PHYTOCHEMICALS OF *ZIZIPHUS CAMBODIANA* PIERRE STEM BARKS

A THESIS

BY

AUDCHARA SAENKHAM

Presented in Partial Fulfillment of the Requirements for the

Master of Science Degree in Chemistry

at Srinakharinwirot University

May 2015

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AN ABSTRACT

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Audchara Saenkham. (2015). Phytochemicals of *Ziziphus cambodiana* Pierre stem barks. Thesis, M.Sc. (Chemistry). Bangkok: Graduate School, Srinakharinwirot University. Advisor Committee: Assoc. Prof. Dr. Sunit Suksamrarn,
Asst. Prof. Dr. Maneekarn Namsa-aid.

From the EtOAc extract obtained from the stem barks of *Ziziphus cambodiana* Pierre, a new triterpene, methyl 3-*O*-protocatechuoylceanothate, along with three known triterpenes and one known phenolic derivative were isolated and identified as betulinic acid, lupeol, ceanothic acid and *p*-hydroxybenzoic acid. Moreover, a new cyclopeptide alkaloid of the 5(14)-scutianine A-type, cambodianine, and a known cyclopeptide of the 5(13)-ziziphine A-type, ziziphine A, were obtained from the MeOH extract. The structures of the known compounds were made by comparison their ^{1}H-NMR and other physical data with the reported values. The structures and stereochemistry of the new compounds were elucidated mainly on the basis of extensive 1D- and 2D-NMR and mass spectroscopic data analysis including by comparison with other related compounds of known stereochemistry. In addition, the stereochemical assignments of cyclopeptides were also established by using CD spectral analysis.

พฤกษเคมีของเปลือกต้นตะครอง

บทคัดย่อ

ของ

อัจฉรา แสนคำ

เสนอต่อบัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ เพื่อเป็นส่วนหนึ่งของการศึกษา
ตามหลักสูตรวิทยาศาสตรมหาบัณฑิต สาขาวิชาเคมี
พฤษภาคม 2558

อัจฉรา แสนคำ (2558). พฤกษเคมีของเปลือกต้นตะครอง. ปริญญานิพนธ์. วท.ม. (เคมี). กรุงเทพฯ: บัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ. คณะกรรมการควบคุม: รองศาสตราจารย์ ดร.สุนิตย์ สุขสำราญ, ผู้ช่วยศาสตราจารย์ ดร. มณีกานต์ น้ำสอาด

จากสารสกัดชั้น EtOAc ของเปลือกต้นตะครอง สามารถแยกสารประกอบไตรเทอร์ปีนชนิดใหม่ 1 สาร คือ methyl 3-*O*-protocatechuoylceanothate ร่วมกับสารประกอบไตรเทอร์ปีนที่มีรายงานไว้แล้ว 3 สาร และอนุพันธ์ของกรดฟีนอลิกที่มีรายงานไว้แล้ว 1 สาร คือ betulinic acid, lupeol, ceanothic acid และ *p*-hydroxybenzoic acid นอกจากนี้ยังพบสารประกอบไซโคลเปบไทด์อัลคาลอยด์ชนิด 5(14)-scutianine A ชนิดใหม่ 1 สาร คือ cambodianine และสารประกอบไซโคลเปบไทด์อัลคาลอยด์ชนิด 5(13)-ziziphine A ที่มีรายงานไว้แล้ว 1 สาร คือ ziziphine A จากสารสกัดชั้น MeOH การพิสูจน์โครงสร้างของสารที่ทราบโครงสร้างแล้วได้จากการเปรียบเทียบข้อมูล ^{1}H-NMR และข้อมูลกายภาพต่างๆ กับที่มีการรายงานไว้แล้ว ส่วนการพิสูจน์โครงสร้าง และสเตอริโอเคมีของสารชนิดใหม่ได้จากวิเคราะห์จากข้อมูล 1D-, 2D-NMR และแมสสเปกโทรสโคปีเป็นหลัก ร่วมกับการเปรียบเทียบข้อมูลที่ได้กับสารประกอบที่มีโครงสร้างใกล้เคียงกัน และทราบสเตอริโอเคมีแล้ว นอกจากนี้ยังใช้การวิเคราะห์ข้อมูล CD สเปกตรัมในการกำหนดสเตอริโอเคมีของสารประกอบไซโคลเปบไทด์

The thesis title

"Phytochemicals of *Ziziphus cambodiana* Pierre stem barks"

by

Audchara Saenkham

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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(Assoc. Prof. Dr. Somchai Santiwatanakul)

May , 2015

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Audchara Saenkham

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CHAPTER 1

INTRODUCTION

Background

Ziziphus is a genus of spiny shrubs and small trees in the bucthorn family, Rhamnaceae (Shah; et al. 1989: 25-29), a family of 58 genera and approximately 900 species worldwide (Bhattacharyya; & Johri. 1998: 326-328), particular in arid and semi-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). There are nine species of *Ziziphus* plants found in Thailand, including *Ziziphus cambodiana* Pierre (Smitinand. 2001: 564-565).

Ethnopharmacological uses

The Rhamnaceous *Ziziphus* species are medicinal plants in several countries. In China, the seeds of *Z. jujuba* Mill var. spinosa were separated and used as a sedative medicine (Cheng; et al. 2000: 8915-8920). Previous studies of the fruit and root bark extracts from *Z. spinachristi* were found to have sedative activity (Zare-Zardini; et al. 2013: 702-709) and decreased neurotransmitter (Waggas; & Al-Hasani. 2009: 263-267). In 2001, Adzu et al. has reported two species of *Z. mauritiana* and *Z. spinachristi* used as traditional medicine. The fruits are used for the treatment of cuts, fresh wounds, ulcers, dysentery, pulmonary and reducing fever. The roots are used to prevent skin diseases (Adzu; et al. 2001: 344-350) and the leaves are reputed to be the most important herbal drug for the treatment of pulmonary, liver troubles, asthma and fever (Michel. 2002: 440). The roots of *Z. nummularia* are bitter and cooling, used to cure coughs, biliousness and headache (Kirtikar; & Basu.1994: 170). The root extracts of *Z. mauritiana* exhibited antimalarial and antimycobacterial activities (Panseeta; et al. 2011: 909-915). The stems of *Z. oxyphylla* showed antibacterial and antimicrobial activities (Kaleem; et al. 2012: 11520-11529). In the Indian system of medicine, *Z. rugosa* is used for the treatment of diarrhea, menorrhagia and infection of teeth (Acharya; et al. 1988: 200-202). In Thailand, the root barks of *Z. oenoplia* have been used for the treatment of ulcers (Bunyapraphatsara; & Chokechaijaroenporn. 2000: 291-292). The spiny of *Z. cambodiana* have been used for the treatment of ulcers

(Bunyapraphatsara; & Chokechaijaroenporn. 1999: 328-329). The stems of *Z. cambodiana* are used traditionally for its antiinfectious ability (Chuakul; & Jaikaew. 1998: 28-55). This plant also commonly used in Cambodian traditional medicine for the treatment of fever (Hout; et al. 2006: 12-18).

Phytochemical studies have shown that the genus *Ziziphus* to be a rich source of cyclopeptides (Gournelis; et al. 1998: 1-179), lupane- and ceanothane-type triterpenes (Suksamrarn; et al. 2006: 535-537). As part of the ongoing research on bioactive compounds from Thai *Ziziphus*, thus *Z. cambodiana* is selected for this study to search for bioactive substances. Our investigation on the root barks of *Z. cambodiana* resulted in isolation of antimicrobial triterpenes (Suksamrarn; et al. 2006: 535-537) and seven new bioactive cyclopeptides (Suksamrarn; et al., manuscript is in preparation). In this project the stem barks of *Z. cambodiana* are selected for further study in order to search for new bioactive compounds.

Objectives of the Study

1. To isolate and purify phytochemicals from the stem barks of *Z. cambodiana* which was collected from Nang Rong district, Buriram Province, Thailand.
2. To elucidate the chemical structures of the isolated triterpenes and /or cyclopeptide alkaloids.

CHAPTER 2

REVIEW OF LITERATURE

Ziziphus cambodiana Pierre is known in Thai as Ta-khrong, other names for this plant are Mak ka than chang, Ma tan dong, Ma mak ma, Mak ma, Nam khom, Nam takhong and Ang krong (Smitinand. 2001: 564-565). *Z. cambodiana* is a thorny Rhamnaceous scandent widely distributed in the north-east of Thailand. *Ziziphus* species have been reported to possess several types of chemical constituents, especially cyclopeptide alkaloids and triterpenes. In addition, cyclopeptide macrocycles and triterpenoids from *Ziziphus* plants exhibited interesting biological properties (Panseeta; et al. 2011, 909-915).

Chemical constituents of *Ziziphus cambodiana*

In 2007, Li; et al., studied on neuraminidase inhibitory activity of the leaf and branch extracts of *Z. cambodiana.* The leaf extract showed stronger activity compared to the branch extract which led to the isolation of flavonoid glycosides, quercitrin (**1**), isoquercitrin (**2**) and quercitrin 3-*O*-*D*-arabinosyl-(1→2)-α-L-rhamnoside (**3**) (Figure 1). All compounds were examined for neuraminidase inhibitory activity and the result was shown in Table 1 (Li; et al. 2007: 1195-1196).

Table 1 IC_{50} values for neuraminidase inhibitory activity of flavonoid glycosides **1-3**

Compounds	neuraminidase inhibitor IC_{50} (μM)
quercitrin (**1**)	7.89 ± 0.96
isoquercitrin (**2**)	9.87 ± 0.68
quercitrin 3-*O*-*D*-arabinosyl-(1→2)-α-L-rhamnoside (**3**)	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

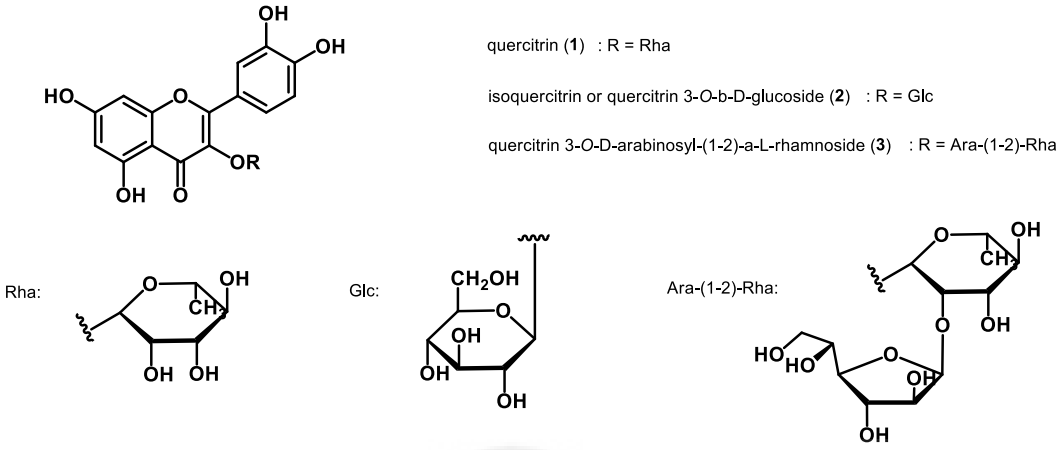


Figure 1 Structures of compounds **1-3**

Arai; et al., reported that the MeOH extract of *Z. cambodiana* leaves inhibited Hh/GLI signaling pathway, which led to the isolation of three active pentacyclic triterpenes, betulinic acid (**4**), alphitolic acid (**5**) and colubrinic acid or zizyberanalic acid (**6**) represented (Figure 2), as potent inhibitors. These compounds demonstrated an important relationship among Hh/GLI signaling inhibition, the decreased expression of the anti-apoptosis protein BCL2, and cytotoxicity against cancer cells. Besides, they also examined the cytotoxicity of these active compounds against PANC1, human prostate cancer cells (DU145) and mouse embryo fibroblast cells (C3H10T1/2) (Table 2). The cytotoxicity against cancer cells (PANC1 and DU145) by betulinic acid (**4**) or colubrinic acid (**6**) would be caused by inhibition of the expression of the anti-apoptosis protein BCL2. These pentacyclic triterpene inhibitors presented an important relationship between Hh/GLI signaling inhibition, the decrease of BCL2, and cytotoxicity against cancer cells (Table 2) (Arai; et al. 2008: 9420-9424).

Table 2 IC_{50} values (μM) of triterpenes **4-8** 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 (**4**)	32	44	37	82
alphitolic acid (**5**)	42	41	70	145
zizyberanalic acid (**6**)	38	43	78	167
ceanothic acid (**7**)	>200	195	>200	>200
ceanothanolic acid (**8**)	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

Suksamrarn; et al., reported a new triterpene ester, 3-*O*-vanillylceanothic acid (**9**), together with five known lupane constituents, betulinic acid (**4**), alphitolic acid (**5**), lupeol (**10**), betulinaldehyde (**11**), and 2-*O*-*trans*-*p*-coumaroyl alphitolic acid (**12**) and three ceanothane triterpenes, zizyberanalic acid (**6**), ceanothic acid (**7**) and zizyberenalic acid (**13**) (Figure 2) as the antiplasmodial and antituberculosis components from the root barks of *Z. cambodiana*. This was the first report of *in vitro* antiplasmodial and antimycobacterial activities from the ceanothane-type triterpenes and the result was shown in Table 3. (Suksamrarn; et al. 2006: 535-537).

Table 3 IC_{50} values for antiplasmodial and MIC values for antimycobacterial activities of triterpenes **4**-**7** and **9**-**13**

Compounds	antiplasmodial IC_{50} (μg/mL)	antimycobacterial MIC (μg/mL)
betulinic acid (**4**)	inactive[a]	25
alphitolic acid (**5**)	inactive[a]	50
zizyberanalic acid (**6**)	inactive[a]	50
ceanothic acid (**7**)	inactive[a]	inactive[b]
3-*O*-vanillylceanothic acid (**9**)	3.7	25
lupeol (**10**)	inactive[a]	inactive[b]
betulinaldehyde (**11**)	6.5	25
2-*O*-*E*-*p*-coumaroylalphitolic (**12**)	0.9	12.5
zizyberenalic acid (**13**)	3.0	100

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

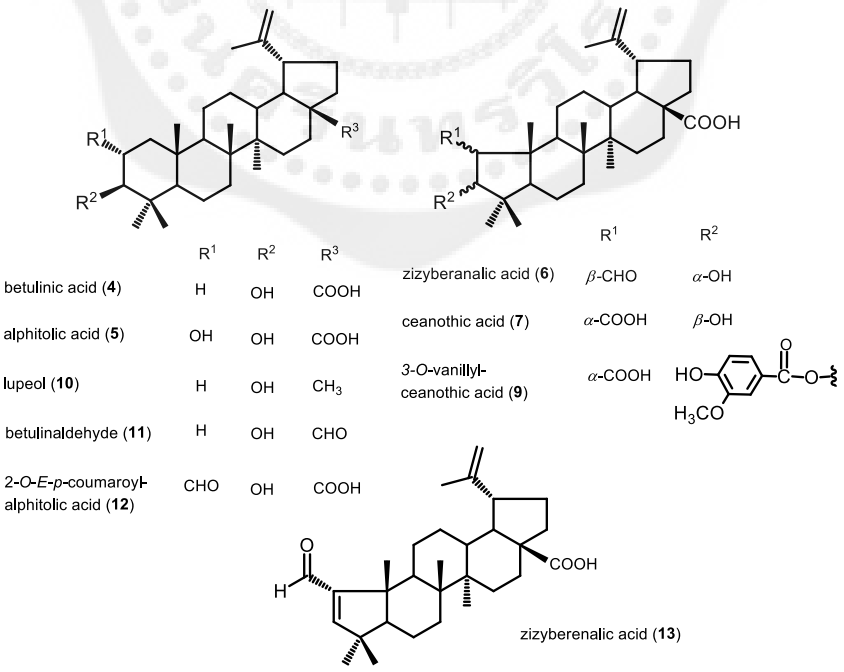


Figure 2 Structures of compounds **4**-**7**, **9**-**13**

Major phytochemicals of *Ziziphus* species

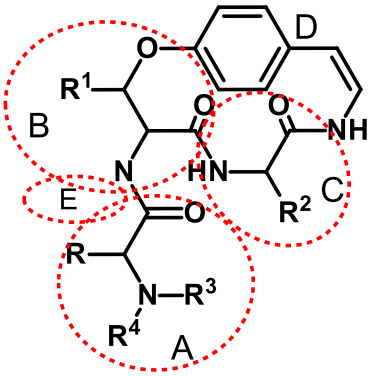
1. Cyclopeptide alkaloids

Cyclopeptide alkaloids are defined as polyamidic basic compounds found in many families of plants, Asteraceae, Celastraceae, Euphorbiaceae, Menispermaceae, Pandaceae, Rubiaceae, Sterculiaceae and Urticaceae, and mainly in species that belong to the family Rhamnaceae (Tschesche; & Kaussmann. 1975: 165). Cyclopeptide alkaloids are natural products that have been found in leaves, stem barks, root barks and seeds (Joullié; & David. 2004: 2011-2015). Among these, more than 100 cyclopeptide alkaloids have been isolated from various plants of the genus *Ziziphus*. They often occur in very minute amounts of complex mixtures. The yield of cyclopeptide alkaloids from dried plant material is about 0.01-1 % (Gournelis; Laskaris; & Verpoorte. 1998: 1-179).

1.1 Classification of cyclopeptide alkaloids

Cyclopeptide alkaloids are defined as basic compounds embodying an ansapeptides structure, in which a 10- or 12-membered peptide type bridge spans the 1,3- (*ortho* and *meta*) or 1,4- (*ortho* and *para*) positions of benzene ring. The general structure of cyclopeptide alkaloids is demonstrated in Figure 3 (Gournelis; Laskaris; & Verpoorte. 1998: 1-179). The general structural element of the cyclopeptide alkaloids consists of two amino acids and stryrylamine unit (Menezes; et al. 1995: 783-786; & Morel; et al, 1979: 473-477).

The cyclopeptide alkaloids are classified according to the size of the macrocycle as 13-, 14- or 15-membered rings which are further divided according to the number of 4 or 5 units in the structural features (Figure 3). Consequently, the cyclopeptide alkaloids are subdivided into groups of 4(13)-, 5(13)-, 4(14)-, 5(14)-, and 5(15)-type. The 4(14)- and 5(14)-type of compounds are further subdivided according to the nature of the β-OH-amino acid (unit B). The β-OH-amino acid of unit B and the size of the ring are important in classifying the various families. The nomenclature of cyclopeptide alkaloids is shown in Table 4 (Gournelis; Laskaris; & Verpoorte. 1998: 1-179).



A = basic terminal (end) amino acid

B = β-hydroxy-amino acid

C = ring-bound amino acid

D = styrylamine unit

An additional (intermediary) amino acid E is sometimes interposed between A and B units.

Figure 3 General structures for cyclopeptide alkaloids

Table 4 Nomenclature of cyclopeptide alkaloids (Gournelis; Laskaris; & Verpoorte. 1998: 1-179)

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			-
	Ziziphine A-type (Pro)	Scutianine A-type (Leu or Phe)		Amphibine B-type (Pro)	

The type of cyclopeptide alkaloids are named according to the first compound found for each type. Cyclopeptide alkaloids have been extensively discovered and reviewed in the period up to 2005 by 3 research groups (Gourenelis; et al. 1998: 1-179, Tan; et al. 2006: 840-895 and El-Seedi; et al. 2007: 143-165). Followings are compounds of each type recently reported during the year of 2006-2014.

1.1.1 The 4(13)-nummularine- C-type cyclopeptide alkaloids

This 13-membered ring of cyclopeptide alkaloid is composed of two amino acids units (A and C), the B unit containing β-OH-proline and the unit D is a styrylamine moiety. Nummularine C was the first compound of this type isolated from *Z. nummularia.* Compounds of this type which were recently reported during the year of 2006-2014 are presented in Table 5.

