CHAPTER 2

LITERATURE REVIEW

Acetylcholine (ACh) is a neurotransmitter at many neuronal synapses. It is synthesised in nerve terminals from acetyl CoA and choline in a reaction catalysed by choline acetyltransferase (CAT) and stored in vesicles in the presynaptic neuron. When the nerve impulse arrives at the nerve ending, ACh, stored there in vesicles, is released and combines with a receptor molecule (muscarinic and nicotinic cholinergic receptor) in the postsynaptic membrane or the end-plate membrane of a muscle fibre (Waymire, 1997). A summary of ACh synthesis and degradation is shown in Figure 2.1.

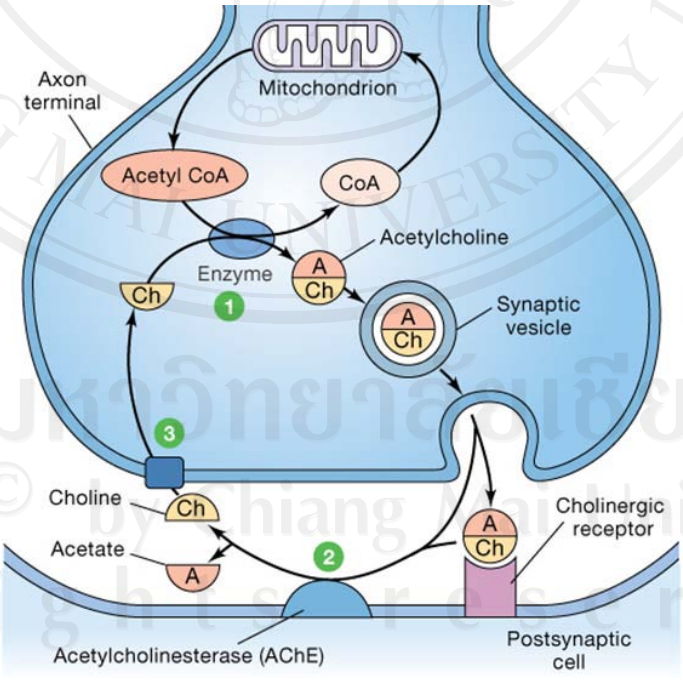


Figure 2.1 A summary of acetylcholine synthesis and degradation.

(http://faculty.pasadena.edu/dkwon/chap%208_files/textmostly/slide58.html.)

Consequently, inhibition of acetylcholinesterase (AChE), the key enzyme in the breakdown of acetylcholine in the nervous system, enhances the accumulation of Ach in insects and causes hyper-stimulation of neurons, resulting in death. This property led to the development of inhibitors of this enzyme for the purposes not only for insecticides but also for the treatment of neurological disorders such as AD (Aldridge, 1950).

Alzheimer's disease (AD) is a neurodegenerative disease primarily affecting people over 65 years of age. Although a definitive cause has not yet been determined, currently available drug therapies are based on increasing the amount of the neurotransmitter acetylcholine (Francis *et al*., 1999). One of the most accepted strategies in AD treatment is the use of acetylcholinesterase inhibitors to accumulate the level of acetylcholine and enhance the function of remaining acetylcholine receptors in the brain (Darvesh *et al*., 2003). Besides these, AD is associated with increased oxidative stress. Although, there are not many investigation, a few studies have found an association between antioxidant intake and reduced incidence of AD.

Oken *et al*. (1998) and Le Bars (2003) have reported that the *Ginkgo biloba* extract can improve or maintain cognitive function in AD patients. Most of the effects of *Ginkgo biloba* have been attributed to its antioxidant properties.

At present, the widely used effective drugs in AD therapy are cholinesterase inhibitors. The first AChE inhibitors (AChEIs) was introduced in 1993 as tacrine (Whitehouse, 1993), followed by donepezil (Kelly *et al*., 1997), rivastigmine (Gottwald and Rozanski, 1999) and galanthamine (Scott and Goa, 2000). Although, all commercial AChEIs are effective in AD treatment they still give many adverse side effect and are very expensive. Therefore, the search for new AChEIs from natural

plant products, with less side effects, is under current investigation in many laboratories.

Stemona aphylla is one of many plants, that have been reported to contain acetylcholinesterase inhibitors of similar activity to galanthamine, a drug widely used in AD treatment (Sastraruji *et al.*, 2011). At the same time many investigations have described the insecticidal properties of the *Stemona* species (Pilli and Ferreira de Oliveira, 2000; Jinwajinda *et al*., 2001; Brem *et al*., 2002, Komalamisra *et al*., 2005; Greger, 2006; Liu *et al.*, 2007; Phattharaphan *et al*., 2010). Moreover, Brem *et al*. (2004) reported the radical scavenging activity of dehydro-δ-tocopherol and dehydro-γ-tocopherol from *Stemona* species showed EC_{50} values of 10 and 9 ppm, respectively.

2.1 *Stemona* plants

The Stemonaceae family consists of three genera. The genus *Stemona*, previously named as *Roxburghia* Roxb. (Prain, 1905), the genus *Croomia* and the genus *Stichoneuron* (Duyfjes, 1993; Pilli and Ferreira de Oliveira, 2000; Tsi *et al*, 2000).

The *Stemona* genus is distributed in Southeast Asia, tropical Australia and only one species in Southeast United States (Duyfjes, 1993; Pilli and Ferreira de Oliveira 2000; Brem *et al*. 2002). *Stemona* plants are called by different names depending upon the regions that they are found for example, ‘Bai bu’ in China, ‘Bach bo’ in Vietnam and ‘Non Tai Yak’ or ‘Pong Mot Ngam’ in Thailand. Although, at least nine species i.e. *S. aphylla* Craib, *S. burkillii* Prain, *S. cochinchinensis*, *S. collinsae* Craib, *S. curtisii* Hook.f., *S. kerrii* Craib, *S. phyllantha* Gagnep, *S. pierrei*

and *S. tuberosae* Lour. and two new species, *S. involuta* and *S. rupestris* in this genus have been found in Thailand, the phytochemical investigations have been studied in only a few species (Maxwell, 1991; Rungrojsakul, 2001; Inthachub *et al*., 2010). However, some species are difficult to identify because of the various aspects and diversity in morphological characters.

***Stemona aphylla* Craib (Inthachub *et al*., 2010)**

Morphology: Twiner, stem 2-4 m long, sometimes precociously flowering on erect shoots. Leaves alternate; petiole 4-6.5 cm long; blade ovate or broadly ovate, base (shallowly) cordate or truncate. Inflorescences borne in the axils scale-like leaves or in the axils of distal leaves. Flowers: sessile or on short peduncles to 5 mm long; bracts 8-10 mm long; pedicel 5-20 mm long; tepals narrowly triangular 4-6 mm; stamens 15-20 mm long; filament 2 mm long; anthers 8-13 mm long, ridge separating the fleshy, smooth and somewhat undulate, petaloid outgrowth of the connective subulate 8-14 mm long, additional appendage lacking. Fruit 13-16 mm. Seeds light brown 4 mm; aril with finger-like lobes. In addition, the tepals are recorded as pink or yellowish pink or greenish purple. The stamens as purple or pink. The tuberous roots as 10-30 cm long and 1 cm thick.

Distribution: Endemic to Thailand (Northern: Chiang Mai, Lampang, Phrae, Nakhon Sawan; North-Eastern: Phetchaboon, Loei, Udon Thani, Khon Kaen).

Habitat and Ecology: In dry evergreen forest, mixed deciduous hardwood forest with much bamboo; on shale bedrock and in rugged limestone terrain and on limestone foothill; 240-700 m altitude. Flowering: February to May, quite frequently encountered flowering precociously on shorter shoots with scale-like leaves only.

***Stemona curtisii* Hook.f. (Inthachub *et al*., 2010)**

Morphology: Glabrous twiner, 1-2 m high, somewhat branched. Leaves alternate, seldom opposite; petiole 4-12 cm long; blade (narrowly) ovate 2.5-12.5 cm long, sometimes slightly rough, base broadly or (shallowly) cordate. Inflorescences many flowered; peduncles 1-10 cm long, not fused with the petiole; bracts 6-20 mm long. Flowers: pedicel 10-20 mm long; tepals narrowly triangular 5-7 mm; stamens 18-25 mm long; filament 2 mm long; anthers 12-15 mm long, with narrow sterile thecal remnants up to the apex, petaloid outgrowth of the connective gradually tapering 6-10 mm long, additional appendage lacking. Fruit 15 mm. Seeds dark red 3-3.5 mm; aril with finger-like lobes. In addition, the tepals are recorded as pink, brown-pink or dark brownish red. The stamens as reddish maroon. The tuberous roots as 80 cm long and 1-1.5 cm thick.

