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INDOLE ALKALOIDS FROM THE STEMS OF *UNCARIA HOMOMALLA*

Miss Kusuma Pasugdee

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Pharmacy

Department of Pharmacognosy

Faculty of Pharmaceutical Sciences

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จากลำต้นเครือโงบ (*Uncaria homomalla* Miq.) สามารถสกัดแยกอินโดลแอลคาลอยด์ได้สองชนิด ชนิดหนึ่งเป็น heteroyohimbine คือ tetrahydroalstonine และอีกชนิดหนึ่งเป็น oxindole คือ isopteropodine, isomitraphylline, mitraphylline และ speciophylline ซึ่งได้พิสูจน์โครงสร้างทางเคมีของอินโดลแอลคาลอยด์ที่แยกได้ด้วยการวิเคราะห์สเปคตรัมของ UV, IR, MS และ NMR ร่วมกับการเปรียบเทียบข้อมูลของแอลคาลอยด์ที่ทราบโครงสร้างแล้ว

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

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KEY WORD : *UNCARIA HOMOMALLA* / INDOLE ALKALOIDS

KUSUMA PASUGDEE : INDOLE ALKALOIDS FROM THE STEMS OF *UNCARIA HOMOMALLA*. THESIS ADVISOR: ASSOC. PROF. DHAVADEE PONGLUX, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. SUMPHAN WONGSERIPIPATANA, Ph.D., 104 pp. ISBN 974-13-1253-9

Five indole alkaloids were isolated from the stems of *Uncaria homomalla* Miq. One of them was identified as a heteroyohimbine namely tetrahydroalstonine and the other four as oxindoles, being isopteropodine, isomitraphylline, mitraphylline and speciophylline. The structures of all isolated alkaloids were determined by extensive spectroscopic studies, including comparison of their UV, IR, MS and NMR spectra with the previously reported data.

Department Pharmacognosy Student's signature…….

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

Academic year 2000 Co-advisor's signature……

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LIST OF ABBREVIATIONS

br	=	Broad (for NMR spectra)
^{o}C	=	Degree Celcius
$CDCl_3$	=	Deuterated chloroform
cm	=	Centimeter
^{13}C NMR	=	Carbon-13 nuclear magnetic resonance
COSY	=	Correlation spectroscopy
1-D	=	One dimentional
2-D	=	Two dimentional
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
DNA	=	Deoxyribonucleic acid
δ	=	Chemical shift
EC_{50}	=	Median effective concentration
EIMS	=	Electron Impact Mass Spectrum
EtOAc	=	Ethyl acetate
g	=	Gram
GABA	=	Gamma-aminobutyric acid
HETCOR	=	Heteronuclear chemical shift correlation
hex	=	Hexane
^{1}H NMR	=	Proton nuclear Magnetic Resonance
5HT	=	5-Hydroxytryptamine (serotonin)
Hz	=	Hertz
IC_{50}	=	Median inhibitory concentration
IL-1	=	Interleukin-1
IL-6	=	Interleukin-6
IR	=	Infrared spectrum
J	=	Coupling constant

KBr	=	Potassium bromide
kg	=	Kilogram
L	=	Liter
λ_{max}	=	Wavelength at maximal absorption
ε	=	Molar absorptivity
M^+	=	Molecular ion
m	=	Multiplet (for NMR spectra)
mg	=	Milligram
MHz	=	MegaHertz
ml	=	Milliliter
mm	=	Millimeter
m/z	=	Mass to charge ratio
MS	=	Mass spectrometry
nm	=	Nanometer
NMR	=	Nuclear Magnetic Resonance
NOESY	=	Nuclear Overhauser Effect Correlation Spectroscopy
ppm	=	part per million
ν_{max}	=	Wave number at maximal absorption
q	=	Quartet (for NMR spectra)
qd	=	Quartet of doublet (for NMR spectra)
s	=	Singlet (for NMR spectra)
t	=	Triplet (for NMR spectra)
TLC	=	Thin layer chromatography
TNF	=	Tumor necrosis factor
UV	=	Ultraviolet

CHAPTER I

Introduction

Uncaria is a genus in the sub-tribe Mitragynineae, tribe Cinchoneae, sub-family Cinchonoideae of the family Rubiaceae. This genus is a woody climber having globose flowering heads and peduncles converted into recurved hooks as outstanding characters. It is widely distributed in tropical regions, its stronghold being South East Asia from Malaysia to the Solomon Island, but it is also located in other part of Asia, in Africa and South America. Difficulties in distinguishing between *Uncaria* species are reflected in the 120 specific names in the Index Kewensis which are now reduced to 34 in Ridsdale's revision. Thirty-four specific names reported to be found are listed below as originally recorded (Ridsdale, 1978; Phillipson *et al*, 1978):-

In America

1. *Uncaria guianensis* (Aubl.) Gmel.
2. *U. tomentosa* (Willd.) DC.

In Africa

3. *U. africana* G. Don
4. *U. donisii* Petit
5. *U. talbotii* Wernh.

In Asia

6. *U. acida* (Hunt.) Roxb.
7. *U. attenuata* Korth.
8. *U. berbata* Merr.
9. *U. bernaysii* F. v. Muell.
10. *U. borneensis* Havil.
11. *U. callophylla* Bl. ex Korth.
12. *U. canescens* Korth.
13. *U. cordata* (Lour.) Merr.
14. *U. elliptica* R. Br. ex G. Don

15. *Uncaria gambir* (Hunt.) Roxb.
16. *U. hirsuta* Havil.
17. *U. homomalla* Miq.
18. *U. kunstleri* King
19. *U. laevigata* Wall. ex G. Don
20. *U. lancifolia* Hunth.
21. *U. lanosa* Wall.
22. *U. longiflora* (Poir.) Merr.
23. *U. macrophylla* Wall.
24. *U. nervosa* Elm.
25. *U. orientalis* Guill.
26. *U. perrotii* (A. Rich.) Merr.
27. *U. roxburghiana* Korth.
28. *U. rhynchophylla* (Miq.) Miq. ex Havil.
29. *U. scandens* (Smith) Hunth.
30. *U. schlenckerae* S. Moore
31. *U. sessilifructus* Roxb.
32. *U. sinensis* (Oliv.) Havil.
33. *U. sterrophylla* Merr. & Perry
34. *U. velutina* Havil.

Uncaria homomalla is known in Thai as "Kreua-ngob" (เครือโงบ), "Khao Khwaai mai wong" (เขาควายไม่ว้อง), "Khao Khwaai mai luup" (เขาควายไม่หลูบ), "Ngob" (โงบ), "Ee ngob" (อีโงบ) (Smitinand, 1980; Botanical Garden Organization, 1996). In Thailand, the leaves of *U. homomalla* have been studied and four indole alkaloids were reported (Ponglux *et al*., 1977). However, there is no study that there are other alkaloids in the stems.

The aim of the present study was to investigate indole alkaloids from the stems of *U. homomalla*. Moreover, this study would reveal some chemotaxonomic significance within this genus.

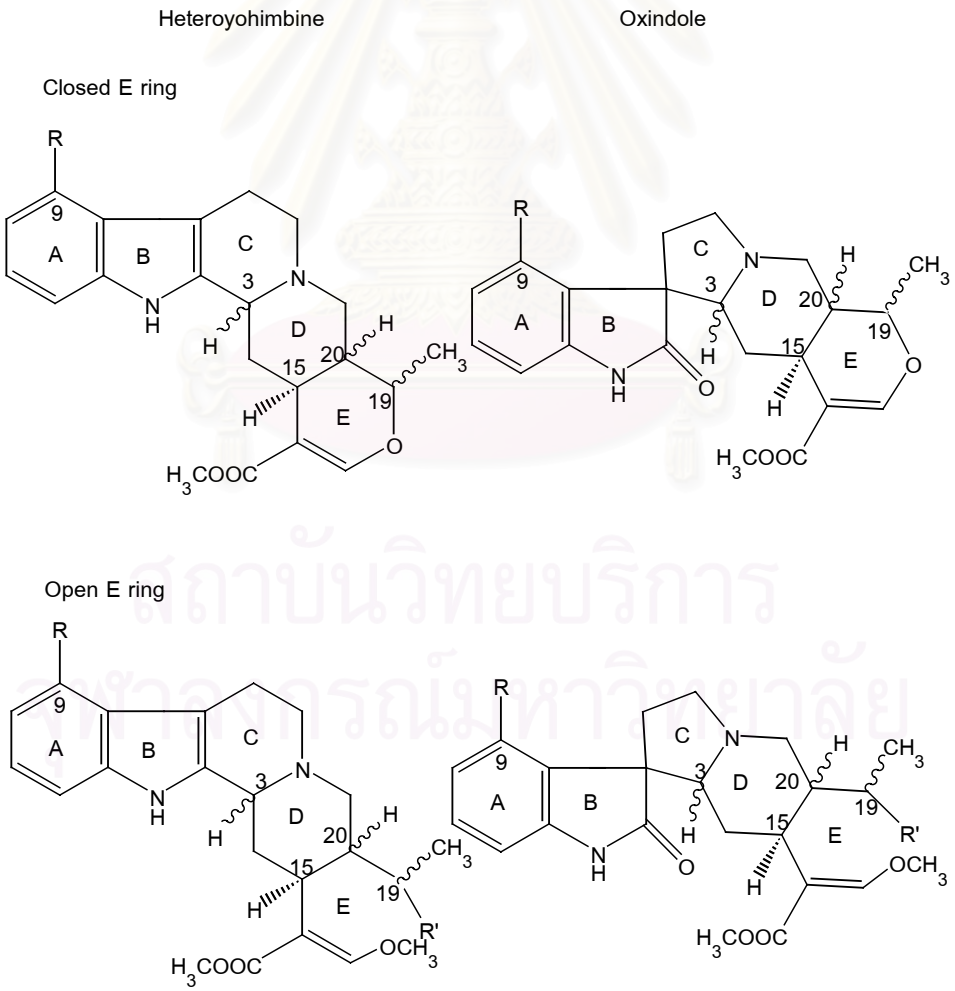
CHAPTER II

Historical

1. Phytochemistry of *Uncaria* species

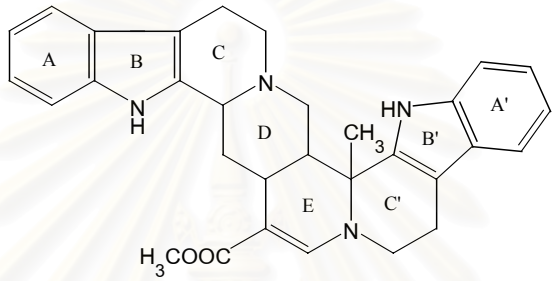
The chemical constituents in *Uncaria* species are indole alkaloids. Most of the indole alkaloids reported are of the pentacyclic and tetracyclic heteroyohimbine-types and the corresponding oxindoles.

There are two types of both heteroyohimbines and oxindoles depending upon the nature of ring E, i.e., closed E ring and open E ring (E-*seco*). The structures are shown below:



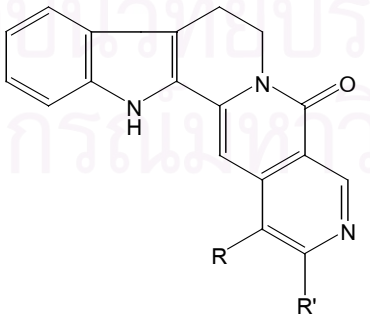
Other types of indole alkaloids

1. Roxburghines represent the first examples of alkaloids derived from two tryptamine moieties and C (10) monoterpene unit (Merlini *et al*., 1970). It must be noted that the arrangement of A, B, C, D and E rings in the roxburghines is the same as that of heteroyohimbines. The four asymmetric centers in both alkaloid types are equivalent at C (3), C (15), C (19) and C (20). The basic structure of these alkaloids is as shown below:



Roxburghines

2. Three pyridino-indolo-quinolizidinone alkaloids, angustine, angustoline and angustidine, originally reported from *Strychnos* species (Loganiaceae) and from *Mitragyna* and *Uncaria* (Rubiaceae) (Phillipson *et al*., 1974). The basic structure of these alkaloids is as shown below:

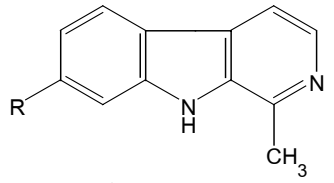


Angustine , R = $CH=CH_2$; R' = H

Angustoline , R = $CH(OH)CH_3$; R' = H

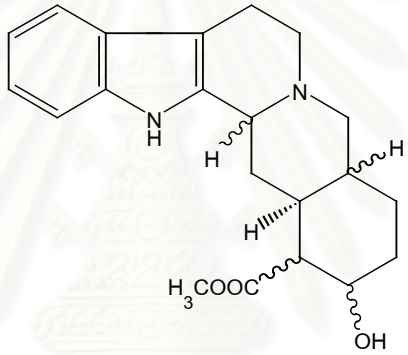
Angustidine , R = H; R' = CH_3

3. The simple β-carboline harmane has been reported from several *Uncaria* species. The basic structure of these alkaloids is as shown below:



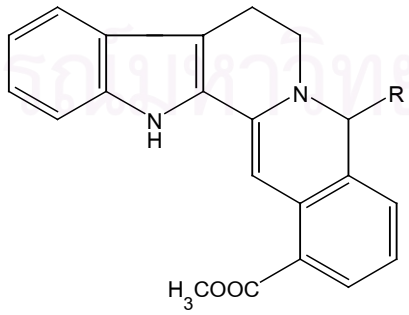
β -Carbolines

4. Yohimbine alkaloids have basic structure as shown below:



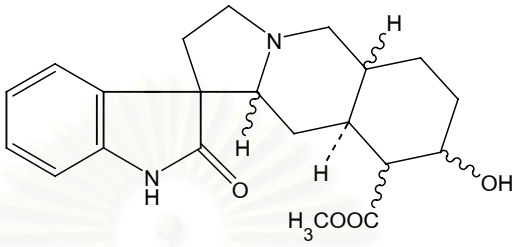
Yohimbines

5. Gambirtannines are yohimbine derivatives with aromatic E ring (Merlini *et al*., 1967). The basic structure of these alkaloids is as shown below:



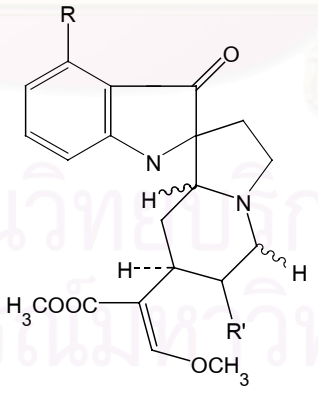
Gambirtannines

6. Yohimbine oxindoles have been reported from one species of *Uncaria* (Phillipson *et al*., 1974). The basic structure of these alkaloids is as shown below:



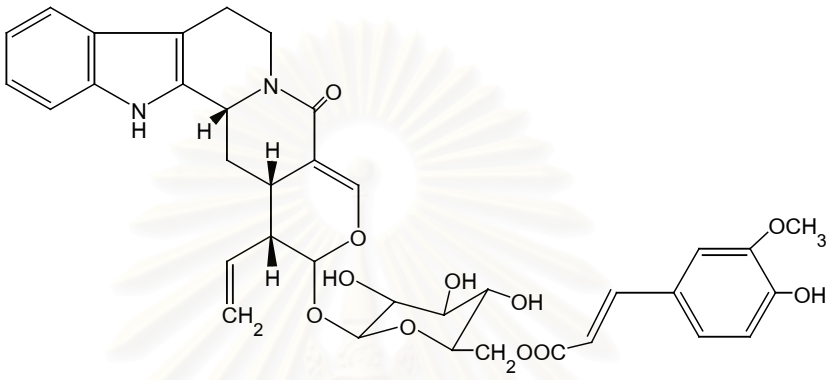
Yohimbine oxindoles

7. Tetracyclic pseudoindoxyls have been reported from *Uncaria africana* (G. Don) Baill. (Phillipson *et al*., 1974) and *U. attenuata* Korth. (Phillipson *et al*., 1975a). The basic structure of these alkaloids is as shown below:



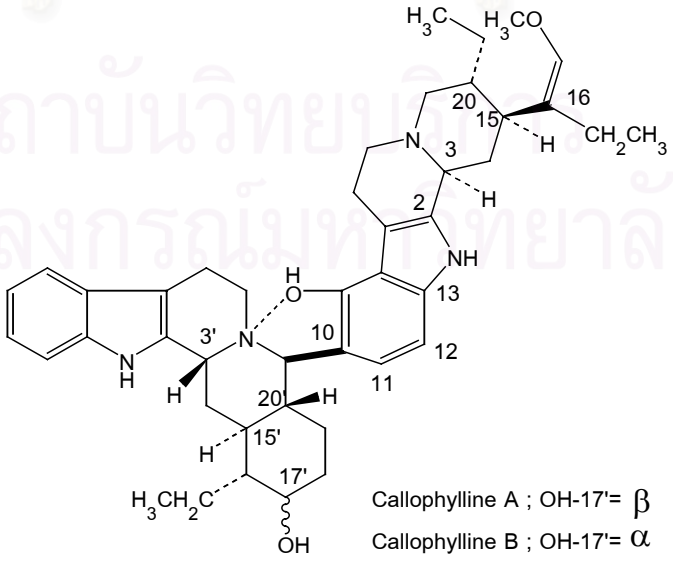
Tetracyclic pseudoindoxyls

8. Indole alkaloid glycosides have been reported from the leaves and stems of *Uncaria rhynchophylla* (Miq.) Miq. ex Havil. (Aimi *et al*., 1982), bark of *U. glabrata* DC. (Arbain *et al*., 1992) and *U. tomentosa* (Willd.) DC. (Kitajima *et al*., 2000). The structure of a sample of these alkaloids are as shown below:



Rhynchophine

9. A few of dimeric indole alkaloids have been reported from *Uncaria* species (Kam *et al*., 1991). The structure of samples of these alkaloids are as shown below:



Callophylline A ; OH-17'= β

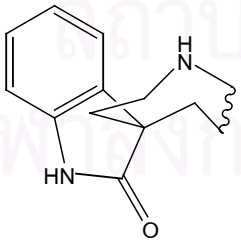
Callophylline B ; OH-17'= α

2. Configuration of heteroyohimbine and oxindole alkaloids

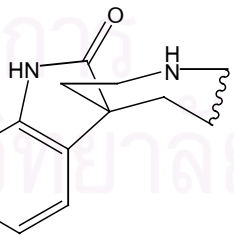
All alkaloids have asymmetric centers at C (3), C (15) and C (20) though all those isolated so far have C (15)-α-H, since these alkaloids are all derived from the monoterpene *seco*-loganin. Four diastereoisomers can thus exist, designated as *normal*, *pseudo*, *allo* and *epiallo*. The closed E ring alkaloids also have an asymmetric center at C (19) which is α in members of the genus *Mitragyna,* but isomers with C (19)-CH_3 is α and β are known to occur in members of the genus *Uncaria*. The E-*seco* alkaloids may show geometric isomerisation because of the double bond between C (16) and C (17). In all known alkaloids the C (17)-H is *cis* to the C (16) carbomethoxy group.

Substitutions in the aromatic ring have been found, but only at C (9), the group being either a hydroxy or a methoxy group for those in *Mitragyna* species. Only 9-hydroxy substituted alkaloids are reported to be present in *Uncaria* species. In the open E ring alkaloids, R_1 may be either an ethyl or a vinyl group.

In addition, the oxindole alkaloids have an asymmetric center at C (7), i.e. ring C attached to ring B at the spiro C-atom, C (7), in two different ways. One of which the lactam carbonyl lies below the plane of C/D ring resulting in the alkaloids termed the A series and those of which the lactam carbonyl lies above the plane of C/D ring giving rise to the alkaloids termed the B series. Thus eight isomers of oxindole are possible.



