2002

Bioactive alkaloids from medicinal plants of Lombok

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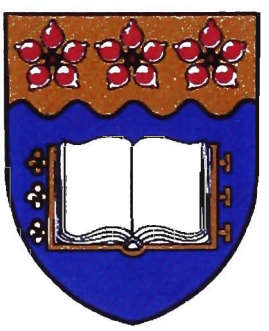
BIOACTIVE ALKALOIDS FROM MEDICINAL PLANTS OF LOMBOK

A Thesis Submitted in Fulfillment of the Requirements for the Award of the Degree

of

DOCTOR OF PHILOSOPHY



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The University of Wollongong

by

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October 2002

CERTIFICATION

I, Surya Hadi, declare that this thesis, submitted in fulfillment of requirement for the award of Doctor of Philosophy, in the Department of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledge. The document has not been submitted for qualifications at any other academic institution.

SURYA HADI
10 October 2002

PUBLICATIONS

1. Initial Studies on Alkaloids from Lombok Medicinal Plants (Molecules)[1]
2. Constituents of Medicinal Plants of Lombok: A New Antibacterial Alkaloid from *Voacanga foetida* (Bl.) Rolfe (Phytochemistry, in preparation)
3. New Bioactive Alkaloids from Young Trees of *Alstonia scholaris* R. Br. (Planta Medica, in preparation)

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(1) Hadi, S.; Bremner, J. B. Initial studies on alkaloids from Lombok medicinal plants. *Molecules [online computer file]* **2001**, *6*, 117-129.

ACKNOWLEDGEMENTS

I would like to give my sincere thanks to my supervisor, Prof. John B. Bremner for his continuous guidance and support during the course of this study. Thanks are extended to AusAid for the scholarship and to the Institute for Biomolecular Science in providing extra funds to assist this project.

My utmost thanks to the following people who extended their kind aid in completing this work: Dr. John Korth for training me to operate the Low Resolution Chemical Ionisation/Electron Impact Mass Spectrometer; Larry Hick for his help in performing High Resolution Chemical Ionisation/Electron Impact Mass Spectrometry; the NMR people, Sandra Chapman, Ellen Manning, and Yoke Berry for their assistance in training me in using the NMR spectrometer; Dr. David Perkins, not only for his kind help in correcting my thesis but also for his warm friendship; Dr. Irawati and Mr. Made Sudana for their great contribution to facilitate the collection of medicinal plants; Mr. Taspin for his help in collecting the plants; Dr. Kirsten Benkendorff for her guidance in the antibacterial testing; Dr. Sumalee Kamchonwongpaisan for the antimalarial testing; and finally to my best friends in the Bremner research group, Susan, Collette, Waya, Siritron, Nisit, Johana, and Burak (Dr. Coban) for their uncountable help and friendship.

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LIST OF ABBREVIATIONS

$[\alpha]_D$	Specific Optical Rotation
^{13}C-NMR	Carbon Nuclear Magnetic Resonance
^{1}H-NMR	Proton Nuclear Magnetic Resonance
CH_3CO_2H	Acetic Acid
CL	Central Lombok
COSY	Correlation Spectroscopy
DCM/CH_2Cl_2	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	Distortionless Enhancement by Polarisation Transfer
DMAPP	Dimethylallyl diphosphate
DNA	Deoxyribonucleic Acid
EL	East Lombok
FDA	Fluorescein Diacetate
GABA	*Gamma*-Aminobutyric Acid
HGPRT	Hypoxanthine Guanine Phosphoribosyl Transferase
HIV	Human Immunodeficiency Virus
HMBC	Heteronuclear Multiple Bond Correlation
HRCIMS	High Resolution Chemical Ionisation Mass Spectrometry
HREIMS	High Resolution Electron Impact Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence

HTS	High-Throughput Screening
IMPDH	Inosine Monophosphate Dehydrogenase
IPP	Isopentenyl Diphosphate
IR	Infrared
K_2HgI_4	Potassium tetraiodomercurate
LRCIMS	Low Resolution Chemical Ionisation Spectrometry
LREIMS	Low Resolution Electron Impact Spectrometry
MeOH	Methanol
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-Resistant *Staphylococcus aureus*
Na_2CO_3	Sodium carbonate
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
NOESY 1D	Nuclear Overhauser Exchange Spectroscopy One Dimension
rRNA	ribosome Ribonucleic Acid
UTIs	Urinary Tract Infections
UV	Ultraviolet
WL	West Lombok
δ	Chemical shift (ppm)

ABSTRACT

The aims of this project were to: 1) assess the efficacy of a combined chemo- and bio-rational approach involving alkaloid occurrence with antimicrobial medicinal plant use, focussing on plants from Lombok island. Lombok has a large population while herbal medicines are widely used with a diverse range of plant species. 2) Investigate the alkaloid constituents of selected plants including isolation, purification, and characterisation, and structure elucidation. 3) Evaluate antibacterial and antimalarial activities of crude alkaloid extracts and major alkaloid compounds isolated from the plants. 4) Identify compounds as possible new drug leads.

A combined chemo- and bio-rational strategy based on alkaloid content and traditional medicinal plant use was demonstrated to be an effective and efficient approach to finding new biologically active compounds in nature. Several new alkaloids were isolated with some potential for development as antibacterial and antimalarial agents. Some previously known alkaloids were also isolated, and in some cases NMR features not previously reported in the literature are presented.

A new optically active indole alkaloid, lombine (major), and the known alkaloid voacangine (minor), were identified from the bark of *Voacanga foetida* (Bl.) Rolfe, used ethnomedically for the treatment of wounds, itches, and swellings. The fruits of the plant produced three known alkaloid compounds, coronaridine (major), voacangine, and

voacristine, together with the new indole alkaloids mataranine A and B, which were also isolated from *Alstonia scholaris* R. Br. in this study. Voacristine was found as a major alkaloid together with the minor alkaloid voacangine from the leaves of *V. foetida* (Bl.) Rolfe. A structural revision for voacristine was also completed.

Initial antibacterial testing of the crude alkaloid extract from *V. foetida* (Bl.) Rolfe (bark and fruits) showed activity against both Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*). The new alkaloids lombine (bark) and mataranine A and B (fruits) also exhibited antibacterial activity. Bactericidal activity was observed for lombine at a concentration of 0.5 mg/ml against *S. aureus* and *E. coli.* At the lower concentration of 0.05 mg/l, it partially inhibited the growth of both *S. aureus* and *E. coli*. The crude alkaloid extract from the leaves was found to be active only against *S. aureus*. Voacristine was considered most likely to be the main active component. These compounds particularly lombine, are thus useful as potential drug leads.

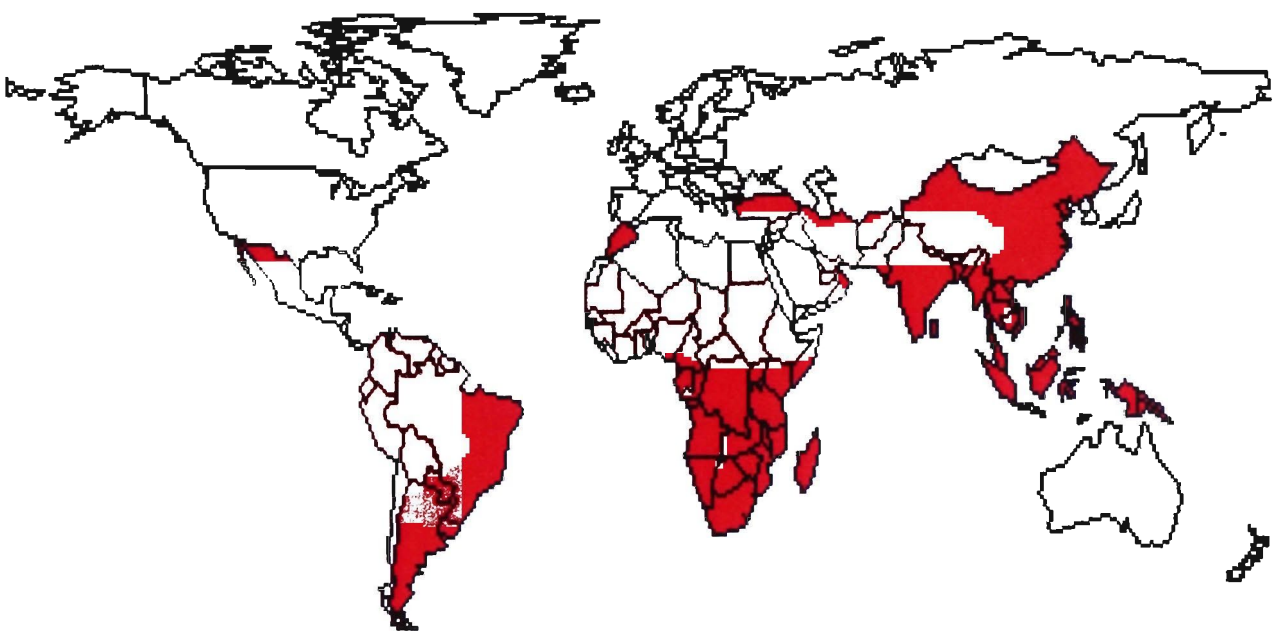
Another new antibacterial agent (LPM-574), a substituted derivative of hodgkinsine, was isolated from *Psychotria malayana* Jack (leaves). This compound, the structure of which was not completely elucidated, was found to have bacteriostatic potency against *S. aureus* and *E. coli* at a concentration of 1.0 mg/ml. The major alkaloid component, hodgkinsine, showed no antibacterial activity at this concentration. However, further structure elucidation and testing are needed for compound LMP-574 before it can be considered as a viable drug lead. Another new indole alkaloid labeled as LPM-186 was isolated as minor compound from this plant.

From the plant *Alstonia scholaris* (leaves; young trees), used in the treatment of malaria, the new indole alkaloids mataranine A and B were isolated. The crude extract of this plant exhibited antimalarial activity against *Plasmodium falciparum*, with EC_{50} values *in vitro* against antifolate resistant parasites (K1 strain) and antifolate sensitive parasites (TM4 strain) of 15.6 μg/ml and 21.0 μg/ml respectively. The new diastereomeric alkaloids mataranine A and B (mixture) had increased potency against K1 with an EC_{50} of 2.6 μg/ml (7.4 μM) and EC_{50} value of 3.4 μg/ml (9.7 μM) against the TM4 strain. Thus, either mataranine A or B, or the mixture, could provide useful new leads not only for antimalarial compounds but also for antibacterial agents. Two other new alkaloids, (*15S*, 16S**)-losbanine and kotarajine, and the known alkaloids alstonamine and (*E*)-akuammidine, were also isolated from leaves of young trees of *A. scholaris*. The antimalarial testing of these compounds has not been carried out as yet.

While there is some indication that the plants, *Clerodendron calamitosum* L. (the leaves were used for the treatment of malaria and wounds) and *C. paniculatum* (the flowers were used to cure sore eyes), based on their traditional medicinal uses, might contain some new alkaloids, full structure elucidation was not achieved due to the very small quantities available. The crude alkaloid extract from each plant did show some antibacterial activity against *E. coli*. The crude alkaloid extract from *C. calamitosum* L. also showed activity against antifolate-resistant *Plasmodium falciparum* parasites (K1) *in vitro* and it was found that the major alkaloid, compound Lcc-3, was responsible for the activity.

Chapter 1

Introduction



Map of endemic areas of malaria (red colour)

Chapter 1

INTRODUCTION

1.1. General

As microbial resistance to current drugs increases in the present day, there has developed an urgent need to discover new antibacterial and antimalarial agents for human and veterinary therapeutic use. It is perhaps readily apparent that the introduction of antibacterial agents, some of which are secondary metabolites (i.e., natural products), has contributed significantly to reducing death rates resulting from infectious diseases. However, as pharmaceutical industries have created newer antimicrobial drugs, the microorganisms have developed mechanisms to overcome the effects of these potent drugs.[1,2]

In 1941, virtually all strains of the bacterium *Staphylococcus aureus* were susceptible to penicillin G, however by 1944, *S. aureus* was reported[3] to have developed a mechanism of destroying penicillin by means of a β-lactamase enzyme named penicillinase. Today, in excess of 95% of *S. aureus* strains worldwide are resistant to penicillin, and its analogues, ampicillin, and antipseudomonas penicillin.[4,5] The pharmaceutical industries responded to this challenge by synthesising methicillin, a semisynthetic penicillin. However, resistance to this new drug was later reported in several hospitals in Europe and the United States.[6] Now, methicillin-resistant *S. aureus* (MRSA) has become the most frequent nosocomial

pathogen in the world.[7,8] Moreover, *Klebsiella pneumoniae*, a common cause of respiratory and urinary tract infections, was also found to have developed a resistance to tobramycin.[1]

These phenomena have also occurred in the biological targets of antimalarial drugs in malarial parasites. For example, strains of *Plasmodium falciparum*, the parasite responsible for many fatalities from malaria, have become resistant to chloroquine.[9-11] Furthermore, other drugs including amidiaquine, sulphadoxine-pyrimethamine, quinine, and mefloquine have been found to be ineffectual in combating infections from strains of *P. falciparum*.[12-14] As a consequence of the above developments, more than two billion people worldwide are at high risk of bacterial and malarial diseases.

The identification of structurally novel natural products with antimicrobial activity might be adopted as a way of tackling this problem. While various approaches to locating such natural products have been undertaken, researchers within the author's group have explored a combined chemo- and bio-rational strategy based on alkaloids extracted from medicinal plants found in Lombok, Indonesia. By targeting alkaloid-containing medicinal plants, it was hoped that novel compounds with the required bioactivity would be found and isolated more efficiently. Such an approach would also eliminate widely distributed tannins and polyphenolics, which often show some biological activity, from consideration.

Alkaloids have a diverse range of structures and many show an array of pharmacological activities including antimicrobial properties.[15,16] They are also normally readily separable from other plant metabolites as a result of their basicity. A further positive feature of

alkaloids is that the nitrogen site (or sites) can be used for further derivatisation and analogue development.

1.2. Resistance to Antibacterial Agents

Due to the rapid emergence of antibiotic resistant bacteria, the worldwide scientific community has concentrated a great deal on the problem. The continuous use and misuse of antibiotics imposes selective pressure on existing microorganisms, leading to highly resistant strains. As previously mentioned, the first example of resistance to an antibiotic was observed shortly after the introduction of penicillin. The 1980s and 1990s has seen the introduction of very potent broad-spectrum antibacterials accompanied by an increase in antibiotic resistance.[17,18]

In general, three main drug-resistance mechanisms are found in bacteria.[19] The first is substrate or biological target modification prohibiting the ability of the antibiotic to bind. This is a very common mechanism of resistance and is used by bacteria against many types of anti-infective agents including beta-lactam antibiotics, glycopeptides, macrolides, tetracycline, chloramphenicol, rifamycin, quinolones, beta-lactamase inhibitors, novobiocin, aminocyclitol, trimethoprim, steroids, and sulfanilamides.[20] The second mechanism involves inactivation of the antimicrobial agents. This mechanism includes enzymatic degradation and is a common mechanism of bacterial resistance to antimicrobial agents of natural origin. Bacteria have used this mechanism, for example, to resist β-lactam antibiotics, in which penicillinases, cephalosporinases, or metallo-β- lactamases can destroy the crucial β-lactam moiety required for antibacterial activity. Other examples, with the

inactivation enzymes involved shown in brackets, include aminoglycosides (*N*-acylases, *O*-nucleotidylases, *O*-phosphorylases, adenylases), aminocyclitol, chloramphenicol (chloramphenicol acetyltransferase), fusidic acid (chlorophenicol acetyltransferase), fosfomycin, and macrolides-lincosamides-streptogramins (macrolide esterases/ phosphotransferase).[18]

The third mechanism for the development of microbial resistance involves reduction in accumulation of the antibacterial agent at the active site. Permeability of the barrier imposed by bacterial cell envelopes is a factor in drug uptake. Antibacterial compounds with biological targets inside the cell must pass through the cell envelope to reach a certain minimum concentration intracellularly and hence be effective. Cell envelopes of Gram-negative bacteria consist of a cytoplasmic membrane, peptidoglycan layer and outer membrane.[21,22] It has been known for some time that the outer membrane of Gram-negative microorganisms, composed mainly of lipopolysaccharide, acts as a permeation barrier and that hydrophilic solutes cross it through water-filled porin channels.[23,24] Porins are outer membrane proteins which constitute the major route of uptake for hydrophilic antibiotics. The rate of diffusion depends on their molecular weight, shape, electronic distribution, and the relative hydrophobicity of the molecule being transported. A further way in which drug concentrations are reduced is via drug efflux pumps. These pumps are membrane proteins, which can pump out a variety of antibiotics, for example tetracycline and furoquinolines, thus reducing their intracellular concentrations to sub-lethal levels.[21]

Frequently, bacteria protect themselves from the lethal action of antibiotics by developing more than one mechanism of resistance. This is particularly noticed in the case of Gram-

negative bacteria, which often use their outer membrane as an additional resistance mechanism against drugs targeting sites located within bacterial cells.[25]

1.3. Resistance to Antimalarial Agents

Malaria is mainly transmitted by the female Anopheline mosquito that carries the *plasmodia* parasite (a protozoan) and in rare cases by transfusion of infected blood. The life cycle of the *plasmodia* parasite involves two stages, a sexual and an asexual reproductive stage. The sexual reproductive phase with multiplication (sporogony) occurs in the gut of the mosquito, while asexual reproduction with multiplication takes place in the host. Sporozoites, the infective stage, are injected during a blood meal from the mosquito and circulate to the liver where there is a period of preerythrocytic development into tissue schizonts.[26,27] Once these tissue schizonts rupture, they each release thousands of merozoites that enter the circulation and invade erythrocytes. In *Plasmodium falciparum* and *P. malariae*, all tissue schizonts rupture simultaneously and none persist in the liver. In contrast, *P. vivax* and *P. ovale* can persist in a dormant exoerythrocytic form called hypnozoites that remain in the liver for months before rupturing, resulting in relapses of erythrocyte infection. The exoerythrocytic phase is clinically asymptomatic and its duration depends on the species. [26,27]

A number of drugs have been introduced to combat malaria, such as the quinolines and artemisinin. However, the hope to eradicate malaria from the world has slowly disappeared with emerging resistance to antimalarial agents. Resistance of *P. falciparum* to chloroquine was first reported in the late 1950s in South East Asia; it proved slow to evolve initially but

then spread rapidly within South East Asia in the late 1960s and early 1970s.[25,28] Chloroquine resistance developed much more slowly in Africa after its first report in the 1960s, however, it has now spread to all parts of sub-Saharan Africa where chloroquine is used.[29]

Chloroquine-resistant parasites accumulate less chloroquine, suggesting that drug resistance results mainly from exclusion of the drug from the site of action rather than an alteration in the chloroquine target,[26,27] although, a decreased sensitivity of the target may be involved.[30] The molecular basis for decreased accumulation of chloroquine by resistant parasites has been the subject of much controversy. Three possible mechanisms have been proposed.[31] The first involves the association of chloroquine resistance with an elevated level of drug efflux. The second is related to ion trapping, and a third possibility entails the action of a protein involved in chloroquine uptake.

There are no known examples of the malaria parasite becoming resistant to an antimalarial drug by developing a mechanism for metabolising the drug.[32]

1.4. The Importance of Alkaloids in Drug Discovery

The following is the rationalisation behind why this project focusses only on one group of natural products, i.e. the alkaloids, as opposed to others such as flavonoids, lignans, or terpenoids. The emphasis of the following discussion is on understanding alkaloids and their significance.

1.4.1. Alkaloids in Plants

Alkaloids were defined initially as basic compounds containing at least one nitrogen atom in their structure. However, as the actual structure and biogenetic origin of alkaloids emerged, the notion that they were largely derived from amino acids and the constraint that the nitrogen was usually situated in a heterocyclic ring were added.

As mentioned in a comprehensive alkaloid review by Cordell *et al.*,[15] alkaloids are distributed in 7,231 species of higher plants, in 1,730 genera (approx. 14.2%) and within 186 families. The total number of plant-derived alkaloids is 21,120 distributed in the monocots, dicots, and gymnosperms. The 20 most important alkaloid-containing plant families are Amaryllidaceae, Annonaceae, Apocynaceae, Asteraceae, Berberidaceae, Boraginaceae, Buxaceae, Celastraceae, Fabaceae, Lauraceae, Liliaceae, Loganiaceae, Menispermaceae, Papaveraceae, Piperaceae, Poaceaeceae, Ranuculaceae, Rubiaceae, Rutaceae, and Solanaceae.

Plant families are able to produce more than one type of alkaloid and alkaloid distribution is not evenly distributed. For example, 164 genera in 47 different plant families are capable of producing both isoquinoline and indole alkaloids.[15] The structural diversity of isolated alkaloids reflects also the genetic diversity within plant families. Certain types of alkaloids are concentrated in the roots, for example reserpine, while others may be located predominantly in the leaves such as nicotine, the fruits (strychnine), the bark (quinine) or latex (morphine).

The biosynthetic apparatus utilised by plants in alkaloid formation is frequently located in more than one site in the plant cell.[33,34] This observation affords significant insight into which parts of a plant to collect for alkaloid extraction, and furthermore, how the genetic apparatus of alkaloid biosynthesis might be determined and possibly relocated to other systems.[35]

1.4.2. Contribution of Alkaloids to Humankind

The use of alkaloid-containing beverages as stimulants, such as tea and coffee that contain caffeine, is one of the oldest known interactions humans have with alkaloids. More recently, alkaloids have been used in such fields as health care and biology, and they have been the subjects of much research in organic chemistry.[15] The first commercial natural product to come from the alkaloid group was morphine, marketed in 1826.[36] At the end of the 20th century, of the 119 compounds isolated from 90 plants to be used as medical agents, 54 were alkaloids.[37] There are also several synthetic alkaloid derivatives used as pharmaceutical agents.[38,39] In addition, there are many pharmaceutical agents with key features based on those in alkaloids such as fendiline (based on atropine), atracurium (based on tubocurarine), and neostigmine (based on physostigmine).[40]

Alkaloids play an important role in the functioning of some biological receptors and ion channels. The numerous and varied ecological roles of various alkaloids have led to a more profound awareness of the relationship humans have with the biome and of the interrelationship between species.[34] Wink and co-workers have extensively reviewed the action of alkaloids.[41-44] They present evidence for alkaloids acting as antagonists at such

sites as the nicotinic acetylcholine receptor, the muscarinic acetylcholine receptor, alpha- and beta-adrenergic receptors, serotonin receptors, dopamine receptors, GABA receptors, and the glutamate and opiate receptors. Their review also summarizes the ability of alkaloids to affect sodium, potassium, and calcium channels. It discusses alkaloids possessing acetylcholinesterase inhibiting activity, and those that inhibit neurotransmitter uptake. Alkaloids binding to DNA and affecting protein synthesis are also mentioned. The review points out that several very potent and structurally different alkaloids may interact with a particular receptor, and some of them are active against more than one molecular target. This was interpreted by Wink *et al.*[41] as being a multi-purpose defence mechanism during evolution.

From a chemical perspective, the study of alkaloids has developed in the field of organic chemistry in three major areas: organic synthesis, drug development, and chirally specific synthesis. The targets of many classical syntheses developed in the 1940s to 1980s were alkaloids. For example, Woodward and co-workers developed streospecific syntheses of reserpine and strychnine.[45] Furthermore, it is worth mentioning the strategies adopted by Barton *et al.*[46] in preparing benzylisoquinoline-derived alkaloids through phenol oxidative coupling. In the area of drug development, many synthetic pharmaceutical products are based on alkaloids (e.g. morphine) or are derived from them (e.g. iprotropium bromide).[47]

1.4.3. Alkaloids and Drug Discovery

According to the NAPRALERT™ database as reported by Cordell,[15] alkaloids derived from plants comprise about 15.6% of known natural products and they constitute almost

50% of the plant-derived natural products of pharmaceutical and biological value. In addition, over one-third of the alkaloids that have been examined biologically in 20 or more assays are pharmaceutically significant. This figure demonstrates the compelling rationale for focussing on alkaloids in the search for new drugs.

In order to better predict a compound's suitability as a drug, Lipinski and co-workers[48] evaluated 2245 compounds which were considered to have superior physicochemical properties and suggested four properties which an orally available drug is very likely to possess. Firstly, the molecule should have fewer than 5 H-bond donor groups expressed as the sum of -OH and NH groups. Secondly there should be fewer than 10 H-bond acceptors (expressed as the sum of nitrogen and oxygen atoms). Thirdly, the molecule should have a molecular weight less than 500 Dalton, and finally log P (a measure of absorption permeability) is less than 5 units. In a study of 60 alkaloids, Cordell *et al*,[15] found an average molecular weight of 348.9 Dalton, the number of -NH and -OH groups per alkaloid was 0.97, and the average number of nitrogen and oxygen atoms for each alkaloid was 5.55. These parameters fall well within the ranges of the criteria suggested by Lipinski[48] for a molecule to be a suitable candidate for use as a drug.

There are other factors contributing to the reasons behind the study of the biological properties of alkaloids. Alkaloids typically have a moderate molecular weight (250-600 Dalton) and are thus amenable to standard purification and analysis techniques in order to meet strict administratively imposed drug standards. Alkaloids also display a wide range of biological responses, can have extremely high potency (nM range), and may serve as biological standards for enzymatic or receptor-based processes.

The basicity of most alkaloids also provides an opportunity for them to be readily made substantially more water soluble through salt formation and therefore perhaps more bioavailable. Finally, the range of functional group diversity permits modifications, enabling the introduction of groups capable of modulating biological activity such as reducing or increasing lipophilicity if necessary.

1.5. Lombok and Natural Products Development

Lombok, a part of the tropical Indonesian archipelago (Figure 1), is a small island (4600 km^2) with 2.4 million inhabitants, which is divided administratively into the three regions of West, Central, and East Lombok (Figure 2). It is situated between Bali and Sumbawa islands, where transition from the western to the eastern Indonesian flora and fauna begins. The northern region of the island is mountainous and is dominated by tall trees and shrubs, while to the south there is a drier region with typical savannah vegetation. As a consequence, Lombok has a rich and varied flora. However, as the population increases 2.2% each year, the need for housing and agricultural land also rises. This has induced a consequent deforestation causing the destruction of several plant species.

The local population has a long historical tradition of using plants for medicinal purposes and more than 70 percent of the population still use them. Aqueous plant extracts, for example, which could contain alkaloid salts with naturally occurring acids, are made for internal medicinal use. For topical applications, plant material is normally crushed and moistened with water before application. From a chemical and pharmacological

INDONESIAN ARCHIPELAGO

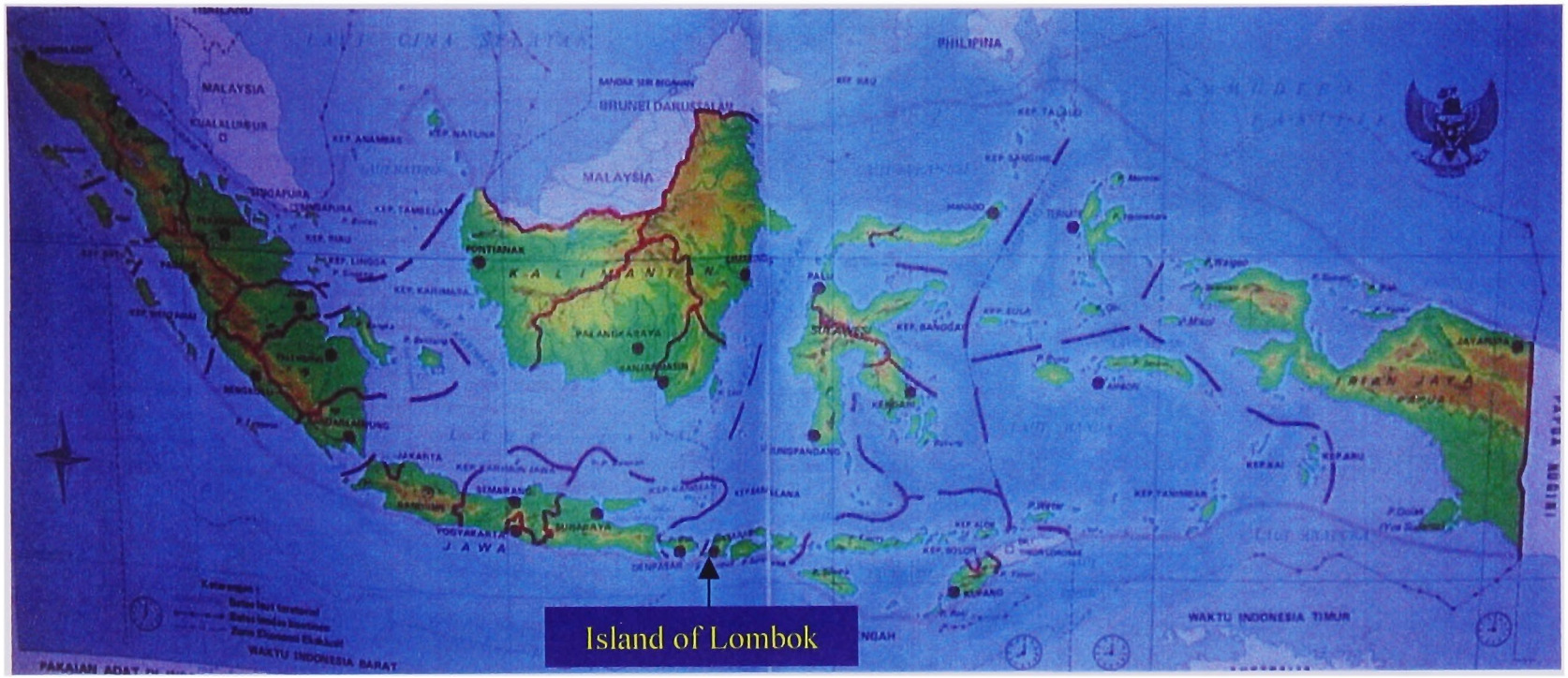


Figure 1. Map of the Indonesian archipelago, showing the position of the island of Lombok

Figure 2. Map of the island of Lombok, Indonesia, showing towns and regions; Lombok Barat (West Lombok), Lombok Tengah (Central Lombok), and Lombok Timur (East Lombok)

perspective, however, most of these medicinal plants remain unstudied, and there is therefore a great potential for the isolation of novel, naturally-occurring bioactive compounds.

The work undertaken during the course of this project initiates an investigation of medicinal plants from Lombok, and establishes an effective approach to locating bioactive plant-derived compounds that may have the potential to be further developed into antimicrobial and antimalarial agents. The further details of targets of this project are given in Section 1.6.

1.6. The Aims of the Study

As mentioned at the beginning of this chapter, there is a requirement for new structurally different compounds, and hence with possible new modes of action, in order to overcome the resistance of pathogenic microbes to current antimicrobial agents, particularly antibacterial and antimalarial compounds. One way to achieve this objective is to search for new natural products with a high natural abundance. The challenge is to locate efficiently the natural sources of such products.

The major aims of this project were thus as follows: firstly to look at a combined chemo- and bio-rational approach involving alkaloid occurrence with medicinal plant use focussing on Lombok island. Lombok has a large population in which herbal medicines are widely used and it has a diverse range of plants species. Secondly, to investigate alkaloid constituents of selected plants by isolation, purification, and structural elucidation. Thirdly,

to evaluate antibacterial and antimalarial activities of crude alkaloid extracts and major alkaloid compounds isolated from the plants, and finally, to identify compounds which may have the potential to be developed into possible new drugs.

To achieve the above goals several series of experiments were carried out by the author, the results of which are discussed in Chapters 2 to 7. The advantages of using a chemo- and bio-rational approach in plant selection and in locating bioactive compounds are discussed in Chapter 2. Chapters 3 and 4 present and discuss the results of alkaloidal investigations from the respective plants *Voacanga foetida* (Bl.) Rolfe and *Psychotria malayana* Jack., which were suggested from ethnomedical use as possible sources of antibacterial agents. Alkaloids from the plant *Alstonia scholaris* R. Br. (young trees), selected as a potential source of new antimalarial agents, are presented in Chapter 5. Chapter 6 documents the exploration of alkaloids from *Clerodendron calamitosum* L. for their potential antimalarial and antibacterial properties, and from *C. paniculatum* for their possible antibacterial properties. The results of antibacterial and antimalarial testing studies are discussed in Chapter 7. The conclusions drawn from the results obtained are presented in Chapter 8 as well as suggested future work on this project. Finally, Chapter 9 contains a summary of the experimental procedures performed during the course of the project.

Chapter 2

Selection of Plant Materials

Clockwise from top left: leaves and fruit of *Voacanga foetida*, young trees of *Alstonia scholaris*, leaves of *Clerodendron calamitosum*, fruit of *Sweitania macrophylla*, leaves and flowers of *Clerodendron paniculatum*, and *Psychotria malayana* trees

Chapter 2

SELECTION OF PLANT MATERIALS

2.1. General

The plant kingdom represents an extraordinary reservoir of novel molecules. Of the estimated two hundred and seventy thousand plant species around the globe, only a small percentage have been investigated phytochemically and the fraction subjected to biological and pharmacological study is even lower.[49] Since the number of metabolites found in any given plant may reach in excess of one thousand, there is currently a resurgence of interest in the plant kingdom as a source of new lead compounds for use in therapeutic screening programs.

Because of the rapid disappearance of tropical forests and other important areas of dense vegetation, it is necessary to develop a systematic method that leads to the rapid isolation and identification of bioactive natural products. The approach adopted in obtaining an exploitable pure plant constituent involves interdisciplinary work in the fields of botany, pharmacognasy, chemistry, and toxicology. During the past 5 years, the program involved in drug discovery has changed substantially.[15] The main steps include: defining strategies, selection and collection, extraction and biological evaluation, de-replication, isolation and structure elucidation, biological evaluation, and information management.

This chapter focusses on the choice of strategy for the efficient selection of plant materials with a view to obtaining new antibacterial and antimalarial agents.

2.2. Approaches to Plant Selection

In order to succeed in locating useful compounds effectively, careful selection of plant material is obviously very important. Random collection, particularly combined with high throughput screening, is one technique but it is more methodical (and arguably more efficient) to base the selection on certain criteria.[50,51] For example, plants used in traditional medicine often provide pharmacologically active compounds.[52]

Choosing plants according to chemotaxonomical classification is another possibility. It is perhaps advisable to start by investigating known plant families to gain some awareness of the type of compounds occurring naturally within the plant family. Another alternative method for plant selection is by field observation (bio-rational approach). If a bush or tree shows no sign of being attacked by pests and has neither pieces eaten out of the leaves nor discolorations due to the presence of a foreign organism, then it is likely that at least one metabolite with antimicrobial or insecticidal properties is present.[49]

It is known that medicinal plants have made a huge contribution as sources of new potent therapeutic agents.[53,54] Mendelsohn and Balick[55] have pointed out that the total of 375 drugs of pharmaceutical significance from the rainforests of the world, represents an estimated one-eighth of the total to be discovered.

The approach to plant selection developed in this project, results from a combination of a chemo- and bio-rational strategy based on alkaloids and medicinal plants from Lombok respectively. The plants were selected by the following procedure: a list of medicinal plants on Lombok was generated, consisting of 100 plant species, which might, on the basis of their use in local medicine, contain antimicrobial or antimalarial agents. These plants were then short-listed to 5 species for initial investigation on the basis of the following further criteria: 1) the presence of alkaloids in the species, 2) the extent of chemical and biological work already undertaken on the alkaloid-positive species, and 3) the limited geographical distribution of the alkaloid-positive species.

2.3. Medicinal Plant Use in Lombok

People on Lombok, as on other islands in Indonesia, have a rich traditional knowledge inherited from their older generations. They have used the plants available, by a process of trial and error, to cure, or treat the symptoms of certain diseases. Knowledge of medicinal plants has also been gained from people living on other islands. Information about the plants, including their characteristics and which parts are known to be effective, has been handed down (verbally) to each succeeding generation. However, as rapid social and economic changes occur, many people of the younger generation in particular, have left their village for school or work resulting in the loss of some useful traditional medical knowledge. Presently, however, the information can still be learned from the aged.

Many species of medicinal plants have been found to be useful in curing external infections such as infected wounds and ulcers. The plant species and the particular parts of the plant

used for such conditions are tabulated in Tables 1 and 2. Traditionally, the treatment of these kinds of diseases is simple: the selected part of the plant is crushed and directly placed on the infected area. For the treatment of internal infections or malaria, a concentrated aqueous extract of the leaves or bark of selected plants is used. These extracts are generally prepared by boiling a mixture of the crushed materials and water.

2.4. Alkaloid Screening

The number of programs screening for alkaloids currently underway in various regions of the world has been particularly stimulated by the pharmacological properties that many alkaloids possess.

For alkaloid screening, many different techniques have been introduced from simple ones such as using certain reagents to modern tools like chromatography and spectroscopic techniques. However, the focus normally is on the ability to qualitatively detect the presence of alkaloids quickly from a natural mixture so that screening can be carried out in the field.

Alkaloids can often be detected by several of the common techniques used to detect organic compounds, such as quenching of UV light on fluorescent plates, iodine vapour or iodine spray reagent and concentrated sulfuric acid. However, these techniques lack selectivity.[56] In order to achieve selectivity, alkaloid-selective reagents are usually used. These are mostly modified or developed from Dragendorff's reagent and potassium iodoplatinate,

which react with tertiary and quaternary nitrogen groups.[57-59] These reagents are used in combination with paper or thin layer chromatography.

Another reagent commonly used for alkaloid detection is Mayer's reagent (potassium tetraiodomercurate; K_2HgI_4). This forms a water insoluble tetraiodomercurate salt with the protonated alkaloid, leading to the formation of a precipitate. Culvenor and Fitzgerald[60] used this reagent in a plant screening procedure for alkaloids, and with modification by Bick *et al.*,[61] it was made very suitable for semi-quantitative field screening.

In any alkaloid screening methodology, one has to be aware of false positive reactions. For instance, protein and peptides can cause precipitates with reagents containing heavy metals. Artifacts giving a positive alkaloid test can be produced by the interaction of ammonium hydroxide and lactones in plant extracts, or by the interaction of ammonia with non-alkaloidal precursors of alkaloids.[62]

2.5. Collection of Lombok Medicinal Plants

A hundred medicinal plants giving negative or positive tests for alkaloids (modified Culvenor and Fitzgerald procedure)[61] are listed in Tables 1 and 2 respectively under family and species; the local uses of the plants are also included in these tables. These uses were obtained from local knowledge and discussion with the author.

Table 1. Lombok medicinal plant species giving a negative test for alkaloids

Family	Species	Locality[a]	Collection Code	Diseases/ Conditions treated[b]	Part Tested[c]
Acanthaceae	*Justicia gendarussa*	Narmada, WL	NWL08	Fever	Lf, **bk**, rt
Agavaceae	*Cordyline fruticosa* L.	Puyung, CL	PCL04	Diarrhoea	Lf, **rh**
	C. rumphii Miq.	Puyung, CL	PCL13	Dysentery	**Lf**, st, rt
Amaryllidaceae	*Crinum latifolium* L.	Kopang, EL	KEL01	Fever	**Lf**, bl
Anacardiaceae	*Bouea burmanica* Griff.	Masbagik, EL	MEL03	Ulcers, abscesses	**Lf, bk**, rt
	Buchanania macrophylla Bl.	Narmada, WL	NWL02	Fever, sore eyes	**Lf, bk**, rt
	Dracontomelon celebicum Kds.	Narmada, WL	NWL01	Fever, sore eyes	**Lf, bk**, rt
	Gluta elegans Kurz.	Puyung, CL	PCL08	Skin diseases	Lf, **bk**, rt
Annonaceae	*Xylopia malayana*	Suranadi, WL	SWL04	Fever, malaria	**Lf, bk**, fl
Apiaceae	*Corriandrum sativum* L.	Mantang, CL	MCL05	Wounds	**Lf**, sd
	Foeniculum vulgare Mill.	Mantang, EL	MLT05	Cough, fever	**Lf**, st, rt, sd
Apocynaceae	*Alstonia macrophylla* Wall.	Puyung, CL	PCL07	Ulcers	Lf, **bk**, rt
	Wrightia spp.	Masbagik, EL	MEL11	Dysentery	Lf, **bk**, rt
Asclepiadaceae	*Cryptostegia madagascariensis* Boj.	Ampenan, WL	AWL04	Dysentery	**Lf, bk**, rt
Asteraceae	*Achillea millefolium L.*	Tetebatu, EL	TEL02	Wounds	**Lf**, st, rt
	Bidens leuchantus Willd.	Tetebatu, EL	TEL05	Swellings	**Lf, bk**, rt,
Balsaminaceae	*Impatiens balsamina* L	Masbagik, EL	MEL04	Swellings, Ulcers	Lf, **rh**

Table 1 continued

Capparidaceae	*Gynandropsis speciosa*	Narmada, WL	NWL06	Gonorrhoea dysentery	**Lf**, st, rt
	Polanisia icosandra	Kotaraja, EL	KEL08	Skin diseases	**Lf**, bk, rt
Compositae	*Pluchea indica* Less.	Kotaraja, EL	KEL07	Fever, dysentery	**Lf, bk**, rt
Erythroxylaceae	*Phyllanthus acidus* Skeels.	Sepakek, CL	SCL02	Fever	Lf, bk, **rt**
Euphorbiaceae	*Aleurites moluccana* Wild.	Suranadi, WL	SWL05	Dysentery, itches	**Lf, bk**, fr
	Antidesma cuspidatum Muell. Arg.	Suranadi, WL	SWL09	Fever	Lf, bk, **rt**
	A. montanum Bl.	Pancor, EL	PEL02	Ulcers, wounds	**Lf, bk**, rt, sd
	Aporosa frutescens Bl.	Puyung, CL	PCL05	Fever	Lf, **bk**, rt
	Baccaurea brevipes Hook. f.	Puyung, CL	PCL06	Fever, dysentery	**Lf, bk**, rt
	B. dulcis Muell. Arg.	Puyung, CL	PCL11	Wounds	Lf, **bk**, rt
	Croton argyratus Bl.	Puyung, CL	PCL09	Dysentery	Lf, **bk**, rt
	Euphorbia. pulcherrima Willd	Puyung, CL	PCL10	Wounds	**Lf**, **bk**, rt
	E. tirucalli L.	Puyung, CL	PCL12	Ulcers	Lf, **bk**, rt
	Sauropus androgynus L.	Kotaraja, EL	KEL11	Fever	**Lf**, **bk**, rt
Gentianaceae	*Canscora decussata* Schult.	Kopang, CL	KCL02	Ulcers, wounds	Lf, st, **rt**
Haloragaceae	*Gunnera macrophylla* Bl.	Suranadi, WL	SWL03	Dysentery, Diarrhoea	**Lf**, **bk**, fr
	Myriophyllum brasiliense Cambess.	Kotaraja, EL	KEL10	Diarrhoea	Lf, **bk**, rt
Hernandiaceae	*Artocarpus anisophylla* Miq.	Mantang, CL	MCL04	Dysentery	**Lf**, **bk**, rt, fr
	A. dadah Miq.	Pancor, EL	PEL05	Dysentery	Lf, **bk**, **rt**

Table 1 continued

	A. champeden Spreng.	Ampenan,WL	AWL02	Sore eyes, Diarrhoea	**Lf**, **bk**
	A. gomeziana Wall.	Kotaraja, EL	KEL09	Dysentery	Lf, **bk**, rt
	Hernandia ovigera L.	Ampenan,WL	AWL01	Sore eyes	**Lf**, st
Lamiaceae	*Coleus ambonicus* Lour.	Tetebatu, EL	TEL04	Diphtheria, tetanus	Lf, **bk**, rt, sd
	Desmodium heterophyllum DC.	Mantang, CL	MCL03	Scabies, itches	**Lf**, st, rt
	Orthosiphon grandiflorus Bold.	Sepakek, CL	SCL01	Syphilis	Lf, st, **rt**
Leguminosae	*Bauhinia variegata* L.	Mataram, WL	MWL05	Fever, cough	**Lf**, bk, rt
	Derris elliptica Benth.	Suranadi, WL	SWL07	Fever, scabies	**Lf**, bk, **rt**
Malvaceae	*Abelmoschus escelentus* Moench.	Pancor, EL	PEL03	Gonorr-hoea	Lf, bk, **rt**
	Gossypium arboreum L.	Pancor, EL	PEL06	Ulcers	**Lf**, bk, rt
Meliaceae	*Dysoxylum sp.*	Suranadi, WL	SWL08	Itches	**Lf**, **bk**, rt
	Sweitenia macrophylla King.	Masbagik, EL	MEL08	Malaria	Lf, bk, rt
Myristicaceae	*Horsfleidia glabra* Warb.	Masbagik, EL	MEL09	Itches	Lf, bK, **rt**
Myrtaceae	*Eugenia cumini* Merr.	Mataram, WL	MWL07	Sore eyes	**Lf**, bk, rt, sd
Oxalidaceae	*Averrhoea bilimbi* L.	Kotaraja, EL	KEL15	Fever, cough	**Lf**, **bk**, rt
	A. carambola L.	Kotaraja, EL	KEL06	Wounds, scabies	**Lf**, bk, rt
Palmae	*Cocos nucifera* L	Narmada, WL	NWL05	Fever, dysentery	Lf, st, **rt**
Pandanaceae	*Pandanus furcatus* Roxb.	Pancor, EL	PEL04	Dysentry	**Lf**, st, rt

Table 1 continued

Papilionaceae	*Abrus fruticulosus* Wall.	Puyung, CL	PCL01	Fever	Lf, fl, **bk**, rt
Pedaliaceae	*Sasamus indicum* L.	Masbagik, EL	MEL06	Diarrhoea	**Lf**, bk, rt
Pinaceae	*Pinus mercusii* Jungh & De. Vr.	Mataram, WL	MWL01	Ulcers	**Lf**, bk, rt
Piperaceae	*Piper baccatum* L.	Mataram, WL	MWL03	Fever, swelling	**Lf**, st
	Peperomia pellucida Kunth.	Pancor, EL	PEL01	Fever	**Lf**, **bk**, rt
Poaceae	*Dendrocalamus asper* Schult. F.	Kekait, WL	KWL03	Fever	**Lf**, st
Rafflesiaceae	*Brugmensia suaveolens*	Mataram, WL	MWL06	Wounds	**Lf**, **bk**, rt
Rhamnaceae	*Alphitonia moluccana* T&B.	Sepakek, CL	SCL03	Fever	**Lf**, bk, rt
Rubiaceae	*Borreria hispida* Schum.	Narmada, WL	NWL11	Wounds, dysentery	**Lf**, st, rt
	B. setidens (Miq.) Bold.	Masbagik, EL	MEL10	Fever	**Lf**, **bk**, rt
	B. ocimoides DC.	Ampenan, WL	AWL05	Ulcers	Lf, **bk**, rt
	Catesbaea spinosa L.	Mantang, CL	MCL01	Fever, swellings	**Lf**, **bk**, rt
Sapindaceae	*Schleichera oleaosa* L.	Masbagik, EL	MEL07	Malaria	**Lf**, bk, rt
Sapotaceae	*Manilkara achras* Fosberg.	Tetebatu, EL	TEL01	Fever, dysentery	Lf, **bk**, rt, fl
Sterculiaceae	*Melochia umbellata* Staff.	Narmada, WL	NWL09	Fever	Lf, **bk**, rt
Thymelaeaceae	*Aquilaria filaria*	Kekait, WL	KWL04	Malaria	**Lf**, **bk**, rt
Verbenaceae	*Callicarpa cuspidata* Roxb.	Tetebatu, EL	TEL03	Fever	Lf, **bk**, rt
	Alpinia galanga Sw.	Puyung, CL	PCL02	Cholera	Lf, **rh**
	A. javanica Bl.	Suranadi, WL	SWL01	Swelling, cholera	Lf, **rh**

Table 1 continued

Zingiberaceae	*Curcuma aerginosa* Roxb.	Tetebatu, EL	TEL 05	Fever	**Lf**, rh
	C. zedoaria Rosc.	Puyung, CL	PCL03	Malaria	**Lf**, rh
	C. domestica Val.	Sepakek, CL	SCL04	Diarrhoea, scabies	Lf, **rh**
	Zingiber officinale Rosc.	Suranadi, WL	SWL02	Itches, cholera, wounds	**Lf, rh**

[a] WL(West Lombok), CL(Central Lombok), EL(East Lombok); [b] Information gathered by interviewing local people and confirmed by Perry.[63] [c] bk (bark), st (stem), rt (root), bl (bulb), rh (rhizome), fl (flower), fr(fruit), sd (seed); plant parts printed in bold are those used medicinally

Table 2. Lombok medicinal plant species giving a positive test for alkaloids

Family	Species	Locality[a]	Collection Code	Diseases/ Conditions Treated[b]	Part tested[c] (Result)[d]
Amaryllidaceae	*Crinum asiaticum* L.	Masbagik, EL	MEL03	Wounds, abscesses	**Lf**(++), **bl**(++)
Annonaceae	*Annona squamosa* L.	Kotaraja, EL	KEL13	Fever	**Lf**(+), bk(++), rt(++)
Apocynaceae	*Alstonia scholaris* R.Br.	Kotaraja,EL	KEL3	Malaria	**Lf**(+++), **bk**(+++), rt(+++)
	Voacanga foetida (Bl.) Rolfe	Kekait, WL	KWL01	Almost all skin diseases	**Lf**(+++), **bk**(++++), fr(+++), sd(+++)
Caesalpiniaceae	*Cassia siamea*	Kotaraja,EL	KEL02	Malaria	Lf(+++), **bk**(++), rt(++)
Caricaceae	*Carica papaya* L.	Narmada, WL	NWL04	Malaria, Ulcers	**Lf**(++), st(-), rt(-), fr(-)
Convolvulaceae	*Ipomoea batatas* Polr.	Narmada, WL	NWL03	Wounds	**Lf**(+), rh(-)

Table 2 continued

Cucurbitaceae	*Momordica charantia* L.	Pancor, EL	PEL07	Malaria	**Lf**(++), st(++), fr(+)
	M. bicolour Bl.	Narmada, WL	NWL10	Malaria	Lf(++), st(++), **rt** (+)
Euphorbiaceae	*Jatropha multifida* L.	Ampenan, WL	AWL03	Swellings, wounds	**Lf** (-), bk(-), sd(+)
Lamiaceae	*Drysophylla auricularia* (L.) Bl.	Masbagik, EL	MEL05	Fever	**Lf**(++), St(++), rt(+)
Loganiaceae	*Strychnos ligustrina* Bl.	Masbagik, MEL	MEL12	Malaria	Lf(-), **bk**(+++), rt(++)
Magnoliaceae	*Michelia champaca* L.	Mataram, WL	MWL06	Fever, wounds	Lf(+++), bk(+++), **rt**(++)
	M. alba DC.	Narmada, WL	NWL07	Malaria	**Lf**(++), bk(++), rt(++)
Meliaceae	*Azadirachta indica* Juss.	Kopang, EL	KCL02	Dysentery malaria	**Lf**(++), **bk**(++), rt(+)
Mimosaceae	*Crotalaria retusa* L.	Kotaraja, EL	KEL14	Fever, wounds	**Lf**(++), st(+), rt(++), fr(-)
Moraceae	*Ficus septica*	Mataram, WL	MWL05	Wounds	**Lf**(+++), bk(+++), rt(+++)
Moringaceae	*Moringa oleifera* Lamk.	Mataram, WL	MWL08	Fever, Wounds	Lf (++), **bk** (++), rt (++)
Rubiaceae	*Psychotria malayana* Jack.	Suranadi, WL	SWL04	Wounds, skin diseases	**Lf**(++), **bk**(++), rt(-), fr(-)
Sterculiaceae	*Sterculia foetida* Linn.	Kotaraja, EL	KEL01	Fever, malaria	**Lf**(++), bk(+++), (rt(+++)

Table 2 continued

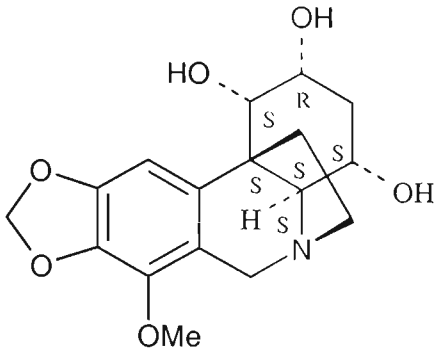
Verbenaceae	*Clerodendron calamitosum* L.	Kotaraja, EL	KEL12	Malaria, wounds	**Lf** (++)
	C. paniculatum L.	Narmada, WL	NWL06	Sore eyes	**Lf**(-), fl(++), rt(-)
Zingiberaceae	*Curcuma xanthorrhiza* Roxb.	Mataram, WL	MWL02	Diarrhoea, malaria	Lf (+), **rh** (+)

[a] WL(West Lombok), CL(Central Lombok), EL(East Lombok); [b] Information gathered by interviewing local people and confirmed by Perry.[63] [c] bk (bark), st (stem), rt (root), bl (bulb), rh (rhizome), fl (flower), fr (fruit), sd (seed); plant parts printed in bold are those used medicinally; [d] A result of (++++) indicates a very heavy precipitate with Mayer's Reagent, (+++) a moderate precipitate, (++) a light precipitate, and (+) a milkiness in the solution

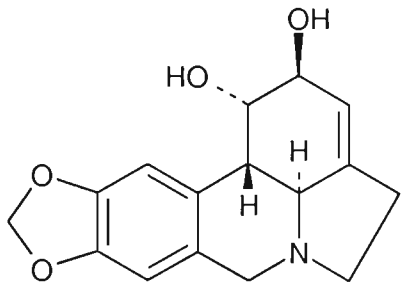
The plants collected were widely distributed in 49 families and 80 genera, giving some indication of the variety of medicinal plants growing on Lombok. Of these plants, twenty-three species (23%) contained alkaloids. In a survey of plants of Tasmania, Australia, focussing mainly on endemic species in this cool temperate environment,[61] 15 % of the plant species gave positive alkaloid tests. However, in a similar alkaloid survey in Queensland, Australia, with many tropical and sub-tropical species, 20% of species were positive.[64]

2.6. Previous Work and Geographical Distribution of Alkaloid Positive Species

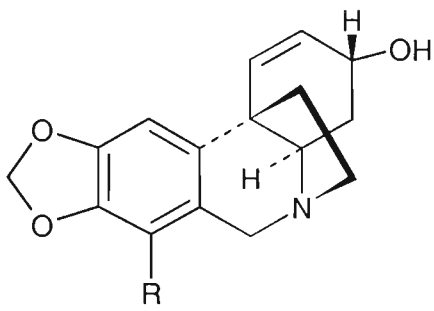
A further brief review on alkaloid-containing plants from Lombok is presented below (Table 2 contains a summary), which concentrates mainly on the characteristics, distribution, local uses, and alkaloidal constituents of the plants discussed.



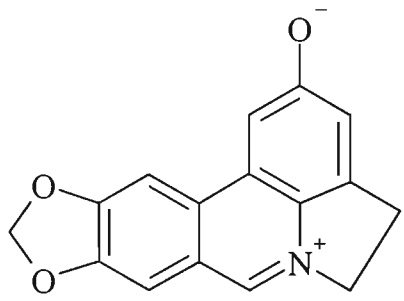
1: Crinisine



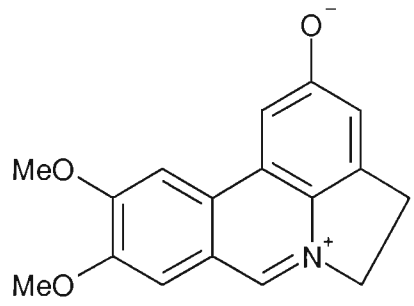
2: Lycorine
7: Palmilycorine, O^1-hexadeconyl
8: Lycoriside, O^1-(6-O-Hexadeconyl-β-D-glucopyranoside)



3: R = Powelline, R = OMe
4: Crinine, R = H

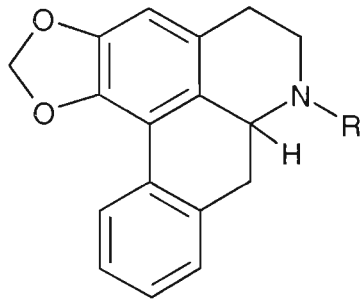


5: Ungeremine

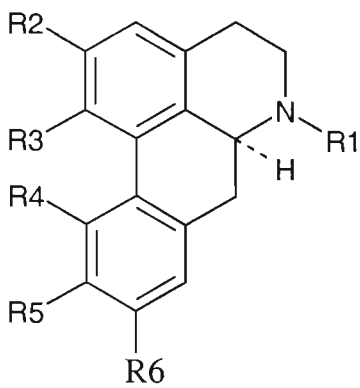


6: Criasbetaine

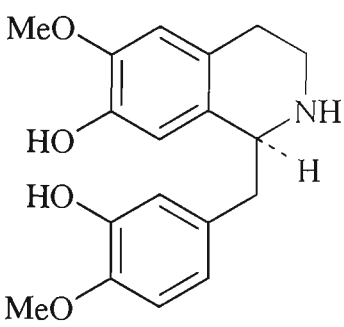
Crinum asiaticum L. (Amaryllidaceae), locally called "bakung", is found largely from Southeast Asia to Polynesia, growing on forest borders at an altitude of 1-700 m. In Lombok, the leaves are used to treat wounds while the bulbs are employed as a remedy for abscesses. Tang *et al.* [65] have reported isolating a new alkaloid, namely crinisine **1** and three known compounds: lycorine **2**, powelline **3**, and crinine **4** from this plant. The pyrrolophenanthridine lycorine **2** has frequently been used as a specific inhibitor to help elucidate the function of ascorbic acid in a wide range of biological processes.[66] Ghosal *et al.*[67] have reported obtaining from this plant, ungeremine **5** and criasbetaine **6**, which have antitumour activity. Palmilycorine **7**, and Lycoriside **8** were also produced by this plant.[68]



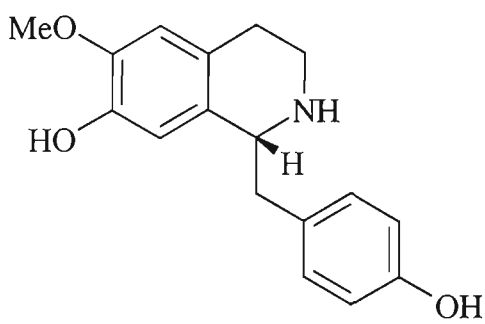
9: Anonaine, R=H
10: Roemerine, R=Me



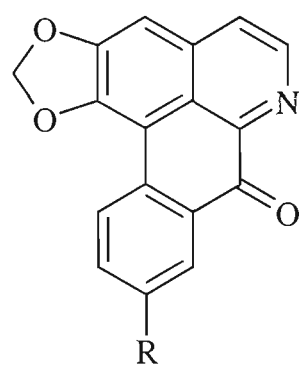
11: Corydine, R1=Me, R2=OMe, R3=OH, R4=OMe, R5=OMe, R6=H
12: Norcoryne, R1=H, R2=OMe, R3=OH, R4=OMe, R5=OMe, R6=H
13: Norisocorydine, R1=H, R2=OMe, R3=OMe, R4=OH, R5=OMe, R6=H
14: Isocorydine, R1=Me, R2=OMe, R3=OMe, R4=OH, R5=OMe, R6=H
15: Glaucine, R1=Me, R2=OMe, R3=OH, R4=H, R5=OMe, R6=OMe



16: Reticuline



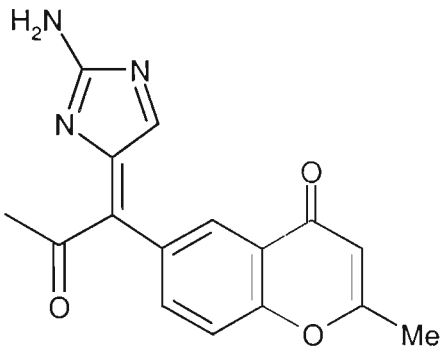
17: Coclaurine
18: *N*-Methylcoclaurine



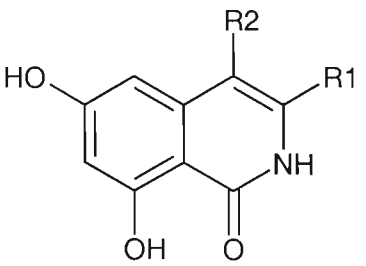
19: Liriodinine, R=H
20: Oxoxylopine, R=OMe

Annona squamosa L. belongs to the family *Annonaceae*, a family of the old world tropics and well known as a source of edible fruits, from which at least 50 genera including some 75 species are known to contain alkaloids.[69] *A. squamosa*, known locally as "srikaya", is a native of tropical America. An extract of its leaves is used to reduce fever. Several aporphine alkaloids, annonaine **9**, roemerine **10**, corydine **11**, norcorydine **12**, norisocorydine **13**, isocorydine **14**, and glaucine **15** were isolated from the plant.[70] Wu *et al.*[71] have investigated the unripe fruits and found three benzylisoquinolines, reticuline **16**, coclaurine **17**, and *N*-methylcoclaurine **18**; two oxoaporphines, liriodenine **19** and oxoxylopine **20**. They reported that coclaurine **18** and oxoxylopine **21** showed significant antiplatelet and cytotoxic activity respectively.

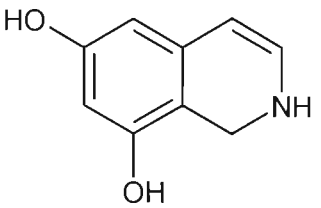
Alstonia sholaris R. Br. and *Voacanga foetida* (Bl.) Rolfe are members of the family Apocynaceae, possibly the most thoroughly investigated family for alkaloidal plants. About a thousand compounds have been isolated from them.[69] Many members of the Apocynaceae are used as ornamentals, some species yield rubber, several are valuable sources for drugs, and many are toxic. So far, there are 215 known genera with more than 2,100 species having been studied. Further information on the *Alstonia* and *Voacanga* species is discussed in Chapters 3 and 5 respectively.



21: Cassiadine



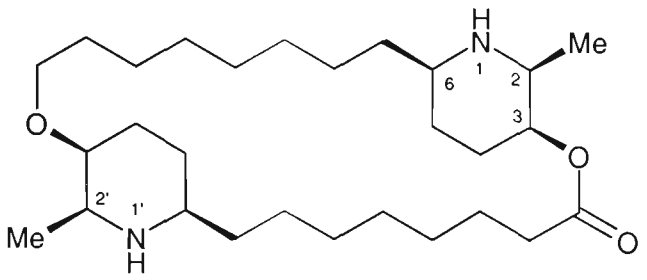
22: Siamine A, R1=Me, R2=Me
23: Siamine B, R1=H, R2=H



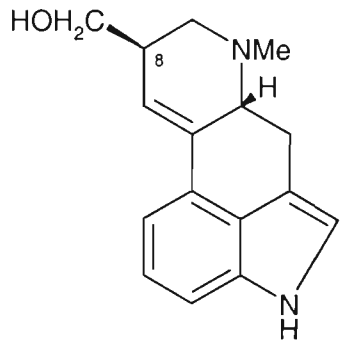
24: Siamine C

Cassia siamea (Caesalpiniaceae), with the local name "johar", is native to Southern Asia. It sometimes grows as a roadside tree. The timber is used for construction materials and a concentrated water extract of the bark is prepared to treat malaria. It was reported to produce a chromone alkaloid, cassiadine **21** and several alkaloids such as, siaminine A **22**, siamine B **23**, and siamine C **24**.[72,73]

Carica papaya L. (Caricaceae) is a native of tropical America and now pantropic through cultivation. *C. papaya* L, with local name "gedang", is collected for commercial purposes from the fruits and all over Southeast Asia. The leaves are used against malaria and ulcers. The latex, found in all parts of the plant, contains an enzyme that aids in the digestion of albuminoidal substances.[63] The presence of alkaloids such as carpaine **25** has been recorded, and this alkaloid is highly active against the bacterium *Bacillus cereus* and moderately active against *Bacillus micoid*.[74,75] Dehydrocarpaine I **26,** dehydrocarpaine II **27**, and pseudocarpaine **28** have also been isolated from this species.[76]



25: Carpaine
26: Dehydrocarpaine I; 1,2-didehydro
27: Dehydrocarpaine II; 1,1',2,2'-tetrahydro
28: Pseudocarpaine; 2-epimer



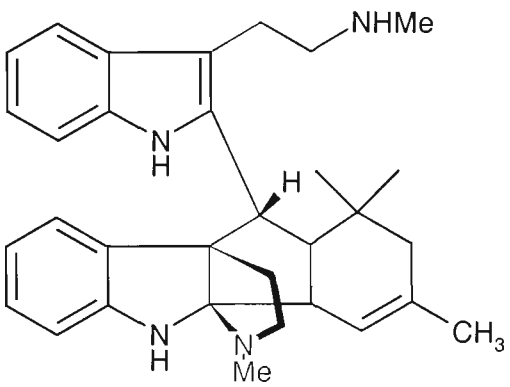
29: Lysergic acid
30: Ergine; lysergic acid amide
31: Isoergine; C8-epimer, amide
32: Lysergic acid α-hydroxyethylamide; α-hydroxyethylamide
33: Isolysergic acid α-hydroxyethylamide; C-8 epimer, α-hydroxyethylamide

Ipomoea batatas Poir. (Convolvulaceae), is cultivated in tropical and sub-tropical regions. The crushed leaves are employed to treat wounds. Constituents reported were starch, sugar, fatty acid, phytosterols, carotene, chlorogenic acid, vitamins A, B, and C.[77] Some alkaloids, derived from lysergic acid **29**, such as ergine **30**, isoergine **31**, isolysergic acid α-hydroxyethylamide **32**, and lysergic acid α-hydroxyethylamide **33**, have also been obtained.[78]

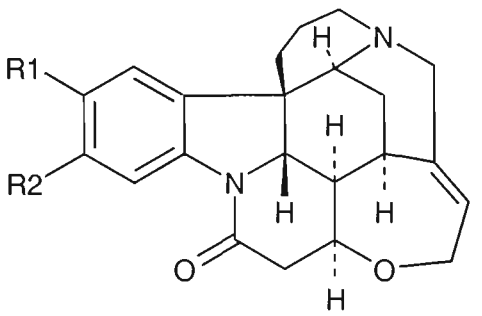
Momordica charantia L. and *M. bicolor* Bl. (*Cochinia cardifolia*) belong to the family Cucurbitaceae, a primarily tropical and semitropical family with members in both Northern and Southern hemispheres. Several alkaloids have been reported, which are mainly pyrazoline derivatives.[79] The family consists of about 121 genera with more than 760 known species. In Lombok, *M. charantia* L (leaves) and *M. bicolor* Bl. (root) are inexpensive sources of antimalarial agents (crude extracts). Lectins from *M. charantia* have been reported to have the ability to inhibit HIV-1 reverse transcriptase.[80] A phytochemical

study found the presence of amino acids in the fruits of the two species and 5-hydroxytryptamine was also obtained.[81]

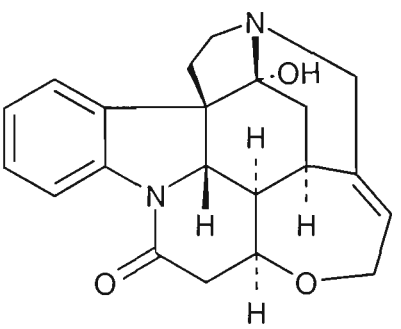
The plant *Jatropha multifida* L. (Euphorbiaceae), locally called "jarak cina", is a species native to tropical America. In Indonesia, it is planted as an ornamental shrub and its leaves are used to cure swelling and wounds. Successive extracts of the root using hexane, ethyl acetate, chloroform, and then methanol were reported as being effective in inhibiting the growth of *B. subtilis* and *S. aureus* at a level of 200 µg/disk.[82] A cyanoglucoside, multifidin, was isolated from the latex of the species by van de Berg *et al.*[83]



34: Auricularine



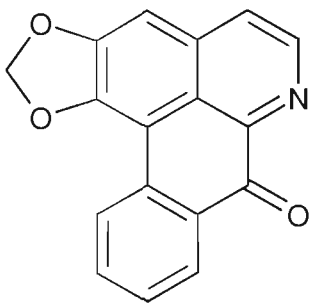
35: Brucine; R_1=R_2=OMe
36: Brucine (3 Amine) *N*-oxide; R_1=R_2=OMe
37: β-Colubrine; R_1=OMe, R_2=H



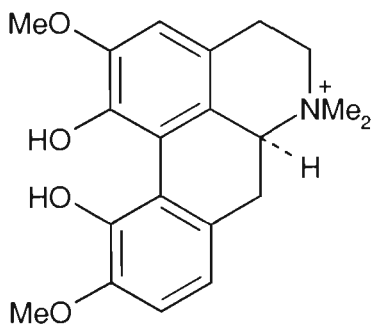
38: Pseudostrychine

Drysophylla auricularia (L.) Bl. (Lamiaceae), known locally as "ketumpang", is easily found in humid localities such as rice fields and dams, at altitudes ranging from 5 to 1,200 metres. A decoction of leaves can be employed to reduce fever. Purushothaman *et al.*[84] reported the presence of the bis-indole alkaloid, auricularine **34**.

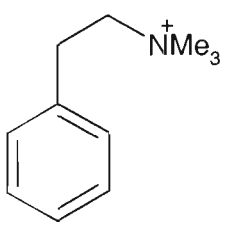
Strychnos ligustrina Bl. (Loganiaceae), locally called "bidara pute", is a shrub growing in brushwood below 1500 m altitude. The bark is employed to cure malaria. Alkaloids of the strychnos type are major alkaloid components of the plant, with brucine **35** being the main alkaloid component.[85] Other strychnos alkaloids obtained were brucine *N*b-oxide **36**, β-colubrine **37**, and pseudostrychine **38**.[86]



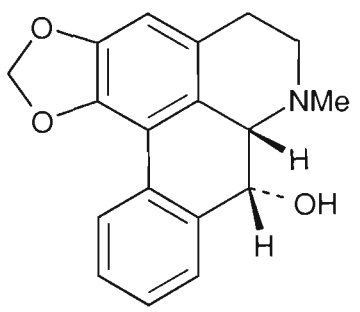
39: Oxoushinsunine



40: Magnoflorine



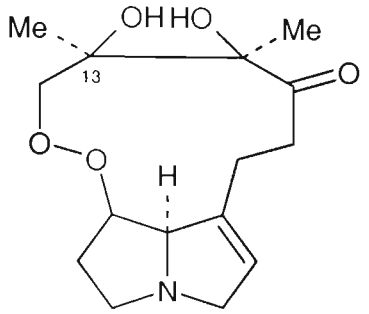
41: Salicifoline



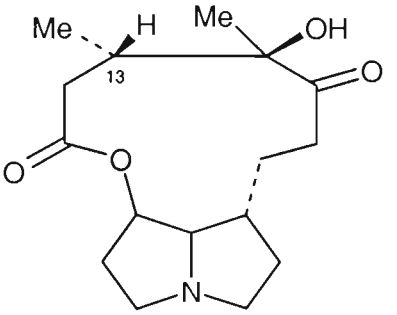
42: Ushinsunine

Michelia champaca L. and *M. alba* DC belong to the family Magnoliaceae. *M. champaca* L., originally from India, and known locally as "cempake kuning", is traditionally used as a remedy for fever and wounds. Extraction of its bark afforded the alkaloids oxoushinsunine **39** and magnoflorine **40**.[87] The leaves of *M. alba* DC, native to China, are traditionally used as a remedy for malaria, and it has been reported to contain the alkaloids salicifoline **41**, ushinsunine **42** and oxoushinsunine **39**.[88]

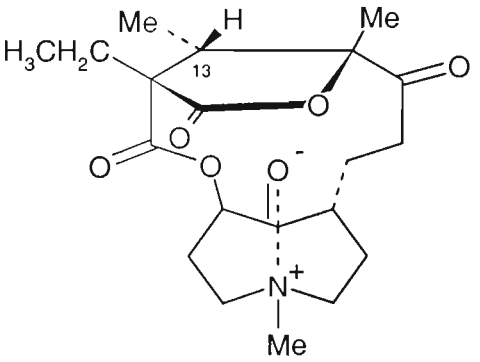
Azadirachta indica Juss. (Meliaceae) ranges from India, Western China, and into Java. This plant, with the local name of "imbe", is a middle-sized tree growing in drier regions, at roadsides, and in light forests. The leaves and bark are used as a medication for dysentery and malaria. An anti-microbial activity study of the alcohol extract of the bark and leaves showed that the bark was more active than the leaves against Gram-positive and Gram-negative bacteria and the fungus *Aspergillus niger*.[89] The main active compound is azadirachtin considered also to be the most potent of all known insect antifeedants.[90] Chemical screening of bark and leaves showed that the plant contains high protein levels, amino acids, alkaloids, and minerals.[89]



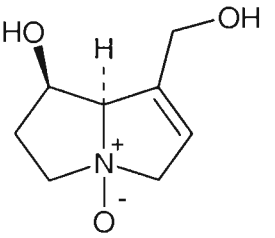
43: Monocrotaline
47: Spectabiline, O^{13}-Acetyl



44: Retusine

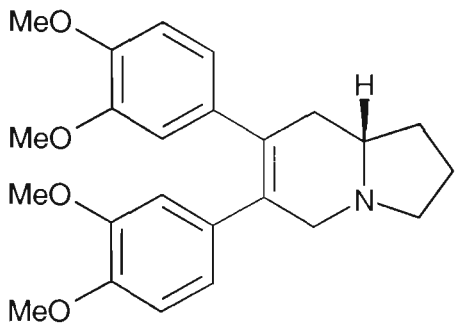


45: Retusamine

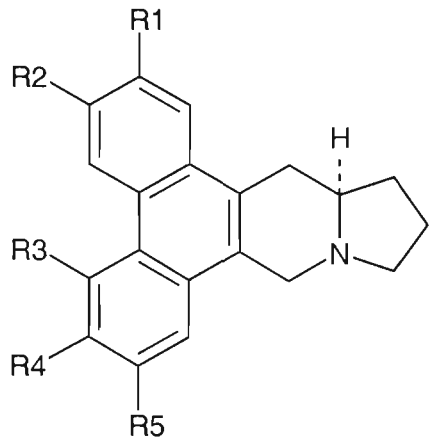


46: Retronecine *N*-oxide

Crotalaria retusa L. (*Mimosaceae*), locally called "orok-orok cine", grows in sandy coastal plains on Lombok. The leaves are applied as a remedy for fever and wounds. Phytochemical studies by the Han group[91] and Culvenor *et al.*[92] confirmed the presence of several alkaloids: monocrotaline **43**, retusine **44**, retusamine **45**, retronecine *N*-oxide **46** and spectabiline **47**. Monocrotaline **43** is a known antitumour agent and less toxic than retusine **44** to HeLa cells.



48: Septicine



49: Antofine; R1=R2=R4=OMe, R3=R5=H
50: Tylophorine; R1=R2=R4=R5=OMe, R3=H
51: Tylocrebine, R1=R2=R3=R4=OMe, R5=H

Ficus septica (*Moraceae*) is very common species in Indonesia with the local name "awar-awar". It is common to roadsides, brushwood, or open forest. The crushed leaves are employed to protect wounds from infection. Baumgartner *et al.*[93] reported that a methanol extract of the leaves displayed intense antibacterial and antifungal activities. Bioactivity-guided fractionation led to the isolation of two indolizine alkaloids, septicine **48** and antofine **49**. Two other alkaloids, (-)-tylophorine **50** and (+)-tylocrebrine **51**, were also isolated from this plant.[94]

Moringa oleifera Lamk. (Moringaceae), having the local name "kelor", is a native of north-west India. In Indonesia, the plant is cultivated at altitudes up to 500 m. The leaves are eaten and the bark is used as a remedy for fever and wounds. Mazumder *et al.* have reported that a methanolic extract was found to contain some alkaloids (0.2%) affecting liver and kidney function at a high dose (7 mg/kg) in mice.[95] Among the other compounds reported to have been isolated from this species are rotenone and a benzylamine.[96]

Sterculia foetida Linn. (Sterculiaceae), which is known locally as "kepuh", grows in very dry areas and has dark red flowers. The concentrated water extract of the leaves is employed to cure fever and malaria. Janzen *et al.*[97] have investigated the toxicity of the secondary metabolites from *S. foetida* and found that the alkaloids were generally the most toxic compounds of those tested.

Curcuma xanthorrhiza Roxb. (Zingiberaceae), locally called "temu lawak", is cultivated in all regions between altitudes of 5-1500 meters. A concentrated water extract from the roots is utilized as a remedy for diarrhoea and malaria. No alkaloids have been reported from the family Zingiberaceae. In some cases, a positive result to a test for the presence of alkaloids is the result of a false positive reaction from non-alkaloid compounds, which may also occur in *C. xanthorrhiza* Roxb.

2.7. Plants Selected for Further Study

On the basis of the criteria mentioned in Section 2.3, the plants selected for further study by the author, were *Alstonia scholaris* R. Br. (Apocynaceae; antimalarial properties), *Voacanga foetida* (Bl.) Rolfe (Apocynaceae; antimicrobial properties), *Psychotria malayana* Jack. (Rubiaceae; antimicrobial properties), *Clerodendron paniculatum* L. (Verbenaceae; antimicrobial properties), and *C. calamitosum* L. (Verbenaceae; antimalarial and antimicrobial properties). Although there are a number of reports on alkaloids from *A. scholaris* R. Br.[98,99] there was particular interest in investigating alkaloids that might be found in the young plants, as the local population have used the plant at this particular

growth stage, traditionally, to treat malaria. The results of the investigations carried out on the above selected plants are discussed in the ensuing chapters.

