CHAPTER 1
INTRODUCTION

1.1 General

Natural products could be any biological molecule, but the term is usually reserved for secondary metabolites or small molecules produced by an organism.[1] They are not strictly necessary for the survival of the organism but they could be for the basic machinery of the fundamental processes of life.[2]

Many phytochemicals have been used as medicines such as for cardiovascular drugs, reserpine and digitoxin which were isolated from *Rauwolfia serpentine* and *Digitalis purpurea*.[3,4] The natural antimalarial drug, quinine from *Cinchona* species together with the synthetic drugs, chloroquine and antabrin are still widely used until today to treat malaria.[5]

Plants are of great importance to many local communities in Malaysia and Asia, either used as health supplements or for therapeutic purposes. Concoctions of several species in the form of *jamu* are consumed daily. Herbal medicine is now becoming more popular as alternative to western medicine and is now available in many forms. As a result, there has been rapid development of herbal industries utilizing locally available plants, either wild or introduced.[6]

Malaysian tropical forest is home to a large number of plants and considered to be one of the oldest and richest in the world. The rich Malaysian flora provides opportunities for the discovery of novel compounds, some of which could have useful bioactivities. The use of plant extracts and phytochemicals, both with known biological activities, can be of great significance in therapeutic treatments. Lauraceae plants are known to be prolific producers of many interesting alkaloids such as the rare proaporphine-tryptamine dimers; phoebegrandines A-B and (-)-phoebescortechiniine isolated from *Phoebe grandis* and *Phoebe scortechinii*, and bisbenzylisoquinoline alkaloids; oxoperakensimines A-C and 3′, 4′-dihydronorstephasubine isolated from *Alseodaphne perakensis* and *Alseodaphne corneri*

respectively. Several antihypertensive alkaloids have been isolated from this family such as dihydronorstephasubine and gyrolidine, which have shown moderate vasorelaxant activity on isolated rat aorta. In continuation of our research on plants from the Lauraceae family, the author has embarked a study on the CH_2Cl_2 extract of the stem bark of *Litsea lancifolia* (locally known as *Medang melukut*) and *Litsea grandis* which were collected in Peninsular Malaysia.[7-11]

1.2 Lauraceae: General appearance and morphology

The Lauraceae family is a green medium size tree found in the tropics especially in India, China, East Africa and South East Asian countries like Malaysia, Indonesia and the Philippines. It is a big family consisting of 62 genera and over 2000 to 2500 species.[12] Among the genus found in Malaysia are *Cinnamomun, Litsea*, *Lindera* and *Nothaphoebe*.[12]

In Malaysia, Lauraceae family was known as "*Medang"* or "*Tejur"* and its growth depends on the type of lowland or highland. In the lowland, it is typically small tress of the lower canopy except for a new species which may reach up to 30 meters tall whereas in the highland the Lauraceae becomes more abundant reaching the top of the forest canopy.

Like the oaks in the mountains, the Lauraceae or Laurel family, become more abundant relative to other kinds of tree and many of them participating in the canopy. Thus the term Oak-Laurel forest is a feature of the mountains of tropical Asia from the Himalayas to New Guinea.[13]

The leaves are spiral, alternate, opposite, sub-opposite, whorled, entire, and leatherly. The color of the new leaves are vary from nearly white to pink, purple, red, or brown. The leaves of many kinds are glaucous beneath and often have closely parallel veinlets between the main veins of the leaf so as to give a spider-web effect.

The flowers of Lauraceae are actinomorphic and usually small, regular, greenish white or yellow, fragrant or with rancid smell, bisexual, or unisexual and united with six sepals in two rows. The fruits are small to large (one seed berry). In some genera the perianth lobes are dropping but the tube is developing into a shallow or deep cup at a base of fruit. The fruit stalks are enlarging and becoming highly colored in some species of *Dehaasia* and *Alseodaphne*. The seeds of Lauraceae are without albumen, with thin testa. The fruit are eaten by monkeys, squirrels, bats, and birds.

1.3 Classification of Lauraceae

Classification of Lauraceae can be illustrated in the list below. The classification included 62 genera, mainly in Southeast Asia and Latin America.[14]

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Laurales

Family: Lauraceae

Genera:

Actinodaphne	*Adenodaphne*	*Aiouea*	*Alseodaphne*
Anaueria	*Aniba*	*Apollonias*	*Aspidostemon*
Beilschmiedia	*Brassiodendron*	*Caryodaphnopsis*	*Cassytha*
Chlorocardium	*Cinnadenia*	*Cinnamomum*	*Clinostemon*
Cryptocarya	*Dahlgrenodendron*	*Dehaasia*	*Dicypelium*
Dodecadenia	*Endiandra*	*Endlicheria*	*Eusideroxylon*
Gamanthera	*Hexapora*	*Hypodaphnis*	*Iteadaphne*
Kubitzkia	*Laurus*	*Licaria*	*Lindera*
Litsea	*Machilus*	*Mezilaurus*	*Mocinnodaphne*
Mutisiopersea	*Nectandra*	*Neocinnamomum*	*Neolitsea*
Nothaphoebe	*Ocotea*	*Paraia*	*Parasassafras*
Persea	*Phoebe*	*Phyllostemonodaphne*	*Pleurothyrium*
Potameia	*Potoxylon*	*Povedadaphne*	*Ravensara*
Rhodostemonodaphne	*Sassafras*	*Sextonia*	*Sinosassafras*
Syndiclis	*Triadodaphne*	*Umbellularia*	*Urbanodendron*
Williamodendron	*Yushunia*		

Scheme 1.1: Classification of Tribes

1.4 Medicinal uses of Lauraceae

From the studies carried out previously, plants from Lauraceae family were known to have various commercial value and medicinal uses. Cinnamon, the dried bark and twig of *Cinnamomum* species is one of the most popular Lauraceae genus and the oldest spices

used for foods, confectionery, and mulled wine. They also an important crude drug frequently prescribed in Chinese and Japanese traditional medicine[15], and it has been used as stomachic, carminative, diaphoretic, astringent, analgesic, and antipyretic in Asian countries. The cinnamon in Asian traditional medicines is represented by *C. cassia* Blume, which is called cassia or Chinese cinnamon, although some other *Cinnamomum* species, such as *C. zeylanicum* Nees, *C. burmanni* Blume, and *C. obtussifolium* Nees, etc. are also used under the name of cinnamon.[16]

In Peninsular Malaysia, the bark of *Cryptocarya massoy* is used by the women after childbirth, also added to tonics and cigarettes. In Indonesia, it is used against diarrhea and spasmodic bowel trouble usually in combination with a cinnamon. Furthermore, it is an ingredient of various native medicines and has a characteristic odor. In New Guinea, the aromatic bark is employed to treat fever and a small plug of the bark is placed so as to close a fresh wound. Meanwhile, for the treatment of the sore eyes usually the bark of *C. griffithiana* Wight and *C. multipaniculata* Teschn is used.[17]

Lindera glauca Sieb is one of Lauraceae species that can be found in the forests at low altitudes in Japan, China and also Taiwan.[18] It has been used for the treatment of stroke, pains of the heart and abdomen.[19]

1.5 The genus *Litsea*

The genus *Litsea* (Lauraceae) comprises nearly 200 species, which are distributed widely throughout tropical and subtropical Asia, North America and subtropical South America. In China, it's represented by 72 species and mostly growing in the south and southwestern parts of the country on mountains 1500 m above sea level or higher,[20] while in Indonesia it's represented about 22 species genera.[21]

In Peninsular Malaysia, the species that can be found are *L. johorensis, L. trunciflora, L. magnifica, L. tomentosa, L.grandis, L. artocarpifolia, L. firma, L. gracilis, L. amara, L. polyantha, L. wrayi, L. cordata, L. glabrifolia, L. angulata, L. quercina, L. perakensis, L. machilifolia, L. panamonja* and also *L. robusta*.

Litsea species are normally trees or shrubs. The leaves are usually alternate or opposite or sub-opposite. The flowers are shortly pedicelled or sessile. The perianth tube in males was small or none while in females it is funnel shaped and have 6 lobes. The fruits were globose ovoid, ellipsoid or cylindric on the perianth tube enlarged in a cup, usually large and often thick.[22]

1.6 Medicinal uses of *Litsea* species

Some of the Litsea are important as medicine in certain countries. For example, Litsea *glutinosa* C. B. Rob., locally known as "Chan Gao Shu" in China and "Jaisanda" in India is an evergreen medium-sized tree. Its bark and leaves are used as a demulcent and mild astringent for diarrhea and dysentery, and the roots are used to poultice sprains and bruises.[23]

Conventionally *Litsea cubeba* has been used as a seasoning and Chinese herbal medicine in China and Taiwan. The leaves contain essential oil and the roots and woods have flavoring and diuretic effects. The seeds and fruits are often used as seasoning by the native Taiwanese. However, it is unclear whether consumption of this plant decreases the prevalence of cardiovascular diseases in the native Taiwanese. The present and previous studies suggest libetamine may have beneficial effects in preventing cardiovascular diseases.[24,25]

Litsea pungens Hemsl. is found mostly in northwestern and eastern China.[26] The fruits of *L. pungens* Hemsl. contains 1-4% essential oil, whereas foliage contains 0.4% and roots contain only 0.2-0.3%[27]. *L. pungens* has long been used in traditional medicine against influenza, stomachache, and other ailments.[28] Research indicates that *L. pungens* essential oil has immunostimulatory, antimicrobial, and antitumor activities.[29,30,31]

Litsea polyantha Juss. also known as *Litsea monopetala* Roxb. is a small to medium sized evergreen tree. The bark of *L. polyantha* Juss. has a long history of medicinal use among the traditional healers of Oraon and Munda community of Jharkhand, India. It is known by the popular names of Pojo, Kakuri, Munga and Barkukuchita. The bark of *L. polyantha* Juss. is mildly astringent and is reported to be used as diarrhea while powdered bark and roots are used for pains, bruises and contusions and also for fractures in animals.[32]

Previous phytochemical studies revealed the presence of alkaloids,[33,34] butanolides, [35,36] and sesquiterpenoid in the genus *Litsea*.[37-40] *Litsea* plants also exhibit a variety of biology activities, including antimicrobial, antimutagenicity, chemo preventative, insecticidal, hypothermic, and antitumor activities.[41-43]

1.7 *Litsea grandis*

Litsea grandis is a medium sized tree up to 15 m tall with about 30 cm diameters. The bark is dark brown, lenticellate, smooth to cracking and scaly. The inner bark is pale yellow brown and strongly aromatic while the young twigs of this species are velvety brown hairy. The leaves are spirally simple, thickly coriaceous from elliptic to broadly oblong or obovate. They are apex rounded, base obtuse with 18.5-25 × 9.5-15 cm. The upper surface is bright green, sparsely hairy on midrib while secondary nerves were placed on the lower surface with pale green colored.[7] The fruit of this species are ellipsoid, 2-3 cm long on a thin obconic tube.[22]

Figure 1.1: Pictures of barks and leaves of *Litsea grandis*

1.8 *Litsea lancifolia*

Litsea lancifolia is a bush or small sized tree about 10 m tall with 12 cm diameters. Its bark is dark brown and smooth texture while the inner bark is pale yellow. The young twigs of this species are densely brownish hairy. *Litsea lancifolia* leaves are chartaceous, opposite, rather long acuminate, thinly coriaceous from lanceolate to elliptic oblanceolate. The apex is shortly acuminate blunt with narrow base, obtuse or rounded. The apex also looks shining dark green above with glaucous color below it. The young leaves is pinkish

red colored, nerves 9-13 pairs which are faint above it.[44] The fruits are shaped as ellipsoid apiculate.[22]

Figure 1.2: Pictures of barks and leaves of *Litsea lancifolia*

1.9 Objective of the study

The objectives of this study are:

1. To extract the crude alkaloids from two Malaysian *Litsea* species; *Litsea grandis* and *Litsea lancifolia* by using dichloromethane (CH_2Cl_2) as a solvent extraction and to purify the known and new alkaloids by using chromatographic methods such as, column chromatography (CC) and preparative thin layer chromatography (PTLC).

2. To elucidate the structure of isolated alkaloids using spectroscopic methods mainly 1D-NMR (^{1}H and ^{13}C, DEPT-135), 2D-NMR (COSY, HSQC, HMBC, NOESY), Ultraviolet (UV), Infrared (IR) and LCMS-IT-TOF.

3. To classify the alkaloid based on its interesting structure especially aporphine, benzylisoquinoline, morphinandienone and bisbenzylisoquinoline types.

4. To study the biological activity of the major product isolated from *Litsea grandis* and *Litsea lancifolia* for antihypertensive activity.

CHAPTER 2

GENERAL AND CHEMICAL ASPECT

2.1 General

Phytochemical studies of Lauraceae plants have produced non-alkaloid (e.g. carbohydrates, lipids, amino acids, proteins, polyphenol, essential oils, terpenes, aromatic compounds) and also alkaloid constituents. The production of those phytochemicals from the Lauraceae plants has been the subject of a number of comprehensive articles.[45-48]

Among all compounds found in the plants, alkaloids are the most powerful as well as very effective. Hence, it is little surprising that the alkaloids have been researched and examined the most by the modern day scientists. The strength or effectiveness of the alkaloids commonly includes everything or all substances that are poisonous in the plants.

Alkaloids are the natural compounds that include nitrogen and are considered to be disintegration of substances that comprise proteins. They are exuded in particular cells or tubes and can be of great use in safeguard against normal enemies.

Alkaloids have significant therapeutic value and form the ingredients of many important medicines. Normally substances like caffeine and theobromine that are closely associated with natural purine (substance that can be converted to uric acid in the body) compounds are categorized as alkaloids.

The alkaloids are one of the most diverse groups of secondary metabolites found in living organisms and have an array of structure types, biosynthethic pathways, and pharmacological activities. Many alkaloids have been used for hundreds of years in medicine and some are still prominent drugs today.

2.2 Alkaloids

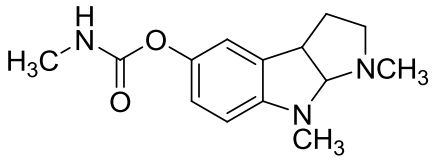
The word alkaloid has been initially drawn from the word ‘vegetable alkali’ and was used to express the alkalinity of a number of initial alkaloidal segregations. Generally speaking, alkaloids comprise almost all alkaline nitrogenous materials having distinct physiological. Colchicine and ricinine are not alkaline in nature, while mescaline, ephedrine and muscarine do not enclose nitrogen. This is despite the fact that alkaloids are conventionally known to be delivered from higher plants, and even animals as well as lower organisms are identified as alkaloid producers.

Normally, the majority of the alkaloids are heterocyclic (compound containing a closed ring of atoms of which at least one is not a carbon atom) in nature. But there are others like mescaline, ephedrine and hordenine that are non-cyclic and these are also often known as ‘protoalkaloids’.

A simple general definition of alkaloids has been suggested by Pelletier (1983), “An alkaloids 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 exception with extracyclic bound nitrogen such as colchicines or capsaicin.[49]

Since ancient times by human, alkaloids have been applied; a prime example of applied alkaloids are poisons for weapons. Poisons derived from plants continue to be utilized not only in hunting food and against the depredations of wild animals, but also in tribal warfare, especially in Africa. In Africa, the extensive use of constituents of the plants providing the hunting poisons as sources of medicines are among the most renowned plants of traditional medicine.

The best known examples are physostigmine **1** for treatment of glaucoma and myasthenia gravis, reserpine **2** as an antihypertensive and phychotropic drug, *d*-tubocurarine **3** as a muscle relaxant in anesthesia, , and ajmaline **4** for cases of cardiac rhythm disturbances.[49] Some well-known examples alkaloids and their physiological activities are listed in Table 2.1.



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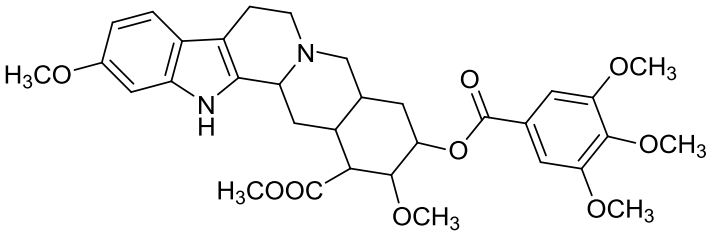

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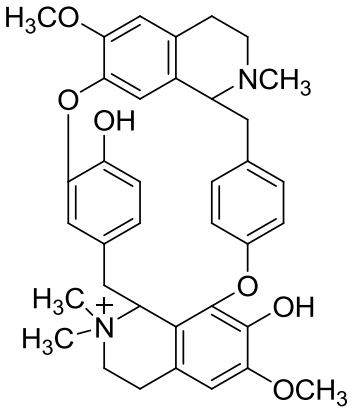
Known Alkaloids with physiological activities



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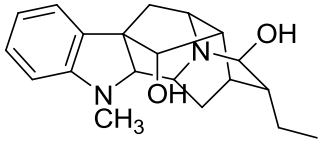

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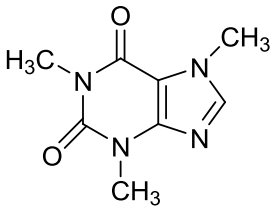

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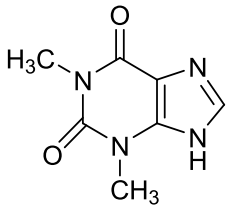

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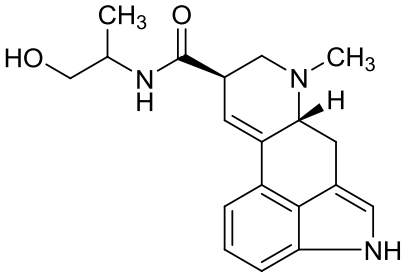

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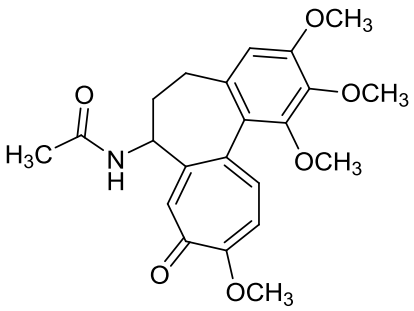
Known Alkaloids with physiological activities



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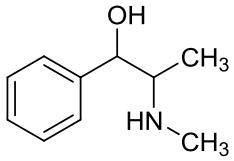

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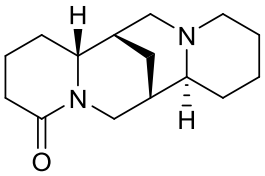

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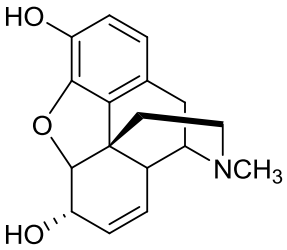

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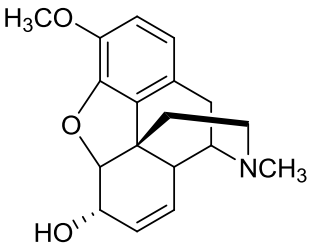

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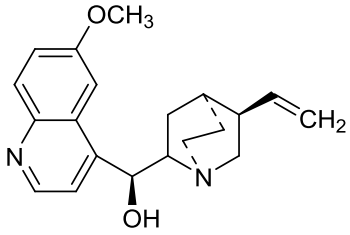

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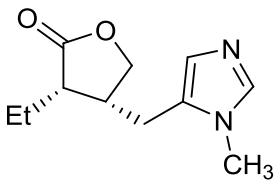
Known Alkaloids with physiological activities



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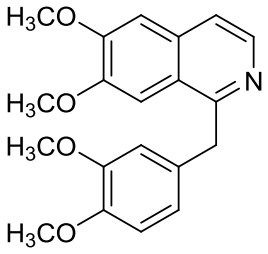

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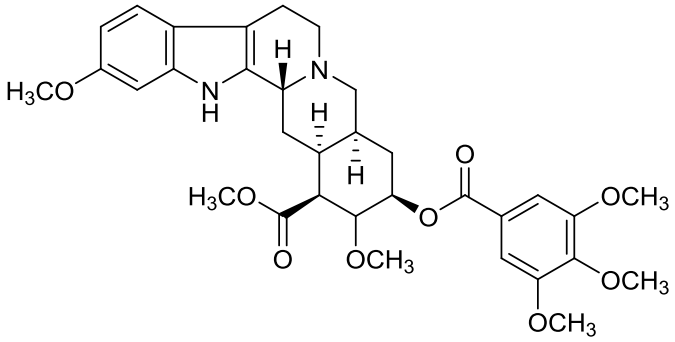

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Known Alkaloids with physiological activities

Table 2.1: Physiological activities of some alkaloids

Alkaloids	Physiological activities	Sources
Caffeine **5**	Acts as a central stimulant and activates circulation and respiration	*Camellia sinensis*
Theophylline **6**	Used in asthma patients	*Camellia sinensis*
Ergometrine **7**	Used in obstetrics	*Claviceps purpurea*
Colchicine **8**	Used in treatment of acute gout and has ability to arrest mitosis at methaphase. It is also used for doubling chromosomes in plant genetics	*Colchicum autumnale*
Ephedrine **9**	Has spasmolytic properties and used to treat asthma and also cough	*Ephedra fragilis*
Lupanine **10**	Used against moles, ulcers, itches, eczema, impetigo and for abortion	*Lupinus albus*
Morphine **11**	For the relief of severe pain. Depressant action on the cough and respiratory centres	*Papaver somniferum*
Codeine **12**	A pain killer and also a cough supressent	*Papaver somniferum*
Quinidine **13**	Used to treat abnormal heart rhythms and malaria	*Cinchona officinalis*
Pilocarpine **14**	Pilocarpine is an agent that affects the nervous system. It increases saliva secretion in the mouth	*Pilocarpus venenosum*
Papaverine **15**	Relaxes veins and arteries, which makes them wider and allows blood to pass through them more easily. It may also be useful in treating conditions involving spasms of the intestines and urinary tract	*Papaver somniferum*
Reserpine **16**	Used to treat hypertension (high blood pressure) and also sometimes used to treat psychotic states such as schizophrenia	*Rauwolfia serpentine*

2.3 Functions of Alkaloids

The role alkaloids in the plants are still unknown, but the researchers suggested that the alkaloids are as the end product of metabolism or waste product. It functions as a protection against predator attack, storage reservoir of nitrogen for protein synthesis, plants stimulants or regulator in many activities, such as growth, metabolism and reproduction and a model for the chemical synthesis of analogue with excellent properties.[50] The function of alkaloids in plants remains the subject of speculation.

2.4 Classification of the Alkaloids

A great variety in the botanical properties, chemical structure, and pharmacological action makes alkaloids unique. Alkaloids has been classified to three main groups by Hegnauer which based on speculation of biogenetic pathways.[51]

- **True Alkaloid**

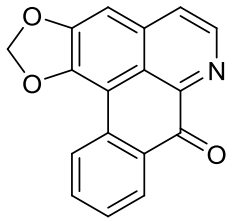
  The true alkaloid are compound in which the nitrogen-containing heterocyclic system is derived from a biogenetic amine which formed by decarboxylation from an amino acid. They are usually found as salts in plant such as liriodenine **17**.

- **Pseudo Alkaloid**

  The pseudo alkaloid are not derived from amino acid precursor and usually basic. They are nitrogen containing in the molecule but they have a carbon skeletons derived from terpenoids, steroidal, hemiterpenoids and other acetate derivatives such as coniine **18** and actinidine **19**.

- **Proto Alkaloid**

These compound like true alkaloids, derived from an amino acids or biogenetic amines but they do not contain any heterocyclic system. They are represented in nature by biogenetic amine themselves and their methylated derivatives such as serotonin **20** and cathinone **21**.



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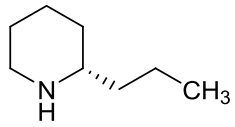

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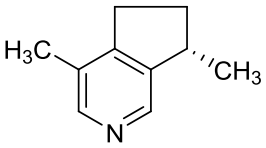

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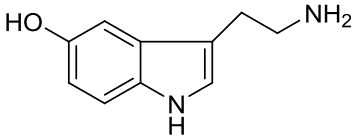

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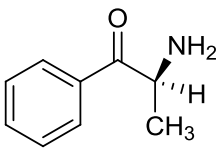


21

2.5 Isoquinoline Type of Alkaloids

In Lauraceae, isoquinolines are the main alkaloidal constituents with aporphine as the large group of this type.[52] There are also a small number of indole alkaloids and quinoline type of alkaloids. The simple isoquinoline alkaloids may be defined as those containing only one aromatic nucleus and no other cyclic structure except a methylenedioxy substituent.

Isoquinoline groups may be further subdivided into several groups such as benzylisoquinolines, bisbenzylisoquinolines, proaporphines, aporphines, berberines, morphinans, aristolactams and micellananeous isoquinoline type alkaloids. These groups were divided based on the skeletal of the structure. Shamma has divided isoquinoline type into 34 categories (Table 2.2).[53] Each type of these isoquinoline alkaloids showed different biosynthesis. Biosynthesis is the experimental study of formation of secondary metabolites.[51]

Alkaloids of isoquinoline type are usually synthesized *in vivo* by a Mannich condensation between two metabolites of phenylalanine or known as tyrosine **22**. Oxidation of tyrosine **22** leads to formation of 3,4-dihydroxyphenylalanine (DOPA) which later forms 3,4-dihydroxyphenylamine or dopamine **23**.

Condensation of dopamine **23** and 4-hydroxylphenylacetaldehyde **24** yields the first alkaloidal intermediate (*S*)-norcoclaurine **25**, which mark the first and central intermediate of isoquinoline alkaloids (Scheme 2.3).[54] Scheme 2.2 shows various type of isoquinoline alkaloids derived from alkaloidal intermediate (*S*)-norcoclaurine **25**.

Bisbenzylisoquinoline can be derived from (*S*)-norcoclaurine **25**. Firstly, (*S*)-norcoclaurine **25** must be change to (*S*)-coclaurine **26** by *O*-methylation for hydroxyl group

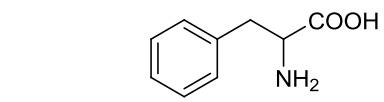
at position 6. Then, condensation between two (*S*)-*N*-methylcoclaurine **27** or coclaurine units produced bisbenzylisoquinoline.[54] The phenolics oxidative reactions with two tetra hydrobenzylisoquinolines moieties are coupled usually proceed with formation of diaryl ether bridge such as berbamunine **28** (Scheme 2.3). Second or third oxidative coupling may occur in this bisbenzylisoquinoline formation as the initial dimerization.[55-57]

Intramolecular oxidative reaction from the (*S*)-norcoclaurine **25** also gives aporphine structure (Scheme 2.4). In morphinan synthesis, morphinandienone was recoqnized as intermediate such as in the morphine **11** biogenesis pathway from norlaudanosoline **29** as shown in Scheme 2.5. Morphine skeleton structure formed when cyclization occur during the electrophilic attack of the benzene nucleus.

Table 2.2: Categories of isoquinoline alkaloids

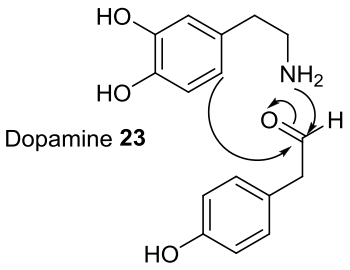
Simple isoquinolines	Benzylisoquinolines
Isoquinolones	Pavines and Isopavines
Bisbenzylisoquinolines	Baluchistanamine
Cularines	Dibenzopyrrocolines
Proaporphines	Aporphines
Pakistanamine	Aporphine-Benzylisoquinoline Dimers
Aporphine-Pavine Dimers	Oxoaporphines
Phenanthrenes	4,5-Dioxoaporphines
Aristolochic Acids and Aristolactams	Dibenzazonines
Protoberberines and Retroprotoberberines	Secoberbines
Benzophenanthridines	3-Arylisoquinolines
Protopines	Phthalideisoquinolines
Spirobenzylisoquinolines	Rhoeadines
Emetine and Related Bases	Phenethylisoquinolines

Homoaporphines and Homoproaporphines	1-Phenylisoquinolines
N-Benzyltetrahydroisoquinolines	Cherylline (A 4-Arylisoquinoline)
Azafluoranthenes and Tropoloisoquinolines	Eupolauridine (A 1,6-Diazafluoranthene)



Tyrosine **22**

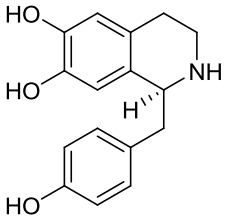
DOPA



Dopamine **23**

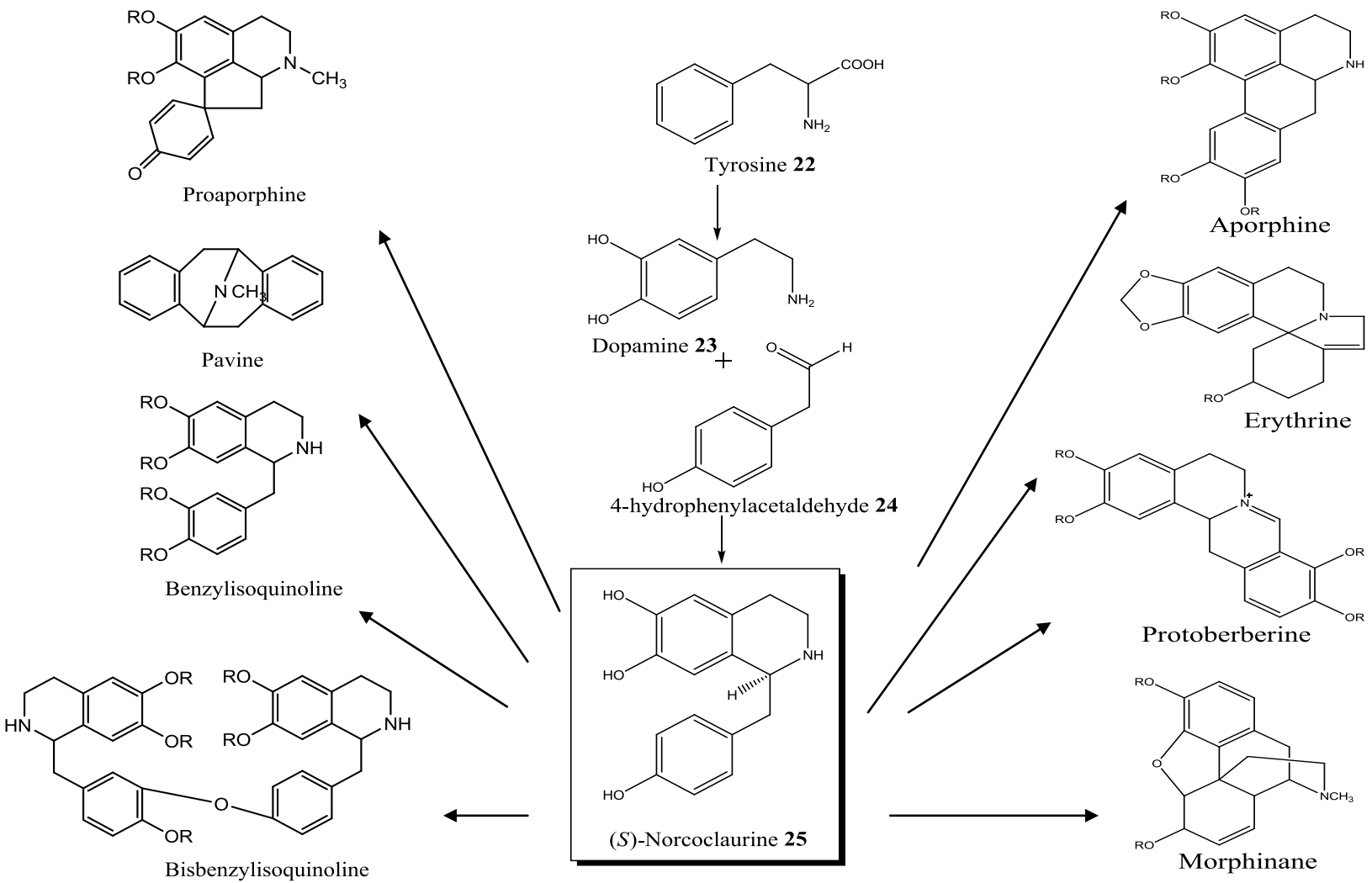
4-hydrophenylacetaldehyde **24**

Mannich
Condensation

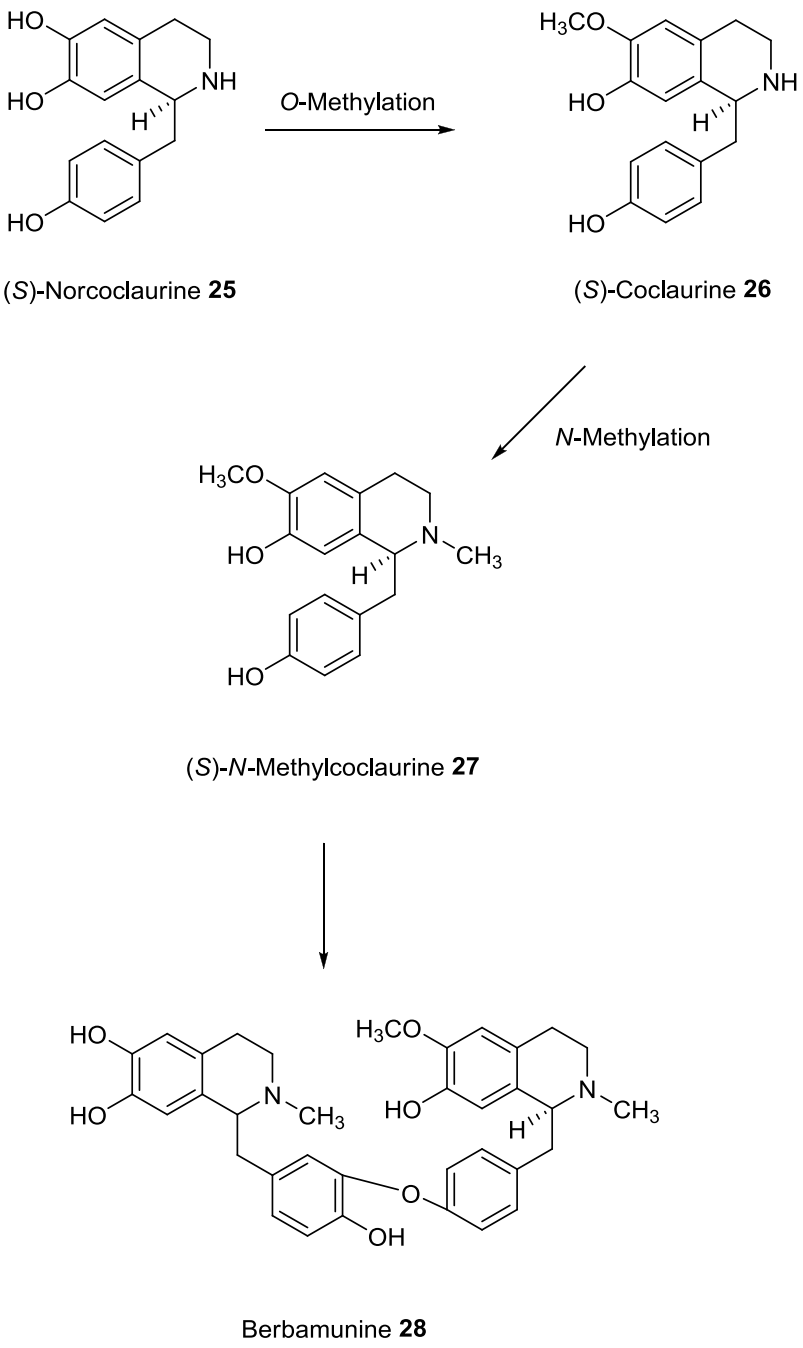


(*S*)-Norcoclaurine **25**

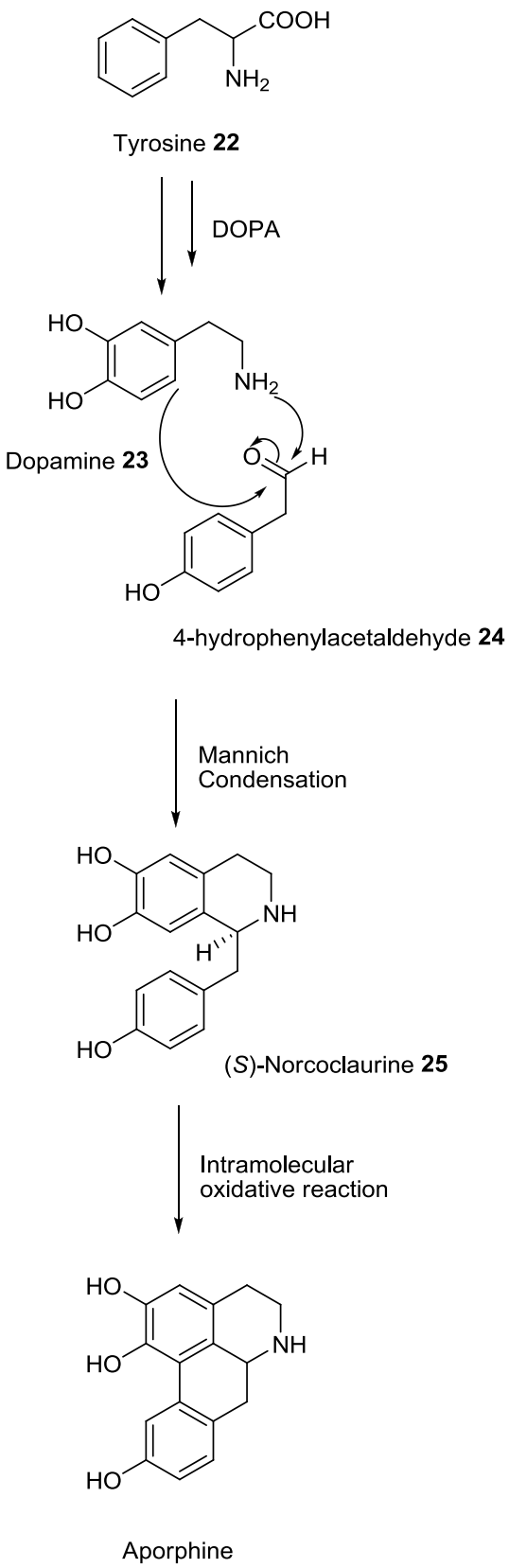
Scheme 2.1: The biogenetic pathway to (*S*)-Norcoclaurine **25**



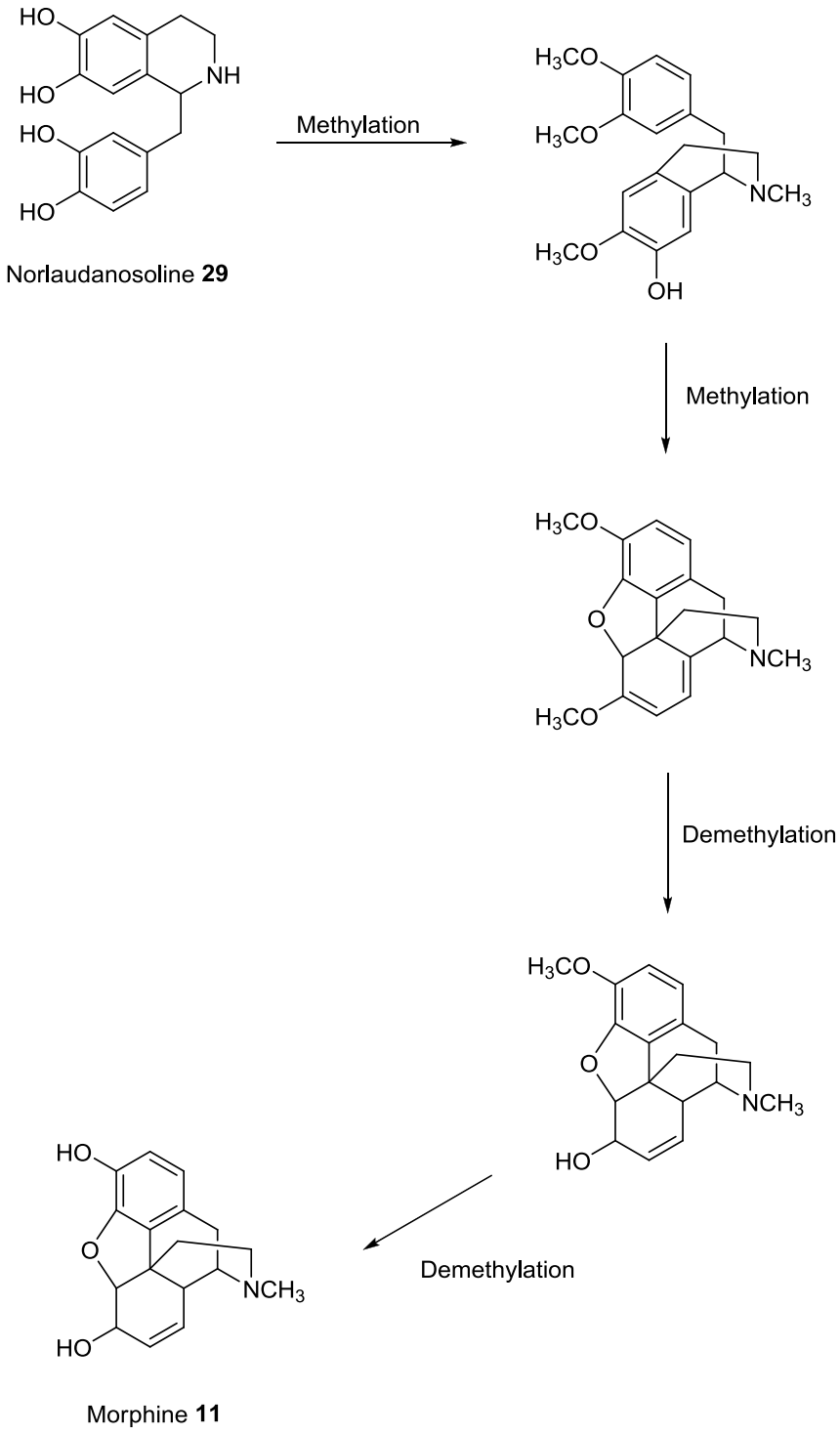
Scheme 2.2 : Biosynthesis of various Isoquinoline Alkaloids from (*S*)-Norcoclaurine **25**



Scheme 2.3: Biosynthesis of Berbamunine **28** from (*S*)-Norcoclaurine **25**



Scheme 2.4: Biosynthesis of Aporphine Alkaloids from Tyrosine **22**



Scheme 2.5: Biosynthetic pathway of Morphine **11**

2.5.1 Simple isoquinolines

As implied by their name, the simple isoquinolines are structurally the simplest of the isoquinoline alkaloids (Figure 2.1). They are usually bicyclic, although tricyclic species such as peyoglutam **30** and mescalotam **31** are also included among them. The nitrogen function in ring B is often tertiary and *N*-methylated, but it may also be secondary, *N*-formylated, *N*-acetylated, *N*-ethylated, or oxidized to the imine stage. Completely aromatic isoquinoline alkaloids such as backebergine **32** are also known.[58]

These alkaloids are derived from tetrahydroisoquinoline and for the most part have a carbon chain attached to C-1, often a one-carbon substituent. Simple isoquinoline derivatives can be further sub-divided based on the differences substituent at C-1 such as those that not bearing a carbon substituent at C-1 such as anhalamine **33**, those with an amide carbonyl group at C-1 like corydaldine **34** and those with a methyl group at C-1 such as salsoline **35**.

Most simple isoquinolines have been obtained from the Cactaceae, but they also occur among the Alangiaceae, Annonaceae, Berberidaceae, Chenopodiaceae, Euphorbiaceae, Fumariaceae, Leguminosae, Menispermaceae, Monimiaceae, Musaceae, Nymphaeaceae, Papaveraceae, Ranunculaceae, Rhamnaceae, and Sterculiaceae.[58]

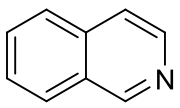
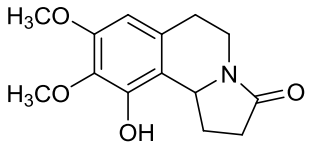


Figure 2.1: Simple isoquinoline skeleton



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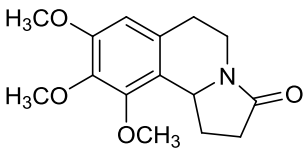

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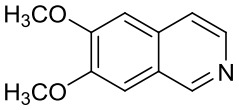

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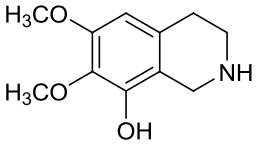

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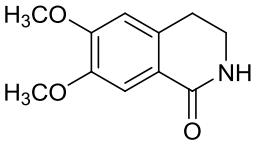

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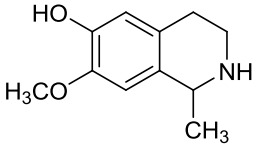


35

Simple isoquinoline alkaloids

2.5.2 Benzylisoquinolines

The benzylisoquinoline types of alkaloids were derived from tyrosine and are the parent skeleton of a wide variety of alkaloids belonging to numerous different ring systems. The benzylisoquinoline alkaloids include both the benzylisoquinoline bases of type **A** and the benzyltetrahydroisoquinolines of type **B** (Figure 2.2).

In a few cases, benzyltetrahydroisoquinolines (type **B**) are aromatic, for example papaverine **15**. Papaverine is the only alkaloid in this group which is currently used in therapeutics.

Benzylisoquinoline alkaloids are widely distributed in the family Anonaceae[59-61], Lauraceae[62-64], Menispermaceae[65], Papaveraceae[66,67], Fumariaceae[68,69], and Ranuculaceae[70,71].

A B

Figure 2.2: The benzylisoquinoline bases of type A and the benzyltetrahydroisoquinolines of type B

2.5.3 Bisbenzylisoquinoline

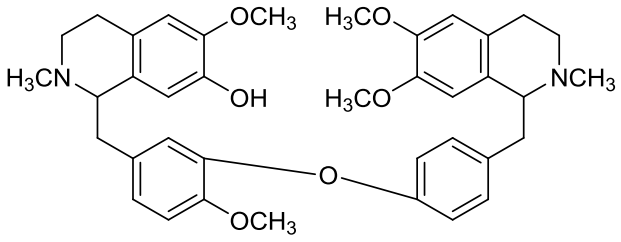
The bisbenzylisoquinoline alkaloids consist of two benzylisoquinoline units attached to each other by one, two or three bonds. In the most structure, the units were joined *via* ether linkage.

The bisbenzylisoquinoline also a complex structure because it consist two symmetric centres that usually presents in either *R* or *S* configuration. Thus, the challenge in the identification of a new bisbenzylisoquinoline is sometimes substantial, given the degree of variation of structural features which may be encountered, differing simply in the nature of the oxygenated substituent, or the oxidation state or degree of substitution of the two nitrogen atoms, or yet in stereochemistry at the two asymmetric centres.

Bisbenzyisoquinoline alkaloids have been classified into 5 groups and 27 subgroups based on the differences of aromatic oxygen substituents, numbers of ether linkages and the nature of ether bridges. Several examples of the subgroups are listed below:

i) Alkaloids containing one diaryl ether linkage between C11 and C12'. This group belongs to the subgroups of type I,Ia,II,III,IV,V bisbenzylisoquinolines.

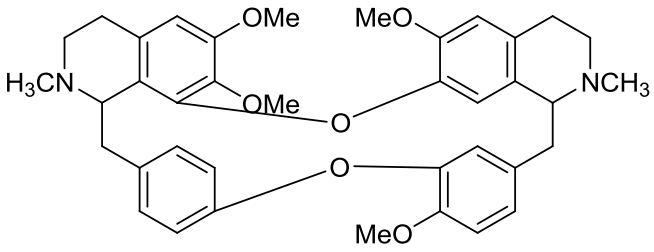
Example : Thalidasine **36**[72]



36

ii) Alkaloids containing two diaryl ether linkages. All these types contain ether linkages between the aromatic rings of tetrahydroisoquinoline component and the benzyl rings. This group belongs to the subgroups of type VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XV, XVI, XVII, XVIII, XIX, XX, XXI bisbenzylisoquinoline.

