CHAPTER 4

RESULTS AND DISCUSSION

4.1 Equipment, materials and chemicals

General experimental procedures

Optical rotations were measured using a JASCO DIP-370 polarimeter. ^{1}H (300 or 500 MHz), ^{13}C (75 or 125 MHz), and 2D NMR spectra were recorded on Varian Mercury 300 and Varian Unity 500 spectrometers. High resolution EIMS were recorded on a Fison/VG Autospec-TOF-oa mass spectrometer (70 eV). High resolution ESIMS (for MH^{+}) were obtained with a Micromass Qtof 2 mass spectrometer using a cone voltage of 30V and polyethyleneglycol (PEG) as an internal reference. TLC was performed on aluminium-backed Merck 60 GF_{254} silica gel and bands were detected by UV light (λ 254 nm) or by straining with Dragendroff's reagent. Column chromatography was performed using Merck GF_{254} flash silica gel (40-63 μm).

Reagent

Dragendroff's Reagent: Bismuth subnitrate (600 mg) was dissolved in 10 M HCl (2 mL) and distilled water (10 mL) was added (solution A). KI (6 g) was dissolved in 10 mL of solution A and diluted with distilled water until 400 mL. The reagent was stored in a dark bottle (Stahl, 1969).

4.2 Collection of plant materials

Three *Stemona* spp. i.e. *Stemona curtisii* Hook F., *Stemona* sp. (unknown 1) and *Stemona* sp. (unknown 2), as showed in Fig. 4.1, were collected from different parts of Thailand and identified by botanical notes on the vascular flora of Chiang Mai (Maxwell, 1991).

***Stemona curtisii* Hook F.**

The roots of *S. curtisii* were collected at Tumbol Kaunmao, Amphur Rasda, in the North of Trang Province, Thailand, in November 2004. A voucher specimen was deposited at the Herbarium (number 17581) of the Department of Biology, Chiang Mai University.

***Stemona* sp. (unknown 1)**

The intact plants of an unidentified *Stemona* species (*Stemona* sp.) were bought at the Tung Kwien market, Lampang Province, Thailand and have been cultivated at Chiang Mai University, Chiang Mai Province since 2000. A voucher specimen was deposited at the Herbarium (number 17582) of the Department of Biology, Chiang Mai University.

***Stemona* sp. (unknown 2)**

The intact plants of an unidentified *Stemona* species (*Stemona* sp.) were bought at the market in Phra Si Rattana Mahathat temple, Amphur Muang, Phitsanulok Province, Thailand, in October 2004. This plant was growing naturally in Amphur Chattrakarn, Phitsanulok Province. A voucher specimen was deposited at the Herbarium (number 25375) of the Department of Biology, Chiang Mai University.

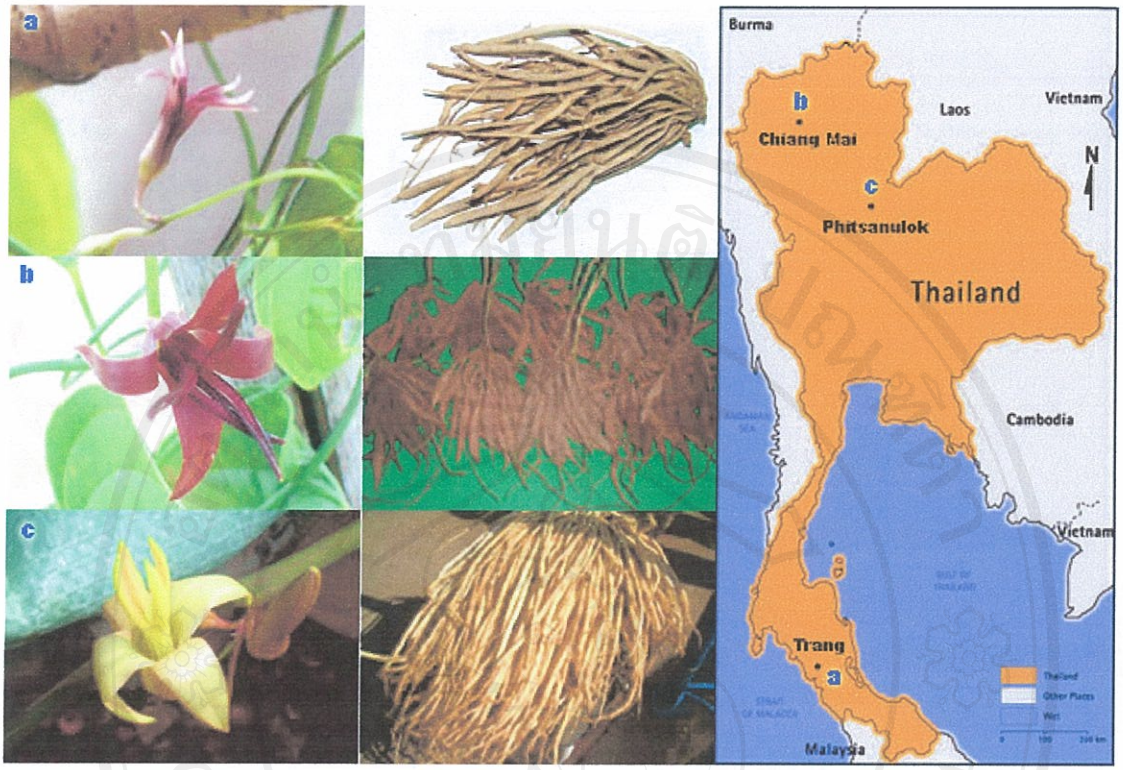


Figure 4.1 Flower and root of *Stemona* species and plant collection areas

(a) *S. curtisii* (b) unknown 1 (c) unknown 2

4.3 Extraction and isolation

The chemical structures and relative configuration of each pure alkaloid isolated from 3 *Stemona* spp. i.e. *S. curtisii*, *S.* sp. (unknown 1) and *S.* sp. (unknown 2) were established or elucidated by spectral data interpretation and from semi-synthetic studies.

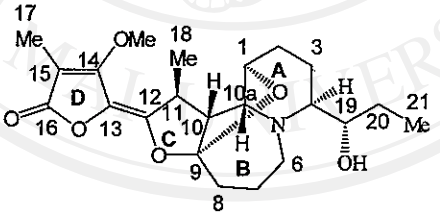
4.3.1 *S. curtisii* extraction and alkaloid isolation

An ethanolic crude extract of the *S. curtisii* roots was partitioned between 5% hydrochloric acid solution and dichloromethane. The aqueous solution was basified with aqueous ammonia and extracted with dichloromethane. Successive purifications of this crude residue by column chromatography and preparative TLC gave pure

samples of 3 known alkaloids namely stemocurtisinol (**63**), oxyprotostemonine (**21**) and stemocurtisine (**58**), respectively. Examination of the ethanolic crude extract by TLC analysis showed the presence of all these alkaloids indicating that these compounds were not being produced *via* an acid catalysed reaction during the acid extraction process.

Stemocurtisinol (63)

Stemocurtisinol (**63**) was isolated as a yellow brown gum; $[\alpha]^{22}_{D}$ +247° (*c* 0.15,$CHCl_3$); lit. (Mungkornasawakul *et al.*, 2004) $[\alpha]^{25}_{D}$ +233° (*c* 0.334, $CHCl_3$). HRMS (EI, *m/z* [M^+] 405.2103, calcd 405.2151) indicated that this compound had the molecular formular $C_{22}H_{31}NO_6$. The structure of stemocurtisinol was established from a comparison of the ^1H and ^{13}C NMR spectroscopic data of stemocurtisinol with that in the literature (Mungkornasawakul *et al.*, 2004). The chemical shifts were in agreement with those previously reported as summarized in Table 4.1.

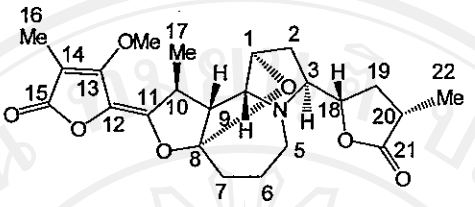


Stemocurtisinol (**63**)

Oxyprotostemonine (21)

Oxyprotostemonine (**21**) was isolated as a yellow brown gum; $[\alpha]^{22}_{D}$ +341° (*c* 0.15,$CHCl_3$); lit. (Kaltenegger *et al.*, 2003) $[\alpha]^{25}_{D}$ +328° (*c* 0.2, MeOH). HRMS (EI, *m/z* [M^+] 431.1894, calcd 431.1944) indicated that this compound had the molecular formular $C_{23}H_{29}NO_7$. The structure of oxyprotostemonine was established from a comparison of the ^1H and ^{13}C NMR spectroscopic data of oxyprotostemonine with

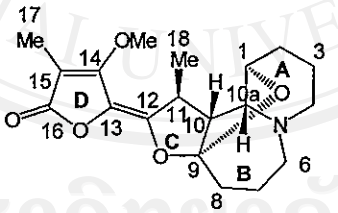
that in the literature (Kaltenegger *et al.,* 2003). The chemical shifts were in agreement with those previously reported as summarized in Table 4.2.



Oxyprotostemonine (**21**)

Stemocurtisine (58)

Stemocurtisine (**58**) was isolated as a yellow brown gum; $[\alpha]^{22}_{D}$ +346° (*c* 0.6,$CHCl_3$); lit. (Mungkornasawakul *et al.*, 2003) $[\alpha]^{25}_{D}$ +334 °(*c* 0.66, $CHCl_3$). HRMS (EI, *m/z* [M^+] 347.1725, calcd 347.1733) indicated that this compound had the molecular formular $C_{19}H_{25}NO_5$. The structure of stemocurtisine was established from a comparison of the ^1H and ^{13}C NMR spectroscopic data of stemocurtisine with that in the literature (Mungkornasawakul *et al.,* 2003). The chemical shifts were in agreement with those previously reported as summarized in Table 4.3.



Stemocurtisine (**58**)

Table 4.1 ^{13}C NMR (75 MHz) and ^{1}H NMR (300 MHz) spectroscopic data of stemocurtisinol and stemocurtisinol (**63**) (Mungkornasawakul *et al.*, 2004) in $CDCl_3$ solution.

Data	Experimental		Literature	
			^{13}C NMR (75 MHz), ^{1}H NMR (300 MHz)	
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)	75.4 (CH)	4.05 (s)
2a	22.4 (CH_2)	1.95(m)	22.4 (CH_2)	1.95(m)
2b		1.73 (dd, J = 12.3, 5.8)		1.73 (dd, J = 12.3, 5.8)
3a	18.4 (CH_2)	1.96 (m)	18.4 (CH_2)	1.96 (m)
3b		1.37 (m)		1.36 (m)
4	65.5 (CH)	2.53 (m)	65.5 (CH)	2.53 (m)
6a	54.8 (CH_2)	3.48 (m)	54.8 (CH_2)	3.48 (m)
6b		2.92 (dd, J = 15.5, 4.5)		2.92 (dd, J = 15.5, 4.5)
7a	25.8 (CH_2)	2.00 (m)	25.8 (CH_2)	1.99 (m)
7b		1.65 (m)		1.65 (m)
8a	33.5 (CH_2)	2.37 (dd, J = 13.0, 4.1)	33.5 (CH_2)	2.36 (dd, J = 13.0, 4.1)
8b		1.76 (dd, J = 13.0, 5.8)		1.76 (dd, J = 13.0, 5.8)
9	120.1 (C)	-	120.1 (C)	-
10	56.9 (CH)	2.70 (d, J = 4.7)	56.9 (CH)	2.70 (d, J = 4.7)
10a	57.5 (CH)	3.40 (s)	57.5 (CH)	3.40 (s)
11	39.3 (CH)	3.06 (quin, J = 6.1)	39.3 (CH)	3.07 (quin, J = 6.1)
12	146.8 (C)	-	146.8 (C)	-
13	125.0 (C)	-	125.0 (C)	-
14	162.7 (C)	-	162.7 (C)	-
15	97.5 (C)	-	97.5 (C)	-
16	169.7 (C)	-	169.7 (C)	-
17	9.2 (CH_3)	2.07 (s)	9.2 (CH_3)	2.07 (s)
18	22.6 (CH_3)	1.38 (d, J = 7.0)	22.6 (CH_3)	1.38 (d, J = 7.0)
19	67.9 (CH)	3.50 (m)	67.9 (CH)	3.50 (m)
20a	26.4 (CH_2)	1.61 (m)	26.4 (CH_2)	1.60 (m)
20b		1.25 (m)		1.25 (m)
21	10.3 (CH_3)	1.02 (t, J = 7.3)	10.3 (CH_3)	1.02 (t, J = 7.3)
OMe	58.9 (CH_3)	4.14 (s)	58.9 (CH_3)	4.15 (s)

Table 4.2 ^{13}C NMR (75 MHz) and ^{1}H NMR (300 MHz) spectroscopic data of oxyprotostemonine and oxyprotostemonine (**21**) (Kaltenegger *et al.*, 2003) in $CDCl_3$ solution.