Chapter 3

Alkaloids from *Voacanga foetida* (Bl.) Rolfe

Leaves of *V. foetida* (Bl.) Rolfe

V. foetida (Bl.) Rolfe trees

Chapter 3

ALKALOIDS FROM *Voacanga foetida* (Bl.) Rolfe

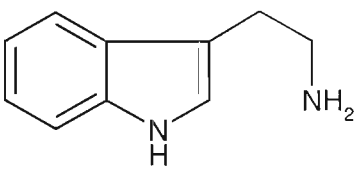
3.1. Introduction

The plant *Voacanga foetida* (Bl.) Rolfe (Apocynaceae), locally known as "kumbi", is distributed throughout Indonesia although Lombok has the highest population density. It grows in areas about 400 m above sea level and reaches 10-15 m in height. In Lombok, an aqueous extract of the leaves or bark is used commonly to treat a wide range of skin conditions such as wounds, itches, and swellings. The leaves of *V. foetida* (Bl.) Rolfe, are also warmed over a fire and then placed on chronic leg sores; this is a common practice in many parts of Indonesia.[63] In Sumatra, the plant's latex has been used externally to treat skin disorders.[63]

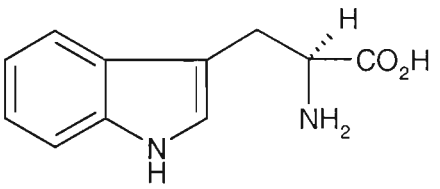
Initial alkaloid screening showed that all parts of the plant contained high concentrations of alkaloids, although a previous report[100] indicated that only small amounts of alkaloids occurred in the bark, fruit rind, and seeds. Moreover, a thorough survey of the relevant literature indicated that no further information concerning structural properties of the alkaloids contained in this plant had been published. Other Voacanga species had been shown to yield a variety of indole alkaloids[101] and a review of this important class of alkaloids follows.

3.2. Indole Alkaloids: Biosynthesis and Classification

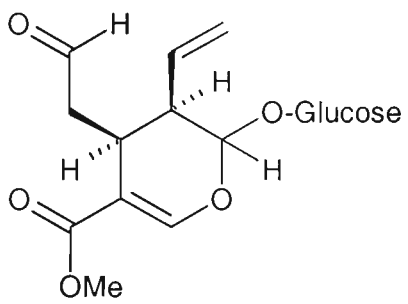
Indole alkaloids are most widely distributed among flowering plants, particularly in the Apocynaceae, Loganiaceae, and Rubiaceae plant families.[102] A few representatives of this significant group of alkaloids have also been found in phylogenetically more remote families such as the Annonaceae, Euphorbiaceae, Sapotaceae, Alangiaceae, and Icacinaceae.[15]



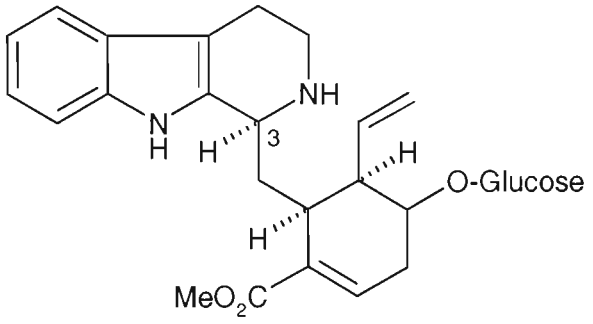
52: Tryptamine



53: Tryptophan



54: Secologanin



55: Strictosidine
56: Vincoside; C-3 epimer

Simple indole alkaloids are analogues of the basic tryptamine unit **52** derived from tryptophan **53** and more complex ones still contain this basic feature in their structures. The tryptamine unit **52**, which is usually unmodified, together with C_9-C_{10} units which initially perplexed researchers with regard to their biosynthetic origin, until it was recognised that they resemble naturally occurring cyclic monoterpenes. They are a major group of indole alkaloids having more than 1000 members.

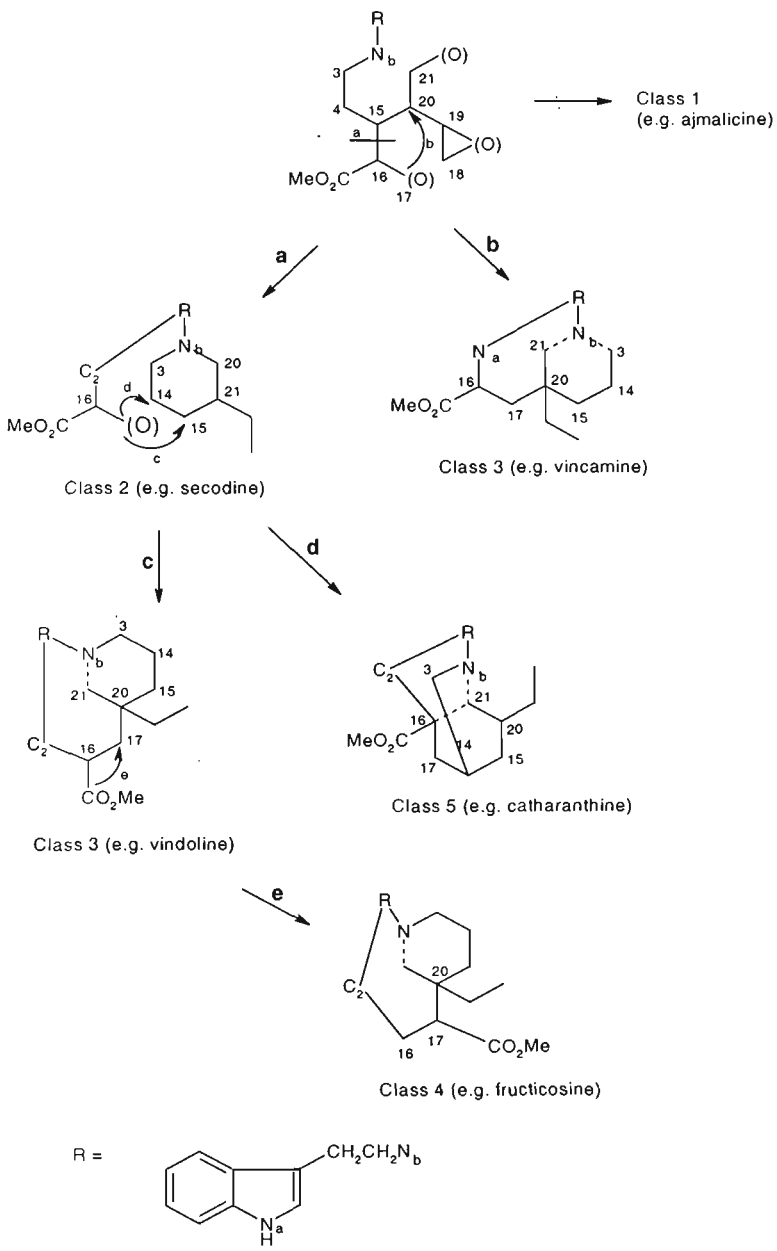
Biosynthetic experiments have established that the fundamental building blocks for the monoterpene indole alkaloids are tryptamine **52** and the iridoid secologanin **54**. Condensation of the two compounds *in vivo* forms the nitrogenous glycosides strictosidine **55** (formerly named isovincoside) and its epimer vincoside **56**, which are the key intermediates in the biosynthetic elaborations of the indole alkaloids.[103]

Because the building blocks for the indole alkaloids have already been established, a secure foundation on which a biogenetically based system for the classification of these bases can be constructed. Kompis *et al.*[104] have presented an approach to the classification of indole alkaloids based on the structural framework established by biosynthetic studies (Scheme 1).

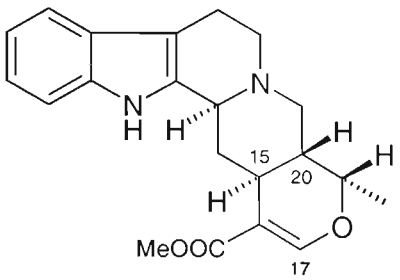
From Scheme 1, it is apparent that the indole alkaloids can be divided into 5 major classes: Class 1, represented by ajmalicine **57**, containing the non-rearranged secologanin unit; Class 2, which arises from pathway **a**, is typified by secodine **58**; Class 3, including vincamine **59**, which involves process **a** and a rearrangement via **b**, vindoline **60** is also typical of class 3 arising from the rearrangement of skeleton class 3 via process **c**; Class 4, typified by fructocosine **61**, which involves the rearrangement of the structural unit of Class 3 via pathway **e**; Class 5, which includes catharanthine **62**, which involves the attachment of C-17 to C-14 via route **d**. These classifications may be of use in the chemotaxonomy of indole alkaloid-bearing plants.

Dalton,[105] in his review on biosynthesis of monoterpenoid indole alkaloids, has renamed the above classes into five widely recognized categories: 1) Corynanthe-Strychnos bases as

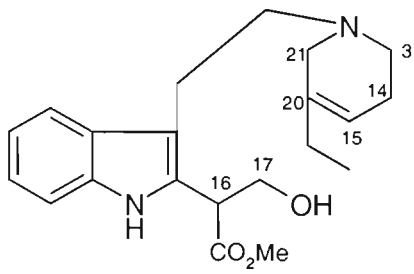
represented by ajmalicine **57**, yohimbine **63**, ajmaline **64** and strychnine **65**; 2) the Cinchona alkaloids typified by quinine **66**; 3) Iboga alkaloids such as catharanthine **62**; 4) the Aspidosperma bases as represented by tabersonine **67**; and 5) the Eburna family including vincamine **59**. These categories have been widely used in discussions concerning monoterpenoid indole alkaloids.



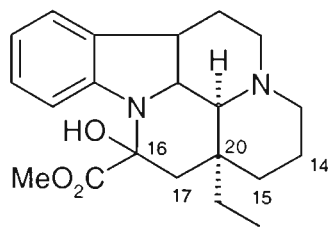
Scheme 1. Classification of indole alkaloids by Kompis *et al.* [104]



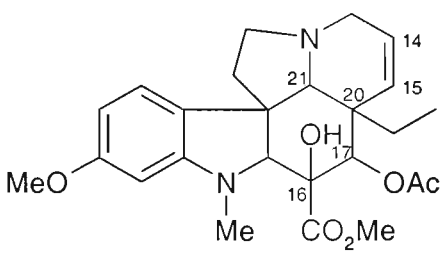
57: Ajmalicine



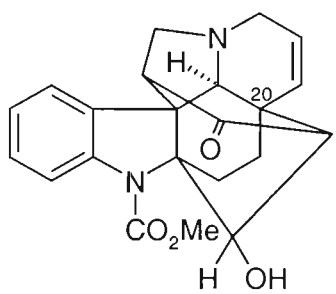
58: Secodine



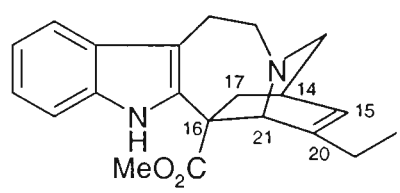
59: Vincamine



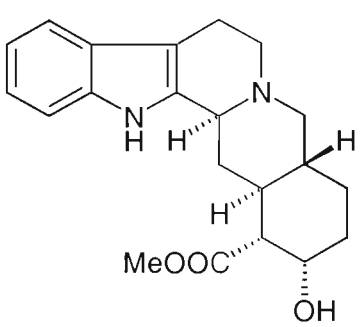
60: Vindoline



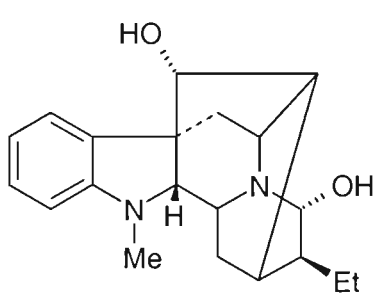
61: Fructocosine



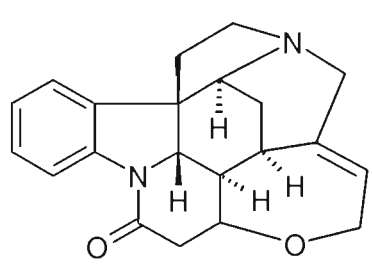
62: Catharanthine



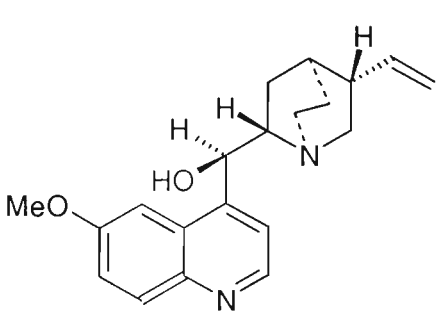
63: Yohimbine



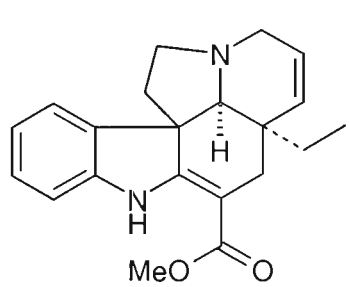
64:Ajmaline



65: Strychnine

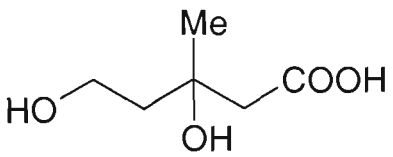


66: Quinine



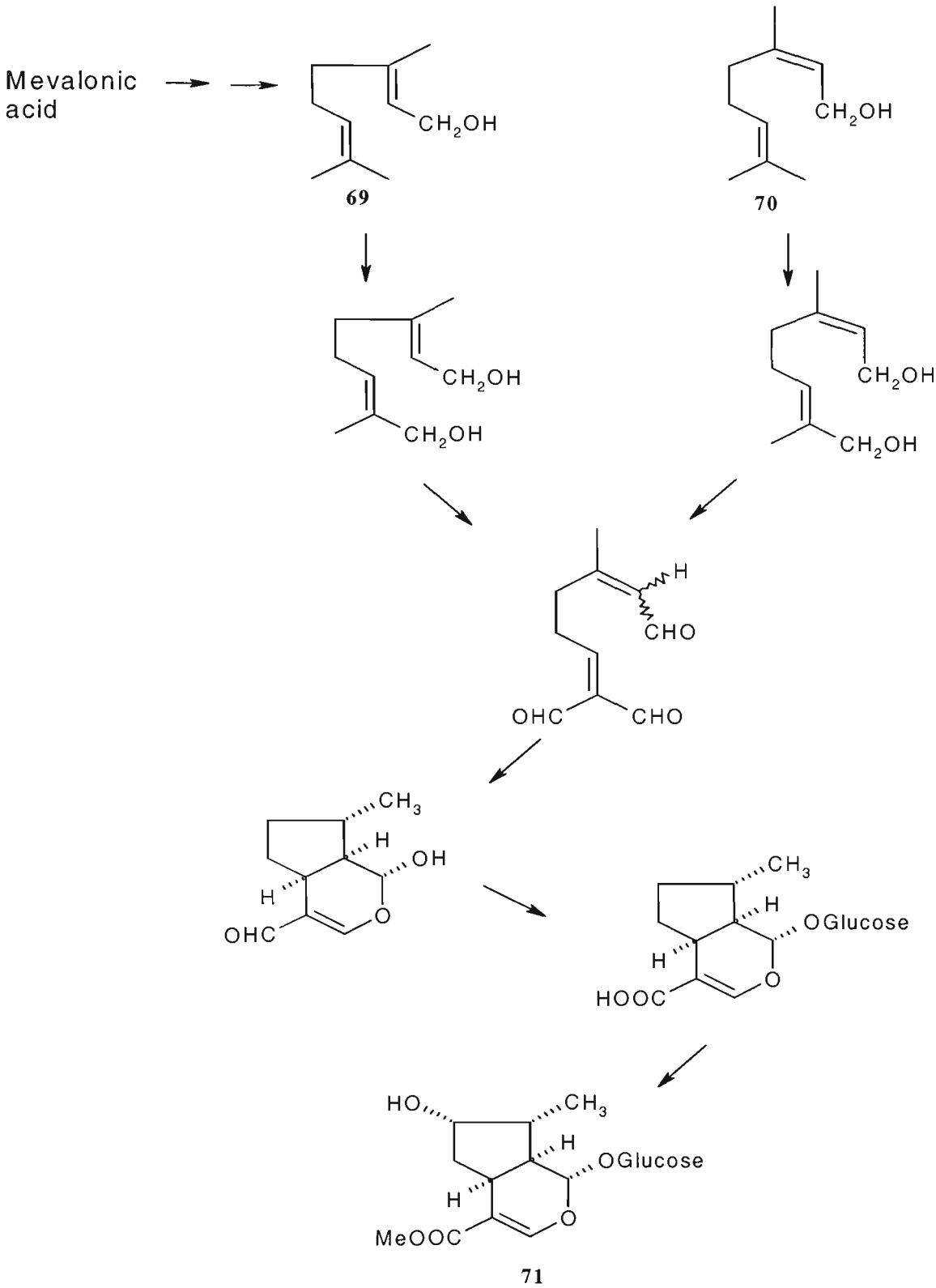
67: Tabersonine

It has been established that secologanin **54** is the source of the C_9-C_{10} unit of monoterpenoid alkaloids. Several studies have proved that the secologanin unit does not come from phenylalanine, tyrosine, melonate, acetate and formate;[106,107] shikimic acid;[108] methionine;[109] or glycine.[110] However, based on the independent investigations of Battersby;[111] Scott;[112] Arigoni;[113] and their co-workers, on a single plant *Vinca roseus*, the C_9-C_{10} unit as represented by the Corynanthe-Strychnos, Aspidosperma, and Iboga skeleton, is derived from two molecules of mevalonic acid **68**.

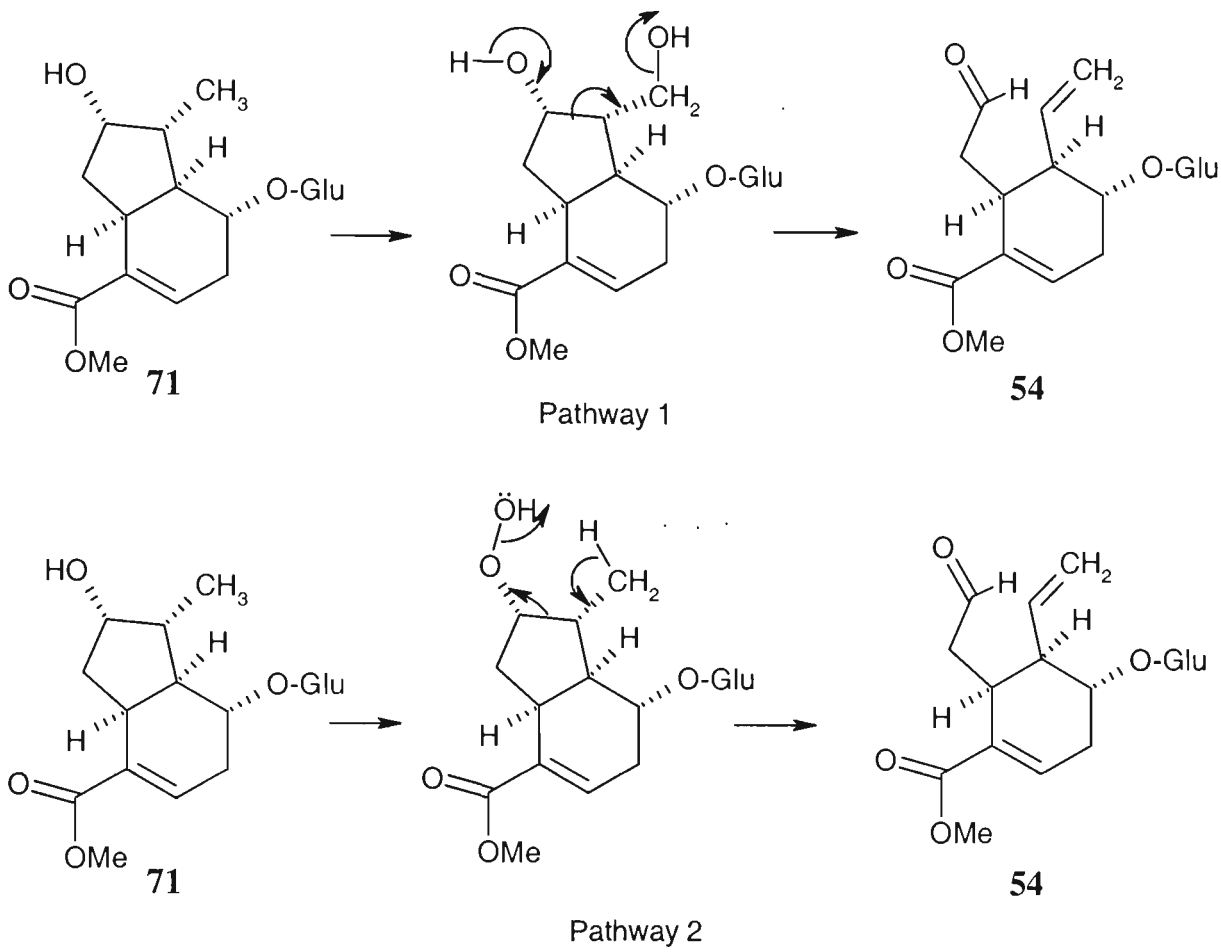


68: Mevalonic acid

As illustrated in Scheme 2, the conversion of mevalonic acid **68** to secologanin **54** involves three distinct transformations: first, the conversion of mevalonate to geraniol **69**, and its *trans* isomer nerol **70**, secondly, the transformation of geraniol **69** and/or nerol **70** into loganin **71**, and finally, the cleavage of loganin **71** to secologanin **54**. Two possible pathways for this last conversion are illustrated in Scheme 3.



Scheme 2. Transformation of geraniol **69** and nerol **70** into loganin **71**[105]

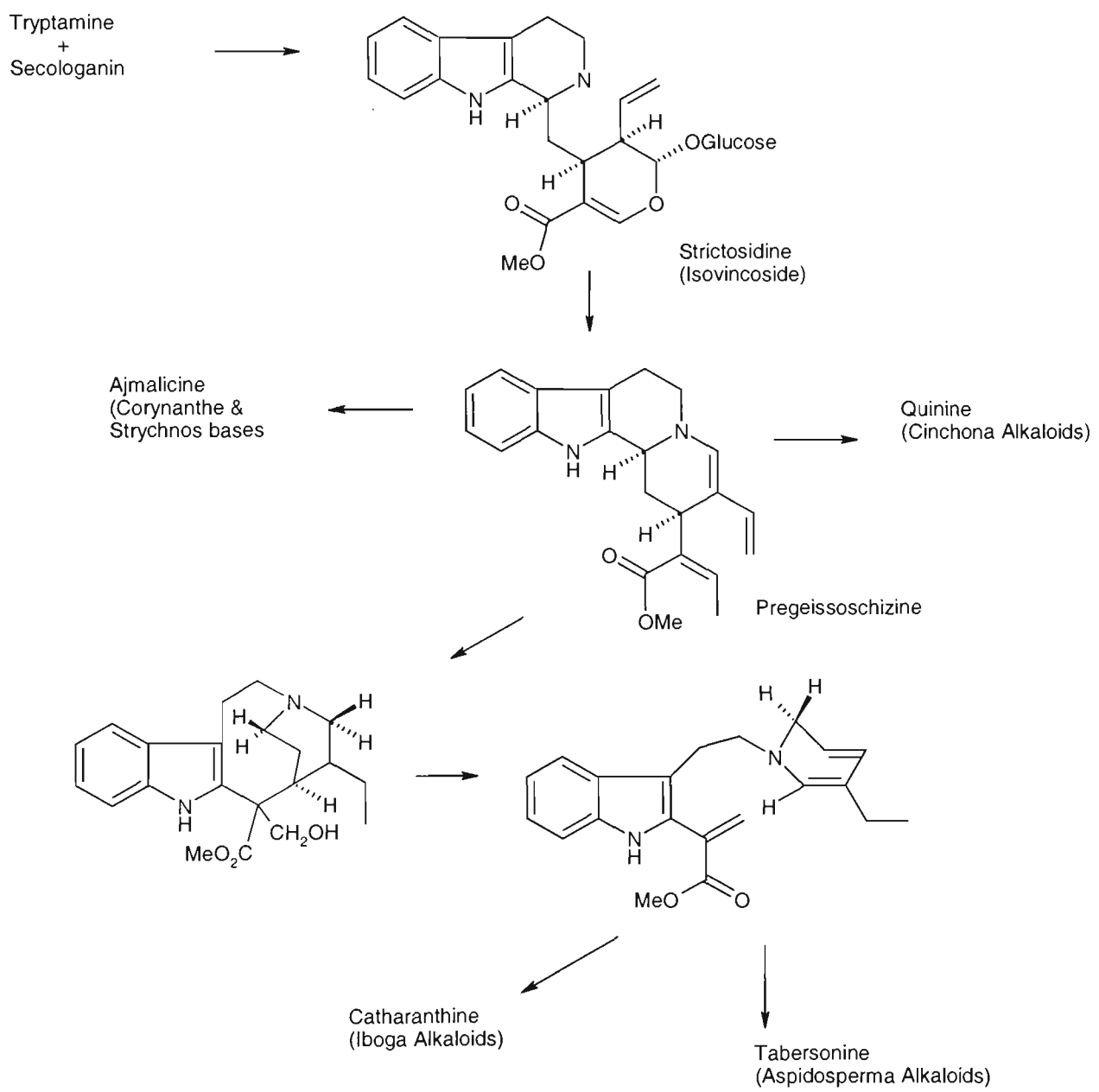


Scheme 3. Two possible pathways of the conversion of loganin **71** to secologanin [104]

Loganin **71** is a key intermediate on the biosynthetic pathway to the complex indole alkaloids and its transformation from geraniol **69** and nerol **70** is crucial in the stereochemical determination of the secologanin unit of the monoterpenoid indole alkaloids. The *trans* isomer nerol **70**, and its hydroxy derivative, are incorporated marginally better than is geraniol **69**.[105]

As previously mentioned, the condensation of tryptamine **52** and secologanin **54** leads to the formation of strictosidine **55**, and its C-3 epimer vincoside **56** (Scheme 4). Initially, it was believed that Corynanthe-Strychnos, Aspidosperma, and Iboga alkaloids were derived

from vincoside **56**, which was considered to be a product of the reaction between tryptamine **52** and secologanin **54**.[114] However, biosynthetic studies on *Catharanthus roseus* cell cultures showed that the sole product of condensation between tryptamine and secologanin **54** was strictosidine **55**.[115,116] The stereochemistry at the C-3 position was eventually resolved with biosynthetic studies conducted on a cell-free extract of *C. roseus*.



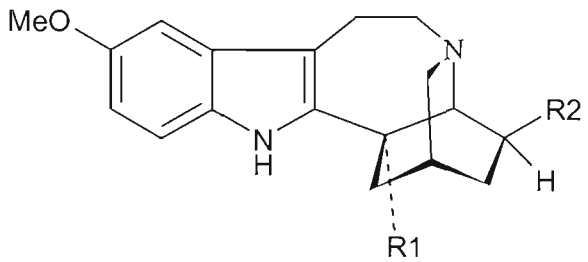
Scheme 4. Sequence of formation of the indole alkaloids as categorised by Dalton[105]

Strictosidine **55** was transformed to ajmalicine **64**, vindoline **60**, and catharanthine **62** with negligible incorporation of vincoside into these alkaloids. A summary of further elaboration of strictosidine **55** into the major classes of monoterpenoid alkaloids can be seen in Scheme 4. A review of alkaloid biosynthesis in plants, including the monoterpenoid indole alkaloids, was recently published by Facchini.[117]

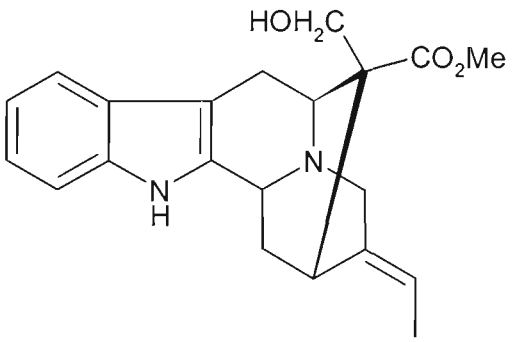
3.3. Alkaloids from the Genus Voacanga

Since the first review of indole alkaloids from the genus *Voacanga* by Taylor[118] in 1968, intensive studies of voacanga alkaloids have been carried out. The alkaloids of eight species of the genus have been reported, namely *Voacanga africana*, *V. bracteata*, *V. chalotiana*, *V. drepei*, *V. globosa*, *V. grandifolia*, *V. schweinfurthii*, and *V. thouarsii*. Alkaloids from *Voacanga* species show quite a large range of indole-based structural types.[101]

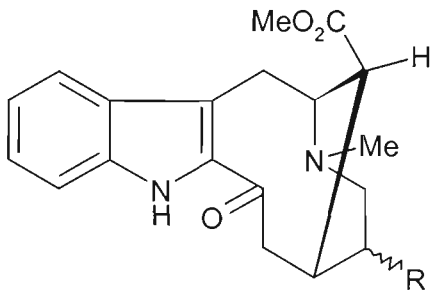
The *Voacanga* genus is a source of iboga-type alkaloids such as ibogaine **72**, voacangine **73**, voacristine **74**, and these are isolated mostly from *V. africana, V. chalotiana, and V. thouarsii*. The alkaloids of this type are also produced by Apocynaceous plants of the genera *Conopharyngia* (*Plumeria*), *Ervatamia*, *Gabunae*, *Stemmadenia*, *Tabernaemontana*, *Vinca*, *Lochnera, Catharanthus, and Tabernanthe*.[118] They are also known to be sources of the sarpagine and vobasine types, for example, voachalotine **75**, vobasine **76**, and dregamine **77**.



72: Ibogaine; R1 = H, R2 = CH_2CH_3
73: Voacangine; R1 = CO_2Me, R2 = CH_2CH_3
74: Voacristine; R1 = CO_2Me, R2 = -H, -OH, and -CH_3



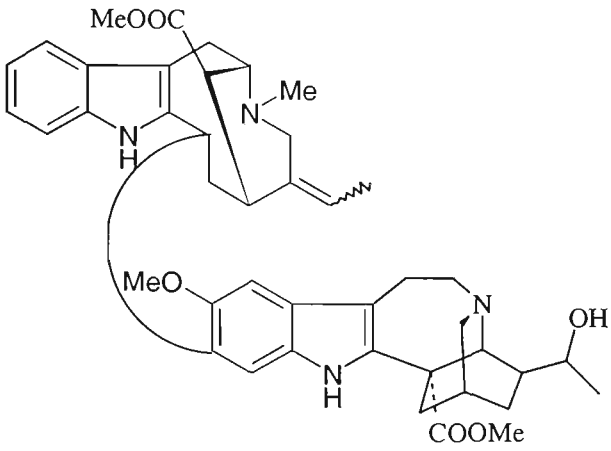
75: Voachalotine



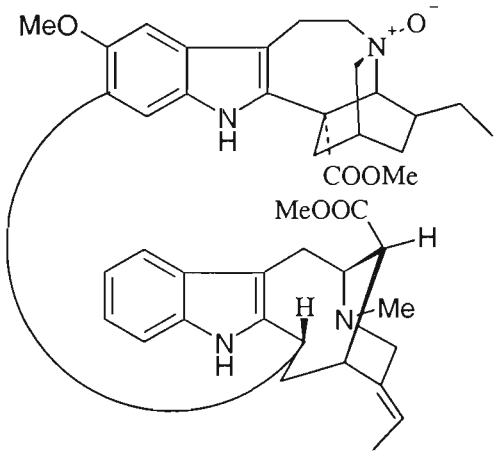
76: Vobasine; R = $CHCH_3$
77: Dregamine; R = Et

The plants *V. africana* and *V. chalotiana* have been found to produce the most significant number of alkaloids among the voacanga species. More than 28 alkaloids have been reported from *V. africana* and 15 alkaloids were found in *V. chalotiana*.[101] The genus voacanga is also well known as a source of dimeric or bisindole alkaloids. The plant *V. bracteata* contained epivoacorine **78** and voacamine N_b-oxide **79**.[119] Three bisindole alkaloids have been isolated from *V. grandifolia*, namely amataine (subsessiline) **80**, goziline (18-oxovobtusine) **81**, and voacinol **82**.[120] The plant *V. thouarsii* produces subsessiline lactone **83**, 3'-oxovobtusine **84**, 12-demethylvobtusine **85**, and 2-deoxy-3'-oxovobtusine **86**.[121,122]

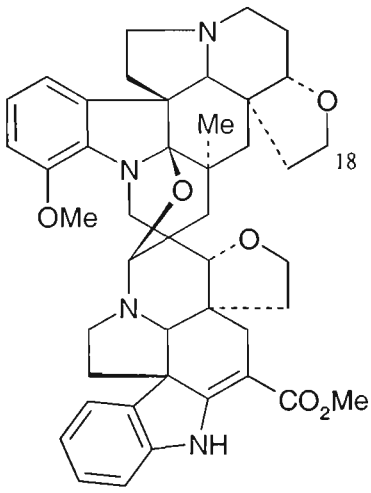
No work has been reported on the alkaloids of *Voacanga foetida* (Bl.) Rolfe, the subject of this study.



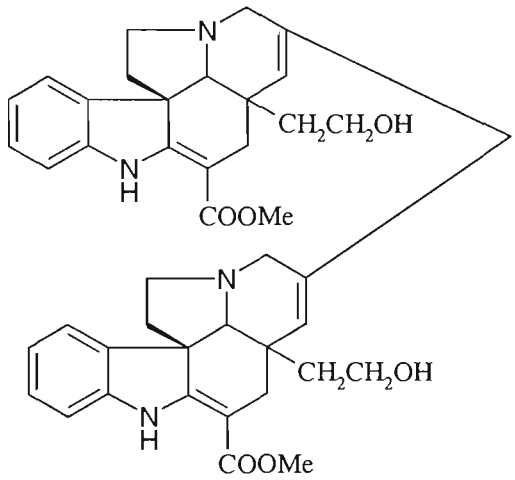
78: Epivoacorine



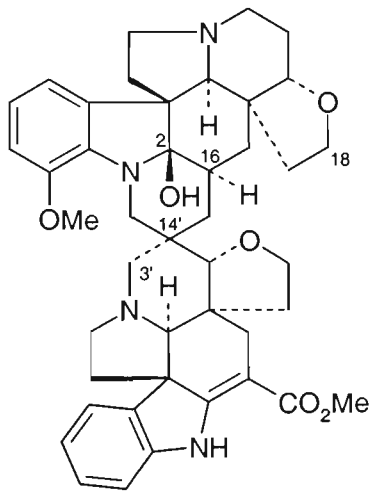
79: Voacamine *N*b-oxide



80: Subsessiline (Amataine)
83: Subsessiline lactone, 18-oxo



82: Voacinol



81: Goziline (18-Oxovobtusine)
84: 3'-Oxovobtusine
85: 12-Demethylvobtusine
86: 2-Deoxy-3'-oxovobtusine

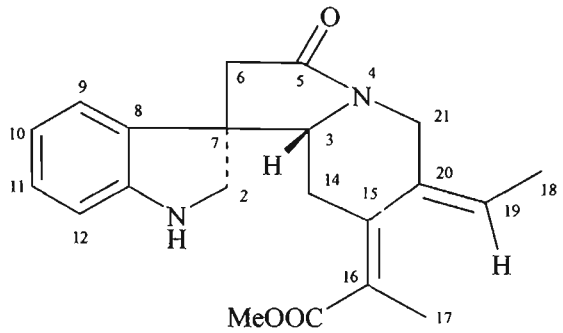
3.4. Isolation and Structural Elucidation of Alkaloids from Voacanga foetida (Bl.) Rolfe

Three different aerial parts of the plant (bark, leaves, and fruit) were subjected to extraction, followed by fractionation into acidic and neutral, water soluble, and basic (alkaloid) materials. The crude alkaloidal extract was then separated by multiple PTLC on silica gel with dichloromethane (CH_2Cl_2), methanol (CH_3OH), and aqueous ammonia (NH_4OH) as a mobile phase in various concentrations. From the bark, a new alkaloid, which the author has named lombine, was isolated together with a known alkaloid voacangine. The fruit produced coronaridine as a major alkaloid component, as well as four minor compounds, voacangine, voacristine, and mataranine A and B. The last two compounds were also isolated as new indole alkaloids from young trees of *Alstonia scholaris* R. Br. as discussed in Chapter 5. The known alkaloid voacristine was found to be a dominant alkaloid in the leaves of *V. foetida* (Bl.) Rolfe while voacangine was a minor component.

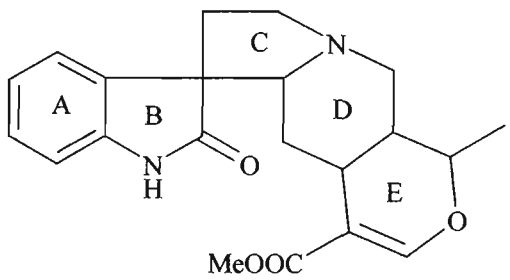
The following section discusses the structural elucidation of the above isolated compounds.

3.4.1. Lombine

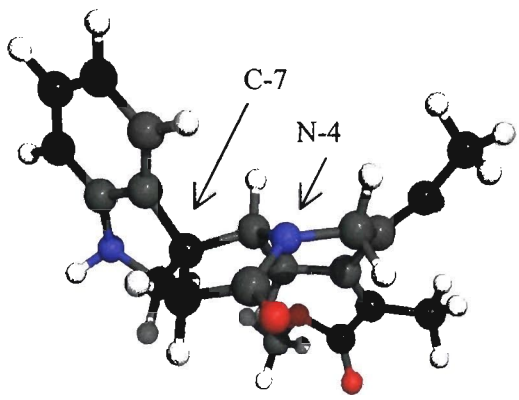
The new optically-active indole alkaloid, named lombine **87**, was obtained as a yellow solid. The new compound was found to have similar spectral properties in some respects to the oxindole uncarine **88**, which has seven known natural stereoisomers, and is one of the major alkaloidal components found in species of *Uncaria* (Rubiaceae).[123-125]



87



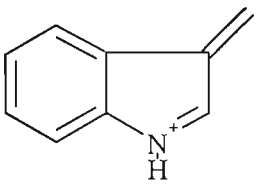
88



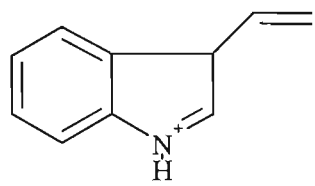
3D-Lombine model (N-blue; O-red; C-black; H-white)

Low resolution chemical ionisation mass spectrometry (LRCIMS) of alkaloid **87** confirmed a molecular mass of 352 (16 amu less than that of uncarine **88**) with the base peak at m/z 353 $[MH]^+$. High-resolution chemical ionisation mass spectrometry (HRCIMS) analysis indicated that the molecular formula was $C_{21}H_{24}N_2O_3$ and hence an index of hydrogen deficiency of eleven. Low resolution electron impact mass spectrometry (LREIMS) (see Appendix 1) confirmed the presence of the unsubstituted indolic moiety with characteristic[126] fragment ions at m/z 130 (**1**) and 144 (**2**) (Figure 3). The presence of methyl and carbomethoxy groups was indicated by the existence of peaks arising from fragment ions in the LREIMS at m/z 337 (M-15 amu) and 293 (M-59 amu) respectively,

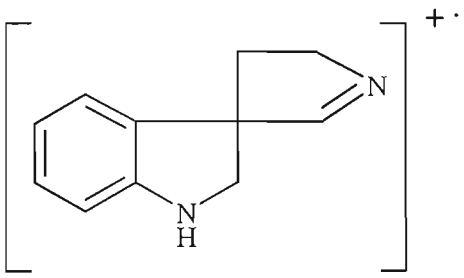
and in the IR spectrum (Appendix 2) a sharp absorption band at 1733 cm^{-1} further supported the presence of an ester functionality. The IR spectrum also suggested the presence of a lactam group in **87**, with a sharp absorption at 1685 cm^{-1}. The structure assigned to alkaloid **87** was also reinforced by two fragment ions at m/z 172 and 208 suggesting the presence of skeleton fragments **3** and **4** (Figure 3) in the compound **87**. These two fragments may possibly arise from ring C opening and further re-arrangement.



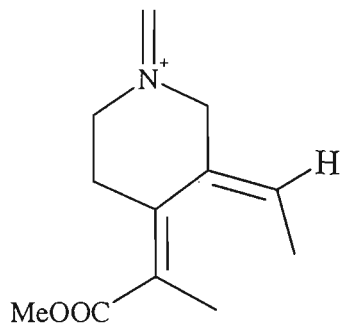
1: m/z 130



2: m/z 144



3: m/z 172



4: m/z 208

Figure 3. Selected fragment ions of lombine **87**

The final structural elucidation of **87** was performed by comparing some characteristics of the NMR spectra of **87** and **88**, and utilizing the combined spectroscopic data obtained from ^{1}H-NMR, gCOSY, selective decoupling, NOESY1D, gHSQC (Heteronuclear Single Quantum Coherence), and gHMBC (Heteronuclear Multiple Bond Correlation)

experiments. Table 3 presents a summary of ^{1}H- and ^{13}C-NMR assignments for **87** obtained by these combined spectra.[a]

The ^{1}H-NMR spectrum (Appendix 3) contained peaks associated with four aromatic protons at δ7.59 (d, 1H, J = 8.5 Hz, H-9), δ7.40 (d, 1H, J = 8.0 Hz, H-12), δ7.35 (t, 1H, H-11), and δ7.15 (t, 1H, H-10). From the gCOSY spectrum (Appendix 4), the correlation of each proton was established. The signal of a doublet at δ7.59 (H-9) showed coupling to a triplet at δ7.15 (H-10). The low field doublet at δ7.59 for proton H-9 was deshielded by the lone pair of electrons on the N-4 nitrogen, and it was thus concluded that the alkaloid possessed the C-7α configuration.[123] Another doublet at δ7.40 (H-12) was found to couple to the triplet at δ7.35 (H-11). The signal for an NH group appeared as a broadened singlet at δ8.92 while the presence of an adjacent methylene at C-2 appeared as two doublets at δ3.62 ($J_{2\alpha,2\beta}$ = 16.0 Hz) and δ2.89 ($J_{2\beta,2\alpha}$ = 16.0 Hz). These were assigned to the respective H-2α and H-2β protons, which correlated in the gCOSY spectrum. A pair of AB doublets at δ3.07 ($J_{6\alpha,\,6\beta}$ = 16.0 Hz) and δ2.44 ($J_{6\beta,\,6\alpha}$ = 16.0 Hz) respectively were attributed to the 6α and 6β protons. Another set of AB doublets occurred at δ3.44 and 2.26 which were assigned to the respective H-21α ($J_{21\alpha,21\beta}$ = 11.5 Hz) and H-21β ($J_{21\beta,21\alpha}$ = 12.0 Hz) protons. The C-3 proton resonated as a doublet at δ 3.53 ($J_{3,14\alpha}$ = 10.5 Hz) which was coupled to a broad triplet at δ 3.10 ($J_{14\alpha,3}$ = 10.5 Hz; $J_{14\alpha,14\beta}$ = 10.5 Hz) which in turn was correlated further to another broadened doublet at δ 2.59 ($J_{14\beta,14\alpha}$ = 10.5 Hz). The C-14 protons resonated at low field since their signals were affected by the presence of the C15-

[a] For consistency, the numbering of atoms in this structure (and all later structures) was established on the basis of those used in known related indole alkaloids as referenced in the Dictionary of Alkaloids[101]

16 double bond and the close proximity to the carbonyl group of the methyl ester. A doublet representing three protons at δ1.59 ($J_{18\text{-}19}$ = 6.5 Hz) was assigned to the C-18 methyl group, which correlated to a one-proton quartet at δ5.43, ascribed to H-19. The carbomethoxy group resonated as a singlet integrating for three protons at δ3.59. The secondary methyl was observed as a singlet at δ2.31 (H-17), with a relative integral appropriate for three protons.

Table 3. ^{1}H- and ^{13}C-NMR data of Lombine **87**

Carbon no.	Chemical shift	Protons	Chemical shift (multiplicity)	Integration	Coupling constant (Hz)
		NH	8.92 bs	1H	
2	31.1	H-2α	3.62 d	1H	$J_{2\alpha,2\beta}$ = 16.0
		H-2β	2.89 d	1H	$J_{2\beta,2\alpha}$ = 15.5
3	34.1	H-3	3.53 d	1H	$J_{3,14\alpha}$ = 10.5
5	193.1				
6	43.9	H-6α	3.07 d	1H	$J_{6\alpha,6\beta}$ = 15.5
		H-6β	2.44 d	1H	$J_{6\beta,6\alpha}$ = 15.5
7	49.2				
8	136.5				
9	120.5	H-9	7.59 d	1H	$J_{9,10}$ = 8.5
10	120.1	H-10	7.15 t	1H	
11	126.4	H-11	7.35 t	1H	
12	112.1	H-12	7.40 d	1H	$J_{12,11}$ = 8.0
13	132.5				
14	61.1	H-14α	3.10 bt	1H	$J_{14\alpha,3}$ = 10.5; $J_{14\alpha,14\beta}$ = 10.5
		H-14β	2.59 bd	1H	$J_{14\beta,14\alpha}$ = 10.5
15	133.5				
16	119.4				
17	45.9	H-17	2.31 s	3H	
18	12.5	H-18	1.59 d	3H	$J_{17,16}$ = 6.5
19	121.3	H-19	5.43 q	1H	$J_{16,17}$ = 7.0
20	136.3				
21	61.7	H-21α	3.44 d	1H	$J_{21\alpha,21\beta}$ = 11.5
		H-21β	2.26 bd	1H	$J_{21\beta,21\alpha}$ = 12.0
OMe	52.4		3.59 s	3H	
COOMe	173.7				

To reinforce the confirmation of the peak assignments in the ^{1}H-NMR spectrum, a comprehensive series of homodecoupling experiments was carried out. Irradiation of the doublet at δ2.89 (H-2β) led to a transformation of the doublet assigned to the H-2α proton (δ3.62) into a singlet. Irradiation of the doublet observed for the C-3 proton (δ 5.53) resulted in the conversion of four doublets at δ 3.10 (H-14α), δ 2.59 (H-14β), δ 3.07 (H-6α), and δ2.44 (H-6β) into singlets. This is thought to have occurred due to an overlapping of the proton signals assigned to H-14α and H-6α. A singlet was observed at δ2.89 (H-2β) when the proton at δ3.62 (H-2α) was irradiated. Irradiation of a quartet representing a single proton at δ5.43 (H-19) caused the doublet at δ1.59 (H-17), attributable to three protons, to become a singlet. No change of ^{1}H-NMR spectra occurred when a three-proton singlet at δ2.31 (H-17) was irradiated, suggesting that the methyl group (H-17) is not coupled directly to any other protons in the compound.

Correlation between protons and carbons was achieved by using gHSQC and gHMBC experiments (see Appendix 5 and 6 respectively for the spectra). This set of data was consistent with the structure **87** suggested for lombine. From the gHMBC spectrum, the proton at δ 2.44 (H-6β) showed cross peaks at δ193.1 (C=O, C-5), δ132.5 (C-13), δ49.2 (C-7), 136.5 (C-8), and 34.1 (C-3), which confirmed the position of C-6 in **87**. From the computer-based 3D-lombine model (see page 51) generated by the Spartan Program, the lactam carbonyl is deshielded by the aromatic ring A of the indole moiety, thus explaining its low field ^{13}C-chemical shift. The location of the ethylidene group on **87** was established by the evidence that a quartet proton at δ 5.43 (H-19) showed a cross peak indicating a

coupling to δ61.7 (C-21), to the methyl (δ12.5, C-18), and to δ34.1 assigned as C-3. Determination of the carbomethoxy locality was a difficult task since the signal at δ3.59 (OMe) overlapped with δ3.62 (H-2α) causing a mixture of HMBC cross peaks to be observed. However, by a process of elimination, the determination of the carbomethoxy position was possible. The signal resonating at δ3.59 (OMe), coupled to 173.7 (C=O), and to δ119.41 assigned as C-16 and to δ133.5 assigned as C-15. The structural position of the other methyl group (C-17) resonating at δ2.31 (H-17) presented another problem due to its proton signal being coupled not only to the carbon at δ61.1 (C-14) but also to the carbon at δ61.7 (C-21). A weak correlation was observed from δ3.10 (H-14α) to δ136.5 (C-8) suggesting a long range coupling occurring between H-14 and C-8. The ion fragmentations previously mentioned (in the mass spectrum) and a possible biosynthetic pathway discussed later give further support to the carbomethoxy and secondary methyl localities and the overall structure suggested for **87**.

From the NOESY 1D spectrum, an NOE was observed between δ1.59 (H-18) and δ3.44 (H-21), suggesting confirmation of the ethylidene position in the proposed structure **87**. Between H-17 and H-14, no NOE was observed which might suggest that H-17 is not close to the C-14 protons as shown on the structure **87**. However, lack of proximity may not be the only possible explanation for the non-existence of any NOE between two protons and this observation should be considered when dealing with NOE experiments.

To complete the stereochemical elucidation of **87**, the IR spectrum was studied. From the IR spectrum on **1**, Bohlmann bands were observed, indicating that the lone pair of electrons

on the *N*-4 nitrogen is *trans* diaxially disposed to at least two hydrogens on adjacent carbons. For most uncarine type alkaloids, the Bohlmann band is also attributed to the C-3 proton being trans to the N-4 lone pair.[127]

The new alkaloid lombine **87** may derive biosynthetically from the uncarine-type alkaloid skeleton (cf. **88**). The pyran ring E could be ring opened by acid hydrolysis, and oxidation process takes place in C-5.

3.4.2. Voacangine

Voacangine **89**, a minor component in the aerial parts of the plant *V. foetida*, was isolated as a yellow solid. It absorbed UV light with maxima at 272 ($\log\varepsilon_{max}$ = 3.789), 286 ($\log\varepsilon_{max}$ = 3.835), 293 ($\log\varepsilon_{max}$ = 3.767), characteristic of a substituted indole moiety.[127] The identification of this compound was based on ion fragmentations by LREIMS (Appendix 7 for the spectrum), which showed most of the simple ion fragments observed for voacangine.[127] Several important fragment ions of voacangine **89** are depicted in structures a-e of Figure 4. From the ^{1}H- NMR spectrum (Appendix 8), the pattern of peaks in the aromatic region was identical to that reported[127] for voacangine suggesting the presence of a 2,3,5-substituted indole nucleus. The other peaks are not presented due to the weakness of the ^{1}H-NMR spectrum observed as a result of the small amount of material available as well as the presence of a significant amount of impurities. The molecular formula of **89** was confirmed by HRCIMS.

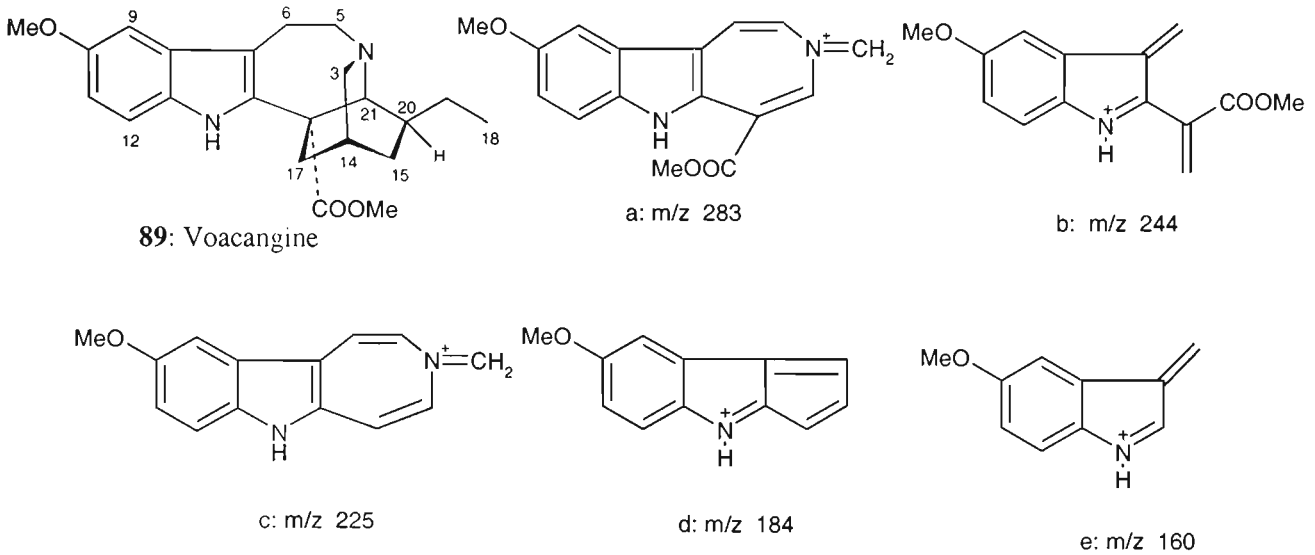
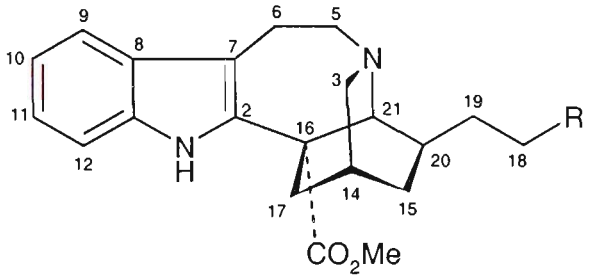


Figure 4. Selected fragment ions of voacangine **89**

Voacangine has been reported to exhibit antimycobacterial activity.[128] It was also shown to have significant analgesic and hypothermic effects in mice at oral doses of 25 mg/kg.[129] Bert *et al.*[130] demonstrated the CNS-stimulating activity of some iboga type alkaloids and suggested that the presence of methoxy substituents increased the activity, while it was lowered by the presence of methoxycarbonyl groups.

3.4.3. Coronaridine

Coronaridine **90** was isolated as a yellow amorphous solid absorbing UV at λmax 283 and 310 nm consistent with an indole chromophore. Initial identification of the sample was achieved using the ion fragmentation patterns observed in the LREIMS spectrum (Appendix 9), which showed the sample's ion fragmentation pattern was identical to that of coronaridine. The molecular formula of coronaridine was obtained by HRCIMS and found to be $C_{21}H_{26}N_2O_2$ (measured 339.2069, calc. 339.2073, for MH^+).



90: Coronaridine (R = H)
91: 18-Hydroxycoronaridine (R = OH)

Further structure elucidation was carried out by utilizing ^{1}H-NMR and gCOSY experiments and by comparing the compound's spectrum with that of an authenticated sample of a known[131] analogous compound, namely 18-hydroxycoronaridine **91**, as shown in Table 4.

From the ^{1}H NMR spectrum (Appendix 10), the compound clearly had an indole moiety, with a characteristic signal for an N-H and a 1,2,3,4-aromatic proton pattern. The signal for the N-H appeared as a broad singlet at δ7.75 while the aromatic signals appeared as a doublet at δ7.48 (H-12) which correlated to a triplet at δ7.08 (H-11); the triplet ascribed to H-11 also coupled to another triplet at δ7.14 (H-10) as shown by the gCOSY spectrum (Appendix 11). Another doublet at δ7.24 (H-9) correlated to the triplet at δ7.14 (H-10). The presence of an ethyl group in **90** was deduced from the appearance of a triplet at δ0.90 (H-18), with an integral appropriate for three protons, coupled to two multiplets resonating at δ1.44 and δ1.32 respectively (H-19, H-19'). Furthermore, a cross peak confirmed coupling between the C-19 protons and a multiplet at δ1.13 (H-20), correlating in turn with a multiplet at δ3.56 (H-21) and two multiplets at δ1.88 and δ1.59 assigned as C-15 protons. The C-3 protons appeared as a multiplet at δ2.93 and a broadened doublet at δ2.81 (J = 8.0 Hz), from which both signals correlated to a broad singlet at δ1.91 (H-14). The proton at C-14 gave a correlation to a broadened singlet at δ1.88 (H-15). A proton signal ascribed to a

proton at C-5 appeared to overlap with a C-6 proton giving a multiplet signal at δ3.15-3.23. Other proton signals of C-5H and C-6H appeared at δ3.38 and δ3.02 respectively.

Table 4. The comparison of ^{1}H-NMR spectrum of coronaridine sample and that of reference **91**

Protons	Chemical shift (δ), multiplicity, *J* in Hz	
	Sample*)	Reference[131]
3	2.93 m	2.95 ddd, 2.5, 3.5, 9.3
	2.81 bd, 8.0	2.81 brd, 9.3
5	3.38 m	3.40 m
	3.15-3.23 m	3.22-2.98 m
6	3.15-3.23 m	3.22-2.98 m
	3.02 m	3.22-2.98 m
9	7.24 d, 8.0	7.25, d, 8
10	7.14 t	7.16, ddd, 8, 8, 1.5
11	7.08 t	7.09, ddd, 8, 8, 1.5
12	7.48 d, 8.0	7.47 d, 8
14	1.91 bs	1.95 bs
15	1.88 bs	1.87-1.77 m
	1.59 m	1.65 m
17	2.58 bd, 13	2.60 brd
	2.09 m	1.97 m
18	0.90 t	**)
19	1.44 m	**)
	1.32 m	**)
20	1.13 m	**)
21	3.56 bs	3.63 brs
$COO\underline{CH_3}$	3.71 s	
NH	7.75 bs	7.89 bs

*) assignment from ^{1}H and gCOSY experiments

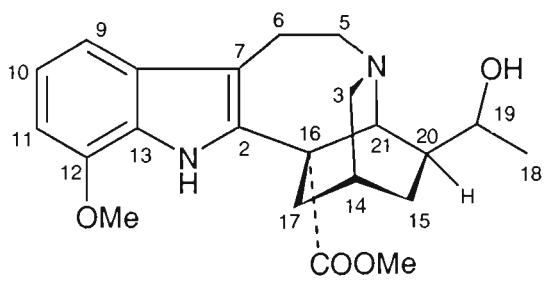
**) not-comparable

Coronaridine **90**, an iboga alkaloid isolated from *Ervatamia* species, was first reported by Gorman *et al.*[132] Iboga alkaloids arise biogenetically from tryptophan or its equivalent and two head-to-tail mevalonate residues.[114] Further details are discussed in Section 3.2 of this chapter.

Coronaridine was reported to have potent antileishmanial activity, inhibiting promastigote and amastigote growth.[133] Some iboga alkaloids including coronaridine have been found showing anti-addictive properties.[134] It has also been reported that coronaridine, like voacangine, produced analgesic and hypothermic effects in mice.[134] However, coronaridine was also found to display cytotoxic activity.

3.4.3. Voacristine

A brown solid was also isolated from the alkaloid mixture, which was found to be the alkaloid, voacristine **92**. This compound absorbed UV light with maxima at 278 and 298 nm, characteristic of the presence of a ring A-substituted indole nucleus. The LRCIMS for voacristine showed a major peak at 385 (MH^+) while EIMS produced peaks at m/z 384, 366, 339, 244, and 184 (Appendix 11a). Initial identification of this compound was based on its ion fragmentation pattern, which was characteristic of iboga type alkaloids. HRCIMS indicated the formula $C_{22}H_{28}N_2O_4$ (found 385.2104, calc. 385.2127, for MH^+), supportive of voacristine **92**. The structural assignment of **92** was then established from ^{1}H-NMR, gCOSY, HSQC, HMBC experiments (see Appendix 12, 13, 14, and 15 respectively for the spectra) and by comparison of the spectra of **92** with the spectra of (*19R*)-voacristine.[131] To this author's knowledge, however, this is the first report to elucidate the structure of voacristine on the basis of 2D-NMR experiments.



92: Voacristine

As a starting point for the structural confirmation of voacristine, focus was placed on the aromatic region. The signal corresponding to the NH proton, existed as a broad singlet at δ7.73. Also in this region of the spectrum, other signals were evident which were typical of a substituted indole moiety. Closer inspection of the aromatic signals permitted the assignment of H-10 as a multiplet at δ7.32 integrating for one proton, and another multiplet centralised at δ6.78 integrating for two protons, and ascribed as H-9 and H-11 respectively. The gCOSY confirmed the connectivity between the two signals, while the gHSQC spectrum provided a straightforward identification of the attached carbons resonating at δ7.32 (H-10)/ δ119.3 (C-10); δ6.78 (H-9) and (H-11)/ δ109.5(C-10 and C-12). The methoxy group was found to be attached at the C-12 position instead of C-10 as reported in the literature;[131,135] It is possible that the alkaloid isolated in our work is an isomer of voacristine, or alternatively the original structure is incorrect. The original data is from a 60 MHz NMR spectrometer and it was difficult to make a detailed comparison of the ^{1}H-NMR spectra in the aromatic region. The position of the methoxy group was confirmed by a gHMBC experiment, as evidenced by a weak cross peak between δ7.37 (H-10) and carbon signal at δ140.6 (C-13). The position of the quaternary carbon, C-13, was determined by comparison with the spectra of analogous compounds.[136] The carbon signal of the methoxy substituent was observed at δ56.0 according to the gHSQC spectrum. Other indole alkaloids, e.g. fuchsiaefoline, are known[268] with a methoxy group in the same position as proposed structure **92**.

Another aid in the confirmation of the structure was the doublet corresponding to a methyl group resonating at δ1.28 (3H, H-18) coupled to a carbon signal at δ22.4 (C-18). The signal

of H-18 showed a cross peak to a multiplet at δ3.91 (1H, H-19) having a correlation to a carbon signal at δ70.8 (C-19). This proton appeared at a relatively low field suggesting its attachment to a carbon with a hydroxyl substituent. The C-19 proton also showed a cross peak to a multiplet at δ1.40 (1H, H-20) connected to δ24.2 (C-20). This pattern indicated the presence of a (CH_3-CH(OH)-CH-) fragment in voacristine. The C-20 proton gave rise to a COSY cross peak indicating a coupling to a multiplet at δ4.08 (H-21) correlated to δ54.5 (C-21) and also to methylene protons at δ1.84 and 1.70 (H-15, H-15'), which had a correlation to δ24.1 (C-15).

The proton signals at δ3.02, which shared a cross peak with a signal at δ2.82, were assigned as H-3, 3' and were connected to a carbon resonating at δ50.8 (C-3), while a peak at δ3.02 (H-3) showed a correlation with a proton signal at δ2.03 (H-14) and also to a carbon signal at δ24.1 (C-14). The C-14 proton gave a cross peak to δ1.96 (H-17) coupled to a proton signal at δ2.55 (H-17), while the gHSQC spectrum showed both signals had cross peaks indicating that they were coupled to a carbon signal at δ36.8 (C-17).

The protons attached to the C-5 carbon, adjacent to N_b, appeared as two multiplets centred around δ3.43 (H-5) and δ3.15 (H-5') and were coupled to the ^{13}C signal at δ52.1. The two protons (H-5) were observed to have a connection to δ3.12 (H-6), which showed a further a cross peak indicating a coupling to the proton signal at δ3.06 (H-6). The gHSQC spectrum showed H-6 was connected to a carbon signal at δ21.7.

Table 5. Assignment of ^{1}H and ^{13}C-NMR data of voacristine **92** by gCOSY, gHSQC and gHMBC

Position	δH (ppm), multiplicity, *J* (Hz)	Reference (δH (ppm), multiplicity, *J*)[131]	δC (ppm)
3	3.02 m	3.02 ddd, 2.4, 3.6, 9.2	50.8
	2.82 bd, 8.5	2.82 bd, 9.2	
5	3.43 m	3.40 m	52.1
	3.15 m	3.25-3.0 m	
6	3.12 m	3.25-3.0 m	21.7
	3.06 m		
7			123.0
8	-		136.2
9	6.78 m	6.92 d, 2.5	109.5
10	7.32 m	-	119.3
11	6.78 m	6.48 dd, 2.5, 8.8	109.5
12		*)	156.9
13	-		140.6
14	2.03 bs	2.03 bs	24.1
15	1.84 m	1.81-1.73 m	28.8
	1.70 m	1.81-1.73 m	
17	2.55 bd, 13.5	2.58 bd, 13.5	36.8
	1.96 m	1.97, ddd, 13.5, 4.0, 2.4	
18	1.28 d, 6.0	1.28 d, 6.6	22.4
19	3.91 m	3.89 dq 2.4, 6.6	70.8
20	1.40 m	1.42 dddd, 10.2, 8.1, 2.4, 0.5	24.2
21	4.08 bs	4.09 bs	54.5
NH	7.73 bs	*)	
OMe	3.73 s	*)	56.0
COOMe	3.83 s	*)	52.4
COOMe		*)	175.0

*) Not mentioned in the reference[131]

The positions of the quaternary carbons were determined by a gHMBC long range carbon coupling experiment. In the aromatic region, the gHMBC spectrum showed the proton signal at δ7.32 (H-10) coupled to carbon signals at δ156.90 (C-9), δ140.6 (weak, C-13), δ136.2 (C-8), and δ109.5 (C-9a and C-11). The presence of a quaternary signal at δ156.9

suggested methoxyl substitution at this carbon in the aromatic ring.[136] The proton signal at δ6.78 correlated to δ156.9, δ136.4, δ123.0, and δ109.5. From here it can be suggested that quaternary carbons at C-7 and C-8 gave rise to signals at δ123.0 and δ136.4 respectively. The signal attributable to the protons associated with the methyl ester moiety was observed as a singlet at δ3.83, which correlated to the peak at δ175.0 (C=O). The NMR spectroscopic data for the compound are summarised in Table 5.

Voacristine obtained from *V. africana*, was first reported by Renner and Thomae in 1957.[137] Tremorigenic activity has been observed in several iboga alkaloids. Singbarti *et al.*[138] studied the change in activity against change in functional groups for various intracerebrally injected tremorigenic indole alkaloids including voacristine and found that the tremorigenic potency was increased by the presence of a methoxyl group and decreased by a hydroxyl or carbomethoxy group.

Chapter 4

Alkaloids from *Psychotria malayana* Jack.

Leaves of *P. malayana* Jack.

P. malayana Jack. trees

Chapter 4

ALKALOIDS FROM *Psychotria malayana* Jack.

4.1. Introduction

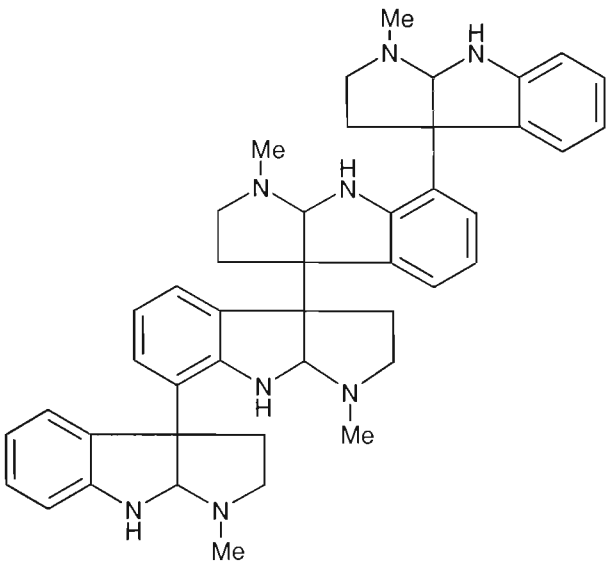
Psychotria (Rubiaceae) is a very large genus, but according to published information available before the 1980s, it appears to be relatively unimportant medicinally, although several species have been used in poultices for sores, ulcers, skin disorders, and also in connection with parturition.[63] However, since the 1990s, several papers have reported that alkaloids found from *Psychotria* species have cytotoxic, antibacterial, and analgesic properties.[139-141] The species recorded are distributed in many tropical regions, such as Indo-China, Malaysia, the Philippines, and the Solomon islands. In Indonesia, on Lombok island in particular, *Psychotria malayana* Jack is found.

Psychotria malayana Jack is a small tree, locally known as "lolon jarum" that grows to a height of 1-4 m, and is largely distributed in the west Indonesian archipelago. In Java, there has been no documentation of local use of this plant for medicinal purposes.[142] However, people in Lombok have utilised it in the form of aqueous extracts of either the leaves or bark, for protecting the skin from infection, for treating open wounds and for other skin diseases. To date, there has not been any published report dealing with the chemical constituents of the plant. From our initial investigation, alkaloids were found to be concentrated in the leaves and bark.[16]

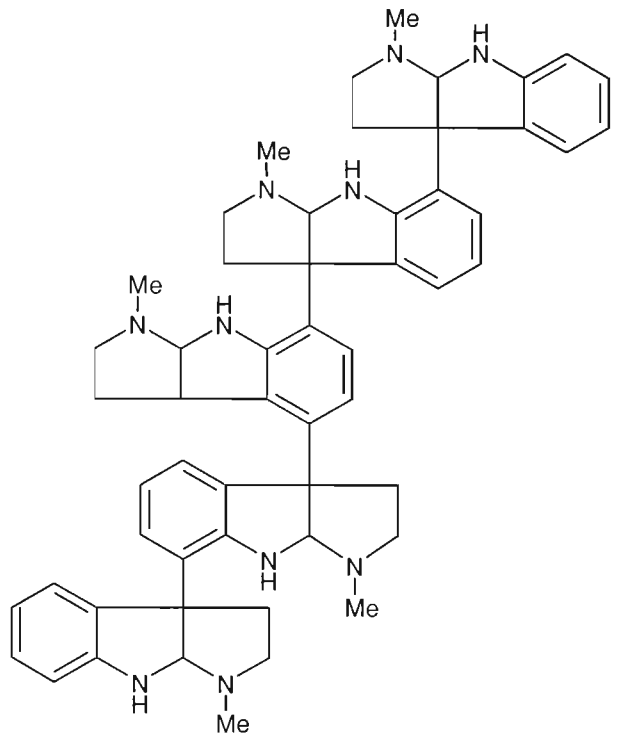
4.2. Alkaloids from the Genus Psychotria

The genus *Psychotria* is well known as a source of pyrrolidinoindoline alkaloids in monomeric, dimeric, trimeric and higher polymeric forms. This genus has contributed several novel indole-type alkaloids with quite a broad spectrum of bioactivities. The alkaloids from at least eight species from the genus have been reported and are reviewed below.

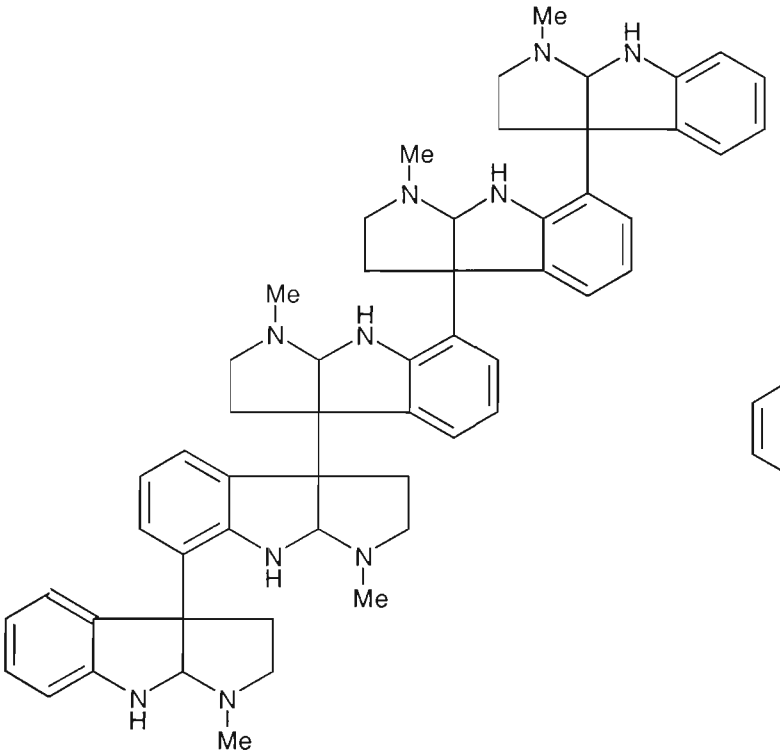
Libot *et al.*[143] have reported the isolation of three new pyrrolidinoindoline alkaloids from *Psychotria oleoides*, which are named quadrigemine C **93**, isopsychotridine B **94**, and isopsychotridine A **95**. Two known alkaloids were also isolated, psychotridine **96** and hodgkinsine **97**. Jannic *et al.*[144] have independently reported that this species produced three other new alkaloids, quadrigemine I **98**, oleoidine **99**, and caledonine **100**. Moreover, following a bioactivity-guided purification of the methanol extract of this plant, a new alkaloid, psycholeine **101**, was isolated by Gueritte-Voegelein *et al.*[145] They reported that psycholeine interacts with somatostatin receptors and exhibits a somatostatin antagonistic activity on growth hormone secretion by pituitary cells in a primary culture.



