1-1-2007

The pyrido[1,2-a]azepine Stemona alkaloids

Stephen G. Pyne
University of Wollongong, spyne@uow.edu.au

Alison T. Ung
University of Wollongong, alison_ung@uow.edu.au

Araya Jatisatienr
Chiang Mai University

Pitchaya Mungkornasawakul
Chiang Mai University, pitchaya@uow.edu.au

Recommended Citation

Pyne, Stephen G.; Ung, Alison T.; Jatisatienr, Araya; and Mungkornasawakul, Pitchaya: The pyrido[1,2-a]azepine Stemona alkaloids 2007, 157-165.
https://ro.uow.edu.au/scipapers/3273

Abstract

This paper reviews the isolation, structure elucidation, proposed biosynthesis and biological activities of the small, but increasing, number of pyrido[1,2-*a*]azepine *Stemona* alkaloids.

Keywords

pyrido, azepine, Stemona, alkaloids, CMMB

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Publication Details

Pyne, S. G., Ung, A. T., Jatisatienr, A. and Mungkornasawakul, P. (2007). The pyrido[1,2-a]azepine Stemona alkaloids. Maejo International Journal of Science and Technology, 1 (2), 157-165.

The Pyrido[1,2-*a*]azepine *Stemona* alkaloids

Stephen G. Pyne,[1*] Alison T. Ung,[1] Araya Jatisatienr[2] and Pitchaya Mungkornasawakul[3]

[1] Department of Chemistry, University of Wollongong, Wollongong, New South Wales, 2522, Australia.

[2] Department of Biology, Chiang Mai University, Chiang Mai, 50202, Thailand.

[3] Department of Chemistry, Chiang Mai University, Chiang Mai, 50202, Thailand.

*Corresponding author, e-mail: spyne@uow.edu.au

Abstract: This paper reviews the isolation, structure elucidation, proposed biosynthesis and biological activities of the small, but increasing, number of pyrido[1,2-*a*]azepine *Stemona* alkaloids.

Keywords: *Stemona*, alkaloids, pyrido[1,2-*a*]azepine, insecticide

Introduction

This paper reviews the isolation, structure elucidation, proposed biosynthesis and biological activities of the small, but increasing, number of pyrido[1,2-*a*]azepine *Stemona* alkaloids.

The extracts of *Stemona* roots have been used in traditional medicine in South East Asia, China and Japan to treat the symptoms of bronchitis, pertussis and tuberculosis and have been used as anti-parasitics on humans and animals.[1,2] The *Stemona* plant is known in the Thai, Chinese and Vietnamese vernacular as, “Non Tai Yak”, “Bai Bu” and “Bach Bo”, respectively.[1,2] Some of the pure alkaloids derived from the extracts of the leaves and roots of *Stemona* species have been shown to have significant antitussive activity in guinea pig after cough induction[3] as well as insect toxicity, antifeedant and repellent activities.[4-6] The *Stemona* group of alkaloids includes more than eighty different natural products that have been structurally classified by Pilli into eight different groups.[1] The pyrrolo[1,2-*a*]azepine nucleus (5,7-bicyclic A,B-ring system) is common to all compounds in six of these groups (as in croomine (Figure 1 (a)), for example). Recently Greger has classified the *Stemona* alkaloids into three skeletal types based on their proposed biosynthetic origins.[2] These are the croomine, stichoneurine (as typified by neotuberostemonine) and the protostemonine skeletal types (Figure 1). Under Pilli's classification the pyrido[1,2-*a*]azepine *Stemona* alkaloids (Figure 2) fall under the stemocurtisine (**1**) structural group. While they are classified as of the protostemonine skeletal type under Greger's classification based on their proposed biosynthesis from protostemonine (see Proposed Biosynthesis section).