Data	Experimental		Literature	
			^{13}C NMR (100 MHz), ^{1}H NMR (400 MHz)	
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 (CH)	4.76 (ddd)
2a	33.0 (CH_2)	2.25 (m)	33.0 (CH_2)	2.25 (m)
2b		1.74 (m)		1.74 (m)
3	66.3 (CH)	3.31 (br s)	66.3 (CH)	3.31 (ddd)
5a	50.8 (CH_2)	3.08 (m)	50.8 (CH_2)	3.08 (m)
5b		2.97 (m)		2.97 (m)
6a	20.9 (CH_2)	1.73 (m)	20.8 (CH_2)	1.70 (m)
6b		1.45 (m)		1.44 (m)
7a	32.4 (CH_2)	2.25 (m)	32.4 (CH_2)	2.25 (m)
7b		1.74 (m)		1.74 (m)
8	120.6 (CH)	-	120.7 (CH)	-
9	57.0 (CH)	2.56 (br s)	57.0 (CH)	2.55 (d)
9a	69.8 (CH)	3.60 (br s)	69.8 (CH)	3.60 (d)
10	39.6 (CH)	3.07 (m)	39.6 (CH)	3.08 (m)
11	146.5 (C)	-	146.7 (C)	-
12	125.6 (C)	-	125.7 (C)	-
13	162.7 (C)	-	162.9 (C)	-
14	97.5 (C)	-	97.6 (C)	-
15	169.8 (C)	-	169.8 (C)	-
16	9.2 (CH_3)	2.08 (s)	9.1 (CH_3)	2.08 (s)
17	22.2 (CH_3)	1.38 (d, J = 6.5)	22.0 (CH_3)	1.37 (d)
18	82.2 (CH)	4.23 (t, J = 4.5)	82.3 (CH)	4.23 (ddd)
19a	34.1 (CH_2)	2.27 (m)	34.1 (CH_2)	2.30 (ddd)
19b		1.80 (m)		1.80 (m)
20	35.9 (CH)	2.68 (m)	35.9 (CH)	2.68 (m)
21	179.1 (C)	-	179.3 (C)	-
22	15.1 (CH_3)	1.30 (d, J = 7.0)	15.0 (CH_3)	1.30 (d)
OMe	58.9 (CH_3)	4.14 (s)	58.9 (CH_3)	4.15 (s)

Table 4.3 ^{13}C NMR (75 MHz) and ^{1}H NMR (300 MHz) spectroscopic data of stemocurtisine and stemocurtisine (**58**) (Mungkornasawakul *et al.*, 2003) in $CDCl_3$ solution.

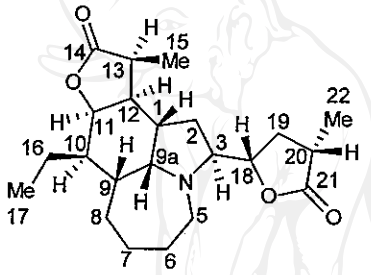
Data	Experimental		Literature	
			^{13}C NMR (75 MHz), ^{1}H NMR (300 MHz)	
position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)
1	75.5 (CH)	4.01 (s)	75.5 (CH)	4.01 (s)
2a	26.9 (CH_2)	2.22 (d, J = 14.5)	26.9 (CH_2)	2.21 (d, J = 14.5)
2b		1.62 (m)		1.62 (m)
3a	18.9 (CH_2)	1.82 (m)	18.9 (CH_2)	1.82 (m)
3b		1.22 (d, J = 13.3)		1.21 (d, J = 13.5)
4a	53.6 (CH_2)	3.02 (m)	53.6 (CH_2)	3.02 (m)
4b		2.86 (m)		2.87 (m)
6a	53.0 (CH_2)	3.38 (t, J = 13)	53.0 (CH_2)	3.38 (t, J = 13)
6b		2.96 (m)		2.96 (m)
7a	27.0 (CH_2)	2.03 (m)	27.0 (CH_2)	2.03 (m)
7b		1.66 (m)		1.66 (m)
8a	33.9 (CH_2)	2.36 (dd, J = 13.5, 4.5)	33.9 (CH_2)	2.36 (dd, J = 13.5, 4.5)
8b		1.75 (m)		1.75 (m)
9	120.4 (C)	-	120.4 (C)	-
10	57.0 (CH)	2.65 (d, J = 4.5)	57.0 (CH)	2.65 (d, J = 4.5)
10a	62.0 (CH)	3.44 (s)	62.0 (CH)	3.44 (s)
11	39.3 (CH)	3.06 (quin, J = 6.5)	39.3 (CH)	3.07 (quin, J = 6.5)
12	147.2 (C)	-	147.2 (C)	-
13	124.9 (C)	-	124.9 (C)	-
14	162.7 (C)	-	162.7 (C)	-
15	97.3 (C)	-	97.3 (C)	-
16	169.8 (C)	-	169.8 (C)	-
17	9.2 (CH_3)	2.08 (s)	9.2 (CH_3)	2.08 (s)
18	22.6 (CH_3)	1.37 (d, J = 7.0)	22.6 (CH_3)	1.37 (d, J = 7.0)
OMe	58.9 (CH_3)	4.14 (s)	58.9 (CH_3)	4.15 (s)

4.3.2 Unknown *S.* sp. 1 extraction and alkaloid isolation

An ethanolic crude extract of the roots of unknown *S.* sp. (unknown 1) was partitioned between 5% hydrochloric acid solution and dichloromethane. The aqueous solution was basified with aqueous ammonia and extracted with dichloromethane. Successive purifications of this crude residue by column chromatography and preparative TLC gave pure samples of 2 new tuberostemonine alkaloids, tuberostemonine L (**69**) and tuberostemonine M (**71**) and a new stemofoline alkaloid, (3′*S*)-hydroxystemofoline (**70**), along with 3 known alkaloids, neotuberostemonine (**13**), (2′*S*)-hydroxystemofoline (**51**) and stemocurtisine. The HRMS analysis of neotuberostemonine (**13**), tuberostemonine L (**69**) and tuberostemonine M (**71**) (ESI, *m/z* $[MH]^+$ 376.2498, 376.2486 and 376.2527, respectively, calcd 376.2488) showed all three compounds had the molecular formula $C_{22}H_{33}NO_4$, indicating their isomeric structural relationship. This structural relationship was further supported from their ^{13}C NMR/DEPT, HSQC and HMBC spectra which indicated the same number of carbons and carbon types. The structure and relative configuration of these new tuberostemonine alkaloids were determined by spectral data interpretation, while the 3′S configuration of (3′*S*)-hydroxystemofoline (**70**) was determined from NMR analysis of its (*R*)- and (*S*)-Mosher esters. Examination of the ethanolic crude extract by TLC analysis showed the presence of all these alkaloids indicating that these compounds were not being produced *via* an acid catalysed reaction during the acid extraction process. However, for the tuberostemonine group of compounds, the bioassays could not be performed due to the chemical instability of its derivatives (Gotz *et al.*, 1968; 1973; Greger *et al.*, 2004).

Neotuberostemonine (13)

Neotuberostemonine (**13**) was isolated as a brown gum; $[\alpha]^{23}_{D}$ +37° (*c* 2.40,$CHCl_3$); lit. (Ye *et al.*, 1994b) $[\alpha]^{18.5}_{D}$ +66° (*c* 1.4, EtOH). HRMS (ESI, *m/z* $[MH]^+$ 376.2498, calcd 345.2488) indicated that this compound had the molecular formular $C_{22}H_{33}NO_4$. The structure of neotuberostemonine was established from a comparison of the ^1H and ^{13}C NMR spectroscopic data of neotuberostemonine with that in the literature (Ye *et al.*, 1994b). The chemical shifts were in agreement with those previously reported as summarized in Table 4.4.

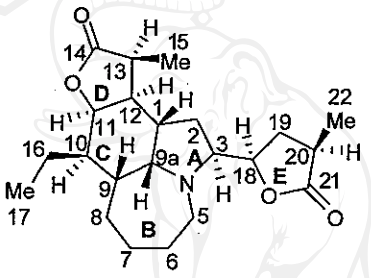


Neotuberostemonine (**13**)

Tuberostemonine L (69) (Sastraruji *et al.*, 2006)

Tuberostemonine L (**69**) was isolated as a brown gum; $[\alpha]^{23}_{D}$ +21° (*c* 0.44,$CHCl_3$). HRMS (ESI, *m/z* $[MH]^+$ 376.2486, calcd 345.2488) indicated that this compound had the molecular formular $C_{22}H_{33}NO_4$. The relative stereochemistry assigned to tuberostemonine L was based on NOESY NMR experiments (Table 4.5). These showed the *syn*-relationship between H-1 and H-9a and H-15, and between H-9a and H-9 and between H-9 and H-16. A *syn*-relationship was also evident between H-10 and H-11 and H-13 and between H-11 and H-12 and H-13. NOESY experiments also showed the *syn*-relationship between H-3 and H-2α and H-12. Thus, clearly defining the relative stereochemistry of the A, B, C and D-rings of tuberostemonine L,

a NOESY cross-peak between H-18 and H-20 indicated their *syn*-stereochemical relationship and therefore the relative stereochemistry of ring-E. The through-space connectivity observed between H-2β and H-18 and H-3 and H-18 then allowed assignment of the entire relative stereochemistry of tuberostemonine L. Thus tuberostemonine L is 18,20-diepituberostemonine. We have named this alkaloid as tuberostemonine L, since a newly reported member of this structural class, 1-epi-neotuberostemonine, was named tuberostemonine K (Jiang *et al.*, 2006).

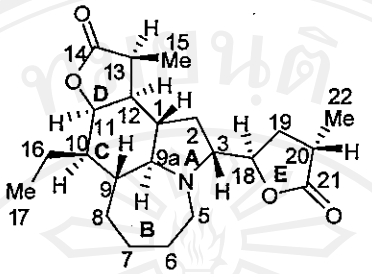


Tuberostemonine L (**69**)

Tuberostemonine M (71) (Sastraruji *et al.*, 2006)

Tuberostemonine M (**71**) was isolated as a brown gum; $[\alpha]^{22}_{D}$ +7° (*c* 0.56,$CHCl_3$). HRMS (ESI, *m/z* $[MH]^+$ 376.2527, calcd 345.2488) indicated that this compound had the molecular formular $C_{22}H_{33}NO_4$. The relative stereochemistry assigned to tuberostemonine M was also based on NOESY NMR experiments (Table 4.6). These showed the *syn*-relationship between H-1 and H-2β H-3, H-9 and H-15 and between H-9 and H-16 and between H-10 and H-11. A *syn*-relationship was also evident between H-10 and H-11 and H-13 and between H-11 and H-12 and H-13. A cross peak between H-18 and H-22 clearly indicated the relative stereochemistry of the E-ring of tuberostemonine M, while cross-peaks between H-19β, in ring-E, and H-

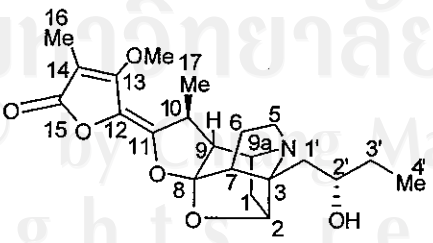
2β and H-3 in ring A allowed the unequivocal assignment of the relative stereochemistry of tuberostemonine M.



Tuberostemonine M (**71**)

(2′*S*)-Hydroxystemofoline (51)

(2′*S*)-Hydroxystemofoline (**51**) was isolated as a yellow brown gum; $[\alpha]^{21}_{D}$ +107° (*c* 0.48,$CHCl_3$); lit.(Brem *et al.*, 2002) $[\alpha]^{20}_{D}$ +197° (*c* 0.5, MeOH) . HRMS (EI, *m/z* [M^+] 403.2030, calcd 403.1995) indicated that this compound had the molecular formular $C_{22}H_{29}NO_6$. The structure of (2′*S*)-hydroxystemofoline was established from a comparison of the ^1H and ^{13}C NMR spectroscopic data of (2′*S*)-hydroxystemofoline with that in the literature (Brem *et al.*, 2002). The chemical shifts were in agreement with those previously reported as summarized in Table 4.7.



(2′*S*)-Hydroxystemofoline (**51**)

(3′*S*)-Hydroxystemofoline (70) (Sastraruji *et al*., 2006)

(3′*S*)-Hydroxystemofoline (**70**) was isolated as a yellow gum; $[\alpha]^{22}_{D}$ +121° (*c* 0.28,$CHCl_3$). The HRMS analysis of (3′*S*)-hydroxystemofoline (ESI, *m/z* $[MH]^+$ 404.2046, calcd 404.2073) showed that this compound had the same molecular formula, $C_{22}H_{29}NO_6$, as (2′*S*)-hydroxystemofoline (**51**). While, (2′*S*)- and (2′*R*)-hydroxystemofoline are known compounds (Sastraruji *et al*, 2005; Brem *et al.,* 2002) the NMR data of (3′*S*)-hydroxystemofoline did not match that of these compounds. The position of the hydroxyl group in (3′*S*)-hydroxystemofoline at C-3′ was clearly evident from ^{1}H NMR analysis which showed a double resonance (δ 1.10 (d, J = 6.0 Hz, 3H)) for the C4′ methyl group. These methyl protons showed a HMBC cross-peak to the secondary C-3′ carbinol carbon at δ 65.9. The large positive specific optical rotation of (3′*S*)-hydroxystemofoline suggested it had the same absolute configuration of the A-D rings as (2′*S*)-hydroxystemofoline (**51**). To determine the absolute configuration of the 3′-hydroxyl group of (3′*S*)-hydroxystemofoline it was treated, in separate experiments, with (*R*)-(-)- and (*S*)-(+)- Mosher's acid chloride ((*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride)) to provide the corresponding (*S*)- and (*R*)-Mosher's esters, derivatives **A** and **B**, respectively (Scheme 1). Due to the diamagnetic effect of the benzene ring, the $H_{A, B, C...}$ NMR signals of the (*R*)-MTPA ester should appear upfield relative to those of the (*S*)-MTPA ester. The reverse should hold true for $H_{X,Y,Z...}$. Therefore, if we calculates $\Delta\delta = \delta_S - \delta_R$, protons on the right side of MTPA plane (Fig. 4.2A) must have positive values ($\Delta\delta > 0$) and protons on the left side of the plane must have negative values ($\Delta\delta < 0$). We calculated $\Delta\delta$ (δ_S-δ_R) for H-2′ (δ1.648 - δ1.569) as 0.079 and $\Delta\delta$ for H-4′ (δ1.310 -

δ1.385) as -0.075. Due to peak overlap, the chemical shifts for H-2′ had to be determined from 1D NOESY experiments with irradiation at H-3′. Using Kakisawa's Model A (Fig. 4.2B) we could then assign the 3′*S*-configuration to (3′*S*)-hydroxystemofoline (Ohtani *et al.,* 1991). The full ^{1}H and ^{13}C NMR spectral assignments for (3′*S*)- hydroxystemofoline are shown in Table 4.8.

