Chapter III

Studies of *Stemona* spp.

3.1 Introduction

Stemona genus

The genus *Stemona,* previously named as *Roxburghia* (Prain, 1905) belongs to the Stemonaceae family (monocotyledon), which is classified under the order of Dioscoreales. This family also includes the genus *Croomia* and *Stichoneuron* (Duyfjes, 1993; Pilli and Ferreira de Oliveira, 2000).

The *Stemona* genus is distributed through Southeastern Asia, Malaysia and Northern Australia (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' in Thailand. There are at least 25 species in this genus and 6 species have been found in Thailand (Rungrojsakul, 2001) but only a few of them have been studied phytochemically (Brem *et al.*, 2002; Seger *et al.*, 2004).

Stemona alkaloids

Most phytochemical studies on the *Stemona* genus focused on isolation and structural elucidation of *Stemona* alkaloids which are very rich in the roots and also

found in leaves, stem and rhizomes (Duyfjes, 1993; Pilli and Ferreira de Oliveira, 2000).

The structures of the *Stemona* alkaloids are relatively complex. Therefore, X-ray crystallographic analysis has been widely used for their structure elucidation (Pilli and Ferreira de Oliveira, 2000).

The structure of *Stemona* alkaloids from at least 7 species have been reported as shown in Table 3.1.

Table 3.1 *Stemona* alkaloids from *Stemona* spp.

***Stemona* spp**	***Stemona* alkaloids**	**References**
S. collinsae	neotuberostemonine **34**	(Pham *et al.*, 2002)
	bisdehydroneotuberostemonine **33**	(Pham *et al.*, 2002)
	isostenine **41**	(Pham *et al.*, 2002)
	2'-hydroxystemofoline **73**	(Seger *et al.*, 2004)
	16,17-didehydro-16(*E*)-stemofoline **72**	(Jiwajinda *et al.*, 2001)
	16,17-didehydro-4(*E*)-16(*E*)- stemofoline **76**	(Jiwajinda *et al.*, 2001)
	Stemofoline **3**	(Jiwajinda *et al.*, 2001)
S. japonica	bisdehydroneostemonine **48**	(Pilli and Ferreira de Oliveira, 2000)
	bisdehydroprotostemonine **50**	(Pilli and Ferreira de Oliveira, 2000)
	isostemoamine **63**	(Pilli and Ferreira de Oliveira, 2000)
	isostemonamide **65**	(Pilli and Ferreira de Oliveira, 2000)
	maistemonine (protostemotinie) **66**	(Pilli and Ferreira de Oliveira, 2000)
	neostemonine **47**	(Pilli and Ferreira de Oliveira, 2000)
	neostemodiol (stemodiol) **54**	(Pilli and Ferreira de Oliveira, 2000)
	oxymaistemonine **67**	(Pilli and Ferreira de Oliveira, 2000)
	protostemonine **49**	(Pilli and Ferreira de Oliveira, 2000)
	protostephanine	(Jiyavoranan, 2001)
	stemonidine **60**	(Pilli and Ferreira de Oliveira, 2000)
	stemoamide **45**	(Pilli and Ferrejra de Oliveira, 2000)
	stemonine **46**	(Pilli and Ferreira de Oliveira, 2000)
	stemoamine **62**	(Pilli and Ferreira de Oliveira, 2000)
	stemofoline **3**	(Pilli and Ferreira de Oliveira, 2000)
	stemospironine **57**	(Pilli and Ferreira de Oliveira, 2000)
	tuberostemonine B **35**	(Zou *et al.*, 1999)

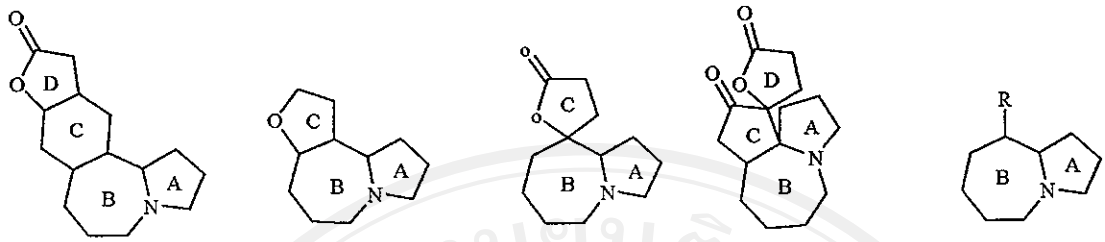
Stemona spp	*Stemona* alkaloids	References
S. mairei	protostemonine **49**	(Pilli and Ferreira de Oliveira, 2000)
	maistemonine (protostemotinine) **66**	(Pilli and Ferreira de Oliveira, 2000)
	oxymaistemonine **67**	(Pilli and Ferreira de Oliveira, 2000)
S. ovata	stemonidine **60**	(Jiyavoranan, 2001)
	stemonine **46**	(Jiyavoranan, 2001)
S. parviflora	parvistemoline **69**	(Pilli and Ferreira de Oliveira, 2000)
	parvistemonine **70**	(Pilli and Ferreira de Oliveira, 2000)
	didehydroparvistemonine **71**	(Pilli and Ferreira de Oliveira, 2000)
	stemofoline **3**	(Pilli and Ferreira de Oliveira, 2000)
	oxystemofoline **74**	(Pilli and Ferreira de Oliveira, 2000)
	methoxystemofoline **75**	(Pilli and Ferreira de Oliveira, 2000)
	parvistemoninine **77**	(Pilli and Ferreira de Oliveira, 2000)
	parvistemoninol **78**	(Pilli and Ferreira de Oliveira, 2000)
	parvistemoamide **81**	(Ke *et al.*, 2003)
S. sessilifolia	oxotuberostemonine **44**	(Pilli and Ferreira de Oliveira, 2000)
	protostemotinine (maistemonine) **66**	(Pilli and Ferreira de Oliveira, 2000)
	stenine **28**	(Pilli and Ferreira de Oliveira, 2000)
	stemoninine **53**	(Pilli and Ferreira de Oliveira, 2000)
	tuberostemonine **29**	(Pilli and Ferreira de Oliveira, 2000)
	tuberostemonine A **30**	(Kakuta *et al.*, 2003)
	stenine **23**	(Kakuta *et al.*, 2003)
	stemoninoamide **82**	(Kakuta *et al.*, 2003)
	tuberostemonone **79**	(Kakuta *et al.*, 2003)
	neotuberostemonol **36**	(Kakuta *et al.*, 2003)
	sessilifoliamides A **83**	(Kakuta *et al.*, 2003)
S. tuberosa	bisdehydroneotuberostemonine **33**	(Pilli and Ferreira de Oliveira, 2000)
	didehydrotuberostemonine **32**	(Pilli and Ferreira de Oliveira, 2000)
	neotuberostemonine **34**	(Pilli and Ferreira de Oliveira, 2000)
	oxotuberostemonine **44**	(Pilli and Ferreira de Oliveira, 2000)
	stemoamide **45**	(Pilli and Ferreira de Oliveira, 2000)
	stemofoline **3**	(Pilli and Ferreira de Oliveira, 2000)
	stenine **28**	(Pilli and Ferreira de Oliveira, 2000)
	stemotinine **58**	(Pilli and Ferreira de Oliveira, 2000)
	tuberostemonone **79**	(Pilli and Ferreira de Oliveira, 2000)
	tuberostemonine **29**	(Pilli and Ferreira de Oliveira, 2000)
	tuberostemonol **31**	(Pilli and Ferreira de Oliveira, 2000)

Stemona spp	*Stemona* alkaloids	References
	tuberostemoamide(stemoninoamide) **52**	(Pilli and Ferreira de Oliveira, 2000)
	tuberostemospironine **55**	(Chung *et al.*, 2003)
	tuberostemonine J **38**	(Chung *et al.*, 2003)
	tuberostemonine H **39**	(Chung *et al.*, 2003)
	epi-bisdehydrotuberostemonineJ **42**	(Chung *et al.*, 2003)
	neostenine **40**	(Jiang *et al.*, 2002)
	neotuberostemonol **36**	(Jiang *et al.*, 2002)
	neotuberostemoninol **37**	(Jiang *et al.*, 2002)

Structural classification

The *Stemona* alkaloid structures contain a characteristic pyrrolo[1,2-*a*]azepine nucleus (Fig 3.1). The *Stemona* alkaloids have been classified according to their structural features into 5 groups, these are stenine **(23)**, stemoamide **(24)**, tuberostemospironine **(25)**, stemoamine **(26)**, and tuberostemoamide **(27)**. Additionally, the *Stemona* alkaloids that lacked this common nucleus were classified into a miscellaneous group. Currently, at least forty-two structures have been reported (Pilli and Ferreira de Oliveira, 2000; Seger *et al.*, 2004)

Figure 3.1 Pyrrolo[1,2-*a*]azepine nucleus

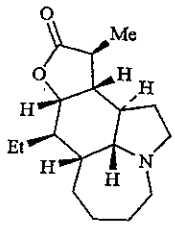


(23) (24) (25) (26) (27)

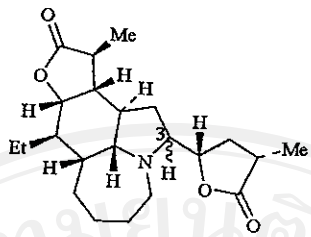
Figure 3.2 The five *Stemona* alkaloid groups

Stenine group

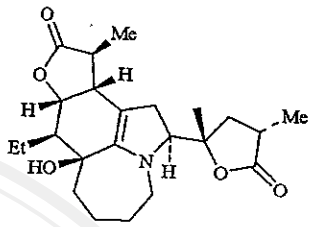
The structural characteristic of this group is the tetracyclic furo[2,3-*h*]pyrrolo[3,2,1-*jk*]benzazepin-10(2H)-one nucleus **(23)**. Currently, seven stemona alkaloids; stenine **(28)**, tuberostemonine **(29)**, tuberostemonine A **(30)**, tuberostemonol **(31)**, didehydrotuberostemonine **(32)**, bisdehydroneotuberostemonine **(33)**, neotuberostemonine **(34)**, tuberostemonine B **(35)**, neotuberostemonol **(36)**, neotuberostemoninol **(37)**, tuberostemonine J **(38)**, tuberostemonine H **(39)**, neostenine **(40)**, isostenine **(41)** and epi-bisdehydrotubrerostemonine J **(42)** and tuberostemonine C **(43)** (Fig. 3.3), has been reported to be members of this group. Additionally, oxotuberostemonine **(44)**, an artefact formed by air oxidation of tuberostemonine **(29)** has been included in this group. The structure of oxotuberostemonine was closely related to this group but with the oxygen atom of the lactone ring D reallocated from C-11 to C-1, keeping the same relative configuration. Its structure also includes a hydroxy group at C-11 and a double bond at C-9 and C-9a.