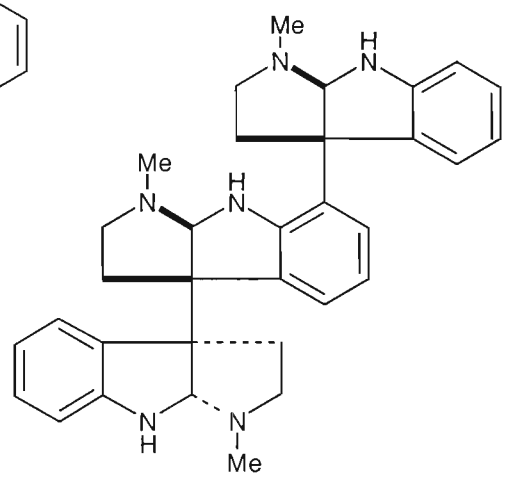
93: Quadrigemine C



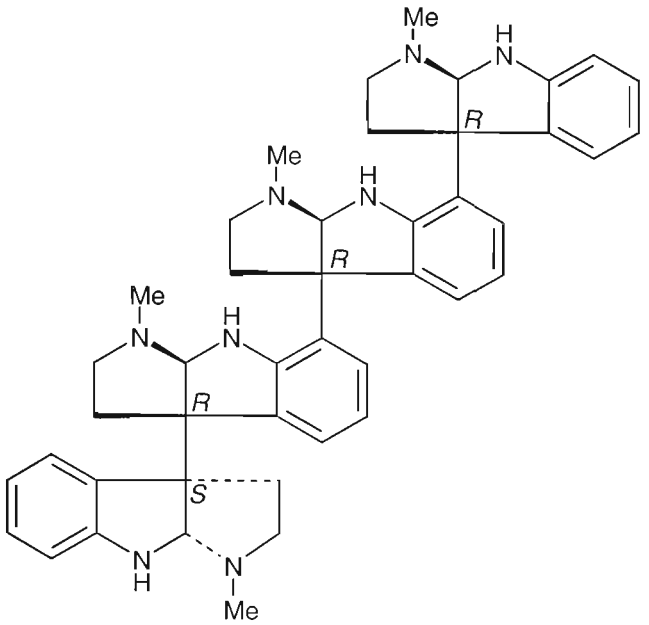
94: Isopsychotridine B
95: Isopsychotridine A



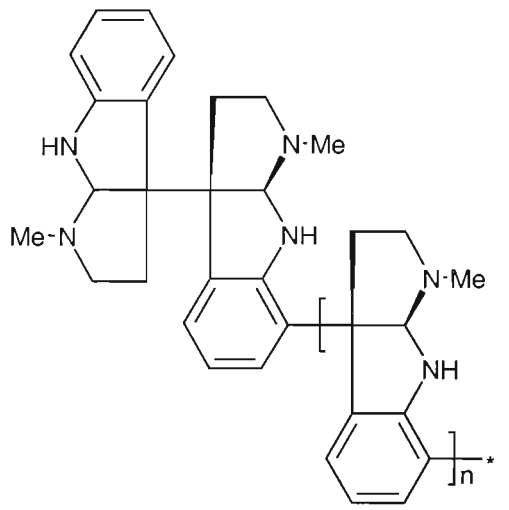
96: Psychotridine



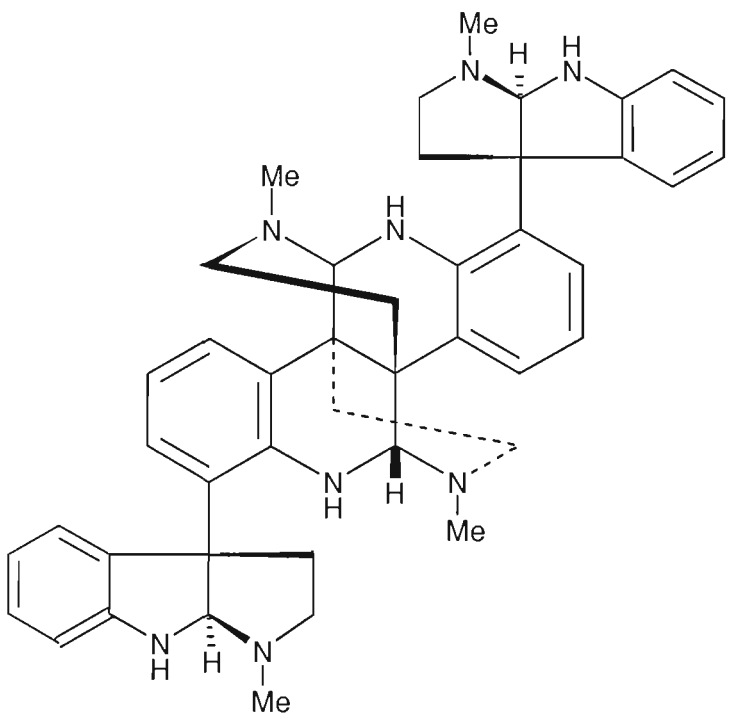
97: Hodgkinsine



98: Quadrigemine I

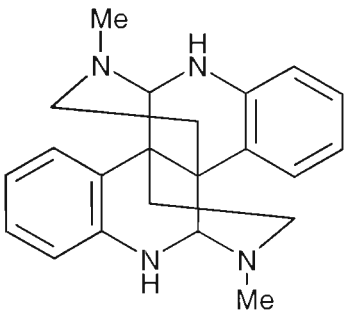


99: n=4, Oleodine
100: n=5, Caledonine

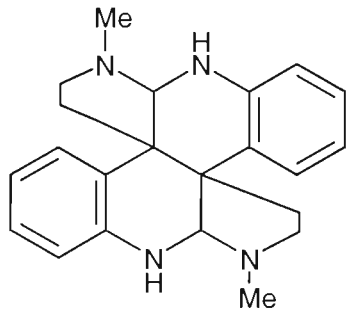


101: Psycholeine

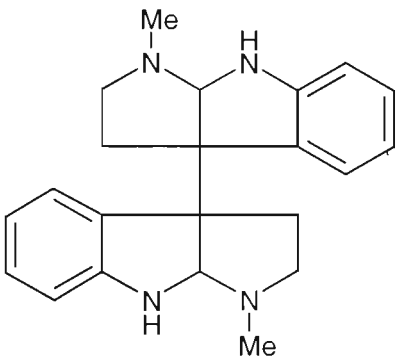
In the plant *Psychotria colorata* dimeric pyrrolidinoindolines predominate, for example, (-)-calycanthine **102**, *iso*-calycanthine **103**, (+)-chimonanthine **104** and (8-8a), (8'-8'a)-tetradehydroisocalycanthine **105**. Trimeric hodgkinsine **97**, tetrameric quadrigemine C **93** and pentameric psychotridine **96** have also been obtained as minor compounds from this species.[146] Analgesic properties have been reported for the alkaloid extract. Further studies by Amador *et al.*[147] reported that psychotridine **96** shows a dose-dependent analgesic effect in the rat tail-flick model and does not induce motor deficits at doses effective in analgesic models. It was also suggested that NMDA receptors participate in psychotridine-induced analgesia. Similar analgesic activity, and with an analogous mechanism, was found with the trimeric alkaloid hodgkinsine.[148] A further bioactivity study was reported on quadrigemine C **93,** which was shown to be cytotoxic towards HEp-2 cells and normal human lymphocytes, with the cytotoxicity being time- and dose-dependent. This compound also exhibited bactericidal activity against *Escherichia coli* and *Staphylococcus aureus*.[149]



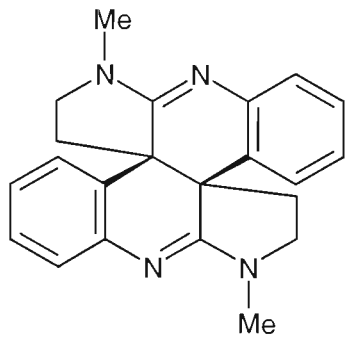
102: Calycanthine



103: *Iso*-Calycanthine

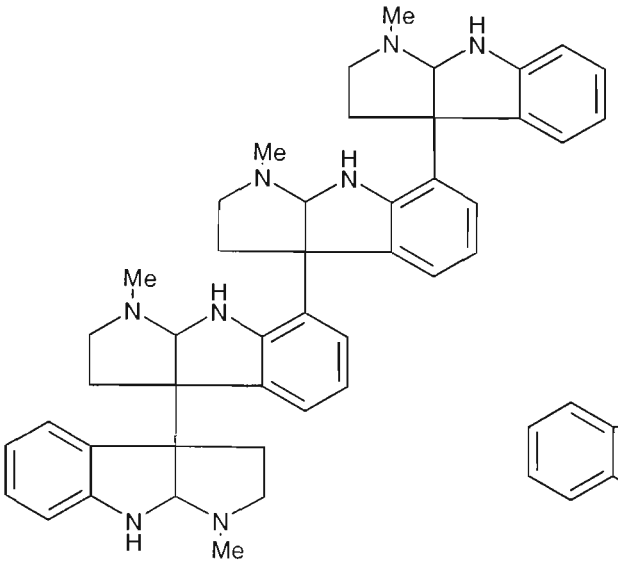


104: Chimonanthine

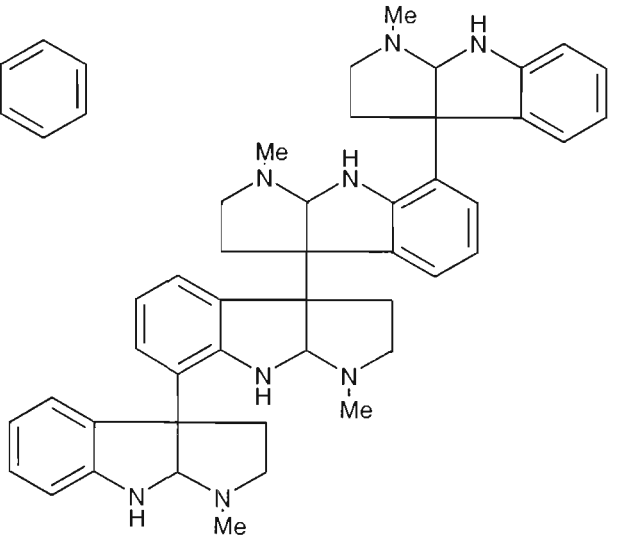


105: (8-8a), (8'-8'a)-Tetradehydroisocalycanthine

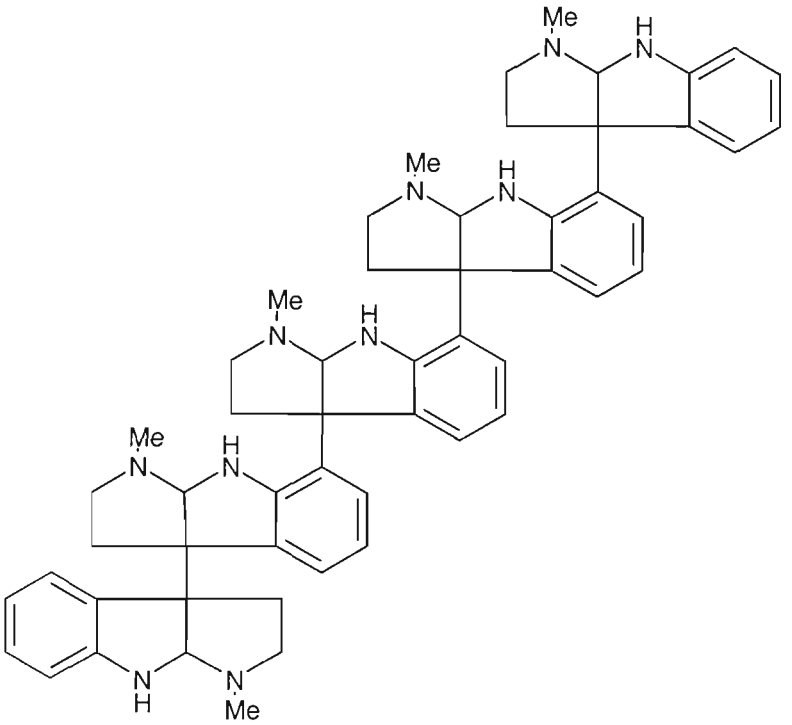
From the leaves of *Psychotria forsteriana*, four optically active alkaloids have been isolated, namely quadrigemine B **106**, quadrigemine A **107**, psychotridine **96** and a new stereoisomer of psychotridine called isopsychotridine C **108**.[150] All four of these compounds were reported to be potent inhibitors of the aggregation of washed human platelets induced by ADP, collagen, or thrombin and appeared to act at a later stage in platelet activation, possibly through an interaction with cytoskeletal proteins.[151]



106: Quadrigemine B

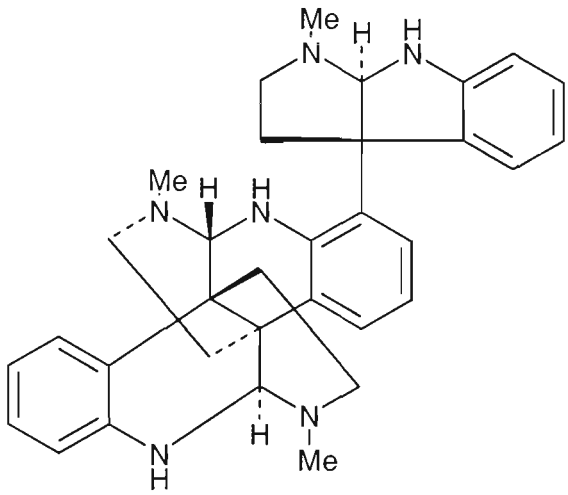


107: Quadrigemine A

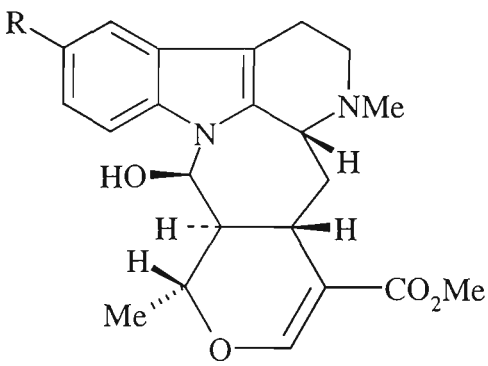


108: Isopsychotridine

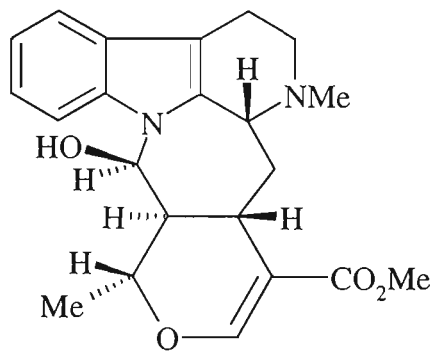
The plant, *Psychotria rostata*, found in Malaysia, and studied by the Zurinah group,[139] was reported to yield quadrigemine B **106** as the main alkaloid component, together with hodgkinsine **97**, (-)-calycanthine **102**, (+)-chimonanthine **104**, and calycosidine **108a** as minor components.



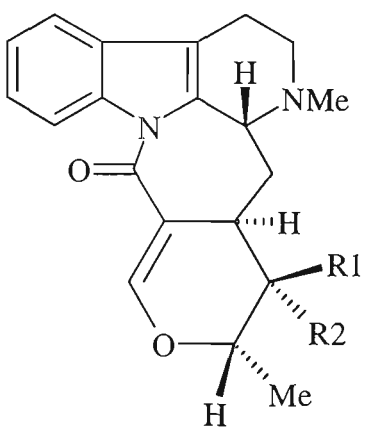
108a: Calycosidine



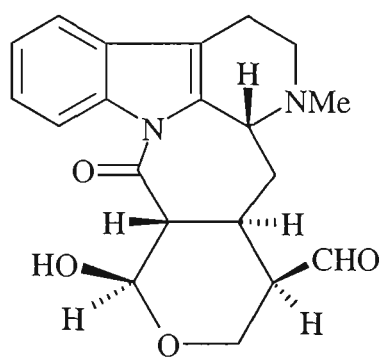
109: Correantoside; R=H
110: 10-Hydroxycorreantoside; R=OH



111: Correantine A

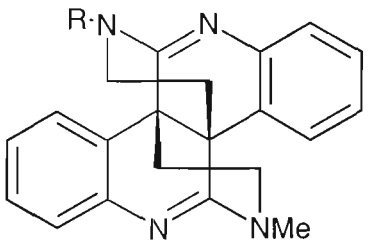


112: Correantine B; R_1=H, R_2=CHO
114: 20-*epi*-Correantine B; R_1=CHO, R_2=H

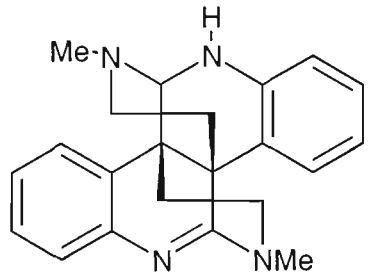


113: Correantine C

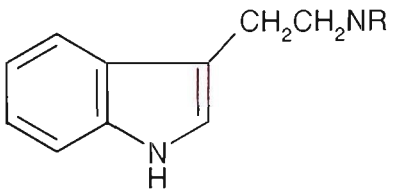
From *Psychotria correae*, six new alkaloids have been obtained, namely correantoside **109**, 10-hydroxycorreantoside **110**, correantine A **111**, B **112**, C **113**, and 20-epi-correatine B **114**.[152] Three new quinoline alkaloids, glomerulatine A **115** (major), B **116**, and C **117**, have also been isolated from aerial parts of *P. glomerulata*. The Amazonian plants *P. viridis* and *P. carthaginensis* are ingredients of a hallucinogenic beverage made by Indian tribes in the Southwestern Amazon basin. Chemical investigations revealed the presence of *N,N*-Dimethyltryptamine **118**, methyltryptamine **119** and 2-methyl-1,2,3,4-tetrahydro-β-carboline **120**.[153,154]



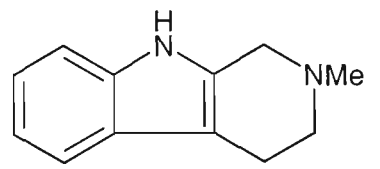
115: Glomerulatine A, R = Me
116: Glomerulatine B, R = H



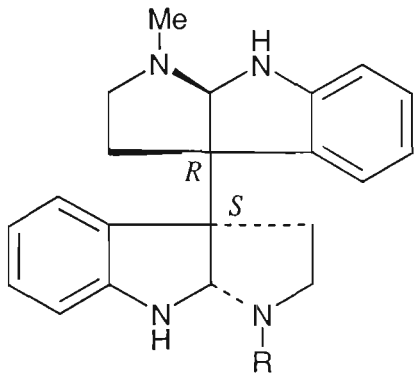
117: Glomerulatine C



118: R = Me_2, *N,N*-dimethyltryptamine
119: R = Me, Methyltryptamine

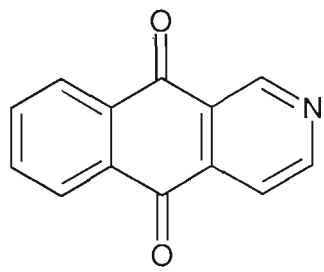


120: 2-Methyl-1,2,3,4-tetrahydro-β-carboline



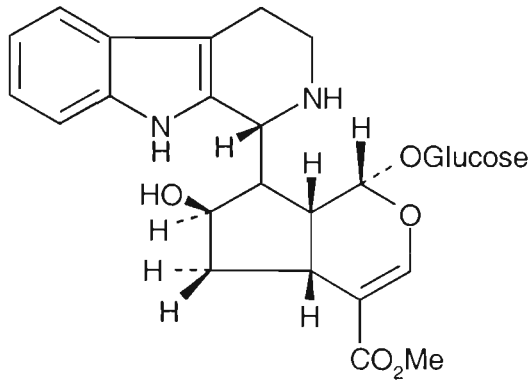
121: R = Me, *meso*-Chimonanthine
122: R = H, *N*b-desmethyl-*meso*-chimonanthine

The plant *Psychotria lyciiflora* from New Caledonia contained two dimers, *meso*-chimonanthine **121** and *N*b-Desmethyl-*meso*-chimonanthine **122**, and hodgkinsine **97** as reported by Jannic *et al.*[144] Psychotridine **96** was obtained as a major alkaloid component of *P. beccarioides*,[155] while the structurally different alkaloid benz[g]isoquinoline-5,10-dione **123** was isolated from the woody parts of *P. computants* based on bioactivity-guided fractionation. The compound showed strong activity *in vitro* against brine shrimp, KB cells, and chloroquine-resistant *Plasmodium falciparum*.[156]



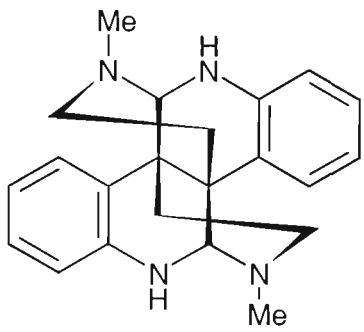
123: Benz[g]isoquinoline-5,10-dione

Another unusual alkaloid, brachycerine **124**, was isolated from *P. brachyceras*. The compound was found to be restricted to shoots in rooted cuttings and accumulation was not affected by root induction treatment with auxin.[157]

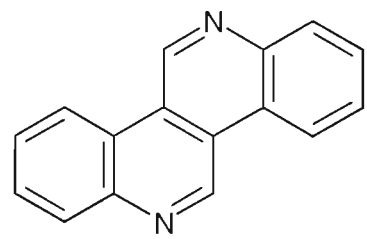


124: Brachycerine

4.3. Biosynthesis of Calycanthine Type Alkaloids



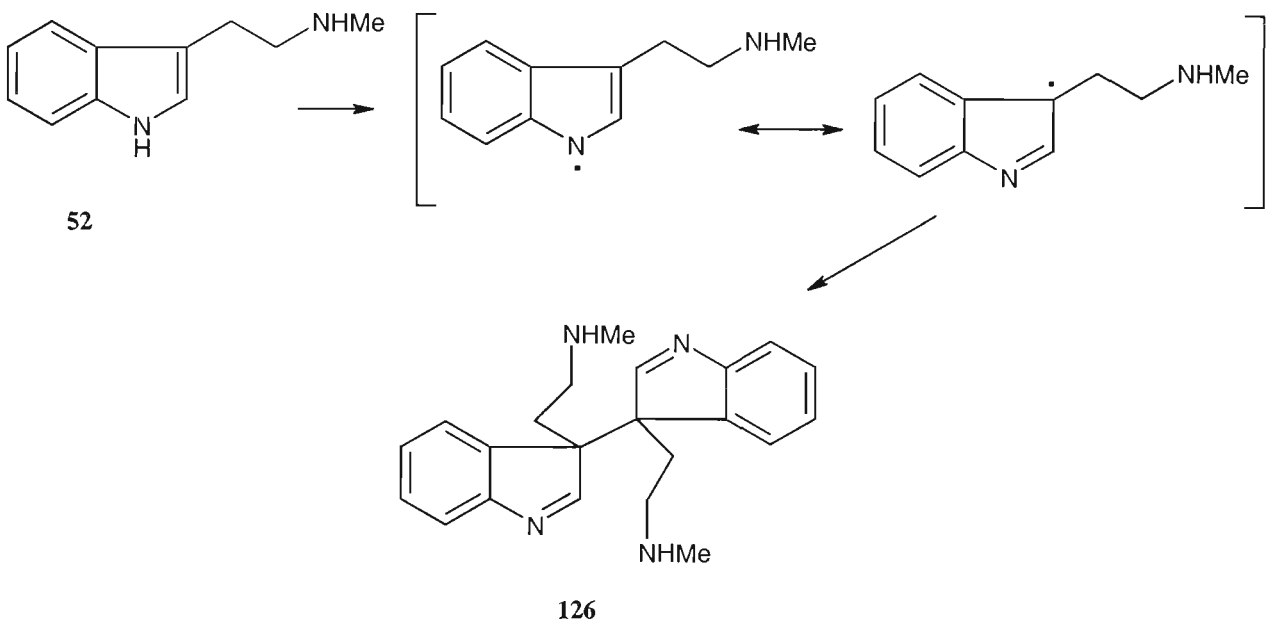
102



125

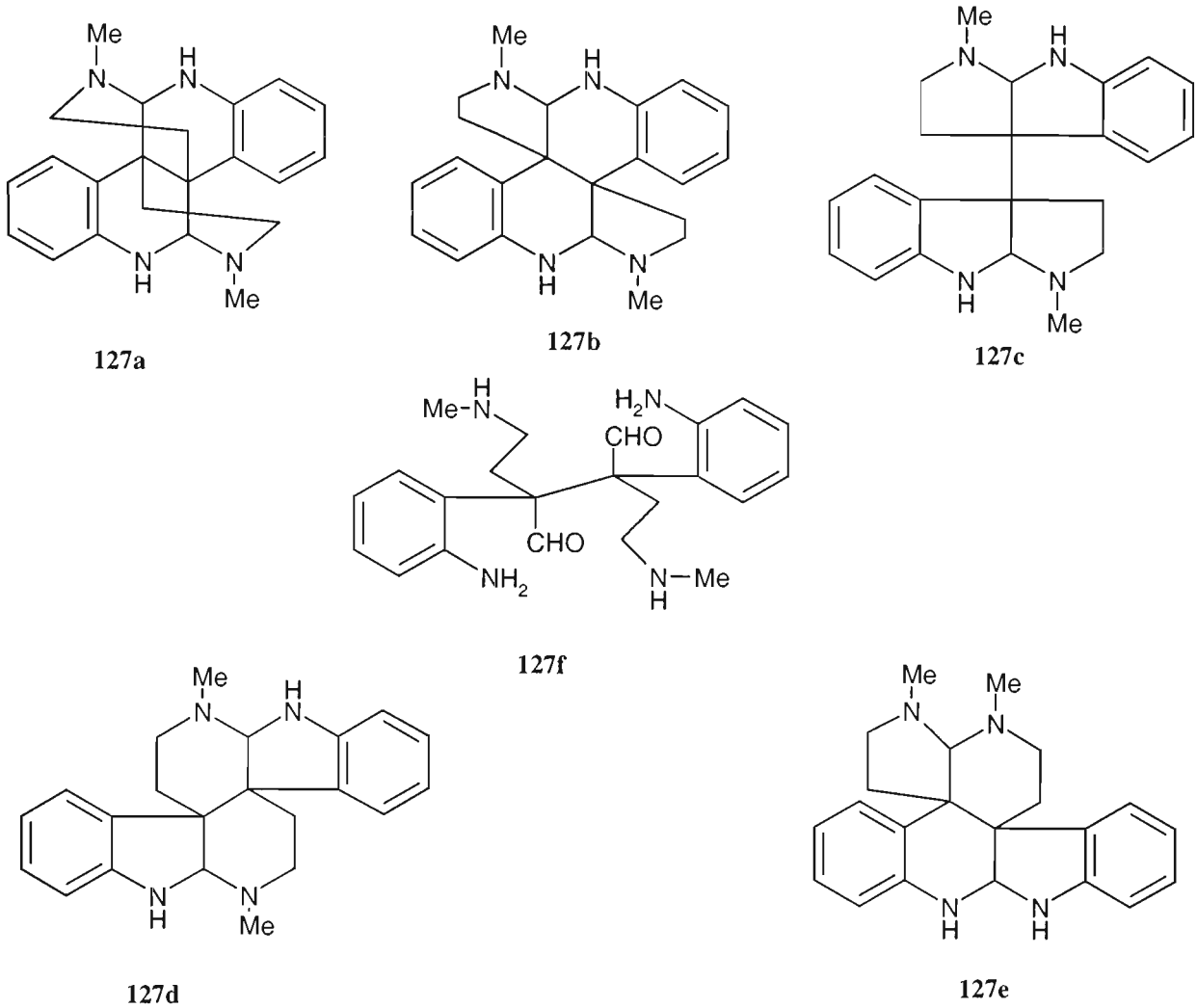
Based on several degradation products of calycanthine **102**, such as calycanine **125** and tryptamine **52**, Woodward[158] and Robinson[159] independently postulated that calycanthine **102** represents one of the five feasible dehydro dimers of tryptamine as a result of coupling of the mesomeric tryptamine radical. The compound **126** (Scheme 5), an intermediate indolenine, could directly cyclise to one of the isomers namely, **127b** (now called chimonanthine **104**), or hydrolyse to the tetra-aminodialdehyde **127f** (Scheme 6) that is

capable of being the progenitor of both **127a** and **127c** and the remaining possible isomers (**127b**, **127d**, **127e**; Scheme 6).



Scheme 5. The formation of the intermediate indolenine **126** from tryptamine **52**[158]

Implicitly, the formulation of the stereochemistry of compounds **127a** - **127e** is dependent on two centers generated in the production of **126** (Scheme 5), thus, each isomer may exist in a *rac*- or *meso*- form. This postulate has been supported by a great deal of experimental evidence[160,161] and the series of compounds found in nature.



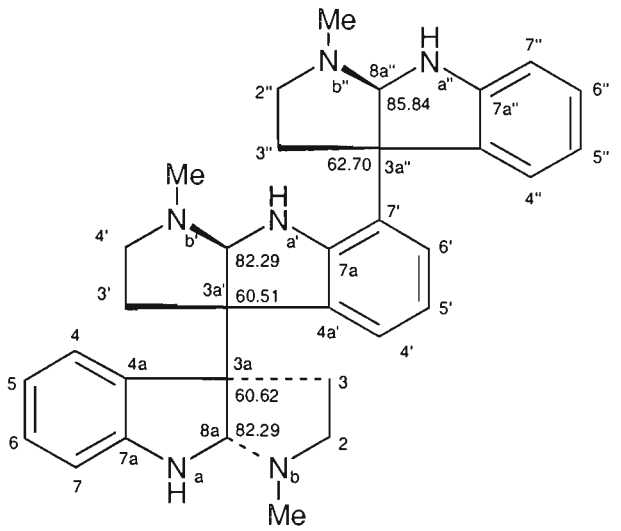
Scheme 6. Possible dehydrodimers of tryptamine

4.4. Isolation and Structure Elucidation of Alkaloids from Psychotria malayana Jack.

Following sequential acid and then base extractions, and multiple PTLC, three known pyrrolidinoindoline alkaloids (hodgkinsine **97**, *meso*-chimonanthine **104** and *iso*-calycanthine **103**), and two possibly new alkaloids designated LPM-574 **128** and LPM-186 **129** were isolated from the leaves of *P. malayana* Jack from Lombok. The crude extract of the bark of the plant yielded only *iso*-calycanthine **103**. The identification of the alkaloids was mainly based on mass spectrometric methods, some NMR data, and literature

comparison. Due to the unavailability of suitable low temperature NMR facilities required to resolve the overlapping signals in the ^{1}H-NMR spectra, these spectra were of limited value.

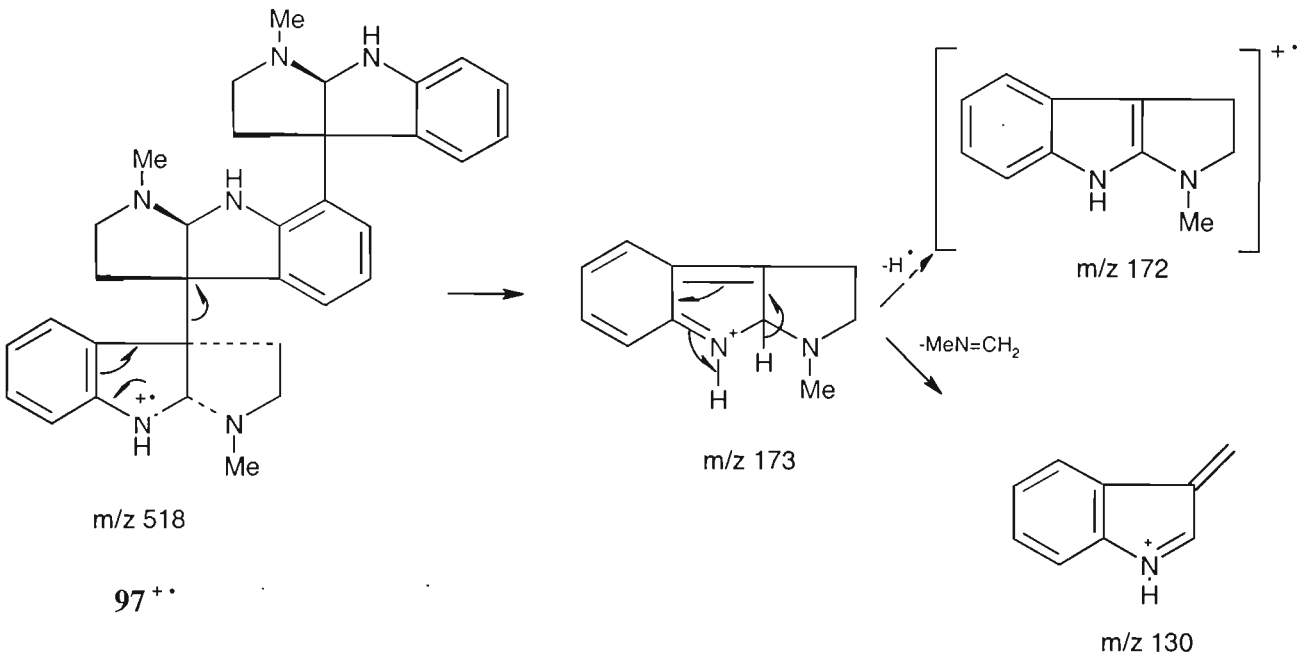
4.4.1. Hodgkinsine



97

The structure of hodgkinsine was determined using LRCIMS, LREIMS, and ^{13}C-NMR. From LRCIMS, the signals from three protonated fragment ions were observed at m/z 519, 345, and 173. Ionization using LREIMS showed five major fragment peaks at m/z 518, 345, 344, 173, 172, and 130 (Appendix 16). Cleavage of the 3a, 3'a-bond in hodgkinsine may occur more readily than that of the 7', 3"a-bond to produce the fragment ion at m/z 173, which then may lose a hydrogen atom to give a radical ion fragment at m/z 172. The remainder of the molecule gave firstly a peak at m/z 345, which then loses a hydrogen atom to give a fragment at m/z 344 by a similar process, as shown in Scheme 7.[155] HRCIMS confirmed the formula $C_{33}H_{38}N_6$ (obtained 519.3237, calc. 519.3236, for MH^+). The ^{13}C-

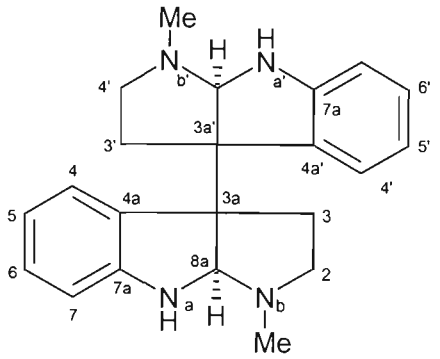
NMR (Appendix 16a) spectrum and physical data (melting point and specific rotation) suggested the structure as reported in the literature.[143,155]



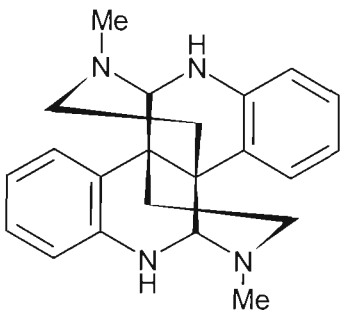
Scheme 7. Proposed mass spectral fragmentation pattern for hodgkinsine **97**

Anet *et al.*[162] initially isolated hodgkinsine **97** following an observation in 1949 by Webb, who obtained a positive test for alkaloids in the leaves of the plant *Hodgkinsonia frutescens*. The X-ray crystal structure was later reported by Fridrichsons *et al.*[163] The presence of two *N*-methyl groups and a significantly different specific rotation from that of chimonanthine (a dimeric compound which was also isolated from this plant), suggested hodgkinsine **97** was a stereoisomer of chimonanthine **104**.[164]

4.4.2. *Meso*-Chimonanthine and *Iso*-Calycanthine

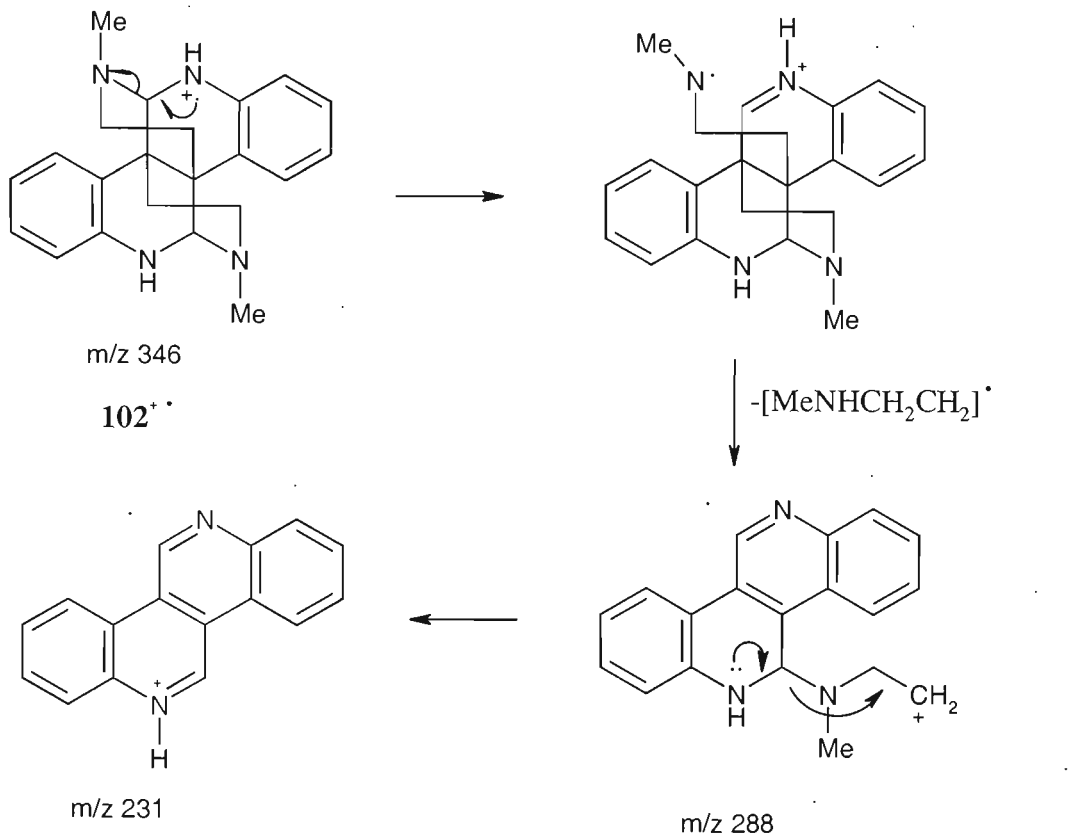


104



102

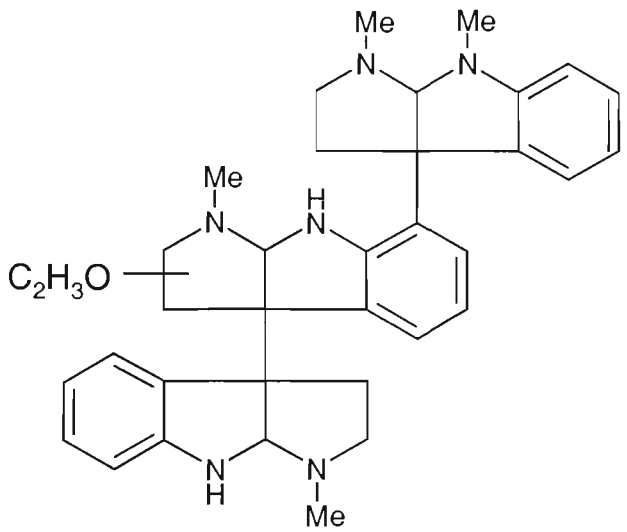
Chimonanthine **104** and calycanthine **102** gave the same molecular mass for the parent ion at m/z 347 (MH^+) in the LRCIMS spectra. However, LREIMS produced a different fragmentation pattern for each. The LREIMS spectrum of chimonanthine **104** suggested the molecule is fragmented symmetrically with the major peak being at m/z 172. Calycanthine **102**, on the other hand, yielded a fragment ion at m/z 231 corresponding to a singly protonated calycanine molecule (Appendix 17). The proposed EIMS fragmentation of this compound is presented in Scheme 8.[165] HRCIMS supported the formula $C_{22}H_{26}N_4$ (found 347.2224, calc. 347.2157, for MH^+) for chimonanthine and gave the same formula $C_{22}H_{26}N_4$ (found 347.2136, calc. 347.2157, for protonated calycanthine). The UV spectra and physical data (melting point and specific rotation) were identical to those for *meso*-chimonanthine and *iso*-calycanthine as reported in the literature.[144,166]



Scheme 8. Proposed mass spectral fragmentation of calycanthine **102**

Speculation about the biosynthesis of chimonanthine **104** and calycanthine **102** suggested that these compounds were derived from the β,β'-oxidative dimerisation of two tryptamine units. This was based on preliminary experiments on the biosynthesis of tryptamine dimers as mentioned in Section 4.3.[159] The dimeric tryptophan derived bases are known as major alkaloids of the order Calycanthaceae.[165]

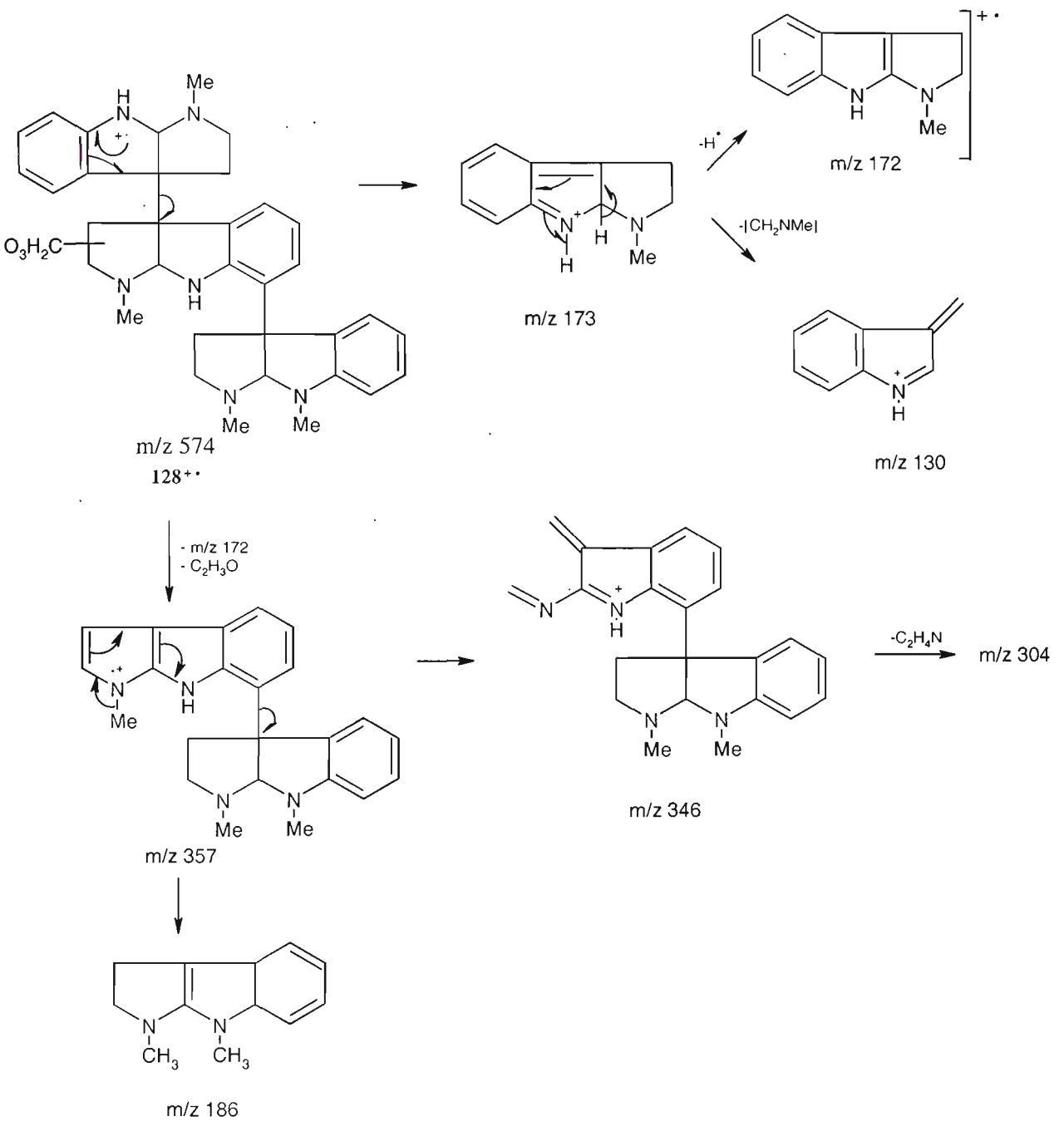
4.4.3. The Alkaloid LPM-574



128: alkaloid LPM-574

The new alkaloid LPM-574 was obtained as a yellow solid with an R_f value of 0.64 on TLC, slightly higher than hodgkinsine (R_f = 0.56). The LRCIMS spectrum showed the main peaks to be similar to those observed from hodgkinsine, with the main difference being the presence of a higher mass peak of low intensity suggesting a parent ion at m/z 574 (a difference of 56 amu from hodgkinsine). However, the fragment ion patterns in the LREIMS spectra of the two compounds were dissimilar. The UV absorption spectrum (Appendix 19) of the new compound showed the same pattern to that of chimonanthine and hodgkinsine suggesting that the compound contains an unsubstituted indole moiety. The presence of intense peaks at m/z 518, 344, 173, and 172 (LREIMS, Appendix 18) suggested that the main skeleton of the compound was identical to that of hodgkinsine. It is probable that the extra 56 amu in the molecular weight of the new compound, relative to hodgkinsine, is made up of a C_2H_3O group situated in the central *N*, N_b-methyltryptamine unit, and a CH_3 group attached to a terminal *N*, N_b-methyltryptamine unit. A peak in the

LREIMS spectrum at m/z 344 is consistent with a dimeric tryptamine fragment containing a C_2H_3O group and an extra methyl group relative to the analogous peak at m/z 288 in the hodgkinsine spectrum.

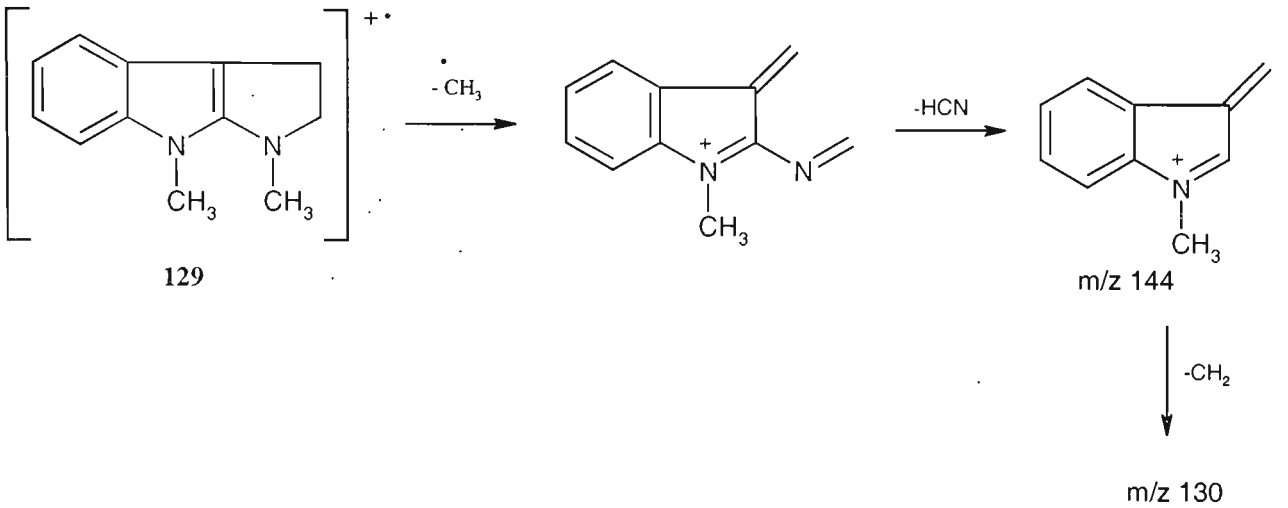


Scheme 9. Proposed ion fragmentation of the new alkaloid LPM-574 **128**

A fragment ion at m/z 186 (14 a.m.u higher than an analogous peak in the EIMS spectrum of hodgkinsine), suggests that the extra methyl group mentioned above is situated on a terminal tryptamine unit. This fragmentation pattern (Scheme 9) is consistent with the tentative structure proposed above. Furthermore the above structure is supported to a certain extent by the isolation of the alkaloid LPM-186 having a molecular weight of 186 a.m.u. (discussed in Section 4.4.4), the biosynthetic origins of which might be expected to be from alkaloid LPM-574. Unfortunately, alkaloid LPM-574 readily decomposed on silica gel. Several efforts to obtain a molecular formula for this compound via HRCIMS, HREIMS and HRESMS failed due to the very low intensity of the MH^+ or M^+ peaks observed. Insufficient pure material was available for detailed NMR studies, so the full structural elucidation could not be completed.

4.4.4. The Alkaloid LPM-186

Another new alkaloid LPM-186, the least-polar alkaloid component of the plant (R_f 0.91; TLC), was isolated as a yellow solid. By LREIMS, it produced principal fragment ions at m/z 130, 144, 157, 171, 172, 173, 174, and a molecular ion at m/z 186 (Appendix 20). From the fragmentation pattern, alkaloid LPM-186 appeared to have an indole moiety present, however, the ^{1}H-NMR spectrum indicated the presence of two singlets at δ2.82 and δ2.93 assigned to two *N*-methyl groups (Appendix 20a). This suggested an extra methyl group in the indole moiety relative to the terminal *N*-methyl moieties found in hodgkinsine.



129

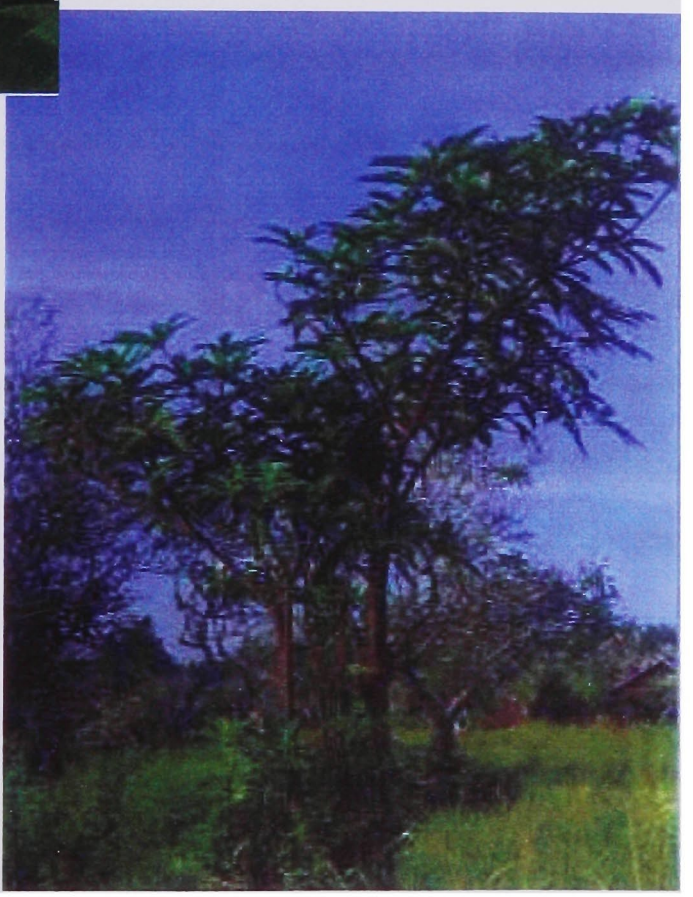
Scheme 10. Proposed ion fragmentation of the alkaloid LPM-186 **129**

Based on this evidence the tentative structure **129** is proposed for this alkaloid and a speculative ion fragmentation scheme is shown in Scheme 10. LRHRMS suggested the formula $C_{12}H_{14}N_2$ (found 187.1229, calc. 187.1235, for the protonated molecule). However, further efforts towards purification by silica gel resulted in decomposition of the compound.

Chapter 5

Alkaloids from *Alstonia scholaris* R. Br.

Leaves of *A. scholaris* R. Br.



Young trees of *A. scholaris* R. Br.

Chapter 5

ALKALOIDS FROM *Alstonia scholaris* R. Br.

5.1. Introduction

The plant *Alstonia scholaris* is the tallest tree belonging to the family Apocynaceae and grows to a height of 20-25 m and a diameter of 40-80 cm. This tree, the most widely distributed species of the genus, occurs in the region from India to South China and the Ryukyu islands south through Indonesia, the Philippines and Solomon islands. *Alstonia scholaris*, known as "nita" in Lombok, is common in areas up to 900 m above sea level. Initial investigations of the constituents of *Alstonia* species were stimulated by knowledge that extracts of the plant were commonly used as a cure for malaria. Until the second review of *Alstonia* alkaloids in 1970,[167] there had been no reports suggesting that *Alstonia* extracts or its pure isolated alkaloids were effective antimalarial agents. In 1999 the Keawpradub group,[168] reported that the methanol extract of the root bark of *A. macrophylla*, collected from Thailand, exhibited antiplasmodial activity. They observed that pronounced antiplasmodial activity occurred mainly among the bisindole alkaloids. Further discussion of this matter will be presented in Section 5.3.

In Lombok, the concentrated aqueous extracts of the leaves or bark of young trees (3-5 years) have been used to treat malaria. A particular point of interest is that young leaves from young trees are used specifically. Antimalarial testing by Yamauchi and Abe[169] with

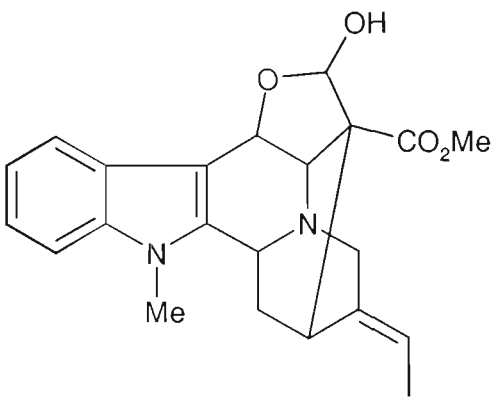
Plasmodium falciparum, revealed that the alkaloids obtained from leaves of *A. scholaris* from Lombok were active, but no specific mention was made of the age of the trees from which the alkaloids were isolated. There was therefore good reason for a thorough investigation of the young trees of this species. Moreover, during the course of this project, it was found that the alkaloids are distributed through the entire young plant including leaves, bark, roots, and fruit. In this study, the investigation of antimalarial agents is focussed only on the leaves.

5.2. Alkaloids from the Genus Alstonia

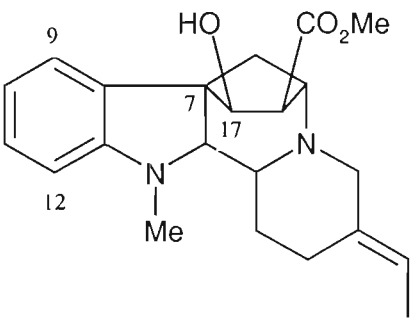
The genus *Alstonia* has long been known as a source of numerous alkaloids and has contributed significantly to the development of alkaloid chemistry. A review of alkaloids from *Alstonia* species was conducted by Saxton in 1960[170] and 1970[167] and a summary of *Alstonia* alkaloids was published in "The Dictionary of Alkaloids" published in 1989.[101] In this thesis, work performed on *Alstonia* alkaloids since 1989 is reviewed, as a means to discuss new and recent alkaloids isolated from this genus and their potency as antimalarial agents.

Since the most recent review on the topic, at least six *Alstonia* species have been reported to produce several novel alkaloids. The plant *A. undulata* has afforded seven new indole alkaloids namely 17*E*-hydroxydehydrovoachalotine **130**, 10-hydroxypericyclivine **131**, *N*(1)-methyl-10-hydroxypericyclivine **132**, 10-methoxypericyclivine **133**, N(1)-methyl-10-methoxypericyclivine **134**, *N*(1)-methyl-16-epipericyclivine **135**, and voachalotinal **136**. The first compound **130** was reported by Morfaux *et al.*,[171] who synthesized 17*E*-

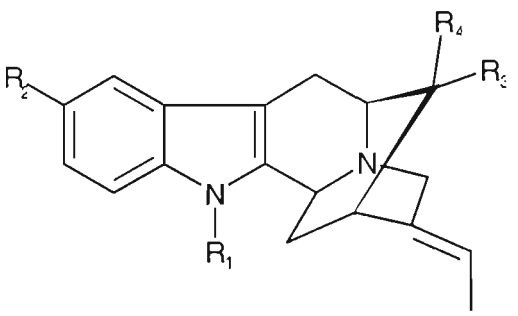
hydroxydehydrovoachalotine from vincamajine **137** by opening the C7-17 bond under oxidising conditions and treating the resulting aldehyde with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). The other six compounds (**131-136**) were isolated from *A. undulata* collected from New Caledonia.[172]



130: 17*E*-Hydroxydehydrovoachalotine



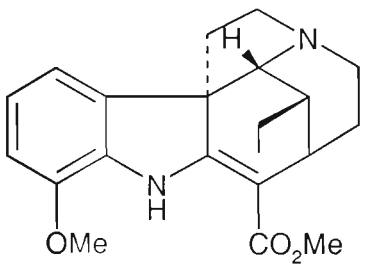
137: Vincamajine



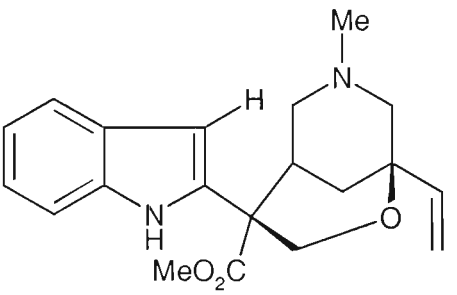
	R_1	R_2	R_3	R_4
131:	H	OH	H	CO_2Me
132:	Me	OH	H	CO_2Me
133:	H	OMe	H	CO_2Me
134:	Me	OMe	H	CO_2Me
135:	Me	H	CO_2Me	H
136:	Me	H	CO_2Me	CHO

Caron *et al.*[173] reported fifteen alkaloids found in the root bark, stem bark, and leaves of *A. congensis*, two of which appear to be new natural products, namely 12-methoxytubotaiwine **138** and 6,7-*seco*-anguistilobine **139**. Two newly observed quaternary indole alkaloids,

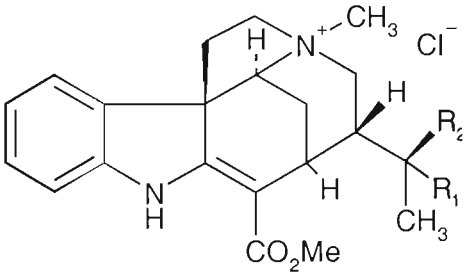
alstogustine **140** and 19-epialstogustine **141**, were isolated from the leaves of *A. angustifolia*.[174] The plant *A. villosa*, collected from Sumbawa island of Indonesia,[175] produced seven new alkaloids, **142-148**. Compound **148** is (19Z)-5α-methoxyrhazimine that has a 3,4-dihydroquinoline nucleus.



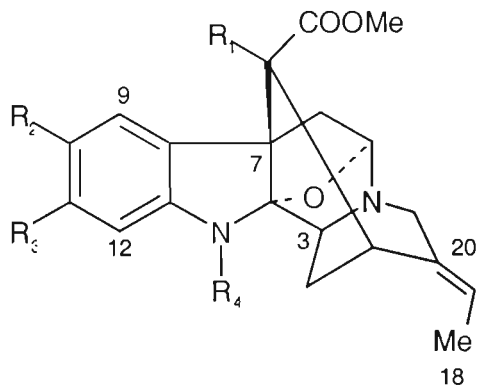
138: 12-Methoxytubotaiwine



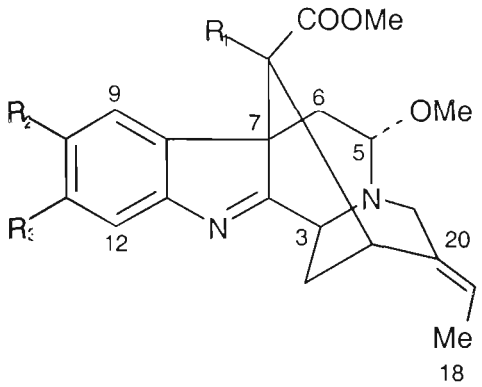
139: 6,7-*Seco*-Anguistilobine

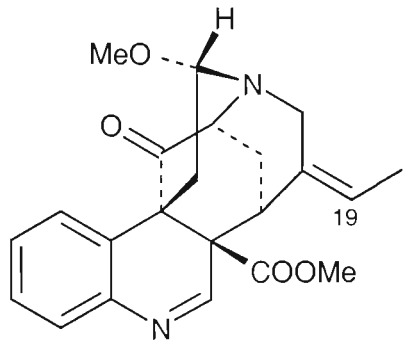


140: Alstogustine R_1=OH, R_2= H
141: Epialstogustine R_1=H, R_2=OH



142: R_1=CHO, R_2=R_3=R_4=H, (19Z)
143: R_1=CH_2O-Benzoyl$(OMe)_3$,
R_2=R_3=R_4=H, (19Z)

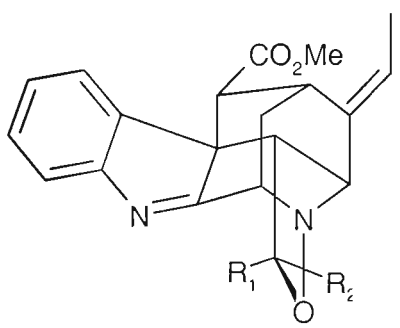


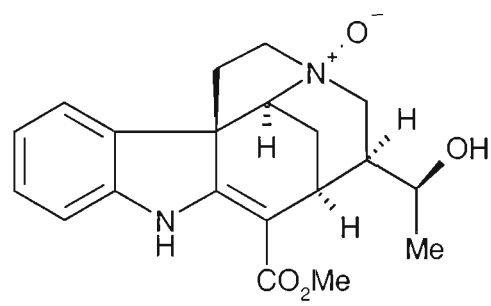


144: R_1=CHO, R_2=R_3=H, (19Z)
145: R_1=R_3=H, R_2=OMe
146: R_1=CH_2O-Benzoyl$(OMe)_3$, R_2=OMe, R_3=H,
147: R_1=CH_2O-Benzoyl, R_2=OMe, R_3=H,

148

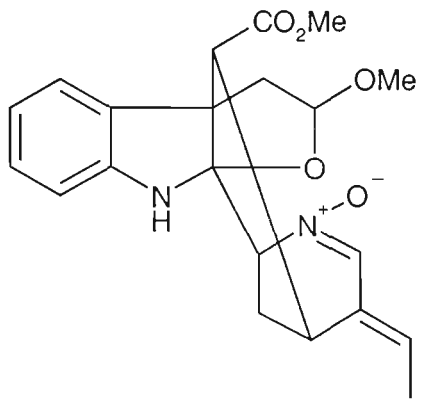
A. scholaris is the most widespread and intensively studied species of the genus. Alkaloids of this plant have been investigated by many groups in Europe,[176-179] India,[180-183] Thailand,[168,184] and Pakistan[185-188] and several alkaloids were reported. Since 1989, four new alkaloids have been reported as having been isolated from the leaves of *A. scholaris*. They were nareline ethyl ether **149**, 5-epi-nareline ethyl ether **150**, scholarine-*N*(4)-oxide **151**, and alschomine **152**.[98,189]





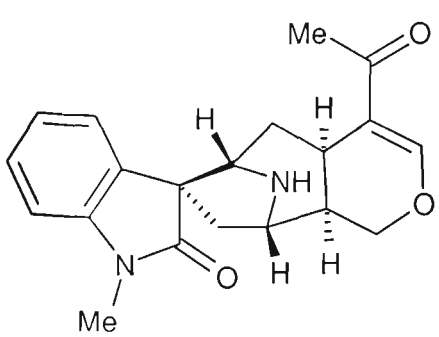
149: Nareline ethyl ether, R_1=H, R_2=OEt
150: 5-*Epi*-nareline ethyl ether, R_1=OEt, R_2=H

151: Scholarine-*N*(4)-oxide

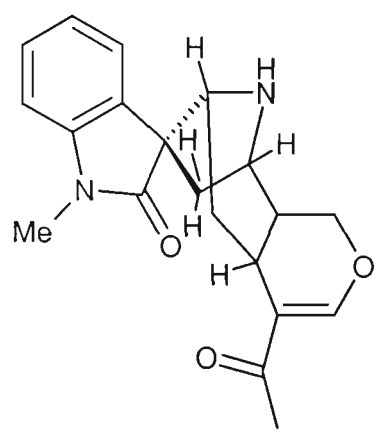


152: Alschomine

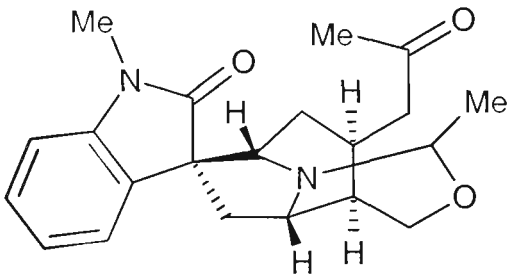
The genus *Alstonia* is characterized by the predominance of macroline-type indole and oxindole alkaloids.[190,191] The first macroline oxindole was alstonisine **153** isolated from *A. muelleriana* as published by Gilman.[192] Among *Alstonia* species, the plant *A. macrophylla* contributed the most significant number of this type of alkaloid. During the last 20 years, 30 new alkaloids were reported from this species. Nine new macroline oxindole alkaloids **154-162**, including several with novel structures, were obtained from the leaves of the Malayan species *A. macrophylla.*[193,194] Two new bisindole alkaloids, alstomacrophylline **163** and alstomacroline **164**, were isolated from the root bark.[195] From the stem bark of this plant, some new indole alkaloids were also found: 10-methoxyaffiniisine **165**, 10-methoxycathafoline **166**, and alstonerinal **167**.[196]



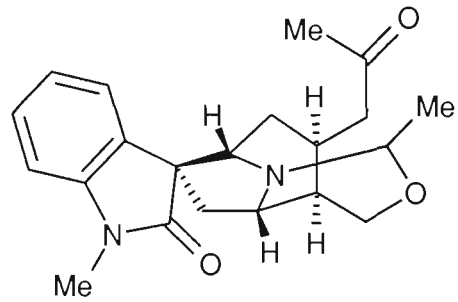
153: Alstonisine



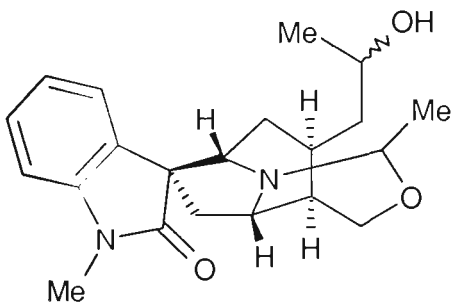
154



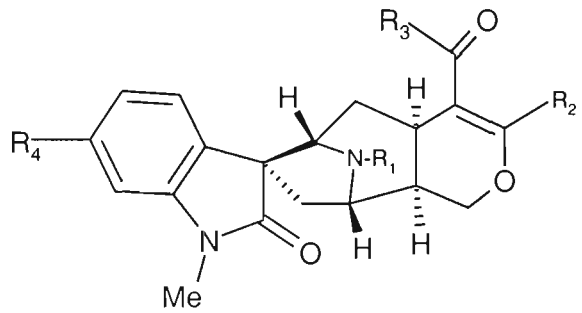
155



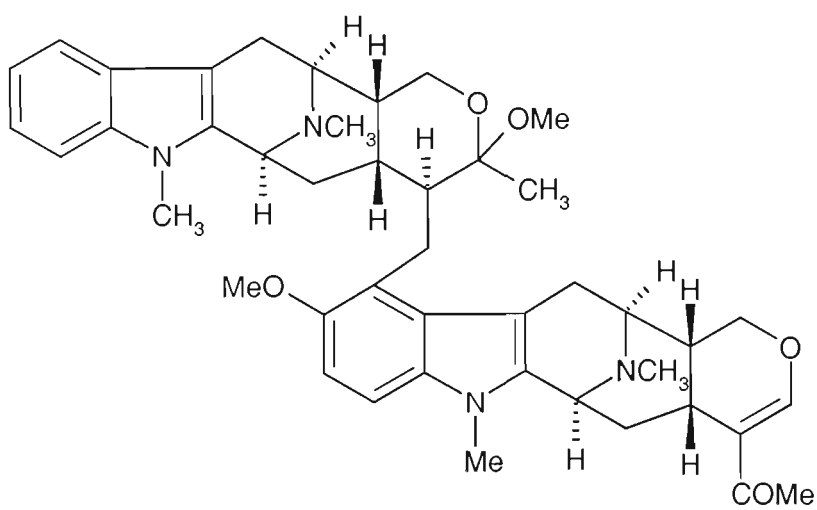
156



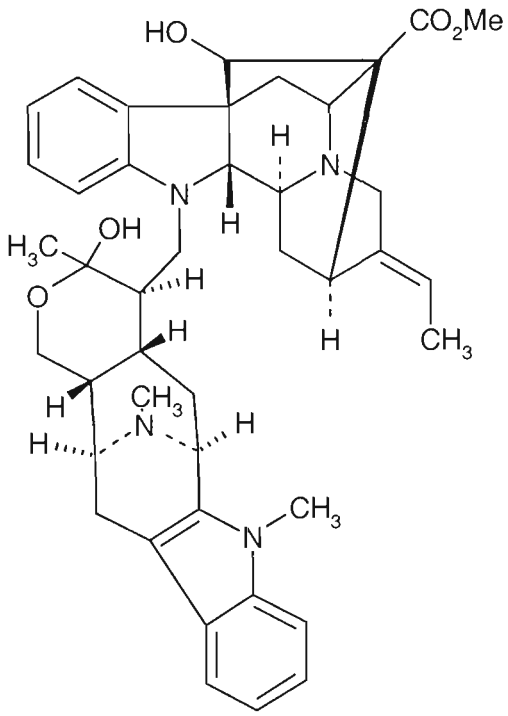
157



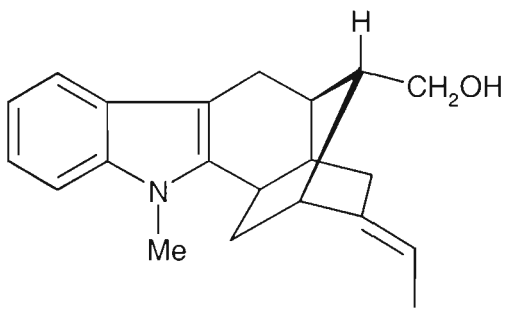
158: R_1=CHO, R_2=H, R_3=Me, R_4=H
159: R_1=H, R_2=H, R_3=Me, R_4=H
160: R_1=H, R_2=Me, R_3=Me, R_4=H
161: R_1=H, R_2=Me, R_3=H, R_4=OMe, *N*b-Desmethylalstophyllal oxindole
162: R_1=H, R_2=Me, R_3=H, R_4=H, Alstonal



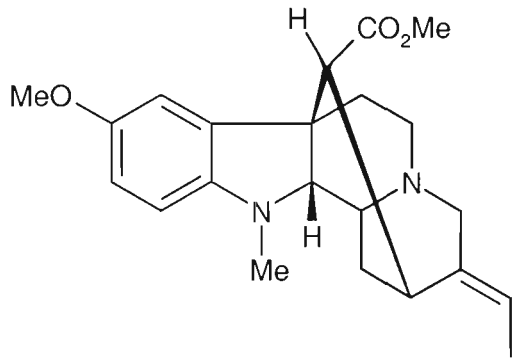
163: Alstomacrophylline



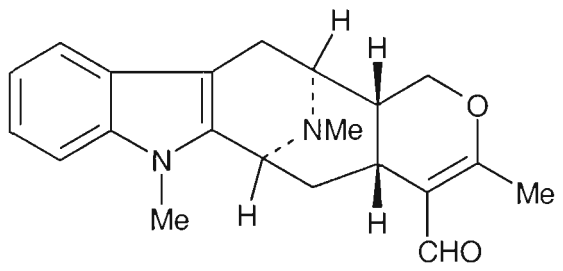
164: Alstomacroline



165: 10-methoxyaffiniisine



166: 10-methoxycathafoline



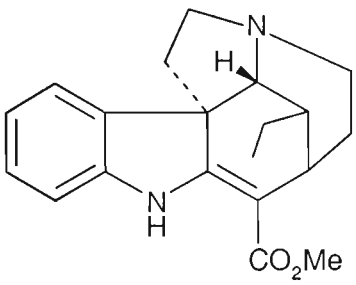
167: Alstonerinal

5.3. Antiplasmodial Activity of Alstonia Alkaloids

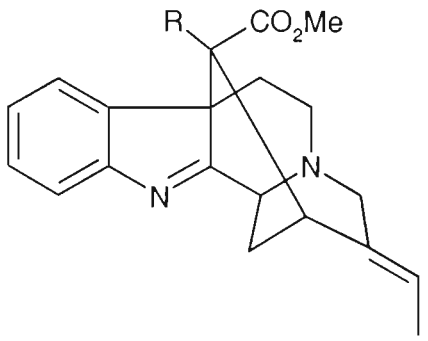
The experimental data dealing with the biological effectiveness of extracts and the alkaloids from *Alstonia* species for the treatment of malaria have been reported by several groups. Yamauchi and Abe,[169] for example, studied alkaloids isolated from *A. scholaris* and *A. macrophylla* from Thailand, Taiwan, Indonesia, and The Philippines. For antimalarial testing, they focussed on alkaloids from plants collected from two locations in Indonesia, Cianjur and Lombok (reported as Lonbok). A remarkable contrast was observed between the two locations, the sample from Cianjur containing scholaricine-type alkaloids, and the latter, tubotaiwine-type alkaloids. The *in vitro* antimalarial activity testing with *Plasmodium falciparum* indicated that all the alkaloids obtained from the Lombok collection (leaves) had potency, except for tubotaiwine-N_4-oxide (see Table 6 for the activity).

Table 6. Antimalarial activity of alkaloids from *Alstonia scholaris* collected on Lombok

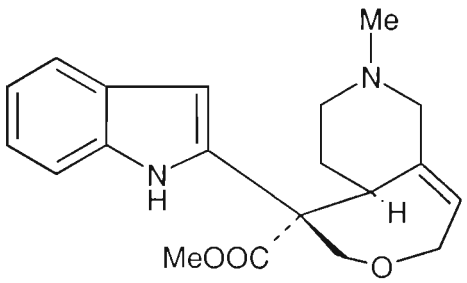
Alkaloids	*Plasmodium falciparum* EC_{50} (M)
Tubotaiwine **168**	7.0×10^{-6}
Tubotaiwine N_4-oxide **169**	(growth 80%)
Akuammiline **170**	1.8×10^{-5}
Deacetylakuammiline **171**	3.6×10^{-5}
6,7-seco-Alstonamine **172**	1.7×10^{-5}
Alstonamine **173**	2.4×10^{-5}



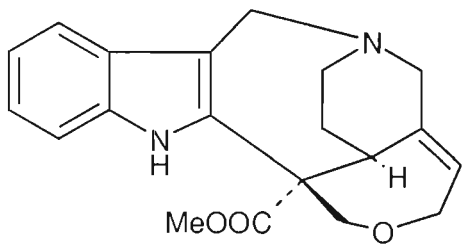
168: Tubotaiwine
169: Tubotaiwine N_4-oxide



170: Akuammiline, R=CH_2OAc
171: Deacetylakuammiline, R=CH_2OH



172: 6,7-*seco*-Alstonamine



173: Alstonamine

Further antiplasmodial activity of *Alstonia* alkaloids was reported by the Keawpradub group in 1999.[168] They assessed methanol extracts prepared from various parts of *A. scholaris*, *A. macrophylla*, and *A. glaucescens* from Thailand. Pronounced activity was exhibited by methanol extracts of the root bark of *A. macrophylla* with an IC_{50} value of 5.5 μg/mL. Further investigation revealed that 13 alkaloids were present in the active extract. These alkaloids, and a semisynthetic bisindole, *O*-acetylmacralstonine **182**, were subsequently tested against the K1 strain of *P. falciparum*. Pronounced antiplasmodial activity was observed mainly among the bisindole alkaloids, particularly villastonine and macrocarpamine, with IC_{50} values of 0.27 and 0.36 μM respectively, which was almost comparable to that of chloroquine diphosphate (see Table 7 for detail). Among the

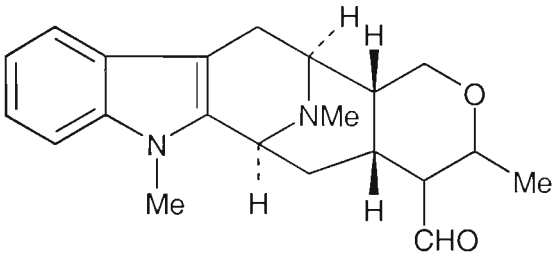
monomeric indoles, pleiocarpamine **175** was the most active alkaloid with an IC_{50} value of 6.44 μM.

Table 7. IC_{50} values of alstonia alkaloids tested against *P. falciparum* (K1 strain)

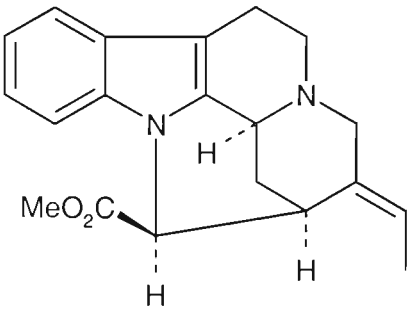
Alkaloid	n	IC_{50} (μM)
Talcarpin **174**	3	40.3 ± 2.9
Pleiocarpamine **175**	2	6.44 ± 0.98
Alstoumerine **176**	2	13.1 ± 2.7
20-*epi*-Antirhine **177**	3	7.51 ± 1.17
Alstonerine **178**	2	9.67 ± 3.57
Alstophylline **179**	2	12.7 ± 1.7
Macralstonine **180**	3	8.92 ± 2.95
O-Methylmacralstonine **181**	2	0.85 ± 0.20
O-Acetylmacralstonine **182**	3	0.53 ± 0.09
Alstomacrophylline **183**	2	1.10 ± 0.30
Villastonine **184**	3	0.27 ± 0.06
Villastonine *N*b-oxide **185**	2	10.7 ± 1.9
Alstomacroline **186**	3	1.12 ± 0.35
Macrocarpamine **187**	3	0.36 ± 0.06
Chloroquine diphosphate	3	0.20 ± 0.07

n = number of independent experiments
(each test was performed in triplicate)

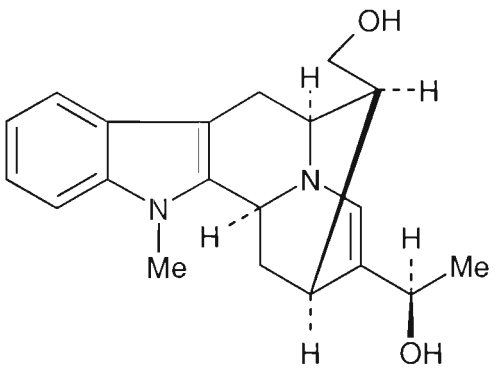
The above activity profile is similar to that previously reported by the Wright group,[197] who tested alkaloids from *A. angustifolia*. They found the bisindoles were the principal active components and they were far more potent than the monomeric indole alkaloids. Based on these two studies, Keawpradub *et al.* [168] pointed out that the lack of high antiplasmodial activity of *A. scholaris* and *A. glaucescens* extracts is most probably due to the fact that only monomeric alkaloids have been isolated from these two species.