Example : Obaberine **37**[73]



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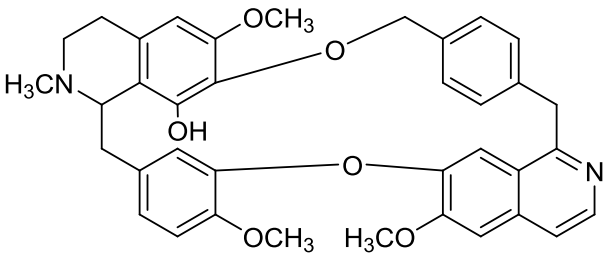
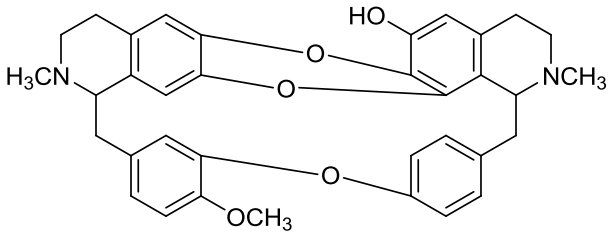
iii) Alkaloids with one diaryl ether and one benzyl phenyl ether linkages. This group belongs to the subgroups of type XXII bisbenzylisoquinoline. Example : Cycleatjehenine **38**[74]



38

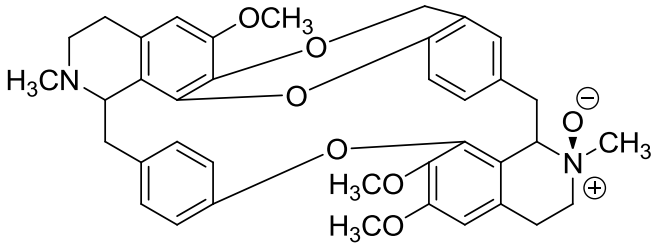
iv) Alkaloids with three ether linkages. This group belongs to the subgroups of type XXIII, XXIV bisbenzylisoquinoline.

Example : 12-*O*-methyltricordatine **39**[75]



39

v) Alkaloids containing two diaryl ether and one diphenyl benzyl ether linkages. This groups belongs to the subgroups of type XXV, XXVI bisbenzylisoquinoline. Example : Insularine 2α-*N*-*Oxide* **40**[52]



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Bisbenzylsoquinolines can be found primarily in Berberidaceae, Menispermaceae, Monimiaceae Ranuculaceae and lately found in Lauraceae[76].

2.5.4 Aporphine

Aporphine alkaloids are the largest group within the isoquinoline alkaloids. All the aporphines alkaloids are based on the 4H-diben[de,g]quinolone structure or its *N*-methyl derivatitive commonly known as the aporphine nucleus. The skeleton of aporphine is showed in Figure 2.3 above. The aporphine alkaloids can be divided into three groups depending upon the degree of methylation at the nitrogen atom. These groups are:

i. The aporphines which contain *N*-methyl function, e.g. isothebaidine **41**

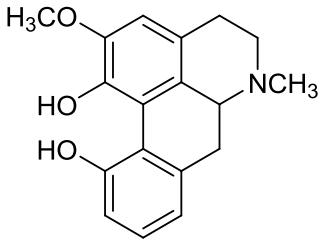
ii. The noraporphines which possess a secondary nitrogen atom, e.g. nornuciferine **42**

iii. The quaternary aporphine salts, e.g. *N*,*N*-dimethylhernovine **43**

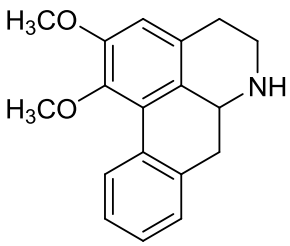
More than 600 aporphinoid alkaloids have been isolated from 19 plants families and many of those plants were used as folk medicines in different parts of the world for the treatment of several kinds of diseases. Examples of the some important families which rich in these alkaloids are Lauraceae, Berberidaceae, Magnoliaceae, Menispermaceae, Ranuculaceae, Monimiaceae, Pavaperaceae and Annonaceae[77,78].

H-6a-(*S*) H-6a-(*R*)

Figure 2.3: Aporphine Skeleton



41



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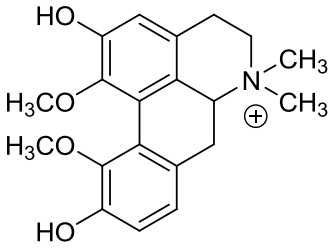


43

Example of aporphine alkaloids

2.5.5 Morphinandienone

Morphinandienones, recognized as intermediates in the biosynthesis of morphine alkaloids, were obtained *in vitro* by processes mimicking the plant synthesis. It also belongs to isoquinoline group and can exist either in *R* or *S* configuration. Morphinandienone skeleton is generally represented by the Figure 2.4. Morphinandienone structures do not contain 4,5-oxide bridge and consists of one ketone functional group at C7.

Bentley has reported new discovery bases of the morphine-sinomenine group which are salutaridine **44** and its 8,14-dihydro-compound, flavinantine **45** and the closely related amurine **46**[79].

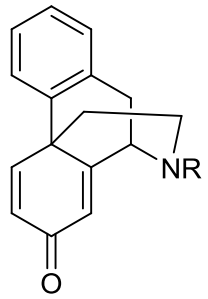
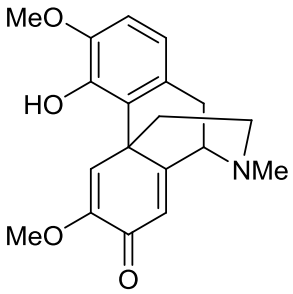
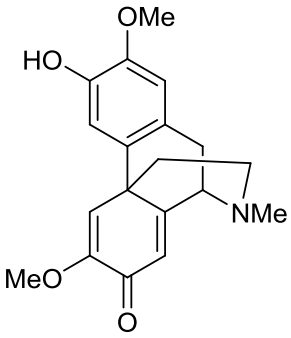


Figure 2.4: The Morphinandienone Skeleton



44



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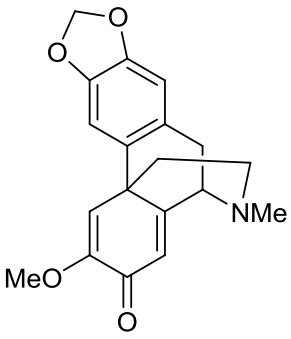


46

Morphinandienone alkaloids

2.6 Structure Elucidation of Alkaloids: General Method and Theory

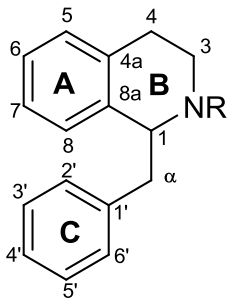
Most common methods applied for structural elucidation of alkaloids are:

^{1}H and ^{13}C NMR	: To detect number and peak of proton and carbon
Infrared spectroscopy	: To detect functional groups
Ultraviolet spectroscopy	: To detect conjugated systems
Mass spectroscopy	: Measures the mass-to-charge ratio of organic ions
Optical rotary dispersion:	Measures the change in rotary power of molecules

In the following section, the general spectral characteristics of benzylisoquinoline, bisbenzylisoquinoline, aporphine and morphinandienone will be discussed briefly.

2.6.1 Benzylisoquinoline

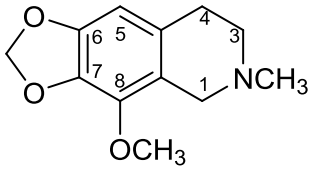
Benzylisoquinoline consists of three rings (A, B and C) and the numbering of benzylisoquinoline skeleton is generally presented by the structure below.



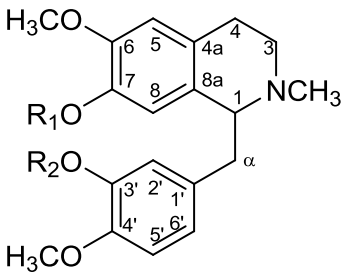
2.6.1.1 ^{1}H NMR Spectroscopy

Due to the one asymmetric centre at C-1, the ^{1}H NMR data of benzylisoquinoline (Table 2.3) shows an interesting feature. Protons at position 1 showed a triplet or doublet of doublet (J_1 = 8-9, J_2 = 1.5-3 Hz) with chemical shift (in $CDCl_3$) between δ 3.6-3.7. Aliphatic proton signals for H-3, H-4 and H-α, normally appeared as multiplet at δ 2.5-3.5.

The methoxyl groups of the benzylisoquinoline, generally resonate at δ 3.50-4.00. Normally, *N*-methyl groups resonate in the region of δ 2.4-2.6. Table 2.3 shows the chemical shifts of ^{1}H NMR for some benzylisoquinolines such as hydrocotarnine **47**, laudanosine **48** and laudanine **49**.



47

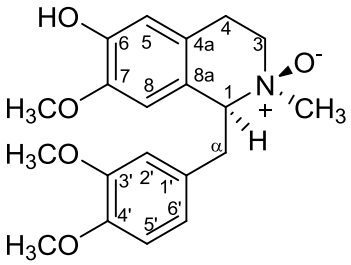


48 R_1= CH_3, R_2= CH_3
49 R_1= CH_3, R_2= H

Table 2.3: ^{1}H NMR (in $CDCl_3$, ppm) for some benzylsioquinolines

	Chemical shifts (δ ppm)		
Position of H	**47**	**48**	**49**
H-1	3.44	3.64	3.64
H-3	2.60	2.73 and 3.12	2.74 and 3.09
H-4	2.80	2.55 and 2.78	2.57 and 2.74
H-5	6.31	6.50	6.45
H-6			
H-7			
H-8		6.02	5.81
H-α		2.17 and 3.10	2.54 and 3.03
H-2’		6.55	6.58
H-3’			
H-4’			
H-5’		6.71	6.60
H-6’		6.58	6.36
6-OCH_3		3.77	3.68
7-OCH_3		3.53	3.37
8-OCH_3	3.98		
3’-OCH_3		3.73	
4’-OCH_3		3.78	3.68
N-CH_3	2.45	2.49	2.40
OCH_2O	5.85		

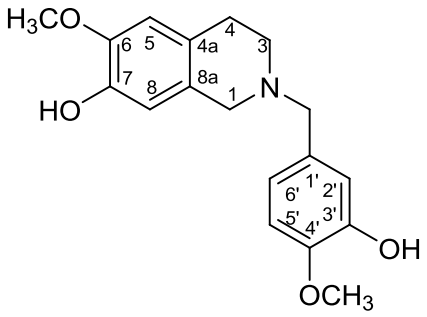
The presence of an *N*-oxide group in alkaloids exert large deshielding effect on C-1, C-3 protons and also on the *N*-methyl group such as in trans-*N*-oxypseudolaudanine **50**.



50

2.6.1.2 ^{13}C NMR Spectroscopy

In the ^{13}C NMR spectra, C-1 normally resonated at δ 52-58, but shifted to higher field such as at δ 60-67 in the presence of *N*-methyl group. Substituted carbon *N*-methyl, methoxyl and methylenedioxy appeared at δ 40-45, δ 54-63 and δ 100-103 respectively. The quaternary carbon at the position 4a, 8a and 1' resonated at δ 115-132 while quaternary carbons with methoxyl and hydroxyl groups appeared at δ 140-152. The unsubstitued sp^2 carbons usually appeared at δ 38-40 and δ 45-46 respectively. The chemical shift of C-4 with *N*-methyl group in the structure appeared at δ 23-24, but it will appear at δ 28-29 without *N*-methyl group. The presence of an *N*-oxide had a large deshielding effect on C-1, C-3 and *N*-methyl groups and normally, it appeared at δ 69-71, δ 60-64 and δ 52-55 respectively. Table 2.4 showed chemical shift of carbon in the ^{13}C NMR for some benzylisoquinolines, such as laudanosine **48**[80], papaverine **15**[81] and capnosine **51**[68].



51

Table 2.4: ^{13}C NMR (in $CDCl_3$ and Acetone D-6, ppm) for some benzylisoquinolines

	^{13}C Chemical shifts (δ ppm)		
Position of C	**48**	**15**	**51**
C-1	65.5	157.5	62.1
C-3	46.8	140.7	50.5
C-4	25.3	118.4	28.7
C-4a	125.8	133.1	127.5
C-5	112.8	105.0	115.5
C-6	146.9	152.1	145.7
C-7	146.9	149.5	145.5
C-8	110.7	103.9	112.3
C-8a	132.2	122.6	125.6
C-1a	40.4	41.9	55.4
C-1’	128.0	132.0	131.5
C-2’	110.7	111.7	110.7
C-3’	148.3	148.8	145.2
C-4’	146.0	147.3	143.7
C-5’	110.7	111.0	110.4

Table 2.4: ^{13}C NMR (in $CDCl_3$ and Acetone D-6, ppm) for some benzylisoquinolines (Continued)

C-6’	121.5	120.2	120.6
6-OCH_3	55.5	55.5	55.9
7-OCH_3	55.5	55.5	
3’-OCH_3	55.3	55.5	
4’-OCH_3	55.3	55.5	55.9
N-CH_3	42.4		

2.6.1.3 Mass Spectroscopy

In the mass spectra of benzylisoquinolines, the main cleavage occurs between C-1 and C-α to form an imine ion. The fragmentation at m/z 192 appeared as a base peak indicated that the carbons C-6 and C-7 was substituted with methoxyl and hydroxyl groups, respectively and in the structure that have *N*-methyl in the structure.

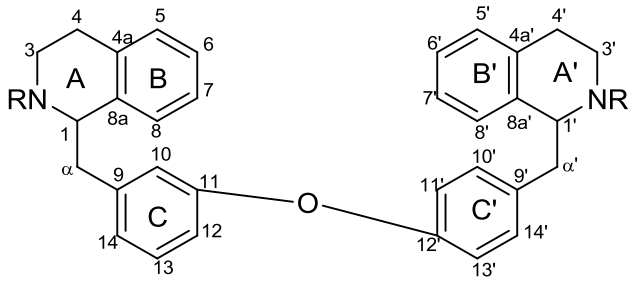
The compounds having a methoxyl and hydroxyl groups in the ring C displayed peak at m/z 137. Two methoxyl groups attached to C-3’ and C-4’ showed the fragmentation peak at m/z 151 and a hydroxyl group in the ring C showed peak at m/z 107[82].

2.6.1.4 Ultraviolet spectroscopy

The ultraviolet spectra of benzylisoquinolines usually show a maximum absorption between 280 and 296 nm effect by additional aromatic substitution. Compounds having methylenedioxy and fully aromatic show increased and more intensity absorption maximum.

2.6.2 Bisbenzylisoquinoline

Since the author has isolated only one type of bisbenzylisoquinoline, which is type 1. Further discussion will be based on this type of bisbenzylisoquinoline which containing one diaryl ether linkages; tail to tail (C11-*O*-C12'). Bisbenzylisoquinoline consists of six rings (A, B, C, A', B' and C') and the numbering of bisbenzylisoquinoline skeleton is generally represented by the structure below.



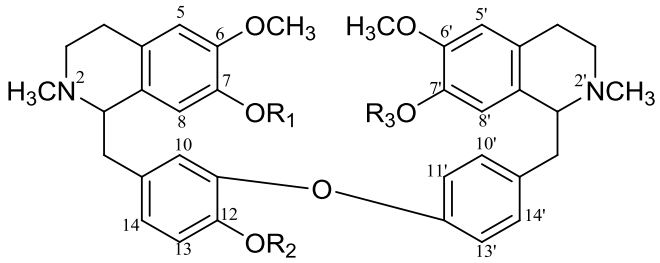
2.6.2.1 ^{1}H NMR Spectroscopy

Generally, ^{1}H NMR spectra of bisbenzylisoquinolines are relatively similar to benzylisoquinolines, and normally the peaks are overlapping and difficult to assign especially if it is identical.

The *N*-methyl group resonated usually near δ 2.50, which showed more upfield signal due to the proton signal of the two *N*-methyl groups. H-8 resonated between δ 5.95 and δ 6.35, corresponds to the substituent at C-7.

If a methoxyl group is present, H-8 appeared at δ 6.00 and more downfield at δ 6.30, if a hydroxyl group is at C-7. The methoxy group at C-7 appeared at δ 3.55, which is the most downfield signal. The same generalization applies to the other side of the dimer. Protons at H-10', H-11', H-13' and H-14' occurred as two doublets, each of which represent

two protons with J~8.2 Hz. Table 2.5 shows the chemical shifts of ^{1}H NMR for some bisbenzylisoquinolines type 1 such as 7'-*O*-methylcuspidaline **52** and temuconine **53**[83].



52 R_1=H, R_2=CH_3, R_3=CH_3
53 R_1=CH_3, R_2=H, R_3=H

Table 2.5: ^{1}H NMR (in $CDCl_3$, ppm) for some bisbenzylisoquinolines

	Chemical shifts (δ ppm)	
Position of H	**52**	**53**
H-5	6.47	6.46
H-8	6.05	6.31
H-10	6.62	6.46
H-13	6.91	6.89
H-14	6.86	6.84
2-*N*-CH_3	2.46	2.56
6-OCH_3	3.81	3.60
7-OCH_3		3.81
12-OCH_3	3.84	
H-5’	6.56	6.58

Table 2.5: ^{1}H NMR (in $CDCl_3$, ppm) for some bisbenzylisoquinolines (Continued)

H-8’	6.36	6.12
H-10’	6.78	7.06
H-11’	6.78	6.84
H-13’	7.00	6.84
H-14’	7.00	7.06
2’-*N*-CH_3	2.54	2.56
6’-OCH_3	3.80	3.85
7’-OCH_3	3.59	

2.6.2.2 ^{13}C NMR Spectroscopy

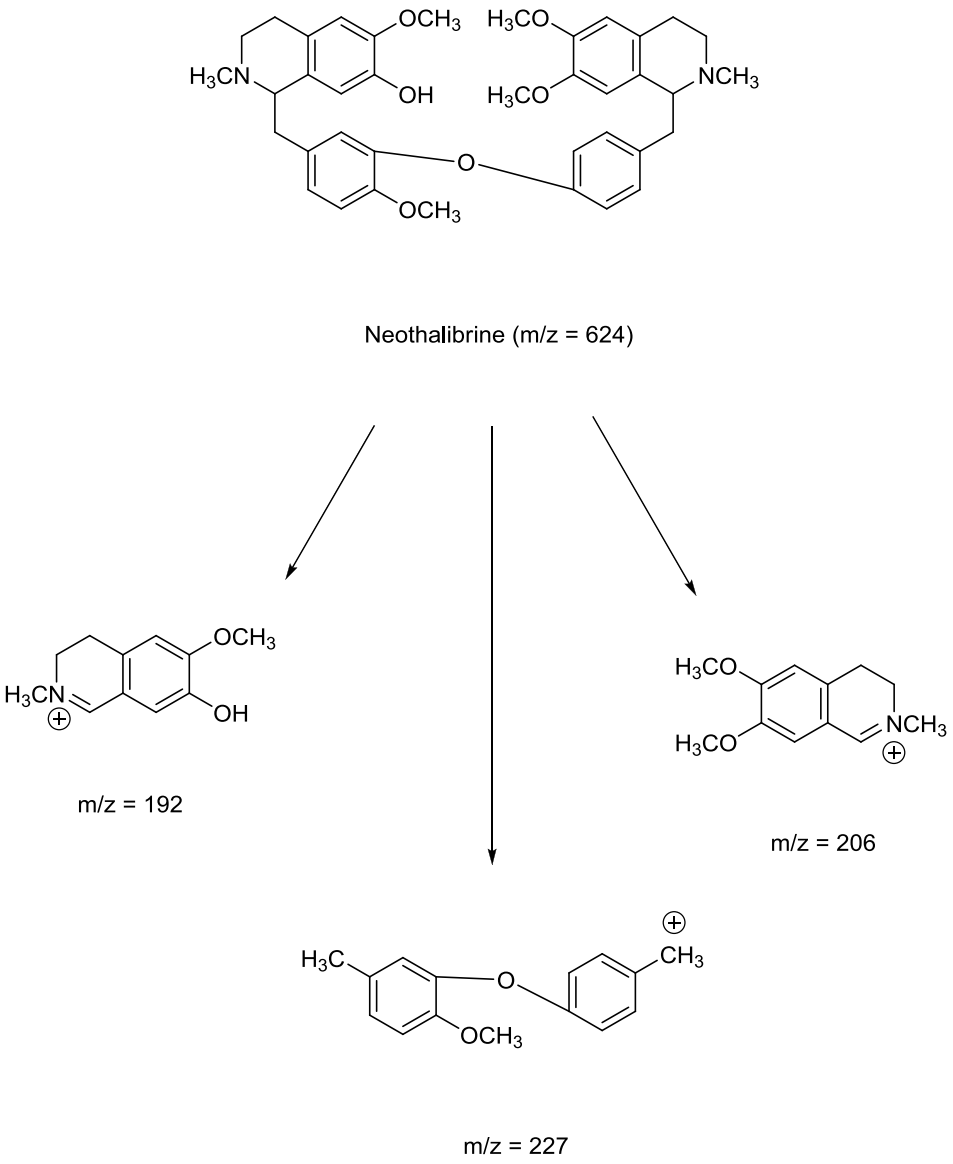
^{13}C NMR provides useful data on the confirmation and configuration of bisbenzylisoquinoline alkaloids. Many signals were grouped in pairs and reflecting the nearly symmetrical nature of the molecule. The sp^3 carbons for position C-4, C-α, C-3 usually appeared at δ 22.0-50.0. The methoxyl carbon signals appeared at normal field near δ 56.0 except for those which attached to C-7 will sterically hindered, cause the signal to appeared further upfield at δ 60.0. The *N*-methyl carbons were at δ 42.0 and it is practically equivalent in all compounds.

The chemical shift of carbon at position 1 and 1’ appeared further downfield at δ 64.0. The presence of an oxygen substituent at position 6, 6’, 7, 7’ and 12 will cause them to resonate further downfield at δ 140-150. The sp^2 carbons (aromatic ring B, B’, C, C’) resonated at δ 111.0-130.0. As an example in ring C resonate at δ 122.7 (C-10), 112.7 (C-13) and 126.2 (C-14).

2.6.2.3 Mass Spectroscopy

Mass spectroscopy is of great importance and has proved valuable in the structural elucidation of bisbenzylisoquinoline alkaloids. Structural classification of bisbenzylisoquinoline alkaloids according to the number and the mode of the diphenyl ether linkages are well correlated with their mass spectrometric gragmentations.

In the mass spectra, all of bisbenzylisoquinoline alkaloids exhibit intense protonated molecular ions ($[M+H]^+$), along with the doubly-protonated molecular ($[M+2H]^{++}$) with 100% relative abundance[84]. The principal fragmentation for this type is shown in Scheme 2.6.



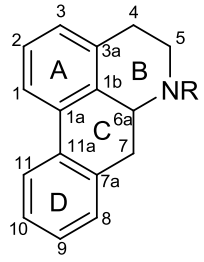
Scheme 2.6: Mass fragmentation pattern of Neothalibrine

2.6.2.4 Ultraviolet Spectroscopy

In UV-VIS spectroscopy the bisbenzylisoquinoline typically exhibit two UV maxima at approximately 283 and 261 nm[85].

2.6.3 Aporphine

Aporphine alkaloids consist of four rings (A, B, C, D). The numbering of aporphine skeleton is generally represented by the structure below.



2.6.3.1 1H NMR Spectroscopy

There are several general features have been observed in the proton shifts for the aromatic protons, methoxyl group, methylenedioxy group and also *N*-methyl group in the ^{1}H NMR spectra of aporphines.

Due to the anisotropic effect generated by π-electron system in the benzene ring, ^{1}H NMR chemical shift of aporphine shows the high field shift of the methoxy group which attached to C-1 and C-11. A proton held above or below the aromatic ring should be shielded where the benzene ring are magnetically very anistropic because of the applied magnetic fields readily generate currents in the π-electron. Usually, C-2 was substituted when position at C-1 and C-11 were substituted; where the methoxyl proton out of the ring plane which was shielded area. Plus, the ring A and ring D are facing each other which

happened to be a shielded zone hence the methoxyl proton can arrange them on the adjacent ring, giving a more up field shift.

The aromatic hydrogen at position 11 is found downfield between δ 8.74–7.68. H–11 usually resonates at lower field δ 8.67–8.43 with respect to the other protons if a methoxyl group substitutes C-1. The low field shift must be attributed not only to deshielding by the neighboring ring, but also to anisotropy effect of the C-O single bond or effects due to C-O electric dipoles as the hydrogen is held very close to the opposite oxygen atom from methoxyl group. H-11 is shifted to δ 8.28–8.04 if they are also *ortho* to a methoxyl group. H-3 normally resonates at a higher field when it is *ortho* to a hydroxyl methoxyl group which due to the inductive effect. Protons at position 8 and 10 are also shifted to a higher field if there is methoxyl substituted at C-9.[86]

The methylenedioxy group shows resonances at range of δ 5.87-6.02. The five possible locations for this group are C-1, 2; C-2, 3; C-9, 10; and C-10, 11. The presence of C-1, 2 methylenedioxy group is proved by an upfield shift of the C-11 proton which appeared in the range of δ 7.47-7.86, and caused the twisted biphenyl system induce magnetic nonequivalent between the methylene proton, which then appeared as doublets at δ 5.90 and 6.10. At position C-9 and C-10, the two protons appear as a singlet whereas at position C-1 and C-2; C-2 and C-3; C-10 and C-11, they appear as two doublets with coupling constant of about 1.5 Hz.

The *N*-methyl group can be typically observed at δ 2.50–2.60 and the aliphatic protons of C-4, C-5 and C-7 displayed a complex absorption pattern between δ 2.40 and δ 4.44. In addition, the methyl group resonated in the region of δ 2.50-2.60. Table 2.6 below summarized the ^{1}H NMR data of aporphine.

Table 2.6: ^{1}H NMR data (δ/ppm) of aporphine alkaloids in $CDCl_3$

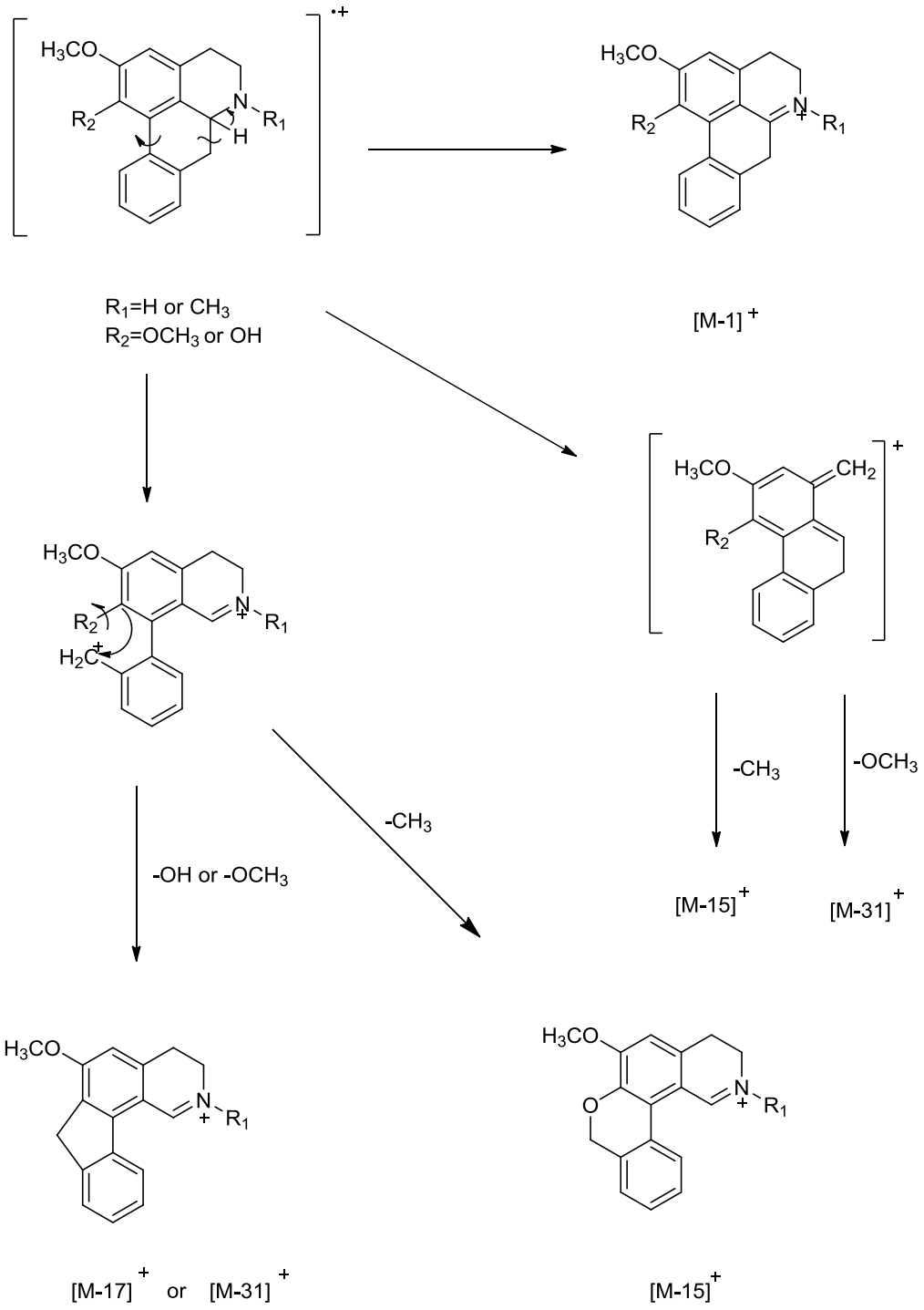
	Chemical shifts (δ ppm)				
Position of substituted	Methoxyl group	Methelenedioxy	Aromatic proton	*N*-methyl group	Aliphatic group
C-1	3.70-3.55				
C-2	4.12-3.75				
C-8			7.00-6.38		
C-9	4.12-3.75		7.00-6.38		
C-10	4.12-3.75				
C-11	3.75-3.65		8.74-8.68		
C-1, 2		6.02-5.87			
C-2, 3		6.02-5.87			
C-8, 9		6.02-5.87			
C-9, 10		6.02-5.87			
C-10, 11		6.02-5.87			
N-Me				4.44-2.50	
C-4					4.00-2.40
C-5					4.00-2.40

2.6.3.2 ^{13}C NMR Spectroscopy

In the ^{13}C NMR sp^2 carbons bearing hydrogen normally resonate at δ 105.0-112.0 while the sp^2 carbons at position 1a, 1b, 3a, 7a, and 11a appeared at δ 119.0-130.0. For sp^3 carbons at position 4 revealed at δ 28.0-30.0; C-7 resonates at δ 35.0; C-5 and C-6a at δ 42.0 and also resonates at δ 52.0 respectively. The substituent carbon *N*-methyl group resonates at δ 43.0 whereas methoxy carbon signal appeared at δ 56.0-62.0.

2.6.3.3 Mass Spectroscopy

In mass spectrum, the fragmentations of the aporphines are mainly because of the loss of hydrogen beside nitrogen [6a-H]. The $[M-1]^+$ peaks always serve as base peak of the molecule. $[M-15]^+$, $[M-17]^+$ and the $[M-31]^+$ peaks will also be observed due to the expulsion of the methyl, hydroxyl, or methoxy group respectively. Aporphine compounds having the *N*-H or *N*-CH_3 groups will display peaks at $[M-29]^+$ and $[M-43]^+$ respectively. The fragments loss is methylene imine group (CH_2=NR) which is expelled via a Retro Diels Alder mechanism. The ion formed can further loose another methyl or methoxyl to produce peaks at $[M-74]^+$, $[M-58]^+$, $[M-60]^+$ and the $[M-44]^+$ peaks. The fragmentation patterns are shown in Scheme 2.7.[55]



Scheme 2.7: The principal Mass Fragmentation of Aporphine Alkaloid

2.6.3.4 Ultraviolet Spectroscopy

The position of the maximum absorptions in the ultraviolet spectra of aporphines depend mainly on the location of the substituents. It is derived from the basic biphenyl system with the added influence of several auxochromes. The approximate absorption for various substitution patterns are listed as in Table 2.7 below.

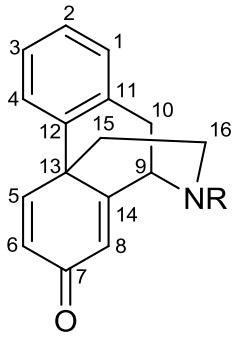
Table 2.7: Ultraviolet Absorption for Aporphine Types

Substitution pattern	Maximum Absorption, λ_{max} (nm)
1, 2	234, 273, 312
1, 2, 9	233, 280, 305
1, 2, 10	226, 266, 275, 305
1, 2, 11	220, 265, 272, 300
1, 2, 9, 10	220, 282, 305
1, 2, 10, 11	220, 270, 305

The shape of the curve and the density of the latter two maxima depend on the substitution in ring D. Furthermore, the monophenolic aporphine position at C-3 and C-9 display a bathochromic shift at 315 nm and 350 nm in the alkaline environment.[55]

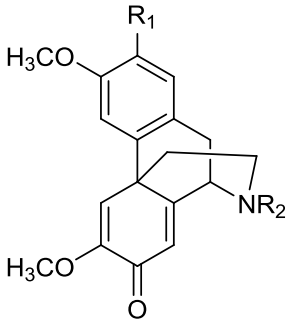
2.6.4 Morphinandienone

Morphinandienone alkaloid consists of a ring of benzene, a cycloalkane, a heterocyclic and a cyclodienone. The numbering of the skeleton is generally represented by the structure below.

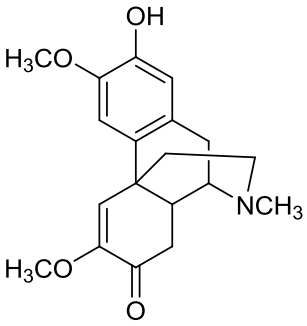


2.6.4.1 ^{1}H NMR Spectroscopy

Morphinandienones possess several general features in the ^{1}H NMR spectroscopy. Three to four singlets of benzene ring and the conjugated cyclohexadienone protons resonated at δ 7.0 and methoxy group appeared at around δ 3.0-4.0. The peak of C-9 proton revealed at around δ 2.5-4.0 as a doublet. Multiplet at around δ 1.0-2.0 and δ 2.0-3.0 are belongs to C-15 and C-16 respectively. The "doublet of doublet" of C-10 protons is observed at δ 2.0-3.50. Table 2.8 showed the chemical shifts of ^{1}H NMR for some morphinandienone alkaloids such as sebiferine **50**[87], norpallidine **51**[88] and pallidinine **52**[89].



50 R_1=OCH_3, R_2=CH_3
51 R_1=OH, R_2=H



52

Table 2.8: ^{1}H NMR (in $CDCl_3$, ppm) for some morphinandienone alkaloids

	Chemical shifts (δ ppm)		
Position of H	**50**	**51**	**52**
1	6.51	6.30	6.72
4	6.71	6.62	6.82
5	6.28	6.73	6.20
8	6.17	6.22	2.44 and 3.36
9	3.60		2.89
10	2.94 and 3.24		2.64 and 3.08
14			2.43
15	1.80		1.50 and 2.12
16	2.48		2.12 and 2.48

2.6.4.2 ^{13}C NMR Spectroscopy

The common fragmentation of this alkaloid are m/z $[M\text{-}Me]^+$, $[M\text{-}CO]^+$, $[M\text{-}COMe]^+$. The peak $[M\text{-}15]^+$ is due to the loss of methyl group which is usually very intense in this type of alkaloid. Initial cleavage at an allylic or benzylic bond follow by the loss of methyl would give the conjugated even-electron ions.

2.6.4.3 Ultraviolet and Infra-red Spectroscopy

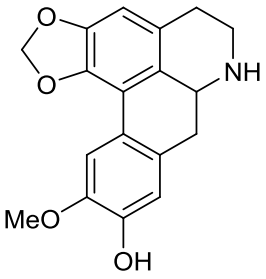
The common positions of the maximum absorptions in ultraviolet spectra of morphinandienone are at 209, 238 and 280 nm. Meanwhile in the infrared spectra, it showed absorption peak at 1620-1670 cm^{-1} which for α methoxy conjugated cyclohexadienone.

2.7 Alkaloids of Genus *Litsea*

Chemical investigation on several species of *Litsea* plants have been conducted before which led to isolation of alkaloids, flavonoids, terpenes, lactones, and also volatile oil including some new compounds. Nevertheless, our main focus was on isolation of alkaloids as it has been reported to possess various biological activities[90]. In *Litsea* species, isoquinoline type of alkaloids was the main alkaloidal constituents, such as aporphines, benzylisoquinolines, phenanthrenes and morphinandienones. Alkaloids isolated from some of the *Litsea* species are shown in Table 2.9 below.

Table 2.9: Alkaloids Isolated from the Genus of *Litsea*

Plant	Alkaloids isolated
Litsea polyantha[91]	Actinodaphnine **53**
Litsea glutinosa[92]	*N*-methyllaurotetanine **54**
	Boldine **55**
	Litseferine **56**
Litsea cubeba[93]	(+)-*N*-(methoxyl-carbonyl)-*N*-norlauroscholtzine **57**
	(+)-*N*-(methoxyl-carbonyl)-*N*-norglaucine **58**
	Atheroline **59**
Litsea chingpingensis[94]	Norisoboldine **60**
Litsea rotundifolia[95]	*N*-acetyllaurolitsine **61**
Litsea garciae[96]	Isodomesticine **62**
Litsea acuminata[97]	Lindcarpine **63**
	Norisocorydine **64**
	Juziphine **65**
Litsea laurifolia[98]	Glaziovine **66**



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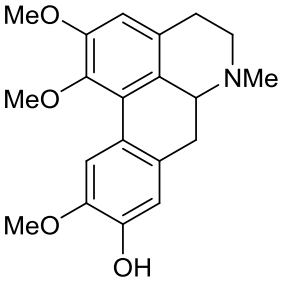

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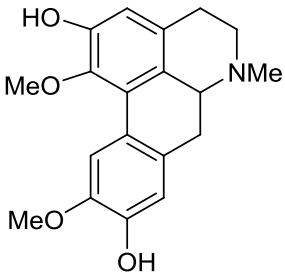

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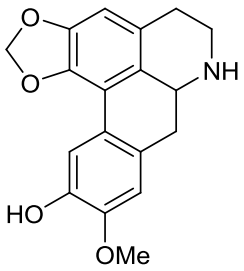

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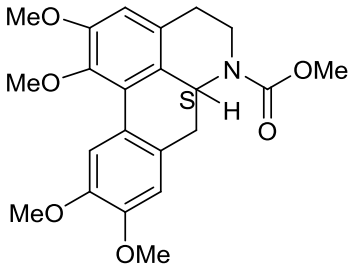

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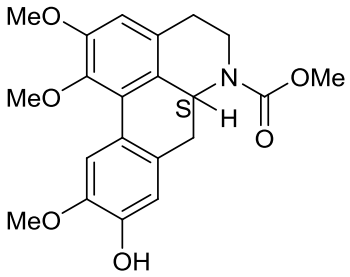

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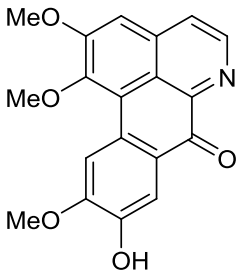

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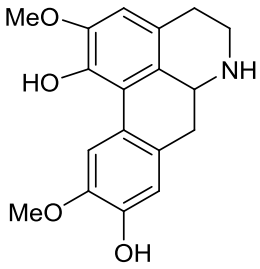

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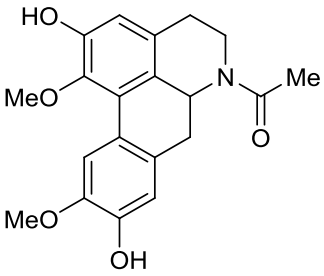

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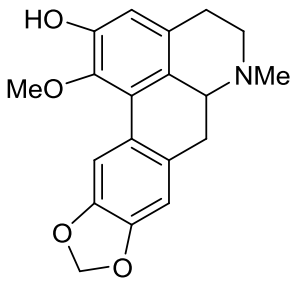

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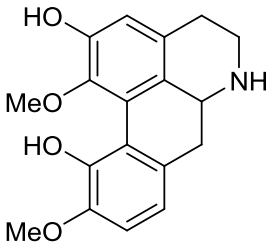

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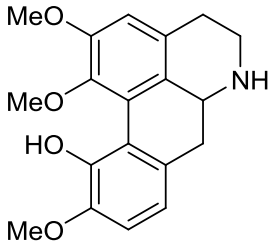

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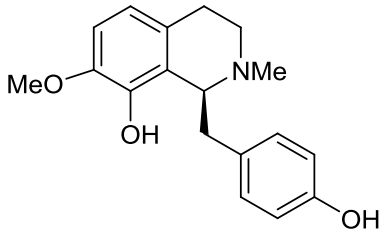

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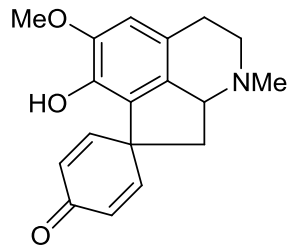


66

CHAPTER 3

RESULTS AND DISCUSSION

3.1 General

Two plant species from the family of Lauraceae; leaves of *Litsea grandis* (KL 5236) and barks of *Litsea lancifolia* (KL 5208) were studied in detail for their alkaloidal contents. The extraction method of alkaloids for these two species is described in chapter 5. The structural elucidations were accomplished through several spectroscopic methods; 1D-NMR (^{1}H, ^{13}C, DEPT), 2D-NMR (COSY, HMQC, HMBC, NOESY), UV, IR, LCMS-IT-TOF and NMR data and also by comparison with literature data with those described in the literatures values.

3.2 Alkaloids from leaves of *Litsea grandis*

Isolation and structural elucidation of the crude alkaloid from the leaves of *Litsea grandis* yielded three known alkaloids. The alkaloids isolated were identified as an aporphine; reticuline **67** and benzylisoquinolines; *N*-methylisococlaurine **68** and laurotetanine **69**. The following sub-chapters discuss the structural elucidation of the isolated compounds.

3.2.1 Reticuline 67

Reticuline **67**, $[\alpha]_D^{27}$ = +28.6º (*c* 1.0, MeOH) was the major alkaloid from the leaves of *Litsea grandis.* This compound was isolated as a brownish amorphous solid. The UV spectrum showed absorption at 293 nm which was a characteristic of a benzylisoquinoline alkaloid.[82] The IR spectrum showed a strong absorption bands at 3418 and 2926 cm^{-1} due to the stretching of hydroxyl group and C-H aromatic, respectively. The LCMS-IT-TOF revealed a pseudomolecular ion peak, $[M+H]^+$ at *m/z* 330.17 consistent with the molecular formula of $C_{19}H_{24}NO_4$.

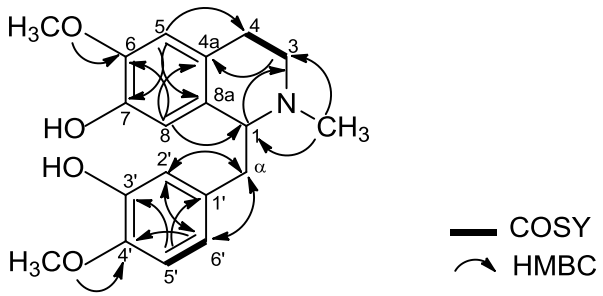
The ^{1}H NMR spectrum of reticuline **67** showed presence of five low field aromatic protons in between δ 6.00-7.00, a characteristic of benzylisoquinoline type of alkaloids.[99] Two of signals appeared as a singlet for the protons in ring A at δ 6.46 (H-5) and 6.30 (H-8) indicating that this ring was tetrasubstituted. Three other protons in ring C were observed as a doublet at δ 6.66 (H-5') with a coupling constant of 8.3 Hz, a doublet of doublets at δ 6.51 (H-6', 2.0 and 8.1 Hz), and a doublet at δ 6.68 (H-2', 2.0 Hz) respectively. This pattern showed that ring C was trisubstituted. Two methoxyl groups appeared as singlet at δ 3.76 and 3.75 could be assigned to 6-OMe and 4'-OMe respectively. A singlet signal at δ 2.37 was also observed indicating the presence of *N*-Me group in ring B. Furthermore, there were seven proton signals observed at a higher field region between δ 2.45-3.61 belonged to the aliphatic protons of H-α, H-1, H-3 and H-4.

The COSY spectrum showed cross peaks between H-1/H-α, H-5'/H-6', and H-4/H-3, as shown in Figure 3.5.

The ^{13}C NMR spectrum showed 19 carbon resonances, which were in agreement with the molecular formula. The DEPT-135 spectrum showed the appearance of three methyls, three methylenes and six methines carbon signals. Two signals of methoxyl groups were observed at δ 56.0 and 55.9 respectively. In addition, the signals for the quaternary carbons appeared at δ 145.1, 143.5, 145.2 and 145.4 which could be assigned to C-6, C-7, C-3' and C-4', bearing the methoxyl and hydroxyl groups.

The assignment of carbons and protons in the structure was further confirmed by HMBC experiments. The HMBC spectrum in Figure 3.7 showed significant cross-peaks of H-5 to C-4, C-8a and the carbon bearing a hydroxyl group, C-7. Meanwhile, H-8 correlated with C-1, C-4a and also C-6. The correlations of H-2' and H-6' to C-α were also seen in the HMBC spectrum. Complete assignment of all protons and carbons are shown in Table 3.1.