Distribution: Sri Lanka, Thailand (South-Western: Kanchanaburi, Ratchaburi, Phetchaburi, Prachuap Khiri Khan; Chumphon, Surat Thani, Krabi, Nakhon Si Thammarat, Phatthalung, Trang, Satun, Songkhla, Pattani, Narathiwat), Sumatra, Peninsular Malaysia.

Habitat and Ecology: Deciduous, evergreen and secondary forest, not far from the coast, on riverbanks, near waterfalls, in thickets and scrub; on sand, limestone and poor granitic soil; from sea level to 600 m altitude. Flowering: December to July; Fruiting: February to November.

2.2 Biological activities

The root extracts of *Stemona* species have been widely used as insecticides on agriculture pests and as anthelmintic agents for domestic animals. Moreover, these extracts have also been used in the treatment of various respiratory diseases and used as anticough agents in China and Japan (Ye *et al.*, 1994a; 1994b; Pilli *et al.*, 2000; Brem *et al*., 2002; Greger, 2006). Some compounds have also been investigated for their antiacetylcholinesterase activities (Mungkornasawakul *et al*., 2009; Sastraruji *et al.*, 2010).

Many investigations have described the insecticidal properties of the *Stemona* species. Sakata *et al.* (1978) have reported that stemonine, stemospironine and stemofoline have insecticidal activity against the fourth instar *Bombyx mori* (silkworm larvae). Additionally, other *Stemona* alkaloids such as neostemonine and isoprotostemonine were also reported to have antifeedant activity against last-instar larvae of *Spodoptera litura*. Jiwajinda *et al*. (2001) found two new alkaloids, 16,17-didehydro-16(*E*)-stemofoline and its isomer at C-4, 16-17-didehydro-4(*E*)-16(*E*)-stemofoline which displayed higher insecticidal and antifeedant activities against the diamondback moth larvae than stemofoline.

Greger (2006) found that the roots of *Stemona* species containing certain protostemonine derivatives, especially didehydrostemofoline exhibited very high toxicity against larvae of *Spodoptera littoralis*, whereas those with dominating stichoneurine or croomine derivatives showed low toxicity but sometimes remarkable repellence due to an accumulation of tuberostemonine. Additionally tuberostemonine also had effects on the motility of helminth worms and could reduce the excitatory transmission at the crayfish neuromuscular junction.

Phattharaphan *et al*. (2010) observed the effect of *Stemona collinsae* extracts on the diamond back moth (*Plutella xylostella*). The results indicated that the dichloromethane extract showed the highest insecticidal activity with a LC_{50} value of 0.60% followed by the methanol and hexane extracts with LC_{50} values of 0.71% and 1.15% respectively, whereas the methanolic crude extracts of *S. cochinchinensis* and *S. curtisii* were the most active with LC_{50} values of 4 and 9 ppm (Kaltenegger *et al*., 2003).

Moreover, Brem *et al*. reported in 2002 that *S. collinsae* extracts showed strong antifeedant activity against fifth instar larvae of *Spodoptera littoralis* in the leaf disk choice assay and the methanolic leaf and root extracts showed very high insect toxicity compared to *Aglaia* species and pyrethrum extract and azadirachtin. Whereas, *S. tuberosa* extracts from the root and leaves showed no toxic activity but demonstrated repellency because of tuberostemonine, which was the major alkaloid in the root.

Additionally, it was found that the anti-insect properties of *S. collinsae* and *S. tuberosa* were based on a pyrrolo[1,2-*a*]azepine alkaloid, namely 1′,2′-didehydrostemofoline which was the major compound of the root of *S. collinsae* and stemofoline that displayed contact toxicity and antifeedant activity. On the other hand, four new stenine-type *Stemona* alkaloids, tuberostemonine JI, tuberostemonine HI, epi-bisdehydrotuberostemonine J and neostenine and also neotuberostemonine, which is a known compound from *S. tuberosa*, displayed antitussive activity in guinea pig (Chung *et al*., 2003; Greger, 2006).

In addition, Greger (2006) studied the structure-activity relationship of seven related compounds and found that the saturated tricyclic pyrrolo[1,2-*a*]azepine nucleus of tuberostemonines is a prerequisite for antitussive activity.

On the other hand Pitiyont *et al*. (2008) and Tikum *et al*. (2008) found that the dichloromethane extract of the root of *Stemona burkillii* was also the most effective against *Spodoptera litura* and *Spodoptera exiqua* with LC_{50} values of 6,204 and 9,589 ppm at 24 hr, respectively.

Mungkornasawakul *et al*., (2004) reported that the pure alkaloids, oxyprotostemonine, stemocurtisine and stemocurtisinol and the ethanolic crude extract of *S. curtisii*, showed strong larvicidal activity on mosquito larvae (*Anopheles minimus* HO).

For fungicidal activity Kostecki *et al*., (2004) found that fifteen new stilbenoids and four dihydrostilbenes isolated from a methanolic extract of *Stemona collinsae* roots showed antifungal activity against *Cladosporium herbarum*, while, other antifungal stilbenoids were found from *S. cf. pierrei*.

For antibacterial activities, twelve dihydrostilbenes, phenanthraquinone, and stemanthraquinone isolated from roots of *Stemona tuberosa* have been studied, most of the tested compounds showed moderate activities while stilbostemine U showed strong activity against *Bacillus pumilus* with a MIC of 12.5–25 μg/mL (Lin *et al*., 2008). Moreover, several stilbenoids from the roots of *Stemona sessilifolia* showed antibacterial activities against *Staphylococcus aureus* and *Staphylococcus epidermidis* (Yang *et al*., 2006).

Akanitapichat *et al.* (2005) investigated the dichloromethane–methanol (DCM-MeOH, 1:1), 95% ethanol and aqueous extracts of *Stemona collinsae* roots for

in vitro antimicrobial, antiviral and anticancer activities. By using a plaque reduction assay, the DCM-MeOH extract showed moderate activity against herpes simplex virus (HSV) type 1 and type 2 with 50% inhibitory concentrations of 105 ± 3.5 and 107 ± 6.2 μg/ml, respectively. Ethanol and aqueous extracts minimally inhibited HSV at 300 μg/ml. All extracts exerted antiproliferative activity against malignant cell lines KB and MCF-7, with 50% cytotoxic concentrations ranging from 85 to 270 μg/ml.

Additionally, ethanolic crude extracts of *Stemona* species, some *Stemona* alkaloids and synthetic compounds were found to have AChE inhibitory activity. For example:

Wang *et al*. (2007) isolated alkaloids from the roots of *Stemona sessilifolia* and found that two new *Stemona* alkaloids, sessilistemonamines A and sessilistemonamines B which were moderately active on AchE with IC_{50} values of 68.8±9.5 and 17.1±2.5 μM, respectively.

Baird *et al.* (2009) indicated that (11*Z*)-1′,2′-didehydrostemofoline from an unidentified *Stemona* sp. showed the highest inhibitory activity of AChE with a minimum inhibitory requirement (MIR) of 5 ng, followed by, (3′*S*)-hydroxystemofoline with a MIR of 10 ng. Whereas, methylstemofoline had a significantly higher activity than its corresponding *E*-isomer, (11*E*)-methylstemofoline with MIR values of 100 and 500 ng, respectively.

Mungkornasawakul *et al*. (2009) studied the AChE inhibitory activity of the root extract of *S. aphylla* and found that the crude extract had higher activity than stemaphylline and stemaphylline-*N*-oxide.

Sastraruji *et al.* (2010) reported the semisynnthesis of the four known *Stemona* alkaloids i.e. oxystemofoline, methoxystemofline, (1′*R*)-hydroxystemofoline

and (1′*S*)-hydroxystemofoline. In a TLC bioautographic assay, (1′*R*)-hydroxystemofoline was found to be the most active AChE inhibitor with a MIR of 5 ng, while (1′*S*)-hydroxystemofoline exhibited slightly weaker inhibitory activity (MIR = 10 ng). All other tested compounds showed moderate activity.

Chaiyong *et al*. (2010) found that the alkaloids from *S. curtisii*, stemocurtisine, stemocurtisinol, oxyprotostemonine and stemocurtisine *N*-oxide were relatively inactive as AChE inhibitors, with MIRs of 500 -1,000 ng.

Sastraruji *et al.* (2011) isolated a new stemofoline alkaloid, (2′*S*)-hydroxy-(11*S*,12*R*)-dihydrostemofoline and known compounds stemofoline and (2′*S*)-hydroxystemofoline from the root extracts of *S. aphylla*. Stemofoline and (2′*S*)-hydroxystemofoline were the most active with MIRs of 10 ng.