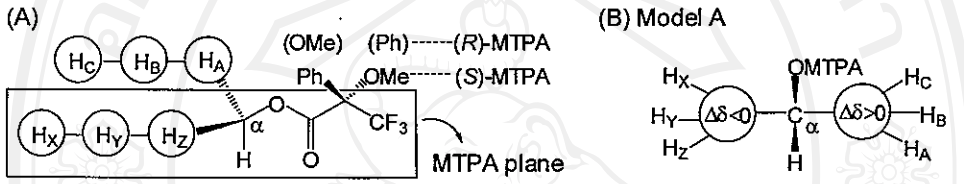
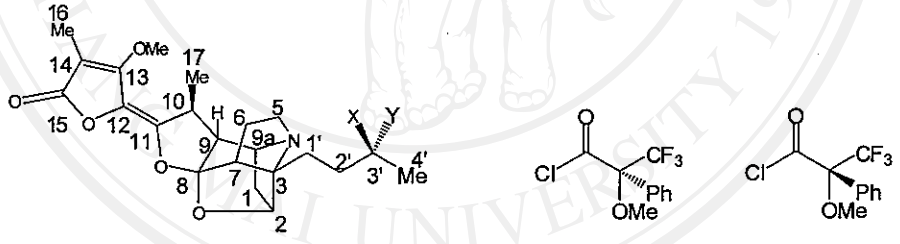


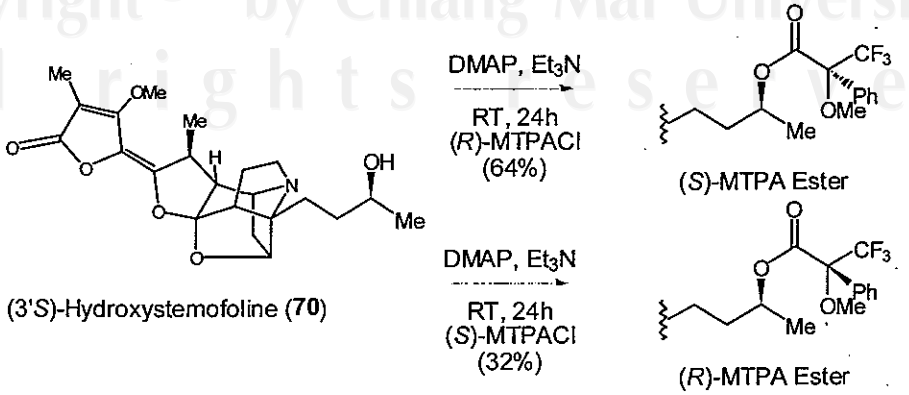
Figure 4.2 Configuration model for the (*R*) and (*S*)-MTPA derivatives proposed by Mosher (A) MTPA plane of an MTPA ester (B) Kakisawa's Model A



(3′*S*)-Hydroxystemofoline (**70**); X=OH, Y=H
Derivative **A**; X=O-(*S*)-MTPA, Y=H
Derivative **B**; X=O-(*R*)-MTPA, Y=H

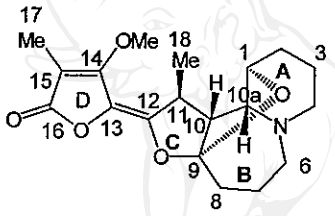
(*R*)-MTPACl (*S*)-MTPACl

Scheme 1



Stemocurtisine (58)

Stemocurtisine (**58**) was isolated as a yellow brown gum; $[\alpha]^{23}_{D}$ +314.2° (*c* 0.16,$CHCl_3$); lit. (Mungkornasawakul *et al.*, 2003) $[\alpha]^{25}_{D}$ +334° (*c* 0.66, $CHCl_3$). HRMS (EI, *m/z* [M^+] 347.1728, calcd 347.1733) indicated that this compound had the molecular formular $C_{19}H_{25}NO_5$. The structure of stemocurtisine was established from a comparison of the ^1H and ^{13}C NMR spectroscopic data of stemocurtisine with that in the literature (Mungkornasawakul *et al.*, 2003). The chemical shifts were in agreement with those previously reported as summarized in Table 4.9.



Stemocurtisine (**58**)

Table 4.4 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of neotuberostemonine and neotuberostemonine (**13**) (Ye *et al.*, 1994b) in $CDCl_3$ solution.

Data	Experimental		Literature	
			^{13}C NMR (100 MHz), ^{1}H NMR (400 MHz)	
position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)
1	37.4 (CH)	1.77 (m)	37.4 (CH)	1.75 (m)
2	32.8 (CH_2)	1.62 (dd, J = 7.3, 3.3)	32.8 (CH_2)	1.65 (m)
3	66.2 (CH)	3.33 (dd, J = 15.8, 7.8)	66.6 (CH)	3.30 (dd, J = 14.0, 7.7)
5a	50.0 (CH_2)	3.08 (dd, J = 12.5, 8.0) β	50.0 (CH_2)	3.05 (dd)
5b		2.97 (dd, J = 13.0, 8.5) α		2.92 (dd)
6a	29.8 (CH_2)	1.72 (m) β	29.8 (CH_2)	1.67 (m)
6b		1.61 (m) α		
7a	23.2 (CH_2)	1.63 (m) β	23.2 (CH_2)	1.64 (m)
7b		1.48 (m) α		1.48 (m)
8a	28.9 (CH_2)	1.93 (m) α	28.9 (CH_2)	1.91 (m)
8b		1.57 (m) β		1.65 (m)
9	34.9 (CH)	1.84 (m)	36.3 (CH)	1.85 (m)
9a	66.6 (CH)	3.20 (br.s)	66.2 (CH)	3.17 (dd, J = 3.9, 3.8)
10	36.3 (CH)	1.75 (m)	34.8 (CH)	1.72 (m)
11	78.9 (CH)	4.58 (br.s)	80.4 (CH)	4.51 (dd, J = 3.3, 3.0)
12	41.8 (CH)	2.10 (ddd, J = 10.0, 4.5, 6.0)	41.8 (CH)	2.07 (ddd, J = 15.0, 6.7, 3.3)
13	42.6 (CH)	2.89 (dq, J = 7.3, 6.5)	42.5 (CH)	2.88 (dq, J = 7.1, 6.7)
14	179.2 (C)	-	179.3 (C)	-
15	10.2 (CH_3)	1.23 (d, J = 7.0)	10.2 (CH_3)	1.23 (d, J = 7.1)
16a	21.0 (CH_2)	1.69 (m)	21.1 (CH)	1.65 (m)
16b		1.38 (m)		1.35 (m)
17	11.2 (CH_3)	1.00 (t, J = 7.3)	11.2 (CH_3)	0.99 (t, J = 7.3)
18	80.4 (CH)	4.41 (br.s)	78.9 (CH)	4.38 (ddd, J = 11.2, 7.7, 5.5)
19a	34.6 (CH_2)	2.38 (ddd, J = 13.3, 8.0, 5.0) β	34.5 (CH_2)	2.36 (ddd, J = 15.2, 13.3, 5.5)
19b		1.44 (m) α		1.45 (dd, J = 15.2, 11.2)
20	34.8 (CH)	2.62 (ddq, J = 15.0, 12.5, 7.5)	34.9 (CH)	2.59 (ddq, J = 12.1, 7.0, 5.3)
21	179.4 (C)	-	179.1 (C)	-
22	14.9 (CH_3)	1.26 (d, J = 7.0)	14.8 (CH_3)	0.88 (t, J = 7.5)

Table 4.5 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of tuberostemonine L (**69**) in $CDCl_3$ solution.

Position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	HMBC	NOESY
1	34.7 (CH)	1.74 (m)	2a, 2b, 11, 13	2a, 9a, 15
2a	33.2 (CH_2)	2.18 (ddd, J = 12.0, 6.0, 6.0) β	1, 3, 9a	1, 2b, 15
2b		1.10 (m) α		2a, 3, 12, 18
3	64.8 (CH)	3.18 (m)	2b, 5b, 19b	2b, 6b, 12, 18
5a	50.8 (CH_2)	3.30 (dd, J = 15.0, 6.5) α	3, 9a	5b
5b		2.80 (dd, J = 14.5, 10.5) β		5a
6a	25.9 (CH_2)	1.48 (m) β	5a	6b, 7b
6b		1.34 (m) α		6a, 7a
7a	29.4 (CH_2)	1.70 (m) α	5a, 5b	7b
7b		1.19 (m) β		7a
8a	32.5 (CH_2)	1.70 (m) β	10	8b, 9
8b		1.53 (m) α		8a
9	39.6 (CH)	1.98 (m)	10, 11	5b, 8a, 9a, 16
9a	64.5 (CH)	3.20 (dd, J = 7.8, 2.8)	2a, 5a, 10, 16	1, 9
10	46.8 (CH)	1.93 (m)	16, 17	8b, 11, 16, 17
11	81.3 (CH)	4.15 (dd, J = 2.0, 1.5)	10, 16	10, 12, 13, 16, 17
12	45.0 (CH)	2.25 (ddd, J = 10.8, 4.5, 6.3)	10, 13, 15	2b, 3, 11, 13
13	41.3 (CH)	2.76 (m)	1, 15	11, 12, 15
14	178.4 (C)	-	13, 15	-
15	11.6 (CH_3)	1.21 (d, J = 5.0)	13	1, 2, 13
16	25.5 (CH_2)	1.35 (m)	10, 17	10, 11, 17
17	12.9 (CH_3)	0.94 (t, J = 7.5)	10, 16	10, 11, 16
18	81.9 (CH)	4.30 (ddd, J = 11.0, 7.0, 7.5)	2b, 3, 19b	2b, 3, 19a, 20(w)
19a	34.1 (CH_2)	2.33 (ddd, J = 13.3, 5.5, 8.5) α	20	18, 19b, 20
19b		1.45 (m) β		19a, 22
20	34.9 (CH)	2.58 (ddq, J = 15.0, 12.0, 8.0)	19a, 19b	18, 19a, 22
21	179.0 (C)	-	19a, 20, 22	-
22	14.7 (CH_3)	1.19 (d, J = 4.5)	19b, 20	19b, 20

Table 4.6 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of tuberostemonine M (**71**) in $CDCl_3$ solution.

Position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	HMBC	NOESY
1	34.9 (CH)	1.85 (m)	2a, 11, 12, 13	2a, 3, 9, 15
2a	33.3 (CH_2)	2.23 (ddd, J = 11.8, 6.0, 6.0) β	-	1, 2b, 3, 19b
2b		1.25 (m) α		2a, 12
3	66.2 (CH)	3.26 (dt, J = 10.0, 7.0)	19b	1, 2a, 19b, 20
5a	51.2 (CH_2)	3.42 (dd, J = 14.8, 6.3)	-	5b, 6
5b		2.94 (dd, J = 14.5, 10.5)		5a, 6
6	29.7 (CH_2)	1.26 (m)	5a, 7b	5a, 5b
7a	29.6 (CH_2)	1.81 (m)	-	7b
7b		1.25 (m)		7a
8a	32.6 (CH_2)	1.78 (m) β	7b	8b
8b		1.66 (m) α		8a, 9a
9	39.6 (CH)	2.08 (m)	11, 16	1, 8a, 16
9a	65.0 (CH)	3.41 (m)	2a, 10(w)	2b, 8b
10	46.9 (CH)	2.03 (m)	16, 17	8b, 11, 16, 17
11	81.3 (CH)	4.21 (dd, J = 4.0, 1.5)	10, 16	10, 12, 13, 16, 17
12	45.3 (CH)	2.33 (ddd, J = 10.8, 4.5, 6.5)	10(w), 13, 15	2b, 11, 13
13	41.5 (CH)	2.84 (dq, J = 7.0, 7.0)	15	11, 12, 15
14	178.3 (C)	-	13, 15	-
15	11.8 (CH_3)	1.28 (d, J = 4.0)	13	1, 2a, 13
16	25.6 (CH_2)	1.44 (m)	10, 17	9, 10, 11, 17
17	13.1 (CH_3)	1.01 (t, J = 7.5)	10, 16	10, 11, 16
18	81.3 (CH)	4.50 (m)	19b	19a, 22
19a	34.5 (CH_2)	2.41 (ddd, J = 13.0, 5.5, 8.3) α	22	18, 19b, 22
19b		1.54 (m) β		3, 19a, 20
20	35.0 (CH)	2.65 (ddq, J = 15.5, 4.0, 7.3)	19a(w), 19b, 22	19b, 22
21	178.4 (C)	-	19a, 20, 22	-
22	14.9 (CH_3)	1.27 (d, J = 4.0)	19b, 20	18, 19a, 20

Table 4.7 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of (2′*S*)-hydroxystemofoline and (2′*S*)-hydroxystemofoline (**51**) (Brem *et al.*, 2002) in $CDCl_3$ solution.