From the analysis of the spectroscopic data obtained and comparison with the literature values, it was identified as reticuline **67**. [100]



67

Figure 3.1: ^{1}H-^{1}H and ^{1}H-^{13}C coupling pattern observed in COSY and HMBC spectrum of reticuline **67**

Table 3.1: ^{1}H NMR, ^{13}C NMR Data and HMBC Correlation for Reticuline **67**

Position	^{1}H NMR (δ, Hz)	^{13}C NMR (δ)	HMBC
1	3.58-3.61, *t*, *J*=6.1	64.6	α, 3, 8a, 1'
3	2.71-3.10, *m*	46.8	4a
4	2.48-2.77, *m*	24.9	
4a		125.2	
5	6.46, *s*	110.5	4, 8a, 7
6		145.1	
7		143.5	
8	6.30, *s*	113.7	1, 4a, 6
8a		130.0	
α	2.65-2.97, *m*	41.0	1, 2', 6', 8a, 1'
1'		133.1	
2'	6.68, *d*, *J*=2.0	115.7	6', 4', α
3'		145.2	
4'		145.4	
5'	6.66, *d*, *J*=8.3	110.6	1', 3'
6'	6.51, *dd*, J_1=2.0, J_2=8.1	121.0	α, 2', 4'
6-OMe	3.76, *s*	56.0	
4'-OMe	3.75, *s*	55.9	
N-Me	2.37, *s*	42.4	1, 3

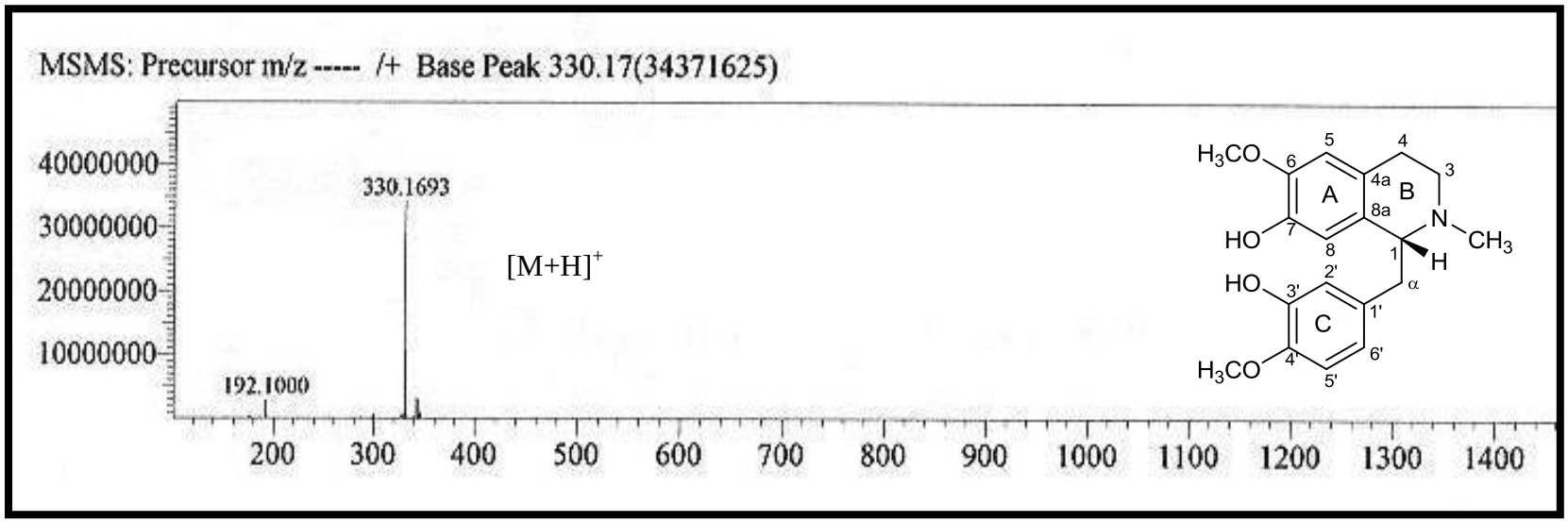


Figure 3.2: LCMS-IT-TOF Spectrum of Reticuline 67

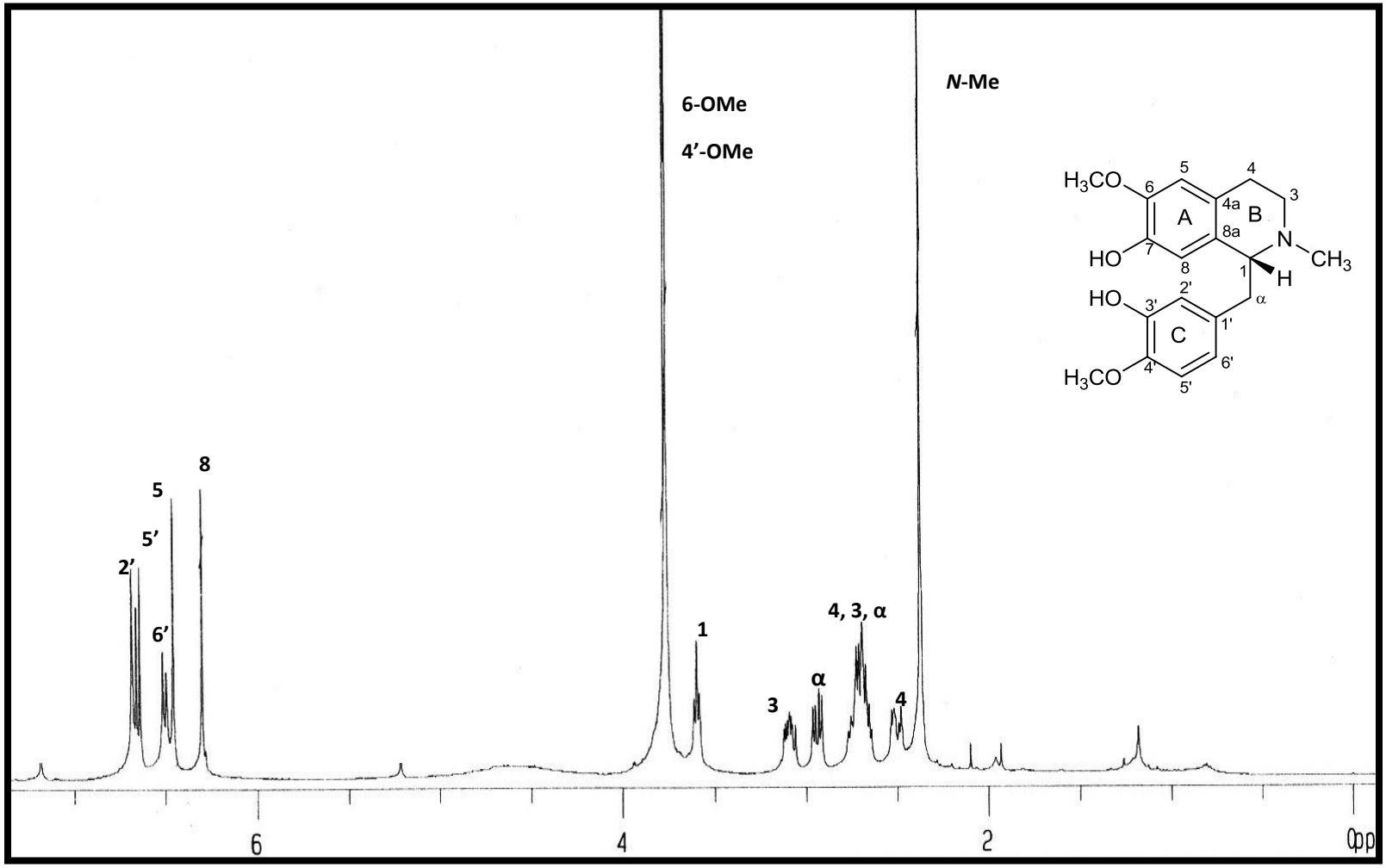


Figure 3.3: ^{1}H NMR Spectrum of Reticuline **67**

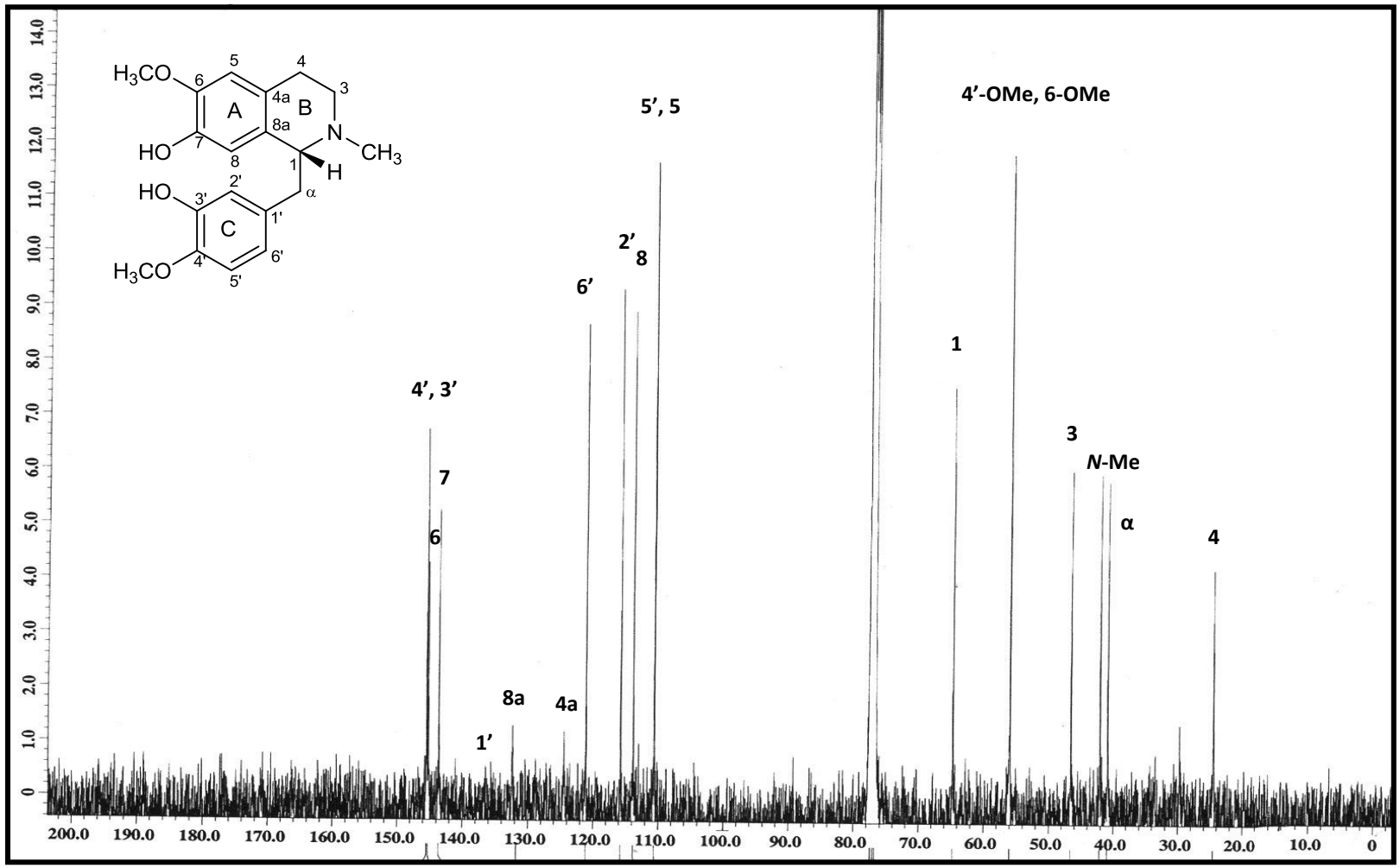


Figure 3.4: ^{13}C NMR Spectrum of Reticuline **67**

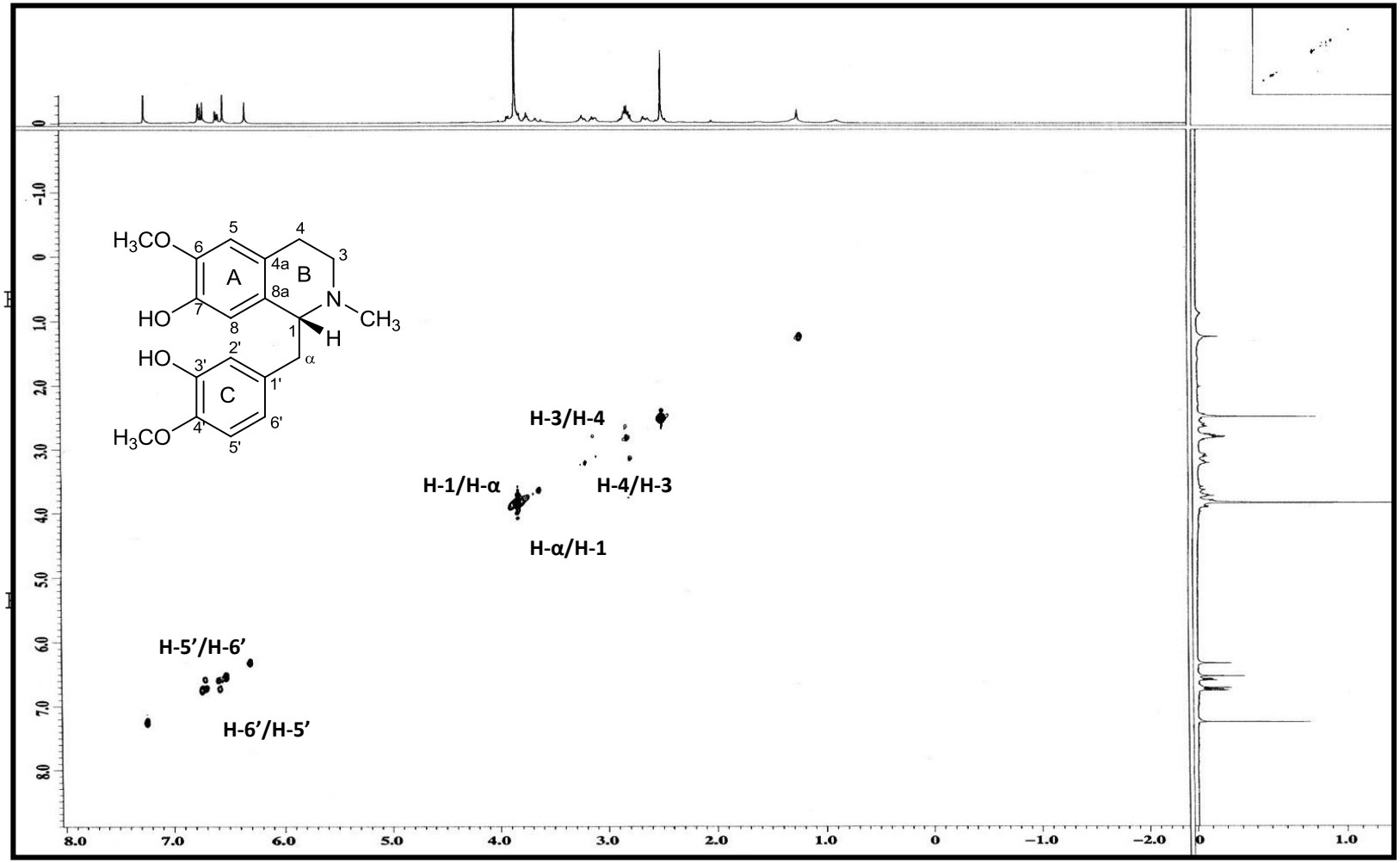


Figure 3.5: COSY Spectrum of Reticuline **67**

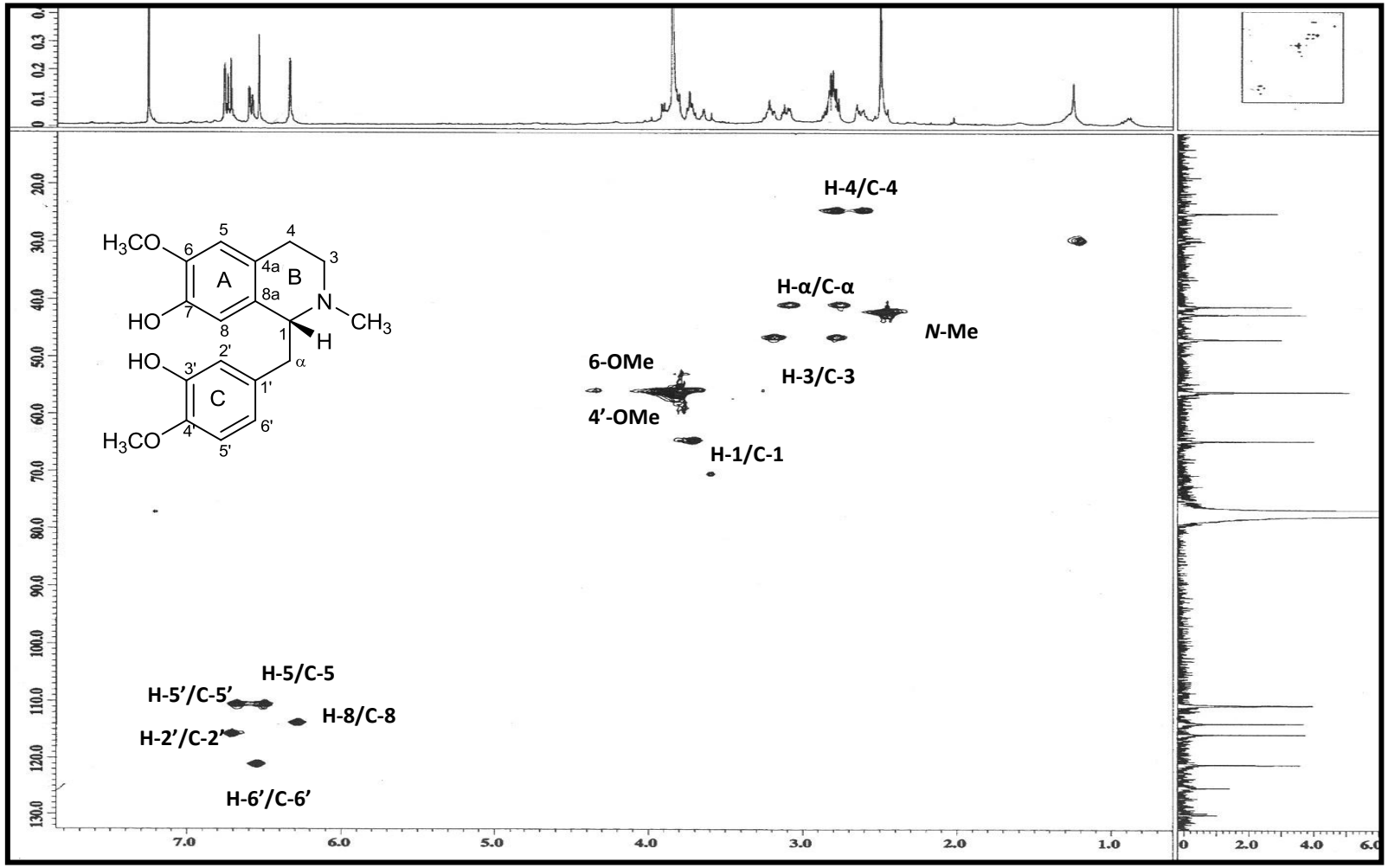


Figure 3.6: HSQC Spectrum of Reticuline **67**

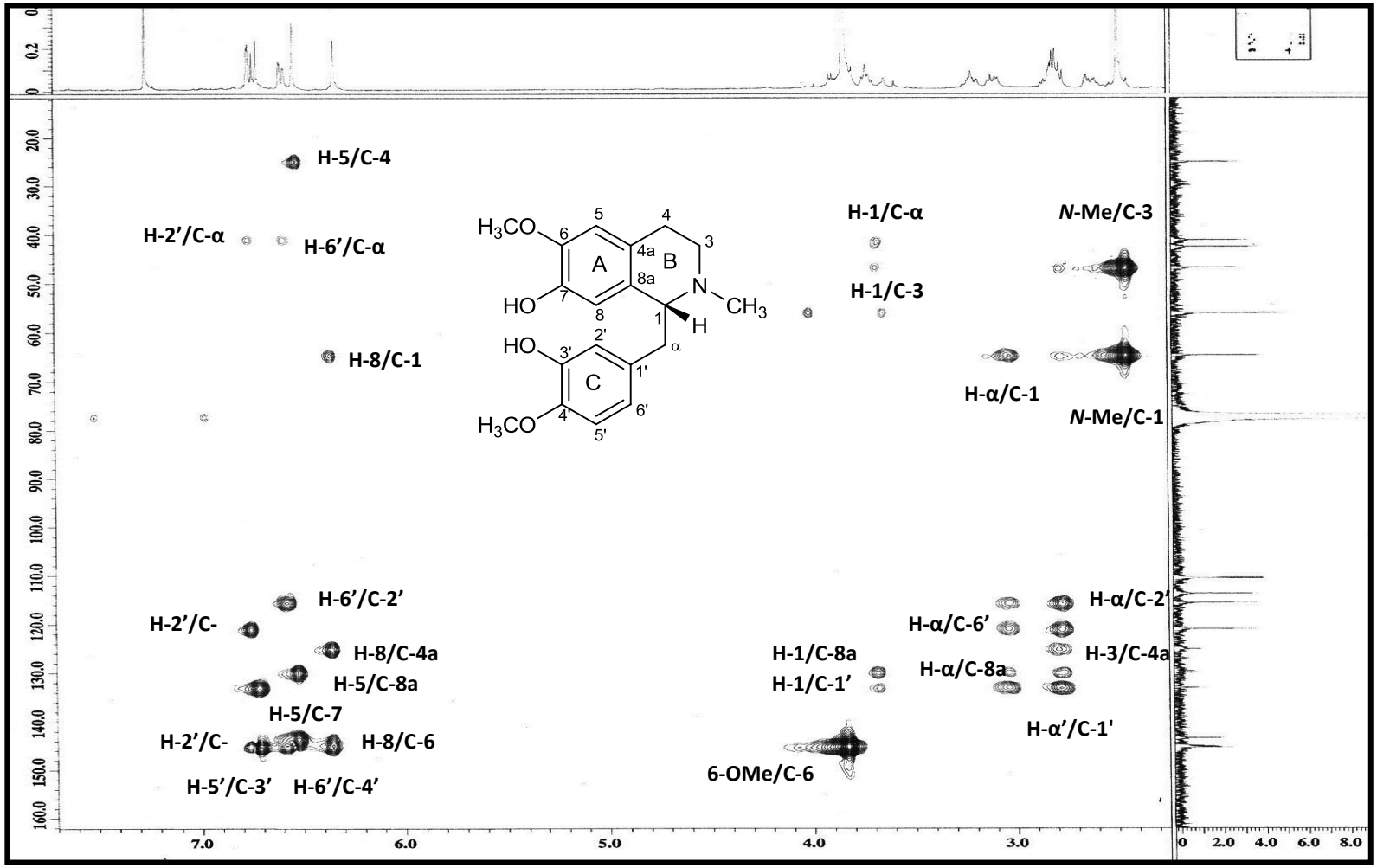


Figure 3.7: HMBC Spectrum of Reticuline **67**

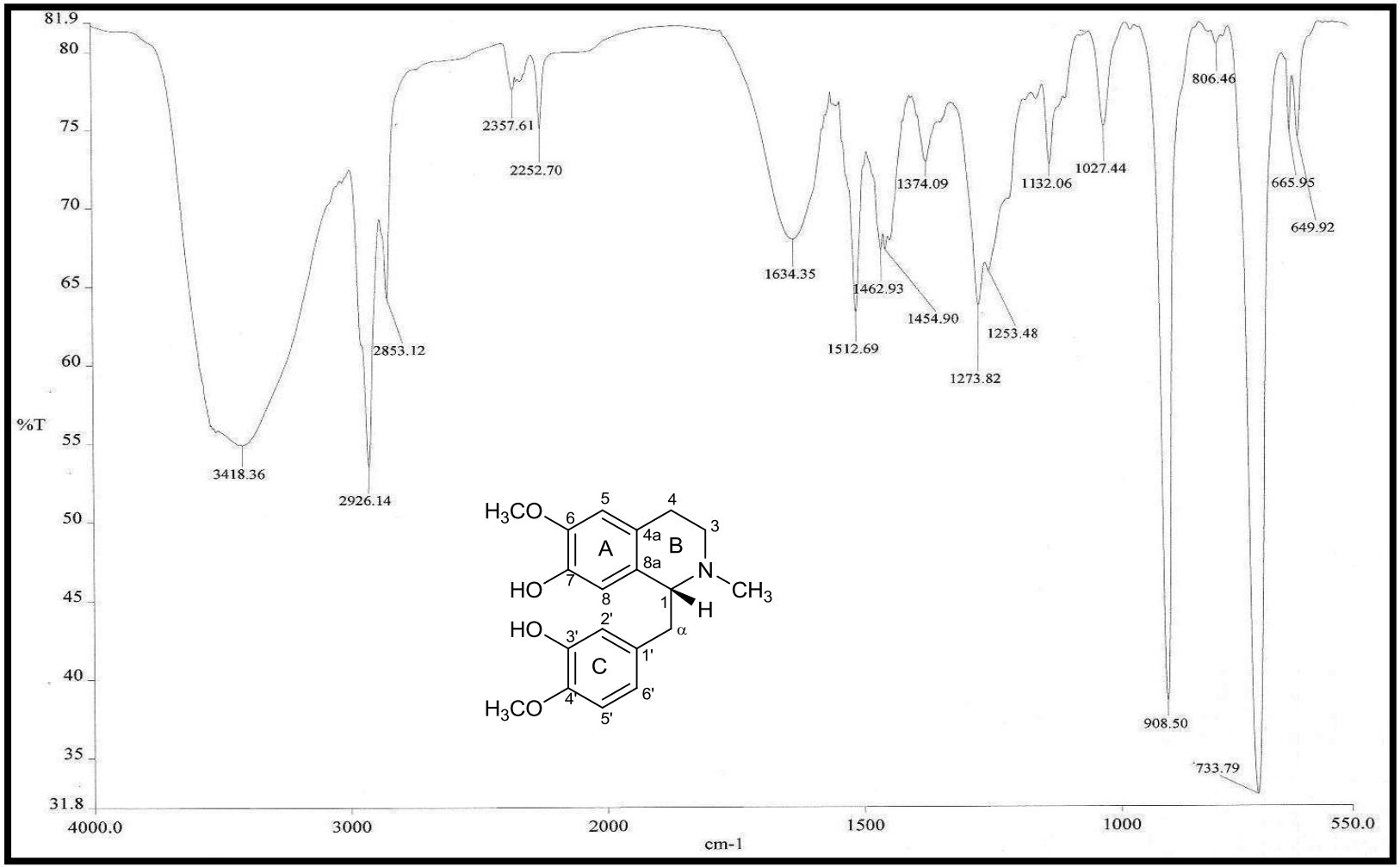


Figure 3.8: IR Spectrum of Reticuline **67**

3.2.2 *N*-Methylisococlaurine 68

N-Methylisococlaurine **68**, $[\alpha]_D^{27}$ = +29.4º (*c* 1.0, MeOH) was isolated as a brownish amorphous solid. The UV spectrum showed an absorption band at 301 nm. The IR spectrum showed a strong absorption bands at 3266 and 2921 cm^{-1} due to the stretching of hydroxyl group and C-H aromatic, respectively. The LCMS-IT-TOF spectrum revealed a pseudomolecular ion peak, $[M+H]^+$ at *m/z* 300.16 consistent with the molecular formula of $C_{18}H_{21}NO_3$.

The ^1H NMR spectrum of *N*-methylisococlaurine **68** exhibited signals for one OMe (δ 3.65), one *N*-Me (δ 2.26), and six aromatic protons, composed of an AB spin system. Protons at position 5 and 8 appeared as a singlet at δ 6.34 and 6.22, respectively, which showed the same pattern with that of the same benzylisoquinoline skeleton, reticuline **67**. Four other aromatic protons resonating as an AB spin system appeared at δ 6.76 (H-2' and H-6', *d*, *J* = 8.3 Hz) and 6.45 (H-3' and H-5', *d*, *J* = 8.3 Hz).

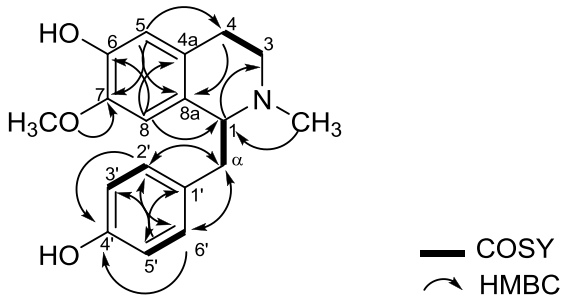
The chemical shift of the aliphatic protons which, appeared as multiplets at the region of δ 2.52-3.01 were determined by using the COSY, HMQC and HMBC experiments. In fact, the correlation of vicinal protons H-1/H-α, H-2'/H-3', H-5'/H-6' and also H-3/H-4 was shown in the COSY spectrum.

The ^{13}C NMR spectrum has shown 18 carbon signals consistent with the molecular formula $C_{18}H_{21}NO_3$. The DEPT 135 spectrum showed the appearance of two methyls, three methylenes, and seven methines respectively. The HMQC spectrum showed the cross-peaks of H5/C-5 and H-8/C-8 at δ_H 6.34/ δ_C 110.8 and δ_H 6.22/ δ_C 113.9 ppm respectively in agreement with reported values for the respective carbon and protons of *N*-methylisococlaurine **68**.[80] For the remaining five methines, the highest field signal in the

^{13}C NMR spectra was observed at δ 64.8 can be assigned to C-1, meanwhile two overlapping signals appeared at δ 130.3 and 115.6 corresponded to the C-2'/C-6' and C-3'/C-5' respectively.

The assignment of carbons and protons in the structure was further confirmed by HMBC experiments. The HMBC spectrum in Figure 3.16 revealed correlation of H-5 to C-4, C-8a and C-7, further supported the position of OMe group at C-7. In the HMBC spectrum, the correlations of H-2' and H-6' to the carbon bearing hydroxyl group, C-4' were also observed. Another hydroxyl group at C-6 was deduced through correlation of H-8 to C-6, C-4a and C-1. Finally, complete ^{1}H and ^{13}C NMR assignments were made thorough analysis of 2D NMR spectra (COSY, HMQC and HMBC) and are listed in Table 3.2.

From the analysis of the spectroscopic data obtained and comparison with the literature values, alkaloid **68** was identified as *N*-methylisococlaurine.[99]



68

Figure 3.9: ^{1}H-^{1}H and ^{1}H-^{13}C coupling pattern observed in COSY and HMBC spectra of *N*-methylisococlaurine **68**

Table 3.2: ^{1}H NMR, ^{13}C NMR Data and HMBC Correlation for *N*-methylisococlaurine **68**

Position	^{1}H NMR (δ, Hz)	^{13}C NMR (δ)	HMBC
1	3.49-3.46, *t*, *J*=6.36	64.8	α, 3, 8a
3	2.96-3.01, *m*	46.1	
4	2.52-2.65, *m*	24.4	3, 4a, 8a
4a		125.0	
5	6.34, *s*	110.8	4, 8a, 7
6		145.3	
7		143.5	
8	6.21, *s*	113.9	1, 4a, 6
8a		129.9	
α	2.77-2.83, *m*	40.8	2', 6', 8a
1'		130.8	
2'	6.76, *d*, *J*=8.28	130.3	4', 6', α
3'	6.45, *d*, *J*=8.28	115.6	1', 5'
4'		154.7	
5'	6.45, *d*, *J*=8.28	115.6	1', 5'
6'	6.76, *d*, *J*=8.28	130.3	4', 6', α
7-OMe	3.65, *s*	55.9	
N-Me	2.26, *s*	42.0	1