Table 5 Examples of 4(13)-nummularine C-type cyclopeptide alkaloids

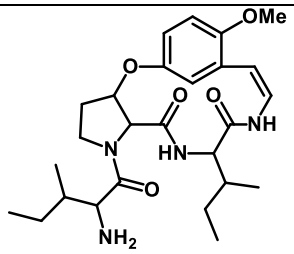
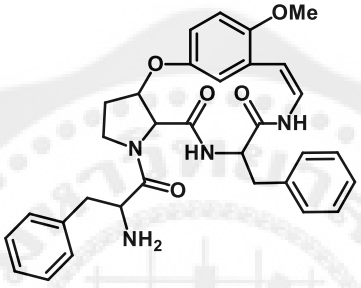
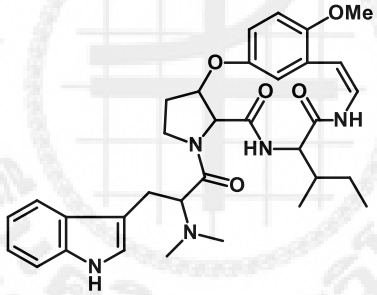
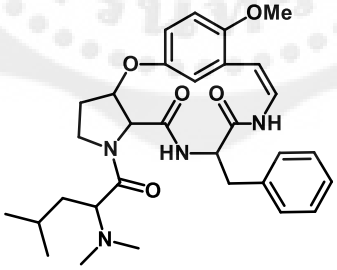
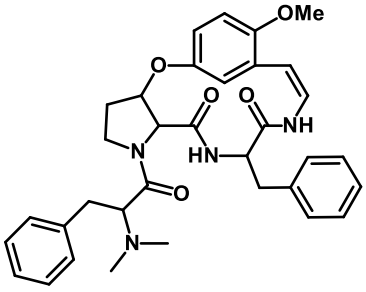
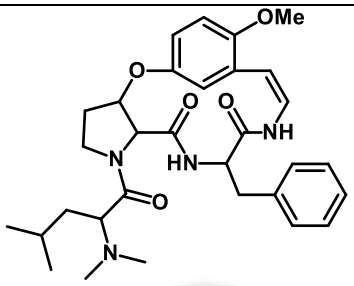
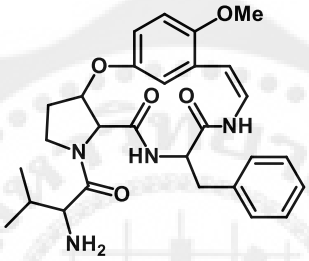
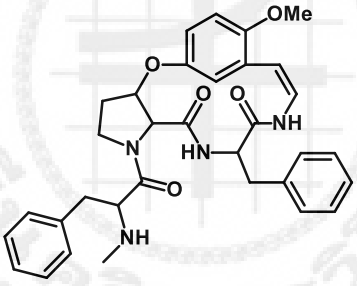
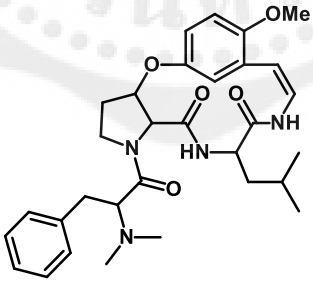
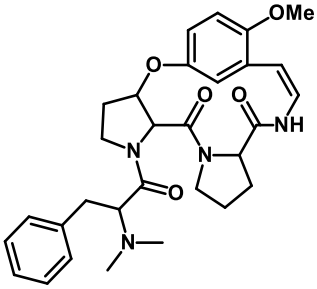
Compounds	Structures	Plant sources
sativanine N (**14**)		The stem barks of *Z. sativa* (Singh; et al. 2006: 733-739).
sativanine O (**15**)		The stem barks of *Z. sativa* (Singh; et al. 2006: 733-739).
nummularine R (**16**)		The stem barks of *Z. oxyphylla* Miq (Inayat-Ur-Rahman; et al. 2007: 243-253).
xylopyrine A (**17**)		The root barks of *Z. xylopyrus* (Willd) (Singh; Raghubanshi; & Yadava. 2007: 1114-1120).
xylopyrine B (**18**)		The root barks of *Z. xylopyrus* (Willd) (Singh; Raghubanshi; & Yadava. 2007: 1114-1120).

Table 5 (Continued)

Compounds	Structures	Plant sources
xylopyrine D (**19**)		The barks of *Z. xylopyra* (Pandey; et al. 2008a: 709-713).
xylopyrine E (**20**)		The barks of *Z. xylopyra* (Pandey; et al. 2008a: 709-713).
jubanine E (**21**)		The barks of *Z. xylopyra* (Pandey; et al. 2008a: 709-713).
nummularine C (**22**)		The roots of *Z. oxyphylla* Miq (Kaleem; et al. 2013: 154-158).
oxyphylline D (**23**)		The roots of *Z. oxyphylla* Miq (Kaleem; et al. 2013: 154-158).

1.1.2 The 4(14)-frangulanine-type cyclopeptide alkaloids

This is the 14-membered ring cyclopeptide alkaloid which composed of the similar A, C and D units as that of 4(13)-nummularine C-type cyclopeptide except for the unit B is β-OH-leucine in place of β-OH-proline in above section (1.1.1). Frangulanine was the first compound of this type isolated from *Rhamnus frangula.* Compounds of this type which were recently reported during the year of 2006-2014 are presented in Table 6.

Table 6 Examples of 4(14)-frangulanine-type cyclopeptide alkaloids

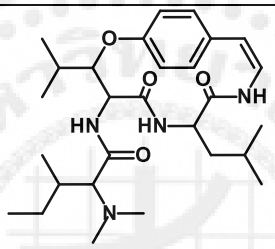
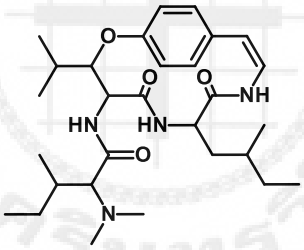
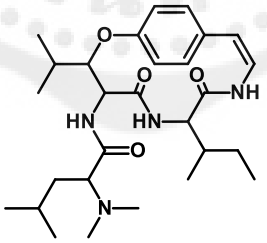
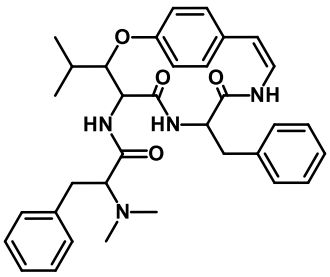
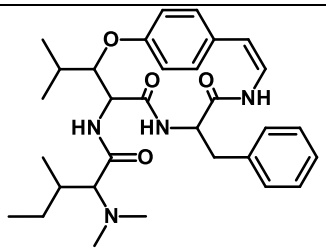
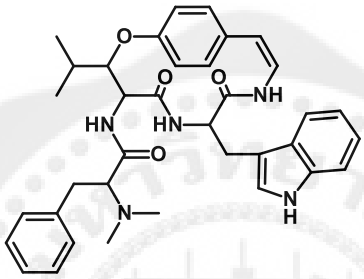
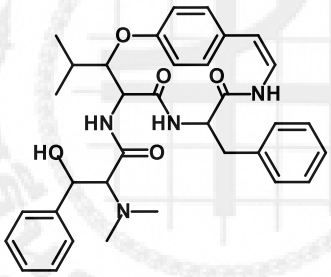
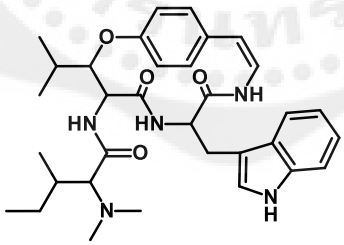
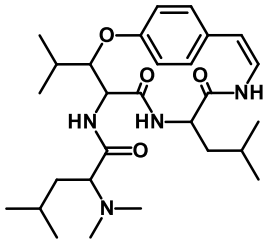
Compounds	Structures	Plant sources
frangulanine (**24**)		The leaves of *Melochia odorata* (Emile; et al. 2007: 398-400).
frangulaline (**25**)		The roots of *Melochia chamaedris* (Sterculiaceae) (Dias; et al. 2007: 668-672).
adouetine X (**26**)		The roots of *Melochia chamaedris* (Sterculiaceae) (Dias; et al. 2007: 668-672).
scutianine B (**27**)		The roots of *Melochia chamaedris* (Sterculiaceae) (Dias; et al. 2007: 668-672).

Table 6 (Continued)

Compounds	Structures	Plant sources
scutianine C (**28**)		The roots of *Melochia chamaedris* (Sterculiaceae) (Dias; et al. 2007: 668-672).
chamaedrine (**29**)		The roots of *Melochia chamaedris* (Sterculiaceae) (Dias; et al. 2007: 668-672).
scutianine D (**30**)		The root barks of *Discaria americana* (Rhamnaceae) (Trevisan; et al. 2009: 608-612).
discarine B (**31**)		The root barks of *Discaria americana* (Rhamnaceae) (Trevisan; et al. 2009: 608-612).
franganine (**32**)		The root barks of *Discaria Americana* (Caro; et al., 2012: 1220-1222).

1.1.3 The 4(14)-integerrine-type cyclopeptide alkaloids

This 14-membered ring of cyclopeptide alkaloid is composed of two amino acids units (A and C). The B unit is β-OH-phenylalanine and the unit D is a styrylamine moiety. Integerrine was the first compound of this type isolated from *Ceanothus integerrimus*. Compounds of this type which were recently reported during the year of 2006-2014 are presented in Table 7.

Table 7 Examples of 4(14)-integerrine-type cyclopeptide alkaloids

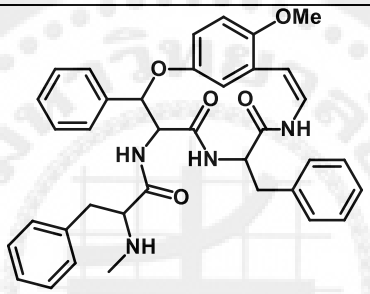
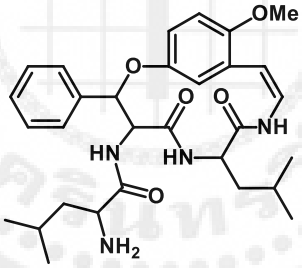
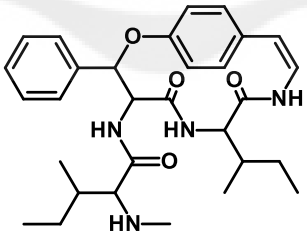
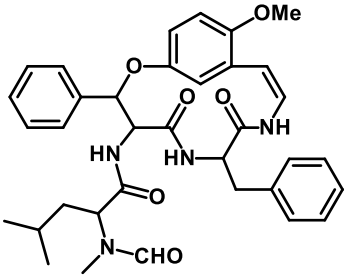
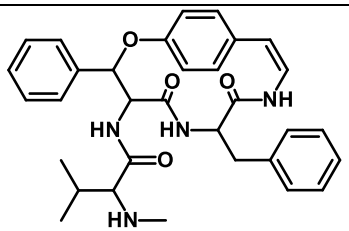
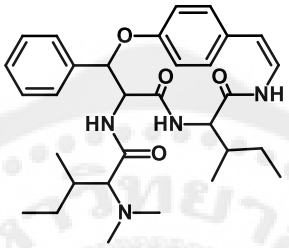
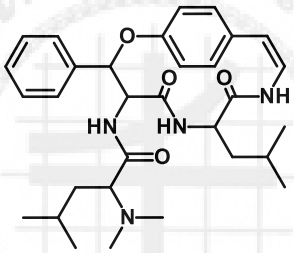
Compounds	Structures	Plant sources
xylopyrine C (**33**)		The root barks of *Z. xylopyra* Willd (Singh; et al. 2008c: 725-728).
xylopyrine F (**34**)		The root barks of *Z. xylopyra* (Pandey; et al. 2008c: 725-728).
mauritine L (**35**)		The root barks of *Z. mauritiana* Lam (Panseeta; et al. 2011: 909-915).
xylopyrine G (**36**)		The barks of *Z. xylopyra* Willd (Pandey; et al. 2012: 836-841).

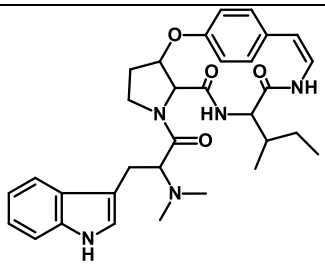
Table 7 (Continued)

Compounds	Structures	Plant sources
xylopyrine H (**37**)		The barks of *Z. xylopyra* Willd (Pandey; et al. 2012: 836-841).
nummularine M (**38**)		The stem barks of *Z. rugosa* Lam (Singh; Raghubanshi; & Yadava. 2013: 994-997).
rugosanine C (**39**)		The stem barks of *Z. rugosa* Lam (Singh; Raghubanshi; & Yadava. 2013: 994-997).

1.1.4 The 4(14)-amphibine F-type cyclopeptide alkaloids

This 14-membered ring of cyclopeptide alkaloid is composed of two amino acids units (A and C). The B unit is β-OH-proline and the unit D is a styrylamine moiety. Amphibine F was the first compound of this type isolated from *Z. amphibia.* Only hemsine A (**40**) was reported since 2011, as shown in Table 8.

Table 8 Examples of 4(14)-amphibine F-type cyclopeptide alkaloids

Compound	Structure	Plant source
hemsine A (**40**)		The root barks of *Z. mauritiana* Lam (Panseeta; et al. 2011: 909-915).

1.1.5 The 4(15)-mucronine A-type cyclopeptide alkaloids

This 15-membered ring of cyclopeptide alkaloid is composed of two amino acids units (A and C). The B unit is an amino acid (not a β-OH amino acid) and the unit D is a styrylamine moiety. Mucronine A was the first compound of this type isolated from *Z. mucronata.* General structure of this type is shown in Figure 4. Unfortunately, there has been no report of this type of alkaloids since 2005.

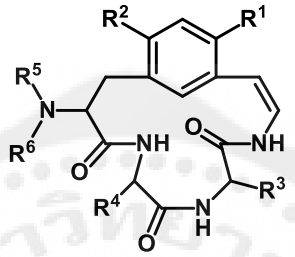


Figure 4 Structure of 4(15)-mucronine A-type cyclopeptide alkaloid

1.1.6 The 5(13)-ziziphine A-type cyclopeptide alkaloids

This 13-membered ring of cyclopeptide alkaloid is composed of three amino acids units (A, C and E). The B unit is β-OH-proline and the unit D is a styrylamine moiety. Ziziphine A was the first compound of this type isolated from *Z. oenoplia.* Compounds of this type which were recently reported during the year of 2006-2014 are presented in Table 9.

Table 9 Examples of 5(13)-ziziphine A-type cyclopeptide alkaloids

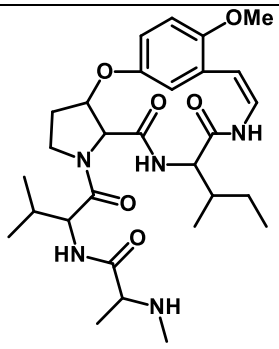
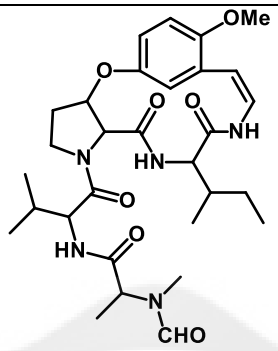
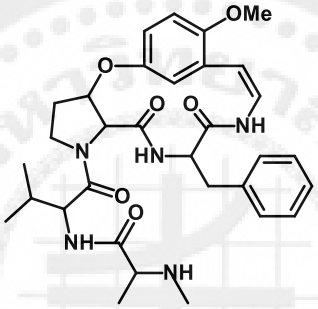
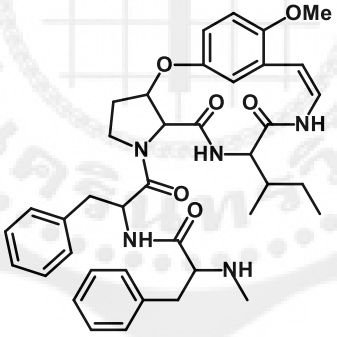
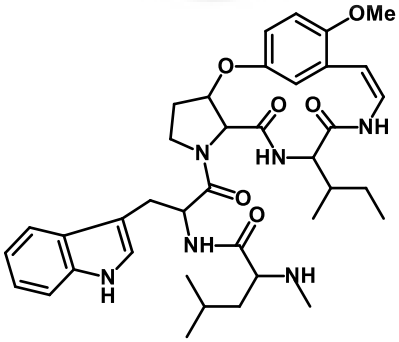
Compound	Structure	Plant source
sativanine C (**41**)		The stem barks of *Z. sativa* (Pandey, et al. 2008b: 219-221).

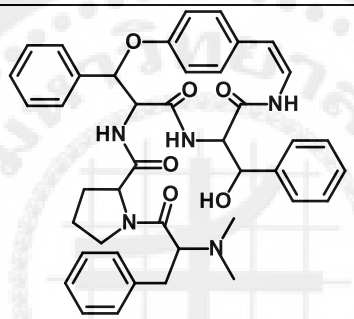
Table 9 (Continued)

Compounds	Structures	Plant sources
sativanine M (**42**)		The stem barks of *Z. sativa* (Pandey, et al. 2008b: 219-221).
nummularine B (**43**)		The roots of *Z. mauritiana* (Panseeta; et al. 2011: 909-915).
nummularine H (**44**)		The roots of *Z. mauritiana* (Panseeta; et al. 2011: 909-915).
mauritine M (**45**)		The roots of *Z. mauritiana* (Panseeta; et al. 2011: 909-915).

1.1.7 The 5(14)-scutianine A-type cyclopeptide alkaloids

This 14-membered type of cyclopeptide alkaloid is composed of two amino acids units (A and C). The B unit is either β-OH-leucine or β-OH-phenylalanine, the intermediary (unit E) is proline and the unit D is a styrylamine moiety. Scutianine A was the first compound of this type isolated from *Scutia buxifolia.* Only oxyphylline A (**46**) has been reported since 2006, as shown in Table 10.

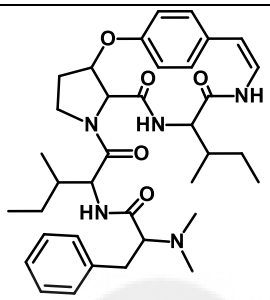
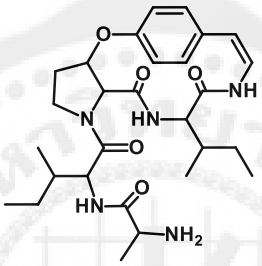
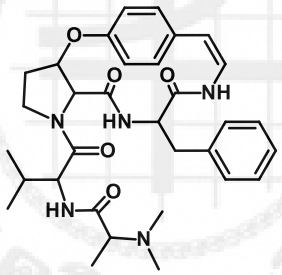
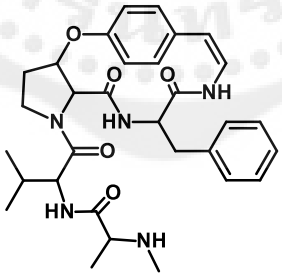
Table 10 Examples of 5(14)-scutianine A-type cyclopeptide alkaloids

Compound	Structure	Plant source
oxyphylline A (**46**)		The stem barks of *Z. oxyphylla* Miq (Inayat-Ur-Rahman; et al. 2007: 243-253).

1.1.8 The 5(14)-amphibine B-type cyclopeptide alkaloids

This 14-membered ring of cyclopeptide alkaloid is composed of three amino acids units (A, C and E). The B unit is β-OH-proline and the unit D is a styrylamine moiety. Amphibine B was the first compound of this type isolated from *Z. amphibia.* Compounds of this type which were recently reported during the year 2006-2014 are presented in Table 11.

Table 11 Examples of 5(14)-amphibine B-type cyclopeptide alkaloids

Compound	Structure	Plant source
amphibine D (**47**)		The seeds of *Z. jujuba* var. *sapinosa* (Liu; Chen; & Yao. 2007: 668-675).
mauritine K (**48**)		The root barks of *Z. muritiana* (Singh; et al. 2008: 781-784).
mauritine A (**49**)		The roots of *Z. apetala* Hook (Han; et al. 2011: 2571-2575).
mauritine F (**50**) or (N-desmethyl-mauritine A)		The roots of *Z. apetala* Hook (Han; et al. 2011: 2571-2575).

1.1.9 The 4(14)-pandamine-type cyclopeptide alkaloids

This 14-membered ring of cyclopeptide alkaloid is composed of two amino acids units (A and C). The B unit is β-OH-leucine and unit D is a 2-alkoxy-2-(*p*-hydroxyphenyl) ethylamine instead of a styrylamine moiety. Pandamine was the first compound of this type isolated from *Panda oleosa* (Pandaceae). General structure of this type is shown in Figure 5. Unfortunately, there has been no report of alkaloid since 2005.

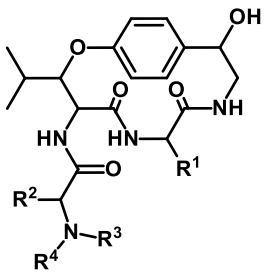


Figure 5 Structure of 4(14)-pandamine-type cyclopeptide alkaloid

1.1.10 Neutral compound related to 14-membered cyclopeptide alkaloids

The cyclopeptide alkaloid of this type is composed of two or three units (B, C and /or E) of amino acids and a neutral moiety such as a coumaroyl as terminal chain (A). Unit D is a styrylamine moiety. Compounds of this type recently reported during the year of 2006-2014 are presented in Table 12.