Data	Experimental		Literature	
			^{13}C NMR (100 MHz), ^{1}H NMR (400 MHz)	
position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)
1a	33.6 (CH_2)	2.05 (m)	33.7 (CH_2)	2.05 (d, J = 15.0)
1b		1.84 (m)		1.85 (m)
2	78.6 (CH)	4.37 (br.s)	78.7 (CH)	4.36 (br.s)
3	82.9 (C)	-	82.7 (C)	-
5a	47.0 (CH_2)	3.26 (ddd, J = 14.5, 10.5, 4.5)	47.1 (CH_2)	3.25 (ddd, J = 13.7, 10.6, 5.3)
5b		3.02 (ddd, J = 13.5, 8.5, 4.5)		3.02 (ddd, J = 13.7, 8.8, 4.8)
6a	26.5 (CH_2)	1.99 (dddd, J = 16.5, 15.0, 11.0, 5.0)	26.6 (CH_2)	1.97 (m)
6b		1.87 (m)		1.89 (m)
7	51.7 (CH)	2.66 (d, J = 6.0)	52.7 (CH)	2.65 (s)
8	111.9 (C)	-	112.0 (C)	-
9	47.5 (CH)	1.83 (dd, J = 10.0, 3.5)	47.6 (CH)	1.82 (dd, J = 9.8, 3.6)
9a	60.8 (CH)	3.57 (br.s)	60.8 (CH)	3.34 (br.s)
10	34.4 (CH)	3.12 (dq, J = 9.8, 6.5)	34.4 (CH)	3.11 (dq, J = 9.8, 6.5)
11	147.8 (C)	-	147.8 (C)	-
12	128.0 (C)	-	128.0 (C)	-
13	162.7 (C)	-	162.7 (C)	-
14	98.7 (C)	-	98.8 (C)	-
15	169.6 (C)	-	169.6 (C)	-
16	9.1 (CH_3)	2.07 (s)	9.2 (CH_3)	2.08 (s)
17	18.2 (CH_3)	1.38 (d, J = 6.5)	18.3 (CH_3)	1.38 (d, J = 6.5)
1′a	36.0 (CH_2)	1.74 (d, J = 14.5)	36.1 (CH_2)	1.74 (dd, J = 14.2, 2.0)
1′b		1.66 (dd, J = 14.0, 10.5)		1.64 (dd, J = 14.2, 10.6)
2′	71.1 (CH)	3.62 (ddd, J = 10.5, 5.5, 5.0)	71.2 (CH)	3.63 (m)
3′a	30.5 (CH_2)	1.52 (q, J = 7.3)	30.6 (CH_2)	1.52 (m)
3′b		1.45 (qd, J = 7.5, 1.5)		1.43 (m)
4′	9.7 (CH_3)	0.95 (t, J = 7.3)	9.7 (CH_3)	0.95 (t, J = 7.3)
OME	58.8 (C)	4.15 (s)	58.8 (C)	4.14 (s)

Table 4.8 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of (3′*S*)-hydroxystemofoline (**70**) in $CDCl_3$ solution.

Position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	HMBC
1a	33.0 (CH_2)	1.91 (m)	1′
1b		1.75 (m)	
2	78.3 (CH)	4.23 (br.s)	1a, 1b, 6a, 1′
3	82.4 (C)	-	1a, 1b, 5b, 6a, 6b, 7, 2′b
5a	47.2 (CH_2)	3.11 (ddd, J = 14.8, 10.5, 5.0)	7
5b		2.96 (ddd, J = 13.8, 9.0, 4.8)	
6a	26.3 (CH_2)	1.89 (m)	7
6b		1.77 (m)	
7	51.0 (CH)	2.62 (d, J = 6.5)	9
8	112.4 (C)	-	2, 6b, 7
9	47.3 (CH)	1.75 (m)	10(w), 17
9a	60.8 (CH)	3.46 (br.s)	2, 5b, 9
10	34.4 (CH)	3.03 (dq, J = 10.0, 6.5)	17
11	148.1 (C)	-	10, 17
12	127.9 (C)	-	16
13	162.7 (C)	-	16, OMe
14	98.5 (C)	-	16
15	169.7 (C)	-	16
16	9.1 (CH_3)	2.00 (s)	-
17	18.2 (CH_3)	1.31 (d, J = 6.5)	9(w), 10
1′	26.4 (CH_2)	1.74 (m)	2′a(w), 2′b
2′a	33.3 (CH_2)	1.58 (m)	1′, 4′
2′b		1.51 (m)	
3′	65.9 (CH)	3.79 (ddq, J = 10.5, 6.0, 6.0)	1′, 2′b, 4′
4′	22.8 (CH_3)	1.10 (d, J = 6.0)	-
OMe	58.8 (CH_3)	4.08 (s)	-

Table 4.9 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of stemocurtisine and stemocurtisine (**58**) (Mungkornasawakul *et al.*, 2003) in $CDCl_3$ solution.

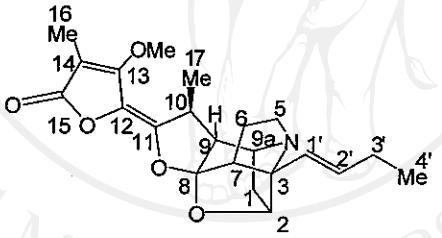
Data	Experimental		Literature	
			^{13}C NMR (75 MHz), ^{1}H NMR (300 MHz)	
position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)
1	75.2 (CH)	3.98 (s)	75.5 (CH)	4.01 (s)
2a	26.3 (CH_2)	2.14 (d, J = 15.0)	26.9 (CH_2)	2.21 (d, J = 14.5)
2b		1.59 (m)		1.62 (m)
3a	18.2 (CH_2)	1.79 (m)	18.9 (CH_2)	1.82 (m)
3b		1.21 (m)		1.21 (d, J = 13.5)
4a	53.3 (CH_2)	3.01 (m)	53.6 (CH_2)	3.02 (m)
4b		2.89 (m)		2.87 (m)
6a	53.0 (CH_2)	3.35 (dd, J = 15.5, 13.0)	53.0 (CH_2)	3.38 (t, J = 13.0)
6b		2.96 (m)		2.96 (m)
7a	26.3 (CH_2)	1.97 (m)	27.0 (CH_2)	2.03 (m)
7b		1.68 (m)		1.66 (m)
8a	33.6 (CH_2)	2.29 (m)	33.9 (CH_2)	2.36 (dd, J = 13.5, 4.5)
8b		1.71 (m)		1.75 (m)
9	120.2 (C)	-	120.4 (C)	-
10	56.7 (CH)	2.64 (d, J = 5.0)	57.0 (CH)	2.65 (d, J = 4.5)
10a	62.2 (CH)	3.44 (s)	62.0 (CH)	3.44 (s)
11	39.2 (CH)	3.01 (quin, J = 6.5)	39.3 (CH)	3.07 (quin, J = 6.5)
12	147.1 (C)	-	147.2 (C)	-
13	125.0 (C)	-	124.9 (C)	-
14	162.9 (C)	-	162.7 (C)	-
15	97.4 (C)	-	97.3 (C)	-
16	169.9 (C)	-	169.8 (C)	-
17	9.1 (CH_3)	2.00 (s)	9.2 (CH_3)	2.08 (s)
18	22.4 (CH_3)	1.31 (d, J = 7.0)	22.6 (CH_3)	1.37 (d, J = 7.0)
OMe	58.9 (CH_3)	4.08 (s)	58.9 (CH_3)	4.15 (s)

4.3.3 Unknown *S.* sp. 2 extraction and alkaloid isolation

An ethanolic crude extract of the roots of the unknown *S.* sp. (unknown 2) was partitioned between 5% hydrochloric acid solution and chloroform. The aqueous solution was basified with aqueous ammonia and extracted with chloroform. Successive purifications of this crude residue by column chromatography and preparative TLC gave pure samples of 6 new stemofoline alkaloids, methylstemofoline (**72**), (2′*R*)-hydroxystemofoline (**73**), (3′*R*)-stemofolenol (**74**), (3′*S*)-stemofolenol (**75**), stemofolinoside (**76**), and 1′,2′-didehydrostemofoline-*N*-oxide (**77**) and 3 known stemofoline alkaloids, (2′*S*)-hydroxystemofoline (**51**), (11*Z*)-1′,2′-didehydrostemofoline (**52**) and(11*E*)-1′,2′-didehydrostemofoline (**53**). The major alkaloid isolated was (11*Z*)-1′,2′-didehydrostemofoline. The structure and relative configuration of these new alkaloids were determined by spectral data interpretation and from semi-synthetic studies. ^{1}H NMR analysis of these alkaloids readily identified them as stemofoline derivatives with characteristic methyl resonances in the range: δ 4.07-4.15 (s), for the C-13 methoxy group; δ 2.00-2.08 (s) for C-16; and δ 1.30-1.39 (d), for C-17. Other diagnostic peaks were the doublet (*J* 5-7 Hz) signal between δ 2.66-2.97 for H-7, and two broad singlets between δ 4.21-4.36 and δ 3.34-3.57 for H-2 and H-9a, respectively. Examination of the ethanolic crude extract by TLC analysis showed the presence of all these alkaloids indicating that these compounds were not being produced *via* an acid catalysed reaction during the acid extraction process.

(11*Z*)- 1′,2′-Didehydrostemofoline (52)

(11*Z*)- 1′,2′-Didehydrostemofoline (**52**) was isolated as a yellow brown gum; $[\alpha]^{20}_{D}$ +195° (*c* 0.22,$CHCl_3$); lit. (Jiwajinda *et al.*, 2001) $[\alpha]^{18}_{D}$ +230° (*c* 0.74,MeOH). HRMS (ESI, *m/z* $[MH]^+$ 386.1963, calcd 386.1967) indicated that this compound had the molecular formular $C_{22}H_{28}NO_5$. The structure of (11*Z*)-1′,2′-didehydrostemofoline was established from a comparison of the ^1H and ^{13}C NMR spectroscopic data of (11*Z*)-1′,2′-didehydrostemofoline with that in the literature (Jiwajinda *et al.*, 2001). The chemical shifts were in agreement with those previously reported as summarized in Table 4.10.

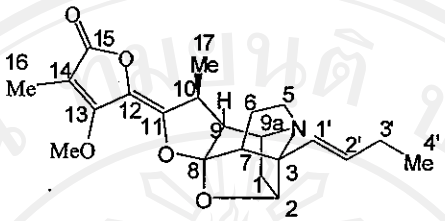


(11Z)-1',2'-Didehydrostemofoline (**52**)

(11*E*)- 1′,2′-Didehydrostemofoline (53)

(11*E*)-1′,2′-Didehydrostemofoline (**53**) was isolated as a yellow brown gum; $[\alpha]^{21}_{D}$ +71° (*c* 0.11,$CHCl_3$); lit. (Jiwajinda *et al.*, 2001) $[\alpha]^{18}_{D}$ +130° (*c* 0.01,MeOH). HRMS (EI, *m/z* $[M^+]$ 385.1884, calcd 385.1889) indicated that this compound had the molecular formular $C_{22}H_{27}NO_5$. The structure of (11*E*)-1′,2′-didehydrostemofoline was established from a comparison of the ^1H and ^{13}C NMR spectroscopic data of (11*E*)-1′,2′-didehydrostemofoline with that in the literature (Jiwajinda *et al.*, 2001).

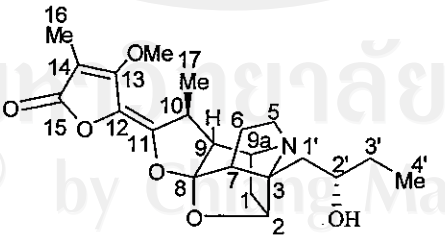
The chemical shifts were in agreement with those previously reported as summarized in Table 4.11.



(11*E*)-1',2'-Didehydrostemofoline (**53**)

(2′*S*)-Hydroxystemofoline (51)

(2′*S*)-Hydroxystemofoline (**51**) was isolated as a yellow brown gum; $[\alpha]^{21}_{D}$ +249° (*c* 0.33,$CHCl_3$); lit. (Brem *et al.*, 2002) $[\alpha]^{20}_{D}$ +197° (*c* 0.5,MeOH). HRMS (ESI, *m/z* $[MH]^+$ 404.2046, calcd 404.2073) indicated that this compound had the molecular formular $C_{22}H_{30}NO_6$. The structure of (2′*S*)-hydroxystemofoline was established from a comparison of the ^1H and ^{13}C NMR spectroscopic data of (2′*S*)-hydroxystemofoline with that in the literature (Brem *et al.*, 2002). The chemical shifts were in agreement with those previously reported as summarized in Table 4.12.