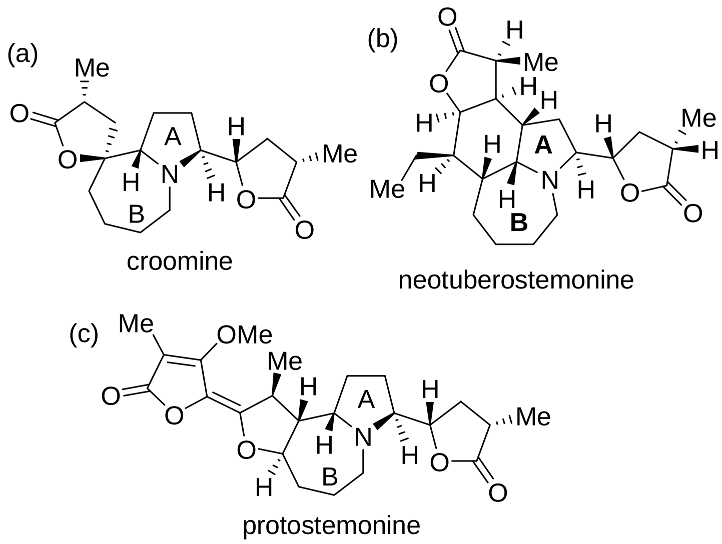


Figure 1. Skeletal types proposed by Greger.[2] (a) Croomine, from the croomine skeletal type; (b) neotuberostemonine, from the stichoneurine skeletal type; (c) protostemonine, from the protostemonine skeletal type.

The pyrido[1,2-*a*]azepine *Stemona* alkaloids

In 2003 we[7] and then Hofer and Greger[5] reported the first structures of *Stemona* alkaloids with a pyrido[1,2-*a*]azepine A,B-ring system. Our group reported the isolation of stemocurtisine **1** from the root extracts of *Stemona curtisii* growing in the northern part of Trang Province in Thailand.[7] The structure of stemocurtisine **1** was established by a single-crystal X-ray structural analysis. While Hofer and Greger reported the structures of stemocurtisine **1** (named pyridostemine in their paper), stemokerrin **2**, oxystemokerrin **3**, oxystemokerrin *N*-oxide **4** and methoxystemokerrin *N*-oxide **5**.[5] The structure of stemokerrin **2** was secured by a single-crystal X-ray structural analysis. These alkaloids were isolated from the root extracts of *S. kerri, S. curtisii* and an unknown species (*S. sp*.), designated as HG915. The *S. kerri* plant material grew in northern Thailand in Doi Suthep near Chiang Mai and in north-western Thailand in Khao Chomphu in Tak. The *S. curtisii* plant material came from southern Thailand in Satan Province while that from *S. sp* (HG 915) was from North-eastern Thailand in Udon Thani. The major alkaloid from the root extracts of both plant samples of *S. kerrii* was stemokerrin **2** with trace amounts of methoxystemokerrin **5** and oxystemokerrin *N*-oxide **4** also isolated, along with other pyrrido[1,2-*a*]azepine *Stemona* alkaloids. The *S. kerrii* species collected from Doi Sutep also provided oxystemokerrin **3** as a minor component. The root and leaf extracts of the *S. curtisii* plant material had trace amounts of oxystemokerrin **3** and its *N*-oxide **4** (in the leaves only) along with other pyrrido[1,2-*a*]azepine *Stemona* alkaloids. The roots and leaves of *S. sp* (HG 915) contained trace amounts of stemocurtisine **1**, oxystemokerrin **3** and its *N*-oxide **4** (in the leaves only).

In 2004 our group reported the isolation of stemocurtisinol **6** and oxyprotostemonine **7** (Figure 3) from the same root extracts of *Stemona curtisii* that earlier provided us with stemocurtisine **1** as the major alkaloid.[6] The structure of stemocurtisinol **6** was also established from a single-crystal X-ray structural analysis (Figure 4 (a)) while that of **7** was described earlier by Hofer and Greger.[5]

