แอลคาลอยด์จากใบทุ้งฟ้า

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ALKALOIDS FROM THE LEAVES OF *ALSTONIA MACROPHYLLA*

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วิมลักษณ์ นพศิริ : แอลคาลอยด์จากใบทุ้งฟ้า (ALKALOIDS FROM THE LEAVES OF *ALSTONIA MACROPHYLLA*) อาจารย์ที่ปรึกษา : รศ. ดร. สัมพันธ์ วงศ์เสรีพิพัฒนา, อาจารย์ที่ปรึกษาร่วม : รศ. ดร. ธาวดี ผ่องลักษณ์, 141 หน้า. ISBN 974-17-1574-9.

จากใบทุ้งฟ้า (*Alstonia macrophylla* Wall. ex G. Don) สามารถสกัดแยกสารบริสุทธิ์ได้ 4 ชนิด ประกอบด้วยอินโดลแอลคาลอยด์ 3 ชนิด เป็นกลุ่ม vincorine 2 ชนิด คือ demethoxyalstonamide และ (-)-1-norvincorine และอีกกลุ่มหนึ่งคือกลุ่ม macroline มี 1 ชนิด คือ alstophylline ส่วนสารบริสุทธิ์อีกชนิดหนึ่งเป็น amide คือ 3,4,5-trimethoxybenzamide ซึ่งได้พิสูจน์โครงสร้างทางเคมีของสารบริสุทธิ์ที่แยกได้ด้วยการวิเคราะห์สเปคตรัมของ UV, IR, MS และ NMR ร่วมกับการเปรียบเทียบข้อมูลของสารประกอบที่ทราบโครงสร้างแล้ว

ภาควิชา เภสัชเวท	ลายมือชื่อนิสิต……....
สาขาวิชา เภสัชเวท	ลายมือชื่ออาจารย์ที่ปรึกษา……....
ปีการศึกษา 2545	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม…….……

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KEY WORD: *ALSTONIA MACROPHYLLA* / INDOLE ALKALOIDS

WIMALUK NOPSIRI : ALKALOIDS FROM THE LEAVES OF *ALSTONIA MACROPHYLLA.* THESIS ADVISOR : ASSOC. PROF. SUMPHAN WONGSEREPIPATANA, Ph.D., THESIS CO-ADVISOR : ASSOC. PROF. DHAVADEE PONGLUX, Ph.D., 141 pp. ISBN 974-17-1574-9.

Four pure compounds were isolated from the leaves of *Alstonia macrophylla* Wall. ex G. Don. Three of them were indole alkaloids identified as two vincorine groups namely demethoxyalstonamide and (-)-1-norvincorine and another one of indole alkaloids as macroline group, being alstophylline. The last one was amide namely 3,4,5-trimethoxybenzamide. The structures of all isolated compounds were determined by extensive spectroscopic studies, including comparison of UV, IR, MS and NMR spectra with the previously reported data.

Department Pharmacognosy	Student's signature……..……....
Field of study Pharmacognosy	Advisor's signature…….……
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LIST OF ABBREVIATIONS

br	=	Broad (for NMR spectra)
c	=	Concentration
°C	=	Degree Celsius
CA	=	Chemical Abstract
$CDCl_3$	=	Deuterated chloroform
CD_3OD	=	Deuterated methanol
$CHCl_3$	=	Chloroform
cm	=	Centimeter
^{13}C NMR	=	Carbon-13 nuclear magnetic resonance
COSY	=	Correlation spectroscopy
1-D	=	One dimensional
2-D	=	Two dimentional
d	=	doublet (for NMR spectra)
dd	=	doublet of doublets (for NMR spectra)
ddd	=	doublet of doublets of doublets (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polariyation Transfer
δ	=	Chemical shift
EC_{50}	=	Median effective concentration
EIMS	=	Electron Impact Mass Spectrum
EtOAc	=	Ethyl acetate
g	=	Gram
^{1}H NMR	=	Proton nuclear Magnetic Resonance
HMBC	=	^{1}H-detected Heteronuclear Multiple Bond Correlation
HMQC	=	^{1}H-detected Heteronuclear Multiple Quantum Correlation
Hz	=	Hertz
IC_{50}	=	Median inhibitory concentration

LIST OF ABBREVIATIONS (continued)

IR	=	Infrared spectrum
J	=	Coupling constant
KBr	=	Potassium bromide
Kg	=	Kilogram
L	=	Liter
λ_{max}	=	Wavelength at maximal absorption
ε	=	Molar absorptivity
μg	=	Microgram
μM	=	Micromolar
M^+	=	Molecular ion
m	=	Multiplet (for NMR spectra)
MeOH	=	Methanol
mg	=	Milligram
MHz	=	MegaHertz
ml	=	Milliliter
m/z	=	Mass to charge ratio
MS	=	Mass spectrometry
N_a	=	Nitrogen atom (a)
N_b	=	Nitrogen atom (b)
NH_4OH	=	Ammonium hydroxide
nm	=	Nanometer
NMR	=	Nuclear magnetic resonance
NOESY	=	Nuclear Overhauser Effect Correlation Spectroscopy
ppm	=	part per million
ν_{max}	=	Wave number at maximal absorption
q	=	Quartet (for NMR spectra)

LIST OF ABBREVIATIONS (continued)

s	=	Singlet (for NMR spectra)
t	=	Triplet (for NMR spectra)
TLC	=	Thin layer chromatography
UV	=	Ultraviolet

CHAPTER I

Introduction

It is from those plants containing alkaloids that a large number of drugs are derived. The group, however, is a very varied one and it is only the chemical properties of a basic nitrogen that unify the many classes of alkaloids. The number of indole alkaloids of known structure today amounts to approximately 1200. Indole alkaloids are defined as the natural organic products containing either the indole nucleus or an oxidized, reduced or substituted equivalent of it (Kisakurek and Hesse, 1980).

Some indole alkaloids exert considerable pharmacological activity, three groups are notable for clinically useful alkaloids: (a) the Ergot alkaloids, ergometrine, with its direct action on the contraction of uterine muscle; ergotamine for migraine relief and modified alkaloid, bromocriptine, which suppresses lactation and has some application for the treatment of mammary carcinoma, (b) the *Rauvolfia* alkaloids and specifically reserpine which was the forerunner of the tranquilizers and hypotensive, (c) the Dimeric anti-leukemic alkaloids of *Catharanthus*, vinblastine and vincristine which are in current clinical use. It might be thought that interest in indole alkaloids had passed their peak as far as new discoveries were concerned. In fact it is logical to assume that after such intensive research efforts, there would be little novelty left in this area (Phillipson and Zenk, 1980). More than 99.8% of the isolations of indole alkaloids are entirely distributed among three families: Loganiaceae, Apocynaceae, and Rubiaceae, belonging to order Gentianales (Kisakurek and Hesse, 1980).

These three families can be recognized and identified easily, as their leaves mostly opposite, simple, pinnately veined, with or without inter-or intrapetiolar stipules. Their flowers mostly 4- or 5-merous, usually actinomorphic, but sometimes zygomorphic and exceptionally irregular. Corolla segments always united, and stamens inserted on the corolla. Style one. Ovary, except in most Rubiaceae, superior and mostly 2-locular. The Apocynaceae can be differentiated from the Loganiaceae by the presence of milky sap. The genus *Alstonia* belongs to the tribe Plumerieae (Alstonieae) in the family

Apocynaceae. They are laticiferous trees or shrubs; leaves: simple, whorled or opposite; inflorescences; terminal, flowers cymose; calyx lobes 5, deeply devided; corolla salver-form, white to yellow or red; stamens: short but distinct filaments, longitudinally dehiscent, without appendages; ovary: apocarpous (bicarpellate), superior or half-superior, ovules numerous, 2-8 seriate; fruits: dry-dehiscent follicles; seeds: numerous, very light, flattened and ciliate (Monachino, 1949).

There are about 40 species of the genus *Alstonia* distributed throughout the tropical and subtropical parts of the world especially in Southeast Asia, Polynesia, Australia, India and Africa (Keawpradub *et al*., 1997). And there are seven species in Thailand. Almost all Malesian Alstonias prefer wet ground, some even peat swamp forests, and tolerate open water. They are frequent to common in lowland rain forests, some in mountain rain forests too. The most widely distributed species, *A. scholaris* and *A. spectabilis*, have a wider range of water requirement, occurring also in sclerophyllous woods and wood patches in savannas (Markgraf, 1974). The leaf shape is extremely variable in this genus. On the same twig, and even in the same whorl, leaves may vary from obovate and obtuse to elliptic and acuminate. Sometimes, after the fruits have shed their seeds, short shoots with much smaller leaves spring between the branches of the inflorescence from the axial of fallen leaves. The inflorescence is terminal, pleiochasial, short-peduncled or often sessile, its branches springing from the axils of small scales; these partial inflorescences have a long peduncle and 1-3 nodes giving rise to secondary cymes.

Alstonia macrophylla Wall. ex G. Don is a tree in the family Apocynaceae, distributes in China, Vietnam, Cambodia, Malaysia, Philippines, Indonesia, New Guinea and Thailand. It was found in evergreen forest, often on sandy soil. In Thailand, this family distributes in Chumphon, Surat Thani, Phangnga, Phuket, Nakhon Si Thammarat. Phattalung, Trang, Satun, Songkhla, Yala (Markgraf, 1974 and Middleton, 1999), and some in central part of Thailand (Abe, Yamauchi and Santisuk,1994b), locally known as Tung fa, Kratung fa hai (Peninsular); Tung fa kai (Chumphon); Tin thian (Songkhla) (Markgraf, 1974 and Middleton, 1999).

It is a tree of 30 m high, 70 cm diameter at breast height, trunk sometimes fluted at base or with small buttresses. ***Bark*** blackish-brown to grey, inner bark cream or green,

sapwood white to yellowish-brown. ***Branchlets*** sparsely pubescent, glabrate; branches sparsely lenticellate or not. ***Leaves*** in whorls of 3 or 4; blade coriaceous, oblong-obovate, 5.5-28 × 3-10.2 cm, apex acuminate, base cuneate; secondary veins 14-21 pairs, widely spaced, ascending; sparsely pubescent on petiole, on underside of leaf blade and on midrib above, rarely completely glabrous. ***Flowers*** *Inflorescence* 3.5-11.5 cm long; sparsely pubescent or rarely glabrous; many flowered; pedicels 2-5 mm long. *Sepals* ovate,1.2-2.2 × 0.8-1.2 mm, apex rounded to acute; pubescent or glabrous, ciliate. *Corolla* white or yellowish; lobes overlapping to the right in bud; tube 4-5.5 mm long; lobes 3.5-6.5 × 1.4-2.5 mm, oblong, apex rounded; lobes ciliate, otherwise glabrous outside, pubescent inside on base of lobes and upper part of tube. ***Fruits*** a pair of follicles; 22-40 cm long, 2.2-3.7 mm wide; glabrous; many seeded. ***Seeds*** pubescent; oblong, one end long acuminate; 4.2-8 × 0.8-1.8 mm, cilia 5-9 mm long (Markgraf, 1974 and Middleton, 1999).

The genus is well-known as a rich source of indole alkaloids, and more than 180 alkaloids have been isolated from this particular genus. In spite of this large number, only a few *Alstonia* alkaloids have been assessed for biological evaluation (Keawpradub and Houghton, 1997). In traditional medicine, methanol extract of root bark of *A. macrophylla* has been used for treatment of malaria, and has been assessed for cytotoxic activity against two human lung cancer cell lines (Keawpradub *et al*., 1997, 1999a and 1999b), also antiplasmodial activity (Keawpradub *et al*., 1999b).

The previous phytochemical studies of the bark, root bark and leaves of *A. macrophylla* were reported by several groups of researchers. However, only one report on the chemical constituents of the leaves in central of Thailand has been published (Abe *et al*., 1994b). Therefore, it is interesting to investigate some other alkaloids from the leaves of this plant.

Figure 1 *Alstonia macrophylla* Wall. ex G. Don

Figure 1 (continued) *Alstonia macrophylla* Wall. ex G. Don

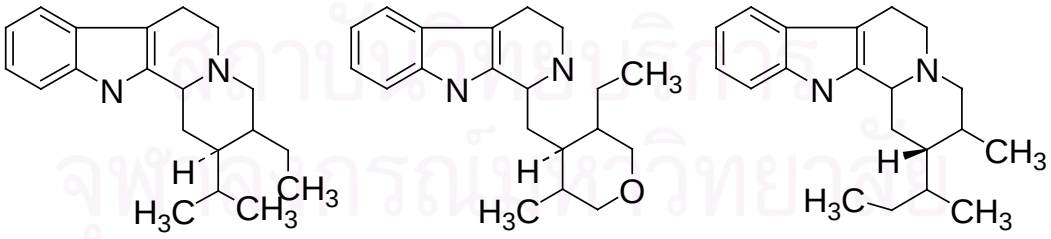
CHAPTER II

Historical

The first isolation of alkaloids in the nineteenth century followed the reintroduction into medicine of number of alkaloid-containing drugs and were coincidental with the advent of the percolation process for extraction of drugs. It was well over a century before the structures were finally elucidated. Modern methods and instrumentation have greatly facilitated these investigations, and it is interesting to note that the yields of minor alkaloids, too small for further investigation, isolated by chemists during the first quarter of this century would now be sufficient, several thousand times over, for a complete structure analysis.

1. Distribution of Indole Alkaloids

All the indole alkaloids with a C_9 – or C_{10} – monoterpene moiety isolated from the plants of the families Apocynaceae, Rubiaceae and Loganiaceae. The huge number of alkaloids (a total of 3450 reports covers the isolation of 1200 different alkaloids) have been classified into eight different skeletal types: corynanthean, vincosan, vallesiachotaman, strychnan, aspidospermatan, eburnan, plumeran and ibogan (Figure 2) (Kisakurek, Leeuwenberg and Hesse, 1983).



Corynanthean Vincosan Vallesiachotaman

Figure 2 Structure of classified eight different skeletal types of alkaloids

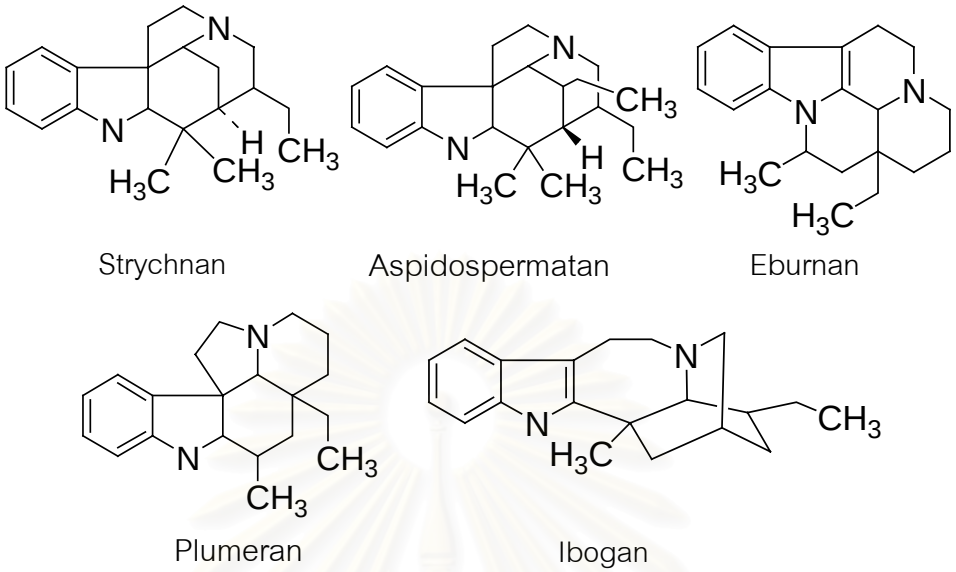


Figure 2 (continued) Structure of classified eight different skeletal types of alkaloids

The botanical classification of the plant families Apocynaceae, Loganiaceae and Rubiaceae which have species containing indole alkaloids are listed below (Kisakurek and Hesse, 1980).

Family Apocynaceae

Subfamily Plumerioideae

Tribe Carisseae

Genus *Carpodinus, Hunteria, Landolphia, Melodinus, Picralima, Pleiocarpa, Ployadoa*

Tribe Tabernaemontaneae

Genus *Anacampta, Bonafousia, Callichilia, Capuronetta, Conopharyngia, Crioceras, Ervatamia, Gabunia, Hazunta, Hedranthera, Muntafara, Pagiantha, Pandaca, Peschiera, Phrissocarpus, Rejoua, Schizozygia, Stemmadenia, Stenosolen, Tabernaemontana, Tabernanthe, Voacanga*

Tribe Plumerieae

Genus *Alstonia, Ammocallis, Amsonia, Aspidosperma, Catharanthus, Craspidospermum, Diplorhynchus, Geissospermum, Gonioma, Haplophyton, Lochnera, Plumeria, Rhazya, Tonduzia, Vinca*

Tribe Rauvolfieae

Genus *Bleekeria, Cabucala, Excavatia, Kopsia, Neiosperma, Ochrosia, Rauvolfia, Vallesia*

Family Loganiaceae

Tribe Strychneae

Genus *Gardneria, Strychnos*

Tribe Gelsemieae

Genus *Gelsemium, Mostuea*

Family Rubiaceae

Subfamily Rubioideae

Tribe Psychotrieae

Genus *Palicourea*

Tribe Urophylleae

Genus *Pauridiantha*

Subfamily Cinchonoideae

Tribe Naucleeae

Genus *Adina, Anthocephalus, Cephalanthus, Haldina, Mitragyna, Nauclea, Neonauclea, Ourouparia, Pertusadina, Sarcocephalus, Uncaria*

Tribe Cinchoneae

Genus *Cinchona, Corynanthe, Pausinystalia, Pseudocinchona, Remijia*

Tribe Mussaendeae

Genus *Isertia*

Subfamily Guettardoideae

Tribe Guettardeae

Genus *Antirhea, Guettarda*

Subfamily Hillioideae

Tribe Hillieae

Genus *Hillia*

2. Distribution of *Alstonia* species

The genus *Alstonia* was named in honour of Charles Alston (1685-1760), a Scottish physician and professor of Botany at the University of Edinburgh. It was first described by Robert Brown in 1811 with four species, namely *A. scholaris* (the type species of the genus), *A costata*, *A. spectabilis*, and *A. venenata* (Monachino, 1949). A systemic revision of the genus was published by Monachino in 1949 with 5 sections, 39 species, and 12 varieties. However, an accumulation of new specimens from recent field studies led to the regional revisions for Malaysia and Australia (Markgraf, 1974). Recently, two new species, i.e. *A. undulifolia* from Malaysia (Kochummen and Wong, 1984) and *A. beatricis* from Irian Jaya, Indonesia (Sidiyasa, 1996) have been described.

According to the taxonomic treatments mentioned earlier, the following 45 species of genus *Alstonia* have been recognized.

2.1 Section *Winchia* (monotypic)

2.1.1 *A. glaucescens* (K. Schum.) Monach.

[syn: *A. pachycarpa* Merrill & Chun, *Winchia calophylla* A. DC.]

2.2 Section *Pala* (Section *Alstonia*)

2.2.1 *A. actinophylla* (A.Cunn.) K. Schum.

[syn: *A. verticillosa* F. Muell.]

2.2.2 *A. angustiloba* Miq.

2.2.3 *A. boonei* De Wild.

2.2.4 *A. congensis* Engl.

[syn: *A. gilletii* De Wild.]

2.2.5 *A. pneumatophora* Backer ex L.G. Den Berger

2.2.6 *A. scholaris* (L.) R. Br.

2.2.7 *A. spatulata* Bl.

2.2.8 *A. undulifolia* Kochum & Wong

2.3 Section *Blaberopus*

2.3.1 *A. curtisii* King & Gamble

2.3.2 *A. mairei* Leveille

2.3.3 *A. neriifolia* D. Don

[syn: *A. sericea* Bl.]

2.3.4 *A. rupestris* Kerr

2.3.5 *A. sebusi* (van Heurck & Muell. Arg.) Monach.

2.3.6 *A. venenata* R. Br.

[syn: *Blaberopus venenatus* A. DC.]

2.3.7 *A. yunnanensis* Diels

2.4 Section *Monuraspermum*

2.4.1 *A. angustifolia* Wall. ex A. DC.

2.4.2 *A. brassii* Monach.

2.4.3 *A. glabriflora* Markgraf

A. linearis Benth.

A. macrophylla Wall. ex G. Don

[syn: *A. batino* Blanco]

2.4.6 *A. muelleriana* Domin

2.4.7 *A. ophioxyloides* F. Muell.

2.4.8 *A. parvifolia* Merrill

2.4.9 *A. spectabilis* R. Br.

[syn: *A. longissima* F. v. Muell., *A. somersetensis* F.M., Bailey, *A. villosa* Bl.]

2.5 Section *Dissuraspermum*

2.5.1 *A. balansae* Guillaum.

2.5.2 *A. boulindaensis* Boit.

2.5.3 *A. constricta* F. Muell.

[syn: *A. mollis* Benth.]

2.5.4 *A. coriacea* Pancher ex S. Moore

[syn: *A. lenormandii* van Heurck & Muell. Arg. var. *coriacea* Monach.]

2.5.5 *A. costata* (Forst. F.) R. Br.

[syn: *A. fragrans* J.W. Moore]

2.5.6 *A. deplanchei* van Heurck & Muell. Arg.

[syn: *A. linearifolia* Guillaum., *A. retusa* S. Moore]

2.5.7 *A. lanceolata* van Heurck & Muell. Arg.

2.5.8 *A. lanceolifera* S. Moore

[syn: *A. lenormandii* van Heurk & Muell. Arg. var.*lanceolifera* (S. Moore) Monach.]

2.5.9 *A. legouixiae* van Heurck & Muell. Arg.

[syn: *A.saligna* S. Moore]

2.5.10 *A. lenormandii* van Heurck & Muell. Arg.

[syn: *A. comptonii* S. Moore, *A. filipes* Schltr. ex Guillaum.]

2.5.11 *A. montana* Turrill

[syn: *A. smithii* Markgraf]

2.5.12 *A. odontophora* Boit.

2.5.13 *A. plumosa* Labill.

[syn: *A. roeperi* van Heurck & Muell. Arg.]

2.5.14 *A. quaternata* van Heurck & Muell. Arg.

2.5.15 *A. reneckeana* Lauterb.

2.5.16 *A. sphaerocapitata* Boit.

2.5.17 *A. undulata* Guillaum.

2.5.18 *A. vieillardii* van Heurck & Muell. Arg.

2.5.19 *A. vitiensis* Seem.

[syn: *A. villosa* Seem.]

2.6 Unknown Section

2.6.1 *A. beatricis* Sidiyasa

There are some interesting points to note on the distribution of native species. Among these 45 species, *A. scholaris* is the most widely distributed species stretching from India through Southeast Asia to Australia and some Eastern Pacific Islands. On the other hand, the occurrence of some species is very restricted. The two species, *A. boonei* and *A. congensis*, have been found exclusively in Africa and are the only two of *Alstonia* reported

from this continent. Nearly all members of section *Blaberopus* are abundant in Southeast Asia and Southern China, for instance, *A. venenata* is native to India while the small shrub *A. yunnanensis* has been found only in the south of China. The only species of the section *Winchia*, *A. glaucescens*, has been reported only from southern China downwards through the Myanmar-Thailand border to Sumatra (Monachino, 1949). The distribution of genus *Alstonia* in Southeast Asia and Australia is dominated by the members of sections *Pala* and *Monuraspermum* particularly *A. scholaris*, *A. macrophylla*, and *A. spectabilis*. All 14 species of *Alstonia* found in New Caledonia belong to the section *Dissuraspermum* (Boiteau, Allorge and Sevenet, 1977).

3. Phytochemistry of *Alstonia* species

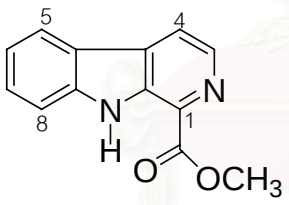
About 246 indole alkaloids have been isolated from the genus *Alstonia.* During the last ten years many more new alkaloids and other compounds have been isolated from the genus *Alstonia* and in order to provide an overall view of *Alstonia* constituents, the following section will deal with all skeletal types of compounds isolated so far from the genus together with corresponding examples. The chemical constituents found in the genus *Alstonia* are arranged in two main classes: indole alkaloids and miscellaneous compounds. The indole alkaloids are further classified on the basis of their biogenesis, according to the skeletal types proposed by Hesse and colleagues (Kisakurek *et al*., 1983; Kisakurek and Hesse, 1980), with slight modifications. Throughout this present work the generally accepted biogenetic numbering system for indole alkaloids proposed by Le Men and Taylor (1965) is used.

3.1 Indole Alkaloids

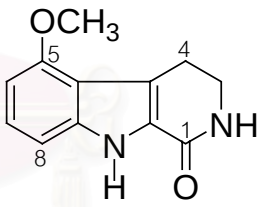
The indole alkaloids are characterized by having the indole nucleus which derives from the amino acid tryptophan and comprise two classes, i.e. simple indole alkaloids and monoterpenoid-derived indole alkaloids. Indole alkaloids can undergo oxidation or dimerization during biosynthesis from which oxindoles and bisindoles are respectively formed.

3.1.1 Simple Indole Alkaloids

Alkaloids of this group do not present a structural uniformity, having only the indole nucleus as a common feature. Only two β-carboline derivatives so far have been isolated from genus *Alstonia*, i.e. 1-carbomethoxy β-carboline from the stem bark of *A. constricta* (Allam, Beutler and Le Quesne, 1987) and 5- methoxy-1-oxo-tetrahydro-β-carboline from the root bark of *A. venenata* (Banerji *et al*., 1982).



1-Carbomethoxy-β-carboline



5-Methoxy-1-oxo-tetrahydro-β-carboline

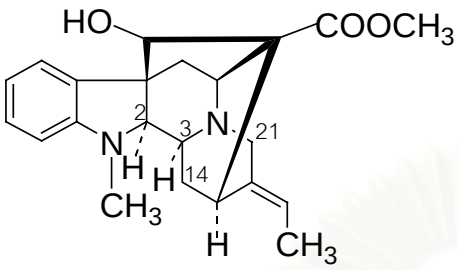
3.1.2 Monoterpenoid-derived indole alkaloids

Indole alkaloids with a C_9- or C_{10}- monoterpene moiety are classified into the following subgroups : see below. And the biologenic relationships of these main skeletal types are shown in Figure 6.

3.1.2.1 Corynanthean-type Indole Alkaloids

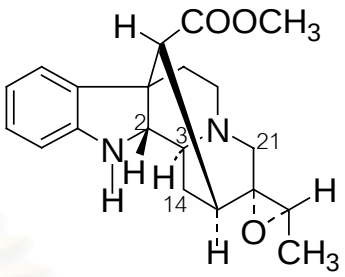
The corynanthean-type includes those alkaloids containing C(2)-C(3)-C(14) unit and N_b-C(21) bond, with the exception of the alstonidine and macroline groups which lack the N_b-C(21) bond. These alkaloids constitute the majority of *Alstonia* alkaloids and can be subdivided into 8 skeletal groups, as shown in Figure 2 with their representative alkaloids. About 135 alkaloids of this type occur throughout many species in five sections of genus *Alstonia*, notably, vincamajine derivatives (ajmaline group) from *A. constricta* (Crow *et al*., 1970) and *A. lanceolifera* (Lewin *et al*., 1975); picraline and picrinine derivatives (akuammiline group) from *A. lanceolata* (Vercauteren *et al*., 1981), *A. lanceolifera* (Ravao *et al*., 1982), *A. scholaris* (Abe *et al*., 1989), *A. venenata* (Majumder and Basu, 1982) and *A. vitiensis* (Mamatas-Kalamaras *et al*., 1975a); pericyclivine derivatives (Sarpagine group) from *A. undulata* (Guillaume *et al*., 1984; Pinchon *et al*., 1990); yohimbine derivatives (yohimbine group) from *A. quaternata* (Mamatas-Kalamaras *et al*., 1975b) and *A. venenata* (Chatterjee, Majumder and Ray, 1965a; Chatterjee, Majumder and Das, 1969; Chatterjee, Roy and Mukhopadhyay, 1981; Govindachari *et al*., 1964, 1965); vincorine and echitamine derivatives (vincorine group) *A. congensis* (Caron *et al*., 1989), *A. glaucescens* (Keawpradub *et al*., 1994), *A. scholaris* (Boonchuay and Court, 1976; Yamauchi *et al*., 1990b) and *A. sphaerocapitata* (Caron *et al*., 1984). On the other hand, the occurrence of the alkaloids belonging to alstonidine, macroline, and pleiocarpamine groups is very restricted. Only three alkaloids of the alstonidine group (Allam *et al*., 1987; Crow *et al*., 1970), six of the macroline group (Burke *et al*., 1973; Cook, Le Quesne and Elderfield, 1969; Ghedira *et al*., 1988; Hart, Johns and Lamberton, 1972; Ratnayake *et al*., 1987) and three of the pleiocarpamine group (Burke *et al*., 1973; Jacquier *et al*., 1982) have been reported from the genus *Alstonia*.

Ajmaline group



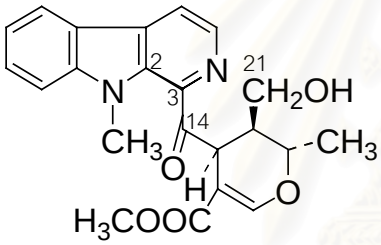
Vincamajine

Akuammiline group



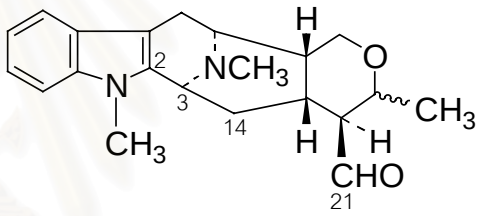
Quaternoxine

Alstonidine group



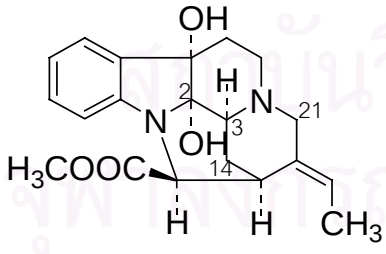
14-Ketoalstonidine

Macroline group



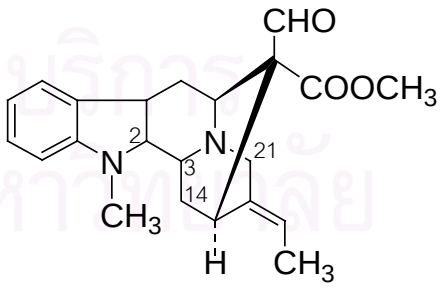
Talcarpine

Pleiocarpamine group



2,7-Dihydropleiocarpamine

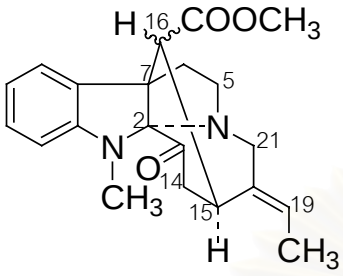
Sarpagine group



Voachalotinal

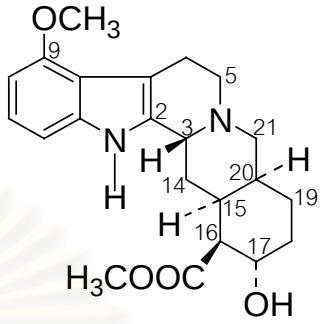
Figure 3 Skeletal group of corynanthean-type indole alkaloids occurring in *Alstonia* species

Vincorine group



Vincoridine

Yohimbine group

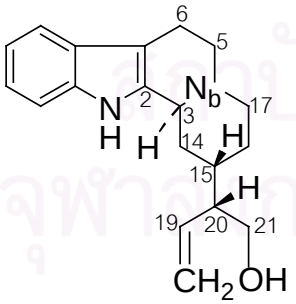


Venenatine

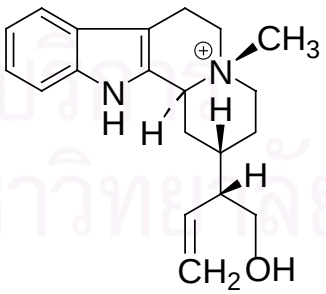
Figure 3 (continued) Skeletal group of corynanthean-type indole alkaloids occurring in Alstonia species

3.1.2.2 Vallesiachotaman-type Indole Alkaloids

Alkaloids of the type are recognized as those containing C(2)-C(3)-C(14) unit with N_b-C(17) bond. Only one skeletal type, the vallesiachotamine group, is found in the genus *Alstonia* of which two alkaloids, antirhine from *A. odontophora* (Vercauteren *et al.*, 1979) and *A. angustifolia* (Ghedira *et al.* 1988) and N_b-β-methylantirhine from *A. angustifolia* (Hu, Zhu and Hesse, 1989), have been reported.