A series



B series

The four isomers of heteroyohimbines and eight of oxindole alkaloids are summarized with their configurations as follows:

Configuration	C (3)-H	C (15)-H	C (20)-H	C (7) series of oxindole
normal	α	α	β	A or B
pseudo	β	α	β	A or B
allo	α	α	α	A or B
epiallo	β	α	α	A or B

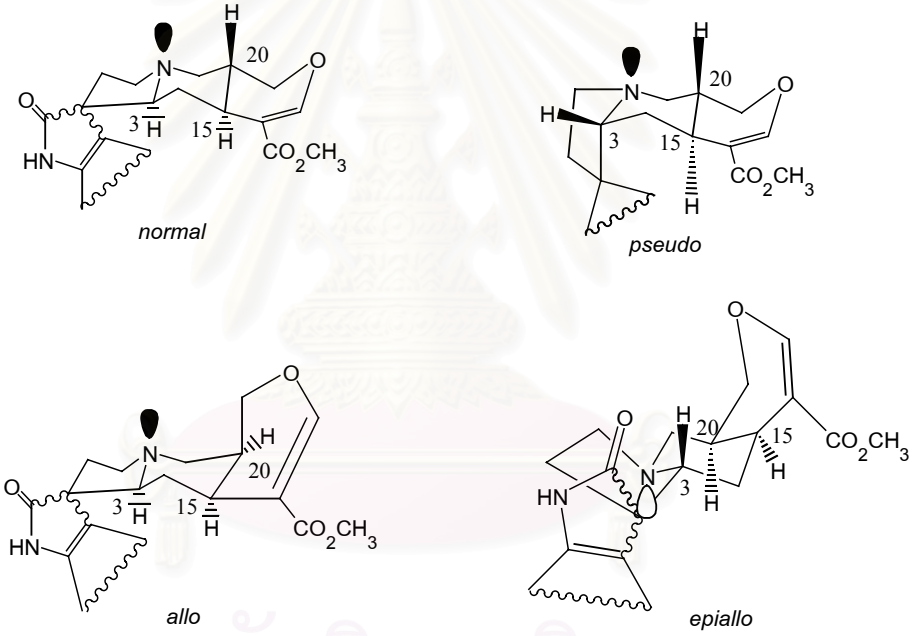
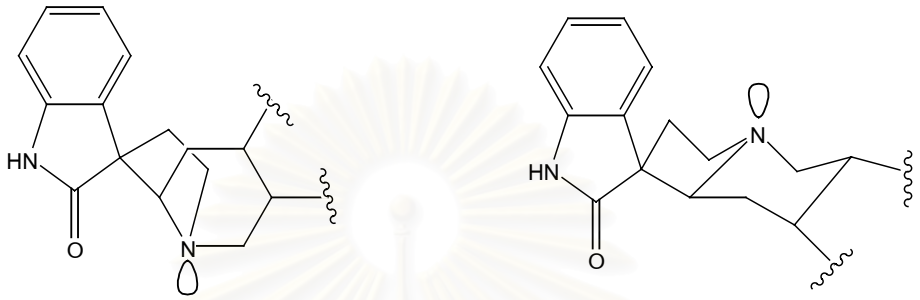


Fig.1 The basic structures of isomers of heteroyohimbine and oxindole alkaloids

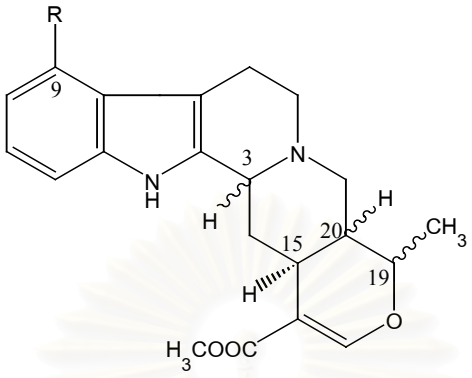
Further, in both types of oxindole alkaloids, the lone pair of electrons on N(4) may be on the same side of the C (7) as the lactam carbonyl group or on the opposite side, the former are known as *syn* and the latter as *anti* alkaloids.



syn *anti*

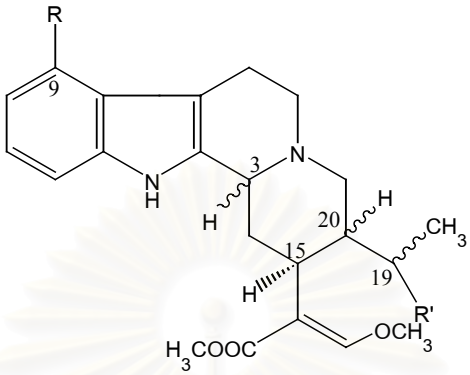
Names of the heteroyohimbine and oxindole alkaloids together with their configurations and substitutions are summarized as follow:

Table 1 Closed E ring heteroyohimbine alkaloids



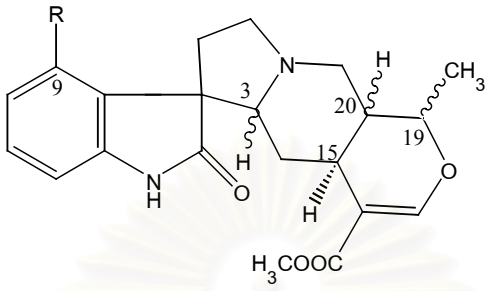
Alkaloid	C (9)-R	C (19)-CH_3	Configuration
Ajmalicine	H	α	*normal*
19-Epi-ajmalicine	H	β	*normal*
Isomitrajavine**	OCH_3	α	*normal*
3- isoajmalicine	H	α	*pseudo*
19-Epi-3-isoajmalicine	H	β	*pseudo*
(Mitrajavine)	OCH_3	α	*pseudo*
Tetrahydroalstonine*	H	α	*allo*
Rauniticine	H	β	*allo*
Akuammigine*	H	α	*epiallo*
(3-Isorauniticine)	H	β	*epiallo*

Table 2 Open E ring heteroyohimbine alkaloids



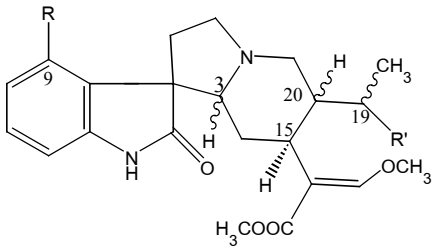
Alkaloid	C (9)-R	R'	Configuration
Dihydrocorynantheine*	H	CH_2CH_3	*normal*
Corynantheine	H	$CH{=}CH_2$	*normal*
Gambirine	OH	CH_2CH_3	*normal*
(Speciogynine)	OCH_3	CH_2CH_3	*normal*
(Paynantheine)	OCH_3	$CH{=}CH_2$	*normal*
Hirsutine*	H	CH_2CH_3	*pseudo*
Hirsuteine	H	$CH{=}CH_2$	*pseudo*
(Isogambirine)	OH	CH_2CH_3	*pseudo*
(Mitraciliatine)	OCH_3	CH_2CH_3	*pseudo*
(Isopaynantheine)	OCH_3	$CH{=}CH_2$	*pseudo*
(Corynantheidine)	H	CH_2CH_3	*allo*
Mitragynine	OCH_3	CH_2CH_3	*allo*
(Isocorynantheidine)	H	CH_2CH_3	*epiallo*
epiallo-Corynantheine	H	$CH{=}CH_2$	*epiallo*
(Speciociliatine)	OCH_3	CH_2CH_3	*epiallo*

Table 3 Closed E ring oxindole alkaloids



Alkaloid	C (9)-R	Configuration	Series	C (19)-CH
Isomitraphylline*	H	*normal*	A	α
Mitraphylline*	H	*normal*	B	α
Uncarine A	H	*normal*	A	β
Uncarine B	H	*normal*	B	β
(Javaphylline)	OCH_3	*normal*	A	α
Isojavaphylline**	OCH_3	*normal*	B	α
Isopteropodine*	H	*allo*	A	α
Pteropodine*	H	*allo*	B	α
(Rauniticine oxindole A)	H	*allo*	A	β
(Rauniticine oxindole B)	H	*allo*	A	β
Speciophylline*	H	*epiallo*	A	α
Uncarine F*	H	*epiallo*	B	α
(Rauniticine *epi*-oxindole A)	H	*epiallo*	A	β
(Rauniticine *epi*-oxindole B)	H	*epiallo*	B	β

Table 4 Open E ring oxindole alkaloids



Alkaloid	C (9)-R	R'	Configuration	Series
Isorhynchophylline*	H	CH_2CH_3	*normal*	A
Rhynchophylline*	H	CH_2CH_3	*normal*	B
Isocorynoxeine	H	$CH{=}CH_2$	*normal*	A
Corynoxeine	H	$CH{=}CH_2$	*normal*	B
(Rotundifoline)	OH	CH_2CH_3	*normal*	A
(Isorotundifoline)	OH	CH_2CH_3	*normal*	B
(Rotundifoleine)	OH	$CH{=}CH_2$	*normal*	A
(Isorotundifoleine)	OH	$CH{=}CH_2$	*normal*	B
(Rhynchociline)	OCH_3	CH_2CH_3	*normal*	A
(Ciliaphylline)	OCH_3	CH_2CH_3	*normal*	B
(Isospecionoxeine)	OCH_3	$CH{=}CH_2$	*normal*	A
(Specionoxeine)	OCH_3	$CH{=}CH_2$	*normal*	B
Corynoxine	H	CH_2CH_3	*allo*	A
Corynoxine B	H	CH_2CH_3	*allo*	B
(Mitrafoline)	OH	CH_2CH_3	*allo*	A
(Isomitrafoline)	OH	CH_2CH_3	*allo*	B
(Mitragynine oxindole A)	OCH_3	CH_2CH_3	*allo*	A
(Mitragynine oxindole B)	OCH_3	CH_2CH_3	*allo*	B
Isospeciofoline	OH	CH_2CH_3	*epiallo*	A
Speciofoline	OH	CH_2CH_3	*epiallo*	B
Speciociliatine oxindole A*	OCH_3	CH_2CH_3	*epiallo*	A
Speciociliatine oxindole B*	OCH_3	CH_2CH_3	*epiallo*	B

* = with its *N*-oxide

** = semi-synthetic

( ) = not yet isolated from *Uncaria* species

3. Indole alkaloids previously found from *Uncaria homomalla* Miq.

Uncaria homomalla Miq. have been studied large number of indole alkaloids. The majority of indole alkaloids found from this species are those of oxindole alkaloids that have closed E ring and their *N* -oxides.

Table 5 Indole alkaloids isolated from *Uncaria homomalla* Miq.

Alkaloids	Part	References
Isopteropodine	leaves	Ponglux *et al*., 1977
Pteropodine	leaves	Ponglux *et al*., 1977
Speciophylline	leaves	Ponglux *et al*., 1977
Uncarine F	leaves	Ponglux *et al*., 1977

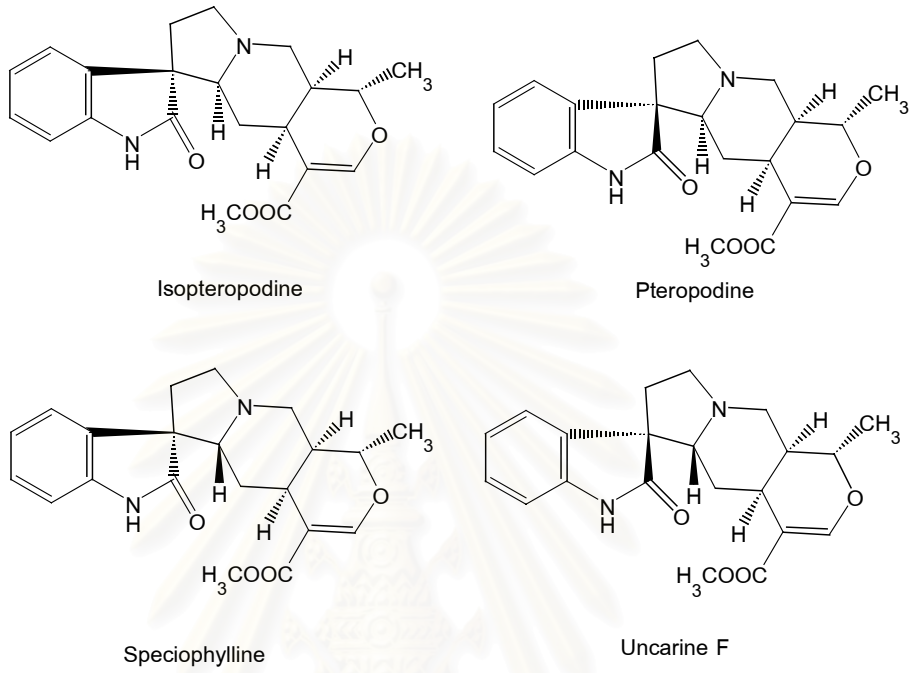


Figure 2 Indole alkaloids previously isolated from *Uncaria homomalla* Miq.

In 1979, Vitayanatpaisan reported that traces of tetrahydroalstonine and 3-isoajmalicine have been noticed from TLC of *U. homomalla* Miq. crude extract comparing with reference alkaloids. Moreover, crude extracts of *U. homomalla* were also compared with authentic alkaloids by TLC technique (Phillipson *et al*., 1978). Other indole alkaloids which are summarized as follows:

Table 6 Indole alkaloids which have been found from *Uncaria homomalla* Miq.

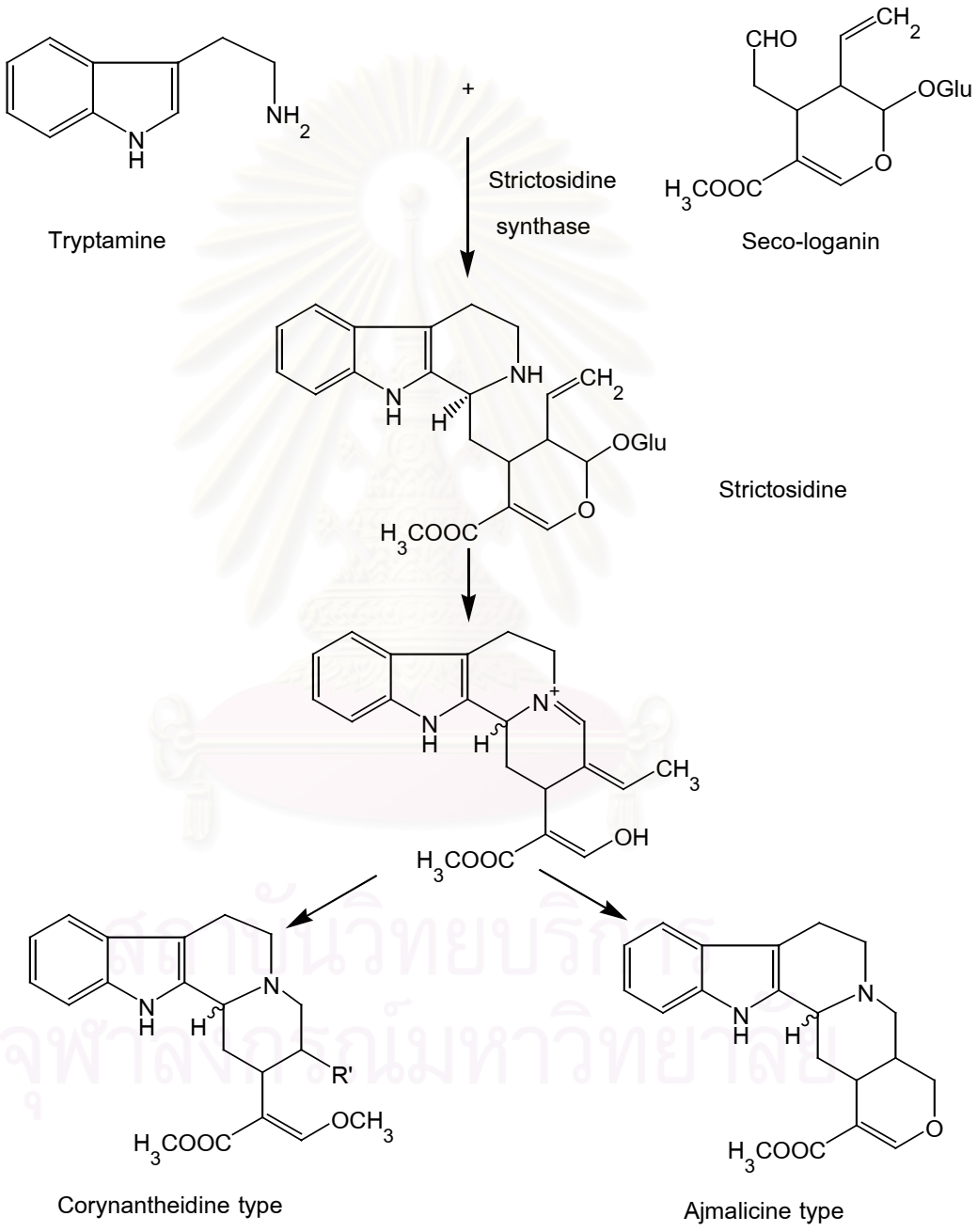
Alkaloid	Part	References
Tetrahydroalstonine	leaves	Phillipson *et al*., 1978; Vityanatpaisan. 1979
3-Isoajmalicine	leaves	Phillipson *et al*., 1978; Vityanatpaisan. 1979
Isomitraphylline	leaves	Phillipson *et al*., 1978
Mitraphylline	leaves	Phillipson *et al*., 1978
Isopteropodine	leaves	Phillipson *et al*., 1978; Ponglux *et al*., 1977
Pteropodine	leaves	Phillipson *et al*., 1978; Ponglux *et al*., 1977
Speciophylline	leaves	Phillipson *et al*., 1978; Ponglux *et al*., 1977
Uncarine F	leaves	Phillipson *et al*., 1978; Ponglux *et al*., 1977
Isopteropodine *N* -oxide	leaves, stem	Phillipson *et al*., 1978
Pteropodine *N* -oxide	leaves, stem	Phillipson *et al*., 1978
Speciophylline *N* -oxide	leaves, stem	Phillipson *et al*., 1978
Uncarine F *N* -oxide	leaves, stem	Phillipson *et al*., 1978
Angustine	leaves, stem	Phillipson *et al*., 1978
Angustidine	leaves, stem	Phillipson *et al*., 1978
Angustoline	leaves, stem	Phillipson *et al*., 1978

4. Biogenetic Pathway of Indole Alkaloids occurring in *Uncaria* species

The hypothesis on biogenesis of indole alkaloids has been reported since early 1900's.

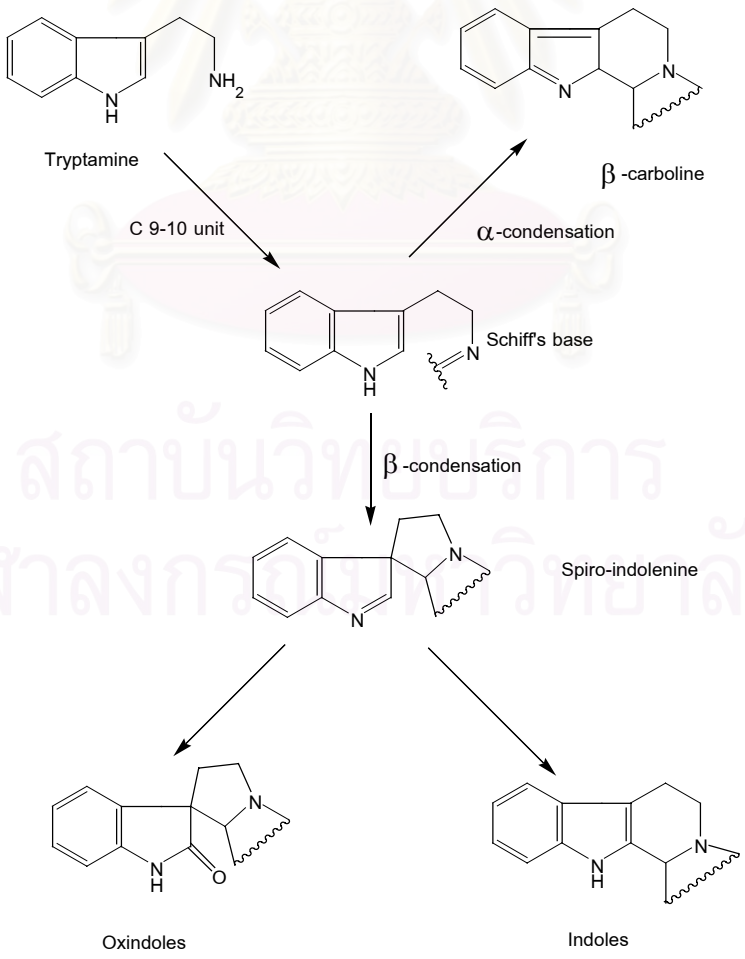
It was accepted that tryptamine provided the β-carboline portion of the alkaloids. Tryptophan, from which tryptamine is derived, is biosynthesized from shikimic acid, having anthranilic acid as an intermediate (Hart *et al*., 1967; Quershi and Scott, 1968). The tryptamine formed then condenses with a C (9-10) unit to give the corresponding indole alkaloids. Several different hypotheses had been postulated but now have been abandoned following the establishment of one theory by the use of radioactive tracer experiments. The theory was independently suggested by Thomas (1961), Wenkert (1962) and confirmed by many other investigators. Battersby *et al.,* (1968) pointed out that it was *seco*-loganin that reacts with the tryptamine. They also proposed a pathway of *seco*-loganin from mevalonic acid, having geraniol, citronellal, irroidial and loganin as intermediates. Battersby *et al.,* (1968) found that vincoside and another intermediate are in the pathway from the reaction of tryptamine and *seco*-loganin to form indole alkaloids. In 1971, Blackstock *et al*., and De Silva *et al*., have shown that vincoside has the C (3)-H β configuration. In 1978, Treimer and Zenk found that strictosidine was derived from the condensation of tryptamine and *seco*-loganin by the aid of enzyme strictosidine synthase. It can be stated that, up to now strictosidine is the central precursor for elaboration of the monoterpenoid indole alkaloids (Nagakura *et al*., 1979; Stockight, 1980).

