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ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

CHEMICAL CONSTITUENTS OF *ALSTONIA ROSTRATA* STEM BARK

Miss Patcharaporn Kositthanasarn

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Pharmacy

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สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Thesis Title	Chemical constituents of *Alstonia rostrata* stem bark
By	Miss Patcharaporn Kositthanasarn
Field of Study	Pharmacognosy
Thesis Advisor	Associate Professor Nijsiri Ruangrungsi, Ph.D.
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การศึกษาพฤกษเคมีของเปลือกต้นน่องขาว สามารถแยกองค์ประกอบทางเคมีจากสิ่งสกัดซึ่งเป็นสารในกลุ่มอินโดลแอลคาลอยด์ได้ 6 ชนิด การพิสูจน์โครงสร้างทางเคมีของสารประกอบที่แยกได้ด้วยการวิเคราะห์เชิงสเปคตรัมของ UV, IR, MS และ NMR ร่วมกับการเปรียบเทียบข้อมูลกับสารที่ทราบโครงสร้างแล้ว พบว่าสารที่แยกได้จากเปลือกต้นน่องขาวเป็นสารที่เคยมีรายงานแล้ว 3 ชนิด คือ echitamidine, echitamine, undolifoline และเป็นสารใหม่ 3 ชนิด คือ 17-carboxyl-*N*(4)-chloromethylechitamidine, *N*(4)-chloromethylechitamidine, 6,7-*seco*-*N*(4)-chloromethyl angustilobine B

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

ภาควิชา เภสัชเวท
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ปีการศึกษา 2549

ลายมือชื่อนิสิต.....พัชรพร โฆษิตธนสาร.....
ลายมือชื่ออาจารย์ที่ปรึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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KEY WORD : *ALSTONIA ROSTRATA* STEM BARK/ INDOLE ALKALOID

PATCHARAPORN KOSITTHANASARN: CHEMICAL CONSTITUENTS OF *ALSTONIA ROSTRATA* STEM BARK. THESIS ADVISOR: ASSOC. PROF. NIJSIRI RUANGRUNGSI, Ph.D., THESIS CO-ADVISOR: PRASAT KITTAKOOP, Ph.D. 95 pp. ISBN 974-14-3497-9

Chemical investigation of *Alstonia rostrata* Fischer led to the isolation of six indole alkaloids. The structure determination of these compounds was accomplished by spectroscopic analyses (UV, IR MS and NMR) and by comparison with previously reported data of known compounds. The stem bark of *Alstonia rostrata* Fischer provided three known compounds: echitamidine, echitamine, undolifoline and three new compounds: 17-carboxyl-*N*(4)-chloromethylechitamidine, *N*(4)-chloromethyl echitamidine, 6,7-*seco*-*N*(4)-chloromethylangustilobine B.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Department: Pharmacognosy Student's signature.....

Field of study: Pharmacognosy Advisor's signature.....

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LIST OF ABBREVIATIONS

br	=	broad (for NMR spectra)
°C	=	degree Celsius
$CDCl_3$	=	deuterated chloroform
CD_3OD	=	deuterated methanol
cm^{-1}	=	wave number
^{13}C NMR	=	carbon-13 nuclear magnetic resonance
COSY	=	correlation spectroscopy
1-D	=	one dimensional
2-D	=	two dimensional
d	=	doublet (for NMR spectra)
dd	=	doublet of doublet (for NMR spectra)
ddd	=	doublet of doublet of doublet (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polarization Transfer
dq	=	doublet of quartet (for NMR spectra)
δ	=	chemical shift (in ppm)
EIMS	=	Electron impact mass spectrometry
ESITOF MS	=	Electrospray-time of flight mass spectrometry
EtOAc	=	ethyl acetate
g	=	gram
^{1}H NMR	=	Proton Nuclear Magnetic Resonance
HMBC	=	^{1}H-detected Heteronuclear Multiple Bond Correlation
HMQC	=	^{1}H-detected Heteronuclear Multiple Quantum Correlation
Hz	=	hertz
IR	=	Infared spectrum

สถาบันวิทยบริการ
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J	=	coupling constant
kg	=	kilogram
l	=	liter
λ_{max}	=	wavelength at maximal absorption
m	=	multiplet
μg	=	microgram
μM	=	micromolar
M^+	=	molecular ion
MeOH	=	methanol
mg	=	miligram
MHz	=	megahertz
min	=	minute
ml	=	mililiter
m/z	=	a value of mass divided by charge
nm	=	nanometer
NMR	=	Nuclear Magnetic Resonance
NOESY	=	Nuclear Overhauser Effect Correlation Spectroscopy
ppm	=	part per million
ν_{max}	=	Wave number of maximal absorption
q	=	quartet (for NMR spectra)
ROESY	=	Rotating frame Overhauser Enhancement Spectroscopy
s	=	singlet (for NMR spectra)
t	=	Triplet (for NMR spectra)
td	=	Triplet of doublet (for NMR spectra)
TLC	=	Thin Layer Chromatography
UV	=	ultraviolet

CHAPTER I

INTRODUCTION

Plants are important sources of alkaloids, and some could be developed to be drugs. However, the group is a very varied one and it is only the chemical properties of basic nitrogen that unify many classes of alkaloids. By 1980, the number of known indole alkaloids had risen to approximately 1200. Indole alkaloids are defined as the natural organic products containing either indole nucleus or an oxidized, reduced or substituted equivalent of it (Kisakurek and Hesse, 1980).

There are many indoles added to the list of naturally occurring alkaloids. These alkaloids include such pharmacologically and structurally diverse compounds as reserpine (tranquilizer, hypotensive agent), strychnine (stimulant-convulsant), harmaline (hallucinogen), ergometrine (oxytocic, migraine reliever), vinblastine and viscristine (antitumor and anti-leukemic agents), tryptophan (essential amino acid), serotonin (anticholinesterase-monoamine oxidase inhibitor), and psilocybin (hallucinogen) (Kisakurek and Hesse, 1980).

The genus *Alstonia* belongs to the tribe Pluemerieae (Alstonieae) of the family Apocynaceae. There are about 40 species in this genus distributed throughout the tropical and subtropical parts of the world especially in Southern Asia, Malaysia, Australia, America, Africa and the eastern Pacific islands (Middleton, 1999). There are seven *Alstonia* species in Thailand as listed below (Middleton, 1999).

1)	*A. angustiloba* Miq.	ตีนเป็ดเล็ก	Tin pet lek
2)	*A. curtisii* King & Gamble	ตีนเป็ดแคระ	Tin pet krae
3)	*A. macrophylla* Wall. ex G.Don	ทุ้งฟ้า	Tung fa
4)	*A. rostrata* Fischer	น่องขาว	Nong khow
5)	*A. rupestris* Kerr	ตีนเป็ดดอย	Tin pet doi
6)	*A. scholaris* (L.) R.Br.	พญาสัตบรรณ	Phayasataban

7) *A. spatulata* Blume ตีนเป็ดพรุ Tin pet phru

Plants of the genus *Alstonia* are lactiferous trees or shrubs; branches: sparsely pubescent or glabrous; leaves: verticillate (sometimes opposite); inflorescence: terminal or axillary, frequently in whorls or umbel-like; sepals: without colleters inside, ovate; corolla: lobes overlapping to the left or right in bud, tube narrow, mouth pubescent; stamen: inserted around the middle or upper half of corolla tube; anthers : lanceolate, without appendages; disk: 2 lobes or small and annular or absent; ovary: 2 separate carpels united into a common style or syncarpous, glabrous or pubescent, ovule numerous; fruits: paired follicles or a solitary follicle; seeds: oblong, ends rounded or acuminate, flattened, pubescent or glabrous on faces of grain, long cilia around margin (Middleton, 1999). The leaf shape is variable in this genus. On the same twig, and even the same whorl, leaves mature vary from ovate and obtuse to elliptic and acuminate. The inflorescence in terminal, pleiochasial, short-peduncled or often sessile, its branches springing from the axils of small scale; these partial inflorescences have along peduncle and 1-3 nodes giving rise to secondary cymes (Middleton, 1999).

Almost all Malaysian Alstonias prefer wet ground; some even peat swamp forests, and tolerate open water. They are frequent to common in lowland rain forests, and some in mountain rain forests. The most widely distributed species, *A. scholaris* and *A. spectabilis*, have a wider range of water requirement, occurring also in sclerophyllous woods and patches in savannas (Markgraf, 1974).

Alstonia rostrata Fischer is found distributing in Burma, China, Vietnam, MalayPenisula, Sumatra and Thailand. It is found in evergreen forest or in disturbed secondary forest to 1300 m. In Thailand, this tree distributes in Mae Hong Son, Chiang Mai, Lampang, Phrae, Phitsanulok, Chaiyaphum, Surat Thani, Nakon Si Thammarat, Satun and Songkhla (Middleton, 1999).

There are four synonymous names for *A. rostrata* Fischer (Middleton, 1999) as:

- *A. glaucescens* (K. Schum.) Monachin
- *A. undulifolia* Kochummen & Wong
- *Winchia calophylla* A.DC.

- *Winchia glaucescens* K. Schum.

It is a tree to 30 m high, *Branchlets* glabrous, *Leaves* in whorls of 3 or 4, petiole 1.1-2.3 cm long, blade coriaceous, oblong or elliptic 5-14 x 1.6-5.5 cm, apex acuminate, base cuneate or decurrent; 35-36 closely parallel pairs of secondary veins; glabrous. *Inflorescence* 2-4.2 cm long; glabrous; many flowered; pedicels 11-20 mm long. *Sepals* ovate, 1.2-1.7 X 0.9-1.2 mm, apex obtuse to rounded; glabrous, ciliate. *Corolla* white; lobes overlapping to the left in bud; tube 4-6 mm long; lobes 2.2-2.5 X 1.4-1.8 mm, oblong, apex rouned; puberulent on top of tube and lobes outside, pubescent inside except at the base of tube. *Stamens* inserted slightly above of middle of tube; anthers 1-1.2 X 0.4mm. *Disk* absent. *Ovary* syncarpous, 0.6-0.9 mm long; style + pistil head 2.7-3.1 mm long. *Fruit* solitary; thick walled; 12-19.5 cm long, 7-8.5 mm wide; glabrous; many seeds. *Seed* surface glabrous; oblong, end rounded; 10 X 2.5 mm; cilia 18 mm long (Middleton, 1999) (Figure 1).

The genus *Alstonia* is well-known as a rich source of indole alkaloids, and more than 180 alkaloids have been isolated from this particular genus. In spite of this large number, only a few *Alstonia* alkaloids have been assessed for biological evaluation (Kaewpradub and Houghton, 1997).

Previous phytochemical studies of *A. rostrata* Fischer under other synonymous scientific names have been reported by several groups of researchers, and one of those has been a study on the stem bark collected from the South of Thailand (Keawpradub *et al*., 1994). This study aims to investigate the chemical constituents of the stem bark of *A. rostrata* Fischer obtained from the Northeast of Thailand.

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องค์การสวนพฤษศาสตร์ สำนักนายกรัฐมนตรี. ไม้ต้นไม้สวน. พิมพ์ครั้งที่ 1. กรุงเทพมหานคร : อักษรสยามการพิมพ์, 2542.

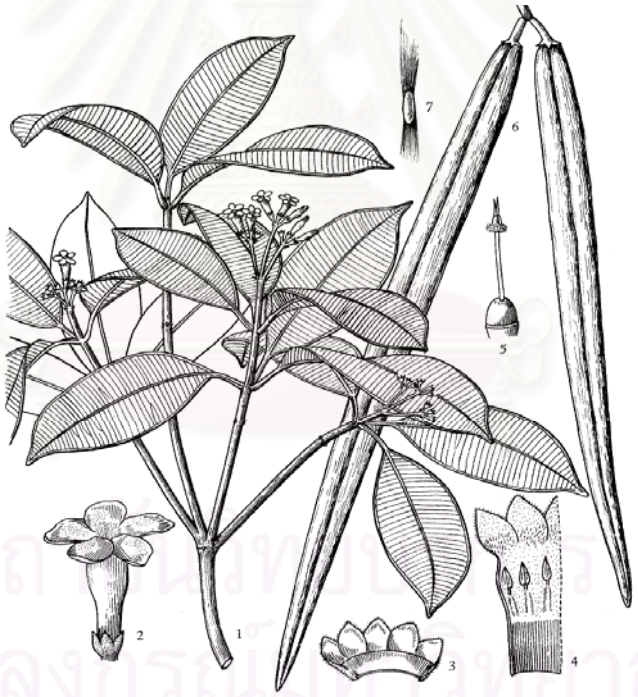


Figure 85. 1–7. **Alstonia rostrata** C. E. C. Fischer, 盆架树 pen jia shu (*Winchia calophylla* A. de Candolle). —1. Flowering branch. —2. Flower. —3. Opened calyx. —4. Portion of opened corolla showing stamens. —5. Pistil. —6. Follicles. —7. Seed. (FOC 155; FRPS 63: 96, pl. 32. 1977. —吴翠云 Wu Cuiyun).

Figure 1

"Apocynaceae: *Alstonia rostrata* Fischer". [Online]. Available: http://www.efloras.org 1997.

CHAPTER II

HISTORICAL

1. Chemical constituents of *Alstonia* spp.

A number of *Alstonia* spp. have been shown to be a good source of indole (Table 1) and other alkaloids, as well as miscellaneous compounds (Table 2). In addition, other classes of natural compounds such as amides, phytosterols, terpenoids, phenolic compounds, lignans and glycosides have been found in this plant genus (Table 3).

Table 1 Examples of indole alkaloids in *Alstonia* spp.

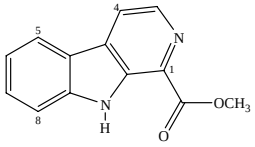
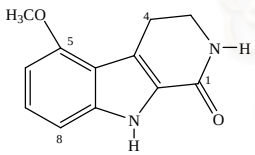
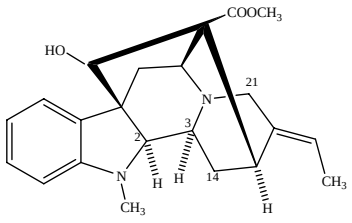
Chemical compounds	Sources	References
1. Simple indole alkaloids		
1-Carbomethoxy-β-carboline 	*A. constrica*: stem bark	Allam, Beutler and Le Quesne, 1987
5-Methoxy-1-oxo-tetrahydro-β-carboline 	*A. venenata*: root bark	Banerji *et al*., 1982
2. Monoterpenoid-derived indole alkaloids 2.1 Corynanthean-type indole alkaloids Ajmaline group Vincamajine 	*A. lanciolifera*: stem bark	Lewin *et al*., 1975

Table 1 (Continued)

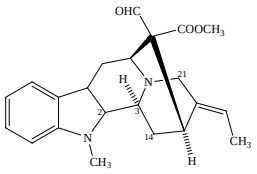
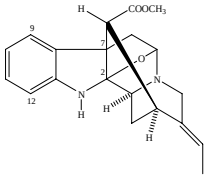
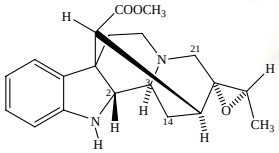
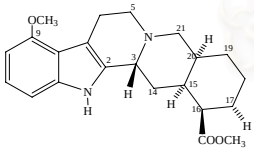
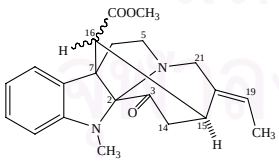
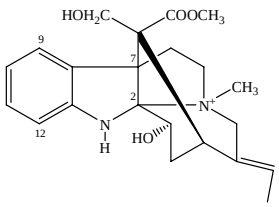
Chemical compounds	Sources	References
Sapagine group Voachalotinal 	*A. undolata*: leaves	Gauillaume *et al*., 1984 Pinchon *et al*., 1990
Akuammiline group Picrinine 	*A. lanceolata*: stem bark *A. lanceolifera*: stem bark	Vercauteren *et al*., 1981 Ravoa *et al*., 1982
Quaternoxine 	*A. vitiensis*: unknown	Mamatas-Kalamaras *et al*., 1975
Yohimbine group Venenatine 	*A. quaternata*: unknown *A. venenata*: root bark	Mamatas-Kalamaras *et al*., 1975 Chatterjee, Roy, and Mukhopadhyay,1981
Vincrorine group Vincoridine 	*A.shaerocapitata* : fruit *A. scholaris*: leaves *A. congensis*: root bark	Caron *et al*., 1984 Yamauchi *et al*., 1990 Caron *et al*., 1989
Echitamine 	*A. undolifolia*: stem bark *A. glaucescens* : stem bark *W. calophylla*: stem bark	Massiot *et al*., 1992 Keawpradub *et al*., 1994 Zhu *et al*., 2005

Table 1 (Continued)

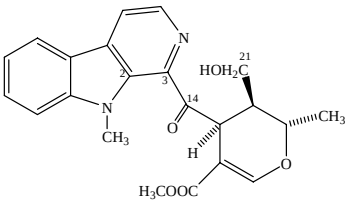
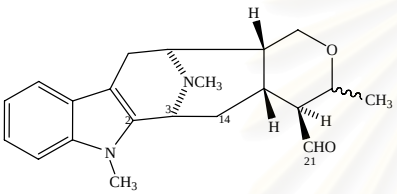
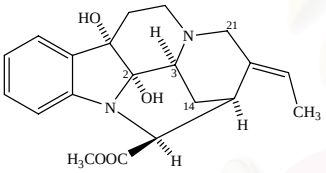
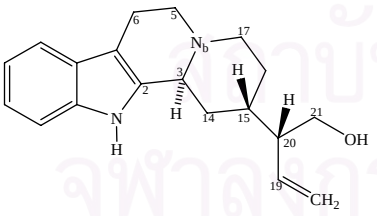
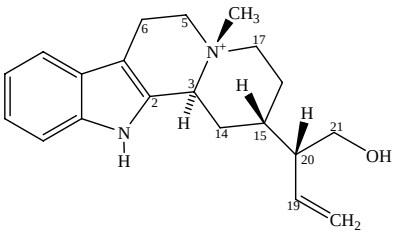
Chemical compounds	Sources	References
Alstonidine group 14-Ketoalstonidine 	*A. constricta*: root bark	Allam *et al*., 1987
Macroline group Talcarpine 	*A. muelleriana*: stem bark	Burke *et al*., 1973
Pleiocarpamine group 2,7-Dihydropleiocarpamine 	*A. muelleriana*: stem bark *A. plumosa*: root bark	Burke *et al*., 1973 Jacquier *et al*., 1982
2.2 Vallesiachotaman-type indole alkaloids Antirhine 	*A. odontophora*: leaves *A. angustifolia*: leaves	Vercauteren *et al*., 1979 Ghedira *et al*., 1988
N_b-β-Methoxylantirhine 	*A. angustifolia*: stem bark	Hu, Zhu and Hesse, 1989

Table 1 (Continued)

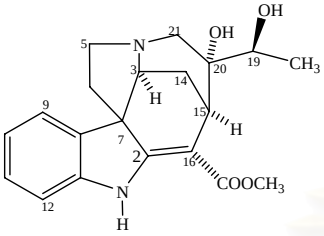
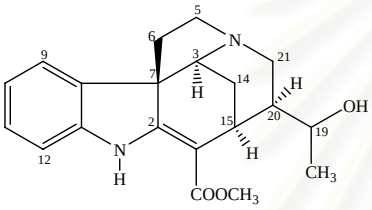
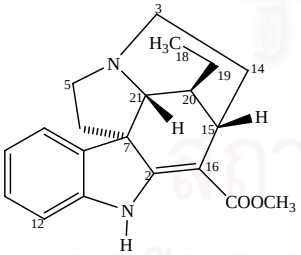
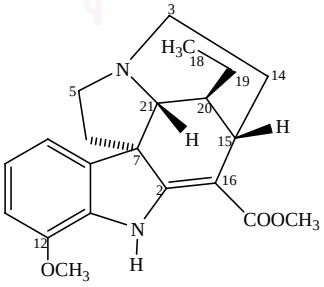
Chemical compounds	Sources	References
2.3 Strychnan-type indole alkaloids		
Compactinervine 	*A. lanceolata*: stem bark	Vercauteren *et al*., 1981
Echitamidine 	*A. congensis*: root bark *A. glaucescens*: stem bark	Caron *et al*., 1989 Keawpradub *et al*., 1994
2.4 Aspidospermatan-type indole alkaloids Tubotaiwine group		
Tubotaiwine 	*A. angustifolia*: stem bark	Hu *et al*., 1988
12-methoxytubotaiwine 	*A. congensis*: root bark	Caron *et al*., 1989

Table 1 (Continued)

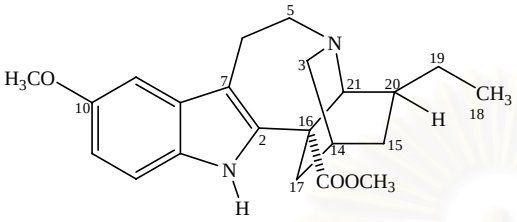
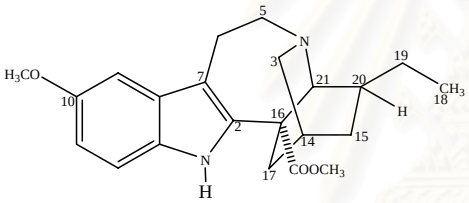
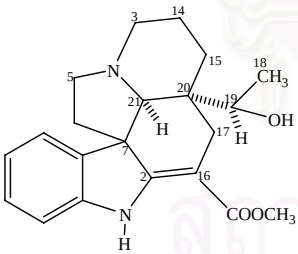
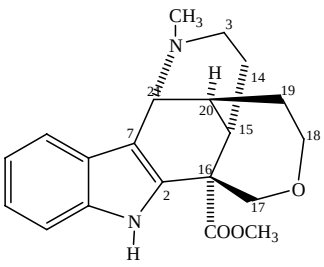
Chemical compounds	Sources	References
2.5 Ibogan-type indole alkaloids Catharanthine group Voacangine 	*A. boonei*: unknown	Croquelois *et al*., 1972
2.6 Plumeran-type indole alkaloids Kosinine group Venalstonidine 	*A. venenata*: root bark	Chatterjee *et al*., 1981
Tabersonine group Minovincinine 	*A. venenata*: stem bark	Majumdr *et al*., 1981
2.7 Uleine-type indole alkaloids Undolifoline 	*A. undolifolia*: stem bark	Massiot *et al*., 1992

Table 1 (Continued)

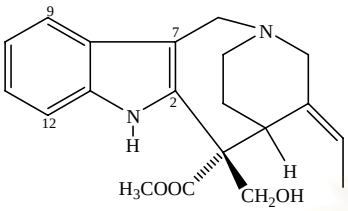
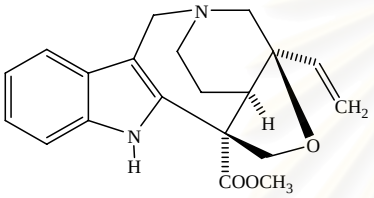
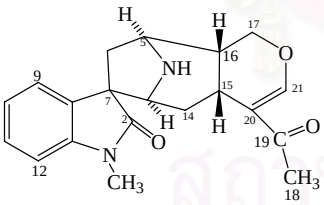
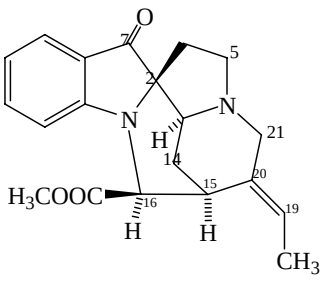
Chemical compounds	Sources	References
2.8 Vellesamine-type indole alkaloids Vellesamine  Angustilobine A 	*A. scholaris*: root bark *W. calophylla*: stem bark *A. congensis*: root bark *A. scholaris*: leaves	Yamauchi *et al*., 1990 Zhu *et al*., 2005 Caron *et al*., 1989 Yamauchi *et al*., 1990
2.9 Oxindole and Psuedoindoxyl alkaloids 2.9.1 Oxindole alkaloid Alstonine 	*A. muelleriana*: leaves *A. angustifolia*: leaves	Elderfield and Gilman, 1972 Ghedira *et al*., 1988
2.9.2 Psuedoindoxyl alkaloids Fluorocarpamine alkaloid 	*A. plumosa*: root bark *A. undulata*: leaves *A. angustifolia*: leaves	Jacquir *et al*., 1982 Guillaume *et al*., 1984 Ghedira *et al*., 1988

Table 1 (Continued)

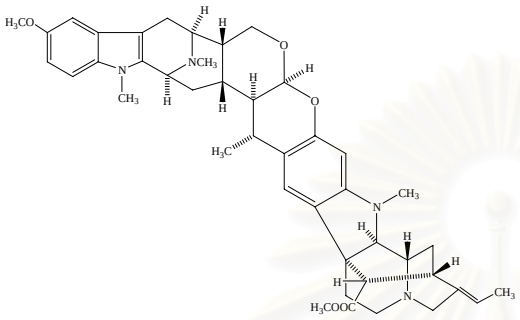
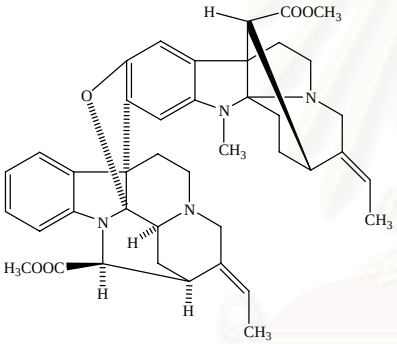
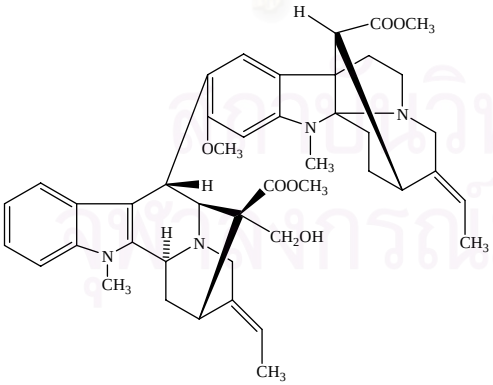
Chemical compounds	Sources	References
2.10 Bisindole alkaloids		
Alstocraline 	*A. angustifolia*: leaves	Ghedira *et al*., 1988
Pleiocorine 	*A.odontophora*: leaves *A. plumosa*: root bark	Vercauteren e*t al*., 1979 Jacquir *et al*., 1982
Undulatine 	*A. undulata*: leaves	Pinchon *et al*., 1990

Table 2 Distribution of other alkaloids and miscellaneous compounds in *Alstonia* spp.

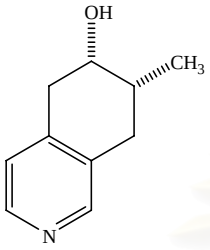
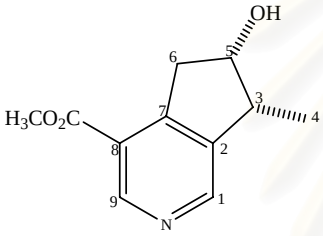
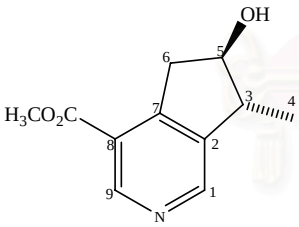
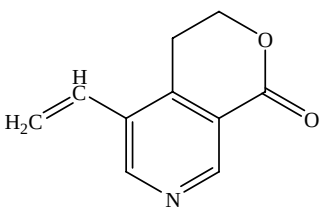
Chemical compounds	Sources	References
1.Alkaloids		
Venoterpine 	*A. venenata*: fruit *A. spatulata*: unknown	Ray and Chatterjee, 1968 Ravao *et al*., 1985
Cantleyine 	*A. undulifolia*: stem bark	Massiot *et al*., 1992
Isocantleyine 	*A. undulifolia*: stem bark	Massiot *et al*., 1992
Tetrahydrocantleyine	*A. angutifolia*: leaves *A. Undulifolia*: stem bark	Ghedira *et al*., 1988 Massiot *et al*., 1992
Gentianine 	*A. lanceolata*: stem bark *A. lenormadii*: leaves	Vercauteren *et al*., 1981 Legseir *et al*., 1986

Table 2 (Continued)

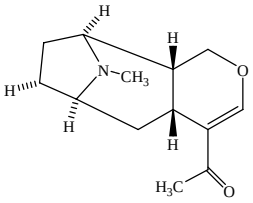
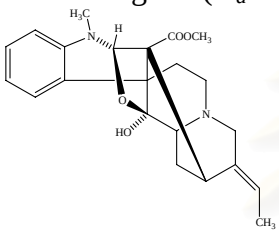
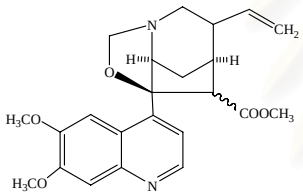
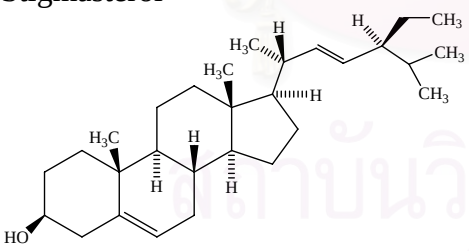
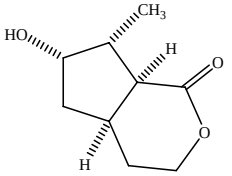
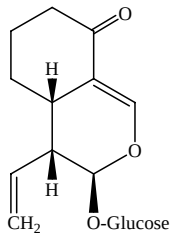
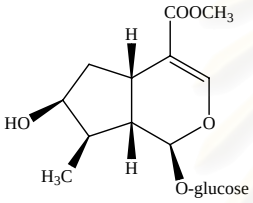
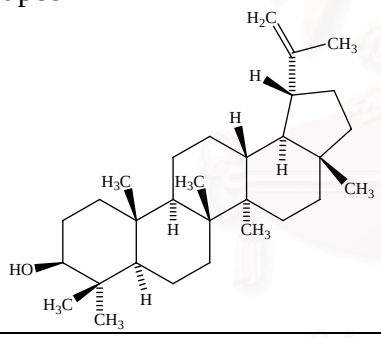
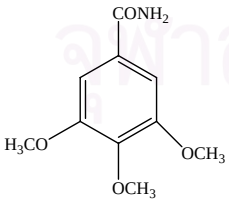
Chemical compounds	Sources	References
Alkaloids Angustimaline 	*A. angustifolia*: stem bark	Kam *et al*., 1997
Lanceomigine (N_a-Methylrhamazine) 	*A. lanceolata*: stem bark	Vercauteren *et al*., 1981
Corialstonine 	*A. coriacea*: stem bark	Cherif *et al*., 1989
2. Phytosterol Stigmasterol 	*A.venenata*: stem bark	Govindachari *et al*., 1964
3. Terpenoids Boonein 	*A. boonei*: stem bark	Marini-Bettolo *et al*., 1983

Table 2 (Continued)

Chemical compounds	Sources	References
Terpenoids Sweroside  	*A. glaucescens*: stem bark	Keawpradub *et al*., 1994
Loganin  	*W. calophylla*: stem bark	Chen *et al*., 1988
Lupeol  	*A. scholaris*: unknown	Mukherjee and Ghosh, 1979
4. Amides 3,4,5-trimethoxybenzamide  	*A. constricta*: stem bark	Allam *et al*., 1987
Cintriamide [3-(3,4,5-Trimethoxyphenyl)-2-propenamide)	*A. lenormandii*: unknown	Legseir *et al*., 1986

2. Previous by reported data of the synonymous names of *Alstonia rostrata* Fischer

The phytochemical studies of *Alstonia rostrata* have been reported under serveral synonymous names such as *Alstonia undolifolia* Kochummen & Wong, *Alstonia glaucescens* (K. Schum.) Monachino, and *Winchia calophylla* A. DC. There have been 31 indole alkaloids, 3 pyridine alkaloids, 1 quinoline alkaloid, 5 terpenoids, 4 phenolic compounds and 2 lignans reported in the literature (Table 3).