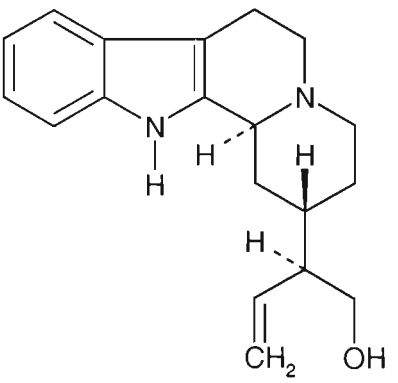
174: Talcarpin



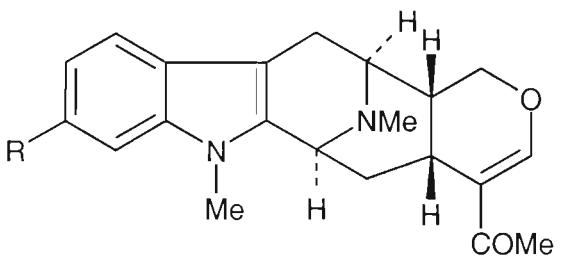
175: Pleiocarpamine



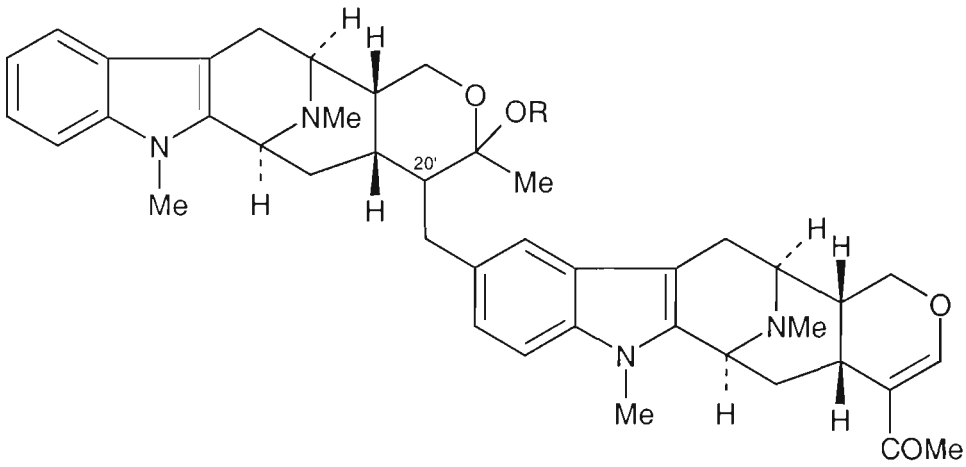
176: Alstoumerine



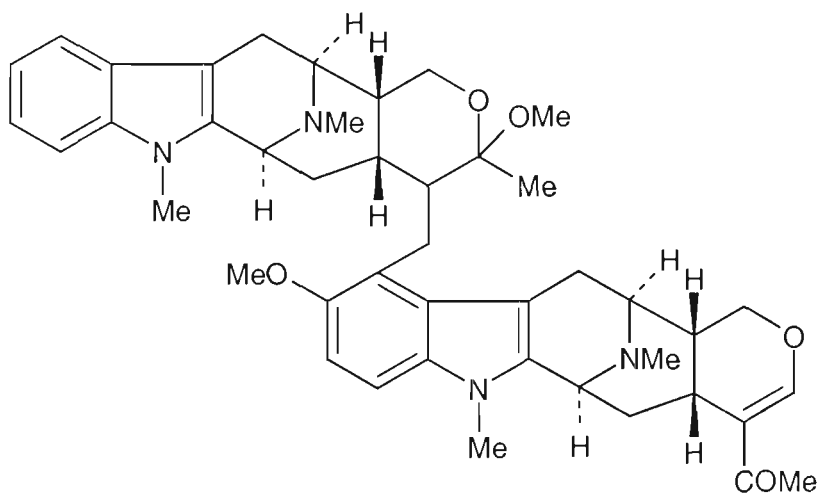
177: 20-*epi*-Antirhine



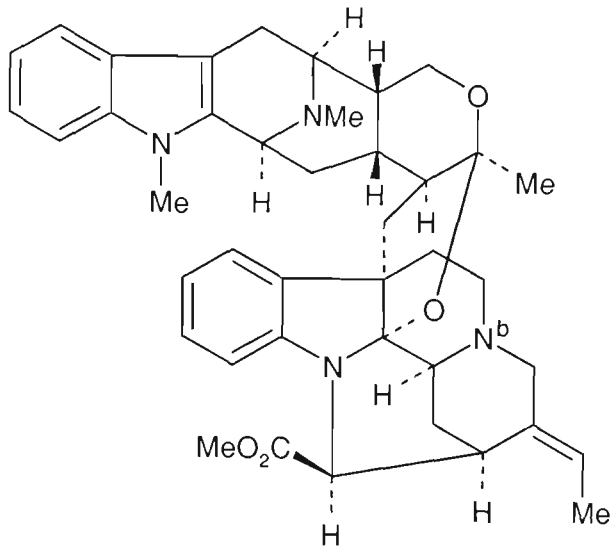
178: Alstonerine, R=H
179: Alstophylline, R=OCH_3



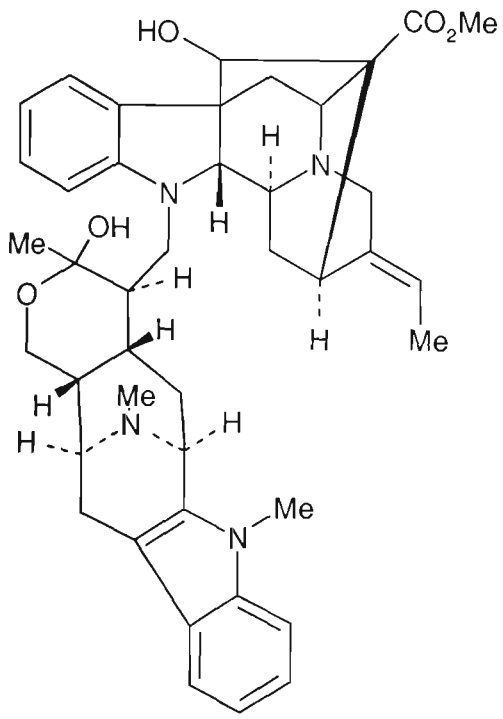
180: Macralstonine, R=H
181: *O*-Methylmacralstonine, R=CH_3, 20'-Hβ
182: *O*-Acetylmacralstonine, R=$COCH_3$



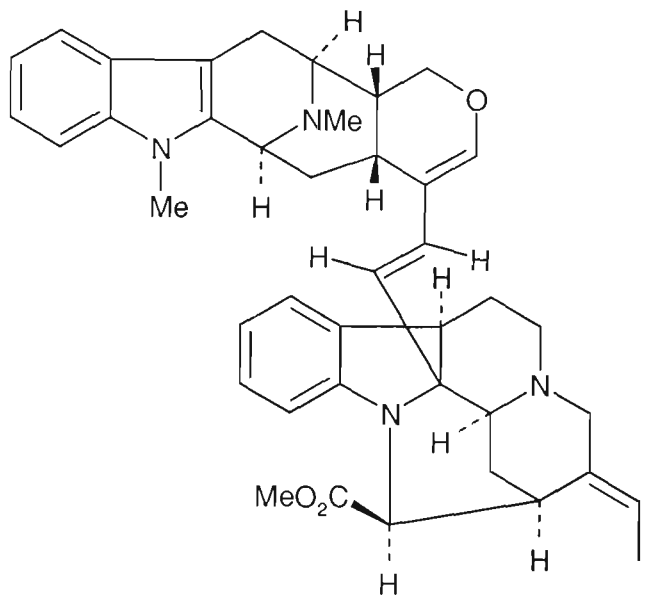
183: Alstomacrophylline



184: Villastonine
185: Villastonine N_b-oxide



186: Alstomacroline



187: Macrocarpamine

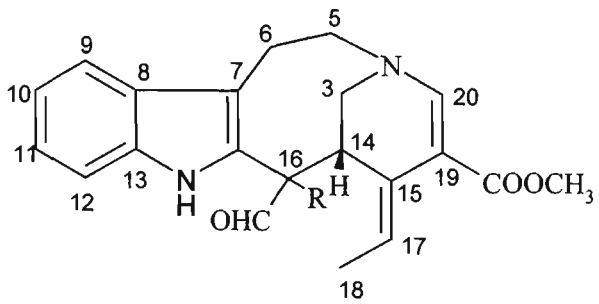
5.4. Isolation and Structure Elucidation of Alkaloids from *A. scholaris* R. Br.

Air-dried leaves from young trees of *Alstonia scholaris* R. Br. (3-5 years, 2 kg) were extracted with methanol. This extract was then separated into neutral, acidic, and basic materials and water solubles, by routine procedures (see Section 9.4). Alkaloid components were separated and purified by repeated preparative thin layer chromatography (PTLC) on silica gel using various combinations of CH_2Cl_2, CH_3OH, and NH_4OH as the eluent. The following alkaloids were isolated by TLC and identified as follows: mataranine A, **188**, and B, **189**, alstonamine **190**, (*15S, 16S)*-losbanine **193**, kotarajine **194**, and akuammidine **195**. The alkaloids **188**, **189**, **193**, and **194** are new, while **188** and **189** are major components found in the leaves of young specimens of *A. scholaris*.

5.4.1. Mataranine A and B

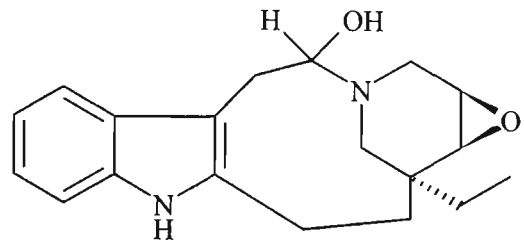
These new alkaloids, that the author has called mataranine A **188** and mataranine B **189**, were obtained as a light yellow oil in the form of a 1:1 diastereomeric mixture, which could not be separated fully by PTLC. However, separating the top and bottom portions of the band on the TLC plate changed the ratio of the resultant two compounds (after extraction from the silica gel), which assisted in differentiating their spectra and hence elucidating their structures. The mixture of **188** and **189** showed absorbance maxima at 283 and 291 nm in the UV spectrum consistent with an indole chromophore being present. The presence of the aldehyde and methyl ester (COOMe) groups was suggested by the LREIMS spectrum with peaks at m/z 321 (M^+-29), and 291 (M^+-59) respectively (Appendix 21).

LRCIMS gave an ion of m/z 351 $[MH]^+$ and the LREIMS spectrum showed the $[M]^+$ ion at m/z 350. The empirical formula was established by HRCIMS as $C_{21}H_{21}N_2O_3$ (measured 351.1711, calc. 351.1709, for MH^+).



188: Mataranine A, R = Hβ

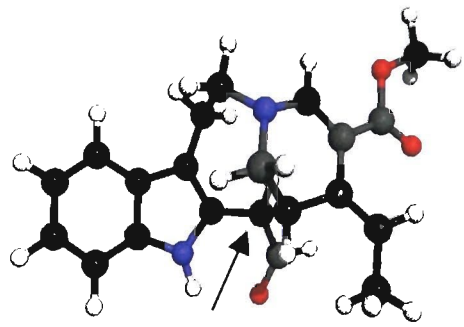
189: Mataranine B, R = Hα



189a: Stapfinine

C-16

3D-Mataranine A model



C-16

3D-Mataranine B model

(N-blue; O-red; C-black, H-white)

To elucidate the structure of the mataranines, ^1H- and ^{13}C-NMR spectra were collected and proton-proton connectivity was deduced from a gCOSY spectrum. The proton-carbon connectivity was assigned by recording gHSQC and gHMBC experiments and these experiments were complemented by DEPT spectra. These results are summarised in Tables 8 and 9.

From the ^{1}H-NMR spectrum (Appendix 22), the significant difference between the two compounds was the chemical shift of the aldehyde proton, which appeared to be dependant on the relative configuration at C-16 with respect to C-14. Mataranine A **188** was assigned the structure with the C-16 proton and C-14 proton being *cis* relative to each other (C-16 Hβ) and mataranine B with the C-16 proton and C-14 proton being *trans* relative to each other (C-16 Hα). Computer-derived 3D-mataranine models (pictured on p.102; modelling based on the Spartan program) indicated that with the C-16 proton in the *beta* position, the aldehyde proton is closer to the lone pair of electrons on N-1, than is the case with the epimer at C-16. This is consistent with a downfield shift of the signal ascribed to aldehyde proton (δ10.28 vs δ9.37) in the former case. The position of the aldehyde functionality (CHO) was determined by the gHMBC spectrum. In mataranine A, the CHO signal at δ10.28 showed cross peaks to signals at δ143.8 (C-2) and δ30.5 (C-14).

Table 8. ^{1}H-NMR data of mataranine A **188** and B **189**

Protons	Mataranine A			Mataranine B		
	δ (ppm)	Integ-ration	*J* (Hz)	δ (ppm) multiplicity	Integ-ration	*J* (Hz)
H-3α	4.25 dd	1H	$J_{3\alpha,\ 3\beta}$ = 12, $J_{3\alpha,\ 14}$ = 4.5	4.48 dd	1H	$J_{3\alpha,\ 3\beta}$ = 12, $J_{3\alpha,\ 14}$ = 4.5
H-3β	4.02 bd	1H	$J_{3\beta,\ 3\alpha}$ = 12	4.02 bd	1H	$J_{3\beta,\ 3\alpha}$ = 12
H-5α	3.70 m	1H		3.70 m	1H	
H-5β	3.59 m	1H		3.59 m	1H	
H-6α	2.91m	1H		2.91 m	1H	
H-6β	2.80 m	1H		2.80 m	1H	
H-9	7.47 d	1H	$J_{9,\ 10}$ = 8.0	7.48 d	1H	$J_{9,\ 10}$ = 8.0
H-10	7.16 t	1H		7.16 t	1H	
H-11	7.11 t	1H		7.11 t	1H	
H-12	7.30 d	1H	$J_{12,\ 11}$ = 8.5	7.30 d	1H	$J_{12,\ 11}$ = 8.5
H-14	1.80 m	1H		1.92 m	1H	
H-16	2.25 bd	1H		2.18 bd		
H-17	6.55 q	1H		6.58 q		

Table 8 continued

H-18	2.19 d	3H	$J_{18,19} = 7.5$	2.09	3H	$J_{18,19} = 7.5$
H-20	7.76 s	1H		7.68	1H	
COOMe	3.64 s	3H		3.64 s	3H	
CHO	10.28 s	1H		9.37 s	1H	
NH	8.20 bs	1H		8.14 bs	1H	

While the following discussion focusses on the structure elucidation of mataranine A **188**, a similar analysis of the spectral data of mataranine B **189** was also carried out; this data can be seen in Tables 8 and 9. In the aromatic region of the ^{1}H-NMR spectrum, six distinct groups of peaks were discerned from which five were readily assigned to the indole nucleus, based on gCOSY and gHSQC spectra (Appendix 23 and 24 respectively). Two doublets centred around δ7.47 and 7.30 were assigned to the H-9 and H-12 protons of the benzo group respectively. Two triplets centred around δ7.16 and 7.11 were assigned to H-10 and H-11 respectively. A broadened singlet was observed at δ8.20, consistent with an indolic NH proton. Another singlet, at δ7.76, was assigned to H-20 with cross peaks in the gHMBC spectrum (Appendix 25) to δ136.4 (C-19), 50.9 (OMe), 47.7 (C-16), and 28.6 (C-14), which is consistent with the proposed structure.

Table 9. ^{13}C-NMR data of mataranine A **188** and B **189**

Carbon No.	Mataranine A		Mataranine B	
	Chemical shift	Carbon type (DEPT)	Chemical shift	Carbon type (DEPT)
2	143.8	-C-	140.03	-C-
3	51.3	CH_2	51.3	CH_2
5	51.3	CH_2	51.3	CH_2
6	22.3	CH_2	22.3	CH_2
7	108.7	-C-	108.7	-C-
8	127.9	-C-	128.9	-C-
9	118.3	CH	118.3	CH
10	120.0	CH	120.0	CH

Table 9 continued

11	122.4	CH	122.4	CH
12	111.3	CH	111.3	CH
13	126.5	-C-	126.5	-C-
14	30.5	CH	28.6	CH
15	146.2	-C-	152.0	-C-
16	47.7	CH	49.5	CH
17	143.7	CH	143.7	CH
18	15.3	CH_3	13.5	CH_3
19	136.4	-C-	136.4	-C-
20	147.7	CH	147.7	-C-
COOMe	153.1		168.4	
COOMe	50.9	CH_3	50.9	CH_3
CHO	196.1	CH	190.6	CH

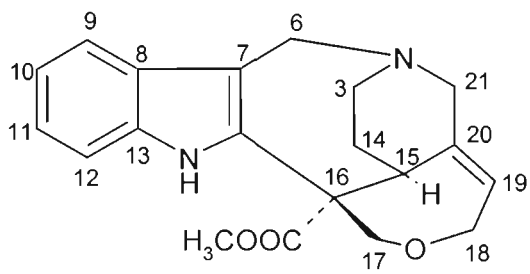
The methyl ester appeared as a three-proton singlet at δ3.64. The observed broad doublet signal at δ2.25 was ascribed to H-16. From the gCOSY spectrum, H-16 (δ2.25) was coupled to a multiplet at δ1.80 (H-14) that also coupled to two broadened downfield doublets at δ4.25 (H-3α) and 4.02 (H-3β), suggesting the presence of a (–CH-CH-CH_2-N-) moiety. The ethylidene group was identified by the observation of a one-proton quartet at δ6.55 (H-17) coupled to a three-proton doublet at δ2.19 (H-18, $J_{18,\ 17}$ = 7.5 Hz). The position of the ethylidene group was confirmed by the gHMBC spectrum, which showed a weak cross peak from δ6.55 (H-17) to δ196.1 (aldehyde) and 30.5 (C-14). The (*Z*) stereochemistry about the double bond was indicated by an NOE observed between H-17 (δ6.55) and the aldehyde proton. Four distinct multiplets centred around δ3.70, 3.59, 2.91, and 2.80 were assigned to the H-5α, H-5β, H-6α, and H-6β protons respectively.

The mataranines are representative of a new indole alkaloid skeleton, which has C-16 linked to C-14 to form an eight membered ring. They may be derived from a cleavamine-

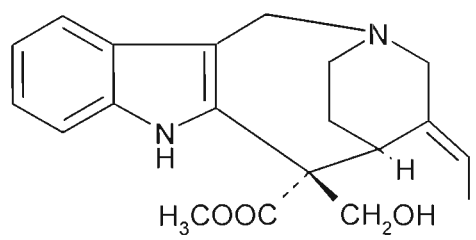
velbanamine alkaloid type (e.g. stapfinine **189a**), which have been isolated from three main species belonging to the family Apocynaceae: *Tabernaemontana eglandulose*,[198] *Ervatamia coronaria*,[199] and *Rhazya stricta*.[200]

5.4.2. Alstonamine (Angustilobine B)

The known alkaloid **190**, called alstonamine or angustilobine B, was obtained as colourless oil. Physical and spectral data of the isolated sample were identical to alstonamine, an alkaloid isolated from *A. scholaris*, and reported by Atta-ur-Rahman and Alvi in 1987.[201] At the same time Zeches *et al.*[202] reported this compound as angustilobine B in *A. angustiloba* Miq. The UV absorption spectrum in chloroform showed absorbance maxima at λ 283 and 291 nm consistent with the presence of an indole ring. LRCIMS gave an m/z at 339 $[MH]^+$ and the molecular formula was established by HRCIMS as $C_{20}H_{21}N_2O_3$.



190



191

The ^{1}H-NMR spectrum (Appendix 28) showed characteristic signals for an indole moiety in the aromatic region. The NH appeared as a broad singlet at δ8.87. Two broadened doublets at δ7.50 and δ7.32 were assigned to H-9 and H-12 respectively. Signals ascribed to H-10 and H-11 resonated as two apparent triplets at δ7.19 and 7.11 respectively. The gCOSY

(Appendix 29) spectrum confirmed the expected *ortho* couplings between H-9 and H-10, and H-12 and H-11, as well as between H-10 and H-11.

Table 10. ^{13}C- and ^{1}H-NMR data of alstonamine **190** (the sample)

Carbon No.	Chemical shift (δ)	Protons No.	δ (ppm), multiplicity	Integration	Coupling constant (*J*, Hz)
2	132.1				
3	43.0	H-3α	3.27 m	1H	
		H-3β	2.93 m	1H	
6	48.8	H-6β	4.00 d	1H	$J_{6\beta, 6\alpha}$ = 16.5
		H-6α	4.85 d	1H	$J_{6\alpha, 6\beta}$ = 17.0
9	117.9	H-9	7.50 d	1H	$J_{9, 10}$ = 7.5
10	120.2	H-10	7.19 t	1H	
11	122.8	H-11	7.11 t	1H	
12	110.9	H-12	7.32 d	1H	$J_{12, 11}$ = 8.0
14	20.0	H-14α	1.75 dd	1H	$J_{14\alpha, 14\beta}$ = 15.5; $J_{14\alpha, 3}$ = 8.5
		H-14β	2.04 m	1H	
15	43.8	H-15	3.68 m	1H	
17	71.2	H-17α	4.37 d	1H	$J_{17\alpha, 17\beta}$ = 12.0
		H-17β	3.77 d	1H	$J_{17\beta, 17\alpha}$ = 11.5
18	77.1	H-18β	4.23 dd	1H	$J_{18\beta, 18\alpha}$ = 12.5; $J_{18\beta, 19}$ = 3.5
		H-18α	4.51 bd	1H	
19	123.8	H-19	5.50 m	1H	
20	134.6				
21	55.3	H-21α	3.43 bd	1H	$J_{21\alpha, 21\beta}$ = 15.5
		H-21β	3.81 bd	1H	$J_{21\beta, 21\alpha}$ = 15.0
COOMe	53.6	COOMe	3.45 s	3H	
COOMe	174.5				
		NH	8.95 bs	1H	

Alstonamine **190** is closely related to vallesamine **191**, but with a 7-membered oxygen-containing ring being present in the former.[201] The ^{1}H-NMR spectrum of **190** showed the absence of the 18-methyl proton signal and the presence of two additional broadened doublets centred at δ 4.23 (H-18β) and δ 4.51 (H-18α), which showed cross peaks to the

multiplet at δ5.50 (H-19) in the gCOSY spectrum. The broadened signals for H-18α were caused by coupling to C-19 proton. The signal ascribed to H-19, a slightly broadened singlet, sharpened on decoupling through irradiation of the signal for H-18. Another AB system appeared at δ4.37 and 3.77 and was assigned to the H-17α and H-17β protons respectively.

We observed a slight difference in the ^{1}H-NMR spectrum (in the same solvent, deuterated chloroform) for the N_b 6-membered ring compared with that reported in the literature. Atta-Ur-Rahman and Alvi[201] reported the C-21 protons resonated as a multiplet at δ3.45 and Zeches *et al.*[202] observed the C-21 protons as a doublet of doublets at δ3.30 and 3.73, while we observed two broad doublets at δ3.43 and 3.81. This observation might be rationalised by protonation equilibria of the nearby tertiary amine. A detailed comparison of the ^{1}H-NMR spectroscopic data is set out in Table 11. It is believed[201] that alstonamine, biosynthetically, arises from a C-18 hydroxylated derivative of **191** through an intramolecular cyclisation reaction.

Table 11. A comparation of ^{1}H-NMR data between the sample, alstonamine and angustilobine B

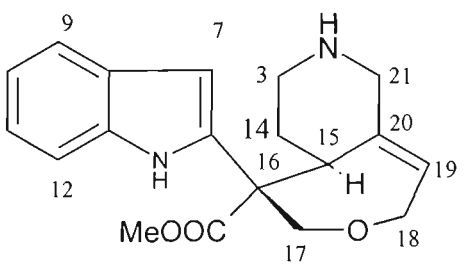
Protons	Chemical shift, multiplicity, *J* (Hz)		
	The sample	Alstonamine[201]	Angustilobine B[202]
H-3α	3.27 m	2.93	3.19 m
H-3β	2.93 m		2.90
H-6β	4.00 d (16.5)	4.02 d (16.29)	3.97 (17)
H-6α	4.85 d (17.0)	4.87 d (16.2)	4.75 d (17)
H-9	7.50 d (7.5)	7.48 d (7.8)	7.46 d (7)
H-10	7.19 t	7.17 ddd (7.8, 7.4, 1.2)	7.15 t
H-11	7.11 t	7.08 ddd (7.68, 7.68, 1.2)	7.06 t

Table 11 continued

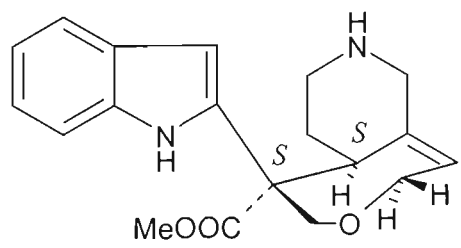
H-12	7.32 d (8.0)	7.31 d	7.30 d (7)
H-14α	1.75 dd (15.5; 8.0)	1.76 dd (15.22, 8.25)	2.0 m
H-14β	2.04 m	2.14 m	1.69 m
H-15	3.68 m	3.69 m	3.60 m
H-17α	4.37 d (12.0)	4.38 d (12.45)	4.37 d (9)
H-17β	3.77 d (9.5)	3.85 d (12.45)	3.85
H-18β	4.23 dd (12.5; 3.5)	4.21 dd (14.25, 3.42)	4.25 dd (16, 4)
H-18α	4.51 bd (15.5)	4.50 dd (14.25, 3.42)	4.47 brd (16)
H-19	5.50 m	5.53 m	5.45 brs
H-21α	3.43 bd (15.5)	3.45 m	3.73 brd (15)
H-21β	3.81 bd (14.0)		3.30 d (15)
COOMe	3.90 s	3.88 s	3.85 s
NH	8.95	9.25	8.65 brs

5.4.3. (*15S*, 16S**)-Losbanine

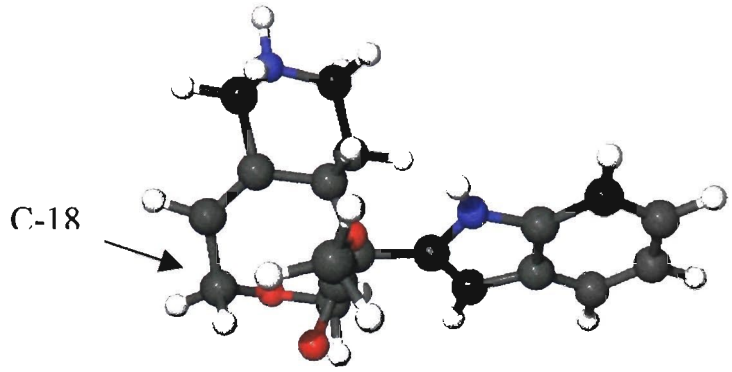
The alkaloid **193** was obtained as a colourless amorphous solid. The UV absorption spectrum was characteristic of the indole chromophore showing maxima at 274, 283 and 291 nm. LRCIMS showed a $[MH]^+$ ion at m/z 327. The molecular formula was established by HRCIMS as $C_{19}H_{22}N_2O_3$ (found 327.1712, calc. 327.1709, for MH^+).



192: Losbanine



193: (*15S*, 16S**)-Losbanine



3D-(*15S*, 16S**)-Losbanine model (N-blue; O-red; C-black; H-white)

The ^{1}H-NMR spectrum of **193** (Appendix 31), was, in some respects, similar to 6,7-*seco*-angustilobine, also called losbanine **192**, an alkaloid isolated from the leaves of *Alstonia scholaris* from the Philippines.[203] Comparison of the ^{1}H-NMR spectroscopic data belonging to **192** and the sample **193** suggested that the sample was a 2-substituted indole derivative. Further structural analysis of compound **193** by the use of the structure simulation capability of the molecular modelling program "Spartan Version 4.1" based on the Karplus equation ($^3J = 7 + (-1 \cos \theta) + 5 \cos 2\theta$), suggested that the sample **193** has the (*15S*, 16S**) configuration (or the mirror image). A key spectroscopic difference between **192** and **193** was the presence of a doublet of doublets for one proton at δ5.67 (J_1 = 17.0 Hz, J_2 = 10.5 Hz) for H-19 instead of the broad singlet observed for this proton in the ^{1}H-NMR spectrum of losbanine **192** (Table 12). This suggested that the position of H-19 was not symmetrical with respect to the two protons at C-18, and that the 7-membered ring adopts a conformation as shown in structure **193**. Further confirmation for this was evident from the signals ascribed to the C-18 protons of **193** appearing at δ5.39 (H-18α) and δ5.17 (H-18β). These shifts are at a lower field than the corresponding signals in **192** at δ4.31 and δ4.17 respectively as a result of the closer proximity of H-18 to the carbonyl group of the carbomethoxy group as shown in the computer-based (Spartan program) 3D-(*15S*, 16S**)-

Losbanine model (p. 110). Further supporting evidence was obtained from the ^{13}C-NMR spectrum (Appendix 33), in which C-18 of **193** was ascribed to the signal at δ115.4 (at very low field), while in **192** the peak at δ69.1 was ascribed to C-18 (Table 13). The proton-carbon connectivity was analysed from the gHSQC spectrum (Appendix 34), and showed that the signals at δ5.39 (H-18α) and δ5.17 (H-18β) correlated to the carbon signal at δ115.4 assigned then as C-18; no H-19 – C-19 correlation was apparent however. The presence of one isolated proton and methylene group in **193** was suggested by a singlet at δ6.38 (H-7) and a pair of doublet signals at 4.96 (H-17α, $J_{17\alpha,\ 17\beta}$ = 9.5 Hz) and 4.57 (H-17β, $J_{17\beta,\ 17\alpha}$ = 9.0 Hz). The C-21 protons appeared as two doublets at δ2.71 (H-21β, $J_{21\beta,\ 21\alpha}$ = 14.5 Hz) and 2.91 (H-21α, $J_{21\alpha,\ 21\beta}$ = 14.5 Hz) that overlapped with a multiplet of H-3α at δ2.89. In the aromatic region five groups of peaks were apparent, consistent with a 2-substituted indole moiety, with a doublet at δ7.54 (H-9), $J_{9,\ 10}$ = 7.5 Hz), another doublet at δ7.30 (d, $J_{12,\ 11}$ = 8.0 Hz, H-12), a triplet at δ7.16 (H-10), another triplet at δ7.08 (H-11), a further singlet at δ6.38 (H-7), and a broad singlet at δ8.33 (NH). Thus **192** is tentatively assigned as (*15S**, *16S**)-Losbanine with the conformation shown.

Table 12. ^{1}H-NMR data (*15S**, *16S**)-losbanine **193** and losbanine **192**

Protons	δ, Multiplicity, Integration, *J* (Hz)	
	(*15S**, *16S**)-losbanine	Losbanine[203]
H-3α	2.89 m, 1H	3.05 bd (10)
H-3β	2.47 m, 1H	2.84 t (10)
H-7	6.38 s, 1H	6.25 s
H-9	7.54 d ($J_{9,\ 10}$ = 7.5)	7.52 d (7)
H-10	7.16 t	7.05 t (7)
H-11	7.08 t	7.34 t (7)
H-12	7.30 d ($J_{12,\ 11}$ = 8.0)	7.33 d (7)
H-14α	1.07 m	1.54 bq

Table 12 continued

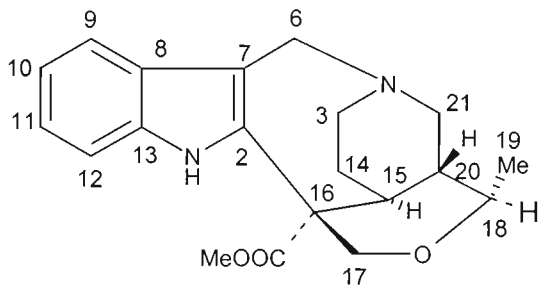
H-14β	1.14 m	1.26 bd
H-15	3.05 dd ($J_{15,14\alpha}$ = 7.0; $J_{15,14\beta}$ = 6.5)	3.49 bd (11)
H-17α	4.96 d ($J_{17\alpha,17\beta}$ = 9.5)	4.71 d (12)
H-17β	4.57 d ($J_{17\beta,17\alpha}$ = 9.0)	4.11 d (12)
H-18α	5.39 d ($J_{18\alpha,19}$ = 17.0)	4.31 bd (15)
H-18β	5.17 d ($J_{18\beta,19}$ = 10.0)	4.17 bd (15)
H-19	5.67 dd ($J_{19,18\alpha}$ = 17.0; $J_{19,18\beta}$ = 10.5)	5.60 bs
H-21α	2.91 d ($J_{21\alpha,21\beta}$ = 14.5)	3.46 bd (12)
H-21β	2.71 d ($J_{21\beta,21\alpha}$ = 14.5)	3.43 bd (12)
COOMe	3.60 s	3.69 s
NH	8.33 bs	9.11

Table 13. ^{13}C-NMR data of (*15S*, 16S**)-losbanine and losbanine

Carbon no.	(*15S*, 16S**)-Losbanine	Losbanine[203]
2	132.3	134.8
3	46.4	44.8
5	-	-
6	-	-
7	101.6	100.8
8	127.5	127.7
9	119.9	120.4
10	120.3	120.0
11	122.3	122.2
12	110.6	111.2
13	140.3	135.8
14	24.2	28.1
15	43.9	45.4
16	59.0	56.2
17	69.5	71.6
18	115.4	69.1
19	127.5	126.5
20	136.1	136.0
21	52.9	54.3
COOMe	51.7	52.8
COOMe	173.5	173.4

5.4.4. Kotarajine

Compound **194**, a new indole alkaloid named kotarajine by the author, was obtained as a colourless amorphous solid. It absorbed UV light with maxima at 275, 284, and 291 nm consistent with an indole chromophore. The LRCIMS spectrum suggested an MH^+ peak at m/z 341 and the exact mass obtained by HRCIMS was found to be m/z 341.1866 corresponding to the molecular formula $C_{20}H_{24}N_2O_3$ (calc. 341.1865, for MH^+).

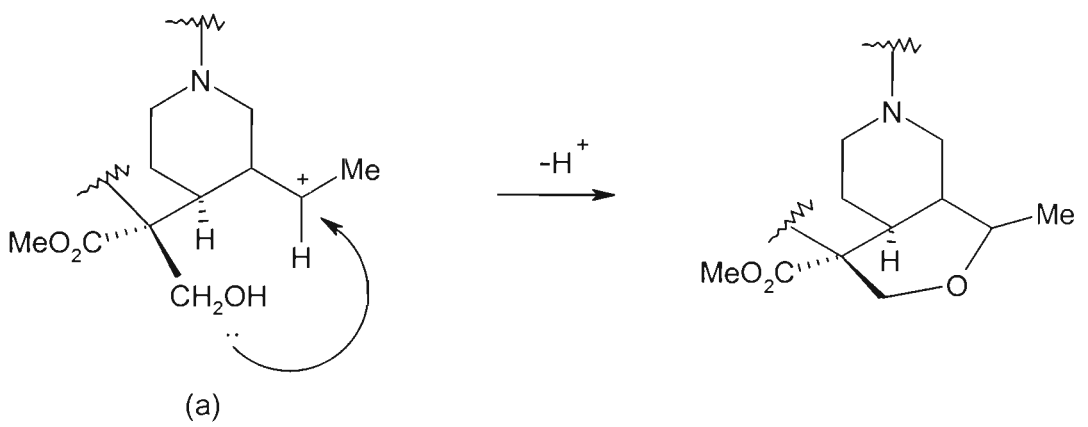


194: Kotarajine

The ^{1}H-NMR spectrum (Appendix 35) of **194** (relative configuration shown) shows some similarity to that of alstonamine, the main difference being the presence of a three-proton doublet at δ1.75 (H-19, J = 6.5 Hz), coupled to a multiplet at δ5.59 (assigned as H-18), indicative of an –O-CH-CH_3 moiety (Table 14). The proton attached to C-20 appeared as a multiplet at δ2.31 while the analogous carbon in the structure of alstonamine has no protons attached. The gCOSY spectrum (Appendix 36) indicated the proton attached to C-20 was coupled to two sets of doublets of doublets resonating at δ3.41 (H-21α, $J_{21\alpha,\ 20}$ = 7.0 Hz; $J_{21\alpha,\ 21\beta}$ = 13.5 Hz) and the other at δ2.16 (H-21β, $J_{21\beta,\ 20}$ = 7.5 Hz; $J_{21\beta,\ 21\alpha}$ = 13.5 Hz) respectively. The signal ascribed to the C-20 proton was also coupled to the signal for the

C-18 proton at δ5.59, and to the signal for H-15 (δ2.92). Two other AB systems were evident and these were assigned to the C-17 and C-6 protons. The C-17α and C-17β protons resonated at δ4.86 and 4.11 ($J_{17\alpha, 17\beta}$ = 17.5 Hz) respectively, while the C-6α and C-6β protons were observed at δ4.20 and 3.82 respectively ($J_{6\beta, 6\alpha}$ = 11.5 Hz). The chemical shifts in the aromatic region were indicative of an indole ring.

It is conceivable that kotarajine, bearing a reduced and rearranged skeleton closely related to altsonamine, is derived biosynthetically from valessamine **191** (see p. 106) via acid catalysed addition of the hydroxymethyl group to the double bond via a carbocation of type (a) as shown in Scheme 11. Kotarajine represents a new alkaloid skeleton.



(a)

Scheme 11. Proposed transformation of valessamine to kotarajine **194**

Table 14. ^{1}H-NMR data of kotarajine **194**

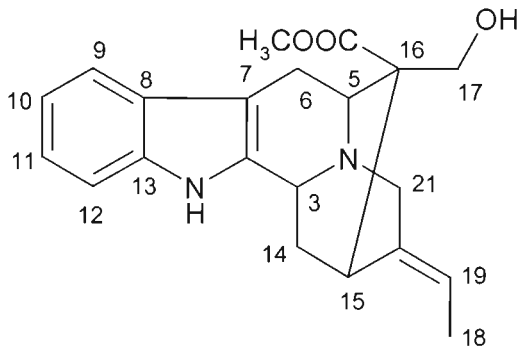
Protons	δ (ppm)	Integration	*J* (Hz)
H-3α	3.29 m	1H	
H-3β	2.38 m	1H	
H-6β	3.82 d	1H	$J_{6\beta, 6\alpha}$ = 11.5
H-6α	4.20 d	1H	$J_{6\alpha, 6\beta}$ = 11.5
H-9	7.50 d	1H	$J_{9, 10}$ = 8.0
H-10	7.09 t	1H	
H-11	7.19 t	1H	

Table 14 continued

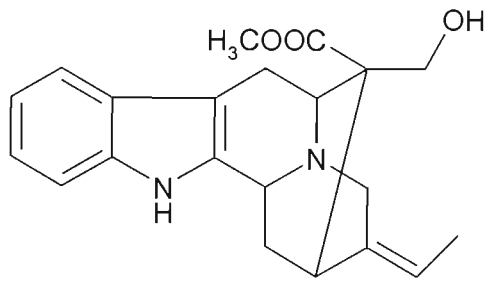
H-12	7.31 d	1H	$J_{12,11} = 8.0$
H-14α	1.58 m	1H	$J_{14\alpha,14\beta} = 15.5$; $J_{14\alpha,3} = 8.5$
H-14β	2.00 m	1H	
H-15	2.92 m	1H	
H-17α	4.86 d	1H	$J_{17\alpha,17\beta} = 17.5$
H-17β	4.11 d	1H	$J_{17\beta,17\alpha} = 17.5$
H-18	5.59 m	1H	
H-19	1.75 d	3H	$J_{19,18} = 6.5$
H-20	2.31 m	1H	
H-21α	3.41 dd	1H	$J_{21\alpha,20} = 7.0$; $J_{21\alpha,21\beta} = 13.5$
H-21β	2.16 dd	1H	$J_{21\beta,20} = 7.5$; $J_{21\beta,21\alpha} = 13.5$
COOMe	3.76 s	3H	
NH	9.44	1H	

5.4.5. (*E*)-Akuammidine

(E)-Akuammidine **195**, was obtained as a minor component in the form of a colourless, amorphous, slightly impure solid. The UV spectrum was characteristic of an indole chromophore, showing absorption maxima at 283 and 293 nm. It was found to have a molecular ion at m/z 353.1852 (MH^+) corresponding to the formula $C_{21}H_{24}N_2O_3$. Initially, this compound was identified based on the LREIMS ion fragmentation that showed an identical pattern to that for akuammidine (Appendix 38). Major peaks were observed at m/z 352 ($M^{+\cdot}$), 338, 337, 321, 293, 249, 182 and 169. The mass spectra of the stereoisomers of the akuammidine group were studied by Ohashi *et al.*[204] The suggested pattern of fragmentation of **195** is shown in Appendix 38.



195: (*E*)-akuammidine



196: (*Z*)-akuammidine

Akuammidine occurs naturally as two stereoisomers, (*E*)-akuammidine **195**, and (*Z*)-akuammidine **196**.[205] To establish the stereochemistry of the compound isolated in our work, the ^{1}H-NMR spectrum (Appendix 37) was recorded and compared to the literature data.[206] It can be seen from Table 15 that the sample spectrum is very similar to that recorded for *(E)*-akuammidine.

Table 15. ^{1}H-NMR spectrum of the sample and (*E*)-akuammidine

Protons	δ (ppm), *J* (Hz)	
	The sample	*(E)*-akuammidine[206]
3	4.23 br d (9.5)	4.24 br d ($J_{3,14\alpha}$ = 11, $J_{3,14\beta}$ = 2)
5	3.06 m	3.1 m
6α	2.92 m	2.94 dd ($J_{6\alpha,5\beta}$ = 5, $J_{17\alpha,17\beta}$ = 17.5
6β	3.30 m	3.30 dd ($J_{6\beta,5}$ = 1.5)
9	7.42 d (7.5)	7.42 d
10	7.05 t	7.05 t
11	7.11 t	7.11 t
12	7.33 d (7.5)	7.28 d
14α	2.67 m	2.67 ddd ($J_{14\alpha,14\beta}$ = 12.5, $J_{14\alpha,15}$ = 2)
14β	1.87 m	1.85 ddd ($J_{14\beta,15}$ = 3)
15	3.06 m	3.1 m
17β	3.67 d (10)	3.67 d ($J_{14\beta,14\alpha}$ = 11)
17α	3.83 d (10.5)	3.83 d
18	1.65 d	1.65 ddd ($J_{18,19}$ = 7, $J_{18,21\alpha}$ = 2, $J_{18,21\beta}$ = 2)
19	5.41 q	5.39 q
21α	3.61 d (14)	3.58 def

Table 15 continued

21β	3.52 d (14)	3.58 def
COOMe	2.95 s	2.94 s
NH	7.79 br s	7.90 br s

Chapter 6

Alkaloids from *Clerodendron calamitosum* L. and *C. paniculatum* L.

Clerodendron calamitosum L

Clerodendron paniculatum L.

Chapter 6

ALKALOIDS FROM *Clerodendron calamitosum* L. and *Clerodendron paniculatum* L.

6.1. Introduction

The genus *Clerodendron* belongs to the family Verbenaceae, and includes more than 500 species and varieties, used medicinally in South-East Asia from Burma to Indonesia. Some species have both internal and external uses while others function only externally as washes and poultices for sores, boils, and skin diseases.[63] This genus contains the most significant number of alkaloids known among members of the family Verbenaceae.[101] In a search of the literature, 5 of the reported 38 species of *Clerodendron*, were known to contain alkaloids. In Lombok, several species have been used for medicinal purposes, and based on their traditional use (treatment of malaria, wounds, and sore eyes), two of these, namely *Clerodendron calamitosum* L. and *Clerodendron paniculatum* L., were predicted to have antimicrobial properties. Both species gave a positive result to alkaloids tests.

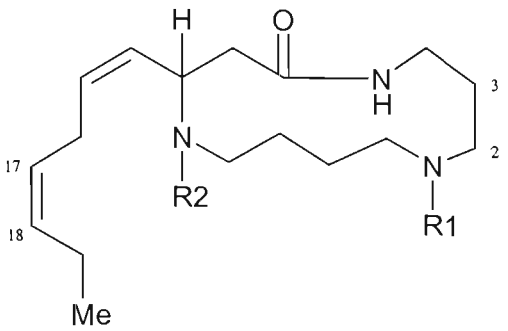
The leaves of the plant *Clerodendron calamitosum* L., locally called "keji beling" on Lombok, have been used commonly to treat malaria and wounds, as well as to destroy kidney stones. It is an erect scrub, which grows in dry, shady areas in villages, coconut groves, and at village borders and roadsides. During our chemical investigation of this species, Cheng *et al,*[207] in 2001, reported the isolation of three pheophorbide compounds from this species. However, in our study, no evidence for the existance of these compounds

was found in alkaloid extracts of specimens of *C. calamitosum* from Lombok. Our initial investigation showed that a Mayer's alkaloid test on whole plants produced a weak positive result. Further testing on separate parts of the plant indicated that alkaloids accumulated mainly in the leaves, therefore, our investigation focussed solely on the leaves.

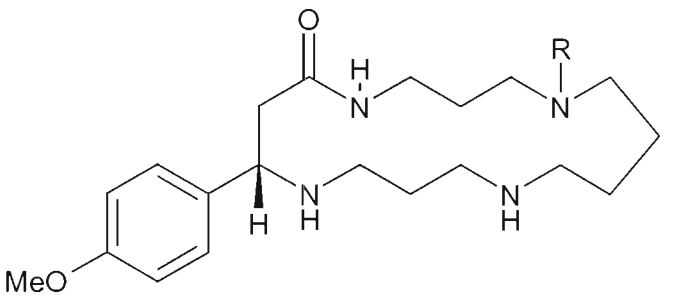
The plant *Clerodendron paniculatum* L, known locally as "kembang aik terjun", possesses beautiful red flowers, and is commonly cultivated in gardens for its ornamental value. Like other species in the Verbenaceae, the plant grows as an erect shrub, with a height up to 1.5 m. The plant is native to South-East Asia, and has been used to treat sore eyes by extracting the leaves with sterile water and applying the extract as eye drops in Lombok. The concentrated water extract of the leaves also has been utilised to treat wounds. Only the mature flowers tested positive for alkaloids, and young flowers (less than two months old) gave a negative alkaloid test result. It is perhaps possible that the leaves also contain alkaloids at very low concentration, but for the purposes of this study it was decided to study extracts from mature flowers only.

6.2. Alkaloids from the Family Verbenaceae

A variety of alkaloid structures have been identified in the family Verbenaceae. Among about 500 species, only relatively few have been shown to contain alkaloids.[79] From the genus *Clerodendron*, spermidine and indole alkaloid types have been identified. Two newly discovered macrocyclic spermidines, called myricoidine **197** and dihydromyricoidine **198**, were isolated from *Clerodendron myricoides*.[208] From *C. buchneri*, two spermine alkaloids, buchnerine **199** and N'-(*Z*)-p-methoxycinnamoylbuchnerine **200**, were isolated.[209]

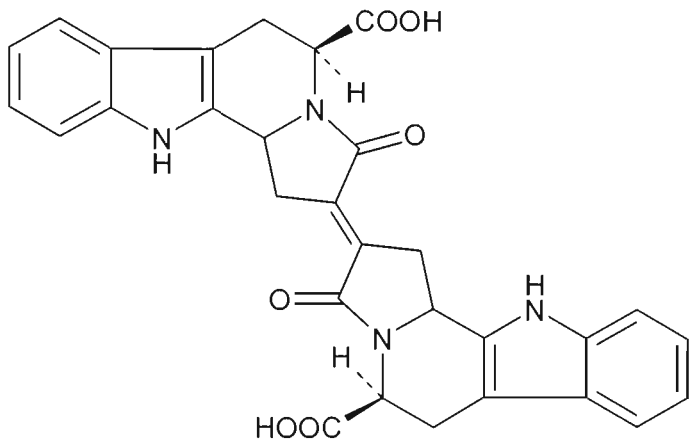


197: Myricoidine, R1=R2=H
198: Dihydromyricoidine,
17,18-dihydro, R1=R2=H

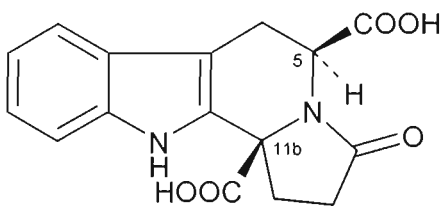


199: Buchnerine, R=H
200: *N*1-(Z)-p-methoxycinnamoylbuchnerine,
R = $COCH{=}CHC_6H_4OMe$ (*Z*)

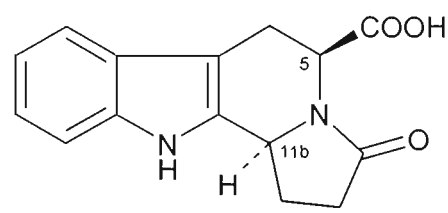
The indole alkaloids, trichotomine **201** and trichotomine G1 **202** and *N,N'*-diglucopyranosyltrichotomine **203**, were obtained from the plant *C. trichotomum*.[210] The absolute configuration of trichotomine was determined by Sasaki *et al.*[211] on the basis of X-ray structural analysis. A biomimetic synthesis of trichotomine, by a one-pot reaction of L-tryptophan and 2-oxoglutaric acid, was reported by Kapadia and Rao.[212] Further chemical studies to investigate the possibility of any precursors of trichotomine in the plant resulted in the isolation of four indolizino[8,7-b]indole-5-carboxylic acids, **204** - **207**.[213]



201: Trichotomine
202: Trichotomine G1, N-b-D-Glucosyl
203: *N,N'*-Di-(glucopyranosyl)-trichotomine, *N,N'*-Diglucosyl



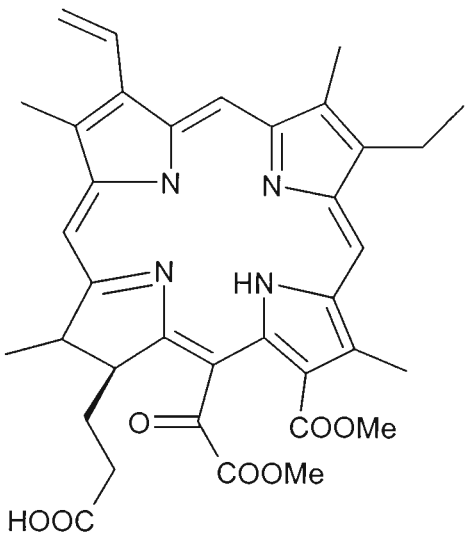
2,3,6,11-Tetrahydro-3-oxo-1*H*-indolizino-[8,7-b]indole-5,11b(5H)-dicarboxylic acid
204: (5*S*,11b*R*)-form
205: (5*S*,11b*S*)-form



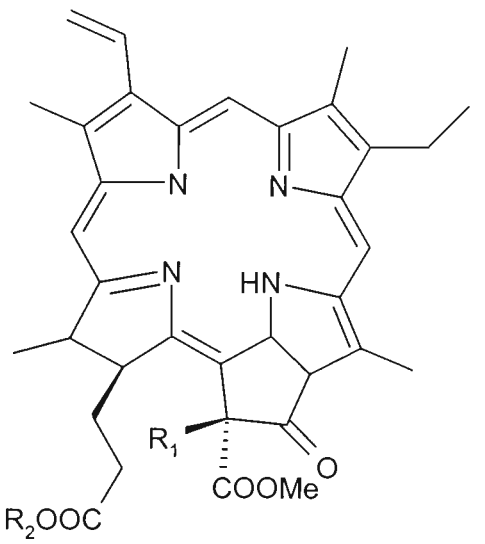
2,3,6,11, 11b-Hexahydro-3-oxo-1*H*-indolizino[8,7-b]indole-5-dicarboxylic acid
206: (*5S,11bS*)-form
207: (*5S,11bR*)-form

The plant *C. calamitosum* was reported[207] to contain three cytotoxic pheophorbide compounds, **208–210**, and from *C. cryptophyllum*, the methyl ester of **208,** namely compound **211** and the known (10*S*)-hydroxypheophytin **212** were isolated.[207] This represents the first reported isolation of compounds **208** and **212** as naturally occurring products. A biological evaluation of the compounds showed that compounds **208** and **209** exhibited strong cytotoxicity against human lung carcinoma, ileocecal carcinoma, renal carcinoma, breast adenocarcinoma, malignant melanoma, ovarian carcinoma, and

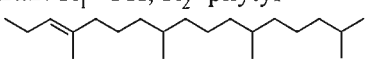
epidermoid carcinoma of the nasopharynx and its etoposide, vincristine, and camptothecine-resistant subclones.[207]



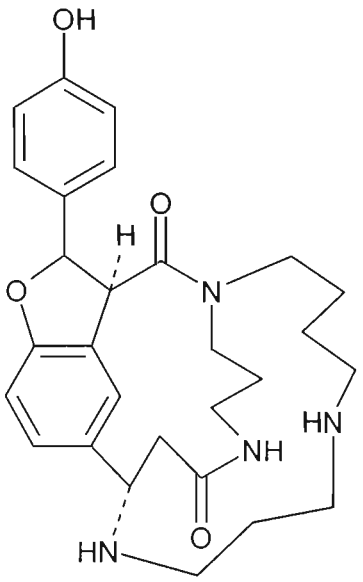
208



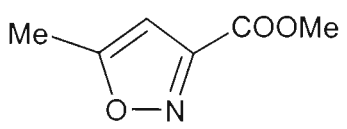
209: R_1=R_2=H
210: R_1=OH, R_2=H
211: R_1=OH, R_2=Me
212: R_1=OH, R_2=phytyl =



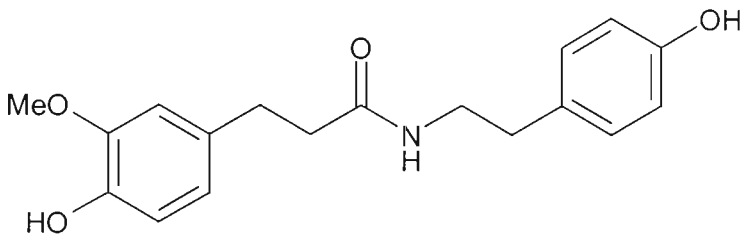
A major spermidine-related alkaloid, aphelandrine **213**, was isolated from *Premna integrifolia* (Verbenaceae) the only species belonging to the genus *Premna* reported to produce alkaloids.[214] Two partially characterized alkaloids, named premnine and ganiarine, were also isolated from this species.[215] From the genus *Gmelina*, only the plant species *Gmelina arborea* has been reported[216] to contain alkaloids. Premnazole **214**, an isoxazole alkaloid, was isolated from this species and this is the first known isoxazole type compound isolated from nature. It exhibits anti-inflammatory activity.[216]



213: Aphelandrine



214: Premnazole



215: *N-trans*-Feruloyltyramine

Species belonging to the genus *Vitex* have been studied intensively both in terms of their biology and chemistry. From this genus, two species, *Vitex leptobotrys* and *V. trifolia* Linn. were identified as sources of alkaloids. The plant *V. leptobotrys* contains the alkaloid derivative *N-trans*-feruloyltyramine **215**.[217] A new alkaloid, vitricine that is not yet fully characterized, was isolated from *V. trifolia* Linn.[218]

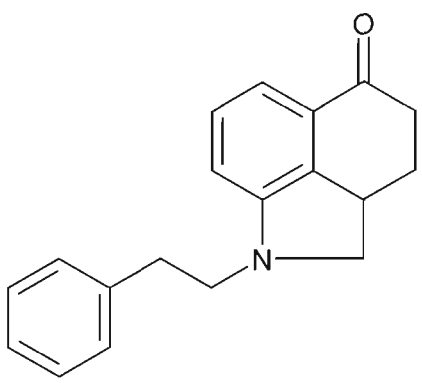
A few species of the genus *Duranta* have been utilized traditionally for medicinal purposes and some investigations of their chemistry have been reported in the literature.[219-224] However, only the species *Duranta repens* was reported to contain alkaloids. An isolation and preliminary identification of an alkaloid from the plant *D. repens* was reported by Yoesef *et al.*[225] and Rocca.[226] No alkaloid structures have yet been reported from the genus *Duranta*.

6.3. Isolation and Structure Elucidation of Alkaloids from *C. calamitosum* L. and *C. paniculatum* L.

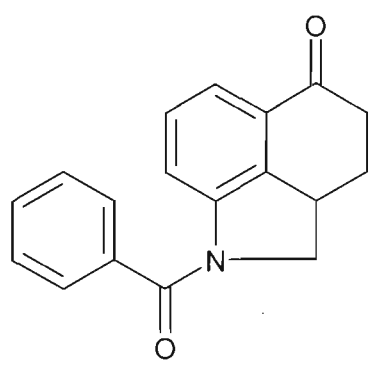
6.3.1. Alkaloids from *C. calamitosum* L.

PTLC separation and subsequent LRCIMS analysis of the alkaloid fraction from the leaves of the plant *C. calamitosum* revealed that at least five alkaloids were present. Unfortunately, most of the compounds were not fully characterised due to the very small amount of sample available. The following discusses some of the characteristics of the isolated compounds presented here in order of increasing polarity according to TLC (silica gel, ethyl acetate : isopropanol : NH_4OH = 95 : 4 : 1 by vol.).

6.3.1.1. Compound Lcc-5



216



217

The compound **216**, the least-polar compound with R_f = 0.91, was isolated as a brown solid. It fluoresced under UV light suggesting the presence of an extended conjugated system in the structure. The parent ion (LRCIMS) gave a peak at m/z 278 (MH^+). Further ion fragmentation (Appendix 39) by LREIMS produced a pattern remarkably similar to that

of the known compound[227] 1-benzoyl-2,2a,3,4-tetrahydro-benz[cd]indole-5(1H)-one **217** ($C_{18}H_{15}NO_2$). HREIMS indicated the sample had the molecular formula $C_{19}H_{18}NO$ (measured 277.1468, calc. 277.1467, M^+). A comparison of the formulae of the two compounds indicates that compound **217** has one more oxygen than **216**, while compound Lcc-5 has an extra carbon and three protons. This observed difference may be rationalised by the substitution of a biosynthetically reasonable phenethyl group for the *N*-benzoyl carbonyl group in **216**, although other variants on this theme (e.g. a tolyl substituent) cannot be excluded. Thus the tentative structure presented in **216** is based on the observation of the similarity in the fragmentation patterns of **216** and **217** as well as the observed molecular formula; biosynthetically, the main skeleton is indicative of a tryptophan origin. In order to elucidate the structure of this novel compound, a larger amount of plant extract is needed.

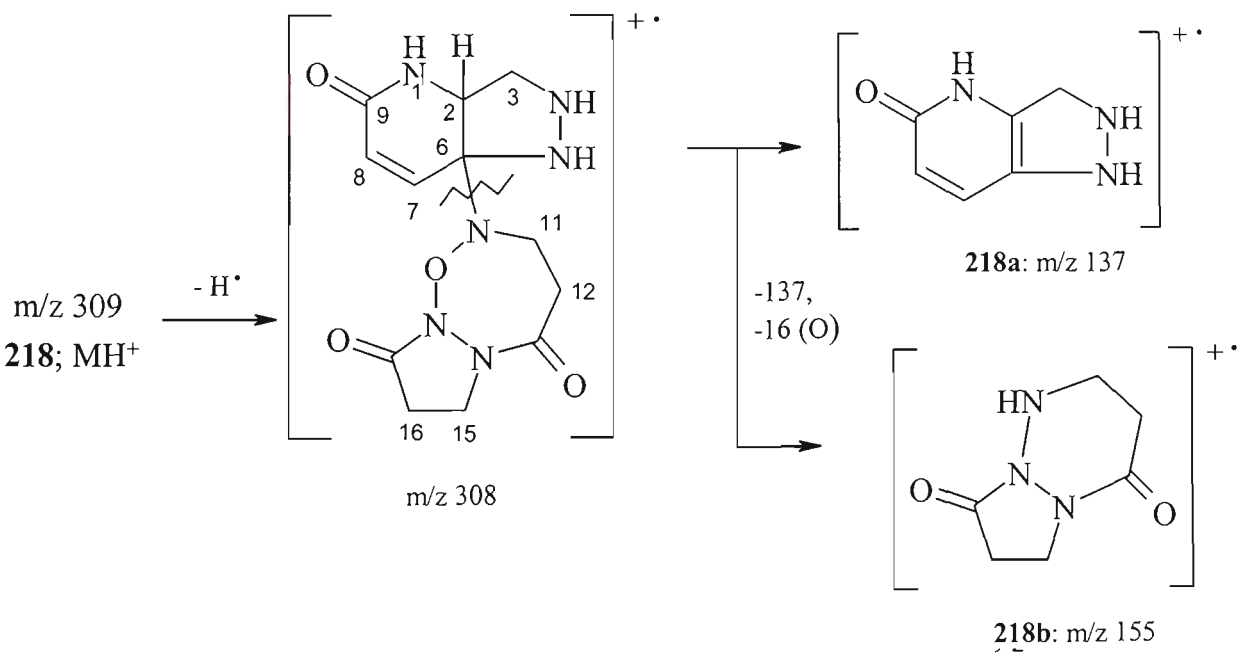
The compound **217**, an ergot alkaloid derivative, was first synthesised by Sailer *et al.* who reported it as being useful as a dopamine agonist for cardiovascular use.[227]

6.3.1.2. Compound Lcc-4

The compound called, for the purposes of this study, Lcc-4 with R_f 0.81, was also isolated as a brown UV-fluorescent solid. The presence of a conjugated system was further supported by several signals appearing in the aromatic region of the ^{1}H-NMR spectrum (Appendix 40). A LRCIMS parent ion for a protonated species at m/z 271 indicated compound Lcc-4 had a molecular weight of 270 a.m.u. However, further efforts to purify the compound by using silica gel-based PTLC resulted in the decomposition of the compound. Further characterisation was therefore not possible for this compound.

6.3.1.3. Compound Lcc-3

Compound Lcc-3, to which the structure **218** is very tentatively ascribed, could be a most unusual alkaloid. It was isolated as a yellow solid, with R_f = 0.56, and it was a major component of the alkaloid extract of the leaves of *C. calamitosum* L. The compound absorbed UV light and yielded positive results to Dragendorff's and Mayer's alkaloid tests. This suggested the presence of an amide or amine functional group in the compound. LRCIMS showed a signal for the parent ion at m/z 309 with another principal peak at m/z 155 (fragment **218a**) and 137 (fragment **218b**). HRCIMS indicated the following formulae for these ions: $C_{12}H_{16}N_6O_4$ (observed 309.1327, calc. 309.1311, for MH^+); $C_6H_8N_3O_2$ (observed 155.0698, calc. 155.0695, for **218b**); and $C_6H_7N_3O$ (observed 137.0604, calc. 137.0589, for **218a**). A proposed ion fragmentation of this compound is shown in Scheme 12. The structure elucidation of this compound is, however, mainly based on NMR data (^{1}H-NMR, gCOSY, gHSQC, and gHMBC). The data summary is shown in Table 16 and the spectra can be seen in the respective Appendices 41 – 44.



Scheme 12. Proposed ion fragmentation of Lcc-3 **218**

^{1}H-NMR, gCOSY, and gHMBC experiments suggested the presence of two main moieties in the structure. In the aromatic region, two doublets at δ6.76 (d, *J*=6.3 Hz, H-8) and δ6.02 (d, *J*=6.3 Hz, H-7) were observed indicating the presence of a HC=CH bond. The two protons, in the gHMBCspectrum, correlated to a carbonyl group (δ197.0, H-9) that is predicted to be attached to a nitrogen according to the chemical shift of the carbonyl group in the ^{13}C-NMR at δ 197.0 consistent with a lactam moiety. The gHMBC spectrum also indicated that the two protons have a cross peak to a quaternary carbon signal at δ75.8 (C-6). This evidence suggested the presence of an –N-CO-CH=CH-C- fragment in the compound. Another observation was the presence of a one-proton multiplet centred around δ4.29 (H–2). This relatively downfield chemical shift suggested the proton was in close proximity to electron-withdrawing groups. Further analysis of the gCOSY spectrum showed the proton at δ4.29 (H-2) was coupled to two proton doublets describing an AB system at δ2.61 (d, *J*=10.2 Hz, H-3) and δ2.79 (d, *J*=10.2 Hz, H-3'). The above three proton signals showed an HMBC correlation to a carbonyl group signal at δ197.0 (C-9) and the quaternary carbon signal at δ75.8 (C-6). It is noted here, that a chemical shift of 197.0 ppm is unusual for an α,β unsaturated lactam. From the above evidence, the pyrazolo-pyridone fragment is proposed. The structure of the second moiety was surmised from the presence of four multiplet signals showing four different AB systems representing a total of eight protons. This suggested the presence of two groups of -CH_2-CH_2- in a very similar environment. Each of the four multiplets appeared as two groups of peaks centred about δ2.21 (m, H-11 and H-15), δ2.35 (m, H-11 and H-15), and δ3.95 (m, H-12 and H-16), δ4.29 (m, H-12 and H-16). The chemical shifts of these multiplets suggested the presence

of adjacent electron withdrawing groups. The two groups of multiplets showed cross peaks in the gCOSY spectrum indicative of coupling to each other. In addition, the gHMBC spectrum indicated that the multiplet at δ2.35 (H-11) correlated to the quaternary carbon at δ75.8 (C-6) in the other portion of the molecule. The gHMBC spectrum also showed a correlation of a multiplet at δ3.95 (H-12) to a carbonyl signal at δ174.79.

Table 16. ^{1}H-NMR data and H-C correlation of Lcc-3 **218**

Proton Number	^{1}H (δ ppm, multiplicity, *J* in Hz)	H-C correlation of gHSQC	H-C correlation of gHMBC
2	4.29m	81.8	197.0 148.6 75.8
3	2.79d (*J*=10.2)	40.3	197.0 81.8 75.8
	2.61d (*J*= 10.2)	40.3	197.0 81.8 75.8
7	6.02d (*J*=6.3)	128.9	75.8
8	6.76d, (*J*=6.3)	148.6	197.0 81.8
11 & 15	2.35m 2.21m	39.6 39.6	75.8
12 & 16	4.11 m 3.95 m	66.3 66.3	 174.8

However, the location of the nitrogens in the structure remains problematic because the small amount of sample prevented the collection of an ^{15}N-NMR spectrum. The presence of nitrogens in the compound is based on the observed fragment ion pattern (Scheme 12) and

accurate mass spectral analysis as mentioned above as well the positive results of the Dragendorff's and Mayer's tests.

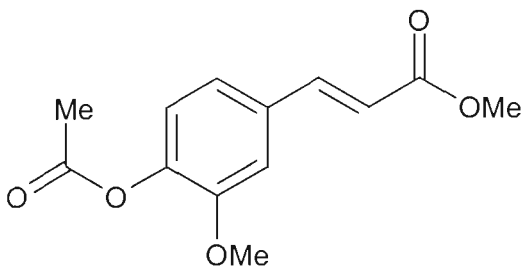
No such structure as tentatively assigned in **218** has been reported as a natural product or from synthesis.

6.3.1.4. Lcc-2

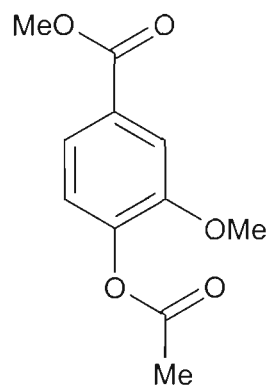
The band with the lowest R_f (0.46) was isolated as a brown solid that was UV absorbing. LRCIMS indicated the presence of two compounds with MH^+ signals appearing at m/z 286 and 310. For alkaloid compounds, this suggests that the compounds responsible for both peaks possess an odd number of nitrogens. The presence of two compounds in the fraction was confirmed by GC/EIMS, with the gas chromatogram showing two peaks with retention times of 20.3 min and 20.9 min (Appendix 45). Unfortunately, there were no similar fragment ion patterns found in the computer library that matched closely to the two compounds. Further efforts to identify the two unknown compounds by using MS/MS experiments to fragment a selected molecular ion in the mixture proved unsuccessful, since again, no ion fragmentation pattern in the computer library matched closely with either of the two compounds present in the fraction (Appendix 45). A thorough search of the literature also failed to find any similar fragmentation patterns. The formulae of the two compounds were determined by HRCIMS as $C_{11}H_{23}N_3O_7$ (measured 310.1615, calc. 310.1614, for MH^+) for the compound with molecular weight 309 amu and $C_{12}H_{19}N_3O_5$ (measured 286.1383, calc. 286.1403, for MH^+) for the compound with molecular weight 285 a.m.u. It is quite likely that the two compounds are new natural products, but they await further characterisation when more material is collected.

6.3.2. Compounds isolated from *C. paniculatum* L.

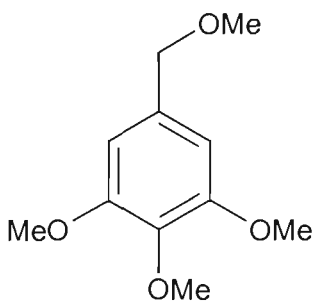
Due to the relatively small quantities of crude alkaloid extract collected, the identification and characterisation of compounds from this species could not be fully carried out. The crude alkaloid extract, obtained from acidic-basic extractions, was chromatographed using PTLC (silica gel, CH_2Cl_2:MeOH:NH_4OH/95:4:1). This resulted in three fractions.



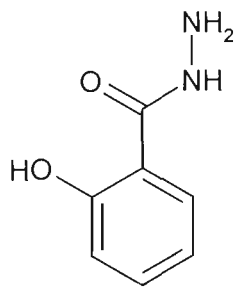
219: Methyl 4-acetoxy-3-methoxycinnamate



220: Methyl 4-acetoxy-3-methoxybenzoate



221: 3,4,5-Trimethoxybenzyl methyl ether



222: Salicylic acid hydrazide

Gas chromatography/mass spectrometric analysis of fraction 3 (R_f = 0.83) indicated the presence of the following non-alkaloidal components: methyl 4-acetoxy-3-methoxycinnamate **219**, methyl 4-methoxy-3-methoxybenzoate **220**, and 3,4,5-

trimethoxybenzyl methyl ether **221** (Appendix 46 & 47). Compound library matches were used for identification purposes.

Compounds **219** and **220** are known natural products isolated from many plant species.[228,229] The biosynthesis of benzoic acid derivatives from cinnamic acid derivatives has been studied in cell cultures of *Hypericum androsaemum* by Ahmed *et al.*[230] Preparation of cinnamic acid derivatives, containing piperidine and benzoic acid groups, as fatty acid synthase inhibitors was also carried out by Gaitanopoulos *et al.*[231] who reported that some cinnamic acid derivatives were active against a wide range microorganisms including both Gram-negative and Gram-positive bacteria. Compound **221** has also been isolated previously as a natural product.[232-234] Derivatives of compound **221** have been used in the preparation of some patented antibiotics such as trimethoprim[235] and vancomycin-type antibiotics.[236,237]

From fraction 2 (R_f = 0.74), salicylic acid hydrazide **222** was identified by GC/MS analysis (Appendix 48). A study had been made of the fate of salicylic acid hydrazide **222** in the rabbit and it was reported[238] that it is metabolised mainly by conjugation with glucuronic acid and partly by hydrolysis to salicylic acid. Reaction of the hydrazides of salicylic, nicotinic and isonicotinic acids with isothiocyanates was used to produce some new disulfide derivatives of triazoles, which have antibacterial activity.[239]

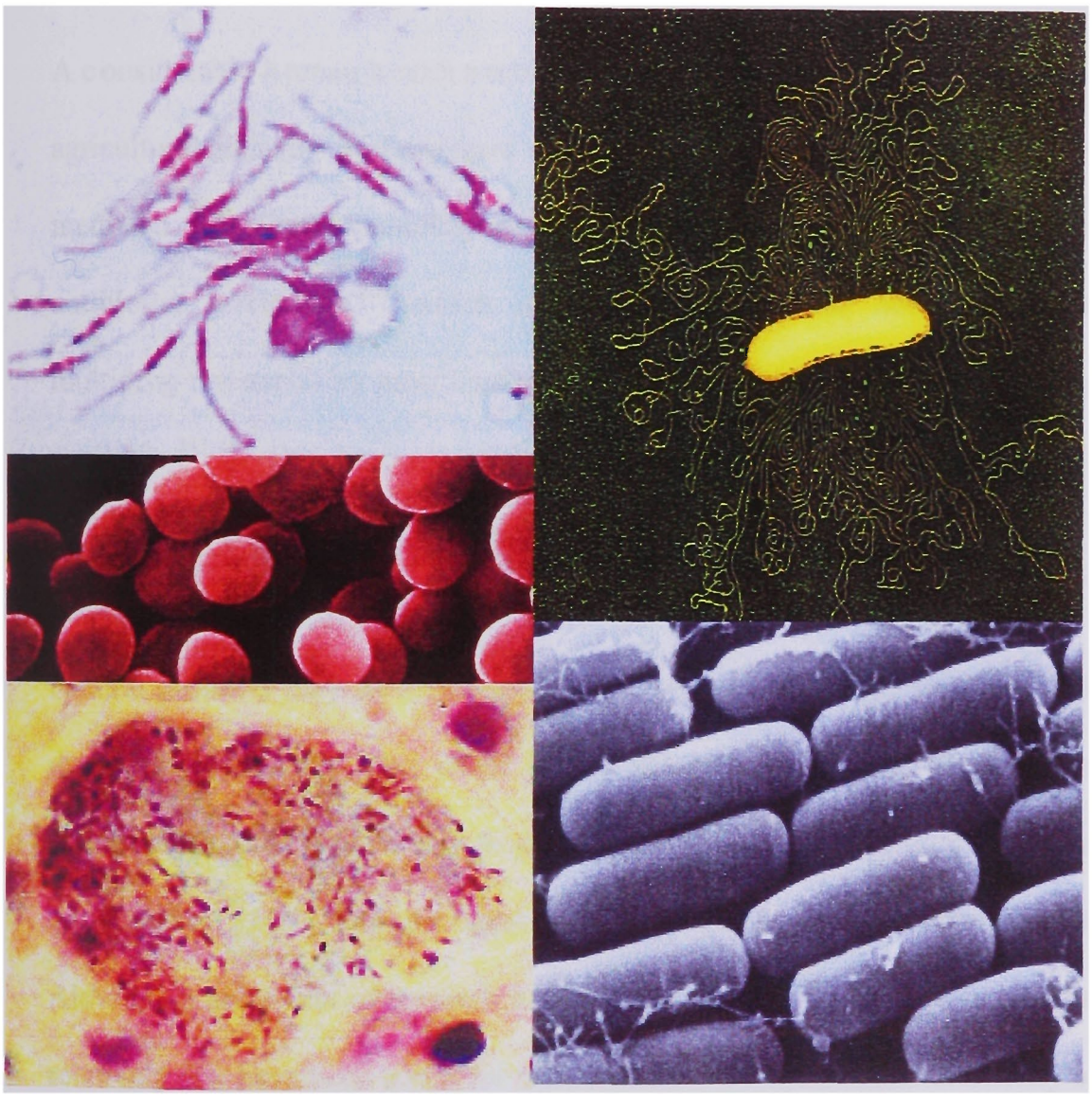
Fraction 1 (R_f = 0.54) from the crude alkaloid extract gave a positive reaction to Dragendorff's reagent, indicative of alkaloids. LRCIMS of fraction 1 gave three significant peaks at 313, 299, and 261 (MH^+) indicating the compounds are perhaps alkaloids with

even numbers of nitrogens present. The formulae of the compounds responsible for the three peaks were subsequently obtained by HRCIMS. These were assigned as $C_{15}H_{24}N_2O_5$ (measured 313.1745, calc. 313.1763, for MH^+), $C_{14}H_{22}N_2O_5$ (measured 299.1610, calc. 299.1607, for MH^+), and $C_{13}H_{28}N_2O_3$ (measured 261.2215, calc. 261.2178, for MH^+) respectively. A search of the relevant literature indicated that these molecular formulae do not correspond to any natural products that have been reported previously from any species of *Clerodendron* or from the family Verbenaceae. Time constraints and sample availability prevented further work on their structural elucidation.

It is worth noting these non-alkaloidal components constituted a very small amount of compound (< 1 mg) and their persistence in the organic phase throughout the extraction process might be due to the fact that they are only weak acids.

Chapter 7

Antimicrobial Testing



Clockwise from top left: Sporozoites of *Plasmodium falciparum*, DNA of *Escherichia coli*, *Escherichia coli* cells, Schizonts of *Plasmodium falciparum*, and *Staphylococcus aureus* cells

Chapter 7
ANTIMICROBIAL TESTING

7.1. Introduction

A considerable human health problem in rural areas of developing countries, and in the agricultural industry of developed countries, is contagious disease caused by microbially mediated infections including malaria.[240,241] This has led to the widespread use of antibiotics worldwide,[242] which in turn, has led to the development of drug and/or multidrug resistance by most human pathogens, for which there are few effective therapies available.[243,244] One way of tackling this problem is by developing new antimicrobial agents or finding new potent drugs from nature.[15]

Natural products play an important role in the process of drug discovery. The majority of drugs currently being used either have a natural origin or are derived from compounds found in nature.[245] For millions of years, terrestrial plants have produced a large number of antimicrobial agents, with the majority being alkaloid compounds (for detail see Chapter 1). Although a great deal of research has concentrated on finding and isolating new alkaloids, relatively few have been tested for their antibacterial or antimalarial activity or potency.

This chapter provides a brief review of recent progress in antimicrobial testing techniques and summarizes the results of antibacterial and antimalarial testing of major alkaloids isolated from the selected medicinal plants of Lombok.

7.2. Assays for Detecting Antibacterial Activity

Recently, a unified approach to antimicrobial screening has been undertaken through a collaboration between natural product chemists, biochemists, molecular pharmacologists, and cell biologists. Recent developments have focussed on a variety of specific inhibition and receptor antagonist bioassays in screening compounds.[246,247] Some pharmacological and biotechnology industries have established several large screening programs and it is difficult for individual researchers to compete with them. However, by focussing on a specific target such as investigating antimicrobial activity for specific compounds like alkaloids, the possibility for success may be increased.

The classification of commonly used assays can be based on whether they are performed on solid media (agar) or in liquid media (broth). Both types of tests have advantages and disadvantages, so the assay method of choice depends on the compatibility of the test compound with the media, the compound's solubility, and its stability under the respective conditions.[248] It must be also considered that the medium constituents may react with the compounds under scrutiny and as a result may influence the results. The pH of the medium could affect either the tested compounds or the response of the microorganisms. The temperature, duration of compound exposure and the type of cells used, are also crucial factors to be considered.[248,249] All of the above factors need to be standardised in antimicrobial screening.

A rapid and facile method for antimicrobial screening of natural extracts is the use of solid media or the agar disk diffusion method.[250,251] But, due to differences in physical properties, such as solubility, volatility, and variable diffusion of antimicrobial agents in agar, any comparison between the relative antimicrobial activity of different samples is essentially meaningless.[252] Sometimes a sharp cut off between bacterial growth and inhibition is produced, and the zone of inhibition cannot be used to determine the minimum inhibitory concentration (MIC). Additionally, the agar/solid method cannot be applied for volatile and unstable compounds, since there is a long incubation time required.[252]

In many cases, antimicrobial assays in liquid media are preferred and they are more direct than agar diffusion assays due to the possibility of diffusion and reaction of the compound with agar. However, these types of assays are generally time consuming.[249,253] The inhibition of microbial growth is observed microscopically and a minimum inhibitory concentration (MIC) is quantitatively determined. These techniques may involve plating out a serial dilution,[253] however, this procedure is laborious and susceptible to contamination.[252] MICs can also be measured in broth from the turbidity of the microbial culture. The turbidity can be estimated visually or spectroscopically by measuring the optical density. But, the solubility and concentration of the test substance can potentially interfere with the turbidity of the microbial culture, thus the accuracy of the MIC is compromised.

The antibacterial activity of a compound or extract is usually measured when it is dissolved in an aqueous solution.[254] However, many extracted compounds and large number of antimicrobial compounds cannot be dissolved appreciably in water. However, this problem

can be solved by using ultrasonication;[248] a water miscible organic solvent;[249,254] or an emulsifying agent[249,255]. These techniques must be used with caution as the final concentration of the solvent or emulsifying agent must not kill the microorganism, causing a false positive reading from the antimicrobial testing. Ethanol (5%) and the common emulsifying agent Tween 80 were found to interfere with cell growth. [249,255] The most suitable solvent for solubilising compounds for antimicrobial assays is acetone, which can be combined with ultrasonication to dissolve non-polar compounds in an aqueous environment.

An accurate and reproducible liquid assay, performed in 96 well microplates, was developed by Chand *et al.* in 1995.[252] This is appropriate for rapid screening of natural products for antimicrobial activity. This method, called the fluorescein diacetate (FDA) assay, depends on the activity of non-specific esterases, which are produced by metabolically active organisms. These metabolic enzymes have been shown to hydrolyse colourless, non-fluorescent fluorescein diacetate to fluorescein, a yellowish green compound, which fluoresces under UV light. The viability of a microbial culture can thus be determined on the basis of a colour change or by detection of fluorescence. This method can solve the problems of interference from the tested compounds in typical turbidometric assays, although autofluorescence may be a problem. The FDA assay is suitable for use in a wide range of different microorganisms, since most organisms produce non-specific esterases.

There is a difference in the terms bacteriostatic and bactericidal activity to report microbial response. Bacteriostasis is defined to be the growth-inhibitory action of a chemical

antagonist on a microorganism under conditions where re-growth can normally occur, while bactericidal activity is applied to the situation where a microorganism subjected to the action of a chemical antagonist is unable to recover.[249] Bactericidal activity can be detected by examining the cells under a microscope or by incubating the culture in fresh media and assessing the ability of the cells to recover.[249] The latter method is more accurate than the former technique. To achieve an accurate and reproducible overall assessment of antimicrobial activity, a combination of the two methods is recommended.