The biogenetic pathway to the indole alkaloids can therefore be represented as shown below:



For the biogenesis of oxindole alkaloids, Jackson and Smith (1968) with the knowledge of C (9-10) unit, have pointed out that the condensation between tryptamine and the C (9-10) unit is more likely to be β condensation than an α condition as proposed by Woodward (1948) as the latter intermediate is far less stable than the former one. Tryptamine reacts with C (9-10) unit to give a Schiff's base. This compound then undergoes cyclisation at either an α or β position of the indole nucleus to give the β-carboline or the spiro-indolenine, respectively. They also pointed out that the β condensation is more favored because the intermediate produced (spiro-indolenine) does not necessitate a re-arrangement of the π electron system of the benzene ring which would be the case with an α condensation.

The spiro-indolenine can readily either isomerise in mild acid condensation to give the β-carboline and hence indole alkaloids, or oxidize to give oxindole alkaloids (Jackson and Smith, 1968).



5. Biological activities of the genus *Uncaria*

From Chinese, Japanese and Peruvian traditional medicine, *Uncaria* species have been used for treatment of wounds, ulcer, fever, febrile convulsions, headache, gastrointestinal disturbances, cancer, arthritis, diabetes, anti-inflammatory, bacterial and fungal infection. Biological activities of *Uncaria* species have been studied for a long time. The major activity was involved in cardiovascular system (CVS), central nervous system (CNS) and immune system; moreover the other activities were anticancer, anti-inflammatory and antioxidant.

Cardiovascular system effects, Akamatsu *et al*., (1928); Hori (1931); Machida, (1931); Usui (1959) and Kuramochi (1994) reported that rhynchophylline and its analogues had only a weak and transient effect on rat hypotensive principle. Yano *et al*., (1987 and 1991); Ozaki (1990); Horie *et al.,* (1992) reported that hirsutine had produced a dilation of the hindlimb, coronary and cerebral arteries in anesthetized dogs. Especially it was as potent as papaverine in dilating hindlimb artery and cerebral artery. Goh *et al.,* (1984); Goh and Aishah (1985); Chang *et al.,* (1989); Ito *et al*., (1990) reported that dihydrocorynantheine can effectively lower the arterial pressure in anesthetized and conscious normotensive rats. Brown and Fraser (1974); Endo *et al.,* (1983) and Aisaka *et al.,* (1985) reported that 3α-dihydrocadambine was exhibited strong and long-lasting hypotensive activity. All of these alkaloids have been isolated from *Uncaria rhynchophylla*, *U. macrophylla* and *U. sinensis*.

Central nervous system effects, Harada *et al.,* (1974); Harada and Ozaki (1976) reported that hirsutine, isorhynchophylline, uncarine* and 2,3-*seco*-yohimbine, indole alkaloids isolated from *U. rhynchophylla*, *U. kawakamii* and *U. florida* have inhibitory effect on rat superior cervical ganglion. Hirsutine and 2,3-*seco*-yohimbine showed a relatively strong inhibitory effect. On the other hand, the inhibitory effect of isorhynchophylline and uncarine* were not potent on the same model. Moreover, hirsutine and isorhynchophylline in a dose of 2 mg showed little effect on neuromuscular contraction of rabbits and rats limb. Sakakibara *et al.,* (1998) reported that four tetracyclic oxindole alkaloids, rhynchophylline, isorhynchophylline, corynoxine and corynoxine B which were isolated from the hooks of *U. macrophylla* exhibited significantly prolongation of thiopental sleeping times in mice at 100 mg/kg. Corynoxine

B is more potent than corynoxine, isorhynchophylline and rhynchophylline, respectively. Recently, Sakakibara *et al* (1999) reported that effect of indole alkaloids from the hooks of *Uncaria* plants, *U. rhynchophylla*, *U. sinensis* and *U. macrophylla* on locomotion. Corynoxine in a dose of 30 mg/kg, corynoxine B, isorhynchophylline and geissoschizine methyl ether in a dose of 100 mg/kg, significantly decreased locomotor activity after oral administration of these alkaloids onto mice. The depression of locomotor activity upon administration of these alkaloids appears to be due to mediating of the central dopaminergic system. Shimada *et al.,* (1999) reported that protective effect of alkaloids isolated from the hooks and stems of *U. sinensis* on glutamate-induced neuronal death in culture cerebellar granule cells from rats. All of these alkaloids in this report are the active compounds which protect against glutamate-induced neuronal death at concentration of 10^{-4}-10^{-3} M. Zhu *et al*., (1996) showed application of radioligand receptor binding assays in the search for CNS active principles from *U. rhynchophylla* extract. In this investigation, the extract exhibited strong binding to α-2 adrenoceptor, 5HT-1, 5HT-1A, opiate, dopamine-1, Ca^{2+} channel, sulphonylureas, $GABA_A$ and $GABA_B$ receptors. These findings are consistent with the known pharmacological activities of *Uncaria* plants. Furthermore, Terasawa *et al.,* (1997) reported that the result of "Choto-san", a kampo formulation was recognized as the most important ingredient in the formula for the treatment of vascular dementia by placebo-controlled study. The evaluated its efficacy using objective criteria, such as spontaneity of conversation, lack of facial expression, decline in simple mathematial ability, global intellectual ability, nocturnal delirium, sleep disturbance, hallucination or delusion and putting and taking off clothes in 139 patients compared with those taking the placebo. These results suggest that "Choto-san" is effective in treatment of vascular dementia.

(* = mixture of uncarine C: uncarine E: uncarine F; 6: 3: 1)

For the effect on immune system, Wagner *et al.,* (1985) has shown a pronounced enhancement effect of isopteropodine, pteropodine, isomitraphylline and isorhynchophylline on phagocytosis determine in two *in vitro* and *in vivo* tests. Furthermore, Wurm *et al.,* (1998) reported that oxindole alkaloids from *U. tomentosa* induced EA hy.926 endothelial cells to released some yet to be determined factors into supernatant, this factor was shown to significantly enhance proliferation of normal

resting or weakly activated B and T lymphocytes. Lemaire *et al.,* (1999) reported that the *U. tomentosa* extract greatly stimulated IL-1 and IL-6 production by rat macrophages. The result suggested a strong immunostimulant action of this plant.

Anticancer activity of oxindole alkaloids from *U. tomentosa* was reported by Stuppner *et al.,* (1993). The most pronounced effect was found for uncarine F with IC_{50} values of 29.0 μmol/L on leukemic cell lines (U 937). At this concentration did not inhibit progenitor cells obtained from normal human bone marrow. This selectivity indicates that uncarine F may be considered as a possible drug for the treatment of patient with acute leukemia. Lee *et al*., (1999) reported that cytotoxicity of isopteropodine and pteropodine, the indole alkaloids from the bark of *U. guinensis*. Both compounds also showed moderate cytotoxicity to several mammalian cell lines, with IC_{50} values ranging from 17-51 μg/ml.

For anti-inflammatory and antioxidant effects, Desmarckeier *et al.,* (1997) reported that methanol extracts of *U. tomentosa* inhibited free radical-mediated DNA-sugar damage. Recently, Sandoval *et al*., (1998 and 2000) reported that water extraction of Cat's claw (*U. tomentosa*) suppressed TNF alpha production by approximately 65-85% ($p<0.01$) but at concentrations considerably lower than its antioxidant activity: freeze-dried EC_{50} = 28 ng/ml. Then, Cat's claw is an effective antioxidant, but perhaps more importantly a remarkably potent inhibitor of TNF alpha production. The primary mechanism for Cat's claw anti-inflammatory action appears to be immunomodulation via suppression of TNF alpha synthesis.

From all of these experimental data on biological evaluation review above, it is reasonable to emphasize that some results reveal the potential of the genus *Uncaria* as a source of several biologically active molecules. Apparently, further work on biological and chemical investigation of this genus is needed to provide further alkaloids with interesting biological activities.

CHAPTER III

Experimental

1. Source of plant materials

The stems of *Uncaria homomalla Miq*. were cellected from Tone-nga-chang waterfall, Songkhla province, Thailand in May 1999. Authentication of plant materials was done by comparison with herbarium specimens at the Royal Forest Department, Ministry of Agriculture and Co-operatives, Bangkok, Thailand.

2. General Technique

2.1 Analytical Thin-layer Chromatography

Technique	:	One dimension, ascending
Adsorbent	:	Silica gel 60 F_{254} (E. Merck) precoated plate
Layer thickness	:	0.2 mm
Distance	:	6.4 cm
Temperature	:	Laboratory temperature (24-30^{o}C)
Detection	:	1. Ultraviolet light at wavelength 254 nm
		2. Dragendorff's spray reagent
		Solution A : bismuth subnitrate (850 mg), distilled water (40 ml) and acetic acid (10 ml)
		Solution B : potassium iodide (8 mg) and distilled water (20 ml)

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

2.2 Column Chromatography

2.2.1 Flash Column Chromatography

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

2.2.2 Gel Filtration Chromatography

Gel filter	:	Sephadex LH 20(Pharmacia)
Packing method	:	Gel filter was suspended in the eluent and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tightly.
Sample loading	:	The sample was dissolved in a small amount of eluent and applied on top of the column.
Detection	:	1. Fractions were examined by TLC under UV light at the wavelength 254 nm. 2. Fractions were examined by TLC using Dragendorff's spray reagent.

2.3 Spectroscopy

2.3.1 Ultraviolet (UV) Absorption Spectra

UV spectra were obtained on a Shimadzu UV-160A UV/vis spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.3.2 Infrared (IR) Absorption Spectra

IR spectra were recorded on a Perkin-Elmer FT-IR 1760X spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University).

2.3.3 Mass Spectra

Electron Impact (EIMS) was measured with FISONS VG TRIO 2000 mass spectrometer (Department of Chemistry, Faculty of Science, Chulalongkorn University).

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

^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) spectra were obtained with a JEOL JNM-EPC 600 NMR spectrometer (Japan). ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were obtained with a JEOL JNM-EPC 500 NMR spectrometer (Japan). ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were obtained with a Bruker Spectrospin 300 (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

Solvents for NMR spectra were deuterated chloroform (chloroform-*d*). Chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signals.

2.4 Physical Properties

2.4.1 Melting Points

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

2.5 Solvents

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

3 Extraction and Isolation

3.1 Extraction

Dried coarsely powdered stems (5.5 kg) were moistened with strong solution of ammonium hydroxide overnight. They were macerated with ethanol twice (2x40 L) and filtered. The marc was remacerated with methanol twice (2x27 L) and filtered. Each filtrate was concentrated under reduced pressure to syrupy mass, 250 g for ethanolic extracts and 100 g for methanolic extracts.

Ethanolic extract was added with glacial acetic acid (150 ml), poured into warm distilled water (3 L) to give about 5% acetic acid solution. The suspension was well shaken and left to stand overnight. The filtered acid extract was washed and made alkaline (pH 8-9) with concentrated ammonia solution and extracted with 5% methanol in chloroform (3x1.5 L). The combined chloroform extract was washed with distilled water, dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to yield dried crude alkaloidal extract I (14.34 g). The methanolic extract was treated with the same procedures as the ethanolic extract and yield of dried crude alkaloidal extract II (1.64 g).

Thin layer chromatograms of these crude extracts indicated that at least 5 alkaloids were present in addition to base-line alkaloids.

3.2 Isolation

The crude alkaloidal extract I (6.00 g) was dissolved in small amount of chloroform, mixed with silica gel (6.00 g), air dried and packed onto the top of silica gel column (4.5x18 cm). The column was eluted with n-hexane: ethyl acetate (1:1), then washed with methanol until no traces of alkaloids could be detected. Fractions of 50 ml were collected and compared by TLC. Those fractions of similar pattern were combined and evaporated to dryness under reduced pressure. By this procedure:-

1) fractions 1-12 were combined and designated as fraction X-1 (0.1439 g)

2) fractions 13-26 were combined and designated as fraction X-2 (2.6114 g)

3) fractions 27-40 were combined and designated as fraction X-3 (0.2517 g)

4) fractions 41-56 were combined and designated as fraction X-4 (0.2687 g)

5) fractions 57-60 were combined and designated as fraction X-5 (2.368 g)

3.2.1 Isolation of alkaloid U-1 from fraction X-1

Fraction X-1 (0.1439 g) was shown by TLC to contain at least two alkaloids. It was dissolved in small amount of chloroform, mixed with silica gel (0.1400 g), air dried and packed onto the top of silica gel column (2x18 cm). The column was eluted with n-hexane: ethyl acetate: chloroform (4:1:0.2). Forty-two fractions, approximately of 5 ml, were collected. The eluates were examined by TLC using n-hexane: ethyl acetate: chloroform (4:1:0.2) as the developing solvent. Fractions with similar chromatographic pattern were combined to yield four fractions: fraction 5 (12 mg), fractions 6-11 (53.9 mg), fractions 12-38 (55.0 mg) and fractions 39-42 (12.4 mg).

Fraction 5 (12 mg) was dried and further separated by gel filtration chromatography, using a column of Sephadex LH 20 (1x52 cm) with methanol as the eluent. Ten fractions were collected (2 ml per fraction) and examined by TLC using n-hexane: ethyl acetate: chloroform (4:1:0.2) as the developing solvent. Fractions showing similar chromatographic pattern were combined. Fractions 3-8 were combined and evaporated under reduced pressure. The TLC chromatogram of fractions 3-8 showed a single spot under UV light at 254 nm, R_f 0.30 on silica gel/ n-hexane: ethyl acetate: chloroform (4:1:0.2). These fractions were combined and evaporated under reduced pressure to give 8 mg of alkaloid U-1 as yellow powder (0.13% based on dried weight of crude alkaloidal extract I). It was later identified as tetrahydroalstonine.

3.2.2 Isolation of alkaloids U-2 and U-3 from fraction X-2

Fraction X-2 (2.6114 g) was shown by TLC to contain at least three alkaloids. It was dissolved in small amount of chloroform, mixed with silica gel (2.600 g), air dried and packed onto the top of silica gel column (4.5x16 cm). The column was eluted with n-hexane: ethyl acetate (1: 1). Sixty- six fractions, approximately of 30 ml were collected. The eluates were examined by TLC using n-hexane: ethyl acetate (1: 1) as the developing solvent. Fractions with similar chromatographic pattern were combined to yield four fractions: fractions 1-5 (11.8 mg), fractions 16-23 (245.3 mg), fractions 24-34 (401.2 mg) and fractions 35-66 (1521.3 mg).

The TLC chromatogram of fractions 24-34 showed a single spot under UV light at 254 nm, R_f 0.26 on silica gel/ n-hexane: ethyl acetate (1: 1). These fractions were combined and evaporated under reduced pressure to give 401.2 mg of alkaloid U-2 as white powder (6.68% based on dried weight of crude alkaloidal extract I). It was later identified as isopteropodine.

Fractions 35-66 (1521.3 mg) were dried and further separated by flash column chromatography, using a column of silica gel 60 (No. 9385, 3x16 cm) with n-hexane: ethyl acetate (1: 1) as the eluent. Ninety-two fractions, approximately of 20 ml were collected. The eluates were examined by TLC using n-hexane: ethyl acetate (1:1) as the developing solvent. Fractions with similar chromatographic pattern were combined to yield five fractions: fractions 1-5 (3.7 mg), fractions 6-12 (102.2 mg), fractions 13-33 (567.0 mg), fractions 34-47 (44.5 mg) and fractions 67-92 (266.1 mg).

The TLC chromatogram of fractions 34-47 showed a single spot under UV light at 254 nm, R_f 0.18 on silica gel/ n-hexan: ethyl acetate (1: 1). These fractions were combined and evaporated under reduced pressure to give 44.5 mg of alkaloid U-3 as pale yellow powder (0.74% based on dried weight of crude alkaloidal extract I). It was later identified as isomitraphylline.

3.2.3 Isolation of alkaloid U-4 from fraction X-3

Fraction X-3 (251.7 mg) was shown by TLC to contain at least three alkaloids. It was dissolved in small amount of chloroform and packed onto the top of silica gel column (2x16 cm). The column was eluted with ethyl acetate: methanol (6: 0.5). Thirty fractions, approximately of 10 ml were collected. The eluates were examined by TLC using ethyl acetate: methanol (6: 0.5) as the developing solvent. Fractions with similar chromatographic pattern were combined to yield five fractions: fractions 1-4 (73.7 mg), fractions 5-7 (51.6 mg), fractions 8-16 (12.5 mg), fractions 17-23 (36.9 mg) and fractions 24-30 (3.5 mg).

The TLC chromatogram of fractions 8-16 showed a single spot under UV light at 254 nm, R_f 0.20 on silica gel/ ethyl acetate: methanol (6: 0.5). These fractions were combined and evaporated under reduced pressure to give 12.5 mg of alkaloid U-4 as white powder (0.208% based on dried weight of crude alkaloidal extract I). It was later identified as mitraphylline.

3.2.3 Isolation of alkaloid U-5 from fraction X-4

Fraction X-4 (268.7 mg) was shown by TLC to contain at least four alkaloids. It was dissolved in small amount of chloroform and packed onto the top of silica gel column (2x17 cm). The column was eluted with n-hexane: ethyl acetate: methanol (4: 4: 1). Twenty-six fractions, approximately each of 10 ml were collected. The eluates were examined by TLC using n-hexane: ethyl acetate: methanol (4: 4: 1) as the developing solvent. Fractions with similar chromatographic pattern were combined to yield five fractions: fractions 1-8 (3.2 mg), fraction 9 (12.8 mg), fractions 10-14 (45.2 mg), fractions 15-17 (23 mg) and fractions 18-26 (82.6 mg).

Fractions 10-14 were dried and further separated by flash column chromatography, using a column of silica gel 60 (No. 9385, 1x20 cm) with ethyl acetate: methanol (6: 0.5) as the eluent. Twenty-two fractions, approximately of 5 ml were collected. The eluates were examined by TLC using ethyl acetate: methanol (6: 0.5) as the developing solvent. Fractions with similar

chromatographic pattern were combined to yield four fractions: fractions 1-3 (4.5 mg), fractions 4-6 (5.5 mg), fractions 7-14 (4.4 mg) and fractions 15-22 (5.2 mg).

The TLC chromatogram of fractions 15-22 showed a single spot under UV light at 254 nm, R_f 0.13 on silica gel/ ethyl acetate: methanol (6: 0.5). These fractions were combined and evaporated under reduced pressure to give 5.2 mg of alkaloid U-5 as white powder (0.086% based on dried weight of crude alkaloidal extract I). It was later identified as speciophylline.

4 Physical and Spectral data of Isolated Alkaloids

4.1 Alkaloid U-1

Alkaloid U-1 was obtained as yellow powder (8 mg). It was soluble in chloroform and ethanol. R_f value on silica gel/n-hexane: ethyl acetate: chloroform (4: 1: 0.2) = 0.30.

