CYCLOPEPTIDE ALKALOIDS FROM SOME *ZIZIPHUS* PLANTS

A THESIS

BY

NATTHAKALN LOMCHOEY

Presented in Partial Fulfillment of the Requirements for the

Master of Science Degree in Chemistry

at Srinakharinwirot University

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AN ABSTRACT

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Natthakaln Lomchoey. (2011). *Cyclopeptide alkaloids from some ziziphus plants*. Thesis, M.Sc. (Chemistry). Bangkok: Graduate School, Srinakharinwirot University. Advisor Committee: Assoc. Prof. Dr. Sunit Suksamrarn, Dr. Prasert Pattanaprateeb.

Phytochemical studies have established the genus *Ziziphus* (Rhamnaceae family) to be a rich source of cyclopeptides. Investigation of selected Thai *Ziziphus* plants (*Z. cambodiana* root bark and *Z. mauritiana* stem bark), led to isolation of two new cyclopeptide alkaloids as 5(14)-scutianine A-type cyclopeptide alkaloid (cambodine) and 5(13)-zizyphine A-type cyclopeptide alkaloid (mauritine M), from *Z. cambodiana* and *Z. mauritiana*, respectively. Moreover, three known cyclopeptides, frangufoline and lotusanine B, as 4(14)-membered cyclopeptides were isolated from *Z. cambodiana*, and nummularine H as 5(13)-cyclopeptide was isolated from *Z. mauritiana*. Their structures and relative stereochemistry of the new compounds were elucidated mainly on the basis of extensive NMR and mass spectroscopic analysis. The stereochemical assignments were established by their CD spectra analysis 2D NMR experiments and by comparison with other related compounds of known stereochemistry.

ไซโคลเปปไทด์อัลคาลอยด์จากพืช *ZIZIPHUS* บางชนิด

บทคัดย่อ

ของ

ณัฏฐกัลย์ ลมเชย

เสนอต่อบัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ เพื่อเป็นส่วนหนึ่งของการศึกษา

ตามหลักสูตรวิทยาศาสตรมหาบัณฑิต สาขาวิชาเคมี

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จากการศึกษาองค์ประกอบทางเคมีจากเปลือกรากตะครองและเปลือกต้นพุทรา สามารถแยกสารไซโคลเปบไทด์อัลคาลอยด์ชนิดใหม่ได้ 2 สาร คือ cambodine จากเปลือกรากตะครอง และ mauritine M จากเปลือกต้นพุทรา นอกจากนี้ยังพบสารประกอบไซโคลเปบไทด์อัลคาลอยด์ที่เคยพบแล้ว 3 สาร ได้แก่ frangufoline และ lotusanine B จากตะครอง และ nummularine H จากพุทรา โครงสร้างของสารทราบได้จากการวิเคราะห์ข้อมูลสเปกโทรสโคปี โดยใช้เทคนิค NMR และแมสสเปกโทรสโคปีเป็นส่วนใหญ่ สำหรับการหาสเตอริโอเคมีของสารใช้เทคนิค CD, 2D NMR และทำการเปรียบเทียบข้อมูลกับสารประกอบอื่นที่มีโครงสร้างใกล้เคียงกัน

The thesis title

"Cyclopeptide alkaloids from some *Ziziphus* plants"

by

Natthakaln Lomchoey

has been approved by the Graduate School as partial fulfillment of the requirements for the Master of Science Degree in Chemistry of Srinakharinwirot University.

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Natthakaln Lomchoey

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CHAPTER 1

INTRODUCTION

Background

The genus *Ziziphus* belongs to the family Rhamnaceae (Smitinand. 2001: 564-565), a family of 58 genera and 900 species worldwide (Bhattacharyya; & Johri. 1998: 326-328), particular in arid regions (Gardner; Sidisunthorn; & Anusarnsunthorn. 2000: 130) with 100 species distributed in the tropical America, Africa, the Mediteranean region, Indo-Malaysia, and Australia, and also the tropical parts of India, Nepal, Pakistan, Bangla Desh and Sri Lanka (Bhattacharyya; & Johri. 1998: 326-328). Only nine species of *Ziziphus* plants are found in Thailand (Smitinand. 2001: 564-565) as follows:

1. *Z. angustifolia* (Miq.) Hatus Ex Steenis, known in Thai as Phutsa bai liam (พุทราใบเหลี่ยม).
2. *Z. attopoensis* Pierre, known in Thai as Kamlang suea khrong (กำลังเสือโคร่ง).
3. *Z. brunoniana* Clarke ex Brand. or *Z. oenoplia* Mill. var. *brunoniana* Tardieu, known in Thai as Nam lep maeo (หนามเล็บแมว).

   Z. oenoplia Mill. var. *oenoplia*, known in Thai as Nam lep yiao (หนาม-เล็บเหยี่ยว).
4. *Z. calophylla* Wall., known in Thai as Chin chi (ชินชี่).
5. *Z. cambodiana* Pierre, known in Thai as Takhrong (ตะครอง).
6. *Z. incurva* Roxb., known in Thai as Ta-chu-mae (ตาฉู่แม).
7. *Z. jujuba* Mill., known in Thai as Phutsa chin (พุทราจีน).
8. *Z. mauritiana* Lam. or *Z. jujube* Lam., known in Thai as Phutsa (พุทรา).
9. *Z. rugosa* Lam., known in Thai as Ma khwat (มะควัด).

Ethnopharmacological uses

The Rhamnaceous *Ziziphus* species are medicinal plants in several countries. The seeds of *Z. vulgaris* var. *spinosus* is reputed to be the most important herbal drug for the treatment of insomnia as sedatives and nerve tonics in Chinese medicine (Huh. 1981: 216) and antiarrythmias in folk medicine (Cho; Ro; & Hong. 1976). In Thailand the root bark of *Z. oenoplia* have been used for treatment of ulcers (Bunyapraphatsara; & Chokechaijaroenporn. 2000: 291-292). The *Z. mauritiana* is used in Thai traditional

medicine; bark and leaf are used for treatment of diarrhea, vomiting and ulcers, fruit is used for treatment of diarrhea and fever (Bunyapraphatsara; & Chokechaijaroenporn. 2000: 328-329). In the Indian system of medicine, *Z. rugosa* is used for treatment of diarrhea, menorrhagia and infection of teeth (Acharya; et al. 1988: 200-202). In Thailand the stem of *Z. cambodiana* is used traditionally for its antiinfectious abilities (Suksamrarn; et al. 2006: 533-537). Moreover, it is commonly used in Cambodian traditional medicine for treatment of fever (Hout; et al. 2006: 12-18).

From the reports on the interesting biological activities of *Ziziphus* plants, it is of interest to study on the chemical constituents of *Ziziphus* plants. Phytochemical studies have established the genus *Ziziphus* to be a rich sources of cyclopeptides (Tschesche; & Kaussmann. 1975: 165-205, Hesham; et al. 2007: 143-165, Tan; & Zhou, 2006: 840), lupane and ceanothane triterpenes (Suksamrarn; et al. 2006: 535-537). Thus, some Thai *Ziziphus* plants (*Z. cambodiana* and *Z. mauritiana*) are selected for this study to search for cyclopeptide alkaloids.

Objectives of the Study

1. To isolate and purify cyclopeptide alkaloids from some *Ziziphus* plants.
2. To elucidate the chemical structures of the isolated cyclopeptide alkaloids.

CHAPTER 2

REVIEW OF LITERATURE

Sources of cyclopeptide alkaloids

Cyclopeptide alkaloids are widely distributed among plants of Rhamnaceae family, but their occurrence has also been confirmed in representatives of Asteraceae, Celastraceae, Euphobiaceae, Menispermaceae, Pandaceae, Rubiaceae, Sterculiaceae and Urticaceae. These compounds are found in leaves, stem bark, root bark and seed. They often occur in minute amounts and as complex mixtures. The total yield from dried plant material is about 0.01-1 % (Gournelis; Laskaris; & Verpoorte. 1998: 2).

Classification of cyclopeptide alkaloids

On the basis of their structural skeletons and distributions in plants, the systematic structural classification of plant cyclopeptides which are divided into two classes according to the skeletons, whether formed with amino acid peptide bonds or not (Tan; & Zhou. 2006: 842).

1. Homocyclopeptides, formed with amino acid peptide bonds.
2. Heterocyclopeptides, formed with amino acid peptide bonds and other compound such as styrylamine moiety.

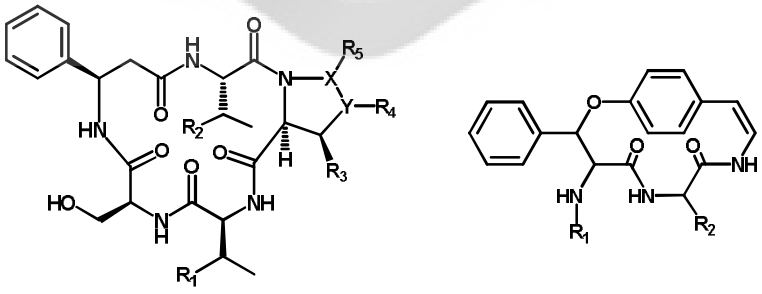
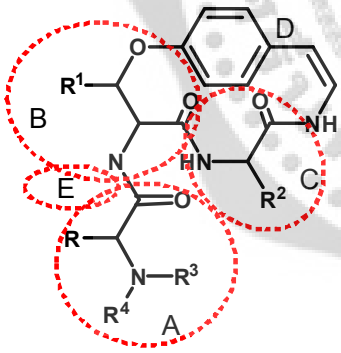


FIGURE 1 Structures of homocyclopeptide and heterocyclopeptide

Nomenclature of cyclopeptide alkaloids

The heterocyclopeptide is defined as basic compound embodying a *p*- or *m*-ansa structure, in which a 10- or 12-membered peptide type bridge spans the 1,3 or 1,4 positions of benzene ring. The 13- and 14-membered rings consist in a 10-membered bridge spans, involve *p*- and *m*-ansa, respectively. Whereas, the 15-membered compound embodying a *m*-ansa structure, with a 12-membered type bridge spans.

The 13-, 14- or 15-membered ring cyclopeptide alkaloids are classified according to the number of 4 (having A, B, C and D) or 5 (with A, B, C, D and E) units in the structural features (FIGURE 2). Consequently the cyclopeptide alkaloids are subdivided into groups with the following annotations: 4(13)-, 5(13)-, 4(14)-, 5(14)-, and 5(15)-. The 4(14)- and 5(14)-compounds are further subdivided according to the nature of the β-OH-amino acid (unit B). The nomenclature is shown in TABLE 1 (Gournelis; Laskaris; & Verpoorte. 1998: 5).



A = basic terminal (end) amino acid

B = β-hydroxy-amino acid

C = ring-bound amino acid

D = styrylamine unit

Sometimes between A and B unit an additional (intermediary) amino acid is interposed and designated as E.

FIGURE 2 General structures for cyclopeptide alkaloid

TABLE 1 Nomenclature of cyclopeptide alkaloids

Number	13 atoms	14 atoms			15 atoms
4 units	4(13)-compounds	4(14)-compounds			4(15)-compounds
	Nummularine C-type (Pro)	Frangulanine-type (Leu)	Integerrine-type (Phe)	Amphibine F-type (Pro)	Mucronine A-type
5 units	5(13)-compounds	5(14)-compounds			-
	Zizyphine A-type (Pro)	Scutianine A-type (Leu or Phe)		Amphibine B-type (Pro)	

The name of the type of cyclopeptide alkaloids according to the first compound has been found for each type. The structures for cyclopeptide alkaloids are described as follows:

Types of cyclopeptide alkaloids

1. 4(13)-Nummularine C-type cyclopeptide alkaloids

The 4(13)-Nummularine C-type cyclopeptide alkaloids are composed of 4 units including 3 units of amino acid (A, B and C) and unit D is a styrylamine moiety, the B unit is β-OH-proline, for examples:

1.1 Nummularine C (**1**) was isolated from the MeOH extract of the root bark of *Z. nummularia* (Tschesche; Elgamal; & Eckhardt. 1977: 2649-2655).

1.2 Daecucyclopeptide I (**2**) (Han, B. H.; Park; & Han, Y.N. 1989: 443-448) or Daechuine S26 (Han B. H.; Park; & Han Y.N. 1989: 443-448) was isolated from the root bark and fruit of *Z. jujuba* var. *inermis* (Han, B. H.; Park; & Han, Y.N. 1989: 443-448).

1.3 Sativanine E (**3**) was isolated from the MeOH extract of the stem bark of *Z. sativa* (Shah; et al. 1985: 555-558).

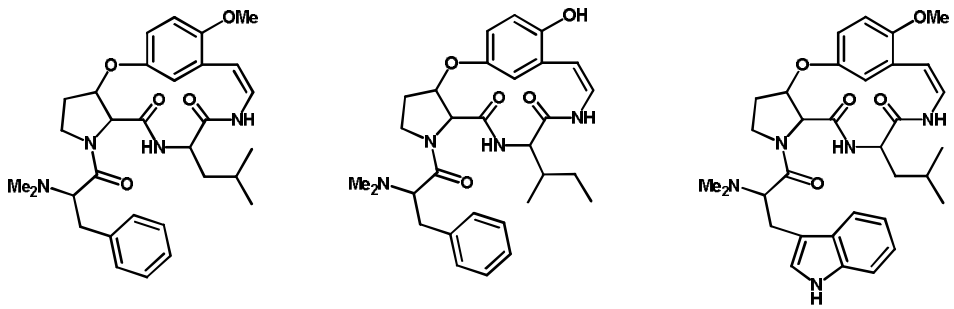


FIGURE 3 Structures of cyclopeptides **1-3**

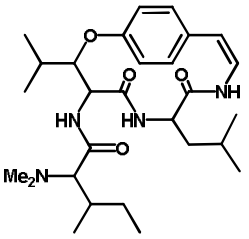
2. 4(14)-Frangulanine-type cyclopeptide alkaloids

The 4(14)-Frangulanine-type cyclopeptide alkaloids are composed of 4 units including 3 units of amino acid (A, B and C) and unit D is a styrylamine moiety, the B unit is β-OH-leucine, for examples:

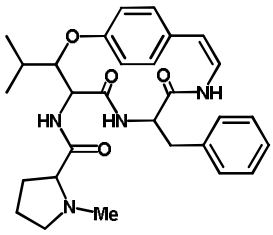
2.1 Frangulanine (**4**) (Mascaretti; et al. 1972: 1133) or ceanothamine A (Servis; et al. 1969: 5619-5624.) or Daechuine S2 (Han, B. H.; Park; & Han, Y.N. 1989: 443-448) was isolated from, such as the MeOH extract of the stem bark of *Z. jujuba* var. *inermis* (Han, B. H.; Park; & Han, Y.N. 1989: 443-448).

2.2 Ceanothine B (**5**) was isolated from, such as the CH_2Cl_2 extract of the root bark of *Ceanothus sanguineus* (family Rhamnaceae) (Lagarias; Goff; & Rapoport. 1979: 663-668).

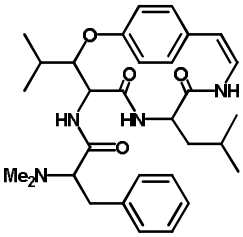
2.3 Frangufoline (**6**) (Zarga; et al. 1995: 504-511) or daechuine S1 (Han, B. H.; Park; & Han, Y.N. 1989: 443-448) or Sanjoinine A (Han, B. H.; Park; & Han, Y.N. 1990: 3315-3319) was isolated from, such as the hexane extract of the seeds of *Z. vulgularis* var. *spinosus* (Han, B. H.; Park; & Han, Y.N. 1990: 3315-3319).



Frangulanine (**4**)
or Ceanothamine A or Daechuine S2)



Ceanothine B (**5**)



Frangufoline (**6**)
(or Sanjoinine A or Daechuine S1)

FIGURE 4 Structures of cyclopeptides **4-6**

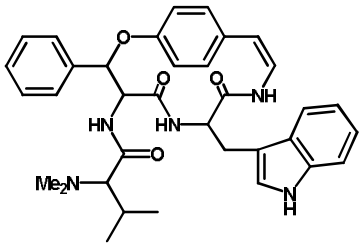
3. 4(14)-Integerrine-type cyclopeptide alkaloids

The 4(14)-Integerrine-type cyclopeptide alkaloids are composed of 4 units including 3 units of amino acid (A, B and C) and unit D is a styrylamine moiety, the B unit is β-OH-phenylalanine, for examples:

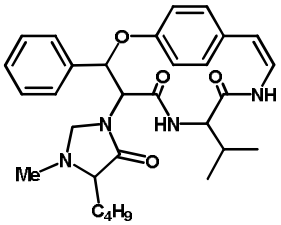
3.1 Integerrine (**7**) was isolated from, such as the CH_2Cl_2 extract of the root of *Ceanothus integerrimus* (Lagarias; et al. 1979: 220-227).

3.2 Sativanine B (**8**) was isolated from the stem bark of *Z. sativa* (Tschesche; Shah; & Eckhardt; 1979: 702-709).

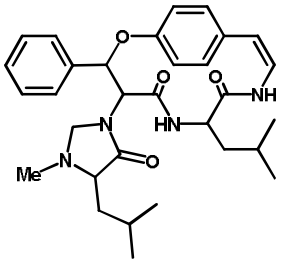
3.3 Nummularine G (**9**) was isolated from the MeOH extract of the stem bark of *Z. nummularia* (Tschesche; Elgamal; & Eckhardt. 1977: 2649-2655).



Integerrine (**7**)



Sativanine B (**8**)



Nummularine G (**9**)

FIGURE 5 Structures of cyclopeptides **7-9**

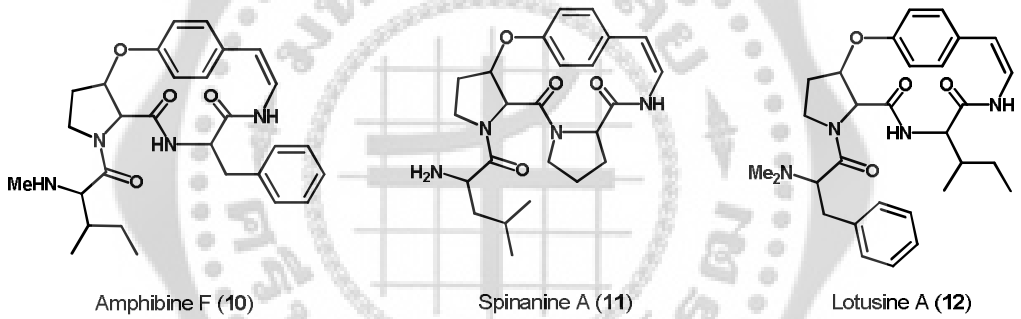
4. 4(14)-Amphibine F-type cyclopeptide alkaloids

The 4(14)-Amphibine F-type cyclopeptide alkaloids are composed of 4 units including 3 units of amino acid (A, B and C) and unit D is a styrylamine moiety, the B unit is β-OH-proline, for examples:

4.1 Amphibine F (**10**) was isolated from, such as the MeOH extract of the stem of *Z. spinachristi* (Tschesche; et al. 1974: 1633).

4.2 Spinanine A (**11**) was isolated from the stem bark of *Z. spinachristi* (Abdel-Galil; & El-Jessry. 1991: 1348-1349).

4.3 Lotusine A (**12**) was isolated from the root bark of *Z. lotus* (Ghedira; et al. 1993: 1591-1594).



Amphibine F (**10**) Spinanine A (**11**) Lotusine A (**12**)

FIGURE 6 Structures of cyclopeptides **10-12**

5. 4(15)-Mucronine A-type cyclopeptide alkaloids

The 4(15)-Mucronine A-type cyclopeptide alkaloids are composed of 4 units including 3 units of amino acid (A, B, and C) and unit D is a styrylamine moiety, the unit B is amino acid without β-OH, for examples:

5.1 Mucronine A (**13**) was isolated from, such as the stem bark of *Z. mucronata* (Fehlhaber; et al. 1972: 195).

5.2 Mucronine G (**14**) was isolated from the stem bark of *Z. mucronata* (Tschesche; et al. 1974: 1915).

5.3 Ziziphine D (**15**) was isolated from the stem bark of *Z. oenoplia* (Cassels; et al. 1974: 2461-2466).

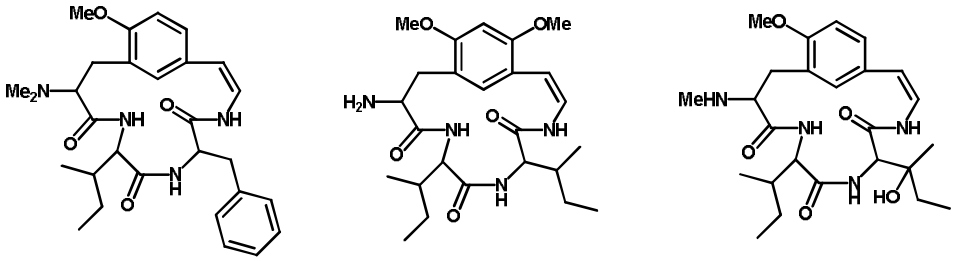


FIGURE 7 Structures of cyclopeptides **13-15**

6. 5(13)-Zizyphine A-type cyclopeptide alkaloids

The 5(13)-Zizyphine A (6)-type cyclopeptide alkaloids are composed of 5 units including 4 units of amino acid (A, B, C and E) and unit D is a styrylamine moiety, the unit B is β-OH-proline, for examples:

6.1 Zizyphine A (**16**) was isolated from the stem bark of *Z. oenoplia* (Tschesche; Kaussmann; & Eckhardt. 1973: 2577-2580).

6.2 Sativanine D (**17**) was isolated from the MeOH extract of the stem bark of *Z. sativa* (Shah; et al. 1985: 2765-2767).

6.3 Lotusine E (**18**) was isolated from the root bark of *Z. lotus* (Ghedira; et al. 1995: 767-772).

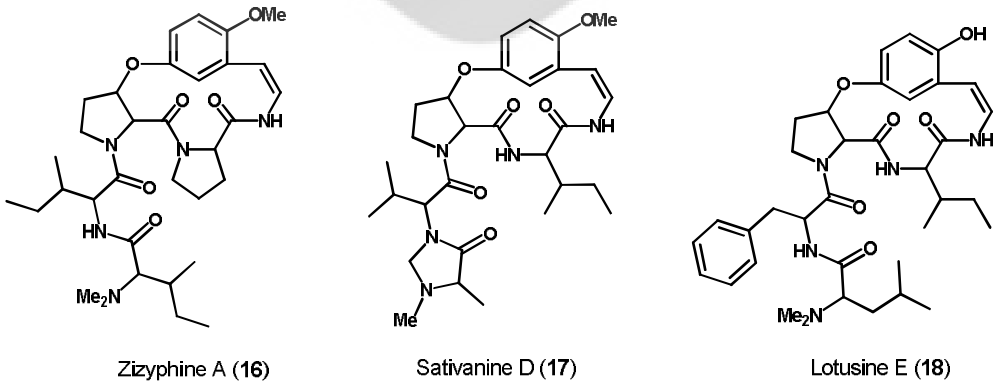


FIGURE 8 Structures of cyclopeptides **16-18**

7. 5(14)-Scutianine A-type cyclopeptide alkaloids

The 5(14)-Scutianine A-type cyclopeptide alkaloids are composed of 5 units including 4 units of amino acid (A, B, C and E) and intermediary (unit E) is proline and unit D is a styrylamine moiety, the B unit is β-OH-leucine or β-OH-phenylalanine, for examples:

7.1 Scutianine A (**19**) and scutianine F (**20**) or *N*-desmethylscutianine A, were isolated from the stem bark of *Scutia boxifolia* (family Rhamnaceae) (Tschesche; Welters; & Fehlhaber. 1967: 323-334).

7.2 Feretine (**21**) or *N*-desmethyladouetine Z was isolated from the leaves of *Feretia apodanthera* (family Rubiaceae) (Bailleul; et al. 1974: 949).

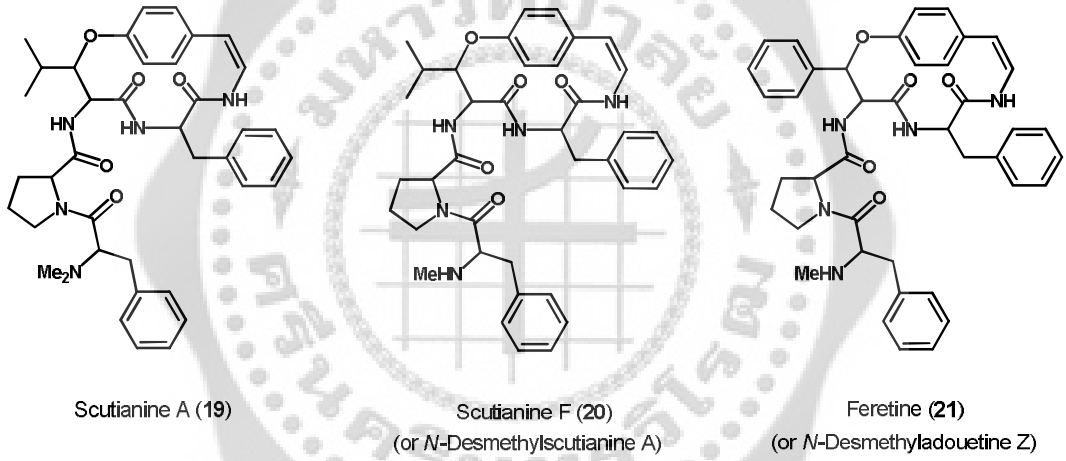


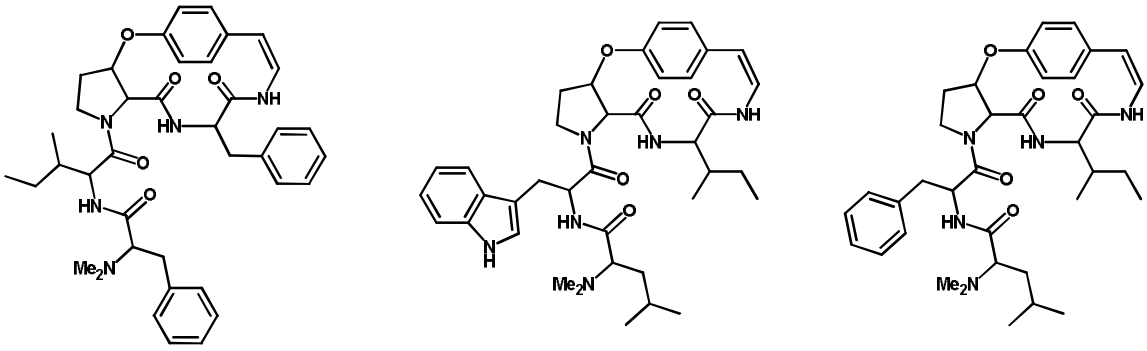
FIGURE 9 Structures of cyclopeptides **19-21**

8. 5(14)-Amphibine B-type cyclopeptide alkaloids

The 5(14)-Amphibine B-type cyclopeptide alkaloids are composed of 5 units including 4 units of amino acid (A, B, C and E) and unit D is a styrylamine moiety, the B unit is β-OH-proline, for examples:

8.1 Amphibine B (**22**) and amphibine E (**23**), were isolated from, such as the stem bark of *Z. amphibia* (Tschesche; Kaussmann; & Fehlhaber. 1972: 3094).

