อินโดลแอลคาลอยด์จากผลพญาสัตบรรณ

นางสาว ลักขณา ฉายศรี

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INDOLE ALKALOIDS FROM THE FRUITS OF *ALSTONIA SCHOLARIS*

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การศึกษาพฤกษเคมีของผลพญาสัตตบรรณ สามารถแยกองค์ประกอบทางเคมี จากสิ่งสกัดได้ 6 ชนิด ประกอบด้วยสารกลุ่ม indole alkaloid 4 ชนิด คือ picrinine, 19-*E*-akuammidine, 19,20-*E*-vallesamine และ 19*S*-scholaricine และพบสารกลุ่ม ester 2 ชนิด คือ dibutyl phthalate และ methyl ferulate ได้พิสูจน์โครงสร้างทางเคมีของสารประกอบที่แยกได้ด้วยการวิเคราะห์สเปคตรัมของ UV, IR, MS และ NMR ร่วมกับการเปรียบเทียบข้อมูลของสารที่ทราบโครงสร้างแล้ว

ภาควิชา เภสัชเวท	ลายมือชื่อนิสิต.....
สาขาวิชา เภสัชเวท	ลายมือชื่ออาจารย์ที่ปรึกษา.....
ปีการศึกษา 2543	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

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LAKHANA CHAISRI : INDOLE ALKALOIDS FROM THE FRUITS OF *ALSTONIA SCHOLARIS*. THESIS ADVISOR : ASSOC. PROF. SUMPHAN WONGSERIPIPATANA, Ph.D., THESIS CO-ADVISOR : ASSOC. PROF. KITTISAK LIKHITWITAYAWUID, Ph.D., 191 pp. ISBN 974-346-363-1.

Phytochemical study of the fruits of *Alstonia scholaris* (L.) R. Br. led to the isolation of six compounds. These compounds are four indole alkaloids picrinine, 19-*E*-akuammidine, 19,20-*E*-vallesamine and 19*S*-scholaricine and two esters which are dibutyl phthalate and methyl ferulate. The structures of all of these isolates were determined by extensive spectroscopic studies, including comparison of their UV, IR, MS and NMR properties with previously reported data.

Department Pharmacognosy	Student's
Field of study Pharmacognosy	Advisor's signature……..
Academic year 2000	Co-advisor's

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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
COLOC	=	Correlation spectroscopy via Long-range Coupling
^{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)
def	=	Deformed (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polarization Transfer
δ	=	Chemical shift
EIMS	=	Electron Impact Mass Spectrum
EtOAc	=	Ethyl acetate
g	=	Gram
HETCOR	=	Heteronuclear Chemical Shift Correlation
^1H NMR	=	Proton nuclear Magnetic Resonance
HMBC	=	^1H-detected Heteronuclear Multiple Bond Correlation
HMQC	=	^1H-detected Heteronuclear Multiple Quantum Correlation
Hz	=	Hertz
IR	=	Infrared spectrum
J	=	Coupling constant

KBr	=	Potassium bromide
Kg	=	Kilogram
L	=	Liter
λ_{max}	=	Wavelength at maximal absorption
ε	=	Molar absorptivity
M^+	=	Molecular ion
m	=	Multiplet (for NMR spectra)
MeOH	=	Methanol
mg	=	Milligram
MHz	=	MegaHertz
ml	=	Milliliter
mm	=	Millimeter
m/z	=	Mass to charge ratio
MS	=	Mass spectrometry
nm	=	Nanometer
NMR	=	Nuclear magnetic resonance
NOE	=	Nuclear Overhauser Effect
NOESY	=	Nuclear Overhauser Effect Correlation Spectroscopy
ppm	=	part per million
pyridine-d_5	=	Deuterated pyridine
ν_{max}	=	Wave number at maximal absorption
q	=	Quartet (for NMR spectra)
s	=	Singlet (for NMR spectra)
t	=	Triplet (for NMR spectra)
TLC	=	Thin layer chromatography
UV	=	Ultraviolet

CHAPTER I

Introduction

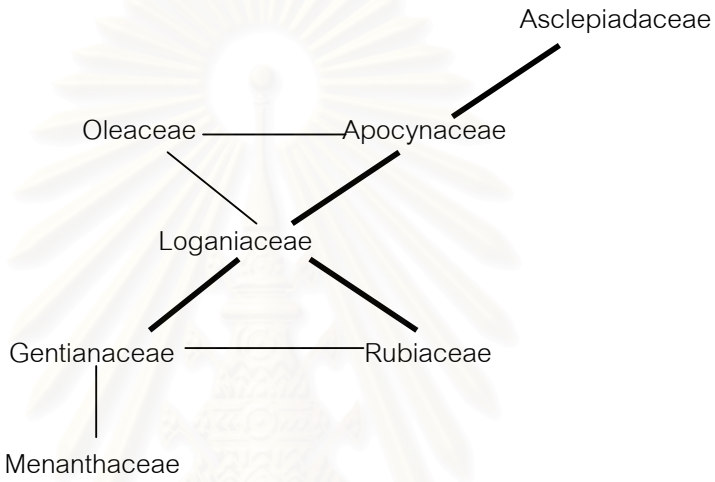
Plants have made a unique contribution to humankind as sources of drugs and for meeting other basic needs since prehistoric times. Almost all cultures in the world have their own expertise concerned with the therapeutic properties of the local flora. About 20,000 plant species are used for medicinal purposes around the world (Phillipson and Anderson, 1989). In addition, it has been estimated by the World Health Organization (WHO) that about 80 % of people in developing countries still rely on plants as the major source of medicine in primary health care (Farnsworth, 1993).

As summarized recently, the active principles of 119 drugs, which represent some 60 therapeutic categories commonly in use in one or more countries are obtained from plants. 74% of these were discovered as a result of chemical and biological studies of plants used in traditional medicine (Farnworth, 1988). Examples include cardiotonic glycosides such as digitoxin and digoxin from *Digitalis* species, the anticholinergic tropane alkaloids, atropine, hyoscyamine, and scopolamine from *Atropa belladonna* and some other members of the Solanaceae, and the analgesic isoquinoline alkaloid, morphine obtained from the Opium poppy *Papaver somniferum*. A full list of such useful drugs is reported in several papers (e.g., Phillipson and Anderson, 1989).

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 plant families: Loganiaceae, Apocynaceae, and Rubiaceae, belonging to order Gentianales (Kisakurek and Hesse, 1980).

The order Gentianales comprises seven plant families. The three mentioned families, having remarkable morphological similarities, have been classified botanically in close relationship, as shown in the following diagram, the thick lines indicate a close degree of relationship (Leeuwenberg, 1980).



The occurrence of indole alkaloids in the families Apocynaceae, Loganiaceae, and Rubiaceae supports the idea given in the above diagram concerning their chemotaxonamy.

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. It is distributed throughout the tropical and subtropical parts of the world especially in Southeast Asia, Polynesia, Australia, India and Africa. 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; Forster, 1992).

Alstonia scholaris is known in Thai as Phayaasattaban (พญาสัตตบรรณ). In Thai traditional medicine, its stem bark and root bark, in dosage forms of decoction or tincture, have been used as remedies for treatment of malaria and some other ailments including chronic diarrhoea, dysentery, menstrual disorders, acute arthritis and fevers (Phuphattanaphong, 1979). In addition, its root has been reported for treatment of cancers (Hartwell, 1967). *A. scholaris* is a large tree measuring up to 40 m. Leaves: in whorls of 4-7; petioles 1-2 cm; blades spatulate, 5-18 cm long and 2-6 cm wide, rounded at apex; lateral nerves 30-40 pairs, 2-6 mm apart. Inflorescence: much-branched panicles; calyx lobes 5, lanceolate-ovate, 1-2 mm long; corolla lobes 5, yellowish white, pubescent, 3-5 mm long, overlapping to the left, corolla tube 5-7 mm long. Stamens 5, anthers 1 mm long, short filaments. Ovary: apocarpous, (bicarpellate). Fruit: follicles in pairs, 25-50 cm long and 0.3-0.4 cm wide. Seed: oblong, 4-6 mm long and 1 mm wide, flattened, with brown cilia 0.7-1.3 cm long (Phuphattanaphong, 1979).

This thesis was under taken in an effort to provide some observations on alkaloidal constituents in certain plant in the tribe Plumerieae (Alstonieae) of the family Apocynaceae. The specific interest was focused on indole alkaloid contents and *Alstonia scholaris* (L.) R. Br. was the subject of study. This plant was studied by several groups of researchers. Up to the present there has been only one phytochemical investigation of the fruits of *Alstonia scholaris*. From the fruits an indole alkaloid named akuammidine has been identified. The author wished to investigate some other possibly remaining interesting indole alkaloids from the fruits of this plant.

Figure 1 *Alstonia scholaris* (L.) R. Br.

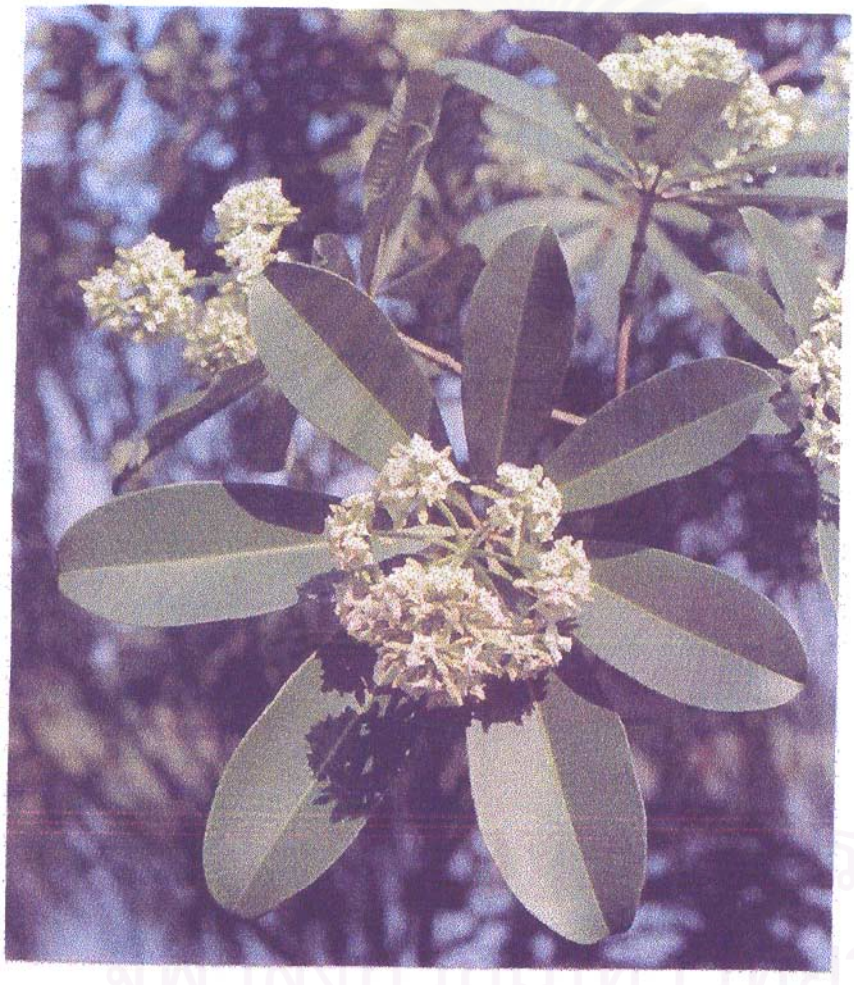


Figure 1 *Alstonia scholaris* (L.) R. Br. (continued)

Figure 1 *Alstonia scholaris* (L.) R. Br. (continued)

CHAPTER II

Historical

1. Distribution of the Genus *Alstonia*

The genus *Alstonia* was named in honour of Charles Alston (1685-1760) , a Scottish physician and Professor of Botany at 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 (Markgraf, 1974), New Caledonia (Boiteau *et al*., 1977), and Australia (Forster, 1992). 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. Mabberley (1987) has suggested that there are three species of *Alstonia* native to Central America, but no details of such species have been provided.

I. Section *Winchia* (monotypic)

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

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

II. Section *Pala* (Section *Alstonia*)

2.1 *A. actinophylla* (A. Cunn.) K. Schum. [syn: *A. verticillosa* F. Muell.]

2.2 *A. angustiloba* Miq.

2.3 *A. boonei* De Wild.

2.4 *A. congensis* Engl. [syn: *A. gilletii* De Wild.]

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

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

[syn: *A. kurzii* Hook. f., *Echites scholaris* L., *Echites pala* Ham.]

2.7 *A. spatulata* Bl.

2.8 *A. undulifolia* Kochum. & Wong

III. Section *Blaberopus*

3.1 *A. curtisii* King & Gamble

3.2 *A. mairei* Leveille

3.3 *A. neriifolia* D. Don [syn: *A. sericea* Bl.]

3.4 *A. rupestris* Kerr

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

3.6 *A. venenata* R. Br. [syn: *Blaberopus venenatus* A. DC.]

3.7 *A. yunnanensis* Diels

IV. Section *Monuraspermum*

4.1 *A. angustifolia* Wall. ex A. DC.

4.2 *A. brassii* Monach.

4.3 *A. glabriflora* Markgraf

4.4 *A. linearis* Benth.

4.5 *A. macrophylla* Wall. ex G. Don [syn: *A. batino* Blanco]

4.6 *A. muelleriana* Domin

4.7 *A. ophioxyloides* F. Muell.

4.8 *A. parvifolia* Merrill

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

V. Section *Dissuraspermum*

5.1 *A. balansae* Guillaum.

5.2 *A. boulindaensis* Boit.

5.3 *A. constricta* F. Muell. [syn: *A. mollis* Benth.]

5.4 *A. coriacea* Pancher ex S. Moore

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

5.5 *A. costata* (Forst. f.) R. Br.

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

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

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

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

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

5.9 *A. legouixiae* van Heurck & Muell. Arg. [syn: *A. saligna* S. Moore]

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

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

5.11 *A. montana* Turrill [syn: A. smithii Markgraf]

5.12 *A. odontophora* Boit.

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

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

5.15 *A. reineckeana* Lauterb.

5.16 *A. sphaerocapitata* Boit.

5.17 *A. undulata* Guillaum.

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

5.19 *A. vitiensis* Seem. [syn: *A. villosa* Seem.]

VI. Unknown Section

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 South 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*. According to Boiteau *et al*. (1977), all 14 species of *Alstonia* found in New Caledonia belong to the section *Dissuraspermum*.

2. Distribution of Indole Alkaloids

The genera of the Loganiaceae, Apocynaceae and Rubiaceae which have species containing indole alkaloids are listed below (Leeuwenberg, 1980).

Family Loganiaceae

Tribe Gelsemieae	*Gelsemium*
	Mostuea
Tribe Strychneae	*Strychnos*
	Gardneria

Family Apocynaceae

Subfamily Plumerioideae

Tribe Carisseae	
Subtribe Carissinae	*Melodinus*
	Leuconotis
Subtribe Landolphiinae	*Landolphia* (*Carpodinus*)
Subtribe Pleiocarpinae	*Picralima*
	Hunteria (*Polyadoa*)
	Pleiocarpa
Tribe Plumerieae (Alstonieae)	
Subtribe Craspidosperminae	*Craspidospermum*
Subtribe Plectaneiinae	*Gonioma*
Subtribe Alstoniinae	*Alstonia*

Tonduzia

Subtribe Aspidospermatinae *Diplorhynchus*

Aspidosperma

Geissosperum

Subtribe Catharanthinae *Rhazya*

Amsonia

Catharanthus

Vinca

Haplophyton

Tribe Rauvolfieae

Subtribe Rauvolfiinae *Cabucala*

Rauvolfia

Subtribe Ochrosiinae *Ochrosia* (*Excavatia*)

Subtribe Vallesiinae *Vallesia*

Kopsia

Subtribe Condylocarpinae *Condylocarpon*

Tribe Tabernaemontaneae *Crioceras*

Callichilia (*Hedranthera*)

Stemmadenia

Capuronetta

Tabernaemontana (*Pagiantha*, *Rejoua*, *Ervatamia*, *Hazunta*, *Peschiera*, *Conopharyngia*, *Pandaca*, *Gabunia*)

Tabernanthe

Voacanga

Scizozygia

Family Rubiaceae

Subfamily Rubioideae

Tribe Chiococceae *Hodgkinsonia*

Tribe Psychotrieae *Psychotria*

Palicourea

Cephaelis

Tribe Urophylleae	*Pauridiantha*
Tribe Ophiorrhizeae	*Ophiorrhiza*
Tribe Hamelieae	*Hamelia*
Tribe Spermacoceae	*Spermacoce* (*Borreria*)
	Richardia (*Richardsonia*)?
Tribe Hedyotideae	*Hedyotis*?
	Manettia?
Subfamily Cinchonoideae	
Tribe Naucleeae	*Nauclea* (*Sarcocephalus*)
	Cephalanthus
	Neonauclea
	Mitragyna
	Uncaria
	Anthocephalus
	Adina
Tribe Cinchoneae	*Cinchona*
	Ladenbergia
	Remijia
	Corynanthe (*Pseudocinchona*)
	Pausinystalia
	Capirona?
	Exostema?
	Coutarea
	Hymenodictyon?
	Crossopteryx?
	Ferdinandusa?
Tribe Rondeletieae	*Pogonopus*?
	Simira (*Sickingia*, *Arariba*)
Tribe Mussaendeae	*Isertia*
Tribe Gardenieae	*Leptactina*
	Tocoyena?

Tribe Coffeeae	*Tarenna*
Subfamily Guettardoideae	
Tribe Guettardeae	*Antirhea*
	Timonius
Subfamily Hillioideae	
Tribe Hillieae	*Hillia*?

(a. Names in brackets represent synonyms; b. question marks indicate that the alkaloids have not definitely been characterized as indole alkaloids)

3. Chemical Constituents of the Genus *Alstonia*

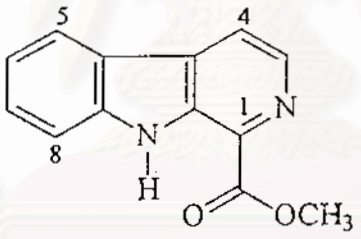
To date, about 246 indole alkaloids have been isolated from the genus *Alstonia*. During the last ten years many more new alkaloids and other compounds have been isolated from the genus *Alstonia* and in order to provide an overall view of *Alstonia* constituents, the following section will deal with 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 (Kompis *et al*., 1971; Kisakurek and Hesse, 1980; Kisakurek *et al*., 1983.), 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

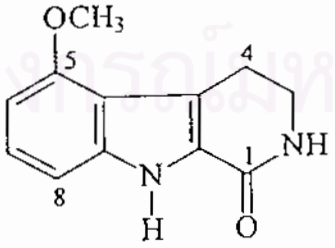
The indole alkaloids are characterized by having the indole nucleus which derives from the amino acid tryptophan and comprise two classes, i.e. simple indole alkaloids and monoterpenoid-derived indole alkaloids. Indole alkaloids can undergo oxidation or dimerization during biosynthesis from which oxindoles 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 (1) from the stem bark of *A. constricta* (Allam *et al.*, 1987) and 5- methoxy-1-oxo-tetrahydro-β-carboline (2) from the root bark of *A. venenata* (Banerji *et al.*, 1982).



1-Carbomethoxy-β-carboline (1)

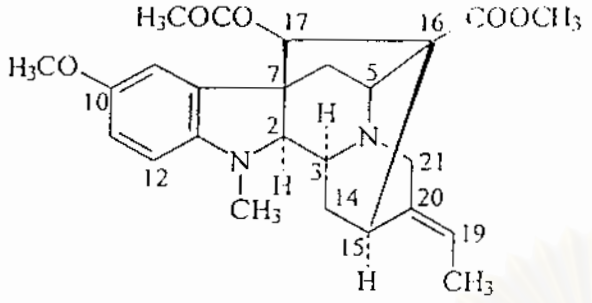


5-Methoxy-1-oxo-tetrahydro-β-carboline (2)

3.1.2 Corynanthean-type Indole Alkaloids

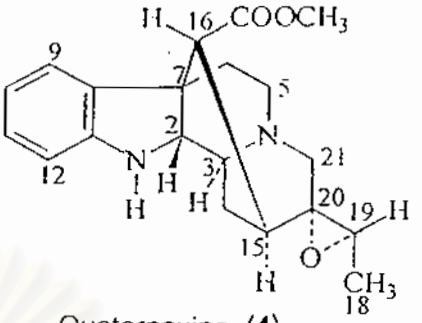
The Corynanthean-type includes those alkaloids containing a C(2)-C(3)-C(14) unit and N_b-C(21) bond, with the exception of the Alstonidine and Macroline groups which lack the N_b-C(21) bond. These alkaloids constitute the majority of *Alstonia* alkaloids and can be subdivided into 8 skeletal groups, as shown in Figure 2 with their representative alkaloids (**3-10**). 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; Morfaux *et al*., 1989; Pinchon *et al*., 1990); yohimbine derivatives (yohimbine group) from *A. quaternata* (Mamatas-Kalamaras *et al*., 1975b) and *A. venenata* (Govindachari *et al*., 1964, 1965; Chatterjee *et al*., 1965a, 1969a, 1981); vincorine and echitamine derivatives (vincorine group) *A. congensis* (Caron *et al*., 1989), *A. glaucescens* (Chen *et al*., 1988; 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 (Crow *et al*., 1970; Allam *et al*., 1987), six of the Macroline group (Cook *et al*., 1969; Hart *et al*., 1972; Burke *et al*., 1973a; Ratnayake *et al*.,1987; Ghedira *et al*., 1988) and three of the Pleiocarpamine group (Burke *et al*., 1973a; Jacquier *et al*., 1982) have been reported from the genus *Alstonia*.

Ajmaline group



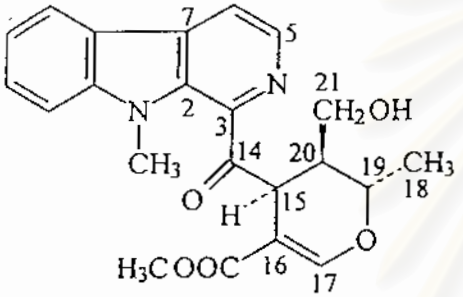
10-Methoxy-*O*-acetylvincamajine (**3**)

Akuammiline group



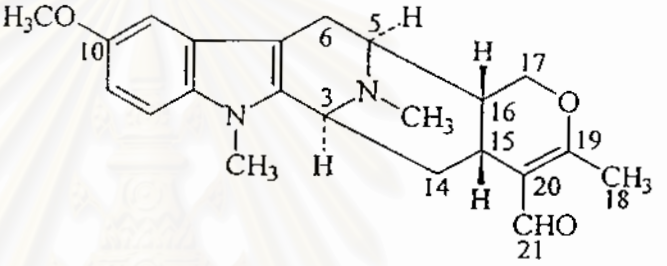
Quaternoxine (**4**)

Alstonidine group



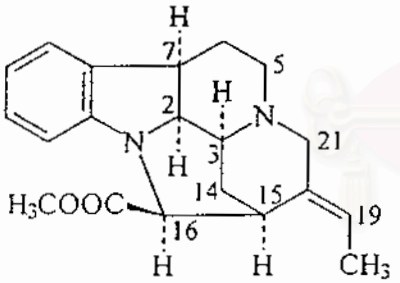
14-Ketoalstonidine (**5**)

Macroline group



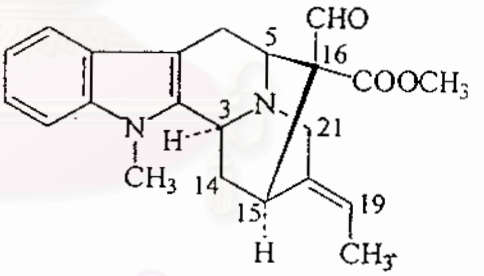
19,20-Dehydro-10-methoxytalcarpine (**6**)

Pleiocarpamine group



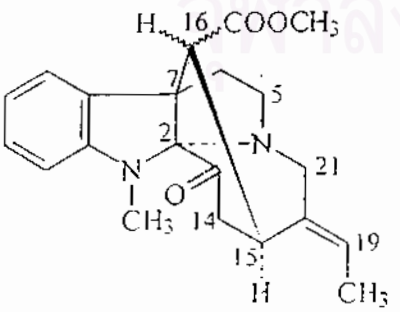
2,7-Dihydropleiocarpamine (**7**)

Sarpagine group



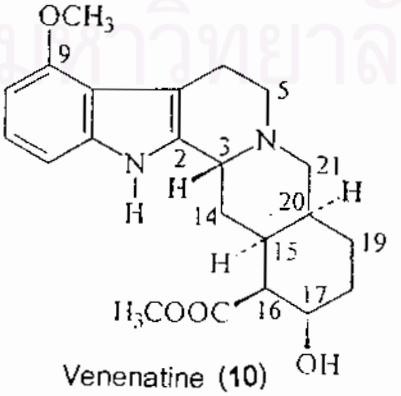
Voachalotinal (**8**)

Vincorine group



Vincoridine (**9**)

Yohimbine group

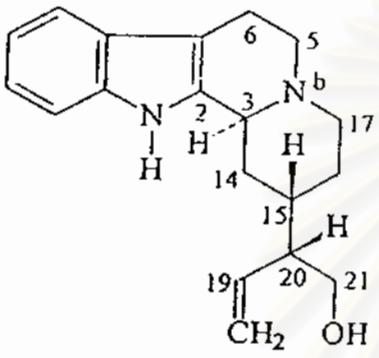


Venenatine (**10**)

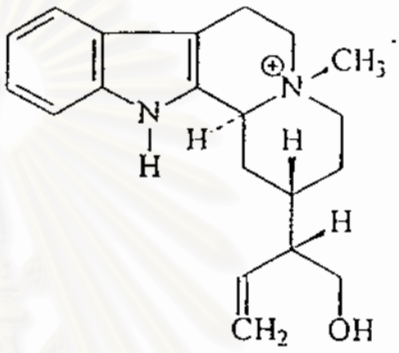
Figure 2 Skeletal groups of Corynanthean-type indole alkaloids occurring in *Alstonia* species

3.1.3 Vallesiachotaman-type Indole Alkaloids

Alkaloids of this type are recognized as those containing A 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 (11) from *A. odontophora* (Vercauteren *et al.*, 1979) and *A. angustifolia* (Ghedira *et al.* 1988) and N_b-β-methylantirhine (12) from *A. angustifolia* (Hu *et al.*, 1989), have been reported.



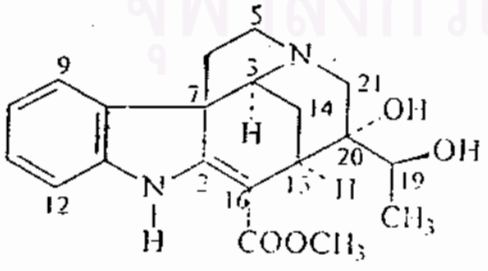
Antirhine (11)



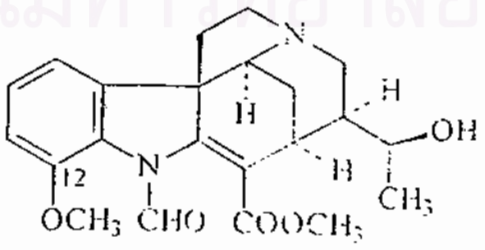
N_b-β-Methylantirhine (12)

3.1.4 Strychnan-type Indole Alkaloids

The Strychnan-type are those alkaloids containing a 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 (13) from *A. lanceolata* (Vercauteren *et al.*, 1981) and N_a-formyl-12-methoxyechitamidine (14) from *A. boonei* (Oguakwa *et al.*, 1983) are illustrated.



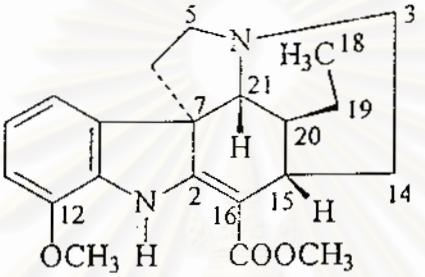
Compactinervine (13)



N_a-Formyl-12-methoxyechitamidine (14)

3.1.5 Aspidospermatan-type Indole Alkaloids

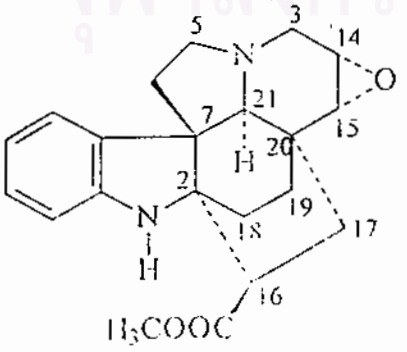
The Aspidospermatan-type alkaloids are those characterized by the forming of a C(2)-C(16)-C(15) unit without a C(3)-C(7) bond, and in some cases with a C(7)-C(21) bond instead. Four alkaloids of this type, belonging to the Tubotaiwine group, have been reported from genus *Alstonia*, for instance, 12-methoxytubotaiwine (15) 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, b).



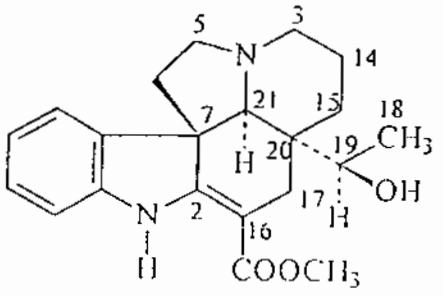
12-Methoxytubotaiwine (**15**)

3.1.6 Plumeran-type Indole Alkaloids

The Plumeran-type are those containing a C(2)-C(16)-C(17)-C(20) unit. Eighteen alkaloids of this 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 (**16**), were isolated only from *A. venenata* (Das and Biemann, 1965; Govindachari *et al.*, 1965; Chatterjee *et al.*,1981). Fourteen alkaloids of the Tabersonine group, for example, minovincinine (**17**), have been isolated mainly from *A. venenata* (Das *et al.*, 1966; Majunder *et al.*, 1973, 1974, 1979, 1981; Majunder and Dinda, 1974) and *A. yunnanensis* (Chen *et al.*, 1985, 1986).



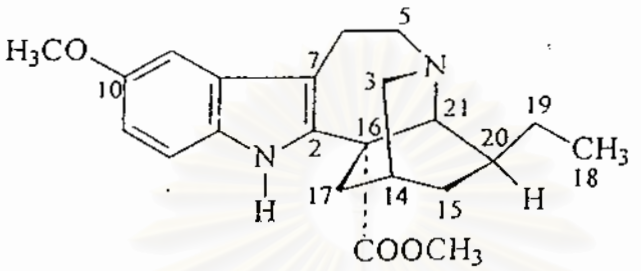
Venalstonidine (**16**)



Minovincinine (**17**)

3.1.7 Ibogan-type Indole Alkaloids

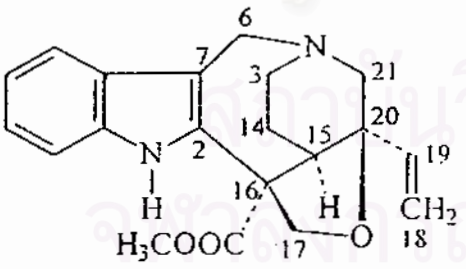
Alkaloids of this type are those containing the C(2)-C(16)-C(17)-C(14) unit. Voacangine (**18**), belonging to the Catharanthine group, from *A. boonei* (Croquelois *et al.*, 1972) is the only one structure of this type which has been reported so far from the genus *Alstonia*.



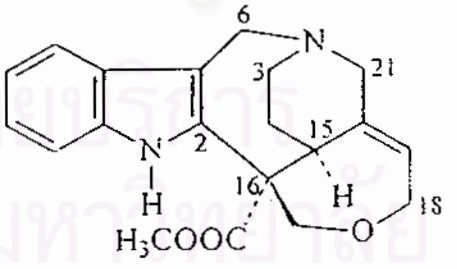
Voacangine (**18**)

3.1.8 Vallesamine-type Indole Alkaloids

Alkaloids of this type are characterized by having a 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. scholaris*. These alkaloids are mainly derived from angustilobine A (**19**) and angustilobine B (**20**) (Zeches *et al.*, 1987; Caron *et al.*, 1989; Yamauchi *et al.*, 1990a, b).



Angustilobine A (**19**)

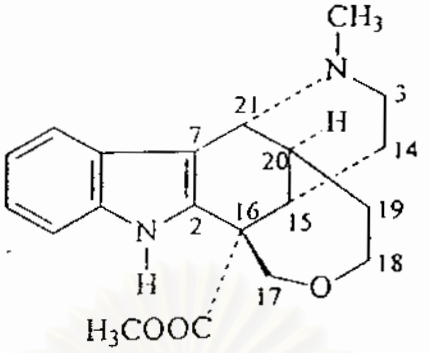


Angustilobine B (**20**)

3.1.9 Uleine-type Indole Alkaloids

The Uleine-type alkaloids are those which possess a C(2)-C(16)-C(15) unit and a 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

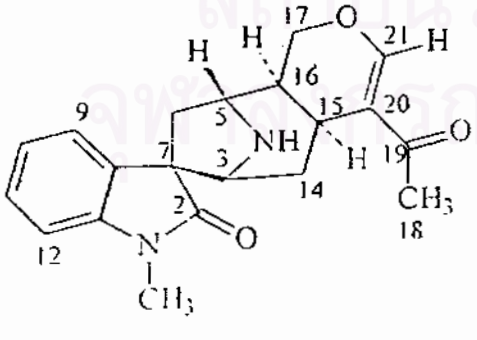
alkaloid of this type, i.e. undulifoline (21) from the stem bark of *A. undulifolia* (Massiot *et al.*, 1992) has been reported.



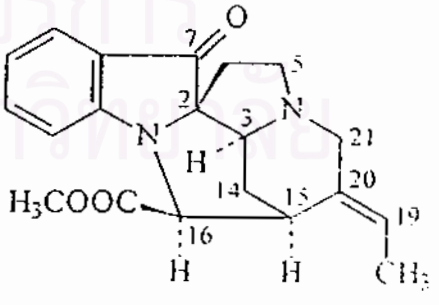
Undulifoline (21)

3.1.10 Oxindole and Pseudoindoxyl Alkaloids

These alkaloids occur as oxidised forms and are typically found to co-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 *et al.*, 1987b, 1988b, 1990, 1991; Abe *et al.*, 1994; Wong *et al.*, 1996) with the exception of alstonisine (22) which was also found in *A. muelleriana* (Elderfield and Gilman, 1972; Burke *et al.*, 1973) and *A. angustifolia* (Ghedira *et al.*, 1988). The only pseudoindoxyl alkaloid reported from genus *Alstonia*, fluorocarpamine (23), was isolated from *A. plumosa* (Jacquier *et al.*, 1982), *A. undulata* (Guillaume *et al.*, 1984) and *A. angustifolia* (Ghedira *et al.*, 1988).



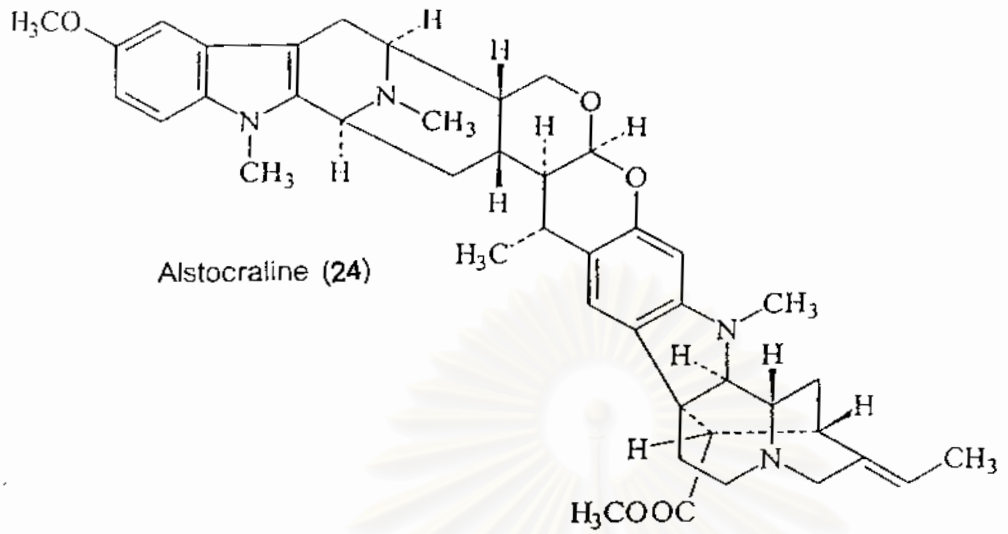
Alstonisine (22)



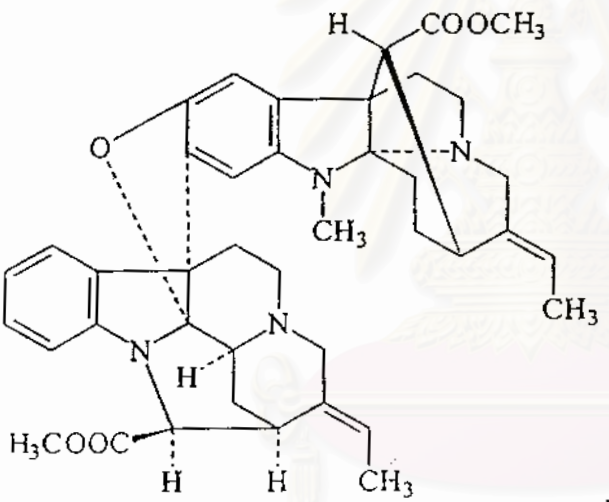
Fluorocarpamine (23)

3.1.11 Bisindole Alkaloids

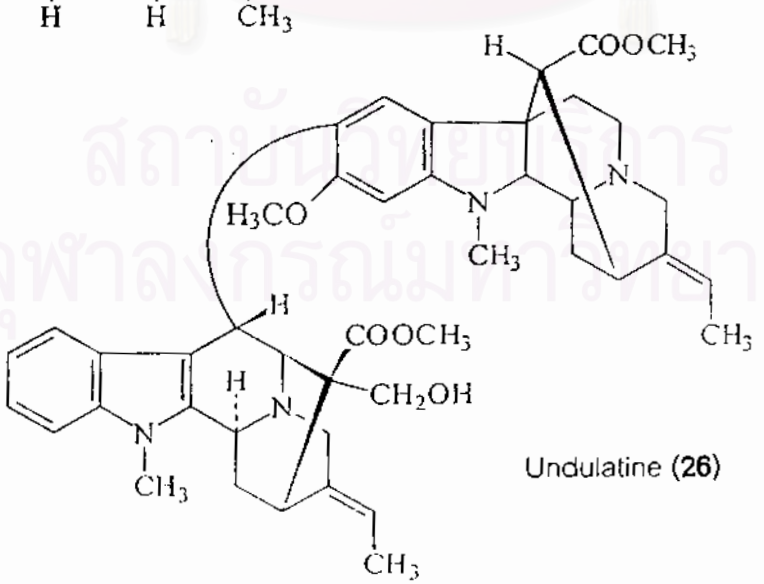
About 28 bisindole alkaloids have been isolated from several species in the sections *Monuraspermum* and *Dissuraspermum* of genus *Alstonia*. The two units of these bisindoles are typically derived from the corresponding Corynanthean-type monomeric alkaloids. It is rare that these alkaloids possess 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 (**24**) from *A. angustifolia* (Ghedira *et al*., 1988); pleiocorine (**25**) from *A. deplanchei* (Das *et al*., 1974), *A. odontophora* (Vercauteren *et al*., 1979) and *A. plumosa* (Jacquier *et al*., 1982); undulatine (**26**) from *A. sphaerocapitata* (Nuzillard *et al*., 1989) and *A. undulata* (Nuzillard *et al*., 1989; Pinchon *et al*., 1990).



Alstocraline (**24**)



Pleiocorine (**25**)



Undulatine (**26**)

Figure 3 Structures of some bisindole alkaloids isolated from *Alstonia* species

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 (**27-36**) are illustrated in Figure 4. Although the occurrence of these compounds is very restricted, some of them such as the terpenoids boonein (**27**), sweroside (**28**), and loganin (**29**) are of interest since they are involved in the biosynthesis of monoterpenoid-derived indole alkaloids. The novel structure of lanceomigine (**33**) 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 (**73**) and secologanin (**74**) but contains a quinoline instead of an indole nucleus, mainly isolated from genera *Melodinus* and *Rhazya* of family Apocynaceae (Hu *et al*., 1987). The other quinoline alkaloid, corialstonine (**34**), from *A. coriacea*, because of its quinine-related structure, is of interest for biological activity as far as the antimalarial activity is concerned.

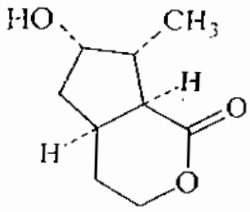
Table 1 Miscellaneous compounds known to occur in *Alstonia* species

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

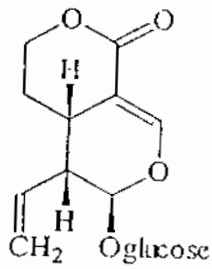
Molecular Weight (Structural Type)	Compound	Source and Reference
149: $C_9H_{11}NO$ (Alkaloid)	Venoterpine (**30**) [Gentialutine]	*A.venenata*: frt (Ray and Chatterjee, 1968); *A. angustiloba*: unk, *A.spatulata*: unk (Ravao *et al*., 1985)
170: $C_9H_{14}O_3$ (Terpenoid)	Boonein (**27**)	*A. boonei*: sb (Marini-Bettolo *et al*., 1983)
175: $C_{10}H_9NO_2$ (Alkaloid)	Gentianine	*A. coriacea*: sb (Cherif *et al*., 1989); *A. lanceolata*: sb (Vercauteren *et al*., 1981); *A. lenormandii*: lvs (Legseir *et al*., 1986)
207: $C_{11}H_{13}NO_3$ (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)
211: $C_{10}H_{13}NO_4$ (Amide)	3,4,5-Trimethoxybenzamide (**31**)	*A. constricta*: sb (Allam *et al*., 1987)

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

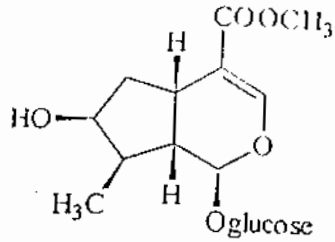
Molecular Weight (Structural Type)	Compound	Source and Reference
237: $C_{13}H_{19}NO_3$ (Alkaliod)	Angustimaline (**32**)	*A. angustifolia*: sb (Kam *et al*., 1997)
237: $C_{12}H_{15}NO_4$ (Amide)	3',4',5'-Trimethoxybenzamide [Cintriamide] [3-(3,4,5-Trimethoxyphenyl)-2-propenamide]	*A. lenormandii*: lvs (Legseir *et al*., 1986)
358: $C_{16}H_{12}O_9$ (Terpenoid)	Sweroside (**28**)	*A. glaucescens*: sb (Keawpradub *et al*., 1994)
382: $C_{22}H_{26}N_2O_4$ (Alkaliod)	Lanceomigine (**33**) [N_a-Methylrhazicine]	*A. lanceolata*: sb (Vercauteren *et al*., 1981)
390: $C_{17}H_{26}O_{10}$ (Terpenoid)	Loganin (**29**)	*A. glaucescens*: roots (Chen *et al*., 1988)
398: $C_{22}H_{26}N_2O_5$ (Alkaloid)	Lanceomigine N_b-oxide	*A. lanceolata*: sb (Vercauteren *et al*., 1981)
410: $C_{23}H_{26}N_2O_5$ (Alkaloid)	Corialstonine (**34**)	*A. coriacea*: sb (Cherif *et al*., 1987, 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)



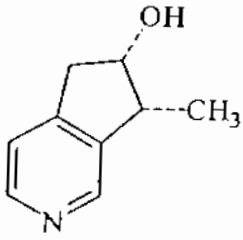
Boonein (27)



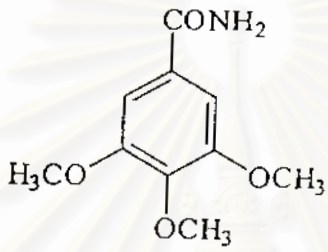
Sweroside (28)



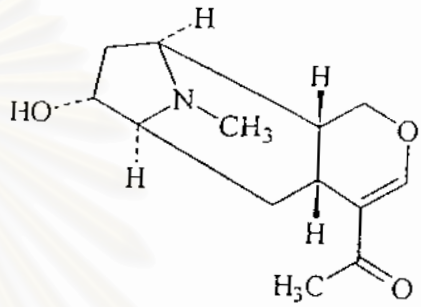
Loganin (29)



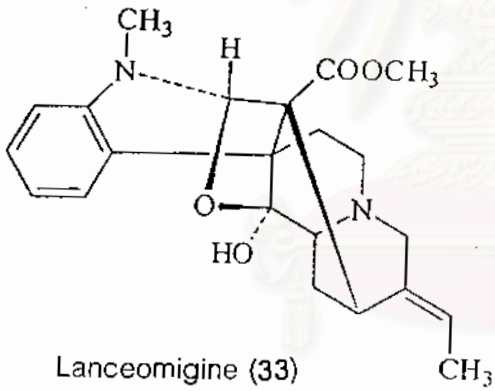
Venoterpine (30)



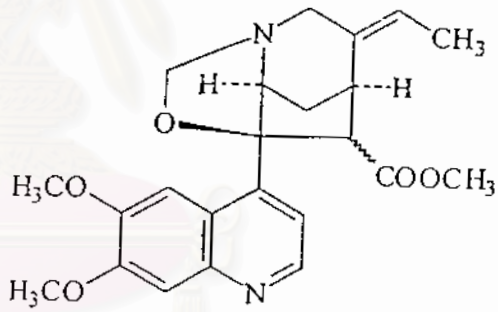
3,4,5-Trimethoxybenzamide (31)



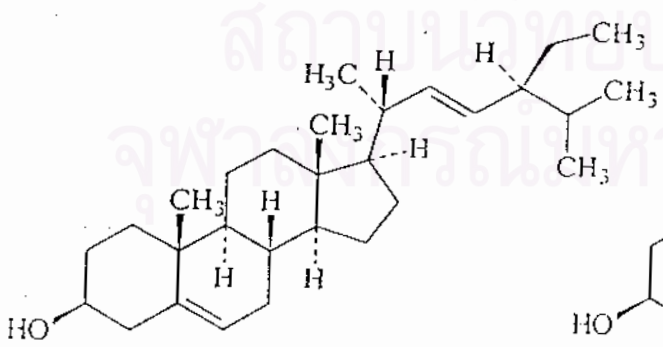
Angustimaline (32)



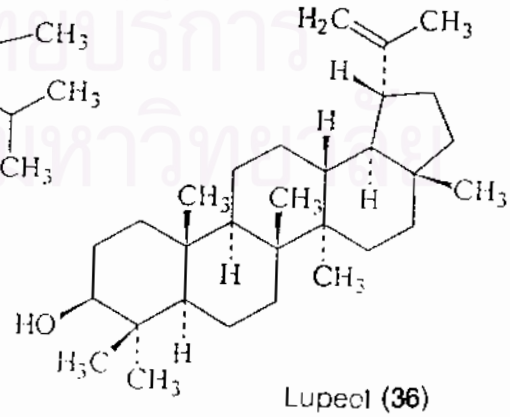
Lanceomigine (33)



Corialstonine (34)



Stigmasterol (35)



Lupeol (36)

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

4. Previous Indole Alkaloids Isolated from *Alstonia scholaris*

Alstonia scholaris has been subject for phytochemical investigation for decades and a large number of alkaloids have been isolated. To date about 36 indole alkaloids (Table 2) and some other compounds (Table 1) have been reported from this species. In general, the majority of alkaloids isolated from this species are those of the Corynanthean-type (mainly Akuammiline and Vincorine groups) and Strychnan-type (Akuammicine group) indole alkaloids (Figure 5).

Table 2 Indole alkaloids isolated from *Alstonia scholaris*

[Abbreviations: Lvs=Leaves, Sb=Stem bark, Rb=Root bark, Fl=flowers, Frt=fruits]

Molecular Weight (Skeletal Type)	Alkaloid	Part and Reference
322: $C_{20}H_{22}N_2O_2$ (Akuammicine group)	Akuammicine (**39**) [Methyl 2,16,19,20-tetradehydrocuran-17-oate]	Rb (Boonchuay and Court, 1976)
322: $C_{20}H_{22}N_2O_2$ (Akuammiline group)	Strictamine (**58**) [Desacetyldesformoakuammiline] [Vincamidine]	Fl (Dutta *et al*., 1976)
324: $C_{19}H_{20}N_2O_3$ (Vallesamine-type)	Angustilobine B acid (**49**)	Lvs (Yamauchi *et al*., 1990b)
324: $C_{20}H_{24}N_2O_2$ (Tubotaiwine group)	Tubotaiwine (**55**)	Lvs (Yamauchi *et al*., 1990a, b); Sb (Boonchuay and Court, 1976)
326: $C_{19}H_{22}N_2O_3$ (Oxindole, ring-opened)	Leuconolam (**72**)	Lvs (Yamauchi *et al*., 1990a)
326: $C_{19}H_{22}N_2O_3$ (vallesamine-type)	Losbanine (**47**)	Lvs (Yamauchi *et al*., 1990b); Sb (Yamauchi *et al*., 1990b)
338: $C_{20}H_{22}N_2O_3$ (Akuammicine group)	Akuammicine N_b-oxide (**40**)	Rb (Boonchuay and Court, 1976)
338: $C_{20}H_{22}N_2O_3$ (Vallesamine-type)	Alstonamine (**50**)	Lvs (Atta-ur-Rahman and Alvi, 1987)
338: $C_{20}H_{22}N_2O_3$ (Akuammiline group)	Picrinine (**59**) [Methyl 2,5-epoxy-1,2-dihydroakuammilan-17-oate] [Vincaridine]	Lvs (Chatterjee *et al*., 1965b; Rastogi *et al*., 1970; Morita *et al*., 1977); Fl (Dutta *et al*., 1976); Sb (Boonchuay and Court, 1976)

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

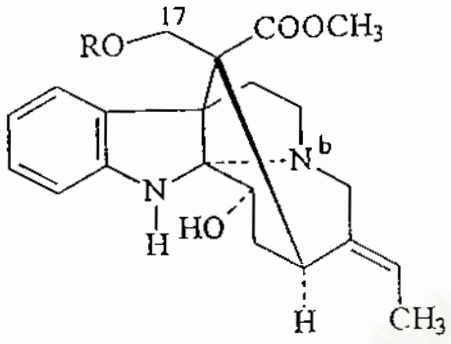
Molecular Weight (Skeletal Type)	Alkaloid	Part and Reference
340: $C_{20}H_{24}N_2O_3$ (Akuammicine group)	Echitamidine (**42**) [19*S*-Hydroxy-19,20*S*-dihydroakuammicine]	Sb (Boonchuay and Court, 1976); Rb (Boonchuay and Court, 1976)
340: $C_{20}H_{24}N_2O_3$ (Tubotaiwine group)	Lagunamine (**56**) [19-Hydroxytubotaiwine]	Lvs (Yamauchi *et al*, 1990b)
340: $C_{20}H_{24}N_2O_3$ (Akuammicine group)	(+)-Lochneridine (**41**) [Methyl 2,16-didehydro-20-hydroxycuran-17-oate]	Lvs (Banerji *et al*., 1984)
340: $C_{20}H_{24}N_2O_3$ (Vallesamine-type)	6,7-Seco-angustilobine B (**48**)	Lvs (Yamauchi *et al*., 1990a, b); Sb (Yamauchi *et al*., 1990b)
340: $C_{20}H_{24}N_2O_3$ (Tubotaiwine group)	Tubotaiwine-N_b-oxide (**57**)	Lvs (Yamauchi *et al*., 1990b); Sb (Yamauchi *et al*., 1990b)
340: $C_{20}H_{24}N_2O_3$ (Vallesamine-type)	19,20-*E*-Vallesamine (**51**)	Lvs (Atta-ur-Rahman *et al*., 1987a)
340: $C_{20}H_{24}N_2O_3$ (Vallesamine-type)	19,20-*Z*-Vallesamine (**52**)	Lvs (Atta-ur-Rahman *et al*., 1987a)
352: $C_{21}H_{24}N_2O_3$ (Sarpagine group)	Akuammidine (**38**) [Methyl 17-hydroxysarpagan-16-carboxylate] [Rhazine]	Lvs (Rastogi *et al*., 1970; Morita *et al*., 1977; Banerji and Siddhanta, 1981); Sb (Boonchuay and Court, 1976); Frt (Chatterjee *et al*., 1969b)
352: $C_{20}H_{20}N_2O_4$ (Akuammiline group)	Nareline (**62**) [Methyl 4,5-epoxy-5-hydroxy-6,21-cyclo-4,5-seco-akuammilan-17-oate]	Lvs (Morita *et al*., 1977; Abe *et al*., 1989; Yamauchi *et al*., 1990a)

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

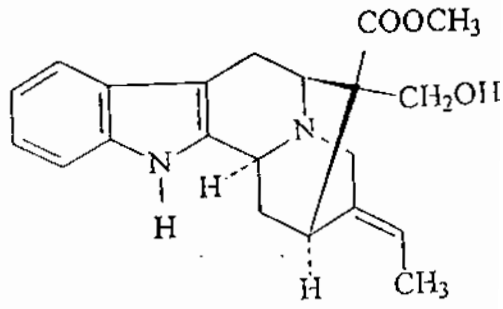
Molecular Weight (Skeletal Type)	Alkaloid	Part and Reference
352: $C_{21}H_{24}N_2O_3$ (Yohimbine group)	Tetrahydroalstonine (**68**)	Fl (Dutta *et al*., 1976)
354: $C_{21}H_{26}N_2O_3$ (Yohimbine group)	Rhazimanine (**69**)	Lvs (Atta-ur-Rahman and Alvi, 1987)
356: $C_{20}H_{24}N_2O_4$ (Akuammicine group)	19-Epi-scholaricine (**45**)	Lvs (Yamauchi *et al*., 1990a)
356: $C_{20}H_{24}N_2O_4$ (Akuammicine group)	19*S*-Scholaricine (**43**) [Demethylscholarine]	Lvs (Atta-ur-Rakman *et al*., 1985)
356: $C_{20}H_{24}N_2O_4$ (Vallesamine-type)	6,7-Seco-19,20-epoxyangustilobine B (**54**)	Lvs (Yamauchi *et al*., 1990a)
356: $C_{20}H_{24}N_2O_4$ (Vallesamine-type)	19,20-*E*-Vallesamine N_b-oxide (**53**)	Lvs (Yamauchi *et al*., 1990a)
366: $C_{21}H_{22}N_2O_4$ (Akuammiline group)	Picralinal (**60**) [Methyl 2,5-epoxy-1,2-dihydro-17-oxo-akuammilan-16-carboxylate]	Lvs (Rastogi *et al*., 1970; Morita *et al*., 1977; Abe *et al*, 1989)
366: $C_{22}H_{26}N_2O_3$ (Akuammiline group)	Pseudoakuammigine (**63**)	Lvs (Morita *et al*., 1977; Yamauchi *et al*., 1990a); Rb (Boonchuay and Court, 1976)
370: $C_{21}H_{26}N_2O_4$ (Vincorine group)	N_b-Demethylechitamine (**71**) [Norechitamine, Norifoline]	Rb (Boonchuay and Court, 1976); Sb (Yamauchi *et al*., 1990b)
370: $C_{21}H_{26}N_2O_4$ (Akuammicine group)	Scholarine (**46**)	Lvs (Banerji and Siddhanta, 1981)
371: $C_{21}H_{27}N_2O_4$ (Akuammicine group)	N_b-Methylscholaricine (**44**)	Lvs (Yamauchi *et al*., 1990a)
382: $C_{22}H_{26}N_2O_4$ (Akuammiline group)	N_a-Methylburnamine (**65**)	Lvs (Yamauchi *et al*., 1990a)

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

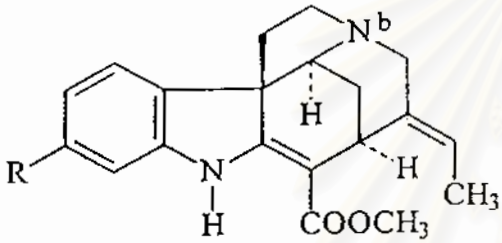
Molecular Weight (Skeletal Type)	Alkaloid	Part and Reference
382: $C_{22}H_{26}N_2O_4$ (Akuammiline group)	Pseudoakuammigine N_b-oxide (**64**)	Lvs (Yamauchi *et al*., 1990a)
384: $C_{21}H_{24}N_2O_5$ (Akuammiline group)	Alschomine (**66**)	Lvs (Abe *et al*., 1989; Yamauchi *et al*., 1990a)
384: $C_{21}H_{24}N_2O_5$ (Akuammiline group)	Isoalschomine (**67**) [5-Epi-alschomine]	Lvs (Abe *et al*., 1989; Yamauchi *et al*., 1990a)
385: $C_{22}H_{29}N_2O_4$ (Vincorine group)	Echitamine (**37**) [Ditaine]	Rb (Boonchuay and Court, 1976); Sb (Yamauchi *et al*., 1990b)
410: $C_{23}H_{26}N_2O_5$ (Akuammiline group)	Picraline (**61**) [Methyl 16-(acetyloxymethyl)-2,5-epoxy-1,2-dihydroakuammilan-17-oate]	Lvs (Yamauchi *et al*., 1990a)
427: $C_{24}H_{31}N_2O_5$ (Vincorine group)	17-*O*-Acetylechitamine (**70**)	Sb (Yamauchi *et al*., 1990b)



Echitamine (**37**), R=H, N_b-CH_3

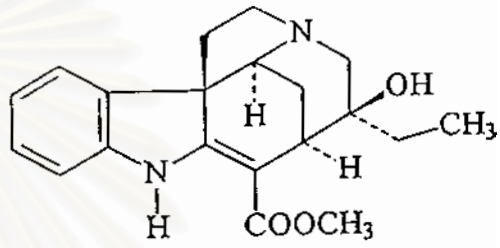


Akuammidine (**38**)

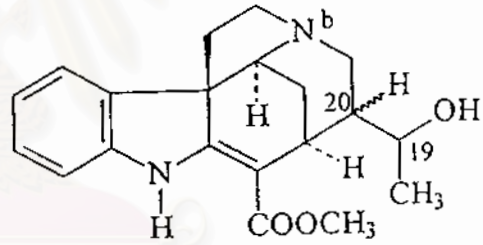


Akuammicine (**39**), R=H

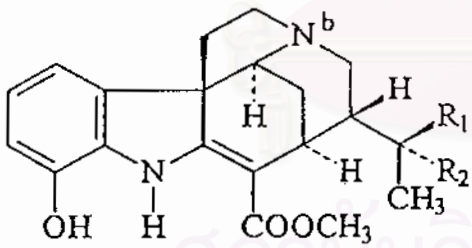
Akummicine N_b-oxide (**40**), R=H, N_b-oxide



(+)-Lochneridine (**41**)



Echitamidine (**42**), C-20*S*, C-19*S*



19*S*-Scholaricine (**43**),

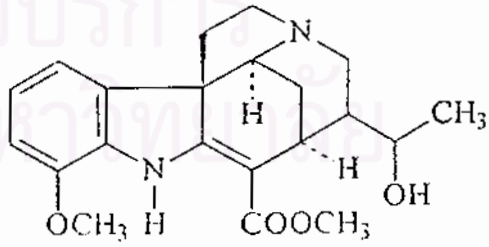
R_1 = OH, R_2 = H

N_b-Methylscholaricine (**44**),

R_1 = OH, R_2 = H, N_b-CH_3

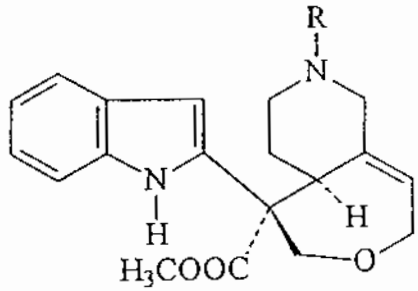
19-Epi-scholaricine (**45**),

R_1 = H, R_2 = OH

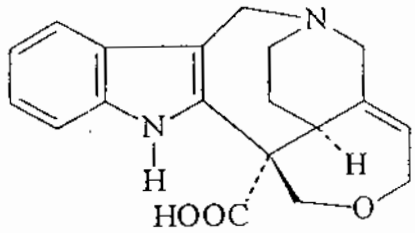


Scholarine (**46**)

Figure 5 Structures of indole alkaloids isolated from *Alstonia scholaris*

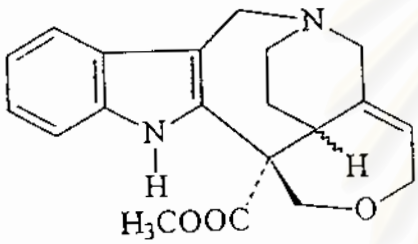


Losbanine (**47**), R = H

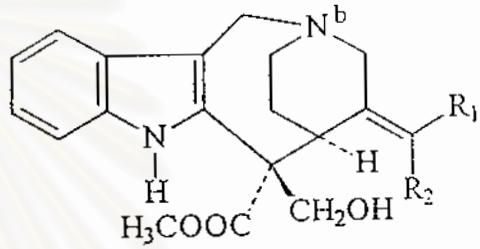


Angustilobine B acid (**49**)

6,7-Seco-angustilobine B (**48**), R = CH_3



Alstonamine (**50**)



19,20-*E*-Vallesamine (**51**),

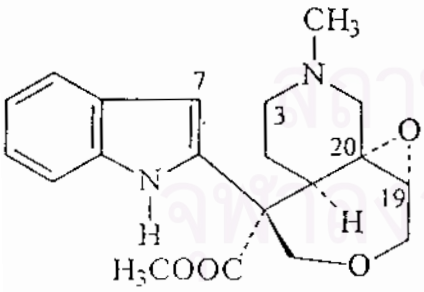
R_1 = H, R_2 = CH_3

19,20-*Z*- Vallesamine (**52**),

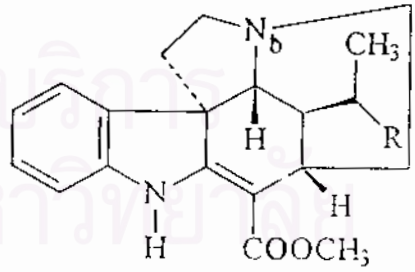
R_1 = CH_3, R_2 = H

19,20-*E*-Vallesamine N_b-oxide (**53**),

R_1 = H, R_2 = CH_3, N_b-oxide



6,7-Seco-19,20-epoxyangustilobine B (**54**)



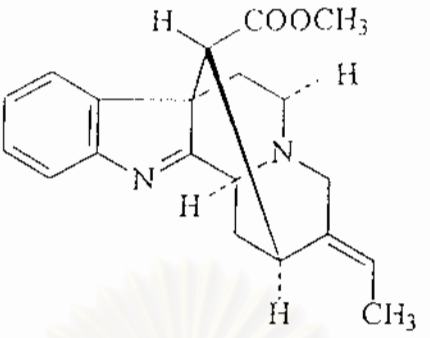
Tubotaiwine (**55**), R = H

Lagunamine (**56**), R = OH

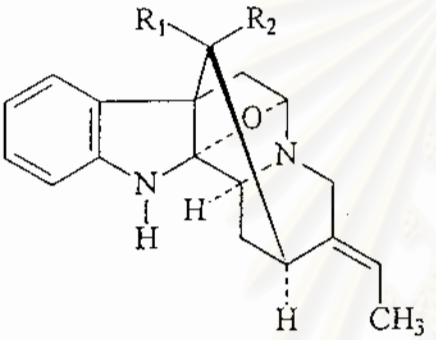
Tubotaiwine N_b-oxide (**57**),

R = H, N_b-oxide

Figure 5 Structures of indole alkaloids isolated from *Alstonia scholaris* (continued)



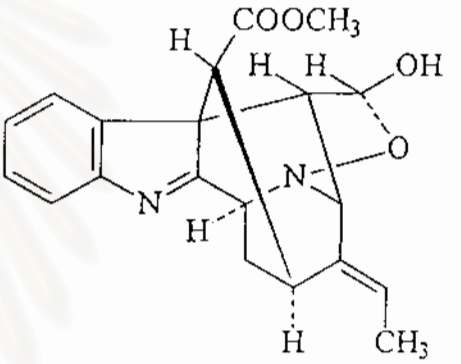
Strictamine (58)



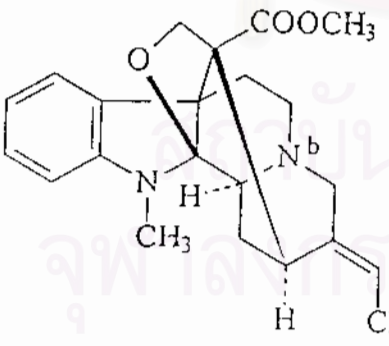
Picrinine (59), R_1 = H, R_2 = $COOCH_3$

Picralinal (60), R_1 = CHO, R_2 = $COOCH_3$

Picraline (61), R_1 = CH_2COOCH_3, R_2 = $COOCH_3$

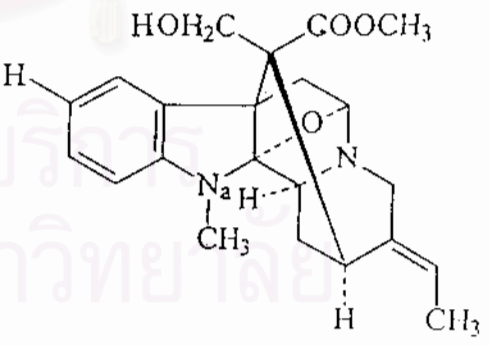


Nareline (62)



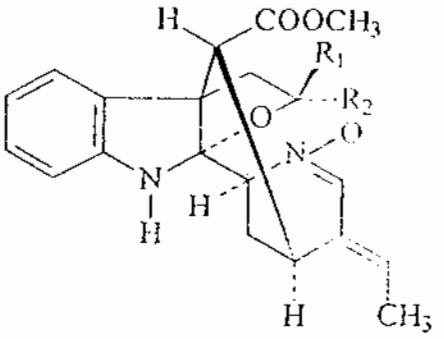
Pseudoakuammigine (63)

Pseudoakuammigine N_b-oxide (64), N_b-oxide



N_a-Methylburnamine (65)

Figure 5 Structures of Indole alkaloids isolated from *Alstonia scholaris* (continued)

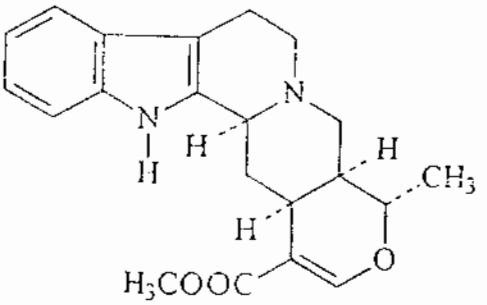


Alschomine (**66**),

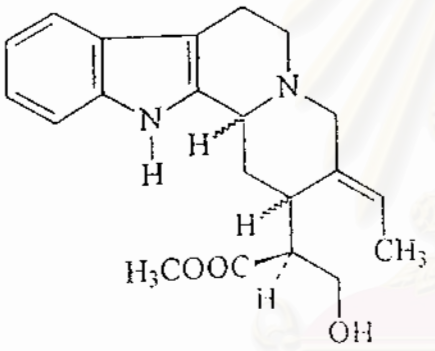
R_1 = H, R_2 = OCH_3

Isoalschomine (**67**),

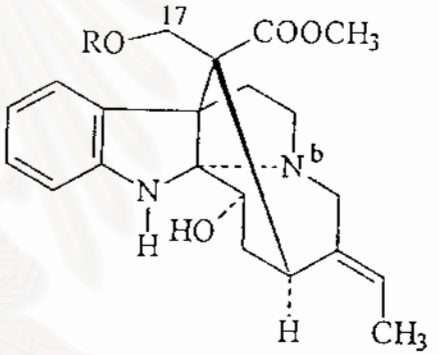
R_1 = OCH_3, R_2 = H



Tetrahydroalstonine (**68**)



Rhazimanine (**69**)

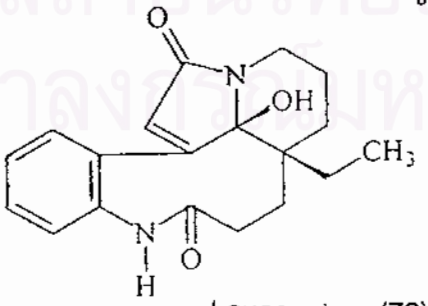


Echitamine (**37**), R = H, N_b-CH_3

17-*O*-Acetylechitamine (**70**),

R = $COCH_3$, N_b-CH_3

N_b-Demethylechitamine (**71**), R = H



Leuconolam (**72**)

Figure 5 Structures of indole alkaloids isolated from *Alstonia scholaris* (continued)

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

Figure 6, based on 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 (**73**), the decarboxylation product of tryptophan, and a monoterpenoid secologanin (**74**) via the key intermediate strictosidine (**75**) (Stockigt and Zenk, 1977; Nagakura *et al*., 1979; Treimer and Zenk, 1979) and the subsequent elaborations of the presumed intermediates 4,21-dehydrogeissoschizine (**76**), stemmadenine-related iminium cation (**77**), and 4,21-dehydrosecodine (**78**) (Cordell, 1974, 1981; Atta-ur-Rahman and Basha, 1983; Van Beek *et al*., 1984).