7.3. Assays for Detecting Antimalarial Activity

As previously mentioned, recent developments in antimicrobial screening, including antimalarial screening, have been directed towards a variety of specific inhibition and receptor antagonist bioassays in screening compounds. Hariharan *et al.*[256] in 1999 studied a mechanism-based inhibitor to develop a high throughput coupled enzyme assay to screen for novel antimalarial agents. They identified potent enzyme inhibitors through a robust high-throughput screening (HTS) assay that is currently thought to be the most efficient way of searching for lead molecules. A developed HTS assay mimics a crucial step in the essential purine salvage pathway of the malarial parasite *Plasmodium falciparum*. Purified recombinant enzymes hypoxanthine guanine phosphoribosyl transferase (HGPRT) and inosine monophosphate dehydrogenase (IMPDH) from respective malarial parasites and human hosts were used in tandem. Through this technique a novel inhibitor that kills the parasite by inhibiting the salvage pathway of the parasites was identified.[256]

A novel screening method based on specifically inhibiting the non-mevalonate pathway by using microorganisms such as *Bacillus sp.* was reported by Seto *et at* in 2001.[257] The organisms are capable of utilisng both mevalonate and non-mevalonate pathways for the biosynthesis of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). This method facilitates screening for antimicrobial, herbicidal or antimalarial agents capable of inhibiting one of the enzyme reactions on the mevalonate pathway responsible for synthesizing IPP and DMAPP without purifying and assaying the enzymes involved.

Another method for screening antimalarial compounds was developed by Clough *et al,*[258] that is based on targeting a plastid DNA of the parasite *Plasmodium falciparum*. The plastid DNA has been sequenced and found to contain a gene encoding an EF-Tu protein. Inhibitors of the protein have been proved effective as antimalarial compounds. Moreover, the 23S rRNA encoded on the malarial parasite's DNA is a target for antimalarial agents and the antibiotic thiostrepton acts as an antimalarial agent by binding to the parasite's RNA.[258]

A high-throughput screen for identification of a new antimalarial pharmocophore based on haematin polymerisation is an advanced method reported by the Kurosawa group.[259] Haematin polymerisation is a parasite-specific process that enables the detoxification of haem following its release in the lysomonal digestive vacuole during haemoglobin degradation. They developed a high-throughput *in vitro* microassay of haematin polymerisation based on the detection of ^{14}C-labelled haematin incorporated into polymeric haemozoin (malaria pigment).

While most developed methods require advanced instruments, the Pandey group[260] was able to produce a simple and reproducible method. The assay utilised the formation of β-haematin produced by the malaria parasite. The assay has been used routinely in the identification of potential antimalarial compounds. Another inexpensive and simple method for screening potential antimalarial drugs relies on the formation of dark pigment precipitation after a solution of tested compound is added synchronously to cultures of *Plasmodium falciparum*. Pigment is produced when a compound is unable to prevent the maturation of rings to *schizont* during incubation. However, in the presence of an active compound, maturation is inhibited, and, consequently, pigment and precipitation is not observed.[261]

Another *in vitro* method of antimalarial testing involves assessment of effects on parasite growth as measured by the incorporation of ^{3}H-hypoxanthine. This activity assay is performed with continuously cultured parasites according to the procedure of Trager and Jensen.[262] This method is economical and simple to perform; however, it is labour intensive and subject to individual variability.

Additional excellent reviews of methods for screening antimalarial agents have been published by Angerhofer *et al.* in 1992,[263] Phillipson in 1991,[264] and Richards in 1984.[265]

7.4. Results of Antibacterial Testing

7.4.1. *Voacanga foetida* (Bl.) Rolfe

The antibacterial testing of the crude alkaloidal extracts used the Fluorescein Diacetate Assay (FDA) procedure presented by Chand *et al.*[252] and Benkendorff *et al.*[266] The procedure is summarised in Section 7.2 above. Most crude alkaloidal extracts from aerial parts of the plant *V. foetida*, bark, fruits and leaves, were found to inhibit the hydrolysis of FDA by bacteria at a concentration of 5 mg/ml (Table 17).

The crude alkaloidal extract from the bark inhibited the growth of the Gram-positive bacterium, *Staphylococcus aureus*, and the Gram-negative bacterium, *Escherichia coli*, at a concentration of 5 mg/ml. Partial growth inhibition was also displayed by the crude extract at a lower concentration (0.5 mg/ml). It was found that the new compound lombine, a major compound isolated from the bark, was active against both *S. aureus* and *E. coli*. The antibacterial activity of lombine was substantially greater than the crude extract, with complete growth inhibition occurring at 0.5 mg/ml for both bacteria. At a concentration 0.05 mg/ml, lombine displayed partial growth inhibition. Bactericidal activity was assessed via replating on agar and undertaking associated dilution studies. Lombine exhibited bactericidal activity against *S. aureus* and *E. coli* causing 94% and 95% cell death respectively at a concentration of 0.5 mg/ml when compared to control cultures. Lombine thus has modest bactericidal potency and could form a useful lead for further antibacterial development.

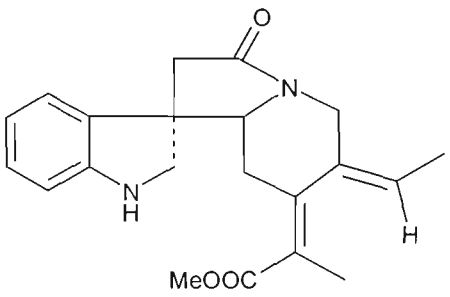
Table 17. Bacteriostatic activity of samples from *Voacanga foetida* (Bl.) Rolfe

Sample	Concentration (mg/ml)	Bacteriostatic activity	
		*S. aureus***	*E. coli***

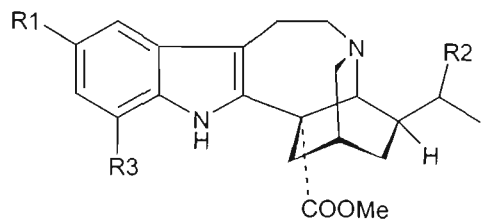
CEA* of bark	5.0	++	++
	0.5	+	+
CEA of fruits	5.0	++	+
	0.5	+	-
CEA of leaves	5.0	++	-
	0.5	+	-
Lombine	0.5	++	++
	0.05	+	+
Coronaridine	1.0	++	-
	0.1	-	-
Mataranine A&B (mixture)	1.0	++	-
	0.1	-	-
Voacristine	1.0	++	-
	0.1	+	-

* CEA = crude extract of alkaloids; ** (++: Complete suppression of FDA hydrolysis; +: partial FDA hydrolysis; -: no antimicrobial properties)

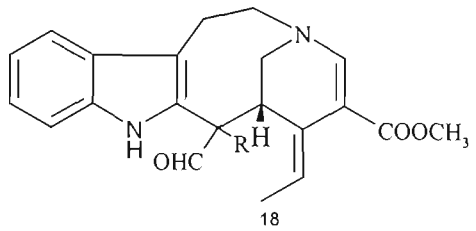
The crude alkaloidal extract of the fruits of *V. foetida* (Bl.) Rolfe at a concentration of 5.0 mg/ml was bacteriolytic, and it killed 100 percent of the *S. aureus* after replating on the agar plates. Further bacterial testing of isolated alkaloidal components from the fruits also indicated activity against *S. aureus*. Coronaridine (1.0 mg/ml), the major compound of the bark, partly reduced FDA hydrolysis suggesting some bacteriostatic activity for this alkaloid. After replating, coronaridine (1.0 mg.ml) was able to kill 57% of the *S. aureus* cells present. Interestingly, mixed mataranine A and B (0.5 mg/ml), the new alkaloids isolated from *A. scholaris* (leaves from young trees) and also found in the bark of the plant *V. foetida* (Bl.) Rolfe, showed stronger bacteriostatic activity, killing 87% of *S. aureus* cells (at 1.0 mg/ml). There was no activity observed following a ten-fold dilution of the above concentrations.



Lombine



Voacangine, R1=OCH_3, R2=H_2, R3=H
Coronaridine, R1=H, R2=H_2, R3=H
Voacristine, R1=H, R2=OH, R3=OMe



Mataranine A: R= Hβ
Mataranine B: R = Hα

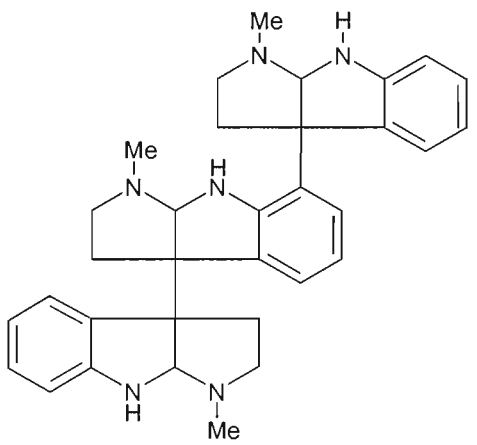
Further testing against *E. coli* indicated that there was no activity observed for coronaridine, mataranine A and B (mixture), and voacristine at the same concentrations used to test against *S. aureus*. However, antimicrobial activity might be possible at higher concentrations because the crude alkaloidal extract from the fruits exhibited activity against *E. coli* at 5.0 mg/ml. However, further tests to find a minimum active concentration could not be performed due to the small amounts of compound available.

The crude alkaloid extract of the leaves (5.0 mg/ml) also showed antibacterial activity against *S. aureus*, but not against *E. coli*. A similar activity trend was also found for voacristine, the major alkaloidal component in the leaves. Voacristine at a concentration of

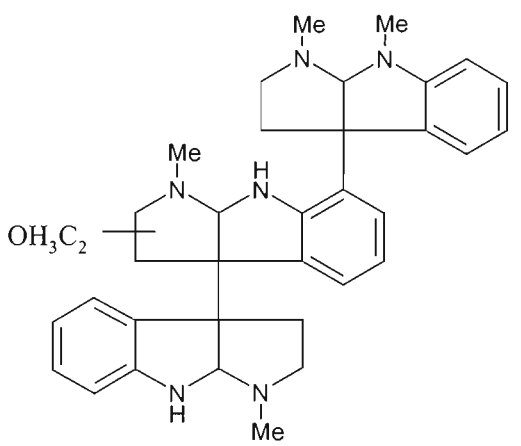
1.0 mg/ml showed bacteriostatic activity against *S. aureus,* killing 87% of the bacterial cells.

From the above observations, it appears that the alkaloid lombine from the bark of the plant *V. foetida* (Bl.) Rolfe, had a broader spectrum of activity, since it killed both Gram positive and Gram negative bacteria. Alkaloids from the fruits and the leaves, on the other hand, tended to have more a specific target in that they were only active against the Gram-positive bacterium *S. aureus*.

7.4.2. *Psychotria malayana* Jack.



Hodgkinsine



LPM-574

Initial antibacterial testing of the crude alkaloidal extract of leaves of the plant *Psychotria malayana* Jack. (5 mg/ml) resulted in inhibition of both *S. aureus* and *E. coli* (Table 18). Subsequent tests using the main alkaloid component of the leaves, hodgkinsine (1.0 mg/ml), had no antibacterial activity. However, antibacterial activity was found in a minor alkaloidal compound, LPM-574, a derivative of hodgkinsine. The active concentration was

observed to be at 1.0 mg/ml. LPM-574 had bacteriolytic potency since *S. aureus* (74%) and *E. coli* (78%) were killed after replating.

Table 18. Bacteriostatic activity of samples from *Psychotria malayana* Jack.

Sample	Concentration (mg/ml)	Bacteriostatic activity	
		*S. aureus***	*E. coli***
CEA* of leaves	5.0	++	++
	0.5	-	-
CEA of bark	5.0	+	+
	0.5	-	-
Hodgkinsine	1.0	-	-
	0.5	-	-
LPM-574	1.0	++	++
	0.1	+	+

*) CEA = crude extract of alkaloids; ** (++: Complete suppression of FDA hydrolysis; +: partial FDA hydrolysis; -: no antimicrobial properties)

7.4.3. *Clerodendron calamitosum* L. and *Clerodendron paniculatum* L.

Antibacterial testing against *S. aureus* and *E. coli* for the crude alkaloidal extract from leaves of the plant *Clerodendron calamitosum* L. and flowers of *C. paniculatum* L. were carried out at two concentrations, 5.0 and 0.5 mg/ml. At a concentration of 5.0 mg/ml both extracts completely suppressed FDA hydrolysis by the *E. coli* and partial inhibition were shown at a concentration of 0.5 mg/ml. This indicates that both extracts had antibacterial activity. However, no activity was found when both alkaloid extracts were tested against *S. aureus* at the two concentrations.

7.5. Results of Antimalarial Testing

Screening for antimalarial activity was carried out using a modified method of the 3H-hypoxanthine incorporation procedure,[262] by using a semiautomated microdilution technique. This work was done in Bangkok, Thailand at the Protein-Ligand Engineering and Antimalarial Screening Laboratories, National Centre for Genetic Engineering and Biotechnology, National Science and Technology Development Agency by Dr. Sumalee Kamchonwongpaisan.

The samples were put into a culture medium, and *Plasmodium falciparum* infected red blood cells with cell suspension of parasitemia was added. The plates were cultured under standard conditions for 24 hours before the addition of ^{3}H-hypoxanthine. A further incubation was done for 18-20 hours, followed by harvesting of the parasites DNA. The change of ^{3}H-hypoxanthine was determined by a radiation counter, from which the inhibitory concentrations of samples were calculated through their dose-response curves. The testing used both an antifolate resistant strain of the parasite (K1) and an antifolate sensitive parasite strain (TM4).

The main focus of this screening was the alkaloidal components from leaves of young trees of *A. scholaris*, since they are commonly used as an antimalarial remedy in Lombok. The crude alkaloid extract exhibited activity against both the K1 and TM4 strains of *Plasmodium falciparum*. The IC_{50} values (concentration for 50% inhibition) less than 25.0 μg/ml were 15.6 μg/ml for K1 and 21.0 μg/ml for TM4.

Further antimalarial testing of the major alkaloids (a mixture of mataranine A and B) showed considerably increased antimalarial activity against both K1 and TM4 with IC_{50} values at 2.6 μg/ml (7.4 μM) and 3.4 μg/ml (9.7 μM) respectively. The IC_{50} value for the K1 strain is similar to that of 20-*epi*-antirhine having a moderate IC_{50} (7.5 μM) against K1, although the most active compounds were found to be the bisindole type alkaloids.[197,168] The mataranines do have considerable potential though as drug leads for new antimalarial agents.

Another target for antimalarial testing was the crude alkaloid extract of *Clerodendron calamitosum* L, since the leaves of this plant are also utilised to treat malaria in Lombok. The crude alkaloid extract from the leaves was active against the K1 strain of *Plasmodium falciparum* with an IC_{50} value of 22.0 μg/ml. Further antimalarial testing on the major isolated alkaloid from the leaves, Lcc-3, indicated that this compound is most likely to be responsible for the activity with an IC_{50} of 13.3 μg/ml (43.1 μM) (K1 strain).

Chapter 8
CONCLUSIONS AND FUTURE WORK

8.1. Conclusions

A combined chemo- and bio-rational strategy, based on alkaloids and medicinal plants respectively, was demonstrated to be an effective and efficient approach in finding new biologically active components from nature. By targeting alkaloid compounds, many of which are known to have biological activity, and a plant selection guided by information of traditional medicinal uses, several new and known antimicrobial alkaloids were isolated. Some of these could be useful as drug leads for antibacterial and antimalarial agents. The overall summary and results of this study are shown in Table 19.

By following the steps of extraction, isolation, purification, and structure elucidation, a new optically active indole alkaloid lombine (major) and the known alkaloid voacangine (minor) were isolated and identified from the bark of *Voacanga foetida* (Bl.) Rolfe, used ethnomedically on Lombok for the treatment of wounds, itches, and swellings. The fruits of the plant produced three known alkaloid compounds, coronaridine (major), voacangine, and voacristine, together with the new alkaloids mataranine A and B, also isolated in this study from *Alstonia scholaris* R. Br. Voacristine was found as a major alkaloid together with the minor alkaloid voacangine from the leaves of *V. foetida* (Bl.) Rolfe. A structural revision for voacristine was also presented.

Initial antibacterial testing of the crude alkaloid extract from *V. foetida* (Bl.) Rolfe (bark and fruits) showed activity against both Gram-positive (*Staphylococcus aureus*) bacteria and Gram-negative (*Escherichia coli*) bacteria. The new alkaloids, lombine (bark) and mataranine A and B (fruits), were found to have antibacterial properties. Bactericidal activity was exhibited by lombine at a concentration of 0.5 mg/ml against *S. aureus* and *E. coli*. At the lower concentration of 0.05 mg/l, it partially inhibited the growth of both *S. aureus* and *E. coli*. The crude alkaloid extract from the leaves was found to have antibacterial activity only against *S. aureus*. Voacristine was most likely to be the main active component. On the basis of testing of the isolated substances, these compounds, particularly lombine, were found to be potentially useful as drug leads for antibacterial agents.

Another new antibacaterial agent, a substituted derivative of hodgkinsine was isolated from *Psychotria malayana* Jack (leaves), namely LPM-574. This compound, whose structure was not completely elucidated, was found to have bacteriostatic potency against *S. aureus* and *E. coli* at a concentration of 1.0 mg/ml. The major alkaloid compound hodgkinsine, showed no antibacterial activity at this concentration. However, further structure elucidation is needed for compound LMP-574 before it can be considered as a drug lead. Another new indole alkaloid labeled as LPM-186 was isolated as a minor compound of this plant.

From the plant *Alstonia scholaris* (young trees), used in the treatment of malaria, the new indole alkaloids mataranine A and B were isolated. Antimalarial activity was confirmed in

the crude alkaloid extract from the plant, with IC_{50} values *in vitro* against antifolate resistant *Plasmodium falciparum* (K1) and antifolate sensitive (TM4) strains of *P. falciparum* of 15.6 μg/ml and 21.0 μg/ml respectively. The new alkaloids mataranine A and B (mixture) gave increased potency against K1 with an IC_{50} of 2.6 μg/ml (7.4 μM) and an IC_{50} value of 3.4 μg/ml (9.7 μM) against the TM4 strain. Thus, either mataranine A or B or a mixture of the two could provide useful structurally novel leads, not only for antimalarial compounds but also for antibacterial agents, especially if human cell toxicity is low. Other new alkaloids, kotarajine and (*15S**, *16S**)-losbanine, and the known alkaloids losbanine and (*E*)-akuammidine, were also isolated from leaves of young trees of *A. scholaris*. The antimalarial testing of these compounds has not been carried out as yet.

While some possible new alkaloids were detected in the alkaloid extract from *Clerodendron calamitosum* L., full characterisation and structure elucidation of the alkaloid content of this plant could not be achieved due to small amounts of compounds isolated. A similar dilemma presented itself in the study of *C. paniculatum* L. in that full structure elucidation of the alkaoids of this plant was not achieved due to the small quantities of compound available. The crude alkaloid extract from each plant did show some antibacterial activity against *E. coli*. The crude alkaloid extract from *C. calamitosum* L also showed antimalarial properties and the major isolated alkaloid, Lcc-3, had increased potency against the K1 strain of *P. falciparum in vitro*.

Table 19. Overall summary and results of work

Plant	Plant part	Use	Alkaloids	Antibacterial activity	Antimalarial activity
Voacanga foetida (Bl.) Rolfe	Bark	Almost all skin diseases	Lombine **87**	*) **)	-
			Voacangine **89**	-	-
	Fruits		Coronaridine **90**	*)	-
			Voacangine **89**	-	-
			Voacristine **92**	*)	-
			Mataranine A **188** and B **189** (mixture)	*)	-
	Leaves	Almost all skin diseases	Voacangine **89**	-	-
			Voacristine **92**	*)	
Psychotria malayana Jack.	Leaves	Wounds, skin diseases	Hodgkinsine **97**	(-)	-
			Iso-calycanthine **102**	-	-
			Meso-chimonanthine **104**	-	-
			LPM-574 **128**	*) **)	-
			LPM-186 **129**	-	-
	Bark	Wounds, skin diseases	*Iso*-calycanthine **102**	-	-

Table 19 continued

Alstonia scholaris R. Br.	Leaves	Malaria	Mataranine A **188** and B **189** (mixture)	-	#) ##)
			Alstonamine **190**	-	-
			(15S, 16S*)*-Losbanine **193**	-	-
			Kotarajine **194**	-	-
			(E-)akuammidine **195**	-	-
Clerodendron calamitosum L.	Leaves	Malaria, wounds	Lcc-1 **216**	-	-
			Lcc-2	-	-
			Lcc-3 **218**	-	#)
			Lcc-4	-	-
			Lcc-5	-	-
Clerodendron calamitosum L.	Flowers	Sore eyes	Methyl 4-acetoxy-3-methoxy-cinnamate **219**	-	-
			Methyl 4-acetoxy-3-methylbenzoate **220**	-	-
			3,4,5-Trimethoxy-benzyl methyl ether **221**	-	-
			Salicylic acid hydrazide **222**	-	-

New alkaloids (red); new alkaloids, tentative structure (blue); known alkaloids, revised structure (green); known alkaloids, first report from species (lavender); non alkaloid, first report from species (brown); known alkaloids, previously reported from the species (black); not known structrures (dark teal); *) active against *Staphylococcus aureus*; **) active against *Escherichia coli*, #) active against K1 strain of *Plasmodium falciparum*; ##) active against TM4 strain of *Plasmodium falciparum*; (-) negative result; - not tested

8.2. Future Work

Future work should be focussed on the synthesis of the new alkaloid lombine (obtained from *V. foetida*), designing and preparing derivatives, and investigating the mode of action against *Staphylococcus aureus* and *Escherichia coli*. Testing against drug resistant strains of *S. aureus* should also be carried out. Further work could also examine synergistic and antagonistic mechanisms between alkaloid components from the plant *V. foetida*.

Separation of the new alkaloids mataranine A and B isolated from *A. scholaris* R. Br. should be undertaken, probably via preparative HPLC and bioactivity testing (antimalarial and antibacterial) for the individual diastereoisomers could be carried out. Further work could then be directed towards synthesis, derivatisation, analogue development, and biological target studies for the mataranines. Collecting more sample from *A. scholaris* (young trees) is important to get further quantities of minor components before an intensive antimalarial investigation can be done.

Further low temperature NMR experiments are required to fully establish the structure elucidation of the alkaloids from *Psychotria malayana* Jack. Intensive biological testing also needs to be done on the new compound LPM-574 after which, synthesis and derivatisation of this compound might prove worthwhile.

Further sample collection is necessary for final identification of the alkaloids from *Clerodendron calamitosum* L and *C. paniculatum* L.

Chapter 9

EXPERIMENTAL SECTION

9.1. General

CI (reactant gas: isobutene) and EI (at 70 eV) mass spectra were obtained on a Shimadzu QP-5000 by the direct insertion technique. HRCIMS were run on a Fisons/VG Autospec-oa-TOF Mass Spectrometer; relative intensities of peaks are given in brackets after the m/z values. ^{1}H, gCOSY, NOESY1D, selective decoupling, gHSQC, and gHMBC NMR spectra were recorded on a Varian Inova-500 MHz NMR spectrometer otherwise stated. ^{13}C-NMR and DEPT spectra were collected on a Varian Unity 300 spectrometer running at 75.42 MHz. The UV absorption spectra (solvent corrected) were recorded on a Shimadzu UV-265 spectrophotometer. IR spectra were recorded on a Perkin Elmer 783 Infrared Spectrophotometer using a KBr disc. Preparative TLC was performed on plates made from Merck silica gel 60 PF_{254}, 0.3 mm thickness, and bands were observed under UV light (λ 360 nm). Solvent ratios are v/v. All solvents were re-distilled before use. Melting points were measured on a Reichert hot stage melting point apparatus and are uncorrected. The optical rotations were measured on solutions with a Jasco Dip-370 Digital Polarimeter. The specific rotation was calculated by the use of equation, $[\alpha]_D = \alpha/l.c$, in which α is observed rotation at t°C, l is length of polarimeter cell (dm), and c is concentration of sample (g/mL). The 3D-structure modelling was performed by minimising conformation energies on Spartan Version 4.1.

9.2. Plant Collection

A hundred candidate plant species were investigated on the basis of their possibility of indicative antimalarial activities based on their local uses as remedies for wounds, malaria, or fever. The required field information was gathered by interviewing the local people living where the plants were collected.

The list of possible plants was then reduced to 5 species based on the following criteria: 1) the traditional medicinal use of the plant for antibacterial or antimalarial purposes, 2) the presence of alkaloids in the species, 3) the extent of chemical and biological work already undertaken on the species, and 4) the geographical distribution of the species. The plants thus selected were *Alstonia scholaris* R. Br. (Apocynaceae; antimalarial properties), *Voacanga foetida* (Bl.) Rolfe. (Apocynaceae; antimicrobial properties), *Psychotria malayana* Jack. (Rubiaceae; antimicrobial properties), *Clerodendron paniculatum* L. (Verbenaceae; antimicrobial properties), and *C. calamitosum* L. (Verbenaceae; antimalarial and antimicrobial properties). Although there are a number of reports on alkaloids from *A. scholaris* R. Br., particular interest was focused on investigating alkaloids in the young plants, as local people have used the plant at this growth stage to treat malaria.

There were thirteen villages involved in this investigation throughout Lombok island, which is divided administratively into three regions: western, central, and eastern Lombok. The villages of Narmada, Suranadi, Ampenan, Kekait, and Mataram are located in the western region, while the villages of Puyung, Kopang, Mantang, and Sepakek are in the

central region. The rest of the villages (Masbagik, Tetebatu, Kotaraja, and Pancor) are in east Lombok (Figure 2, Chapter 1).

The plant collection was carried out with the appropriate permission of the local government and in collaboration with the University of Mataram. All plants were collected between April and June 1999, and were identified with the assistance of Mr. Made Sudana, a botanist at the University of Mataram, Lombok. In addition, plant specimens were sent to the Research and Development Centre for Biology, Bogor, Indonesia, for confirmatory identification. A local herbarium collection was created and stored in a pest-proof wooden container at the Laboratory of Biology, the University of Mataram. The collection codes noted in Tables 1 and 2 in Chapter 2 are also the voucher specimen codes used in this herbarium.

9.3. Alkaloid Screening

Semi-quantitative alkaloid testing was carried out either in the field or in the laboratory according to the procedure described by Culvenor and Fitzgerald,[60] and Bick *et al.* [61] A sample of the plant part (~ 5 g) was ground in a mortar with a small amount of acid-washed sand, or the sample was pre-ground in a coffee grinder. Ammoniacal CH_2Cl_2 (10.0 ml) was added and the mixture was stirred for about one minute before filtering the CH_2Cl_2 into a small tube. Sufficient recovery of the solvent was obtained by pressing the material in the filter with a cork. Dilute H_2SO_4 (1.0 M, 0.5 ml) was then added and the test tube was sealed with a cork and shaken, and the phases allowed separating. The aqueous phase was removed with a dropper, the tip of which was fitted with a cotton wool plug. Three drops of

aqueous solution were then placed in a small rimless tube for testing with Mayer's reagent (K_2HgI_4). The density of the diagnostic precipitate formed was finally assessed on a + to ++++ scale, a + result was indicated by a milky turbidity, and ++++ by a heavy white to cream precipitate.

Ammoniacal CH_2Cl_2 was made up by adding three drops of concentrated aqueous ammonia solution in CH_2Cl_2 (10 ml) and the mixture was shaken. Excess water was removed with anhydrous Na_2SO_4. Dilute H_2SO_4 (1.0 M) was prepared by diluting concentrated H_2SO_4 (10.0 ml) in a 100 ml volumetric flask with water. Mayer's reagent was freshly made up by dissolving a mixture of mercuric chloride (1.36 g) and of potassium iodide (5.00 g) in water (100.0 ml).

In the laboratory, some selected samples were tested with Dragendorff's reagent prepared by mixing acetic acid (10 ml) with a stock solution (5 ml) prepared by mixing a solution of bismuth subnitrate (2.5 g) in water (20 ml) and acetic acid (5.0 ml) with a solution of potassium iodide (4 g) in water (10.0 ml) and diluting the resulting mixture to 100 ml with water.[267] The alkaloids in this study reacted to give an orange colour with this reagent on TLC analysis.

9.4. Alkaloid Isolation Procedure

Before extraction, parts of the plants were prepared by air-drying in the shade at room temperature (*ca.* 27°C) followed by grinding separately in a coffee grinder. In the case of plants giving a positive result when fresh and a negative result after drying (such as

Clerodendron calamitosum L.), fresh material was ground and extracted to reduce the loss of alkaloid.

The procedure for extraction of the alkaloid from the five plants selected is outlined in Figure 5. Ground plant material was extracted with cold distilled MeOH with occasional swirling. Methanol extraction was continued until the plant material gave a negative alkaloid test (Culvenor and Fitzgerald Procedure[60]). After filtration, the solvent was removed under reduced pressure at 40°C, to minimise any thermal degradation of the alkaloids.

The crude alkaloid mixture was then separated from neutral and acidic materials, and water-soluble materials, by initial extraction with aqueous acetic acid (CH_3CO_2H) followed by dichloromethane (CH_2Cl_2) extraction and then basification of the aqueous solution and further CH_2Cl_2 extraction.

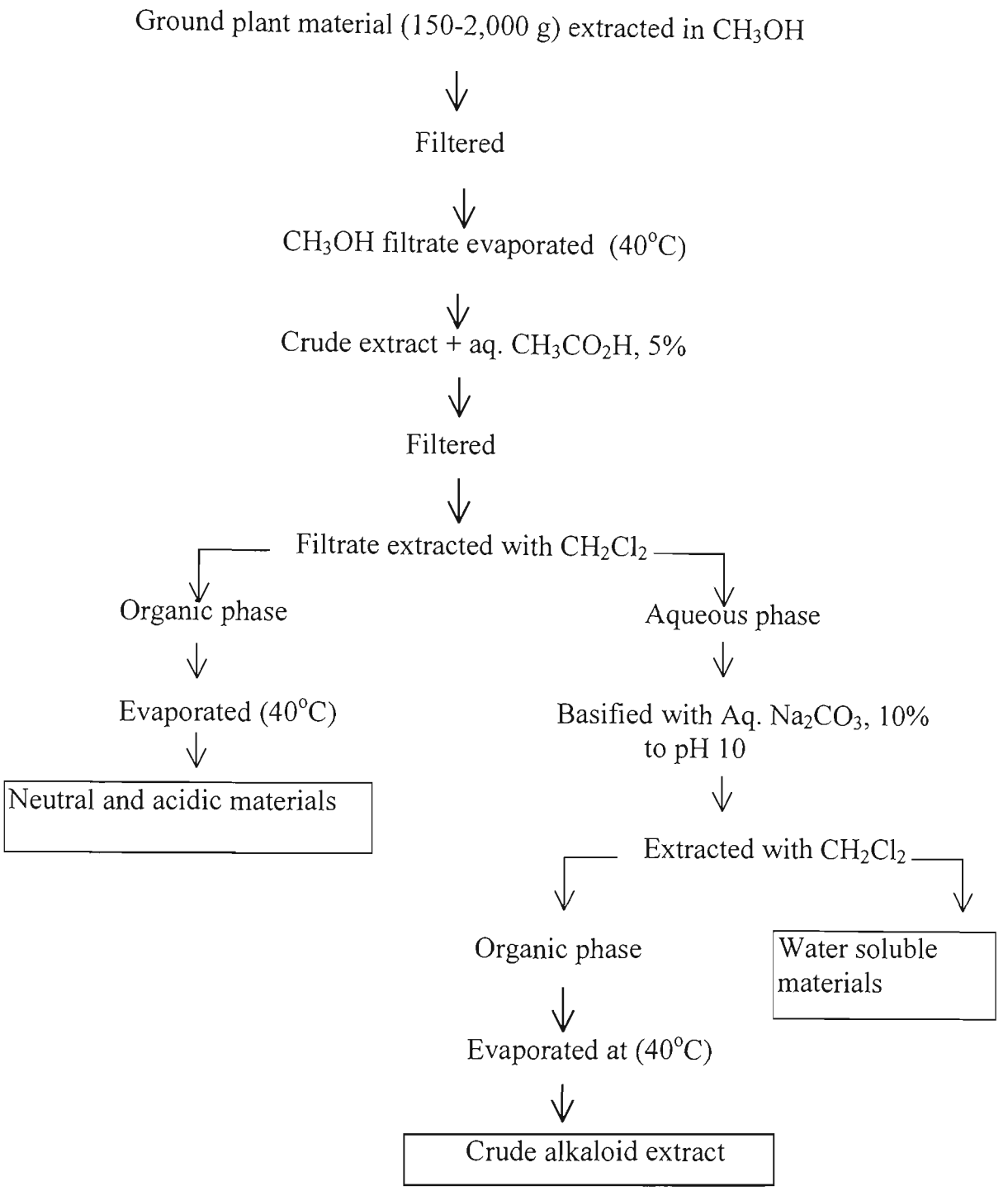
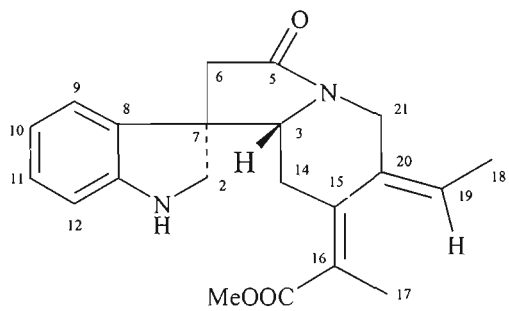


Figure 5. Outline of the extraction procedure for alkaloids carried out for the selected medicinal plants of Lombok

9.4.1. Isolation and Purification of Alkaloids from *Voacanga foetida* (Bl.) Rolf.

9.4.1.1. Bark

Finely-powdered, air-dried bark (1.0 kg) was extracted with cold MeOH (3 x 2.5 litres) with occasional swirling. Methanol extractions continued until the residual plant material gave a negative alkaloid test result. After filtration, the solvent was removed under reduced pressure at 40°C. The crude alkaloid mixture was then separated from neutral, acidic, and water soluble material, by initial extraction with aqueous acetic acid (250 ml, 5%, v/v) followed by dichloromethane (DCM) extraction (3 x 500 ml) of the aqueous acid extract which was basified by aqueous sodium carbonate solution (10%) to pH 10 and further extracted with DCM (3 x 250 ml). The combined alkali DCM extracts were dried (Na_2SO_4), and the solvent was evaporated under reduced pressure to give a crude alkaloid residue (378 mg). This mixture was chromatographed (PTLC) on silica gel (DCM:MeOH:conc. NH_4OH/9:1:1) with multiple development. Two compounds were isolated: a new compound called lombine (15.7 mg) and the known alkaloid voacangine (1.7 mg).



Lombine

Lombine 87: yellow solid; m.p. 82-84 °C; $[\alpha]_D$ = +75° (c 0.1; $CHCl_3$); **UV** λ_{max}nm ($CHCl_3$): 235 ($\log\varepsilon_{max}$ = 4.126), 310 ($\log\varepsilon_{max}$ = 4.183); **IR** (KBr) ν_{max} (cm^{-1}) 2780, 1733, 1685, 1559, 1497; **^{1}H-NMR** ($CDCl_3$, 500 MHz), 1.59 (d, 3H, $J_{17,16}$ = 6.5 Hz, H-18), 2.26

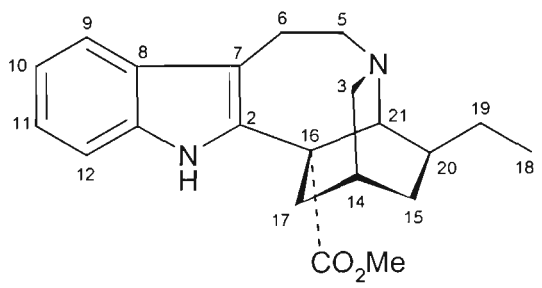
(bd, 1H, $J_{21\beta, 21\alpha}$ = 12.0 Hz, H-21β), 2.31 (s, 3H, H-17), 2.44 (bd, 1H, $J_{6\beta, 6\alpha}$ = 10.5 Hz, H-6β), 2.59 (bd, 1H, $J_{14\beta, 14\alpha}$ = 15.5 Hz, H-14β), 2.89 (d, 1H, $J_{2\beta, 2\alpha}$ = 15.5 Hz, H-2β), 3.07 (bd, 1H, $J_{14\alpha,3}$ = 10.5 Hz, $J_{6\alpha, 6\beta}$ = 10.5 Hz, H-6α), 3.10 (d, 1H, $J_{14\alpha, 14\beta}$ = 15.5 Hz, H-14α), 3.44 (d,1H, $J_{21\alpha, 21\beta}$ = 11.5 Hz, H-21α), 3.53 (d, 1H, $J_{3, 14\alpha}$ = 10.5 Hz, H-3), 3.59 (s, 3H, OMe), 3.62 (d, 1H, $J_{2\alpha, 2\beta}$ = 16.0 Hz, H-2α), 7.15 (t, 1H, H-10), 5.43 (q, 1H, $J_{16, 17}$ = 7.0 Hz, H-19), 7.35 (t, 1H, H-11), 7.40 (d, 1H, $J_{12, 11}$ = 8.0 Hz, H-12), 7.59 (d, 1H, $J_{9,10}$ = 8.5 Hz, H-9), 8.92 (bs, 1H, NH); **^{13}C-NMR** ($CDCl_3$, 75 MHz), 12.5 (C-18), 31.1 (C-2), 34.1 (C-3), 43.9 (C-6), 45.9 (C-17), 49.2 (C-7), 52.4 (OMe), 61.1 (C-14), 61.7 (C-21), 112.1 (C-12), 120.1 (C-10), 120.5 (C-9), 121.3 (C-19), 126.4 (C-11), 127.2 (C-16), 132.5 (C-13), 133.5 (C-15), 136.3 (C-20), 136.5 (C-8), 173.7 (COOMe), 193.1 (C-5); **gHSQC** ($CDCl_3$, 500 MHz), 3.62 (H-2α) & 2.89 (H-2β)/31.1 (C-2), 3.53 (H-3)/34.1 (C-3), 3.07 (H-6α) & 2.22 (H-6β)/43.9 (C-6), 7.59 (H-9)/120.5 (C-9), 7.15 (H-10)/120.1 (C-10), 7.35 (H-11)/126.4 (C-11), 7.40 (H-12)/112.1 (C-12), 3.10 (H-14α) & 2.59 (H-14β)/61.1 (C-14), 2.31 (H-17)/45.9 (C-17), 1.59 (H-18)/12.5 (C-18), 5.43 (H-19)/121.3 (C-19), 3.44 (H-21α) & 2.26 (H-21β)/61.7 (C-21), 3.59 (OMe)/52.4 (OMe); **gHMBC** ($CDCl_3$, 500 MHz), 7.59 (H-9)/136.5 (C-8), 120.1 (C-10); 7.40 (H-12)/126.4 (C-11), 120.5 (C-9); 7.35 (H-11)/136.5 (C-8), 120.1 (C-10); 7.15 (H-10)/126.4 (C-11), 112.1 (C-12); 5.43 (H-19)/61.7 (C-21), 34.1 (C-3), 12.5 (C-18); {3.59 (OMe)+3.62 (H-2α)}/173.7 (COOMe), 132.5 (C-13), 127.2 (C-16), 120.5 (C-9), 49.2 (C-7), 34.1 (C-3); 3.53 (H-3)/193.1 (C-5), 49.2 (C-7); (H-21α)/193.1 (C-5), 61.1 (C-14), 34.1 (C-3); 3.10 (H-14α)/136.3 (C-20), 61.7 (C-21), 49.2 (C-7), 34.05 (C-3); 3.07 (H-6α)/193.1 (C-5), 61.7 (C-21), 49.2 (C-7), 34.1 (C-3); 2.44 (H-6β)/193.1 (C-5), 136.5 (C-8), 132.5 (C-13), 49.2 (C-7), 34.1 (C-3); 2.31 (H-17)/61.7 (C-21), 61.1 (H-14); 1.59 (H-18)/136.3 (H-20), 121.3 (H-19); **LREIMS**: m/z (relative intensity, %) 352 (26),

337 (7), 323 (7), 309 (7), 293 (11), 234 (4), 220 (7), 208 (7), 195 (11), 194 (24), 181 (24), 180 (100), 172 (13), 168 (7), 167 (7), 166 (14), 144 (6), 130 (14), 97 (14), 82 (38). **HRCIMS**, (MH^+), found 353.1784, $C_{21}H_{25}N_2O_3$ requires 353.1786.

Voacangine 89: The spectroscopic data were identical to those for voacangine isolated from the fruit (see below).

9.4.1.2. Fruit

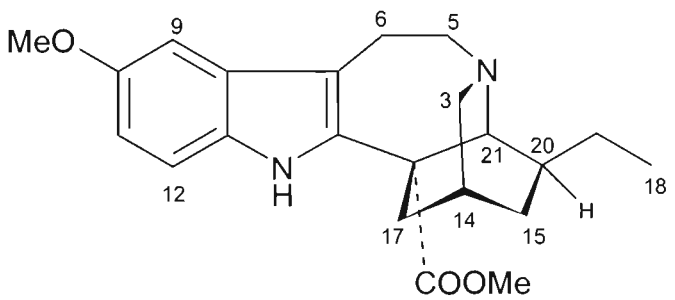
Following the procedure outlined in Figure 5, the air-dried blended fruit materials (2.0 kg) of the plant produced a dark brown MeOH (3 x 4 litres) extract (257.8 g). Further sequential acid and base extractions yielded a dark brown crude extract containing alkaloids (724.5 mg). Repeated separation by PTLC on silica gel (DCM:MeOH:conc.NH_4OH/92:7:1) resulted in the isolation of the major component coronaridine (7.2 mg), and four minor compounds, voacangine (1.7 mg), voacristine (2.4 mg), and mataranine A and B (3.6 mg). The characteristics of these compounds are described below.



Coronaridine

Coronaridine 90: yellow amorphous solid; m.p. 236-238°C (lit.[132] m.p. 237-239°C); **UV** λ_{max}nm ($CHCl_3$): 283 ($\log\varepsilon_{max}$ = 3.616), 310 ($\log\varepsilon_{max}$ = 3.799); **^{1}H-NMR** ($CDCl_3$, 500 MHz), 0.90 (t, 1H, H18), 1.13 (m, 1H, H-20), 1.32 (m, 1H, H-19), 1.44 (m, 1H, H-19), 1.59 (m, 1H, H-15), 1.88 (bs, 1H, H-15), 1.91 (bs, 1H, H-14), 2.09 (m, 1H, H-17), 2.58 (bd, 1H,

13 Hz, H-17), 2.81 (bd, 1H, J = 8.0 Hz, H-3), 2.93 (m, 1H, H-3), 3.02 (m, 1H, H-6), 3.15-3.23 (m, 1H, H-5), 3.15-3.23 (m, 1H, H-6), 3.38 (m, 1H, H-5), 3.56 (bs, 1H, H-21), 3.71 (s, 3H, COOMe), 7.08 (t, 1H, H-11), 7.14 (t, 1H, H-10), 7.24 (d, J = 8.0, 1H, H-9), 7.48 (d, 1H, J = 8.0 Hz, H-12), 7.75 (bs, 1H, NH); **LRCIMS**, m/z 339 (MH^+), **LREIMS**, m/z (relative intensity, %) 338 (34), 323 (7), 279 (7), 253 (6), 214 (17), 208 (11), 195 (7), 194 (7), 180 (13), 169 (40), 168 (24), 166 (34), 154 (34), 149 (36), 136 (51); HRCIMS, $C_{21}H_{27}N_2O_2$ (measured 339.2069, calc. 339.2073, for MH^+).



Voacangine

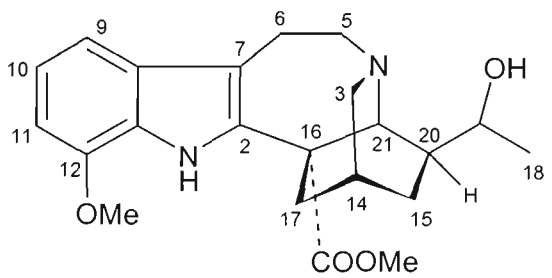
Voacangine 89: yellow solid; m.p 135-136°C (lit.[127] m.p. 136-137°C); **UV** λ_{max}nm ($CHCl_3$): 272 ($\log\varepsilon_{max}$ = 3.789), 286 ($\log\varepsilon_{max}$ = 3.835) 293 ($\log\varepsilon_{max}$ = 3.767); **LRCIMS**, 369 (MH^+), **LREIMS**, m/z (relative intensity, %) 368 (48), 253 (11), 338 (20), 323 (8), 309 (7), 283 (9), 245 (9), 244 (15), 225 (7), 208 (20), 195 (12), 184 (33), 167 (20), 160 (31), 154 (41), 136 (100); **HRCIMS**, $C_{22}H_{29}N_2O_3$ (measured 369.2162, calc. 369.2178, for MH^+).

Voacristine 92: yellow solid; physical and chemical properties were identical to those for voacristine isolated from the leaves (see voacristine isolated from the leaves below).

Mataranine A 188 and B 189: physical and chemical data were identical to those for Mataranine A and B isolated from *A. scholaris* R. Br. (Section 9.4.3).

9.4.1.3. Leaves

Finely-powdered, air-dried leaves (2.0 kg) of *V. foetida* (Bl.) Rolfe extracted with cold MeOH (3 x 4 litres) with occasional swirling produced a dark green extract (290.5 g). Further steps used to isolate alkaloids from this extract followed the procedure outlined in Figure 5 and produced a dark-green, crude alkaloid extract (692.8 mg). Two known alkaloids, voacristine (18.3 mg, major) and voacangine (2.1 mg, minor), were isolated from the crude extract by repeated PTLC on silica gel (DCM:MeOH:conc.NH_4OH(aq) / 90:9:1).



Voacristine

Voacristine 92: brown solid; m.p 168-169°C (lit.[137] m.p. 167-169°C); **UV** λ_{max}nm ($CHCl_3$): 278 ($\log\varepsilon_{max}$ = 3.885), 298 ($\log\varepsilon_{max}$ = 3.821); **^{1}H-NMR** ($CDCl_3$, 500 MHz), 1.28 (d, 1H, *J* = 6.0 Hz, H-18), 1.40 (m, 1H, H-20), 1.70 (m, 1H-15), 1.84 m, 1H, H-15), 1.96 (m, 1H, H-17), 2.03 (bs, 1H, H-14), 2.55 (bd, 1H, *J* = 13.5 Hz, H-17), 2.82 (bd, 1H, *J* = 8.5 Hz, H-3), 3.02 (m, 1H, H-3), 3.06 (m, 1H, H-6), 3.12 (m, 1H, H-6), 3.15 (m, 1H, H-6), 3.43 (m, 1H, H-5), 3.73 (s, 3H, OCH_3), 3.83 (s, 3H, $COOCH_3$), 3.91 (m, 1H, H-19), 4.08 (bs, 1H, H-21), 6.78 (m, 1H, H-9), 6.78 (m, 1H, H-11), 7.32 (m, 1H, H-10), 7.73 (bs, 1H, NH); **gHSQC**

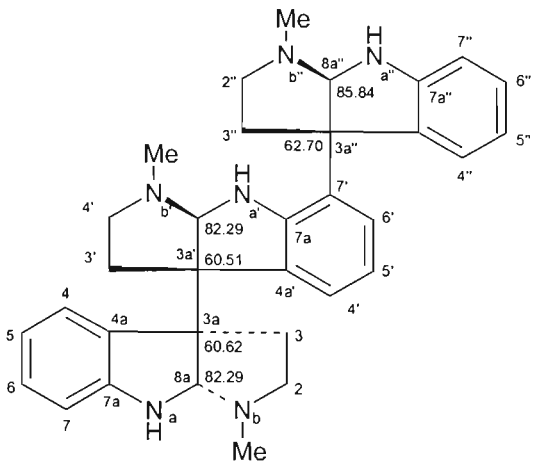
($CDCl_3$, 500 MHz), 3.02 & 2.82/50.8(C-3), 3.43 & 3.15/52.1 (C-5), 3.12 & 3.06/21.7 (C-6), 6.78/109.5 (C-9), 7.32/119.3 (C-10), 6.78/109.5 (C-11), 2.03/28.8 (C-14), 1.84 & 1.70/24.1 (C-15), 2.55 & 1.96/ 36.8 (C-17), 1.28/22.39 (C-18), 3.91/70.8 (C-19), 1.40/24.2 (C-20), 4.08/54.5 (C-21), 3.73/56.0 ($O\underline{C}H_3$), 3.83/52.4 ($COO\underline{C}H_3$), LRCIMS, m/z 385 (MH^+); **LREIMS**, m/z (relative intensity, %) 384 (46), 369 (20), 367 (20), 366 (60), 339 (10), 323 (7), 297 (9), 297 (10), 279 (10), 265 (9), 245 (10), 244 (34), 225 (16), 224 (20), 212 (20), 198 (10), 184 (46), 160 (46), 152 (57), 140 (41); **HRCIMS**, $C_{22}H_{29}N_2O_4$ (measured 385.2105, calc. 385.2127, for MH^+).

Voacangine, see voacangine isolated from the fruit.

9.4.2. Isolation and Purification of Alkaloids from *Psychotria malayana* Jack.

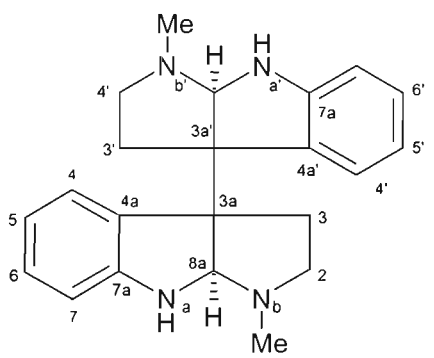
9.4.2.1. Leaves

Finely-powdered, air-dried, leaves (1.0 kg) were extracted with cold MeOH (3 x 2.5 litres) with occasional swirling to give a dark-green extract (178.9 g) after evaporation of the solvent. Further stages for isolation of the alkaloid fraction followed the procedure outlined in Figure 5 and produced a dark green crude alkaloid extract (406.5 mg). Three known alkaloids, hodgkinsine (23.6 mg, major), *meso*-chimonanthine (2.7 mg, minor), and *iso*-calycanthine (1.9 mg) and two new indole alkaloids, LPM-574 and LPM-186, were isolated from the crude alkaloid extract by the use of PTLC on silica gel (DCM:MeOH:conc. NH_4OH/92:7:1).



Hodgkinsine

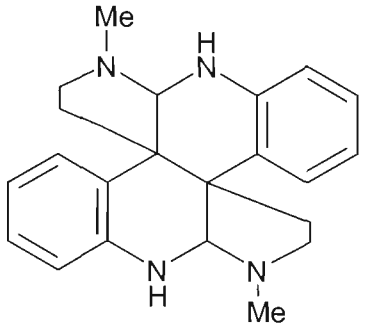
Hodgkinsine 97: colourless needles recrystallised from benzene; m.p 127-128°C (lit.[143] m.p 127-128°C); **UV** λ_{max}nm ($CHCl_3$): 214 ($\log\varepsilon_{max}$ = 4.306), 248 ($\log\varepsilon_{max}$ = 3.995), 310 ($\log\varepsilon_{max}$ = 3.574); **LREIMS**, m/z (relative intensity, %) 518 (1), 345 (20), 344 (70), 173 (19), 172 (54), 130 (21); **HRCIMS** $C_{33}H_{39}N_6$ (measured 519.3237, calc. 519.3236, for MH^+).



Meso-Chimonanthine

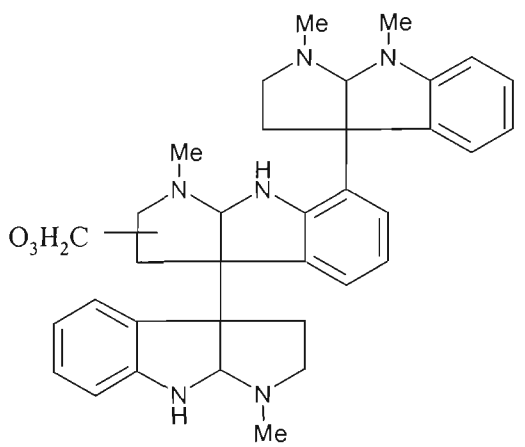
***Meso*-Chimonanthine 104**: yellow solid, m.p. 177-178°C (lit.[166] m.p. 176°C); $[\alpha]_D = 0^o$ (c 0.1 in EtOH; lit.[166] $[\alpha]_D = 0^o$); **UV** λ_{max}nm ($CHCl_3$): 214 ($\log\varepsilon_{max}$ = 4.298), 248 ($\log\varepsilon_{max}$ = 3.978), 310 ($\log\varepsilon_{max}$ = 3.398); **LREIMS**, m/z (relative intensity, %) 173 (43), 172 (100),

171 (17), 144 (7), 143 (11), 131 (16), 130 (60); **HRCIMS**, $C_{22}H_{27}N_4$ (measured 347.2224, calc. 347.2157, for MH^+).



103: *Iso*-Calycanthine

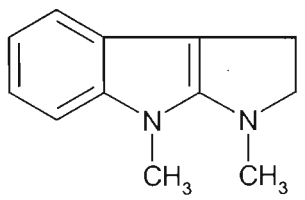
***Iso*-Calycanthine 102**: yellow solid; m.p: 252-253°C (lit.[144] m.p. 253 °C); $[\alpha]_D = -148°$ (c 0.1 in EtOH; lit.[166] $[\alpha]_D = -150°$); **UV** λ_{max}nm ($CHCl_3$): 214 ($\log\varepsilon_{max} = 4.067$), 248 ($\log\varepsilon_{max} = 3.708$), 310 ($\log\varepsilon_{max} = 3.255$); **LREIMS**, m/z (relative intensity) 346 (57), 302 (14), 288 (13), 246 (7), 245 (13), 232 (14), 231 (21), 217 (7), 185 (11), 173 (13), 172 (16), 145 (7), 144 (10), 130 (21); **HRCIMS**, $C_{22}H_{27}N_4$ (measured 347.2136, calc. 347.2157, MH^+).



LPM-574

LPM-574 128: yellow solid; m.p. 69-70°C; **UV** λ_{max}nm ($CHCl_3$): 214, 248, 304; **LREIMS**, m/z (relative intensity, %) 530 (14), 518 (12), 358 (21), 357 (31), 356 (38), 355 (24), 346

(24), 345 (38), 344 (71), 315 (29), 314 (57), 313 (64), 312 (29), 302 (26), 301 (38), 300 (29), 288 (14), 286 (14), 285 (17), 272 (23), 271 (48), 260 (22), 258 (20), 245 (29), 244 (19), 187 (45), 186 (71), 185 (38), 174 (100), 173(100) 172 (100), 171 (100), 158 (55), 157 (59), 145 (59), 144 (100), 143 (83), 132 (83), 131 (100), 130 (100), 129 (83), 128 (83), 127 (85).



LPM-186

LPM-186: yellow solid, **UV** λ_{max}nm ($CHCl_3$): 248, 304**, LRCIMS**, m/z 187 (MH^+); **HREIMS**, m/z (relative intensity) 186 (3), 174 (9), 173 (40), 172 (73), 171 (13), 157 (3), 144 (7), 130 (36); **HRCIMS**, $C_{12}H_{15}N_2$ (measured 187.1229, calc. 187.1235, MH^+)

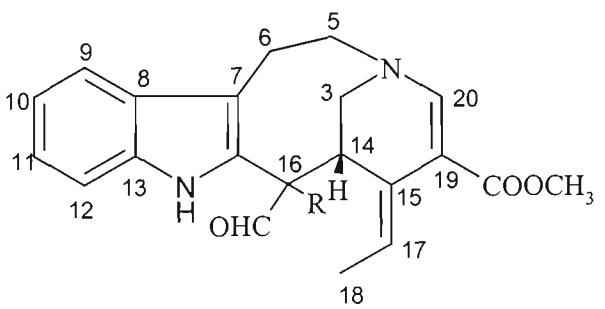
9.4.2.2. Bark

By the use of the procedure outlined in Figure 5, air dried and finely-powdered bark (350 g), after extraction with MeOH (3 x 1.0 litre) produced a dark-brown extract (87.5 g). Further acidic-basic extraction yielded a dark brown crude alkaloid extract (106.8 mg). Alkaloid separation by PTLC on silica gel (DCM:MeOH:conc. NH_4OH/92:7:1) resulted in the isolation of a major known indole alkaloid, *iso*-calycanthine (5.4 mg).

***Iso*-Calycanthine**; The spectroscopic data were identical to those for *iso*-calycanthine isolated from the leaves (9.4.2.1).

9.4.3. Isolation and Purification of Alkaloids from *Alstonia scholaris* R. Br.

Finely-powdered, air-dried young leaves (1.5 kg) from young trees (up to 2.0 m high) were extracted with cold MeOH (3 x 3.0 litres) with occasional swirling. Methanol extraction was discontinued when the residual plant material gave a negative result for Mayer's test for alkaloids. After filtration, the solvent was removed under reduced pressure at 40°C yielding a dark-blue MeOH extract (218.7 g). The crude alkaloid mixture was then separated from neutral and acidic material, and water solubles, by initial extraction with aqueous acetic acid (300 ml; 5%, v/v) followed by dichloromethane (DCM) extraction (3 x 500 ml) of the aqueous acid extract, which was then basified with aqueous sodium carbonate solution (10%) to pH 10, and then extracted with dichloromethane (3 x 200 ml). The solvent was evaporated under reduced pressure to give a crude alkaloid residue (753.6 mg) which was chromatographed (PTLC) on silica gel (DCM:MeOH:conc. NH_4OH/17:2.5:0.5) with multiple development resulting in the isolation of Mataranine A and B as a mixture (28.3 mg; major), alstonamine (4.6 mg), (*15S*, 16S**)-losbanine (4.4 mg), kotarajine (3.1 mg), and (*E*)-akuammidine (2.3 mg).

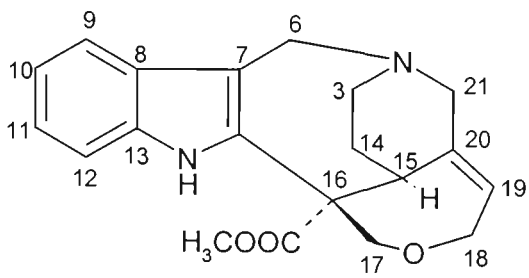


Mataranine A, R=Hα
Mataranine B, R=Hβ

Mataranine A 188: a light yellow oil mixed with mataranine B (approximate ratio A:B from ^{1}H-NMR 1:1); **UV** λ_{max}nm ($CHCl_3$), 283, 291; **^{1}H-NMR** ($CDCl_3$, 500 MHz), 1.80 (m, 1H, H-14), 2.19 (d, 1H, $J_{18, 19}$ = 7.5, H-18), 2.25 (bd, 1H, H-16), 2.80 (m, 1H, H-6β), 2.91 (m, 1H, H-6α), 3.59 (m , 1H, H-5β), 3.64 (s, 3H, $COOC\underline{H}_3$), 3.70 (m,1H, H-5α), 4.02 (bd, 1H, H-3β), 4.25 (dd, 1H, $J_{3\alpha, 3\beta}$ = 12, $J_{3\alpha, 14}$ = 4.5-H-3α), 6.55 (q, 1H, H-17), 7.11 (t, 1H, H-11), 7.16 (t, 1H, H-10), 7.30 (d, 1H, $J_{12, 11}$ = 8.5, H-12), 7.47 (d, 1H, $J_{9, 10}$ = 8.0, H-9), 7.76 (s, 1H, H-20), 8.20 (bs, 1H, NH), 10.28 (s, 1H, $C\underline{H}O$); **^{13}C-NMR** ($CDCl_3$, 75 MHz), 15.3 (C-18), 22.3 (C-6), 30.5 (C-14), 47.7 (C-16), 50.9 ($COO\underline{C}H_3$), 51.3 (C-3), 51.3 (C-5), 108.7 (C-7), 111.3 (C-12), 118.3 (C-9), 120.0 (C-10), 122.4 (C-11), 126.5 (C-13), 127.9 (C-8), 136.4 (C-19), 143.7 (C-17), 143.8 (C-2), 146.2 (C-15), 147.7 (C-20), 153.1 ($\underline{C}OOCH_3$), 196.1 ($\underline{C}HO$). **LRCIMS**, m/z 351 (MH^+); **LREIMS**, m/z (relative intensity, %), 350 (43), 335 (11), 322 (46), 321 (13), 307 (31), 291 (61), 279 (100), 265 (39), 264 (26), 263 (71), 249 (20), 238 (20), 221 (58), 209 (31), 208 (26), 193 (14), 184 (23), 169 (50), 156 (30), 155 (21), 154 (25), 145 (23), 144 (23), 143(22), 130 (21), 129 (33), 128 (21); **HRCIMS**, $C_{21}H_{22}N_2O_3$ (measured 351.1711, calc. 351.1709, for MH^+).

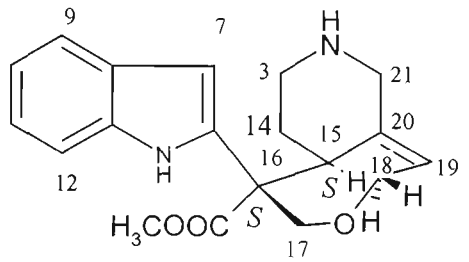
Mataranine B 189: **^{1}H-NMR** ($CDCl_3$, 500 MHz), 1.92 (m, 1H, H-14), 2.09 (d, 1H, $J_{18, 19}$ = 7.5, H-18), 2.18 (bd, H-16), 2.80 (m, 1H, H-6β), 2.91 (m, 1H, H-6α), 3.59 (m, 1H, H-5β), 3.64 (s, 3H, $COOC\underline{H}_3$), 3.70 (m, 1H, H-5α), 4.02 (bd, 1H, H-3β), 4.48 (bd, 1H, $J_{3\alpha, 3\beta}$ = 12, $J_{3\alpha, 14}$ = 4.5, H-3α), 6.58 (q, 1H, H-17), 7.11 (t, 1H, H-11), 7.16 (t, 1H, H-10), 7.30 (d, 1H, $J_{12, 11}$ = 8.5, H-12), 7.48 (d, 1H, $J_{9, 10}$ = 8.0, H-9), 7.68 (s, 1H, H-20), 8.14 (bs, 1H, NH), 9.37 (s, 1H, $C\underline{H}O$); **^{13}C-NMR** ($CDCl_3$, 75 MHz), 13.5 (C-18), 22.3 (C-6), 28.6 (C-14), 49.5 (C-16), 50.9 ($COOC\underline{H}_3$), 51.3 (C-3), 51.3 (C-5), 108.7 (C-7), 111.3 (C-12), 118.3 (C-9),

120.0 (C-10), 122.4 (C-11), 126.5 (C-13), 128.9 (C-8), 136.4 (C-19), 140.0 (C-2), 143.7 (C-17), 147.7 (C-20), 152.0 (C-15), 168.4 ($\underline{C}OOCH_3$), 190.6 ($\underline{C}HO$).



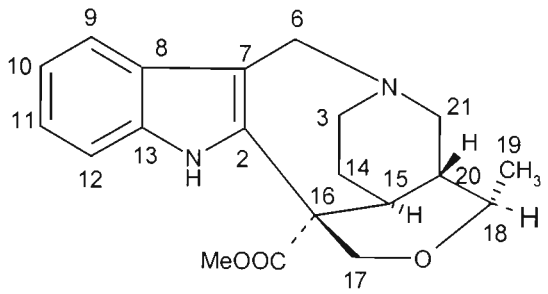
Alstonamine

Alstonamine 190: a colourless oil; **UV** λ_{max}nm ($CHCl_3$): 283 ($\log\varepsilon_{max}$ = 3.892), 291 ($\log\varepsilon_{max}$ = 3.829); ^{1}**H-NMR** ($CDCl_3$, 500 MHz), 1.75 (dd, 1H, $J_{14\alpha,\,14\beta}$ = 15.5; $J_{14\alpha,\,3}$ = 8.5, H-14α), 2.04 (m, 1H, H-14β), 2.93 (m, 1H, H-3β), 3.27 (m, 1H, H-3α), 3.43 (bd, 1H, $J_{21\alpha,\,21\beta}$ = 15.5, H-21α), 3.45 (s, 1H, COO$\underline{C}H_3$), 3.68 (m, 1H, H-15), 3.77 (d, 1H, $J_{17\beta,\,17\alpha}$ = 9.5, H-17β), 3.81 (bd, 1H, $J_{21\beta,\,21\alpha}$ = 14.0, H-21β), 4.00 (d, 1H, $J_{6\beta,\,6\alpha}$ = 16.5, H-6β), 4.23 (dd, 1H, $J_{18\beta,\,18\alpha}$ = 12.5; $J_{18\beta,\,19}$ = 3.5, H-18β), 4.37 (d, 1H, $J_{17\alpha,\,17\beta}$ = 12.0 -H-17α), 4.51 (bd, 1H, H-18α), 4.85 (d, 1H, $J_{6\alpha,\,6\beta}$ = 17.0, H-6α), 5.50 (m, 1H, H-19), 7.11 (t, 1H, H-11), 7.19 (t, 1H, H-10), 7.32 (d, 1H, $J_{12,\,11}$ = 8.0, H-12), 7.50 (d, 1H, $J_{9,\,10}$ = 7.5, H-9), 8.95 (bs, 1H, NH); ^{13}**C-NMR** ($CDCl_3$, 75 MHz), 19.9 (C-14), 42.9 (C-3), 43.8 (C-15), 48.8 (C-6), 53.7 (COO$\underline{C}H_3$), 55.3 (C-21), 71.2 (C-17), 77.1 (C-18), 110.9 (C-12), 117.9 (C-9), 120.2 (C-10), 122.8 (C-11), 123.8 (C-19), 132.1 (C-2), 134.6 (C-20), 174.5 ($\underline{C}OOCH_3$); **LRCIMS**, m/z 339 (MH^+); **HRCIMS**, $C_{20}H_{22}N_2O_3$ (measured 339.1730, calc. 339.1709, for MH^+).



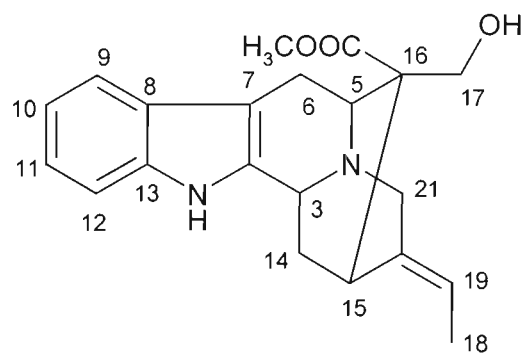
(*15S*, 16S**)-Losbanine

(*15S*, 16S)-Losbanine 192**: a colourless amorphous solid, m.p 130-132°C; $[\alpha]_D = 67^\circ$ (c 0.1 in $CHCl_3$); **UV** λ_{max}nm ($CHCl_3$): 274 ($\log\varepsilon_{max} = 3.813$), 283 ($\log\varepsilon_{max} = 3.839$), 291 ($\log\varepsilon_{max} = 3.796$); **^{1}H- NMR** ($CDCl_3$, 500 MHz), 1.07 (m, 1H, H-14α), 1.14 (m, 1H, H-14β), 2.47 (m, 1H, H-3β), 2.71 (d, $J_{21\beta, 21\alpha} = 14.5$, H-21β), 2.91 (d, 1H, $J_{21\alpha, 21\beta} = 14.5$, H-21α), 2.89 (m, 1H, H-3α), 3.05 (dd, $J_{15, 14\alpha} = 7.0$; $J_{15, 14\beta} = 6.5$, H-15), 3.60 (s, 3H, COOC$\underline{H}_3$), 4.57 (d, 1H, $J_{17\beta, 17\alpha} = 9.0$), 4.96 (d, $J_{17\alpha, 17\beta} = 9.5$, H-17α), H-17β), 5.17 (bd, 1H, $J_{18\beta, 18\alpha} = 10.5$,H-18β), 5.39 (bd, 1H, $J_{18\beta, 18\alpha} = 9.0$; $J_{18\beta, 19} = 3.5$, H-18α), 5.67 (dd,1H, $J_{19, 18\alpha} = 17.0$; $J_{19,18\beta} = 10.5$, H-19), 6.38 (s, 1H, H-7), 7.08 (t, 1H, H-11), 7.16 (t, 1H, H-10), 7.30 (d, 1H, $J_{12, 11} = 8.0$, H-12), 7.54 (d, $J_{9, 10} = 7.5$, H-9), 8.33 (bs,1H, NH); **^{13}C-NMR** ($CDCl_3$, 75 MHz), 24.2 (C-14), 43.9 (C-15), 46.4 (C-3), 51.7 (COO$\underline{Me}$), 52.9 (C-21), 59.0 (C-16), 69.5 (C-17), 101.6 (C-7), 110.6 (C-12), 115.4 (C-18), 119.9 (C-9), 120.3 (C-10), 122.3 (C-11), 127.5 (C-8), 127.5 (C-19), 132.3 (C-2), 136.1 (C-20), 140.3 (C-13), 173.5 ($\underline{C}$OOMe); LRCIMS, m/z 327 (MH^+); **HRCIMS**, $C_{19}H_{23}N_2O_3$ (measured 327.1712, calc. 327.1709, MH^+).



Kotarajine

Kotarajine 193: a colourless amorphous solid, mp 145-147°C; $[\alpha]_D$ = -124° (c 0.1 in $CHCl_3$); **UV** λ_{max}nm ($CHCl_3$): 275 ($\log\varepsilon_{max}$ = 3.734), 284 ($\log\varepsilon_{max}$ = 3.755), 291 ($\log\varepsilon_{max}$ = 3.713); **^{1}H-NMR** ($CDCl_3$, 500 MHz), 1.58 (m, 1H, $J_{14\alpha,\ 14\beta}$ = 15.5; $J_{14\alpha,\ 3}$ = 8.5, H-14α), 1.75 (d, 1H, $J_{19,\ 18}$ = 6.5, H-19), 2.00 (m, 1H, H-14β), 2.16 (dd, 1H, $J_{21\beta,\ 20}$ = 7.5; $J_{21\beta,\ 21\alpha}$ = 13.5, H-21β), 2.31 (m, 1H, H-20), 2.38 (m, 1H, H-3β), 2.92 (m, 1H, H-15), 3.29 (m, 1H, H-3α), 3.41 (dd, 1H, $J_{21\alpha,\ 20}$ = 7.0; $J_{21\alpha,\ 21\beta}$ = 13.5, H-21α), 3.76 (s, 3H, $COOC\underline{H}_3$), 3.82 (d, 1H, $J_{6\beta,\ 6\alpha}$ = 11.5, H-6β), 4.11 (d, 1H, $J_{17\beta,\ 17\alpha}$ = 17.5, H-17β), 4.20 (d, 1H, $J_{6\alpha,\ 6\beta}$ = 11.5, H-6α), 4.86 (d, 1H, $J_{17\alpha,\ 17\beta}$ = 17.5, H-17α), 5.59 (m, 1H, H-18), 7.09 (t, 1H, H-10), 7.19 (t, 1H, H-11), 7.31 (d, 1H, $J_{12,\ 11}$ = 8.0, H-12), 7.50 (d, 1H, $J_{9,\ 10}$ = 8.0, H-9), 9.44 (bs, 1H, NH), **LRCIMS**, m/z 341 (MH^+), **HRCIMS**, $C_{20}H_{25}N_2O_3$ (measured 341.1867, calc. 341.1865, MH^+).



(E)-Akuammidine

***(E)*-Akuammidine 195**: a colourless amorphous slightly impure solid; **UV** λ_{max}nm ($CHCl_3$): 283 ($\log\varepsilon_{max}$ = 3.835), 293 ($\log\varepsilon_{max}$ = 3.896); **^{1}H NMR** ($CDCl_3$, 500 MHz), 1.65 (d, 1H, H-18), 1.87 (m, 1H, H-14β), 2.67 (m, 1H, H-14α), 2.92 (m, 1H, H-6α), 2.95 (s, 3H, $COO\underline{C}H_3$), 3.06 (m, 1H, H-15), 3.06 (m, 1H, H-5), 3.30 (br d, 1H, H-6β), 3.52 (d, 1H, *J* = 14 Hz, H-21β), 3.61 (d, 1H, *J* = 14 Hz, H-21α), 3.67 (d, 1H, *J* = 10 Hz, H-17β), 3.83 (d, 1H, *J* = 10.5 Hz, H-17α), 4.23 (brd, 1H, *J* = 9.5 Hz, H-3), 5.41 (q, 1H, H-19), 7.05 (t, 1H, H-10), 7.11 (t, 1H, H-11), 7.33 (d, 1H, *J* = 7.5 Hz, H-12), 7.42 (d, 1H, *J* = 7.5 Hz, H-9), 7.79 (bs, 1H, NH); **LREIMS**, m/z 352, 338, 337, 321, 294 293, 249, 236, 220, 206, 194, 193, 182, 180, 169, 168, 167, 154, 143, 130; **HRCIMS**, $C_{21}H_{25}N_2O_3$ (measured 353.1852, calc. 353.1865).

9.4.4. Isolation and Purification of Alkaloids from *Clerodendron calamitosum* L. and *C. paniculatum* L.

9.4.4.1. Clerodendron calamitosum L.

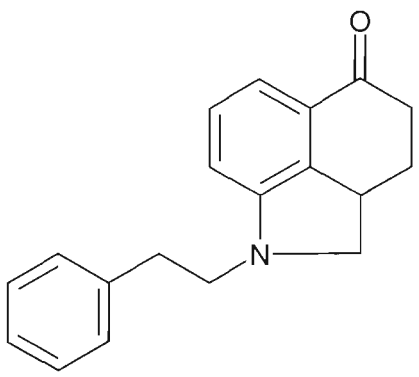
Fresh ground leaves of *C. calamitosum* (500 g) were extracted with MeOH (3 x 1.5 ml) until the fresh extract gave a negative result for Mayer's test. The solvent was evaporated *in vacuo* yielding a dark-greenish material (45.0 g). This was subjected to sequential acid and base extraction as described in Section 9.4. The basic alkaloid fraction (155.0 mg) so obtained was then chromatogaphed using PTLC (silica gel, ethyl acetate : isopropanol : NH_4OH = 95 : 10 : 5 v/v); five main bands were isolated and some of their characteristics are noted in Table 20.

Table 20. Some characteristics of components in the crude alkaloidal extract of *C. calamitosum* L.

Faction Code (weight in mg)	Rf*	UV light (333 nm)**	Iodine Test***	Dragendorff Test****	MH^+ (CIMS)*****
Lcc-1 (5.2)	0.34	abs.	brown	yellow	286 &310
Lcc-2 (2.3)	0.41	abs.	brown	yellow	286
Lcc-3 (5.7)	0.56	abs.	brown	yellow	309
Lcc-4 (1.8)	0.81	blue fl.	-	-	271
Lcc-5 (1.7)	0.91	blue fl.	-	-	278

* PTLC (silica gel, ethyl acetate : isopropanol : NH_4OH = 95 : 10 : 5 by vol.); ** Abs (absorbance), fl (fluorescence); *** - no colouration; **** - no colouration; ***** LRCIMS

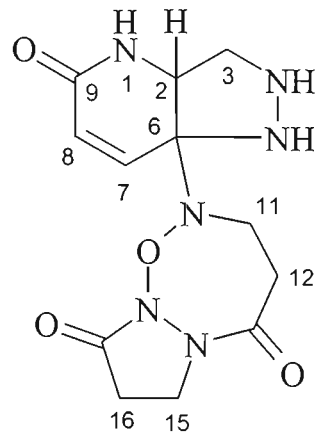
Further spectroscopic data of the fraction mentioned in above table as follows.



Lcc-5

Lcc-5 216: brown solid; **LRCIMS**, m/z 278 (MH^+); **LREIMS**, m/z (relative intensity, %) 277 (13), 235 (10), 149 (20), 132 (27), 131 (27), 105 (100), 77 (100); **HREIMS**, $C_{19}H_{19}NO$ (found 277.1468, calc. 277.1467, M^+).

Lcc-4: a brown solid; **LRCIMS**, 271 (MH^+); the sample decomposed before full spectroscopic characterisation could be performed.



Lcc-3

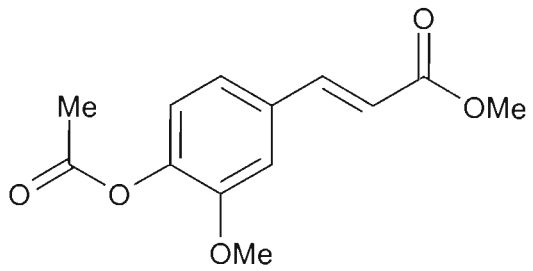
Lcc-3 218, a yellow solid; m.p 69-70°C; **LRCIMS** (MH^+), 309, 155, 137. **HRCIMS**, $C_{12}H_{17}N_6O_4$ (found 309.1327, calc. 309.1311, MH^+), $C_6H_9N_3O_2$ (found 155.0698, calc. 155.0695, MH^+), $C_6H_7N_3O$ (found 137.0604, calc. 137.0589, MH^+); **^{1}H-NMR** ($CDCl_3$, nanoprobe-300 MHz) 2.21 (m, 2H, H-11 and H-15), 2.35 (m, 2H, H-11 and H-15), 2.61 (d, *J*= 10.2, 1H, H-3), 2.79 (d, *J*=10.2, 1H, H-3), 3.95 (m, 2H, H-12 and H-16), 4.11 (m, 2H, H-12 and H-16), 4.29 (m,1H, H-2), 6.02 (d, *J*=6.3, 1H, H-7), 6.76 (d, *J*=6.3, 1H, H-8); **gHSQC**, 4.29/81.8 (C-2), 2.79/40.3 (C-3), 2.61/40.3 (C-3), 6.02/128.9 (C-7), 6.76/147.9 (C-8), 2.35/39.6 (C-11 & 15), 2.21/39.6 (C-11 & 15), 4.11/66.3 (C-12 & 16), 3.95/66.3 (C-12 & 16); **gHMBC** ($CDCl_3$, 500 MHz), 4.29/197.0 (C-9), 148.6 (C-8), 75.8 (C-6), 2.79/197.0 (C-9), 81.8 (C-2), 75.8 (C-6), 2.61/197.0 (C-9), 81.8 (C-2), 75.8 (C-6), 6.02/75.8 (C-6), 6.76/197.0 (C-9), 81.8 (C-2), 2.35/75.8 (C-6), 3.95/174.8 (C-13 & C-17).

