ALKALOIDS ISOLATED FROM THE BARK OF *ALSEODAPHNE PEDUNCULARIS* (WALL. EX NEES) MEISN AND THE ROOTS OF *ALSEODAPHNE CORNERI* KOSTERM

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ABSTRACT

The objectives of this study are to extract and isolate the alkaloids from two species of *Alseodaphne*; *Alseodaphne peduncularis* (Wall. ex Nees) Meisn from Kluang-Mersing, Johor and *Alseodaphne corneri* Kosterm from University Malaya, Kuala Lumpur. The extraction process of the plant material started by cold percolation process using hexane to remove non-polar organic compounds, waxes and fats. The plant material then re-extracted by dichloromethane using soxhlet extractor followed by acid-base extraction to get the alkaloid crude extract. The isolation and purification of alkaloids from the crude extract were done by using various chromatographic techniques including column chromatography and preparative thin layer chromatography. The elucidation of the isolated alkaloids were determine by using various spectroscopic methods such as 1D NMR (^{1}H, ^{13}C and DEPT) and 2D NMR (COSY, HMQC and HMBC), ultraviolet (UV), infrared (IR) and mass spectrometry (MS). The structures were further confirmed by comparison with other literature data. Isolation and purification of alkaloids from the bark of *Alseodaphne peduncularis* (KL 5165) yielded four aporphines; boldine **69**, norpredicentrine **90**, norlirioferine **91** and norboldine **78**. In addition, seven alkaloids were successfully isolated from the roots of *Alseodaphne corneri* (KL 4928). The isolated alkaloids include two aporphines; laetanine **30**, boldine **69** and five bisbenzylisoquinolines; gyrolidine **47**, stephasubine **92**, 2-norobaberine **93**, 3′,4′-dihydrostephasubine **94** and *O*-methyllimacusine **95**. The bioactivity study on the bark crude extract of *Alseodaphne peduncularis* and three isolated aporphines; boldine **69**, norlirioferine **91** and norboldine **78** showed good to moderate antiplasmodial activity against *Plasmodium falciparum* after compared to the standard (chloroquine; 0.087 μg/ml) with IC_{50} value of 2.135, 1.067, 2.786 and 2.228 μg/ml, respectively. It was found that boldine **69** showed the most potent activity with an IC_{50} value of 1.067 μg/ml and it showed potential for antiplasmodial drug. The extraction and isolation of alkaloids from this species will be continued to determine various type of alkaloids contents and new alkaloids findings. Moreover, the isolated alkaloids should be tested with other biactivity test such as cytotoxicity, antibacterial and antifungal activities for new drugs discovery.

Alkaloid dari Kulit Kayu Pokok *Alseodaphne peduncularis* (Wall. ex Nees) Meisn dan Akar Pokok *Alseodaphne corneri* Kosterm

ABSTRAK

Objektif kajian ini adalah mengestrak dan mengasingkan sebatian alkaloid daripada dua spesies *Alseodaphne*; *Alseodaphne peduncularis* (Wall. ex Nees) Meisn dari Kluang-Mersing, Johor dan *Alseodaphne corneri* Kosterm dari Universiti Malaya, Kuala Lumpur. Pengekstrakan sampel tumbuhan dimulakan dengan proses serapan sejuk menggunakan heksana untuk mengeluarkan sebatian organik tidak polar, lilin dan lemak. Sampel tumbuhan kemudiannya diekstrak semula oleh diklorometana menggunakan pemerah *soxhlet* diikuti oleh pengekstrakan asid-bes untuk mendapatkan ekstrak mentah alkaloid. Pengasingan dan penulenan alkaloid dari ekstrak mentah telah dilakukan dengan menggunakan pelbagai teknik kromatografi termasuk kromatografi turus dan kromatografi lapisan nipis. Struktur alkaloid dikenalpasti dengan kaedah kombinasi spektroskopi seperti resonan magnet nukleus satu dimensi; 1D NMR (^{1}H, ^{13}C dan DEPT), dua dimensi; 2D NMR (COSY, HMQC dan HMBC), ultralembayung (UV), inframerah (IR) dan spektrometri jisim (MS). Struktur-struktur alkaloid disahkan melalui perbandingan dengan data daripada kajian-kajian lepas. Pengasingan dan penulenan alkaloid daripada kulit kayu pokok *Alseodaphne peduncularis* (KL 5165) menghasilkan empat sebatian aporfina; boldina **69**, norpredicentrina **90**, norlirioferina **91** dan norboldina **78**. Tambahan pula, tujuh sebatian alkaloid berjaya diasingkan daripada akar pokok *Alseodaphne corneri* (KL 4928). Sebatian alkaloid tersebut termasuklah dua aporfina; laetanina **30**, boldina **69** dan lima bisbenzilisokuinolina; girolidina **47**, stephasubina **92**, 2-norobaberina **93**, 3´,4´-dihidrostephasubina **94** dan *O*-metillimacusina **95**. Kajian bioaktiviti ke atas ekstrak mentah kulit kayu daripada *Alseodaphne peduncularis* dan tiga sebatian aporfina; boldina **69**, norlirioferina **91** dan norboldina **78** menunjukkan aktiviti baik sehingga sederhana terhadap aktiviti antiplasmodial ke atas *Plasmodium falciparum* selepas dibandingkan dengan standard (chloroquine; 0.087 µg/ml) dengan nilai IC_{50} masing-masing iaitu 2.135, 1.067, 2.786 dan 2.228 µg/ml. Didapati boldina **69** menunjukkan aktiviti yang paling baik dengan nilai IC_{50} iaitu 1.067 µg/ml dan berpotensi digunakan sebagai ubat antiplasmodial. Proses pengekstrakan dan pengasingan alkaloid daripada spesies ini juga akan diteruskan untuk menentukan pelbagai jenis kandungan alkaloid di dalamnya termasuk penemuan sebatian alkaloid baru. Selain itu, alkaloid yang berjaya diasingkan juga perlu diuji untuk kajian bioaktiviti lain seperti kajian sitotoksik, antibakteria dan antikulat untuk digunakan dalam penemuan ubat-ubatan baru.

CONTENTS

LIST OF TABLES

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

LIST OF FIGURES

LIST OF SCHEMES

ABBREVIATIONS

α	Alpha
β	Beta
λ	Maximum wavelength
δ	Chemical shift
g	Gram
kg	Kilogram
cm^{-1}	per centimeter
ml	Mililitre
nm	Nanometer
MHz	Mega Hertz
Hz	Hertz
UV	Ultraviolet
IR	Infrared
ppm	Part per million
MeOH	Methanol
CH_2Cl_2	Dichloromethane
$CHCl_3$	Chloroform
OCH_3	Methoxyl group
OH	Hydroxyl group
NH_3	Ammonia
HCl	Hydrochloric acid
Na_2SO_4	Sodium sulphate
$MgSO_4$	Magnesium sulphate
NaCl	Sodium chloride
KCl	Potassium chloride

$CDCl_3$	Deuterated chloroform
pH	Power of hydrogen
TLC	Thin layer chromatography
CC	Column chromatography
PTLC	Preparative thin layer chromatography
NMR	Nuclear magnetic resonance
J	Coupling constant
d	Doublet
dd	Doublet of doublet
s	Singlet
br s	Broad singlet
m	Multiplet
t	Triplet
1D NMR	One dimension nuclear magnetic resonance
2D NMR	Two dimension nuclear magnetic resonance
COSY	Correlation spectroscopy
HMQC	Heteonuclear multiple quantum coherence
HMBC	Heteronuclear multiple bond coherence
GCMS	Gas chromatography spectrometry
m/z	Mass to charge ratio

CHAPTER 1

INTRODUCTION

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1.1 General

In Southeast Asia, there are rich jungles in Cambodia and Malaysia region due to the hot climate and humid all year round and it supports some of the most complex and species-rich ecosystem on the globe (Croix, 2008). Malaysia known as a green country with 60% of the land surface covered by forest various type of flora and fauna. The forest of East Malaysia are estimated around 2,000 tree species and known as one of most biodiverse areas in the world with 240 difference species in every hectare (Wikipedia, 2014).

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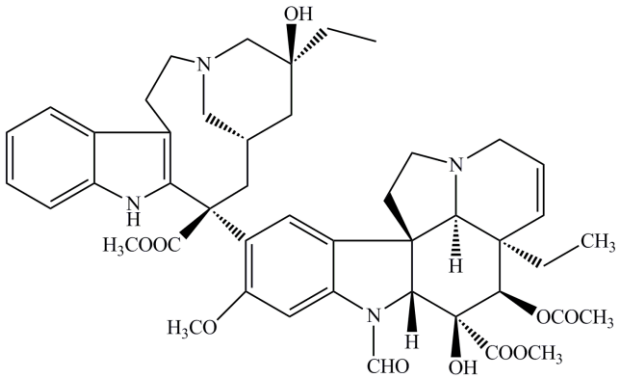
Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand for plant-based

medicines, health products, pharmaceuticals, food supplements and cosmetics. It was estimated that about 80% of all world's medicine are originally derived from plant sources (Cseke et al., 2006). Therefore, plants have contributed to the varieties of medicinal products since the past years.

Higher plants are important sources of natural products and are still used commercially to produce a wide range of chemicals as drugs, flavors, enzymes, perfumes, insecticides and emulsifying agents. Hence, many tropical plants from Malaysia has been extensively studied as well as their biological activity such as *Artocarpus* and *Actinodaphne* species (Hashim et al., 2012; Rachmatiah et al., 2009a).

Plants continue to be a major source of medicines. Among the most important are the physiologically active alkaloids including vincristine **1** and vinblastine **2** from *Catharanthus roseus*, codeine **3** and morphine **4** from *Papaver somniferum*, hyoscyamine **5** and scopolamine **6** from *Datura* species, quinine **7** and quinidine **8** from *Cinchona ledgeriana* and reserpine **9** from *Rauwolfia* species.



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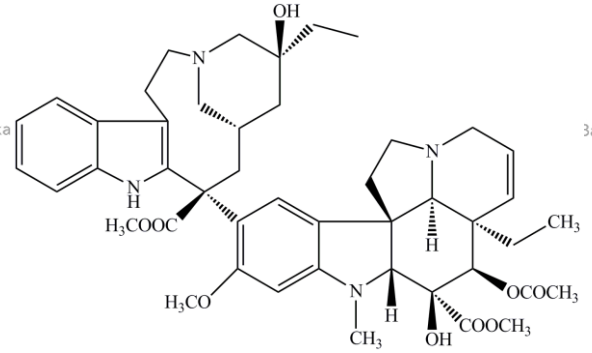

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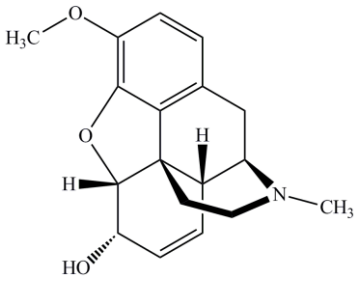

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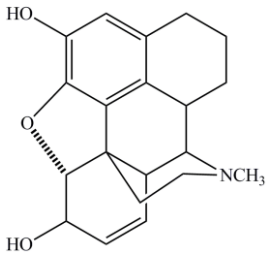

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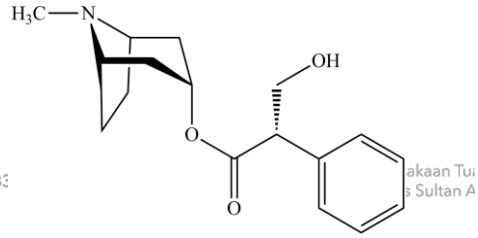

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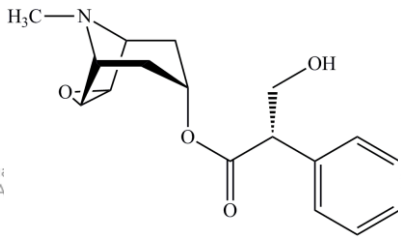

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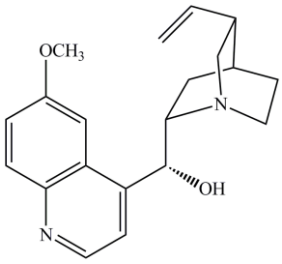

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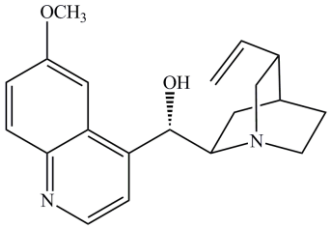

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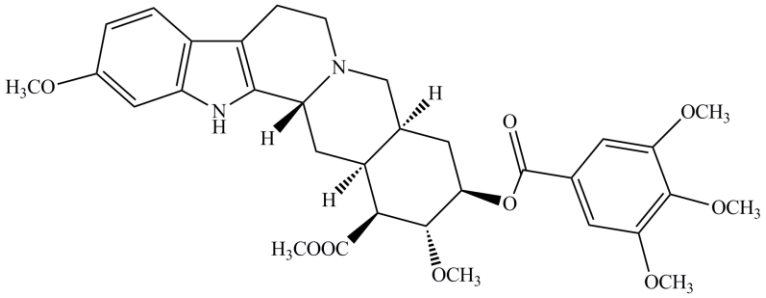

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1.2 Objectives of study

A research work on the species of *Alseodaphne*; *Alseodaphne peduncularis* and *Alseodaphne corneri* were carried out with the following objectives:

1) To extract and isolate the alkaloids.
2) To elucidate the structures of the isolated compounds using modern spectroscopic methods such as 1D-NMR, 2D-NMR, UV, IR and MS.
3) To test the antiplasmodial activity of crude extract and pure alkaloids.

1.3 Lauraceae

Lauraceae form a large family of woody plants with about 50 genera and 2,500 to 3,000 species distributed throughout tropical to subtropical latitudes (Chanderbali, Werff & Renner, 2001). There are 20 genera and more than 420 species of Lauraceae in China which are mainly distributed in areas south of the Qinling Mountain-Huaihe River (Kuo et al., 2012).

In Malaysia, Lauraceae also known as '*Medang*' or '*Tejur*'. About 16 genera and 213 species of Lauraceae family can be found in Malaysia (Omar et al., 2013). Lauraceae distributed in the lowland and becoming more abundant in the mountains between 1,200 and 1,600 m altitude. Major producing states in Peninsular Malaysia including Kelantan, Perak, Terengganu, Negeri Sembilan and Kedah (Gan & Lim, 2004).

The Lauraceae family is known to contain alkaloids which explain the positive alkaloid tests towards *Dehaasia caesia* (leaves 2+ and bark 3+) and *Litsea Elliptibacea* (leaves 2+ and bark 4+) (Ismail & Din, 1995a). Previous works showed that aporphine alkaloids have been isolated from 18 genera of these plants including *Actinodaphne*, *Alseodaphne*, *Beilschmiedi*, *Cassytha*, *Cinnamomum*, *Cryptocarya*, *Dehaasia*, *Laurus*, *Lindera*, *Litsea*, *Machilus*, *Mezilaurus*, *Nectandra*, *Neolitsea*, *Ocotea*, *Phoebe*, *Ravensara* and *Sassafras* (Kuo et al., 2012).

1.3.1 Anatomical features and wood characteristics of Lauraceae

Growth rings absent but the presence of terminal parenchyma in some species may stimulate growth rings. Vessels medium-sized, solitary, radial pairs and multiples of up to 4, tyloses usually present (Figure 1.1). Wood parenchyma mainly as incomplete border to the vessels with ill-developed aliform to confluent (Gan & Lim, 2004).

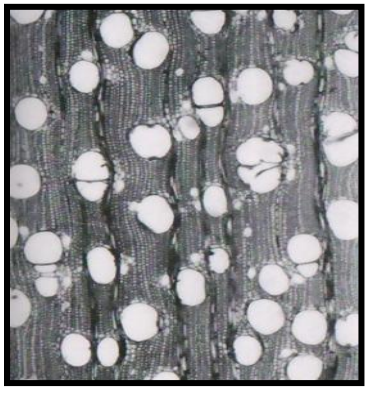

Figure 1.1. Anatomical features of Lauraceae. Adapted from "Common Commercial Timbers of Peninsular Malaysia," by K. S. Gan and S. C. Lim, 2004, *Research Pamphlet No. 125*, p. 38.

Some species with irregularly spaced bands. Rays fine or medium-sized and not distinct to naked eye. The characteristics of heartwood very variable, light-straw, red-brown to olive brown. Sapwood ill-defined and the surface is dull while the texture is moderately fine but even (Figure 1.2). Grain interlocked or wavy (Gan & Lim, 2004).

Figure 1.2. Wood characteristic of Lauraceae. Adapted from "Common Commercial Timbers of Peninsular Malaysia," by K. S. Gan and S. C. Lim, 2004, *Research Pamphlet No. 125*, p. 38.

1.3.2 Taxonomy of Lauraceae

The classification of Lauraceae illustrated as listed below:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Laurales

Family: Lauraceae

Genera:

Actinodaphne
Aiouea
Alseodaphne
Aniba
Apollonias
Aspidostemon
Beilschmiedia
Caryodaphnopsis
Cassytha
Chlorocardium
Cinnadenia
Cinnamomum
Cryptocarya
Dehaasia
Dicypellium
Dodecadenia
Endiandra
Endlicheria
Eusideroxylon
Gamanthera
Hexapora
Hufelandia
Hypodaphnis
Iteadaphne
Kubitzkia
Laurus
Licaria
Lindera
Litsea
Machilus
Malapoenna
Mezilaurus
Misanteca
Mocinnodaphne
Mutisiopersea
Nectandra
Neocinnamomum
Neolitsea
Notaphoebe
Nothaphoebe
Ocotea
Oreodaphne
Parasassafras
Parthenoxylon
Paraia
Persea
Phoebe
Phyllostemonodaphne
Pleurothyrium
Polyadenia
Potameia
Potoxylon
Povedadaphne
Ravensara
Rhodostemonodaphne
Sassafras
Schauera
Sextonia
Sinopora
Sinosassafras
Syndiclis
Tetranthera
Tylostemon
Umbellularia
Urbanodendron
Williamodendron

1.3.3 Uses of Lauraceae

In Sabah, a species namely, *Cinnamomum* from Lauraceae family had been used for traditional medicine. Dusun people of Sabahan called the *Cinnamomum* species as '*Lamau-Lamau*'. They boiled the roots and make as a tonic to heal the headache (Ismail & Din, 1995b).

One of the major product of Lauraceae is in timber industry (Gan & Lim, 2004). The timber classification of Lauraceae is light hardwood and it is a large family of medium-sized to large trees. Some species of Lauraceae family are commercial

importance in Malaysia. The important genera which produce the timber of '*Medang*' include *Actinodaphne, Alseodaphne, Beilschmiedia, Cinnamomum, Cryptocarya, Dehaasia, Litsea, Nothaphoebe* and *Phoebe.*

The wood density is within 400 to 800 kg/m^3 air dry. Its suitable for decorative work such as interior finishing, paneling, furniture and cabinet making. It also suitable for plywood manufacture and the heavier species are suitable for medium construction under cover (Gan & Lim, 2004).

Besides that, the family's great economic has another sources such as the high content of ethereal oils in the woods and leaves of many Lauraceae which are sources of perfumes, spices and flavourings such as camphor and cinnamon (Renner, 2011).

1.4 Genus *Alseodaphne*

From the *World Dictionary of Plant Names* state that *Alseodaphne Ness* (Lauraceae) origins name is from Greek. The *alsos* is "a grove" and *daphne* is "bay laurel" (Umberto, 2002). *Alseodaphne* is one of genus in Lauraceae family. It can be found in South East Asia countries such as China, Philippines, Borneo, Indonesia, Malaysia, New Guinea and Burma. *Alseodaphne* species also known as '*gemor*' in Indonesia and grows in Kalimantan Tengah and Kalimantan Selatan. Each year, it has become the major product of timber with about 250-300 kg/tree to 500-600 kg/tree (Budi & Andri, 2011).

The genus has 96 species of evergreen large trees to shrubs. The species of genus *Alseodaphne* including *Alseodaphne bancana*, *Alseodaphne peduncularis*, *Alseodaphne gracilis*, *Alseodaphne hainanensis*, *Alseodaphne perakensis*, *Alseodaphne corneri* and *Alseodaphne yunnanensis*. The *Alseodaphne* genus is well known for their alkaloid bearing plants that have the isoquinoline structures (Zahari, 2010; Ahmat, 2008).

1.4.1 Characteristics of genus *Alseodaphne*

The genus of *Alseodaphne* is evergreen trees. Terminal buds scaly. Leaves alternate and always clustered near apex of branchlet, pinninerved and often turning black when dry. Inflorescence axillary, paniculate or racemose; bracts and bracteoles deciduous.

The flowers are bisexual. Perianth tube short; perianth lobes 6, subequal or outer 3 smaller, slightly dilated after anthesis but absent in fruit. Fertile stamens 9, in 3 whorls; filaments of first and second whorls glandless, those of third whorl each with 2 glands at base; anthers 4-celled; cells of first and second whorls introrse, those of third whorl extrorse or upper 2 lateral and lower 2 extrorse staminodes 3, of innermost whorl, very small, nearly sagittate.

Furthermore, the ovary partly immersed into shallow perianth tube; style often as long as ovary; small stigma, inconspicuous, discoid. The fruit is black or purplish black when mature, ovoid, oblong or subglobose; fruit stalk is red, green or yellow,

sometimes nearly cylindric, fleshy, pulpy, always warty, truncate at apex (Xiwen, Jie & Werff, 2008).

1.4.2 *Alseodaphne peduncularis* (Wall. ex Nees) Meisn

Alseodaphne peduncularis (Figure 1.3) is a small tree up to 12 metre tall. The twigs color is whitish. The leaves are green colour and stalk slender sized to 0.5-1 cm long. The flowers are sub equal or outer 3 lobes slightly smaller. The fruits shapes are ellipsoid or globose with purple color and on enlarged red perianth tube. Common found in lowland and hill forest in Kedah, Perak, Kelantan, Terengganu, Pahang, Selangor, Negeri Sembilan, Johor and Sumatera (Ng, 1989).

Figure 1.3. Alseodaphne peduncularis.

1.4.3 *Alseodaphne corneri* Kosterm

Alseodaphne corneri (Figure 1.4) is a tree of moderate size growing in Singapore, Malaysia, Jawa, Sumatra and Borneo (Corner, 1988). It is 6 metre tall trees. The leaves are green colour and closely spirally arranged at the ends of twigs with stalk about 3 to 4 cm long. The twigs are stout and grey with prominent leaf scars. The flowers are up to 14 mm long and the fruit are ellipsoid up to 3x2 cm placed on thick and rough pedicles (Ng, 1989).

Figure 1.4. Alseodaphne corneri.

CHAPTER 2

GENERAL AND CHEMICAL ASPECTS OF ALKALOIDS

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2.0 Introduction

Plants contain more than 100,000 known natural organic constituents, many of which are valuable phytopharmaceuticals (Robinson, 1991; Kaufman, Cseke, Warber, Duke & Brielmann, 1999). Natural products chemistry as defined today, involves many studies on biosynthesis, isolation, structure determination, and investigation of biological properties of secondary metabolites (Torsell, 1997).

Since today, a lots of natural products especially alkaloids have been a source of highly effective conventional drugs for the treatment of many types of cancer. The major source of alkaloids in the past has been the flowering plants, the Angiospermae, where about 20% contain these constituents. In recent years, an increasing number of

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examples of alkaloids have come from animals, insects, marine organisms, microorganisms and lower plants (Roberts & Wink, 1998).

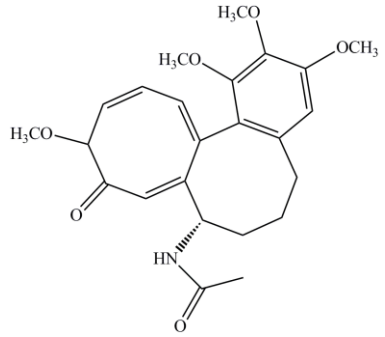
Phytochemical surveys are now seen as the first step towards the discovery of useful drugs now that the tropical rain forest has been identified as a potential source due to its diverse richness in flora. Moreover, the study of alkaloids constituents is always interesting and important in chemistry of natural products. New discovery of alkaloids have high potential to be used in medicinal area.

2.1 Definition of Alkaloid

The term alkaloid is derived from the Arabic word "*al-qali*" that refers to potassium carbonate-containing ashes from plant material, from which the term "alkali" is derived. Traditionally alkaloids are defined as heterocyclic nitrogen compounds biosynthesized from amino acids. Later, in 1819, the term "alkaloid" was first suggested by Meiser and it usually defined as basic nitrogen-containing compounds widely distributed in different plant groups (Cordell, 1983).

Nearly all alkaloids are alkaline, and most are optically active. Alkaloids are classically defined as being plant-derived, pharmacologically active, basic compounds derived from amino acids that contain one or more heterocyclic nitrogen atoms. Most nitrogen-containing secondary metabolites are considered alkaloids, unless they may be readily classified otherwise such as amines or glucosinolates.

Another simple general definition of an alkaloid has been suggested by Pelletier (1983): "An alkaloid is a cyclic compound containing nitrogen in a negative oxidation state which is of limited distribution in living organisms". This definition includes both alkaloids with nitrogen as part of a heterocyclic system as well as the many exceptions with extra cyclic bound nitrogen such as colchicines **10** or capsaicin **11**.



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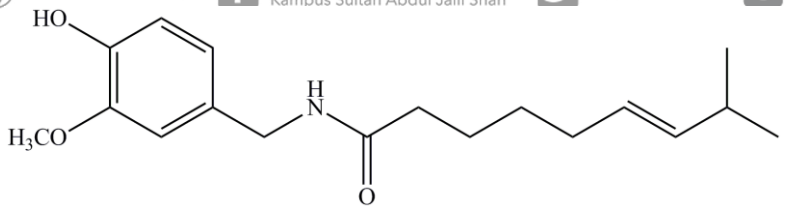


11

The definition of an alkaloid has always been rather problematical. In a taxonomic context it is probably best to restrict what is recognized as an alkaloid to the following (Roberts & Wink, 1998) :

1. a nitrogenous compound in which at least one nitrogen atom is derived directly from an amino acid and
2. a compound with limited distribution

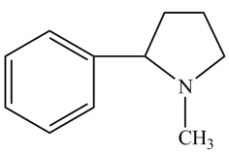
Thus nonbasic and noncyclic structures such as simple amines and amides (commonly called protoalkaloids) would be considered as alkaloids for taxonomic purpose.

2.2 Classification of Alkaloid

Alkaloids are generally classified by their common molecular precursors, based on the biological pathway used to construct the molecule. From a structural point of view, alkaloids are divided according to their shapes and origins. There are three main types of alkaloids: (1) true alkaloids, (2) protoalkaloids and (3) pseudoalkaloids (Aniszewski, 2007). True alkaloids and protoalkaloids are derived from amino acids, whereas pseudoalkaloids are not derived from these compounds.

2.2.1 True alkaloids

True alkaloids derive from amino acid and share a heterocylic ring with nitrogen. These alkaloids have a bitter taste and appear as a white solid, with the exception of nicotine **12** which has a brown liquid. True alkaloids may occur in plants in free state, salts and *N*-oxides. These alkaloids occur in a limited number of species and families. Examples of true alkaloids are morphine **4**, quinine **7** and cocaine **13**.



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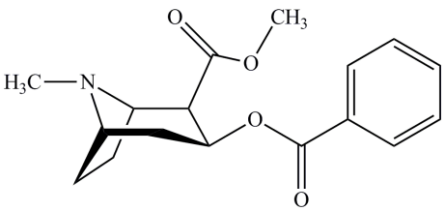

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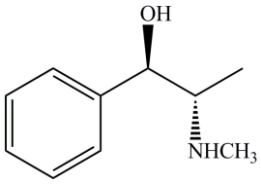
2.2.2 Protoalkaloids

Protoalkaloids are amino acid related compounds whose nitrogen atom is not part of a heterocylic system but has remained biogenetically "inert" (Fattorusso & Taglialatela-Scafati, 2008). Protoalkaloids are those with a closed ring, being perfect but structurally simple alkaloids. Some examples of these alkaloids are ephedrine **14** and mescaline **15**.



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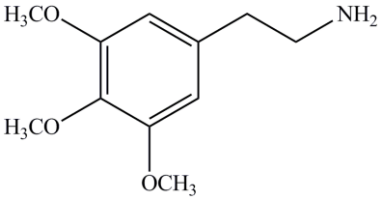

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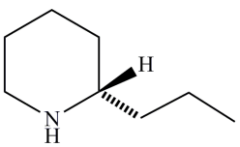
2.2.3 Pseudoalkaloids

Pseudoalkaloids are compounds, the basic skeletons of which are not derived from amino acids. From book 'Modern Alkaloids' stated that pseudoalkaloids are compounds unrelated biogenetically to amino acids and whose cyclic nitrogen derives from the formal incorporation of ammonia into a carbon skeleton, generally of terpenoid or polyketide origin (Fattorusso & Taglialatela-Scafati, 2008). In reality, pseudoalkaloids are connected with amino acid pathways. They are derived from the precursors or postcursors (derivatives the indegradation process) of amino acids. Examples of these alkaloids include capsaicin **11**, coniine **16** and caffeine **17**.



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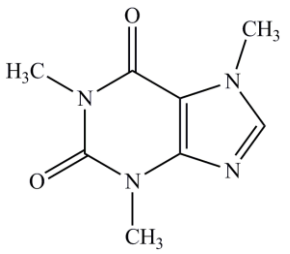

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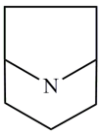
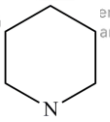
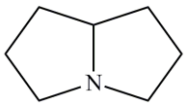
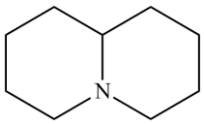
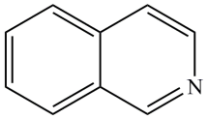
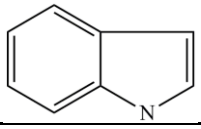
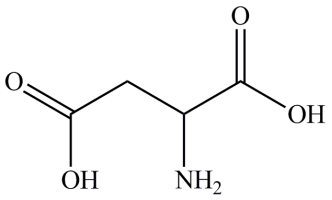
Moreover, there are many categories of alkaloids including pyrrolidine, tropane, piperidine, pyrrolizidine, quinolizidine, isoquinoline and indole alkaloids (Micheal 2003, 2004). Table 2.1 shows the major alkaloid classes and their biosynthetic precursors.

Table 2.1

Major Classes of Alkaloids, Their Chemical Structures, Their Biosynthetic Precursors and Well Known Examples of Each Class

Alkaloid class	Structure	Biosynthetic precursor	Examples
Pyrrolidine		Aspartic acid **18**	Nicotine **12**
Tropane		Ornithine **19**	Cocaine **13**
Piperidine		Lysine **20**	Coniine **16**
Pyrrolizidine		Ornithine **19**	Retrorsine **23**
Quinolizidine		Lysine **20**	Lupinine **24**
Isoquinoline		Tyrosine **21**	Codeine **3**
Indole		Tryptophan **22**	Reserprine **9**

Note. Adapted from "Quinoline, Quinazoline, and Acridone Alkaloids," by J. P. Michael, 2004, *Nat. Prod Rep*, 21, p. 650-668.



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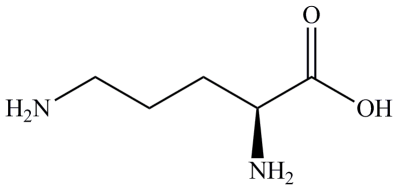

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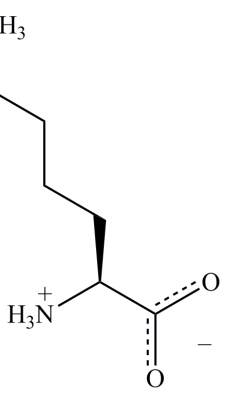

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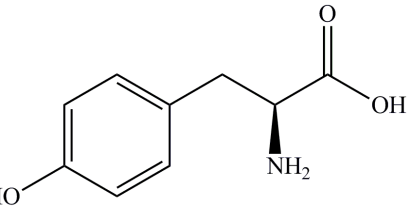

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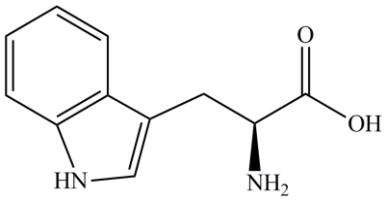

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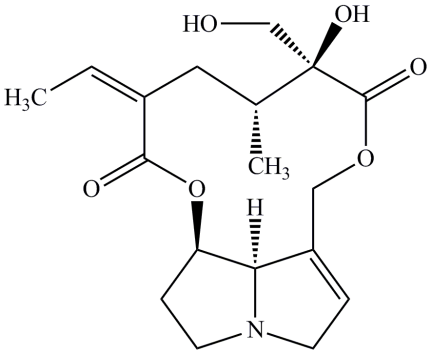

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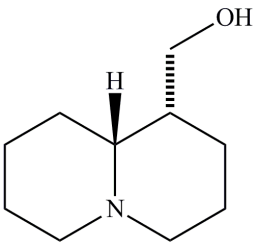


24

2.3 Alkaloids in Pharmaceutical

Alkaloids are toxic to both herbivores and humans, yet they have some very important medicinal properties for mankind. The alkaloids are structurally the most diverse class of secondary metabolites and over 5000 compounds are known such as coniine **16** from hemlock (Mann et al., 1996). Traditionally, some plants have been used as poisons for hunting and murder (euthanasia **25**) or as medicines (ephedrine **14**).

.

Furthermore, alkaloid that have other pharmacological activities including antiarrhythmic effects (quinidine **8**), antimalarial activity (quinine **7**) and anticancer actions (vincristine **1** and vinblastine **2**) (Cordell, 1983). Other examples of pharmaceutically important alkaloids from plants also can be seen in Table 2.2 (Hay et al., 1988).

Table 2.2

Examples of Pharmaceutically Important Alkaloids Extracted from Plants

Natural product	Pharmacological activity
Reserpine **9**	Central nervous system
Caffeine **17**	Autonomic nervous system
Ephedrine **14**	Adrenergic
Nicotine **12**	Ganglion blocking
Vincristine **1**	Anticancer
Quinine **7**	Antimalarial
Morphine **4**, codeine **3**	Analgesics
Cocaine **13**	Local anesthetic

Note. Adapted from "Alkaloid Production by Plant Cell Culture in A. Misrahi, A. L. Van Wezel (Eds.)," by C.A. Hay, L.A. Anderson, M.F. Roberts and J.D. Phillipson, 1988, *Biotechnology in Agricultures*, p. 97-140.

2.4 Aporphine

About 250 aporphine alkaloids isolated from plants of 20 families. Plants of the families of Annonaceae Araceae, Aristolochiaceae, Magnoliaceae and Lauraceae are known as known widely to have aporphine type alkaloid (Wu & Huang, 2006).

The numbering of the skeleton (Figure 2.1) is according to the accepted ruling (Guinaudeau, Leboeuf & Cavé, 1975). All aporphine alkaloids are based on the skeleton in Figure 2.1 and consist of di-, tri-, tetra-, penta- and hexasubstituted derivatives, the substituents being hydroxyl, methoxy or methylenedioxy groups.

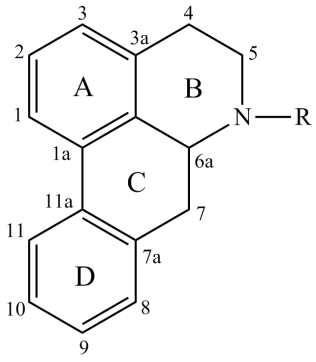


Figure 2.1. The numbering of the aporphine skeleton. Adapted from "Aporphine Alkaloids," by H. Guinaudeau, M. Leboeuf and A. Cavé, 1975, I. *Lloydia*, *38*, p. 275-338.

The substituents in the aporphine alkaloids may be located in all four rings with the exception of the methylenedioxy group, which is found only in rings A and D. In the case of all the disubstituted aporphines isolated, the substituents are present in positions 1 and 2 in ring A (Shamma, 1972). The most widespread in nature are the 1,2,9,10- and 1,2,10,11-tetrasubstituted bases, but pentasubstituted aporphines in

which functional groups occupy positions at various carbon atoms are found fairly frequently (Israilov, Karimova, M. Yunusov & Y. Yunusov, 1980).

2.4.1 UV Spectra of Aporphine

In the last 20 years, spectroscopic methods have been widely used giving a large amount of information on the structure of aporphine. According to the nature of the substitution in the aporphine skeleton, the UV spectra are divided into three groups (Sangster & Stuart, 1965):

1. The spectra of unsubstituted alkaloids or those monosubstituted in ring D have a single maximum at 270-280 nm and a weak shoulder at 310-320 nm.
2. In the spectra of 1,2,9,10-substituted alkaloids absortion maxima are observed at 280-284 and 303-310 nm which are characterized by approximately equal intensities.
3. The spectra of aporphine substituted in positions 1,2,10 and 11 each have a maximum at 268-272 nm with a maximum of lower intensity at 303-310 nm.

In addition, the spectra of 1,2,10,11-tetrasubstituted dehydroaporphines absorption maxima are observed at 220, 310 and 340 nm.

2.4.2 IR Spectra of Aporphine

The feature of IR spectra of aporphine is the presence in the aporphine nucleus of a biphenyl system, giving rise to three bands at about 1500, 1580 and 1600 cm^{-1}. A difference in the type of substitution of the aromatic rings is demonstrated by a scatter of the frequencies of the maximum of each band (Israilov et al., 1980).

The dehydroaporphine alkaloids have absorption bands in their IR spectra in the 1570-1610 cm^{-1} region. In the IR spectrum of the oxoaporphine alkaloids the absorption band of the carbonyl group is observed in the form of a sharp peak in the 1640-1675 cm^{-1} region (Israilov et al., 1980).

2.4.3 NMR Spectra of Aporphine

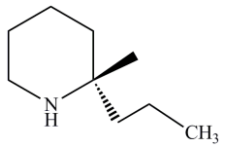
The nuclear magnetic resonance method gives a a large amount of information on the mutual positions of the substituting groups in the aporphine alkaloids (Israilov et al., 1980). Previous research showed that a lot of aporphine alkaloids had been isolated from Lauraceae family (Rachmatiah et al., 2009b; Mukhtar, 1996).

Generally, aporphine from Lauraceae family are dehydroaporphine, oxoaporphine and methylenedioxyaporphine such as pulchine **26**, litseferine **27** and oxophoebine **28** (Sivakumaran & Gopinath, 1976; Castro, López & Stermitz, 1986). Based on the ^{1}H NMR spectrum, methoxyls groups always appear at region 3.00-3.90 ppm (Karimova & Sadykov, 1981; Zarga & Shamma, 1982). If the methoxy and

hydroxy groups present at C-2, C-3, C-9 and C-10, the protons of aromatic rings at H-3, H-8 and H-11 each appear as a singlet at 6.60-8.00 ppm as shown in lirioferine **29** and laetanine **30** (Chen, Chang, Cowling, Huang & Gates, 1976; Borthakur & Rastogi, 1979).

In oxoaporphine, the present of carbonyl carbon (C=O) can be seen in the ^{13}C NMR spectrum near 180 ppm. The methoxy groups of oxoaporphine will appear at region 3.90-4.25 ppm as shown in oxoglaucine **31** and *O*-methylmoschatoline **32** (Marsaioli, Magalhães, Ruveda & Reis, 1980; Leboeuf, Cortes, Hocquemiller & Cavé, 1983). The aromatic protons of this aporphine always appear at 7.05-9.00 ppm as a singlet or doublet of doublets (Kunitomo, Murakami & Sugisakon, 1979; Menachery & Cava, 1981).

The *N*-methyl signal appear as singlets with integral of three protons at region 2.35-2.55 ppm depends on substituents near the *N*-methyl group (Hara, Hoshino, Isihige & Umezawa, 1981; Karimova & Sadykov, 1981; Pharadai, Tantisewie, Ruchirawat, Hussain & Shamma, 1981). Furthermore, the signals of methylene protons mainly appear at 2.5-4.0 ppm (Roblot, Hocquemiller, Cavé & Moretti, 1983).



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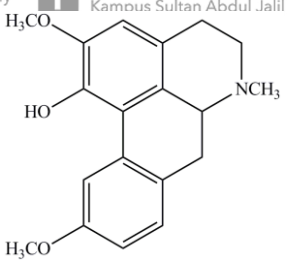

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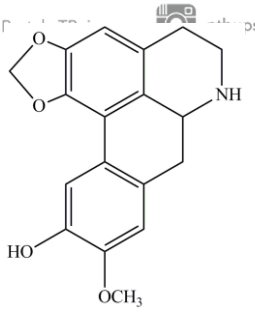

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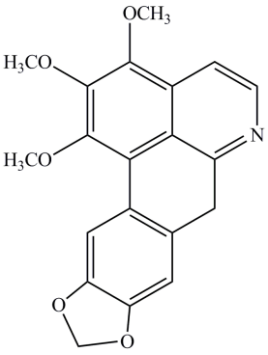

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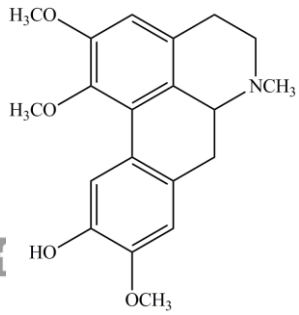

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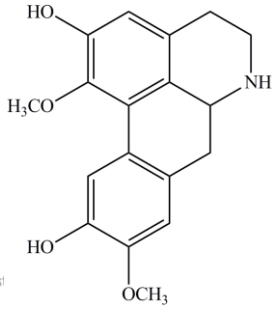

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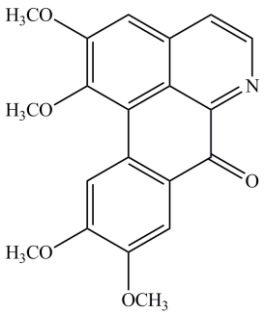

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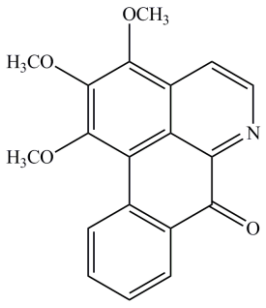

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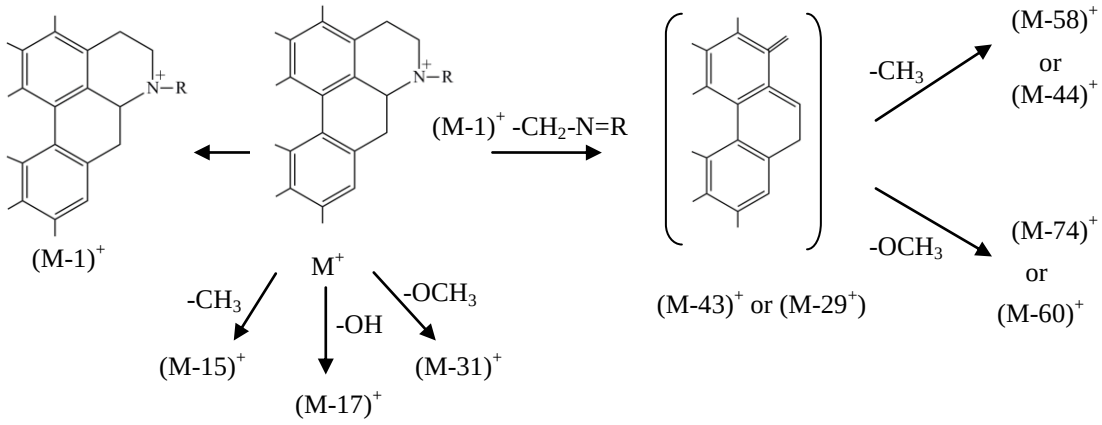
2.4.4 Mass Spectra of Aporphine

The mass spectra of the aporphine shows characteristic peaks of the M^+ ions, $(M-1)^+$, $(M-29)^+$ or $(M-43)^+$ ions (Israilov et al., 1980). The presence of methoxyl and hydroxyl groups in the molecule is responsible for the appearance in the spectrum of peaks of the ions M-CH_3, M-OH and M-OCH_3 and also for the subsequent ejection of these fragments from the M-CH_2-N=CH_2 ion.

The nature of the fragmentation of the aporphine alkaloids depends on the type of their substitution. For example, $(M-1)^+$ ion is the main peak in the spectra of the 1,2,9,10-tetrasubstituted aporphines while 1,2,10,11-tetrasubstituted bases the main peak is that of the molecular ion and as a rule the $(M-1)^+$ ion does not exceed 50%. Scheme 2.1 shows the overall fragmentations of aporphine (Israilov et al., 1980).



Scheme 2.1. Fragmentations of aporphine. Adapted from "Aporphine Alkaloids," I.A., Israilov, S. U. Karimova, M. S. Yunusov and S. Y. Yunusov, 1980, *Chemistry of Natural Compounds*, *16*(3), p. 197-225.

2.5 Bisbenzylisoquinoline

Bisbenzylisoquinoline form a large group of natural bases found in plants of the families of Menispermaceae, Berberidaceae, Ranunculaceae, Lauraceae, Annonaceae, Hernandiaceae, Magnoliaceae and Nymphaeaceae (Tolkachev, Nakova & Evstigneeva, 1977). More than 250 structures of bisbenzylisoquinoline bases are known which two benzylisoquinoline fragments are connected by one, two or three ether bonds.

Bisbenzylisoquinoline are built up of two benzylisoquinoline units linked by ether bridges. Shamma and Moniot (1976) had established the numbering of the skeleton and the systematic numerical classification describing oxygenation and dimerization patterns of the alkaloids as shown in Figure 2.2.

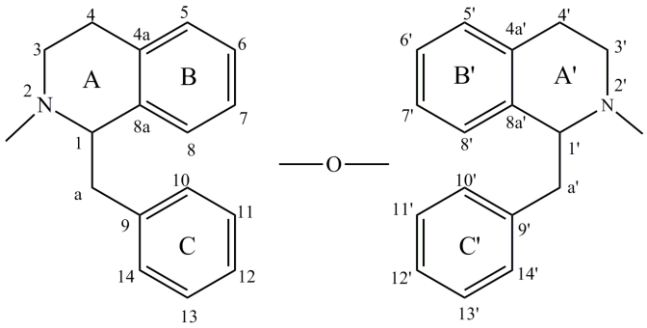


Figure 2.2. Systematic numbering system in bisbenzylisoquinoline. Adapted from "The Systematic Classification of Bisbenzylisoquinoline," by M. Shamma and J. L. Moniot, 1976, *Heterocycles*, 4, p. 1817.

The bisbenzylisoquinolines are the largest sub-group among the isoquinoline alkaloids, numbering over 260 members (Tanaka, Harada, Ichino & Ito, 1981). The principal structural variations found in bisbenzylisoquinoline alkaloids as follows:

1. The number of oxygen substituents present in the aromatic rings.
2. The number of ether linkages present in the molecule.
3. The nature of ether linkage;
    a) Diphenyl ether
    b) Benzyl phenyl ether
    c) The site of the two benzylisoquinoline units at which the ether or the C-C bond originates.

Based on these differences, the bisbenzylisoquinoline are classified into the groups and subgroups as shown in Table 2.3 (Shamma & Moniot, 1976). Individual members in each group differ in:

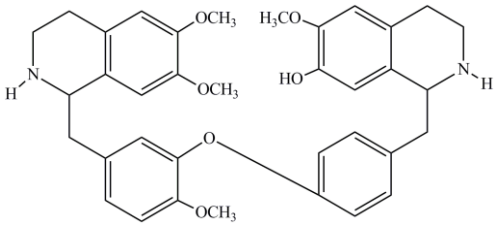
a) The nature of the oxygenated substituents (OH, OCH_3, OCH_2O);
b) The nature of the substitution of the two nitrogen atoms (NH, NCH_3, $N´CH_3$, NO).
c) The degree of unsaturation of the hetero rings.
d) The stereochemistry of the two asymmetric centers.

Table 2.3

Types of Skeleton in Bisbenzylisoquinoline

Skeleton	Classification	Group of skeleton	Example
One diphenyl ether linkage	1. Tail to tail	I, Ia, II, III	7-*O*-methyllindoldhamine **33**
	2. Head to head	IV	Antioquine **34**
	3. Head to tail	V	Neferine **35**
Two diphenyl ether linkages	1. Head to head and tail to tail	VI –XVII	2-norlimacusine **36**
	2. Only head to head	XVIII, XIX	Tilianangine **37**
	3. Head to tail	XX, XXI	Sciadoline **38**
One diphenyl ether and one benzyl phenyl ether linkages	-	XXII	Cissampareine **39**
Three diphenyl ether linkages	-	XXIII, XXIV	Kurramine **40**
Two diphenyl ether and one phenyl-benzyl ether linkages	-	XXV, XXVI	Insularine **41**

Note. Adapted from "The Systematic Classification of Bisbenzylisoquinoline," by M. Shamma and J. L. Moniot, 1976, *Heterocycles*, 4, p. 1817-1824.