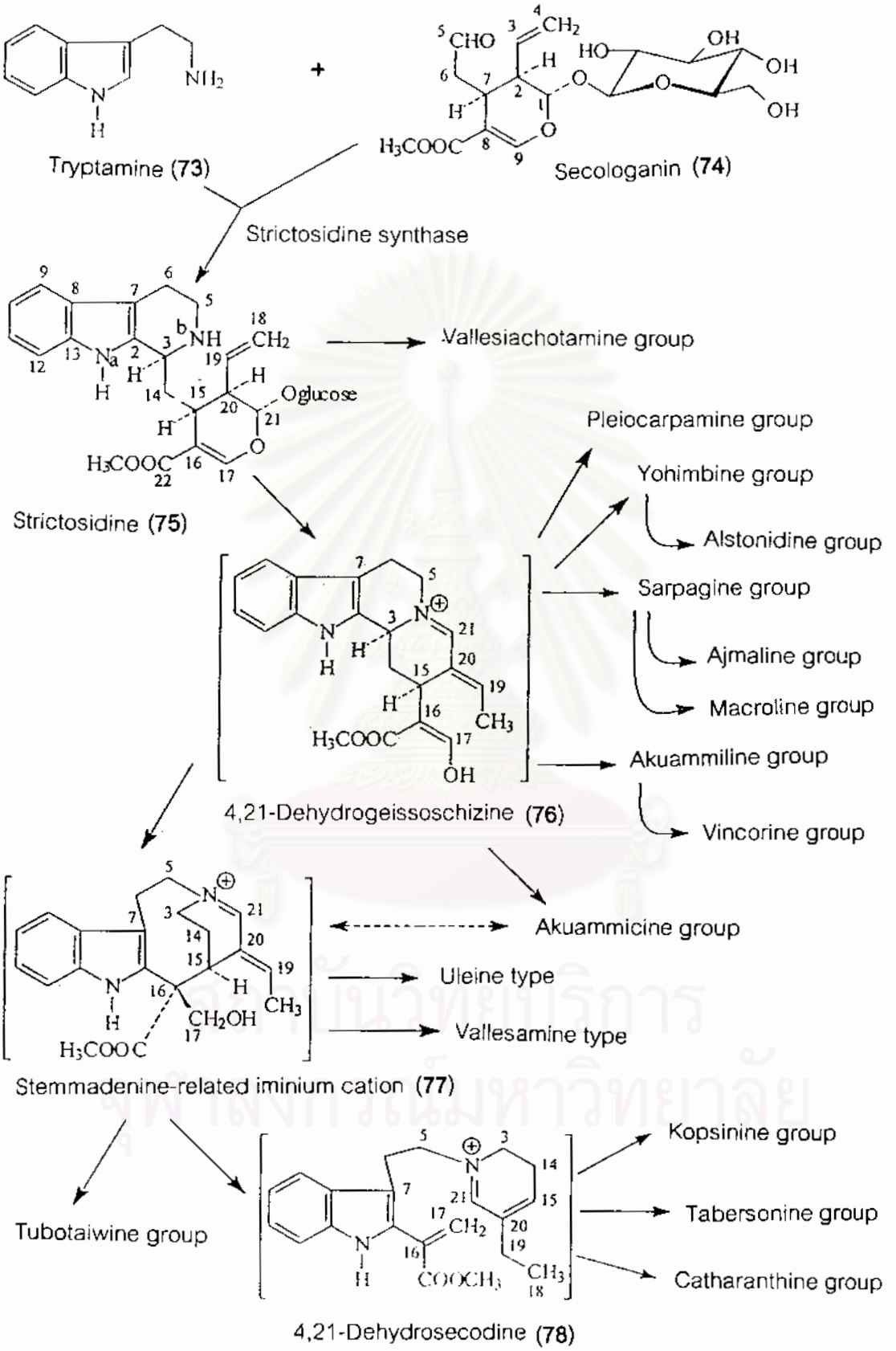
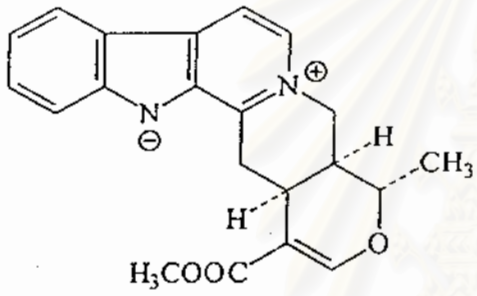


Figure 6 Plausible biogenetic interrelationships of various structural groups of monoterpenoid-derived indole alkaloids occurring in *Alstonia* species

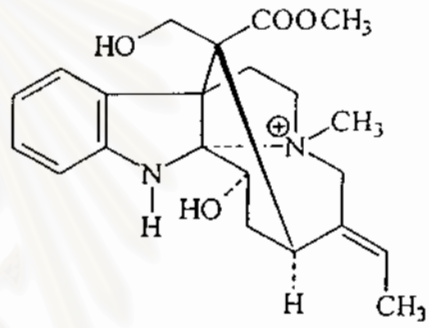
6. Biological Activity of the Genus *Alstonia*

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 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 coworker (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 hydrochlorides. Alstonine (**79**), 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 (**37**), 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 (**79**), 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 berghei* in mice (Awe and Opeke, 1990).

The extract, when given orally in 4-day 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 chloroquine 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 brucei* at a dose of 100 mg/kg (i.p.), for 5 days.



Alstonine (79)



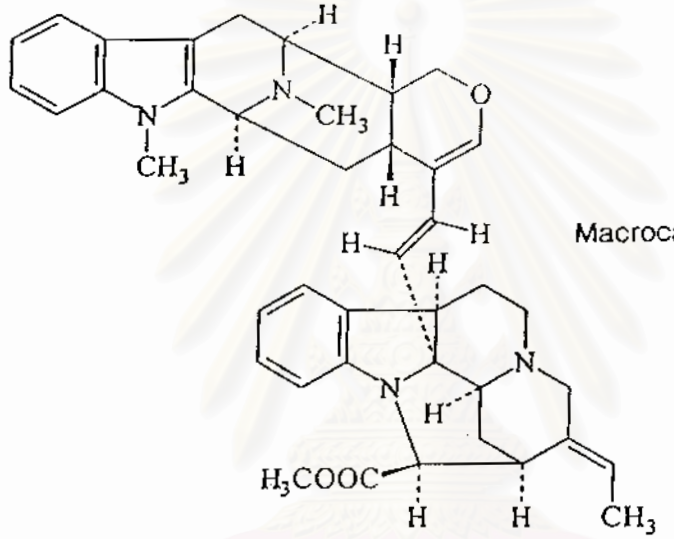
Echitamine (37)

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 (80) and villastonine (81), 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 (37) and the quinoline alkaloid from *A. coriacea*, corialtonine (34), were investigated for *in vitro* antiprotozoal activity (Wright *et al.*, 1993). Echitamine (37) 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

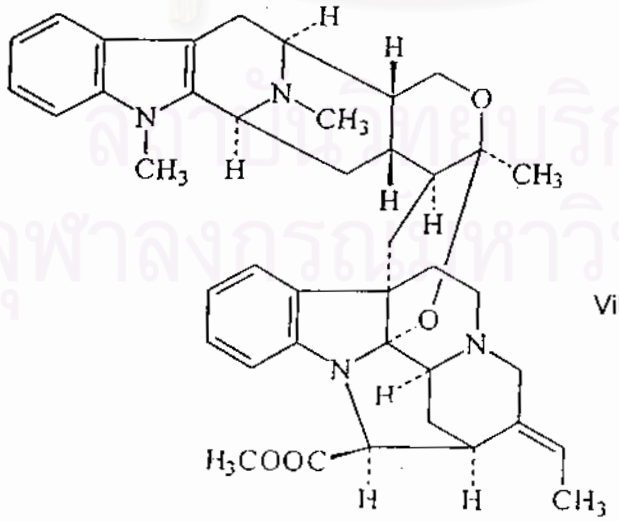
quinine. Disappointingly, the two alkaloids were inactive against *Giardia intestinalis* at 60 μM.

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 (**81**) and macrocarpamine (**80**) with IC_{50} values of 0.27 and 0.36 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} values 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.

The 50% ethanol extract from the stem bark of *A. scholaris* has been reported to possess antileishmanial activity against *Leishmania donovani* in golden hamsters with an increased survival period (Singha *et al.*, 1992). The aqueous extract from the bark of *A. scholaris* also showed a promising hepatoprotective effect in various experimental liver injury models (hepatotoxin-induced acute liver damage) in mice and rats (Lin *et al.*, 1996). It was also found that the LD_{50} of the extract was higher than 2000 mg/kg (orally) in mice.



Macrocarpamine (80)



Villalstonine (81)

Based on the limited experimental data on biological evaluation reviewed above, it is likely that evidence in support of effectiveness of extracts and alkaloids from *Alstonia* species in the treatment of malaria is still controversial. On the other hand, it is reasonable to emphasize that some results reveal the potential of the genus *Alstonia* as a source of biologically active molecules. Apparently, further work on biological and chemical investigation of this genus is needed and would provide further compounds with interesting biological activity.

CHAPTER III

Experimental

1. Sources of Plant Materials

The fruits of *Alstonia scholaris* (L.) R. Br. were collected from Phukae Botanical Garden, Saraburi province, Thailand, in February 2000. 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	:	0.2 mm
Distance	:	6.4 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 g) and distilled water (20 ml)

Solution A and Solution 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 Preparative Thin-Layer Chromatography

Technique	:	One dimension, ascending
Adsorbent	:	Silica gel 60 F_{254} (E. Merck) precoated plate
Layer thickness	:	1 mm
Distance	:	18 cm
Temperature	:	Laboratory temperature (24-30°C)
Detection	:	Ultraviolet light at wavelength 254 nm

2.3 Column Chromatography

2.3.1 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.

2.3.2 Gel Filtration Chromatography

Gel filter	:	Sephadex LH 20 (Pharmacia)
Packing method	:	Gel filter was suspended in the eluent and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tightly.
Sample loading	:	The sample was dissolved in a small amount of eluent and applied 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.

2.4 Spectroscopy

2.4.1 Ultraviolet (UV) Absorption Spectra

UV (in methanol, ethanol and chloroform) 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) or a Perkin Elmer FT-IR 1760X spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University).

2.4.3 Mass Spectra

Electron Impact (EIMS) were measured with a JEOL JMS-AM 20 instrument (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 (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained with a JEOL JNM-EPC 400 NMR spectrometer (Japan). ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were obtained with a JEOL JNM-A 500 NMR spectrometer (Japan). ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were obtained with a JEOL JNM-EPC 600 NMR spectrometer (Japan).

Solvents for NMR spectra were deuterated methanol (CD_3OD) and deuterated chloroform (chloroform-*d*). 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 Rotation

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 were redistilled prior to use.

3. Extraction and Isolation

3.1 Extraction

The fresh fruits of *A. scholaris* (60.7Kg) were chopped and blended into small pieces. They were extracted with methanol four times (4 x 40 L) and filtered. The combined filtrate was concentrated to syrupy mass under reduced pressure, mixed with glacial acetic acid (500 ml) then poured into a large volume of distilled water to give about 5% acetic acid solution (10 L). The suspension was well shaken and left to stand overnight. The acidic filtrate was washed with portions of petroleum ether (1x400 ml), then made alkaline (pH 10) with strong solution of ammonium hydroxide and extracted with chloroform (3x300 ml). The combined chloroform extract was washed with distilled

water, dried over anhydrous sodium sulfate and evaporated under reduced pressure to yield dry crude alkaloidal extract (15.28 g).

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

3.2 Isolation

The crude methanolic alkaloidal extract (7.64 g) was dissolved in a small amount of chloroform and packed onto the top of wet silica gel column (9 x16 cm). The column was eluted with methanol : chloroform (1:9), methanol : chloroform (4:6), methanol : chloroform (6:4), methanol : chloroform (8:2) and then washed with methanol until no traces of compounds could be detected. Fractions of 75 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. The mentioned solvent systems afforded 78, 13, 20 and 15 fractions, respectively. Those fractions of similar pattern were combined and evaporated to dryness under reduced pressure. By this procedure :-

1. Fractions 1-50 were combined and designated as Fraction a (3.0408 g).
2. Fractions 51-72 were combined and designated as Fraction b (1.2984 g).
3. Fractions 73-78 were combined and designated as Fraction c (0.2397 g).
4. Fractions 79-91 were combined and designated as Fraction d (0.304 g).
5. Fractions 92-111 were combined and designated as Fraction e (1.1597 g).
6. Fractions 112-126 were combined and designated as Fraction f (0.3661 g).
7. Methanolic fractions were combined and designated as Fraction g (0.4676 g).

3.2.1 Isolation of Compounds from Fraction a and Fraction a-1

3.2.1.1 Isolation of Compounds from Fraction a

Fraction a (3.0408 g) was shown by TLC to contain at least four compounds. It was dissolved in a small amount of chloroform and packed onto the top of wet silica gel column (4.5 x16 cm). The column was eluted with methanol : chloroform (0.5:9.5), methanol : chloroform (1:9) and then washed with methanol : chloroform

(1.5:8.5) until no traces of compounds could be detected. Thirty ml fractions were collected. The volumes of eluent were 840 ml, 720 and 1000 ml, respectively. The fractions were examined by TLC and the liked fractions were combined to give the following portions:-

1. Fractions 1-13 were combined and designated as Fraction a-1 (1.7747 g).
2. Fractions 14-20 were combined and designated as Fraction a-2 (0.3912 g).
3. Fractions 21-28 were combined and designated as Fraction a-3 (0.1540 g).
4. Fractions 29-41 were combined and designated as Fraction a-4 (0.1773 g).
5. Fractions 42-52 were combined and designated as Fraction a-5 (0.1165 g).
6. Methanol : chloroform (1.5:8.5) fractions were combined and designated as Fraction a-6 (0.0607 g).

3.2.1.2 Isolation of Compounds from Fraction a-1

Fraction a-1 (1.7747 g) was shown by TLC to contain at least two compounds. It was dissolved in a small amount of chloroform and packed onto silica gel column (3 x 16 cm). The column was eluted with chloroform and methanol : chloroform (0.5 : 9.5). Fractions of 20 ml were collected, until no traces of compounds could be detected. The volumes of eluent were 1000 ml and 40 ml, respectively. The fractions were examined by TLC and the liked fractions were combined to give the following portions :-

1. Fractions 1-10 were combined and designated as a-10 (0.1910 g).
2. Fractions 11-21 were combined and designated as a-11 (0.9379 g).
3. Fractions 22-26 were combined and designated as a-12 (0.4040 g).
4. Fractions 27-31 were combined and designated as a-13 (0.2513 g).
5. Fractions 32-35 were combined and designated as a-14 (0.1158 g).
6. Fractions 36-39 were combined and designated as a-15 (0.0268 g).

7. Fractions 40-46 were combined and designated as a-16 (0.0177 g).

3.2.2 Isolation of Compounds D-1 and D-2 from Fraction a-10

Fraction a-10 (191.0 mg) was shown by TLC to contain a mixture of at least four compounds. It was dissolved in a small amount of chloroform and packed onto a silica gel column (1.5 x 16 cm). The column was eluted with chloroform. Ten ml fractions were collected, until no traces of compounds could be detected. The volumes of eluent were 500 ml. The fractions were examined by TLC and the liked fractions were combined to give the following portions :-

1. Fractions 1-4 containing mixture of compounds (14.9 mg)

2. Fractions 5-7 (11.9 mg) were combined and further separated by flash column chromatography, using a column of silica gel 60 (No. 9385, 1 x 16 cm) with chloroform as the eluent. Ten fractions were collected (3 ml per fraction) and examined by TLC using chloroform as the developing solvent. The TLC chromatogram of fractions 5-10 showed only one spot under UV light at 254 nm, R_f 0.87 [silica gel, methanol : chloroform (1:9)]. These fractions were combined and evaporated under reduced pressure to give 7.5 mg of compound D-1 as yellow oil (0.098% base on dried weight of crude alkaloidal extract). It was later identified as dibutyl phthalate (**82**).

3. Fractions 8-14 containing mixture of compounds (26.7 mg)

4. Fractions 15-19 (24 mg) were combined and further separated by preparative TLC on precoated silica gel F_{254} (1 mm, 18 x 20 cm) plates with development in methanol : chloroform (0.25 : 9.75)(double development). Two dark bands were observed under UV light at 254 nm, with the upper band [R_f 0.64, methanol : chloroform (0.25 : 9.75)] and the lower band [R_f 0.55, methanol : chloroform (0.25 : 9.75)]. Extraction of the lower band with 5% methanol in chloroform gave compound D-2 (6.5 mg) as yellowish brown oil (0.085% based on dried weight of crude alkaloidal extract). It was later identified as methyl ferulate (**83**).

5. Fractions 20-34 containing mixture of compounds (55.3 mg)

6. Fractions 35-47 containing traces of mixture compounds (8.1 mg)

3.2.3 Isolation of Compound D-3 from Fraction a-12

Fraction a-12 (404.0 mg) was fractionated on a column using silica gel 60 (No. 9385, 2.2 x 16 cm) as the adsorbent. Elution was performed with methanol : ethyl acetate (1 : 9). Seven fractions, approximately of 30 ml, were collected. The eluates were examined by TLC using methanol : ethyl acetate (1:9) as the developing solvent. Fractions showing similar chromatographic pattern were combined. Fractions 1-2 (209.7 mg) were combined and further separated by flash column chromatography, using a column of silica gel 60 (No. 9385, 2.2 x 16 cm) with methanol : ethyl acetate (1:9) as eluent. Twelve fractions were collected (15 ml per fraction) and examined by TLC using methanol : ethyl acetate (1:9) as the developing solvent. Fractions showing similar chromatographic pattern were combined. Fractions 7-12 (90.4 mg) were combined and further separated by flash column chromatography, using a column of silica gel 60 (No. 9385, 1.5 x 16 cm) with methanol : ethyl acetate (1:9) as eluent. Six fractions were collected (10 ml per fraction) and examined by TLC using methanol : chloroform (1:9) as the developing solvent. Fractions showing similar chromatographic pattern were combined. Fractions 3-6 (60.6 mg) were combined and further separated by flash column chromatography, using a column of silica gel 60 (No. 9385, 1 x 16 cm) with methanol : chloroform (0.5:9.5) as eluent. Four fractions were collected (10 ml per fraction) and examined by TLC using methanol : chloroform (0.5:9.5) as the developing solvent. The TLC chromatogram of fractions 2-3 showed only one spot under UV light at 254 nm, R_f 0.27 [silica gel, methanol : chloroform (1:9)]. These fractions were combined and evaporated under reduced pressure to give 14.9 mg of compound D-3 as a white powder (0.195% based on dried weight of crude alkaloidal extract). It was later identified as picrinine (**84**).

3.2.4 Isolation of Compound D-4 from Fraction a-13

Fraction a-13 (251.3 mg) was fractionated on a column using silica gel 60 (No. 9385, 2.2 x 19 cm) as the adsorbent. Elution was performed with acetone : chloroform (1:1). Thirty - two fractions, approximately of 15 ml, were collected. The eluates were examined by TLC using acetone : chloroform (1:1) as the

developing solvent. Fractions showing similar chromatographic pattern were combined. Fractions 6-18 (107.6 mg) were combined and further separated by gel filtration chromatography, using a column of sephadex LH 20 (1x 39 cm) with methanol as the eluent. Fourteen fractions were collected (10 ml per fraction) and examined by TLC using methanol : chloroform (1:9) as the developing solvent. Fractions showing similar chromatographic pattern were combined. Fractions 2-4 (73.2 mg) were combined and further separated by flash column chromatography, using a column of silica gel 60 (No. 9385, 1 x 25 cm) with acetone : ethyl acetate : hexane (5:4:1) as the eluent. Sixteen fractions were collected (5 ml per fractions) and examined by TLC using acetone : ethyl acetate : hexane (5:4:1) as the developing solvent. The TLC chromatogram of fractions 8-16 showed a single spot under UV light at 254 nm, R_f 0.24 [silica gel, methanol : chloroform (1:9)]. These fractions were combined and evaporated under reduced pressure to give 8.8 mg of compound D-4 as a white powder (0.115% based on dried weight of crude alkaloidal extract). It was later identified as 19-*E*-akuammidine (**85**).

3.2.5 Isolation of Compounds D-5 and D-6 from Fraction b

Fraction b (1.2984 g) was fractionated on a column using silica gel 60 (No. 9385, 3.2 x 15 cm) as the adsorbent. Elution was performed in a polarity gradient manner with methanol, acetone and chloroform. Fourty five fractions, approximately of 50 ml, were collected. The eluates were examined by TLC using methanol : acetone : chloroform (0.5 : 4.5 : 4.5) as the developing solvent. Fractions with similar chromatographic pattern were combined to yield nine fractions: fractions 1-2 (47 mg), fractions 3-6 (246.2 mg), fractions 7-15 (199.3 mg), fractions 16-18 (81.6 mg), fractions 19-24 (168.5 mg), fractions 25-28 (99 mg), fractions 29-34 (137.2 mg), fractions 35-37 (44.9 mg) and fractions 38-45 (108.5 mg).

Fractions 19-24 (168.5 mg) were pooled, dried and further separated by flash column chromatography, using a column of silica gel 60 (No. 9385, 2.2 x 30 cm) with methanol : acetone : chloroform (0.5 : 4.5 : 4.5) as the eluent. Fourty six fractions were collected (10 ml per fraction) and examined by TLC using methanol : acetone : chloroform (0.5 : 4.5 : 4.5) as the developing solvent. Fractions with similar chromatographic pattern were combined. Fractions 29-35 (36.6 mg) were combined

and further separated by flash column chromatography, using a column of silica gel 60 (No. 9385, 1.5 x 18 cm) with methanol : acetone : chloroform (0.5 : 4.5 : 4.5) as the eluent. Fifty fractions were collected (5 ml per fraction) and examined by TLC using methanol : acetone : chloroform (0.5 : 4.5 :4.5) as the developing solvent. The TLC chromatogram of fractions 41-50 showed a single spot under UV light at 254 nm, R_f 0.14 [silica gel, methanol : acetone : chloroform (0.5 : 4.5 : 4.5)]. These fractions were combined and evaporated under reduced pressure to give 10 mg of compound D-5 as a white powder (0.131% based on dried weight of crude alkaloidal extract). It was later identified as 19,20-*E*-vallesamine (**87**).

Fractions 29-34 (137.2 mg) were further separated by flash column chromatography, using a column of silica gel 60 (No. 9385, 2.2 x 17 cm) with methanol : chloroform (1:9) as the eluent. Twenty-seven fractions were collected (15 ml per fraction) and examined by TLC using methanol : chloroform (1:9) as the developing solvent. The TLC chromatogram of fractions 12 showed only one spot under UV light at 254 nm, R_f 0.11 [silica gel, methanol : chloroform (1:9)]. This fraction was evaporated under reduced pressure to give 5.9 mg of compound D-6 as a white powder (0.077% based on dried weight of crude alkaloidal extract). It was later identified as 19*S*-scholaricine (**89**).

4. Physical and Spectral data of Isolated Compounds

4.1 Compound D-1

Compound D-1 was obtained as yellow oil (7.5 mg). It was soluble in chloroform.

UV : λ_{max} nm (log ε), in chloroform; Figure 7: 298 (0.66), 283 (0.78), 244 (1.26)

IR : ν_{max} cm^{-1}, Film; Figure 8: 3428, 2931, 2870, 1730, 1642, 1463, 1380, 1286

EIMS : *m/z* (% relative intensity) ; Figure 9
278 (M^+, 4.88), 258 (4.85), 240(4.26), 166 (14.69), 148 (100),
128 (48.05), 112 (29.26), 100 (8.88), 70 (25.55)

^{1}H NMR : δ_{ppm}, 500 MHz, in $CDCL_3$; see Figures 10a - 10b and Table 3

^{13}C NMR : δ_{ppm}, 100 MHz, in $CDCl_3$; see Figure 11 and Table 3

4.2 Compound D-2

Compound D-2 was obtained as yellowish brown oil (6.5 mg). It was soluble in chloroform.

UV : λ_{max} nm (log ε), in chloroform; Figure 12: 320 (1.67), 288 (1.38), 244 (1.16)

EIMS : *m/z* (% relative intensity) ; Figure 13
208 (M^+, 70.26), 192 (2.87), 176 (100), 144 (82.77), 133 (58.90), 117 (48.89), 105 (79.08)

^{1}H NMR : δ_{ppm}, 500 MHz, in $CDCl_3$; see Figures 14a - 14b and Table 4

^{13}C NMR : δ_{ppm}, 100 MHz, in $CDCl_3$; see Figures 15a - 15b and Table 4

4.3 Compound D-3

Compound D-3 was obtained as a white powder (14.9 mg). It was soluble in chloroform.

Melting Point : 220-222°C

$[\alpha]^{20}_D$: -51.94° (*c* 0.129 g/100 ml, in methanol)

UV : λ_{max} nm (log ε), in methanol; Figure 16: 288 (0.25), 235 (0.64), 206 (2.22)

IR : ν_{max} cm^{-1}, KBr disc; Figure 17: 3391, 1724, 1611, 1464, 1168

EIMS : *m/z* (% relative intensity) ; Figure 18
338 (M^+, 26.81), 279 (2.38), 239 (89.03), 206 (16.24), 180 (57.49), 156 (22.82), 130 (63.71), 108 (73.57), 77 (100), 59 (61.68)

^{1}H NMR : δ_{ppm}, 500 MHz, in $CDCL_3$; see Figures 19a - 19b and Table 5

^{13}C NMR : δ_{ppm}, 125 MHz, in $CDCl_3$; see Figures 20a - 20b and Table 5

4.4 Compound D-4

Compound D-4 was obtained as a white powder (8.8 mg). It was soluble in methanol.

Melting point : 235-237°C

$[\alpha]^{20}_D$: +22.04° (*c* 0.313 g/100 ml, in methanol)

UV : λ_{max} nm (log ε), in ethanol; Figure 26: 282 (0.31), 227 (1.64)

IR : ν_{max} cm^{-1}, KBr disc; Figure 27: 3419 (br), 3265, 1715, 1622, 1456, 1223, 1063

EIMS : *m/z* (% relative intensity) ; Figure 28

352 (M^+, 81.32), 321 (44.51), 293 (16.48), 281 (6.04), 249 (80.22), 221 (15.93), 182 (23.08), 169 (100)

^{1}H NMR : δ_{ppm}, 600 MHz, in $CDCl_3$; see Figures 29a - 29d and Table 6

^{13}C NMR : δ_{ppm}, 150 MHz, in $CDCL_3$ - CD_3OD; see Figures 30a - 30c and Table 6

4.5 Compound D-5

Compound D-5 was obtained as a white powder (10 mg). It was soluble in methanol and chloroform.

Melting Point : 160-162°C

$[\alpha]^{20}_D$: +118.50° (*c* 0.573 g/100 ml, in methanol)

UV : λ_{max} nm (log ε), in ethanol; Figure 36: 284 (0.27), 221 (1.35)

IR : ν_{max} cm^{-1}, KBr disc; Figure 37: 3431 (br), 1725

EIMS : *m/z* (% relative intensity) ; Figure 38

340(M^+, 5.59),339(2.48),310(10.56),309 (3.73),208 (6.83),201 (9.32), 199 (11.18),194 (10.56),180 (7.14),170 (10.56),169 (12.42),167 (11.18), 154 (12.73),143 (6.21),130 (9.32),122 (24.84),109 (4.35),108 (8.70),58 (100)

^{1}H NMR : δ_{ppm}, 500 MHz, in $CDCl_3$; see Figures 39a - 39b and Table 7

^{13}C NMR : δ_{ppm}, MHz, in $CDCl_3$; see Figures 40a - 40c and Table 7

4.6 Compound D-6

Compound D-6 was obtained as a white powder (5.9 mg). It was soluble in methanol and chloroform.

Melting Point : 177-180°C

$[\alpha]^{20}_D$: -339.26° (*c* 0.978 g/100 ml, in methanol)

UV : λ_{max} nm (log ε), in ethanol; Figure 45: 340 (1.04), 286 (0.33), 236 (1.09), 211 (1.65)

IR : ν_{max} cm^{-1}, KBr disc; Figure 46: 3432 (br), 1597

EIMS : *m/z* (% relative intensity) ; Figure 47

356 (M^+, 27.95), 257 (83.23), 139 (22.98), 94 (61.49), 44 (100)

^{1}H NMR : δ_{ppm}, 500 MHz, in $CDCl_3$; see Figures 48a - 48b and Table 8

^{13}C NMR : δ_{ppm}, MHz, in $CDCl_3$; see Figures 49a - 49c and Table 8

CHAPTER IV

Results and Discussion

The fresh fruits of *Alstonia scholaris* (L.) R. Br. (60.7 kg) were extracted with methanol. The obtained methanolic extract, after acid - basic treatment (15.28 g), was then separated using several chromatographic techniques to afford six pure compounds.

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

1. Structure Determination of Isolated Compounds

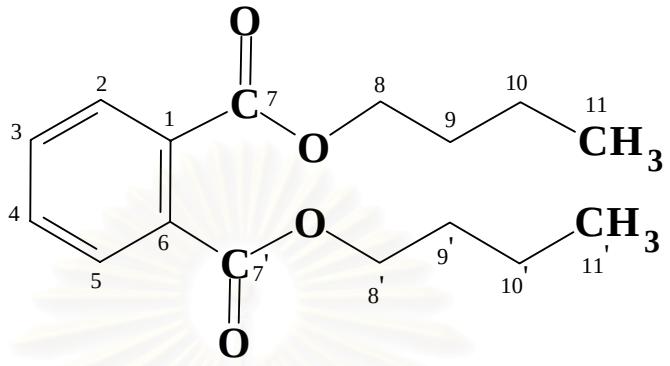
1.1 Structure Determination of Compound D-1

Compound D-1 was obtained as yellow oil. The UV spectrum of D-1 (Figure 7) possessed maxima at 244, 283 and 298 nm suggesting the presence of a benzene ring chromophore. The IR spectrum (Figure 8) of compound D-1 indicated absorption bands for C-H stretching of alkene at 3428 cm^{-1}, C-H stretching of alkanes at 2931 and 2870 cm^{-1}, C=O stretching of ester at 1730 cm^{-1}, C=C stretching of aromatic ring at 1642 and 1463 cm^{-1} and C-O stretching of esters at 1380 and 1286 cm^{-1}. The EIMS (Figure 9) showed a molecular ion peak $[M^+]$ at *m/z* 278, consistent with the molecular formula $C_{16}H_{22}O_4$ (D.B. E. = 6). D-1 was determined as dibutyl phthalate (**82**).

The ^1H NMR spectrum (Figures 10a-10b) showed one triplet (6H) for two methyl groups at δ 0.96, one sextet (4H) for two methylene groups at δ 1.44, one quintet (4H) for two methylene groups at δ 1.72 and one triplet (4H) for two methylene groups at δ 4.31. The signals at δ 7.5-7.75 were assigned to four aromatic protons.

The ^{13}C NMR spectrum (Figure 11) suggested the presence of two methyl carbons, six methylene carbons, four methine carbons and four quaternary carbons. The most downfield signal at δ 167.78 was assigned to C-7 and C-7′. The signal at δ 65.64 was assigned to C-8 and C-8′. This compound was previously isolated from

Aloe vera (Liliaceae) by Lee and co-workers (2000), although it is a plasticizer. The complete carbon assignments of D-1 (dibutyl phthalate) are depicted in Table 3.



(82)

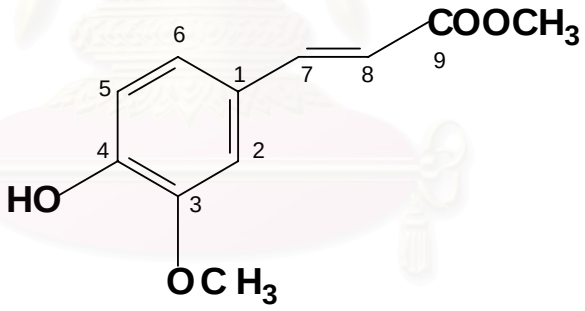
Table 3 ^{1}H and ^{13}C NMR Assignments of Compound D-1 (in $CDCl_3$)

Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)
1,6	-	132.4
2,5	7.72 (dd, 6.5, 3.7)	128.9
3,4	7.53 (dd, 6.5, 3.7)	131.0
7, 7′	-	167.8
8, 8′	4.31 (triplet, 7.2)	65.6
9, 9′	1.72 (quintet, 7.2)	30.7
10, 10′	1.44 (sextet, 7.2)	19.3
11, 11′	0.96 (triplet, 7.2)	13.8

1.2 Structure Determination of Compound D-2

Compound D-2 was obtained as brownish yellow oil. The UV spectrum of compound D-2 (Figure 12) possessed maxima at 244, 288 and 320 nm suggesting the presence of a benzene ring chromophore. The EIMS (Figure 13) showed a molecular ion peak $[M^+]$ at *m/z* 208 , consistent with the molecular formula $C_{11}H_{12}O_4$ (D. B. E. = 6).

The ^{1}H NMR spectrum (Figures 14a - 14b) showed two singlets (3H each) for two methoxyl groups at δ 3.81 and 3.95 ppm, and three aromatic protons at δ 6.92 (1H), 7.03 (1H) and 7.07 (1H). The hydroxyl proton (4-OH) showed a singlet signal δ 5.87 ppm. In addition, the spectrum showed two one-proton doublets at δ 6.29 and 7.63 ppm (*J* = 16 Hz), revealing a trans olefinic structure. By comparing the ^{13}C-NMR spectral data of D-2 (Figures 15a - 15b) with ferulic acid (Kelley et al., 1976), D-2 was determined as methyl ferulate (**83**). The carbon assignments of D-2 (methyl ferulate) are depicted in Table 4.



(**83**)

Table 4 ^{1}H and ^{13}C-NMR Assignments of Compound D-2 [in $CDCl_3$] and ^{13}C-NMR Assignments of Ferulic acid [in acetone-d_6-D_2O (9:1)]

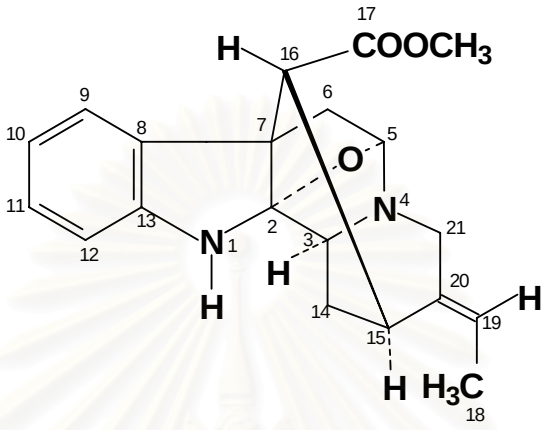
Position	Compound D-2		Ferulic acid
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_C (ppm)
1	-	127.0	127.7
2	7.03 (d, 2)	109.4	110.5
3	-	148.0	147.1
4	-	146.8	146.4
5	6.92 (d, 8)	115.3	115.3
6	7.07 (dd, 8, 2)	123.1	121.9
7	7.63 (d, 16)	145.0	141.3
8	6.29 (d, 16)	114.8	121.1
9	-	167.8	175.8
4-OH	5.87 (s)	-	-
3-O$\underline{C}H_3$	3.95 (s)	56.0	55.6
COO$\underline{C}H_3$	3.81 (s)	51.7	-

1.3 Structure Determination of Compound D-3

Compound D-3 was obtained as a white powder. The UV spectrum of D-3 (Figure 16) possessed maxima at 206, 235 and 288 nm suggesting the presence of an indoline chromophore (Grossmann and Sefcovic, 1973). The IR spectrum (Figure 17) of compound D-3 indicated absorption bands for an N-H functionality at 3391 cm^{-1}, a methoxycarbonyl functional group at 1724 cm^{-1}, an aromatic ring at 1611 and 1464 cm^{-1}, and an ether linkage at 1168 cm^{-1}. The EIMS (Figure 18) showed a molecular ion $[M^+]$ at *m/z* 338 , consistent with the molecular formula $C_{20}H_{22}N_2O_3$ (D. B. E. = 11). The intense ion peak at *m/z* 279, $[M-59]^+$, was attributed to the loss of an ester group ($COOCH_3$). This was confirmed by the presence of the signals of a carbonyl carbon and its corresponding methoxyl carbon at δ 172.4 and 51.1 ppm, respectively in the ^{13}C-NMR spectrum (Figures 20a and 20b). The information obtained from the ^{1}H-^{1}H COSY spectrum (Figures 22a - 22c) and the DEPT spectra (Figures 21a and 21b) suggested the existence of one methoxyl group, one methyl group, three methylene groups, and nine methine groups in the structure. The chemical shifts and the coupling patterns of the four aromatic protons of D-3 indicated a lack of substitution on the benzene ring of indole nucleus. Moreover, the signals for H-16 (d, *J* = 3.7 Hz) at δ 2.45 ppm, and the vinyl proton (H-19) and the corresponding methyl group (18-CH_3) of the ethylidene side-chain at δ 5.40 and 1.48 ppm observed in the ^{1}H-NMR spectrum of D-3 (Figures 19a and 19b) are characteristics of indole alkaloids belonging to the Akuammiline group of the Corynanthean-type. By comparing these spectral data with those earlier published (Grossmann and Sefcovic, 1973; Batista *et al*., 1996), D-3 was determined as picrinine (**84**). This alkaloid was first isolated from *Rauvolfia vomitoria* (Apocynaceae) by Britten and Smith (1963) and its ^{1}H-NMR (300 MHz) and ^{13}C-NMR assignments (75 MHz) were studied by Batista and co-workers (1996).

Although the ^{13}C-NMR data of this alkaloid have previously been reported, this study re-investigate the ^{13}C-NMR properties of D-3 using 2D-NMR experiments. The hydrogen-bonded carbons of D-3 were straightforwardly assigned by the one-bond ^{13}C-^{1}H correlations (HETCOR, Figure 23) and the DEPT experiments. The long-range couplings observed in the ^{13}C-^{1}H COLOC spectrum of D-3 (Figure 24 and Table 5) at δ

2.14 (H-14)/106.3 ($^3J_{CH}$) and δ 3.77 (H-21)/136.3 ($^2J_{CH}$) enabled us to assign the quaternary carbons at δ 106.3 and 136.3 as C-2 and C-20, respectively. The typical chemical shifts of C-7, C-8 and C-13 of the substituted indole nucleus of D-3 were shown at δ 51.8, 135.2 and 147.5 ppm, respectively. The one - proton broad quartet (*J* = 7.0 Hz) signal at δ 5.40 ppm and its corresponding carbon signal at δ 120.3 ppm were attributed to the 19-CH function. This proton signal, in the NOESY spectrum (Figures 25a and 25b), displayed through space interactions with those at δ 3.09 and 1.48 ppm, which were accordingly assigned to H-21 and H_3-18, respectively. The one - proton broad doublet (*J* = 2.8 Hz) signal at δ 3.28 ppm and its corresponding carbon signal at δ 31.0 ppm were attributed to the 15-CH function. This proton signal, in the NOESY spectrum (Figures 25a and 25b), displayed space interactions with a proton at δ 1.48 ppm, which was accordingly assigned to H_3-18. Inspection of the ^{1}H-^{1}H COSY spectrum of D-3 (Figure 22a - 22c), revealed the correlations between H-3 and H-14α, and between H-15 and 16-CH. A doublet at δ 4.82 ppm (H-5) showed vicinal coupling with H-6 (δ 2.26 ppm). The chemical shift of the methylene carbon at δ 46.3 indicated that this carbon (C-21) was directly attached to the nitrogen (N_b). The H-21 proton showed a cross peak with the H-19 proton in the NOESY spectrum. Our results confirmed the earlier ^{1}H and ^{13}C NMR assignments of picrinine (**84**) (Batista *et al*., 1996). This alkaloid was also previously isolated from the flowers of *Alstonia scholaris* (Dutta *et al*, 1976).



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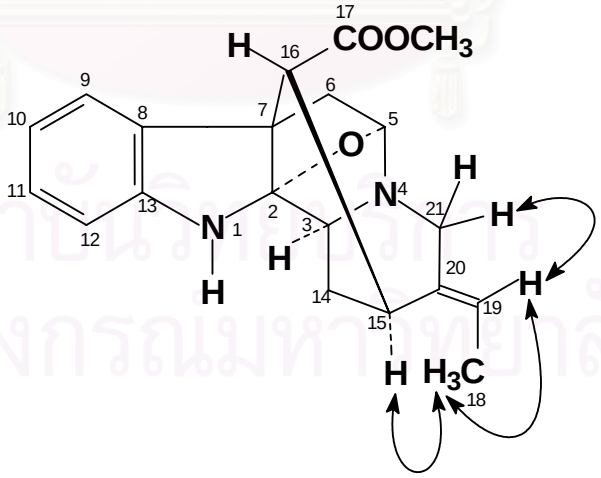


Significant NOE relationships observed in NOESY of D-3 (Picrinine)

Table 5 ^{1}H and ^{13}C NMR Assignments of Compound D-3 (in $CDCl_3$) and Picrinine (in $CDCl_3$) with long-range correlation observed in COLOC spectrum

Position	Compound D-3		Picrinine		COLOC (correlation with proton)
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	
1	4.84 (br s)	-	4.90 (br s)	-	-
2	-	106.3	-	106.3	H-14 and H-3*
3	3.60 (d, 4.7)	52.0	3.60 (d, 4.7)	52.0	H-14*, H-21 and H-5
5	4.82 (d, 2.2)	87.3	4.84 (d, 2.2)	87.3	H-6* and H_2-21
6	3.41 (d, 13.8)	40.6	3.41 (d, 13.8)	40.5	-
	2.26 (dd, 13.8, 2.8)	-	2.26 (dd, 13.8, 2.8)	-	-
7	-	51.8	-	51.8	H-6*, H-16* and H-5
8	-	135.2	-	135.1	H-6, H-16, H-12 and H-10
9	7.14 (dd, 7.6, 1.3)	125.1	7.14 (dd, 7.6, 1.3)	125.0	-
10	6.78 (ddd, 7.6, 7.6, 1.2)	120.7	6.78 (ddd, 7.6, 7.6, 1.2)	120.7	H-12 and H-11*
11	7.08 (ddd, 7.6, 7.6, 1.4)	127.9	7.08 (ddd, 7.6, 7.6, 1.4)	127.9	H-10* and H-9
12	6.75 (d, 7.8)	110.5	6.75 (d, 7.8)	110.6	H-10 and H-11*
13	-	147.5	-	147.4	H-11 and H-9
14	2.14 (ddd, 14.2)	26.0	2.14 (ddd, 14.2)	25.9	H-15*
14	1.85 (dd, 14.5, 3.4)	-	1.85 (dd, 14.5, 3.4)	-	-

Table 5 ^{1}H and ^{13}C NMR Assignments of Compound D-3 (in $CDCl_3$) and Picrinine (in $CDCl_3$) with long-range correlation observed in COLOC spectrum (continued)

Position	Compound D-3		Picrinine		COLOC (correlation with proton)
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	
15	3.28 (br d, 2.8)	31.0	3.28 (br d, 2.8)	31.1	-
16	2.45 (d, 3.7)	51.4	2.45 (d, 3.7)	51.7	H-14
17	-	172.4	-	172.4	H-16* and 17-OCH_3
18	1.48 (dd, 7.0, 2.4)	12.7	1.48 (dd, 7.0, 2.4)	12.7	-
19	5.40 (br q, 7.0)	120.3	5.40 (br q, 7.0)	120.4	H-18*, H-15 and H_2-21
20	-	136.3	-	136.1	H_2-21*, H-15*, H-18, H-14 and H-16
21	3.77 (br d, 17.8)	46.3	3.77 (br d, 17.8)	46.3	-
	3.09 (d, 17.8)	-	3.09 (d, 17.8)	-	-
17-OMe	3.65 (s)	51.1	3.65 (s)	51.4	-

* Two-bond coupling

1.4 Structure Determination of Compound D-4

Compound D-4 was obtained as a white powder. The UV spectrum of D-4 (Figure 26) suggested the presence of an indole chromophore, with absorption maxima at 227 and 282 nm. Its IR spectrum (Figure 27) exhibited absorption bands for O-H stretching at 3419 cm^{-1}, N-H stretching at 3265 cm^{-1}, C=O stretching 1715 cm^{-1}, C=C stretching of aromatic ring at 1622 and 1456 cm^{-1}, C-N stretching at 1223 cm^{-1} and C-O stretching of alcohol at 1063 cm^{-1}. The EIMS of D-4 (Figure 28) gave a molecular ion $[M^+]$ at *m/z* 352, suggesting the molecular formula $C_{21}H_{24}N_2O_3$ (D. B. E. = 11). An intense ion peak at *m/z* 321, $[M-31]^+$, was attributed to the loss of a methylenehydroxyl group (CH_2OH) in the molecule. This was confirmed by the presence of the signals of a carbonyl carbon and its corresponding methoxyl carbon at δ 174.1 and 50.6 ppm, respectively in the ^{13}C-NMR spectrum (Figures 30a - 30c). The information obtained from the ^{1}H-^{1}H COSY (Figures 32a - 32c) and DEPT spectra (Figures 31a - 31b) revealed the presence of one methoxyl group, one methyl group, four methylene groups and eight methine groups in the structure. The chemical shifts and splitting patterns of the four aromatic protons of D-4 indicated the lack of substitution on positions 9, 10, 11, and 12 of the indole nucleus. The signals for the vinyl proton (H-19) and the corresponding methyl group (18-CH_3) of the ethylidene side-chain at δ 5.41 and 1.65 ppm were observed in ^{1}H-NMR spectrum of D-4.

The one-proton doublet (*J* = 11 Hz) signal at δ 3.84 ppm and its corresponding carbon signal at δ 68.7 ppm were attributed to 17$'$-CH function. This proton signal, in an NOE difference experiment (Figures 35b and 35c), displayed space interaction with a proton at δ 3.1 ppm, which was accordingly assigned to H-5. The one-proton broad quartet (*J* = 7 Hz) signal at 5.41 ppm and its corresponding carbon signal at δ 116.7 ppm were attributed to 19-CH function. This proton displayed NOE interactions with the protons at δ 3.59 and 1.65 ppm, which were accordingly assigned to H-21 and H-18, respectively. In the ^{1}H-^{1}H COSY spectrum, the correlations between H-3 and H_2-14 provided the assignments for H-14. In addition, a COSY correlation (Figures 32a - 32c) was observed between H-15 and H-14β. The OH-bearing carbon at δ 68.7 ppm was assigned to C-17 on the basis of the one-bond ^{13}C-^{1}H correlations

(HMQC, Figure 33d). A multiplet at δ 3.1 ppm was assigned to H-5 since it showed vicinal coupling with H-6α and H-6β (δ 2.91 and 3.29 ppm). These spectral data suggested that D-4 should belong to the sarpagine group.

The chemical shift of the C-21 methylene carbon (δ 51.4 ppm) indicated that it was directly attached to the nitrogen (N_b). The assignment of H-17′ was obtained by irradiation of H-17′ (δ 3.84) which led to the enhancement of H-5 (δ 3.1) in the NOE difference spectrum (Figures 35b - 35c). The methylene protons at δ 3.59 ppm, which showed the enhancement (NOE) with the H-19 vinyl proton, was assigned to H_2-21. The methyl-protons at δ 1.65 ppm (J = 7, 2, 2 Hz) was assigned to 18-CH_3. The long-range couplings observed in the ^{13}C-^{1}H HMBC spectrum of D-4 (Figures 34a - 34f) at δ 1.86 (H-14)/137.2 ($^{3}J_{CH}$) and δ 3.58 (H-21)/137.4 ($^{2}J_{CH}$) enabled us to assign the quaternary carbons at δ 137.2 and 137.4 as C-2 and C-20, respectively. Moreover, the typical chemical shifts of C-7, C-8 and C-13 of an unsubstituted indole nucleus of D-4 were observed at δ 106.2, 127.0 and 136.6 ppm, respectively. The carbon assignments of D-4 are depicted in Table 6.

These spectral data of D-4 were in good agreement with those of 19-*E*-akuammidine (**85**), the structure of which was revised in 1996 by Jokela and Lounasmaa. This alkaloid has been earlier reported from the fruits of *A. scholaris* (Chatterjee *et al*., 1969b).

It has been reported that the ^{1}H-NMR and ^{13}C-NMR spectra of 19-*E*-akuammidine (**85**) and 19-*Z*-akuammidine (**86**) are similar but not completely identical (Ponglux *et al*., 1988). The ^{13}C-NMR chemical shifts (Table 6) of C-15 (29.4 ppm, upper field than *Z* form) and C-21 (55.5 ppm, lower field than *Z* form) of the isolated 19-*E*-akummidine (D-4) compared with those of 19-*Z*-akuammidine can be reasonably interpreted in terms of γ-gauche effect due to C-18 on the double bond having *E*-configuration. Our difference NOE experiment on D-4 (Figures 35a - 35c) confirmed the configuration of the ethylidene side chain, as previously described.

Table 6 ^{1}H and ^{13}C NMR Assignments of Compound D-4 (in $CDCl_3$-CD_3OD) and 19-*E*- Akuammidine (in $CDCl_3$) with long-range correlation observed in HMBC spectrum

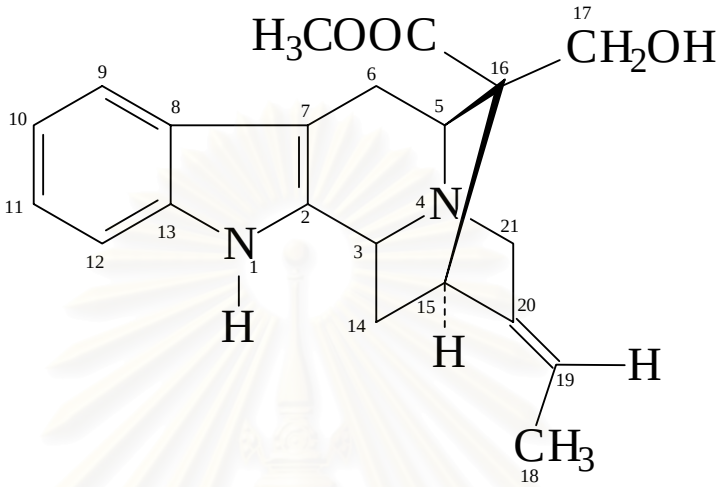
Position	Compound D-4		19-*E*-Akuammidine		HMBC (correlation with proton)
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	
1	7.70 (br s)	-	7.90 (br s)	-	-
2	-	136.6[a]	-	136.6[b]	H-3*
3	4.22 (br d, 11, 2)	51.4	4.24 (br d, 11, 2)	51.4	H-14α*
5	3.1 (m, 5, 1.5)	58.0	3.1 (m, 5, 1.5)	58.0	H-6α*, H-6β*, H-3, H-17 and H-17$'$
6α	2.91 (dd, 16, 5)	24.8	2.94 (dd, 16, 5)	24.7	H-5*
6β	3.29 (dd, 16, 1.5)	-	3.30 (dd, 16, 1.5)	-	-
7	-	106.2	-	106.2	H-6α*, H-6β*, H-5 and H-9
8	-	127	-	126.9	H-9*, H- 6β, H-10 and H-12
9	7.43 (d, 7.3)	118.1	7.42 (d, 7.3)	118.0	H-11
10	7.05 (t, 10.3)	119.4	7.05 (t, 10.3)	119.4	H-12
11	7.11 (t, 7.3)	121.5	7.11 (t, 7.3)	121.5	H-9
12	7.28 (d, 10.3)	110.9	7.28 (d, 10.3)	110.9	H-10
13	-	137.2[a]	-	137.0[b]	H-11 and H-9
14α	1.86 (ddd, 12.5, 11, 2)	29.2	1.85 (ddd, 12.5, 11, 2)	29.2	H-15*
14β	2.66 (ddd, 12.5, 3, 2)	-	2.67 (ddd, 12.5, 3, 2)	-	-
15	3.1 (m, 3, 2)	29.4	3.1 (m, 3, 2)	29.4	H-14β*
16	-	49.9	-	50.3	H-5*
17	3.68 (d, 11)	68.7	3.67 (d, 11)	68.8	H-5

Table 6 ^{1}H and ^{13}C NMR Assignments of Compound D-4 (in $CDCl_3$-CD_3OD) and 19-*E*- Akuammidine (in $CDCl_3$) with long-range correlation observed in HMBC spectrum (continued)

Position	Compound D-4		19-*E*-Akuammidine		HMBC (correlation with proton)
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	
17′	3.84 (d, 11)	-	3.83 (d, 11)	-	-
18	1.65 (ddd, 7, 2, 2)	13.0	1.65 (ddd, 7, 2, 2)	13.0	H-19*
19	5.41 (br q, 7)	116.7	5.39 (br q, 7)	116.8	-
20	-	137.4[a]	-	137.1[b]	H-15*, H-21α*, H-21β*, H-18, H-14α and H-14β
21α	3.59 (def, 2)	55.5	3.58 (def, 2)	55.5	H-15 and H-5
21β	3.59 (def, 2)	-	3.58 (def, 2)	-	-
$CO_2\underline{Me}$	2.94 (s)	50.6	2.94 (s)	50.6	-
$\underline{C}O_2Me$	-	174.1	-	173.8	$COO\underline{CH_3}$, H-5, H-17 and H-17′

[a, b,] Assignments for these signals within a vertical column may be reversed.

* Two bond coupling.



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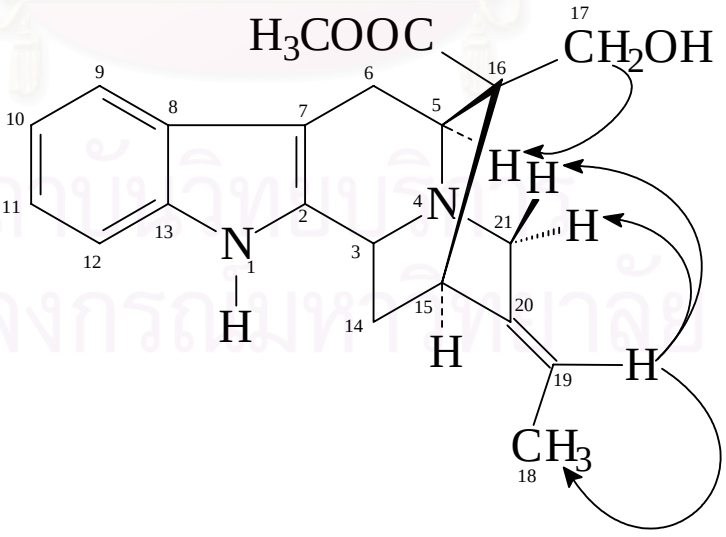
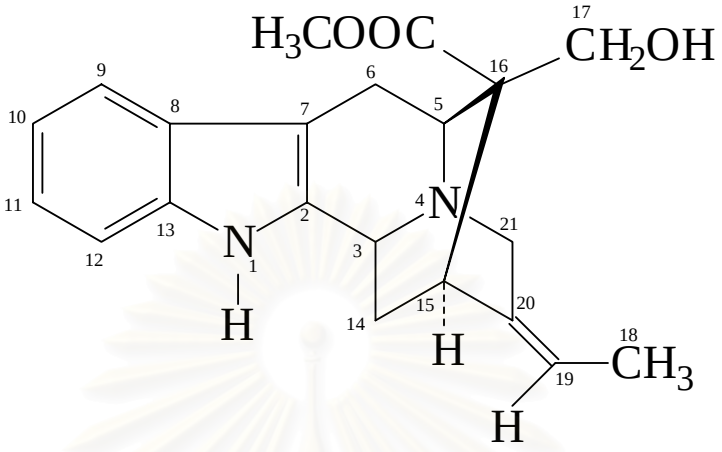


Significant NOE relationships observed in NOE difference of D-4 (19-*E*-Akuammidine)



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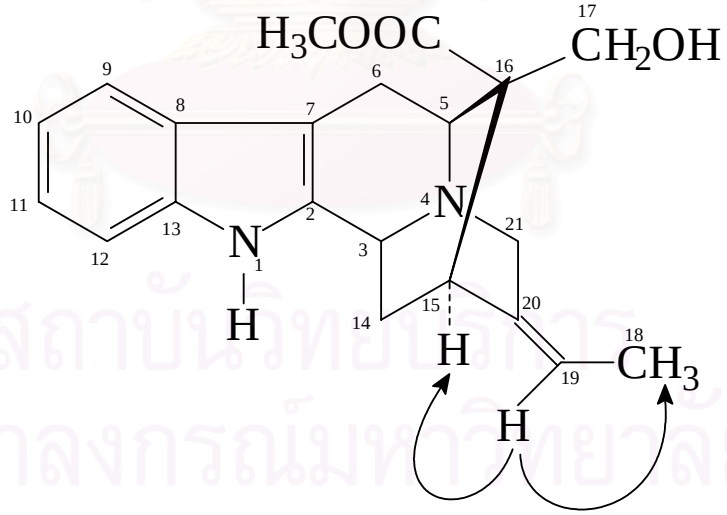


Significant NOE relationships observed in NOE difference of 19-*Z*-Akuammidine

1.5 Structure Determination of Compound D-5

Compound D-5 was obtained as a white powder. It was identified as 19,20-*E*-vallesamine (**87**). This alkaloid was previously isolated from the leaves of *Alstonia scholaris* (Atta-ur-Rahman *et al*., 1987a; Yamauchi *et al*., 1990a) and the fruits of *Tabernaemontana dichotoma* (Apocynaceae) (Perera *et al*., 1984). The UV spectrum (Figure 36) was found to be characteristic for the indole chromophore, showing absorption maxima at 221 and 284 nm. The IR spectrum (Figure 37) showed absorptions at 3431 cm^{-1} (NH) and 1725 cm^{-1} (ester C=O). The EI mass spectrum (Figure 38) gave a molecular ion [M^+] at *m/z* 340, corresponding to the molecular formula $C_{20}H_{24}N_2O_3$ (D. B. E. = 10). Other significant peaks were observed at *m/z* 208, 143 and 122.