Lcc-2, brown solid, **LRCIMS**, m/z 286 and 310 (MH^+); **CIMS/MS** for m/z 286 (relative intensity), 286 (100), 200, 172, 158, 131, 117, 115, 105, 77; **HRCIMS**, $C_{12}H_{20}N_3O_5$ (found 286.1383, calc. 286.1403, MH^+). **CIMS/MS** for m/z 310 (100), 154, 112, 111, 83, 82; **HRCIMS**, $C_{11}H_{24}N_3O_7$ (found 310.1615, calc. 310.1614, MH^+).

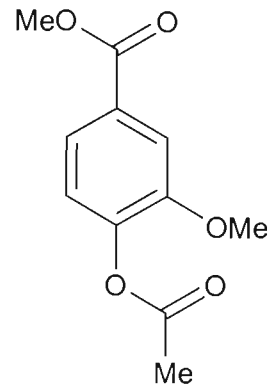
9.4.4.2. Clerodendron paniculatum L.

By using the procedure outlined in Figure 5, finely-powdered, air-dried flowers (150 g) yielded a dark brown extract (17.2 g) after extraction with MeOH (3 x 350 ml). Further sequential acidic and basic extractions afforded a dark brown crude extract containing alkaloids (26.9 mg). Alkaloid separation by PTLC on silica gel (ethyl acetate:isopropanol:NH_4OH = 93: 6.5:0.5, v/v) resulted in three bands which were subjected to GC/MS for further separation and identification.

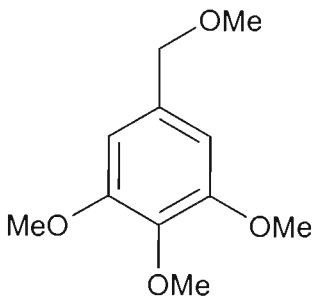
The GC/MS experiment in this study involved a GC-17A (Shimadzu) gas chromatograph coupled to a QP-5000 (Shimadzu) mass spectrometer. The sample was run in the splitless mode, with a split ratio of 1:20. The sample (50 µl, dissolved in DCM) was injected, at an estimated concentration of 5 mg/ml. The following GC parameters were used: the injector temperature was set at 260°C; the oven temperature was adjusted at 40°C for 2 min then ramped to 290°C at a rate of 4°C per min; the final oven temperature was kept at 290°C for 10 min, and the carrier gas was helium with a flow rate of 1.4 ml/min. The electron beam energy in the mass spectrometer was 70 eV and the source temperature was 200°C. The compounds were identified from their mass spectral fragmentation patterns. The fragmentation pattern was compared to that of a known compound contained in the mass spectrometer library.



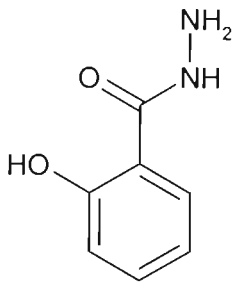
219: Methyl 4-acetoxy-3-methoxycinnamate



220: Methyl 4-acetoxy-3-methoxybenzoate



221: 3,4,5-Trimethoxybenzyl methyl ether



222: Salicylic acid hydrazide

Fraction 1, brown solid, $R_f = 0.54$, which gave a positive reaction to Dragendorff's reagent, but no peaks in the GC/MS. **LRCIMS**, m/z 313, 299, and 261 (MH^+). **HRCIMS**, $C_{15}H_{25}N_2O_5$ (measured 313.1745, calc. 313.1763, MH^+), $C_{14}H_{23}N_2O_5$ (measured 299.1610, calc. 299.1607, MH^+), $C_{13}H_{29}N_2O_3$ (measured 261.2215, calc. 261.2178, MH^+).

Fraction 2, isolated as a brown solid; identified as predominantly salicylic acid hydrazide; **LREIMS** for **222**, m/z (relative intensity, %) 152 (31), 121 (100), 93 (38), 65 (81), 53 (19), 39 (73).

Fraction 3, a brown solid, in which three compounds were identified by GC/MS: methyl 4-acetoxy-3-methoxycinnamate **219**, methyl 4-acetoxy-3-methylbenzoate **220**, and 3,4,5-trimethoxybenzyl methyl ether **221**. **LREIMS for 219**, m/z (relative intensity, %) 208

(100), 117 (69), 150 (16), 145 (63), 133 (25), 117 (78), 105 (19), 89 (37), 77 (41); **LREIMS for 220**, m/z (relative intensity, %) 182 (53), 151 (100), 123 (18), 108 (9), 79 (12), 52 (41); **LREIMS for 221**, m/z (relative intensity, %) 212 (100), 197 (6), 181 (82), 167 (7), 141 (24), 123 (12), 108 (18), 95 (12), 79 (18), 78 (18), 77 (18).

9.5. Antibacterial Assay

9.5.1. Sample Preparation

The isolated alkaloidal compounds were dissolved in enough acetone to achieve a concentration as stated in Sections 7.3 and 7.4 of Chapter 7. The crude alkaloid extracts, which could not completely dissolved in acetone, were mixed in an ultrasonic bath (Unisonics, frequency: 50 Hz).

9.5.2. Antibacterial Test Procedure

The crude alkaloid extracts and isolated alkaloidal compounds were tested using the FDA assay described by Chand and co-wokers.[252,266] The microorganisms, cultured overnight, were diluted to an absorbance of 0.12 at 600 nm and grown (37°C, 30 min) to absorbance of 0.18. The culture (175.0 μl) was filled to wells of tissue culture plates. A solution of the test substance (20.0 μl) or appropriate control was added to each well in triplicate. Before FDA (5.0 μl, 0.2% solution in acetone) was added, the microtitre plate was incubated at 37°C for 30 min. The incubation was then continued for three hours or until fluorescence was easily observed under UV light (λ254 nm). The results were recorded as positive (+ to ++) or negative according to the fluorescence detected.

To determine any possible effect of acetone on the viability of the cells, three replications of controls consisting of acetone (20.0 µl) with FDA were added to each test plate. Additional controls to ascertain whether the tested compound hydrolysed FDA were made by including tested compounds in broth solution (175.0 µl) with FDA.

After the FDA assay was completed, the culture (20.0 µl, in triplicate) from all the wells that did not show fluorescence, were spread on to agar to observe whether the cells were able to recover. The plates were then incubated (overnight, 37°C). The visibly growing colonies were counted and compared to a dilution series of a control culture from the FDA plate containing acetone.

9.6. Antimalarial Assay

9.6.1. Sample Preparation

A 3-5 mg sample was dissolved in ethanol to a stock concentration of 0.01 mg/ml. The sample was then diluted with culture medium to $9x10^{-5}$ g/ml and a 10-fold serial dilution was made up as needed. Care was taken that the final concentration of the organic solvent in each dilution at this step did not exceed 0.9%.

9.6.2. Procedure of Antimalarial Activity Testing

A modified method of ^{3}H-hypoxanthine incorporation was applied for this antimalarial testing and the procedure was as follows. The sample (25µl) in the culture medium was

placed in triplicate in a 96-well plate. *Plasmodium falciparum* infected red cells (200 μl) with a cell suspension (1.5%) of parasitemia (0.5-1%) was added to the wells. Final concentrations of the samples ranged from 1×10^{-5} to 1×10^{-8} g/ml with 0.1% of the organic solvent. The plates were cultured under standard conditions for 24 hours prior to the addition of ^{3}H-hyphoxanthine (25 μl, 0.5mCi). After an additional incubation for 18-20 hours, the parasites' DNA was harvested onto glass fibre filters. A radiation counter determined the amount of ^{3}H-hypoxanthine. The inhibitory concentration of the sample was determined from its dose-response curves or by calculation.

Plasmodium falciparum K1 strain was cultured according to the method of Trager and Jensen in 1976.[262] The parasites were maintained in human red blood cells in a culture medium. RPMI 1640 was supplemented with 25mM HEPES, 0.2% sodium bicarbonate, and 8% human serum, at 37°C in a CO_2 incubator.

This *in vitro* testing was carried out in Bangkok, Thailand at the Protein-Ligand Engineering and Antimalarial Screening Laboratories, National Centre for Genetic Engineering and Biotechnology, National Science and Technology Development Agency by Dr. Sumalee Kamchonwongpaisan.

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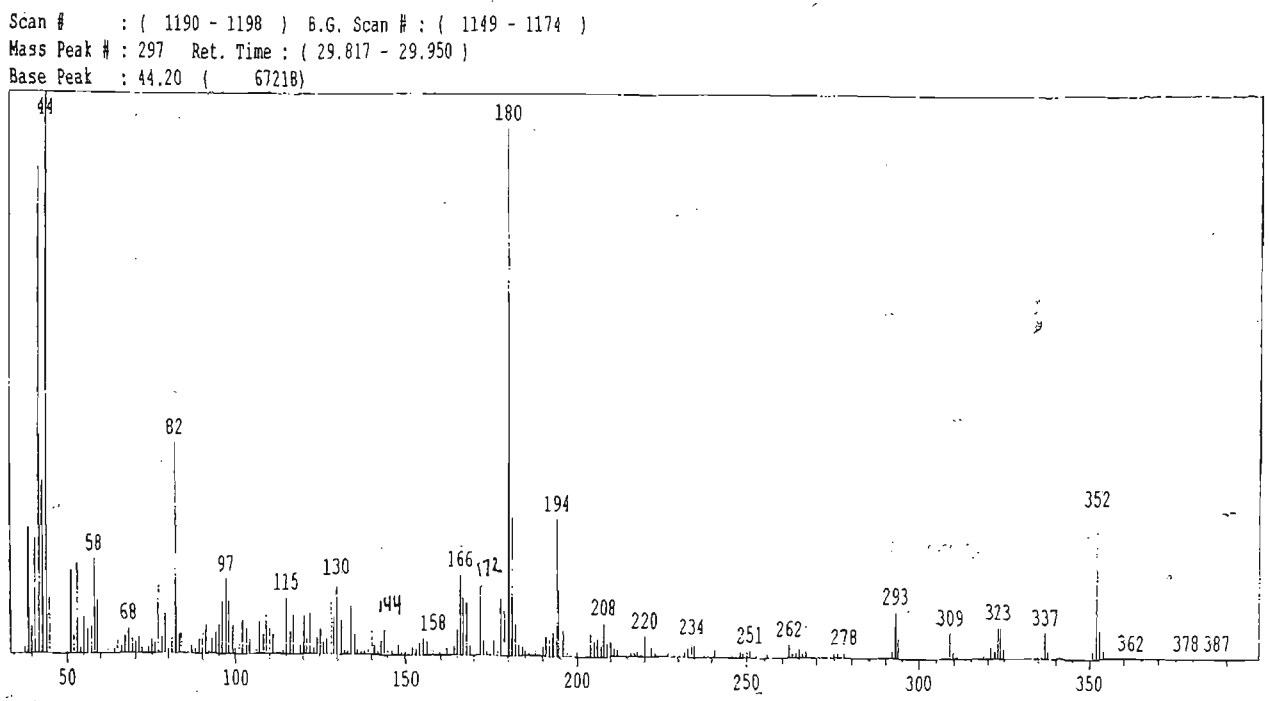
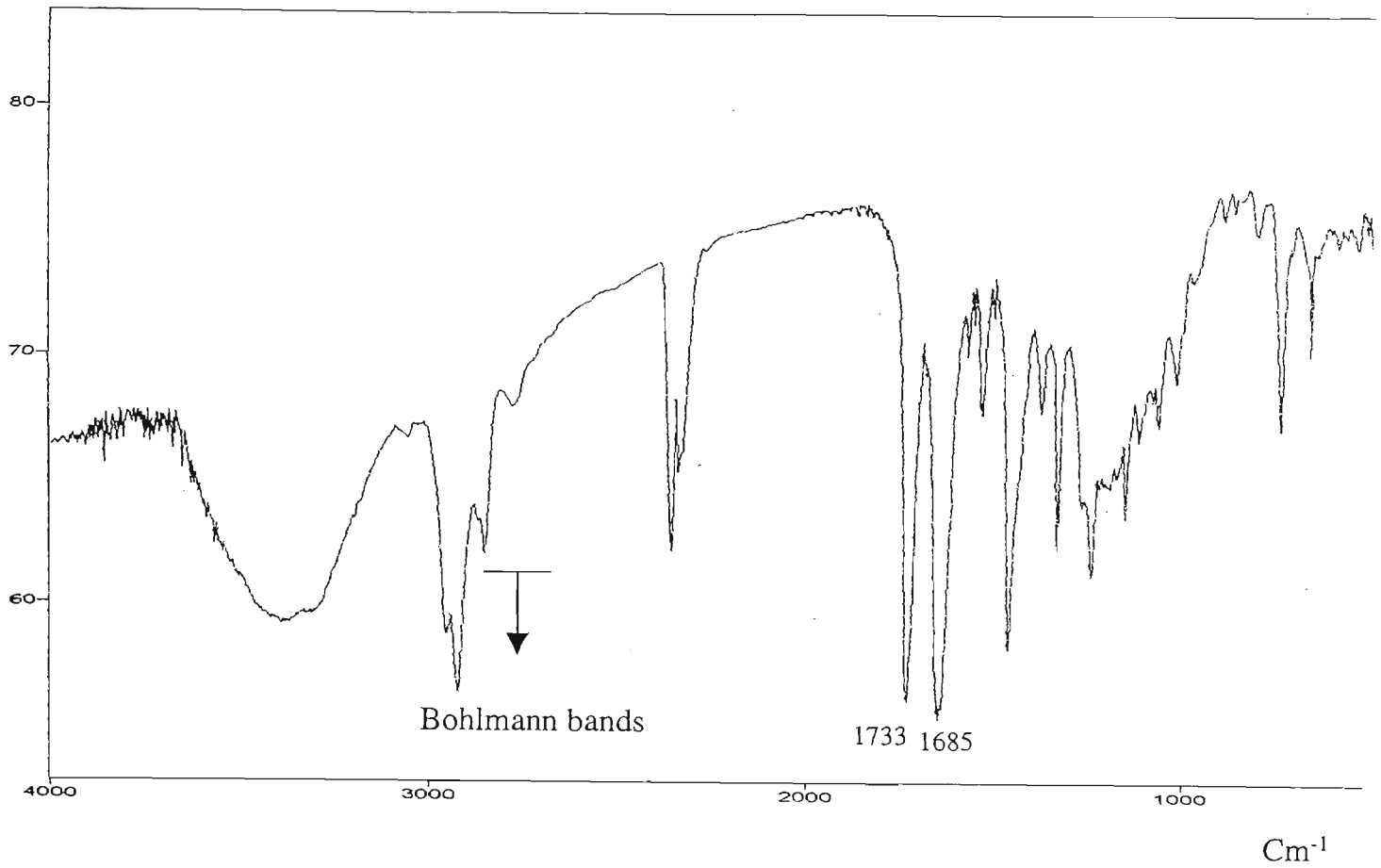
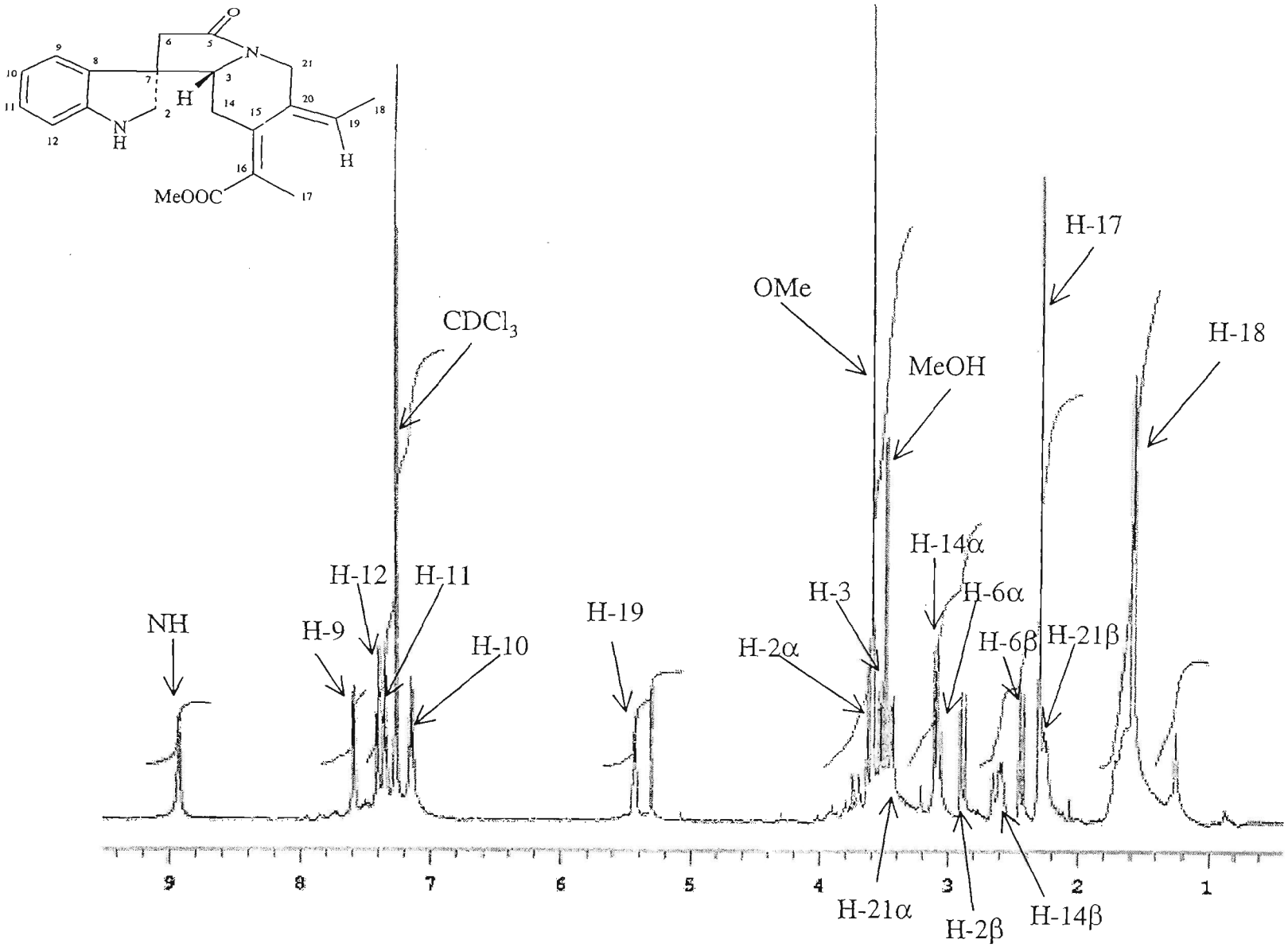
APPENDICES



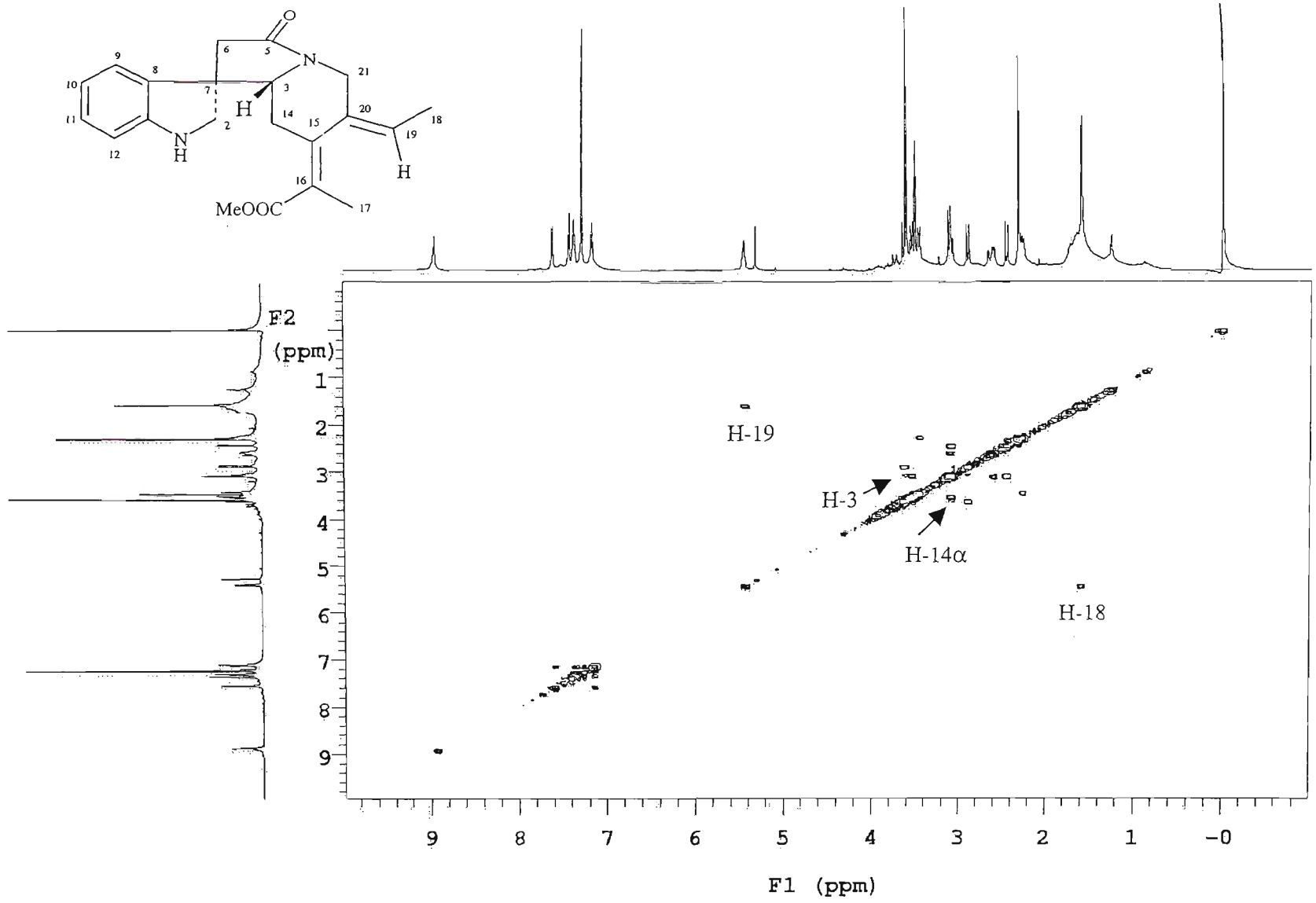
Appendix 1. LREIM spectrum of lombine **87**



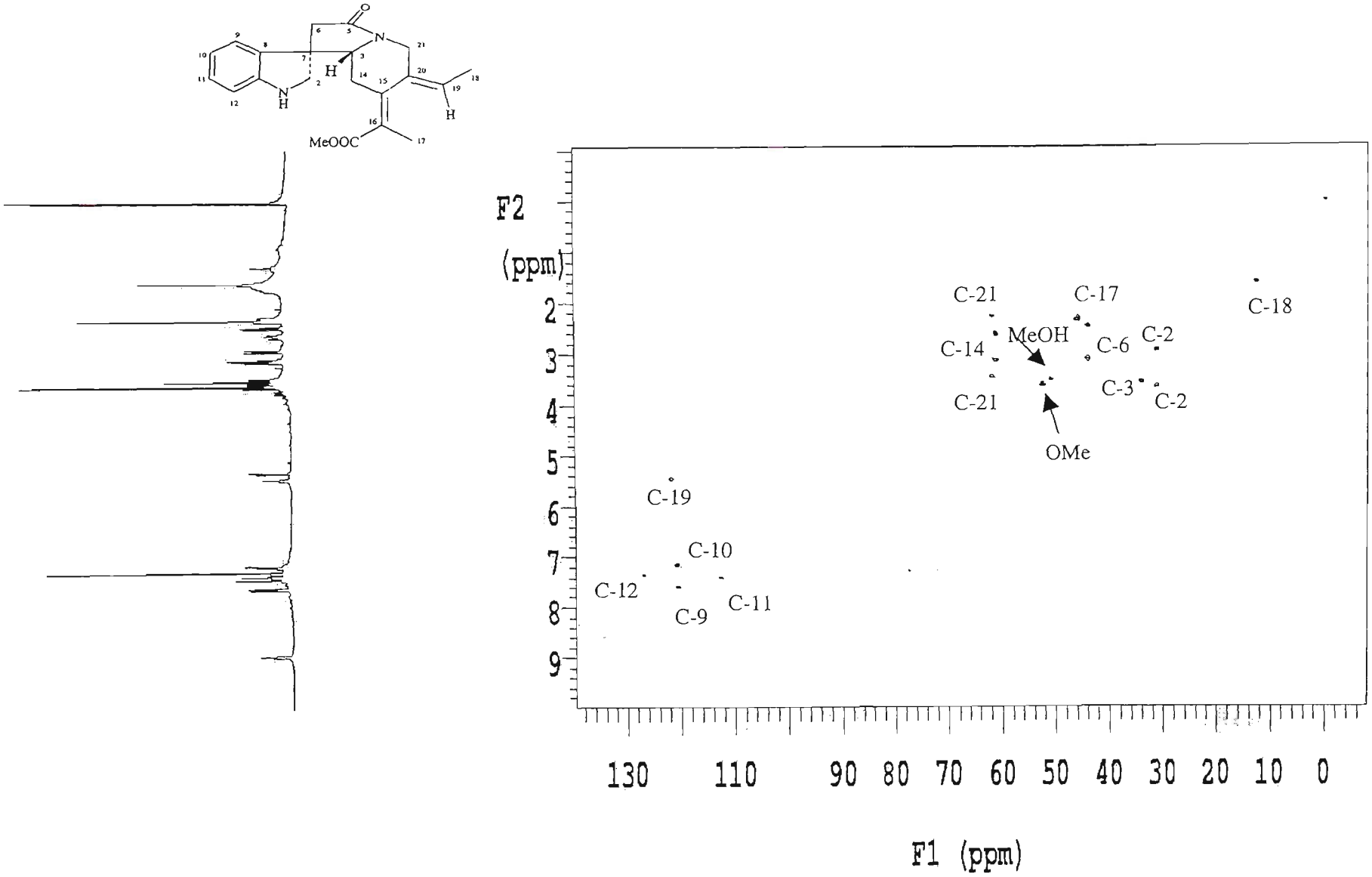
Appendix 2. IR-spectrum of lombine **87**



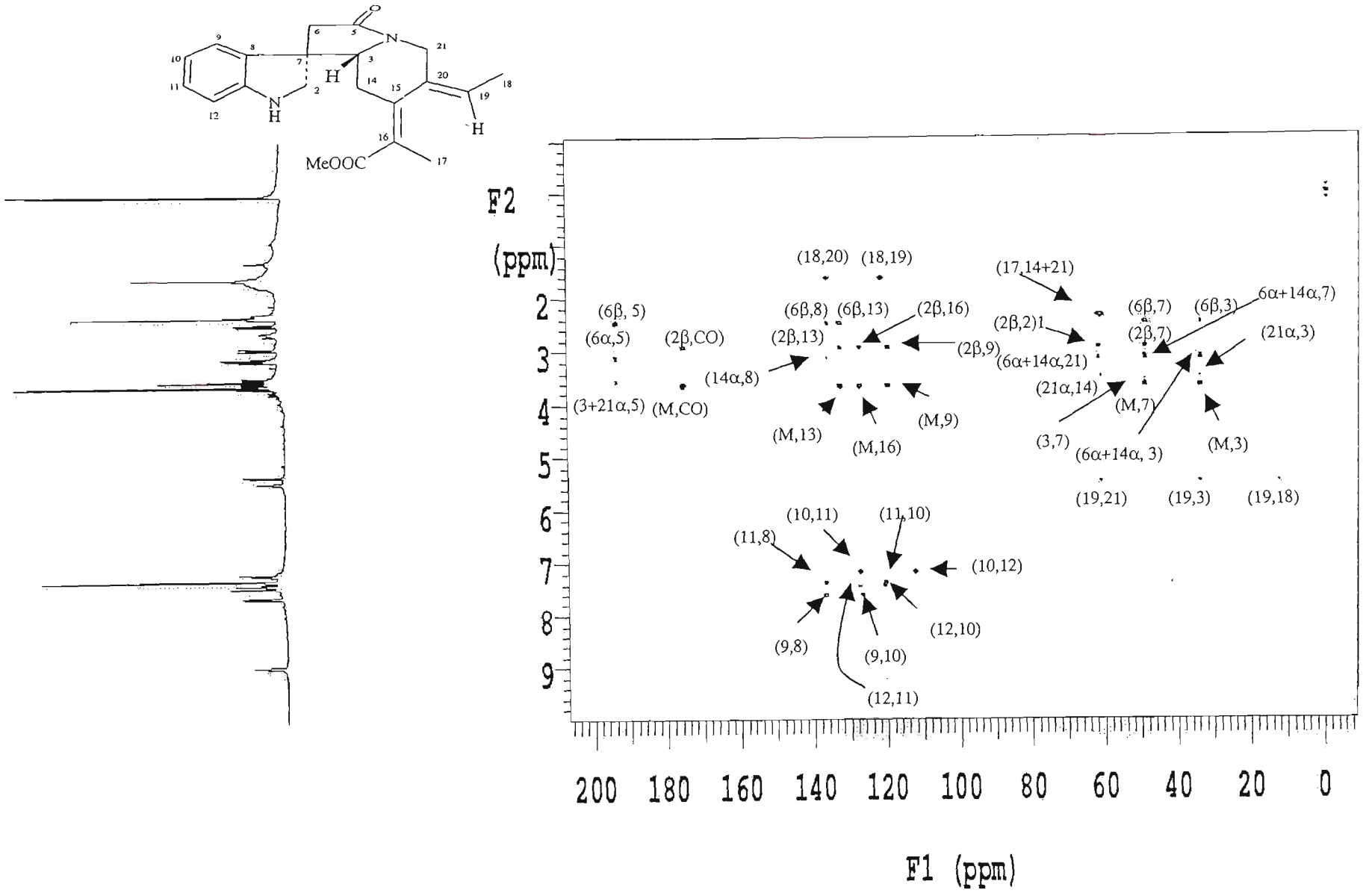
Appendix 3. ^{1}H-NMR spectrum of lombine **87** ($CDCl_3$, 500 MHz, TMS as reference)



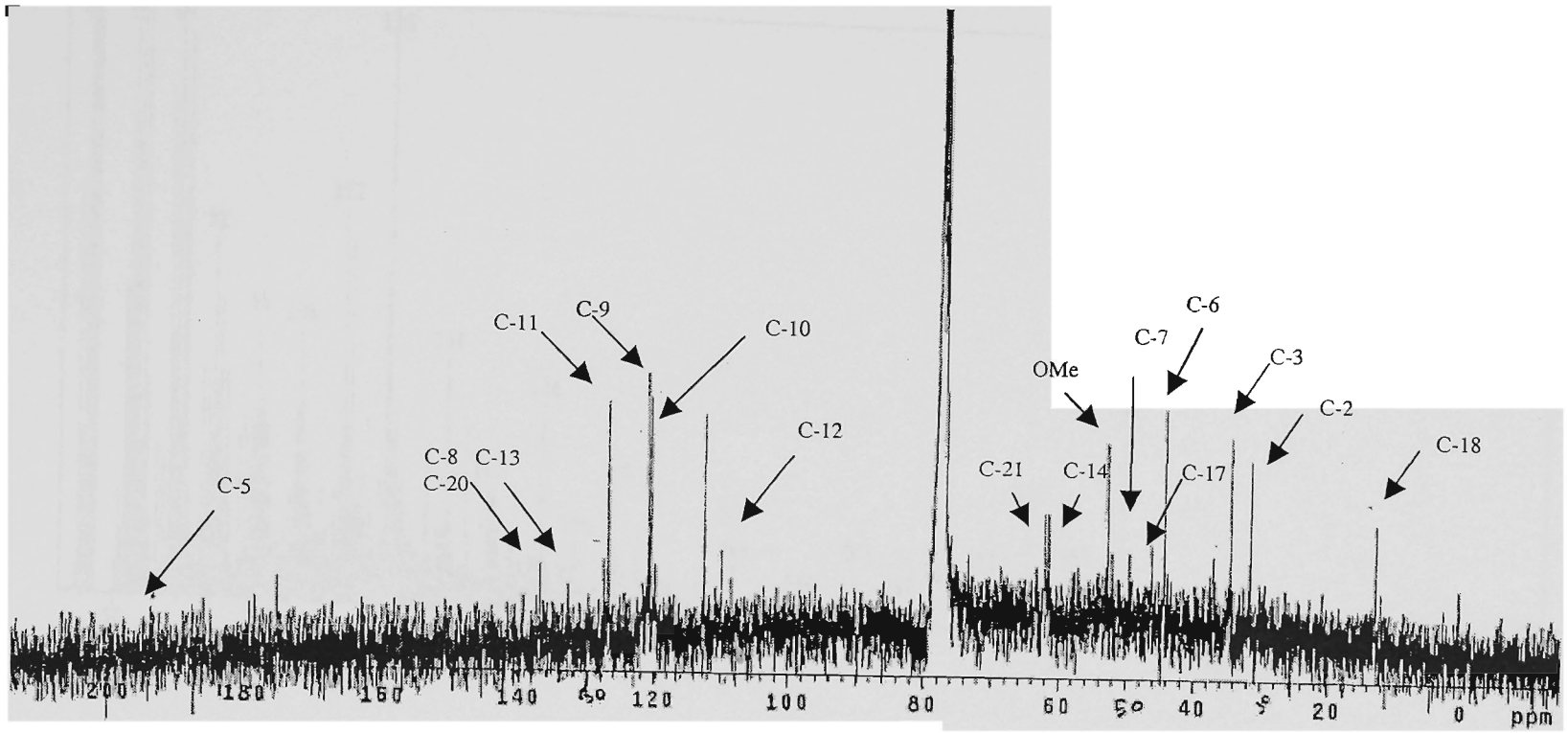
Appendix 4. gCOSY spectrum of lombine **87** ($CDCl_3$, 500 MHz, TMS as reference)



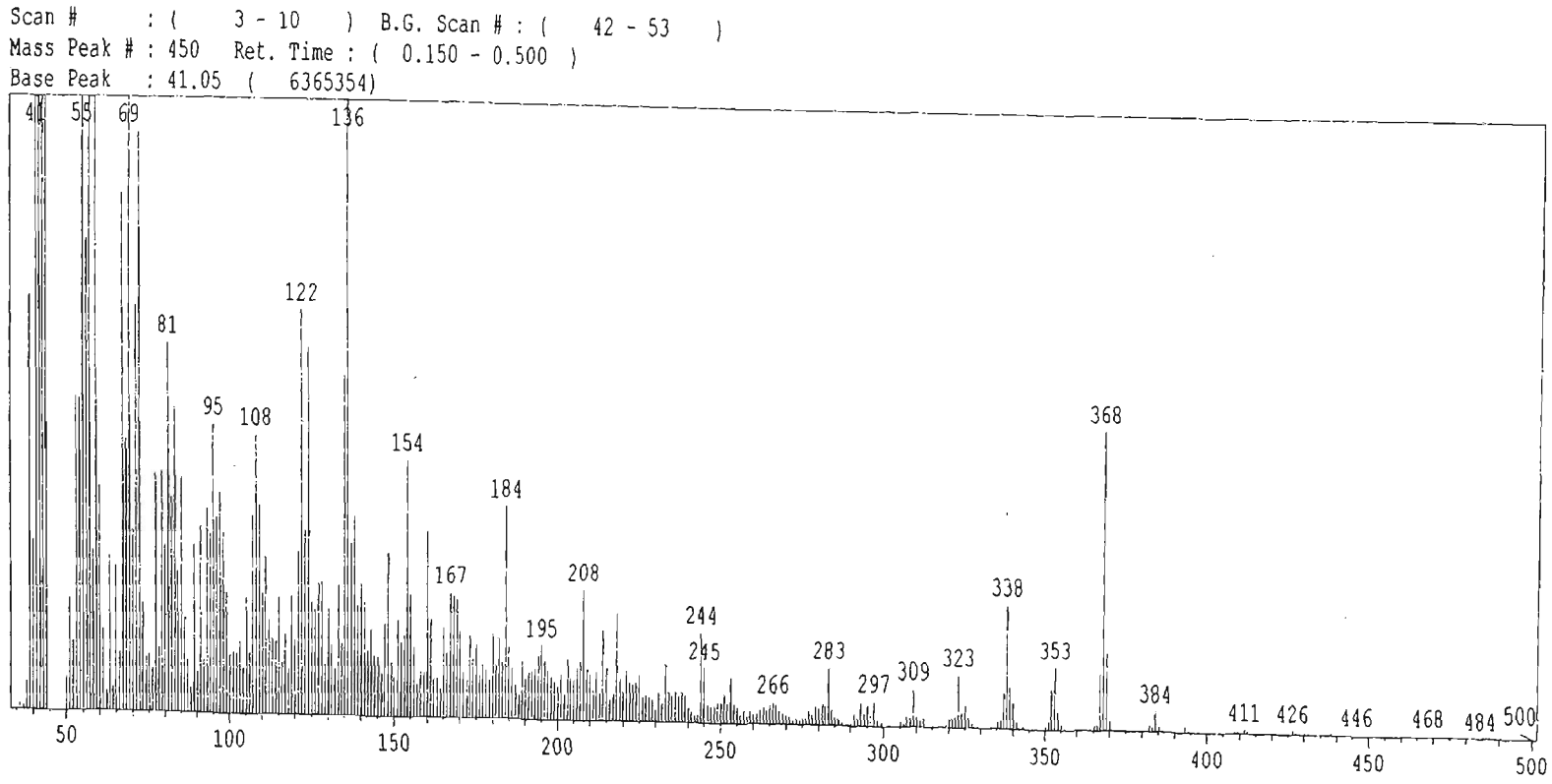
Appendix 5. gHSQC spectrum of lombine **87** ($CDCl_3$, 500 MHz, TMS as reference)



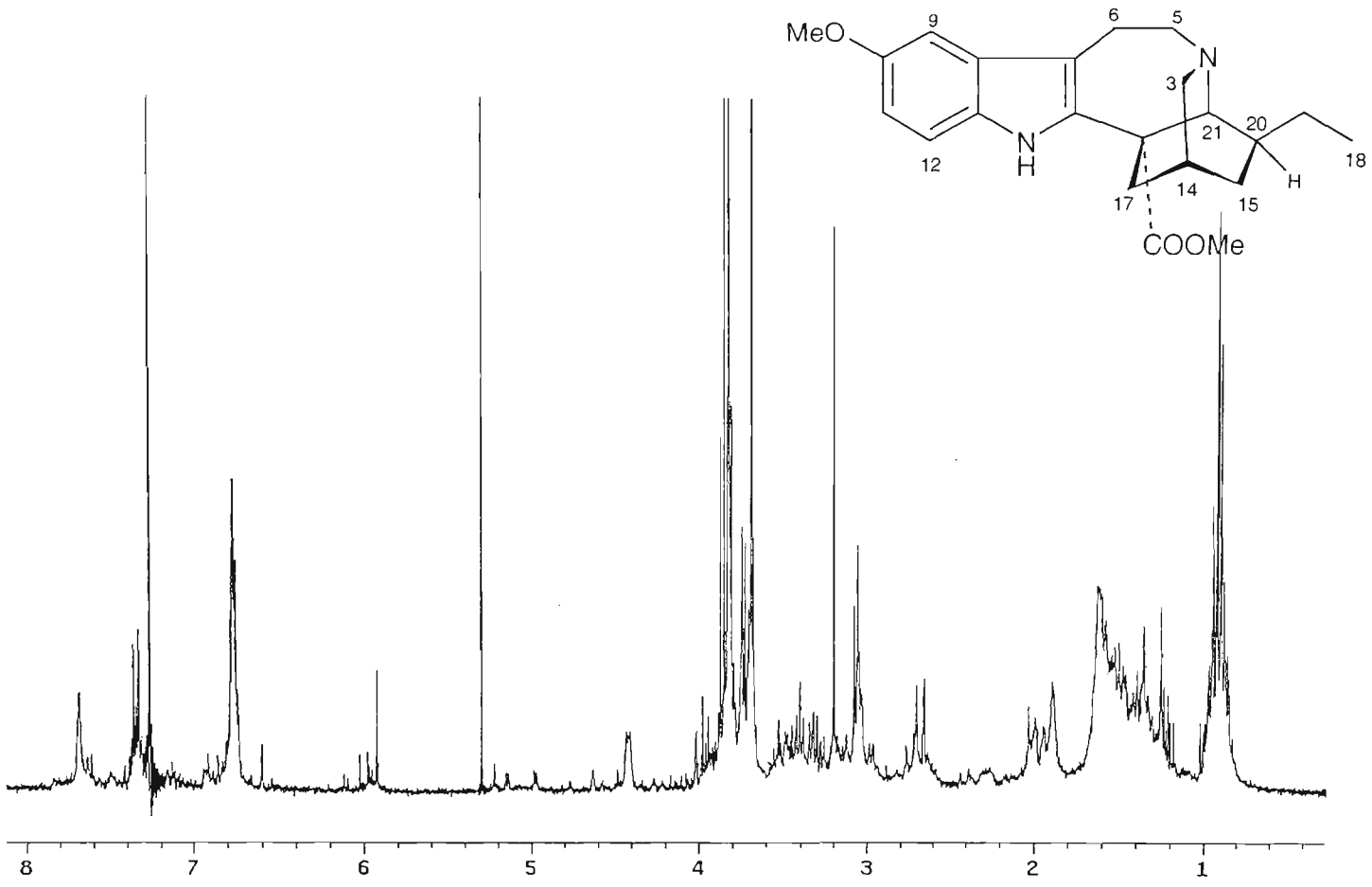
Appendix 6. gHMBC spectrum of lombine **87** ($CDCl_3$, 500 MHz, TMS as reference, M = overlap spectrum of OMe and H-2α)



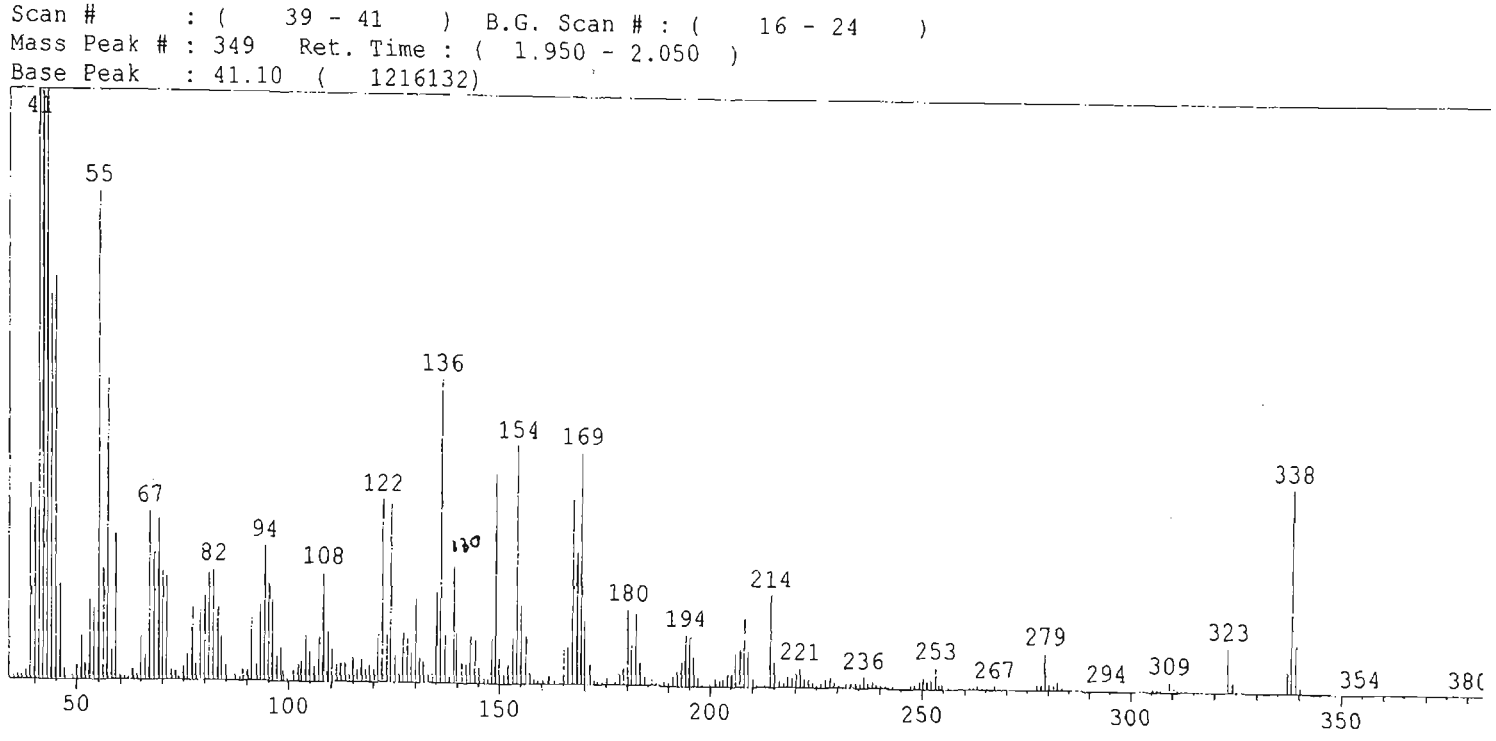
Appendix 6a. ^{13}C-NMR spectrum of lombine **87** ($CDCl_3$, 75 MHz, TMS as reference)



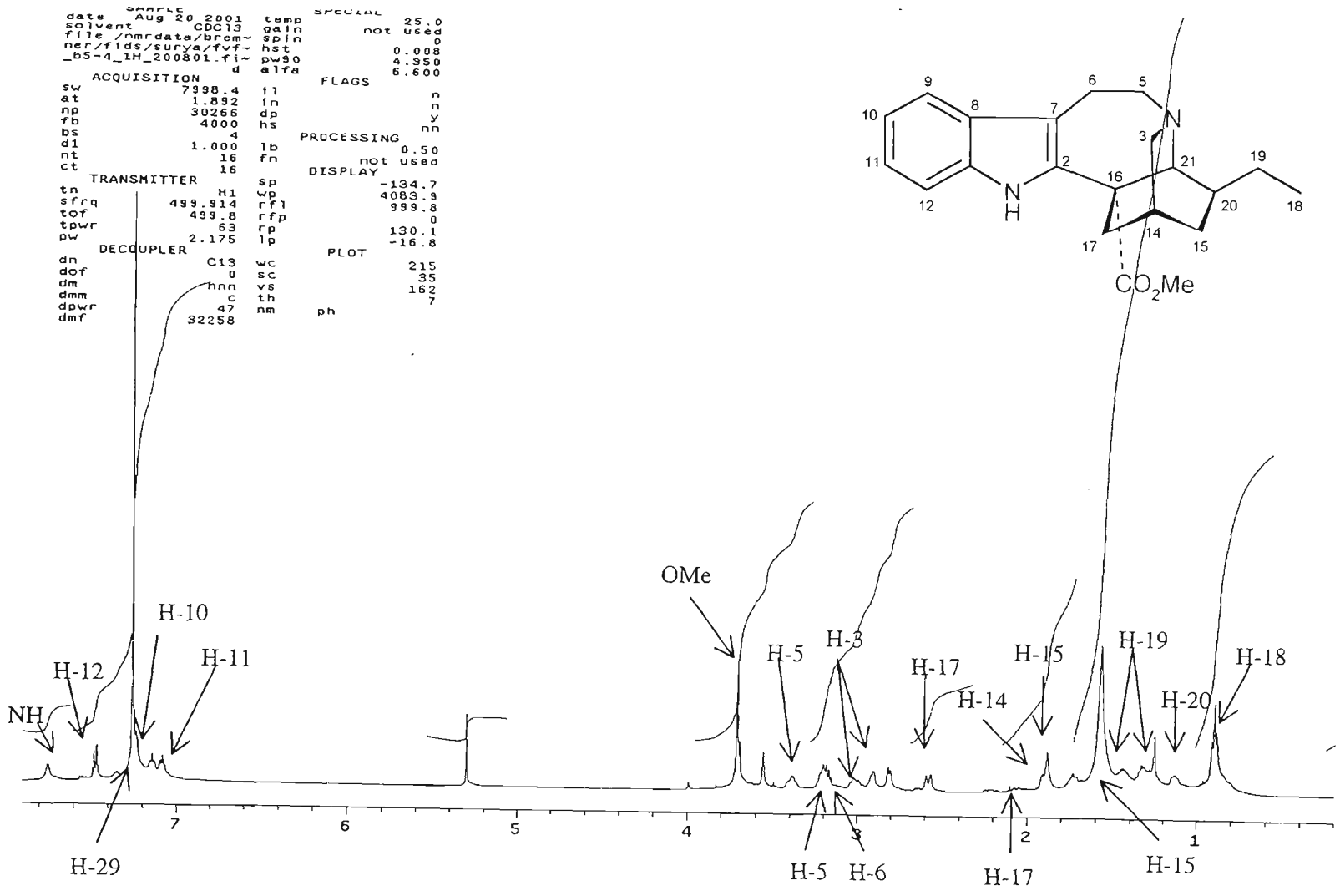
Appendix 7. LREIMS spectrum of voacangine **89**



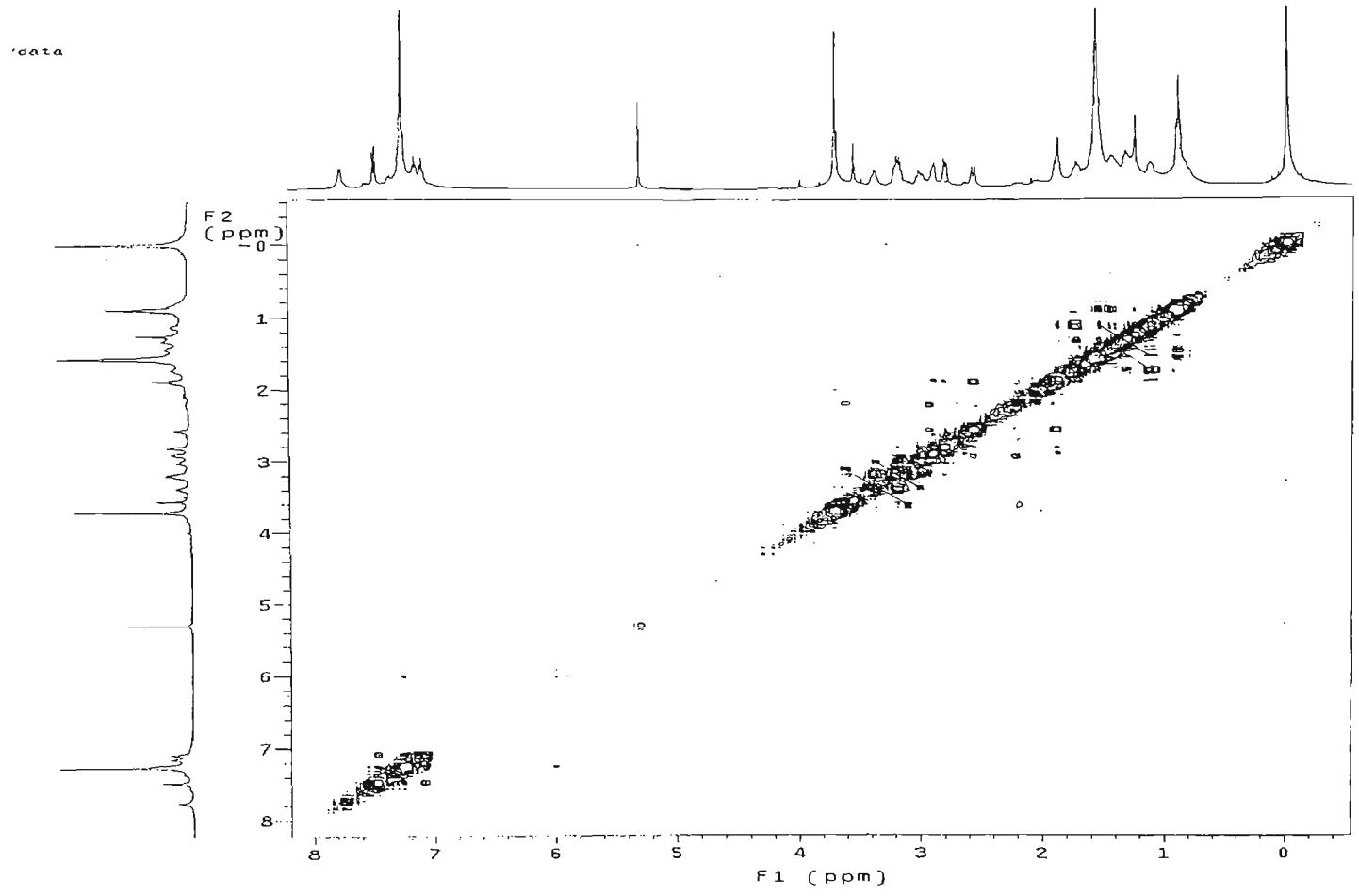
Appendix 8. ^{1}H-NMR spectrum of voacangine **89** with impurities ($CDCl_3$, 500MHz TMS as reference)



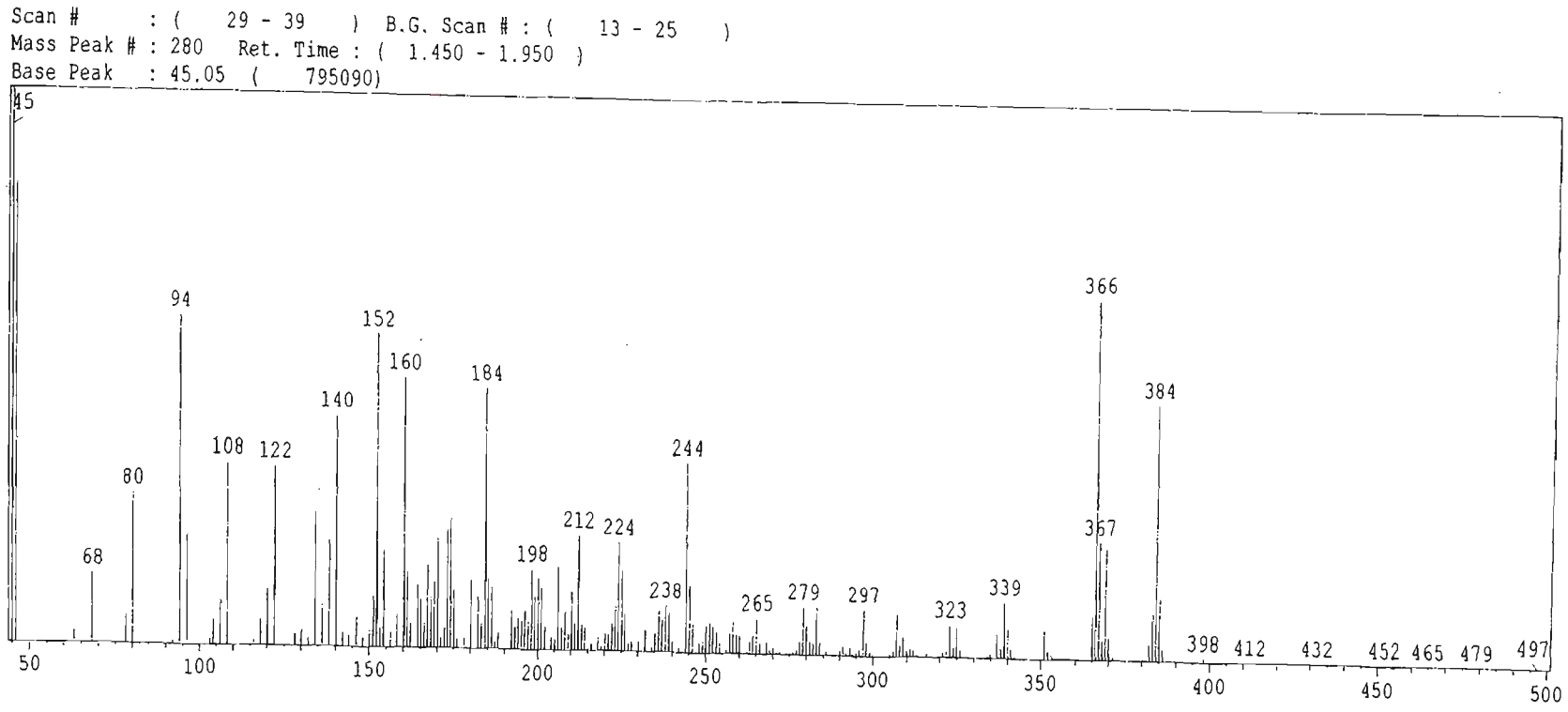
Appendix 9. LREIMS spectrum of coronaridine **90**



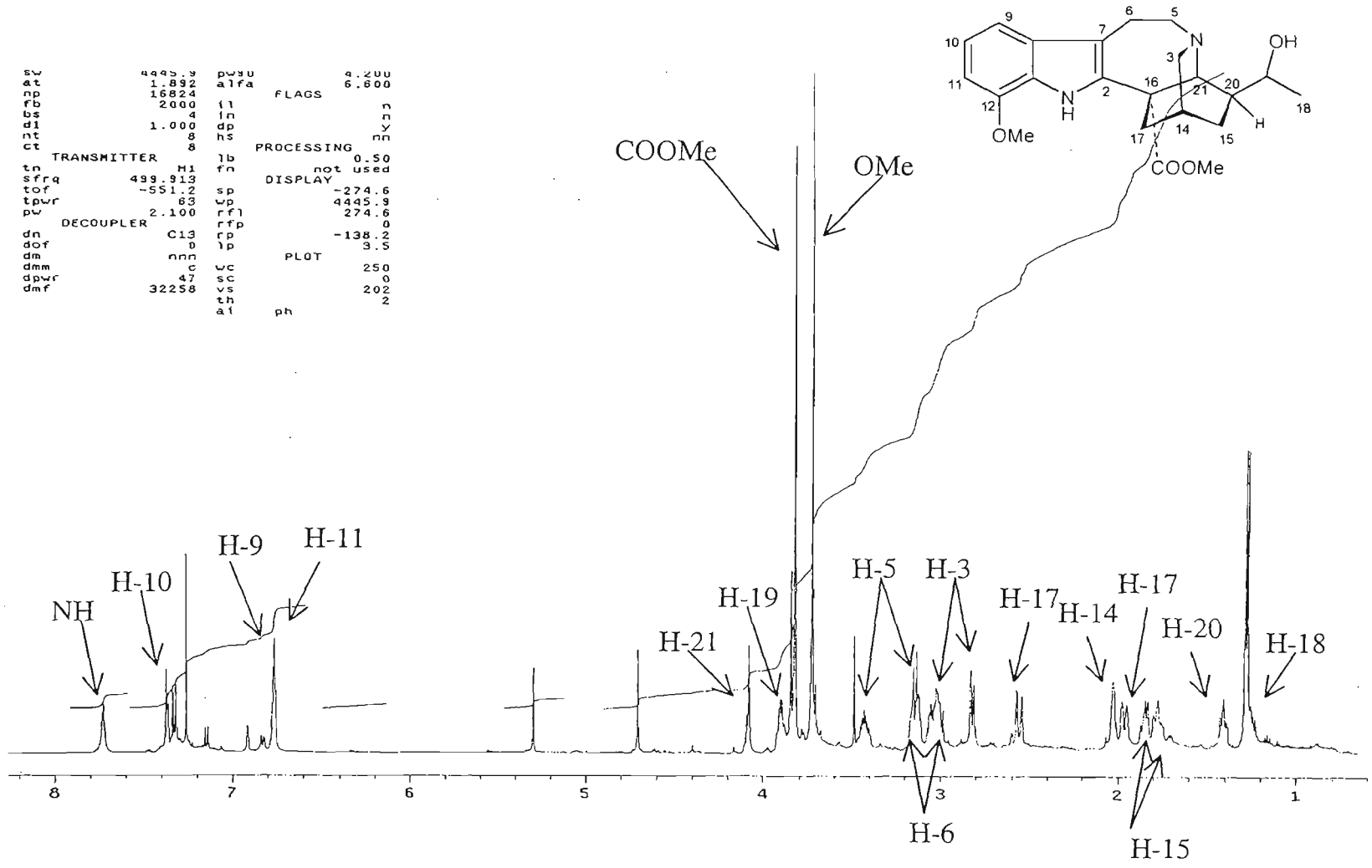
Appendix 10. ^{1}H-NMR spectrum of coronaridine **90** ($CDCl_3$, 500 MHz, TMS as reference)



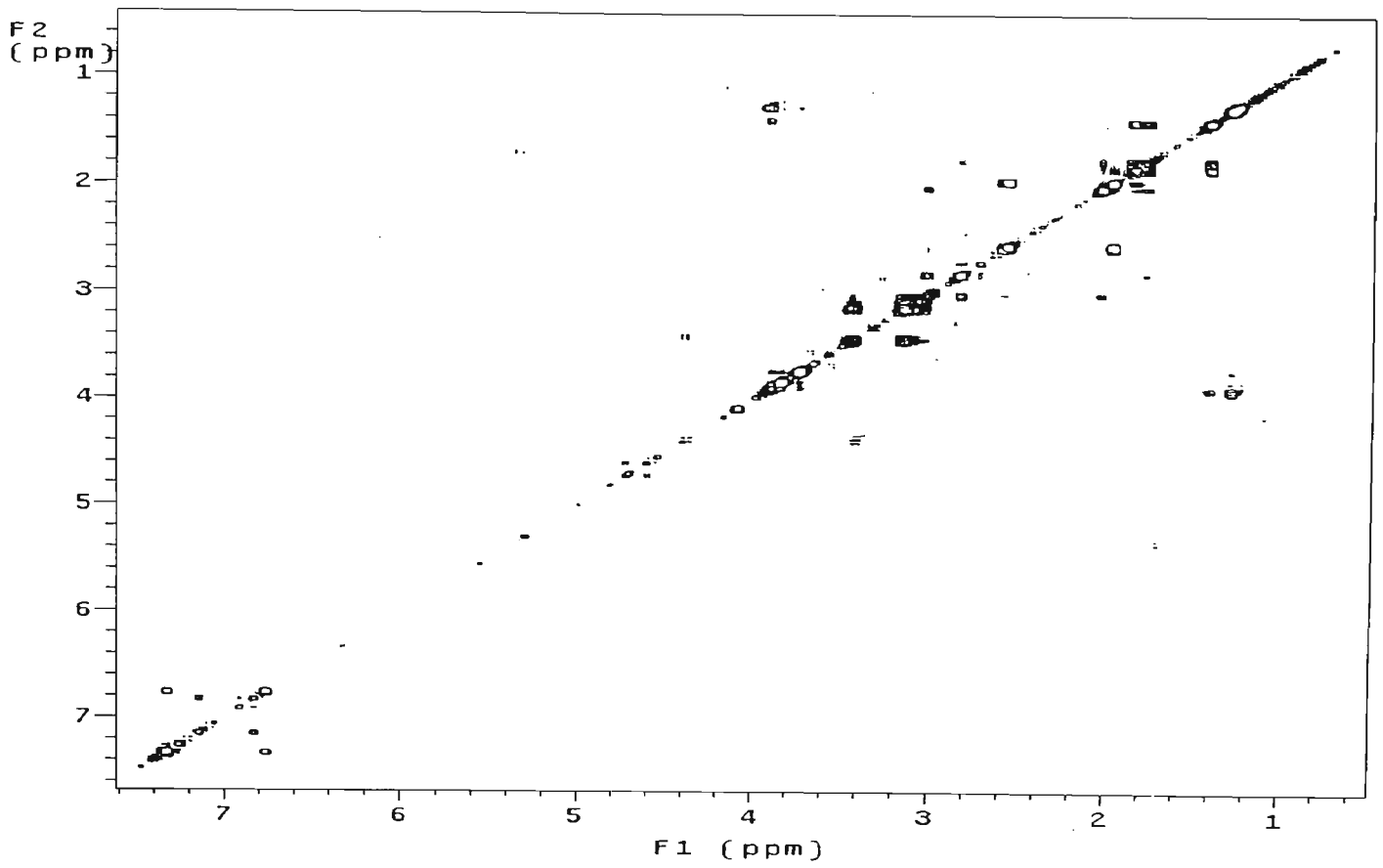
Appendix 11. gCOSY spectrum of coronaridine **90** ($CDCl_3$, 500 MHz TMS as reference)



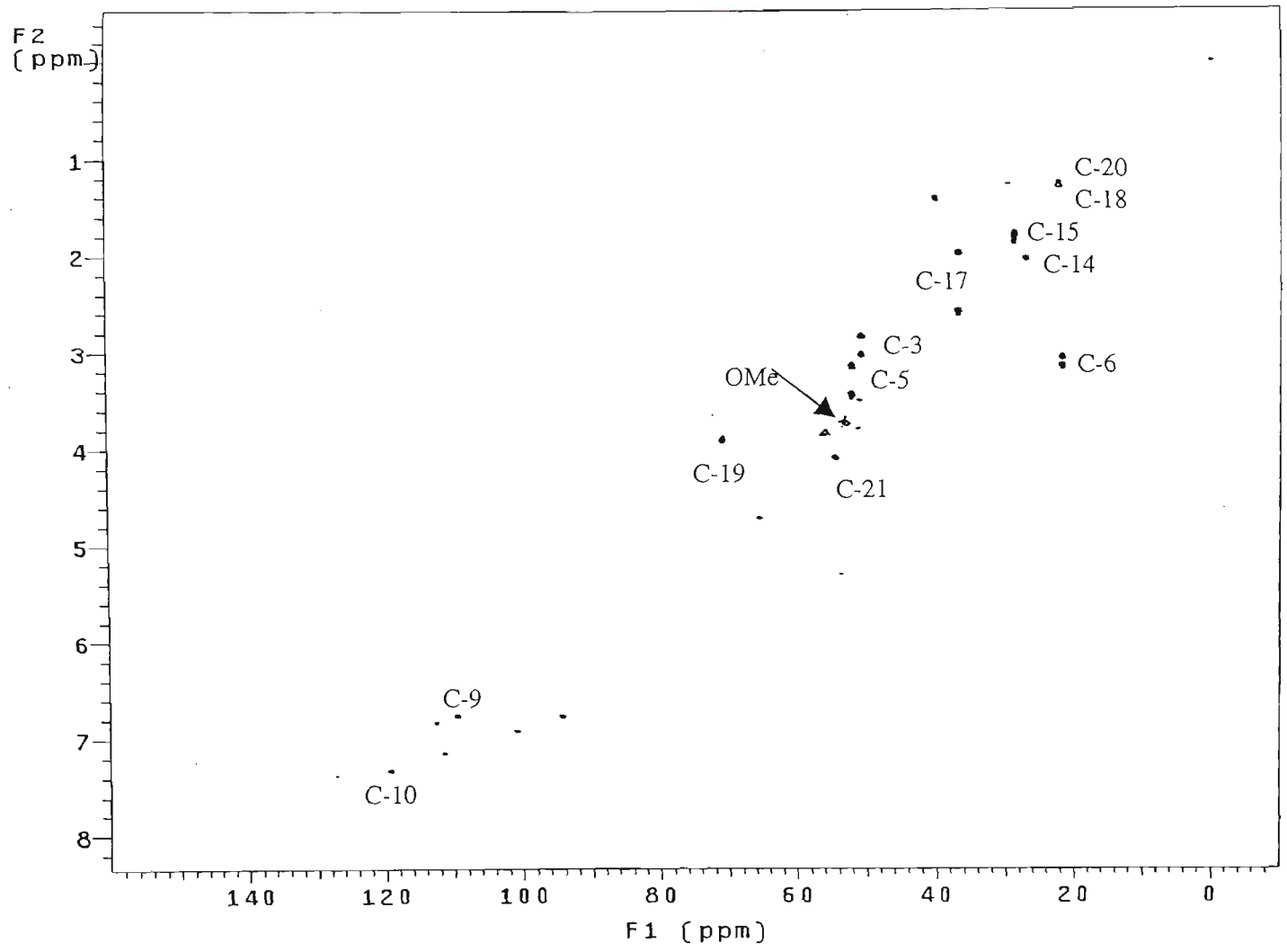
Appendix 11a. LREIMS spectrum of voacristine **92**



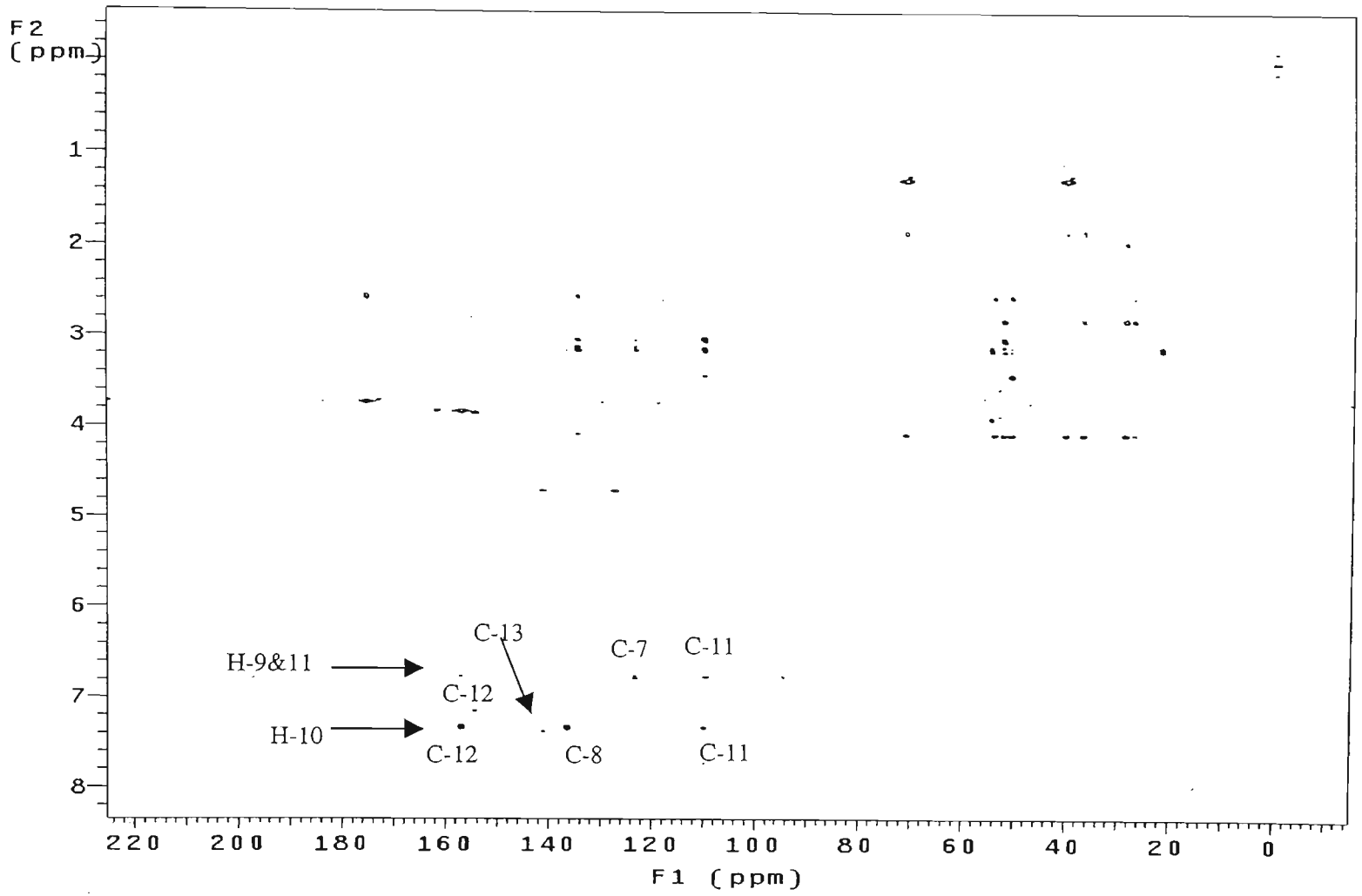
Appendix 12. ^{1}H-NMR spectrum of voacristine **92** ($CDCl_3$, 500 MHz TMS as references)



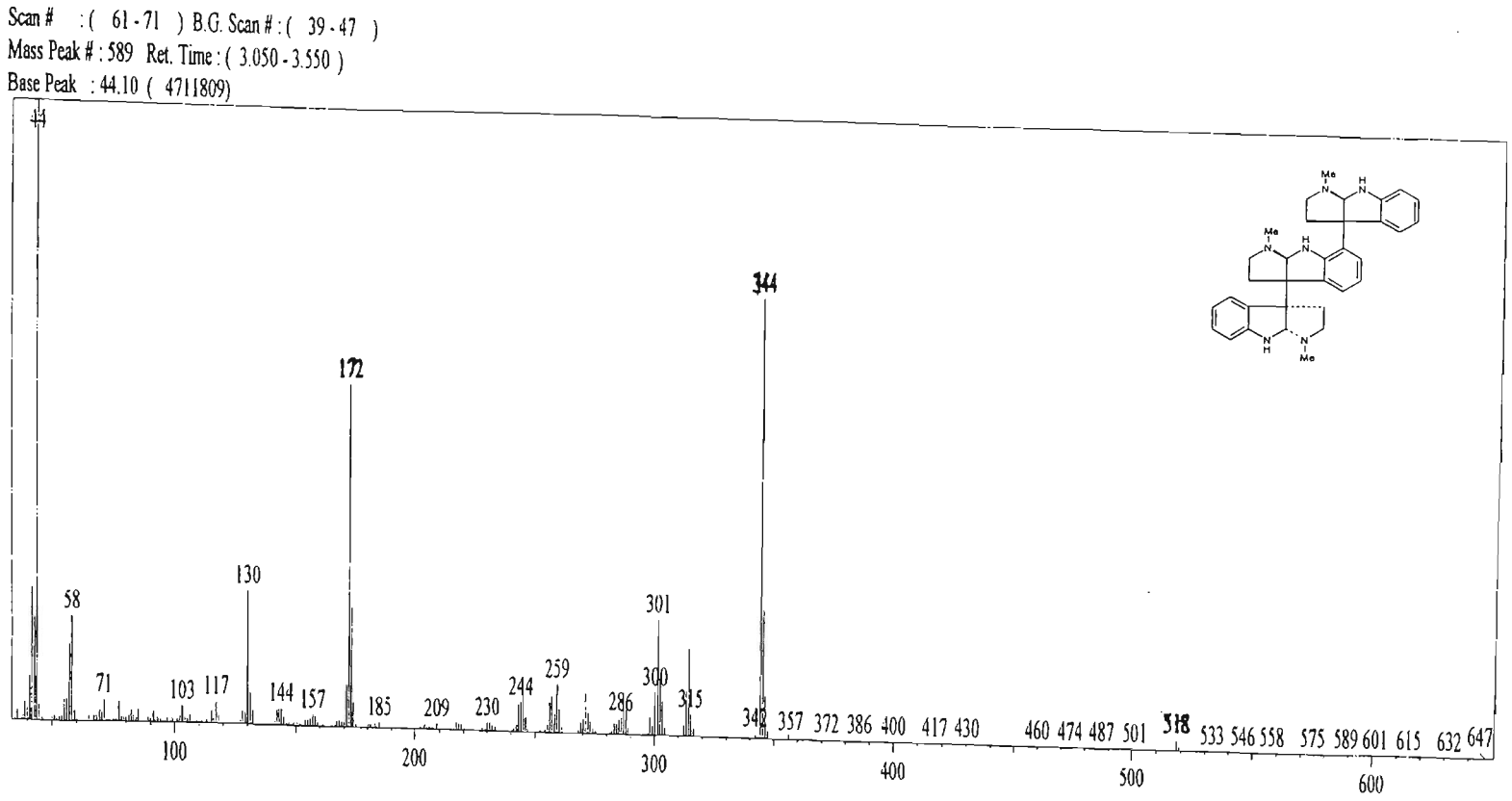
Appendix 13. gCOSY spectrum of voacristine **92** ($CDCl_3$, 500 MHz TMS as references)