Melting point : 228 ^{o}C

UV : λmax nm (log ε), in ethanol ; Figure 3: 282(0.36), 224(1.00), 205(1.88)

IR : νmax CM^{-1}, KBr disc ; Figure 4: 3400 (br), 2933, 1699, 1628, 1447, 1210, 1086

EIMS : m/z (% relative intensity); Figure 5: 352 (M^+, 25), 351 (M^+-1, 100), 251 (42), 225 (5), 223 (51), 184 (18), 170 (20), 169 (30), 156 (55)

^{1}H-NMR : δppm, 600 MHz, in $CDCl_3$; see figure 6 and Table 7

^{13}C-NMR : δppm, 150 MHz, in $CDCl_3$; see figure 7 and Table 8

4.2 Alkaloid U-2

Alkaloid U-2 was obtained as white powder (401.2 mg). It was soluble in chloroform and ethanol. R_f value on silica gel/n-hexane: ethyl acetate (1: 1) = 0.26.

Melting point : 198-199 °C

UV : λmax nm (log ε), in ethanol ; Figure 8: 283 (0.075), 245 (0.790), 208 (1.474)
λmin nm (log ε), in ethanol ; Figure 8: 225 (0.47)

IR : νmax CM^{-1}, KBr disc ; Figure 9: 3445 (br), 2949, 1707, 1628, 1472, 1212, 1087

EIMS : m/z (% relative intensity); Figure 10: 368 (M^+, 100), 223 (51), 159 (12), 146 (16), 145 (15), 140 (5), 130 (28), 69 (38)

^{1}H-NMR : δppm, 600 MHz, in $CDCl_3$; see figure 11 and Table 9

^{13}C-NMR : δppm, 150 MHz, in $CDCl_3$; see figure 12 and Table 10

4.3 Alkaloid U-3

Alkaloid U-3 was obtained as pale yellow powder (44.5 mg). It was soluble in chloroform and ethanol. R_f value on silica gel/ n-hexane: ethyl acetate (1: 1) = 0.18.

Melting point : 117 °C

UV : λmax nm (log ε), in ethanol ; Figure 14: 281 (0.089), 242 (0.830), 207 (1.530)
λmin nm (log ε), in ethanol ; Figure 14: 224 (0.578)

IR : νmax CM^{-1}, KBr disc ; Figure 15: 3256 (br), 2932, 1704, 1619, 1472, 1212, 1095

EIMS : m/z (% relative intensity); Figure 16: 368 (M^+, 78), 223 (93), 159 (15), 146 (21), 145 (17), 140 (5), 130 (32), 69 (33)

^{1}H-NMR : δppm, 600 MHz, in $CDCl_3$; see figure 17 and Table 11

^{13}C-NMR : δppm, 150 MHz, in $CDCl_3$; see figure 18 and Table 12

4.4 Alkaloid U-4

Alkaloid U-4 was obtained as white powder (12.5 mg). It was soluble in chloroform and ethanol. R_f value on silica gel/ethyl acetate: methanol (6: 0.5) = 0.20.

Melting point : 269-270 °C

UV : λmax nm (log ε), in ethanol ; Figure 20: 281 (0.076), 243 (0.767), 207 (1.461) λmin nm (log ε), in ethanol ; Figure 20: 224 (0.485)

IR : νmax CM^{-1}, KBr disc ; Figure 21: 3380 (br), 2979, 1706, 1620, 1194, 1122

EIMS : m/z (% relative intensity); Figure 22: 368 (M^+, 60), 223 (100), 159 (4), 146 (8), 145 (6), 140 (4), 130 (10), 69 (33)

^{1}H-NMR : δppm, 600 MHz, in $CDCl_3$; see figure 23 and Table 13

^{13}C-NMR : δppm, 150 MHz, in $CDCl_3$; see figure 24 and Table 14

4.5 Alkaloid U-5

Alkaloid U-5 was obtained as white powder (5.2 mg). It was soluble in chloroform and ethanol. R_f value on silica gel/ethyl acetate: methanol (6: 0.5) = 0.13.

Melting point : 183 °C

UV : λmax nm (log ε), in ethanol ; Figure 26: 285 (0.179), 244 (1.076), 208 (2.187) λmin nm (log ε), in ethanol ; Figure 26: 225 (0.800)

IR	:	νmax CM^{-1}, KBr disc ; Figure 27: 3202 (br), 2946, 1706, 1621, 1192, 1087
EIMS	:	m/z (% relative intensity); Figure 28: 368 (M^+, 43), 223 (68), 159 (10), 146 (31), 145 (20), 140 (5), 130 (45), 69 (100)
^{1}H-NMR	:	δppm, 600 MHz, in $CDCl_3$; see figure 29 and Table 15
^{13}C-NMR	:	δppm, 150 MHz, in $CDCl_3$; see figure 30 and Table 16

CHAPTER IV

Results and Discussion

Dried stems of *Uncaria homomalla* Miq. (5.5 Kg) were extracted with ethanol and methanol. The alkaloidal extract I was obtained from ethanolic extract and alkaoidal extract II from methanolic extract, after acid-basic treatment, were 14.34 g and 1.64 g, respectively. The alkaloidal extract I was then separated using several chromatographic techniques to afford five pure alkaloids.

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

1. Structure Determination of Isolated Alkaloids

1.1 Structure Determination of Alkaloid U-1

Alkaloid U-1 (8 mg) was obtained as yellow powder, which R_f value as 0.70 (silica gel/ EtOAc), 0.72 (silica gel/ 5% MeOH in $CHCl_3$) and 0.30 (silica gel/ EtOAc: MeOH; 4: 1: 0.2). It was identified as tetrahydroalstonine. This alkaloid was previously isolated from *Vinca rosea* (Shimizu and Uchimaru, 1958), from the leaves and root of *Catharanthus lanceus* (Maloney *et al*., 1965) and from the leaves of *Uncaria attenuata* (Ponglux *et al*., 1980).

The UV spectrum (Figure 3) of alkaloid U-1 showed maximum absorptions at 205, 224, 282 nm, respectively suggesting the presence of indole chromophore (Shimizu and Uchimaru, 1958; Phillipson and Hemingway, 1975b). The IR spectrum (Figure 4) gave absorption bands at 3400 cm^{-1} (NH) and 1699 cm^{-1} (C=O of ester functional group). The EI mass spectrum (figure 5) gave a molecular formula ion [M^+] at m/z 352, corresponding to the molecular formula $C_{21}H_{24}N_2O_3$ (D.B.E. = 9) and found strong M^+-1 ions due to the loss of C-3 hydrogen that specific of closed E ring heteroyohimbine alkaloids at m/z 351 ion. Other major fragments appeared at m/z 251, 223, 169, 170 and 156. The relative intensities ion fragment at m/z 251 and 223 are more abundant than ion fragment at m/z 209 and 225 and ion fragment at m/z 169 and 170 are similar or lower abundance than the m/z 184 ion (Phillipson and Hemingway,

1975b). All of the relative intensities suggested the *allo* and *epiallo* isomers of heteroyohimbine alkaloids. The indole nucleus fragments occur at m/z 156, 169, 170, 184 and 225.

The ^{1}H chemical shifts from NMR spectrum of alkaloid U-1 (Figure 6 and Table 7) were compared with those of alkaloid tetrahydroalstonine which were reported in 1980 by Lounasmaa and Kan and found to be similar. The ^{1}H chemical shifts of alkaloid U-1 on C-18 (CH_3; δ=1.40), C-23 (OCH_3; δ=3.75), C-19 (δ=4.49), C-11 (δ=7.12), C-10 (δ=7.07), C-9 (δ=7.45), C-12 (δ=7.26), C-17 (δ=7.56), and proton on N (δ=7.83) are straightforward. The ^{13}C NMR (Figure 7 and Table 8) of alkaloid U-1 have one carbonyl carbon at δ=168.08 that was ester carbonyl and have amine carbon at C-2 (δ=134.62). From the coupling constant observed for the protons on the D-ring, 3-H (δ=3.35), 14-H (14α; δ=2.50, 14β; δ=1.53), 15-H (δ=2.76), 20-H (δ=1.70), and 21-H (21α; δ=2.73, 21β; δ=3.06) support the chair conformation of the D-ring in the *allo*-type heteroyohimbine alkaloids.

Table 7 ^{1}H-NMR Assignment of Alkaloid U-1 (in $CDCl_3$) and Tetrahydroalstonine (in $CDCl_3$)

position	Alkaloid U-1	Tetrahydroalstonine (Lounasmaa and Kan,1980)
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_H (ppm) (multiplicity, *J* in Hz)
3	3.35 (br d, 11.5)	3.32 (br d, 12.0)
5α	2.55 (ddd, 13.1, 4.3, 10.7)	2.54 (ddd, 12.5, 4.0, 11.0)
5β	2.92 (ddd, 11.5, 1.6, 6.3)	2.92 (ddd, 11.0, 1.0, 6.0)
6α	2.69 (br d, 15.3)	2.67 (br d, 15.0)
6β	2.82 (ddd, 11.7, 6.0, 14.8)	2.87 (ddd, 11.0, 6.0, 15.0)
9	7.45 (d, 7.6)	7.42 (-)
10	7.07 (ddd, 7.6, 7.6, 1.3)	7.05 (-)
11	7.12 (ddd, 7.5, 7.5, 1.3)	7.10 (-)
12	7.26 (ddd, 11.5, 7.9, 3.5)	7.24 (-)
14α	2.50 (dt, 12.9, 3.6)	2.50 (dt, 12.0, 4.0)
14β	1.53 (q, 12.3)	1.52 (q, 12.0)
15	2.76 (dt, 0.8, 4.3)	2.76 (dt, 0.5, 4.0)
17	7.56 (s)	7.55 (d, 0.5)
18 (Me)	1.40 (d, 6.0)	1.38 (d, 6.5)
19	4.49 (qd, 12.3, 6.3)	4.48 (qd, 12.0, 6.5)
20	1.70 (br d, 10.4)	1.68 (br d, 11.0)
21α	2.73 (dd, 12.3, 3.3)	2.72 (dd, 12.0, 5.0)
21β	3.06 (dd, 3.2, 11.7)	3.08 (dd, 3.0, 12.0)
23 (Me)	3.75 (s)	3.74 (s)
NH	7.83 (br s)	7.86 (br s)

Table 8 ^{13}C-NMR Assignment of Alkaloid U-1 (in $CDCl_3$)

Position	Alkaloid U-1
	δ_C (ppm)
2	134.62
3	59.89
5	53.63
6	21.84
7	109.61
8	127.27
9	118.13
10	119.47
11	121.46
12	110.87
13	136.06
14	34.34
15	31.43
16	108.17
17	155.83
18	18.61
19	72.56
20	38.50
21	56.38
22	168.08
23	51.23

1.2 Structure Determination of Alkaloid U-2

Alkaloid U-2 was obtained as white powder, which R_f value as 0.58 (silica gel/ EtOAc), 0.66 (silica gel/ 5% MeOH in $CHCl_3$) and 0.26 silica gel/ n-hexane : EtOAc (1: 1). It was identified as isoptreopodine. This alkaloid previously isolated from the leaves of *U. homomalla* (Ponglux *et al*., 1977) and the stem bark of *U. quadrangularis* (Tantivatana *et al*., 1979).

The UV spectrum (Figure 8) of alkaloid U-2 showed minimum absorption at 224 nm, maximum absorption at 208 and 245 nm and shoulder at 283 nm suggesting the presence of oxindole chromophore (Yeoh *et al*., 1966; Phillipson and Hemingway, 1975b; Tantivatana *et al*., 1979). The IR spectrum (Figure 9) gave absorption bands at 3445 cm^{-1} (NH) and 1707 cm^{-1} (C=O of lactam and ester functional groups). The EI mass spectrum (figure 10) gave a molecular formula ion [M^+] at m/z 368, corresponding to the molecular formula $C_{21}H_{24}N_2O_4$ (D.B.E. = 9) but do not produce M^+-1 fragment which is the difference between heteroyohimbine and oxindole alkaloids. Other fragments appeared at m/z 130, 140, 145, 146 and 159 ions, exhibited oxindole moiety. The relative intensities of M^+ at m/z 368 of greater intensity than ion at m/z 223, that exhibited *allo* and *epiallo* isomers of oxindole alkaloids (Phillipson and Hemingway, 1975b).

The ^1H and ^{13}C chemical shifts from NMR spectrum of alkaloid U-2 (figures 11-16 and Tables 9-10) were compared with those of alkaloid isopteropodine which were reported in 1993 by Seki and co-workers. The chemical shifts of alkaloid U-2 were similar to those reported in this literature. The coupling constants observed for the protons on the D ring, 3-H (δ = 2.48), 14-H (14α; δ = 1.54, 14β; δ = 0.82), 15-H (δ = 2.48), 20-H (δ = 1.53) and 21-H (21α; δ = 2.40, 21β; δ = 3.21) presented the chair conformation of the D ring in the *allo*-type oxindole alkaloids. In order to confirm the relative stereochemistry at C-20, NOESY measurement (Figure 16) between 20-H and 15-H showed α conformation. This result confirmed the *allo* configuration of alkaloid U-2 as previously described (Seki *et al*., 1993).

Table 9 ^{1}H-NMR Assignment of Alkaloid U-2 (in $CDCl_3$) and Isopteropodine (in $CDCl_3$)

position	Alkaloid U-2	Isopteropodine (Seki *et al*., 1993)
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_H (ppm) (multiplicity, *J* in Hz)
3	2.48 (dd, 11.7 , 2.4)	2.57 (dd, 11.7, 2.8)
5α	2.43 (ddd, 7.5 ,7.5 ,7.5)	2.46 (ddd, 7.6, 7.6, 7.6)
5β	3.16 (ddd, 7.5, 7.5, 2.4)	3.22 (ddd, 7.6, 7.6, 2.4)
6α	1.92 (ddd, 11.7, 7.4, 7.4)	2.00 (ddd, 11.9, 7.6, 7.6)
6β	2.34 (ddd, 11.7, 7.4, 2.4)	2.39 (ddd, 11.9, 7.6, 2.4)
9	7.20 (dd, 7.2, 0.7)	7.27 (dd, 7.7, 0.7)
10	6.94 (ddd, 7.2, 7.2, 1.3)	7.02 (ddd, 7.7, 7.7, 1.1)
11	7.12 (ddd, 7.5, 7.5, 1.5)	7.19 (ddd, 7.7, 7.7, 1.3)
12	6.83 (d, 7.8)	6.89 (d, 7.7)
14α	1.54 (ddd, 11.1, 2.4, 2.4)	1.62 (ddd, 11.7, 2.8, 2.8)
14β	0.82 (ddd, 11.9, 11.9, 11.9)	0.88 (ddd, 11.7, 11.7, 11.7)
15	2.48 (ddd, 11.7, 2.4, 2.4)	2.51 (ddd, 11.7, 2.8, 2.8)
17	7.34 (s)	7.41 (s)
18 (Me)	1.34 (d, 6.0)	1.41 (d, 6.2)
19	4.28 (qd, 6.3, 10.6)	4.36 (qd, 6.2, 10.4)
20	1.53 (br m)	1.59 (br m)
21α	2.40 (dd, 11.6, 3.6)	2.42 (dd, 11.9, 3.8)
21β	3.21 (dd, 11.4, 1.8)	3.29 (dd, 11.9, 1.8)
23 (OMe)	3.53 (s)	3.60 (s)
NH	8.57 (br s)	8.42 (br s)

Table 10 ^{13}C-NMR Assignment of Alkaloid U-2 (in $CDCl_3$) and Isopteropodine (in $CDCl_3$)

Position	Alkaloid U-2	Isopteropodine (Seki *et al.*, 1993)
	δ_C (ppm)	δ_C (ppm)
2	181.09	181.25
3	71.23	71.24
5	54.14	54.10
6	34.92	34.83
7	56.98	56.92
8	133.59	133.75
9	124.37	124.54
10	122.35	122.49
11	127.51	127.66
12	109.58	109.62
13	140.09	140.21
14	30.27	30.16
15	30.57	30.47
16	109.73	109.94
17	154.76	154.94
18	18.74	18.62
19	72.13	72.13
20	37.97	37.89
21	53.54	53.50
22	167.37	167.59
23	51.01	50.95

1.3 Structure Determination of Alkaloid U-3

Alkaloid U-3 was obtained as pale yellow powder and subsequently identified as isomitraphylline, which R_f value as 0.52 (silica gel/ EtOAc), 0.26 silica gel/ $CHCl_3$: EtOAc (3: 2) and 0.18 silica gel/ n-hexane: EtOAC (1: 1). It was previously isolated from *Uncaria attenuata* (Phillipson and Hemingway, 1975a) and from the leaves of *U. quadrangularis* (Tantivatana *et al*., 1979).

The UV spectrum (Figure 17) of alkaloid U-3 showed minimum absorption at 224 nm, maximum absorption at 207 and 242 nm and shoulder at 281 nm suggesting the presence of oxindole chromophore (Yeoh *et al*., 1966; Phillipson and Hemingway, 1975b; Tantivatana *et al*., 1979). The IR spectrum (Figure 18) gave absorption bands at 3256 cm^{-1} (NH) and 1704 cm^{-1} (C=O of lactam and ester functional groups). The EI mass spectrum (figure 19) gave a molecular formula ion [M^+] at m/z 368, corresponding to the molecular formula $C_{21}H_{24}N_2O_4$ (D.B.E. = 9) but do not produce M^+-1 fragment that the difference between heteroyohimbine and oxindole alkaloids. Other fragments appeared at m/z 130, 140, 145, 146 and 159 ions, exhibited oxindole moiety. The relative intensities of M+ at m/z 368 is less than that of m/z 223 fragment, that exhibited *normal* isomers of oxindole alkaloids (Phillipson and Hemingway, 1975b).

The ^1H and ^{13}C chemical shifts from NMR spectrum of alkaloid U-3 (figures 20-22 and Tables 11-12) were compared with those of alkaloid isomitraphylline which were reported in 1993 by Seki and co-workers. The chemical shifts of alkaloid U-3 are similar to those reported in this literature. Coupling constants of the proton on C-3 (δ=2.60), C-14 (14α; δ=2.2, 14β; δ= 0.61), C-15 (δ=2.13), C-20 (δ=1.71) and C-21 (21α; δ=1.92, 21β; δ= 3.12) supported the chair conformation of the D-ring in the normal-type oxindole alkaloids. In order to confirm the relative stereochemistry at C-20, NOESY measurement (Figure 22) between H-20 (δ=1.71) and H-15 (δ=2.13) showed different conformation and the C-9 signals of U-3 (isomitraphylline) appear down field by 0.2, compared with U-4 (mitraphylline) and the signals of C-14 proton in U-3 (isomitraphylline) resonate at higher field than those of C-14 proton in U-4 (mitraphylline).