The ^{1}H NMR spectrum (Figures 39a and 39b and Table 7) showed one doublet at δ 1.74 ($J_{18,19}$ = 7.0 Hz) for the ethylidene methyl group. An AB double doublet at δ 4.82 (d, $J_{6\alpha,6\beta}$ = 17.1 Hz) and δ 4.08 (d, $J_{6\beta,6\alpha}$ = 17.1 Hz) were assigned to H-6α and H-6β protons respectively. The H-15 proton appeared as a multiplet centered at δ 3.63 while H-21α and H-21β protons resonated together at δ 3.61 as multiplets. The H-14α and H-14β protons appeared as multiplets at δ 2.33 and 1.90, respectively. Another set of AB doublets resonating at δ 4.19 and δ 3.81 ($J_{17\alpha,17\beta}$ = 10.8 Hz) were assigned to H-17α and H-17β protons, respectively. The ester methyl group appeared as a singlet at δ 3.75 while the olefinic proton resonated at δ 5.55 as a quartet ($J_{19,18}$ = 7.0 Hz) (Table 7). The coupling interactions were determined through COSY 45° spectrum (Figures 41a-41d). The one – bond correlations between proton and carbon nuclei observed in the HMQC spectrum (Figures 42a-42d) indicated the presence of two methyl carbons, five methylene carbons and six methine carbons. The other seven remaining carbons were assigned as quaternary carbon including the C=O function resonating at δ 175.2. The cross-peaks of the ^{13}C – ^{1}H long range correlations obtained from an HMBC experiment (Figures 43a-43g and Table 7) allowed the various fragments to be connected together. The ^{13}C - NMR spectrum of D-5 (Figures 40a-40c and Table 7) showed 20 carbon resonances. The chemical shifts of D-5 were similar to those reported in the literature (Atta-ur-Rahman *et al*., 1987a) for "19-*Z*-

vallesamine (**88**)". The major difference appeared at the C-19 and C-20 carbons which were shifted by 3.02 ppm upfield and 4.72 ppm downfield, respectively which indicated a change in the stereochemistry at the 19, 20 double bond. In order to confirm the relative stereochemistry at C-19, an NOE difference measurement (Figure 44) was carried out. Irradiation of the H-19 quartet at δ 5.55 gave an NOE enhancement of the multiplet at δ 3.61 for the H-21 α and H-21β protons, and an NOE enhancement of the doublet at δ 1.74 for the H-18 proton. This established the proximity of H-18 and H_2-21 protons. This result showed that the 19,20 double bond has *E* configuration, confirming the stereochemistry at the 19,20 double bond as previously described (Atta-ur-Rahman *et al*., 1987a).

Table 7 ^{1}H and ^{13}C NMR Assignments of Compound D-5 (in $CDCl_3$) and 19,20-*E*-Vallesamine (in $CDCl_3$) with long-range correlation observed in HMBC spectrum

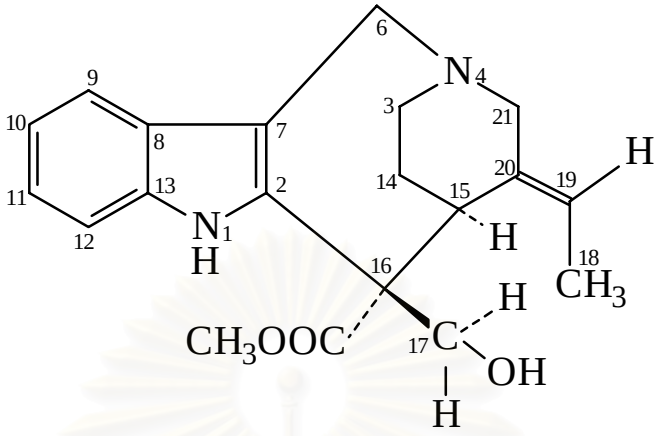
Position	Compound D-5		19,20-*E*-Vallesamine		HMBC Correlation with proton
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C(ppm)	δ_H (ppm) (mulyiplicity, *J* in Hz)	δ_C (ppm)	
1	9.55 (br s)	-	9.50 (br s)	-	-
2	-	133.6[a]	-	133.6[b]	-
3α	2.94-2.85 (m)	47.5	2.96-2.85 (m)	47.5	H-6α and H-6β
3β	2.94-2.85 (m)	-	2.96-2.85 (m)	-	-
6α	4.82 (d, 17.1)	51.0	4.82 (d, 17.1)	51.2	H-21α, H-21β, H-3α and H-3β
6β	4.08 (d, 17.1)	-	4.09 (d, 17.1)	-	-
7	-	109.2	-	109.2	H-9 , H-6α* and H-6β*
8	-	128.1	-	128.1	H-10 , H-12 , H-9*, H-6α and H-6β
9	7.48 (br d, 7.9)[c]	118.3[d]	7.17 (br d, 6.9)[e]	118.4[f]	H-11 and H-10*
10	7.07 (t, 7.9)	119.2	7.07 (t, 7.0)	119.1	H-12
11	7.18 (t, 7.9)	122.4	7.30 (t, 7.9)	122.4	H-12* and H-9
12	7.30 (br d, 7.9)[c]	110.7[d]	7.30 (br d, 7.9)[e]	110.7[f]	H-11* and H-10
13	-	135.4[a]	-	137.4[b]	H-11 and H-9
14α	2.33 (m)	23.9	2.33 (m)	23.8	H-15*
14β	1.90 (m)	-	1.89 (m)	-	-
15	3.63 (m)	36.2	3.63 (m)	36.3	H-21α, H-21β, H-17α, H-19, H-3α and H-3β
16	-	58.5	-	48.5	H-17α*, H-17β* and H-15*

Table 7 ^{1}H and ^{13}C NMR Assignments of Compound D-5 (in $CDCl_3$) and 19,20-*E*-Vallesamine (in $CDCl_3$) with long-range correlation observed in HMBC spectrum (continued)

Position	Compound D-5		19,20-*E*-Vallesamine		HMBC Correlation with proton
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C(ppm)	δ_H (ppm) (mulyiplicity, *J* in Hz)	δ_C (ppm)	
17α	4.19 (d, 10.8)	70.4	4.19 (d, 10.8)	70.2	-
17β	3.81 (d, 10.8)	-	3.81 (d, 10.8)	-	-
18	1.74 (d, 7.0)	14.1	1.74 (d, 6.9)	14.0	H-19*
19	5.55 (q, 7.0)	124.4	5.56 (q, 6.6)	124.1	H-18*, H-21α and H-21β
20	-	132.5[a]	-	132.4[b]	H-18 , H-21α*, H-21β* and H-15*
21α	3.61 (m)	53.9	3.60 (m)	54.0	H-15, H-6β, H-19, H-3α and H-3β
21β	3.61 (m)	-	3.60 (m)	-	-
$\underline{C}OOCH_3$	-	175.2	-	175.2	H-15 , $COO\underline{C}H_3$, H-17β and H-17α
$COO\underline{C}H_3$	3.75 (s)	53.0	3.74 (s)	52.9	-

[a,b,c,d,e,f] Interchangeable within the same column.

* Two bond coupling.



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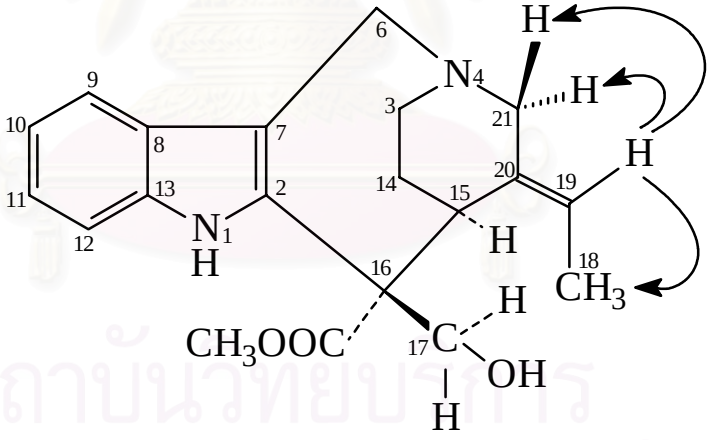
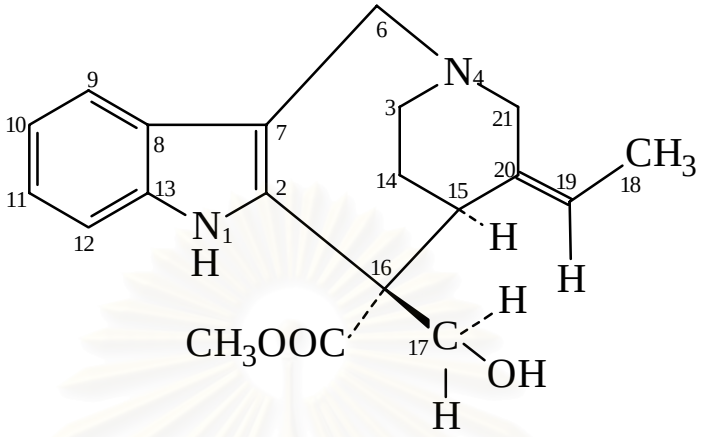


Significant NOE relationships observed in NOE difference of D-5 (19,20-*E*-Vallesamine)



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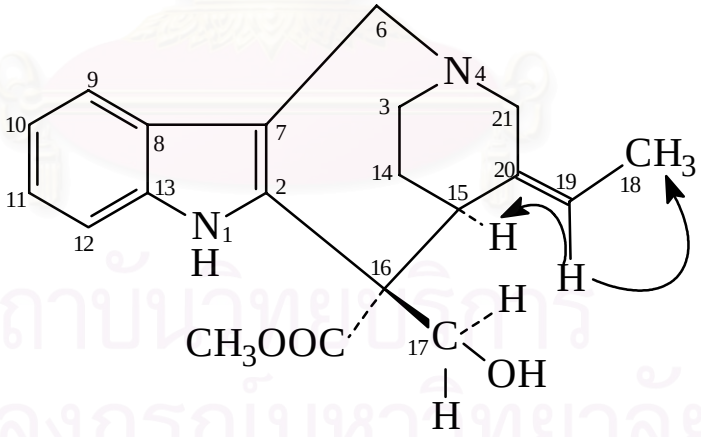

Significant NOE relationships observed in NOE difference of 19,20-*Z*-Vallesamine

1.6 Structure Determination of Compound D-6

Compound D-6 was obtained as a white powder. The UV spectrum of D-6 (Figure 45) was characteristic of an anilino-acrylate chromophore, with absorption maxima at 211, 236, 286 and 340 nm. This suggested the presence of a phenolic group in the molecule. The IR spectrum (Figure 46) gave absorptions at 3500 cm^{-1} (OH), 3432 cm^{-1} (NH) and 1597 cm^{-1} (α, β unsaturated ester, C=O). The EI mass spectrum (Figure 47) afforded a molecular ion $[M]^+$ at *m/z* 356, corresponding to the molecular formula $C_{20}H_{24}N_2O_4$ (D. B. E. = 10) while other major fragments appeared at *m/z* 257, 139 and 94. Compound D-6 was identified as 19*S*-scholaricine (**89**).

The ^1H NMR spectrum (Figures 48a - 48b) showed a three-proton singlet at δ 3.88 which could be assigned to the ester Me group. The NH proton appeared as a singlet at δ 8.58. The signal of methyl protons appearing as a doublet at δ 1.17 (J = 6.0 Hz) suggested the presence of a –CH-Me moiety. Integration of the aromatic region showed the presence of only three protons, which indicated the existence of a substituent in the benzene ring. The aromatic protons gave a complex ABC type multiplet in the region δ 6.65 to 6.85. This suggested that the phenolic group was present at C-9 or C-12, as location of the OH group at C-10 or C-11 would have afforded a readily recognizable ABX pattern.

Further spectroscopic studies were done by examination of the ^{13}C NMR spectrum [150 MHz, $CDCl_3$] (Figures 49a - 49b and Table 8) of compound D-6. The Me group of the –CH(OH)Me moiety resonated at δ 19.8 while the OH bearing methine carbon appeared at δ 68.5. The peaks at δ 52.1 and δ 169.2 were assigned to the Me and carbonyl carbons of the ester group. Three aromatic methine carbons appearing at δ 111.5, δ 122.4 and δ 115.6 were discernible in the spectrum. These were assigned to C-9, C-10 and C-11, respectively. The chemical shifts were consistent with the location of the phenolic group at C-12 rather than at C-9. If the OH had been located at C-9, then an upfield shift would have been expected at C-10 and C-12 in comparison to the unsubstituted compound (Atta-ur-Rahman *et al*., 1985). The ^1H-^1H coupling information obtained from the ^1H-^1H COSY spectrum (Figures 50a - 50c) and the one - bond correlations between proton and carbon nuclei gained from the HMQC spectrum

(Figures 51a and 51b) indicated the presence of two methyl, four methylene and seven methine functions. The other seven remaining carbons were assigned as quaternary carbons including the C=O function resonating at δ 169.2. The cross-peaks of the ^{13}C – ^{1}H long range correlations obtained from the HMBC experiments (Figures 52a - 52e and Table 8) allowed the various fragments to be connected. The stereochemistry at C-19 of D-6 was determined by comparison of the ^{1}H and ^{13}C chemical shifts of compound D-6 with those of compounds 19*S*-scholaricine and 19-epi-scholaricine which were reported in 1990 by Yamauchi and co-workers. This alkaloid was also previously isolated from the leaves of *Alstonia scholaris* (Atta-ur-Rahman *et al*., 1985; Yamauchi *et al*., 1990a).

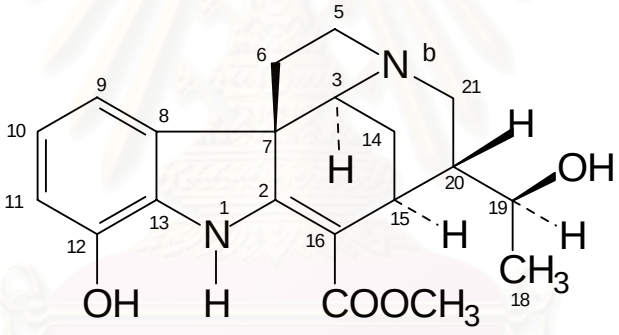

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Table 8 ^{1}H and ^{13}C NMR Assignments of Compound D-6 (in $CDCl_3$) and 19*S*-Scholaricine (in pyridine-d_5) with long-range correlation observed in HMBC spectrum

Position	Compound D-6		19*S*-Schlaricine		HMBC (correlation with proton)
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	
1	8.58 (s)	-	8.58 (s)	-	-
2	-	172.2	-	172.7	H-6, H-3 and H-15
3	3.91 (br s)	61.0	3.96 (br s)	61.5	H-15 , H-21 and H_2-6
5	2.92 (m)	54.0	2.94 (m)	54.4	H-21
	3.1 (m)	-	3.08 (m)	-	-
6	1.90 (m)	43.4	1.87 (m)	44.1	H-5*
	2.85 (m)	-	2.73 (m)	-	-
7	-	57.9	-	58.5	H-5 , H_2-6*, H-14 and H-3
8	-	137.1	-	138.1	H-3 and H-6
9	6.77 (d, 8)	111.5	6.92 (d, 8)	111.4	H-11
10	6.82 (t, 8)	122.4	7.00 (t, 8)	122.7	H-11*
11	6.68 (d, 8)	115.6	7.08 (d, 8)	115.6	H-9 and H-10*
12	-	141.5	-	143.1	H-10 and H-11*
13	-	131.9	-	132.7	H-9 and H-11
14	2.02 (br d, 13)	31.0	2.01 (br d, 13)	31.6	-
	1.42 (dt, 13, 3)	-	1.34 (dt, 13, 3)	-	-
15	3.35 (br s)	28.9	3.54 (br s)	29.6	H-19 and H_2-21
16	-	96.8	-	97.9	-
17	-	169.2	-	169.3	17-OCH_3 and H-15
18	1.17 (d, 6)	19.8	1.24 (d, 6)	20.6	H-19*

Table 8 ^{1}H and ^{13}C NMR Assignments of Compound D-6 (in $CDCl_3$) and 19*S*-Scholaricine (in pyridine-d_5) with long-range correlation observed in HMBC spectrum (continued)

Position	Compound D-6		19*S*-Schlaricine		HMBC (correlation with proton)
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	
19	3.28 (m)	68.5	3.43 (m)	68.5	H-21
20	1.78 (m)	45.9	1.87 (m)	46.8	H_2-21*
21	1.98 (dd, 13, 11)	48.1	1.98 (dd, 13, 11)	48.5	H-5
	2.95 (dd, 13, 6)	-	3.01 (dd, 13, 6)	-	-
17-OMe	3.88 (s)	52.1	3.66 (s)	51.3	-

* Two-bond coupling

CHAPTER V

Conclusion

In this investigation, six pure compounds were isolated from the fruits of *Alstonia scholaris* (L.) R. Br. These compounds are the indole alkaloids picrinine (D-3), 19-*E*-akuammidine (D-4), 19,20-*E*-vallesamine (D-5) and 19*S*-scholaricine (D-6). The others are the esters dibutyl phthalate (D-1) and methyl ferulate (D-2). All of the isolated compounds except 19-*E*-akuammidine (D-4) have never been reported from the fruits of this species before.

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APPENDIX

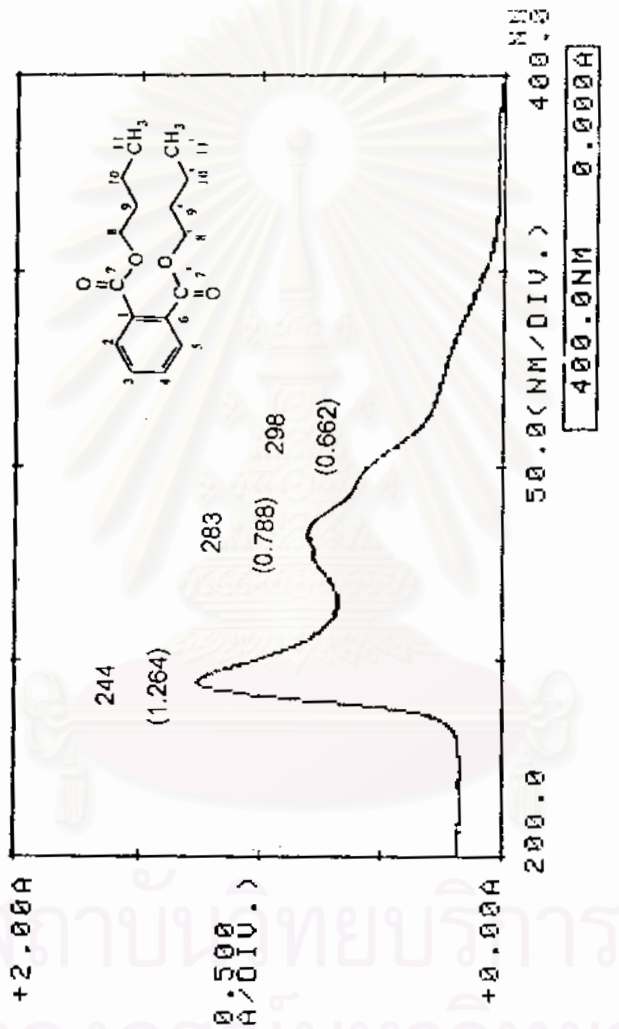


Figure 7 UV spectrum of compound D-1 (in $CDCl_3$)

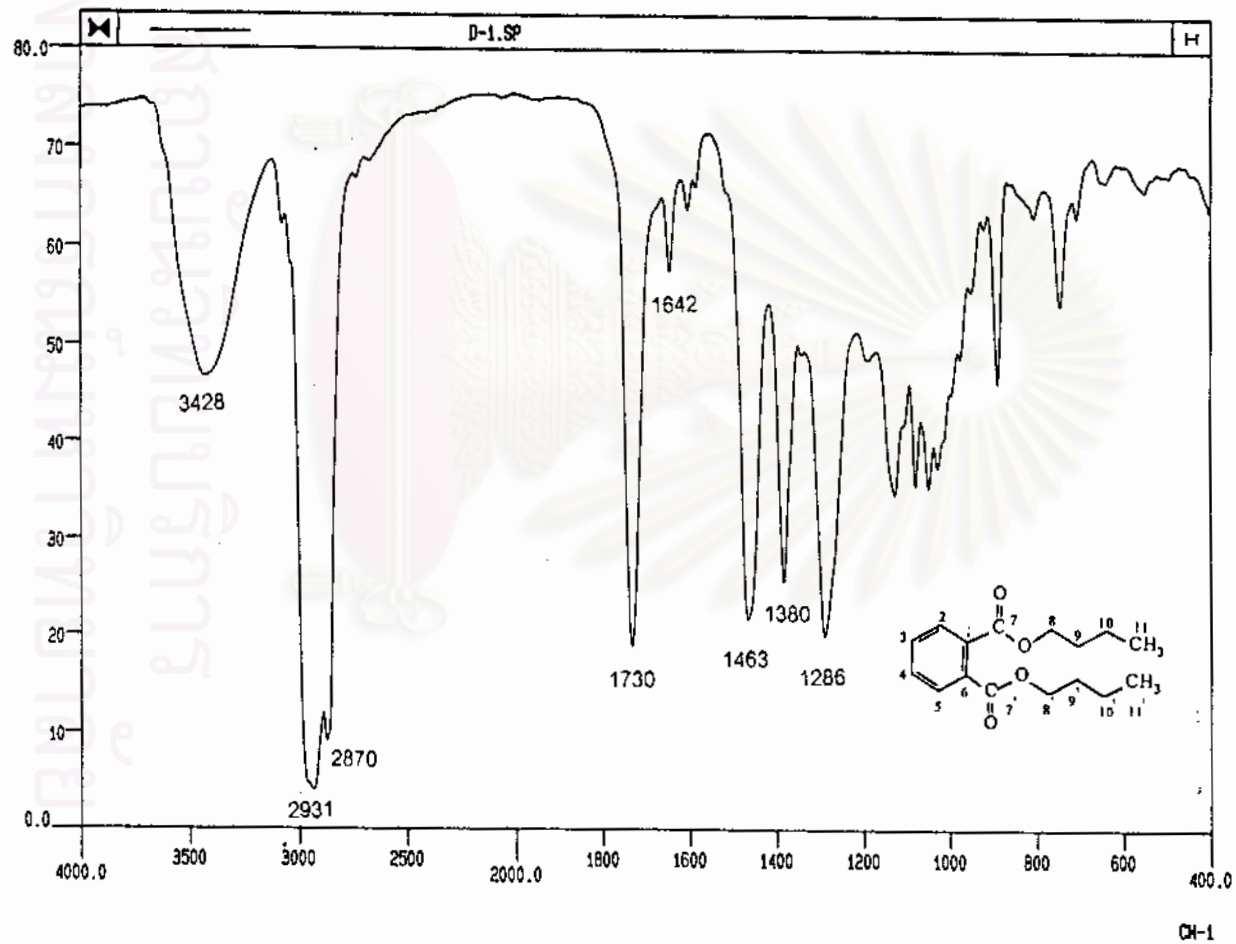


Figure 8 IR spectrum of compound D-1 (film)

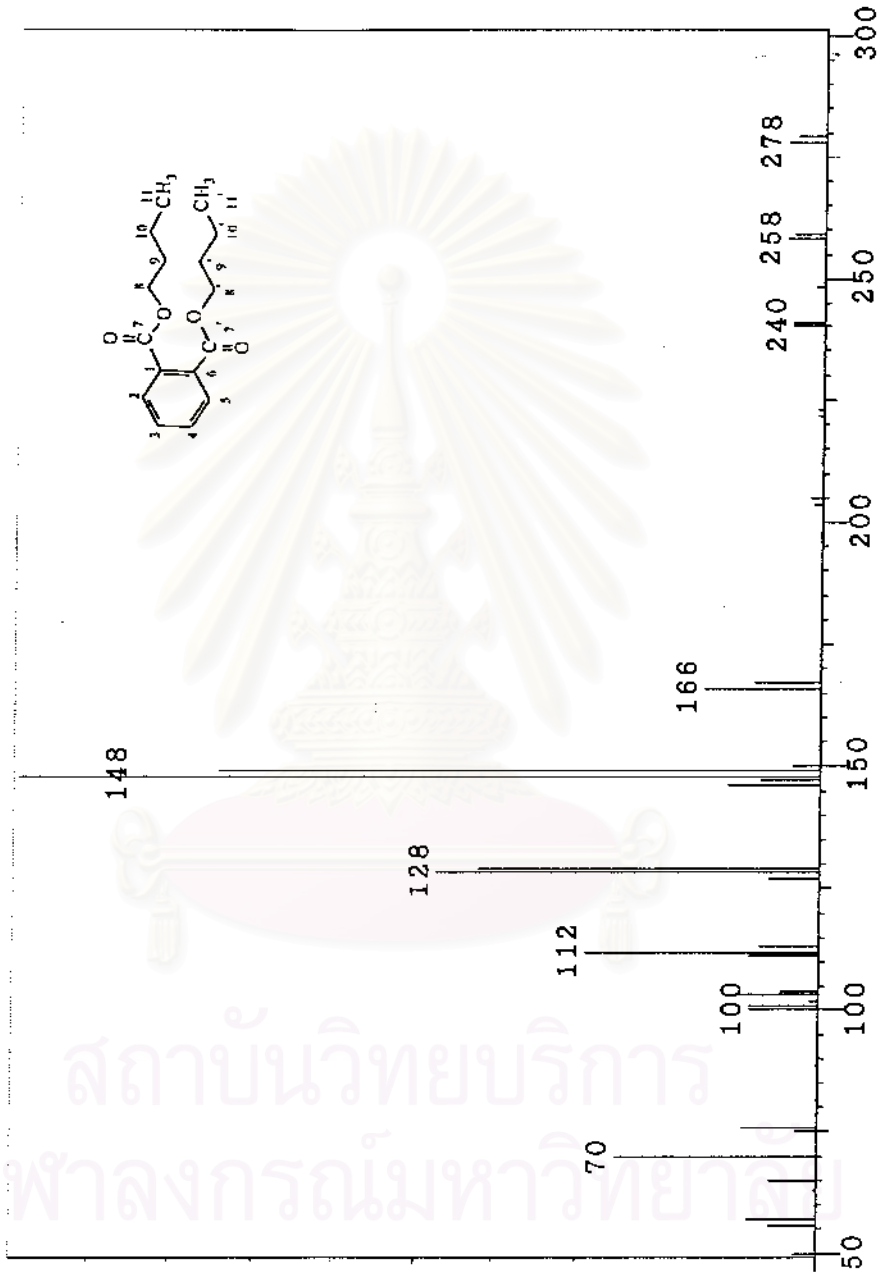


Figure 9 EI mass spectrum of compound D-1

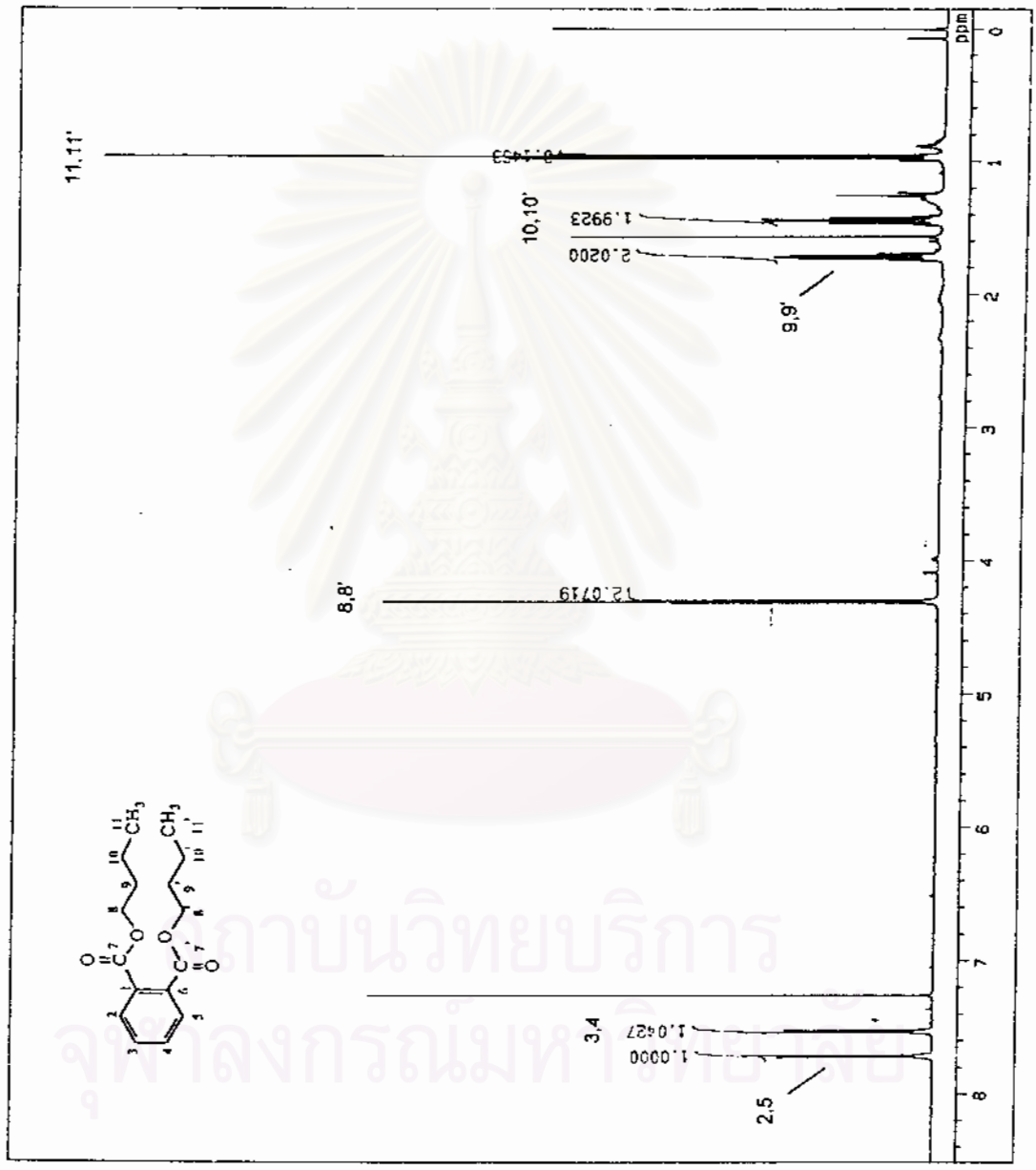


Figure 10a 500 MHz ^{1}H NMR spectrum of compound D-1 (in $CDCl_3$)

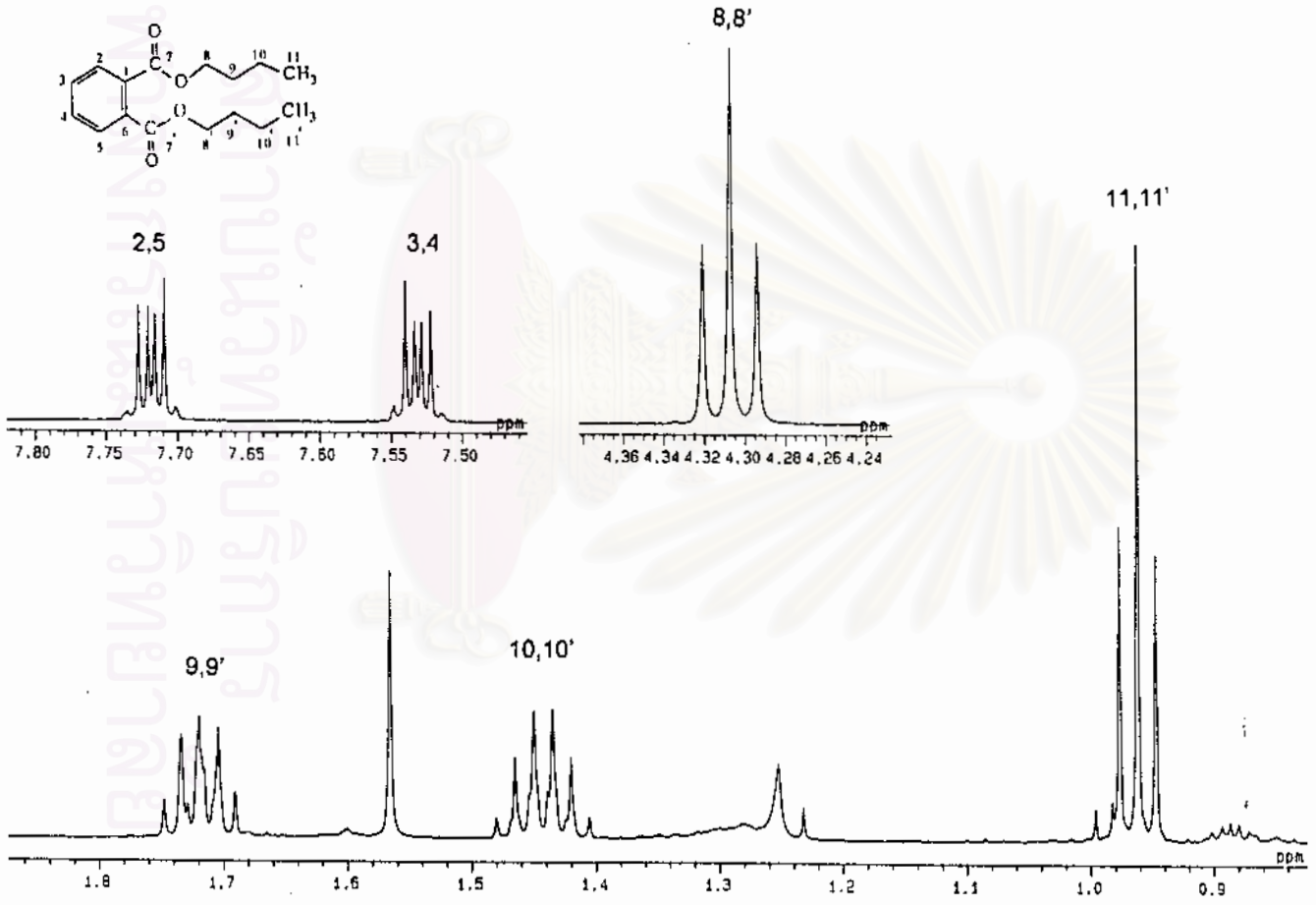


Figure 10b 500 MHz ^{1}H NMR spectrum of compound D-1 (in $CDCl_3$) [δ_H 0.90 -1.80, 4.24 – 4.36, 7.50 -7.80 ppm]

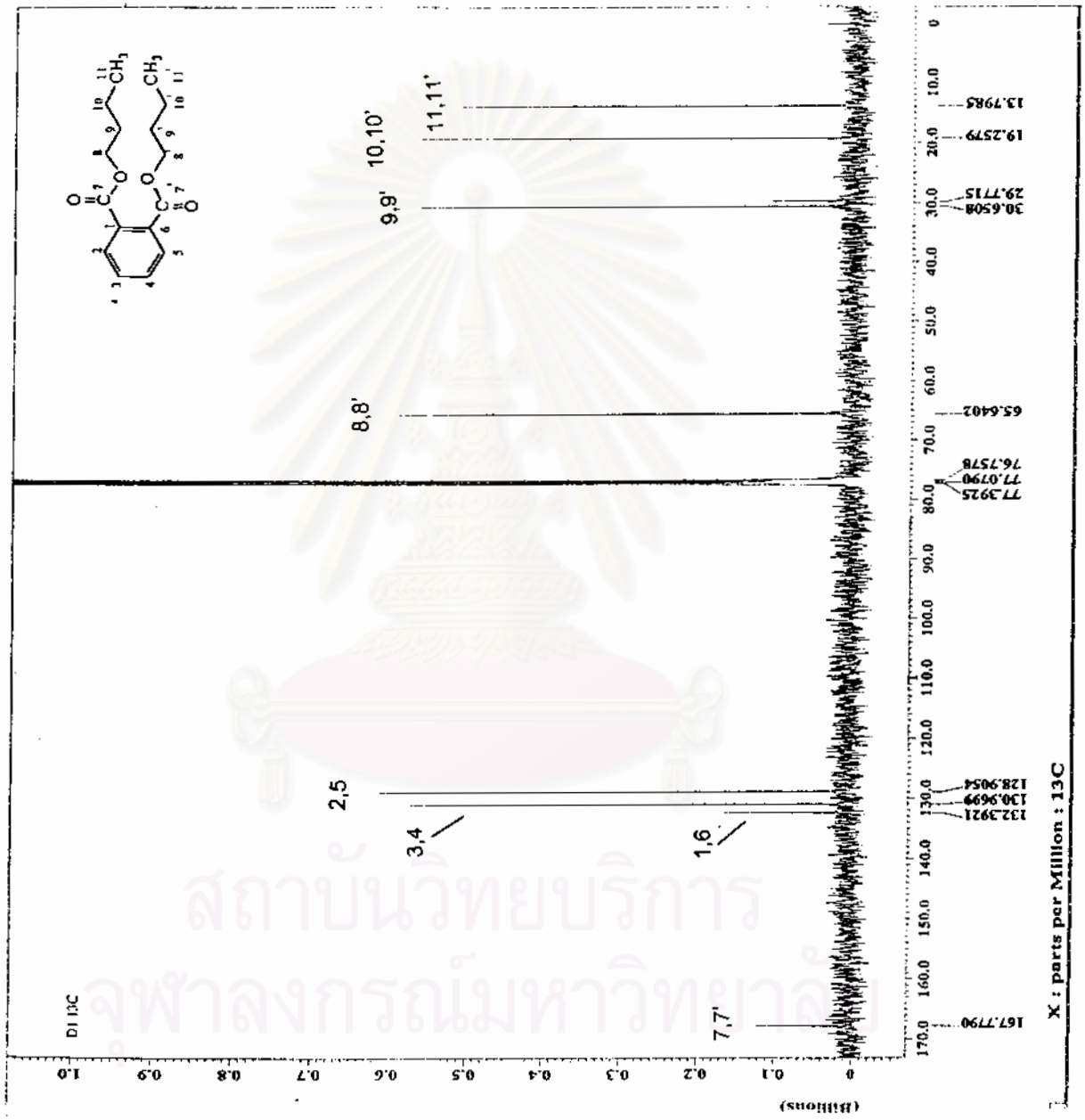


Figure 11 100 MHz ^{13}C NMR spectrum of compound D-1 (in CDCl$_3$)

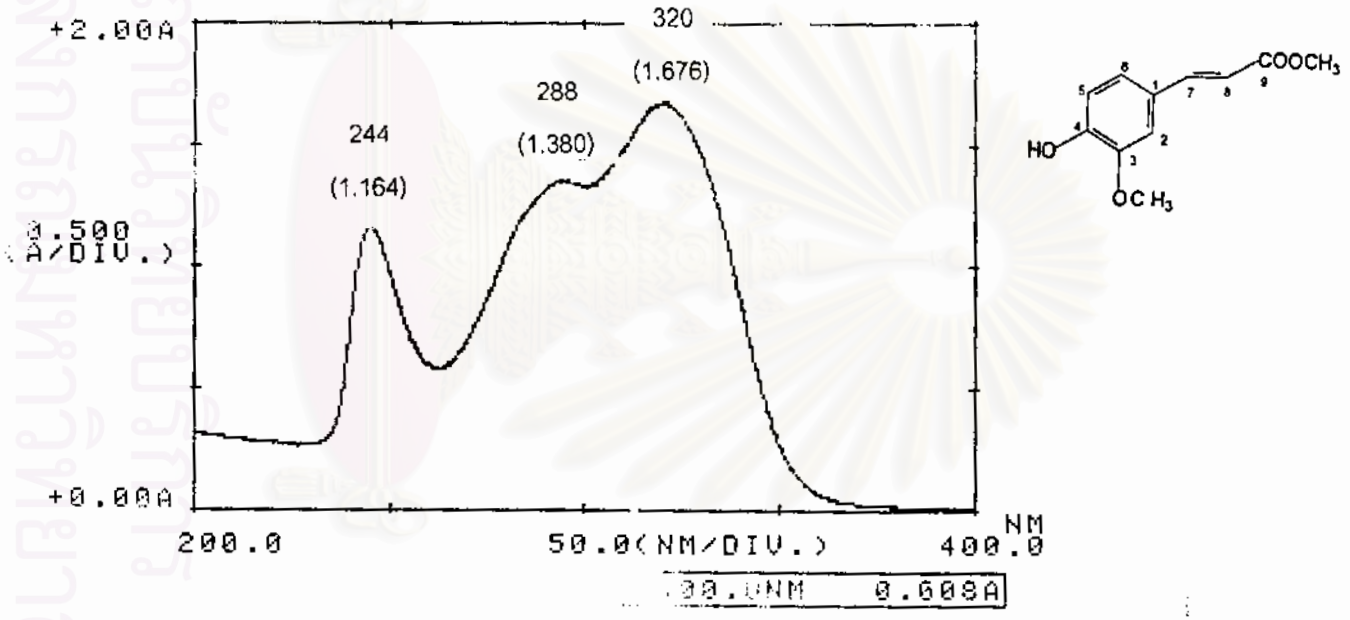


Figure 12 UV spectrum of compound D-2 (in $CDCl_3$)

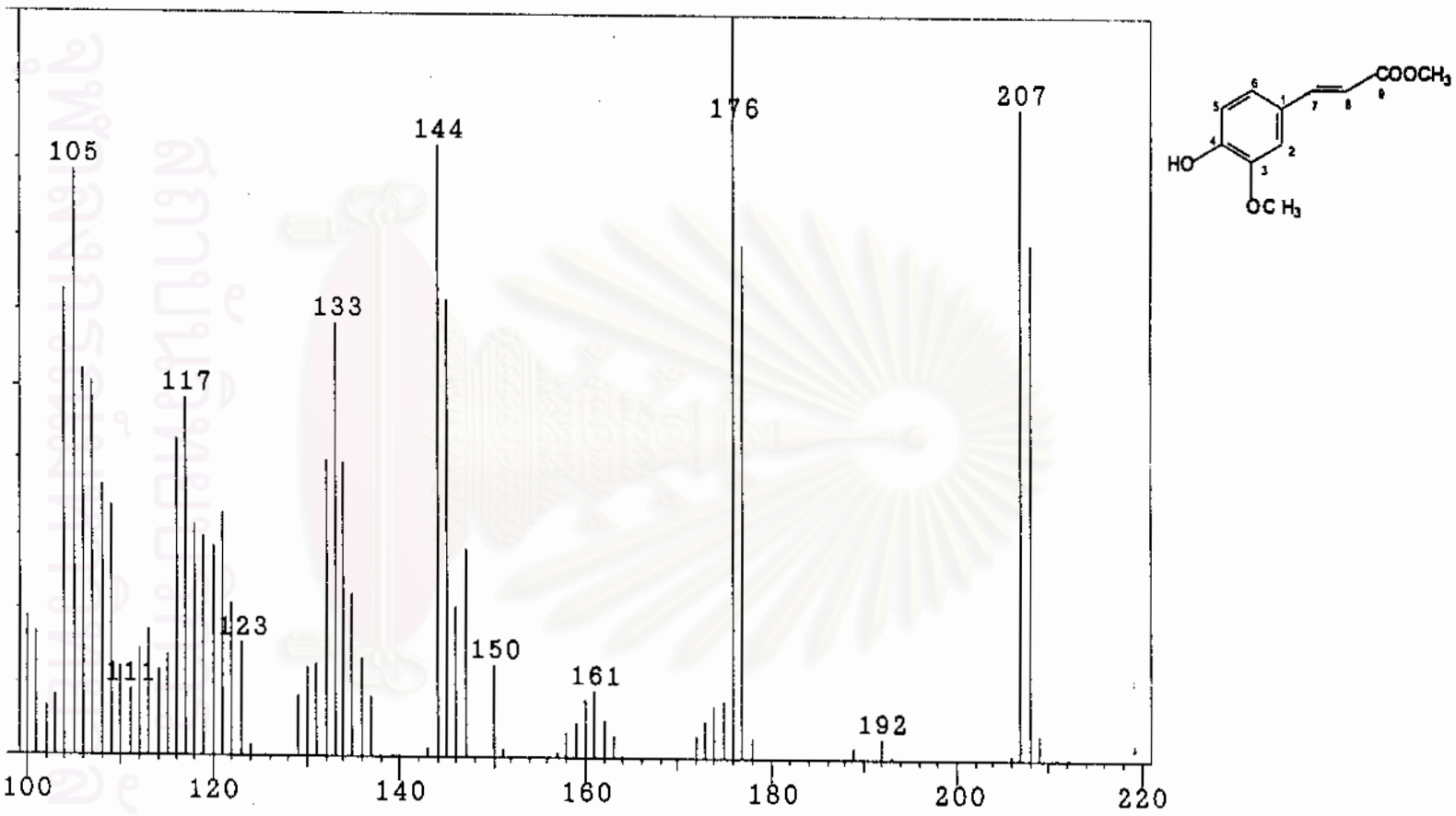


Figure 13 EI mass spectrum of compound D-2

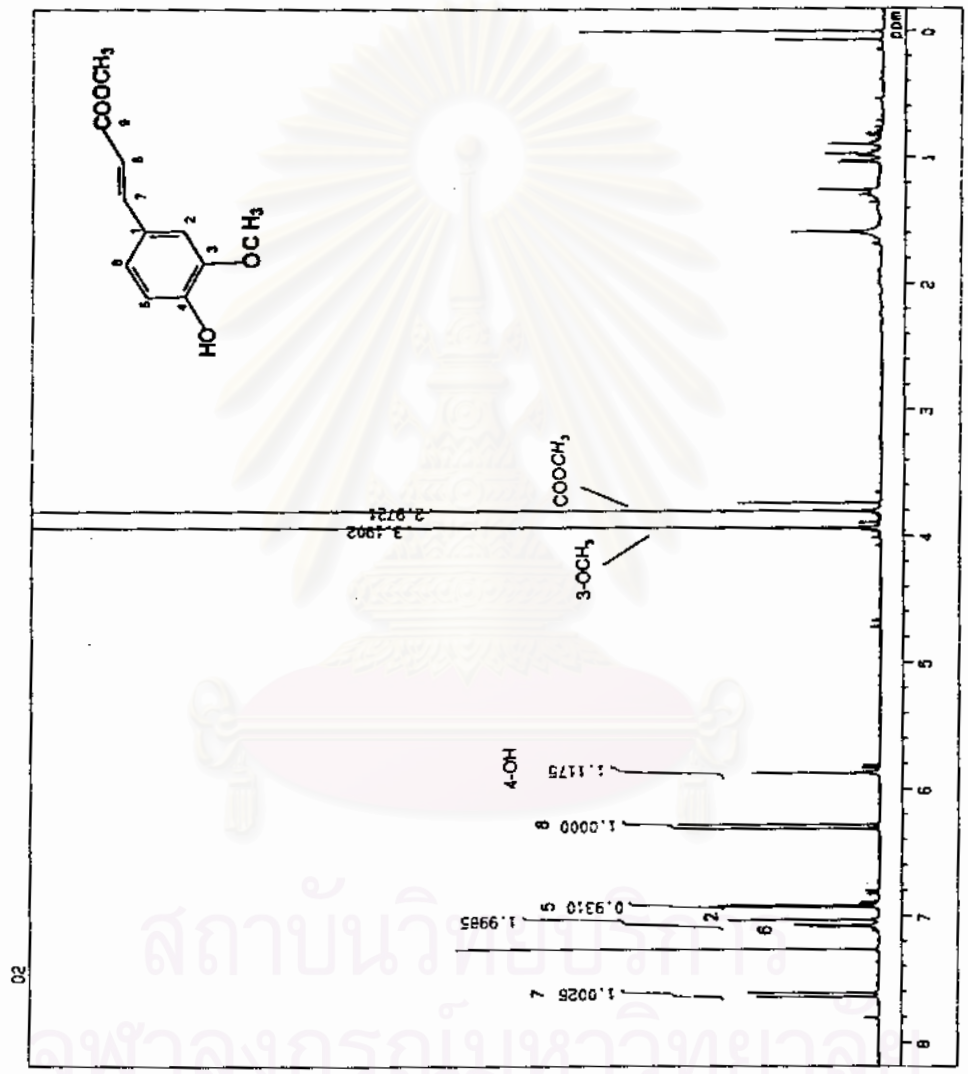


Figure 14a 500 MHz ^{1}H NMR spectrum of compound D-2 (in $CDCl_3$)

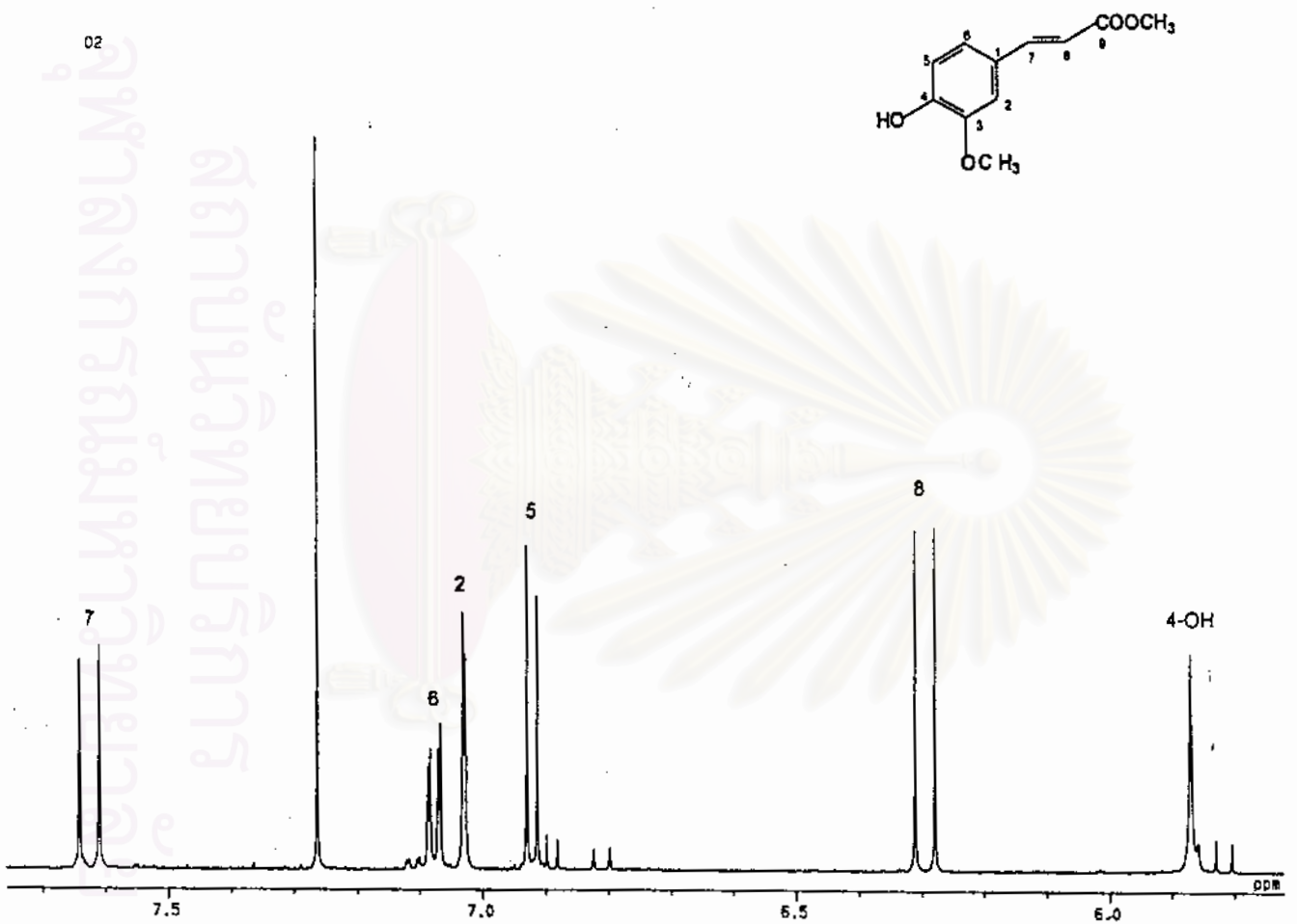


Figure 14b 500 MHz ^{1}H NMR spectrum of compound D-2 (in $CDCl_3$) [δ_H 5.80 -7.70 ppm]

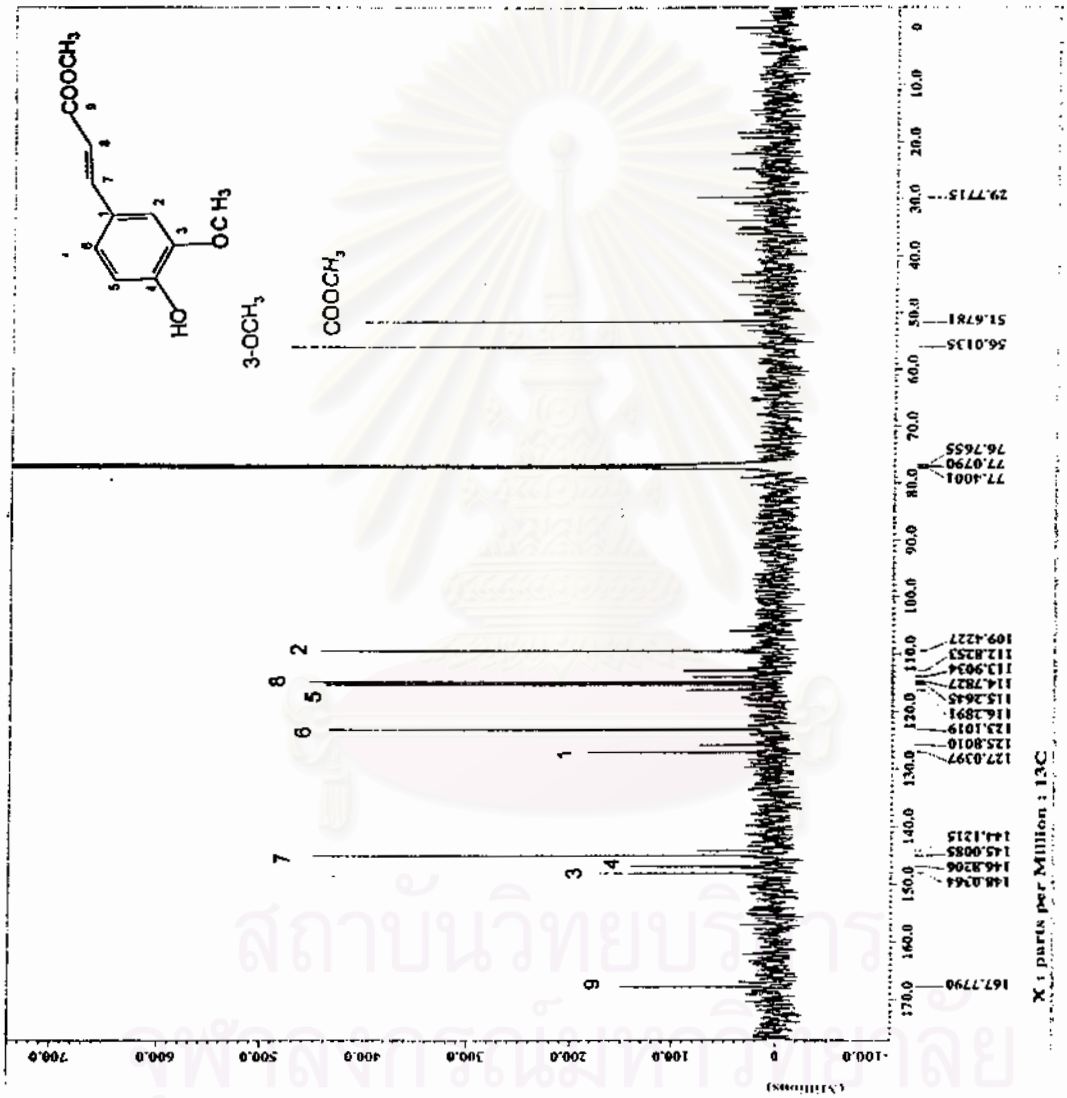


Figure 15a 100 MHz ^{13}C NMR spectrum of compound D-2 (in $CDCl_3$)

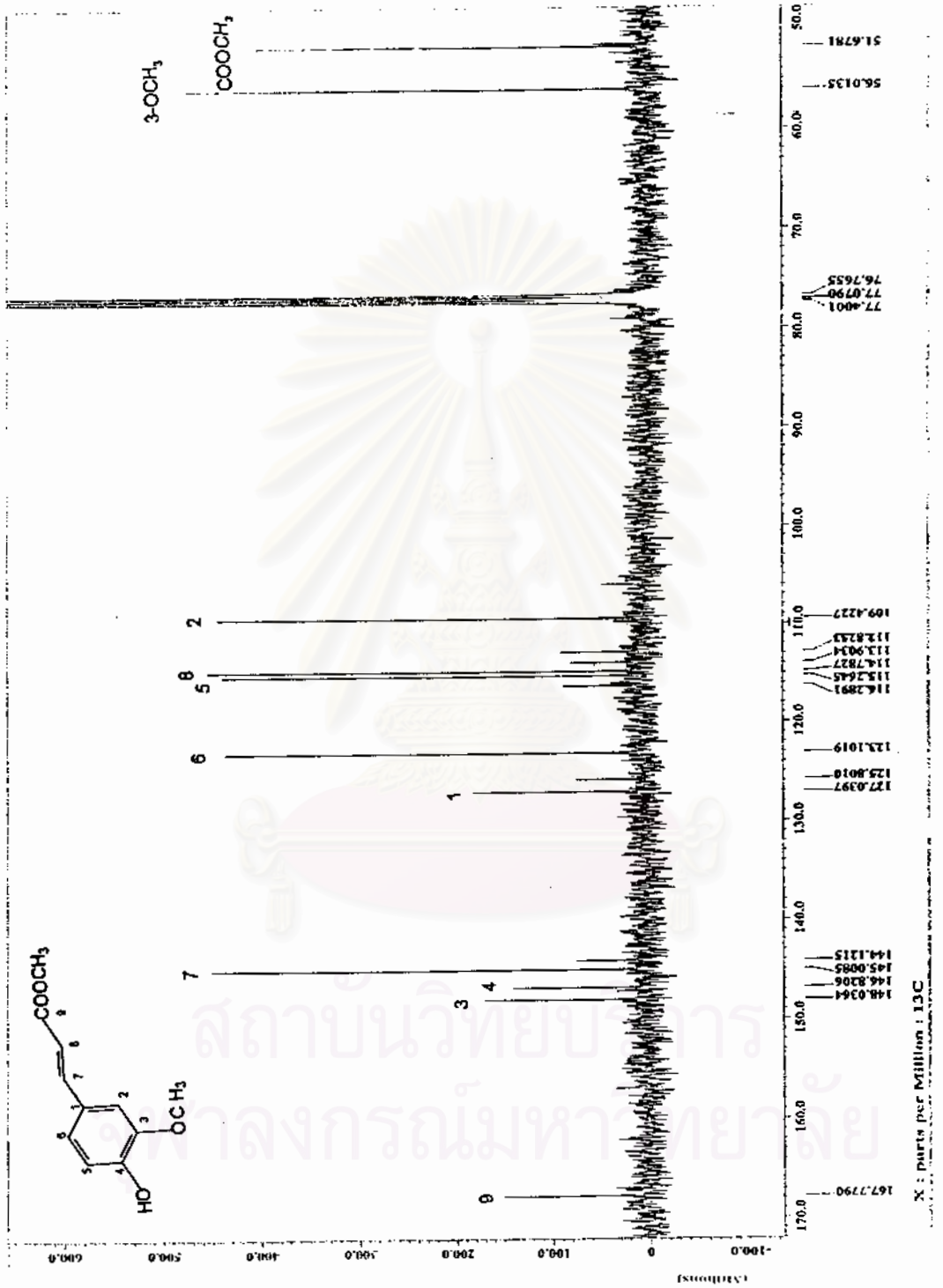


Figure 15b 100 MHz ^{13}C NMR spectrum of compound D-2 (in $CDCl_3$) [δ_c 50.0 – 170.0 ppm]

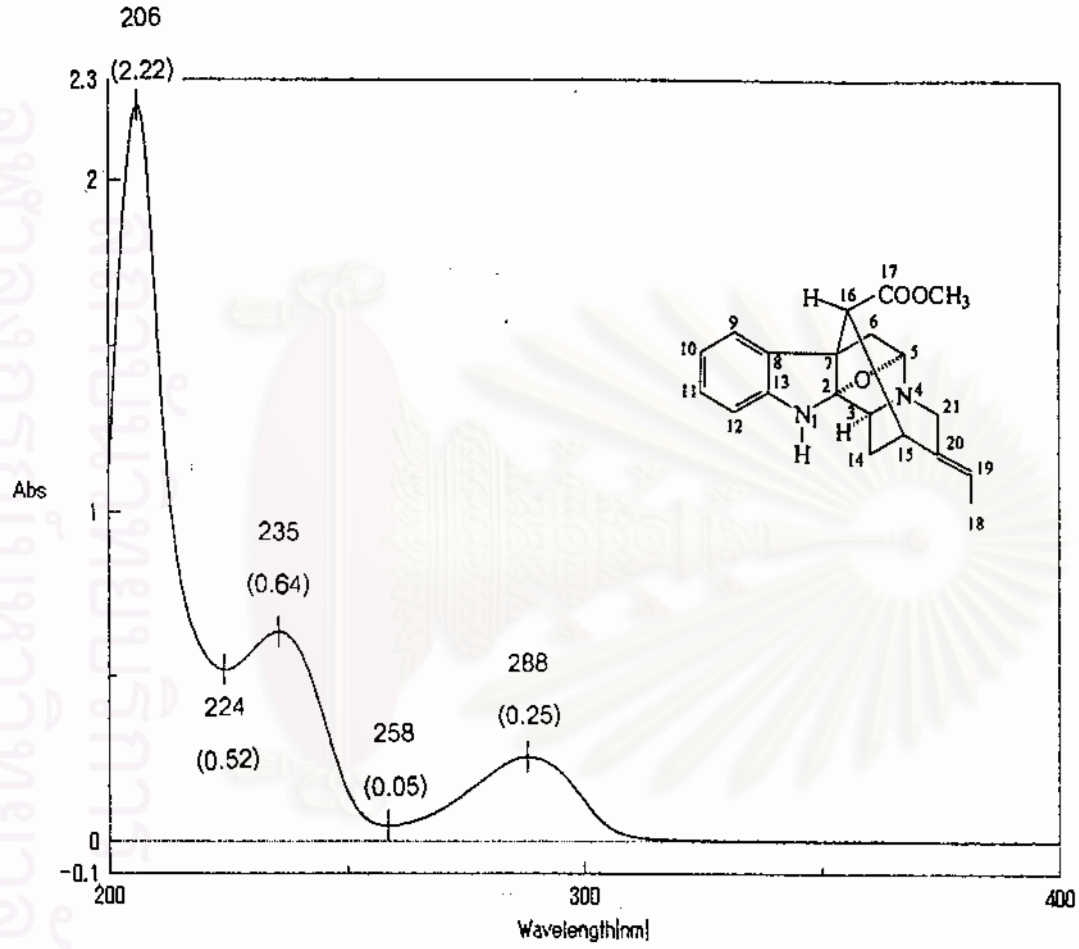


Figure 16 UV spectrum of compound D-3 (in methanol)