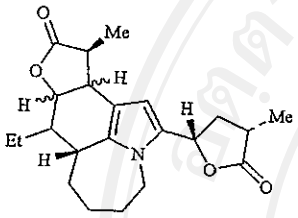
(28)



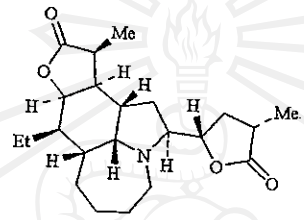
(29) H3= α
(30) H3= β



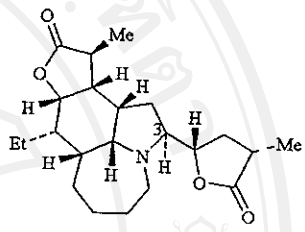
(31)



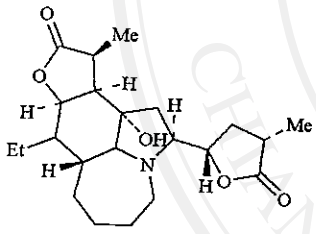
(32) H11=β, β=Et
(33) H11=α, H12=α



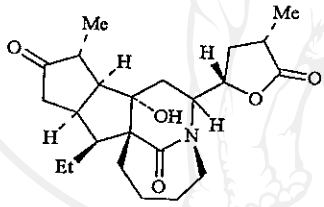
(34)



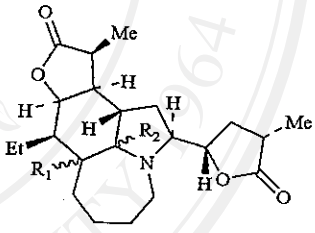
(35)



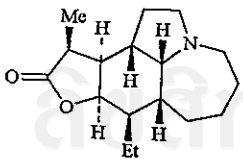
(36)



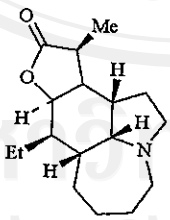
(37)



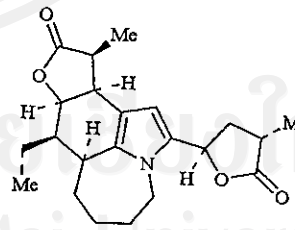
(38) R1=R2=α-H
(39) R1=β–H, R2=α-H



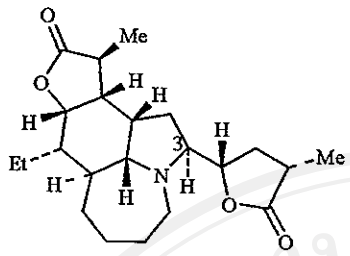
(40)



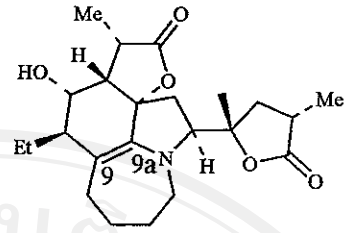
(41)



(42)



(43)

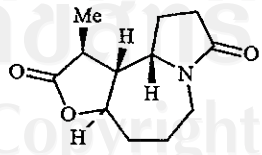


(44)

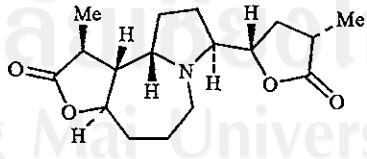
Figure 3.3 *Stemona* alkaloids of the stenine group

Stemoamide group

The *Stemona* alkaloids which have the tricyclic 2H-furo[3,2-*c*]pyrrolo[1,2-*a*]azepine nucleus **(24)** are classified as belonging to the stemoaminde group. Initially, nine *Stemona* alkaloids; stemoamide **(45)**, stemonine **(46)**, neostemonine **(47)**, bisdehydroneostemonine **(48)**, protostemonine **(49)**, didehydroprotostemonine **(50)**, isoprotostemonine **(51)**, tuberostemoamide **(52)** and stemoninine **(53)** (Fig. 3.4) were members of this group. Recently, neostemodiol **(54)** or stemodiol has been included in this group, because, although lacking ring C, it can be related to neostemonine via dehydration to form ring C.



(45)



(46)

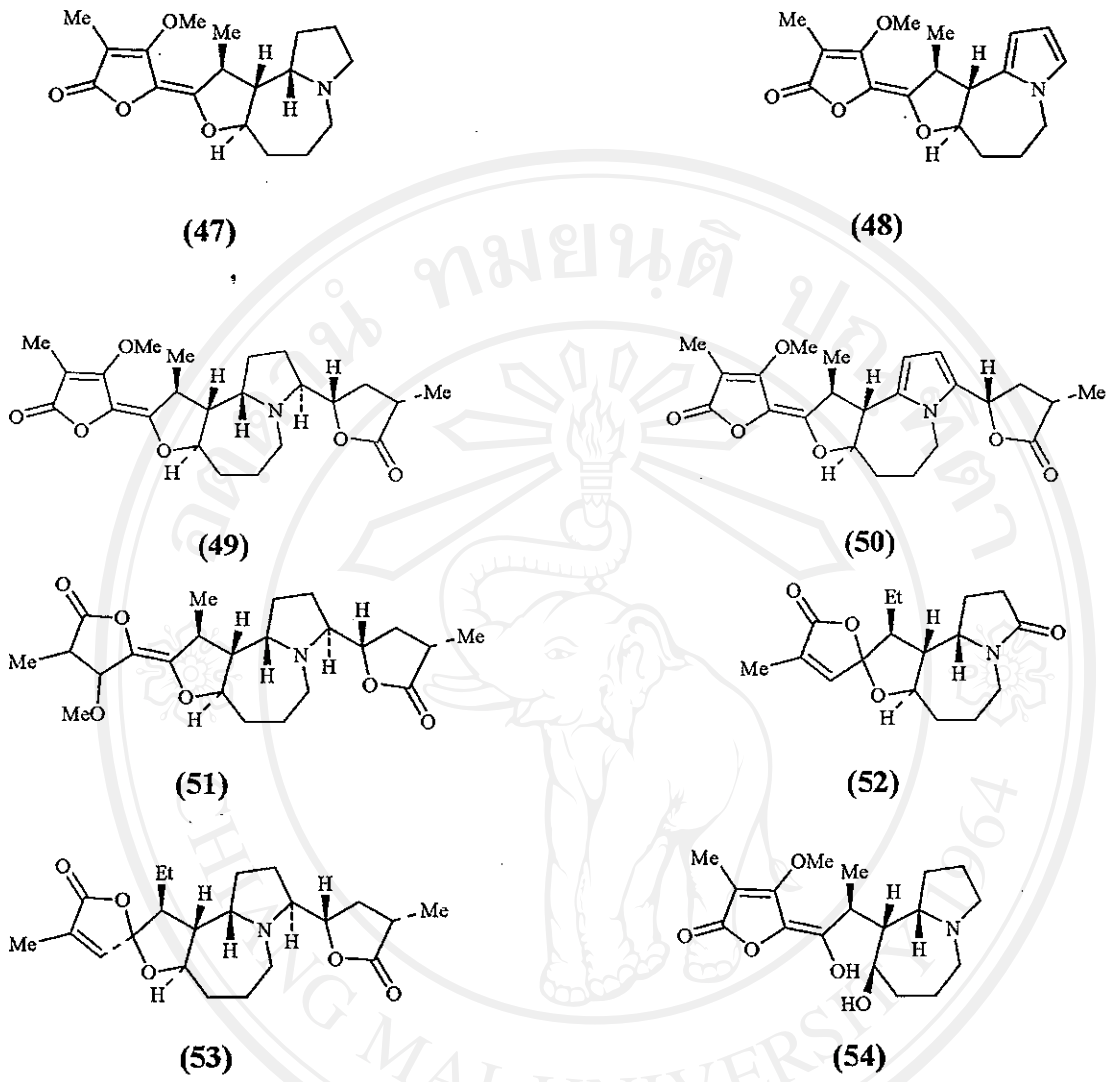
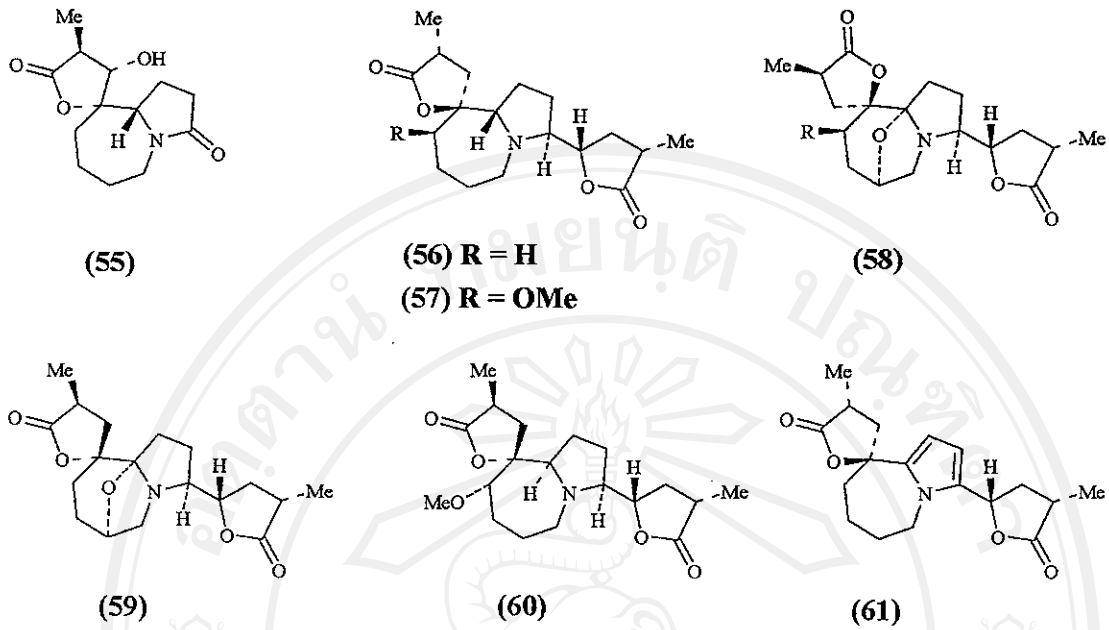