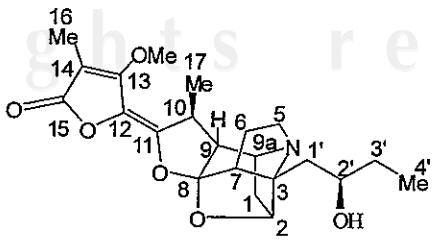


(2′*S*)-Hydroxystemofoline (**51**)

(2′*R*)-Hydroxystemofoline (**73**) (Sastraruji *et al.*, 2005)

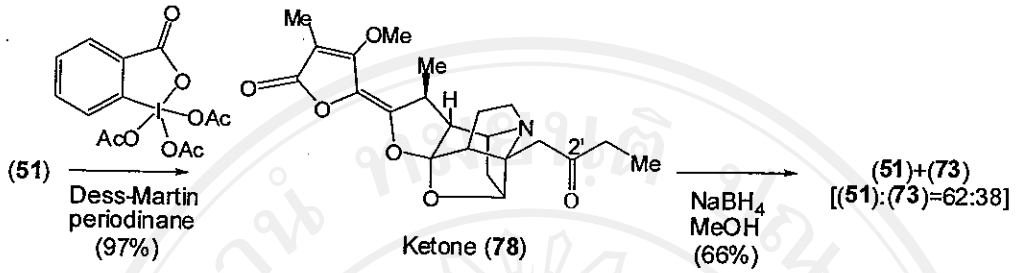
(2′*R*)-Hydroxystemofoline (**73**) was isolated as a yellow brown gum; $[\alpha]^{22}_{D}$ +168° (*c* 0.29,$CHCl_3$). The HRMS analysis of (2′*R*)-hydroxystemofoline (EI, *m/z*

[M^+] 403.1993, calcd 403.1995) showed that this compound had the same molecular formula, $C_{22}H_{29}NO_6$, as (2′*S*)-hydroxystemofoline (**51**). A new alkaloid (2′*R*)-hydroxystemofoline (**73**), the C-2′ epimer of (2′*S*)-hydroxystemofoline (**51**), was also isolated and had the same molecular formula as (2′*S*)-hydroxystemofoline (**51**) (HRMS (ESI +ve, *m/z* $[M+H]^+$ 404.2046., calcd for $C_{22}H_{30}NO_6$ 404.2073)). These epimeric alkaloids had different mobilities on silica gel, (2′*R*)-hydroxystemofoline was more polar than (2′*S*)-hydroxystemofoline, which their difference in polarity occurred because of the internal H-bonding of (2′*S*)-hydroxystemofoline. The similar but different ^1H and ^{13}C NMR spectra were shown in Table 4.13. To unequivocally show that these compounds were C-2′ epimers, (2′*S*)-hydroxystemofoline (**51**) was oxidized to the ketone (**78**) using the Dess-Martin periodinane reagent showed in Scheme 2 (Dess *et al.,* 1991). Ketone (**78**) showed the expected downfield shifts for the ^1H and ^{13}C NMR resonances of the C-3 butyl side chain, especially for C-2′ which appeared at a typical aliphatic ketone carbonyl chemical shift (δ 208.6, Table 4.14). Sodium borohydride reduction of ketone (**78**) gave a 62: 38 mixture of the epimeric carbinols (2′*S*)-hydroxystemofoline and (2′*R*)-hydroxystemofoline, respectively. The full ^1H and ^{13}C NMR spectral assignments for (2′*R*)-hydroxystemofoline are shown in Table 4.15.



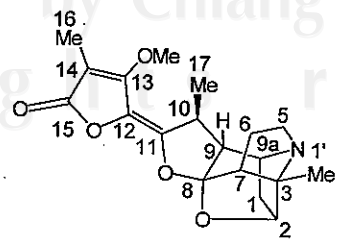
(2′*R*)-Hydroxystemofoline (**73**)

Scheme 2



Methylstemofoline (72) (Sastraruji *et al.*, 2005)

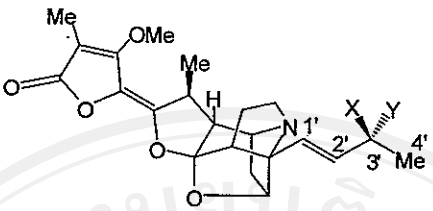
Methylstemofoline (**72**) was isolated as a yellow gum; $[\alpha]^{23}_{D}$ +125° (*c* 0.24,$CHCl_3$). HRMS (EI +ve, *m/z* [M^+] 345.1561, calcd 345.1576) indicated that this compound had the molecular formular $C_{19}H_{23}NO_5$. The first C_{19} stemofoline derivative, methylstemofoline, had 3 carbons less than a normal stemofoline derivative. The ^{1}H NMR spectrum of methylstemofoline showed a methyl singlet at δ 1.35, for the C-3 methyl, while the ^{13}C/DEPT NMR spectra of methylstemofoline showed 19 carbons, 4 of which were methyl groups. The 3 methylenes of the C-3 butyl side chain of stemofoline were clearly absent. Furthermore, the new methyl resonance at δ 1.35 showed HMBC correlations to both C-2 and C-7, indicative of a C-3 methyl substituent. The full ^{1}H and ^{13}C NMR spectral assignments for methylstemofoline are shown in Table 4.16.



Methylstemofoline (**72**)

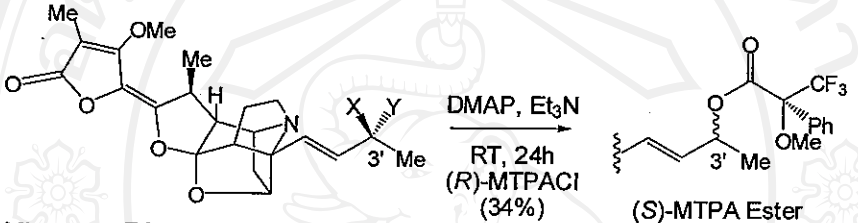
(3′*R*) and (3′*S*)-stemofolenol (74 and 75) (Sastraruji *et al.*, 2005)

(3′*R*)-Stemofolenol and (3′*S*)-stemofolenol (**74** and **75**) were isolated as a mixture (50 : 50, not necessarily respectively) that was homogeneous by TLC analysis. HRMS (EI +ve, *m/z* [M^+] 401.1854, calcd 401.1838) indicated that these isomer compounds had the molecular formular $C_{22}H_{27}NO_6$ and indicated that (3′*R*)-stemofolenol and (3′*S*)-stemofolenol were 1′,2′-didehydrohydoxystemofoline derivatives. The ^{1}H NMR spectrum (500 MHz) of this mixture indicated an apparent single diastereomer with the C-3, 3′-hydroxy-(1′*E*)-butenyl side chain clearly evident (δ 5.87 (dd, *J* = 15.9, 5.1 Hz, H-2′); 5.76 (d, *J* = 15.9 Hz, H-1′); 4.36 (quin, *J* = 6.5 Hz, H-3′); 1.28 (d, *J* = 6.5 Hz, H-4′). The ^{13}C NMR spectrum, however, showed 23 carbon signals with 2 closely resolved methyl resonances at δ 23.41 and δ 23.35 that indicated 2 epimeric compounds at C-3′. This was further substantiated by treatment of the mixture of (3′*R*)-stemofolenol and (3′*S*)-stemofolenol with (*R*)-Mosher's acid chloride ((*R*)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride ((*R*)-MTPACl)), Scheme 3, that gave a 44 : 56 mixture of diastereomeric esters as evident by the doubling up of all methyl resonances in the derivatives **C** and **D**. Further evidence, Scheme 4, that this sample was a mixture of C-3' epimers was obtained from its oxidation with the Dess-Martin periodinane reagent (Dess *et al.,* 1991) to give the ketone (**79**) (Table 4.17) as a single diastereomer which upon reduction with $NaBH_4/CeCl_3.H_2O$ (Luche *et al.,* 1978) provided a 45: 55 mixture of (3′*R*)-stemofolenol and (3′*S*)-stemofolenol, not necessarily respectively, from ^{13}C NMR analysis. The full ^{1}H and ^{13}C NMR spectral assignments for (3′*R*)-stemofolenol and (3′*S*)-stemofolenol are shown in Table 4.18.



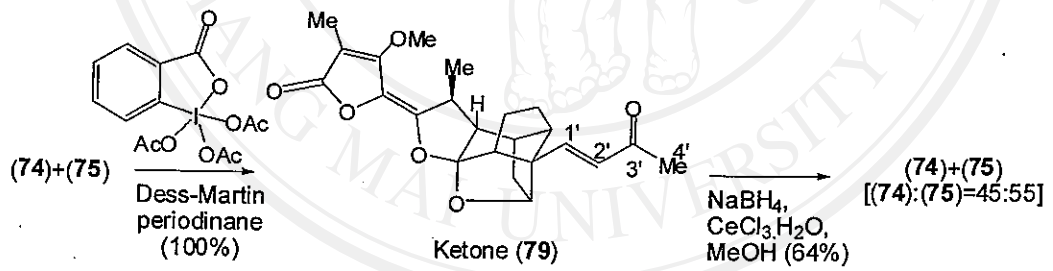
(3'*R*)-Stemofolenol (**74**); X=OH, Y=H
(3'*S*)-Stemofolenol (**75**); X=H, Y=OH
Derivative **C**; X=O-(*S*)-MTPA, Y=H
Derivative **D**; X=H, Y=O-(*S*)-MTPA

Scheme 3



Mixture of (**74**) and (**75**)
(3'*R*)-Stemofolenol (**74**); X=OH, Y=H
(3'*S*)-Stemofolenol (**75**); X=H, Y=OH

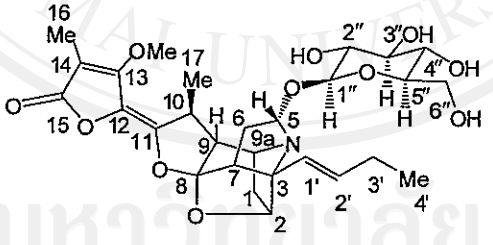
Scheme 4



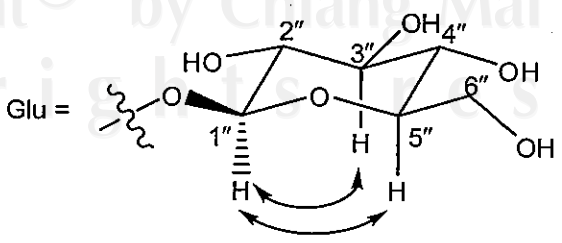
Stemofolinoside (76) (Sastraruji *et al.*, 2005)

Stemofolinoside (**76**) was isolated as a yellow brown amorphous solid; $[\alpha]^{22}_{D}$ +138° (*c* 0.16,$CHCl_3$). HRMS (ESI, *m/z* $[MH]^+$ 564.2469, calcd 564.2445) indicated that this compound had the molecular formular $C_{28}H_{38}NO_{11}$ (stemofoline + $C_6H_8O_6$) which indicated a glycosylated 1′,2′-didehydrostemofoline derivative. This was further confirmed from ^{13}C NMR analysis that showed 2 alkene carbons (δ 129.1 (C-1′) and 134.4 (C-2′)) and 5 new CH-O (δ 102.4, 78.4, 77.6, 74.3 and 71.5) resonances

and a new CH_2-O (δ 62.6) resonance for the glycoside. Furthermore, the typical C-5 methylene resonance of the stemofolines at *ca.* δ 48 had been replaced by a methine resonance at δ 92.2, which indicated hydroxylation had occurred at this position. The position of the glycoside at C-5 was evident from HMBC correlations between H-5 (δ 5.21) and the anomeric carbon of the glycoside (C-1″) at δ 102.4. In the ^{1}H NMR spectrum, the relatively large $J_{1'',2''}$ of 8.5 Hz indicated the β-anomeric configuration of the glycoside (Agrawal, 1992). NOESY NMR experiments showed cross peaks between the anomeric proton (H-1″) and the H-3″ and H-5″ protons, which indicated that the glycoside was either a β-D-glucopyranoside or a β-D-galatopyranoside (Agrawal, 1992). This distinction however was readily made on the basis of the ^{13}C NMR chemical of the glycoside that indicated a D-glucopyranoside (Agrawal, 1992). The full ^{1}H and ^{13}C NMR spectral assignments for stemofolinoside are shown in Table 4.19.



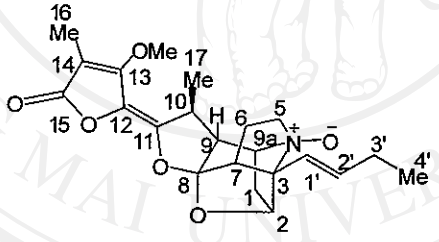
Stemofolinoside (**76**)



NOESY cross peak

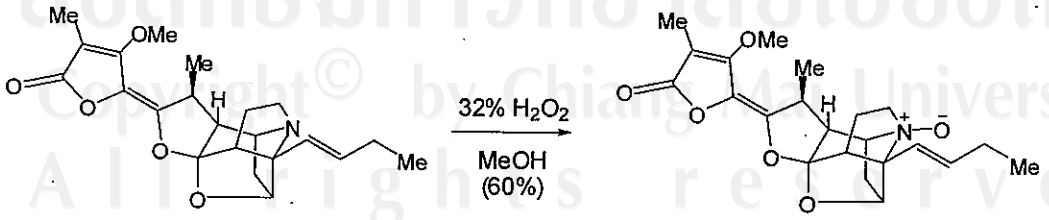
1′,2′-Didehydrostemofoline-*N*-oxide (77) (Sastraruji *et al.*, 2005)

1′,2′-Didehydrostemofoline-*N*-oxide (**77**) was isolated as a yellow brown gum; $[\alpha]^{25}_{D}$ +99° (*c* 0.28,$CHCl_3$). HRMS (EI, *m/z* [M^+] 401.1832, calcd 401.1838) indicated that this compound had the molecular formular $C_{22}H_{27}NO_6$. The very polar *N*-oxide, 1′,2′-didehydrostemofoline-*N*-oxide, was isolated and was readily synthesised, as a single diastereomer, by oxidation of (11*Z*)-1',2'-didehydrostemofoline (**52**) with 32% H_2O_2 in MeOH (Scheme 5). Not unexpectantly the chemical shifts of the protons and carbons close to the *N*-oxide moiety in 1′,2′-didehydrostemofoline-*N*-oxide were shifted significantly downfield relative to those in (11*Z*)-1′,2′-didehydrostemofoline (**52**). The full ^1H and ^{13}C NMR spectral assignments for 1′,2′-didehydrostemofoline-*N*-oxide are shown in Table 4.20.