8.2 Lotusine B (**24**) was isolated from the root bark of *Z. lotus* (Ghedira; et al. 1995: 767-772).



Amphibine B (**22**) Amphibine E (**23**) Lotusine B (**24**)

FIGURE 10 Structures of cyclopeptides **22-24**

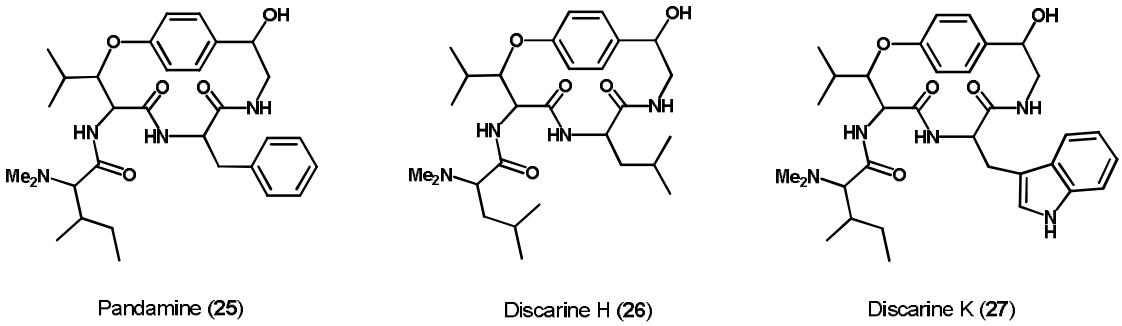
9. 4(14)-Pandamine-type cyclopeptide alkaloids

The 4(14)-Pandamine-type cyclopeptide alkaloids are composed of 4 units including 3 units of amino acid (A, B and C), and unit D is a 2-alkoxy-2-(*p*-hydroxyphenyl)ethylamine, instead of styrylamine moiety, the B unit is β-OH-leucine, for examples:

9.1 Pandamine (**25**) was isolated from the root bark of *Panda oleosal* (family Pandaceae) (Pais; et al. 1964: 817).

9.3 Discarine H (**26**) was isolated from the root bark of *Discaria febrifuga* (family Rhamnaceae) (Herzog; et al. 1984: 406).

9.3 Discarine K (**27**) was isolated from the root of *Discaria febrifuga* (Voelter; et al. 1987: 467).



Pandamine (**25**) Discarine H (**26**) Discarine K (**27**)

FIGURE 11 Structures of cyclopeptides **25-27**

10. Neutral compound related to 14-membered cyclopeptide alkaloids

The neutral compound related to 14-membered cyclopeptide alkaloids are composed of 4 units including 2 units of amino acid (B and C) and a coumaroyl as terminal chain (A), instead of amino acid, unit D is a styrylamine moiety, for examples:

10.1 Sanjoinenine (**28**) was isolated from, such as the MeOH extract of the seeds of *Z. vulgaris* var. *spinosus* (Han, B. H.; Park; & Han, Y.N. 1990: 3315-3319).

10.2 Scutianine C (**29**) was isolated from, such the root of *Scutia buxifolia* (family Rhamnaceae) (Gonzalez; et al. 1974: 2865-2869).

10.3 Lotusanine B (**30**) was isolated from the C_6H_6 extract of the whole plant of (except the roots) of z. *lotus* (Zarga; et al. 1995: 504-511).

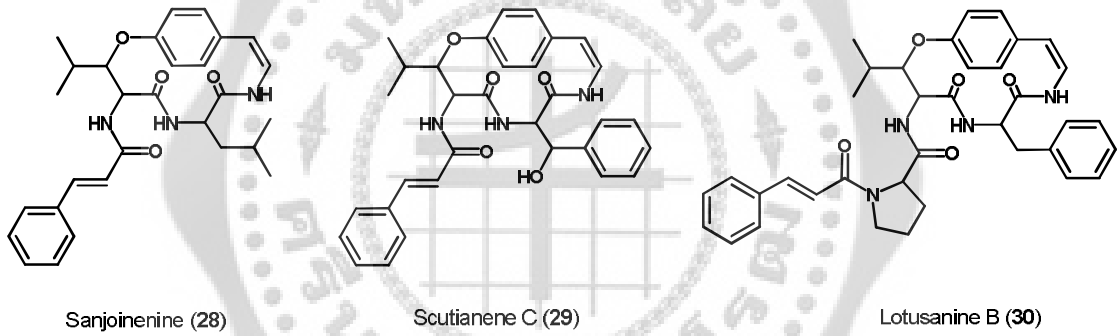


FIGURE 12 Structures of cyclopeptides **28-30**

Literature reviews of *Ziziphus cambodiana* Pierre

In 2006, our group reported one new triterpene ester, 3-*O*-Vanillylceanothic acid (**31**) together with five known lupane constituents, lupeol (**32**), betulinaldehyde (**33**), betulinic acid (**34**), 2-*O*-*trans*-*p*-coumaroyl alphitolic acid (**35**) and alphitolic acid (**36**), and three ceanothane triterpenes, zizyberanalic acid (**37**), zizyberenalic acid (**38**) and ceanothic acid (**39**) (FIGURE 13) from EtOAc extract, as the antiplasmodial and antituberculosis constituents of the root bark of this plant species (TABLE 2). This was the first report of *in vitro* antiplasmodial and antimycobacterial activities from the ceanothane-type triterpenes (Suksamrarn; et al. 2006: 535-537).

TABLE 2 IC_{50} values for antiplasmodial and MIC values for antimycobacterial activities of triterpenes **31-39**

Compounds	IC_{50} (μg/mL)	MIC (μg/mL)
3-*O*-Vanillylceanothic acid (**31**)	3.7	25
Lupeol (**32**)	Inactive[a]	Inactive[b]
Betulinaldehyde (**33**)	6.5	25
Betulinic acid (**34**)	Inactive[a]	25
2-*O*-*E*-*p*-Coumaroylalphitolic (**35**)	0.9	12.5
Alphitolic acid (**36**)	Inactive[a]	50
Zizyberanalic acid (**37**)	Inactive[a]	50
Zizyberenalic acid (**38**)	3.0	100
Ceanothic acid (**39**)	Inactive[a]	Inactive[b]

a = Inactive at 10 μg/mL, b = Inactive at >200 μg/mL

In addition, Hout; et al. reported on antiplasmodial investigation for 28 species of Cambodian plants used by traditional healers. The CH_2Cl_2, MeOH and MeOH extracts were tested for *in vitro* activity against a chloroquine resistant *Plasmodium falciparum* strain (W2). The CH_2Cl_2 extract obtained from the stem of *Z. cambodiana* showed IC_{50} values of 19.0 μg/mL, the MeOH and aqueous extract also showed the same IC_{50} values of > 50 μg/mL. Moreover, all extract of *Z. cambodiana* stem showed *in vitro* antiproliferative activity, which assessed on human monocytic THP1 cells, with the same IC_{50} values of > 50 μg/mL (Hout; et al. 2006: 12-18).

In 2007, Li; et al. studied on neuraminidase inhibitory activity of the leaf- and branch extracts of *Z. cambodiana.* The leaf extract, which showed stronger activity (96% inhibition at 50 μg/mL) compared to the branch extract (46% inhibition at 50 μg/mL), led to isolation of flavonoid glycosides, quercitrin (**40**) (FIGURE 13), isoquercitrin or quercitrin 3-*O*-β-D-glucoside (**41**) and quercitrin 3-*O*-D-arabinosyl-(1$\rightarrow$2)-α-L-rhamnoside (**42**). All compounds were tested for neuraminidase inhibitory activity and resulted was shown in TABLE 3 (Li; et al. 2007: 1195-1196).

TABLE 3 IC_{50} values for neuraminidase inhibitory activity of flavonoid glycosides **40**-**42**

Compounds	IC_{50} (μM)
quercitrin (**40**)	7.89 ± 0.96
isoquercitrin (**41**)	9.87 ± 0.68
quercitrin 3-*O*-D-arabinosyl-(1→2)-α-L-rhamnoside (**42**)	4.82 ± 1.06
Neu5Ac2en (5-acetamido-2,6-anhydro-3,5-dideoxy-D-glycero-D-galacto-non-2-enonic acid)	44.47 ± 0.87

In 2008, Arai; et al. reported that the CH_2Cl_2 extract of *Z. cambodiana* stem inhibited Hh/GLI signaling pathway, which led to the isolation of three pentacyclic triterpenes, betulinic acid (**34**), alphitolic acid (**36**) and colubrinic acid or zizyberanalic acid (**37**), as potent Hh/GLI signaling inhibitors. These compounds showed an important relationship among Hh/GLI signaling inhibition, the decreased expression of the anti-apoptosis protein BCL2, and cytotoxicity against cancer cells. These compounds might become good tools and/or leads to new agents in the investigation of Hh/GLI signaling pathway inhibitors (Arai; et al. 2008: 9420-9424).

Moreover, Arai; et al. also examined the cytotoxicity of these active compounds against PANC1, human prostate cancer cells (DU145) and mouse embryo fibroblast cells (C3H10T1/2) (TABLE 4). The cytotoxicity against cancer cells (PANC1 and DU145) by betulinic acid (**34**) or colubrinic acid (**37**) would be caused by inhibition of the expression of the anti-apoptosis protein BCL2. These pentacyclic triterpene inhibitors showed an important relationship between Hh/GLI signaling inhibition, the decrease of BCL2, and cytotoxicity against cancer cells and resulted was shown in TABLE 4 (Arai; et al. 2008: 9420-9424).

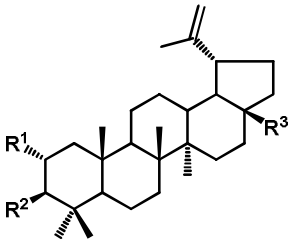
TABLE 4 IC_{50} values (μM) of triterpenes **34**, **36**-**37**, **39** and **43** with GLI1-mediated transcriptional inhibitory activity, and cytotoxicity against PANC1, DU145, and C3H10T1/2 cells

Compounds	GLI1 transcriptional inhibition (IC_{50}: μM)	Cytotoxicity (IC_{50}: μM)		
		PANC1[a]	DU145[b]	C3H10T1/2[c]
Betulinic acid (**34**)	32	44	37	82
Alphitolic acid (**36**)	42	41	70	145
Colubrinic acid or zizyberanalic acid (**37**)	38	43	78	167
Ceanothic acid (**39**)	>200	195	>200	>200
Ceanothanolic acid (**43**)	133	>200	>200	>200

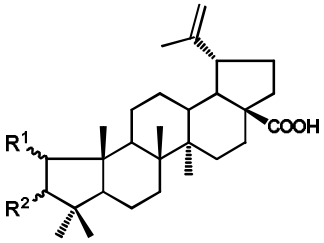
a = HaCaT cells with exogenous GLI1, or human pancreatic cancer cells

b = human prostate cancer cells

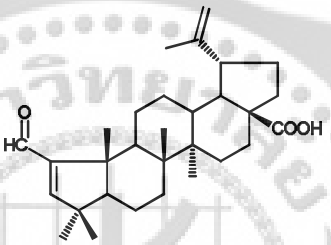
c = mouse embryo fibroblast cells



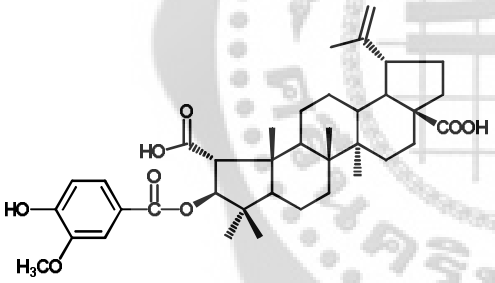
	R1	R2	R3
Lupeol (**32**) :	H	OH	CH_3
Betulin aldehyde (**33**) :	H	OH	CHO
Betulinic acid (**34**) :	H	OH	COOH
Alphitolic acid (**36**) :	OH	OH	COOH



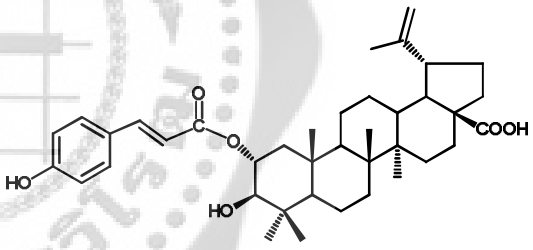
	R1	R2
Colubrinic acid or zizyberanalic acid (**37**) :	β–CHO	α–OH
Ceanothic acid (**39**) :	α–COOH	β–OH
Ceanothanolic acid (**43**) :	β–CH_2OH	α–OH



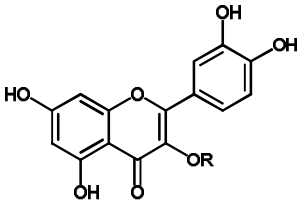
Zizyberenalic acid (**38**)



3-*O*-vanillylceanothic acid (**31**)



2-*O*-*trans*-*p*-coumaroylalphitolic acid (**35**)

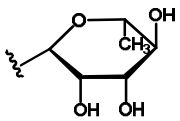


Quercitrin (**40**) : R = Rha

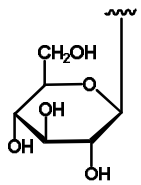
Isoquercitrin or quercitrin 3-*O*-β-D-glucoside (**41**): R = Glc

Quercitrin 3-*O*-D-arabinosyl-(1-2)-α-L-rhamnoside (**42**): Ara-(1-2)-Rha

Rha:



Glc:



Ara-(1-2)-Rha:

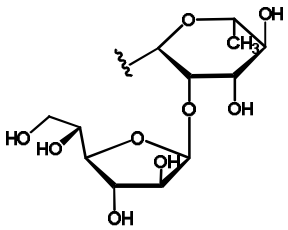
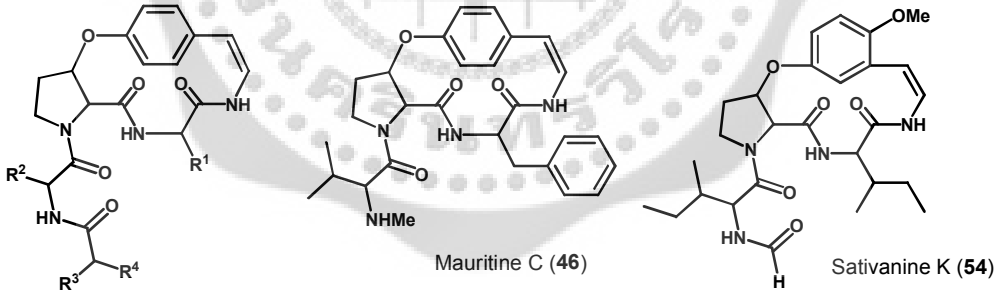


FIGURE 13 Structures of compounds **31-43**

Literature reviews of cyclopeptides from *Ziziphus mauritiana* Lam.

Previous phytochemical studies of *Z. mauritiana* revealed the 14-membered ring cyclopeptides to be the largest subgroup of alkaloid obtained, whereas only one 13-membered macrocyclic alkaloid isolated from this plant (Gournelis; et al. 1998: 1-179). These included mauritine A (**44**), mauritine B (**45**), mauritine C (**46**), mauritine D (**47**), mauritine E (**48**), mauritine F (**49**) amphibine B (**22**), amphibine D (**50**), amphibine F (**10**) and frangufoline (**6**) (Tschesche; Wilhelm; Fehlhaber. 1972: 2609-2612, Tschesche; et. al. 1974; 10: 1694-1701), mauritine H (**51**) (Tschesche; et. al. 1977: 1025-1028), were isolated from bark. Mauritine C (**46**), mauritine D (**47**), mauritine E (**48**) and mauritine F (**49**) belong to the same structural type with a 14-membered ring system configuration *trans*-3-OH-proline, 4-OH-styrylamine and one α-amino acid (Tschesche ; et. al. 1974: 1694-1701). Amphibine E (**23**), mauritine J (**52**) (Jossang; Zahir; & Diakite. 1996: 565-567), mauritine K (**53**) and 13-membered macrocyclic alkaloid sativanine K (**54**) were isolated from the root bark. It had shown that mauritine K (**53**) exhibited significant antifungal activity (Singh; et. al. 2007: 781-784).



	R^1	R^2	R^3	R^4
Mauritine A (**44**) :	D	C	Me	NMe_2
Mauritine B (**45**) :	D	C	A	NMe_2
Mauritine D (**47**) :	A	B	A	NMe_2
Mauritine E (**48**) :	E	C	Me	NMe_2
Mauritine F (**49**) :	D	C	Me	NHMe
Amphibine D (**50**) :	A	A	D	NMe_2
Mauritine H (**51**) :	D	B	Me	NMe_2
Mauritine J (**52**) :	D	F	B	NHMe
Mauritine K (**53**) :	A	A	A	NMe_2

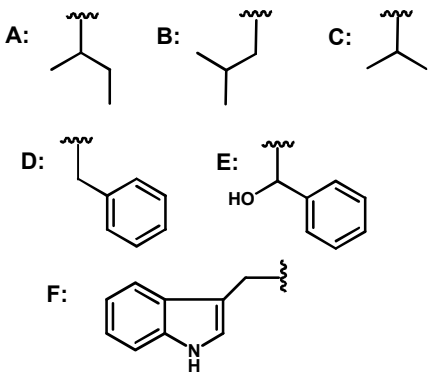
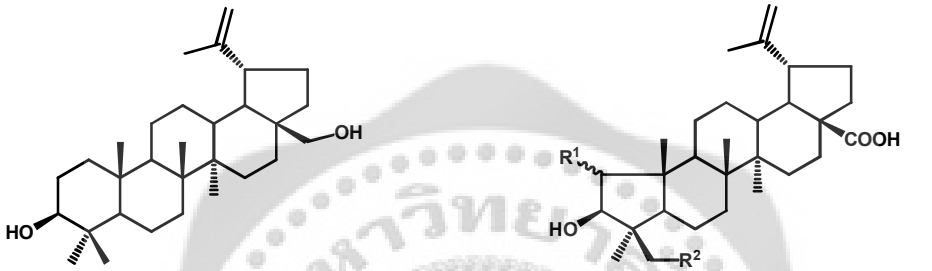


FIGURE 14 Structures of cyclopeptides **44**-**54**

Our previous investigation of Thai *Z. mauritiana* root bark resulted in the isolation of three known lupane-type triterpenes, [lupeol (**32**), betulinic acid (**34**) and betulin (**55**)] and four known ceanothane-type [zizyberenalic acid (**38**), ceanothic acid (**39**), epiceanothic acid (**56**) and 24-hydroxyceanothic acid (**57**)] compounds from EtOAc extract (Panseeta. 2010: 51).



Betulin (**55**)

		R^1	R^2
Epiceanothic acid (**56**)	:	β–COOH	H
24-OH-Ceanothic acid (**57**)	:	α–COOH	OH

FIGURE 15 Structures of triterpenes **55-57**

CHAPTER 3

EXPERIMENTAL

Sources of plant materials

The air-dried root bark of *Z. cambodiana* was collected from Chamni District, Buriram Province, Thailand, in March, 2007. A voucher specimen (Wicharn Wisetsri 001) was identified by James F. Maxwell and has been deposited at the CMU Herbarium, Faculty of Science, Chiang Mai University, Thailand.

The air-dried stem bark of *Z. mauritiana* was collected from Samchuk District, Suphanburi Province, Thailand, in June 2005 and a herbarium sample (Jessada Netsawangwicha 002) was identified by Nopporn Damrongsiri and has been deposited at the Faculty of Science, Ramkhamhaeng University, Thailand.

General techniques

1. Thin-layer chromatography (TLC)

1.1 Technique: One dimension, ascending

1.1.1 Adsorbent: Silica gel 60 F_{254} precoated on aluminium plate (Merck 1.05554)

1.1.2 Layer Thickness: 1.25 mm

1.1.3 Plate size: 1 x 5 cm and 2 x 5 cm

1.2 Detection:

1.2.1 Spots on TLC were visualized under ultraviolet light at wavelengths of 254 and 365 nm.

1.2.1 Developing agent: Anisaldehyde-sulphuric reagent (2.5% (v/v) in absoluted ethanol containing 3.4% (v/v) sulphuric acid and 1.0% (v/v) glacial acetic acid). After heating of TLC plate at 100-110 ^{o}C for 1-2 minutes, the spots of organic compounds will give specific colors with this reagent.

2. Column chromatography (CC)

2.1 Absorbent:

2.1.1 Silica gel 60 particle size < 0.063 mm (Merck 1.07729)

2.1.2 Silica gel 60 particle size 0.040-0.063 mm (Merck 1.09385)

2.1.3 Silica gel 60 particle size 0.063-0.200 mm (Merck 1.07734)

2.2 Packing method: Slurry and dry packing method for particle size < 0.063 mm and 0.040-0.063 mm, respectively.

2.3 Sample loading: The sample was dissolved in a small volume of suitable organic solvent. The solution was mixed with silica gel particle size 0.063-0.200 mm or 0.040-0.063 mm, depending on the type of silica gel in column. The sample was evaporated under reduced pressure and added onto the top of column.

2.4 Elution: After loading of sample onto the column and appropriate solvent system will be used as a mobile phase in the isocratic or gradient systems.

3. Quick Column chromatography (QCC)

3.1 Absorbent: Silica gel 60 GF_{254} for thin-layer chromatography (Merk 1.07730).

3.2 Packing method: Dry vacuum packing method.

3.3 Sample loading: The sample was dissolved in a small volume of suitable organic solvent. The solution was mixed with silica gel 60 GF_{254}. The sample was evaporated under reduced pressure and added onto the top of column.

3.4 Elution: After loading of sample onto the column, and appropriate solvent systems were used as a mobile phase in the gradient systems.

4. Size-exclusion gel column chromatography

4.1 Absorbent: Sephadex LH-20

4.2 Packing method: Slurry packing method.

4.3 Sample loading: The sample was dissolved in a small volume of MeOH and added onto the top of column.

4.4 Elution: The column was eluted with MeOH.

Physical properties

1. Optical rotations: Optical rotations was recorded in MeOH or $CHCl_3$ on a JASCO-1020 digital polarimeter

2. Melting points: Melting points was measured on Griffin melting point apparatus in degree Celsius of temperature.

Spectroscopy

1. Infrared (IR) absorption spectra

IR spectra was measured on Perkin Elmer FT-IR spectrum BX spectrometer by using potassium bromide (KBr) disc or film.

2. Ultraviolet (UV) absorption spectra

UV spectra were obtained on a Shimadzu UV-2401PC UV-VIS Recording Spectrophotometer.

3. Mass spectra:

3.1 Electrospray ionization (ESI) mass spectra were measured on Finnigan LC-Q mass spectrometer.

3.2 Electrospray ionization-time-of-flight (ESI-TOF-MS) mass spectra (Bruker Daltonics GmbH, Bremen, Germany) was measured on micrOTOF-Q II, an orthogonal acceleration quadrupole time-of-flight (Q-TOF) mass spectrometer equipped with electrospray interface.

4. Nuclear Magnetic Resonance (NMR) spectra

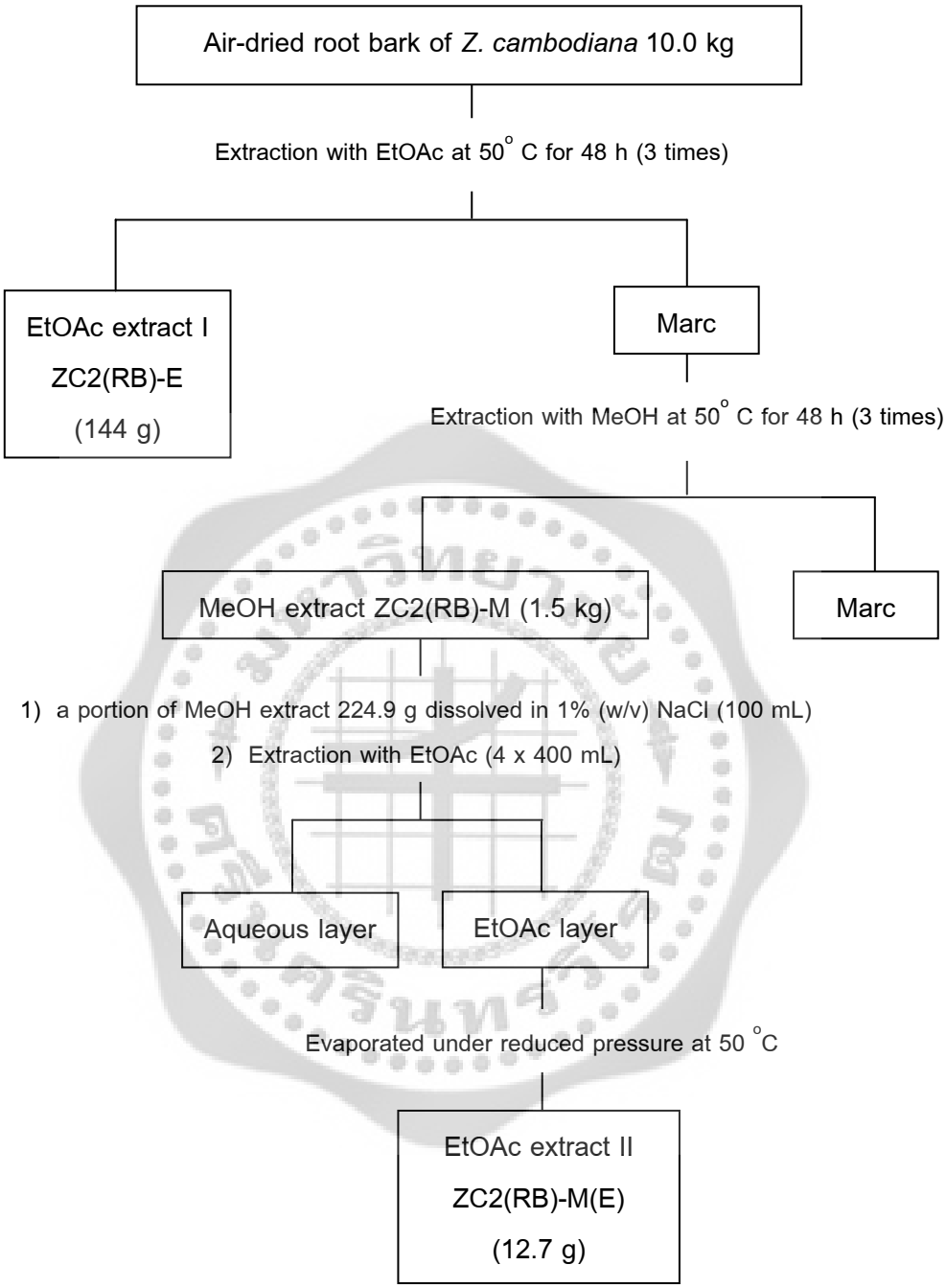
^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were determined on a Bruker Avance 300 FT-NMR spectrometer.

Extraction and isolation procedures

1. *Z. cambodiana*

1.1 Extraction of the dried root bark of *Z. cambodiana.*

The air-dried root bark of *Z. cambodiana* (10 kg) was extracted with EtOAc (3 x 20 L) at 50 ^{o}C for each 48 hours and then with MeOH (3 x 20 L) at 50 ^{o}C for each 48 hours. Each extract was evaporated under reduced pressure at 50 ^{o}C to afford EtOAc I and MeOH extracts for 144 g and 1.5 kg, respectively. A portion of the MeOH extract (224.9 g) was dissolved in 1% (w/v) NaCl (100 mL) then extracted with EtOAc (4 x 400 mL). The EtOAc layers were combined then evaporated under reduced pressure at 50 ^{o}C to provide EtOAc extract II 12.7 g. The extraction procedure is shown in SCHEME 1.