Figure 3.4 *Stemona* alkaloids of the stemoamide group

Tuberostemospironine group

There are seven *Stemona* alkaloids in this group; tuberostemospironine **(55)**, croomine **(56)**, stemospironine **(57)**, stemotinine **(58)**, isostemotinine **(59)**, stemonidine **(60)** and didehydrocroomine **(61)** (Fig 3.5), which have a 2H-spiro[furan-2,9'[9H]pyrrolo[1,2-*a*]azepin]-5-one nucleus and a spiro γ-lactone at C-9 of the basic ring **(25)**.



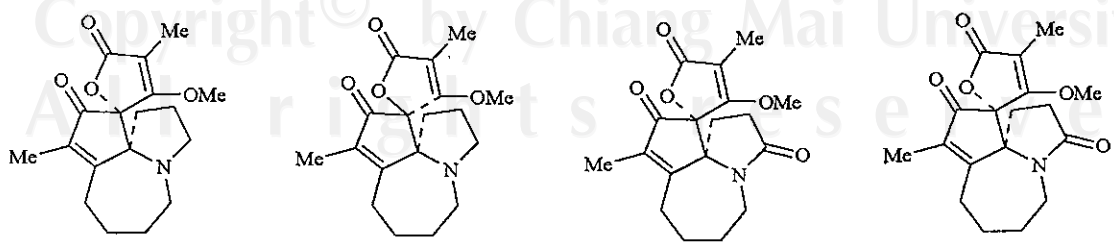
(55) (56) R = H (57) R = OMe (58)

(59) (60) (61)

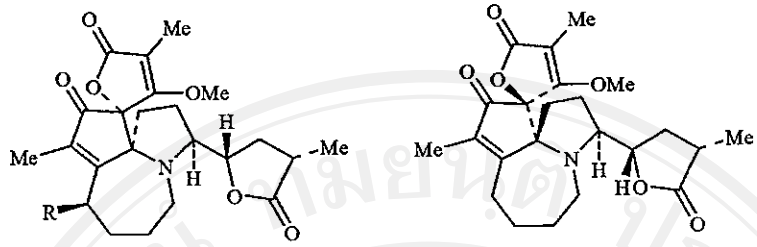
Figure 3.5 *Stemona* alkaloids of the tuberostemospironine group

Stemonamine group

The structural characteristic of this group is a tetracyclic 2'H, 11H-spiro[1H-cyclopenta-[b]pyrrolo[1,2-*a*]azepine-11,2'-furan]-5',10-dione nucleus with a spirolactone ring at C-12 **(26)**. The *Stemona* alkaloids in this group are stemonamine **(62)**, isostemonamine **(63)**, stemonamide **(64)**, isostemonamide **(65)**, maistemonine **(66)**, oxymaistemonine **(67)** and bisdehydrotuberostemonine B **(68)** (Fig 3.6).



(62) (63) (64) (65)



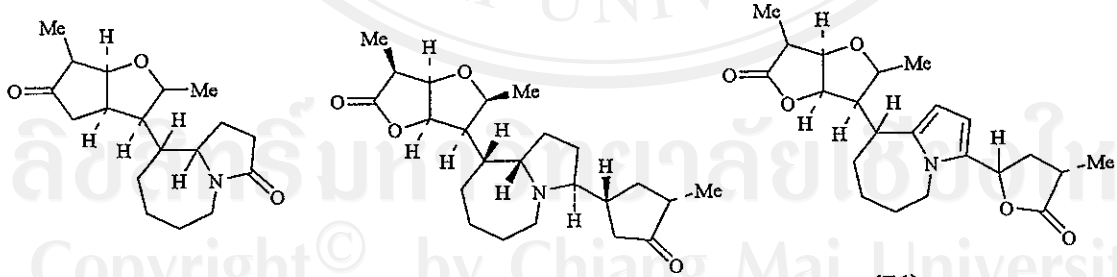
(66) R = H
(67) R =OH

(68)

Figure 3.6 *Stemona* alkaloids of the stemonamine group

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 **(27)**. Members in this group are parvistemoline **(69)**, parvistemonine **(70)** and didehydroparvistemonine **(71)** (Fig 3.7).



(69) **(70)** **(71)**

Figure 3.7 *Stemona* alkaloids of the parvistemoline group

Miscellaneous group

Thirteen *Stemona* alkaloids are represented in this group. These members consist of stemofoline **(3)**, 16,17-didehydro-16(E)-stemofoline **(72)**, 2'-hydroxystemofoline **(73)**, oxystemofoline **(74)**, methoxystemofoline **(75)**, 16,17-didehydro-4(E)-16(E)-stemofoline **(76)** parvistemoninine **(77)**, parvistemoninol **(78)** tuberostemonone **(79)**, tuberostemoninol **(80)**, parvistemoamide **(81)**, stemoniamide **(82)** and sessilifoliamide A **(83)**. Most of their structures have been elucidated by single-crysal X-ray analysis.

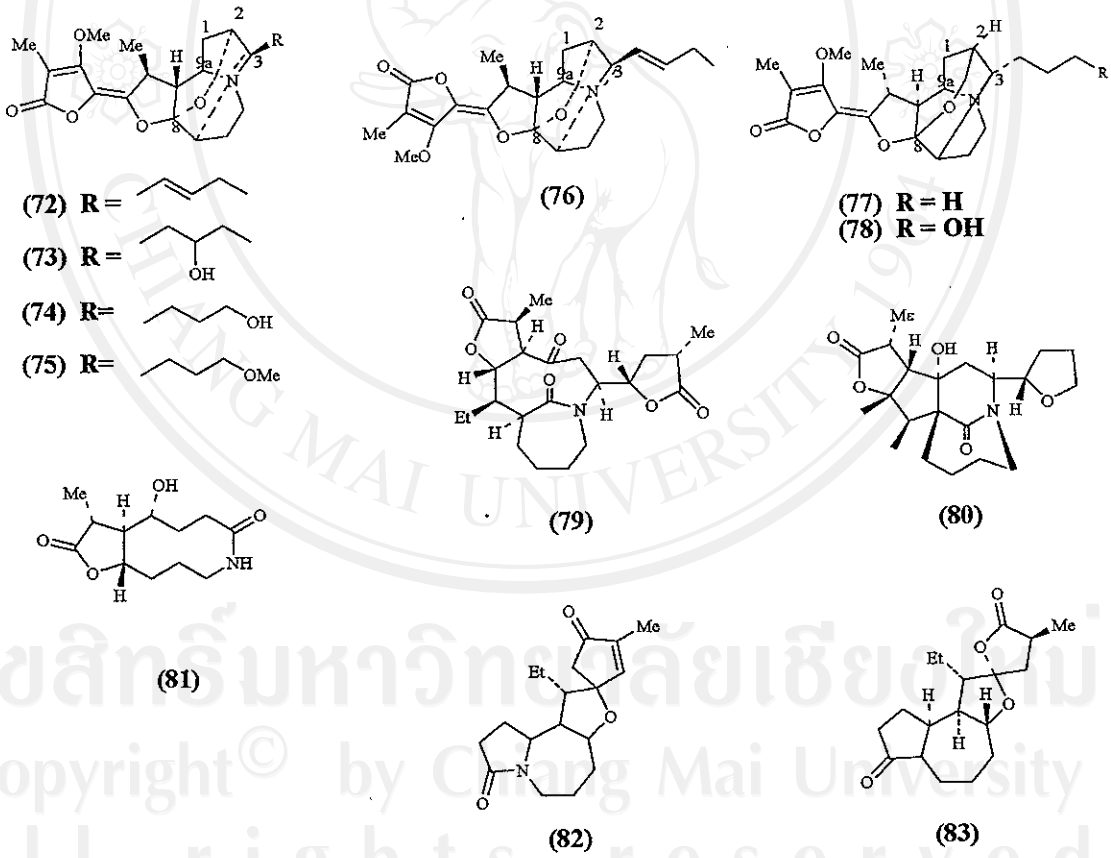


Figure 3.8 *Stemona* alkaloids of the miscellaneous group

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 used in the treatment of various respiratory diseases and used as anticough agents in China and Japan (Pilli and Ferreira de Oliveira, 2000; Brem *et al.*, 2002; Ye, Qin and Xu, 1994; Qin and Xu, 1998 and Terada *et al.*, 1982).