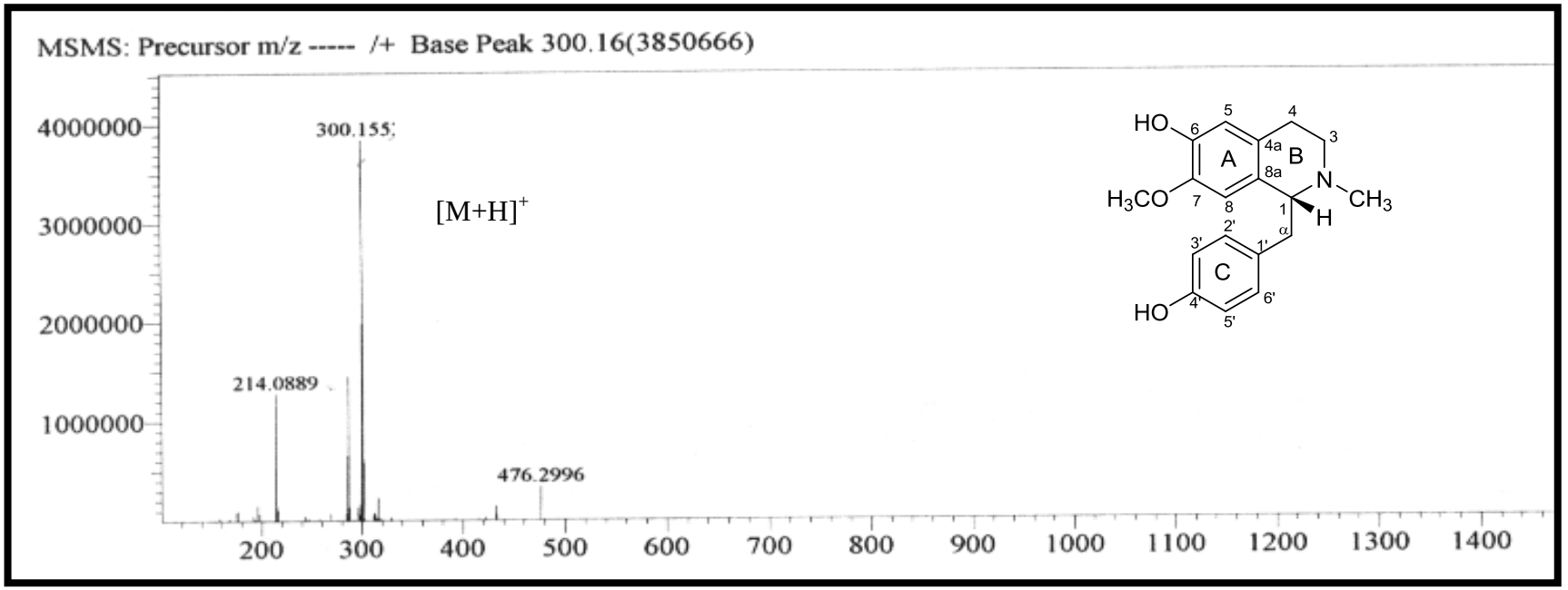


Figure 3.10: LCMS Spectrum of *N*-methylisococlaurine **68**

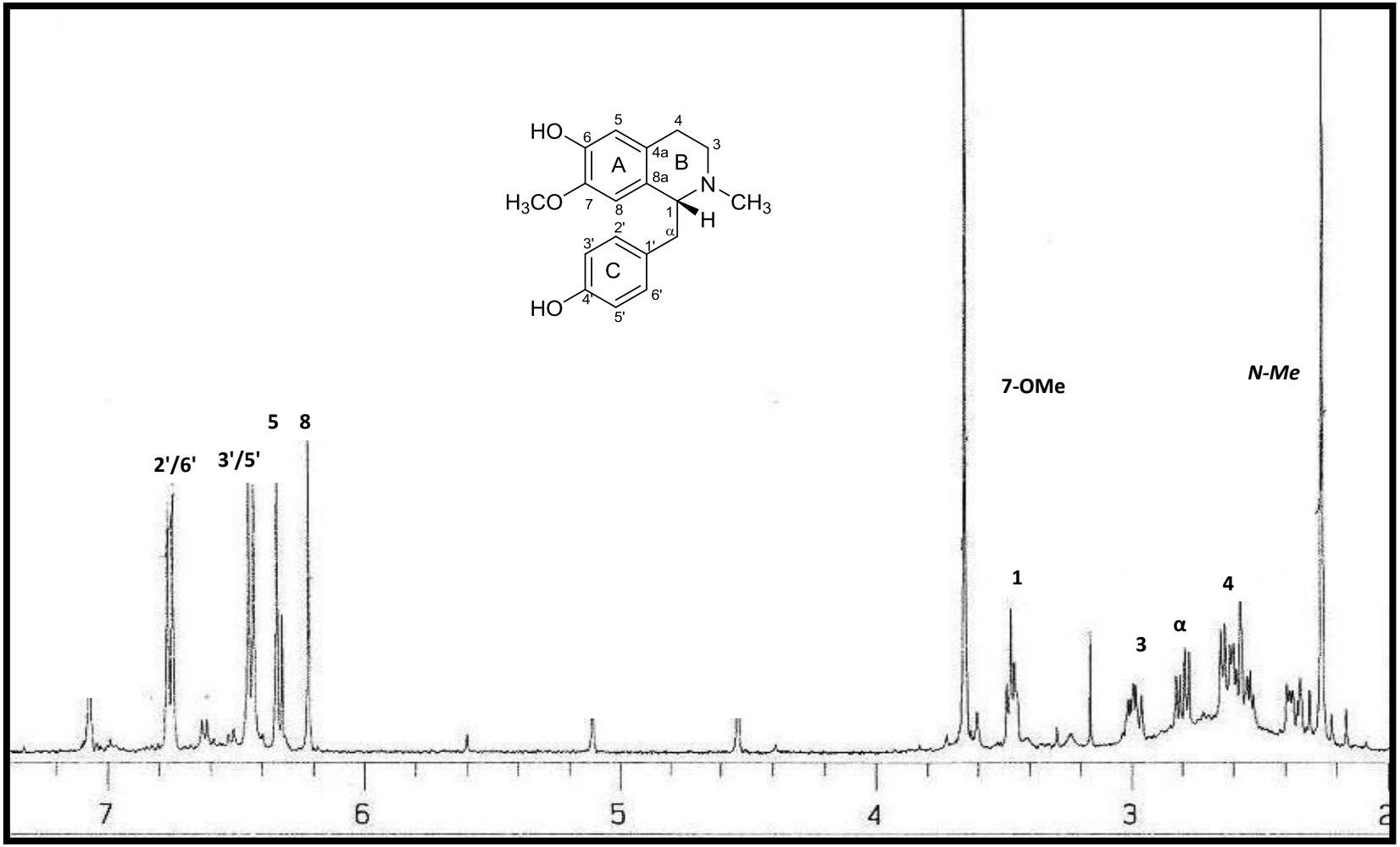


Figure 3.11: ^{1}H NMR Spectrum of *N*-methylisococlaurine **68**

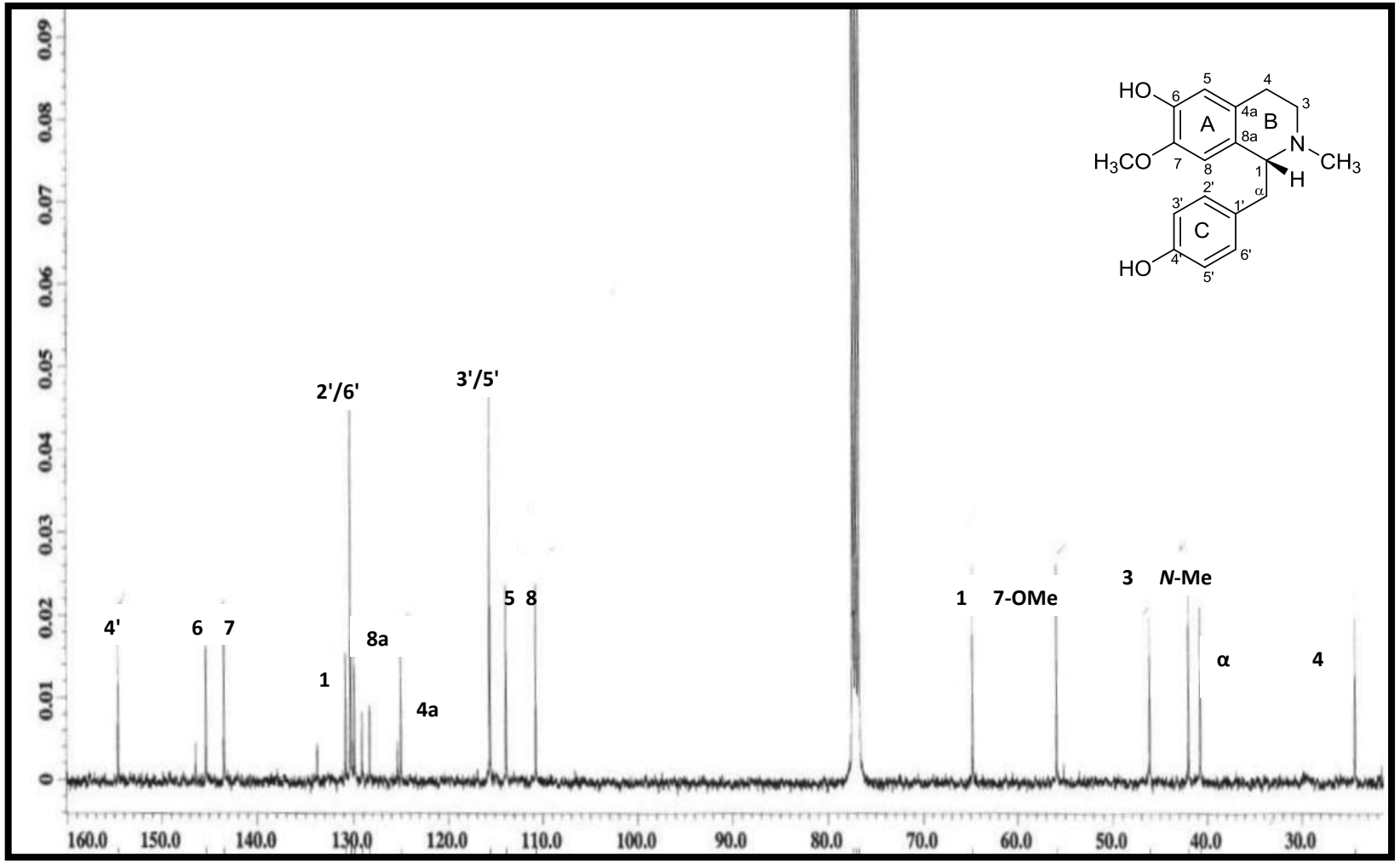


Figure 3.12: ^{13}C NMR Spectrum of *N*-methylisococlaurine **68**

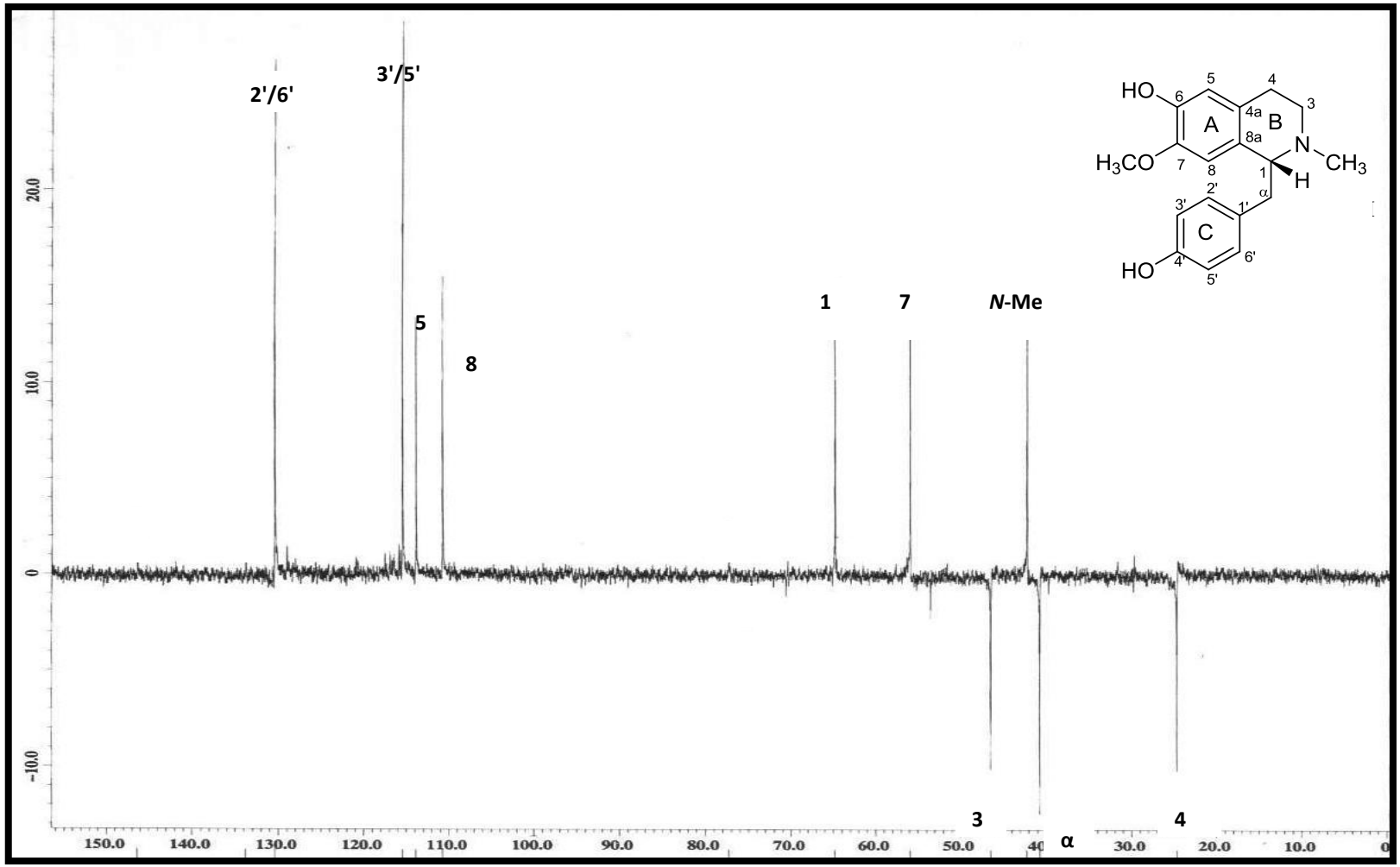


Figure 3.13: 135 DEPT NMR Spectrum of *N*-methylisococlaurine **68**

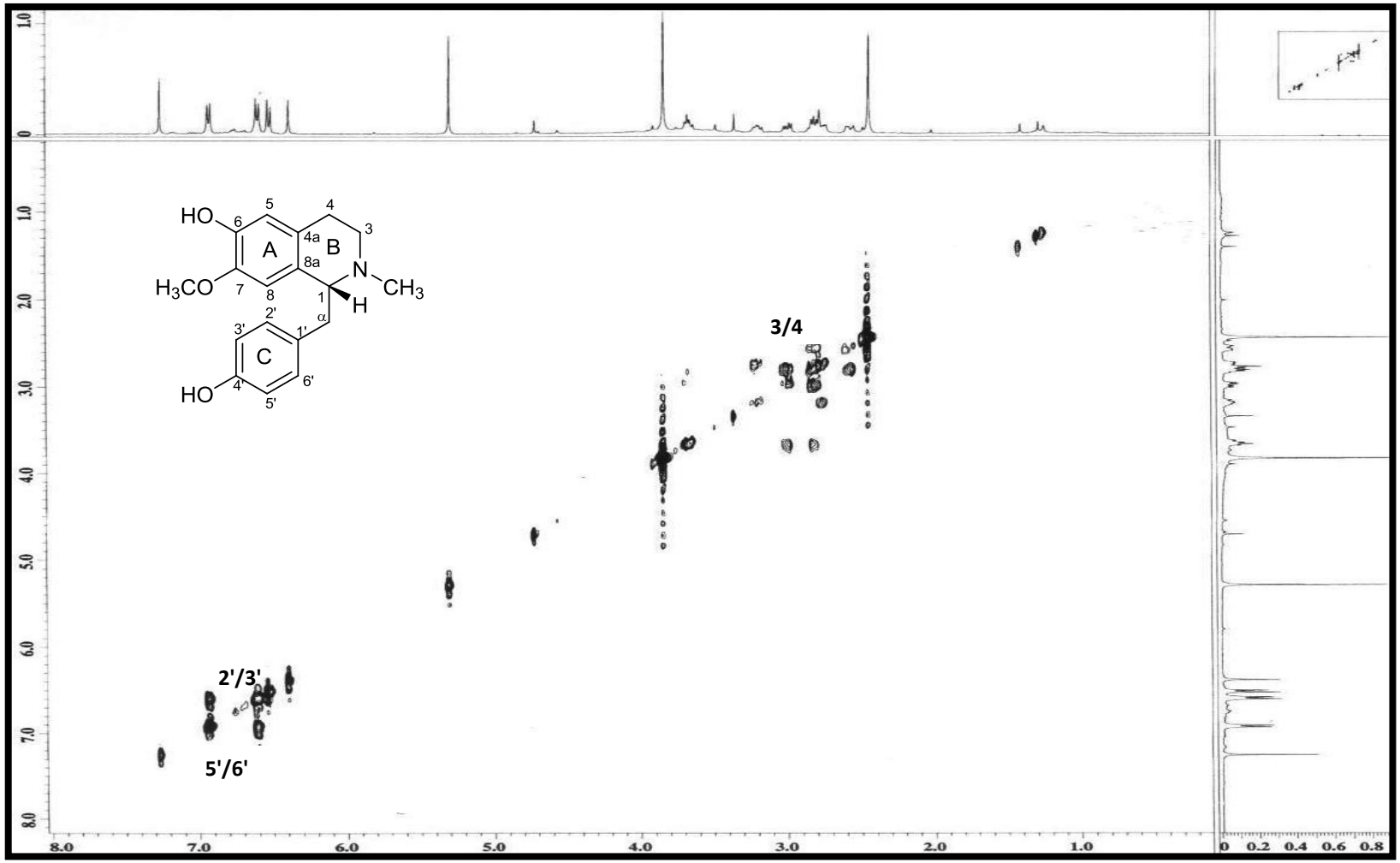


Figure 3.14: COSY Spectrum of *N*-methylisococlaurine **68**

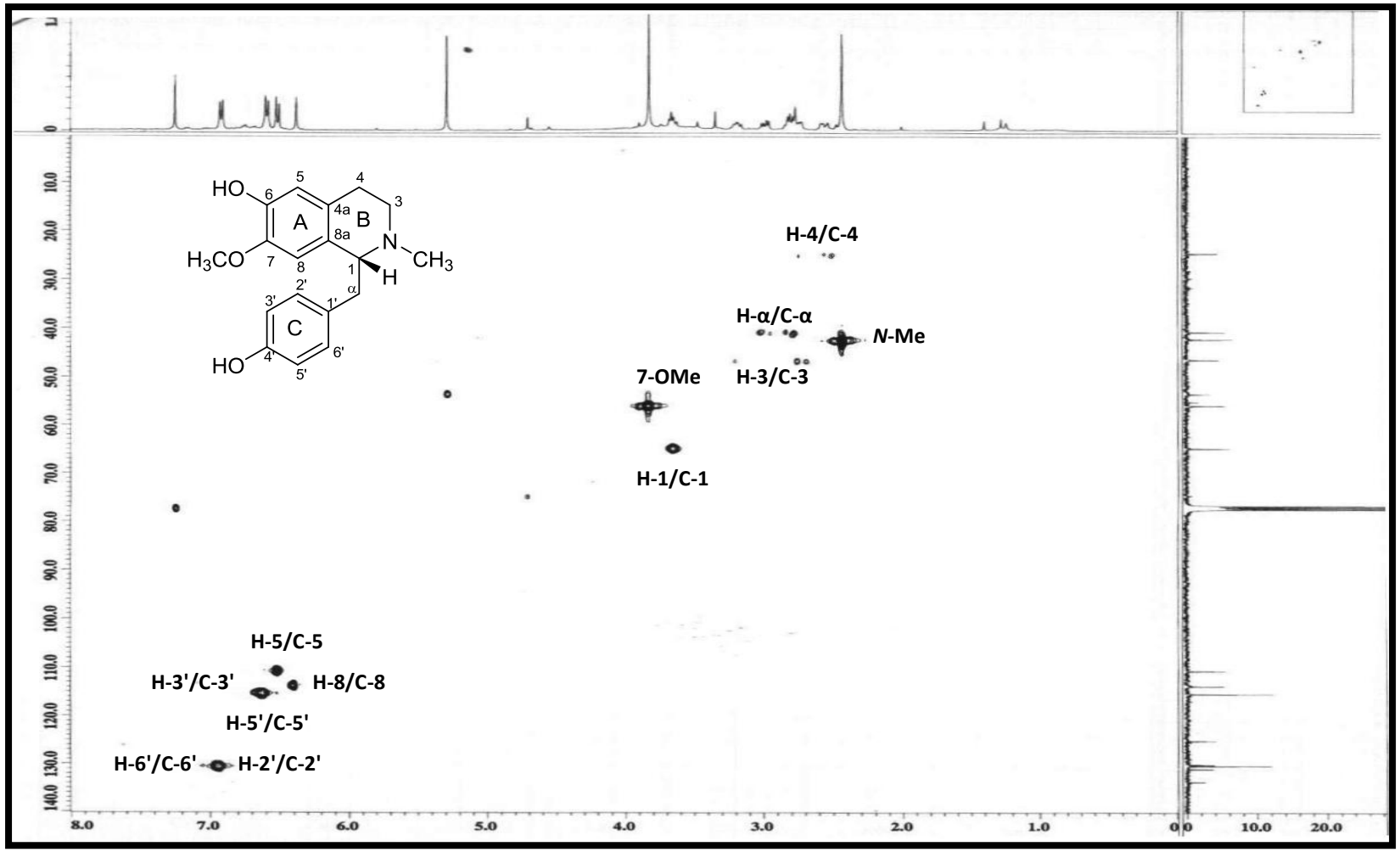


Figure 3.15: HSQC Spectrum of *N*-methylisococlaurine **68**

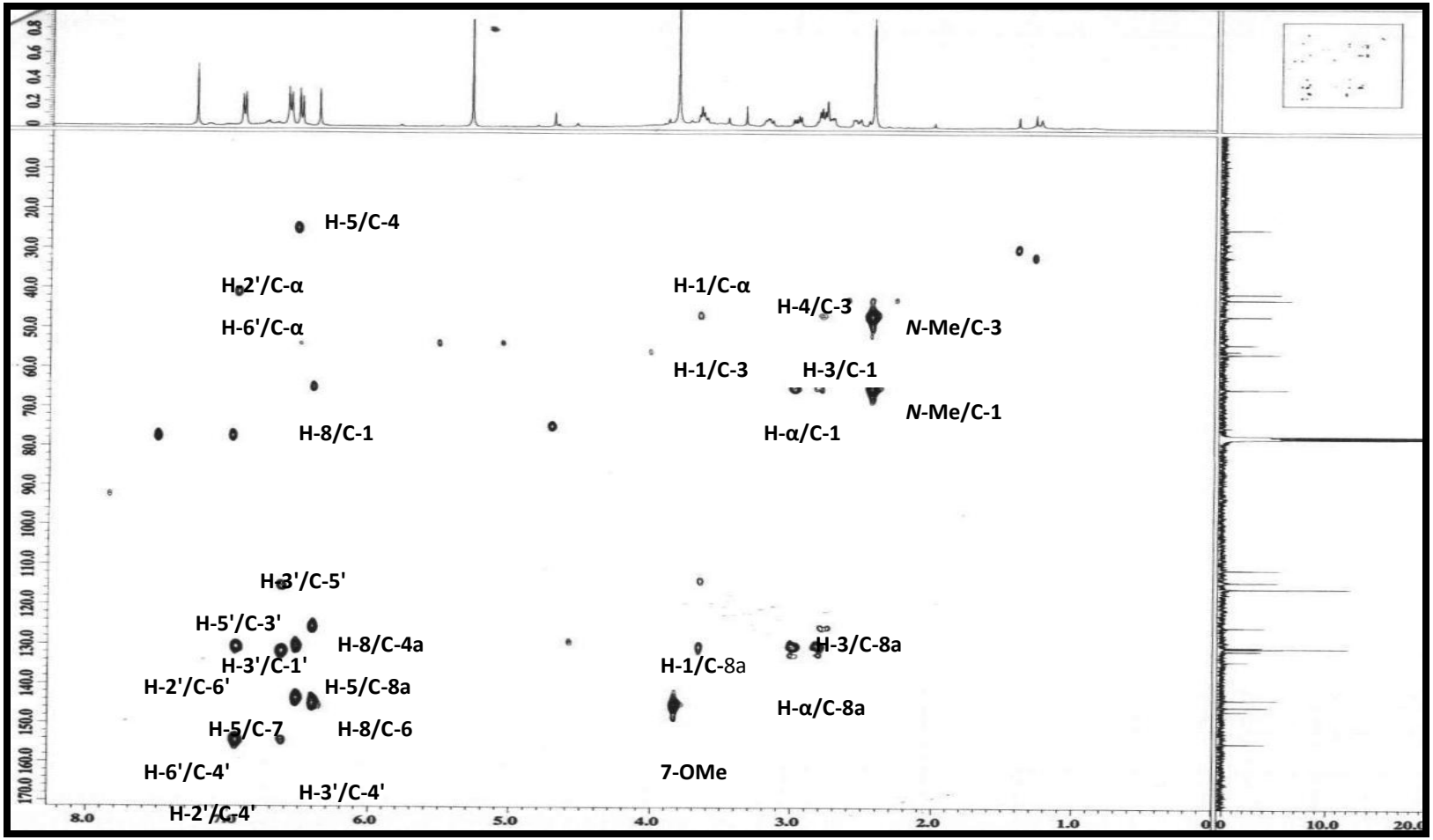


Figure 3.16: HMBC Spectrum of *N*-methylisococlaurine **68**

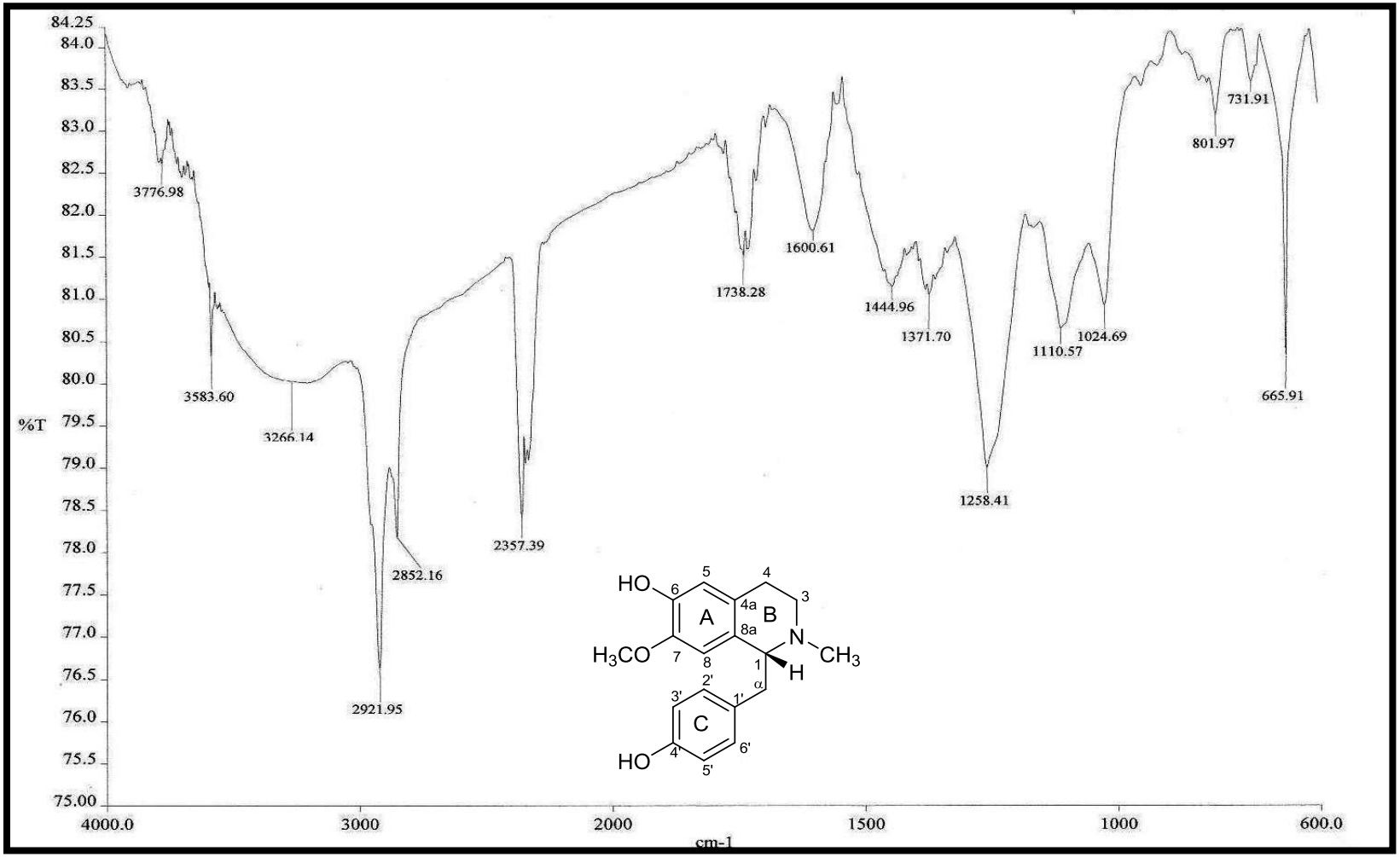


Figure 3.17: IR Spectrum of *N*-methylisococlaurine **68**

3.2.3 Laurotetanine 69

Laurotetanine **69**, $[\alpha]_D^{27}$ = -29.4º (*c* 1.0, MeOH) was isolated as a brownish amorphous solid. The UV spectrum showed absorptions at 221, 278 and 305 nm, thus suggested a 1,2,9,10-tetrasubstituted aporphine skeleton.[101] The IR spectrum showed an absorption bands at 3583 and 3429 cm^{-1} due to the stretching of NH group and also hydroxyl group. Other absorption peaks were at 1508, 1238 and 1464 cm^{-1} which indicated the C-H aromatic, C-O and CH_2 stretching respectively. A pseudomolecular ion peak, $[M+H]^+$ at *m/z* 328.15 was observed in the LCMS-IT-TOF spectrum consistent with the molecular formula of $C_{19}H_{21}NO_4$.

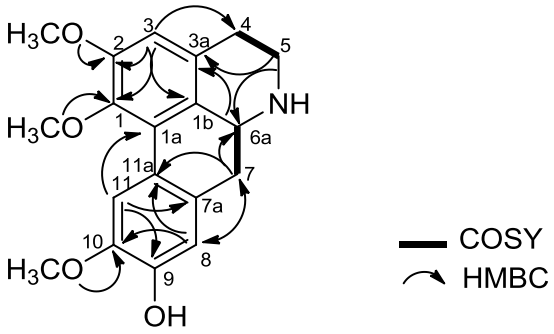
The ^{1}H NMR spectrum of alkaloid **69** showed the presence of three aromatic protons, which appeared as singlet at δ 8.07 (H-11), 6.82 (H-8) and 6.59 (H-3). Three methoxyl groups were also observed as a singlet at δ 3.65, 3.87 and 3.89 could be assigned to 1-OMe, 2-OMe and 10-OMe respectively. Furthermore, there are seven proton signals observed at a high field region between δ 2.71- 3.81 belonging to the aliphatic protons of H-4, H-5, H-6a and H-7.

The DEPT 135 spectrum showed the appearance of three methyls, three methylenes, and four methines. The nine quaternary carbon signals which did not appear in the DEPT 135 spectrum were appeared in ^{13}C NMR spectrum consistent with the number of carbon in the molecular formula $C_{19}H_{21}NO_4$. In addition, four oxygenated aromatic carbon signals were observed at δ 129.9, 144.3, 145.4 and 152.2 which could be assigned to C-9, C-1, C-10 and C-2 indicated the presence of hydroxyl and methoxyl groups.

The HMBC spectrum in Figure 3.24 revealed the cross-peaks of a long range coupling of the proton in the ^{1}H NMR spectrum with ^2J and ^3J interaction in the ^{13}C NMR

spectrum. Figure 3.18 shows the selected correlations of H-8 to C-10, and C-11a, and the correlation of H-3 to C-1b, C-1 and C-2, suggested the position of OMe groups at C-1, C-2 and C-10 respectively. Further assignment of all protons and carbons are shown in Table 3.3.

From the analysis of the spectroscopic data obtained and comparison with the literature values, it was identified as laurotetanine **69**.



69

Figure 3.18: ^{1}H-^{1}H and ^{1}H-^{13}C coupling pattern observed in COSY and HMBC spectrum of Laurotetanine **69**

Table 3.3: ^{1}H NMR, ^{13}C NMR Data and HMBC Correlation for Laurotetanine **69**

Position	^{1}H NMR (δ, Hz)	^{13}C NMR (δ)	HMBC
1		144.3	
1a		126.8	
1b		127.4	
2		152.2	
3	6.59, *s*	110.8	1, 2, 1b
3a		128.9	
4	3.02, *m*	29.1	
5	3.39, *s*	43.1	3a, 6a
6a	3.81, *m*	53.8	3a
7	2.71, *m*	36.6	6a, 11a
7a		129.8	
8	6.82, *s*	113.9	7, 10, 11a
9		129.9	
10		145.4	
11	8.08, *s*	111.4	1a, 7a, 9
11a		124.0	
1-OMe	3.65, *s*	60.3	
2-OMe	3.89, *s*	56.1	
10-OMe	3.87, *s*	55.9	

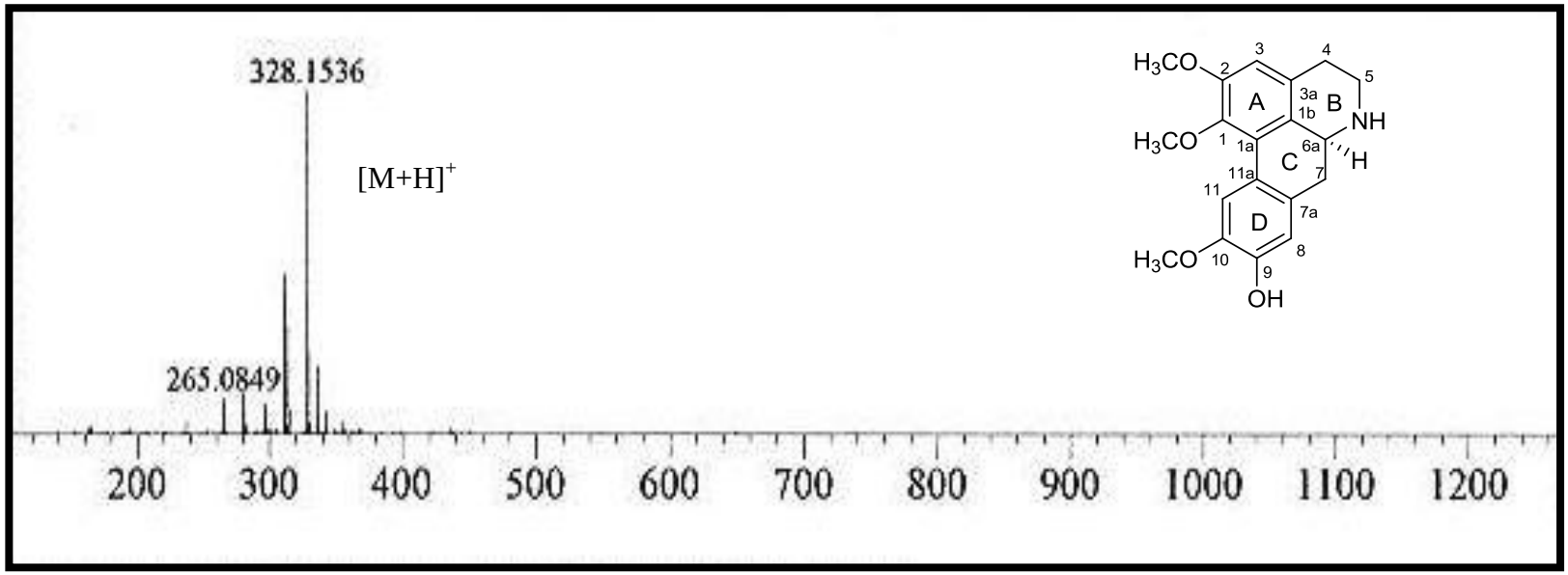


Figure 3.19: LCMS Spectrum of Laurotetanine **69**

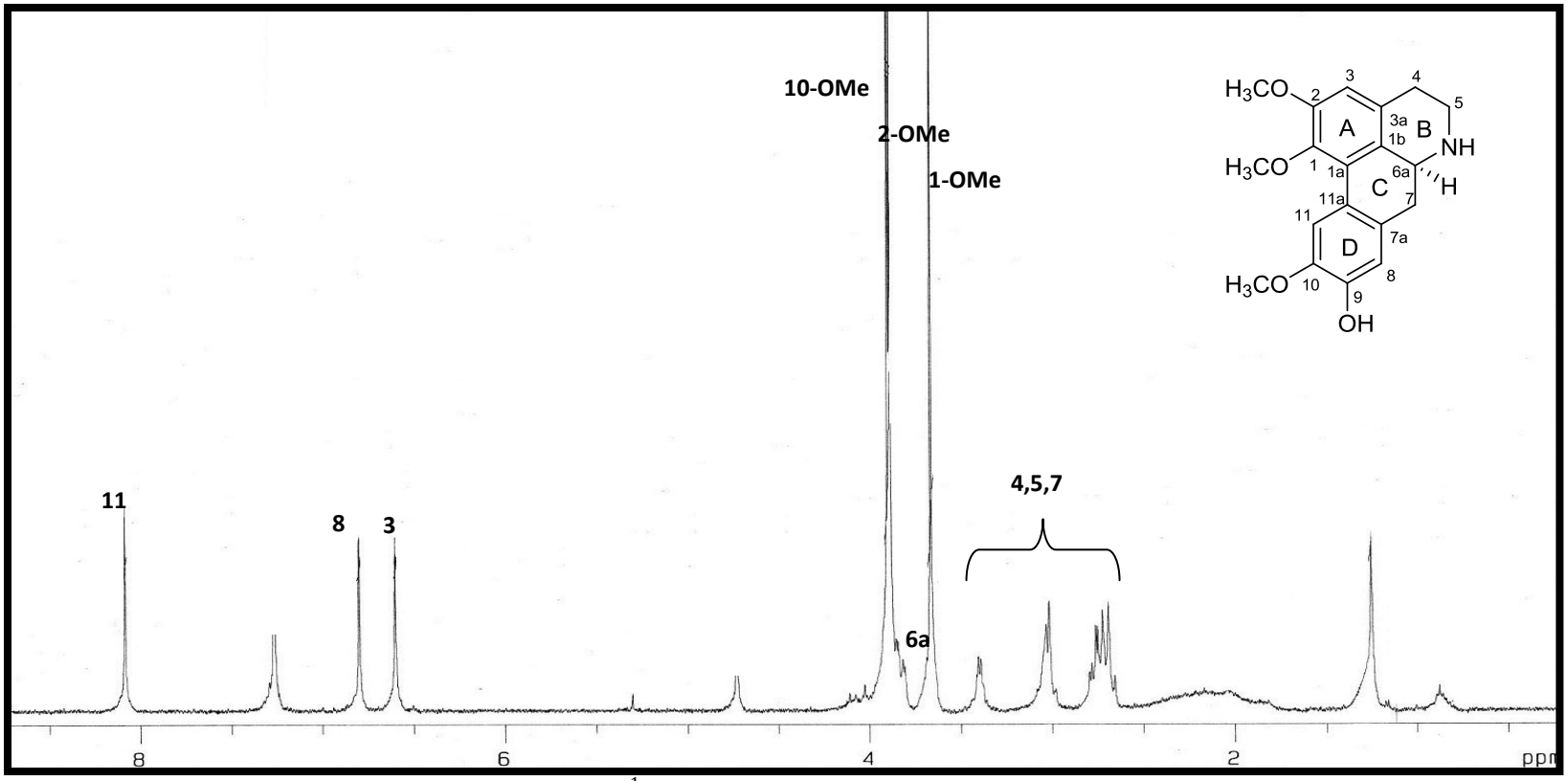


Figure 3.20: ^{1}H NMR Spectrum of Laurotetanine **69**

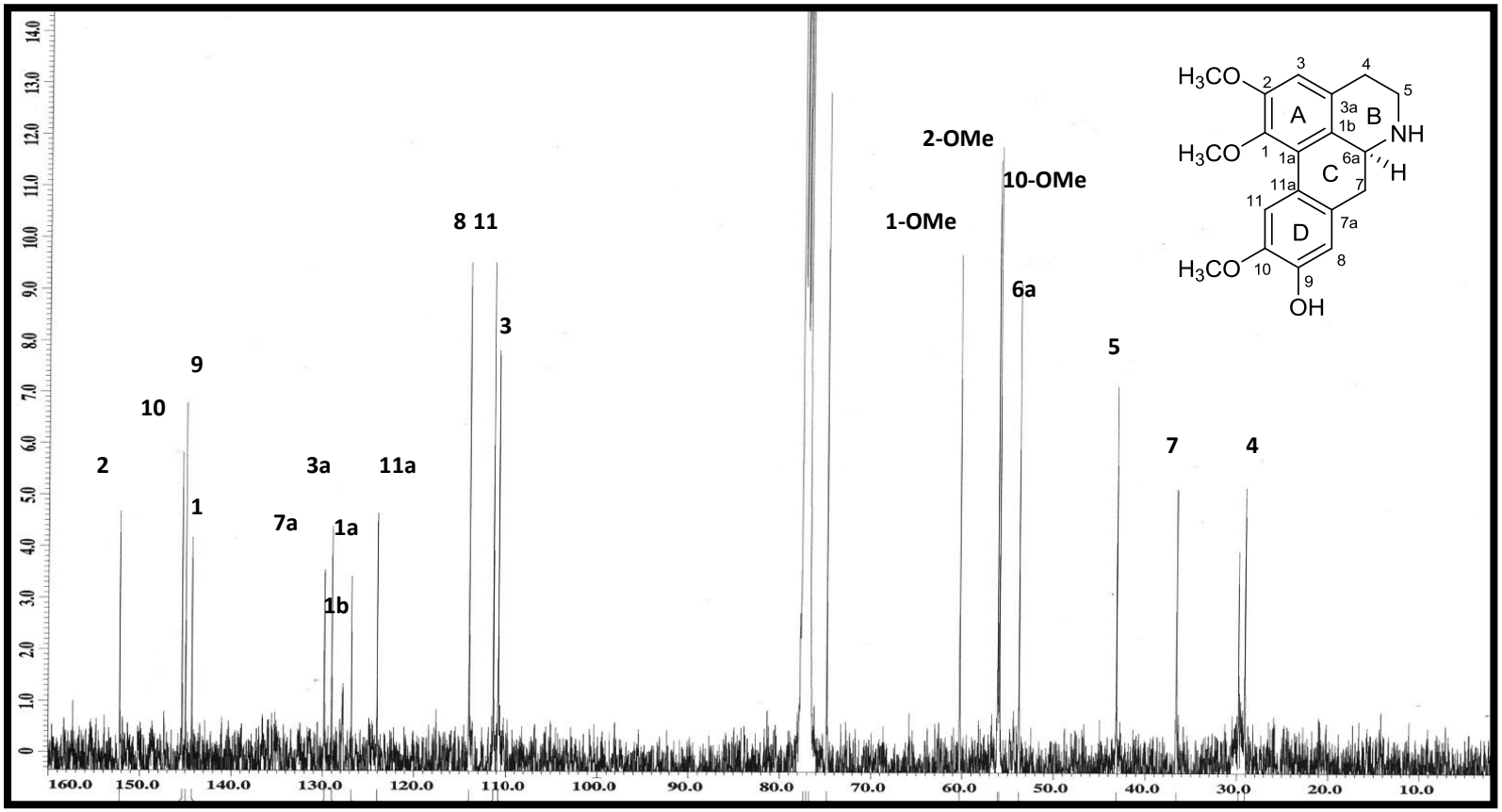


Figure 3.21: ^{13}C NMR Spectrum of Laurotetanine **69**

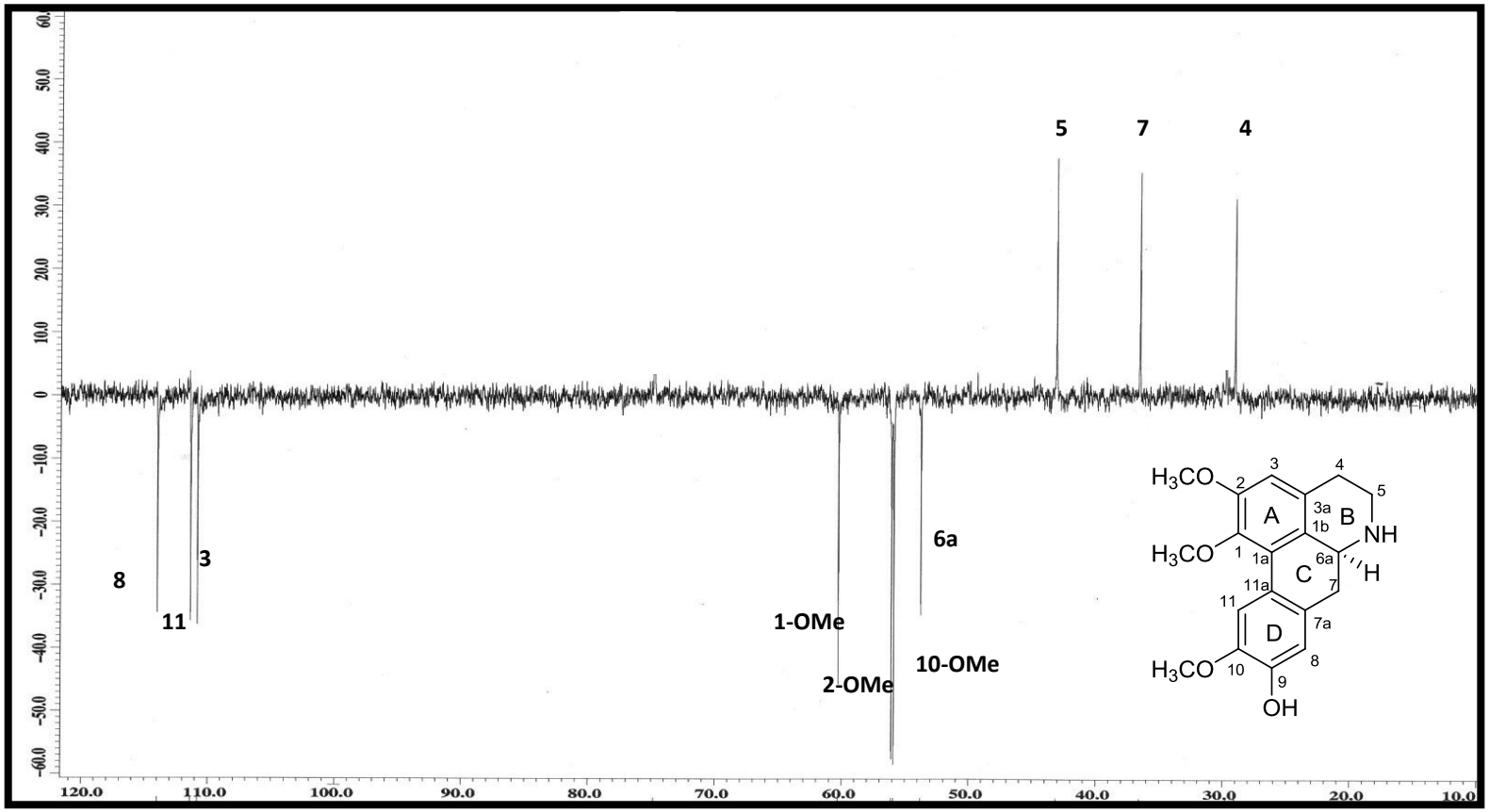


Figure 3.22: 135 DEPT NMR Spectrum of Laurotetanine **69**

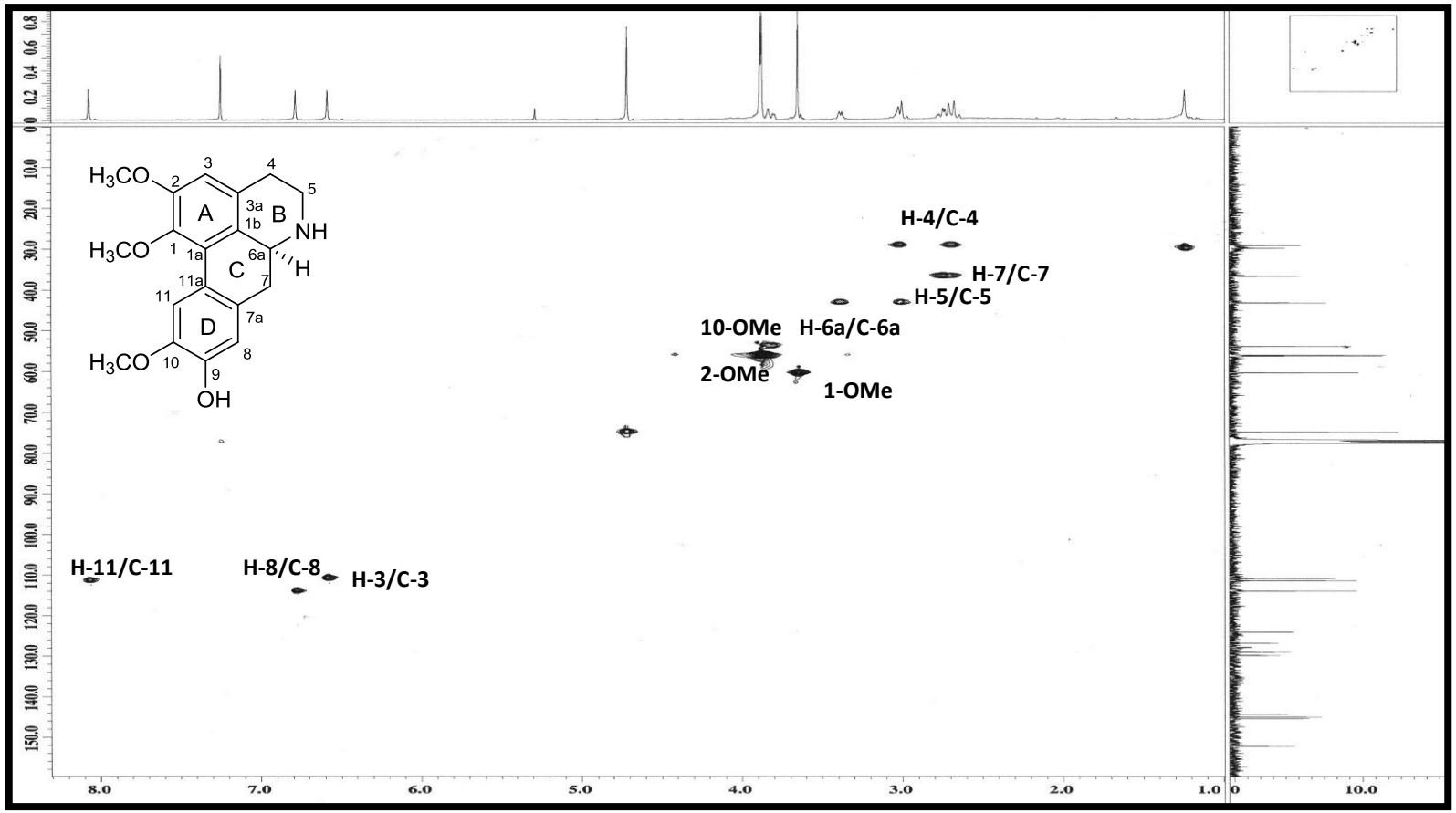


Figure 3.23: HSQC Spectrum of Laurotetanine **69**

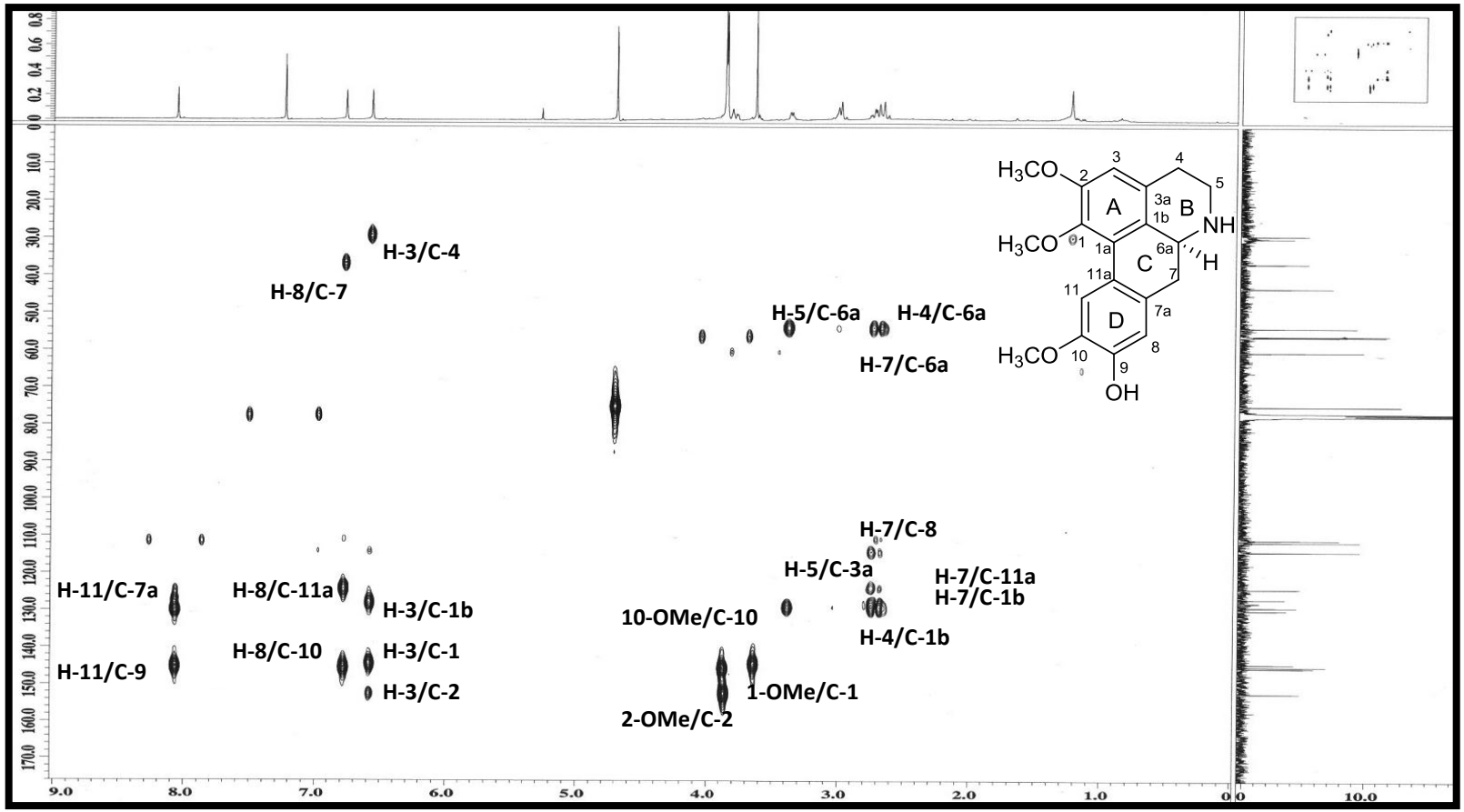


Figure 3.24: HMBC Spectrum of Laurotetanine **69**

3.3 Alkaloids from barks of *Litsea lancifolia*

Phytochemical study on the stem barks of *Litsea lancifolia* collected from Hutan Simpan Tembat Ulu Terengganu, Terengganu, Malaysia has resulted in the isolation of aporphine, morphinandienone, bisbenzylisoquinoline and benzylisoquinoline type of alkaloids. Those alkaloids were *N*-allyllaurolitsine **70**, lancifoliaine **71**, boldine **55**, pallidine **72**, *O*-methylarmepavine **73**, actinodaphnine **53**, cassythicine **74** and norboldine **75**. The following sub-chapters discuss briefly the structural elucidation of the isolated compounds.

3.3.1 *N*-Allyllaurolitsine 70

N-Allyllaurolitsine **70**, $[\alpha]_D^{27}$ = +33.9º (*c* 1.0, MeOH) was isolated as a brownish amorphous solid. The LCMS-IT-TOF spectrum revealed a pseudomolecular ion peak, $[M+H]^+$ at *m/z* 354.18, corresponding to the molecular formula of $C_{21}H_{24}NO_4$, with eleven degrees of unsaturation. This is the first report on *N*-allyllaurolitsine as a natural product compound isolated from the stem bark of *Litsea lancifolia* (Lauraceae).

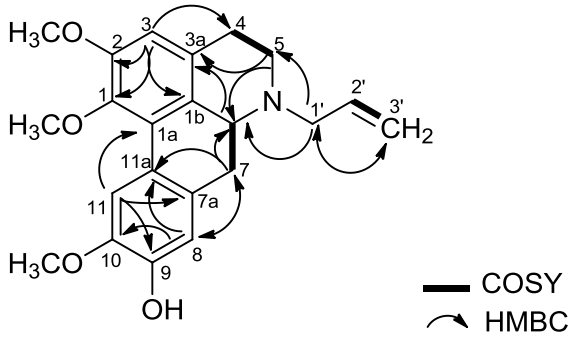
The ^{1}H NMR spectrum of *N*-allyllaurolitsine **70** showed the presence of three aromatic protons at δ 7.88 (H-11), 6.82 (H-8) and 6.64 (H-3), two methoxyl groups at δ 3.58 (1-OMe) and 3.91 (10-OMe), which showed a similar NMR pattern with norboldine **75**, suggesting a close structural relationship between these two compounds. However, a significant different was observed in the alkaloid **70** at δ 5.93- 6.03 (H-2', *J*=6.6, 10.1, 17.2 Hz), a set of broad doublet peak at δ 5.19 and 5.26 which were assigned to two protons of C-3' with coupling constant of 10.1 Hz (*cis*) and *J*=17.2 Hz (*trans*) respectively for *N*-allyl moiety. Meanwhile, a broad doublet signal was observed at δ 3.69 for the proton at position C-1'. The aliphatic protons gave a multiplet between δ 2.43- 3.31.

The ^{13}C NMR spectrum showed 21 carbon resonances for *N*-allyllaurolitsine **70**. The DEPT 135 spectrum showed five negative methylene signals and seven positive methine signals, including two signals of methoxyl groups. Two methoxy signals were observed at δ 56.2 and 60.3. In addition, four oxygenated aromatic carbon signals were observed at δ 148.2, 145.8, 145.2 and 142.2 could be due to those carbons attached to hydroxyl and methoxyl substituents in each of the benzene ring.

The position of the *N*-allyl group was confirmed through HMBC experiment. The HMBC cross peaks of H-1' (δ 3.69, 3.10) to C-5 (δ 49.1) and C-6a (δ 59.7) indicated the connection among C-1', C-5 and C-6a through *N*-6. Furthermore, H-1'

also, showed correlation to C-2' (δ 134.3) and C-3' (δ 118.5). Meanwhile, both germinal protons of C-3', were observed to correlate to C-1' (δ 57.3). Details of ^{1}H, ^{13}C NMR and HMBC data are summarized in Table 3.4.