Table 12 Examples of neutral compound related to 14-membered cyclopeptide alkaloids

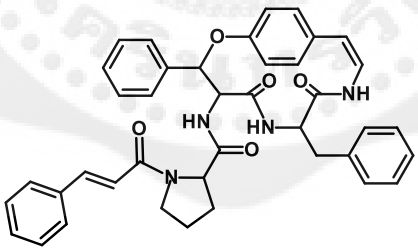
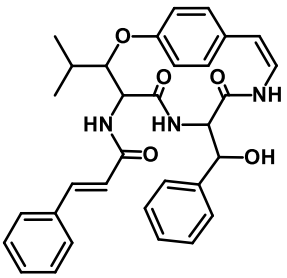
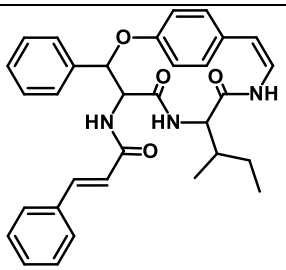
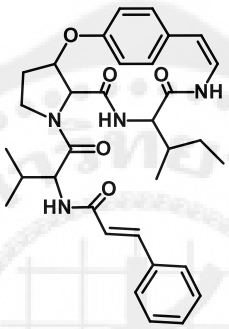
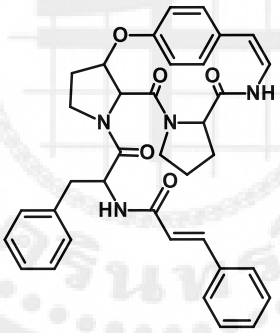
Compounds	Structures	Plant sources
amaiouine (**51**)		The leaves of *Amaioua guianensis* (de Oliveira; et al. 2009: 1195-1197).
scutianene E (**52**)		The barks of *Scutia buxifolia* var. *acutifolia* Reissek (Rhamnaceae) (Maldaner; et al. 2011: 804-809).

Table 12 (Continued)

Compounds	Structures	Plant sources
scutianene L (**53**)		The barks of *Scutia buxifolia* var. *acutifolia* Reissek (Rhamnaceae) (Maldaner; et al. 2011: 804-809).
oxyphylline B (**54**)		The roots of *Z. oxyphylla* Miq (Kaleem; et al. 2013: 154-158).
oxyphylline C (**55**)		The roots of *Z. oxyphylla* Miq (Kaleem; et al. 2013: 154-158).

1.2 Biological activities of cyclopeptide alkaloids

Cyclopeptide alkaloids have been used for centuries as traditional medicines in many parts of the world. Literature reviews on biological activities of cyclopeptide have been extensively reported up to 2005 (Tan; et al. 2006: 840-895; & El-Seedi; et al. 2007: 143-165). Followings cover the recent biological activities reported during the period 2006-present.

1.2.1 Antimicrobial activity

Frangulanine (**24**), a 14-membered cyclopeptide alkaloid isolated from the leaves of *Melochia odorata*, exhibited moderate activity against fungi (*C. albicans* at MIC of 25 μg/mL, *C. neoformans* at MIC >50 μg/mL, and *S. cerevisiae* at MIC of 50 μg/mL) by using a TLC bioautographic method (Emile; et al. 2007: 398-400).

Investigation of the MeOH extract obtained from the roots of the *Z. mauritiana* grown in Thailand resulted in the isolation of two 14- and 13-membered cyclic alkaloids, mauritines L (**35**) and M (**45**), and three known cyclopeptide alkaloids, hemsine A (**40**), nummularines B (**43**) and H (**44**). The isolated alkaloids exhibited potent antiplasmodial activity against the parasite *Plasmodium falciparum* with the inhibitory concentration (IC_{50}) ranging from 3.7 to 10.3 μM. Furthermore, nummularine H (**44**) and mauritine M (**45**) also demonstrated antimycobacterial activity against *Mycobacterium tuberculosis* with the MIC of 4.5 and 72.8 μM, respectively. The relatively high antiplasmodial activity of hemsine A (**40**), nummularine H (**44**) and mauritine M (**45**), could possibly be due to the presence of hydroxyproline unit in the macrocycle ring and the terminal *N*-methylated or *N,N*-dimethylated amino acid residues. (Panseeta; et al. 2011: 909-915).

The 14-membered cyclopeptide alkaloid, oxyphylline B (**54**) and C (**55**) were tested for antibacterial activity. Oxyphylline B (**54**) showed antibacterial activities against *Escherichia coli* at MIC of 5 μg/mL) better than oxyphylline C (**55**). In addition, oxyphylline B also exhibited weak antimicrobial activities against *Staphylococcus aureus* at MIC of 25 μg/mL, *Pseudomonas aeruginosa* at MIC of 50 μg/mL and *Salmonella typhi* at MIC of 50 μg/mL. Oxyphylline C (**55**) showed weak antibacterial activities against *E. coli* (MIC 10 μg/mL), *S. aureus* (MIC 10 μg/mL), *P. aeruginosa* (MIC 50 μg/mL) and *S. typhi* (MIC 50 μg/mL) (Kaleem; et al. 2012: 11520-11529).

1.2.2 Antinociceptive effect

The 14-membered ring cyclopeptide alkaloids were isolated from the root barks of *Discaria americana* (Rhamnaceae). The analgesic potentials of six compounds, franganine (**32**), adouetine X (**26**), scutianines B (**27**), C (**28**), D (**30**) and discarine B (**31**), have been investigated. Among the theses tested, only franganine (**32**) and adouetine X (**26**) displayed antinociceptive effects in a mouse model of acute pain, without inducing undesirable side effects. Furthermore, adouetine X (**26**) also exhibited a pronounced

analgesic effect in a chronic neuropathic pain model in mice (Trevisan, et al. 2009: 608-612).

1.2.3 Urease inhibitory activity

Cyclopeptide alkaloids isolated from *Z. oxyphylla*, named nummularines C (**22**), R (**16**) and oxyphylline D (**23**), were reported as urease inhibitor. Moreover, oxyphylline D (**23**), nummularine C (**22**) and nummularine R (**16**) exhibited weak activity with % inhibition of 58.2%, 35.7% and 29.3%, respectively (Kaleem; et al. 2013: 154-158).

1.2.4 Cholinesterase inhibitory activity

Snakin-Z, a new peptide isolated from *Z. jujube* fruits, exhibited acetylcholinesterase and butyrylcholinesterase with IC_{50} values of 0.58 $\pm$ 0.08 and 0.72 $\pm$ 0.085 mg/mL, respectively. The compound also showed the high antioxidant activity at IC_{50} value of 0.75 $\pm$ 0.09 mg/mL (Lee; et al. 2013: 299-306).

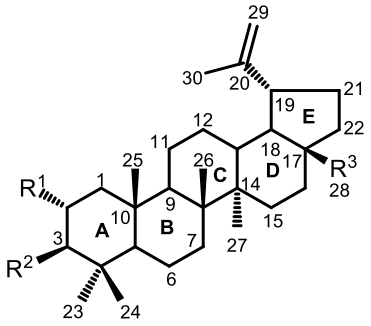
2. Triterpenoids

The triterpenoids comprise a group of diverse C30 from C30 precursors. Triterpenoids are classified according to number of cyclic ring in their chemical structure. Most triterpenoids are 6-6-6-5 tetracycles, 6-6-6-6-5 pentacycles (Xu; Fazio; & Matsuda. 2004: 261-291). Triterpenes of *Ziziphus* plants are lupane-, ceanothan-, oleanane- and ursane-type structures.

2.1 Lupane-type

Lupane-type triterpene is series of 6-6-6-6-5 pentacyclic, which composed of an isopropenyl ($CH_3C=CH_2$) moiety at C-19. Lupeol, betulin, and betulinic acid are common natural pentacyclic triterpenes of this type isolated from *Ziziphus* and other plant sources.

Table 13 Examples of lupane-type triterpenes



Common name	R^1	R^2	R^3	Plant sources
Betulinic acid (**4**)	H	OH	COOH	Roots of *Z. cambodiana* (Suksamrarn; et al. 2006: 535-537) and *Z. oenoplia* (Aunchai. 2002: 1-116), stems of *Z. mauritiana* (Chauhan; & Srivastava. 1978: 6).
lupeol (**10**)	H	OH	CH_3	Root barks of *Z. cambodiana* (Suksamrarn; et al. 2006: 535-537) and *Z. rugosa* (Netsawangwicha. 2005: 1-106), barks of *Z. rugosa* (Nahar; et al. 1997: 151-158) and stems of *Z. oenoplia* (Nahar; et al. 1997: 151-158).
betulin (**56**)	H	OH	CH_2OH	Root barks of *Z. rugosa* (Netsawangwicha. 2005: 1-106) and *Z. jujuba* Mill var. *spinosa* (Lee; Lin; & Chen. 1995: 511-519), barks of *Z. rugosa*, *Z. oenoplia* (Nahar; et al. 1997: 151-158), *Z. rugosa* (Prashith Kekuda; Raghavendra; & Vinayaka. 2011: 887-890) and *Z. mauritiana* (Chauhan; & Srivastava. 1997: 6).

Table 13 (Continued)

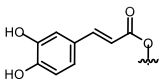
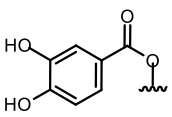
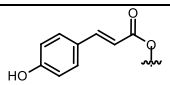
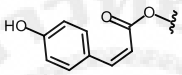
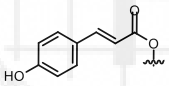
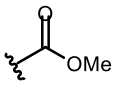
Common name	R^1	R^2	R^3	Plant sources
betulinaldehyde (**11**)	H	OH	CHO	Root barks of *Z. cambodiana* (Suksamrarn; et al. 2006: 535-537) and *Z. rugosa* (Netsawangwicha. 2005: 1-106), barks of *Z. rugosa* and stems of *Z. oenoplia* (Nahar; et al. 1997: 151-158).
alphitolic acid (**5**)	OH	OH	COOH	Root barks of *Z. cambodiana* (Suksamrarn; et al. 2006: 535-537), *Z. rugosa* (Kulshreshtha; & Rastogi. 1972: 152-154) and *Z. rugosa* (Netsawangwicha. 2005: 1-106), fruits of *Z. jujube* (Yagi; et al. 1978b: 1798-1802; Lee; et al. 2004: 1883-1886) and leaves of *Z. mauritiana* (Sharma; & Kumar. 1982: 809-810).
2-*O*-protocatechuoyl-alphitolic acid (**57**)		H	COOH	Roots of *Z. jujuba* var. *spinosa* (Lee; Lin; & Liu.1996: 847-851).
2-α-hydroxy-pyracrenic acid (**58**)	OH		COOH	Roots of *Z. jujuba* var. *spinosa* (Lee; Lin; & Liu.1996: 847-851).

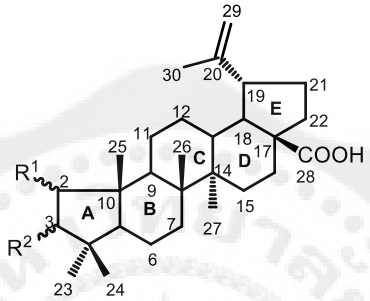
Table 13 (Continued)

Common name	R^1	R^2	R^3	Plant sources
2-*O*-*trans*-*p*-coumaroyl-alphitolic acid (**12**)		H	COOH	Root barks of *Z. cambodiana* (Suksamrarn; et al. 2006: 535-537) and *Z. rugosa* (Netsawangwicha. 2005: 1-106), and fruits of *Z. jujube* (Yagi; et al. 1978b: 1798-1802).
3-*O*-*cis*-*p*-coumaroyl-alphitolic acid (**59**)	H		COOH	Fruits of *Z. jujube* (Yagi; et al. 1978b: 1798-1802) and root barks of *Z. jujuba* Mill var *spinosa* (Lee; Lin; & Chen. 1995: 511-519).
3-*O*-*trans*-*p*-coumaroyl-alphitolic acid (**60**)	H		CH_2OH	Fruits of *Z. jujube* (Yagi; et al. 1978b: 1798-1802) and root barks of *Z. jujuba* Mill var. *spinosa* (Lee; Lin; & Chen. 1995: 511-519).
betulonic acid (**61**)	H	C=O	COOH	The root barks of *Z. jujuba* Mill var. *spinosa* (Lee; Lin; & Chen. 1995: 511-519) and fruits *of Z. jujube* (Huang; et al. 2001: 179-184).
alphitolic acid methyl ester (**62**)	OH	OH		The seeds of *Z. jujuba* var. *spinosa* (He; Pan; & Min. 2006: 168-171).

2.2 Ceanothane-type

Ceanothane-type triterpene is series of 5-6-6-6-5 pentacyclic, which composed of an isopropenyl ($CH_3C=CH_2$) moiety at C-19 similar to the lupane-type, but ceanothane-type triterpene has a five-membered ring in the ring A.

Table 14 Examples of ceanothane-type triterpenes



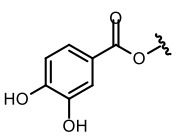
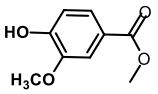
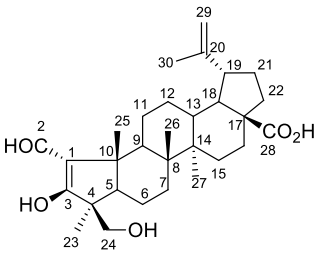
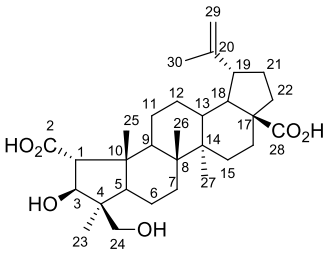
Common name	R^1	R^2	Plant sources
ceanothic acid (**7**)	α-COOH	β-OH	Roots of *Z. jujube* Mill var. *spinosa* (Lee; Lin; & Chen. 1995: 511-519) and *Z. mauritiana* (Panseeta; et al. 2011: 909-915), root barks of *Z. cambodiana* (Suksamrarn; et al. 2006: 535-537) and leaves of *Z. glabrata* (Ganapaty; et al. 2006: 87-92).
ceanothanolic acid (**8**)	α-CH_2OH	α-OH	Root barks of *Z. rugosa* (Netsawangwicha. 2005: 1-106) and *Z. cambodiana* (Arai; et al. 2008: 9420-9424).
3-*O*-protocatechuoyl ceanothic acid (**63**)	α-CH_2OH		Root of *Z. jujuba var. spinosa* (Lee; Lin; & Liu. 1996: 847-851)

Table 14 (Continued)

Common name	R^1	R^2	Plant sources
3-*O*-vanillyl ceanothic acid (**9**)	α-COOH	β-*O*-vanillyl 	Root barks of *Z. cambodiana* (Suksamrarn; et al. 2006: 535-537).
zizyberanalic acid (**6**)	β-CHO	α-OH	Fruits of *Z. jujube* Mill (Lee; et al. 2004:1883-1886) and root barks of *Z. cambodiana* (Suksamrarn; et al. 2006: 535-537).
zizyberenalic acid (**13**)	-	-	Fruits of *Z. jujube* Mill (Lee; et al. 2004:1883-1886) and root barks of *Z. cambodiana* (Suksamrarn; et al. 2006: 535-537).
epiceanothic acid (**64**)	β-COOH	β-OH	Roots of *Z. mauritiana* (Panseeta; et al. 2011: 909-915).
a-norlupa-2,20-diene-14,17-dicarboxylic acid (**65**)	H	COOH	The both barks and roots of *Z. jujube* (Mondal; & Kundu, et al. 1998: 384-385).
24-hydroxyceanothic acid (**66**)	-	-	Roots of *Z. mauritiana* (Panseeta; et al. 2009: 1-149).



Zizyberenalic acid (**13**)

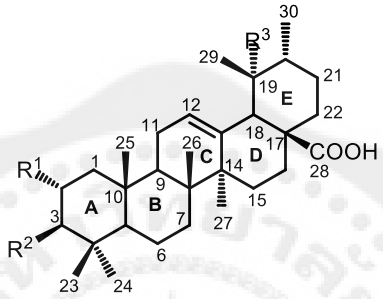


24-Hydroxyceanothic acid (**66**)

2.3 Ursane-type

Ursane-type triterpene is series of 6-6-6-6-6 pentacyclic, which contain a double bond at C12-C13 and additional of two methyl groups at carbon C-19 and C-20 in the ring E.

Table 15 Examples of ursane-type triterpenes



Common name	R^1	R^2	R^3	Plant sources
ursolic acid (**67**)	H	β-OH	H	Roots of *Z. jujube* Mill var. *spinosa* (Lee; Lin; & Chen. 1995: 511-519) and the fruits *Z. jujube* (Guo; et al. 2009: 1296-1302).
2-α-hydroxy-ursolic acid (**68**)	α-OH	β-OH	H	Roots of *Z. jujube* Mill var. *spinosa* (Lee; Lin; & Chen. 1995: 511-519) and *Z. rugosa* (Kulshreshrehtha; & Rastogi. 1972: 152-154).
ursolonic acid (**69**)	H	C=O	H	Fruits of *Z. jujube* (Huang; et al. 2001: 178-184).
pomonic acid (**70**)	H	C=O	OH	Fruits of *Z. jujube* var. *spinosa* (Guo; et al. 2011: 880-882).

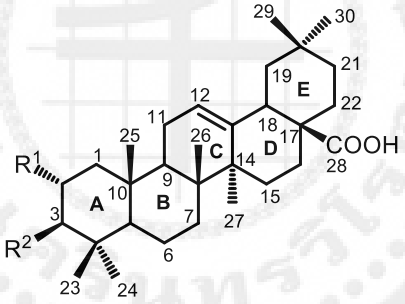
Table 15 (Continued)

Common name	R^1	R^2	R^3	Plant sources
pomolic acid (**71**)	H	OH	OH	Fruits of *Z. jujube* var. *spinosa* (Guo; et al. 2011: 880-882).

2.4 Oleanane-types

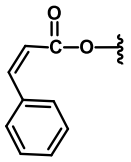
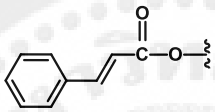
Oleanane-types triterpene is series of 6-6-6-6-6 pentacyclic, which contain a double bond at C12-C13 and additional of two methyl groups at carbon C-20 in the ring E.

Table 16 Examples of oleanane-types triterpenes



Common name	R^1	R^2	Plant sources
oleanolic acid (**72**)	H	OH	Roots of *Z. rugosa* (Kulshreshrehtha; & Rastogi. 1972: 152-154) and the fruits of *Z. jujuba* Mill (Lee; et al. 2004: 1883-1886).
oleanonic acid (**73**)	H	C=O	Fruits of *Z. jujuba* Mill (Lee; et al. 2004: 1883-1886), *Z. jujube* (Huang; et al. 2001: 178-184).
maslinic acid (**74**)	OH	OH	Fruits of *Z. jujuba* Mill (Lee; et al. 2004: 1883-1886) and *Z. jujube* (Yagi; et al. 1978b: 1798-1802).

Table 16 (Continued)

Common name	R^1	R^2	Plant sources
3-O-cis-p-coumaroyl maslinic acid (**75**)	OH	*Z-p*-coumaroyl 	Fruits of *Z. jujuba* Mill (Lee; et al. 2004: 1883-1886) and *Z. jujube* (Yagi; et al. 1978b: 1798-1802).
3-O-trans-p-coumaroyl maslinic acid (**76**)	H	*E-p*-coumaroyl 	Fruits of *Z. jujuba* Mill (Lee; et al. 2004: 1883-1886) and *Z. jujube* (Yagi; et al. 1978b: 1798-1802).

CHAPTER 3

EXPERIMENTAL

Plant materials

The air-dried stem barks of *Z. cambodiana* were collected from Nang Rong District, Buriram Province, Thailand, in April, 2011. A voucher specimen (Napaporn Charoenran 001) has been deposited at the Chemistry Department of Srinakharinwirot University.

