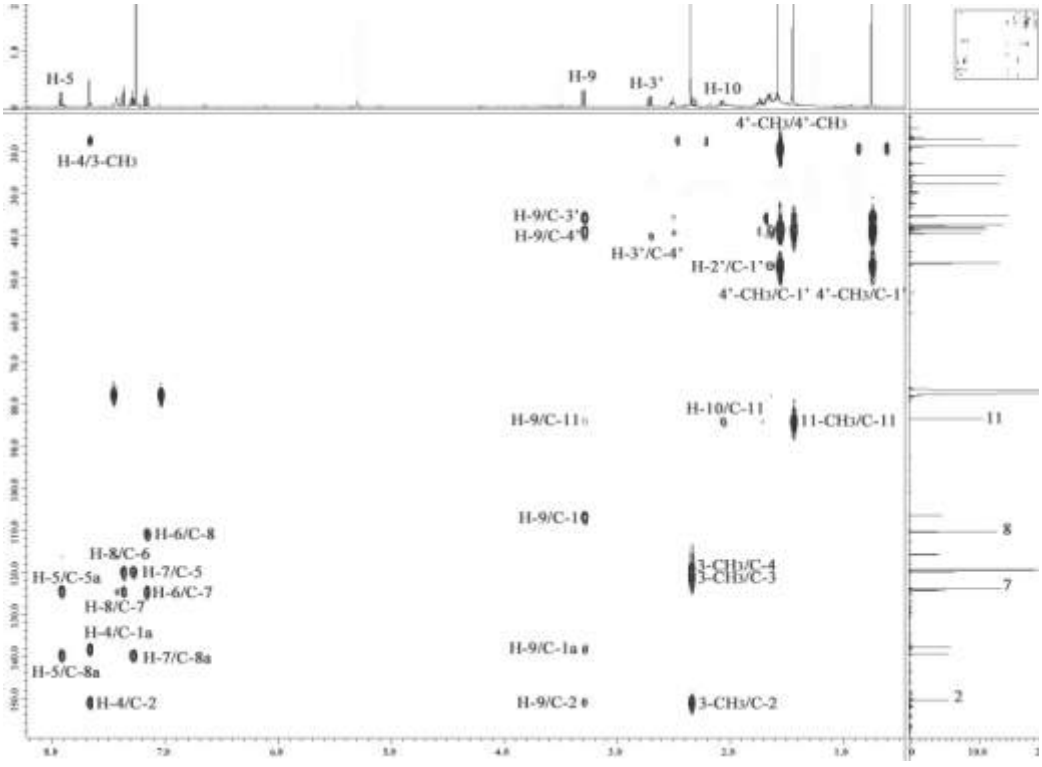


Figure 4.4.1f. HMBC Spectrum ($CDCl_3$, 500 MHz) of **M1**

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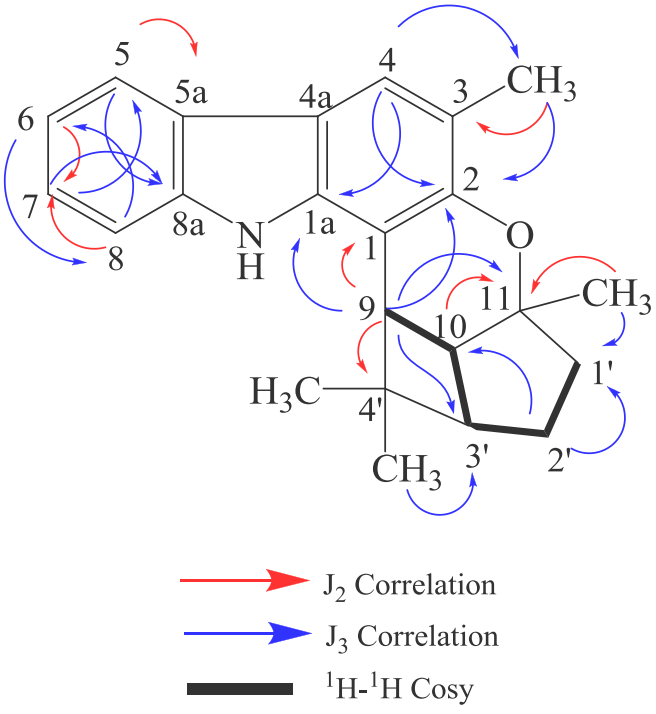


Figure 4.4.1g. Selected ^{1}H-^{1}H COSY and HMBCs of **M1**

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Table 4.4.2

1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of ***M2****

Position	δ ^{13}C	DEPT	δ ^{1}H	HMBC correlations	COSY
1	105.3	qC		H-10	
1a	138.4	qC		H-4	
2	153.7	qC		H-4, 3-CH_3	
3	117.7	qC		3-CH_3	
4	119.6	CH	7.64 (*s*)	3-CH_3	
4a	114.7	qC			
5	119.1	CH	7.89 (*d*, 8.0 Hz)		H-6
5a	124.3	qC		H-8	
6	119.2	CH	7.14 (*td*, 8.0, 1.1 Hz)	H-7, H-8	H-5, H-7
7	123.6	CH	7.27 (*td*, 8.0,1.1 Hz)	H-6, H-5	H-7, H-8
8	110.3	CH	7.32 (*d*, 8.0 Hz)	H-6	H-7
8a	139.4	qC		H-5, H-7	
9	36.3	CH	3.41 (*d*, 2.9 Hz)		H-10, H-3'
10	37.4	CH_2	2.02 (*dd*, 12.6, 2.9 Hz) 1.91 (*m*)	11-CH_3	H-9
11	74.0	qC		11-CH_3	
N-H			7.72 (*br s*)		
3-CH_3	16.9	CH_3	2.33 (*s*)	H-4	
11-CH_3	29.1	CH_3	1.44 (*s*)	H-1'	
1'	40.0	CH_2	2.08 (*m*) 1.62 (*m*)	H-2', 11-CH_3	H-2'
2'	23.2	CH_2	2.04 (*m*) 1.62 (*m*)		H-1', H-3'
3'	48.8	CH	2.58 (*m*)	H-5', H-1'	H-9, H-2'
4'	150.2	qC		4'-CH_3	
5'	112.1	CH_2	4.74 (*d*, 1.7 Hz) 4.81 (*d*, 1.7 Hz)	4'-CH_3	
4'-CH_3	21.7	CH_3	1.50 (*s*)	H-5'	

Note. *Solvent used was $CDCl_3$; assignments based on COSY, HMQC and HMBC.

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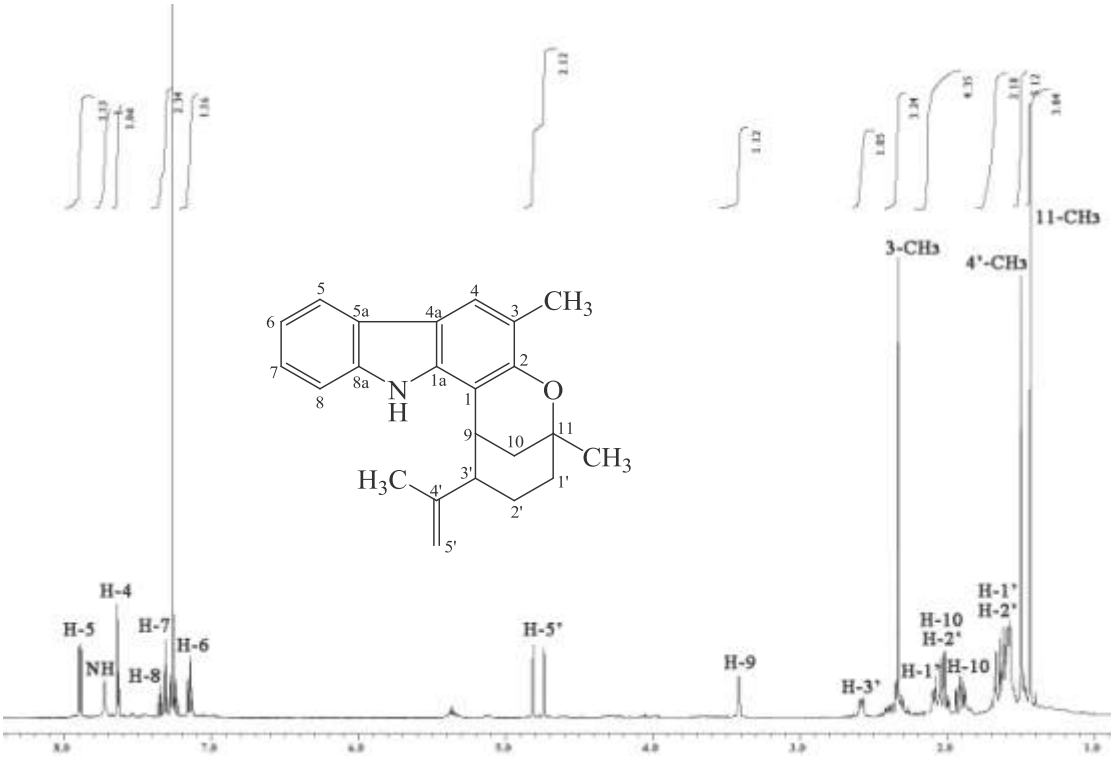


Figure 4.4.2a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of **M2**

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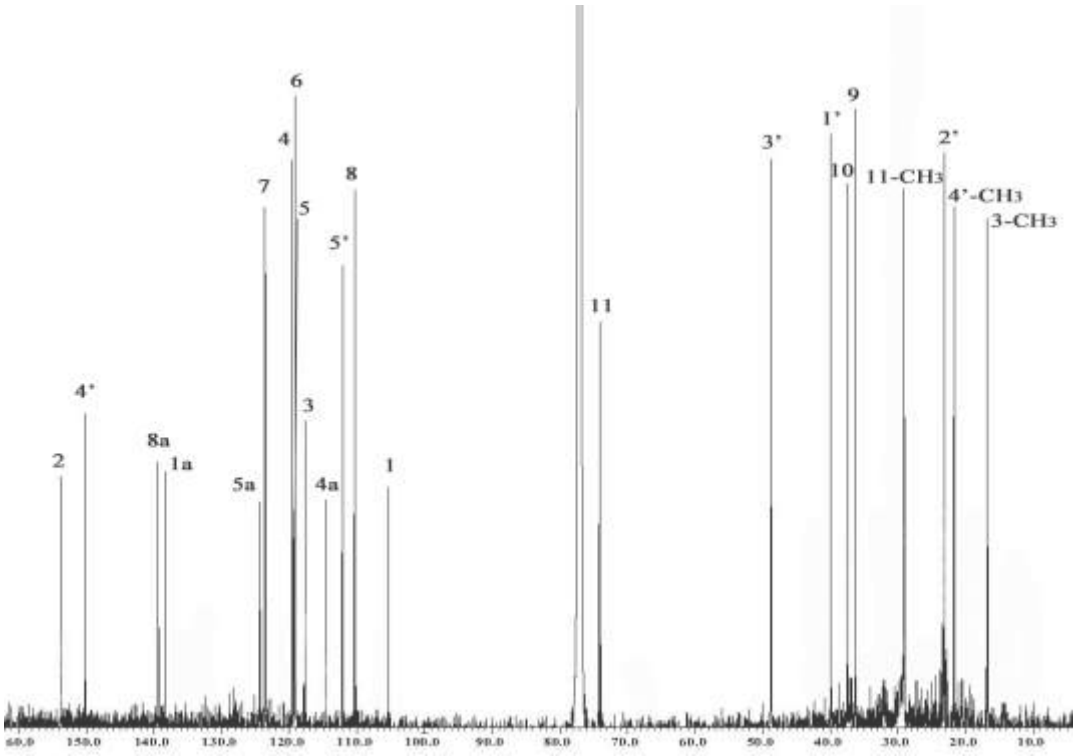


Figure 4.4.2b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of **M2**

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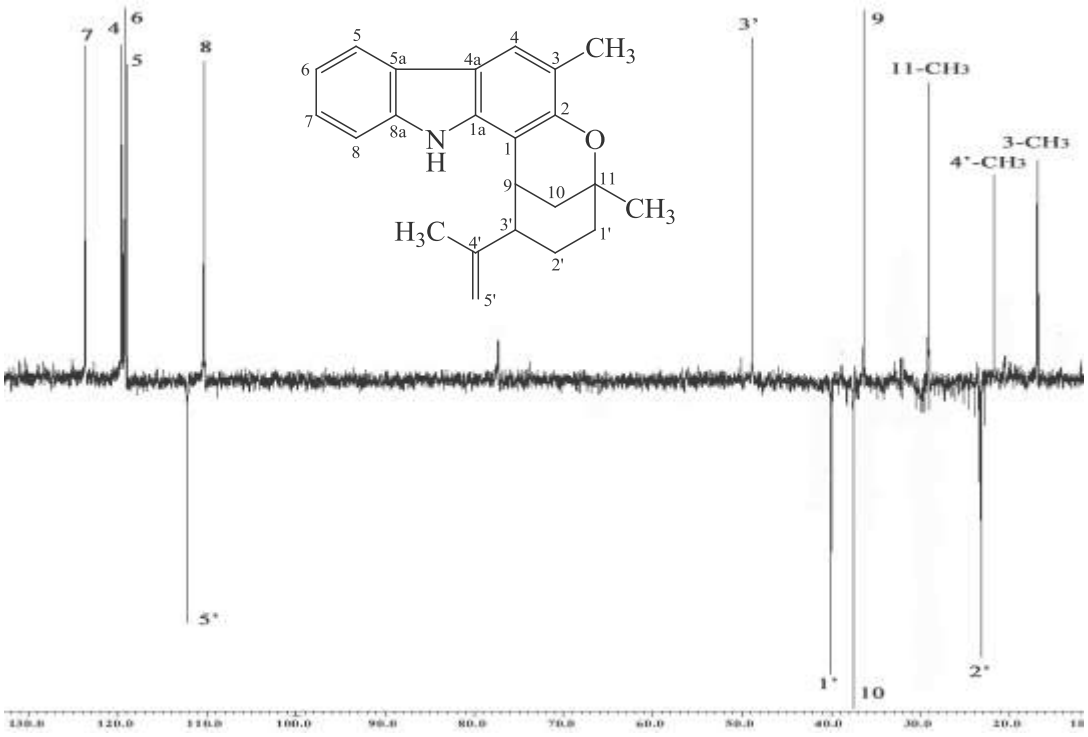