SCHEME 1 Extraction procedure of the root bark of *Z. cambodiana*

1.2 Isolation of compounds from the EtOAc extract II of *Z. cambodiana* root bark.

The EtOAc extract II (12.7 g) was subjected to CC, eluting with CC, eluted with a gradient of EtOAc-CH_2Cl_2 and MeOH-EtOAc. The fractions were examined by TLC and combined. The isolation procedure is shown in SCHEME 3.

1.2.1 Isolation of compound ZC-M1 (frangufoline, sss4449)

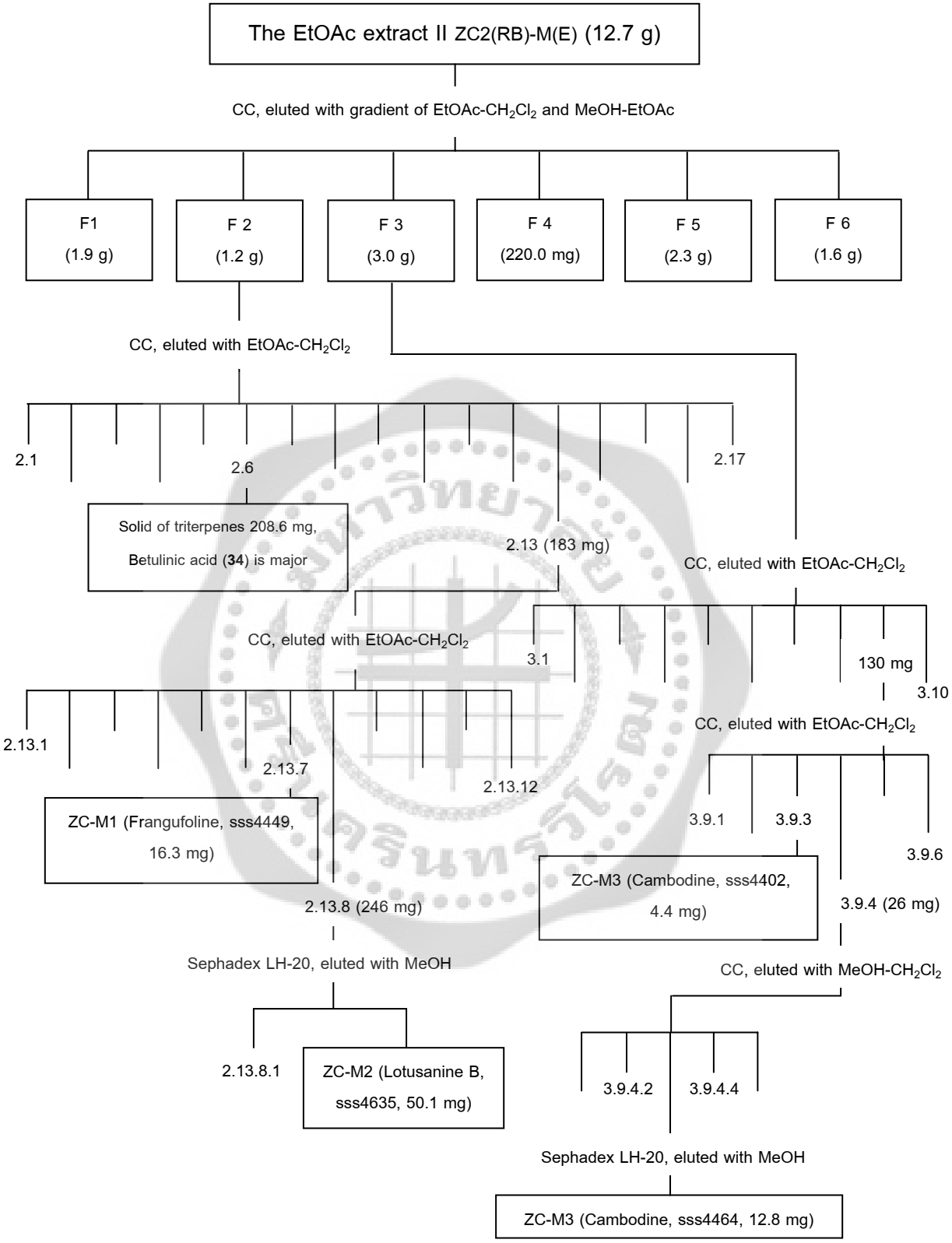
Fraction 2 (1.2 g) was chromatographed on a silica gel column (finer than 0.063 mm, 16 g) eluted with gradient of EtOAc-CH_2Cl_2 (1% increment of EtOAc, each 100 mL). Eighty-nine fractions (10 mL per fraction) were collected and combined the fraction according to their TLC behavior to yield seventeen subfractions. Subfraction 2.13 was further chromatographed to give compound ZC-M1 (frangufoline) as colorless needles (16.3 mg) from subfraction 2.13.7.

1.2.1 Isolation of compound ZC-M2 (lotusanine, sss4635)

Subgroup 2.13.8 was subjected to size-exclusion column chromatography over sephadex LH-20 eluted with MeOH to yield compound ZC-M2 (lotusanine B) as a colorless amorphous solid (50.1 mg).

1.2.3 Isolation of compound ZC-M3 (cambodine, sss4402)

Fraction 3 (3.0 g) was rechromatographed over silica gel (finer than 0.063 mm, 60 g) with *n*-hexane-EtOAc as eluting solvent to give ten subfractions. Subfraction 3.9 was further chromatographed (eluted with gradient of EtOAc-CH_2Cl_2) followed by size-excluded over sephadex LH-20 (eluted with MeOH) to yield compound ZC-M3 (cambodine) as colorless needles (17.2 mg).



SCHEME 2 Isolation of compounds from the EtOAc extract II of *Z. cambodiana* root bark.

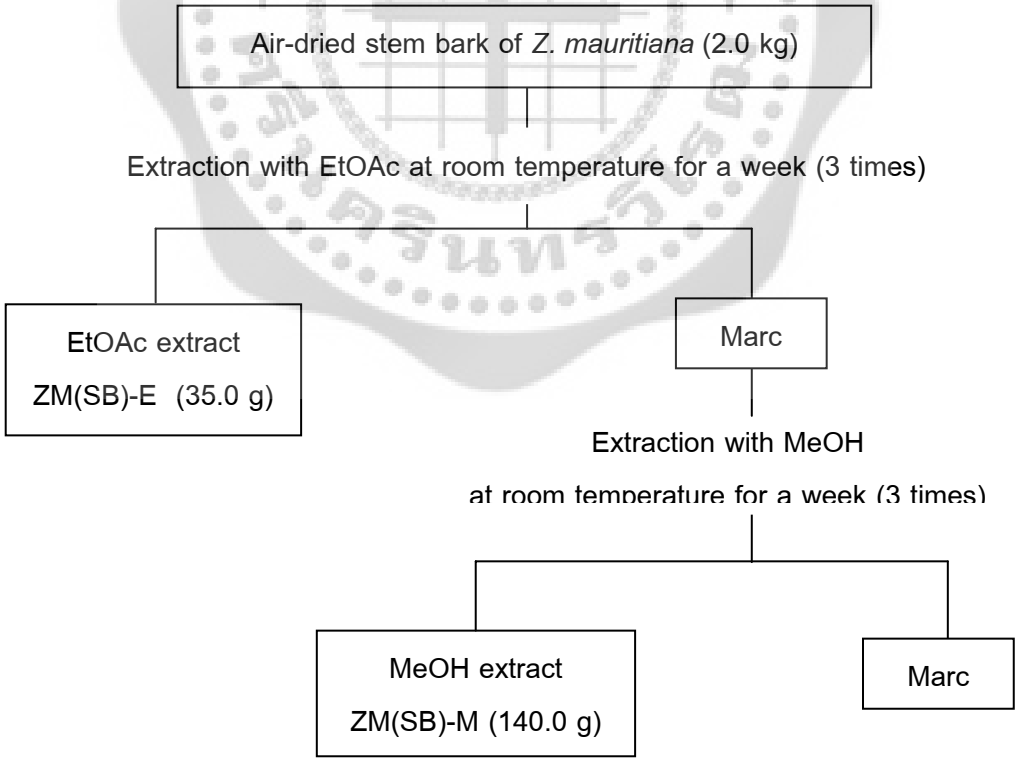
2. *Z. mauritiana*

2.1 Extraction of the dried stem bark of *Z. mauritiana*

The air-dried stem bark of *Z. mauritiana* (2.0 kg) was extracted with EtOAc (3 x 8 L) at room temperature for a week and then with MeOH (3 x 8 L) at room temperature for a week. Each extract was evaporated under reduced pressure at 50 ^{o}C to yield EtOAc and MeOH extracts for 35.0 g and 173.3 g, respectively. The isolation procedure is shown in SCHEME 3.

2.2 Isolation of compounds from the MeOH extract

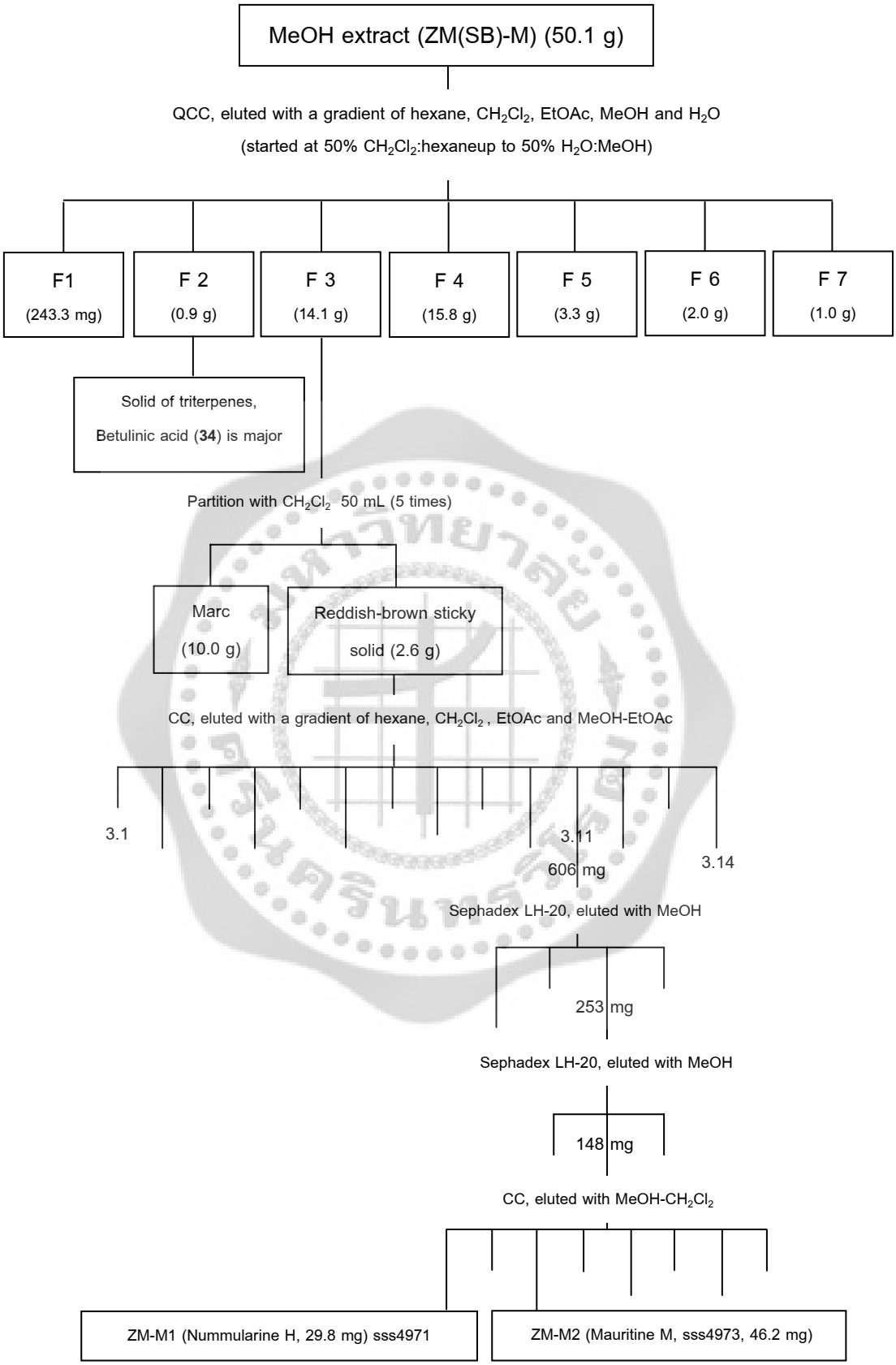
The MeOH extract (85.3 g) was fractionated by QCC techniques, eluting with hexane, CH_2Cl_2, EtOAc, MeOH and H_2O with increasing amounts of the more polar solvent (started at 50% CH_2Cl_2:hexaneup to 50% H_2O:MeOH). The all fractions were examined by TLC and combined.



SCHEME 3 Extraction procedure of the stem bark of *Z. mauritiana*

Isolation of compound ZM-M1 (nummularine H, sss4971) and compound ZM-M2 (mauritine M, sss4973)

Fraction 3 (14.6 g) was partly dissolved by using CH_2Cl_2 then combined CH_2Cl_2 soluble residue followed by evaporated under reduced pressure at 50 ^{o}C afford reddish-brown sticky solid (2.6 g). The CH_2Cl_2 residue was subjected to column chromatography on silica gel by using gradient of hexane, CH_2Cl_2, EtOAc and MeOH-EtOAc and fourteen subfractions were obtained. Subfraction 3.11 was further several chromatographed and size excluded sephadex LH-20 (eluted with MeOH) to yield a colorless amorphous solid of compound ZM-M1 (28.9 mg, sss4971) and compound ZM-M2 (46.2 mg, sss4973). Compound ZM-M1 and compound ZM-M2 were elucidated as nummularine H and mauritine M, respectively.



Scheme 4 Isolation of compounds from the MeOH extract of *Z. mauritiana* stem bark

Physical and spectral data of the isolated compounds from the root bark of *Z. cambodiana*

1. Compound ZC-M1 (frangufoline, sss4449)

Colorless needle 16.3 mg, soluble in CH_2Cl_2

mp : 216-218 °C [lit. 234-236 °C, Merkuza; & et al. 1974: 1279-1282]

R_f : 0.36 (20% EtOAc-CH_2Cl_2), a light-blue coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{27}$: -218° (c 0.20, $CHCl_3$) [lit. $[\alpha]_D^{22}$: -299° (c 0.1, $CHCl_3$), Tan; & Zhou. 2006: 845]

UV $[\lambda]_{\max}^{MeOH}$ nm (log ε) : 223 (*sh*) [lit. 280 (4.49), Zarga; et al. 1995: 504-511]

IR $\nu_{\max}^{neat}$ cm^{-1} : 3446, 3266, 2956, 2923, 2852, 1645, 1632, 1521, 1508, 1237, 1125, 701

ESIMS (+ve) *m/z* (% rel. intensity) : 535 $[M+H]^+$ (100)

ESIMS (-ve) *m/z* (% rel. intensity) : 533 $[M-H]^-$ (100)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; TABLE 5, FIGURE 28

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; TABLE 5, FIGURE 29

2. Compound ZC-M2 (lotusanine B, sss4635)

Colorless solid 50.1 mg, soluble in CH_2Cl_2

mp : 185-187 °C [lit. not recorded (Zarga; et al. 1995: 504-511)]

R_f : 0.43 (20% EtOAc-CH_2Cl_2), a violet-blue coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{23}$: -117° (c 0.20, $CHCl_3$) [lit. isolated as racemic mixture (Zarga; et al. 1995: 504-511)

UV $[\lambda]_{\max}^{MeOH}$ nm (log ε) : 216 (4.45), 224 (4.35), 279 (4.26) and 305 (*sh*) [lit. 244 (3.74), 280(3.12), Zarga; et al. 1995: 504-511]

IR $\nu_{\max}^{neat}$ cm^{-1} : 3456, 3391, 3277, 3065, 3030, 2956, 2925, 2853, 2853, 1671, 1646, 1624, 1602, 1507, 1455, 1419, 1238, 1168, 985, 762, 699

ESIMS (-ve) *m/z* (% rel. intensity) : 619.5 $[M-H]^-$ (100)

ESIMS (+ve) *m/z* (% rel. intensity) : 620 $[M^+]$ (84), 643 $[M+Na]^+$ (24), 1263 $[2M+Na]^+$ (100)

[lit. EIMS (+ve) *m/z* (% rel. intensity) : 631 [M] + (0.9), 574 (0.2), 519 (2), 427 (62), 274 (0.3), 243 (0.4), 227 (1), 209 (0.5), 203 (1.6), 200 (1), 190 (4), 148 (100), 135 (11), 134 (12), 114 (100), 96 (4), 91 (9), 86 (9), 84 (II), 72 (27), 68 (17)]

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; TABLE 8, FIGURE 30

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; TABLE 8, FIGURE 31

3. Compound ZC-M3 (cambodine, sss4402)

Colorless needle 17.2 mg, soluble in CH_2Cl_2

mp : 225-227 ^{o}C

R_f : 0.27 (4% MeOH-CH_2Cl_2), a violet-blue coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{27}$: = -198^{o} (c = 0.190, $CHCl_3$)

CD $[\lambda]_{max}^{MeOH}\Delta\varepsilon$: 220 (+15.46), 236 (-41.88), 280 (+6.44)

UV $[\lambda]_{max}^{MeOH}$ nm (log ε) : 227 (*sh*)

IR $[\nu]_{max}^{KBr}$ cm^{-1} : 3449, 3261, 3056, 3030, 2966, 2934, 1685, 1631, 1539, 1512, 1382, 1242, 1230, 746, 713, 701

ESIMS (+ve) *m/z* (% rel. intensity) : 668.3 $[M+H]^+$ (100)

ESIMS (-ve) *m/z* (% rel. intensity) : 666.5 $[M-H]^-$ (100)

HRTOFMS (APCI$^+$) : *m/z* 668.38079 $[M+H]^+$ (calcd. 668.38116 for $C_{39}H_{49}N_5O_5$ + H)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; TABLE 12, FIGURE 31

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; TABLE 12, FIGURE 32

Physical and spectral data of the isolated compounds from the stem bark of *Z. mauritiana*

1. Compound ZM-M1 (nummularine H, sss4971)

Colorless solid 16.3 mg, soluble in CH_2Cl_2

mp : 128-129 ^{o}C (lit. 194-196 ^{o}C, Tschesche; Elgamal; & Eckhardt. 1977: 2649-2655)

R_f : 0.48 (20% MeOH-CH_2Cl_2), a blue coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{26}$: -296^{o} (c 0.22, $CHCl_3$) [lit. -343^{o} (c 0.27, MeOH), Tschesche; Elgamal; & Eckhardt. 1977: 2649-2655]

UV $[\lambda]_{max}^{MeOH}$ nm (log ε) : 268 (4.05), 319 (3.89) [lit. 268 (4.05), 320 (3.65), Tschesche; Elgamal; & Eckhardt. 1977: 2649-2655]

IR $[\nu]_{max}^{KBr}$ cm^{-1} : 3402, 3338, 2959, 2927, 2878, 1668, 1642, 1508, 1453, 1221, 1032, 701

ESMS (+ve) *m/z* (% rel. intensity) : 682 $[M+H]^+$(100)

ESMS (-ve) *m/z* (% rel. intensity) : 680 $[M-H]^-$ (5)

HRTOFMS (APCI, +ve) *m/z* : 682.3586 $[M+H]^+$ (calcd 682.3599 for $C_{39}H_{47}N_5O_6$ + H)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; TABLE 14

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; TABLE 14

2. Compound ZM-M2 (mauritine M, sss4973)

Colorless solid 50.1 mg, soluble in CH_2Cl_2

mp : 188-189 °C

R_f : 0.31 (20% MeOH-CH_2Cl_2), an orange coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{27}$: -385° (c 0.28, MeOH)

CD $[\lambda]_{\max}^{MeOH}\Delta\varepsilon$: 319 (-13.00), 264 (-27.58), 231 (+2.19), 217 (-21.81)

UV $[\lambda]_{\max}^{MeOH}$ nm (log ε) : 219 (4.78), 272 (4.27), 279 (4.23), 289 (4.07), 318 (3.95)

IR $[\nu]_{\max}^{KBr}$ cm^{-1} : 3345, 2963, 2929, 2878, 1679, 1664, 1637, 1508, 1224, 1186, 1038, 741

EIMS (+ve) *m/z* (% rel. intensity) : 687 $[M+H]^+$ (0.5), 550 (31), 549 (100), 324 (71), 248 (76), 170 (90), 130 (87), 100 (47), 44 (30)

ESMS (+ve) *m/z* (% rel. intensity) : 687 $[M+H]^+$ (100)

ESMS (-ve) *m/z* (% rel. intensity) : 685 $[M-H]^-$ (15) , 721 (100)

HRTOFMS (APCI, +ve) *m/z* : 687.3856 $[M+H]^+$ (calcd 687.3865 for $C_{38}H_{50}N_6O_6$ + H)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; TABLE 16

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; TABLE 16

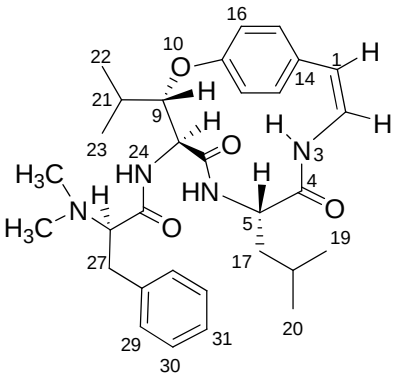
CHAPTER 4

RESULTS AND DISCUSSION

1. Cyclopeptide alkaloids from the MeOH extract of *Z. cambodiana* root bark

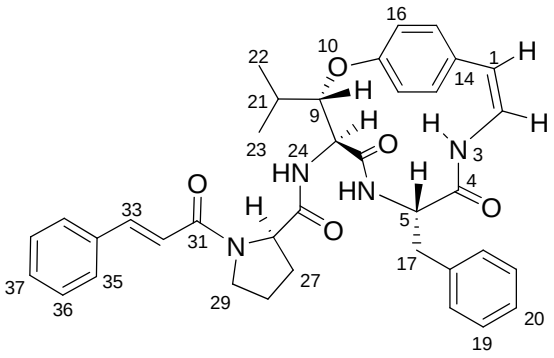
In 2006, Suksamrarn; et al. studied on the EtOAc extract (80.0 g) of Thai *Z. cambodiana* root bark, which showed a purple coloration with anisaldehyde-H_2SO_4 reagent, resulted in the isolation of one new triterpene ester, 3-*O*-vanillylceanothic acid (**31**) together with five known lupane constituents, lupeol (**32**), betulinaldehyde (**33**), betulinic acid (**34**), 2-*O*-*trans*-*p*-coumaroyl alphitolic acid (**35**) and alphitolic acid (**36**), and three ceanothane triterpenes, zizyberanalic acid (**37**), zizyberenalic acid (**38**) and ceanothic acid (**39**) (FIGURE 13), as the antiplasmodial and antituberculosis constituents of the root bark of this plant species (TABLE 2). This was the first report of *in vitro* antiplasmodial and antimycobacterial activities of the ceanothane-type triterpenes (Suksamrarn; et al. 2006: 535-537).

In this work, the pulverized, dried root bark of *Z. cambodiana* was extracted successively with EtOAc and MeOH for 144 g and 1.5 kg, respectively. A typical intense blue coloration with anisaldehyde-H_2SO_4 reagent for the MeOH extract indicated the presence of cyclopeptide alkaloid (Suksamrarn; et al. 2005: 1175-1180), whereas the EtOAc extract gave very weak blue color development. Thus, the MeOH soluble extract (224.9 g) was therefore selected for further chromatographic separations and resulted in the isolation of 14-membered ring cyclopeptide alkaloid including a new cyclopeptide ZC-M3 as 5(14)-scutianine A-type (cambodine) together with two known alkaloids [ZC-M1 (frangufoline) and ZC-M2 (lotusanine B)] (FIGURE 16). Due to the strain of the ring system in the molecule, the 14-membered cyclopeptide alkaloid showed end absorption band. Their IR spectra exhibited diagnostic peaks for amino (3261-3338 cm^{-1}), amide (1631-1685 cm^{-1}), and aryl ether (1237-1242 cm^{-1}) functions. The structures of these compounds were determined mainly based on their NMR data, MS analysis and by comparison to previously reported data.



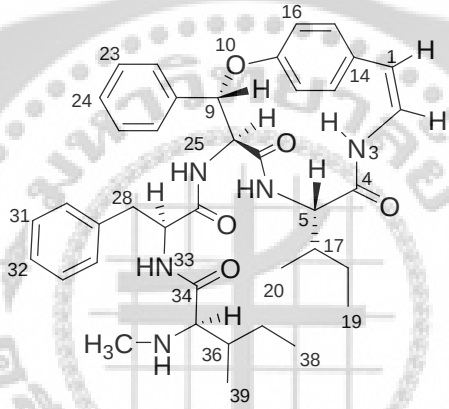
ZC-M1 or Frangufoline

sss4449



ZC-M2 or Lotusanine B

sss4635



ZC-M3 or Cambodine

sss4402

FIGURE 16 Structures of cyclopeptide alkaloids from the root bark of *Z. cambodiana*

1.1 Structure determination of compound ZC-M1 (frangufoline, sss4449)

Compound ZC-M1 was obtained as colorless needles, mp 216-218 ^{o}C [lit. 234 - 236 ^{o}C (Merkuza; & et al. 1974: 1279-1282)] and gave light-blue coloration with anisaldehyde-H_2SO_4 reagent. On the basis of its ESIMS (*m/z* 535 $[M+H]^+$, *m/z* 533 $[M+H]^-$), a molecular formula of ZC-M1 was established as $C_{31}H_{42}N_4O_4$ with support of ^{13}C NMR spectrum. The UV spectrum showed end absorption band, which was consistent with the 14-membered type cyclopeptide (Gournelis; Laskaris; & Verpoorte. 1998: 7). Their IR spectra exhibited diagnostic peaks for amino (3266 cm^{-1}), amide (1632-1645 cm^{-1}) and aryl ether (1237 cm^{-1}) functions.

