CHAPTER I

INTRODUCTION

Strychnos genus consists of climbing shrubs or trees. The large genus *Strychnos* (about 185-190 species) is distributed throughout the tropics and subtropics, the greater part of the species occurring in Africa (75 species), and Central and South America (65-70 species), and about 45 species in Asia including Australia and Polynesia.[1] *Strychnos* species have several ethnobotanical uses. A few species are well known for their incorporation into arrow and ordeal poison, but play more a role in ethnomedicine against fever, rheumatism, worms, ulcers, leprosy, snake-bites, and so forth.[2] *Strychnos* alkaloids are in fact an example of molecular and pharmacological biodiversity. More than 300 different *Strychnos* alkaloids have been isolated to date and they present various biological activities in several fields: parasitology, cancer, neurology, inflammation, and so on.[3]

The *Strychnos* genus (about 45 species) is distributed in Australia and Asia. Many species are known in Thailand. There are various Thai names for each species[4,5], as shown below;

- *Strychnos nux-blanda* A.W. Hill

  Klo-Wu-Sae, Klo-Ue, Kla Ue (Karen Mae Hong Son); Khi Ka (Northeastern); Tumka Khao (Central); Plu-Wiat (Khmer); Mating, Mating Ton, Mating Mak (Northern)

- *Strychnos axillaris* Colebr.

  Khwak Kai, Nam Khem (Eastern); Tueng Khruea Dam Tua Mae (Northern); Kho Bet, Ben, Ben Kho (Northeastern); Mak Ta Kai (Loei); Lep Khrut (Southeastern); Khieo Ngnu, Lep Rok (Peninsular)

- *Strychnos vanprukii* Craib

  Thao Chang (Northern)

- *Strychnos minor* Dennst.

  Tum Ka Khao, Tum Ka Daeng (Lampang)

- *Strychnos ignatii* Berg

  Phaya Mue Lek (Krabi)

- *Strychnos rupicola* Pierre ex Dop.

  Khika Khruea (Prachin Buri)

- *Strychnos lucida* R. Br.

  Phaya Mue Lek, Phaya Mun Lek (Central); Ya Mue Lek (Peninsular); Sieo Duk (Northern)

- *Strychnos nux-vomica* L.

  Krachi, Ka Kling, Tumka Daeng, Salaeng Chai (Central); Salaeng Thom, Salaeng Buea (Eastern); Saeng Buea (Ubon Ratchathani)

- *Strychnos nitida* G. Don

  San Di Lok (Northern)

- *Strychnos thorelii* Pierre ex Dop

  Khieo Ngu, Lum Nok (Chumphon); Chong La A (Chanthaburi); Thao-Sa Em, Sa Eng (Trat)

- *Strychnos kerrii* A. W. Hill

  Kluai Khiao (Nakorn Ratchasima)

1.1 *Strychnos nux-blanda*

1.1.1 Botany of *Strychnos nux-blanda*

Scientific name : *Strychnos nux-blanda* A.W. Hill

Family : Loganiaceae (Strychnaceae)

Morphology[4]

Tree to 15 m; twigs yellowish grey without lenticels; glabrous, ± terete; axillary spines sometimes present; tendrils absent. *Leaves*: petiole 5-17 mm, glabrous or sparsely tomentose; lamina elliptic, broadly ovate, orbicular, 4.9-15 by 2.3-12 cm; base acute to rounded to subcordate, apex blunt to acuminate, often mucronulate, thinly chartaceous, glabrous or sometimes spasely tomentose along the lower nerves; 3-5 plinerved, midrib slightly sunken to flat above, rounded below. *Inflorescence* 3-5.5 cm, terminal or on ends of short side-branches, fairly lax to dense, tomentose, many flowered; peduncle 1-3 cm, pedicels < 2.5 mm. *Calyx* 1.5-2.2 mm, sepals narrowly ovate to lanceolate, outside sparsely tomentose to glabrous, inside glabrous. *Corolla* 9.4-13.6 mm, green to white; tube ca 3-times as long as lobes; outside glabrous to finely papillose; inside sparsely woolly in lower part of tube; lobes densely papillose; lobes thickened at the tip. *Stamens* sessile; anthers 1.5-2 mm, oblong, glabrous, minutely apiculate or not. *Ovary* and *style* 8.1-13 mm, glabrous, stigma capitate, papillose. *Fruit* 5-8 cm diam., red to orange when ripe, globose,

thick-walled, scabrous, glabrous, with 4-15 seeds. *Seeds* 1.5-2.2 cm, 5-15 mm thick, irregularly ellipsoid, surface sericeous. **(Fig. 1)**

Vernacular Names[4]

Burma (Myanmar) : Khabaung

Thailand : Klo-Wu-Sae, Klo-Ue, Kla Ue (Karen Mae Hong-Son); Khi Ka (Northeastern); Tumka Khao (Central); Plu-Wiat (Khmer); Mating, Mating Ton, Mating Mak (Northern)

Distribution[4]

The *Strychnos nux-blanda* is probably from India, Burma (Myanmar), Thailand, Cambodia, Laos and Vietnam. In Thailand, it is distributed in the north: Chiang Mai; east: Khon Kaen, and south-western: Kanchanaburi.

Ecology[4]

The tree is chiefly in the dipterocarp or mixed deciduous forests and dry savanna.

Figure 1 *Strychnos nux-blanda* (Chayamarit, K. and Phuphatanapong, L., *Medicinal Plants of Thailand (part seven: Strychnaceae* (*Loganicaeae*).[6]

1.1.2 Chemical Components of *Strychnos nux-blanda*

There has been only a single report by Bisset, N.G.[7], in 1971, about the isolation of the alkaloid components in the leaves and seeds of *Strychnos nux-blanda*, as shown below;

seeds : strychnine, brucine, strychnine *N*-oxide, brucine *N*-oxide

leaves : icajine, vomicine, novacine

1.1.3 Medicinal properties of *Strychnos nux-blanda*[6,8]

This plant, *Strychnos nux-blanda* has been recognized as medicinal plant whose parts have been used as components in traditional medicine of various purposes;

roots : antimalarial, cathartic and external use as anti-inflammatory for snake bite

wood : relief of muscular pain and fevers

leaves : external use as anti-inflammatory for swelling

stem : topically apply for sprains and antiphlogistic for snake bite

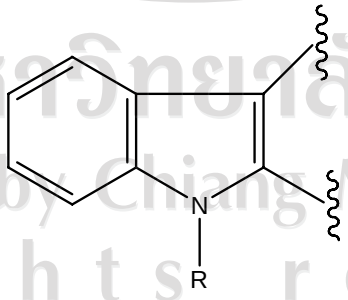
bark : in high doses, used as a poison which stimulates a central nervous system that can cause violent muscular convulsions

1.2 Review of Chemical Constituents of *Strychnos* genus

Strychnos plants are well-known as rich sources of various bioactive indole alkaloids.[9] The Asian species are sources of strychnine and brucine, whereas the South American species are better known as the sources of certain types of curare, where the active constituents are dimeric *Strychnos* alkaloids.[10]

1.2.1 Indole Alkaloids

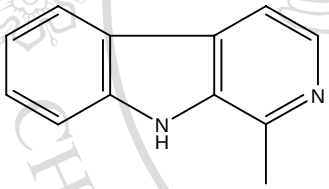
Indole alkaloids is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring which can be substituted, either in oxidized or reduced forms, for example, *N*-acylindole, 2-acylindole, oxindole, pseudoindoxyl (ψ-indoxyl), indoline, *N*-acylindoline, methyleneindoline, indolenine, 7-hydroxy indolenine.[11]



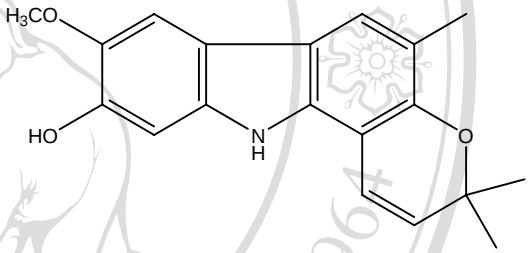
Indole alkaloid

The indole alkaloids have been classified by their molecular skeletons into two types; simple indole alkaloids and complex or monoterpenoid indole alkaloids.