Figure 4.4.2c. DEPT-135 Spectrum ($CDCl_3$, 125 MHz) of **M2**

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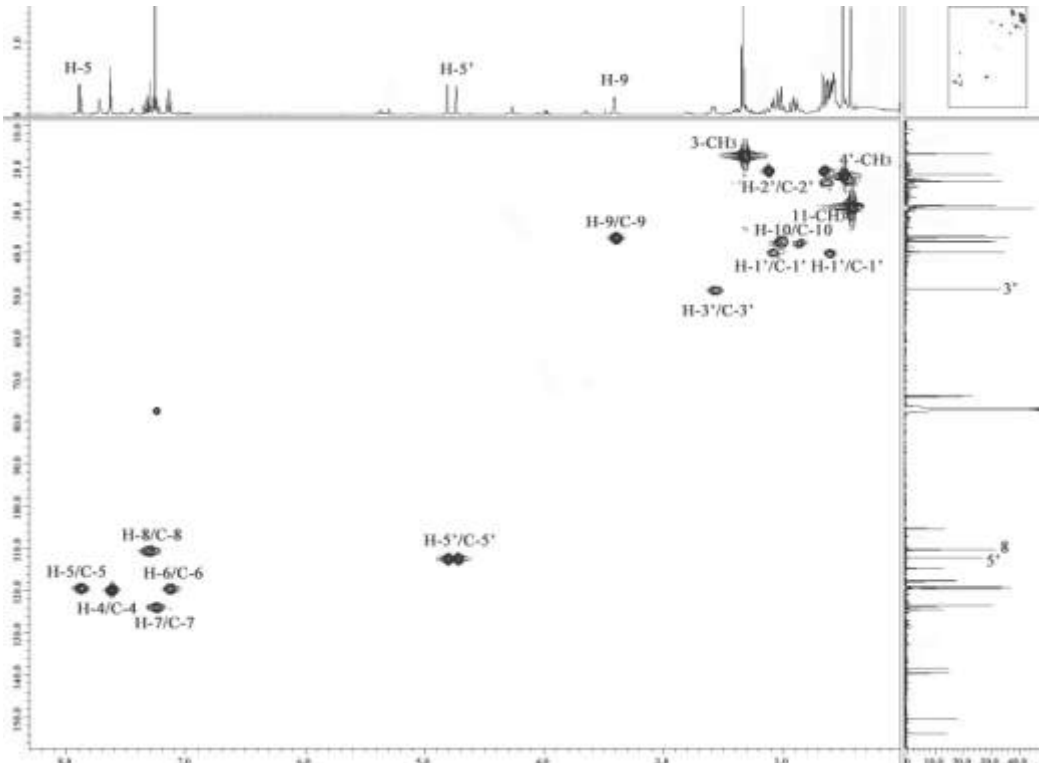


Figure 4.4.2d. HMQC Spectrum ($CDCl_3$, 500 MHz) of **M2**

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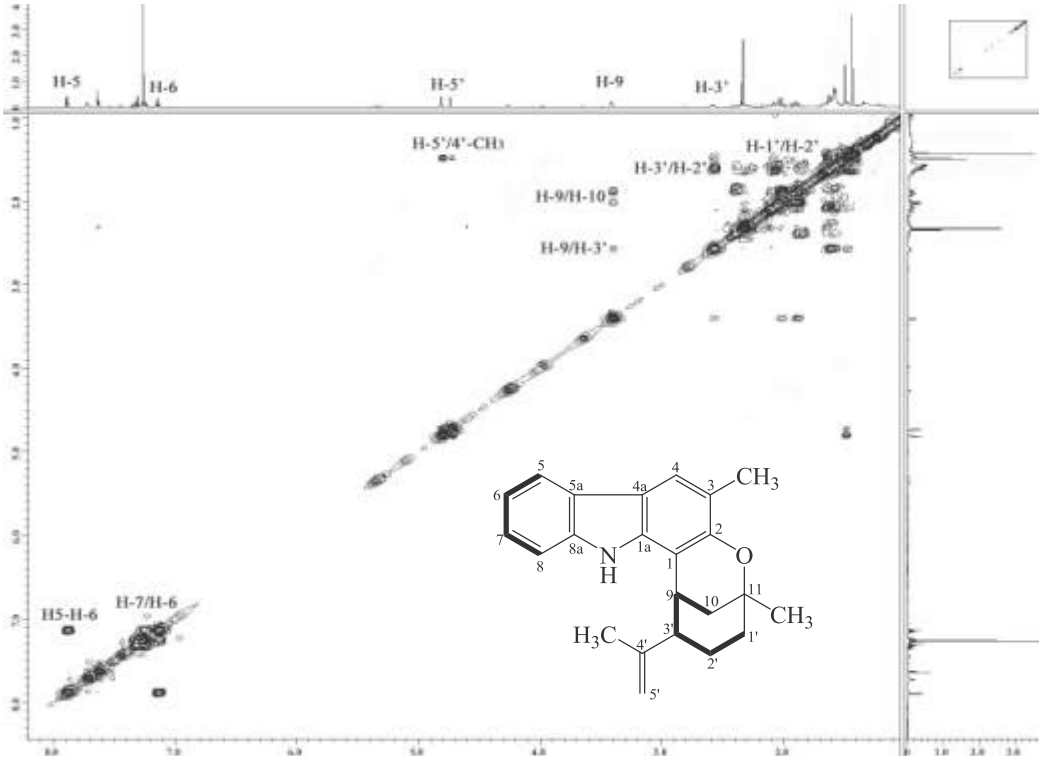


Figure 4.4.2e. ^{1}H-^{1}H COSY Spectrum ($CDCl_3$, 500 MHz) of **M2**

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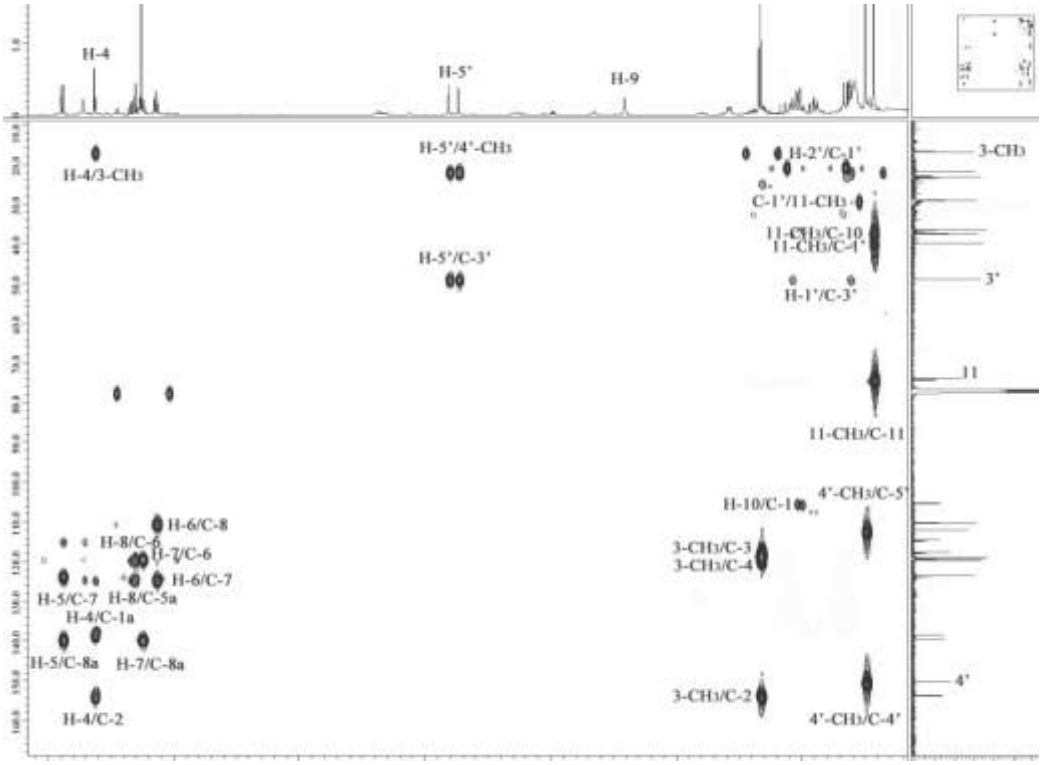


Figure 4.4.2f. HMBC Spectrum ($CDCl_3$, 500 MHz) of **M2**

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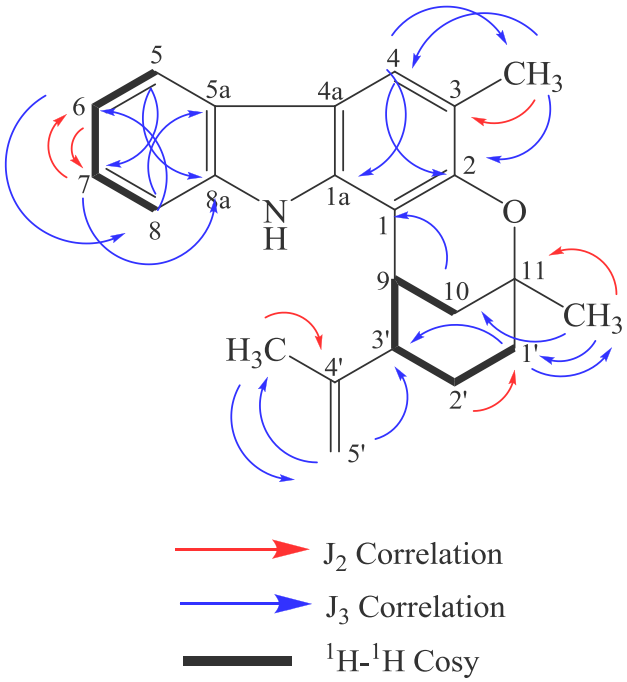


Figure 4.4.2g. Selected ‘H-‘H COSY and HMBCs of **M2**

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Table 4.4.3

1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR Spectral Data of ***M3****

Position	δ ^{13}C	DEPT	δ ^{1}H	HMBC correlations	COSY
1	105.9	qC		H-9, H-10	
1a	139.0	qC		H-4, H-9	
2	153.7	qC		H-4, H-9, 3-CH_3	
3	117.5	qC			
4	119.5	CH	7.66 (*s*)		
4a	114.8	qC		H-5	
5	119.1	CH	7.91 (*d*, 8.0 Hz)		H-6
5a	124.4	qC		H-4, H-6, H-8	
6	118.7	CH	7.14 (*td*, 8.0, 1.2 Hz)	H-7, H-8	H-5, H-7
7	123.4	CH	7.27 (*td*, 8.0,1.2 Hz)	H-5	H-6, H-8
8	110.6	CH	7.37 (*d*, 8.0 Hz)	H-6	H-7
8a	139.8	qC		H-5, H-7	
9	29.3	CH	3.80 (*m*)	H-3'	H-10, H-3'
10	38.3	CH_2	1.85 (*q*, 2.9 Hz)	H-9, H-1', H-3'	H-9
11	74.4	qC		H-10, H-1', 11-CH_3	
N-H			9.59 (*br s*)		
3-CH_3	16.9	CH_3	2.34 (*s*)	H-4	
11-CH_3	29.0	CH_3	1.43 (*s*)	H-10, H-1'	
1'	40.5	CH_2	2.09 (*m*)		H-2'
			1.62 (*m*)		
2'	22.9	CH_2	1.45 (*m*)	H-9	H-1', H-3'
3'	52.8	CH	1.90 (*m*)	H-9, 4'-CH_3	H-9, H-2'
4'	74.4	qC		4'-CH_3, H-3'	
4'-CH_3	33.4	CH_3	1.32 (*s*)	H-3', 4'-CH_3	
4'-CH_3	23.3	CH_3	0.55 (*s*)	H-3', 4'-CH_3	
4'-OH			1.72 (*br s*)		

Note. *Solvent used was $CDCl_3$; assignments based on COSY, HMQC and HMBC.

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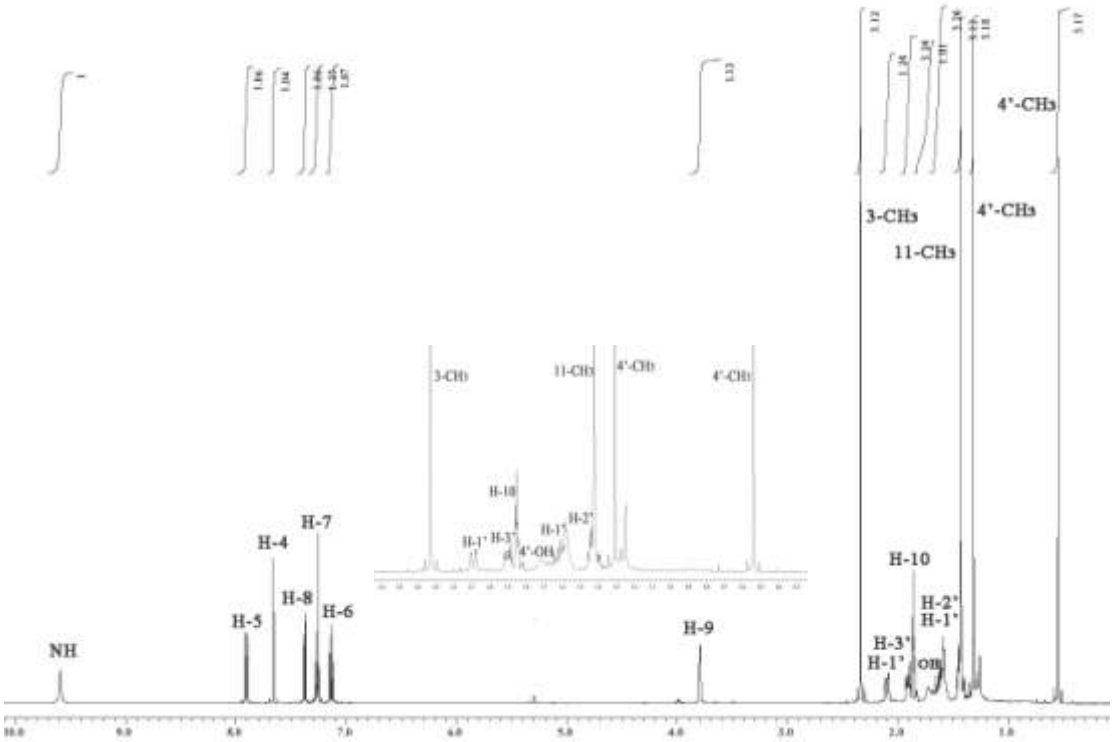