The ^{1}H and ^{13}C NMR (TABLE 5, FIGURE 28-29), DEPT and HMQC spectra of compound ZC-M1 indicated the presence of 31 carbon resonances, which provided signals for *N,N*-dimethyl carbons, four methyls, two methylenes, seventeen methines and six quaternary carbons including three carbonyl carbons. The ^{1}H spectrum ($CDCl_3$, 300 MHz) of ZC-M1 showed four methyl doublets, including two methyl doublets at δ_H 1.28 (*J* = 6.7 Hz) and 1.01 (*J* = 6.7 Hz) of the β-OH-leucine and two methyl doublets at δ_H 0.65 (*J* = 6.5 Hz) and 0.60 (*J* = 6.5 Hz) of the ring-bound leucine unit. The C-8 (δ_C 55.2) and C-9 (δ_C 81.7) methine protons appeared as double of doublets at δ_H 4.50 (*J* = 7.3, 10.0 Hz) and δ_H 5.0 (*J* = 7.3, 1.5 Hz), which were due to the α- and β-protons of the β-OH-leucine, respectively. The C-5 methine proton and the C-17 methylene protons of the leucine appeared as double doublet of doublets at δ_H 4.04 (*J* = 11.3, 7.5, 3.3 Hz) and two multiplets at δ_H 1.69 (17α) and 1.23-1.29 (17β), respectively. The C-26 (δ_C 70.4) methine proton of the *N,N*-dimethylated leucine appeared as overlapped signal at 3.16-3.23. The diastereotopics protons at C-27 (δ_C 30.6) are identified as multiplets at overlapping with the signal of the proton at δ_H 3.16-3.23 of H-27α and H-25β showed as doublet of doublets at δ_H 2.85 (*J* = 15.8, 8.3 Hz). The C-1 olefinic proton appeared at δ_H 6.36 *d* (*J* = 7.5 Hz), the second olefinic proton at C-2 (δ_C 125.6) and NH-3, appeared as doublet of doublets at δ_H 6.67 (*J* = 10.6, 7.5 Hz) and 6.46 *d* (*J* = 10.6 Hz), respectively. The NMR data allowed the assignment for amide protons at δ_H 5.78 *d* (*J* = 7.8 Hz, NH-6), δ_H 7.90 *d* (*J* = 10.0 Hz, NH-24). Unambiguous assignments of all protons of compound ZC-M1 were made by a series of 2D NMR experiments and are reported in TABLE 5 and FIGURE 17.

TABLE 5 ^{1}H, ^{13}C NMR and 2D NMR data of compound ZC-M1 in $CDCl_3$

Position	δ_H	δ_C	HMBC correlations	NOESY correlations
1	6.36 *d* (*J* = 7.5 Hz)	115.6	C-2, C-14, C-15	H-2, H-15, NH-3
2	6.67 *dd* (*J* = 10.6, 7.5 Hz)	125.6	C-1, C-14, C-15	H-1, NH-3
NH-3	6.46 *d* (*J* = 10.6 Hz)	-	-	H-1, H-2, NH-6
4	-	167.4	-	-
5	4.04 *ddd* (*J* = 11.3, 7.8, 3.3 Hz)	52.6		H-17, H-19
NH-6	5.78 *d* (*J* = 7.8 Hz)	-	C-17 (weak)	NH-3, H-8
7	-	171.6	-	-
8	4.50 *dd* (*J* = 7.3, 10.0 Hz)	55.2	C-9	NH-6, H-12, H-22, H-24, NMe_2
9	5.0 *d* (*J* = 7.3, 1.5 Hz)	81.7	C-7, C-8, C-11, C-21, C-22, C-23	H-16, H-21, H-23
11	-	155.9	-	-
12	7.13 *dd* (*J* = 8.1, 2.6)	123.0	C-11, C-14, C-15	-
13	7.04 *m*	130.2	C-11	-
14	-	131.8	-	-
15	7.07 *m*	131.7	C-1, C-11, C-13	H-1
16	7.19 *m*	122.7	C-11, C-14, C-15	-
17	α 1.69 *m* , β 1.23-1.29[a] (signal overlapped)	39.1	-	H-5, H-18
18	0.60-0.65[a]	23.1	-	H-17, H-19, H-20
19	0.65 *d* (*J* = 6.5 Hz)	24.3	C-17, C-18, C-20	H-5, H-18
20	0.60 *d* (*J* = 6.5 Hz)	20.4	C-17, C-18, C-19	H-18
21	3.16-3.23[a] (signal overlapped)	29.3	-	H-9, H-22, H-23
22	1.28 *d* (*J* = 6.7 Hz)	20.3	C-9, C-21, C-23	H-21, H-23
23	1.01 *d* (*J* = 6.7 Hz)	15.0	C-9, C-21, C-22	H-9, H-21, H-22
NH-24	7.90 *d* (*J* = 10.0 Hz)	-	-	H-8, H-21, NMe_2
25	-	172.6	-	-
26	3.16-3.23[a] (signal overlapped)	70.4	C-27, C-28	H-27, H-29, H-29′, NMe_2
27	α 3.16-3.23[a] (signal overlapped) β 2.85 *dd* (*J* = 15.8, 8.3 Hz)	30.6	C-25, C-26, C-28, NMe_2	H-26, NMe_2
28	-	140.3	-	-
29,29'	7.26 *m*	128.5	C-27, C-28, C-31	H-27
30,30'	7.25 *m*	128.9	C-29, C-31	-
31	7.22 *m*	126.1	C-30	-
NMe_2	2.25 *s*	41.8	C-26	H-8, H-26, H-27

[a] Assignments confirmed by COSY.

Connections among these subgroups were provided by analysis of its HMBC and NOESY spectra (FIGURE 17). The NOE correlations were observed for styrylamine proton at δ_H 6.46 (NH-3) to H-5 (δ_H 4.04) of leucyl proton together with HMBC correlations of H-2 (δ_H 6.67) to C-1 (δ_C 115.6) and C-14 (δ_C 131.8), and H-1 (δ_H 6.36) to C-2 (δ_C 125.6), C-14 and C-15 (δ_C 131.7) indicating that the leucine amino acid was attached to the styrylamine unit. HMBC correlations from H-8 and H-9 to β-OH-leucine carbonyl C-7 (δ_C 171.6) confirmed the connection between the ring-bound leucine unit and β-OH-leucine amino acids. A strong NOE enhancement displayed in the NOESY spectrum between the proton at δ_H 4.50 (H-8) and NH-24 (δ_H 7.90) of *N,N*-dimethylphenylalanine unit, and HMBC correlation observed between NH-24 and terminal carbonyl C-25 (δ_C 172.6) confirm that the *N,N*-dimethylphenylalanine unit attached to the β-OH-leucine amino acids. Furthermore, the NOE correlation of the *N,N*-dimethyl proton δ_H 2.25 to H-8, and HMBC correlations of H-26 to C-25, and *N,N*-dimethyl carbon, of H-27 to C-25 and of H-29 and H-30 to C-28, supported the *N*-methyl phenylalanine as the end amino acid (TABLE 6). These evidences led to the conclusion that the structure of ZC-M1 was frangufoline.

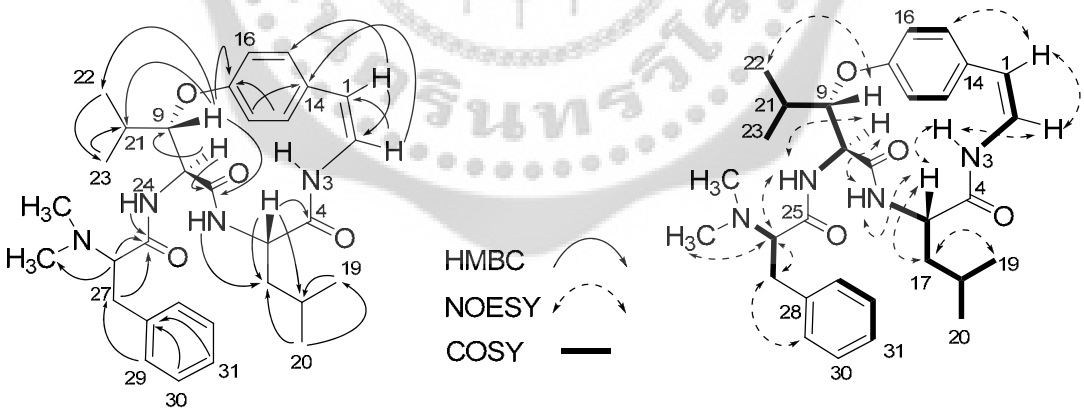


FIGURE 17 Selected HMBC, COSY and NOESY correlations for ZC-M1

The NMR data of ZC-M1 was similar to that of frangufoline (**6**) (Zarga, M. A.; et al. 1995: 504-511) except for C-1 (TABLE 6). However, carbon chemical shift of ZC-M1 at C-1 (δ_C 115.6) was comparable with that of discarine M (**61**) (δ_C 116.8) (Giacomelli; 2004: 933-937), in which both compounds composed of the same cyclic part (TABLE 7).

The *Z*-geometry of 1,2 double bond was established on the basis of the coupling constant value of 7.5 Hz for H-1 and H-2 (Morel; et al. 1999: 473-477). The coupling constant value of 10.6 Hz between H-2 and NH-3 protons and a small NOE interaction observed among them indicated *trans*-coplanar of these two protons (Morel; et al. 1999: 473-477). The NH-6 and H-5 protons showed the coupling constant of 7.8 Hz and a small NOE cross peak was observed between both protons suggesting they were in the opposite orientation. In contrast, strong NOE enhancement observed between NH-6 and H-8 allowing these both protons resided on the same side. The vicinal coupling constant value 7.3 Hz of the methine protons H-8 and H-9 indicated a *trans*- configuration (Gournelis; Laskaris; & Verpoorte. 1998: 6) together with weak significant NOE observed between these two protons in its NOESY spectrum further supported the *trans*- relationship between H-8 and H-9.

The stereochemistry of discarine M (**61**) was assigned as 5*S*, 8*S* and 9*S* configuration by analysis of the hydrolyzed residue (Giacomelli; et al. 2004: 933-937). The methine protons chemical shift and their coupling constant at H-8 and H-9 of ZC-M1 were similar to that of discarine M (TABLE 7). The β-OH-leucine moiety of discarine M was characterized as *erythro* relative configuration with vicinal coupling constant of H-8/H-9 *J* = 7.6 Hz (Giacomelli; 2004: 933-937). In turn, the ^{1}H and ^{13}C NMR chemical shift together with the coupling constant of H-8/H-9 (*J* = 7.3 Hz) for compound ZC-M1 followed the similar values for that of discarine M, These evidences and its levorotatory optical rotation, $[\alpha]_D^{27}$: -218° similar to discarine M $[\alpha]_D^{20}$ = -176° (Giacomelli; et al. 2004: 933-937), led to conclusion that this cyclopeptide displayed 5*S*, 8*S* and 9*S* configurations (FIGURE 17).

Frangufoline (**6**) (Zarga; & et al. 1995: 504-511) or daechuine S1 (Han, B. H.; Park; & Han, Y.N. 1989: 443-448) or Sanjoinine A (Han, B. H.; Park; & Han, Y.N. 1990: 3315-3319) was isolated from several *Ziziphus* plants, such as the hexane extract of the seeds of *Z. vulgularis* var. *spinosus* (Han, B. H.; Park; & Han, Y.N. 1990: 3315-3319).

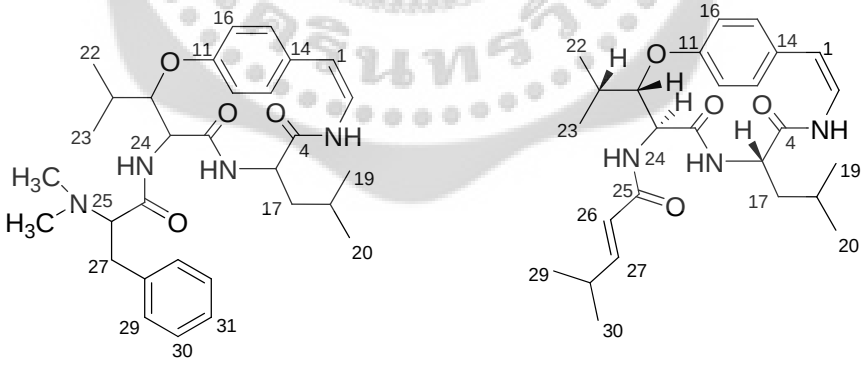
TABLE 6 Comparison of ^{1}H and ^{13}C NMR data of compound ZC-M1 with frangufoline (**6**)

Position	δ_H		δ_C	
	ZC-M1 (300 MHz)	**6** (500 MHz)	ZC-M1 (75 MHz)	**6** (125 MHz)
1	6.36 *d* (*J* = 7.5 Hz)	6.38 *d* (*J* = 7.7 Hz)	115.6	123.1
2	6.67 *dd* (*J* = 10.6, 7.5 Hz)	-	125.6	125.9
NH-3	6.46 *d* (*J* = 10.6 Hz)	-	-	-
CO-4	-	-	167.4	167.6
5	4.04 *ddd* (*J* = 11.3, 7.8, 3.3 Hz)	4.05 *m*	52.6	52.7
NH-6	5.78 *d* (*J* = 7.8 Hz)	-	-	-
CO-7	-	-	171.6	171.5
8	4.50 *dd* (*J* = 7.3, 10.0 Hz)	4.65 *m*	55.2	55.4
9	5.0 *d* (*J* = 7.3, 1.5 Hz)	4.92 *dd* (*J* = 7.5,1.9 Hz)	81.7	81.9
11	-	-	155.9	156.2
12	7.13 *dd* (*J* = 8.1, 2.6)	7.00-7.50 *m*	123.0	122.8
13	7.04 *m*	7.00-7.50 *m*	130.2	115.9
14	-	-	131.8	131.8
15	7.07 *m*	7.00-7.50 *m*	131.7	130.3
16	7.19 *m*	7.00-7.50 *m*	122.7	132.0
17	α 1.69 *m* , β 1.23-1.29[a] (signal overlapped)	3.10 *m*	39.1	39.4
18	0.60-0.65[a]	2.02 *m*	23.1	24.5
19	0.65 *d* (*J* = 6.5 Hz)	1.24 *d* (*J* = 6.8 Hz)	24.3	20.6
20	0.60 *d* (*J* = 6.5 Hz)	1.14 *d* (*J* = 6.8 Hz)	20.4	23.1
21	3.16-3.23[a] (signal overlapped with H-21, H-27)	2.05 *m*	29.3	29.4
22	1.28 *d* (*J* = 6.7 Hz)	0.71 *d* (*J* = 6.6 Hz)	20.3	15.1
23	1.01 *d* (*J* = 6.7 Hz)	0.57 *d* (*J* = 6.6 Hz)	15.0	20.4
NH-24	7.90 *d* (*J* = 10.0 Hz)	-	-	-
CO-25	-	-	172.6	172.7
26	3.16-3.23[a] (signal overlapped with H-21, H-27)	-	70.4	70.6
27	α 3.16-3.23[a] (signal overlapped with H-21, H-26) β 2.85 *dd* (*J* = 15.8, 8.3 Hz)	3.34 *br d* (*J* = 14.8 Hz)	30.6	30.9
28	-	-	140.3	140.4
29,29'	7.26 *m*	-	128.5	128.6
30,30'	7.25 *m*	-	128.9	129.0
31	7.22 *m*	-	126.1	126.2
NMe_2	2.25 *s*	2.97*s* , 2.85*s*	41.8	41.9

[a] Assignments confirmed by COSY.

TABLE 7 Comparison of ^{1}H and ^{13}C NMR data of compound ZC-M1 with discarine M (**61**)

Position	δ_H (300 MHz)		δ_C (75 MHz)	
	ZC-M1	**61** (DMSO-d_6, 400 MHz)	ZC-M1 (75 MHz)	**61** (100 MHz)
1	6.36 *d* (*J* = 7.5 Hz)	6.38 *d* (*J* = 7.5 Hz)	115.6	116.8
2	6.67 *dd* (*J* = 10.6, 7.5 Hz)	6.51 *dd* (*J* = 9.7, 7.5 Hz)	125.6	125.7
NH-3	6.46 *d* (*J* = 10.6 Hz)	7.62 *d* (*J* = 9.7 Hz)	-	-
4	-	-	167.4	168.0
5	4.04 *ddd* (*J* = 11.3, 7.8, 3.3 Hz)	3.78 *m*	52.6	55.1
NH-6	5.78 *d* (*J* = 7.8 Hz)	6.72 *d* (*J* = 8.0 Hz)	-	-
7	-	-	171.6	171.4
8	4.50 *dd* (*J* = 7.3, 10.0 Hz)	4.55 *dd* (*J* = 10.0, 7.6 Hz)	55.2	54.9
9	5.0 *d* (*J* = 7.3, 1.5 Hz)	4.89 *dd* (*J* = 7.7, 7.6 Hz)	81.7	82.2
11	-	-	155.9	155.8
12	7.13 *dd* (*J* = 8.1, 2.6)	7.11 *d* (*J* = 8.3 Hz)	123.0	121.4
13	7.04 *m*	6.93 *d* (*J* = 8.3 Hz)	130.2	130.9
14	-	-	131.8	131.1
15	7.07 *m*	6.98 *dd* (*J* = 10, 2 Hz)	131.7	130.3
16	7.19 *m*	7.00 *d* (*J* = 8.7 Hz)	122.7	122.8
NH-24	7.90 *d* (*J* = 10.0Hz)	8.42 *d* (*J* = 10.0 Hz)	-	-



Frangufoline (**6**) Discarine M (**61**)

FIGURE 18 Structures of frangufoline and discarine M

1.2 Structure determination of compound ZC-M2 (lotusanine B, SSS4635)

Compound ZC-M2 was obtained as colorless needles, mp 185-187 ^{o}C and gave violet-blue coloration with anisaldehyde-H_2SO_4 reagent. On the basis of its ESIMS *m/z* 619.5 $[M-H]^-$, a molecular formula of ZC-M2 was established as $C_{37}H_{40}N_4O_5$ with support of ^{13}C NMR spectrum. The UV absorption maxima at 216, 224 and 279 nm were found, which was consistent with the 14-membered type cyclopeptide alkaloid with coumaroyl moiety (Zarga; et al. 1995: 504-511, Han, B. H.; Park; & Han Y. N. 1990: 3315-3319, Gonzalez; et al. 1974: 2865-2869). Their IR spectra exhibited diagnostic peaks for amino (3277 cm^{-1}), amide (1624-1646 cm^{-1}) and aryl ether (1238 cm^{-1}) functions.

The ^{1}H, ^{13}C NMR (TABLE 8, FIGURE 30-31), DEPT and HMQC spectra of compound ZC-M2 indicated the presence of 31 carbon resonances, which provided signals for four methyls, four methylenes, twenty three methines and eight quaternary carbons including four carbonyl carbons. The ^{1}H NMR spectrum ($CDCl_3$ 300 MHz) of ZC-M2 exhibited signals for *trans*- double bonds at 6.75 *d* (*J* = 15.4 Hz) and 7.77 *d* (*J* = 15.4 Hz) which were assigned to the *trans*-coumaroyl protons, H-32 (δ_C 116.9) and H-33 (δ_C 144.1), respectively, the carbonyl carbon (CO-31) of coumaroyl group displayed at δ_C 166.5, [δ_H 7.56 *dd* (*J* = 5.9, 2.0 Hz, H-35,35′), 7.41 *t* (*J* = 3.5, 2.9 Hz, H-36,36′) and δ_C 134.6 (C-34), 128.5 (C-35,35′), 128.9 (C-36,36′) and 130.3 (C-37)] (de Oliveira; et al. 2009: 1195-1197). The C-26 methine proton of the proline appeared as doublet at 4.70 (*J* = 7.4 Hz) (TABLE 10 and FIGURE 31) and three methylene group of proline showed at δ_H 2.53 *m* (H-27α), 1.80 *m* (H-27β), 2.13 *m* (H-28), 3.70 *quin* (*J* = 8.7 Hz, H-29α) and 3.67 *quin* (*J* = 8.7 Hz, H-29β). Two sets of doublets methyl protons of β-OH-leucine H-22 and H-23 showed at δ_H 0.69 (*J* = 6.6 Hz) and 1.14 (*J* = 6.7 Hz). The C-8 and C-9 methine protons appeared as double doublets at δ_H 4.44 (*J* = 9.4, 6.9 Hz) and 5.02 (*J* = 6.9, 1.7 Hz), respectively, which were due to the α- and β-protons of the β-OH-leucine, respectively. The C-5 methine proton appeared at δ_H 4.62 *ddd* (*J* = 11.0, 7.9, 4.1 Hz) and the C-17 methylene protons of the phenylalanine showed at δ_H 3.42 *dd* (*J* = 16.0, 4.0 Hz, H-17α) and δ_H 2.75 *dd* (*J* = 16.0, 11.0 Hz, H-17β). The C-1 olefinic proton identified as doublet at δ_H 6.36 *d* (*J* = 7.6 Hz), the second olefinic proton at C-2 and NH-3, appeared at δ_H 6.73 *dd* (*J* = 10.4, 7.6 Hz) and 6.56 *d* (*J* = 10.4 Hz), respectively. The doublet of NH proton were exhibited at δ_H 6.09 *d* (*J* = 7.9 Hz, H-6), 8.09 *d* (*J* = 9.4 Hz, NH-24). Moreover, the

unambiguous assignments of all protons of compound ZC-M2 were made by a series of 2D NMR experiments and are reported in TABLE 8.

Connections among four subgroups were provided by analysis of its HMBC and NOESY spectra. The NOESY correlations were observed for styrylamine proton at δ_H 5.56 (NH-3) to H-5 (δ_H 4.62) of phenylalanine proton together with HMBC correlations of H-2 (δ_H 6. 37) to C-1 (δ_C 115.5) and C-15 (δ_C 131.8), and H-1 (δ_H 6.36) to C-2 (δ_C 125.5), C-14 (δ_C 136.6) and C-15 indicating that phenylalanine amino acid was attached to the styrylamine unit. HMBC correlations from H-8 and H-9 to β-OH-leucine carbonyl C-7 (δ_C 171.1) confirmed the connection between the ring-bound leucine unit and β-OH-leucine amino acids. The NOESY correlations of H-29 (δ_H 3.58) to *trans*-double bond proton H-32 (δ_H 6.69) supported that the *trans*-coumaroyl connected to the *N*-proline unit. The strong NOE effects between the proton at δ_H 8.09 (NH-24) to H-8 (δ_H 4.32) and H-26 confirmed that the *N*-coumaroylproline unit attached to the β-OH-leucine amino acids (FIGURE 19). These evidences led to the conclusion that the structure of compound ZC-M2 deduced to be lotusanine B.

Lotusanine B (**30**) was isolated from the C_6H_6 extract of the whole plant (except the roots) of *Z. lotus* (Zarga; et al. 1995: 504-511).

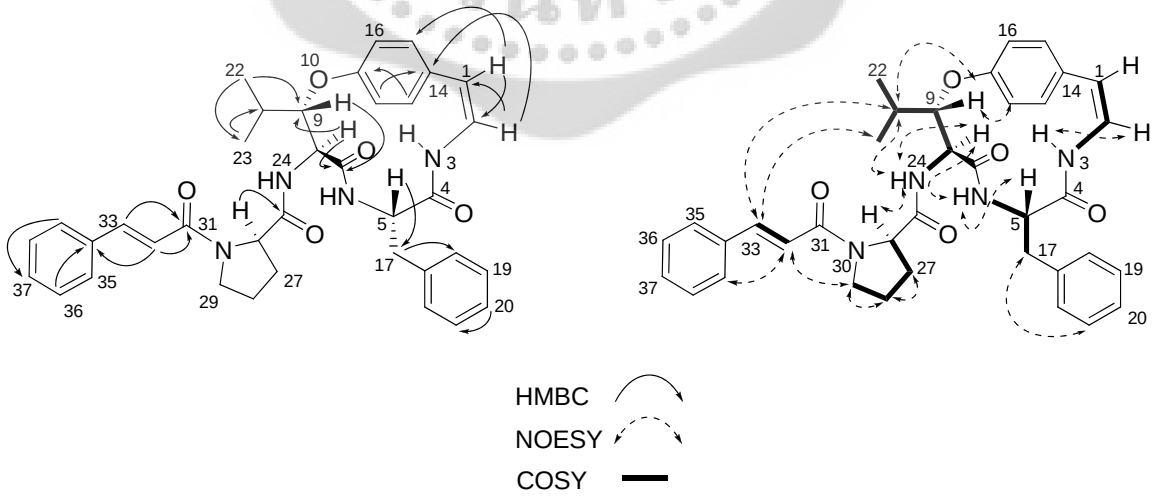


FIGURE 19 Selected HMBC, COSY and NOESY correlations for ZC-M2

TABLE 8 ^{1}H, ^{13}C NMR and 2D NMR data of compound ZC-M2 in $CDCl_3$

Position	δ_H	δ_C	HMBC correlations	NOESY correlations
1	6.36 *d* (*J* = 7.6 Hz)	115.5	C-2, C-15	-
2	6.73 *dd* (*J* = 10.4, 7.5 Hz)	125.5	C-1, C-15	NH-3, H-5,
NH-3	6.56 *d* (*J* = 10.4 Hz)	-		H-2
4	-	166.9	-	-
5	4.62 *ddd* (*J* = 11.0, 7.9, 4.1 Hz)	53.4		NH-3, NH-6, H-17, H-19
NH-6	6.09 *d* (*J* = 7.9 Hz)	-	-	H-5, H-17
CO-7	-	171.1	-	-
8	4.32 *dd* (*J* = 9.4, 6.9 Hz)	55.1	C-7, C-9	H-21, NH-24
9	4.94 *dd* (*J* = 6.9, 1.6 Hz)	81.8	C-8	H-21, H-22, H-23
11	-	156.0	-	-
12	7.22 *m*[a]	123.4	C-14	-
13	7.04 *m*[a]	126.5	C-11	-
14	-	136.6	-	-
15	7.05 *m*[a]	131.8	C-16	-
16	7.17 *m*[a]	123.4		-
17	α 3.42 *dd* (*J* = 16.0, 4.0 Hz)	35.9	C-17a, C-18	H-19
	β 2.75 *dd* (*J* = 16.0, 11.0 Hz)			
17a	-	136.6	-	-
18,18'	7.27 *m*[a]	128.2	-	-
19,19'	7.07 *m*[a]	128.0	-	H-5, H-17
20	7.02 *m*[a]	130.1	C-19	
21	1.82 *ddd* (*J* = 6.7, 6.6, 1.6 Hz)	29.0	-	H-8, H-9, H-22, H-23, NH-24, H-32
22	1.14 *d* (*J* = 6.7 Hz)	14.5	C-9, C-21, C-23	H-21, H-23
23	0.69 *d* (*J* = 6.6 Hz)	20.4	C-9, C-21, C-22	H-21, H-22, H-32
NH-24	8.09 *d* (*J* = 9.4 Hz)	-	-	H-21, H-26, H-32
CO-25	-	171.4		
26	4.16 *d* (*J* = 7.6 Hz)	59.1	C-25	NH-24, H-27
27	27α 2.26 *dd* (*J* = 12.2, 4.4 Hz)	25.8	-	H-26, H-28
	27β 1.56 *m*			
28	2.03 *m*	24.9	-	H-27, H-29
29	3.58 *m*	47.1	-	H-28, H32
31	-	166.5	C-31, C-32, C-33	
32	6.69 *d* (*J* = 15.4 Hz)	116.9	C-30, C-31, C-33, C-34, C-35	H-29, H-33, H-35
33	7.73 *d* (*J* = 15.4 Hz)	144.1	-	H-21, H-23, NH-24, H-31
34	-	134.6	-	-
35	7.56 *dd* (*J* = 5.9, 2.0 Hz)	128.5	C-37	H-32
36	7.41 *t* (*J* = 3.5, 2.9 Hz)	128.9	C-34	H-32
37	7.02 *m*	130.3	C-35, C-36	-

[a] Assignments confirmed by DEPT, HMQC and HMBC.