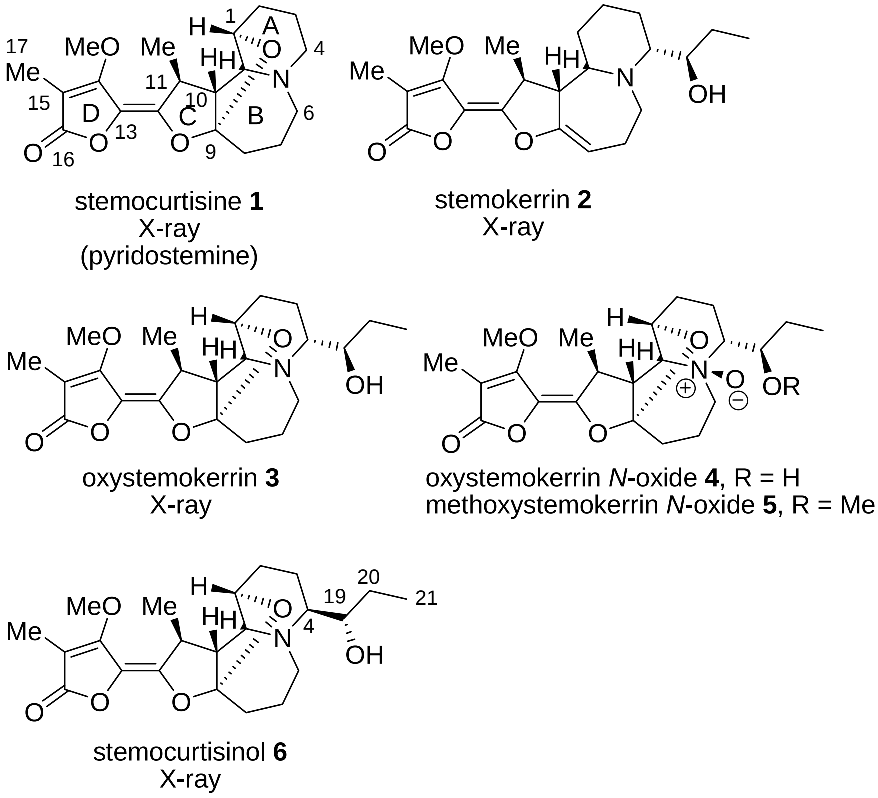


Figure 2. The first isolated pyrido[1,2-*a*]azepine *Stemona* alkaloids.

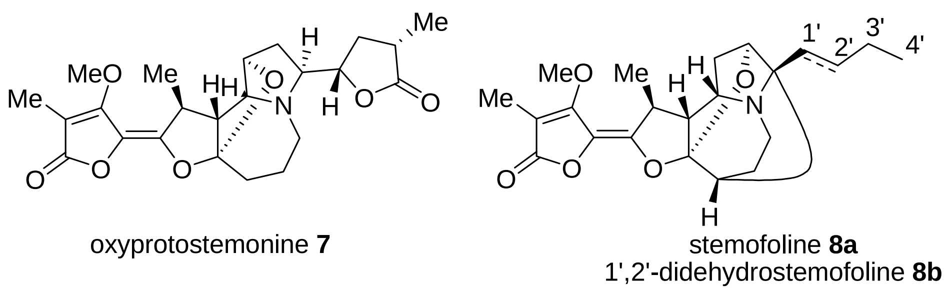
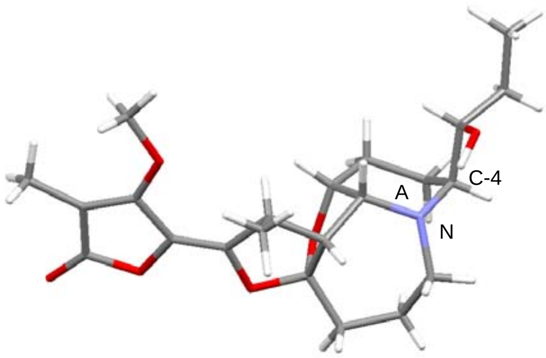


Figure 3. The structures of oxyprotostemonine **7** and stemofoline **8a** and 1’,2’-didehydrostemofoline **8b**.

Surprisingly, extracts of our *S. curtisii* plant sample and that of Hofer and Greger gave different pairs of pyrido[1,2-*a*]azepine *Stemona* alkaloids, **1** and **6** and **3** and **4**, respectively. In our plant sample, the pyrido[1,2-*a*]azepine *Stemona* alkaloids **1** and **6** were the major alkaloid components.[6,7] In the case of Hofer and Greger,[5] the pyrrolo[1,2-