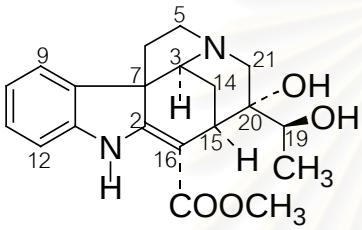
Antirhine



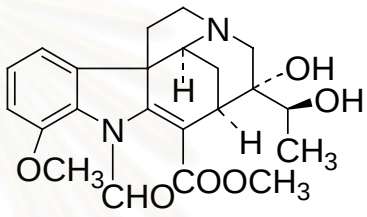
N_b-β-Methylantirhine

3.1.2.3 Strychnan-type Indole Alkaloids

The strychnan-type alkaloids are those containing C(2)-C(16)-C(15) unit with C(3)-C(7) bond. About 30 strychnan-type alkaloids isolated from genus *Alstonia* are derived from the curan stereoparent and are known as the akuammicine group. Two representative alkaloids, compactinervine from *A. lanceolata* (Vercauteren *et al*., 1981) and N_a-formyl-12-methoxyechitamidine from *A. boonei* (Oguakwa *et al.,* 1983) are illustrated.



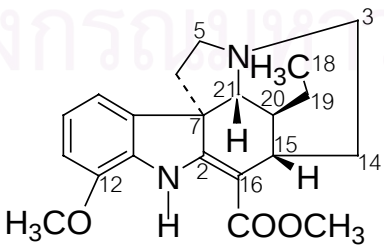
Compactinervine



N_a-formyl-12-methoxyechitamidine

3.1.2.4 Aspidospermatan-type Indole Alkaloids

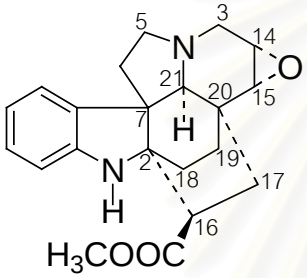
The aspidospermatan-type alkaloids are those characterized by the forming of C(2)-C(16)-C(15) unit without C(3)-C(7) bond, and in some cases with C(7)-C(21) bond instead. Four alkaloids of this type, belonging to the tubotaiwine group, have been reported from genus *Alstonia*, for instance, 12-methoxytubotaiwine from the leaves of *A. congensis* (Caron *et al*., 1989). The other remaining three were isolated exclusively from *A. scholaris* (Boonchuay and Court, 1976; Yamauchi *et al*., 1990a, 1990b).



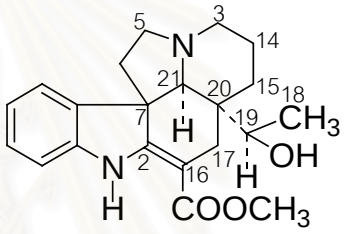
12-methoxytubotaiwine

3.1.2.5 Plumeran-type Indole Alkaloids

The plumeran-type are those containing C(2)-C(16)-C(17)-C(20) unit. Eighteen alkaloids of the type so far have been isolated from genus *Alstonia* which can be subdivided into two groups, the kopsinine group and the tabersonine group. Four kopsinine derivatives, for instance, venalstonidine, were isolated only from *A. venenata* (Chatterjee *et al.*,1981; Das and Biemann, 1965; Govindachari *et al.*, 1965). Fourteen alkaloids of the tabersonine group, for example, minovincinine, have been isolated mainly from *A. venenata* (Das *et al.*, 1966; Majumder, Chanda and Dinda, 1973; ; Majumder, *et al.,* 1981).



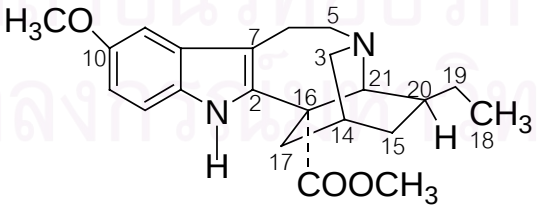
Venalstonidine



Minovincinine

3.1.2.6 Ibogan-type Indole Alkaloids

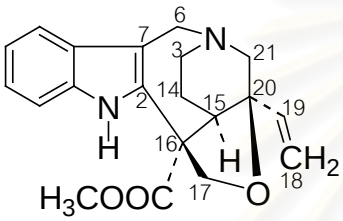
Alkaloids of the type are those containing the C(2)-C(16)-C(17)-C(14) unit. Voacangine, belonging to the catharanthine group, from *A. boonei* (Croquelois *et al.,* 1972) is the only one structure of this type which has been reported so far from the genus *Alstonia*.



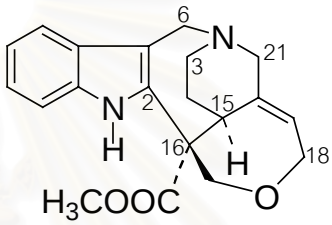
Voacangine

3.1.2.7 Vallesamine-type Indole Alkaloids

Alkaloids of this type are characterized by having C(2)-C(16)-C(15) unit with C(7)-C(6) and N_b-C(21) bonds, but lack of the typical two-carbon tryptamine bridge. Among *Alstonia* species, seventeen vallesamine-type alkaloids occur exclusively in the section *Pala* such as *A. angustiloba*, *A. congensis* and *A. scholaria*. These alkaloids are mainly derived from angustilobine A and angustilobine B (Caron *et al*., 1989; Yamauchi *et al*., 1990a,1990b).



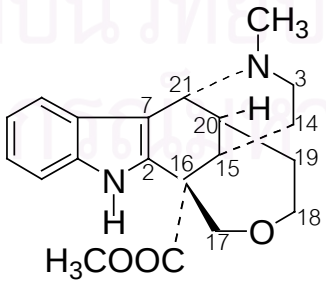
Angustilobine A



Angustilobine B

3.1.2.8 Uleine-type Indole Alkaloids

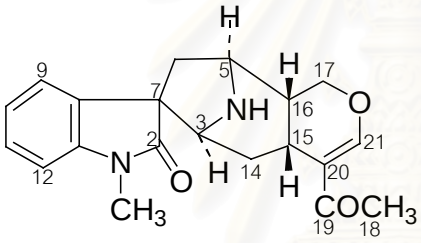
The uleine-type alkaloids are those which possess C(2)-C(16)-C(15) unit and C(7)-C(21) bond, but lack the original tryptamine side chain, having only one carbon atom between N_b and the indole nucleus. Of the genus *Alstonia*, only one alkaloid of the type, i.e. undulifoline from the stem bark of *A. undulifolia* (Massiot *et al*., 1992) has been reported.



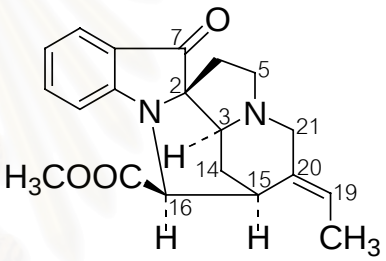
Undulifoline

3.1.2.9 Oxindole and Pseudoindoxyl Alkaloids

These alkaloids occur as oxidised forms and are typically found to occur with their corresponding indole analogues. Seven oxindole alkaloids have been reported from genus *Alstonia*, almost all of which were isolated from *A. macrophylla* (Atta-ur-Rahman, Qureshi and Muzaffar, 1988c; Atta-ur-Rahman *et al*., 1987; 1991b; Abe *et al*., 1994b; Wong, Lim and Chuah, 1996) with the exception of alstonisine which was also found in *A. muelleriana* (Elderfield and Gilman, 1972; Burke *et al*., 1973) and *A. anguslifolia* (Ghedira *et al*., 1988). The only pseudoindoxyl alkaloid reported from genus *Alstonia*, fluorocarpamine, was isolated from *A. plumosa* (Jacquier *et al*., 1982), *A. undulata* (Guillaume *et al*., 1984) and *A. angustifolia* (Ghedira *et al*., 1988).



Alstonisine

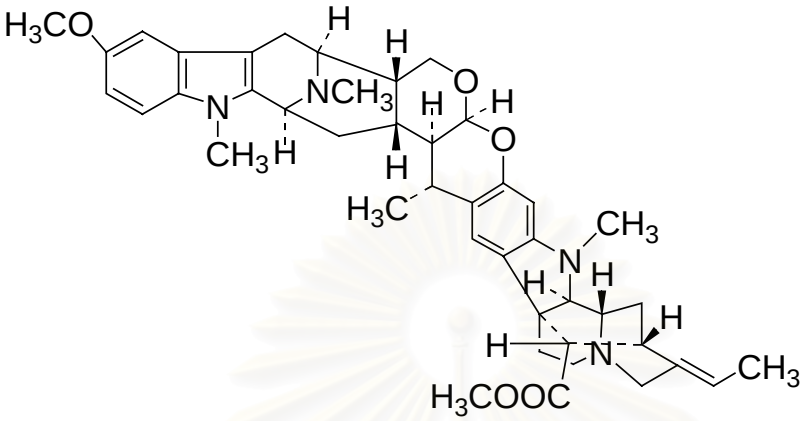


Fluorocarpamine

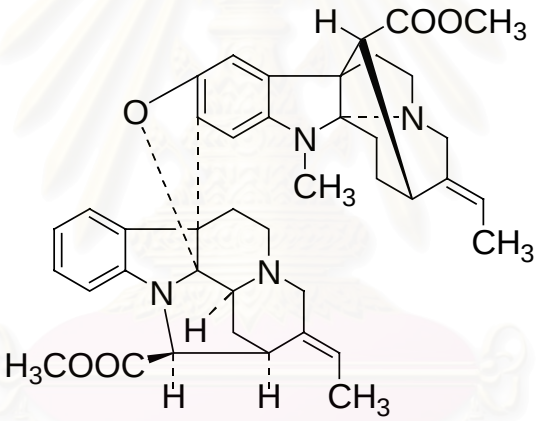
3.1.2.10 Bisindole Alkaloids

About 28 bisindole alkaloids have been isolated from several species in the sections *Monuraspermum* and *Dissuraspermum* of genus *Alstonia*. The two units of these bisindoles are typically derived from the corresponding corynanthean-type monomeric alkaloids. It is rare that these alkaloids possess two identical monomeric units. More often, different structural groups are involved in the two portions particularly those derived from macroline, akuammiline, pleiocarpamine, and sarpagine groups. Three representative bisindoles are illustrated in Figure 3, namely alstocraline from *A. angustifolia* (Ghedira *et al*., 1988); pleiocorine from *A. deplanchei* (Das *et al*., 1974), *A. odontophora*

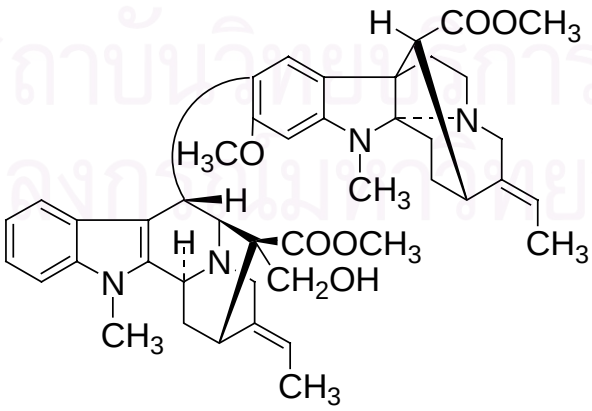
(Vercauteren *et al*., 1979) and *A. plumosa* (Jacquier *et al*., 1982); and undulatine from *A. undulata* (Pinchon *et al*., 1990).



Alstocraline



Pleiocorine



Undulatine

3.2 Miscellaneous Compounds

Apart from indole alkaloids, there are some other types of compounds including alkaloids, amides, phytosterols, and terpenoids which have been isolated from the genus *Alstonia* as listed in Table 1, and some representative compounds are illustrated in Figure 4. Although the occurrence of these compounds is very restricted, some of them such as the terpenoids boonein, sweroside, and loganin are of interest since they are involved in the biosynthesis of monoterpenoid-derived indole alkaloids. The novel structure of lanceomigine from *A. lanceolata* is the first example of an indole-derived quinoline alkaloid isolated from the genus *Alstonia*. This structural type is built up biogenetically from tryptamine and secologanin but contains a quinoline instead of an indole nucleus, mainly isolated from genera *Melodinus* and *Rhazya* of the family Apocynaceae (Hu *et al*., 1987). The quinine-related alkaloid, corialstonine, from *A. coriacea* is of interest for biological activity as far as the antimalarial activity is concerned.

Table 1 **Miscellaneous compounds known to occur in *Alstonia* species**

[Abbreviations: lvs=leaves, sb=stem bark, rb=root bark, frt=fruits, unk=unknown plant part]

Molecular Weight (Structural Type)	Compound	Source and Reference
149: $C_9 H_{11} NO$ (Alkaloid)	Venoterpine (27) [Gentialutine]	*A. venenata*: frt (Ray and Chatterjee, 1968): *A. angustiloba*: unk, *A. spatulata*: unk (Ravao *et al*., 1985)
170: $C_9 H_{14} O_3$ (Terpenoid)	Boonein (28)	*A. boonei*: sb (Marini-Bettolo *et al*., 1983)
175: $C_{10} H_9 O_2$ (Alkaloid)	Gentianine	*A. coriacea*: sb (Cherif *et al*., 1989); *A. lanceolata*: sb (Vercauteren *et al*., 1981); *A. lenormandii*: lvs (Legseir *et al*., 1986)
207: $C_{11} H_{13} O_3$ (Alkaloid)	Cantleyine	*A. angustiloba*: unk, *A. pneumatophora*: unk, *A. spatulata*: unk (Ravao *et al*., 1985) *A. undulifolia*: sb (Massiot *et al*., 1992)
211: $C_{11} H_{17} NO_3$ (Alkaloid)	Tetrahydrocantleyine	*A. angustifolia*: lvs (Ghedira *et al*., 1988) *A. undulifolia*: sb (Massiot *et al*., 1992)

Table 1 (continued) **Miscellaneous compounds known to occur in *Alstonia* species**

Molecular Weight (Structural Type)	Compound	Source and Reference
211: $C_{10}H_{13}NO_4$ (Amide)	3,4,5-Trimethoxybenzamide (29)	*A. constricta*: sb (Allam *et al*., 1987)
237: $C_{13}H_{19}NO_3$ (Alkaloid)	Angustialine (30)	*A. angustifolia*: sb (Kam *et al*., 1997)
237: $C_{12}H_{15}NO_4$ (Amide)	Cintriamide [3-(3,4,5-Trimethoxyphenyl)-2-Propenamide]	*A. lenormandii*: lvs (Legseir *et al*., 1986)
358: $C_{16}H_{12}O_9$ (Terpenoid)	Sweroside (31)	*A. glaucescens*: sb (Keawpradub *et al*., 1994)
382: $C_{22}H_{26}O_4$ (Alkaloid)	Lanceomigine (32) [N_a-Methylrhazicine]	*A. lanceolata*: sb (Vercauteren *et al*., 1981)
390: $C_{17}H_{26}O_{10}$ (Terpenoid)	Loganin (33)	*A. glaucescens*: roots (Chen *et al*., 1988)
398: $C_{22}H_{26}N_2O_5$ (Amide)	Lanceomigine N_b-oxide	*A. lanceolata*: sb (Vercauteren *et al*., 1981)
410: $C_{23}H_{26}N_2O_5$ (Amide)	Corialstonine (34)	*A. coriacea*: sb (Cherif, Massiot and Le Man-Olivier, 1987; Cherif *et al*., 1989)
412: $C_{29}H_{48}O$ (Phytosterol)	Stigmasterol (35)	*A. venenata*: bark (Govindachari *et al*., 1964)
426: $C_{30}H_{50}O$ (Terpenoid)	α-Amyrin	*A. scholaris*: unk (Mukherjee And Ghosh, 1979)
426: $C_{30}H_{50}O$ (Terpenoid)	Lupeol (36)	*A. scholaris*: unk (Mukherjee and Ghosh, 1979)

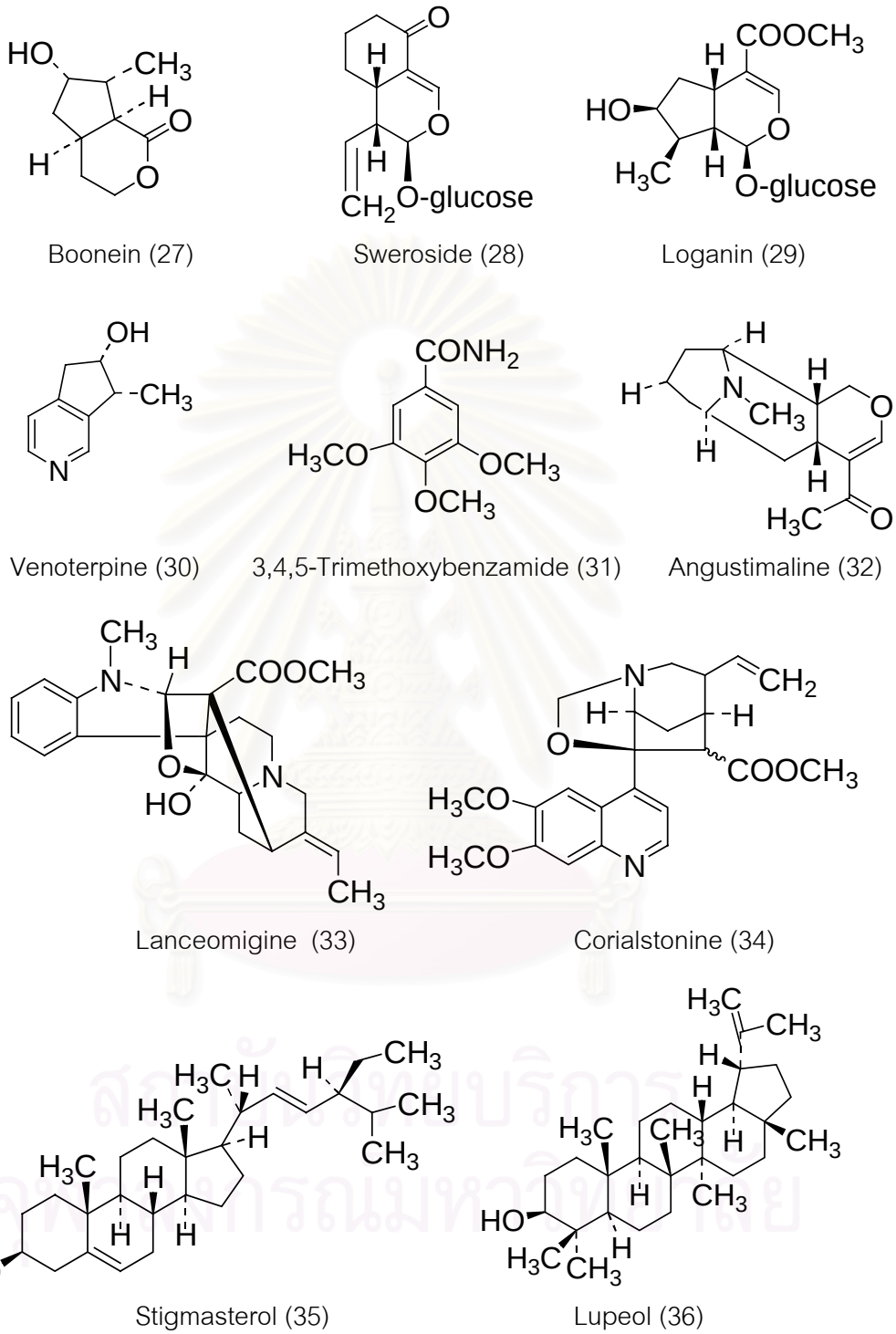


Figure 4 Structures of some miscellaneous compounds occurring in *Alstonia* species

4. Previous Indole Alkaloids Isolated from *Alstonia macrophylla*

Alstonia macrophylla has been subjected for phytochemical investigation for decades and a large number of alkaloids have been isolated. To date, about 44 indole alkaloids (Table 2) have been reported from this species.

Table 2 **Indole alkaloids isolated from *Alstonia macrophylla***

[Abbreviations: lvs=leaves, sb=stem bark, rb=root bark, frt=fruits, unk=unknown plant part]

Molecular Weight (Structural Type)	Compound	Plant Part and Reference
308: $C_{20}H_{24}N_2O$ (Sarpagine group)	Affinisine (37) [1-Methylsarpagan-1-ol]	: lvs (Banerji *et al*., 1972)
322: $C_{20}H_{22}N_2O_2$ (Pleiocarpamine group)	Pleiocarpamine (38) [Methyl 19,20-didehydro-1,16-cyclocorynan-17-oate]	: unk (Manalo *et al*., 1968)
322: $C_{20}H_{22}N_2O_2$ (Akuammiline group)	Strictamine (39) [Desacetyldesformoakuammiline] [Vincamidine]	: lvs (Abe *et al*., 1994a)
324: $C_{20}H_{24}N_2O_2$ (Sarpagine group)	Alstoumerine (40)	: lvs (Atta-ur-Rahman *et al*., 1991a)
336: $C_{21}H_{24}N_2O_2$ (Macroline group)	Alstonerine (41)	: sb (Ratnayake *et al*., 1987)
338: $C_{20}H_{22}N_2O_3$ (Oxindole)	Alstonal (42) [11-Demethoxy-N_b-demethylalstophyllal oxindole]	: bark (Wong *et al*., 1996)
338: $C_{20}H_{22}N_2O_3$ (Oxindole)	Alstonisine (43)	: lvs, bark (Atta-ur-Rahman *et al*., 1990b; Wong *et al*., 1996)
338: $C_{21}H_{26}N_2O_2$ (Akuammiline group)	Cathafoline (44) [Methyl 1,2-dihydro-1-methylakuammilan-17-oate]	: lvs (Abe *et al*., 1994a, 1994b)
338: $C_{20}H_{22}N_2O_3$ (Akuammiline group)	10-Hydroxystrictamine (45)	: lvs (Atta-ur-Rahman *et al*., 1994)

Table 2 (continued) **Indole alkaloids isolated from *Alstonia macrophylla***

Molecular Weight (Structural Type)	Compound	Plant Part and Reference
338: $C_{20}H_{22}N_2O_3$ (Akuammiline group)	Picrinine (46) [Methyl 2,5-epoxy-1,2-dihydroakuammilan-17-oate] [Vincaridine]	: lvs (Banerji *et al*., 1972)
338: $C_{21}H_{26}N_2O_2$ (Macroline group)	Talcarpine (47) [20,21-Dihydro-21-methyl-18-noralstophyllan-19-al]	: bark (Ratnayake *et al*., 1987; Wong *et al*., 1996)
339: $C_{21}H_{27}N_2O_2$ (Macroline group)	Macrosalhine (48)	: sb (Khan, Hesse and Schmid, 1967)
340: $C_{21}H_{28}N_2O_2$ (Macroline group)	Alstomacrocine	: lvs (Atta-ur-Rahman *et al*., 1994)
342: $C_{20}H_{26}N_2O_3$ (Oxindole)	Macroxine (49)	: lvs (Atta-ur-Rahman *et al*., 1991b)
352: $C_{21}H_{24}N_2O_3$ (Akuammiline group)	11-Methoxyakuammicine (50)	: lvs (Abe *et al*., 1994b)
352: $C_{21}H_{24}N_2O_3$ (Ajmaline group)	Quebrachidine (51) [Methyl 19,20-didehydro-1-demethyl-17-hydroxyajmalan-16-carboxylate]	: lvs (Atta-ur-Rahman *et al*., 1990b)
354: $C_{21}H_{26}N_2O_3$ (Akuammiline group)	Cathafoline N_b-oxide (52)	: lvs (Abe *et al*., 1994b)
354: $C_{21}H_{26}N_2O_3$ (Akuammiline group)	Strictaminolamine (53)	: lvs (Atta-ur-Rahman *et al*., 1988a)

Table 2 (continued) **Indole alkaloids isolated from *Alstonia macrophylla***

Molecular Weight (Structural Type)	Compound	Plant Part and Reference
366: $C_{22}H_{26}N_2O_3$ (Macroline group)	Alstophylline (54) [11-Methoxyalstonerine]	: lvs, sb (Abe *et al*., 1994b; Kishi *et al*., 1965; Ratnayake *et al*., 1987)
366: $C_{22}H_{26}N_2O_3$ (Ajmaline group)	Vincamajine (55)	: lvs (Abe *et al*., 1994b; Atta-ur-Rahman, Nighat and Choudhary, 1988b; Ratnayake *et al*., 1987)
368: $C_{21}H_{24}N_2O_4$ (Akuammiline group)	Alstolagumine (56) [11-Methoxy-19α,20α-epoxyakuammicine]	: lvs (Abe *et al*., 1994a)
368: $C_{22}H_{28}N_2O_3$ (Akuammiline group)	Cabucraline (57) [11-Methoxycathafoline]	: sb (Ratnayake *et al*., 1987)
368: $C_{21}H_{24}N_2O_4$ (Akuammiline group)	11-Methoxyakuammicine N_b-oxide (58)	: lvs (Abe *et al*., 1994b)
368: $C_{21}H_{24}N_2O_4$ (Oxindole)	N_b-Demethylalstophyllal oxindole (59)	: bark (Wong *et al*., 1996)
368: $C_{21}H_{24}N_2O_4$ (Oxindole)	N_b-Demethylalstophylline oxindole (60)	: lvs (Abe *et al*., 1994b; Atta-ur-Rahman *et al*., 1987; Wong *et al*., 1996)
368: $C_{22}H_{28}N_2O_3$ (Vincorine group)	Vincorine (61) [Methyl 10-methoxy-1-methyl-2,4(1*H*)-cyclo-3,4-secoakuammilan-17-oate]	: lvs (Abe *et al*., 1994b; Ratnayake *et al*., 1987)
382: $C_{22}H_{26}N_2O_4$ (Vincorine group)	Demethoxyalstonamide (62)	: lvs (Atta-ur-Rahman *et al*., 1991a)

Table 2 (continued) **Indole alkaloids isolated from *Alstonia macrophylla***

Molecular Weight (Structural Type)	Compound	Plant Part and Reference
384: $C_{21}H_{24}N_2O_5$ (Oxindole)	16-Hydroxy-N_b-demethylalstophylline oxindole (63)	: lvs (Atta-ur-Rahman *et al*., 1988c)
384: $C_{22}H_{28}N_2O_4$ (Ajmaline group)	19-Hydroxyvincamajine (64)	: lvs (Ratnayake *et al*., 1987)
384: $C_{21}H_{24}N_2O_5$ (Akuammiline group)	Lagumidine (65) [11-Methoxy-19-oxo-20α-hydroxyakuammicine]	: lvs (Abe *et al*., 1994a)
398: $C_{22}H_{26}N_2O_5$ (Akuammiline group)	Norquaternine (66) [10,11-Dimethoxypicrinine] [Volkensine]	: lvs (Abe *et al*., 1994a)
400: $C_{22}H_{28}N_2O_5$ (Akuammiline group)	Alstozine N_b-oxide	: lvs (Atta-ur-Rahman *et al*., 1990a)
412: $C_{23}H_{28}N_2O_5$ (Vincorine group)	Alstonamide (67)	: lvs (Atta-ur-Rahman *et al*., 1991a)
412: $C_{23}H_{28}N_2O_5$ (Akuammiline group)	Alstopicralamine (68)	: lvs (Atta-ur-Rahman *et al*., 1988b)
412: $C_{23}H_{28}N_2O_5$ (Akuammiline group)	Quaternine (69) [10,11-Dimethoxy-N_a-methylpicrinine]	: lvs (Abe *et al*., 1994a)
412: $C_{23}H_{28}N_2O_5$ (Akuammiline group)	5α,10,11-Trimethoxystrictamine (70)	: lvs (Abe *et al*., 1994a)
470: $C_{29}H_{30}N_2O_4$ (Ajmaline group)	*O*-Benzoylvincamajine	: lvs (Mukherjee *et al*., 1969)
516: $C_{30}H_{32}N_2O_6$ (Akuammiline group)	10-Methoxy-N_a-methylburnamine-17-*O*-benzoate	: lvs (Abe *et al*., 1994a)

Table 2 (continued) **Indole alkaloids isolated from *Alstonia macrophylla***

Molecular Weight (Structural Type)	Compound	Plant Part and Reference
530: $C_{31}H_{34}N_2O_6$ (Ajmaline group)	Vincamajine-17-*O*-veratrate [*O*-(3,4-Dimethoxybenzoyl)-vincamajine]	: lvs (Abe *et al*., 1994b)
546: $C_{31}H_{34}N_2O_7$ (Ajmaline group)	N_a-Demethylvincamajine-N_a-tri-*O*-methylgallate	: lvs (Abe *et al*., 1994b)
576: $C_{32}H_{36}N_2O_8$ (Akuammiline group)	10-Methoxy-N_a-methylburnamine-17-*O*-veratrate	: lvs (Abe *et al*., 1994a)
642: $C_{41}H_{46}N_4O_3$ (Bisindole)	Macrocarpamine (71) (macroline-pleiocarpamine group)	: bark (Mayerl and Hesse, 1978)
644: $C_{41}H_{48}N_4O_3$ (Bisindole)	Macralstonidine (macroline-sarpagine group)	: bark (Sharp, 1934; Waldner *et al*., 1967)
660: $C_{41}H_{48}N_4O_4$ (Bisindole)	Villalstonine (72) (macroline-pleiocarpamine group)	: bark (Hesse *et al*., 1965; Sharp, 1934)
704: $C_{43}H_{52}N_4O_5$ (Bisindole)	Macralstonine (73) (macroline-macroline group)	: bark (Kishi *et al*., 1966; Ratnayake *et al*., 1987)

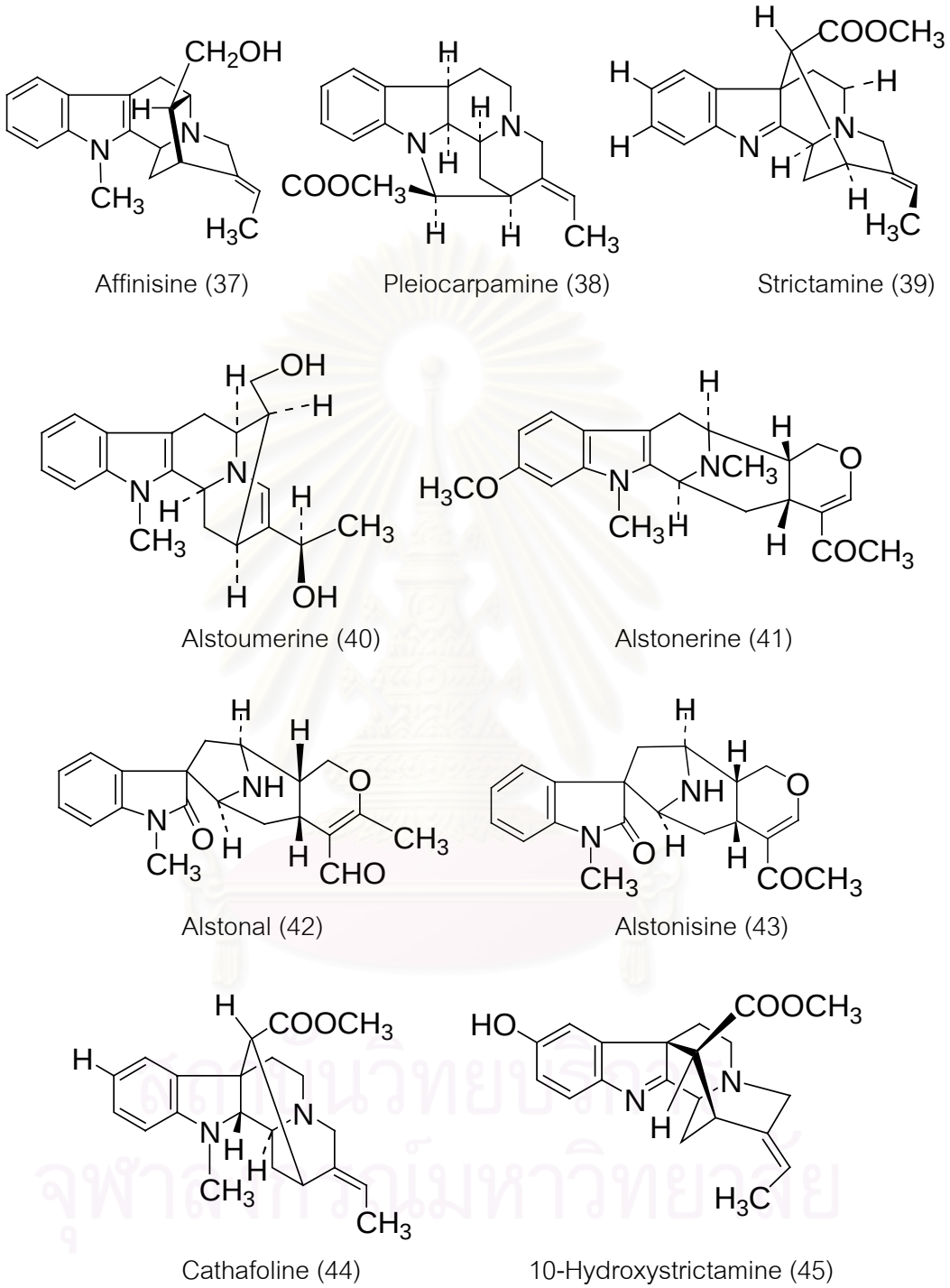
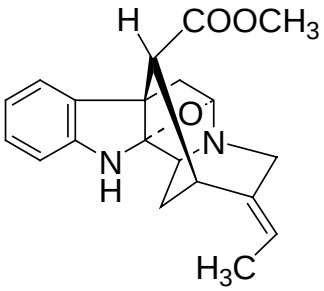
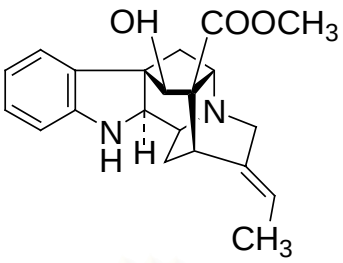