The comparison of ^{1}H and ^{13}C NMR data (TABLE 9) of cyclic part of compound ZC-M2 with lotusanine B (**24**) (Zarga; et al. 1995: 504-511) showed the similar chemical shift in both compound, except for C-1 [ZC-M2: δ_C 115.5, δ_H 6.36 *d* (*J* = 7.6 Hz); lotusanine B: δ_C 130.9, δ_H 6.02 *d* (*J* = 7.8 Hz) (Zarga; & et al. 1995: 504-511)] (TABLE 8). However, the comparison of ^{1}H and ^{13}C NMR data at C-1 of compound ZC-M2 with waltherine A (**62**) [δ_C 119.0, δ_H 6.40 *d* (*J* = 7.0 Hz)], a cyclopeptide with the same cyclic part as ZC-M2, gave the better result (Morel; et al. (1999: 473-477). Moreover, the chemical shift of C-27 at 59.1 ppm was corrected to be 29.1 ppm in lotusanine B (**24**) (Zarga; et al. 1995: 504-511) by Tan and Zhou (Tan; & Zhou. 2006: 840-895) and this was comparable to our result.

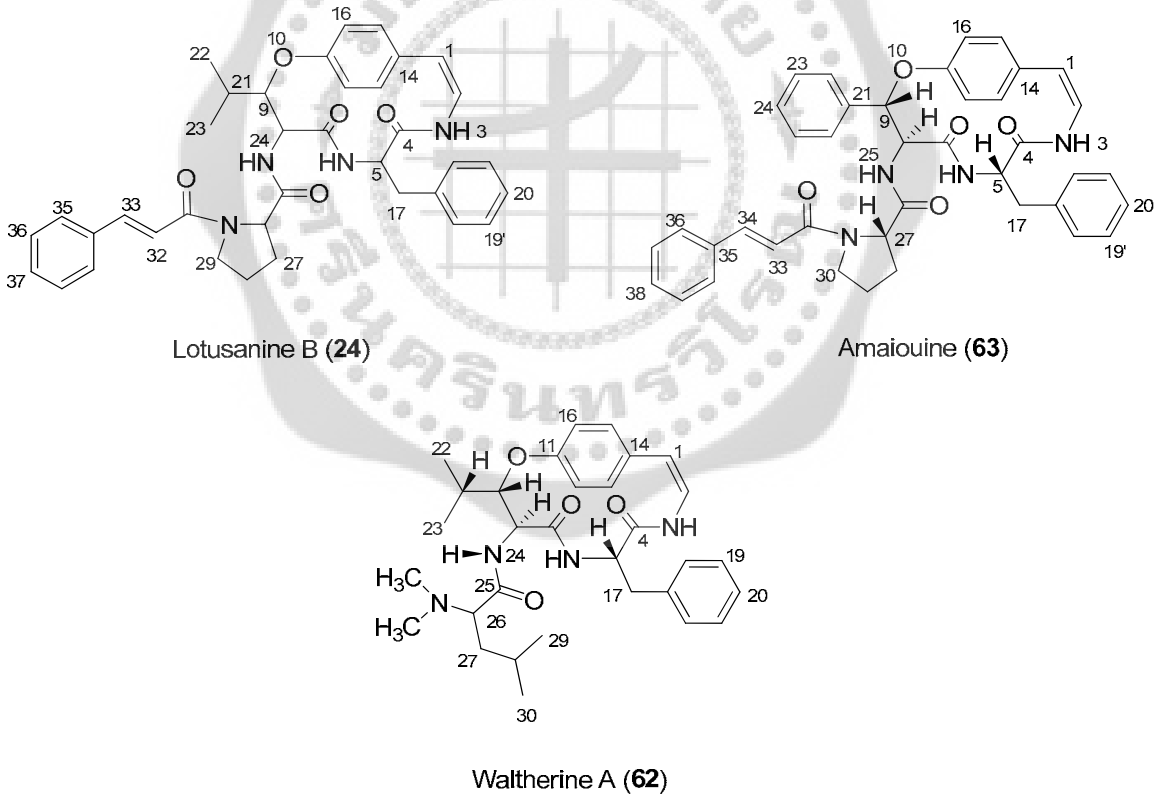


FIGURE 20 Structures of lotusanine B, amaiouine and waltherine A

TABLE 9 Comparison of ^{1}H and ^{13}C NMR data of compound ZC-M2 with lotusanine (**24**)

Position	δ_H		δ_C	
	ZC-M2 (300 MHz)	**24** (500 MHz)	ZC-M2 (70 MHz)	**24** (125 MHz)
1	6.36 *d* (*J* = 7.6 Hz)	6.02 *d* (*J* = 7.8 Hz)	115.5	130.9
2	6.73 *dd* (*J* = 10.4, 7.6 Hz)	6.32 *d* (*J* = 7.6 Hz)	125.5	131.8
NH-3	6.56 *d* (*J* = 10.4 Hz)	-	-	-
4	-	-	166.9	166.6
5	4.62 *ddd* (*J* = 11.0, 7.9, 4.0 Hz)	4.30 *dd* (*J* = 9.2, 6.7 Hz)	53.4	53.9
NH-6	6.09 *d* (*J* = 7.9 Hz)	-	-	-
CO-7	-	-	171.1	167.7
8	4.32 *dd* (*J* = 9.4, 6.9 Hz)	4.60 *m*	55.1	55.2
9	4.94 *dd* (*J* = 6.9, 1.6 Hz)	5.00 *dd* (*J* = 6.6, 1.9 Hz)	81.8	81.9
11	-	-	156.0	156.0
12	7.22 *m*[a]	7.38-7.54 *m*	123.4	123.5
13	7.04 *m*[a]	7.38-7.54 *m*	126.5	117.1
14	-	7.38-7.54 *m*	136.6	126.6
15	7.05 *m*[a]	7.38-7.54 *m*	131.8	123.4
16	7.17 *m*[a]	7.38-7.54 *m*	123.4	130.1
17	α 3.42 *dd* (*J* = 16.0, 4.0 Hz)	7.38-7.54 *m*	35.9	36.0
	β 2.75 *dd* (*J* = 16.0, 11.0 Hz)			
17a	-	-	136.6	131.8
18,18'	7.27 *m*[a]	7.38-7.54 *m*	128.2	128.8
19,19'	7.07 *m*[a]	7.38-7.54 *m*	128.0	129.0
20	7.02 *m*[a]	7.38-7.54 *m*	130.1	125.5
21	1.82 *m*	1.80 *m*	29.0	28.9
22	1.14 *d* (*J* = 6.7 Hz)	0.66 *d* (*J* = 6.6 Hz)	20.4	14.0
23	0.69 *d* (*J* = 6.6 Hz)	1.12 *d* (*J* = 6.6 Hz)	14.5	19.7
NH-24	8.09 *d* (*J* = 9.4 Hz)	-	-	-
CO-25	-	-	171.4	171.5
26	4.16 *br d* (*J* = 7.6 Hz)	3.39-3.55 *m*	59.1	59.1
27	α 2.26 *dd* (*J* = 12.2, 4.4 Hz)	3.39-3.55 *m*	25.8	59.1[b]
	β 1.56 *m*			
28	2.03 *m*	3.39-3.55 *m*	24.9	28.1
29	3.58 *m*	3.39-3.55 *m*	47.1	47.2
CO-31	-	-	166.5	171.4
32	6.69 *d* (*J* = 15.4 Hz)	6.68 *d* (*J* = 15.4 Hz)	116.9	128.2
33	7.73 *d* (*J* = 15.4 Hz)	7.72 *d* (*J* = 15.4 Hz)	144.1	144.1
34	-	-	134.6	136.7
35	7.56 *dd* (*J* = 5.9, 2.9 Hz)	7.3- 7.54 *m*	128.5	128.1
36	7.41 *t* (*J* = 3.5, 2.9 Hz)	7.3- 7.54 *m*	128.9	130.9
37	7.02 *m*	7.3- 7.54 *m*	130.3	126.6

[a] Assignments confirmed by DEPT, HMQC and HMBC.

[b] Data were corrected to 29.1 ppm by Tan and Zhou (Tan; & Zhou. 2006: 840-895).

TABLE 10 Comparison of ^{1}H and ^{13}C NMR data of compound ZC-M2 with waltherine A (**62**)

Position	δ_H		δ_C (75 MHz)	
	ZC-M2 (300 MHz)	**62** (400 MHz)	ZC-M2 (75 MHz)	**62** (100 MHz)
1	6.36 *d* (*J* = 7.6 Hz)	6.40 *d* (*J* = 7.0 Hz)	115.5	119.0
2	6.73 *dd* (*J* = 10.4, 7.6 Hz)	6.60 *dd* (*J* = 7.5,7.0 Hz)	125.5	125.5
NH-3	6.56 *d* (*J* = 10.4 Hz)	6.39 *d* (*J* = 7.5 Hz)	-	-
CO-4	-	-	166.9	167.0
5	4.62 *ddd* (*J* = 11.0, 7.9, 4.0 Hz)	4.46 *m*	53.4	54.5
NH-6	6.09 *d* (*J* = 7.9 Hz)	6.07 *d* (*J* = 8.0 Hz)	-	-
CO-7	-	-	171.1	171.5
8	4.32 *dd* (*J* = 9.4, 6.9 Hz)	4.38 *dd* (*J* = 10, 7 Hz)	55.1	54.7
9	4.94 *dd* (*J* = 6.9, 1.6 Hz)	4.96 *dd* (*J* = 7, 2 Hz)	81.8	80.7
11	-	-	156.0	156.0
12	7.22 *m*[a]	7.16 *dd* (*J* = 10, 2 Hz)	123.4	122.5
13	7.04 *m*[a]	7.10 *dd* (*J* = 10, 2 Hz)	126.5	131.5
14	-	-	136.6	131.6
15	7.05 *m*[a]	6.98 *dd* (*J* = 10, 2 Hz)	131.8	130.0
16	7.17 *m*[a]	7.07 *dd* (*J* = 10, 2 Hz)	123.4	123.0
17	α 3.42 *dd* (*J* = 16.0, 4.0 Hz)	α 3.06 *dd* (*J* = 15, 4 Hz)	35.9	36.4
	β 2.75 *dd* (*J* = 16.0, 11.0 Hz)	β 2.94 *dd* (*J* = 15, 8 Hz)		
17a	-	-	136.6	136.0
18,18'	7.27 *m*[a]	7.10-7.30[b]	128.2	128.3
19,19'	7.07 *m*[a]	7.10-7.30[b]	128.0	128.7
20	7.02 *m*[a]	7.10-7.30[b]	130.1	127.6
21	1.82 *m*	1.88 *m*	29.0	29.1
22	1.14 *d* (*J* = 6.7 Hz)	1.25 *d* (*J* = 7 Hz)	20.4	20.2
23	0.69 *d* (*J* = 6.6 Hz)	0.92 *d* (*J* = 7 Hz)	14.5	15.0
NH-24	8.09 *d* (*J* = 9.4 Hz)	7.60 *d* (*J* = 10 Hz)	-	-

[a] Assignments confirmed by DEPT, HMQC and HMBC.

[b] Peaks occur in the given range, no assignment.

The stereochemistry of compound ZC-M2 was determined by comparison of the corresponding chemical shift with those of amaiouine (**63**), a cyclopeptide with related structure as that of compound ZC-M2 (de Oliveira; et al. 2009:1195-1197). The X-ray analysis of amaiouine confirmed all *S* configurations at amino acid residues in its structure. The levorotatory optical rotation $[\alpha]_D^{27}$ = -117° similar to amaiouine $[\alpha]_D^{25}$ = -87° (de Oliveira; et al. 2009:1195-1197), thus ZC-M2 should have displayed all *S* configurations as shown in FIGURE 19.

TABLE 11 Comparison of ^{1}H and ^{13}C NMR data of compound ZC-M2 with amaiouine (**63**)

Position	ZC-M2		amaiouine (**63**)	
	δ_H (300 MHz)	δ_C	δ_H (300 MHz)	δ_C
1	6.36 *d* (*J* = 7.6 Hz)	115.5	6.40 *d* (*J* = 7.6 Hz)	116.3
2	6.73 *dd* (*J* = 10.4, 7.6 Hz)	125.5	6.74 *dd* (*J* = 10.0, 7.6 Hz)	125.8
NH-3	6.56 *d* (*J* = 10.4 Hz)	-	6.59 *d* (*J* = 10.0 Hz)	-
4	-	166.9	-	167.1
5	4.62 *ddd* (*J* = 11.0, 7.9, 4.0 Hz)	53.4	4.58 *m*	54.3
NH-6	6.09 *d* (*J* = 7.9 Hz)	-	5.89 *d* (*J* = 10.5 Hz)	-
CO-7	-	171.1	-	171.1
8	4.32 *dd* (*J* = 9.4, 6.9 Hz)	55.1	4.76 *dd* (*J* = 7.2, 2.7 Hz)	56.1
9	4.94 *dd* (*J* = 6.9, 1.6 Hz)	81.8	5.92 *d* (*J* = 7.2 Hz)	82.2
11	-	156.0	-	155.3
12	7.22 *m*[a]	123.4	7.08 *m*	130.5
13	7.04 *m*[a]	126.5	7.24 *m*	123.7
14	-	136.6	-	132.6
15	7.05 *m*[a]	131.8	7.24 *m*	123.7
16	7.17 *m*[a]	123.4	7.18 *m*	128.6
17	α 3.42 *dd* (*J* = 16.0, 4.0 Hz)	35.9	α 2.78 *dd* (*J* = 15.0, 10.2 Hz),	36.3
	β 2.75 *dd* (*J* = 16.0, 11.0 Hz)		β 3.37 *dd* (*J* = 15.0, 3.6 Hz)	
17a	-	136.6	-	136.6
18,18'	7.27 *m*[a]	128.2	7.07 *m*	128.8
19,19'	7.07 *m*[a]	128.0	7.20 *m*	128.7
20	7.02 *m*[a]	130.1	7.15 *m*	132.1
21	1.82 *m*	29.0	-	137.5
22	1.14 *d* (*J* = 6.7 Hz)	20.4	7.54 *m*	128.1
23	0.69 *d* (*J* = 6.6 Hz)	14.5	7.07 *m*	126.9
	-	-	7.09 *m*	128.9
NH	8.09 *d* (*J* = 9.4 Hz)	-	-	-
	terminal part (proline-coumaroyl moiety)			
CO-25	-	171.4	-	170.6
26	4.16 *br d* (*J* = 7.6 Hz)	59.1	3.90 *d* (*J* = 6.9 Hz)	59.0
27	α 2.26 *dd* (*J* = 12.2, 4.4 Hz)	25.8	α 1.42 *m*, β 2.20 *dd* (11.5, 5.1 Hz)	26.0
	β 1.56 *m*			
28	2.03 *m*	24.9	α 1.46 *m*, β 1.82 *dd* (*J* = 11.5, 5.1 Hz)	24.7
29	3.58 *m*	47.1	3.02 *t* (*J* = 8.4 Hz) 3.21 *m*	47.0
31	-	166.5	-	166.2
32	6.69 *d* (*J* = 15.4 Hz)	116.9	6.27 *d* (*J* = 15.6 Hz)	117.5
33	7.73 *d* (*J* = 15.4 Hz)	144.1	7.50 *d* (*J* = 15.6 Hz)	143.6
34	-	134.6	-	135.0
35	7.56 *dd* (*J* = 5.9, 2.9 Hz)	128.5	7.44 *m*	129.3
36	7.41 *t* (*J* = 3.5, 2.9 Hz)	128.9	7.20 *m*	128.6
37	7.02 *m*	130.3	7.09 *m*	130.4

[a] Assignments confirmed by DEPT, HMQC and HMBC.

1.3 Structure determination of compound ZC-M3 (cambodine, sss4402)

Compound ZC-M3 was obtained as colorless needles, mp 225-227 ^{o}C and gave violet-blue coloration with anisaldehyde-H_2SO_4 reagent. On the basis of its HRTOFMS (APCI^{+}) *m/z* 668.38079 $[M+H]^{+}$ (calcd. 668.38116 $C_{39}H_{49}N_5O_5$ + H), a molecular formula of ZC-M3 was established as $C_{39}H_{49}N_5O_5$ with support of ^{13}C NMR spectrum. The UV spectrum showed end absorption band, which was consistent with the 14-membered type cyclopeptide (Gournelis; Laskaris; & Verpoorte. 1998: 7). Their IR spectra exhibited diagnostic peaks for amino (3338 cm^{-1}), amide (1631-1685 cm^{-1}) and aryl ether (1230-1242 cm^{-1}) functions.

The ^{1}H, ^{13}C NMR (TABLE 12, FIGURE 32-33), DEPT and HMQC spectra of compound ZC-M3 indicated the presence of 39 carbon resonances, which provided signals for four methyls, one *N*-methyl, three methylenes, twenty three (including one oxygenated and two olefinic) methines and eight quaternary carbons, four of which corresponded to the carbonyl groups. The ^{1}H NMR spectrum of compound ZC-M3 displayed signals corresponding to *Z*-olefinic protons of styrylamine at δ_H 6.45 *d* (*J* = 7.1 Hz) and 6.66 *dd* (*J* = 9.6, 7.1 Hz), a number of aromatic, methine, methylene and methyl protons including a singlet of *N*-methyl proton at δ_H 2.11. From the COSY and HMQC spectra of compound ZC-M3 and comparison with the reported values led to a conclusion for the presence of *p*-oxystyrylamine group [δ_H 7.37 *m* (H-12), 7.36 *m* (H-16), 7.17 *br d* (*J* = 7.7 Hz, H-13), 7.12 *br d* (*J* = 9.1 Hz, H-15), 6.72 *d* (*J* = 9.6 Hz, NH-3), 6.66 *dd* (*J* = 9.6, 7.1 Hz, H-2) and 6.45 *d* (*J* = 7.1 Hz, H-1); δ_C 155.1 (C-11), 132.5 (C-14), 130.1 (C-13), 125.5 (C-2), 122.6 (C-12) and 117.5 (C-1)], β-OH-phenylalanine or β-phenylserine [δ_H 7.45 *m* (H-24), 7.42 *m* (H-23,23′),7.10 *br dd* (*J* = 7.5, 1.0 Hz, 22,22′), 6.85 *d* (*J* = 8.9 Hz, NH-25), 6.10 *d* (*J* = 6.9 Hz, H-9) and 4.79 *dd* (*J* = 8.9, 6.9 Hz, H-8); δ_C 171.0 (CO-7), 136.8 (C-21), 128.9 (C-24), 128.8 (C-23,23′), 128.1 (C-22,22′), 81.5 (C-9) and 56.7 (C-8)], isoleucine [δ_H 6.20 *d* (*J* = 8.3 Hz, NH-6), 4.09 *dd* (*J* = 8.3, 4.2 Hz, H-5), 2.17 *m* (H-17), 1.25 *m* (H-18α), 0.96 *m* (H-18β), 0.87 *t* (*J* = 7.2 Hz, H-19) and 0.75 *d* (*J* = 6.8 Hz, H-20); δ_C 167.2 (CO-4), 59.3 (C-5), 35.2 (C-17), 23.9 (C-18), 11.9 (C-19) and 15.8 (C-20)], phenylalanine [δ_H 6.98 *br d* (*J* = 6.1 Hz, H-30,30′), 7.24 *br d* (*J* = 7.5 Hz, H-31,31′), 7.20 *m* (H-32), 4.20 *ddd* (*J* = 10.4, 7.1, 4.8 Hz, H-27), 2.89 *dd* (*J* = 14.2, 4.8 Hz, H-28α) and 2.46 *dd* (*J* = 14.2, 10.4 Hz,

H-28β) ; δ_C 170.9 (CO-26), 136.3 (C-29), 128.9 (30,30′), 128.6 (31,31′), 127.0 (C-32), 54.7 (C-27) and 36.6 (C-28)] and *N*-methylisoleucine [δ_H 2.50 *d* (*J* = 4.1 Hz, H-35), 2.11 *s* (NMe), 1.52 *m* (H-36), 0.69 *m* (H-37), 0.66 *m* (H-38) and 0.62 *d* (*J* = 6.9 Hz, H-39); δ_C 174.2 (CO-34), 69.1 (C-35), 37.6 (C-36), 36.5 (NMe), 24.2 (C-37), 11.7 (C-38) and 15.5 (C-39)] units.

Analysis of the COSY, HMBC and NOESY spectra provided the connections among these subunits (TABLE 12). The signal for *p*-oxystyrylamine proton at NH-3 (δ_H 6.72) showed weak NOESY correlation to signal at δ_H 4.09 (H-5) and the HMBC correlations of the resonance at δ_H 6.66 (H-2) to C-14 and of H-1 (δ_H 6.45) to C-2 allowed the placement of an isoleucine moiety next to the styrylamine group. The correlations of H-8 (δ_H 4.79, *dd, J* = 8.9, 6.9 Hz) to NH-25 (δ_H 6.85, *d, J* = 8.9) in the NOESY and of NH-6 (δ_H 6.20, *d, J* = 8.3 Hz) to C-7 (δ_C 171.0), H-8 (δ_H 4.79) to C-7 (δ_C 171.0), C-11 (δ_C 155.1), C-21 (δ_C 136.8) and C-22 (δ_C 128.1) in the HMBC experiments revealed the connection of isoleucine to β-OH-phenylalanine fragments in the macrocyclic ring. The β-OH-phenylalanine moiety was characterized as *erythro* relative configuration by the vicinal coupling constant (*J* = 6.8 Hz) between H-8 and H-9 (Gournelis; Laskaris; & Verpoorte (1998: 7). NOE enhancements displayed between NH-25 and H-27 in the NOESY spectrum, and the HMBC of the H-8 with the carbonyl carbon signal at δ_C 170.9 (C-26), H-27 to C-29 (δ_C 136.3) supported that the phenylalanine unit was attached to the β-OH-phenylalanine at N-25. HMBC correlations of H-27 with the carbonyl carbon signal at δ_C 174.28 (C-34) and NOESY correlation of *N*-methyl proton at δ_H 2.11 with H-35 (δ_H 2.50*, d, J* = 4.1 Hz) supported that the *N*-methylisoleucine unit was attached to the phenylalanine at N-33. HMBC correlations of H-35 to ^{13}C signals at δ_C 15.5 (C-39), δ_C 24.5 (C-37) and δ_C 37.6 (C-36), of H-38 (δ_H 0.66) to C-36 and NOESY correlation of NMe to the signal at δ_H 0.62 (H-39) were also observed.

These evidences led to the conclusion that the structure of ZC-M3 was elucidated as new cyclopeptide alkaloid in 5(14)-scutianine A-type and named cambodine after its plant origin.

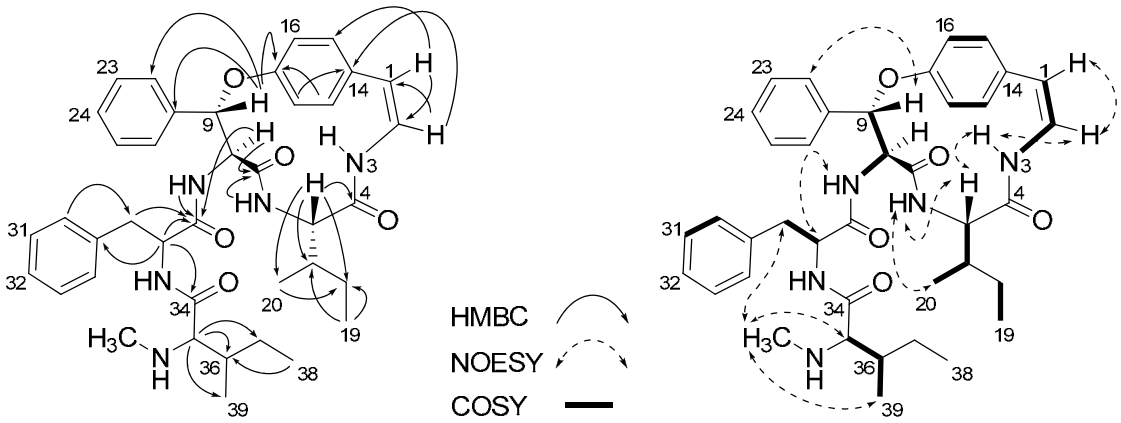
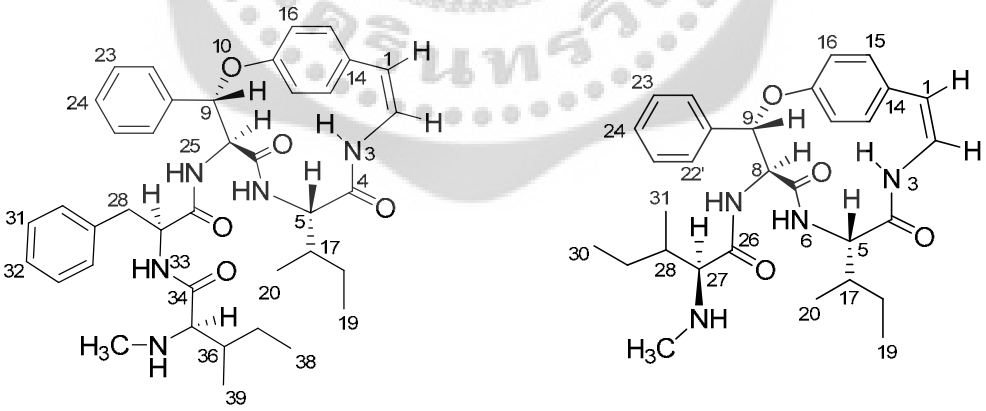


FIGURE 21 Selected HMBC COSY and NOESY correlations for ZC-M3

The comparison of ^{1}H and ^{13}C NMR data of cyclic part of compound ZC-M2 with mauritine L (**64**), which consistent with the isoleucine as ring-bound amino acid, β-OH-phenylalanine and styrylamine moiety showed the similarly chemical shift, that compound ZC-M3 displayed the similar cyclic part as that of mauritine L (TABLE 13).



Cambodine Mauritine L (**64**)

FIGURE 22 Structures of cambodine and mauritine L

TABLE 12 ^{1}H, ^{13}C NMR and 2D NMR data of compound ZC-M3 in $CDCl_3$

Position	δ_H (300 MHz)	δ_C (75 MHz)	HMBC correlations	NOESY correlations
1	6.45 *d* (*J* = 7.1 Hz)	117.5	C-2, C-14, C-15	H-2, NH-3
2	6.66 *dd* (*J* = 9.6, 7.1 Hz)	125.5	C-1, C-14	H-1, H-3, H-5
NH-3	6.72 *d* (*J* = 9.6 Hz)	-	-	H-2, H-5, NH-6
CO-4	-	167.2	-	-
5	4.09 *dd* (*J* = 8.3, 4.2 Hz)	59.3	C-4, C-7, C-17, C-18, C-20	NH-3, H-6, H-17
NH-6	6.20 *d* (*J* = 8.3 Hz)	-	C-5, C-7	NH-3, H-5, H-8, H-20
CO-7	-	171.0	-	
8	4.79 *dd* (*J* = 8.9, 6.9 Hz)	56.7	C-7, C-9, C-26	NH-6, H-23, NH-25
9	6.10 *d* (*J* = 6.9 Hz)	81.5	C-7, C-8, C-11, C-21, C-22, C-22′	H-22, H-22′, H-23
11	-	155.1	-	-
12	7.37 *m*	122.6	C-11, C-14	-
13	7.17 *br d* (*J* = 7.7 Hz)	130.1	C-11, C-15	-
14	-	132.5	-	-
15	7.12 *br d* (*J* = 9.1 Hz)	131.9	C-13	-
16	7.36 *m*	123.1	C-11	-
17	2.17 *m*	35.2	-	H-5
18	α 1.25 *m*[a], β 0.96 *m*[a]	23.9	C-29	-
19	0.87 *t* (*J* = 7.2 Hz)	11.9	C-17, C-18	-
20	0.75 *d* (*J* = 6.8 Hz)	15.8	C-5, C-17, C-18	H-6,
21	-	136.8	-	-
22,22′	7.10[b] *br dd* (*J* = 7.5, 1.0 Hz)	128.1	C-9, C-21, C-23	H-9
23,23′	7.42 *m*[b]	128.8	-	H-8
24	7.45 *m*[b]	128.9	C-23	-
NH-25	6.85 *d* (*J* = 8.9 Hz)	-	C-7, C-26	H-8, H-27
CO-26	-	170.9	-	-
27	4.20 *ddd* (*J* = 10.4, 7.1, 4.8 Hz)	54.7	C-26, C-28, C-29, C-34	NH-25, H-28, H-30, H-31
28	α 2.89 *dd* (*J* = 14.2, 4.8 Hz) β 2.46 *dd* (*J* = 14.2, 10.4 Hz)	36.6	C-26, C-27, C-29	H-27, H-30, H-31, NMe
29	-	136.3	C-28	-
30,30′	6.98 *b rd*[b] (*J* = 6.1 Hz)	128.9	C-26, C-28, C-34, NMe	H-27, H-28
31,31′	7.24 *b rd*[b] (*J* = 7.5 Hz)	128.6	C-27, C-34	H-27, H-28
32	7.20 *m*[b]	127.0	-	-
CO-34	-	174.2	-	-
35	2.50 *d* (*J* = 4.1 Hz)	69.1	C-36, C-37, C-39, NMe	H-36, H-39, NMe
36	1.52 *m*	37.6	-	H-35, H-39
37	0.69 *m*	24.2	C-38	-
38	0.66 *m*	11.7	C-36, C-37	-
39	0.62 *d* (*J* = 6.9 Hz)	15.5	C-35, C-36	H-35, H-36, NMe
NMe	2.11 *s*	36.5	C-26	H-28, H-39, H-35

[a] Assignments confirmed by 1H-1H COSY and NOESY.