a]azepine *Stemona* alkaloids, stemofoline **8a** and 2'-hydroxystemofoline **8b** (Figure 3) were the major components. Interestingly, oxystemokerrin **3** and stemocurtisinol **6** were diastereomers at C-4 and C-19 (Figure 2). To further confirm the structure of **3** we isolated it from the root extracts of *S. kerrii* that were collected at Tambol Mae Hea, Amphur Maung, Chiang Mai, in August 2003. Fortunately we were able to grow crystals of this compound and verified its structure by a single-crystal X-ray structural analysis (Figure 4 (b)).[8] Figure 4 shows that the A-ring of both stemocurtisinol **6**[6] and oxystemokerrin **3**[8] adopts a chair conformation in which the C-4 1'-hydroxypropyl substituent is axially and equatorially positioned, respectively. Furthermore, it is clear that these compounds have the opposite configurations at the secondary carbinol carbon (C-19). In the solid state the C-19 hydroxyl group is intramolecularly H-bonded to the nitrogen atom in both alkaloids. Our X-ray analysis confirmed the structural assignments made for **3** by Hofer and Greger using 2D NMR spectroscopic methods.

(a)



(b)

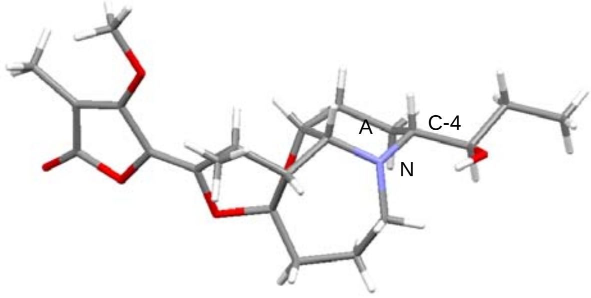


Figure 4. X-ray crystal structures of (a) stemocurtisinol **6**[6] and (b) oxystemokerrin **3**.[8]

In a more recent study Greger examined the alkaloid components of *S. curtisii* growing in different provinces in southern Thailand.[9] Plant collections from Krabi, Satun and Narathiwat showed stemofoline **8a** as the major alkaloid component with oxystemokerrin **3** and its *N*-oxide **4** present in varying amounts. While two different *S. cutisii* plant specimens from Chumphon were found to be rich in the pyrido[1,2-*a*]azepine *Stemona* alkaloids. One plant sample showed stemocurtisine **1** and stemocurtisinol **6** as the predominant alkaloid components, while the other showed oxystemokerrin **3** and its *N*-oxide **4** as the major components and stemocurtisine **1** and stemocurtisinol **6** as more minor components. Interestingly, this latter plant contained both of the diastereomeric alkaloids, stemocurtisinol **6** and oxystemokerrin **3**. This study clearly indicated the variability of the alkaloid profiles of *S. curtisii* samples within different regions of southern Thailand. The phytochemical profile of the former *S. curtisii* plant sample from Chumphon is consistent with our sample of *S. curtisii* collected in the southern Thai province of Trang.[6,7]

In work published in 2007, Ye[10,11] reported the isolation and identification of four new pyrido[1,2-*a*]azepine *Stemona* alkaloids from *S. sp.* from Vietnam (Figure 5). The root extracts of *S. cochinchinensis*, collected from the Sonla province of northern Vietnam, gave the alkaloid cochinchistemonine **9**, having a novel spirocyclic ring structure.[10] This structure was established by a single-crystal X-ray structural analysis. While the root extracts of *S. saxorum*, collected from the Hanam province of northern Vietnam, yielded the new alkaloids, cochinchistemoninone **10**, stemokerrin-*N*-oxide **11** and oxystemokerrilactone **12** along with oxystemokerrin **3**, its *N*-oxide **4** and stemokerrin **2**.[11] Other known pyrrolo[1,2-*a*]azepine *Stemona* alkaloids were also isolated. Cochinchistemonine **9** and cochinchistemoninone **10** and stemokerrin **2** and stemokerrin-*N*-oxide **11** are clearly related by oxidation-reduction chemical processes, while oxystemokerrilactone **12** is a truncated form of oxystemokerrin **3**.