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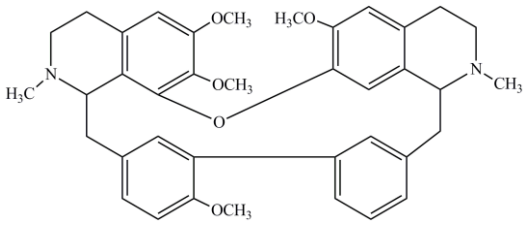

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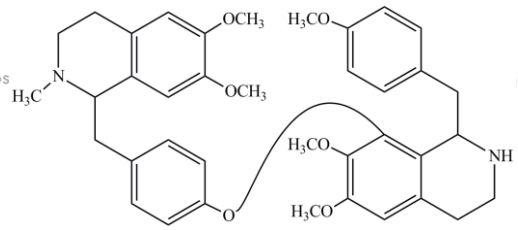

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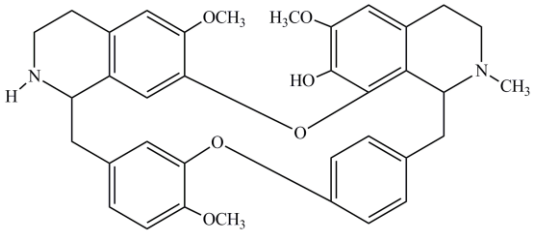

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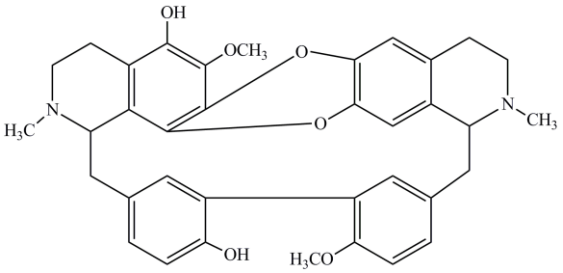

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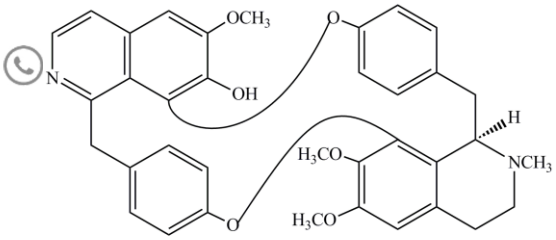

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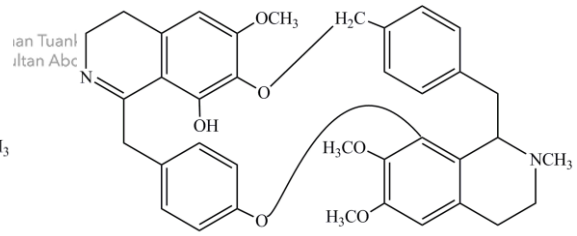

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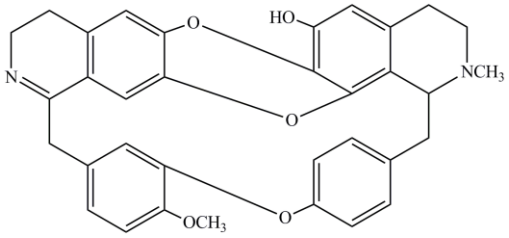

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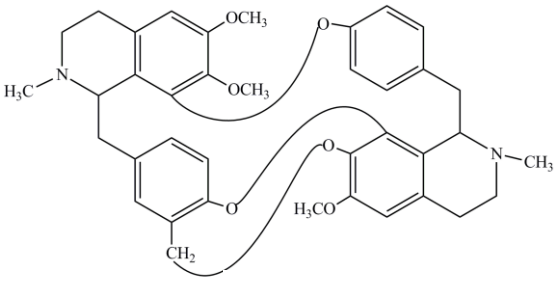

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2.5.1 UV and IR Spectra of Bisbenzylisoquinoline

Bisbenzylisoquinoline have UV spectra with an absorption maximum at approximately 283 nm and a minimum at 260 nm. Strong absorption is also observed in the 225 nm region. IR spectra of bisbenzylisoquinoline depends on the structure, particularly when they contain characteristic functional groups such as carbonyl, hydroxyl and imino (Tolkachev et al., 1977).

2.5.2 NMR Spectra of Bisbenzylisoquinoline

Bisbenzylisoquinoline show difference characteristics spectral behaviour depends on the quantity or connection of ether bridge and substituents present in the rings. The substituents on the aromatic rings may be hydroxyl or methoxyl or methylenedioxy groups (Nelofar, 1989). Some examples of generalization on the basis of their spectral characteristics as follows:

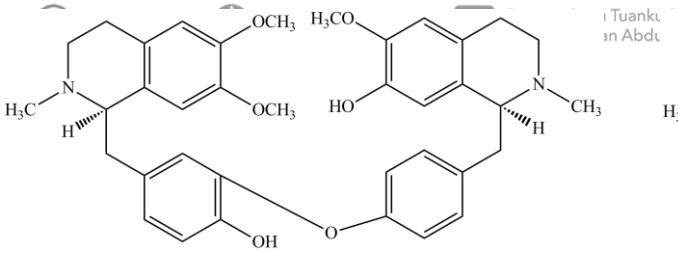
a) Tail to tail bisbenzylisoquinoline with one diaryl ether bridge (11-12´)

The ^{1}H NMR spectrum of this compounds such as temuconine **42** and 7´-*O*-methylcuspidaline **43** show the presence of eleven aromatic protons, four of which appear as singlets belongs to H-5, H-8, H-5´ and H-8´ (Guinaudeau, Cassels & Shamma, 1982; El-Sebakhy & Waterman, 1984). Furthermore, H-10´, H-11´, H-13´ and H-14´ appear as doublets each of which represents two protons with J = ~8 Hz like thaligrisine **44** (Guinaudeau, Freyer, Shamma & Baser, 1984). The *N*-methyl

groups usually appear near δ 2.5 with the most upfield signal assignable to *N´*-methyl group such as guattegaumerine **45** and isodaurisoline **46** (Dehaussy, Tits & Angenot, 1984; Jossang, Leboeuf, Cabalion & Cavé, 1984). The signal for H-8 appear at upfield region between δ 5.95-6.35. The actual chemical shift for this proton depends upon the substituent at C-7 either hydroxyl or methoxyl groups.

b) Tail to tail bisbenzylisoquinoline with two diaryl ether bridges (7-8´, 11-12´)

The peaks for the two *N*-methyl groups appear close to each other or even superimposed near δ 2.55 with the more upfield signal belongs to *N´*-methyl group. H-10 generally appears upfield as a broad singlet in range of δ 5.55-6.66. The signal for H-8 appears in the range of δ 6.45-6.50. Signal for H-1 is usually close to δ 3.55 while that for H-1´ is near δ 4.20 just like gyrolidine **47** and 2´-noroxyacanthine **48** (Chalandre, Bruneton, Cabalion, & Guinaudeau, 1986; Herath, Hussain, Freyer, Guinaudeau, & Shamma, 1987). The absorption for aromatic protons H-10´, H-11´, H-13´ and H-14´ is spread out over a range of more than 1 ppm. Among these four protons, H-14´ appear at the farthest downfield near δ 7.40 and above such as in gyrocarpine **49** and gyrocarpusine **50** (Chalandre et al., 1986).



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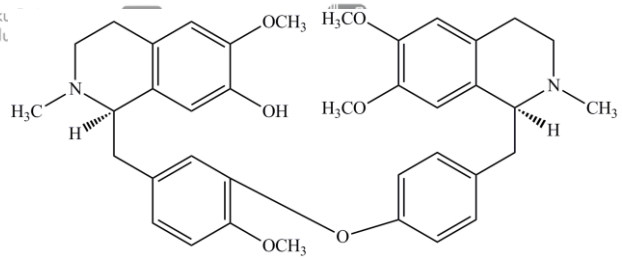

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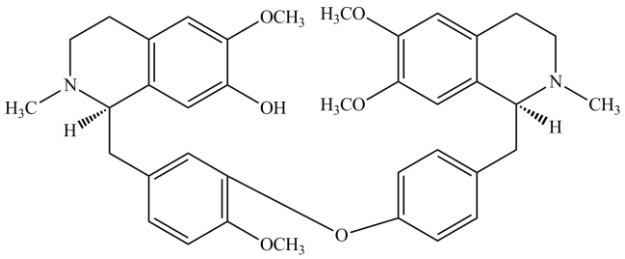

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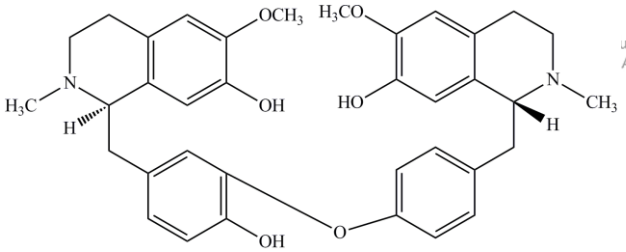

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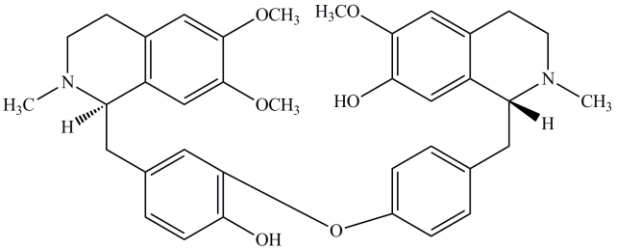

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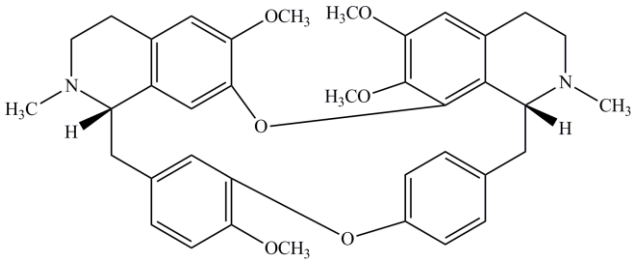

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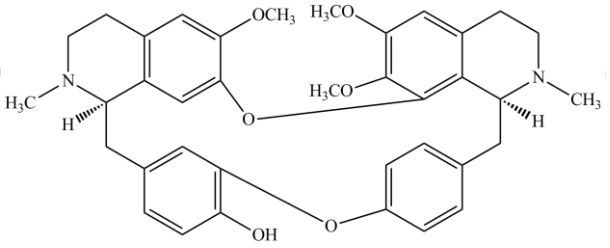

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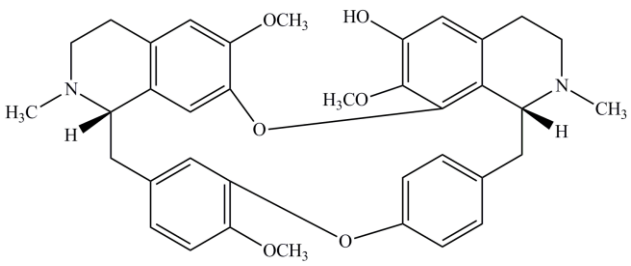

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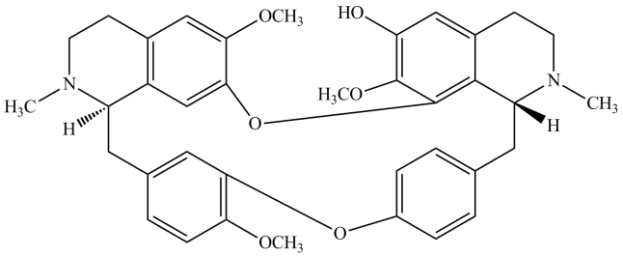

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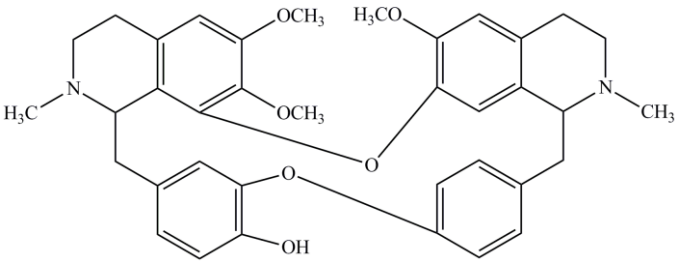
2.5.3 Mass Spectra of Bisbenzylisoquinoline

Mass spectrum provides extremely useful information for the structure elucidation of bisbenzylisoquinoline. The spectra are most conveniently discussed in terms of groups of alkaloids with similar skeletal (Nelofar, 1989). So far, the major type of bisbenzylisoquinoline alkaloid from Lauraceae family is similar to berbamine **51** type which tail to tail bisbenzylisoquinoline with two diaryl ether bridges such as gyrocarpine **49** and dehatridine **52** (Chalandre et al., 1986; Lu, Tsai, & Leou, 1989). The favoured fission is always at the doubly benzylic positions at C-1 and C-1´. The mass spectra of this bensylisoquinoline can be summarized as shown in Scheme 2.2 (Tomita et al., 1966).

A typical member of berbamine **51** group is isotetrandine **53**. Scheme 2.2 shows the characteristic fragments of berbamine **51** type alkaloids. Isotetrandine **53** (M^+ 622) reveals a characteristic doubly charged ion at m/z 198 (**54**), which then eliminates a methoxyl and a methyl radical to give an ion at m/z 175 (**55**). A peak at m/z 396, represented by **56**, is probably a key intermediate ion for further fragmentation. Loss of a hydrogen from **56** gives an ion at m/z 395 (**57**) and in turn fragment **57** affords a fragment ion at m/z 364 (**58**) and at m/z 349 (**59**) by loss of methoxyl and successive loss of methyl radicals, respectively. Loss of a methyl radical from **56** gives an ion at m/z 381 (**60**) (Tomita et al., 1966).



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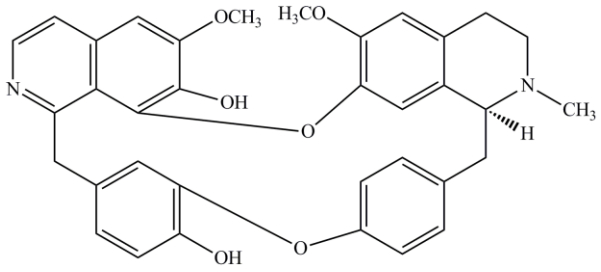

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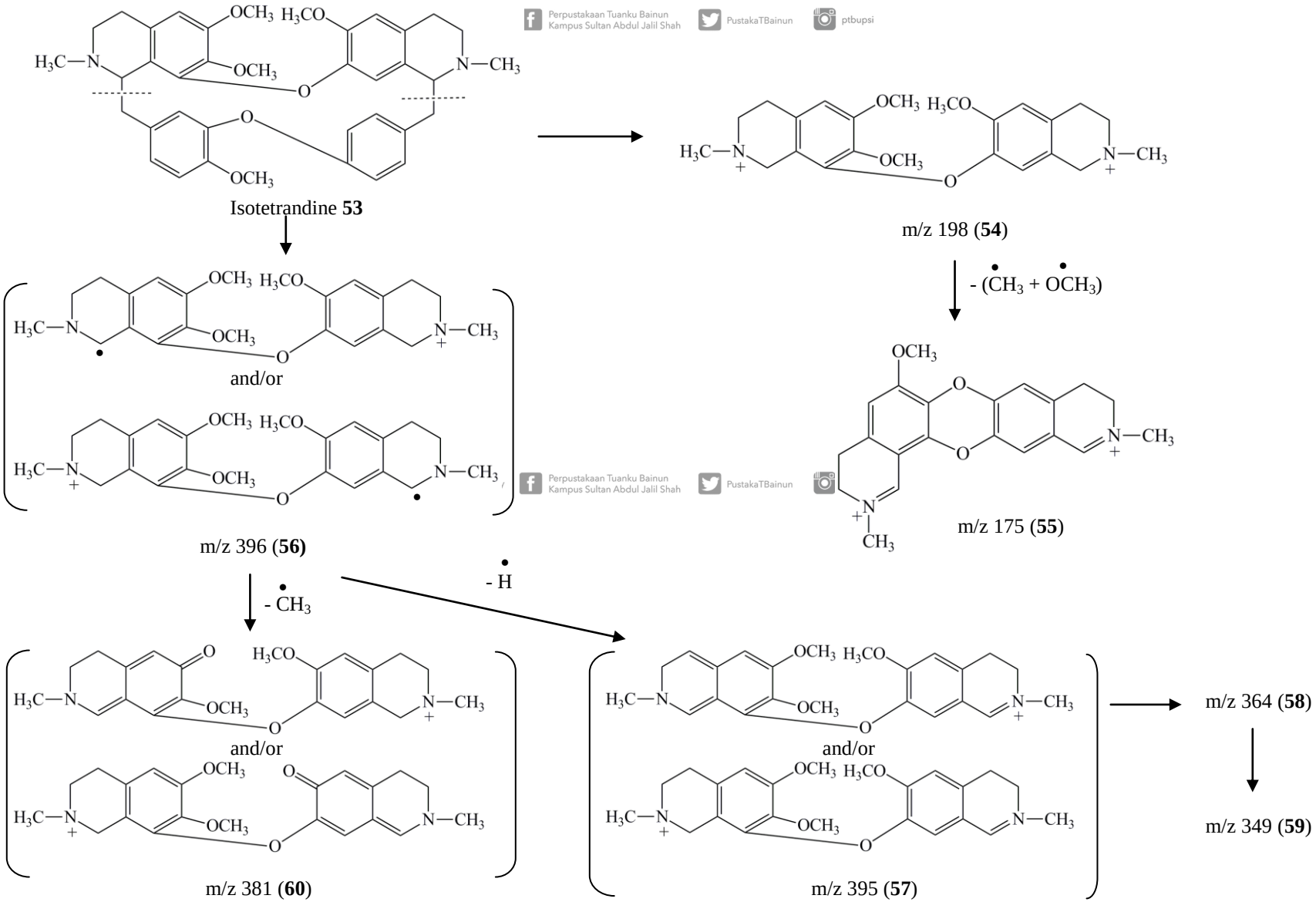
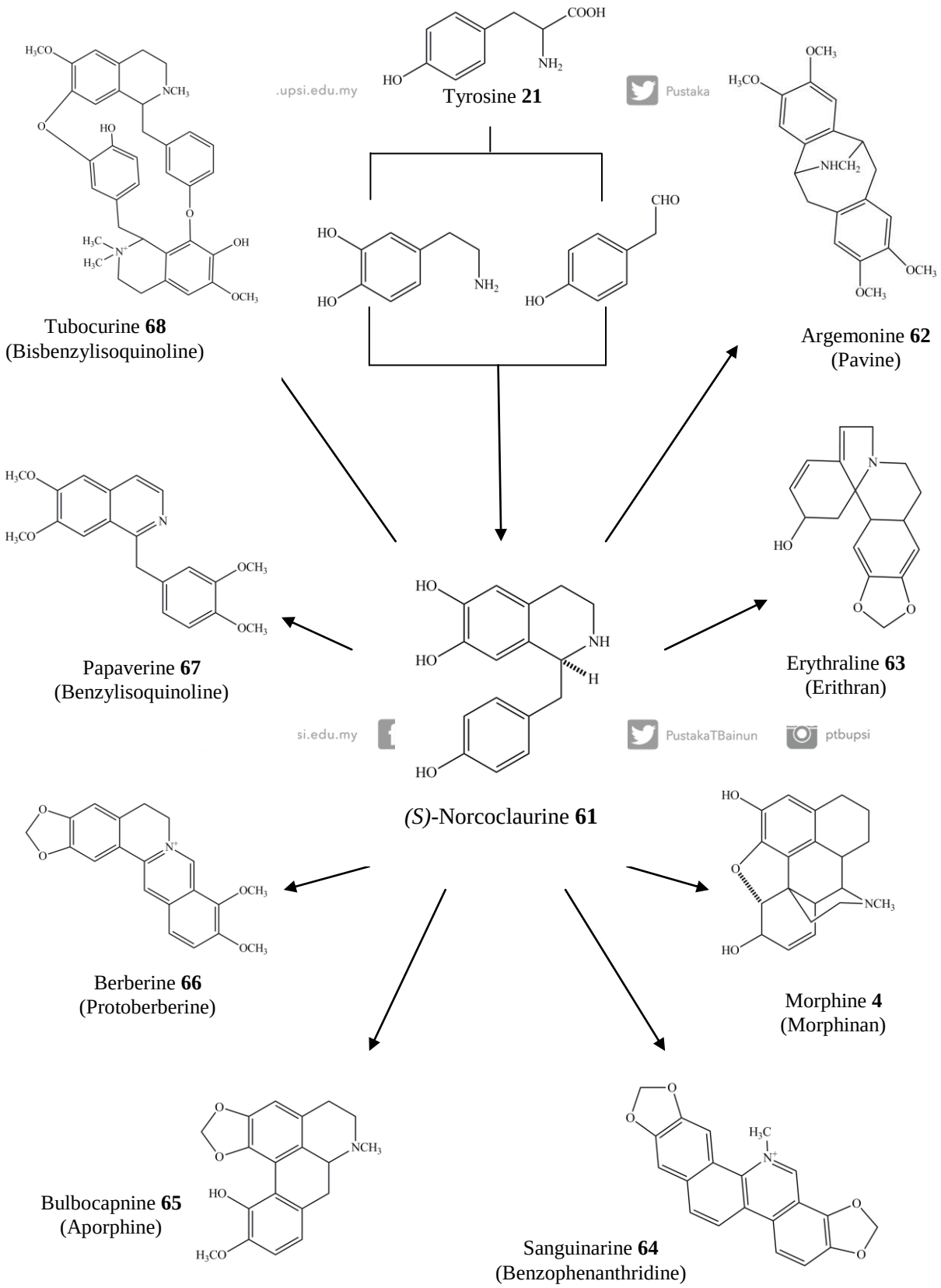


Scheme 2.2. Characteristic fragments of berbamine **51** type alkaloids. Adapted from "Mass Spectrometry of Bisbenzylisoquinoline Alkaloids by M. Tomita, T. Kikuchi, K. Fujitani, A. Kato, H. Furukawa, Y. Aoyagi, M. Kitano and T. Ibuka, 1966, *Tetrahedron Letters, 8*, p. 857-864.

2.5.4 Biosynthesis of Benzylisoquinoline

Benzylisoquinoline are formed from two molecules of tyrosine which are elaborated to form *(S)*-norcoclaurine **61** (Rueffer & Zenk, 1987). This alkaloid is an important precursor of a variety of pathways that lead to a series of diverse structures, some of which are shown in Scheme 2.3. Excellent progress has therefore been made in unravelling the route to *(S)*-norcoclaurine **61** and the sequences leading to some of the more important groups of isoquinolines.



Scheme 2.3. The various groups of benzylisoquinoline alkaloids derived from *(S)*-norcoclaurine **61**. Adapted from "Distant Precursors of Benzylisoquinoline Alkaloids and their Anzymatic Formation," M. Rueffer and M. H. Zenk, 1987, *Z. Naturforsh, 42c*, p. 319-332.

2.6 Alkaloids of Lauraceae Family

Aporphine-type alkaloids, a class of secondary metabolites widely occurring in members of the Lauraceae family (Cseke et al., 2006). Furthermore, plants belonging to Lauraceae also known to produce various bisbenzylisoquinoline with pharmacological activities. A large number of bisbenzylisoquinoline have been isolated from various plant resources and the medicinal properties such as antimalarial, antihypotensive and antitumor. Table 2.4 shows the variety of alkaloids isolated from Lauraceae family.

Table 2.4

Alkaloids of Lauraceae Family

Lauraceae	Part of tree	Isolated alkaloids
Litsea machilifolia (Medang Katuko)	leaf	boldine **69**, actinodaphine **70**, *N*-methylactinodaphine **71** and cryptodorine **72** (Abidin, Awang, Hadi & Mukhtar, 2009).
Litsea petiolata Hk.f	bark	harman **73**, reticuline **74** and thalifoline **75** (Omar, Nafiah, Mukhtar, Awang & Hadi, 2009).
Actinodaphne Pruinosa Nees	bark	(-)-dauricine **76** and (+)-thaligrisine **77** (Rachmatiah et al., 2009b).
Phoebe grandis (Nees) Merr.	bark	boldine **69**, norboldine **78**, laurotetanine **79** and lindcarpine **80** (Mukhtar, 1996)
Phoebe tavoyana (Meissn.) Hk.f.	bark	laetanine **30**, boldine **69**, norboldine **78**, roemerine **81**, (+)-tavoyanine **82**, sebiferine **83** (Omar, 2009)
Lindera pipericarpa Boerl	bark	*N*-methyllaurotetanine **84** and isocorydine **85** (Lajis, Sharif, Kiew, Khan & Samadi, 1992)

2.7 Alkaloids of Genus *Alseodaphne*

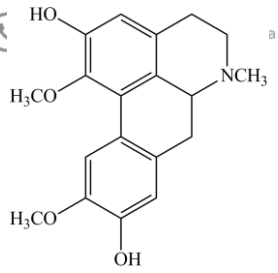
Alseodaphne species also had been reported containing various types of aporphine and bisbenzylisoquinoline alkaloids as shown in Table 2.5.

Table 2.5

Alkaloids of Genus Alseodaphne

Alseodaphne species	Part of tree	Isolated alkaloids
Alseodaphne corneri	leaves and bark	gyrolidine **47**, *N*- methyllaurotetanine **84**, isocorydine **85**, norisocorydine **86**, stephasubimine **87** (Zahari, 2010)
Alseodaphne corneri	bark	3´, 4´-dihydronorstephasubine **88** (Mukhtar et al., 2009)
Alseodaphne perakensis	roots	boldine **69**, sebiferine **83** and laurolitsine **89** (Ahmat, 2008)
Alseodaphne perakensis	bark	norboldine **78**, *N*-methyllaurotetanine **84** and *N*-cyanomethylnorboldine **90** (Nafiah et al., 2011)
Alseodaphne archboldiana	*	reticuline **74** and (-)-*N*-norarmepavine **91** (Johns, Lamberton & Sioumis, 1967)
Alseodaphne hainanensis	bark	(6,7-dimethoxyisoquinolinyl)-(4′-methoxyphenyl) methanone **92** (Haitao, Lian & Pengfei, 2000)
Alseodaphne hainanensis	roots	xylopinine **93** and armepavine **94** (Zhang, Liu, Li & Mia, 1988)

Note. * = not available.



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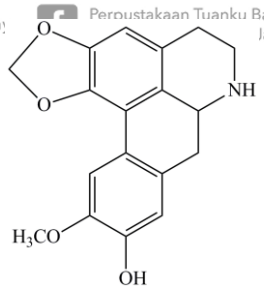

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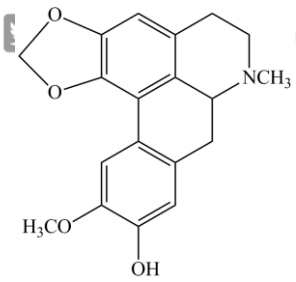

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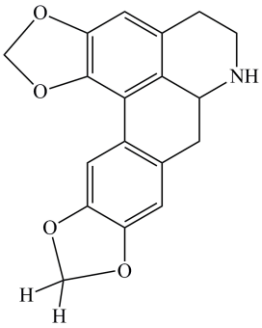

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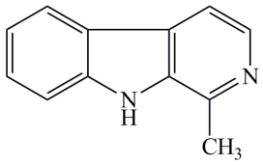

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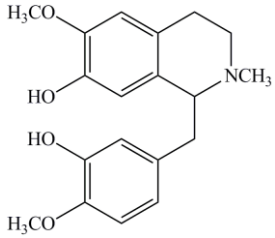

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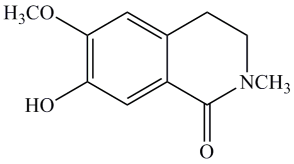

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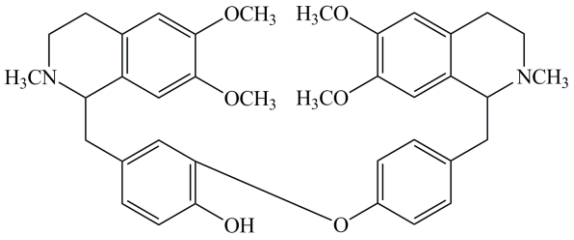

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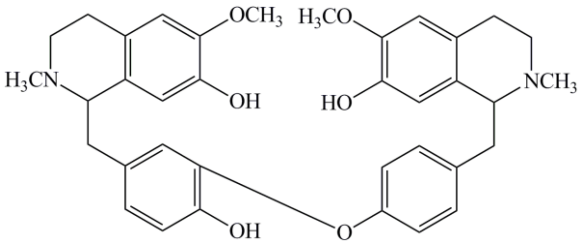

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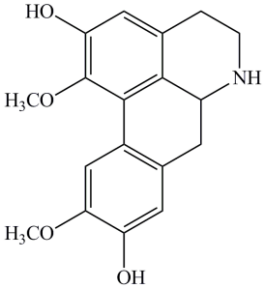

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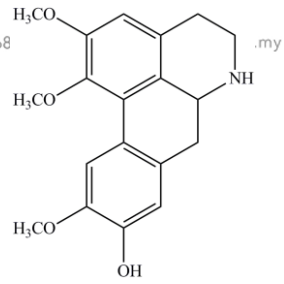

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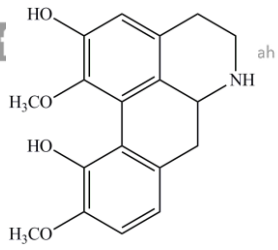

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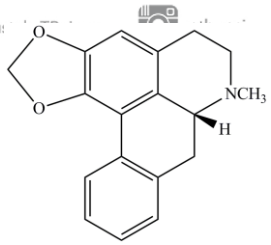

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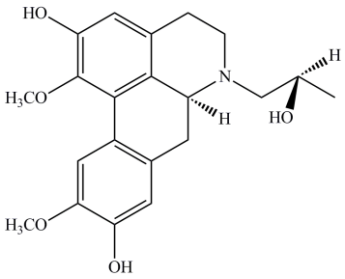

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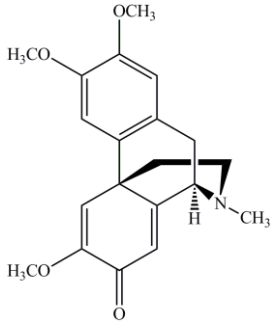

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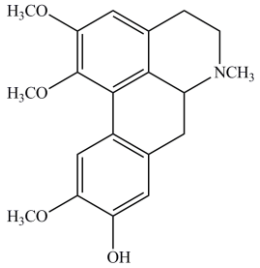

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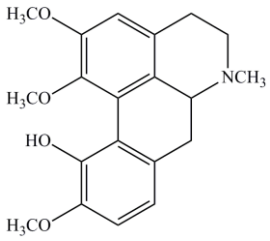

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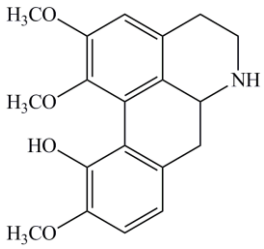

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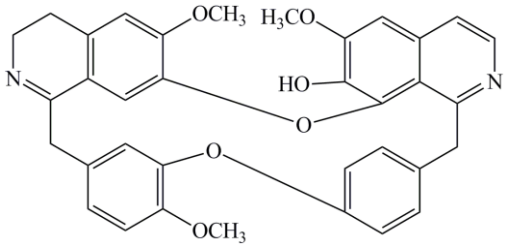

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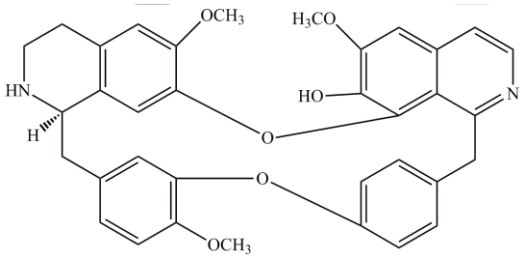

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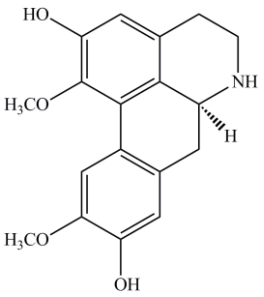

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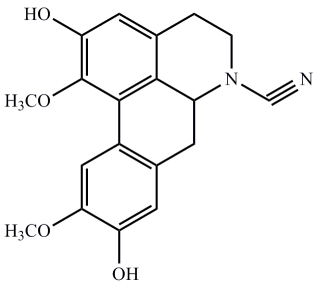

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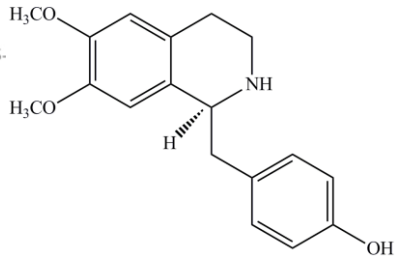

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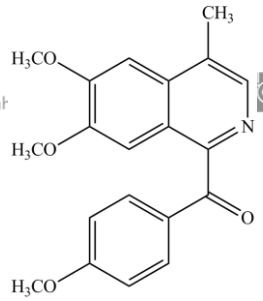

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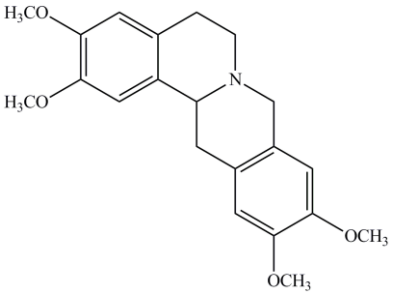

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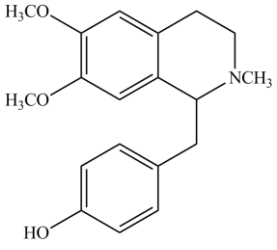

94

CHAPTER 3

EXPERIMENTAL

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3.1 Plant Material

The bark of *Alseodaphne peduncularis* (Wall. ex Nees) Meisn (KL 5165) was collected from Kluang-Mersing, Johor. The roots of *Alseodaphne corneri* Kosterm (KL 4928) was collected from University of Malaya, Malaysia. Both species were identified by Herbarium Group of Chemistry Department, University of Malaya, Malaysia.

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3.2 Instrumentation

The 1D (^{1}H, ^{13}C and DEPT) and 2D NMR (COSY, NOESY, HMQC and HMBC) spectra were obtained using JEOL ECX 500 MHz spectrometer system using deuterated chloroform as solvent. The chemical shift were reported in ppm or δ scale and the coupling constants are given in the Hz unit.

Mass spectrum was obtained on JEOL JMS 700 TZ spectrometer. The EIMS spectrum was obtained on Shimadzu GC-MS-QP2000A Mass Spectrometer 70 eV.

UV spectra was obtained by using Perkin Elmer UV-Visible spectrophotometer with methanol as solvent.

The infrared spectra was obtained on Nicolet 6700 FTIR spectrophotometer with chloroform as solvent.

3.3 Chromatography

3.3.1 Thin Layer Chromatography (TLC)

Aluminium supported silica gel 60 F_{254} (Merck 1.05554.0001) plates were used to monitor the spots on the TLC. The TLC spots were visualized under ultraviolet lights of 254 and 365 nm.

3.3.2 Column Chromatography (CC)

The solvents used in this experiment were hexane, dichloromethane and methanol. Silica gel 60, 70-230 mesh ASTM (Merck 1.07734.1000) and silica gel 60, 200-400 mesh ASTM (Merck 1.09385.1000) were used for column chromatography. A slurry of silica gel 60 in hexane solvent was poured into a glass column of appropriate size. The crude extract was initially dissolved in minimum amount of solvent and loaded on top of the packed column. The extract was eluted with gradient solvent system at a certain flow rate.

3.3.3 Preparative Thin Layer Chromatography (PTLC)

PTLC glass plates of size 20x20 cm containing silica gel 60 F_{254} with gypsum (Merck 1.07749.1000) was used for separation of compounds that cannot be separated by conventional column. UV Light Model UVGL-58 was used to examine bands on the PTLC.

3.4 Reagents

3.4.1 Mayer's Reagent (Potassium mercuric iodide)

About 1.4 g mercuric iodide in 60 ml distilled water was mixed with solution of 5.0 g potassium iodide in 10 ml distilled water. The mixture then made up to 100 ml

solution. The positive result was indicated by the formation of white precipitate when the aqueous layer (acidified) is treated with 2 to 3 drops of Mayer's reagent.

3.4.2. Dragendorff's Reagent (potassium bismuth iodide)

Bismuth (III) nitrates (0.85 g) are dissolved in a mixture of glacial acetic acid (10 ml) and distilled water (40 ml) for solution A. While for solution B; potassium iodides (8.0 g) are dissolved in distilled water (20 ml). To prepare the stock solution, solution A (20 ml) and B (20 ml) mixed with equal volumes (1:1). The stock solution (20 ml) then was diluted in the mixture acetic acid (20) ml and distilled water (60 ml) for spray agent. The formation of orange spots on the thin layer chromatography indicated the presence of alkaloids.

3.5 Extraction

Extraction was carried out by cold percolation to remove non-polar organic compounds, waxes and fats. The dried bark were soaked with hexane for three days at room temperature. The extract was then filtered and dried using rotary evaporator. The plant material was dried and wetted with 27% ammonia solution (NH_4OH) and left overnight to aggregate the nitrogen-containing compounds from the plant.

It was then re-extracted with dichloromethane (CH_2Cl_2) by exhaustively using soxhlet extractor. The dichloromethane extract was evaporated to 500 ml followed by

acid-base extraction using 5% HCl until Mayer's test is negative. The HCl extract was basified with concentrated ammonia to pH 11 and re-extracted with dichloromethane. The dichloromethane extract was washed with distilled water and dried over anhydrous sodium sulphate (Na_2SO_4). Finally the extract was evaporated to dryness to give alkaloid crude extract. The extraction process of the samples are shown in Scheme 3.1.

3.6 Isolation

The alkaloid crude extracts was subjected to column chromatography over silica gel (70-230 mesh ASTM, Merck 7734). The column was eluted with solvent mixtures of increasing polarity (CH_2Cl_2, CH_2Cl_2/MeOH and MeOH) to give several fractions. Fractions that having spots with the same R_f value were grouped together by TLC. Each series of fractions was then treated separately by extensive column chromatography and preparative TLC to purify the alkaloids.

Structural identification of the isolated compounds were carried out by using spectroscopic methods such as ^{1}H NMR, ^{13}C NMR, COSY, DEPT, HMQC, HMBC, IR, UV and mass spectroscopy.

3.6.1 Isolation of *Alseodaphne peduncularis*

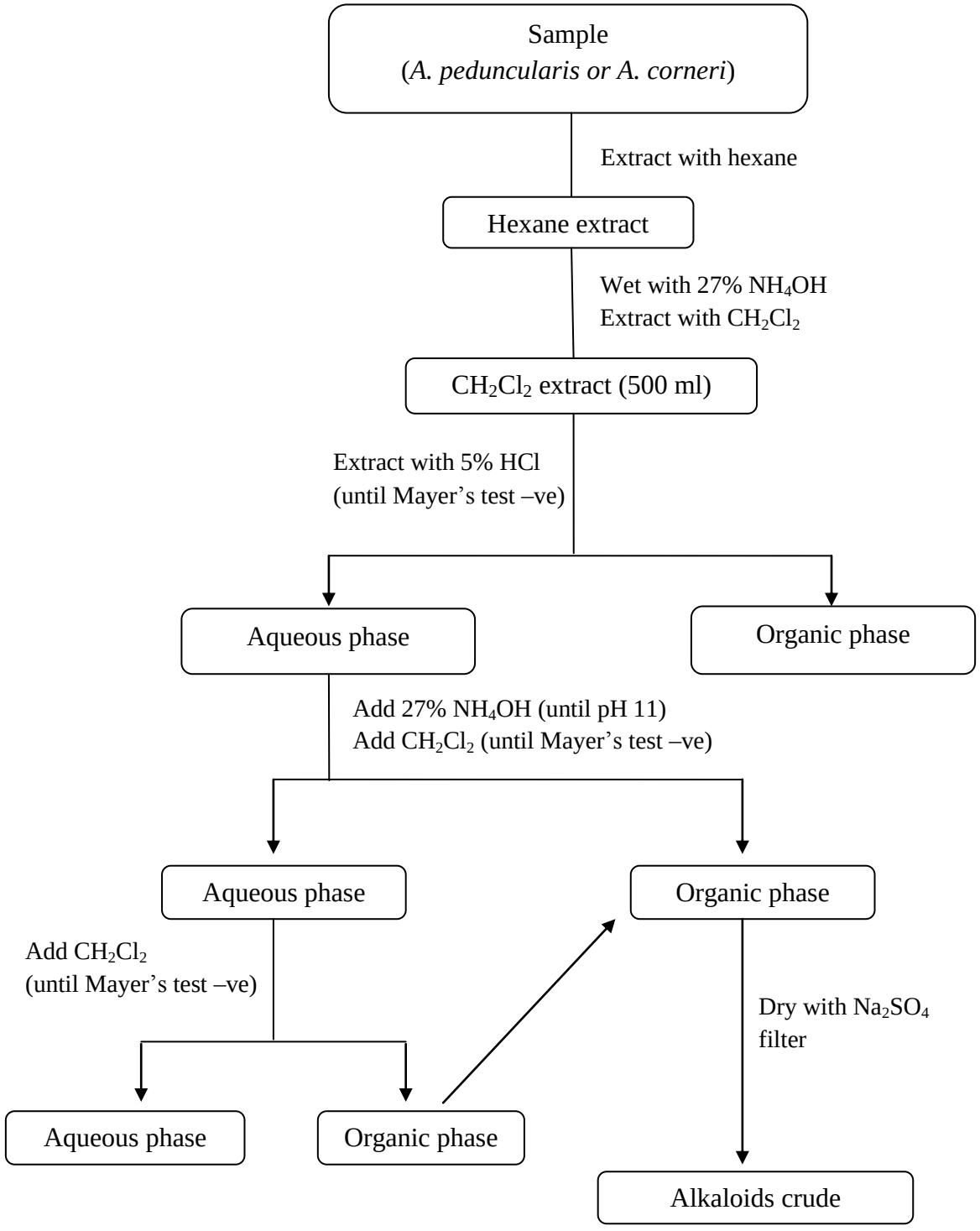
The alkaloid extract of *Alseodaphne peduncularis* (8.28 g) was subjected to column chromatography over silica gel using hexane, dichloromethane and methanol solvents.

The fractions collected from the column chromatography that having same R_f value were grouped into series of fractions. Two potential fractions that contain alkaloid; fractions 169-225 and 226-242 were subjected to another extensive column chromatography.

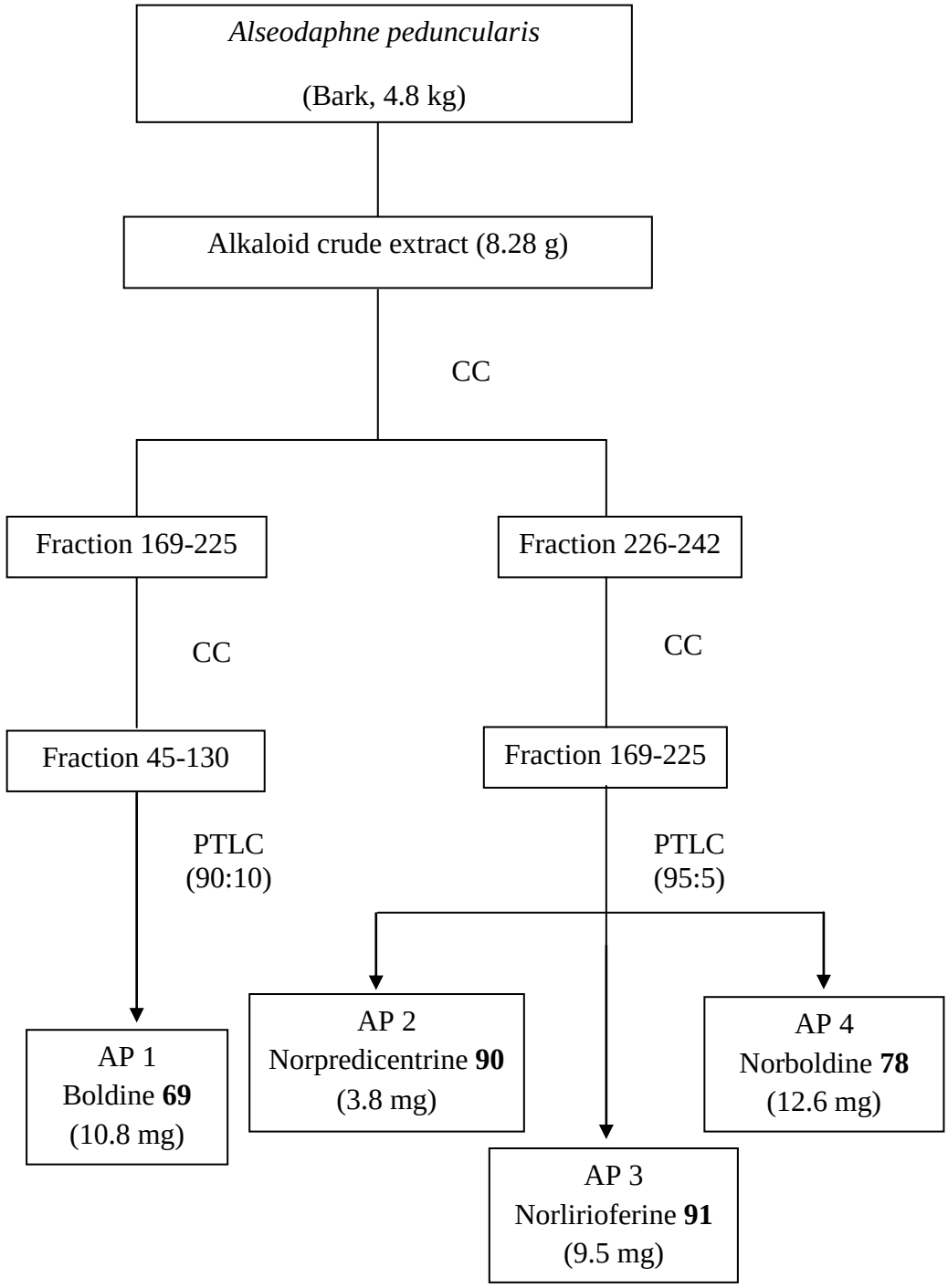
Then, the purification of alkaloids continue with preparative TLC with suitable solvent system of CH_2Cl_2:MeOH. Finally, AP 1 (boldine **69)**, AP 2 (norpredicentrine **90**), AP 3 (norlirioferine **91**) and AP 4 (norboldine **78**) were successfully isolated from *A. peduncularis*. Scheme 3.2 shows the isolation process of *A. peduncularis*.

3.6.2 Isolation of *Alseodaphne corneri*

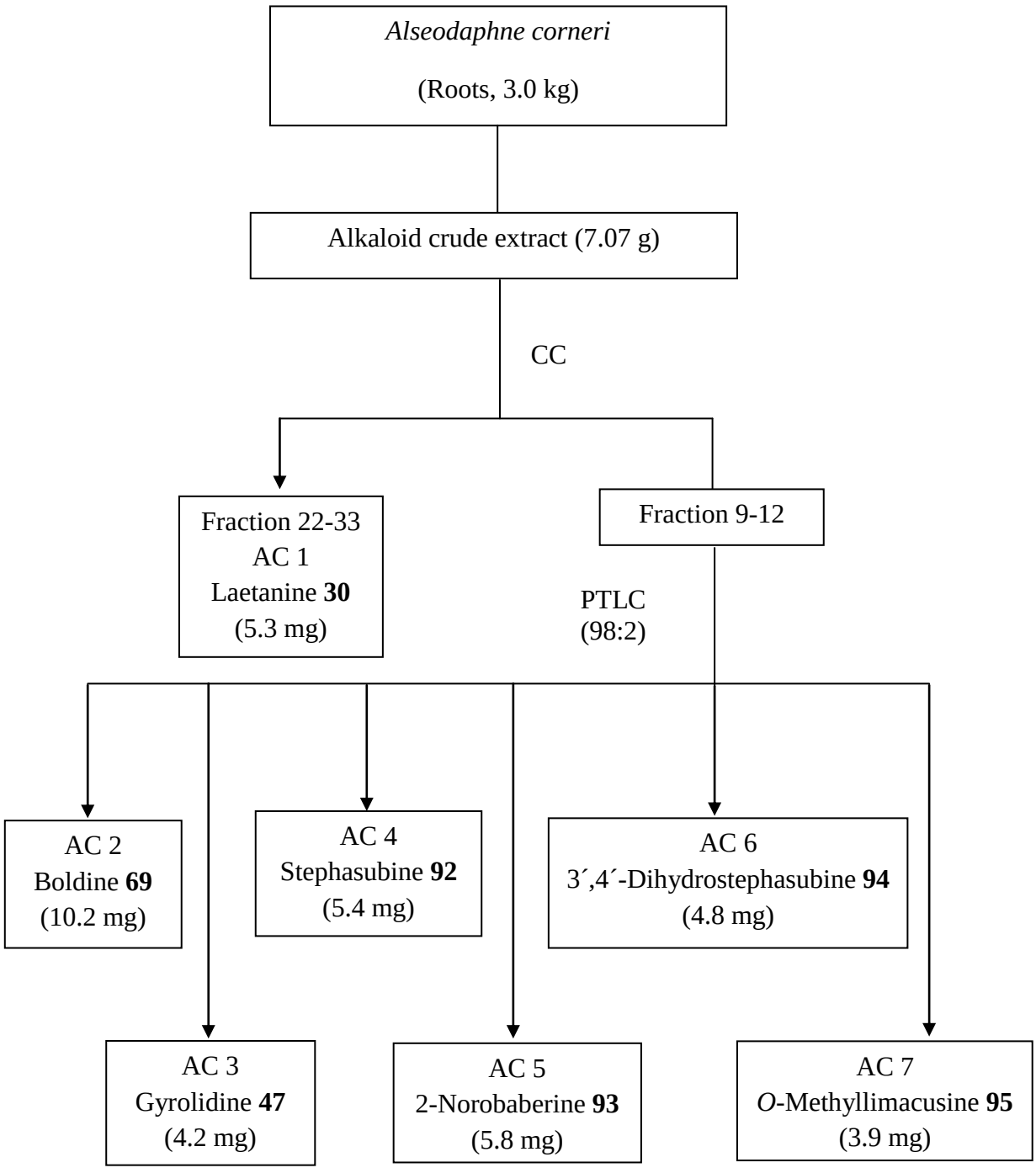
Scheme 3.3 shows the isolation process of alkaloids isolated from *A. corneri*. 7.07 g of the crude was subjected to column chromatography with various solvent system of hexane, dichloromethane and methanol. Series of fractions were collected. AC 1 (laetanine **30**) was successfully isolated from fraction 22-33. Then, fraction 9-12 from the column chromatography was undergoing the preparative TLC process with solvent system of 98:2 of CH_2Cl_2:MeOH. Finally, another six alkaloids were isolated known as AC 2 (boldine **69**), AC 3 (gyrolidine **47**), AC 4 (stephasubine **92**), AC 5 (2-norobaberine **93**), AC 6 (3´,4´-dihydrostephasubine **94**) and AC 7 (*O*-methyllimacusine **95**).