[b] Assignments confirmed by DEPT, HMQC and HMBC.

TABLE 13 Comparison of ^{1}H and ^{13}C NMR data of cyclic part of compound ZC-M3 with mauritine L (**64**)

Position	δ_H (300 MHz)		δ_C (75 MHz)	
	ZC-M3	mauritine L (**64**)	ZC-M3	**64**
1	6.45 *d* (*J* = 7.1 Hz)	6.36 *d* (*J* = 7.3 Hz)	117.5	115.5
2	6.66 *dd* (*J* = 9.6, 7.1 Hz)	6.71 *dd* (*J* = 9.7, 7.3 Hz)	125.5	125.5
NH-3	6.72 *d* (*J* = 9.6 Hz)	6.57 *br d* (*J* = 9.7 Hz)	-	-
CO-4	-	-	167.2	167.1
5	4.09 *dd* (*J* = 8.3, 4.2 Hz)	4.03 *dd* (*J* = 7.8, 3.1 Hz)	59.3	59.6
NH-6	6.20 *d* (*J* = 8.3 Hz)	6.42 *d* (*J* = 7.8 Hz)	-	-
CO-7	-	-	171.0	171.5
8	4.79 *dd* (*J* = 8.9, 6.9 Hz)	*4.64* dd (*J* = 8.4, 6.3)	56.7	56.4
9	6.10 *d* (*J* = 6.9 Hz)	*6.17 d* (*J* = 6.3)	81.5	81.4
11	-	-	155.1	155.1
12	7.37 *m*	7.34 *m*[b]	122.6	123.5
13	7.17 *br d* (*J* = 7.7 Hz)	7.12 *br t* (*J* = 7.8 Hz)	130.1	130.1
14	-	-	132.5	132.2
15	7.12 *br d* (*J* = 9.1 Hz)	7.12 *br t* (*J* = 7.8 Hz)	131.9	132.2
16	7.36 *m*	7.34 *m*[b]	123.1	123.6
17	2.17 *m*	2.15 *m*	35.2	35.0
18	α 1.25 *m*[a], β 0.96 *m*[a]	α 1.61 *m* , β 0.95 *m*	23.9	24.0
19	0.87 *t* (*J* = 7.2 Hz)	0.81 *t* (*J* = 7.2 Hz)	11.9	12.1
20	0.75 *d* (*J* = 6.8 Hz)	0.66 *d* (*J* = 6.4 Hz)	15.8	16.0
21	-	-	136.8	137.2
22,22′	7.10[b] *br dd* (*J* = 7.5, 1.0 Hz)	7.50 *br d* (*J* = 7.4 Hz)	128.1	127.5
23,23′	7.42 *m*[b]	7.40 *m*[b]	128.8	128.9
24	7.45 *m*[b]	7.40 *m*[b]	128.9	128.7
NH-25	6.85 *d* (*J* = 8.9 Hz)	7.43[b]	-	-

[a] Assignments confirmed by 1H-1H COSY and NOESY.

[b] Signals without multiplicity was assigned from COSY.

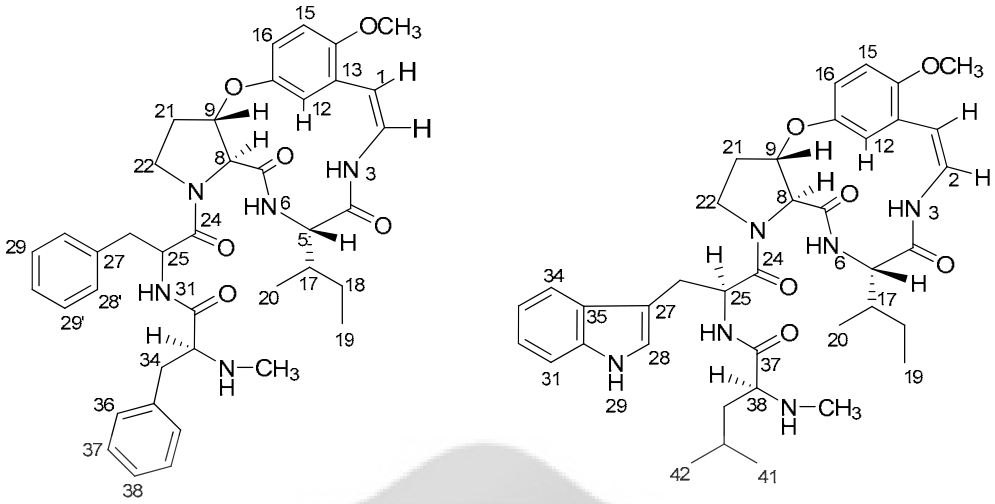
The levorotatory optical rotation $[\alpha]_D^{27}$ = -198° and CD spectrum of compound ZC-M3 displayed an intense negative and a weak positive Cotton effect bands at 236 (-41.88), 280 (+6.44) nm, respectively, consistent with the 5*S*, 8*S* and 9*S* configurations presented in the 14-membered ring nucleus (Gournelis; Laskaris; & Verpoorte. 1998: 7).

2. Cyclopeptide alkaloids from the MeOH extract of *Z. mauritiana* stem bark

The pulverized, dried stem bark of *Z. mauritiana* was extracted successively with EtOAc and MeOH. The EtOAc extract gave very weak blue color development. TLC investigation of the EtOAc extract and the comparison with authentic compounds, resulted in the similarly coloration and R_f of the known triterpenes 3-*O*-vanillylceanothic acid (**31**), lupeol (**32**), betulinic acid (**34**), 2-*O*-*trans*-*p*-coumaroyl alphitolic acid (**35**) and alphitolic acid (**36**), and ceanothic acid (**39**) (FIGURE 13).

However, a typical intense blue or orange coloration with anisaldehyde-H_2SO_4 reagent for the MeOH extract indicated the presence of cyclopeptide alkaloid (Suksamrarn; et al. 2005), that MeOH soluble extract was therefore selected for further chromatographic separations and resulted in the isolation of one new, in addition to two known, cyclopeptide alkaloids. The UV absorption bands at around 270 and 320 nm were observed for the 13-membered cyclopeptides, which is the characteristic styrylamine chromophore. The presence of tryptophan moiety showed the absorption bands at around 220, 270 and 290 nm (Gournelis; Laskaris; & Verpoorte. 1998: 7). Their IR spectra exhibited diagnostic peaks for amino (3338-3345 cm^{-1}), amide (1637-1679 cm^{-1}) and aryl ether (1221-1224 cm^{-1}) functions.

In this work, the isolation and structure elucidation of one new cyclopeptide alkaloid of the 5(13)-zizyphine A-type, mauritine M (**60**), together with a known alkaloid nummularine H (**59**) from the stem bark of *Z. mauritiana*. Their structures were elucidated on the basis of spectroscopic analysis and by comparison their spectroscopic data with the literature values.



ZM-M1 or Nummularine H (**59**) sss4971

ZM-M2 or Mauritine M (**60**) sss4973

FIGURE 23 Structures of cyclopeptide alkaloids from stem bark of *Z. mauritiana*

2.1 Structure determination of compound ZM-M1 (nummularine H, sss4971)

Compound ZM-M1 was a colorless solid, mp 128-129 ^{o}C and gave a blue coloration with anisaldehyde-H_2SO_4 reagent. The HRTOFMS displayed a pseudomolecular ion at m/z 682.3586 $[M+H]^+$ and in combination with the ^{13}C NMR data (TABLE 14) suggesting that ZM-M1 had a molecular formular of $C_{39}H_{47}N_5O_6$. Its IR absorption spectrum showed the presence of amide units at 3338 (NH stretch), 1668, 1642 (C=O stretch of secondary amide) and 1221 (C-N stretching of secondary amide). The UV absorption maxima at 268 and 319 nm suggested that compound ZM-M1 was a 13-membered-type cyclopeptide alkaloid (Suksamrarn; et al. 2005: 1175-1180).

The ^{13}C NMR (TABLE 14) and DEPT spectra of compound ZM-M1 displayed 39 carbon signals including one *N*-methyl, one methoxyl, two methyls, five methylenes, 21 methines, nine quaternary carbons, four of which corresponded to the carbonyl groups. The meta-oxygenated styrylamine [δ_H 6.68 (*d, J* = 2.9 Hz), 6.88 (*d, J* = 9.1 Hz), 6.80 (*dd, J* = 9.1, 2.9 Hz), 5.93 (*d, J* = 8.9 Hz) and 6.96 (*dd, J* = 11.3, 8.9 Hz)] appeared as an ABX coupling (Lee; Su; & Liu. 2001: 1271-1276) further supported that compound ZM-M1 was a 13-membered cyclopeptide which corresponded to its UV and IR data. The methoxyl signal

at δ_H 3.76 showed a cross-peak with a quaternary aromatic carbon signal at δ_C 151.4 (C-14) in the HMBC spectrum in addition to a strong NOE effect shown between this methoxyl group and H-15 in the NOESY experiment confirmed that the methoxyl group was placed at C-14 of the 13-membered cyclopeptide feature.

The ^{1}H, ^{13}C NMR, DEPT and 2D-NMR (COSY and HMQC) spectra of compound ZM-M1 together with a comparison with the literature reported value (Tschesche; Elgamal; & Eckhardt. 1977: 2649-2655, Lee; Su; & Liu. 2001: 1271-1276) led to the assignments for four amino acid units of isoleucine [δ_H 4.31 *t* (H-5), 2.07 (*m*, H-17), 1.42 *m* (H-18α) and 1.18 *m* (H-18β), 0.92 *t* (H-19) and 1.01 *d* (H-20); δ_C 60.3, 35.5, 24.7, 11.8 and 16.1], 3-oxygenated proline [δ_H 4.41 *d* (H-8), 5.43 *dt* (H-9), 2.41 *m* (H-21α) and 2.21 *m* (H-21β), 3.96 *m* (H-22α) and 2.76 *m* (H-22β); δ_C 64.5, 76.5, 32.3 and 46.2] (Lee; Su; & Liu. 2001: 1271-1276), phenylalanine [δ_H 4.98 *m* (H-25), 2.93 *t* (H-26), ca 7.18-7.34 *m* (aromatic protons); δ_C 50.9, 39.4, 135.3, 129.0 and 128.7] (Lee; Su; & Liu. 2001: 1271-1276), and *N*-methyl phenylalanine [δ_H 3.22 *dd* (H-33), 2.67 and 3.12 *dd* (H-34), 7.03-7.34 (*m*, aromatic protons) and 2.27 (3H, *s*, *N*Me); δ_C 65.8, 38.9, 137.3, 129.1, 128.7, 126.9 and 35.3] (Lee; Su; & Liu. 2001: 1271-1276).

Connecting these subgroups were provided by HMBC and NOESY experiments (TABLE 14, FIGURE 24), as follow : the styrylamine proton at δ_H 8.42 (NH-3) and isoleucyl proton at δ_H 4.31 (H-5) showed HMBC cross peaks with C-4 (δ_C 167.0) along with correlations of H-2 (δ_H 6.96) to C-13, and H-1 (δ_H 5.93) to C-2, C-12 and C-14 indicating that isoleucine amino acid was attached to the styrylamine unit. The correlations of H-5, H-8 and H-9 to β-OH-proline carbonyl C-7 (δ_C 169.6) revealed the connection between the isoleucine and β-OH-proline amino acids. In the NOESY spectrum, a strong NOE effect displayed between the proline proton at δ_H 3.96 (H-22) and H-25 (δ_H 4.98) of phenylalanine unit, but no HMBC correlation observed between these protons. The HMBC correlation from H-26 (δ_H 2.93) to C-24, C-25, C-27, C-28 ; NH-31 (δ_H 7.78) to C-32; H-33 (δ_H 3.22) to C-32, C-35 and NMe confirmed the phenylalanine as the intermediate unit connected between the β-OH-proline amino acid and *N*-methyl phynylanine (as the terminal amino acid). The *Z*-geometry of 1,2 double bond was established on the basis of the coupling constant value of 8.9 Hz for H-1 and H-2 (Suksamrarn; et al. 2005: 1175-1180). The styrylamine proton NH-3 showed strong NOE effect with aromatic proton H-12. Strong

NOE enhancement from H-9 to H-12 and H-21β, whist H-8 showed a cross peak with NH-6.

The ^{1}H and ^{13}C NMR data of compound ZM-M1 were closely resembled with that of nummularine H (**59**) (TABLE 14) in which the absolute stereochemistry was determined by its CD (MeOH) spectrum analysis and assigned to be 5*S*, 8*S* and 9*S* configurations for the macrocyclic ring (Lee; Su; & Liu. 2001: 1271-1276, Gournelis; Laskaris; & Verpoorte. 1998: 7). Compound ZM-M1 showed levorotatory optical rotation ($[\alpha]_D^{26}$ = -296^{o}) similar to that of nummularine H ($[\alpha]_D^{20}$ = -343^{o}) (Lee; Su; & Liu. 2001: 1271-1276), thus the stereochemical structure of ZM-M1 was thus established as shown in FIGURE 24.

Nummularine H (**59**) has been isolated from *Z. nummularia* (Tschesche; Elgamal; & Eckhardt, G. 1977: 2649-2655) and the stem of *Paliurus ramossisimus* (Rhamnaceae) (Lee; Su; & Liu. 2001: 1271-1276).

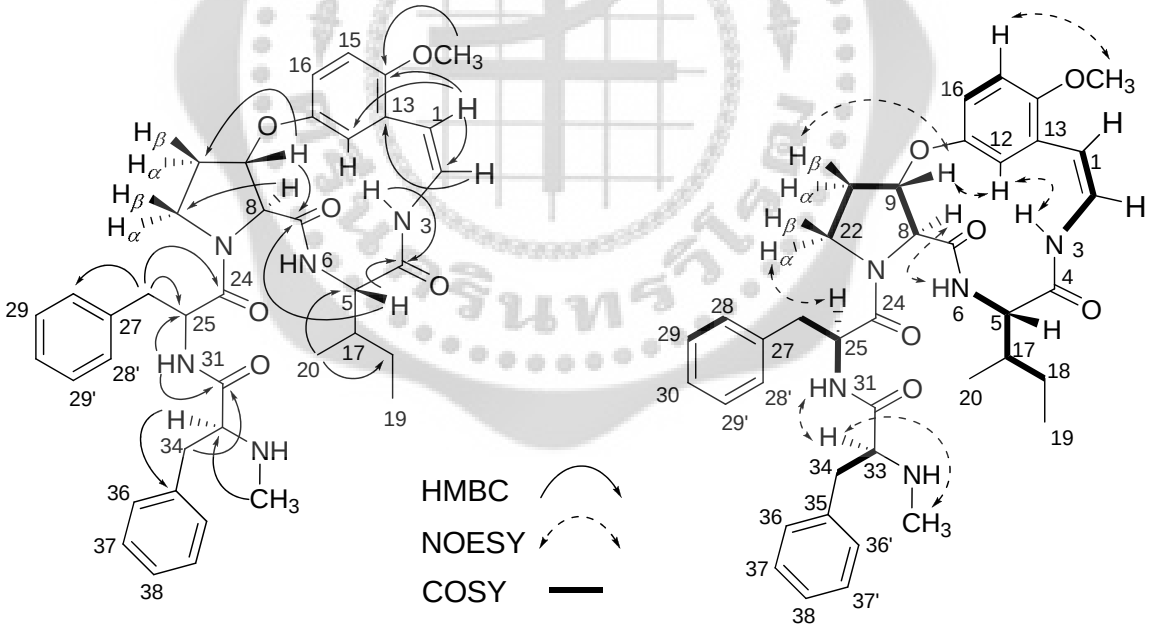


FIGURE 24 Selected HMBC, COSY and NOESY correlations for compound ZM-M1

TABLE 14 ^{1}H, ^{13}C NMR and 2D NMR data of compound ZM-M1 in $CDCl_3$

position	δ_H	δ_C	HMBC correlations	NOESY correlations
1	5.93 *d* (*J* = 8.9 Hz)	106.7	C-2, C-12, C-14	-
2	6.96 *dd* (*J* = 11.3, 8.9 Hz)	121.4	C-13	-
NH-3	8.42 *d* (*J* = 11.3 Hz)	-	C-4	H-12
CO-4	-	167.0	-	-
5	4.31 *t* (*J* = 4.4 Hz)	60.3	C-4, C-7, C-17, C-18, C-20	H-17, H-20
NH-6	7.26 *m*	-	-	H-8
CO-7	-	169.6	-	-
8	4.41 *d* (*J* = 3.1 Hz)	64.5	C-7, C-9, C-22	NH-6, H-9 (weak), H-21α (weak)
9	5.43 *dt* (*J* = 6.6, 3.1 Hz)	76.5	C-7, C-21	H-8 (weak), H-12, H-21β
11		150.9	-	-
12	6.68 *d* (*J* = 2.9 Hz)	111.2	C-14, C-16	NH-3, H-8 (weak), H-9
13	-	124.3	-	-
14	-	151.4	-	-
15	6.88 *d* (*J* = 9.1 Hz)	113.7	C-11, C-13	OMe
16	6.80 *dd* (*J* = 9.1, 2.9 Hz)	117.6	C-14	-
17	2.07 *m*	35.4	C-4	-
18	1.18 *m* and 1.42 *m*	24.7	-	-
19	0.92 *t* (*J* = 7.2 Hz)	11.8	C-17, C-18	-
20	1.01 *d* (*J* = 6.9 Hz)	16.1	C-5, C-17, C-18	-
21	α 2.21 *m*, β 2.41 *m*	32.3	-	H-22α
22	α 3.96 *m*, β 2.76 *m*	46.3	-	H-8 (weak), H-21α, H-25
CO-24	-	170.6	-	-
25	4.98 *m*	50.9	-	H-22α, H-26, NH-31
26	2.93 *t* (*J* = 6.5 Hz)	39.4	C-24, C-25, C-27, C-28	H-25, H-31, H-36
27	-	135.3	-	-
28, 28'	7.18 *m*	129.0	C-26, C-30	-
29, 29'	7.29-7.34 *m*	128.7	C-27	-
30	7.21 *m*	127.2	C-28	-
NH-31	7.78 *d* (*J* = 8.4 Hz)	-	C-32	H-25, H-26, H-33, H-34
CO-32	-	173.2	-	-
33	3.22 *dd* (*J* = 9.2, 4.3 Hz)	65.8	C-32, C-35, NMe	H-31, NMe
34	3.12 *dd* (*J* = 13.4, 4.3 Hz), 2.67 *dd* (*J* = 13.4, 9.2 Hz)	38.9	C-32, C-33, C-35, C-37	-
35	-	137.3	-	-
36, 36'	7.03 *m*	129.1	C-38	H-20
37, 37'	7.29-7.34 *m*	128.3	C-35	-
38	7.21	126.9	C-36	-
NMe	2.27 *s*	35.3	C-33	H-31, H-33
OMe	3.79 *s*	56.0	C-14	H-15

TABLE 15 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-M1 with nummularine H (**59**)

position	δ_H		δ_C	
	ZM-M1	nummularine H (**59**)	ZM-M1	nummularine H
1	5.93 *d* (*J* = 8.9 Hz)	5.90 *d* (*J*= 9.1 Hz)	106.7	106.6
2	6.96 *dd* (*J* = 11.3, 8.9 Hz)	6.93 *dd* (*J*= 11.3, 9.1 Hz)	121.4	121.6
NH-3	8.42 *d* (*J* = 11.3 Hz)	8.40 *d* (*J*= 11.3 Hz)	-	-
CO-4	-	-	167.0	167.0
5	4.31 *t* (*J* = 4.4 Hz)	4.29 *t* (*J* = 4.4 Hz)	60.3	60.3
NH-6	7.26 *m*	7.27	-	-
CO-7	-	-	169.6	169.8
8	4.41 *d* (*J* = 3.1 Hz)	4.39 *d* (*J*= 3.1 Hz)	64.5	65.0
9	5.43 *dt* (*J* = 6.6, 3.1 Hz)	5.41 *dt* (*J*= 7.3, 3.1 Hz)	76.5	76.7
11	-	-	150.9	151.2
12	6.68 *d* (*J* = 2.9 Hz)	6.66 *d* (*J*= 2.9 Hz)	111.2	111.4
13	-	-	124.3	124.8
14	-	-	151.4	151.7
15	6.88 *d* (*J* = 9.1 Hz)	6.86 *d* (*J*= 9.1 Hz)	113.7	114.2
16	6.80 *dd* (*J* = 9.1, 2.9 Hz)	6.77 *dd* (*J*= 9.1, 2.9 Hz)	117.6	117.7
17	2.07 *m*	2.05 *m*	35.4	35.7
18	α 1.18 *m*, β 1.42 *m*	1.12 *m* , 1.42 *m*	24.7	25.0
19	0.92 *t* (*J* = 7.2 Hz)	0.90 *t* (*J*= 7.2 Hz)	11.8	11.8
20	1.01 *d* (*J* = 6.9 Hz)	0.99 *d* (*J*= 6.9 Hz)	16.1	16.2
21	α 2.21 *m*, β 2.41 *m*	α 2.19 *m*, β 2.41 *dddd* (*J* = 13.6, 7.3, 6.5, 2.6 Hz)	32.3	32.4
22	α 3.96 *m*, β 2.76 *m*	α 3.94 *ddd* (*J* = 10.8, 8.2, 2.6 Hz), β 2.73 *dt* (*J* = 6.5, 10.8 Hz)	46.3	46.4
CO-24	-	-	170.6	170.9
25	4.98 *m*	4.96 *dt* (*J*= 8.2, 6.2 Hz)	50.9	51.1
26	2.93 *t* (*J* = 6.5 Hz)	2.94 *dd* (*J*= 12.5, 6.2 Hz)	39.4	39.5
27	-	-	135.3	135.6
28, 28'	7.18 *m*	7.16-7.32 *m*	129.0	129.2
29, 29'	7.29-7.34 *m*	7.16-7.32 *m*	128.7	128.7
30	7.21 *m*	7.16-7.32 *m*	127.2	127.2
NH-31	7.78 *d* (*J* = 8.4 Hz)	7.78 *d* (*J* = 8.4 Hz)	-	-
CO-32	-	-	173.2	173.1
33	3.22 *dd* (*J* = 9.2, 4.3 Hz)	3.19 *dd* (*J*= 9.1, 4.3 Hz)	65.8	66.0
34	3.12 *dd* (*J* = 13.4, 4.3 Hz), 2.67 *dd* (*J* = 13.4, 9.2 Hz)	3.09 *dd* (*J*= 13.7, 4.1 Hz)	38.9	39.0
35	-	-	137.3	137.5
36, 36'	7.03 *m*	7.16-7.32 *m*	129.1	129.1
37, 37'	7.29-7.34 *m*	7.16-7.32 *m*	128.3	128.7
38	7.21	7.16-7.32 *m*	126.9	126.9
NMe	2.27 *s*	2.24 *s*	35.3	35.3
OMe	3.79 *s*	3.76 *s*	56.0	56.2

2.2 Structure determination of compound ZM-M2 (mauritine M, sss4973)

Compound ZM-M2 was obtained as a colorless amorphous solid, mp 188-189 ^{o}C and gave an orange coloration with anisaldehyde-H_2SO_4 reagent. Its molecular formula was established as $C_{38}H_{50}N_6O_6$ by HRTOFMS (m/z 687.3856, $[M+H]^+$), in combination with analysis of the ^{13}C NMR spectrum that showed 38 carbon resonances. The IR spectrum of compound ZM-M2 showed absorption bands at 3345 (amino), 1679, 1664, 1637 (amide), 1508 (aromatic) and 1224 cm^{-1} (phenol ether) functions. The UV spectrum revealed absorption maxima at 219, 272, 279, 289 and 318 nm, which was consistent with the 13-membered cyclopeptide alkaloid containing a tryptophan moiety (Gournelis; Laskaris; & Verpoorte. 1998: 1-179, Jossang; Zahir; & Diakite. 1996: 565-657).

The ^{1}H, ^{13}C NMR (TABLE 16), DEPT and HMQC spectra of compound ZM-M2 indicated the presence of 38 carbon resonances, which provided signals for one *N*-methyl carbon, four methyls, one methoxyl, five methylenes, 17 methines and ten quaternary carbons including four carbonyl carbons. The observation of an ABX coupling for a meta-oxygenated styryl [δ_H 6.61 (*d, J* = 2.9 Hz), 6.74 (*dd, J* = 9.0, 2.9 Hz), 6.84 (*d, J* = 9.0 Hz), 5.91 (*d, J* = 9.0 Hz) and 6.91 (*dd, J* = 11.2, 9.0 Hz)] indicated that compound ZM-M4 was a 13-membered cyclopeptide (Lee; Su; & Liu. 2001: 1271-6), which corresponded to its UV and IR data. The methoxyl signal at δ_H 3.75 showed a cross-peak with a quaternary aromatic carbon signal at δ_C 151.5 (C-14) in the HMBC spectrum in addition to a strong NOE effect shown between this methoxyl group and H-15 in the NOESY experiment confirming that the methoxyl group was placed at C-14 of the 13-membered cyclopeptide feature.