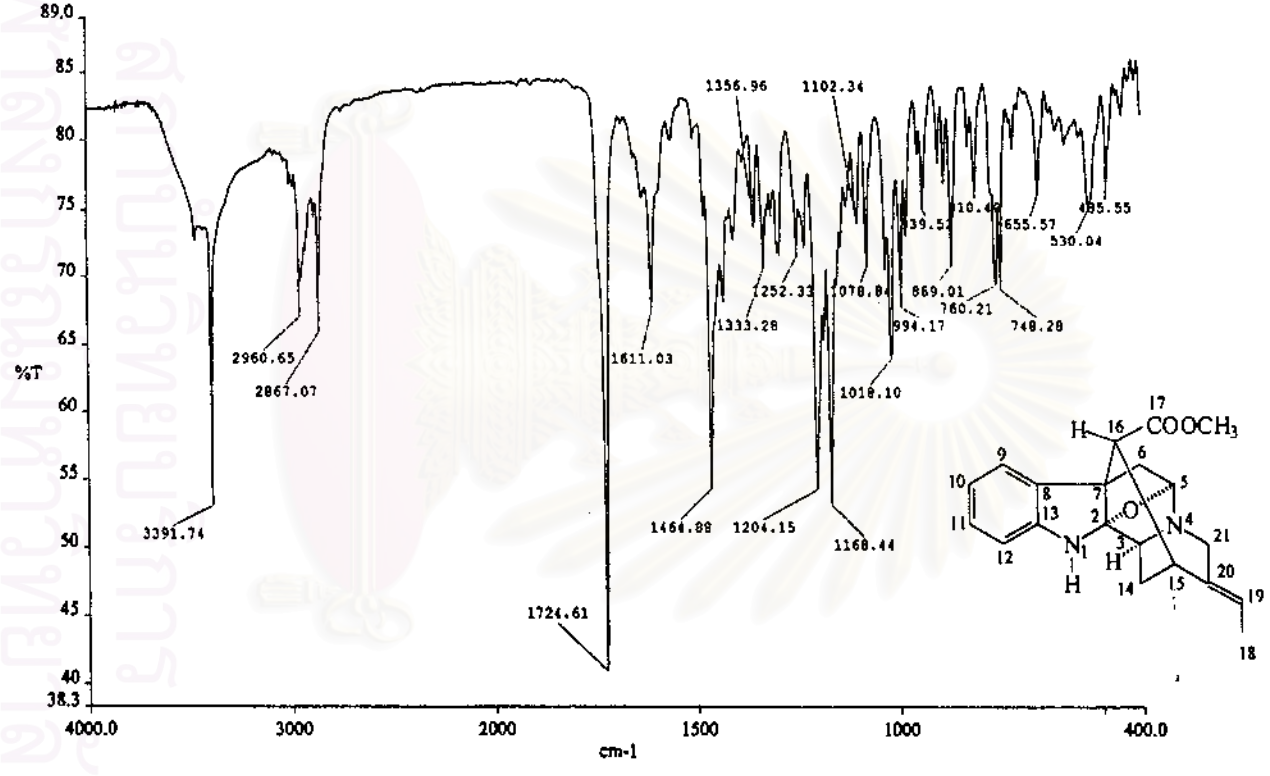


Figure 17 IR spectrum of compound D-3 (KBr disc)

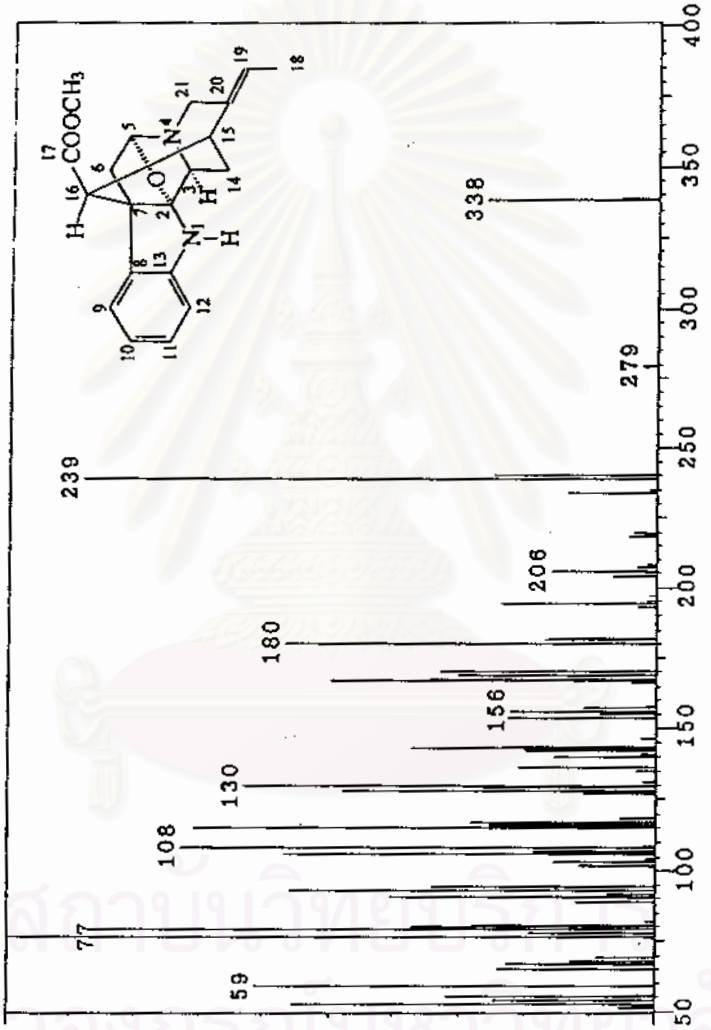


Figure 18 EI mass spectrum of compound D-3

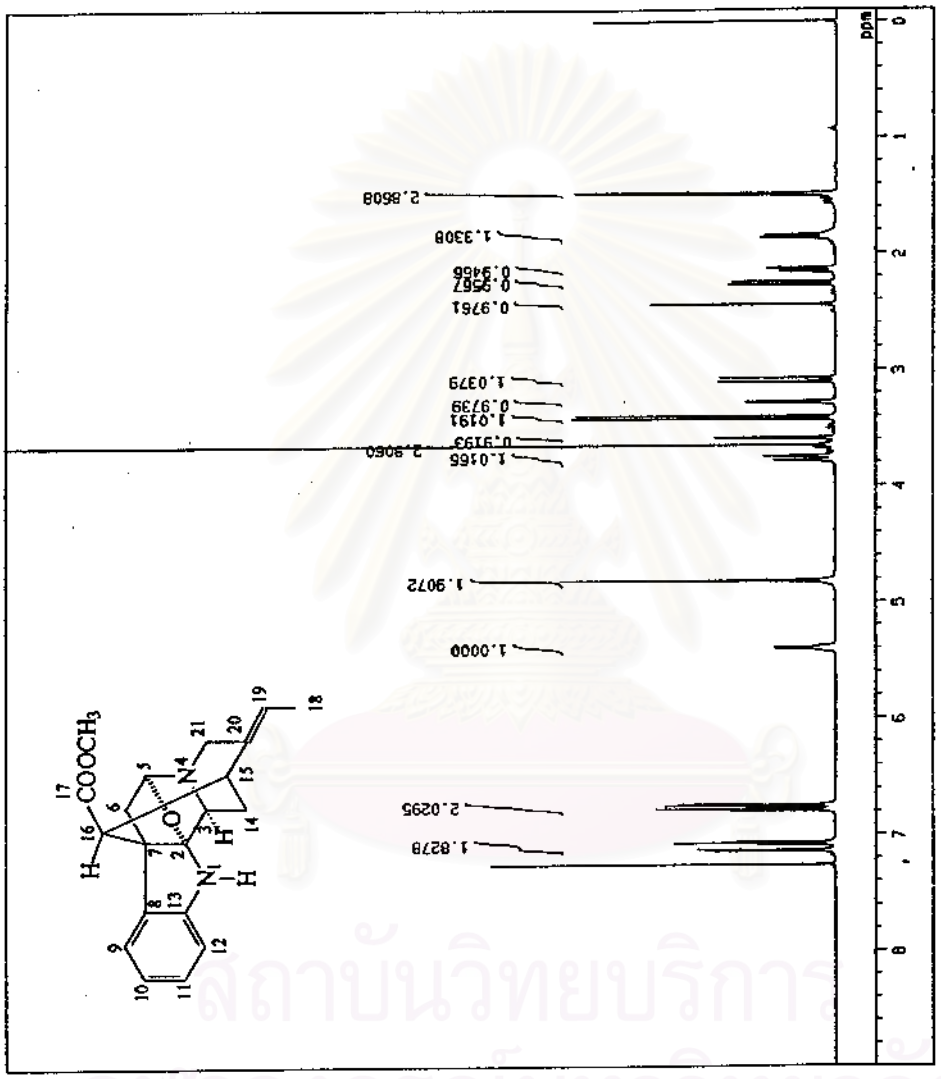


Figure 19a 500 MHz ^{1}H NMR spectrum of compound D-3 (in $CDCl_3$)

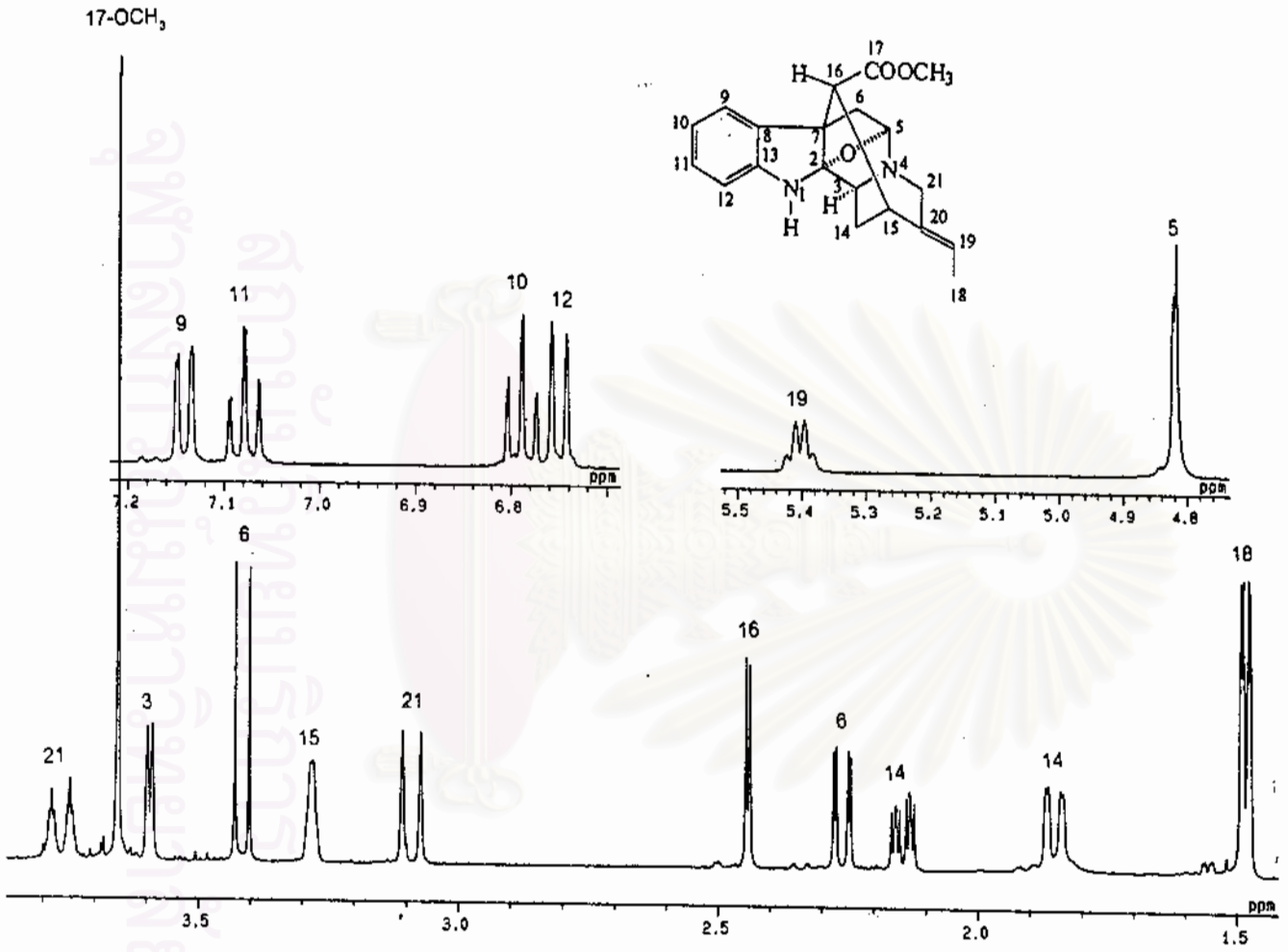


Figure 19b 500 MHz ^{1}H NMR spectrum of compound D-3 (in $CDCl_3$) [δ_H 1.40 – 3.80, 4.80 – 5.50, 6.70 – 7.20 ppm]

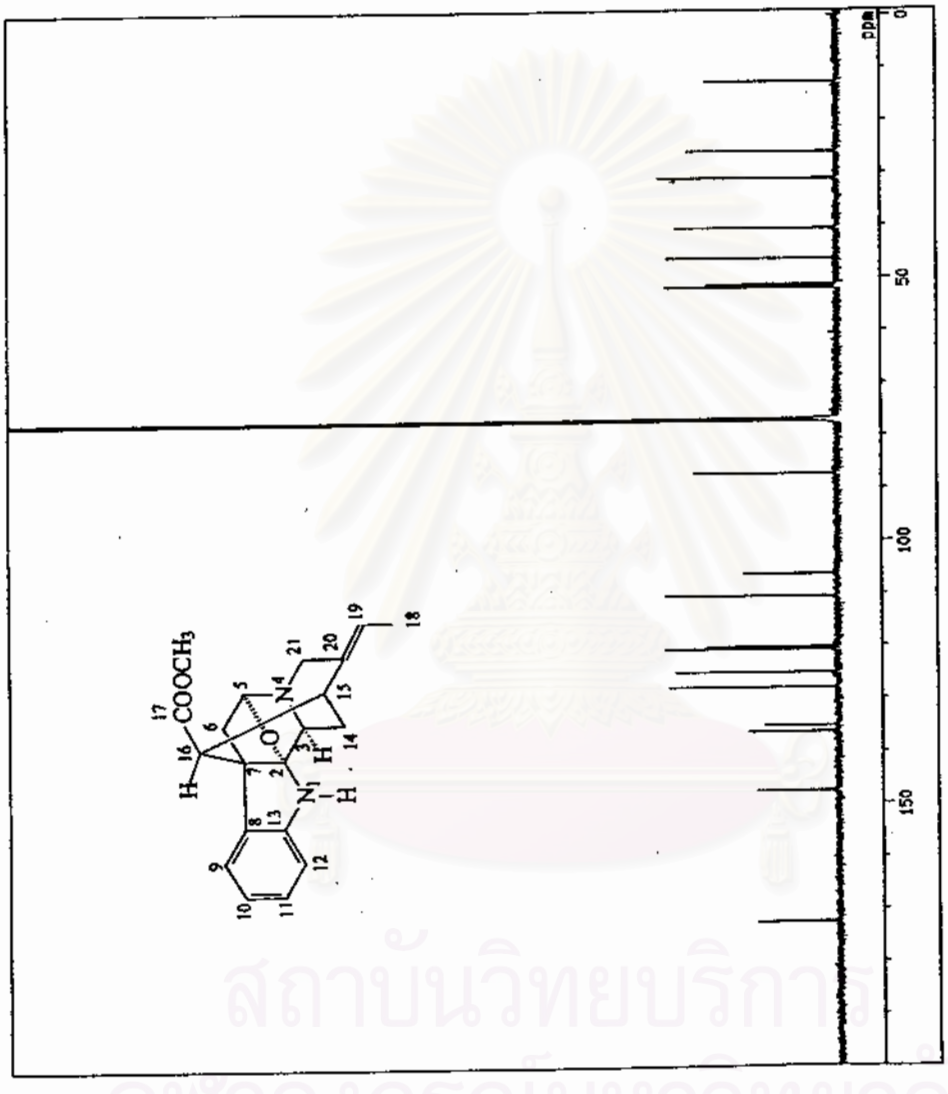


Figure 20a 125 MHz ^{13}C NMR spectrum of compound D-3 (in $CDCl_3$)

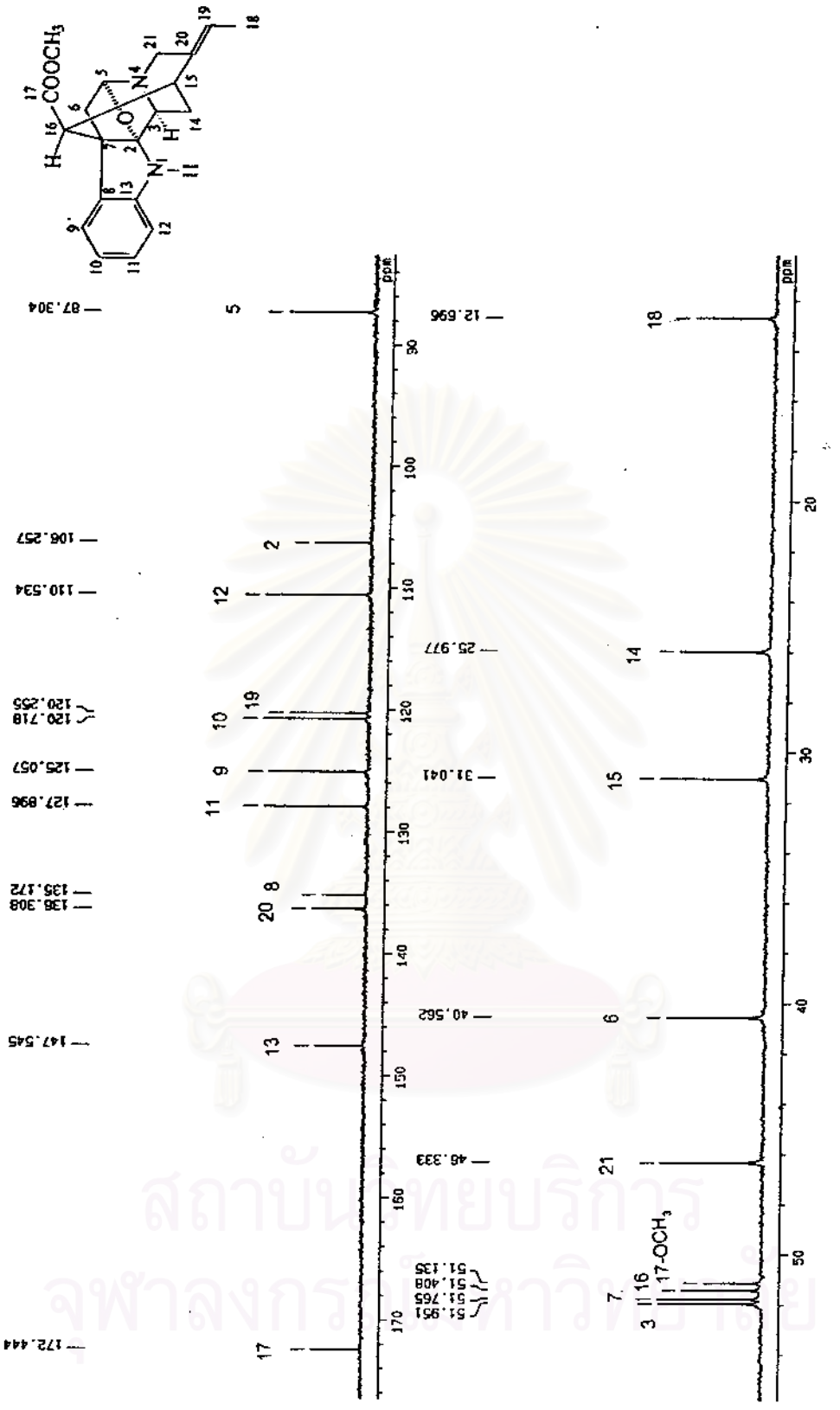


Figure 20b 125 MHz ^{13}C NMR spectrum of compound D-3 (in $CDCl_3$) [δ_C 10.0 – 52.0, 84.0 –176.0 ppm]

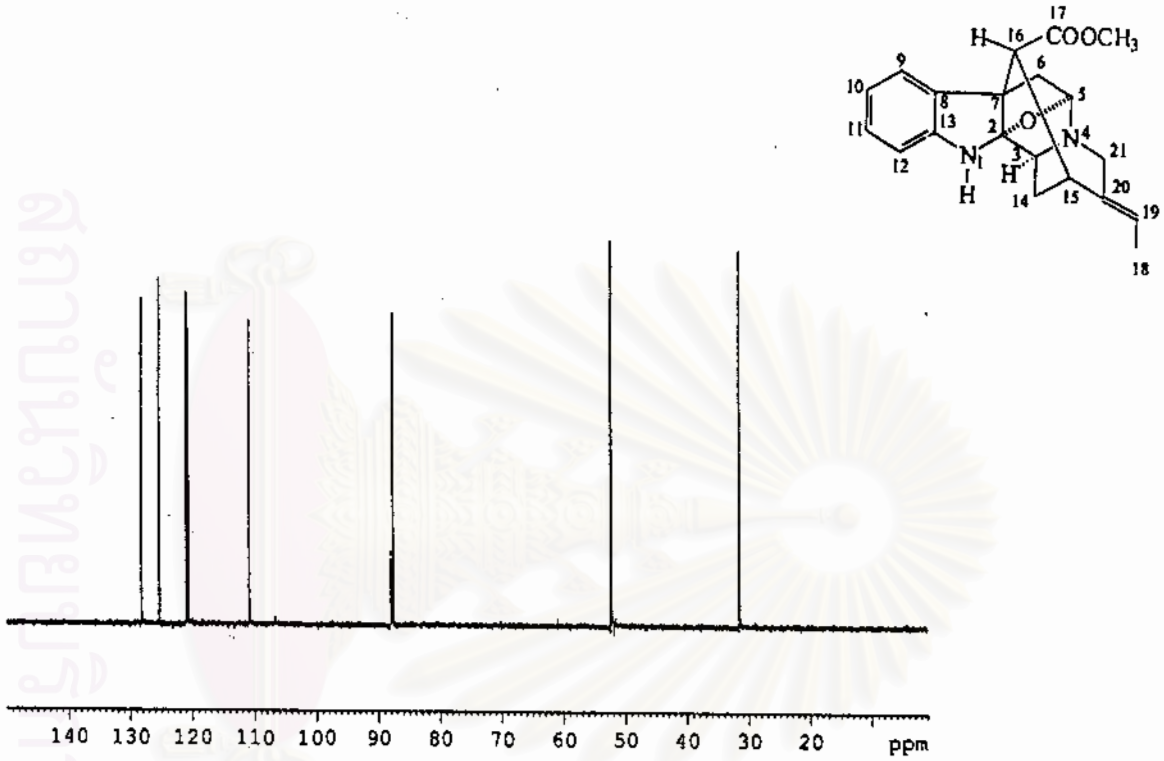


Figure 21a DEPT 90 spectrum of compound D-3 (in $CDCl_3$)

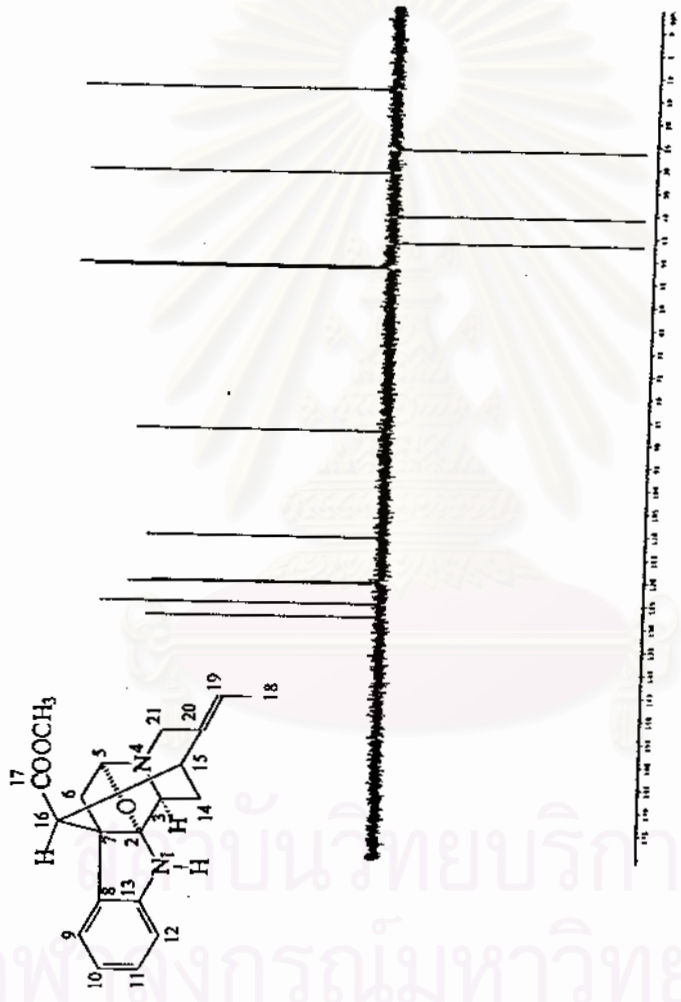


Figure 21b DEPT 135 spectrum of compound D-3 (in $CDCl_3$)

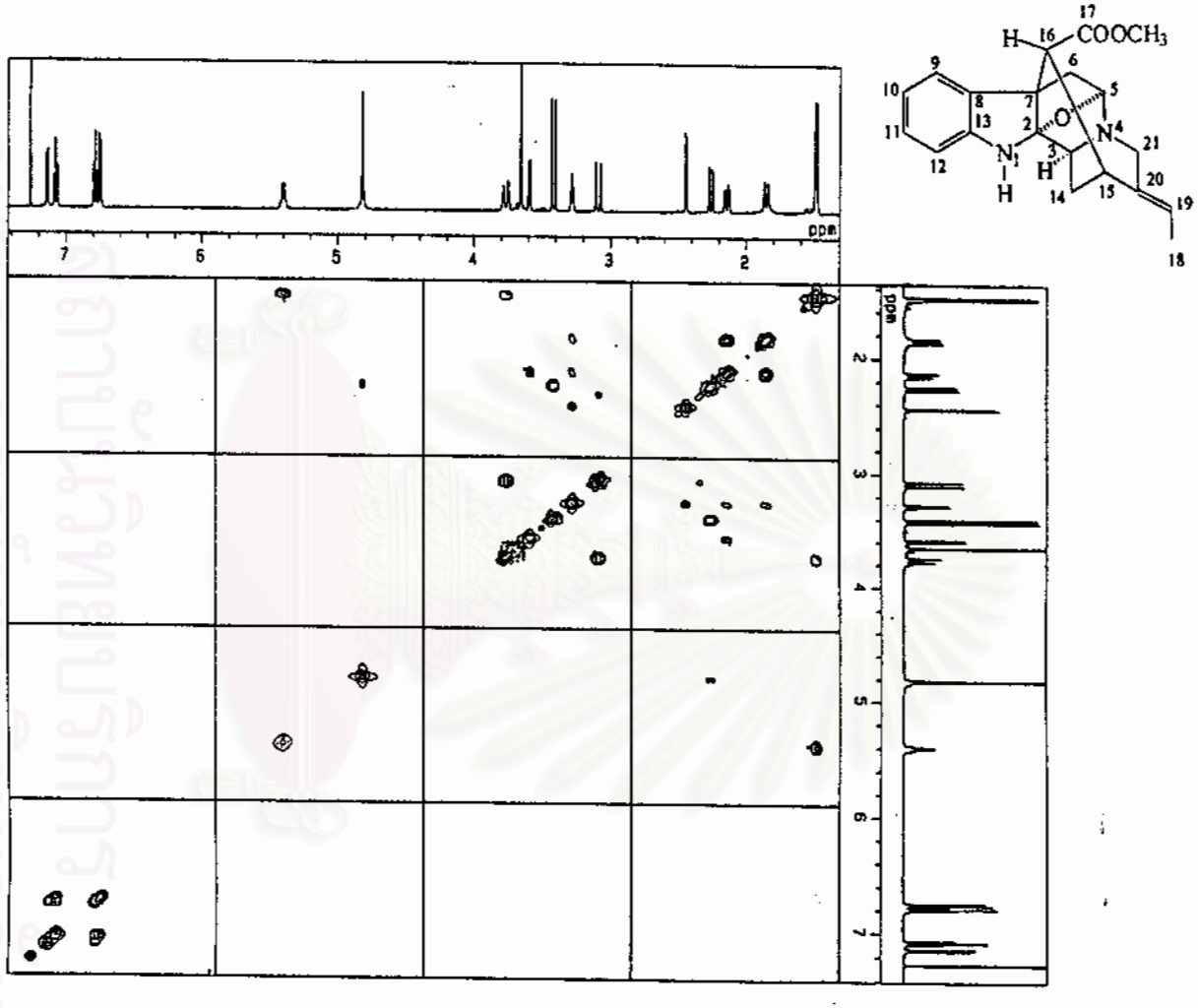


Figure 22a ^{1}H-^{1}H COSY spectrum of compound D-3 (in $CDCl_3$)

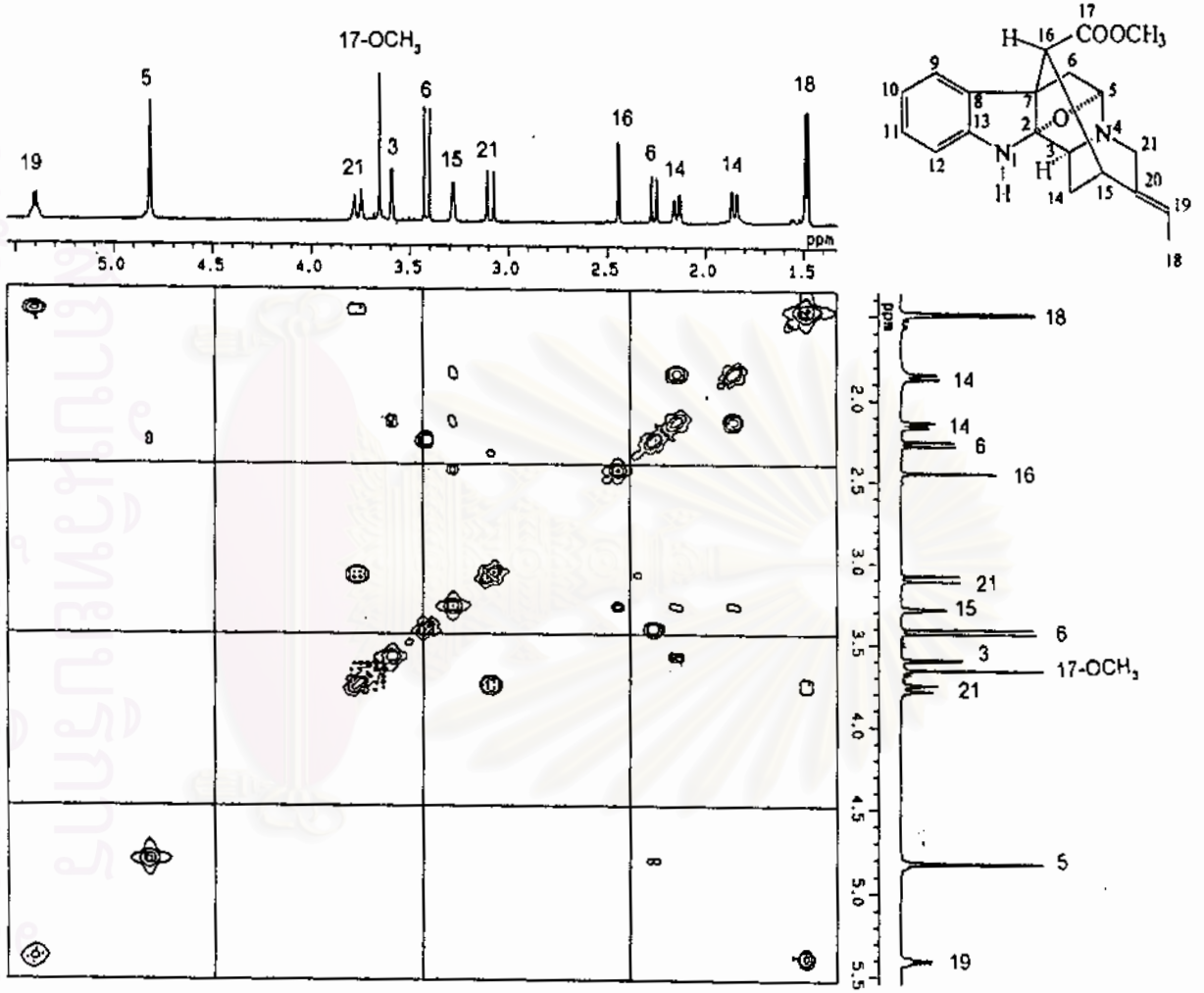


Figure 22b ^{1}H-^{1}H COSY spectrum of compound D-3 (in $CDCl_3$) [δ_H 1.40 – 5.50 ppm, δ_H 1.40 – 5.50 ppm]

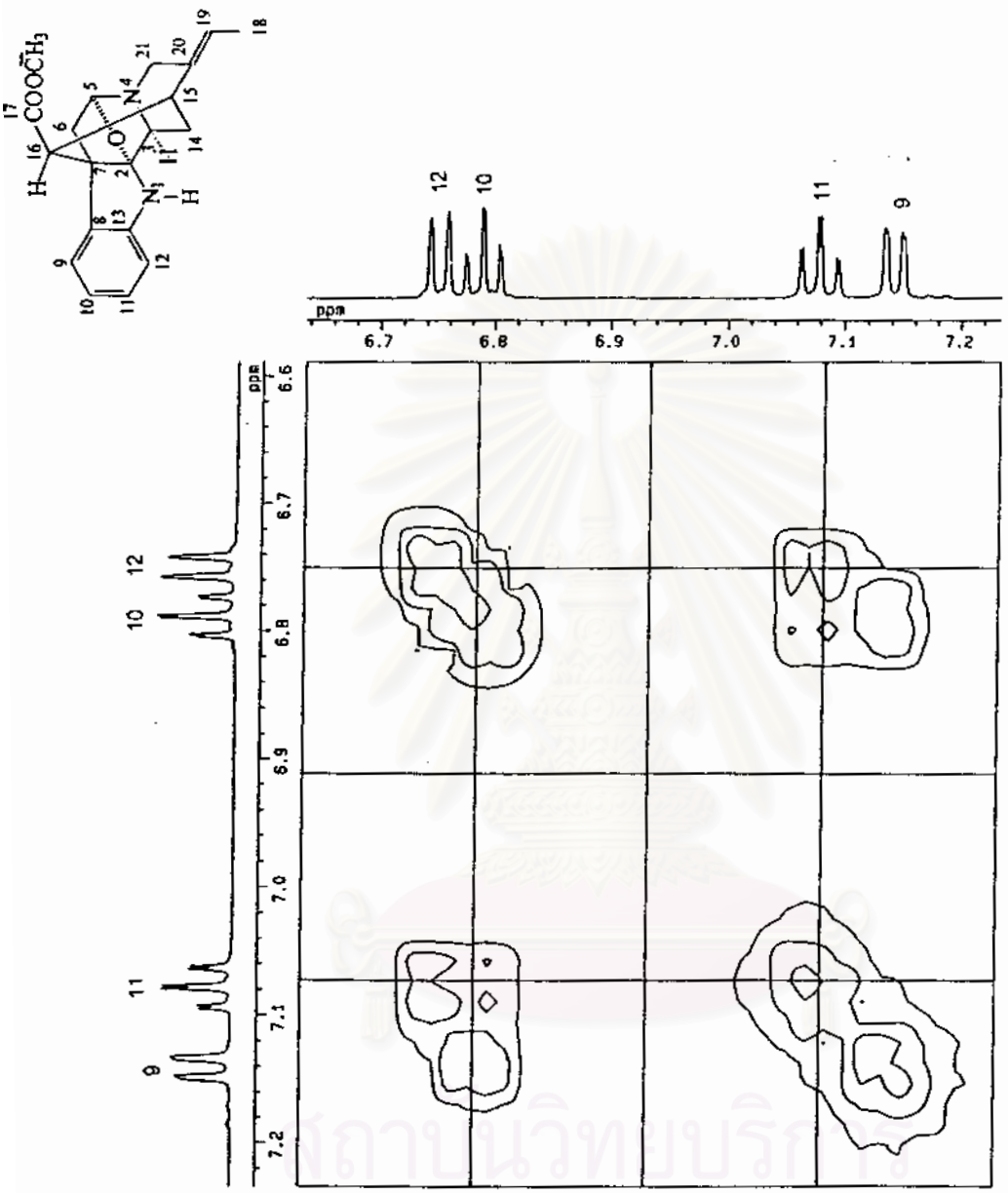


Figure 22c ^{1}H-^{1}H COSY spectrum of compound D-3 (in $CDCl_3$) [δ_H 6.60 – 7.20 ppm, δ_H 6.60 – 7.20 ppm]

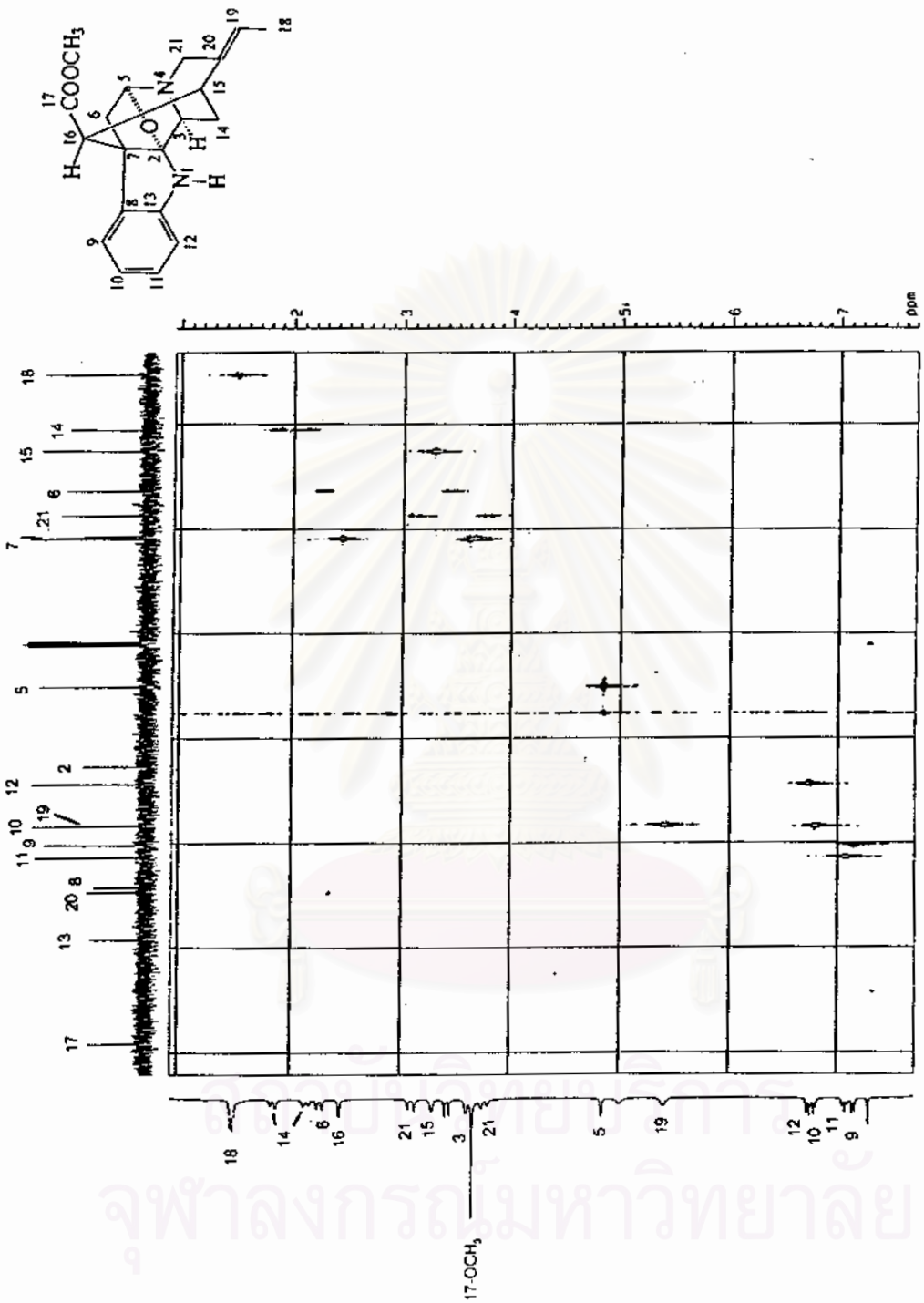
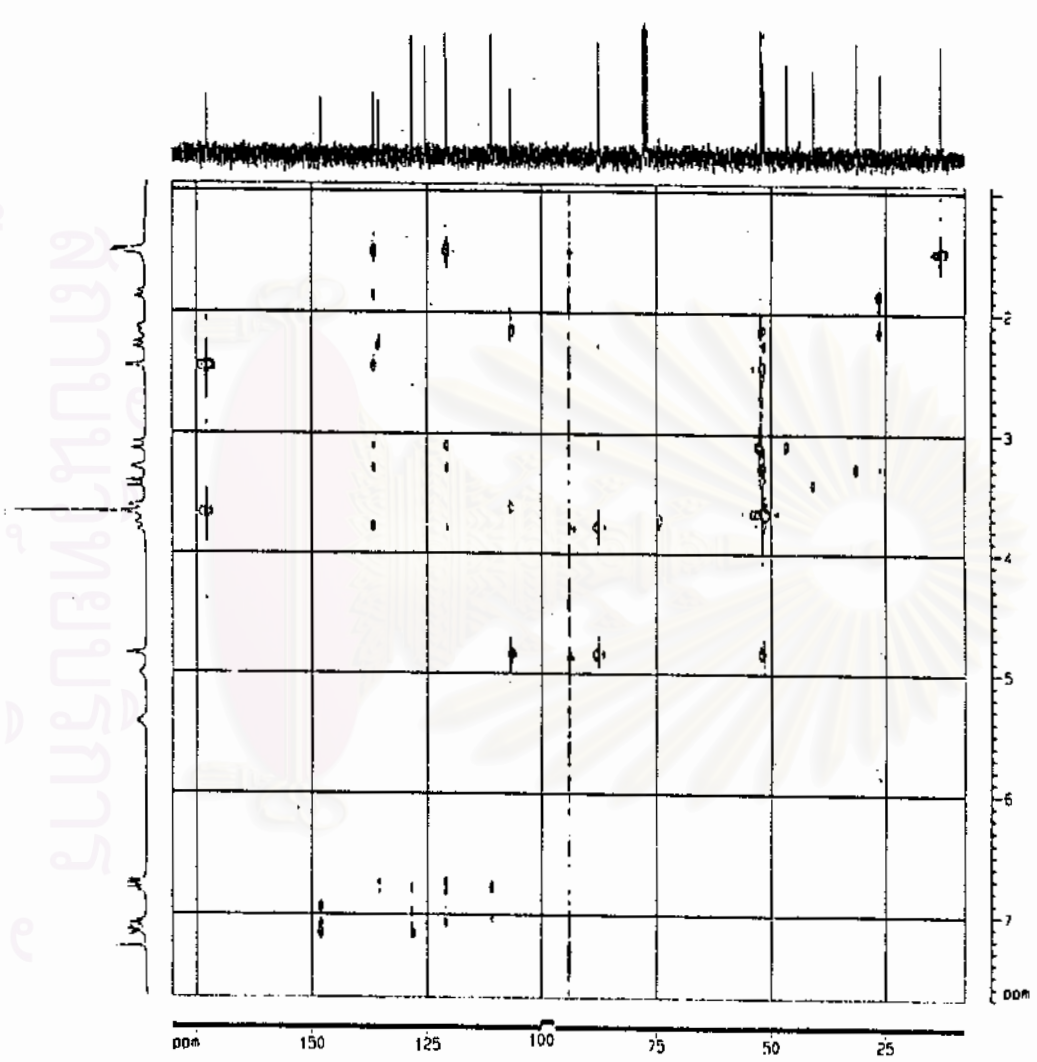


Figure 23 HETCOR spectrum of compound D-3 (in $CDCl_3$)



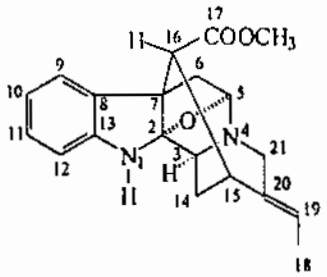


Figure 24 COLOC spectrum of compound D-3 (in $CDCl_3$)

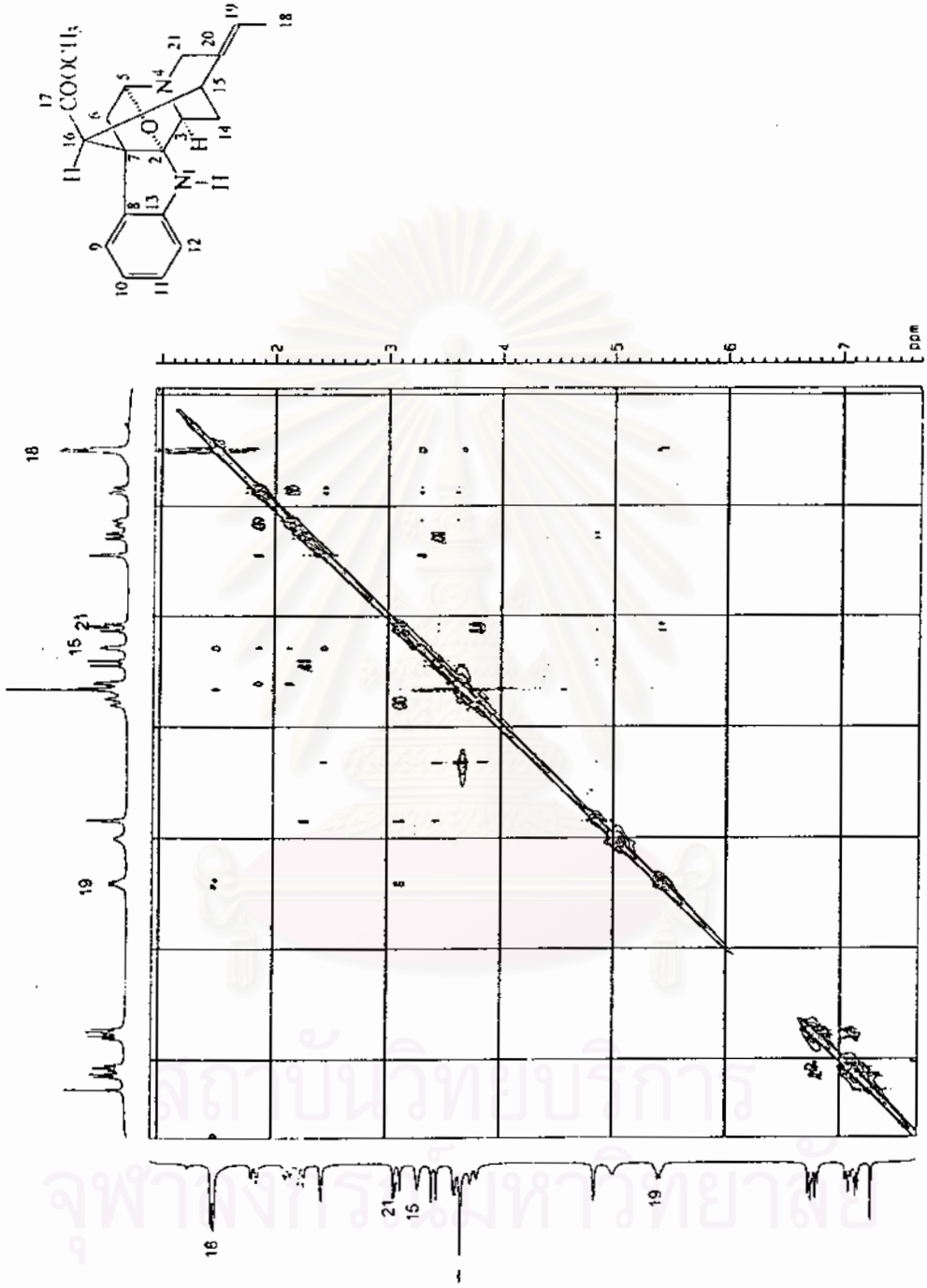


Figure 25a NOESY spectrum of compound D-3 (in $CDCl_3$)

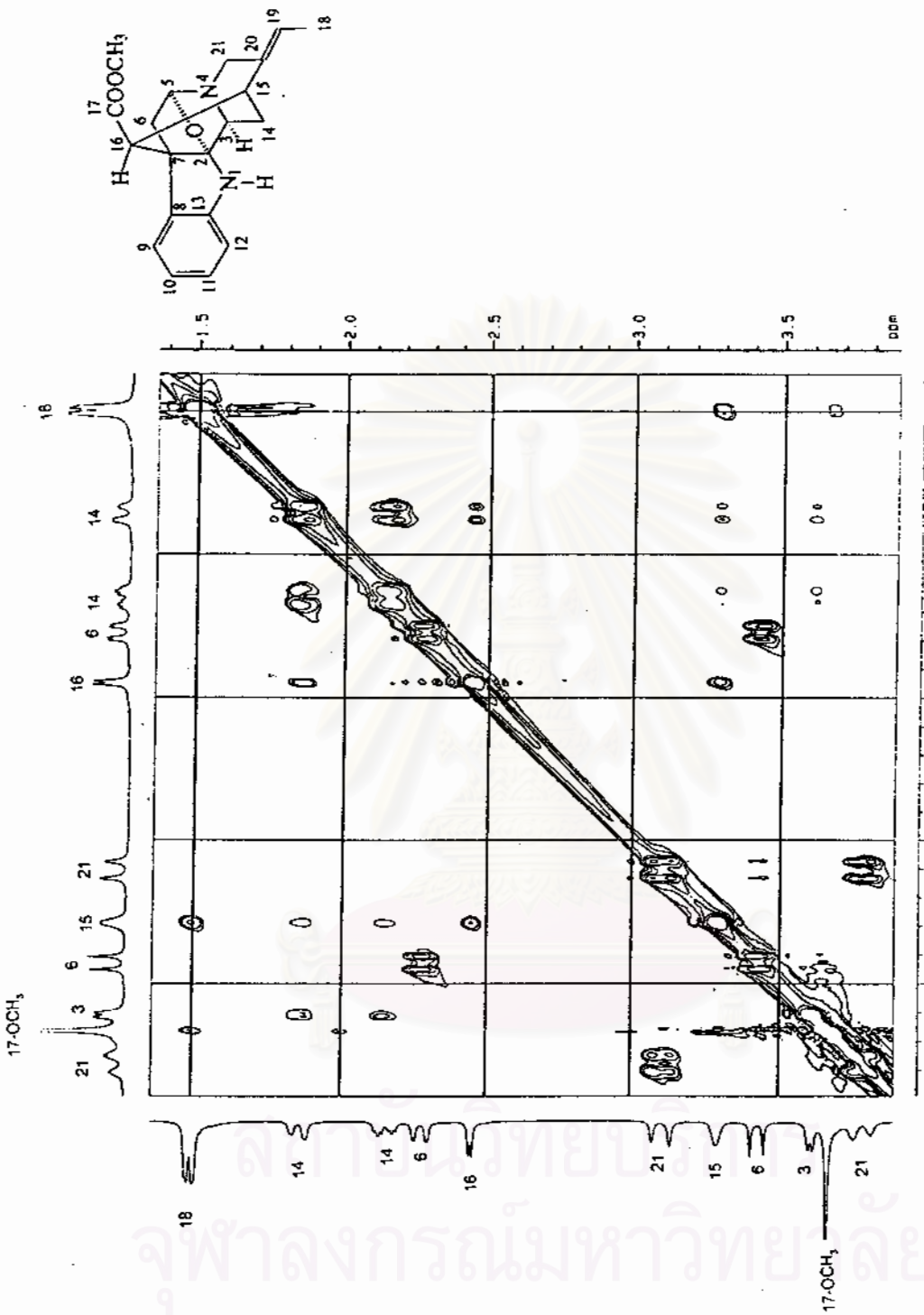


Figure 25b NOESY spectrum of compound D-3 (in $CDCl_3$) [δ_H 1.40 – 3.90 ppm, δ_H 1.40 – 3.90 ppm]

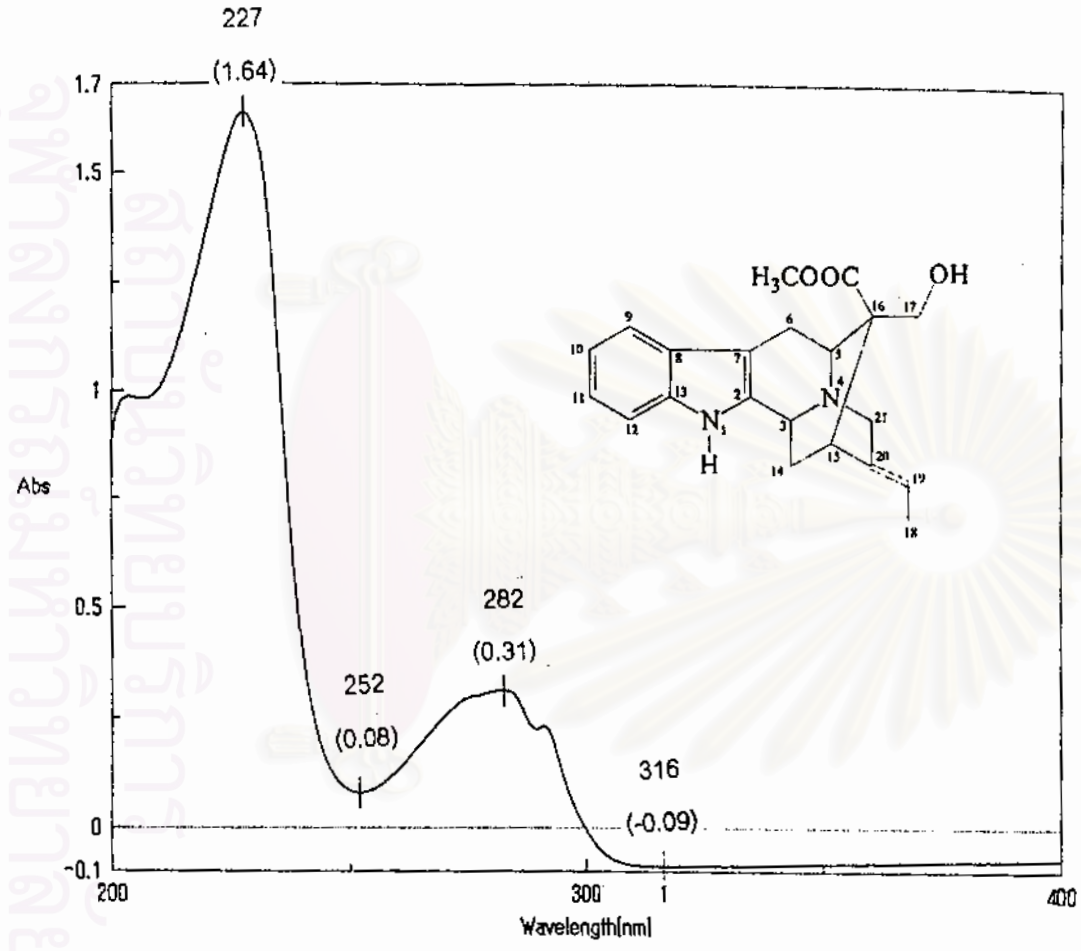


Figure 26 UV spectrum of compound D-4 (in ethanol)

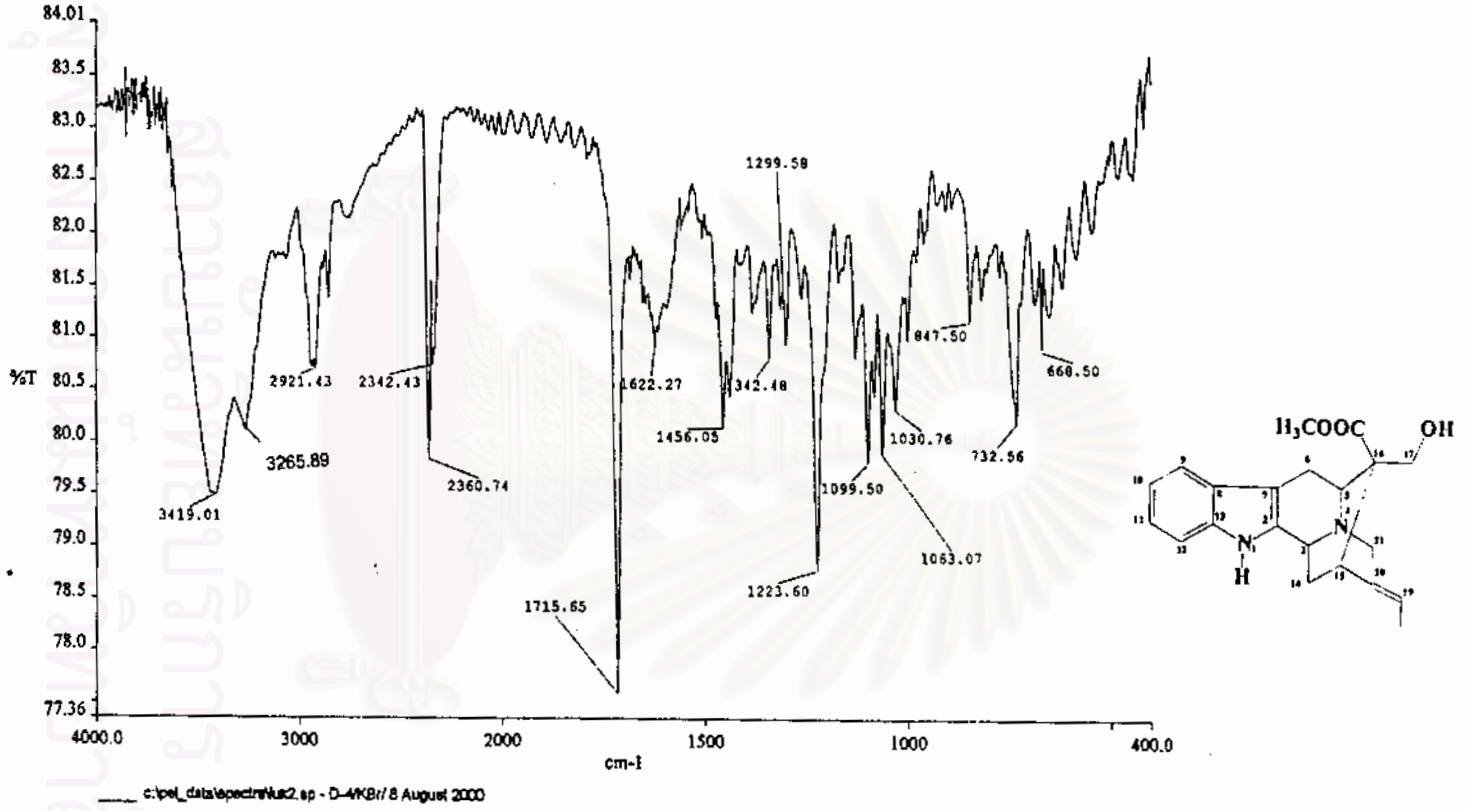


Figure 27 IR spectrum of compound D-4 (KBr disc)

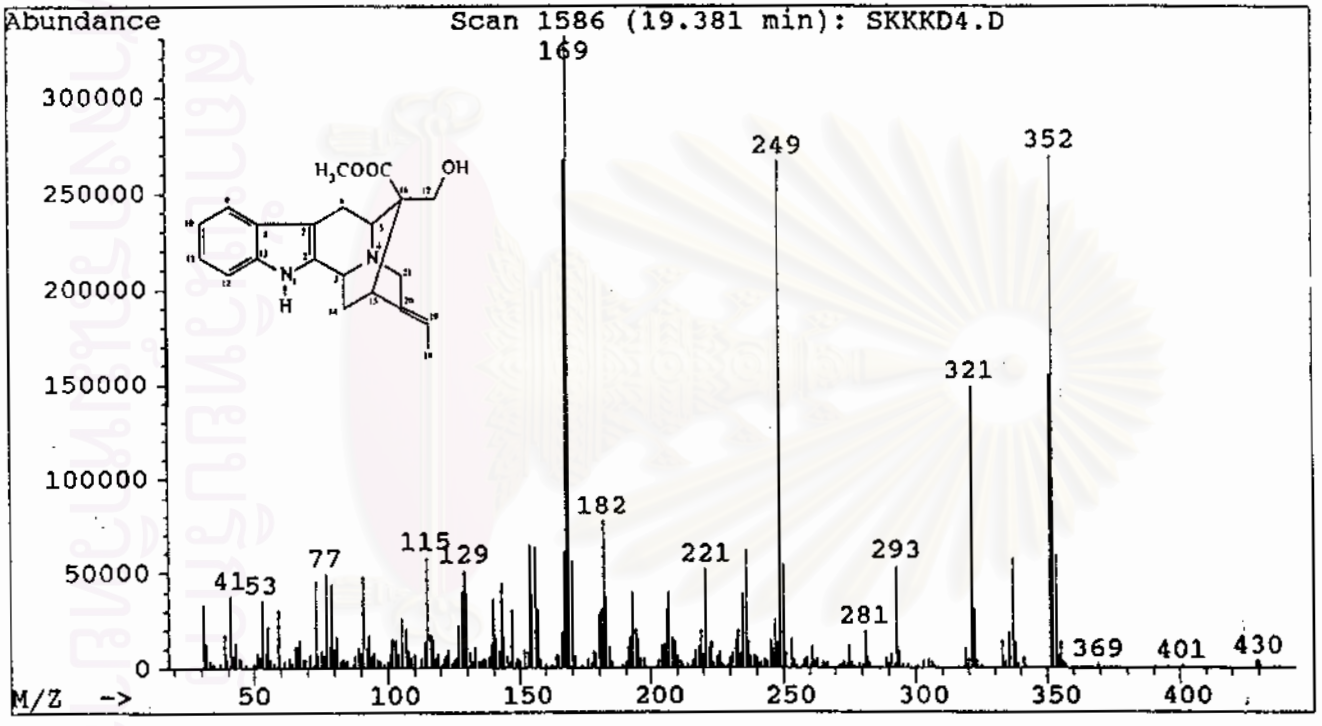


Figure 28 EI mass spectrum of compound D-4

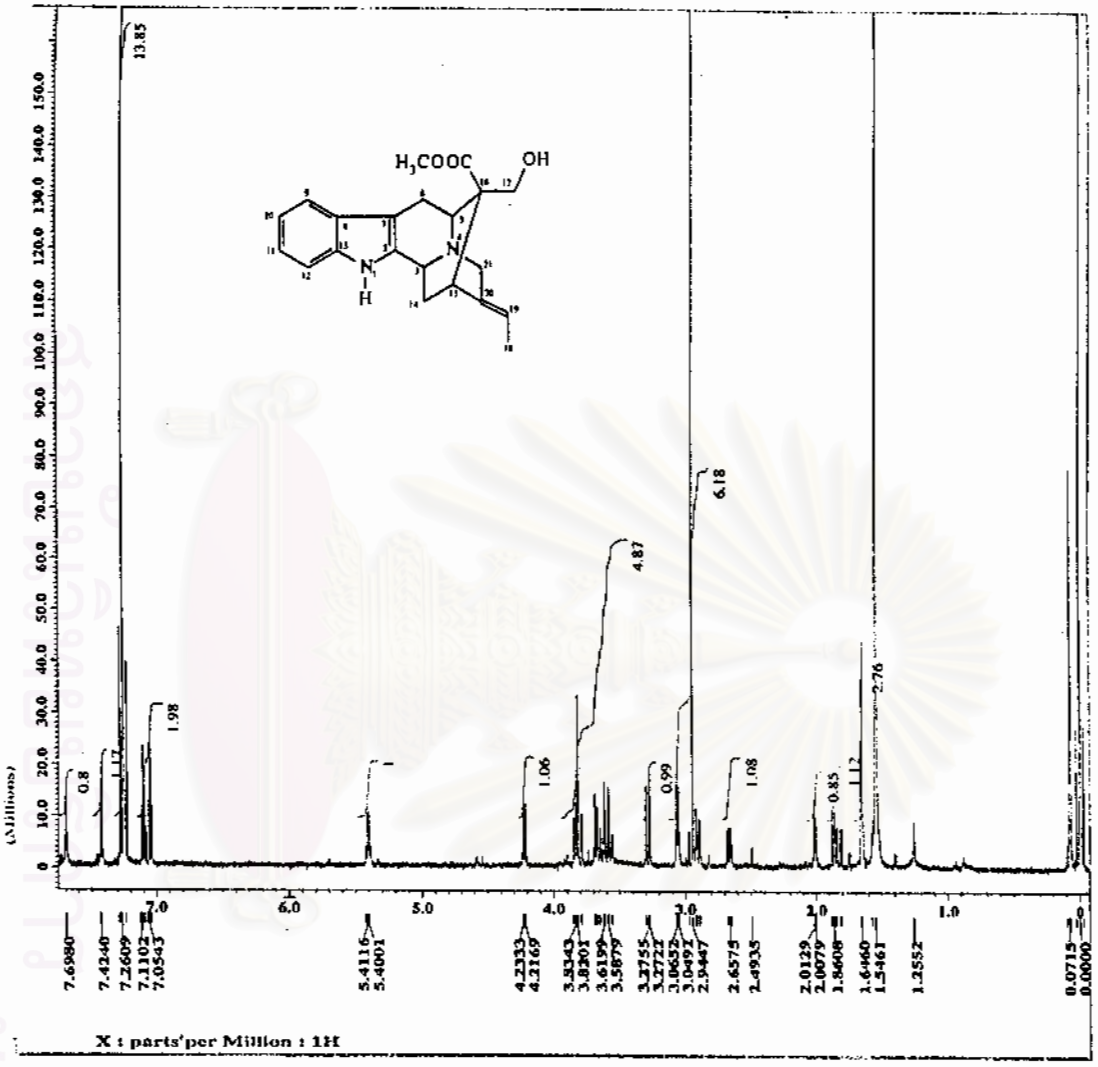


Figure 29a 600 MHz ^{1}H NMR spectrum of compound D-4 (in $CDCl_3$-CD_3OD)