Many investigations have described the insecticidal properties of the *Stemona* species. Pilli and Ferreira de Oliveira (2000) 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 antifeeding 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. Brem *et al.* (2002) indicated that the methanolic leaf and root extracts from *S. collinsae* 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 activity. Moreover, *S. collinsae* extracts also showed strong antifeedant activity against fifth instar larvae in leaf disk choice, whereas *S. tuberosa* showed repellency activity. Additionally, it was found that the antiinsect properties of both species were based on pyrrolo[1,2-*a*]azepine alkaloids, namely didehydrostemofoline which was the major compound of the root of *S. collinsae* and stemofoline that displayed contact toxicity and feeding assay. While, tuberostemonine was the major alkaloid in the root of *S. tuberosa* which

demonstrated repellency but no toxic effects. On the other hand, four new stenine-type *Stemona* alkaloids that are 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).

For fungicidal activity from *Stemona* species, the literature indicated that fifteen new stilbenoids and four dihydrostilbenes were extracted from a methanolic extract of *Stemona collinsae* roots and showed antifungal activity against *Cladosporium herbarum*. While, other antifungal stilbenoids were found from *S.* cf. *pierrei* (Kosteck *et al.*, 2004).

Synthetic studies

Many synthetic approaches to the *Stemona* alkaloids were also studied. The last review demonstrated that the total synthesis of stenine, stemoamide and croomine have been studied by various researchers (Pilli and Ferreira de Oliveira, 2000). Recently, the first total synthesis of (±)-didehydrostemofoline (I) and (±)-isodidehydrostemofoline were reported by Brueggemann *et al.* (2003). The other *Stemona* alkaloids synthesis were described in the literature (Subramaian *et al.*, 2003; Ginn *et al.*, 2002; Wipf *et al.*, 2002; Velazquez *et al.*, 2002; Hinman and Heathcock, 2001; Morimoto *et al.*, 2001; Jacobi *et al.*, 2000; Pilli and Ferreira de Oliveira, 2000).

Stemona curtisii Hook F.

S. curtisii is distributed in Sri-Lunka (rare), Thailand and Malaysia. It is a glabrous twiner and has 10 cm long tuberous roots, which form a bundle. It has

alternate, seldom opposite leaves. Flowers (Fig 3.9) are tepars pink, brown pink and brownish red. This species can be found near waterfalls, the shore and on the riverbanks (Duyfjes, 1993). There have been no phytochemical studies and a few biological activities have been reported. The effect of the crude root extract on the action potential of the frog sciatic nerve and its toxicity on house fly larvare (*Culex p. fatigans* and *Aedes aegypti*) have been reported (Prucksunand *et al.*, 1977).

Figure 3.9 Flower of *S. curtisii*

Stemona burkillii Prain.

S. burkillii was found in the northern part of Mienma and in the north of Thailand. It has stout and hypogeous rootstock and ovate, acute, slightly cordate leaves. Flowers (Fig. 3.10) are externally greenish-red and within dull-red (Maxwell, 1991) There has been no phytochemical or biological activity studies done on this species.

Figure 3.10 Flower of *S. burkillii*

Stemona kerrii Craib.

S. kerrii was found in the north of Thailand. Its characteristic is similar to *S. burkillii*, but can be classified by having pubescent stems, leaves, inflorescence axes and tepals (Fig 3.11). Additionally, S. *kerrii* is less vigorous and always twines or sprawls (Duyfjes, 1993). At the start of this project there were no phytochemical or biological activity studies on this species.

Figure 3.11 Flower of *S. kerrii*

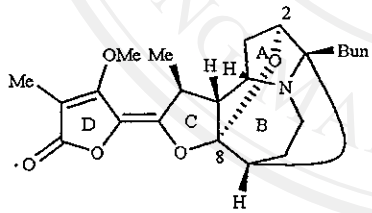
3.2 Structure determination of alkaloids from *S. curtisii*

A crude ethanol extract of the roots of *S. curtisii* was partitioned between 5% hydrochloric acid solution and chloroform. The aqueous solution was made basic with aqueous ammonia and extracted with chloroform. The crude residue was subjected to flash column chromatography and preparative TLC to provide the new alkaloids **84, 85** and **86**, namely stemocurtisine, stemocurtisinol and stemocurtisinolide (oxyprotostemonine). Examination of the crude ethanol extract by TLC analysis showed the presence of all these new alkaloids indicating that these compounds was not being produced *via* an acid catalysed reaction during the acid extraction process.

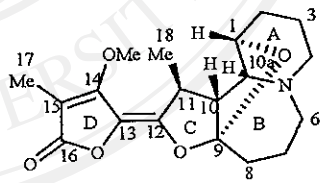
Stemocurtisine (84)

Stemocurtisine **84** was obtained as colourless prismatic crystals (mp 149-151 °C) by careful and slow evaporation of a solution of **84** in ethyl acetate and diethyl ether. HRMS (EI +ve, *m/z* $[M]^+$ 347.1727, calcd 347.1733) indicated that **84** has the molecular formula $C_{19}H_{25}NO_5$. The ^1H and ^{13}C NMR specta of **84** (see Table 6.1, Chapter VI) indicated the presence of the C- and D-ring system that is typically found in the stemoamide group (Pilli and Ferreira de Oliveira, 2000) of alkaloids, including stemofoline (**3**). The ^{13}C/DEPT NMR spectra of **84** indicated four methine carbons, and six methylene carbons and, unlike the other *Stemona* alkaloids, except **3** and its didehydro and 2'-hydroxy derivatives (Seger *et al.*, 2004) a quaternary carbon at δ 120.4 was apparent, indicative of a acetal-like structure (C-9). Indeed the corresponding acetal carbon (C-8) in **3** occurs further upfield at δ 112.7 (Seger *et al.*, 2004). The X-ray structural analysis confirmed the molecular formula of **84** and

revealed its connectivity and relative stereochemistry and showed that this new alkaloid has a novel pentacyclic structure based on a unique pyrido[1,2-*a*]azepine A,B-ring system (that is a 6,7-bicyclic A,B-ring system) and not the typical pyrrolo[1,2-*a*]azepine A,B-ring system (5,7-bicyclic A,B-ring system). This is the first *Stemona* alkaloid to have this type of base structure. We named this alkaloid stemocurtisine based on its botanical origin. The absolute configuration of **84** is not known but is assumed based on the known configurations of *Stemona* alkaloids with similar C,D-ring structures (Ye *et al.*, 1994; Jiwajinda *et al.*, 2001). The X-ray structure of **84** also showed that the piperidine A-ring adopts a chair-like conformation and is connected to the B- and C-rings through an ether bridge between C-1 and the quaternary acetal carbon C-9, readily identified in the ^{13}C NMR spectrum.



3



84

The full ^{1}H and ^{13}C NMR spectral assignments for **84**, based on extensive COSY, TOCSY, NOESY, HSQC, and HMBC experiments, are shown in Table 6.1 of Chapter VI. Of significance in the NOESY spectra of **84** were cross peaks between H-10 and H-6β, H-6β and H-8β, H-7α and H-3α, H-3β and H-2β, and H-2β and H-4β (Fig 3.12). These cross peaks allowed the unequivocal assignment of all methylene protons in the ^{1}H NMR spectrum of **84** (Table 6.1, Chapter VI) and indicated that the

solution-structure of **84** is similar to the solid-state structure. That is, the A-ring adopts a chair-like conformation in which the axial proton H-3α is in close proximity to H-7α in the seven-membered ring on the concave face of the molecule (Fig 3.12).

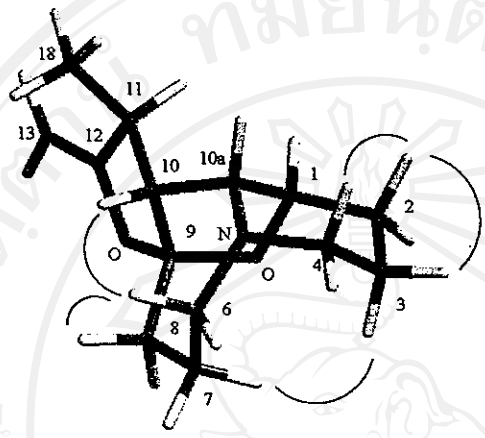


Figure 3.12 Molecular model (AM1, Spartan) of the A,B,C-ring substructure of **84** showing significant NOESY cross peaks (curved lines).

Stemocurtisinol (85)

Compound **85** was obtained as colorless prismatic crystals (mp 209-211 °C) by careful and slow evaporation of a solution of **85** in ethyl acetate. HRMS (EI +ve, *m/z* [M^+] 405.2100, calcd 405.2151) indicated that **85** had the molecular formula $C_{22}H_{31}NO_6$. The ^1H and ^{13}C NMR spectra of **85** indicated the presence of the ABCD-ring system of stemocurtisine (**84**), including the ether bridging structure between C-1 and C-9 and the C-4, 1-hydroxypropyl A-ring side chain. NOESY experiments showed significant cross peaks between H-19 and H-10a and H-19 and H-2β, revealing the β-configuration (axial orientation) of the C-4 substituent relative to the axial protons H-2β and H-10a. X-ray structural analysis confirmed the molecular formula of **85** and revealed its connectivity and relative stereochemistry (Fig 3.13).

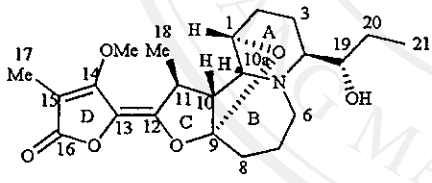
We have named this new alkaloid stemocurtisinol based on its hydroxyl functional group and its botanical origin. In the crystal, the hydroxyl hydrogen, H(19-O), is hydrogen-bonded intramolecularly to N(5) at a distance of 2.04(3) Å (Fig. 6.5, Chapter VI). The absolute configuration of **85** was not established but is assumed, based on the known configurations of *Stemona* alkaloids with similar C,D-ring structures (Irie, 1970).