Analysis of ^{1}H, ^{13}C NMR, DEPT and 2D-NMR (^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC) spectra of compound ZM-M2 together with a comparison with the literature reported value (Jossang; Zahir; & Diakite. 1996: 565-657, Lee; Su; & Liu. 2001: 1271-1276) led to the assignments for four amino acid units of isoleucine [δ_H 4.23 *t* (H-5), 2.00 *m* (H-17), 1.43 *m* (H-18α) and 1.15 *m* (H-18β), 0.87 *t* (H-19) and 0.96 *d* (H-20); δ_C 60.5 (C-5), 35.4 (C-17), 24.7 (C-18), 11.7 (C-19) and 16.0 (C-20)] (Jossang; Zahir; & Diakite. 1996: 565-657), 3-oxygenated proline [δ_H 4.38 *d* (H-8), 5.31 *dt* (H-9), 2.25 and 2.08 *m* (H-21), 3.79 *m* (H-22α) and 2.46 *m* (H-22β); δ_C 64.3 (C-8), 76.5 (C-9), 32.3 (C-21) and 46.2 (C-22)] (Lee; Su; & Liu. 2001: 1271-1276), tryptophan [δ_H 5.09 *ddd* (H-25), 3.21 (H-26α) and 3.02 (H-26β),

6.76 (H-28), 8.58 *br s* (NH-29), 7.31 *d* (H-31), 7.15 br *t* (H-32), 7.09 br *t* (H-33), 7.65 *d* (H-34); δ_C 50.2 (C-25), 29.4 (C-26), 109.5 (C-27), 122.8 (C-28), 136.0 (C-30), 111.4 (C-31), 122.2 (C-32), 119.7 (C-33), 118.4 (C-34), 127.3 (C-35)] (Jossang; Zahir; & Diakite. 1996: 565-657), and *N*-methyl leucine [δ_H 3.06 *t* (H-38), 1.56 and 1.41 *m* (H-39), 1.63 *m* (H-40), 0.90 *d* (H-41), 0.92 *d* H-42), 2.37 *s* (NMe); δ_C 63.3 (C-38), 42.7 (C-39), 25.1 (C-40), 21.9 (C-41), 23.2 (C-42), 35.4 (NMe)] (Jossang; Zahir; & Diakite. 1996: 565-657).

Connections among these subgroups were provided by analysis of its HMBC and NOESY spectra. The HMBC correlations were observed for styrylamine proton at δ_H 8.35 (NH-3) to C-5 (δ_C 60.5) of isoleucyl carbonyl C-4 (δ_C 167.1) together with correlations of H-2 (δ_H 6.91) to C-1, C-4 and C-13, and H-1 (δ_H 5.91) to C-2, C-12 and C-14 indicating that isoleucine amino acid was attached to the styrylamine unit. HMBC correlations from H-5, H-8 and H-9 to 3-oxygenated proline carbonyl C-7 (δ_C 170.2) confirmed the connection between the isoleucine and β-OH-proline amino acids. A strong NOE effects displayed in the NOESY spectrum between the proline proton at δ_H 3.79 (H-22) and H-25 (δ_H 5.09) of tryptophan unit, but no HMBC correlation observed between these two amino acids. In addition, the latter proton H-25 showed HMBC cross peaks to the carbonyl of leucine at δ_C 174.7 (C-37) confirmed the tryptophan as the intermediate side chain amino acid connected between the leucine terminal amino acid, and the β-OH-proline of the macro molecule. Furthermore, the HMBC correlations of the leucyl proton NH-36 to C-37, and of H-38 to C-37, C-39, C-40 and *N*-methyl carbon, along with an intense peak at *m/z* 100 observed in its ESI mass spectrum also supported the *N*-methyl leucine as the end amino acid. The *Z*-geometry of 1,2 double bond was established on the basis of the coupling constant value of 9.0 Hz for H-1 and H-2 (Suksamrarn; & 2005: 1175-1180). The coupling constant value of 11.2 Hz between H-2 and NH-3 protons and a small NOESY interaction observed among them indicated *trans* coplanar of these two protons (Suksamrarn; et al. 2005: 1175-1180, Lee; Su; & Liu. 2001: 1271-1276). The NH-6 and H-5 protons showed the coupling constant of 6.0 Hz and a small NOE cross peak was observed between both protons suggesting they were in the opposite orientation. In contrast, strong NOESY interaction observed between NH-6 and H-8 allowing these both protons resided on the same side. Strong NOE enhancement between H-3 and the aromatic proton H-12, and the latter with H-9 were observed in NOESY spectrum. The small vicinal coupling constant

value 3.2 Hz of the methine protons H-8 and H-9 indicated a *trans* configuration together with no significant NOE observed between these two protons in its NOESY spectrum further supported the *trans* relationship between H-8 and H-9. Strong NOESY correlations were observed between H-9 and H-21β but not with H-21α indicating that H-9 and H-21β were on the same side of the pyrrolidine ring.

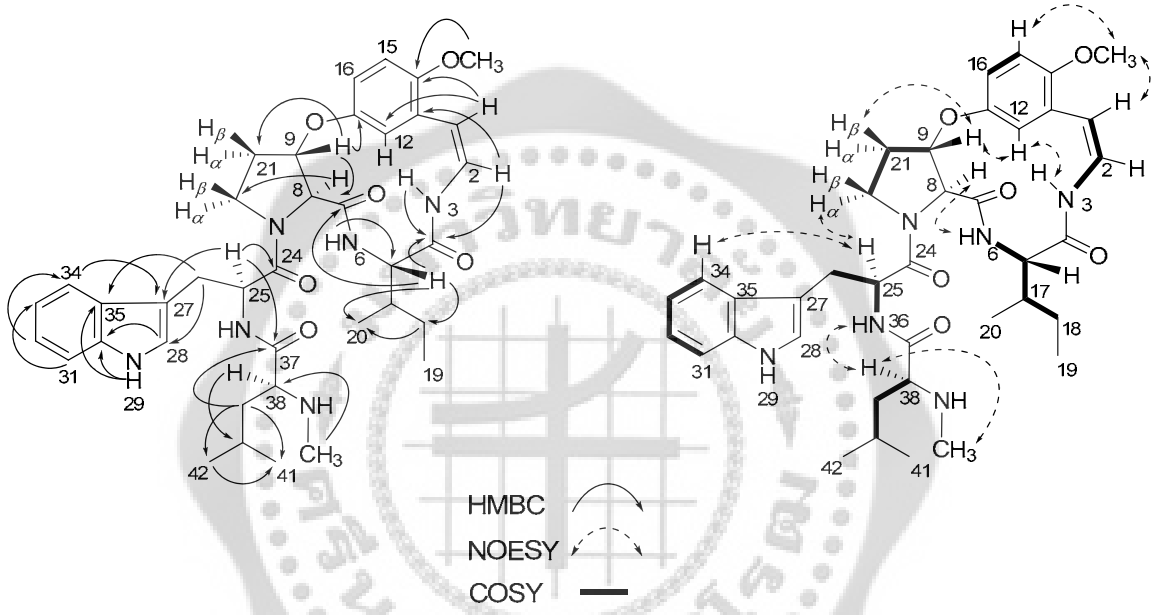


FIGURE 25 Selected HMBC, COSY and NOESY correlations for compound ZM-M2

TABLE 16 ^{1}H, ^{13}C NMR and 2D NMR data of compound ZM-M2 in $CDCl_3$

position	δ_H	δ_C	HMBC correlations	NOESY correlations
1	5.91 *d* (*J* = 9.0 Hz)	107.2	C-2, C-12, C-14	OMe, H-2
2	6.91 *dd* (*J* = 11.2, 9.0 Hz)	121.3	C-1, C-4, C-13	H-1
NH-3	8.35 *d* (*J* = 11.2 Hz)	-	C-1, C-2, C-4	H-12
CO-4	-	167.2	-	-
5	4.23 *t* (*J* = 4.5 Hz)	60.5	C-4, C-7, C-17, C-18, C-20	NH-3 (small), H-20
NH-6	7.25 *d* (*J* = 6.0 Hz)	-	C-4, C-5, C-7, C-17	H-8
CO-7	-	170.2	-	-
8	4.38 *d* (*J* = 3.2 Hz)	64.3	C-7, C-9, C-21, C-22	NH-6
9	5.31 *dt* (*J* = 7.2, 3.2 Hz)	76.4	C-7, C-11, C-21	H-12, H-21β
11		151.9	-	-
12	6.61 *d* (*J* = 2.9 Hz)	111.2	C-1, C-11, C-14, C-16	NH-3, H-9
13	-	124.1	-	-
14	-	151.4	-	-
15	6.84 *d* (*J* = 9.0 Hz)	113.7	C-11, C-13, C-14, C-16	OMe
16	6.74 *dd* (*J* = 9.0, 2.9)	117.6	C-11, C-12, C-14	-
17	2.00 *m*	35.4	-	-
18	α 1.43 *m*, β 1.15 *m*	24.6	C-19, C-20	-
19	0.87 *t* (*J* = 7.3 Hz)	11.7	C-17, C-18	-
20	0.96 *d* (*J* = 6.9 Hz)	16.0	C-5, C-17, C-18	-
21	α 2.08 *m*, β 2.25 *m*	32.3	C-8, C-9, C-22	-
22	α 3.79 *m*, β 2.46 *m*	46.2	C-9, C-21	H-21α, H-25
CO-24	-	171.3	-	-
25	5.09 *ddd* (*J* = 8.4, 8.0, 5.1 Hz)	50.3	C-24, C-26, C-27, C-37	H-22α, H-34
26	3.21 *dd* (*J* = 14.0, 5.1 Hz)	39.3	C-24, C-25, C-27, C-28, C-35	-
27	-	109.3	-	-
28	ca 6.76 obscured signal	122.9	C-26, C-27, C-30, C-35	H-29
NH-29	8.58 *br s* 1H	-	C-27, C-28, C-30, C-35	H-28, H-31
30	-	136.0	-	-
31	7.31 *d* (*J* = 7.8 Hz)	111.4	C-33, C-35	H-29
32	7.15 *br t* (*J* = ca 7.6 Hz)	122.1	C-30, C-31, C-33, C-34	-
33	7.09 *br t* (*J* = ca 7.3 Hz)	119.6	C-31, C-32, C-34, C-35	-
34	7.65 *d* (*J* = 7.5 Hz)	118.4	C-27, C-30, C-32, C-35	H-25
35	-	127.2	-	-
NH-36	7.86 *d* (*J* = 8.0 Hz)	-	C-25, C-37	H-25, H-38
37	-	174.0	-	-
38	3.06 *t* (*J* = 8.5 Hz)	63.1	C-37, C-39, C-40, NMe	NH-36, H-41, H-42, NMe
39	α 1.56 *m*, β 1.41 *m*	42.3	C-37, C-38, C-40, C-41, C-42	-
40	1.63 *m*	25.0	C-38, C-39, C-41, C-42	-
41	0.90 *d* (*J* = 7.0 Hz)	21.9	C-39, C-40, C-42	-
42	0.92 *d* (*J* = 7.0 Hz)	23.0	C-39, C-40, C-41	-
NMe	2.37 *s*	35.0	C-38	H-38
OMe	3.75 *s*	55.9	C-14	H-1, H-15

TABLE 17 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-M2 with paliurine G (**65**)

position	δ_H		δ_C	
	ZM-M2	paliurine G (**65**)	ZM-M2	paliurine G
1	5.91 *d* (*J* = 9.0 Hz)	5.92 *d* (*J* = 9.1 Hz)	107.2	106.6
2	6.91 *dd* (*J* = 11.2, 9.0 Hz)	6.93 *dd* (*J* = 11.3, 9.1 Hz)	121.3	121.5
NH-3	8.35 *d* (*J* = 11.2 Hz)	8.45 *d* (*J* = 11.3 Hz)	-	-
CO-4	-	-	167.2	167.0
5	4.23 *t* (*J* = 4.5 Hz)	4.26 *dd* (*J* = 5.0, 4.5 Hz)	60.5	60.3
NH-6	7.25 *d* (*J* = 6.0 Hz)	7.14 *d* (*J* = 4.5 Hz)	-	-
CO-7	-	-	170.2	170.2
8	4.38 *d* (*J* = 3.2 Hz)	4.42 *d* (*J* = 3.2 Hz)	64.3	64.4
9	5.31 *dt* (*J* = 7.2, 3.2 Hz)	5.52 *dt* (*J* = 7.4, 3.2 Hz)	76.4	76.7
11			151.9	151.0
12	6.61 *d* (*J* = 2.9 Hz)	6.69 *d* (*J* = 2.9 Hz)	111.2	111.2
13	-	-	124.1	124.2
14	-	-	151.4	151.4
15	6.84 *d* (*J* = 9.0 Hz)	6.89 *d* (*J* = 9.1 Hz)	113.7	113.8
16	6.74 *dd* (*J* = 9.0, 2.9)	6.82 *dd* (*J* = 9.1, 2.9 Hz)	117.6	117.7
17	2.00 *m*	2.08	35.4	35.1
18	1.43 *m*, 1.15 *m*	α 1.35, β 1.10	24.6	24.4
19	0.87 *t* (*J* = 7.3 Hz)	0.86 *t* (*J* = 6.9 Hz)	11.7	11.7
20	0.96 *d* (*J* = 6.9 Hz)	0.94 *d* (*J* = 6.9 Hz)	16.0	16.2
21	α 2.08 *m*, β 2.25 *m*	α 2.10 *m* , β 2.60 *m*	32.3	32.5
22	α 3.79 *m*, β 2.46 *m*	α 4.28 *dt* (*J* = 8.8, 2.7 Hz) β 3.55 *dt* (*J* = 10.7, 6.6)	46.2	46.7
CO-24	-	-	171.3	171.5
25	5.09 *ddd* (*J* = 8.4, 8.0, 5.1	4.49 *dd* (*J* = 8.8, 7.7 Hz)	50.3	54.9
26	3.21 *dd* (*J* = 14.0, 5.1 Hz)	1.93 *d sept* (*J* = 8.8, 6.7 Hz)	39.3	31.4
	ca 3.02 obscured signal			
27	-	0.83 *d* (*J* = 6.7 Hz)	109.3	19.1
28	ca 6.76 obscured signal	0.86 *d* (*J* = 6.7 Hz)	122.9	18.1
NH-29	8.58 *br s* 1H	7.26 *d* (*J* = 7.7 Hz)	-	
30	-		136.0	172.4
31	7.31 *d* (*J* = 7.8 Hz)	3.29 *dd* (*J* = 6.9, 6.3 Hz)	111.4	71.0
32	7.15 *br t* (*J* = ca 7.6 Hz)	3.17 *dd* (*J* = 13.9, 6.9 Hz)	122.1	32.9
33	7.09 *br t* (*J* = ca 7.3 Hz)		119.6	139.7
34	7.65 *d* (*J* = 7.5 Hz)	7.25 *m*	118.4	129.1
35		7.25 *m*	127.2	128.3
36	7.86 *d* (*J* = 8.0 Hz)	7.25 *m*	-	126.1
37		-	174.0	-
38	3.06 *t* (*J* = 8.5 Hz)	-	63.1	-
39	α 1.56 *m*, β 1.41 *m*	-	42.3	-
40	1.63 *m*	-	25.0	-
41	0.90 *d* (*J* = 7.0 Hz)	-	21.9	-
42	0.92 *d* (*J* = 7.0 Hz)	-	23.0	-
NMe	2.37 *s*	2.30 *s*	35.0	42.3
OMe	3.75 *s*	3.78 *s*	55.9	56.0

TABLE 18 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-M2 with mauritine J (**52**)

position	δ_H		δ_C	
	ZM-M2	mauritine J (**52**)	ZM-M2	mauritine J
1	5.91 *d* (*J* = 9.0 Hz)	6.30 *d* (*J* = 7.7 Hz)	107.2	114.5
2	6.91 *dd* (*J* = 11.2, 9.0 Hz)	6.73 *dd* (*J* = 10.5, 7.7 Hz)	121.3	125.4
NH-3	8.35 *d* (*J* = 11.2 Hz)	6.51 *d* (*J* = 10.5 Hz)	-	-
CO-4	-	-	167.2	166.9
5	4.23 *t* (*J* = 4.5 Hz)	4.20 *dd* (*J* = 8.5, 3.0 Hz)	60.5	59.0
NH-6	7.25 *d* (*J* = 6.0 Hz)	6.58 (*d, J* = 8.5 Hz)	-	-
CO-7	-	-	170.2	170.5
8	4.38 *d* (*J* = 3.2 Hz)	4.25 *d* (*J* = 5.5 Hz)	64.3	63.9
9	5.31 *dt* (*J* = 7.2, 3.2 Hz)	5.43 *m*	76.4	83.4
11		-	151.9	157.4
12	6.61 *d* (*J* = 2.9 Hz)	7.18 *m*	111.2	122.6
13	-	7.05 *m*	124.1	130.1
14	-	-	151.4	132.5
15	6.84 *d* (*J* = 9.0 Hz)	7.09 *m*	113.7	132.4
16	6.74 *dd* (*J* = 9.0, 2.9)	7.24 *m*	117.6	122.3
17	2.00 *m*	2.22 *m*	35.4	35.2
18	1.43 *m*, 1.15 *m*	1.30 *m*	24.6	23.7
19	0.87 *t* (*J* = 7.3 Hz)	0.88 *t* (*J* = 6.5 Hz)	11.7	12.2
20	0.96 *d* (*J* = 6.9 Hz)	0.82 *d* (*J* = 7.0 Hz)	16.0	16.0
21	α 2.08 *m*, β 2.25 *m*	α 2.00 *m*, β 2.32 *m*	32.3	31.8
22	α 3.79 *m*, β 2.46 *m*	α 3.79 *dd* (*J* = 11.5, 8.3 Hz), β 2.50 *m*	46.2	46.4
CO-24	-	-	171.3	171.4
25	5.09 *ddd* (*J* = 8.4, 8.0, 5.1 Hz)	5.00 *ddd* (*J* = 8.5, 8.0, 6.2 Hz)	50.3	50.0
26	3.21 *dd* (*J* = 14.0, 5.1 Hz)	3.13 *dd* (*J* = 14.5, 6.2 Hz)	39.3	29.3
27	-	-	109.3	110.0
28	ca 6.76 obscured signal	6.76 *d* (*J* = 2.5 Hz)	122.9	122.7
NH-29	8.58 *br s*	8.12 *br s*	-	
30	-	-	136.0	135.9
31	7.31 *d* (*J* = 7.8 Hz)	7.31 *d* (*J* = 8.0 Hz)	111.4	111.2
32	7.15 *br t* (*J* = ca 7.6 Hz)	7.10 *m*	122.1	122.8
33	7.09 *br t* (*J* = ca 7.3 Hz)	7.12 *m*	119.6	119.8
34	7.65 *d* (*J* = 7.5 Hz)	7.68 *d* (*J* = 8.0 Hz)	118.4	118.5
35	-	-	127.2	127.4
NH-36	7.86 *d* (*J* = 8.0 Hz)	7.73 *d* (*J* = 8.5 Hz)	-	-
37	-	-	174.0	174.8
38	3.06 *t* (*J* = 8.5 Hz)	2.98 *dd* (*J* = 9.2, 5.0 Hz)	63.1	63.3
39	α 1.56 *m*, β 1.41 *m*	α 1.42 *m*, β 1.25 *m*	42.3	42.5
40	1.63 *m*	1.59 *m*	25.0	25.0
41	0.90 *d* (*J* = 7.0 Hz)	0.87 *d* (*J* = 6.5 Hz)	21.9	21.8
42	0.92 *d* (*J* = 7.0 Hz)	0.89 *d* (*J* = 6.5 Hz)	23.0	23.1
NMe	2.37 *s*	2.38 *s*	35.0	35.4
OMe	3.75 *s*	-	55.9	-

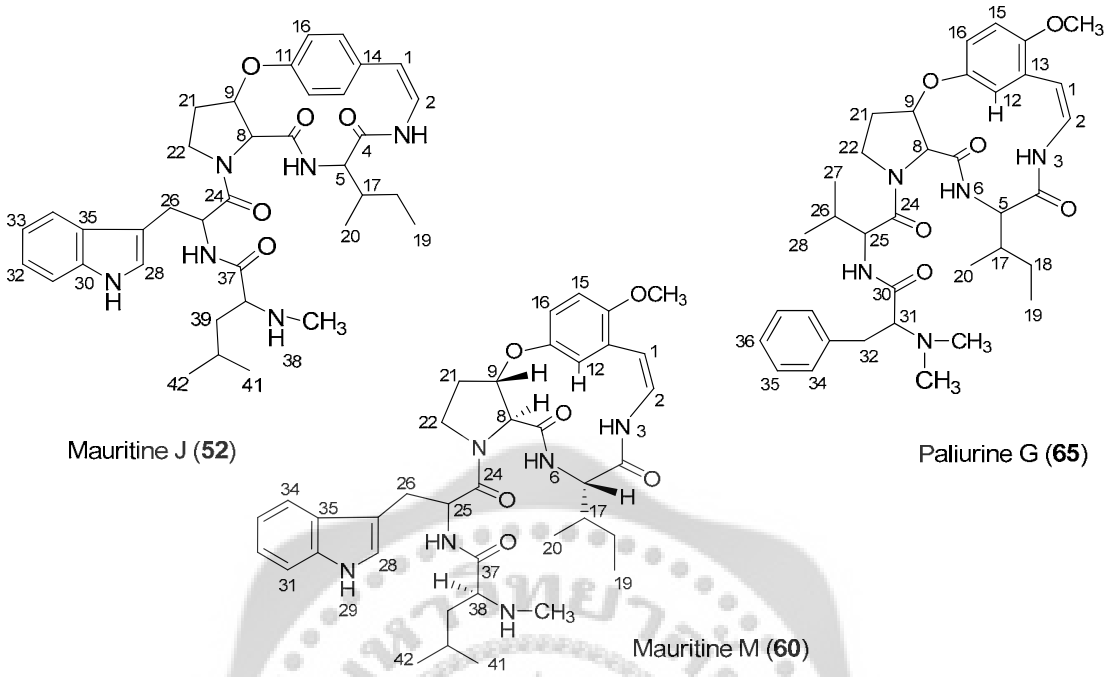


FIGURE 26 Structures of mauritine J, paliurine G and mauritine M

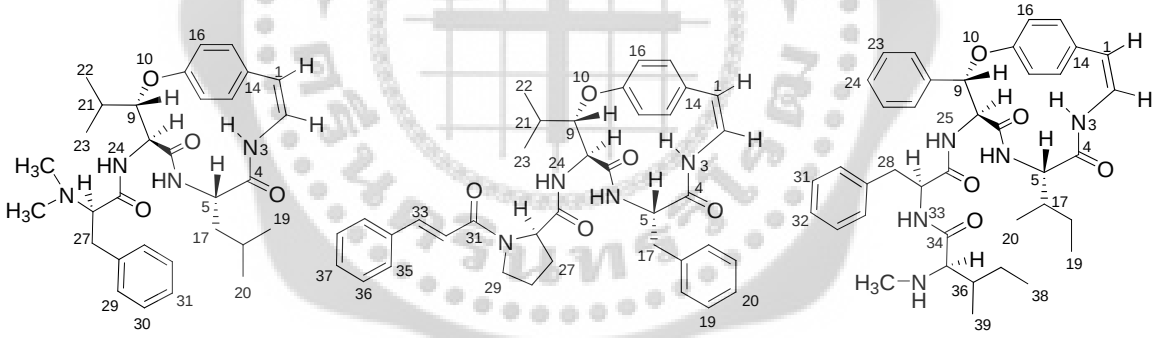
The CD spectrum of compound ZM-M2 (319 (-13.00), 264 (-27.58) and 217 (-21.81) nm) was similar to that of paliurine G (**65**) which constructed with the same amino acid units for the cyclic part, suggested the 5*S*, 8*S* and 9*S* configurations for the macrocyclic unit (Lee; Su; & Liu. 2001: 1271-1276). The ^{1}H and ^{13}C NMR chemical shift together with the coupling pattern of H-8/H-9 and H-5/H-6 for compound ZM-M2 followed the similar values as that of paliurine G (**65**) (Lee; Su; & Liu. 2001: 1271-1276), thus compound ZM-M2 should have the same *S*-configuration at the position C-5, C-8 and C-9 (TABLE 17). ^{1}H and ^{13}C NMR data (TABLE 18) of the acyclic part of ZM-M2 which attached to the macrocyclic ring at N-22, were very similar to that of mauritine J (**52**), a 14-membered ring cyclopeptide constructed with the same amino acid units (i.e. tryptophan as the intermediate and leucine as the end amino acids) connected to the macrocyclic ring and had L configuration (Jossang; Zahir; & Diakite. 1996: 565-657). The stereochemistry of these two acyclic amino acid units in compound ZM-M2 was thus tentatively inferred to be L. Therefore, compound ZM-M2 is a new cyclopeptide and named mauritine M (**60**) after its plant origin as shown in FIGURE 25.

CHAPTER 5

CONCLUSION

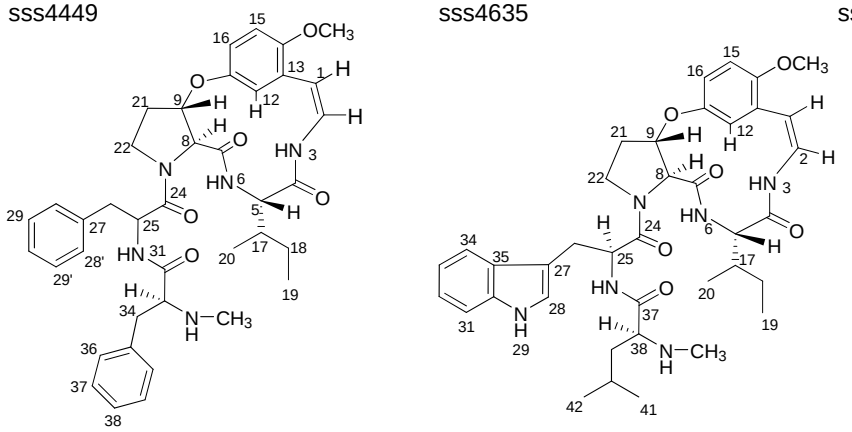
Phytochemical investigation of the root of *Z. cambodiana* led to the isolation of one new 5(14)-scutianine A-type cyclopeptide alkaloid, named cambodine (**58**). In addition, two known cyclopeptides as 4(14)-frangulanine-type cyclopeptide alkaloid, frangufoline (**6**) and neutral compound related to 14-membered cyclopeptide alkaloids, lotusanine B (**24**).

Investigation of the chemical constituents of the stem bark of *Z. mauritiana* led to the isolation of the 5(13)-zizyphine A-type with one new cyclopeptide alkaloid, named mauritine M (**60**) together with one known alkaloid, nummularine H (**59**), from MeOH extract. The structure of the new cyclopeptide alkaloid was elucidated by spectroscopic techniques, whilst the known compounds were identified by comparisons of spectroscopic data with those of reported values and chromatographic comparison with an authentic sample in several solvent systems.