Table 11 ^{1}H-NMR Assignment of Alkaloid U-3 (in $CDCl_3$) and Isomiraphylline (in $CDCl_3$)

position	Alkaloid U-3	Isomitraphylline (Seki *et al*., 1993)
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_H (ppm) (multiplicity, *J* in Hz)
3	2.60 (dd, 11.6, 2.4)	2.60 (dd, 11.5, 3.0)
5α	2.52 (ddd, 8.8, 8.8, 8.8)	2.54 (ddd, 8.9, 8.9, 8.9)
5β	3.31 (ddd, 8.2, 8.2, 2.1)	3.30 (ddd, 8.9, 8.6, 2.3)
6α	2.04 (ddd, 13.7, 8.5, 8.2)	2.04 (ddd, 13.0, 8.9, 8.6)
6β	2.42 (ddd, 12.5, 7.0, 2.3)	2.41 (ddd, 13.0, 8.9, 2.3)
9	7.35 (d, 7.3)	7.35 (d, 7.6)
10	6.99 (ddd, 7.3, 7.3, 0.6)	7.00 (ddd, 7.6, 7.6, 0.8)
11	7.17 (ddd, 7.6, 7.6, 1.3)	7.17 (ddd, 7.6, 7.6, 1.2)
12	6.87 (d, 7.6)	6.85 (d, 7.6)
14α	2.22 (ddd, 11.5, 3.0, 3.0)	2.21 (ddd, 11.5, 3.0, 3.0))
14β	0.61 (ddd, 11.4, 11.4, 11.4)	0.60 (ddd, 11.5, 11.5, 11.5)
15	2.13 (br ddd, 11.3, 11.3, 3.0)	2.18 (br ddd, 11.5, 11.5, 3.0)
17	7.38 (d, 1.2)	7.38 (d, 1.7)
18 (Me)	1.20 (d, 7.0)	1.12 (d, 6.9)
19	4.35 (qd, 6.3, 6.3)	4.37 (qd, 6.9, 3.7)
20	1.71 (m)	1.91 (m)
21α	1.92 (dd, 11.0, 11.0)	1.94 (dd, 11.0, 11.0)
21β	3.12 (dd, 11.0, 7.3)	3.12 (dd, 11.0, 7.3)
23 (OMe)	3.56 (s)	3.57 (s)
NH	8.36 (br s)	7.74 (br s)

Table 12 ^{13}C-NMR Assignment of Alkaloid U-3 (in $CDCl_3$) and Isomitraphylline (in $CDCl_3$)

Position	Alkaloid U-3	Isomitraphylline (Seki *et al.*, 1993)
	δ_C (ppm)	δ_C (ppm)
2	181.33	180.73
3	71.72	71.85
5	53.35	53.40
6	35.41	35.45
7	56.37	56.32
8	133.78	133.82
9	124.82	124.99
10	122.30	122.43
11	127.52	127.58
12	109.58	109.35
13	140.21	139.98
14	29.11	29.16
15	30.00	30.07
16	107.32	107.38
17	153.80	153.85
18	14.83	14.89
19	73.97	74.03
20	40.87	40.95
21	54.24	54.31
22	167.04	167.07
23	50.73	50.76

1.4) Structure Determination of Alkaloid U-4

Alkaloid U-4 was obtained as white powder, which was identified as mitraphylline that has R_f value as 0.24 (silica gel/ EtOAc), 0.34 (silica gel/ 5% MeOH in $CHCl_3$) and 0.20 [silica gel/ EtOAc: MeOH (6: 0.5)]. It was previously isolated from *Uncaria attenuata* (Phillipson and Hemingway, 1975a) and from the leaves of *U. quadrangularis* (Tantivatana *et al*., 1979)

The UV spectrum (Figure 23) of alkaloid U-4 showed minimum absorption at 224 nm, maximum absorption at 207 and 243 nm and shoulder at 281 nm suggesting the presence of oxindole chromophore (Phillipson and Hemingway, 1975b; Tantivatana *et al*., 1979). The IR spectrum (Figure 24) gave absorption bands at 3380 cm^{-1} (NH) and 1706 cm^{-1} (C=O of lactam and ester functional groups). The EI mass spectrum (figure 25) gave a molecular formula ion [M^+] at m/z 368, corresponding to the molecular formula $C_{21}H_{24}N_2O_4$ (D.B.E. = 9) but do not produce M^+-1 fragment that the difference between heteroyohimbine and oxindole alkaloids. Other fragments appeared at m/z 130, 140, 145, 146 and 159 ions, exhibited oxindole moiety. The relative intensities of M^+ at m/z 368 is less than that of m/z 223 fragment, that exhibited *normal* isomers of oxindole alkaloids (Phillipson and Hemingway, 1975b).

The ^1H and ^{13}C chemical shifts from NMR spectrum of alkaloid U-4 (figures 26-28 and Tables 13-14) were compared with those of alkaloid mitraphylline which were reported in 1993 by Seki and co-workers and found to be similar. From the coupling constants of the proton on C-3 (δ = 2.40), C-14 (14α; δ = 2.39, 14β; δ = 1.21), C-15 (δ = 2.09), C-20 (δ = 2.09) and C-21 (21α; δ = 1.85, 21β; δ = 3.22) supported the chair conformation of the D-ring in the normal-type oxindole alkaloids. In order to confirm the relative stereochemistry at C-20, NOESY measurement (Figure 28) between H-20 (δ=2.09) and 21β (δ = 3.22) showed different conformation and the C-9 signals of U-3 (isomitraphylline) appear up field by 0.2, compared with U-4 (mitraphylline) and the signals of C-14 proton in U-3 (isomitraphylline) resonate at lower field than those of C-14 proton in U-4 (mitraphylline). From NMR specrum data of U-3 and U-4 are showed different series oxindole alkaloids, U-3 is in A series and U-4 is in B series.

Table 13 ^{1}H-NMR Assignment of Alkaloid U-4 (in $CDCl_3$) and Mitraphylline (in $CDCl_3$)

position	Alkaloid U-4	Mitraphylline (Seki *et al*., 1993)
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_H (ppm) (multiplicity, *J* in Hz)
3	2.40 (dd, 10.1, 2.7)	2.41 (dd, 11.1, 2.4)
5α	2.50 (m)	2.50 (m)
5β	3.38 (m)	3.39 (m)
6α	2.04 (m)	2.03 (m)
6β	2.49 (m)	2.49 (m)
9	7.19 (d, 7.6)	7.19 (d, 7.8)
10	7.04 (ddd, 7.3, 7.3, 0.9)	7.03 (ddd, 7.8, 7.8, 0.9)
11	7.19 (ddd, 7.6, 7.2, 0.9)	7.18 (ddd, 7.8, 7.6, 0.9)
12	6.85 (d, 7.3)	6.89 (d, 7.6)
14α	2.39 (ddd, 11.4, 2.7, 2.4)	2.38 (ddd, 11.1, 2.7, 2.4)
14β	1.21 (ddd, 10.9, 10.9, 10.9)	1.21 (ddd, 11.1, 11.1, 11.1)
15	2.09 (br m)	2.10 (br m)
17	7.42 (d, 1.4)	7.43 (d, 1.3)
18 (Me)	1.08 (d, 6.4)	1.11 (d, 6.6)
19	4.36 (qd, 6.0, 3.6)	4.37 (qd, 6.6, 3.2)
20	2.09 (br m)	2.10 (br m)
21α	1.85 (dd, 10.6, 10.0)	1.85 (dd, 10.5, 10.4)
21β	3.22 (dd, 10.7, 2.4)	3.22 (dd, 10.5, 2.2)
23 (OMe)	3.58 (s)	3.58 (s)
NH	7.78 (br s)	8.40 (br s)

Table 14 ^{13}C-NMR Assignment of Alkaloid U-4 (in $CDCl_3$) and Mitraphylline (in $CDCl_3$)

Position	Alkaloid U-4	Mitraphylline (Seki *et al.*, 1993)
	δ_C (ppm)	δ_C (ppm)
2	180.91	181.33
3	74.58	74.58
5	54.27	54.28
6	35.14	35.16
7	55.51	55.56
8	133.32	133.35
9	123.02	122.89
10	122.64	122.54
11	128.01	128.00
12	109.55	109.74
13	140.65	140.87
14	28.36	28.37
15	30.44	30.39
16	106.90	106.92
17	154.08	154.05
18	14.88	14.84
19	73.85	73.81
20	40.49	40.48
21	54.37	54.33
22	167.12	167.10
23	50.74	50.71

1.5) Structure Determination of Alkaloid U-5

Alkaloid U-5 was obtained as pale yellow powder, which was identified as speciophylline that has Rf value as 0.16 (silica gel/ EtOAc), 0.26 (silica gel/ 5% MeOH in $CHCl_3$) and 0.13 [silica gel/ EtOAc: MEOH (6: 0.5)]. This alkaloid previously isolated from the leaves and stems of *Uncaria bernaysia* (Phillipson and Hemingway, 1973) and from the leaves of *U. homomalla* (Ponglux *et al*., 1977)

The UV spectrum (Figure 29) of alkaloid U-5 showed minimum absorption at 225 nm, maximum absorption at 208 and 244 nm and shoulder at 285 nm suggesting the presence of oxindole chromophore (Yeoh *et al*., 1966; Phillipson and Hemingway, 1975b; Tantivatana *et al*., 1979). The IR spectrum (Figure 30) gave absorption bands at 3202 cm^{-1} (NH) and 1706 cm^{-1} (C=O of lactam and ester functional groups). The EI mass spectrum (figure 31) gave a molecular formula ion [M^+] at m/z 368, corresponding to the molecular formula $C_{21}H_{24}N_2O_4$ (D.B.E. = 9) but do not produce M^+-1 fragment that the difference between heteroyohimbine and oxindole alkaloids. Other fragments appeared at m/z 130, 140, 145, 146 and 159 ions, exhibited oxindole moiety. The relative intensities of M^+ at m/z 368 of greater intensity than ion at m/z 223, that exhibited *allo* and *epiallo* isomers of oxindole alkaloids (Phillipson and Hemingway, 1975b).

The ^1H and ^{13}C chemical shifts from NMR spectrum of alkaloid U-5 (figures 32-33 and Tables 15-16) were compared with those of alkaloid speciophylline which were reported in 1993 by Seki and co-workers. The chemical shifts of alkaloid U-5 were similar to those reported in this literature. The coupling constants observed for the protons on the D ring, 3-H (δ = 2.15), 14-H (14α; δ = 1.59, 14β; δ = 0.82), 15-H (δ = 2.19), 20-H (δ = 2.06) and 21-H (21α; δ = 3.09, 21β; δ = 2.06) suggesting the boat conformation of the D ring in the *epiallo*-type oxindole alkaloids.

Table 15 ^{1}H-NMR Assignment of Alkaloid U-5 (in $CDCl_3$) and Speciophylline (in $CDCl_3$)

position	Alkaloid U-5	Speciophylline (Seki *et al*., 1993)
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_H (ppm) (multiplicity, *J* in Hz)
3	2.15 (br dd, 12.2, 2.0)	2.16 (br dd, 12.3, 2.0)
5α	3.36 (br dd, 6.8, 6.8)	3.38 (br dd, 7.1, 7.1)
5β	2.42 (m)	2.44 (m)
6α	2.42 (m)	2.42 (m)
6β	2.02 (m)	2.02 (m)
9	7.13 (d, 7.4)	7.12 (d, 7.3)
10	6.99 (ddd, 7.4, 7.4, 1.0)	7.17 (ddd, 7.6, 7.6, 1.2)
11	7.16 (ddd, 7.7, 7.7, 1.3)	7.17 (ddd, 7.6, 7.6, 1.2)
12	6.86 (d, 7.7)	6.90 (d, 7.6)
14α	1.59 (ddd, 12.2, 12.2, 4.6)	1.61 (ddd, 12.3, 12.3, 4.6)
14β	2.19 (ddd, 12.2, 2.3, 2.3)	2.20 (ddd, 12.3, 2.5, 2.5)
15	2.82 (br m)	2.85 (br m)
17	7.36 (d, 1.9)	7.37 (d, 1.9)
18 (Me)	1.24 (d, 6.6)	1.24 (d, 6.6)
19	4.18 (br q, 6.6, 1.0)	4.19 (br q, 6.6, 1.0)
20	2.06 (m)	2.09 (m)
21α	3.09 (dd, 13.2, 6.6)	3.10 (dd, 13.2, 6.6)
21β	2.06 (m)	2.09 (m)
23 (OMe)	3.36 (s)	3.36 (s)
NH	8.06 (br s)	8.79 (br s)

Table 16 ^{13}C-NMR Assignment of Alkaloid U-5 (in $CDCl_3$) and Speciophylline (in $CDCl_3$)

Position	Alkaloid U-5	Speciophylline (Seki *et al.*, 1993)
	δ_C (ppm)	δ_C (ppm)
2	181.27	181.63
3	70.55	70.45
5	55.01	54.90
6	34.20	34.06
7	55.75	55.71
8	133.30	133.24
9	122.88	122.67
10	122.50	122.30
11	127.92	127.79
12	109.49	109.61
13	140.78	140.93
14	26.35	26.22
15	25.13	25.01
16	104.96	104.86
17	153.75	153.65
18	18.85	18.74
19	74.86	74.77
20	36.49	36.38
21	53.45	53.35
22	167.55	167.46
23	50.69	50.57

CHAPTER V

Conclusion

In this investigation, five alkaloids were isolated from the stems of *Uncaria homomalla* Miq. All of them are the indole alkaloids, one of which being heteroyohimbine namely tetrahydroalstonine. The other four are oxindoles being identified as isopteropodine, isomitraphylline, mitraphylline and speciophylline.

This is the first isolation of tetrahydroalstonine from this plant. The other alkaloids have never been isolated from the stems of this species, except isopeteropodine.

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APPENDIX

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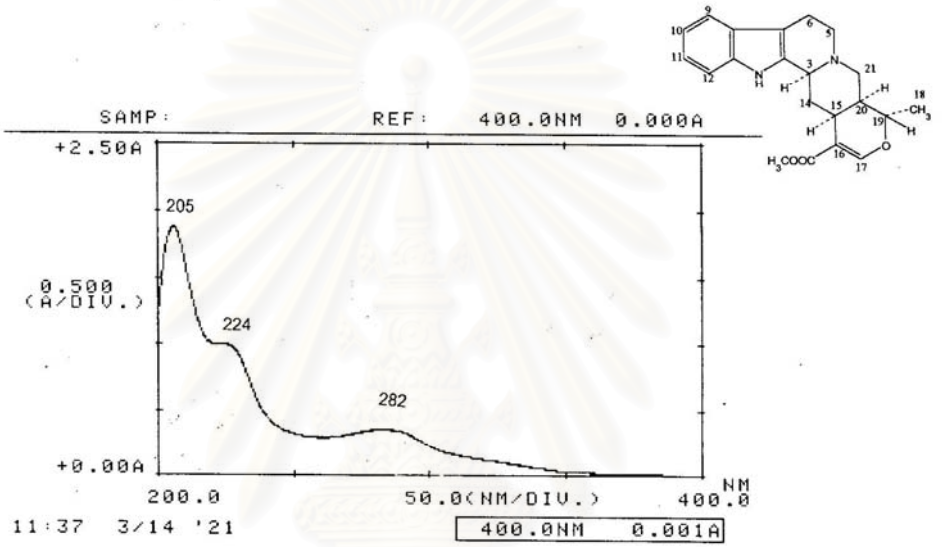

Figure 3 UV spectrunm of alkaloid U-1 (in ethanol)

Figure 3 UV spectrum of alkaloid U-1 (in ethanol)

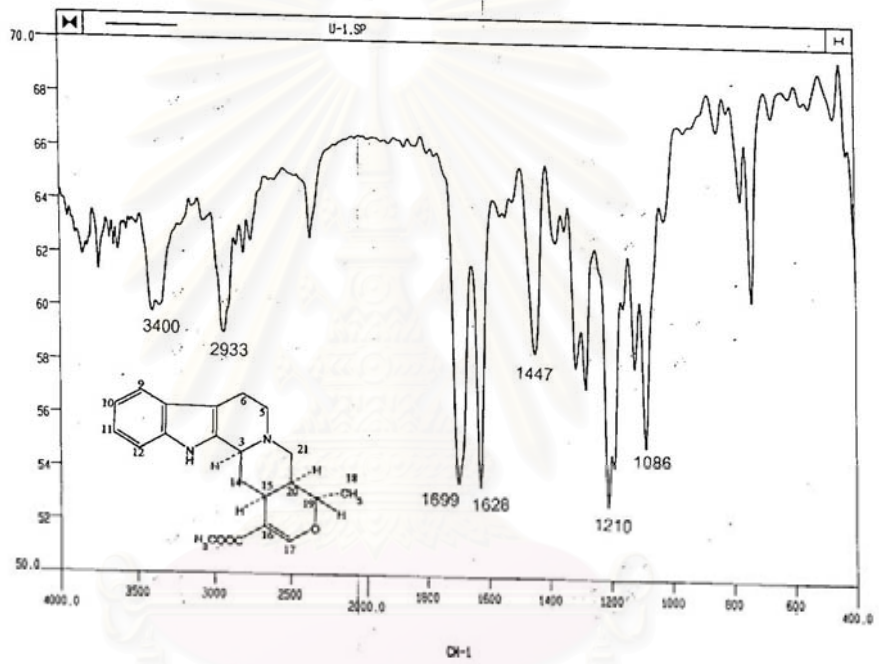

Figure 4 IR spectrum of alkaloid U-1 (KBr disc)

Figure 4 IR spectrum of alkaloid U-1 (KBr disc)

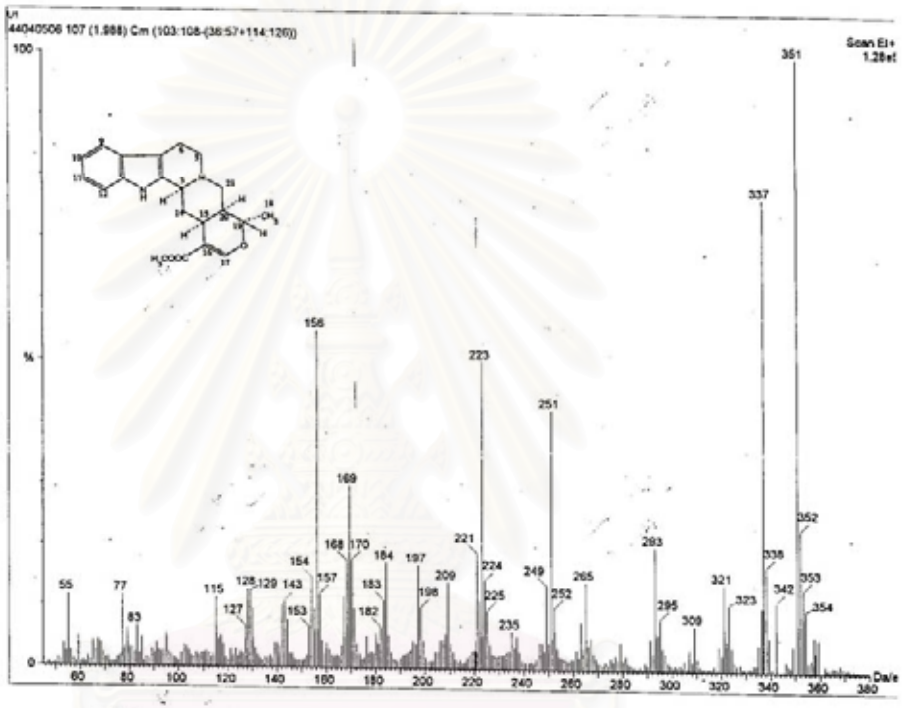

Figure 5 EI mass spectrum of alkaloid U-1

Figure 5 EI mass spectrum of alkaloid U-1

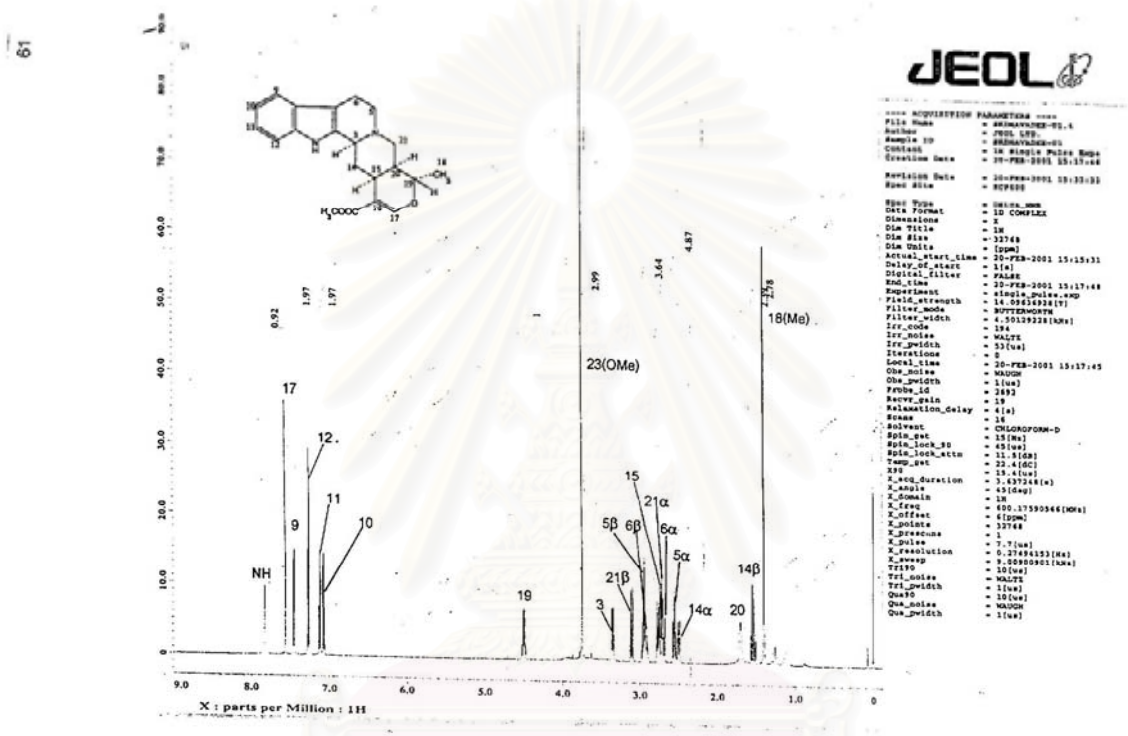

Figure 6 500 MHz ^{1}H NMR spectrum of alkaloid U-1 (in $CDCl_3$)