Physical properties measurements

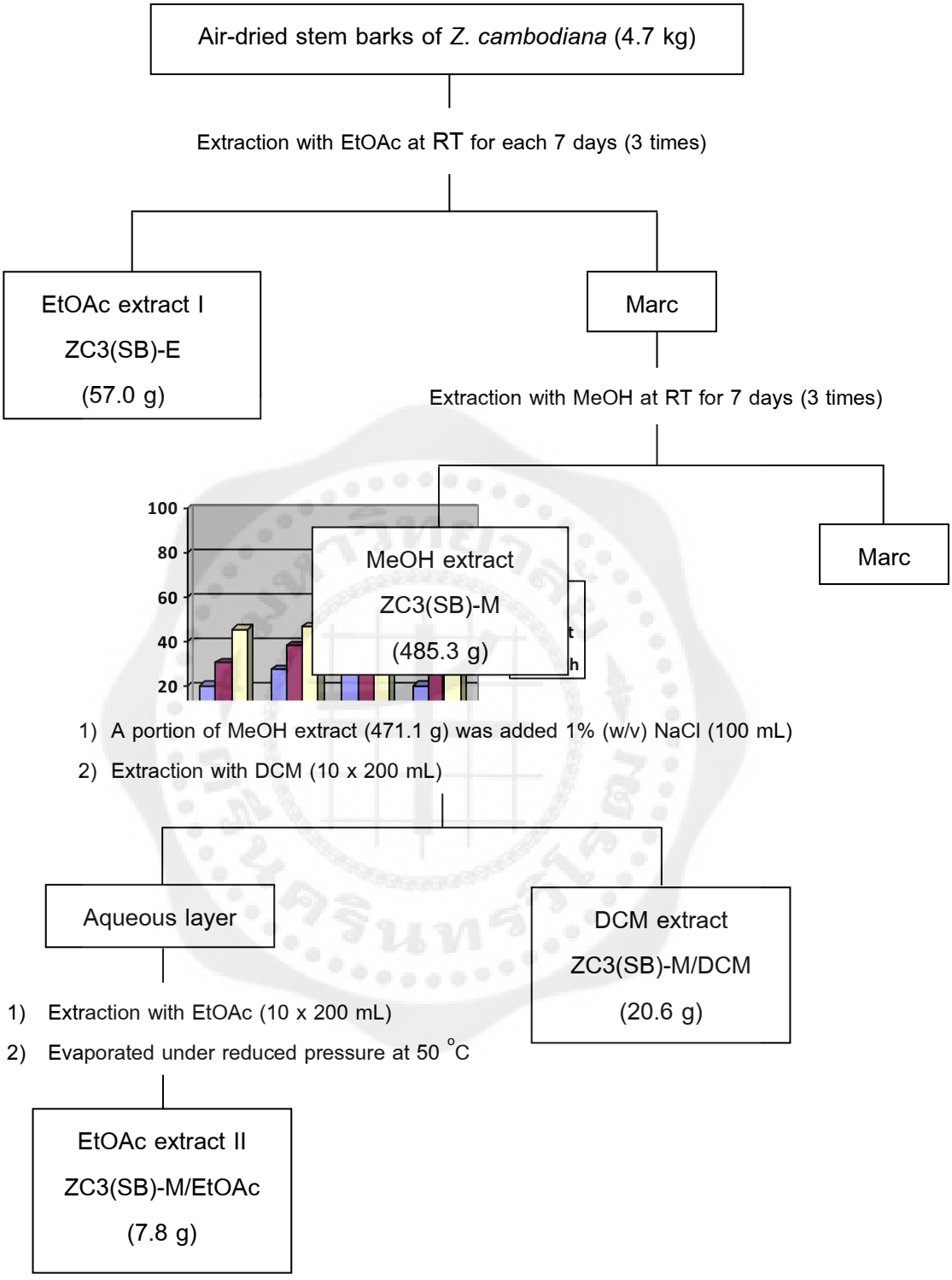
Melting points were determined using a Griffin melting point apparatus. Optical rotations were determined on a JASCO-1020 digital polarimeter. UV spectra were obtained on a Shimadzu UV-2401PC UV-VIS Spectrophotometer. IR spectra were measured on a Perkin Elmer FT-IR Spectrum BX spectrophotometer using potassium bromide (KBr) disc or $CHCl_3$ film. ^{1}H- and ^{13}C-NMR spectra were determined on a Bruker AVANCE 300 FT-NMR spectrometer operating at 300 MHz (^{1}H) and 75 MHz (^{13}C). For the spectra taken in $CDCl_3$ and CD_3OD, the residual nondeuterated solvent signals at δ_H 7.24 and δ_H 3.35 and the solvent signals at δ_C 77.0 and δ_C 49.0 were used as references for ^{1}H- and ^{13}C-NMR spectra, respectively. Electrospray ionization (ESI) mass spectra were measured on Thermo Finnigan LC-Q and a Bruker micrOTOF mass spectrometer. Unless indicated otherwise, column chromatography were carried out using Merck silica gel 60 (<0.063 and 0.063-0.200 mm) or Silicycle silica gel 60 (40-63 μm) and Merck precoated silica gel 60 F_{254} plates for TLC. Spots on TLC were visualized under UV light and by spraying with anisaldehyde-H_2SO_4, followed by heating.

Extraction and isolation procedures

1. *Z. cambodiana*

1.1 Extraction of the dried stem barks of *Z. cambodiana.*

Pulverized, dry stem barks (4.7 kg) of *Z. cambodiana* were extracted with EtOAc (3 x 20 L) and then MeOH (3 x 20 L) at room temperature for each one week. Solvents were then evaporated under reduced pressure at 50 $^{\circ}$C to afford an EtOAc extract I (as green-brown residue, 57.0 g) and MeOH extract (as reddish brown sticky residuce, 485.3 g), respectively. A portion of the MeOH extract (471.1 g) was added with 1% NaCl (200 mL) and then the mixture was further extracted with DCM (10 x 200 mL) to yield the DCM extract soluble fraction (reddish brown residue, 20.6 g, 4.3 %) after removal of solvent. The aqueous soluble fraction was further extracted with EtOAc (10 x 200 mL) to yield the EtOAc II (reddish brown residue, 7.8 g, 1.6 %) residue. The extraction procedure is shown in Scheme 1.



Scheme 1 Extraction procedure of the stem barks of *Z. cambodiana*

1.2 Isolation of compounds from the EtOAc extract I of *Z. cambodiana* stem barks.

A portion of the EtOAc extract I (52.6 g) was separated by QCC technique (150 g of silica gel), eluted with *n*-hexane, DCM, DCM-EtOAc, EtOAC-MeOH and MeOH with increasing amounts of the more polar solvent. The fractions were examined by TLC and 6 combined fractions (Fr.1-6) were obtained: Fr.1 (1.5 g) as dark yellow viscous oil, Fr.2 (26.5 g) as dark green viscous material, Fr.3 (2.7 g) as brown solid, Fr.4 (16 g) as green solid, Fr. 5 (0.7 g) as dark brown solid, Fr.6 (0.8 g) as dark brown solid. TLC and IR spectrum of Fr. 1 showed the presence of fatty acid.

1.2.1 Isolation of compound A (**Betulinic acid**, sss5971)

Fraction 2 (26.5 g) was chromatographed over silica gel column (finer than 40 µm, 150 g) with *n*-hexane, DCM and MeOH as eluting solvent, with increasing amount of the more polar solvent to give 7 subfractions. Subfraction 4 (11.9 g) was separated by CC (eluted with *n*-hexane, DCM, EtOAc and MeOH), to afford 5 subfractions (fr. 4a-4e). Fraction 4b (10.7 mg) was reCC (eluted with *n*-hexane, EtOAc and MeOH), to give 4 subfractions (fr. 4b.1-4b.4). Fraction 4b.1 (3.2 g) was reCC (eluting with *n*-hexane, EtOAc and MeOH), to obtain 3 subfractions (fr. 4b.1.1-4b.1.3). Fraction 4b.1.2 (2.5 g) was further CC eluting with *n*-hexane-EtOAc (1% increment of EtOAc, each 100 mL), to afford compound **A** (fractions 235-276, 500 mg) as major compound. This compound was a colorless solid and subsequently identified as betulinic acid. TLC of fractions 3-5 showed the presence of the major triterpene, betulinic acid. Recrystallization of these fractions in DCM-MeOH (1:1) gave colorless needles of betulinic acid (20 g) (see Scheme 2).

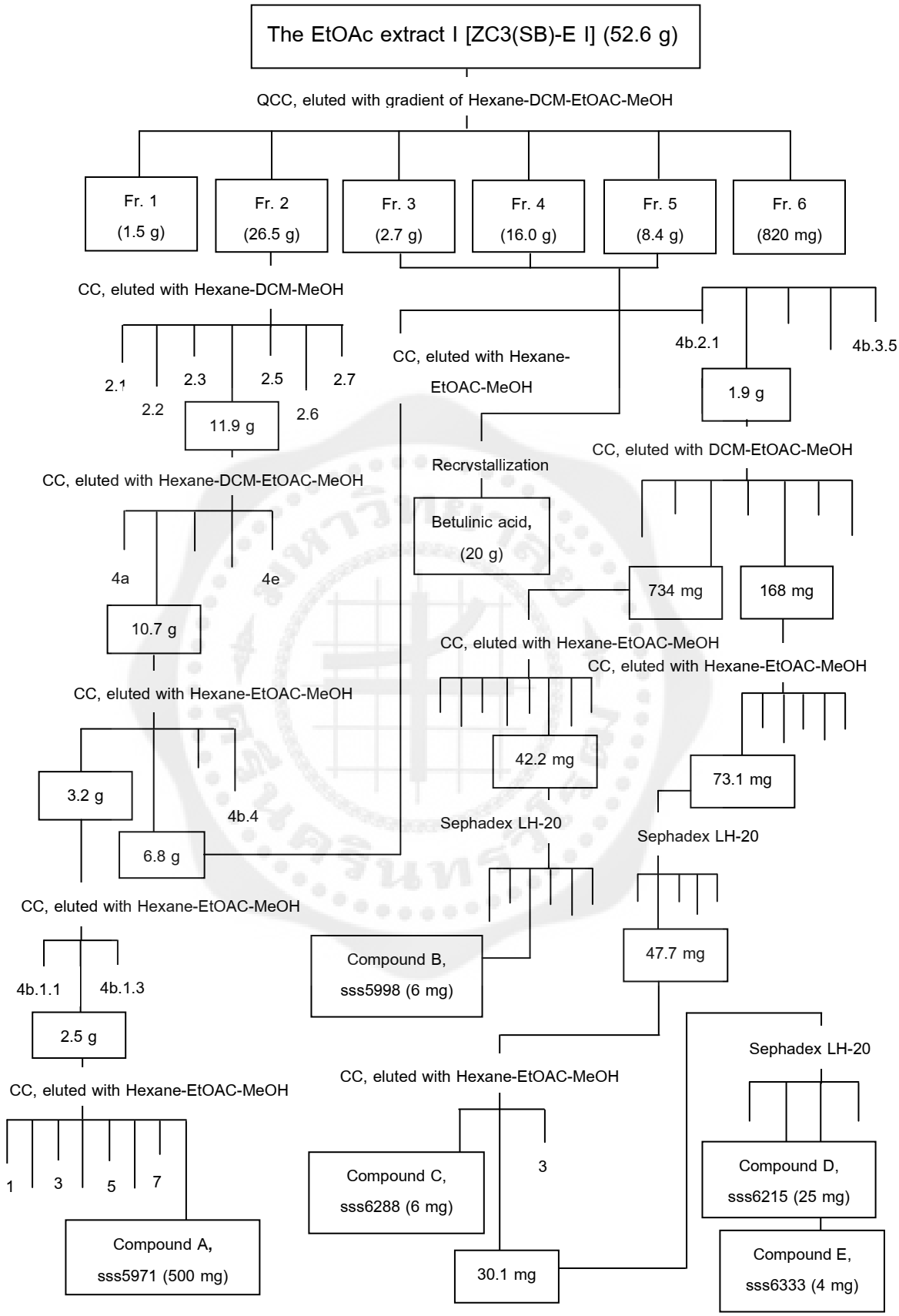
1.2.2 Isolation of compound B (**Methyl 3-*O*-protocatechuoylceanothate**, sss5998)

Subgroup 4b.2 (6.8 g) was rechromatographed over silica (finer than 40 µm, 60 g) with DCM, EtOAc and MeOH as eluting solvent to give 5 subfractions (fr. 4b.2.1-4b.2.5). Subfraction 4b.2.2 (1.9 g) was reCC (eluted with *n*-hexane, EtOAc and MeOH), to provide 7 subfraction (fr. 4b.2.2.1-4b.2.2.7). Subfractions 4b.2.2.3 (734 mg) was reCC using *n*-hexane, EtOAc and MeOH as eluents, with increasing amount of the more polar solvent to obtain 8 subfractions (fr. 4b.2.2.3.1-4b.2.2.3.8). Subfractions 4b.2.2.3.6 (42.2 mg) was subjected to size-exclusion column chromatography over sephadex LH-20 eluted with MeOH to yield compound **B** (fractions 32-35, 6 mg) as a colorless amorphous

solid. Compound **B** was identified as Methyl 3-*O*-protocatechuoylceanothate (see Scheme 2).

1.2.3 Isolation of compounds C, D and E (**Lupeol**, sss6288; **Ceanothic** acid, sss6215 and ***p*-hydroxybenzoic acid**, sss6333)

Subgroup 4b.2.2.5 (168.4 g) was rechromatographed over silica gel (less than 0.063 mm, 5 g) with *n*-hexane, EtOAc and MeOH as eluting solvent to give 6 subfractions (4b.2.2.5.1-4b.2.2.5.6). Subfraction 4b.2.2.5.1 (73.1 mg) was then separated by sephadex LH-20 (eluted with MeOH) to give 5 subfractions (4b.2.2.5.1.1-4b.2.2.5.1.5). Subfraction 4b.2.2.5.1.2 (47.7 mg) was further reCC (eluted with gradient of DCM, EtOAc and MeOH) to obtain 3 fractions (4b.2.2.5.1.2.1-4b.2.2.5.1.2.3). Subfraction 4b.2.2.5.1.2.1 to yield compound **C** (fractions 1-8, 6 mg) as a colorless amorphous solid. Compounds **C** was identified as lupeol. Subfraction 4b.2.2.5.1.2.2 (30.1 mg) followed by size-excluded over sephadex LH-20 (eluted with MeOH) to afford compound **D** (fractions 9-18, 25 mg) and compounds **E** (fractions 19-23, 4 mg) as colorless solid. Compound **D** and **E** were identified as ceanothic acid and *p*-hydroxybenzoic acid, repectively (see Scheme 2).



Scheme 2 Isolation of compounds from the EtOAc extract I of *Z. cambodiana* stem barks

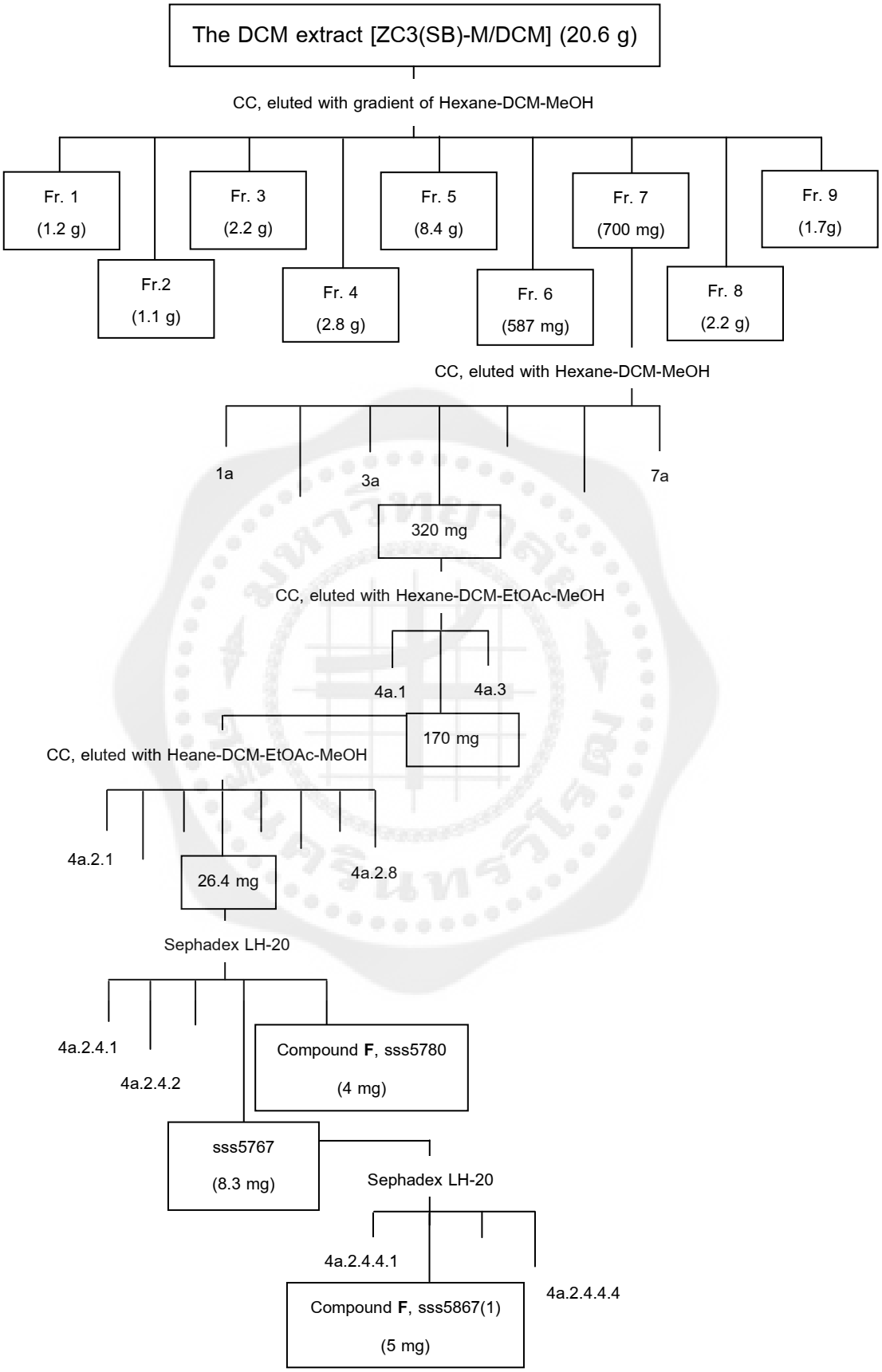
1.3 Isolation of compounds from the DCM extract of *Z. cambodiana* stem barks.

The DCM extract (20.6 g) was separated by CC (150 g of silica gel), eluted with a gradient systems of *n*-hexane-DCM, DCM-MeOH and MeOH (5% increment of more polar solvent, each 300 mL). The eluates were examined by TLC and 9 combined fractions (Fr.1-9) were obtained.

1.3.1 Isolation of compound F (**Cambodianine**, sss5867 (1) and sss5780)

Fraction 7 (700 mg) was subjected to silica gel chromatography (less than 0.063 mm, 20 g), eluted with DCM and MeOH (1% increment of MeOH, each 100 mL), to give 7 subfractions (1a-7a) as shown in Scheme 3.

Subfraction 4a (320.1 mg) was chromatographed on a silica gel column (10 g) eluted with *n*-hexane, DCM and MeOH with increasing amounts of the more polar solvent to afford 3 fractions (fr. 4a.1-4a.3). Subfraction 4a.2 (170 mg) was reCC (eluted with *n*-hexane, DCM, EtOAc and MeOH), to obtain 8 fractions (4a.2.1-4a.2.8). Subfraction 4a.2.4 (26.4 mg) followed by size-excluded over sephadex LH-20 (eluted with MeOH) to afford 5 subfractions (4a.2.4.1-4a.2.4.5). Subfraction 4a.2.4.5 was obtained as a colorless solid (fractions 82-92, 4 mg), this compound **F** was identified as cambodianine. Subfractions 4a.2.4.4 (8.3 mg) followed by sephadex LH-20 (eluted with MeOH) to yield compound **F** (fractions 15-37) as colorless solid (5 mg) cambodianine (see Scheme 3).



Scheme 3 Isolation of compounds from the DCM extract of *Z. cambodiana* stem barks

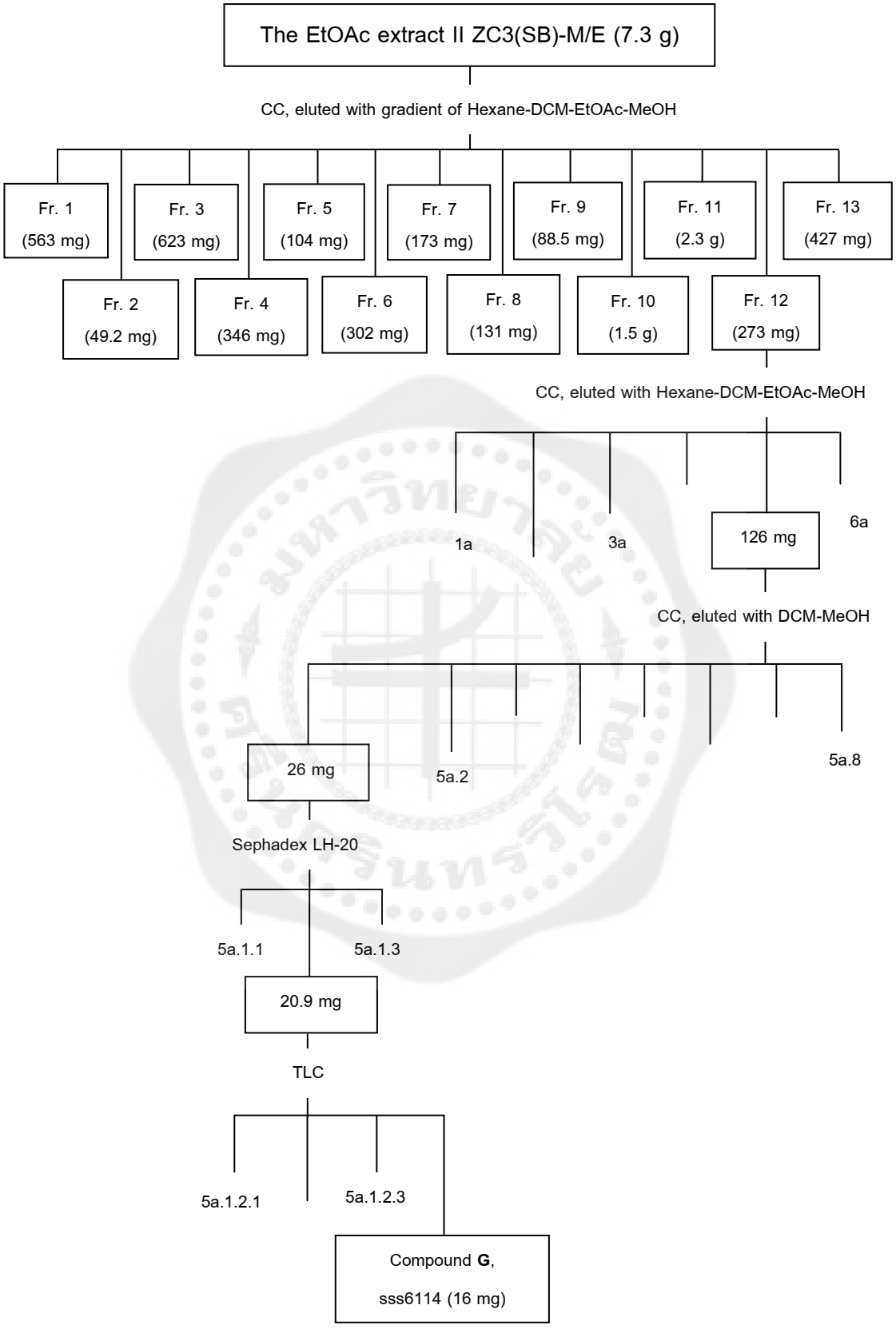
1.4 Isolation of compounds from the EtOAc extract II of *Z. cambodiana* stem barks.