Table 3 Previous by reported data of the synonymous names of *Alstonia rostrata*

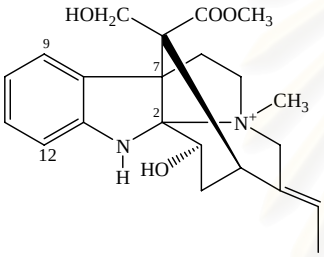
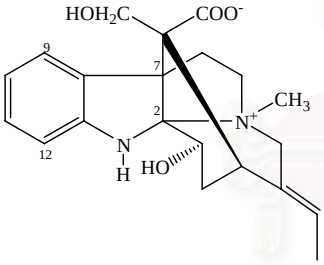
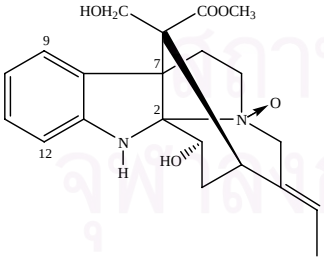
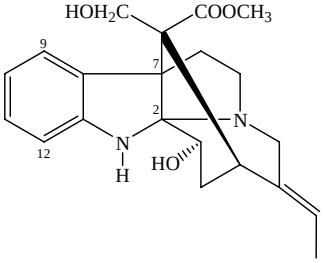
Chemical compounds	Sources	References
1. Indole alkaloids		
Echitamine 	*A. undolifolia*: stem bark *A. glaucescens*: stem bark *W. calophylla*: stem bark	Massiot *et al*., 1992 Keawpradub *et al*., 1994 Zhu *et a*l., 2005
Echitamic acid 	*A. glaucescens*: stem bark	Keawpradub *et al*., 1994
N_b-demethylechitamine N-*oxide* 	*A. glaucescens*: stem bark	Keawpradub *et al*., 1994
N_b-demethylechitamine 	*A. glaucescens*: stem bark	Keawpradub *et al*., 1994

Table 3 (Continued)

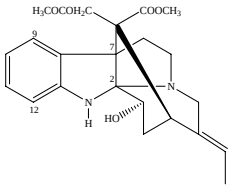
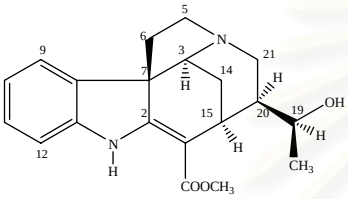
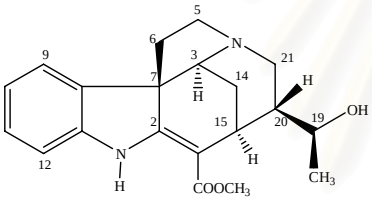
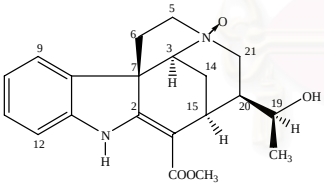
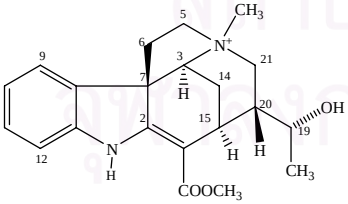
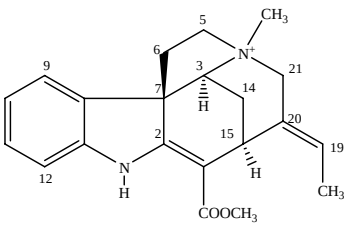
Chemical compounds	Sources	References
Indole alkaloids		
17-O-acethyl-Nb-demethylechitamine 	*A. glaucescens*: stem bark	Keawpradub *et al*., 1994
Echitamidine 	*A. undolifolia*: stem bark *A. glaucescens*: stem bark *W. calophylla*: stem bark	Massiot *et al*., 1992 Keawpradub *et al*., 1994 Zhu *et al*., 2005
20-epi-19ζ-echitamidine 	*A. undolifolia*: stem bark *A. glaucescens*: stem bark	Massiot *et al*., 1992 Keawpradub *et al*., 1994
Echitamine N-oxide 	*A. glaucescens*: stem bark	Keawpradub *et al*., 1994
Alstoqustine 	*W. calophylla*: stem bark	Zhu *et al*., 2005
N(4)-demethyl akuammicine 	*W. calophylla*: stem bark	Zhu *et al*., 2005

Table 3 (Continued)

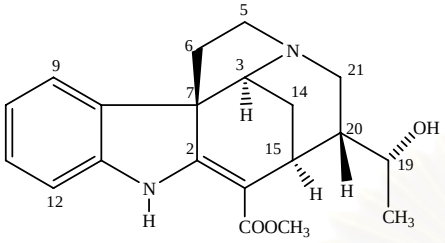
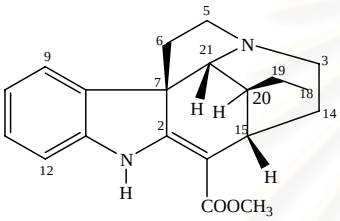
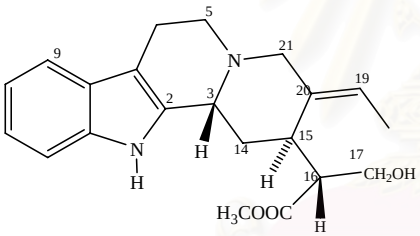
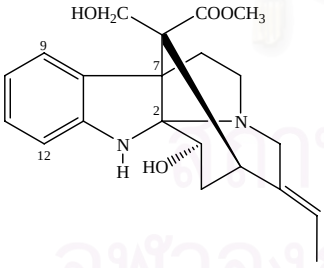
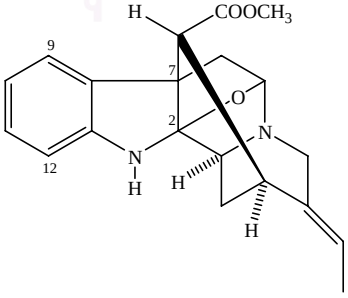
Chemical compounds	Sources	References
Indole alkaloids		
N(4)-demethyl alstoqustine 	*W. calophylla*: stem bark	Zhu *et al*., 2005
Fubotaine 	*W. calophylla*: stem bark	Zhu *et al*., 2005
Rhazimanine 	*W. calophylla*: stem bark	Zhu *et al*., 2005
17-O-acetylechitamine 	*W. calophylla*: stem bark	Zhu *et al*., 2005
Picrinine 	*W. calophylla*: stem bark	Zhu *et al*., 2005

Table 3 (Continued)

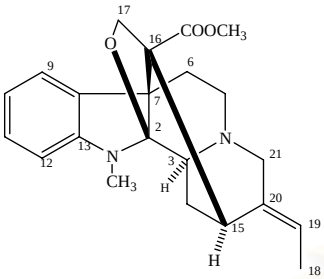
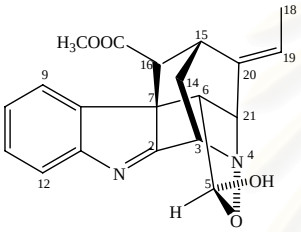
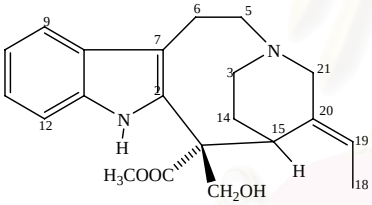
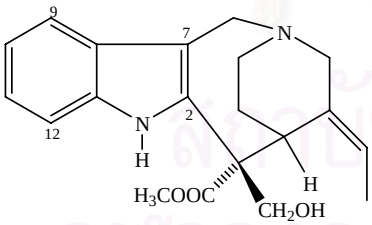
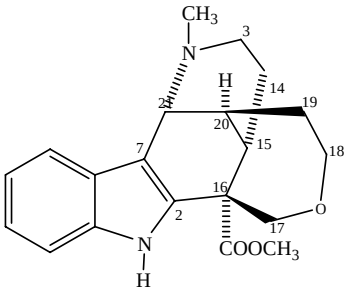
Chemical compounds	Sources	References
Indole alkaloids		
Pseudoakuammigine 	*W. calophylla*: stem bark	Zhu *et al*., 2005
Nareline 	*W. calophylla*: stem bark	Zhu *et al*., 2005
Stemmadenine 	*W. calophylla*: stem bark	Zhu *et al*., 2005
Vellesamine 	*W. calophylla*: stem bark	Zhu *et al*., 2005
Undolifoline 	*A. undolifolia*: stem bark	Massiot *et al*., 1992

Table 3 (Continued)

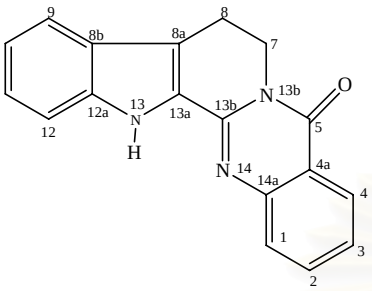
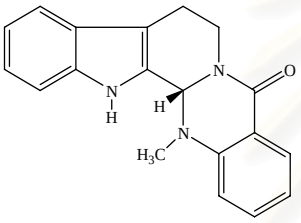
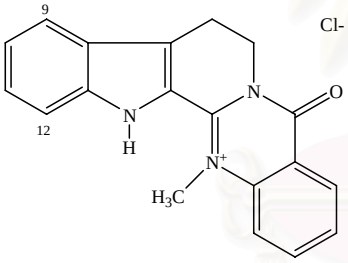
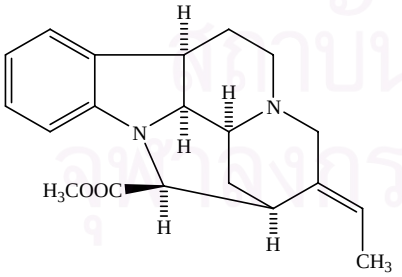
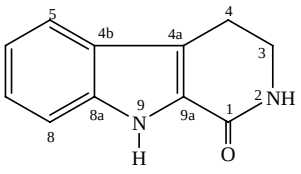
Chemical compounds	Sources	References
Indole alkaloids		
Rutaecarpine 	*W. calophylla*: stem bark	Zhu *et al*., 2005
Evodiamine 	*W. calophylla*: stem bark	Zhu *et al*., 2005
Dehydroevodiamine hydrochloride 	*W. calophylla*: stem bark	Zhu *et al*., 2005
Pleiocarpamine 	*A. undolifolia*: stem bark *W. calophylla*: stem bark	Massiot *et al*., 1992 Zhu *et al*., 2005
1,2,3,4,-tetrahydro-1-oxo-carboline 	*W. calophylla*: stem bark	Zhu *et al*., 2005

Table 3 (Continued)

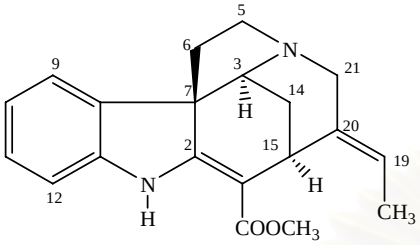
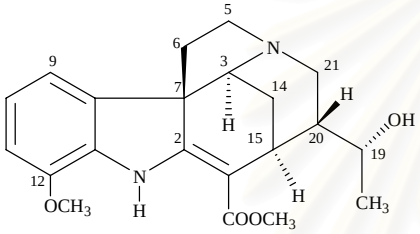
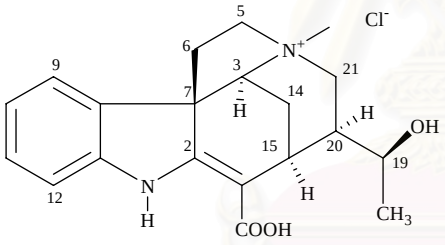
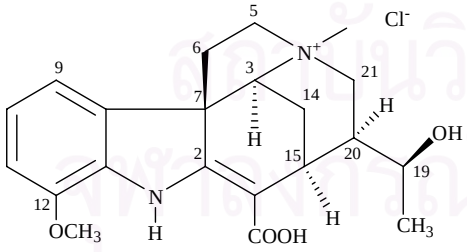
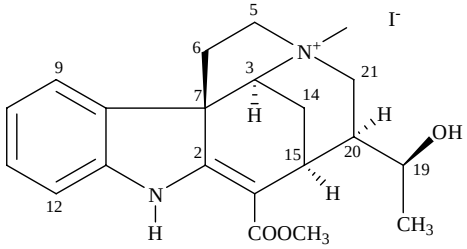
Chemical compounds	Sources	References
Indole alkaloids		
(-)-Akuammicine 	*A. undolifolia*: stem bark *W. calophylla*: stem bark	Massiot *et al*., 1992 Zhu *et al*., 2005
N(4)-demethyl-12-methoxyalstogustine 	*W. calophylla*: stem bark	Gan *et al*., 2006
17-carboxyl-*N*(4)-methylechitamidine 	*W. calophylla*: stem bark	Gan *et al*., 2006
17-carboxyl-12-methoxy-*N*(4)-methylechitamidine 	*W. calophylla*: stem bark	Gan *et al*., 2006
N(4)-methylechitamidine iodide 	*W. calophylla*: stem bark	Gan *et al*., 2006

Table 3 (Continued)

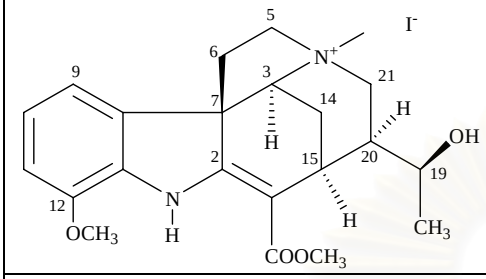
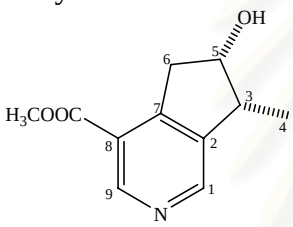
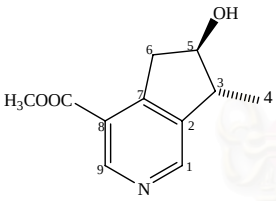
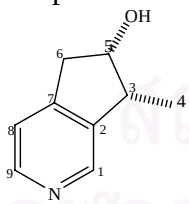
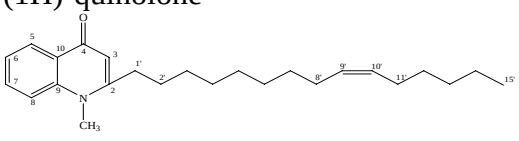
Chemical compounds	Sources	References
Indole alkaloids 12-Methoxy- *N*(4)-methylechitamidine iodide 	*W. calophylla*: stem bark	Gan *et al*., 2006
2. Pyridine alkaloids		
Cantleyine 	*A. undolifolia*: stem bark *W. calophylla*: stem bark	Massiot *et al*., 1992 Zhu *et al*., 2005
Isocantleyine 	*W. calophylla*: stem bark	Zhu *et al*., 2005
Venoterpine 	*W. calophylla*: stem bark	Zhu *et al*., 2005
3. Quinoline alkaloids		
1-Methyl-2[10Z]-10-pentadecanenyl-4 (1H)-quinolone 	*W. calophylla*: stem bark	Zhu *et al*., 2005

Table 3 (Continued)

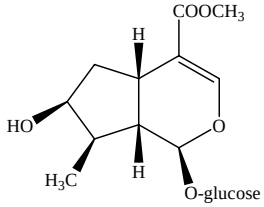
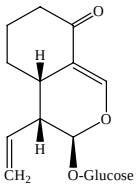
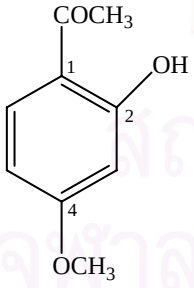
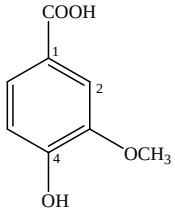
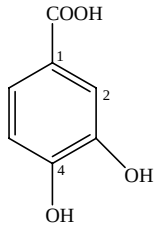
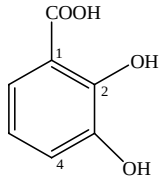
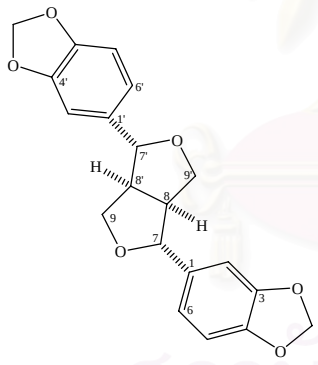
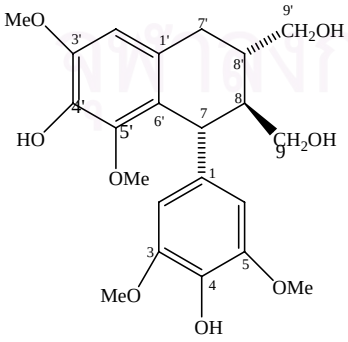
Chemical compounds	Sources	References
4. Terpenoids		
Loganin 	*W. calophylla*: stem bark	Zhu *et al*., 2005
Sweroside 	*A. glaucescens*: stem bark	Keawpradub *et al*., 1994
wincaloside A wincaloside B winchiepoxide	*W. calophylla*: stem bark	Zhu *et al*., 2002
5. Phenolic compounds		
Paeonol 	*W. calophylla*: stem bark	Zhu *et al*., 2005
4-Hydroxy-3-methoxybenzoic acid 	*W. calophylla*: stem bark	Zhu *et al*., 2005

Table 3 (Continued)

Chemical compounds	Sources	References
Phenolic compounds		
3,4-Dihydroxybenzoic acid 	*W. calophylla*: stem bark	Zhu *et al*., 2005
2,3-Dihydroxybenzoic acid 	*W. calophylla*: stem bark	Zhu *et al*., 2005
6. Lignan		
Sesamin 	*W. calophylla*: stem bark	Zhu *et al*., 2005
(-)-Lyoniresinol 	*W. calophylla*: stem bark	Zhu *et al*., 2005

3. Traditional uses and biological activities of *Alstonia* spp.

Alstonia plants have been used in traditional medicine in many countries with several proposes. In India and the Philippines, the stem bark of *Alstonia scholaris* is used in homoeopathy for its tonic bitter and astringent properties; it is particularly useful for chronic diarrhea and dysentery (Yamauchi *et al*., 1990). In Andaman Island, India, ethnobotanical literature states that decoction of *A. macrophylla* leaves and stem bark is widely used to treat stomach-ache, skin diseases and urinary infections (Bhargava *et al*., 1983). Moreover, *A. macrophylla* leaves are reported to have anticholeretic and vulnerary effect, and greased with hot coconut oil for sprains, bruises and dislocated joints as poultice and used as febrifuge (Asolkar *et a*l., 1992). Recently, there are reports on the moderate antibacterial, limited antifungal and strong anti-inflammatory activity of *A. macrophylla* leaves extract (Chattopadhyay *et al*., 2001). Futhermore, the leaf extract of *A. macrophylla* at oral doses of 200-300 mg/kg and *n*-butanol fractons of the extract at 50 mg/kg showed significant reduction in normal body temperature of Wistar rats and yeast-provoked elevated temperature in dose dependent manner comparable to the standard antipyretic drug paracetamol (Chattopadhyay *et al*., 2005). Being synonymous with *A. rostrata*, *Winchia calophylla*, distributed in Yunnan and Hainan provinces of China, India, Myanmar, and Indonesia, is used in the treatment of cough, asthma, and chronic bronchitis. Components of stem bark of *Winchia calophylla*, loganin, paeonol, N(4)-methylakuammicine and cantleyine exhibited a moderate relaxation effect on isolated smooth muscles of guinea-pig tracheal spirals and lung strips (Zhu *et al*., 2005).

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CHAPTER III

EXPERIMENTAL

1. Source of Plant Material

The stem bark of *Alstonia rostrata* Fischer was collected from Phurue, Loei province, Thailand in April 2005. The plant was authenticated by comparison with the herbarium specimens (SN 117972, BKF 121644), at the Royal Forest Department, Bangkok, Thailand.

2. General Techniques

2.1 Analytical Thin-Layer Chromatography

Technique : One dimension, ascending

Adsorbent : Silica gel 60 F_{254} (E.Merck) precoated plate

Layer thickness : 250 μm

Distance : 5 cm

Temperature : Laboratory temporary (24-30 °C)

Detection : 1. Ultraviolet light at wavelengths 254 nm and 356 nm

2. Dragendorff's spray reagent

Solution A: bismuth subnitrate (850 mg), distilled water (40ml) and acetic acid (10 ml)

Solution B: potassium iodide (8 mg) and distilled water (20 ml)

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

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2.2 Column Chromatography

2.2.1 Vacuum Liquid Column Chromatography

Adsorbent : Silica gel 60 (NO. 7734) particle size 0.063-0.200 nm (E. Merck)

Packing Method : Dry packing

Sample loading : The sample was dissolved in a small amount of organic solvent, mixed with a small quantity of adsorbent, triturated, dried and then placed gently on top of the column.

Detection : 1. Fractions were examined by TLC under UV light at the wavelength 254 nm and 356 nm.

2. Fractions were examined by TLC using Dragendorff's reagent and Anisaldehyde reagent.

2.2.2 Flash Column Chromatography

Adsorbent : 1. Silica gel 60 (NO. 7734) particle size 0.063-0.200 nm (E. Merck)

2. Silica gel 60 (NO. 9385) particle size 0.040-0.63 nm (E. Merck)

Packing method : Wet packing

Sample loading : The sample was dissolved in a small volume of eluent and then applied gently on the top of the column.

Detection : Fractions were examined in the same way as described in section 2.2.1.

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2.2.3 Gel Filtration Chromatography

Gel filter	:	Sephadex LH 20 (Pharmacia)
Packing method	:	Gel filter was suspended in the eluent and left standing to swell for 4 hours prior to use. It was then poured into column and allowed to set tightly.
Sample loading	:	The sample was dissolved in a small volume of eluent and applied on the top of the column.

2.3 Recrystallization technique

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

2.4 Spectroscopy

2.4.1 Ultraviolet (UV) Absorption Spectra

UV (in MeOH) spectra were obtained on Shimadzu UV-160A spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand).

2.4.2 Infared (IR) Absorption Spectra

IR spectra were recorded with UATR on a Perkin-Elmer Spectrum One FT-IR spectrometer (Chulabhorn Research Institute).

2.4.3 Mass Spectra

Electron Spray Impact Mass Spectra (ESIMS) were measured with a Mass Finnigan mat GCQ-Mass spectrometer (Chulabhorn Research Institute).

High Resolution mass spectra were obtained in the Time-of-Flight (TOF) manner with a Bruker Datonics mass spectrometer (Chulabhorn Research Institute).

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

^{1}H- NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were obtained with a Bruker Avance DPX-300 FT-NMR spectrometer (Faculty of Pharmaceutical Sciences, Chulalongkorn University) and ^{1}H- NMR (400 MHz) and ^{13}C-NMR (100MHz) spectra were obtained with a Bruker Avance DPX-400 FT-NMR spectrometer (Chulabhorn Research Institute).

The solvent for NMR spectra was deuterated chloroform, and deuterated dimethyl sulfoxide. 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 Optical Rotations

Optical Rotations were measured on a Perkin Elmer 341 polarimeter (Pharmaceutical Sciences, Chulalongkorn University).

2.5.2 Melting Points

Melting points were measured on Gallenkamp Melting Point Apparatus (Department of Pharmaceutical Botany, Chulalongkorn University)

2.6 Solvents

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

3. Extraction and Isolation

3.1 Extraction and Isolation of Compounds from *Alstonia rostrata*

3.1.1 Extraction

The dried stem bark of *Alstonia rostrata* Fischer (2.5 kg) was chopped, ground and then extracted with hexane (5 X 8 l), dicholoromethane (CH_2Cl_2, 6 X 8 l), and then methanol (MeOH, 5 X 8 l) to give, after removal of organic solvent, a hexane extract

(110 g), a dichloromethane extract (45.5 g), and a methanol extract (440 g), respectively (Scheme 1).

3.1.2 Isolation of Compounds from CH_2Cl_2 Extract

The CH_2Cl_2 extract (45.5 g) was dissolved in a small amount of CH_2Cl_2, triturated with silica gel 60 (NO. 7734) and dried under room temperature. It was then fractionated by vacuum liquid column chromatography using sintered glass filter column of silica gel (No. 7734). Elution was performed in a polarity gradient manner with mixtures of hexane, EtOAc and MeOH. The eluate was collected 1,000 ml per fraction and examined by TLC (Silica gel, 10 % MeOH in CH_2Cl_2). Fractions (25 fractions) with similar chromatographic pattern were combined to yield 10 fractions: Fractions AR1 (3.8 g), AR2 (2.9 g), AR3 (3.3 g), AR4 (3.5g), AR5 (3.9 g), AR6 (3.0 g), AR7 (3.7 g), AR8 (4.2 g), AR9 (7.9 g), and AR10 (6.5 g).

3.1.2.1 Isolation of Compound **1** (echitamidine)

Fraction AR7 (3.7 g) was purified on a silica gel column eluting with 0-20% MeOH in CH_2Cl_2. Fractions with similar chromatographic pattern were combined to yield 8 fractions (AR71-AR78). Fraction AR75 (210 mg) was subjected to elute with 10% MeOH in EtOAc on a silica gel column, and fraction AR754 (20 mg) was recrystallized from CH_2Cl_2-MeOH mixture to give colorless needle crystals of compound **1** (12 mg). This compound was eventually identified as **echitamidine** (Scheme 2).

3.1.2.2 Isolation of Compound **2** (echitamine)

Fraction AR8 (4.2 g) was fractionated on a silica gel column using gradient elution with 0-50% MeOH in CH_2Cl_2 to give 10 fractions (AR81-AR810). Fraction AR88 (480 mg) was separated with 20 % MeOH in CH_2Cl_2 by a silica gel column to yield 8 fractions and fraction AR887 was recrystallized from CH_2Cl_2-MeOH mixture to give yellowish crystals of compound **2** (58 mg). It was later identified as **echitamine** (Scheme 3).

3.1.2.3 Isolation of Compound **3** (Undolifoline)

Fraction AR9 (7.9 mg) was separated by silica column chromatography using gradient elution with 0-30% MeOH in CH_2Cl_2 to give 11 fractions (AR91-AR911). Fraction AR95 was further separated by silica column chromatography using isocratic elution with 10% MeOH in CH_2Cl_2 to yield 9 fractions (AR951-AR959). Fraction AR957 was obtained as white crystals and was further purified on Sephadex LH 20 by using 3:7 of MeOH in CH_2Cl_2 to give 4 fractions (AR9571-AR9574). Finally, fraction AR9573 was purified with 5 % MeOH in CH_2Cl_2 on a silica gel column to give white needle crystals of compound **3** (52 mg). It was identified as **undolifoline** (Scheme 4).

3.1.2.3 Isolation of Compound **4**, Compound **5** and Compound **6**

Fraction AR10 (6.5 mg) was fractionated on silica gel column with 0-50% MeOH in EtOAc as eluent to give 10 fractions (AR101-AR1010).

Fraction AR109 was subjected to column chromatography eluting with 0-50 % MeOH in CH_2Cl_2 to give 6 fractions (AR1091-AR1096) and fraction AR1095 was further purified with 3:7 of MeOH-CH_2Cl_2 on Sephadex LH 20 for 3 times to yield 4 fractions (AR10951-AR10954). Finally, fraction AR10953 was further separated by a silica column using 0-40 % MeOH-CH_2Cl_2 to give colorless solids of compound **4** (2.1 mg).

Fraction AR107 was separated by a silica column using gradient elution with 0-30% MeOH in EtOAc to give 7 fractions (AR1071-AR1077). Fraction AR1074 was further purified on Sephadex LH 20 with 3:7 of MeOH-CH_2Cl_2 to yield 4 fractions (AR10741-AR10744). Fraction AR10743 was finally separated on a silica gel column by using 12 % MeOH-CH_2Cl_2 to give colorless solids of compound **5** (9.6 mg).

Fraction AR105 was fractionated on silica gel column by using gradient elution with 0-30% MeOH in CH_2Cl_2 to give 6 fractions (AR1051-AR1056). Fraction AR1054 was subjected to separation on a silica column with 15 % MeOH in CH_2Cl_2 to give yellow needle crystals of compound **6** (9.8 mg) (Scheme 5).

4. Physical and Spectral data of Isolated compounds

4.1 Compound **1** (Echitamidine)

Compound **1** was obtained as colorless needle crystals, soluble in $CHCl_3$ (12 mg, 4.8 x 10^{-4} % base on dried weight of the stem bark).

$[\alpha]^{20}_{d}$: -470˚

EIMS : $[M^+]$ *m/z* 340: 340(37), 296(16), 241(100), 225(28), 180(23) 139(8); Figure 23

UV : λ_{max} nm (log ε), in MeOH; 235(3.47) and 330(3.59); Figure 22

^{1}H NMR : δ_H ppm, 400 MHz, in $CDCl_3$, Table 4; Figure 24

^{13}C NMR : δ_C ppm, 150 MHz, in $CDCl_3$, Table 4; Figure 25

4.2 Compound **2** (Echitamine)

Compound **2** was obtained as yellowish crystals, soluble in DMSO (58 mg, 1.92 x 10^{-3} % base on dried weight of the stem bark).

$[\alpha]^{20}_{d}$: -55˚

IR : ν_{max} cm^{-1}, UATR: 3284, 3153, 1725, 1606, 1472; Figure 31

EIMS : $[M-H]^+$ *m/z* 384: 385(61), 384(81), 252(53), 232(68), 194(42) 152(35); Figure 32

UV : λ_{max} nm (log ε), in MeOH; 236(3.83) and 293(3.43); Figure 30

^{1}H NMR : δ_H ppm, 400 MHz, in DMSO-*d_6*, Table 5, Figure 33

^{13}C NMR : δ_C ppm, 150 MHz, in DMSO-*d_6*, Table 5, Figure 34

4.3 Compound **3** (Undolifoline)

Compound **3** was obtained as white needle crystals, soluble in MeOH (52 mg, 3.12 x 10^{-3} % base on dried weight of the stem bark).

$[\alpha]^{20}_{d}$: -33˚

ESITOF : $[M+H]^+$ *m/z* 341.1854; Figure 40

UV : λ_{max} nm (log ε), in MeOH; 220(4.16), 281(3.52), and 288(3.44); Figure 39

^{1}H NMR : δ_H ppm, 400 MHz, in DMSO-*d*$_6$, Table 6, Figure 41

^{13}C NMR : δ_C ppm, 150 MHz, in DMSO-*d*$_6$, Table 6, Figure 43

4.4 Compound **4**

Compound **4** was obtained as colorless solids, soluble in MeOH (2.1 mg, 8.4 x 10^{-5} % base on dried weight of the stem bark).

$[\alpha]^{20}_{d}$: -154º

Melting point : 180-182 °C

IR : ν_{max} cm^{-1}, UATR : 3298, 2923, 1638, 1603, 1557, 1462; Figure 48

ESITOF : $[M]^+$ *m/z* 375.1471(100), $[M+2]^+$ *m/z* 377.1445(34); Figure 49

UV : λ_{max} nm (log ε), in MeOH; 220(3.75) and 286(3.59); Figure 47

^{1}H NMR : δ_H ppm, 400 MHz, in DMSO-*d*$_6$, Table 7, Figure 50

^{13}C NMR : δ_C ppm, 150 MHz, in DMSO-*d*$_6$, Table 7, Figure 51

4.5 Compound **5**

Compound **5** was obtained as colorless solids, soluble in MeOH (9.6 mg, 3.84 x 10^{-4} % base on dried weight of the stem bark).

$[\alpha]^{20}_{d}$: -228º

Melting point : 182-184 °C

IR : ν_{max} cm^{-1}, UATR : 3356, 2924, 1659, 1597, 1464, 1438; Figure 56

ESITOF : $[M]^+$ *m/z* 389.1623(100), $[M+2]^+$ *m/z* 391.1606(33); Figure 57

UV : λ_{max} nm (log ε), in MeOH; 232(3.66), 291(3.52) and 329(3.63); Figure 55

^{1}H NMR : δ_H ppm, 400 MHz, in DMSO-*d*$_6$, Table 8, Figure 58

^{13}C NMR : δ_C ppm, 150 MHz, in DMSO-*d*$_6$, Table 8, Figure 59

4.6 Compound **6**

Compound **6** was obtained as yellow needle crystals, soluble in MeOH (9.8 mg, 3.92 x 10^{-4} % base on dried weight of the stem bark).

$[\alpha]^{20}_{d}$: +46º

Melting point : 170-172 °C

IR : ν_{max} cm^{-1}, UATR : 3377, 2923, 1732, 1670, 1608, 1457; Figure 65

ESITOF : $[M]^+$ *m/z* 389.1635(100), $[M+2]^+$ *m/z* 391.1618(35); Figure 66

UV : λ_{max} nm (log ε), in MeOH; 220(3.96) and 269(3.4); Figure 64

^{1}H NMR : δ_H ppm, 400 MHz, in DMSO-*d*$_6$, Table 9, Figure

^{13}C NMR : δ_C ppm, 150 MHz, in DMSO-*d*$_6$, Table 9, Figure

5. Evaluation of biological Activity

5.1 Anticholinesterase activity

An important approach to treat Alzeimer's disease is directed to inhibition of acetylcholinesterarse enzyme (AchE). Some indole alkaloids which are AchE inhibitors such as physostigmine or tacrine are known to have limitation such as short half life or side effects like hepatotoxicity.

5.1.1 Materials

5.1.1.1 Buffer : 50 m*M* Tris-HCL, pH 8

5.1.1.2 Enzyme : Acetylcholinesterase from electric eel type VI-s was purchased from Sigma. It was diluted with 50 m*M* Tris-HCL to give 3 U/ml

5.1.1.3 Subtrate: 1 m*M* Acetylthiocholine iodide (ACTI)

5.1.1.4 Ellman's reagent: 1 m*M* 5,5'-Dithiobis-2-nitrobenzoic acid (DTNB)

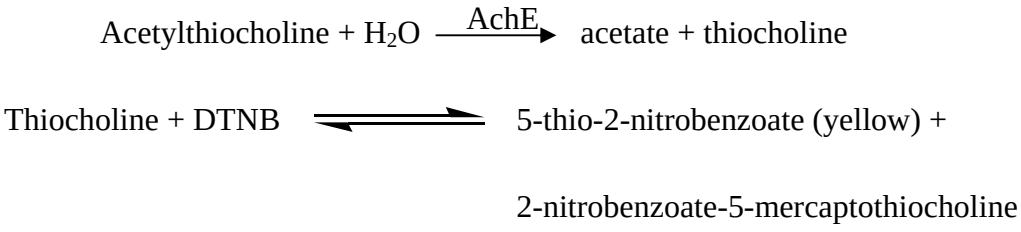
5.1.1.5 Reference AchE inhibitor: Physostigmine

5.1.1.6 TCL plate: Silica gel 60 F254

5.1.1.7 Indole alkaloids of the stem bark of *Alstonia rostrata*

5.1.2 Method

Six indole alkaloids from the stem bark of *Alstonia rostrata* and physostigmine, each, were dissolved in methanol to a concentration of 1 mg/ml. Then 2.5 μl of each sample was spotted on the silica gel TLC plate and developed in the solvent dichloromethane:methanol 8:2. Then, the enzyme inhibitory activity of the developed spots was detected by spraying the substrate, dye and enzyme base on Ellman's method (Kornkanok *et al*., 2003).



The plate was sprayed with ACTI and DTNB and waited for 45 minutes, then sprayed with 3 U/ml of enzyme solution. A yellow background appeared, with the white spots for inhibiting compounds becoming visible after 5 minutes.