Figure 6 600 MHz ^{1}H NMR spectrum of alkaloid U-1 (in $CDCl_3$)

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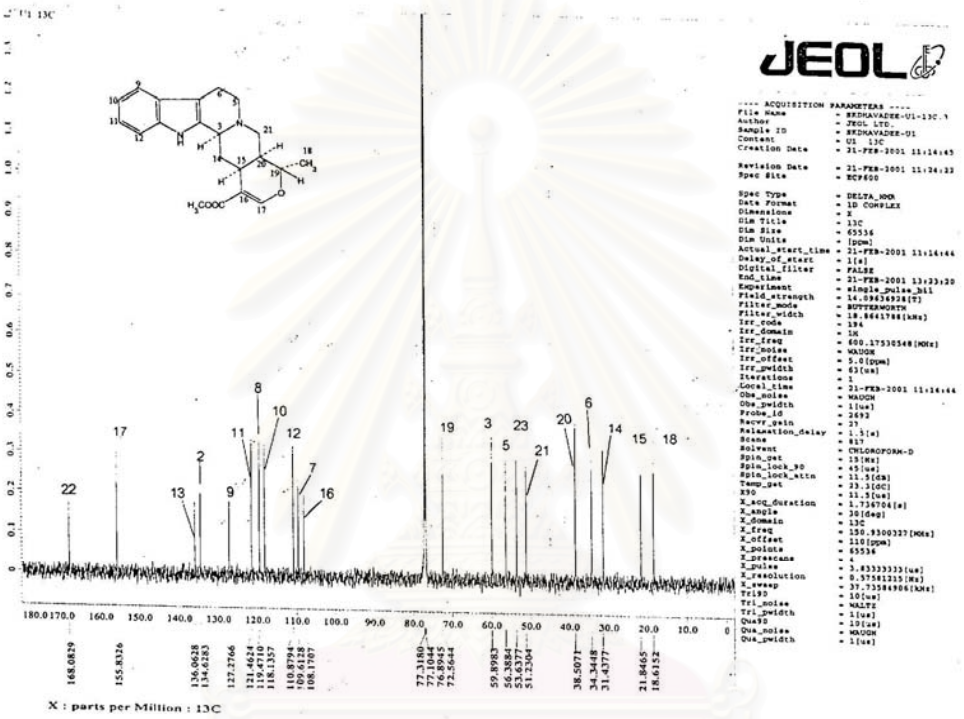

Figure 7 150 MHz ^{13}C NMR spectrum of alkaloid U-1 (in $CDCl_3$)

Figure 7 150 MHz ^{13}C NMR spectrum of alkaloid U-1 (in $CDCl_3$)

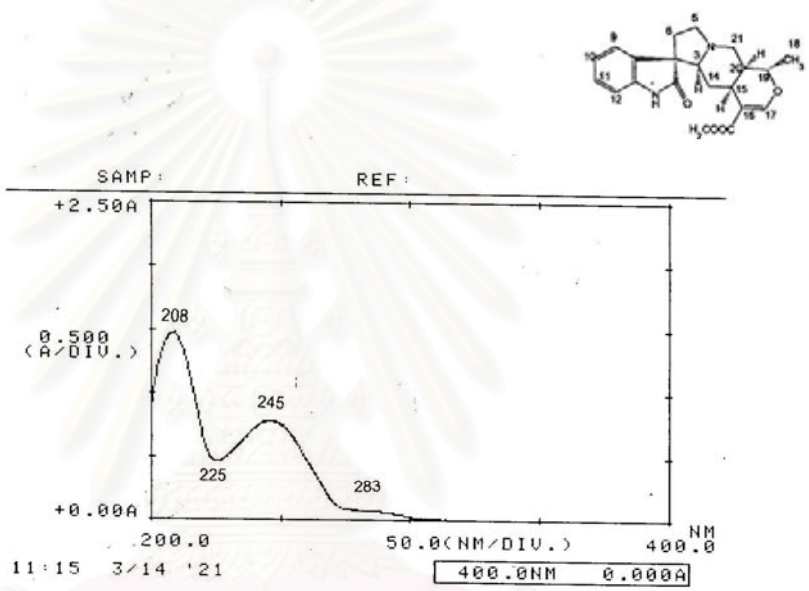

Figure 8 UV spectrunm of alkaloid U-2 (in ethanol)

Figure 8 UV spectrum of alkaloid U-2 (in ethanol)

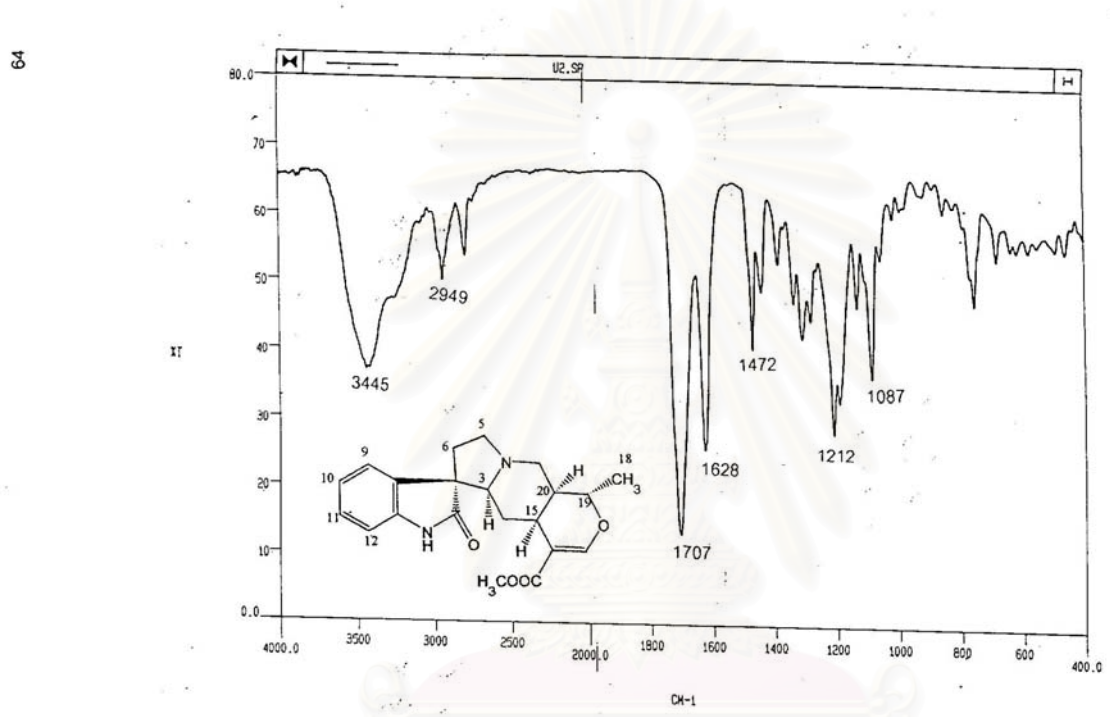

Figure 9 IR spectrum of alkaloid U-2 (KBr disc)

Figure 9 IR spectrum of alkaloid U-2 (KBr disc)

65

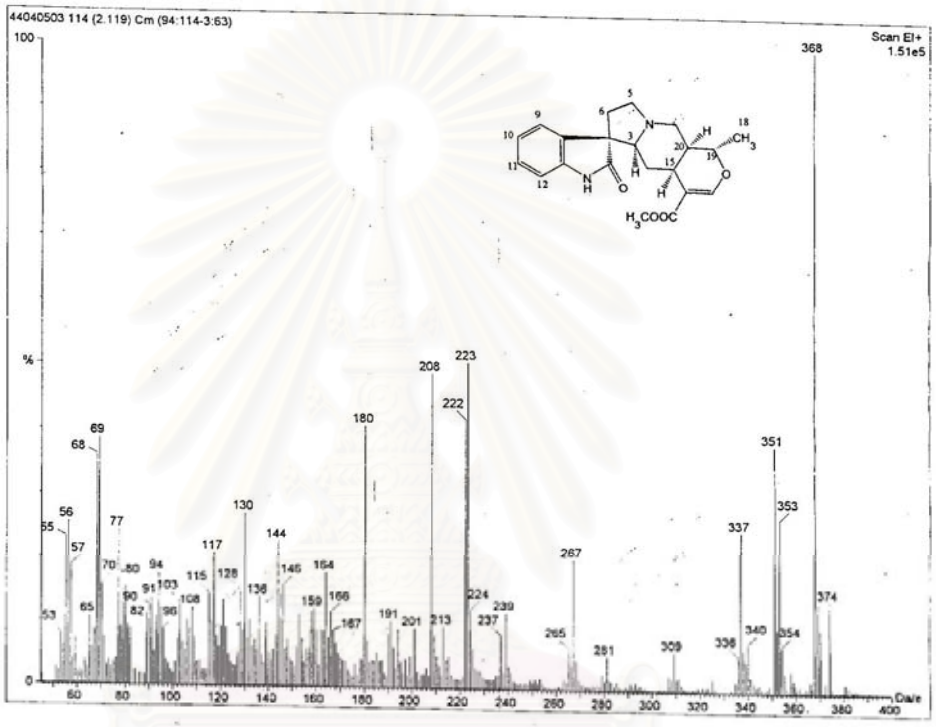

Figure 10 EI mass spectrum of alkaloid U-2

Figure 10 EI mass spectrum of alkaloid U-2

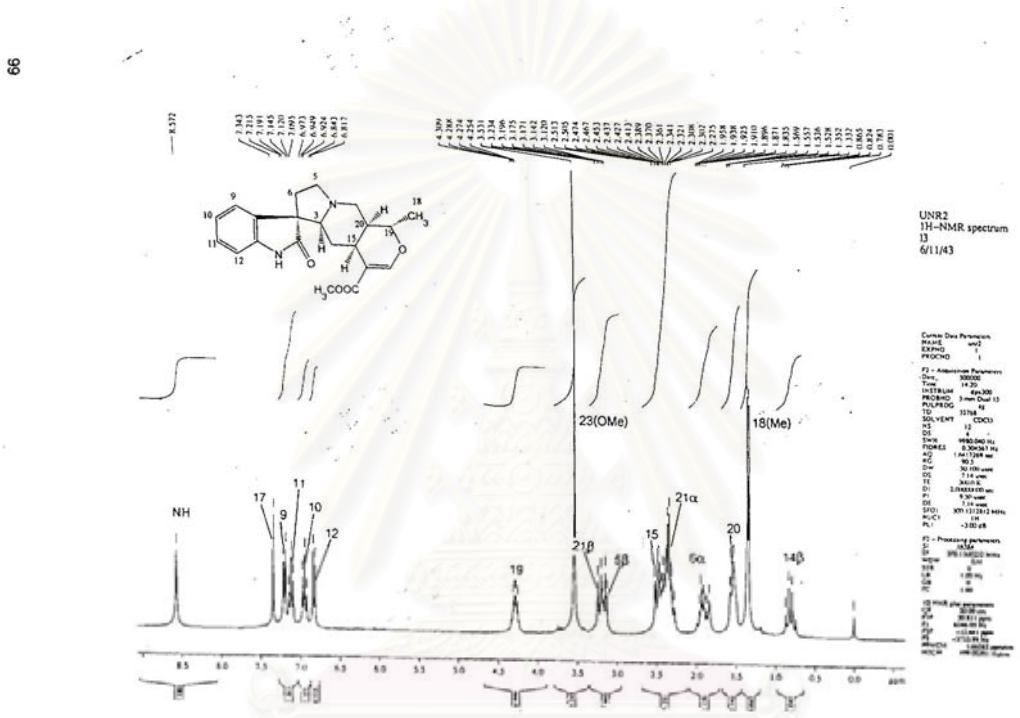

Figure 11 300 MHz ^{1}H NMR spectrum of alkaloid U-2 (in $CDCl_3$)

Figure 11 300 MHz ^{1}H NMR spectrum of alkaloid U-2 (in $CDCl_3$)

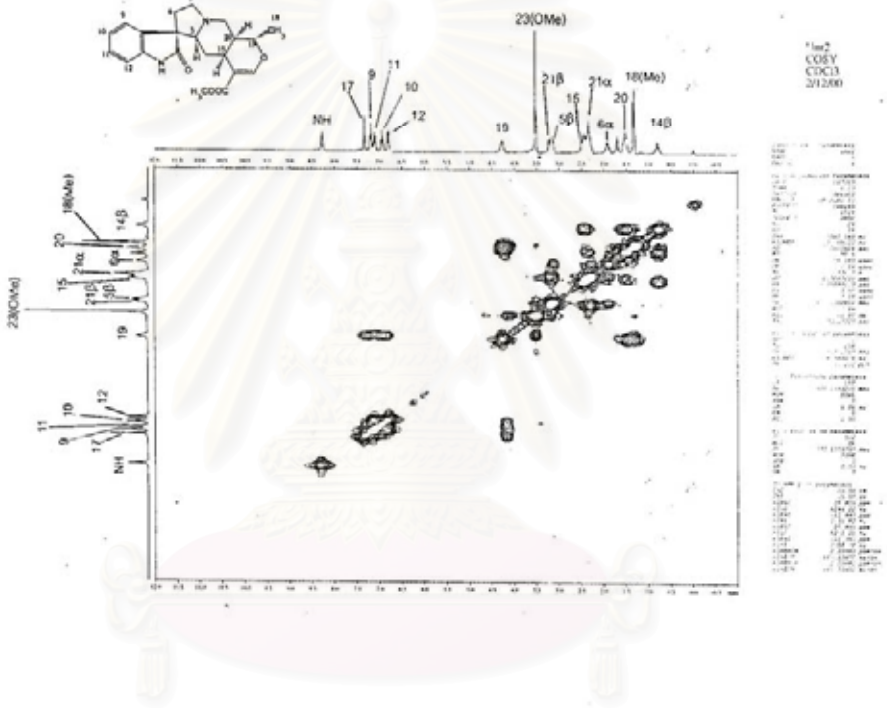

Figure 12 COSY spectrum of alkaloid U-2 (in $CDCl_3$)

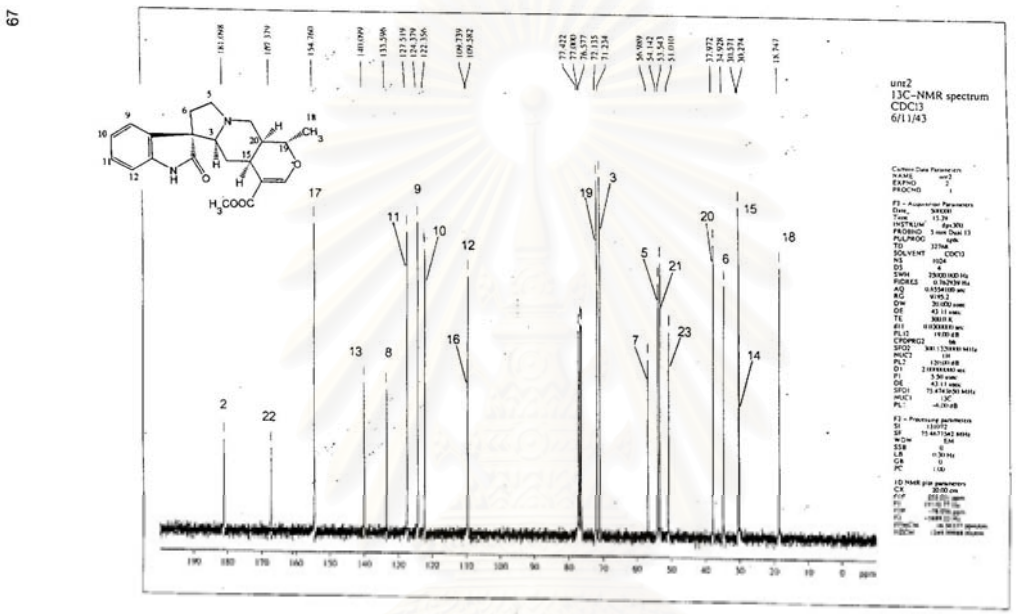

Figure 12 75 MHz ^{13}C NMR spectrum of alkaloid U-2 (in $CDCl_3$)

Figure 13 75 MHz ^{13}C NMR spectrum of alkaloid U-2 (in $CDCl_3$)

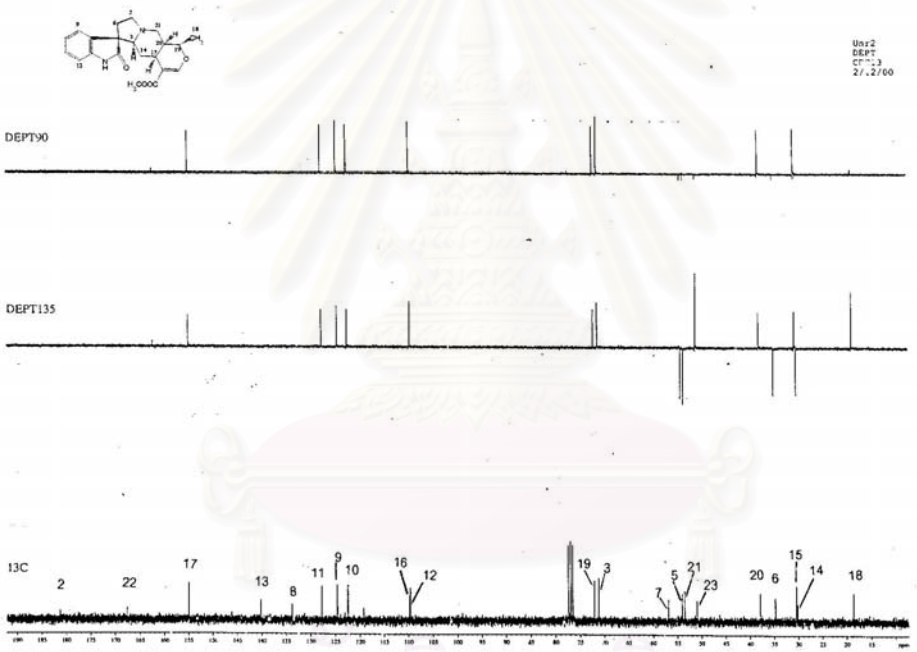

Figure 14 DEPT spectrum of alkaloid U-2 (in $CDCl_3$)