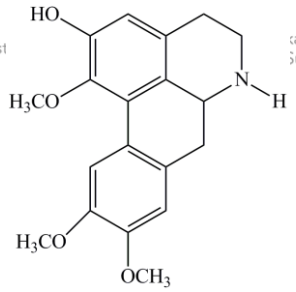
Scheme 3.1. Extraction process of samples.



Scheme 3.2. Isolation of alkaloids from the bark of *Alseodaphne peduncularis*.



Scheme 3.3. Isolation of alkaloids from the roots of *Alseodaphne corneri*.



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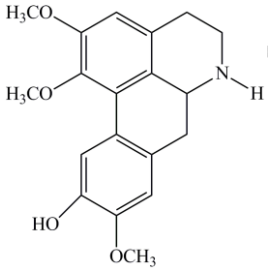

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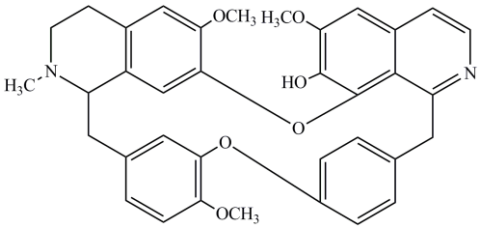

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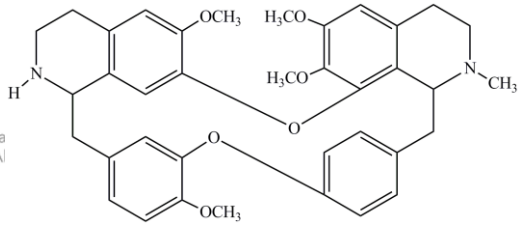

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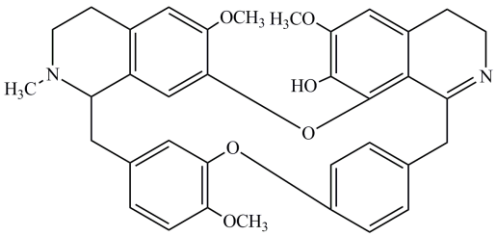

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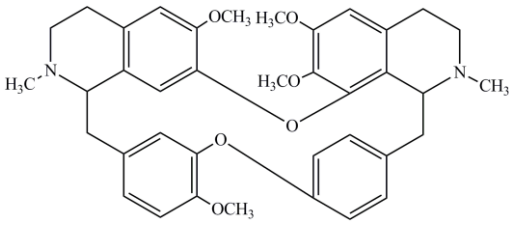

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3.7 Antiplasmodial Assay

For bioactivity assay, the samples were sent to Unit Bioassay, Herbal Medicine Research Center Institute for Medical Research, Kuala Lumpur. The samples were tested for antimalaria *in-vitro* drug screening by using HRP2 assay.

The antimalarial drug screening test was done by using HRP2 (histidine-rich protein II) in a simple enzyme-linked immunosorbent assay (ELISA) that first published in 2002 and was developed to diagnose *falciparum* malaria rapidly and reliably without the need of a microscope (Noedl, Wernsdorfer, Miller & Wongsrichanalai, 2002). The screening test is on the measurement of a histidine- and analine-rich protein produce by *Plasmodium falciparum* in the course of its growth and multiplication (Noedl, Wongsrichanalai & Wernsdorfer, 2003). If parasite growth

is inhibited by antimalarial drugs, the inhibition is reflected in HRP2 levels and may therefore easily be quantified. This assay also faster than other malaria *in-vitro* sensitivity test, easy and rapid to perform (Noedl et al., 2002).

The antiplasmodial activity of the extracts was determined against the chloroquine-resistant (K1) strains of *Plasmodium falciparum* that were continuously cultured according to the methods described by Trager and Jensen (1976). Plant extracts were assessed for antiplasmodial activity *in-vitro* in human blood using parasite lactate dehydrogenase method (pLDH) with slight modifications (Makler & Hinrichs, 1993; Makler, Ries, Williams & Bancroft, 1993).

Microtitration techniques were used to measure the activity of samples over a

wide range of concentrations. A solution of chloroquine diphosphate and artemisinin

served as positive control in all experiments. All tests were performed in duplicate. Crude extract was dissolved in DMSO to produce a stock solution of 20 mg/ml. The stock solutions were subsequently diluted with deionized water at 20 concentrations of two-fold dilutions into two 96-well microtiter plates. 10µl of each concentration was transferred into another 96-well microtiter plates. 190µl of parasitised red blood cell suspensions (1% parasitaemia) were next added to each well.

For the infected control, parasitised red blood cells were devoid of plant extracts whereas only non-parasitised red blood cells were prepared for the non-infected control plates were incubated for 24 hours at 37°C in a candle jar and were subsequently cooled at −20°C to lyse the red blood cells. The plates were next allowed to reach room temperature, and 20µl of the blood suspension was dispensed into a new microtiter plate containing 100µL MALSTAT™ reagent and 20µl nitroblue tetrazolium and phenazine ethosulfate mixture. Absorbance was measured with an ELISA plate reader at 630 nm. The percentage inhibition at each concentration was determined and the mean of IC_{50} values of parasite viability was calculated using probit analysis (Chan, Choo, Abdullah & Ismail, 2004).

3.8 Physical and Spectral Data of Isolated Alkaloids

AP 1, Boldine **69** : $C_{19}H_{21}NO_4$ (Brownish amorphous)

UV λ max : 282, 302

IR ν max cm^{-1} : 2945

Mass spectrum m/z : 327 $[M]^+$, 326 [M-1]$^+$

^{1}H NMR ($CDCl_3$), δ : 2.58 (3H, *s*, *N*-CH_3), 2.60 (1H, *d*, *J*= 5.8 Hz, H_{ax}-5), 2.64 (1H, *br s*, H_{ax}-7), 2.67 (1H, *m*, H_{ax}-4), 2.97 (1H, *dd*, *J*=13.8 Hz and 4.0, H_{eq}-7), 3.01 (1H, *m*, H-6a), 3.12 (1H, *br s*, H_{eq}-5), 3.16 (1H, *br s*, H_{eq}-4), 3.59 (3H, *s*, 1-OCH_3), 3.91 (3H, *s*, 10-OCH_3), 6.64 (1H, s, H-3), 6.82 (1H, *s*, H-8), 7.89 (1H, *s*, H-11).

^{13}C NMR ($CDCl_3$), δ : 142.2 (C-1), 60.4 (1-OCH_3), 126.1 (C-1a), 126.1 (C-1b), 148.3 (C-2), 113.3 (C-3), 129.5(C-3a), 28.5 (C-4), 53.3 (C-5), 62.5 (C-6a), 33.9 (C-7), 129.8 (C-7a), 114.3 (C-8), 145.2 (C-9), 145.7 (C-10), 56.2 (10-OCH_3), 110.2 (C-11), 123.6 (C-11a), 43.5 (*N*-CH_3).

AP 2, Norpredicentrine **90** : $C_{19}H_{21}NO_4$ (brownish amorphous)

UV λ max : 217, 281, 301

IR ν max cm^{-1} : 3000-3500 (broad)

Mass spectrum m/z : 327 $[M]^+$

^{1}H NMR ($CDCl_3$), δ : 3.09 (1H, *m*, H_{ax}-5), 2.93 (2H, *m*, H-7), 2.75 (1H, *d*, *J*=14.9 Hz, H_{ax}-4), 3.07 (1H, *m*, H_{eq}-4), 3.96 (1H, *br s*, H-6a), 3.49 (1H, *s*, H_{eq}-5), 3.61 (3H, *s*, 1-OCH_3), 3.91 (6H, *s*, 9,10-OCH_3), 6.68 (1H, s, H-3), 6.78 (1H, *s*, H-8), 7.94 (1H, *s*, H-11).

^{13}C NMR ($CDCl_3$), δ : 142.4 (C-1), 60.5 (1-OCH_3), 125.4 (C-1a), 129.1 (C-1b), 148.8 (C-2), 113.8 (C-3), 125.7(C-3a), 27.8 (C-4), 42.7 (C-5), 53.6 (C-6a), 35.7 (C-7), 128.3 (C-7a), 111.1 (C-8), 148.5 (C-9), 148.0 (C-10), 56.1 (10-OCH_3), 56.0 (9-OCH_3), 110.7 (C-11), 123.9 (C-11a).

AP 3, Norlirioferine **91** : $C_{19}H_{21}NO_4$ (dark brown amorphous)

UV λ max : 220, 280, 302

IR ν max cm^{-1} : 1642, 3453

Mass spectrum m/z : 327 $[M]^+$, 326 $[M-1]^+$

^{1}H NMR ($CDCl_3$), δ : 3.05 (1H, *m*, H_{ax}-5), 2.79 (2H, *m*, H-7), 2.73 (1H, *m*, H_{ax}-4), 3.05 (1H, *m*, H_{eq}-4), 3.85 (1H, *br s*, H-6a), 3.43 (1H, *d*, *J*=8.6 Hz, H_{eq}-5), 3.66 (3H, *s*, 1-OCH_3), 3.88 (6H, *s*, 2,10-OCH_3), 6.59 (1H, s, H-3), 6.78 (1H, *s*, H-8), 8.07 (1H, *s*, H-11).

^{13}C NMR ($CDCl_3$), δ : 144.4 (C-1), 60.3 (1-OCH_3), 126.9 (C-1a), 127.2 (C-1b), 152.3 (C-2), 110.8 (C-3), 128.7 (C-3a), 28.8 (C-4), 42.9 (C-5), 53.7 (C-6a), 36.2 (C-7), 129.5 (C-7a), 114.1 (C-8), 145.5 (C-9), 145.1 (C-10), 56.1 (10-OCH_3), 55.9 (2-OCH_3), 111.4 (C-11), 129.5 (C-11a).

AP 4, Norboldine **78** : $C_{18}H_{19}NO_4$ (brownish amorphous)

UV λ max : 216, 282, 302

IR ν max cm^{-1} : 3300

Mass spectrum m/z : 313 $[M]^+$, 312 $[M-1]^+$, 298 $[M-CH_3]^+$

^{1}H NMR ($CDCl_3$), δ : 3.00 (1H, *dd*,*J*=12.3 and 3.5 Hz, H_{ax}-5), 2.65 (1H, *m*, H_{ax}-7), 2.72 (1H, *dd*, *J*=14.3 and 4.6 Hz, H_{eq}-7), 2.96 (1H, *m*, H_{ax}-4), 2.67 (1H, *m*, H_{eq}-4), 3.77 (1H, *dd*, *J*= 13.8 and 4.6 Hz, H-6a), 3.33 (1H, *m*, H_{eq}-5), 3.61 (3H, *s*, 1-OCH_3), 3.91 (3H, *s*,10-OCH_3), 6.65 (1H, *s*, H-3), 6.81 (1H, *s*, H-8), 7.91 (1H, *s*, H-11).

^{13}C NMR ($CDCl_3$), δ : 141.9 (C-1), 60.4 (1-OCH_3), 125.6 (C-1a), 128.1 (C-1b), 148.1 (C-2), 113.7 (C-3), 130.2 (C-3a), 29.1 (C-4), 43.3 (C-5), 53.8 (C-6a), 36.8

(C-7), 130.1 (C-7a),114.2 (C-8), 145.1 (C-9), 145.6 (C-10), 56.2 (10-OCH_3), 110.2 (C-11), 123.8 (C-11a).

AC 1, Laetanine **30** : $C_{18}H_{19}NO_4$ (dark brownish amorphous)

UV λ max : 282

IR ν max cm^{-1} : 3000-3500 (broad)

Mass spectrum m/z : 313 $[M]^+$

^1H NMR ($CDCl_3$), δ : 2.99 (1H, *s*, H_{ax}-5), 2.68 (1H, *m*, H_{ax}-7), 2.77 (1H, *dd*, *J*=15.5 and 5.2 Hz, H_{eq}-7), 2.68 (1H, *m*, H_{ax}-4), 2.97 (1H, *s*, H_{eq}-4), 3.81 (1H, *dd*, *J*= 11.1 and 5.8 Hz, H-6a), 3.39 (1H, *m*, H_{eq}-5), 3.59 (3H, *s*, 1-OCH_3), 3.88 (3H, *s*, 9-OCH_3), 6.62 (1H, *s*, H-3), 6.78 (1H, *s*, H-8), 7.89 (1H, *s*, H-11).

^{13}C NMR ($CDCl_3$), δ : 142.3 (C-1), 60.3 (1-OCH_3), 125.9 (C-1a), 126.1 (C-1b), 148.6 (C-2), 113.8 (C-3), 129.3 (C-3a), 28.1 (C-4), 42.8 (C-5), 53.6 (C-6a), 35.9 (C-7), 129.3 (C-7a), 114.4 (C-8), 145.9 (C-9), 145.3 (C-10), 56.2 (9-OCH_3), 110.5 (C-11), 123.5 (C-11a).

AC 2, Boldine **69** : refer AP 1

AC 3, Gyrolidine **47** : $C_{38}H_{42}N_2O_6$ (yellow amorphous)

UV λ max : 283

IR ν max cm^{-1} : 1012, 1639

Mass spectrum m/z : 622 $[M]^+$

^1H NMR ($CDCl_3$), δ : 3.75 (1H, *br s*, H-1), 2.61 (3H, *s*, *N*-CH_3), 2.45 (1H, *m*, H_{ax}-3), 2.76 (1H, *m*, H_{eq}-3), 2.39 (2H, *m*, H-4), 6.32 (1H, *s*, H-5), 3.63 (3H, *s*, 6-OCH_3), 6.65 (1H, *s*, H-8), 2.90 (1H, *dd*, *J*=14.9 and 3.5 Hz, H_{ax}-α), 3.24 (1H, *m*, H_{eq}-

α), 5.41 (1H, *d*, *J*=2.3 Hz, H-10), 3.89 (3H, *s*, 12-OCH_3), 6.87 (1H, *d*, *J*=7.5 Hz, H-13), 6.78 (1H, *d*, *J*= 8.6 Hz, H-14), 4.29 (1H, *d*, *J*=6.3 Hz, H-1′), 2.69 (3H, *s*, *N*′-CH_3), 3.00 (1H, *m*, H_{ax}-3′), 3.26 (1H, *m*, H_{eq}-3′), 2.75 (1H, *m*, H_{ax}-4′), 3.07 (1H, *m*, H_{eq}-4′), 6.37 (1H, *s*, H-5′), 3.78 (3H, *s*, 6′-OCH_3), 3.19 (3H, *s*, 7′-OCH_3), 2.80 (1H, *m*, H_{ax}-α′), 3.42 (1H, *m*, H_{eq}-α′), 6.94 (1H, *d*, *J*=2.3 Hz, H-10′), 6.40 (1H, *d*, *J*= 4.6 Hz, H-11′), 6.95 (1H, *d*, *J*=2.3, H-13′), 7.48 (1H, *d*, *J*=8.1 Hz, H-14′).

^{13}C NMR ($CDCl_3$), δ : 63.8 (C-1), 43.3 (*N*-CH_3), 50.1 (C-3), 29.7 (C-4), 130.9 (C-4a), 110.9 (C-5), 148.6 (C-6), 55.0 (6-OCH_3), 143.8 (C-7), 116.7 (C-8), 127.1 (C-8a), 37.6 (C-α), 130.9 (C-9), 116.3 (C-10), 149.0 (C-11), 146.7 (C-12), 55.9 (12-OCH_3), 110.9 (C-13), 123.5 (C-14), 61.6 (C-1′), 41.7 (*N*′-CH_3), 45.1 (C-3′), 24.9 (C-4′), 127.1(C-4′a), 105.8 (C-5′), 151.9 (C-6′), 56.0 (6′-OCH_3), 137.2 (C-7′), 60.5 (7′-OCH_3), 147.5 (C-8′), 138.2 (C-8′a), 39.8 (C-α′), 128.1 (C-9′), 131.4 (C-10′), 121.1 (C-11′), 152.3 (C-12′), 122.4 (C-13′), 128.1 (C-14′).

AC 4, Stephasubine **92** : $C_{36}H_{34}N_2O_6$ (yellow amorphous)

UV λ max : 207, 236

IR ν max cm^{-1} : 3286

Mass spectrum m/z : 590 $[M]^+$

^{1}H NMR ($CDCl_3$), δ : 3.64 (1H, *br s*, H-1), 2.67 (3H, *s*, *N*-CH_3), 2.45 (1H, *d*, *J*=4.1 Hz, H_{ax}-3), 2.68 (1H, *m*, H_{eq}-3), 2.25 (1H, *m*, H_{ax}-4), 2.50 (1H, *s*, H_{eq}-4), 6.54 (1H, *s*, H-5), 4.03 (3H, *s*, 6-OCH_3), 5.98 (1H, *s*, H-8), 2.42 (1H, *d*, *J*=2.9 Hz, H_{ax}-α), 3.00 (1H, *d*, *J*=12.6, H_{eq}-α), 4.77 (1H, *s*, H-10), 3.87 (3H, *s*, 12-OCH_3), 6.71 (1H, *d*, *J*=9.1 Hz, H-13), 6.70 (1H, *d*, *J*=8.1 Hz, H-14), 8.43 (1H, *d*, *J*=5.8 Hz, H-3′), 7.47 (1H, *d*, *J*=5.8 Hz, H-4′), 6.98 (1H, *s*, H-5′), 4.03 (3H, *s*, 6′-OCH_3), 4.50 (1H, *d*, *J*= 13.7 Hz, H_{ax}-α′), 5.36 (1H, *d*, *J*=13.8, H_{eq}-α′), 7.0 (1H, *br s*, H-10′), 6.62 (1H, *dd*, *J*=

8.6 and 2.3 Hz, H-11′), 6.47 (1H, *dd*, *J*=8.1 and 2.3 Hz, H-13′), 7.40 (1H, *d*, *J*=8.1 Hz, H-14′).

^{13}C NMR ($CDCl_3$), δ : 63.6 (C-1), 43.2 (*N*-CH_3), 49.0 (C-3), 27.0 (C-4), 129.1 (C-4a), 111.9 (C-5), 147.7 (C-6), 56.2 (6-OCH_3), 144.3 (C-7), 111.8 (C-8), 119.3 (C-8a), 38.1 (C-α), 130.0 (C-9), 117.0 (C-10), 150.0 (C-11), 146.6 (C-12), 56.7 (12-OCH_3), 111.1 (C-13), 123.4 (C-14), 157.3 (C-1′), 140.8 (C-3′), 119.2 (C-4′), 133.9 (C-4′a), 102.1 (C-5′), 151.3 (C-6′), 56.1 (6′-OCH_3), 135.6 (C-7′), 144.3 (C-8′), 137.3 (C-8′a), 45.4 (C-α′), 137.6 (C-9′), 129.1 (C-10′), 123.0 (C-11′), 152.8 (C-12′), 122.4 (C-13′), 131.5 (C-14′).

AC 5, 2-Norobaberine **93** : $C_{37}H_{40}N_2O_6$ (brownish amorphous)

UV λ max : 284

IR ν max cm^{-1} : 3274

Mass spectrum m/z : 608 $[M]^+$

^{1}H NMR ($CDCl_3$), δ : 4.34 (1H, *br s*, H-1), 2.90 (1H, *m*, H_{ax}-3), 3.01 (1H, *m*, H_{eq}-3), 2.38 (1H, *m*, H_{ax}-4), 2.53 (1H, *m*, H_{eq}-4), 6.36 (1H, *s*, H-5), 3.63 (3H, *s*, 6-OCH_3), 6.69 (1H, *s*, H-8), 2.91 (1H, *m*, Hz, H_{ax}-α), 3.25 (1H, *m*, H_{eq}-α), 5.56 (1H, *d*, *J*=2.3 Hz, H-10), 3.90 (3H, *s*, 12-OCH_3), 6.86 (1H, *d*, *J*= 2.3 Hz, H-13), 6.80 (1H, *d*, *J*=8.6 Hz, H-14), 4.25 (1H, *d*, *J*=4.6 Hz, H-1′), 2.69 (3H, *s*, *N*′-CH_3), 2.92 (1H, *m*, H_{ax}-3′), 3.23 (1H, *m*, H_{eq}-3′), 2.74 (1H, *m*, H_{ax}-4′), 3.04 (1H, *m*, H_{eq}-4′), 6.37 (1H, *s*, H-5′), 3.79 (3H, *s*, 6′-OCH_3), 3.22 (3H, *s*, 7′-OCH_3), 2.84 (1H, *dd*, *J*=14.9 and 5.8 Hz, H_{ax}-α′), 3.36 (1H, *m*, H_{eq}-α′), 6.85 (1H, *d*, *J*=2.3 Hz, H-10′), 6.30 (1H, *d*, *J*= 8.1 and 2.9 Hz, H-11′), 6.98 (1H, *dd*, *J*=8.6 and 2.3 Hz, H-13′), 7.50 (1H, *d*, *J*=8.1 Hz, H-14′).

^{13}C NMR ($CDCl_3$), δ : 54.7 (C-1), 41.0 (C-3), 29.0 (C-4), 126.8 (C-4a), 111.8 (C-5), 148.8 (C-6), 55.1 (6-OCH_3), 114.2 (C-7), 116.0 (C-8), 130.5 (C-8a), 38.9 (C-

α), 147.6 (C-9), 115.9 (C-10), 149.7 (C-11), 147.3 (C-12), 55.9 (12-OCH_3), 123.5 (C-13), 110.9 (C-14), 61.8 (C-1′), 41.9 (*N*′-CH_3), 45.3 (C-3′), 25.1 (C-4′), 127.2(C-4′a), 105.9 (C-5′), 137.3 (C-6′), 56.1 (6′-OCH_3), 151.7 (C-7′), 60.6 (7′-OCH_3), 128.5 (C-8′), 122.4 (C-8′a), 40.0 (C-α′), 139.2 (C-9′), 131.3 (C-10′), 121.0 (C-11′), 151.9 (C-12′), 122.4 (C-13′), 128.1 (C-14′).

AC 6, 3′,4′-Dihydrostephasubine **94** : $C_{36}H_{36}N_2O_6$ (yellow amorphous)

UV λ max : 284

IR ν max cm^{-1} : 1646, 3308

Mass spectrum m/z : 593 $[M]^+$

^{1}H NMR ($CDCl_3$), δ : 3.54 (1H, *br s*, H-1), 2.48 (3H, *s*, *N*-CH_3), 2.39 (1H, *m*, H_{ax}-3), 3.71 (1H, *m*, H_{eq}-3), 2.39 (1H, *m*, H_{ax}-4), 2.40 (1H, *s*, H_{eq}-4), 6.46 (1H, *s*, H-5), 3.87 (3H, *s*, 6-OCH_3), 6.09 (1H, *s*, H-8), 2.51 (1H, *m*, H_{ax}-α), 2.99 (1H, *d*, *J*=13.2, H_{eq}-α), 4.88 (1H, *s*, H-10), 3.86 (3H, *s*, 12-OCH_3), 6.71 (2H, *br s*, H-13/H-14), 3.60 (1H, *m*, H_{ax}-3′), 3.90 (1H, m, H_{eq}-4′), 2.66 (1H, *m*, H_{ax}-4′), 2.68 (1H, *m*, H_{eq}-4′), 6.55 (1H, *s*, H-5′), 3.94 (3H, *s*, 6′-OCH_3), 4.50 (1H, *d*, *J*= 13.8 Hz, H_{ax}-α′), 3.97 (1H, *d*, *J*=13.8 Hz, H_{eq}-α′), 7.36 (1H, *d*, *J*=8.6 Hz, H-10′), 6.47 (1H, *d*, *J*=2.9 Hz, H-11′), 6.74 (1H, *dd*, *J*=8.6 and 2.3 Hz, H-13′), 7.40 (1H, *d*, *J*=8.6 Hz, H-14′).

^{13}C NMR ($CDCl_3$), δ : 63.6 (C-1), 43.3 (*N*-CH_3), 49.7 (C-3), 27.9 (C-4), 130.2 (C-4a), 111.6 (C-5), 144.3 (C-6), 55.9 (6-OCH_3), 147.5 (C-7), 113.1 (C-8), 135.0 (C-8a), 38.1 (C-α), 130.2 (C-9), 116.9 (C-10), 146.3 (C-11), 149.7 (C-12), 56.2 (12-OCH_3), 110.6 (C-13), 123.2 (C-14), 164.6 (C-1′), 46.8 (C-3′), 27.0 (C-4′), 135.6 (C-4′a), 105.6 (C-5′), 132.0 (C-6′), 55.8 (6′-OCH_3), 149.3 (C-7′), 140.5 (C-8′), 116.5 (C-8′a), 44.7 (C-α′), 135.0 (C-9′), 131.9 (C-10′), 122.0 (C-11′), 152.8 (C-12′), 122.5 (C-13′), 128.3 (C-14′).

AC 7, *O*-Methyllimacusine **95** : $C_{38}H_{42}N_2O_6$ (brownish amorphous)

UV λ max : 282

IR ν max cm^{-1} : 1015, 1650

Mass spectrum m/z : 622 $[M]^+$

^{1}H NMR ($CDCl_3$), δ : 3.48 (1H, *d*, *J*=7.5 Hz H-1), 2.56 (3H, *s*, *N*-CH_3), 2.75 (1H, *m*, H_{ax}-3), 3.04 (1H, *m*, H_{eq}-3), 2.65 (1H, *m*, H_{ax}-4), 2.85 (1H, *m*, H_{eq}-4), 6.45 (1H, *s*, H-5), 3.42 (3H, *s*, 6-OCH_3), 6.40 (1H, *s*, H-8), 2.57 (1H, *m*, H_{ax}-α), 3.11 (1H, *m*, H_{eq}-α), 6.96 (1H, *s*, H-10), 3.95 (3H, *s*, 12-OCH_3), 6.95 (1H, *d*, *J*= 5.8 Hz, H-13), 6.65 (1H, *d*, *J*=2.3 Hz, H-14), 4.28 (1H, *d*, *J*=10.3 Hz, H-1′), 2.56 (3H, *s*, *N*′-CH_3), 2.99 (1H, *m*, H_{ax}-3′), 3.51 (1H, *m*, H_{eq}-3′), 2.73 (1H, *m*, H_{ax}-4′), 2.77 (1H, *m*, H_{eq}-4′), 6.39 (1H, *s*, H-5′), 3.76 (3H, *s*, 6′-OCH_3), 3.01 (3H, *s*, 7′-OCH_3), 2.83 (1H, *m*, H_{ax}-α′), 3.32 (1H, *d*, *J*= 11.5 Hz, H_{eq}-α′), 6.80 (1H, *d*, *J*=7.5 Hz, H-10′), 6.81 (1H, *d*, *J*= 2.4 Hz, H-11′), 7.13 (1H, *dd*, *J*= 8.6 and 1.7 Hz, H-13′), 7.35 (1H, *d*, *J*=8.1 Hz, H-14′).

^{13}C NMR ($CDCl_3$), δ : 65.5 (C-1), 42.2 (*N*-CH_3), 46.5 (C-3), 26.3 (C-4), 127.5 (C-4a), 112.5 (C-5), 148.6 (C-6), 55.4 (6-OCH_3), 144.6 (C-7), 120.6 (C-8), 131.0 (C-8a), 40.7 (C-α), 134.0 (C-9), 133.6 (C-10), 148.7 (C-11), 149.4 (C-12), 56.3 (12-OCH_3), 112.9 (C-13), 120.3 (C-14), 60.5 (C-1′), 41.5 (*N*′-CH_3), 44.2 (C-3′), 22.9 (C-4′), 127.5(C-4′a), 106.8 (C-5′), 152.2 (C-6′), 55.8 (6′-OCH_3), 138.1 (C-7′), 59.8 (7′-OCH_3), 148.8 (C-8′), 135.5 (C-8′a), 43.8 (C-α′), 136.0 (C-9′), 131.7 (C-10′), 120.4 (C-11′), 155.6 (C-12′), 122.0 (C-13′), 130.3 (C-14′).

CHAPTER 4

RESULTS AND DISCUSSION

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4.0 Introduction

The extraction and isolation of the bark of *Alseodaphne peduncularis* (Wall. ex Nees) Meisn and *Alseodaphne corneri* Kosterm yielded two types of isoquinoline alkaloids. There are aporphine and bisbenzylisoquinoline alkaloids. The isolation process and the spectral data of alkaloids isolated had previously explained in chapter 3. The structural elucidations were done by several spectroscopic methods such as NMR, UV, IR, MS and also confirmation by comparison with previous works.

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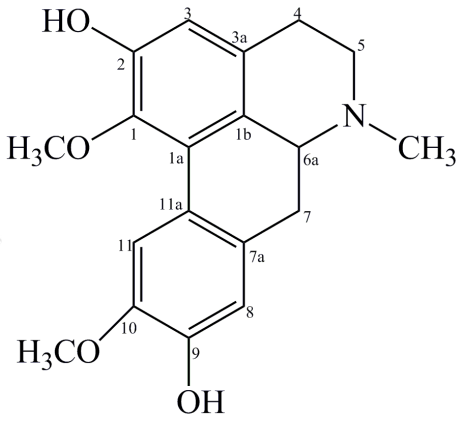
4.1 Isolation of *Alseodaphne peduncularis* (Wall. ex Nees) Meisn

The sample (4.8 kg) was collected in Kluang-Mersing, Johor and was identified by Herbarium group of Chemistry Department of Universiti of Malaya. Extraction and further isolation process yielded four aporphine alkaloids. There are boldine **69**, norpredicentrine **90** and norlirioferine **91** and norboldine **78**.

4.1.1 AP 1, Boldine 69



69

Alkaloid AP 1 (10.8 mg) was isolated as brownish amorphous. The UV spectrum showed absorption at 282 and 302 nm suggested 1,2,9,10-tetrasubstituted aporphine skeleton (Sangster & Stuart, 1965). The IR spectrum gave a broad band and showed absorption at 3280 cm^{-1} due to the presence of a highly conjugated hydroxyl group and strong absorption at 2945 cm^{-1} due to the stretching of CH aromatic (Williams & Fleming, 1989). The molecular ion peak was observed at m/z 327 giving the possibility of molecular formula of $C_{19}H_{21}NO_4$. The base peak at m/z 326 $[M-1]^+$ indicated the loss of proton.

The ^{1}H NMR spectrum (Figure 4.1) displayed two singlet peaks at δ 3.59 and δ 3.91 corresponding to two methoxyl groups attached to C-1 and C-10. The methoxyl group at C-1 is more shielded due to the anisotropic effect of ring D (Kanokmedhakul, S., Kanokmedhakul, K., Yodbuddee & Phonkerd, 2003). The *N*-methyl group resonated at δ 2.58 as a singlet. Another three singlets appeared at downfield region corresponding to three aromatic protons at δ 6.64, 6.82 and 7.89 attributed to H-3, H-8 and H-11, respectively. H-11 was found more deshielded due to the anistropic effect caused by the ring A. The aliphatic protons appeared as multiplets at region δ 2.60-3.16 (Omar et al., 2013).

The ^{13}C NMR spectrum (Figure 4.2) of AP 1 showed the presence of nineteen peaks corresponding to nineteen carbons. There are three methyls, three methylenes, four methines and nine quaternary carbon signals. The signals of three methyls carbons were observed at δ 43.5, 56.2 and 60.4 corresponding to *N*-CH_3, 10-OCH_3 and 1-OCH_3, respectively. In addition, three methylenes carbons resonated at δ 28.5, 33.9 and 53.3 attributed to C-4, C-7 and C-5. The signal for C-6a appeared at δ 62.5 while three methines signals of C-3, C-8 and C-11 appeared at δ 113.3, 114.3 and 110.2. Finally, nine quaternary carbon signals deshielded to downfield region due to diamagnetic anisotropy effects at δ 123.6, 126.1, 129.5, 129.8, 142.2, 145.2, 145.7 and 148.3 were attributed to (C-11a), (C-1a/C-1b), (C-3a), (C-7a), (C-1), (C-9), (C-10) and (C-2), respectively.

The COSY spectrum (Figure 4.3) showed correlation of aliphatic protons between H-4/H-5 and H-7/H-7. The ^{1}H-^{13}C direct correlations are shown in HMQC spectrum in Figure 4.4.

Furthermore, the ^{1}H-^{13}C long range correlations were determined by HMBC spectrum as shown in Figure 4.5. The HMBC correlation revealed cross peaks between H-3 with C-1, C-1b and C-2 while H-8 with C-7, C-10 and C-11a, respectively. The selected COSY and HMBC correlations are illustrated in Figure 4.6.

The complete NMR spectral data of ^{1}H, ^{13}C, HMQC and HMBC of AP 1 are summarized in Table 4.1 while the comparison of the spectral data with literature values are tabulated in Table 4.2 and 4.3.

The comparison between the observed data and literature value (Table 4.3) confirmed that alkaloid AP 1 is boldine **69** (Johns, Lamberton & Sioumis, 1969; Mukhtar, 1996; Guinaudeau, Leboeuf & Cave, 1979).

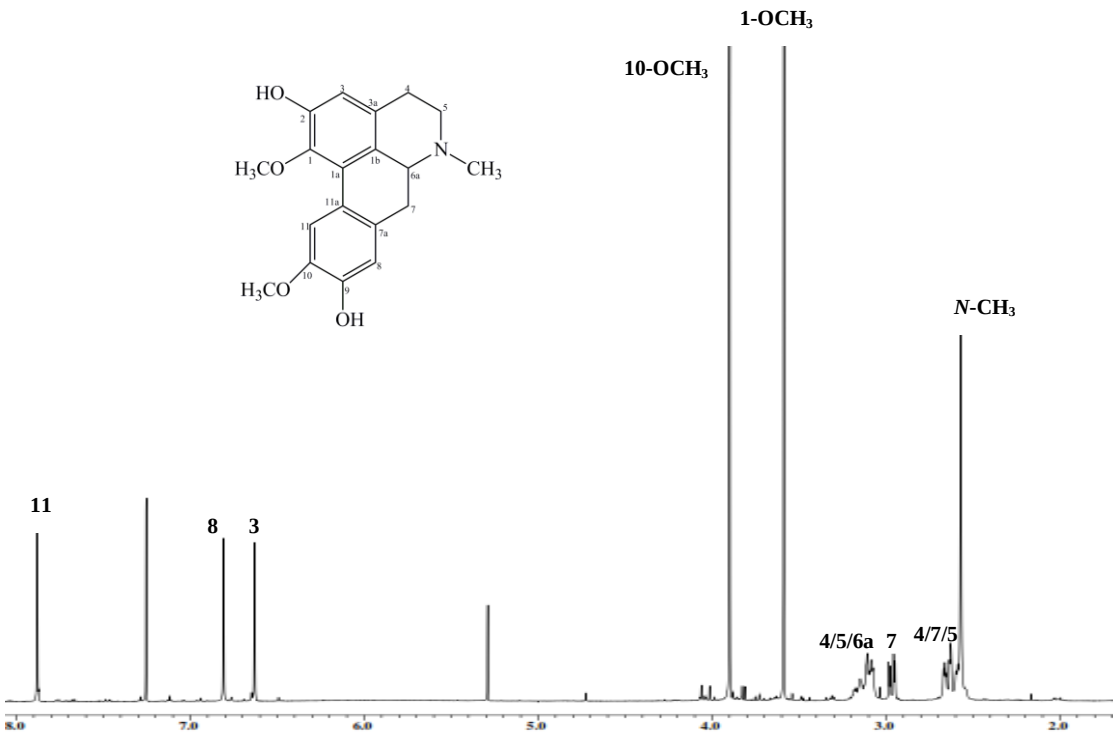


Figure 4.1. ^{1}H NMR spectrum of AP 1.

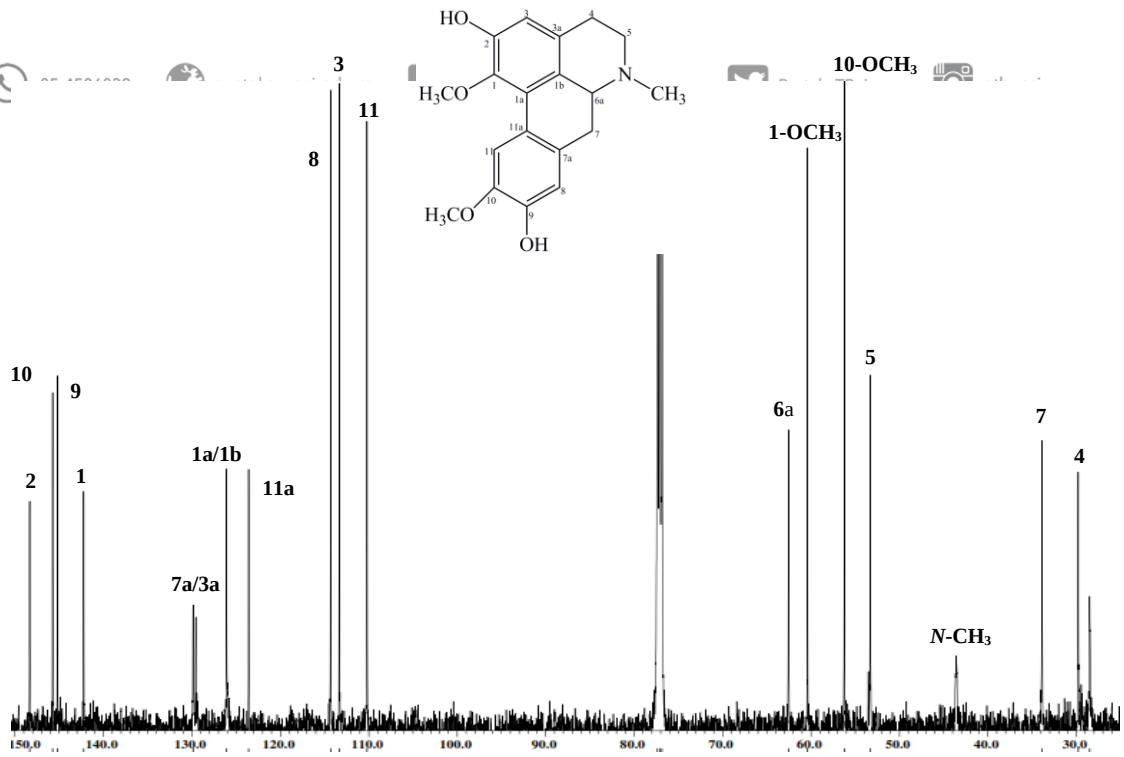


Figure 4.2. ^{13}C NMR spectrum of AP 1.

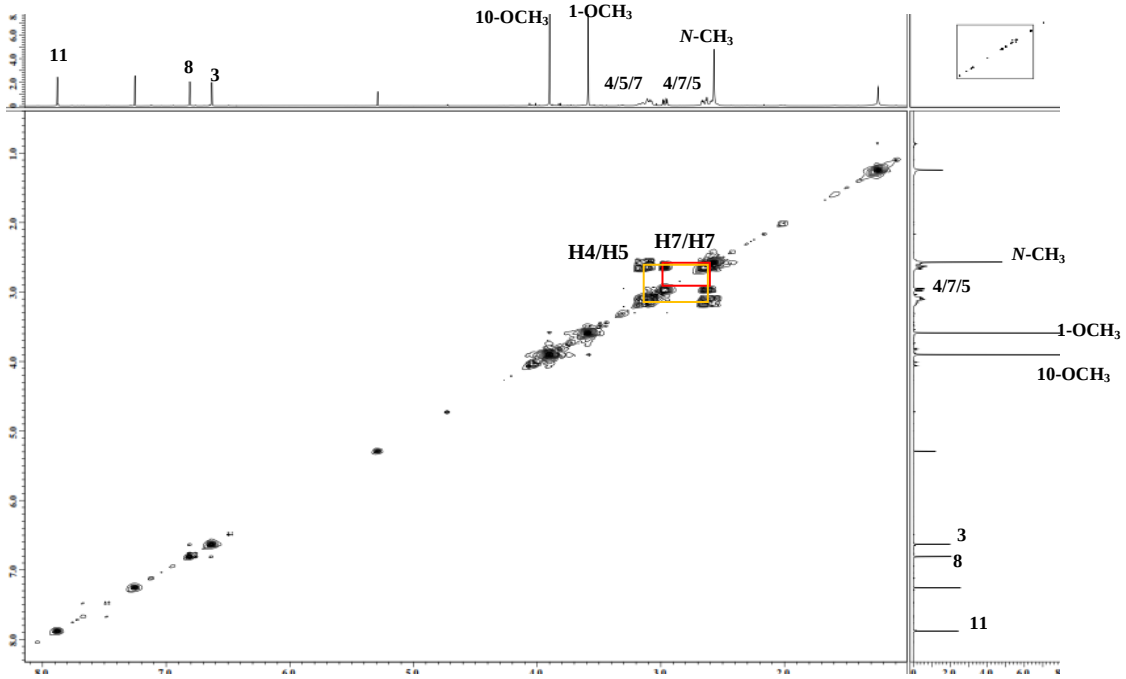


Figure 4.3. COSY spectrum of AP 1.

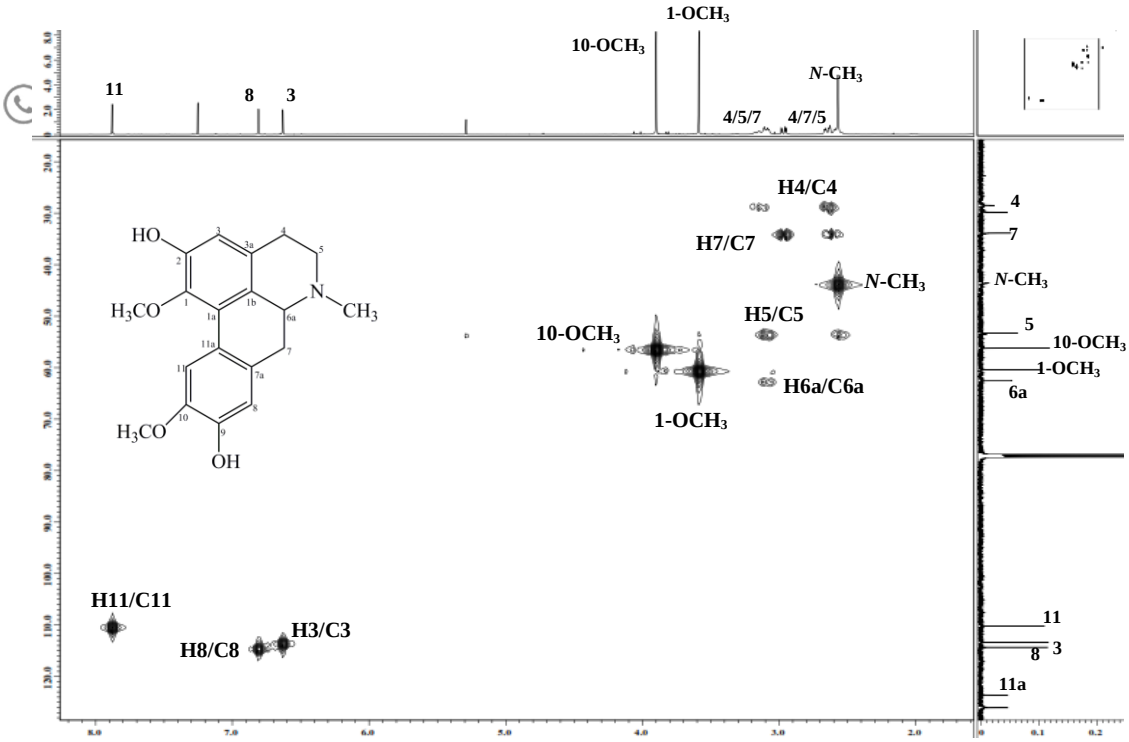


Figure 4.4. HMQC spectrum of AP 1.

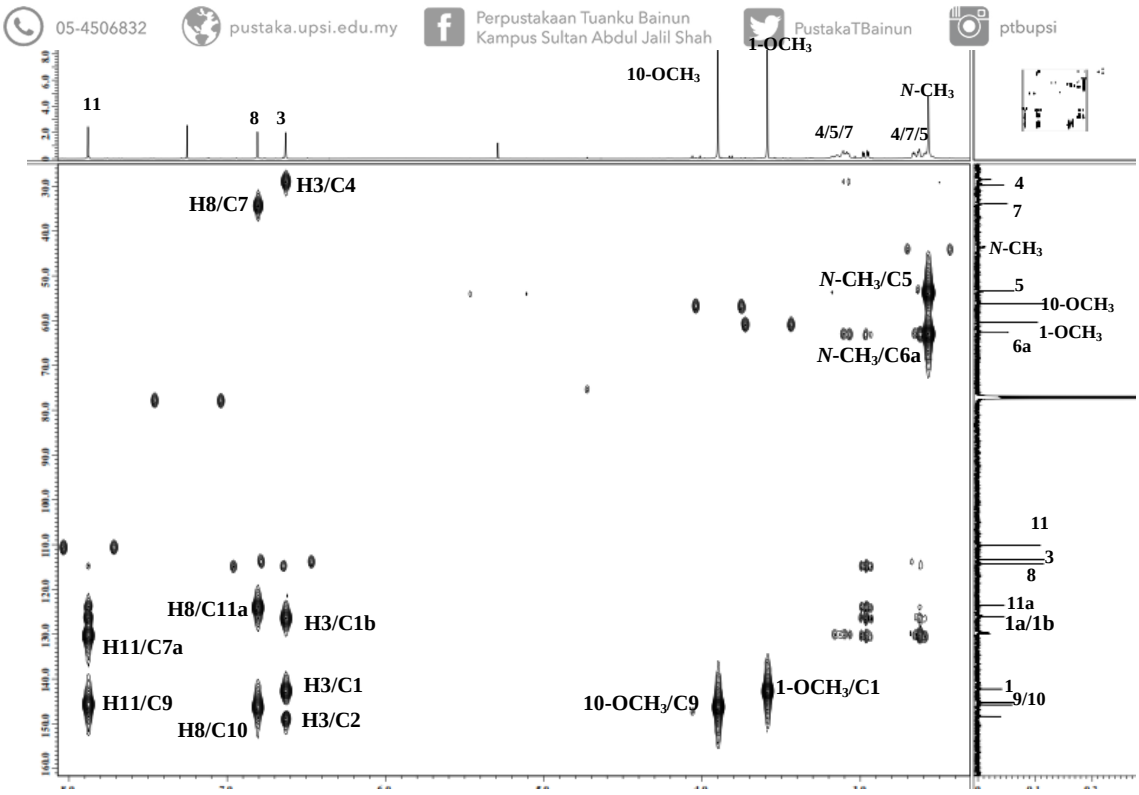


Figure 4.5. HMBC spectrum of AP 1.

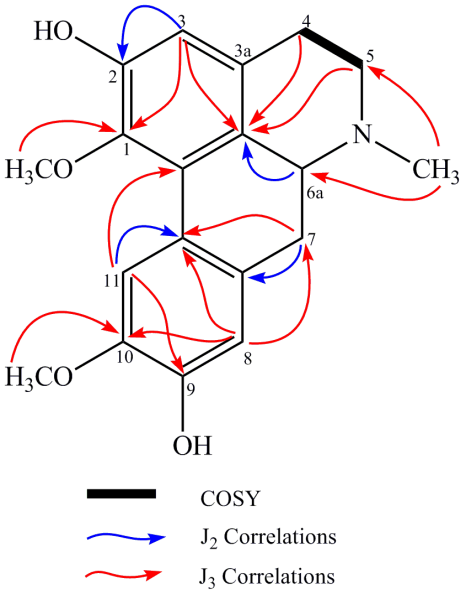


Figure 4.6. Selected COSY and HMBC correlation in AP 1.