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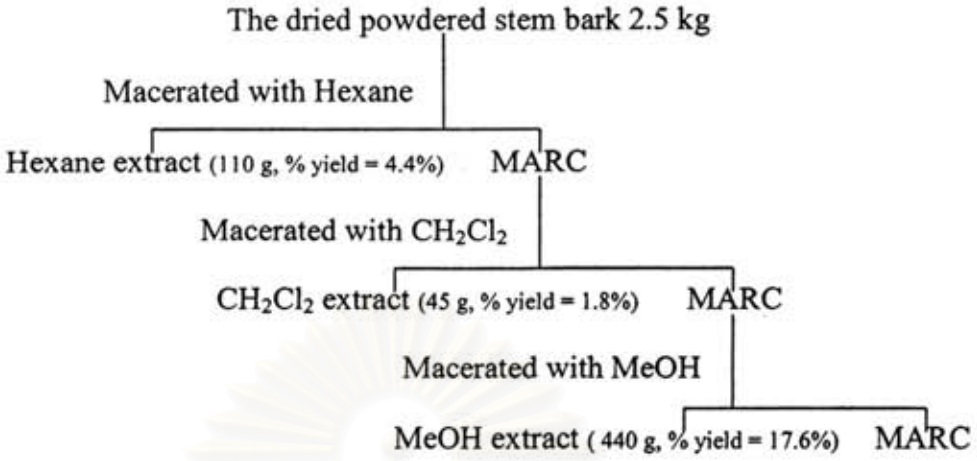
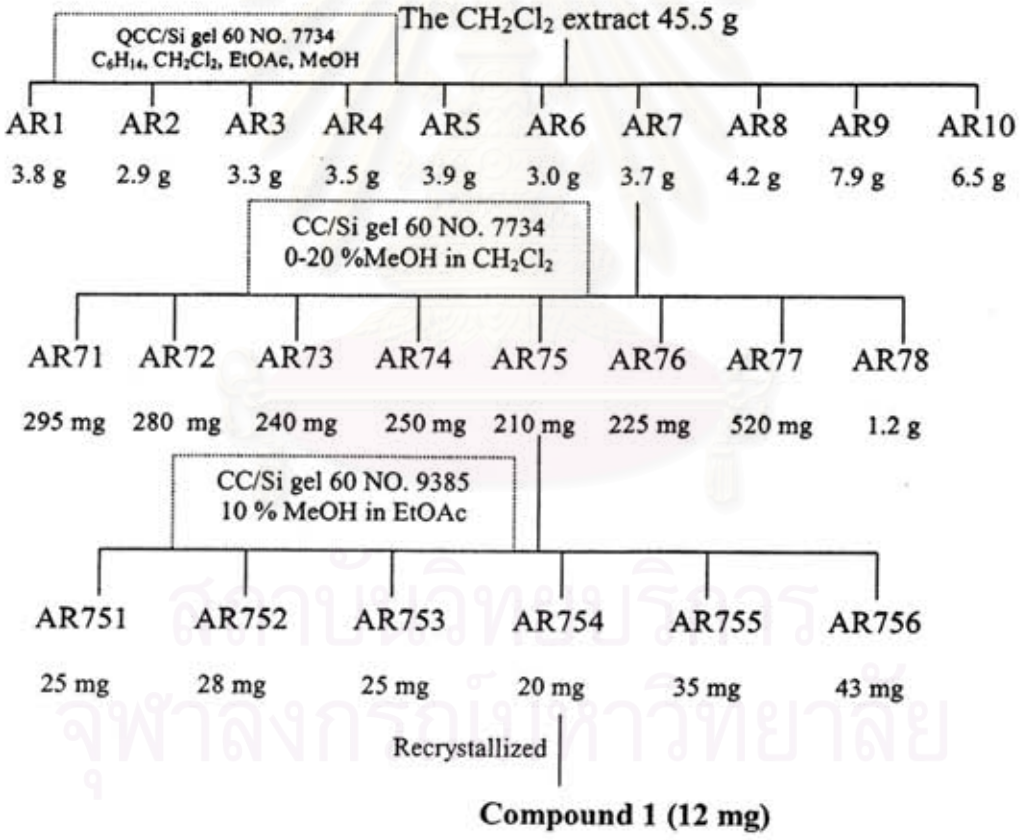
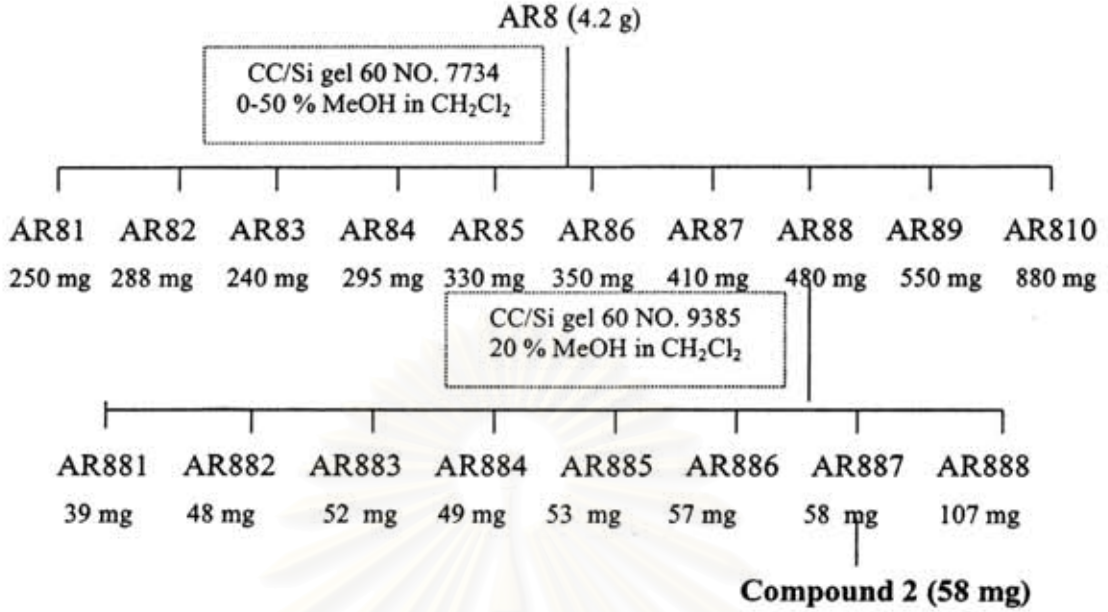


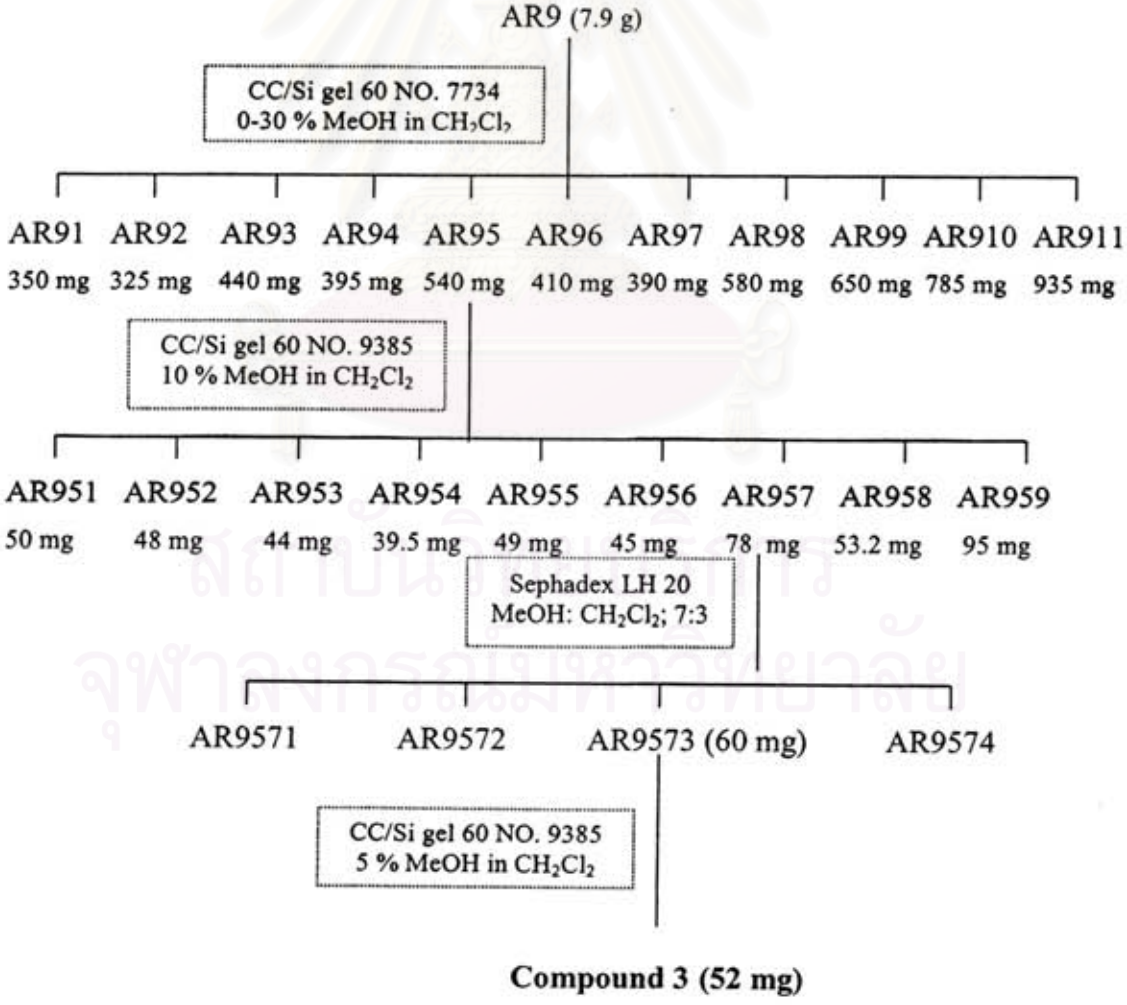
Schemes 1: Extraction scheme of the stem bark of *Alstonia rostrata*



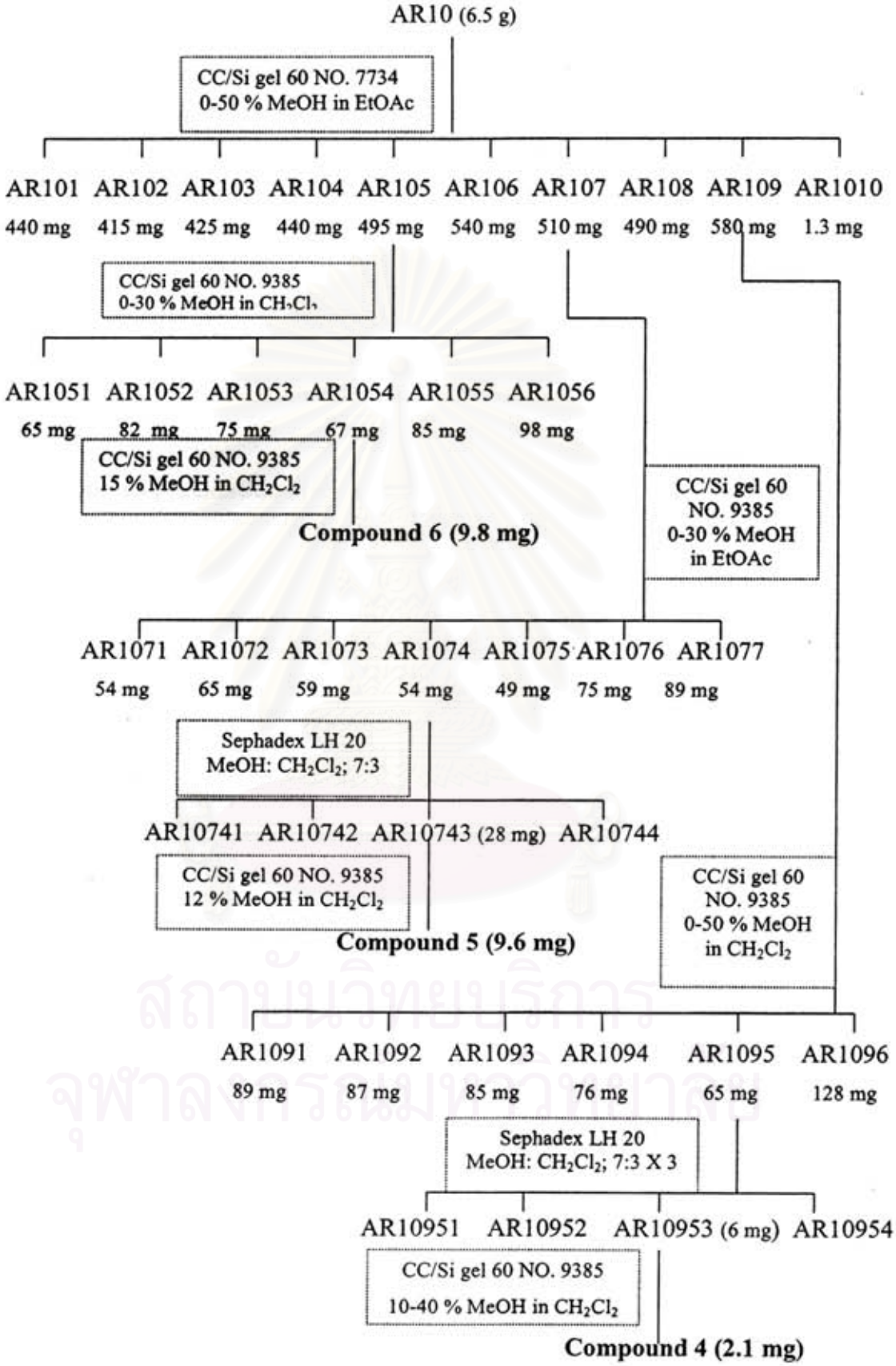
Schemes 2: Isolation scheme of Compound **1**



Schemes 3: Isolation scheme of Compound **2**



Schemes 4: Isolation scheme of Compound **3**



Schemes 5: Isolation scheme of Compound **4, 5,** and **6**

CHAPTER IV

RESULTS AND DISCUSSION

The crude extract of the stem bark of *Alstonia rostrata* Fischer was separated by repeated chromatography using silica gel and Sephedex LH 20 to give six pure compounds. The structures of the isolated compounds were determined by interpretation of their UV, IR, NMR and MS data and by comparison of the spectral data with literature values. The antiacetylcholinesterase activity of these compounds was also determined.

1. Structure Elucidation of Compound 1

Compound **1** was obtained as colorless needle crystals. The R_f values are 0.55 (silica gel / 15% MeOH in CH_2Cl_2) and 0.35 (silica gel / 15% MeOH in EtOAc). It was identified as ecthitamidine (Figure 2). This compound was previously isolated from *A. congensis* (Caron *et al*., 1989), *A. undolifolia* (Massiot *et al*., 1992), *A. glaucescens* (Keawpradub *et al*., 1994) and *W. calophylla* (Zhu *et al*., 2005).

The UV spectrum of compound **1** (Figure 22) showed maximum absorptions at 330 and 235 nm, which was characteristic of an anilino-acrylate chromophore. The EI mass spectrum (Figure 23) afforded a molecular peak $[M]^+$ (rel. int.) at *m/z* 340(37) which agreed with the molecular formula $C_{20}H_{24}N_2O_3$ (D.B.E. = 10), while other major fragments appeared at *m/z* 241 (100), 225 (28), 180 (23), 139 (8).

The ^{1}H-NMR spectrum of compound **1** (Figure 24, Table 4) showed a three-proton singlet signal at δ 3.87 which could be assigned to the methoxy group. The NH proton signal appeared at δ 8.64. The signal of methyl protons appearing as a doublet at δ 1.16 (*J*=6.1 Hz) showed vicinal coupling with the adjacent H-19. On the other hand, H-19 signal resonated at δ 3.27 (*J*=11.1, 6.3 Hz) as a split doublet of quartet showing vicinal coupling with the H-20 and H-18, respectively. In the aromatic region, there are four protons of a 1, 2-substituted benzene ring.

Further spectroscopic studies were done by examination of the ^{13}C NMR spectrum [100 MHz, $CDCl_3$] (Figure 25, Table 4) of compound **1**. The methyl group signal of the –CH(OH)CH_3 moiety resonated at δ 19.8 while the OH bearing methine

carbon signal appeared at δ 68.4. The peaks at δ 51.8 and δ 172.6 were assigned to methoxy and carbonyl carbons of the ester group. Four aromatic methine carbon signals appearing at δ 127.6, δ 121.4, δ 119.8, and δ 109.6 were assigned to C-11, C-9, C-10, and C-12, respectively. The ^{1}H-^{1}H coupling information obtained from the ^{1}H-^{1}H COSY spectrum (Figure 26) and the one-bond correlations between proton and carbon gained from the HMQC spectrum (Figure 27) indicated the presence of two methyl, four methylene, and eight methine carbons in **1**. The other six remaining carbons were assigned as quaternary carbons including the C=O function. The cross-peaks of the ^{13}C-^{1}H long range correlations obtained from HMBC experiment (Figure 28, Table 4) allowed various fragments to be connected, as shown in figure 4. The stereochemistry at C-19 and C-20 were determined by comparison of the ^{1}H and ^{13}C chemical shifts of compound **1** with those of compounds ecthitamidine and 20-epi-19ζ-echitamidine which were reported in 1994 by Keawpradub and co-workers, and also confirmed by the NOESY experiment (Figure 3 and Figure 29).

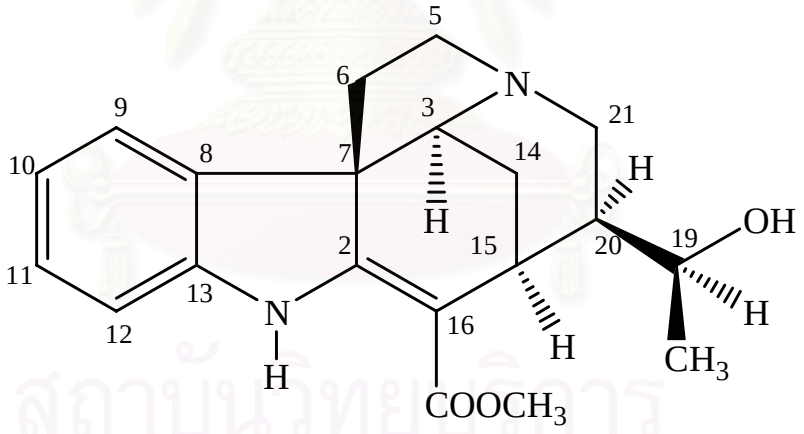


Figure 2: Structure of compound **1**

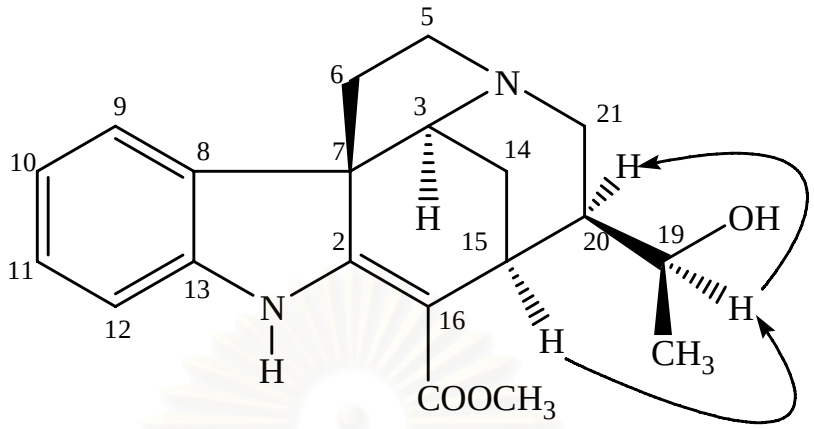


Figure 3: NOESY correlation of compound **1**

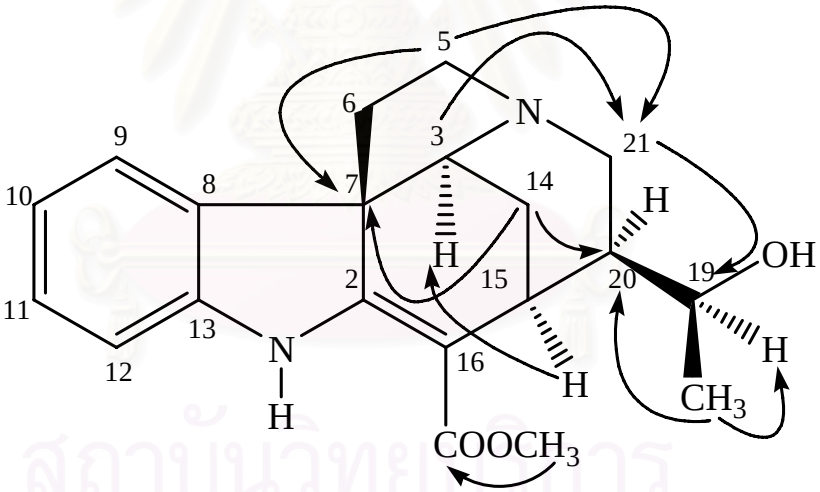


Figure 4: HMBC correlations of compound **1**

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Table 4 ^{1}H, ^{13}C and HMBC spectral data of compound **1** (in $CDCl_3$) and echitamidine (in $CDCl_3$) (Keawpradub *et al*., 1994)

Position	Compound **1**		Echitamidine		HMBC Correlation
	δ_H(ppm), (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H(ppm), (multiplicity, *J* in Hz)	δ_C (ppm)	
2	-	168.9	-	168.8	
3	3.88 (1H, *br s*)	61.0	3.91 (1H, *br s*)	60.9	C-15, C-21
5	3.08 (1H, *m*)	54.2	3.10 (1H, *m*)	54.0	C-6
5	2.87 (1H, *dd*, 13.5, 1.6)	-	2.87 (1H, *dd*, 13.0,1.6)	-	C-21, C-7
6	2.82 (1H, *m*)	43.7	2.82 (1H, *m*)	43.4	C-21, C-7
6	1.84 (1H, *m*)	-	1.86 (1H, *m*)	-	
7	-	57.3	-	57.1	
8	-	135.8	-	135.5	
9	7.19 (1H, *br d*, 7.5)	121.4	7.19 (1H, *br d*,7.6)	121.4	C-7, C-11
10	6.93 (1H, *td*, 7.5, 1.0)	119.8	6.93 (1H, *td*, 7.6, 1.0)	119.8	C-12, C-8
11	7.15 (1H, *td*, 7.5, 1.0)	127.6	7.15 (1H, *td*, 7.6, 1.0)	127.6	C-10, C-13
12	6.84 (1H, *br d*, 7.5)	109.6	6.85 (1H, *br d*, 7.6)	109.6	C-9, C-8
13	-	143.8	-	147.7	
14	2.02 (1H, *ddd*, 13.0,3.0, 2.0)	31.1	2.04 (1H, *ddd*, 13.0, 3.0, 1.8)	31.0	C-20, C-7 C-16,
14	1.41 (1H, *ddd*, 13.0, 3.0, 2.0)	-	1.42 (1H, *ddd*, 13.0, 3.0, 2.0)	-	
15	3.32 (1H, *br d*, 1.6)	28.9	3.33 (1H,*br d*,1.7)	28.8	C-21, C-3
16	-	96.9	-	96.9	
18(CH_3)	1.16 (3H, *d*, 6.3)	19.8	1.16 (3H, *d*, 6.1)	19.8	C-19, C-20
19	3.27 (1H, *dq*, 11.1, 6.3)	68.4	3.27 (1H, *dq*, 11.8, 6.1)	68.4	
20	1.74 (1H, *m*)	46.0	1.77 (1H, *m*)	45.8	C-21
21	2.93 (1H, *dd*, 11.4, 4.3)	48.2	2.91 (1H, *dd*, 11.4, 4.3)	48.1	C-5, C-19 C-20
21	1.93 (1H, *br t*, 11.4)	-	1.94 (1H, *br t*, 11.4)	-	
COO	-	172.6	-	172.3	
OCH_3	3.87 (3H, *s*)	51.8	3.88 (3H, *s*)	51.9	C=O
NH	8.64 (1H, *brs*)	-	8.64 (1H, *br s*)	-	

2. Structure Elucidation of Compound 2

Compound **2** was obtained as yellowish crystals. The R_f values are 0.35 (silica gel / 15% MeOH in CH_2Cl_2) and 0.15 (silica gel / 15% MeOH in EtOAc). By comparison with published spectral data, compound **2** was identified as echitamine (Figure 5). This compound was previously isolated from the stem bark of *A. congensis*(Caron *et al*., 1989), *A. undolifolia* (Massiot *et al*., 1992), *A. glaucescens* (Keawpradub *et al*., 1994), and *W. calophylla* (Zhu *et al*., 2005).

The UV spectrum of compound **2** (Figure 30) showed maximum absorptions at 293 and 236 nm suggesting the presence of an indoline chromophore. The IR spectrum (Figure 31) indicated absorption bands for an N-H functionality at 3405 cm^1, a methoxycarbonyl group at 1735 cm^{-1} and an aromatic ring at 1606 and 1472 cm^{-1}. The EI mass spectrum (Figure 32) of compound **2** measured under EI condition was characterized by its thermal decomposition product at *m/z* 384 $[M-H]^+$ formed by a Hofman degradation and the molecular ion peak at *m/z* 385 corresponding to the molecular formula $C_{22}H_{29}N_2O_4$ (D.B.E.=9.5).

The ^{1}H-NMR spectrum of compound **2** (Figure 33, Table 5), the chemical shifts and splitting patterns of the four aromatic protons of compound **2** indicated a lack of substitution on positions 9, 10, 11 and 12 of 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 5.74 and 1.79 ppm were observed in ^{1}H-NMR spectrum The signal for methoxy protons resonated at δ 3.77 and the signal for N-CH_3 protons appeared at δ 3.29 ppm. A multiplet signal at δ 3.34 was assigned to H-5 since it showed vicinal coupling with H-6α and H-6β (δ 2.03 and 2.24). The information obtained from the HMQC correlations (Figure 37) and DEPT spectra (Figure 35) revealed the presence of three methyl groups, five methylene groups, seven methine, and seven quaternary carbons in the structure of **2**. The signal due to the C-2 quaternary carbon located between two nitrogen atoms was resonated at δ 98.0. 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 (Figure 36) was observed between H-14β and H-15. The downfield methylene carbon signals at δ 69.2 and δ 65.0 were assigned to C-3 and C-17, respectively, on the basis of the one-bond ^{13}C-^{1}H correlations. The long-range coupling observed in the ^{13}C-^{1}H HMBC spectrum (Figure 38, Table 5) allowed the various fragments to be connected (Figure 2). The chemical shifts of compound **2**

were similar to those reported in the literature (Keawpradub *et al*., 1994) for "echitamine".

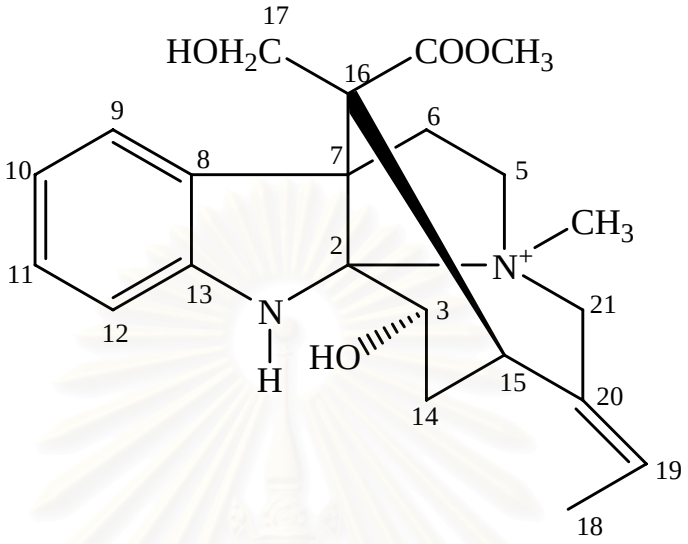

Figure 5: Structure of compound **2**

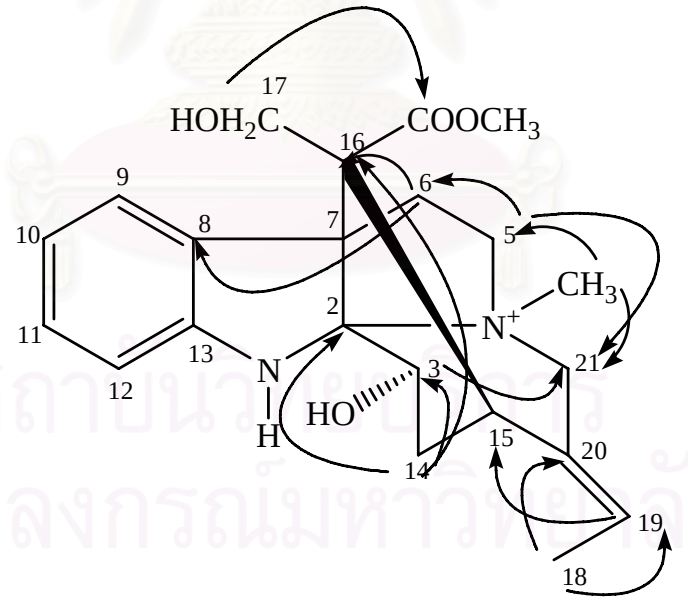

Figure 6: HMBC correlations of compound **2**

Table 5 ^{1}H, ^{13}C and HMBC spectral data of compound **2** (in DMSO-d_6) and echitamine (in DMSO-d_6) (Keawpradub *et al*., 1994)

Position	Compound **2**		Echitamine		HMBC Correlation
	(multiplicity, *J* in Hz)	δ_C (ppm)	(multiplicity, *J* in Hz)	δ_C (ppm)	
2	-	100.4	-	100.0	
3	4.36 (1H, *dd*, 10.6, 5.3)	69.2	4.36 (1H, *dd*, 10.6, 5.5)	68.8	21
5	3.64 (1H, *dd*, 12.0, 8.3)	62.2	3.63 (1H, *dd*, 12.7, 8.5)	61.8	C-6, C-21
5	3.34 (1H, *m*)	-	3.34 (1H, *m*)	-	
6	2.24 (1H, *dt*,14.2, 8.3)	41.5	2.24 (1H, *dt*, 14.2, 8.5)	41.1	C-5, C-7
6	2.03 (1H, *dd*,14.2, 8.6)	-	2.02 (1H, *dd*, 14.2, 8.4)	-	C-8, C-16
7	-	61.0	-	60.6	
8	-	129.2	-	128.7	
9	7.75 (1H, *dd*, 7.6, 1.0)	127.1	7.74 (1H, *dd*, 7.6, 1.0)	126.7	C-7, C-11 C-13
10	6.76 (1H, *td*, 7.6, 1.0)	119.9	6.75 (1H, *td*, 7.6, 1.0)	119.9	C-11,C-12
11	7.11 (1H, *td*, 7.6, 1.2)	129.1	7.10 (1H, *td*, 7.6, 1.0)	128.7	C-9, C-13
12	6.74 (1H, *dd*,7.6, 1.0)	111.1	6.73 (1H, *dd*, 7.9, 1.0)	110.6	C-10
13	-	147.9	-	147.5	
14_β	2.60 (1H, *ddd*, 15.1, 10.6, 5.5)	31.2	2.59 (1H, *ddd*, 15.0, 10.6, 5.5)	30.7	C-2, C-3 C-15, C-16
14_α	1.52 (1H, *ddd*, 14.7, 5.7, 1.0)	-	1.52 (1H, *ddd*, 14.7, 5.7, 1.0)	-	C-20
15	3.86 (1H, *d*, 4.9)	34.9	3.86 (1H, *d*, 4.7)	34.4	C-3, C-14 C-16, C-17
16	-	56.2	-	55.7	
17	3.74 (1H, *d*, 10.2)	65.0	3.74 (1H, *d*, 10.2)	64.5	C=O
17	3.16 (1H, *d*, 5.2)		3.16 (1H, *d*, 5.2)		
18(CH_3)	1.13 (3H, *d*, 5.8)	15.4	1.16 (3H,*d*, 6.1)	15.4	C-19, C-20
19	5.74 (1H, *q*, 6.4)	130.2	5.73 (1H, *q*, 6.4)	129.8	C-15, C-18
21_α	4.44 (1H, *br d*, 14.9)	65.1	4.42 (1H, *br d*, 14.9)	64.7	C-15, C-19
21_β	4.25 (1H, *d*, 14.9)	-	4.25 (1H, *d*, 14.9)		C-20
NH	7.61 (1H, *br s*)	-	7.61 (1H, *br s*)	-	
COO		173.6		173.1	
OCH_3	3.73 (3H, *s*)	52.4	3.73 (3H, *s*)	51.9	C=O, C-16
NCH_3	3.29 (3H, *s*)	50.0	3.29 (3H, *s*)	49.6	C-2, C-5 C-21

3. Structure Elucidation of Compound 3

Compound **3** was obtained as white needle crystals. The R_f values are 0.40 (silica gel / 15% MeOH in CH_2Cl_2) and 0.20 (silica gel / 15% MeOH in EtOAc). By comparison with published spectral data, compound **3** was identified as undolifoline (Figure 7). This compound was previously isolated from the stem bark of *A. undolifolia* (Massiot *et al*., 1992).

The UV spectrum of compound **3** (Figure 39) was found to be characteristic for the indole chromophore, showing maximum absorptions at 288, 281, 220 nm. Its psuedomolecular ion peak at *m/z* 341.1854 $[M+H]^+$ was observed from the electrospray time of flight mass spectrometry (ESITOF MS) (Figure 40), suggesting the molecular formula $C_{20}H_{24}N_2O_3$ (D.B.E. = 10), a smaller ion at *m/z* 282 was attributed to the loss of a methoxy carbonyl.