Figure 4.4.3a. ^{1}H NMR Spectrum ($CDCl_3$, 500 MHz) of **M3**

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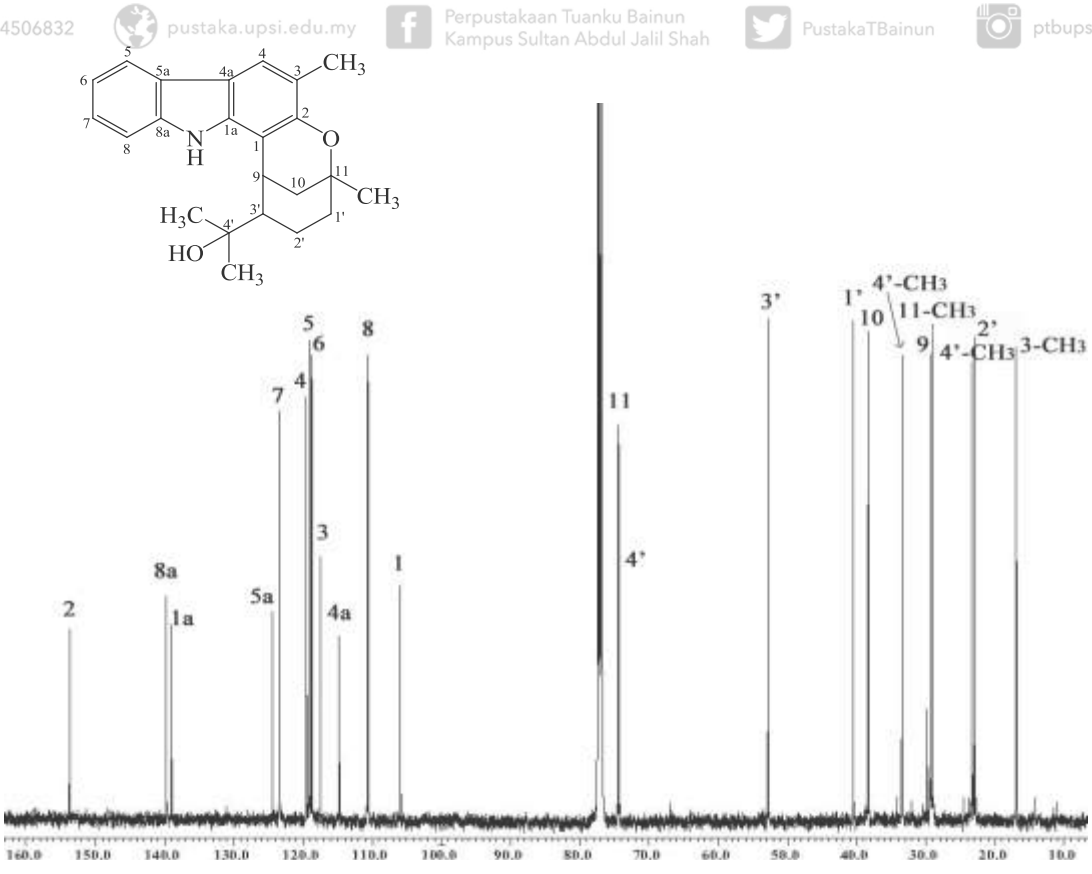


Figure 4.4.3b. ^{13}C NMR Spectrum ($CDCl_3$, 125 MHz) of **M3**

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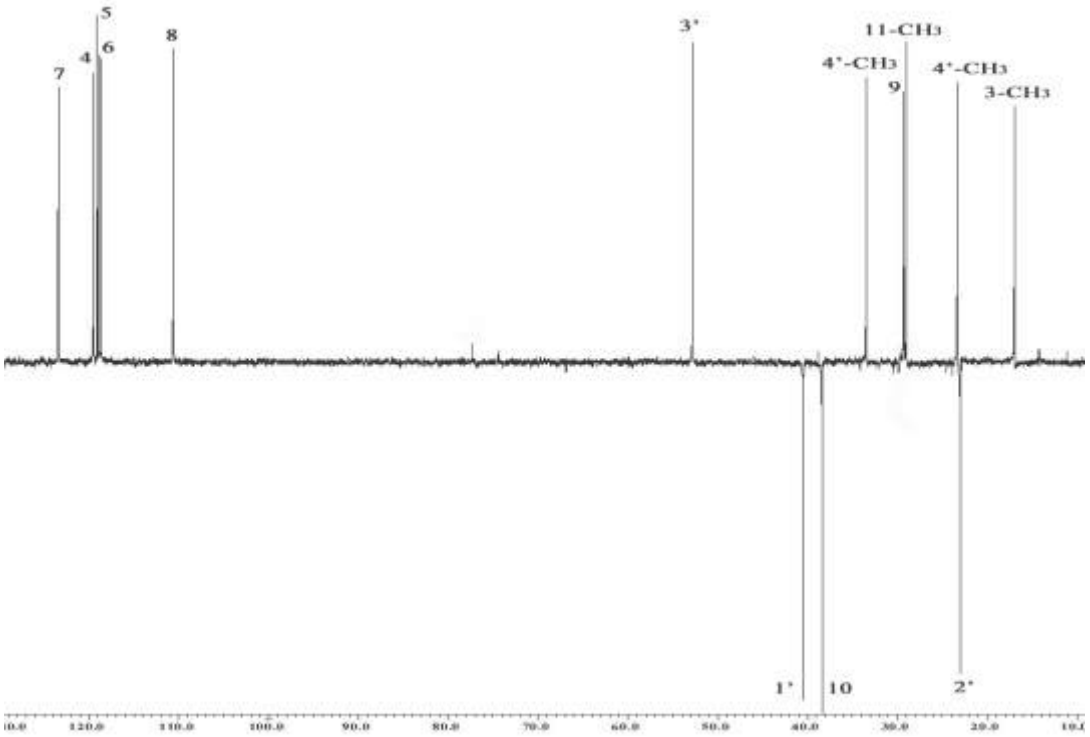


Figure 4.4.3c. DEPT-135 Spectrum ($CDCl_3$, 125 MHz) of **M3**

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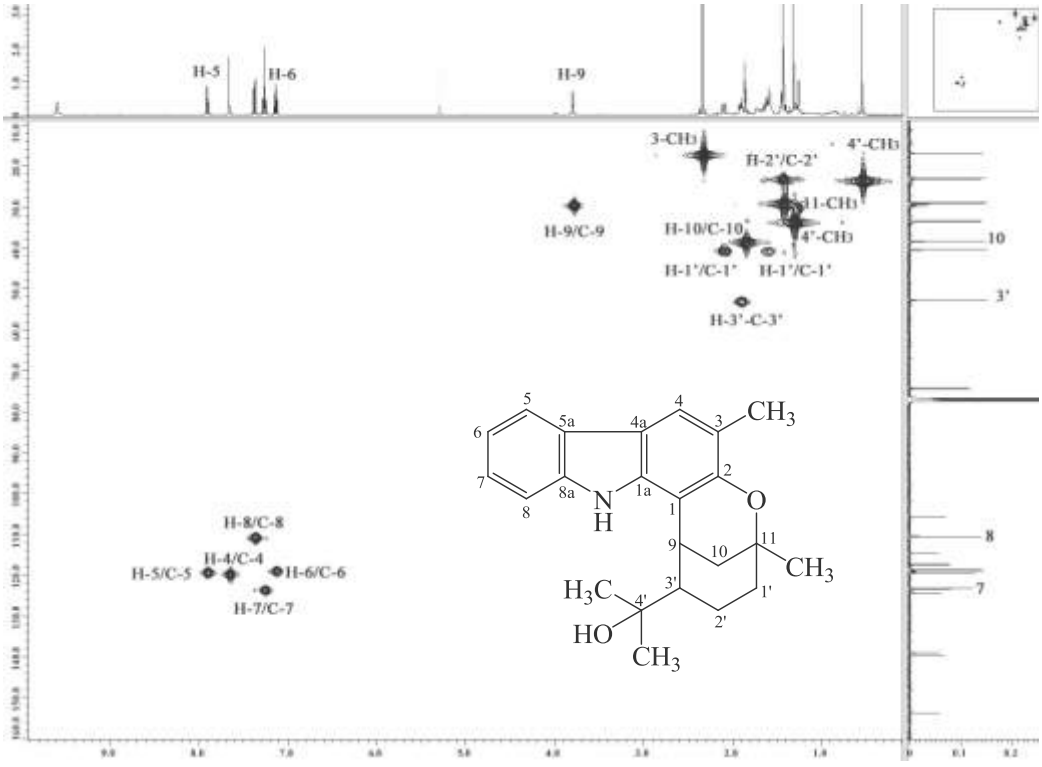


Figure 4.4.3d. HMQC Spectrum ($CDCl_3$, 500 MHz) of **M3**

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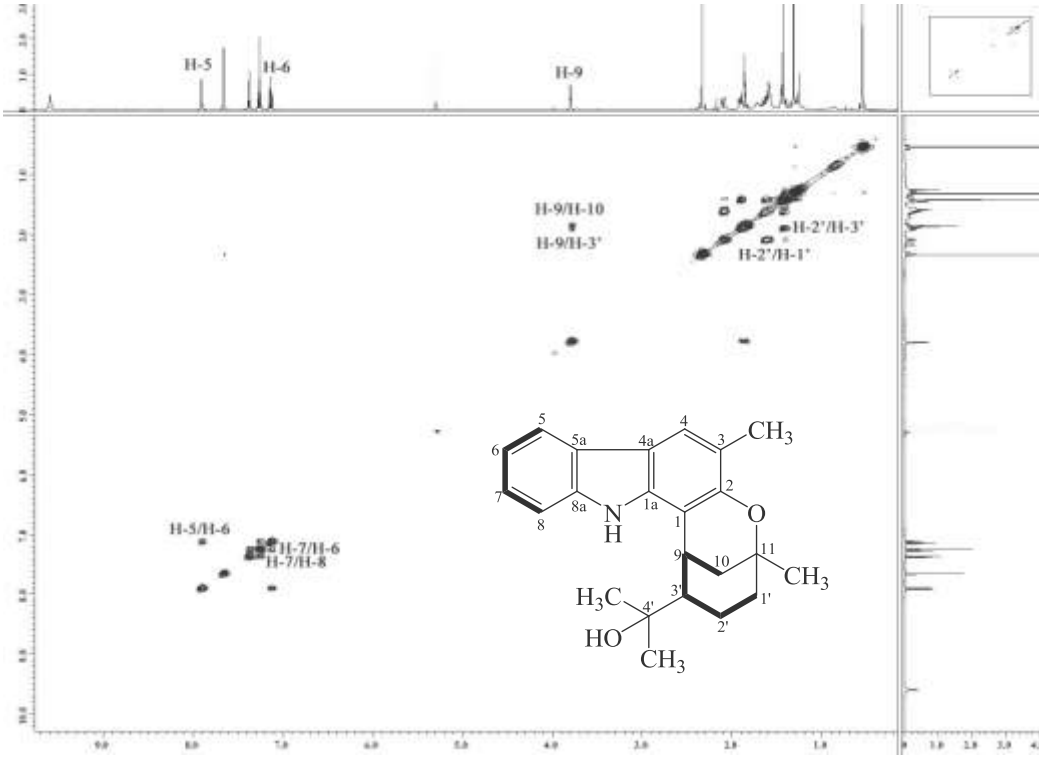


Figure 4.4.3e. ^{1}H-^{1}H COSY Spectrum ($CDCl_3$, 500 MHz) of **M3**

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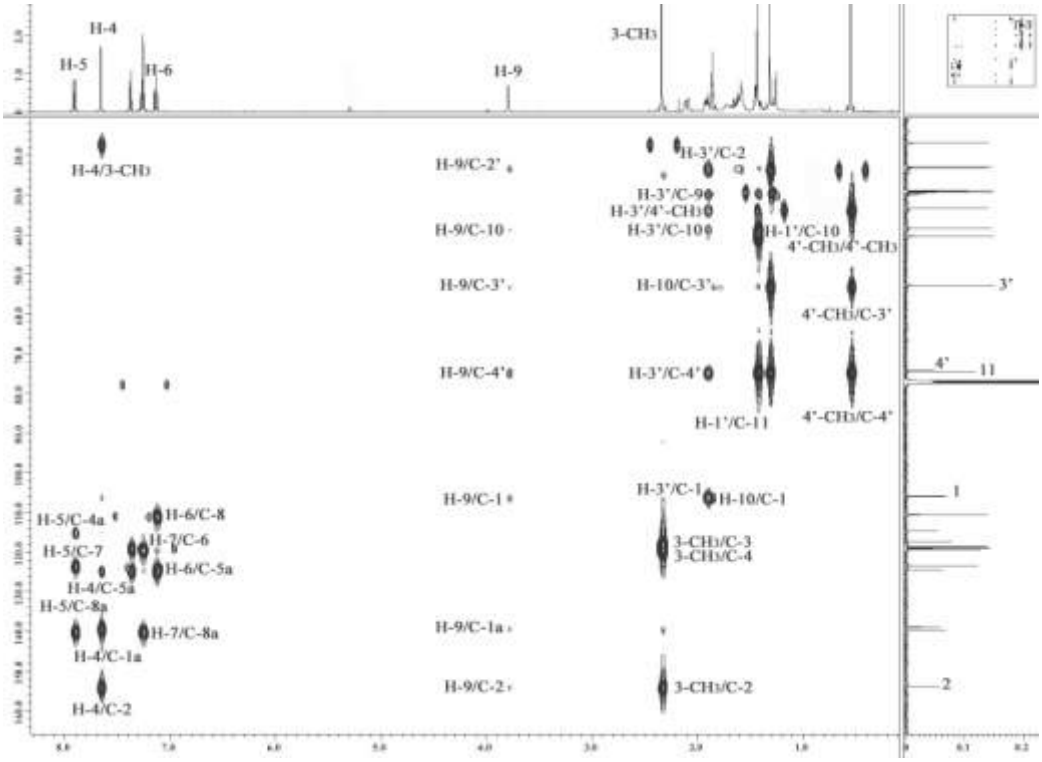