From the analysis of the spectroscopic data obtained and comparison with the literature values, it was identified as *N*-allyllaurolitsine **70**.[102]



70

Figure 3.25: ^{1}H-^{1}H and ^{1}H-^{13}C coupling pattern observed in COSY and HMBC spectrum of *N*-allyllaurolitsine **70**

Table 3.4: ^{1}H NMR, ^{13}C NMR Data and HMBC Correlation for *N*-allyllaurolitsine

70

Position	1H NMR (δ, Hz)	^{13}C NMR (δ)	HMBC
1		142.2	
1a		126.3	
1b		127.3	
1-OMe	3.58, *s*	60.3	1
2		148.2	
3	6.64, *s*	113.3	4, 1b, 1,2
3a		130.4	
4	3.05, *m*	28.8	
5	3.20, *m*	49.1	3a
6a	3.28, br *d*	59.7	4, 3a
7	2.99, *m*	33.9	7a, 11a
7a		130.4	
8	6.82, *s*	114.2	7, 11a, 10
9		145.2	
10		145.8	
10-OMe	3.91, *s*	56.2	10
11	7.88, *s*	110.1	1a, 9, 11a
11a		123.8	
1’ (N-C**H$_2$**CH=CH$_2$)	3.69, 3.10, br *d*, *J*= 15.2	57.3	6a, 2', 3'
2’ (N-CH$_2$C**H**=CH$_2$)	5.96, *m*	134.3	
3’		118.5	1'
(N-CH$_2$CH=C**H$_E$** CH$_Z$)	5.30, br *d*, *J*= 15.2		
(N-CH$_2$CH=CH$_E$**CH$_Z$**)	5.23, br *d*, *J*= 6.6		

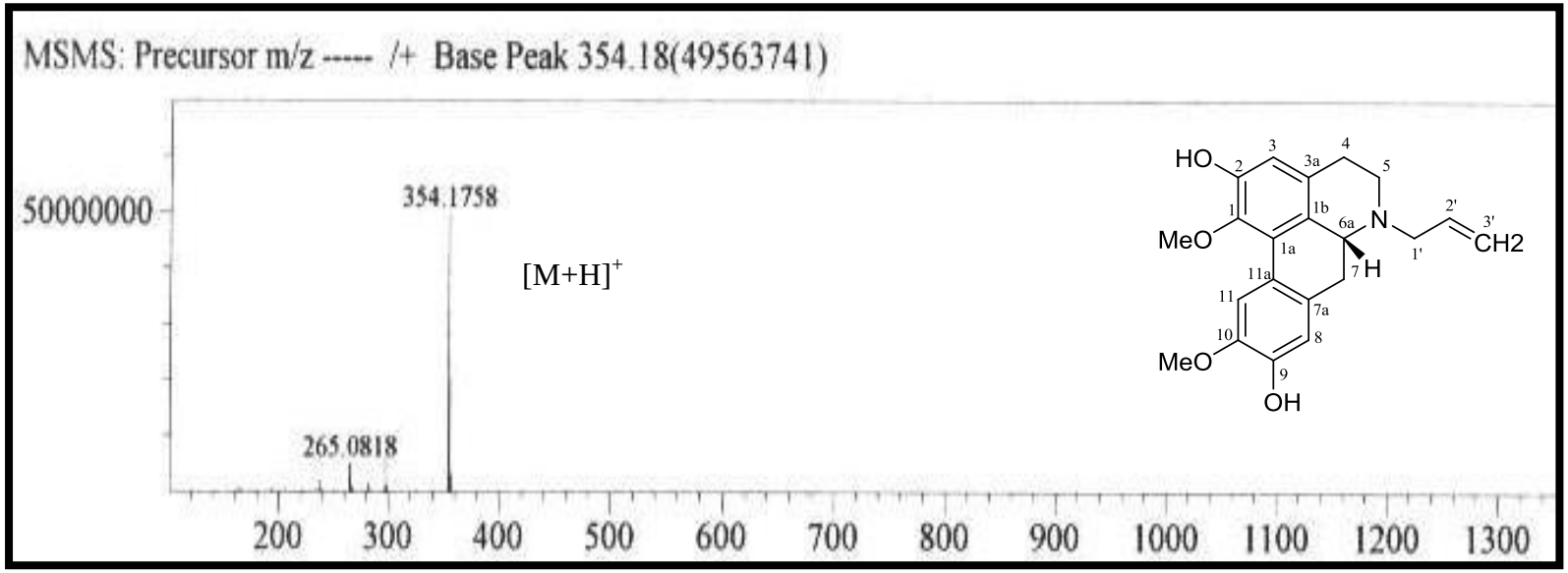


Figure 3.26: LCMS Spectrum of *N*-allyllaurolitsine **70**

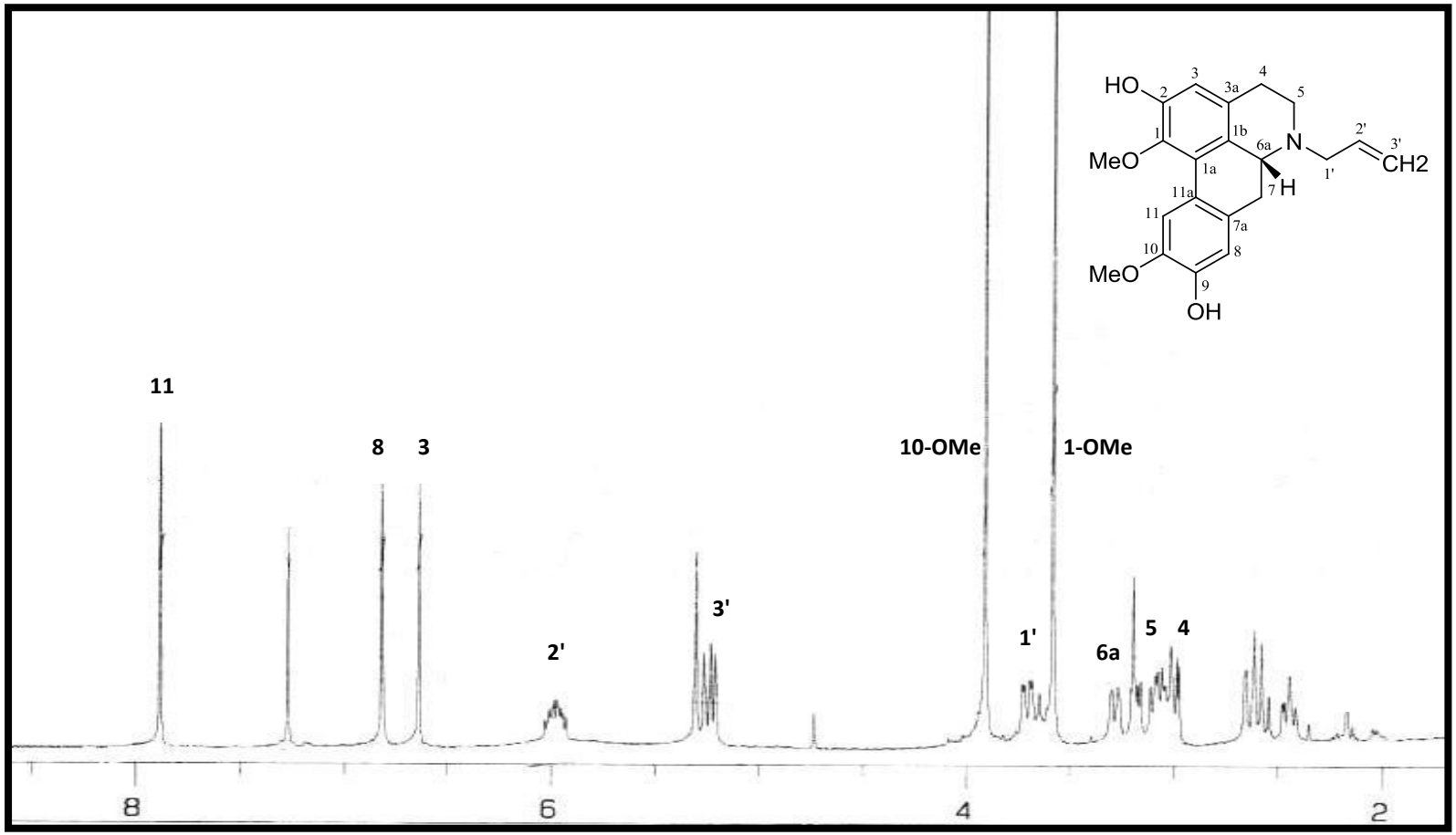


Figure 3.27: ^{1}H NMR Spectrum of *N*-allyllaurolitsine **70**

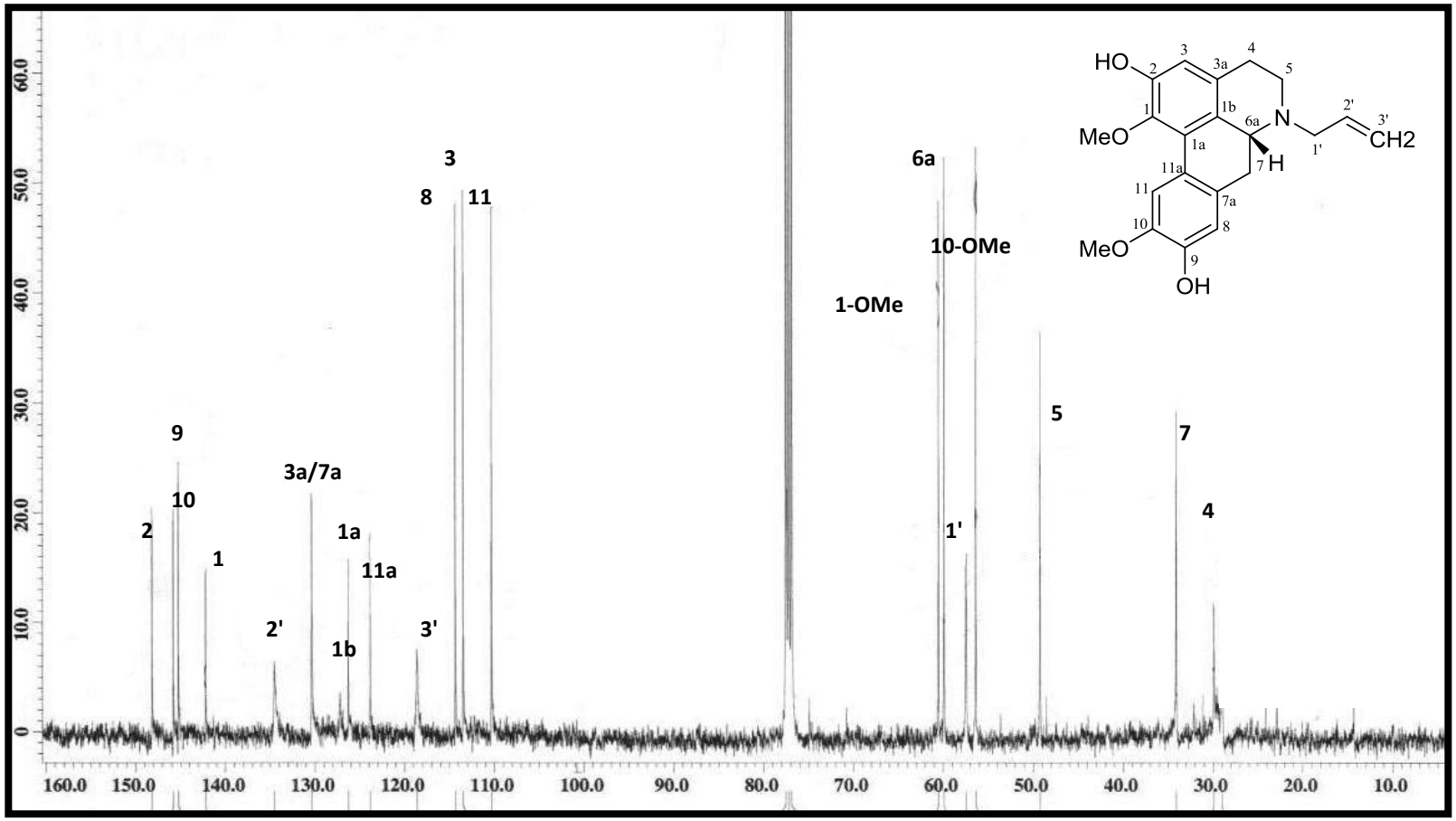


Figure 3.28: ^{13}C NMR Spectrum of *N*-allyllaurolitsine **70**

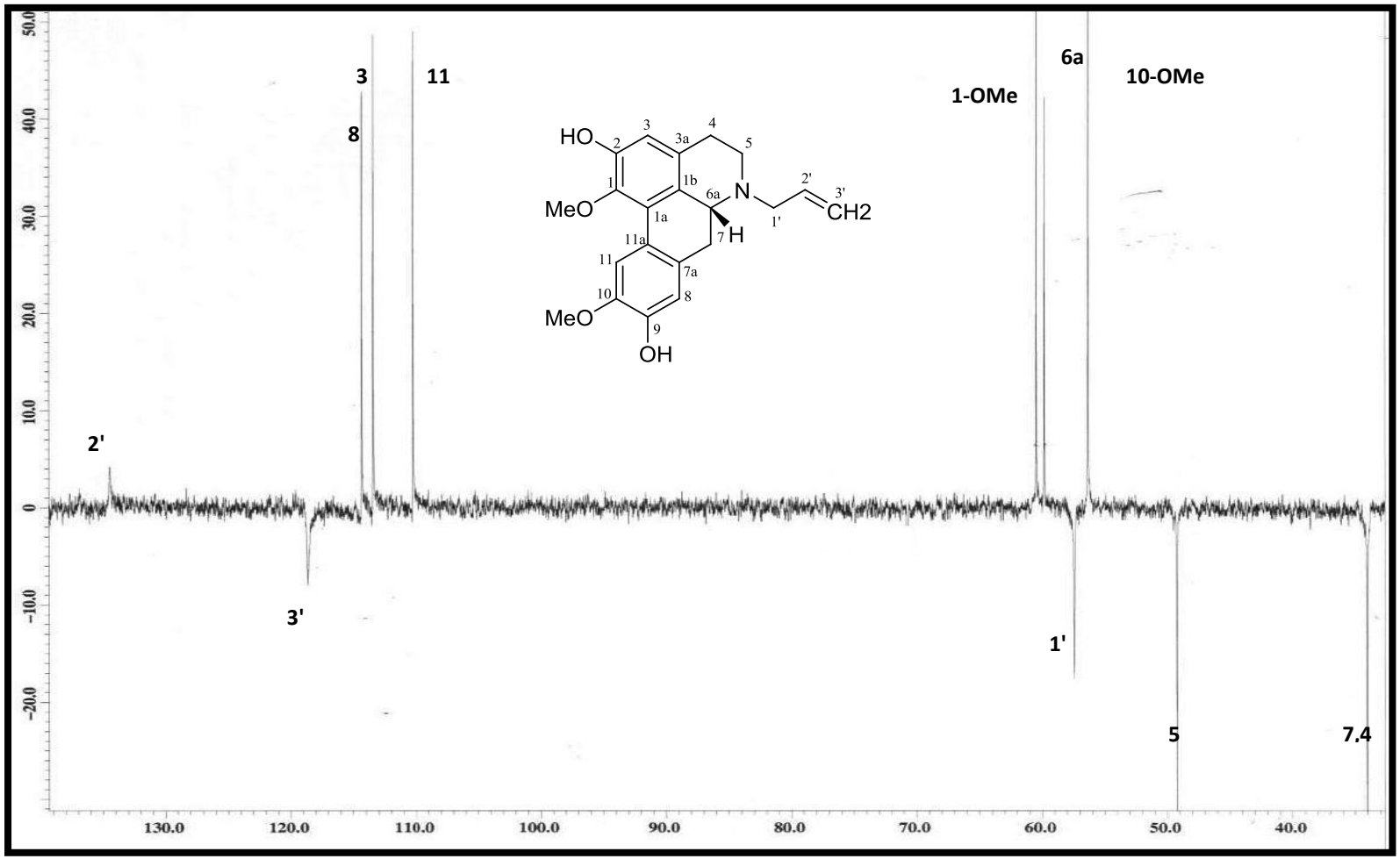


Figure 3.29: DEPT 135 NMR Spectrum of *N*-allyllaurolitsine **70**

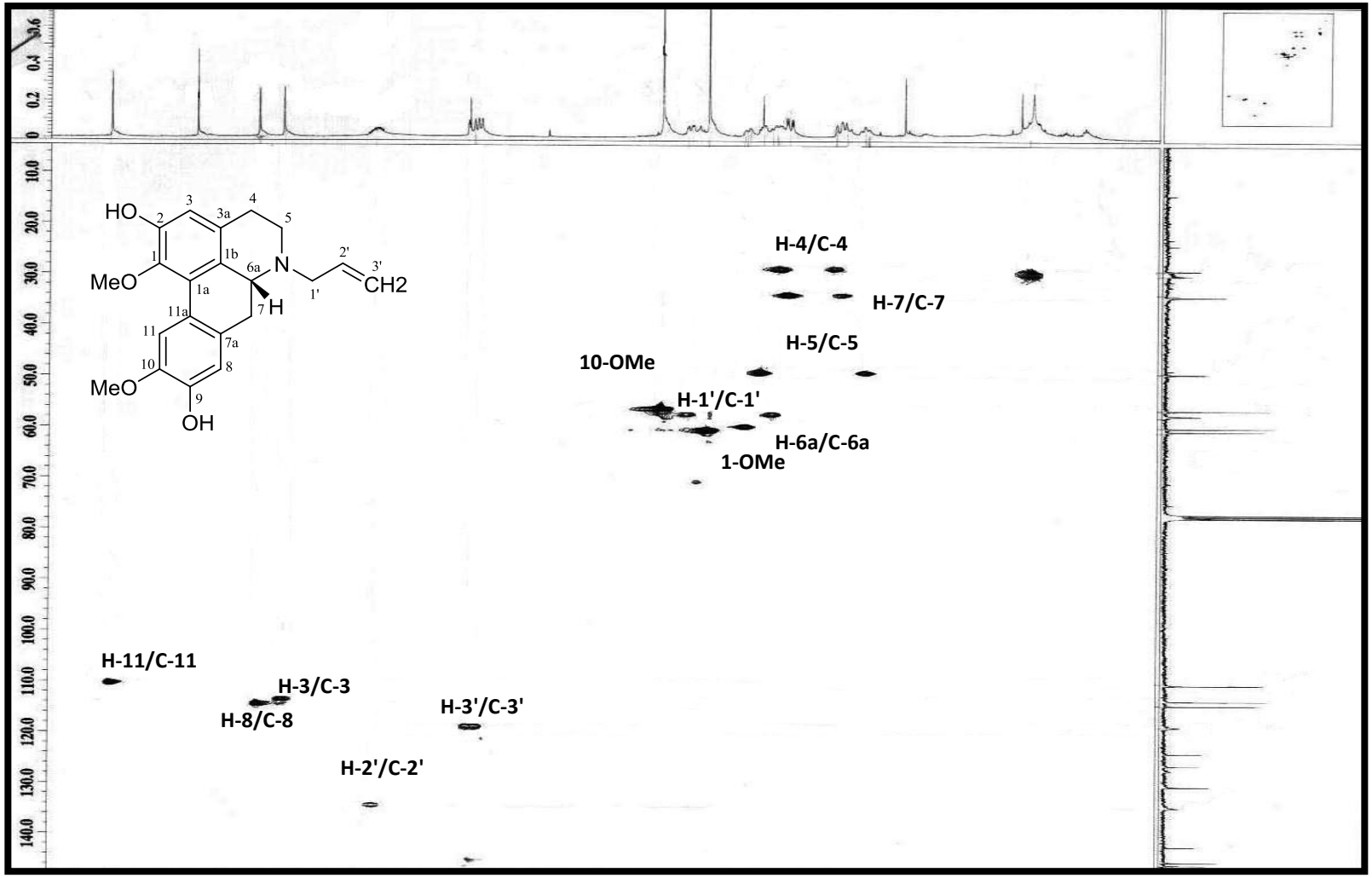


Figure 3.30: HSQC Spectrum of *N*-allyllaurolitsine **70**

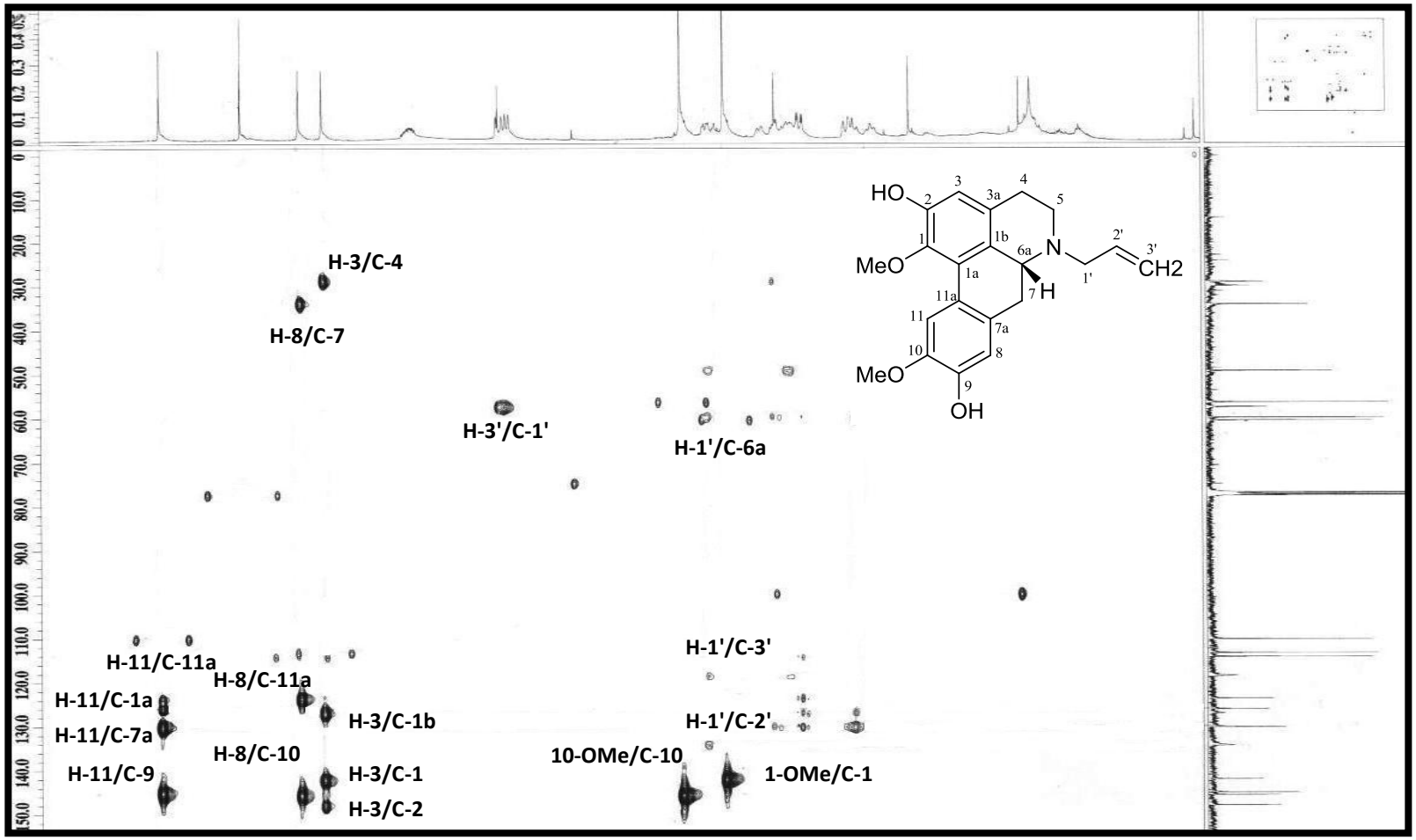


Figure 3.31: HMBC Spectrum of *N*-allyllaurolitsine **70**

3.3.2 Lancifoliaine 71

Lancifoliaine **71** was isolated as a brown amorphous solid. The LCMS-IT-TOF spectrum of **71** showed a pseudomolecular ion peak, $[M+H]^+$ at *m/z* 607.22, corresponding to the molecular formula of $C_{35}H_{31}N_2O_8$. Absorption bands in the IR spectrum at 1599 and 1665 cm^{-1} were typical of an imine and carbonyl stretching bands.[103]

In the ^1H NMR spectrum, signals for eleven aromatic protons due to three methoxy singlets and two $–CH_2-CH_2-N-$ groups were observed, thus suggesting a bisbenzylisoquinoline type of skeleton.[103,104] Among the eleven aromatic proton signals, four singlets representing H-5, H-5′, H-8 and H-8′ appeared at δ 6.69, 6.71, 6.89 and 6.88 respectively. H-10 resonated as a doublet at δ 7.71 (*J* = 2.0 Hz) while H-14 appeared as a doublet of doublets at δ 7.95 (*J* = 8.8, 2.0 Hz) and H-13 appeared as a doublet at δ 7.03 (*J* = 8.8 Hz), thus implying that ring C was trisubstituted. Ring C′ showed signals of four aromatic protons; H-10′ (*dd*, δ 7.95, *J* = 8.8, 2.0 Hz), H-14′ (*dd*, δ 7.95, *J* = 8.8, 2.0 Hz), H-11′ (*d*, δ 6.86, *J* = 8.8 Hz) and H-13′ (*d*, δ 6.89, *J* = 8.8 Hz). This pattern suggest that it was a *para* disubstituted ring system.[105] In addition, three methoxy groups appeared as singlets at δ 3.92 (6-OCH_3), 3.84 (12-OCH_3) and 3.91 (6′-OCH_3).

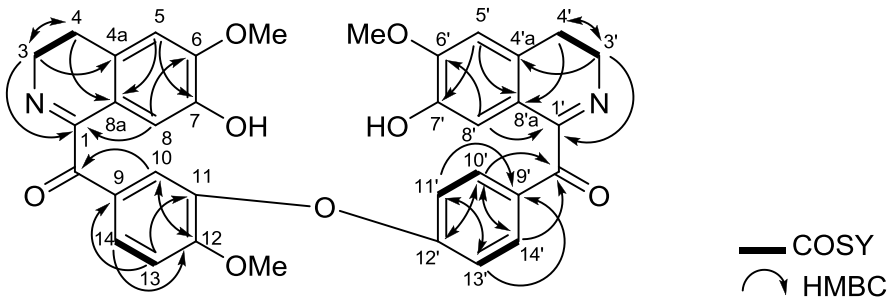
The ^{13}C NMR spectrum showed 35 carbon resonances, in agreement with the molecular formula. The presence of two carbonyl carbons was observed at δ 191.9 (C-α) and 192.7 (C-α′). The signals at δ 165.1 and 164.9 could be assigned as the two imines C-1 and C-1′ carbons, respectively.

The position of $\Delta^{1\text{-}N}$ and $\Delta^{1'\text{-}N'}$ double bonds were confirmed by the HMBC correlation of H-8 to C-1 (δ 165.1) and correlation of H-8′ to C-1′ (δ 164.9). The most downfield signal at δ 162.6 was assignable to the oxygenated C-12′ by the HMBC correlations of H-10′ and H-14′ (^3J) to C-12′.[106] The presence of carbonyl groups at C-α and C-α′ were also confirmed

based on the HMBC correlation of H-10 (δ_H 7.71) to C-α (δ 191.9), and H-10′ (δ 7.95) and H-14′ (δ 7.95) to C-α′ (δ 192.7) respectively. In addition, the position of the three methoxy groups, were assigned based on the NOESY cross-peaks between H-5/6-OCH_3, H-5′/6′-OCH_3 and H-13/12-OCH_3 respectively. Selected NOESY correlations are shown in Figure 3.33.

The ^{1}H and ^{13}C NMR spectral assignments performed by extensive 2D NMR experiments (COSY, NOESY, HMQC and HMBC) were summarized in Table 3.5.

From the analysis of the spectroscopic data obtained from various spectral assignments, it was identified as a new bisbenzylisoquinoline alkaloid namely lancifoliaine **71**.



71

Figure 3.32: ^{1}H-^{1}H and ^{1}H-^{13}C coupling pattern observed in COSY and HMBC spectrum of Lancifoliaine **71**

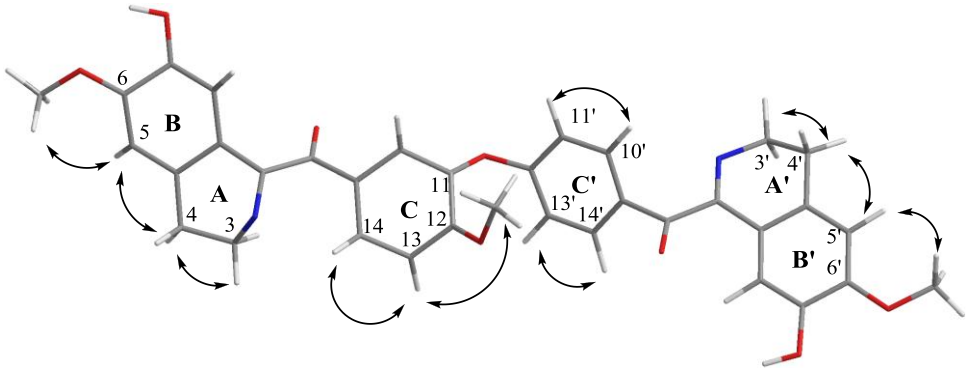


Figure 3.33: Selected NOESY correlations of lancifoliaine **71**

Table 3.5: ^{1}H NMR, ^{13}C NMR Data and HMBC Correlation for Lancifoliaine **71**

Position	^{1}H NMR (δ, Hz)	^{13}C NMR (δ)	HMBC
1		165.1	
3	3.88, *m*	47.3	1, 4, 4a
4	2.76, *m*	25.5	3, 5, 8a, 4a
4a		130.2	
5	6.69, *s*	110.1	4, 8a, 7, 6
6		149.5	
6-OMe	3.92, *s*	56.3	6
7		144.4	
8	6.89, *s*	113.1	6, 7, 1
8a		119.9	
α		191.9	
9		129.8	
10	7.71, *d*, *J*=2.0 Hz	124.4	9, 11, 12, α
11		143.0	
12		156.6	
12-OMe	3.84, *s*	56.3	12
13	7.03, *d*, *J*=8.8 Hz	112.2	9, 11, 12
14	7.95, *dd*, *J*=8.8, 2.0 Hz	132.7	10, 12
1'		164.9	
3'	3.88, *m*	47.3	1', 4', 4a'
4'	2.76, *m*	25.5	3', 5', 4a', 8a'
4'a		130.2	
5'	6.71, *s*	110.1	4', 8a', 7', 6'
6'		149.4	
6'-OMe	3.91, *s*	56.1	6'
7'		144.4	
8'	6.88, *s*	112.2	1', 7', 6'
8'a		119.9	
α'		192.7	
9'		129.8	
10'	7.95, *dd*, *J*=8.8, 2.0 Hz	132.7	14', 12', α'
11'	6.86, *d*, *J*=8.8 Hz	116.1	13', 9', 12'
12'		162.6	
13'	6.89, *d*, *J*=8.8 Hz	116.1	9', 12'
14'	7.95, *dd*, *J*=8.8, 2.0 Hz	132.7	10', 12', α'

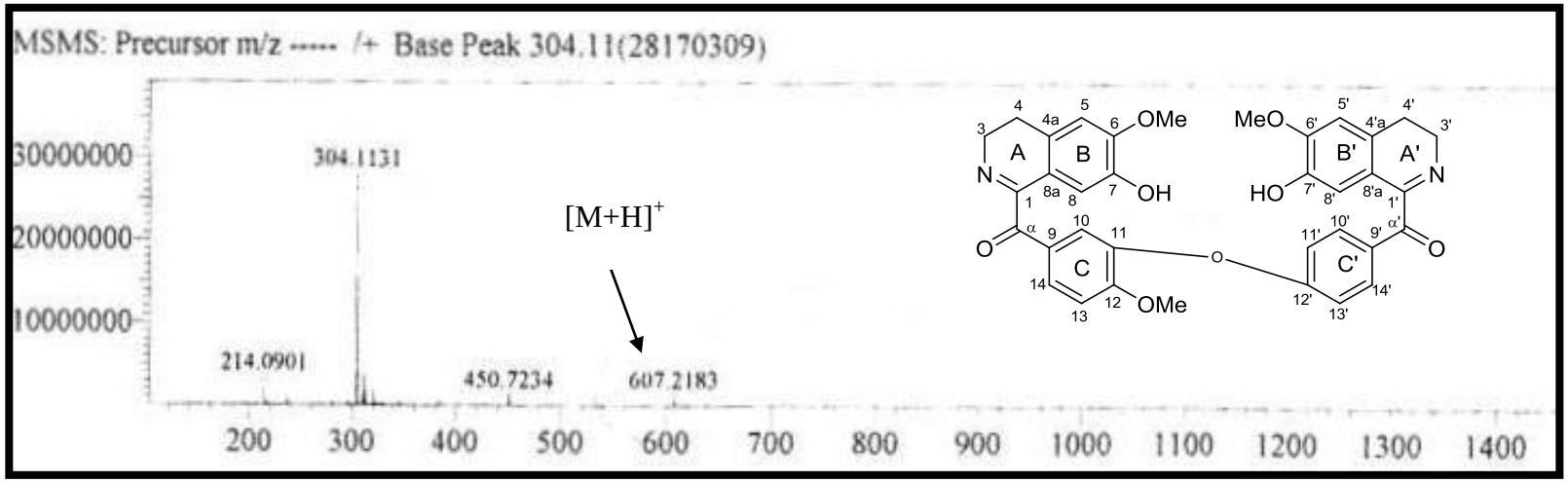


Figure 3.34: LCMS Spectrum of Lancifoliaine **71**

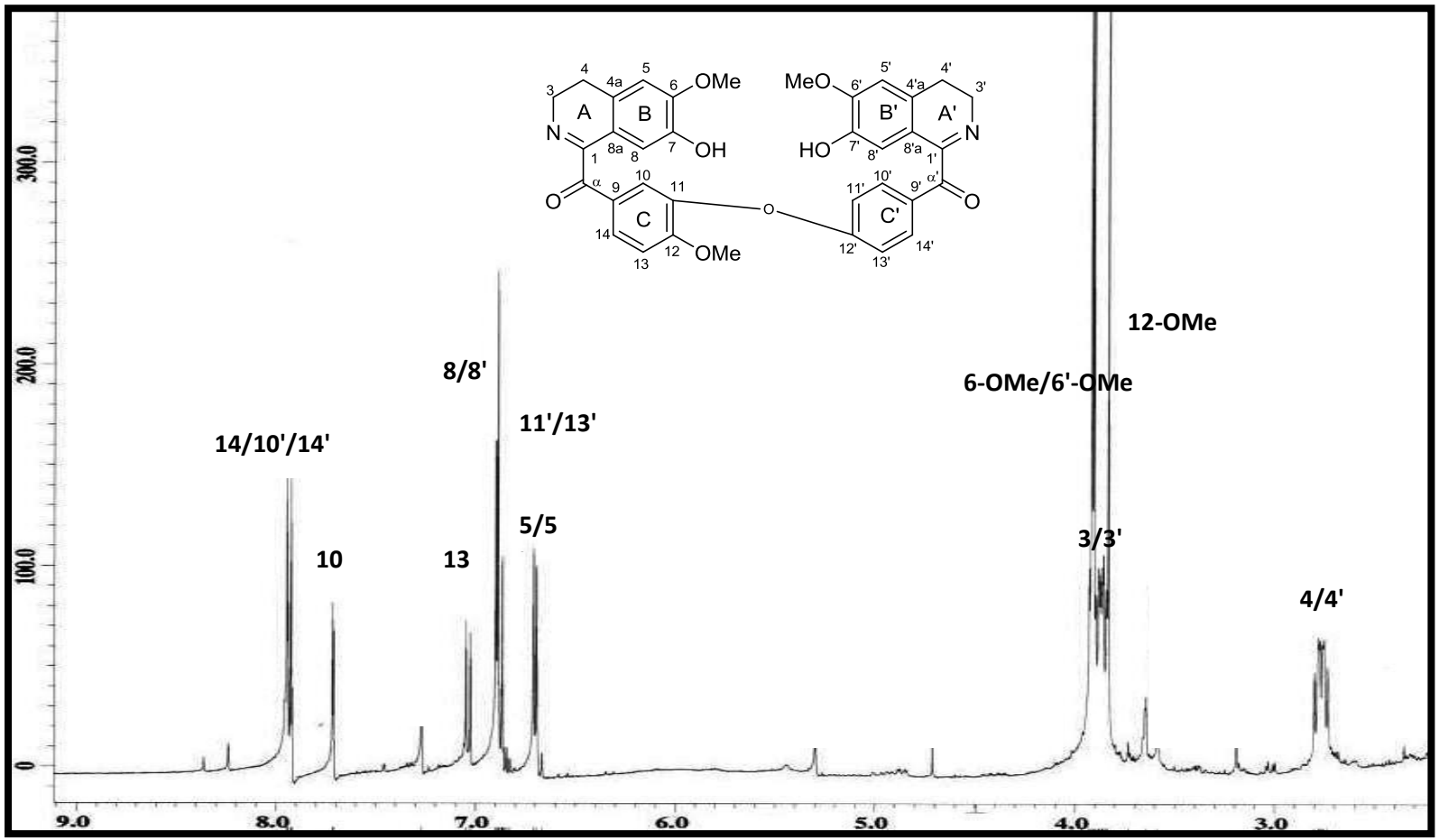


Figure 3.35: ^{1}H NMR Spectrum of Lancifoliaine **71**

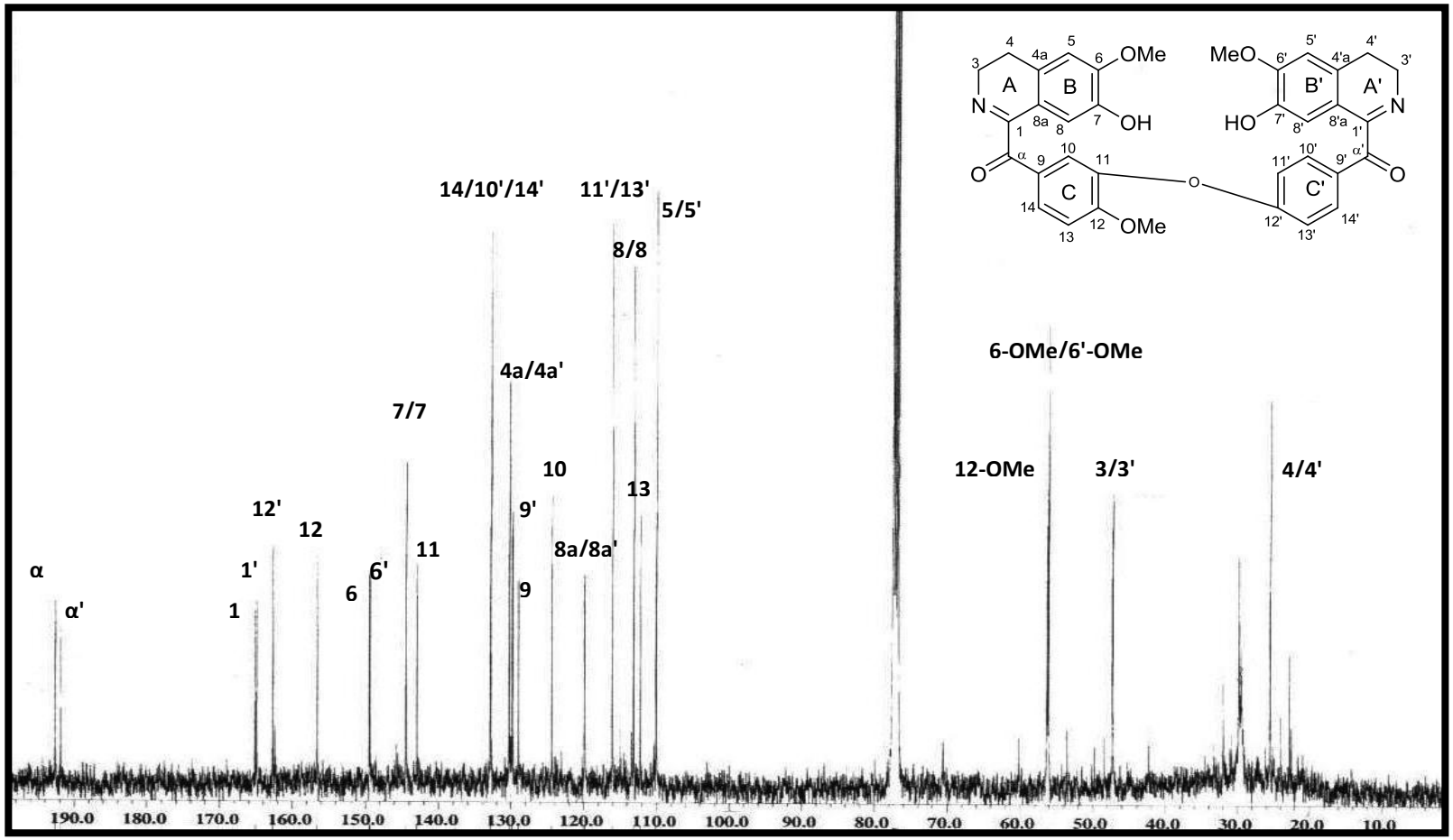


Figure 3.36: ^{13}C NMR Spectrum of Lancifoliaine **71**

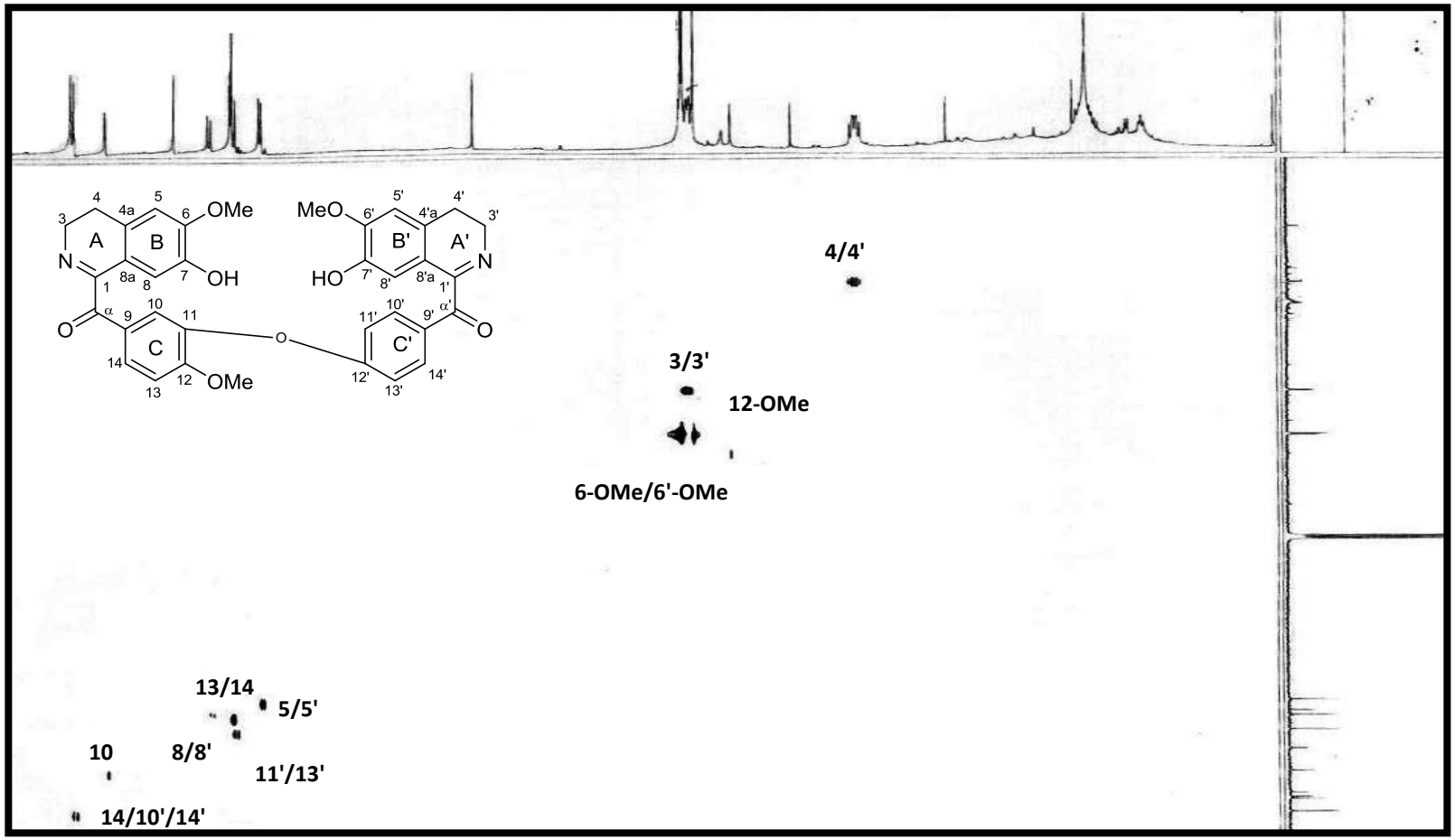


Figure 3.37: HSQC Spectrum of Lancifoliaine **71**

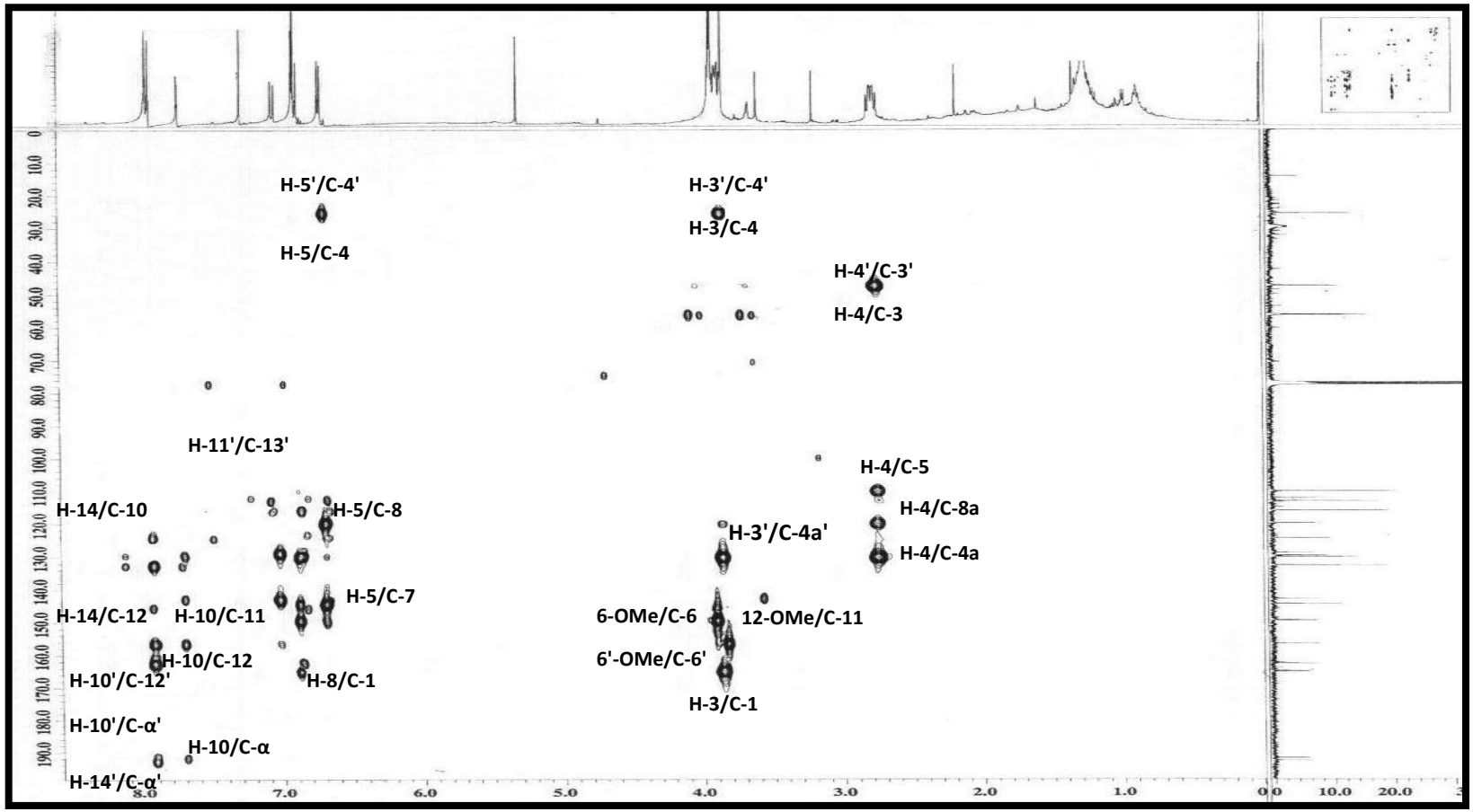


Figure 3.38: HMBC Spectrum of Lancifoliaine **71**

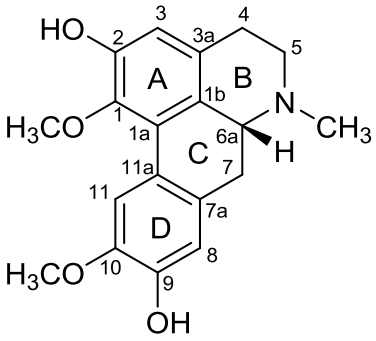
3.3.3 Boldine 55

Boldine **55**, $[\alpha]_D^{27}$ = +15º (*c* 1.0, MeOH) was isolated as a brownish amorphous solid. The UV spectrum showed absorptions typical of an aporphine type at 282 and 302 nm, significant for a 1,2,9,10-substituted aporphine.[100,101] In addition, the IR spectrum showed an absorption band at 3350 cm^{-1} due to the stretching of a hydroxyl group. Other absorption peaks were at 2930 and 1598 cm^{-1} which indicated the C-H aromatic and C-O stretching respectively. The LCMS-IT-TOF revealed a pseudomolecular ion peak, $[M+H]^+$ at *m/z* 328.16 consistent with the molecular formula of $C_{19}H_{21}NO_4$.

The ^1H NMR spectrum of boldine **55** showed three singlet signals representing three aromatic protons at δ 6.61 (H-3), 6.81 (H-8) and 7.87 (H-11) respectively, which has shown the same pattern with that of laurotetanine **69** and *N*-allyllaurolitsine **70**. However, the former alkaloid has one *N*-methyl singlet observed at δ 2.51 and two distinct methoxyl peaks at δ 3.57 and 3.89, most probably attached to C-1 and C-10 respectively. In addition, the aliphatic protons were observed as multiplets at the region of δ 2.54-3.12.

The ^{13}C NMR spectrum of boldine **55** showed 19 carbon resonances consistent with the number of carbon in the molecular formula $C_{19}H_{21}NO_4$. Furthermore, four oxygenated aromatic carbon signals were observed at δ 148.2 (C-2), 145.6 (C-10), 145.1 (C-9) and 142.1 (C-1) indicated the presence of hydroxyl and methoxyl groups in each of the benzene ring. C-3 and C-8 resonated at δ 113.3 and 114.3 due to the fact that it attached to the carbon bearing the hydroxyl group[107]. It was reported that, the hydroxyl group exhibited a lesser *ortho* shielding effect compared to the methoxyl group[103].

Complete ^{1}H and ^{13}C NMR assignments for boldine **55** were made by analyzing all the spectroscopic data and comparison with the literature values, finally it was identified as boldine **55**.[103] ^{1}H NMR and ^{13}C NMR data for boldine **55** are listed in the Table 3.6.