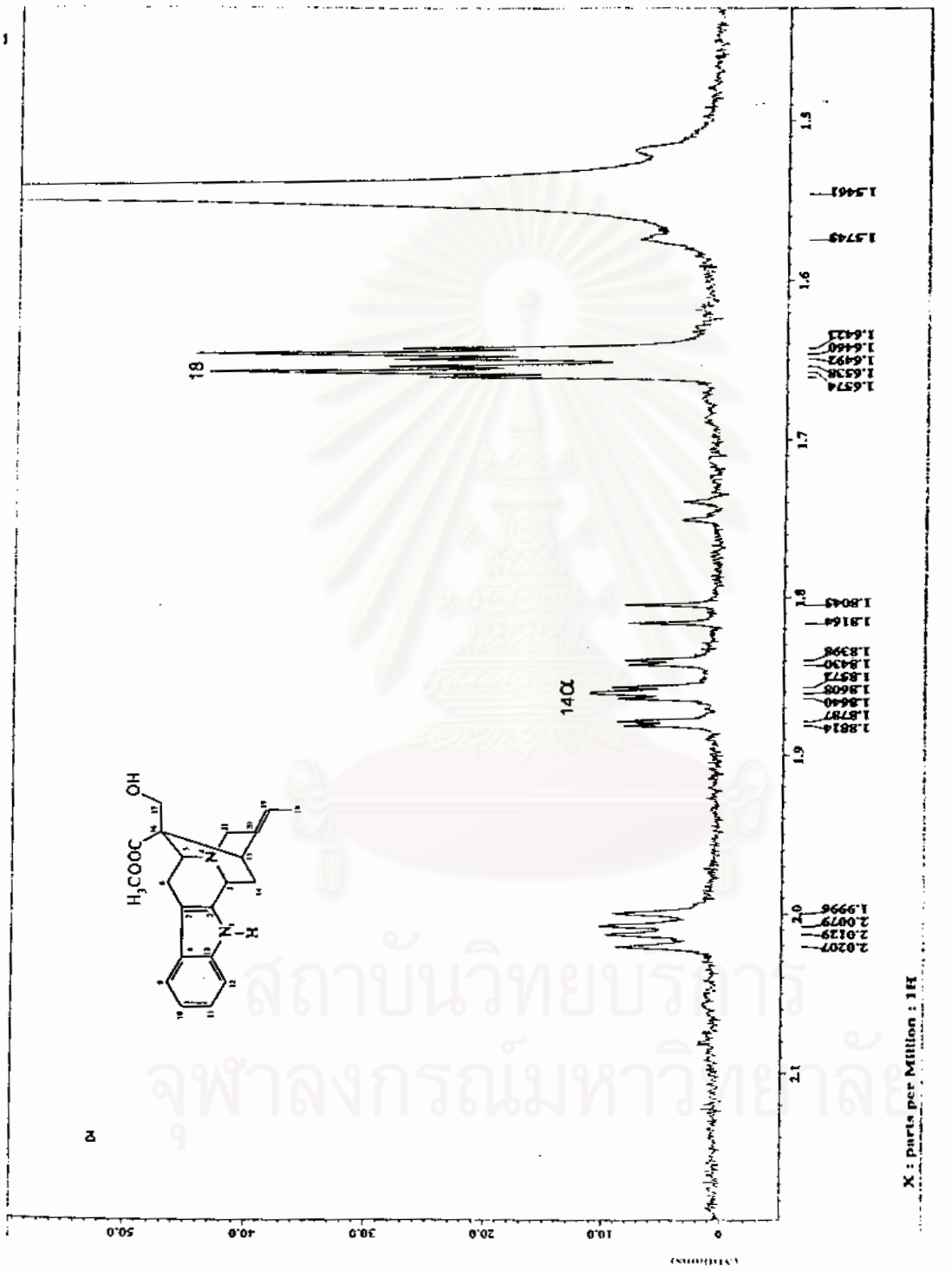


Figure 29b 600 MHz ^{1}H NMR spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_H 1.50 – 2.10 ppm]

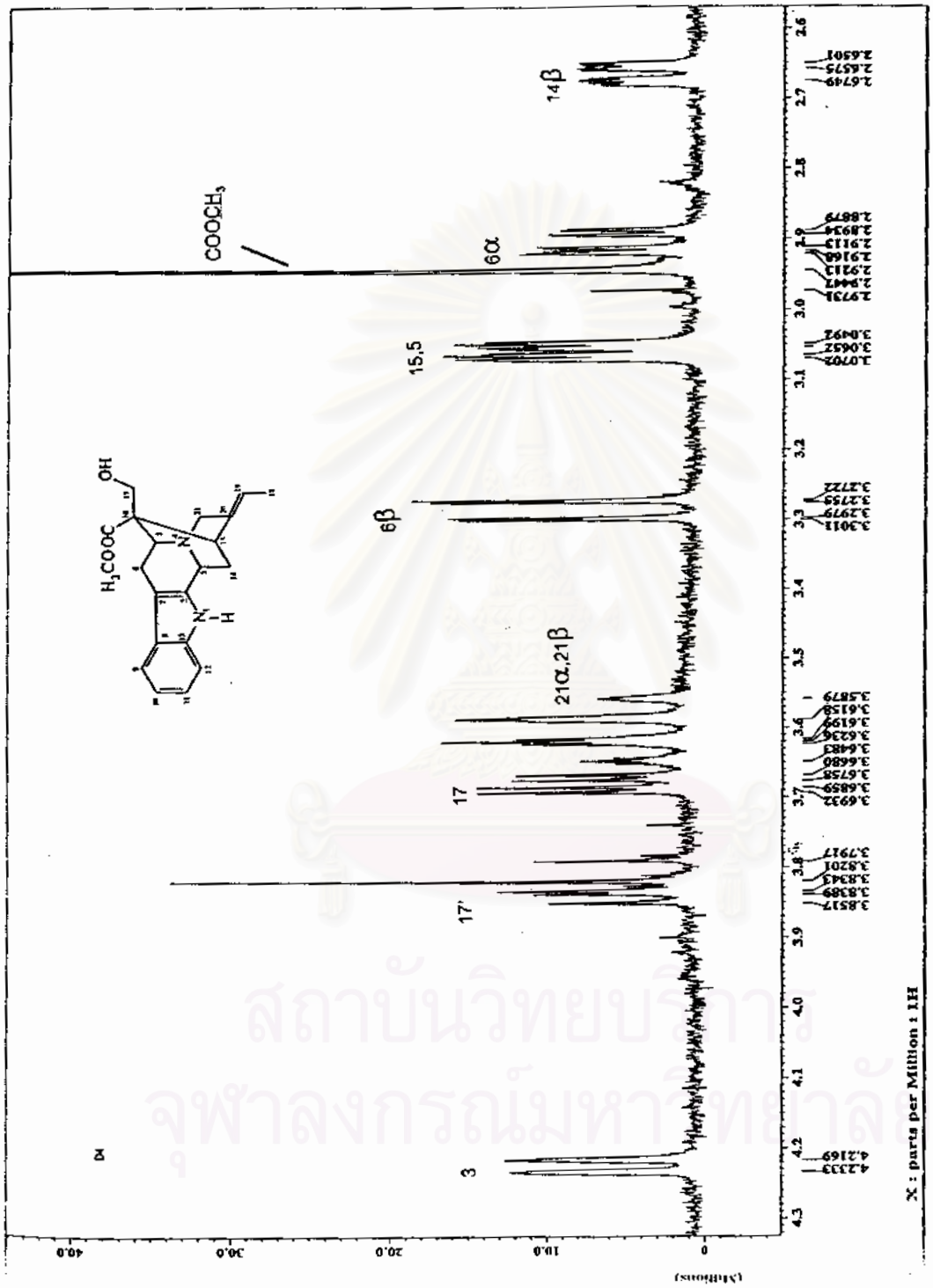


Figure 29c 600 MHz ^{1}H NMR spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_H 2.60 – 4.30 ppm]

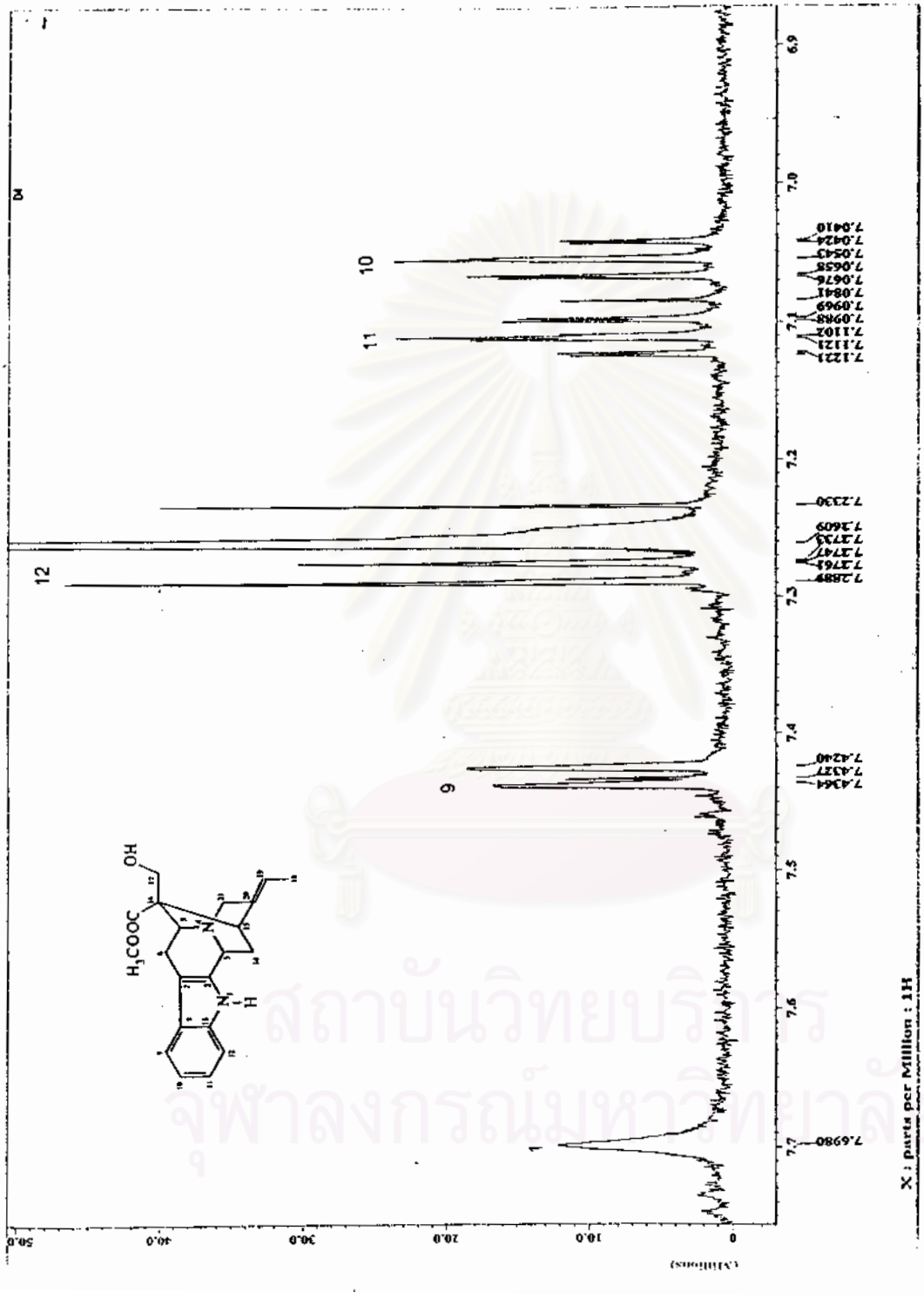


Figure 29d 600 MHz ^{1}H NMR spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_H 7.00 – 7.75 ppm]

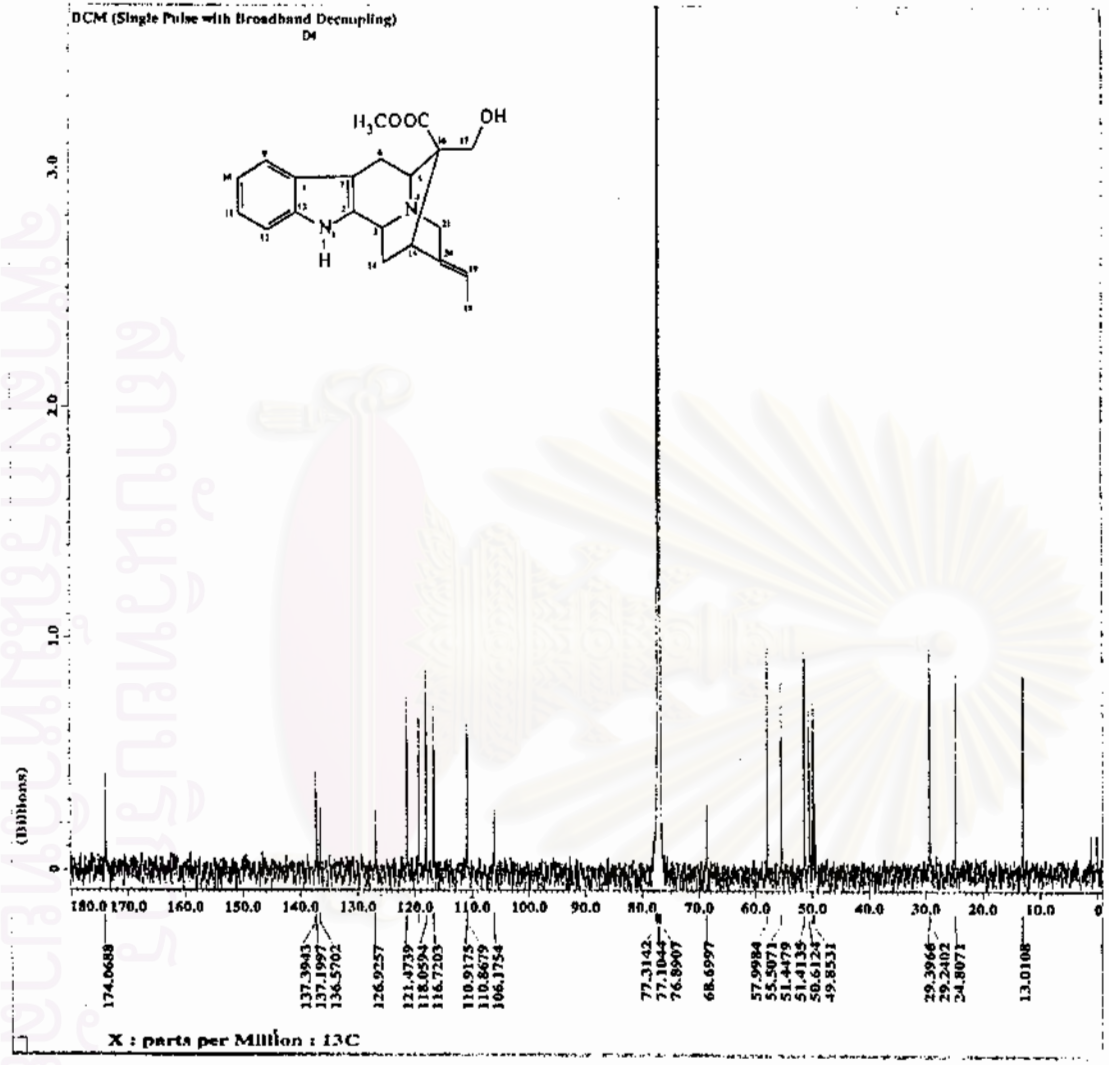


Figure 30a 150 MHz ^{13}C NMR spectrum of compound D-4 (in $CDCl_3$-CD_3OD)

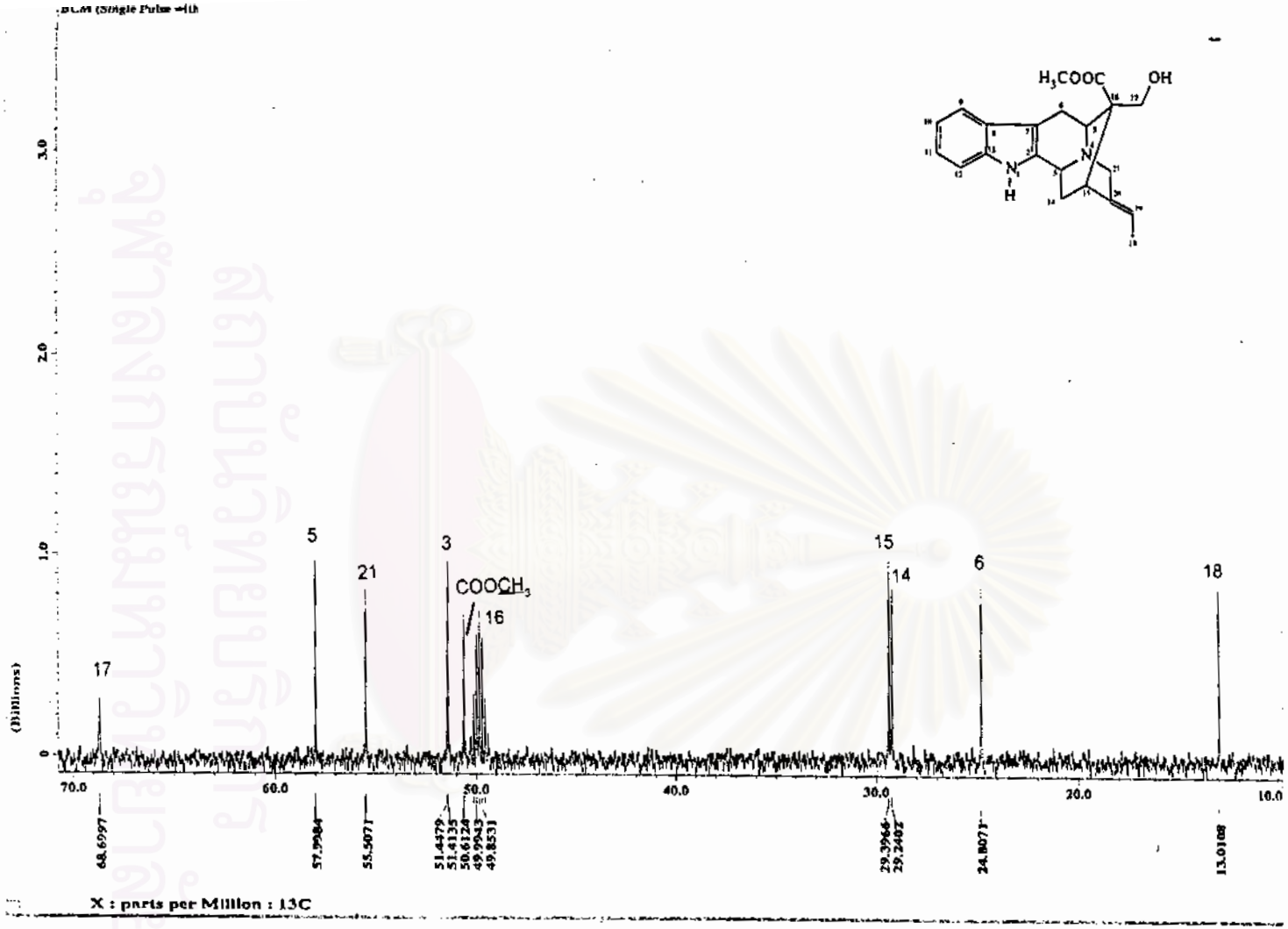


Figure 30b 150 MHz ^{13}C NMR spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_c 10.0 – 70.0 ppm]

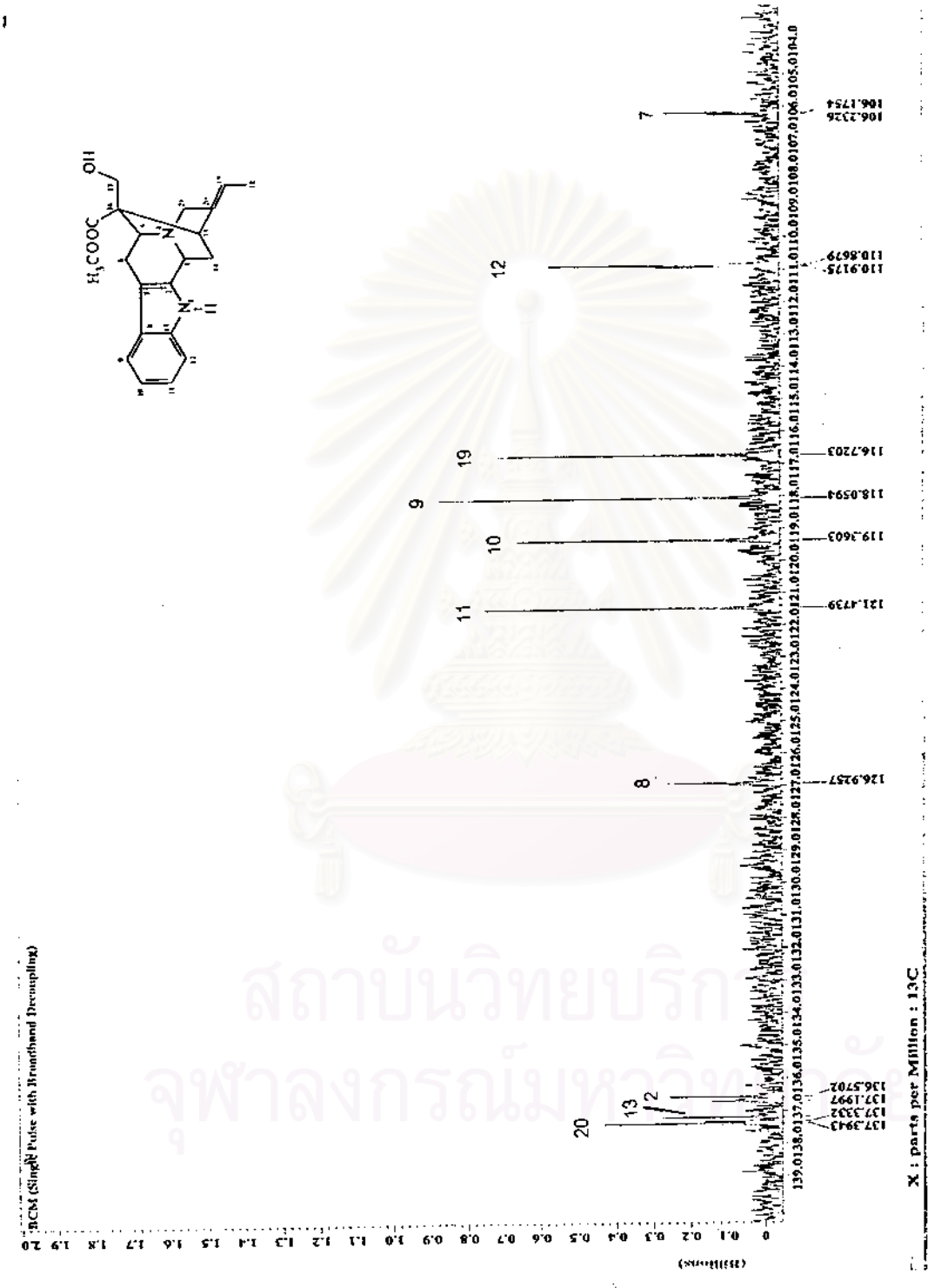


Figure 30c 150 MHz ^{13}C NMR spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_c 104.0 – 139.0 ppm]

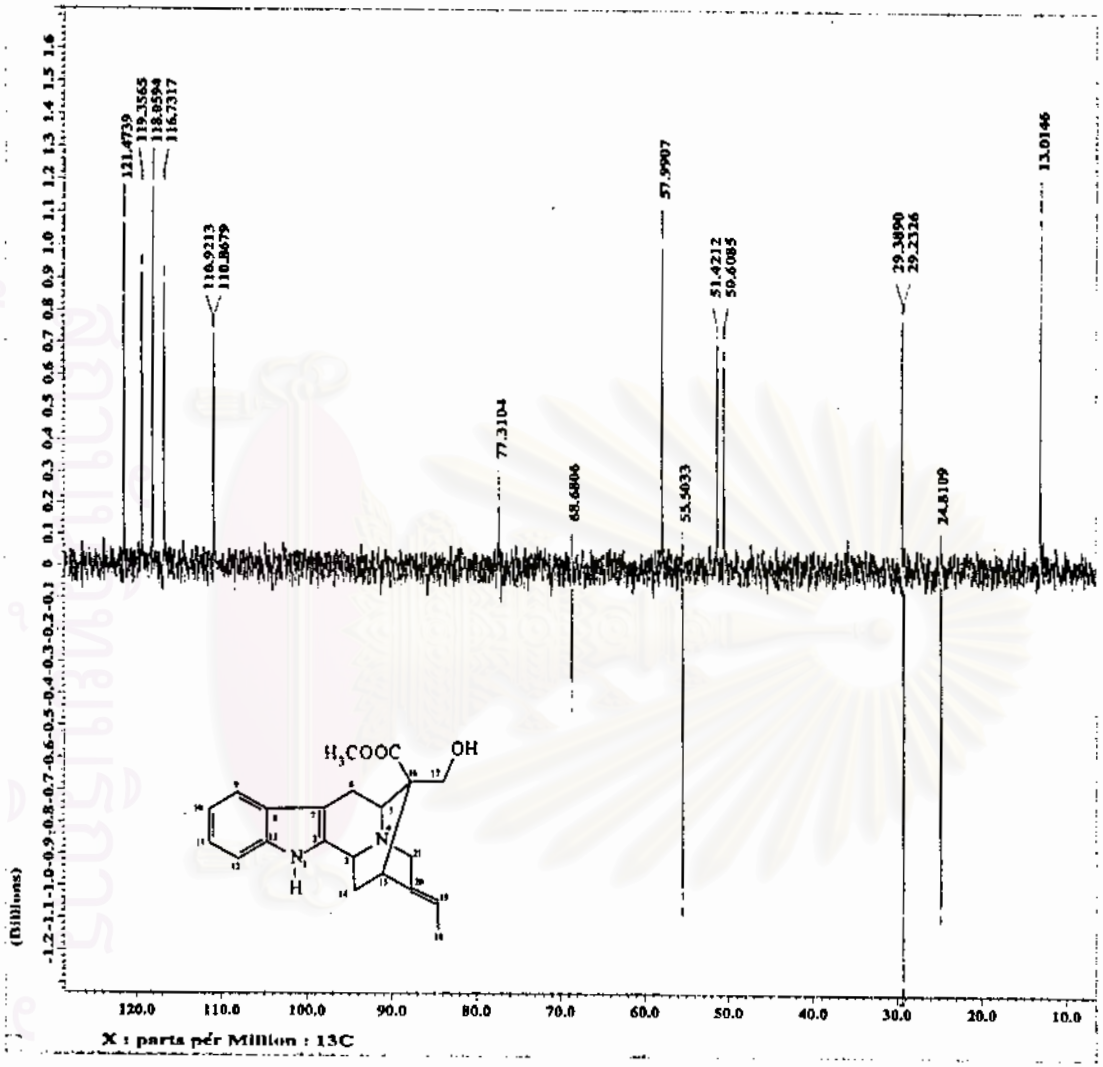


Figure 31a DEPT 135 spectrum of compound *D*-4 (in $CDCl_3$-CD_3OD)

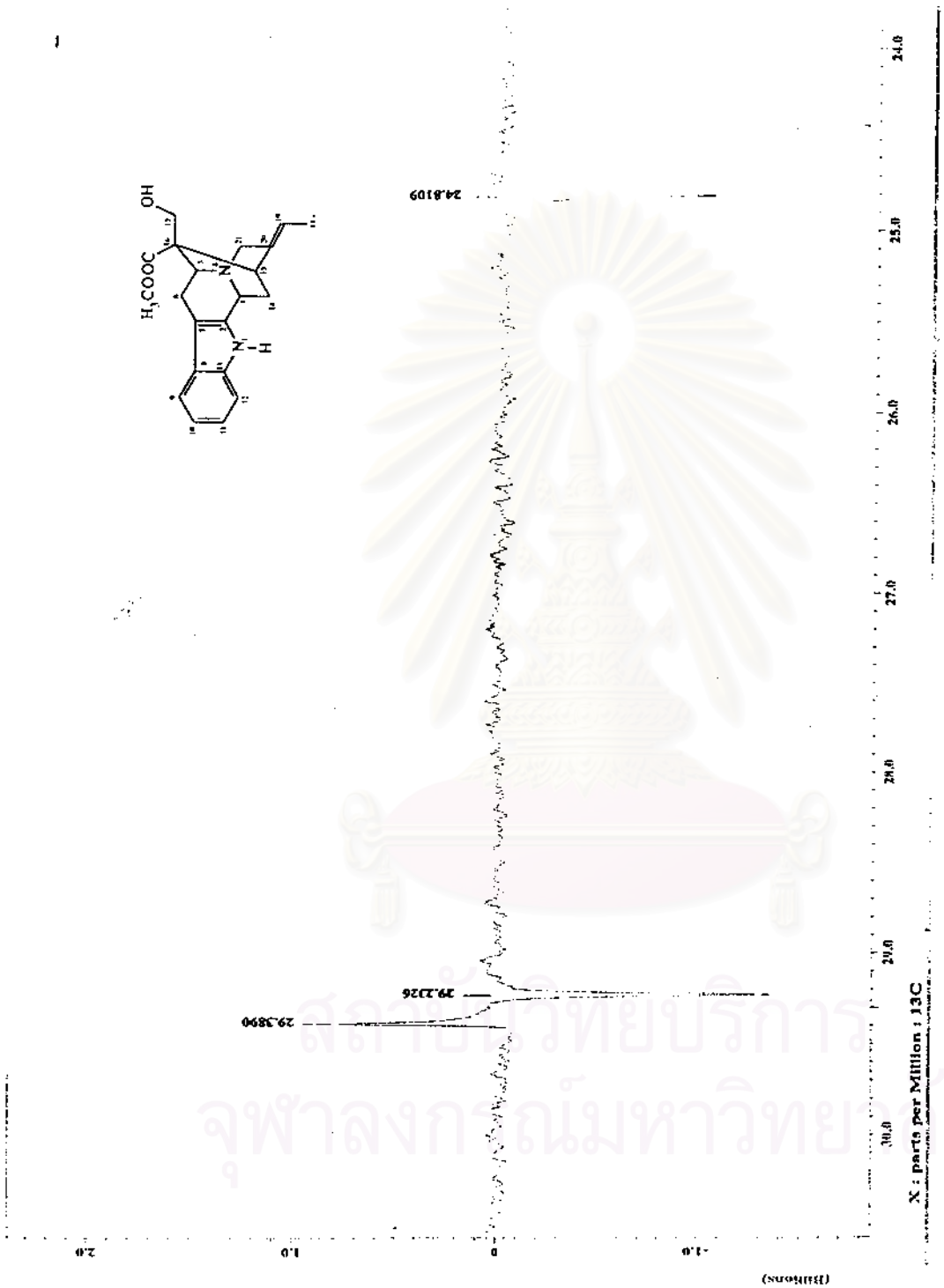


Figure 31b DEPT 135 spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_c 24.0 – 30.0 ppm]

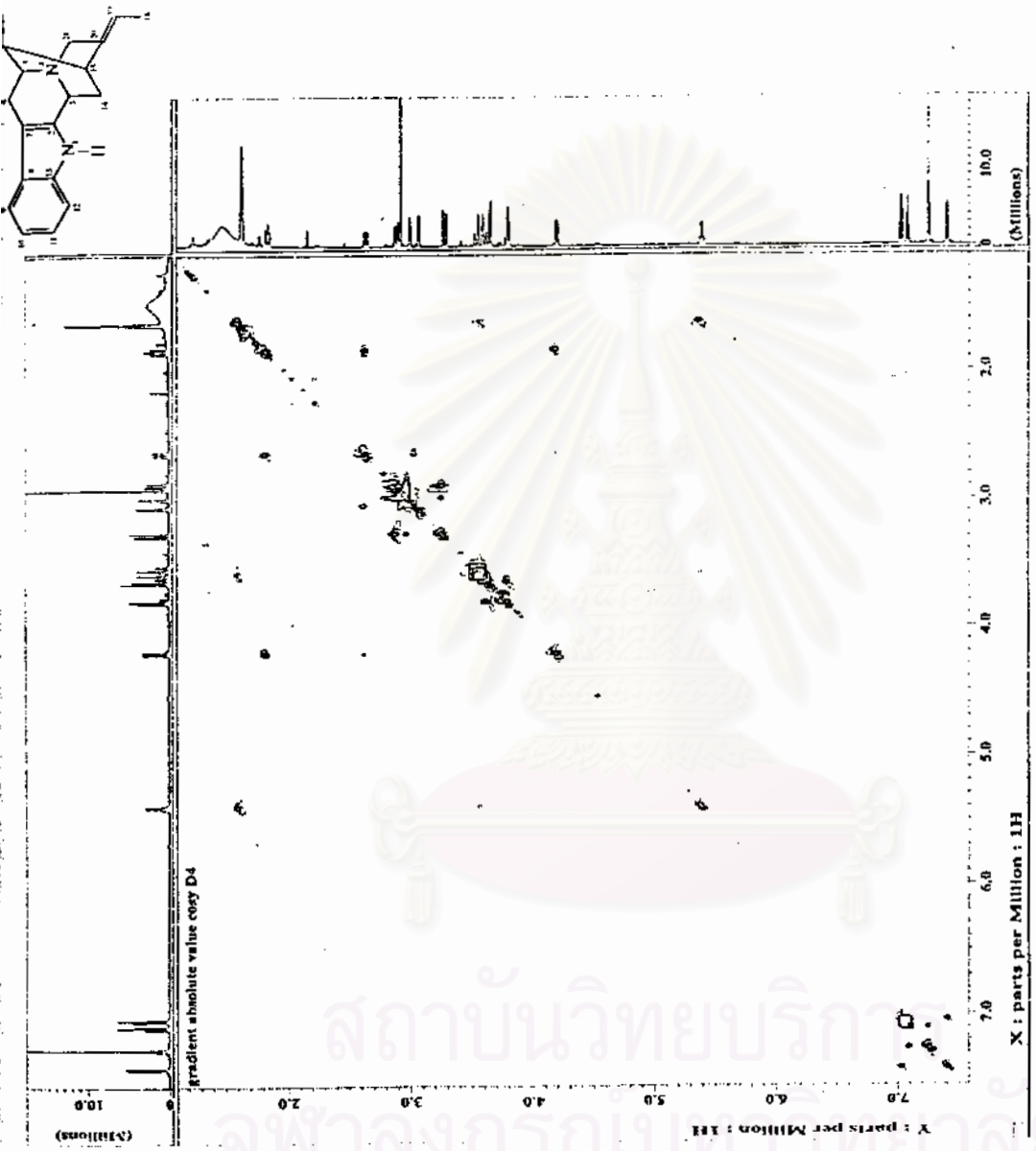


Figure 32a ^{1}H-^{1}H COSY spectrum of compound D-4 (in $CDCl_3$-CD_3OD)

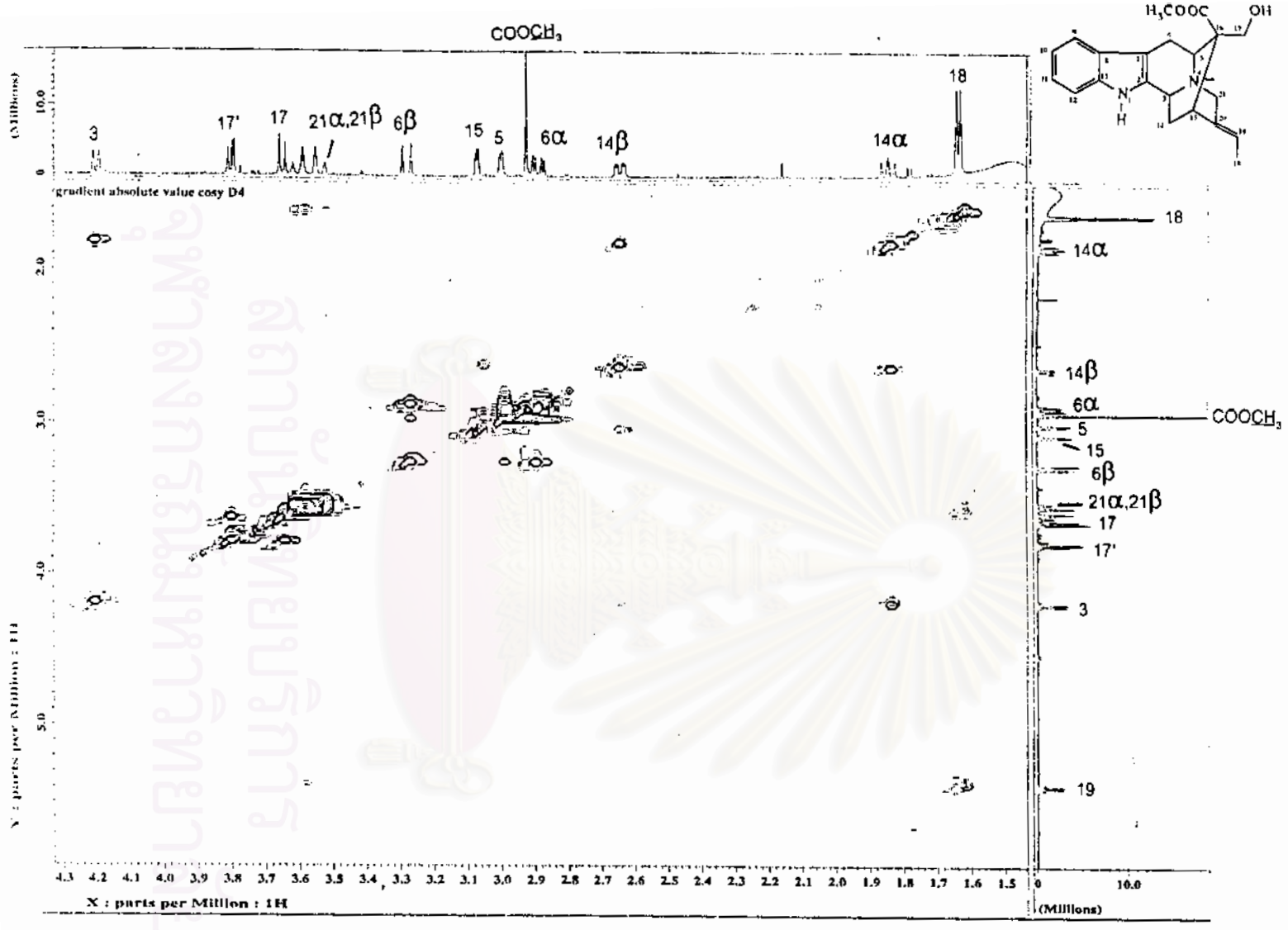


Figure 32b ^{1}H-^{1}H COSY spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_H 1.50 – 4.30 ppm, δ_H 1.40 – 5.90 ppm]

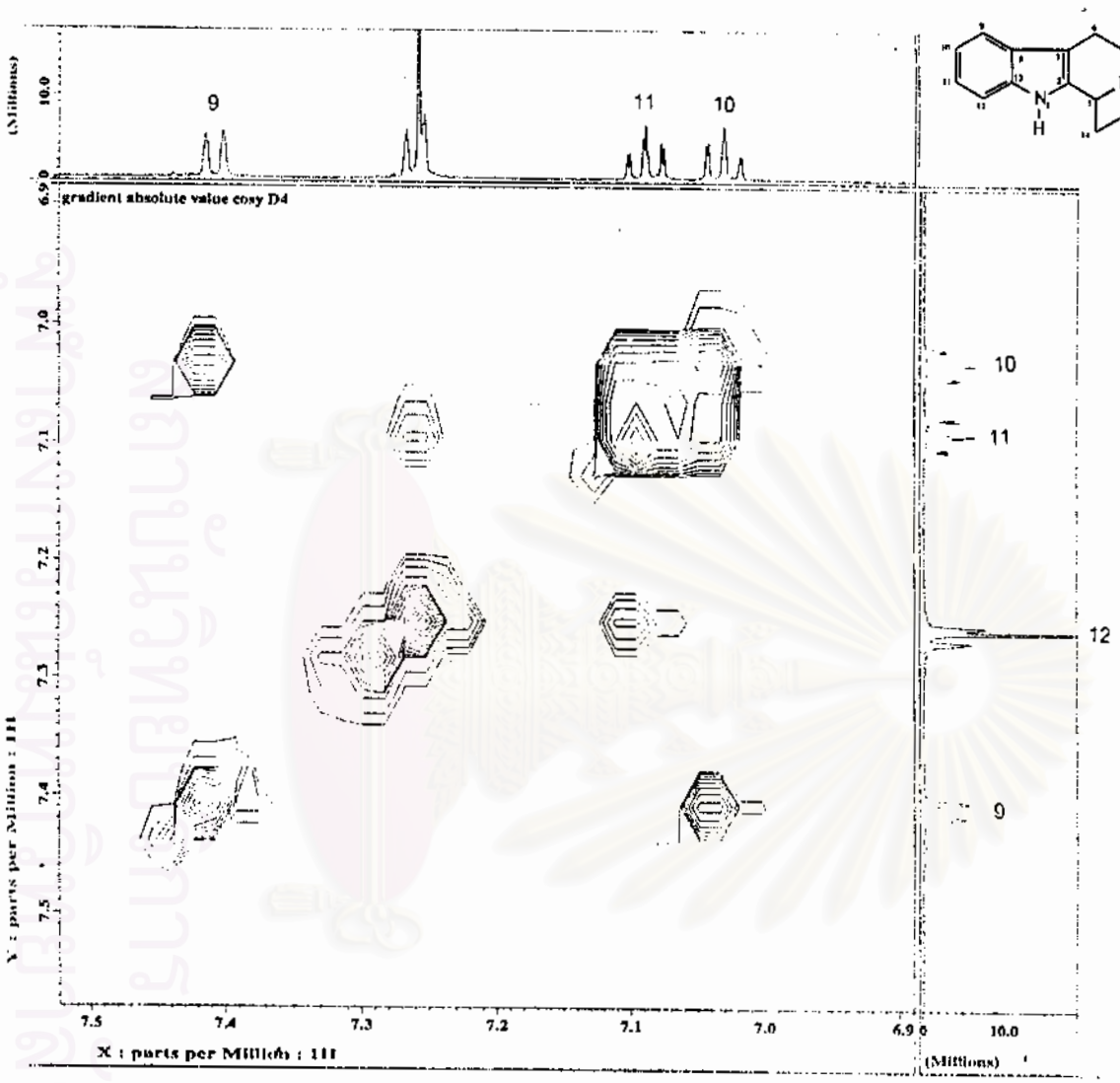


Figure 32c ^{1}H-^{1}H COSY spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_H 6.90 – 7.50 ppm, δ_H 6.90 – 7.50 ppm]

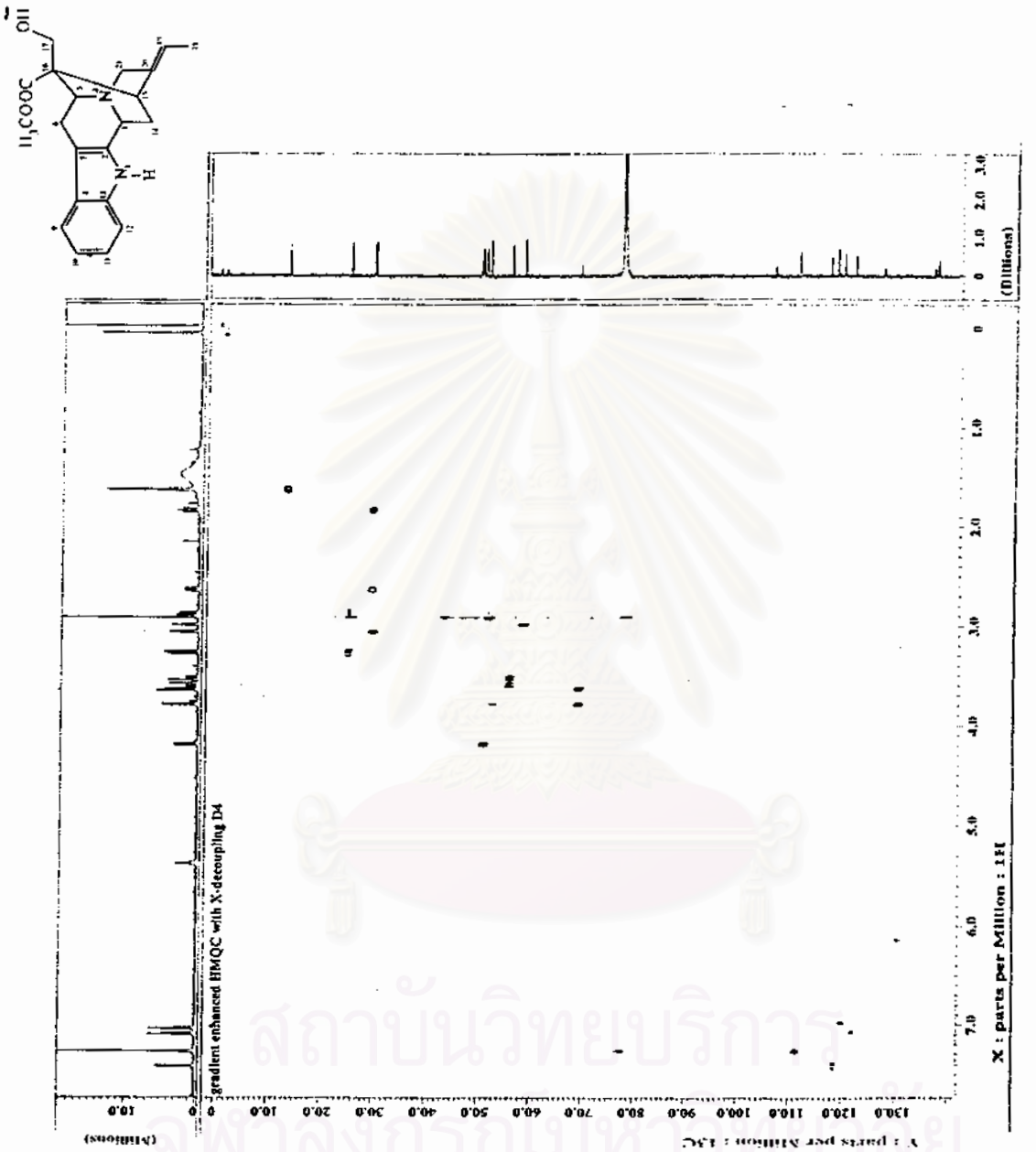


Figure 33a HMQC spectrum of compound D-4 (in $CDCl_3$-CD_3OD)

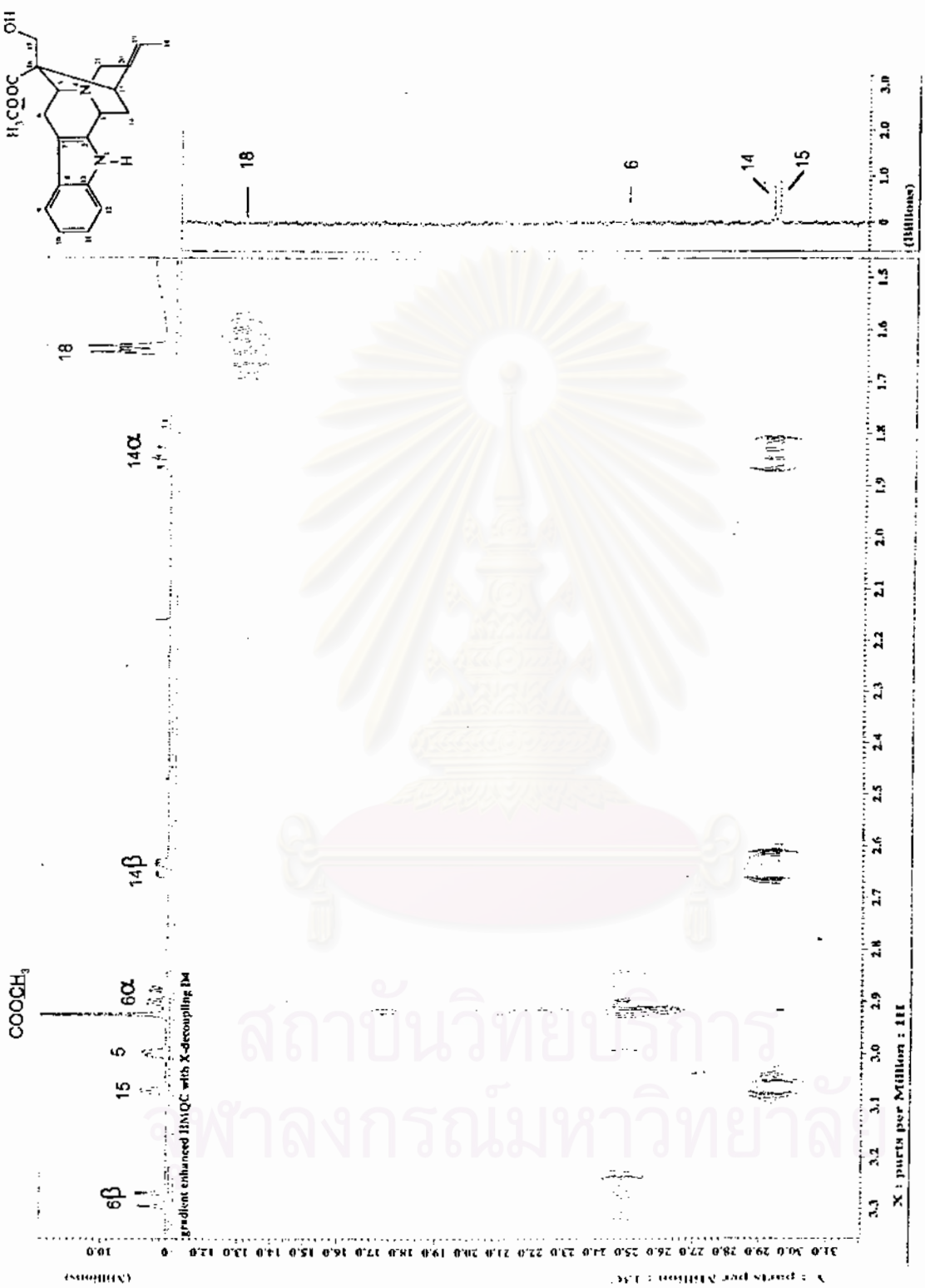


Figure 33b HMQC spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_H 1.50 – 3.35 ppm, δ_C 12.0 – 31.0 ppm]

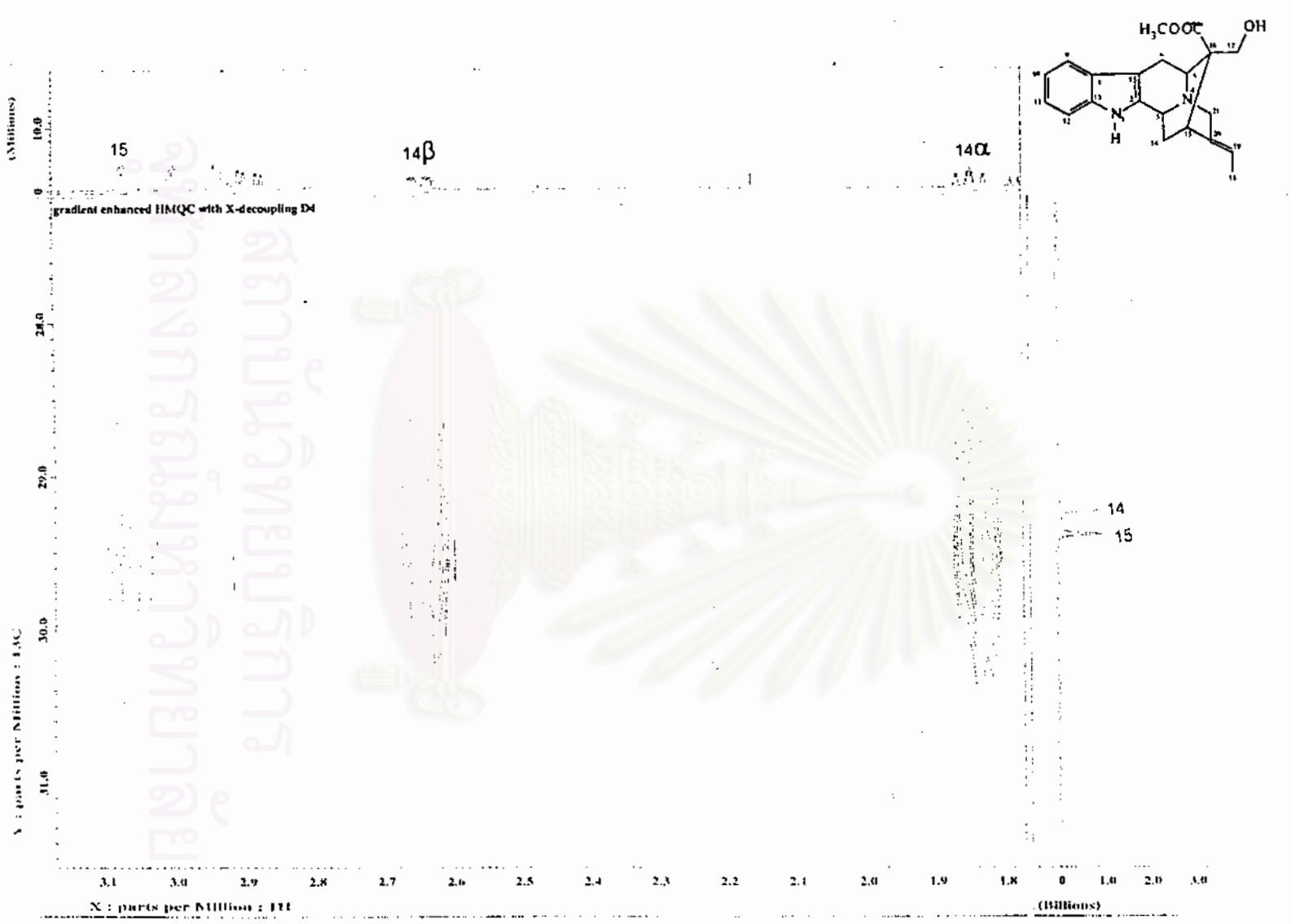


Figure 33c HMQC spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_H 1.80 – 3.17 ppm, δ_C 27.2 – 31.5 ppm]

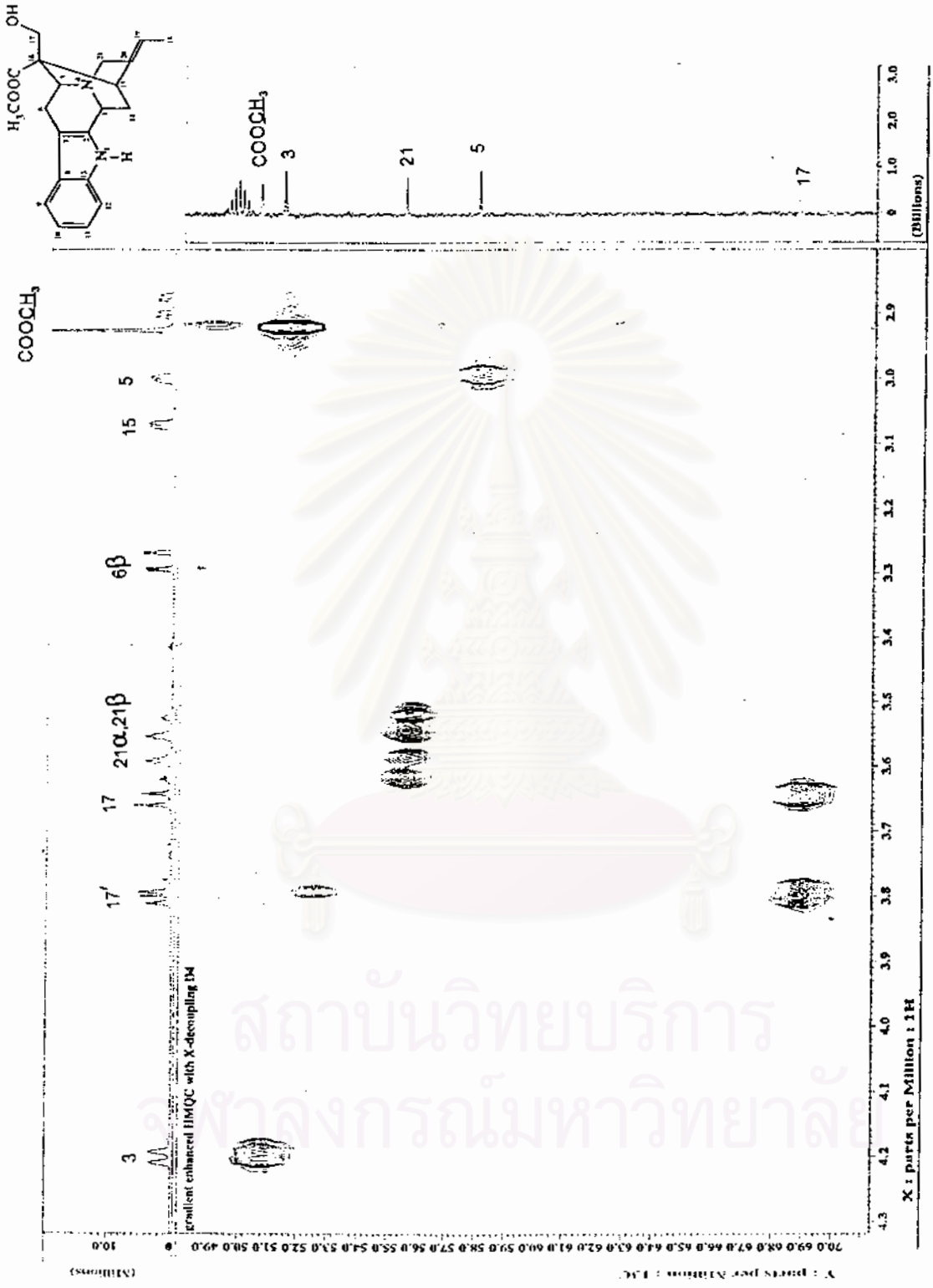


Figure 33d HMQC spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_H 2.80 – 4.30 ppm, δ_C 48.0-71.0 ppm]

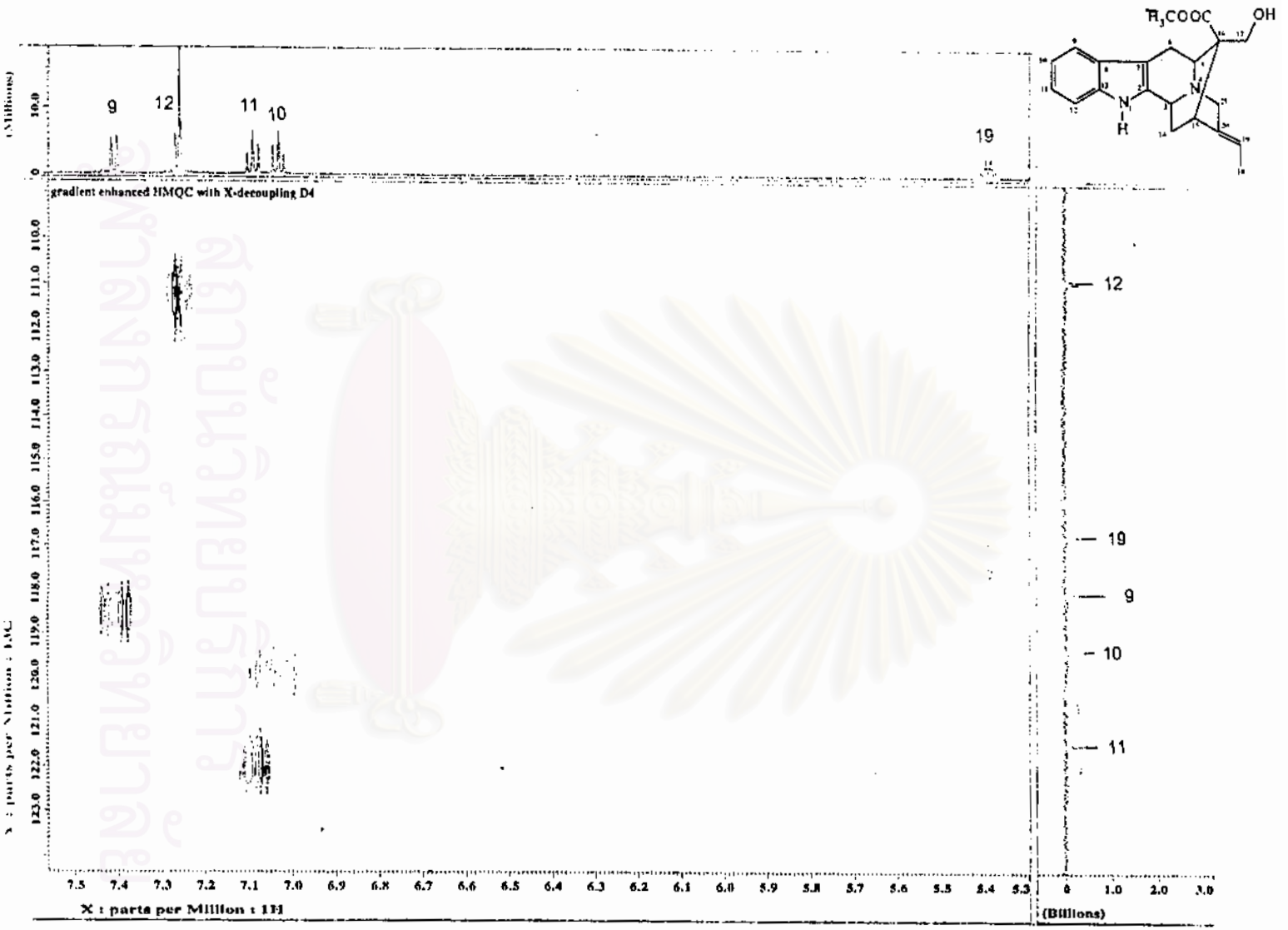


Figure 33e HMQC spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_H 5.30 – 7.50 ppm, δ_C 109.0 – 124.0 ppm]