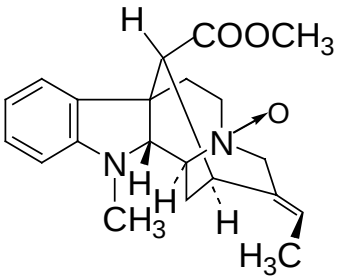
Figure 5 Structure of some indole alkaloids isolated from *Alstonia macrophylla*



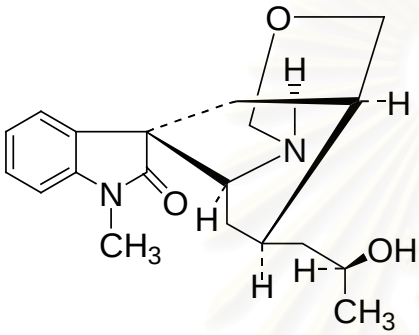
Picrinine (46)



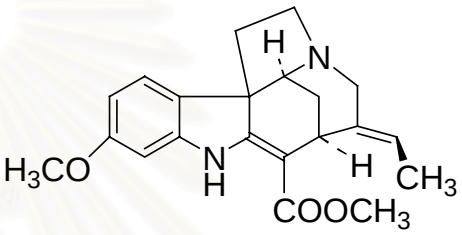
Quebrachidine (51)



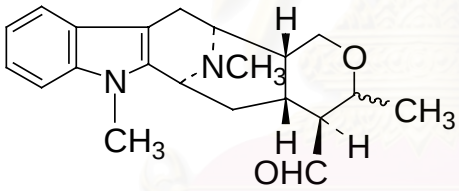
Cathafoline N_b-oxide (52)



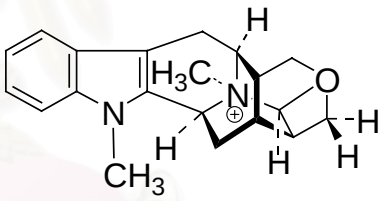
Macroxine (49)



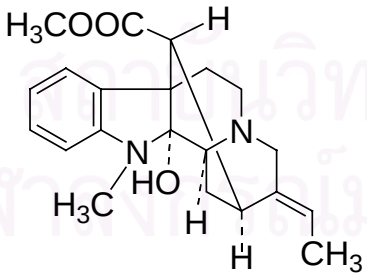
11-Methoxyakuammicine (50)



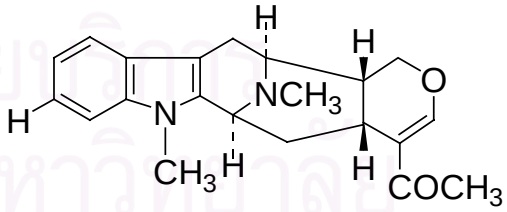
Talcarpine (47)



Macrosalhine (48)

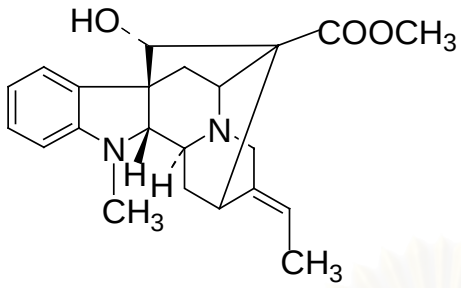


Strictaminolamine (53)

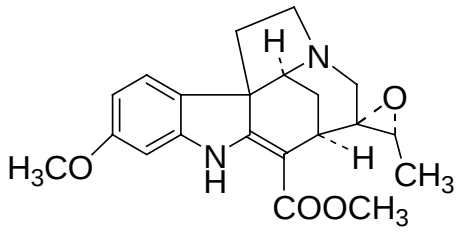


Alstophylline (54)

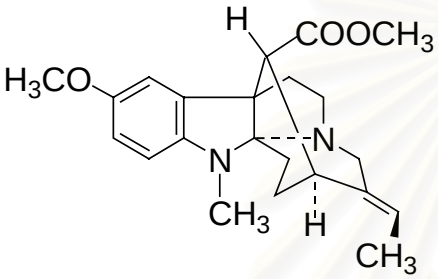
Figure 5 (continued) Structure of some indole alkaloids isolated from *Alstonia macrophylla*



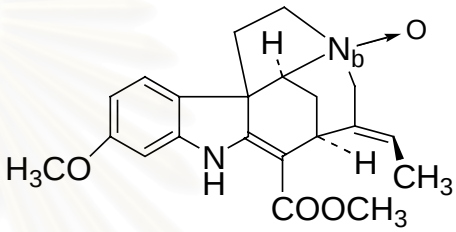
Vincamajine (55)



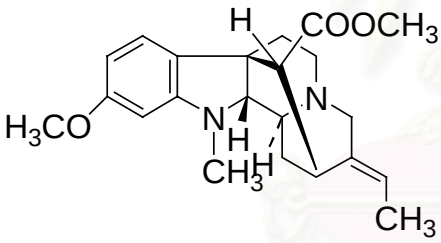
Alstolagumine (56)



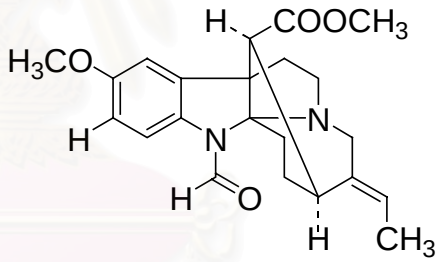
Cabucraline (57)



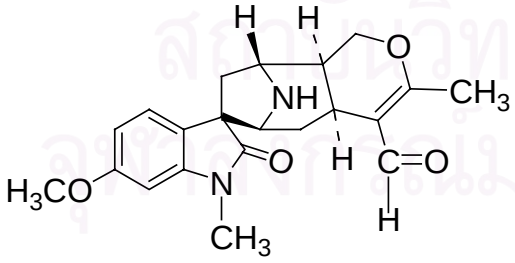
11-Methoxyakuammicine N_b-oxide (58)



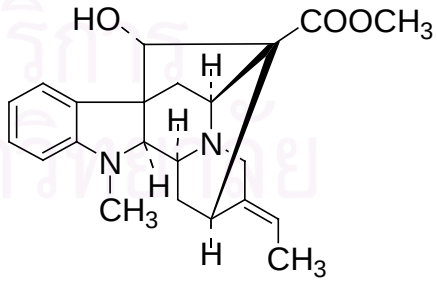
Vincorine (59)



Demethoxyalstonamide (62)

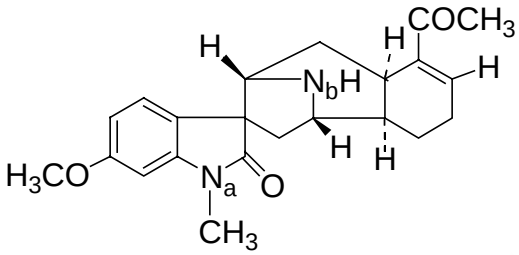


N_b-Demethylalstophyllal oxindole (59)

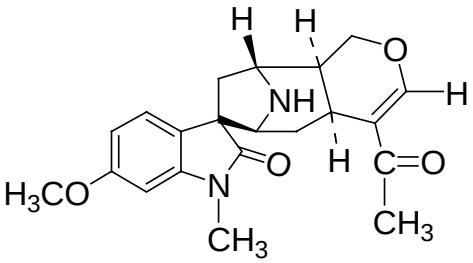


N_b-Demethylalstophylline oxindole (60)

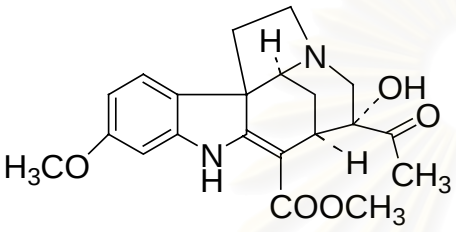
Figure 5 (continued) Structure of some indole alkaloids isolated from *Alstonia macrophylla*



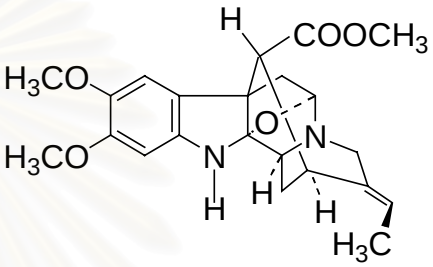
16-Hydroxy-N_b-demethylalstophylline oxindole (63)



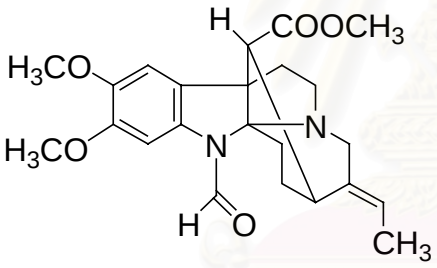
19-Hydroxyvincamajine (64)



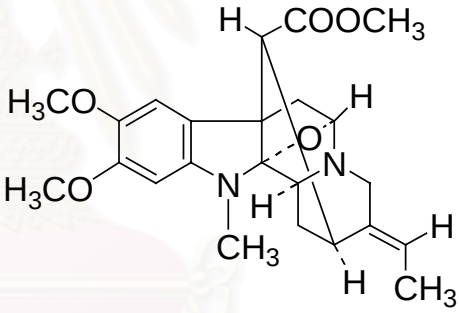
Lagumidine (65)



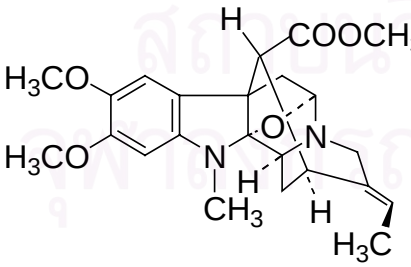
Norquaternine (66)



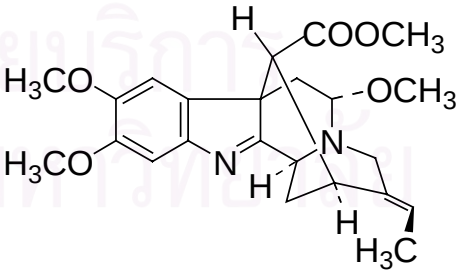
Alstonamide (67)



Alstopicralamine (68)

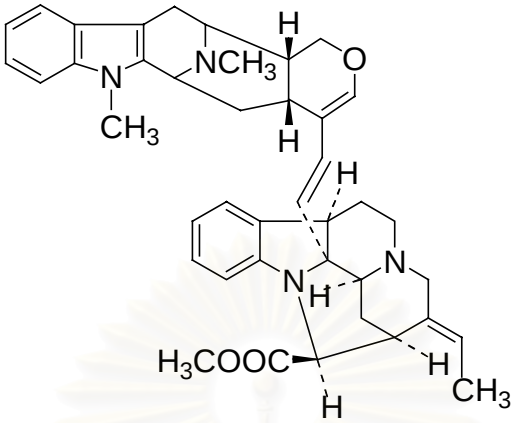


Quaternine (69)

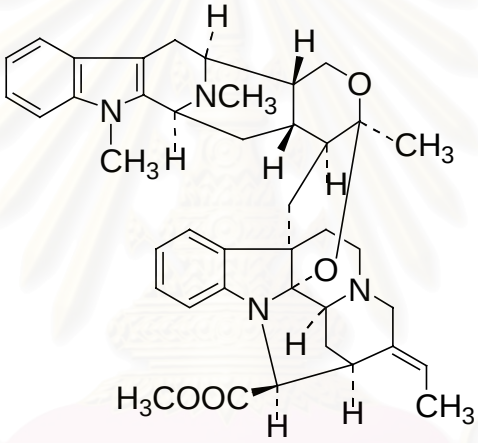


5α,10,11-Trimethoxystrictamine (70)

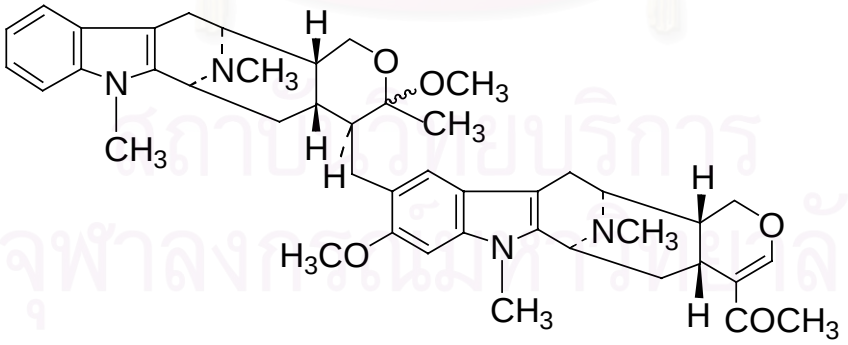
Figure 5 (continued) Structure of some indole alkaloids isolated from *Alstonia macrophylla*



Macrocarpamine (71)



Villastonine (72)



Macralstonine (73)

Figure 5 (continued) Structure of some indole alkaloids isolated from *Alstonia macrophylla*

5. Plausible Biogenetic Pathway of Monoterpenoid-derived Indole Alkaloids occurring in *Alstonia* species

The well-established knowledge on biogenesis of indole alkaloids, illustrates the plausible biogenetic pathways leading to the different structural groups of the indole alkaloids so far isolated from the genus *Alstonia*. With the exception of simple indole alkaloids, monoterpenoid-derived indole alkaloids are biogenetically derived from tryptamine (74), the decarboxylation product of tryptophan, and a monoterpenoid secologanin (75) via the key intermediate strictosidine (76) (Stockigt and Zenk, 1977) and the subsequent elaborations of the presumed intermediates 4,21-dehydrogeissoschizine (77), stemmadenine-related iminium cation (78), and 4,21-dehydrosecodine (79) (Atta-ur-Rahman and Basha, 1983; Cordell, 1981; Van Beek *et al.,* 1984).

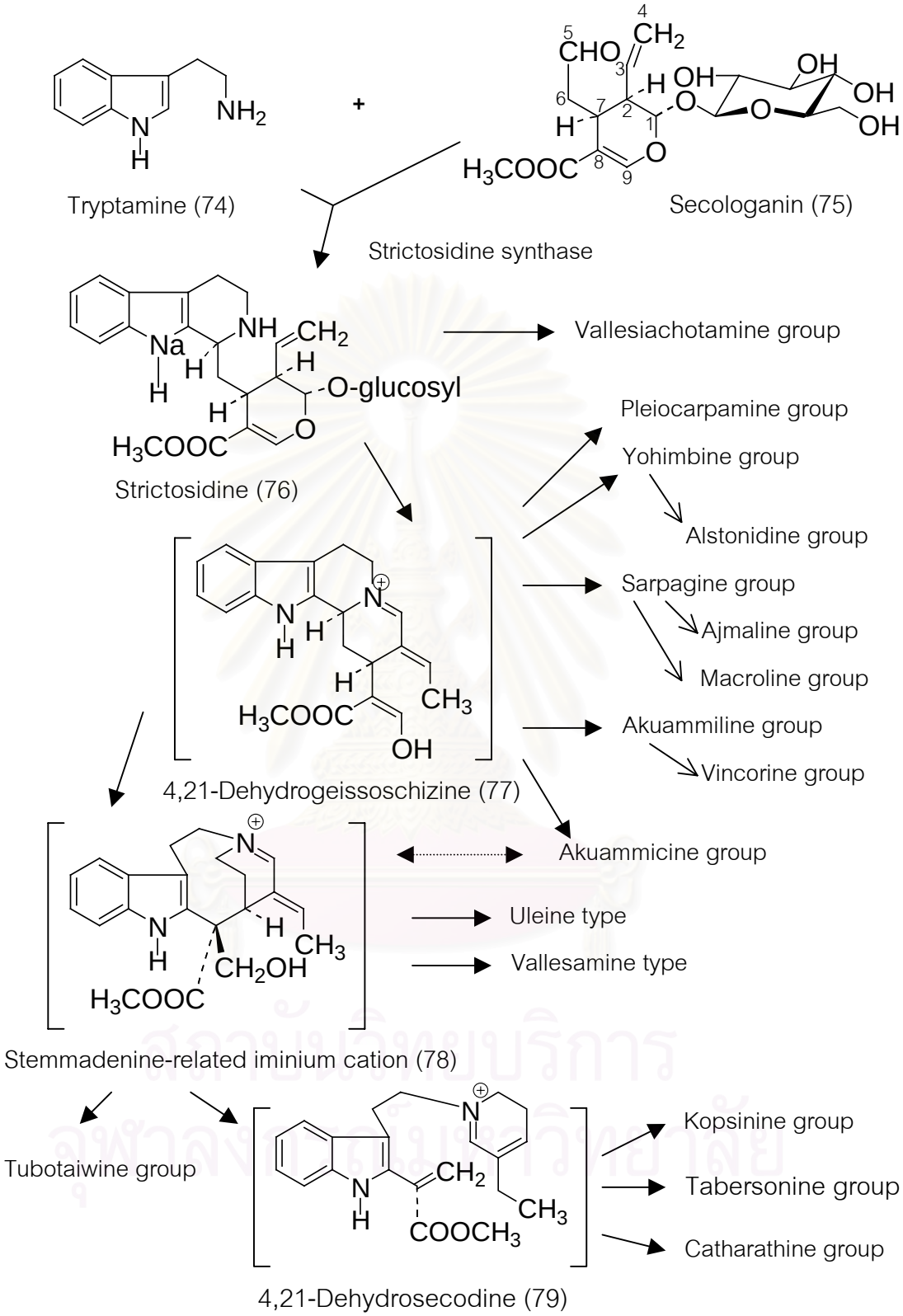


Figure 6 Plausible biogenetic interrelationships of various structural groups of monoterpenoid-derived indole alkaloids occurring in *Alstonia* species

6. Biological Activity of the Genus *Alstonia*

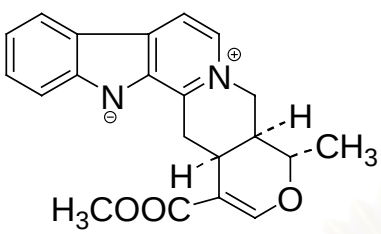
In contrast to the enormous amount of phytochemical work, relatively little is known about the biological activity of extracts or alkaloids of genus *Alstonia*. Almost all of previous investigations have been focused on antimalarial activity of some extracts and alkaloids from a few species. In 1930, the results of alkaloid extracts from the barks of four *Alstonia* species tested against avian malaria, *Plasmodium inconstans*, were published by Goodson and co-worker (cited by Wright *et al*., 1993). Slight activity was observed for the total alkaloids of *A. scholaris* and for *A. constricta* at daily oral doses of 125 mg/kg and 500 mg/kg, respectively, for 6 days. On the other hand, the total alkaloids of *A. congensis* and *A. macrophylla* were inactive in the same tests. Hawkins and Elderfield (1942) also reported that finely ground bark of *A. constricta* was inactive when fed to birds infected with avian malaria at a dose of 150 mg/day and the total alkaloid extract of the bark was inactive at doses of 60-120 mg/day both as the free bases and hydrochorides. Alstonine (80), the major alkaloid found in the bark of *A. constricta*, was also inactive against avian malaria at a dose of 35 mg/day (Leonard and Elderfield, 1942). Furthermore, Mukerji (1946) reported that *A. scholaris* alkaloids exhibited no antiplasmodial effect in fowls, but a pronounced febrifugal activity was noticed. More recent investigation by Gandhi and Vinayak (1990) has demonstrated that both petroleum ether and methanol extracts of the bark of *A. scholaris* were inactive orally against *Plasmodium berghei* in mice. However, a dose-dependent improvement of conditions and delayed mortality was noticed amongst animals treated with methanol extract even though it had no direct antiplasmodial activity. In contrast to these findings, it was reported that echitamine (81), the main alkaloid of *A. scholaris*, given subcutaneously as a chloride salt, was effective against *P. berghei* in mice at a dose of 1.6 mg/kg. However, its LD_{50} in mice by intravenous route was 13.7 mg/kg (Vasanth *et al*., 1990). The same authors also reported that alstonine (80), as a hydrochloride salt, was active against *P. lophurae* in ducks and was about 2-3 times more effective than quinine dihydrochloride, but it was found to be more toxic. Various doses of methanol extract from

the leaves of the African species *A. congensis* were screened for antimalarial activity using *P. berghei* in mice (Awe and Opeke, 1990). The extract, when given orally in 4 days suppressive test of blood schizontocidal action, produced a dose-dependent suppressive effect in the early infection with a maximum of 75% at a dose of 200 mg/kg/day while 90% suppression of parasitaemia was demonstrated by chloroguine at a dose of 5 mg/kg/day. However, the extract had no significant activity against the established infection. It was also reported by Asuzu and Anaga (1991) that the aqueous extract of *A. boonei*, the other African species, noticeably reduced the level of parasitaemia in mice infected with *Trypanosoma brucei* at a dose of 100 mg/kg (i.p.), for 5 days.

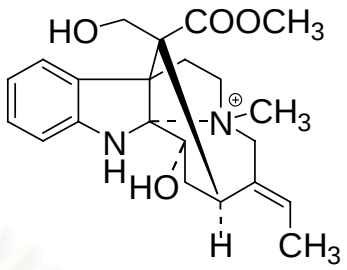
It is of interest to note that the investigations for antimalarial activity of extracts and alkaloids of *Alstonia* species prior to 1990 were carried out *in vivo* and none of the test organisms were infected with *P. falciparum*, the human malaria parasite. In recent years *in vitro* testing procedures for antiprotozoal activity have been developed. Two bisindole alkaloids isolated from the roots of *A. angustifolia*, macrocarpamine (82) and villastonine (83), have been reported to possess pronounced antiprotozoal activity (*in vitro*) against *P. falciparum* and *Entamoeba histolytica* with IC_{50} values in the ranges of 2.9-11.8 μM (Wright *et al*., 1992). Also, echitamine (81) and the quinoline alkaloid from *A. coriacea*, corialtonine (34), were investigated for *in vitro* antiprotozoal activity (Wright *et al*., 1993). Echitamine (81) exhibited slight antiplasmodial activity against *P. falciparum* with an IC_{50} value of 42.6 μM, but corialstonine (34) was found to be more active with an IC_{50} value of 5.7 μM which was about 10 times less potent than that of quinine. Disappointingly, the two alkaloids were inactive against *Giardia intestinalis* at 60 μM.

Recently, Keawpradub *et al*. (1999b) reported that methanol extracts prepared from various parts of *Alstonia scholaris*, *A. macrophylla* and *A. glaucescens*, collected from Thailand, have been assessed for antiplasmodial activity against multidrug-resistant K1 strain of *Plasmodium falciparum* cultured in human erythrocytes. Pronounced antiplasmodial activity was exhibited by methanol extract of the root bark of *A. macrophylla* with an IC_{50} value of 5.7 μg/ml. Thirteen indole alkaloids were isolated from the active

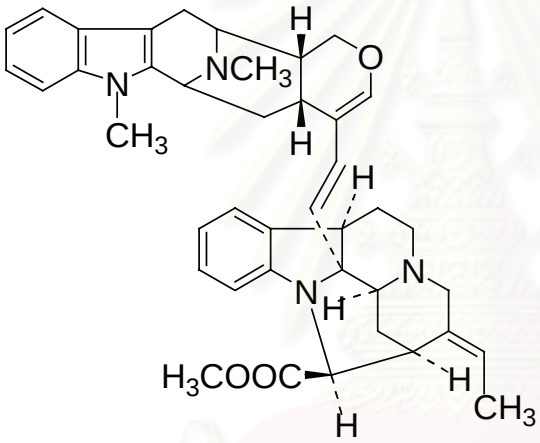
extract. These alkaloids and a semisynthetic bisindole *O*-acetylmacralstonine were subsequently tested against the K1 strain of *P. falciparum*. Pronounced antiplasmodial activity was observed mainly among the bisindole alkaloids, particularly villalstonine (83) and macrocarpamine (82) with IC_{50} value of 0.27 and 0.35 μM, respectively. The potent alkaloids were further tested against T9-96, the chloroquine-sensitive strain of *P. falciparum*. It has been found that the active alkaloids, in contrast to chloroquine, have significantly higher affinity to K1 strain than to the T9-96 strain. Furthermore, Keawpradub *et al*. (1999a) reported that thirteen indole alkaloids isolated from the root bark of *Alstonia macrophylla* and a semisynthetic bisindole *O*-acetylmacralstonine have been assessed for cytotoxic activity against two human lung cancer cell lines, MOR-P (adenocarcinoma) and COR-L23 (large cell carcinoma), using the SRB assay. Pronounced cytotoxic activity was exhibited by the bisindoles on both cell lines. This suggests that, in comparison with the corresponding monomeric indoles, at least part of both the ring systems present in the bisindoles is essential for cytotoxic activity. The potent alkaloids were further tested against a human normal cell line (breast fibroblasts) and other human cancer cell lines including StMl1 1a (melanoma), Caki-2 (renal cell carcinoma), MCF7 (breast adenocarcinoma), and LS174T (colon adenocarcinoma). The bisindoles *O*-acetylmacralstonine, villalstonine and macrocarpamine were found to possess pronounced activity against cancer cell lines with IC_{50} value in the range of 2-10 μM with no discernible cell-type selectivity. However, *O*-acetylmacralstonine displayed discernibly less toxicity against the normal breast fibroblasts.



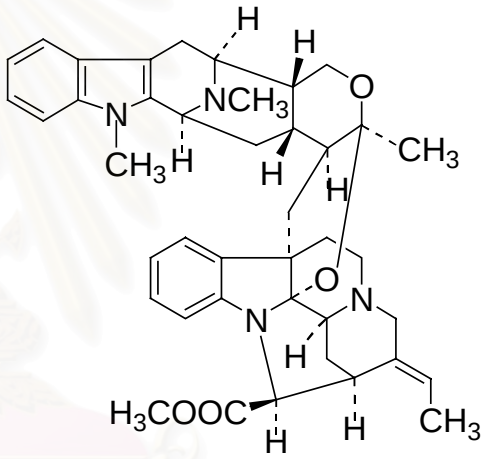
Alstonine (80)



Echitamine (81)



Macrocarpamine (82)



Villastonine (83)

Figure 7 Structures of investigated alkaloids from genus *Alstonia* that have interesting biological activities

CHAPTER III

Experimental

1. Source of Plant Materials

The leaves of *Alstonia macrophylla* Wall. ex G. Don were collected from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand, in June 2001. Authentication of plant materials was done by comparison with herbarium specimens at the Royal Forest Department, Ministry of Agriculture and Co-operatives, Bangkok, Thailand.

2. General Techniques

2.1 Analytical Thin-Layer Chromatography

Technique	:	One dimension, ascending
Adsorbent	:	Silica gel 60 F_{254} (E. Merck) precoated plate
Layer thickness	:	250 μm
Distance	:	5 cm
Temperature	:	Laboratory temperature (24-30 °C)
Detection	:	1. Ultraviolet light at wavelength 254 nm
		2. Dragendorff's spray reagent
		Solution A: bismuth subnitrate (850 mg), distilled water (40 ml) and acetic acid (10 ml)
		Solution B: potassium iodide (8 mg) and distilled water (20 ml)

Solutions A and B, each of 5 ml were mixed. Then 20 ml of glacial acetic acid and 70 ml of distilled water were added and used as spray reagent. The alkaloids give orange spots as positive test.

2.2 Column Chromatography

2.2.1 Flash Column Chromatography

Adsorbent	:	Silica gel 60 (No. 9385) particle size 0.040-0.063 mm (230-400 mesh ASTM) (E. Merck)
Packing method	:	Wet packing
Sample loading	:	The sample was dissolved in a small amount of eluent and then applied gently on top of the column.
Detection	:	1. Fractions were examined by TLC under UV light at the wavelength 254 nm. 2. Fractions were examined by TLC using Dragendorff's spray reagent.

2.3 Crystallization technique

The compounds were crystallized from the unsoluble differently solvents. Each compound was dissolved in selected solvent until saturated and let standing at room temperature until amorphous powder or crystals were formed.

2.4 Spectroscopy

2.4.1 Ultraviolet (UV) Aborption Spectra

UV (in methanol) spectra were obtained on a JASCO V-560 instrument (Japan) or a Shimadzu UV-160A UV/vis spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.4.2 Infrared (IR) Absorption Spectra

IR spectra (KBr disc and film) were recorded on a Perkin-Elmer Spectrum 2000 FT-IR spectrometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.4.3 Mass Spectra

Electron Impact (EIMS) were measured with a JEOL GCmate (Japan) or a FISONS VG TRIO 2000 mass spectrometer (Department of Chemistry, Faculty of Science, Chulalongkorn University).

2.4.4 Proton and Carbon-13 Nuclear Magnetic Resonance (^{1}H and ^{13}C-NMR) Spectra

^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were obtained with a JEOL JNM-EPC 600 NMR spectrometer (Japan). ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained with a Bruker Avance DPX-300 FT-NMR spectrometer (Faculty of Pharmaceutical Sciences, Chulalongkorn University).

The solvent for NMR spectra was deuterated chlorofom (chloroform-α). Chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal.

2.5 Physical Properties

2.5.1 Melting Points

Melting Points were obtained on a Fisher/Johns melting point apparatus (Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.5.2 Optical Rotations

Optical Rotations were measured on a Perkin Elmer 341 polarimeter (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.6 Solvents

Throughout this work, all organic solvents were of commercial grade and redistilled prior to use.

3. Extraction and Isolation

3.1 Extraction

The dried and powdered leaves of *Alstonia macrophylla* (6.75 kg) were moistened with strong solution of ammonium hydroxide overnight. They were macerated with methanol for one day four times (4×12 L) and filtered. Each filtrate was concentrated under reduced pressure to syrupy mass, added with glacial acetic acid (100 ml) poured into distilled water (1900 ml) to give about 5% glacial acetic acid solution. The suspension was well shaken and left to stand overnight. The filtered acid extract was washed and made alkaline (pH 8-9) with concentrated ammonium hydroxide solution and extracted with 5% methanol in chloroform (4×2 L). The combined chloroform extract was washed with distilled water, dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to yield dried crude alkaloidal extract (77.41 g).

Thin layer chromatograms of these crude extracts indicated that at least 7 compounds were present in addition to base-line alkaloids.

3.2 Isolation

The crude methanolic alkaloidal extract (7.25 g) was dissolved in a small amount of chloroform and packed onto top of wet silica gel column (4.5×16 cm). The column was eluted with each 300 ml of chloroform, chloroform : ethyl acetate (9:1), chloroform : ethyl acetate (4:1), chloroform : ethyl acetate (1:1), ethyl acetate, ethyl acetate : methanol (20:1), ethyl acetate : methanol (9:1), ethyl acetate : methanol (4:1), ethyl acetate : methanol (1:1) and then washed with methanol until no traces of alkaloids could be detected. Fractions of 50 ml were collected and compared by TLC. The eluting solvents were altered to give more polar solvent systems when the difference of alkaloidal patterns on TLC were observed. Those fractions of similar pattern were combined and evaporated to dryness under reduced pressure. By this procedure :-

1. Fractions 1-8 were combined and designated as fraction A (0.042 g).
2. Fractions 9-19 were combined and designated as fraction B (0.075 g).
3. Fractions 20-27 were combined and designated as fraction C (1.687 g).
4. Fractions 28-45 were combined and designated as fraction D (1.823 g).
5. Fractions 46-68 were combined and designated as fraction E (3.861 g).
6. Fractions 69-126 were combined and designated as fraction F (1.376 g).

3.2.1 Isolation of compounds AM-1 and AM-2 from fraction C, fraction C-3 and fraction C-33

3.2.1.1 Isolation of fraction C-3 from fraction C

Fraction C (1.687 g) was shown by TLC to contain at least three compounds. It was dissolved in small amount of n-hexane and packed onto the top of wet silica gel column (3×16 cm). The column was eluted with n-hexane : ethyl acetate (8:2), n-hexane : ethyl acetate (1:9) and then washed with methanol until no traces of compounds could be detected. Ninety-eight fractions, each of approximately 30 ml, were collected. The volumes of eluent were 1600, 850 and 500 ml, respectively. The fractions were examined by TLC and the liked fractions were combined to give the following portions :-

1. Fractions 8-24 were combined and designated as fraction C-1 (0.022 g).
2. Fractions 25-27 were combined and designated as fraction C-2 (0.003 g).
3. Fractions 28-60 were combined and designated as fraction C-3 (0.169 g).
4. Fractions 61-90 were combined and designated as fraction C-4 (1.259 g).
5. Fractions 91-98 were combined and designated as fraction C-5 (0.183 g).

3.2.1.2 Isolation of fraction C-33 from fraction C-3

Fraction C-3 (0.169 g) was shown by TLC to contain at least seven compounds. It was dissolved in small amount of chloroform and packed onto the top of wet silica gel column (1.5×18 cm). The column was eluted with n-hexane : ethyl acetate (1:2). Then washed with methanol until no traces of compounds could be detected. Twenty-four fractions, each of approximately 20 ml, were collected. The

volumes of eluent were 250 ml and 200 ml, respectively. The fractions were examined by TLC and the liked fractions were combined to give the following portions :-

1. Fractions 1-3 were combined and designated as fraction C-31 (0.002 g).
2. Fractions 4 was designated as fraction C-32 (0.011 g).
3. Fractions 5-6 were combined and designated as fraction C-33 (0.118 g).
4. Fractions 7-8 were combined and designated as fraction C-34 (0.004 g).
5. Fractions 9-16 were combined and designated as fraction C-35 (0.008 g).
6. Fractions 17-20 were combined and designated as fraction C-36 (0.021 g).
7. Fractions 21-24 were combined and designated as fraction C-37 (0.044 g).