55

Table 3.6: ^{1}H NMR and ^{13}C NMR Data for Boldine **55**

Position	^{1}H NMR (δ, Hz)	^{13}C NMR (δ)
1		142.1
1a		126.4
1b		125.9
2		148.2
3	6.61, *s*	113.3
3a		129.6
4	2.96, *m*	28.6
5	3.12, *m*	53.2
6a	3.81, *m*	62.5
7	3.03, *m*	33.9
7a		129.9
8	6.80, *s*	114.3
9		145.1
10		145.6
11	7.87, *s*	110.2
11a		123.5
1-OMe	3.57, *s*	60.2
10-OMe	3.89, *s*	56.1
N-Me	2.51, *s*	43.7

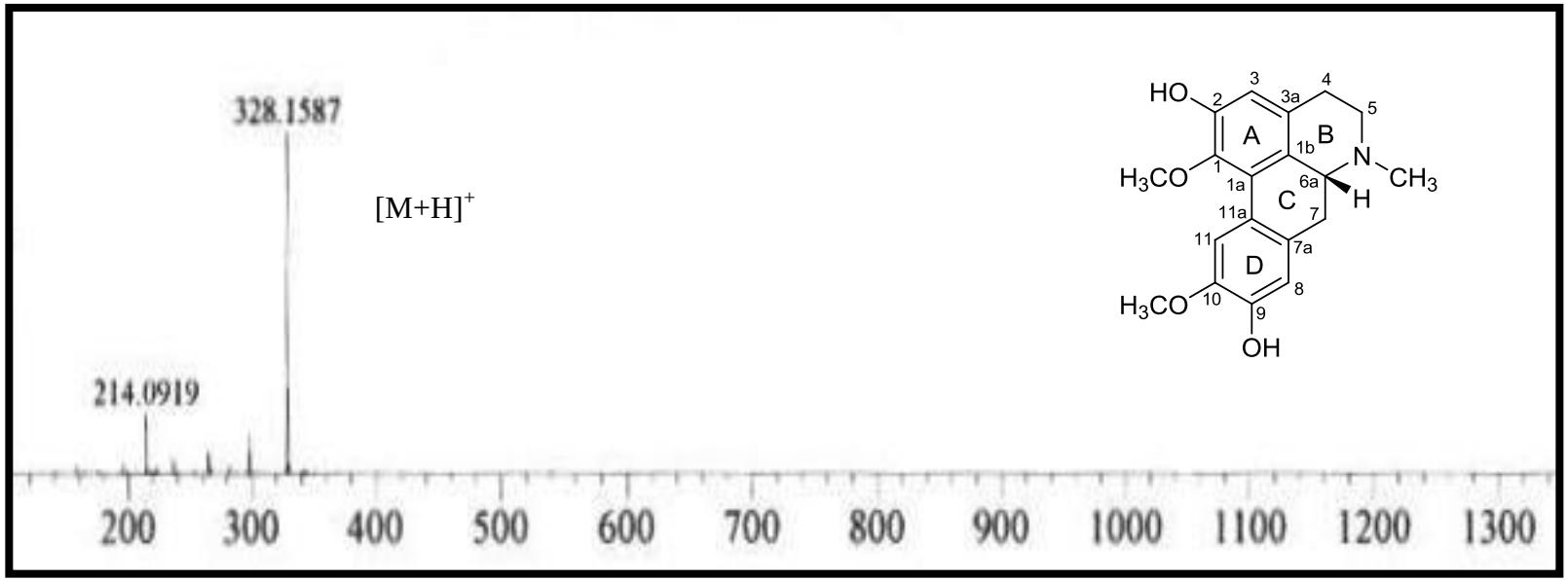


Figure 3.39: LCMS-IT-TOF Spectrum of Boldine **55**

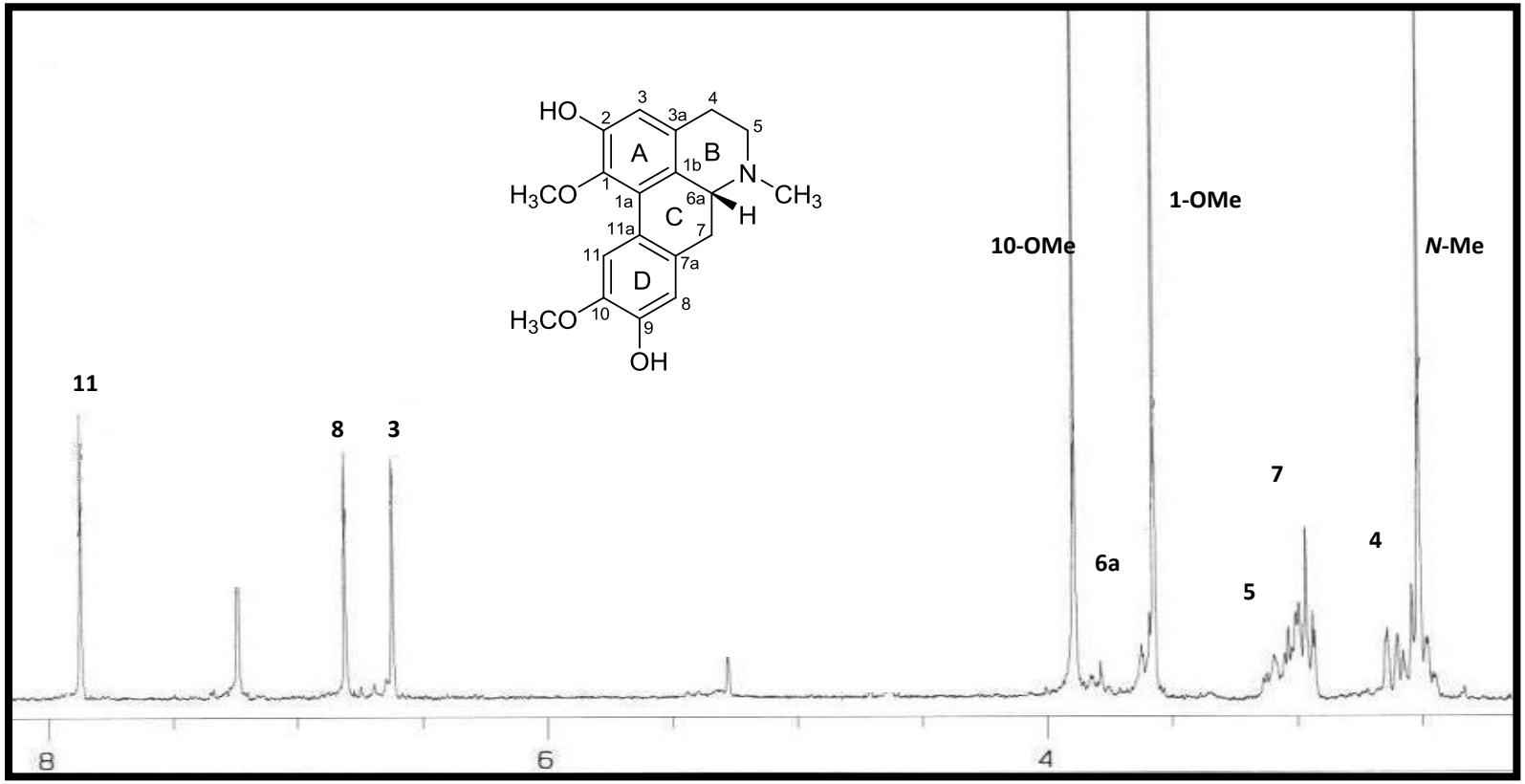


Figure 3.40: ^{1}H NMR Spectrum of Boldine **55**

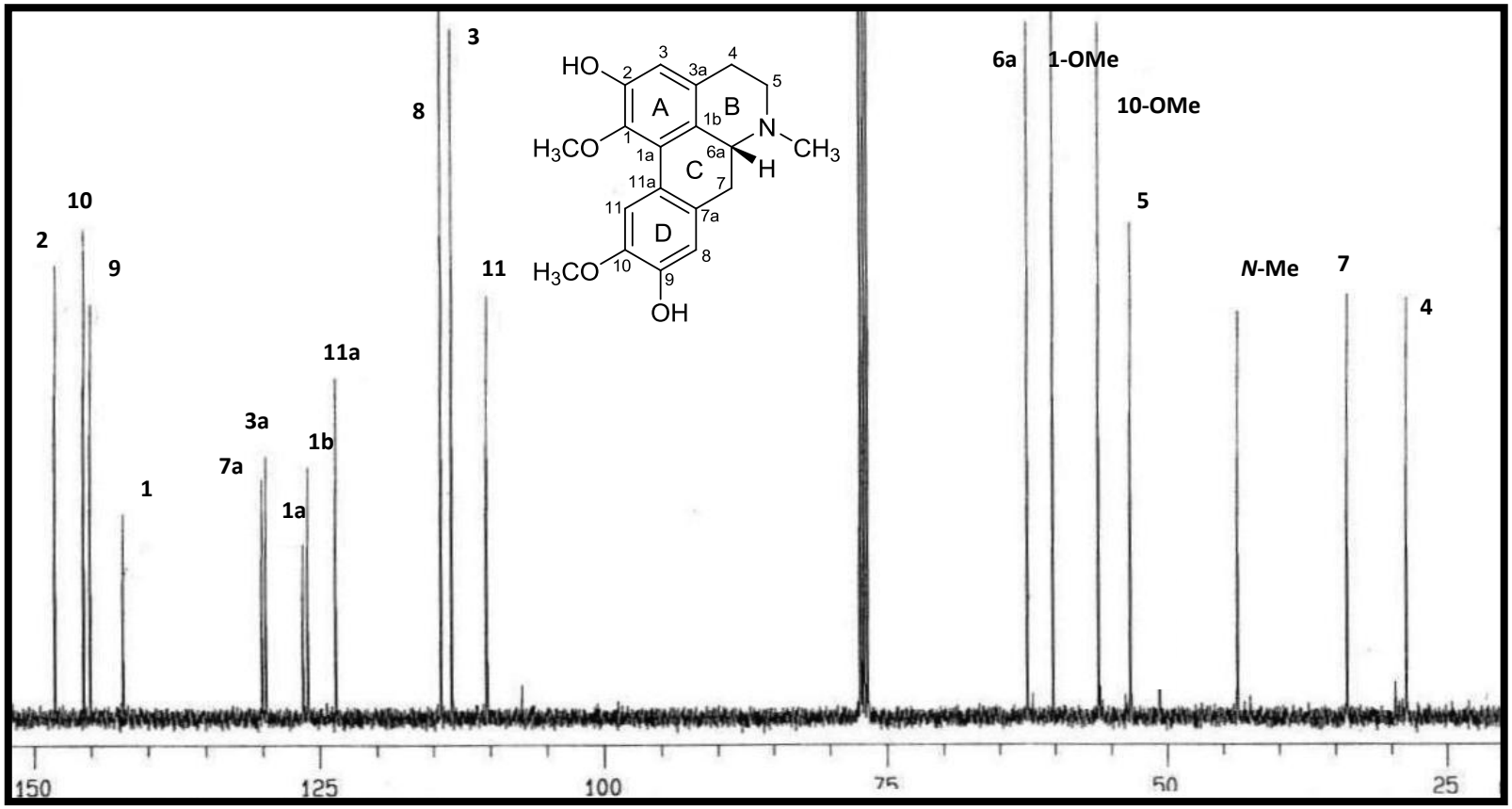


Figure 3.41: ^{13}C NMR Spectrum of Boldine **55**

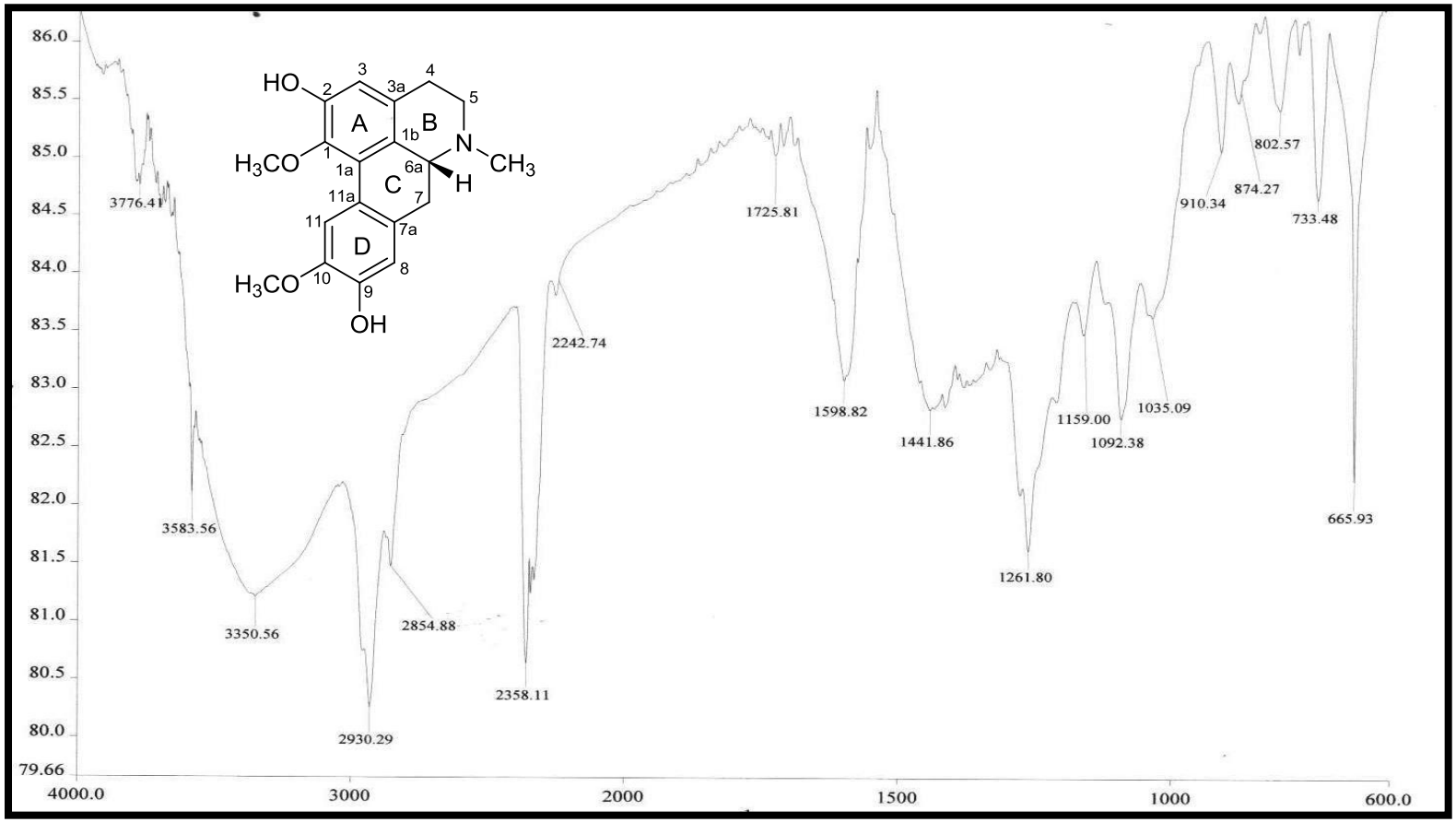


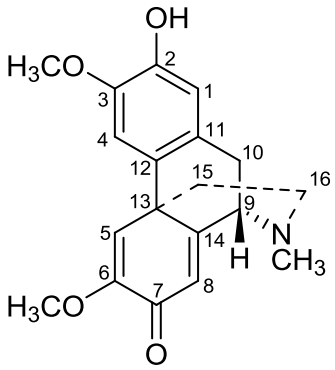
Figure 3.42: IR Spectrum of Boldine **55**

3.3.4 Pallidine 72

Pallidine **72**, with $[\alpha]_D^{27}$ = -22.8º (*c* 1.0, MeOH) was isolated as a yellowish amorphous solid. The UV spectrum showed absorptions at 206, 235 and 283 nm, which were the features of the morphinandienones.[108] The IR spectrum showed absorption bands at 3370 and 1649 cm^{-1} due to the stretching of conjugated hydroxyl and carbonyl groups. Its IR spectrum also showed a strong absorption at 2927 cm^{-1} due to the stretching of CH aromatic, respectively. The LCMS-IT-TOF revealed a pseudomolecular ion peak, $[M+H]^+$ at *m/z* 328.16 consistent with the molecular formula of $C_{19}H_{22}NO_4$.

^{1}H NMR spectrum of pallidine **72** clearly showed its morphinandienone nature by displaying the characteristic four low field singlets at δ 6.78 (H-4), 6.71 (H-1), 6.34 (H-5) and 6.33 (H-8)[109] together with two methoxy groups at δ 3.90 and 3.81, most probably attached to C-3 and C-6 respectively. Another characteristic signals of morphinandienone type was at δ 3.34 (*d*, *J*=17.6 Hz) and δ 3.03 (*dd*, *J*=11.2, 17.6 Hz) which corresponding to H-10β and H-10α.[87] The position of 3-OMe at δ 3.90 was determined by using NOE difference experiment, which showed that upon irradiation of H-4 peak enhanced the methoxyl singlet at δ 3.90 and H-5 at δ 6.34, thus proved the position of OMe at C-3 and another OMe group at C-6.

Therefore, based on the above results and comparison with the reported values from Shamma and Chang as listed in the Table 3.7, alkaloid **72** was identified as pallidine.[109,110]



72

Table 3.7: ^{1}H NMR Data for Pallidine **72**

Position	^{1}H NMR (δ, Hz) (observed)	^{1}H NMR (δ, Hz) (Shamma *et al.*)[109]	^{1}H NMR (δ, Hz) (Chang *et al.*)[110]
1	6.71, *s*	6.67, *s*	6.60, *s*
4	6.78, *s*	6.87, *s*	6.73, *s*
5	6.34, *s*	6.48, *s*	6.35, *s*
8	6.33, *s*	6.30, *s*	6.28, *s*
3-OMe	3.90, *s*	2.82, *s*	3.74, *s*
6-OMe	3.81, *s*	2.89, *s*	3.82, *s*
N-Me	2.47, *s*	2.44, *s*	2.42, *s*

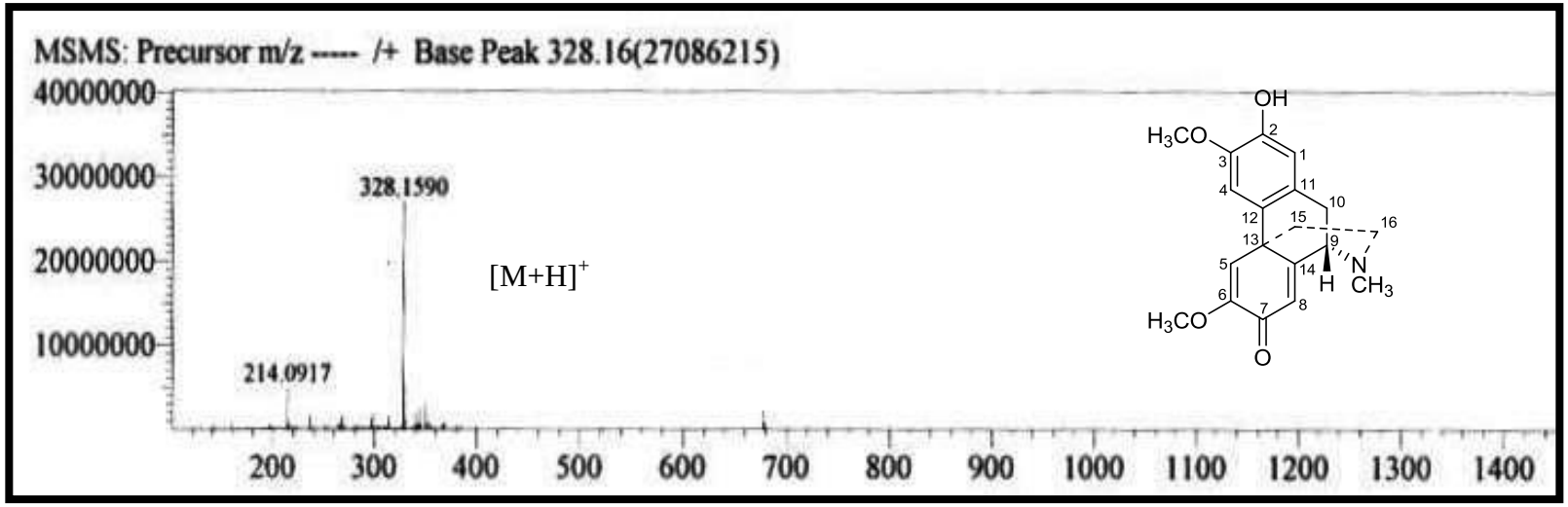


Figure 3.43: LCMS-IT-TOF Spectrum of Pallidine **72**

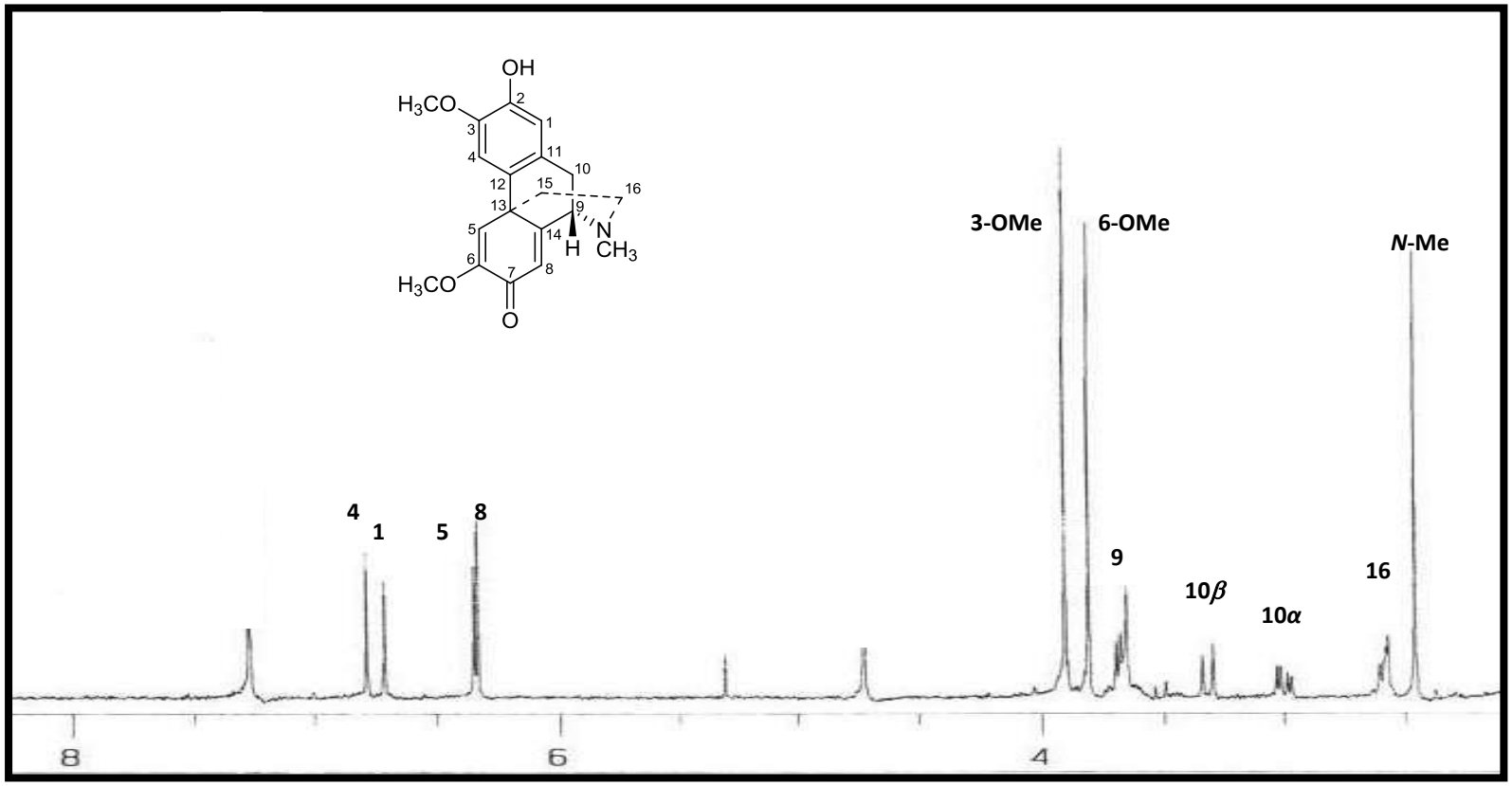


Figure 3.44: ^{1}H NMR Spectrum of Pallidine **72**

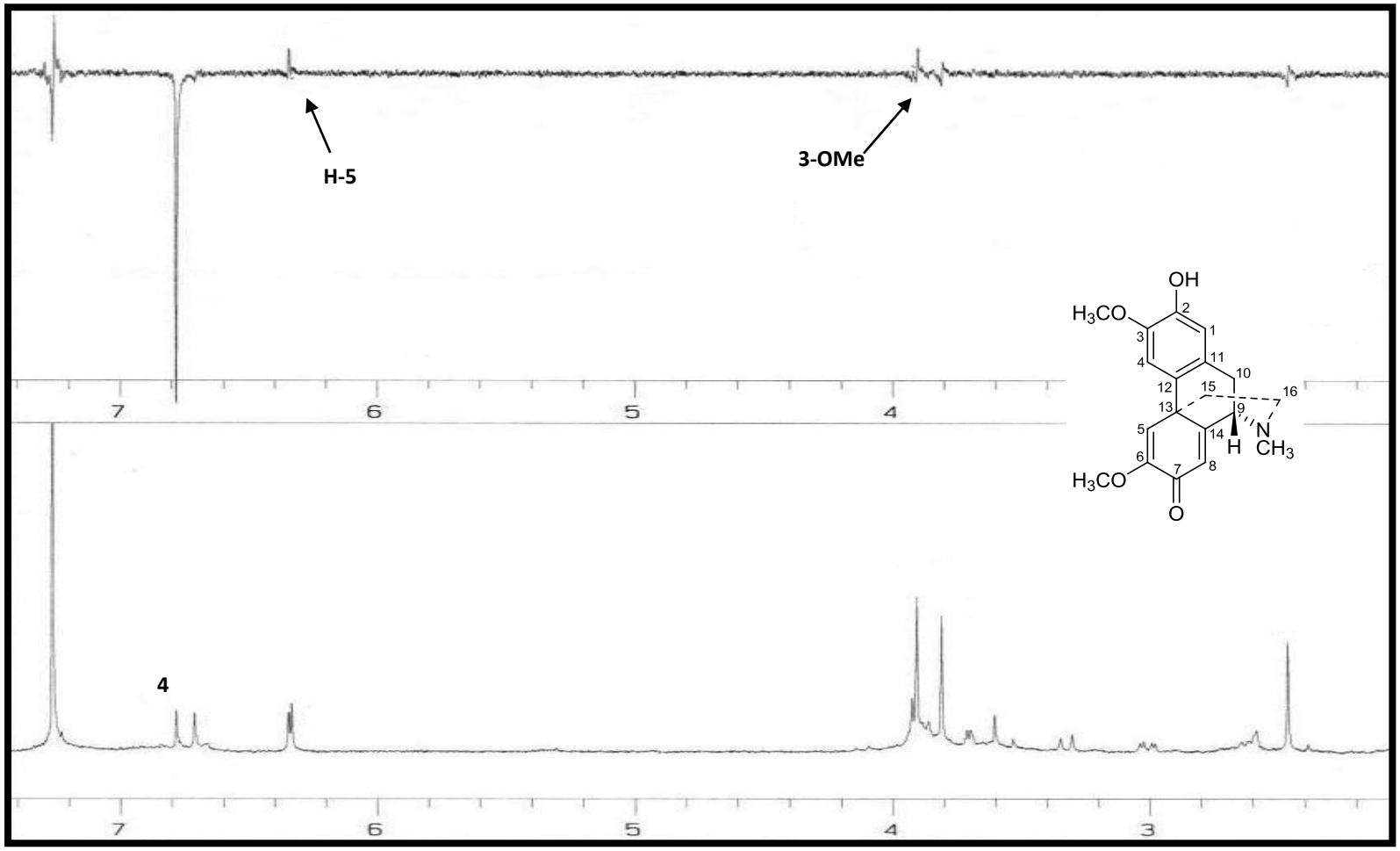


Figure 3.45: NOE-Diff Spectrum of Pallidine **72**

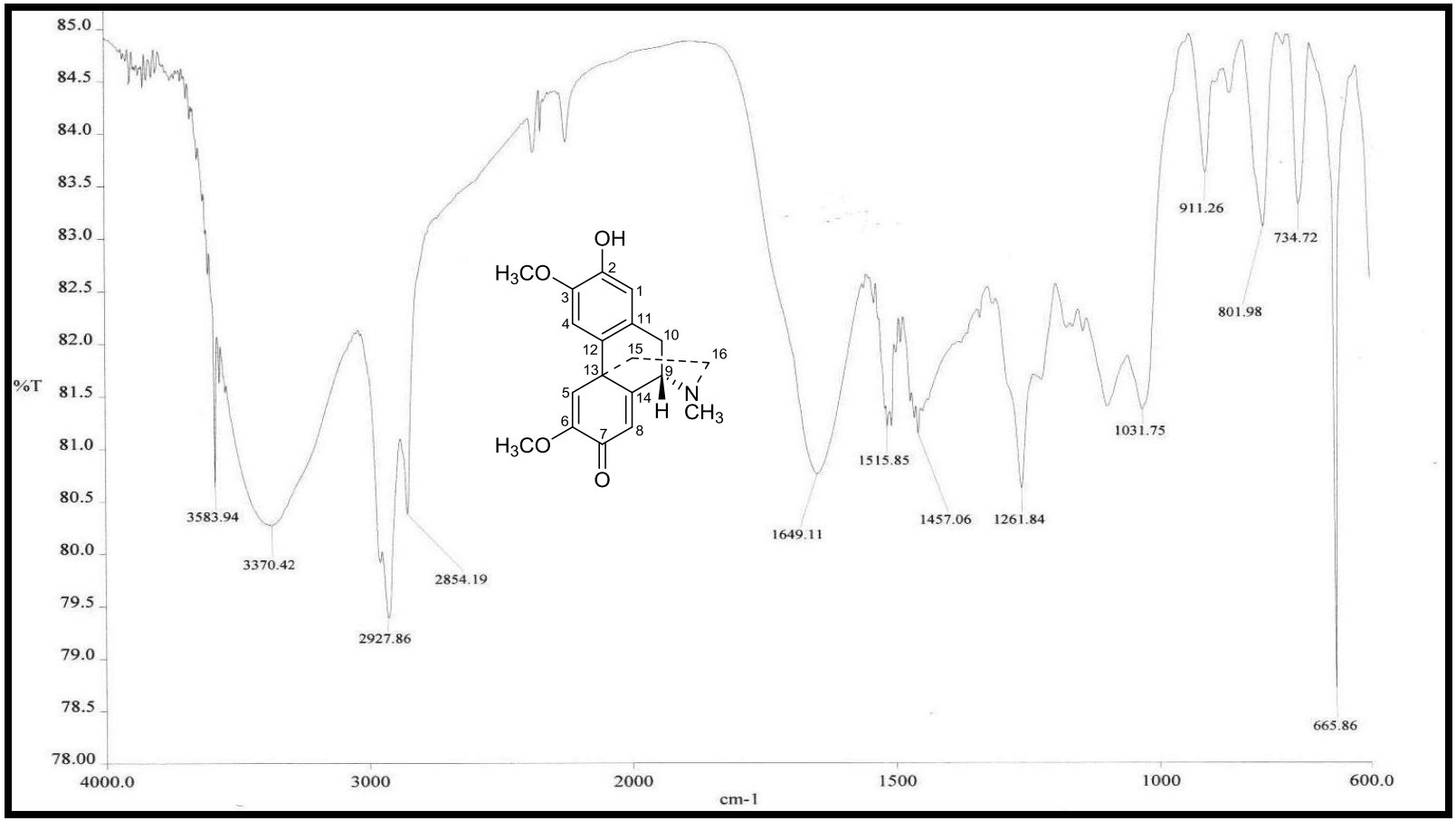


Figure 3.46: IR Spectrum of Pallidine **72**

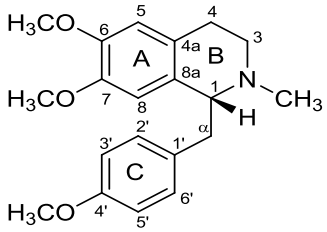
3.3.5 *O*-Methylarmepavine 73

O-Methylarmepavine **73**, $[\alpha]_D^{27}$ = +65º (*c* 1.0, MeOH) was identified as a minor alkaloid from the bark of *L. lancifolia*. The UV spectrum showed absorption at 301 nm. The IR spectrum showed a strong absorption band at 2921 cm^{-1} due to the stretching of C-H aromatic, respectively. The LCMS-IT-TOF revealed a pseudomolecular ion peak, $[M+H]^+$ at *m/z* 328.16 corresponding to the molecular formula of $C_{20}H_{26}NO_3$ with nine degrees of unsaturation.

The ^{1}H NMR spectrum of *O*-methylarmepavine **73** exhibited signals for three methoxyl groups (δ 3.74, 3.78 and 3.83), one *N*-Me (δ 2.26) and six aromatic protons respectively. Four aromatic protons in ring C showed the same pattern, an AB spin system with that of *N*-methylisococlaurine **68**, resonated at δ 6.67 (H-2' and H-6', *d*, *J* = 8.3 Hz) and δ 6.63 (H-3' and H-5', *d*, *J* = 8.3 Hz). Meanwhile, the other two aromatic protons were resonated as singlet at δ 6.46 (H-5) and δ 6.34 (H-8), and as usual the aliphatic protons appeared as a multiplet at the region of δ 2.45-3.61.

The ^{13}C NMR spectrum showed the presence of 20 carbon resonances, which was in agreement with the molecular formula of *O*-methylarmepavine **73**. The assignments of carbons in the skeleton are determined by comparing the obtained spectral data with the ^{13}C NMR data reported previously.[80]

From the analysis of the spectroscopic data obtained and comparison with the literature values, it was identified as *O*-methylarmepavine **73**.[80]



73

Table 3.8: ^{1}H NMR, ^{13}C NMR Data for *O*-methylarmepavine **73**

Position	^{1}H NMR (δ, Hz)	^{13}C NMR (δ)	^{13}C NMR (δ) [80]
1	3.61, *t*, *J*=6.12	64.6	64.4
3	2.89-2.94, *m*	46.9	46.3
4	2.45-2.53, *m*	25.2	25.0
4a		125.4	125.3
5	6.46, *s*	113.6	113.0
6		145.8	146.6
7		145.1	145.7
8	6.34, *s*	110.6	110.7
8a		129.4	128.6
α	2.69-2.74, *m*	41.0	39.7
1'		133.3	131.3
2'	6.67, *d*, *J*=8.28	129.6	130.1
3'	6.63, *d*, *J*=8.28	113.6	113.0
4'		161.9	157.3
5'	6.63, *d*, *J*=8.28	113.6	113.0
6'	6.67, *d*, *J*=8.28	129.6	130.1
6-OMe	3.73, *s*	56.2	55.2
7-OMe	3.77, *s*	56.0	55.0
4'-OMe	3.83, *s*	55.2	54.6
N-Me	2.38, *s*	42.3	42.1

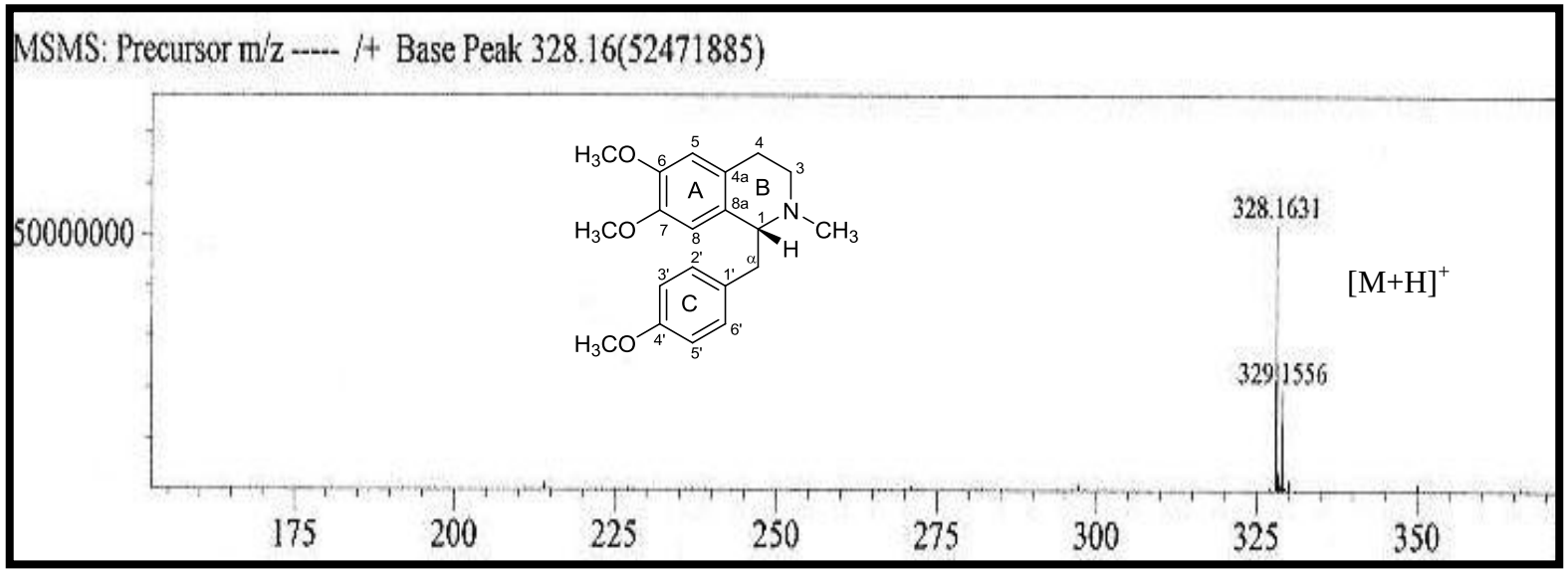


Figure 3.47: LCMS-IT-TOF Spectrum of *O*-methylarmepavine **73**

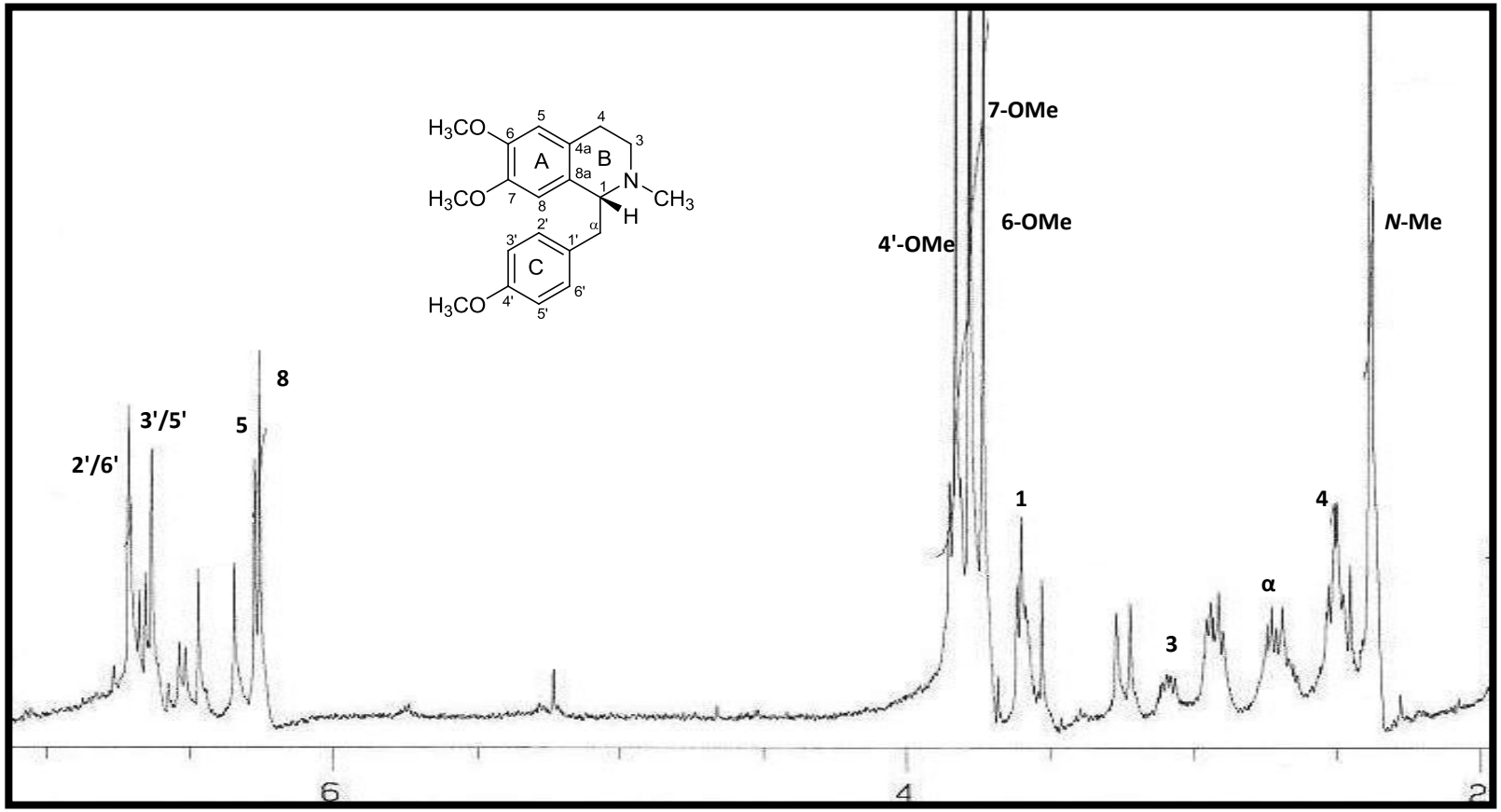


Figure 3.48: ^{1}H NMR Spectrum of *O*-methylarmepavine **73**

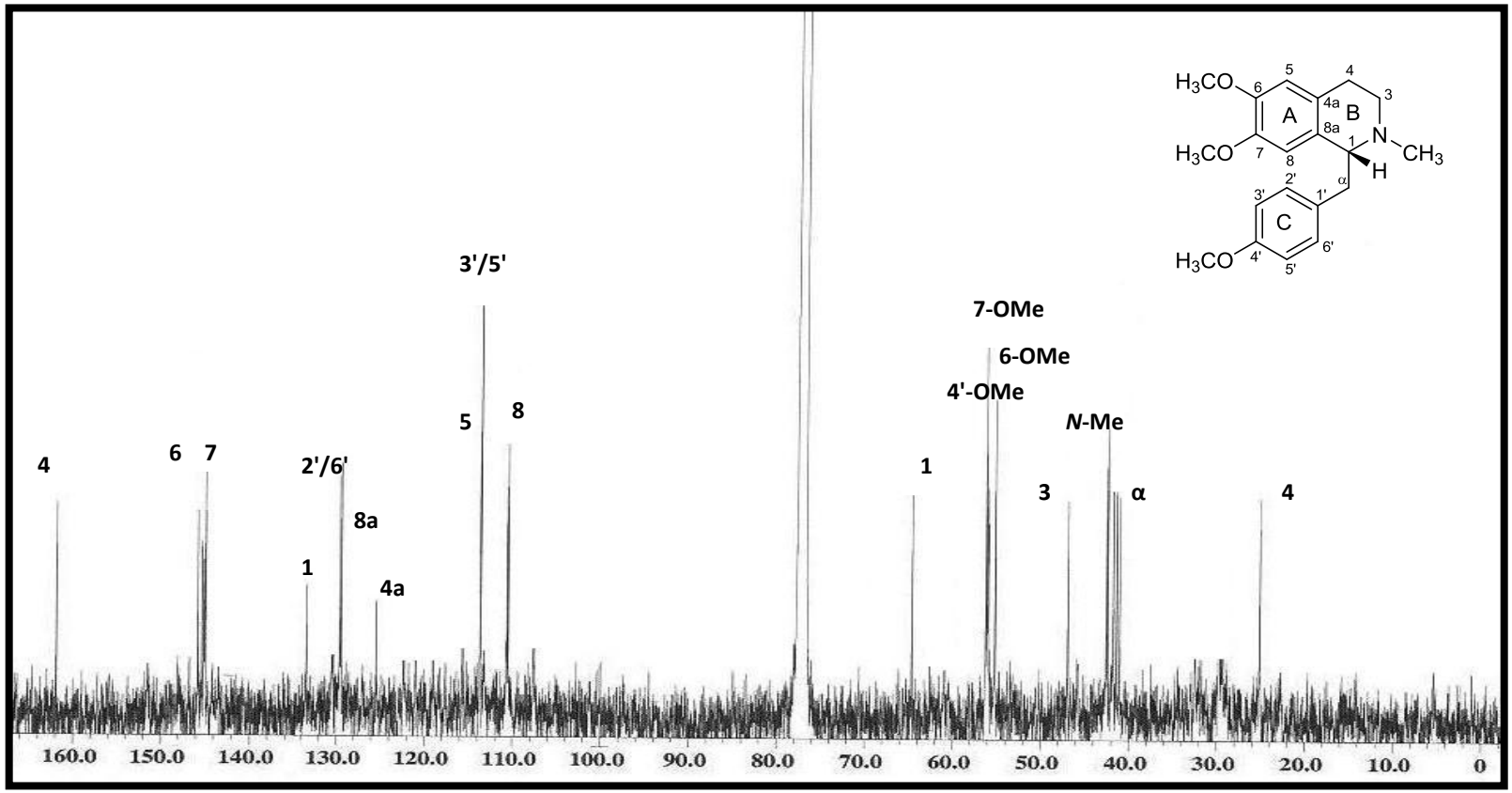


Figure 3.49: ^{13}C NMR Spectrum of *O*-methylarmepavine **73**

3.3.6 Actinodaphnine 53

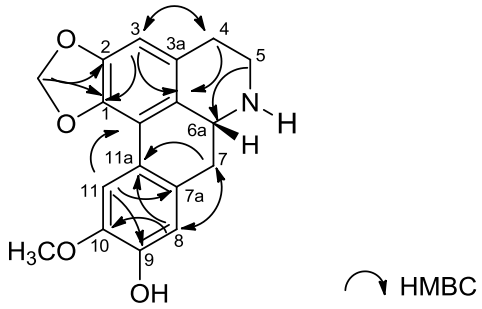
Actinodaphnine **53**, $[\alpha]_D^{27}$ = +18.2° (*c* 1.0, MeOH) was isolated as a brownish amorphous solid. The UV spectrum showed absorption bands typical of an aporphine at 221, 285 and 308 nm, due to the degree of resonance in the biphenyl system and indicated that this base is 1,2,9,10-substituted aporphine.[100,111]In addition, the IR spectrum showed broad band absorption at 3350 cm^{-1} due to the stretching of the hydroxyl group. Other absorption band was at 1580 cm^{-1} for the stretching of C-O. The LCMS-IT-TOF revealed a pseudomolecular ion peak, $[M+H]^+$ at *m/z* 312.12 consistent with the molecular formula of $C_{18}H_{18}NO_4$ with eleven degrees of unsaturation.

The ^{1}H NMR spectrum of actinodaphnine **53** showed a similar pattern with that of norboldine **75** in the aromatic region. In fact, the same substituents could be characterized in both compounds namely methoxyl group (C-10) and hydroxyl group at position C-9. However, the former spectrum peaks of one methoxyl group and *N*-methyl group in ^{1}H NMR spectrum at the range of δ 3.50 and 2.00-2.50 respectively. Instead, the additional doublet peak was observed at δ 6.01 with a coupling constant of 1.48 Hz in ^{1}H NMR spectrum of actinodaphnine **53**, corresponding to the replacement of 1,2-dimethoxyisoquinoline system in norboldine **75**.

The ^{13}C NMR spectrum showed 18 carbon signals and the DEPT 135 spectrum showed the appearance of one methyl, four methylenes and four methines signals. A signal of methoxyl group was observed at δ 56.1. In addition, four oxygenated aromatic carbon signals were observed at δ 146.6, 145.4, 145.1 and 141.5. The latter group signal was observed at δ 100.5 may be assigned to a methylenedioxyisoquinoline (O-CH_2-O) group.

The long range of ^{1}H-^{13}C correlations were observed in the HMBC experiment (Figure 3.50) allowed the assignment of carbon atoms and further confirmed the methylenedioxyisoquinoline type moiety. The HMBC spectrum showed correlations between H-3 (δ 6.45) to C-1 (δ 141.5), and methylenedioxy protons at δ 6.01 with the quaternary carbons (C-1 and C-2), thus confirmed the existence of a methylenedioxy (O-CH_2-O) group at C-1 and C-2. Other long range ^{1}H-^{13}C correlations were revealed correlations of H-8 (δ 6.72) to C-10 and C-11a, and OMe (δ 3.86) to C-10, suggesting attachment of methoxy group to C-10. Complete ^{1}H and ^{13}C NMR data of alkaloid **53** are listed in table 3.9.