2.3 Structural classification of *Stemona* alkaloids

The *Stemona* alkaloid structures contain a characteristic pyrrolo[1,2-*a*]azepine nucleus. The *Stemona* alkaloids have been divided into 8 groups according to their structural features i.e. stenine (**I**), stemoamide (**II**), tuberostemospironine (**III**), stemonamine (**IV**), parvistemoline (**V**), stemofoline (**VI**), containing the pyrrolo[1,2-*a*]azepine nucleus characteristic of the majority of the *Stemona* alkaloids, stemocurtisine (**VII**) which displays the pyrido[1,2-*a*]azepine nucleus, and a miscellaneous group (**VIII**) either lacking or featuring a hidden pyrrolo[1,2-*a*]azepine moiety (Fig. 2.2) (Pilli *et al*., 2010).

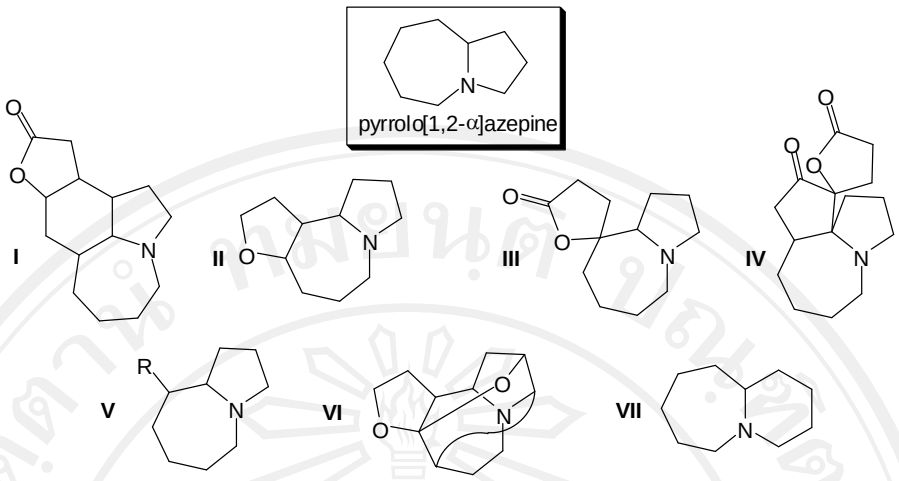


Figure 2.2 *Stemona* alkaloid groups.

Stenine group

The structural characteristic of this group is the tetracyclic furo[2,3-*h*]pyrrolo[3,2,1-*jk*]benzazepin-10(2*H*)-one nucleus (**I**, Fig. 2.2). Currently, fourteen *Stemona* alkaloids: stenine (**1**), 2-oxostenine (**2**), isostenine (**3**), also named as neostenine, sessilifoline B (**4**), tuberostemonine J (**5**), tuberostemonine H (**6**), tuberostemonine N (**7**), tuberostemonine K (**8**), neotuberostemonol (**9**), *epi*-bisdehydroneotuberostemonine J (**10**), 9α-bisdehydrotuberostemonine (**11**), 9α-bisdehydrotuberostemonine A (**12**), tridehydrotuberostemonine (**13**), and sessilifoline A (**14**) (Fig. 2.3) had been reported.

Stemoamide group

The *Stemona* alkaloids which have the tricyclic 2*H*-furo[3,2-*c*]pyrrolo[1,2-*a*]azepine nucleus (**II**, Fig. 2.2) are classified as belonging to the stemoaminde group. Twenty six alkaloids (Fig. 2.4): stemoamide (**15**), protostemonamide (**16**), saxorumamide (**17**), isosaxorumamide (**18**), neostemocochinine (**19**),

isoneostemocochinine (**20**), stemocochinin (**21**), 13-demethoxy-(11*S**,12*R**)-dihydroprotostemonine (**22**), bisdehydrostemocochinine (**23**), isobisdehydrostemocochinine (**24**), dehydroprotostemonine (**25**), oxyprotostemonine (**26**), neostemofoline (**27**), sessilifoliamide A (**28**), bisdehydroneostemoninine (**29**), bisdehydrostemoninine A (**30**), bisdehydrostemoninine B (**31**), bisdehydrostemoninine (**32**), isobisdehydrostemoninine (**33**), stemoninine A (**34**) stemoninine B (**35**), dihydrostemoninine (**36**), oxystemoninine (**37**), stemoenonine (**38**), 9a-*O*-methylstemoenonine (**39**), and oxystemoenonine (**40**) are members of this group.

Tuberostemospironine group

The tuberostemospironine group of *Stemona* alkaloids is characterized by a spiro[furan-2-(5*H*),9′[9*H*]pyrrolo[1,2-*a*]azepin]-5-one nucleus which displays a spiro γ-lactone at C9 of the basic ring system (**III**, Fig. 2.2) and comprises six members: tuberostemospironine (**41**), 10-hydroxycroomine (**42**), 6-hydroxycroomine (**43**), dehydrocroomine (**44**), croomine (**45**), 7-methoxycroomine (**46**), tuberospironine (**47**), and sessilifoliamine A (**48**) (Fig. 2.5).

Stemonamine group

The structural characteristic of this group is the tetracyclic spiro[1*H*-cyclopenta[*b*]pyrrolo[1,2-*a*]azepine-11(10*H*),2′(5′*H*)-furan]-5′,10-dione skeleton with a spirolactone ring at C12 (**IV**, Fig. 2.2), which may be found in both absolute configurations. The stemonamine group includes the following *Stemona* alkaloids: stemonamine (**49**), sessilistemonamines A (**50**), sessilistemonamines B (**51**),

sessilistemonamines C (**52**), isooxymaistemonine (**53**), and isomaistemonine (**54**) (Fig. 2.6).

Parvistemoline group

The parvistemoline alkaloids lack the B-C ring fusion and have a hexahydro-2,6-dimethyl-5-oxofuro[3,2-*b*]furan-3-yl moiety attached to C-9 in the pyrrolo[1,2-*a*]azepine nucleus (**V**, Fig. 2.2). This group comprises the alkaloids parvistemoline (**55**), sessilifoliamide B (**56**), sessilifoliamide C (**57**), sessilifoliamide D (**58**), stemaphylline (**59**), stemaphylline *N*-oxide (**60**), stichoneurine A (**61**), stichoneurine B (**62**), and protostemodiol (**63**) (Fig. 2.7).

Stemofoline group

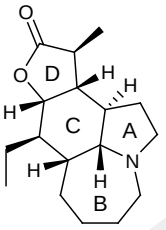
This group features a pentacyclic skeleton with an oxygen bridge between C2 and C8 and a carbon-carbon bond between C3 and C7 of the parent pyrrolo[1,2-*a*]azepine ring system with a β-methoxy-α-methyl-α,β-unsaturated γ-butyrolactone appended at C11 (**VI**, Fig. 2.2). Fifteen alkaloids have been reprorted: stemofoline (**64**), (11*S*,12*R*)-dihydrostemofoline (**65**), methylstemofline (**66**), (2′*R*)-hydroxystemofoline (**67**), (2′*S*)-hydroxystemofoline (**68**), (3′*R*)-stemofolenol (**69**), (3′*S*)-stemofolenol (**70**), stemofolinoside (**71**), 16,17-didehydro-16(*E*)-stemofoline (**72**), 1′,2′-didehydrostemofoline-*N*-oxide (**73**), 16,17-didehydro-(4*E*)-(16*E*)-stemofoline (**74**), stemoburkilline (**75**), 6β-hydroxystemofoline (**76**), 16-hydroxystemofoline (**77**), and isostemofoline (**78**) (Fig. 2.8).

Stemocurtisine group

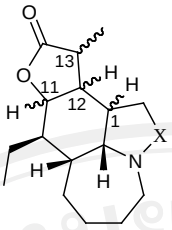
The stemocurtisine group is characterized by the presence of the pyrido[1,2-*a*]azepine nucleus (**VII**, Fig. 2.2). This group comprises twelve alkaloids: stemocurtisine (**79**), also named as pyridostemin, stemocochinamine (**80**), oxystemokerrilactone (**81**), cochinchistemonine (**82**), cochinchistemoninone (**83**), stemokerrin (**84**), stemokerrin-*N*-oxide (**85**), methylstemokerrin-*N*-oxide (**86**), stemosessifoine (**87**), stemocurtisinol (**88**), oxystemokerrin (**89**), and oxystemokerrin-*N*-oxide (**90**) (Fig. 2.9).