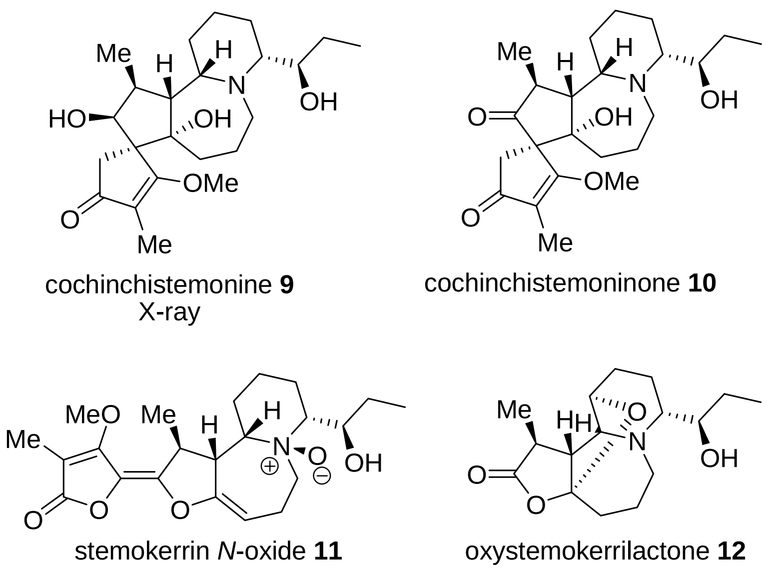
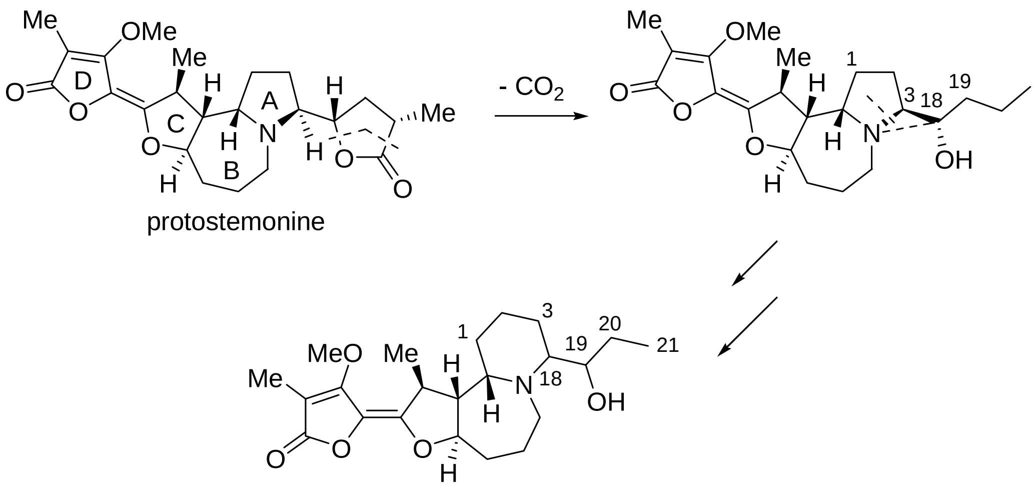


Figure 5. Pyrido[1,2-*a*]azepine *Stemona* alkaloids isolated from plants in Vietnam.[10,11]