Figure 4.4.3f. HMBC Spectrum ($CDCl_3$, 500 MHz) of **M3**

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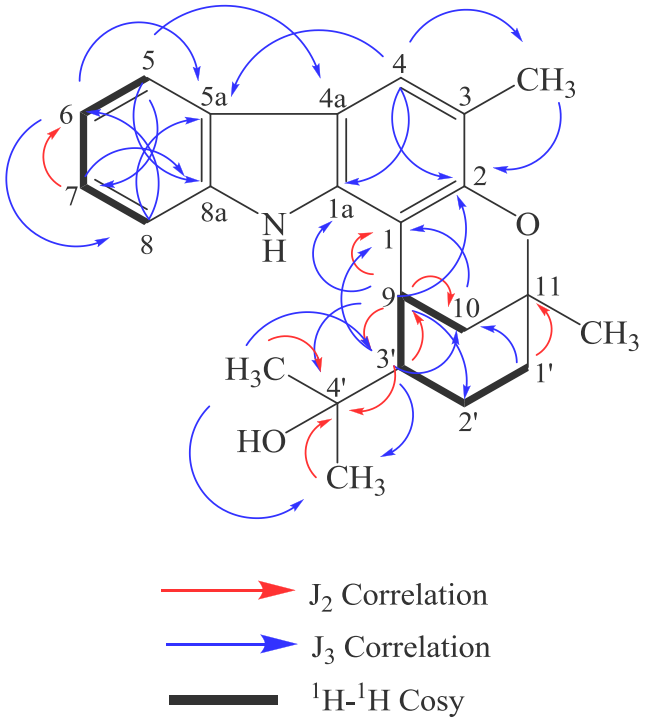


Figure 4.4.3g. Selected ^{1}H-^{1}H COSY and HMBCs of **M3**

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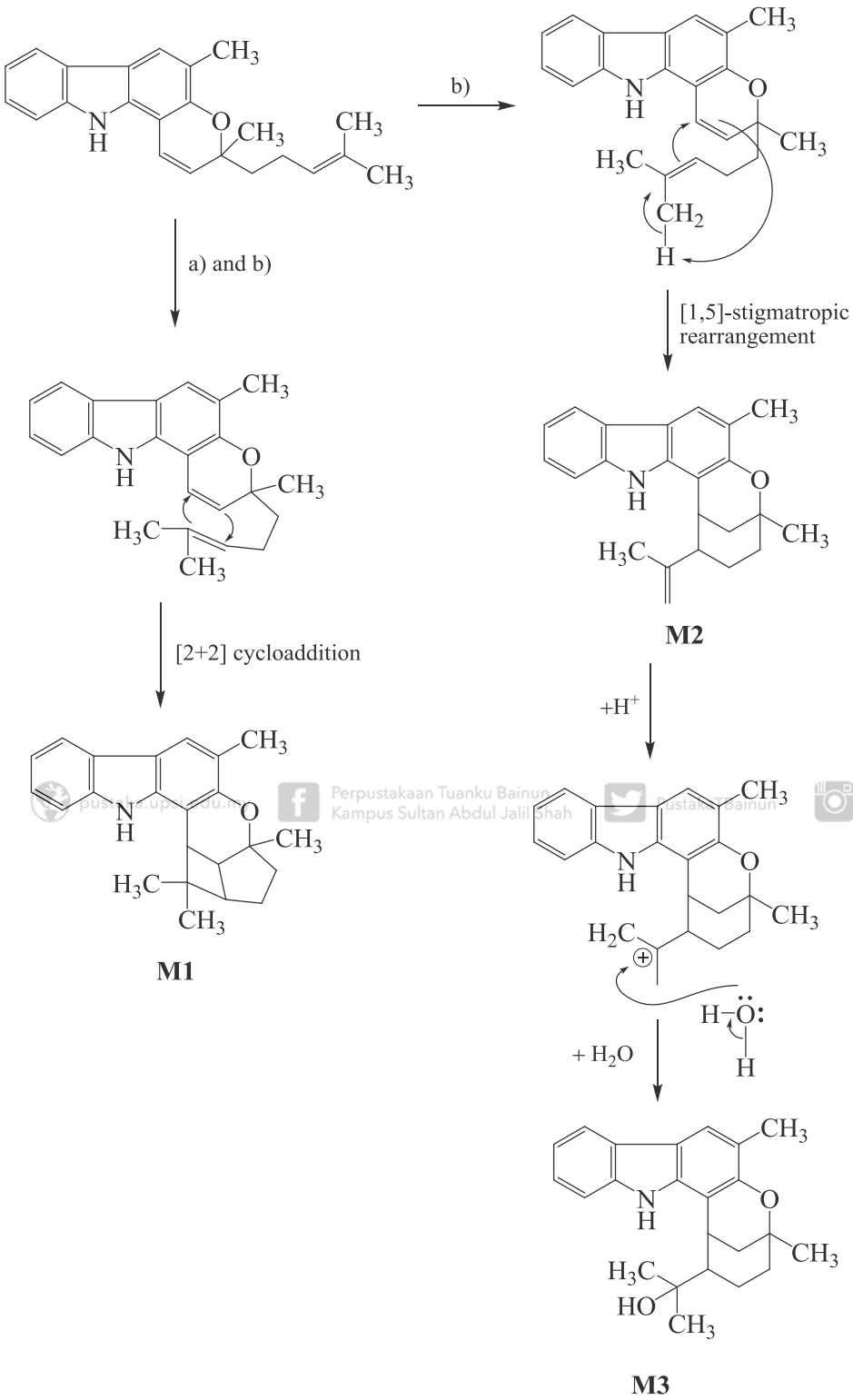
4.4.2 Mechanistic Studies

On the biogenetic pathway, mahanimbine **(Mk15)** is proposed to be derived from 2-hydroxy-3-methylcarbazole **39**, an important precursor, through geranylation followed by pyran annulation (Hesse et al., 2013). In an approach suggested by Kureel et al. (1969) and Cordell (2008), the intramolecular cyclization of mahanimbine leads to the formation of various cyclized monoterpenoid pyrano[3,2a]carbazoles such as cyclomahanimbine **17**, mahanimbidine **19** and bicyclomahanimbine **44**.

An efficient biomimetic synthetic route to the cyclized monoterpenoid pyrano[3,2a]carbazole with mahanimbine **(Mk15)** as starting material were developed in the study. In the approach using anhydrous $FeCl_3$ afforded only bicyclomahanimbine **(M1)** with moderate yields (1 step, up to overall 41.7 %). This reaction was suggested to be a Lewis acid-catalyzed [2+2]cycloaddition reaction according to Woodward-Hoffmann Rules (Woodward & Hoffmann, 1965). However, in the latter approach using BF_3-$O(C_2H_5)_2$ afforded total of three cyclized carbazoles, *viz.*, bicyclomahanimbine **(M1)**, cyclomahanimbine **(M2)** and murrayazolinine **(M3)**. The probable synthetic transformation was suggested that the hexacyclic **M1** was formed through Lewis acid-catalyzed [2+2]cycloaddition, the similar pathway with the reaction using anhydrous $FeCl_3$. The formation of another two pentacyclics **M2** and **M3** were initiated by [1,5]-stigmatropic rearrangement (to **M2**) via Lewis acid-mediated reaction (Brichacek, Lee, & Njardarson, 2008), and finally followed by a combination protonation and hydrolysis reactions (to **M3**) (Scheme 4.4.1).

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Scheme 4.4.1. A Possible Mechanistic Pathway to **M1**, **M2** and **M3**. a) Anhydrous $FeCl_3$, 25 °C; b) BF_3-$O(C_2H_5)_2$, 25 °C.

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4.5 Biological Activity

In our continuing interest for the search of biological active compounds derived from Malaysian plants, this section described the results of a further examination on the *in vitro* cytotoxic potential of the isolated carbazole alkaloids and the synthetic analogues of girinimbine **(Mk12)** and mahanimbine **(Mk15)** via MTT assay against targeted cancer cell lines such as the human promyelocytic leukemia cell lines (HL-60), cervical cancer cell lines (HeLa) and normal mouse embryonic fibroblasts (NIH/3T3) cell lines.

The cytotoxic activity was tested against targeted cell lines by measuring the cytotoxic dose that kill 50% of cell population (CD_{50}) as compared to the untreated control and a positive control by using a standard cancer chemotherapy drug known as vincristine (VCR) via colorimetric cytotoxicity assay (MTT assay). The cytotoxic dose (CD_{50}) was then determined from the standard curve of cell number by plotting a scattergram of the percentage of cell viability (%) against the test sample concentrations (μg/mL). According to Wibowo et al. (2011), cytotoxicity activity needed 5 μg/mL or less sample to kill 50% of the cell is considered as very strong activities; strong activities for 5–10 μg/mL; moderate is between 10–20 μg/mL; weak is between 20–100 μg/mL and not active for those activity more than 100 μg/mL of sample needed to kill the cells.

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4.5.1 Cytotoxic Assay on the Isolated Chemical Constituents of *M. koenigii*

The percentage of viability of NIH/3T3 cell line showed almost linear pattern at 10 to 60 μg/mL concentration on the isolated carbazole alkaloids and the positive control used (Figure 4.5.1). The results have proven that the isolated chemical compounds from *M. koenigii* were not active against normal cell.

The cytotoxicity effects of isolated chemical compounds against HL-60 cell line and HeLa cell lines were determined by measuring the cytotoxic dose that kill 50% of cell population as compared to the untreated control via MTT assay. The results showed the effectiveness of 0 to 60.0 μg/mL concentrations of all the isolated compounds of *M. koenigii* towards cell viability of HL-60 and HeLa to 50% of control cells (1×10^5 cells) responses after 72 hours exposure. Many of the graphs showed the drastically declined in percentage of cell viability from 100% of the selected cancer cell lines to 50% or below after exposed to 60 μg/mL of the compounds (Figure 4.5.1).

The results obtained indicated that the compounds isolated from *M. koenigii* inhibited the growth of HL-60 and HeLa when compared to VCR (CD_{50} < 1.0 μg/mL). The results showed that some of the compounds isolated *M. koenigii* may led to develop potential anticancer agents against HL-60 and HeLa cancer cells when these compounds showed very strong to weak effectiveness in cytotoxic activity (Table 4.5.1). In consequence of the results, the isolated chemical compounds were shown not active against the normal cell lines. The study again suggested that *M koenigii* can be considered as valuable source for anticancer drug discovery. In fact, it also suggested to consume orally as a cancer preventive action.

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Table 4.5.1

CD_{50} Values (μg/mL) for Cytotoxic Activity of Carbazole Alkaloids Isolated from M. koenigii

Compound	Cell Lines		
	HL-60	HeLa	NIH/3T3
Murrayanine **(Mk1)**	40.4	> 60	NA
Murrayafoline-A **(Mk2)**	8.5	4.6	NA
Koenoline **(Mk3)**	41.1	47.8	NA
3-Formylcarbazole **(Mk4)**	44.2	28.7	NA
2-Hydroxy-3-methylcarbazole **(Mk5)**	35.7	39.1	NA
Mahanimbilol **(Mk6)**	> 60	39.1	NA
Euchrestine-B **(Mk7)**	41.4	19.8	> 60
Mukoenine-B **(Mk8)**	52.1	> 60	NA
Murrastanine-A **(Mk9)**	51.1	41.6	NA
Murrayaquinone-A **(Mk10)**	16.0	36.7	NA
Murrayaquinone-B **(Mk11)**	39.0	6.6	> 60
Girinimbine **(Mk12)**	34.8	12.2	NA
Koenimbine **(Mk13)**	15.8	40.6	NA
Murrayacine **(Mk14)**	47.4	45.0	NA
Mahanimbine **(Mk15)**	38.2	21.4	NA
Mahanine **(Mk16)**	12.1	12.8	NA
Isomahanine **(Mk17)**	> 60	27.7	NA
Murrayamine-B **(Mk18)**	46.8	36.9	NA
Murrayamine-J **(Mk19)**	5.1	7.7	> 60
Murrastinine-A **(Mk20)**	46.9	44.5	NA
Murrastinine-B **(Mk21)**	49.5	23.6	NA
Murrastinine-C **(Mk22)**	16.9	1.2	NA
Murrayazolinol **(Mk23)**	42.2	46.0	> 60
Murrayakoeninol **(Mk24)**	46.6	18.1	> 60
Bicyclomahanimbine **(Mk25)**	31.7	19.6	NA
Murrayatanine-A **(Mk26)**	11.2	4.6	> 60
Bismurrayafoline-E **(Mk27)**	42.9	> 60	NA
Bispyrayafoline **(Mk28)**	> 60	45.7	> 60

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Table. *Continued.*

Compound	Cell Lines		
	HL-60	HeLa	NIH/3T3
Bisisomahanine **(Mk29)**	> 60	> 60	> 60
Bismahanimboline **(Mk30)**	21.9	35.6	> 60
β-Sitosterol **(Mk31)**	8.6	0.9	NA
Stigmast-4-ene-3-one **(Mk32)**	> 60	44.6	NA
Squalene **(Mk33)**	NA	> 60	NA
α-Tocopherol **(Mk34)**	> 60	> 60	NA
2-Hydroxy-4-methoxy-3,6-dimethylbenzoic acid **(Mk35)**	1.5	11.2	NA
Selin-11-en-4α-ol **(Mk36)**	53.1	28.2	> 60
Vincristine (VCR)	0.8	0.5	NA

Note. NA = Not Active.