Miscellaneous group

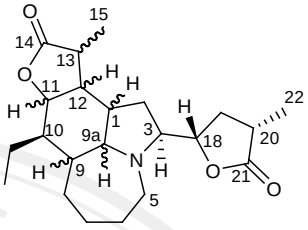
The miscellaneous group comprises those *Stemona* alkaloids that do not display the pyrrolo[1,2-*a*]azepine nucleus. Sixteen new alkaloids belonging to this class have being reported: neotuberostemoninol (**91**), maireistemoninol (**92**), sessilifoliamide I (**93**), sessilifoliamide E (**94**), sessilifoliamide F (**95**), tuberostemoline (**96**), sessilifoliamide G (**97**), sessilifoliamide H (**98**), neotuberostemonone (**99**), epoxytuberostemonone (**100**), sessilifoliamide J (**101**), tuberocrooline (**102**), parvineostemonine (**103**), sessilistemonamine D (**104**), 1,9a-seco-stemoenonine (**105**), and tuberostemonone (**106**) (Fig. 2.10).



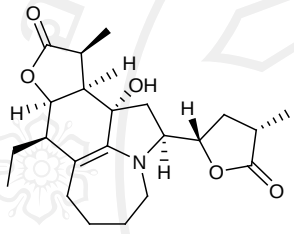
Stenine (**1**)



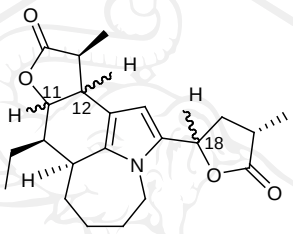
2- Oxostenine (**2**)
H1-α, H11-β, H12-β, H13-α, X= CO
Isostenine (**3**)
H1-β, H11-α, H12-α, H13-α, X= CH_2
Sessilifoline B (**4**)
H1-α, H11-β, H12-β, H13-β, X= CH_2



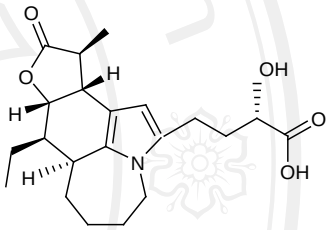
Tuberostemonine J (**5**)
H1-β, H9-α, H9a-α, H11-α, H12-α, H13-α
Tuberostemonine H (**6**)
H1-β, H9-β, H9a-α, H11-α, H12-α, H13-α
Tuberostemonine N (**7**)
H1-α, H9-β, H9a-β, H11-β, H12-β, H13-β
Tuberostemonine K (**8**)
H1-α, H9-β, H9a-β, H11-α, H12-α, H13-α



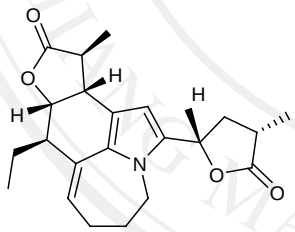
Neotuberostemonol (**9**)



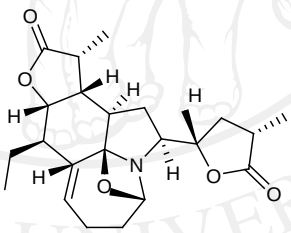
epi-Bisdehydroneotuberostemonine J (10)
H11-α, H12-α, H18-α
9α-Bisdehydrotuberostemonine (11)
H11-β, H12-β, H18-β



9α-Bisdehydrotuberostemonine A (12)

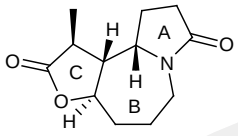


Tridehydrotuberostemonine (13)

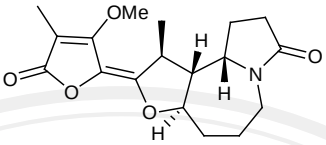


Sessilifoline A (**14**)

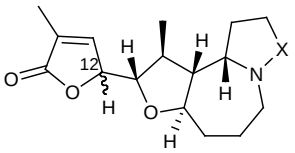
Figure 2.3 *Stemona* alkaloids of the stenine group.



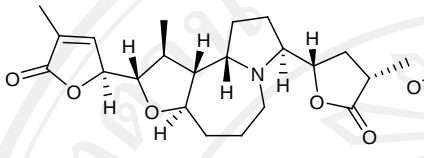
Stemoamide (**15**)



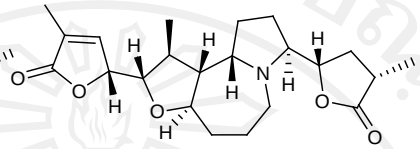
Protostemonamide (**16**)



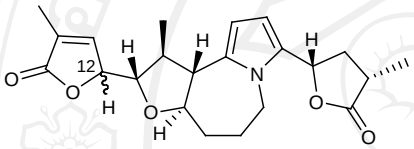
Saxorumamide (**17**)
H12-α, X= CO
Isosaxorumamide (**18**)
H12-β, X= CO
Neostemocochinine (**19**)
H12-β, X= CH_2
Isoneostemocochinine (**20**)
H12-α, X= CH_2



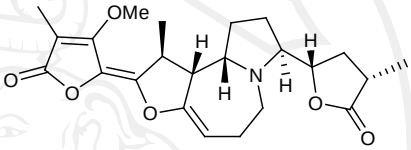
Stemocochinin (**21**)



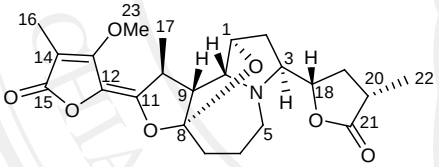
13-demethoxy-(11*S*,12R**)-dihydroprostemonine (**22**)



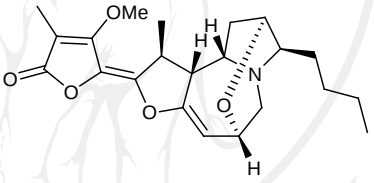
Bisdehydrostemocochinine (**23**) H12-β
Isobisdehydrostemocochinine (**24**) H12-α



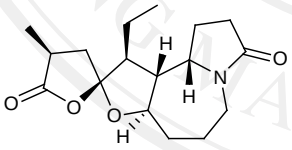
Dehydroprotostemonine (**25**)



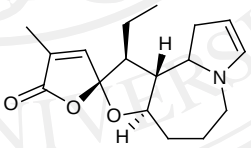
Oxyprotostemonine (**26**)



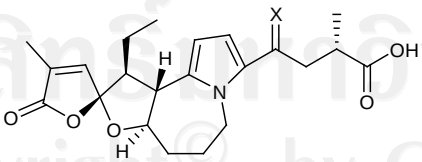
Neostemofoline (**27**)



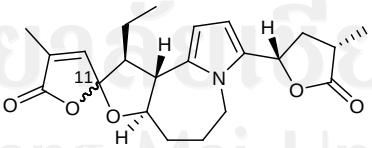
Sessilifoliamide A (**28**)



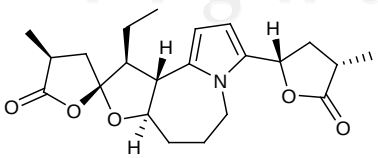
Bisdehydroneostemoninine (**29**)



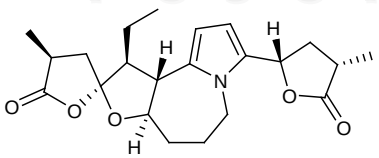
Bisdehydrostemoninine A (**30**) X= O
Bisdehydrostemoninine B (**31**) X= H_2



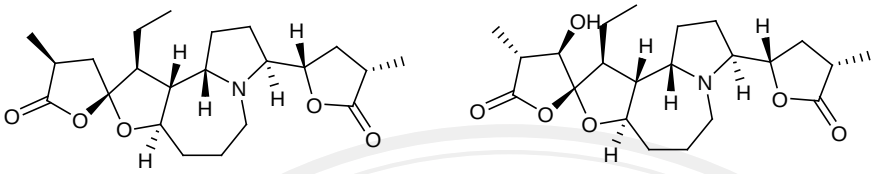
Bisdehydrostemoninine (**32**) C11-O-β
Isobisdehydrostemoninine (**33**) C11-O-α



Stemoninine A (**34**)

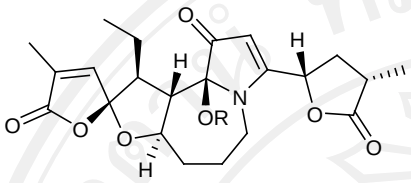


Stemoninine B (**35**)

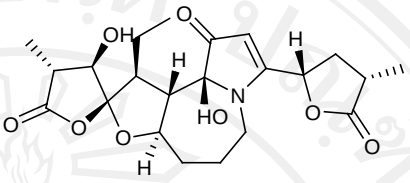


Dihydrostemoninine (**36**)

Oxystemoninine (**37**)

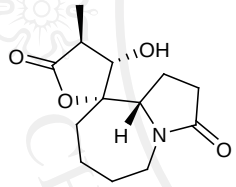


Stemoenonine (**38**) R=H
9a-*O*-Methylstemoenonine (**39**) R=CH_3

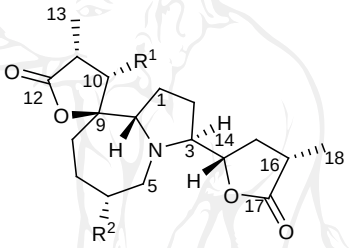


Oxystemoenonine (**40**)

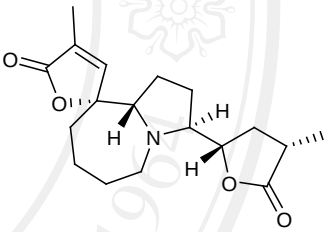
Figure 2.4 *Stemona* alkaloids of the stemoamide group.