The EtOAc extract II (7.8 g) was separated by CC, eluted with *n*-hexane, DCM, DCM-EtOAc and EtOAC-MeOH (2-5% increment of more polar solvent, each 200 mL). The eluates were examined by TLC and 13 combined fractions (Fr.1-13) were obtained.

1.4.1 Isolation of compound G (Ziziphine A, sss6114)

Fraction 12 (273.9 mg) was subjected to silica gel chromatography (less than 0.063 mm, 10 g), eluted with *n*-hexane, DCM and MeOH (5% increment of MeOH, each 100 mL), to give 6 subfractions (1a-6a) as shown in Scheme 4.

Subfraction 5a (126.2 mg) was reCC eluted with *n*-hexane, DCM and MeOH with increasing amounts of the more polar solvent to afford 8 fractions (fr. 5a.1-5a.8). Subfraction 5a.1 (26 mg) was then separated further by size-excluded over sephadex LH-20 (eluted with MeOH) to afford 3 subfractions (5a.1.1-5a.1.3). Subfraction 5a.1.2 was separated by TLC to yield compound **G** as colorless solid (16 mg) of ziziphine A.



Scheme 4 Isolation of compounds from the EtOAc extract II of *Z. cambodiana* stem barks

Physical and spectral data of the isolated compounds in EtOAc I extract

1. Compound A [**Betulinic acid**, sss5971 ZC3(SB)-E(I)-65/F8 (235-276)]

Colorless solid 20.5 g, soluble in DCM and a few drops of MeOH

mp : 280-282 °C [lit. 280-282 °C, (Panseeta and Suksamrarn, 2553)]

R_f : 0.76 (30% EtOAc-hexane), a violet coloration with anisaldehyde-H_2SO_4 reagent

^{1}H-NMR : δ ppm, 300 MHz, in $CDCl_3$ + CD_3OD 3 drops, Figure 21

δ_H 4.73 and 4.60 (2 X 1H, 2 x *br s*, H-29), 3.19 (1H, *dd*, *J* = 10.7, 5.6 Hz, H-3), 2.99 (1H, *m*, H-19), 1.68 (3H, *s*, H-30), 0.97 (3H, *s*, H-27), 0.96 (3H, *s*, H-23), 0.94 (3H, *s*, H-26), 0.82 (3H, *s*, H-25), 0.78 (3H, *s*, H-24), 0.68 (1H, *d*, *J* = 9.2 Hz, H-5)

2. Compound B [**Methyl 3-*O*-protocatechuoylceanothate**, sss5998 ZC3(SB)-E(I)-74/F3 (32-35)]

Colorless solid 6 mg, soluble in DCM and a few drops of MeOH

mp : 180-185 °C

R_f : 0.36 (30% EtOAc-hexane), a violet coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{27}$: -16.74 ° (c 0.16, MeOH)

UV (MeOH) λ_{max} (log ε) nm : 294 (3.98), 263(4.10), 216 (4.56) nm

IR (film) ν_{max} cm^{-1} : 3369, 2947, 1696, 1604, 1512, 1446, 1375, 1292, 1219, 981, 886 and 759

ESIMS (+ve) *m/z* (% rel. intensity) : 659.8 $[M+Na]^+$ (100)

HRTOFMS ($APCI^+$) : 659.3554 $[M+Na]^+$ (Calcd 659.3554 for $C_{38}H_{52}O_8$ + Na)

^{1}H-NMR : δ ppm, 300 MHz, in $CDCl_3$ + CD_3OD 2 drops; Table 19, Figure 22

^{13}C-NMR : δ ppm, 75 MHz, in $CDCl_3$ + CD_3OD 2 drops; Table 19, Figure 23

3. Compound C [**Lupeol**, sss6288 ZC3(SB)-E(I)-113/F1 (1-8)]

Colorless solid 6 mg, soluble in DCM or MeOH

mp : 198-200 °C [lit. mp 204-206 °C (Panseeta, 2009: 1-149)]

R_f : 0.86 (30% EtOAc-hexane), a violet coloration with anisaldehyde-H_2SO_4 reagent

^{1}H-NMR : δ ppm, 300 MHz, in $CDCl_3$, Figure 24

δ_H 4.68 and 4.56 (2 x 1H, 2 x br *s*, H-29), 3.19 (1H, *dd*, *J* = 10.8 Hz, 5.5 Hz, H-3), 2.36 (1H, *dt*, *J* = 11.1 Hz, 5.5 Hz, H-19), 1.68 (3H, *s*, H-30), 1.03 (3H, *s*, H-26), 0.97 (3H, *s*,

H-23), 0.94 (3H, *s*, H-27), 0.83 (3H, *s*, H-25), 0.79 (3H, *s*, H-28), 0.76 (3H, *s*, H-24), 0.68 (1H, *d*, *J* = 8.9 Hz, H-5)

4. Compound D [**Ceanothic acid**, sss6215 ZC3(SB)-E(I)-117/F2 (9-18)]

Colorless solid 25 mg, soluble in DCM and a few drops of MeOH

mp : 327-330 ^{o}C [lit. mp 326-329 ^{o}C (Panseeta, 2547: 1-73)]

R_f : 0.48 (30% EtOAc-hexane), a violet coloration with anisaldehyde-H_2SO_4 reagent

^{1}H-NMR : δ ppm, 300 MHz, in $CDCl_3$ + CD_3OD 4 drops, Figure 25

δ_H 4.70 and 4.57 (2 X 1H, 2 x br *s*, H-29), 4.14 (1H, *s*, H-3), 2.98 (1H, *m*, H-19), 2.52 (1H, *s*, H-1), 2.22 (1H, *m*, H-13), 1.66 (3H, *s*, H-30), 1.51 (1H, *m*, H-18), 1.10 (3H, *s*, H-23), 1.07 (3H, *s*, H-25), 0.95 (3H, *s*, H-24), 0.94 (3H, *s*, H-26), 0.91 (3H, *s*, H-27)

5. Compound E [***p*-Hydroxybenzoic acid**, sss6333 ZC3(SB)-E(I)-117/F3 (19-23)]

Colorless solid 4 mg, soluble in DCM and a few drops of MeOH

mp : 213-217 ^{o}C [lit. mp 214 ^{o}C (Yayli, et al. 2003: 749-755)]

R_f : 0.30 (30% EtOAc-hexane), colorless with anisaldehyde-H_2SO_4 reagent

IR (film) ν_{max} cm^{-1} : 3399, 2923, 2853, 1682, 1607, 1594, 1513, 1453, 1246, 1168, 937, 851, 796 and 618

ESMS (-ve) *m/z* (% rel. intensity) : 137.6 $[M-H]^-$(100), 275.3 $[2M-H]^-$(60)

^{1}H-NMR : δ ppm, 300 MHz, in $CDCl_3$ + CD_3OD 3 drops, Figure 26

δ_H 7.93 (2H, *d*, *J* = 8.6 Hz, H-3 and H-3'), 6.85 (2H, *d*, *J* = 8.6 Hz, H-4 and H-4')

^{13}C-NMR : δ ppm, 75 MHz, in $CDCl_3$ + CD_3OD 3 drops, Figure 27

δ_C 169.0 (C-1), 161.4 (C-5), 132.1 (C-3 and C-3'), 121.5 (C-2), 115.0 (C-4 and C-4')

Physical and spectral data of the isolated compounds from MeOH/DCM extract

6. Compound F [**Cambodianine**, sss5780 ZC3(SB)-M/DCM-42/F5 (82-92) and sss5867 (1) ZC3(SB)-M/DCM-42/F2 (15-37)]

Colorless solid 5 mg and 4 mg, soluble in DCM and a few drops of MeOH

mp : 244-246 ^{o}C

R_f : 0.48 (4% MeOH-DCM), a blue coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{29}$: -202 ^{o} (c 0.16, MeOH)

UV (MeOH) λ_{max} (log ε) nm : end absorption

IR (KBr) ν_{max} cm^{-1} : 3440, 3279, 2966, 2929, 1637, 1544, 1513, 1383, 1241, 989, 762, 708, 686

CD (MeOH) $\Delta\varepsilon$: 277 (+2.06), 236 (-17.65), 202 (-72.61)

ESIMS sss5867 (1) (+ve) *m/z* (% rel. intensity) : 584.9 $[M+H]^+$(100)

ESIMS sss5780 (+ve) *m/z* (% rel. intensity) : 584.9 $[M+H]^+$(100)

HRTOFMS (APCI, +ve) *m/z* : 584.3802 $[M+H]^+$ (Calcd 584.3806 for $C_{32}H_{49}N_5O_5$ + H)

^{1}H-NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 21, Figure 28

^{13}C-NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 21, Figure 29

Physical and spectral data of the isolated compounds from MeOH/EtOAc II extract

7. Compound G [**Ziziphine A**, sss6114 ZC3(SB)-M/E(II)-99 /F4]

Colorless solid 16 mg, soluble in DCM or MeOH

mp : 121-124 ^{o}C [lit. mp.121-126 ^{o}C (Cassel, B. K.; Eckhard, E. U- and Tschesch, R. 1973: 2461-2466)]

R_f : 0.64 (4% MeOH-DCM), a blue coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{30}$: -418.4 ^{o} (c 0.19, MeOH)

CD (MeOH) $\Delta\varepsilon$: 318 (-14.60), 259 (-32.99), 215 (-9.27)

UV (MeOH) λ_{max} (log ε) nm : 319 (4.45) and 268 (4.60) nm

IR (film) ν_{max} cm^{-1} : 3396, 3289, 2962, 2930, 2874, 1689, 1643, 1510, 1419, 1223, 1105, 1053, 754, 665

EIMS (+ve) *m/z* (% rel. intensity) : 634.9 $[M+Na]^+$ (87), 612.9 $[M+H]^+$(68)

^{1}H-NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 24, Figure 30

^{13}C-NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 24, Figure 31

CHAPTER 4

RESULTS AND DISCUSSION

The ethyl acetate extract of the dried stem barks of *Z. cambodiana* was investigated by column chromatographic methods to give a new triterpene ester (compound **B**), two known lupane-type triterpenes betulinic acid (**A**) and lupeol (**C**), a known ceanothane-type, ceanothic acid (**D**) and *p*-hydroxybenzoic acid (**E**) (Table 17). From the methanol extract a previously reported cyclopeptide, ziziphine A (**G**) and a new alkaloid (**F**) (Table 18) were obtained. The known compounds were determined mainly based on their NMR data analysis, and by comparison with the previous reported data.

The structures of the new compounds were established based on their spectroscopic (UV, IR, MS and NMR) data analysis and by comparison their data with the related compounds.

Table 17 Compounds isolated from the EtOAc extract of *Z. cambodiana*

Compounds		**Weight (mg)**
Compound **A** [sss5971, ZC3(SB)-E(I)-65/F8 (235-276)]	Betulinic acid	20.5 g
Compound **B** [sss5998, ZC3(SB)-E(I)-74/F3 (32-35)]	Methyl 3-*O*-protocatechuoylceanothate	6 mg
Compound **C** [sss6288, ZC3(SB)-E(I)-113/F1 (1-8)]	Lupeol	6 mg
Compound **D** [sss6215, ZC3(SB)-E(I)-117/F2 (9-18)]	Ceanothic acid	25 mg
Compound **E** [sss6333, ZC3(SB)-E(I)-117/F3 (19-23)]	*p*-Hydroxybenzoic acid	4 mg

Table 18 Compounds isolated from the MeOH extract of *Z. cambodiana*

Compounds		**Weight (mg)**
Compound **F** [sss5867 (1), ZC3(SB)-M/DCM 42/F2 (15-37)] [sss5780, ZC3(SB)-M/DCM 32/F2 (82-92)]	Cambodianine	9 mg
Compound **G** [sss6114, ZC3(SB)-M/E(II)-99/F4]	Ziziphine A	16 mg

1. Structure determination of the known compounds, triterpenes (A and C-D), the new triterpene (B) and a phenolic acid, *p*-hydroxybenzoic acid (E)

1.1 Compound A [**Betulinic acid**, sss5971 ZC3(SB)-E(I)-65/F8 (235-276)]

The major ompound **A** was obtained as colorless solid. It gave a violet spot with anisaldehyde reagent on TLC. The ^{1}H-NMR spectrum showed three characteristic singlet signals for isopropenyl group at δ_H 4.73 (1H, br *s*), 4.60 (1H, br *s*) of H-29 and 1.68 (methyl-30) and five additional singlet methyls (δ_H 0.78, 0.82, 0.94, 0.96 and 0.97 ppm) of the ring triterpene. A carbinol proton H-3 at δ_H 3.19 (*dd, J* = 10.7, 5.6 Hz), including a number of methine and methylene protons suggested that compound **A** was a lupane-type triterpene. This compound was identified as betulinic acid by comparison of its spectroscopic data with those reported in literature (Panseeta; & Suksamrarn, 2010: 106-118), and by TLC comparison with the authentic betulinic acid. The structure of compound **A** was thus identified as 3β-hydroxy-lup-20(29)-en-28-oic acid or betulinic acid.

Betulinic acid was a widely distributed pentacyclic lupane-type triterpene in *Ziziphus* plants and commonly isolated as the major triterpene of *Z. oenoplia* (Aunchai, 2002: 1-116), *Z. rugosa* (Netsawangwicha. 2005: 1-106), *Z. cambodiana* (Panseeta. 2004: 1-74) and *Z. mauritiana* (Panseeta. 2009: 1-149).

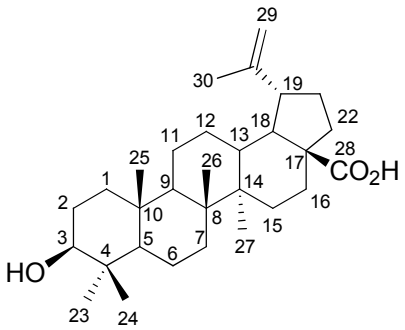


Figure 6 Structure of compound **A**

1.2 Compound C [**Lupeol**, sss6288 ZC3(SB)-E(I)-113/F1 (1-8)]

The minor compound **C** was obtained as a colorless solid, mp 198-200 °C, with the R_f value of 0.86 (30% EtOAc-hexane) and also gave a violet coloration with anisaldehyde-H_2SO_4 reagent on TLC, indicating it was a triterpene. The ^{1}H-NMR spectrum of compound **C** showed a lupane-type triterpene pattern for six singlet methyls at δ_H 0.76, 0.79, 0.83, 0.94, 0.97 and 1.03 ppm, a isopropenyl group at δ_H 4.68, 4.56 and 1.68 ppm. From the ^{1}H-NMR spectrum pattern and chromatographic comparisons with the authentic lupeol in several solvent systems, the structure of compound **C** was identified as lup-20(29)-en-3β-ol or lupeol. Lupeol was a commonly triterpene found in plants including *Ziziphus* such as *Z. rugosa* (Netsawangwicha. 2005: 1-106), *Z. cambodiana* (Panseeta. 2004: 1-74; Suksamrarn, et al. 2006: 535-537), and *Z. mauritiana* (Panseeta. 2009: 1-149).

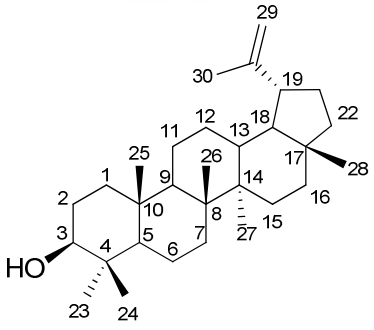


Figure 7 Structure of compound **C**

1.3 Compound D [**Ceanothic acid**, sss6215 ZC3(SB)-E(I)-117/F2 (9-18)]

Compound **D** was obtained as a colorless solid, with high polarity [R_f 0.48 (30% EtOAc-hexane)] and also gave a violet coloration with anisaldehyde-H_2SO_4 reagent. The ^{1}H-NMR spectrum revealed the presence of three singlets of isopropenyl group [δ_H 4.70, 4.14 and 1.66], five singlet methyls (δ_H 0.91, 0.94, 0.95, 1.07 and 1.10 ppm) and two singlet signals for carbinol protons at δ_H 4.14 (H-3) and 2.52 (H-1), which were characterized of with a ceanothane-type triterpene found in *Ziziphus* species. The ^{1}H-NMR data of compound **D** is the same as that of ceanothic acid, the major ceanothane-type triterpene. From the physical data and chromatographic comparison with the authentic ceanothic acid in several solvent systems, led to conclude that the structure of compound **D** was assigned as ceanothic acid (**7**). Ceanothic acid or 2α-carboxy-3β-hydroxy-A(1)-norlup-20(29)-en-28-oic acid has been isolated from the root of *Z. jujuba* Mill var. *spinosa* (Lee; Lin; & Chen. 1995: 511-519), *Z. rugosa* (Netsawangwicha. 2005: 1-106) and *Z. cambodiana* (Panseeta. 2004:1-73; Suksamrarn, et al. 2006: 535-537).

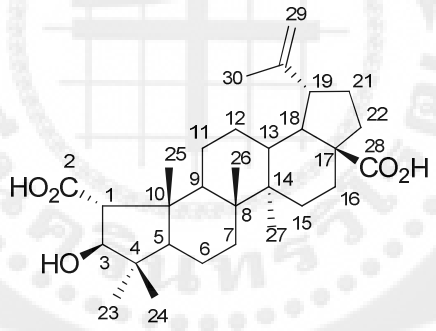


Figure 8 Structure of compound **D**

1.4. Compound B [**Methyl 3-*O*-protocatechuoylceanothate**, sss5998 ZC3(SB)-E(I)-74/F3 (32-35)]

Compound **B** was the most polar triterpene obtained from this plant with the R_f value of 0.46 (30% EtOAc-hexane) as a colorless solid. It also gave a violet coloration with anisaldehyde-H_2SO_4 reagent on TLC indicating of a triterpenoid-type compound. Its HRTOFMS showed a pseudomolecular ion at *m/z* 659.3554 $[M+Na]^+$ corresponding to the formula of $C_{38}H_{52}O_8$. The IR absorption bands for hydroxyl (3369 cm^{-1}), carbonyl ester conjugated (1696 cm^{-1}) and aromatic (1604-1512 cm^{-1}) functionalities were observed. Its ^{1}H-NMR spectrum (Table 19) revealed a typical ceanothane-type triterpene for an isopropenyl group (δ_H 4.71, 4.58, 1.65), five singlet methyls (δ_H 1.23, 1.11, 0.96, 0.94, 0.94), as well as two broad singlet methine protons at δ_H 5.26 and 2.68 coupled to each other as suggested by its COSY spectrum. In the ^{13}C-NMR and DEPT spectra, 38 carbon signals were observed, including six methyls (δ_C 14.7, 16.5, 18.4, 19.4, 19.8 and 30.4), one methoxyl (δ_C 51.3), nine methylenes, ten methines, and ten quaternary carbons, as well as two ester carbonyls (δ_C 176.9 and 166.6) and one carboxylic acid (δ_C 176.4) carbon signals (Table 19). The presence of an AA'X trisubstituted phenyl type of the 3,4-dihydroxyphenyl moiety in the molecule was supported by a broad singlet at δ_H 7.45 (δ_C 116.1) and two doublets at δ_H 6.85 (δ_C 114.6) and 7.44 (δ_C 116.1) with *J* coupling constant of 8.7 Hz in its NMR data. The NMR spectral data of compound **B** is similar to those of ceanothic acid, except for a down field shift of H-3 (δ_H 5.26) in **B** compared with that of H-3 (δ_H 4.14) of ceanothic acid, as well as from the HMBC correlations exhibited between H-3 and C-7' (δ_C 166.6), C-1 (δ_C 64.4) and C-2 (δ_C 176.4) in its HMBC spectrum, suggesting the 3,4-dihydroxybenzoyl moiety located at C-3 in compound **B**. In the NMR data, the presence of a carbonyl methyl ester was evidenced at δ_H 3.67 and δ_C 51.3 and δ_C 176.9 (CO) together with its HMBC connection displayed between C-28 and the methoxyl group in the HMBC spectrum supporting the placement of this ester group at the C-28. Selected COSY, NOESY and HMBC correlations were shown in Figure 10 and Table 19. NMR data of **B** was also similar to that of 3-*O*-vanillyl ceanothic acid, a related ceanothic acid derivative (Table 20) found

the in root bark of this plant (Suksamrarn, et al. 2006: 535-537). Complete ^{1}H- and ^{13}C-NMR data assignments for compound **B** were made by 1D- and 2D-NMR spectral data analysis and by comparison with those of ceanothic acid (Lee; Su; & Liu. 1991: 615-618) and 3-*O*-vanillylceanothic acid (**9**) (Figure 12, Table 20). Compound **B**, therefore, was elucidated as methyl 3-*O*-(3,4-dihydroxybenzoyl)ceanothate or methyl of 3-*O*-protocatechuoylceanothate. Ceanothane-type triterpenes such as ceanothic acid (**7**) (Lee; Su; & Liu. 1991: 615-618), 3-*O*-vanillylceanothic acid (**9**) (Suksamrarn, et al. 2006: 535-537) and 3-*O*-protocatechuoylceanothic acid (**63**) (Lee; Lin; & Liu. 1996: 847-851) exhibit levorotatory optical rotation, including that of compound **B** ($[\alpha]_D^{27}$ -16.74°). This evidence led to conclusion that compound **B** shares the same stereochemistry as those of ceanothic acid derivatives.