A few months after we published the structure of stemocurtisine a publication appeared describing the isolation of the same compound from *S. sp.* (HG 915), an unknown *Stemona* species found in Northeast Thailand (Nong Wua So near Udon Thani). The authors named this alkaloid pyridostemonine. The NMR data for this compound matched very closely that of stemocurtisine and we therefore concluded that they were the same compound. Fortunately we published our structure first. The same paper also described the isolation of oxyprotostemonine from *S. curtisii* an alkaloid that we also isolated, however we did not include this structure in our first publication. They also published the structure of oxystemokerrin from *S. kerrii* which had a different absolute configuration at C-4 and C-19 to stemocurtisinol. This prompted us to isolate oxystemokerrin and attempt to get its crystal structure. This is described in this section.

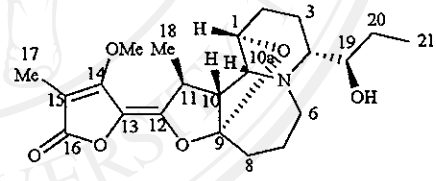
The NMR spectroscopic data of **85** and oxystemokerrin (Kaltenegger *et al.*, 2003) were different, especially their ^{13}C NMR chemical shifts for the carbons near the C-4 and the 1'-hydroxypropyl side chain, consistent with these compounds being epimeric at C-4. Significant differences were observed in the chemical shifts for C-6 (δ 42.8 in oxystemokerrin and δ 54.8 in **85**) and C-19 (δ 70.5 in oxystemokerrin and δ 67.9 in **85**). The differences in the C-6 chemical shifts are consistent with the C-4

substituent having an equatorial disposition in oxystemokerrin and an axial disposition in **85**. Indeed the ^{13}C NMR chemical shift for C-6 in **84** and **85** were almost identical, whereas that in oxystemokerrin is about 12 ppm upfield due to the γ-gauche effect (Barfield, 1995) of the C-4 substituent on C-6. Compounds **85** and oxystemokerrin also have opposite configurations at the carbinol carbon C-19.

The full ^{1}H and ^{13}C NMR spectral assignments for **85**, based on extensive COSY, TOCSY, NOESY, HSQC, and HMBC experiments, are shown in Table 6.2, Chapter VI. NOESY experiments were used to determine the relative α or β orientation of the protons. In addition, the ^{1}H and ^{13}C NMR comparison of **85** and oxystemokerrin are shown in Tables 3.2 and 3.3.



85



oxystemokerrin (90)

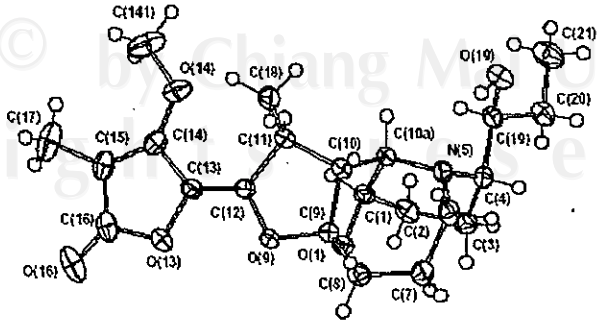


Figure 3.13 X-ray structure of **85**

Table 3.2 ^{13}C NMR (75 MHz)and ^{1}H NMR (500 MHz) spectroscopic data of **85** and oxystemokerrin (Kaltenegger *et al.*, 2003) in $CDCl_3$ solution.

	85		oxystemokerrin	
position	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**
1	75.4 (CH)	4.05 (s)	-	4.02 (m)
2a	22.4 (CH_2)	1.73 (dd, 5.8, 12.3, β)	-	2.0-1.3 (m)
b		1.95 (m, α)		2.23 (m)
3a	18.4 (CH_2)	1.36 (m, β)	65.8	2.0-1.3
b		1.96 (m, α)		
4	65.5 (CH)	2.53 (m)	42.8	2.43 (ddd,12,9,2)
6a	54.8 (CH_2)	2.92 (dd, 4.5, 15.5, α)	-	2.94 (br m)
b		3.48 (m, β)		3.20 (br m)
7a	25.8 (CH_2)	1.65 (m, β)	-	2.0-1.3 (m)
b		1.99 (m, α)		
8a	33.5 (CH_2)	1.76 (dd, 5.8, 13, β)	119.9	2.0-1.3 (m)
b		2.36 (dd, 4.1, 13, α)		2.28 (m)
9	120.1 (C)		56.5	-
10	56.9 (CH)	2.70 (d, 4.7)	-	2.70 (d,5)
10a	57.5 (CH)	3.40 (s)	39.7	3.39 (br s)
11	39.3 (CH)	3.07 (quin, 6.1)		3.03 (br m)
12	146.8 (C)	-		-
13	125.0 (C)	-	162.9	-
14	162.7 (C)	-	97.5	-
15	97.5 (C)	-	169.9	-
16	169.7 (C)	-		-
17	9.2 (CH_3)	2.07 (s)		2.08 (s)
18	22.6 (CH_3)	1.38 (d, 7)	70.5	1.4 (d, 7)
19	67.9 (CH)	3.50 (m)	26.9	3.53 (br m)
20a	26.4 (CH_2)	1.25 (m)	1.02 (t, 7.4)	2.0-1.3 (m)
b		1.60 (m)		
21	10.3 (CH_3)	1.02 (t, 7.3)	58.9	1.02 (t, 7.4)
OMe	58.9 (CH_3)	4.15 (s)		4.15 (s)

Table 3.3 ^{13}C NMR (75MHz)and ^{1}H NMR(300MHz) spectroscopic data of **85** and oxystemokerrin (Kaltenegger *et al.*, 2003) in CD_3OD solution.

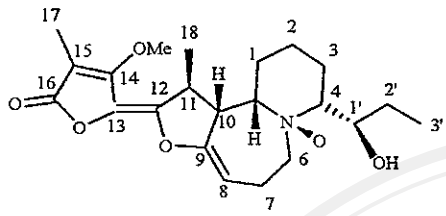
	85		**. oxystemokerrin**	
position	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**
1	77.6(CH)	4.03 (s)	76.8	4.03 (ddd)
2a	23.6(CH_2)	1.75 (dd)	28.1	1.81 (m)
b		1.84 (m)		2.19 (m)
3a	19.5CH_2)	1.36 (m)	30.8.	1.64 (m)
b		1.87 (m)		1.34 (m)
4	66.4 (CH)	2.54 (m)	65.8	2.59 (ddd)
6a	55.2 (CH_2)	2.86 (dd)	44.2	3.36 (ddd)
b		3.44 (m)		3.06 (ddd)
7a	26.6 (CH_2)	1.62 (m)	27.4	1.95 (m)
b		1.95 (m)		1.74 (m)
8a	34.1 (CH_2)	1.77 (m)	34.8	2.22 (m)
b		2.18 (dd)		1.87 (m)
9	121.9 (C)	-	121.8	-
10	57.5 (CH)	2.82 (d)	57.4	2.88 (d)
10a	58.8(CH)	3.26 (s)	66.4	3.50 (br s)
11	40.5 (CH)	3.12 (quin)	41.0	3.20 (dq)
12	149.7 (C)	-	150.2	-
13	126.2 (C)	-	126.2	-
14	165.1 (C)	-	165.4	-
15	97.9 (C)	-	97.9	-
16	172.6 (C)	-	172.8	-
17	8.9 (CH_3)	2.01 (s)	8.9	2.10 (s)
18	22.5 (CH_3)	1.36(d)	22.6	1.45 (d)
19	70.3 (CH)	3.49 (m)	73.1	3.59 (ddd)
20a	27.0 (CH_2)	1.24 (m)	28.2	1.72 (ddq)
b		1.59 (m)		1.43 (ddq)
21	10.3 (CH_3)	0.95 (t)	10.4	1.04 (t)
OMe	59.9 (CH_3)	4.17 (s)	60.0	4.25 (s)

Other novel pyrido[1,2-*a*]azepine compounds, namely stemokerrin **(87)**, methoxystemokerrin-*N*-oxide **(88)**, oxystemokerrin-*N*-oxide **(89)** were also isolated from *S. kerrii* and were reported in the same 2003 paper (Kaltenegger *et al.*, 2003). The ^{13}C NMR data in $CDCl_3$ of **(87)**, **(88)**, stemocurtisinol **(85)** and stemocurtisine **(84)** are compared in Table 3.4 while the ^{13}C NMR data in CD_3OD of **89** and **85** are compared in Table 3.5.

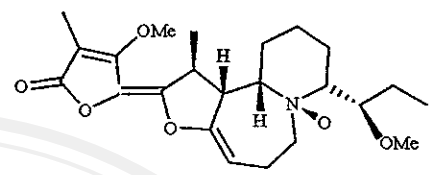
Alkaloids **87** and **88** displayed some similarities to the corresponding data of **85** and **84**. The difference was the lack of olefinic CH group in **85** and **84** that caused the quaternary carbons C-9 and C-8 to be more upfield than those in stemokerrin and methoxystemokerrin-*N*-oxide. The strongly deshielded C-1 of **85** and **84** is due to the oxygen bridge between C-1 and C-9 which causes their ^{13}C NMR chemical shifts to be more downfield (shifted by ~ 50 ppm) than **87** and **88**. In addition, these two compounds from *S. kerrii* and oxystemokerrin have the opposite configurations at C-4 and C-19 when compared to that of **85**.