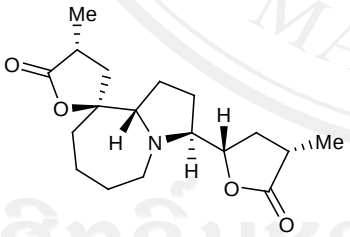
Tuberostemospironine (**41**)



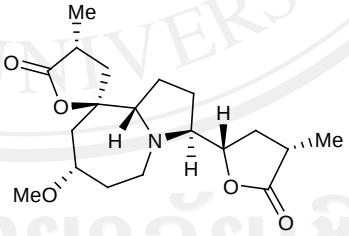
10-Hydroxycroomine (**42**)
R^1= OH, R^2= H
6-Hydroxycroomine(4**3**)
R^1= H, R^2= OH



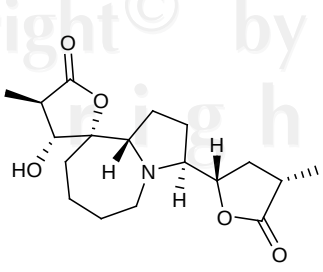
Dehydrocroomine (**44**)



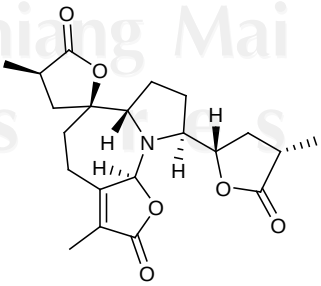
Croomine (**45**)



7-MethoxyCroomine (**46**)

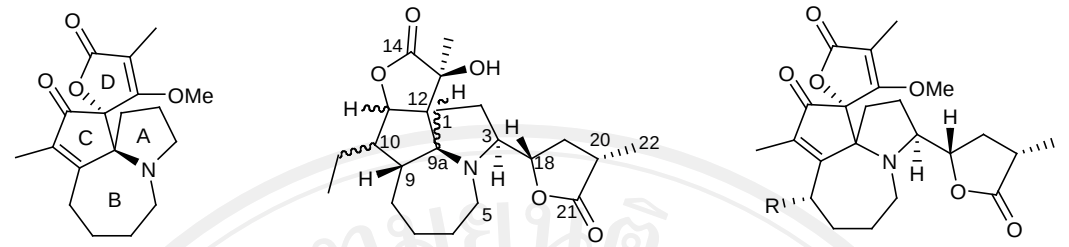


Tuberospironine (**47**)



Sessilifoliamine A (**48**)

Figure 2.5 *Stemona* alkaloids of the tuberostemospironine group.

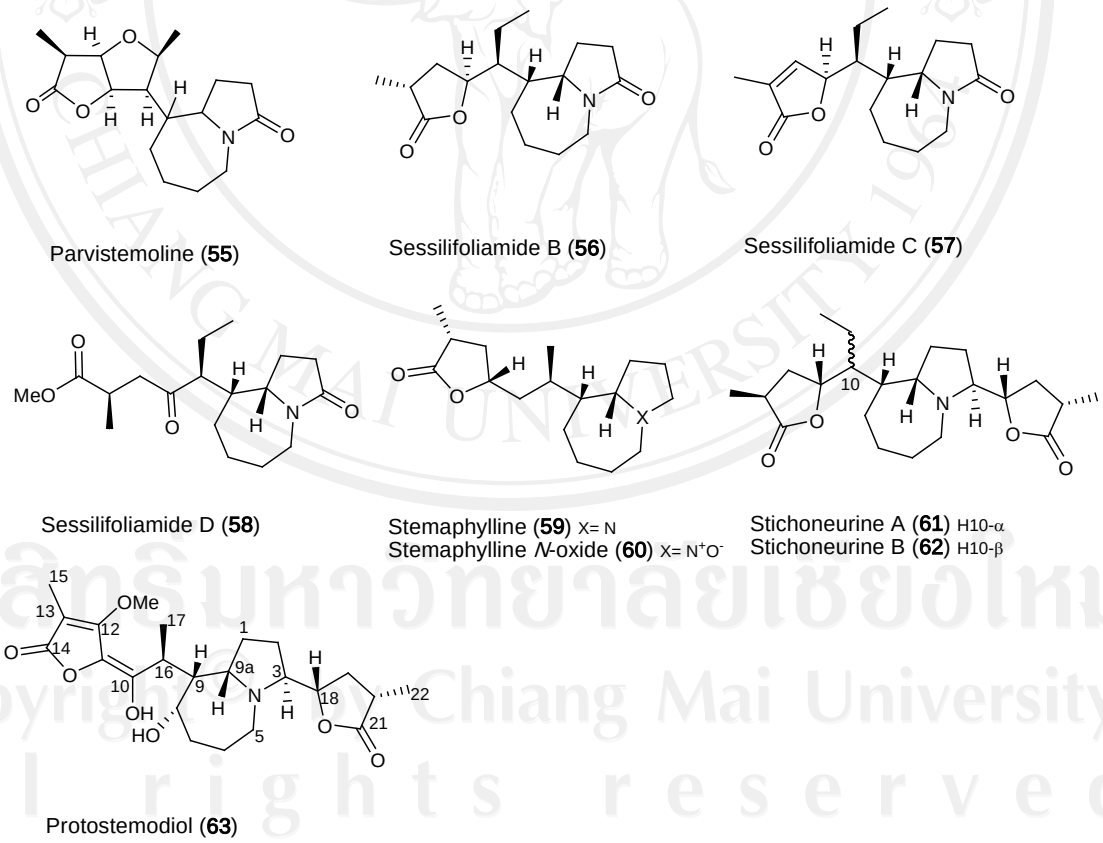


Stemonamine (**49**)

Sessilistemonamine A (**50**)
H10-β, H11-α, H12-α, C9a-C1-β
Sessilistemonamine B (**51**)
H10-α, H11-β, H12-β, C9a-C1-α
Sessilistemonamine C (**52**)
H10-α, H11-α, H12-α, C9a-C1-β

Isooxymaistemonine (**53**) R= OH
Isomaistemonine (**54**) R= H

Figure 2.6 *Stemona* alkaloids of the stemonamine group.



Parvistemoline (**55**)

Sessilifoliamide B (**56**)

Sessilifoliamide C (**57**)

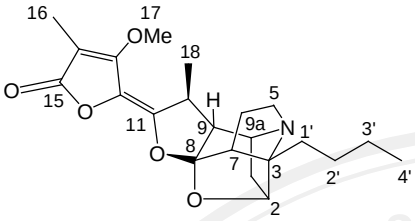
Sessilifoliamide D (**58**)

Stemaphylline (**59**) X= N
Stemaphylline *N*-oxide (**60**) X= N^+O^-

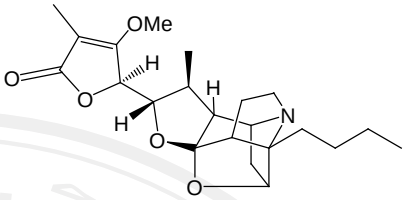
Stichoneurine A (**61**) H10-α
Stichoneurine B (**62**) H10-β

Protostemodiol (**63**)

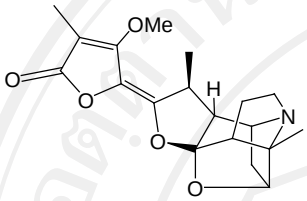
Figure 2.7 *Stemona* alkaloids of the parvistemoline group.