3.2.1.3 Isolation of compounds AM-1 and AM-2 from fraction C-33

Fraction C-33 (0.118 g) was shown by TLC to contain at least five compounds. It was dissolved in small amount of chloroform, mixed with a small amount of silica gel, vacuum dried and packed onto the top of silica gel column (1.5×22 cm). The column was eluted with n-hexane : ethyl acetate (1:1). Thirty-two fractions, each of approximately 10 ml were collected, until no traces of compounds could be detected. The volumes of eluent were 400 ml. The fractions were examined by TLC and the liked fractions were combined to give the following portions :-

1. Fractions 1-8 were combined and designated as fraction C-331 (0.005 g).

2. Fractions 9-10 were combined and the TLC chromatogram shown only one spot under UV light at 254 nm, R_f 0.37 [silica gel / n-hexane : ethyl acetate (1:1)] and 0.57 [silica gel / chloroform : ethyl acetate (1:1)]. These fractions were evaporated under reduced pressure to give 0.022 g of compound AM-1 as colorless rosette (0.303% based on dried weight of crude alkaloidal extract). It was later identified as demethoxyalstonamide.
3. Fractions 11-15 containing mixture of compounds (0.050 g).
4. Fractions 16-25 were combined and the TLC chromatogram shown only one spot under UV light at 254 nm, R_f 0.28 [silica gel / n-hexane : ethyl acetate (1:1)] and 0.32 [silica gel / n-hexane : ethyl acetate (2:1)]. These fractions were evaporated under reduced pressure to give 0.025 g of compound AM-2 as pale yellow powder (0.345% based on dried weight of crude alkaloidal extract). It was later identified as (-)-1-norvincorine.
5. Fractions 26-29 were combined and designated as fraction C-335 (0.003 g).
6. Fractions 30-32 were designated as fraction C-336 (0.024 g).

3.2.2 Isolation of compound AM-3 from fraction C-4 and fraction C-47

3.2.2.1 Isolation of fraction C-47 from fraction C-4

Fraction C-4 (1.259 g) was shown by TLC to contain at least three compounds. It was dissolved in small amount of ethyl acetate and packed onto the top of wet silica gel column (3×23 cm). The column was eluted with ethyl acetate and fifty- three fractions, each of approximately 30 ml, were collected. The eluates were examined by TLC using ethyl acetate. Then washed with methanol until no traces of compounds could be detected and thirty-seven fractions, each of approximately 50 ml, were collected and compared with TLC. The volumes of eluent were 1500 and 2000 ml, respectively. The fractions were examined by TLC and the liked fractions were combined to give the following portions :-

1. Fractions 1-3 were combined and designated as fraction C-41 (0.0175 g).
2. Fractions 4-7 were combined and designated as fraction C-42 (0.0382 g).
3. Fractions 8 and 9 were designated as fraction C-43 (0.0088 g) and fraction C-44 (0.0079 g), respectively.
4. Fractions 10-13 were combined and designated as fraction C-45 (0.2858 g).
5. Fractions 14-19 were combined and designated as fraction C-46 (0.5827 g).
6. Fractions 20-30 were combined and designated as fraction C-47 (0.5183 g).
7. Fractions 31 was designated as fraction C-48 (0.0030 g).
8. Fractions 32-37 were combined and designated as fraction C-49 (0.0059 g).

9. Fractions 38-46 were combined and designated as fraction C-410 (0.0066 g).
10. Fractions 47-53 were combined and designated as fraction C-411 (0.0087 g).
11. Fractions 54-56 were combined and designated as fraction C-412 (0.0930 g).
12. Fractions 57 and 58 were designated as fraction C-413 (0.0165 g) and fraction C-414 (0.0135 g), respectively.
13. Fractions 59-62 were combined and designated as fraction C-415 (0.0163 g).
14. Fractions 63-90 were combined and designated as fraction C-416 (1.0261 g).

3.2.2.2 Isolation of compound AM-3 from fraction C-47

Fraction C-47 (0.5183 g) was shown by TLC to contain at least two compounds. It was dissolved in small amount of chloroform and packed onto the top of wet silica gel column (3×25 cm). The column was eluted with n-hexane : ethyl acetate (1:4). Fifty-one fractions, each of approximately 30 ml were collected, until no traces of compounds could be detected. The volumes of eluent were 1600 ml. Then washed with methanol until no traces of compounds could be detected and seven fractions, each of approximately 50 ml were collected. The volumes of eluent were 400 ml. The fractions were examined by TLC using n-hexane : ethyl acetate (1:4) and the liked fractions were combined to give the following portions :-

1. Fractions 1-4 were combined and designated as fraction C-471 (0.0070 g).
2. Fractions 5-16 were combined and designated as fraction C-472 (0.0485 g).

3. Fractions 17-34 were combined and the TLC chromatogram shown only one spot under UV light at 254 nm, R_f 0.12 [silica gel / n-hexane : ethyl acetate (1:4)] and 0.18 [silica gel / n-hexane : ethyl acetate (1:9)]. These fractions were evaporated under reduced pressure to give 0.046 g of compound AM-3 as pink amorphous powder (0.634% based on dried weight of crude alkaloidal extract). It was later identified as alstophylline.
4. Fractions 35-51 containing mixture of compounds (0.0439 g).
5. Fraction 52 was designated as fraction C-475 (0.0033 g).
6. Fractions 53-58 were combined and designated as fraction C-476 (0.0072 g).

3.2.3 Isolation of compound AM-4 from fraction D, fraction D-9 and fraction D-95

3.2.3.1 Isolation of fraction D-9 from fraction D

Fraction D (1.823 g) was shown by TLC to contain at least three compounds. It was dissolved in small amount of chloroform and ethyl acetate and packed onto the top of wet silica gel column (3.5×17 cm). The column was eluted with n-hexane : ethyl acetate (8:2), chloroform : ethyl acetate (1:1), n-hexane : ethyl acetate (9:1) and chloroform : ethyl acetate : methanol (5:4:1). Then washed with methanol until no traces of compounds could be detected. Fifty-six fractions, each of approximately 50 ml, were collected. The volumes of eluent were 200, 1600, 200, 600 and 500 ml, respectively. The fractions were examined by TLC and the liked fractions were combined to give the following portions :-

1. Fractions 1-4 were combined and designated as fraction D-1 (0.016 g).
2. Fractions 5, 6, 7 and 8 were designated as fraction D-2 (0.002 g), D-3 (0.009 g), D-4 (0.020 g), D-5 (0.019 g), respectively.
3. Fractions 9-11 were combined and designated as fraction D-6 (0.303 g).
4. Fractions 12-13 were combined and designated as fraction D-7 (0.116 g).
5. Fractions 14-16 were combined and designated as fraction D-8 (0.088 g).
6. Fractions 17-41 were combined and designated as fraction D-9 (0.219 g).
7. Fractions 42-49 were combined and designated as fraction D-10 (0.548 g).
8. Fractions 50-56 were combined and designated as fraction D-37 (0.291 g).

3.2.3.2 Isolation of fraction D-95 from fraction D-9

Fraction D-9 (0.219 g) was shown by TLC to contain at least three compounds. It was dissolved in small amount of chloroform and packed onto the top of wet silica gel column (2.5×18 cm). The column was eluted with chloroform : methanol (40:1). Then washed with methanol until no traces of compounds could be detected. Forty-four fractions, each of approximately 30 ml, were collected. The volumes of eluent were 1100 ml and 350 ml, respectively. The fractions were examined by TLC and the liked fractions were combined to give the following portions :-

1. Fractions 1-3 were combined and designated as fraction D-91 (0.004 g).
2. Fractions 4-9 were combined and designated as fraction D-92 (0.002 g).

3. Fractions 10-11 were combined and designated as fraction D-93 (0.005 g).
4. Fractions 12-14 were combined and designated as fraction D-94 (0.070 g).
5. Fractions 15-31 were combined and designated as fraction D-95 (0.100 g).
6. Fractions 32-40 were combined and designated as fraction D-96 (0.009 g).
7. Fractions 41-44 were combined and designated as fraction D-97 (0.045 g).

3.2.3.3 Isolation of compound AM-4 from fraction D-95

Fraction F-95 (0.100 g) was crystallized from chloroform to yield 0.061 g of white needle crystals. It was shown by TLC to contain at least two compounds. It was dissolved in small amount of chloroform and packed onto the top of wet silica gel column (1.5×30 cm). The column was eluted with ethyl acetate. Then washed with methanol until no traces of compounds could be detected. Forty-one fractions, each of approximately 30 ml were collected. The volumes of eluent were 1050 ml and 350 ml, respectively. The fractions were examined by TLC and the liked fractions were combined to give the following portions :-

1. Fractions 1-6 were combined and designated as fraction D-951 (0.007 g).
2. Fraction 7 was designated as fraction D-952 (0.003 g).

3. Fraction 8 was shown by the TLC chromatogram only one spot under UV light at 254 nm, R_f 0.22 [silica gel / ethyl acetate] and 0.12 [silica gel / n-hexane : ethyl acetate (1:20)]. These fractions were evaporated under reduced pressure to give 0.011 g of compound AM-4 as colorless needle (0.152% based on dried weight of crude alkaloidal extract). It was later identified as 3,4,5-trimethoxybenzamide.
4. Fractions 9-28 were combined and designated as fraction D-954 (0.047 g).
5. Fractions 29-37 were combined and designated as fraction D-955 (0.005 g).
6. Fractions 38-41 were combined and designated as fraction D-956 (0.049 g).

4. Physical and Spectral data of Isolated Compounds

4.1 Compound AM-1

Compound AM-1 was obtained as colorless rosette (0.022 g). It was soluble in chloroform and methanol. R_f value on silica gel / n-hexane : ethyl acetate (1:1) = 0.37 and silica gel / chloroform : ethyl acetate (1:1) = 0.57

Melting point : 141-146° C

UV : λ_{max} nm (log ε), in methanol ; Figure 11: 30 (0.24), 294 (0.26), 263 (0.56), 205 (1.51)

IR : ν_{max} cm^{-1}, KBr disc ; Figure 12: 2923, 1730, 1669, 1596

EIMS : *m/z* (% relative intensity); Figure 13: 382 (M^+,35.37), 354 (100), 339 (26.52),295 (8.54), 199 (5.43), 186 (7.62), 174 (7.32)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; see Figure 14 and Table 3

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; see Figure 16 and Table 4

4.2 Compound AM-2

Compound AM-2 was obtained as pale yellow powder (0.025 g). It was soluble in chloroform and methanol. R_f value on silica gel / n-hexane : ethyl acetate (1:1) = 0.28 and silica gel / n-hexane : ethyl acetate (2:1) = 0.32

$[\alpha]^{20}_D$: -165° ($CHCl_3$; *c* 0.1 g/100 ml)

Melting point : 219-222° C

UV : λ_{max} nm (log ε), in methanol ; Figure 20: 306 (0.31), 247 (0.64), 206 (1.73)

IR : ν_{max} cm^{-1}, KBr disc ; Figure 21: 3447, 2926, 1734, 1668, 1594, 1490, 1281, 1158, 1033

EIMS : *m/z* (% relative intensity); Figure 22: 354 (M^+,11.55), 339 (3.34), 326 (6.99), 295 (1.82), 281 (1.22)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; see Figure 23 and Table 5

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; see Figure 25 and Table 6

4.3 Compound AM-3

Compound AM-3 was obtained as pink amorphous powder (0.046 g). It was soluble in chloroform and methanol. R_f value on silica gel / n-hexane : ethyl acetate (1:4) = 0.12 and silica gel / n-hexane : ethyl acetate (1:9) = 0.18

Melting point : 155-158°C

UV : λ_{max} nm (log ε), in methanol ; Figure 29: 289 (0.24), 264 (0.39), 232 (1.05)

IR : ν_{max} cm^{-1}, KBr disc ; Figure 30: 3447, 2926, 1653, 1621, 1473, 1385, 1242

EIMS : *m/z* (% relative intensity); Figure 31: 366 (M^+, 100), 297 (10.3), 254 (7.2), 227 (80.6), 212 (19.3), 211 (27.9), 200 (55.0)

^{1}H NMR : δ ppm, 600 MHz, in $CDCl_3$; see Figure 32 and Table 7

^{13}C NMR : δ ppm, 150 MHz, in $CDCl_3$; see Figure 34 and Table 8

4.4 Compound AM-4

Compound AM-4 was obtained as colorless needle (0.011 g). It was soluble in chloroform and methanol. R_f value on silica gel / ethyl acetate = 0.22 and silica gel / n-hexane : ethyl acetate (1:20) = 0.12

Melting point : 168-169°C

UV : λ_{max} nm (log ε), in methanol ; Figure 38: 281 (0.22), 257 (0.43), 210 (1.59)

IR : ν_{max} cm^{-1}, KBr disc ; Figure 39: 3420, 2924, 2856, 1661, 1617, 1580, 1385, 1262, 1127

EIMS : *m/z* (% relative intensity); Figure 40: 211 (M^+, 100), 196 (26.23), 140 (25), 109 (6.59)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; see Figure 41 and Table 9

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; see Figure 43 and Table 10

CHAPTER IV

Results and Discussion

Dried leaves of *Alstonia macrophylla* Wall. ex G. Don (6.75 kg) were extracted with methanol. The obtained methanolic extract, after acid-basic treatment (77.41 g), was then separated using several chromatographic techniques to afford four pure compounds.

The structure determinations of all isolates were performed by interpretation of their UV, IR, MS and NMR data, and then confirmed by comparison with literature values.

1. Structure Determination of Isolated Compounds

1.1 Structure Determination of Compound AM-1

Compound AM-1 was obtained as colorless rosette. The R_f values are 0.37 [silica gel / n-hexane : ethyl acetate (1:1)] and 0.57 [silica gel / chloroform : ethyl acetate (1:1)]. It was identified as demethoxyalstonamide. This compound was previously isolated from leaves of *Alstonia macrophylla* (Atta-Ur-Rahman *et al*., 1991a).

The UV spectrum of compound AM-1 (Figure 11) showed maximum absorption at 205, 263, 294 and 302 nm, typical of a vincorine-type system (Atta-Ur-Rahman *et al*., 1991a). The IR spectrum of AM-1 (Figure 12) indicated absorption bands at 1596 cm^{-1} (C=C), 1669 cm^{-1} (*N*-formyl C=O) and 1730 cm^{-1} (ester C=O). The EIMS (Figure 13) afforded a molecular ion peak [M^+] at *m/z* 382, consistent with the molecular formula $C_{22}H_{26}N_2O_4$ (D.B.E.= 11), while other major fragments appeared at *m/z* 354, 339, 295, 281, 199, 186 and 174. This fragmentation pattern is characteristic of the vincorine-type alkaloids (Mamatas-Kalamaras *et al*., 1975b and Proksa *et al*., 1987)

The ^1H- NMR spectrum of compound AM-1 (Figure 14 and Table 3), 2-D COSY-45 (Figure 15) and *J*-resolved measurements helped in assigning each proton and their relative stereochemistries. The ethylidene methyl protons (H-18) appeared as a doublet at δ 1.57 showing vicinal coupling ($J_{18,19}$ = 6.3 Hz) with the adjacent C-19 olefenic

proton.The C-19 olefenic proton, on the other hand, resonated at δ 5.41 as a split quartet showing vicinal coupling ($J_{19,18}$ = 6.6 Hz) with the ethylidene methyl protons (H-18). The ester methyl protons resonated at δ 3.79. A three-proton singlets at δ 3.75 was assigned to the 10-OCH_3. A one-proton singlet at δ 8.47 was assigned to the *N*-formyl proton. In the aromatic region where the three aromatic protons were found to resonate, indicating the presence of one substituent on the benzene ring.

The ^{13}C-NMR spectrum (Figure 16 and Table 4) indicated the presence of seven methine, five methylene, three methyl and seven quaternary carbons. The signal due to the C-2 quaternary carbon was found to resonate at δ 94.6. In the aromatic region three methine carbons were found to resonate at δ 111.9, 110.9 and 116.8 and were assigned to C-9, C-11 and C-12, respectively. The *N*-formyl carbon resonated at 160.1. The two OCH_3 carbons resonated at δ 55.6 and 51.9. All of the above spectral studies led us to assign this structure to be demethoxyalstonamide.

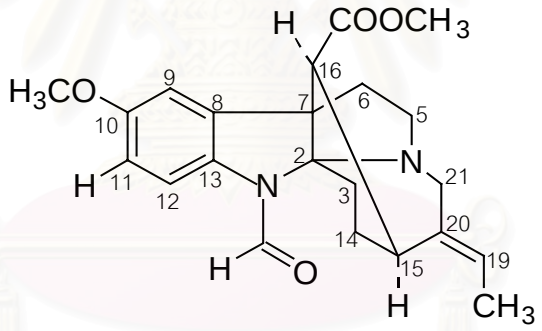


Figure 8 Structure of Demethoxyalstonamide

Table 3 ^{1}H NMR Assignments of Compound AM-1 (in $CDCl_3$) and Demethoxyalstonamide (in $CDCl_3$)

Position	Compound AM-1	Demethoxyalstonamide (Atta-Ur-Rahman *et al*., 1991a)
	δ_H (ppm)(multiplicity, *J* in Hz)	δ_H (ppm)(multiplicity, *J* in Hz)
6α	3.18 (q, 8.7)	3.20 (m)
6β	2.76 (m)	2.80 (m)
9	6.98 (d, 2.1)	7.00 (d, 2.6)
11	6.71 (dd, 2.3, 8.5)	6.72 (dd, 2.6, 8.7)
12	7.98 (d, 8.7)	8.00 (d, 8.7)
15α	2.34 (m)	2.40 (m)
16α	1.96 (d, 5.7)	1.98 (d, 8.4)
18	1.57 (d, 6.3)	1.60 (dd, 7.0, 1.8)
19	5.41 (q, 6.6)	5.43 (split q, 7.0)
21α	2.98 (d, 15.3)	3.00 (d, 15.4)
21β	3.88 (d, 15.6)	3.92 (d, 15.4)
CHO	8.47 (s)	8.47 (s)
10-OCH_3	3.75 (s)	3.81 (s)
$COOCH_3$	3.79 (s)	3.88 (s)

Table 4 ^{13}C NMR Assignments of Compound AM-1 (in $CDCl_3$) and Demethoxyalstonamide (in $CDCl_3$)

Position	Compound AM-1	Demethoxyalstonamide (Atta-Ur-Rahman *et al.*, 1991a)
	δ_C (ppm)	δ_C (ppm)
2	94.6	94.2
3	26.4	26.4
5	54.3	54.3
6	41.4	41.3
7	58.3	58.4
8	139.5	139.7
9	111.9	112.0
10	156.7	157.0
11	110.9	111.1
12	116.8	117.0
13	132.4	133.0
14	27.8	27.4
15	35.5	35.4
16	49.9	49.9
17	172.9	173.3
18	13.6	13.5
19	123.0	123.3
20	135.2	138.0
21	58.0	58.0
N-CHO	159.8	160.1
OCH_3	55.6	56.2
$COOCH_3$	51.9	51.9

1.2 Structure Determination of Compound AM-2

Compound AM-2 was obtained as pale yellow powder. The R_f values are 0.28 [silica gel / n-hexane : ethyl acetate (1:1)] and 0.32 [silica gel / n-hexane : ethyl acetate (2:1)]. It was identified as (-)-1-norvincorine. This compound was previously isolated from leaves of *Vinca minor* (Proksa *et al*., 1987)

The UV spectrum of compound AM-2 (Figure 20) showed maximum absorption at 206, 247and 306 nm, this compound belongs to the dihydroindole group of alkaloids (Proksa *et al*., 1987). The IR spectrum of AM-2 (Figure 21) indicated absorption bands for an N-H functionality at 3447 cm^{-1}, C-H stretching at 2926 cm^{-1}, a methoxycarbonyl functional group at 1734 cm^{-1}, C=C stretching of aromatic ring at 1668, 1594, 1490 cm^{-1}, C-C stretching of ester at 1281 cm^{-1}. The EIMS (Figure 22) showed a molecular ion peak [M^+] at *m/z* 354, corresponding to the molecular formula $C_{21}H_{26}N_2O_3$ (D.B.E.= 10), while other major fragments appeared at *m/z* 339, 326, 295 and 281. This fragmentation pattern is characteristic of the vincorine-type alkaloids (Mamatas-Kalamaras *et al*., 1975b and Proksa *et al*., 1987).

The ^{1}H chemical shifts from NMR spectrum of compound AM-2 (Figure 23 and Table 5), 2-D COSY-45 (Figure 24) and *J*-resolved measurements helped in assigning each proton and their relative stereochemistries. The ethylidene methyl protons (H-18) appeared as a doublet at δ 1.56 showing vicinal coupling ($J_{18,19}$ = 6.6 Hz) with the adjacent C-19 olefenic proton. The C-19 olefenic proton, on the other hand, resonated at δ 5.36 as a quartet showing vicinal coupling ($J_{19,18}$ = 6.6 Hz) with the ethylidene methyl protons (H-18). The ester methyl protons resonated at δ 3.77. A three-proton singlets at δ 3.71 was assigned to the 10-OCH_3. In the aromatic region where the three aromatic protons were found to resonate, indicating the presence of one substituent on the benzene ring.

The ^{13}C-NMR spectrum (Figure 25 and Table 6) indicated the presence of six methine, five methylene, four methyl and seven quaternary carbons. The signal due to the C-2 quaternary carbon located between two nitrogen atoms was found to resonate at δ 98.0. In the aromatic region three methine carbons were found to resonate at δ 112.6, 111.7 and 105.4 and were assigned to C-9, C-11 and C-12, respectively. The two OCH_3

carbons resonated at δ 56.5 and 52.0. All of the above spectral studies led us to assign this structure to be (-)-1-norvincorine.

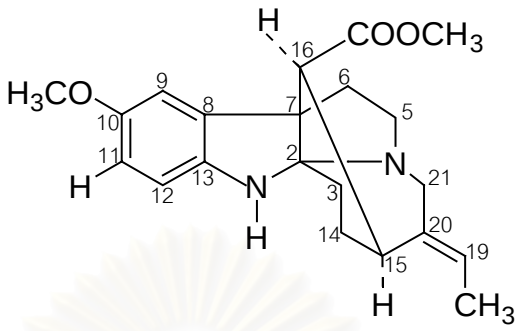


Figure 9 Structure of (-)-1-Norvincorine

Table 5 **^{1}H NMR Assignments of Compound AM-2 (in $CDCl_3$) and (-)-1-norvincorine (in $CDCl_3$)**

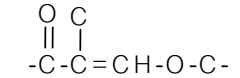
Position	Compound AM-2	(-)-1-norvincorine (Proksa *et al.*, 1987)
	δ_H (ppm)(multiplicity, *J* in Hz)	δ_H (ppm)(multiplicity, *J* in Hz)
5α	3.57 (s)	3.51 (m)
5β	2.71 (m)	2.74 (m)
6α	2.43 (m)	2.49 (m)
6β	1.72 (m)	2.02 (m)
9	6.92 (d, 2.1)	6.92 (d, 2.2)
11	6.60 (dd, 2.3, 8.3)	6.57 (dd, 2.2, 8.4)
12	6.15 (d, 8.4)	6.42 (d, 8.4)
18	1.56 (d, 6.6)	1.58 (dd, 7.0)
19	5.36 (q, 6.6)	5.40 (q, 7.0)
10-OCH_3	3.71 (s)	3.72 (s)
$COOCH_3$	3.77 (s)	3.79 (s)

Table 6 ^{13}C NMR Assignments of Compound AM-2 (in $CDCl_3$) and (-)-1-norvincorine (in $CDCl_3$)

Position	Compound AM-2	(-)-1-norvincorine (Proksa *et al*., 1987)
	δ_C (ppm)	δ_C (ppm)
2	97.7	94.4
3	26.4	26.6
5	55.0	56.0
6	40.9	41.3
7	58.3	57.8
8	138.8	139.1
9	112.1	112.2
10	151.9	153.1
11	111.3	111.9
12	105.0	109.3
13	143.5	141.8
14	28.1	26.6
15	34.9	35.3
16	50.8	50.6
17	173.4	173.5
18	13.7	13.5
19	122.4	122.0
20	138.4	138.2
21	57.2	58.1
OCH_3	56.1	54.6
$COOCH_3$	51.7	51.7

1.3 Structure Determination of Compound AM-3

Compound AM-3 was obtained as pink amorphous powder. The R_f values are 0.12 [silica gel / n-hexane : ethyl acetate (1:4)] and 0.18 [silica gel / n-hexane : ethyl acetate (1:9)]. It was identified as alstophylline. This compound was previously isolated from leaves of *Alstonia macrophylla* (Kishi *et al*., 1965, Cook *et al*., 1969 and Abe *et al*., 1994b).

The UV spectrum of compound AM-3 (Figure 29) showed maximum absorption at 234, 257 and 295 nm suggesting the presence of summation of exo-enol function,  and an indole nucleus (Cook *et al*., 1969). The IR spectrum of AM-3 (Figure 30) indicated absorption bands at 1653 and 1621 cm^{-1} suggest the presence of an oxo-enol ether function (Cook *et al*., 1969). The EIMS (Figure 31) afforded a molecular ion peak [M^+] at *m/z* 366, consistent with the molecular formula $C_{22}H_{26}N_2O_3$ (D.B.E.=11), while other major fragments appeared at *m/z* 297, 254, 227, 212, 211 and 200 (Kishi *et al*.,1965). And the mass spectral fragmentation pattern was virtually identical with that of the ajmaline derivative (Kishi *et al*.,1965).

In the ^1H NMR spectrum of compound AM-3 (Figure 32 and Table 7), a one-proton singlet at δ 7.53 arises from the enol ether vinyl proton. The aromatic region contains three protons from the indole nucleus, resonating at δ 7.33 with no pyrrole N-H or indole α-H signals. A three-proton singlets at δ 2.09 is consonant with a CH_3CO-group, permitting the expansion of the oxo-enol ether structure element to CH_3CO-C (C)=CH-O-C-. Two further methyl group signals, at δ 2.32 and δ 3.60, are assigned to aliphatic and indolic N-CH_3 groups respectively. This evidence, taken together with the absence from the NMR spectrum of any signals at higher field than δ 2.00, suggests a polycyclic structure resembling that of the unusual indolohomotropane alkaloid alstophylline.

The ^{13}C NMR spectrum of compound AM-3 is shown on Figure 34. And the carbon assignments of AM-3 (alstophylline) are depicted in table 8.

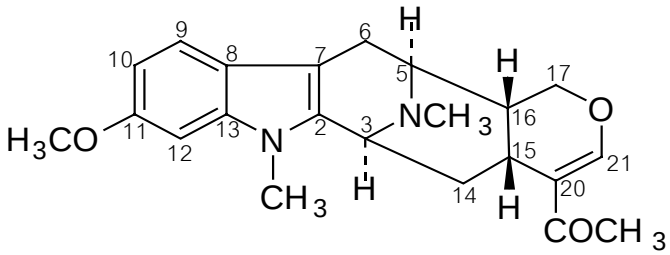


Figure 10 Structure of Alstophylline

Table 7 ^{1}H NMR Assignments of Compound AM-3 (in $CDCl_3$) and Alstophylline (in $CDCl_3$)

Position	Compound AM-3	Alstophylline (Abe *et al.*, 1994b)
	δ_H (ppm)(multiplicity, *J* in Hz)	δ_H (ppm)(multiplicity, *J* in Hz)
3	3.84 (br s)	3.83 (br s)
5	3.07 (br d, 6.8)	3.05 (br d, 7)
6	2.46 (d, 16.2) 3.27 (dd, 16.6, 6.8)	2.45 (dd, 17, 2) 3.28 (dd, 17, 7)
9	7.33 (d, 8.5)	7.33 (d, 8)
10	6.77 (dd, 8.5, 2.2)	6.75 (dd, 8, 2)
12	6.81 (d, 2.2)	6.80 (d, 2)
14	1.80 (m) 2.10 (m)	1.80 (m) 2.10 (m)
15	2.62 (m)	2.61 (m)
16	1.89 (m)	1.89 (m)
17	4.17 (ddd, 11.0, 3.8, 1.7) 4.39 (t, 11.4)	4.15 (dd, 11, 2) 4.43 (t, 11)
18	2.09 (s)	2.08 (s)
21	7.53 (s)	7.52 (s)
N_a-CH_3	3.60 (s)	3.59 (s)
N_b-CH_3	2.32 (s)	2.32 (s)
OCH_3	3.89 (s)	3.88 (s)

Table 8 ^{13}C NMR Assignments of Compound AM-3 (in $CDCl_3$) and Alstophylline (in $CDCl_3$)

Position	Compound AM-3	Alstophylline (Abe *et al.*, 1994b)
	δ_C (ppm)	δ_C (ppm)
2	132.0	132.0
3	53.9	53.9
5	54.8	54.8
6	22.9	22.9
7	105.9	105.9
8	121.2	121.2
9	118.4	118.3
10	108.3	108.2
11	156.0	156.0
12	93.4	93.5
13	138.0	138.0
14	32.5	32.5
15	23.0	23.0
16	38.6	38.6
17	67.9	67.8
18	25.1	25.0
19	195.6	195.4
20	121.2	121.2
21	157.5	157.3
N_a-CH_3	29.2	29.1
N_b-CH_3	41.8	41.8
OCH_3	56.0	56.0

1.4 Structure Determination of Compound AM-4

Compound AM-4 was obtained as colorless needle. The R_f values are 0.22 [silica gel / ethyl acetate] and 0.12 [silica gel / n-hexane : ethyl acetate (1:20)]. It was identified as 3,4,5-trimethoxybenzamide. This compound was previously isolated from the stem bark of *Alstonia constricta* (Allam *et al*., 1987 and Crow *et al*., 1970).

The UV spectrum of compound AM-4 (Figure 38) showed maximum absorption at 210, 257 and 286 nm suggesting the presence of a benzene ring chromophore. The IR spectrum of AM-4 (Figure 39) indicated absorption bands for an N-H functionality at 3420 and 3196 cm^{-1}, C-H stretching of alkanes at 2924 and 2856 cm^{-1}, C=O stretching of ester at 1735 cm^{-1}, C=C stretching of aromatic ring at 1661 and 1617 cm^{-1}, N-H bending of primary amine at 1580 cm^{-1}, and C-O stretching of esters at 1385 and 1262 cm^{-1}. The EIMS (Figure 40) afforded a molecular ion peak [M^+] at *m/z* 211,consistent with the molecular formula $C_{10}H_{13}NO_4$ (D.B.E.= 5), while other major fragments appeared at *m/z* 196, 181, 165, 140 and 109.

The ^1H chemical shifts from NMR spectrum of compound AM-4 (Figure 41 and Table 9) showed signals from three methoxy groups at δ 3.87 (6H, s, 2×OCH_3) and 3.90 (3H, s, OCH_3) and from two aromatic protons which are coincident in chemical shift at δ 7.03 (2H, s, aromatic proton of trimethoxybenzoyl). From these signals, all of which can be assigned as the 3,4,5-trimethoxybenzoyl derivative.

The ^{13}C NMR spectrum of compound AM-4 is shown on Figure 43. And the carbon assignments of AM-4 (3,4,5-,trimethoxybenzamide) are depicted in table 10.

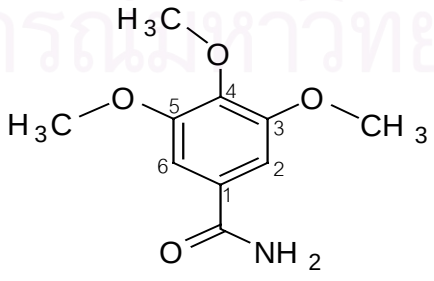


Figure 11 Structure of 3,4,5-trimethoxybenzamide

Table 9 ^{1}H NMR Assignments of Compound AM-4 (in $CDCl_3$) and 3,4,5-trimethoxybenzamide (in $CDCl_3$)

Position	Compound AM-4	3,4,5-trimethoxybenzamide	
		(Crow *et al*., 1970)	(Schaller, 1999)
	δ_H (ppm)(multiplicity, *J* in Hz)	δ_H (ppm)(multiplicity, *J* in Hz)	δ_H (ppm)(multiplicity, *J* in Hz)
2	7.03 (s)	7.13 (s)	6.91 (s)
6	7.03 (s)	7.13 (s)	6.91 (s)
3-OCH_3	3.87 (s)	3.88 (s)	3.73 (s)
4-OCH_3	3.90 (s)	3.91 (s)	3.73 (s)
5-OCH_3	3.87 (s)	3.88 (s)	3.73 (s)
NH_2	-	-	Not estimated

Table 10 ^{13}C NMR Assignments of Compound AM-4 (in $CDCl_3$) and 3,4,5-trimethoxybenzamide (in $CDCl_3$)

Position	Compound AM-4	3,4,5-trimethoxybenzamide (Schaller, 1999)
	δ_C (ppm)	δ_C (ppm)
1	120.1	127.8
2	105.0	106.2
3	153.2	148.7
4	128.7	136.6
5	153.2	148.7
6	105.0	106.2
3-OCH_3	56.1	56.3
4-OCH_3	56.4	56.6
5-OCH_3	56.1	56.3
NH_2	169.1	169.7

CHAPTER V

Conclusion

In this investigation, four pure compounds were isolated from the leaves of *Alstonia macrophylla* Wall. ex G. Don. These compounds are the indole alkaloids being identified as demethoxyalstonamide (AM-1), (-)-1-norvincorine (AM-2) and alstophylline (AM-3). Another one is amide namely 3,4,5-trimethoxybenzamide (AM-4). Two of the isolated compounds demethoxyalstonamide (AM-1) and alstophylline (AM-3) have been reported from the leaves of this species before, except (-)-1-norvincorine (AM-2) and 3,4,5-trimethoxybenzamide (AM-4).