The ^1H and ^{13}C NMR spectra (DMSO-d_6) confirmed the presence of an intact indole nucleus. The ^1H NMR spectrum (Figure 41, Table 6) showed the signal for methoxy protons at δ 3.71 and the signal for N-methyl protons at 2.55 ppm. The ^1H NMR spectrum of **3** revealed the presence of a 1, 2-disubstituted benzene ring. The downfield part of ^{13}C NMR spectrum (Figure 43, Table 6) showed signals for an indole nucleus and for the carbomethoxy at δ 171.9 and DEPT spectra (Figure 44), the upfield part displayed signals for three methine, five methylene, two methyl and one quaternary carbon. Two of the methylenes bearing an oxygen atom and a CH_2-O-CH_2 coupling were observed in COSY spectrum (Figure 42). Beside this long range coupling in HMBC spectrum (Figure 46, Table 6), the proton of one of the oxymethylene showed no other coupling than the germinal coupling (*J*=11.8 Hz), and thus, this methylene was linked to a quaternary carbon atom. The other oxymethylene was linked to a highfield methylene, as displayed in figure 8. In the COSY spectrum, except H_2-17, all other protons were linked, and it was therefore possible to assemble an O-CH_2-CH_2-CH-(CH)-CH-CH_2-CH_2 substructure. The existence of an ether bridge between C-17 and C-18 fixed the relative configurations of C-16 and C-20. The stereochemistry of compound **3** was determined by comparison of the optical rotation values of compound **3** and undolifoline which was reported in 1992 by Massiot and colleagues

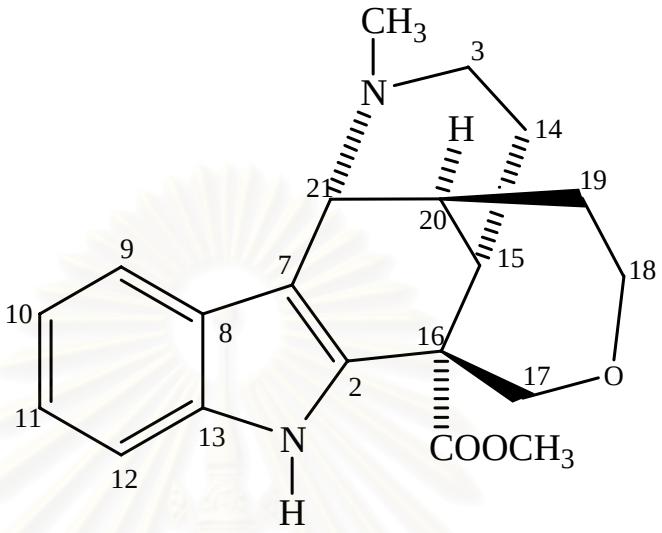

Figure 7: Structure of compound **3**

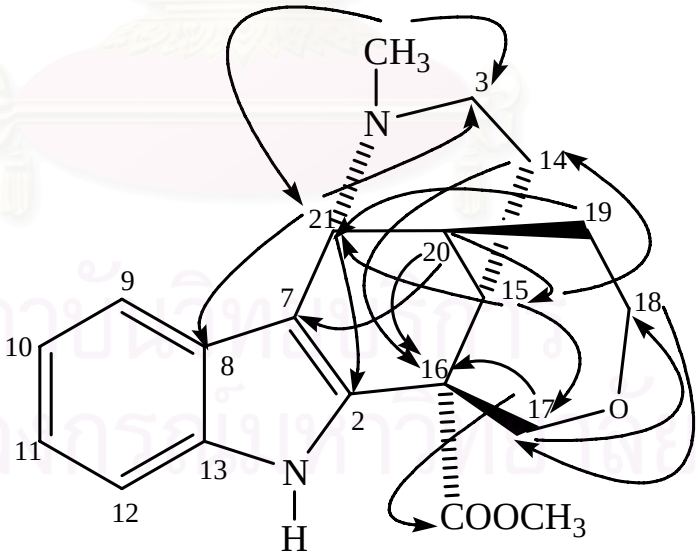

Figure 8: HMBC correlations of compound **3**

Table 6 ^{1}H, ^{13}C and HMBC spectral data of compound **3** (in DMSO-d_6) and undolifoline (in $CDCl_3$) (Massiot *et al*., 1992)

Position	Compound **3**		Undolifoline		HMBC Correlation
	(multiplicity, *J* in Hz)	δ_C (ppm)	(multiplicity, *J* in Hz)	δ_C (ppm)	
2	-	138.3	-	134.6	
3	3.94 (1H, *dd*, 12.2, 3.9)	45.7	2.45 (1H, *m*)	46.1	C-14, C-15 C-21, N-CH_3
3	2.54(1H, *m*)	-	2.12-1.91 (1H, *m*)	-	
5	-	-	-	-	
6	-	-	-	-	
7	-	101.4	-	107.4	
8	-	127.8	-	129.0	
9	7.69 (1H, *d*, 7.8)	118.7	7.36 (1H, *d*, 8.0)	119.7	C-7, C-8, C-10, C-13
10	7.06 (1H, *td*, 7.8, 1.0)	119.9	7.2-7.07 (1H, *m*)	118.9	C-12, C-8
11	7.14 (1H, *td*, 7.8, 1.0)	122.1	7.2-7.07 (1H, *m*)	121.9	C-9, C-13
12	7.40 (1H, *d*, 7.8)	111.9	7.55 (1H, *d*, 8.0)	111.2	C-8, C-10
13	-	136.9	-	136.9	
14	2.15 (1H, *m*)	28.1	2.12-1.91 (1H, *m*)	30.7	C-15, C-16
14	1.53 (1H, *d*, 17.8)		1.65 (1H, *m*)		
15	2.85 (1H, *br q*, 2.1)	36.3	2.78 (1H, *br* q, 3.3)	37.9	C-2, C-3, C-14 C-17, C-20,C-21
16	-	54.5	-	55.4	
17	4.34 (1H, *d*, 11.8)	77.4	4.18 (1H, *d*, 11.8)	77.4	C=O, C-2, C-15, C-18
17	3.84 (1H, *d*, 11.8)	-	3.89 (1H, *d*, 11.8)	-	
18	3.52 (2H, *m*)	68.6	3.72 (1H, *dt*, 12.6, 6.0)	69.6	C-17, C20
		-	3.50 (1H, *br t*, 12.6)	-	
19	1.95 (1H, *m*)	32.5	2.12-1.91 (1H, *m*)	33.1	C-18, C-21
19	0.98 (1H, *ddd*, 15, 11.8, 5.1, 3.5)	-	1.35 (1H, *ddd*, 15, 11.8, 5.1, 3.5)	-	
20	3.05 (1H, *m*)	37.1	2.73 (1H, *m*)	44.0	C-7, C-15, C-16
21	4.60 (1H, *br d* , 1.9)	58.4	3.95 (1H, *br d* , 2.6)	58.7	C-2, C-3, C-7 C-8, C-15
COO	-	171.9	-	172.7	
OCH_3	3.71 (3H, *s*)	52.8	3.88 (3H, *s*)	52.3	C=O
NH	11.70 (1H, *br s*)	-	8.30 (1H, *br s*)	-	C-2, C-7, C-8
NCH_3	2.55 (3H, *br s*)	41.3	2.30 (3H, *s*)	40.4	C-3, C-21

4. Structure Elucidation of Compound 4

Compound **4** (Figure 9) was obtained as colorless solids. The R_f values are 0.15 (silica gel / 15% MeOH in CH_2Cl_2) and 0.05 (silica gel / 20% MeOH in EtOAc). The UV absorptions at 220 and 288 nm of compound **4** (Figure 47) were characteristic of an aniline acrylate chromophore. The IR spectrum (Figure 48) showed the presence of hydroxol (3298 cm^{-1}) and carbonyl (1638 cm^{-1}). The ESITOF mass spectrum of **4** (Figure 49) showed a $[M]^+$ peak at *m/z* 375.1471, consistent with the tentative molecular formula $C_{20}H_{24}ClN_2O_3$ (D. B. E.=9.5).

The ^1H NMR spectrum (Figure 50, Table 7) showed the NH proton signal at δ 10.2, and the signal for methyl protons at δ 1.03 (H-18) which showed vicinal coupling with a neighboring proton at δ 3.46 (*d*, *J*= 6.1 Hz, H-19). In the aromatic region, there are four protons of a 1, 2-substituted benzene ring. The ^{13}C NMR spectrum (Figure 51, Table 7) displayed the peak of the carbonyl carbon of carboxylic acid functional group at δ 171.9. The methyl carbon signal at δ 20.5 ppm could be assigned to C-18 while the downfield methine carbon signal at δ 66.2 ppm could be assigned to C-19. Furthermore, in the downfield region of HMQC spectrum (Figure 53), the signals for methylene group (N-CH_2) at δ 5.65 ppm and 5.88 ppm correlated to the carbon at δ 65.6 ppm, and showed long range correlation to C-5 and C-21 in HMBC spectrum (Figure 54). Furthermore three bonds correlation from H-3 to this methylene carbon was also observed. However, there were no correlations between the methylene proton (N-CH_2) and other protons in the ^1H-^1H COSY spectrum (Figure 52). Based on these spectral data the methylene protons at δ 5.65 and 5.88 ppm may attach to the quaternary ammonium salt adjacent to C-5, C-21, as shown. On the other hand, the other side of this mehylene group should be connected with heteroatom, according to its resonances in the ^1H and ^{13}C NMR spectra. The ESITOF MS spectrum suggested that the heteroatom should be chlorine. The information obtained from the ^1H-^1H COSY spectrum and the one bond correlations between proton and carbon nuclei gained from HMQC spectrum indicated the presence of one methyl, five methylene, and eight methine functions. The other six remaining carbons were assigned as quaternary carbons including the C=O function. The ^1H-^1H COSY correlations, in the aromatic region showed cross peaks from δ 7.37 (H-9) to δ 6.79 (H-10) and from δ 7.09 (H-11) to δ 6.79 (H-10) and δ 6.89 (H-12). Moreover, there were cross peaks from δ 2.15 (H-14) to δ 4.71 (H-3) and from δ 1.35 (H-14) to δ 3.26

(H-15), from δ 3.10 (H-19) to δ 1.92 (H-20) and δ 1.03 (H-18), from δ 1.92 (H-20) to δ 2.98 (H-21), and between H_2-5 and H_2-6, as shown in figure11. The cross-peaks of ^{1}H-^{13}C long range correlations obtained from HMBC experiment allowed various fragments to be connected (Figure 10). The stereochemistry at C-19 and C-20 were determined by comparison of ^{1}H and ^{13}C chemical shifts of compound **4** with those of 17-Carboxyl-*N*(4)-methylechitamidine chloride (Figure 12) which was reported in 2006 by Gan and colleagues. Therefore compound **4** was identified as a new compound namely 17-carboxyl-*N*(4)-chloromethylechitamidine.

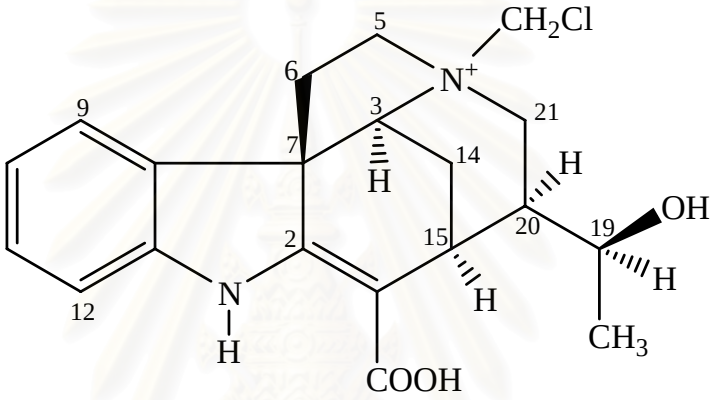

Figure 9: Structure of compound **4**

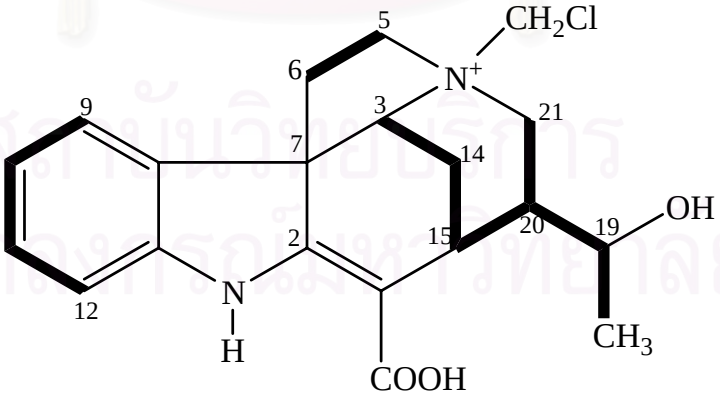

Figure 10: ^{1}H-^{1}H COSY correlations of compound **4**

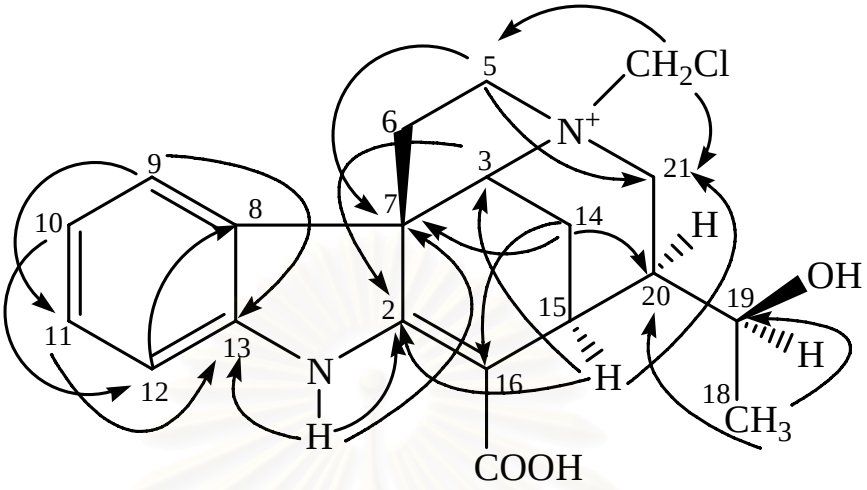

Figure 11: HMBC correlations of compound **4**

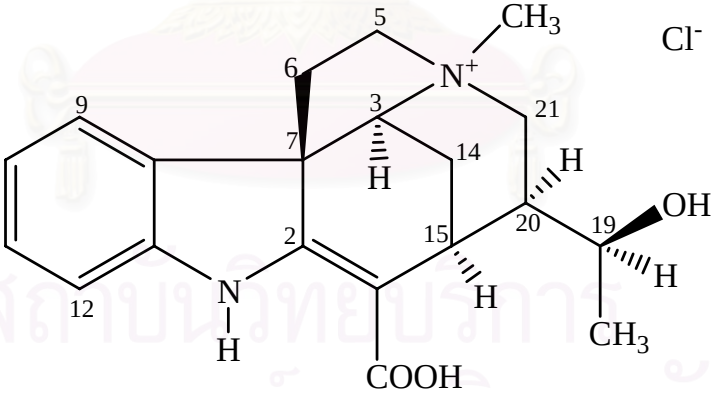

Figure 12: 17-Carboxyl-*N*(4)-methylechitamidine chloride from *Winchia calophylla*

By Gan *et al*.

Table 7 ^{1}H, ^{13}C and HMBC spectral data of compound **4** (in DMSO-d_6)

Position	Compound **4**		HMBC Correlation
	δ_H(ppm), (multiplicity, *J* in Hz)	δ_C (ppm)	
2	-	159.1	
3	4.71 (1H, *br s*)	70.6	C-2, C-14, C-21
5	3.80 (1H, *m*); 3.57 (1H, *m*)	60.3	C-6, C-7, C-21
6	1.94 (1H, *m*); 2.84 (1H, *m*)	37.8	C-2, C-7, C-8, C-21
7	-	52.9	
8	-	132.9	
9	7.37 (1H, *d*, 7.5)	120.6	C-7, C-11,C-13
10	6.79 (1H, *td*, 7.5, 1.2)	119.8	C-8, C-12
11	7.09 (1H, *td*, 7.5, 1.2)	128.7	C-9, C-12, C-13
12	6.89 (1H, *br d*, 7.5)	110.3	C-8, C-10
13	-	146.3	
14	1.35 (1H, *br d*, 14.1); 2.15 (1H, *br d*, 14.1)	27.2	C-7, C-16, C-20
15	3.26 (1H, *d*, 4.8)	28.0	C-2, C-3, C-16 C-20, C-21
16	-	108.9	
18 (CH_3)	1.03 (3H, *d*, 6.1)	20.5	C-19, C-20
19	3.10 (1H, *m*)	66.2	
20	1.92 (1H, *m*)	41.4	
21	3.48 (1H, *m*); 2.98 (1H, *br t*, 13.8)	52.5	C-14, C-19, C-20 C-22
COO	-	171.9	
N-CH_2Cl	5.88 (1H, *d*, 9.9); 5.65 (1H, *d*, 9.9)	65.6	C-5, C-21
NH	10.2 (1H, *br s*)		C-7, C-8, C-13

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5. Structure Elucidation of Compound 5

Compound **5** (Figure 13) was obtained as colorless solids. The R_f values are 0.32 (silica gel / 15% MeOH in CH_2Cl_2) and 0.18 (silica gel / 20% MeOH in EtOAc). The UV spectrum of compound **5** (Figure 55) showed similar UV spectral data to those of compound **1**, with absorption maxima at 232, 291, and 329 nm, which was characteristic of an anilino-acrylate chromophore. The IR spectrum (Figure 56) gave absorptions at 3356 cm^{-1} (NH) and 1659 cm^{-1} (α, β unsaturated ester, C=O). The ESITOF mass spectrum of compound **5** (Figure 57) showed a $[M]^+$ peak at *m/z* 389.1623, consistent with the tentative molecular formula $C_{21}H_{26}ClN_2O_3$ (D. B. E.=9.5).

The ^1H NMR spectrum (Figure 58, Table 8) showed a proton singlet signal of the carbomethoxy group at δ 3.87, the NH proton signal at δ 8.64, and the signal for methyl protons at δ 1.08 (H-18) which showed vicinal coupling with the adjoining H-19 (δ 3.46). The chemical shift and splitting patterns of the four aromatic protons indicated the lack of substitution on positions 9, 10, 11, and 12. The ^{13}C NMR spectrum (Figure 59, Table 8) displayed the peaks of the methyl ester and carbonyl ester carbons at δ 52.0 and δ 167.7, respectively. The methyl carbon signal at δ 20.3 ppm was assigned to C-18 while the downfield methine carbon signal at δ 66.5 ppm which connected with oxygen could be assigned to C-19. Furthermore, in the downfield region of HMQC spectrum (Figure 62), the methylene group ($N\text{-}CH_2$) signal at δ 5.95 ppm correlated to the carbon at δ 65.6 ppm, and showed long range correlation to C-5 and C-21 in HMBC spectrum (Figure 63). However, there were no correlations between the methylene at δ 5.95 ($N\text{-}CH_2$) and other protons in the ^1H-^1H COSY spectrum (Figure 61). Based on these spectral data the methylene at δ 5.95 may attach to the quaternary ammonium salt adjacent to C-5 and C-21 as shown. On the other hand, the other side of this mehylene group should be connected with heteroatom, according to its resonances in the ^1H and ^{13}C NMR spectra. The ESITOF MS spectrum suggested that the heteroatom should be chlorine. The information obtained from the ^1H-^1H COSY spectrum and the one bond correlations between proton and carbon nuclei gained from HMQC spectrum indicated the presence of two methyl, five methylene, and eight methine functions. The other six remaining carbons were assigned as quaternary carbons including the C=O function. The ^1H-^1H COSY correlations, in the aromatic region showed cross peaks from δ 6.85 (H-10) to δ 7.68

(H-9) and from δ 7.18 (H-11) to δ 6.85 (H-10) and δ 7.06 (H-12). Moreover, there were cross peaks from δ 2.28 (H-14) to δ 5.00 (H-3) and δ 3.43 (H-15), from δ 2.15 (H-20) to δ 3.43 (H-15), δ 3.46 (H-19) and δ 3.21 (H-21), from δ 1.08 (H-18) to δ 3.46 (H-19), and between H_2-5 and H_2-6, as shown in figure14. The cross-peaks of ^{1}H-^{13}C long range correlations obtained from HMBC experiment allowed various fragments to be connected (Figure 15). The stereochemistry at C-19 and C-20 were determined by comparison of ^{1}H and ^{13}C chemical shifts of compound **5** with those of echitamidine *N*-oxide (Figure 16) which was reported in 1994 by Keawpradub and co-workers. Compound **5** was newly identified as *N*(4)-chloromethylechitamidine.

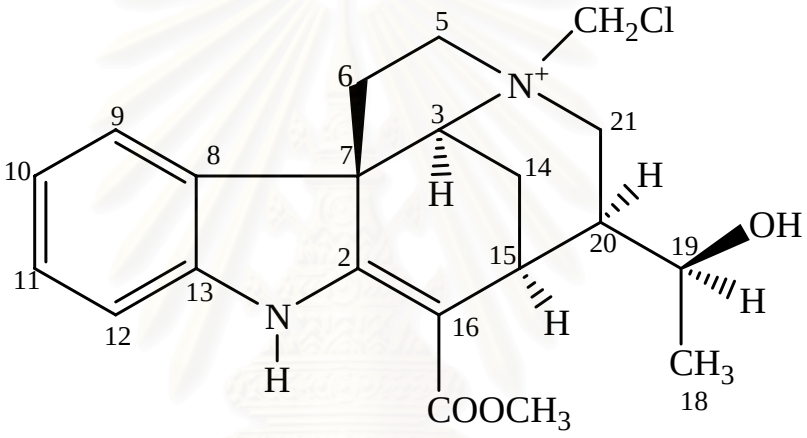

Figure 13: Structure of compound **5**

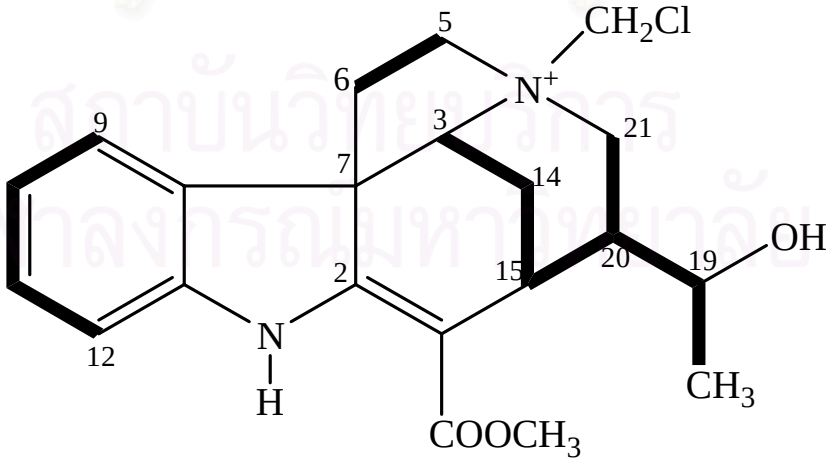

Figure 14: ^{1}H-^{1}H COSY correlations of compound **5**

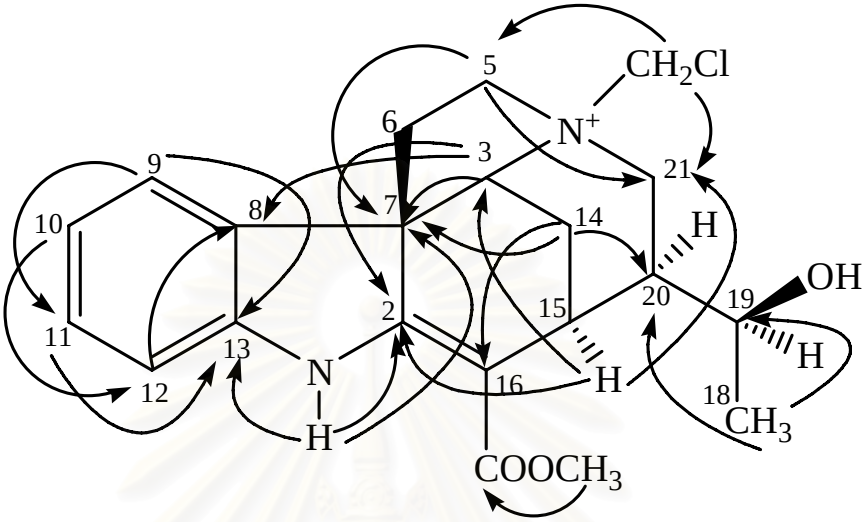

Figure 15: HMBC correlations of compound **5**

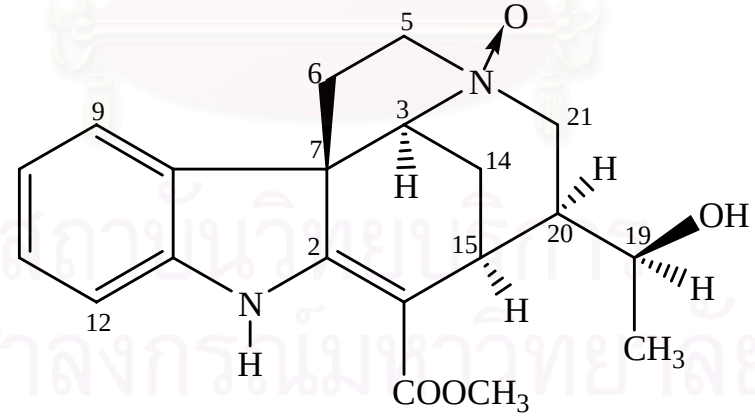

Figure 16: Echitamidine *N*-oxide from *Alstonia glaucescen* by Keawpradub *et* al.

Table 8 ^{1}H, ^{13}C and HMBC spectral data of compound **5** (in DMSO-d_6)

Position	Compound **5**		HMBC Correlation
	δ_H(ppm), (multiplicity, *J* in Hz)	δ_C (ppm)	
2	-	165.5	
3	5.00 (1H,*br s*)	70.6	C-2, C-7, C-8 C-15, C-21
5	4.00 (1H, *m*); 3.65 (1H, *m*)	60.4	C-3, C-7, C-21
6	2.93 (1H, *dd*, 14.4, 7.5,); 2.05 (1H, *m*)	39.1	C-2, C-5
7	-	55.2	-
8	-	132.6	-
9	7.68 (1H, *br d*, 7.5)	120.9	C-7, C-11, C-13
10	6.85 (1H, *td*, 7.5, 0.9)	121.2	C-8, C-9, C-12
11	7.18 (1H, *td*, 7.5, 0.9)	129.1	C-10, C-13
12	7.06 (1H, *br d*, 7.5)	111.2	C-8, C-10
13	-	144.4	-
14	2.28 (1H, *m*); 1.42 (1H, *br d*, 13.1)	27.4	C-7, C-16, C-20
15	3.43 (1H, *br s*,)	27.6	C=O, C-3, C-16 C-20, C-21
16	-	99.1	-
18 (CH_3)	1.08 (3H, *d*, 6.2)	20.3	C-19, C-20
19	3.46 (1H, *m*)	66.5	C-15
20	2.15 (1H, *m*)	41.2	
21	3.47 (1H, *m*); 3.21 (1H, *m*)	52.0	C-20, NCH_2
COO	-	167.7	-
OCH_3	3.87 (3H, *s*)	52.0	C=O
NCH_2Cl	5.95 (2H, *m*)	65.6	C-5, C-21
NH	8.64 (1H, *br s*)		C-2, C-8, C-13

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6. Structure Elucidation of Compound 6

Compound **6** (Figure 17) was obtained as yellow needle crystals. The R_f values are 0.38 (silica gel / 15% MeOH in CH_2Cl_2) and 0.18 (silica gel / 20% MeOH in EtOAc). The UV spectrum (Figure 64) was found to be the characteristic for the indole chromophore, showing absorptions maxima at 220 and 269 nm. The IR spectrum (Figure 65) showed absorptions at 3377 cm^{-1} (NH) and 1732 cm^{-1} (C=O). The ESITOF MS of compound **6** (Figure 66) showed a protonated molecular ion $[M]^+$ peak at *m/z* 389.1635 corresponding to a tentative molecular formula $C_{21}H_{26}ClN_2O_3$ (D. B. E.=9.5).

The ^{1}H NMR spectrum (DMSO-d_6) (Figure 67, Table 9) showed a broad singlet proton signal (δ 6.22) in the downfield region, which could be assigned to the proton (H-7) of an indole framework, based on the cross peak with the N-H in the indole nucleus. There was a single vinylic proton signal as a broad singlet at δ 5.82, which could be assigned to H-19. On the other hand, two extra doublets were observed in the 4-5 ppm region, pointing to the presence of an extra –CH_2-O-R part in the molecule. In the ^{13}C-NMR (Figure 68, Table 9), the two isolated methylene signals were in the downfield region resonated at δ 70.2 and δ 68.9, assigning to C-17 and C-18, respectively, in accordance with the C-17-O-C-18 bond. The one bond correlations between proton and carbon nuclei observed in the HMQC spectrum (Figure 70) indicated the presence of two methyl carbons, six methylene carbons, and seven methine carbons. The other six remaining carbons were assigned as quaternary carbons including the C=O function resonating at δ 172.4. The ^{1}H-^{1}H COSY spectrum (Figure 69, Table 9), in the aromatic region showed cross peaks from δ 7.05 (H-10) to δ 7.34 (H-9) and δ 6.96 (H-11), and from δ 7.45 (H-12) to δ 6.96 (H-11). In addition, there were cross peaks among H_2-14, H-3, and H-15, and connecting an olefenic proton (H-19) with H_2-18, as shown in figure 18. The cross peaks of the ^{1}H-^{13}C long range correlations obtained from an HMBC experiment (Figure 71, Table 9) allowed various fragments to be connected, as shown in figure 19. The unique signals for methylene protons resonating at δ 5.44 and 5.46 showed the correlations to C-3, C-21, and N-CH_3. Therefore, the methylene protons at δ 5.44 and 5.46 may attach to the quaternary ammonium adjacent to C-3, C-21 and N-CH_3 as shown. On the other hand, the other side of this mehylene group should be connected with heteroatom, according to its resonances in the ^{1}H and ^{13}C NMR spectra. The ESITOF MS spectrum

suggested that the heteroatom should be chlorine. The stereochemistry of compound **6** was determined by comparison of the ^{1}H, ^{13}C chemical shifts and specific optical rotation with those of 6, 7-*seco*-angustilobine B, previously isolated from the Indonesian *A. scholaris* in 1990 by Yamauchi and his colleagues (Figure 20). Compound **6** was newly identified as 6, 7-*seco*-*N*(4)-chloromethylangustilobine B.

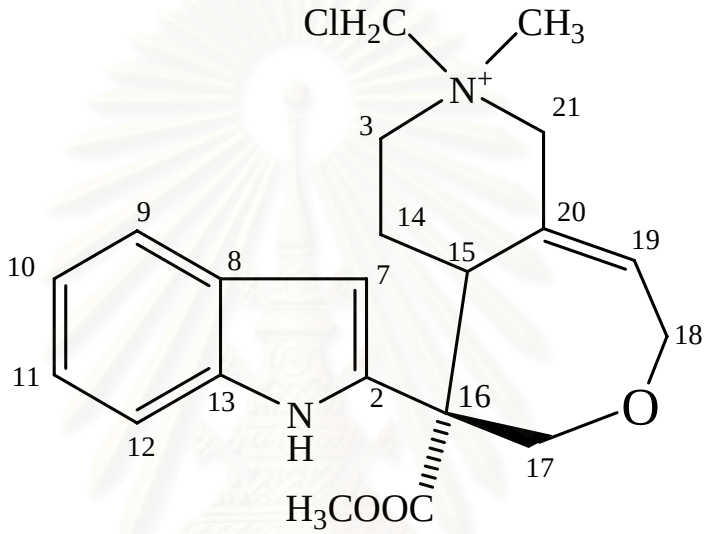

Figure 17: Structure of compound **6**

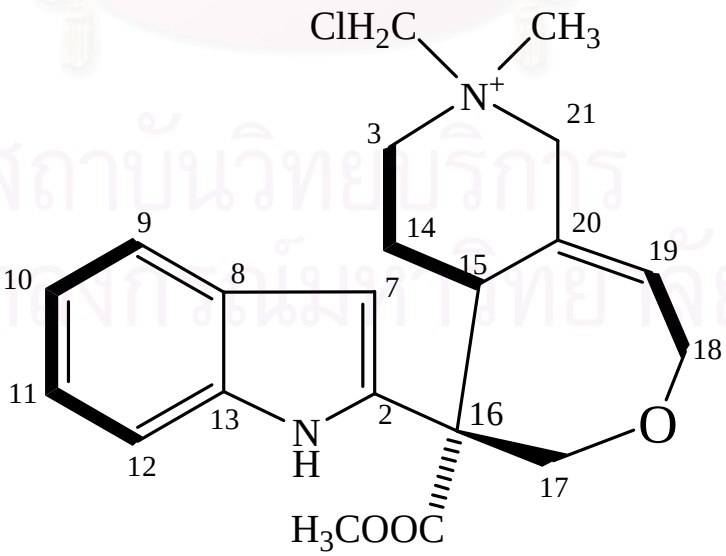

Figure 18: ^{1}H-^{1}H COSY correlations of compound **6** (indicated by bold lines)

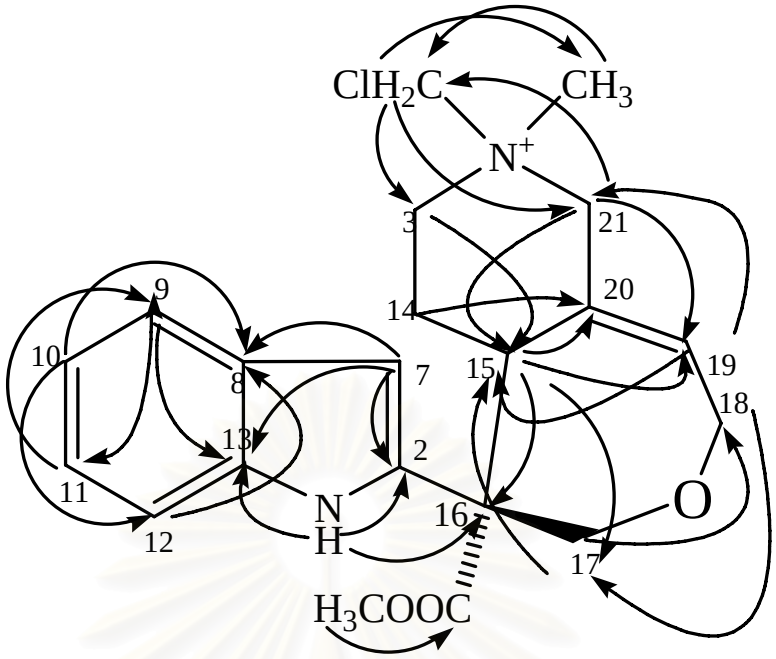

Figure 19: HMBC correlations of compound **6**

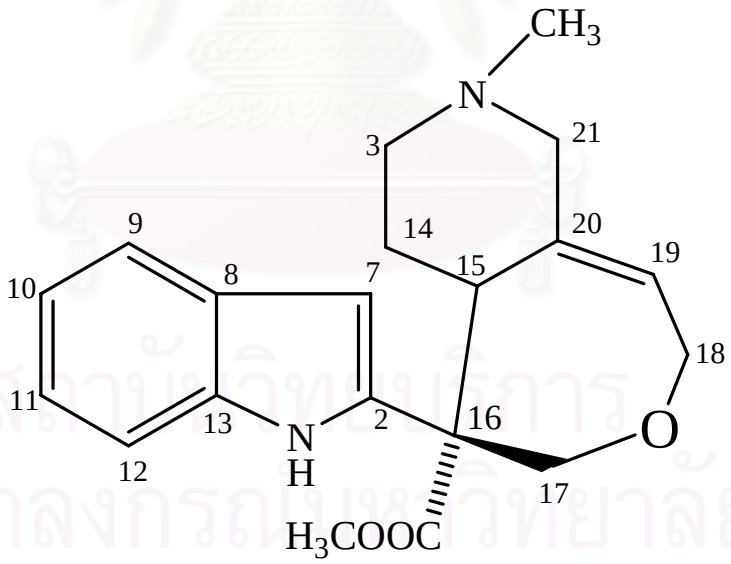

Figure 20: 6, 7-*seco*-angustilobine B from *A. scholaris* by Yamauchi *et* al.