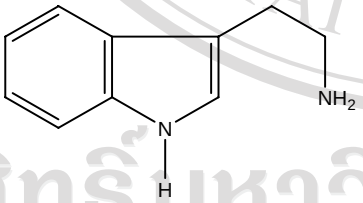
The simple indole alkaloids have uniformity structure. There are only indole nucleus or derivatived indole nucleus, for example, harman and koenigine. Whereas the complex or monoterpenoid indole alkaloids are the derivation from a single precursor derived by the joining of an amino acid, tryptamine, and a monoterpenoid, secologanin.[12]



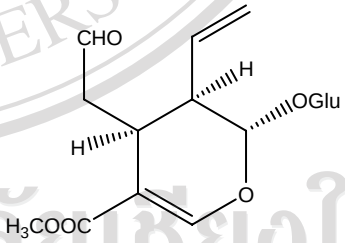
harman



koenigine



tryptamine



secologanin

The review of these complex indole alkaloids including monoterpenoid-, bismonoterpenoid-, and trismonoterpenoid indole alkaloids are summarised, according to the plants they have been found, in **Table 1.1**

Table 1.1 Indole Alkaloids from *Strychnos* genus

Plant	Plant part	Compounds	References
		Monoindole alkaloids	
Strychnos nux-vomica	seeds	strychnine (**1**)	13-19
		brucine (**2**)	14-18
		β-colubrine (**3**)	18
		pseudostrychnine (**4**)	18, 20-21
		pseudobrucine (**5**)	18
		strychnine *N*-oxide (**6**)	14, 18
		brucine *N*-oxide (**7**)	14-16, 18
		16-hydroxy-α-colubrine (**8**)	18
		2-hydroxy-3-methoxystrychnine (**9**)	18
		icajine (**10**)	17-18, 21
		vomicine (**11**)	17, 18
		novacine (**12**)	17, 18
		isostrychnine (**13**)	15-16, 18
		isobrucine (**14**)	18
		isostrychnine *N*-oxide (**15**)	18
		isobrucine *N*-oxide (**16**)	18
Strychnos icaja	root bark	strychnine (**1**)	19

Table 1.1 Indole Alkaloids from *Strychnos* genus (continued)

Plant	Plant part	Compounds	References
Strychnos icaja	roots	pseudostrychnine (**4**)	20
	roots	protostrychnine (**17**)	19, 22
Strychnos henning	root bark	diaboline (**18**)	21
Strychnos variabilis	root bark	retuline (**19**)	23, 24
Strychnos panganensis	root bark	*N*-desacetylisoretuline (**20**)	25
		N-desacetylretuline (**21**)	25
		12-hydroxy-11-methoxy-*N*-acetyl-nor-C-fluorocurarimine (**22**)	25
		12-hydroxy-11-methoxy-nor-C-fluorocurarine (**23**)	25
		N-desacetylspermostrychnine (**24**)	25
Strychnos guianensis	stem bark	9-methoxy-N_b-methylgeissochizol (**25**)	26
		C-alkaloid O (**26**)	26
		fluorocurine (**27**)	27
		C-profluorocurine (**28**)	28-30
		mavacurine (**29**)	27
		macusine B (**30**)	31-34

Table 1.1 Indole Alkaloids from *Strychnos* genus (continued)

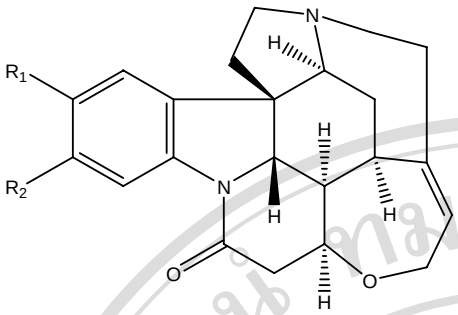
Plant	Plant part	Compounds	References
Strychnos myrtoides	stem bark	strychnobrasiline (**31**)	35, 36
		malagashanine (**32**)	35, 36
		12-hydroxymalagashanine (**33**)	35
		12-hydroxy-19-epi-malagashanine (**34**)	35
		malagashanol (**35**)	35
		myrtoidine (**36**)	35, 37
		11-demethoxymyrtoidine (**37**)	35, 37
Strychnos diplotricha	stem bark	myrtoidine (**36**)	35, 37
		11-demethoxymyrtoidine (**37**)	35, 37
		3-epi-myrtoidine (**38**)	37
		11-demethoxy-3-epi-myrtoidine (**39**)	37
		11-demethoxy-12-hydroxy-3-epi-myrtoidine (**40**)	37
		<u>Bisindole alkaloids</u>	
Strychnos usambarensis	root bark, leaves	usambarensine (**41**)	38, 39
		dihydrousambarensine (**42**)	38, 39
		10'-hydroxyusambarensine (**43**)	38
		Methylusambarensine (**44**)	38

Table 1.1 Indole Alkaloids from *Strychnos* genus (continued)

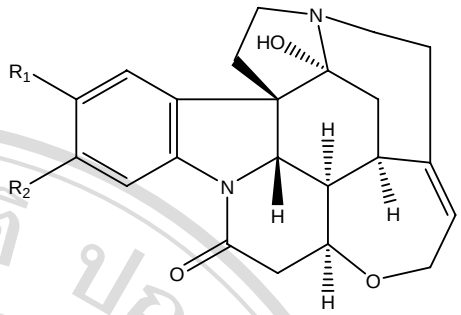
Plant	Plant part	Compounds	References
Strychnos usambarensis	root bark, leaves	usambarine (**45**)	38, 39
		dihydrousambarine (**46**)	38
		11-hydroxyusambarine (**47**)	38
		10-hydroxyusambarine (**48**)	38
		10-hydroxydihydrousambarine (**49**)	38
		11-hydroxydihydrousambarine (**50**)	38
		strychnopentamine (**51**)	38, 42
		isostrychnopentamine (**52**)	38-40, 42
	leaves	chrysopentamine (**53**)	42
	root bark, leaves	tetradehydrolongicaudatine Y (**54**)	38, 41
		longicaudatine (**55**)	39
		longicaudatine F (**56**)	39
	root bark	C-dihydrotoxiferine (**57**)	43
Strychnos icaja	roots, root bark	bisnordihydrotoxiferine (**58**)	44, 45
	roots	C-dihydrotoxiferine (**57**)	21
		C-toxiferine (**59**)	21

Table 1.1 Indole Alkaloids from *Strychnos* genus (continued)