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APPENDIX

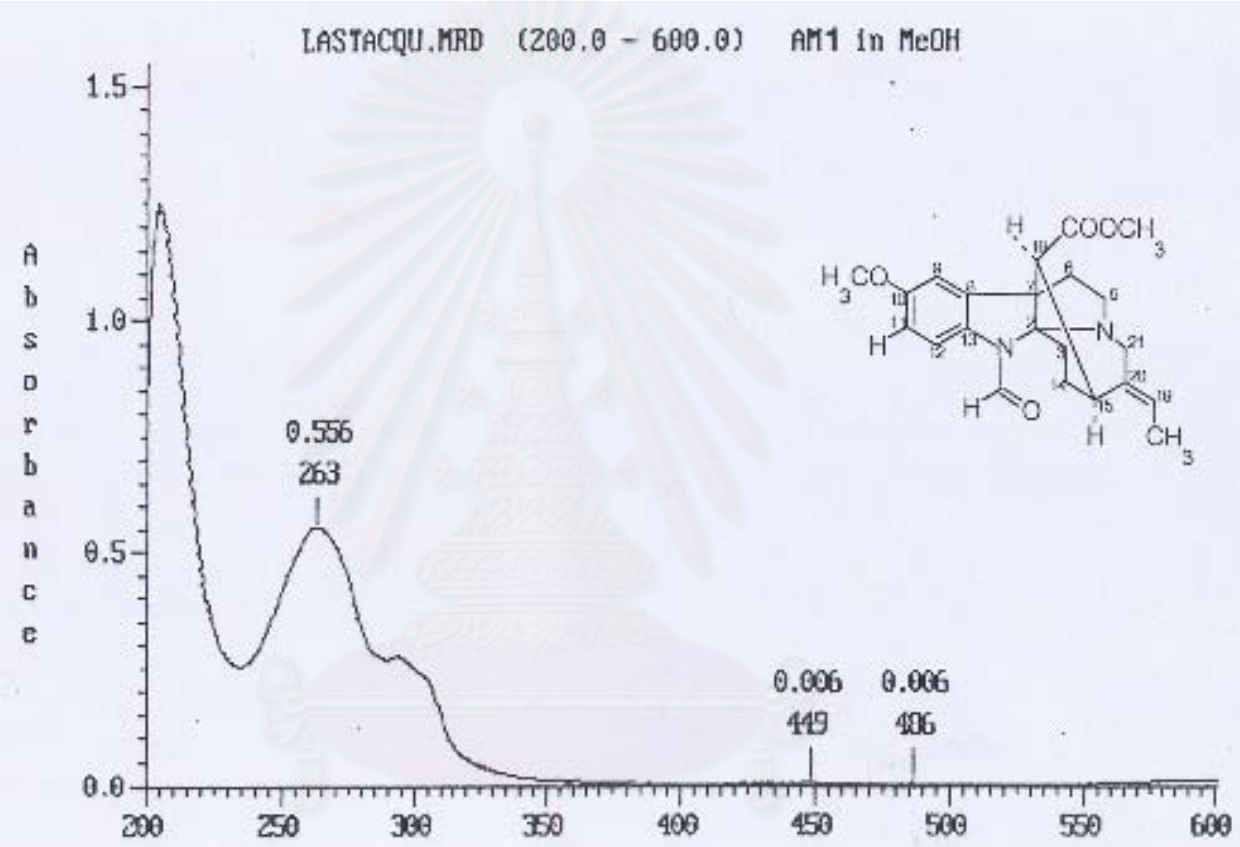


Figure 12 UV spectrum of compound AM-1 (in $CDCl_3$)

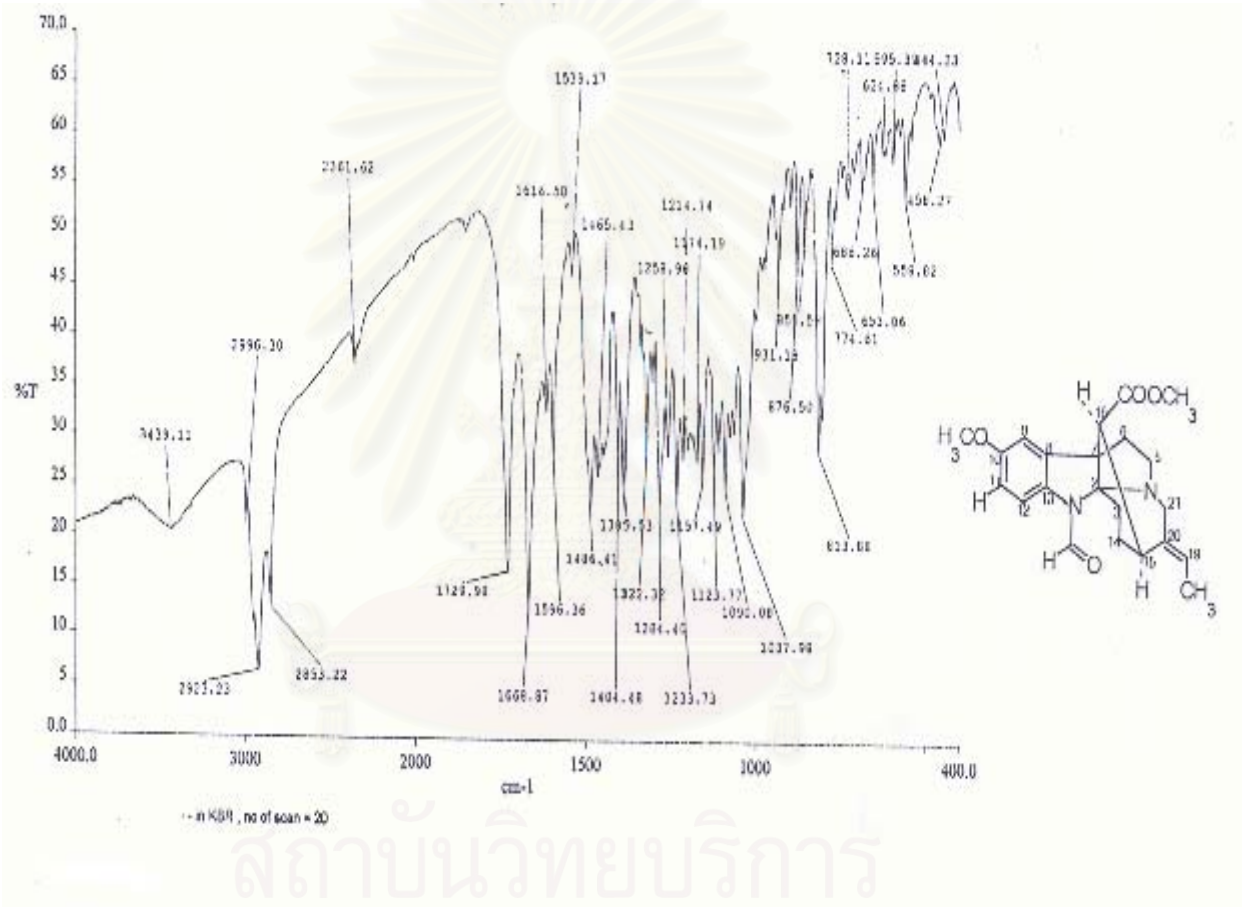


Figure 13 IR spectrum of compound AM-1 (KBr disc)

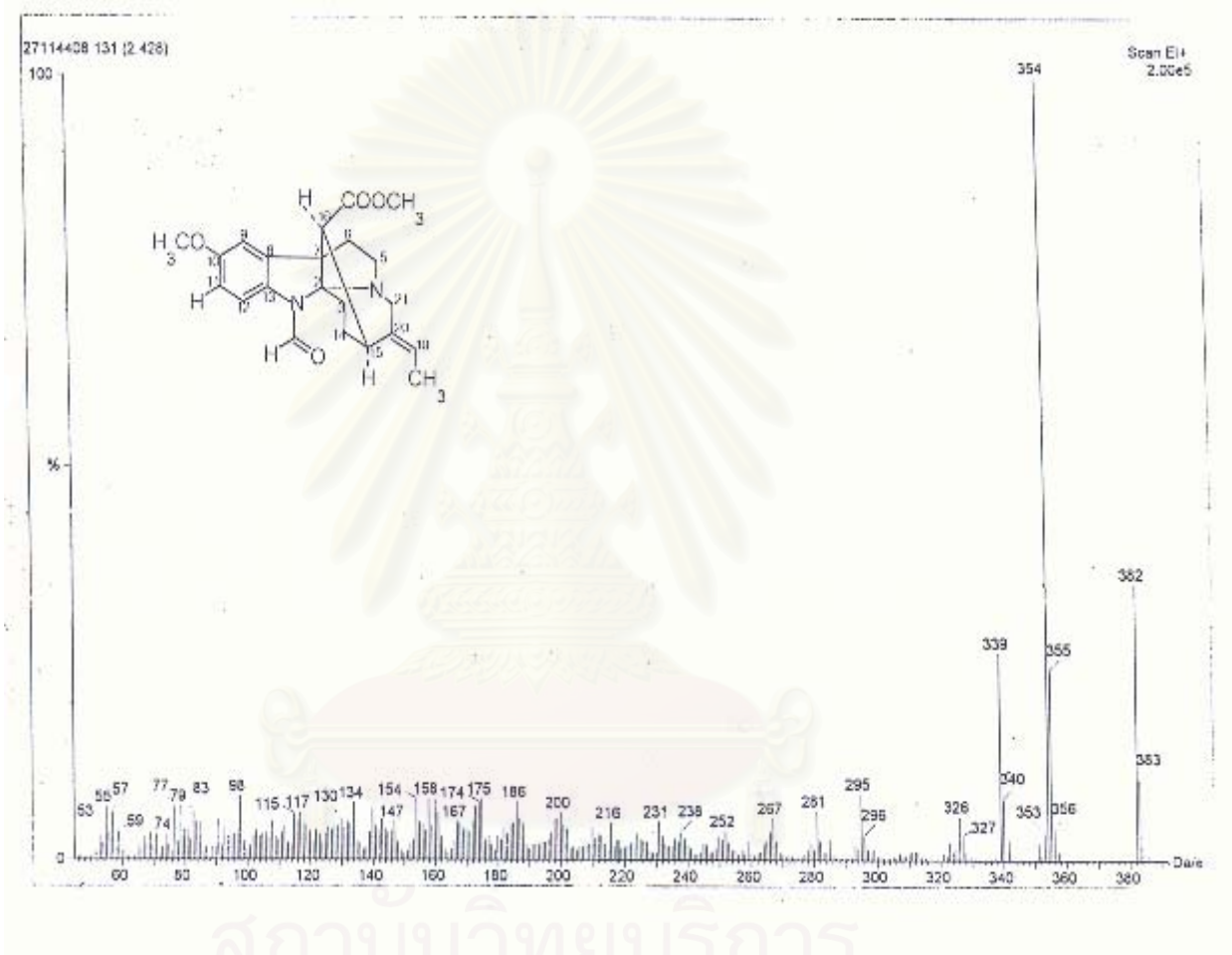


Figure 14 EI mass spectrum of compound AM-1

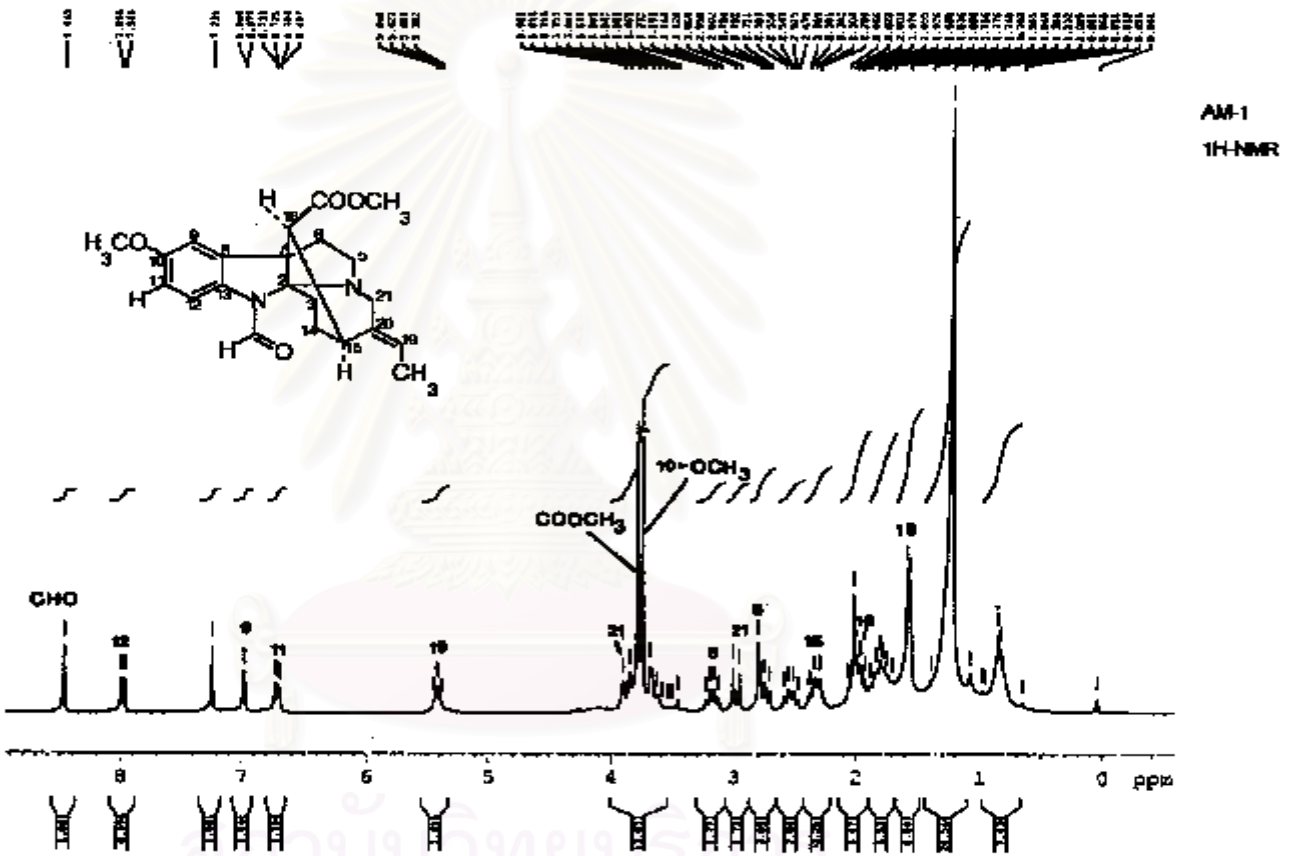


Figure 15 300 MHz ^{1}H NMR spectrum of compound AM-1 (in $CDCl_3$)

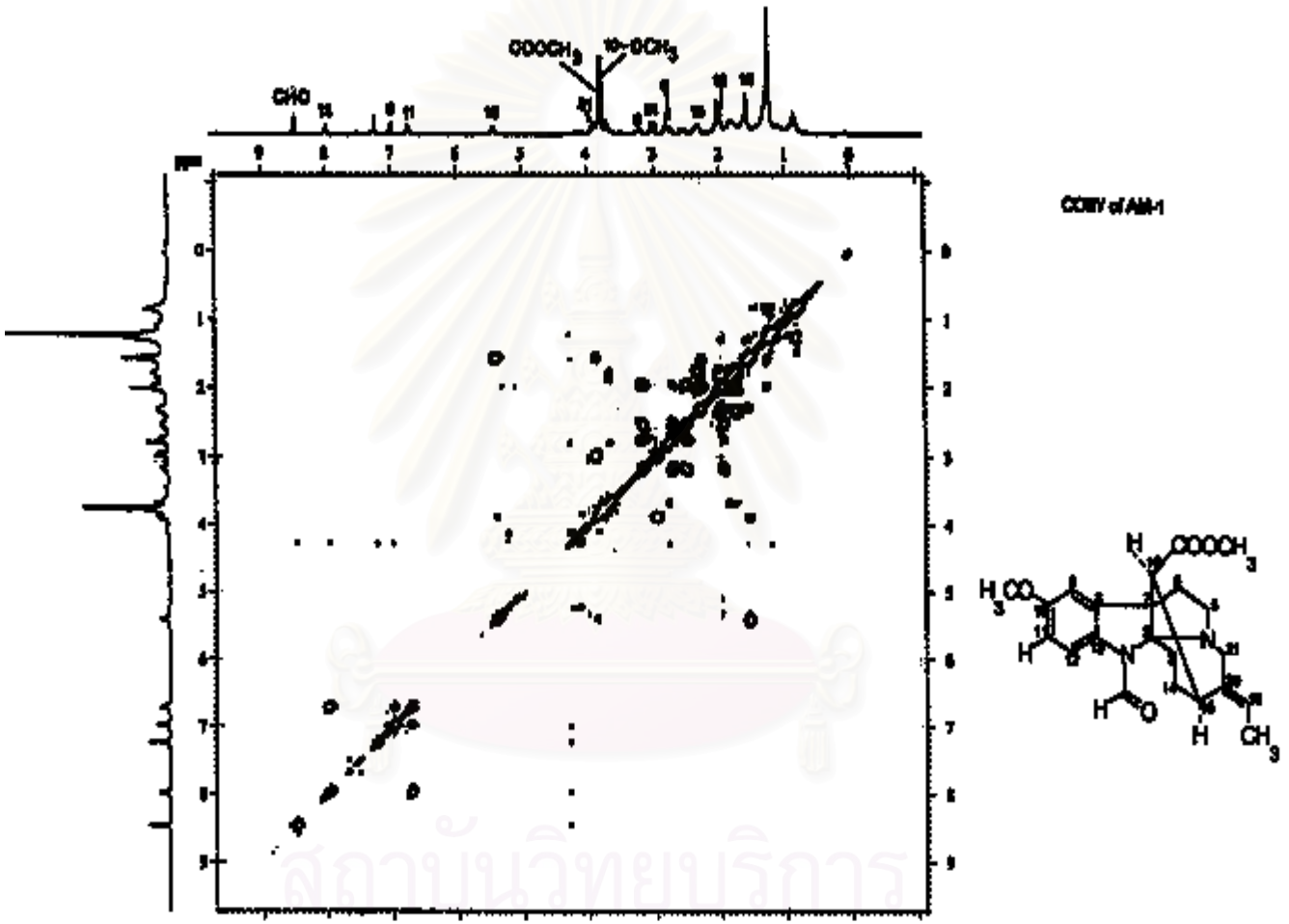


Figure 18 ^{1}H-^{1}H COSY spectrum of compound AM-1 (in $CDCl_3$)

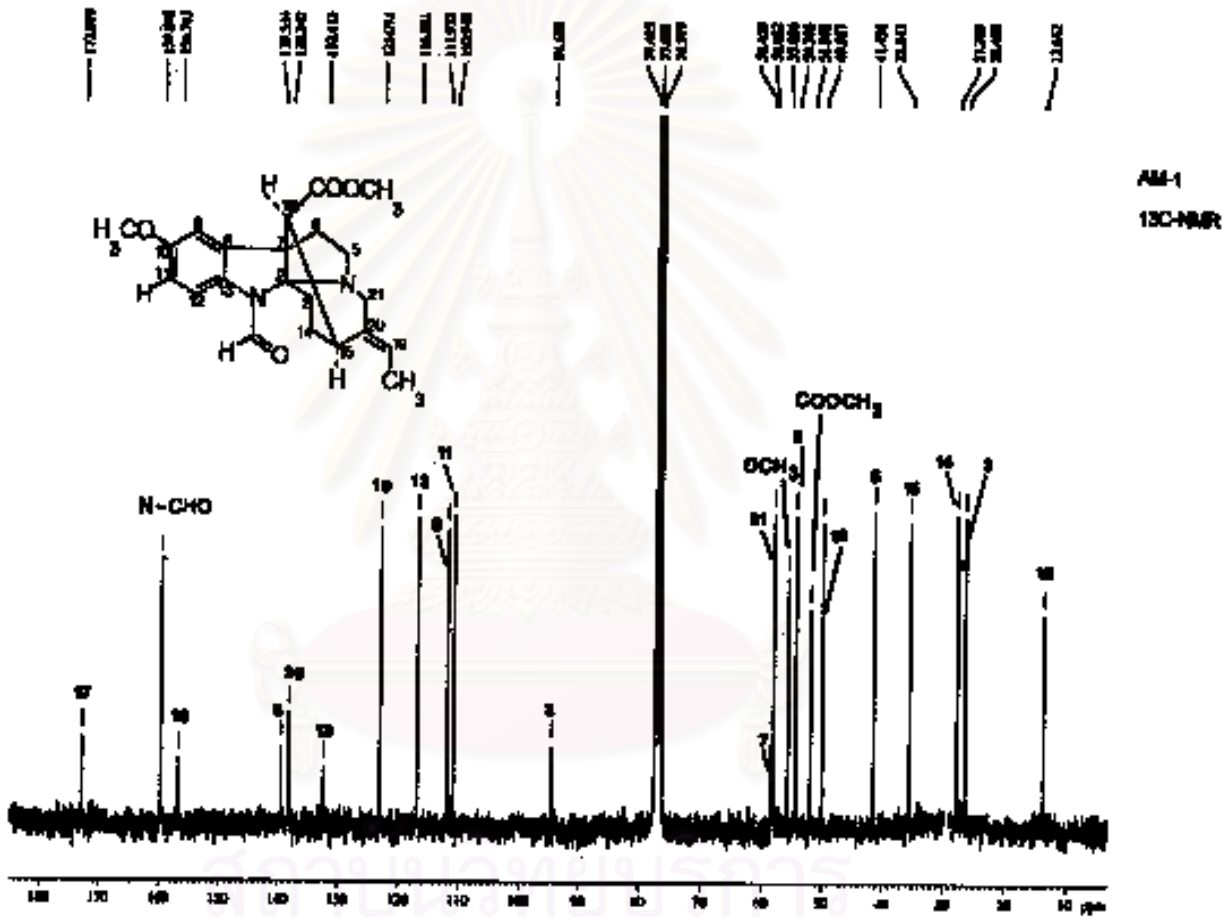


Figure 17 75 MHz ^{13}C NMR spectrum of compound AM-1 (in $CDCl_3$)

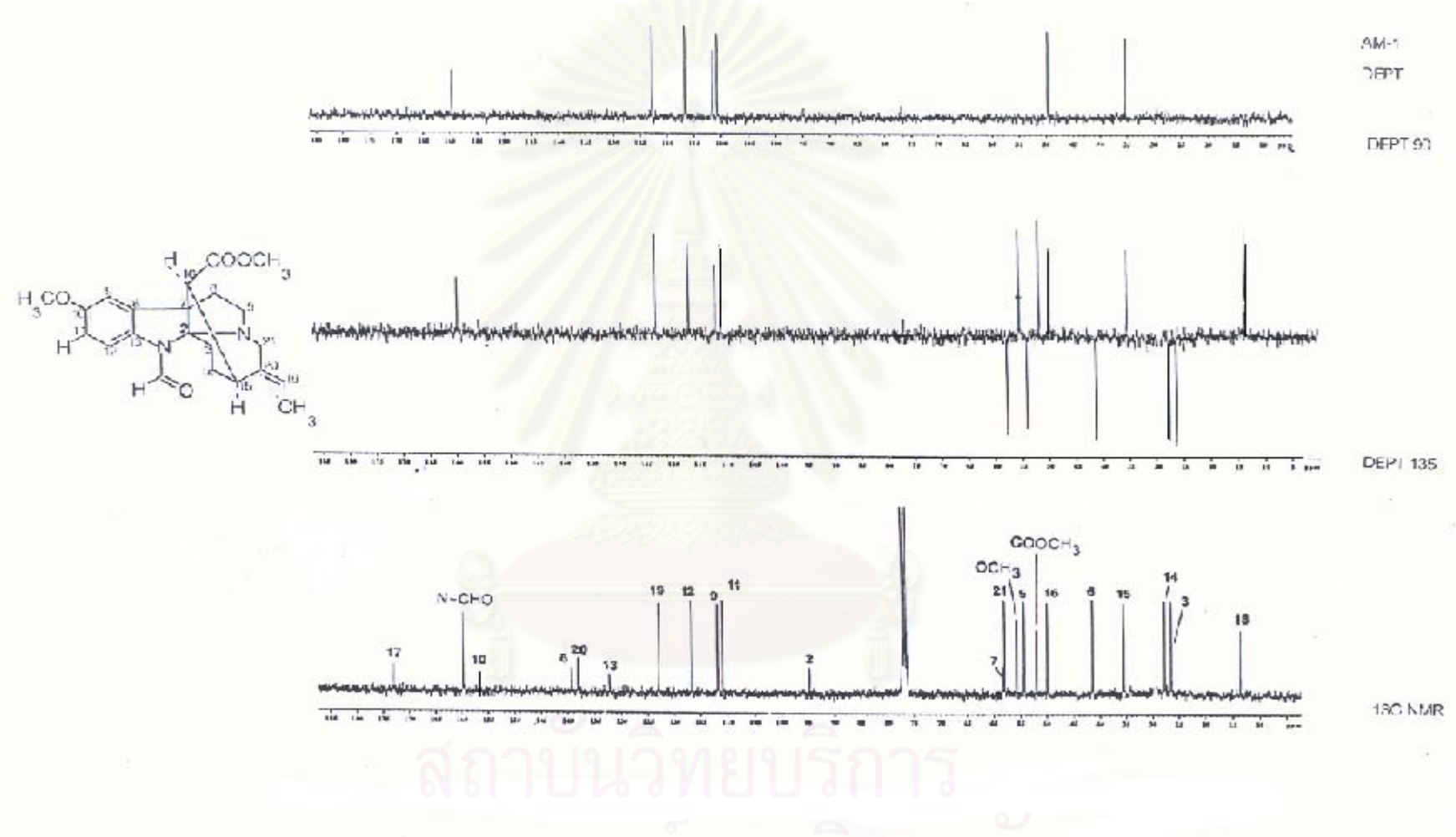


Figure 18 75 MHz ^{13}C NMR, DEPT 90 and DEPT 135 spectrum of compound AM-1 (in $CDCl_3$)

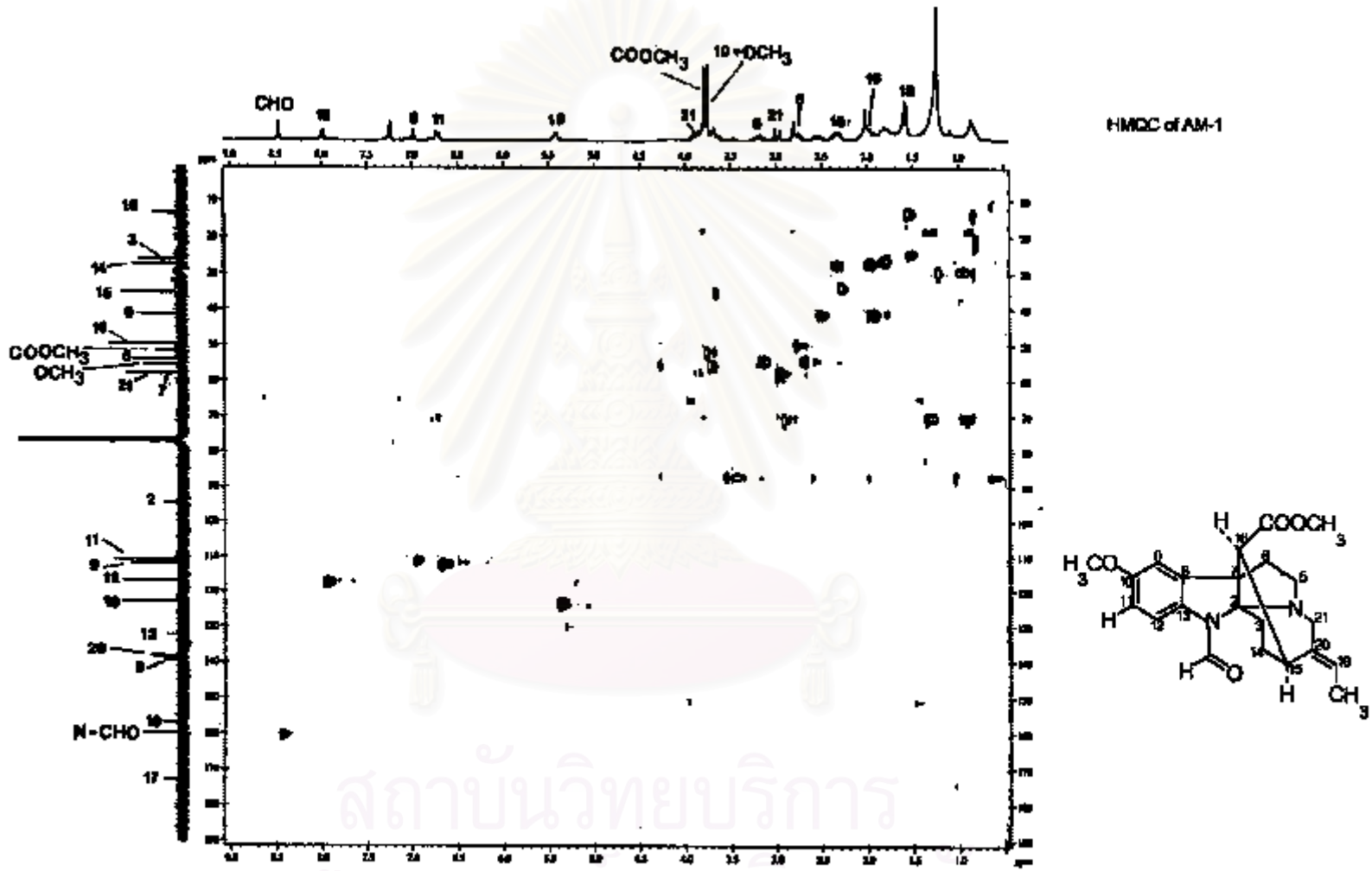


Figure 19 HMQC spectrum of compound AM-1 (in $CDCl_3$) [δ_H 0.5-9.0 ppm, δ_C 0-190.0 ppm]

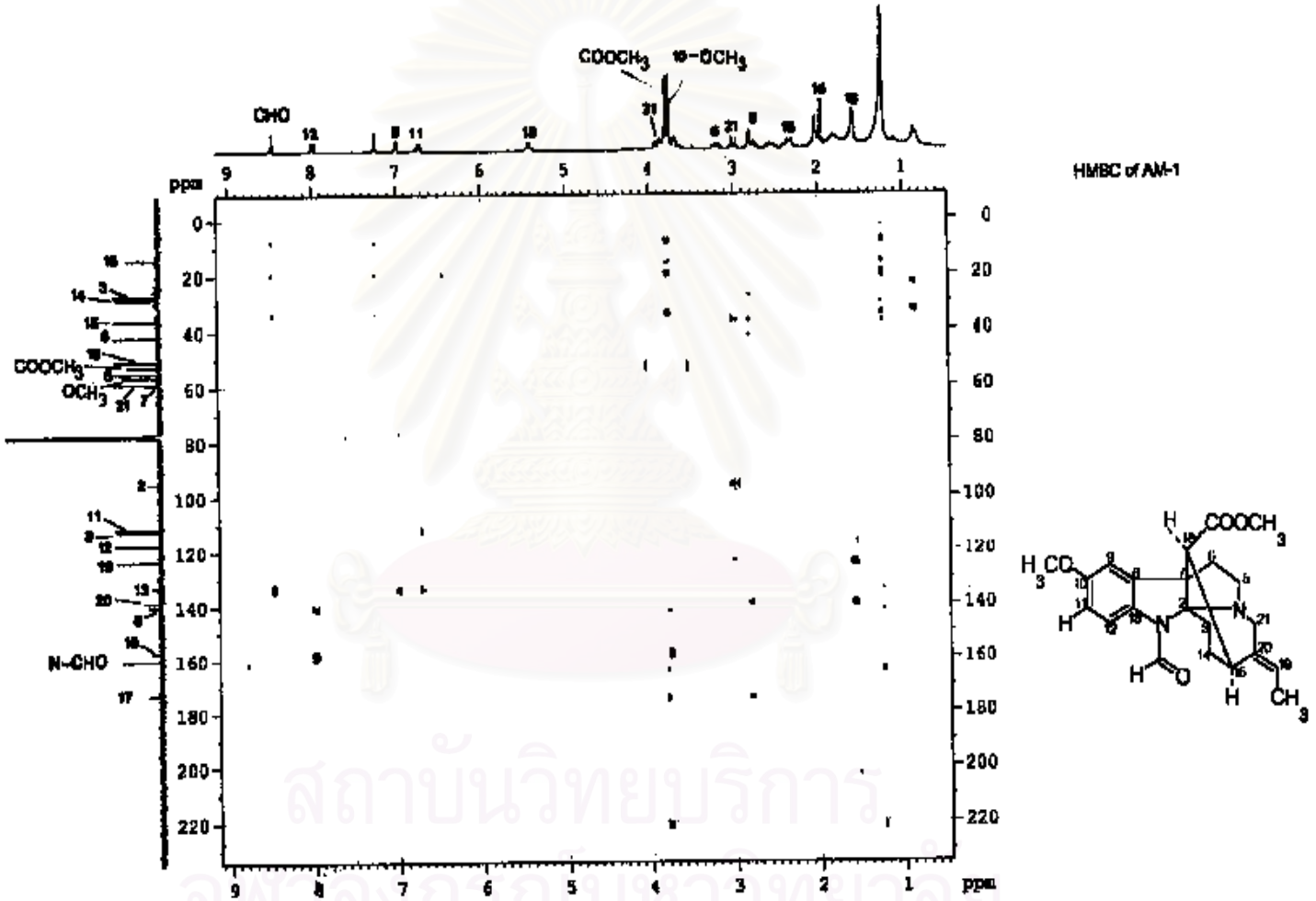


Figure 20 HMBC spectrum of compound AM-1 (in $CDCl_3$) [δ_H 0.5-9.0 ppm, δ_C 0-230.0 ppm]