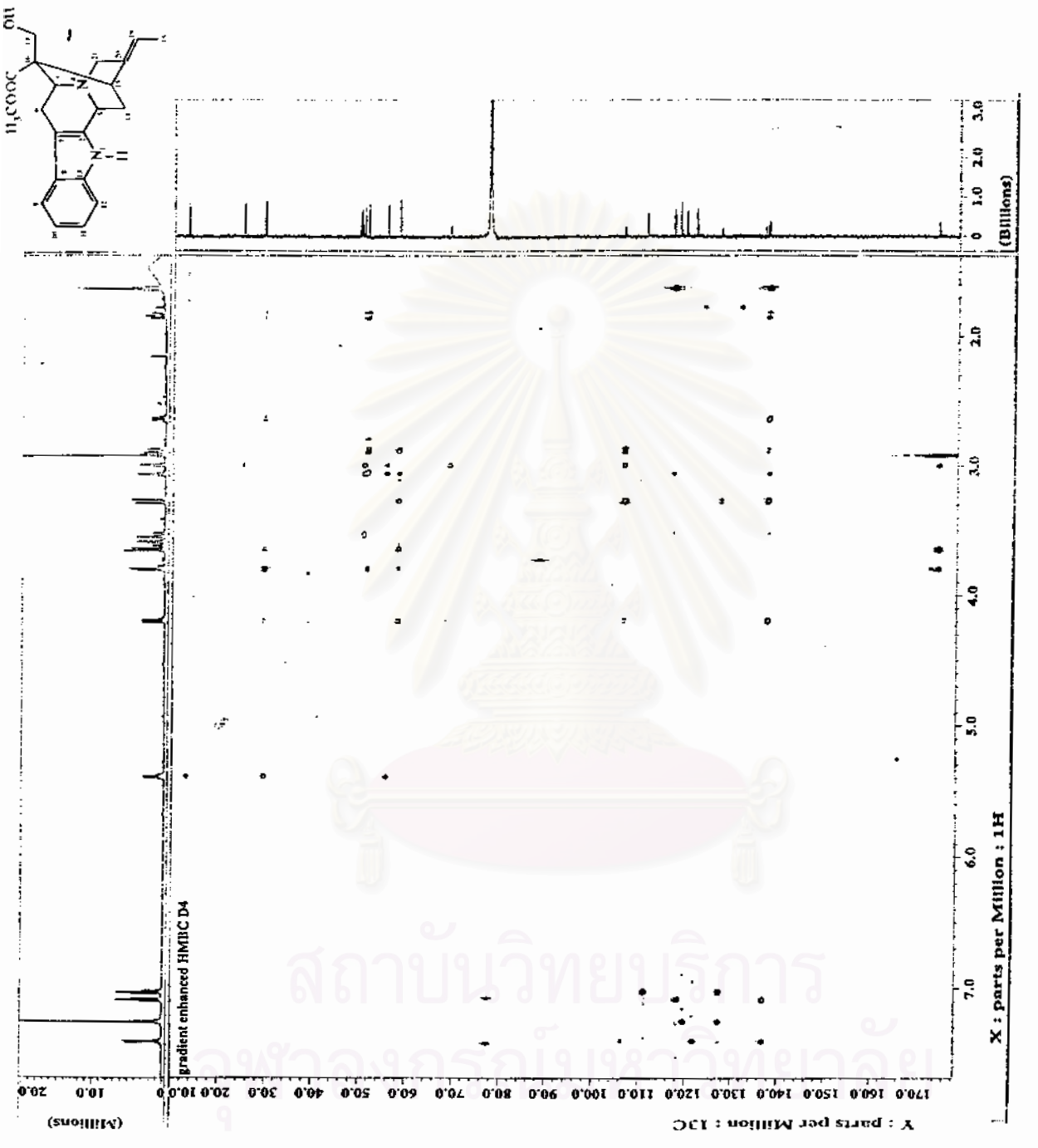


Figure 34a HMBC spectrum of compound D-4 (in $CDCl_3$-CD_3OD)

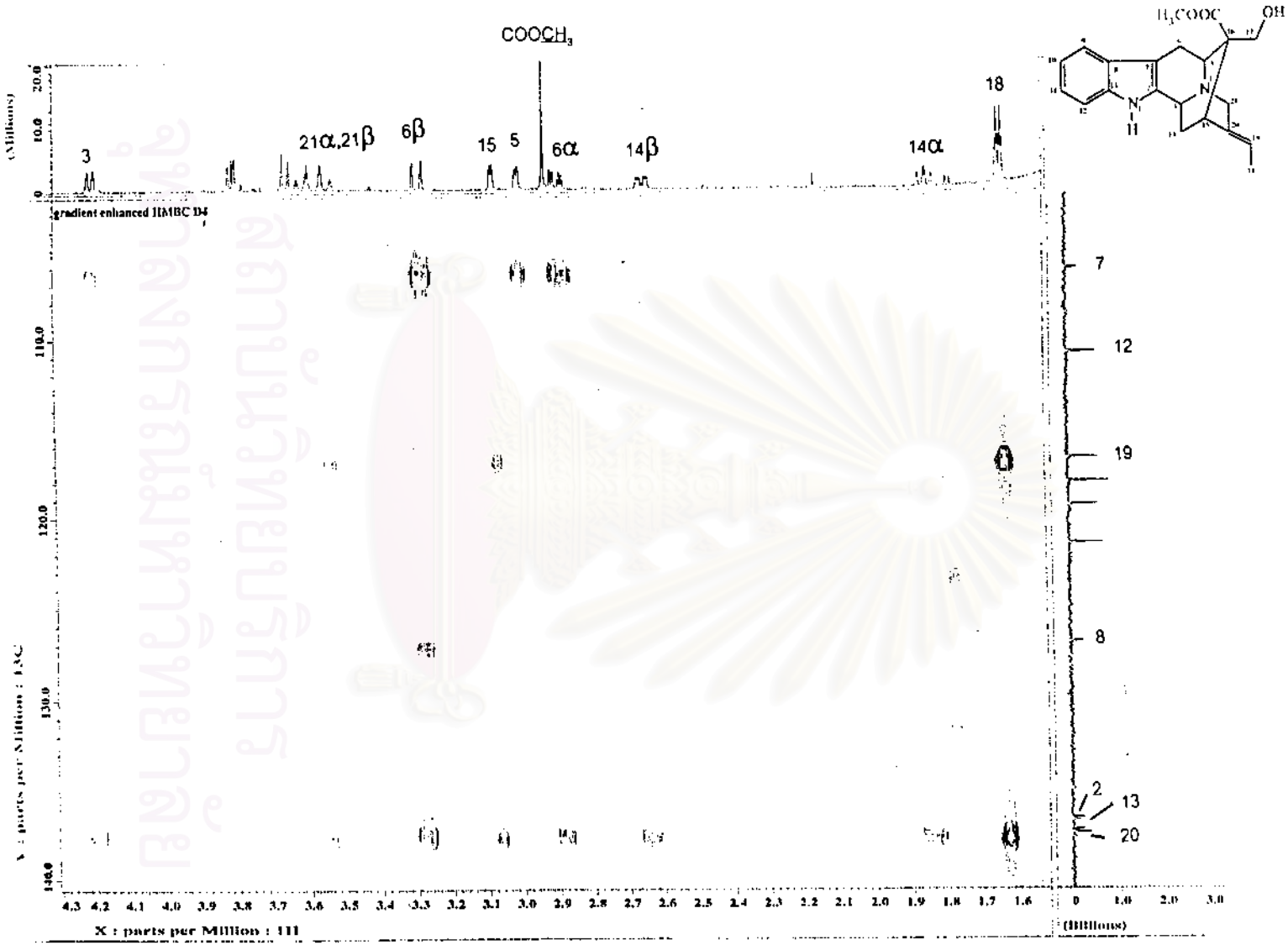


Figure 34b HMBC spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_H 1.52 – 4.30 ppm, δ_C 102.0 – 140.0 ppm]

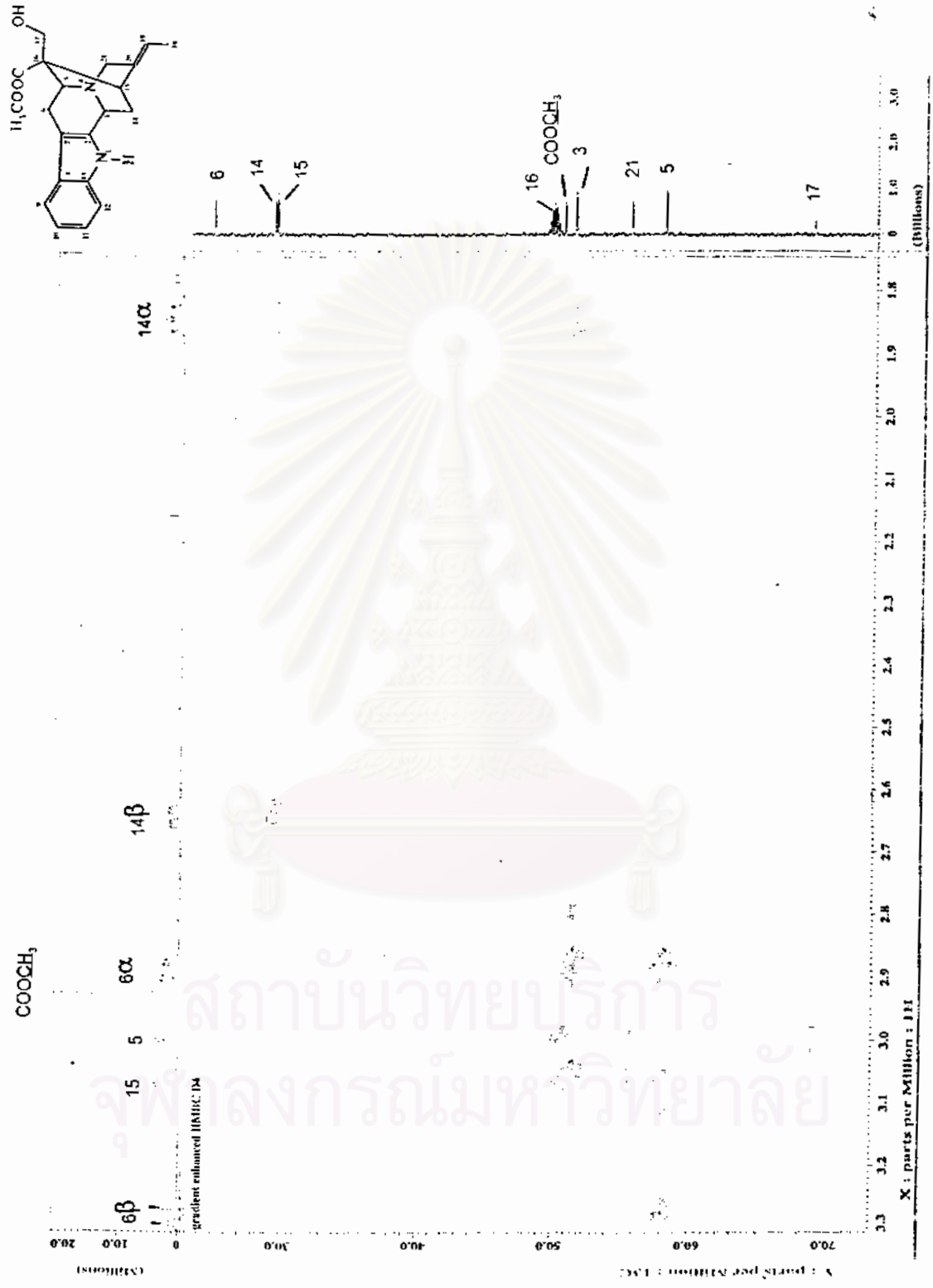


Figure 34c HMBC spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_H 1.80 – 3.30 ppm, δ_C 29.3 – 72.0 ppm]

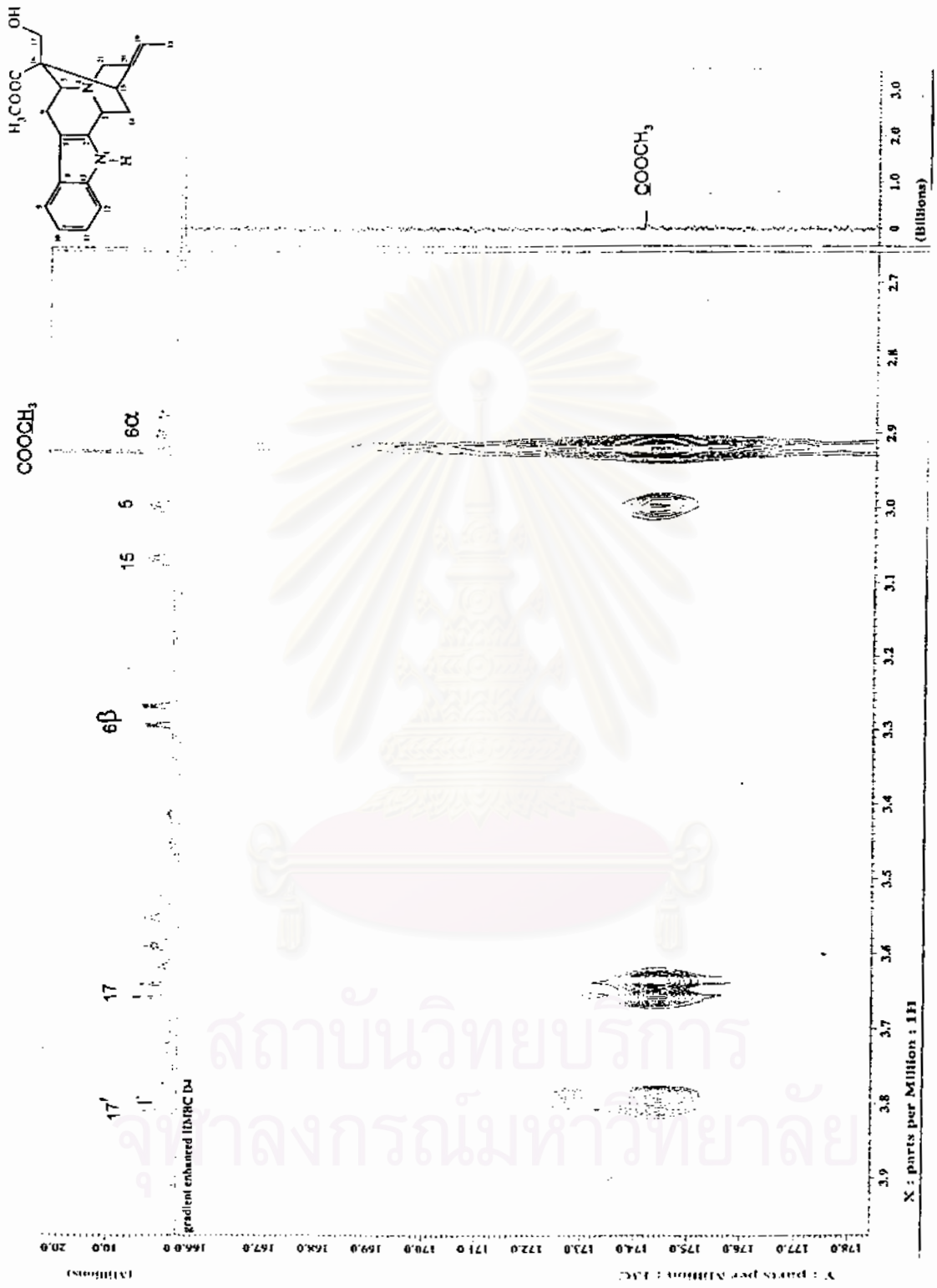


Figure 34d HMBC spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_H 2.70 – 3.90 ppm, δ_C 166.0 – 178.0 ppm]

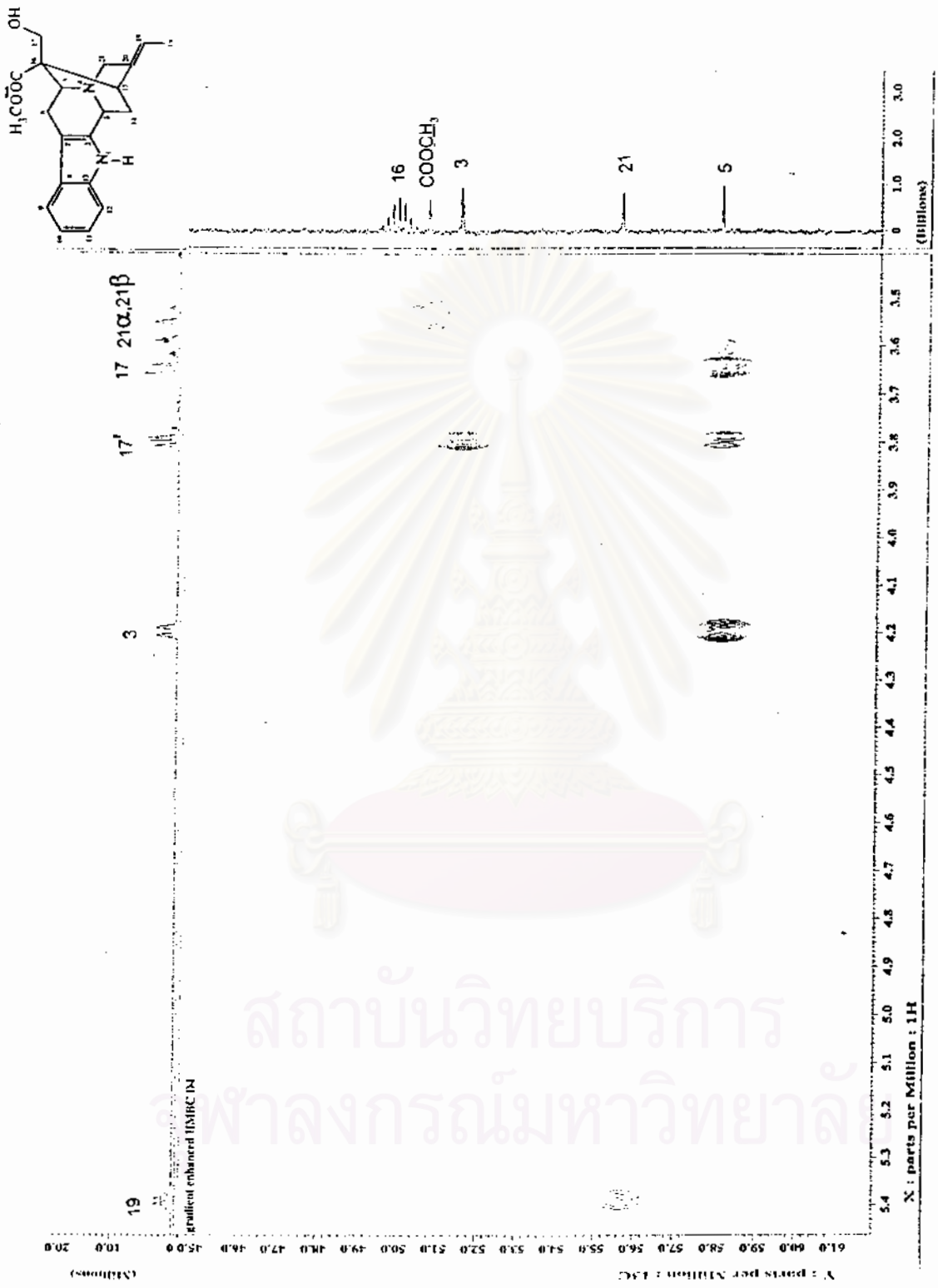


Figure 34e HMBC spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_H 3.50 – 5.40 ppm, δ_C 45.0 – 61.0 ppm]

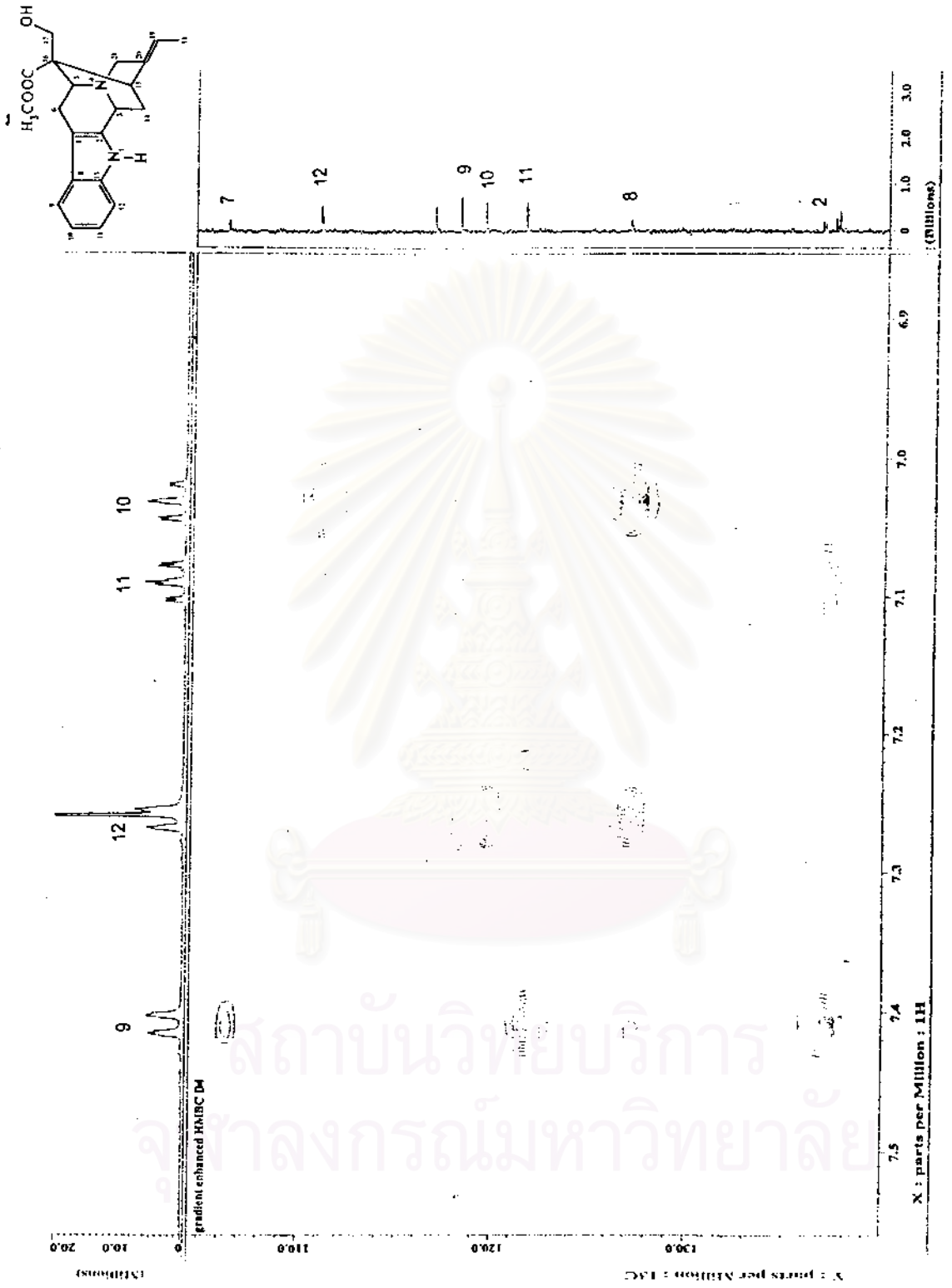


Figure 34f HMBC spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [δ_H 6.85 – 7.56 ppm, δ_C 105.0 – 140.0 ppm]

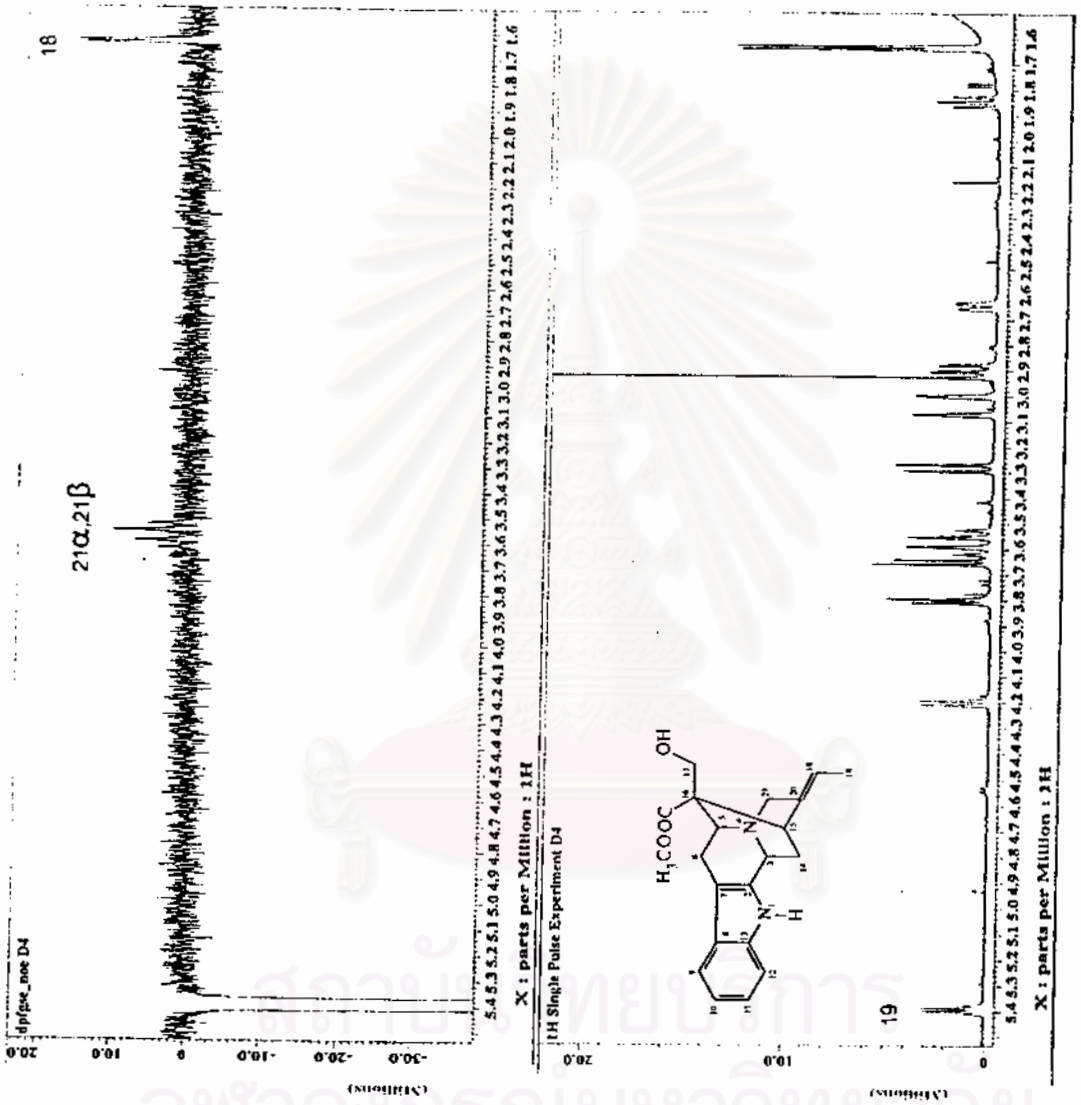


Figure 35a NOE difference spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [irradiated H-19, δ_H 1.50-5.50 ppm]

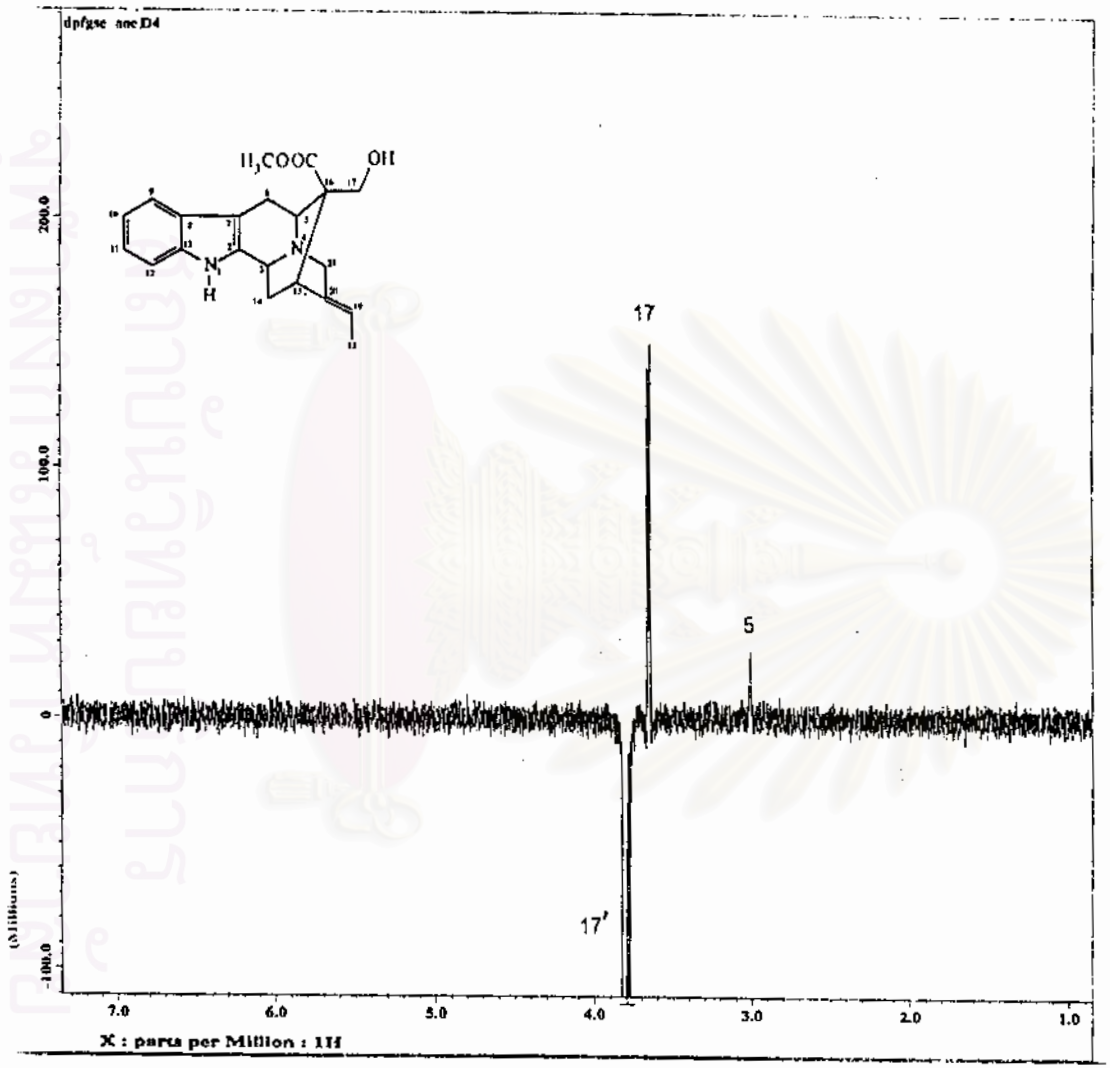


Figure 35b NOE difference spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [irradiated H-17′, δ_H 1.00-7.00 ppm]

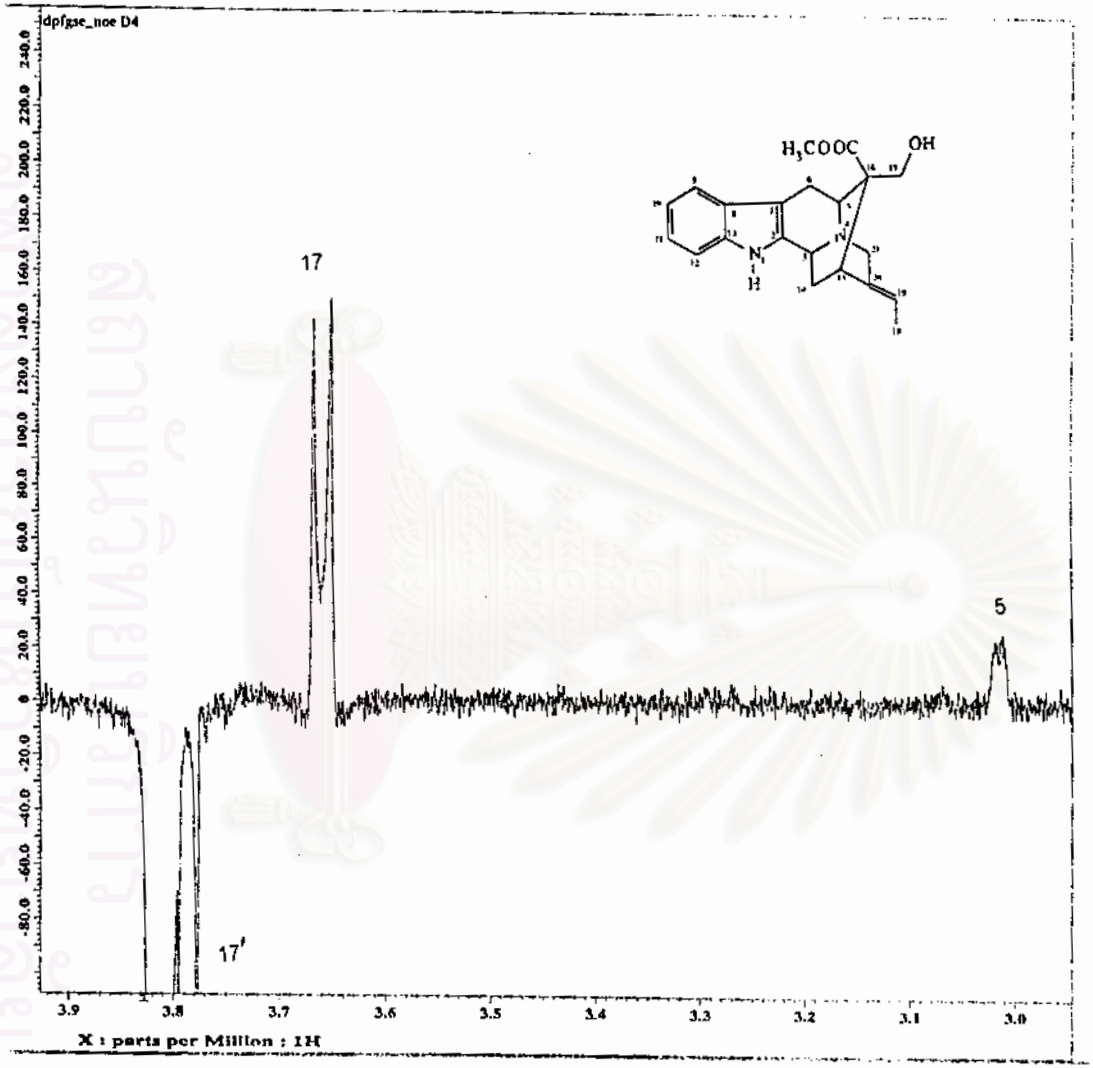


Figure 35c NOE difference spectrum of compound D-4 (in $CDCl_3$-CD_3OD) [irradiated H-17′, δ_H 3.00-3.90 ppm]

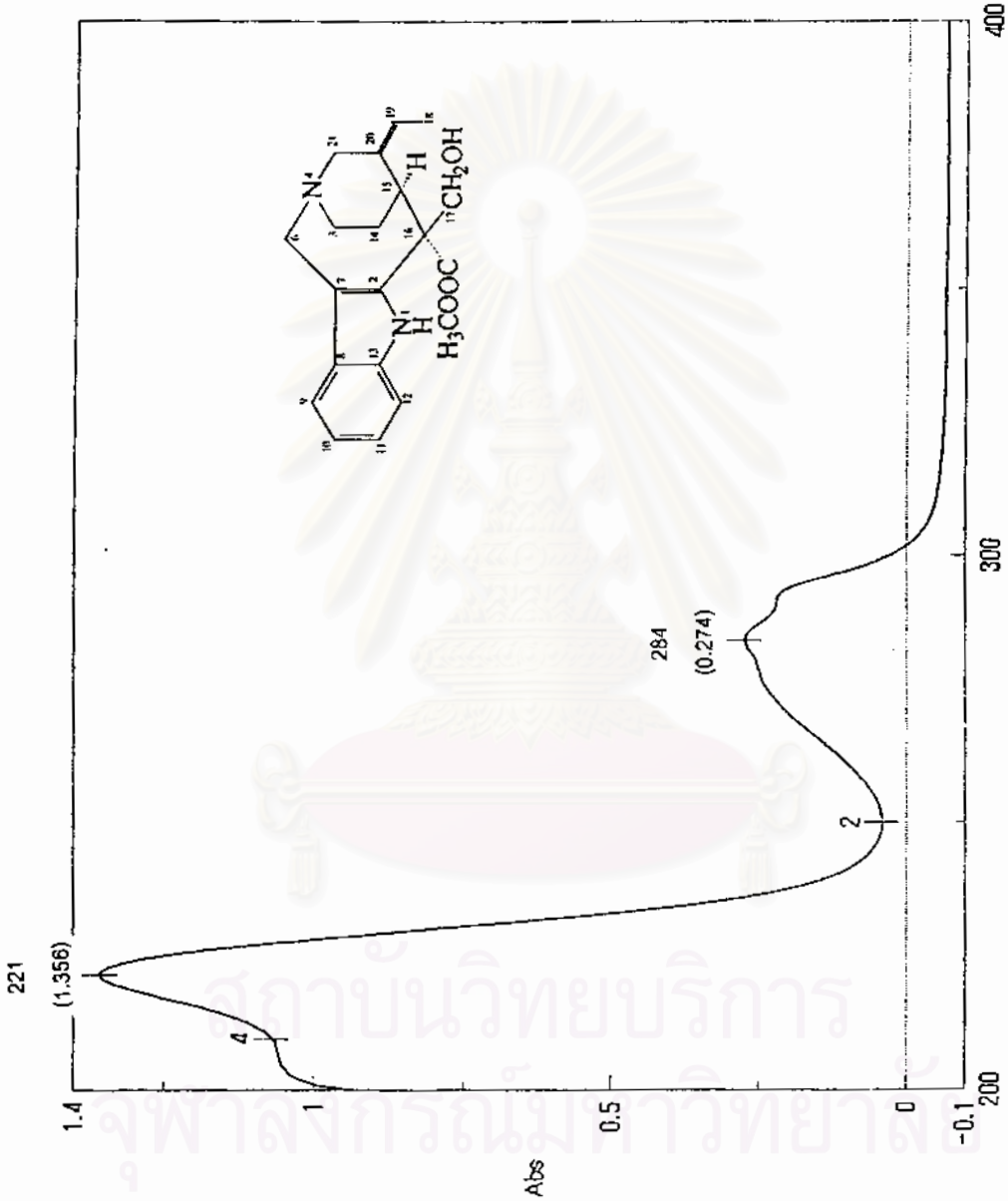


Figure 36 UV spectrum of compound D-5 (in ethanol)

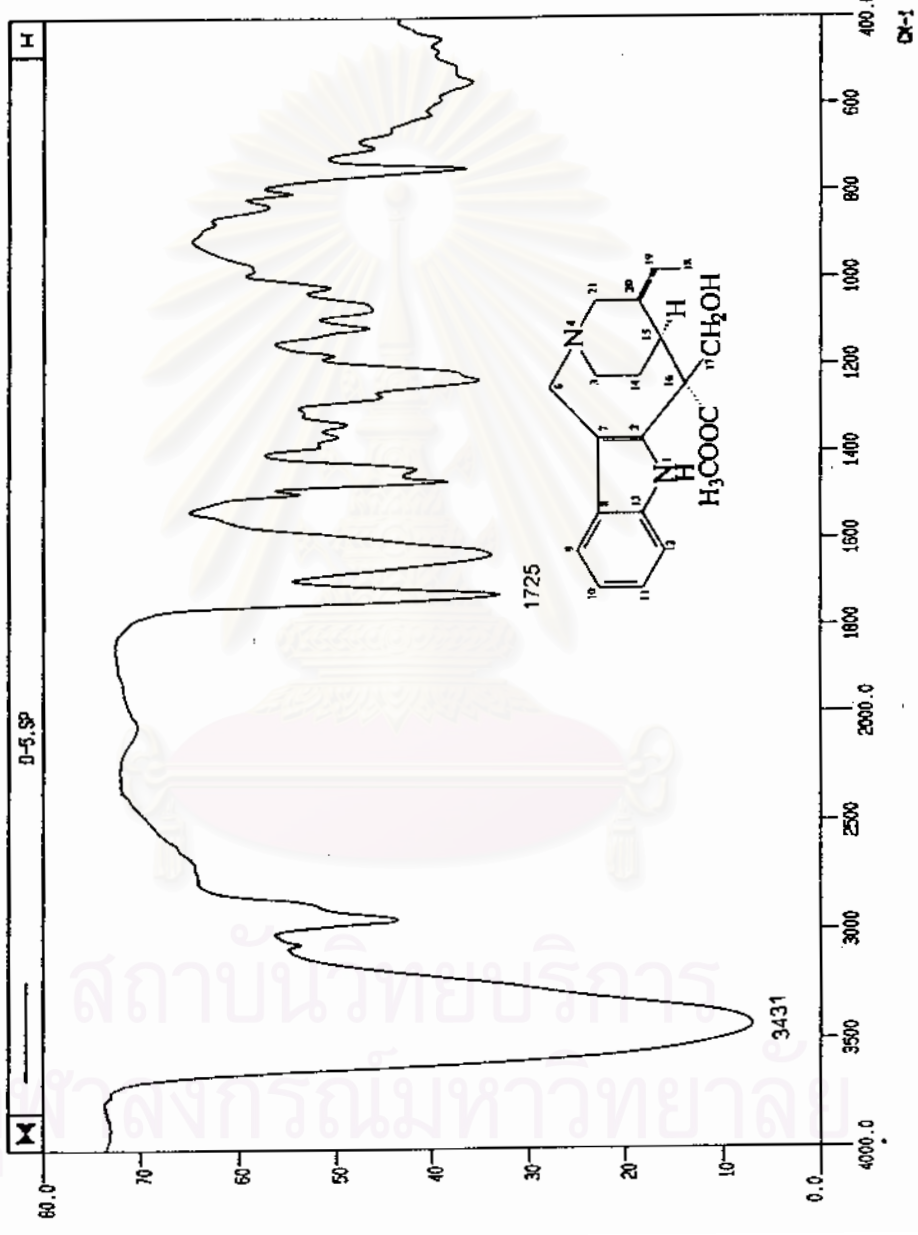


Figure 37 IR spectrum of compound D-5 (KBr disc)

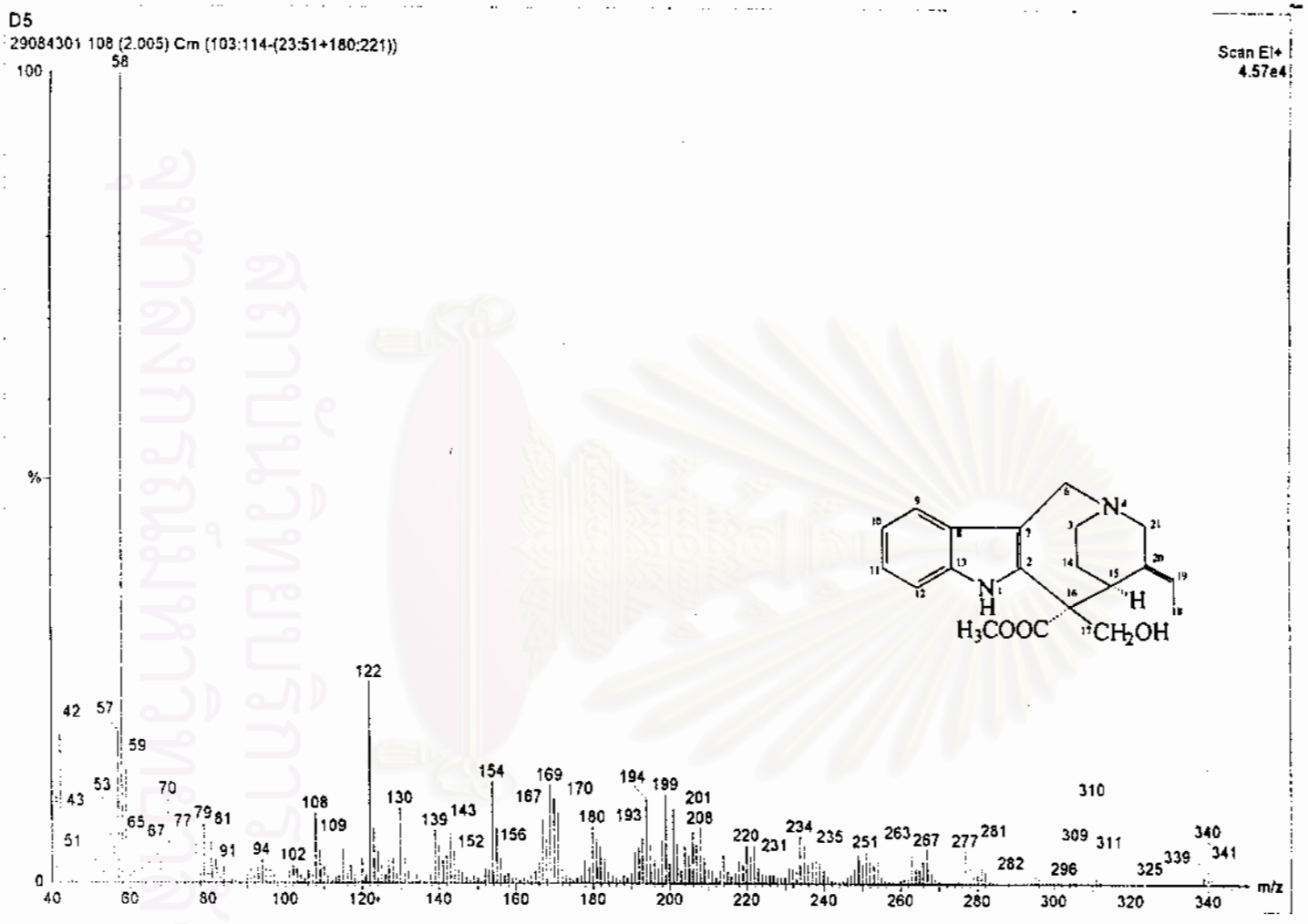


Figure 38 EI mass spectrum of compound D-5

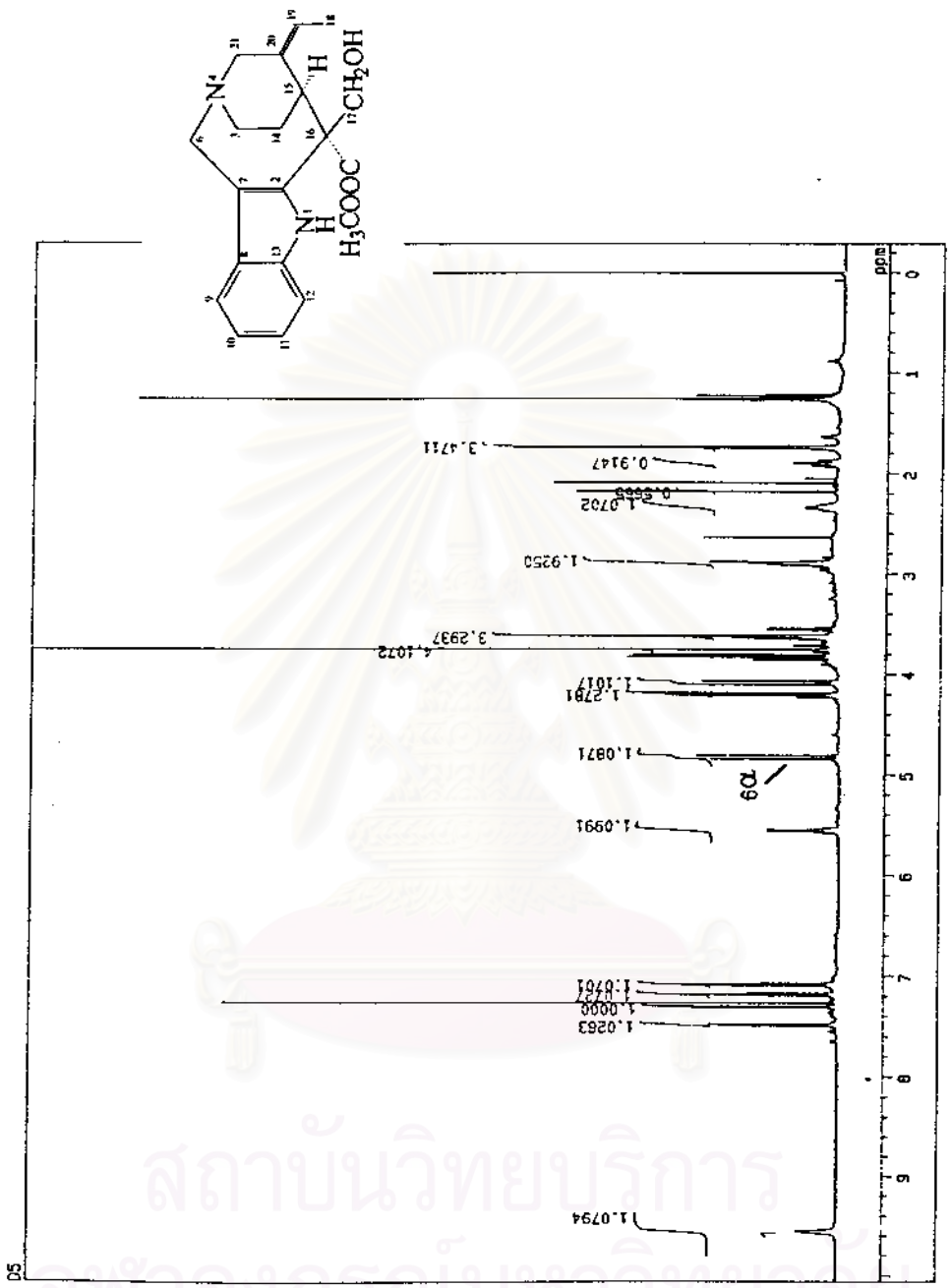


Figure 39a 500 MHz ^{1}H NMR spectrum of compound D-5 (in $CDCl_3$)

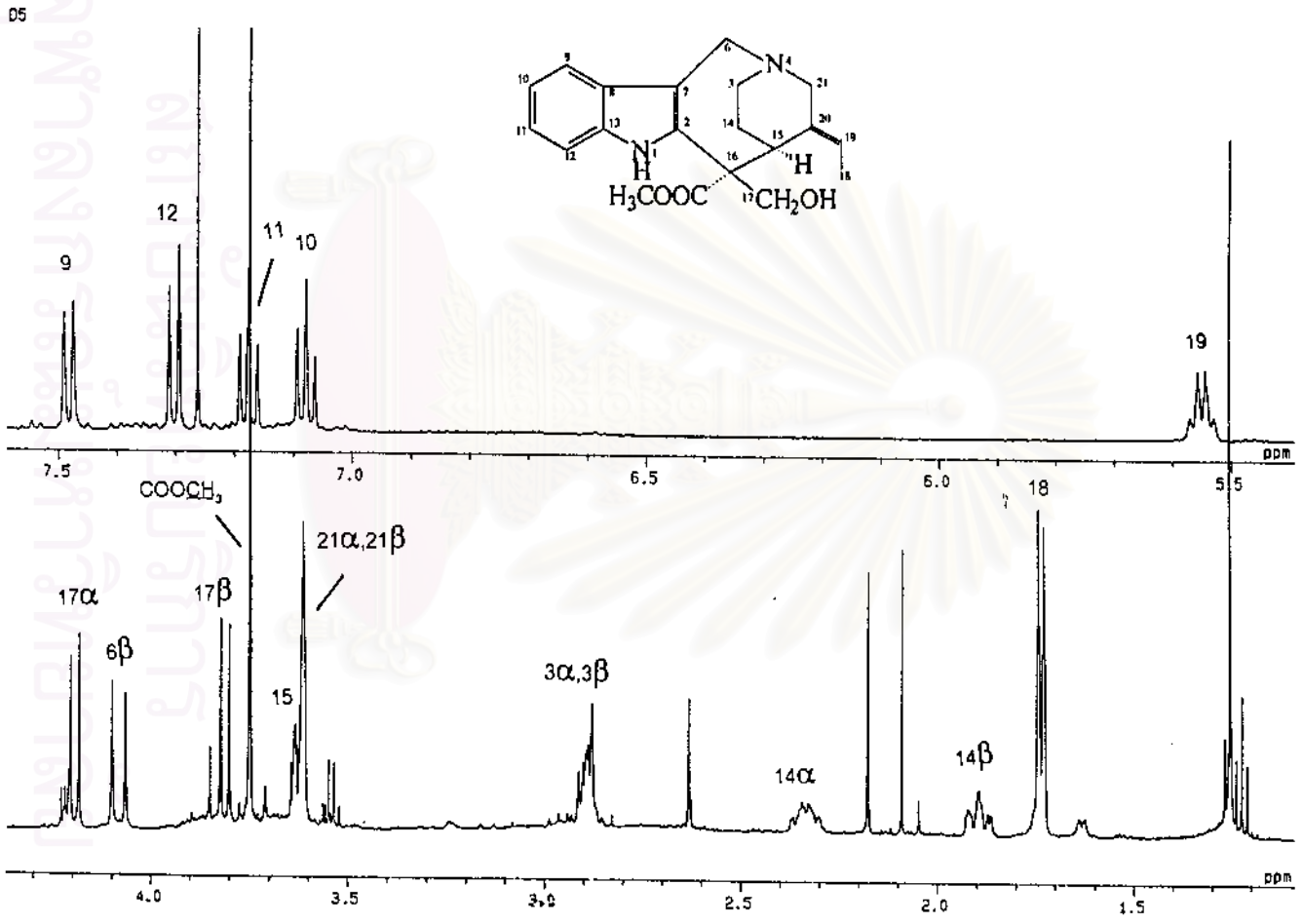


Figure 39b 500 MHz ^{1}H NMR spectrum of compound D-5 (in $CDCl_3$) [δ_H 1.20 – 4.30, 5.50 – 7.50 ppm]

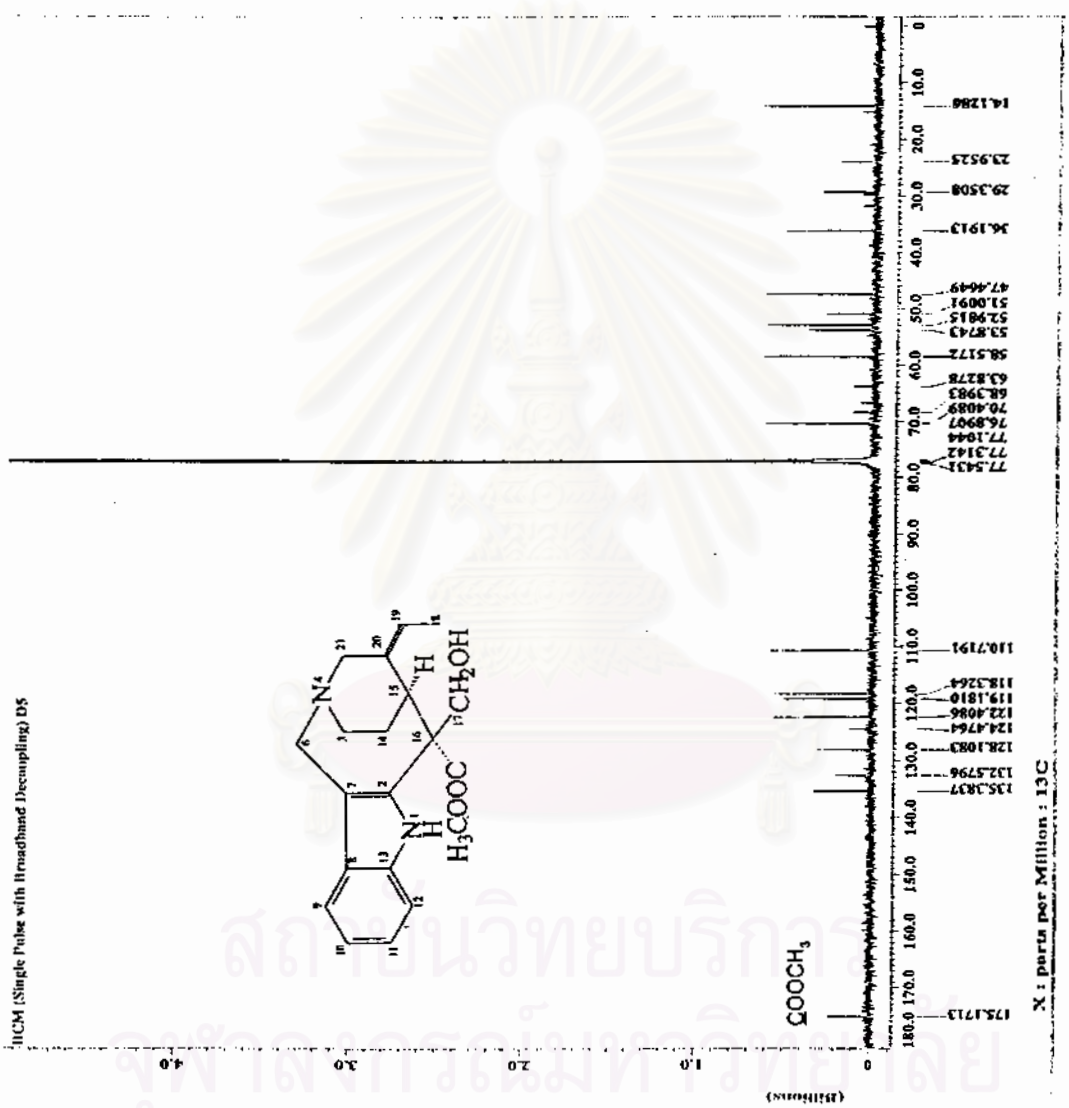


Figure 40a 150 MHz ^{13}C NMR spectrum of compound D-5 (in $CDCl_3$)

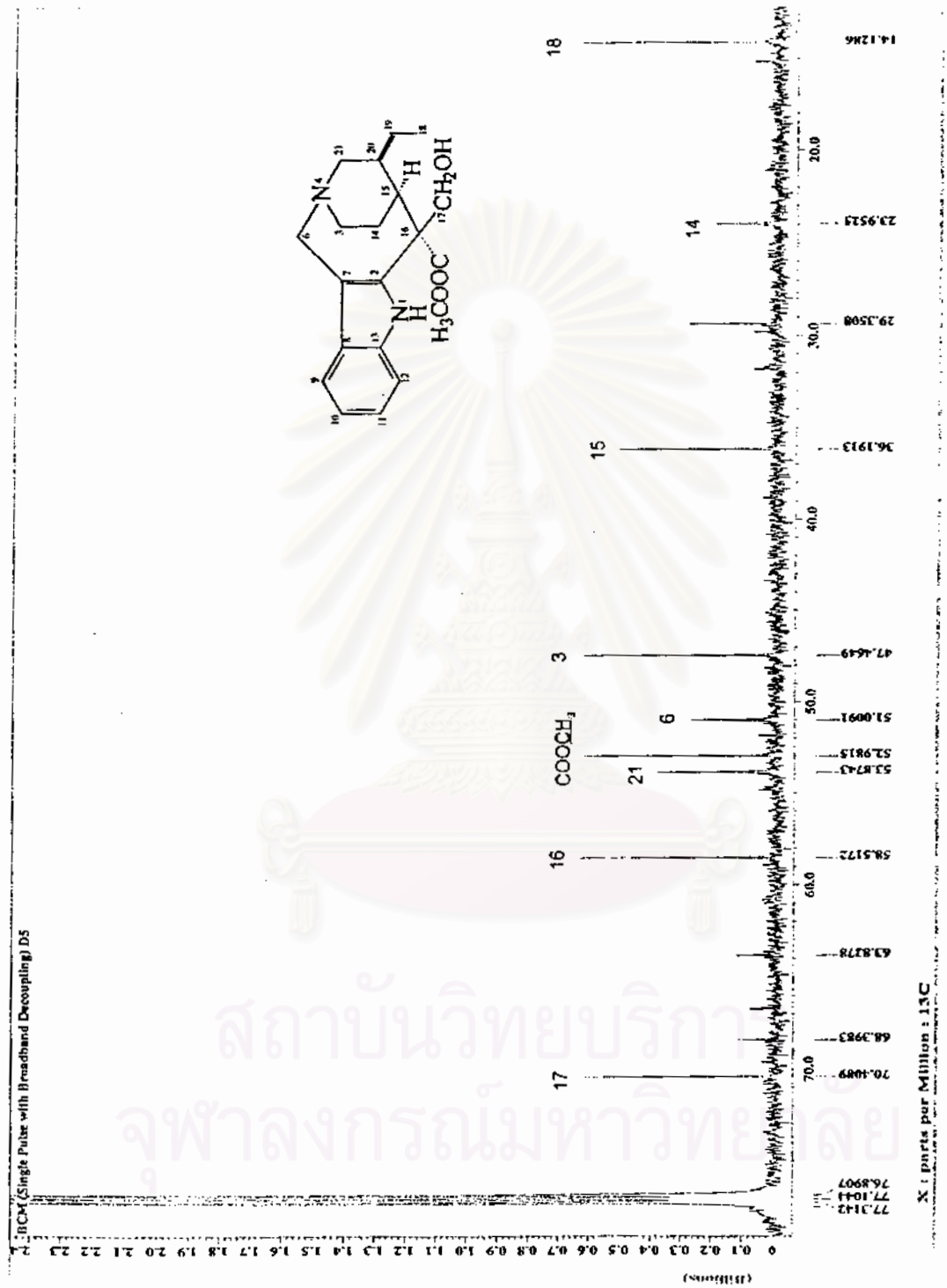


Figure 40b 150 MHz ^{13}C NMR spectrum of compound D-5 (in $CDCl_3$) [δ_C 14.1 – 77.3 ppm]