Table 4.1

1D (^1H and ^{13}C) and 2D (HMQC and HMBC) NMR Spectral Data of AP 1

Position	^1H $CDCl_3$ (*J*, Hz)	^{13}C (δ, $CDCl_3$)	HMQC	HMBC
1		142.2		
1a		126.1		
1b		126.1		
2		148.3		
3	6.64 (*s*)	113.3	H_3	1,1b,2
3a		129.5		
4	2.67 (*m*) 3.16 (*br s*)	28.5	H_4	1b
5	2.60 (*d*, 5.8) 3.12 (*br s*)	53.3	H_5	1b
6a	3.01 (*m*)	62.5	H_{6a}	1b
7	2.64 (*br s*) 2.97 (*dd*, 13.8, 4.0)	33.9	H_7	1a,1b,7a,11a
7a		129.8		
8	6.82 (*s*)	114.3	H_8	7,10,11a
9		145.2		
10		145.7		
11	7.89 (*s*)	110.2	H_{11}	1b,7a,9,11a
11a		123.6		
1-OCH_3	3.59 (*s*)	60.4	$3H_{1\text{-}OCH_3}$	1
10-OCH_3	3.91 (*s*)	56.2	$3H_{10\text{-}OCH_3}$	10
N-CH_3	2.58 (*s*)	43.5	$3H_{N\text{-}CH_3}$	5,6a

Table 4.2

^{1}H NMR Spectral Data of AP 1 and Boldine ***69***

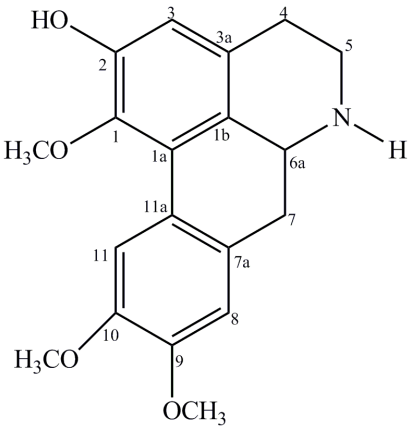
Positions	^{1}H $CDCl_3$ (Hz)	
	AP 1	Boldine (Mukhtar, 1996)
3	6.64 (*s*)	6.63 (*s*)
4, 5, 6a, 7	2.64-3.16 (*m*)	2.58-3.20 (*m*)
8	6.82 (*s*)	6.83 (*s*)
11	7.89 (*s*)	7.89 (*s*)
1-OCH_3	3.59 (*s*)	3.60 (*s*)
10-OCH_3	3.91 (*s*)	3.91 (*s*)
N-CH_3	2.58 (*s*)	2.52 (*s*)

Table 4.3

^{13}C NMR Spectral Data of AP 1 and Boldine ***69***

Positions	^{13}C (δ, $CDCl_3$)	
	AP 1	Boldine (Guinaudeau et al., 1979)
1	142.2	142.0
1a	126.1	126.8
1b	126.1	125.9
2	148.3	148.1
3	113.3	113.3
3a	129.5	129.9
4	28.5	28.9
5	53.3	53.4
6a	62.5	62.6
7	33.9	34.2
7a	129.9	130.2
8	114.3	114.2
9	145.2	145.1
10	145.7	145.6
11	110.2	110.1
11a	123.6	123.6
1-OCH_3	60.4	60.2
10-OCH_3	56.2	56.1
N-CH_3	43.5	44.0

4.1.2 AP 2, Norpredicentrine 90



90

Alkaloid AP 2 (3.8 mg) was obtained as brownish amorphous. Its molecular formula was assigned as $C_{19}H_{21}NO_4$ as the EIMS data showed the presence of ion peak at m/z 327 relevant to the formula assigned. The UV spectrum showed maxima absorption at 217, 281 and 301 nm, indicating the presence of an aporphine substituted at position 1,2,9 and 10 (Sangster & Stuart, 1965). The IR spectrum showed broad band between 3000 to 3500 cm^{-1} due to the presence of OH and NH functional groups in AP 2 (Pretsch, Bühlmann & Affolter, 2000).

The ^1H NMR spectrum (Figure 4.7) of AP 2 have similar pattern with AP 1, thus predicted to be aporphine skeleton structure. Its showed three singlets of aromatic protons at δ 6.68, 6.78 and 7.94 and were assigned to three aromatic protons of H-3, H-8 and H-11, respectively. A singlet at δ 3.61 corresponding to a methoxyl group at position C-1 and another two methoxyl groups signals were overlapping with each other at δ 3.91 assignable to 9-OCH_3 and 10-OCH_3, respectively. The other

aliphatic protons appeared as multiplets at the region δ 2.75-3.09. The COSY spectrum (Figure 4.8) showed cross peaks between H-4/H-5 and H-6a/H-7.

The ^{13}C NMR spectrum (Figure 4.9) gave a total of nineteen carbon signals. The aromatic carbons of C-3, C-8 and C-11 resonated at δ 113.8, 111.1 and 110.7, respectively. Three methoxyl carbons appeared at δ 56.0, 56.1 and 60.5 belongs to 9-OCH_3, 10-OCH_3 and 1-OCH_3. Another four aliphatic carbon signals of C-4, C-5, C-6a and C-7 resonated at δ 27.8, 42.7, 53.6 and 35.7. Finally, the other nine quarternary carbons appeared at downfield region at δ 123.9, 125.4, 125.7, 128.3, 129.1, 142.4, 148.0, 148.5 and 148.8 assignable to C-7a, C-1a, C-3a, C-11a, C-1b, C-1, C-10, C-9 and C-2, respectively as the diamagnetic anisotropy effect increases in the benzene rings.

Direct correlations between carbon and hydrogen can be seen in HMQC spectrum in Figure 4.10. The HMBC spectrum can be seen in Figure 4.11. The HMBC spectrum showed cross peaks of H-3 with C-1, C-1a, C-2 and C-4; H-11 with C-1a, C-7a, C-10; and H-6a with C-7. The selected COSY and HMBC correlations are illustrated in Figure 4.12, further confirmed the structure of AP 2.

The 1D (^1H and ^{13}C) and 2D (HMQC and HMBC) NMR spectral data of AP 2 are tabulated in Table 4.4. Analysis of the spectroscopic data obtained and comparison with literature values (Table 4.5) alkaloid AP 2 was identified as norpredicentrine **90** which previously isolated from *Guatteria* (Hocquemiuer, Rasamizafy, Cavé & Moretti, 1983).

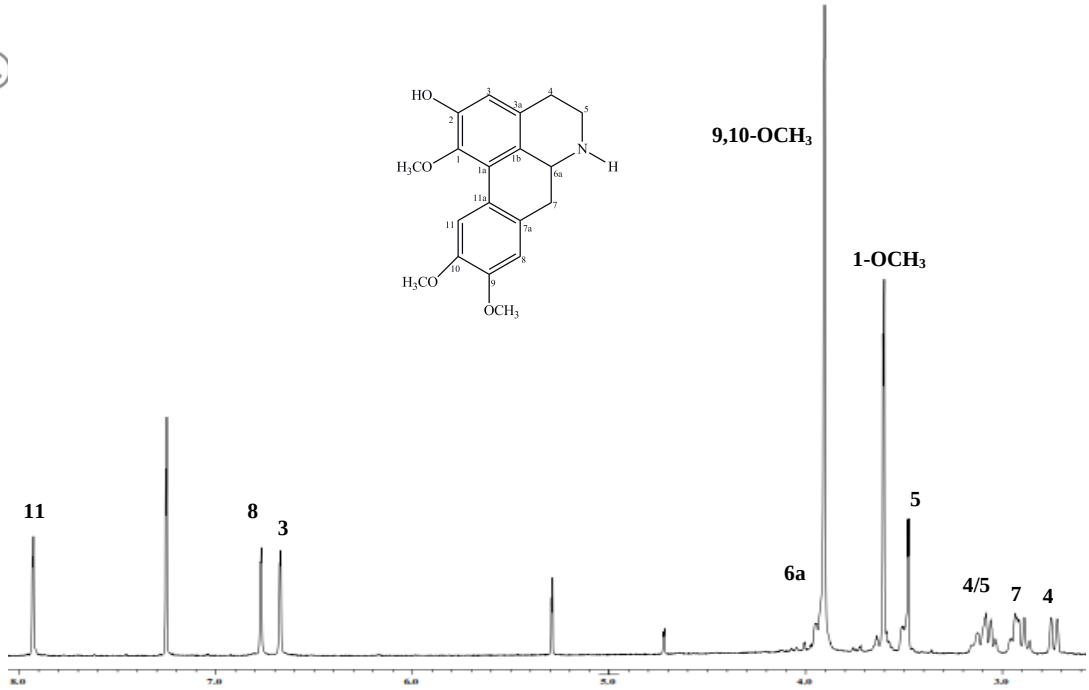


Figure 4.7. ^{1}H NMR spectrum of AP 2.

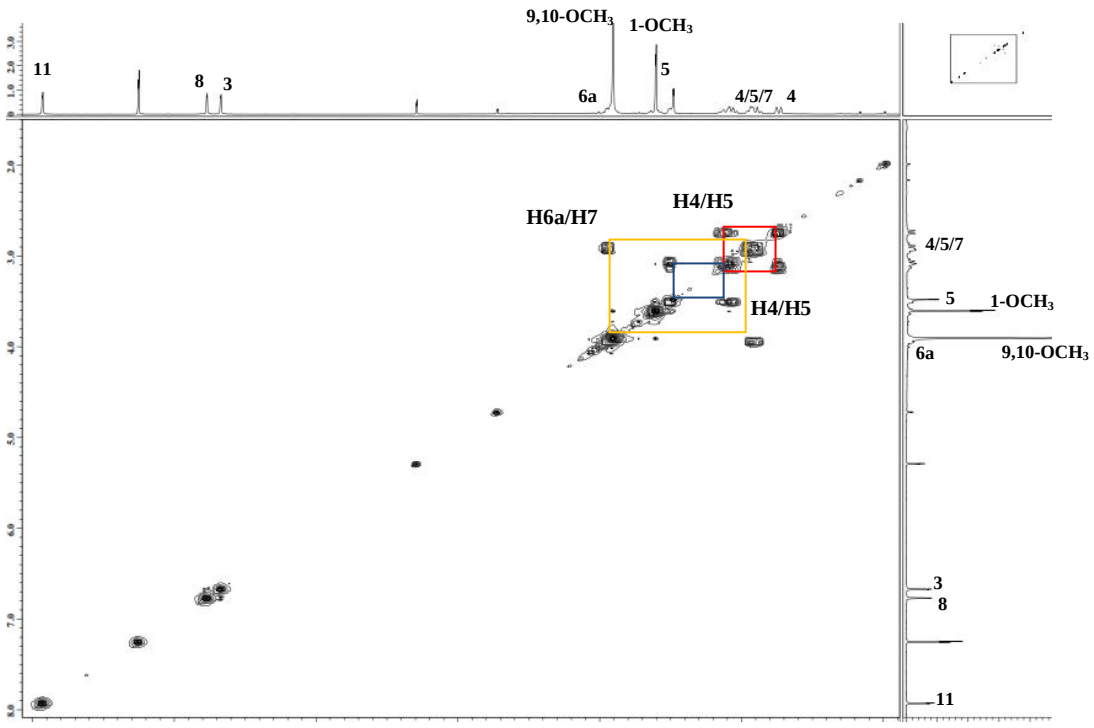


Figure 4.8. COSY spectrum of AP 2.

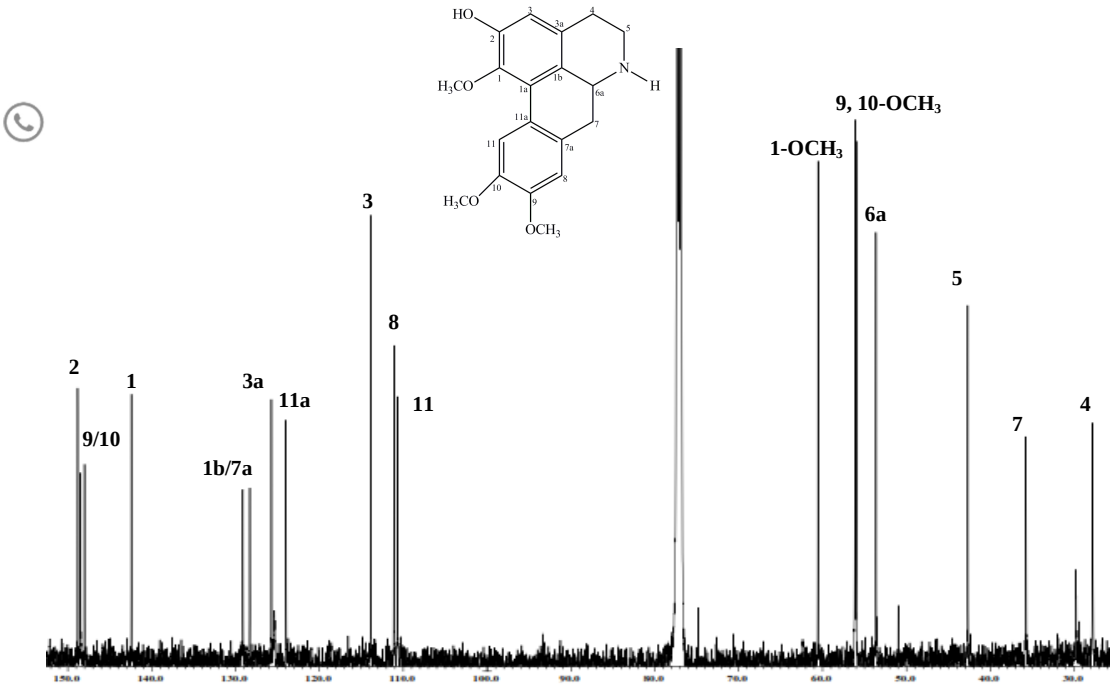


Figure 4.9. ^{13}C NMR spectrum of AP 2.

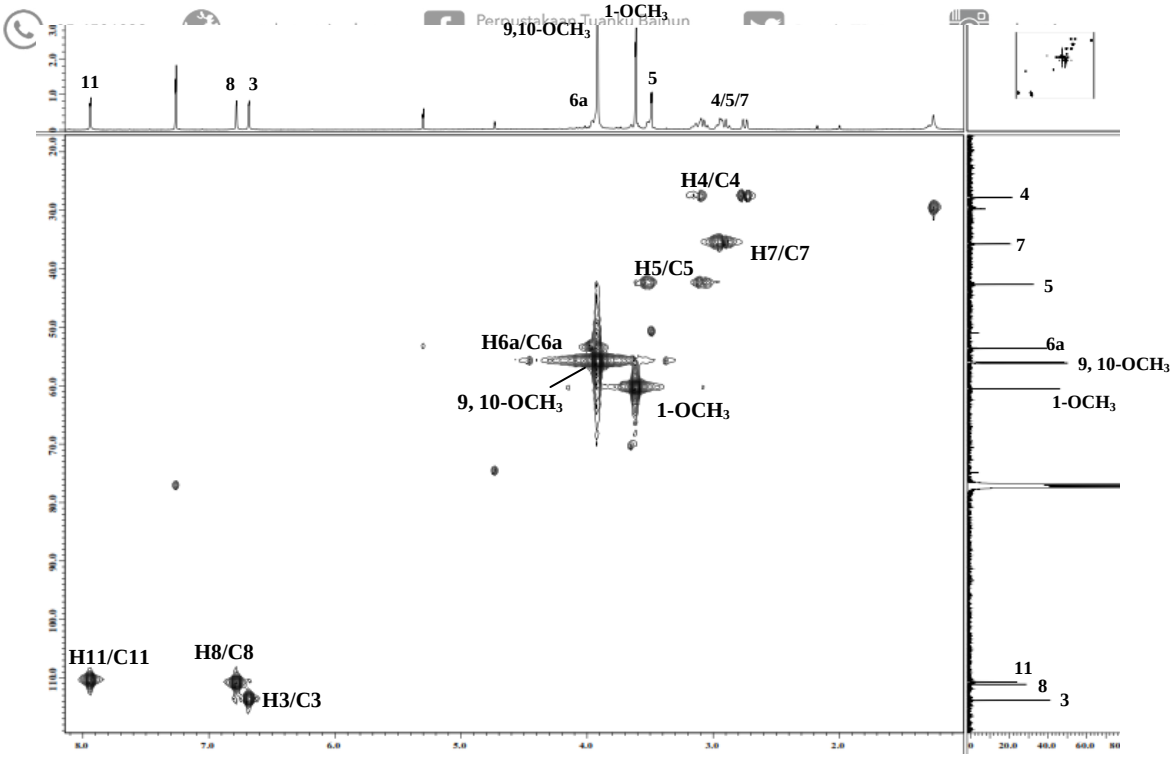


Figure 4.10. HMQC spectrum of AP 2.

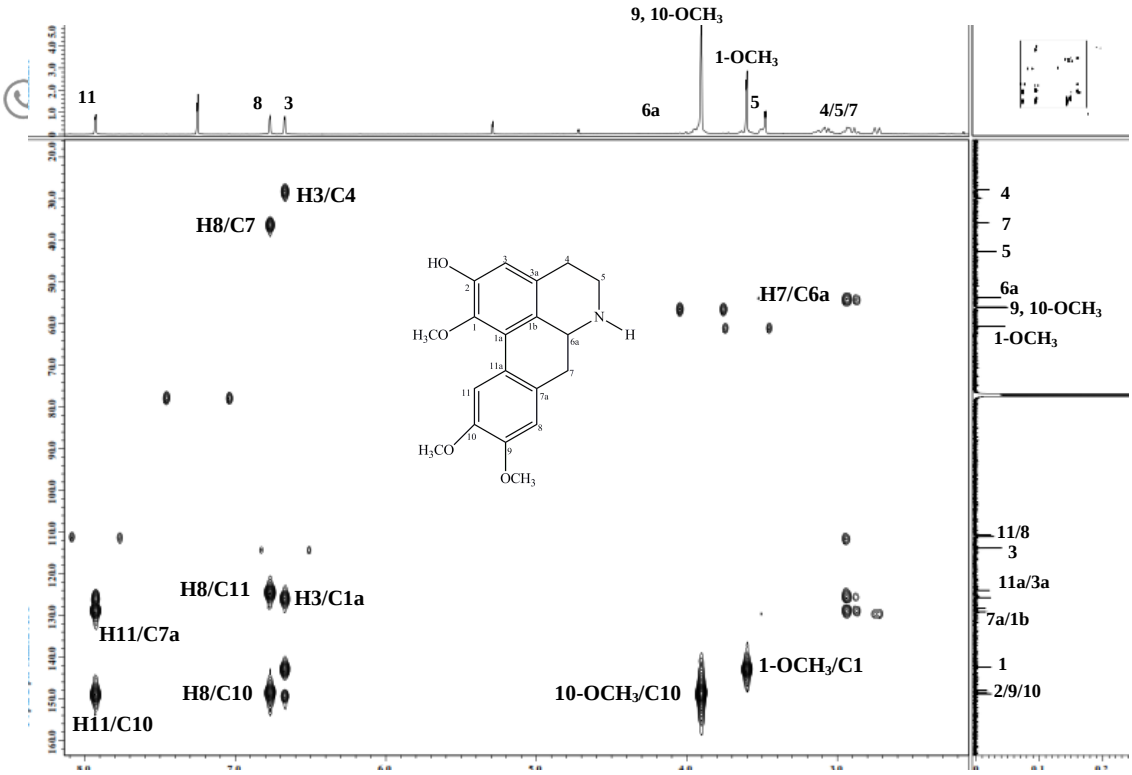


Figure 4.11. HMBC spectrum of AP 2.

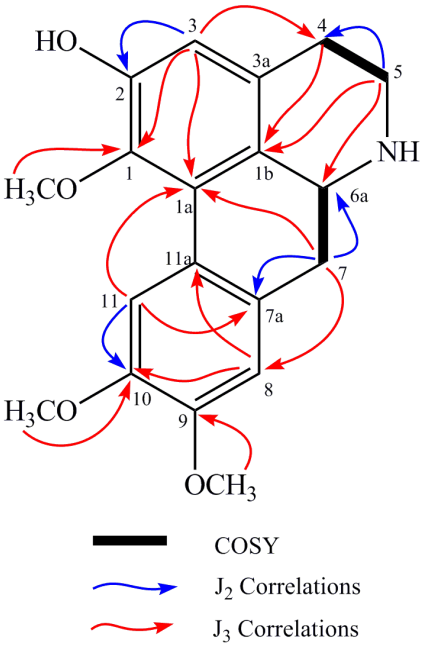


Figure 4.12. Selected COSY and HMBC correlation in AP 2.

Table 4.4

1D (^{1}H and ^{13}C) and 2D (HMQC and HMBC) NMR Spectral Data of AP 2

Position	^{1}H $CDCl_3$ (*J*, Hz)	^{13}C (δ, $CDCl_3$)	HMQC	HMBC
1		142.4		
1a		125.4		
1b		129.1		
2		148.8		
3	6.68 (*s*)	113.8	H_3	1,1a,2,4
3a		125.7		
4	2.75 (*d*, 14.9) 3.07 (*m*)	27.8	H_4	1b,3
5	3.09 (*m*) 3.49 (*s*)	42.7	H_5	1b,4,6a
6a	3.96 (*br s*)	53.6	H_{6a}	7
7	2.93 (*m*)	35.7	H_7	1b,6a,7a,8
7a		123.9		
8	6.78 (*s*)	111.1	H_8	7,10,11a
9		148.5		
10		148.0		
11	7.94 (*s*)	110.7	H_{11}	1a,7a,10
11a		128.3		
1-OCH_3	3.61 (*s*)	60.5	$3H_{1\text{-}OCH_3}$	1
9-OCH_3	3.91 (*s*)	56.0	$3H_{9\text{-}OCH_3}$	9
10-OCH_3	3.91 (*s*)	56.1	$3H_{10\text{-}OCH_3}$	10

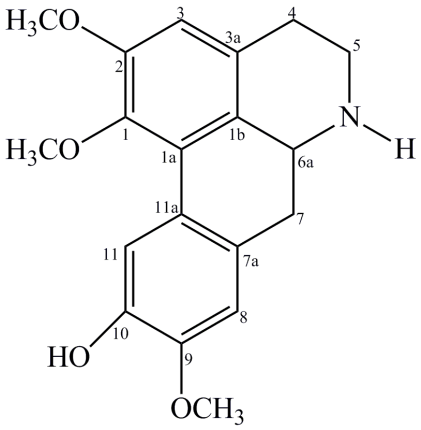
Table 4.5

*^{1}H NMR Data of AP 2 and Norpredicentrine **90***

Position	^{1}H $CDCl_3$ (*J*, Hz)	
	AP 2	Norpredicentrine (Hocquemiuer et al., 1983)
3	6.68 (*s*)	6.64
4	2.75 (*d*, 14.9) 3.07 (*m*)	*
5	3.09 (*m*) 3.49 (*s*)	*
6a	3.96 (*br s*)	*
7	2.93 (*m*)	*
8	6.78 (*s*)	6.73
11	7.94 (*s*)	8.06
1-OCH_3	3.61 (*s*)	3.60
9-OCH_3	3.91 (*s*)	3.86
10-OCH_3	3.91 (*s*)	3.86

Note. * = not available

4.1.3 AP 3, Norlirioferine 91



91

Alkaloid AP 3 (9.5 mg) was obtained as a dark brown amorphous. The UV spectrum showed absorption bands at 220, 280 and 302 nm, thus suggesting a 1,2,9,10-tetrasubstituted aporphine skeleton (Sangster & Stuart, 1965). Moreover, the IR spectrum exhibited the presence of a highly conjugated hydroxyl group at 3453 cm^{-1}. Its also showed strong absorption at 1642 cm^{-1} due to the presence of aromatic rings (Pretsch et al., 2000). The EIMS data revealed a molecular ion peak at m/z 327 suggesting the molecular formula of $C_{19}H_{21}NO_4$.

The ^1H NMR spectrum (Figure 4.13) displayed two singlets at δ 3.66 (3H) and 3.88 (6H) that overlapping attributed to the three methoxyl groups at C-2, C-9 and C-1, respectively. At the downfield region, three singlets assigned to the aromatic protons of H-3 (δ 6.59), H-8 (δ 6.78) and H-11 (δ 8.07). This observation proved that C-1, C-2, C-9 and C-10 are substituted. From the prior observation and comparison with the literature value, confirmed the aromatic ring A and D are substituted by hydroxyl and methoxyl groups (Castro, López & Vergara, 1985), thus deshielding the aromatic protons to the lower region. Three sets of methylene protons of H-4, H-5 and

H-7 appeared as multiplet at δ 2.73-3.43 as appeared in ring B and C. A broad singlet at δ 3.85 corresponding to a single proton of H-6a. The COSY spectrum (Figure 4.14) showed cross-peak between H4/H5 and H6a/H7.

The ^{13}C NMR spectrum (Figure 4.15) exhibited nineteen carbons equal to the structure proposed. From the ^{13}C NMR data together with ^{1}H-^{13}C correlation observed in the HMQC experiment (Figure 4.16) indicated the presence of nine quaternary carbons. The other signals were assigned to three aromatic carbons of C-3 (δ 110.8), C-8 (δ 114.1) and C-11 (δ 111.4). Three methylene carbons were observed at δ 28.8 (C-4), 36.2 (C-5) and 42.9 (C-7). Another three carbons signals at δ 55.9, 56.1 and 60.3 are belongs to 2-OCH_3, 9-OCH_3 and 1-OCH_3, respectively.

The structure of alkaloid AP 3 was finally assigned by ^{1}H-^{13}C long range correlations in the HMBC spectrum (Figure 4.17). The position of methoxy groups were further confirmed due to cross peaks between 1-OCH_3 with C-1 (δ 144.4); 2-OCH_3 with C-2; and 9-OCH_3 with C-9, respectively.

The other selected COSY and HMBC correlations are illustrated in Figure 4.18. The NMR spectral data (^{1}H, ^{13}C, HMQC and HMBC) are tabulated in Table 4.6. Comparison of the spectral data with the literature values (Table 4.7 and 4.8) confirmed the alkaloid AP 3 is norlirioferine **91** (Castedo, Saá, Suau & Villaverde, 1980; Castro et al., 1985).

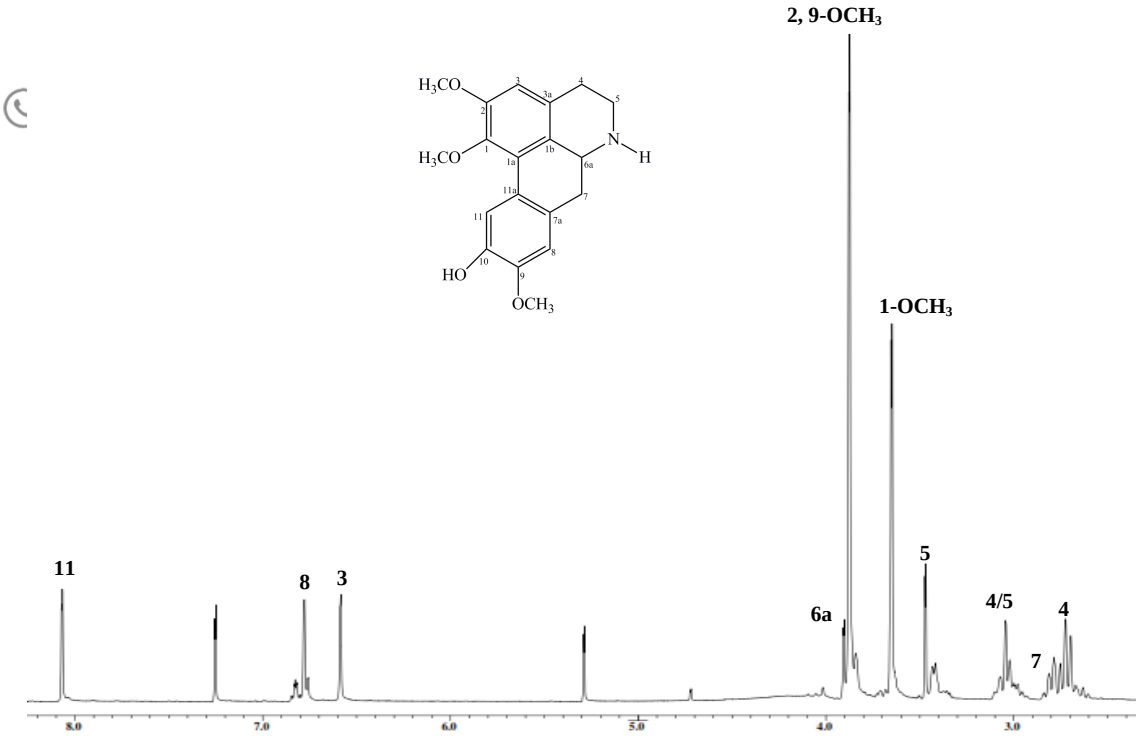


Figure 4.13. ^{1}H NMR spectrum of AP 3.

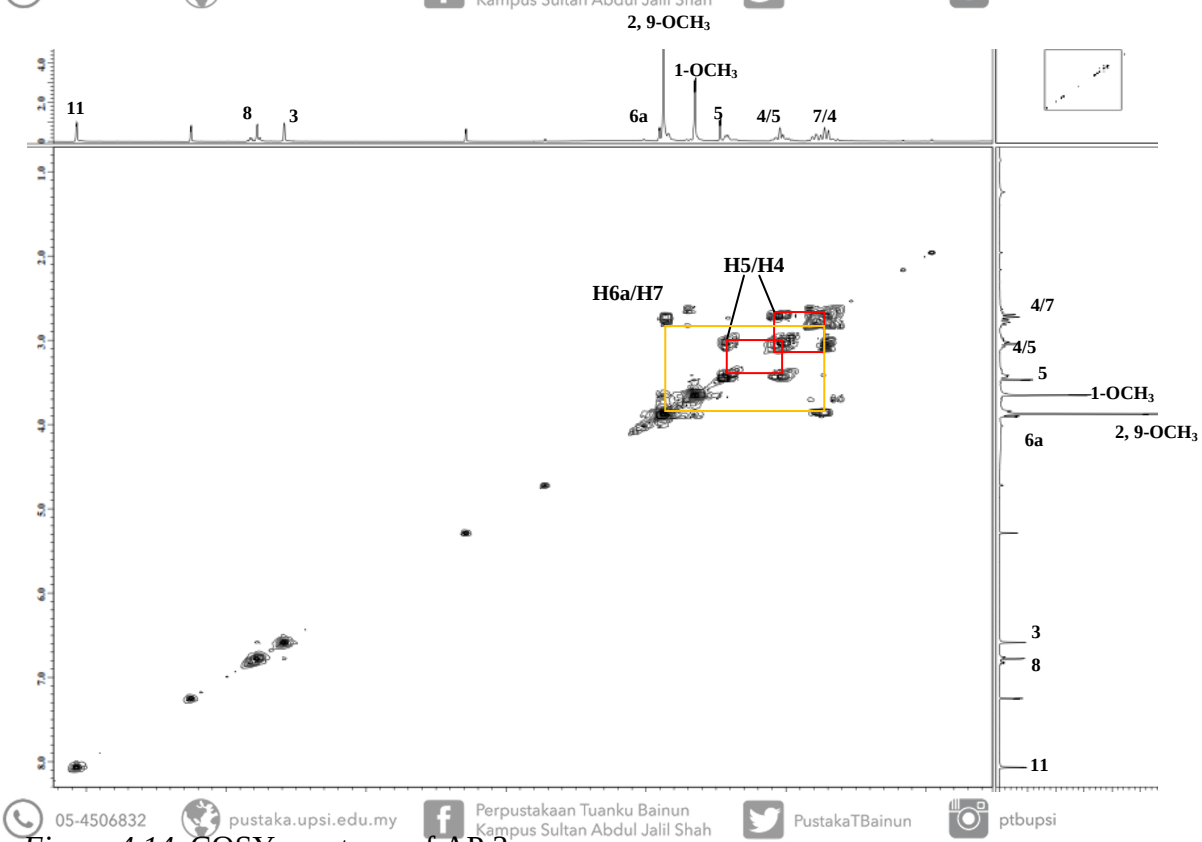


Figure 4.14. COSY spectrum of AP 3.

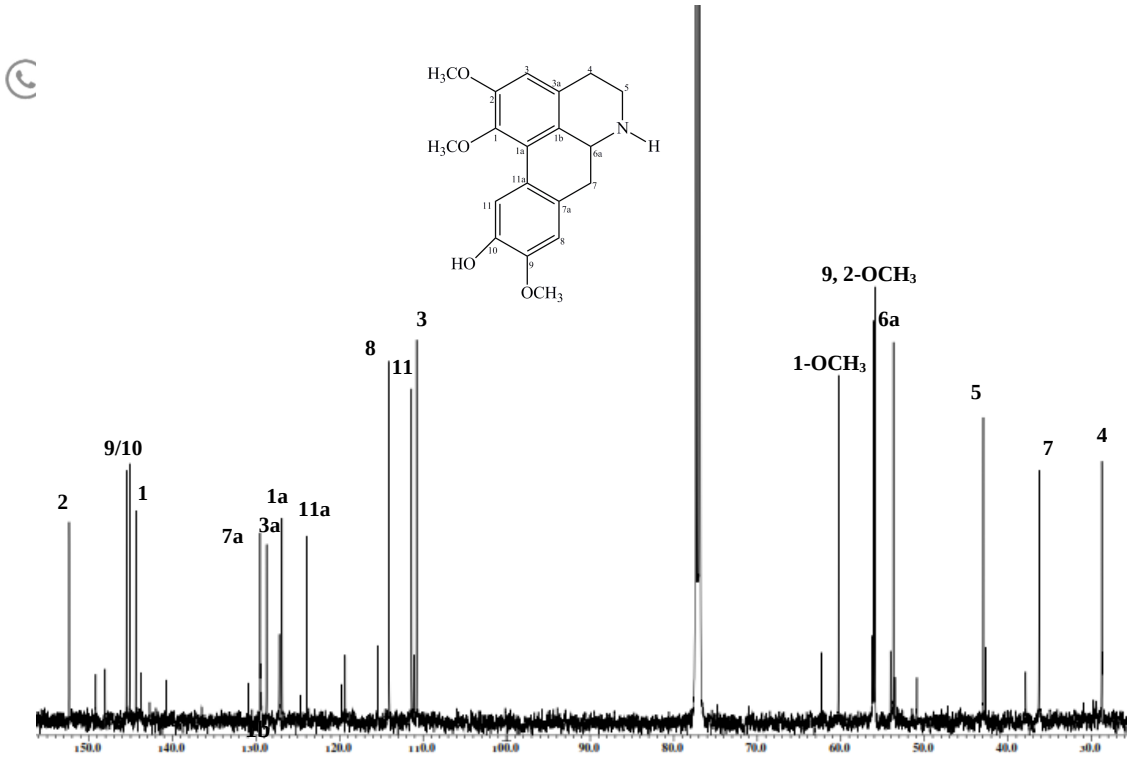


Figure 4.15: ^{13}C NMR spectrum of AP 3.

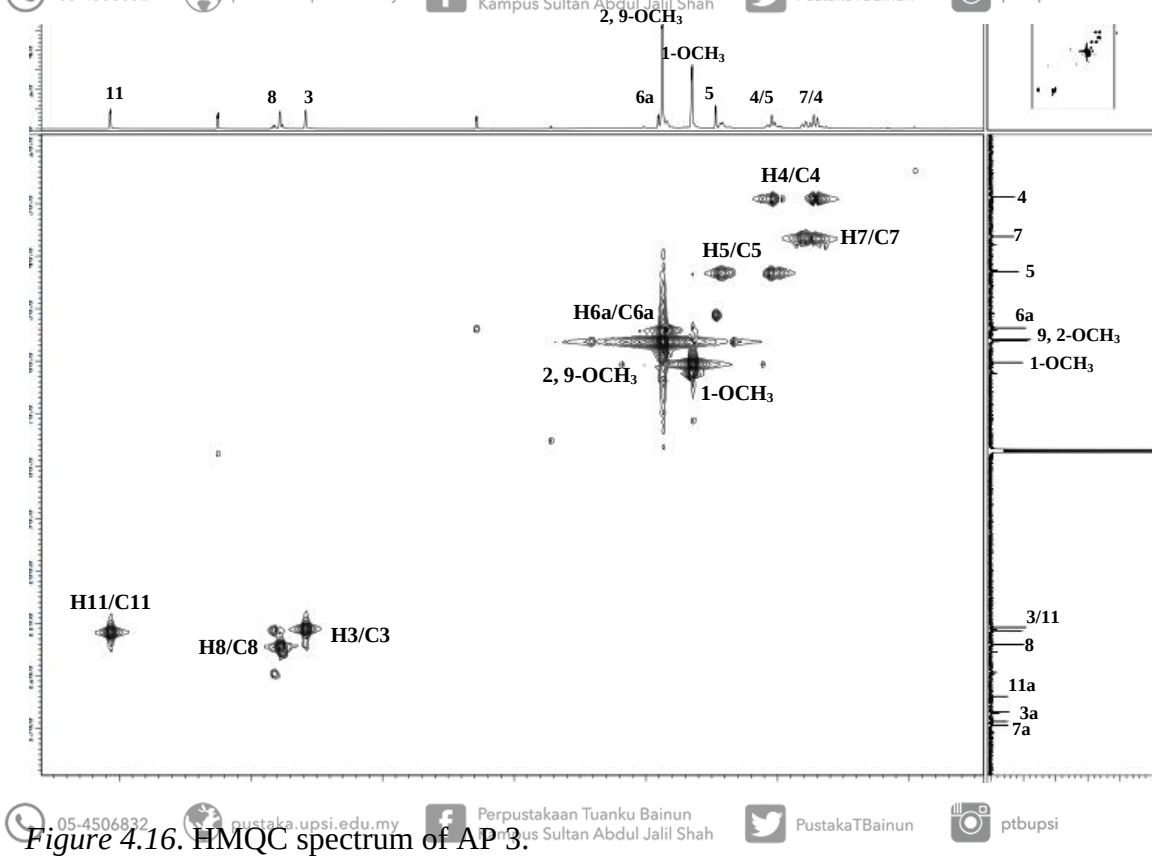


Figure 4.16. HMQC spectrum of AP 3.

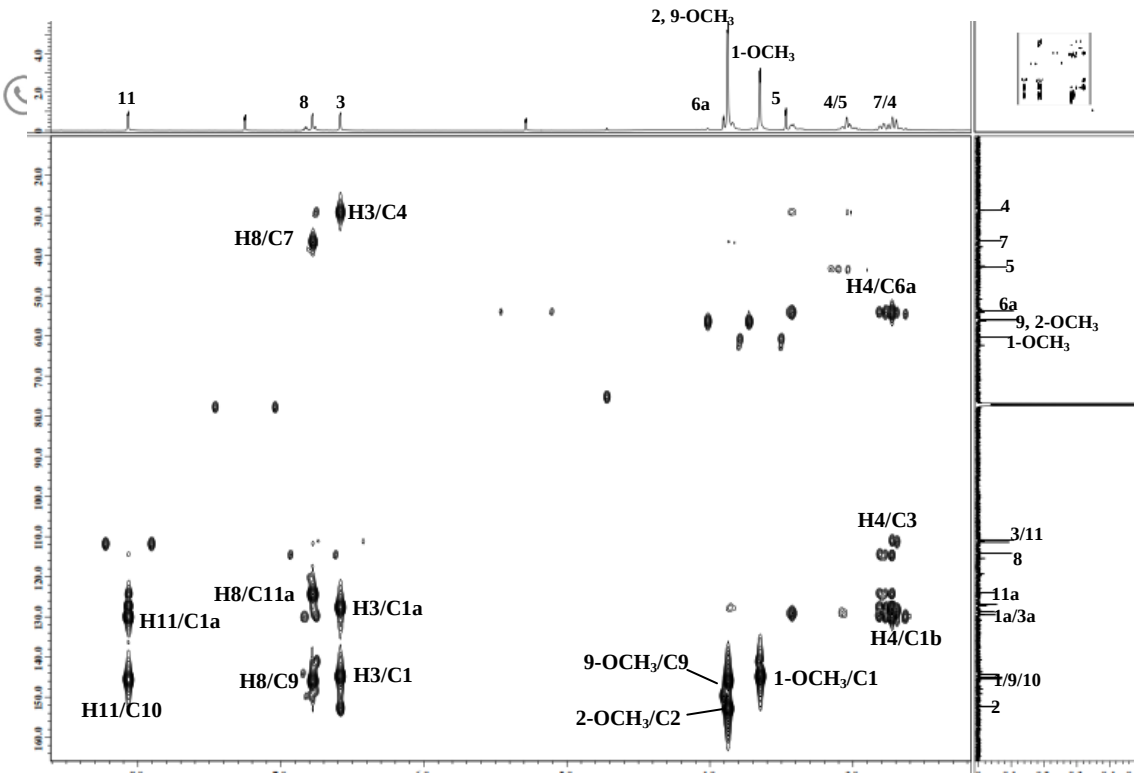


Figure 4.17. HMBC spectrum of AP 3.

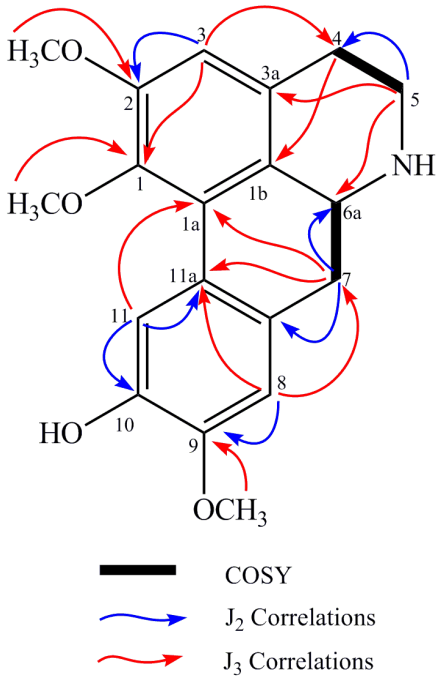


Figure 4.18. Selected COSY and HMBC correlation in AP 3.

Table 4.6

1D (^1H and ^{13}C) and 2D (HMQC and HMBC) NMR Spectral Data of AP 3

Position	^1H $CDCl_3$ (*J*, Hz)	^{13}C (δ, $CDCl_3$)	HMQC	HMBC
1		144.4		
1a		126.9		
1b		127.2		
2		152.3		
3	6.59 (*s*)	110.8	H_3	1,1a,2,4
3a		128.7		
4	2.73 (*m*) 3.05 (*m*)	28.8	H_4	1b,3,6a
5	3.05 (*m*) 3.43 (*d*, 8.6)	42.9	H_5	3a,4,6a
6a	3.85 (*br s*)	53.7	H_{6a}	
7	2.79 (*m*)	36.2	H_7	1a,6a,7a,8,11a
7a		129.5		
8	6.78 (*s*)	114.1	H_8	7,9,11a
9		145.5		
10		145.1		
11	8.07 (*s*)	111.4	H_{11}	1a,10,11a
11a		123.9		
1-OCH_3	3.66 (*s*)	60.3	$3H_{1\text{-}OCH_3}$	1
2-OCH_3	3.88 (*s*)	55.9	$3H_{2\text{-}OCH_3}$	2
9-OCH_3	3.88 (*s*)	56.1	$3H_{9\text{-}OCH_3}$	9

Table 4.7

*^1H NMR Data of AP 3 and Norlirioferine **91***

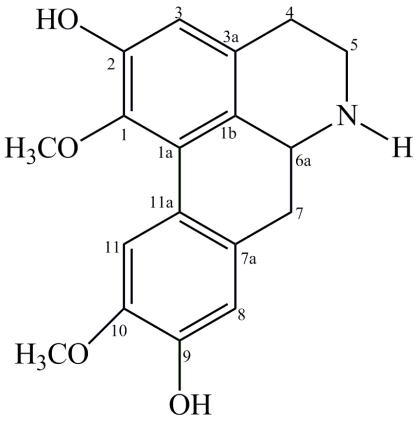
Position	^1H $CDCl_3$ (*J*, Hz)	
	AP 3	Norlirioferine (Castedo et al., 1980)
3	6.59 (*s*)	6.55 (*s*)
8	6.78 (*s*)	6.69 (*s*)
11	8.07 (*s*)	7.99 (*s*)
1-OCH_3	3.66 (*s*)	3.66 (*s*)
2-OCH_3	3.88 (*s*)	3.89 (*s*)
9-OCH_3	3.88 (*s*)	3.85 (*s*)

Table 4.8

^{13}C NMR Data of AP 3 and Norlirioferine ***91***

Position	^{13}C (δ, $CDCl_3$)	
	AP 3	Norlirioferine (Castro et al., 1985)
1	144.4	144.1
1a	126.9	126.6
1b	127.2	127.9
2	152.3	151.9
3	110.8	110.7
3a	128.7	128.8
4	28.8	29.2
5	42.9	43.1
6a	53.7	53.8
7	36.2	36.7
7a	129.5	129.7
8	114.1	111.3
9	145.5	145.2
10	145.1	144.9
11	111.4	114.0
11a	123.9	123.7
1-OCH_3	60.3	60.1
2-OCH_3	55.9	55.9
9-OCH_3	56.1	56.0

4.1.4 AP 4, Norboldine 78



78

Alkaloid AP 4 (12.6 mg) was obtained as a brownish amorphous solid. The UV spectrum showed absorption band at 216, 282 and 302 nm due to the degree of resonance in the biphenyl system that existed in ring A and ring D. Thus suggesting the characteristic of 1,2,9,10-tetrasubstituted aporphine (Goodwin, Shooley & Johnson, 1958). The EIMS spectrum revealed base peak at m/z 312 indicated the loss of proton. Another peak at m/z 313 showed the molecular formula of $C_{18}H_{19}NO_4$. The IR spectrum showed broad band at 3300 cm^{-1} due to the presence of NH functional groups in this compound (Williams & Fleming, 1989).

The ^1H NMR spectrum (Figure 4.19) data were somewhat similar to AP 1 except the absence of *N*-CH_3 group in AP 4. Two signals appeared as singlets at δ 3.61 and 3.91. Its showed the presence of two methoxyls groups attached to C-1 and C-10 at ring A and ring D. Three singlets corresponding to three aromatic protons revealed at δ 6.65, 6.81 and 7.91 which can be assigned to H-3, H-8 and H-11, respectively. Proton of H-11 is more deshielded due to the anisotropic effect caused

by the ring A. The COSY spectrum (Figure 4.20) showed the exact position of aliphatic protons between H4/H5 and H6a/H7. The aliphatic protons appeared as multiplets at δ 2.65-3.76 (Kanokmedhakul et al., 2003).

The ^{13}C NMR spectrum (Figure 4.21) established eighteen signals which nine of the signals belongs to nine quarternary carbons. The methoxyl carbons were observed at δ 56.2 (C-10) and 60.4 (C-1) meanwhile three methylene carbons appeared at δ 29.1 (C-4), 36.8 (C-7) and 43.3 (C-5). The other signals representing four methines of C-3 (δ 113.7), C-6a (δ 53.8), C-8 (δ 114.2) and C-11 (δ 110.2).

The HMQC and HMBC spectrum of alkaloid AP 4 are shown in Figure 4.22 and Figure 4.23. The HMBC spectrum revealed cross peaks between 1-OCH_3/C-1 and 10-OCH_3/C-10 further confirmed the position of 1-OCH_3 and 10-OCH_3. Other HMBC correlations can be seen in Figure 4.24.

The NMR spectral data of AP 4 are shown in Table 4.9. Finally, the alkaloid AP 4 was confirmed to be norboldine **78** by comparison of its spectral data with the literature values in Table 4.10 (Mukhtar, 1996; Zahari, 2010).

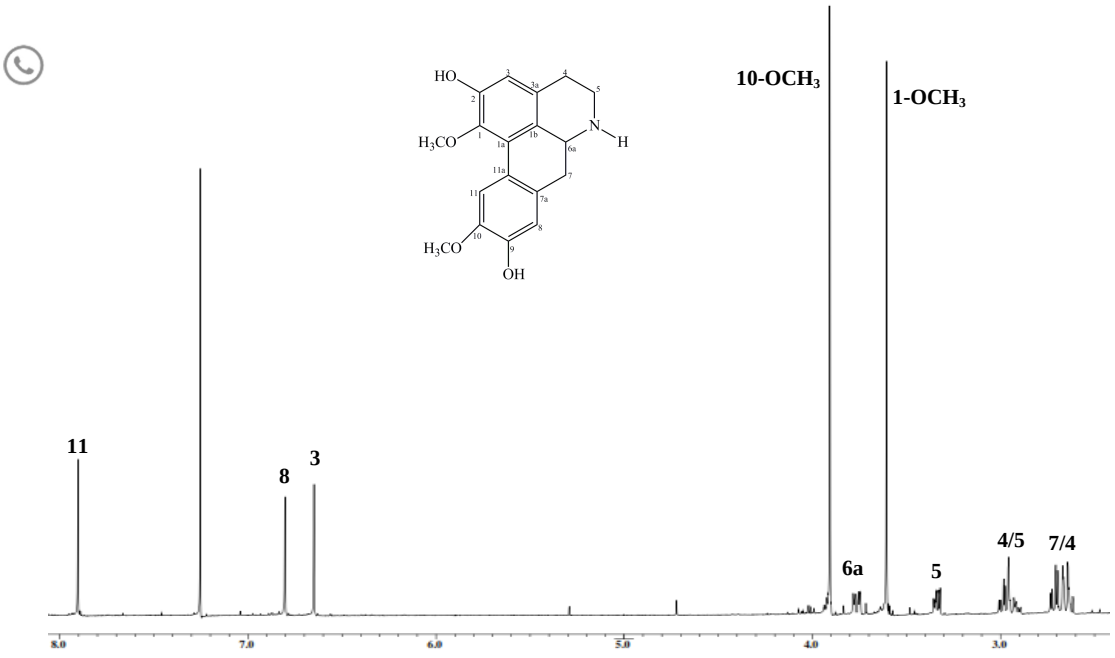


Figure 4.19. ^{1}H NMR spectrum of AP 4.