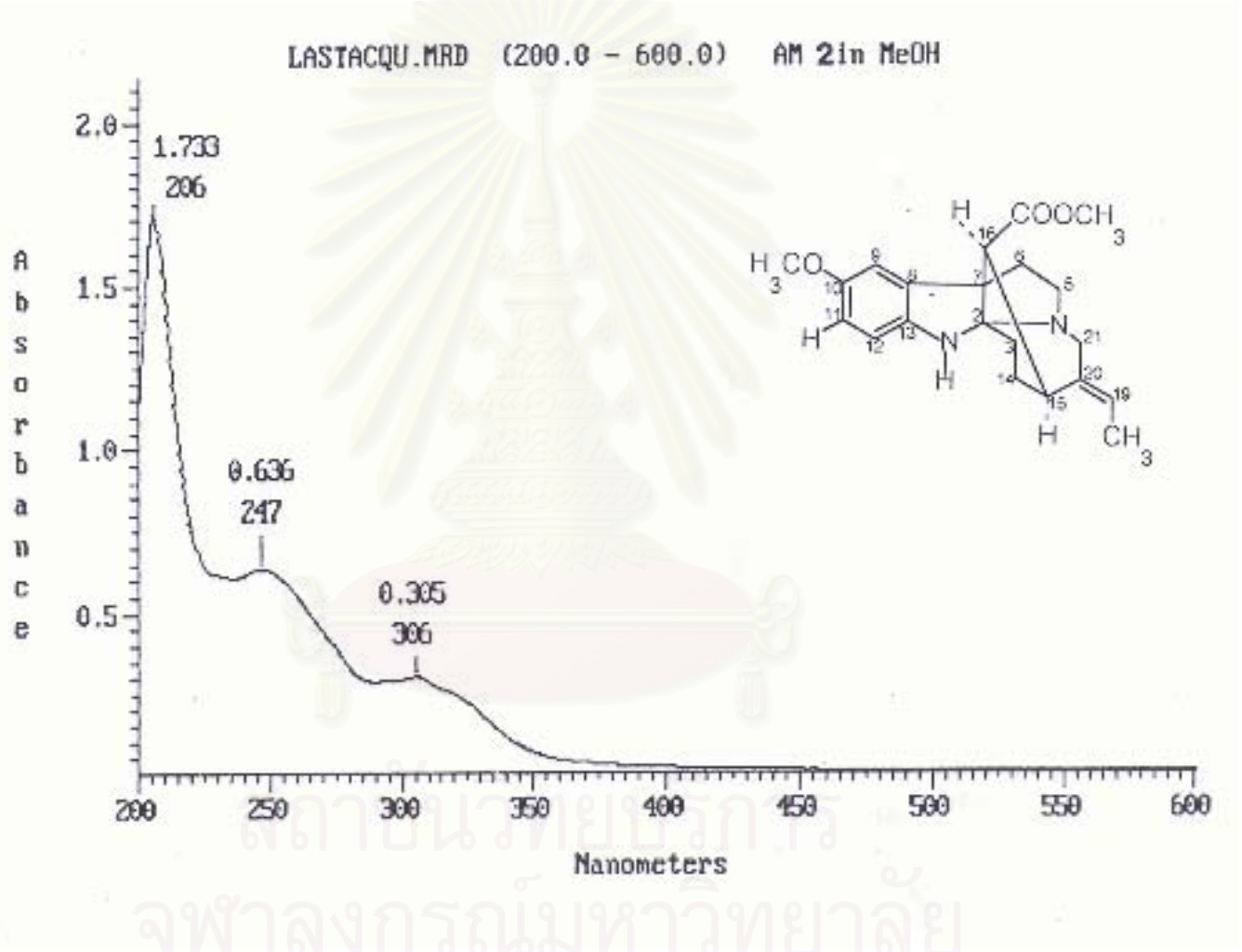


Figure 21 UV spectrum of compound AM-2 (in $CDCl_3$)

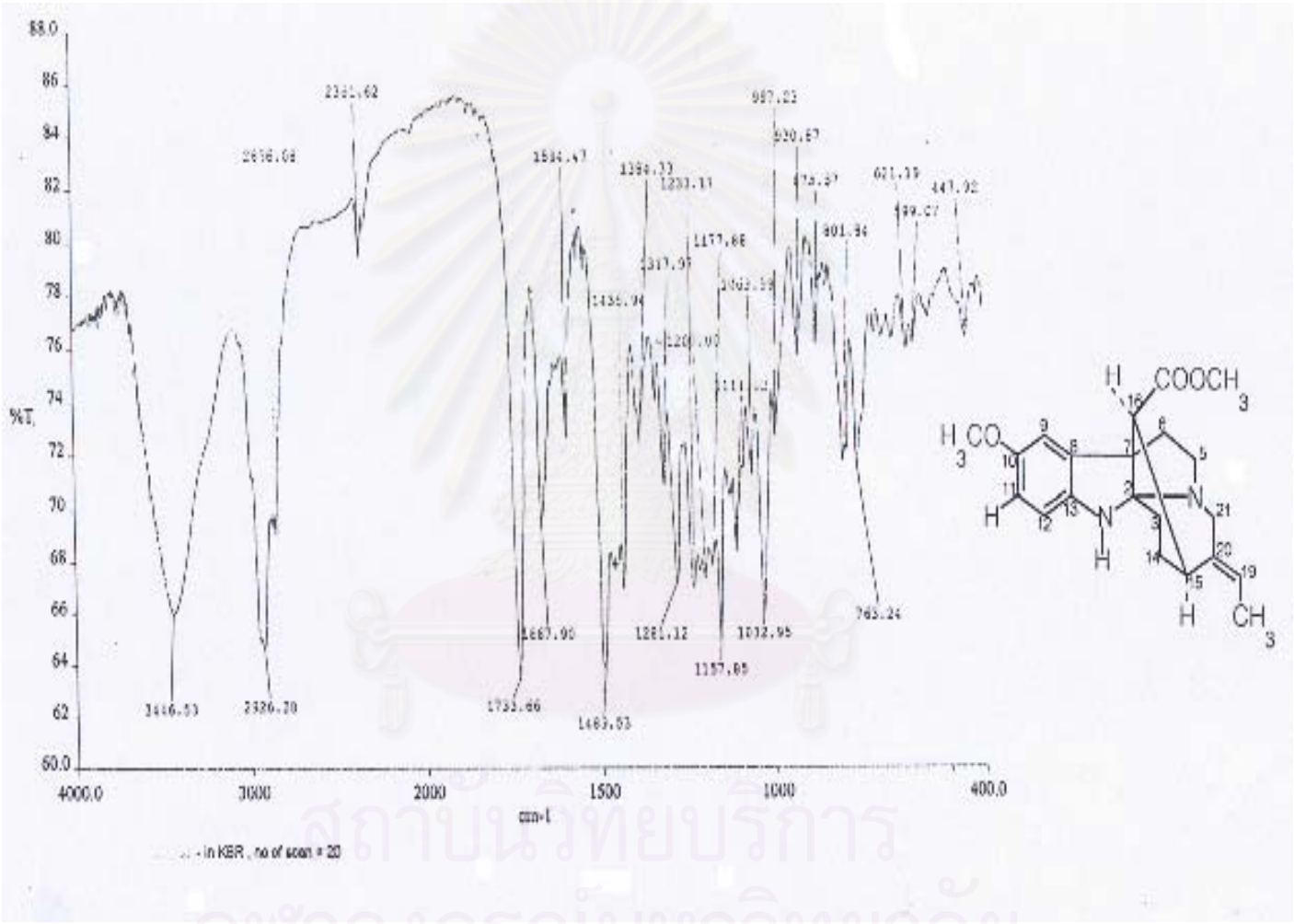


Figure 22 IR spectrum of compound AM-2 (KBr disc)

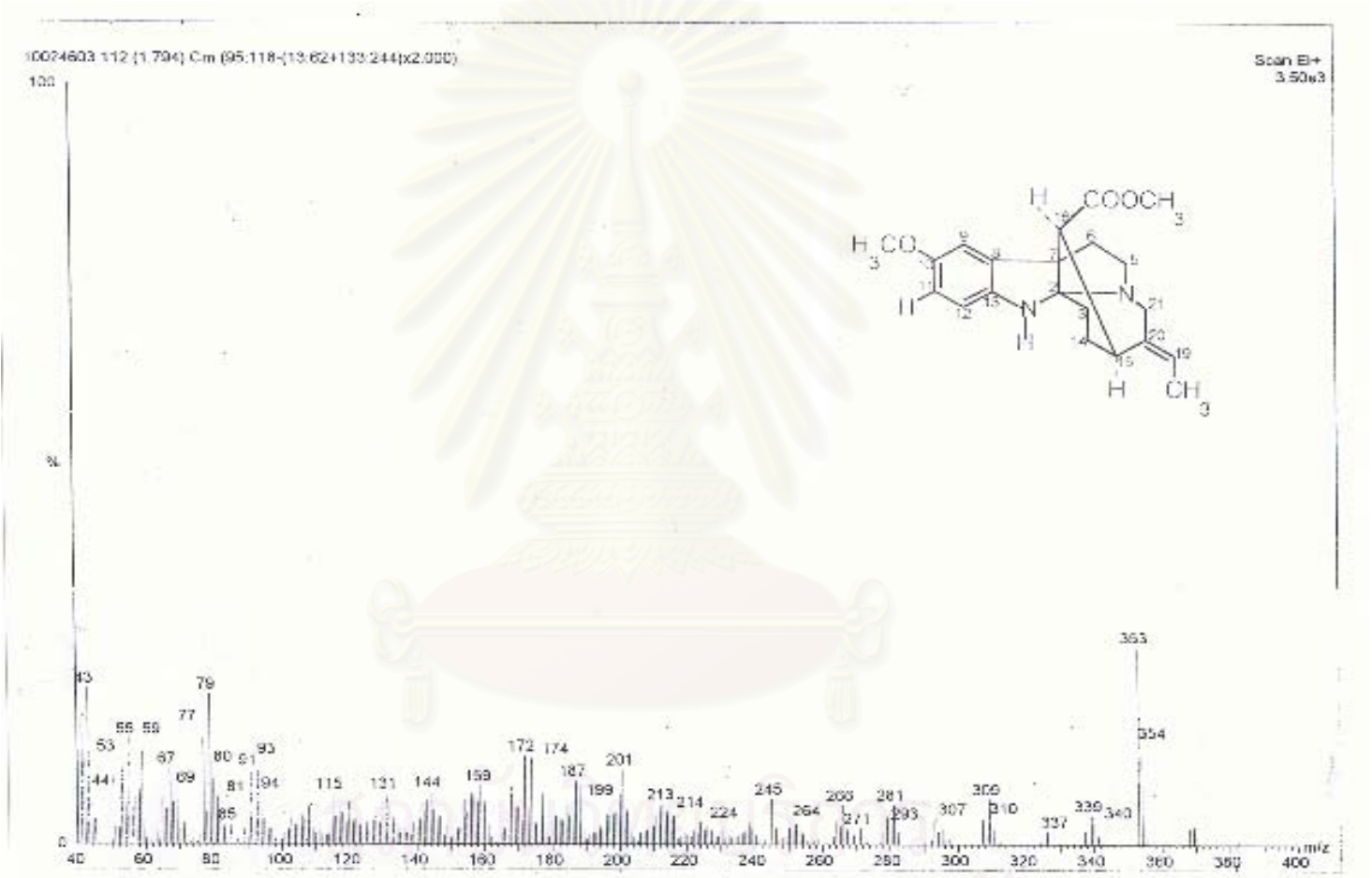


Figure 23 EI mass spectrum of compound AM-2

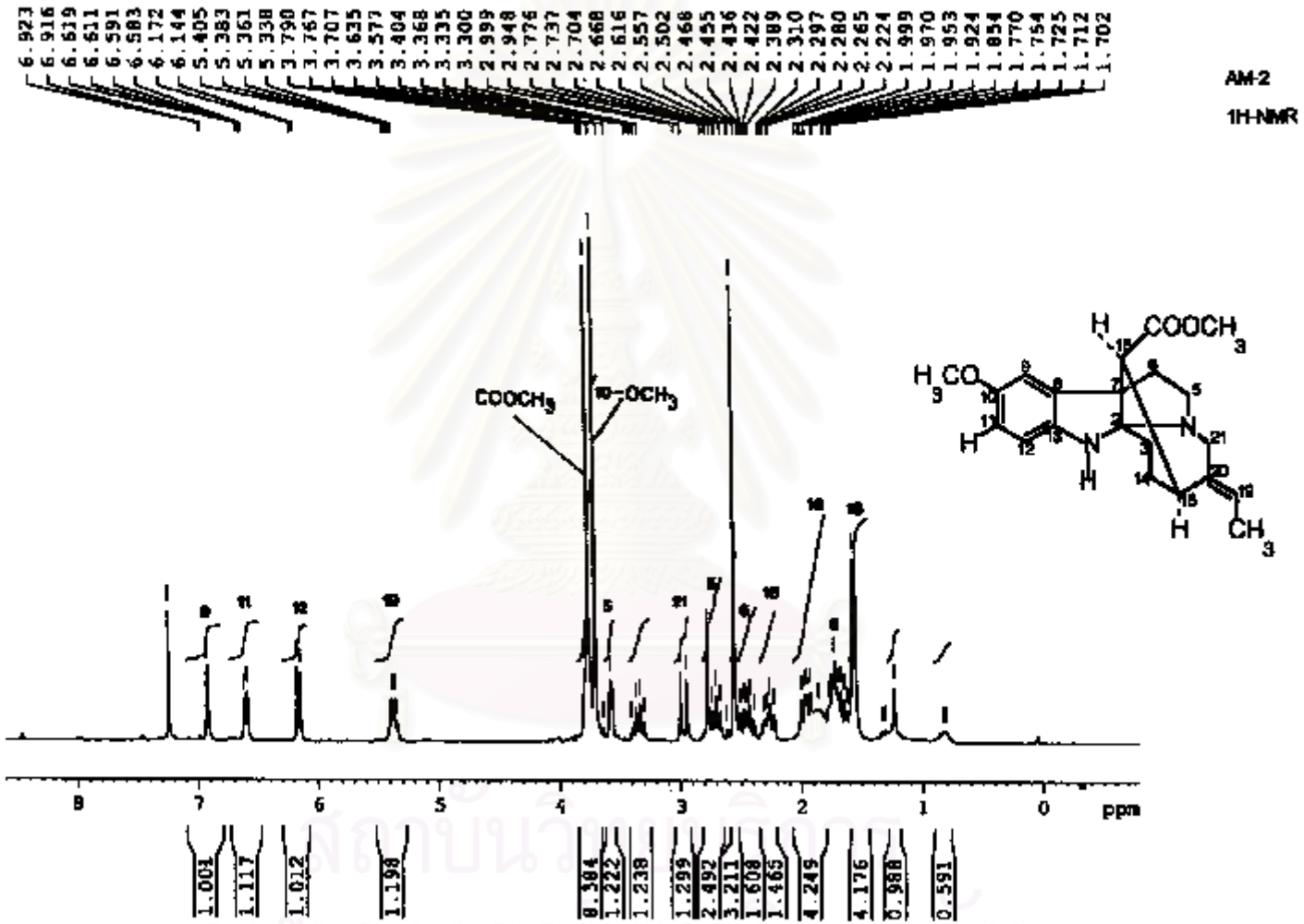


Figure 24 300 MHz ^{1}H NMR spectrum of compound AM-2 (in $CDCl_3$)

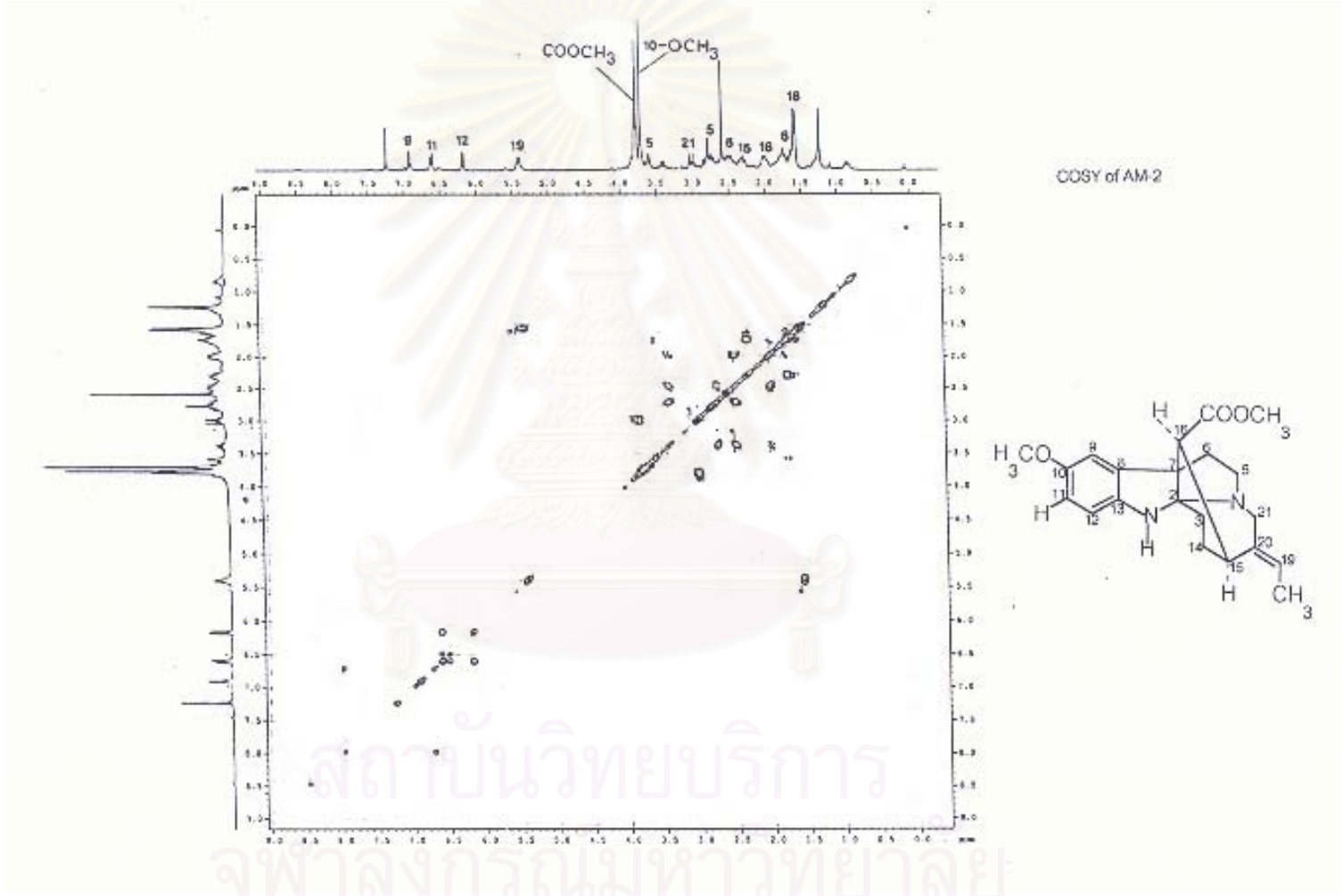


Figure 25 ^{1}H-^{1}H COSY spectrum of compound AM-2 (in $CDCl_3$)

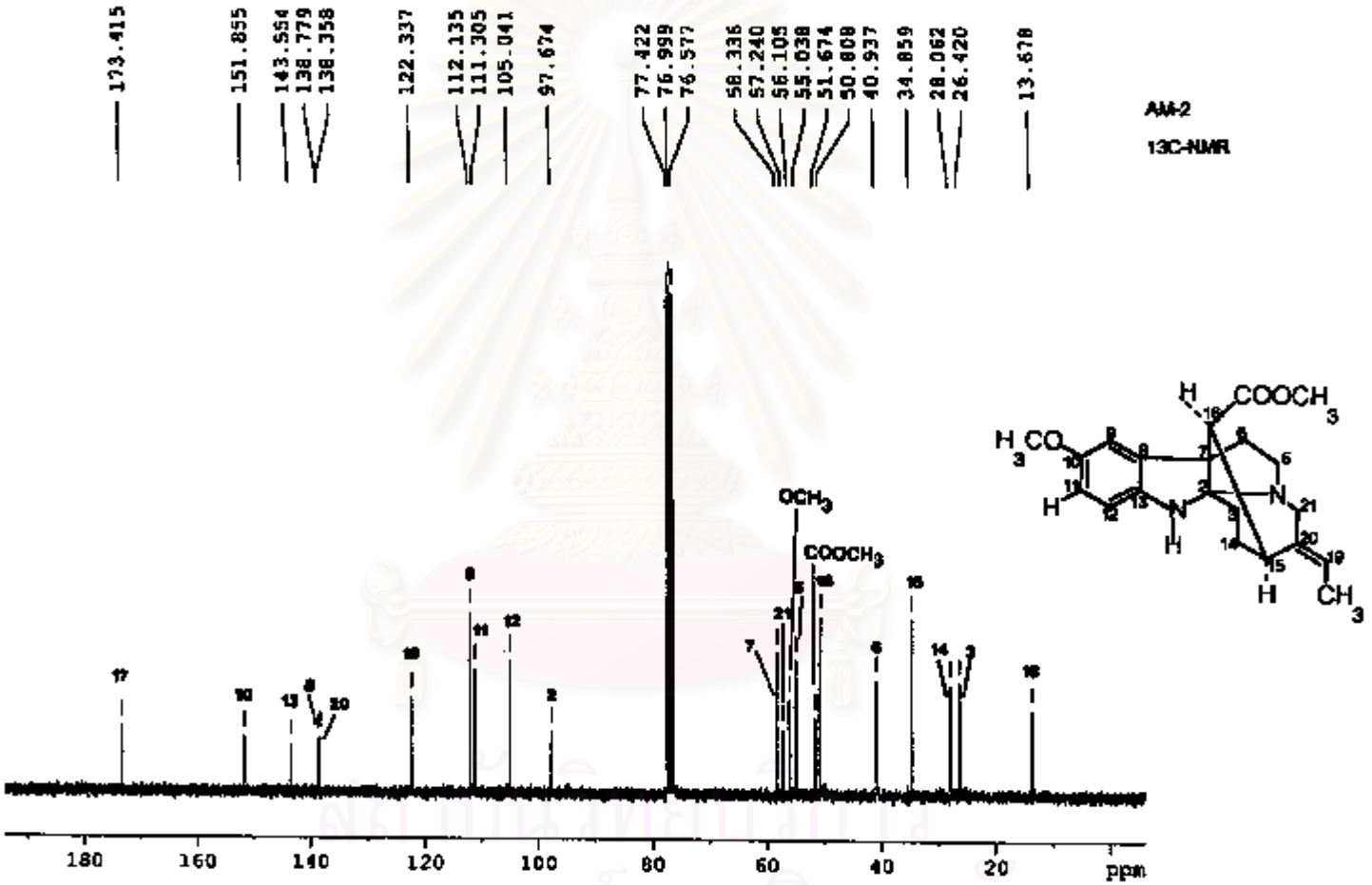


Figure 26 75 MHz ^{13}C NMR spectrum of compound AM-2 (in $CDCl_3$)

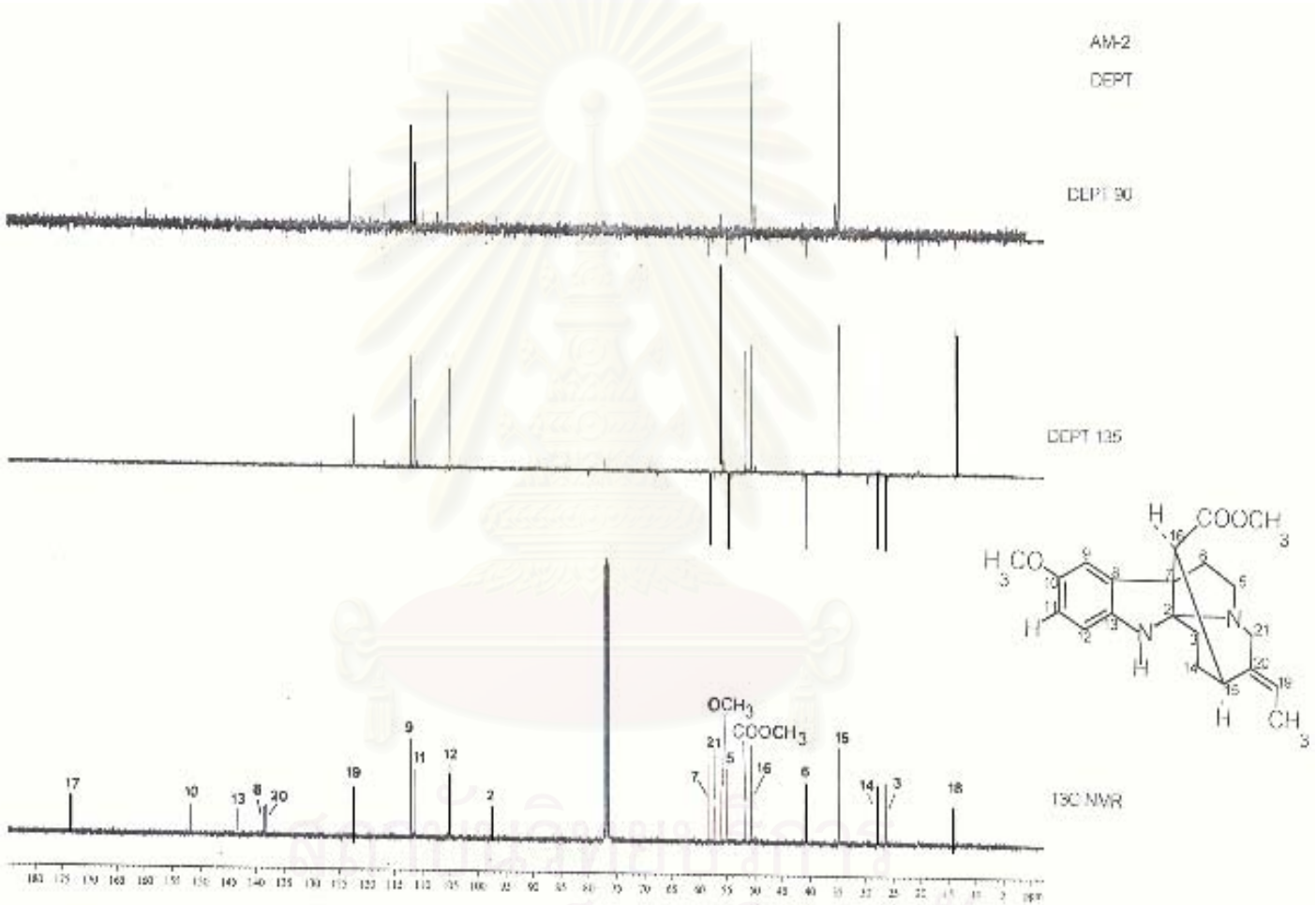


Figure 27 75 MHz ^{13}C NMR, DEPT 90 and DEPT 135 spectrum of compound AM-2 (in $CDCl_3$)

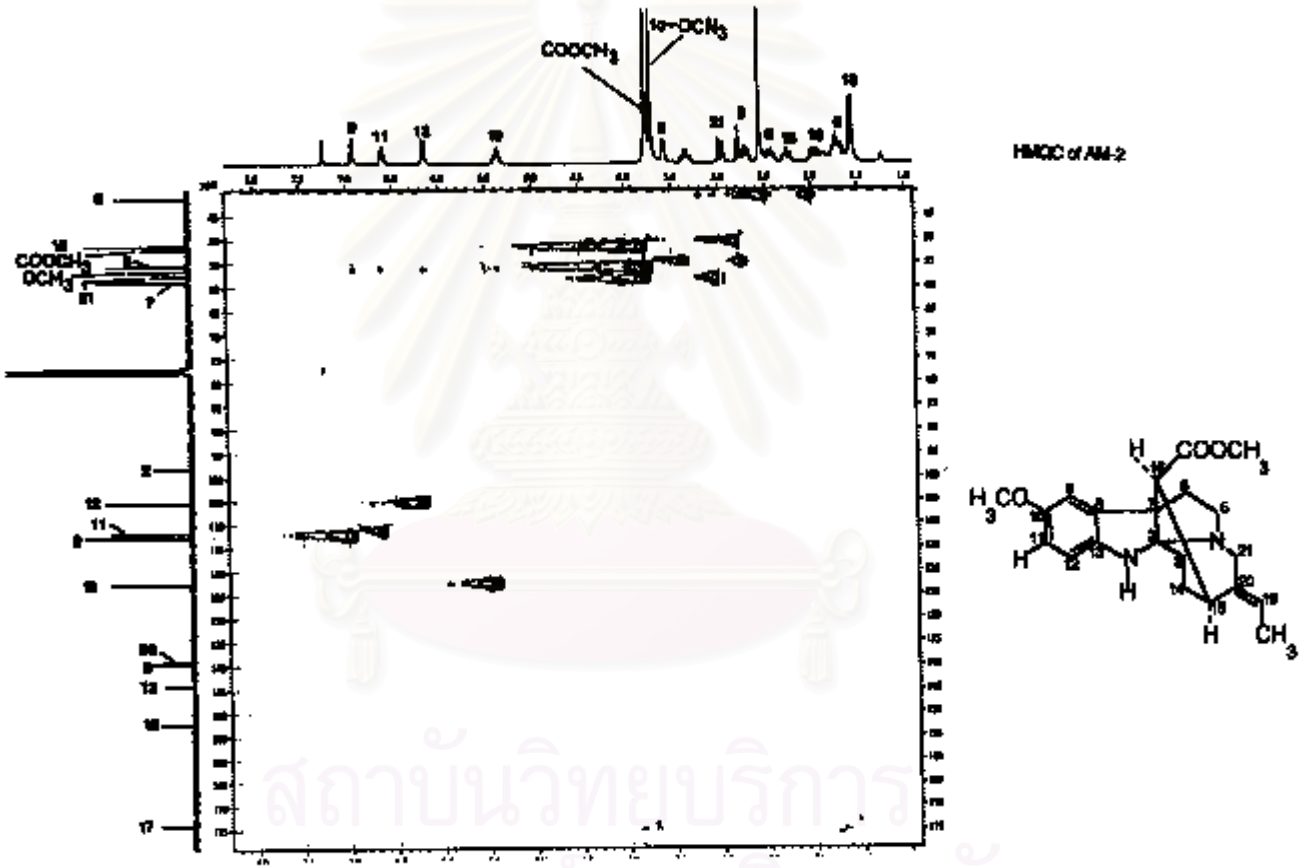


Figure 28 HMQC spectrum of compound AM-2 (in $CDCl_3$) [δ_H 1.0-8.2 ppm, δ_C 40.0-178.0 ppm]