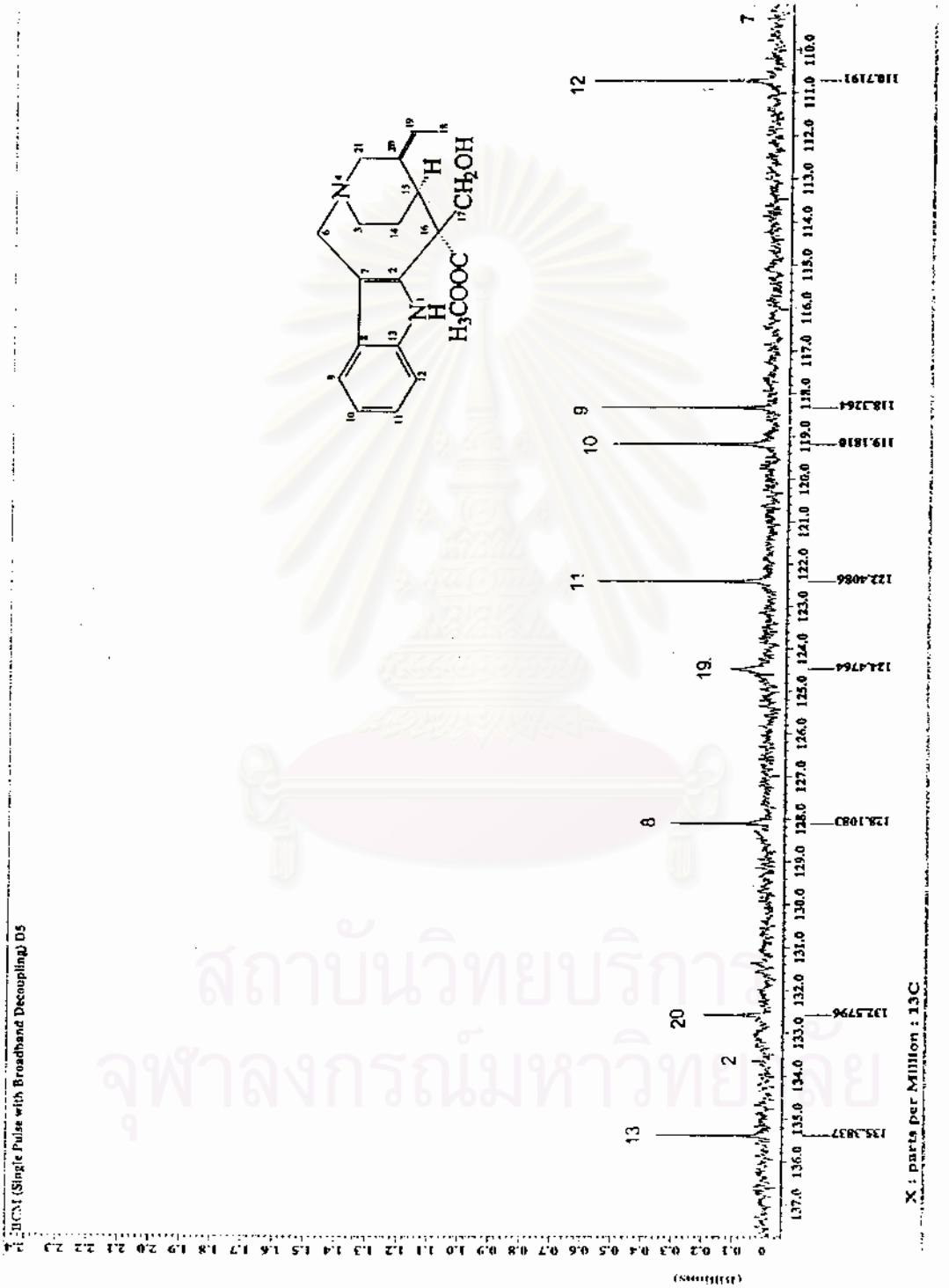


Figure 40c 150 MHz ^{13}C NMR spectrum of compound D-5 (in $CDCl_3$) [δ_c 110.0 – 137.0 ppm]

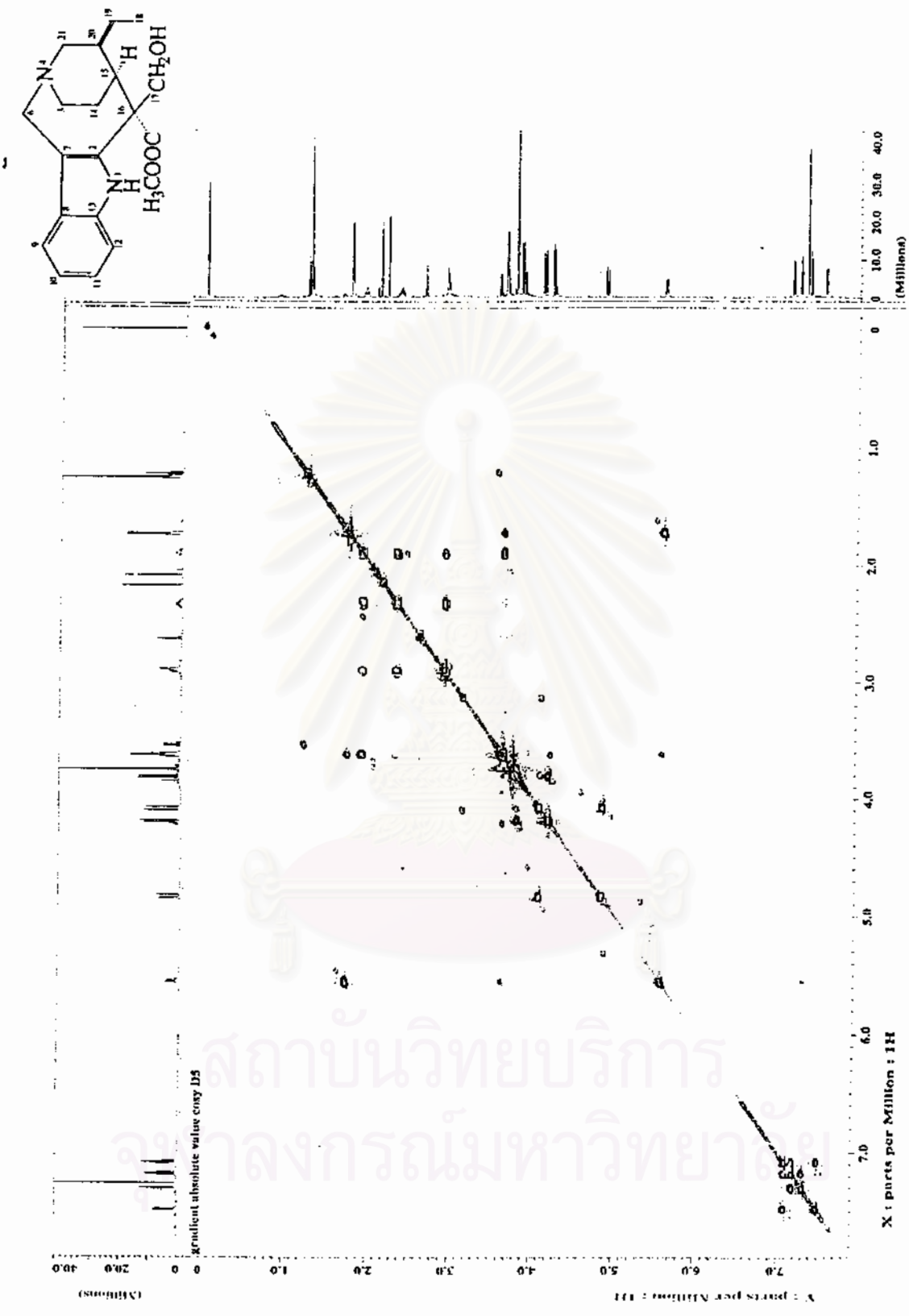


Figure 41a ^{1}H-^{1}H COSY spectrum of compound D-5 (in $CDCl_3$)

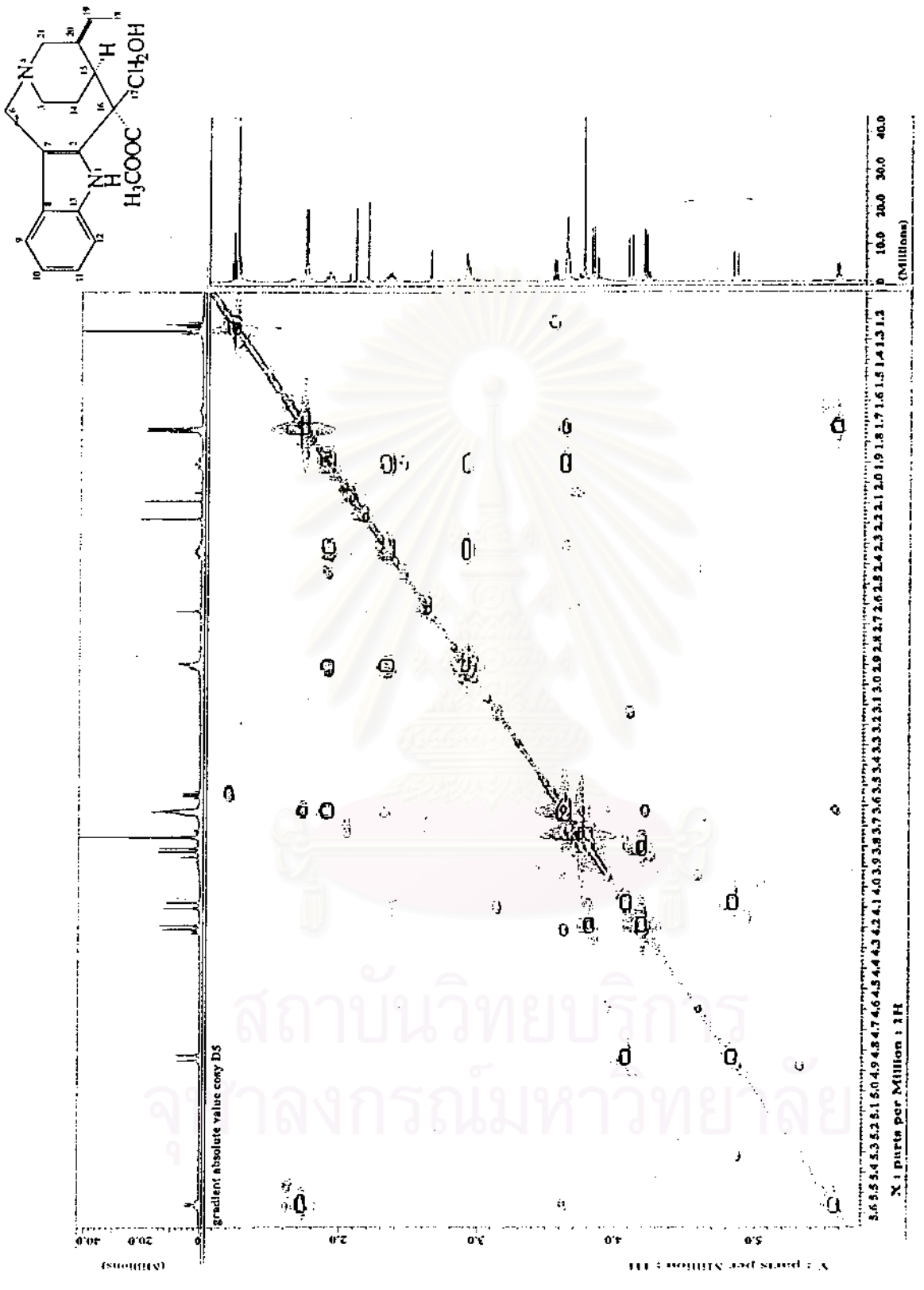


Figure 41b ^{1}H-^{1}H COSY spectrum of compound D-5 (in $CDCl_3$) [δ_H 1.20 – 5.60 ppm, δ_H 1.00 – 5.80 ppm]

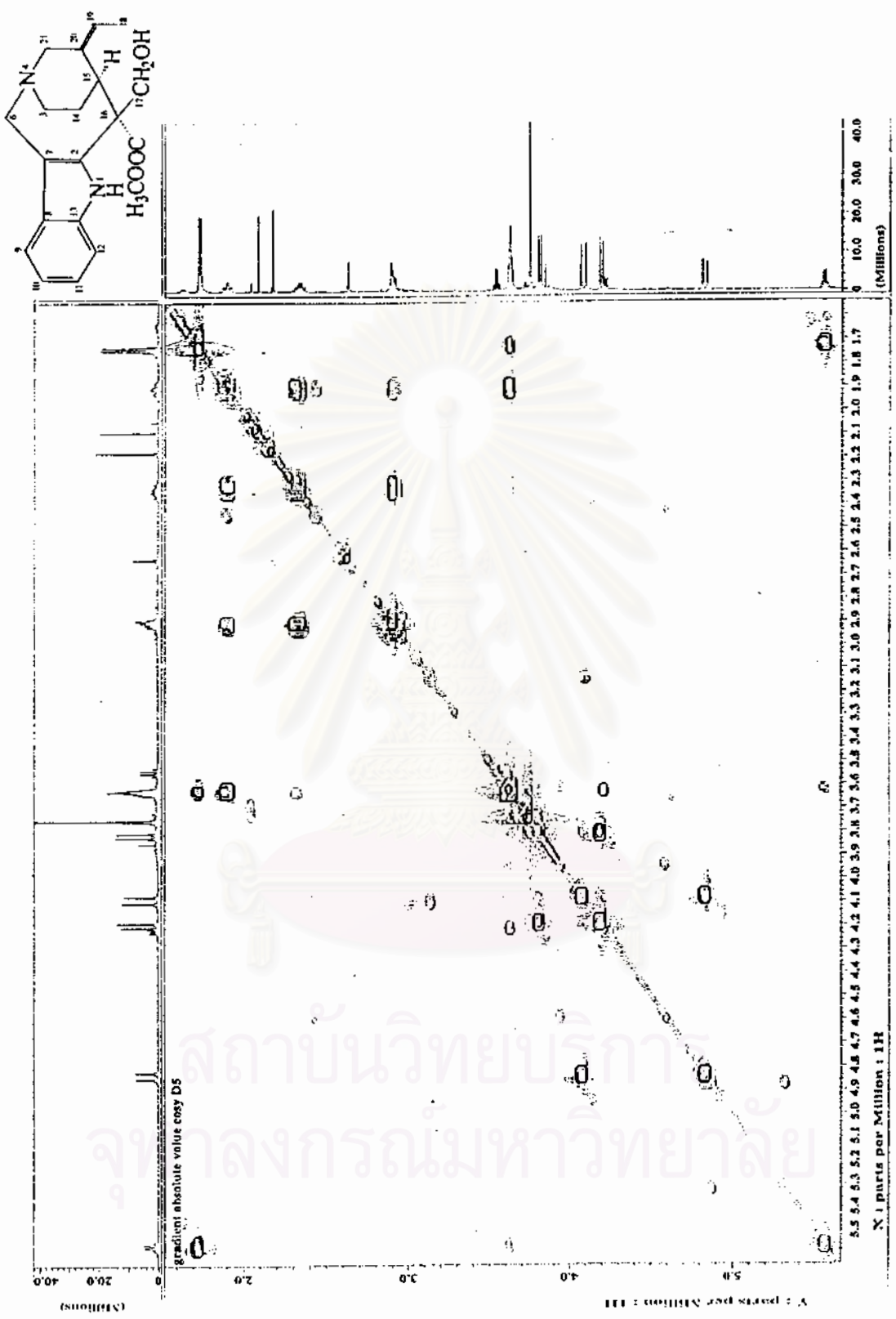


Figure 41c ^{1}H-^{1}H COSY spectrum of compound D-5 (in $CDCl_3$) [δ_H 1.60 – 5.60 ppm, δ_H 1.50 – 5.60 ppm]

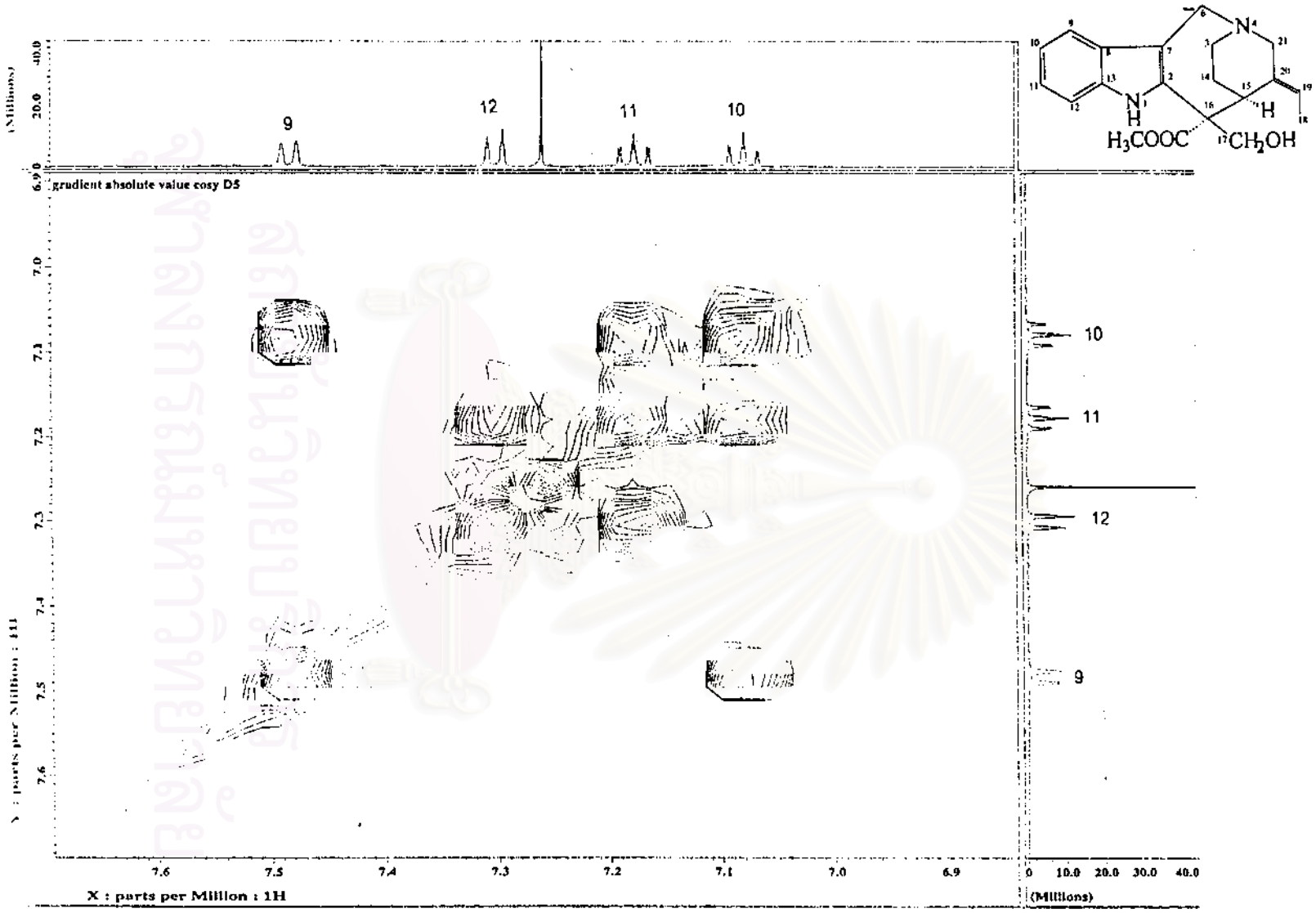


Figure 41d ^{1}H-^{1}H COSY spectrum of compound D-5 (in $CDCl_3$) [δ_H 6.84 – 7.69 ppm, δ_H 6.90 – 7.69 ppm]

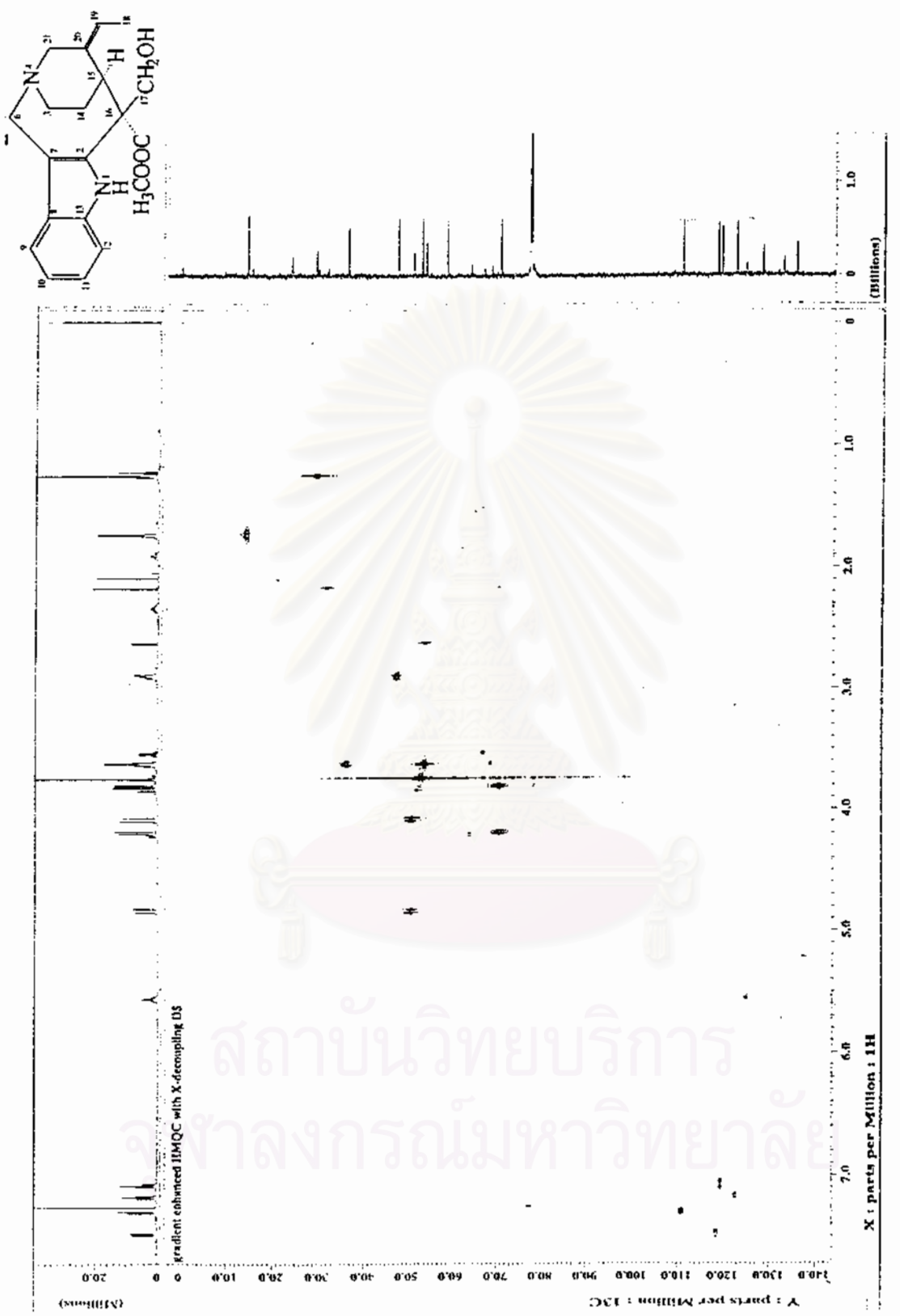


Figure 42a HMQC spectrum of compound D-5 (in $CDCl_3$)

Figure 42b HMQC spectrum of compound D-5 (in $CDCl_3$) [δ_H 1.10 – 4.90 ppm, δ_C 10.0 – 74.0 ppm]

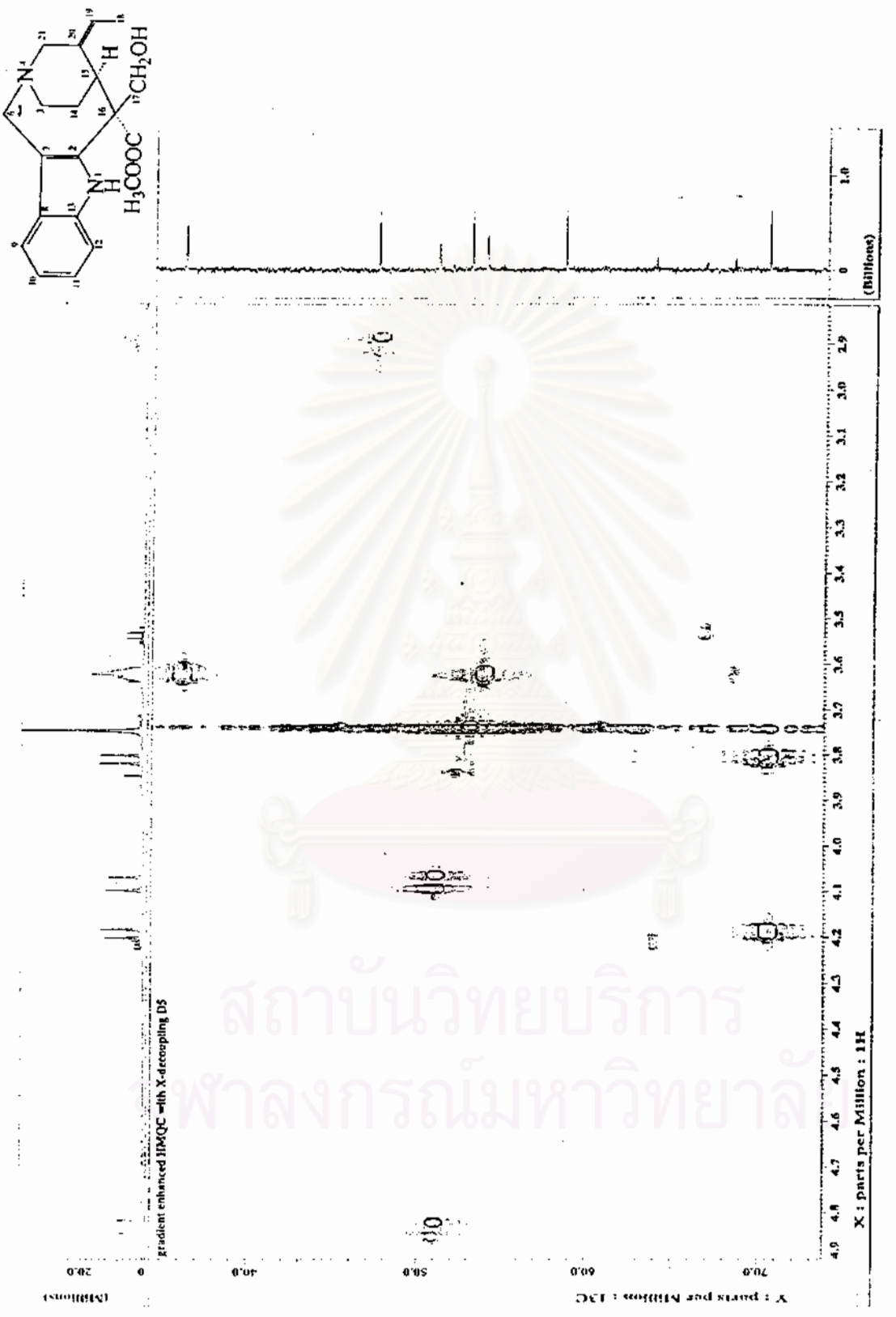


Figure 42c HMQC spectrum of compound D-5 (in $CDCl_3$) [δ_H 2.81 – 4.90 ppm, δ_c 34.0 – 74.0 ppm]

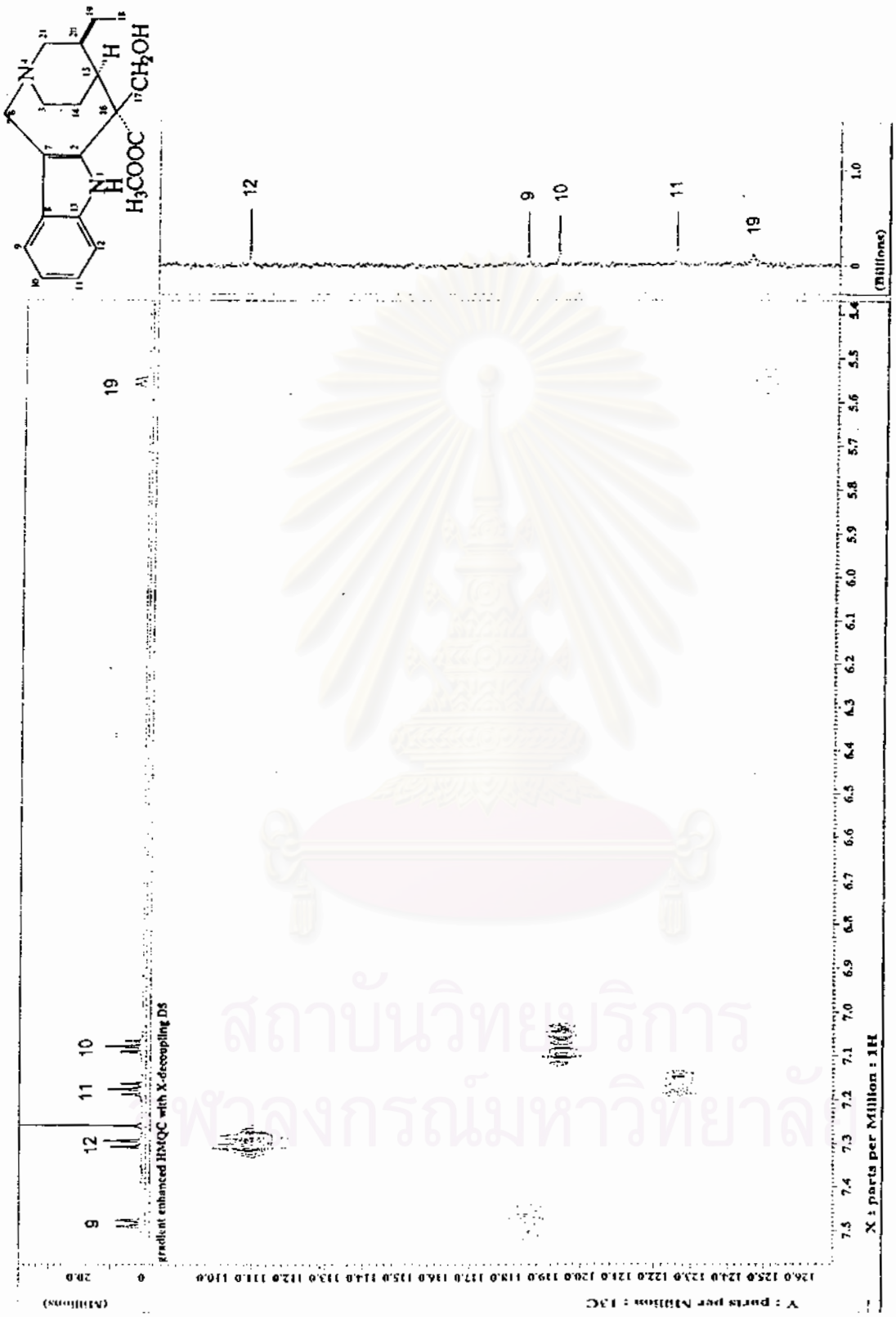


Figure 42d HMQC spectrum of compound D-5 (in $CDCl_3$) [δ_H 5.40 – 7.50 ppm, δ_C 110.0 – 126.0 ppm]

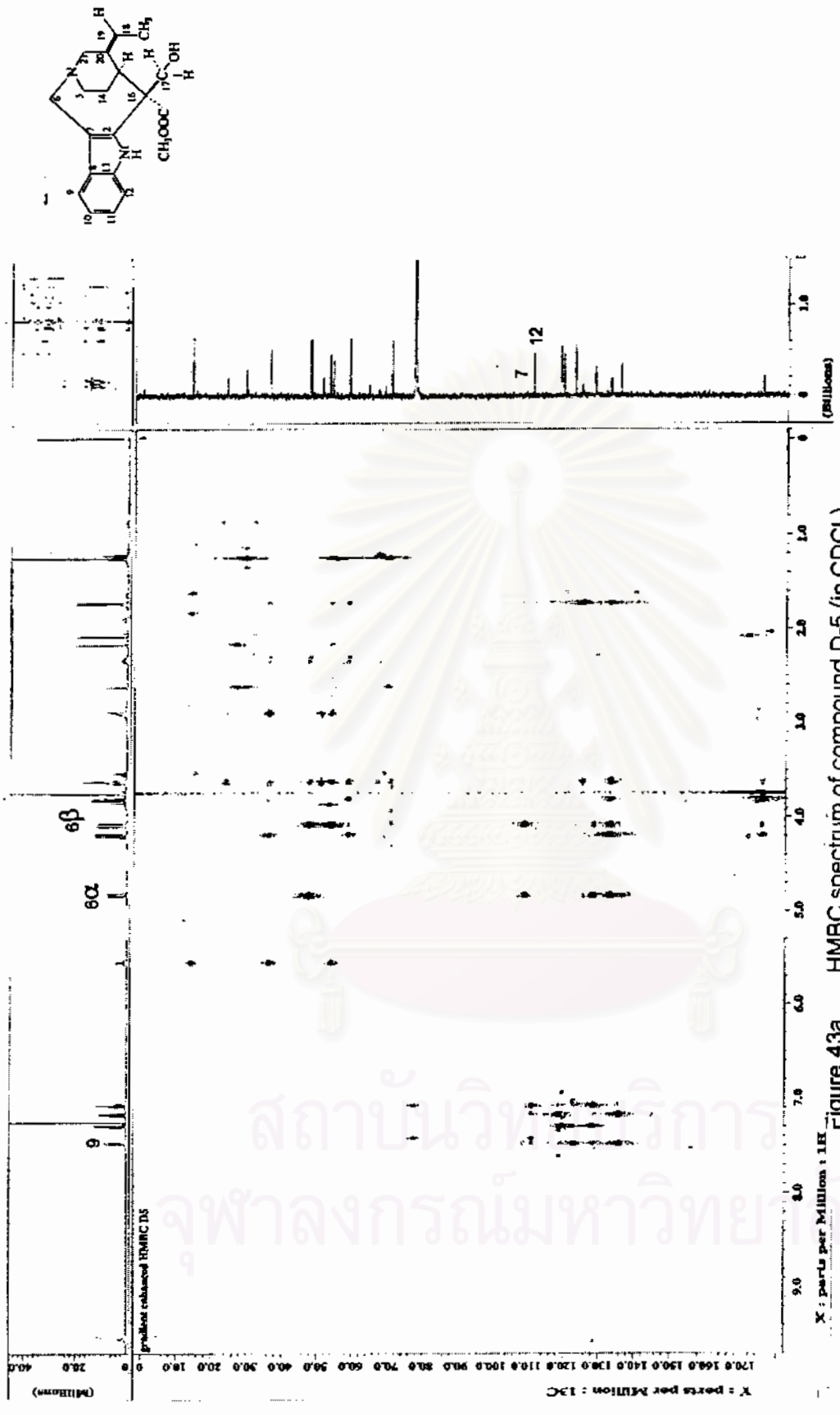


Figure 43a HMBC spectrum of compound D-5 (in $CDCl_3$)

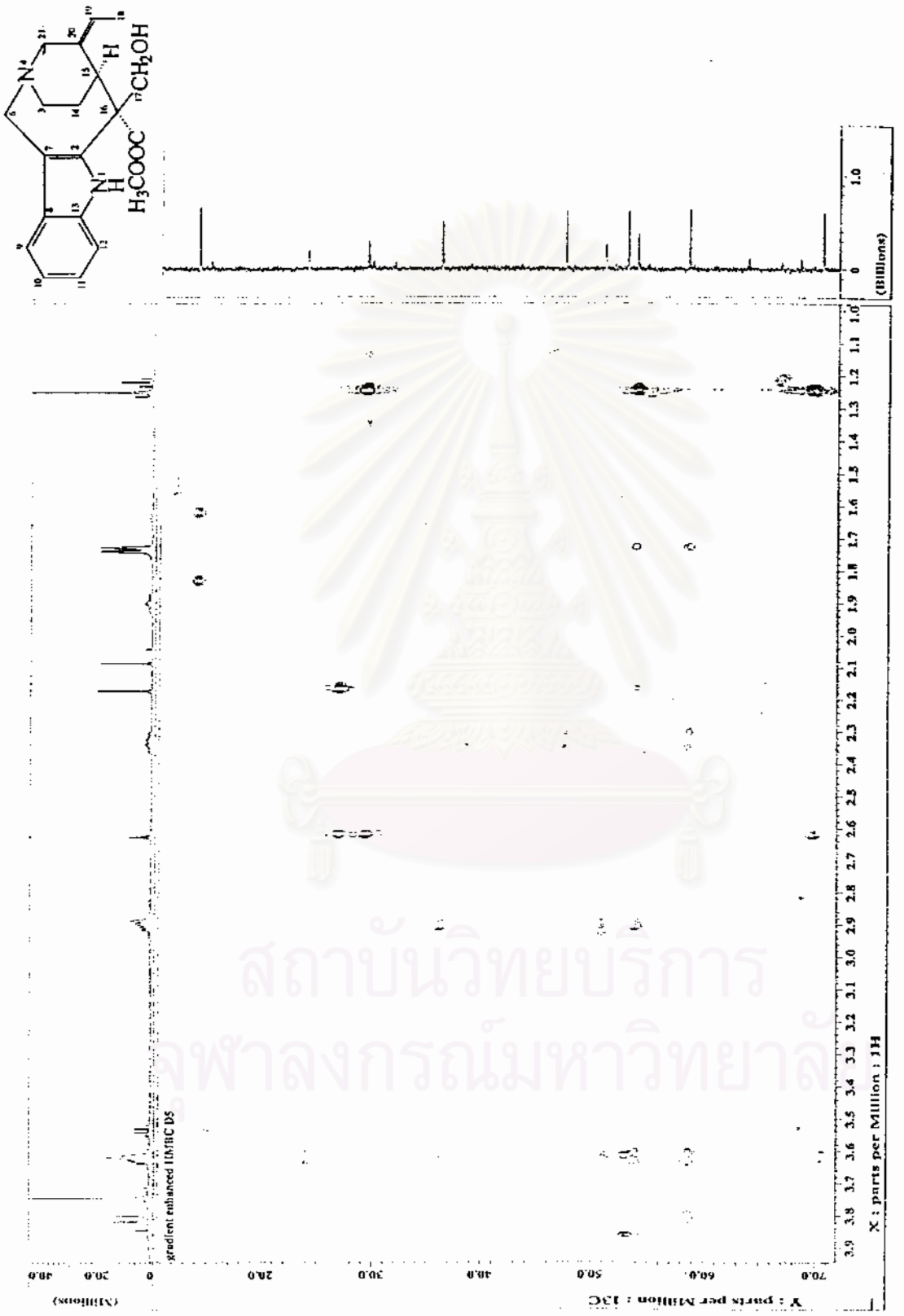


Figure 43b HMBC spectrum of compound D-5 (in $CDCl_3$) [δ_H 1.00 – 3.90 ppm, δ_C 10.0 – 70.0 ppm]

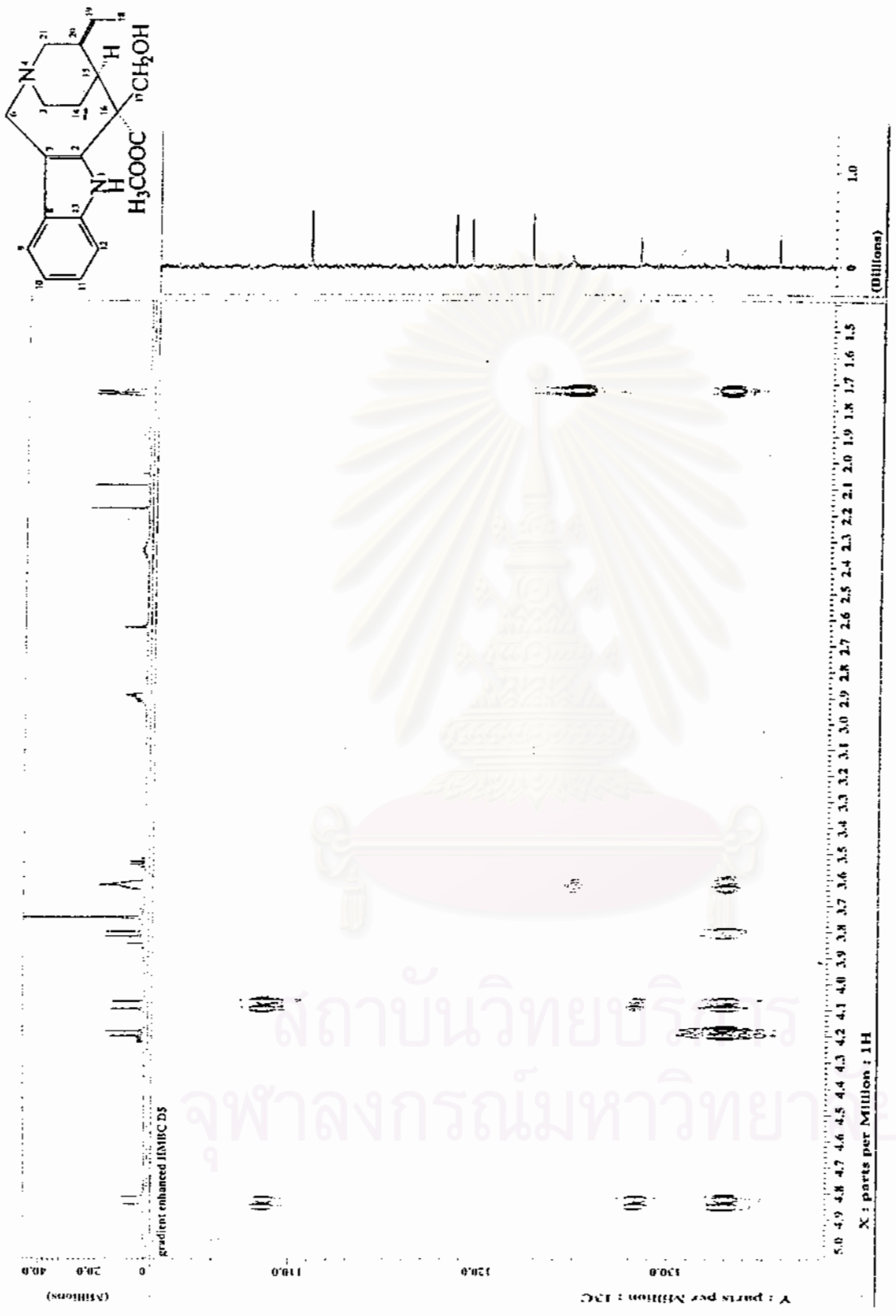


Figure 43c HMBC spectrum of compound D-5 (in $CDCl_3$) [δ_H 1.50 – 5.00 ppm, δ_C 103.0 – 138.0 ppm]

Figure 43d HMBC spectrum of compound D-5 (in $CDCl_3$) [δ_H 2.80 – 4.40 ppm, δ_C 168.0 – 180.0 ppm]

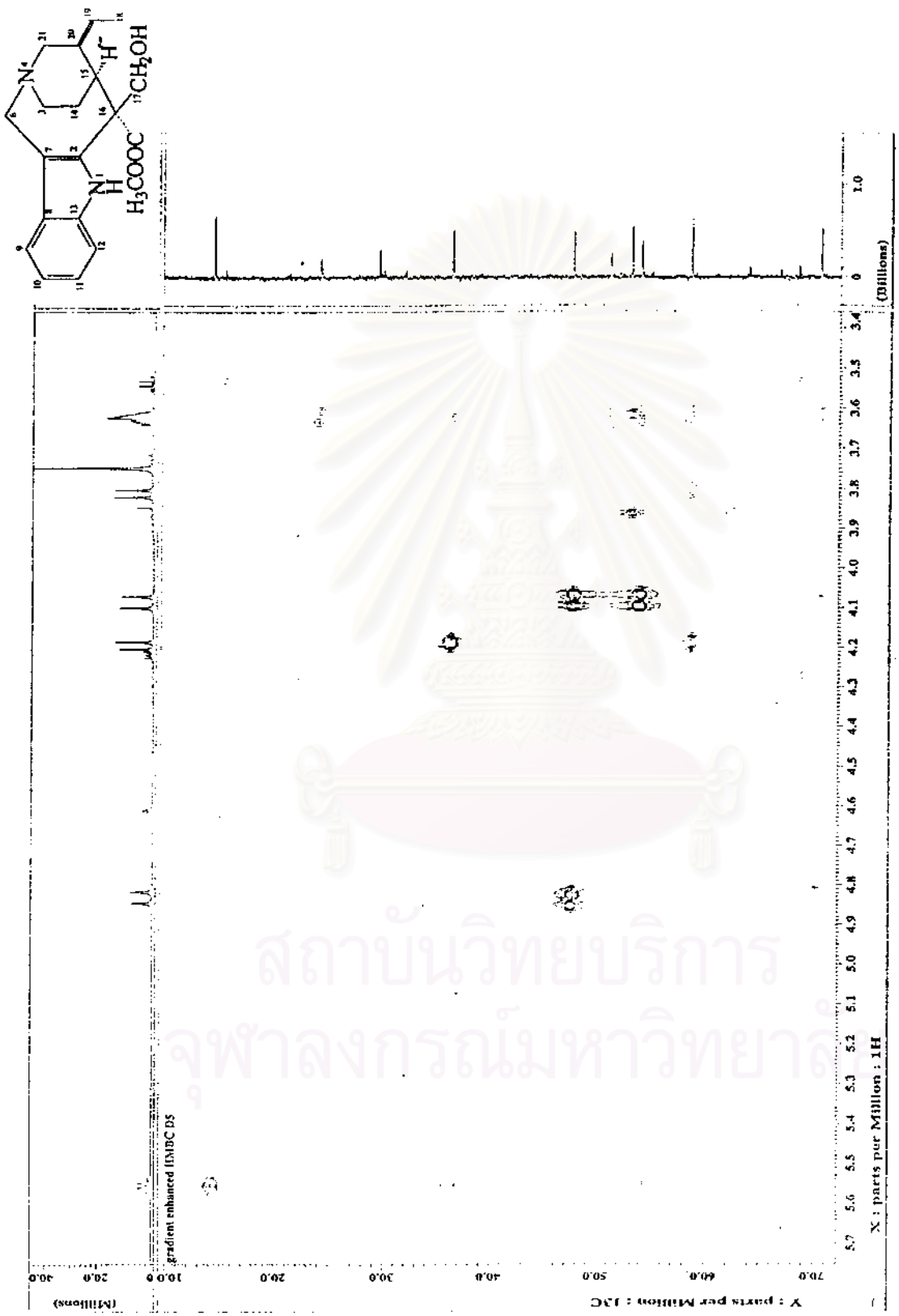
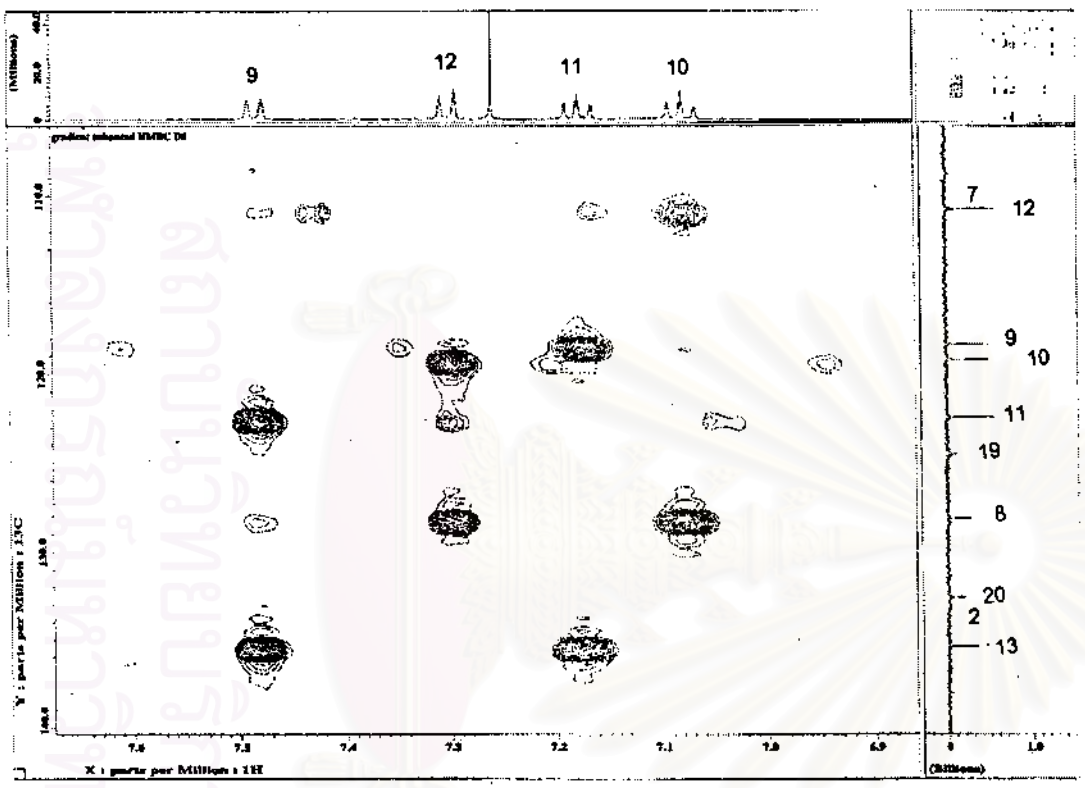


Figure 43e HMBC spectrum of compound D-5 (in $CDCl_3$) [δ_H 3.40 – 5.70 ppm, δ_C 10.0 – 70.0 ppm]



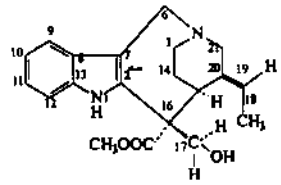


Figure 43f HMBC spectrum of compound D-5 (in $CDCl_3$) [δ_H 6.90-7.60 ppm, δ_C 110.0-140.0 ppm]

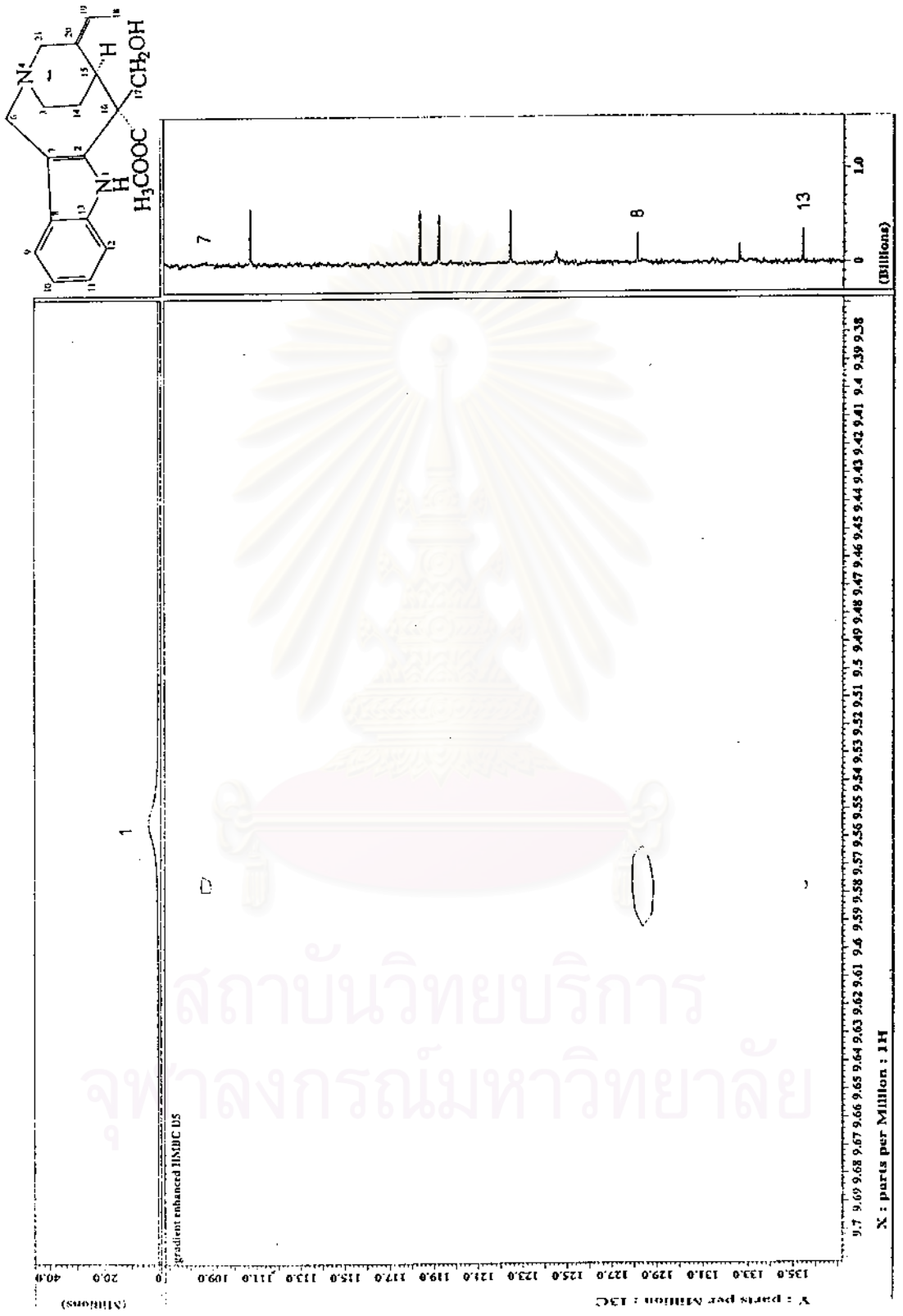


Figure 43g HMBC spectrum of compound D-5 (in $CDCl_3$) [δ_H 9.38 – 9.70 ppm, δ_C 107.0 – 137.0 ppm]

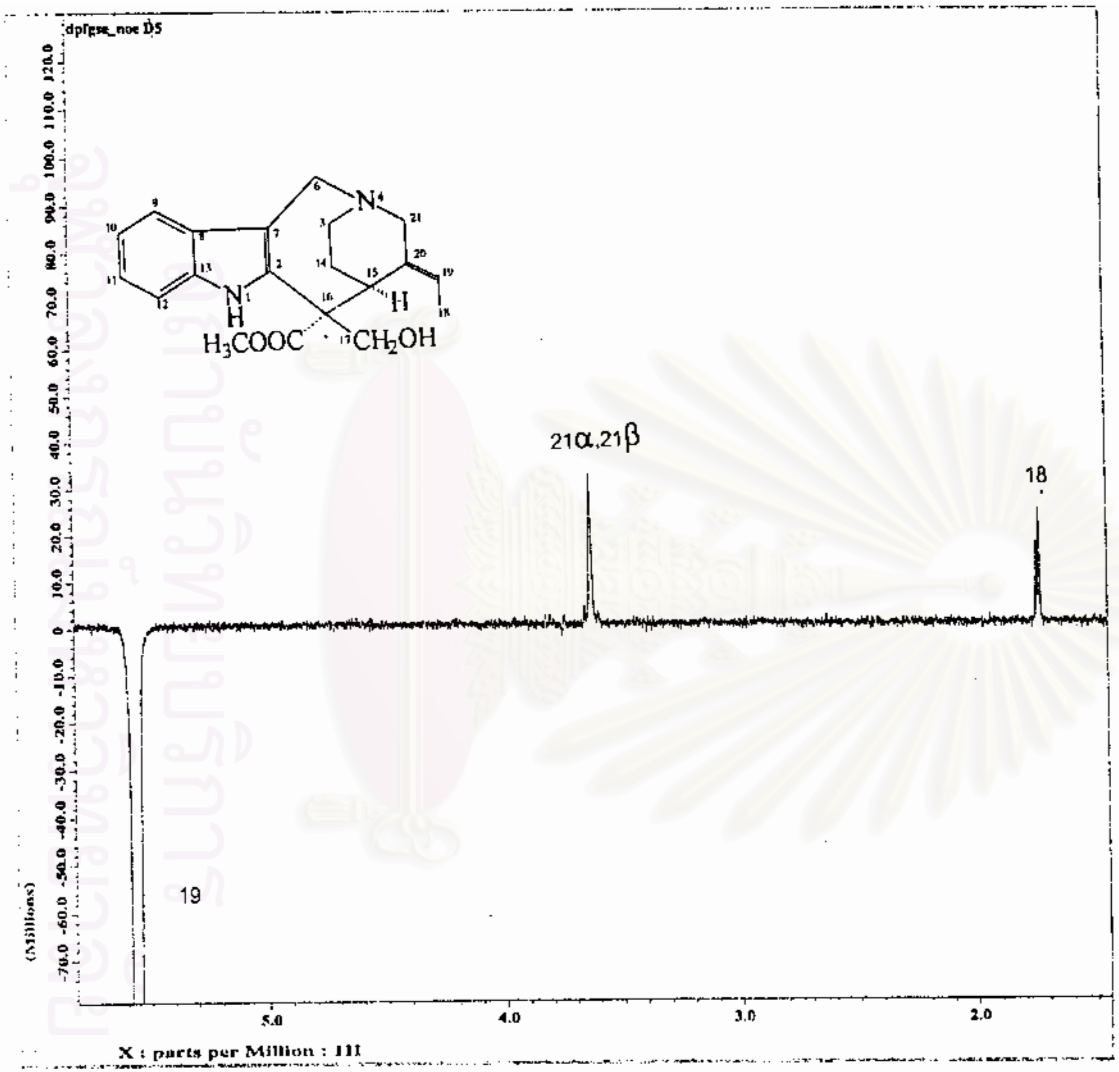


Figure 44 NOE difference spectrum of compound D-5 (in $CDCl_3$) [irradiated H-19, δ_H 1.50-5.80 ppm]

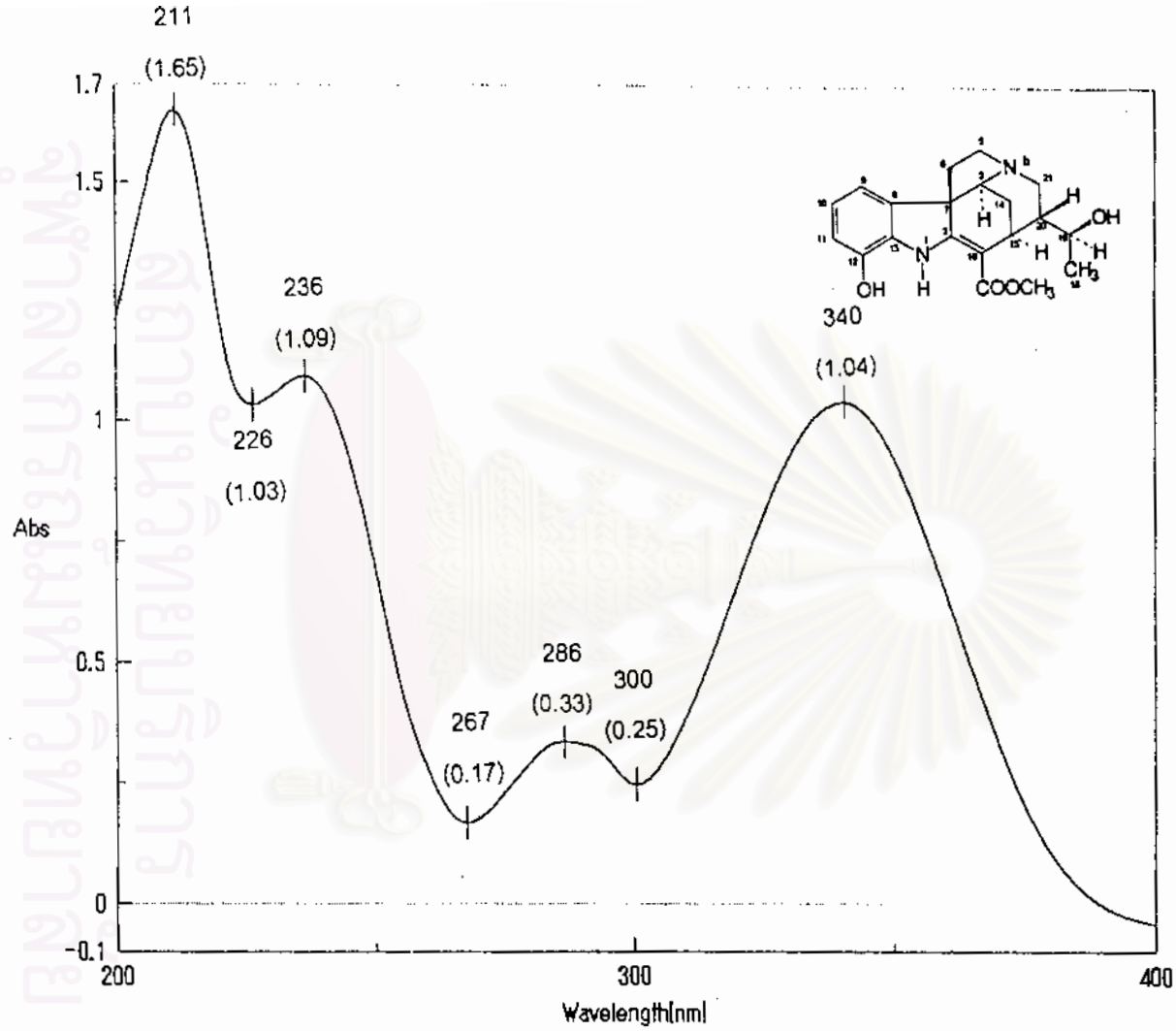


Figure 45 UV spectrum of compound D-6 (in ethanol)

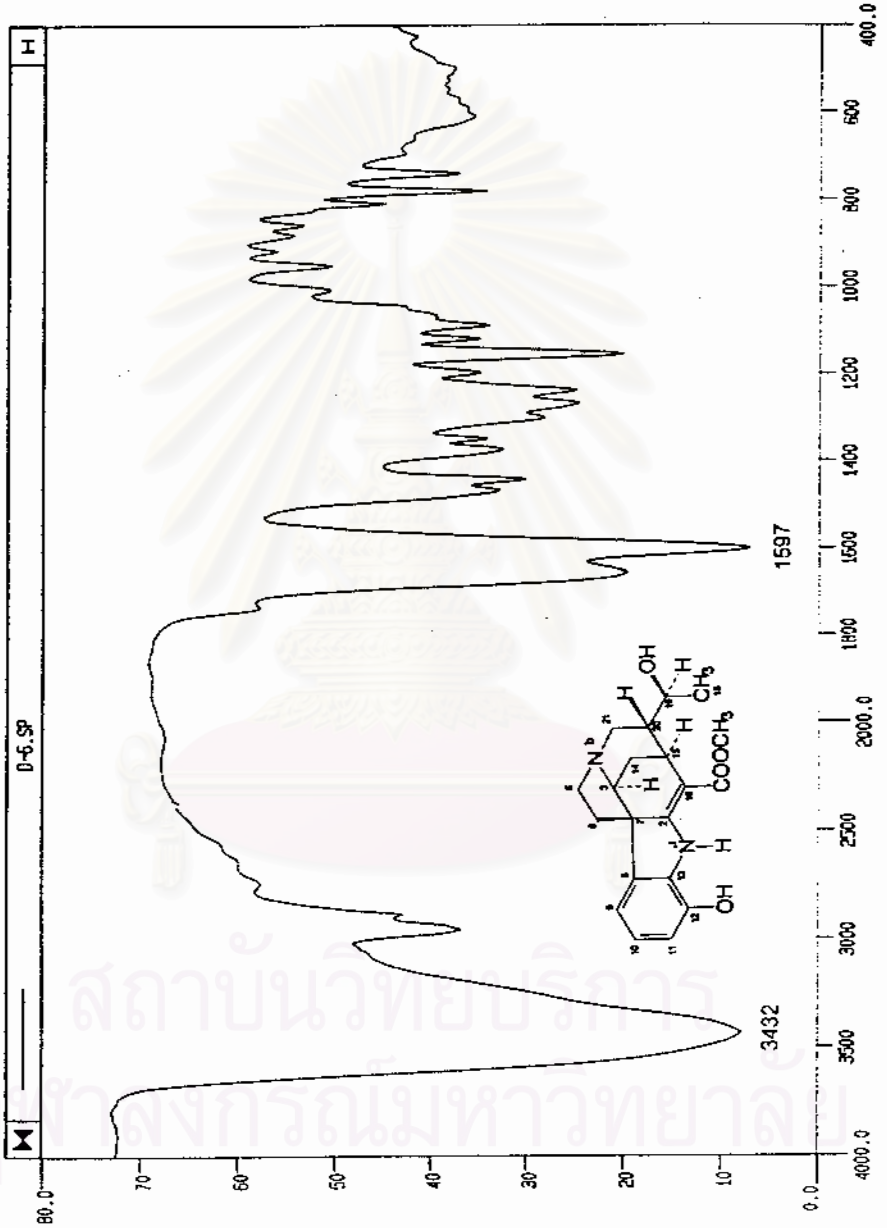


Figure 46 IR spectrum of compound D-6 (KBr disc)