Table 9 ^{1}H, ^{13}C and HMBC spectral data of compound **6** (in DMSO-d_6)

Position	Compound **6** (multiplicity, *J* in Hz)	δ_C (ppm)	HMBC Correlation
2	-	136.1	
3	3.75 (2H, *m*)	60.2	C-14, C-15, N-CH_2
5	-		
6	-		
7	6.22 (1H, *br s*)	99.9	C-2, C-8, C-13
8	-	127.7	
9	7.34 (1H, *d*, 7.8)	120.2	C-11, C-13
10	7.05 (1H, *td*, 7.8, 1.0)	119.7	C-8, C-12
11	6.96 (1H, *td*, 7.8, 1.0)	121.7	C-9, C-13
12	7.45 (1H, *br d*, 7.8)	111.9	C-8, C-10
13	-	136.7	
14	1.85 (1H, *m*); 0.88 (1H, *m*)	23.7	C-20
15	3.56 (1H, *br d*, 12.0)	44.2	C-16, C-17, C-19, C-20
16	-	56.8	
17	4.55 (1H, *br d*, 12.0); 4.38 (1H, *br d*, 12.0)	70.2	C-15, C-16, C-18, C-19
18	4.28 (2H, *m*)	68.9	C-15, C-17, C-19
19	5.82 (1H, *br s*)	134.4	C-15, C-18, C-21
20	-	127.2	
21	4.18 (2H, *m*)	68.6	C-15, C-19,C-20, N-CH_2
COO	-	172.4	
OCH_3	3.58 (3H, *s*)	52.7	C=O, C-16
NCH_3	3.12 (3H, *s*)	50.1	C-3, C-21 N-CH_2
NH	11.12 (1H, *br s*)	-	C-2, C-13, C-16
NCH_2Cl	5.44 (1H, *d*, 9.8); 5.46 (1H, *d*, 9.8)	65.0	NCH_3, C-3, C-21

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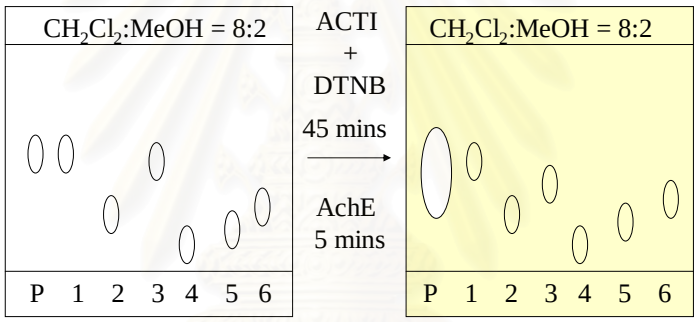
7. Biological Activity of Isolated Compounds

Anticholinesterase activity

Anticholinesterase activity of six isolated compounds was detected by spraying the substrate, dye and enzyme base on Ellman's method and comparing with physostigmine which was the positive control. The result of anticholinesterase activity was shown in figure 21, and it was not inhibited by six isolated compounds.

Figure 21: The result of anticholinesterase activity of 6 isolated compounds was displayed on TLC plate by comparing with physostigmine (P).



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CHAPTER V

CONCLUSION

In this investigation, six pure compounds were isolated from the stem bark of *Alstonia rostrata* Fischer. These compounds are indole alkaloid compounds. There are three known compounds: echitamidine (compound **1)**, echitamine (compound **2**), undolifoline (Compound **3**) and three new indole alkaloids: 17-carboxyl-*N*(4)-chloromethylechitamidine (compound **4**), *N*(4)-chloromethylechitamidine (Compound **5**), 6,7-*seco*-*N*(4)-chloromethylangustilobine B (compound **6**).

In anticholinesterase activity of 6 isolated compounds were not active by comparing with positive control, which was physostigmine.

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APPENDIX

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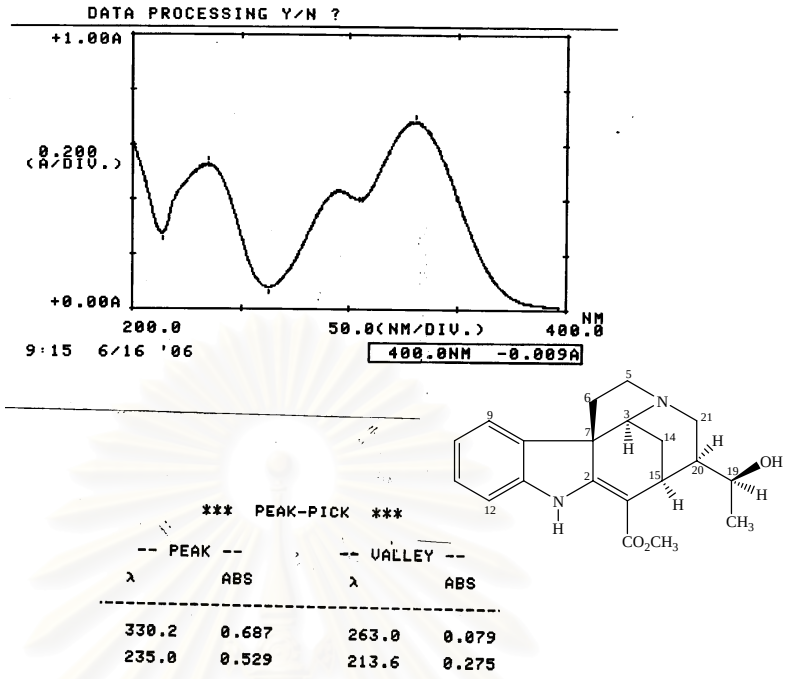


Figure 22: UV spectrum of compound **1**

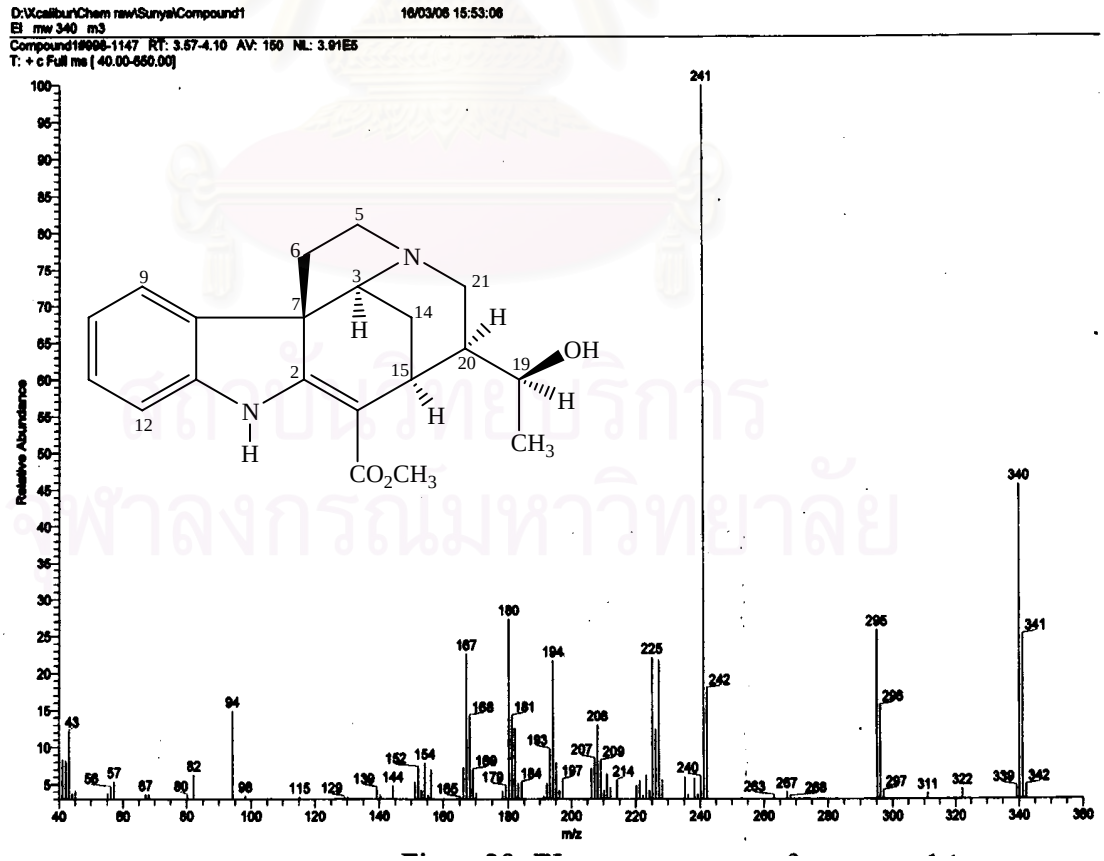


Figure23: EI mass spectrum of compound **1**

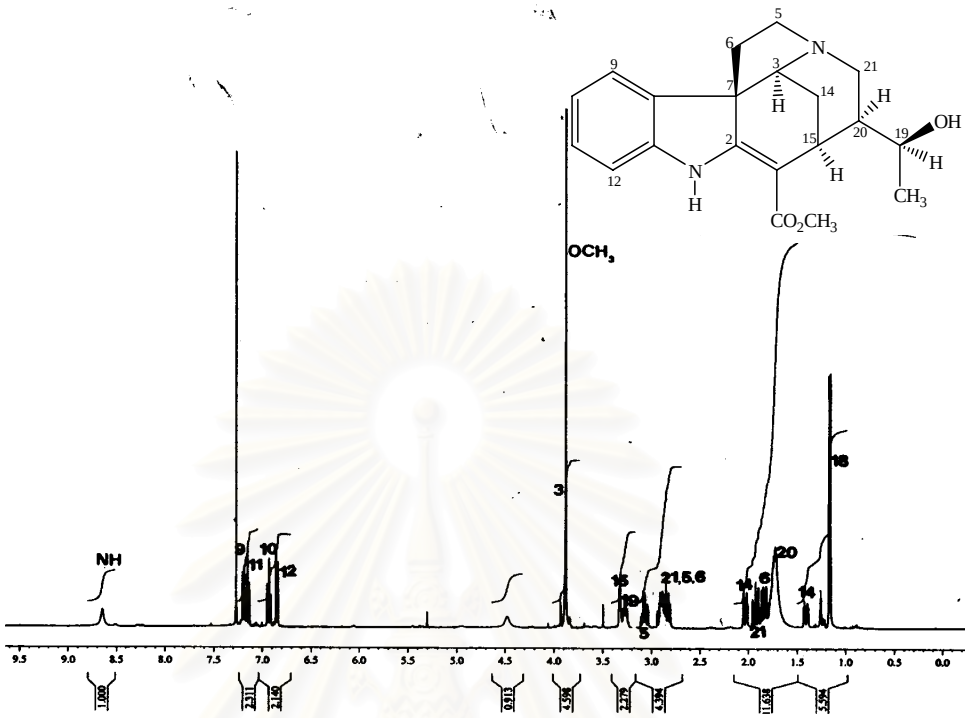


Figure 24: ^{1}H NMR (400 MHz) spectrum of compound **1** ($CDCl_3$)

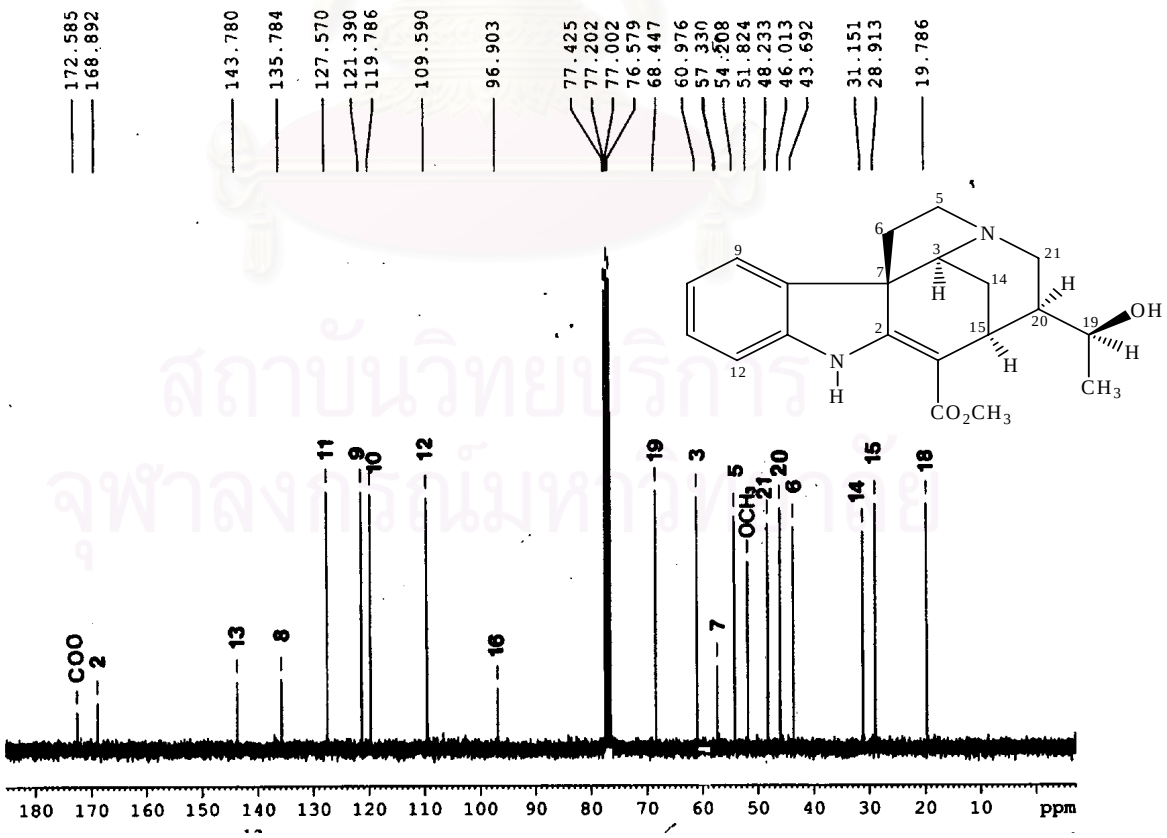


Figure 25: ^{13}C NMR (100 MHz) spectrum of compound **1** ($CDCl_3$)

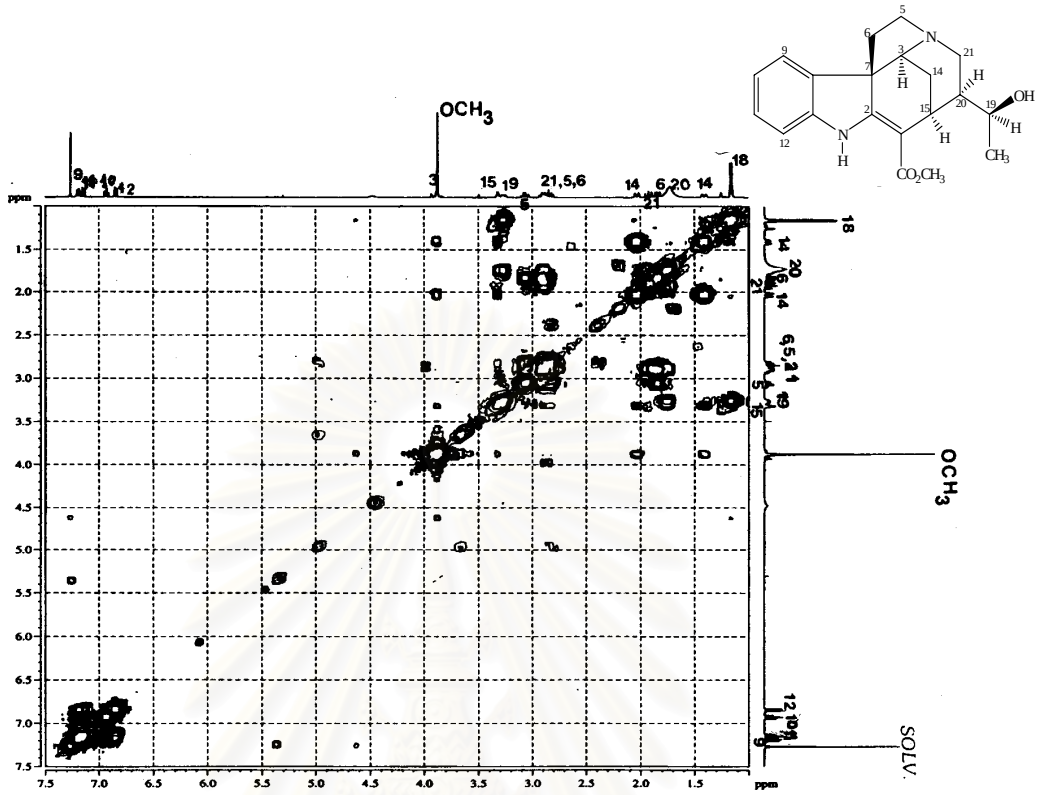


Figure 26: COSY NMR spectrum of compound **1** ($CDCl_3$)

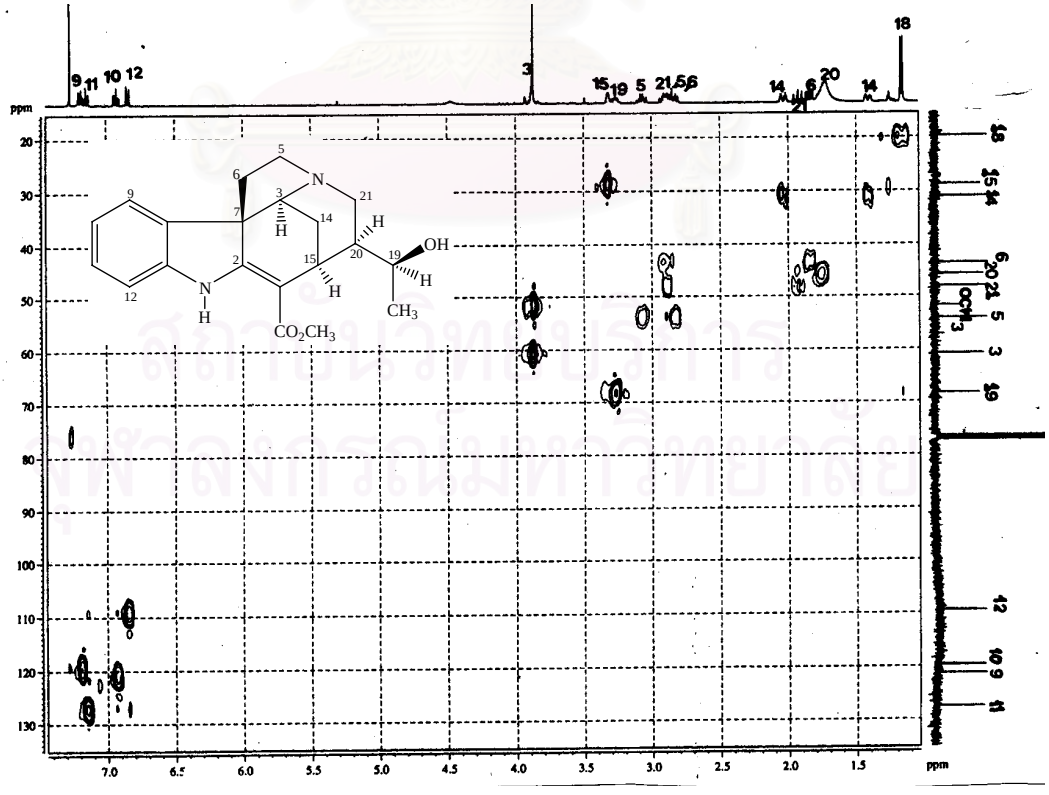


Figure 27: HMQC NMR spectrum of compound **1** ($CDCl_3$)

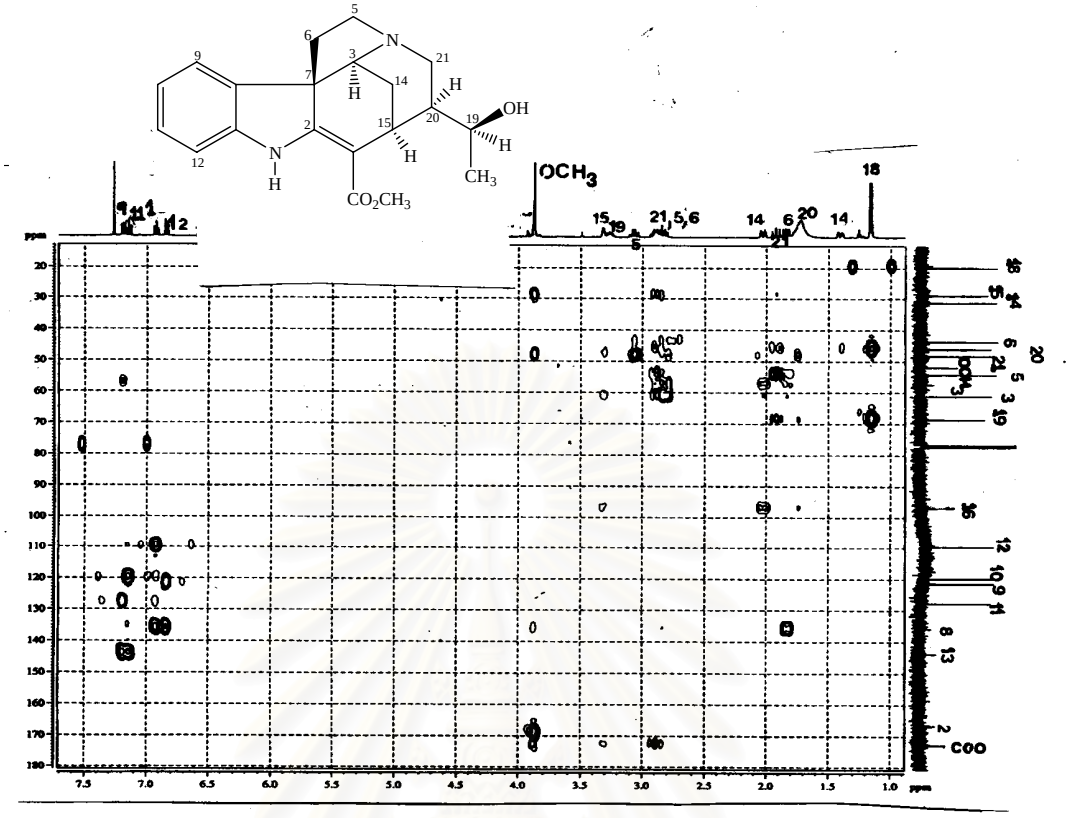


Figure 28: HMBC NMR spectrum of compound **1** ($CDCl_3$)

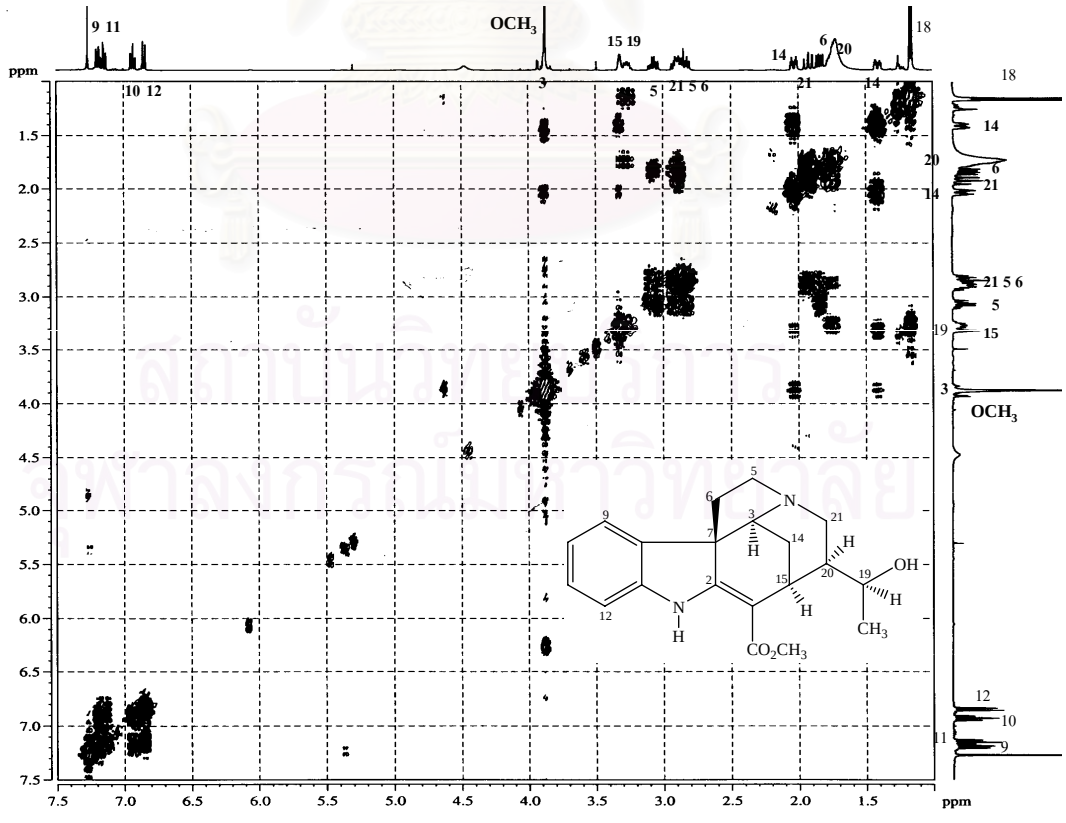
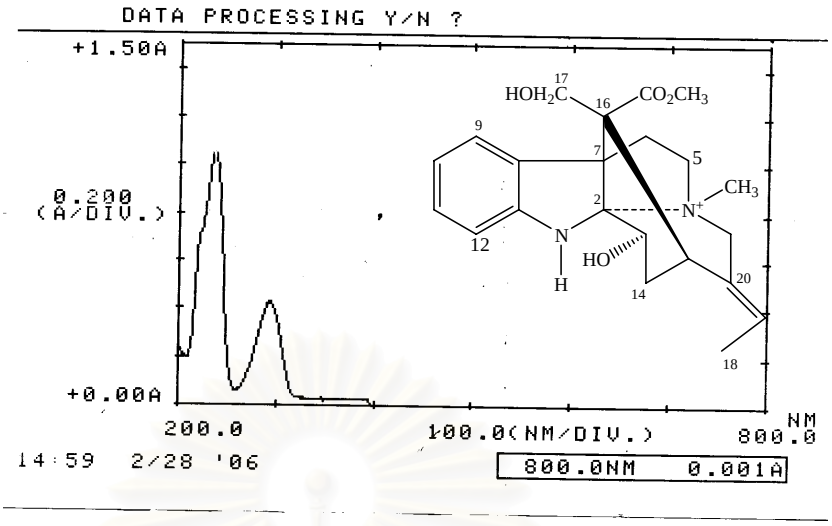


Figure 29: NOESEY NMR spectrum of compound **1** ($CDCl_3$)



*** PEAK-PICK ***

-- PEAK --		-- VALLEY --	
λ	ABS	λ	ABS
293.0	0.428	716.0	0.001
236.0	1.057	258.0	0.062
		209.0	0.197

Figure 30: UV spectrum of compound **2**

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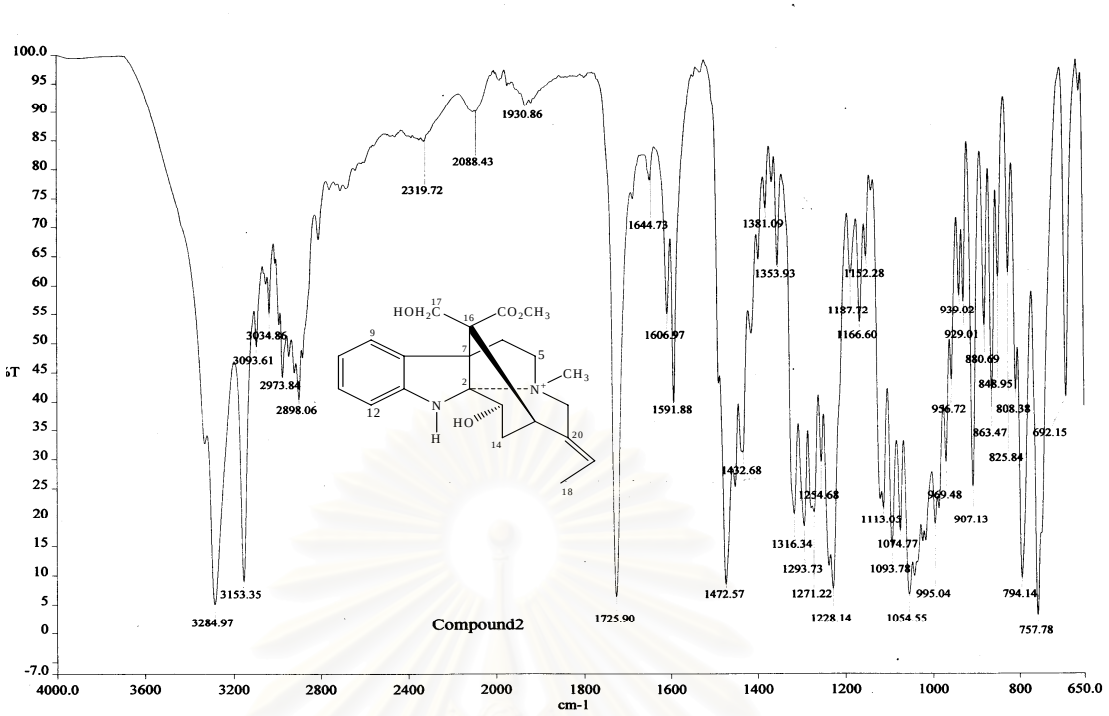


Figure 31: IR spectrum of compound **2** (UATR)

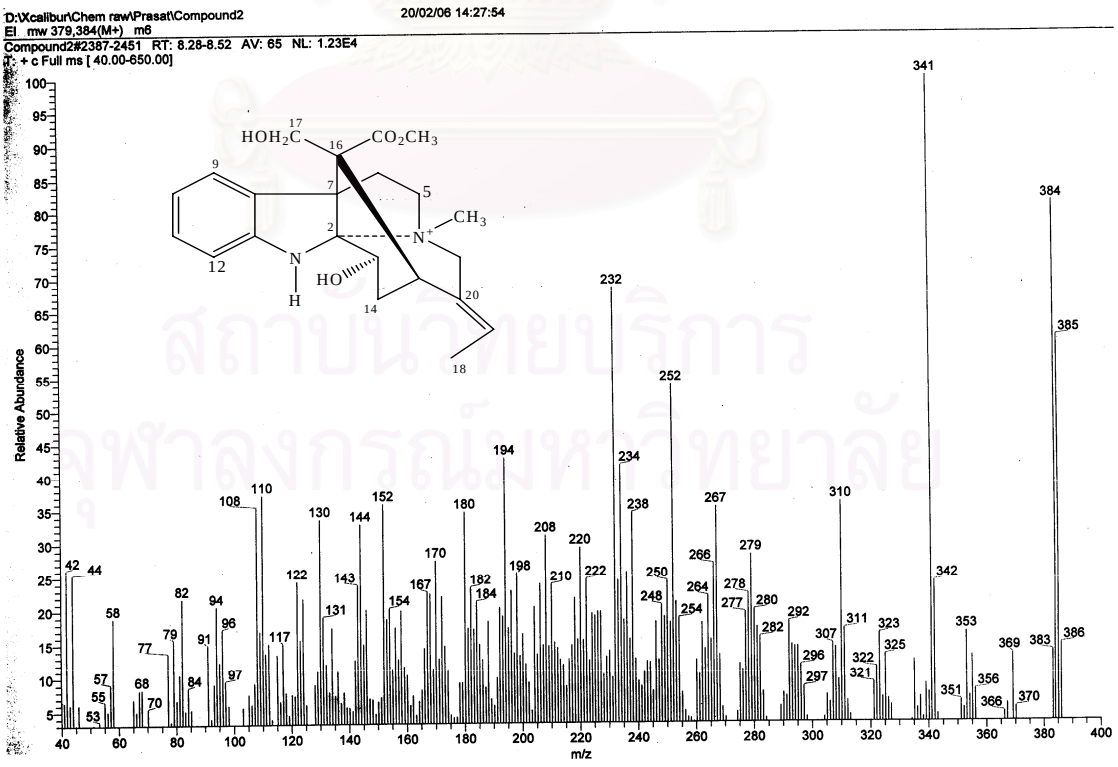


Figure 32: EI mass spectrum of compound **2**

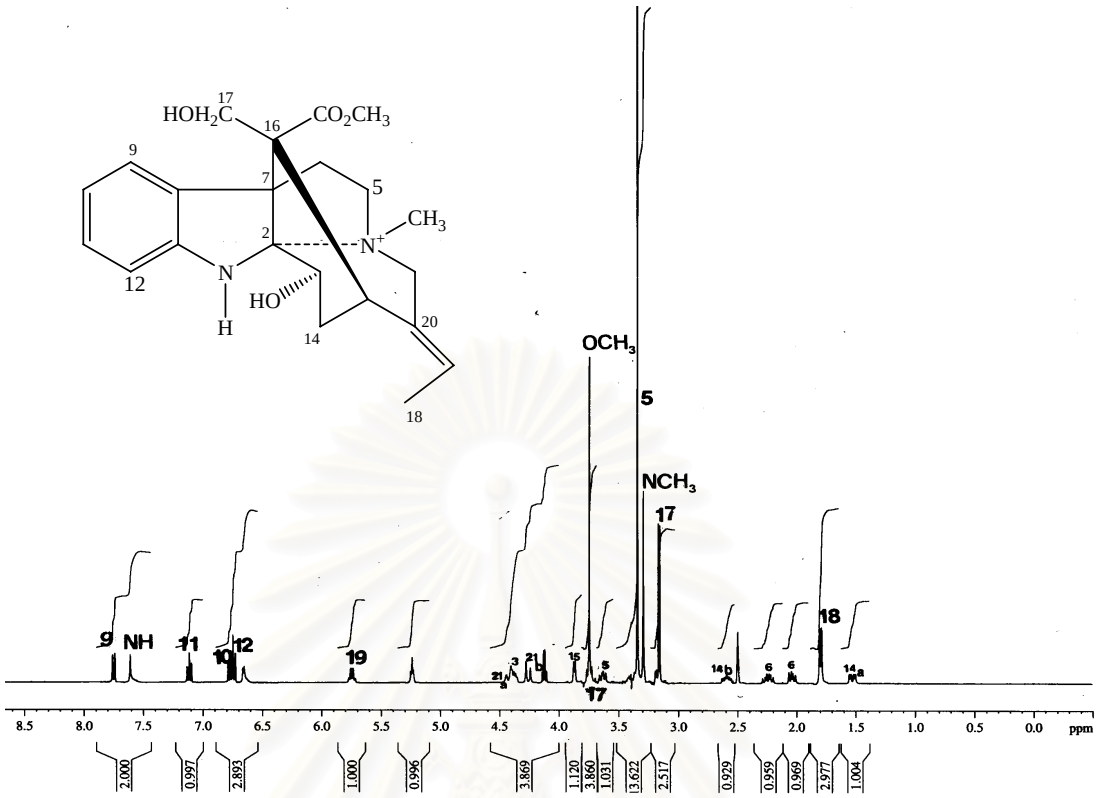


Figure 33: ^{1}H NMR (400 MHz) spectrum of compound **2** (DMSO-d_6)