1',2'-Didehydrostemofoline-*N*-oxide (**77**)

Scheme 5



(11Z)-1',2'-Didehydrostemofoline (**52**) 1',2'-Didehydrostemofoline-*N*-oxide (**77**)

Table 4.10 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of (11*Z*)-1′,2′-didehydrostemofoline and (11*Z*)-1′,2′-didehydrostemofoline (**52**) (Jiwajinda *et al.*, 2001) in $CDCl_3$ solution.

Data	Experimental		Literature	
			^{13}C NMR (100 MHz), ^{1}H NMR (400 MHz)	
position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)
1a	32.8 (CH_2)	1.96 (d, J = 12.0)	32.9 (CH_2)	1.7-2.0 (m)
1b		1.81 (m)		
2	80.6 (CH)	4.21 (br.s)	79.7 (CH)	4.20 (br s)
3	83.1 (C)	-	83.1 (C)	-
5a	48.0 (CH_2)	3.10 (m)	48.0 (CH_2)	~2.95-3.1 (m)
5b		2.99 (ddd, J = 13.0, 8.8, 4.5)		
6a	26.8 (CH_2)	1.88 (m)	26.9 (CH_2)	1.7-2.0 (m)
6b		1.80 (m)		
7	51.1 (CH)	2.86 (d, J = 6.0)	51.2 (CH)	2.84 (d, J = 5.8)
8	112.8 (C)	-	112.8 (C)	-
9	47.6 (CH)	1.79 (m)	47.6 (CH)	1.7-2.0 (m)
9a	60.8 (CH)	3.52 (br.s)	60.9 (CH)	3.50 (br.s)
10	34.6 (CH)	3.11 (dd, J = 7.0, 3.0)	34.6 (CH)	~3.1 (m)
11	148.4 (C)	-	148.4 (C)	-
12	127.9 (C)	-	128.0 (C)	-
13	162.8 (C)	-	162.8 (C)	-
14	98.6 (C)	-	98.6 (C)	-
15	169.7 (C)	-	169.7 (C)	-
16	9.1 (CH_3)	2.07 (s)	9.2 (CH_3)	2.07 (s)
17	18.3 (CH_3)	1.38 (d, J = 6.0)	18.3 (CH_3)	1.38 (d, J = 6.4)
1′	126.5 (CH)	5.51 (d, J = 15.8)	126.5 (CH)	5.50 (d, J = 15.6)
2′	133.3 (CH)	5.78 (dt, J = 15.8, 6.5)	133.4 (CH)	5.78 (dt, J = 15.6, 6.4)
3′	25.3 (CH_2)	2.08 (m)	25.3 (CH_2)	~2.05 (m)
4′	13.4 (CH_3)	1.00 (t, J = 7.5)	13.5 (CH_3)	0.99 (t, J = 7.3)
OMe	58.8 (CH_3)	4.14 (s)	58.8 (CH_3)	4.14 (s)

Table 4.11 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of (11*E*)-1′,2′-didehydrostemofoline and (11*E*)-1′,2′-didehydrostemofoline (**53**) (Jiwajinda *et al.,* 2001) in $CDCl_3$ solution.

Data	**Experimental**		**Literature**	
			^{13}C NMR (100 MHz), ^{1}H NMR (400 MHz)	
position	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**
1a	32.7 (CH_2)	1.94 (d, J = 12.0)	32.9 (CH_2)	1.7-2.0 (m)
1b		1.79 (m)		
2	80.8 (CH)	4.23 (br.s)	79.7 (CH)	4.22 (br s)
3	83.3 (C)	-	83.1 (C)	-
5a	47.9 (CH_2)	3.12 (m)	48.0 (CH_2)	~3.0-3.2 (m)
5b		3.02 (ddd, J = 13.3, 8.8, 4.5)		
6a	26.7 (CH_2)	1.86 (m)	26.9 (CH_2)	1.7-2.0 (m)
6b		1.78 (m)		
7	51.2 (CH)	2.88 (d, J = 6.0)	51.2 (CH)	2.86 (d, J = 6.1)
8	113.6 (C)	-	112.8 (C)	-
9	45.9 (CH)	1.77 (dd, J = 11.0, 3.5)	47.6 (CH)	1.7-2.0 (m)
9a	60.9 (CH)	3.57 (br.s)	60.9 (CH)	3.52 (br.s)
10	36.2 (CH)	3.16 (dq, J = 11.0, 6.8)	34.6 (CH)	~3.1 (m)
11	149.8 (C)	-	148.4 (C)	-
12	128.9 (C)	-	128.0 (C)	-
13	163.3 (C)	-	162.8 (C)	-
14	98.5 (C)	-	98.6 (C)	-
15	170.5 (C)	-	169.7 (C)	-
16	8.8 (CH_3)	2.04 (s)	9.2 (CH_3)	2.07 (s)
17	16.2 (CH_3)	1.46 (d, J = 7.0)	18.3 (CH_3)	1.46 (d, J = 6.7)
1′	126.0 (CH)	5.53 (d, J = 15.5)	126.5 (CH)	5.50 (d, J = 15.6)
2′	133.7 (CH)	5.80 (dt, J = 15.5, 6.5)	133.4 (CH)	5.78 (dt, J = 15.6, 6.4)
3′	25.3 (CH_2)	2.08 (m)	25.3 (CH_2)	~2.05 (m)
4′	13.4 (CH_3)	0.99 (t, J = 7.5)	13.5 (CH_3)	0.99 (t, J = 7.9)
OMe	59.4 (CH_3)	4.11 (s)	58.8 (CH_3)	4.13 (s)

Table 4.12 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of (2′*S*)-hydroxystemofoline and (2′*S*)-hydroxystemofoline (**51**) (Brem *et al.*, 2002) in $CDCl_3$ solution.

Data	**Experimental**		**Literature** ^{13}C NMR (100 MHz), ^{1}H NMR (400 MHz)	
position	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**	**δ_C (DEPT)**	**δ_H (mult., *J* (Hz), assign.)**
1a	33.6 (CH_2)	2.04 (m)	33.7 (CH_2)	2.05 (d, J = 15.0)
1b		1.87 (m)		1.85 (m)
2	78.6 (CH)	4.37 (br.s)	78.7 (CH)	4.36 (br.s)
3	82.9 (C)	-	82.7 (C)	-
5a	47.0 (CH_2)	3.27 (ddd, J = 14.3, 10.5, 5.1)	47.1 (CH_2)	3.25 (ddd, J = 13.7, 10.6, 5.3)
5b		3.03 (ddd, J = 14.3, 8.9, 5.1)		3.02 (ddd, J = 13.7, 8.8, 4.8)
6a	26.5 (CH_2)	1.97 (m)	26.6 (CH_2)	1.97 (m)
6b		1.90 (m)		1.89 (m)
7	51.7 (CH)	2.66 (d, J = 5.7)	52.7 (CH)	2.65 (s)
8	112.0 (C)	-	112.0 (C)	-
9	47.5 (CH)	1.82 (dd, J = 9.9, 3.9)	47.6 (CH)	1.82 (dd, J = 9.8, 3.6)
9a	60.8 (CH)	3.57 (br.s)	60.8 (CH)	3.34 (br.s)
10	34.3 (CH)	3.12 (dq, J = 9.9, 6.3)	34.4 (CH)	3.11 (dq, J = 9.8, 6.5)
11	147.7 (C)	-	147.8 (C)	-
12	128.0 (C)	-	128.0 (C)	-
13	162.7 (C)	-	162.7 (C)	-
14	98.7 (C)	-	98.8 (C)	-
15	169.6 (C)	-	169.6 (C)	-
16	9.1 (CH_3)	2.08 (s)	9.2 (CH_3)	2.08 (s)
17	18.2 (CH_3)	1.38 (d, J = 6.6)	18.3 (CH_3)	1.38 (d, J = 6.5)
1′a	36.0 (CH_2)	1.75 (dd, J = 14.3, 2.1)	36.1 (CH_2)	1.74 (dd, J = 14.2, 2.0)
1′b		1.66 (dd, J = 14.3, 9.9)		1.64 (dd, J = 14.2, 10.6)
2′	71.1 (CH)	3.62 (m)	71.2 (CH)	3.63 (m)
3′a	30.5 (CH_2)	1.54 (q, J = 7.2)	30.6 (CH_2)	1.52 (m)
3′b		1.45 (qd, J = 7.2, 1.8)		1.43 (m)
4′	9.7 (CH_3)	0.95 (t, J = 7.2)	9.7 (CH_3)	0.95 (t, J = 7.3)
OME	58.8 (CH_3)	4.15 (s)	58.8 (C)	4.14 (s)

Table 4.13 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of (2′*R*)-hydroxystemofoline (**73**) comparing with (2′*S*)-hydroxystemofoline (**51**) in $CDCl_3$ solution.

Data	(2′*R*)-hydroxystemofoline (**73**)		(2′*S*)-hydroxystemofoline (**51**)	
position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)
1a	32.8 (CH_2)	1.88 (d, J = 12.5)	33.6 (CH_2)	2.04 (m)
1b		1.81 (m)		1.87 (m)
2	79.8 (CH)	4.42 (br.s)	78.6 (CH)	4.37 (br.s)
3	81.9 (C)	-	82.9 (C)	-
5a	47.6 (CH_2)	3.09 (ddd, J = 14.3, 10.0, 5.0)	47.0 (CH_2)	3.27 (ddd, J = 14.3, 10.5, 5.1)
5b		2.95 (ddd, J = 14.3, 8.8, 5.0)		3.03 (ddd, J = 14.3, 8.9, 5.1)
6a	26.5 (CH_2)	1.88 (m)	26.5 (CH_2)	1.97 (m)
6b		1.77 (m)		1.90 (m)
7	51.8 (CH)	2.64 (d, J = 6.0)	51.7 (CH)	2.66 (d, J = 5.7)
8	111.8 (C)	-	112.0 (C)	-
9	47.3 (CH)	1.72 (dd, J = 9.0, 4.0)	47.5 (CH)	1.82 (dd, J = 9.9, 3.9)
9a	60.9 (CH)	3.43 (br.s)	60.8 (CH)	3.57 (br.s)
10	34.4 (CH)	3.02 (dq, J = 9.0, 6.8)	34.3 (CH)	3.12 (dq, J = 9.9, 6.3)
11	148.3 (C)	-	147.7 (C)	-
12	127.8 (C)	-	128.0 (C)	-
13	162.8 (C)	-	162.7 (C)	-
14	98.4 (C)	-	98.7 (C)	-
15	169.7 (C)	-	169.6 (C)	-
16	9.0 (CH_3)	2.00 (s)	9.1 (CH_3)	2.08 (s)
17	18.2 (CH_3)	1.30 (d, J = 7.0)	18.2 (CH_3)	1.38 (d, J = 6.6)
1′a	36.7 (CH_2)	1.76 (dd, J = 15.2, 3.8)	36.0 (CH_2)	1.75 (dd, J = 14.3, 2.1)
1′b		1.70 (dd, J = 15.2, 7.3)		1.66 (dd, J = 14.3, 9.9)
2′	71.0 (CH)	3.69 (m)	71.1 (CH)	3.62 (m)
3′a	31.1 (CH_2)	1.43 (quin, J = 7.5)	30.5 (CH_2)	1.54 (q, J = 7.2)
3′b				1.45 (qd, J = 7.2, 1.8)
4′	10.2 (CH_3)	0.88 (t, J = 7.5)	9.7 (CH_3)	0.95 (t, J = 7.2)
OME	58.8 (CH_3)	4.07 (s)	58.8 (CH_3)	4.15 (s)

Table 4.14 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of Ketone (**78**) in $CDCl_3$ solution.

Position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	HMBC
1a	32.7 (CH_2)	2.01 (d, J = 12.5)	9a(w)
1b		1.96 (d, J = 12.5)	
2	78.9 (CH)	4.60 (br.s)	1, 1′
3	80.9 (C)	-	1, 5b, 6a, 6b, 1′
5a	47.6 (CH_2)	3.23 (ddd, J = 14.8, 10.5, 5.5)	7
5b		3.14 (m)	
6a	26.2 (CH_2)	1.98 (m)	-
6b		1.87 (m)	
7	51.2 (CH)	2.73 (d, J = 5.5)	6b
8	111.7 (C)	-	2, 6b, 7
9	47.2 (CH)	1.78 (dd, J = 9.7, 3.5)	7(w), 10, 17
9a	61.3 (CH)	3.56 (br.s)	2, 10
10	34.4 (CH)	3.10 (dq, J = 9.7, 6.5)	9(w), 17
11	148.0 (C)	-	10, 16(w), 17
12	128.0 (C)	-	10(w), 16
13	162.7 (C)	-	16, OMe
14	98.7 (C)	-	16
15	169.6 (C)	-	16
16	9.1 (CH_3)	2.05 (s)	-
17	18.2 (CH_3)	1.35 (d, J = 6.5)	9, 10
1′a	42.9 (CH_2)	2.94 (d, J = 16.3)	7(w)
1′b		2.65 (d, J = 16.3)	
2′	208.3 (C)	-	1′, 3′, 4′
3′	37.6 (CH_2)	2.46 (m)	4′
4′	7.3 (CH_3)	1.01 (t, J = 7.3)	3′
OMe	58.9 (CH_3)	4.11 (s)	-

Table 4.15 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of (2′*R*)-hydroxystemofoline (**73**) in $CDCl_3$ solution.