The ^{13}C NMR chemical shifts of C-4, C-6, C-10a, the atoms which are directly attached to the N-5 nitrogen in **88** and **89** showed a strong downfield shift of 10-30 ppm due to oxidation at N-5 (*N*-oxide) when compared with **85** and **84**.

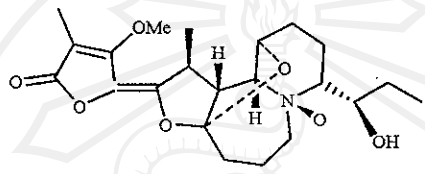
The ^{13}C NMR chemical shifts of **89** also displayed similarities to the corresponding data of **85**, the only difference was the presence of an additional oxygen attached to nitrogen N-5.



87



88



89

Table 3.4 ^{13}C NMR spectroscopic data of **87**, **88** (Kaltenegger *et al.*, 2003), **85** and **84** (75 MHz) in $CDCl_3$ solution.

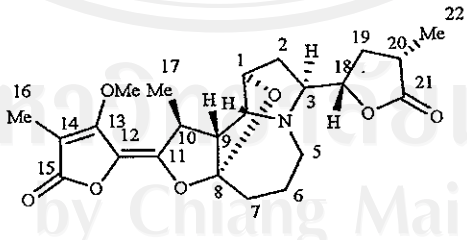
compound	87	88	85	84
position	δ_C (DEPT)	δ_C (DEPT)	δ_C (DEPT)	δ_C (DEPT)
1	16.9 (CH)	23.8 (CH)	75.4 (CH)	75.5 (CH)
2	24.8 (CH_2)	23.3 (CH)	22.4 (CH_2)	26.9 (CH_2)
3	19.2 (CH_2)	23.2 (CH_2)	18.4 (CH_2)	18.9 (CH_2)
4	69.9 (CH)	84.3 (CH)	65.5 (CH)	53.6 (CH_2)
6	389.7 (CH_2)	56.2 (CH_2)	54.8 (CH_2)	53.0 (CH_2)
7	25.8 (CH_2)	18.7 (CH_2)	25.8 (CH_2)	27.0 (CH_2)
8	100.2 (CH_2)	98.2 (CH_2)	33.5 (CH_2)	33.9 (CH_2)
9	157.4 (C)	157.9 (C)	120.1 (C)	120.4 (C)
10	52.8 (CH)	44.2 (CH)	56.9 (CH)	57.0 (CH)
10a	62.4 (CH)	78.5 (CH)	57.5 (CH)	62.0 (CH)
11	38.9 (CH)	38.5 (CH)	39.3 (CH)	39.3 (CH)
12	146.8 (C)	146.1 (C)	146.8 (C)	147.2 (C)
13	123.2 (C)	123.6 (C)	125.0 (C)	124.9 (C)
14	163.1(C)	162.9 (C)	162.7 (C)	162.7 (C)
15	97.2 (C)	97.6 (C)	97.5 (C)	97.3 (C)
16	169.8 (C)	169.7 (C)	169.7 (C)	169.8 (C)
17	9.2 (CH_3)	9.2 (CH_3)	9.2 (CH_3)	9.2 (CH_3)
18	22.1 (CH_3)	21.7 (CH_3)	22.6 (CH_3)	22.6 (CH_3)
1'	70.1 (CH)	78.7 (CH)	67.9 (CH)	-
2'	26.9 (CH_2)	26.0 (CH_2)	26.4 (CH_2)	-
3'	9.8 (CH_3)	11.1 (CH_3)	10.3 (CH_3)	-
14-OMe	59.0 (CH_3)	59.2 (CH_3)	58.9 (CH_3)	58.9 (CH_3)
1'-OMe	-	57.9 (CH_3)	-	-

Table 3.5 ^{13}C NMR spectroscopic data of **89** (Kaltenegger *et al.*, 2003) and **85** (75MHz) in CD_3OD solution.

compound	89	85
position	δ_C (DEPT)	δ_C (DEPT)
1	77.0 (CH)	75.4 (CH)
2	22.5 (CH_2)	22.4 (CH_2)
3	23.0 (CH_2)	18.4 (CH_2)
4	79.4 (CH)	65.5 (CH)
6	61.9 (CH_2)	54.8 (CH_2)
7	19.7 (CH_2)	25.8 (CH_2)
8	32.6 (CH_2)	33.5 (CH_2)
9	154.1 (C)	120.1 (C)
10	52.3 (CH)	56.9 (CH)
10a	85.4 (CH)	57.5 (CH)
11	40.2 (CH)	39.3 (CH)
12	148.2 (C)	146.8 (C)
13	120.7(C)	125.0 (C)
14	165.2 (C)	162.7 (C)
15	98.3 (C)	97.5 (C)
16	172.6 (C)	169.7 (C)
17	8.9 (CH_3)	9.2 (CH_3)
18	22.8 (CH_3)	22.6 (CH_3)
1'	73.7 (CH)	67.9 (CH)
2'	28.5 (CH_2)	26.4 (CH_2)
3'	8.8 (CH_3)	10.3 (CH_3)
14-OMe	60.0 (CH_3)	58.9 (CH_3)

Oxyprotostemonine (86)

Oxyprotostemonine **86** was obtained as a white amorphous solid. HRMS (EI +ve, *m/z* [M]+, 431.1892 calcd 431.1944. indicated that **86** has the molecular formula $C_{23}H_{29}NO_7$. The identification of this compound was based on 1D and 2D NMR spectroscopic data (Table 6.3, Chapter VI) and corresponded with the structure reported in the literature (Kaltenegger *et al.*, 2003). When we isolated this compound it was a new alkaloid, unfortunately this structure was published during our investigations. The mass spectrum base peak at *m/z* 332 $[M\text{-}C_5H_7O_2]^+$ indicated the presence of a saturated α-methyl-γ-lactone ring annexed to the C-3 of the pyrrolidine ring (Ye *et al.*, 1994) and an oxygen bride C(8)-O-C(1) could be proved by comparing the ^1H and ^{13}C NMR spectroscopic data with dehydroprotostemonine which was described in the literature (Kaltenegger *et al.*, 2003). IR absorptions at 1743 (C=O) and 1621 (C=C) cm^{-1} indicated the presence of an unsaturated-γ-lactone ring. The ^1H and ^{13}C NMR spectroscopic data compared to those reported are shown in Table 3.6.



86

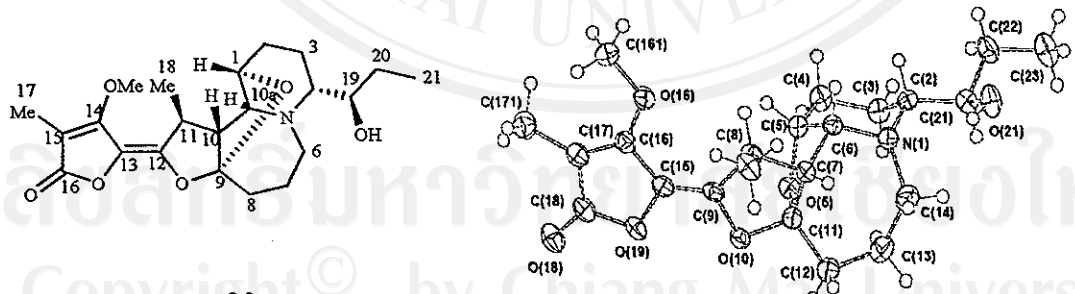
Table 3.6. ^{13}C NMR (75MHz) and ^{1}H NMR (300 MHz) spectroscopic data of **86** and oxyprotostemonine (Kaltenegger *et al.*, 2003) in $CDCl_3$ solution.

	86		**oxyprotostemonine**	
position	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**
1	87.8 (CH)	4.67 (s)	87.9	4.76 (ddd)
2a	33.0 (CH_2)	2.25 (m)	33.0	2.25 (m)
b		1.74 (m)		1.74 (m)
3	66.3 (CH)	3.31 (br s)	66.3	3.31 (ddd)
5a	50.8 (CH_2)	3.08 (m)	50.8	3.08 (m)
b		2.97 (m)		2.97 (m)
6a	20.9 (CH_2)	1.74 (m)	20.8	1.70 (m)
b		1.45 (m)		1.44 (m)
7a	32.4 (CH_2)	2.25 (m)	32.4	2.25 (m)
b		1.74 (m)		1.74 (m)
8	120.6 (CH)	-	120.7	-
9	57.0 (CH)	2.56 (br s)	57.0	2.55 (d)
9a	69.8 (CH)	3.60 (br s)	69.8	3.60 (d)
10	39.6 (CH)	3.08 (m)	39.6	3.08 (m)
11	146.5 (C)	-	146.7	-
12	125.6 (C)	-	125.7	-
13	162.7 (C)	-	162.9	-
14	97.5 (C)	-	97.6	-
15	169.8 (C)	-	169.8	-
16	9.2 (CH_3)	2.08 (s)	9.1	2.08 (s)
17	22.2 (CH_3)	1.38 (d, 6.5)	22.0	1.37 (d)
18	82.2 (CH)	4.23 (t, 4.5)	82.3	4.23 (ddd)
19a	34.1 (CH_2)	2.27 (m)	34.1	2.30 (ddd)
b		1.80 (m)		1.8 (m)
20	35.9 (CH)	2.68 (m)	35.9	2.68 (m)
21	179.1 (C)	-	179.3	-
22	15.1 (CH_3)	1.30 (d, 7)	15.0	1.30 (d)
OMe	58.9 (CH_3)	4.15 (s)	58.9	4.15 (s)

3.3 X-ray crystal structure of oxystemokerrin (90)

In order to establish the unequivocal structure of oxystemokerrin by single crystal X-ray structural analysis, this compound was isolated from *S. kerrii* according to section 6.4.3. Oxystemokerrin **(90)** was isolated as colourless prismatic crystals (mp 134-136°C) by careful and slow evaporation of a solution of ethanol. HRMS (EI+ve, *m/z* [M+] 406.2250, calcd 406.2209) indicated that this compound had molecular formular $C_{22}H_{32}NO_6$. The structure of this compound was confirmed by comparing the ^{1}H and ^{13}C NMR spectroscopic data with the literature (Kaltenegger *et al.*, 2003) and the chemical shifts were in agreement with those previously reported. These are shown in Table 3.7. Importantly we were able to prove the structure by X-ray crystallography (Fig 3.14).