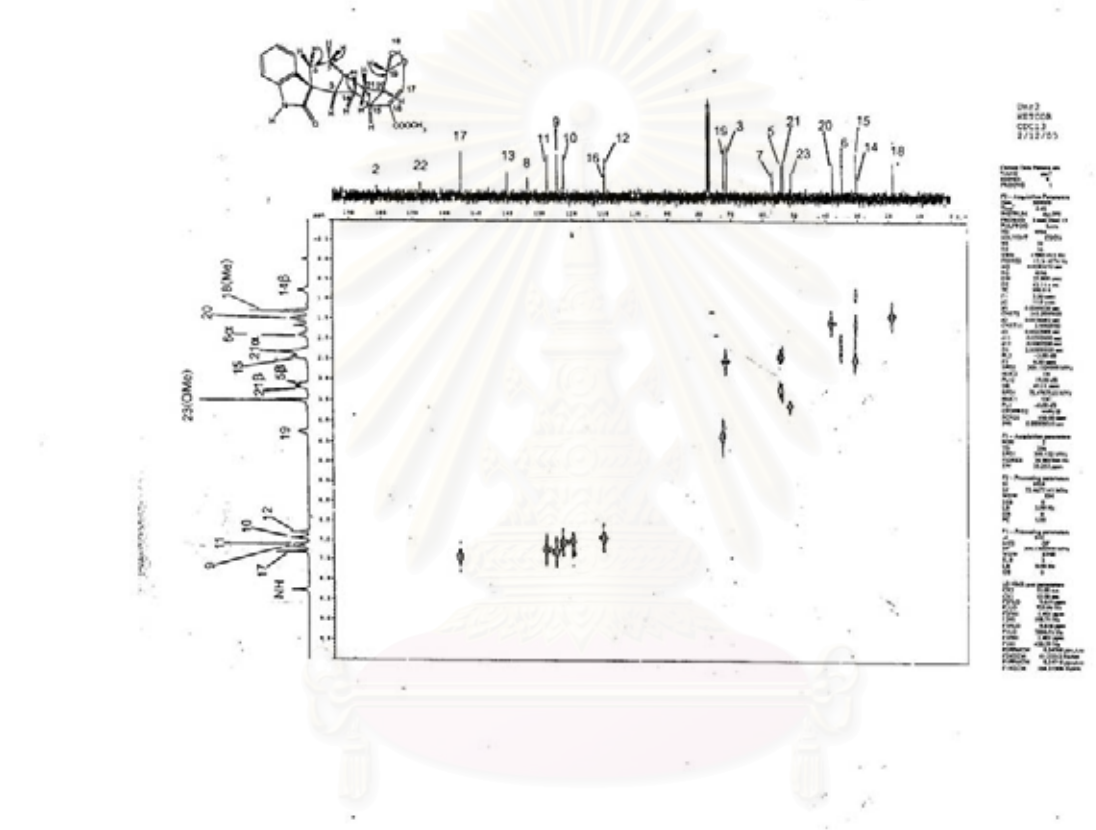

Figure 15 HETCOR spectrum of alkaloid U-2 (in $CDCl_3$)

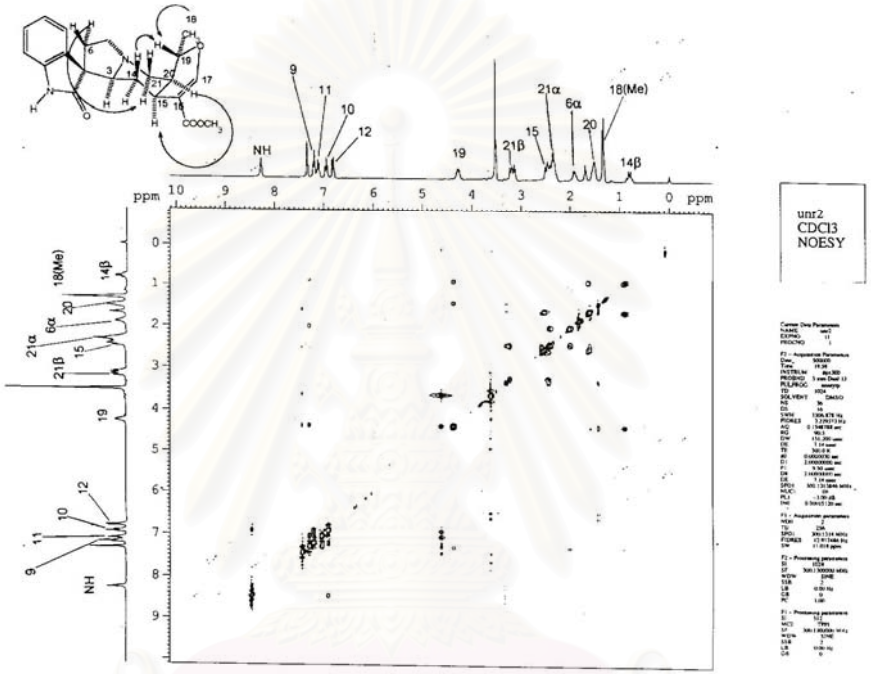

Figure 13 NOESY spectrum of alkaloid U-2 (in $CDCl_3$)

Figure 16 NOESY spectrum of alkaloid U-2 (in $CDCl_3$)

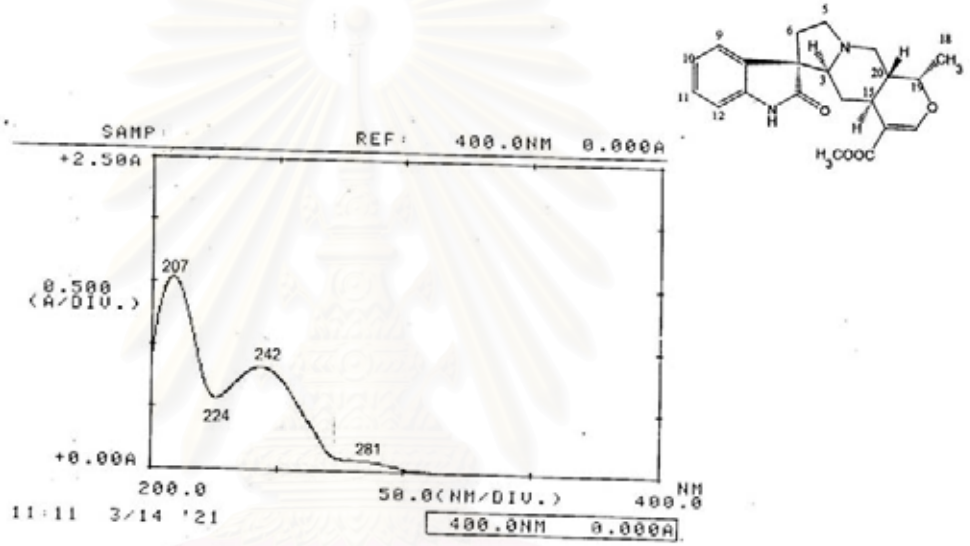

Figure 14 UV spectrum of alkaloid U-3 (in ethanol)

Figure 17 UV spectrum of alkaloid U-3 (in ethanol)

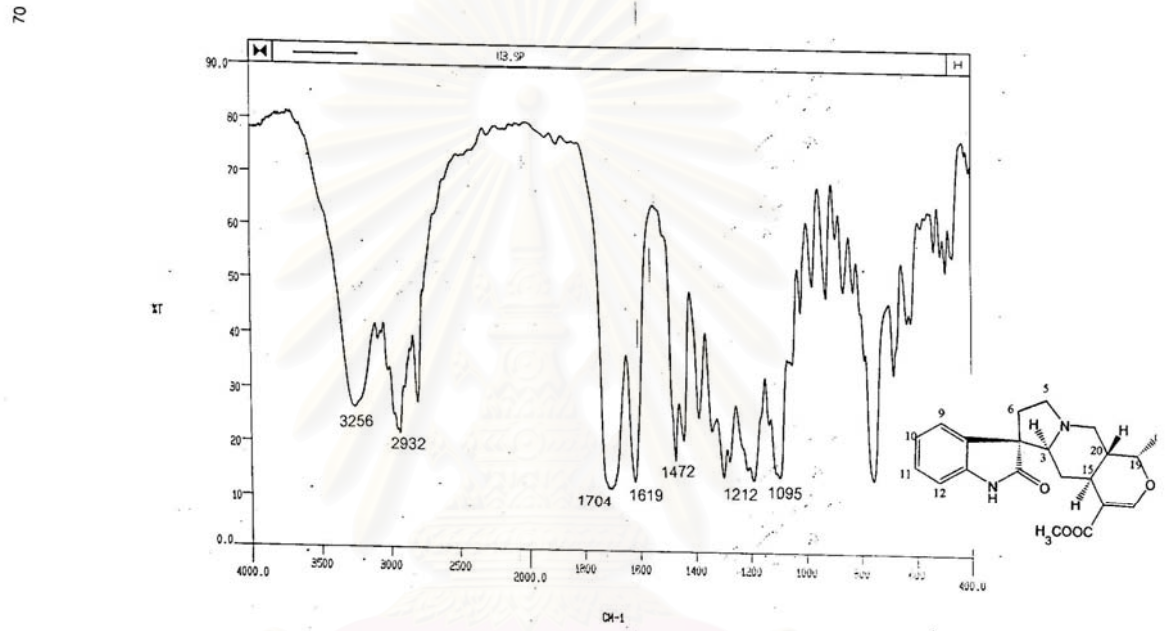

Figure 15 IR spectrum of alkaloid U-3 (KBr disc)

Figure 18 IR spectrum of alkaloid U-3 (KBr disc)

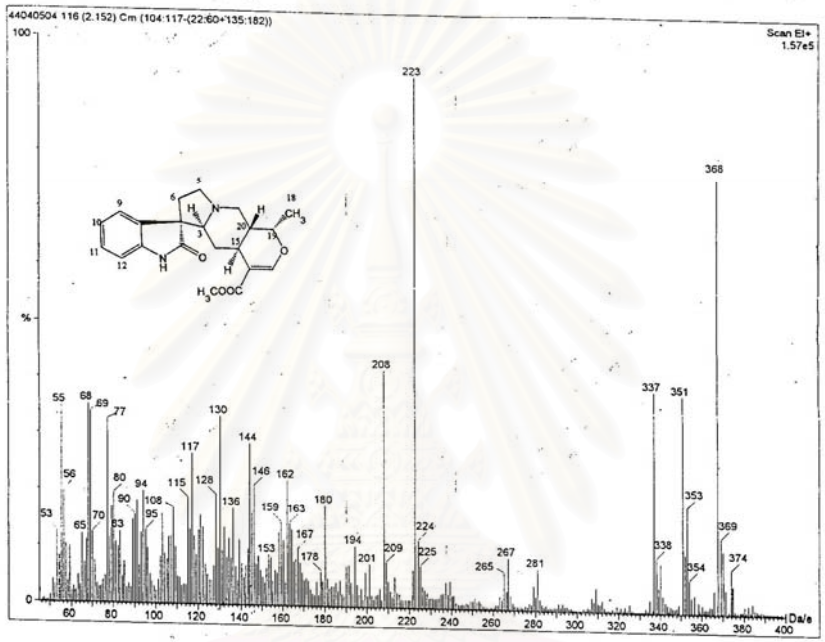

Figure 16 EI mass spectrum of alkaloid U-3

Figure 19 EI mass spectrum of alkaloid U-3

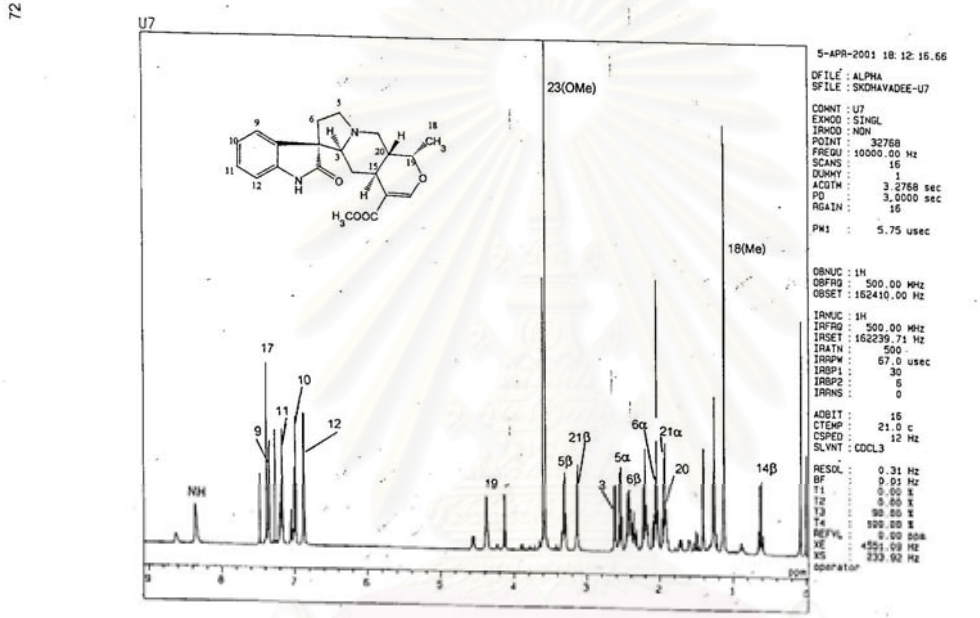

Figure 17 500 MHz ^{1}H NMR spectrum of alkaloid U-3 (in $CDCl_3$)

Figure 20 500 MHz ^{1}H NMR spectrum of alkaloid U-3 (in $CDCl_3$)

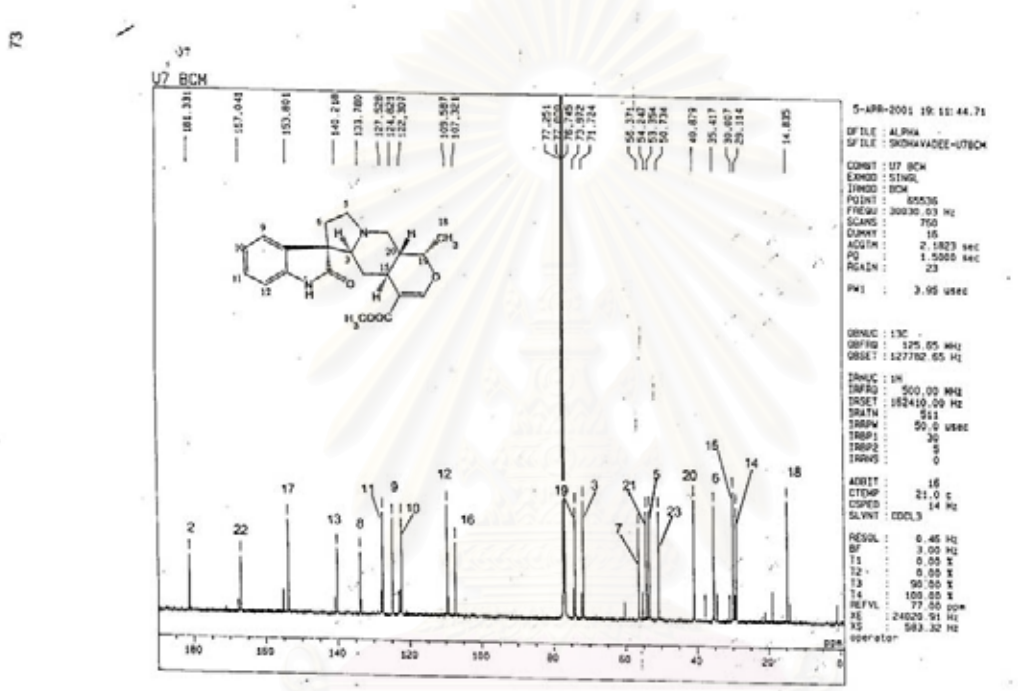

Figure 18 150 MHz ^{13}C NMR spectrum of alkaloid U-3 (in $CDCl_3$)

Figure 21 125 MHz ^{13}C NMR spectrum of alkaloid U-3 (in $CDCl_3$)

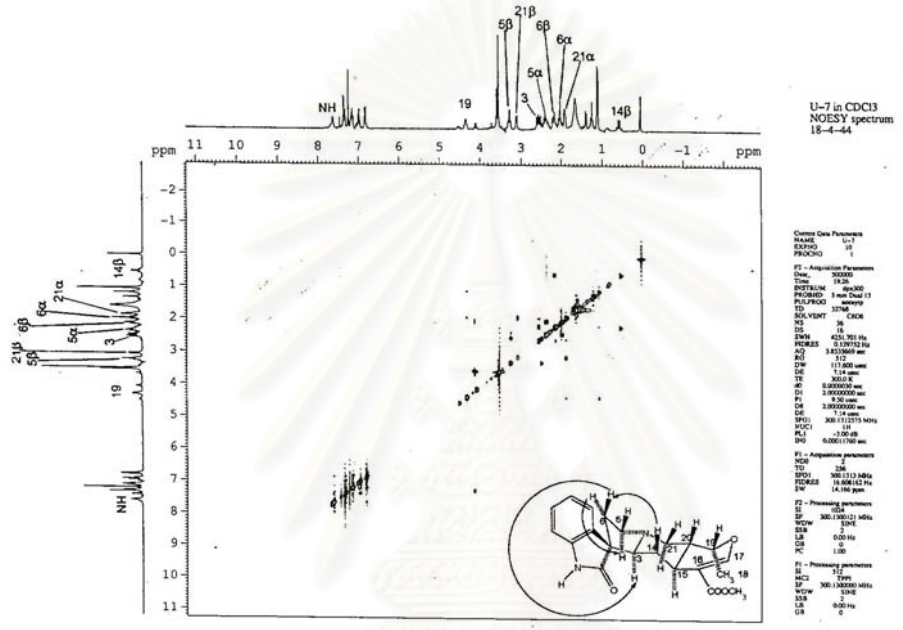

Figure 19 NOESY spectrum of alkaloid U-3 (in $CDCl_3$)

Figure 22 NOESY spectrum of alkaloid U-3 (in $CDCl_3$)

75

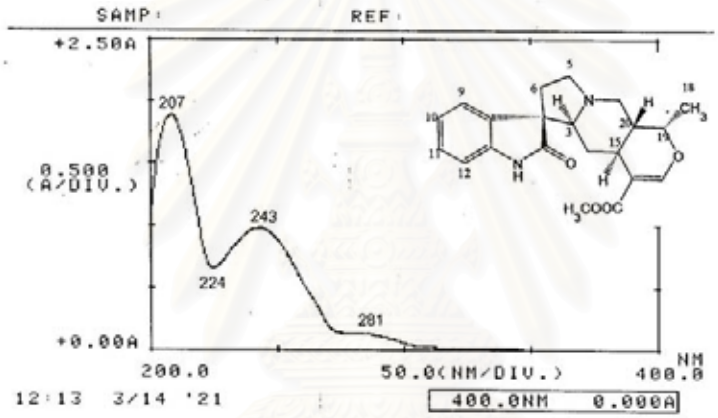

Figure 20 UV spectrum of alkaloid U-4 (in ethanol)

Figure 23 UV spectrum of alkaloid U-4 (in ethanol)

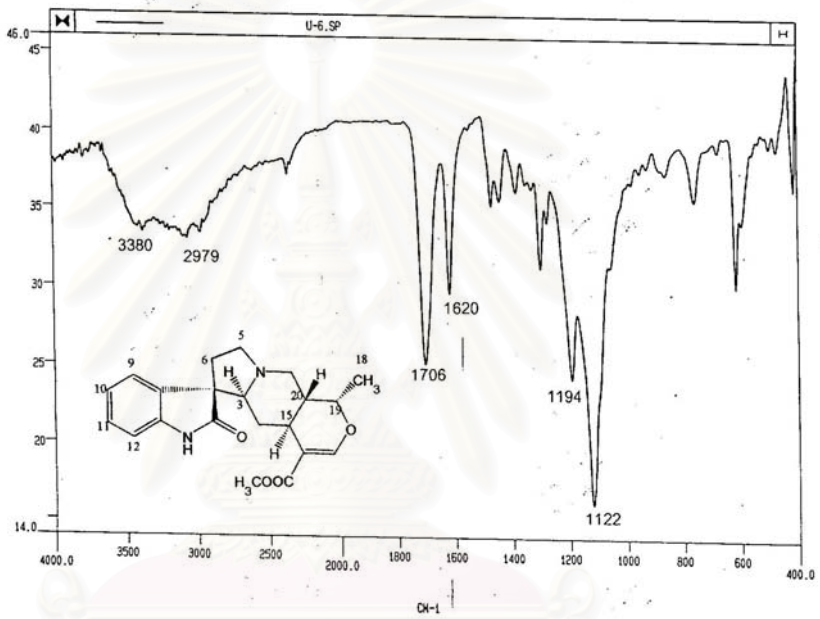

Figure 21 IR spectrum of alkaloid U-4 (KBr disc)

Figure 24 IR spectrum of alkaloid U-4 (KBr disc)