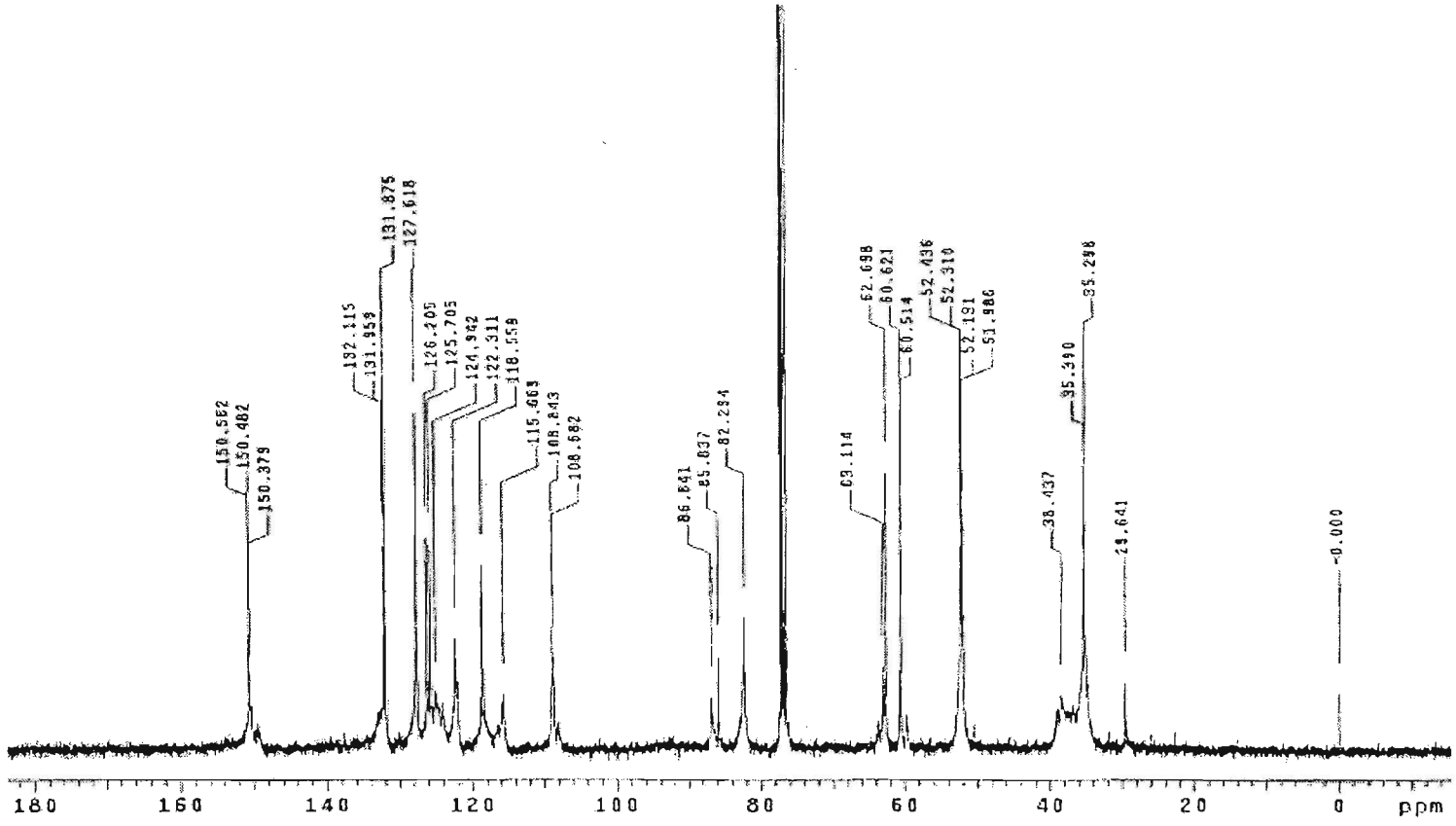
Appendix 14. gHSQC spectrum of voacristine **92** ($CDCl_3$, 500 MHz TMS as references)



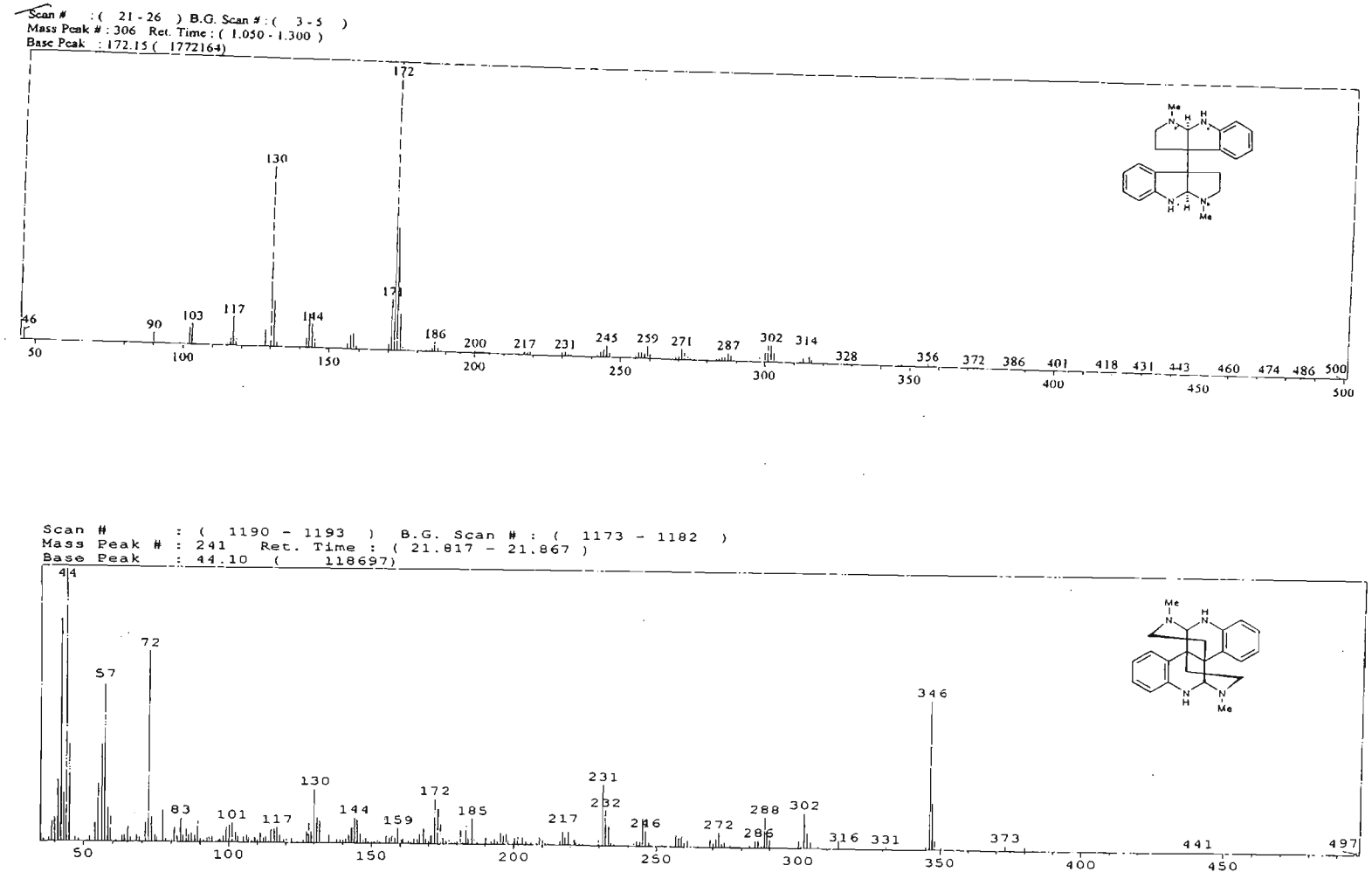
Appendix 15. gHMBCspectrum of voacristine **92** ($CDCl_3$, 500 MHz, TMS as references)



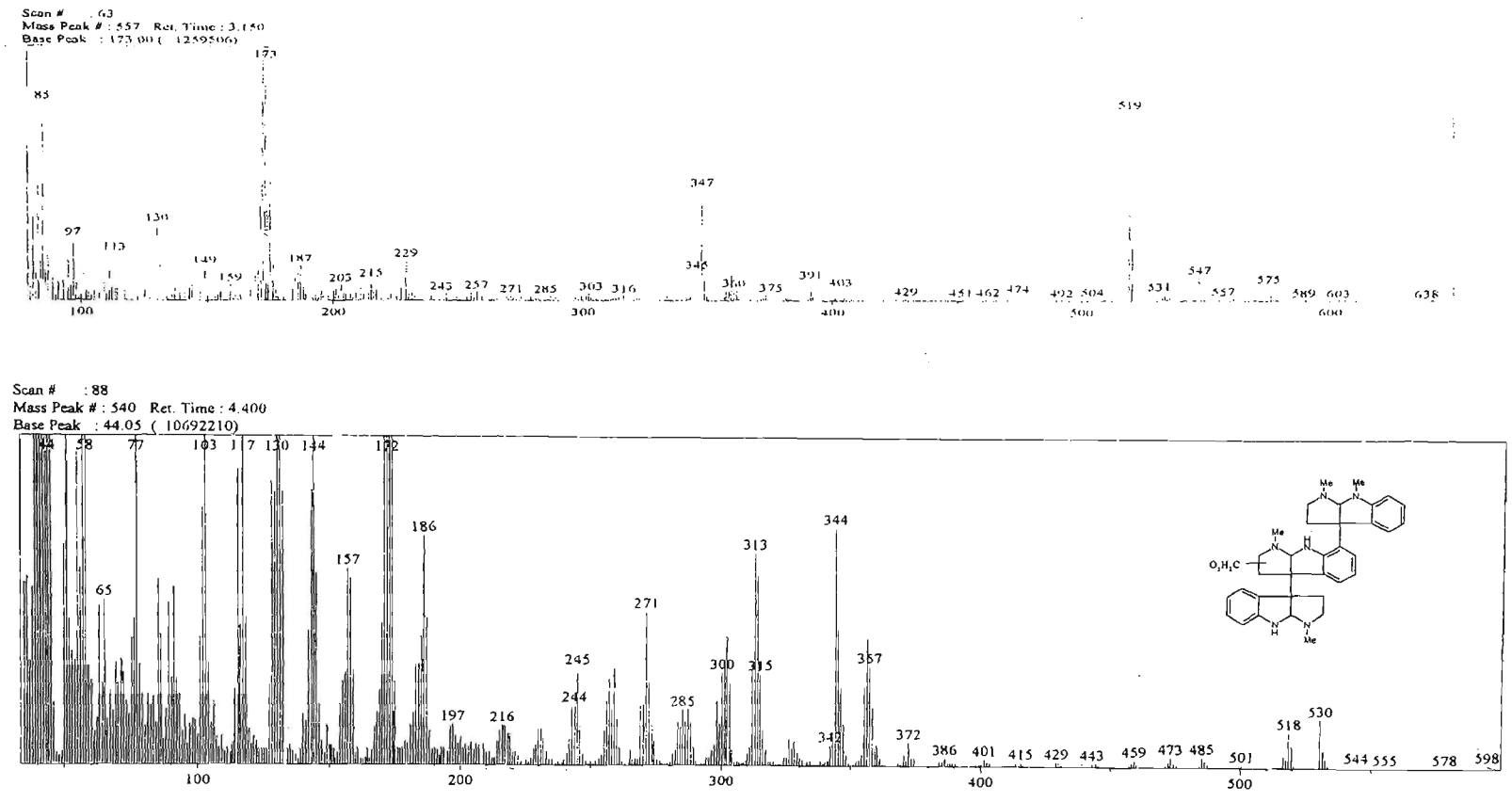
Appendix 16. LREIMS spectrum of hodgkinsine **97**



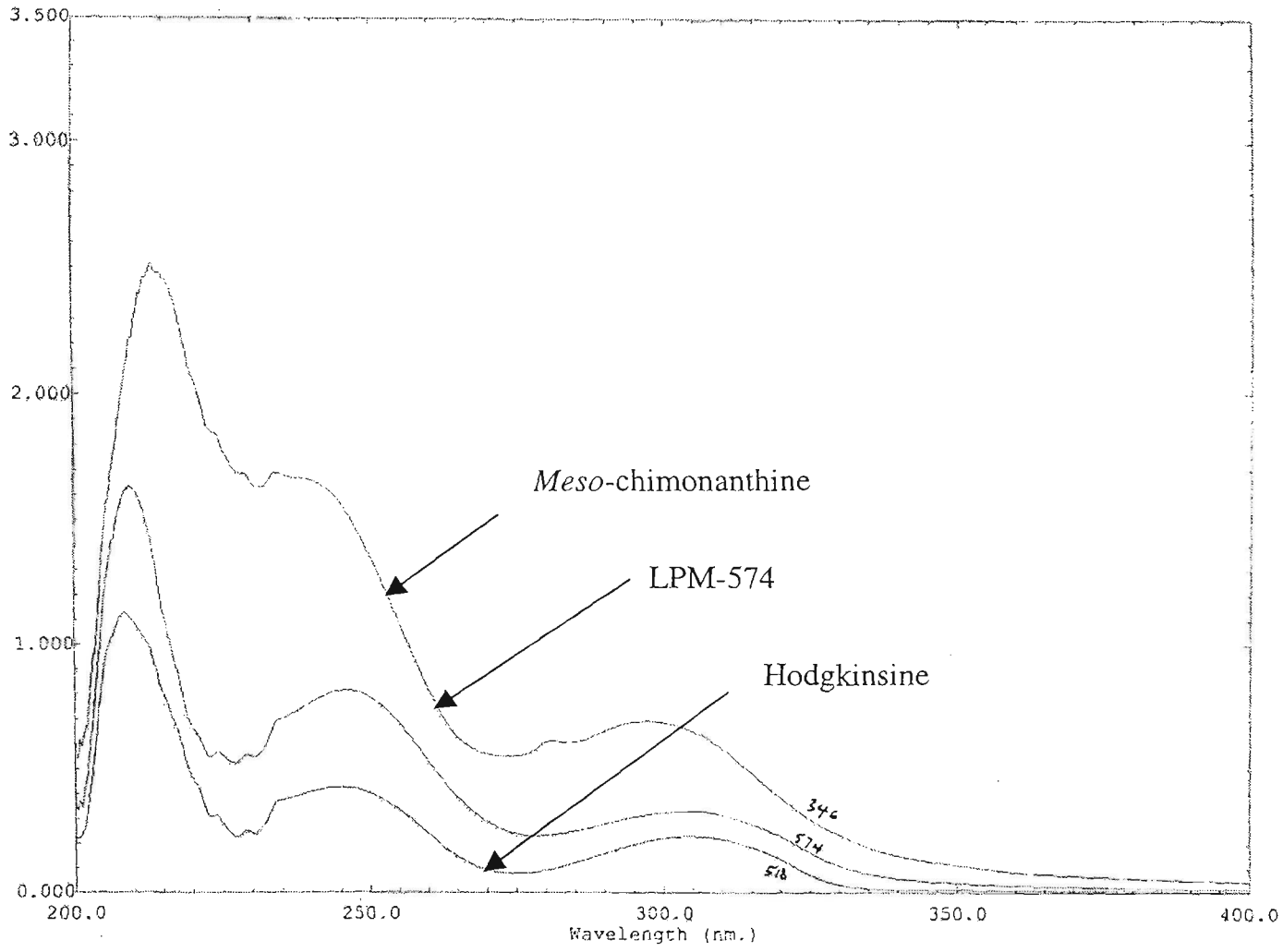
Appendix 16a. ^{13}C-NMR spectrum of hodgkinsine **97** ($CDCl_3$, 75MHz TMS as reference)



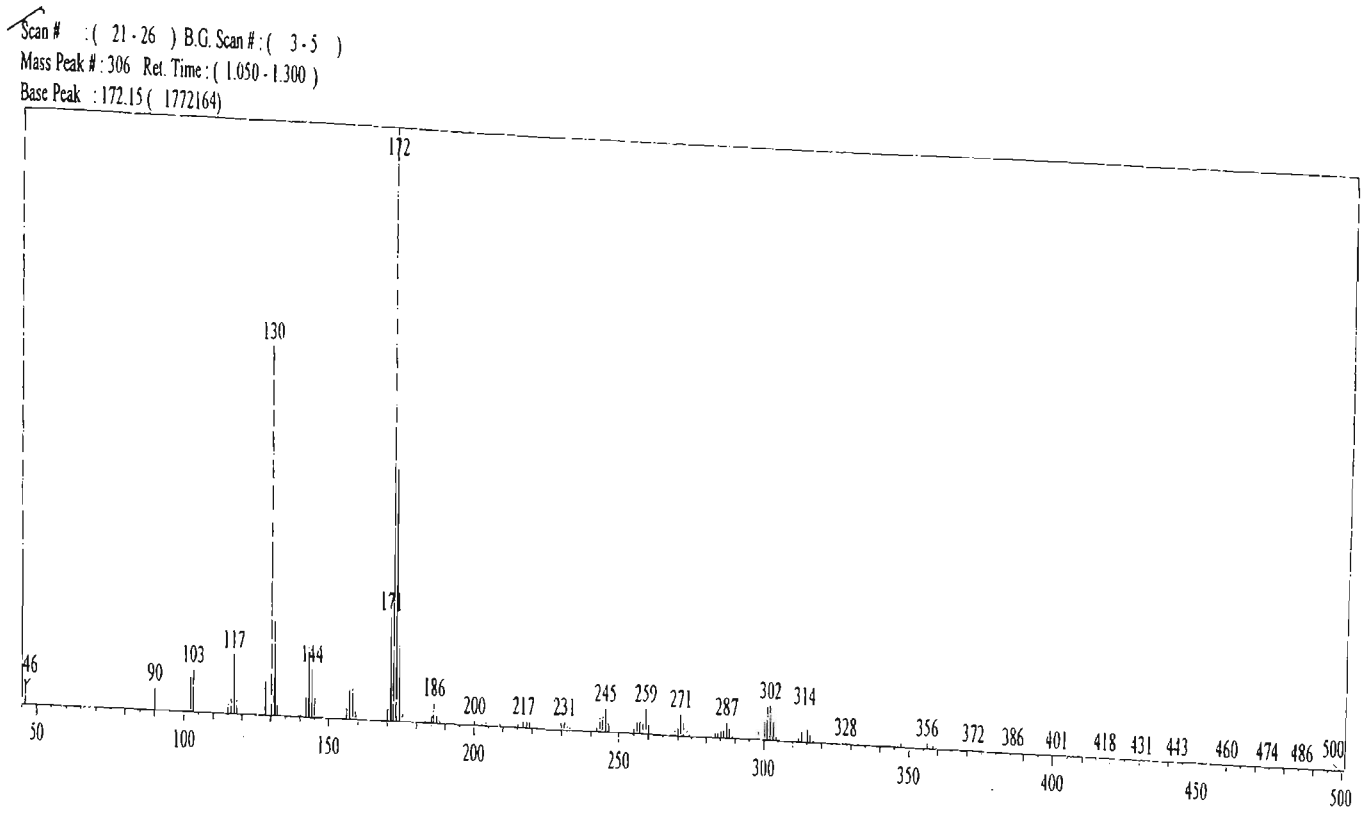
Appendix 17. LREIMS spectra of *meso*-chimonanthine **104** (top) and *iso*-calycanthine **102** (below)



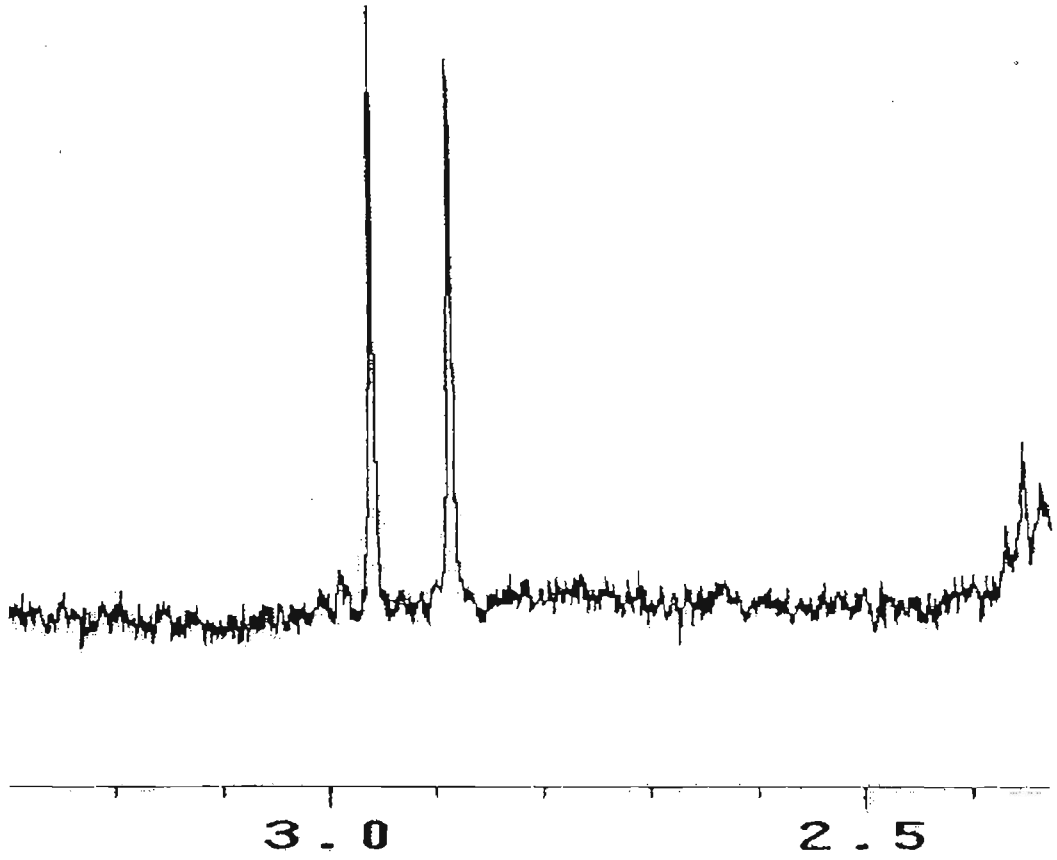
Appendix 18. LRCIMS (above) and LREIMS (below) spectra of compound LPM-574 **128**



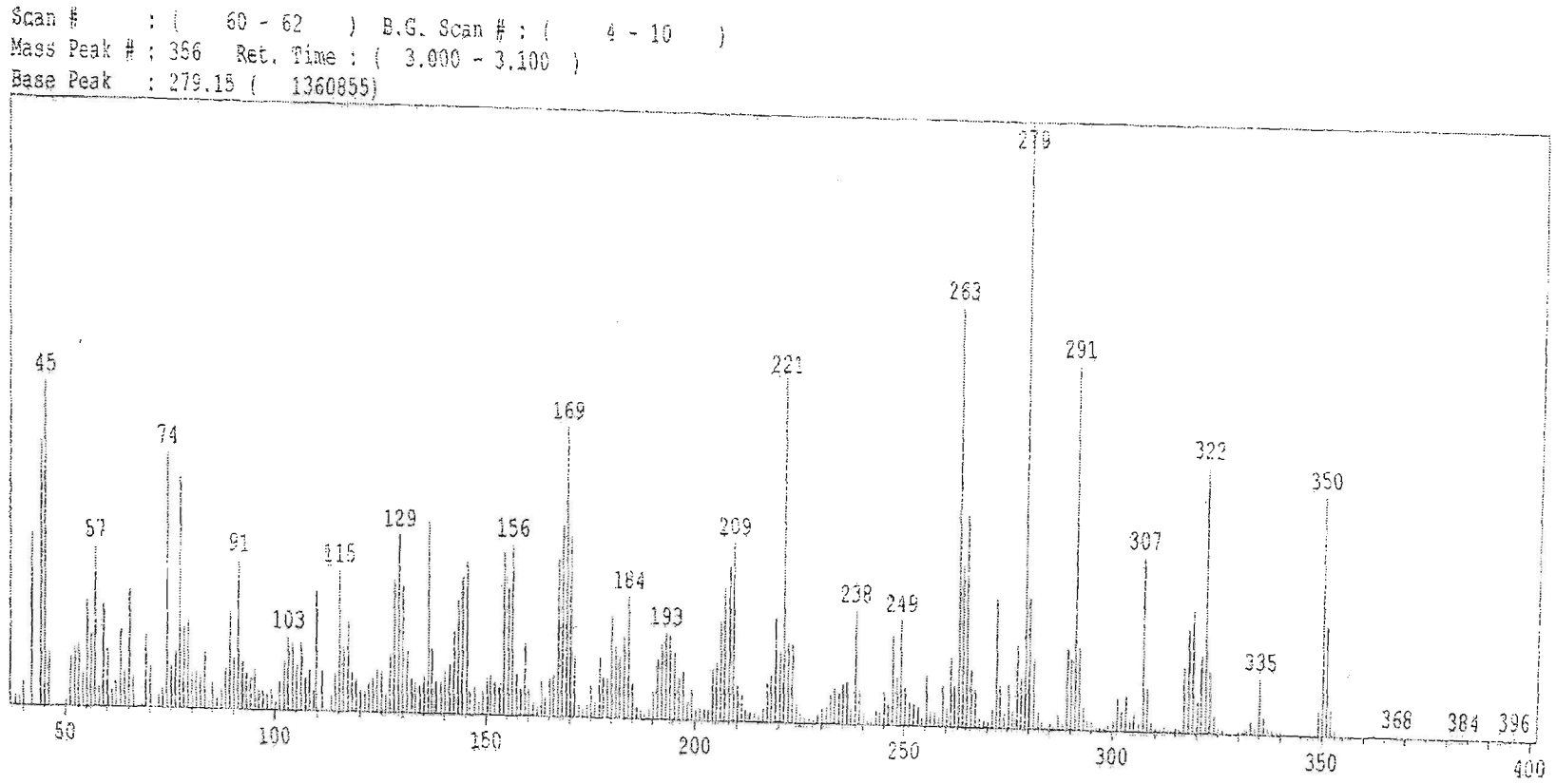
Appendix 19. UV spectra of *meso*-chimonanthine, LPM-574 and Hodgkinsine



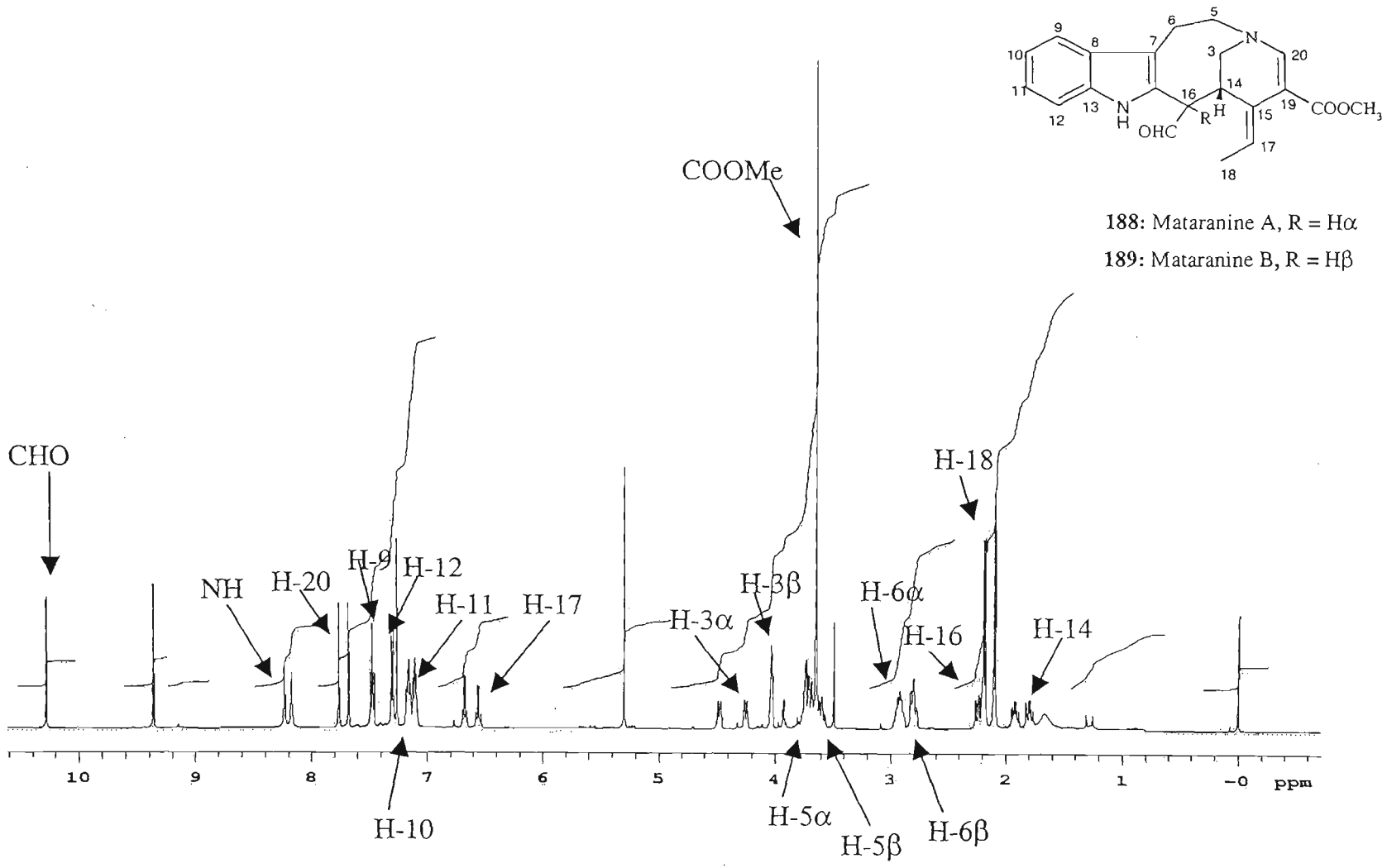
Appendix 20. LREIMS spectrum of compound LPM-186 **129**



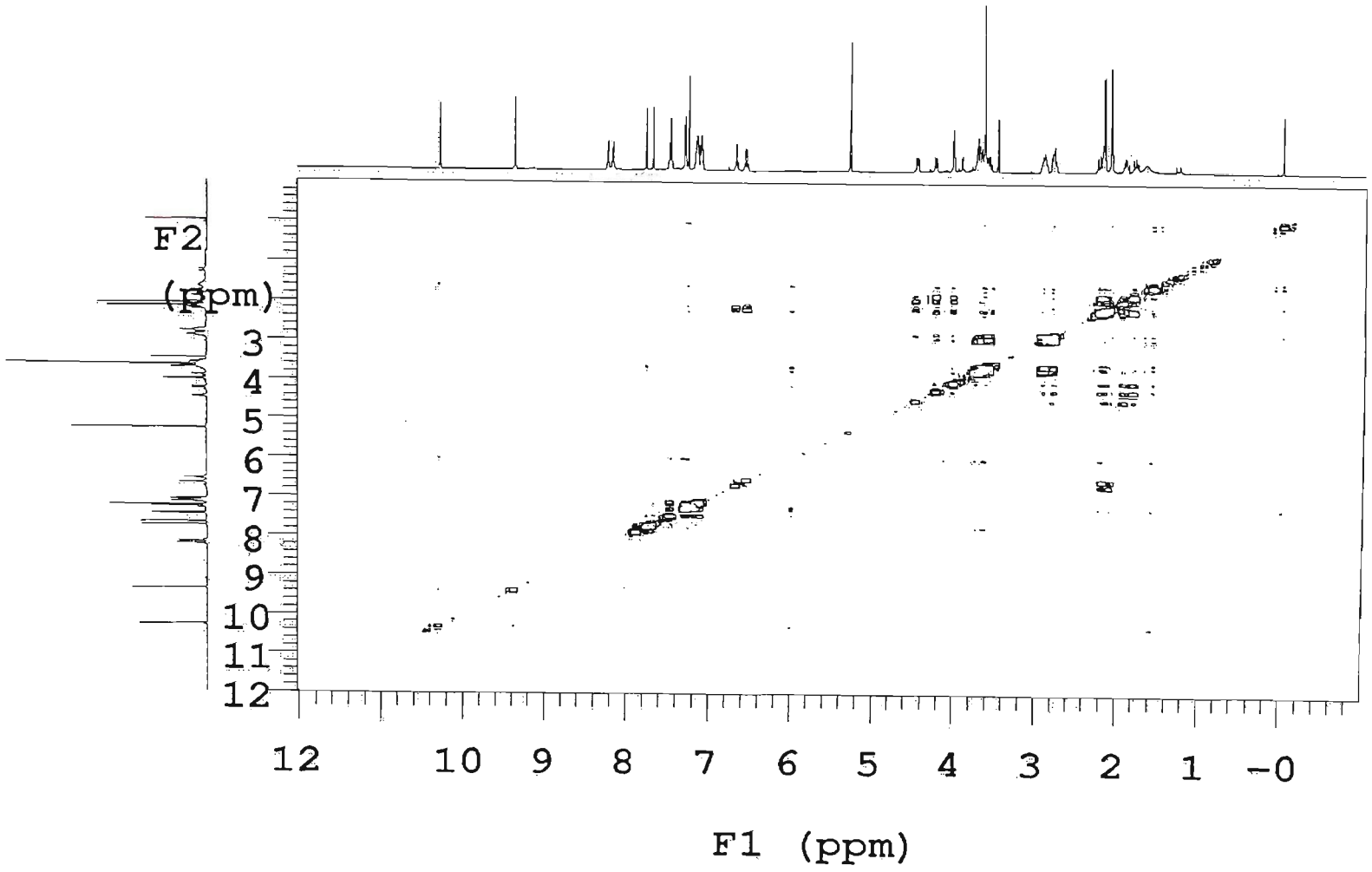
Appendix 20a. Selected 1H-NMR spectrum of compound LPM-186 **129**, showing presence of two N-Me ($CDCl_3$, 500 MHz, TMS as reference)



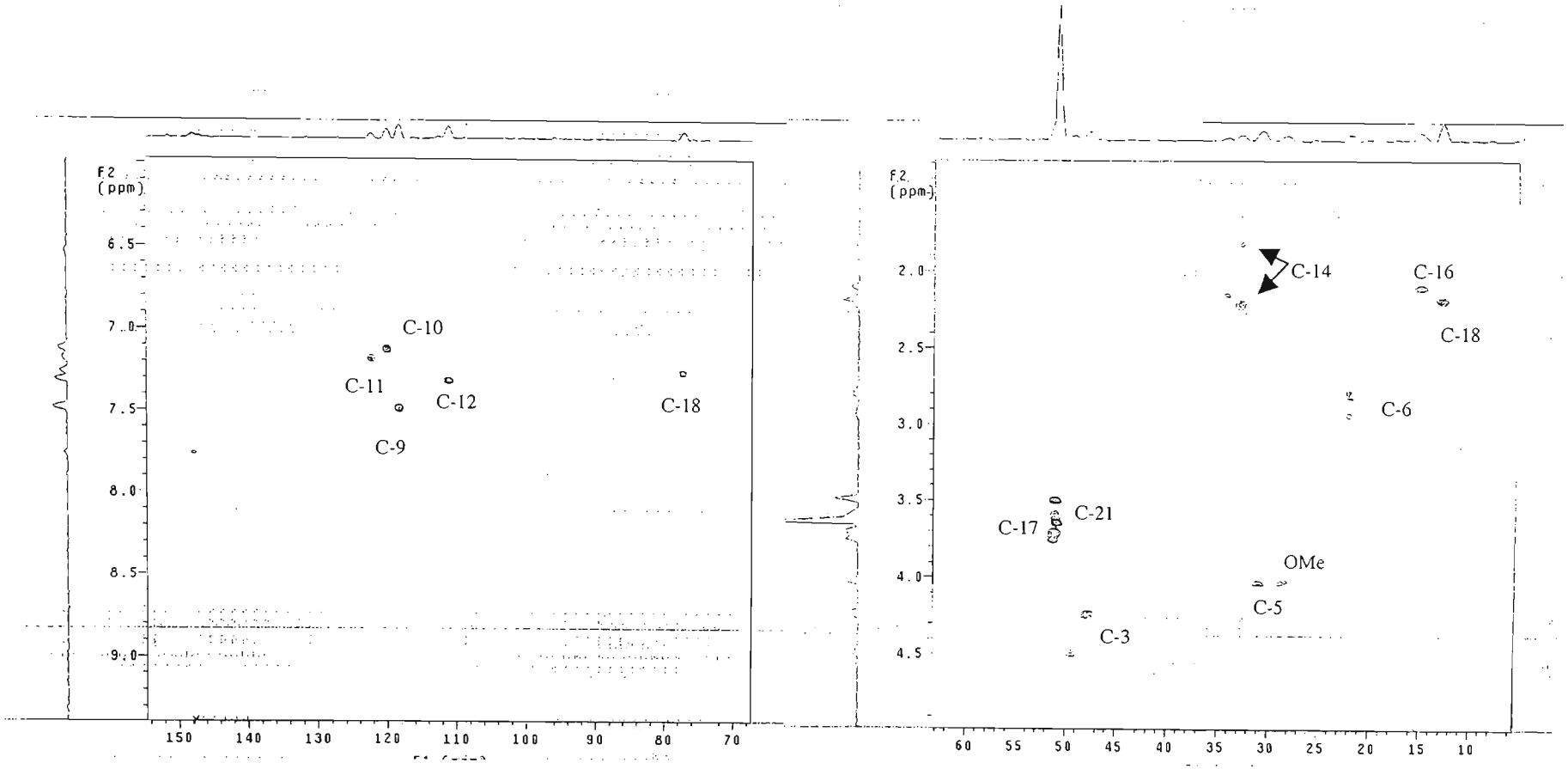
Appendix 21. LREIMS spectrum of Mataranine A **188** and B **189** (mixture)



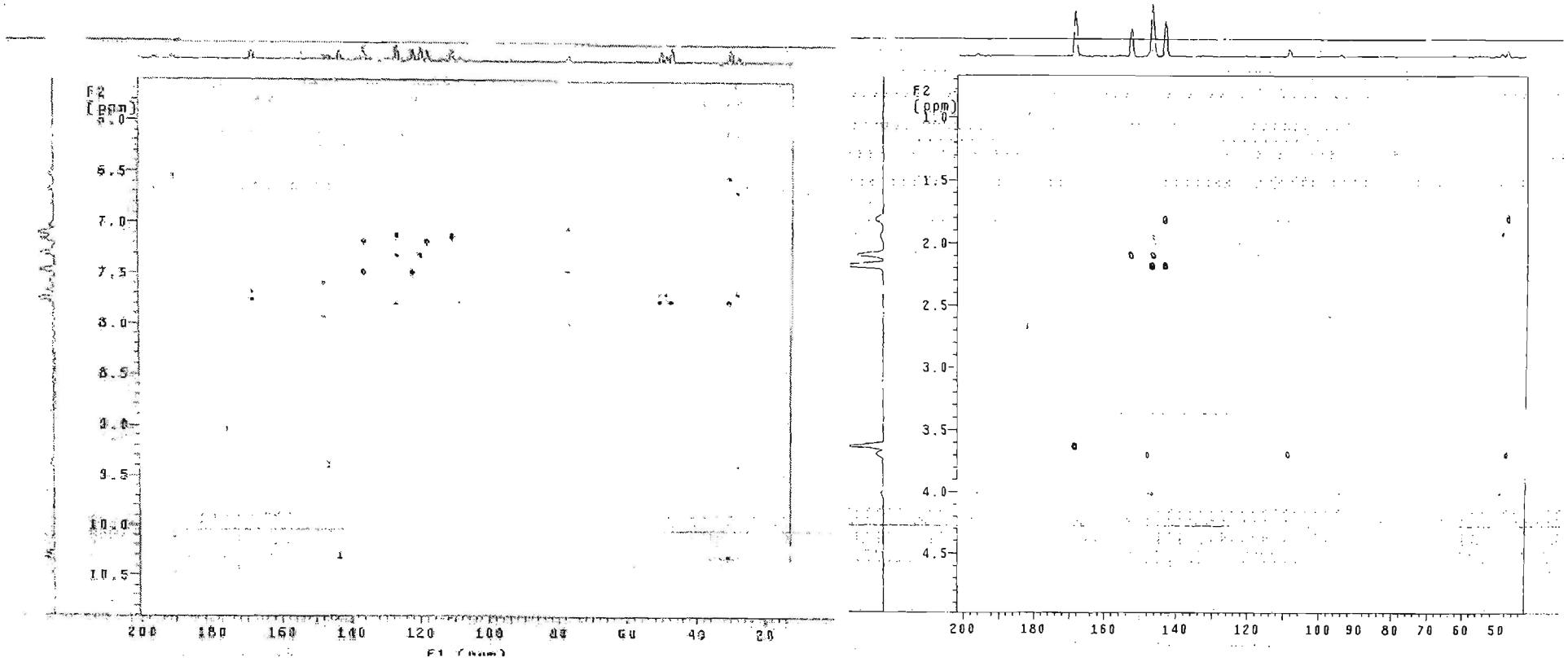
Appendix 22. ^{1}H-NMR spectrum of Mataranine A **188** and B **189** (mixture, assign for **188**, $CDCl_3$, 500 MHz, TMS as reference)



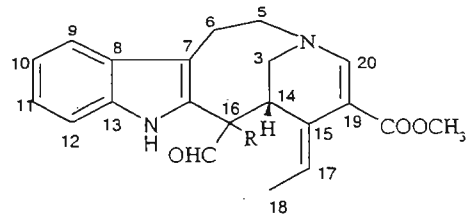
Appendix 23. gCOSY spectrum of Mataranine A **188** and B **189** (mixture, $CDCl_3$, 500 MHz, TMS as reference)



Appendix 24. gHSQC spectrum of Mataranine A **188** and B **189** (mixture, $CDCl_3$, 500 MHz, TMS as reference)

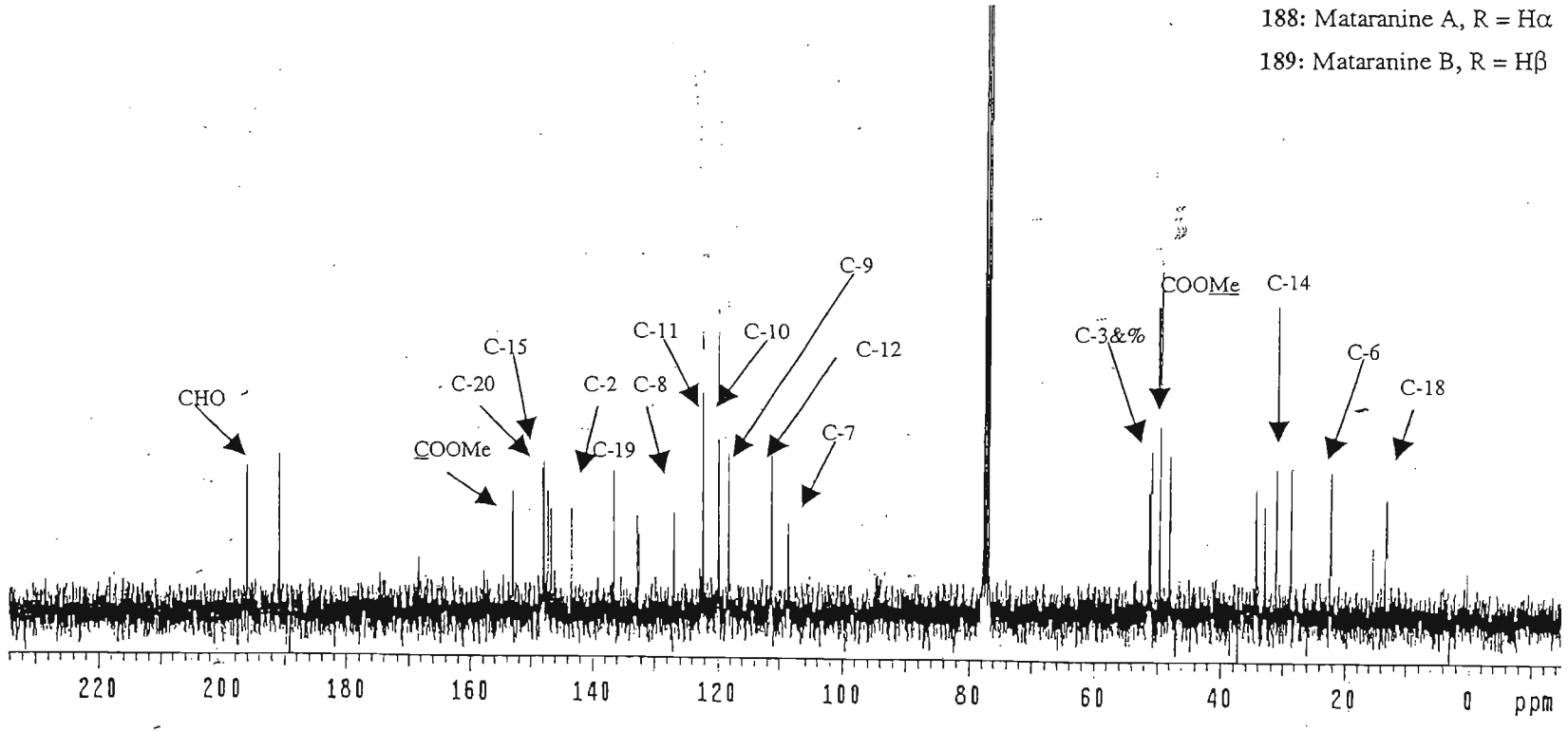


Appendix 25. gHMBC spectrum of Mataranine A **188** and B **189** (mixture, $CDCl_3$, 500 MHz, TMS as reference)

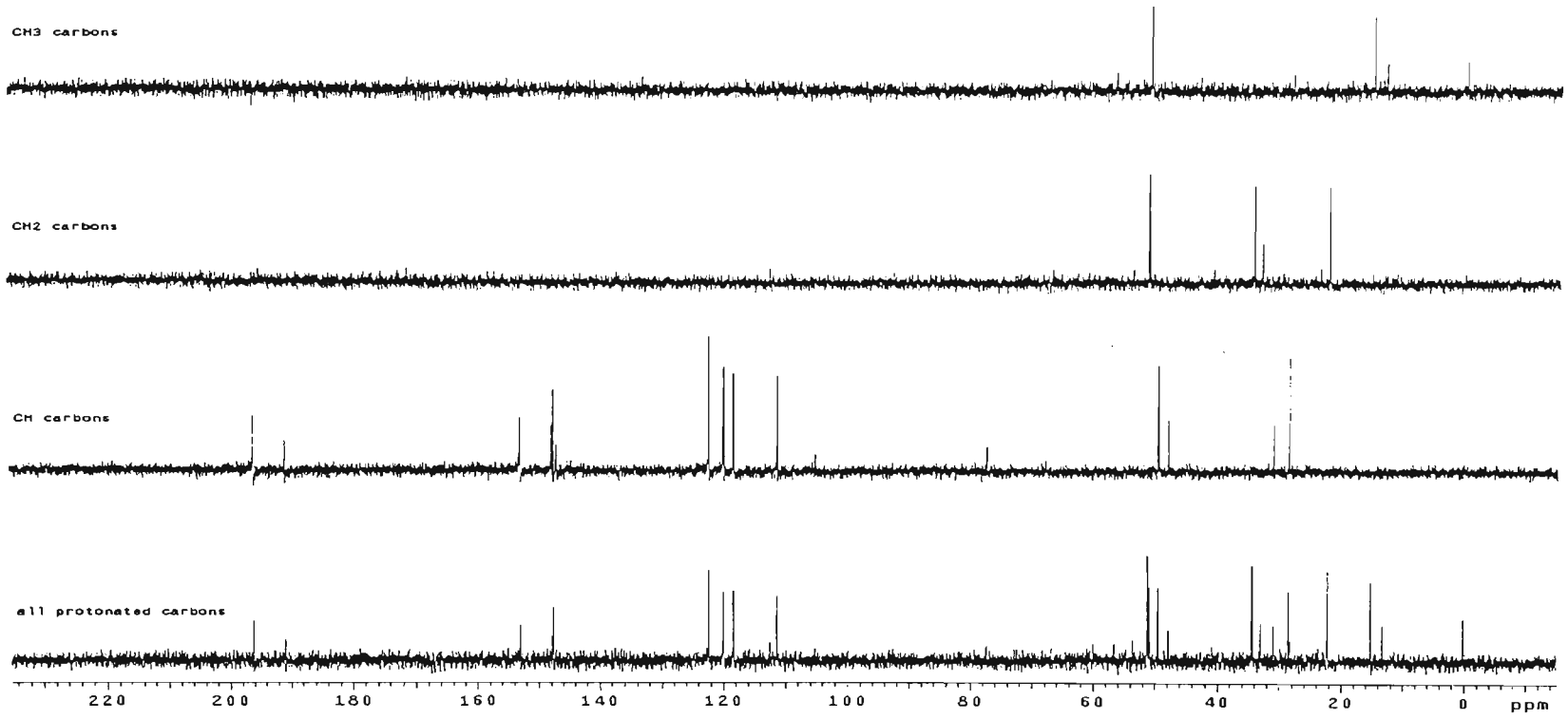


188: Mataranine A, R = Hα

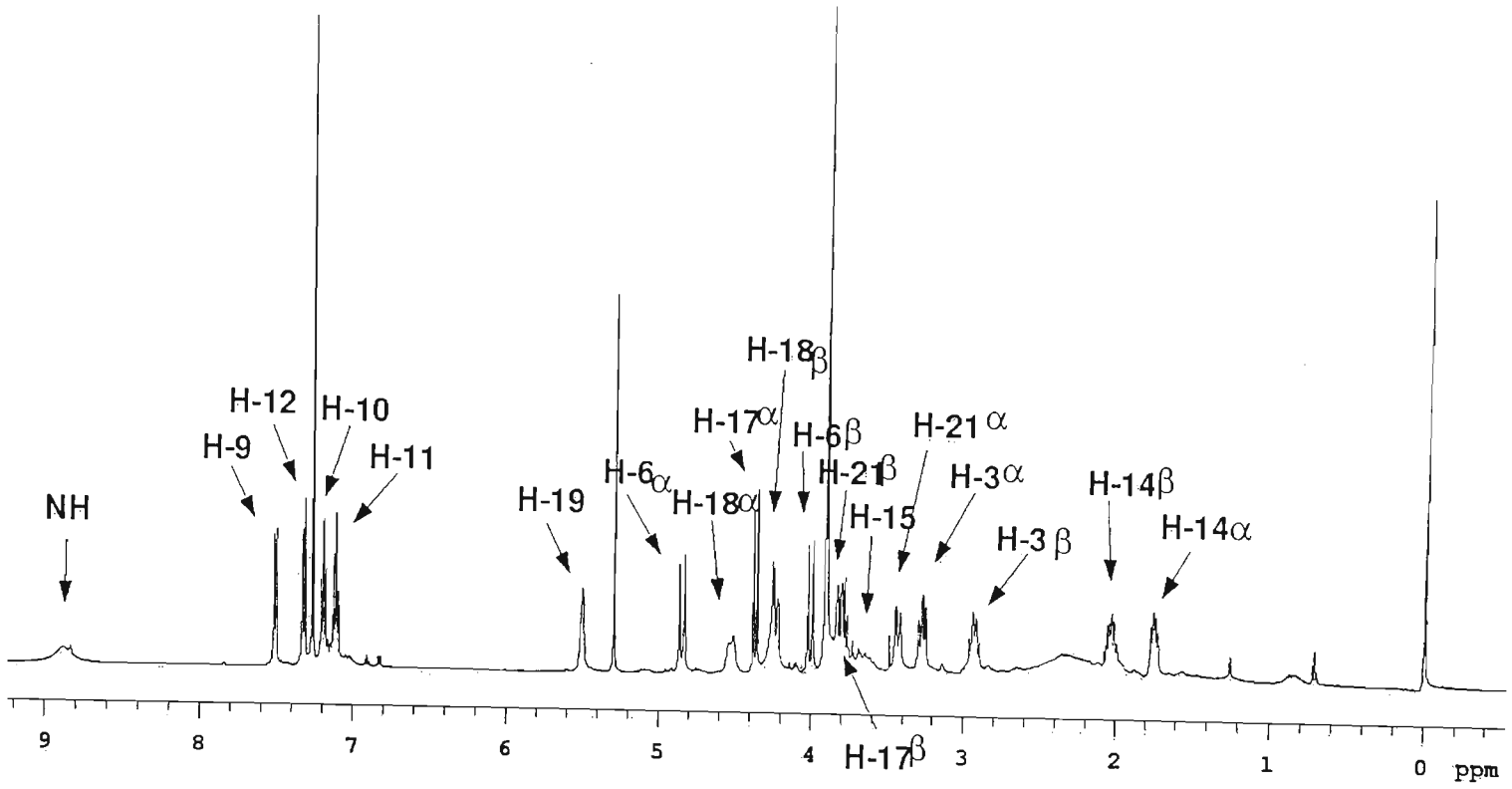
189: Mataranine B, R = Hβ



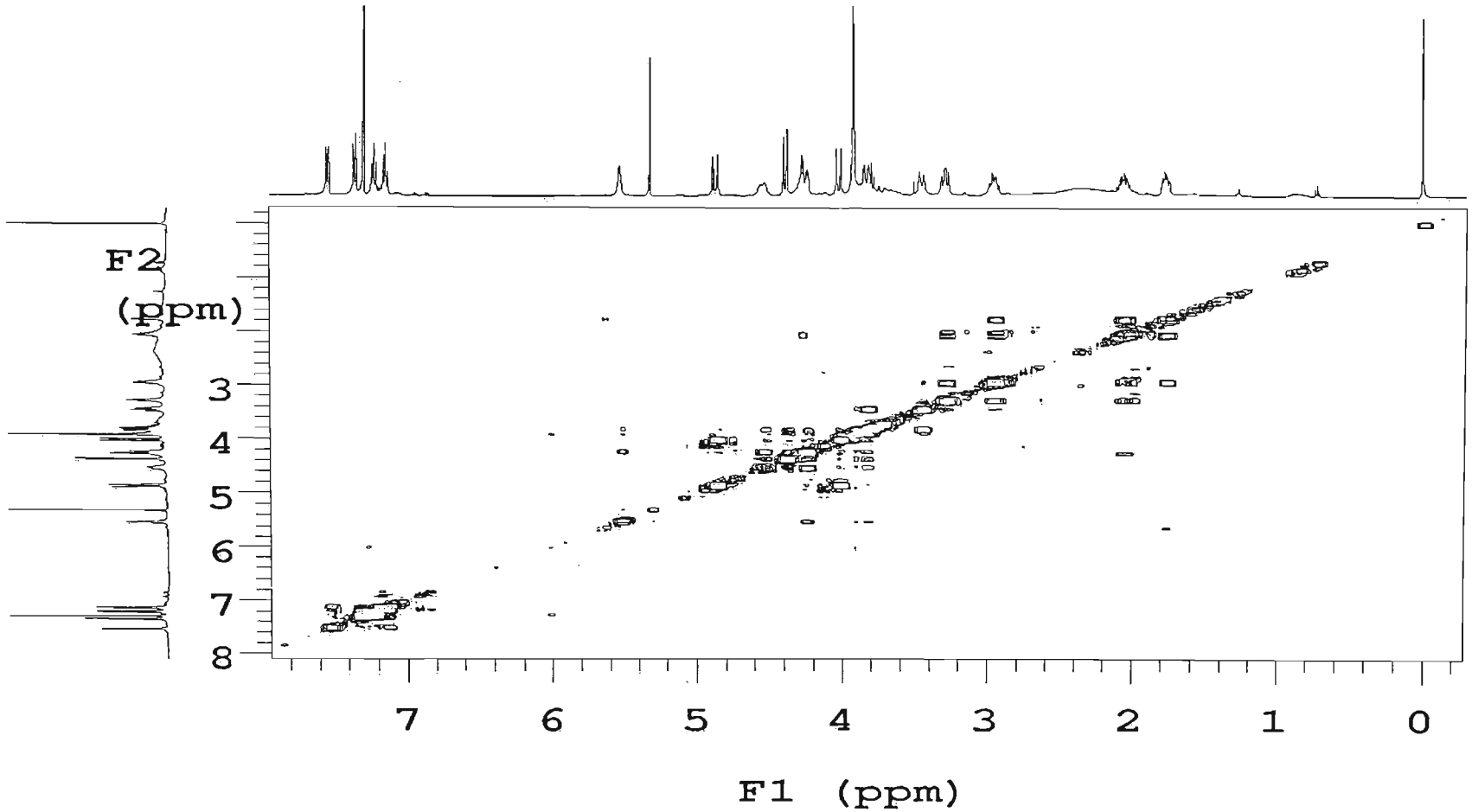
Appendix 26. ^{13}C-NMR spectrum of Mataranine A **188** and B **189** (mixture, $CDCl_3$, 500 MHz, TMS as reference, assigned for **188**)



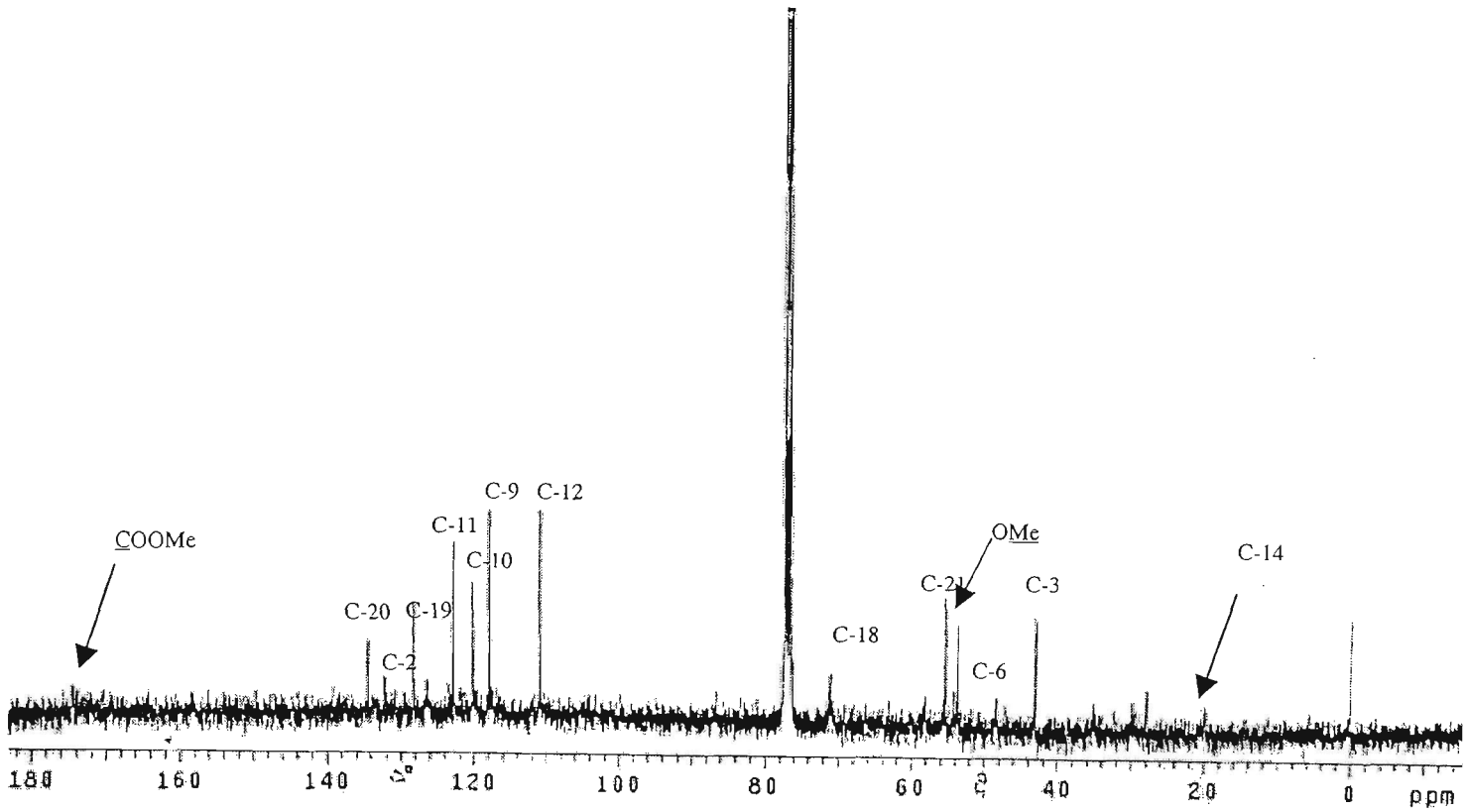
Appendix 27. DEPT spectrum of Mataranine A **188** and B **189** (mixture, $CDCl_3$, 500 MHz, TMS as reference)



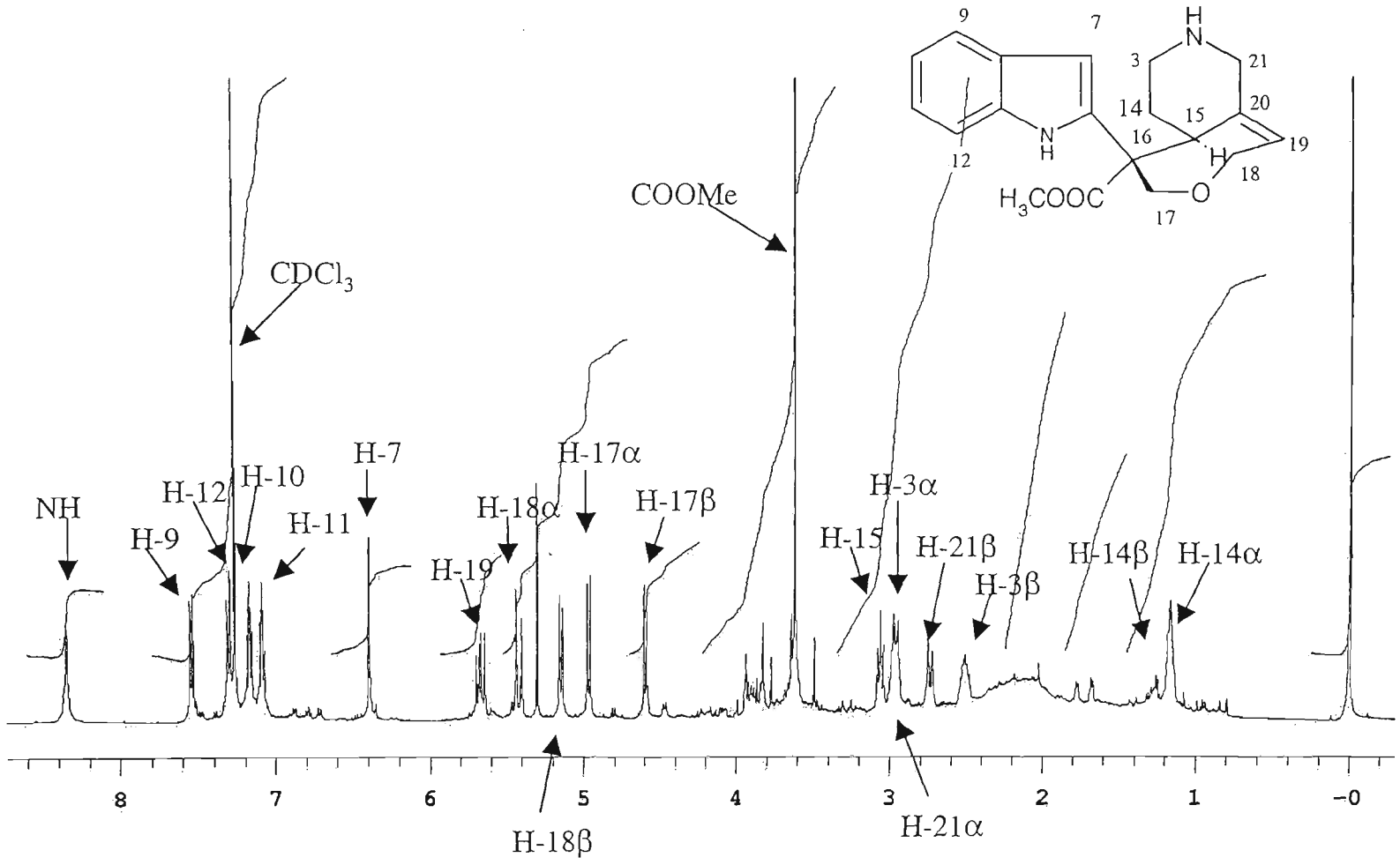
Appendix 28. ^{1}H-NMR spectrum of alstonamine **190** ($CDCl_3$, 500 MHz, TMS as reference)



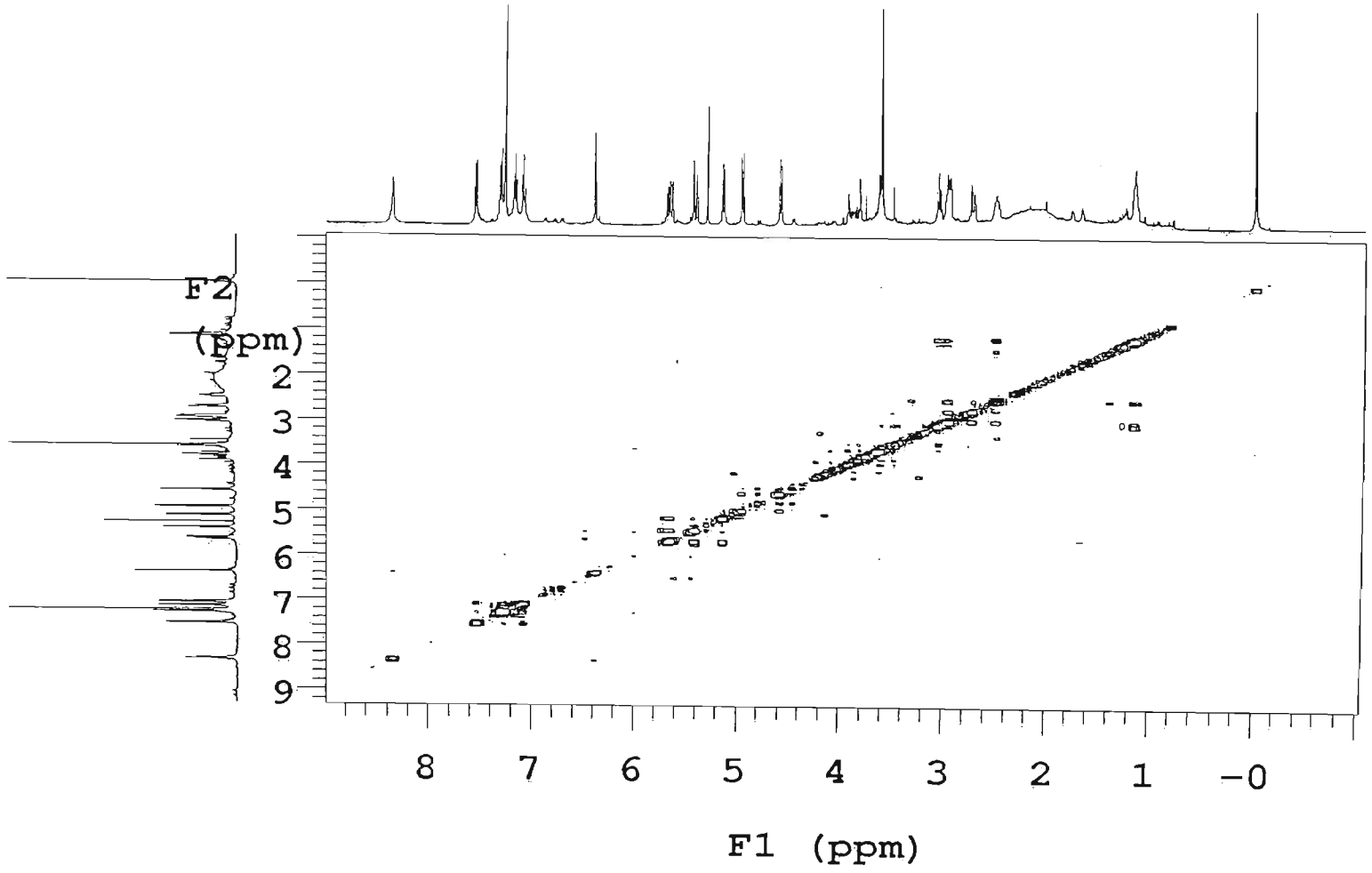
Appendix 29. gCOSY spectrum of alstonamine **190** ($CDCl_3$, 500 MHz, TMS as reference)



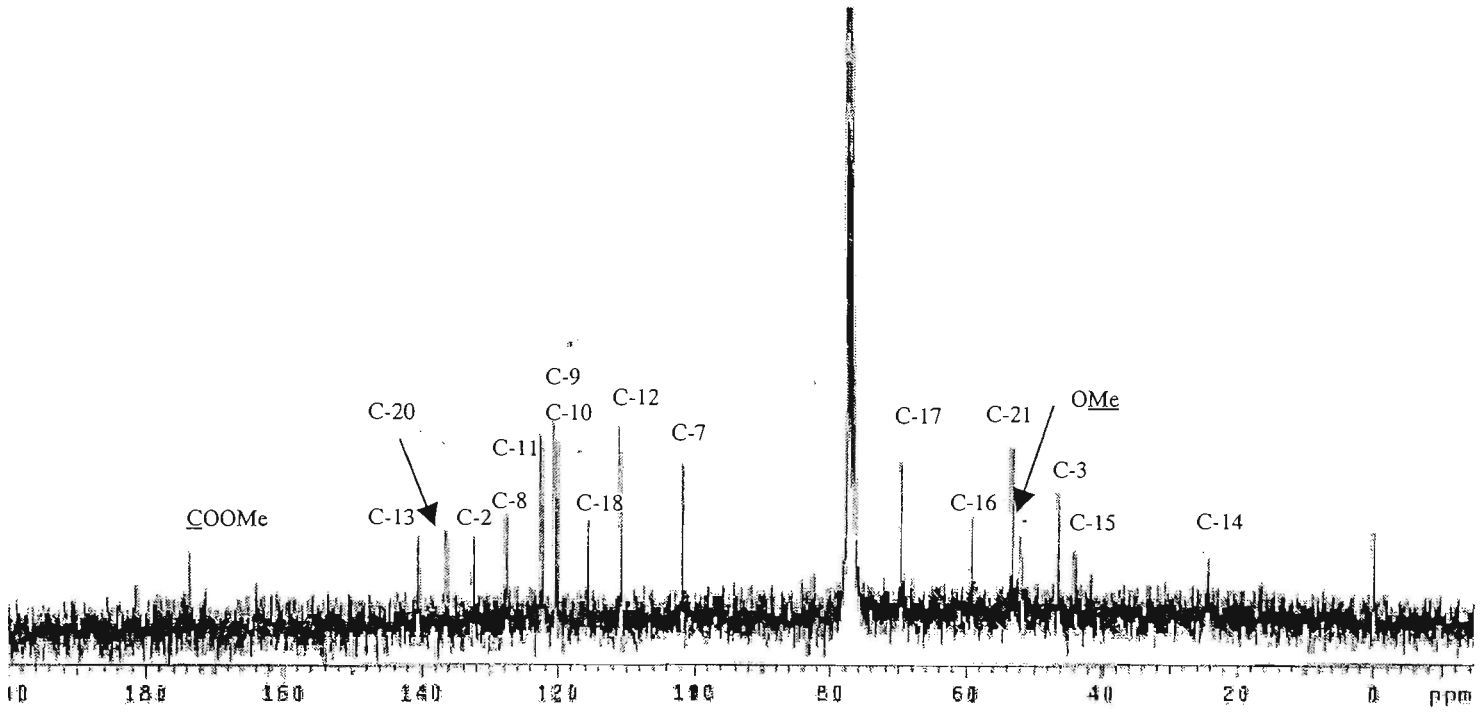
Appendix 30. ^{13}C-NMR spectrum of alstonamine **190** ($CDCl_3$, 75 MHz, TMS as reference)



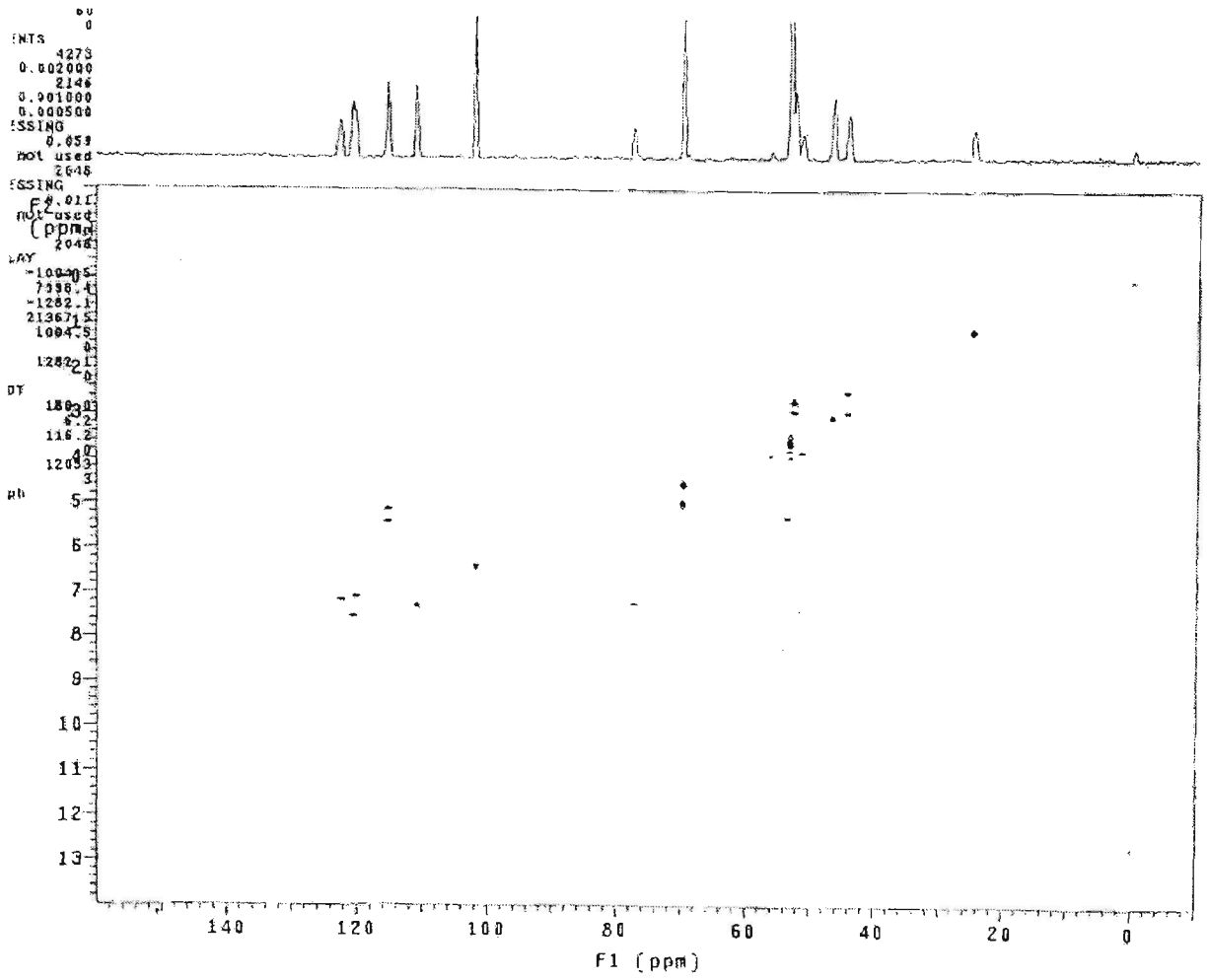
Appendix 31. ^{1}H-NMR spectrum of (*15S, 16S*)-losbanine ($CDCl_3$, 500 MHz, TMS as reference)



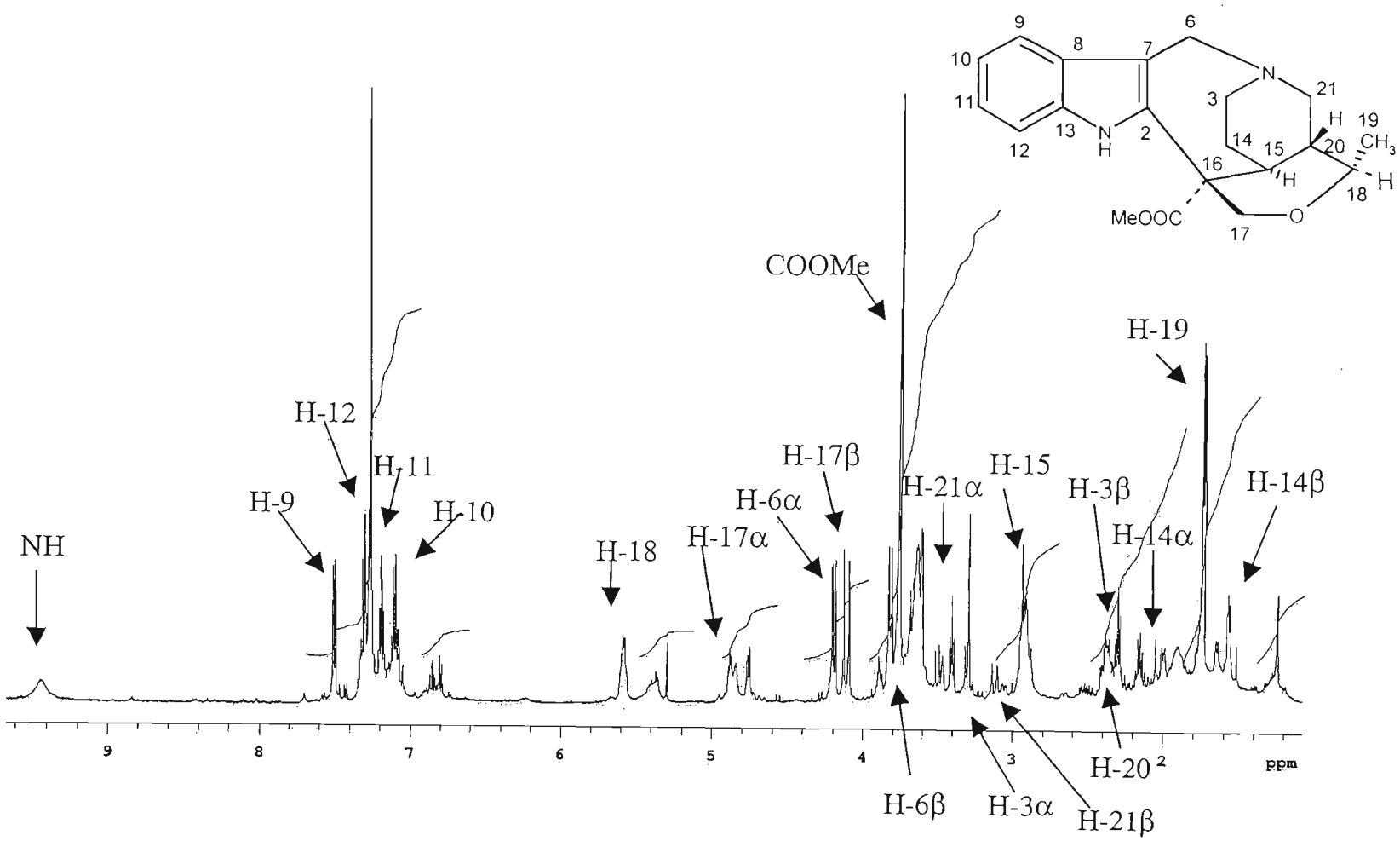
Appendix 32. gCOSY spectrum of (*15S, 16S*)-losbanine ($CDCl_3$, 500 MHz, TMS as reference)