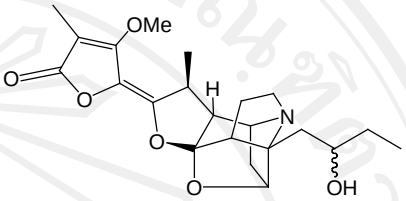
Stemofoline (**64**)



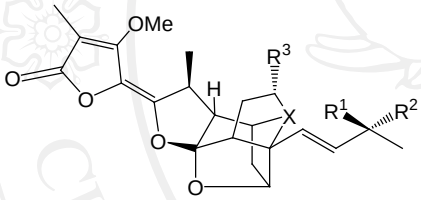
(11*S*,12*R*)-Dihydrostemofoline (**65**)



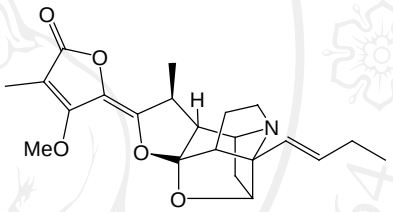
Methylstemofoline (**66**)



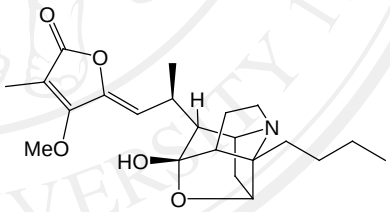
(2'*R*)-Hydroxystemofoline (**67**)
(2'*S*)-Hydroxystemofoline (**68**)



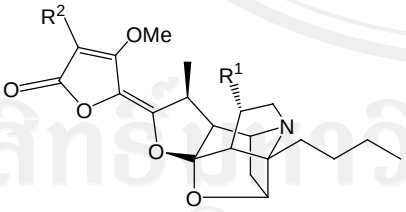
(3'*R*)-Stemofolenol (**69**)
R^1= H, R^2= OH, R^3= H, X= N
(3'*S*)-Stemofolenol (**70**)
R^1= OH, R^2= H, R^3= H, X= N
Stemofolinoside (**71**)
R^1= H, R^2= H, R^3= OGlu, X= N
16,17-Didehydro-16(*E*)-stemofoline (**72**)
R^1= H, R^2= H, R^3= H, X= N
1',2'-Didehydrostemofoline-*N*-oxide (**73**)
R^1= H, R^2= H, R^3= H, X= N^+O^-



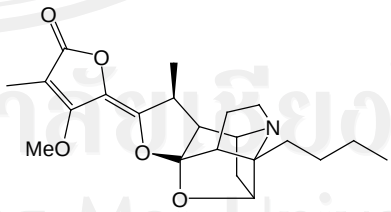
16,17-Didehydro-4(*E*)-16(*E*)-stemofoline (**74**)



Stemoburkilline (**75**)

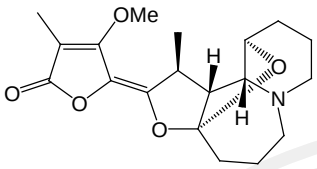


6β-Hydroxystemofoline (**76**)
R^1= OH, R^2= Me
16-Hydroxystemofoline (**77**)
R^1= H, R^2= CH_2OH

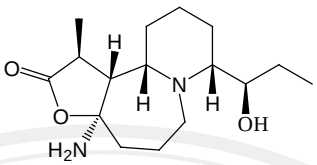


Isostemofoline (**78**)

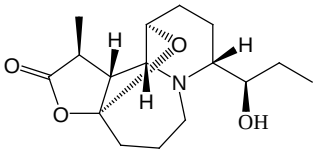
Figure 2.8 *Stemona* alkaloids of the stemofoline group.



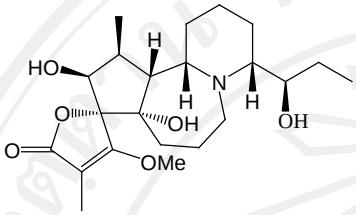
Stemocurtisine (**79**)



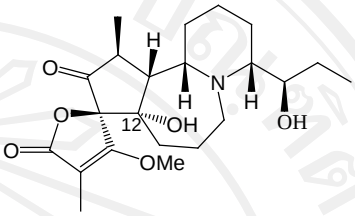
Stemocochinamine (**80**)



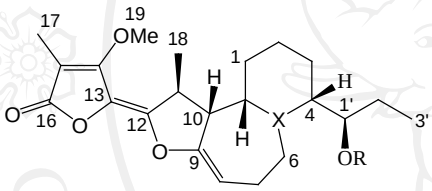
Oxystemokerrilactone (**81**)



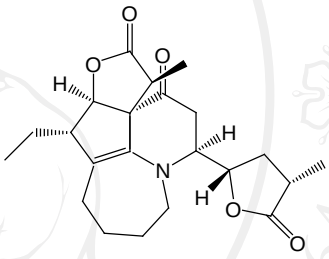
Cochinchistemonine (**82**)



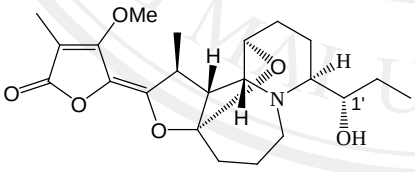
Cochinchistemoninone (**83**)



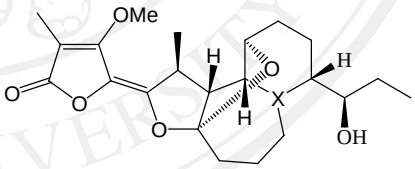
Stemokerrin (**84**)
X= N, R= H
Stemokerrin-*N*-oxide (**85**)
X= N^+O^-, R= H
Methoxystemokerrin-*N*-oxide (**86**)
X= N^+O^-, R= Me



Stemosessifoine (**87**)

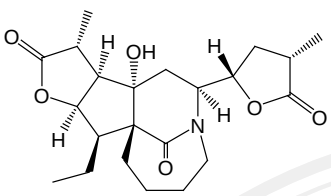


Stemocurtisinol (**88**)

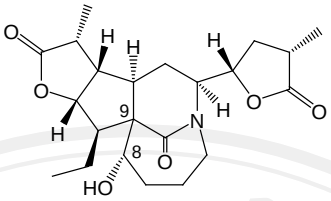


Oxystemokerrin (**89**)
X= N
Oxystemokerrin-*N*-oxide (**90**)
X= N^+O^-

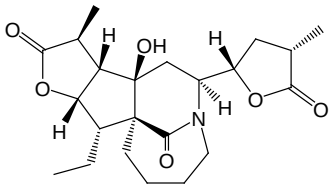
Figure 2.9 *Stemona* alkaloids of the stemocurtisine group.



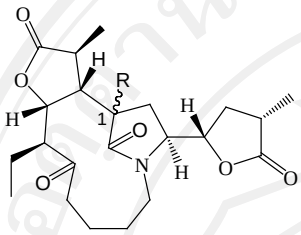
Neotuberostemoninol (**91**)



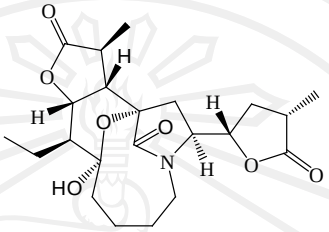
Maireistemoninol (**92**)



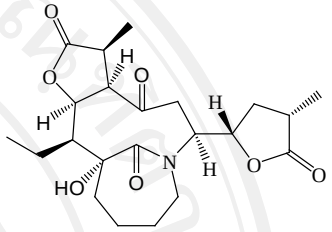
Sessilifoliamide (**93**)



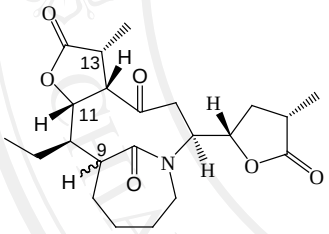
Sessilifoliamide E (**94**) R= H-β
Sessilifoliamide F (**95**) R= OH-β
Tuberostemoline (**96**) R= OH-α



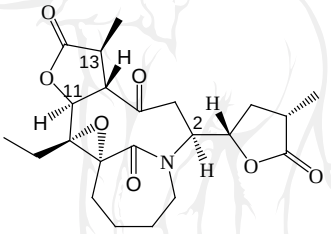
Sessilifoliamide G (**97**)



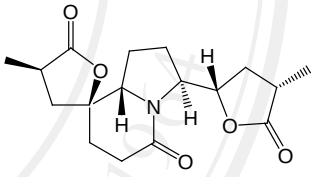
Sessilifoliamide H (**98**)



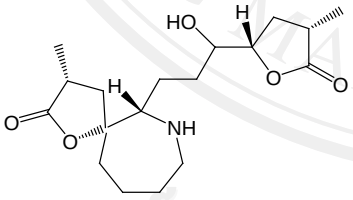
Neotuberostemonone (**99**)



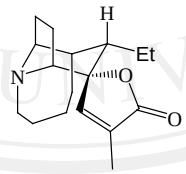
Epoxytuberostemonone (**100**)



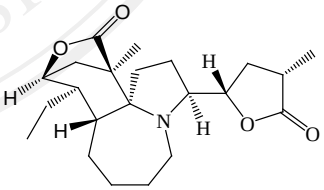
Sessilifoliamide J (**101**)



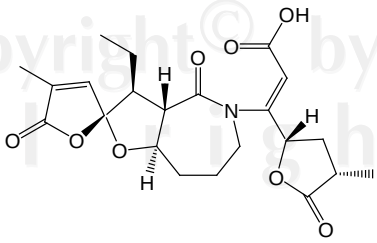
Tuberocrooline (**102**)



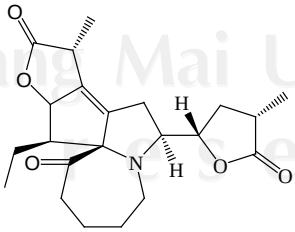
Parvineostemonine (**103**)



Sessilistemonamine D (**104**)



1,9a-seco-stemoenonine (**105**)



Tuberostemoenone (**106**)

Figure 2.10 *Stemona* alkaloids of the miscellaneous group**.**

2.4 Phytochemical studies

Phytochemical investigations of *Stemona* species, which involved their roots, rhizomes, leaves and stems have mostly resulted in the isolation of alkaloids that are shown in Table 2.1 (Pilli *et al*., 2010) whereas stilbenoid were the main non-alkaloid components shown in Table 2.2.