To our knowledge, the triterpene ester compound **B** is the new naturally occurring aromatic acid ester of the ceanothane-type triterpene. The previous ones being 2-*O*-*E*-*p*-coumaroylceanothanolic acid (**78**), 3-*O*-protocatechuoylceanothic acid (**63**), 3-*O*-(3,4-dimethoxybenzoyl)ceanothic acid dimethyl ester (**77**) (Lee; Lin; & Liu. 1996: 847-851) and 3-*O*-vanillylceanothic acid (**9**) (Suksamrarn, et al. 2006: 535-537) are presented in Figure 11.

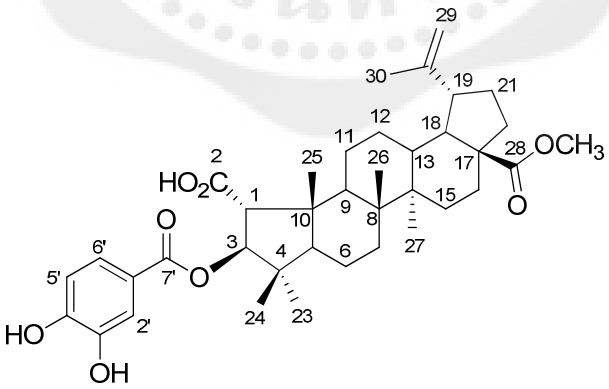


Figure 9 Structure of compound **B**

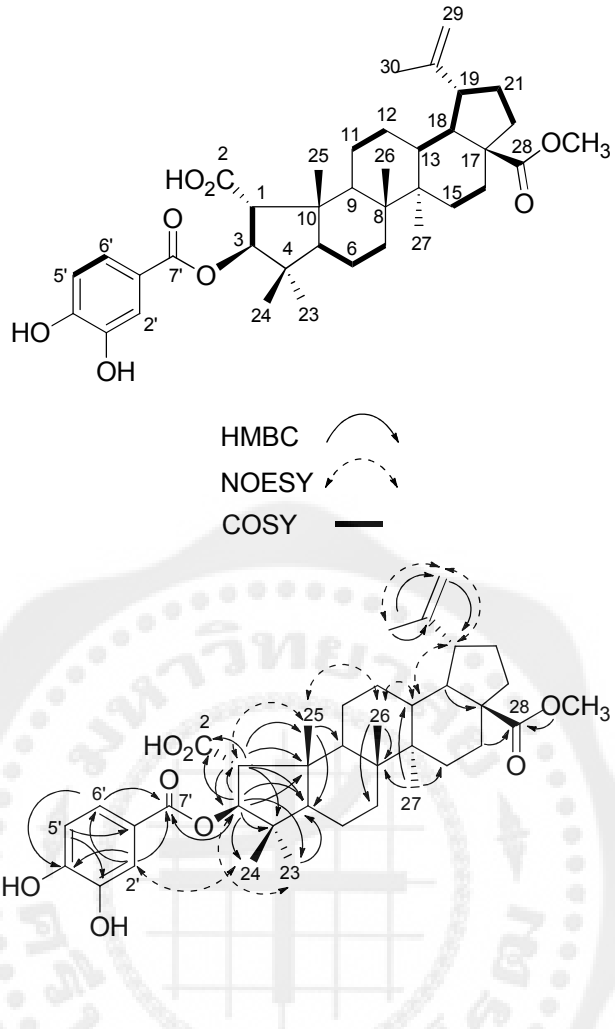
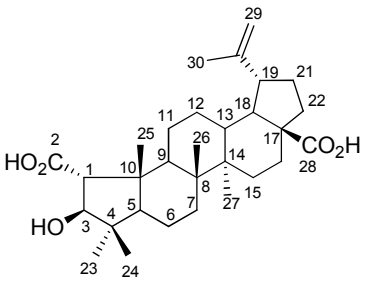
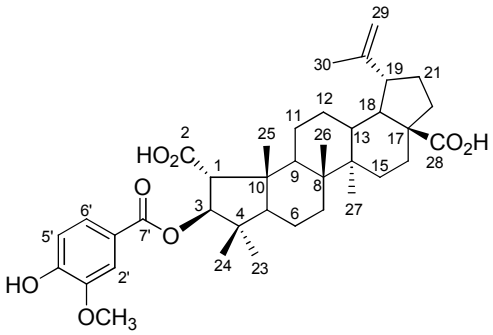


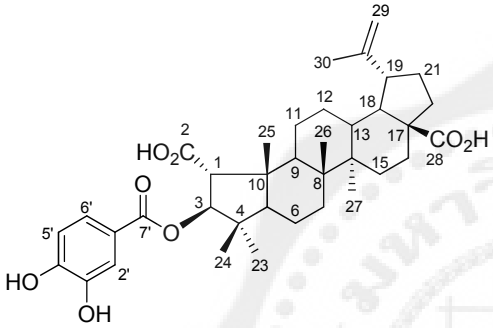
Figure 10 Selected COSY, HMBC and NOESY correlations of compound **B**



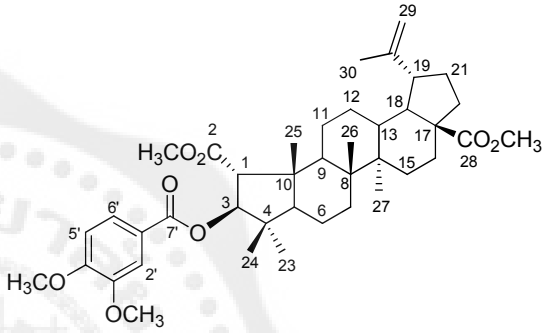
Ceanothic acid (**7**)



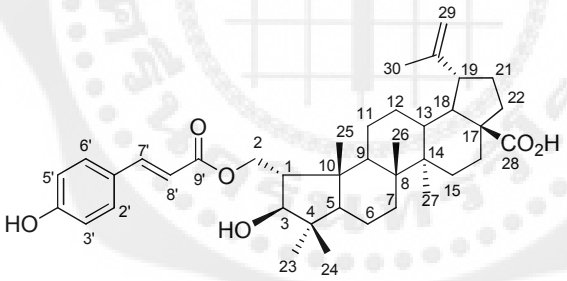
3-*O*-Vanillylceanothic acid (**9**)



3-*O*-Protocatechuoylceanothic acid (**63**)



3-*O*-(3,4-Dimethoxybenzoyl) ceanothic acid dimethyl ester (**77**)



2-*O*-*E*-*p*-Coumaroylceanothanolic acid (**78**)

Figure 11 Structure of ceanothic acid (**7**) and its ester derivatives (**9**, **63**, **77** and **78**)

Table 19 ^{1}H-, ^{13}C-NMR and 2D NMR data of compound **B** in $CDCl_3$ + CD_3OD 2 drops

Position	δ_H	δ_C	HMBC correlations	NOESY
1	2.68 *s*	63.4	C-2, C-3, C-4, C-5, C-10, C-25	H-25
2	-	176.4	-	-
3	5.26 *s*	85.9	C-2, C-4, C-5, C-10, C-23, C-24, C-7'	H-1, H-23
4	-	43.3	-	-
5	1.77 *m*	56.5	-	-
6	1.37 *m*	18.4	-	-
7	1.31 *m*	34.0	-	-
8	-	43.0	-	-
9	1.77 *m*	44.6	-	-
10	-	49.5	-	-
11	α 1.72 *m*, β 1.52 *m*	23.5	-	-
12	α 1.72 *m*, β 1.05 *m*	25.4	-	-
13	2.18 *m*	38.7	-	H-19, H-26
14	-	41.7	-	-
15	α 1.42 *m*, β 0.96 *m*	29.7	-	-
16	α 2.23 br *d* (*J* = 9.8 Hz) β 1.17 *m*	32.3	C-28	-
17	-	56.6	-	-
18	1.56 *m*	49.5	C-13, C-17, C-19, C-20, C-28	H-27
19	2.93 *m*	47.0	C-21, C-19, C-30	H-13, H-29
20	-	149.7	-	-
21	α 1.68 *m*, β 1.30 *m*	30.7	-	-
22	α 1.90 *m*, β 1.20 *m*	37.0	-	-
23	1.23 *s*	30.4	C-3, C-4, C-5, C-24	H-3, H-5
24	0.96 *s*	19.8	C-3, C-4, C-5, C-23	-
25	1.11 *s*	18.4	C-1, C-5, C-9, C-10	H-26
26	0.94 *s*	16.5	C-7, C-8, C-14	H-13
27	0.94 *s*	14.7	C-8, C-13	-
28	-	176.9	-	-
29	α 4.71 br *s*, β 4.58 br *s*	109.6	C-19, C-20, C-30	H-18, H-19, H-30
30	1.65 *s*	19.4	C-19, C-20, C-29	H-19, H-29
1'	-	121.9	-	-
2'	7.44 br *s*	116.1	C-3', C-4', C-7'	H-1, H-24, H-25
3'	-	144.1	-	-
4'	-	150.4	-	-
5'	6.85 br *d* (*J* = 8.7 Hz)	114.6	C-1', C-3', C-4	-
6'	7.45 br *d* (*J* = 8.7 Hz)	123.0	C-2', C-4'	H-5
7'	-	166.6	-	-
OCH_3	3.67 *s*	51.3	C-28	-

Table 20 Comparison of ^{1}H- and ^{13}C-NMR data of compound **B** with 3-*O*-vanillylceanothic acid (**9**)

Position	δ_H		δ_C	
	3-*O*-Vanillylceanothic acid[a] (C_5D_5N)	Compound **B** ($CDCl_3$ + 2 drops CD_3OD)	3-*O*-Vanillylceanothic acid[a]	Compound **B**
1	3.15 *s*	2.68 *s*	64.3	63.4
2	-	-	176.8	176.4
3	5.94 *s*	5.26 *s*	86.0	85.9
4	-	-	43.8	43.3
5	2.18 *m*	1.77 *m*	56.5	56.5
6	1.43 *m,*	1.37 *m*	18.8	18.4
7	1.38 *m*	1.31 *m*	34.6	34.0
8	-	-	43.5	43.0
9	2.12 *m*	1.77 *m*	45.4	44.6
10	-	-	49.6	49.5
11	α 2.15 *m*[b], β 1.58 *m*	α 1.68 *m*[c], β 1.52 *m*	24.2	23.5
12	α 1.97 *m*[b], β 1.23 *m*	α 1.72[c], β 1.05 *m*	26.3	25.4
13	2.74 (1H, br *t, J* = 10.1Hz)	2.18 *m*	39.1	38.7
14	-	-	42.2	41.7
15	α 1.86 *m,* β 1.20 *m*	α 1.39 *m,* β 1.08 *m*	30.5	29.7
16	α 2.57 br *d* (*J* = 12.3 Hz) β 1.4 *m*	α 2.23 br *d* (*J* = 9.8 Hz) β 1.17 *m*	32.9	32.3
17	-	-	56.6	56.6
18	1.66 *m*	1.56 *m*	49.6	49.5
19	3.47 *m*	2.93 *m*	47.6	47.0
20	-	-	151.1	149.7
21	α 2.17 *m*, β 1.20 *m*	α 1.68 *m*, β 1.30 *m*	31.3	30.7
22	α 2.18 *m,* β 1.51 *m*	α 1.90 *m*, β 1.20 *m*	37.6	37.0
23	1.53 *s*	1.23 *s*	30.7	30.4
24	1.11 *s*	0.96 *s*	20.3	19.8
25	1.23 *s*	1.11 *s*	18.5	18.4
26	0.10 *s*	0.94 *s*	17.1	16.5
27	1.02 *s*	0.94 *s*	15.0	14.7
28	-	-	179.1	176.9
29	α 4.82 br *s*, β 4.62 br *s*	α 4.71 br *s*, β 4.58 br *s*	109.8	109.6
30	1.63 *s*	1.65 *s*	19.7	19.4
1’	-	-	121.9	121.9
2’	7.86 br *s*	7.44 br *s*	116.4	116.1
3’	-	-	148.5	144.1
4’	-	-	153.4	150.4
5’	7.28 br *d* (*J* = 8.2 Hz)	6.85 br *d* (*J* = 8.7 Hz)	113.5	114.6

Table 20 (Continued)

Position	δ_H		δ_C	
	3-*O*-vanillylceanothic acid [a]	Compound **B**	3-*O*-Vanillylceanothic acid [a]	Compound **B**
6'	7.90 br *d* (*J* = 8.2 Hz)	7.45 br *d* (*J* = 8.7 Hz)	124.6	123.0
7'	-	-	166.3	166.6
OCH_3	3.75 *s*	3.67 *s*	55.8	51.3

[a] Suksamrarn, et al. 2006: 535-537

[b,c] Interchangeable within the column.

1.5 Compound E [***p*-Hydroxybenzoic acid**, sss6333 ZC3(SB)-E(I)-117/F3 (19-23)]

Compound **E** was obtained as a yellow solid, with high polarity [R_f 0.30 (30% EtOAc-hexane)]. Its ESMS spectrum showed the molecular ion at *m/z* 137.6 $[M-H]^-$, corresponding to the formula $C_7H_6O_3$. This compound exhibited IR absorption band for hydroxyl (3399 cm^{-1}), carboxyl (1682 cm^{-1}), aromatic (1607-1453 cm^{-1}) and a *para* substituted (851cm^{-1}) aromatic functionalities. Its ^{1}H-NMR spectrum displayed two AX doublet signals (*J* = 8.6 Hz), at δ_H 7.93 and 6.85, suggesting for the presence of a 1,4-disubstituted aromatic system in the structure. The ^{13}C-NMR, DEPT and HMQC spectra of compound **E** indicated the presence of 7 carbon resonances, providing signals for one carboxylic acid at δ_C 169.0, two quaternary carbons at δ_C 161.4 and 121.5, two methine aromatic carbon signals at δ_C 132.1 (C-3/C-3') and 115.0 (C-4/C-4'). The HMBC correlation of H-3/3' to C-1 confirmed that the carboxyl was place at C-2, as well as correlations of H-3/3' to C-3/3' and of C-5; H-4/4' to C-4/4', C-2 and C-5 (Figure 14) were also observed. From the NMR data analysis compound **E** was characterised as a *para*-disubstituted phenolic acid. Comparison of its NMR data with that of the literature values (Yayli. 2003: 749-755), led to identify that the structure of compound **E** was *p*-hydroxybenzoic acid. *p*-Hydroxybenzoic acid has been isolated from the fruits and seeds of *Z. mauritiana* (Memon, et al. 2012: 123-128) and fruits of *Z. jujube* (Siriamornpun, et al. 2015: 246-255). This is the first report of *p*-hydroxybenzoic acid found in *Z. cambodiana*.

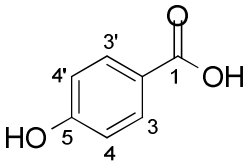


Figure 12 Structure of compound **E**

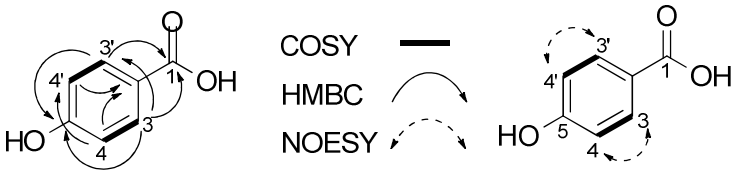


Figure 13 Selected HMBC, COSY and NOESY correlations for compound **E**

2. Structure determination of cyclopeptide alkaloid (compounds F and G) the MeOH extract of *Z. cambodiana*

2.1 Compound F [**Cambodianine**, sss5867 (1) ZC3(SB)-M/DCM-42/F2 (15-37) and sss5780 ZC3(SB)-M/DCM-42/F5 (82-92)]

Compound **F** was isolated as a colorless solid, mp 244-246 ^{o}C and gave violet-blue coloration with anisaldehyde-H_2SO_4 reagent indicating for a cyclopeptide alkaloid. On the basis of its ESIMS *m/z* 584.9 $[M+H]^+$(100) and HRTOFMS at m/z 584.3802 $[M+H]^+$, the molecular formula $C_{32}H_{49}N_5O_5$ was then established. No UV absorption band was observed which was consistent with the 14-membered type cyclopeptide alkaloid (Gournelis; Laskaris; & Verpoorte. 1998: 1-179). Their IR spectra exhibited characteristic peaks for amino (3279 cm^{-1}), amide (1637 cm^{-1}) and aryl ether (1241 cm^{-1}) functions. The ^{1}H-, ^{13}C-NMR (Table 21), DEPT and HMQC spectra of compound **F** indicated for the presence of 32 carbon resonances, which provide signals for six methyls, five methylenes, fourteen methines and six quaternary carbons including four carbonyls. Its ^{1}H-NMR spectrum exhibited signals corresponding to *Z*-olefinic protons of a styrylamine at 6.*43 d* and 6.58 with a *J* couling constant of 7.3 Hz, a number of aromatic, methine, methylene and methyl protons including a singlet of *N*-methyl proton at δ_H 2.28. From the ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC and HMBC data analysis of compound **F** and comparison with the reported values led to a conclusion for the presence of *p*-oxystyrylamine group [δ_H 6.42 (H-1), 6.58 (H-2),

7.11 (H-12), 7.04 (H-13), 7.02 (H-15), 7.15 (H-16); δ_C 118.4 (C-1), 125.5 (C-2), 155.9 (C-11), 122.6 (C-12), 130.0 (C-13), 131.6 (C-14), 131.5 (C-15), 121.9 (C-16)], β-OH-leucine [δ_H 4.49 (H-8), 4.95 (H-9), 1.98 (H-21), 0.91 (H-22), 1.22 (H-23), 7.53 (H-24); δ_C 171.7 (C-7), 60.0 (C-8), 81.2 (C-9), 29.2 (C-21), 14.7 (C-22), 20.4 (C-23)], isoleucine [δ_H 3.98 (H-5), 5.75 (H-6), 2.08 (H-17), 1.18 and 0.80 (H-18), 0.82 (H-19), 0.73 (H-20); δ_C 167.3 (C-4), 59.3 (C-5), 35.1 (C-17), 23.9 (C-18), 11.8 (C-19), 15.9 (C-20)], proline [δ_H 4.50 (H-26), 2.33 and 1.82 (H-27), 2.12 and 2.02 (H-28), 3.55 (H-29) ; δ_C 170.9 (C-25), 55.7 (C-26), 27.3 (C-27), 25.3 (C-28), 47.7 (C-29)], and *N*-methylisoleucine amino acid [δ_H 3.13 (H-32), 1.65 (H-33), 1.65 and 0.87 (H-34), 0.92 (H-35), 0.97 (H-36), 2.28 (NCH_3); δ_C 175.6 (C-31), 66.3 (C-32), 37.7 (C-33), 24.7 (C-34), 11.4 (C-35), 15.7 (C-36), 35.0 (NCH_3)] units (Table 21). Analysis of the COSY, HMBC and NOESY spectra data provided the connections among these subunits (Figure 16) as follows:

1. The signal for styrylamino NH-3 (δ_H 6.45) showed NOESY correlations to signals at δ_H 3.98 (H-5) and δ_H 5.75 (NH-6) and the HMBC correlations of the resonances at δ_H 6.58 (H-2) to C-14 (δ_C 131.6), H-3 to C-4 (δ_C 167.3) and of H-5 to C-7 (δ_C 171.7) allowed the placement of an isoleucine moiety next to the styrylamine group.

2. The NOESY connectivities of H-8 to H-13 (δ_H 7.04) and of H-9 to H-16 (δ_H 7.15) and NH-24 (δ_H 7.53) revealed the location of hydroxyl leucine next to styrylamine unit. The NOESY cross-peak for methine proton H-9 to aromatic signal at δ_H 7.11 (H-12) was also observed.

3. The NOESY correlation of NH-6 (δ_H 5.75) to H-8 (δ_H 4.49) and H-23 (δ_H 1.22) and of NH-24 (δ_H 7.53) to H-20 (δ_H 0.73) permitted the connection between β-OH-leucine and isoleucine units.