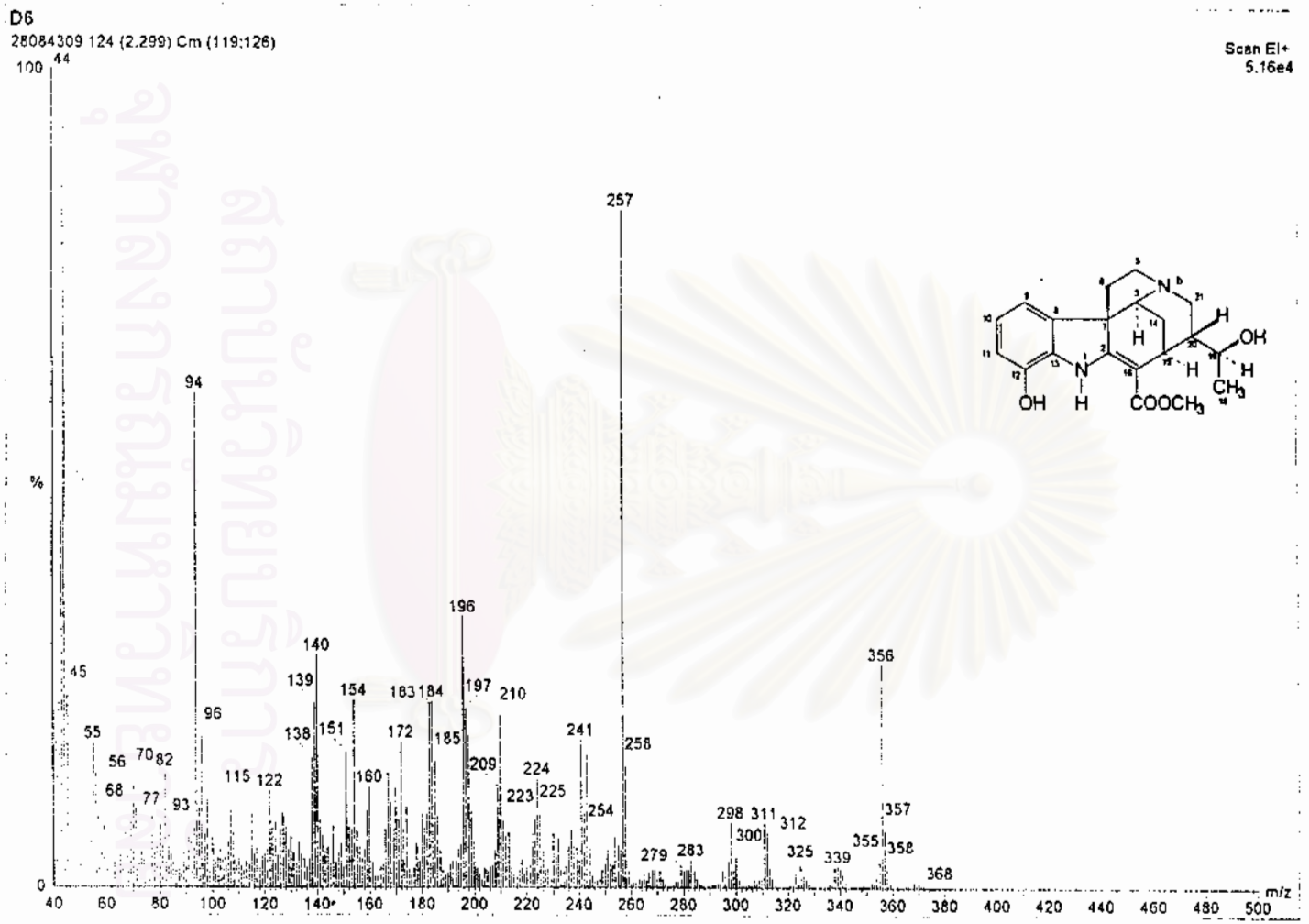


Figure 47 EI mass spectrum of compound D-6

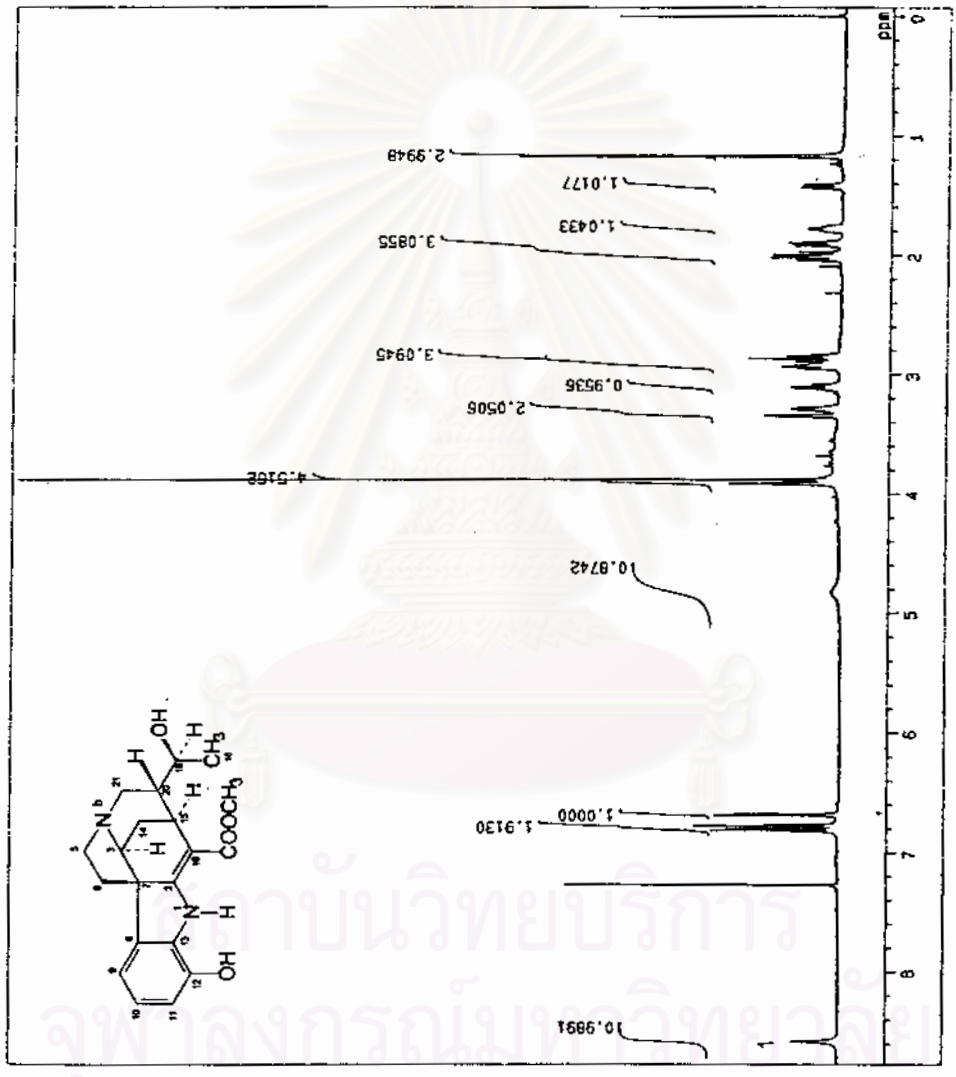


Figure 48a 500 MHz ^{1}H NMR spectrum of compound D-6 (in $CDCl_3$)

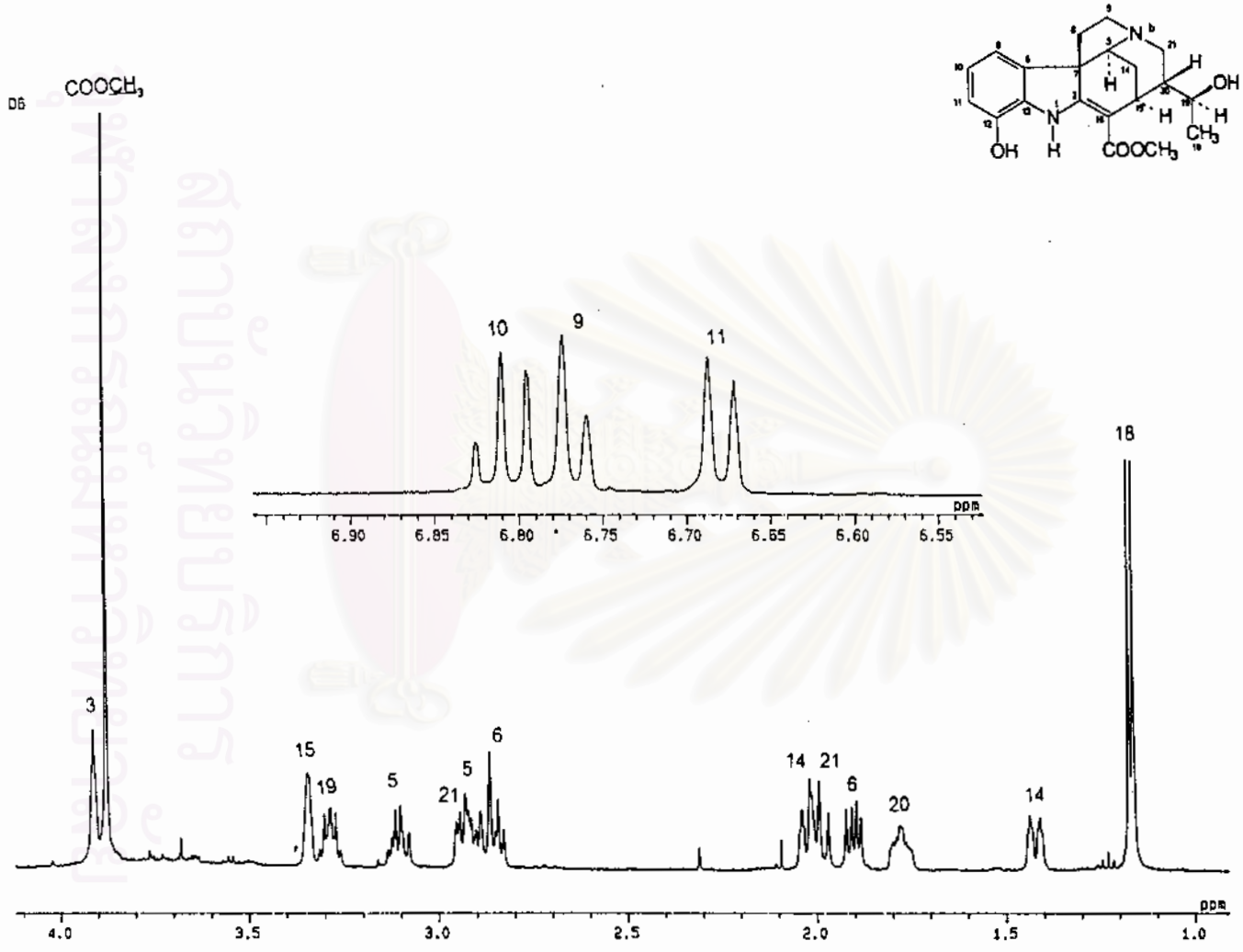


Figure 48b 500 MHz ^{1}H NMR spectrum of compound D-6 (in $CDCl_3$) [δ_H 1.00 – 4.00, 6.55 – 6.90 ppm]

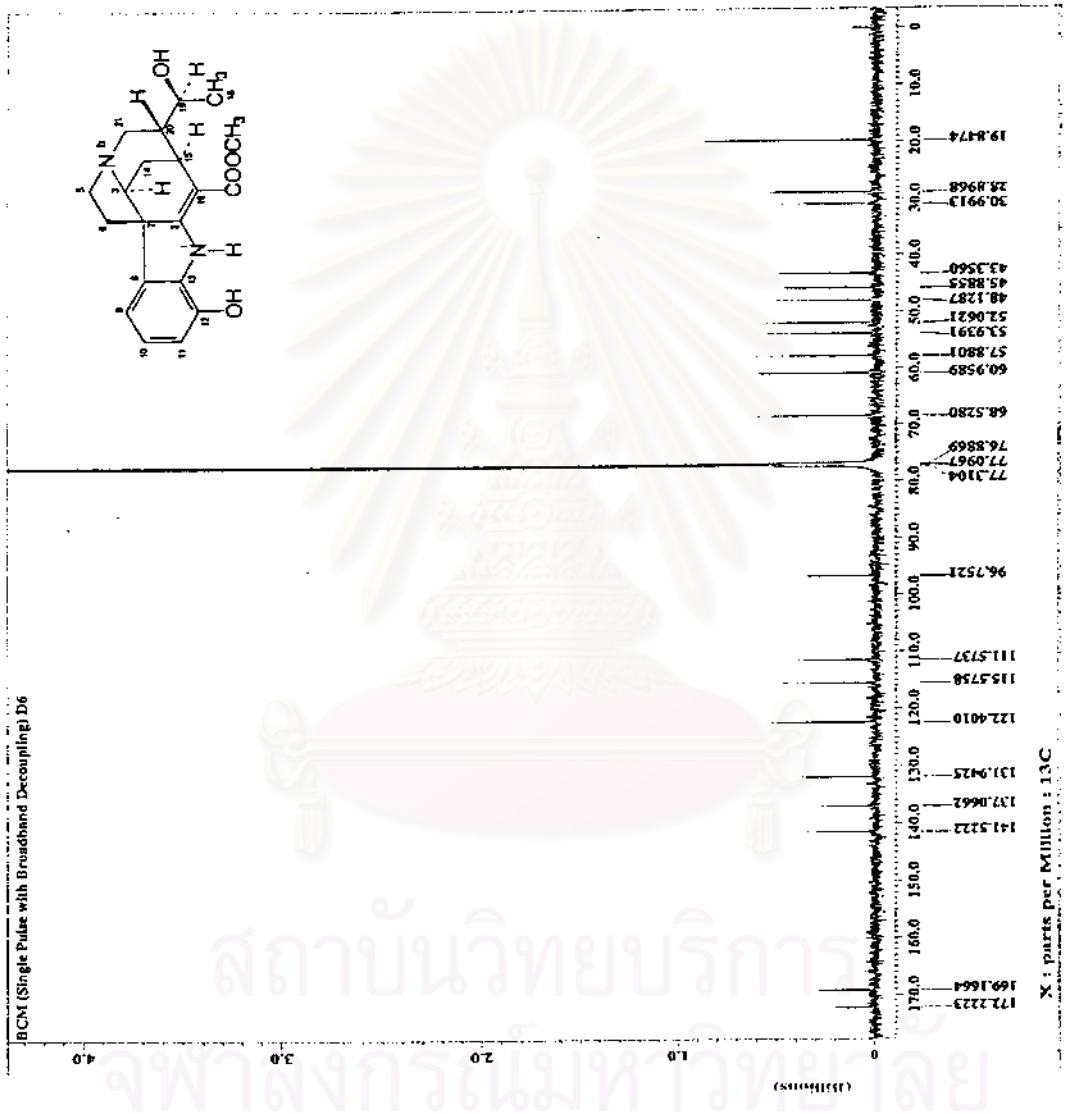


Figure 49a 150 MHz ^{13}C NMR spectrum of compound D-6 (in $CDCl_3$)

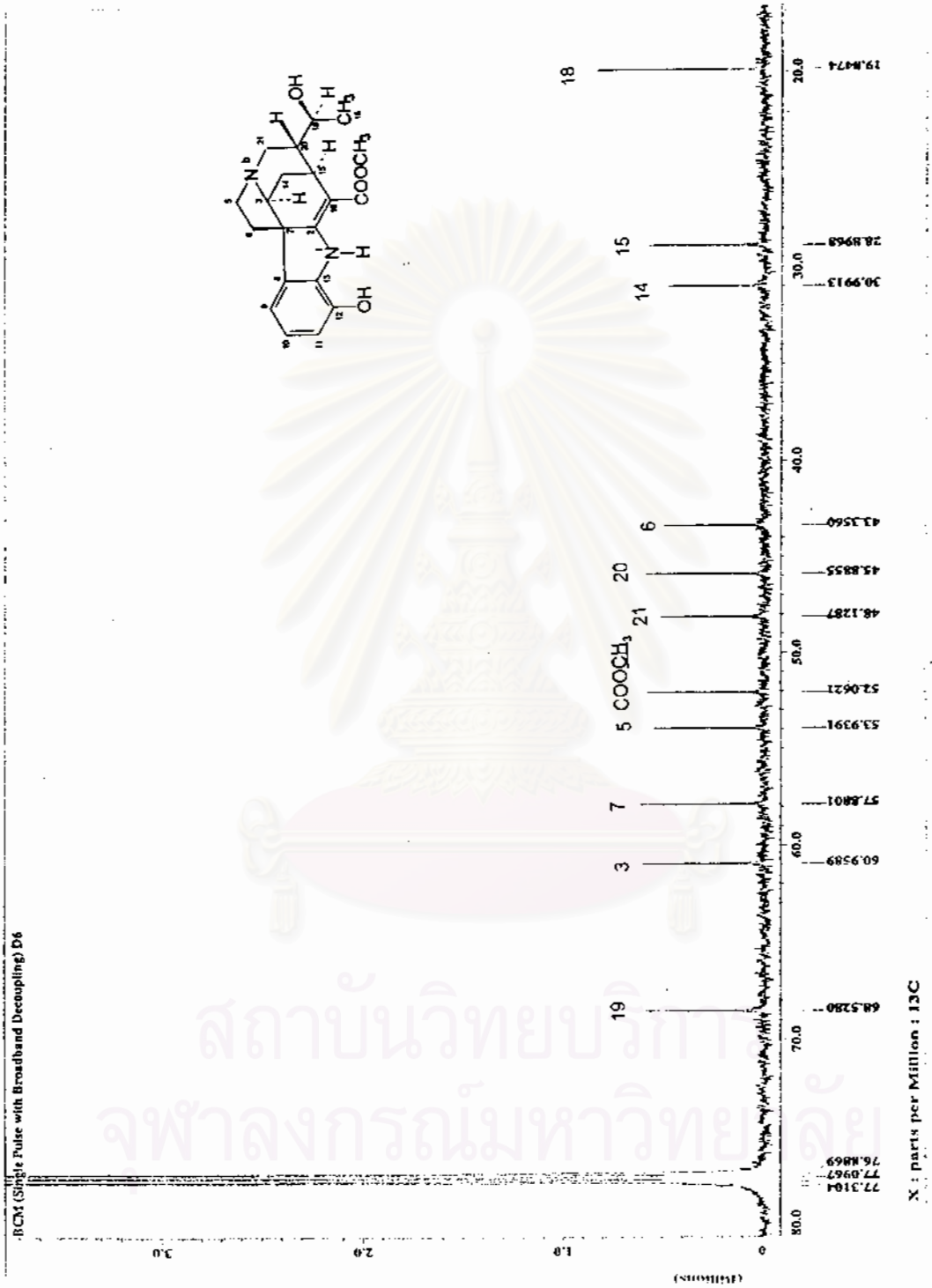


Figure 49b 150 MHz ^{13}C NMR spectrum of compound D-6 (in $CDCl_3$) [δ_c 17.0 – 80.0 ppm]

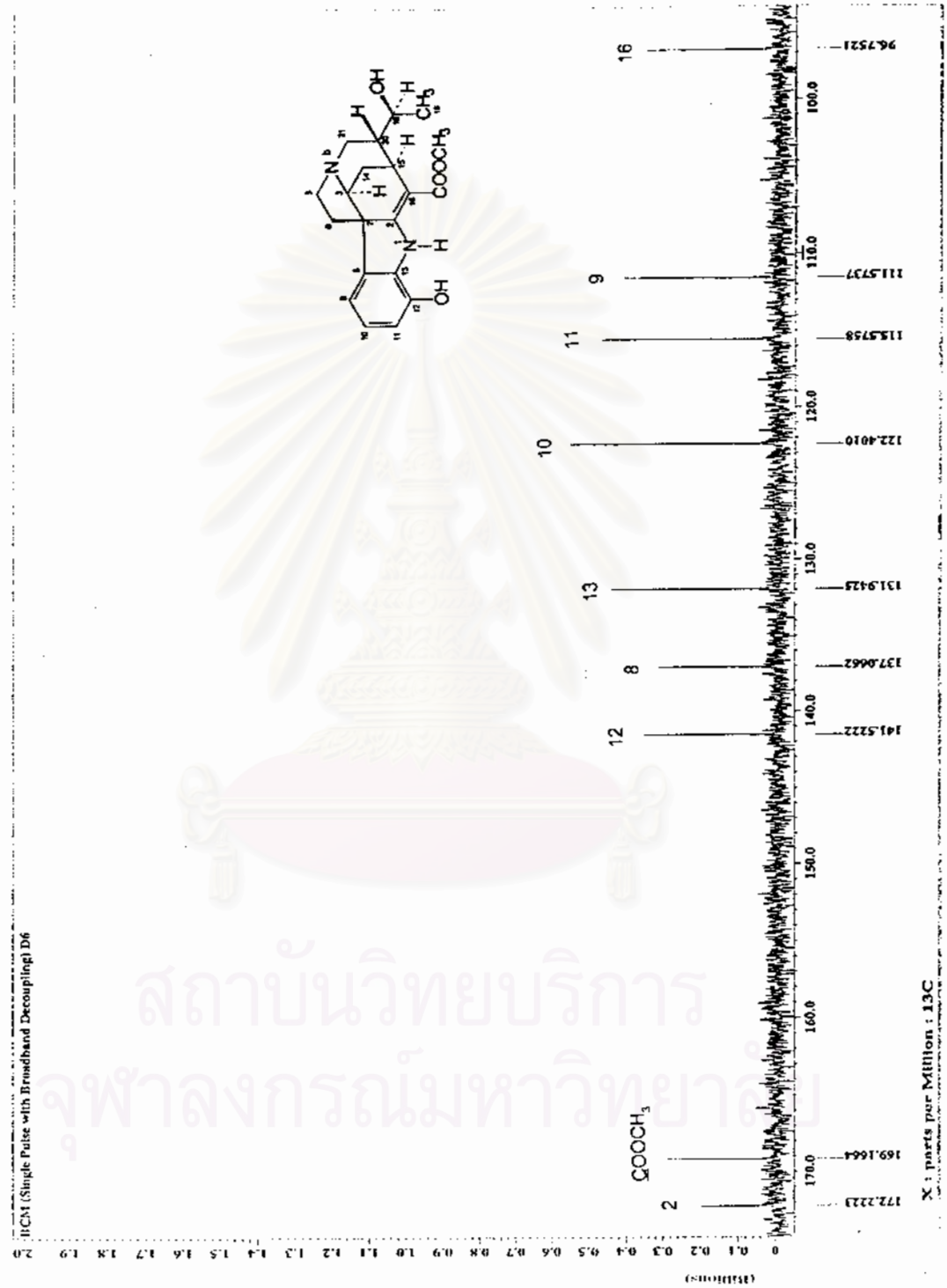


Figure 49c 150 MHz ^{13}C NMR spectrum of compound D-6 (in $CDCl_3$) [δ_c 94.0 – 174.0 ppm]

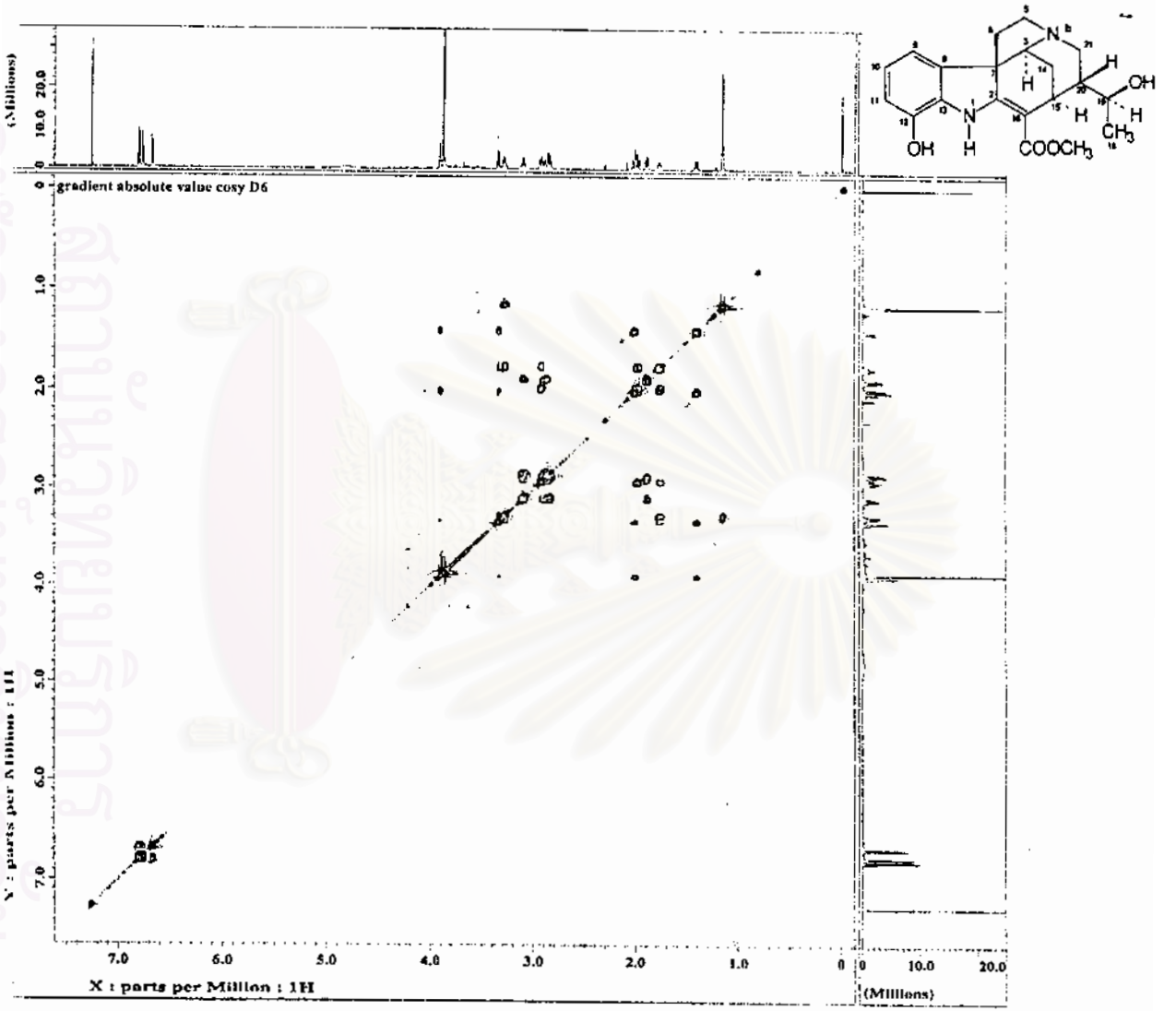


Figure 50a ^{1}H-^{1}H COSY spectrum of compound D-6 (in $CDCl_3$)

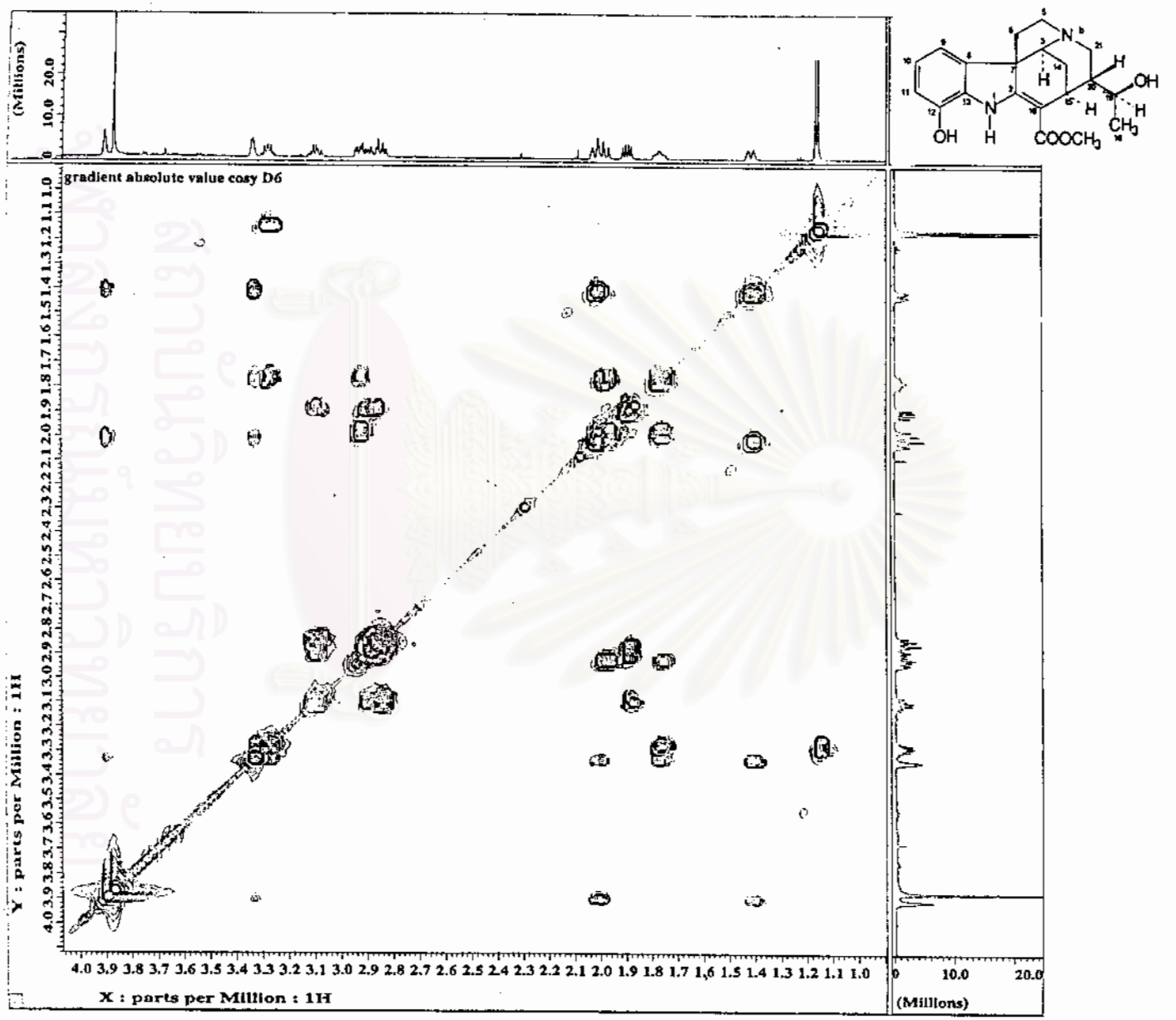


Figure 50b ^{1}H-^{1}H COSY spectrum of compound D-6 (in $CDCl_3$) [δ_H 1.00 – 4.00 ppm, δ_H 1.00 –4.00 ppm]

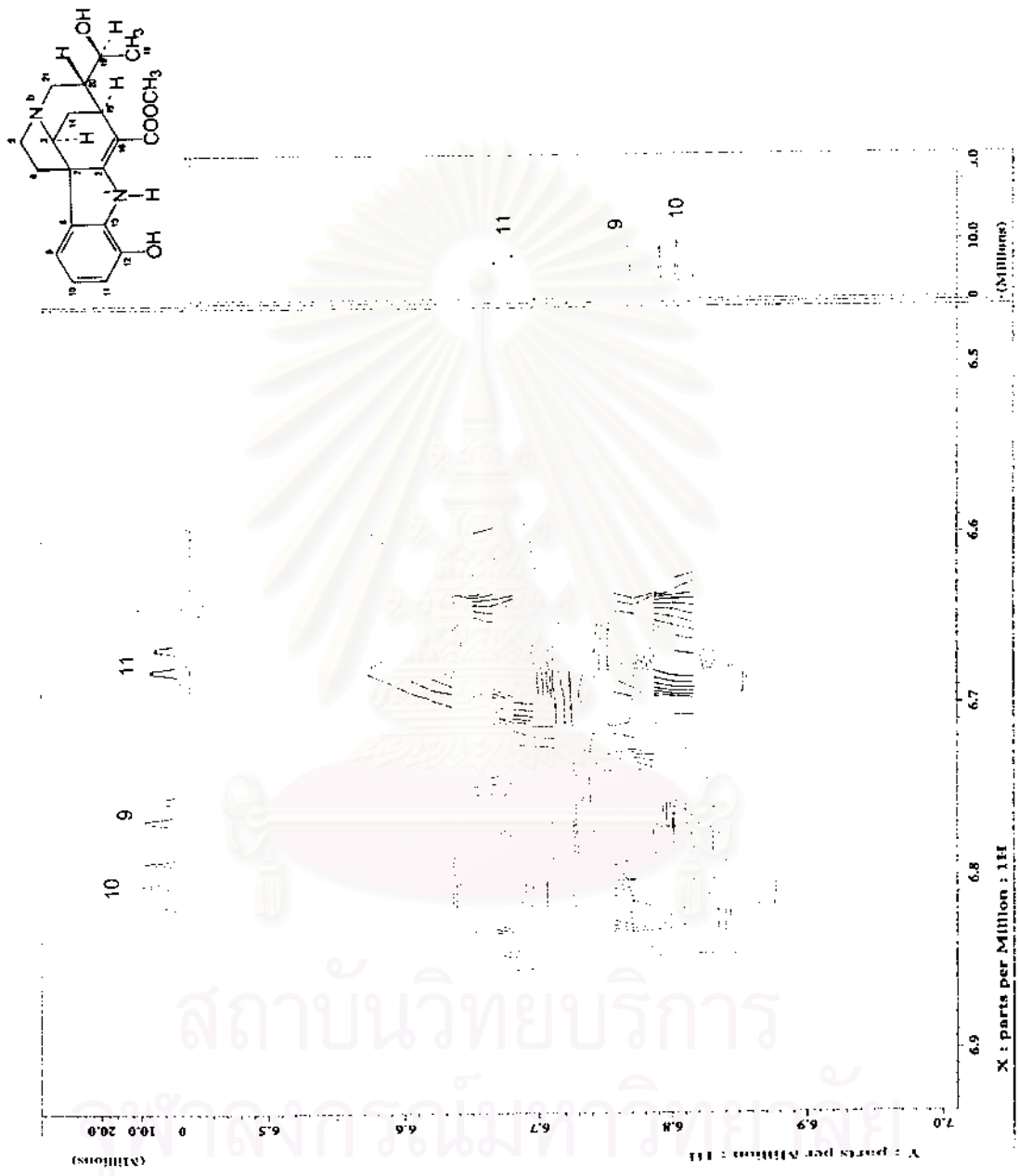


Figure 50c ^1H-^1H COSY spectrum of compound D-6 (in $CDCl_3$) [δ_H 6.50 – 6.90 ppm, δ_H 6.50 – 7.00 ppm]

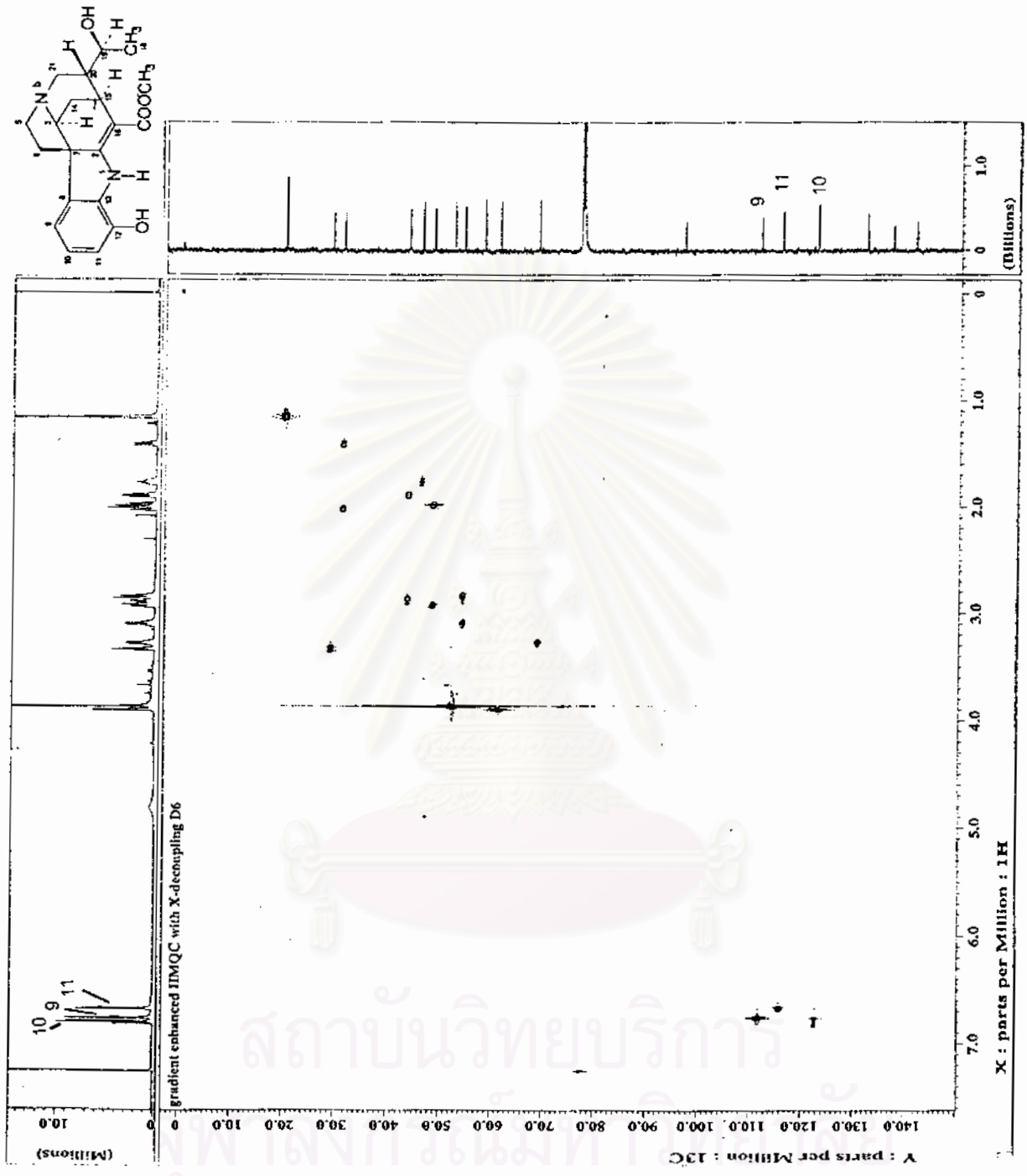


Figure 51a HMQC spectrum of compound D-6 (in $CDCl_3$)

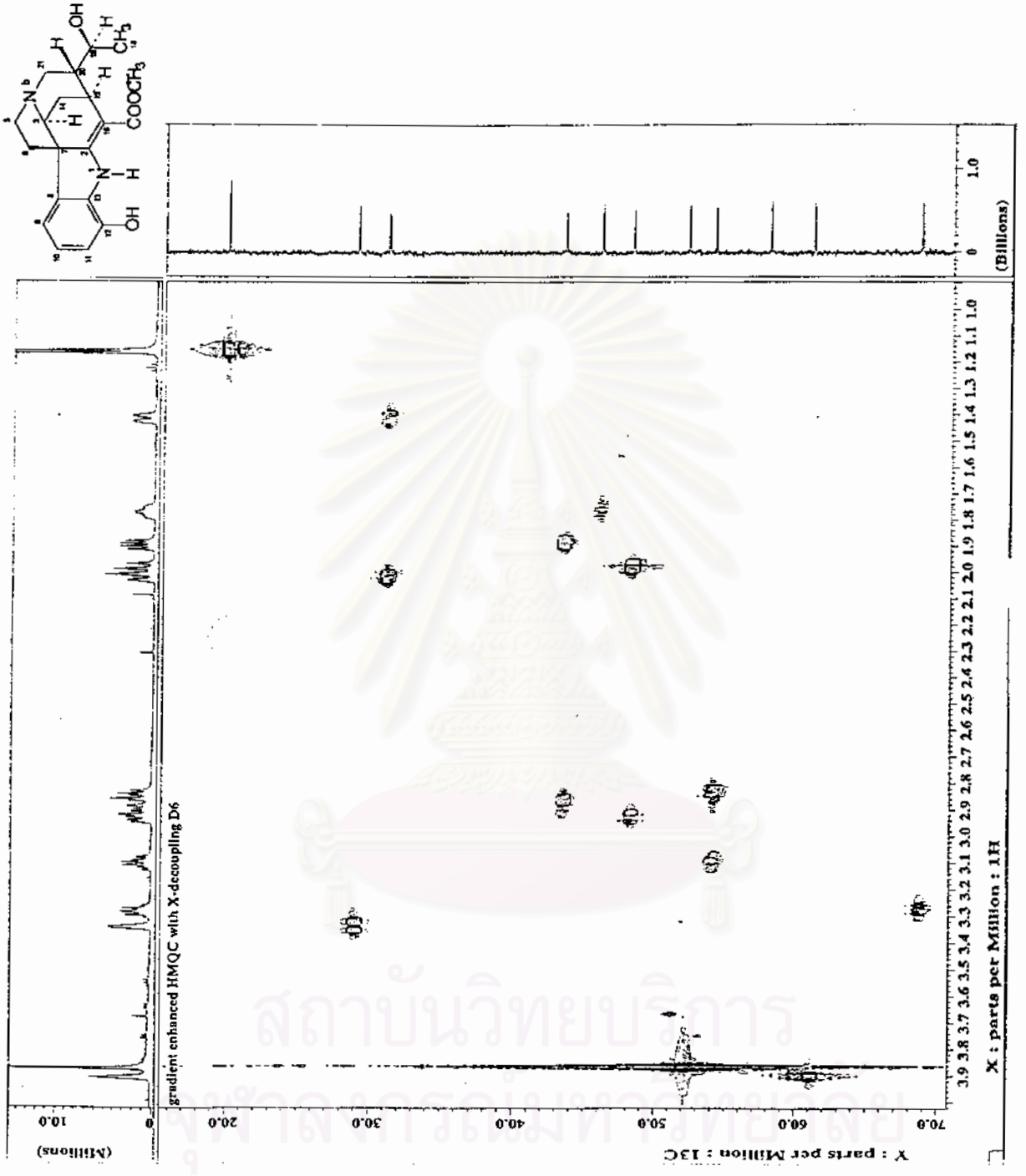


Figure 51b HMQC spectrum of compound D-6 (in $CDCl_3$) [δ_H 1.00 –4.00 ppm, δ_C 15.0 – 70.0 ppm]

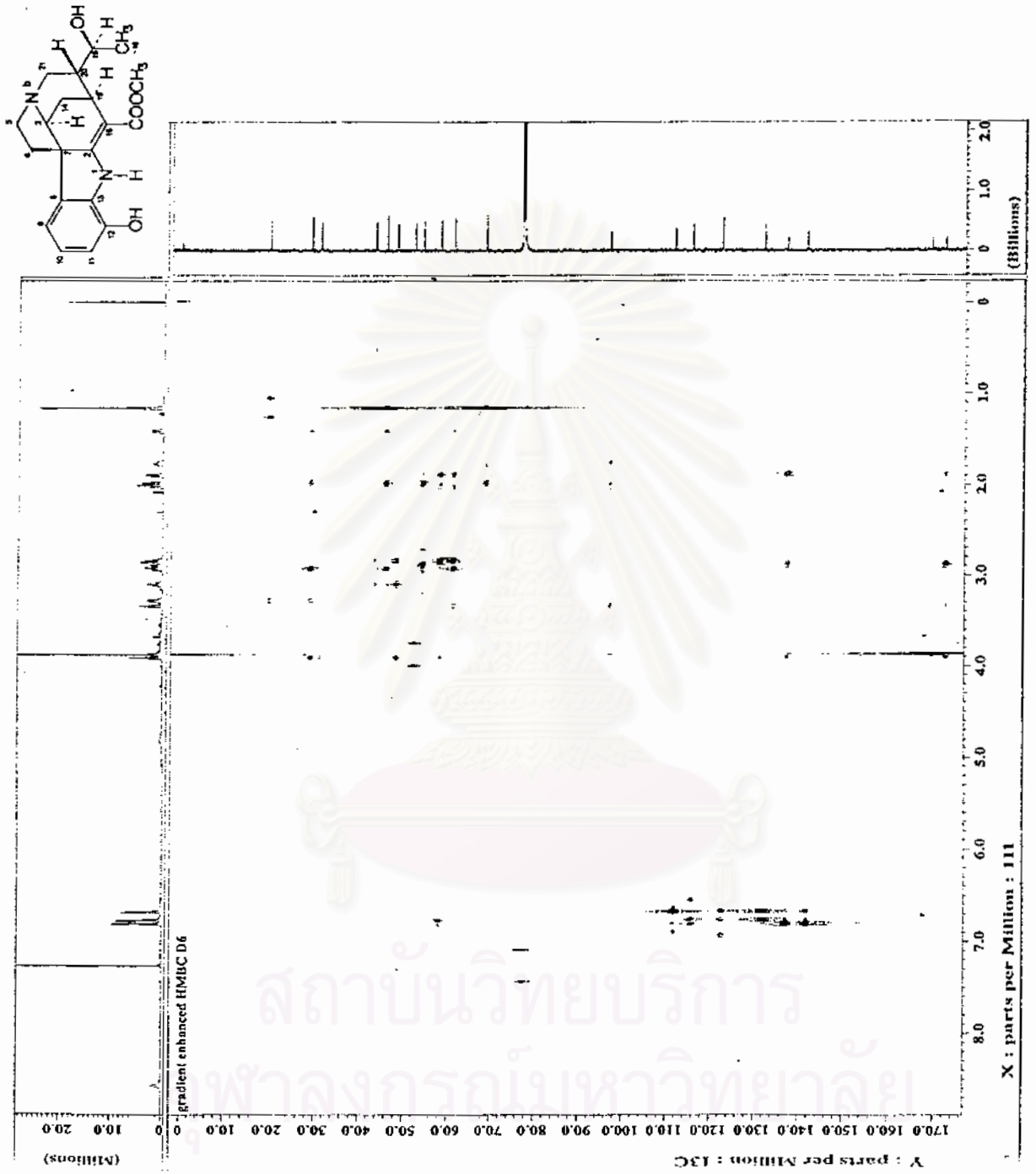


Figure 52a HMBC spectrum of compound D-6 (in $CDCl_3$)

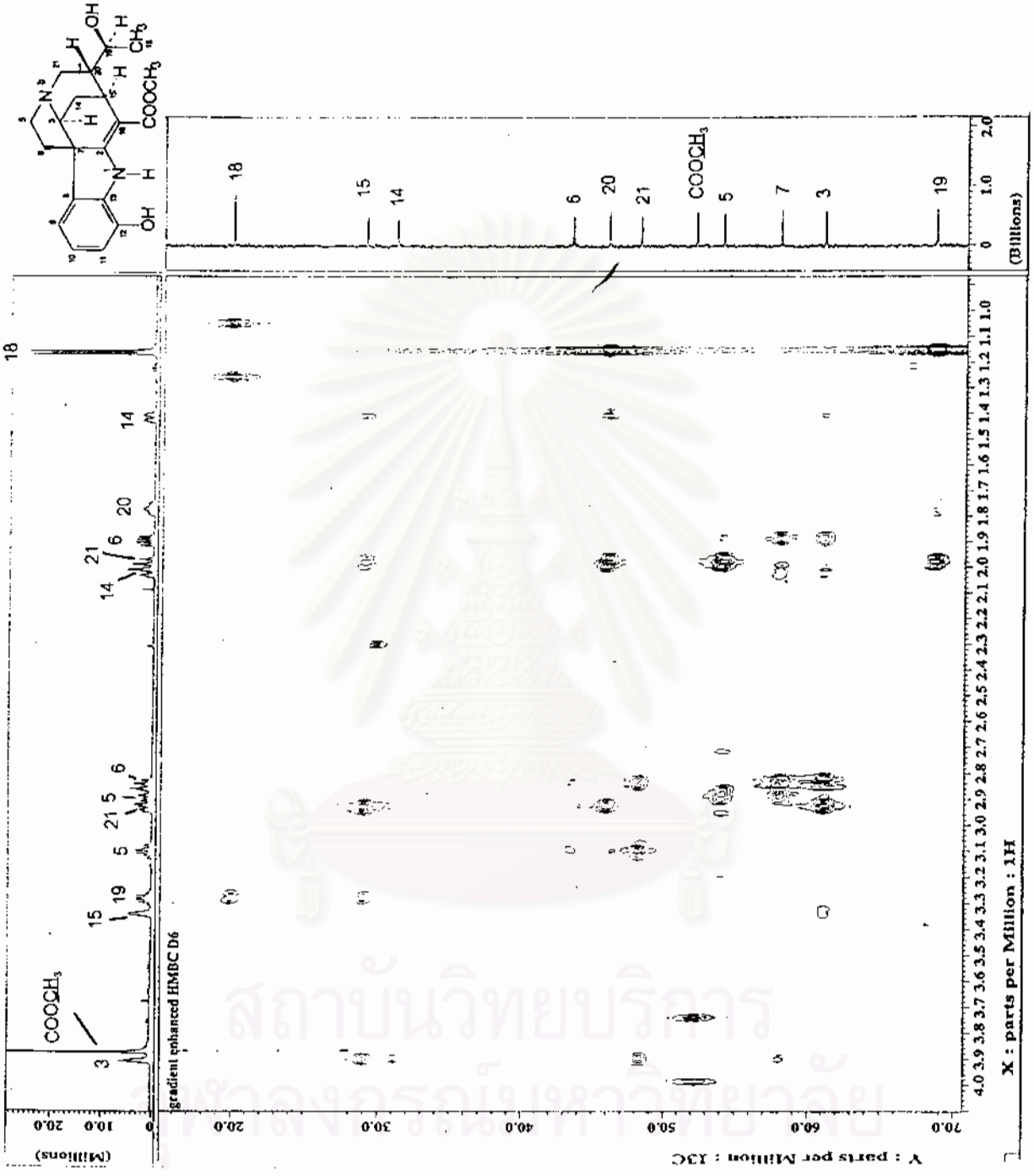


Figure 52b HMBC spectrum of compound D-6 (in $CDCl_3$) [δ_H 0.90 – 4.10 ppm, δ_C 15.0 – 70.0 ppm]

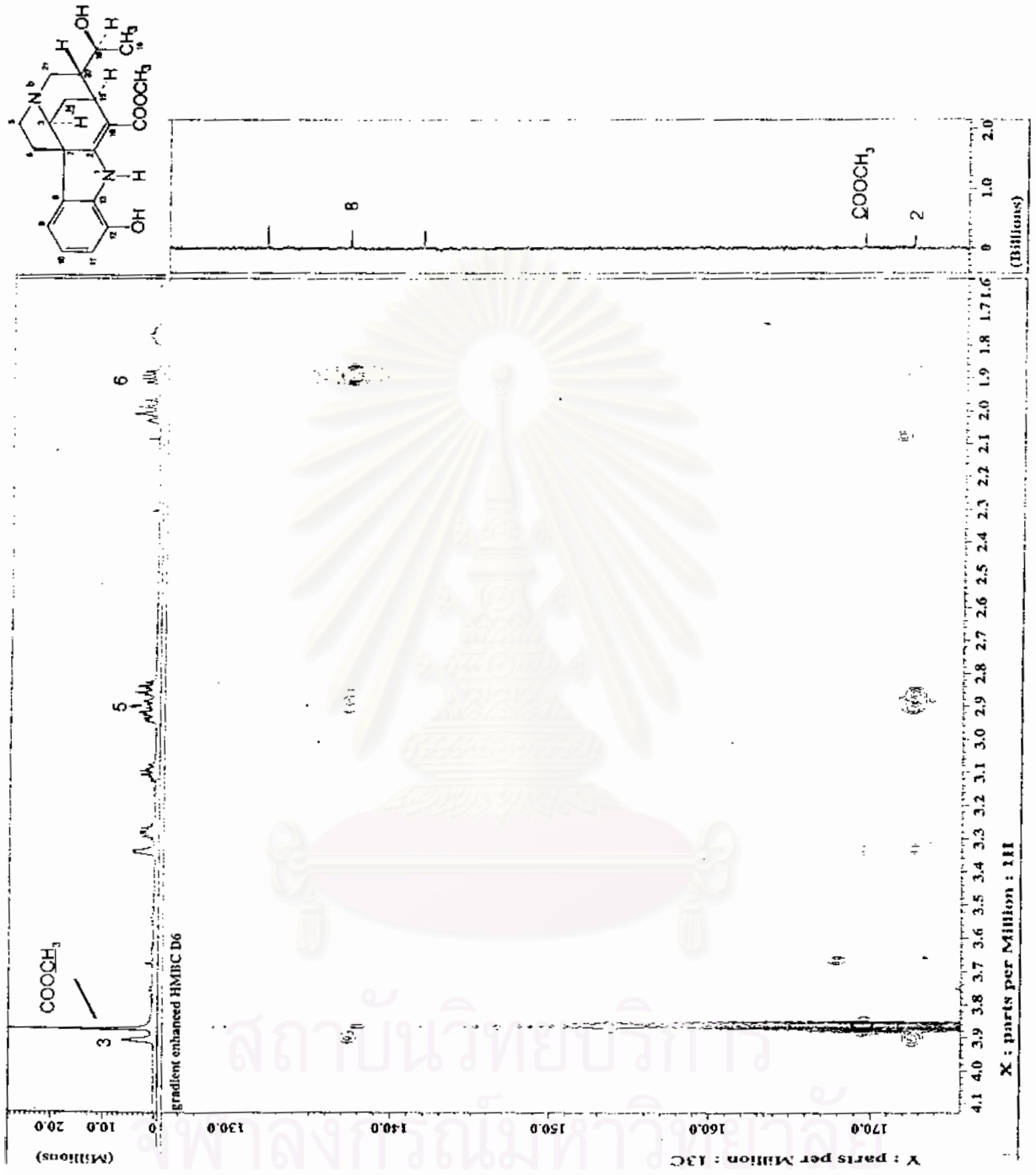


Figure 52c HMBC spectrum of compound D-6 (in $CDCl_3$) [δ_H 1.60 – 4.10 ppm, δ_C 126.0 – 175.0 ppm]

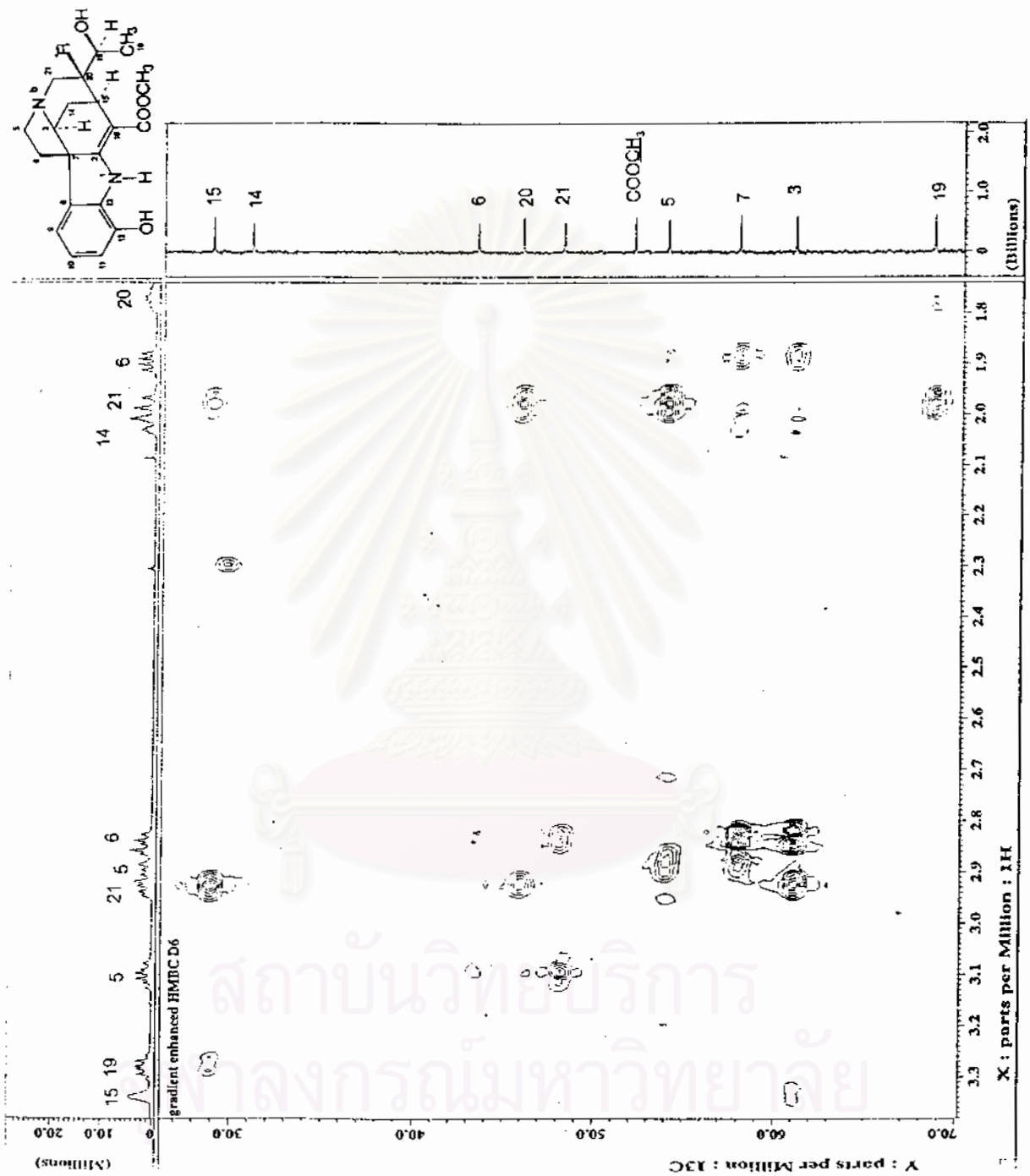


Figure 52d HMBC spectrum of compound D-6 (in $CDCl_3$) [δ_H 1.74 – 3.38 ppm, δ_C 26.0 – 70.0 ppm]

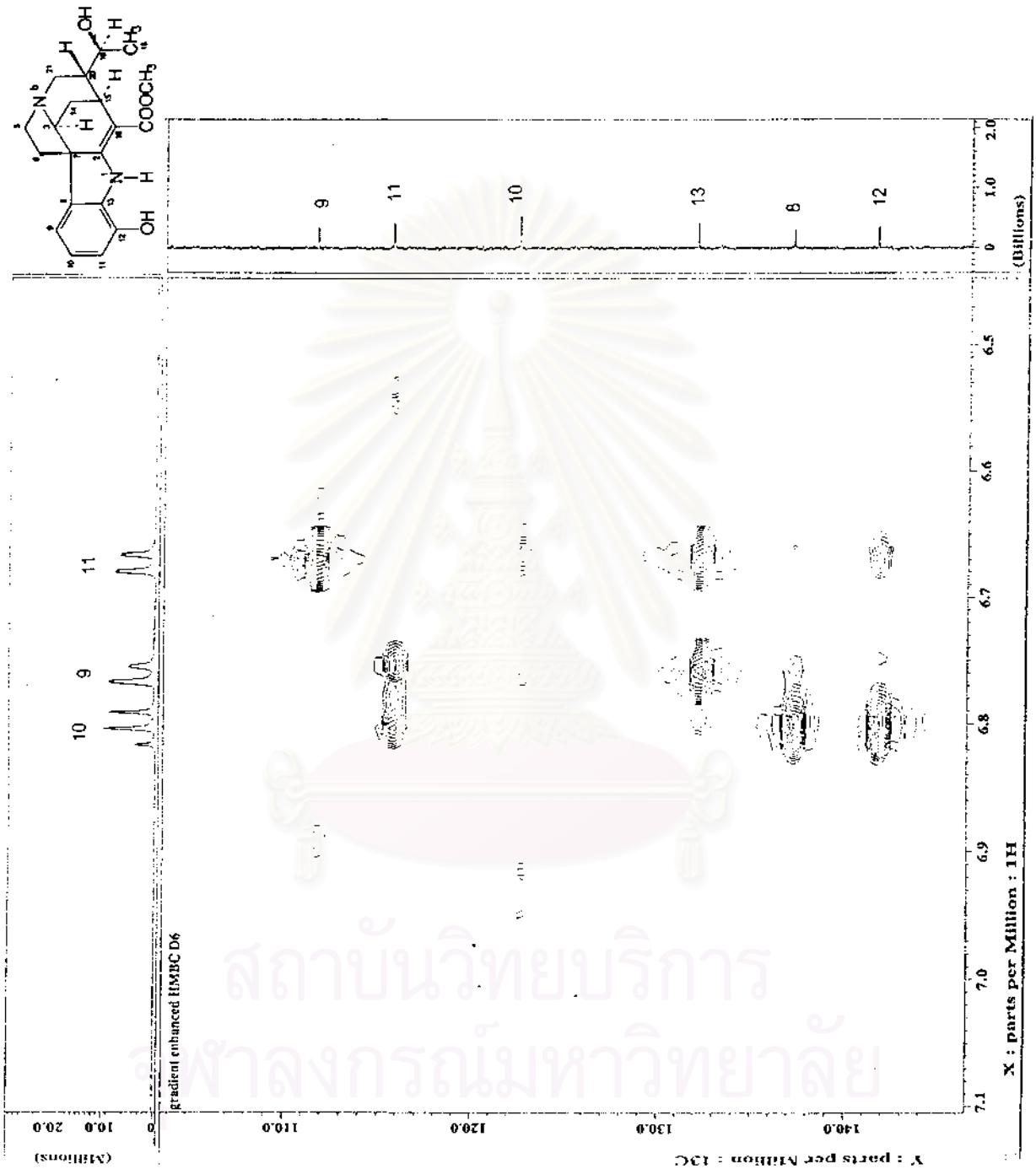


Figure 52e HMBC spectrum of compound D-6 (in $CDCl_3$) [δ_H 6.45 – 7.10 ppm, δ_C 104.0 – 146.0 ppm]

VITA

Miss Lakhana Chaisri was born on March 13, 1972 in Phichit, Thailand. She received her Bachelor's degree of Science in Pharmacy in 1994 from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

At present, she works at Tungphotalae Hospital in Kumpangpetch, Public Health Ministry.