Proposed Biosynthesis

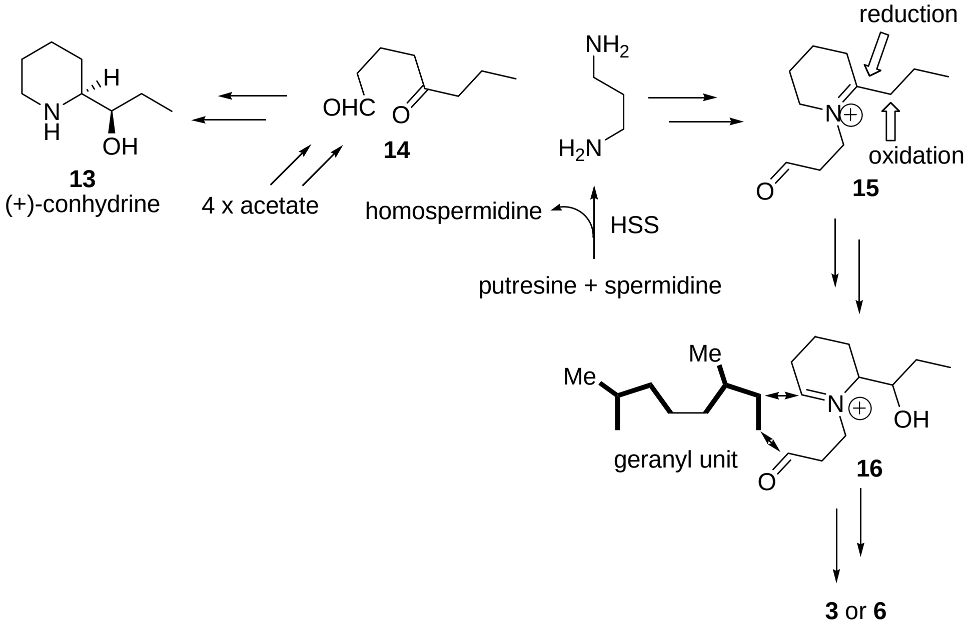
While biosynthetic studies on *Stemona* alkaloids have not been reported, a proposed biosynthetic pathway leading to the pyrrolo[1,2-*a*]azepine *Stemona* alkaloids has been made by Seger *et al.*[12] The terpenoid origin of the C- and D-ring carbons (see Scheme 1 for ring numbering) has been postulated by Seger, while the A-ring of these alkaloids has been postulated to arise from spermidine. A ring expansion of the pyrrolidine ring (A-ring) of protostemonine to a piperidine ring has been proposed by Hofer and Greger to account for the biosynthesis of the pyrido[1,2-*a*]azepine *Stemona* alkaloids (Scheme 1).[5] This proposed mechanism does not account for the different stereochemistries at C-4 and C-19 in oxystemokerrin **2** and stemocurtisinol **6**.

Scheme 1. Proposed biosynthesis of pyrido[1,2-*a*]azaepine alkaloids.[5]



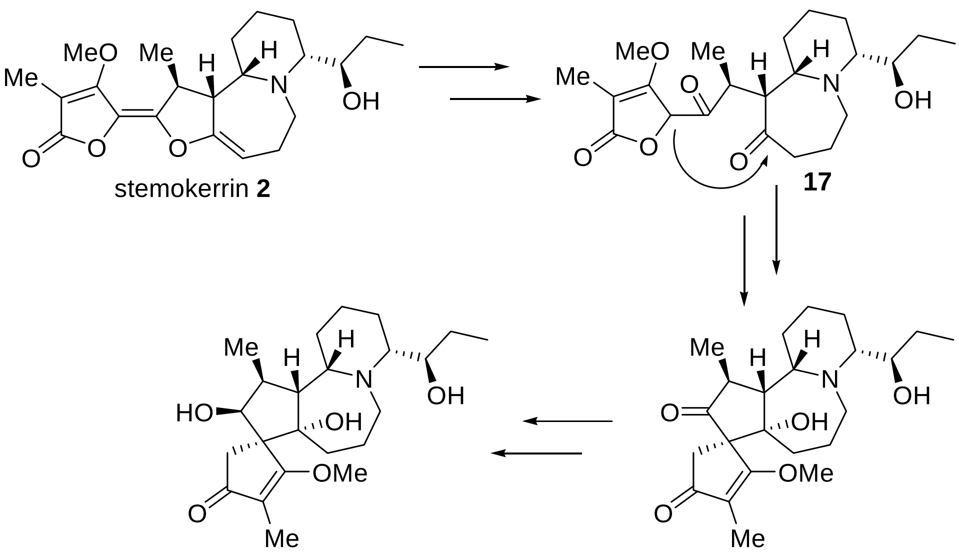
We have proposed an alternative biosynthesis as shown in Scheme 2.[8] Our proposed biosynthesis of the A-ring of **2** and **6** is based on the known biosynthesis of the hemlock alkaloid (+)-conhydrine **13** from the acetate-derived polyketide derivative **14**.[13,14] Condensation of **14** with 1,4-diaminopropane, a biosynthetic product from the homospermidine synthase (HSS) production of homospermidine,[15] could provide the piperidine A-ring precursor intermediate **15** (Scheme 2). A stereoselective reduction of the cyclic iminium ion intermediate **15** and a stereoselective oxidation at C-1 of the propyl side-chain of **15** may lead to intermediate iminium ion **16**. Coupling of **16** to a geranyl unit, as propsed by Seger *et al.*,[12] could then provide alkaloids **3** or **6** (Scheme 2). Whatever the biosynthetic pathway may be, however, it is clear that at least three *Stemona* species of plants have evolved to produce enzymes that give opposite stereochemical outcomes in their biosynthetic reactions, to produce stereoselectively either oxystemokerrin (**3**) or stemocurtisinol (**6**).