4. NOE enhancements displayed between NH-24 (δ_H 7.53) to H-8 and H-26 in the NOESY spectrum, and the HMBC of the H-26 to C-27 (δ_C 27.3) supported that the proline unit was attached to the β-OH-leucine at NH-24.

5. HMBC correlation of H-32 to carbonyl group at C-31 (δ_C 175.6) and NCH_3 signal (δ_C 35.0) in addition to NOE enhancement of H-32 to H-29 in the NOESY spectrum confirmed the place unit of N-methyl isoleucine fragment next to proline amino acid.

The NMR data of the cyclic part in **F** was very similar to that of discarine E (**79**) (Machado; Filho; & Orel. 1995: 548-553) (table 22) which has the same subunits of the macrocyclic ring.

The CD spectrum of compound **F** displayed a negative and a weak positive Cotton effect bands at 236 and 277 nm, respectively, consistent with the 5*S*, 8*S*, 9*S*-configurations presented in the 14-membered ring nucleus (Gournelis; Laskaris; & Verpoorte. 1998: 1-179). In general, Rhamnaceous cyclopeptide alkaloids compose of L amino acid subunits and display levorotatory optical rotations (Panseeta, et al. 2011: 909-915; Suksamrarn, et al. 2005: 1175-1180) including compound **F** ($[\alpha]_D^{29}$ -202 °). These evidences led to the conclusion that the structure and stereochemistry of compound **F** was elucidated as shown and named cambodianine after its plant origin.

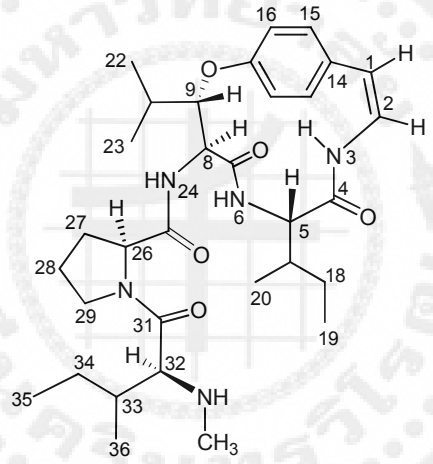


Figure 14 Structure and stereochemistry of compound **F**

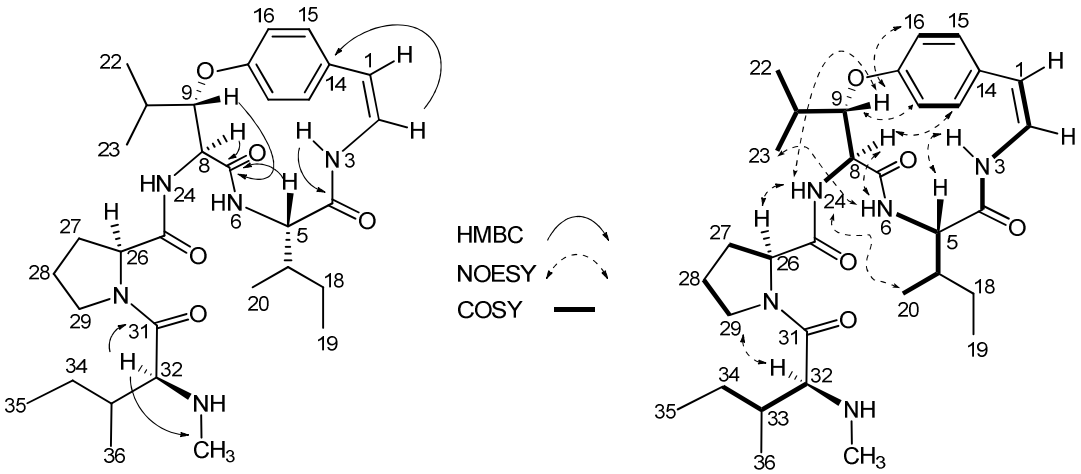


Figure 15 Key HMBC, COSY and NOESY correlations for compound **F**

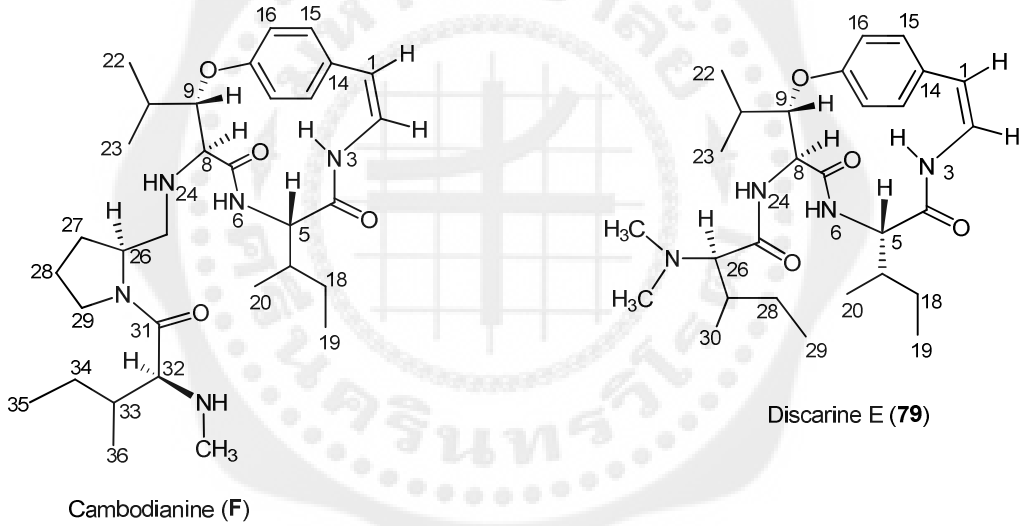


Figure 16 Structures of compound **F** and discarine E (**79**)

Table 21 ^{1}H-, ^{13}C-NMR and 2D NMR data of compound **F** in $CDCl_3$ + CD_3OD 2 drops

Position	δ_H	δ_C	HMBC correlations	NOESY correlations
1	6.42 *d* (*J* = 7.3 Hz)	118.4	C-2, C-14, C-15	-
2	6.58 br*dd* (*J* = 8.1, 7.3 Hz)	125.5	C-14	-
NH-3	6.45 *d* (*J* = 8.1 Hz)	-	C-4	H-5, NH-6
4	-	167.3	-	-
5	3.98 *dd* (*J* = 8.1, 4.5 Hz)	59.3	C-7	H-17, H-20
NH-6	5.75 *d* (*J* = 8.0 Hz)	-	-	NH-3, H-5, H-8, H-13
7	-	171.7	-	-
8	4.49 *m*	60.0	C-7	H-22
9	4.95 br*d* (*J* = 6.7 Hz)	81.2	C-7	H-16, H-21, H-23, NH-24
11	-	155.9	-	-
12	7.11 *dd* (*J* = 6.9, 1.2)	122.6	C-11	H-9
13	7.04 *dd* (*J* = 6.9, 1.4)	130.0	C-14	H-3, H-8, H-9
14	-	131.6	-	-
15	7.02 *dd* (*J* = 6.9, 1.4)	131.5	-	H-1
16	7.15 *dd* (*J* = 6.9, 1.2)	121.9	-	-
17	2.08 *m*	35.1	-	H-5, H-19, H-20
18	α 1.18 *m* [a]	23.9	-	-
	β 0.80 *m* [a]		-	-
19	0.82 *t* (*J* = 7.0 Hz)	11.8	C-17, C-18	H-17
20	0.73 *d* (*J* = 6.8 Hz)	15.9	C-17, C-18	H-5, H-17
21	1.98 *m*	29.2	-	H-9, H-22, H-23
22	0.91 *d* (*J* = 7.0 Hz)	14.7	C-9, C-21, C-23	H-21, H-23
23	1.22 *d* (*J* = 6.7 Hz)	20.4	C-9, C-21, C-22	H-9, H-21
NH-24	7.53 *d* (*J* = 9.5 Hz)	-	-	H-8, H-9, H-21, H-26
25	-	170.9	-	-
26	4.50 *m*	55.7	C-27	H-27
27	α 2.33 *m*	27.3	-	H-26
	β 1.82 *m*		-	-
28	α 2.12 *m*	25.3	-	H-29
	β 2.02 *m*		-	-
29	3.55 *dd* (*J* = 7.8, 5.3 Hz)	47.7	-	H-28, H-32, H-33
30	-	-	-	-
31	-	175.6	-	-
32	3.13 *d* (*J* = 6.1 Hz)	66.3	C-31, C-33, NCH_3	H-34, H-36, NCH_3
33	1.65 *m* [a]	37.8	-	H-29
34	α 1.65 *m* [a]	24.7	-	H-35
	β 0.87 *m* [a]		-	-
35	0.92 *t* (*J* = 8.7 Hz)	11.4	C-36, C-32	-
36	0.97 *d* (*J* = 6.8 Hz)	15.7	C-34	H-32
NCH_3	2.28 *s*	35.0	C-32	H-20, H-32, H-36

[a] Assignments confirmed by COSY.

Table 22 Comparison of ^{1}H-and ^{13}C-NMR data of compound **F** with discarine E (**79**)

Position	δ_H		δ_C	
	Compound **F**	Discarine E	Compound **F**	Discarine E
1	6.42 *d* (*J* = 7.3 Hz)	6.65 *dd*	118.4	119.5
2	6.58 br*dd* (*J* = 8,1, 7.3	6.20 *dd*	125.5	
NH-3	6.45 *d* (*J* = 8.1 Hz)	7.65 *d*	-	-
4	-	-	167.3	168.8
5	3.98 *dd* (*J* = 8.1, 4.5 Hz)	3.90 *dd*	59.3	51.6
NH-6	5.75 *d* (*J* = 8.0 Hz)	7.25 *d*	-	-
7		-	171.7	171.9
8	4.49 *m*	4.40 *dd*	60.0	55.9
9	4.95 br*d* (*J* = 6.7 Hz)	4.80 *dd*	81.2	82.0
11	-	-	155.9	155.9
12	7.11 *dd* (*J* = 6.9, 1.2)	6.80-7.00 *m*	122.6	125.5
13	7.04 *dd* (*J* = 6.9, 1.4)	6.80-7.00 *m*	130.0	129.1
14	-	-	131.6	130.8
15	7.02 *dd* (*J* = 6.9, 1.4)	6.80-7.00 *m*	131.5	128.6
16	7.15 *dd* (*J* = 6.9, 1.2)	6.80-7.00 *m*	121.9	121.5
17	2.08 *m*	1.28 *m*	35.1	33.5
18	α 1.18 *m*, β 0.80 *m* [a]	1.40 *m*	23.9	24.0
19	0.82 *t* (*J* = 7.0 Hz)	0.75 *t*	11.8	10.6
20	0.73 *d* (*J* = 6.8 Hz)	0.70 *d*	15.9	14.5
21	1.98 *m*	2.25 *d*	29.2	
22	0.91 *d* (*J* = 7.1 Hz)	0.90 *d*	14.7	14.2
23	1.22 *d* (*J* = 6.7 Hz)	1.10 *d*	20.4	19.5
NH-24	7.53 *d* (*J* = 9.5 Hz)	8.10 *d*	-	-
25	-	-	170.8	172.8
26	4.50 *m*	2.75 *d*	55.8	62.0
27	α 2.33 *m*, β 1.82 *m*	1.70 *dd*	27.3	33.9
28	α 2.12 *m*, β 2.02 *m*	1.50 *m*, 1.60 *m*	25.3	26.5
29	3.55 *dd* (*J* = 7.8, 5.3 Hz)	0.80 *t*	47.7	11.9
30	-	0.65 *d*	-	14.6
31	-	-	175.6	-
32	3.13 *d* (*J* = 6.1 Hz)	-	66.3	-
33	1.65 *m* [a]	-	37.7	-
34	α 1.65 *m*, β 0.87 *m* [a]	-	24.7	-
35	0.92 *t* (*J* = 8.7 Hz)	-	11.4	-
36	0.97 *d* (*J* = 6.8 Hz)	-	15.7	-
$N(CH_3)_2$	-	2.20 *s*	-	41.9
NCH_3	2.28 *s*	-	35.0	-

2.2 Compound G [Ziziphine A, sss6114 ZC3(SB)-M/E(II)-99 /F4]

The major cyclopeptide, compound **G,** was obtained as a colorless solid, mp 121-124 ^{o}C and also gave a violet-blue coloration with anisaldehyde-H_2SO_4 reagent. Its IR spectrum showed absorption band for amino (3396-3289 cm^{-1}), amide (1643 cm^{-1}), aromatic (1510 cm^{-1}) and phenol ether (1223 cm^{-1}) functional groups. The UV spectrum showed absorption maxima at 268 and 319 nm, which are typical bands of 13-membered ring cyclopeptide alkaloids (Suksamrarn; et al. 2005: 1175-1180). Its molecular formula was established as $C_{33}H_{46}N_5O_6$ by ESIMS at *m/z* 612.9 $[M+H]^+$, and in combination with the ^{13}C-NMR data (Table 23). Compound **G** exhibited the following signals in its ^{1}H-NMR spectrum: two olefinic, three aromatic, one methoxyl and a number of methine and methylene protons (Table **23**). Thirty-three carbon signals were observed in its ^{13}C-NMR and DEPT spectra including four methyls, one *N*,*N*-dimethyl, one methoxyl, seven methylenes, twelve methines (one oxygenated and two olefinic) and seven quaternary carbons (four amide carbonyl groups). The assignments of the spin systems for amino acid units were conducted by analysis of ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC spectra of compound **G** and comparison with the reported value (Suksamrarn; et al. 2005: 1175-1180). These were identified as proline [δ_H 4.38 (H-8), 5.25 (H-9), 2.35 and 2.44 (H-20), 4.30 and 3.62 (H-21); δ_C 171.5 (C-7), 62.7 (C-8), 78.7 (C-9), 32.7 (C-20), 45.6 (C-21)], 3-oxygenated proline [δ_H 4.53 (H-5), 4.24 and 3.31 (H-19), 2.35 and 2.44 (H-20), 4.30 and 3.62 (H-21); δ_C 167.8 (C-4), 62.2 (C-5), 47.9 (C-19), 32.7 (C-20), 45.6 (C-21)], isoleucine [δ_H 4.56 (H-24), 1.81 (H-25), 1.55 and 1.20 (H-26), 0.94 (H-27), 0.90 (H-28); δ_C 172.3 (C-23), 53.8 (C-24), 37.1 (C-25), 27.7 (C-26), 12.0 (C-27), 15.1(C-28)] and *N,N*-dimethylisoleucine [δ_H 2.57 (H-31), 1.79 (H-32), 1.16 and 0.85 (H-33), 0.87 (H-34), 0.88 (H-35), 3.80 (OCH_3); δ_C 74.6 (C-31), 34.4 (C-32), 25.0 (C-33), 10.6 (C-34), 14.7 (C-35), 56.0 (OCH_3)] (Table 23). Analysis of HMBC and NOESY spectra (Figure 18 and Table 23) provided connections among these subgroups. The *m*-oxystyrylamino-NH signal showed HMBC cross peaks with C-1 and C-4. HMBC cross-peaks of H-2 to C-1, C-4 and C-13 and of H-1 to C-2, C-12 and C-14 were also observed. The connection between the proline and β-oxyproline amino acids was revealed by the observed HMBC correlations of H-5, H-8 and H-9 to C-7. Furthermore, HMBC correlation from H-9 to C-11 confirmed that the β-oxyproline unit was attached to the aryl group. The methoxyl group at δ_H 3.80 was confirmed to place at C-14 (δ_C151.3) in the HMBC experiment. The H-8 proton (δ_H 4.38) and the isoleucyl protons H-24 (δ_H 4.56) showed

connectivities with C-23 (δ_C 172.3) in which the latter signal proton also exhibited cross-peaks with C-30 (δ_C 171.1) in HMBC spectrum. In the NOESY spectrum, the NOE interactions displayed between H-24 (δ_H 4.56) and H-20α (δ_H 2.35) further confirmed the placement of the isoleucine unit attached to the hydroxyproline amino acid. The HMBC correlations of NH-29 (δ_H 6.91) to C-24 (δ_C 53.8) and C-30 (δ_C 171.1) and of the isoleucyl proton H-31 to C-30, C-32, C-35 and *N,N*-dimethyl carbon were also observed. Thus the connections between the intermediate isoleucyl group and the hydroxyprolyl *N* (N-22) as well as the *N,N*-dimethylisoleucyl unit were established. NMR data of compound **G** are very similar to that of ziziphine A (Gournelis; Laskaris; & Verpoorte. 1998: 1-179 and Thchesche; Kaussmann; & Eckhardt. 1973: 2577-2580). Based on these findings the structural framework of compound **G** was proved to be ziziphine A.

The coupling constant value of 8.8 Hz for H-1 and H-2 established the *Z*-double bond. The small vicinal coupling constant value of 6.1 Hz for the methine protons H-8 and H-9 together with no significant NOE observed in NOESY spectrum between both protons indicated a *trans* configuration (Auvin, et al.1996: 676-678; Jossang; Zahir; & Diakite. 1996: 565-567). The CD spectrum of compound **G** displayed three negative Cotton effect bands at 318, 259 and 215 nm which was similar to that of mauritine M (**45**) (Panseeta, 2011:909-915), a 13-membered ring alkaloid found in *Z.mauritiana,* and consistent with the 5*S*, 8*S* and 9*S*-configurations determination in the 13-membered ring cyclopeptide alkaloid (Gournelis; Laskaris; & Verpoorte. 1998:1-179). Furthermore, most 5(13)-cyclopeptide alkaloids of *Ziziphus* plants compose of L-amino acids and show levorotary optical rotations (Cassels; Eckhardt; Kaussmann; & Tschesche: 1974: 2461-2466) including that of compound **G** ($[\alpha]_D^{30}$ = -418.4 $^{\circ}$). Thus the stereochemical structure of **G** was established as shown in Figure 19 which composed of all L-amino acid subunits. Ziziphine A has been previously isolated from *Z. oenoplia* (Cassels; Eckhardt; Kaussmann; & Tschesche: 1974: 2461-2466).