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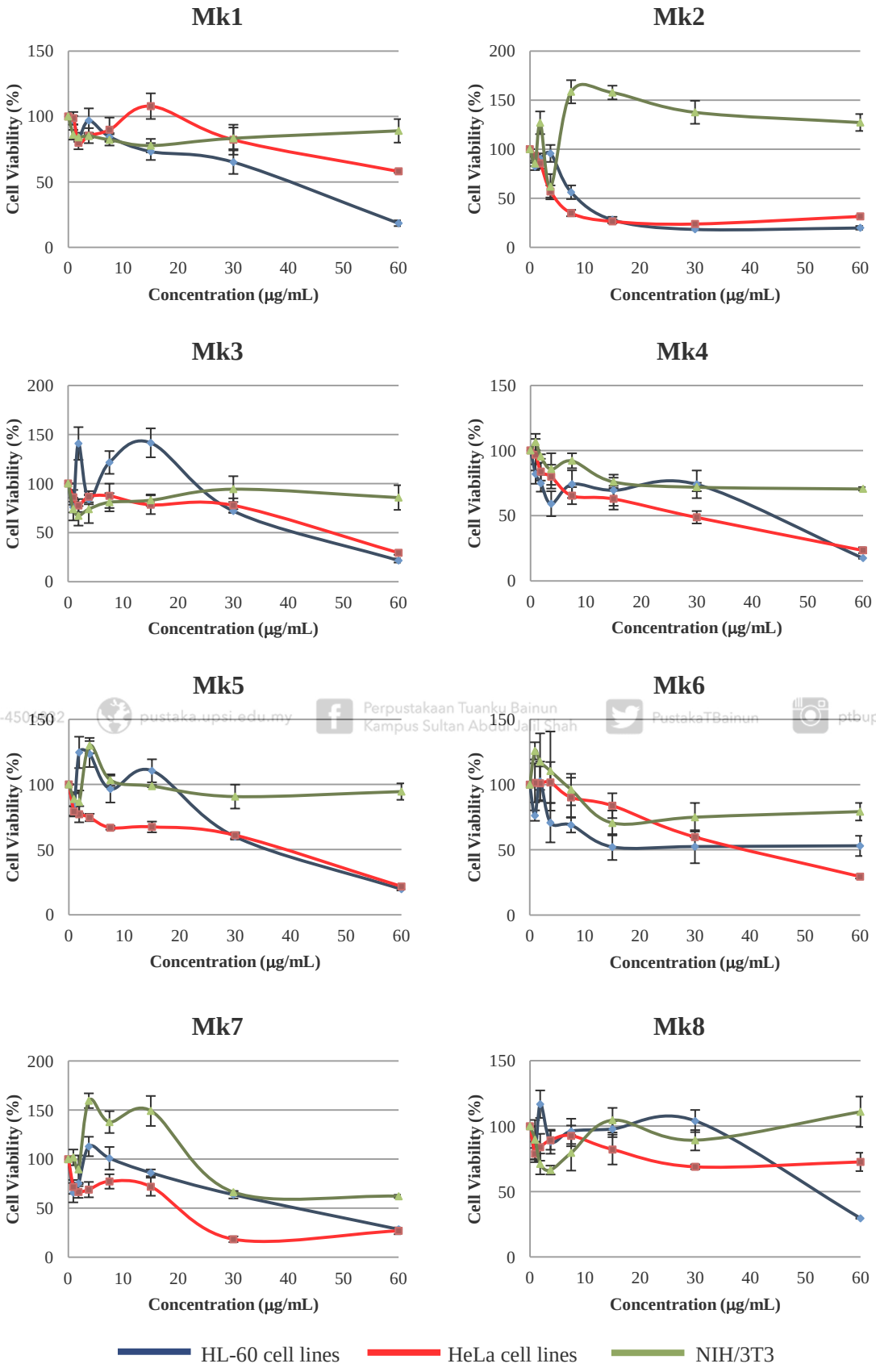


Figure 4.5.1. Cytotoxic Effects (CD_{50}) of Isolated Carbazole Alkaloids against HL-60 and HeLa along with NIH/3T3 Cell Lines via MTT Assay

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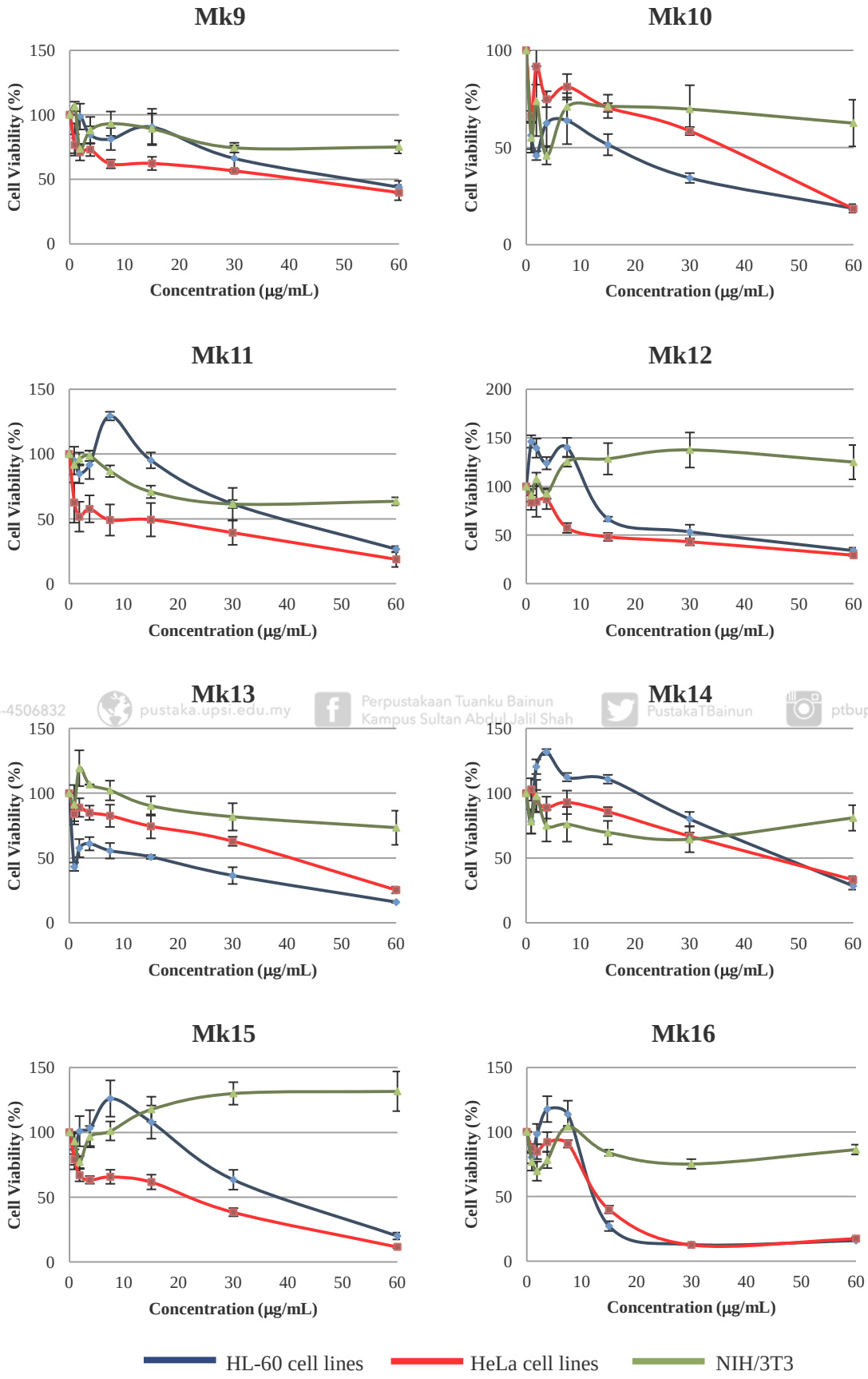


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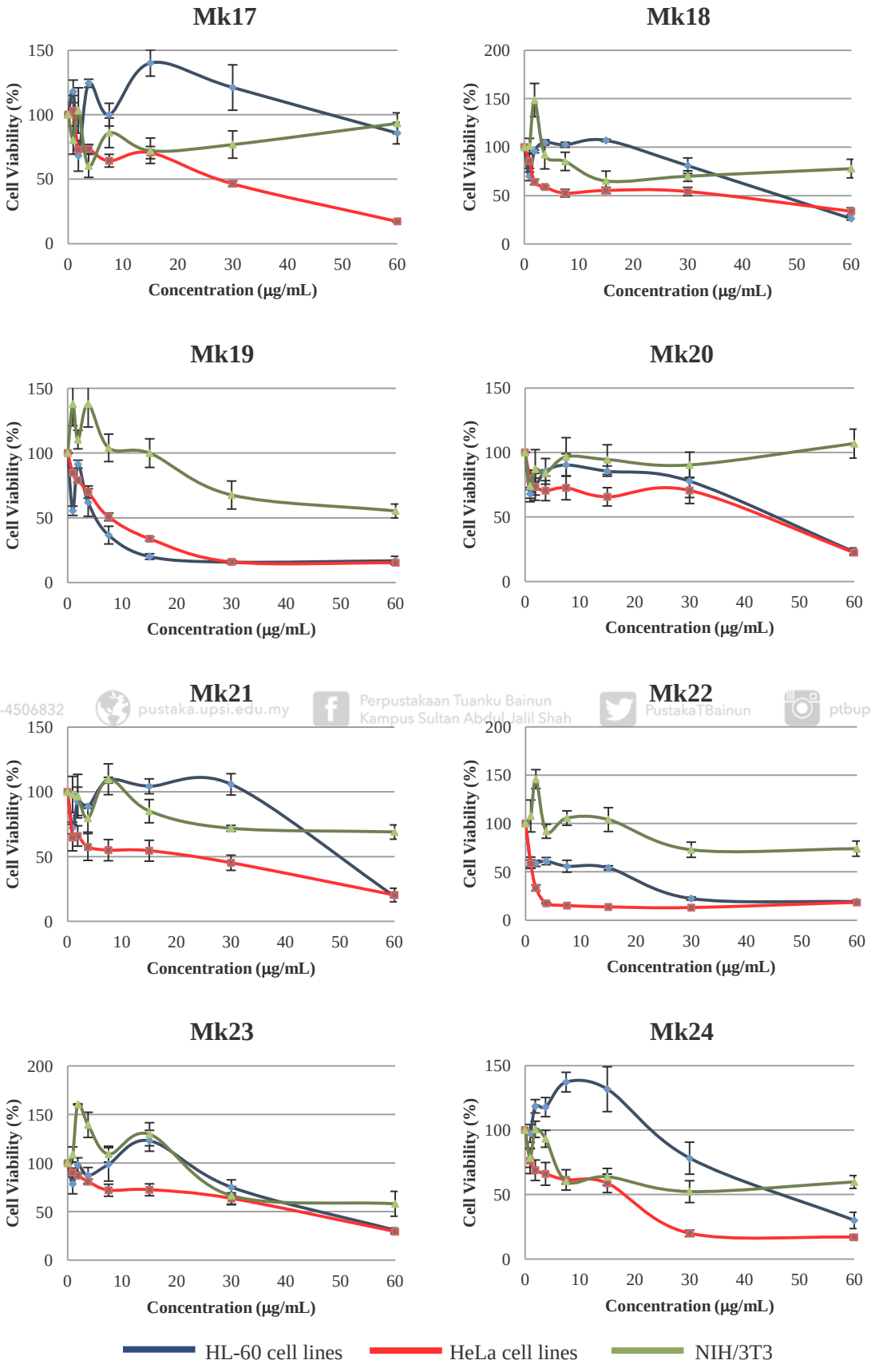