The full ^{1}H and ^{13}C NMR spectral assignments for **90** based on extensive COSY, TOCSY, NOESY, HSQC, and HMBC experiments, are shown in Table 6.9, Chapter VI.



90

Figure 3.14 X-ray structure of **90**

Table 3.7 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of **90** and oxystemokerrin (Kaltenegger *et al.*, 2003) in CD_3OD solution.

	90		**oxystemokerrin**	
position	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**
1	76.5 (CH)	4.08 (m)	76.8	4.03 (ddd)
2a	28.1 (CH_2)	1.81 (m)	28.1	1.81 (m)
b		2.19 (m)		2.19 (m)
3a	30.3 (CH_2)	1.33 (m)	30.8	1.64 (m)
b		1.37 (m)		1.34 (m)
4	65.8 (CH)	2.53 (m)	65.8	2.59 (ddd)
6a	44.4 (CH_2)	3.40 (m)	44.2	3.36 (ddd)
b		3.13 (m)		3.06 (ddd)
7a	22. 4(CH_2)	1.95 (m)	27.4	1.95 (m)
b		1.74 (m)		1.74 (m)
8a	34.2 (CH_2)	2.22 (m)	34.8	2.22 (m)
b		1.90 (m)		1.87 (m)
9	121.8 (C)	-	121.8	-
10	57.1 (CH)	2.92 (d)	57.4	2.88 (d)
10a	66.2 (CH)	3.61 (br s)	66.4	3.50 (br s)
11	40.9 (CH)	3.22 (m)	41.0	3.20 (dq)
12	150.2 (C)	-	150.2	-
13	126.2 (C)	-	126.2	-
14	165.4 (C)	-	165.4	-
15	97.9 (C)	-	97.9	-
16	172.8 (C)	-	172.8	-
17	8.9 (CH_3)	2.10 (s)	8.9	2.10 (s)
18	22.6 (CH_3)	1.45 (d, 7)	22.6	1.45 (d)
19	72.7 (CH)	3.6 (ddd)	73.1	3.59 (ddd)
20a	28.2 (CH_2)	1.72 (m)	28.2	1.72 (ddq)
b		1.42 (m)		1.43 (ddq)
21	10.4 (CH_3)	1.04 (t, 7.3)	10.4	1.04 (t)
OMe	60.0 (CH_3)	4.15 (s)	60.0	4.25 (s)

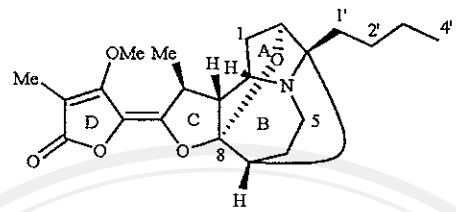
3.4 Structure determination of alkaloids from *S. burkillii*

A crude ethanol extract (10.4 g) of the roots of *S. burkillii* was partitioned between 5% hydrochloric acid solution and dichloromethane. The aqueous solution was made basic with aqueous ammonia and extracted with dichloromethane to afford

0.224 g of crude alkaloid material. Successive purifications of this material by preparative TLC gave pure samples of stemofoline **(3)** (6.8 mg), 2'-hydroxystemofoline **(73)** (3.7 mg), 11(*S*),12(*R*)-dihydrostemofoline **(91)** (2.1 mg) and stemoburkilline **(92)** (1.5 mg). The former two known alkaloids were identified from a comparison of their spectral data (NMR and MS) with those reported in the literature (Jiwajinda *et al.*, 2001; Kaltenegger *et al.*, 2003; Seger *et al.*, 2004). Compounds **91** and **92** are new compounds. We named **92** as stemoburkilline, based on its botanical origin. Examination of the crude ethanol extract by TLC and ^{1}H NMR analysis showed the presence of all four alkaloids indicating that these compounds were not being produced *via* an acid catalysed reaction during the acid extraction process.

Stemofoline (3)

Stemofoline (**3**) was isolated as the major component in this species and was obtained as a yellow brown gum. HRMS (EI +ve, *m/z* [M]+, 387.2048 calcd 387.2045) indicated that **3** has the molecular formula $C_{22}H_{29}NO_5$. The ^{1}H and ^{13}C NMR spectroscopic data were compared with that reported for stemofoline (Jiwajinda *et al.*2001; Brem *et al.*, 2002)) and it was found that these spectral data were in good agreement. However, a difference in the ^{1}H chemical shift at H-6 and H-1' between **3** and the literature values were observed. The position of H-1'in **3** was confirmed by HMBC experiments which showed that H-1' (δ 1.97, 1.94) correlated with C2', C3' and C4'. The ^{1}H chemical shifts assignments for **3** were also aided by HSQC experiments.



3

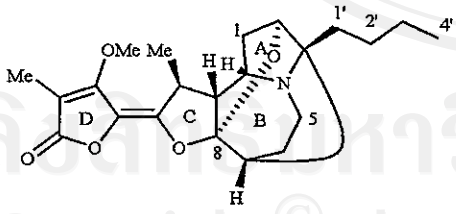
Table3.8 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of **3** and stemofoline (Jiwajinda *et al.*, 2001; Brem *et al.*, 2002; Seger *et al.*, 2004) in $CDCl_3$ solution.

	3		**stemofoline**	
Position	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**
1a	32.9 (CH_2)	2.02 (m)	33.3 (CH_2)	1.7-2.0 (m)
b		1.78 (m)		
2	78.2 (CH)	4.28 (br s)	78.6 (CH)	4.25 (br s)
3	83.2 (C)	-	82.8 (C)	-
5a	47.3 (CH_2)	3.21 (m)	47.6 (CH_2)	3.1 (m)
b		3.06 (m)		3.0 (m)
6a	27.1 (CH_2)	1.42 (m)	27.3 (CH_2)	1.7-2.0 (m)
b		1.25 (m)		
7	49.7 (CH)	2.74 (d, 6.5)	49.9 (CH)	2.70 (d, 6.4)
8	112.4 (C)	-	112.7 (C)	-
9	47.3 (CH)	1.87 (m)	47.6 (CH)	1.7-2.0 (m)
9a	61.0 (CH)	3.58 (br s)	60.9 (CH)	3.49 (br s)
10	34.3 (CH)	3.12 (m)	34.6 (CH)	3.1 (m)
11	148.14 (CH)	-	148. 4 (CH)	-
12	127.8 (CH)	-	127.9 (CH)	-
13	162.7 (C)	-	162.8 (C)	-
14	98.5 (C)	-	98.6 (C)	-
15	169.6 (C)	-	169.7(C)	-
16	9.04 (CH_3)	2.07 (s)	9.20 (CH_3)	2.07 (s)
17	18.1 (CH_3)	1.41 (d, 6.5)	18.3 (CH_3)	1.37 (d, 6.4)
1'a	26.1 (CH_2)	1.97 (m)	26.7(CH_2)	1.5-1.6 (m)
b		1.94 (m)		
2'	31.0 (CH_2)	1.62 (t, 8)	31.0 (CH_2)	1.5-1.6 (m)
3'	22.9 (CH_2)	1.38 (d, 6.5)	22.9 (CH_2)	1.5-1.6 (m)
4'	13.9 (CH_3)	0.92 (t, 7)	13.9 (CH_3)	0.91 (t, 7.0)
OMe	58.8 (CH_3)	4.14 (s)	4.13 (CH_3)	4.13

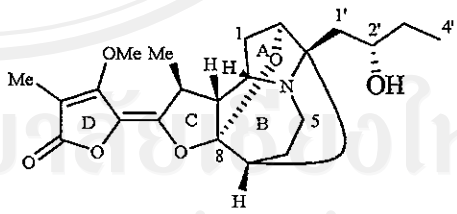
Stemofoline is found in many *Stemona* species such as *S. tuberosa*, *S. Japonica* and *S. collinsae*. The absolute configuration of this molecule was established by X-ray crystallographic analysis of the hydrobromide monohydrate derivative (Irie, 1970).

2'-Hydroxystemofoline (73)

2'-Hydroxystemofoline (**73**) was isolated as a yellow brown gum. HRMS (EI+ve, *m/z* [M+] 403.1999, calcd 403.1995) indicated that this compound had molecular formular $C_{22}H_{29}NO_6$. The structure of this compound was similar to stemofoline, the difference was the OH substituent group at C-2' in **73**. The structure of **73** was established from a comparison of the ^{1}H and ^{13}C NMR spectroscopic data of **73** with that in the literature (Seger *et al.*, 2004). The chemical shifts were in agreement with those previously reported, except for the chemical shift of C-1 (Table 3.9).



3



73

Table 3.9. ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of compound **73** and 2'-hydroxystemofoline (Seger *et al.*, 2004) in $CDCl_3$ solution.