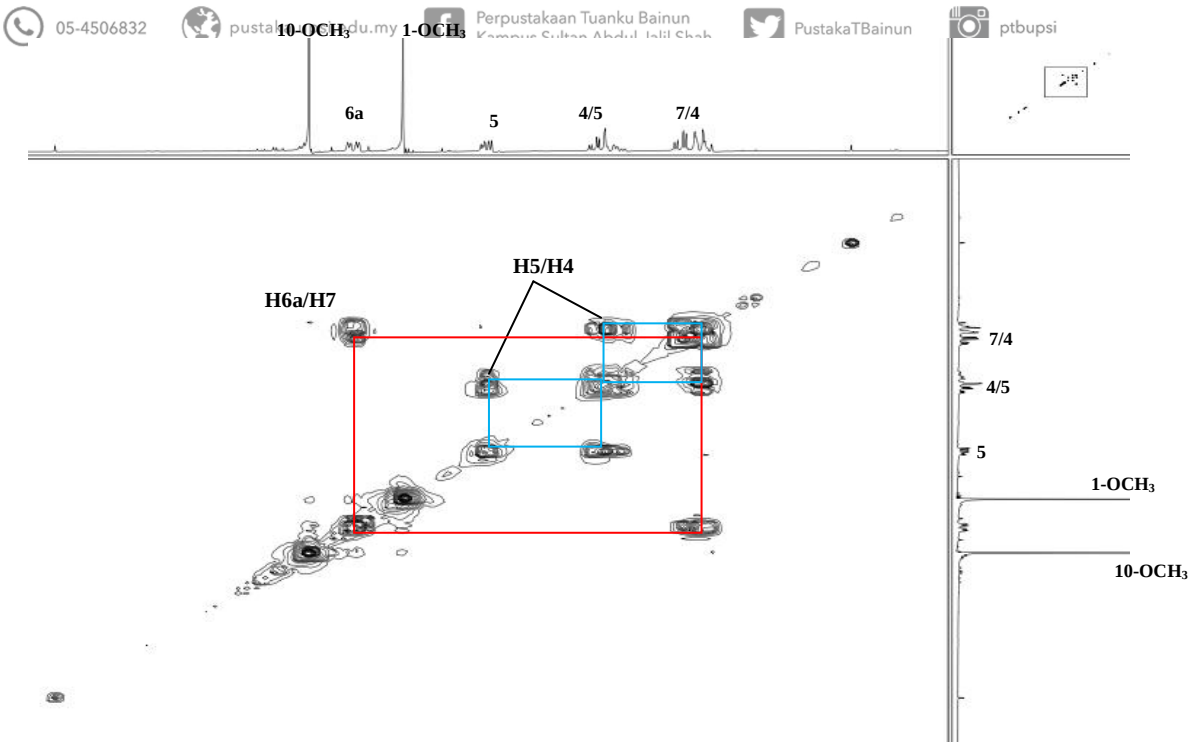


Figure 4.20. COSY spectrum of AP 4.

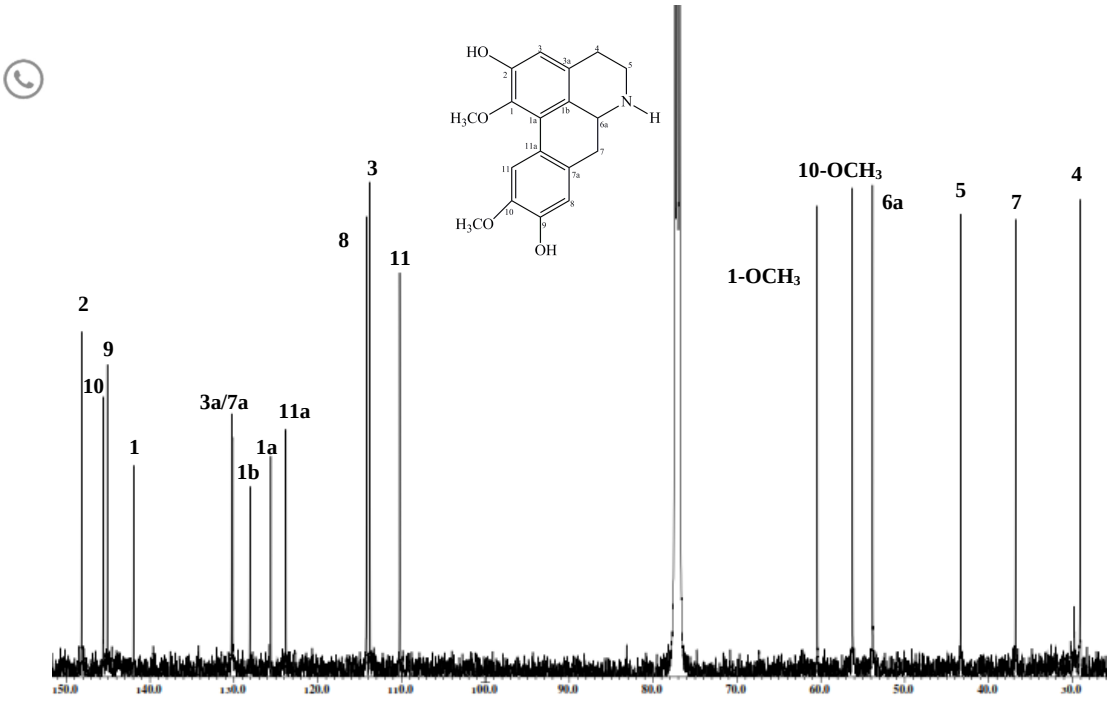


Figure 4.21. ^{13}C NMR Spectrum of AP 4.

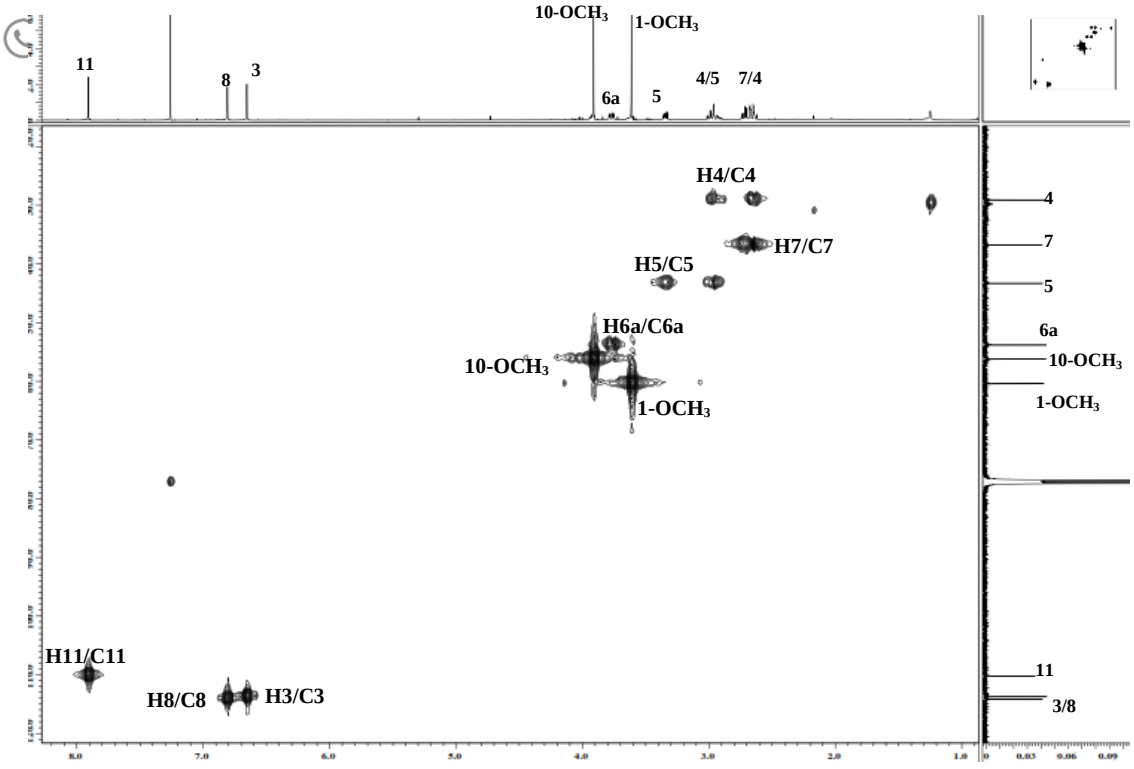


Figure 4.22. HMQC spectrum of AP 4.

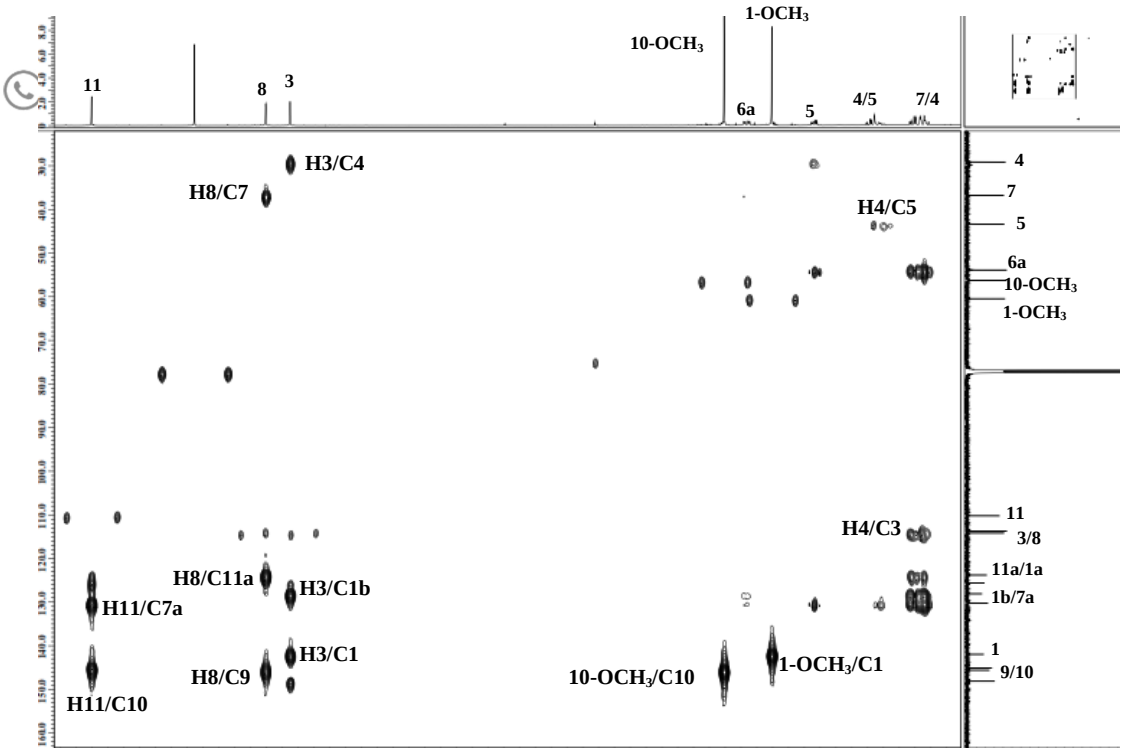


Figure 4.23. HMBC spectrum of AP 4.

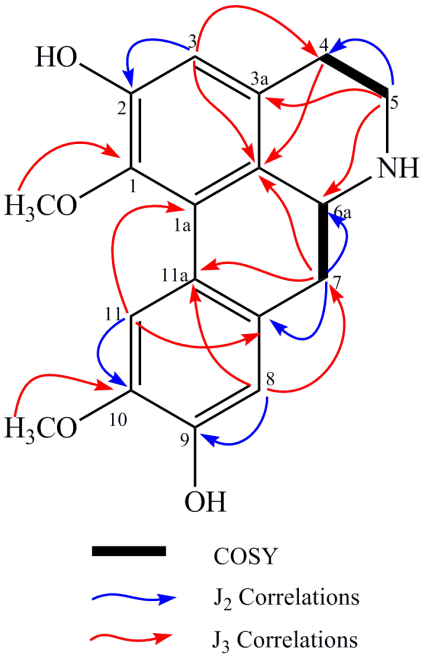


Figure 4.24. Selected COSY and HMBC correlation in AP 4.

Table 4.9

1D (^{1}H and ^{13}C) and 2D (HMQC and HMBC) NMR Spectral Data of AP 4

Position	^{1}H $CDCl_3$ (*J*, Hz)	^{13}C (δ, $CDCl_3$)	HMQC	HMBC
1		141.9		
1a		125.6		
1b		128.1		
2		148.1		
3	6.65 (*s*)	113.7	H_3	1,1b,4
3a		130.2		
4	2.96 (*m*) 2.67 (*m*)	29.1	H_4	1b,3,5
5	3.33 (*m*) 3.00 (*dd*,12.3, 3.5)	43.3	H_5	3a,4,6a
6a	3.77 (*dd*, 13.8, 4.6)	53.8	H_{6a}	1b,7
7	2.65 (*m*) 2.72 (*dd*, 14.3, 4.6)	36.8	H_7	1b,6a,7a,11a
7a		130.1		
8	6.81 (*s*)	114.2	H_8	7,9,11a
9		145.1		
10		145.6		
11	7.91 (*s*)	110.2	H_{11}	1a,7a,10
11a		123.8		
1-OCH_3	3.61 (*s*)	60.4	$3H_{1\text{-}OCH_3}$	1
10-OCH_3	3.91 (*s*)	56.2	$3H_{10\text{-}OCH_3}$	10

Table 4.10

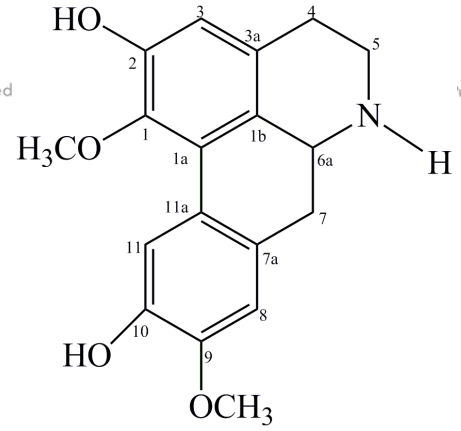
*^{1}H NMR Data of AP 4 and Norboldine **78***

Position	^{1}H $CDCl_3$ (*J*, Hz)		
	AP 4	Norboldine (Zahari, 2010)	Norboldine (Mukhtar, 1996)
3	6.65 (*s*)	6.60 (*s*)	6.65 (*s*)
4	2.96 (*m*) 2.67 (*m*)	2.60 (*m*) 2.90 (*m*)	2.80-3.20
5	3.33 (*m*) 3.00 (*dd*)	2.95 (*m*) 3.31 (*m*)	
6a	3.77 (*dd*)	3.74 (*dd*)	
7	2.65 (*m*) 2.72 (*dd*)	2.68 (*m*)	
8	6.81 (*s*)	6.73 (*s*)	6.67 (*s*)
11	7.91 (*s*)	7.90 (*s*)	7.91 (*s*)
1-OCH_3	3.61 (*s*)	3.58 (*s*)	3.60 (*s*)
10-OCH_3	3.91 (*s*)	3.85 (*s*)	3.80 (*s*)

4.2 Isolation of *Alseodaphne corneri* Kosterm

The roots of *Alseodaphne corneri* (3.0 kg) was collected in University of Malaya and going through further extraction and isolation process by various chromatographic techniques. The structural elucidation yielded two aporphines; laetanine **30** and boldine **69** and five bisbenzylisoquinoline alkaloids; gyrolidine **47**, stephasubine **92**, 2-norobaberine **93**, 3′,4′-dihydrostephasubine **94** and *O*-methyllimacusine **95**.

4.2.1 AC 1, Laetanine 30



30

Alkaloid AC 1 (5.3 mg) was obtained as a dark brownish amorphous solid which showed positive result on Dragendroff's tests. Its UV spectrum showed absorption band at 282 nm suggested 1,2,9,10-tetrasubstituted aporphine skeleton (Sangster & Stuart, 1965). The IR spectrum showed broad band between 3000 to 3500 cm^{-1} due to the presence of OH and NH functional groups in the structure. Its also showed strong absorption at 1015 cm^{-1} due to stretching of C-N (amine) group (Williams & Fleming,

1989). The EIMS spectrum exhibited ion peak at m/z 313 relevant with suggested molecular formula of $C_{18}H_{19}NO_4$.

The ^{1}H NMR spectrum (Figure 4.25) showed two singlets at δ 3.59 and 3.88 proving the existence of two methoxyl groups at C-1 and C-9 at ring A and ring D. Only three aromatic singlet protons were observed and confirmed to be H-3, H-8 and H-11 at δ 6.62, 6.78 and 7.89. This observation indicated that ring A and ring D were di-substituted with hydroxyl and methoxyl groups. In addition, the downfield shift of H-3 and H-11 protons had proved that the OH groups are located at C-2 and C-10 positions, respectively. The aliphatic protons of H-4, H-5, H-6a and H-7 resonated as multiplets at δ 2.68-3.81. The COSY spectrum (Figure 4.26) showed cross-peak between H4/H5 and H6a/H7.

The ^{13}C NMR spectrum (Figure 4.27) showed presence of eighteen carbons in the molecule and showed two peaks at δ 56.2 and 60.3 belongs to 9-OCH_3 and 1-OCH_3, respectively. In addition, a signal at δ 53.6 was attributable to C-6a. Another three methylene carbons were resonated at δ 28.1, 35.9 and 42.8 may assigned to C-4, C-7 and C-5. Carbons in the aromatic region (C-3, C-8 and C-11) were resonated at δ 113.8, 114.4 and 110.5, respectively.

The HMQC spectrum of AC 1 was shown in Figure 4.28. The structure of AC 1 was further confirmed by the HMBC experiment as shown in Figure 4.29. The correlations between 1-OCH_3/C-1 and 9-OCH_3/C-9 were observed and further confirmed the position of the methoxyl groups in the molecule. The spectrum also

showed cross peaks of H-3 with C-1, C-1b, C-2 and C-4; H-4 with C-3 and C-3a; and H-8 with C-7, C-9 and C-11a (Figure 4.29 and 4.30).

Complete spectral data of ^{1}H NMR, ^{13}C NMR, HMQC and HMBC are tabulated in Table 4.11. moreover, Table 4.12 shows the comparison of those spectral data with the literature of laetanine **30** that previously isolated from *Phoebe tavoyana* and *Litsea laeta* (Omar, 2009; Borthakur & Rastogi, 1979).

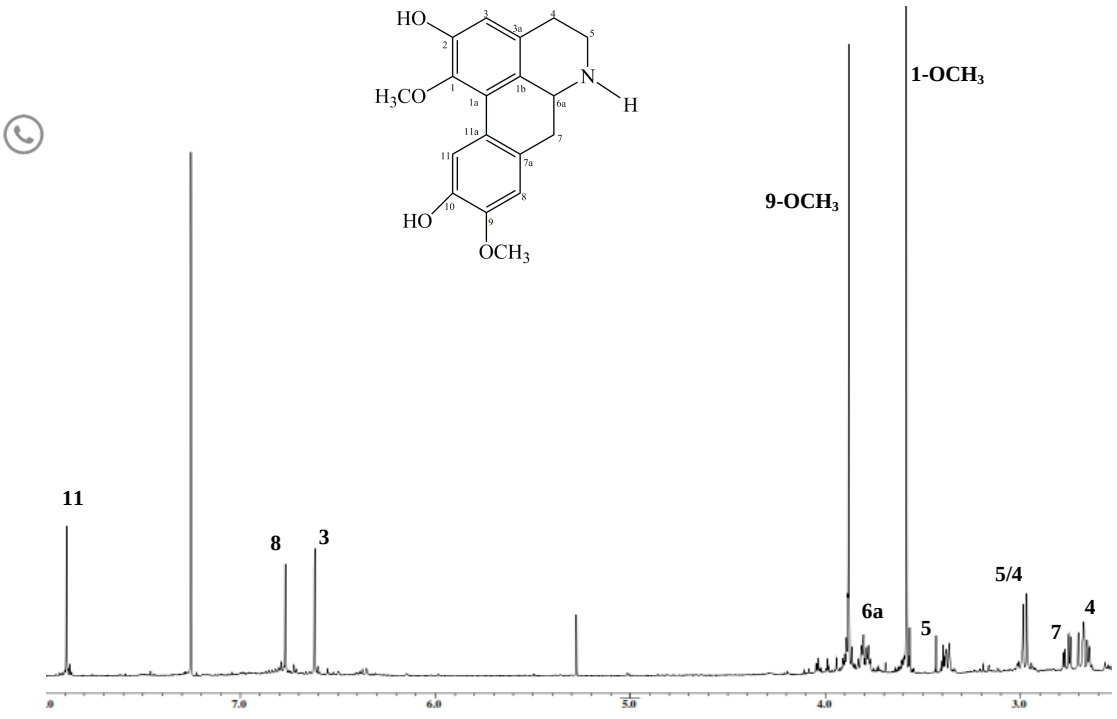


Figure 4.25. ^{1}H NMR spectrum of AC 1.

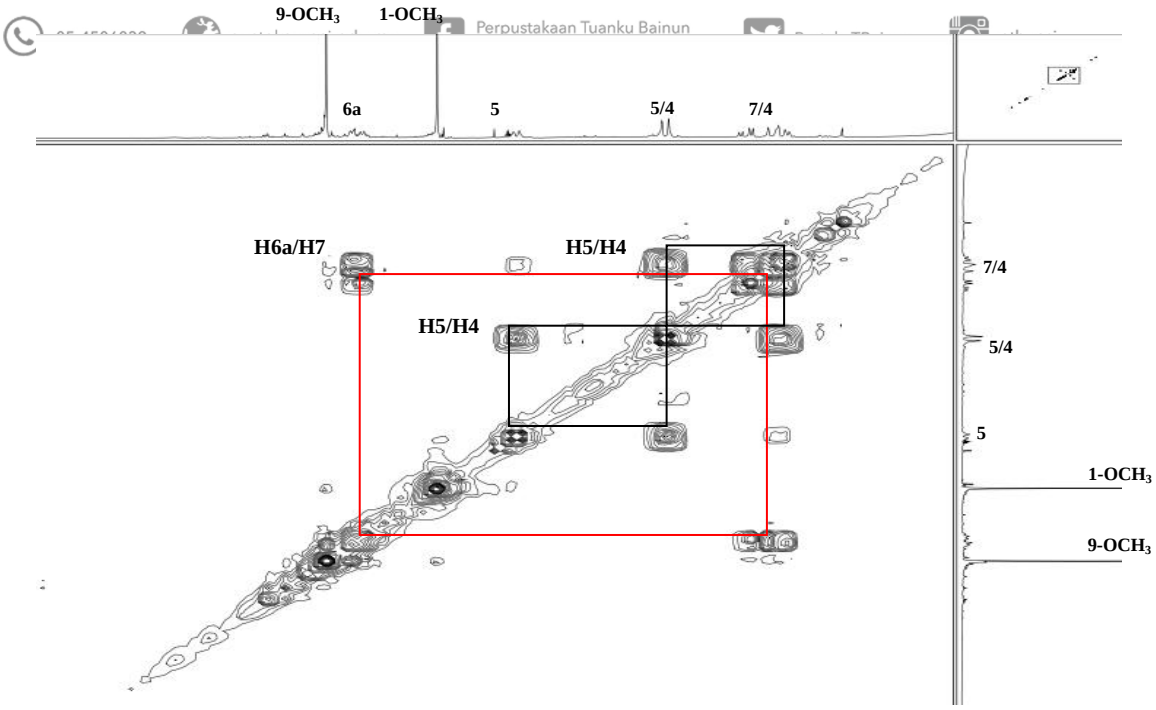


Figure 4.26. COSY spectrum of AC 1.

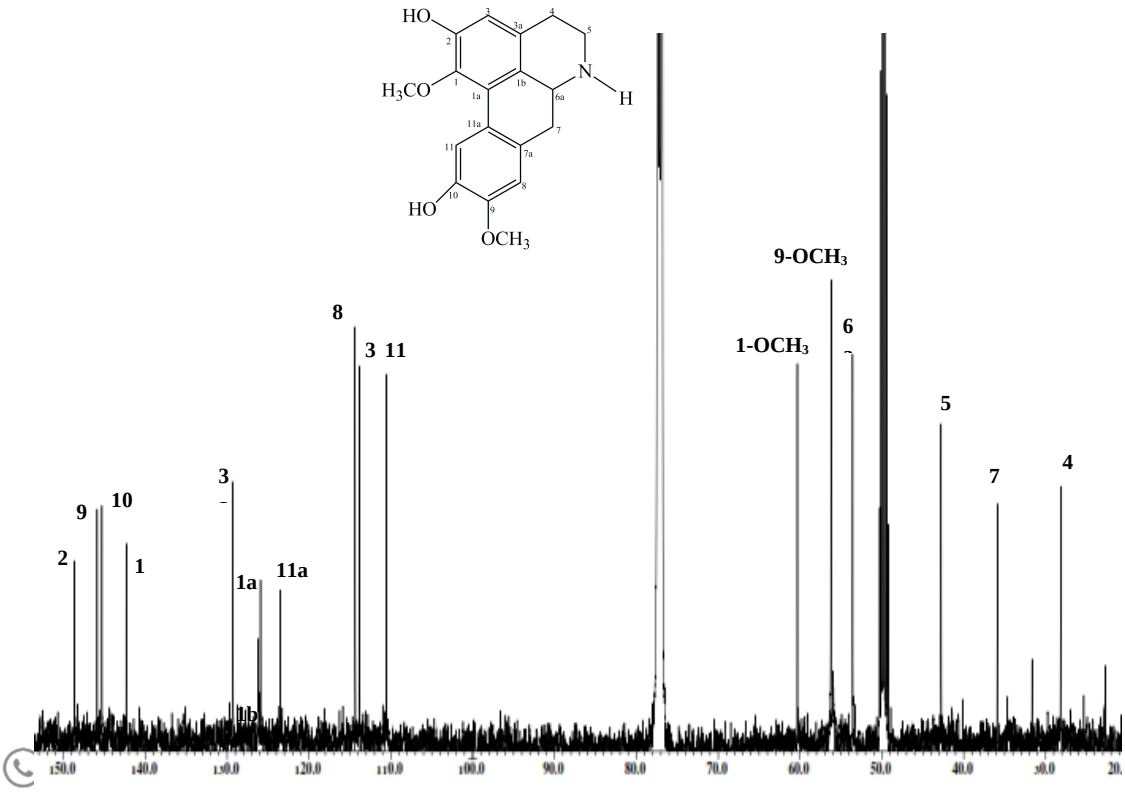


Figure 4.27. ^{13}C NMR spectrum of AC 1.

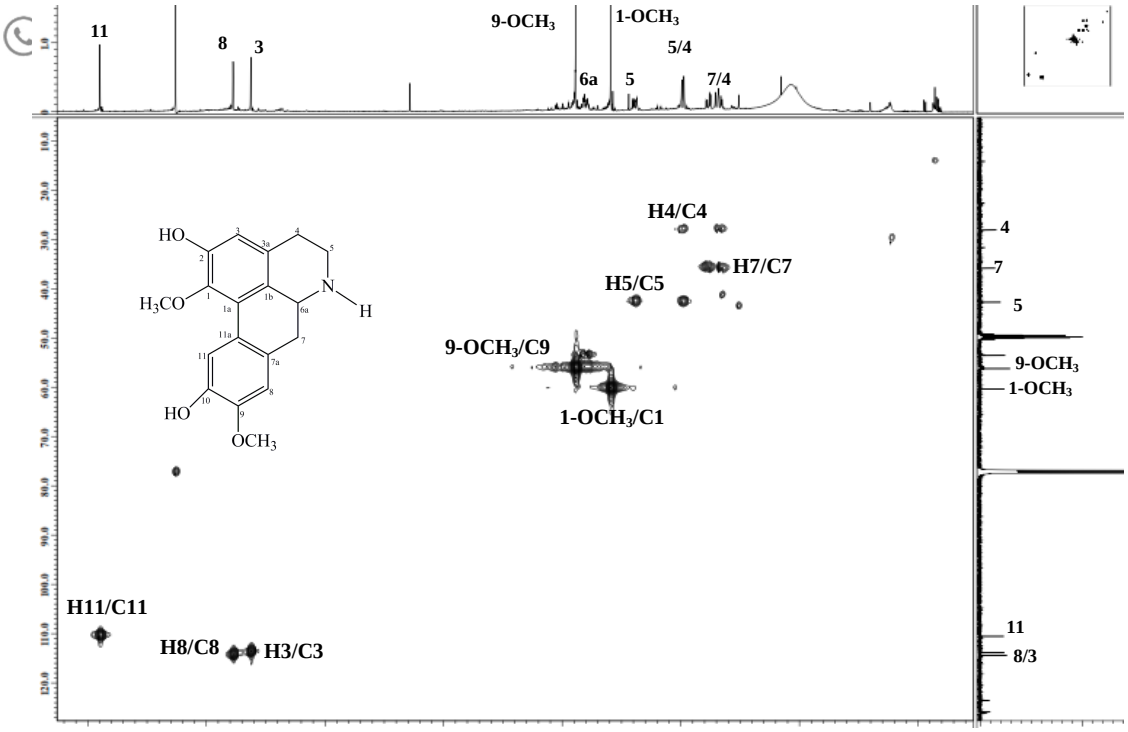


Figure 4.28. HMQC spectrum of AC 1.

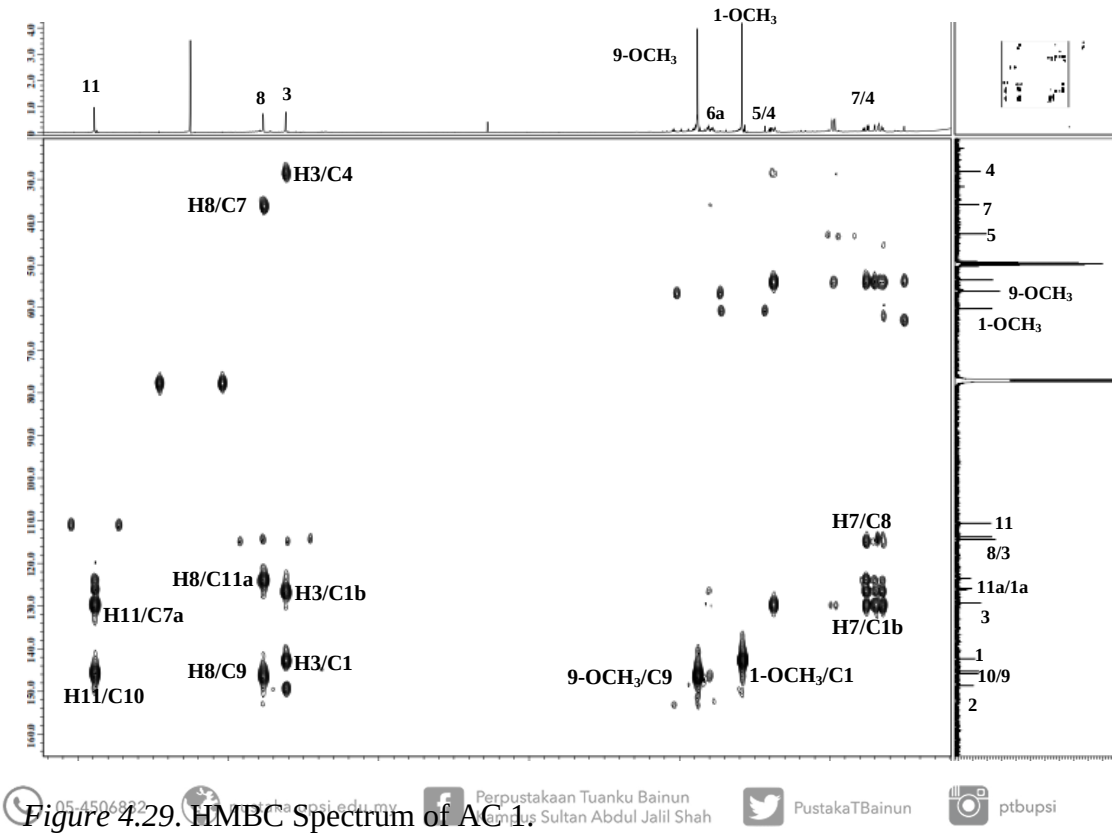


Figure 4.29. HMBC Spectrum of AC 1.

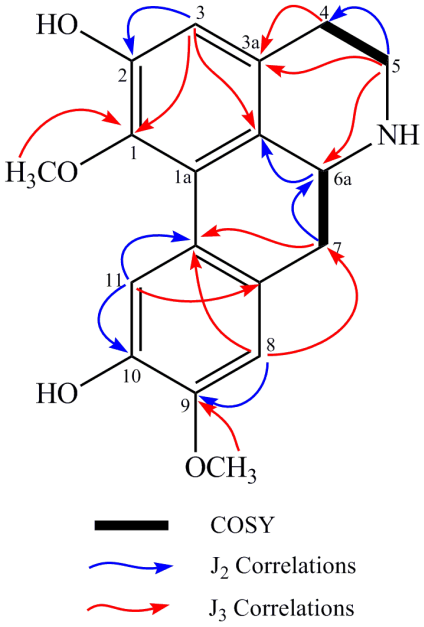


Figure 4.30. Selected COSY and HMBC correlation in AC 1.

Table 4.11

1D (^{1}H and ^{13}C) and 2D (HMQC and HMBC) NMR Spectral Data of AC 1

Position	^{1}H $CDCl_3$ (*J*, Hz)	^{13}C (δ, $CDCl_3$)	HMQC	HMBC
1		142.3		
1a		126.1		
1b		125.9		
2		148.6		
3	6.62 (*s*)	113.8	H_3	1,1b,2,4
3a		129.3		
4	2.68 (*m*) 2.97 (*s*)	28.1	H_4	3,3a
5	2.99 (*s*) 3.39 (*m*)	42.8	H_5	3a,4,6a
6a	3.81 (*dd*,11.1, 5.8)	53.6	H_{6a}	1b
7	2.68 (*m*) 2.77 (*dd*, 15.5, 5.2)	35.9	H_7	1a,6a,7a,8,11a
7a		129.3		
8	6.78 (*s*)	114.4	H_8	7,9,11a
9		145.9		
10		145.3		
11	7.89 (*s*)	110.5	H_{11}	7a,10,11a
11a		123.5		
1-OCH_3	3.59 (*s*)	60.3	$3H_{1\text{-}OCH_3}$	1
9-OCH_3	3.88 (*s*)	56.2	$3H_{9\text{-}OCH_3}$	9

Table 4.12

^{1}H NMR Data of AC 1 and Laetanine ***30***

Position	^{1}H $CDCl_3$ (*J*, Hz)		
	AC 1	Laetanine (Omar, 2009)	Laetanine (Borthakur & Rastogi, 1979)
3	6.62 (*s*)	6.56 (*s*)	6.65 (*s*)
4	2.68 (*m*) 2.97 (*s*)	2.67 (*m*) 2.96 (*m*)	*
5	2.99 (*s*) 3.39 (*m*)	2.94 (*m*) 3.32 (*m*)	*
6a	3.81 (*dd*)	3.77 (*dd*)	4.15 (*dd*)
7	2.68 (*m*) 2.77 (*dd*)	2.62 (*dd*) 2.75 (*dd*)	*
8	6.78 (*s*)	6.71 (*s*)	6.77 (*s*)
11	7.89 (*s*)	7.87 (*s*)	7.91 (*s*)
1-OCH_3	3.59 (*s*)	3.54 (*s*)	3.60 (*s*)
9-OCH_3	3.88 (*s*)	3.83 (*s*)	3.80 (*s*)

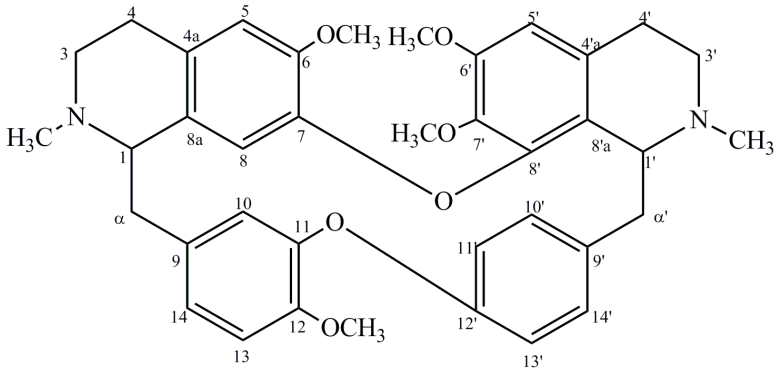
Note. * = not available

4.2.2 AC 2, Boldine 69

Alkaloid AC 2 (10.2 mg) was isolated as brownish amorphous and the UV spectrum revealed absorption at 282 and 303 nm giving possible skeleton of 1,2,9,10-tetrasubstituted aporphine (Sangster & Stuart, 1965). The IR spectrum showed absorption at 3450 cm^{-1} due to the presence of conjugated hydroxyl group in the compound. AC 2 was confirmed to be boldine **69** after detailed analysis on the NMR spectrum obtained. The NMR spectral data of AC 2 was similar to AP 1 that also previously reported as boldine **69** (page 63).

Please refer 4.1.1 for further explanation on the structural elucidation.

4.2.3 AC 3, Gyrolidine 47



47

Alkaloid AC 3 (4.2 mg) was obtained as yellow amorphous solid. The EIMS spectrum showed existence of molecular ion peak at m/z 622 approved the suggested molecular formula of $C_{38}H_{42}N_2O_6$. The UV spectrum displayed maximum absorption at 283 nm while the IR spectrum revealed absorption bands due to phenyl ether groups (1012 cm^{-1}) and aromatic rings (1639 cm^{-1}).

The ^{1}H NMR spectrum (Figure 4.31) showed four singlets owned by four methoxyl groups at δ 3.19 (7′-OCH_3), 3.63 (6-OCH_3), 3.78 (6′-OCH_3) and 3.89 (12-OCH_3), respectively. The singlet for 7′-OCH_3 was observed at the higher region due to the presence of the adjacent bulky substituents. A characteristic of two singlets with integration of three each at δ 2.61 and 2.69 assignable to *N*-CH_3 and *N*′-CH_3 protons.

The ^{1}H NMR spectrum also displayed a singlet at δ 5.49 corresponding to an aromatic proton attached to C-10 and another two doublets at δ 6.78 (*J*=8.6 Hz, H-13)

and 6.87 (*J*=7.5 Hz, H-14). From this observation, ring C was confirmed to be *meta-para* trisubstituted ring. Moreover, another four protons signals at the aromatic region resonated as doublet at δ 6.40 (*J*=4.6 Hz), 6.94 (*J*=2.3 Hz), 6.95 (*J*=2.3 Hz) and 7.48 (*J*=8.1 Hz) belongs to vicinal proton of H-11´, H-10´, H-13´and H-14´ approved that ring C´ was *para* disubstituted (Mukhtar et al., 2009).

The ^{13}C NMR spectrum (Figure 4.32) showed two signals at δ 63.8 and 61.6 belongs to C-1 and C-1´. Those signals have similar pattern to the type VI bisbenzylisoquinoline with two diaryl ether linkages that previously reported in Chapter 2 which is 2´-noroxyacanthine **48** (Herath et al., 1987). Four methoxyl carbon signals resonated at δ 55.0, 55.9, 56.0 and 60.5 which corresponding to C-6, C-12, C-6´and C-7´, respectively. The signals of carbons that contained nitrogen atoms (*N*-CH_3 and *N*´-CH_3) were observed at δ 41.7 and 43.3.

Among fourteen quaternary carbons, eight of them are substituted quaternary carbons which are C-6, C-7, C-11, C-12, C-6´, C-7´, C-8´ and C-12´ that resonated to much lower region compared to non-substituted carbons at δ 148.6, 143.8, 149.0, 146.7, 151.9, 137.2, 147.5 and 152.3. This situation occur due to the presence of activating groups as the substituents bring the inductive effects to the adjacent carbons and increase the chemical shifts. The methylene carbons for C-α and C-α´were observed at δ 37.6 and 39.8 which typical for methylene positions (Zahari, 2010).

The COSY spectrum (Figure 4.33) showed the correlations of vicinal proton between H-1´/H-α´, H-3/H-4, H-1/H-α and H-3´/H-4´. In the NOESY spectrum, the cross peaks were observed between H-13/12-OCH_3, H-5/6-OCH_3 and H-5´/6´-OCH_3

thus confirming the positions of 12-OCH_3, 6-OCH_3 and 6´-OCH_3. Another two signals in the spectrum also showed the correlation between H-1 with *N*-CH_3 and H-1´ with *N´*-CH_3.

The structure of this alkaloid was further analyzed by the HMQC and HMBC experiments (Figure 4.34 and 4.35). Furthermore, the other selected COSY and HMBC correlations are shown in Figure 4.36.

The complete NMR spectral data are shown in Table 4.13. Finally, Table 4.14 and 4.15 show the comparison of the spectral data with the literature, thus confirmed that AC 3 is gyrolidine **47** (Chalandre et al., 1986; Zahari, 2010) .

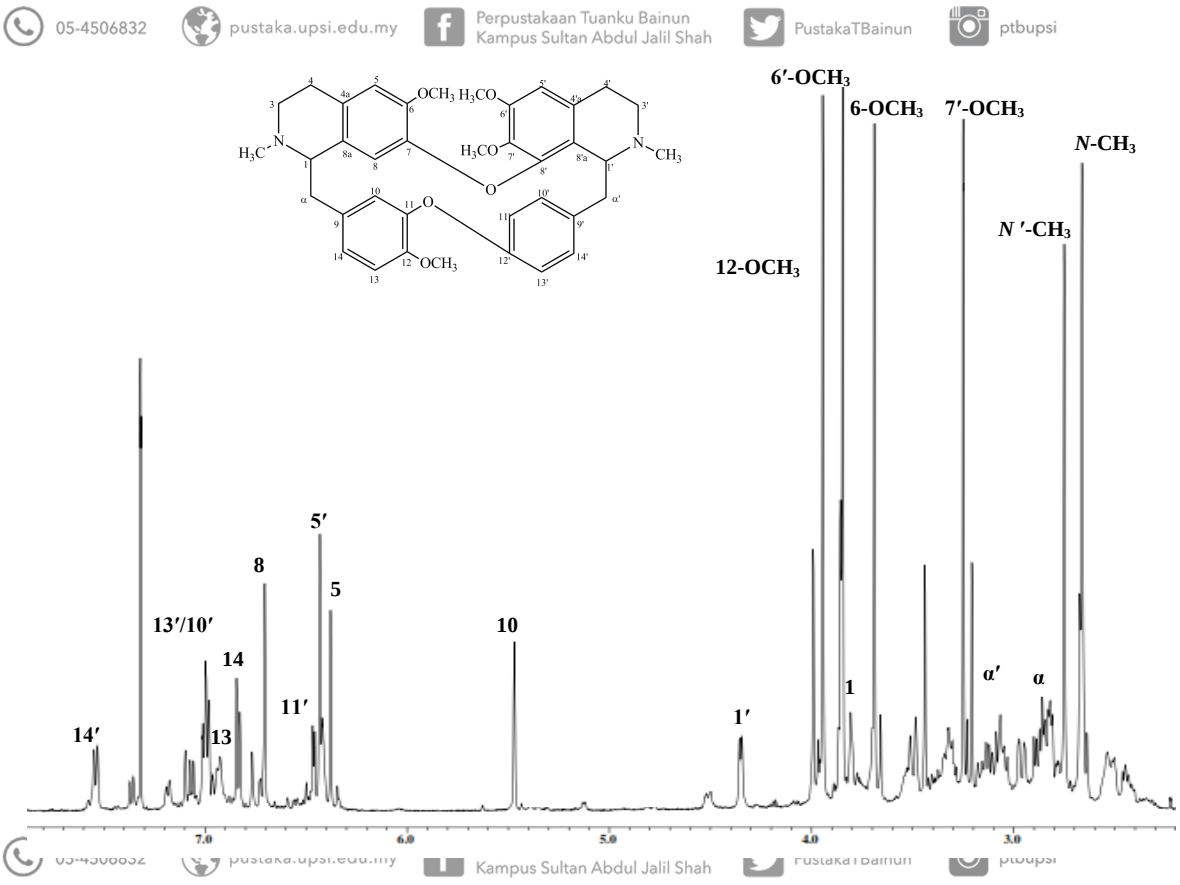


Figure 4.31. ^{1}H NMR spectrum of AC 3.

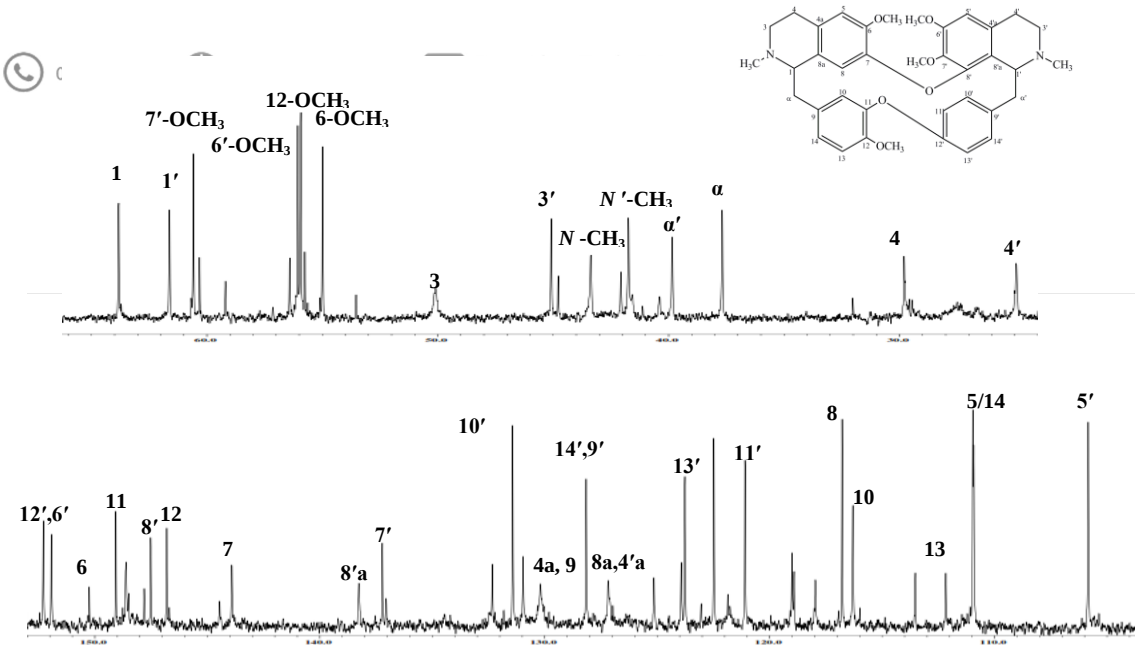


Figure 4.32. ^{13}C NMR spectrum of AC 3.

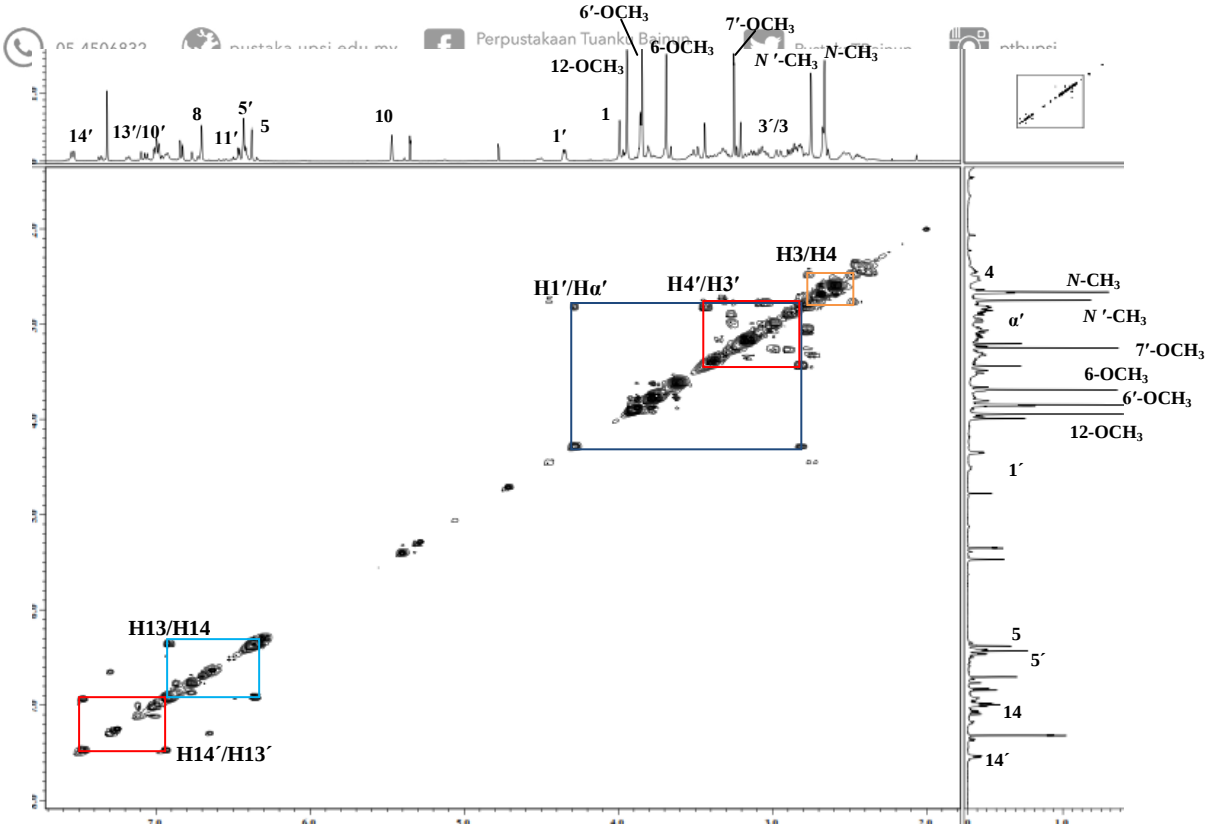


Figure 4.33. COSY spectrum of AC 3.