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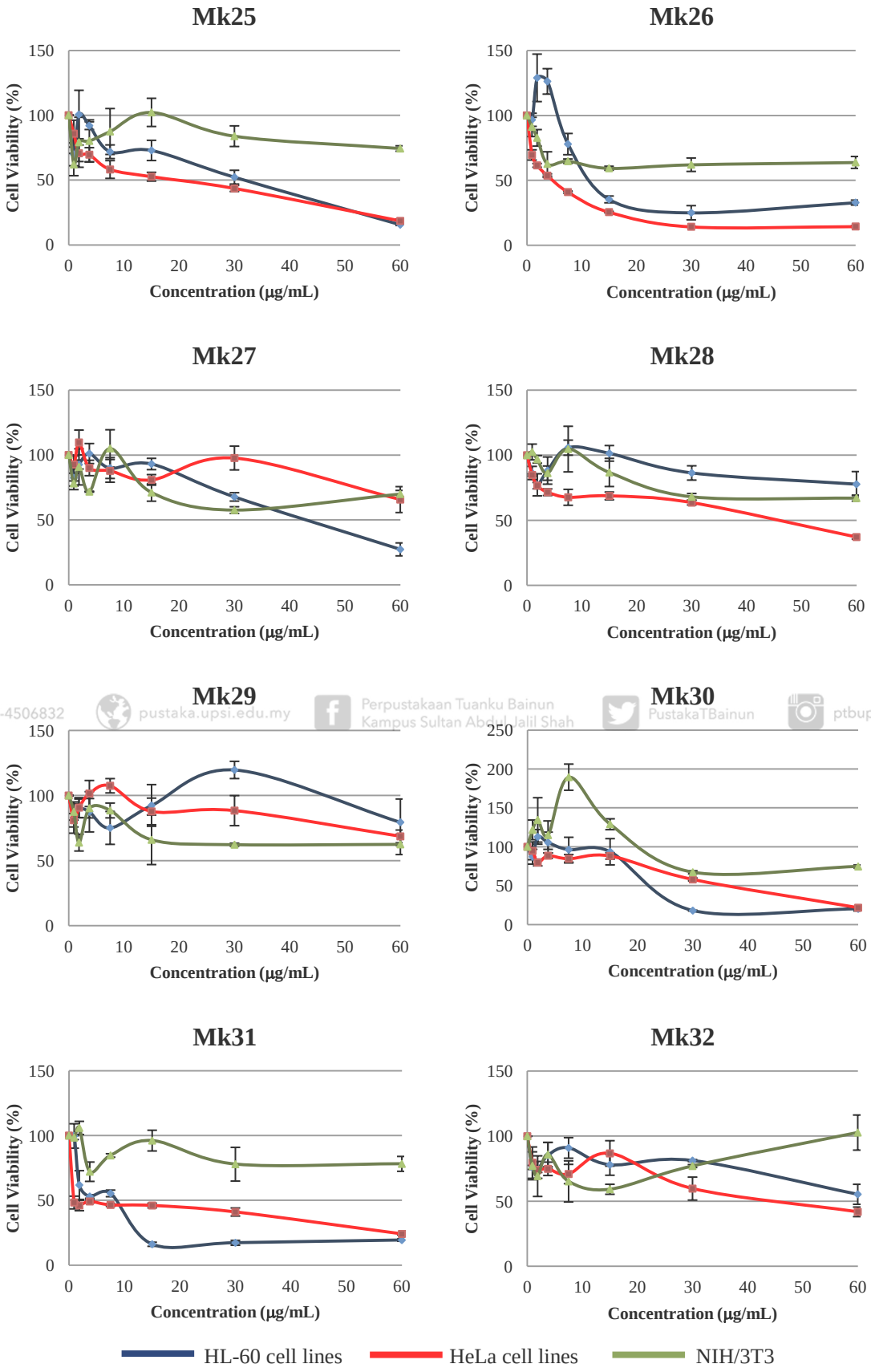
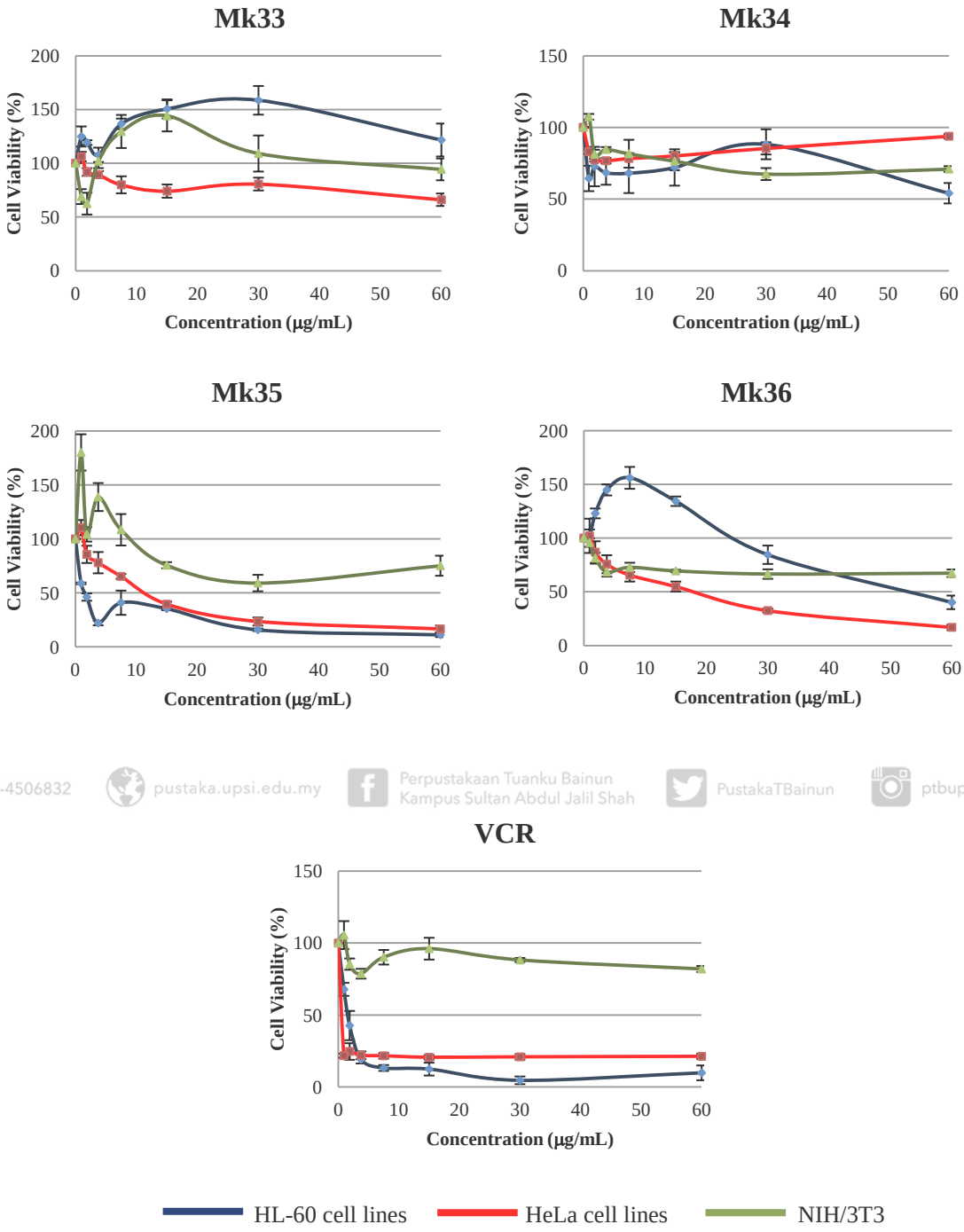


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Figure 4.5.1. Continued.

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4.5.2 Cytotoxic Assay on the Synthetic Analogues of Girinimbine and Mahanimbine

The cytotoxic effect of the synthetic analogues of girinimbine and mahanimbine against HL-60 and HeLa cell lines were also determined by measuring the cytotoxic dose that kill 50% of cell population (1×10^5 cells) after 72 hours exposure as compared to the untreated and positive control via MTT assay. Some of the graphs showed significant declined in percentage of cell viability from 100% of the selected cancer cell lines to 50% or below after exposed to 60 μg/mL of the compounds (Figure 4.5.2). The results obtained indicated that the synthetic compounds derived from girinimbine and mahanimbine also inhibited the growth of HL-60 and HeLa cancer cells when compared to VCR (CD_{50} < 1.0 μg/mL). However, the percentage of viability of NIH/3T3 cell line showed almost linear pattern at 0 to 60 μg/mL concentration of all the synthetic analogues of girinimbine and mahanimbine (Figure 4.5.2) as well as the positive control. The results again have proven that the synthetic analogues of girinimbine and mahanimbine were not active against normal NIH/3T3 cell lines.

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Table 4.5.2

CD_{50} Values (μg/mL) for Cytotoxic Activity of the Synthetic Analogues of Girinimbine and Mahanimbine

Compound	Cell Lines		
	HL-60	HeLa	NIH/3T3
G1	49.2	36.6	NA
G2	17.6	36.5	NA
M1	28.7	36.9	NA
M2	> 60	1.0	NA
M3	51.6	29.6	NA
Vincristine (VCR)	0.8	0.5	NA

Note. NA = Not Active.

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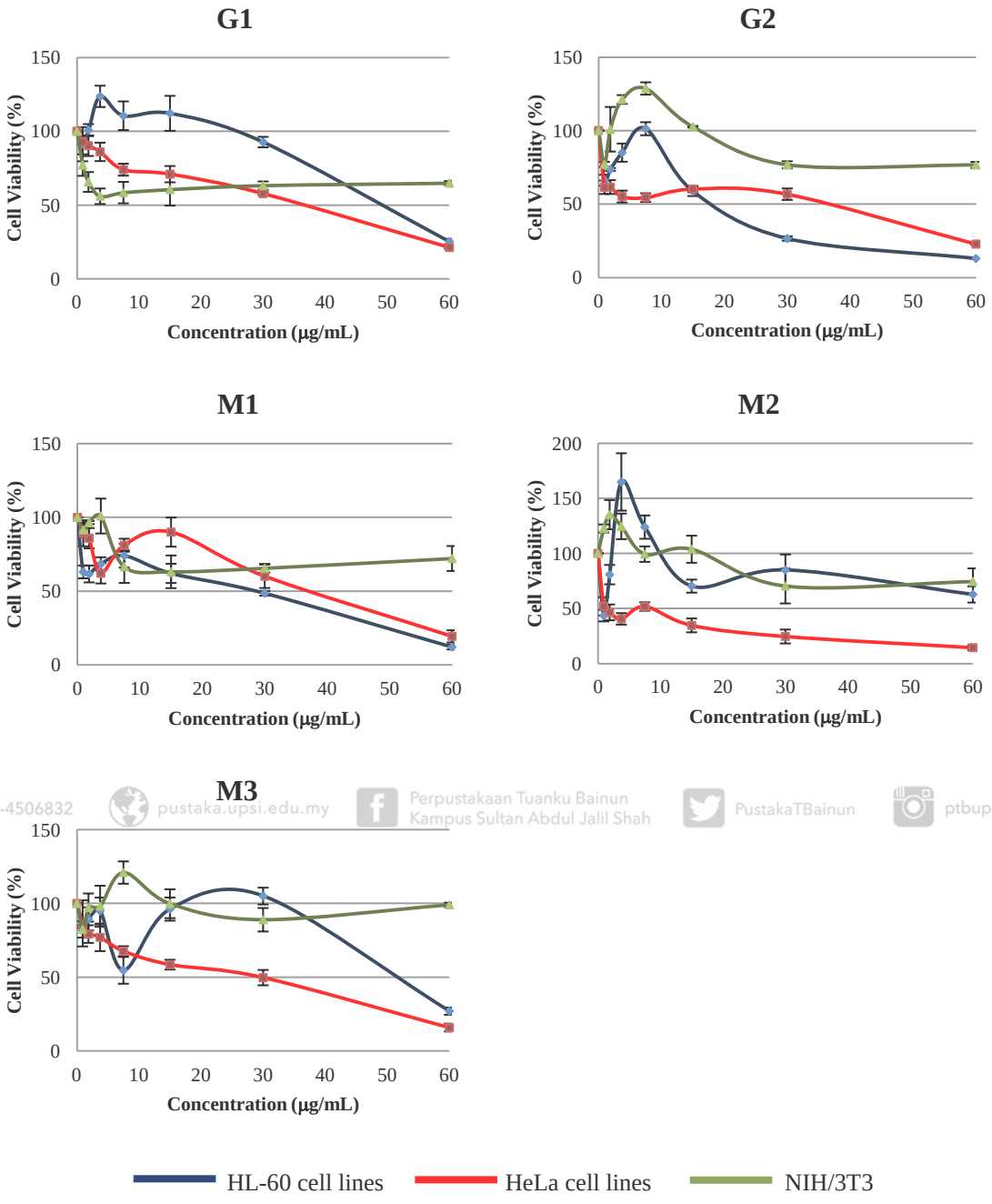


Figure 4.5.2. Cytotoxic Effects (CD_{50}) of Synthetic Analogues of Girinimbine and Mahanimbine against HL-60 and HeLa along with NIH/3T3 Cell Lines via MTT Assay

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4.5.3 Structure-activity Relationship (SAR) Analysis on Carbazole Alkaloids

The structure-activity relationship (SAR) analysis on carbazole alkaloids (**Mk1** to **Mk30**) that isolated from the bark and leaves of *Murraya koenigii* was performed in order to understand the relationship between the diverse structural features of carbazole alkaloids and their potency in cytotoxic activities.

In comparison of the *in vitro* cytotoxic activities against HL-60 and HeLa cancer cell lines of tricyclic carbazoles **Mk1** to **Mk9**, it was clearly showed that the introduction of various substituents to the ring-A and/or ring-C of the carbazole nucleus had no significant effect on the cytotoxic activities (Figure 4.5.3). On the basic of the investigations, comparison was made between monosubstituted tricyclic carbazole alkaloid **(Mk4)**, disubstituted tricyclic carbazole alkaloids (**Mk1**, **Mk2**, **Mk3**, **Mk5**, and **Mk9**) at either *ortho*-, *meta*- or *para*-position, and trisubstituted (**Mk6** and **Mk8**), as well as the tetrasubstituted **(Mk7)** tricyclic carbazole alkaloids, no specific trend was observed in cytotoxic activities against both HL-60 and HeLa cell lines. All these compounds showed weak activities against both cancer cell lines, except for **Mk2**. The presence of two electron-donating groups (-OCH_3 and -CH_3) in **Mk2** at *ortho*- and *para*-position, respectively, remarkably enhanced the activities. On contrary, the presence of two electron-donating groups at *meta*- and *para*-position **(Mk5)** and the presence of both the electron-donating and electron-withdrawing groups at either position in the carbazole nucleus led to decrease the activities in **Mk1**, **Mk3** and **Mk5**. Meanwhile, a *p*-OH, a stronger electron-donating group, in **Mk9** was also decrease the activities when compared to a weaker electron-donating group (*p*-CH_3) in **Mk2**.

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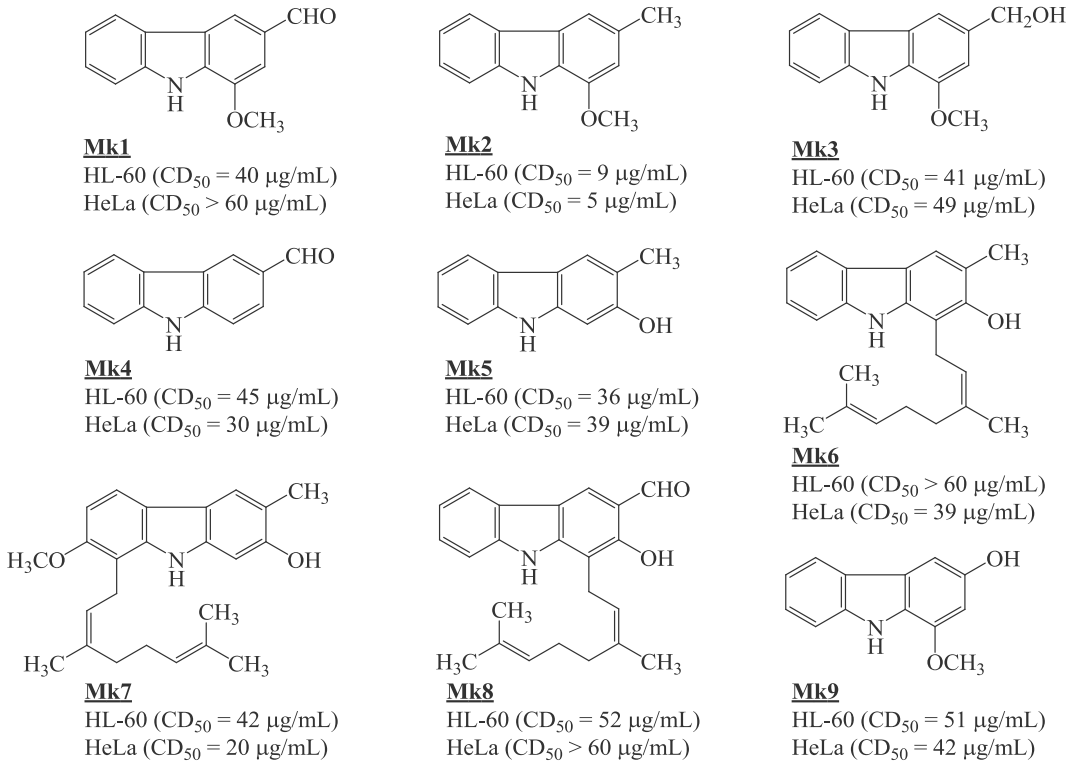


Figure 4.5.3. Structure of Tricyclic Carbazole Alkaloids and Their Corresponding CD_{50} Values against HL-60 and HeLa Cell Lines

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Comparison was also made to determine the structure-activity relationship on quinone-type of tricyclic carbazole alkaloids (**Mk10** and **Mk11**). The presence of quinone ring system showed increase in cytotoxic activities against HL-60 and HeLa cell lines (Figure 4.5.4), but there was no specific trend observed in the activities. The result suggested that the presence of quinone ring system is important in determining the potency of a tested compound.