ZC-M1 or Frangufoline (**6**) ZC-M2 or Lotusanine B (**24**) ZC-M3 or Cambodine (**58**)

sss4449 sss4635 sss4402



ZM-M1 or Nummularine H (**59**), sss4971 ZM-M2 or Mauritine M (**60**), sss4973

FIGURE 27 Cyclopeptide alkaloids from selected Thai *Ziziphus* plants

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APPENDIX

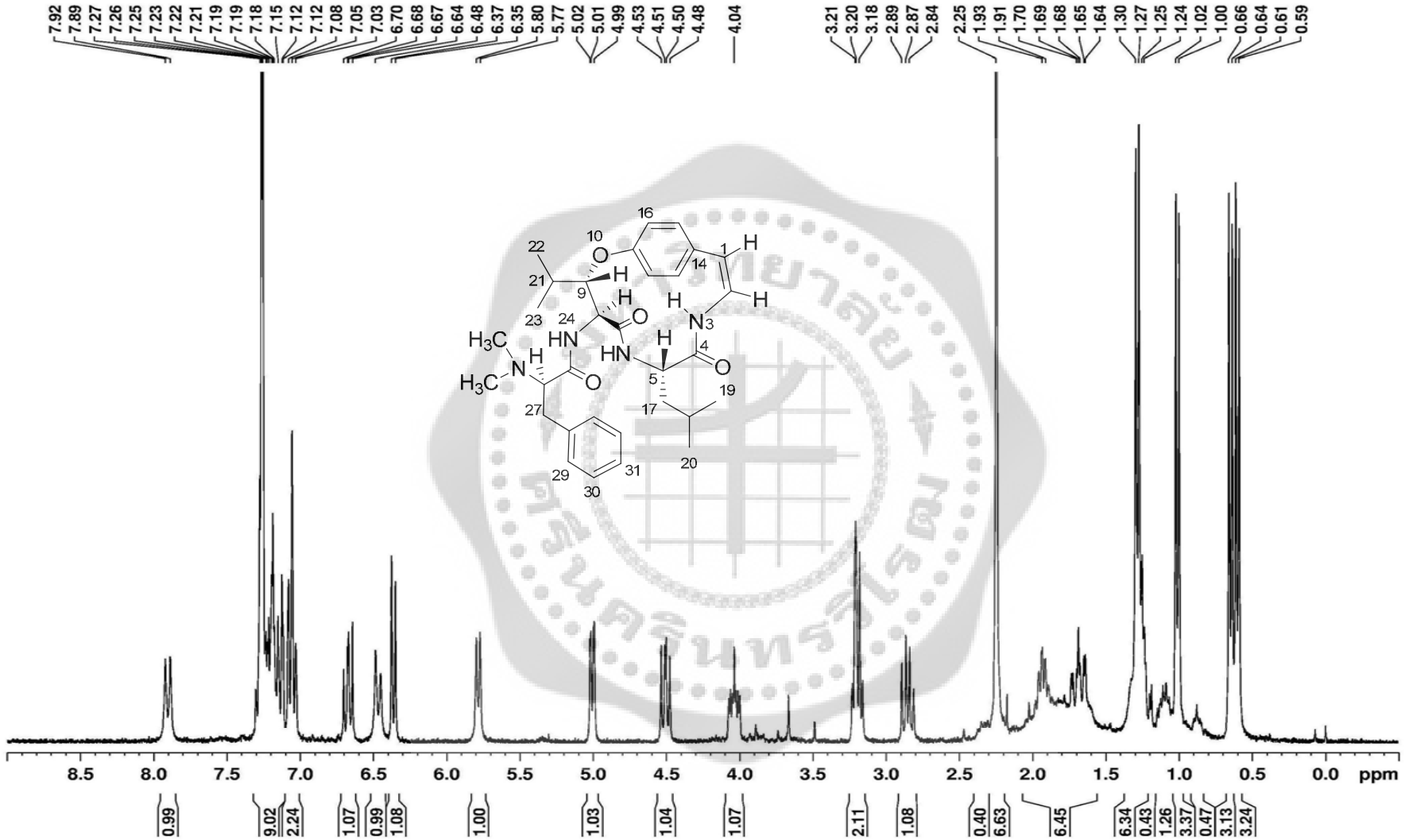

FIGURE 28 ^{1}H NMR of compound ZC-M1 (frangufoline (**6**), sss4449) in $CDCl_3$

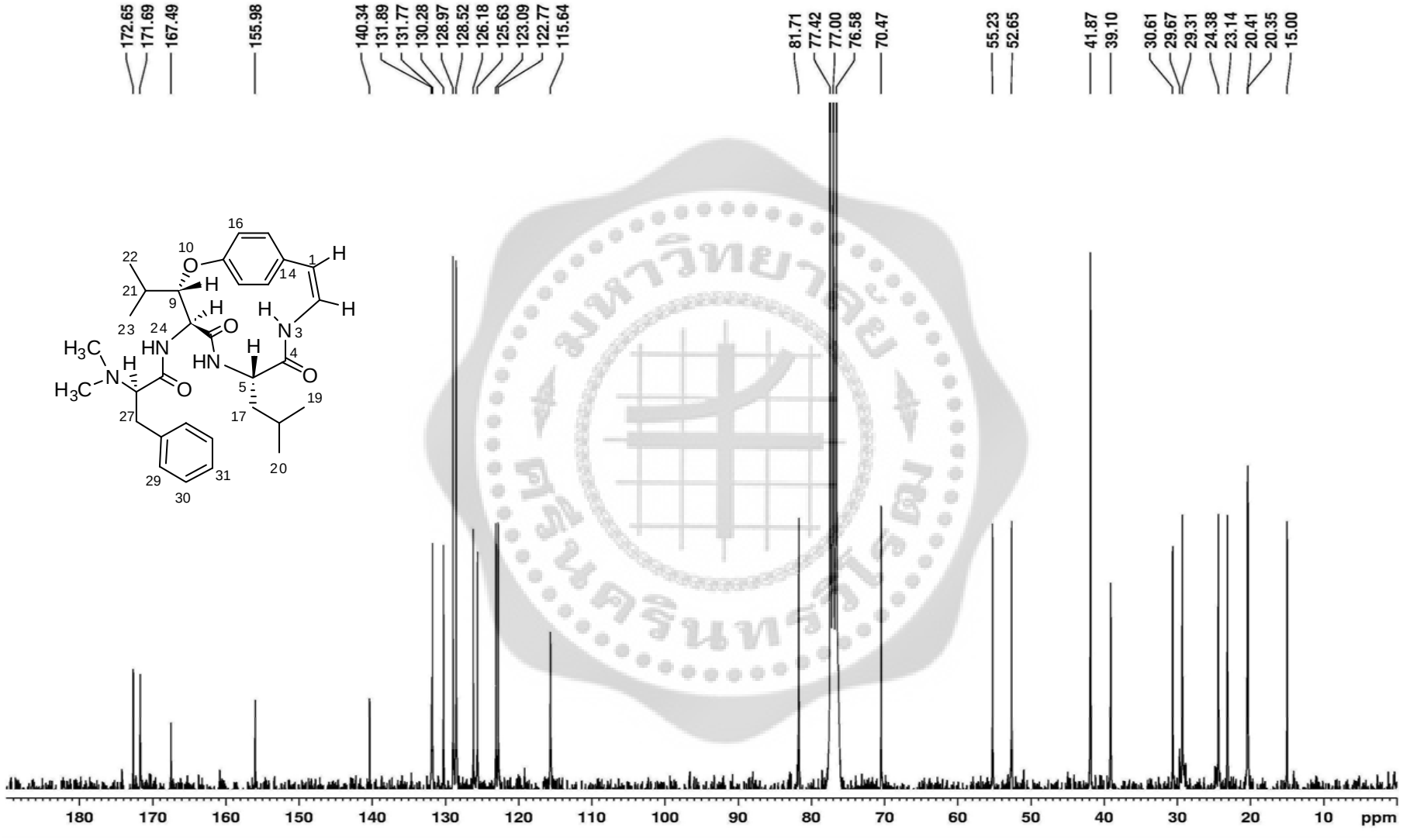

FIGURE 29 ^{13}C NMR of compound ZC-M1 (frangufoline (**6**), sss4449) in $CDCl_3$

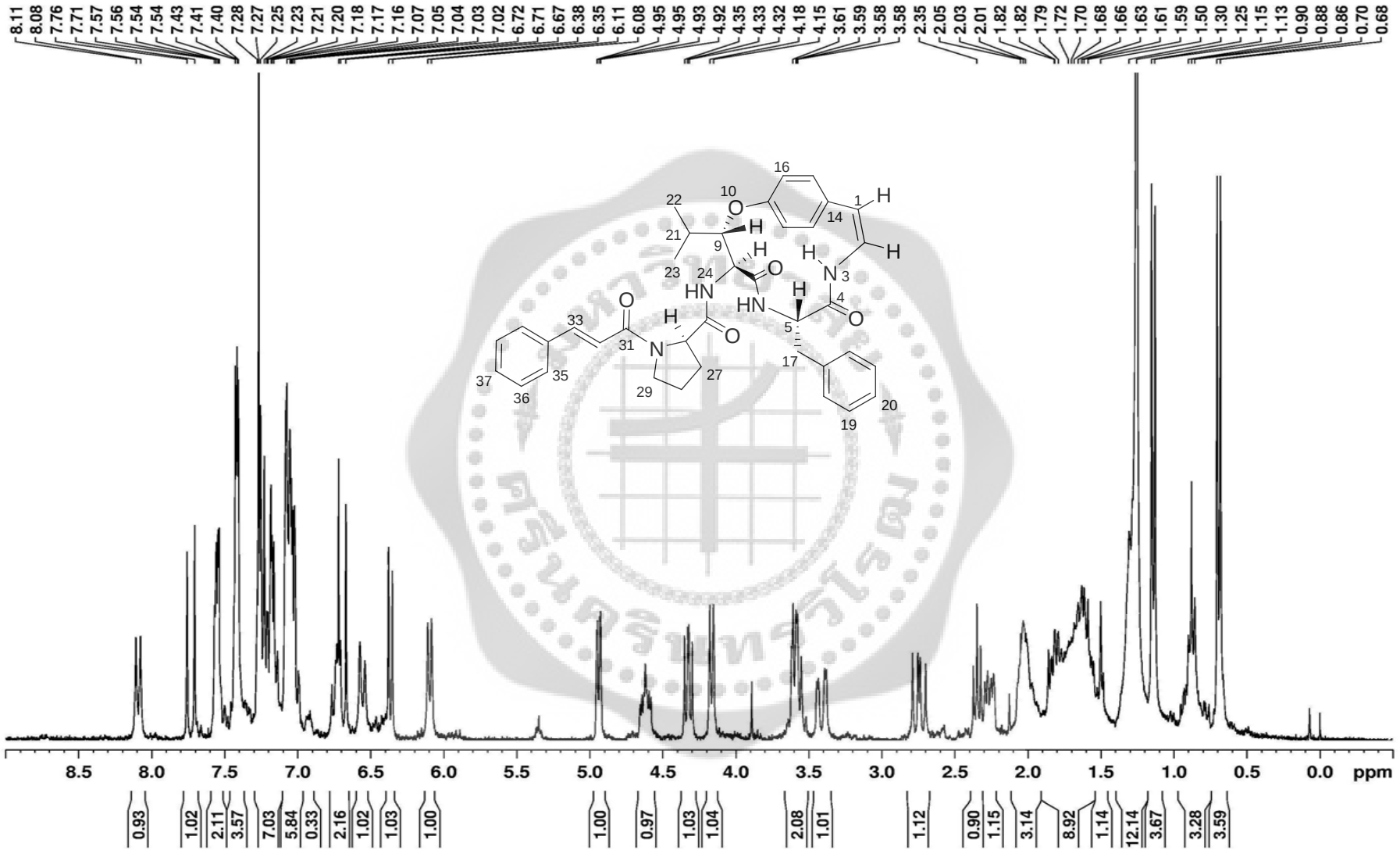

FIGURE 30 ^{1}H NMR of compound ZC-M2 (lotusanine B (**24**), sss4635) in $CDCl_3$

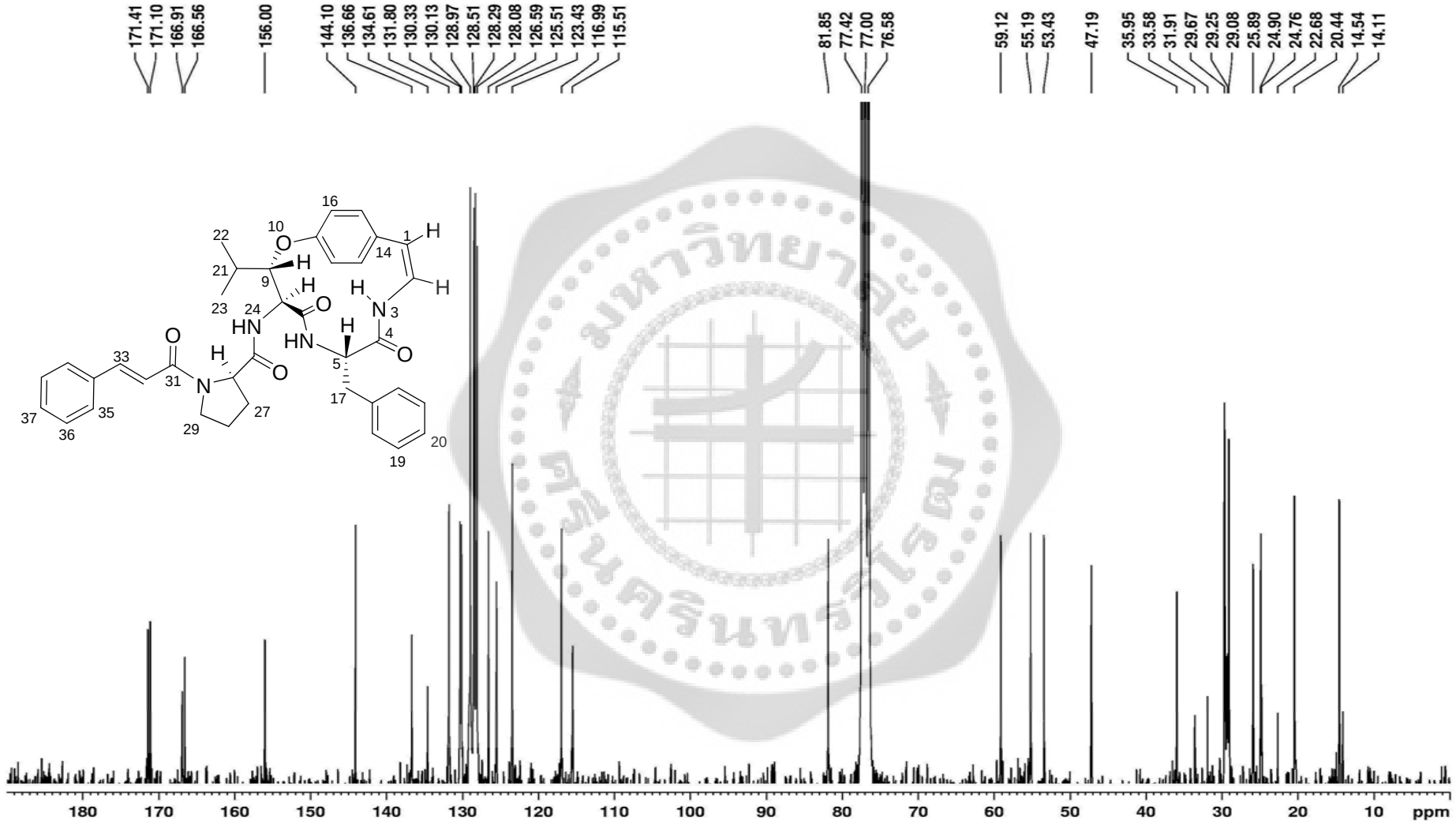

FIGURE 31 ^{13}C NMR of compound ZC-M2 (lotusanine B (**24**), sss4635) in $CDCl_3$

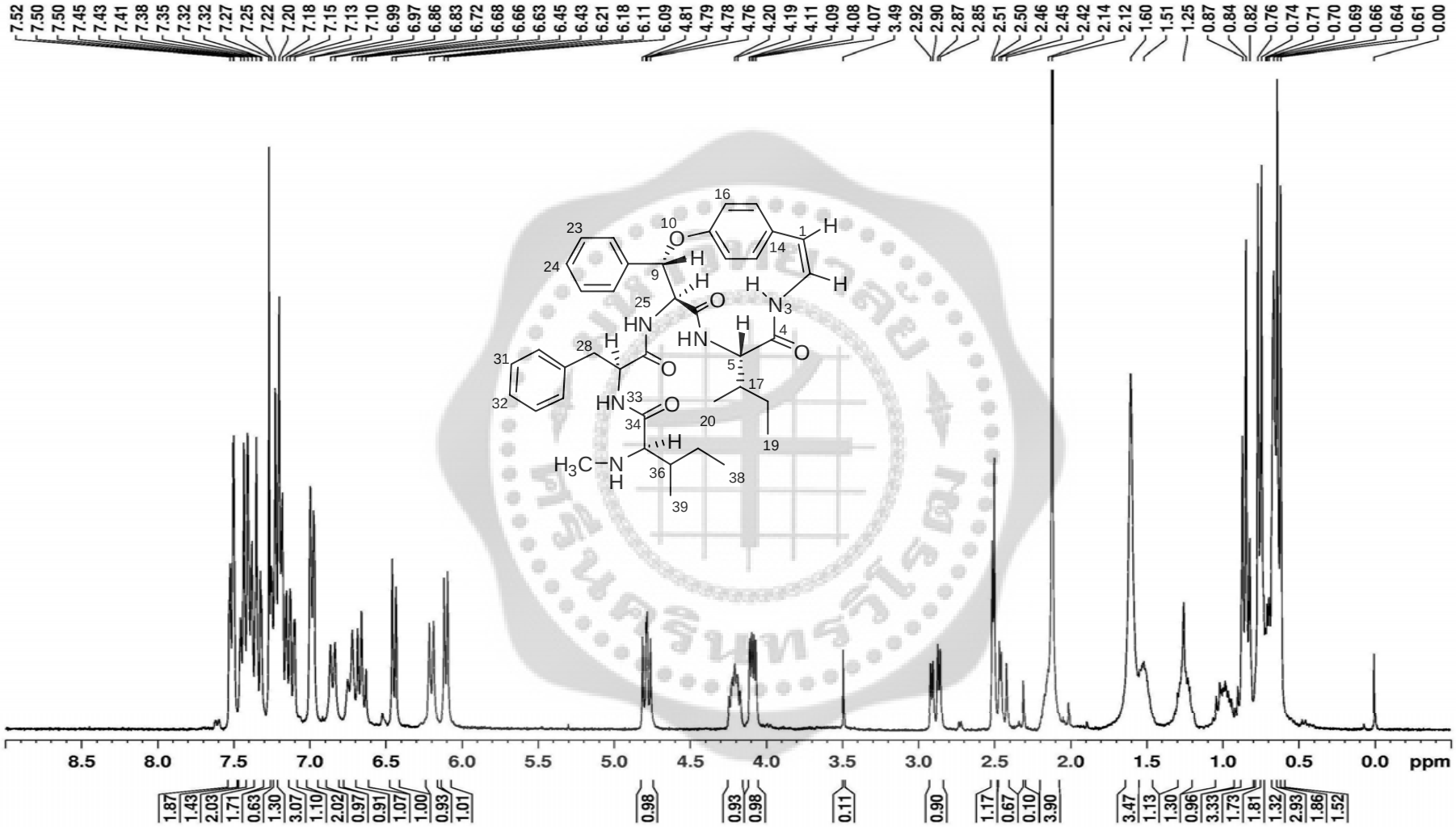

FIGURE 33 ^{1}H NMR of compound ZC-M3 (cambodine (**58**), sss4402) in $CDCl_3$

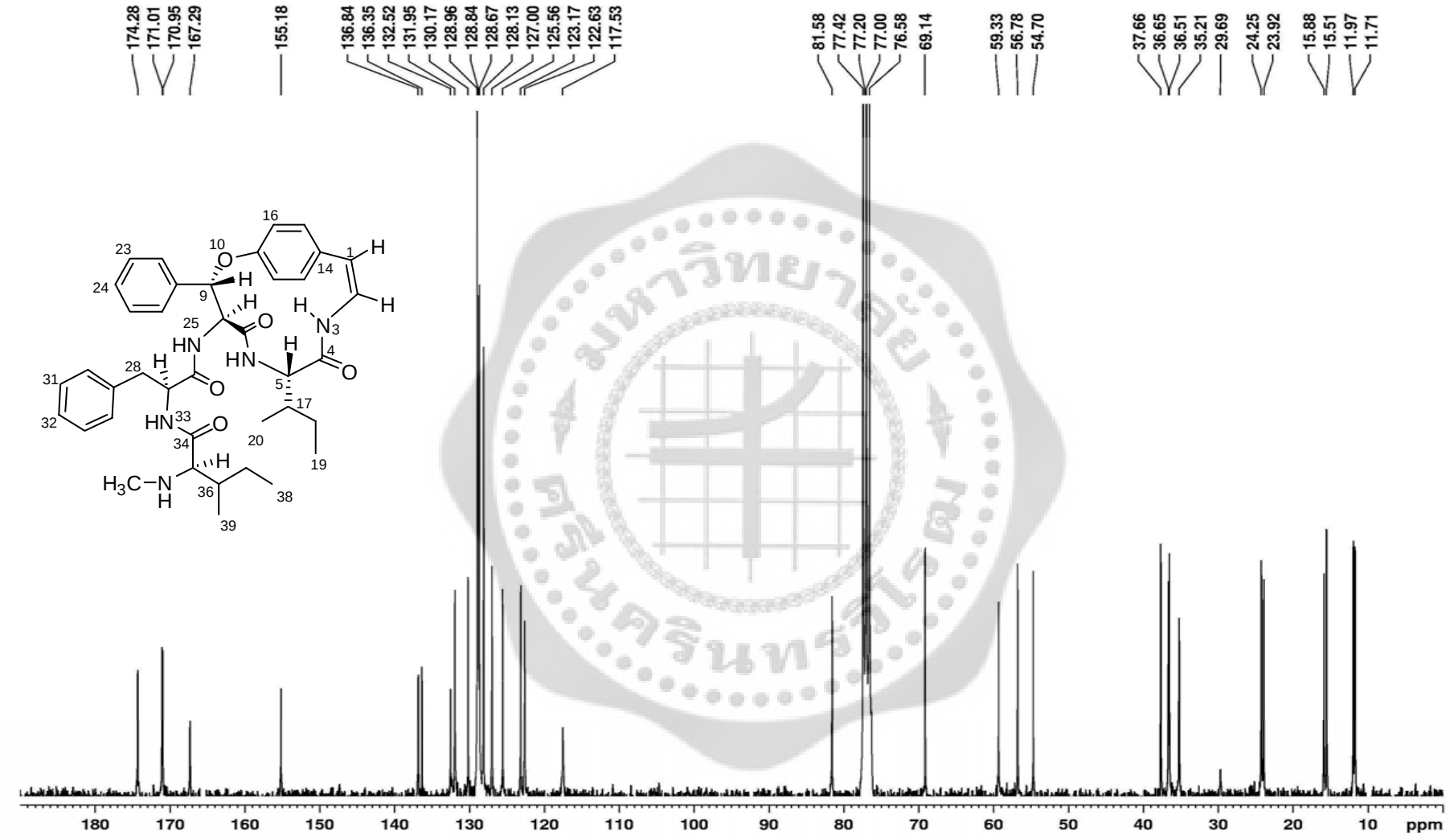

FIGURE 33 ^{13}C NMR of compound ZC-M3 (cambodine (**58**), sss4402) in $CDCl_3$

GLOSSARY

LIST OF ABBREVIATIONS AND SYMBOLS

$[\alpha]_D^{27}$

Specific rotation at 27° C and sodium D line

δ

Chemical shift (for NMR data)

ε

Molar absorptivity

μL

Microliter

μM

Micromolar

λ_{max}

Wavelength at maximal absorption

ν_{max}

Wave number at maximal absorption

$[M+H]^+$

Protonated molecular ion

^{13}C NMR

13-Carbon Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR

Proton Nuclear Magnetic Resonance Spectroscopy

^{1}H-^{1}H COSY

Homonuclear (Proton-Proton) Correlation Spectroscopy

ara-(1-2)-rha

Arabinosyl-(1→2)-α-L-rhamnoside

br d

Broad doublet (for NMR data)

br s

Broad singlet (for NMR data)

br t

Broad triplet (for NMR data)

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

calcd

Calculated

C_6H_6

benzene

CC

Column chromatography

$CDCl_3$

Deuterated chloroform

CH_2Cl_2

Dichloromethane

$CHCl_3$

Chloroform

cm

Centimeter

cm^{-1}

Reciprocal centimeter (unit of wave number)

d

Doublet (for NMR data)

dd

Doublet of doublets (for NMR data)

ddd

Double doublet of doublets (for NMR data)

DEPT

Distortionless Enhancement by Polarization Transfer

dq

Doublet of quartets (for NMR data)

dt

Doublet of triplets (for NMR data)

EIMS

Electron Impact Ionization Mass Spectrometry

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

ESIMS

Electrospray Ionization Mass Spectrometry

EtOAc

Ethyl acetate

g

Gram

Glc

Glucoside

h

Hour

H_2O

Water

HMBC

^{1}H-Detected Heteronuclear Multiple Bond Coherence

HMQC

^{1}H-Detected Heteronuclear Multiple Quantum Coherence

HRTOFMS

High Resolution Time of Flight Mass Spectrometry

Hz

Hertz

IC_{50}

50% Inhibitory Concentration

IR

Infrared

J

Coupling constant

KBr

Potassium bromide

kg

Kilogram

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

L

Liter

m

Multiplet (for NMR data)

MeOH

Methanol

mg

Milligram

MIC

Minimum Inhibitory Concentration

mL

Milliliter

mm

Millimeter

NMe

N-methyl

NMe_2

N,N-dimethyl

NMR

Nuclear Magnetic Resonance Spectroscopy

NOESY

Nuclear Overhauser Effect Spectroscopy

°C

Degree Celsius

QCC

Quick column chromatography

Rha

Rhamnoside

s

Singlet (for NMR data)

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

sh

shoulder

t

Triplet (for NMR data)

TLC

Thin Layer Chromatography

UV

Ultraviolet

α

Alpha

β

Beta

CURRICULUM VITAE

CURRICULUM VITAE

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Panseeta, P.; **Lomchoey, K.**; Prabpai, S.; Kongsaeree, P.; Suksamrarn, A.; Ruchirawat, S.; Kongsaeree, P.; Suksamrarn, A.; Ruchirawat, S.; Suksamrarn, S. (2011). Antiplasmodial and antimycobacterial cyclopeptide alkaloids from the root of *Ziziphus mauritiana*. *Phytochemistry* 72: 909-915.

Scholarships :

2009-2010 The Center of Excellence for Innovation in Chemistry (PERCH-CIC)

2011 Research assistantship

Oral presentation :

Lomchoey, N.; Panseeta, P.; Prabpai, S.; Kongsaeree, P.; Suksamrarn, A.; Ruchirawat, S.; Suksamrarn, S. New Cyclopeptide Alkaloids with Antiplasmodial and Antimycobacterial Activities from the Root of *Ziziphus mauritiana* Lam. International

Congress for Innovation in Chemistry (PERCH-CIC Congress VII), Jomtien Palm Beach Hotel & Resort, Thailand, May 4-7, 2011. Presentation number S2A-08.

Poster presentations :

1. **Lomchoey, K.**; Panseeta, P.; Thitivorn, Y.; Kongsomboon, P.; Suksamrarn, A.; Suksamrarn, S. Cyclopeptide alkaloids from *Ziziphus cambodiana* Pierre. Pure and Applied Chemistry International Conference 2011. Miracle Grand Hotel, Thailand, January 5-7, 2011. Poster number OM_O0062.
2. Panseeta, P.; **Lomchoey, K.**; Prabpai, S.; Kongsaeree, P.; Suksamrarn, A.; Ruchirawat, S.; Suksamrarn, S. Antiplasmodial and antimycobacterial cyclopeptide alkaloids from the root of *Ziziphus mauritiana* Lam. Pure and Applied Chemistry International Conference 2011. Miracle Grand Hotel, Thailand, January 5-7, 2011. Poster number OM_O0055.