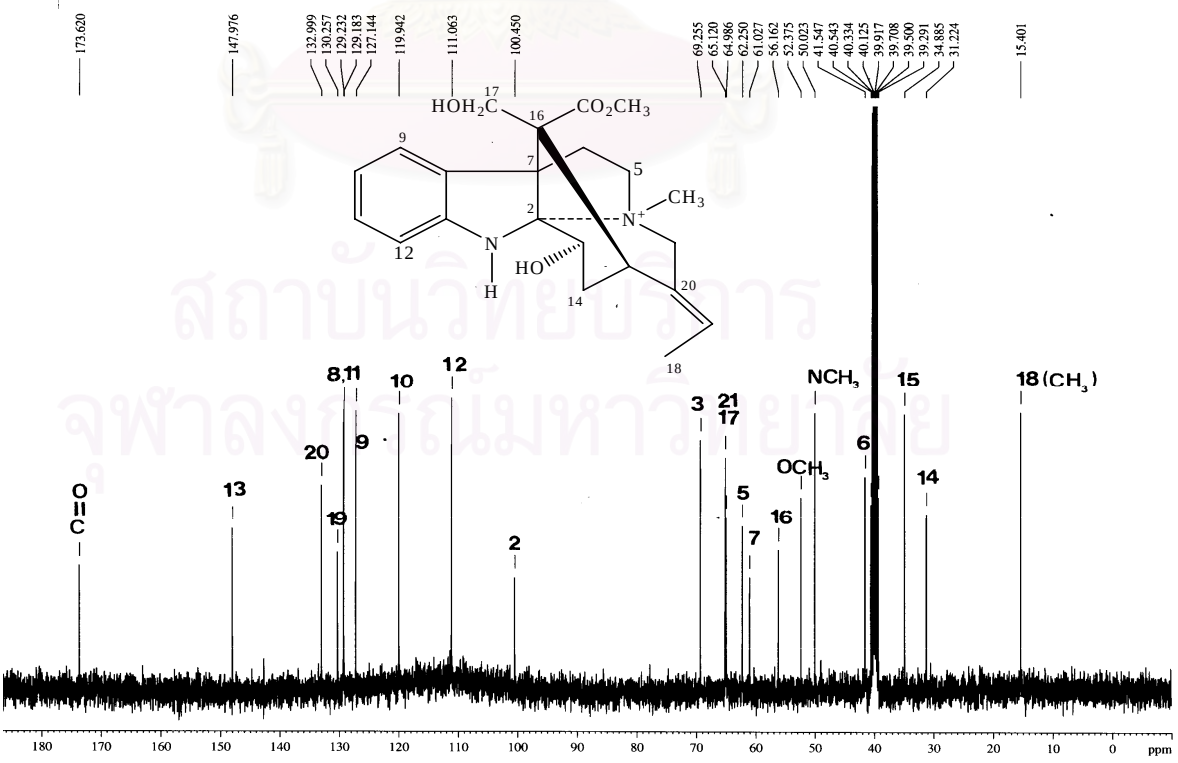


Figure 34: ^{13}C NMR (100 MHz) spectrum of compound **2** (DMSO-d_6)

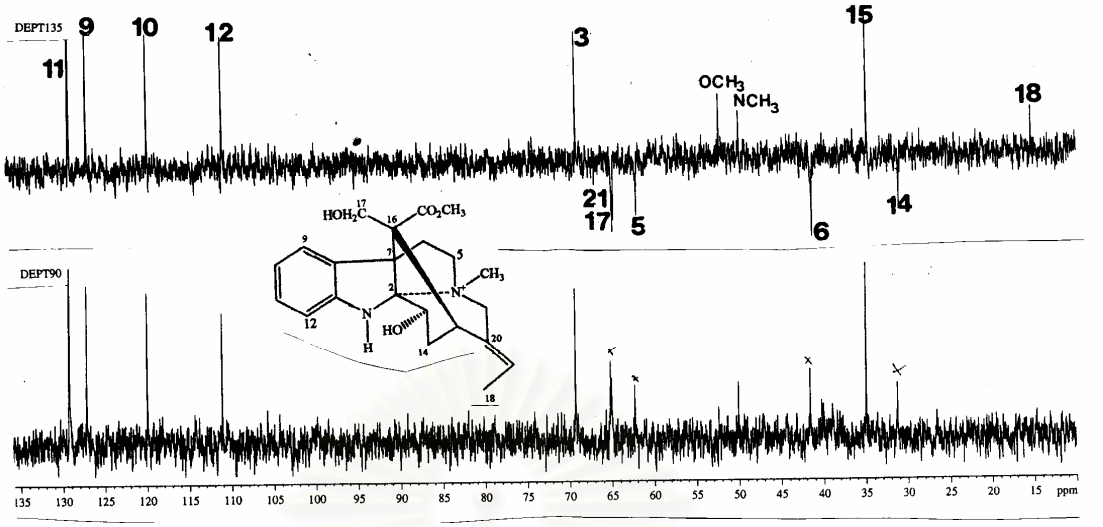


Figure 35: DEPT NMR (100 MHz) spectrum of compound **2** (DMSO-d_6)

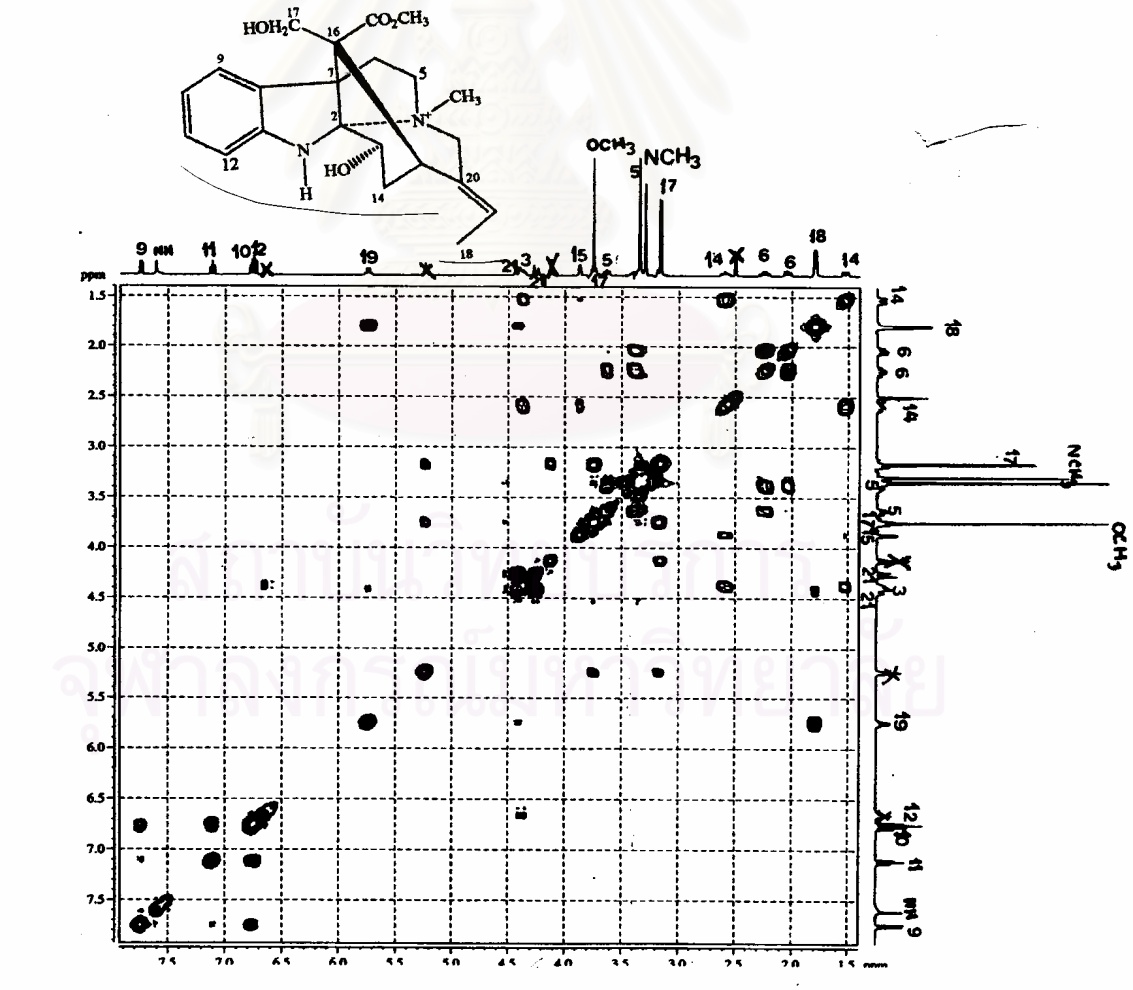


Figure 36: COSY NMR spectrum of compound **2** (DMSO-d_6)

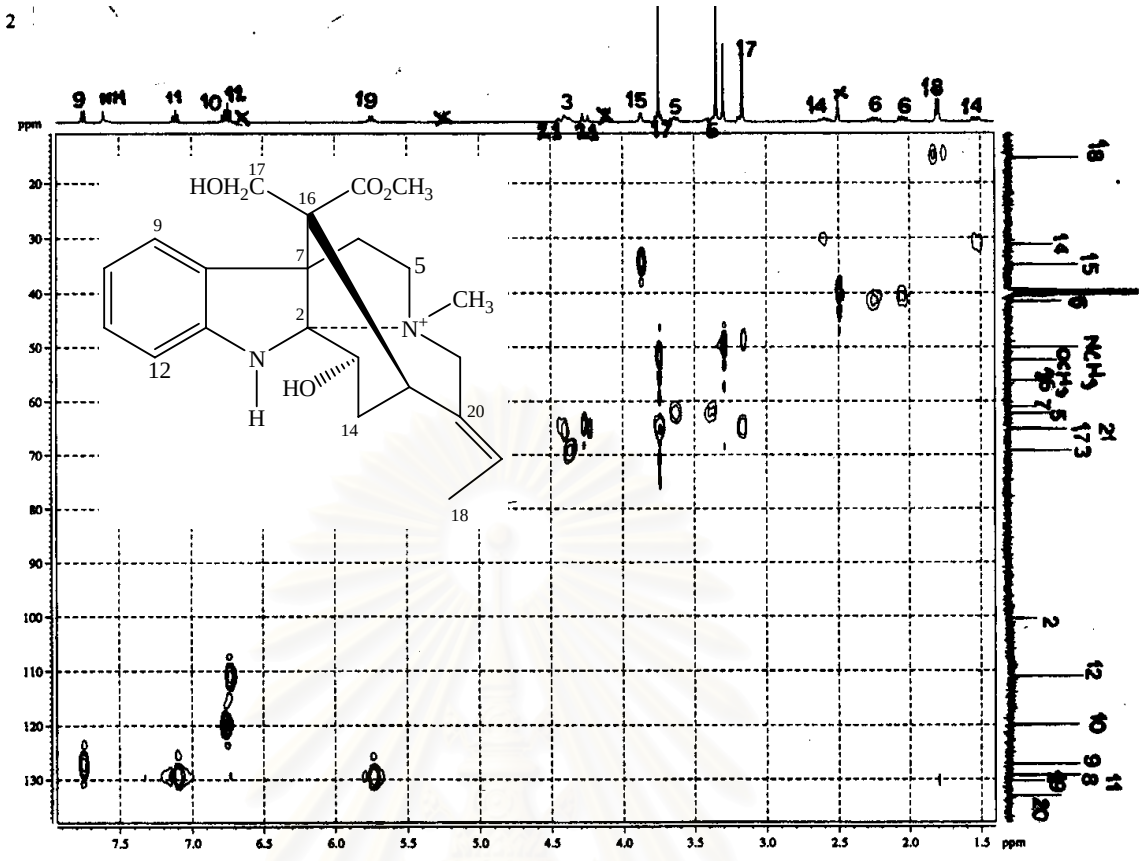


Figure 37: HMQC NMR spectrum of compound **2** (DMSO-d_6)

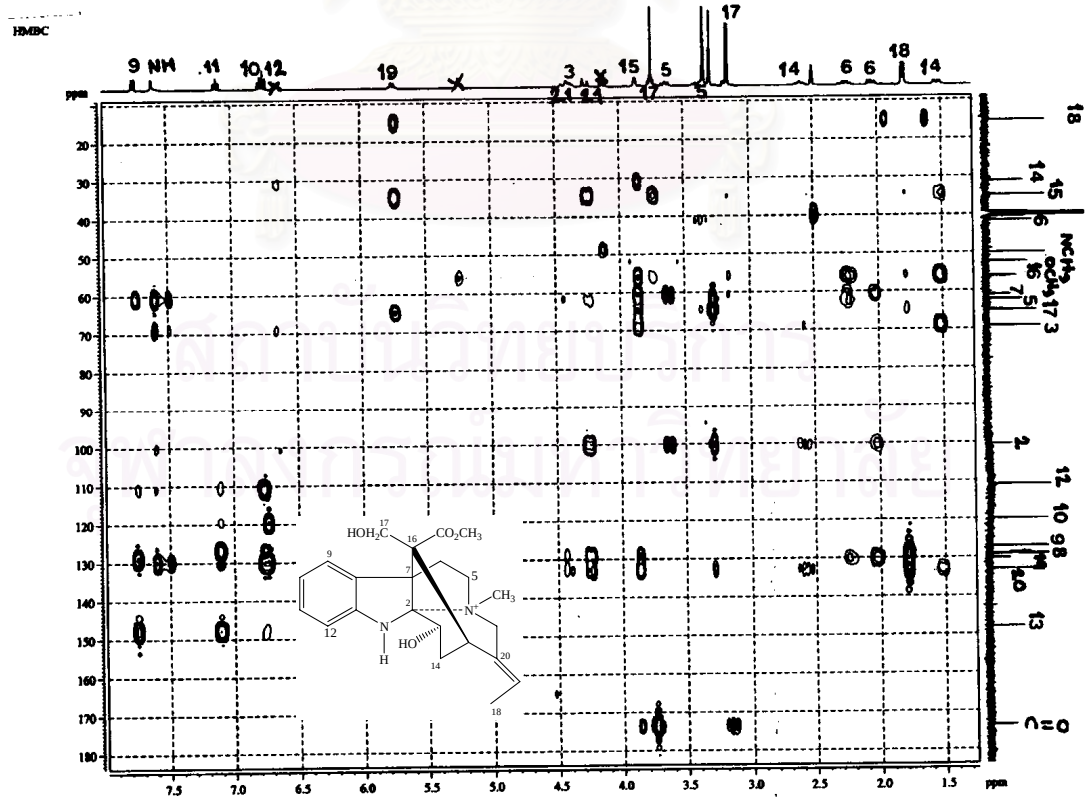
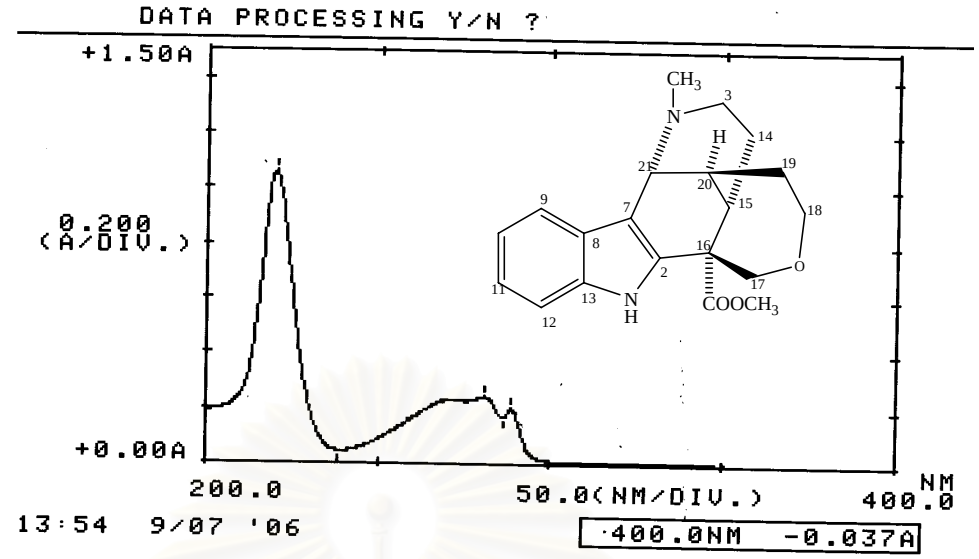


Figure 38: HMBC NMR spectrum of compound **2** (DMSO-d_6)



*** PEAK-PICK ***

-- PEAK --		-- VALLEY --	
λ	ABS	λ	ABS
288.6	0.202	286.2	0.169
281.0	0.246	238.6	0.044
220.0	1.060		

Figure 39: UV spectrum of compound **3**

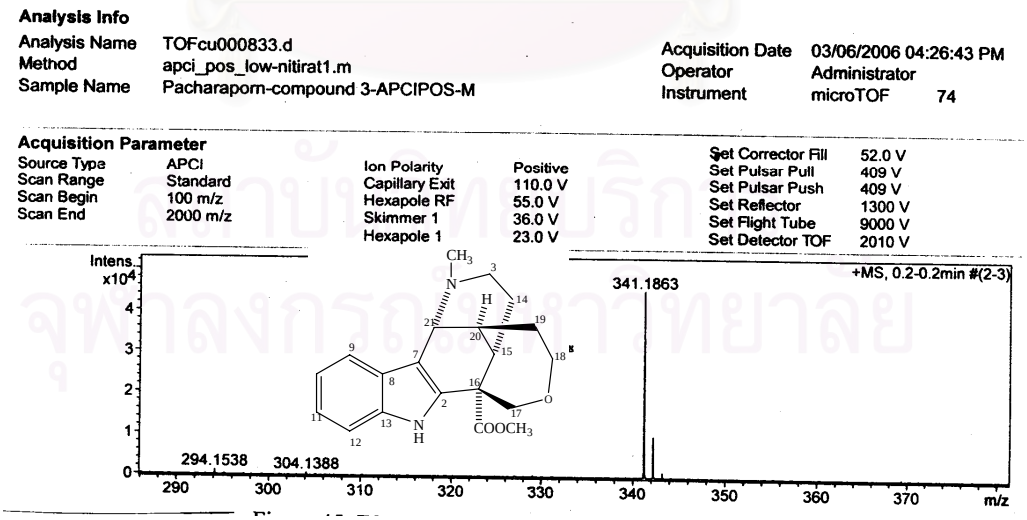


Figure 40: ESITOF mass spectrum of compound **3**

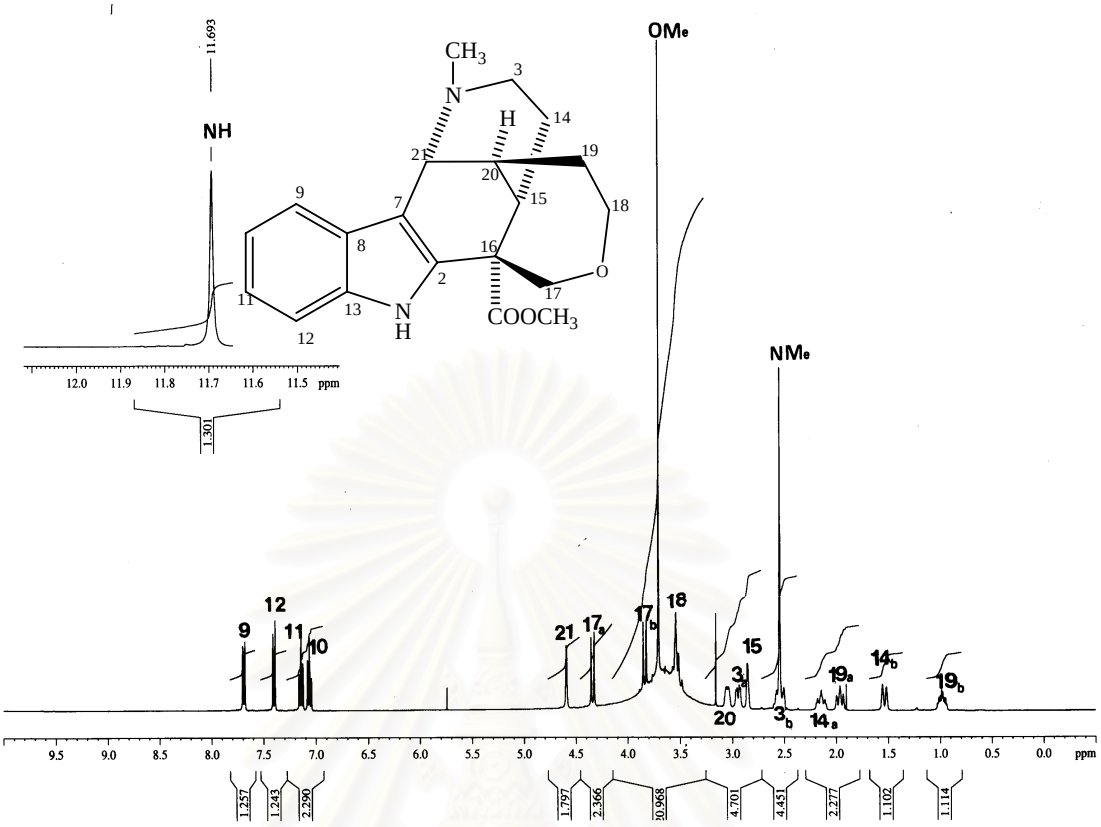


Figure 41: ^{1}H NMR (400 MHz) spectrum of compound **3** (DMSO-d_6)

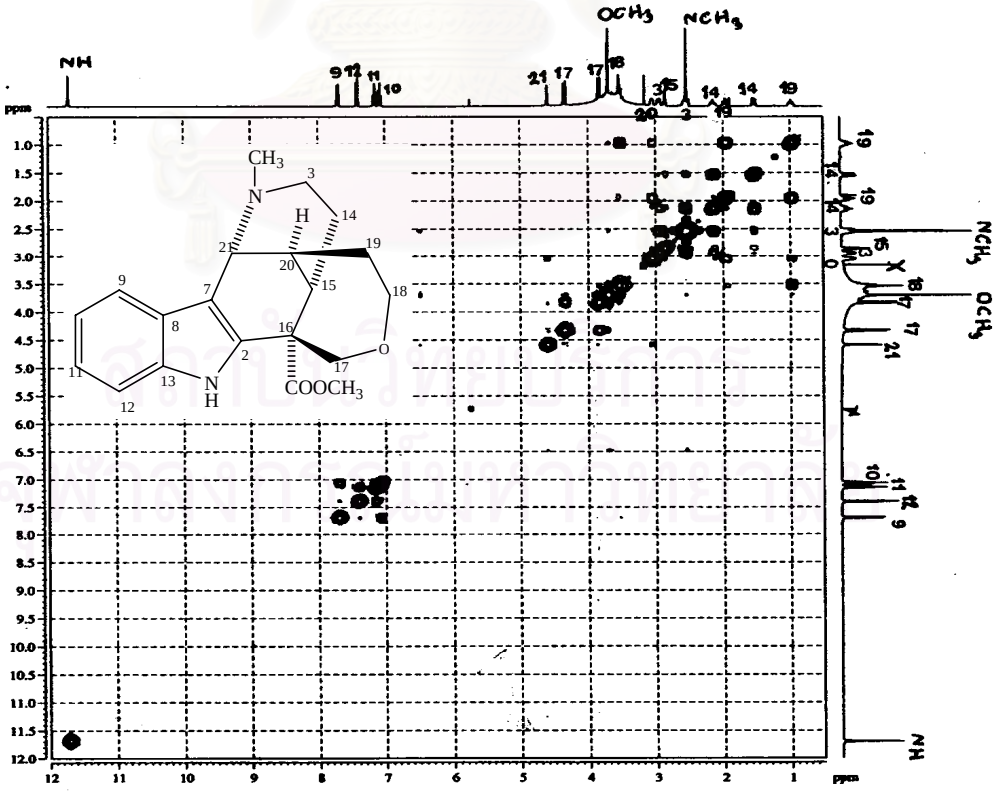


Figure 42: COSY NMR spectrum of compound **3** (DMSO-d_6)

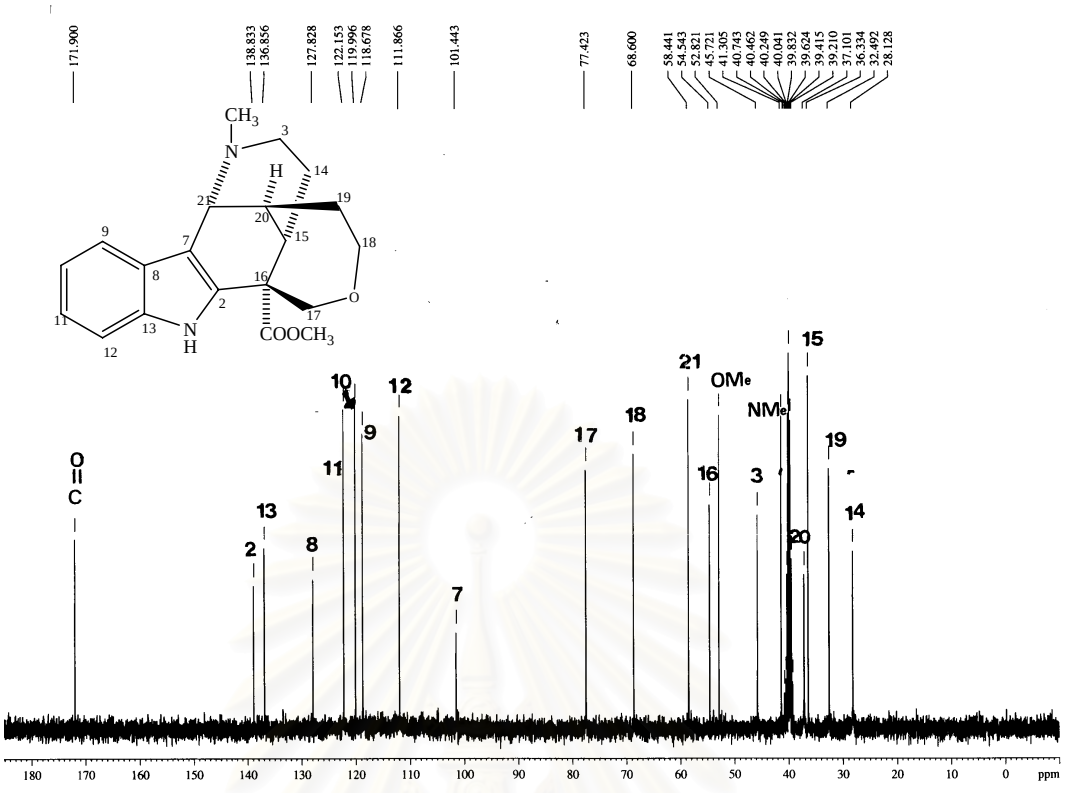


Figure 43: ^{13}C NMR (100 MHz) spectrum of compound **3** (DMSO-d_6)

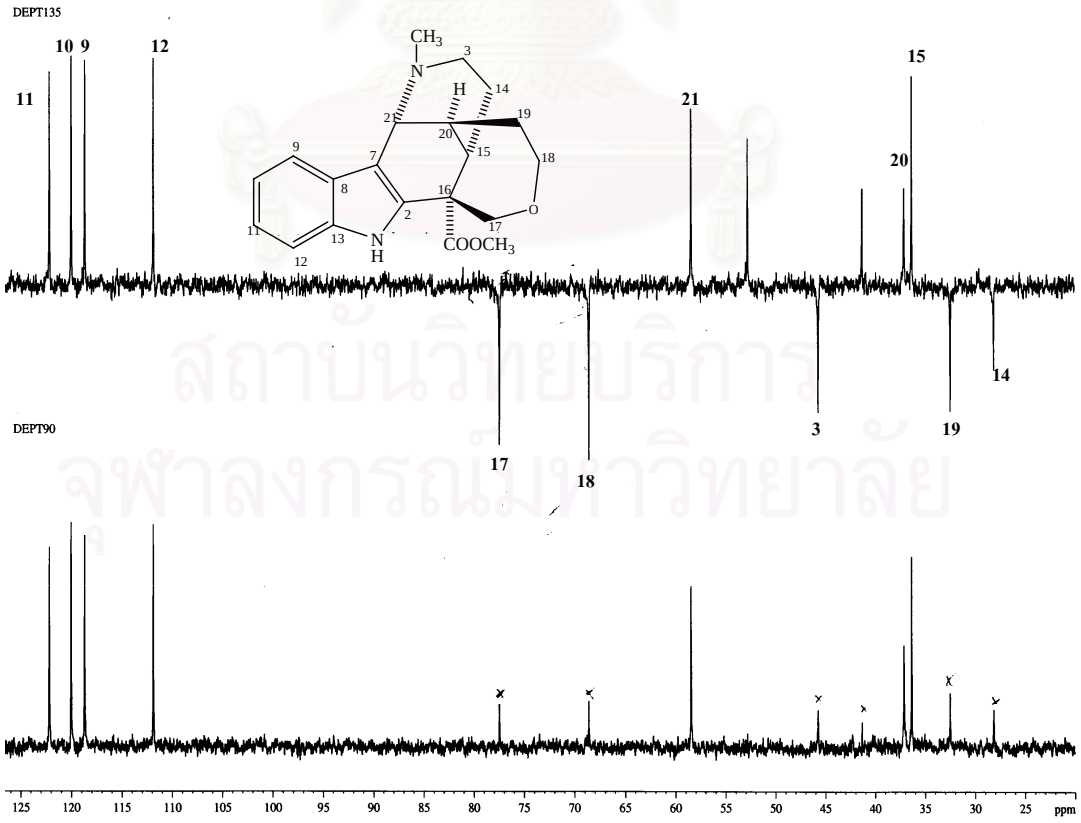


Figure 44: DEPT NMR (100 MHz) spectrum of compound **3** (DMSO-d_6)

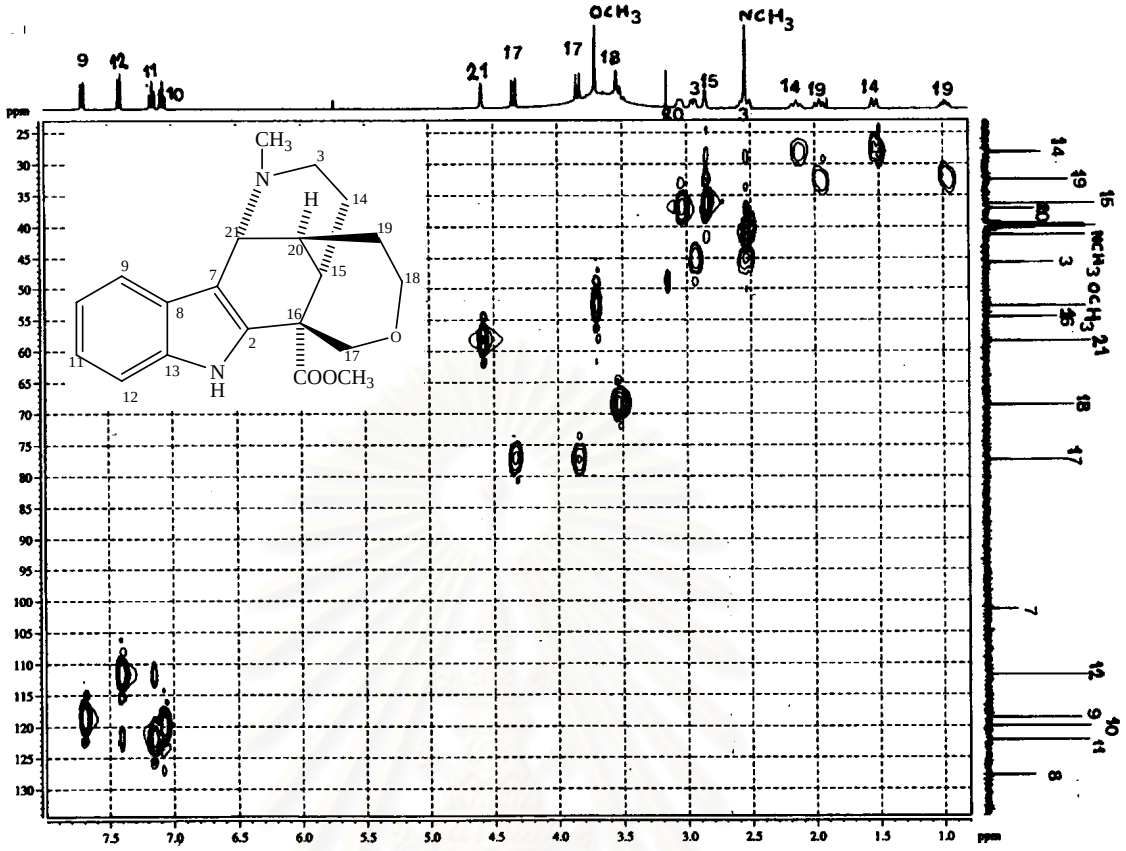


Figure 45: HMQC NMR spectrum of compound **3** (DMSO-d_6)