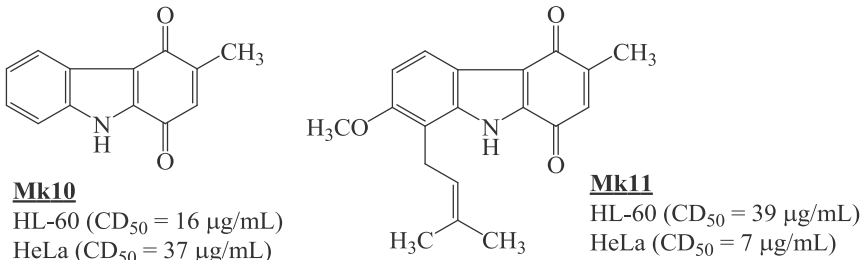


Figure 4.5.4. Structure of Carbazolequinone Alkaloids and Their Corresponding CD_{50} Values against HL-60 and HeLa Cell Lines

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On the other hand, in comparison of the activities of various tetracyclic pyrano[3,2-a]carbazoles (**Mk12** to **Mk22**) against HL-60 and HeLa cancer cell lines (Figure 4.5.5), the presence of electron-donating or electron-withdrawing group on either ring of the carbazole nucleus showed differences in activities.

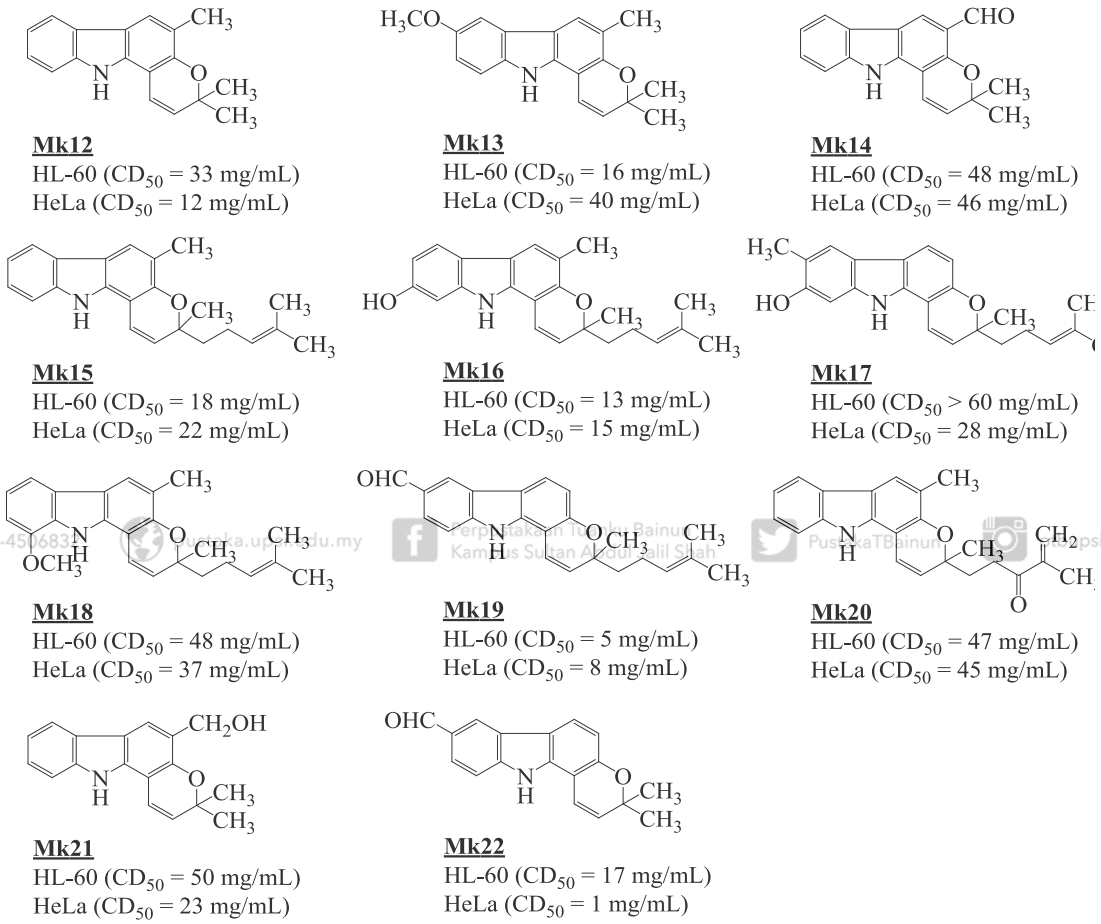


Figure 4.5.5. Structure of Pyrano[3,2-a]carbazole Alkaloids and Their Corresponding CD_{50} Values against HL-60 and HeLa Cell Lines

There was no specific trend but gave significant effect of the position of the substituents was observed in cytotoxic activities of all the tested pyrano[3,2-a]carbazole alkaloids. The pyrano[3,2-a]carbazoles with monosubstituted either an electron-donating group (**Mk12**, **Mk15** and **Mk20**) or an electron-withdrawing group

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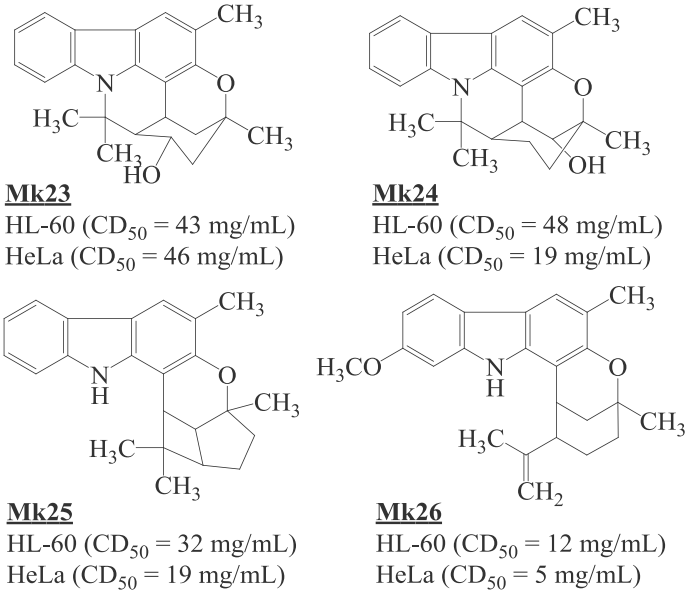
(**Mk14** and **Mk21**) at position 3 of the carbazole nucleus showed moderate to weak activities. Meanwhile, the presence of an electron-withdrawing group (-CHO) at position 6 in the carbazole nucleus (**Mk19** and **Mk22**) showed very strong activities against both tested cancer cells. Pyrano[3,2-a]carbazole alkaloids with disubstituted electron-donating groups at either C-3/C-6 **(Mk13)** or C-3/C-7 **(Mk16)** showed stronger activities when compared to those at either C-3/C-8 **(Mk18)** or C-6/C-7 **(Mk17)**. These results revealed that the pyrano[3,2-a]carbazole alkaloids containing an electron-withdrawing group (-CHO) at position-6 in the carbazole nucleus play an important role in cytotoxic activities.

All tested cyclic monoterpenoid pyrano[3,2-a]carbazole-type of alkaloids (**Mk23** to **Mk25**) that possessing a pyranocarbazole moiety with a five/six cyclic ring system showed weak activities against both the tested cancer cells, except for **Mk26** (Figure 4.5.6). The presence of an additional electron-donating group (-OCH_3) at position-7 of the carbazole nucleus of **Mk26** showed strong activities against each tested cancer cell lines. The results confirmed that the presence of methoxyl group at C-7 was beneficial for the improvement of cytotoxic activities.

Finally, the isolated biscarbazoles or binary carbazoles (**Mk27** to **Mk30**) showed weak activities against both the tested cancer cell lines (Figure 4.5.7). The results indicated that the symmetrical dimers of carbazole alkaloids cause an obvious decrease in cytotoxic activities which is similar to those report from the literature (Tachibana et al. 2003).

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Mk23
HL-60 (CD_{50} = 43 mg/mL)
HeLa (CD_{50} = 46 mg/mL)

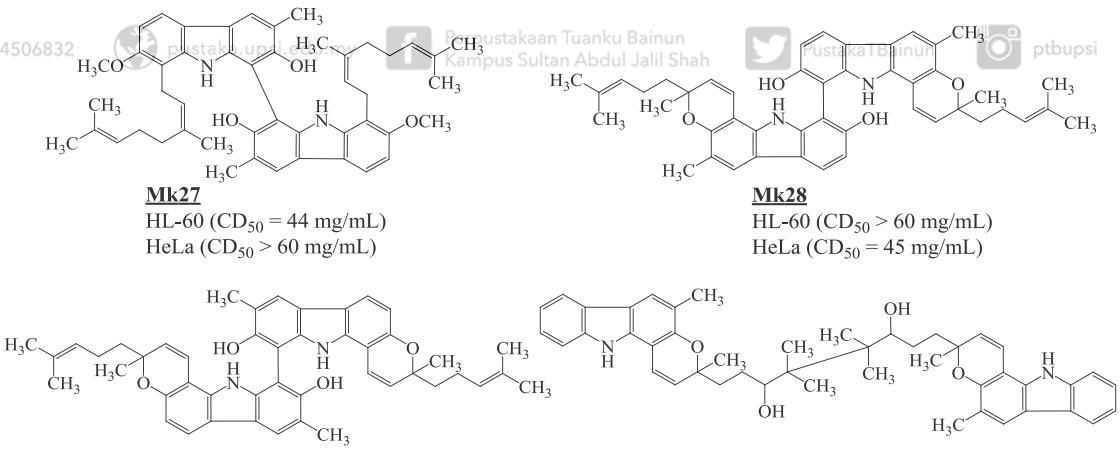
Mk24
HL-60 (CD_{50} = 48 mg/mL)
HeLa (CD_{50} = 19 mg/mL)

Mk25
HL-60 (CD_{50} = 32 mg/mL)
HeLa (CD_{50} = 19 mg/mL)

Mk26
HL-60 (CD_{50} = 12 mg/mL)
HeLa (CD_{50} = 5 mg/mL)

Figure 4.5.6. Structure of Cyclic Monoterpenoid Pyrano[3,2-a]carbazole Alkaloids and Their Corresponding CD_{50} Values against HL-60 and HeLa Cell Lines

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Mk27
HL-60 (CD_{50} = 44 mg/mL)
HeLa (CD_{50} > 60 mg/mL)

Mk28
HL-60 (CD_{50} > 60 mg/mL)
HeLa (CD_{50} = 45 mg/mL)

Mk29
HL-60 (CD_{50} > 60 mg/mL)
HeLa (CD_{50} > 60 mg/mL)

Mk30
HL-60 (CD_{50} = 22 mg/mL)
HeLa (CD_{50} = 37 mg/mL)

Figure 4.5.7. Structure of Biscarbazole Alkaloids and Their Corresponding CD_{50} Values against HL-60 and HeLa Cell Lines

Throughout the SAR investigation on five different groups of carbazole alkaloids, it has revealed that the presence of electron-donating groups (EDG) and the electron-withdrawing groups (EWG) have greatly influenced the cytotoxic activities

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against HL-60 and HeLa cancer cells, whereas the position of the substituents in the carbazole nucleus play an important role in the activities. Moreover, the higher the cytotoxic activity resulted the more potential of the carbazole alkaloids as the lead compounds for the therapeutic drugs. The important structural features of carbazole alkaloids that essential for cytotoxic effect against HL-60 and HeLa cancer cells were summarised in Figure 4.5.8.

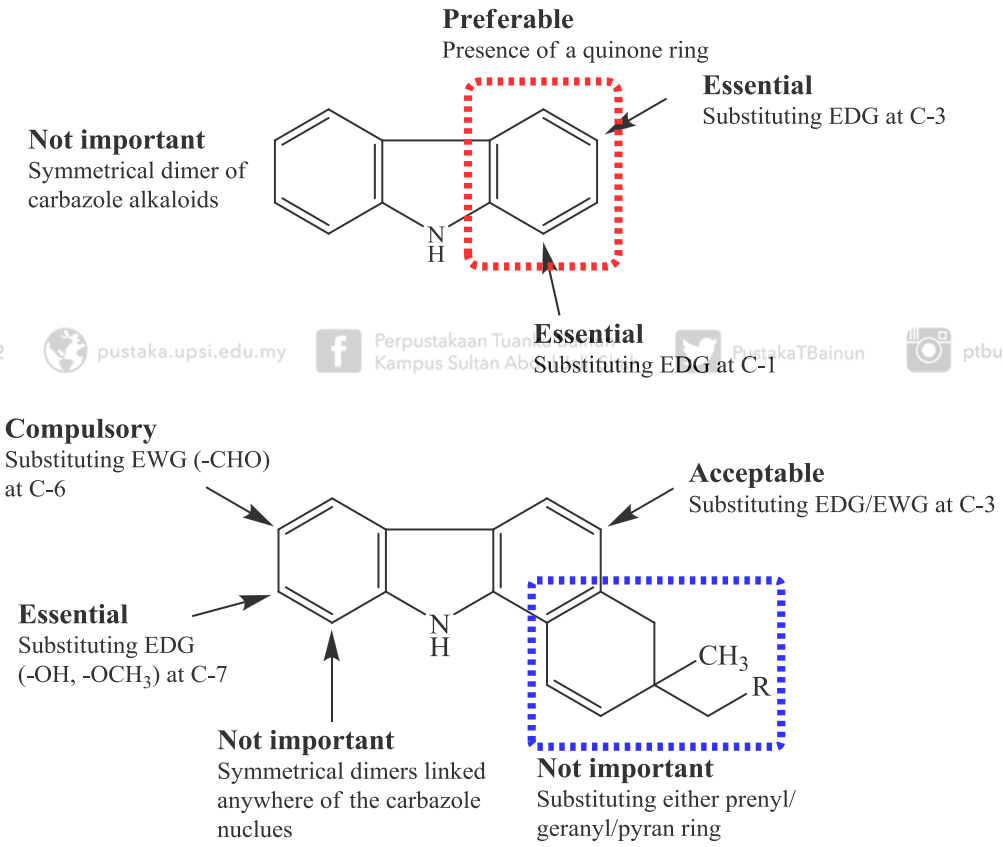


Figure 4.5.8. Important Structural Features of Carbazole Alkaloids for Cytotoxic Activities against HL-60 and HeLa Cell Lines

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CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

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5.1 INTRODUCTION

In this chapter, the main findings with regard to the research questions are summarised with conclusions. Recommendations based on the findings and conclusions of the study were also included which future research would be beneficial.