Position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	HMBC
1a	32.8 (CH_2)	1.88 (d, J = 12.5)	1′
1b		1.81 (m)	
2	79.8 (CH)	4.42 (br.s)	1a, 1b, 1′
3	81.9 (C)	-	1a, 1b, 5b, 6a, 6b, 7
5a	47.6 (CH_2)	3.09 (ddd, J = 14.3, 10.0, 5.0)	7
5b		2.95 (ddd, J = 14.3, 8.8, 5.0)	
6a	26.5 (CH_2)	1.88 (m)	-
6b		1.77 (m)	
7	51.8 (CH)	2.64 (d, J = 6.0)	6b
8	111.8 (C)	-	2, 6b, 7, 9a(w),1′
9	47.3 (CH)	1.72 (dd, J = 9.0, 4.0)	10, 17
9a	60.9 (CH)	3.43 (br.s)	2, 5a, 5b, 9
10	34.4 (CH)	3.02 (dq, J = 9.0, 6.8)	17
11	148.3 (C)	-	10, 17
12	127.8 (C)	-	16(w)
13	162.8 (C)	-	16, OMe
14	98.4 (C)	-	16
15	169.7 (C)	-	16, OMe(w)
16	9.0 (CH_3)	2.00 (s)	-
17	18.2 (CH_3)	1.30 (d, J = 7.0)	10
1′a	36.7 (CH_2)	1.76 (dd, J = 15.2, 3.8)	7, 3′
1′b		1.70 (dd, J = 15.2, 7.3)	
2′	71.0 (CH)	3.69 (m)	3′, 4′
3′	31.1 (CH_2)	1.43 (quin, J = 7.5)	4′
4′	10.2 (CH_3)	0.88 (t, J = 7.5)	3′
OMe	58.8 (CH_3)	4.07 (s)	-

Table 4.16 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of methylstemofoline (**72**) in $CDCl_3$ solution.

Position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	HMBC
1a	32.6 (CH_2)	1.93 (d, J = 12.5)	-
1b		1.84 (m)	
2	80.1 (CH)	4.15 (br.s)	1a, 1b, 1′
3	79.9 (C)	-	5b, 7, 1
5a	47.1 (CH_2)	3.20 (ddd, J = 14.0, 10.5, 5.0)	7
5b		3.03 (m)	
6a	25.8 (CH_2)	1.97 (m)	-
6b		1.82 (m)	
7	51.4 (CH)	2.69 (d, J = 5.0)	6a, 1′
8	112.3 (C)	-	1b, 2, 6b, 7
9	46.6 (CH)	1.78 (dd, J = 9.8, 3.5)	10, 17
9a	61.4 (CH)	3.55 (br.s)	2, 5a, 10
10	34.3 (CH)	3.03 (dq, J = 9.8, 6.0)	9, 17
11	148.0 (C)	-	10, 17
12	127.9 (C)	-	16, OMe(w)
13	162.7 (C)	-	16, OMe
14	98.5 (C)	-	16
15	169.6 (C)	-	16, OMe
16	9.0 (CH_3)	2.00 (s)	-
17	18.1 (CH_3)	1.32 (d, J = 6.5)	9, 10
1′	18.6 (CH_3)	1.35 (s)	5b, 7
OMe	58.8 (CH_3)	4.08 (s)	-

Table 4.17 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of Ketone (**79**) in $CDCl_3$ solution.

Position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	HMBC
1a	32.6 (CH_2)	2.00 (d, J = 12.0)	-
1b		1.78 (dt, J = 12.0, 3.3)	
2	80.0 (CH)	4.32 (br.s)	1a
3	83.2 (C)	-	1a, 5b(w), 1′, 2′
5	48.2 (CH_2)	3.08 (m)	-
6	26.7 (CH_2)	1.86 (m)	-
7	52.3 (CH)	2.97 (t, J = 3.3)	2′(w)
8	112.6 (C)	-	2(w), 6(w)
9	47.6 (CH)	1.87 (dd, J = 6.6, 3.3)	17
9a	61.0 (CH)	3.56 (br.s)	9, 10(w)
10	34.5 (CH)	3.12 (m)	9, 17
11	147.9 (C)	-	17
12	129.3 (C)	-	16
13	162.7 (C)	-	16, OMe
14	98.7 (C)	-	16
15	169.6 (C)	-	16
16	9.2 (CH_3)	2.08 (s)	-
17	18.3 (CH_3)	1.40 (d, J = 6.6)	9
1′	143.8 (CH)	6.82 (d, J = 15.8)	2′, 4′ (w)
2′	130.6 (CH)	6.38 (d, J = 15.8)	4′
3′	197.9 (C)	-	1′, 2′, 4′
4′	27.7 (CH_3)	2.28 (s)	-
OMe	58.9 (CH_3)	4.15 (s)	-

Table 4.18 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of (3′*R*)-stemofolenol (**74**) and (3′*S*)-stemofolenol (**75**) in $CDCl_3$ solution.

Position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	HMBC
1a	32.7 (CH_2)	1.98 (dd, J = 12.0, 3.0)	-
1b		1.80 (m)	
2	80.3 (CH)	4.25 (br.s)	1a
3	83.0 (C)	-	1a, 5b(w), 6(w), 1′, 2′
5a	47.9 (CH_2)	3.05 (m)	-
5b		3.01 (m)	
6	26.6 (CH_2)	1.83 (m)	-
7	51.3 (CH)	2.89 (d, J = 5.4)	-
8	112.6 (C)	-	1b, 7
9	47.4 (CH)	1.85 (dd, J = 5.9, 3.0)	7, 17
9a	60.9 (CH)	3.57 (br.s)	9
10	34.5 (CH)	3.08 (m)	17
11	148.2 (C)	-	10, 16, 17
12	127.9 (C)	-	16
13	162.8 (C)	-	16, OMe
14	98.6 (C)	-	16
15	169.7 (C)	-	16,OMe(w)
16	9.1 (CH_3)	2.07 (s)	-
17	18.2 (CH_3)	1.39 (d, J = 6.3)	10
1′	126.5 (CH)	5.76 (d, J = 15.9)	2′, 3′
2′	135.7 (CH)	5.87 (dd, J = 15.9, 5.1)	1′, 4′
3′	67.9 (CH)	4.36 (quin, J = 6.5)	1′, 2′, 4′
4′	23.41, 23.35 (CH_3)	1.28 (d, J = 6.5)	3′
OMe	58.8 (CH_3)	4.15 (s)	-

Table 4.19 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of stemofolinoside (**76**) in $CDCl_3$ solution.

Position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	HMBC	NOESY
1a	33.5 (CH_2)	2.00 (d, J = 12.5)	-	1b, 2(w), 10
1b		1.78 (dd, J = 12.5, 3.5)		1a, 2
2	82.3 (CH)	4.28 (br.s)	-	1a(w), 1b
3	83.4 (C)	-	1a, 6a, 1′, 2′	-
5	92.2 (CH)	5.21 (dd, J = 6.0, 3.0)	7, 1″	6a, 9, 9a(w), 1″
6a	36.5 (CH_2)	2.35 (dd, J = 14.0, 6.0)	-	6b, 7(w), 9
6b		2.15 (m)		6a, 7
7	55.0 (CH)	2.92 (d, J = 5.5)	6a	6a(w), 6b
8	113.3 (C)	-	2(w), 6a, 7	-
9	49.8 (CH)	1.85 (d, J = 10.0)	7(w), 17	9a(w), 17
9a	59.9 (CH)	3.76 (br.s)	2(w)	5, 9(w)
10	36.2 (CH)	3.10 (m)	17	1a, 17
11	150.9 (C)	-	10(w), 17	-
12	129.0 (C)	-	16	-
13	165.1 (C)	-	16, OMe	-
14	99.2 (C)	-	16	-
15	172.3 (C)	-	16	-
16	13.8 (CH_3)	2.06 (s)	-	OMe
17	18.0 (CH_3)	1.40 (d, J = 6.5)	-	9, 10
1′	129.1 (CH)	5.79 (d, J = 16.0)	3′	-
2′	134.4 (CH)	5.88 (dt, J = 16.0, 6.0)	3′, 4′	3′(w)
3′	26.6 (CH_2)	2.10 (quin, J = 7.3)	1′, 2′, 4′	2′(w), 4′
4′	9.0 (CH_3)	1.02 (t, J = 7.3)	-	3′
1″	102.4 (CH)	4.41 (d, J = 8.5)	3″	5, 3″, 5″(w)
2″	78.4 (CH)	3.29 (m)	-	-
3″	74.3 (CH)	3.31 (m)	-	1″
4″	71.5 (CH)	3.32 (m)	5″	6″a, 6″b(w)
5″	77.6 (CH)	3.37 (m)	3″	1″
6″a	62.6 (CH_2)	3.85 (d, J = 11.8)	-	4″
6″b		3.68 (dd, J = 11.8, 5.0)		4″(w)
OMe	59.9 (CH_3)	4.21 (s)	-	16, 17(w)

Table 4.20 ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectroscopic data of 1′,2′-didehydrostemofoline-*N*-oxide (77) in $CDCl_3$ solution.

Position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	HMBC
1a	31.6 (CH_2)	2.98 (d, J = 12.8)	-
1b		2.08 (d, J = 12.8)	
2	80.0 (CH)	4.29 (br.s)	1b, 9a
3	91.8 (C)	-	1b, 9a, 2′, 3′
5a	63.5 (CH_2)	4.04 (m)	1b, 7
5b		3.66 (m)	
6a	21.9 (CH_2)	2.26 (m)	-
6b		1.84 (m)	
7	48.2 (CH)	3.13 (d, J = 6.5)	10, 1′
8	110.8 (C)	-	1b, 2, 6b, 7, 9a
9	48.5 (CH)	2.25 (d, J = 9.9)	7, 10, 17
9a	77.0 (CH)	4.08 (br.s)	2, 9(w)
10	34.7 (CH)	3.19 (dq, J = 9.9, 6.5)	9, 17
11	147.1 (C)	-	10, 17
12	128.2 (C)	-	16
13	162.3 (C)	-	16, OMe
14	98.9 (C)	-	16(w), OMe
15	169.3 (C)	-	16, OMe(w)
16	9.0 (CH_3)	2.02 (s)	-
17	17.9 (CH_3)	1.39 (d, J = 6.5)	9, 10
1′	119.6 (CH)	6.06 (d, J = 16.0)	3′, 4′
2′	137.1 (CH)	5.83 (dt, J = 16.0, 6.0)	3’
3′	25.7 (CH_2)	2.15 (quin, J = 7.2)	1′, 2′, 4′
4′	12.9 (CH_3)	1.00 (t, J = 7.2)	2′, 3′
OMe	58.8 (CH_3)	4.10 (s)	-

4.4 Determination of bioactive compounds

Brine shrimp assays

The toxic activities of the pure alkaloids from *Stemona* spp. on *Artemia salina* Leach (brine shrimp) were determined. The brine shrimp were treated with the crude extracts from *S. curtisii* and the unknown *S.* spp. unknown 1 and unknown 2. They were also treated with pure samples of stemocurtisinol (**63**), oxyprotostemonine (**21**), stemocurtisine (**58**), (11*Z*)-1′,2′-didehydrostemofoline (**52**), (2′*S*)-hydroxystemofoline (**51**) and the mixture of 3′-stemofolenol (**74**+**75**). The ratio of dead/alive nauplii was analysed to determine the LC_{50} by probit analysis (Table 4.21).

Table 4.21 LC_{50} of *stemona* spp. compounds on *Artemia salina* Leach (brine shrimp)

***Stemona* spp. compounds**	**LC_{50} (ppm)**
Ethanolic crude	
S. curtisii	37
Unknown 1	98
Unknown 2	50
Pure compounds	
Oxyprotostemonine (**21**)	18
(2′*S*)-Hydroxystemofoline (**51**)	82
(11*Z*)-1′,2′-Didehydrostemofoline (**52**)	58
Stemocurtisine (**58**)	27
Stemocurtisinol (**63**)	57
Mixture of 3′-stemofolenol (**74**+**75**)	155

The ethanolic crudes extracts of *Stemona* spp. were evaluated for their insecticidal activities. The preliminary bioassay experiment results for lethality against brine shrimp of 3 *Stemona* spp. are shown in Table 4.21. The crude extract of *S. curtisii*, which have been reported as insecticidal compounds against *S. littoralis* with a LC_{50} value of 9 ppm (Kaltenegger *et al.*, 2003), was the most effective against brine shrimps with a LC_{50} value of 37 ppm, followed by unknown 2 and unknown 1 with LC_{50} values of 50 and 98 ppm, respectively. The preliminary bioassay experiment was also studied on *Stemona* spp. pure compounds and the results showed that oxyprotostemonine (**21**), *S. curtisii* pure alkaloid, had the highest lethality against brine shrimp with a LC_{50} value of 18 ppm, followed by stemocurtisine (**58**), stemocurtisinol (**63**), (11*Z*)-1′,2′-didehydrostemofoline (**52**), (2′*S*)-hydroxystemofoline (**51**) and mixture of 3′-stemofolenol (**74**+**75**) with LC_{50} values of 27, 57, 58, 82 and 155 ppm, respectively. Pure alkaloids from *S. curtisii* showed the high lethality against brine shrimp. These alkaloids showed a good correlation between their lethality on brine shrimp and their larvicidal activities on mosquito larvae (*Anopheles minimus* HO) from Mungkornasawakul *et al.*, 2004 which showed LC_{50} values of 4, 18 and 39 ppm for oxyprotostemonine, stemocurtisine and stemocurtisinol, respectively.

Either the ethanolic crude extract or pure compounds from *S. curtisii* showed the highest insecticidal activities. This specie was therefore selected for producing a bioinsecticidal formulation.

4.5 Formulation of the bioinsecticide

Different solvents and other supplement substances such as some surfactants and emulsifiers were used for producing the bioinsecticidal formulation. The percentage of crude extract, solvents and supplement substances for bioinsecticidal formulation are reported in Table 4.22.