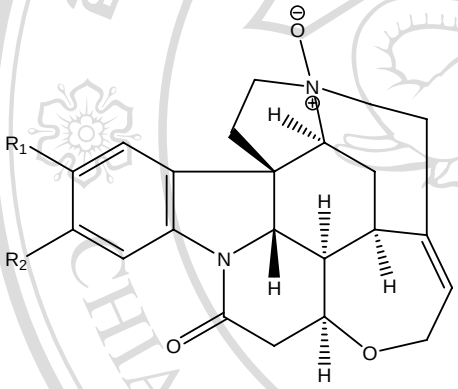
Plant	Plant part	Compounds	References
Strychnos icaja	roots, root bark	sungucine (**60**)	19, 44-49
	roots	isosungucine (**61**)	38, 45-49
		18-hydroxyisosungucine (**62**)	38, 46-49
		strychnogucine A (**63**)	45-48
		strychnogucine B (**64**)	19, 45-48
		strychnogucine C (**65**)	48
Strychnos matopensis	roots	matopensine (**66**)	39
Strychnos kasengaensis	root bark	matopensine *N*-oxide (**67**)	39
Strychnos guianensis	stem bark	guiaflavine (**68**)	50, 51
		5',6'-dehydroguiaflavine (**69**)	50
		guiachrysine (**70**)	26
		<u>Trisindole alkaloids</u>	
Strychnos icaja	roots	strychnohexamine (**71**)	19, 45



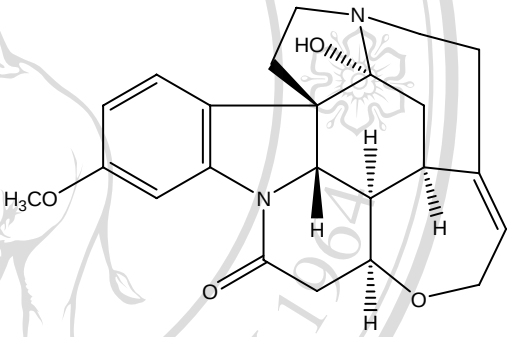
(1) $R_1 = H, R_2 = H$
(2) $R_1 = OCH_3, R_2 = OCH_3$
(3) $R_1 = OCH_3, R_2 = H$



(4) $R_1 = H, R_2 = H$
(5) $R_1 = OCH_3, R_2 = OCH_3$



(6) $R_1 = H, R_2 = H$
(7) $R_1 = OCH_3, R_2 = OCH_3$



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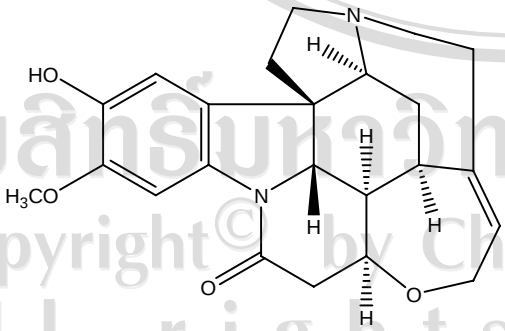

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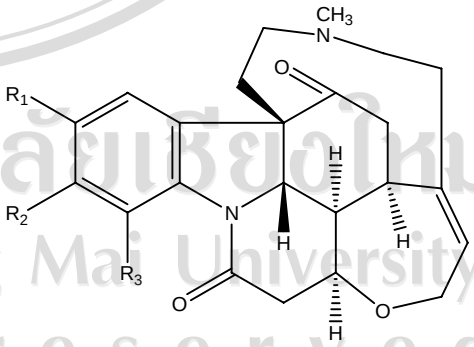
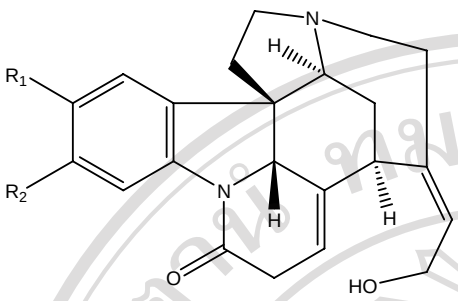
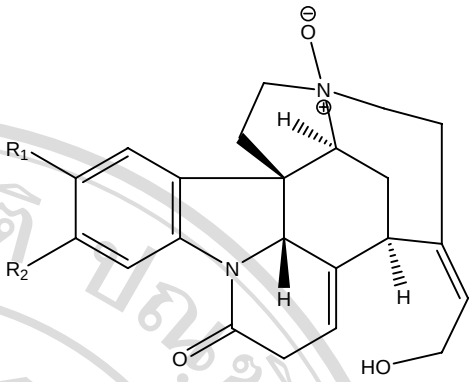


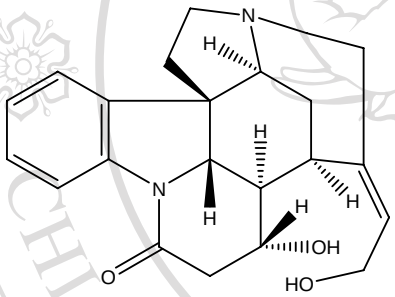
(10) $R_1 = H, R_2 = H, R_3 = H$
(11) $R_1 = H, R_2 = H, R_3 = OH$
(12) $R_1 = OCH_3, R_2 = OCH_3, R_3 = H$



(13) $R_1 = H, R_2 = H$
(14) $R_1 = OCH_3, R_2 = OCH_3$



(15) $R_1 = H, R_2 = H$
(16) $R_1 = OCH_3, R_2 = OCH_3$



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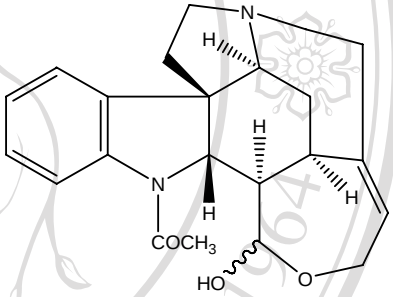

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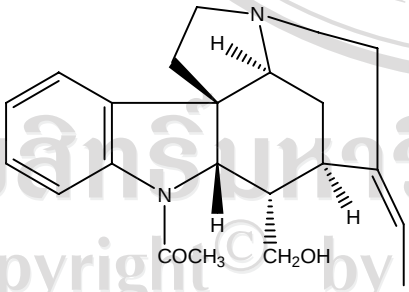

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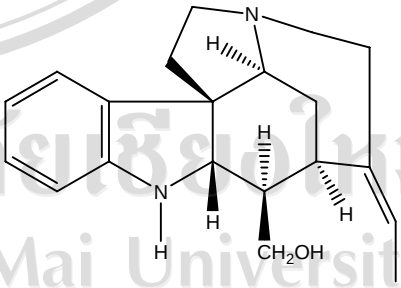

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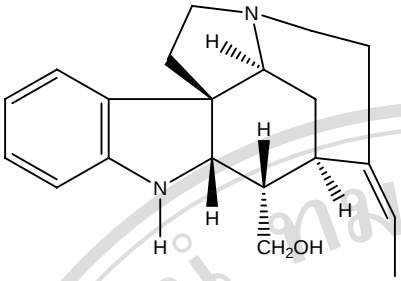

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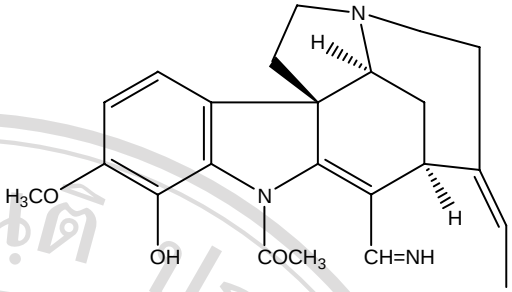

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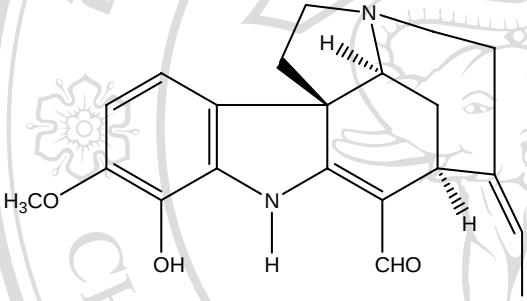