77

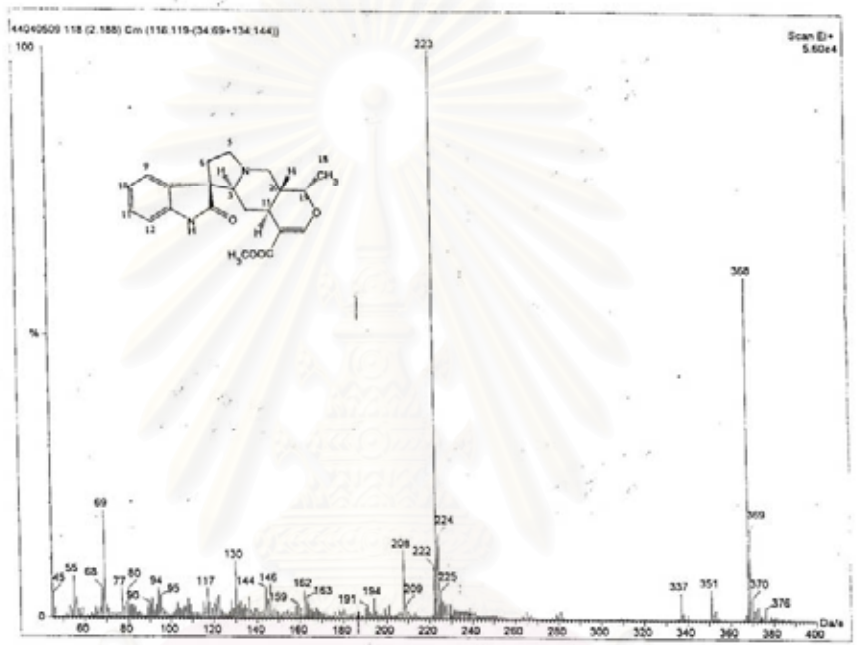

Figure 22 EI mass spectrum of alkaloid U-4

Figure 25 EI mass spectrum of alkaloid U-4

78

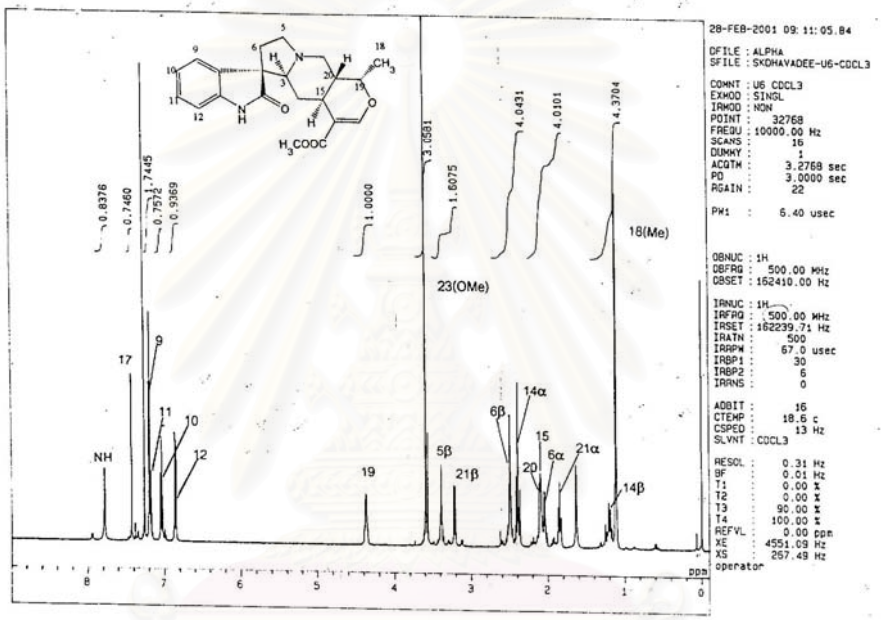

Figure 23 500 MHz ^{1}H NMR spectrum of alkaloid U-4 (in $CDCl_3$)

Figure 26 500 MHz ^{1}H NMR spectrum of alkaloid U-4 (in $CDCl_3$)

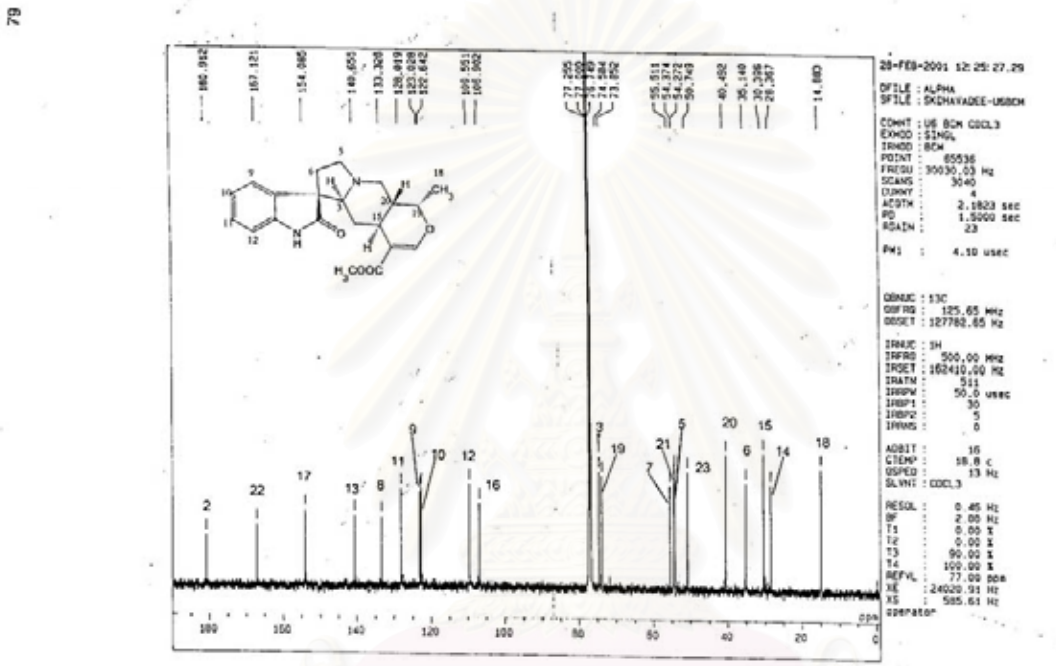

Figure 24 150 MHz ^{13}C NMR spectrum of alkaloid U-4 (in $CDCl_3$)

Figure 27 125 MHz ^{13}C NMR spectrum of alkaloid U-4 (in $CDCl_3$)

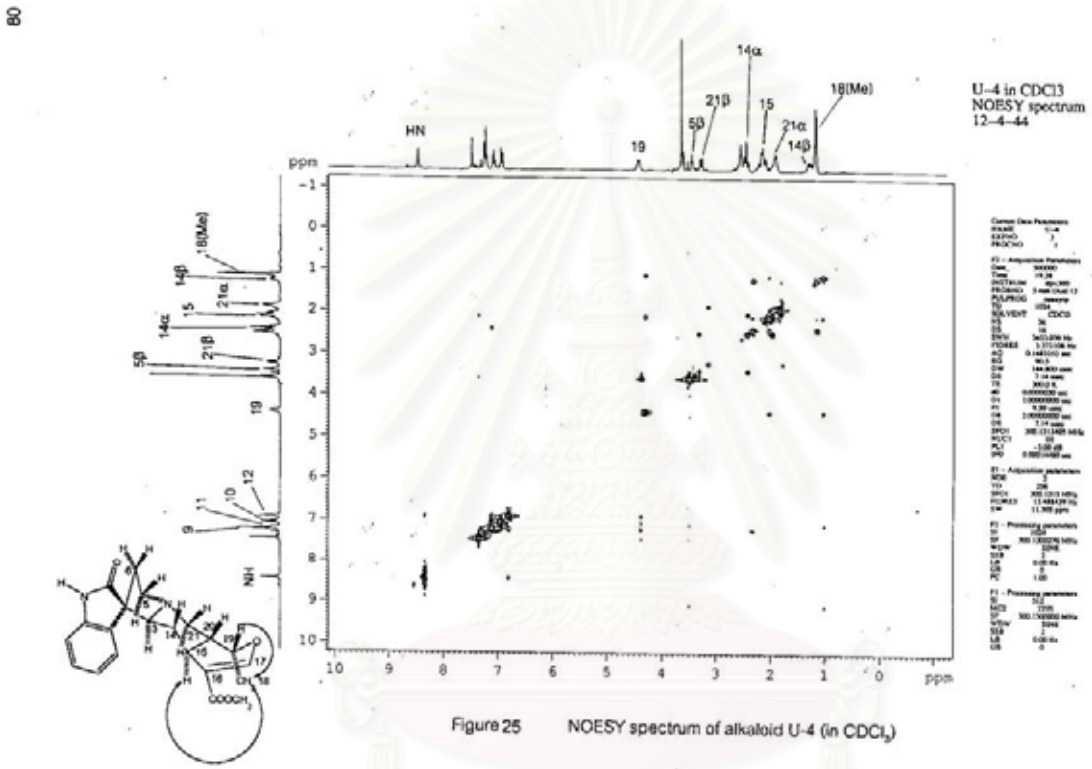

Figure 25 NOESY spectrum of alkaloid U-4 (in $CDCl_3$)

Figure 28 NOESY spectrum of alkaloid U-4 (in $CDCl_3$)

81

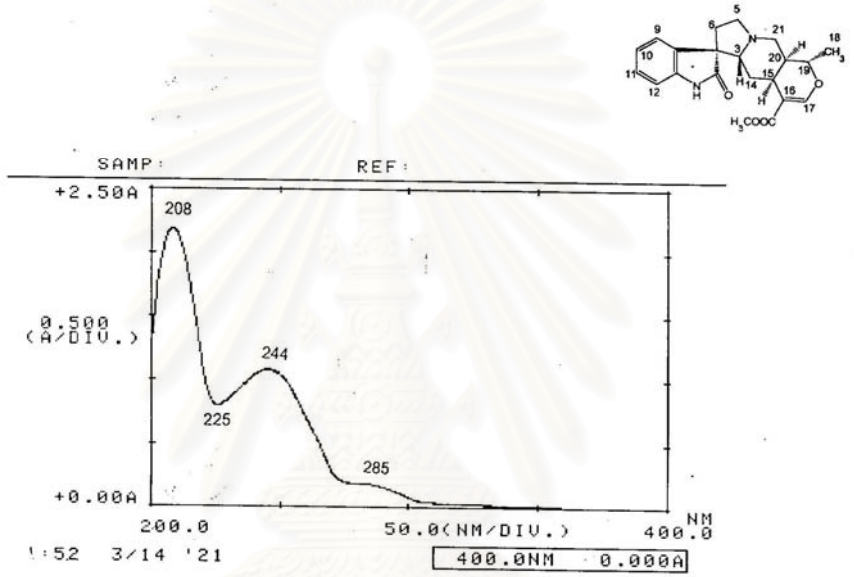

Figure 26 UV spectrum of alkaloid U-5 (in ethanol)

Figure 29 UV spectrum of alkaloid U-5 (in ethanol)

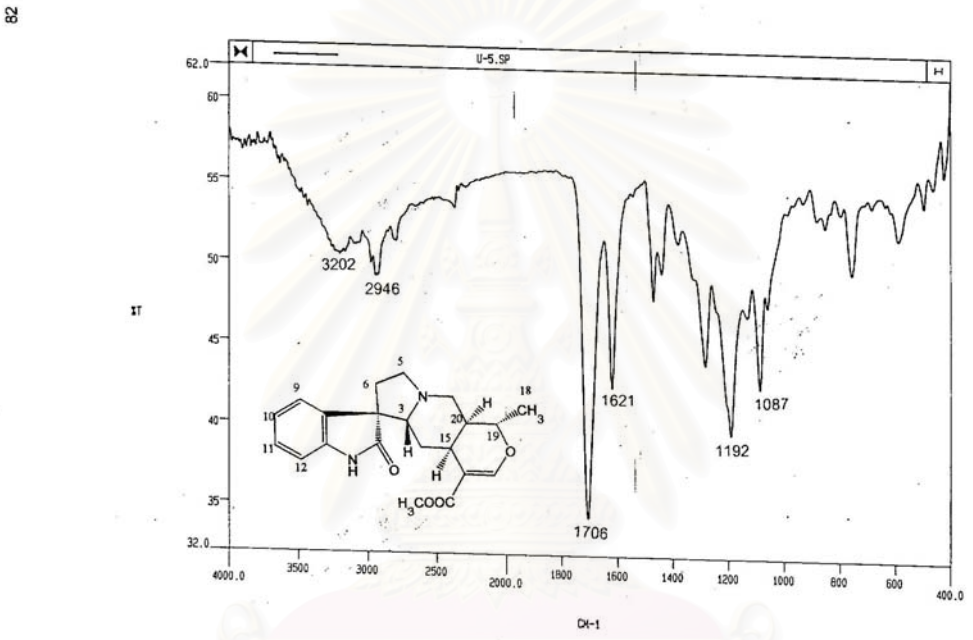

Figure 27 IR spectrum of alkaloid U-5 (KBr disc)

Figure 30 IR spectrum of alkaloid U-5 (KBr disc)

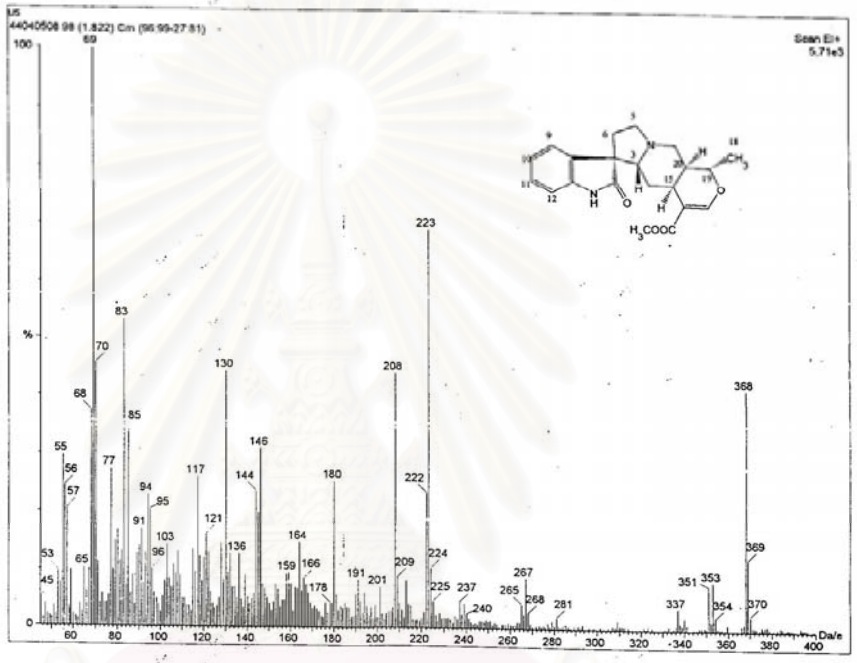

Figure 28 EI mass spectrum of alkaloid U-5

Figure 31 EI mass spectrum of alkaloid U-5

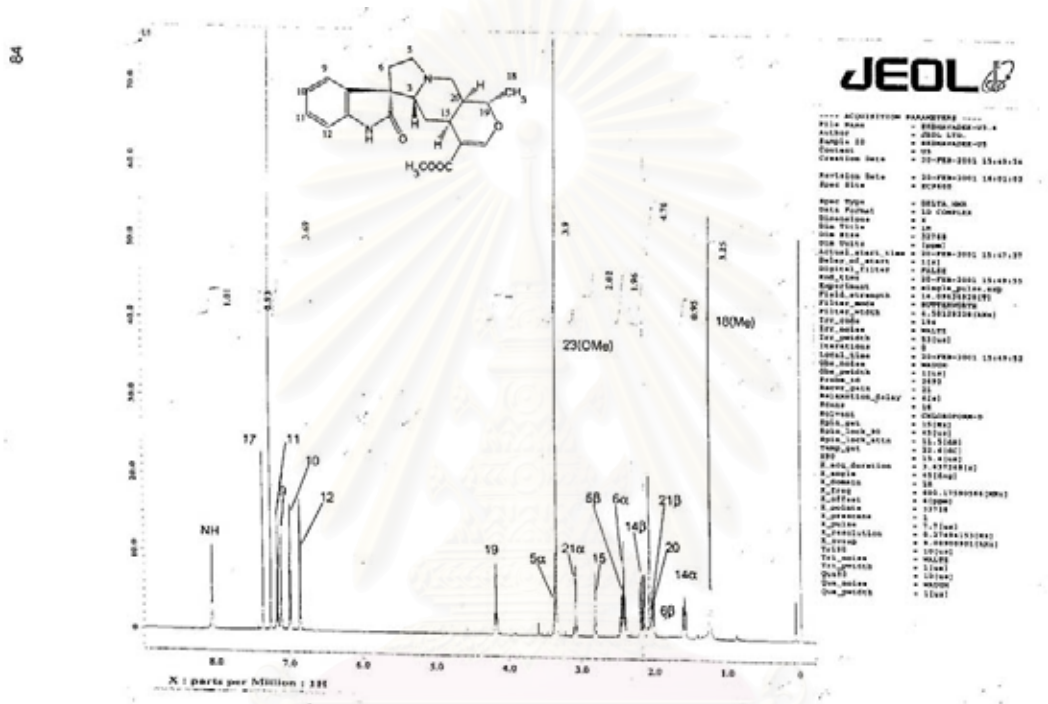

Figure 29 500 MHz ^{1}H NMR spectrum of alkaloid U-5 (in $CDCl_3$)

Figure 32 600 MHz ^{1}H NMR spectrum of alkaloid U-5 (in $CDCl_3$)

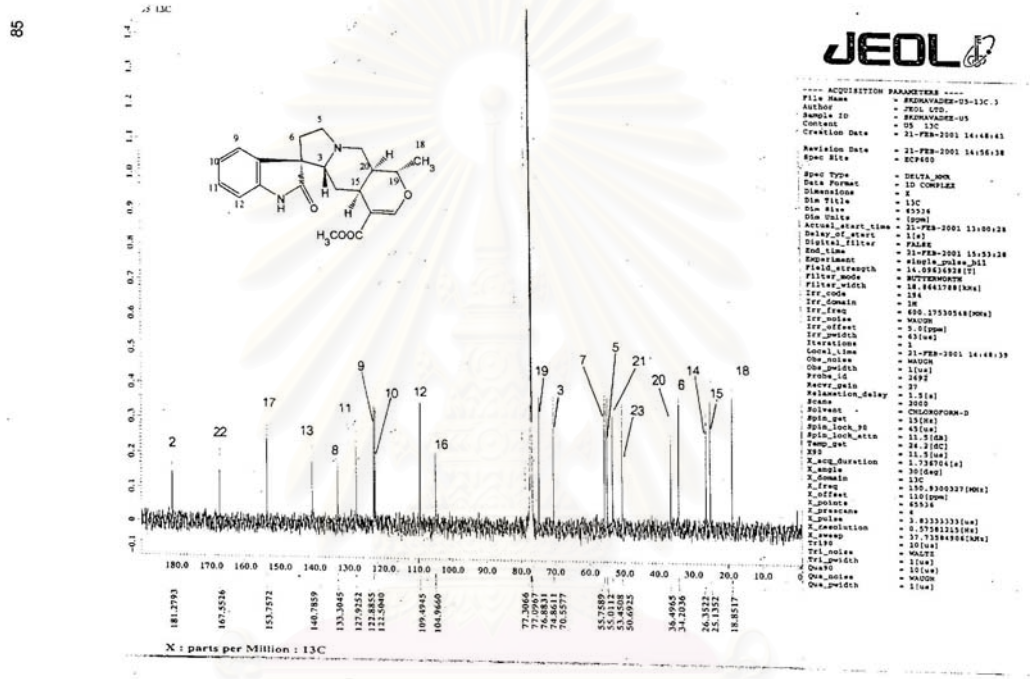

Figure 30 150 MHz ^{13}C NMR spectrum of alkaloid U-5 (in $CDCl_3$)

Figure 33 150 MHz ^{13}C NMR spectrum of alkaloid U-5 (in $CDCl_3$)

VITA

Miss Kusuma Pasugdee was born on August 22, 1975 in Saraburi, Thailand. She received her Bachelor's degree of Science in Pharmacy in 1999 from the Faculty of Pharmaceutical Sciences, Huachiew Chalermprakiet University, Thailand.