	73		**2'-hydroxystemofoline**	
Position	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**
1a	35.9 (CH_2)	2.06 (m)	33.7 (CH_2)	2.05 (d, 15)
b		1.89 (m)		1.85 (m)
2	78.6 (CH)	4.36 (br s)	78.7(CH)	4.36 (br s)
3	82.8 (C)	-	82.7 (C)	-
5a	47.4 (CH_2)	3.25(m)	47.1 (CH_2)	3.25 (ddd, 13.7, 10.6,
b		3.03(m)		5.3)
				3.02 (ddd, 13.7, 8.8,
				4.8)
6a	26.5 (CH_2)	2.04 (m)	26.6 (CH_2)	1.97 (m)
b		1.88 (m)		1.89 (m)
7	51.6 (CH)	2.65 (d, 6)	52.7 (CH)	2.65 (s)
8	111.9 (C)	-	112.0 (C)	-
9	47.0 (CH)	1.82 (m)	47.6 (CH)	1.82 (dd, 9.8, 3.6)
9a	60.7 (CH)	3.55 (br s)	60.8 (CH)	3.34 (br s)
10	34.3 (CH)	3.12 (dq, 13,6.5)	34.4 (CH)	3.11 (dq, 9.8, 6.5)
11	147.8 (CH)	-	147.8 (CH)	-
12	127.9 (CH)	-	128.0 (CH)	-
13	162.7 (C)	-	162.7 (C)	-
14	98.6 (C)	-	98.8 (C)	-
15	169.6 (C)	-	169.6 (C)	-
16	9.1 (CH_3)	2.07 (br s)	9.2 (CH_3)	2.08 (br s)
17	18.2 (CH_3)	1.38 (d, 6.5)	18.3 (CH_3)	1.38 (d, 6.5)
1'	35.9 (CH_2)	1.81 (m)	36.1 (CH_2)	1.74 (d, 14.2)
		1.67 (m)		1.64 (dd, 14.2, 10.6)
2'	71.1 (CH_2)	3.62 (ddd, 10.5,5.5,5)	71.2 (CH_2)	3.63 (ddd, 10.5,5.5,5)
3'	30.5 (CH_2)	1.52 (m)	30.6 (CH_2)	1.52 (q, 7.3)
		1.44 (m)		1.43 (qd, 7.3, 1.7)
4'	9.7 (CH_3)	0.94 (t, 7)	9.7 (CH_3)	0.95 (t, 7.3)
OMe	58.8 (CH_3)	4.14 (s)	58.8 (CH_3)	4.14 (s)

11(*S*),12(*R*)-Dihydrostemofoline (91)

The HRMS (EI +ve, *m/z* $[M]^+$ 389.2202, calcd 389.2202) of **91** showed it had the molecular formula $C_{22}H_{31}NO_5$ and indicated that it was a dihydrostemofoline

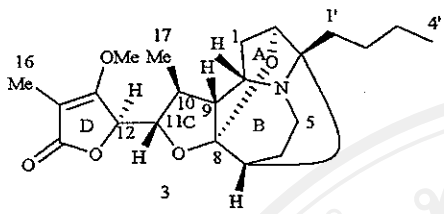
derivative. The ^{1}H and ^{13}C NMR specta of **91** indicated the presence of the A,B,C,D-ring system of stemofoline (**3**) (Ye *et al.*, 1994; Seger *et al.*, 2004).

A comparison of the ^{13}C/DEPT NMR spectra of **91** with that of **3**, showed that **91** had two additional methine carbons (C-11 [δ 86.3] and C-12 [δ 76.5]), and was missing the two quaternary carbons at δ 148.4 and 127.9 and for C-11 and C-12, respectively, of stemofoline (Jiwajinda *et al.*, 2001; Brem *et al.*, 2002; Seger *et al.*, 2004). Furthermore, the ^{1}H NMR spectrum of **91** showed to new signals at δ 3.79 (dd, J = 3, 9 Hz, H-11) and 4.60 (br s, H-12) indicating compound **91** was an 11,12-dihydrostemofoline. NOESY experiments showed a significant cross peak between the C-10 methyl protons (H-17) and H-11 indicating their *syn*-relationship. Thus, assuming that **66** had the same absolute stereochemistry as stemofoline in the rings A-C, we have assigned the 11(*S*) stereochemistry to this new compound, **91**. Unfortunately, these experiments did not allow us to assign the stereochemistry at C-12. In 2003, Velten (Ye and Velten, 2003) reported the synthesis of 11(*S*),12(*S*)-dihydrostemofoline (**93**) from the *syn*-hydrogenation of stemofoline, this compound also showed a significant cross peak between the C-10 methyl protons (H-17) and H-11, consistent with the 11(*S*) stereochemistry. Compound **93** was prepared from hydrogenation of stemofoline **3** and the NMR data for this compound are shown in Table 6.5. The ^{1}H and ^{13}C NMR spectra of **91** and **93** are similar but not the same as shown in Table 3.10. Indeed, there is a significant difference in the chemical shifts and coupling constants for the signals for H-11 and H-12 in the ^{1}H NMR spectra of these two compounds, especially $J_{11,12}$ which was 3 Hz in **91** and 7 Hz in **93**. Based on these differences we have assigned the 12(*R*) stereochemistry to **91**. The full ^{1}H and ^{13}C NMR spectral assignments for **91** and **93** based on extensive COSY, TOCSY,

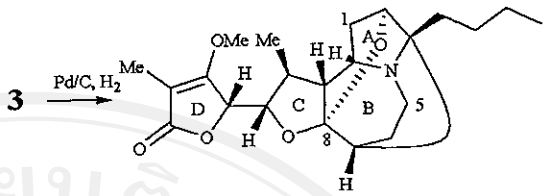
NOESY, HMQC, and HMBC experiments, are shown in Tables 6.6 and 6.7, respectively. A NOESY cross peak between H-9 and H-5b allowed for the unequivocal assignment of the H-5 protons (Table 6.6, Chapter VI).

Table 3.10 The ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of **91** and **93**.

	91		93	
Position	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**
1a	33.4 (CH_2)	1.99 (m)	33.4 (CH_2)	1.97 (m)
b		1.63 (dd, $J_{1a,1b}$=3, $J_{1b,2}$=7.5)		1.62 (m)
2	78.2 (CH)	4.22 (br s)	78.6 (CH)	4.21 (br s)
3	82.2 (C)	-	82.1 (C)	-
5a	47.4 (CH_2)	3.14 (m) α	47.5 (CH_2)	3.09 (m)
b		3.01 (m) β		2.96 (m)
6a	26.5 (CH_2)	1.82 (m)	33.4(CH_2)	1.85 (m)
b		1.72 (m)		1.73 (m)
7	50.5 (CH)	2.45 (d, 6)	50.5 (CH)	2.47 (d, J=6)
8	111.8 (C)	-	112.8 (C)	-
9	47.3 (CH)	1.64 (dd, $J_{9,9a}$=3, $J_{9,10}$=12)	47.2 (CH)	1.60 (m)
9a	61.0 (CH)	3.44 (br s)	61.1 (CH)	3.38 (br s)
10	33.1 (CH)	2.61 (m)	35.1 (CH)	2.48 (m)
11	86.3 (CH)	3.79 (dd, $J_{10,11}$=9, $J_{11,12}$=3)	87.8 (CH)	3.69 (t, J=7)
12	76.5 (CH)	4.60 (br s)	78.7(CH)	4.75 (d, J=6.5)
13	170.3 (C)	-	173.3 (C)	-
14	98.5 (C)	-	98.8 (C)	-
15	174.5 (C)	-	174.3 (C)	-
16	8.7 (CH_3)	2.01 (br s)	8.2 (CH_3)	1.95 (br s)
17	14.8 (CH_3)	1.08 (d, 6.5)	16.9 (CH_3)	1.12 (d, J= 6.3)
1'	31.5 (CH_2)	1.56 (t, 8)	31.8 (CH_2)	1.54 (m)
2'	27.2 (CH_2)	1.40 (m)	27.4 (CH_2)	1.40 (m)
		1.23 (m)		1.23 (m)
3'	23.2 (CH_2)	1.33 (m)	23.4 (CH_2)	1.35 (q, J=6.8)
4'	13.9 (CH_3)	0.87(t, 7)	14.1 (CH_3)	0.92 (t, J= 6.8)
OMe	58.8 (CH_3)	4.11 (s)	59.4 (CH_3)	4.10 (s)



91

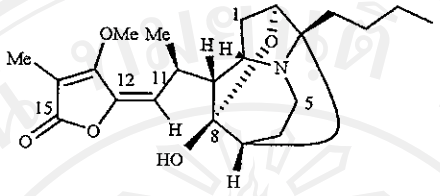


93

Stemoburkilline (92)

The HRMS (EI +ve, *m/z* $[M]^+$ 389.2194, calcd 389.2202) of stemoburkilline **92** showed that it also had the molecular formula $C_{22}H_{31}NO_5$. Its ^1H NMR spectrum indicated the presence of an olefinic proton (δ 5.5, 1H, d, J = 10 Hz, H-11) coupled to an adjacent CH group (H-10) while its ^{13}C/DEPT NMR spectrum, in comparison with that of **91**, showed the C-11 and C-12 methines in **91** had been replaced by two olefinic carbons (one quaternary and one methine). The full ^1H and ^{13}C NMR spectral assignments for **92** based on extensive COSY, TOCSY, NOESY, HMQC, and HMBC experiments, are shown in Table 6.7 and indicated that **92** was formally the C-ring opened product of **91**. Our attempts to induce ring opening of **91** to produce **92** were not successful using either base catalysis (excess DBU, RT 16 h) or acid catalysis (5% aqueous HCl, RT 1h). The ^{13}C NMR spectrum of **92** showed this compound existed in the hemi-acetal form (δ 105.7, C-8, quaternary) and this was further supported from its IR spectrum (3382 cm^{-1}, br) that showed a hydroxyl group. Unfortunately NOESY experiments did not allow us to confidently assign the stereochemistry of the C-11, C-12 alkene group. We have assigned the (*E*)-

stereochemistry to **92** based upon the assumption that **92** arises from **91** via an *anti*-elimination process.



92