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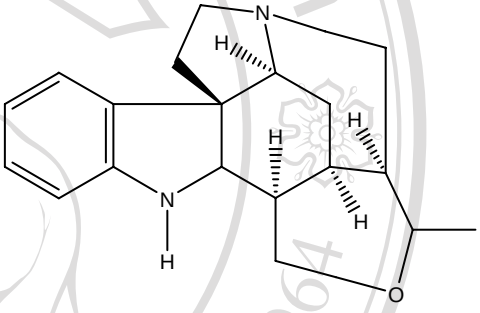

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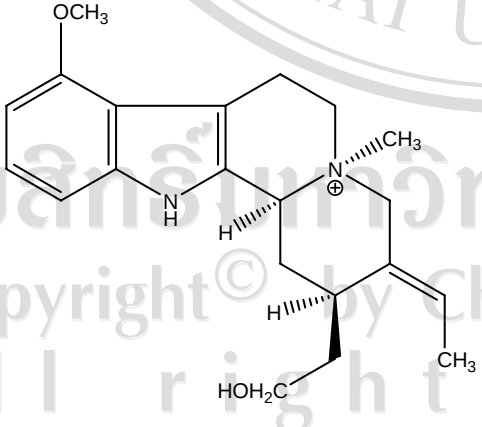

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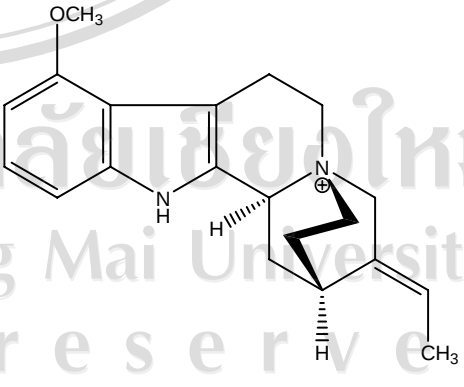

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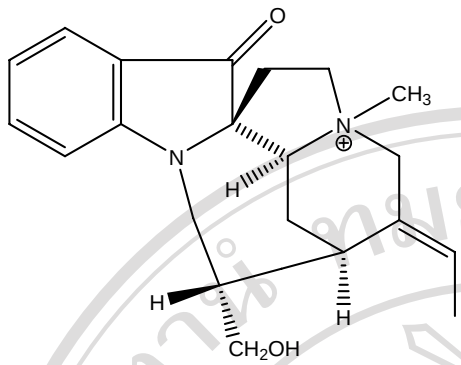

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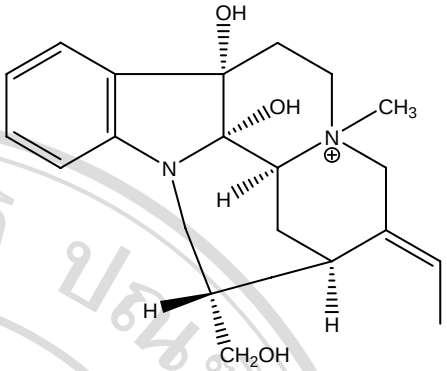

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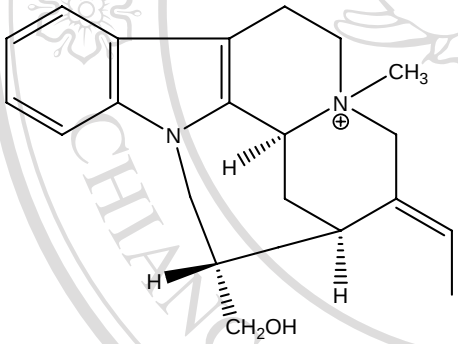

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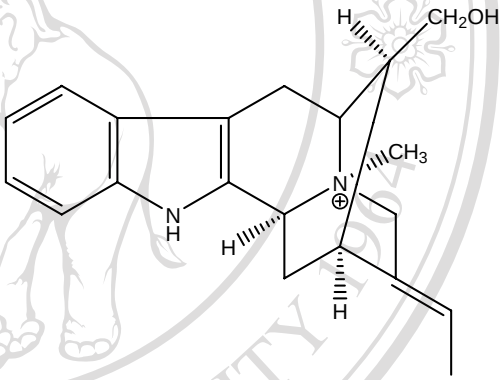

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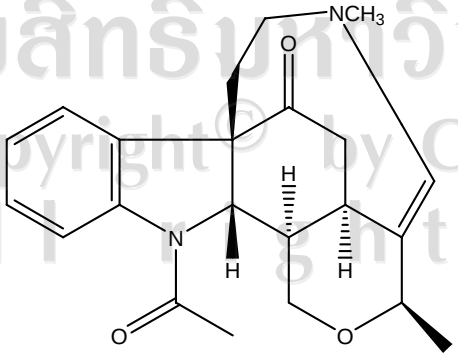

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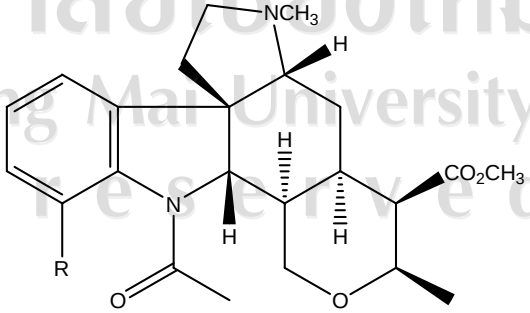
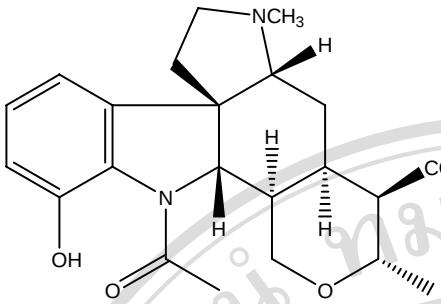


(32) R = H
(33) R = OH



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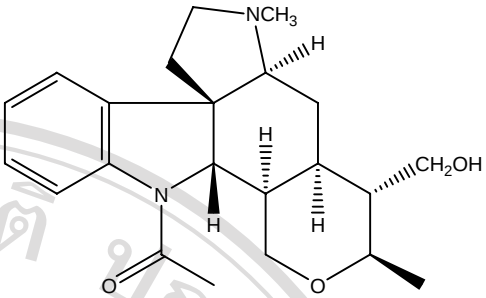

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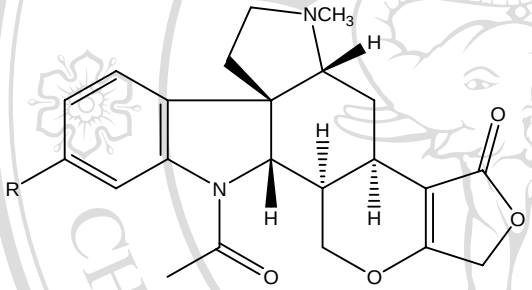
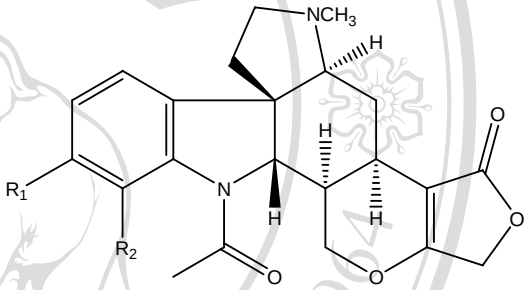
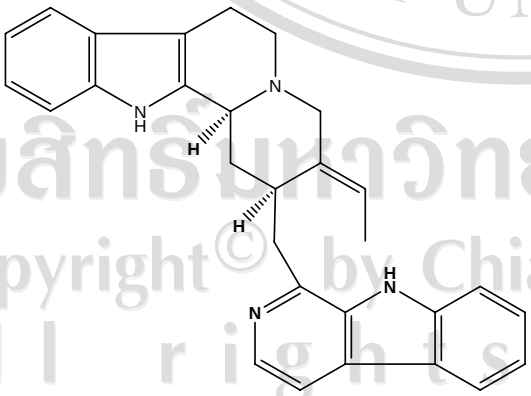