From the analysis of the spectroscopic data obtained and comparison with the literature values, it was identified as actinodaphnine **53**.[110]



53

Figure 3.50: ^{1}H-^{13}C coupling pattern observed in HMBC spectrum of Actinodaphnine **53**

Table 3.9: ^{1}H NMR, ^{13}C NMR Data and HMBC Correlation for Actinodaphnine **53**

Position	^{1}H NMR (δ, Hz)	^{13}C NMR (δ)	HMBC
1		141.5	
1a		116.5	
1b		127.6	
2		146.6	
3	6.45, *s*	107.1	1, 1b, 2, 4
3a		126.8	
4	2.71, *m*	29.4	3, 3a
5	3.32, *m*	43.3	3a, 6a
6a	3.83, *d*, *J*=6.08	53.7	
7	2.94, *m*	36.4	1b, 6a, 7a, 11a
7a		130.0	
8	6.72, *s*	110.1	7, 10, 11a
9		145.4	
10		145.1	
11	7.58, *s*	114.2	1a, 7a, 9, 11a
11a		123.1	
10-OMe	3.86, *s*	56.2	10
1,2-OCH_2O	6.01, *d*, *J*=1.48	100.5	1, 2

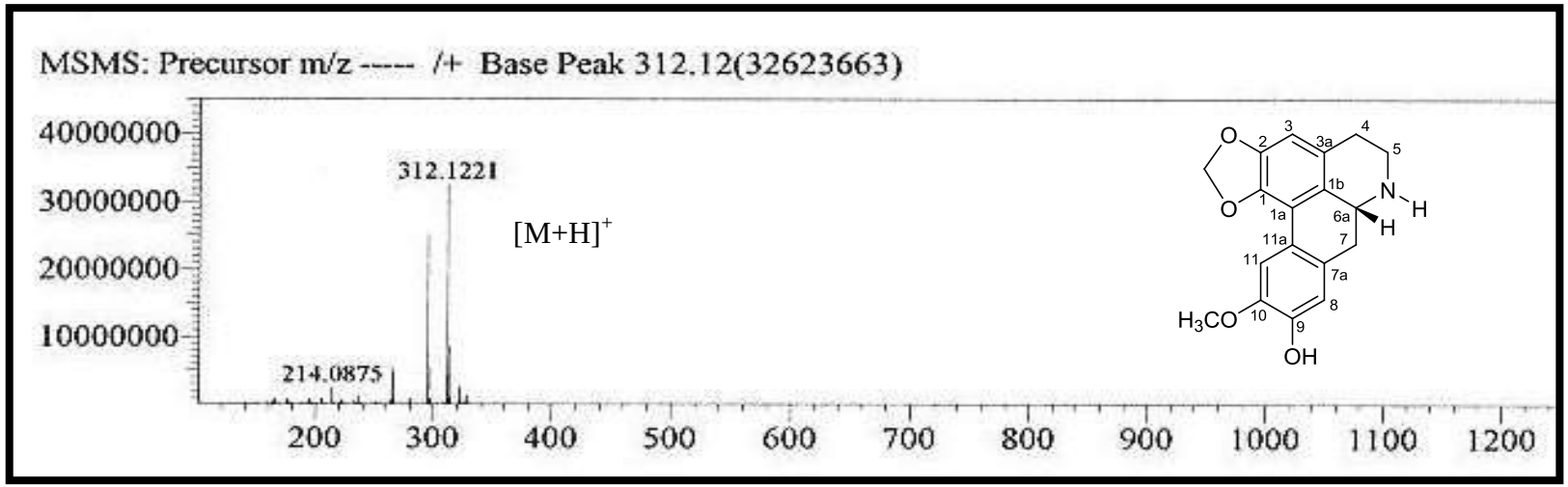


Figure 3.51: LCMS-IT-TOF Spectrum of Actinodaphnine **53**

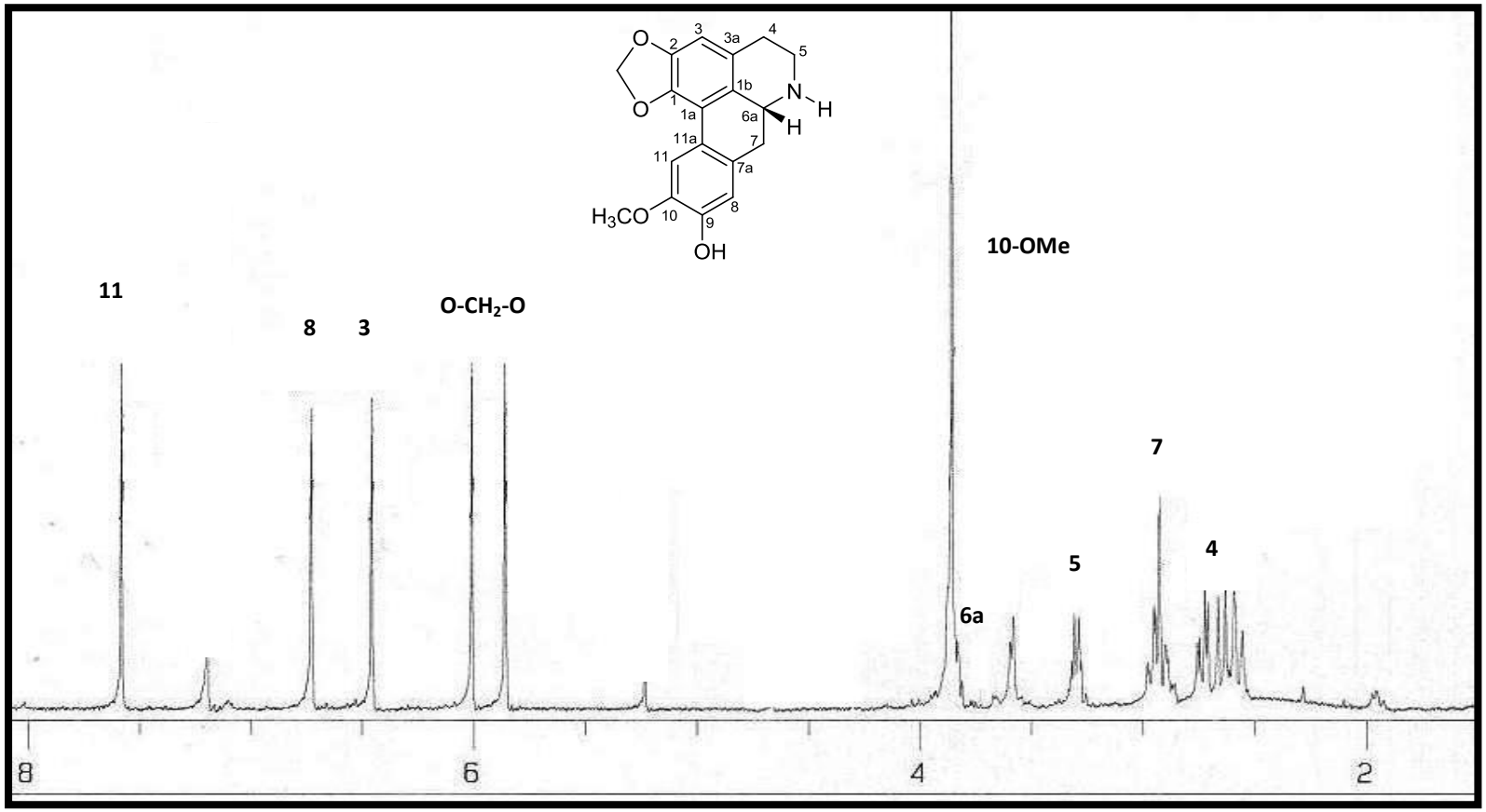


Figure 3.52: ^{1}H NMR Spectrum of Actinodaphnine **53**

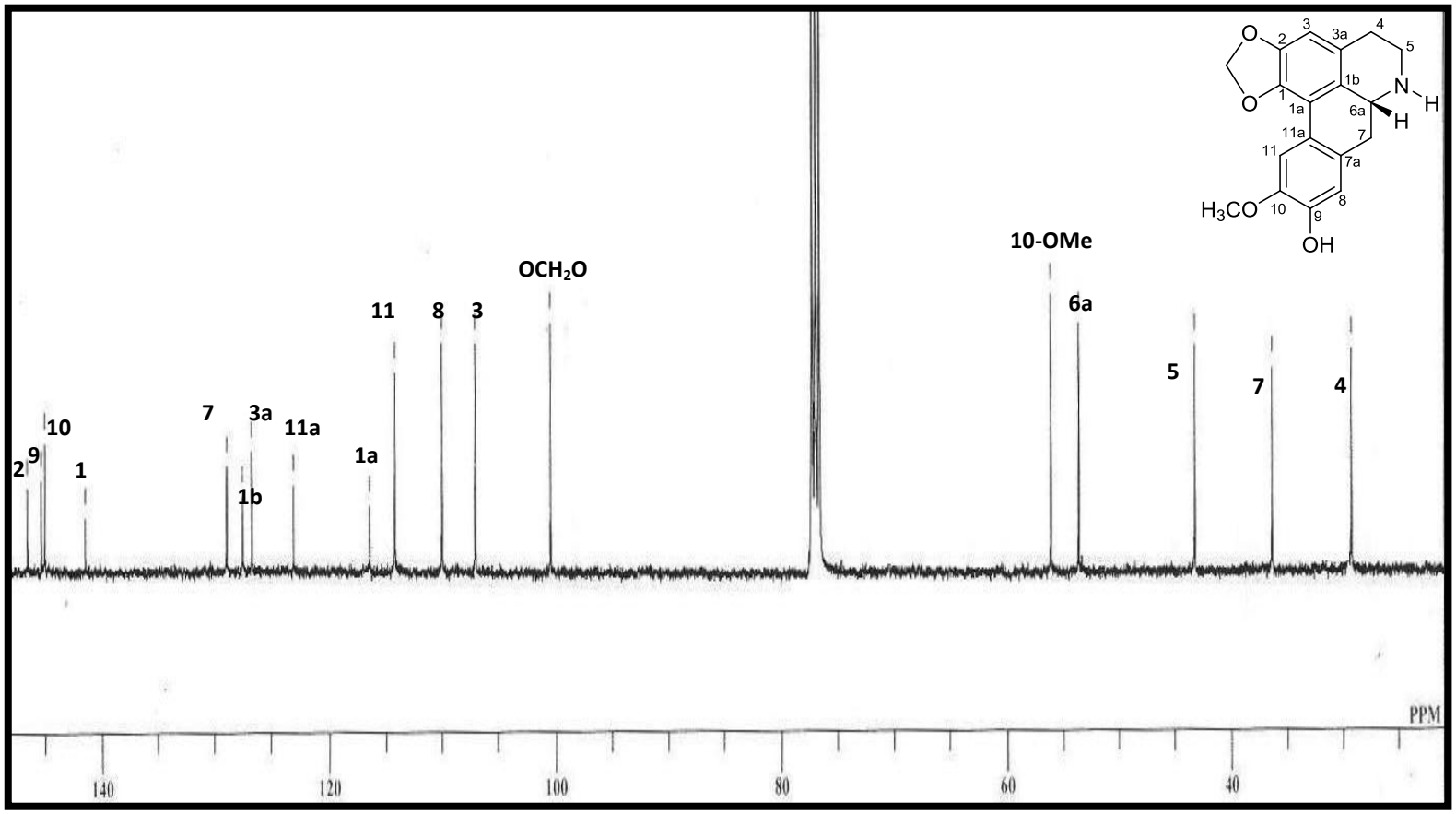


Figure 3.53: ^{13}C NMR Spectrum of Actinodaphnine **53**

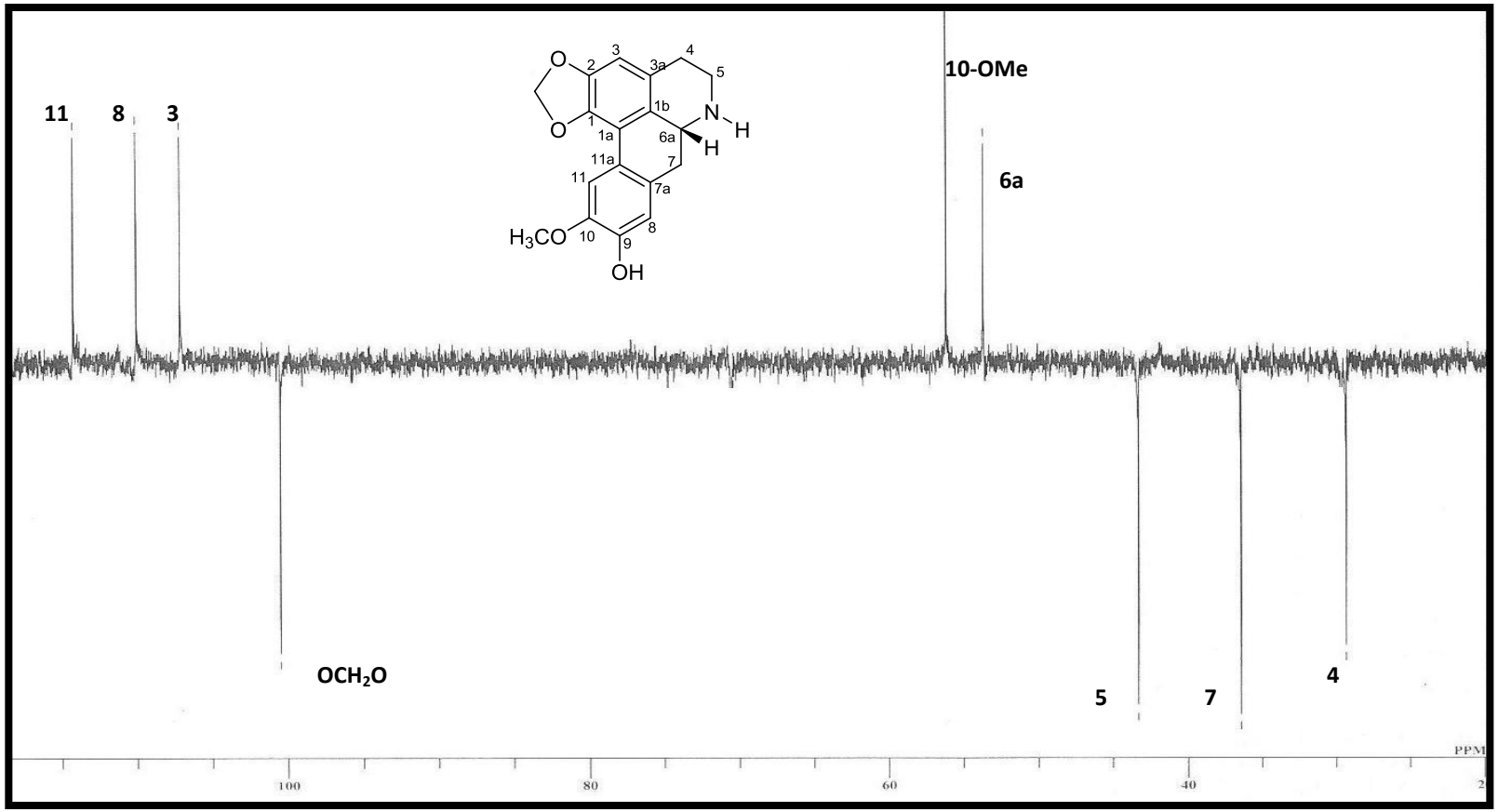


Figure 3.54: DEPT 135 NMR Spectrum of Actinodaphnine **53**

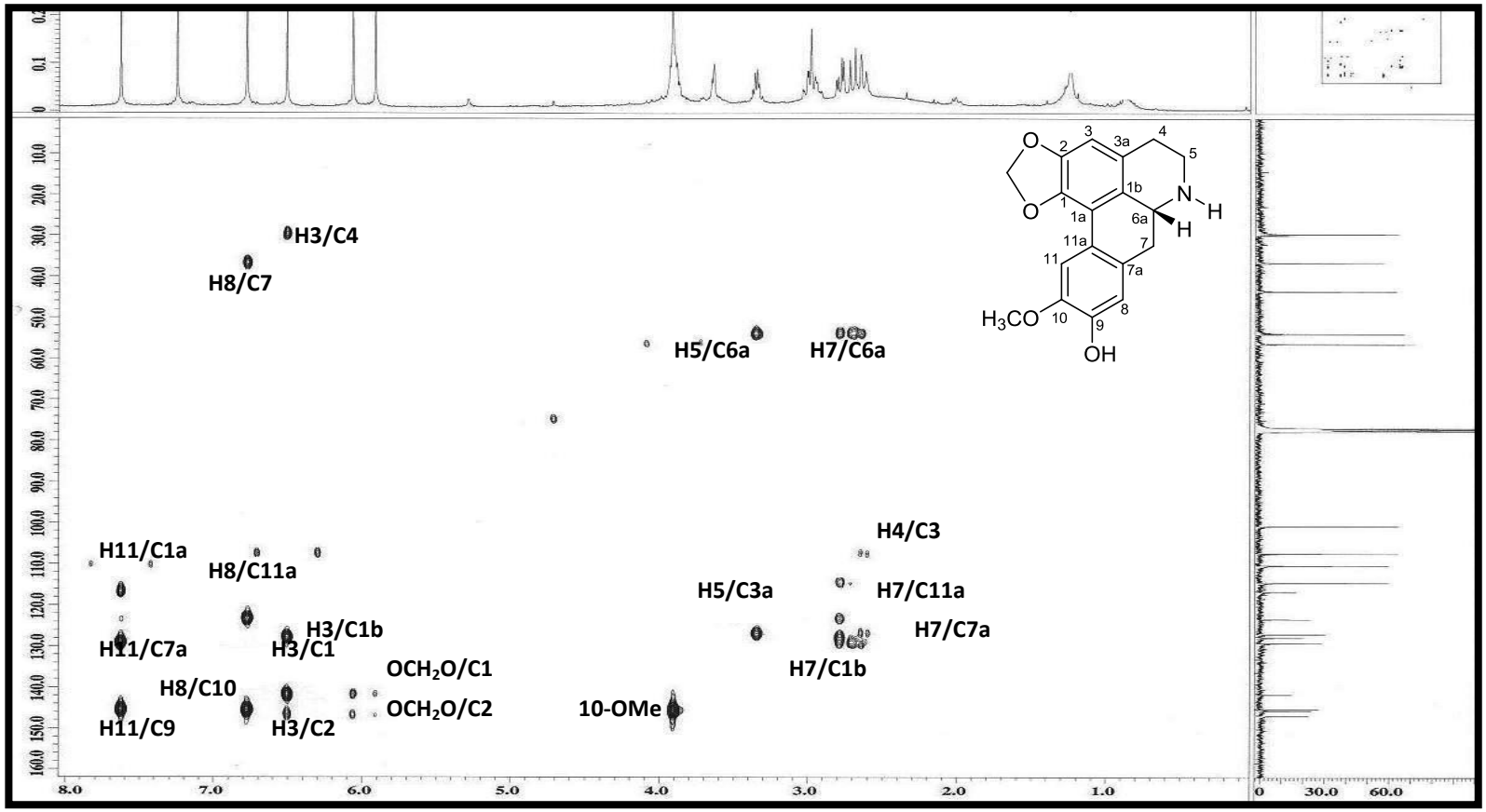


Figure 3.55: HMBC Spectrum of Actinodaphnine **53**

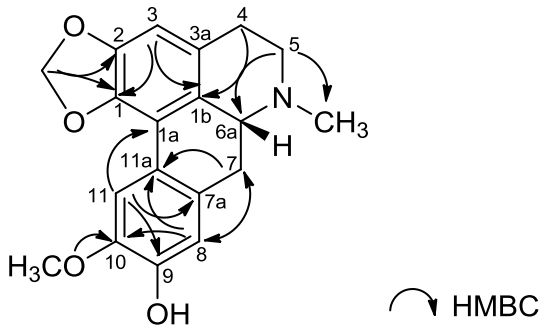
3.3.7 Cassythicine 74

Cassythicine **74**, $[\alpha]_D^{27}$ = +64.3º (*c* 1.0, MeOH) was isolated as a brownish amorphous solid. The UV spectrum showed absorption typical of an aporphine at 221, 285 and 308 nm.[111] In the IR spectrum, important absorption was noted at 3350 cm^{-1} for an OH group. The LCMS-IT-TOF spectrum revealed a pseudomolecular ion peak, $[M+H]^+$ at *m/z* 326.15 consistent with the molecular formula of $C_{19}H_{20}NO_4$ with eleven degrees of unsaturation.

The ^1H NMR and ^{13}C NMR spectra of this alkaloid were similar to the previous alkaloid (actinodaphnine **53**) which suggested a close structural relationship between these two compounds. In fact, the same substituents in rings A and D could be characterized in both compounds namely methylenedioxy group (C-1, 2), methoxyl group (C-10) and hydroxyl group (C-9). However, this alkaloid exhibited the *N*-methyl group signal in ^1H NMR and ^{13}C NMR spectra at δ 2.47 and 43.2 respectively.

The presence of a methylenedioxyisoquinoline (O-CH_2-O) carbon was further supported with the occurrence of a low field peak at δ 100.9, which was seen to correlate with C-1 and C-2 in the HMBC spectrum. The exact position of methoxyl, hydroxyl and *N*-methyl group was also determined by the correlation observed in the HMBC spectrum (Figure 3.62). The connectivity's of methoxyl was confirmed by ^3J interaction observed between its signal at δ 3.86 and quaternary aromatic carbon at δ 145.50 (C-10) in the HMBC spectrum. Meanwhile, correlation of *N*-methyl group was observed between its signal at δ 2.47 and quaternary aromatic carbon at δ 53.5 (C-5) and 62.4 (C-6a). In addition, the position of hydroxyl group at C-9 was confirmed with the correlation of H-11 to C-1a, C-7a and C-9.

Complete ^{1}H and ^{13}C NMR assignments were listed in the Table 3.10. Comparison of these spectral data with the literature,[110] led to the assignment of alkaloid **74** identified as cassythicine.



74

Figure 3.56: ^{1}H-^{13}C coupling pattern observed in HMBC spectrum of Cassythicine **74**

Table 3.10: ^{1}H NMR, ^{13}C NMR Data and HMBC Correlation for Cassythicine **74**

Position	^{1}H NMR (δ, Hz)	^{13}C NMR (δ)	HMBC
1		142.1	
1a		117.0	
1b		126.3	
2		146.6	
3	6.44, *s*	106.9	1, 1b, 2
3a		126.3	
4	3.04, *m*	28.5	1b, 5
5	2.98, *m*	53.5	6a
6a	3.08, *m*	62.4	
7	2.95, *m*	33.8	8, 11a
7a		128.8	
8	6.86, *s*	114.5	7, 10, 11a
9		145.8	
10		145.5	
11	7.57, *s*	110.0	1a, 7a, 9
11a		123.0	
10-OMe	3.86, *s*	56.3	10
N-Me	2.47, *s*	43.2	5, 6a
1,2-OCH_2O	6.01, *d*, *J*=1.24	100.9	1, 2

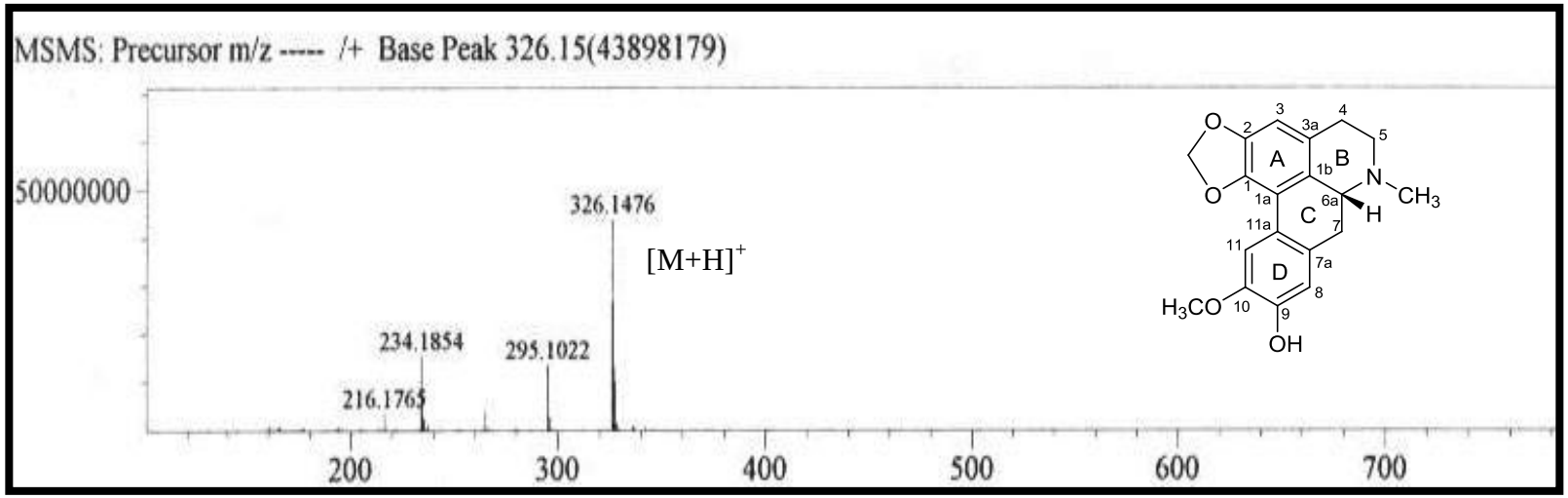


Figure 3.57: LCMS-IT-TOF Spectrum of Cassythicine **74**

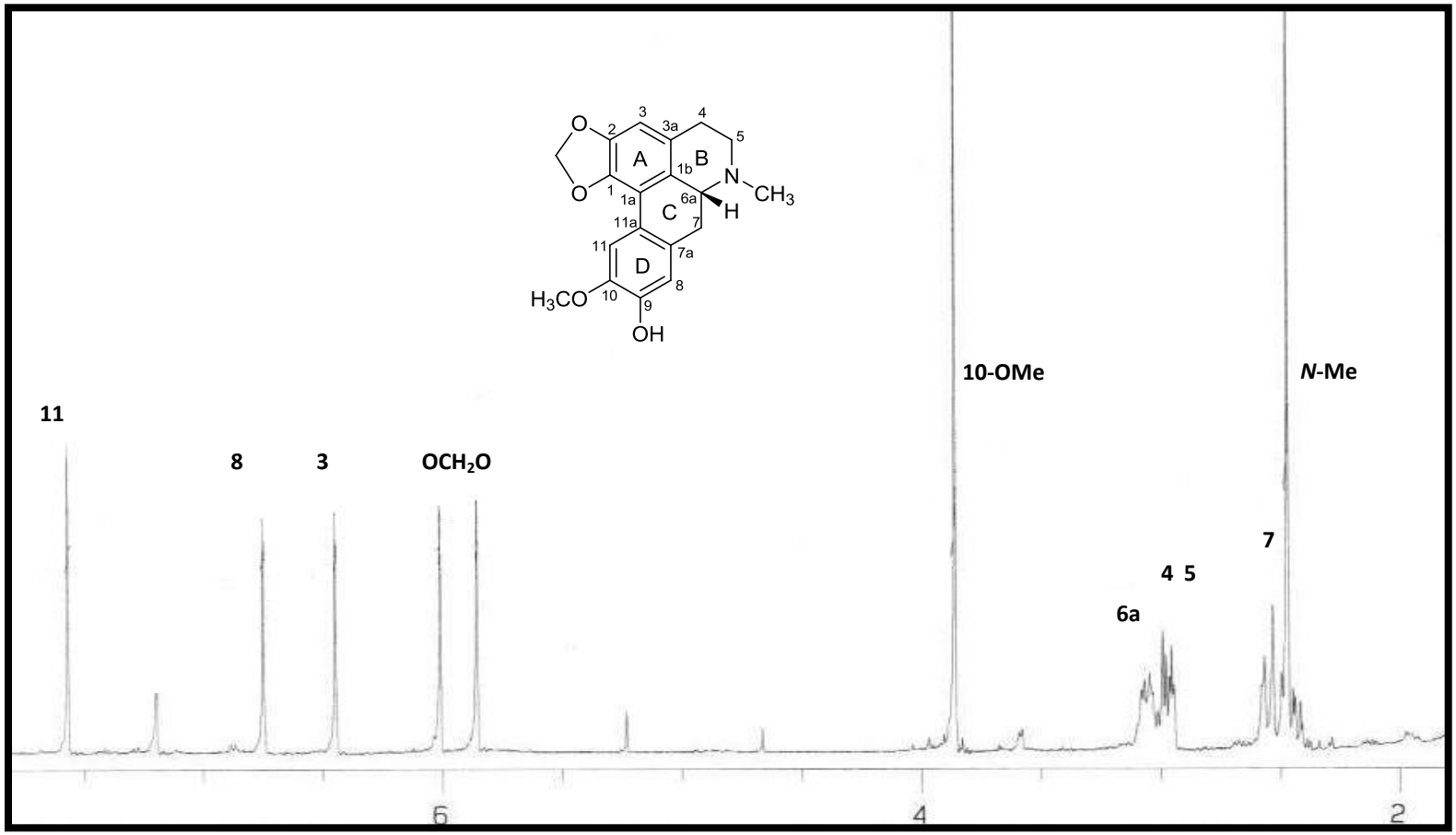


Figure 3.58: ^{1}H NMR Spectrum of Cassythicine **74**

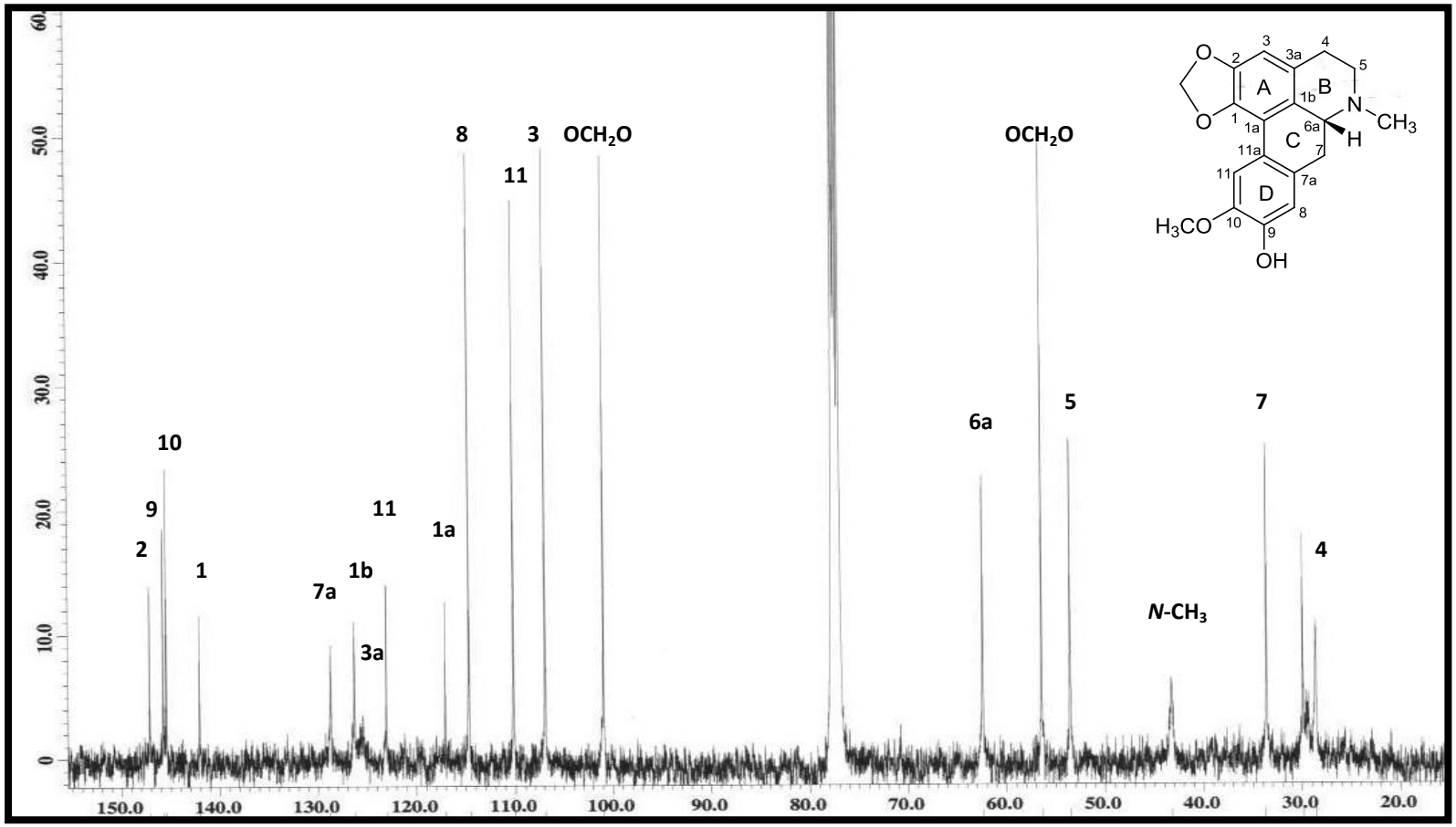


Figure 3.59: ^{13}C NMR Spectrum of Cassythicine **74**

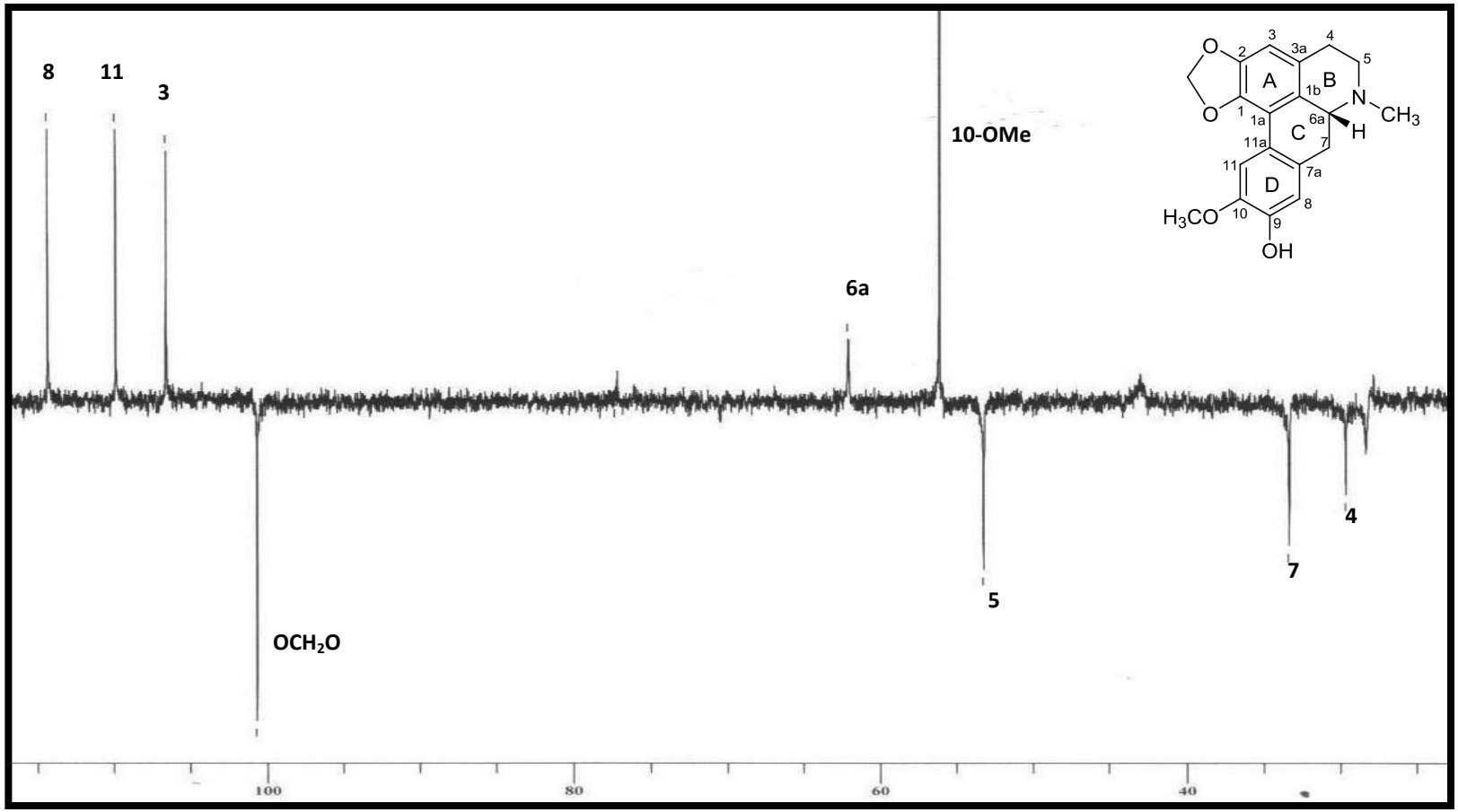


Figure 3.60: 135 DEPT NMR Spectrum of Cassythicine **74**

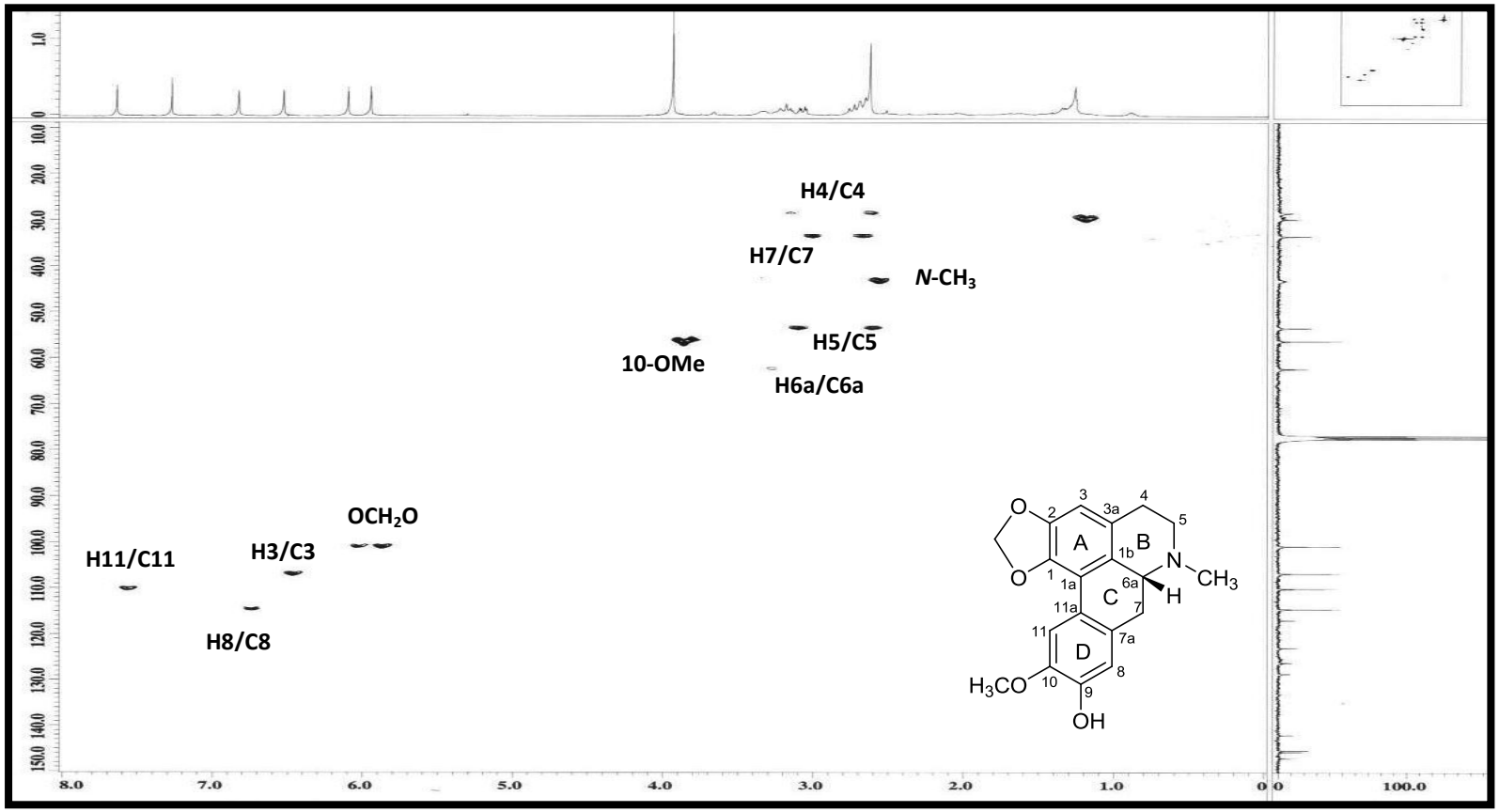


Figure 3.61: HSQC Spectrum of Cassythicine **74**

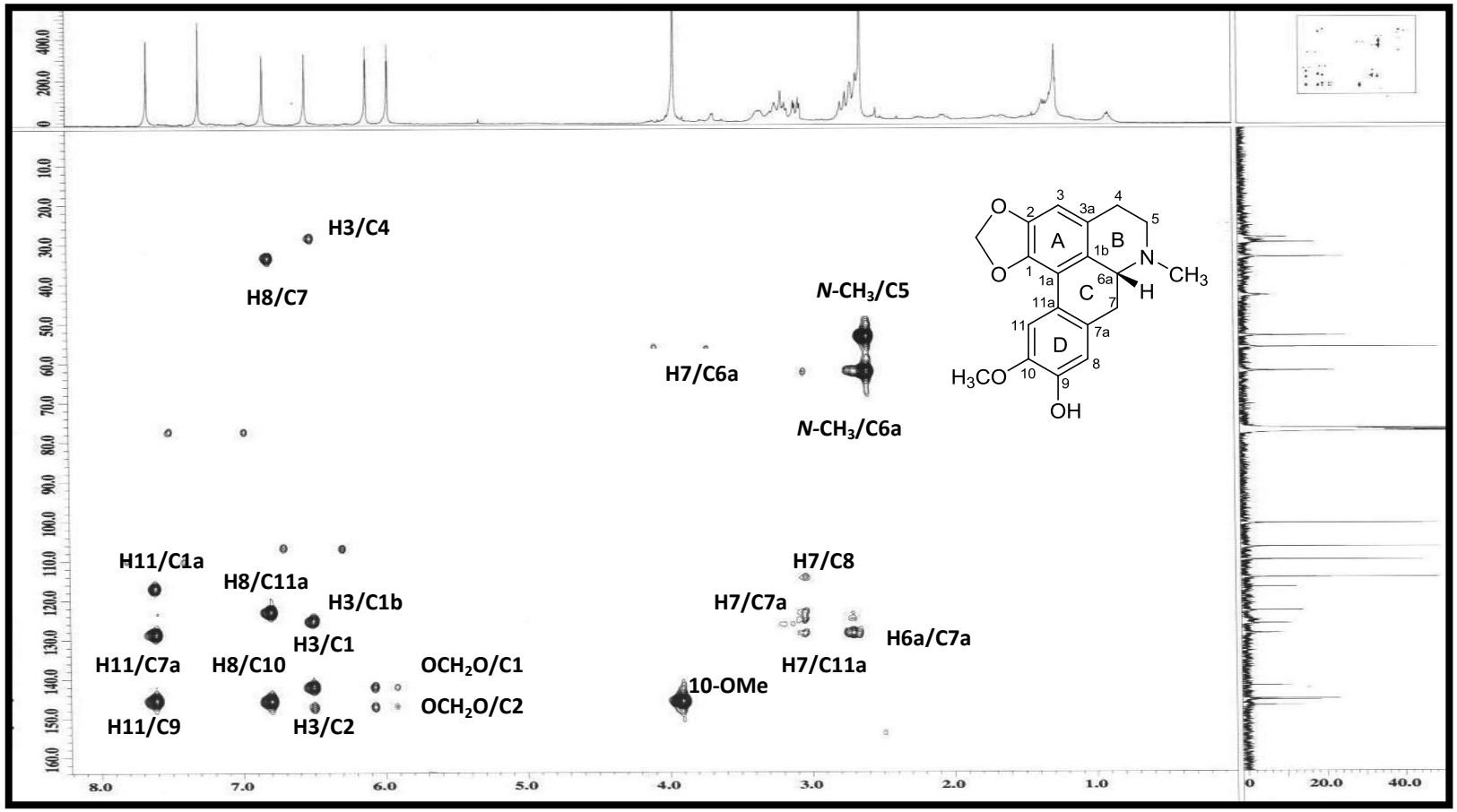


Figure 3.62: HMBC Spectrum of Cassythicine **74**

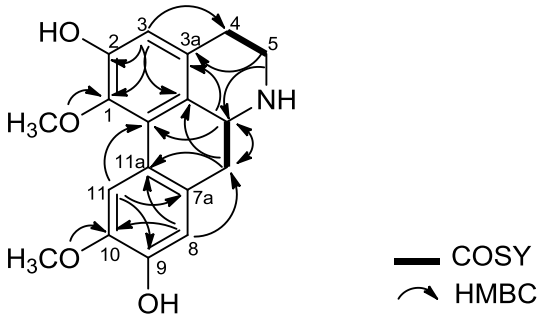
3.3.8 Norboldine 75

Norboldine **75**, $[\alpha]_D^{27}$ = +33.3º (*c* 1.0, MeOH) was isolated as a brownish amorphous solid. The UV spectrum showed typical absorption of an aporphine at 282 and 302 nm.[100] The IR spectrum showed absorption bands at 3288 and 2357 cm^{-1} indicated the streching of NH and OH group. Its IR spectrum also showed a strong band at 2932 cm^{-1} due to the stretching of CH aromatic, respectively. The LCMS-IT-TOF revealed a pseudomolecular ion peak, $[M+H]^+$ at *m/z* 314.14 consistent with the molecular formula of $C_{18}H_{20}NO_4$.

The ^1H NMR and ^{13}C NMR spectra of norboldine **75** were almost similar to boldine **55**, which suggested a close structural relationship between these two compounds. Indeed, the same substituent could be characterized in both compounds namely methoxyl groups (C-1 and C-10) and hydroxyl groups at position C-2 and C-9. However, norboldine **75** exhibited the absent of *N*-methyl group signal in ^1H NMR and ^{13}C NMR spectra at range of δ 2.00-2.50 and 44.0-45.0 respectively.

In the HMBC spectrum in Figure 3.69 revealed correlation of H-11 to C-7, C-8, C-1a, C-7a and C-9, further supported the position of OH group at C-9. The correlation of H-3 to C-1 which bearing a methoxyl group was also observed. In addition, the exact positions of two methoxyl groups was confirmed by ^3J interaction observed between signal at δ 3.57 and quaternary aromatic carbon at δ 142.1 (C-1); and between signal at δ 3.89 and quaternary aromatic carbon at δ 145.6 (C-10). Other significant correlations were shown in Figure 3.63. Complete ^1H and ^{13}C NMR assignments for norboldine **75** were made by analysis of 2D NMR spectra (HMQC and HMBC) are listed in the table 3.11.