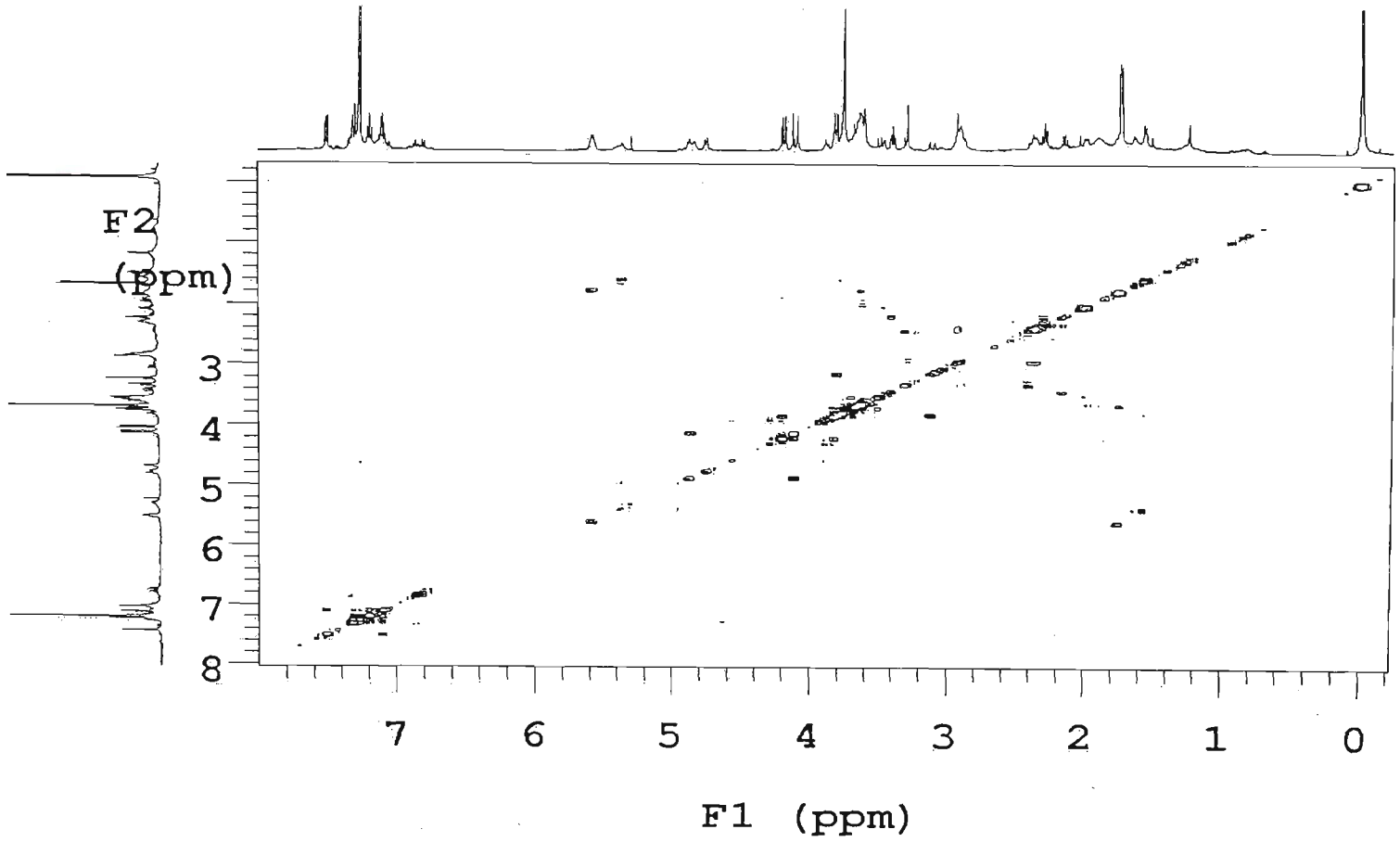
Appendix 33. ^{13}C-NMR spectrum of (*15S**, *16S**)-losbanine ($CDCl_3$, 75 MHz, TMS as reference)



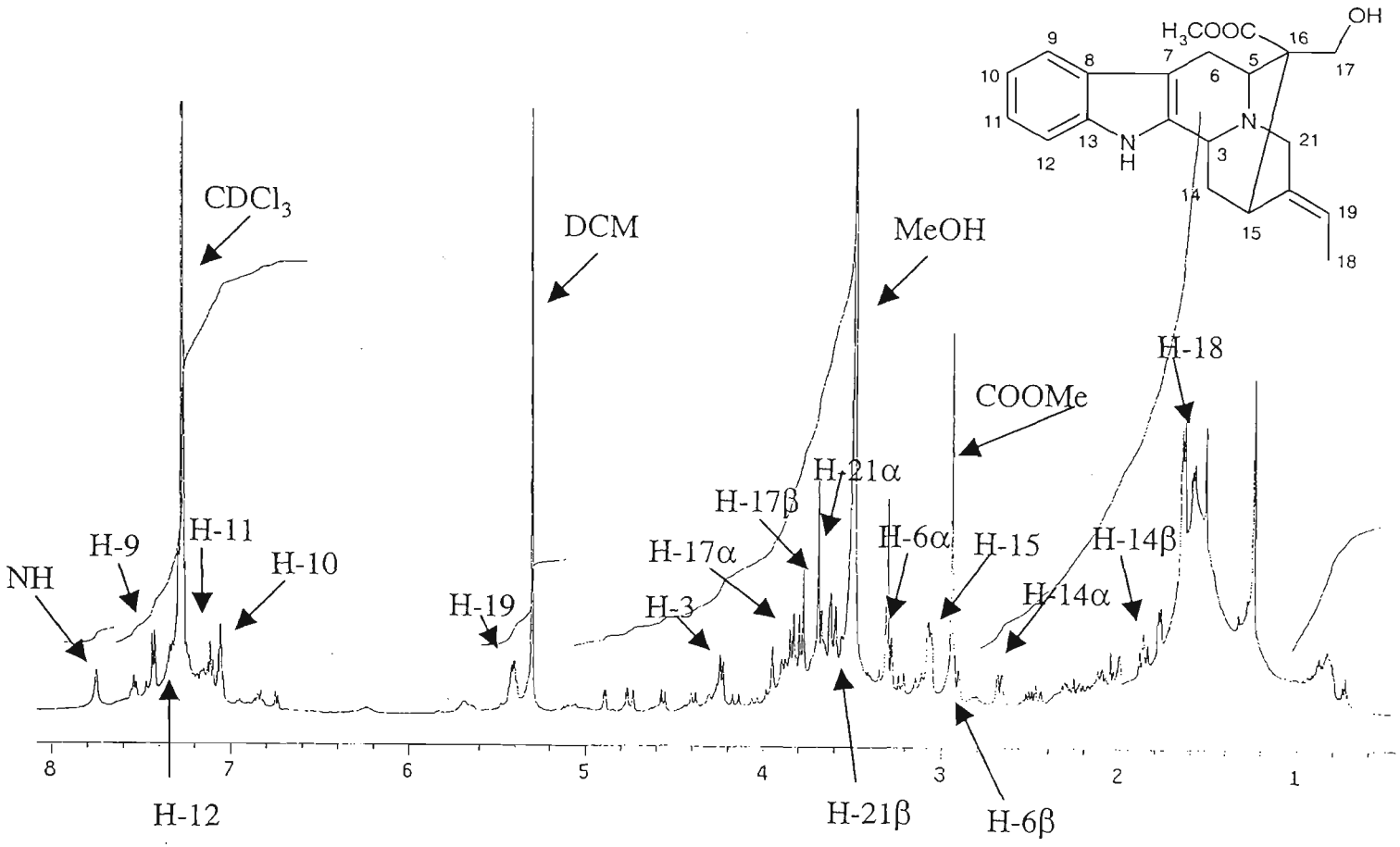
Appendix 34. gHSQC spectrum of (*15S*, 16S**)-losbanine ($CDCl_3$, 500 MHz, TMS as reference)



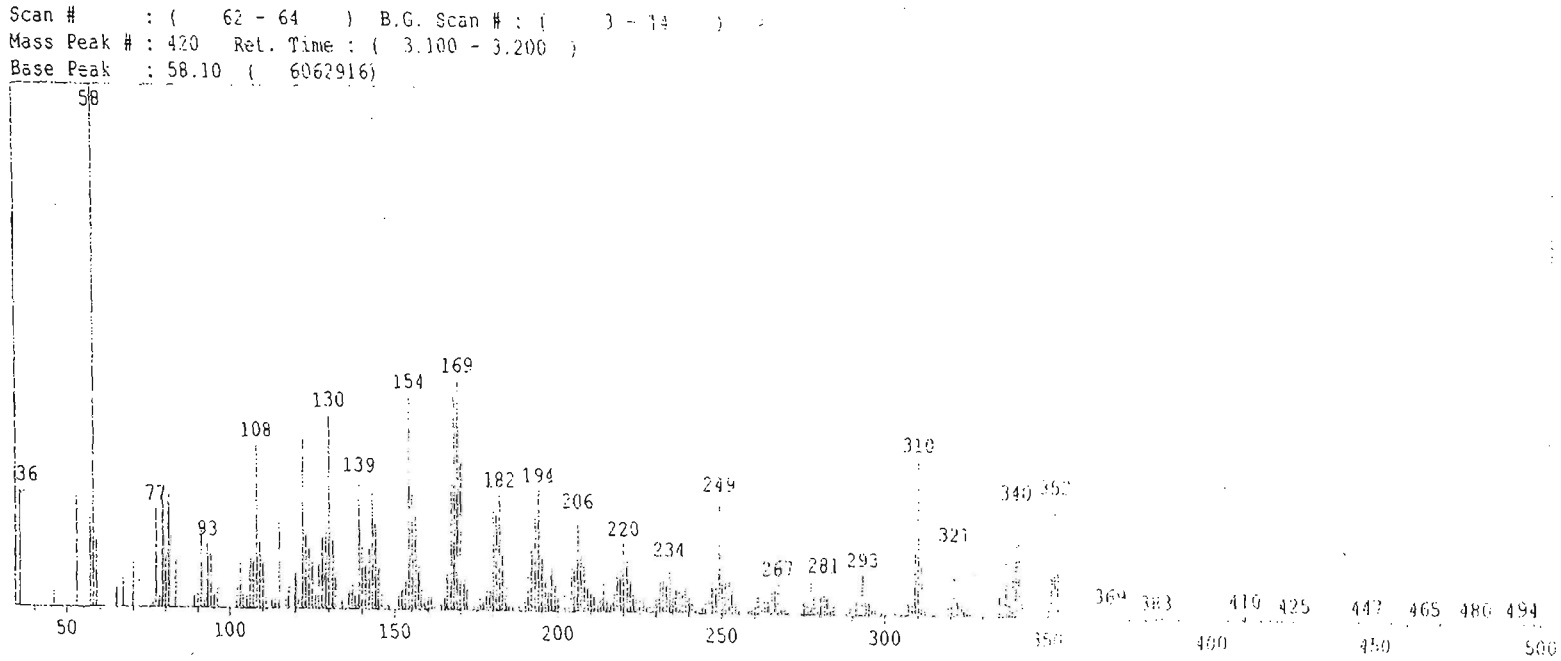
Appendix 35. ^{1}H-NMR spectrum of kotarajine **194** ($CDCl_3$, 500 MHz, TMS as reference)



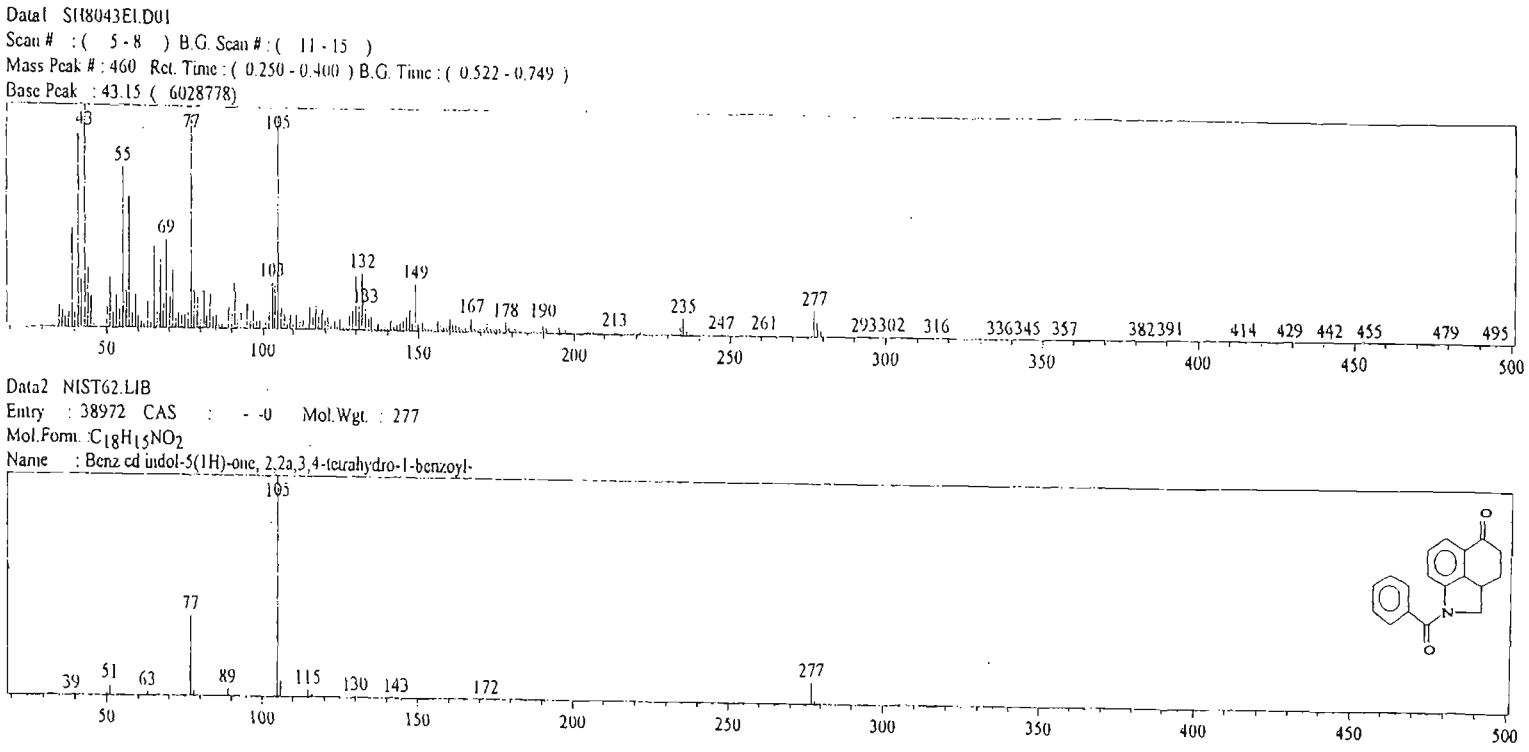
Appendix 36. gCOSY spectrum of kotarajine **194** ($CDCl_3$, 500 MHz, TMS as reference)



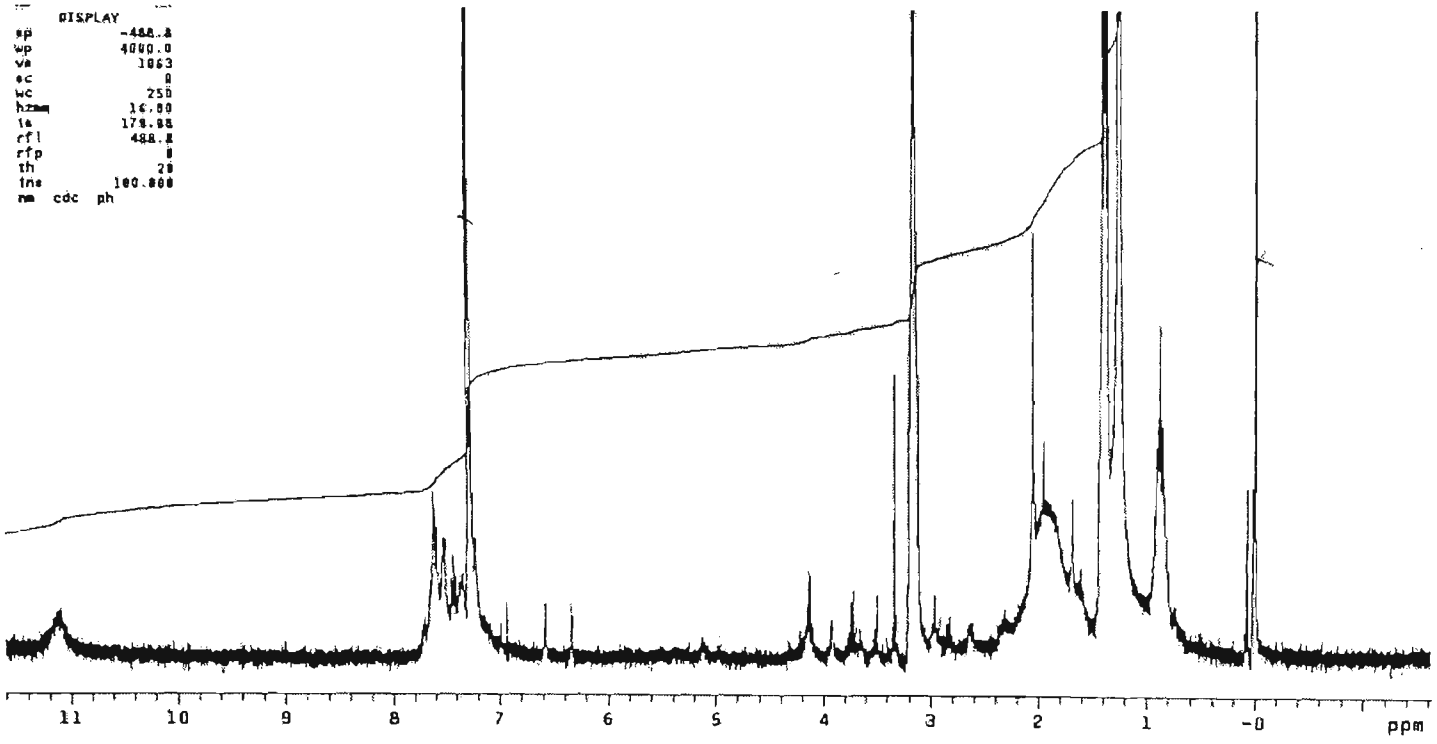
Appendix 37. ^{1}H-NMR spectrum of *E*-akuammidine **195** ($CDCl_3$, 500 MHz, TMS as reference)



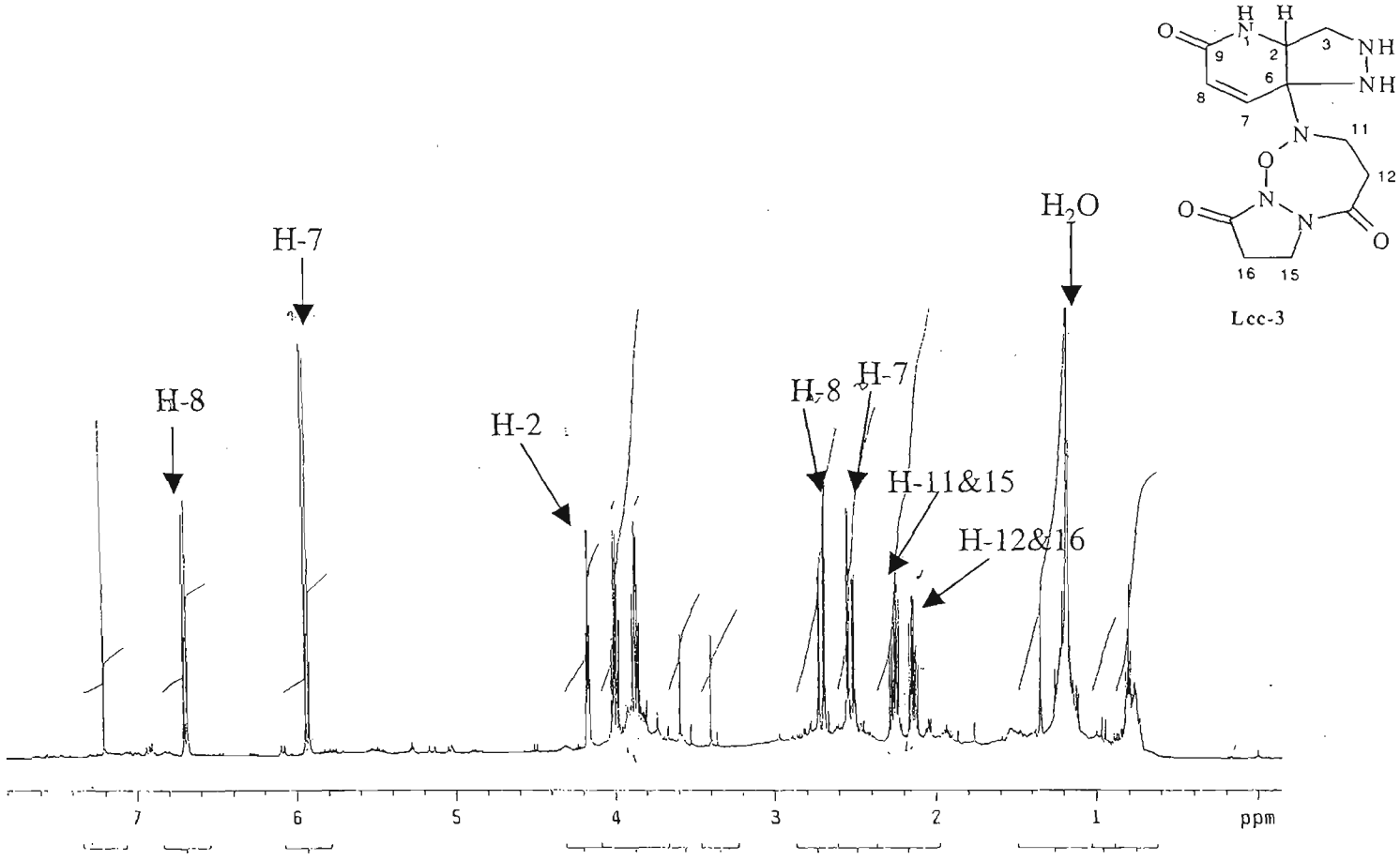
Appendix 38. LREIMS spectrum of *E*-akuammidine **195**



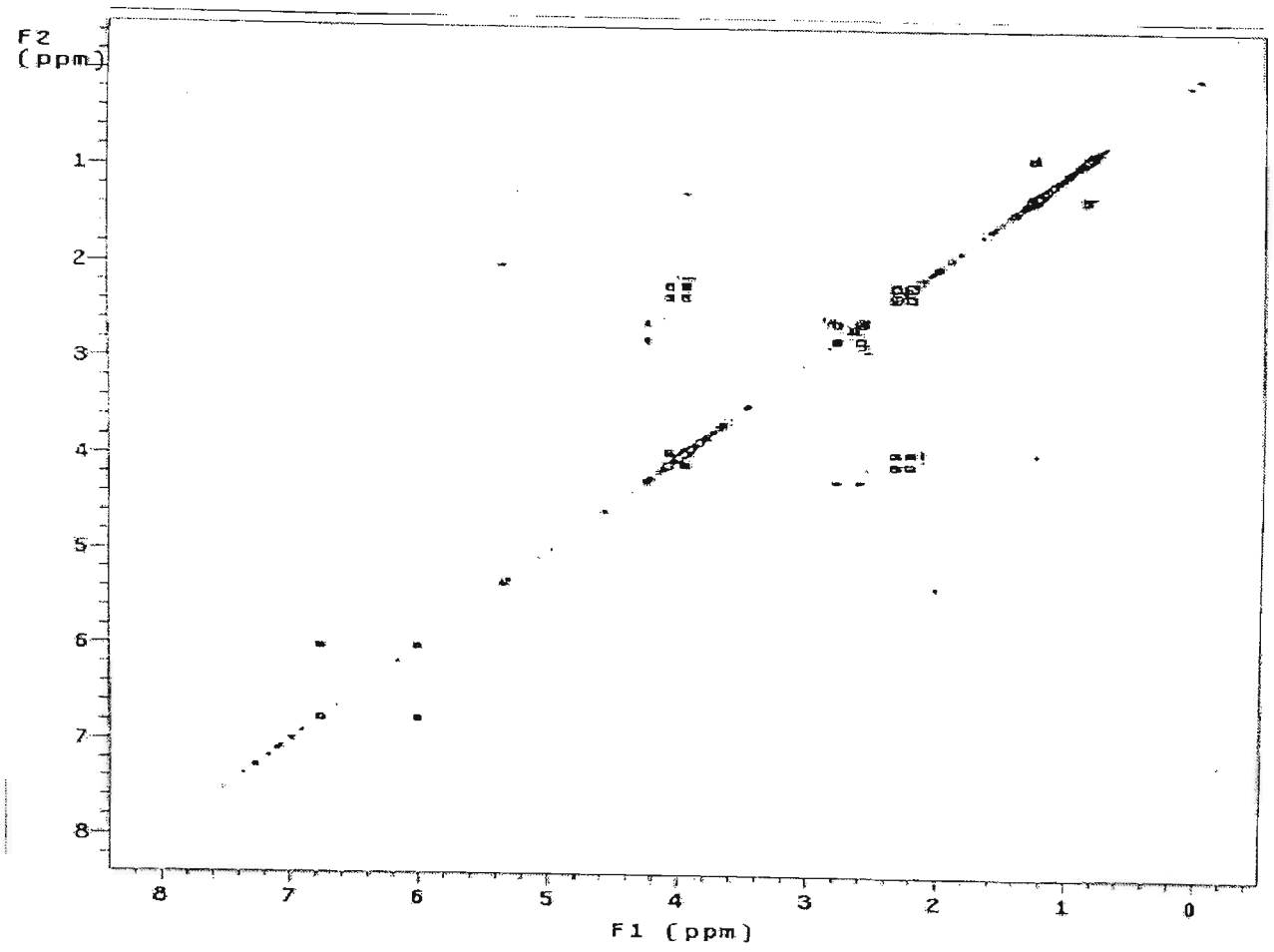
Appendix 39. LREIMS spectra of compounds **216** (top) and compound **215** (below)



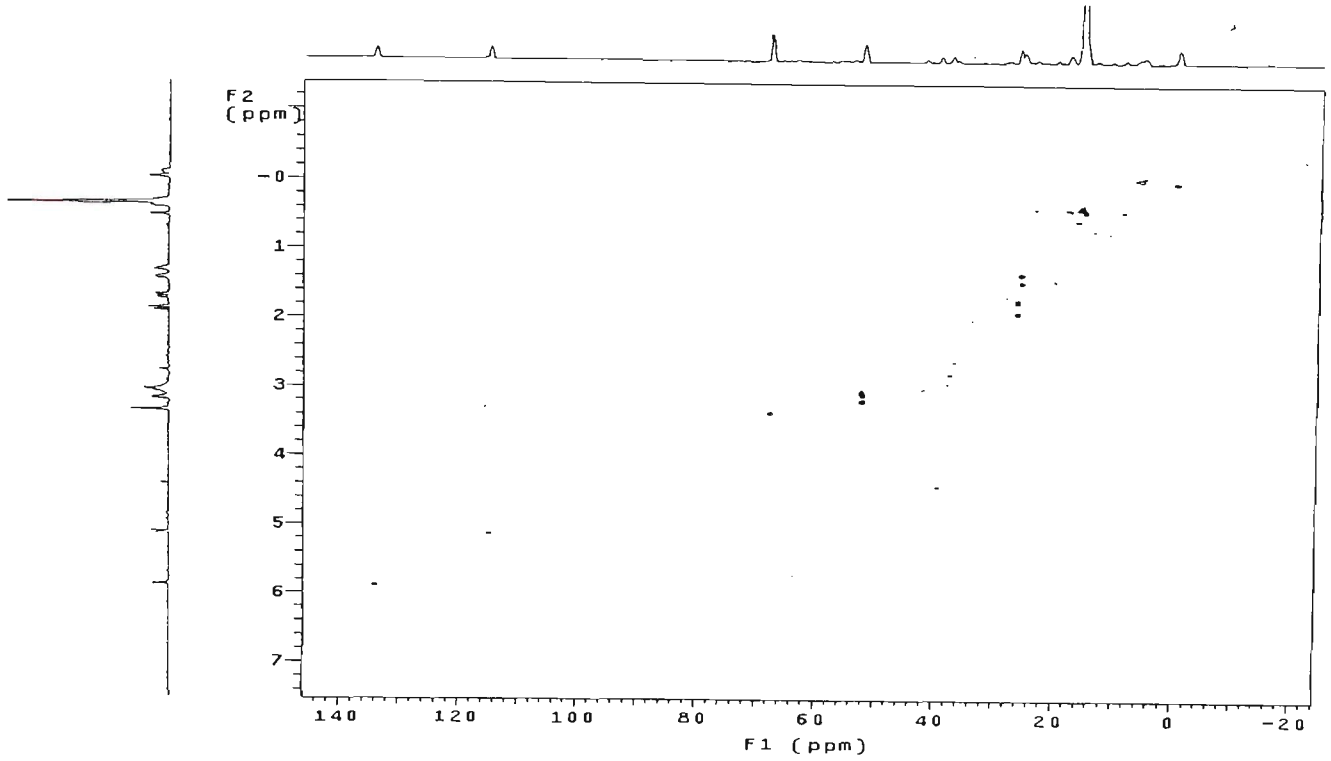
Appendix 40. 1H-NMR spectrum of Lcc-4 ($CDCl_3$, 500 MHz, TMS as reference)



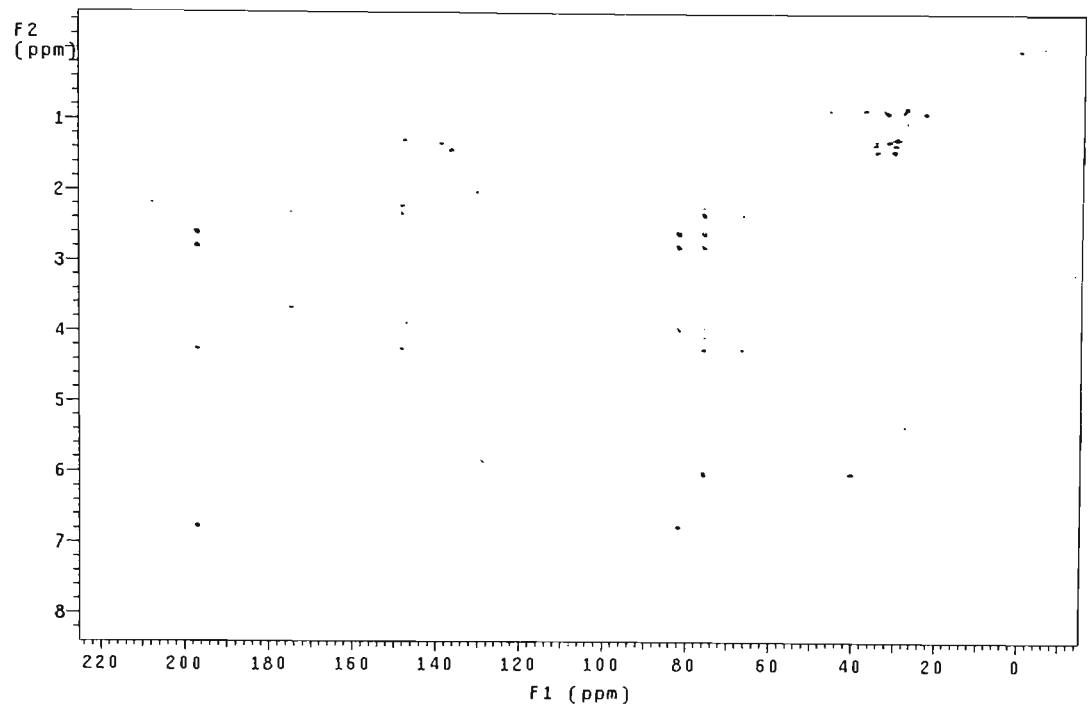
Appendix 41. ^{1}H-NMR spectrum of Lcc-3 **218** (nanoprobe, $CDCl_3$, 500MHz)



Appendix 42. gCOSY spectrum of Lcc-3 **218** (nanoprobe, $CDCl_3$, 500MHz)



Appendix 43. gHSQC spectrum of Lcc-3 **218** (nanoprobe, $CDCl_3$, 500 MHz, TMS as reference)

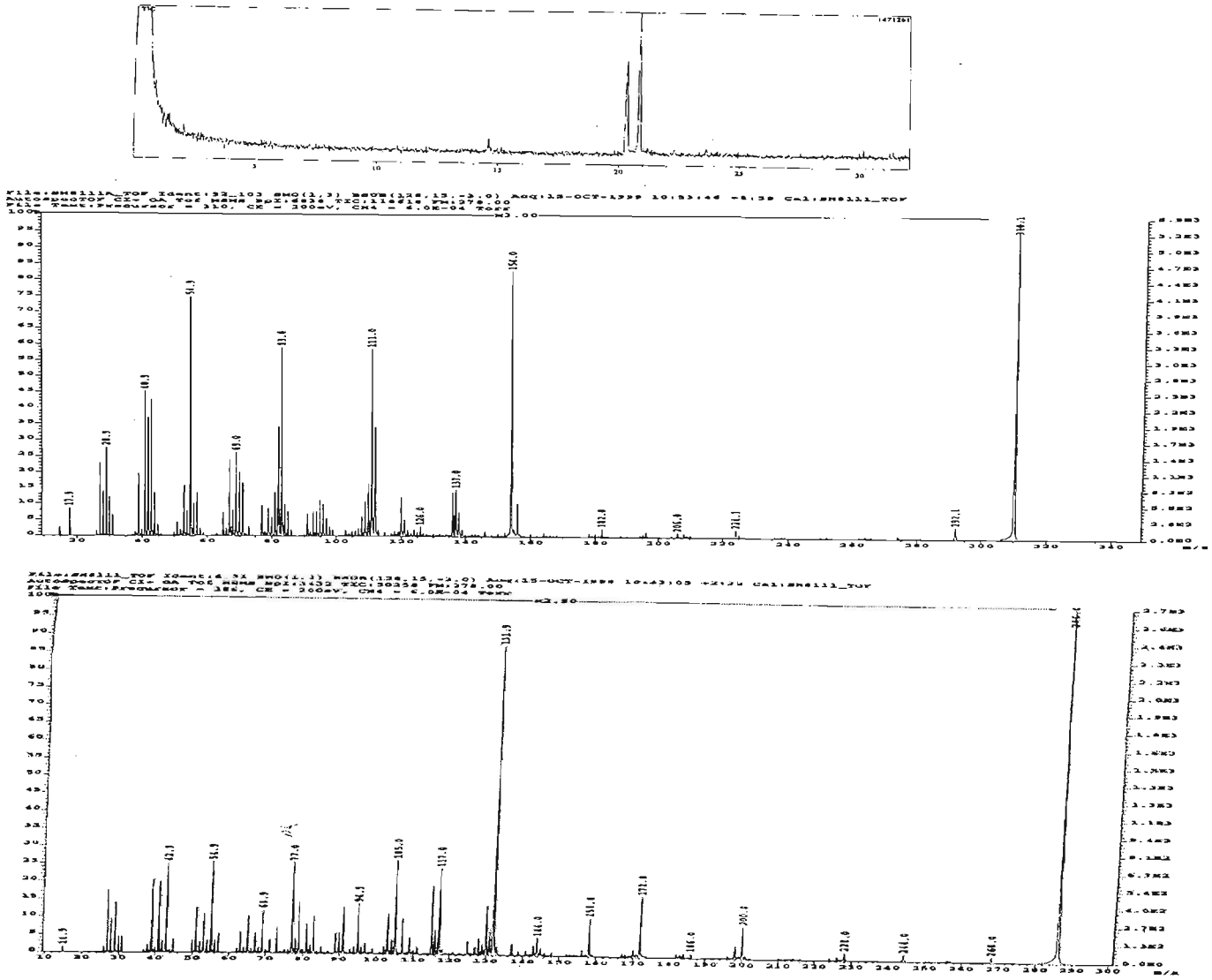


Appendix 44. gHMBC spectrum of Lcc-3 **218** (nanoprobe, $CDCl_3$, 500MHz, TMS as reference)

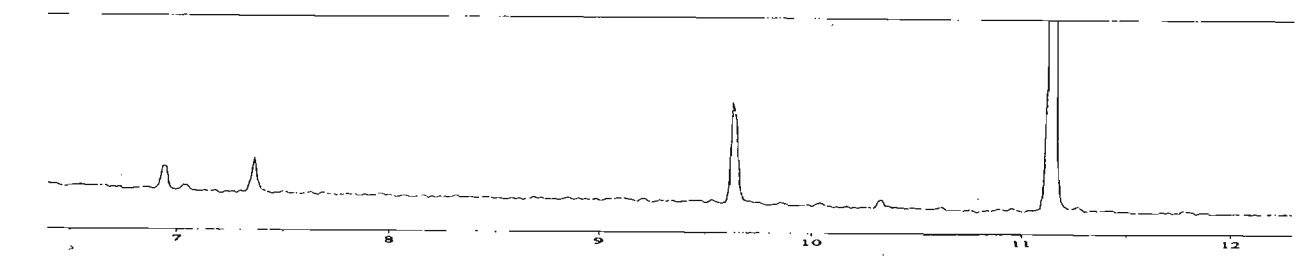
(1)

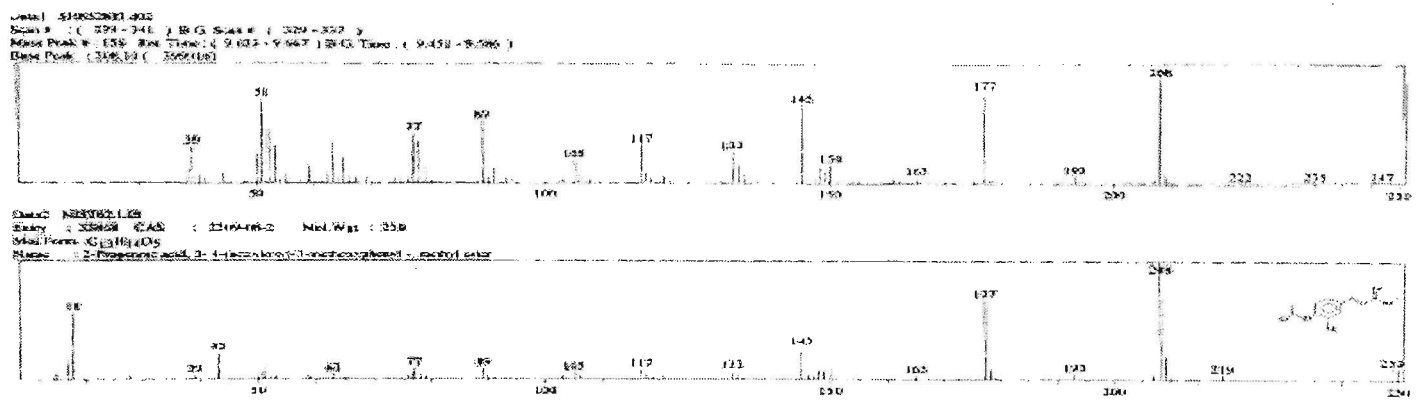
(2)

(3)

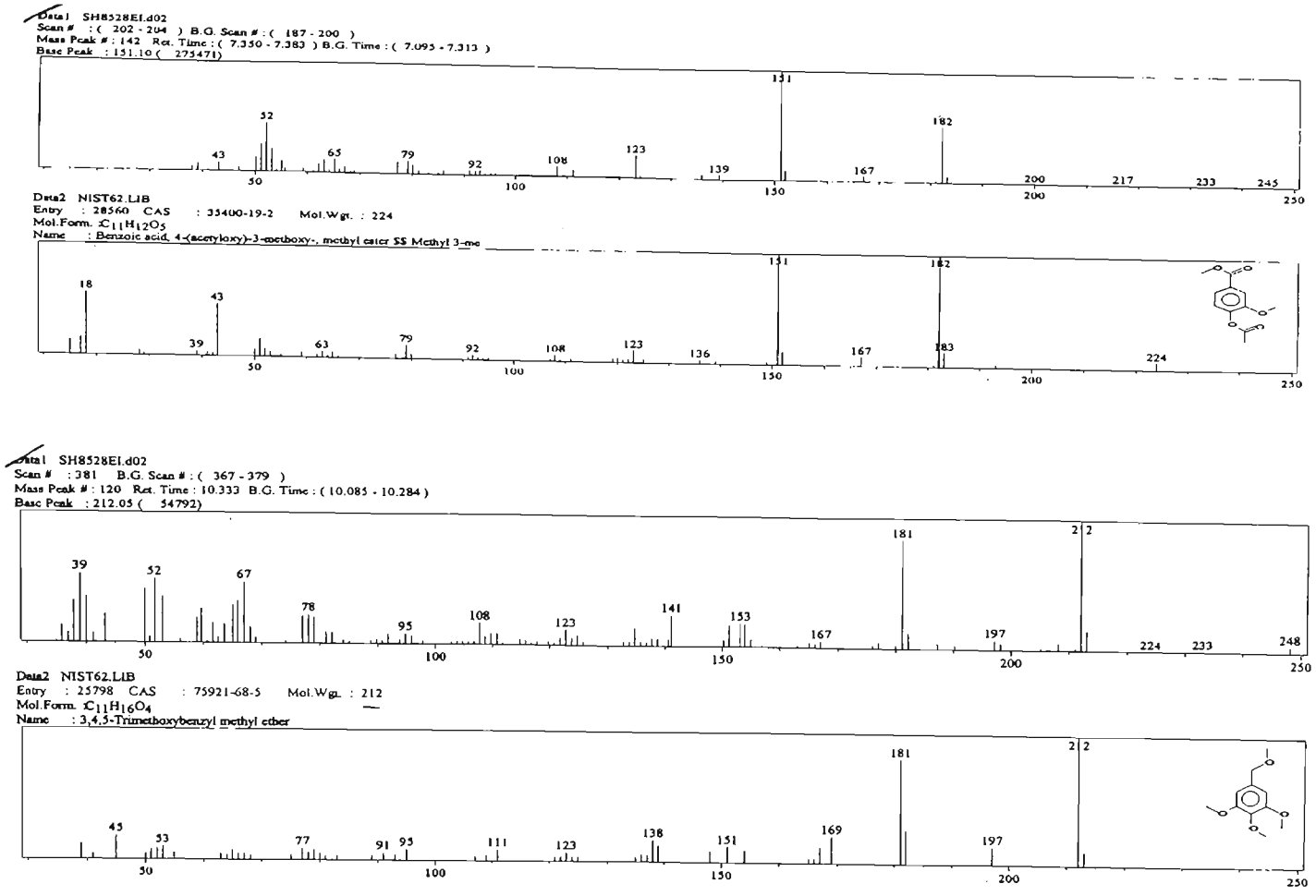


Appendix 45. Chromatogram of fraction Lcc-2 from *Clerodendron calamitosum* L. and CIMS/MS spectra of the two uncharacteristic compounds (1) and (2)

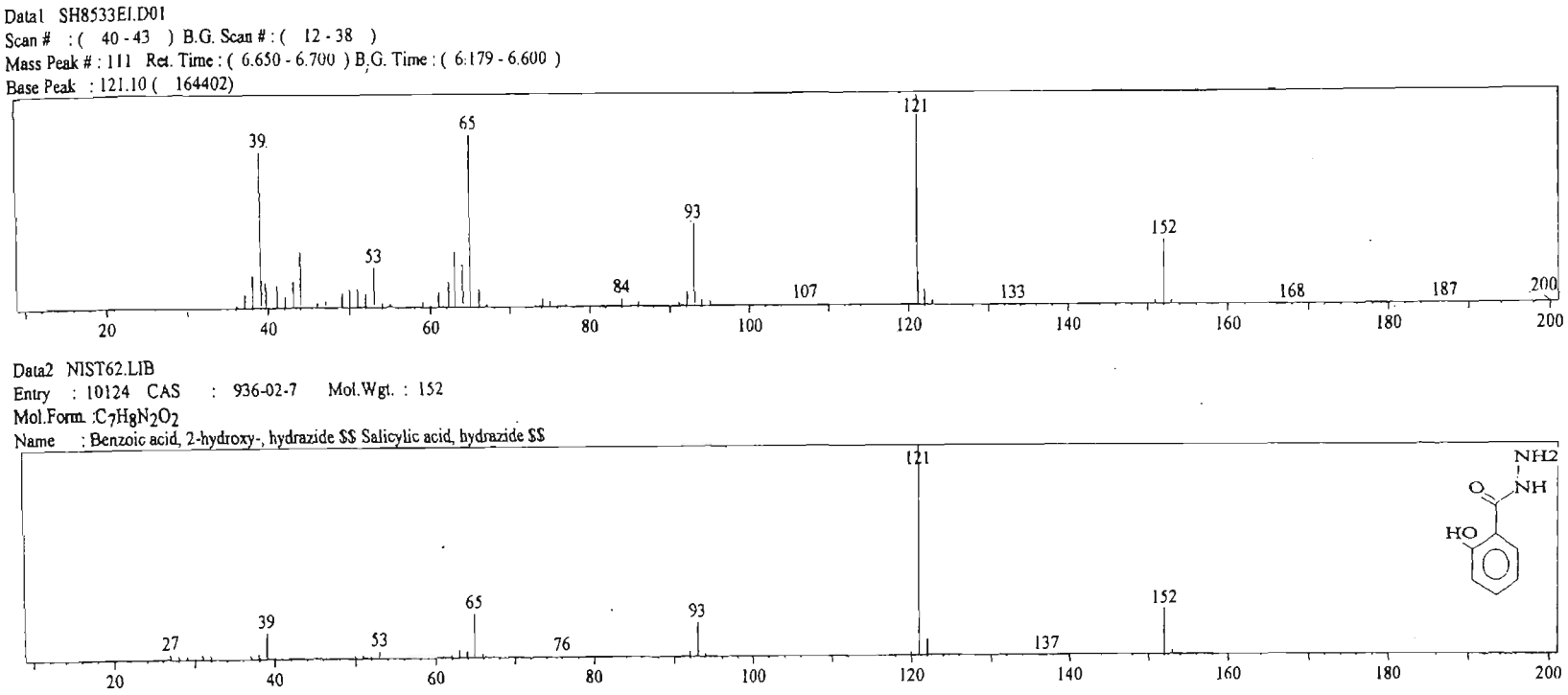




Appendix 46. Chromatogram of fraction 3 from crude extract of alkaloid isolated from *Clerodendron paniculatum* L. (above) and LREIMS sepctrum of compound **219** (below)



Appendix 47. LREIMS sepctrum of compound **220** (above) and compound **221** (below)



Appendix 48. LREIMS spectrum of compound **222**

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molecules

ISSN 1420-3049

http://www.mdpi.org

Initial Studies on Alkaloids from Lombok Medicinal Plants

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Received: 12 May 2000 / Accepted: 1 October 2000 / Published: 16 January 2001

Abstract: Initial investigation of medicinal plants from Lombok has resulted in the collection of 100 plant species predicted to have antimicrobial, including antimalarial, properties according to local medicinal uses. These plants represent 49 families and 80 genera; 23% of the plants tested positively for alkaloids. Among the plants testing positive, five have been selected for further investigation involving structure elucidation and antimicrobial testing on the extracted alkaloids. Initial work on structural elucidation of some of the alkaloids is reported briefly.

Keywords: Alkaloids, Medicinal Plants, Lombok

Introduction

There is an urgent need to discover new antimicrobial agents for human and veterinary therapeutic uses, as resistance to current drugs increases in severity and extent. For instance, many *Plasmodium falciparum* strains, the parasite responsible for many fatalities from malaria, have become resistant to chloroquine [1]. This phenomenon has also occurred with some important pathogenic bacteria [2]. As a result, more than two billion people worldwide are at high risk of malarial/bacterial diseases. The identification of new and structurally novel natural products with antimicrobial activity, and hopefully new modes of action, is one of the ways of tackling this problem. While various approaches to locating such natural products have been undertaken, we are exploring a combined chemo- and bio-rational strategy based on alkaloids and medicinal plants respectively. By targeting alkaloid-containing medicinal plants, it is hoped that structural novelty with the required bioactivity will be achieved more efficiently. Alkaloids have diverse structures and many show a range of pharmacological activities

including antimicrobial activity [19]. They are also normally readily separable from the other plant metabolites as a result of their basicity.

Lombok, part of the tropical Indonesian archipelago, is a small island (4600 km^2) with 2.4 million inhabitants which administratively is divided into three regions of West, Central, and East Lombok. It is situated between Bali and Sumbawa Islands, where transition from the western to the eastern Indonesian flora and fauna begins. The northern region of the island is mountainous and is dominated by tall trees and shrubs, while the south is a drier region with savanna vegetation. As a consequence, Lombok has a rich and varied flora. Local people have a long tradition of using plants for medicinal purposes and more than 70 percent are still utilising them. Aqueous plant extracts, which could extract alkaloid salts with naturally occurring acids, are made for internal medicinal use. For topical applications, plant material is normally crushed and applied directly or crushed in the presence of water before application. However, most of the plants have not been studied chemically and pharmacologically, and there is great potential for the isolation of novel, naturally-occurring bioactive compounds.

The overall aims of this current study were to locate, isolate, identify and evaluate novel bioactive alkaloids from plants traditionally used for medicinal purposes in Lombok, Indonesia. The plants investigated were those predicted to have antimicrobial and antimalarial properties based on the local uses of the plants. This study has involved plant specimen collection, field screening for alkaloids, extraction, purification, structure elucidation, and later will include bioactivity testing. The first three steps have been carried out at the University of Mataram, Lombok and further work is continuing at the University of Wollongong. Presently, we would like to report the results of the plants selected for alkaloid screening, as well as the results of initial work on molecular elucidation.

Results and Discussion

Plant Selection

A list of medicinal plants on Lombok was generated, consisting of 100 plant species based on the possibility of them having antimicrobial or antimalarial activities inferred from their local uses in medicine. The list was then reduced to 5 species for initial investigation on the basis of the following criteria: 1) the medicinal use of the plant and the possibility of it containing antibacterial, or antimalarial compounds, 2) the presence of alkaloids in the species, 3) the extent of chemical and biological work already undertaken on the species, and 4) the geographical distribution of the species. The plants thus selected were *Alstonia scholaris* R. Br. (Apocynaceae; antimalarial properties), *Voacanga foetida* (Bl.) Rolfe. (Apocynaceae; antimicrobial properties), *Psychotria malayana* Jack. (Rubiaceae; antimicrobial properties), *Clerodendron paniculatum* L. (Verbenaceae; antimicrobial properties), and *C. calamitosum* L. (Verbenaceae; antimalarial and antimicrobial properties). Although there are a number of reports on alkaloids from *A. scholaris* R. Br. [3,4], we were particularly interested in investigating alkaloids in the young plants, as local people have used the plant at this growth stage to treat malaria.

Alkaloid Screening

A hundred medicinal plants giving negative or positive tests for alkaloids (modified Culvenor and Fitzgerald procedure [5]) are listed in Table 1 and 2 respectively under family and species; the local uses of the plants are also included in these tables.

Table 1. Lombok medicinal plant species giving a negative test for alkaloids.

Family	Species	Locality*	Collection Code	Diseases/ Conditions treated**	Part Tested***
Acanthaceae	*Justicia gendarussa*	Narmada, WL	NWL08	Fever	Lf, bk, rt
Agavaceae	*Cordyline fruticosa L.*	Puyung, CL	PCL04	Diarrhoea	Lf, rh
	C. rumphii Miq.	Puyung, CL	PCL13	Dysentery	Lf, st, rt
Amaryllidaceae	*Crinum latifolium L.*	Kopang, EL	KEL01	Fever	Lf, bl
Anacardiaceae	*Bouea burmanica Griff.*	Masbagik, EL	MEL03	Ulcers, abscesses	Lf, bk, rt
	Buchanania macrophylla Bl.	Narmada, WL	NWL02	Fever, sore eyes	Lf, bk, rt
	Dracontomelon celebicum Kds.	Narmada, WL	NWL01	Fever, sore eyes	Lf, bk, rt
	Gluta elegans Kurz.	Puyung, CL	PCL08	Skin diseases	Lf, bk, rt
Annonaceae	*Xylopia malayana*	Suranadi,WL	SWL04	Fever, malaria	Lf, bk, fl
Apiaceae	*Corriandrum sativum L.*	Mantang, CL	MCL05	Wounds	Lf, sd
	Foeniculum vulgare Mill.	Mantang,EL	MLT05	Cough, fever	Lf, st, rt, sd
Apocynaceae	*Alstonia macrophylla Wall.*	Puyung, CL	PCL07	Ulcers	Lf, bk, rt
	Wrightia spp.	Masbagik, EL	MEL11	Dysentery	Lf, bk, rt
Asclepiadaceae	*Cryptostegia madagascariensis Boj.*	Ampenan, WL	AWL04	Dysentery	Lf, bk, rt
Asteraceae	*Achillea millefolium L.*	Tetebatu, EL	TEL02	Wounds	Lf, st, rt
	Bidens leuchantus Willd.	Tetebatu, EL	TEL05	Swellings	Lf, bk, rt,
Capparidaceae	*Gynandropsis speciosa*	Narmada,WL	NWL06	Gonorrhoea, dysentery	Lf, st, rt
	Polanisia icosandra	Kotaraja, EL	KEL08	Skin diseases	Lf, bk, rt
Compositae	*Pluchea indica Less.*	Kotaraja, EL	KEL07	Fever, dysentery	Lf, bk, rt
Erythroxylaceae	*Phyllanthus acidus Skeels.*	Sepakek, CL	SCL02	Fever	Lf, bk, rt
Euphorbiaceae	*Aleurites moluccanaWild.*	Suranadi, WL	SWL05	Dysentery, itches	Lf, bk, fr
	Antidesma cuspidatum Muell. Arg.	Suranadi, WL	SWL09	Fever	Lf, bk, rt
	A. montanum Bl.	Pancor, EL	PEL02	Ulcers, wounds	Lf, bk, rt, sd

Family	Species	Locality*	Collection Code	Diseases/ Conditions treated**	Part Tested***
Euphorbiaceae	*Aporosa frutescens Bl.*	Puyung, CL	PCL05	Fever	Lf, bk, rt
	Baccaurea brevipes Hook. f.	Puyung, CL	PCL06	Fever, dysentery	Lf, bk, rt
	B. dulcis Muell. Arg.	Puyung, CL	PCL11	Wounds	Lf, bk, rt
	Croton argyratus Bl.	Puyung, CL	PCL09	Dysentery	Lf, bk, rt
	Euphorbia. pulcherrima Willd.	Puyung, CL	PCL10	Wounds	Lf, bk, rt
	E. tirucalli L.	Puyung, CL	PCL12	Ulcers	Lf, bk, rt
	Sauropus androgynus L.	Kotaraja, EL	KEL11	Fever	Lf, bk, rt
Gentianaceae	*Canscora ecussata Schult.*	Kopang, CL	KCL02	Ulcers, wounds	Lf, st, rt
Haloragaceae	*Gunnera macrophylla Bl.*	Suranadi, WL	SWL03	Dysentery, Diarrhoea	Lf, bk, fr
	Myriophyllum brasiliense Cambess.	Kotaraja, EL	KEL10	Diarrhoea	Lf, bk, rt
Hernandiaceae	*Artocarpus anisophylla Miq.*	Mantang, CL	MCL04	Dysentery	Lf, bk, rt, fr
	A. dadah Miq.	Pancor, EL	PEL05	Dysentery	Lf, bk, rt
	A. champeden Spreng.	Ampenan,WL	AWL02	Sore eyes, Diarrhoea	Lf, bk
	A. gomeziana Wall.	Kotaraja, EL	KEL09	Dysentery	Lf, bk, rt
	Hernandia ovigera L.	Ampenan,WL	AWL01	Sore eyes	Lf, st
Lamiaceae	*Coleus ambonicus Lour.*	Tetebatu, EL	TEL04	Diphtheria, tetanus	Lf, bk, rt, sd
	Desmodium heterophyllum DC.	Mantang, CL	MCL03	Scabies, itches	Lf, st, rt
	Orthosiphon grandiflorus Bold.	Sepakek, CL	SCL01	Syphilis	Lf, st, rt
Leguminosae	*Bauhinia variegata L.*	Mataram, WL	MWL05	Fever, cough	Lf, bk, rt
	Derris elliptica Benth.	Suranadi, WL	SWL07	Fever, scabies	Lf, bk, rt
Malvaceae	*Abelmoschus escelentus Moench.*	Pancor, EL	PEL03	Gonorr-hoea	Lf, bk, rt
	Gossypium arboreum L.	Pancor, EL	PEL06	Ulcers	Lf, bk, rt
Meliaceae	*Dysoxylum sp.*	Suranadi, WL	SWL08	Itches	Lf, bk, rt
	Sweitenia macrophylla King.	Masbagik, EL	MEL08	Malaria	Lf, bk, rt
Myristicaceae	*Horsfleidia glabra Warb.*	Masbagik, EL	MEL09	Itches	Lf, bK, rt
Myrtaceae	*Eugenia cumini Merr.*	Mataram, WL	MWL07	Sore eyes	Lf, bk, rt, sd
Oxalidaceae	*Averrhoea bilimbi L.*	Kotaraja, EL	KEL15	Fever, cough	Lf, bk, rt
	A. carambola L.	Kotaraja, EL	KEL06	Wounds, scabies	Lf, bk, rt
Palmae	*Cocos nucifera L.*	Narmada, WL	NWL05	Fever, dysentery	Lf, st, rt

Family	Species	Locality*	Collection Code	Diseases/ Conditions treated**	Part Tested***
Pandanaceae	*Pandanus furcatus Roxb.*	Pancor, EL	PEL04	Dysentry	Lf, st, rt
Papilionaceae	*Abrus fruticulosus Wall.*	Puyung, CL	PCL01	Fever	Lf, fl, bk, rt
Pedaliaceae	*Sasamus indicum L.*	Masbagik, EL	MEL06	Diarrhoea	Lf, bk, rt
Pinaceae	*Pinus mercusii Jungh & De. Vr.*	Mataram, WL	MWL01	Ulcers	Lf, bk, rt
Piperaceae	*Piper baccatum L.*	Mataram, WL	MWL03	Fever, swelling	Lf, st
	Peperomia pellucida Kunth.	Pancor, EL	PEL01	Fever	Lf, bk, rt
Poaceae	*Dendrocalamus asper Schult. F.*	Kekait, WL	KWL03	Fever	Lf, st
Rafflesiaceae	*Brugmensia suaveolens*	Mataram, WL	MWL06	Wounds	Lf, bk, rt
Rhamnaceae	*Alphitonia moluccana T&B.*	Sepakek, CL	SCL03	Fever	Lf, bk, rt
Rubiaceae	*Borreria hispida Schum.*	Narmada, WL	NWL11	Wounds, dysentery	Lf, st, rt
	B. setidens (Miq.) Bold.	Masbagik, EL	MEL10	Fever	Lf, bk, rt
	B. ocimoides DC.	Ampenan, WL	AWL05	Ulcers	Lf, bk, rt
	Catesbaea spinosa L.	Mantang, CL	MCL01	Fever, swellings	Lf, bk, rt
Sapindaceae	*Schleichera oleaosa L.*	Masbagik, EL	MEL07	Malaria	Lf, bk, rt
Sapotaceae	*Manilka raachras Fosberg.*	Tetebatu, EL	TEL01	Fever, dysentery	Lf, bk, rt, fl
Sterculiaceae	*Melochia umbellata Staff.*	Narmada, WL	NWL09	Fever	Lf, bk, rt
Thymelaeaceae	*Aquilaria filaria*	Kekait, WL	KWL04	Malaria	Lf, bk, rt
Verbenaceae	*Callicarpa cuspidata Roxb.*	Tetebatu, EL	TEL03	Fever	Lf, bk, rt
	Alpinia galanga Sw.	Puyung, CL	PCL02	Cholera	Lf, rh
	A. javanica Bl.	Suranadi, WL	SWL01	Swelling, cholera	Lf, rh
Zingiberaceae	*Curcuma aerginosa Roxb.*	Tetebatu, EL	TEL 05	Fever	Lf, rh
	C. zedoaria Rosc.	Puyung, CL	PCL03	Malaria	Lf, rh
	C. domestica Val.	Sepakek, CL	SCL04	Diarrhoea, scabies	Lf, rh
	Zingiber fficinale Rosc.	Suranadi, WL	SWL02	Itches, cholera, wounds	Lf, rh

*WL(West Lombok), CL(Central Lombok), EL(East Lombok); ** Information gathered by interviewing local people and confirmed by Perry (1980) [9]; ***bk(bark), st (stem), rt(root), bl(bulb), rh(rhizome), fl(flower), fr(fruit), sd(seed); plant parts printed in bold are those used medicinally

Table 2. Lombok medicinal plant species giving a positive test for alkaloids.

Family	Species	Locality*	Collection Code	Diseases/ Conditions treated**	Part tested(Result)
Amaryllidaceae	*Crinum asiaticum L.*	Masbagik, EL	MEL03	Wounds, abscesses	Lf(++), bl(++)
Annonaceae	*Annona squamosa L.*	Kotaraja, EL	KEL13	Fever	Lf(+), bk(++), rt(++)
Apocynaceae	*Alstonia cholaris R.Br.*	Kotaraja,EL	KEL3	Malaria	Lf(+++), bk(+++), rt(+++)
	Voacanga foetida (Bl.) Rolfe	Kekait, WL	KWL01	Almost all skin diseases	Lf(+++), bk(++++), fr(+++), sd(+++)
Caesalpiniaceae	*Cassia siamea*	Kotaraja,EL	KEL02	Malaria	Lf(+++), bk(++), rt(++)
Caricaceae	*Carica papaya L.*	Narmada, WL	NWL04	Malaria, Ulcers	Lf(++), st(-), rt(-), fr(-)
Convolvulaceae	*Ipomoea batatas Polr.*	Narmada, WL	NWL03	Wounds	Lf(+), rh(-)
Cucurbitaceae	*Momordica charantia L.*	Pancor, EL	PEL07	Malaria	Lf(++), st(++), fr(+)
	M. bicolor Bl.	Narmada, WL	NWL10	Malaria	Lf(++), st(++), rt (+)
Euphorbiaceae	*Jatropha multifida L.*	Ampenan, WL	AWL03	Swellings, wounds	Lf (-), bk(-), sd(+)
Lamiaceae	*Drysophylla auricularia (L.) Bl.*	Masbagik, EL	MEL05	Fever	Lf(++), St(++), rt(+)
Loganiaceae	*Strychnos ligustrina Bl.*	Masbagik, MEL	MEL12	Malaria	Lf(-), bk(+++), rt(++)
Magnoliaceae	*Michelia champaca L.*	Mataram, WL	MWL06	Fever, wounds	Lf(+++), bk(+++), rt(++)
	M. alba DC.	Narmada, WL	NWL07	Malaria	Lf(++), bk(++), rt(++)
Meliaceae	*Azadirachta indica Juss.*	Kopang, EL	KCL02	Dysentery malaria	Lf(++), bk(++), rt(+)
Mimosaceae	*Crotalaria retusa L.*	Kotaraja, EL	KEL14	Fever, wounds	Lf(++), st(+), rt(++), fr(-)
Moraceae	*Ficus septica*	Mataram, WL	MWL05	Wounds	Lf(+++), bk(+++), rt(+++)
Moringaceae	*Moringa oleifera Lamk.*	Mataram, WL	MWL08	Fever, Wounds	Lf (++), bk (++), rt (++)
Rubiaceae	*Psychotria malayana Jack.*	Suranadi, WL	SWL04	Wounds, skin diseases	Lf(++), bk(++), rt(-), fr(-)
Sterculiaceae	*Sterculia foetida Linn.*	Kotaraja,EL	KEL01	Fever, malaria	Lf(++), k(+++), rt(+++)

Family	Species	Locality*	Collection Code	Diseases/ Conditions treated**	Part tested(Result)
Verbenaceae	*Clerodendron alamitosum L.*	Kotaraja, EL	KEL12	Malaria, wounds	Lf (++)
	C. paniculatum L	Narmada, WL	NWL06	Sore eyes	Lf(-), fl(++), rt(-)
Zingiberaceae	*Curcuma xanthorrhiza Roxb.*	Mataram, WL	MWL02	Diarrhoea, malaria	Lf (+), rh (+)

*WL(West Lombok), CL(Central Lombok), EL(East Lombok); ** Information gathered by interviewing local people and confirmed by Perry (1980) [7]; ***bk(bark), st (stem), rt(root), bl(bulb), rh(rhizome), fl(flower), fr(fruit), sd(seed); plant parts printed in bold are those used medicinally

The plants collected were widely distributed in 49 families and 80 genera, and this is an indication of the variety of Lombok medicinal plants. Of these plants, twenty-three species (23%) contained alkaloids. In a survey of plants of Tasmania, Australia, which focused mainly on endemic species in this cool temperate environment [6], 15 % of the plant species gave positive alkaloid tests. However, in a similar alkaloid survey in Queensland, Australia, with many tropical and sub-tropical species, 20% of species were positive [10]. Further details on the five selected plants are as follows:

Alstonia scholaris R.Br. This is the tallest tree belonging to the family Apocynaceae growing to a height of 20-25 m and 40-80 cm in diameter. In Lombok, this tree, known as 'nita', is common in areas up to 900 m above sea level. The concentrated aqueous extract of leaves or bark of the young tree has been used to treat malaria in Lombok. Antimalarial testing with *Plasmodium falciparum*, revealed that the alkaloids obtained from leaves of *A. scholaris* from Lombok were active [8], but no specific mention was made on the age of the trees from which the alkaloids were isolated. We have found that alkaloids are distributed through the whole plant including leaves, bark, roots, and fruit.

Clerodendron calamitosum L. The leaves of this plant in the Verbenaceae, under the local name 'keji beling', have been used commonly to treat malaria and wounds, as well as to destroy kidney stones. It is an erect scrub, which grows in dry, shady areas in villages, coconut groves, and at village borders and roadsides.

No alkaloid studies appear to have been reported on this plant. A Mayer's alkaloid test on whole plants produced a weak positive result (+). Further testing on separated parts indicated that the alkaloid accumulated mainly in the leaves (++), therefore, isolation of the alkaloid was focused on this part. At least five major alkaloids have been shown to be present in the crude alkaloidal extract (0.04% based on fresh material, 250 g) and some of their characteristics are noted in Table 3.

Table 3. Some characteristics of components in the crude alkaloidal extract of *C. calamitosum* L.

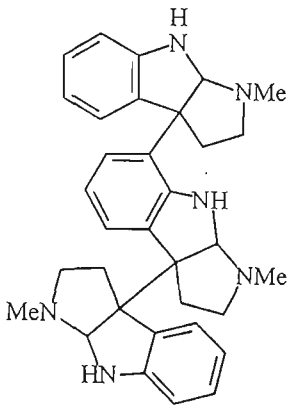
Component No.	Rf*	UV light (333 nm)**	Iodine Test***	Dragendorff Test****	M^+ (CIMS)*****
1	0.34	abs.	brown	yellow	285 &309
2	0.41	abs.	brown	yellow	285
3	0.56	abs.	brown	yellow	308
4	0.81	blue fl.	-	-	270
5	0.91	blue fl.	-	-	277

* TLC (silica gel, ethyl acetate : isopropanol : NH_4OH = 95 : 10 : 5 by vol.); **Abs (absorbance), fl (fluorescence); ***By using iodine vapour [9], - no colouration; **** Reference [9], - no colouration; *****CIMS(Chemical Ionisation Mass Spectrometry)

By comparing molecular weights of the few known indole-based alkaloids isolated previously from other species in the genus [11-13], all the components appear to be new alkaloids from the genus *Clerodendron*. Molecular elucidation of these components is currently in progress.

Clerodendron paniculatum L. Having a beautiful red flower, the plant is commonly cultivated in gardens for ornamental value. Like other species in the Verbenaceae, the plant, known locally as 'kembang aik terjun', grows as an erect shrub, with a height up to 1.5 m. The plant is reported [14] as a native in South East Asia, and has been used to treat sore eyes by extracting the leaves with sterile water and applying the extract as eye drops. The concentrated water extract of the leaves also has been utilised to treat wounds. Only the mature flowers tested positive for alkaloids, and young flowers (less than two months old) gave a negative alkaloid test. It is possible that the leaves contain alkaloids at very low concentration, but in this study it was decided to extract mature flowers only.

Psychotria malayana Jack. This small tree, locally known as 'lolon jarum', and which grows to a height of 1-4 m, is largely distributed in the west Indonesia archipelago. In Java, there have been no reports indicating local uses [14], however, people in Lombok have utilised this plant (aqueous extracts of either leaves or bark) for protecting skin from infection from open wounds and for other skin diseases. The alkaloids are concentrated in the leaves and bark. Further separation of the crude alkaloid extract from the leaves (0.9% based on air dried material, 100 g) by the use of preparative TLC (silica gel; solvent system CH_2Cl_2: CH_3OH: NH_4OH/ 90 : 15 : 1) showed at least four alkaloids to be present; hodgkinsine **1** (Rf 0.56), a trimeric N_b-methyltryptamine alkaloid, was the major constituent. It was readily identified by electron impact mass spectrometry (EIMS) with three principal peaks at m/z 172, 344, and 518 for the trimeric structure (Chemical Ionisation HRMS m/z 519.3200, calc 519.3236 for $C_{33}H_{38}N_6$+H). The other minor alkaloid found was chimonanthine **2** (Rf 0.63), a dimeric N_b-methyltryptamine, having two main fragments at m/z 172 and 130 (Chemical Ionisation HRMS m/z 347.2224, calc 347.2235 for $C_{22}H_{26}N_4$+H) in the EI mass spectrum. Polymeric tryptamines are commonly found in *Psychotria* species [15-18]. Two other constituents with molecular weights of 186 (Chemical Ionisation HRMS m/z 187.1230, calc 187.1235 for $C_{12}H_{14}N_2$+H) and 574 are currently under investigation and will be reported separately. Alkaloids with these molecular weights have not been reported from *Psychotria* previously.



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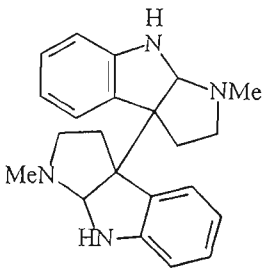

2

Voacanga foetida (Bl.) Rolfe. Locally called 'kumbi', this plant in the Apocynaceae is distributed throughout Indonesia but Lombok is the main region. It grows in areas about 400 m above sea level and reaches 10-15 m in height. The aqueous extract of the leaves or bark is used commonly to treat a wide range of skin conditions such as wounds, itches, and swellings. Initial alkaloid screening showed that all parts of the plant contained high concentrations of alkaloids, although a previous report indicated that only small amounts of alkaloids occurred in the bark [7].

Conclusions

Initial work on Lombok medicinal plants has resulted in a hundred species being collected and screened for the presence of alkaloids and the five selected plants are being investigated for determination of the alkaloid structures and antimicrobial activity. At least five novel alkaloids appear to be present in *C. calamitosum* L. It has also been found that hodgkinsine is the major alkaloid in *Psychotria malayana* Jack. Further work on the isolation and structural elucidation of alkaloids from the selected plants, and testing of the alkaloids for antibacterial and antimalarial properties, will be reported separately.

Experimental

General

Chemical Ionisation and Electron Impact (at 70 eV) mass spectra were obtained on a Shimadzu QP-5000 by the direct insertion technique. High resolution CI mass spectra were obtained on a Fisons/VG Autospec-TOF-oa Mass Spectrometer. Preparative TLC was performed on Merck silica gel 60 PF_{254}, 0.3 mm thick plates, and observed under UV light (333 nm). All solvents were distilled before use.

Plant Collection

The plants were collected througout Lombok with permission of the Local Government and in collaboration with the University of Mataram. All plants were collected between April and June 1999, and were identified with the assistance of Mr. Made Sudana, a botanist at the University of Mataram, Lombok. In addition, plant specimens were sent to the Research and Development Centre for Biology, Bogor, Indonesia, for confirmatory identification. A local herbarium collection was created and stored in a pest-proof wooden container at the Laboratory of Biology, the University of Mataram. The collection codes noted in Tables 1 and 2 are also the voucher specimen codes in this herbarium.

Alkaloid Testing

The method of alkaloid testing followed the procedure of Culvenor and Fitzgerald [5]. In the procedure, finely ground plant material is rapidly extracted with ammoniacal chloroform ($CHCl_3$) or dichloromethane (CH_2Cl_2). After filtration, the solution is extracted in turn with aqueous sulphuric acid (0.1M, H_2SO_4). The presence of most alkaloids in the aqueous phase can be detected by the formation of a precipitate on addition of Mayer's reagent (K_2HgI_4). The semi-quantitative results of the method have been rated from + for faint turbidity to ++++ for a heavy curdy precipitate. Modifications to the procedure introduced by Bick *et. al.*[6], notably the use of a domestic coffee grinder to grind the plant material, were also used in this study.

In general, the procedure is rapid and produces consistent results. Water soluble alkaloids, however, are not detected, since they are not extracted by the ammoniacal $CHCl_3$ or CH_2Cl_2. Separate water extractions of plant material were not undertaken in this investigation.

Isolation of Alkaloids

Before extraction, parts of the plants were prepared by air drying at room temperature (*ca.* 27°C) followed by grinding separately in a coffee grinder. In the case of plants giving a negative alkaloid result after drying, although giving a positive test when fresh (such as *Clerodendron calamitosum* L.), fresh material was ground and extracted to reduce the loss of alkaloid.

The procedure for extraction of the alkaloid from the five plants selected is outlined in Figure 1. Ground plant material was extracted with cold distilled methanol (CH_3OH) with occasional swirling. Methanol extraction was continued until the plant material gave a negative test for alkaloids (Mayer's test). After filtration, the solvent was removed under reduced pressure at 40°C, to minimise any thermal degradation of the alkaloids.

The crude alkaloid mixture was then separated from neutral and acidic materials, and water solubles, by initial extraction with aqueous acetic acid (CH_3CO_2H) followed by dichloromethane extraction and then basification of the aqueous solution and further dichloromethane extraction.

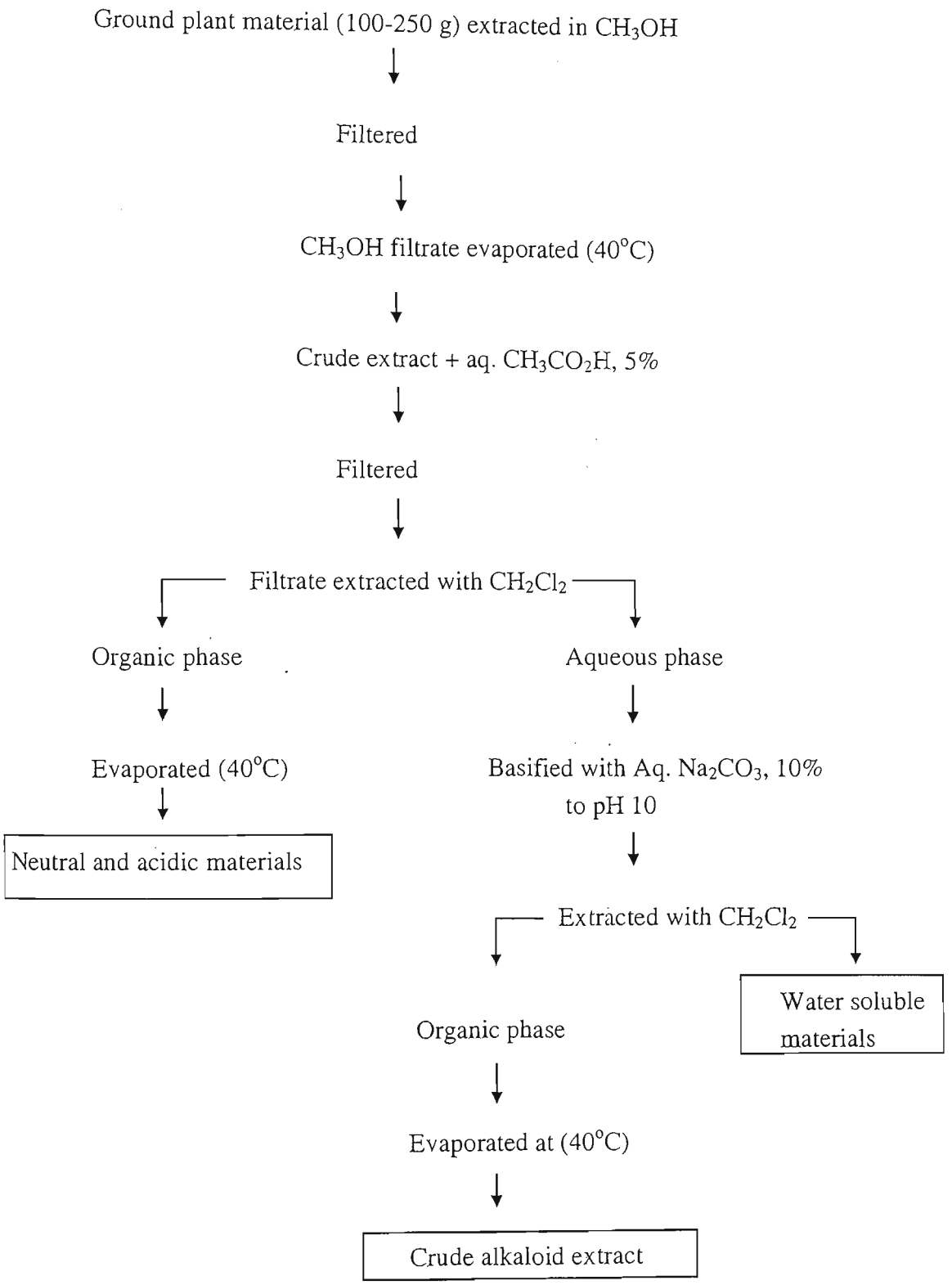

Figure 1. Outline of the extraction procedure for alkaloids carried out for selected medicinal plants of Lombok.

Acknowledgments

We would like to thank Dr. Irawati from the Research and Development Centre for Biology, Bogor, and Mr. Made Sudana from University of Mataram, Lombok for the identification of the plants. We also thank Mr. Taspin (University of Mataram) for his kind help in providing technical assistance and to AusAid for providing a scholarship and support (to S.H).

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Sample availability: Samples available from the authors.