5.2 CONCLUSIONS

The main objective of the study is to isolate secondary metabolites with potential cytotoxic properties from the bark and leaves of Malaysian *Murraya koenigii* by screening through targeted cancer cell lines such as the human promyelocytic

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leukemia cell lines (HL-60) and cervical cancer cell lines (HeLa), as well as the normal mouse embryonic fibroblasts (NIH/3T3) cell lines.

The study was designed to identify the types of chemical constituents which can be extracted, isolated and purified from the selected plant species by details fractionation process using various chromatography methodologies such as column chromatography, thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC). The isolated chemical constituents were structurally identified by combination of spectroscopic techniques such as 1D NMR (^{1}H, ^{13}C and DEPT), 2D NMR (^{1}H-^{1}H COSY, NOESY, TOCSY, HMQC and HMBC), IR, UV and mass spectrometry.

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Phytochemical study on the bark and leaves of *Murraya koenigii* afforded 36 chemical constituents, included six new carbazoles and six non-carbazole entities. These chemical entities were made up of nine tricyclic carbazoles, of which eight were known carbazoles, *viz.*, murrayanine **(Mk1)**, murrayafoline-A **(Mk2)**, koenoline **(Mk3)**, 3-formylcarbazole **(Mk4)**, 2-hydroxy-3-methylcarbazole **(Mk5)**, mahanimbilol **(Mk6)**, euchrestine-B **(Mk7)** and mukoenine-B **(Mk8)**, along with a new carbazole, namely murrastanine-A **(Mk9)**; two known carbazolequinone alkaloids, namely murrayaquinone-A **(Mk10)** and murrayaquinone-B **(Mk11)**; eleven pyrano[3,2-a]carbazole type of alkaloids were also isolated from the bark and leaves of the plant, of which eight of these carbazoles were known, *viz.*, girinimbine **(Mk12)**, koenimbine **(Mk13)**, murrayacine **(MK14)**, mahanimbine **(Mk15)**, mahanine **(Mk16)**, isomahanine **(Mk17)**, murrayamine-B **(Mk18)** and murrayamine-J **(Mk19)**, along with three new carbazoles, *viz.*, murrastinine-A **(Mk20)**, murrastinine-B **(Mk21)** and

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murrastinine-C **(Mk22)**. Four cyclic monoterpenoid pyrano[3,2-a]carbazole alkaloids, namely murrayazolinol **(Mk23)**, murrayakoeninol **(Mk24)**, bicyclomahanimbine **(Mk25)**; and a new pyranocarbazole with cyclic monoterpenoid framework, *viz.*, murrayatanine-A **(Mk26)**, were also isolated. Four biscarbazole alkaloids were also obtained, of which three of them were known carbazoles, *viz.*, bismurrayafoline-E **(Mk27)**, bispyrayafoline **(Mk28)** and bisisomahanine **(Mk29)**, as well as a new biscarbazole, namely bismahanimboline **(Mk30)**. Six non-carbazoles such as β-sitosterol **(Mk31)**, stigmast-4-ene-3-one **(Mk32)**, squalene **(Mk33)**, α-tocopherol **(Mk34)**, 2-hydroxy-4-methoxy-3,6-dimethylbenzoic acid **(Mk35)** and an eudesmane sesquiterpene, selin-11-en-4α-ol **(Mk36)**, were also isolated from the bark and leaves of *M. koenigii*.

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The isolated chemical entities were then evaluated for their *in vitro* cytotoxic activities against selected cell lines via MTT assay. Among the isolated compounds, five carbazoles and two non-carbazoles were found to be very strong to moderate active in cytotoxic activity against both HL-60 and HeLa cancer cell line with CD_{50} less than 20 μg/mL such as murrayafoline-A **(Mk2)**, mahanine **(Mk16)**, murrayamine-J **(Mk19)**, murrastinine-C **(Mk22)**, murrayatanine-A **(Mk26)**, β-sitosterol **(Mk31)** and 2-hydroxy-4-methoxy-3,6-dimethylbenzoic acid **(Mk35)**. Others were found to be weakly active to both HL-60 and HeLa with CD_{50} between 20 to 60 μg/mL when compared to vinscritine, the positive control, with CD_{50} value less than 1.0 μg/mL. Most importantly, all the isolated compounds were not active against NIH/3T3 cell lines.

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The structure-activity relationship (SAR) evaluation on isolated carbazole alkaloids has suggested that the presence of electron-donating groups (EDG) and the electron-withdrawing groups (EWG) have greatly influenced the cytotoxic activities against HL-60 and HeLa cancer cells, whereas the position of the substituents in the carbazole nucleus play an important role in the activities. In fact, the higher the cytotoxic activity resulted the more potential of the carbazole alkaloids as the lead compounds for the therapeutic drugs.

Further from the approach, the study was continued to develop novel synthetic analogues from girinimbine **(Mk12)** and mahanimbine **(Mk15)**, the major carbazole alkaloids isolated from the selected plant species, via Lewis acid-mediated oxidative coupling/cyclization reaction by using anhyrous $FeCl_3$- and BF_3-$O(C_2H_5)_2$. Two analogues were successfully derived from girinimbine **(Mk12)**, *viz.*, murranimbine **(G1)** and epoxygirinimbine **(G2)**. Meanwhile, three mahanimbine analogues were also successfully synthesized, *viz.*, bicyclomahanimbine **(M1)**, cyclomahanimbine **(M2)** and murrayazolinine **(M3)**. All these structures were unambiguously confirmed by 1D-NMR (^1H, ^{13}C and DEPT), 2D-NMR (^1H-^1H COSY, NOESY, HMQC and HMBC), IR, UV and mass spectrometry. These synthetic analogues of girinimbine and mahanimbine were also evaluated for their *in vitro* cytotoxic activities against selected cell lines via MTT assay. The results showed that most of these synthetic derivatives were showed weak activity against both HL-60 and HeLa cell lines, but inactive against NIH/3T3 cell lines.

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Herein, we can conclude that the isolated chemical constituents from Malaysian *M. koenigii* are remarkably similar to those of Indian species when direct comparison with those listed in literature, except for the following findings:

(i) Six new carbazoles were successfully isolated from the bark and leaves from Malaysian *M. koenigii*;

(ii) Two known carbazoles, *viz.*, murrayamine-J **(Mk19)** and bisisomahanine **(Mk29)** were also first time isolated from *M. koenigii*;

(iii) Six non-carbazole compounds were first time reported from Malaysian *M. koenigii*;

(iv) Biscarbazoles were able to be isolated as minor compounds from the bark and leaves of Malaysian *M. koenigii* which is hardly isolated from Indian species; and

(v) The isolated compounds from the bark of leaves of Malaysian *M. koenigii* were found to possess potential *in vitro* cytotoxic activity against HL-60 and HeLa cancer cell lines, of which most of these results were first time reported.

The knowledge obtained from the phytochemical study and the development of synthetic analogues of carbazole alkaloids, as well as their cytotoxic screening have served to provide an important source for therapeutic discovery and may lead to the development of potential drugs.

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5.3 RECOMMENDATIONS FOR FUTURE RESEARCH

The study conducted showed a promising future for Malaysia *Murraya koenigii*. Although there are limitations, Malaysia *M. koenigii* provides a potential source for the discovery of anti-cancer leads. The investigations performed in this thesis covered only one Malaysia *Murraya* species. Thus, future research is needed to understand and expand further the knowledge of Malaysia *Murraya* as potential anticancer agents.

Study of other Malaysian Rutaceae species is recommended, since most of the studies were focused on Indian species and limited information are available on Malaysian Rutaceae species. In fact, this family have proven to offer an intriguing structural features with promising biological properties which may lead to the development of potential plant-based drug candidates.

In the phytochemical study of the selected plant species, the conventional extraction, isolation and purification process have provided us board range of carbazole alkaloids. In fact, the extraction methods that used in this study can be improved by using exhaustive extraction such as Soxhlet extraction method, which usually carried out with different solvents of increasing polarity in order to extract as much as possible the active components with potential biological activities.

Bioassay-guided isolation and identification of bioactive chemical constituents is highly recommended since some of the isolated compounds of *M. koenigii* showed very strong cytotoxic activity against selected cancer cells. Besides, it is highly recommended to establish in-house bioassays in phytochemistry laboratories which do

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not really require a specialized knowledge of biochemistry, biology or pharmacology. This may also expand the applications until molecular mechanism of action on strongly active compounds.

With the increasing interest of many promising drug candidates in the current drug development and also the findings obtained from the structure-activity relationship investigation on the isolated carbazole alkaloids, total synthesis of bioactive carbazole alkaloids is highly recommended in order to develop potential bioactive leads which may beneficial to the society.

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APPENDICES

Articles Publication

1. **Siow-Ping Tan**, Mohd Azlan Nafiah and Kartini Ahmad. C_{23}-Carbazole Alkaloids from Malayan *Murraya koenigii* (L.) Spreng. *Journal of Chemical and Pharrnaceutical Research*, 2014, *6*(4), 1093-1098.

2. **Tan Siow Ping**, Mohd Azlan Nafiah, and Kartini Ahmad. C_{23}-Carbazole Alkaloids from the Bark of *Murraya koenigii* (L.) Spreng. *EDUCATUM - Journal of Science, Mathematics and Technology*, 2014, *1*(1), 1-9.

3. Kartini Ahmad, **Siow-Ping Tan**, Mohd Aspollah Sukari, Abdul Manaf Ali and Mohd Azlan Nafiah. Cytotoxic and Anti-tumour Promoting Activities of Carbazole Alkaloids from Malayan *Murraya koenigii* (L.) Spreng. *American Journal of Plant Sciences*, 2014, *5*(19), 2869-2877.

4. **Siow-Ping Tan**, Abdul Manaf Ali, Mohd Azlan Nafiah, Khalijah Awang, Kartini Ahmad. Isolation and Cytotoxic Investigation of New Carbazole Alkaloids from *Murraya koenigii* (Linn.) Spreng. *Tetrahedron*, 2015, *71*, 3946-3953.

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Articles in Seminar, Proceeding and Conferences

1. Pyranocarbazole Alkaloids from Malayan *Murraya koenigii* (Rutaceae)
Tan Siow Ping, Mohd Azlan Nafiah, Kartini Ahmad
International Conference on Natural Products 2013, UiTM, 4-6 March 2013.
- Oral Presentation
The Open Conference Proceedings Journal, ISSN: 2210-2892

2. C_{23}-Carbazole Alkaloids from Bark of *Murraya koenigii* (L.) Spreng
Tan Siow Ping, Mohd Azlan Nafiah, and Kartini Ahmad
International Post Graduate Conference on Science and Mathematics 2013, Research in Science and Mathematics Catalyse Sustainable Future, 5 October 2013.
- Poster Presentation

3. C_{23}-Carbazole Alkaloids from Malayan *Murraya koenigii* (L.) Spreng
Tan Siow Ping, Mohd Azlan Nafiah, Kartini Ahmad
5th Global Submit on Medicinal and Aromatic Plants 2013, Marriot Resort and Spa, Miri, Malaysia, 8-12 December, 2013.
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4. **Bioactive Carbazole Alkaloids from Malayan *Murraya koenigii* (L.) Spreng**
   Kartini Ahamd, Tan Siow Ping, Mohd Aspollah Sukari, Abdul Manaf Ali and Mohd Azlan Nafiah.
   5th Global Submit on Medicinal and Aromatic Plants 2013, Marriot Resort and Spa, Miri, Malaysia, 8-12 December, 2013.
   - Poster Presentation

5. **Tricyclic Carbazole Alkaloids from Bark of *Murraya koenigii* (L.) Spreng**
   Tan Siow Ping, Mohd Azlan Nafiah, Kartini Ahmad. International Post Graduate Conference on Science and Mathematics 2014, 18-19 October 2014.
   - Oral Presentation

6. **Study on Chemical Constituents from Roots of *Murraya koenigii* (Rutaceae)**
   Adilah Shahidan, Tan Siow Ping, Kartini Ahmad, Mohd Azlan Nafiah, Aspollah Sukari. International Post Graduate Conference on Science and Mathematics 2014, 18-19 October 2014.
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7. ***In Vitro* Cytotoxicity of Isolated Carbazole Alkaloids from the bark of *Murraya koenigii* (L.) Spreng**
   Siow-Ping Tan, Abdul Manaf Ali, Mohd Azlan Nafiah, Kartini Ahmad
   18th Malaysian International Chemical Congress (18MICC) 2014, 3-5 November 2014.
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8. **Chemical Constituents from the Roots of *Murraya koenigii* (Rutaceae)**
   Adilah Shahidan, Tan Siow Ping, Aspollah Sukari, Mohd Azlan Nafiah, Kartini Ahmad
   18th Malaysian International Chemical Congress (18MICC) 2014, 3-5 November 2014.
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