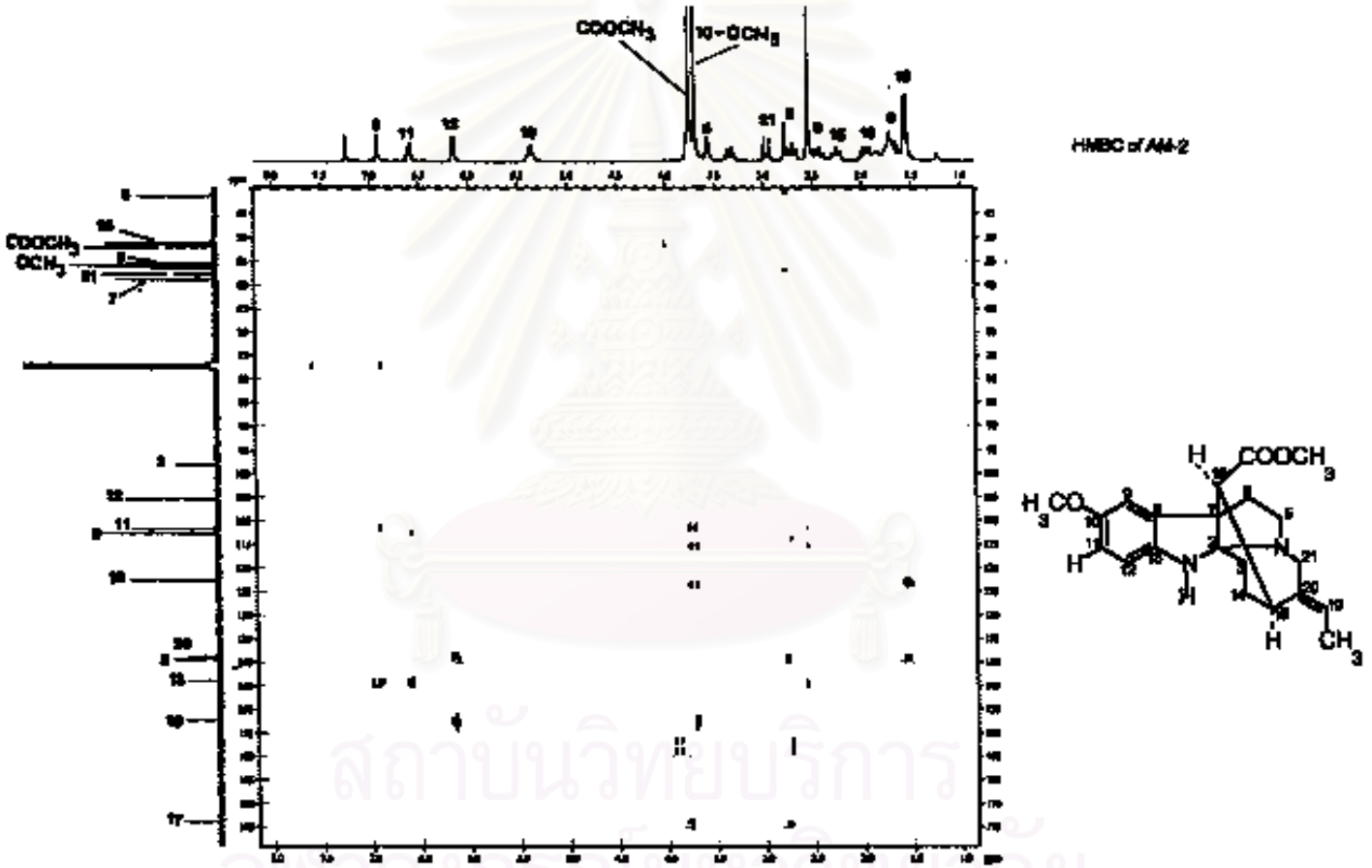


Figure 29 HMBC spectrum of compound AM-2 (in $CDCl_3$) [δ_H 1.0-6.0 ppm, δ_C 40.0-178.0 ppm]

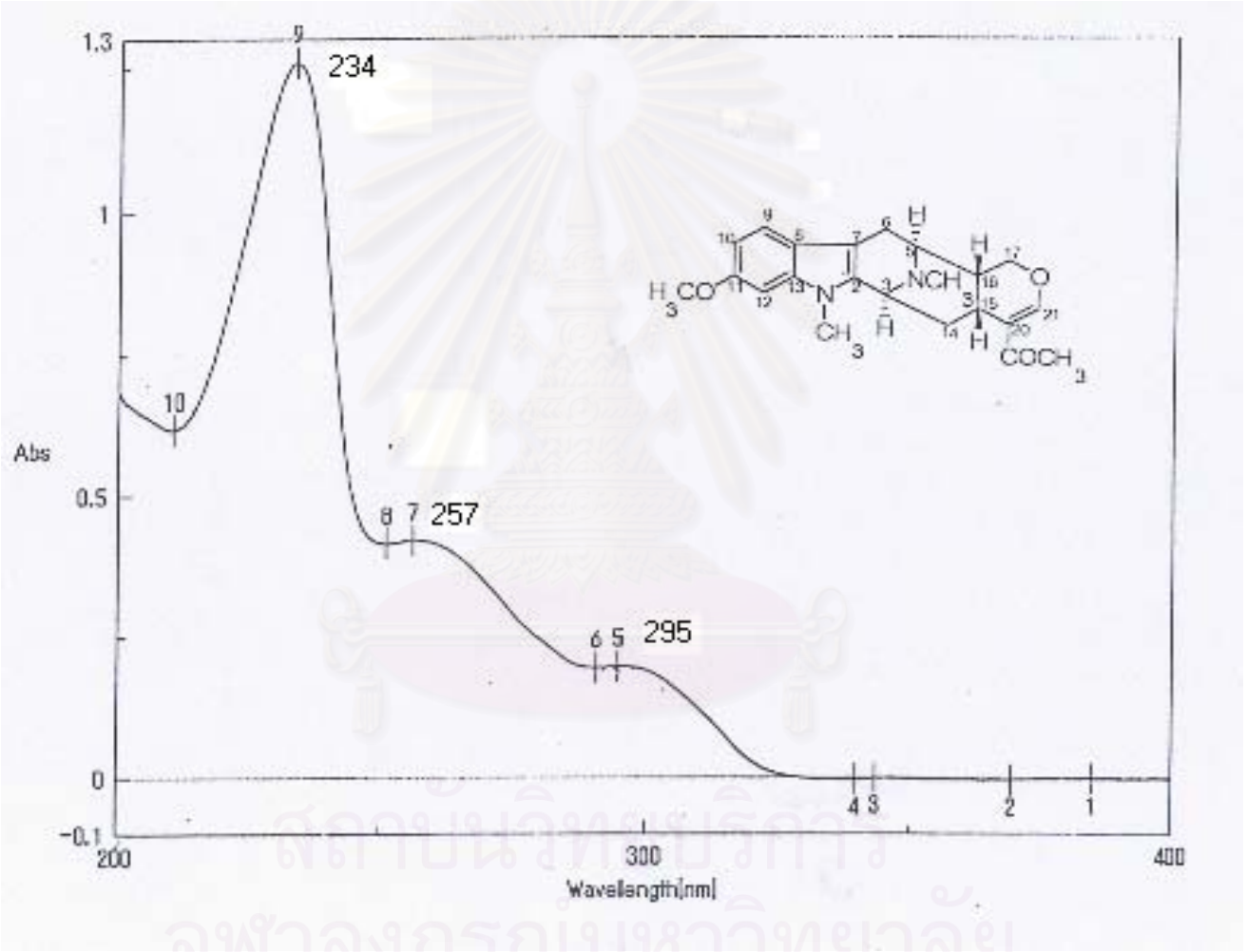


Figure 30 UV spectrum of compound AM-3 (in $CDCl_3$)

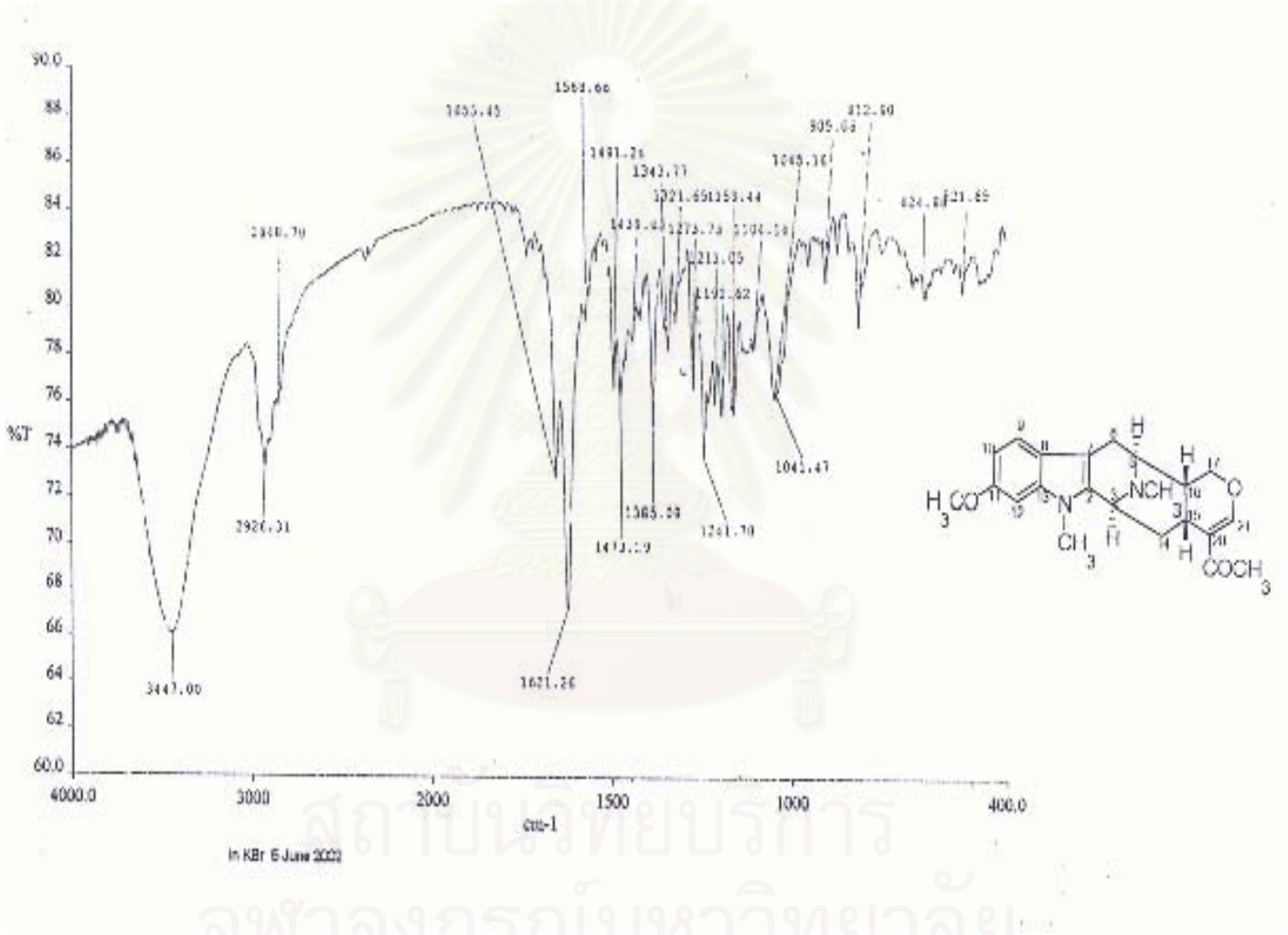


Figure 31 IR spectrum of compound AM-3 (KBr disc)

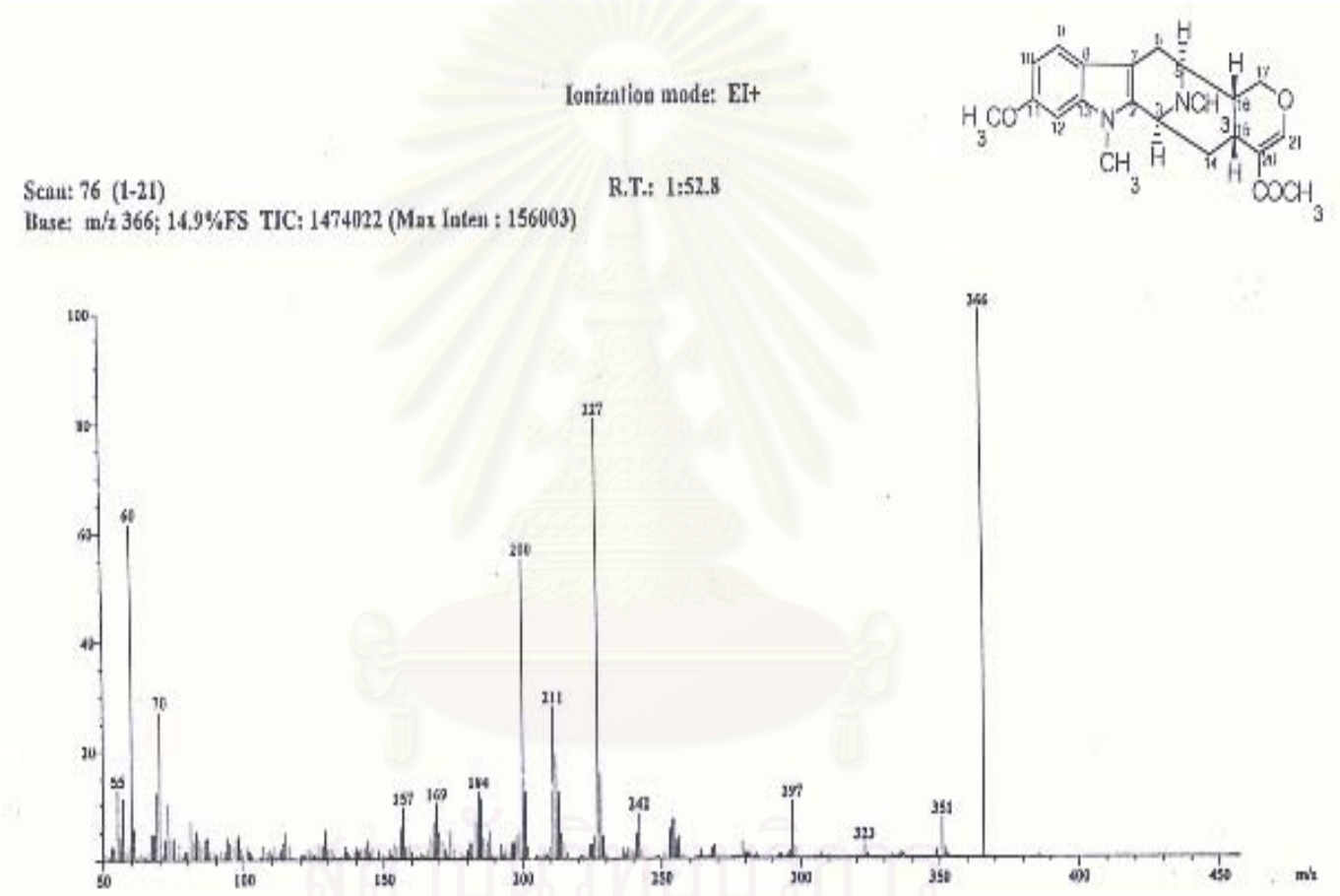


Figure 32 EI mass spectrum of compound AM-3

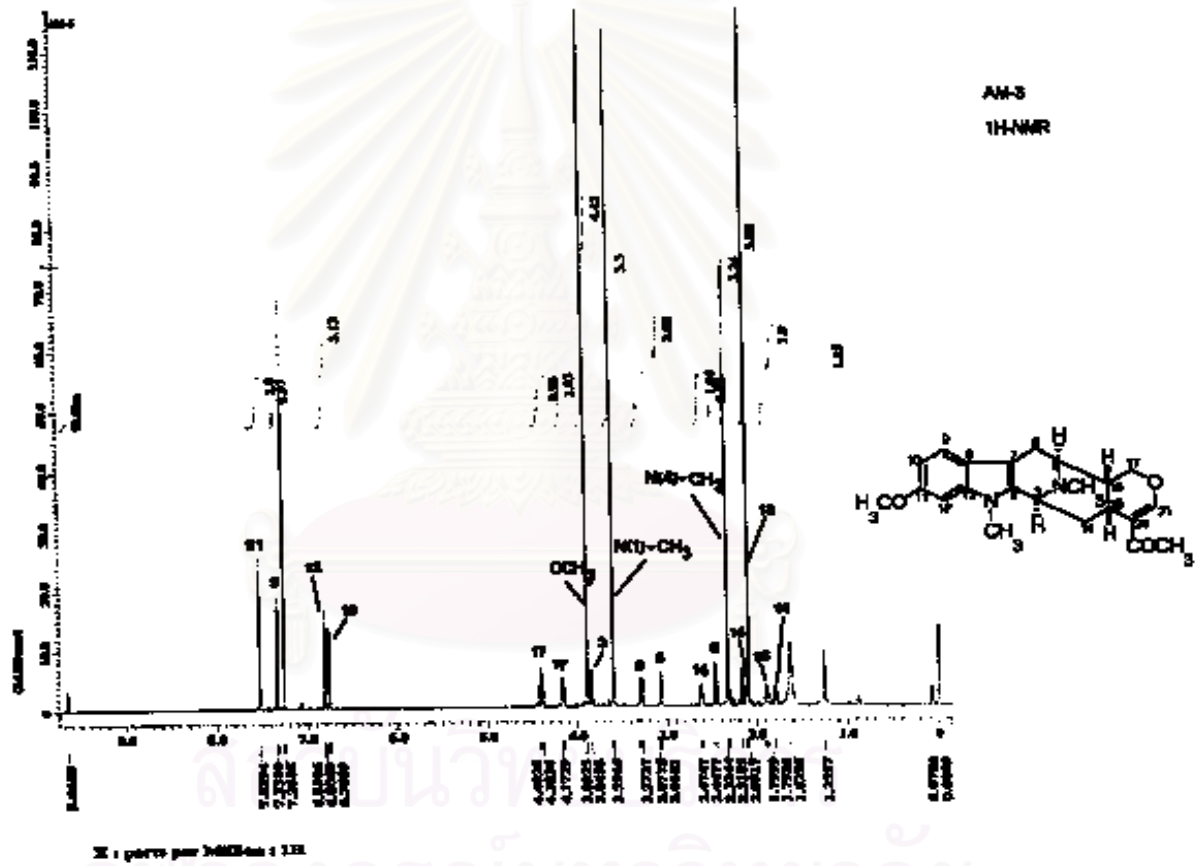


Figure 33 500 MHz ^{1}H NMR spectrum of compound AM-3 (In $CDCl_3$)

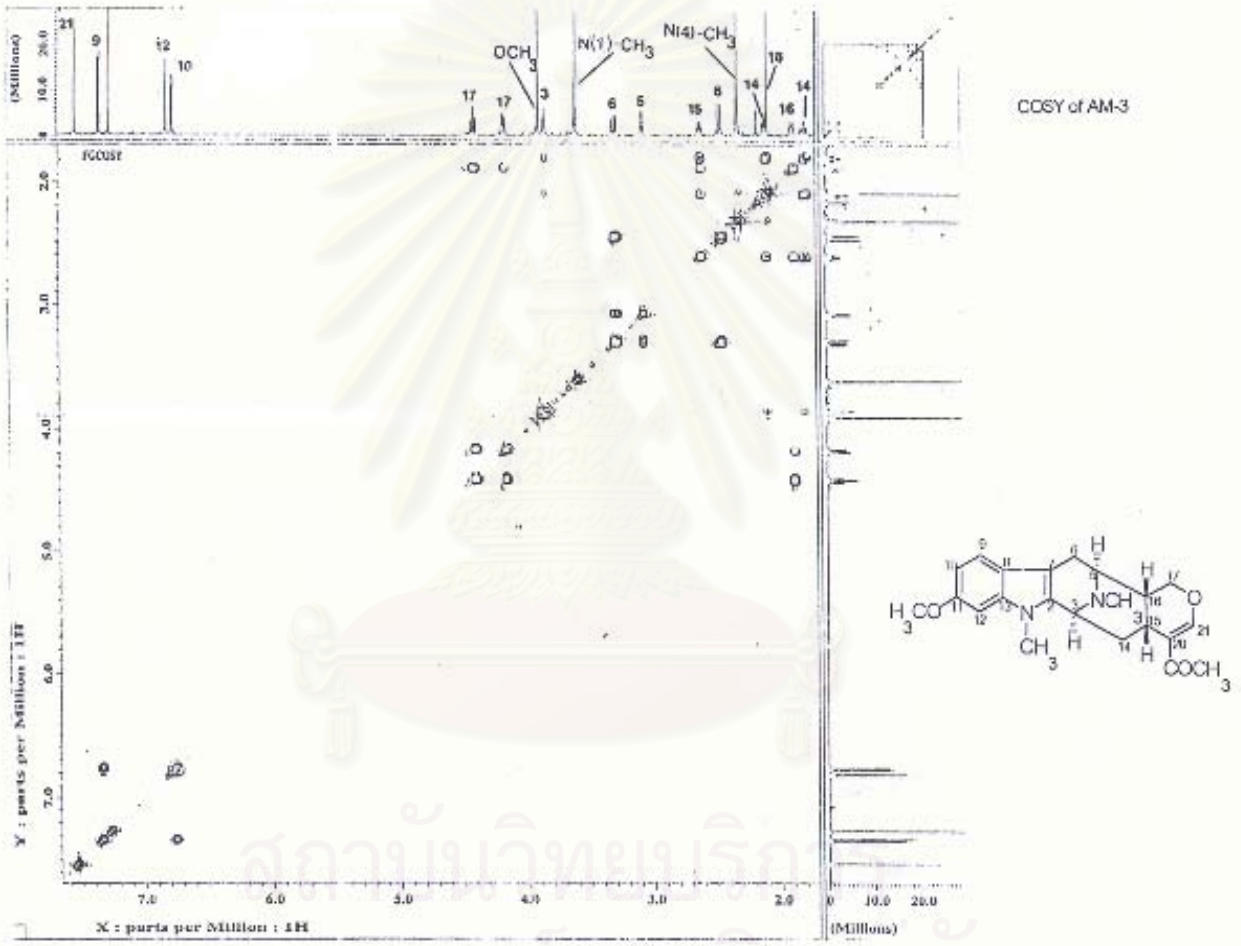


Figure 34 ^{1}H-^{1}H COSY spectrum of compound AM-3 (in $CDCl_3$)

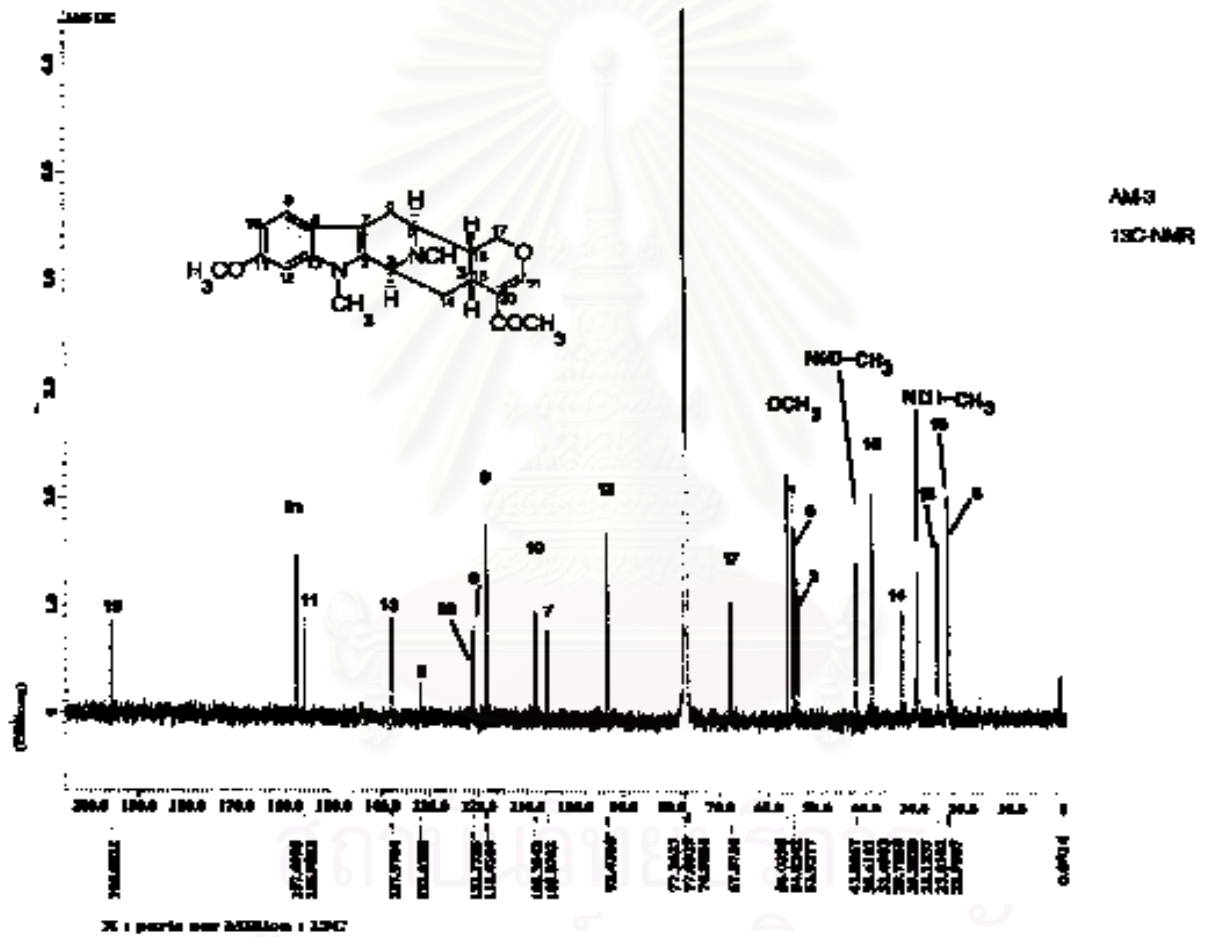


Figure 35 125 MHz ^{13}C NMR spectrum of compound AM-3 (in $CDCl_3$)

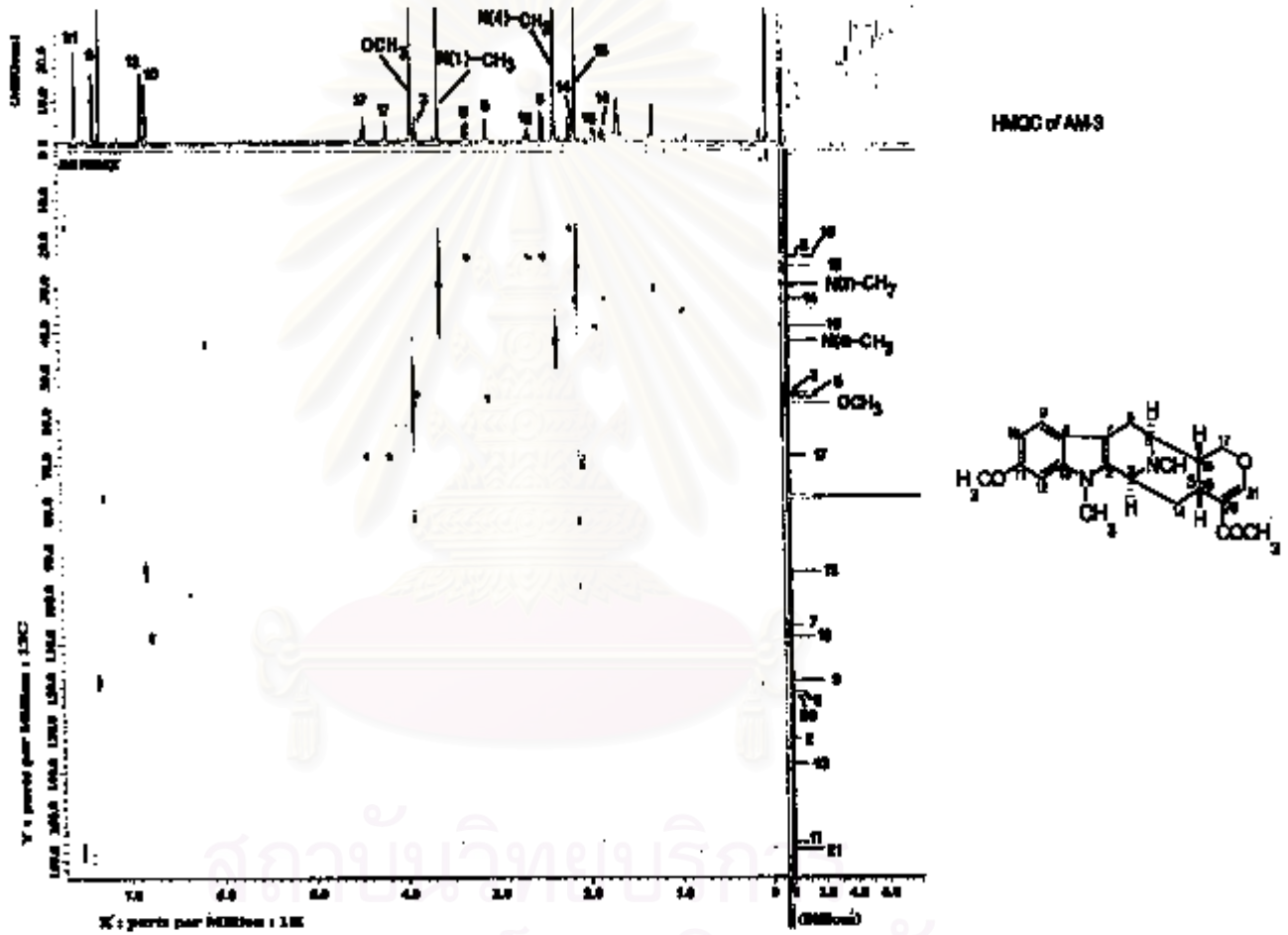


Figure 38 HMQC spectrum of compound AM-3 (in $CDCl_3$) [δ_H 0-7.7 ppm, δ_C 0-161.8 ppm]

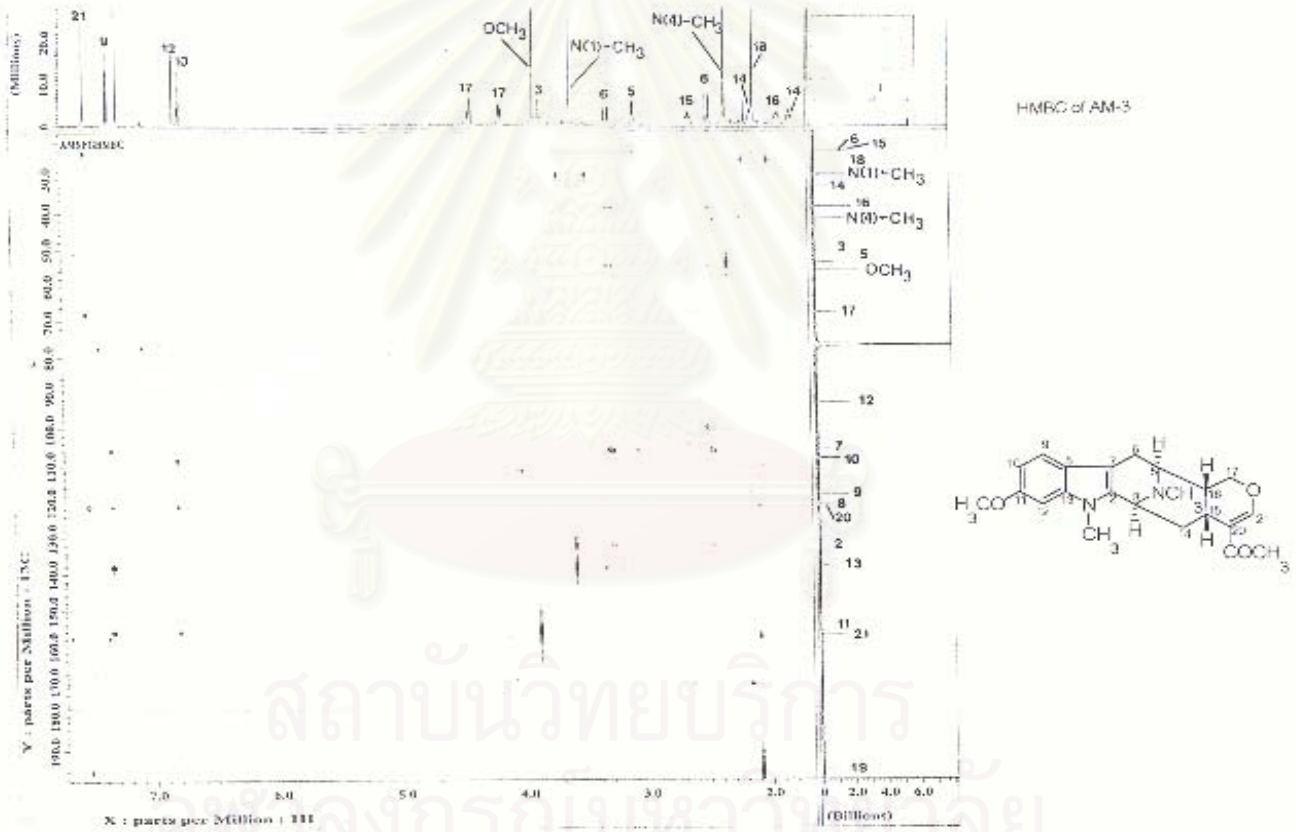


Figure 37 HMBC spectrum of compound AM-3 (in $CDCl_3$) [δ_H 1.7-7.7 ppm, δ_C 18.0-198.0 ppm]