Scheme 2. Proposed biosynthesis of alkaloids **3** and **6**.[8]



In the case of the spirocyclic ring structure types, Ye has proposed that cochinchistemonine **9** and cochinchistemoninone **10** are derived biosynthetically from stemokerrin **2**.[10] Hydrolysis of the enol ether group of stemokerrin **2** would be expected to give the diketone **17** which could undergo an intramolecular aldol reaction to give cochinchistemoninone **10**. A diastreoselective reduction of the ketone group of cochinchistemoninone **10** would then give cochinchistemonine **9** (Scheme 3).

Scheme 3. Proposed biosynthesis of alkaloids **9** and **10**.[10]



Biological studies

We have examined the larvicidal activity of our crude root extract of *S. curtisii* and that of compounds **1**, **6** and **7** on mosquito larvae (*Anopheles minimus* HO), using the WHO method to determine the LC_{50}.[6] While the crude ethanol extract showed a LC_{50} of 81 ppm, the individual alkaloid components were significantly more potent (LC_{50} values of **1** and **6** were, 18 and 39 ppm, respectively). The most potent compound was oxyprotostemonine **7** having a LC_{50} of 4 ppm. We have also demonstrated that the crude root extracts of *S. curtisii* shows strong insect antifeedant activities against third instar larvae of *Spodoptera littoralis* Boisduval.[16] The crude extract has been formulated into a “biopesticide” that shows great potential in agricultural field trials as an effective “natural pesticide”.[16] Hofer and Greger also demonstrated that their crude extract of *S. curtisii* had insecticidal activity against neonate larvae of *Spodoptera littoralis* with a LC_{50} of 9 ppm.[5] While their crude extracts of *S. kerrii* showed less insecticidal activity with LC_{50} values of 48 (from Doi Sutep sample) and 89 (from Khao Chomphu sample) ppm. The individual alkaloids were also tested. Amongst the pyrido[1,2-*a*]azepine *Stemona* alkaloids tested (**1-5**) the most potent was oxystemokerrin **3** (LC_{50} of 5.9 ppm), whereas stemocurtisine **1** was the least active (LC_{50} of 149 ppm). Two of the pyrrolo[1,2-*a*]azepine *Stemona* alkaloids also tested were much more potent, especially 1',2'-didehydrostemofoline **8b** (LC_{50} of 0.8 ppm) and stemofoline **8a** (LC_{50} of 2.0 ppm). These latter two compounds caused hyperactivity of the larvae resulting in their sudden death. Whereas the pyrido[1,2-*a*]azepine *Stemona* alkaloids **1-5** only led to paralysis and the softening of the larval bodies.[5]

A recent study by Limtrakul,[17] showed that the crude root extract of *S. curtisii*, collected from Udon Thani in Thailand, inhibited the drug-efflux pump, P-glycoprotein. This study indicated the potential application of these extracts for the treatment of multidrug-resistant cancers. The alkaloid components of this extract were not determined. Future studies may involve the testing of the individual pure components.

Conclusions

In conclusion, the pyrido[1,2-*a*]azepine *Stemona* alkaloids represent a small subset (about 10%) of the increasing number of *Stemona* alkaloids that have been discovered. In this paper the isolation, structure elucidation, proposed biosynthesis and biological activities of these alkaloids is reviewed from the time that they were first reported in 2003.

Acknowledgments: We are grateful to the staff and students at the Universities of Wollongong and Chiang Mai for their valuable contributions to this project. Their names are indicated in our publications. We are also grateful to the Universities of Wollongong and Chiang Mai and the Department of Environmental Quality Promotion, Ministry of Natural Resources and Environment, Thailand for supporting this project.

References

1. Pilli, R. A.; Rosso, G. B. de Oliveira, M. C. F. "The *Stemona* alkaloids", *The Alkaloids*, (Editor G. A. Cordell) **2005**, *Vol 62*, Chapter 2, pp 77-173, Elsevier, Amsterdam.
2. Greger, H., "Structural relationships, distribution and biological activities of *Stemona* alkaloids", *Planta Medica* **2006**, *72*, 99-113.