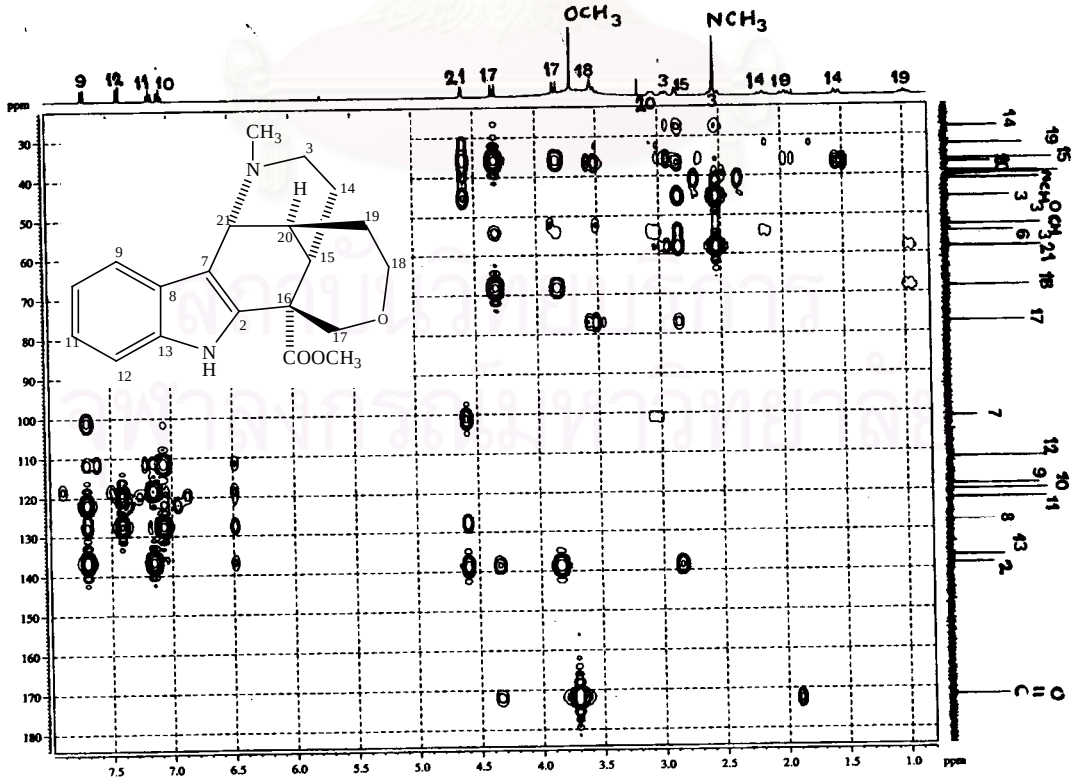


Figure 46: HMBC NMR spectrum of compound **3** (DMSO-d_6)

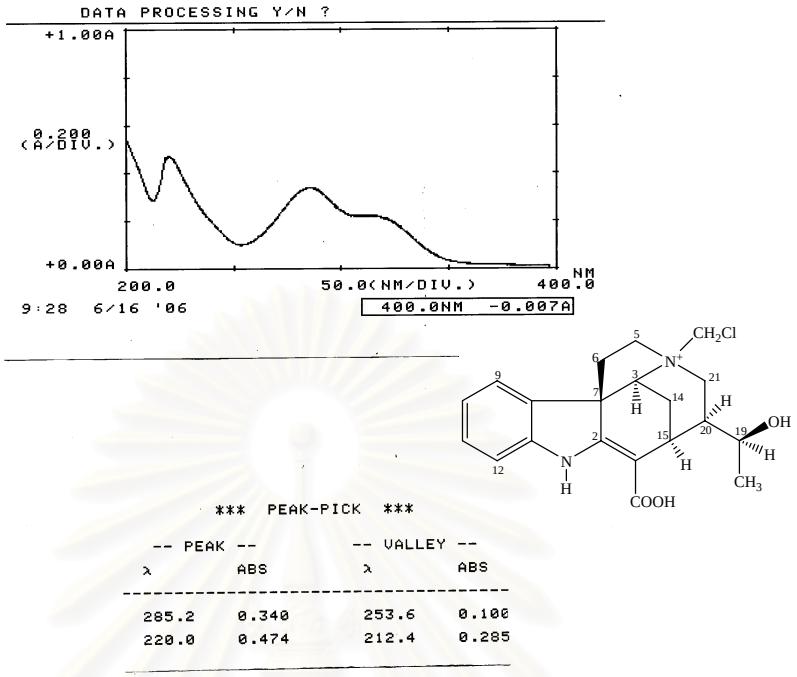


Figure 47: UV spectrum of compound **4**

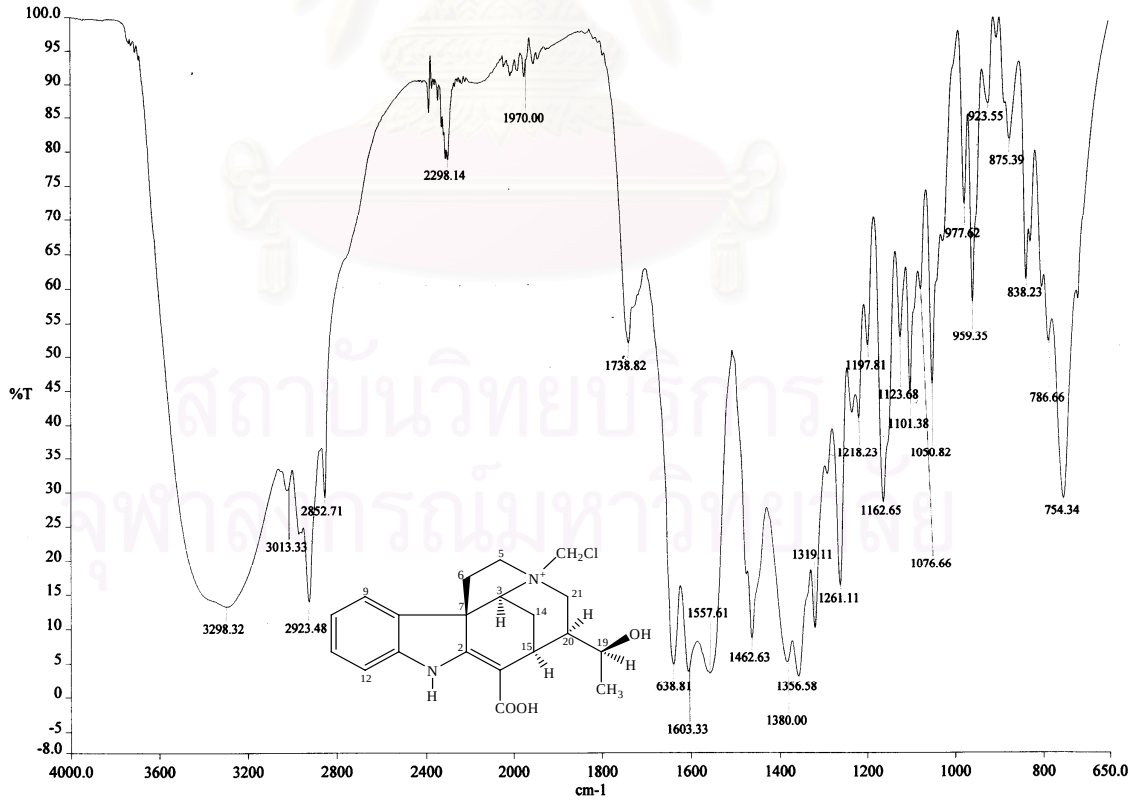


Figure48: IR spectrum of compound **4** (UATR)

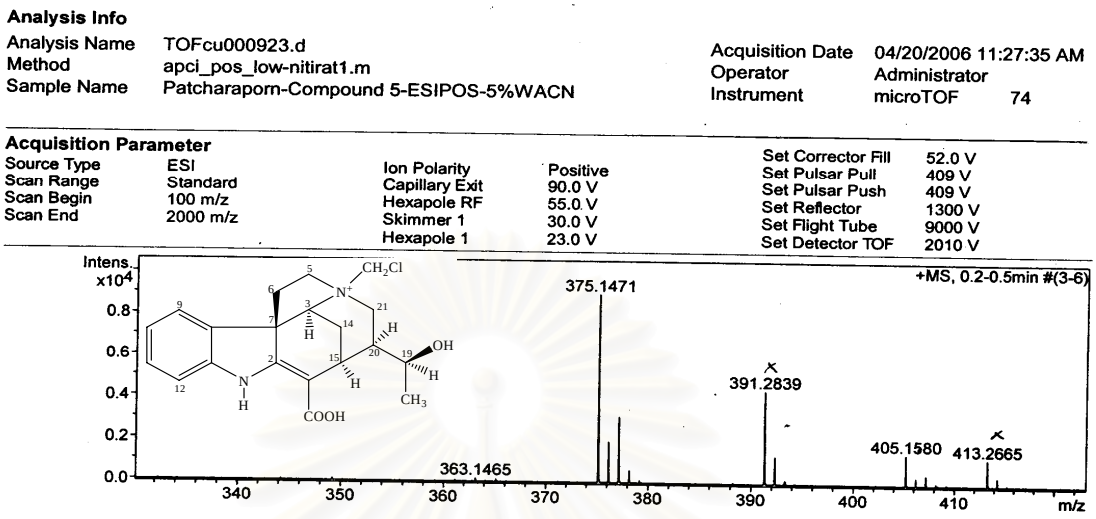


Figure 49: ESITOF mass spectrum of compound **4**

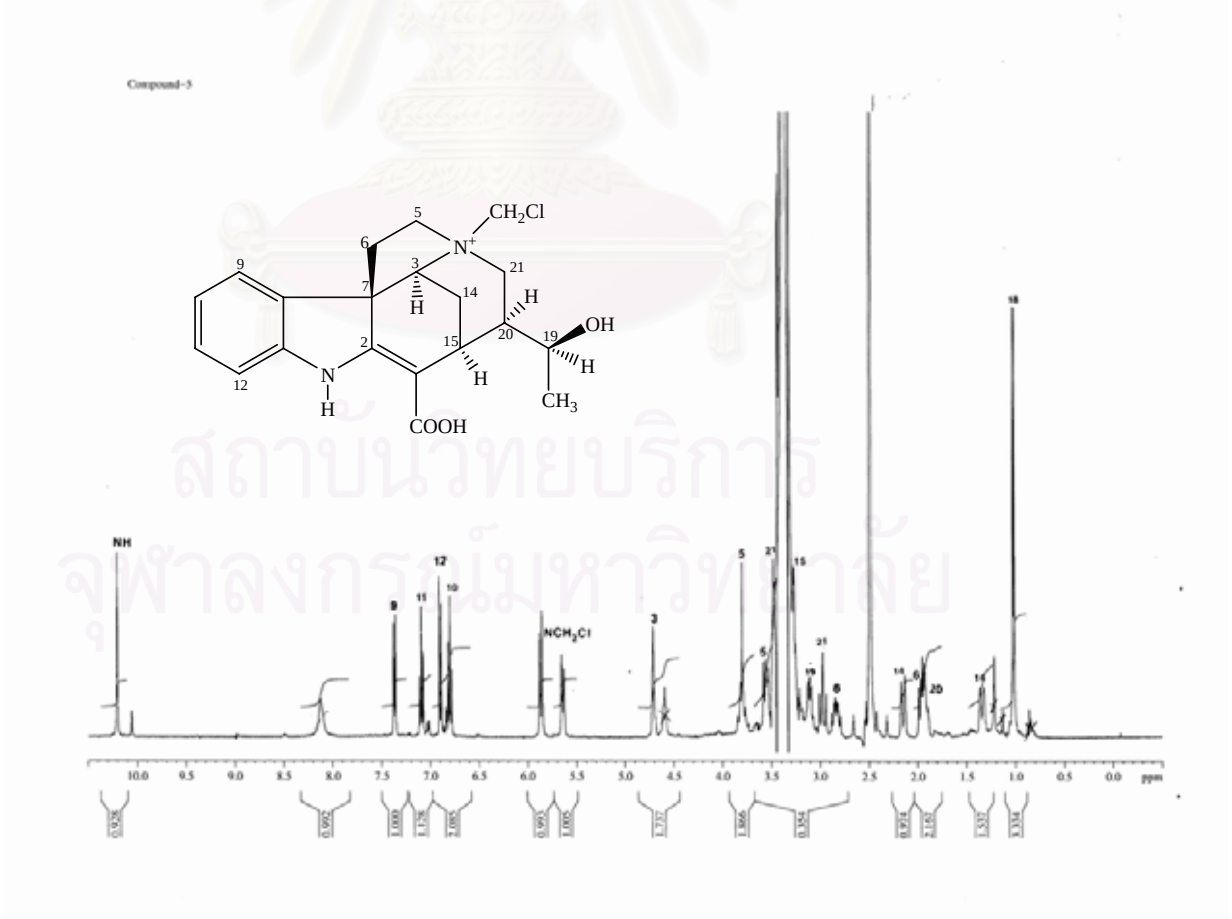


Figure 50: ^{1}H NMR (400 MHz) spectrum of compound **4** (DMSO-d_6)

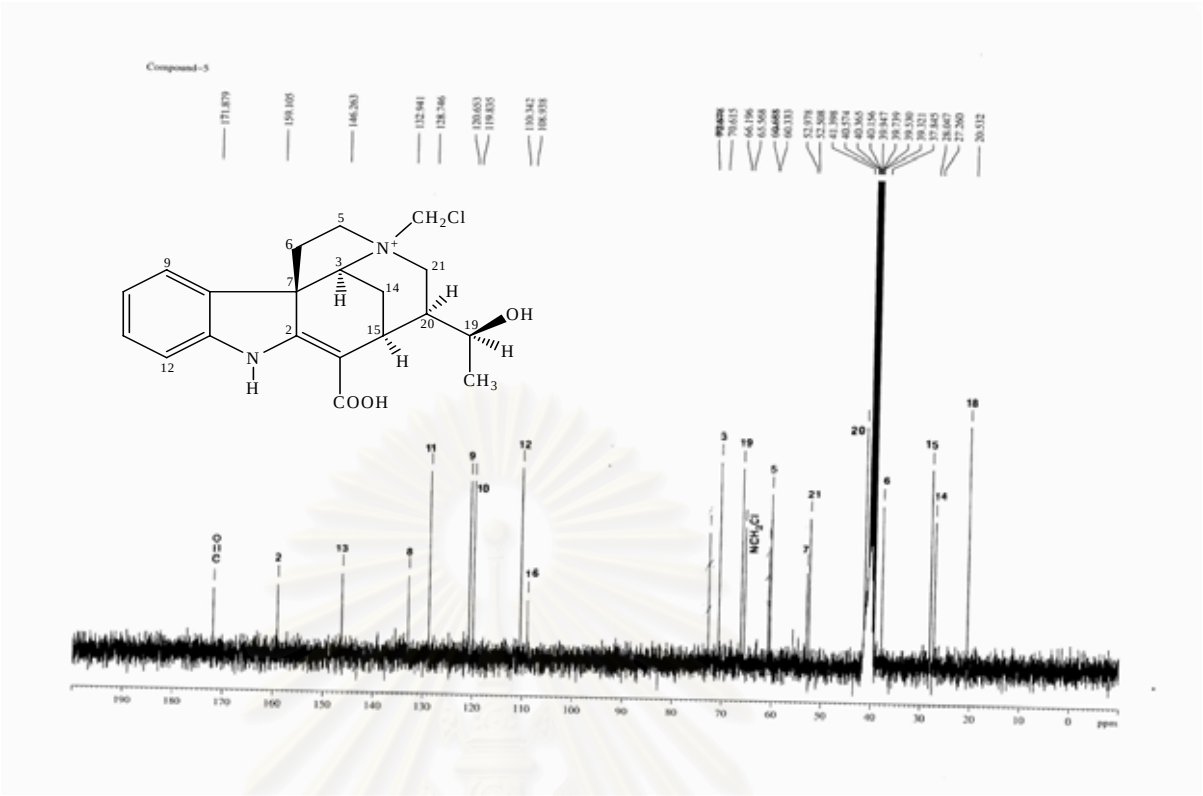


Figure 51: ^{13}C NMR (100 MHz) spectrum of compound **4** (DMSO-d_6)

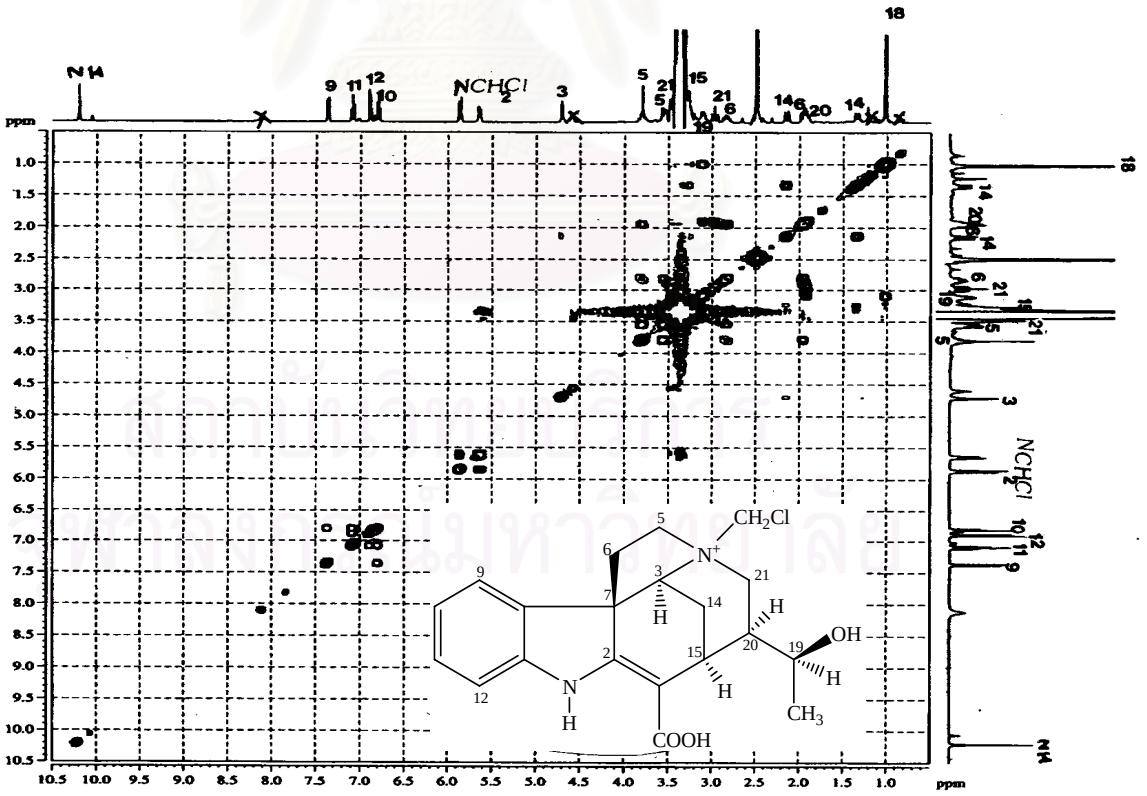


Figure 52: COSY NMR spectrum of compound **4** (DMSO-d_6)

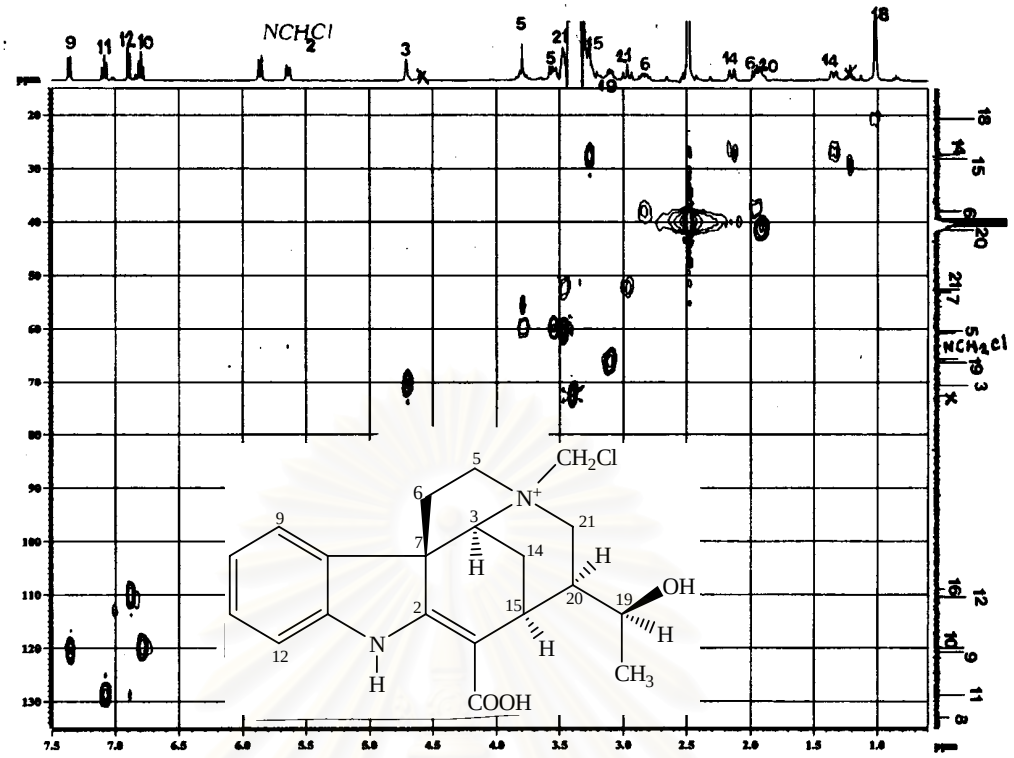


Figure 53: HMQC NMR spectrum of compound **4** (DMSO-d_6)

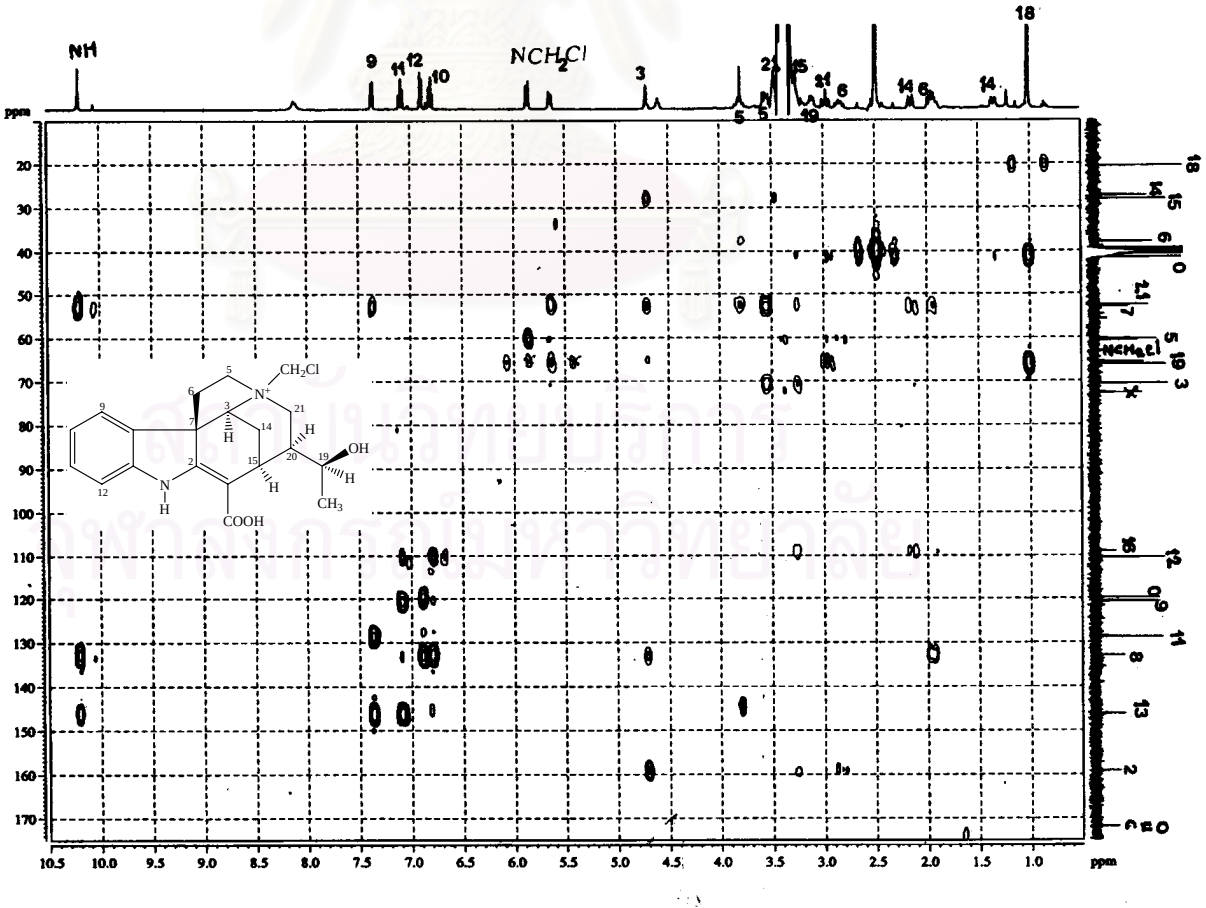
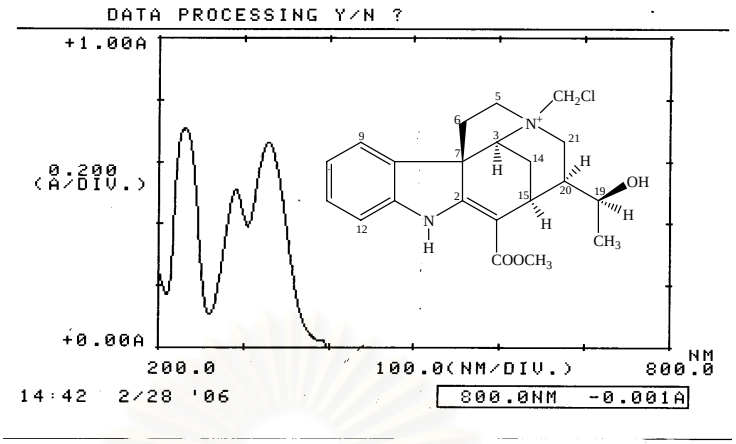


Figure 54: HMBC NMR spectrum of compound **4** (DMSO-d_6)



*** PEAK-PICK ***

-- PEAK --		-- VALLEY --	
λ	ABS	λ	ABS
329.0	0.665	305.0	0.387
291.0	0.509	260.0	0.108
232.0	0.708	210.0	0.170

Figure 55: UV spectrum of compound **5**

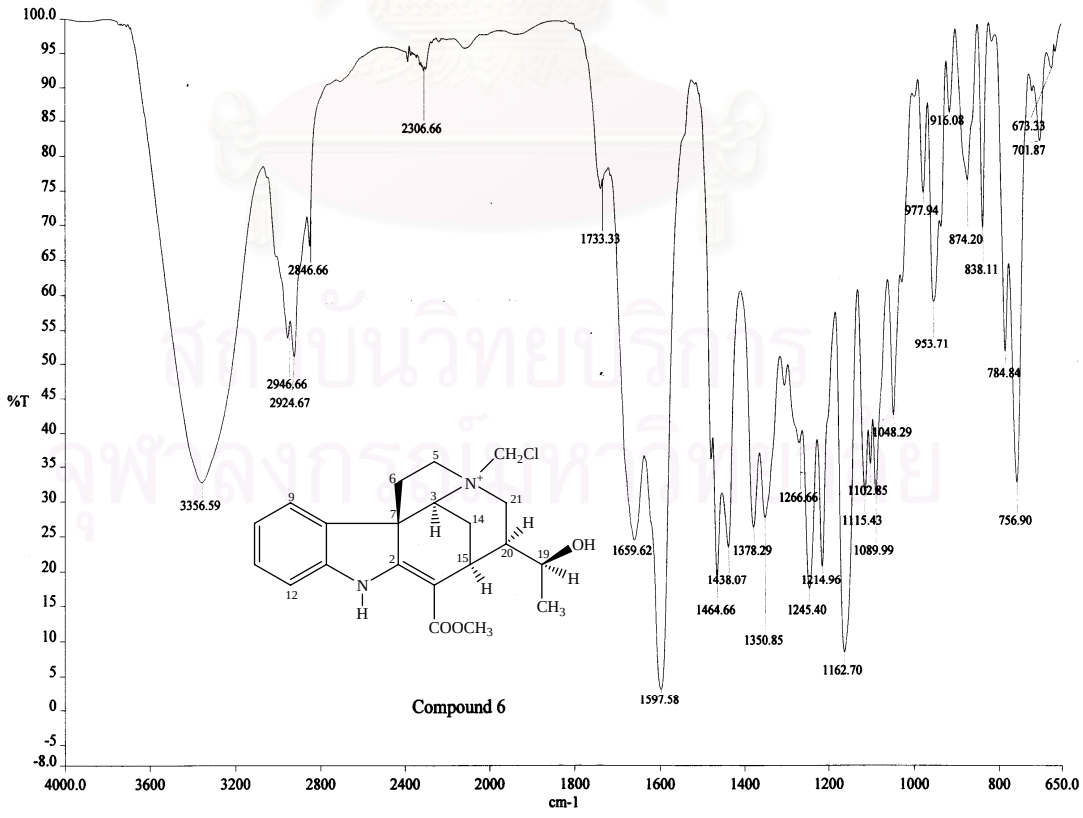


Figure 56: IR spectrum of compound **5** (UATR)

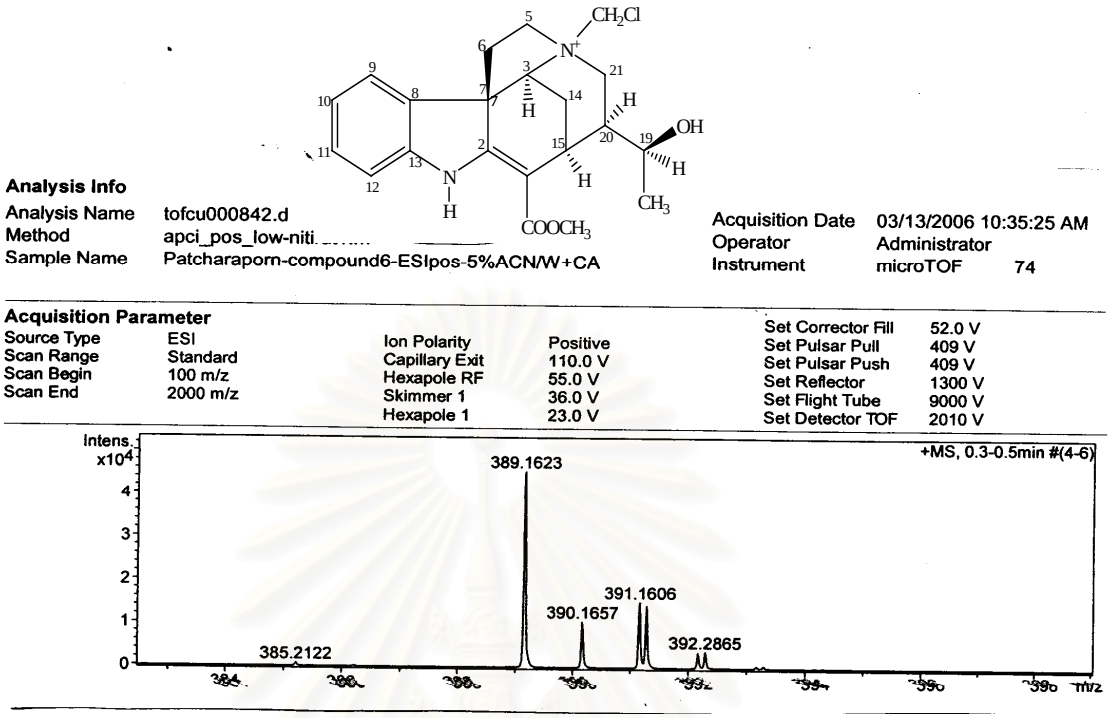


Figure 57: ESITOF mass spectrum of compound **5**

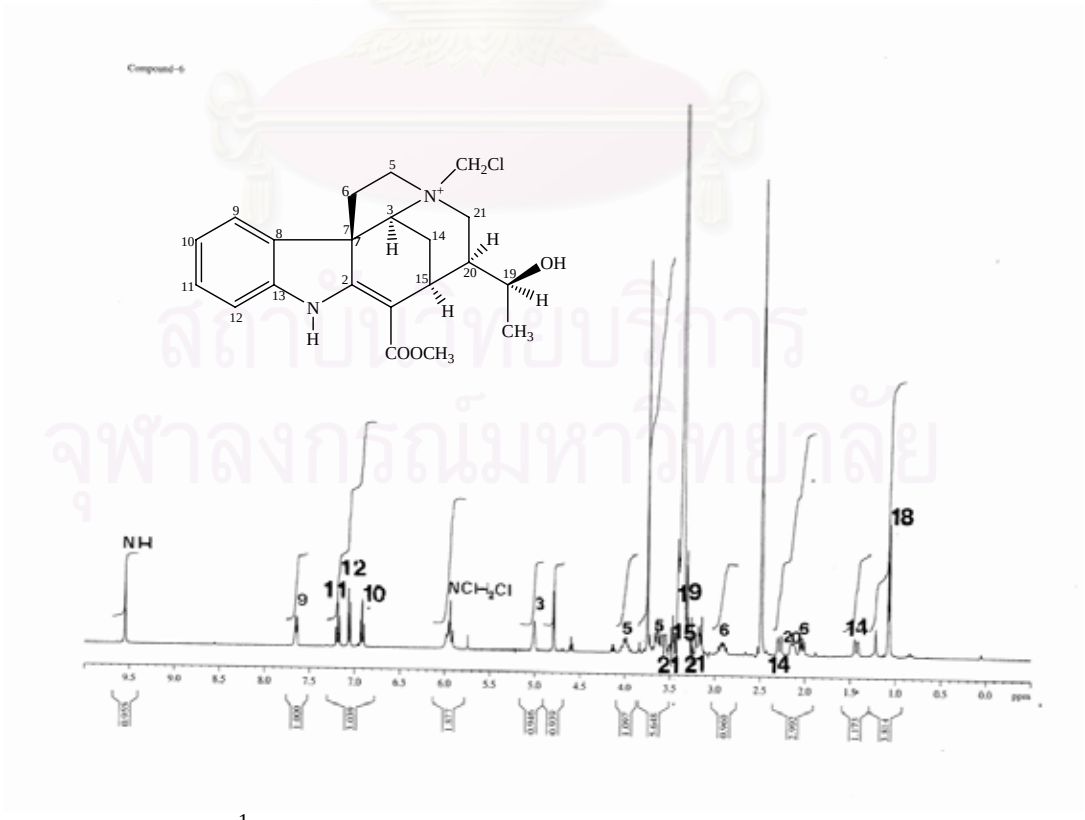


Figure 58: ^{1}H NMR (400 MHz) spectrum of compound **5** (DMSO-d_6)