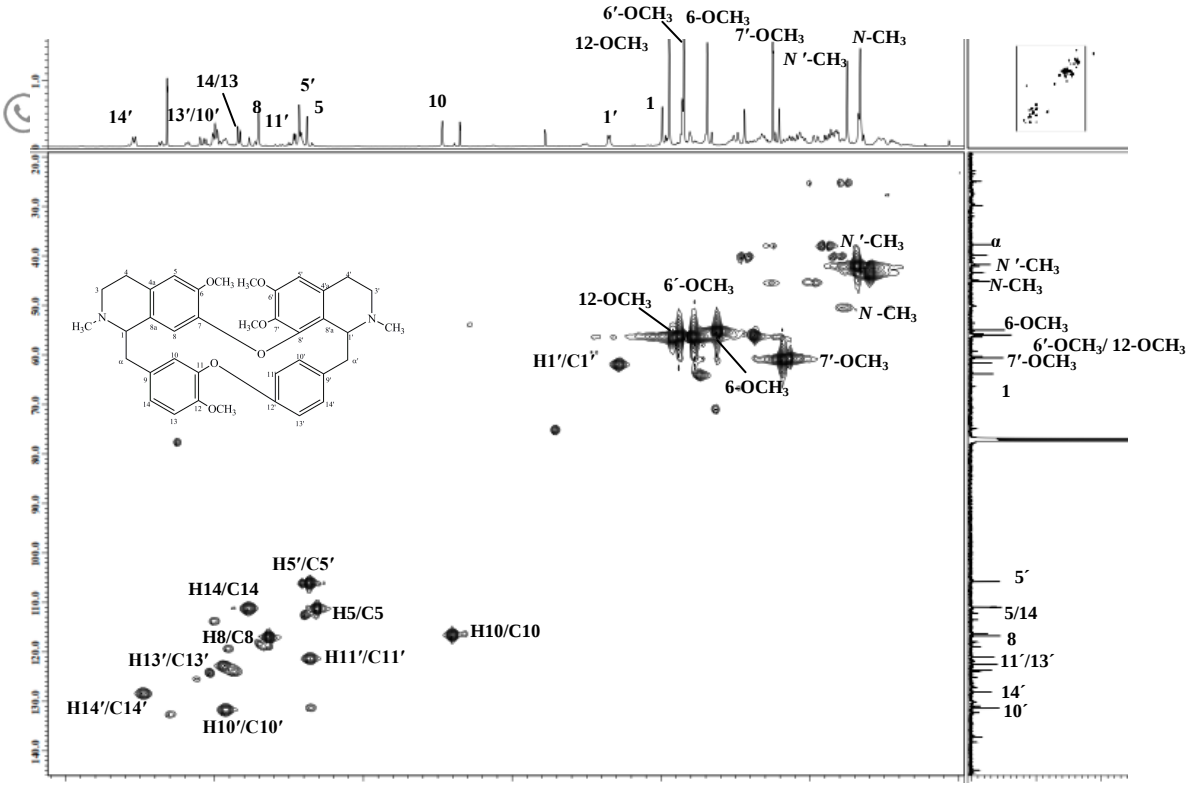


Figure 4.34. HMQC spectrum of AC 3.

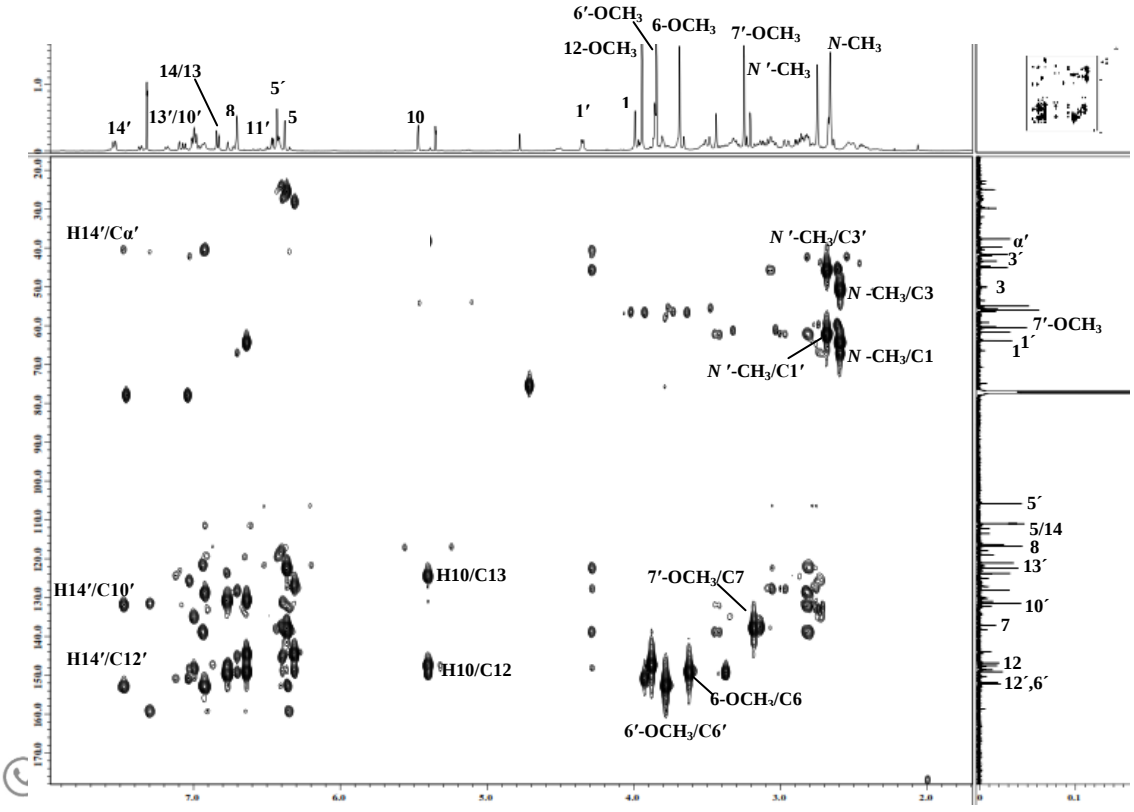


Figure 4.35. HMBC spectrum of AC 3.

Table 4.13

1D (^{1}H and ^{13}C) and 2D (HMBC) NMR Spectral Data of AC 3

Position	^{1}H $CDCl_3$ (*J*, Hz)	^{13}C (δ, $CDCl_3$)	HMBC
1	3.75 (*br s*)	63.8	
N-CH_3	2.61 (s)	43.3	1,3
3	2.45 (*m*) 2.76 (*m*)	50.1	
4	2.39 (*m*)	29.7	
4a		130.9	
5	6.32 (*s*)	110.9	4,6,7
6		148.6	
6-OCH_3	3.63 (*s*)	55.0	6
7		143.8	
8	6.65 (*s*)	116.7	1,6,7
8a		127.1	
α	2.90 (*dd*, 14.9,3.5) 3.24 (*m*)	37.6	14
9		130.9	
10	5.41 (*s*)	116.3	α,12,14
11		149.0	
12		146.7	
12-OCH_3	3.89 (s)	55.9	12
13	6.87 (*d*, 7.5)	110.9	12
14	6.78 (*d*, 8.6)	123.5	11
1′	4.29 (*d*, 6.3)	61.6	α′,3′,8′
N′-CH_3	2.69 (*s*)	41.7	1′,3′
3′	3.00 (*m*) 3.26 (*m*)	45.1	
4′	2.75 (*m*) 3.07 (*m*)	24.9	8′a
4′a		127.1	
5′	6.37 (*s*)	105.8	4′,6′,7′
6′		151.9	
6′-OCH_3	3.78 (*s*)	56.0	6′
7′		137.2	
7′-OCH_3	3.19 (*s*)	60.5	
8′		147.5	
8′a		138.2	
α′	2.80 (*m*) 3.42 (*m*)	39.8	9′,8′a
9′		128.1	
10′	6.94 (*d*, 2.3)	131.4	α′,12′,14′
11′	6.40 (*d*, 4.6)	121.1	
12′		152.3	
13′	6.95 (*d*, 2.3)	122.4	11′
14′	7.48 (*d*, 8.1)	128.1	α′,10′,12′

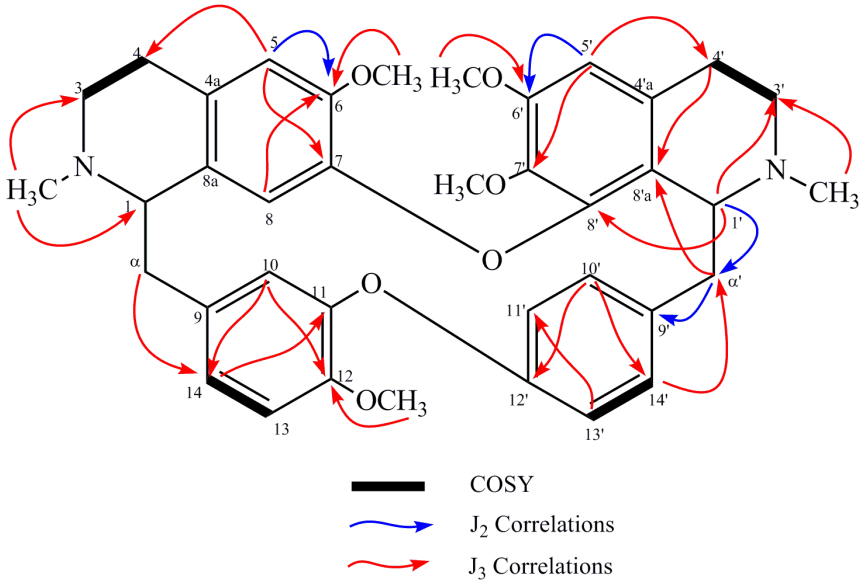


Figure 4.36. Selected COSY and HMBC correlation in AC 3.

Table 4.14

^{1}H NMR Data of AC 3 and Gyrolidine ***47***

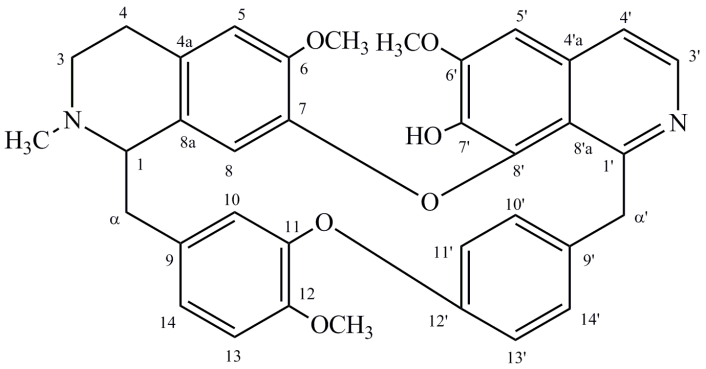
Position	^{1}H CDCl$_3$ (*J*, Hz)	
	AC 3	Gyrolidine (Chalandre et al., 1986)
N-CH$_3$	2.61 (*s*)	2.57
5	6.32 (*s*)	6.36
6-OCH$_3$	3.63 (*s*)	3.63
8	6.65 (*s*)	6.65
10	5.41 (*s*)	5.47
12-OCH$_3$	3.89 (*s*)	3.89
13	6.87 (*d*, 7.5)	6.78
14	6.78 (*d*, 8.6)	6.78
N′-CH$_3$	2.69 (*s*)	2.66
5′	6.37 (*s*)	6.32
6′-OCH$_3$	3.78 (*s*)	3.79
7′-OCH$_3$	3.19 (*s*)	3.19
11′	6.40 (*d*, 4.6)	6.37
13′	6.95 (*d*, 2.3)	6.95
14′	7.48 (*d*, 8.1)	7.42

Table 4.15

^{13}C NMR Data of AC 3 and Gyrolidine **47**

Position	^{13}C (δ, $CDCl_3$)	
	AC 3	Gyrolidine (Zahari, 2010)
1	63.8	63.9
N-CH_3	43.3	43.7
3	50.1	50.8
4	29.7	28.4
4a	130.9	130.7
5	110.9	110.9
6	148.6	148.3
6-OCH_3	55.0	54.9
7	143.8	143.8
8	116.7	116.7
8a	127.1	127.3
α	37.6	37.5
9	130.9	130.7
10	116.3	116.4
11	149.0	149.0
12	146.7	146.6
12-OCH_3	55.9	55.9
13	110.9	110.7
14	123.5	123.5
1′	61.6	61.5
N′-CH_3	41.7	42.1
3′	45.1	45.3
4′	24.9	25.4
4′a	127.1	127.2
5′	105.8	105.7
6′	151.9	151.6
6′-OCH_3	56.0	56.0
7′	137.2	137.0
7′-OCH_3	60.5	60.5
8′	147.5	147.5
8′a	138.2	138.9
α′	39.8	39.5
9′	128.1	127.7
10′	131.4	131.4
11′	121.1	121.1
12′	152.3	152.2
13′	122.4	122.3
14′	128.1	127.8

4.2.4 AC 4, Stephasubine 92



92

Alkaloid AC 4 (5.4 mg) was obtained as a yellow amorphous. The EIMS spectrum showed a significant molecular ion peak at m/z 590 giving a possible molecular formula of $C_{36}H_{34}N_2O_6$. The UV spectrum showed maximum absorption at 207 and 236 nm. In addition, the IR spectrum gave a broad band and showed absorption at 3286 cm^{-1} due to the presence of a highly conjugated hydroxyl group (Pretsch et al., 2000).

The ^{1}H NMR spectrum (Figure 4.37) showed the presence of one *N*-CH_3 singlet at δ 2.67. The six methoxyl protons of 6-OCH_3 and 6-OCH_3 were overlapped at δ 4.03 and appeared as higher singlets than other methoxyl of 12-OCH_3 (δ 3.87). Two sets of doublets were observed at farthest downfield at δ 8.43 and 7.47 corresponding to H-3′ and H-4′ due to the presence of adjacent nitrogen atom with coupling constant of 5.8 Hz. Moreover, due to the same circumstance, the H-α′ dishielded to lower field as doublets of doublets at δ 4.50 and 5.36 compared to H-α at δ 2.42 and 3.00.

The spectrum also exhibited four vicinal protons of H-10′, H-11′, H-13′ and H-14′ at δ 7.0, 6.62, 6.47 and 7.40. The signals of H-14′ at δ 7.40 showed a characteristic peak of tail to tail bisbenzylisoquinoline with two diaryl ether bridges (7-8′, 11-12′) (Nelofar, 1989). Furthermore, the methylenes protons of H-3 and H-4 resonated as multiplets at δ 2.25-2.68.

The assignment of aromatic protons were supported by COSY spectrum (Figure 4.38) which showed the cross peaks between H-3′/H-4′, H-10′-H-11′ and H-13′/H-14′.

The ^{13}C NMR spectrum (Figure 4.39) revealed the presence of thirty six carbons signals. The methoxyls C-6′, C-6 and C-12 resonated at δ 56.1, 56.2 and 56.7, respectively. In addition, a methyl group attached to the nitrogen atom (*N*-2) resonated at δ 43.2. The spectrum also showed the chemical shifts of fifteen aromatic carbons between δ 119.3 to 157.3. Moreover, C-1′ resonated far from the other aromatic carbons at δ 157.3 as it is near to the nitrogen atom which is the electronegative element and potent to have low electron density and shifts the signals downfield.

The HMQC spectrum (Figure 4.40) showed direct correlations of carbons and hydrogens in the compound. The HMBC spectrum (Figure 4.41) revealed cross peaks of H-3′ with C-4′, C-4′a and C-1′; H-14′ with C-α′, C-10′ and C-12′; and H-10 with C-α, C-11 and C-14, respectively. In addition, from the HMBC spectrum also confirmed the characteristic of tail to tail bisbenzylisoquinoline with two diaryl ether bridges (7-8′, 11-12′) by cross peaks between H-10 with C-11; H-11′ with C-12′; and

H-8 with C-7. The other selected COSY and HMBC correlations are illustrated in Figure 4.42.

The spectral data of AC 4 are recorded in Table 4.16. Comparison of the collected data with the literature (Table 4.17) further confirmed that alkaloid AC 4 is stephasubine **92** (Patra et al., 1986).

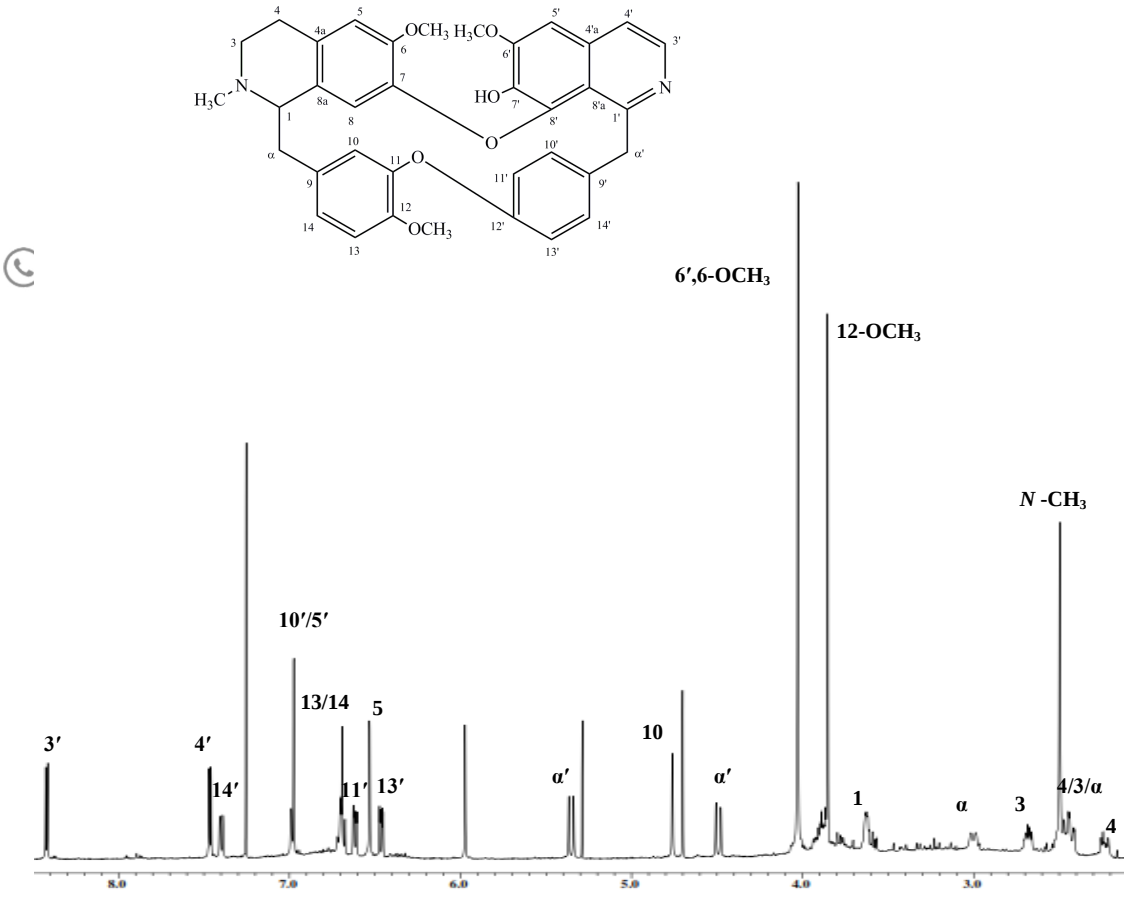


Figure 4.37. ^{1}H NMR spectrum of AC 4.

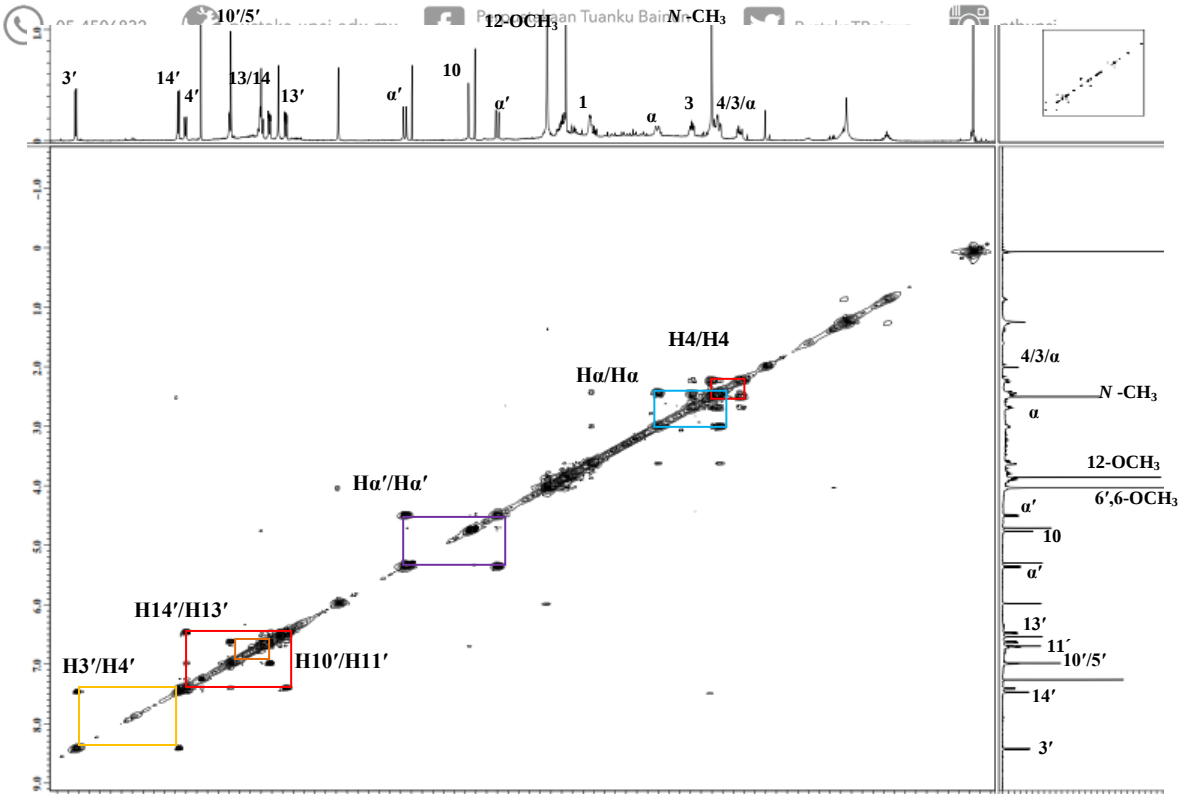


Figure 4.38. COSY spectrum of AC 4.

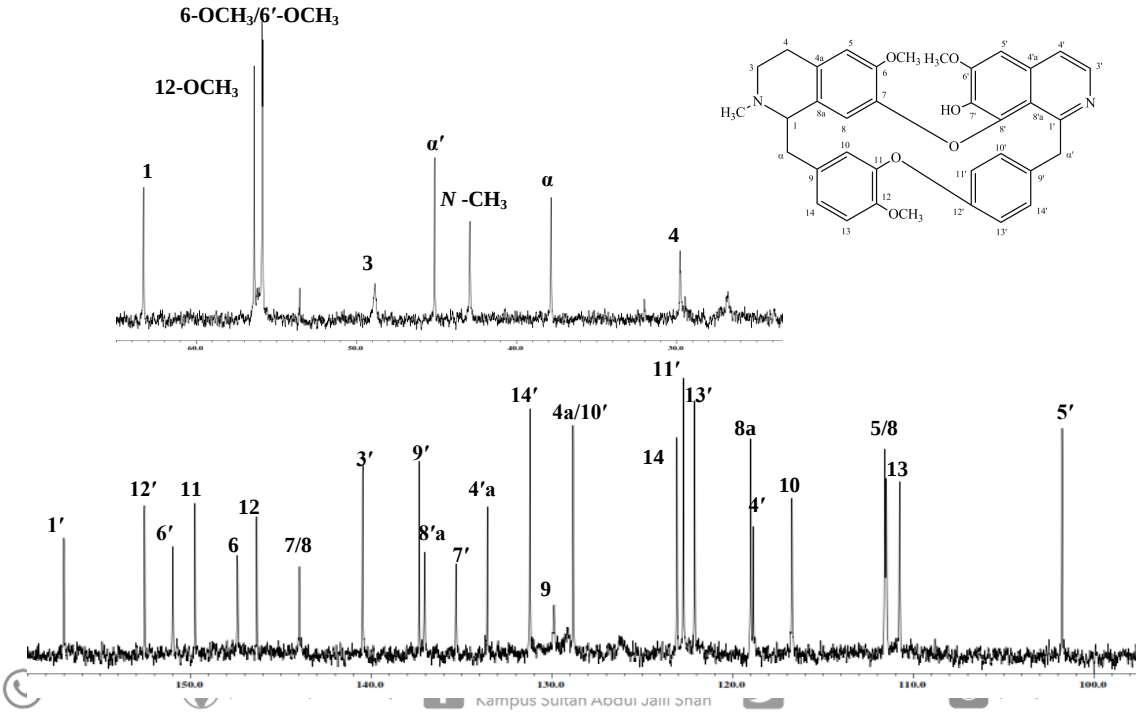


Figure 4.39. ^{13}C NMR spectrum of AC 4.

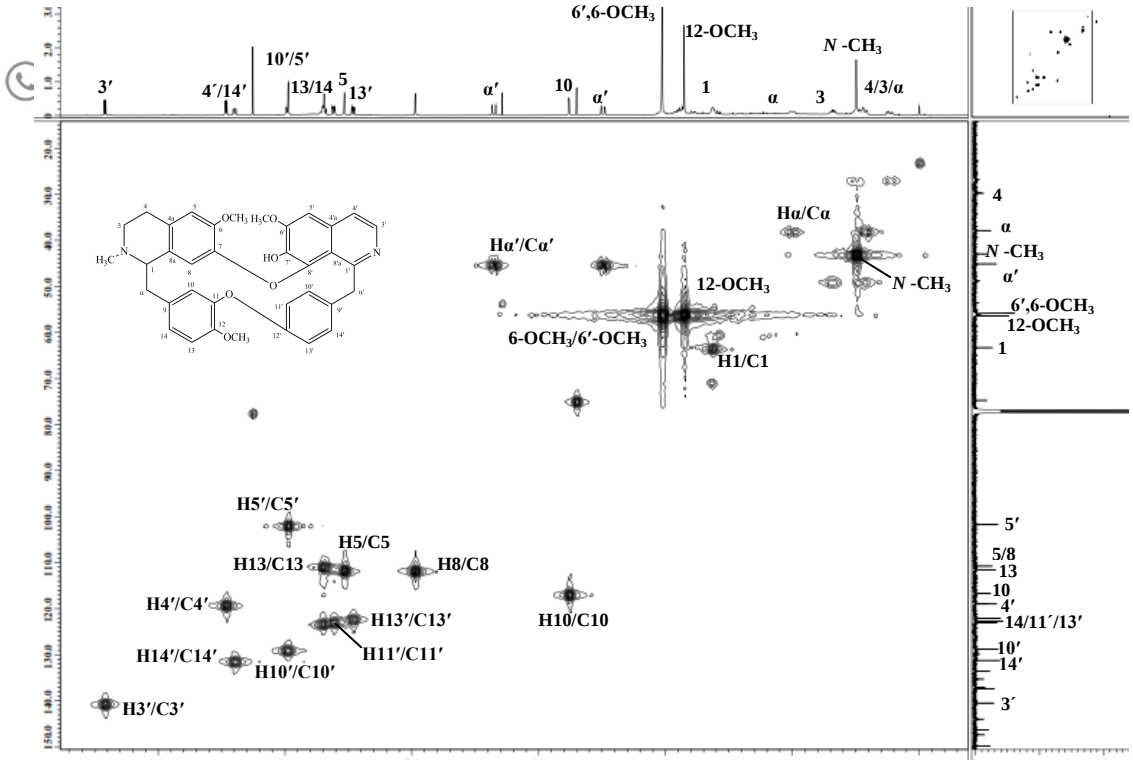


Figure 4.40. HMQC spectrum of AC 4.

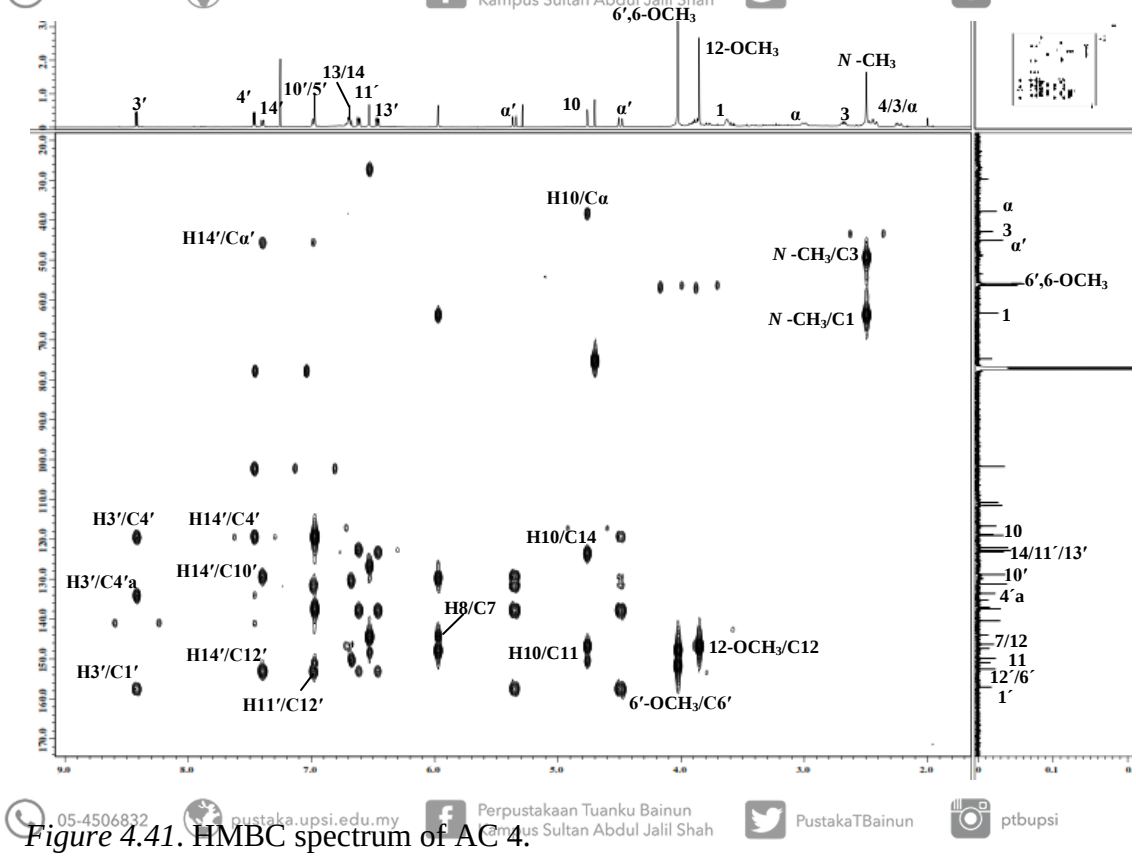


Figure 4.41. HMBC spectrum of AC 4.

Table 4.16

1D (^{1}H and ^{13}C) and 2D (HMBC) NMR Spectral Data of AC 4

Position	^{1}H $CDCl_3$ (*J*, Hz)	^{13}C (δ, $CDCl_3$)	HMBC
1	3.64 (*br s*)	63.6	
N-CH_3	2.67 (*s*)	43.2	1,3
3	2.45 (*d*, 4.1) 2.68 (*m*)	49.0	
4	2.25 (*m*) 2.50 (*m*)	27.0	
4a		129.1	
5	6.54 (*s*)	111.9	4,6,7
6		147.7	
6-OCH_3	4.03 (*s*)	56.2	6
7		144.3	
8	5.98 (*s*)	111.8	1,4a,6,7
8a		119.3	
α	2.42 (*d*, 2.9) 3.00 (*d*, 12.6)	38.1	
9		130.0	
10	4.77 (*s*)	117.0	α,11,12,14
11		150.0	
12		146.6	
12-OCH_3	3.87 (*s*)	56.7	12
13	6.71 (*d*, 9.1)	111.0	α,9,11
14	6.70 (*d*, 8.1)	123.4	
1′		157.3	
3′	8.43 (*d*, 5.8)	140.8	1′,4′,4′a
4′	7.47 (*d*, 5.8)	119.2	3′,4′a,5′
4′a		133.9	
5′	6.98 (*s*)	102.1	
6′		151.3	
6′-OCH_3	4.03 (*s*)	56.1	6′
7′		135.6	
8′		144.3	
8′a		137.3	
α′	4.50 (*d*, 13.7) 5.36 (*d*, 13.8)	45.4	1′,8′a,10′,14′
9′		137.6	
10′	7.0 (*br s*)	129.1	α′,12′,14′
11′	6.62 (*dd*, 8.6, 2.3)	123.0	9′,12′,13′
12′		152.8	
13′	6.47 (*dd*, 8.1, 2.3)	122.4	
14′	7.40 (*d*, 8.1)	131.5	α′,10′,12′

Table 4.17

^{1}H NMR Data of AC 4 and Stephasubine 92

Position	^{1}H $CDCl_3$ (*J*, Hz)	
	AC 4	Stephasubine (Patra et al., 1986)
1	3.64 (*br s*)	3.56 (*m*)
N-CH_3	2.67 (*s*)	2.51
3	2.45 (*d*, 4.1) 2.68 (*m*)	2.35 (*m*) 2.72 (*m*)
4	2.25 (*m*) 2.50 (*s*)	2.18 (*m*) 2.27 (*m*)
5	6.54 (*s*)	6.56
6-OCH_3	4.03 (*s*)	4.07 (*s*)
8	5.98 (*s*)	5.99
α	2.42 (*d*, 2.9) 3.00 (*d*, 12.6)	2.25 (*m*) 2.97 (*m*)
10	4.77 (*s*)	4.79 (*br s*)
12-OCH_3	3.87 (*s*)	3.88
13	6.70 (*d*, 9.1)	6.71 (*br s*)
14	6.71 (*d*, 8.1)	6.71 (*br s*)
3′	8.43 (*d*, 5.8)	8.45 (*d*, 5.6)
4′	7.47 (*d*, 5.8)	7.48 (*d*, 5.6)
5′	6.98 (*s*)	7.01
6′-OCH_3	4.03 (*s*)	4.07 (*s*)
α′	4.50 (*d*, 13.7) 5.36 (*d*,13.8)	4.52(*d*, 13.8) 5.37 (*d*, 13.8)
10′	7.0 (*br s*)	7.03 (*dd*, 8.4, 2.0)
11′	6.62 (*dd*, 8.6, 2.3)	6.65 (*dd*, 8.4, 2.0)
13′	6.47 (*dd*, 8.1, 2.3)	6.49 (*dd*, 8.4, 2.0)
14′	7.40 (*d*, 8.1)	7.43 (*dd*, 8.4, 2.0)

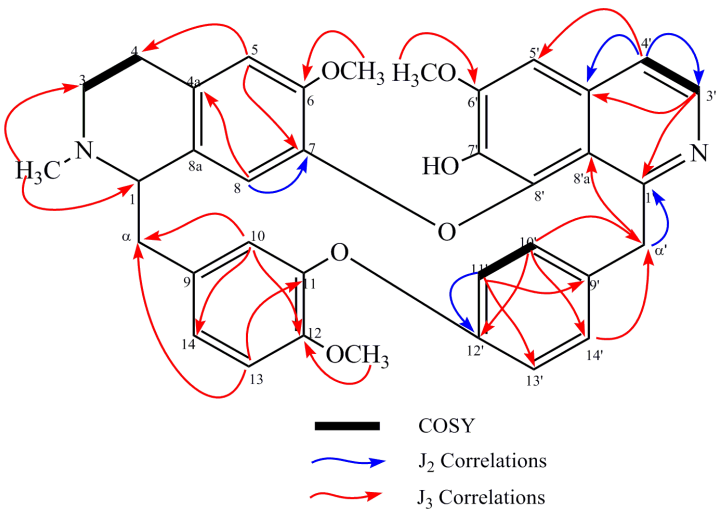
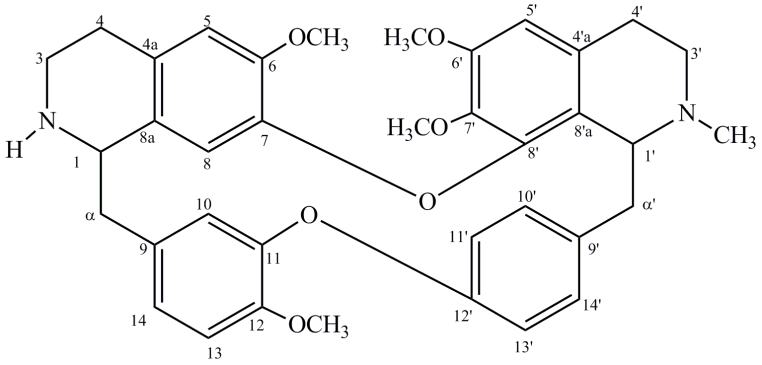


Figure 4.42. Selected COSY and HMBC correlation in AC 4.

4.2.5 AC 5, 2-Norobaberine 93



93

Alkaloid AC 5 (5.8 mg) was obtained as a brownish amorphous. The UV spectrum showed absorption maxima at 284 nm. The IR spectrum showed broad band at 3274 cm^{-1} due to the presence of NH functional groups in the structure. Its also showed strong absorption due to C=C stretching in the aromatic rings (Williams & Fleming, 1989). The EIMS data showed the presence of molecular ion peak at m/z 608 suggesting the molecular formula of $C_{37}H_{40}N_2O_6$.

The ^{1}H NMR spectrum (Figure 4.43) showed one singlet belongs to *N´*-CH_3 at δ 2.69. The bulky substituents within area between ring B and ring B´ deshielded 7´-OCH_3 at δ 3.22 compared to the other methoxyl groups of 6-OCH_3 (δ 3.63), 6´-OCH_3 (δ 3.79) and 12-OCH_3 (δ 3.90). The presence of germinal protons belongs to H-α and H-α´ as doublets of doublets and multiplets between δ 2.84-3.36. The presence of another three proton singlets at the downfield region at δ 6.36, 6.37 and 6.69 related to H-5, H-5´ and H-8, respectively. In addition, the *para* disubstituted ring C´ was confirmed by the presence of doublets and doublets of doublets signals at δ 6.85, 6.30, 6.98 and 7.50 corresponding to H-10´, H-11´, H-13´ and H-14´,

respectively. H-1 and H-1′ together appeared as a broad singlet and a doublet at δ 4.34 and 4.25.

The ^{13}C NMR spectrum (Figure 4.44) showed presence of thirty seven carbons resonances and showed four signals at δ 55.1, 55.9, 56.1 and 60.6 belongs to 6-OCH_3, 12-OCH_3, 6′-OCH_3 and 7′-OCH_3, respectively. A signal for a carbon attached to a nitrogen atom was observed at δ 41.9 corresponding to *N*′-CH_3. In addition, the DEPT spectrum showed the presence of six methylene carbons were observed at δ 25.1 (C-4′), 29.0 (C-4), 38.9 (C-α), 40.0 (C-α′), 41.0 (C-3) and 45.3 (C-3′). Finally, fourteen quaternary carbons appeared at downfield region at δ 123.9-148.8 as the diamagnetic anisotropy effect increases in the rings.

The assignment of protons was further confirmed by COSY experiment (Figure 4.45). The spectrum showed correlations of Hα/Hα, Hα′/Hα′, H1′/Hα′and H-4′/H-4′. The NOESY spectrum approved the position of methoxy groups when cross peaks were observed between H-14/12-OCH_3, H-5/6-OCH_3 and H-5′/6′-OCH_3.

The HMQC and HMBC spectrum (Figure 4.46 and 4.47) further confirmed the structure of AC 5. The spectrum revealed cross peaks between H-5 with C-4, C-4a, C-6 and C-7; H-13 with C-10 and C-12; and H-10′ with C-α′, C-12′ and C-14′. Furthermore, based on the HMBC correlation of H-13′and H-10′, its confirmed that C-12′ resonated at δ 151.9. The other HMBC and selected COSY correlations of AC 5 are illustrated in Figure 4.48.

The NMR spectral data of AC 5 are shown in Table 4.18. After comparison with the literature (Table 4.19), finally assigned alkaloid AC 5 as 2-norobaberine **93** (Tantisewie, Amurrio, Guinaudeau & Shamma, 1989).

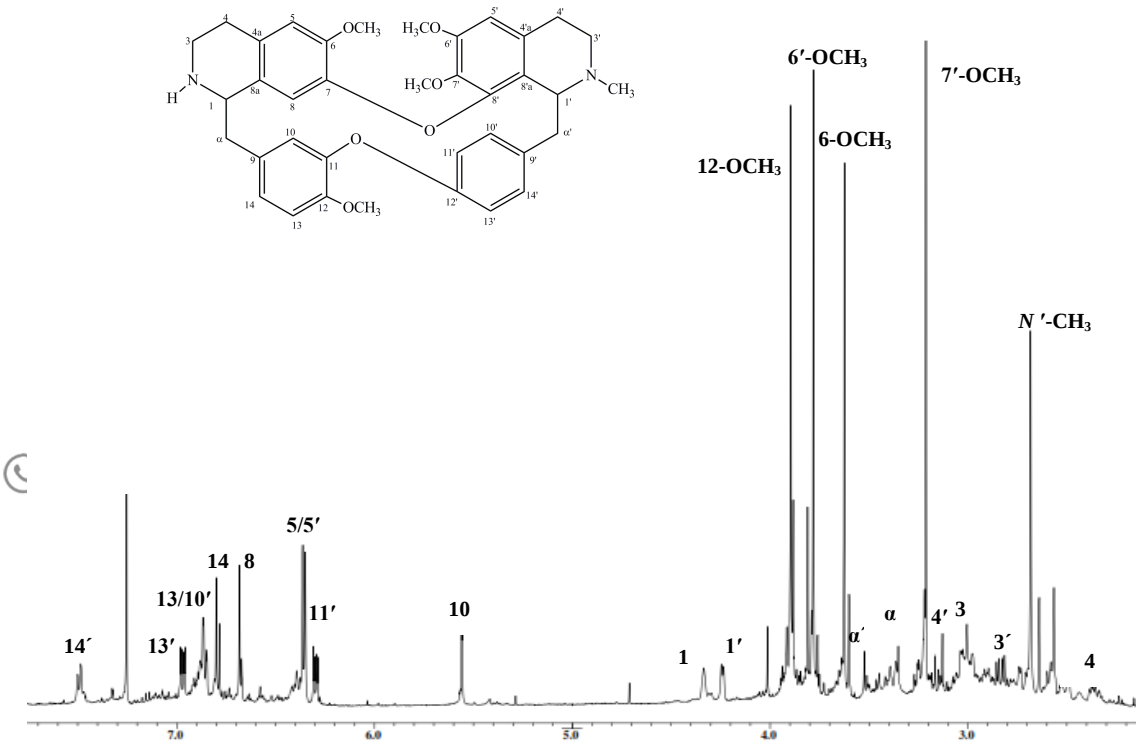


Figure 4.43. ^{1}H NMR spectrum of AC 5.

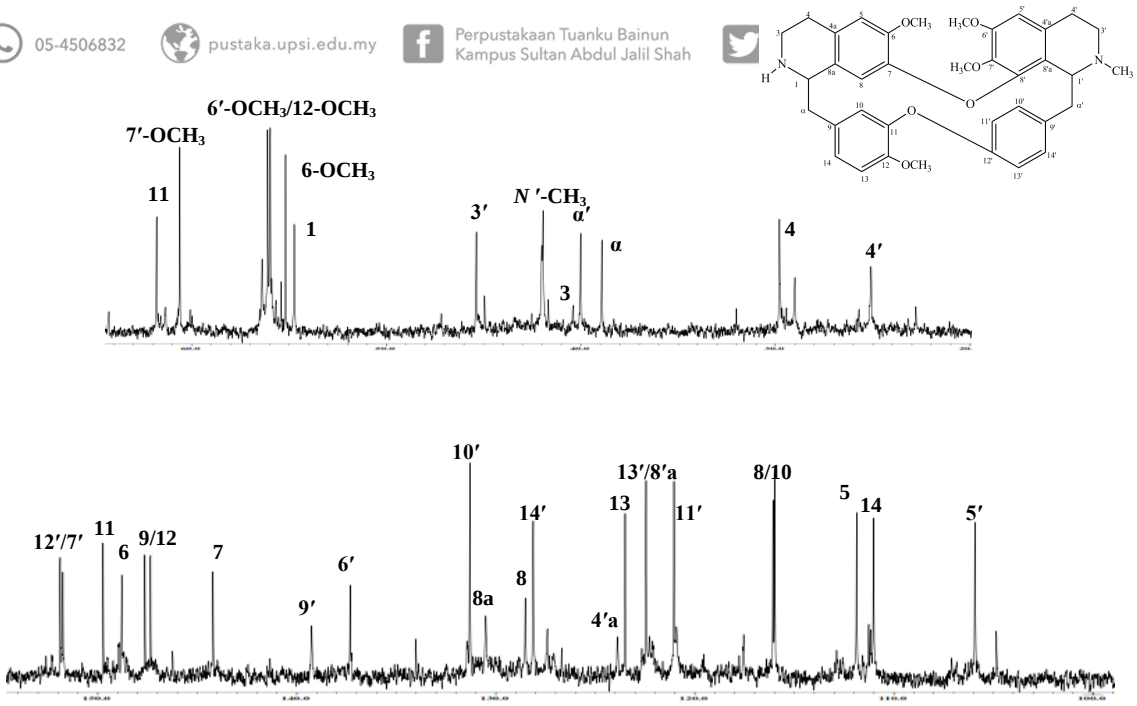


Figure 4.44. ^{13}C NMR spectrum of AC 5.

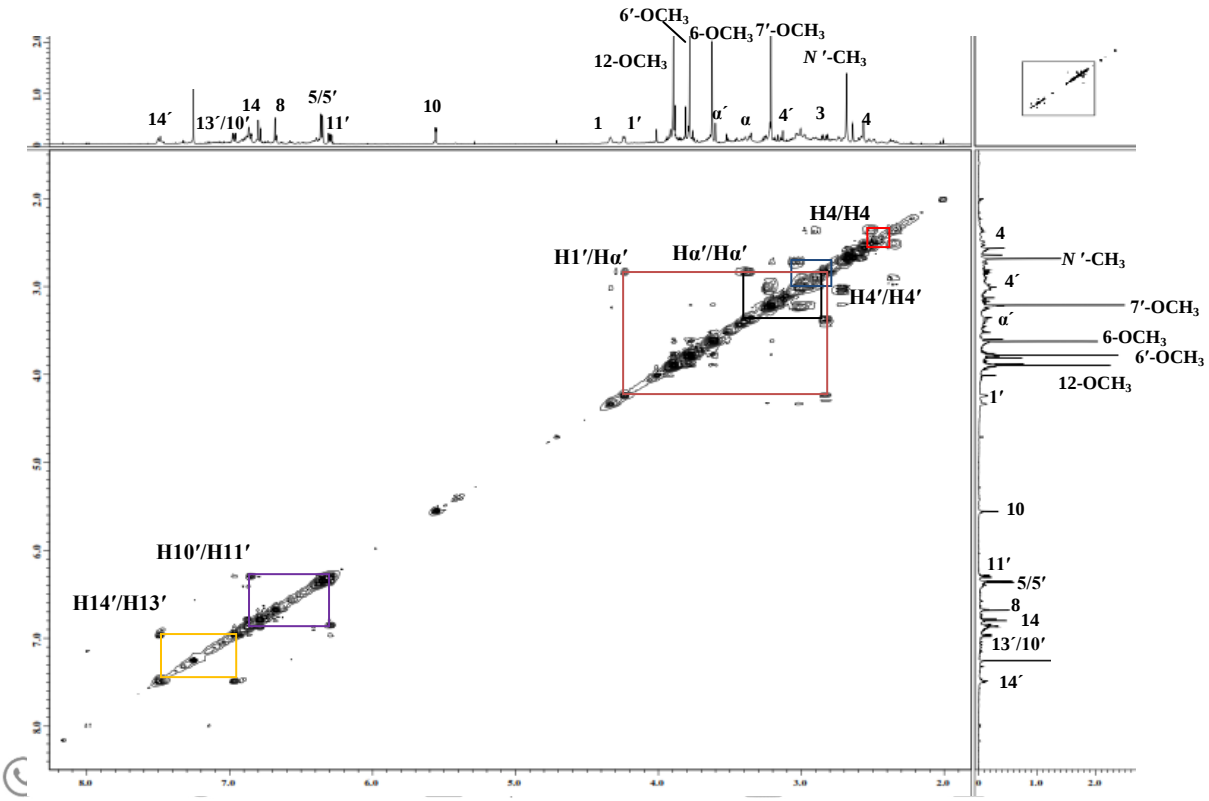


Figure 4.45. COSY spectrum of AC 5.