Table 4.22 The percentage of crude extract, solvents and supplement substances for the bioinsecticidal formulation

Ingredient	Percentage (%w/w)
S. curtisii ethanolic crude extract	30
Ethanol	30
Methanol	10
Water	10
Pine oil	10
Tween 80	10

4.6 Efficiency of bioinsecticidal formulation

4.6.1 Leaf disk choice test

Leaf disk assays were carried out with third instar larvae of *Spodoptera littoralis* Boisduval (*S. littoralis*) to determine the mode of action of bioinsecticidal formulation. The results showed that the bioinsecticidal formulation had a strong antifeedant activity at 0.015 % of the total formulation, while repellent activity was observed at higher concentrations (Table 4.23).

Table 4.23 Feeding inhibitory activities of the bioinsecticidal formulation

sample	Concentration of the total formulation (%v/v)			
	0.015	0.030	0.15	0.30
Bioinsecticidal formulation	A	R	R	R
Control solvent	-	-	-	-

R = repellent activity, feeding inhibition without testing treated leaf disk

A = strong antifeedant activity, less than 5% of total area of treated leaf disks in each Petri dish was consumed

a = antifeedant activity, 5-20% of total area of treated leaf disks in each Petri dish was consumed

4.6.2 Topical application method

Different concentrations of the bioinsecticidal formulation with a 0.15, 1.5, 3.0 and 15% formulation were topically applied to the dorsal thorax of the third instar of *S. littoralis* and compared with a 0.15% formulation of the chemical pesticide, Methomyl. The percent mortality of the bioinsecticidal formulations after application for 24 hours are shown in Table 4.24.

Table 4.24 The percent mortality after 24 hours

sample	Concentration (%v/v)	Percent mortality
Bioinsecticidal formulation	0.15	0
	0.30	0
	3.0	0
	15.0	0
Chemical pesticide (Methomyl)	0.15	100

Efficiency of bioinsecticidal formulation was studied in the laboratory on third instar larvae of *Spodoptera littoralis* Boisduval, which are a major pest problem in horticultural fields. The leaf disk choice test and the topical application method were used and the results are shown in Tables 4.23 and 4.24. For the leaf disk choice test, the bioinsecticidal formulation had a strong antifeedant activity against *S. littoralis* at 0.015%, while repellent activity against *S. littoralis* was presented at higher concentrations above 0.030%. In addition, the *S. curtisii* crude extract showed strong antifeedant activity against *S. littoralis* at 25 ppm while repellent activity against *S. littoralis* was observed at higher concentrations above 100 ppm (Muangkornasawakul, 2004). For the topical application method, the previous study of Kaltenegger *et al.*, 2003 showed that *S. curtisii* crude extract had toxic activity against the neonate larvae of *S. littoralis* with a LC_{50} value of 9 ppm while, in this study, the percent mortality of *S. littoralis*, which were treated with various concentrations of the bioinsecticidal formulation was equal to 0%, but the chemical pesticide gave the percent mortality equal to 100%. The previous study showed that the numerous synthetic pyrethroid and other insecticides have been used with *S. littoralis*, with appearance of resistance in many cases (Issa *et al.*, 1984a; 1984b; Abo-El-Ghar *et al.*, 1986). Therefore, the reason might be the resistance to compound in *S. curtisii* developed. The other reason might be the same *S. curtisii* specie contained not the same alkaloids components (comparing between Pitchaya *et al.*, 2003 and Kaltenegger *et al.*, 2004). Thus, toxic activities were also not the same. Moreover, the larvae in this study might be older than third instar which could be the reason for the different results.

Consequently, the mode of action of the bioinsecticidal formulation in this study worked as a repellent substance which did not kill the pests. Therefore, the

usefulness of this bioinsecticidal formulation does not destroy the balance of the ecosystem unlike chemical pesticides which often kill both pests and predators.

4.7 Formulation of bioinsecticide on a pilot scale

The formulation process on a pilot scale was also studied to find out the proper conditions for the scale up to at least 30 litres. The 30%w/w stemona crude extract was put in the inner chamber of the formulation machine and the solvents i.e. 30%w/w ethanol, 10%w/w methanol, 10%w/w water and 10%w/w pine oil were added. The water temperature in the outer chamber was set around 40 °C to increase the solubility. A surfactant, 10%w/w Tween 80, was then added and the motor was turned on to stir until all the components were homogenized.

The bioinsecticidal formulation was then analysed using the HPTLC technique. A mixture of dichloromethane, methanol and ammonia solution in a ratio of 95: 4: 1 was used as the mobile phase and the active bands on TLC were detected under UV light at 254 nm. The fingerprint of the formulation produced was established for quality control by using HPTLC quantitative analysis densitometry (Fig. 4.3). The first three bands were the bands of the *S. curtisii* alkaloids, stemocurtisinol, oxyprotostemonine and stemocurtisine, respectively, while the five latter bands were the bands of the bioinsecticidal formulation which contained the ethanolic soluble crude components of *S. curtisii*, and other supplement substances such as surfactants and emulsifiers. The fingerprint was the specific character of the formulation and also used to control the quality of product in each batch. The 3D view of 8 tracks under UV light at 254 nm irradiation are shown in Fig. 4.4.

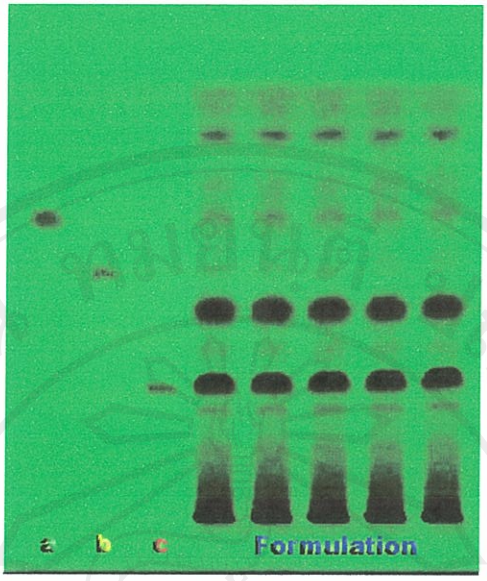


Figure 4.3 The bioinsecticidal formulation fingerprint from HPTLC

(a) stemocurtisinol (b) oxyprotostemonine (c) stemocurtisine

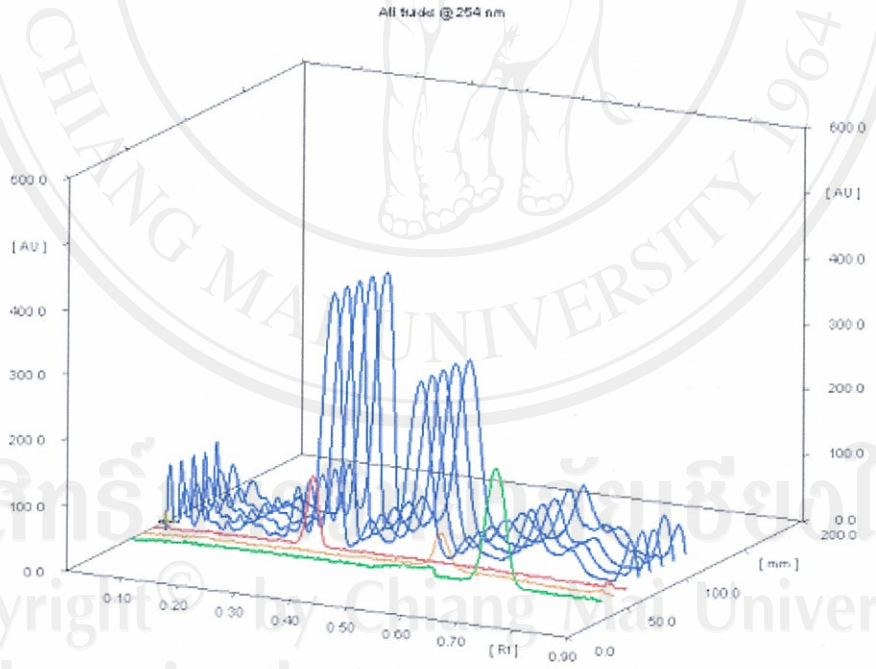


Figure 4.4 3D view of fingerprint stemocurtisinol(green), oxyprotostemonine(yellow) , stemocurtisine(red) and bioinsecticidal formulation (blue)

4.8 Efficiency of bioinsecticide produced on a pilot scale

The effect of the bioinsecticidal formulation on *Brassica oleracea* L. CV. (Chinese kale) in the field

The efficiency of the bioinsecticide produced on a pilot scale was determined by a field trial at the Department of Agronomy (Fig. 4.5) by a comparison with a commercial chemical pesticide and a control treatment (water). The results for quality and quantity of crop production are shown in Table 4.25. Many of the pests and predators found in the field are shown in Fig. 4.6 and Fig. 4.7, respectively.

Figure 4.5 The field trial of the Department of Agronomy, Chiang Mai University (28 June 2006)

Table 4.25 Quality and quantity of crop production

Plant yield	Bioinsecticide	Control	Chemical pesticide
Quantitative plant growth			
Height (cm)	26.08 a	26.34 a	27.83 a
Weight (g)	44.20 a	38.13 a	44.89 a
Number of leaves	6.59 a	5.78 b	6.14 b
Amount of insects found (numbers)			
Leaf eating beetle, *Phyllotreta chontanica*	0.28 a	0.24 a	0.18 a
Diamondback moth, *Plutella xylostella*	0.13 a	0.18 a	0.18 a
Green aphid, *Lipaphis erysimi*	13.16 b	15.38 b	3.09 a
Cabbage looper, *Trichoplusia ni*	0.01 a	0.03 a	0.07 a
Common cutworm, *Spodoptera littoralis*	0.02 a	0.14 b	0.03 a
Predators	0.80 a	0.07 b	0.00 b
Total plant quality	6.73 a	6.28 b	6.95 a

Notice: Different letters in the same row indicated the statistical significant difference at $\alpha \geq 0.05$ (a showed the better results than b)

The efficiency of the bioinsecticidal formulation produced on a pilot scale on Chinese kale was studied in the agronomy field and the results for both quality and quantity were analyzed and interpreted. Quantity items comprised an analysis of the height, the weight, the number of leaves, and the amount of insects found i.e. leaf eating beetles, diamondback moths, green aphids, cabbage loopers, common cutworms and predators. For the height and the weight of vegetables, the chemical

pesticide and the bioinsecticidal formulation gave better results than the control but the results were not statistically significant difference. For the number of leaves, the bioinsecticide gave a better result in terms of both value and the statistically significant difference. The chemical pesticide gave slightly better results of the amount of leaf eating beetles and total quality compared to the bioinsecticidal formulation and the control but the results were not of a statistically significant difference. The bioinsecticidal formulation gave a slightly better result for the amount of diamondback moths, cabbage loopers and common cutworms but the results were also not of a statistically significant difference. For the amount of predators, the bioinsecticidal formulation gave a statistically significant better result than both the chemical pesticide and the control. Predators did not feed on or harm plants. Many of these were just passing through or had innocuous habits. Others feed on and destroy pest species. Therefore, the activities of these beneficial species, the predators, can prevent or greatly limit pest problems. Unfortunately, the bioinsecticidal formulation was ineffective to green aphids compared to the chemical pesticide in a statistically significant way. However, this bioinsecticidal formulation was more or less the same or better than the chemical pesticide for controlling pests considering both the quality and the quantity of the product. The bioinsecticidal formulation appeared to be an alternative solution for controlling insect pests which was more safe than the chemical pesticide. Moreover, the benefits of using a bioinsecticidal formulation, although not fully tested in this study, may have less adverse effects on the users health, the ecosystem and the environment.

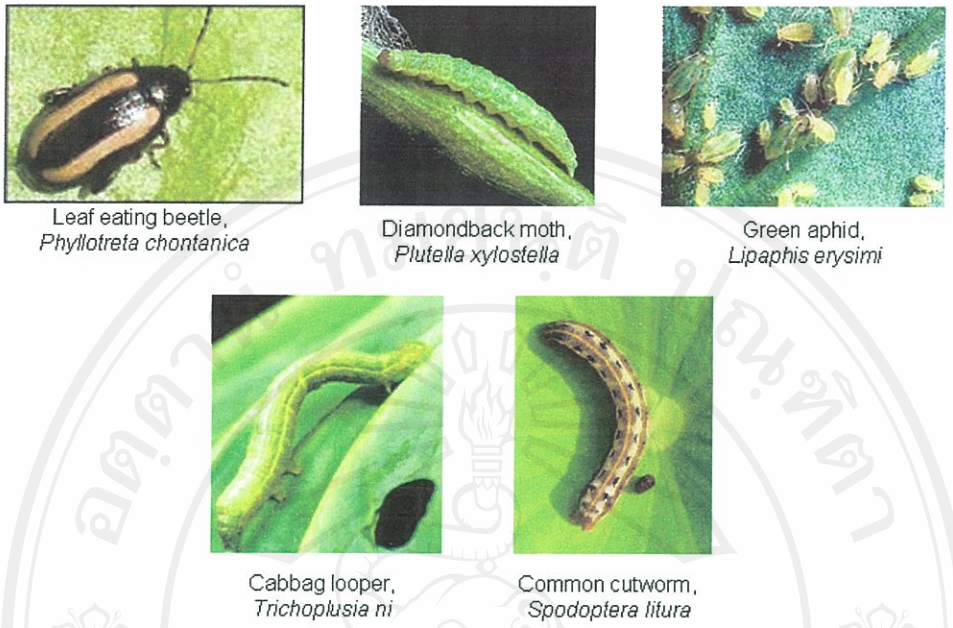


Figure 4.6 Pests found in experimental field

Figure 4.7 Predators found in experimental field