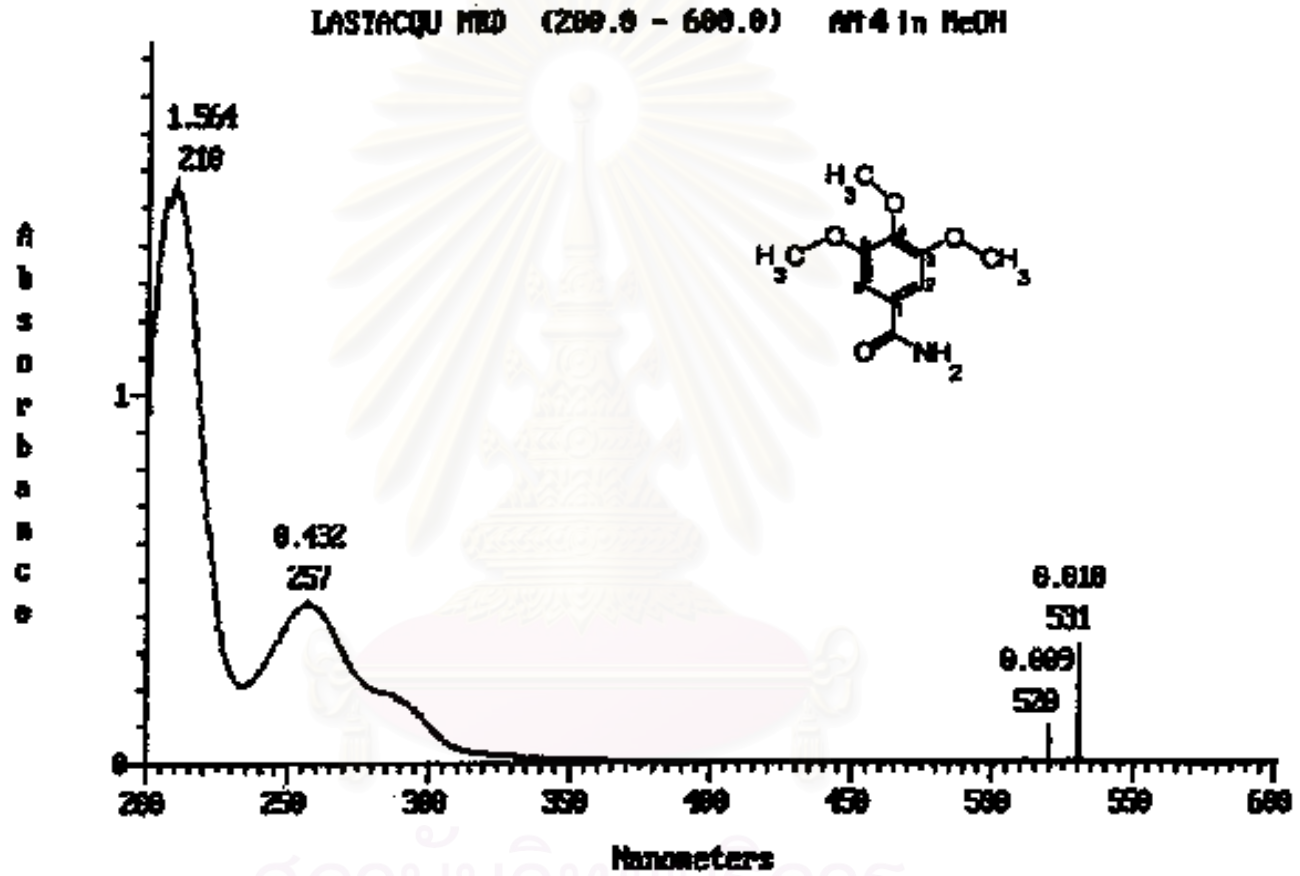
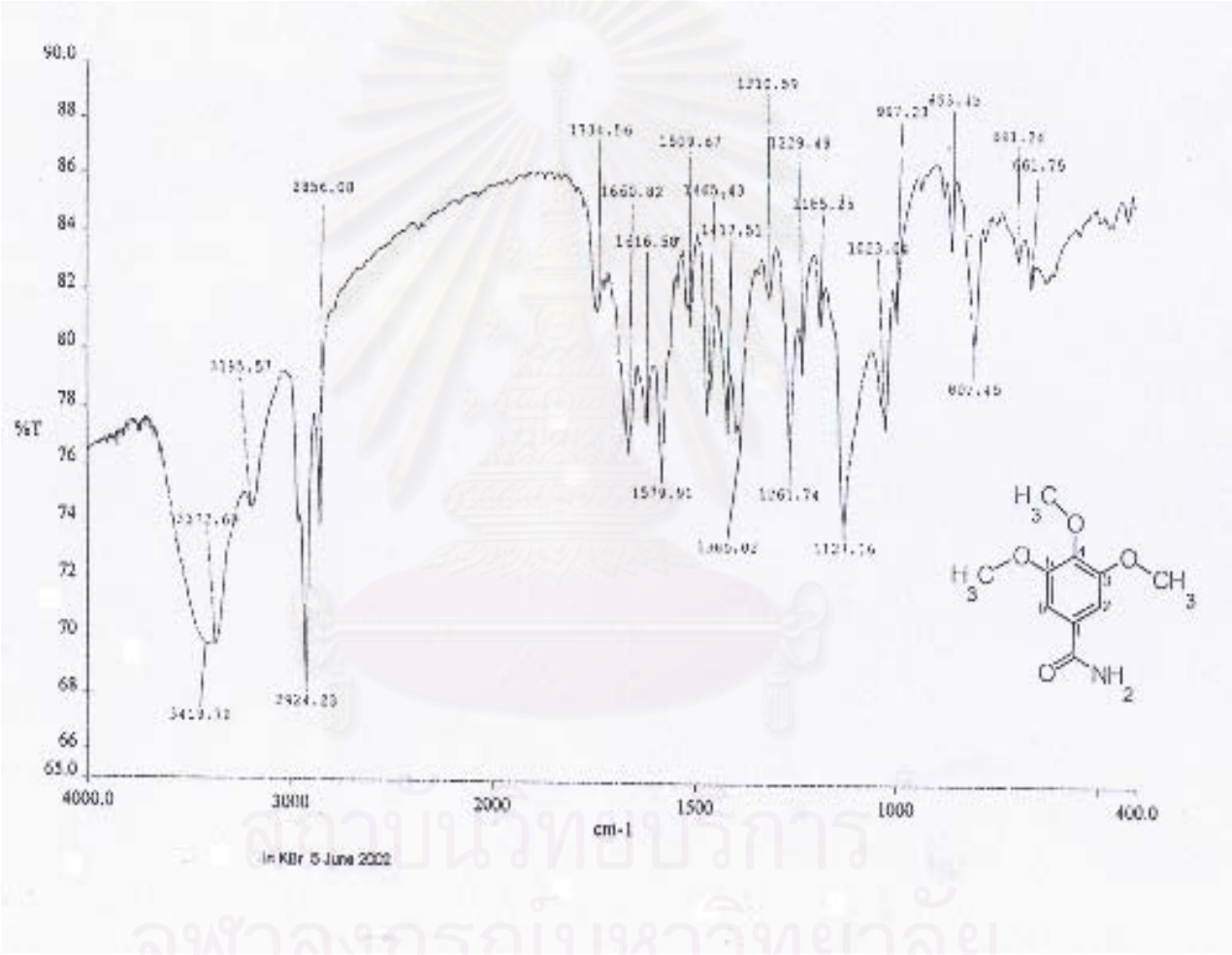


Figure 38 UV spectrum of compound AM-4 (in $CDCl_3$)

Figure 39 IR spectrum of compound AM-4 (KBr disc)



Fingure 39 IR spectrum of compound AM-4 (KBr

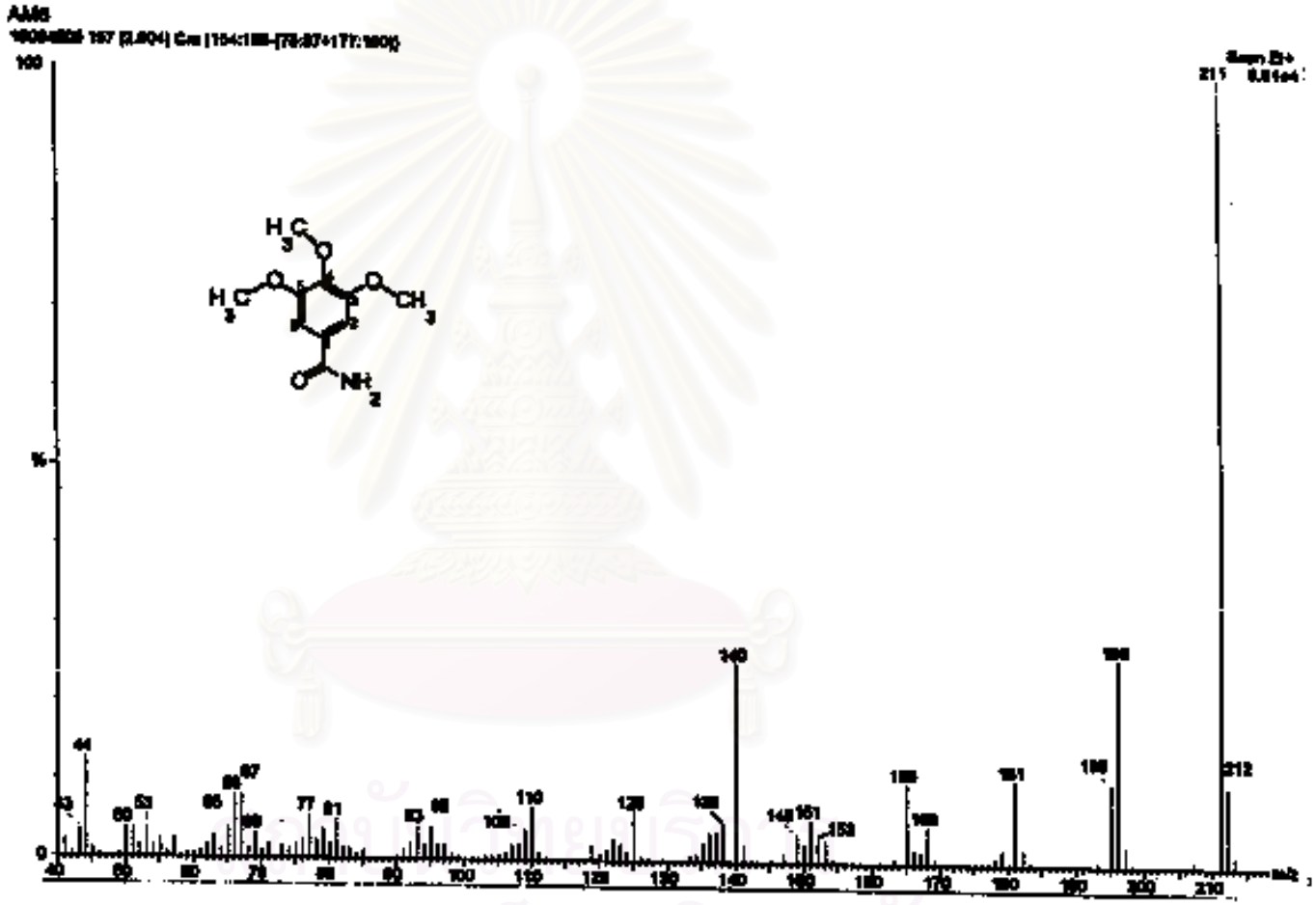


Figure 40 EI mass spectrum of compound AM-4

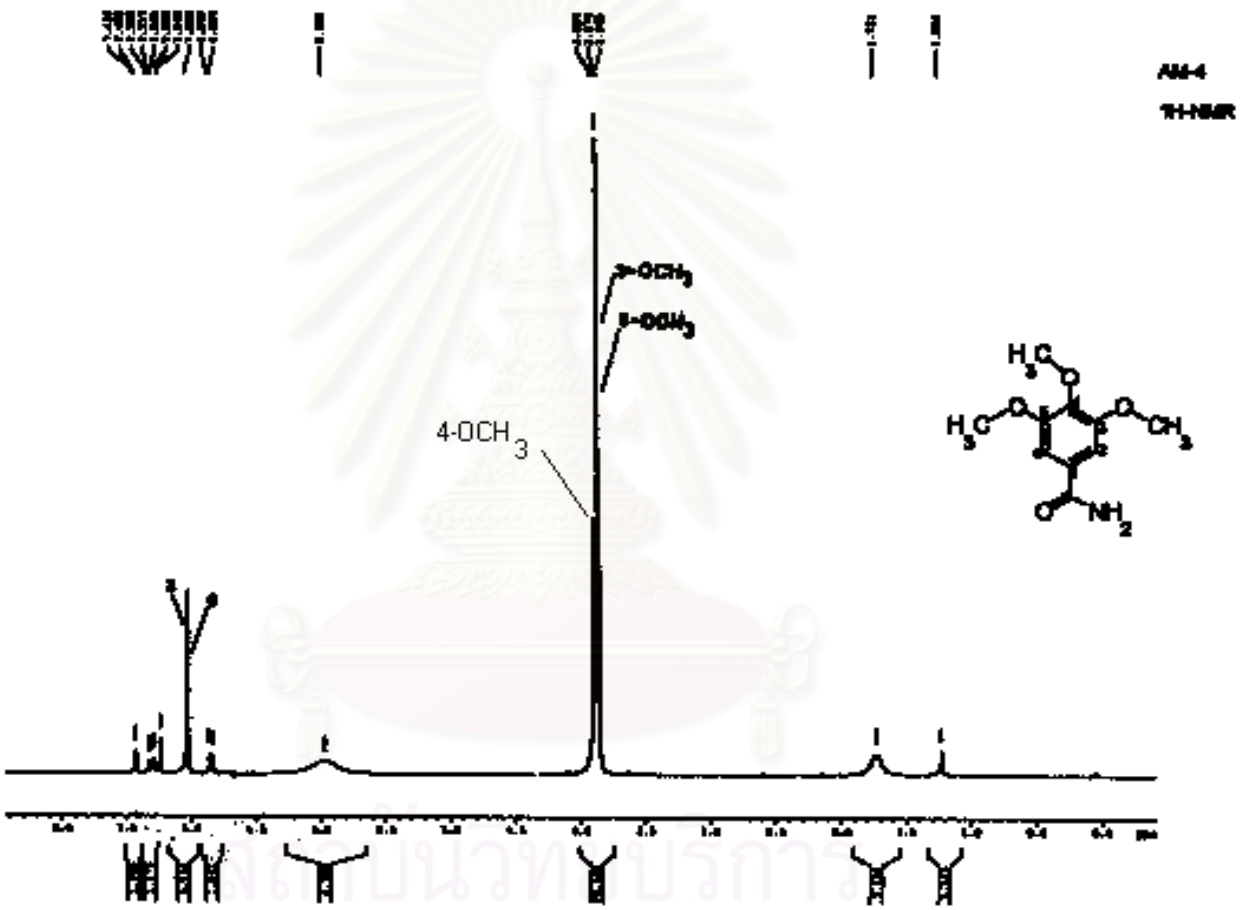


Figure 41 300 MHz ^{1}H NMR spectrum of compound AM-4 (in $CDCl_3$)

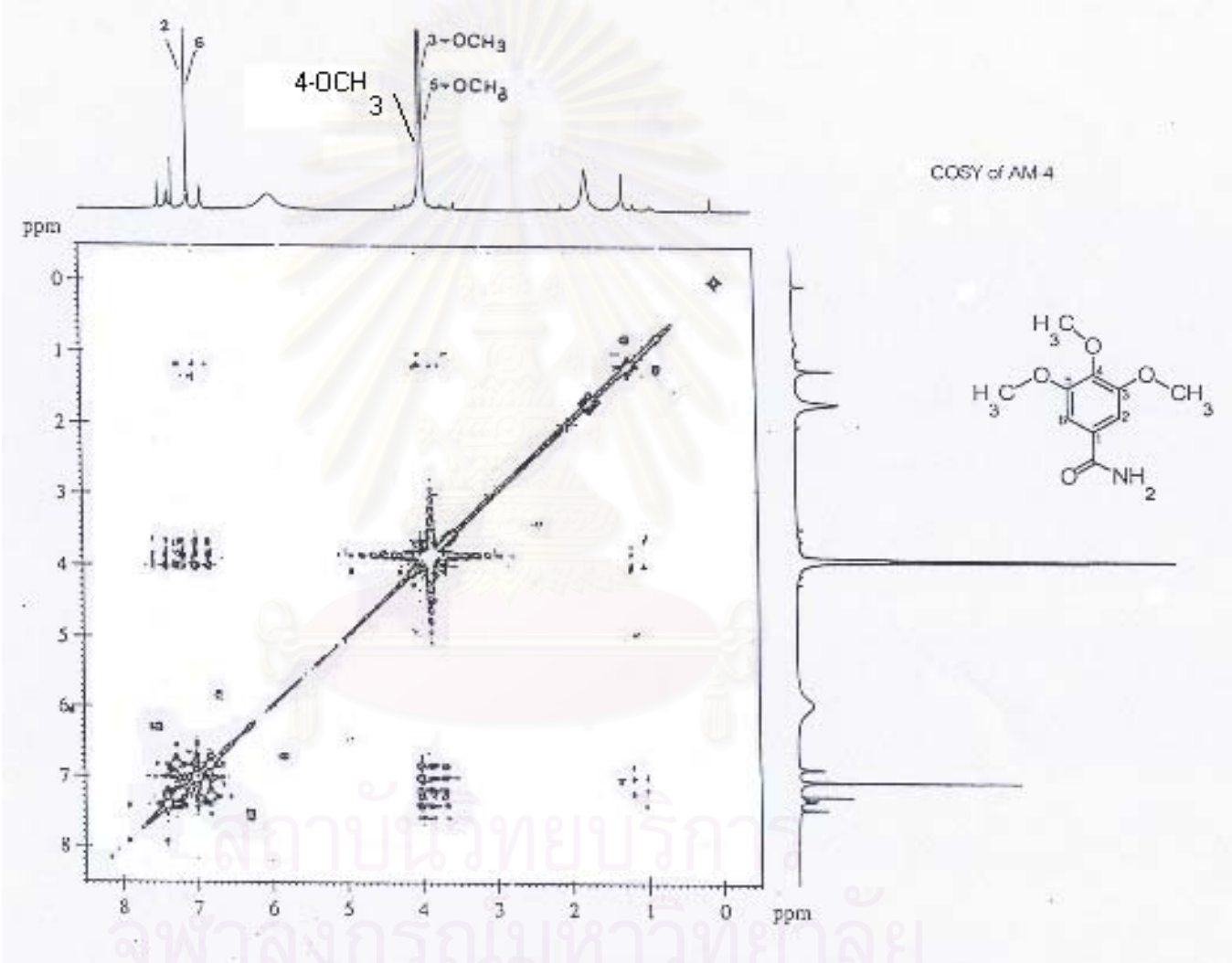


Figure 42 ^{1}H-^{1}H COSY spectrum of compound AM-4 (in $CDCl_3$)

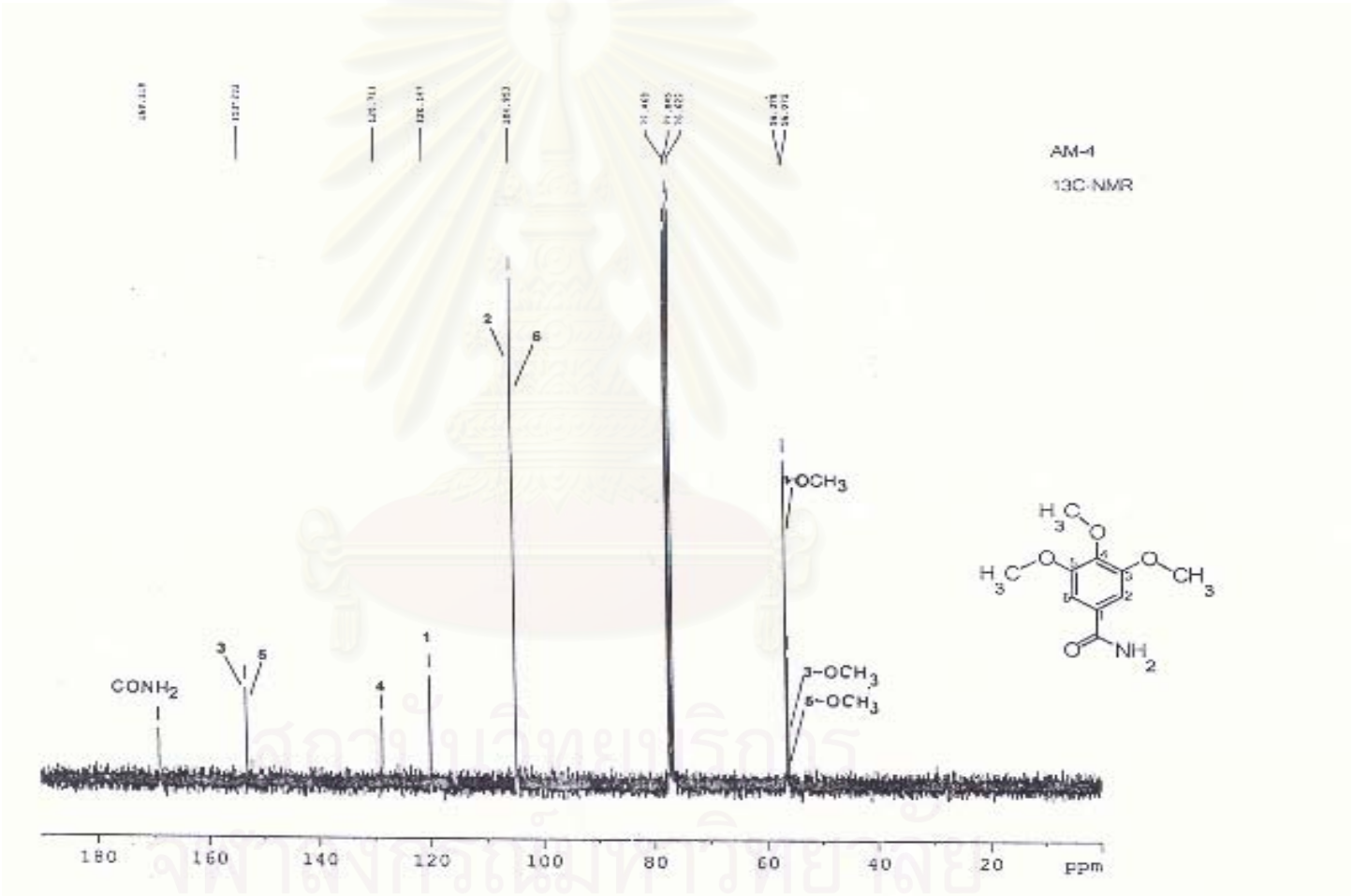


Figure 43 75 MHz ^{13}C NMR spectrum of compound AM-4 (in $CDCl_3$)

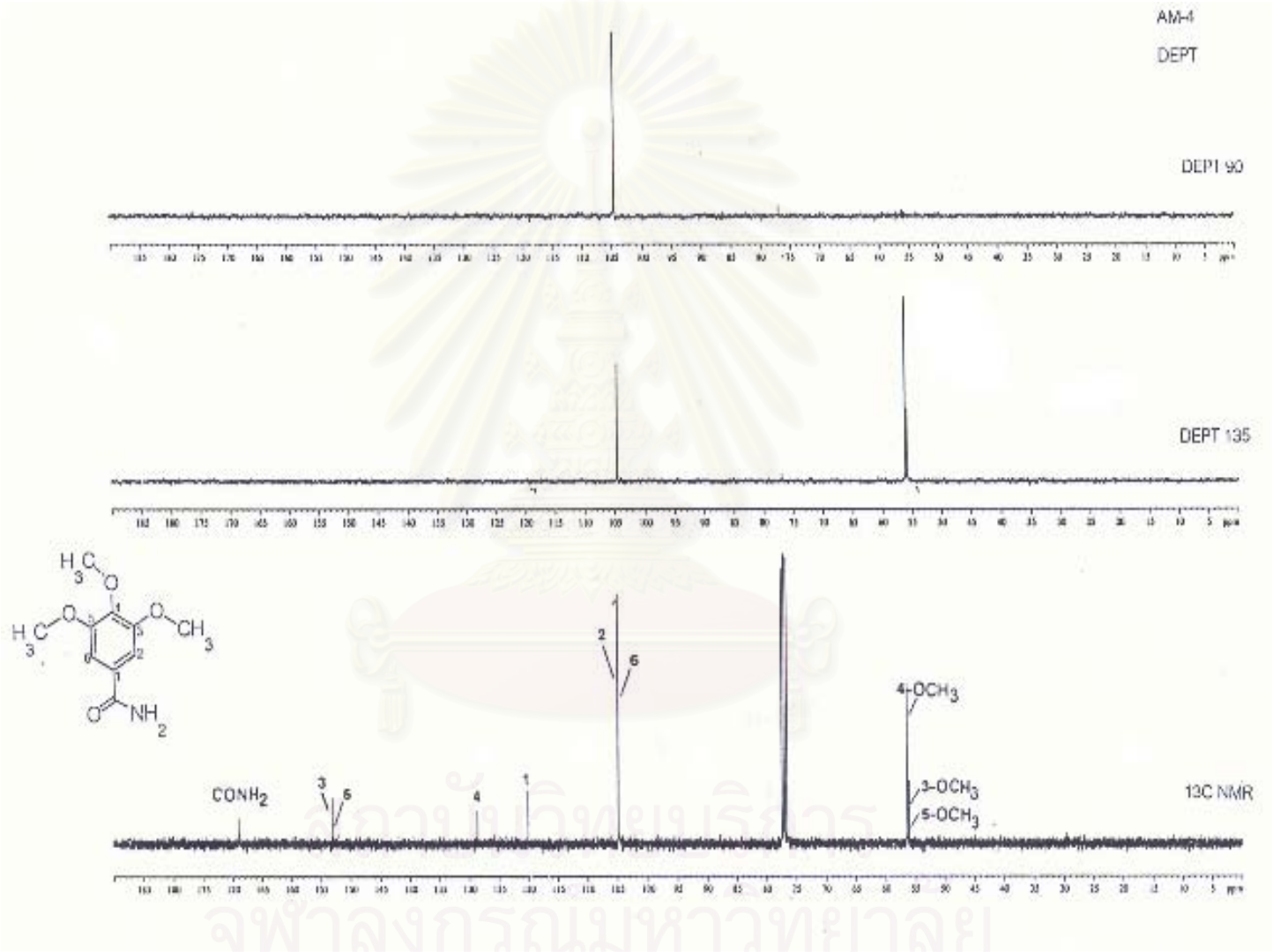


Figure 44 78 MHz ^{13}C NMR, DEPT 90 and DEPT 135 spectrum of compound AM-4 (in $CDCl_3$)

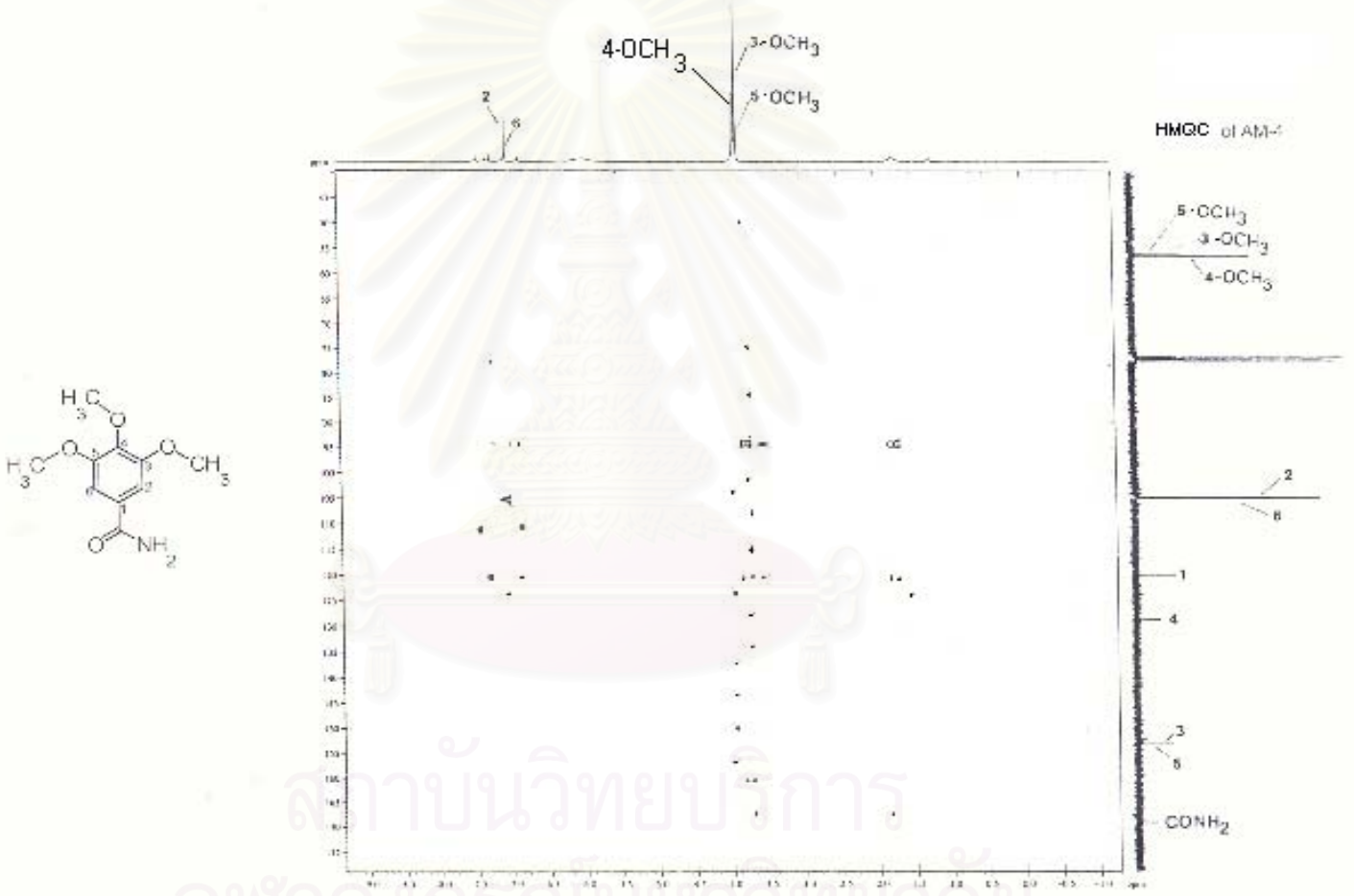


Figure 45 HMQC spectrum of compound AM-4 (in $CDCl_3$) [δ_H 1.2-9.3 ppm, δ_C 40.0-178.0 ppm]

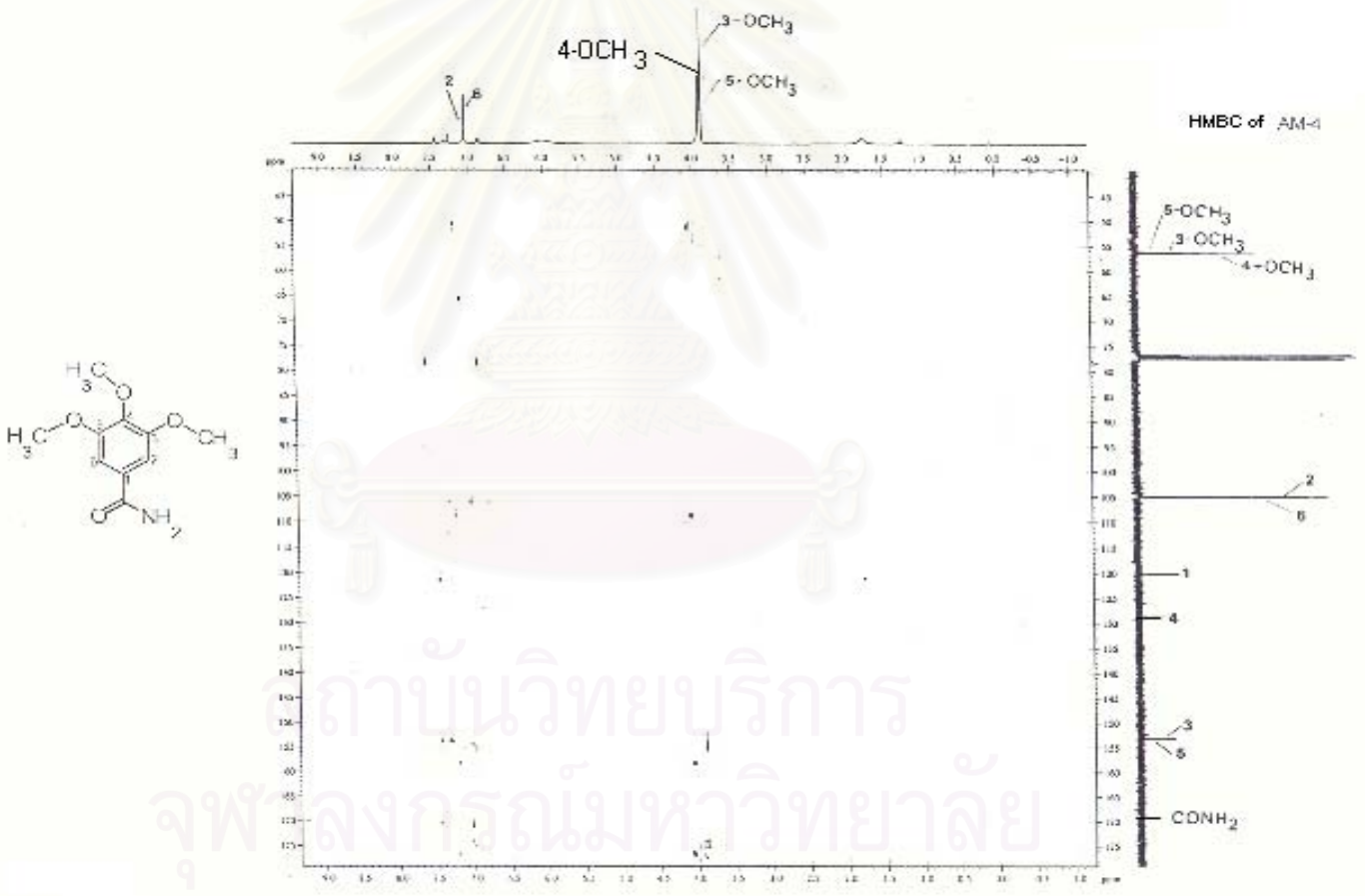


Figure 46 HMBC spectrum of compound AM-4 (in $CDCl_3$) [δ_H -1.2-9.3 ppm, δ_C 40.0-178.0 ppm]

VITA

Miss Wimaluk Nopsiri was born on October 18, 1974 in Phrae, Thailand. She received her Bachelor' s degree of Science in Pharmacy in 1998 from the Faculty of Pharmceutical Sciences, Chiang Mai University, Thailand.