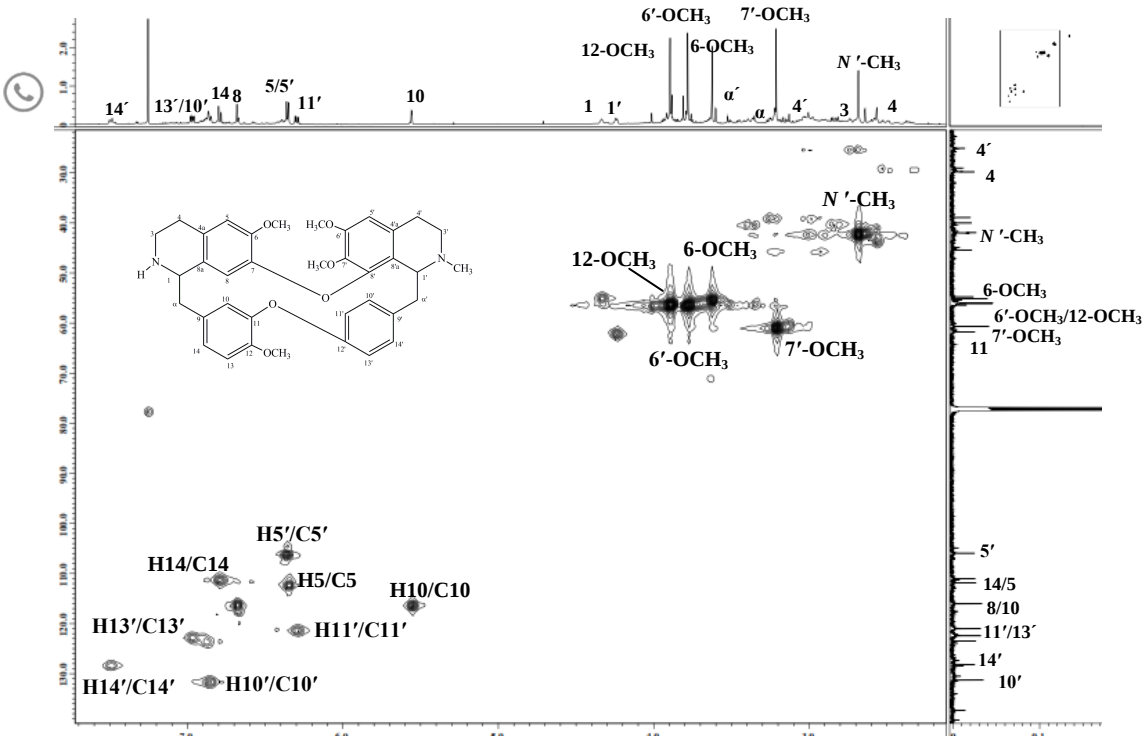


Figure 4.46. HMQC spectrum of AC 5.

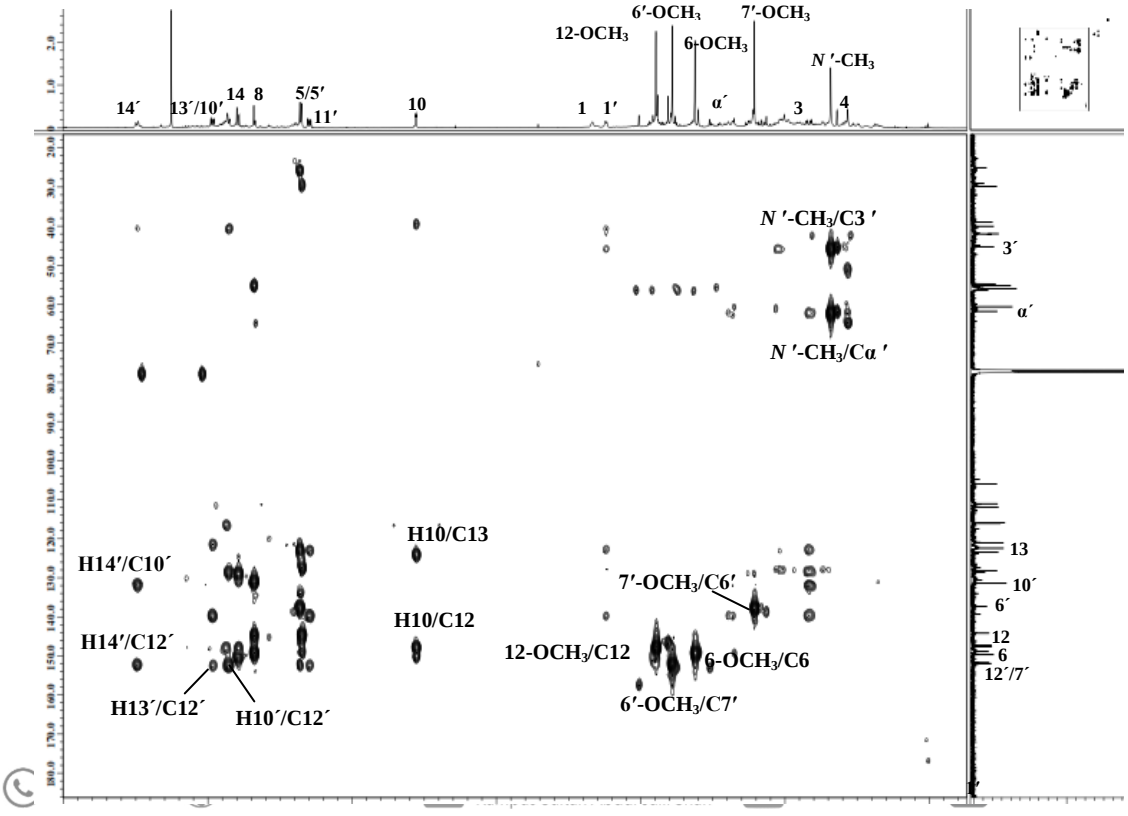


Figure 4.47. HMBC spectrum of AC 5.

Table 4.18

1D (^{1}H and ^{13}C) and 2D (HMBC) NMR Spectral Data of AC 5

Position	^{1}H $CDCl_3$ (*J*, Hz)	^{13}C (δ, $CDCl_3$)	HMBC
1	4.34 *(br s)*	54.7	
3	2.90 (*m*) 3.01 (*m*)	41.0	
4	2.38 (*m*) 2.53 (*m*)	29.0	8a
4a		126.8	
5	6.36 (*s*)	111.8	4,4a,6,7
6		148.8	
6-OCH_3	3.63 (*s*)	55.1	6
7		114.2	
8	6.69 (*s*)	116.0	1,6,7,8a
8a		130.5	
α	2.91 (*m*) 3.25 (*m*)	38.9	
9		147.6	
10	5.56 (*d*, 2.3)	115.9	α,11,12,13
11		149.7	
12		147.3	
12-OCH_3	3.90 (*s*)	55.9	12
13	6.86 (*d*, 2.3)	123.5	10,12
14	6.80 (*d*, 8.6)	110.9	11,12
1′	4.25 (*d*, 4.6)	61.8	α′,3′,9′
N′-CH_3	2.69 (*s*)	41.9	1′,3′
3′	2.92 (*m*) 3.23 (*m*)	45.3	4′a
4′	2.74 (*m*) 3.04 (*m*)	25.1	4′a ,6′
4′a		127.2	
5′	6.37 (*s*)	105.9	4′,6′,7′,8′a
6′		137.3	
6′-OCH_3	3.79 (*s*)	56.1	7′
7′		151.7	
7′-OCH_3	3.22 (*s*)	60.6	6′
8′		128.5	
8′a		122.4	
α′	2.84 (*dd*, 14.9, 5.8) 3.36 (*m*)	40.0	1′,9′,10′,13′ ,14′
9′		139.2	
10′	6.85 (*d*, 2.3)	131.3	α′,12′,14′
11′	6.30 (*dd*, 8.1, 2.9)	121.0	
12′		151.9	
13′	6.98 (*dd*, 8.6, 2.3)	122.4	9′,11′,12′
14′	7.50 (*d*, 8.1)	128.1	α′,10′,12′

Table 4.19

^{1}H NMR Data of AC 5 and 2-Norobaberine ***93***

Position	^{1}H $CDCl_3$ (*J*, Hz)	
	AC 5	2-Norobaberine (Tantisewie et al., 1989)
1	4.34 (*br s*)	4.23 (*m*)
5	6.36 (*s*)	6.37
6-OCH_3	3.63 (*s*)	3.64
8	6.69 (*s*)	6.69
10	5.56 (*d*, 2.3)	5.61 (*br s*)
12-OCH_3	3.90 (*s*)	3.92
13	6.86 (*d*, 2.3)	6.81 (*br s*)
14	6.80 (*d*, 8.6)	6.81 (*br s*)
1′	4.25 (*d*, 4.6)	4.23 (*m*)
N′-CH_3	2.69 (*s*)	2.69
5′	6.37 (*s*)	6.36
6′-OCH_3	3.79 (*s*)	3.79
7′-OCH_3	3.22 (*s*)	3.23
10′	6.85 (*d*, 2.3)	6.87 (*dd*, 8.2, 2.2)
11′	6.30 (*dd*, 8.1, 2.9)	6.31 (*dd*, 8.2, 2.2)
13′	6.98 (*dd*, 8.6, 2.3)	6.99 (*dd*, 8.2, 2.2)
14′	7.50 (*d*, 8.1)	7.47 (*dd*, 8.2, 2.2)

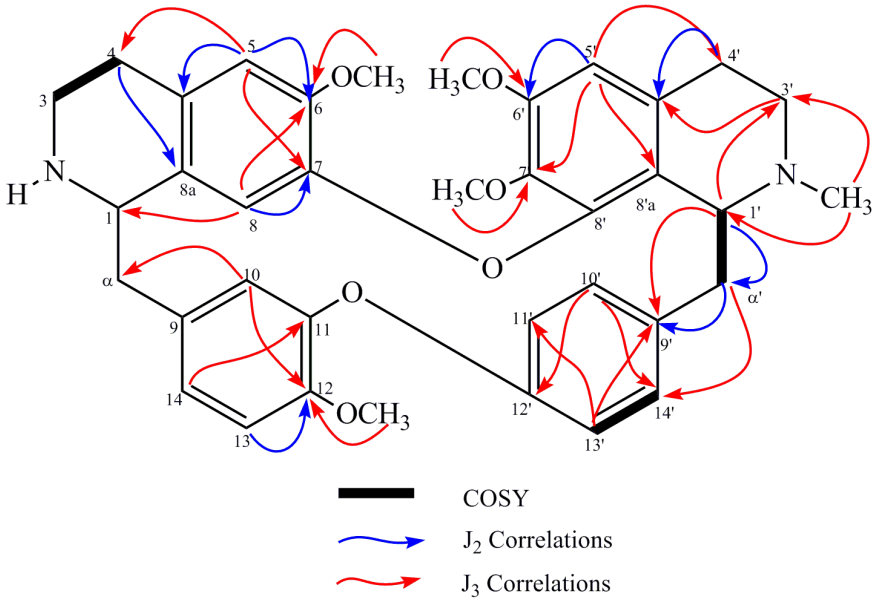
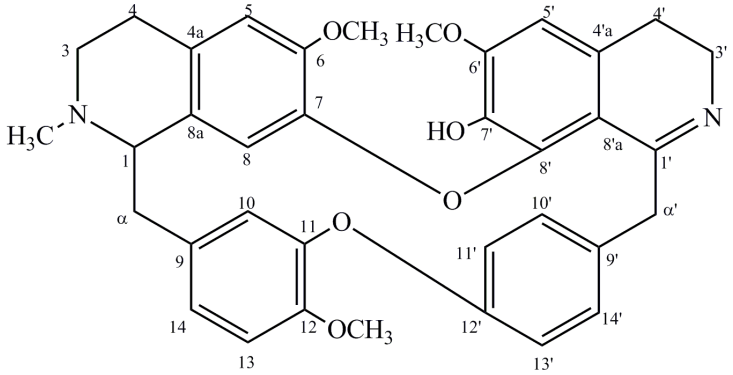


Figure 4.48. Selected COSY and HMBC correlation in AC 5.

4.2.6 AC 6, 3′, 4′-Dihydrostephasubine 94



94

Alkaloid AC 6 (4.8 mg) was obtained as brownish amorphous. The EIMS spectrum showed the presence of molecular ion peak at m/z 592 corresponding to the molecular formula of $C_{36}H_{36}N_2O_6$. The UV spectrum showed maxima absorption at 284 nm. The IR spectrum showed a broad band of hydroxyl group at 3308 cm^{-1} and aromatic rings at 1646 cm^{-1}, respectively.

The prior analysis of ^{1}H NMR spectrum (Figure 4.49) of AC 6 showed that it has similar pattern as 3´,4´-dihydronorstephasubine **88** that previously isolated from the bark of *Alseodaphne corneri* (Mukhtar et al., 2009). The ^{1}H NMR spectrum exhibited the presence of one *N*-CH_3 signal assignable to *N*-2 proton at δ 2.48. The signals for three methoxyls appeared at δ 3.86, 3.87 and 3.94 corresponding to C-12, C-6 and C-6´, respectively. The downfield signal of H-14´ at δ 7.40 showed the VI type of bisbenzylisoquinoline skeleton (Nelofar, 1989).

The vicinal protons of aromatic ring B, C and B´ appeared as a singlet and broad singlet at δ 3.54 (H-1), 4.88 (H-10), 6.09 (H-8), 6.46 (H-5), 6.55 (H-5´) and 6.71 (H-13, H-14). In addition, the protons H-10´ and H-11´ resonated at δ 7.36 and 6.47 as a doublet. Another doublet of doublet signal at δ 6.74 corresponding to H-13´. The COSY spectrum (Figure 4.50) showed cross peaks between H-10´/H-11´ and H-13´/H-14´ confirmed the position of structure proposed.

The ^{13}C NMR spectrum (Figure 4.51) and DEPT spectrum revealed a *N*-CH_3 carbon signal at δ 43.3 and three methoxyl carbon signals appeared at δ 55.8 (6´-OCH_3), 55.9 (6-OCH_3) and 56.2 (12-OCH_3), respectively. The spectrum also exhibited signals of six methylenes, eleven methines and fifteen quaternary carbons. A signal of C-1´ deshielded to lower region at δ 164.6 due to adjacent nitrogen atom that formed double bond. Moreover, C-α´ signal also deshielded due to the same circumstance at δ 44.7 compared to C-α at δ 38.1. The other methylenes carbon signals at δ 27.0, 27.9, 46.8 and 49.7 were assigned to C-4´,C-4, C-3´and C-3, respectively. The substituted aromatic carbons signals resonated at higher chemical shifts at δ 144.3 (C-6), 147.5 (C-7), 132.0 (C-6´), 149.3 (C-7´), 140.5 (C-8´), 146.3 (C-11), 149.7 (C-12) and 152.8 (C-12´) due to electronegative substituents effects that induce the carbons to shifts to lower region.

The HMQC spectrum (Figure 4.52) showed direct ^{1}H-^{13}C correlations while the HMBC spectrum (Figure 4.53) showed the long range correlation of ^{1}H-^{13}C of the structure. From the HMBC spectrum, the correlation of H-8 with C-7 and H-5 with C-7 confirmed the position of C-7 at δ 147.5. Furthermore, another correlation of H-13´, H-14´ and H-10´ with C-12 also confirmed the position of C-12 at δ 152.8. The

position of *N*-CH_3 in the structure was further confirmed due to cross peaks between *N*-CH_3 itself with H-1 and H-3, respectively. The other selected COSY and HMBC correlations are illustrated in Figure 4.54.

The NMR spectral data are shown in Table 4.20 while Table 4.21 shows the comparison of the observed data with the literature value (Patra, Mandal, Mukhopadhyay & Ranu, 1988), further confirmed that alkaloid AC 6 is 3′,4′-dihydrostephasubine **94**.

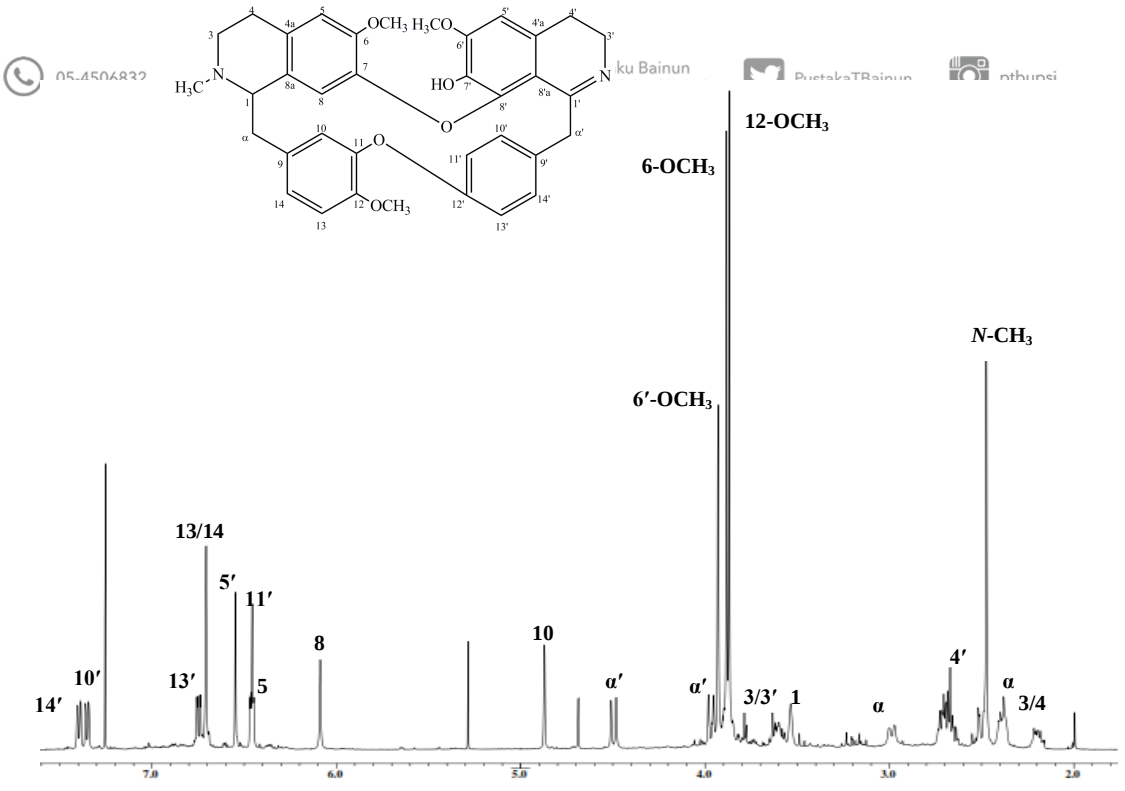


Figure 4.49. ^{1}H NMR spectrum of AC 6.

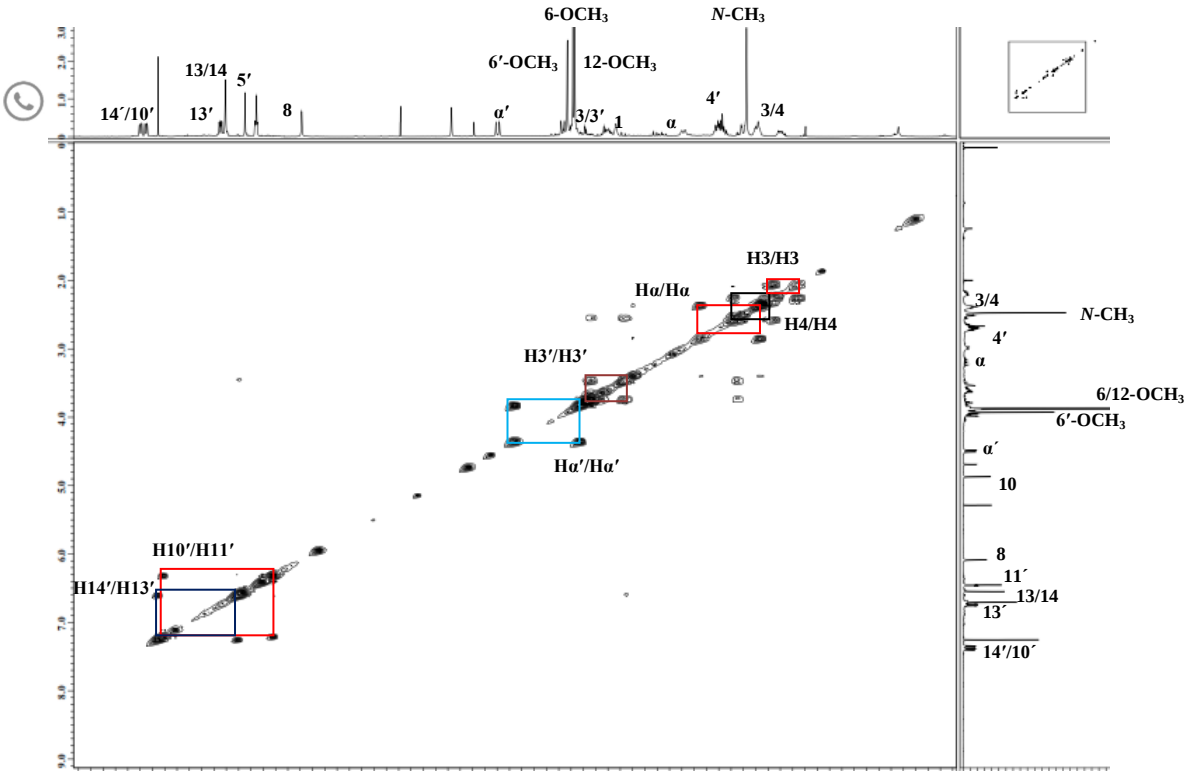


Figure 4.50. COSY spectrum of AC 6.

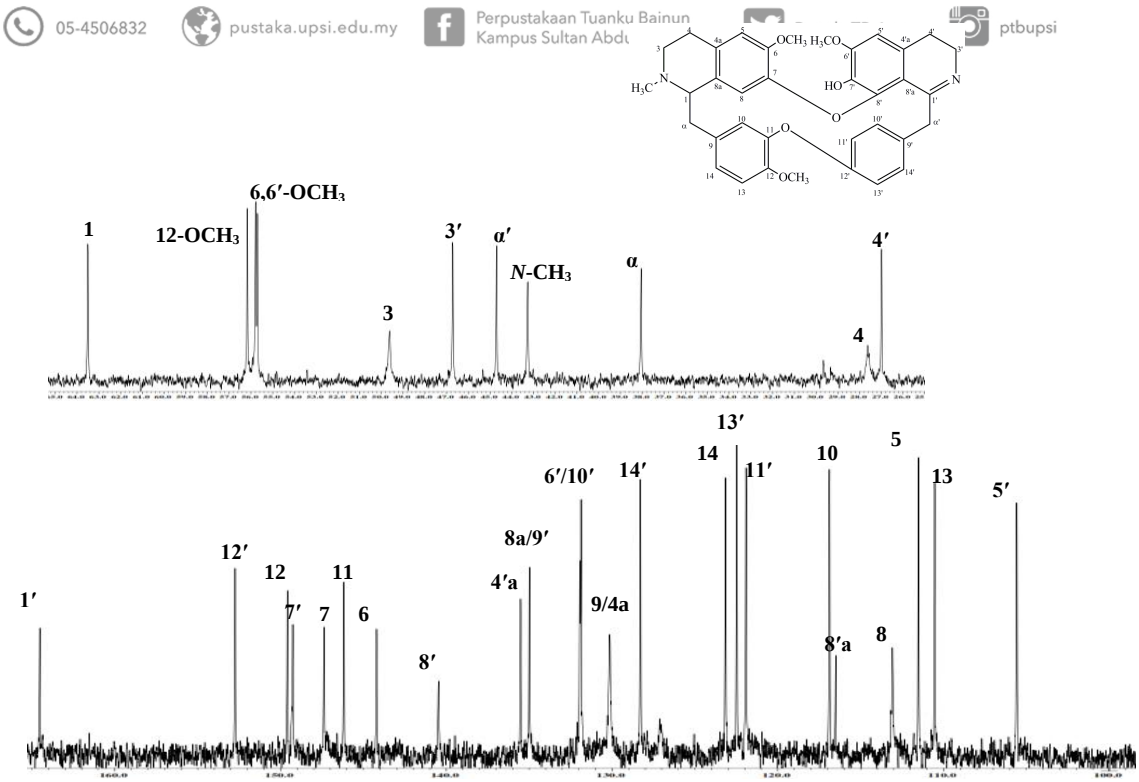


Figure 4.51. ^{13}C NMR spectrum of AC 6.

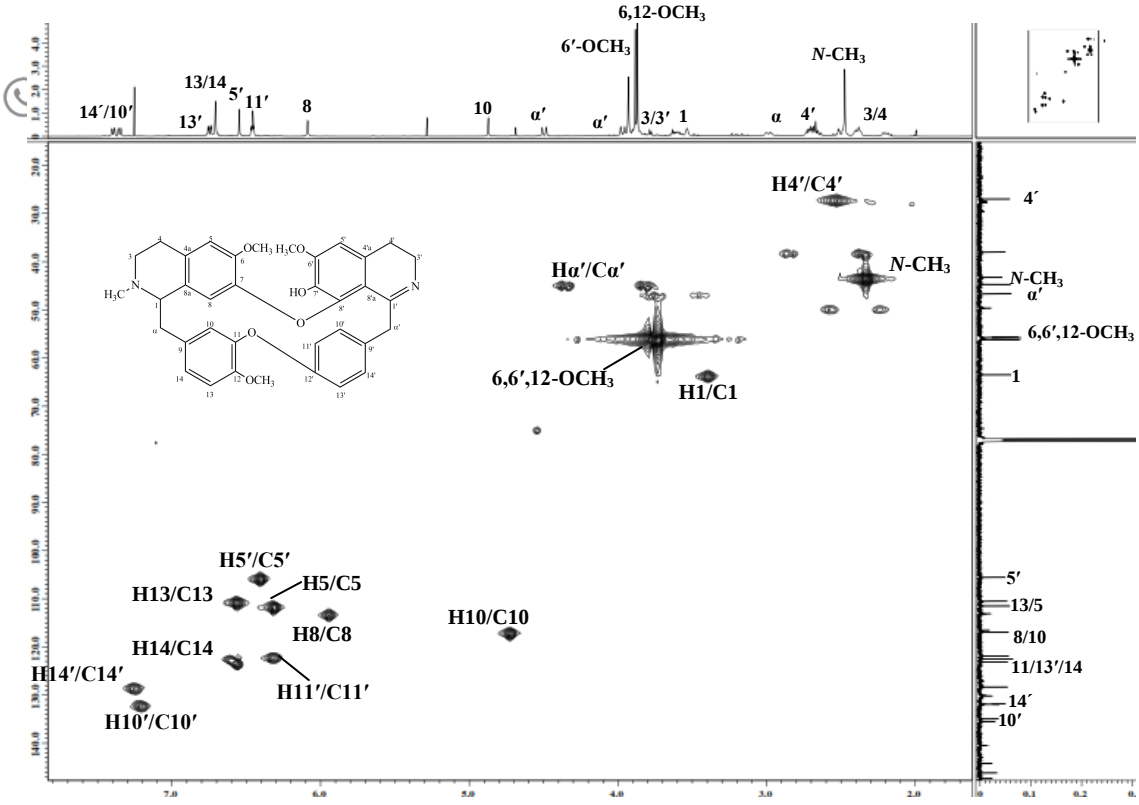


Figure 4.52. HMQC spectrum of AC 6.

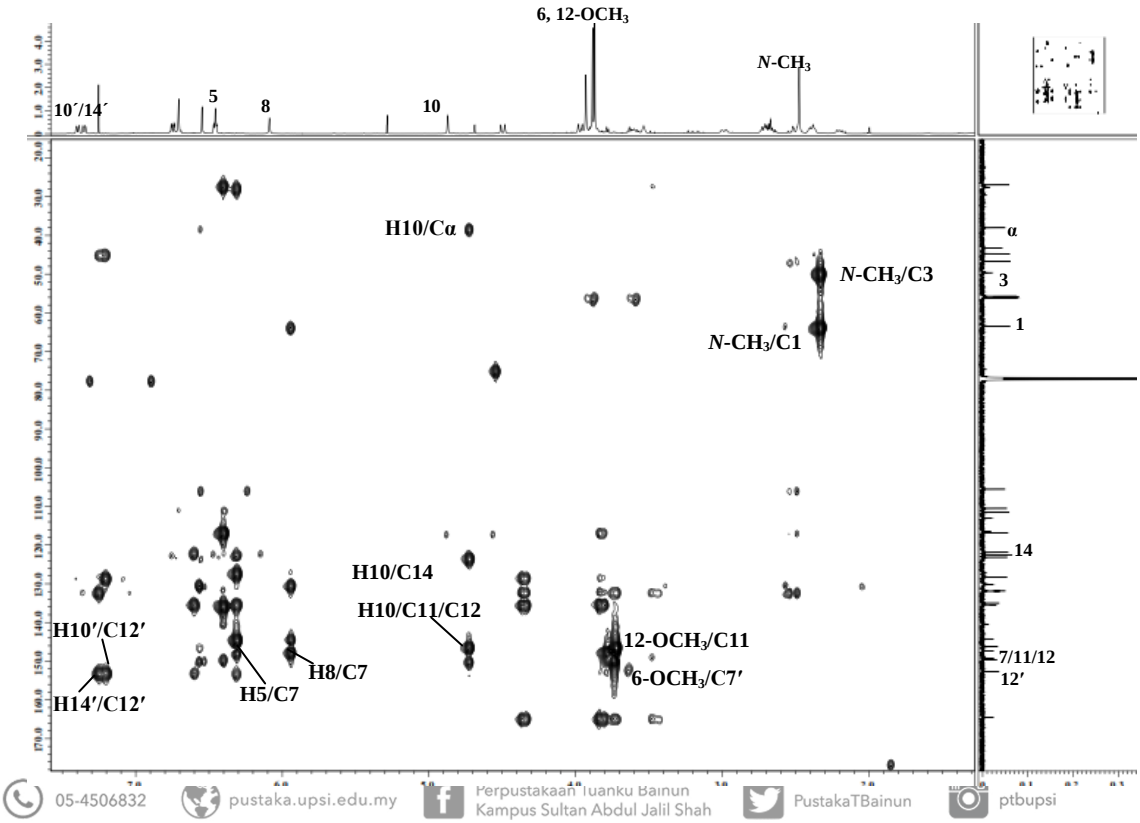


Figure 4.53. HMBC spectrum of AC 6.

Table 4.20

1D (^{1}H and ^{13}C) and 2D (HMBC) NMR Spectral Data of AC 6

Position	^{1}H $CDCl_3$ (*J*, Hz)	^{13}C (δ, $CDCl_3$)	HMBC
1	3.54 *(br s)*	63.6	4a
N-CH_3	2.48 (*s*)	43.3	1,3
3	2.39 (*m*) 3.71 (*m*)	49.7	
4	2.39 (*m*) 2.40 (*m*)	27.9	4a
4a		130.2	
5	6.46 (*s*)	111.6	4,6,7,8a
6		144.3	
6-OCH_3	3.87 (*s*)	55.9	7′
7		147.5	
8	6.09 (*s*)	113.1	1,4a,6,7
8a		135.0	
α	2.51 (*m*) 2.99 (*d*, 13.2)	38.1	
9		130.2	
10	4.88 (*s*)	116.9	α,11,12
11		146.3	
12		149.7	
12-OCH_3	3.86 (*s*)	56.2	11
13	6.71 (*br s*)	110.6	α,9,12,14
14	6.71 (*br s*)	123.2	9
1′		164.6	
3′	3.60 (*m*) 3.90 (*m*)	46.8	1′
4′	2.66 (*m*) 2.68 (*m*)	27.0	6′
4′a		135.6	
5′	6.55 (*s*)	105.6	4′,4′a,7′,8′a
6′		132.0	
6′-OCH_3	3.94 (*s*)	55.8	7
7′		149.3	
8′		140.5	
8′a		116.5	
α′	3.97 (*d*, 13.8) 4.50 (*d*, 13.8)	44.7	1′,8′a,9′,10′,14′
9′		135.0	
10′	7.36 (*d*, 8.6)	131.9	α′,12′,14′
11′	6.47 (*d*, 2.9)	122.0	11′,12′,14′,9′
12′		152.8	
13′	6.74 (*dd*, 8.6, 2.3)	122.5	9′,11′,12′
14′	7.40 (*d*, 8.6)	128.3	10′,12′

Table 4.21

^{1}H NMR Data of AC 6 and 3′,4′-Dihydrostephasubine ***94***

Position	^{1}H CDCl$_3$ (*J*, Hz)	
	AC 6	3′,4′-Dihydrostephasubine (Patra et al., 1988)
1	3.54 (*br s*)	3.59 (*br s*)
N-CH$_3$	2.48 (*s*)	2.51
5	6.46 (*s*)	6.50
6-OCH$_3$	3.87 (*s*)	3.88
8	6.09 (*s*)	6.08
10	4.88 (*s*)	4.91 (*br s*)
12-OCH$_3$	3.86 (*s*)	3.91
13	6.71 (*br s*)	6.73 (*d*, 8.3)
14	6.71 (*br s*)	6.84 (*dd*, 8.3, 1.0)
5′	6.55 (*s*)	6.60
6′-OCH$_3$	3.94 (*s*)	3.95
α′	3.97 (*d*, 13.8) 4.50 (*d*, 13.8)	4.08 (*d*, 14.0) 4.52 (*d*, 14.0)
10′	7.36 (*d*, 8.6)	7.36 (*dd*, 8.2, 2.0)
11′	6.47 (*d*, 2.9)	6.48 (*dd*, 8.2, 2.2)
13′	6.74 (*dd*, 8.6, 2.3)	6.77 (*dd*, 8.2, 2.0)
14′	7.40 (*d*, 8.6)	7.40 (*dd*, 8.2, 2.0)

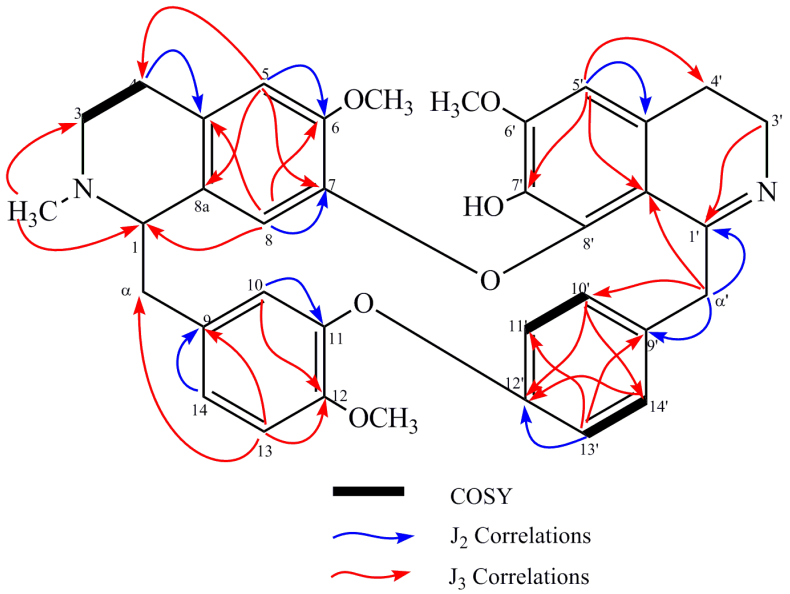
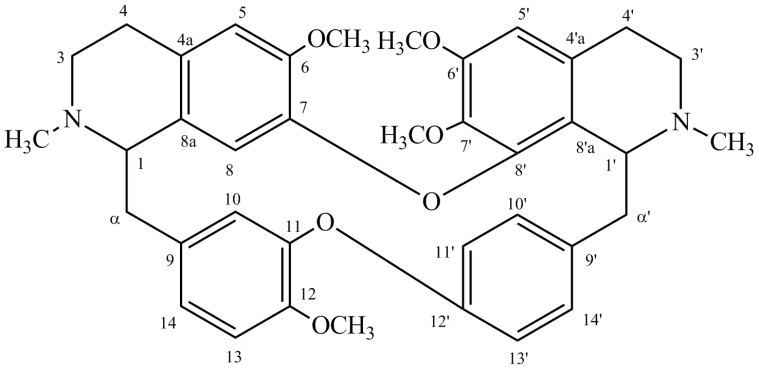


Figure 4.54. Selected COSY and HMBC correlation in AC 6.

4.2.7 AC 7, *O*-Methyllimacusine 95



95

Alkaloid AC 7 (3.9 mg) was isolated as a brownish amorphous. The UV spectrum revealed maximum absorption at 282 nm. The absorption bands at 1015 and 1650cm^{-1} in the IR spectrum are typical of C-N and C=C absorption bands (Williams & Fleming, 1989). The EIMS data showed molecular ion peak at m/z 622 giving the possibility of molecular formula of $C_{38}H_{42}N_2O_6$.

The ^{1}H NMR spectrum (Figure 4.55) showed four singlets at δ 3.01, 3.42, 3.76 and 3.95 corresponding to four methoxyls groups at C-7′, C-6, C-6′ and C-12. The former methoxyl (7′-OCH_3) resonated much higher region due to the bulky substituents and compressed position surrounding the atmosphere compared to the latter methoxyls. A singlet at δ 2.56 with integral value of six attributable to two *N*-CH_3 groups at ring A and ring A′, respectively.

Moreover, H-5′, H-10, H-5 and H-8 also appeared as a singlet at δ 6.39, 6.96, 6.45 and 6.40. The vicinal protons appeared as doublet signals were observed at δ 6.65, 6.80, 6.81, 6.95 and 7.35 corresponding to H-14, H-10′, H-11′, H-13 and H-14′,

respectively. These observations showed that ring C *meta-para* trisubstituted while ring C´ is *para* disubstituted. Proton H-1 and H-1´ appeared at δ 3.47 and 4.28 as a doublet with coupling constant of 7.5 and 10.3 Hz. The aliphatic protons of H-3, H-3´, H-4, H-4´, H-α and H-α´ appeared as multiplets at region δ 2.57-3.51.

The COSY spectrum (Figure 4.56) showed cross-peak between H-1´/H-α´ and H-3a/H-3b. The ^{13}C NMR spectrum (Figure 4.57) revealed thirty eight carbon signals in the molecule and showed two methyl groups attached to the nitrogen atoms belongs to *N*-2 and *N*-2´ at δ 42.2 and 41.5, respectively. The four methoxyl groups of C-7, C-6´, C-12 and C-7´ resonated at δ 55.4, 55.8, 56.3 and 59.8, respectively. In addition, DEPT spectrum showed presence of fourteen quaternary carbons, twelve methines and six methylenes of C-3 (δ 46.5), C-4 (δ 26.3), C-α (δ 40.7), C-3´ (δ 44.2), C-4´ (δ 22.9) and C-α´ (δ 43.8). The quaternary carbons resonated at the lower regions area at δ 106.0-155.6 due to the diamagnetic anisotropy effect increases in the benzene rings.

The HMQC spectrum is shown in Figure 4.58. The HMBC spectrum (Figure 4.59) showed cross peak of H-1 with C-4, C-4a and C-8; H-14 with C-α and C-10; H-5´ with C-6´ and C-4´ ; and H-13´ with C-9´ and C-12´. Furthermore, the cross peaks between 12-OCH_3 with C-12; 6-OCH_3 with C-6; 6´-OCH_3 with C-6´; and 7´-OCH_3 with C-7´ further confirmed the positions of methoxyl groups in the structure. The other HMBC correlations are illustrated in Figure 4.60.

The NMR spectral data of ^{1}H NMR, ^{13}C NMR and HMBC correlations are tabulated in Table 4.22. Finally, structure of alkaloid AC-7 was further confirmed as

O-methyllimacusine **95** by comparison with other literature in Table 4.23 (Chalandre et al., 1986) .

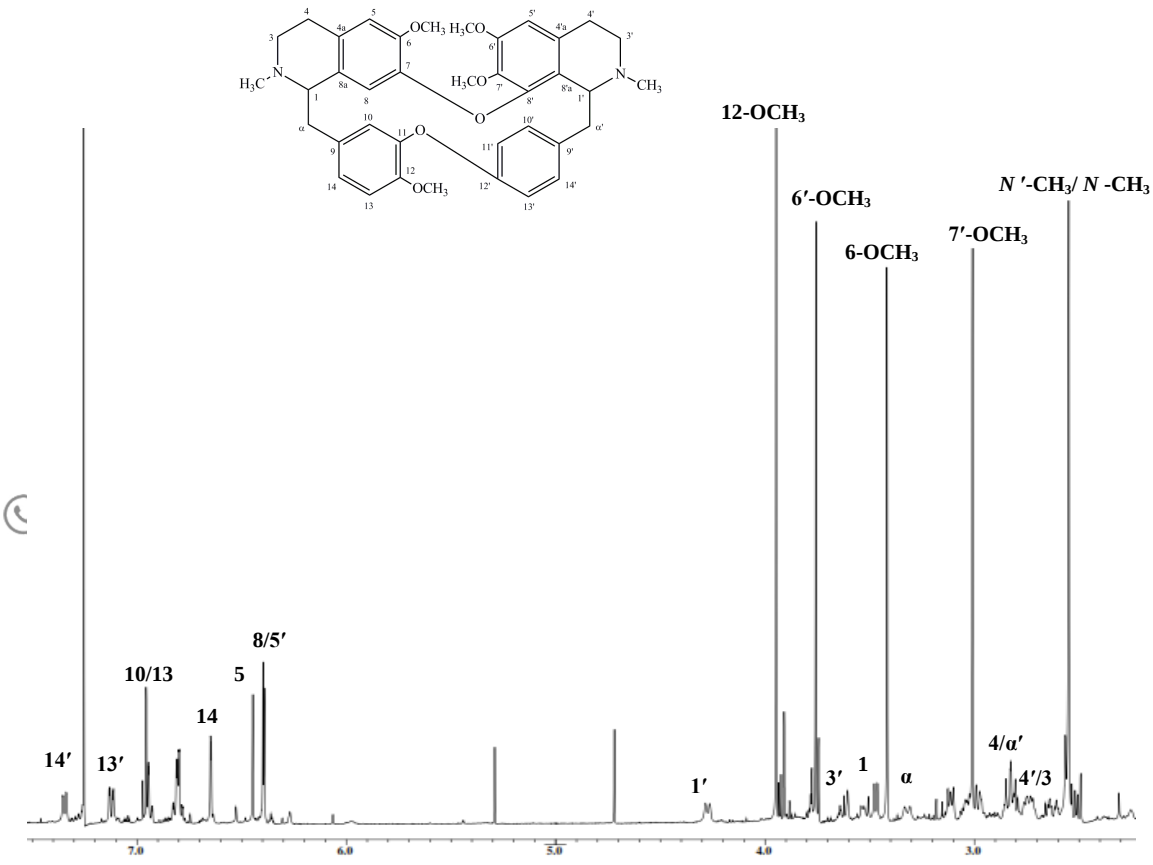


Figure 4.55. ^{1}H NMR spectrum of AC 7.

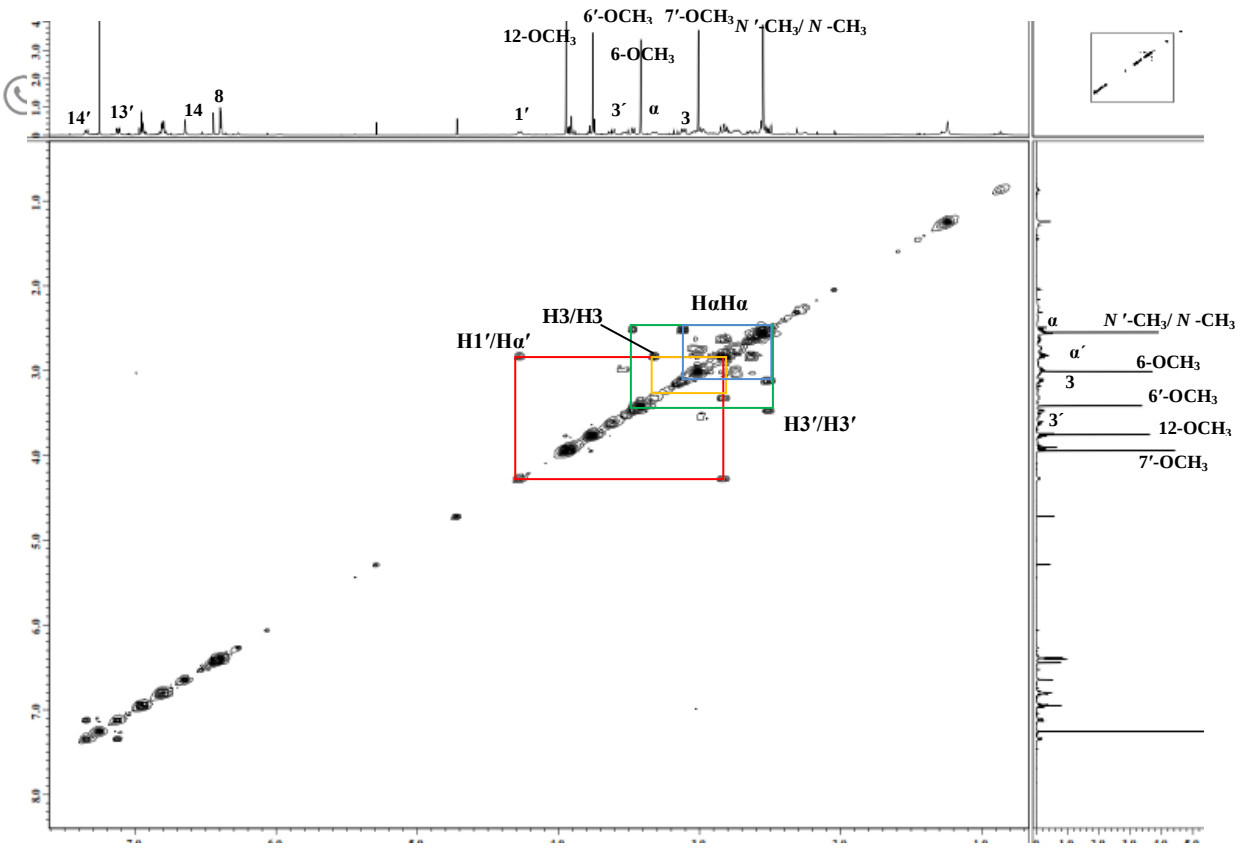


Figure 4.56. COSY spectrum of AC 7.

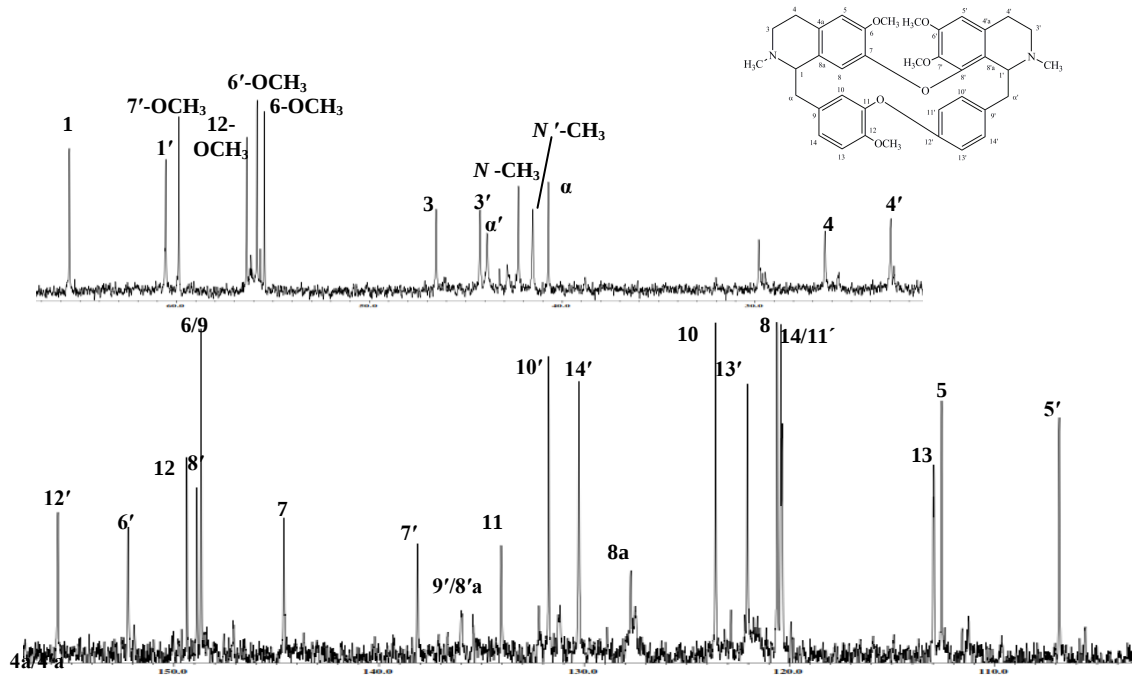


Figure 4.57. ^{13}C NMR spectrum of AC 7.