(36) R = OCH_3
(37) R = H



(38) R_1 = OCH_3, R_2 = H
(39) R_1 = H, R_2 = H
(40) R_1 = H, R_2 = OH



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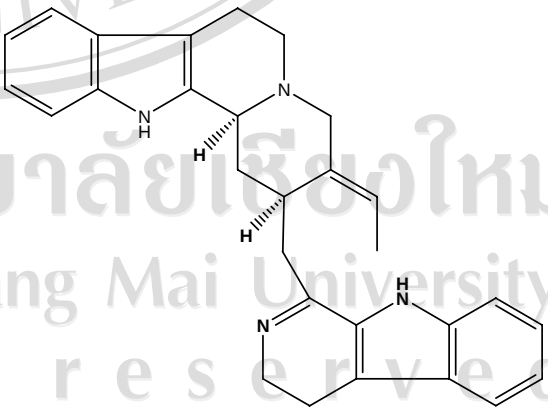

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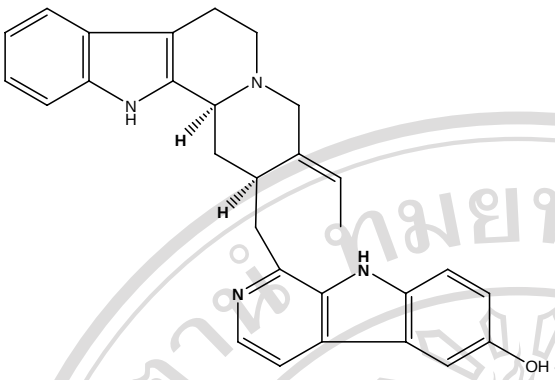

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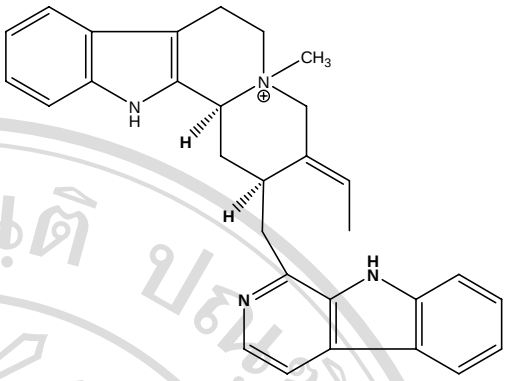

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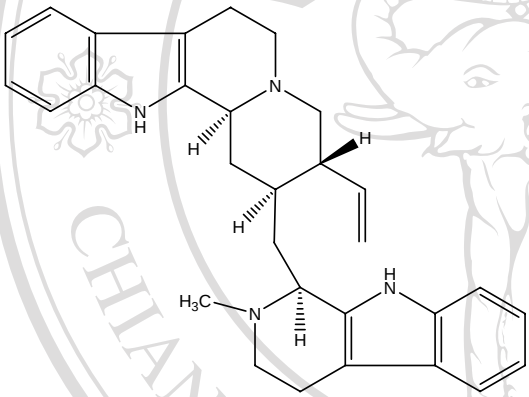

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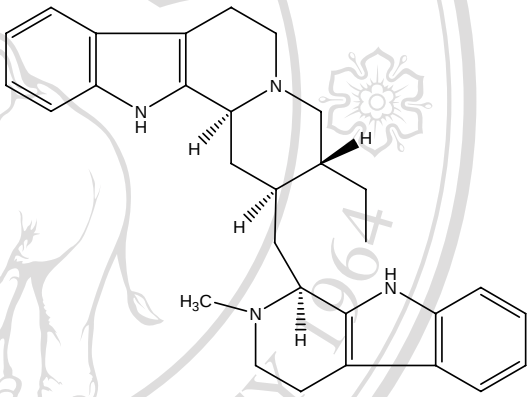

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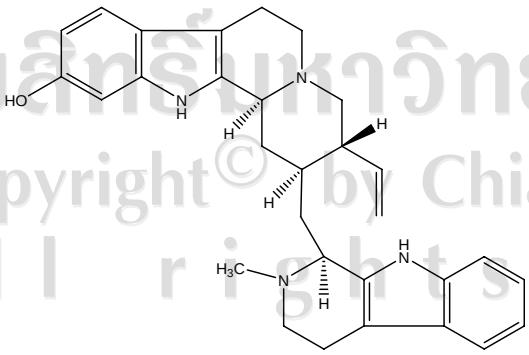

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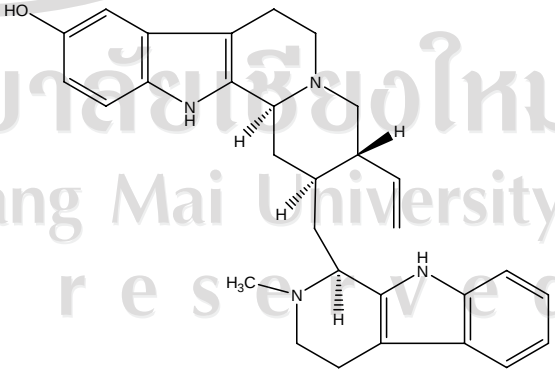

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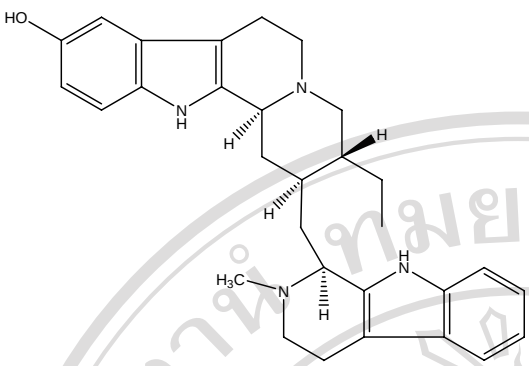

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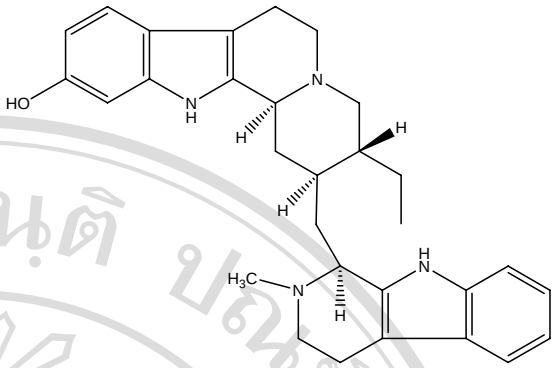

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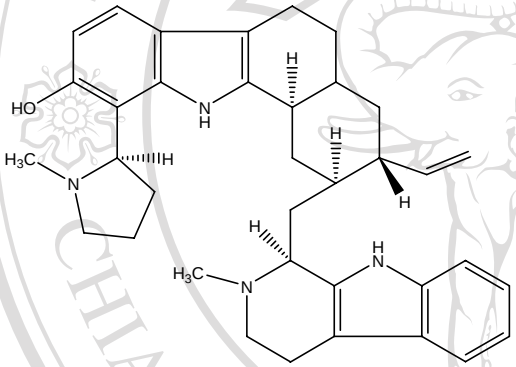