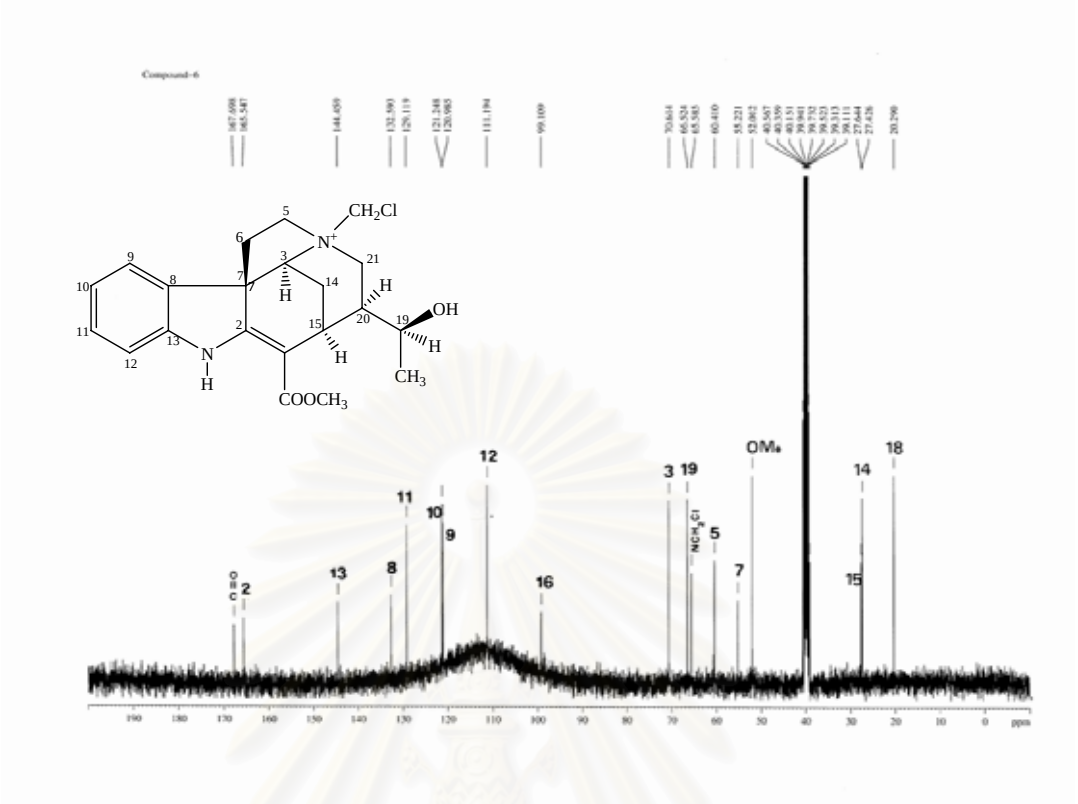


Figure 59: ^{13}C NMR (100 MHz) spectrum of compound **5** (DMSO-d_6)

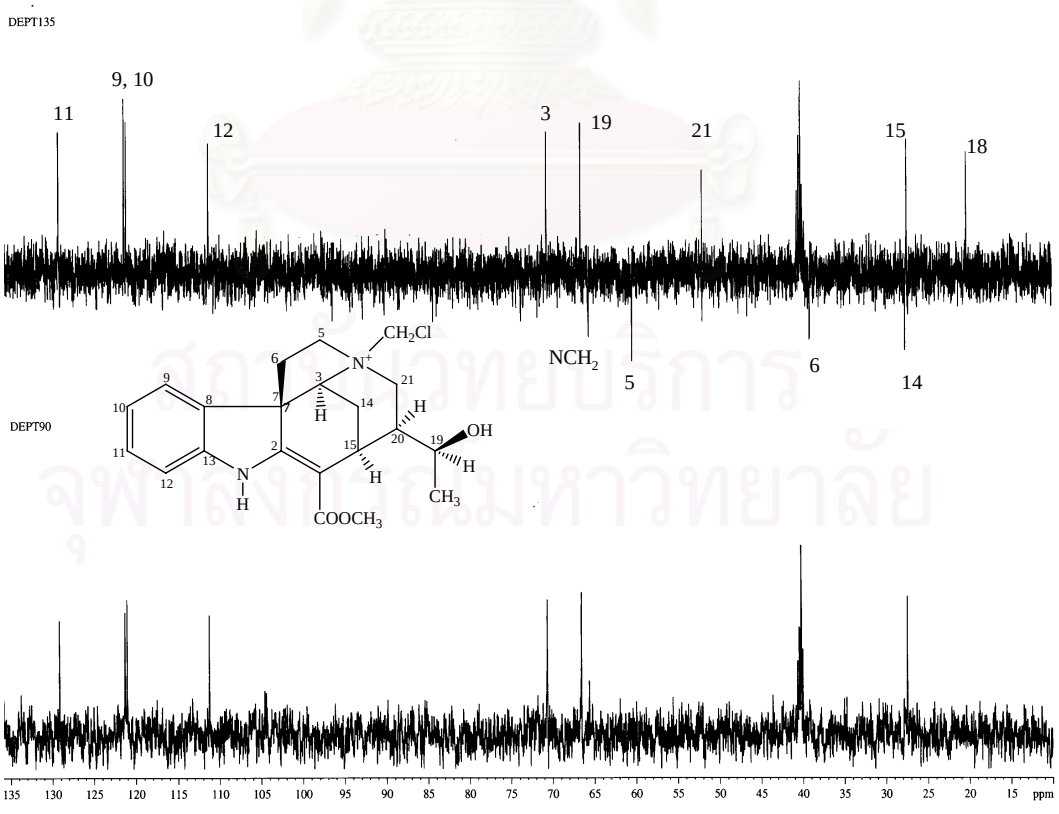


Figure 60: DEPT NMR (100 MHz) of compound **5** (DMSO-d_6)

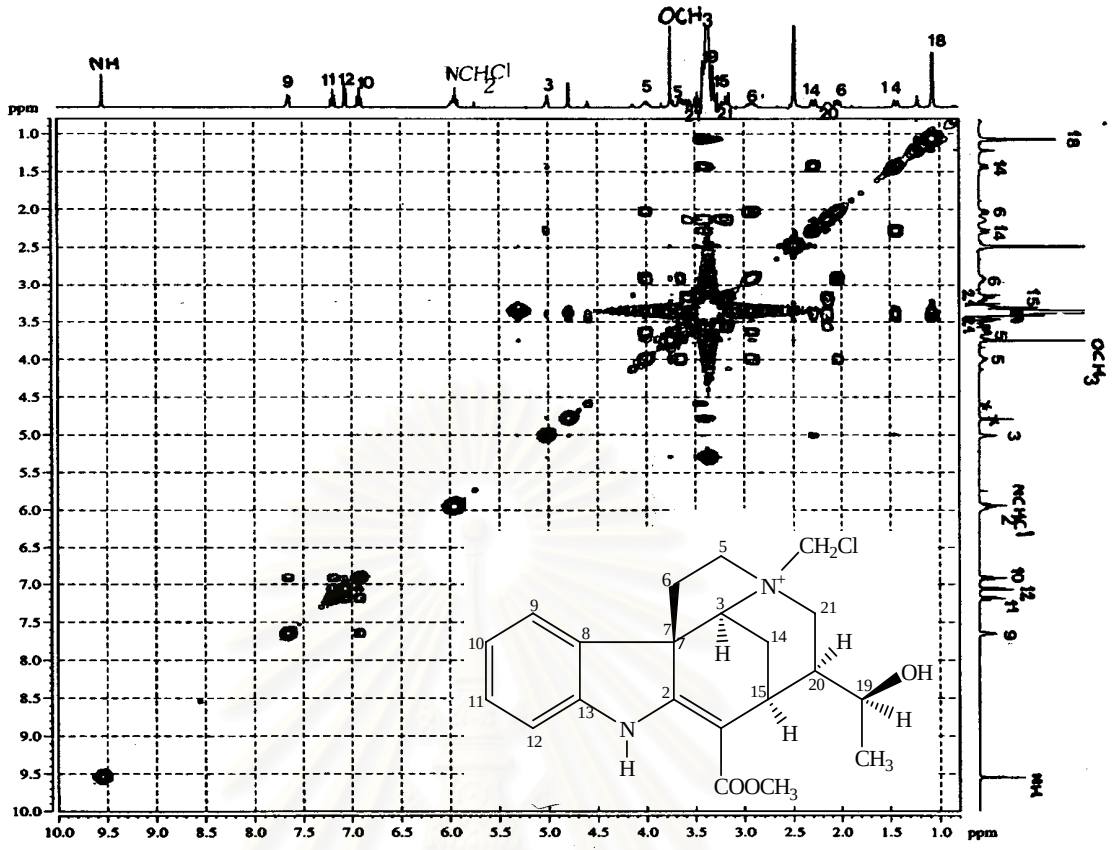


Figure 61: COSY NMR spectrum of compound **5** (DMSO-d_6)

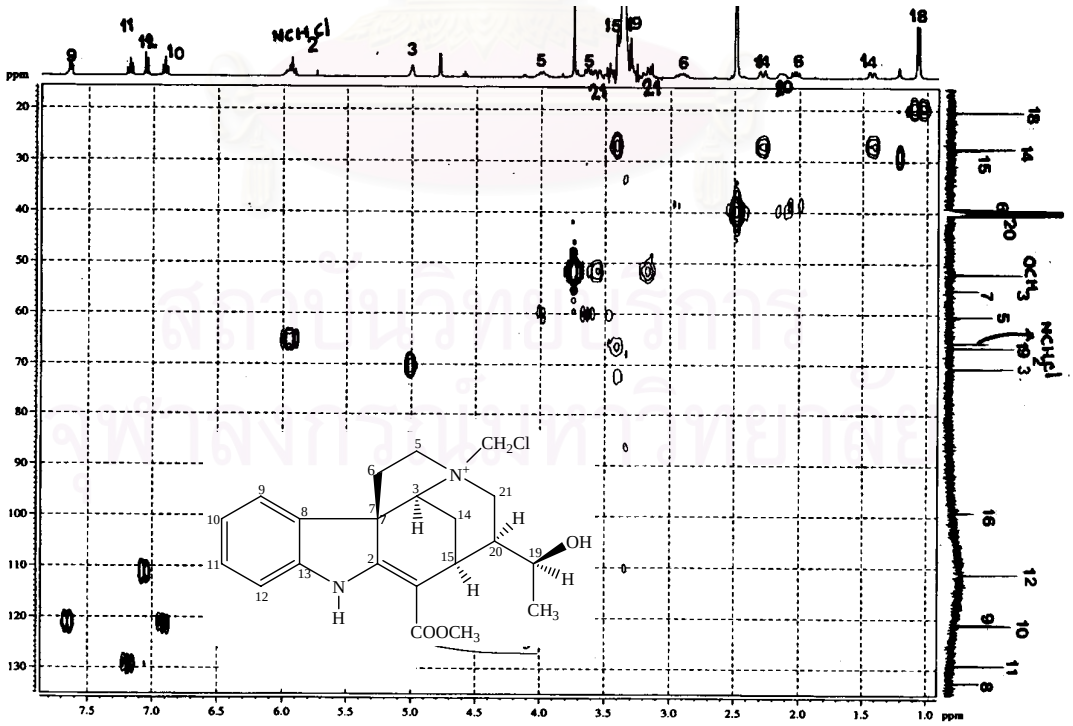


Figure 62: HMQC NMR spectrum of compound **5** (DMSO-d_6)

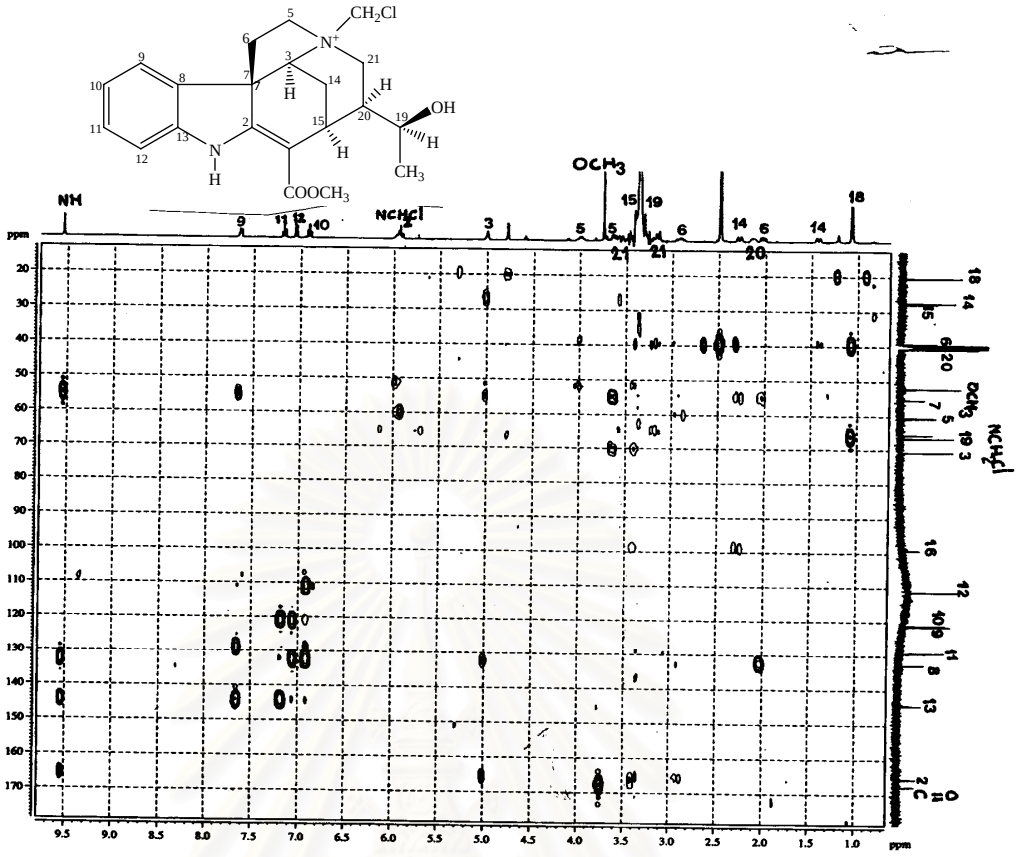


Figure 63: HMBC NMR spectrum of compound **5**(DMSO-d_6)

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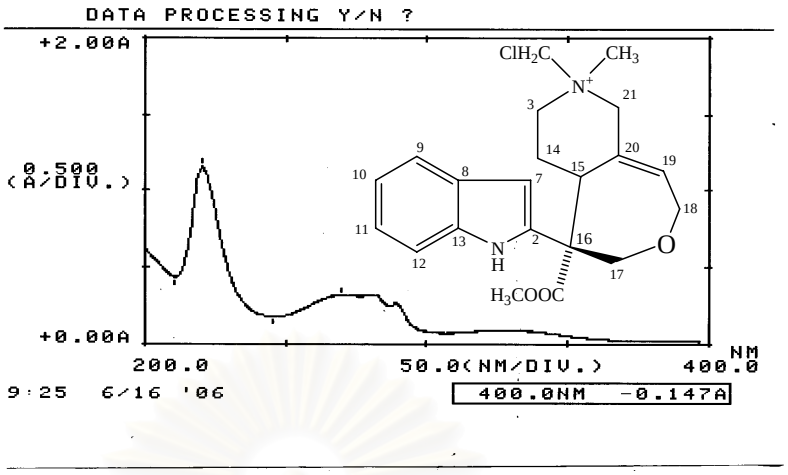

*** PEAK-PICK ***

-- PEAK --		-- VALLEY --	
λ	ABS	λ	ABS
269.4	0.325	245.6	0.179
220.2	1.161	210.8	0.437

Figure 64: UV spectrum of compound **6**

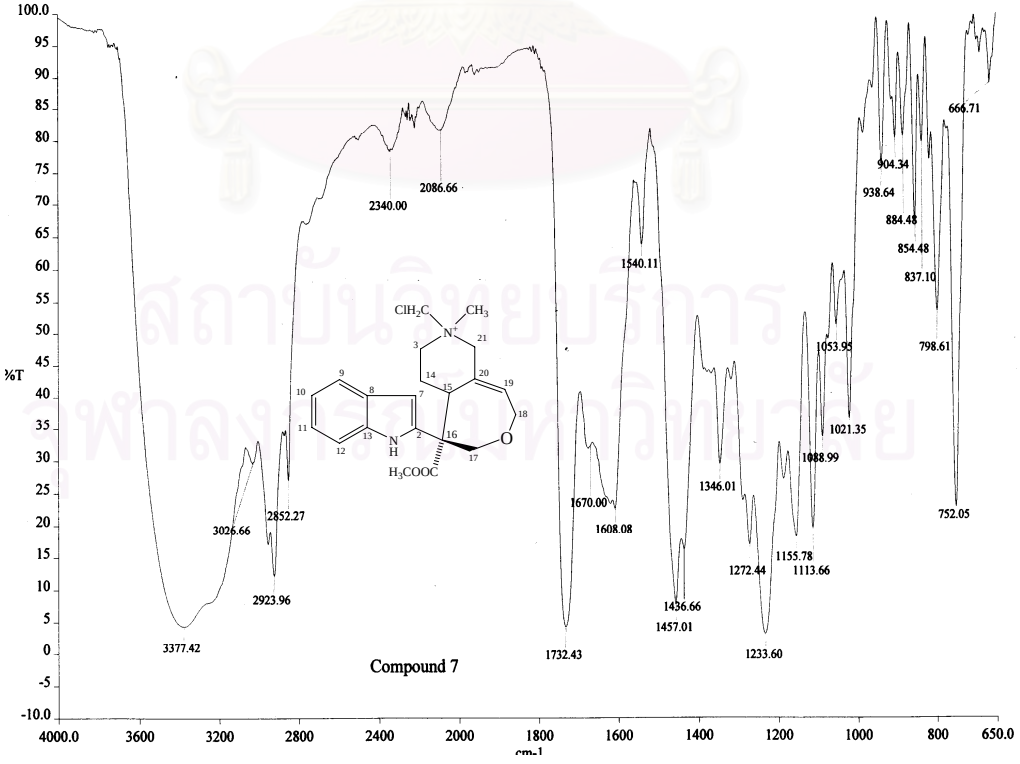

Figure 65: IR spectrum of compound **6** (UATR

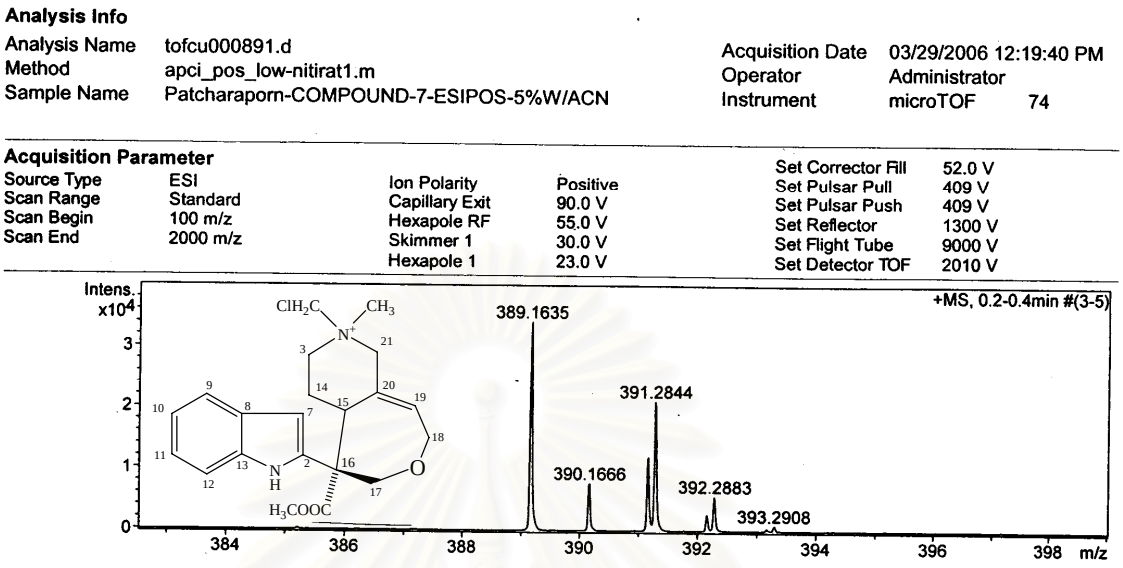

Figure 66: ESITOF mass spectrum of compound **6**

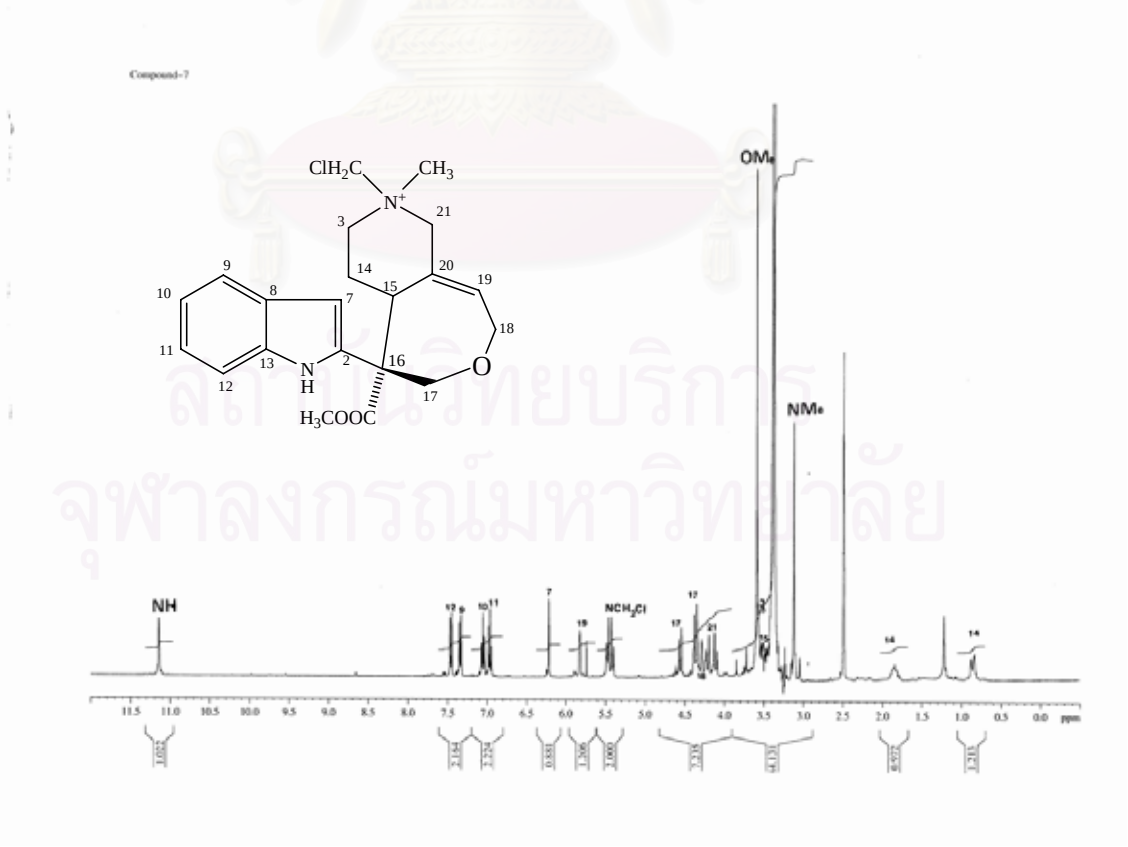

Figure 67: ^{1}H NMR (400 MHz) spectrum of compound **6** (DMSO-d_6)

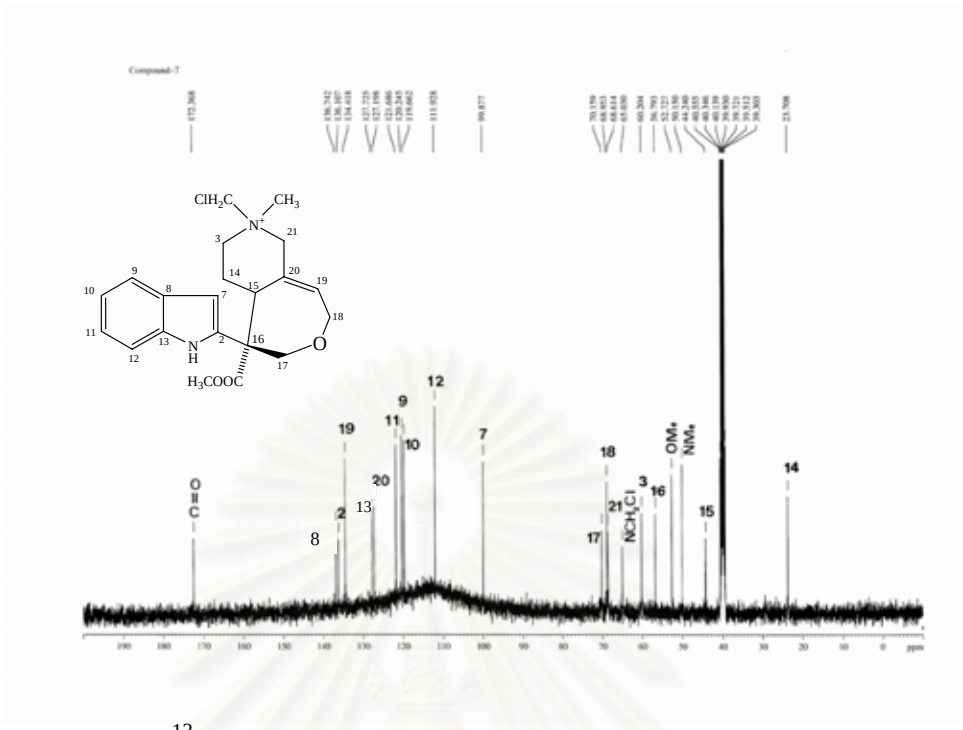

Figure 68: ^{13}C NMR (100 MHz) spectrum of compound **6** (DMSO-d_6)

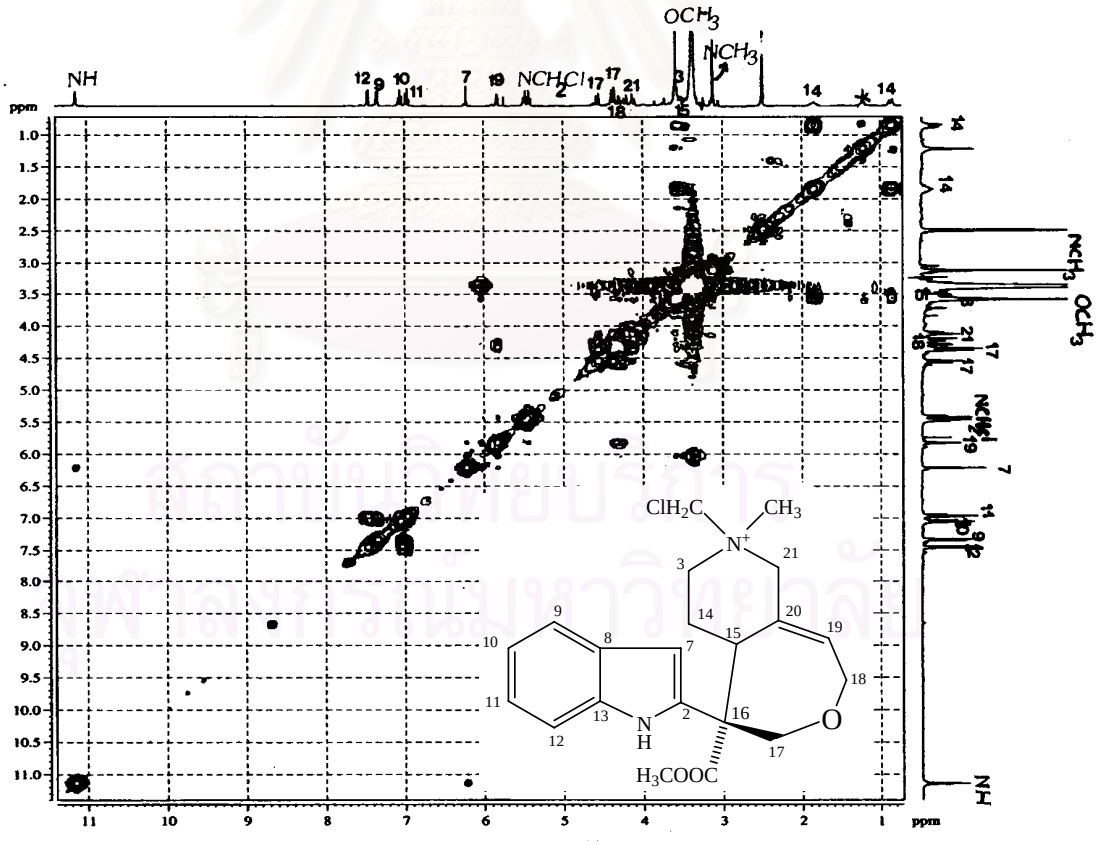

Figure 69: COSY NMR spectrum of compound **6** (DMSO-d_6)

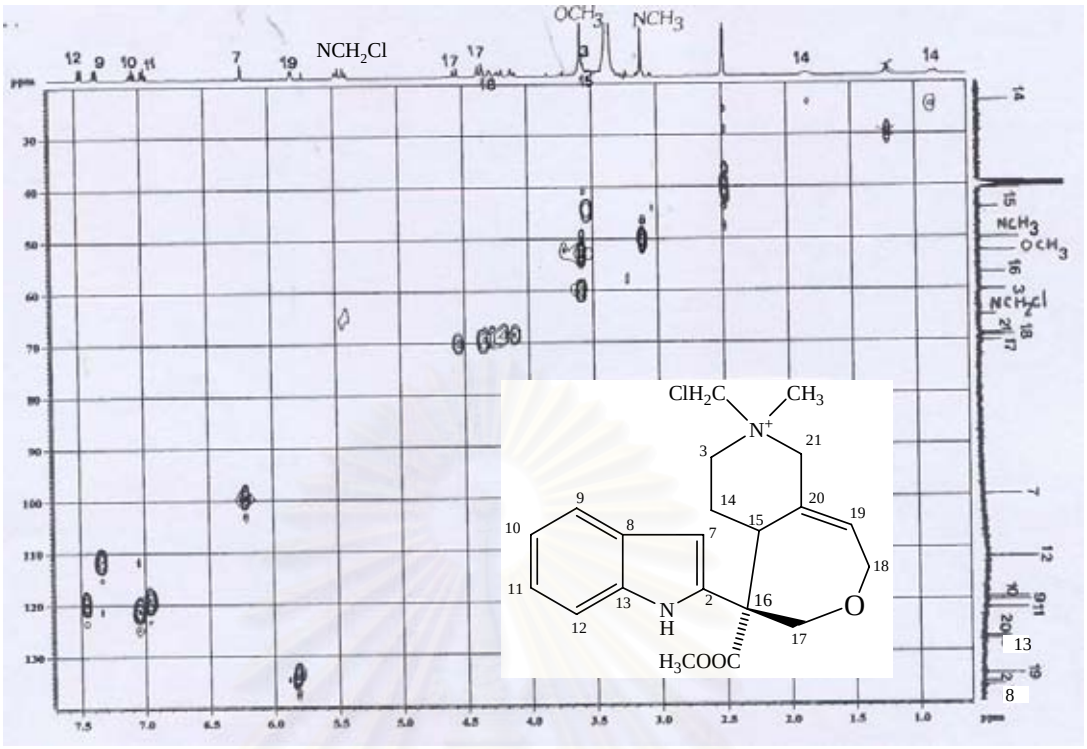

Figure 70: HMQC NMR spectrum of compound **6** (DMSO-d_6)

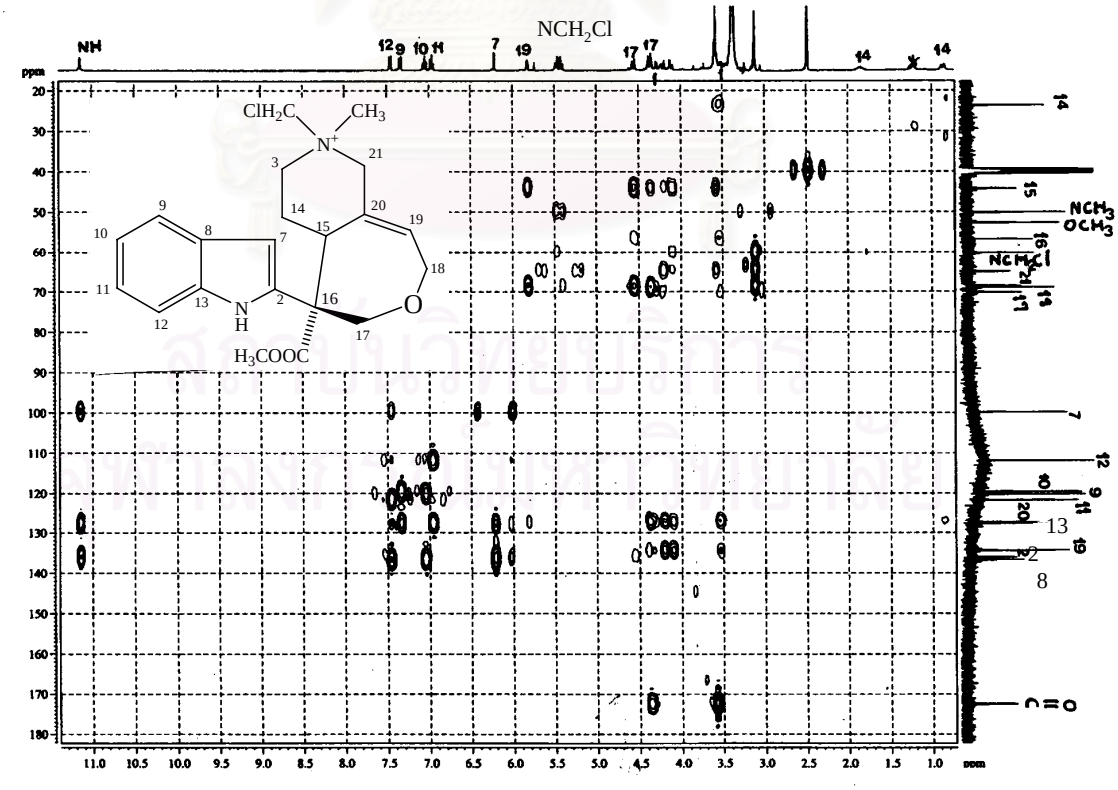

Figure71: HMBC NMR spectrum of compound **6** (DMSO-d_6)

VITA

Miss Patcharaporn Kositthanasarn was born on May 27, 1979 in Chaiyaphum, Thailand. She received her Bachelor's Degree of Science in Pharmacy in 2002 from the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. After graduation, she worked as a pharmacist and the head of Pharmacy department for 2 years at Nongbuadaeng Hospital in Chaiyaphum province.

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