From the analysis of the spectroscopic data obtained and comparison with the literature values, it was identified as norboldine **75**.[34]



75

Figure 3.63: ^{1}H-^{1}H and ^{1}H-^{13}C coupling pattern observed in COSY and HMBC spectrum of norboldine **75**

Table 3.11: ^{1}H NMR, ^{13}C NMR Data and HMBC Correlation for Norboldine **75**

Position	^{1}H NMR (δ, Hz)	^{13}C NMR (δ)	HMBC
1		142.1	
1a		126.4	
1b		125.9	
2		148.2	
3	6.61, *s*	113.3	1, 1b, 2, 4
3a		129.6	
4	2.96, *m*	28.6	3a, 5
5	3.12, *m*	53.2	3a, 4, 6a
6a	3.81, *m*	62.5	1a, 1b, 7
7	3.03, *m*	33.9	1b, 6a, 11a
7a		129.9	
8	6.80, *s*	114.3	7, 10, 11a
9		145.1	
10		145.6	
11	7.87, *s*	110.2	1a, 7, 7a, 8, 9
11a		123.5	
1-OMe	3.57, *s*	60.2	1
10-OMe	3.89, *s*	56.1	10

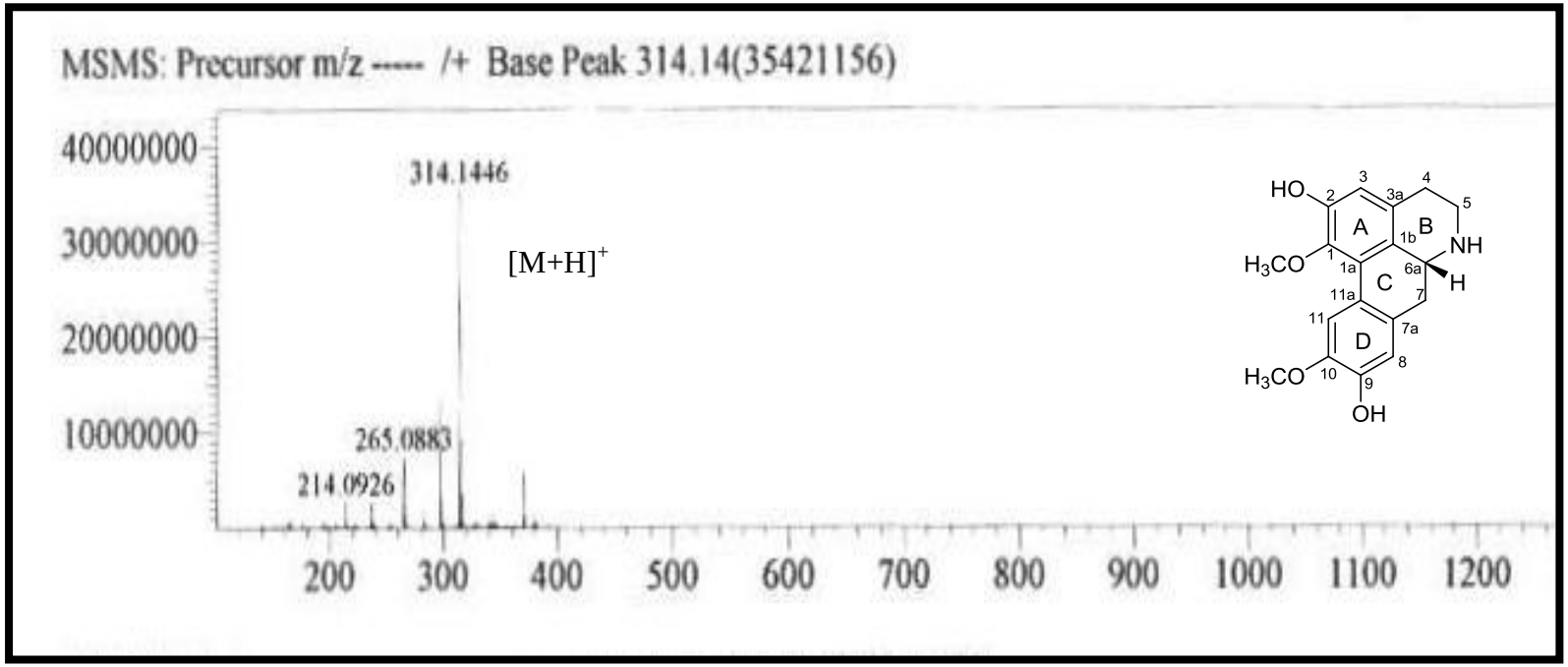


Figure 3.64: LCMS-IT-TOF Spectrum of Norboldine **75**

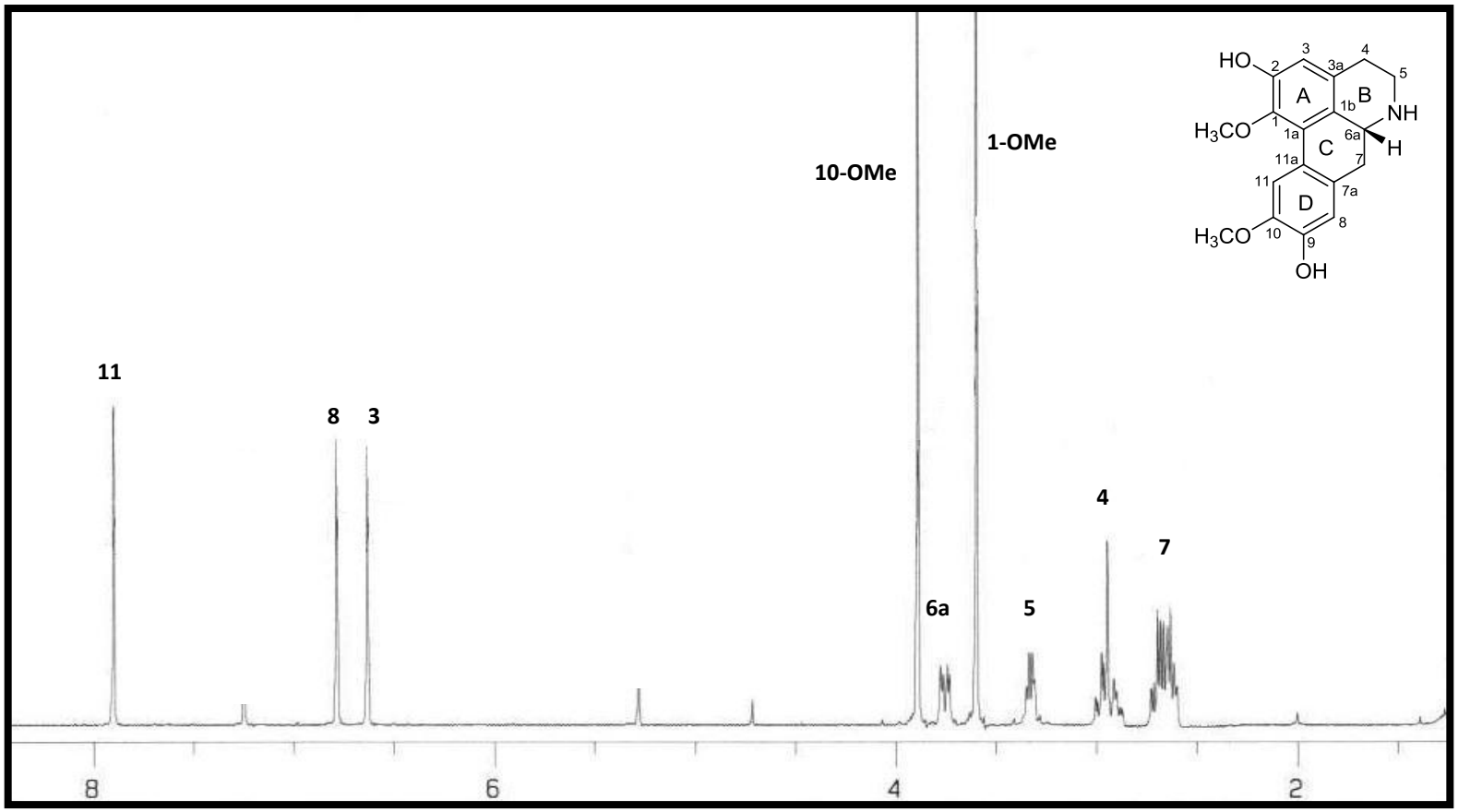


Figure 3.65: ^{1}H NMR Spectrum of Norboldine **75**

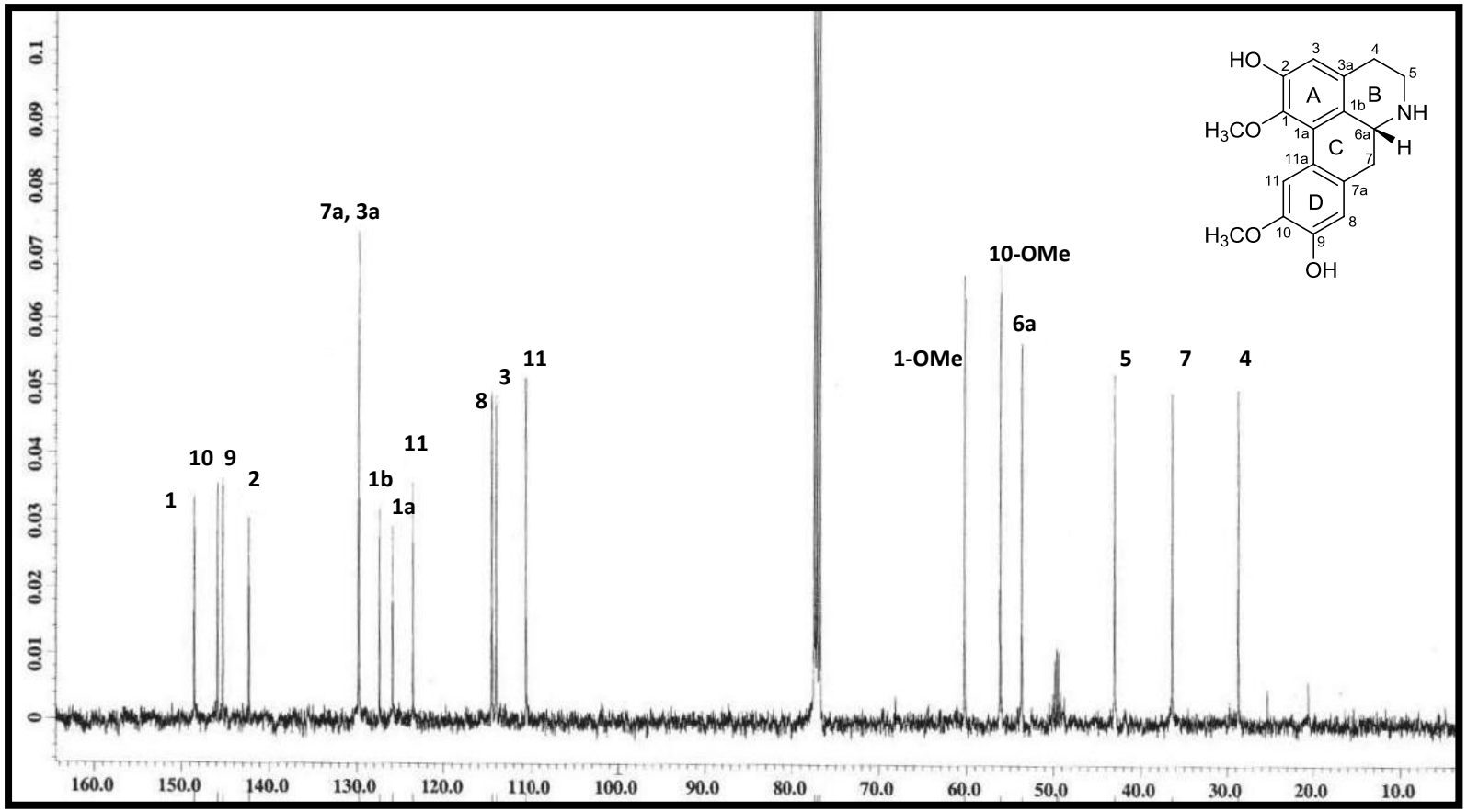


Figure 3.66: ^{13}C NMR Spectrum of Norboldine **75**

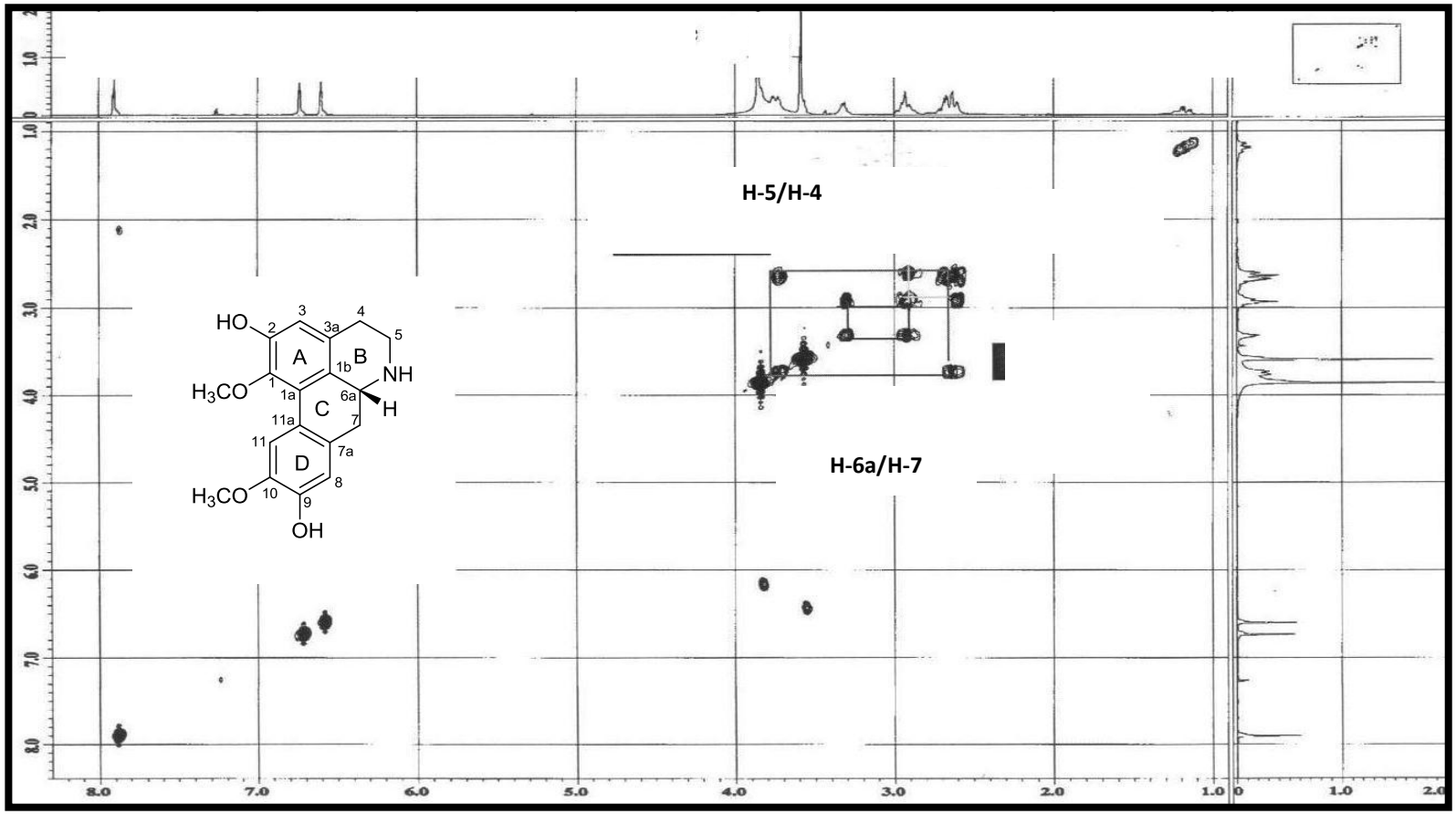


Figure 3.67: COSY Spectrum of Norboldine **75**

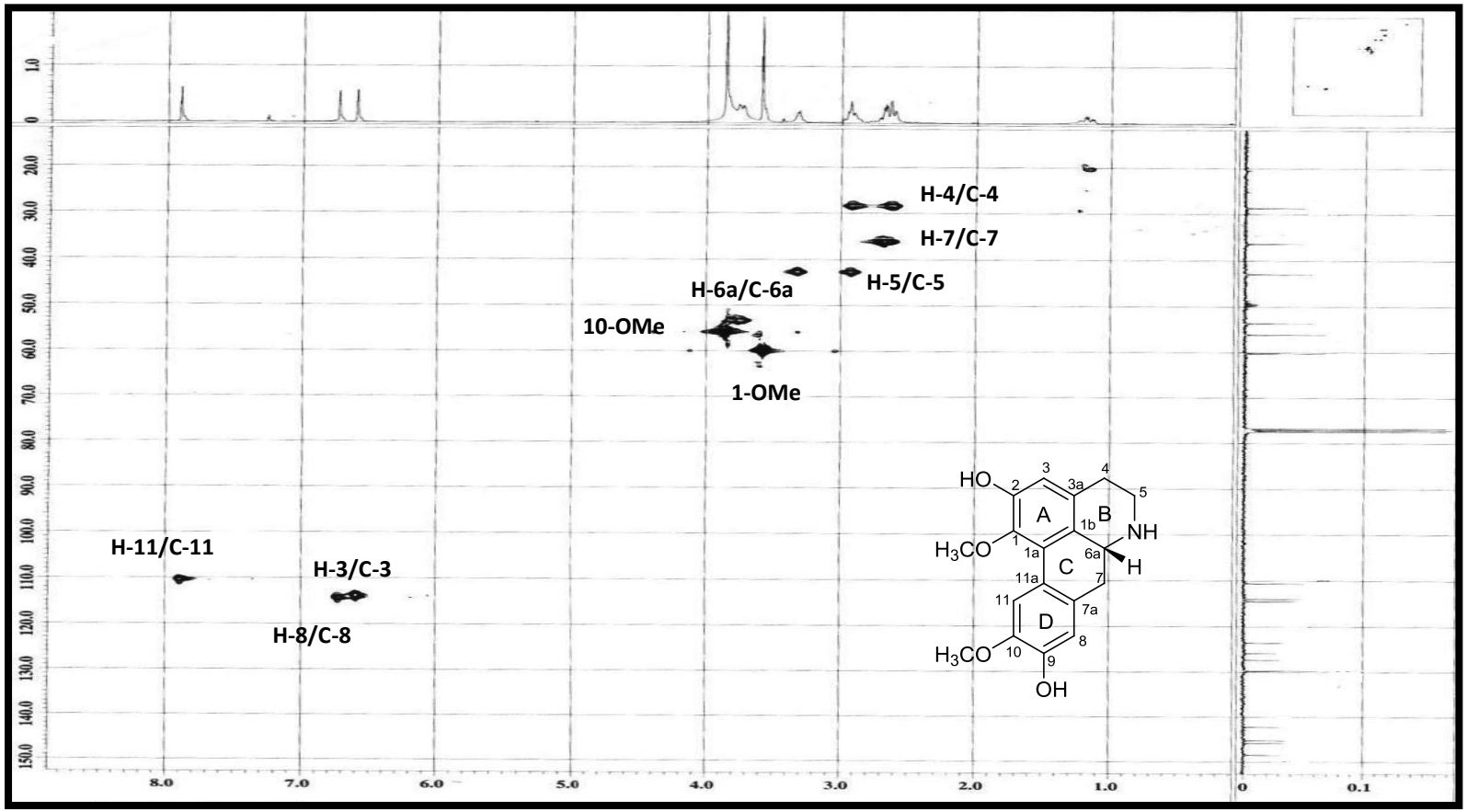


Figure 3.68: HSQC Spectrum of Norboldine **75**

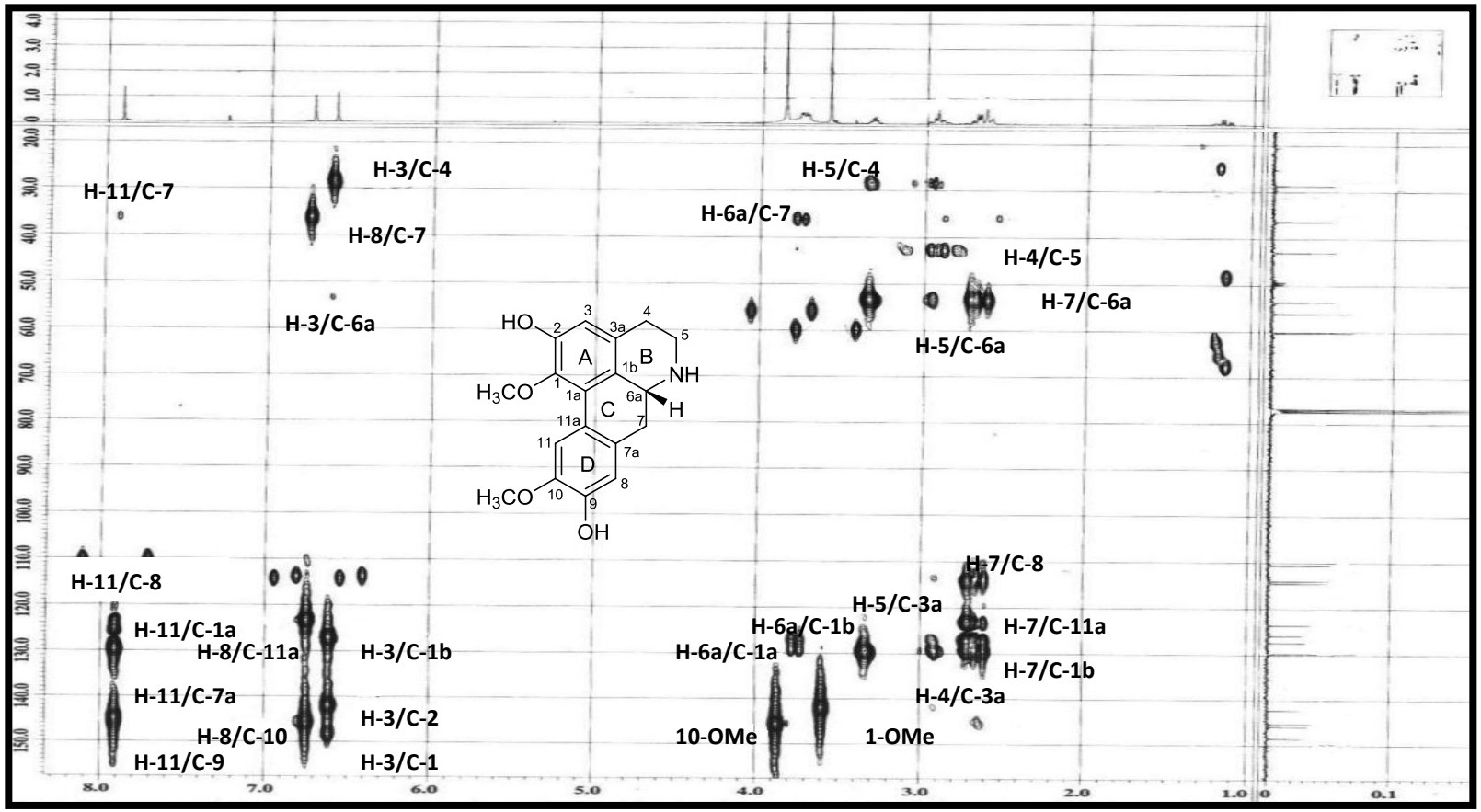


Figure 3.69: HMBC Spectrum of Norboldine **75**

CHAPTER 4

VASORELAXANT ACTIVITY

4.1 General

In the year 2000 it is estimated that nearly one billion people or ~26% of the adult population had hypertension worldwide.[112] The American Heart Association estimates high blood pressure affects approximately one in three adults in the United State of America and about 73 million peoples were affected. High blood pressure or hypertension means high pressure in the arteries. Arteries are vessels that carry blood from the pumping heart to all the tissues and organs of the body.

Before pharmacological treatment for hypertension became possible, three treatment modalities were used, all with numerous side-effects: strict sodium restriction, sympathectomy (surgical ablation of parts of the sympathetic nervous system), and pyrogen therapy (injection of substances that caused a fever, indirectly reducing blood pressure).[113]

Therefore a biological assay; vasolidator was useful for safe treatment of cerebral vasospasm and hypertension, and for improvement of peripheral circulation.[114]

4.2 Vasorelaxant Effects on Isolated Rat Aorta

This study was to examine the action of the compounds on the smooth muscles of the rat aorta and to elucidate the mechanism of the relaxant effect. These animal experimental studies were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University and under the supervision of the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports Culture, and Technology of Japan.

4.3 Preparation of the Aorta Strips[115]

A male Wistar rat weighting 260 g was sacrificed by bleeding from carotid arteries under an anesthetization. A section of the thoracic aorta between the aortic arch and the diaphragm was removed and placed in oxygenated, modified Krebs-Henseleit solution (KHS: 118.0 mM NaCl, 4.7 mM KCl, 25.0 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, and 11.0 mM glucose). The aorta was cleaned of loosely adhering fat and connective tissue and cut into ring preparations 3 mm in length. The tissue was placed in a well-oxygenated (95% O_2, 5% CO_2) bath of 5 mL KHS solution at 37 °C with one end connected to a tissue holder and the other to a force-displacement transducer (Nihon Kohden, TB-611T). The tissue was equilibrated for 60 min under a resting tension of 1.0 g. During this time the KHS in the tissue bath was replaced every 20 min.

After equilibration, each aortic ring was contracted by treatment with 3×10^{-7} M PE. The presence of functional endothelial cells was confirmed by demonstrating relaxation to 10^{-5} M acetylcholine (ACh), and aortic ring in which 80% relaxation occurred, were regarded as tissues with endothelium. When the PE-induced contraction reached a plateau, each sample was added.

4.4 Results and Discussion

When phenylephrine (PE) 3×10^{-7} M was applied to thoracic aortic rings with endothelium after achieving a maximal response, we added three major alkaloids isolated from *Litsea lancifolia*; a new bisbenzylisoquinoline alkaloid, lancifoliaine **71**, a new natural products *N*-allyllaurolitsine **70**, and reticuline **67** as a related benzylisoquinoline alkaloid. *N*-allyllaurolitsine **70** showed a moderate vasorelaxant activity on isolated rat aorta (85% relaxation at 1×10^{-4}M), whereas lancifoliaine **71** and reticuline **67** did not show any

significant vasorelaxant activity (30% relaxation at 1×10^{-4}M). The same vasorelaxant activities of some bisbenzylisoquinoline alkaloids such as α′-oxoperakensimines A–C from *Alseodaphne perakensis* and *A. corneri* have been reported.[116,117] Incidently, both *Litsea* and *Alseodaphne* belong to the family of Lauraceae. Vasodilation activity of the bisbenzylisoquinoline alkaloids of the latter species may seem to be influenced by the asymmetric chirality of C-1 while the latter species seems to be influenced by substitution of a nitrogen atom. The results were shown in Table 4.1 and Figure 4.1.

Table 4.1: Relaxation Response on 3 x 10^{-7}M PE Induced Contraction of Rat Aorta

Compounds/concentration (10^{-4})	Time of Incubation				
	10 min	20 min	30 min	40 min	50 min
Lancifoliaine **71**	27.4	39.4	46.7	47.8	46.9
N-allyllaurolitsine **70**	72.9	83.3	84.9	85.3	83.7
Reticuline **67**	33.7	28.3	28.9	29.3	27.9

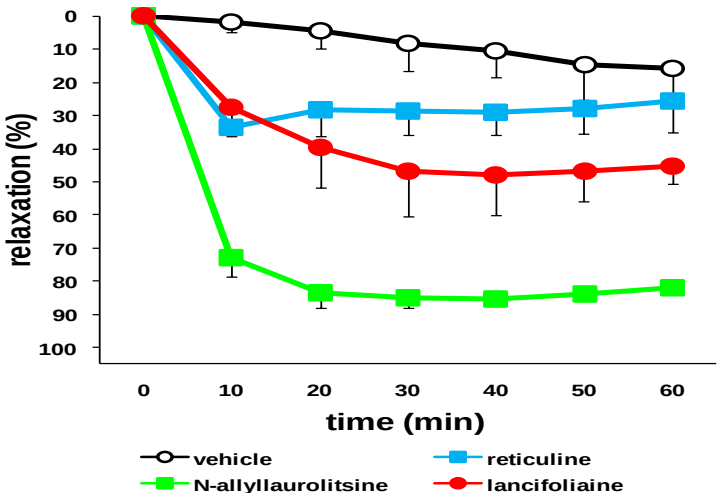


Figure 4.1: Relaxation responses induced by lancifoliaine (**71**; 10^{-4}M), *N*-allyllaurolitsine (**70**; 10^{-4}M), and reticuline (**67**; 10^{-4}M) in aortic rings precontracted with 3×10^{-7} M phenylephrine (PE). Values are the mean ± S.E (n=3)

CHAPTER 5

CONCLUSION

CONCLUSION

Two plants species from the family of Lauraceae were studied; *Litsea grandis* (leaves) and *Litsea lancifolia* (bark). The species were collected from two different localities; *Litsea grandis* (KL 5236) - Hutan Simpan Bukit Berangi, Sintok, Kedah and *Litsea lancifolia* (KL 5208) - Hutan Simpan Tembat Ulu Terengganu, Terengganu. The alkaloidal screening of the leaves of *Litsea grandis* and the barks of *Litsea lancifolia* has shown the presence of an alkaloid by giving the positive Mayer test. Thus, these two parts of the species have been chosen and phytochemical studies on these species led to isolation of four types of alkaloids namely aporphine, morphine, benzylisoquinoline and also bisbenzylisoquinoline as shown in Table 5.1.

The chemical study on the leaves of *Litsea grandis* gave three alkaloids; one aporphine (laurotetanine **69**) and two benzylisoquinoline alkaloids (reticuline **67,** *N*-methylisococlaurine **68)**.

The investigation of the bark of *Litsea lancifolia* afforded a known benzylisoquinoline; *O*-methylarmepavine **73**; four known aporphines; boldine **55**, actinodaphnine **53**, cassythicine **74** and norboldine **75**; one morphinandienone type; pallidine **72**; one new natural product compound; *N*-allyllaurolitsine **70**; and one new bisbenzylisoquinoline; lancifoliaine **71**. The structures of all these alkaloids were determined by the extensive spectroscopic methods namely 1D-NMR (^{1}H, ^{13}C, DEPT) and 2D-NMR(COSY, HMQC, HMBC, NOESY), UV, IR, LCMS-IT-TOF and NMR data, and the comparison with previously reported data in the literature.

To the knowledge of the author, the new compound; lancifoliaine **71** was the first occurrence of bisbenzylisoquinoline alkaloid in the *Litsea* species. In fact, this was the

second report on bisbenzylisoquinoline alkaloid (lancifoliaine **71)** with both α and α' positions oxidized forming carbonyl groups. The first related compound was reported previously as a synthetic compound, 1,1′-[oxybis(p-phenylenecarbonyl)]bis[3,4-dihydro-6,7-dimethoxy-isoquinoline]. Meanwhile, a new natural product compound; *N*-allyllaurolitsine **70** was synthesized previously by Chiou *et al.* using norboldine as a starting material. The first natural product compound, (+)-*N*-(2-hydroxypropyl)lindcarpine having the similar number of carbon attached to an *N* atom was reported by Tiah *et al.* from the species of *Actinodaphne pruinosa* Ness (Lauraceae), and this new *N*-allyllaurolitsine **70** could be formed by the dehydration of *N*-isopropyl alcohol moiety of *N*-(2-hydroxypropyl)laurolitsine.

Vasorelaxant activity study revealed that the new bisbenzylisoquinoline alkaloid, lancifoliaine **71** and reticuline **67** from the bark of *Litsea lancifolia*; did not show any significant vasorelaxant activity (30% relaxation at 1×10^{-4}M). Meanwhile, the new natural product compound, *N*-allyllaurolitsine **70** showed a moderate vasorelaxant activity on isolated rat aorta (85% relaxation at 1×10^{-4}M).

In conclusion, the study of alkaloids from the species of *Litsea grandis* and *Litsea lancifolia* has yielded eleven alkaloids of which two are new alkaloids. These species can be a source of pharmacological interesting molecules. In connection with this, more biological activities will be performed on the isolated alkaloids.

CHAPTER 6

EXPERIMENTAL

6.1 Instrumentation

The (^{1}H, ^{13}C and 2D) NMR spectra were obtained using JEOL LA 400 FT NMR and JEOL ECA 400 FT NMR spectrometers using deuterated chloroform as solvent. The chemical shifts were reported in δ ppm and the coupling constants are given in the Hz.

Mass spectra were obtained by using Shimadzu LCMS-IT-TOF spectrometer. Meanwhile, UV spectra were obtained by using Shimadzu UV-250 uv-visible spectrophotometer with methanol as solvent.

In addition, the infrared spectra were obtained with methanol as solvent on a Perkin Elmer 16000 spectrometer and for optical rotation JASCO (J-815) polarimeter was used.

6.2 Chromatographies

6.2.1 Thin Layer Chromatography (TLC)

Aluminium supported silica gel 60 F_{254} TLC plates were used to visualized isolated compounds based on the spot of TLC. TLC spots were visualize under ultra-violet light (245-365 nm) using the model UVGL-58 after spraying with the required reagents.

6.2.2 Column Chromatography (CC)

The solvents used in this experiment were hexane, dichloromethane, chloroform, acetone and methanol. All solvents are AR grade except those used for bulk extractions distilled which were from technical grade solvent. Silica gel 60, 70-230 mesh ASTM (Merck 7734) was used for column chromatography. A slurry of silica gel 60 (approximately ratio of 30:1, silica gel: sample) in dichloromethane 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.

6.2.3 Preparative Thin Layer Chromatography (PTLC)

PTLC silica gel 60 F_{254} glass plates of size 20x20 cm were 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.

6.3 Reagents

6.3.1 Mayer's Test (Potassium Mercuric Iodide)

For Mayer's test, 1.4 g mercuric iodide in 60 ml distilled water is 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 2-3 drops of Mayer's reagent.

6.3.2 Dragendorff's Reagent (Potassium Bismuth Iodide)

Bismuth (III) nitrate (0.85 g) in a mixture glacial acetic acid (10 ml) and distilled water (40 ml) for solution A. While for solution B; Potassium iodide (8.0 g) dissolves in distilled water (20 ml). To prepare the stock solution, solution A and B mixed with equal volumes. The stock solution (20 ml) then was diluted in the mixture acetic acid (20 ml) and distilled water (60 ml) for spray agent. A positive result was indicated by the formation of orange spots.

6.4 Plant Materials

The plant materials were collected and identified by the phytochemistry group of the Chemistry Department University of Malaya. The locality of the plant species is shown in the Table 6.1 below.

Table 6.1: Plant species and locality

Herbarium Specimen No.	Species	Part of Plant	Locality and Date Collected
KL 5236	*Litsea grandis*	Leaf	Hutan Simpan Bukit Berangi, Sintok, Kedah, Malaysia. 28 April 2006.
KL 5208	*Litsea lancifolia*	Bark	Hutan Simpan Tembat Ulu Terengganu, Malaysia. 9 March 2006.

6.5 Extraction of Plant Material

Plant extractions were carried out by cold percolation or exhaustive extraction using the soxhlet extractor, following the general procedure describe below.

6.5.1 Extraction of *Litsea grandis*

Dried, grounded leaves of the plant (1.8 kg) were first defatted with hexane for twice for 3-days period at room temperature. The hexane extract were evaporated to dryness by using rotary evaporator. The plant material was filtered and dried in room temperature. After being dried, the plant material was moistened with 25% NH_4OH for 2 hours.

They were then re-extracted with dichloromethane (CH_2Cl_2) solvent for another 3 days. The CH_2Cl_2 extract were concentrated to about 500 ml using the rotary evaporator.

Then it was re-extracted with 5% hydrochloric acid (HCl). Later, it was basified with 25% NH_3 solution to pH 11 and then re-extracted with CH_2Cl_2 until a negative Mayer test was obtained. The CH_2Cl_2 then were washed with distilled water and sodium chloride solution and dried with sodium sulfate. Finally, it was evaporated to dryness to give crude alkaloid (1.5 g, 0.083%).

6.5.2 Extraction of *Litsea lancifolia*

2.0 kg of dried and milled barks of this plant were not subjected to the normal acid-base extraction. This plant was soaked three times with hexane for 3-days period by cold extraction. Then the sample were filtered and dried. After the dried sample was basified with 25% NH_4OH for 2 hours, it was then extracted with CH_2Cl_2 by cold extraction for three days. The CH_2Cl_2 extract was dried under reduce pressure to get crude CH_2Cl_2 in dark solid residue form. The percentage of yield obtained was 23.51 g (1.18%).

6.6 Isolation and Purification of the Alkaloids

The crude was subjected to column chromatography over silica gel as stationary phase. The solvent system used for chromatography was dichloromethane with increasing portion of methanol (gradient elution system). The ratio of the solvent between CH_2Cl_2 and MeOH were 100:0, 99:1, 98:2, 96:4, 93:7, 90:10, 85:15, 80:20, 50:50 and finally with pure MeOH. Fractions were collected every 100 ml and each fraction was tested with aluminium TLC plate for their alkaloids. The alkaloid spots were first detected by UV light (254 and 356 nm) and confirmed by spraying with Dragendroff's reagent. Fraction having spots with the same R_f values and stains were combined and treated as a group. The combined groups were then treated separately to isolate and purify its alkaloid content either by extensive

column chromatography or preparative TLC. The purified alkaloids were controlled by a single spot on the TLC.

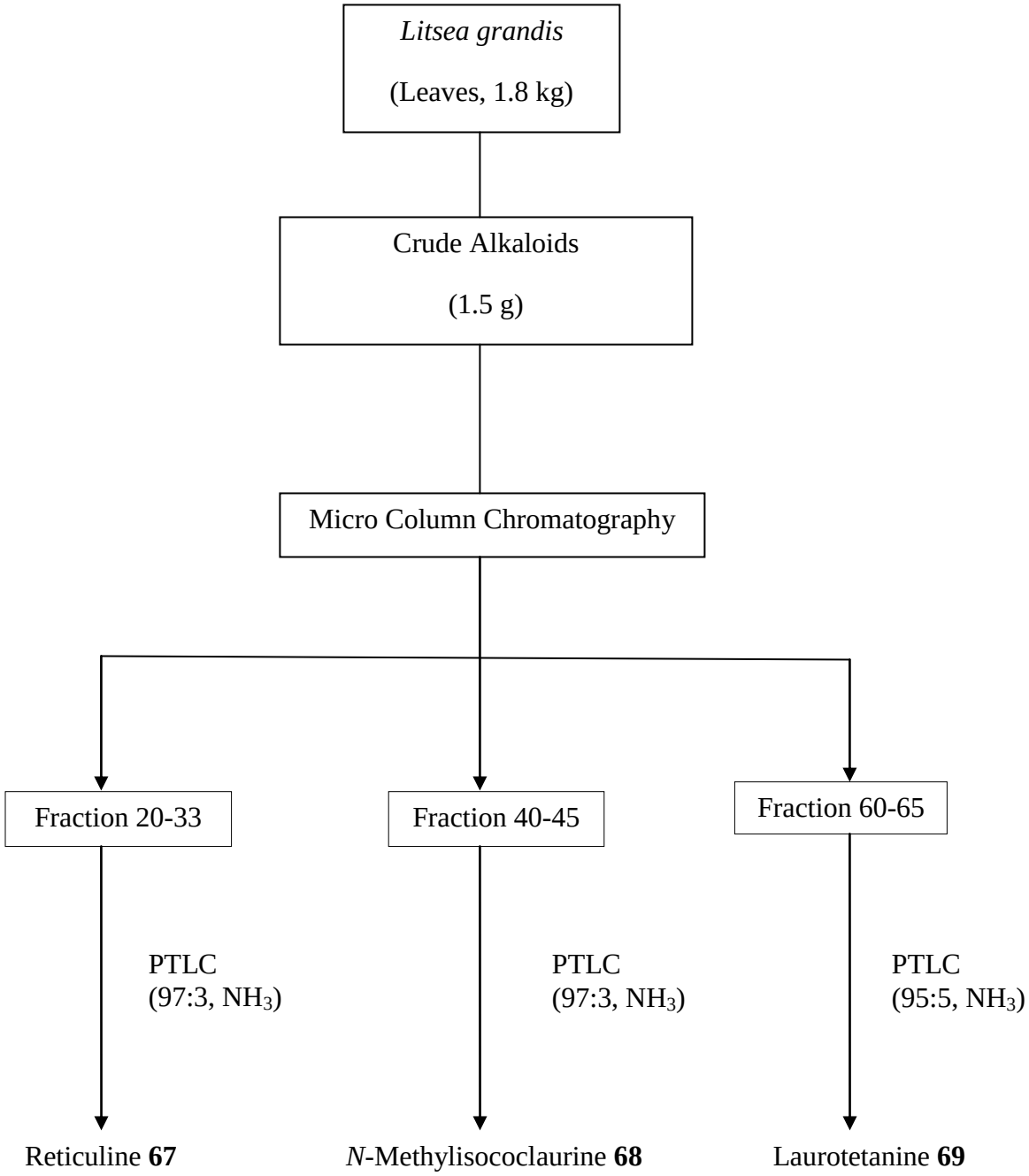
Structural identification of the isolated compounds were carried out by using spectroscopic methods; ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, DEPT, IR, UV, optical rotation and mass spectroscopy. Compounds isolated from the leaves of *Litsea grandis* and barks of *Litsea lancifolia* were listed in Table 6.1 and 6.2. The isolation of alkaloids was summarized in the flow diagram shown in Scheme 6.1 (leaves *Litsea grandis*) and Scheme 6.2 (barks of *Litsea lancifolia*).

Table 6.2: List of Eluent and Fractions of Respective Alkaloids from the Leaves of *Litsea grandis*

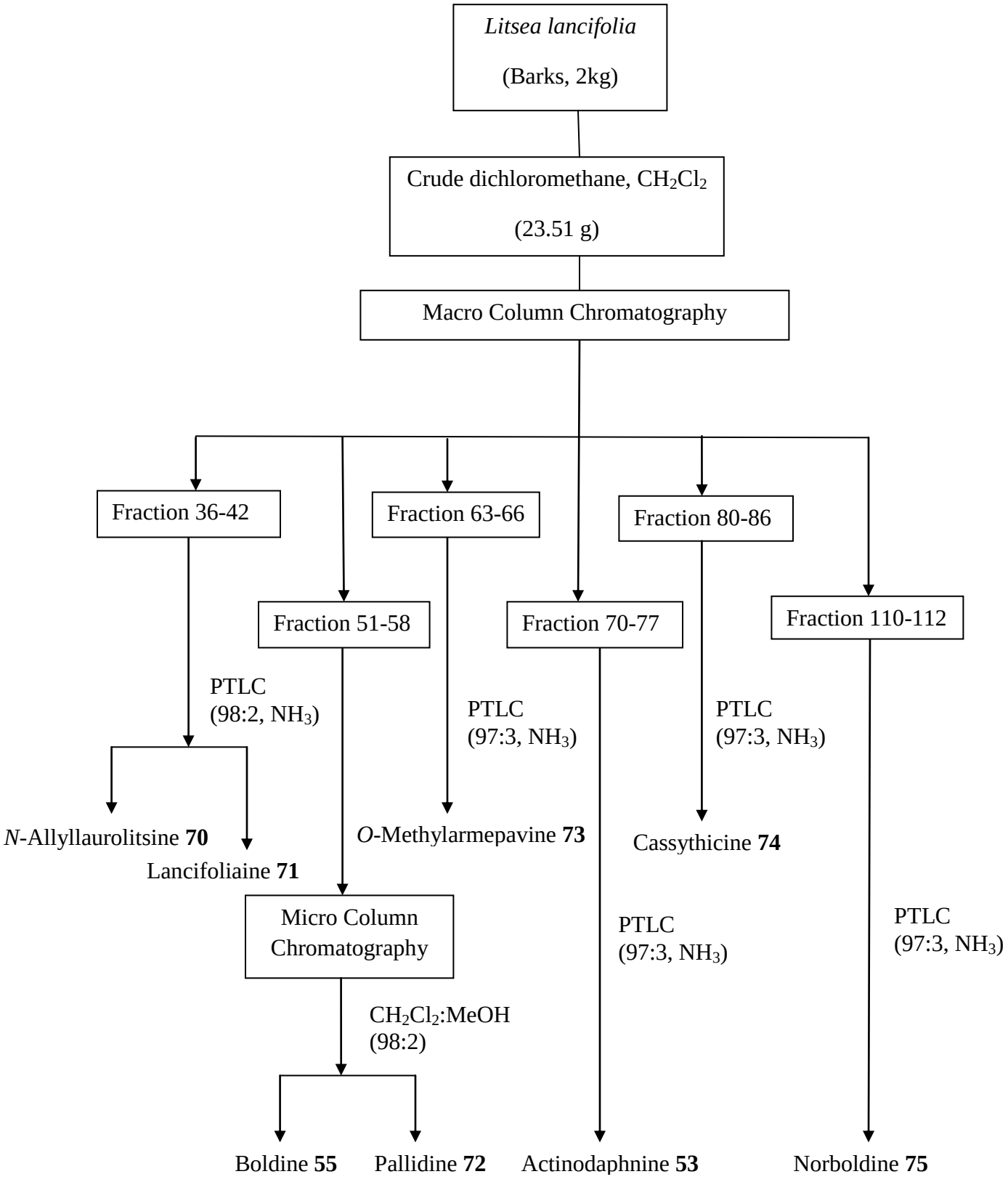
Eluent CH_2Cl_2 : MeOH	Fraction	Alkaloids Isolated
97:3	20-33	Reticuline **67**
97:3	40-45	*N*-Methylisococlaurine **68**
95:5	60-65	Laurotetanine **69**

Table 6.3: List of Eluent and Fractions of Respective Alkaloids from the Barks of *Litsea lancifolia*

Eluent CH_2Cl_2 : MeOH	Fraction	Alkaloids Isolated
98:2	36-42	*N*-Allyllaurolitsine **70**
98:2	36-42	Lancifoliaine **71**
98:2	51-58	Boldine **55**
98:2	51-58	Pallidine **72**
97:3	63-66	*O*-Methylarmepavine **73**
97:3	70-77	Actinodaphnine **53**
97:3	80-86	Cassythicine **74**
96:4	110-112	Norboldine **75**



Scheme 6.1: Isolation of Alkaloids from the Leaves of *Litsea grandis*



Scheme 6.2: Isolation of Alkaloids from the Barks of *Litsea lancifolia*

6.7 Physical and Spectral Data of the Isolated Compound

6.7.1 *Litsea grandis*

Reticuline 67

Molecular formula : $C_{19}H_{23}NO_4$

UV λ_{max} nm : 293

IR v_{max} cm^{-1} : 3418, 2926

$[\alpha]_D^{27}$: +28.6˚ (*c* 1.0, MeOH)

Mass spectrum, $[M+H]^+$ *m/z* : 330.17

^1H NMR ($CDCl_3$) δ : See Table 3.1

^{13}C NMR ($CDCl_3$) δ : See Table 3.1

***N*-Methylisococlaurine 68**

Molecular formula : $C_{18}H_{21}NO_3$

UV λ_{max} nm : 301

IR v_{max} cm^{-1} : 3266, 2921

$[\alpha]_D^{27}$: +29.4º (*c* 1.0, MeOH)

Mass spectrum, $[M+H]^+$ *m/z* : 300.16

^1H NMR ($CDCl_3$) δ : See Table 3.2

^{13}C NMR ($CDCl_3$) δ : See Table 3.2

Laurotetanine 69

Molecular formula : $C_{19}H_{21}NO_4$

UV λ_{max} nm : 221, 278, 305

IR v_{max} cm^{-1} : 1238, 1464, 1508, 3429

$[\alpha]_D^{27}$: -29.4º (*c* 1.0, MeOH)

Mass spectrum, $[M+H]^+$ *m/z* : 328.15

^1H NMR ($CDCl_3$) δ : See Table 3.3

^{13}C NMR ($CDCl_3$) δ : See Table 3.3

6.7.2 *Litsea lancifolia*

_N_-Allyllaurolitsine 70

Molecular formula : $C_{21}H_{23}NO_4$

UV λ_{max} nm : 221, 278

IR v_{max} cm^{-1} : 1238, 1464

$[\alpha]_D^{27}$: +33.9º (*c* 1.0, MeOH)

Mass spectrum, $[M+H]^+$ *m/z* : 354.18

^1H NMR ($CDCl_3$) δ : See Table 3.4

^{13}C NMR ($CDCl_3$) δ : See Table 3.4

Lancifoliaine 71

Molecular formula : $C_{35}H_{30}N_2O_8$

UV λ_{max} nm : -

IR v_{max} cm^{-1} : 1599, 1665

$[\alpha]_D^{27}$: -

Mass spectrum, $[M+H]^+$ *m/z* : 607.22

^1H NMR ($CDCl_3$) δ : See Table 3.5

^{13}C NMR ($CDCl_3$) δ : See Table 3.5

Boldine 55

Molecular formula	: $C_{19}H_{21}NO_4$
UV λ_{max} nm	: 282, 302
IR v_{max} cm^{-1}	: 1598, 2930, 3350
$[\alpha]_D^{27}$	: +15º (*c* 1.0, MeOH)
Mass spectrum, $[M+H]^+$ *m/z*	: 328.16
^1H NMR ($CDCl_3$) δ	: See Table 3.6
^{13}C NMR ($CDCl_3$) δ	: See Table 3.6

Pallidine 72

Molecular formula	: $C_{19}H_{21}NO_4$
UV λ_{max} nm	: 206, 235, 283
IR v_{max} cm^{-1}	: 1649, 2927, 3370
$[\alpha]_D^{27}$	: -22.8º (*c* 1.0, MeOH)
Mass spectrum, $[M+H]^+$ *m/z*	: 328.16
^1H NMR ($CDCl_3$) δ	: See Table 3.7
^{13}C NMR ($CDCl_3$) δ	: See Table 3.7

***O*-methylarmepavine 73**

Molecular formula	: $C_{20}H_{25}NO_3$
UV λ_{max} nm	: 301
IR v_{max} cm^{-1}	: 2921
$[\alpha]_D^{27}$	: +65º (*c* 1.0, MeOH)
Mass spectrum, $[M+H]^+$ *m/z*	: 328.16
^1H NMR ($CDCl_3$) δ	: See Table 3.8

^{13}C NMR ($CDCl_3$) δ : See Table 3.8

Actinodaphnine 53

Molecular formula : $C_{18}H_{17}NO_4$

UV λ_{max} nm : 221, 285, 308

IR v_{max} cm^{-1} : 1580, 3350

$[\alpha]_D^{27}$: +18.2º (*c* 1.0, MeOH)

Mass spectrum, $[M+H]^+$ *m/z* : 312.12

^{1}H NMR ($CDCl_3$) δ : See Table 3.9

^{13}C NMR ($CDCl_3$) δ : See Table 3.9

Cassythicine 74

Molecular formula : $C_{19}H_{19}NO_4$

UV λ_{max} nm : 221, 285, 308

IR v_{max} cm^{-1} : 3350

$[\alpha]_D^{27}$: +64.3º (*c* 1.0, MeOH)

Mass spectrum, $[M+H]^+$ *m/z* : 326.15

^{1}H NMR ($CDCl_3$) δ : See Table 3.10

^{13}C NMR ($CDCl_3$) δ : See Table 3.10

Norboldine 75

Molecular formula : $C_{18}H_{19}NO_4$

UV λ_{max} nm : 282, 302

IR v_{max} cm^{-1} : 2357, 2932, 3288

$[\alpha]_D^{27}$: +33.3º (*c* 1.0, MeOH)

Mass spectrum, $[M+H]^+$ *m/z*	: 314.14
^1H NMR ($CDCl_3$) δ	: See Table 3.11
^{13}C NMR ($CDCl_3$) δ	: See Table 3.11

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