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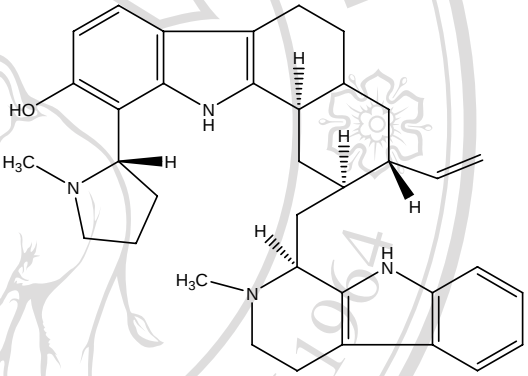

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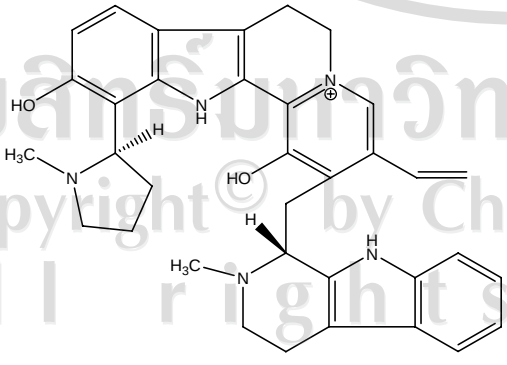

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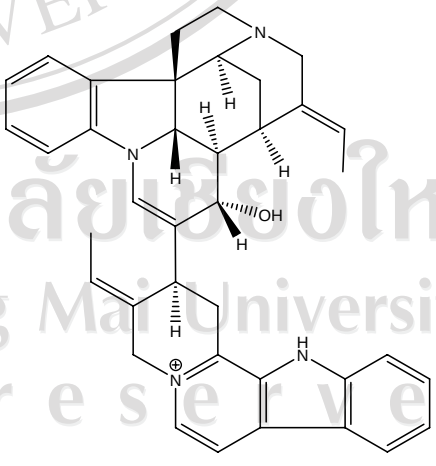

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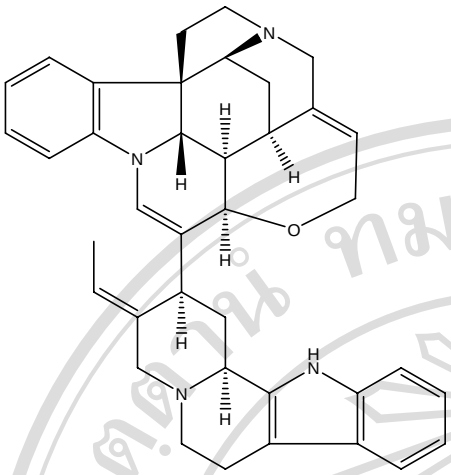

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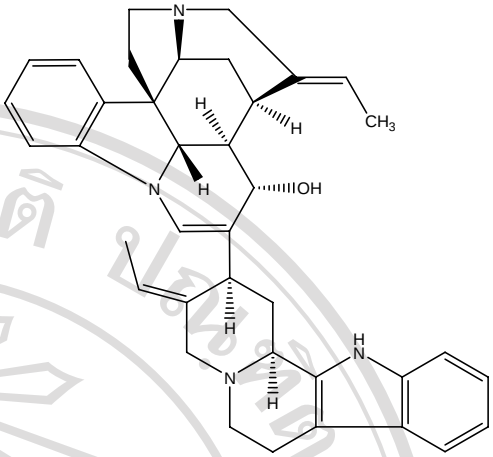

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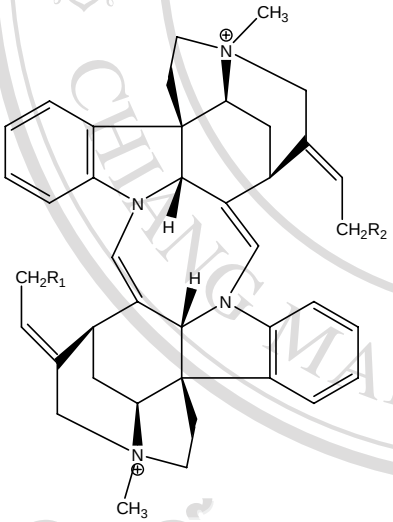
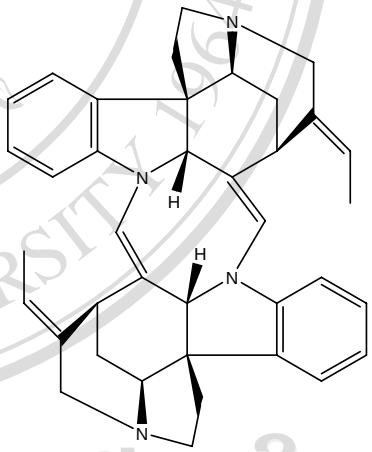


(57) R_1 = H, R_2 = H
(59) R_1 = OH, R_2 = OH



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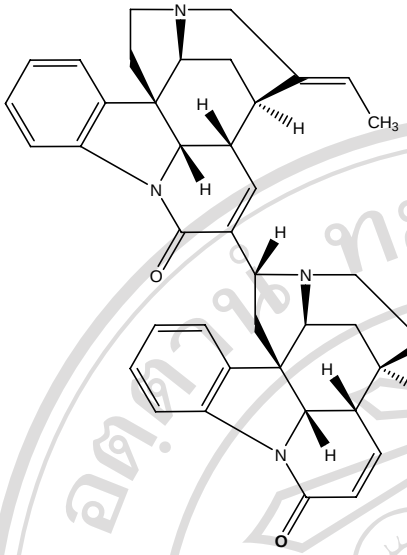

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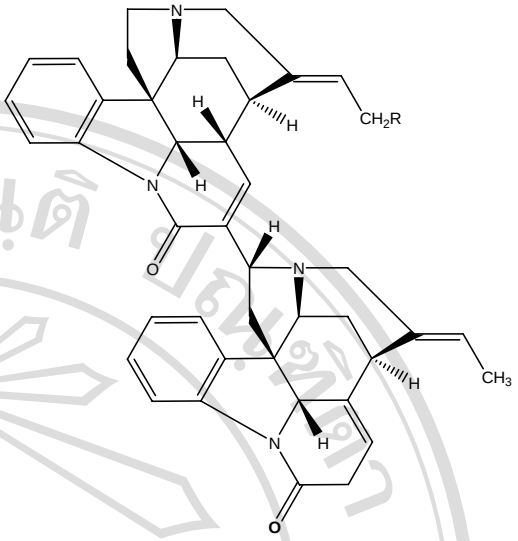
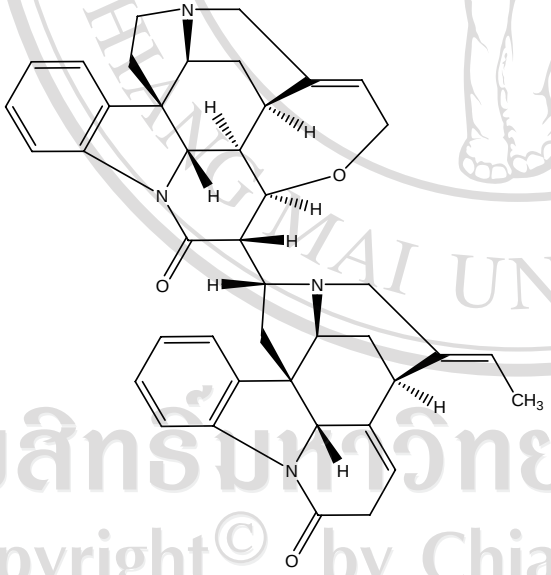