Table 2.1 *Stemona* alkaloids from *Stemona* spp. (Pilli *et al*., 2010)

***Stemona* species**	**Group**	**Alkaloids**
Stemona sp.	Parvistemoline (**V**)	Parvistemonine
	Stemofoline (**VI**)	Methylstemofoline
		(2′*R*)-Hydroxystemofoline
		(2′*S*)-Hydroxystemofoline
		(3′*R*)-Stemofolenol
		(3′*S*)-Stemofolenol
		Stemofolinoside
		(11*Z*)-1′, 2′-Dihydrostemofoline/
		16,17-didehydro-16(*E*)-stemofoline
		1′, 2′-Dihydrostemofoline-*N*-oxide
		(11*E*)-1′, 2′-Dihydrostemofoline
	Stemocurtisine (**VII**)	Stemocurtisine/ Pyridostemin
		Oxystemokerrin
S. aphylla	Parvistemoline (**V**)	Stemaphylline
		Stemaphylline *N*-oxide
	Stemofoline (**VI**)	Stemofoline
		(2′*S*)-Hydroxystemofoline
		(11*Z*)-1′, 2′-Dihydrostemofoline

Table 2.1 (Continued)

***Stemona* species**	**Group**	**Alkaloids**
S. burkillii	Stemofoline (**VI**)	Stemofoline
		(11*S*),12(*R*)-Dihydrostemofoline
		(2′*S*)-Hydroxystemofoline
		Stemoburkilline
S. cochinchinensis	Stemoamide (**II**)	Neostemocochinine
		Isoneostemocochinine
		Stemocochinin
		Bisdehydrostemocochinine
		Isobisdehydrostemocochinine
		Dehydroprotostemonine
		Oxyprotostemonine
		Bisdehydroprotostemonine
		Protostemonine
	Stemonamine (**IV**)	Isomaistemonine
		Maistemonine/ Protostemotinine
	Stemofoline (**VI**)	Stemofoline
		(2′*R*)-Hydroxystemofoline
		(2′*S*)-Hydroxystemofoline
	Stemocurtisine (**VII**)	Stemocochinamine
		Cochinchistemonine
S. collinsae	Stenine (**I**)	Isostenine/ Neostenine
		Bisdehydroneotuberostemonine
		Neotuberostemonine/ Tuberostemonine LG
	Stemofoline (**VI**)	Stemofoline
		(2′*S*)-Hydroxystemofoline
		(11*Z*)-1′, 2′-Dihydrostemofoline

Table 2.1 (Continued)

***Stemona* species**	**Group**	**Alkaloids**
S. collinsae	Stemofoline (**VI**)	(11*E*)-1′, 2′-Dihydrostemofoline
S. curtisii	Stemoamide (**II**)	Stemocochinin
		Dehydroprotostemonine
		Oxyprotostemonine
	Stemofoline (**VI**)	Stemofoline
		(2′*R*)-Hydroxystemofoline
		(2′*S*)-Hydroxystemofoline
	Stemocurtisine (**VII**)	Stemocurtisine/ Pyridostemin
		Oxystemokerrin
S. japonica	Stemoamide (**II**)	Stemocochinin
		13-Demethoxy-(11S^*,12R^*)-dihydroprotostemonine
		Neostemofoline
		Isoprotostemonine
		Protostemonine
	Tuberostemospironine (**III**)	Croomine
	Stemonamine (**IV**)	Stemonamine
		Isomaistemonine
		Isostemonamine
		Maistemonine/ Protostemotinine
	Parvistemoline (**V**)	Protostemodiol
	Stemofoline (**VI**)	Stemofoline
		6β-Hydroxystemofoline
		16-Hydroxystemofoline

Table 2.1 (Continued)

***Stemona* species**	**Group**	**Alkaloids**
S. kerrii	Stemoamide (**II**)	Stemocochinin
		Dehydroprotostemonine
		Oxyprotostemonine
		Protostemonine
	Stemocurtisine (**VII**)	Stemokerrin
		Methoxystemokerrin-*N*-oxide
		Oxystemokerrin
		Oxystemokerrin-*N*-oxide
S. mairei	Stenine (**I**)	2-Oxostenine
		Bisdehydroneotuberostemonine
		Neotuberostemonine
	Tuberostemospironine (**III**)	Stemotinine
	Miscellaneous (**VIII**)	Neotuberostemoninol
		Maireistemoninol
		Neotuberostemonone
		Epoxytuberostemonone
S. parviflora	Miscellaneous (**VIII**)	Parvineostemonine
S. cf. pierrei	Stemoamide (**II**)	Protostemonine
		Stemonine
S. saxorum	Stemoamide (**II**)	Saxorumamide
		Isosaxorumamide
		Stemocochinin
		Dehydroprotostemonine
		Oxyprotostemonine
		Isoprotostemonine
		Protostemonine

Table 2.1 (Continued)

***Stemona* species**	**Group**	**Alkaloids**
S. saxorum	Stemonamine (**IV**)	Stemonamine
		Isomaistemonine
		Maistemonine/ Protostemotinine
	Stemocurtisine (**VII**)	Oxystemokerrilactone
		Cochinchistemoninone
		Stemokerrin
		Stemokerrin-*N*-oxide
		Oxystemokerrin
		Oxystemokerrin-*N*-oxide
S. sessilifolia	Stenine (**I**)	Stenine
		2-Oxostenine
		Sessilifoline B
		Neotuberostemonol
		Sessilifoline A
		Bisdehydrotuberostemonine
		Tuberostemonine
	Stemoamide (**II**)	Stemoamide
		Protostemonamide
		Sessilifoliamide A
		Bisdehydrostemoninine
		Isobisdehydrostemoninine
		Stemoninine A
		Stemoninine B
		Dihydrostemoninine
		Bisdehydroprotostemonine
		Isoprotostemonine

Table 2.1 (Continued)

***Stemona* species**	**Group**	**Alkaloids**
S. sessilifolia	Stemoamide (**II**)	Neostemonine
		Protostemonine
		Stemoninine
		Tuberostemoamide/ Stemoninoamide
	Tuberostemospironine (**III**)	Sessilifoliamine A
		Stemospironine
	Stemonamine (**IV**)	Stemonamine
		Sessilistemonamines A
		Sessilistemonamines B
		Sessilistemonamines C
		Isooxymaistemonine
		Isomaistemonine
		Protostemotinine
		Isostemonamide
		Maistemonine
	Parvistemoline (**V**)	Sessilifoliamide B
		Sessilifoliamide C
		Sessilifoliamide D
	Stemocurtisine (**VII**)	Stemosessifoine
	Miscellaneous (**VIII**)	Sessilifoliamide I
		Sessilifoliamide E
		Sessilifoliamide F
		Sessilifoliamide G
		Sessilifoliamide H
		Sessilifoliamide J
		Sessilistemonamine D

Table 2.1 (Continued)

***Stemona* species**	**Group**	**Alkaloids**
S. sessilifolia	Miscellaneous (**VIII**)	Tuberostemonone
S. tuberosa	Stenine (**I**)	Isostenine/ neostenine
		Tuberostemonine J
		Tuberostemonine H
		Tuberostemonine N
		Tuberostemonine K
		Neotuberostemonol
		epi-Bisdehydroneotuberostemonine J
		9α-Bisdehydrotuberostemonine
		9α-Bisdehydrotuberostemonine A
		Tridehydrotuberostemonine
		Bisdehydrotuberostemonine
		Neotuberostemonine
		Tuberostemonine
		Tuberostemonine A
	Stemoamide (**II**)	Bisdehydroneostemoninine
		Bisdehydrostemoninine A
		Bisdehydrostemoninine B
		Bisdehydrostemoninine
		Isobisdehydrostemoninine
		Oxystemoninine
		Stemoenonine
		9a-*O*-Methylstemoenonine
		Oxystemoenonine
		Stemoninoamide
		Tuberostemoninoamide

Table 2.1 (Continued)

***Stemona* species**	**Group**	**Alkaloids**
S. tuberosa	Tuberostemospironine (**III**)	Tuberospironine
		Croomine
		Isostemotinine
		Tuberostemospironine
		10-Hydroxycroomine
		6-Hydroxycroomine/ 6α-hydroxycroomine
		Dehydrocroomine
		Stemotinine
	Miscellaneous (**VIII**)	Neotuberostemoninol
		Sessilifoliamide F
		Tuberostemoline
		Tuberocrooline
		1,9a-seco-Stemoenonine
		Tuberostemonone

Table 2.2 Non-alkaloid constituents from *Stemona* spp.