3. Chung, H.-S.; Hon, P.-M., Lin, G.; But, P. P,-H., Dong, H., "Antitussive activity of *Stemona* alkaloids from *Stemona tuberosa*", *Planta Med*. **2003**, *69*, 914-920.
4. Brem, B.; Seger, C.; Pacher, T.; Hofer, O.; Vajrodaya, S.; Greger, H., "Feeding deterrence and contact toxicity of *Stemona* alkaloids - a source of potent natural insecticides", *J. Agric. Food Chem*. **2002**, *50*, 6383-6388.
5. Kaltenegger, E.; Brem, B.; Mereiter, K.; Kalchhauser, H.; Kahlig, H.; Hofer, O.; Vajrodaya, S.; Greger, H., "Insecticidal pyrido[1,2-*a*]azepine alkaloids and related derivatives from *Stemona* species", *Phytochemistry* **2003**, *63*, 803-816.
6. Mungkornasawakul, P.; Pyne, S. G.; Jatisatienr, A.; Supyen, D.; Jatisatienr, C.; Lie, W.; Ung, A. T.; Skelton, B. W.; White, A. H., "Phytochemical and larvicidal studies on *Stemona curtisii*: structure of a new pyrido[1,2-*a*]azepine *Stemona* alkaloid", *J. Nat. Prod*. **2004**, *67*, 675-677.
7. Mungkornasawakul, P.; Pyne, S. G.; Jatisatienr, A.; Supyen, D.; Lie, W.; Ung, A. T.; Skelton, B. W.; White, A. H., "Stemocurtisine, the first pyrido[1,2-*a*]azepine *Stemona* alkaloid", *J. Nat. Prod*. **2003**, *66*, 980-982.
8. Mungkornasawakul, P.; Matthews, H.; Ung, A. T.; Pyne, S. G.; Jatisatienr, A.; Lie, W.; Skelton, B. W.; White, A. H., "Confirmation of the structure of oxystemokerrin by single crystal X-ray structural analysis and a proposed biosynthesis", *ACGC Chem. Res. Commun.* **2005**, *19*, 30-33.
9. Schinnerl, J.; Brem, B.; But, P. P.-H.; Vajrodaya, S.; Hofer, O.; Greger, H., "Pyrrolo- and pyridoazepine alkaloids as chemical markers in *Stemona* species", *Phytochemistry* **2007**, *68*, 1417-1427.
10. Lin, L.-G.; Tang, C.-P.; Dien, P.-H.; Xu, R.-S.; Ye, Y., "Cochinchistemonine, a novel skeleton alkaloid from *Stemona cochinchinensis*", *Tetrahedron Lett.* **2007**, *48*, 1559-1561.
11. Wang, Y.-Z.; Tang, C.-P.; Dien, P.-H.; Ye, Y., "Alkaloids from the Roots of *Stemona saxorum*", *J. Nat. Prod*. **2007**, *70*, 1356-1359.
12. Seger, C.; Mereiter, K.; Kaltenegger, E.; Pacher, T.; Greger, H.; Hofer, O. *Chem. & Biodiversity* **2004**, *1*, 265-279.
13. Leete, E., Biosynthesis of the hemlock alkaloids. The incorporation of acetate-1-C14 into coniine and conhydrine. *J. Am. Chem. Soc*. **1964**, *86*, 2509-2513.

14. ROMPP Encyclopedia, Natural Products, Steglich, W.; Fugmann, B.; Lang-Figmann, Ed. George Thieme Verlag, Stuttgart, 1997, p 285-286.
15. Ober D; Hartmann T., "Homospermidine synthase, the first pathway-specific enzyme of pyrrolizidine alkaloid biosynthesis, evolved from deoxyhypusine synthase", *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 14777-14782.
16. Sastraruji, T.; "Bioinsecticide production from *Stemona* extract and its application in agriculture use", PhD thesis, Chiang Mai University, Thailand, November 2006, pp 96-104.
17. Limtrakul, P.; Siwanon, S.; Yodkeeree, S.; Duangrat, C., "Effect of *Stemona curtisii* root extract on P-glycoprotein and MRP-1 function in multidrug-resistant cancer cells", *Phytomedicine* **2007**, *14*, 381-389.