(61) R = H
(62) R = OH



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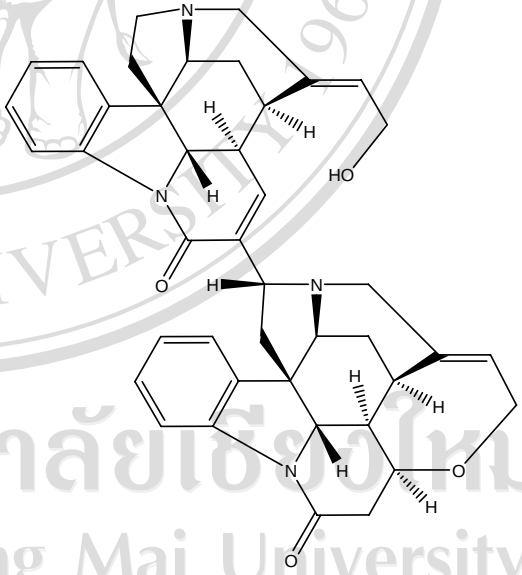

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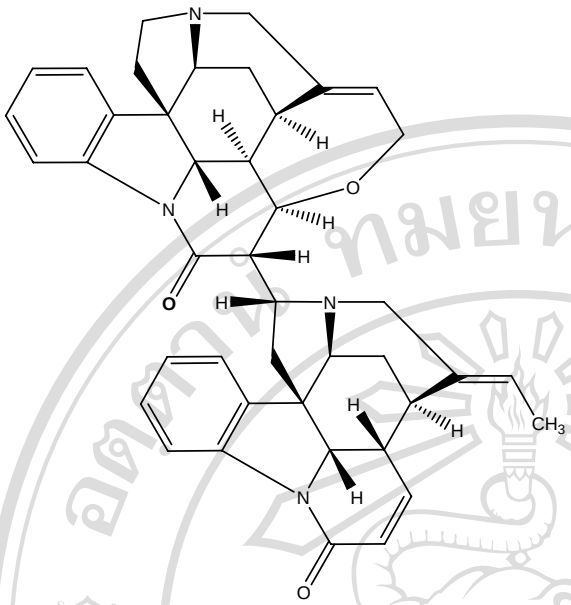

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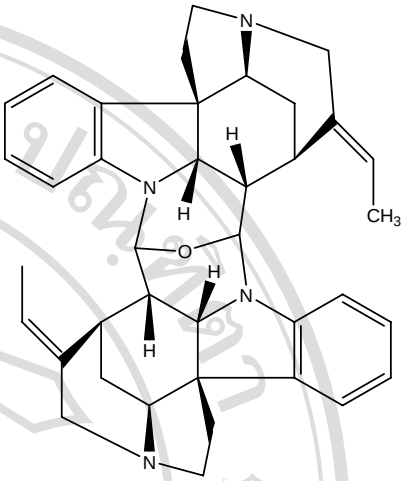

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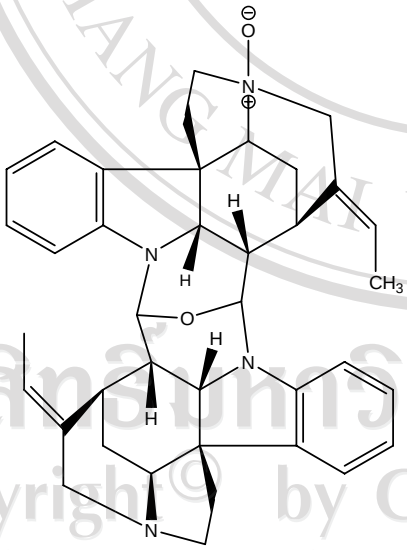

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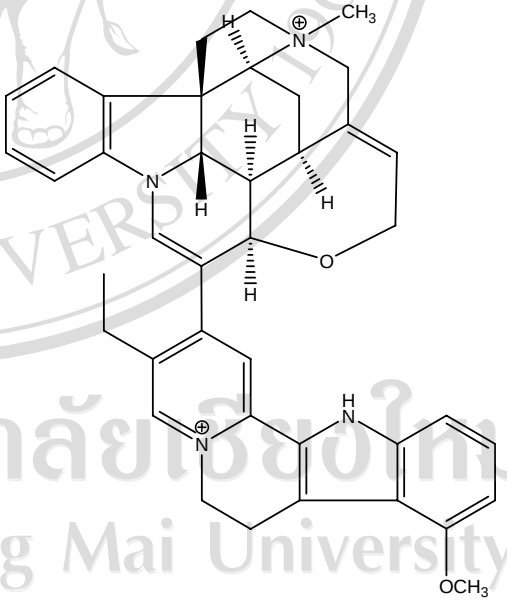

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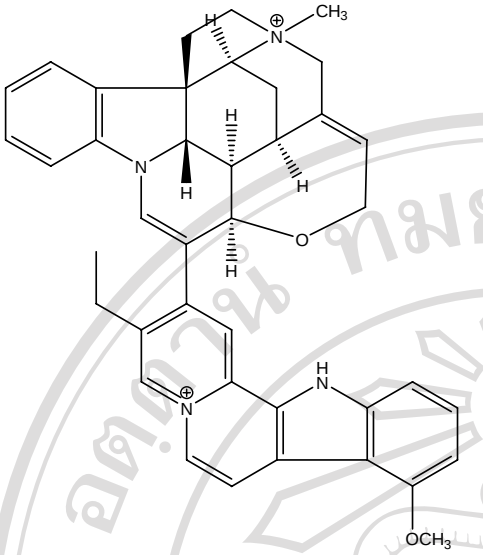

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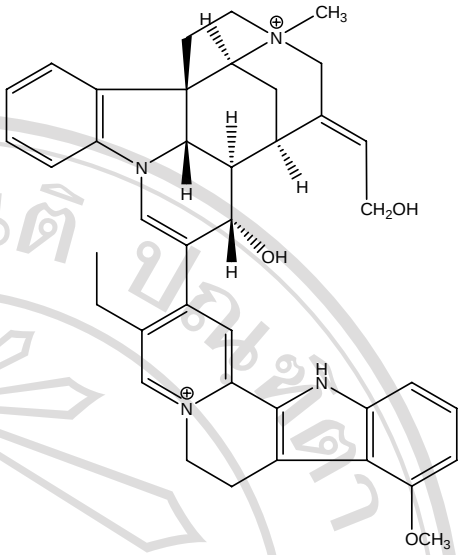

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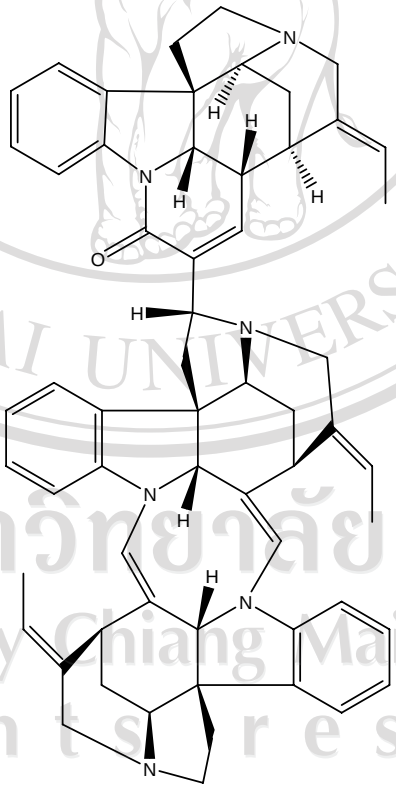

(71)

1.2.2 Miscellaneous Compounds

Although the indole alkaloids are abundant in *Strychnos* plants, there have been reports that these plants also contain other compounds such as pimarane and lignan glucosides (in stem of *Strychnos vanprukii*).[52,53] Phenolic and irridoid glucosides have been isolated from bark and wood of *Strychnos axillaris*.[54] In addition, there are irridoid glucosides in seeds of *Strychnos nux-vomica* too.[55] Quinic acid ester was also found in bark and wood of *Strychnos lucida*.[56]

1.3 Review of Bioactive Compounds from *Strychnos* genus

According to earlier studies of the chemical constituents, it was found that some of them displayed biological activities. There were various types of bioactive compounds isolated from *Strychnos* plants as shown below.