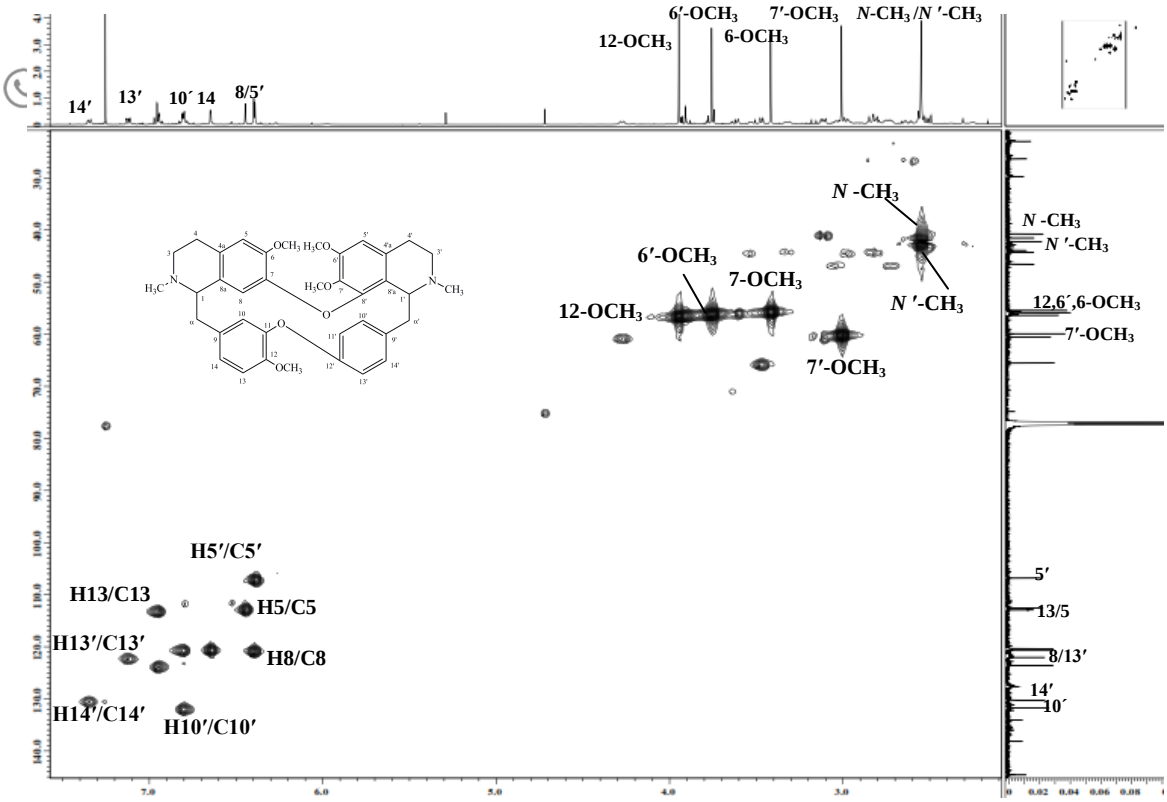


Figure 4.58. HMQC spectrum of AC 7.

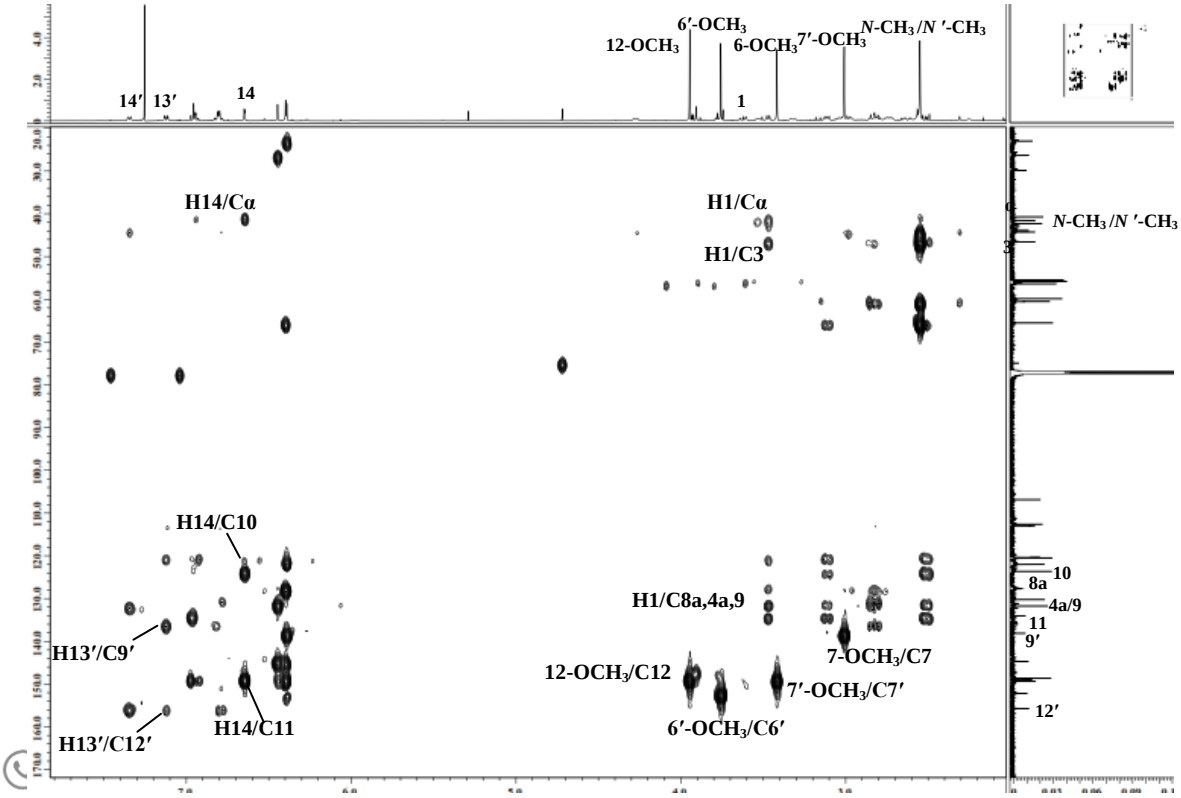


Figure 4.59. HMBC spectrum of AC 7.

Table 4.22

1D (^{1}H and ^{13}C) and 2D (HMBC) NMR Spectral Data of AC 7

Position	^{1}H $CDCl_3$ (*J*, Hz)	^{13}C (δ, $CDCl_3$)	HMBC
1	3.48 (*d*, 7.5)	65.5	α,3,4a,8a,8
N-CH_3	2.56 (*s*)	42.2	1,3
3	2.75 (*m*) 3.04 (*m*)	46.5	
4	2.65 (*m*) 2.85 (*m*)	26.3	4a, 8a
4a		127.5	
5	6.45 (*s*)	112.5	4,8a,7
6		148.6	
6-OCH_3	3.42 (*s*)	55.4	6
7		144.6	
8	6.40 (*s*)	120.6	1,4a,6,7
8a		131.0	
α	2.57 (*m*) 3.11 (*m*)	40.7	1,10,14
9		134.0	
10	6.96 (*s*)	123.6	9,11
11		148.7	
12		149.4	
12-OCH_3	3.95 (*s*)	56.3	12
13	6.95 (*d*, 5.8)	112.9	α,11,14
14	6.65 (*d*, 2.3)	120.3	α, 10,11
1′	4.28 (*d*,10.3)	60.5	
N′-CH_3	2.56 (*s*)	41.5	1′,3′
3′	2.99 (*m*) 3.51 (*m*)	44.2	*N*′-CH_3
4′	2.73 (*m*) 2.77 (*m*)	22.9	
4′a		127.5	
5′	6.39 (*s*)	106.8	4′,6′
6′		152.2	
6′-OCH_3	3.76 (*s*)	55.8	6´
7′		138.1	
7′-OCH_3	3.01 (*s*)	59.8	7′
8′		148.8	
8′a		135.5	
α′	2.83 (*m*) 3.32 (*d*, 11.5)	43.8	9′
9′		136.0	
10′	6.80 (*d*, 7.5)	131.7	12′,14′
11′	6.81 (*d*, 2.4)	120.4	9′
12′		155.6	
13′	7.13 (*dd*, 8.6,1.7)	122.0	9′,12′
14′	7.35 (*d*, 8.1)	130.3	α′,10′,12′

Table 4.23

^{1}H NMR Data of AC 7 and O-Methyllimacusine ***95***

Position	^{1}H $CDCl_3$ (*J*, Hz)	
	AC 7	*O*-Methyllimacusine (Chalandre et al., 1986)
N-CH_3	2.56 (*s*)	2.55
5	6.45 (*s*)	6.40
6-OCH_3	3.42 (*s*)	3.43
8	6.40 (*s*)	6.45
10	6.96 (*s*)	6.65
12-OCH_3	3.95 (*s*)	3.96
13	6.95 (*d*, 5.8)	6.99
14	6.65 (*d*, 2.3)	6.96
N′-CH_3	2.56 (*s*)	2.57
5′	6.39 (*s*)	6.40
6′-OCH_3	3.76 (*s*)	3.77
7′-OCH_3	3.01 (*s*)	3.02
10′	6.80 (*d*, 7.5)	6.81
11′	6.81 (*d*, 2.4)	6.81
13′	7.13 (*dd*, 8.6, 1.7)	7.13
14′	7.35 (*d*, 8.1)	7.36

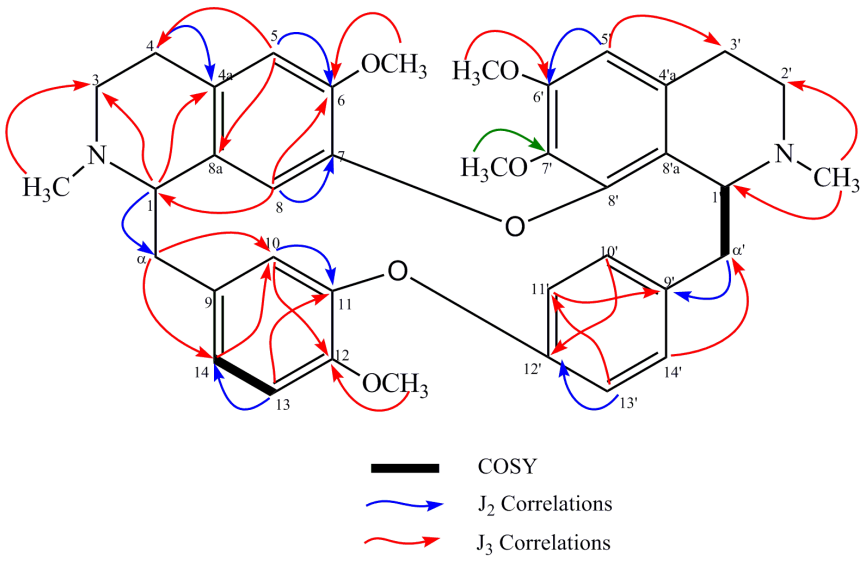


Figure 4.60. Selected COSY and HMBC correlation in AC 7.

4.3 Antiplasmodial activity

Malaria is a major global public health problem and responsible for the death of over one million people annually, with more than 90% of cases found in sub-Sarahan Africa. Every year malaria disease kills between one and two million people with as many as 300-500 million people being infected. It is estimated that nearly half of the world population is at risk, with fatal rates increase among young children below 5 years of age (Saxena, Pant, Jain & Bhakuni, 2003).

The *in-vitro* antiplasmodial activity was performed as method reported previously (Trager & Jensen, 1976). Then, the IC_{50} value of the samples were compared with the standard reference drug of chloroquine, 0.087 μg/ml (Omoregie, Sisodia, 2012).

For this experiment, the IC_{50} value less than 10 μg/ml for crude extract and IC_{50} value less than 5 μg/ml for compound were considered having good activity. The alkaloid crude extract of *Alseodaphne peduncularis* (bark) showed good to moderate active to *Plasmodium falciparum*, K1 isolate (resistant strain) with IC_{50} value of 2.135 μg/ml. Therefore, further isolation and purification of alkaloid compound from this species was done to investigate the active pure compound towards *Plasmodium falciparum* that have potential as antimalarial drug.

Three isolated compounds; boldine **69**, norlirioferine **91** and norboldine **78** showed good to moderate activity against *Plasmodium falciparum*. Among them,

boldine **69** showed most potent antiplasmodial activity with IC_{50} value of 1.067 µg/ml. Table 4.24 showed the IC_{50} value of each samples.

Table 4.24

Results of HRP2 test for antimalaria in-vitro drug screening

Samples	IC_{50} (µg/ml)
Chloroquine (standard)	0.087
Crude extract of *Alseodaphne peduncularis* (bark)	2.135
Boldine (AP 1)	1.067
Norlirioferine (AP 3)	2.786
Norboldine (AP 4)	2.228

CHAPTER 5

CONCLUSION

Two *Alseodaphne* species namely *Alseodaphne peduncularis* (Wall. ex Nees) Meisn from Kluang-Mersing, Johor and *Alseodaphne corneri* Kosterm from University of Malaya, Kuala Lumpur were used to investigate the alkaloids contents. The phytochemical study on the bark of *Alseodaphne peduncularis* and the leaves of *Alseodaphne corneri* led to discovery of two types of alkaloids; aporphine and bisbenzylisoquinoline. The alkaloids were identical by comparing the spectral data with published report. The list of isolated alkaloid compounds are shown in Table 5.1.

Four aporphines were isolated from the bark of *Alseodaphne peduncularis* namely boldine **69**, norpredicentrine **90**, norlirioferine **91** and norboldine **78**. From this research, boldine **69** shows as a major alkaloid product from *Alseodaphne* species when it was successfully isolated from both species of *Alseodaphne peduncularis* and

Alseodaphne corneri. Furthermore, norpredicentrine **90**, norlirioferine **91** and norboldine **91** showed another significant value for future researcher to determine the aporphine-type alkaloid presence in this species.

Moreover, the roots of *Alseodaphne corneri* afforded two aporphines and five bisbenzylisoquinolines alkaloids. The two aporphines were boldine **69** laetanine **30**. To our knowledge, this is the first report on the isolation of laetanine **30** from *Alseodaphne corneri*. The spectral data of AC1 identical with laetanine **30** from literatures, thus confirming the presence of laetanine **30** in *Alseodaphne corneri* (Omar, 2009; Borthakur & Rastogi, 1979).

Another five bisbenzylisoquinoline alkaloids known as gyrolidine **47**, stephasubine **92**, 2-norobaberine **93**, 3′,4′-dihydrostephasubine **94** and *O*-methyllimacusine **95**. Differ from aporphine, bisbenzylisoquinoline is a dimer of benzylisoquinolines shows that the structure is more complicated and the mass double or higher than aporphine. Gyrolidine **47**, stephasubine **92** and 3′,4′-dihydrostephasubine **94** were previously isolated from *Dehaasia incrassata* (Mukhtar et al., 2005). Moreover, 2-norobaberine **93** and *O*-methyllimacusine **95** were also had been isolated since 1980s (Tantisewie et al., 1989; Chalandre et al., 1986). The comparison of the spectral data with the previous data concluded the presence of these bisbenzylisoquinoline alkaloids in *Alseodaphne corneri*.

The crude of *Alseodaphne peduncularis* and three isolated aporphines; boldine **69**, norlirioferine **91** and norboldine **78** were tested for antiplasmodial activity

against *Plasmodium falciparum*. Among samples, the result stated that boldine **69** showed most potent activity with IC_{50} value of 1.067 µg/ml.

In conclusion, the phytochemical study of these *Alseodaphne* species proved the existence of two types of alkaloids; aporphine and bisbenzylisoquinoline. It is believed that this species also have strong potential to show interesting bioactivities such as cytotoxicity, vasorelaxant activity and many more in the future.

Table 5.1

Alkaloids Isolated from Alseodaphne peduncularis and Alseodaphne corneri

Alseodaphne species	Types of alkaloids	Isolated alkaloids
Alseodaphne peduncularis (Wall. ex Nees) Meisn	aporphine	boldine **69**
	aporphine	norpredicentrine **90**
	aporphine	norlirioferine **91**
	aporphine	norboldine **78**
Alseodaphne corneri Kosterm	aporphine	laetanine **30**
	aporphine	boldine **69**
	bisbenzylisoquinoline	gyrolidine **47**
	bisbenzylisoquinoline	stephasubine **92**
	bisbenzylisoquinoline	2-norobaberine **93**
	bisbenzylisoquinoline	3′,4′-dihydrostephasubine **94**
	bisbenzylisoquinoline	*O*-methyllimacusine **95**

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Extraction and Isolation of Alkaloids from The Leaves of *Alseodaphne corneri* Kosterm

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Abstract : The isolation and purification of the leaves extract of *Alseodaphne corneri* Kosterm yielded four alkaloids; norisocorydine **1**, isocorydine **2**, 2-norobamegine **3** and obamegine **4**. This phytochemical study involves extraction, separation by using various chromatographic methods and structural determination by spectroscopic technique such as ultraviolet spectroscopy (UV), infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy (NMR) including 1D-NMR (^{1}H, ^{13}C and DEPT), 2D-NMR (COSY, NOESY, HMQC/HSQC, and HMBC) and mass spectrometry (MS). The IC_{50} value of antiplasmodial activity for isocorydine **2** and obamegine **4** are 0.50 µmolL^{-1} and 0.14 µmolL^{-1} respectively.

Keywords : Alkaloids, *Alseodaphne corneri*, *Lauraceae*.

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Introduction

The Lauraceae are nearly all woody trees and shrubs comprising of 30 to 50 genera with about 2,000 species. There is about fifty or more *Alseodaphne* species that can be found in Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Philipines, Sri Lanka, Thailand and Vietnam [1]. *Alseodaphne corneri* Kosterm of Lauraceae, grows as wild plant, 6-8 m high. In Malaysia, the plant is also known as Medang [2].

Based on literature review, both aporphine and bisbenzylisoquinoline alkaloids showed interesting biological bioactivities such as vasorelaxants effect [3], cytotoxic action [4] and cardiovascular pharmacological effects [5].

This paper reports the isolation and identification of four alkaloids which are aporphine and bisbenzylisoquinoline types from leaves extract of the plant species. The structural elucidation was performed by various spectroscopic methods; nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR), ultraviolet spectroscopy (UV) and mass spectroscopy (MS).

In this study, the isolated compounds were then tested for *in vitro* inhibitory activity against *Plasmodium falciparum*.

Experimental

General methods

^{1}H and ^{13}C and 2D NMR were recorded in $CDCl_3$ with TMS as internal reference on a JEOL JNM-FX100 (400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical shifts were reported in ppm or δ scale and the coupling constant are given in Hz. Mass spectra obtained using JEOL JMS 700 TZ spectrometer. The infrared spectra were obtained with chloroform as a solvent on a Perkin Elmer 2000 spectrometer. UV spectra were recorded on a Shimadzu UV-310 IPC Ultraviolet-Visible NIR Scanning Spectrophotometer. All solvents used are AR grade except those that are used for bulk extraction (distilled). Column chromatography (CC) was carried out using Merck silica gel 230-400 mesh and TLC was performed on silica gel 60 F_{254}, Merck.

Plant material

The leaves of *Alseodaphne corneri* was obtained and identified by the team of the Herbarium of Chemistry Department, University of Malaya, Kuala Lumpur, Malaysia on 2008. Voucher specimens (KL 4928) were deposited at the Herbarium of Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia.

Extraction and Isolation

4.0 kg of the air dried leaves of *Alseodaphne corneri* were moistened with 25% ammonia solution and soaked in dichloromethane (CH_2Cl_2) for 3 days (cold extraction). The CH_2Cl_2 extract was evaporated to 500 ml followed by extraction using 5% hydrochloric acid (HCl) until Mayer's test is negative. The HCl extract was basified with concentrated ammonia to pH 11 and re-extracted with CH_2Cl_2. The CH_2Cl_2 was washed with distilled water and dried over anhydrous sodium sulphate.

Finally, the extract was evaporated to dryness to give crude alkaloid (10.5g). The crude

alkaloid was introduced to column chromatography over silica gel with the solvent systems of CH_2Cl_2 (100%), CH_2Cl_2: MeOH (99:1, 98:2, 97:3, 95:5) and finally 100% MeOH. Further purification was done by using the preparative thin layer chromatography (PTLC). The purified alkaloids were indicated by a single spot on thin layer chromatography (TLC).

In vitro antiplasmodial activity

The antimalaria activity of isolated compounds was determined by the procedure described by Budimulya et al [6]. In brief, each sample was separately dissolved in dimethyl sulfoxide (DMSO; 10^{-2} mol L^{-1}) and kept at -20 ^{o}C until use. The malaria parasite *Plasmodium falciparum* 3D7 clone was propagated in a 24 well culture plate in the presence of wide range of concentrations of each sample. The growth of the parasite was monitored by making a blood smear fixed with MeOH and stained with Geimsa. The half maximal inhibitory concentration, IC_{50} value, used to measure the effectiveness of isolated compound in antiplasmodial activity was calculated.

Result and Discussion

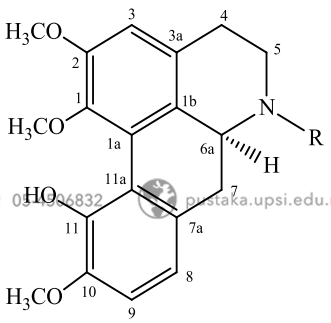
Four known alkaloids have been isolated from the leaves of *Alseodaphne corneri*. They are norisocorydine **1**, isocorydine **2**, 2-norobamegine **3** and obamegine **4**.

Compound **1** was isolated as brownish amorphous solid. Its UV spectrum showed an absorption bands at 223, 267 and 308 nm, thus suggesting a 1,2,10,11-tetrasubstituted aporphine skeleton [7,8]. In addition, the IR spectrum gave a broad band between 3500 and 2936 cm^{-1} due to the presence of OH and NH groups [9,10]. In its mass spectrum, the base peak $[M-1]^+$, m/z 326 was formed by the loss of a hydrogen atom from the molecular ion. The $[M]^+$ occurred at m/z 327 suggesting a molecular formula of $C_{19}H_{21}NO_4$. In addition, the peak at m/z 312 $[M-CH_3]^+$ and m/z 296 $[M-OCH_3]^+$ suggested the fragmentation of a methyl and methoxyl groups, respectively. The ^{1}H NMR spectrum showed the presence of an aromatic proton appeared as a singlet at δ 6.65, attributable to H-3. The spectrum also revealed two doublets belonging toH-8 at δ 6.73 (*J*= 8.0 Hz) and H-9 at δ 6.78 (*J*= 8.0 Hz) which formed an AB spin system. Three singlets at δ 3.65 (1-OCH_3), 3.83 (2-OCH_3) and 3.84 (10-OCH_3) were detected, which corresponded to the three methoxyl groups.

The ^{13}C and DEPT experiments further confirmed the presence of nineteen carbons, which consisted of three aromatic methines, four sp^3 aliphatic carbons, nine sp^2 quaternary carbons and three methoxyl carbons. The full assignment of the 1D-NMR (^{1}H and ^{13}C) spectral data are given in Table 1. Finally comparison of this spectroscopic data with those reported in the literature, showed significantly that alkaloid obtained was norisocorydine [11].

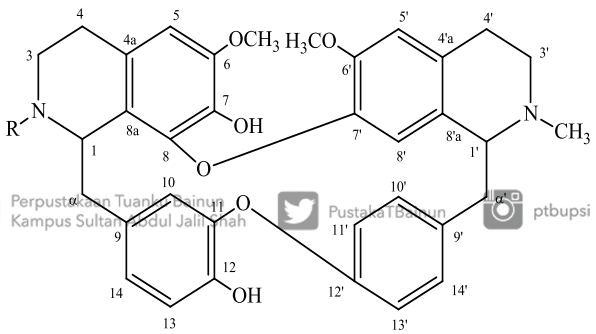
Compound **2** was obtained as a dark brown amorphous solid. The UV spectrum showed absorption bands at 283 and 304 nm, which were typical of the aporphine skeleton [7,8]. The IR spectrum showed the presence of hydroxyl group at about 3450 cm^{-1}. The molecular ion peak was observed at *m/z* 341 proposed the molecular formula of $C_{20}H_{23}NO_4$. The base peak at *m/z* 340 indicated the loss of a proton. The high intensity fragment ions at *m/z* 326 $[M-CH_3]^+$ and *m/z* 310 $[M-OCH_3]^+$ indicated the loss of a methyl and methoxyl group, respectively.

The ^{1}H NMR spectrum showed a singlet at δ 3.68 and another six proton singlet at δ 3.89, corresponding to the three methoxyl groups. The former was attributed to methoxyl on C-1 and the latter to C-2 and C-10, respectively. The C-1 methoxyl signal was rather shielded compared to the normal aromatic methoxyls since the protons of the methoxyl were forced to place themselves on top of ring A where the electron density was high. Another singlet at δ 2.51 was attributed to *N*-methyl group which differentiates compound **2** with compound **1** due to the absence of this peak in the ^{1}H NMR spectrum of compound **1**. The spectrum also revealed two doublets assigned to H-8 at δ 6.82 (*J*= 8.0 Hz) and H-9 at δ 6.83 (*J*= 8.0 Hz) which formed an AB system. In ring A, the C-3 aromatic proton served as singlet at δ 6.68.



1: R = H

2: R =CH_3



3: R = H

4: R =CH_3

Table 1 : ^{1}H and ^{13}C NMR Spectral Data of Compound **1** and **2**

Position	^{1}H δ, $CDCl_3$ (*J*, Hz)		^{13}C (δ, $CDCl_3$)	
	1	**2**	**1**	**2**
1			141.8	142.2
1-OCH_3	3.65 (*s*)	3.68 (*s*)	61.9	62.0
1a			125.4	125.9
1b			130.0	129.6
2			151.2	151.4
2-OCH_3	3.83 (*s*)	3.89 (*s*)	55.9	55.5
3	6.65 (*s*)	6.68 (*s*)	111.5	110.0
3a			130.1	128.5
4	2.63 (*d*, 13.4) 2.86 (*dd*, 11.2, 2.9)	2.66 (*d*, 16.0) 3.15 (*m*)	29.0	28.7
5	2.94 (*dd*, 15.8, 4.8) 3.27 (*t*, 5.4)	2.50 (*m*) 3.01 (*d*, 3.2)	42.5	52.5
N-CH_3		2.51 (*s*)		43.4
6a	3.58 (*dd*, 13.2, 3.9)	3.30 (*m*)	53.8	62.7
7	2.50 (*t*, 13.2) 2.70 (*dd*, 13.1, 4.1)	2.41 (*t*, 13.2) 3.04(*d*, 3.6)	38.1	35.4
7a			129.6	129.5
8	6.73 (*d*, 8.0)	6.82 (*d*)	118.7	119.0
9	6.78 (*d*, 8.0)	6.83 (*d*)	110.8	111.0
10			149.2	149.5
10-OCH_3	3.84 (*s*)	3.89 (*s*)	55.7	55.8
11			143.9	143.9
11a			119.9	120.0

The ^{13}C NMR and DEPT experiments further confirmed the presence of twenty carbons, which consist of three aromatic methines, four sp^3 aliphatic carbons; nine sp^2 quaternary carbons, three methoxyl carbons and one *N*-methyl carbon. The correlations of 2D NMR of compound **2** are similar to 2D NMR of norisocorydine. Comparison of the spectral data with the literature values confirmed that alkaloid obtained was isocorydine [12,13]. The full assignment of the ^{1}H and ^{13}C NMR spectral data is given in Table 1.

Compound **3** was obtained as a brownish amorphous solid with $[\alpha]_D^{27}$ +290.0^o (*c* = 0.5, MeOH). Its UV spectrum exhibited absorption maxima at 295 nm which is characteristic of a bisbenzylisoquinoline [14, 15].

The IR spectrum revealed absorption peaks at 3394, 2930, 1514 and 1264 cm^{-1} corresponding to the stretching of O-H, C-H, C=C ring and C-O-C; diphenyl ether groups, respectively [16]. It showed a molecular ion $[M+H]^+$ peak at m/z 581 corresponding to the molecular formula of $C_{35}H_{36}N_2O_6$.

The ^{1}H NMR spectrum revealed one *N*-methyl signal at δ 2.51. The spectrum also exhibited the presence of two methoxyl groups, with one in the upfield region, δ 3.53 which was the characteristic for methoxyl on C-6´ substituted and the other peaks at δ 3.73 were located at C-6. The presence of three protons singlet at δ 6.27, 6.31 and 6.64 were related to the H-5, H-5´ and H-8´, respectively. In addition, H-10 resonated as a broad singlet at δ 5.48. H-11´, H-14 and H-10´ each appeared as doublets at δ 6.21 (*J* = 6.8 Hz), 6.69 (*J* = 7.6 Hz) and 6.78 (*J* = 7.6 Hz), respectively; and another three signals corresponding to H-13, H-13´and H-14´ resonated as a doublet of doublets at δ 6.55 (*J* = 8.0 and 1.6 Hz), δ 6.87 (*J* = 8.4 and 2.8 Hz) and δ 7.38 (*J* = 10.4 and 1.6 Hz), respectively. A

broad singlet and a doublet signal corresponding to two protons, H-1 and H-1´ were observed at δ 4.09 and 4.13, respectively.

The ^{13}C NMR spectrum of this alkaloid showed thirty-five carbons. There were fourteen quarternary carbons, two methoxyl, twelve methines, six methylenes and one methyl group which attached to nitrogen atom (*N*´-CH_3), consistent with the structure proposed.

Compound **4** was obtained as a brownish amorphous state with $[\alpha]_D^{26}$ +140° (*c* = 8.28, MeOH). The UV spectrum revealed absorbance band at 283 nm, while the IR spectrum exhibited absorption for aromatic ring and diphenyl ether at 1500 and 1220 cm^{-1} respectively. Another significant peak was also observed at 3400 cm^{-1} corresponding to the phenolic function [17,18]. The EIMS mass spectrum revealed the $[M+H]^+$ peak at m/z 594 thus suggesting a molecular formula of $C_{36}H_{38}N_2O_6$.

Table 2 : ^{1}H and ^{13}C NMR Spectral Data of Compound **3** and **4**

Position	^{1}H δ, $CDCl_3$ (*J*, Hz)		^{13}C (δ, $CDCl_3$)	
	3	**4**	**3**	**4**
1	4.09 (*br s*)	4.06 (*d*, 10.1)	54.7	60.6
N-CH_3	-	2.31 (*s*)		42.4
3	2.69 (*m*) 2.97 (*m*)	2.79 (*d*, 11.0) 3.28 (*m*)	42.1	44.1
4	2.29 (*m*) 2.29 (*m*)	2.42 (*m*) 2.82 (*m*)	29.2	23.0
4a			133.7	132.1
5	6.27 (*s*)	6.34 (*s*)	104.7	121.5
6			146.1	147.0
6-OCH_3	3.73 (*s*)	3.77 (*s*)	56.2	56.2
7			141.1	136.2
8			144.2	143.9
8a			122.1	124.2
α	2.72 (*d*, 2.8) 3.10 (*d*, 14.8)	2.66 (*d*, 15.5) 2.93 (*m*)	38.7	38.9
9			127.5	132.5
10	5.48 (*br s*)	6.21 (*br s*)	116.0	114.6
11			148.7	148.4
12			148.1	143.6
13	6.55 (*dd*, 8.0, 1.6)	6.75 (*d*, 8.2)	123.6	115.2
14	6.69 (*d*, 7.6)	6.67 (*d*, 7.3)	114.9	122.8
1′	4.13 (*d*, 4.8)	3.72 (*dd*, 11.8, 6.8)	61.1	64.8
N′-CH_3	2.51 (*s*)	2.53 (*s*)	41.4	42.7
3′	2.85 (*dd*, 12.8, 6.8) 3.15 (*m*)	2.88 (*m*) 3.51 (*m*)	44.8	45.5
4′	2.56 (*dd*, 16.4, 4.8) 3.93 (*m*)	2.96 (*m*) 2.96 (*m*)	23.8	24.9
4′a			130.6	130.3
5′	6.31 (*s*)	6.73 (*s*)	112.0	112.3
6′			148.7	149.6
6′-OCH_3	3.53 (*s*)	3.87 (*s*)	55.3	56.1
7′			144.1	143.5
8′	6.64 (*s*)	6.05 (*s*)	116.7	121.5
8′a			128.3	129.3
α′	2.78 (*d*, 5.6) 3.20 (*d*, 14.8)	2.83 (*m*) 3.37 (*dd*, 13.2, 4.1)	40.3	38.5
9′			139.3	135.1
10′	6.78 (*d*, 7.6)	6.42 (*dd*, 8.2, 1.8)	131.2	132.1
11′	6.21 (*d*, 6.8)	6.78 (*d*, 8.2)	120.8	122.8
12′			151.9	154.4
13′	6.87 (*dd*, 8.4, 2.8)	7.05 (*dd*, 8.2, 2.7)	122.3	122.7
14′	7.38 (*dd*, 10.4, 1.6)	7.32 (*dd*, 8.2, 2.2)	128.1	130.1

Table 3 : Results of *Plasmodium falciparum* Inhibition Screening Assay

Compound	Compound name	IC_{50} ($\mu molL^{-1}$)
1	Norisocorydine	*
2	Isocorydine	0.50
3	2-norobamegine	*
4	Obamegine	0.14

**Not available*

The ^{1}H-NMR spectrum particularly revealed two *N*-methyl singlets, which were at δ 2.31 and 2.53 corresponding to *N*-2 and *N*-2′ methyl protons, respectively. It also showed another two singlets attributed to two methoxyl groups appeared at δ 3.77 and 3.87 which were attached to C-6 and C-6′, respectively. The absence of signals positioned between δ 2.95 to δ 3.20 characteristic of a C-7′ methoxyl indicated that C-7′ was phenyl ether linkage instead substituted with hydroxyl or methoxyl group [19]. The spectrum also showed three singlets at δ 6.05, 6.34 and 6.73 which were assignable to H-8′, H-5 and H-5′, respectively. In addition, H-10 resonated as a broad singlet at δ 6.21. The spectrum also displayed three doublet of doublets attributable to H-10′, H-13′ and H-14′ were present at δ 6.42 (*J* = 8.2 and 1.8 Hz), 7.05 (*J* =8.2 and 2.7 Hz) and 7.32 (*J* = 8.2 and 2.2 Hz), respectively.

In addition, H-14, H-13 and H-11' appeared as a doublet at δ 6.67 (*J* = 7.3 Hz), 6.75 (*J* = 8.2 Hz) and 6.78 (*J* = 8.2 Hz), respectively. A doublet (*J* = 10.1 Hz) and doublet of doublets (*J* = 11.8 and 6.8 Hz) signals corresponding to two protons, H-1 and H-1′ were observed at δ 4.06 and 3.72, respectively.

The ^{13}C NMR spectrum revealed thirty-six carbons. There were fourteen quarternary carbons, two methoxyls, twelve methines, six methylenes, and two methyl groups attached to two different nitrogen atoms consistent with the structure proposed. Signals for C-1 (δ 60.6), C-3 (δ 44.1) and C-8a (δ 124.2) shifted to a lower field due to the presence of methyl group at *N*-2 position when compared with compound **3**. Furthermore, in the HMBC spectrum for compound **4**, long-range correlation at *N*-2 connected with C-1 and C-3 was observed and this is to further confirm the position of methyl. The full assignment of the 1D-NMR (^{1}H and ^{13}C) spectral data are given in Table 2.

The isolated alkaloids were tested for *in-vitro* inhibitory activity against *Plasmodium falciparum*. The IC_{50} value of compound **2** and **4** were tabulated in Table 3 and then compared with standard chloroquine (IC_{50}, 0.0069 $\mu molL^{-1}$) [20]. Both compounds showed weak inhibitory activity against *Plasmodium falciparum*.

Conclusions

Study on the leaves of *Alseodaphne corneri* has resulted in the isolation and the identification of norisocorydine **1**, isocorydine **2**, 2-norobamegine **3** and obamegine **4**. Antiplasmodial activity test showed that isocorydine **2** and obamegine **4** exhibited weak inhibitory activity against *Plasmodium falciparum* compared with standard chloroquine.

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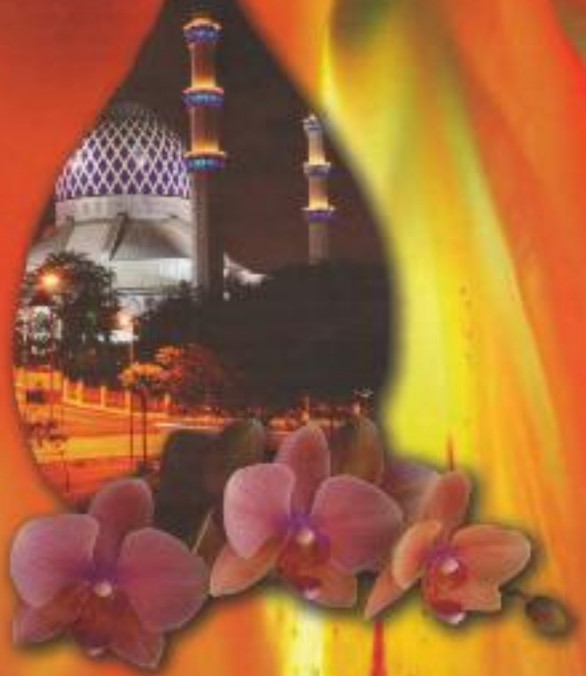

have never been reported. Our recent study on the chemical constituents of the stem bark of both *Calophyllum benjaminum* and *Calophyllum javanicum* has yielded three xanthones. These were identified as fuscaxanthone C (1), β-mangostin (2), thwaitesixanthone (3), and dombakinaxanthone (4) together with four triterpenes known as friedelin, β-sitosterol, γ-sitosterol and stigmasterol. The hexane, chloroform, ethyl acetate and methanol extracts of *Calophyllum benjaminum* were tested for antioxidant properties by DPPH free radical scavenging test. Only the methanol extract shows significant antioxidant activity.

Keywords: Clusiaceae; *Calophyllum benjaminum; Calophyllum javanicum;* xanthone; Antioxidant

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P-30

Isolation of Stilbenes from the Stem of *Gnetum microcarpum*

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Over the last 15 years, plant stilbenes have received considerable interest, due to their biological activities and possible pharmacological applications. Large numbers of natural stilbenes isolated from plants are oligomers which include dimers, trimers and tetramers. Gnetaceae is a family of the most advanced members of tropical gymnosperms in the order Gnetales (division Gnetophyta). It composed of only one genus, Gnetum and there are about 30 to 40 species in the tropical lowlands of the world, from northeastern South America, tropical West Africa, and south China to Southeast Asia. Various species in the family have been used as folk medicine for the treatment of arthritis, bronchitis and asthma. The leaves and the fruits are also used as food in many parts of the tropics [1]. The plants of Gnetaceae are known to contain stilbene oligomers as their major chemical constituents, in which their structural formations are unique [2]. In this research, the lianas of *Gnetum microcarpum* has been investigated. *Gnetum microcarpum* Blume grows in Malaysia and is not recorded in folk medicines. The standard procedures of extraction, fractionation, isolation and elucidation were used for the accomplishment of this research. The stem of *Gnetum microcarpum* was chopped, air dried, grind into powder and extracted using acetone. The crude extract obtained was fractioned with vacuum liquid chromatography (VLC) and each fraction was subjected to multiple column and radial chromatography techniques for isolation and purification process. Four known stilbenes were successfully isolated from the stem of Gnetum microcapum namely resveratrol (1), gnetol (2), gnetucleistol C (3) and gnetucleistol D (4). The structures of these stilbenes were determined using several spectroscopic methods which were 1D and 2D NMR, UV, IR and MS.

Keywords: Gnetaceae, Gnetum microcarpum, stilbenes

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P-31

Aporphine and Bisbenzylisoquinoline Alkaloids from Roots of *Alseodaphne corneri* Kosterm (Lauraceae)

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In Malaysia, there about 15 genera and 212 species of Lauraceae family and one of the species that reported contained various aporphine and bisbenzylisoquinoline types alkaloids is *Alseodaphne corneri* Kosterm. The plant of this family growth in moderate size in Singapore, Malaysia, Jawa, Sumatra and Borneo. The phytochemical study of the roots of *Alseodaphne corneri* (Lauraceae) had been carried out. Chromatographic separation of the alkaloid extract led to the isolation of four isoquinoline alkaloids namely laetanine (1), boldine (2), *O*-methyllimacusine (3) and stephasubine (4). The isolation and purification of the alkaloids were achieved using column chromatography (CC) and preparative thin layer chromatography (PTLC). The structural elucidation

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PETRONAS

NAT-P8

ALKALOIDS FROM THE BARKS OF *ALSEODAPHNE PEDUNCULARIS* (WALL. EX. NESS) MEISSN

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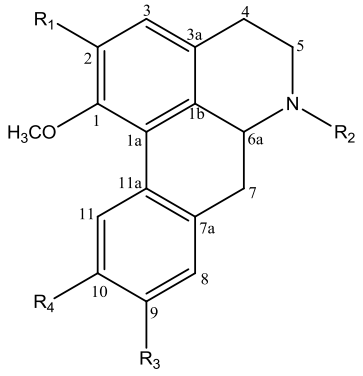
In Malaysia, Lauraceae is known as '*Medang*' or '*Tejur*' which distributed in the lowland and becoming more abundant in the mountains between 1200 and 1600 m altitude. The phyochemical study of *Alseodaphne peduncularis* had been carried out. The alkaloid extract produced three aporphines namely boldine **1**, norboldine **2**, norpredicentrine **3** and norlirioferine **4**. The isolation and purification of the alkaloids were achieved using column chromatography (CC) and preparative thin layer chromatography (PTLC). The structural elucidation was performed by spectral methods mainly UV, IR, NMR including 1D-NMR (^{1}H and ^{13}C) and 2D-NMR (COSY, HMQC and HMBC).

Keywords: alkaloids, *Alseodaphne peduncularis*, *Alseodaphne corneri*, Lauraceae.



Alkaloid	R_1	R_2	R_3	R_4
1	OH	CH_3	OH	OCH_3
2	OH	H	OH	OCH_3
3	OH	H	OCH_3	OCH_3
4	OCH_3	H	OCH_3	OH

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Bisbenzylisoquinoline alkaloids from leaves of *Alseodaphne Corneri* Kosterm (Lauraceae)

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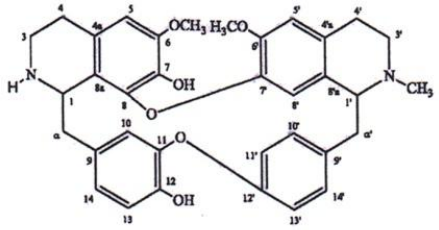
A phytochemical study on the leaves of *Alseodaphne corneri* (Lauraceae) has been carried out. The dichloromethane extract produced two bisbenzylisoquinoline alkaloids namely 2-norobamegine **1** and obamegine **2**. The isolation and purification of the alkaloids were achieved using column chromatography (CC) and preparative thin layer chromatography (PTLC). The structural elucidation was performed by spectral methods mainly 1D and 2D NMR, IR, UV and MS, and in comparison with published literature.



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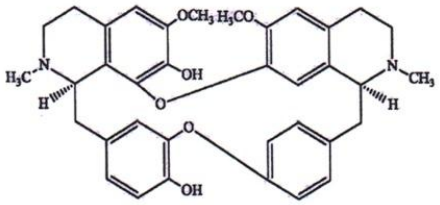

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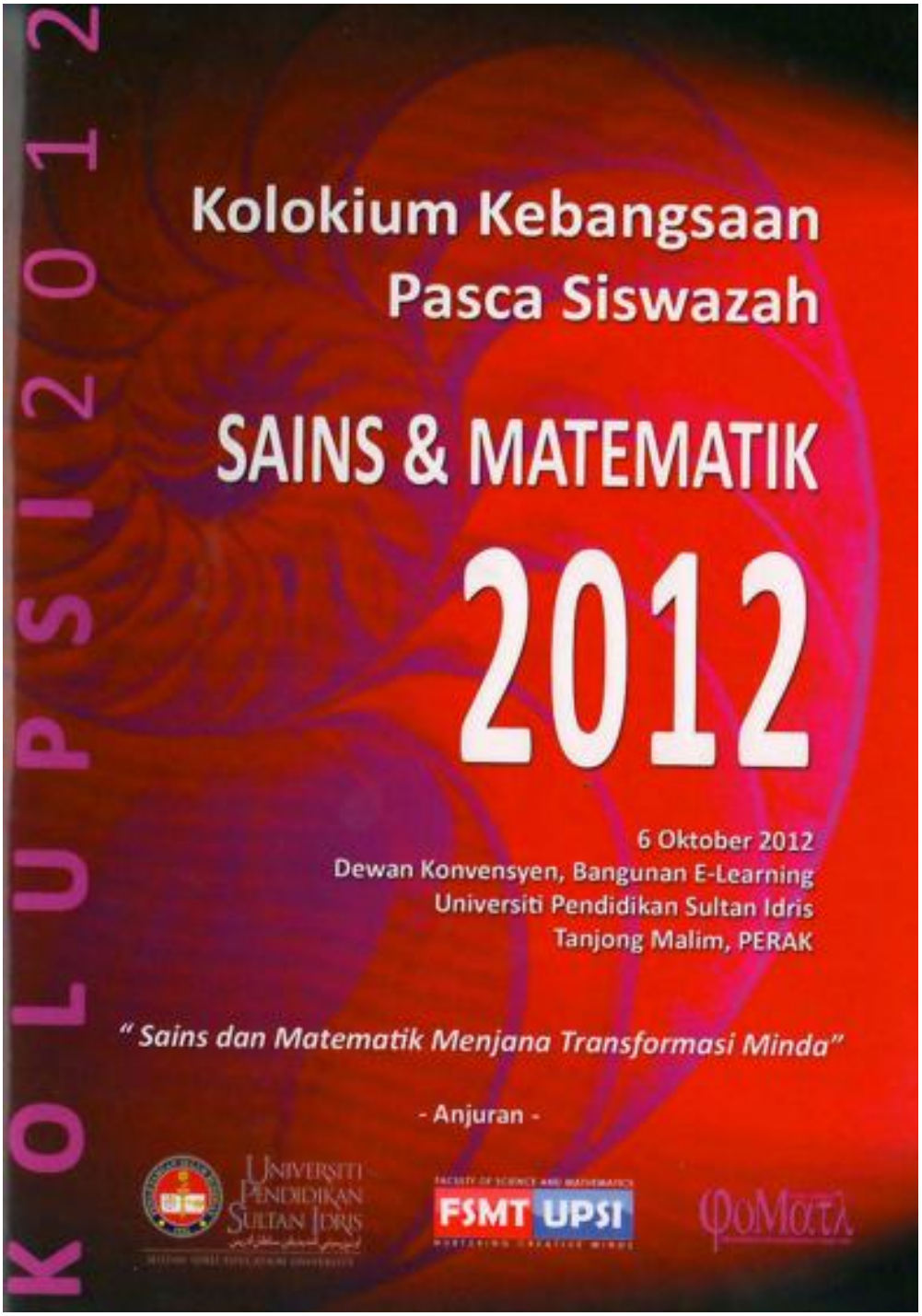

KIMIA

ISOLATION OF BOLDINE FROM *ALSEODAPHNE PEDUNCULARIS* (WALL. EX. NESS) MEISSN (LAURACEAE)

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Abstract

A phytochemical investigation of the bark of *Alseodaphne peduncularis* (Wall. Ex. Ness) Meissn (Lauraceae) has resulted in the isolation of a known aporphine alkaloid; boldine (**1**). The isolation and purification of the alkaloids were achieved using column chromatography (CC) and preparative thin layer chromatography (PTLC). The structural elucidation was performed by spectral methods mainly 1D and 2D NMR, IR, UV and MS, and in comparison with data from other literature.

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P-005

ALKALOIDS FROM ROOTS OF *Alseodaphne Corneri* Kosterm (Lauraceae)

Siti Nor Fadzilah Mohammad[1], Kartini Ahmad[1], Muhammad Hafiz Husna Hasnan[1]
Jennie Khoo Hui Chen[1], A. Hamid A. Hadi[2], Khalijah Awang[2], Mohd Azlan Nafiah[1]
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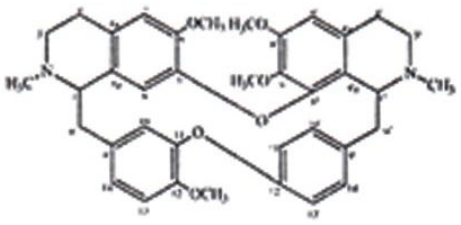
Lauraceae distributed in the lowland and becoming more abundant in the mountains between 1200 and 1600 m altitude. Major producing states in Peninsular Malaysia including Kelantan, Perak, Terengganu, Negeri Sembilan and Kedah (Gan & Lim, 2004) *Alseodaphne corneri* Kosterm belongs to Lauraceae family. The *Alseodaphne* genus is well known for their alkaloid bearing plants that have the isoquinoline structures. A phytochemical study on the roots of *Alseodaphne corneri* had been carried out. The roots were air dried, grinded and extracted with dichloromethane. The extract was then proposed to further isolation and separation process such as column chromatography and preparative thin layer chromatography to obtain pure alkaloid compound. Two bisbenzylisoquinoline alkaloids were successfully isolated from the roots of *Alseodaphne corneri* namely gyrolidine **(1)** and 2-norobaberine **(2)**. The structural elucidation was performed by spectral methods mainly NMR, UV, IR and MS. Finally, determination of compounds was further confirmed by comparison with previous works.



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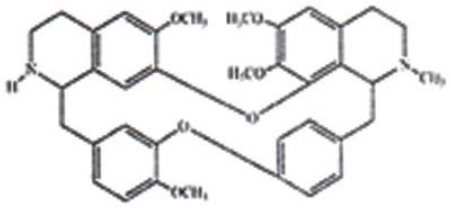

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