***Stemona* species**	**Non-alkaloids**	**References**
S. aphylla	Stilbostemin R	Mungkornasawakul *et al*., 2009
	Stilbostemin F	Sastraruji *et al*., 2011
	Stemofuran E	
	Stemofuran F	
	Stemofuran J	
	Stemofuran M	
	Stemofuran N	
	Stemofuran O	
	Stemofuran P	
	Stemofuran Q	
	Stemofuran R	
S. collinsae	Stemofuran A	Pacher *et al*., 2002
	Stemofuran B	
	Stemofuran C	
	Stemofuran D	
	Stemofuran E	
	Stemofuran F	
	Stemofuran J	
	Stemofuran K	
	Racemosol	
	Pinosylvin	
	4'-Methylpinosylvin	
	Stilbostemins A	
	Stilbostemins B	
	Stilbostemins C	

Table 2.2 (Continued)

***Stemona* species**	**Non-alkaloids**	**References**
S. collinsae	Stilbostemins D	Pacher *et al*., 2002
	Stilbostemins E	
	Stilbostemins F	
	Dehydro-δ-tocopherol	Brem *et al*., 2004
	Dehydro-β-tocopherol	
	Dehydro-α-tocopherol	
S. cochinchinensis	3,3'-Bis(3,4-dihydro-4-hydroxy-6,8-dimethoxy-2*H*-1-benzopyran)	Dien *et al*., 2008
	8'-Methoxy-3,3'-bis(3,4-dihydro-4-hydroxy-6-methoxy-2*H*-1-benzopyran)	
	3,3'-Bis(3,4-dihydro-4-hydroxy-6-methoxy-2*H*-1-benzopyran)	
S. curtisii	Dehydro-γ-tocopherol	Brem *et al*., 2004
	Stemofuran F	Chaiyong *et al*., 2010
	Stemofuran J	
	Stemofuran K	
	Stemofuran L	
	Dehydro-δ-tocopherol	
	Stigmasterol	
S. parviflora	Parvistemin A	Yang *et al*., 2007
	Parvistemin B	
	Parvistemin C	
	Parvistemin D	

Table 2.2 (Continued)

***Stemona* species**	**Non-alkaloids**	**References**
S. japonica	Japonin A	Yang *et al*., 2006
	Japonin B	
	Japonin C	
	Japonin D	
	Stilbostemin M	
	Stilbostemin C	
	Stilbostemin J	
	Stilbostemin K	
	Stilbostemin L	
	Stemanthrene F	
	3,5-Dihydroxy-4-methylbibenzyl	
	3,5-dihydroxy-2'-methoxy-4-methylbibenzyl	
	4-Hydroxybenzaldehyde	Pacher *et al*., 2002
	3,5-Dihydroxy-4-methylbibenzyl	
	Stigmasterol	
	4-Methoxybenzoic acid	
	Sesamin	
	lyciumamide	Noguchi *et al*., 1984
S. sessilifolia	Stilbostemin M	Zhang *et al*., 2007
	Stilbostemin N	
	Stilbostemin O	
	6-Methoxy-3,4-dehydro-δ-tocopherol	
	3,5-Dihydroxy-2'-methoxy bibenzyl	

Table 2.2 (Continued)

***Stemona* species**	**Non-alkaloids**	**References**
S. sessilifolia	3,5-Dihydroxy bibenzyl	Zhang *et al*., 2007
	β-tocopherol	
	γ-tocopherol	
	Stilbostemin H	Tong *et al*., 2007
	Stilbostemin I	
	Stilbostemin B	
	Stilbostemin D	
	Stilbostemin G	
	Stemanthrene A	
	Stemanthrene C	
S. tuberosa	Stilbostemin N	Lin *et al*., 2008
	Stilbostemin O	
	Stilbostemin P	
	Stilbostemin Q	
	Stilbostemin R	
	Stilbostemin S	
	Stilbostemin T	
	Stilbostemin U	
	Stilbostemin V	
	Stilbostemin X	
	Stilbostemin Y	
	Stemanthraquinone	
	Stilbostemin B 3'-*β*-D-glucopyranoside	Lee *et al*., 2006
	Stilbostemin H 3'-*β*-D-glucopyranoside	
	Stilbostemin I 2''-*β*-D-glucopyranoside	

2.5 Proposed biosynthesis of *Stemona* alkaloid

Only few publications have reported about the biosynthesis of any compounds from *Stemona* spp. Seger *et al*. (2004) has proposed the biosynthetic pathway leading to the pyrrolo[1,2-*a*]azepine *Stemona* alkaloids. Pyne *et al*. (2007) mentioned in his work that the terpenoid origin of the C- and D-ring carbons (Fig. 2.11) has been accepted by Seger, while the A-ring of these alkaloids has been arised from spermidine. Moreover, Kaltenegger *et al*. (2003) has proposed the biosynthesis of the pyrido[1,2-a]azepine *Stemona* alkaloids by an expansion of the pyrrolidine ring (A-ring) of protostemonine to a piperridine ring. This proposed mechanism does not account for the different stereochemistry at C-4 and C-19 in oxystemokerrin and stemocurtisinol.

Considering the insecticidal property correlate to chemical structure, oxystemokerrin was found to give the strongest activity with the LC_{50} value of 5.9 ppm. *N*-Oxidation in oxystemokerrin-*N*-oxide or insertion of double bond in stemokerrin produced less activity to LC_{50} values of 12.5 and 58 ppm, respectively. Loss of side chain in pyridostemin (LC_{50} = 149) or *O*-methylation in methoxystemokerrin-*N*-oxide (LC_{50} > 100 ppm) brought to the biggest drop in activity. In general compounds stemokerrin, oxystemokerrin, oxystemokerrin-*N*-oxide, pyridostemin and protostemonine were characterized by the paralysis and softening of the larval bodies (Brem *et al*., 2004).

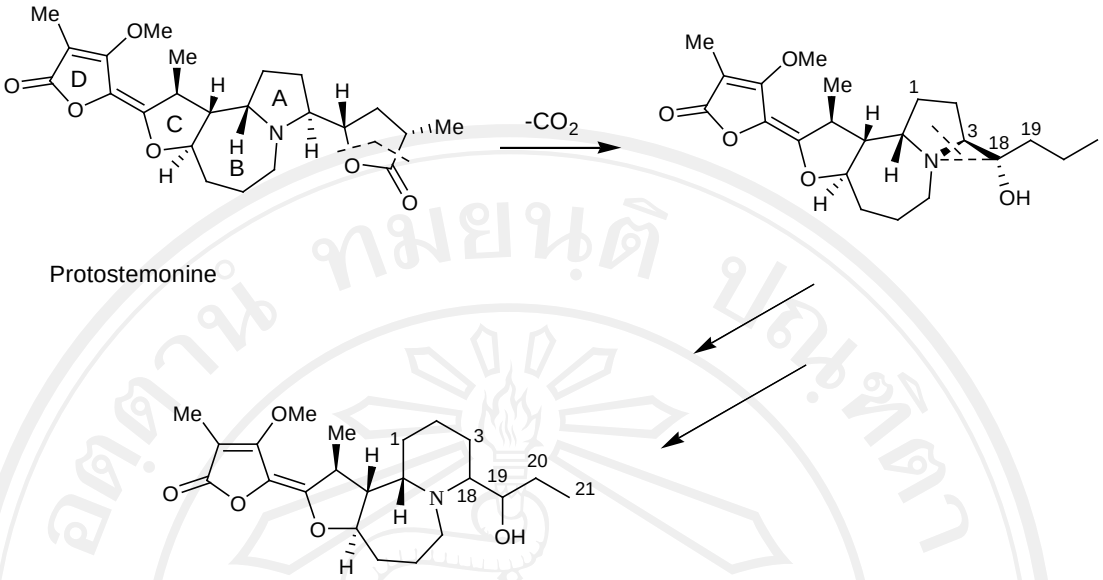


Fig. 2.11 Proposed biosynthesis of pyrido[1,2-*a*]azaepine alkaloids.