1.3.1 Antimalarial Compounds

There have been reports that plants in *Strychnos* genus contain alkaloids as major compounds especially bisindole alkaloids that show antimalarial activities.[38] The details of the study of antimalarial activity of alkaloids isolated from various *Strychnos* species are as follows.

In 1999, Frederich and co-workers[38] evaluated the *in vitro* antimalarial activities of 46 alkaloids and extracts from *Strychnos* species and found that two types of quasidimeric alkaloids exhibited high and selective activities against *Plasmodium falciparum*, which is a protozoa that causes malaria. Strychnopentamine (**51**) and isostrychnopentamine (**52**) were active against chloroquine-sensitive and resistant strains (IC_{50} 0.15 μM), while dihydrousambarensine (**42**) exhibited a 30-fold higher activity against the chloroquine-resistant strain (IC_{50} 0.03 μM) than it did against the chloroquine sensitive one.

Two years later, the same researchers reported[47] the isolation of strychnogucines A (**63**) and B (**64**) from the roots of *Strychnos icaja*. Strychnogucine B was more active against a chloroquine-resistant strain (IC_{50} 80 nM against the W2 strain) than the chloroquine-sensitive one and showed a selective antiplasmodial activity with 25-

180 times greater toxicity towards *Plasmodium falciparum*, in comparison with cultured human cancer cell (KB) or human fibroblasts (WI38).

The antimalarial activities of *Strychnos* alkaloids were further investigated in 2002 by Frederich and co-workers.[39] Sixty-nine alkaloids from various *Strychnos* species were subjected to *in vitro* antiplasmodial activities against chloroquine-resistant and chloroquine-sensitive lines of *Plasmodium falciparum*. The compounds, comprising mainly indolomonoterpenoid alkaloids, exhibited a wide range of biological potencies in the antiplasmodial assays. The most active alkaloids were also tested for cytotoxicity against HCT-116 colon cancer cells to determine their antiplasmodial selectivity. As a result of these studies, the alkaloids representing four types of bisindole skeleton exhibited potent and selective activities against *Plasmodium*. They were sungucine-type (**60-62**) (IC_{50} values ranging from 80 nM to 10 μM), longicaudatine-type (**54-56**) (IC_{50} values ranging from 0.5 to 10 μM), matopensine-type (**66-67**) (IC_{50} values ranging from 150 nM to 10 μM), and usambarine-type alkaloids (**45-50**).

In 2003, Philippe and co-workers[48] isolated a bisindole alkaloid, named strychnogucine C (**65**), and the first naturally occurring trimeric indolomonoterpenic alkaloid: strychnohexamine (**71**) from the roots of *Strychnos icaja*. The *in vitro* antiplasmodial activities of these alkaloids have been determined against the FCA chloroquine-sensitive strain of *Plasmodium falciparum*. It was found that strychnogucine C possessed a weak activity (IC_{50} 16.1±0.76 μM), which was notably less active than other sungucine type alkaloids: strychnogucine A (IC_{50} 2.3±0.30 μM) and strychnogucine B (IC_{50} 0.6±0.07 μM). On the other hand, strychnohexamine

presented a strong antiplasmodial activity with an IC_{50} of 1.1±0.10 μM, which was about two times more potent than bisnordihydrotoxiferine (**58**) (IC_{50} 2.8±1.1 μM).

In 2004, Frederich and co-workers[42] isolated a derivative of strychnopentamine from the leaves of *Strychnos usambarensis*. This compound, namely chrysopentamine (**53**), exhibited strong antiplasmodial properties (IC_{50} less than 1 μM). In the same year, the same group reported[40] the finding that isostrychnopentamine (**52**) isolated from the leaves of *Strychnos usambarensis* possessed an *in vitro* antiplasmodial activities against five *Plasmodium falciparum* cell lines (chloroquine-resistant and chloroquine-sensitive lines) (IC_{50} near 0.1 μM) and showed antiplasmodial selectivity in comparison with the cytotoxicity on human cell lines. In addition, *in vivo* antimalarial activities of isostrychnopentamine against the *P. berghei* NK173 and *P. vinckei petteri* murine strains were also determined. It was found that the ED_{50} *in vivo* was about 30 mg/kg/day by the intraperitioneal route (after 4 days treatment).

1.3.2 Cytotoxic Compounds[3]

There has been a report on the study of the cytotoxic activity of two bisindolomonoterpenic alkaloids, viz. sungucine (**60**) isolated from the roots of *Strychnos icaja* and isostrychnopentamine (**52**) from the leaves and root bark of *Strychnos usambarensis*. Whereas isostrychnopentamine was found to induce apoptosis in HCT-116 colon cancer cells by classical pathways, it was found that sungucine was able to induce apoptosis in HL-60 leukemia cells. This has been observed by several apoptosis tests: morphology, induction of caspase 3, cleavage of RARP, and fragmentation of DNA.

1.3.3 Antagonists of Neuromuscular Transmission Compounds

In 2003, Wins and co-workers[57] presented the effective antagonists of nicotinic acetylcholine receptors in cultured human TE671 cells of constituents from the stem bark of *Strychnos guianensis*. It was found that the most effective antagonist, guiachrysine (**70**), had an IC_{50} of 0.43 μM whereas another bisindole alkaloid, guiaflavine (**68**) (IC_{50} 0.70 μM) was slightly less effective. Moreover, monoindole compounds were 10 to 100 times less potent than bisindole alkaloids.

1.3.4 Analgesic and Anti-inflammatory Compounds

Two monoterpenoid indole alkaloids, burcine (**2**) and brucine *N*-oxide (**7**) from seeds of *Strychnos nux-vomica* were reported by Yin and co-workers[58] in 2003 that they possessed analgesic and anti-inflammatory activities. Both compounds significantly inhibited the released of prostaglandin E_2 in inflammatory tissue, reduced acetic acid-induced vascular permeability and the content of 6-keto-PGF_{1a} in Freund's complete adjuvant (FCA) induced arthritis rat's blood plasma.

1.4 Aims of the Research

As a part of our study on ethnobotany of Thai medicinal plants which have interesting components and/or exhibit biological activities, we have been interested in the investigation of the chemical constituents from the roots of *Strychnos nux-blanda*. To the best of our knowledge, at present, the phytochemical and pharmacological information on the roots of this plant has not been available. Moreover, there have been reports on the chemical components of *Strychnos* genus, and the plants in this genus are a rich source of indole alkaloids that show interesting bioactivities such as anti-inflammatory, cytotoxicity, and most importantly antimalarial properties.

Therefore, in this work, we focused on the isolation and purification of the crude alkaloid extract from the roots of *Strychnos nux-blanda* to search for some alkaloid components which possess interesting molecular structures which may have potential for further medicinal applications.