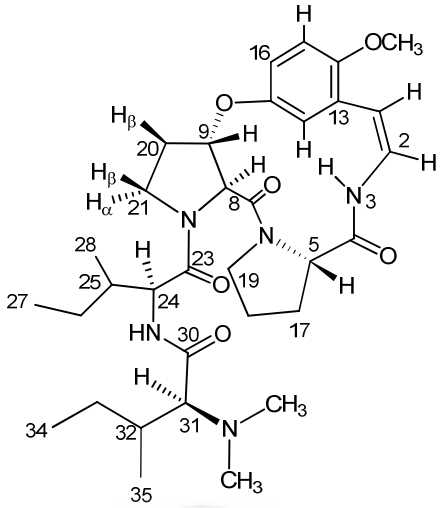


Figure 17 Structure and stereochemistry of compound **G**

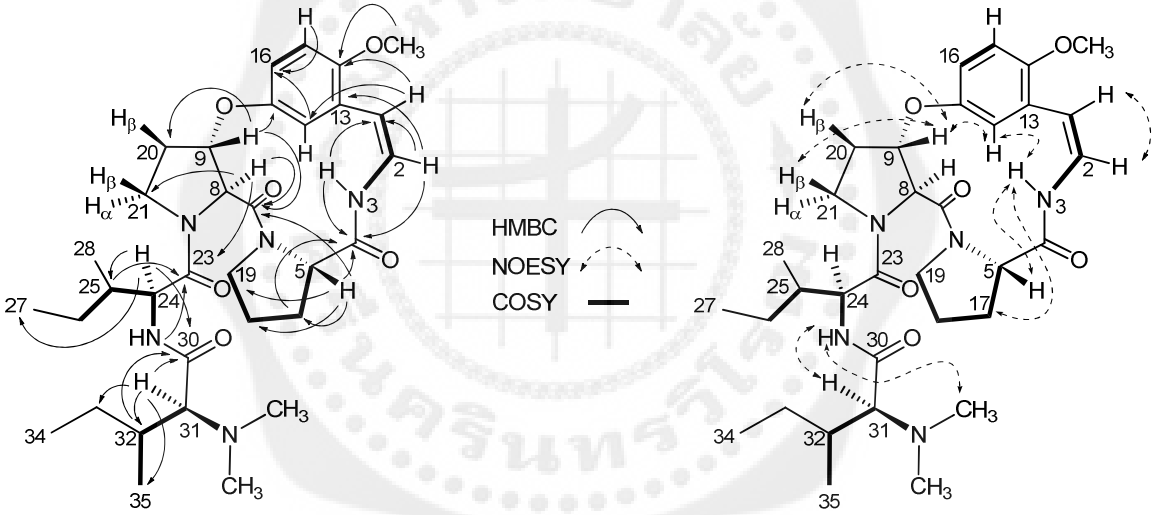


Figure 18 Selected HMBC, COSY and NOESY correlations for compound **G**

Table 23 ^{1}H-, ^{13}C-NMR and 2D NMR data of compound **G** in $CDCl_3$

position	δ_H	δ_C	HMBC correlations	NOESY correlations
1	5.95 *d* (*J* = 8.8 Hz)	106.7	C-2, C-12, C-14	H-2, OCH_3,
2	6.93 *dd* (*J* = 11.3, 8.8 Hz)	121.7	C-1, C-4, C-13	H-1
NH-3	8.36 *d* (*J* = 11.3 Hz)	-	C-1, C-4	H-1, H-5, H-12, H-17
4	-	167.8	-	-
5	4.53 br *t* (*J* = 8.5, 2.5 Hz)	62.2	C-4, C-7, C-17, C-18	H-17, H-20
6	-	-	-	-
7	-	171.5	-	-
8	4.38 *d* (*J* = 6.1 Hz)	62.7	C-7, C-9, C-21, C-23	-
9	5.25 *dt* (*J* = 9.7, 6.1 Hz)	78.7	C-7, C-11, C-15, C-20	H-12, H-20, H-21
11	-	151.0	-	-
12	6.82 *d* (*J* = 2.6 Hz)	110.9	C-1, C-11, C-16	H-9
13	-	124.2	-	-
14	-	151.3	-	-
15	6.89 *d* (*J* = 6.0 Hz)	113.9	C-12, C-16	OCH_3
16	6.81 *d* (*J* = 6.0 Hz)	116.9	C-12	-
17	α 2.22 *m*, β 1.96 *m*	29.0	C-18	-
18	α 1.99 *m*, β 1.53 *m*	25.0	-	H-5, H-19
19	α 4.24 *m*, β 3.31 *m*	47.9	C-17, C-18	-
20	α 2.35 *m*, β 2.44 *m*	32.7	C-8, C-9	H-9, H-21
21	α 4.30 *m*, β 3.62 *m*	45.6	C-8, C-9, C-20	H-20
22	-	-	-	-
23	-	171.1	-	-
24	4.56 br *t* (*J* = 8.8)	53.8	C-25, C-27, C-30	H-20, H-25
25	1.81 *m* [b]	34.4 [c]	C-27, C-28	H-24
26	α 1.55 *m*, β 1.20 *m*	27.0	C-24, C-27, C-28	H-24
27	0.94 *t* (*J* = 14.4, 7.3 Hz)	12.0	-	-
28	0.90 *d* (*J* = 6.2 Hz)	15.1	C-25, C-26	H-26
NH-29	6.91 *d* (*J* = 8.6 Hz)	-	C-24, C-30	H-23, H-31, $N(CH_3)_2$
30	-	172.3	C-23	-
31	2.57 *d* (*J* = 5.5 Hz)	74.6	C-26, C-30, C-32, C-35, $N(CH_3)_2$	H-25, H-26, H-32, $N(CH_3)_2$
32	1.79 *m* [b]	37.7 [c]	-	H-34
33	α 1.16 *m*, β 0.85 *m*	25.0	C-31, C-35	-
34	0.87 *t* (*J* = 10.4, 7.5 Hz)	10.6	C-31, C-32, C-33	H-32
35	0.88 *d* (*J* = 6.9 Hz)	14.7	-	-
$N(CH_3)_2$	2.23 *s*	43.1	C-31	H-31, H-32
OCH_3	3.80 *s*	56.0	C-14	H-1, H-15

[b,C] Interchangeable within the same column

Table 24 Comparison of ^{1}H- and ^{13}C-NMR data of compound **G** with ziziphine A in $CDCl_3$

position	δ_H		δ_C	
	Compound **G**	Zizyphine A	Compound **G**	Ziziphine A [a]
1	5.95 *d* (*J* = 8.8 Hz)	-	106.7	106.9
2	6.93 *dd* (*J* = 11.3, 8.8 Hz)	-	121.7	121.9
NH-3	8.36 *d* (*J* = 11.3 Hz)	-	-	-
4	-	-	167.8	167.9
5	4.53 br *t* (*J* = 8.5, 2.5 Hz)	-	62.2	62.3
6	-	-	-	-
7	-	-	171.5	171.2
8	4.38 *d* (*J* = 9.7 Hz)	-	62.7	63.1
9	5.25 *dt* (*J* = 9.7, 6.1 Hz)	-	78.7	79.1
11	-	-	151.0	151.7
12	6.82 *d* (*J* = 2.6 Hz)	-	110.9	111.3
13	-	-	124.2	124.8
14	-	-	151.3	151.4
15	6.89 *d* (*J* = 6.0 Hz)	-	113.9	111.4
16	6.81 *d* (*J* = 6.0 Hz)	-	116.9	117.2
17	α 2.22 *m*, β 1.96 *m*	-	29.0	29.2
18	α 1.99 *m*, β 1.53 *m*	-	25.0	25.1
19	α 4.24 *m*, β 3.31 *m*	-	47.9	48.0
20	α 2.35 *m*, β 2.44 *m*	-	32.7	32.9
21	α 4.30 *m*, β 3.62 *m*	-	45.6	45.9
22	-	-	-	-
23	-	-	171.1	171.6
24	4.56 br *t* (*J* = 8.8)	-	53.8	54.1
25	1.81 *m* [b]	-	34.4 [c]	34.6
26	α 1.55 *m*, β 1.20 *m*	-	27.0	25.1
27	0.94 *t* (*J* = 14.46, 7.33 Hz)	-	12.0	10.8
28	0.90 *d* (*J* = 6.28 Hz)	-	15.1	15.0
29	6.91 *d* (*J* = 8.64 Hz)	-	-	-
30	-	-	172.3	171.9
31	2.57 *d* (*J* = 5.50 Hz)	-	74.6	74.5
32	1.79 *m* [b]	-	37.7 [c]	37.4
33	α 1.16 *m*, β 0.85 *m*	-	25.0	27.1
34	0.87 *t* (*J* = 10.4, 7.5 Hz)	-	10.6	11.9
35	0.88 *d* (*J* = 6.91 Hz)	-	14.7	15.4
N(CH_3)	2.23 *s*	-	43.1	43.0
OCH_3	3.80 *s*	-	56.0	56.3

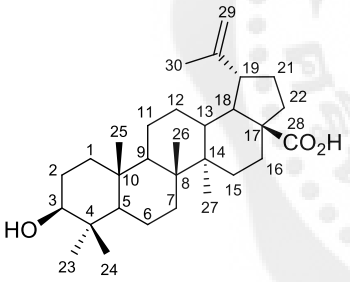
[a] (Cassels; Eckhardt; Kaussmann; & Tschesche: 1974: 2461-2466)

[b,c] Interchangeable within a column

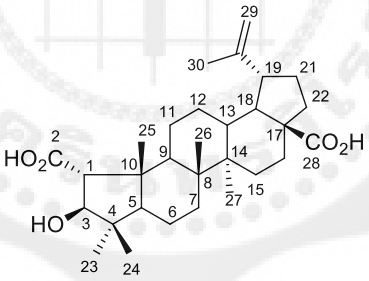
CHAPTER 5

CONCLUSION

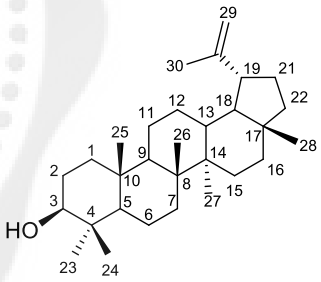
Phytochemicals investigation of the *Z. cambodiana* stem barks led to the isolation from ethyl acetate extract of one new triterpene ester, named methyl 3-*O*-protocatechuoylceanothate (compound **B**). In addition, two known lupane-type triterpenes, betulinic acid (compound **A**) and lupeol (compound **C**), a ceanothane-type, ceanothic acid (compound **C**) and *p*-hydroxybenzoic acid (compound **E**) were obtained. From the methanol extract, a new 5(14)-scutianine A-type cyclopeptide alkaloid, named cambodianine (compound **F**), together with a known 5(13)-ziziphine A-type alkaloid, zizyphine A (compound **G**) were isolated. The structures of the new compounds were elucidated by spectroscopic techniques, whilst the known compounds were identified by comparisons of spectroscopic data with those of reported values and by chromatographic comparison (TLC) with authentic samples in several solvent systems.



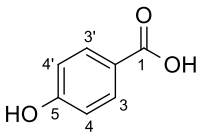
Compound **A** (Betulinic acid)



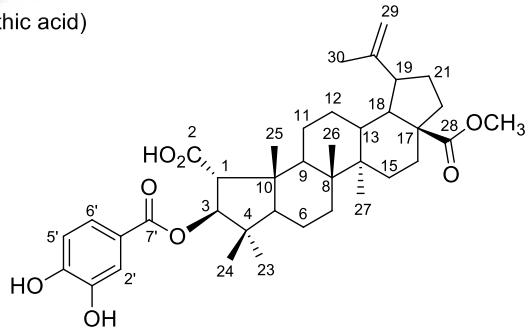
Compound **D** (Ceanothic acid)



Compound **C** (Lupeol)

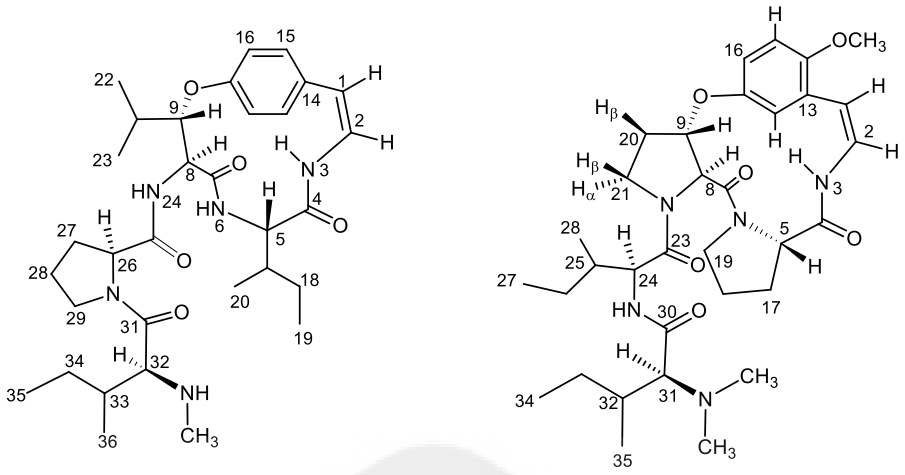


Compound **E** (*p*-Hydroxybenzoic acid)



Compound **B** (Methyl 3-*O*-protocatechuoylceanothate)

Figure 19 Compounds obtained from the EtOAc extract of isolated *Ziziphus cambodiana* stem barks



Compound **F** (Cambodianine) Compound **G** (Ziziphine A)

Figure 20 Compounds isolated from the MeOH extract of *Ziziphus cambodiana* stem barks

BIBLIOGRAPHY

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Acharya, S. B.; Tripathi, S. K.; Tripathi, Y. C.; Pandey, V. B. (1988). Some pharmacological studies on *Ziziphus rugosa* saponins. *Indian J. Pharmacol*. 20: 200-202.

Adzu, B.; Amos, S.; Wambebe, C.; Gamaniel, K. (2001). Antinociceptive activity of *Ziziphus spina-christi* root bark extract. *Fitoterapia*. 72: 344-350.

Arai, M. A.; Tateno, C.; Hosoya, T.; Koyano, T.; Kowithayakorn, T.; Ishibashi, M. (2008). Hedgehog/GLI-mediated transcriptional inhibitors from *Zizyphus cambodiana*. *Bioorg. Med. Chem*. 16(21): 9420-9424.

Aunchai, N. (2002). Study on the chemical constituents of *Ziziphus oenoplia* (Linn.) Mill. root [Dissertation M.Sc. Biological Chemistry]. Bangkok: Srinakharinwirot University; p. 1-116.

Auvin, C.; Lezenven, F.; Blond, A.; Augeven-Bour, I.; Pousset , J-. L.; Bodo, B. (1996). Mucronine J, a 14-membered cyclopeptide alkaloid from *Ziziphus mucronata*. *J Nat Prod*. 59: 676-678.

Bhattachachryya, B.; & Johri, B. M. (1998). *Flowering plants: Taxonomy and phylogeny*. New Delhi: Narosa Publishing House; pp. 326-328.

Bunyapraphatsara, N.; & Chokechaijaroenporn, O. (1999). In: *Thai medicinal plants* vol.3. Bangkok: Mahidol University and National Center for Genetic Engineering and Biotechnology. p. 328-329.

Bunyapraphatsara, N.; & Chokechaijaroenporn, O. (2000). In: *Thai medicinal plants* vol.4. Bangkok: Mahidol University and National Center for Genetic Engineering and Biotechnology. p. 291-292.

Caro, M. S. B.; de Oliveira, L. H.; Ilha, V.; Burrow, R. A.; Dalcol, I. I.; & Morel, A. F. (2012). Absolute configuration of franganine. *J. Nat. Prod*. 75: 1220-1222.

Cassels, B. K.; et al. (1974). Cyclopeptide alkaloids of *Zizyphus oenoplia*. *Tetrahedron.* 30(15): 2461-2466.

Chauhan J. S.; & Srivastava, S. K. (1978). Chemical investigation of the stem of *Zizyphus mauritiana* (N.O. Rhamnaceae). *Proc Natl Acad Sci, India*, Sect A. 48(1): 6

Cheng, G.; Bai, Y.; Zhao, Y.; Tao, J.; Liu, Y.; Tu, G.; Ma, L.; Liaoc, N.; Xu, X. (2000). Flavonoids from *Ziziphus jujuba* Mill *var. spinosa*. *Tetrahedron*. 56: 8915-8920.

Chuakul, W.; & Jaikaew, C. (1998). *Thai J. Phytopharmacy*. 5: 28-55.

de Oliveira, P. L.; Tanaka, C. M. A.; Kato, L. I.; da Silva, C. C.; Medina, R. P.; Moraes, A. P.; Sabino, J. R.; de Oliveira, C. I. M. A. (2009). Amaiouine, a cyclopeptide alkaloid from the leaves of *Amaioua guianensis*. *J. Nat. Prod.* 72: 1195-1197.

Dias, G. C. D.; Gressler, V.; Hoenzel, S. C. S. M.; Silva, U. F.; Dalcol, I. I.; Morel, A. F. (2007). Constituents of the roots of *Melochia chamaedrys. Phytochemistry*. 68: 668-672.

El-Seedi, H. R.; Ulf Goransson, Z. M. H.; & Verpoorte, R. (2007). Cyclopeptide alkaloids. *Phytochemistry Rev*. 6: 143-165.

Emile, A.; Waikedre, J.; Herrenknecht, C.; Fourneau, C.; Gantier, J. C.; Hnawia, E.; Cabalion, P.; Hocquemiller, R.; Fournet, A.; (2007). Bioassay-guided isolation of antifungal alkaloids from *Melochia odorata. Phytother. Res.* 21(4): 398-400.

Ganapaty, S.; Thomas, P. S.; Ramana, K. V.; Karagianis, G.; Waterman, P. G. (2006). Dammarane and ceanothane triterpenes from *Zizyphus glabrata*. *Z. Naturforsch*. 61b: 87-92.

Gardner, S.; Sidisunthorn, P.; & Anusarnsunthorn, V. (2000). *A field guide to forest trees of northern Thailand*. Bangkok: Kobfai Publishing Project. p.130.

Gournelis, D. C.; Laskaris, G. G.; & Verpoorte, R. (1998). *Progress in the Chemistry of Organic Natural Products*; Herz, H. F. W., Kirby, G. W., Moore, R. E., Tamm, C., Eds.; Springer: New York. 75: 1-179.

Guo, S.; Duan, J.-a.; Tang, Y.; Su, S.; Shang, E.; Ni, S.; Qian, D. (2009). High-performance liquid chromatography-Two wavelength detection of triterpenoid acids from the fruits of *Ziziphus jujuba* containing various cultivars in different regions and classification using chemometric analysis. *J. Pharm. Biomed. Anal.* 49: 1296-1302.

Guo, S.; Duan, J.-a.; Tang, Y.; Qian, Y.; Zhao, J.; Qian, D. (2011).Triterpenoids from the fruits of *Ziziphus jujuba* var. *spinosa*. *Biochem. Sys. Ecol.* 39: 880-882.

Han, J.; Ji, C. J.; He, W. J.; Shen, Y.; Leng, Y.; Xu, W. Y.; Fan, J. T.; Zeng, G. Z.; Kong, L. D.; Tan, N. H. (2011). Cyclopeptide alkaloids from *Ziziphus apetala. J. Nat. Prod.* 74: 2571-2575.

He, F.; Pan, Q.; & Min, Z. (2006). A novel lupane triterpene from the seeds of *Ziziphus jujube* var. *spinosa*. *Zhongcaoyao*. 37: 168-171.

Huang, R. L.; Wang, W. Y.; Kuo, Y. H.; Lin, Y. L. (2001). Cytotoxic triterpenes from the fruit of *Ziziphus jujuba* [abstract]. *Chin. Pharm. J.* (Taipei); 53(4): 179-84, from SciFinder Scholar CAN 136:131533.

Hout, S.; Chea, A.; Bun, S. S.; Elias, R.; Gasquet, M.; Timon-David, P.; Balansard, G.; Azas, N. (2006). Screening of selected indigenous plants of Cambodia for antiplasmodial activity. *J. Ethnopharm*. 107: 12-18.

Inayat-ur-Rahman; Khan, M. A.; Arfan, M.; Akhtar, G.; Khan, L.; Ahmad, V. U. (2007). A new 14-membered cyclopeptide alkaloid from *Zizyphus oxyphylla*. *Nat. Prod. Res*. 21(3): 243-253.

Jossang, A.; Zahir, A.; & Diakite, D. (1996). Mauritine J, a cyclopeptide alkaloid from *Zizyphus mauritiana*. *Phytochemistry* 42(2): 565-567.

Joullié, M. M.; & David, J. R. (2004). Cyclopeptide alkaloids: chemistry and biology. *Chem. Commun.* 18: 2011-2015.

Kaleem, W. A.; Muhammad, N.; Qayum, M.; Khan, H.; Khan, A.; Aliberti, L.; De Feo, V. (2013). Antinociceptive activity of cyclopeptide alkaloids isolated from *Ziziphus oxyphylla* Edgew (Rhamnaceae), *Fitoterapia.* 91: 154-158.

Kaleem, W. A.; Nisar, M.; Qayum, M.; Zia-Ul-Haq, M.; Choudhary, M.; ERCİŞLİ, S. (2013). Urease inhibitory potential of *Zizyphus oxyphylla* Edgew. Extracts and isolated compounds. *Turk. J. Med. Sci.* 43: 497-500

Kaleem, W. A.; Nisar, M.; Qayum, M.; Zia-Ul-Haq, M.; Adhikari, A.; De Feo, V. (2012). New 14 membered cyclopeptide alkaloids from *Zizyphus oxyphylla* Edgew. *Int. J. Mol. Sci.* 13: 11520-11529.

Kirtikar, K. R.; & Basu B. D. (1994). Indian Medicinal Plants. *Dehradun, India*. 3: 170.

Kulshreshtha, M. J.; & Rastogi, R. P. (1972). Chemical constituents of *Zizyphus rugosa*. *Indian J. Chem.* 10: 152-154.

Lee, S. M.; Park, J. G.; Lee, Y. H.; Lee, C. G.; Min, B. S.; Kim, J. H.; Lee, H. K. (2004). Anti-complementary activity of triterpenoides from fruits of *Zizyphus jujuba*. *Biol. Pharm. Bull.* 27: 1883-1886.

Lee, S.-S.; Lin, B. F.; Chen, K. C. S. Chemical constituents from the roots of *Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu.(II). Chiness Pharmaceutical Journal (Taipei). 1995; 47(6): 511-519.

Lee, S.-S.; Lin, B. F.; & Liu, K. C. (1996). Three triterpenes esters from *Zizyphus jujuba*. *Phytochemistry*. 43(4): 847-851.

Lee, H. E.; Lee, S. Y.; Kim, J. S.; Park, S. J.; Kim, J. M.; Lee, Y. W.; Jung, J. M.; Kim, D. H.; Shin, B. Y.; Jang, D. S.; Kang, S. S.; Ryu, J. H. (2013). Ethanolic extract of the seed of *Zizyphus jujuba* var. spinosa ameliorates cognitive impairment induced by cholinergic blockade in mice. *Biomol. Ther.* 21(4): 299-306.

Lee, S.-S.; Su, W.-C.; & Liu, K. C. S. C. (2001). Cyclopeptide alkaloids from *Paliurus ramossisimus*. *Phytochemistry* 58: 1271-1276.

Lee, S. -S.; Su, W.-C.; & Liu, K. C. (1991). Two new tritrtpene glucosides from *Paliurus ramosissimus*. *J. Nat. Prod.* 58(2): 615-618.

Li, X.; Ohtsuki, T.; Shindo, S.; Sato, M.; Koyano, T.; Preeprame, S.; Kowithayakorn, T.; Ishibashi, M. (2007). Mangiferin identified in a screening study guided by neuraminidase inhibitory activity. *Planta Med*. 73(11): 1195-1196.

Liu, J.; Chen, B.; & Yao, S. (2007). Simultaneous analysis and identification of main bioactive constituents in extract of *Zizyphus jujuba* var. *sapinosa* (*Zizyphi spinosi* semen) by high-performance liquid chromatography-photodiode array detection-electrospray mass spectrometry. *Talanta*. 71: 668-675.

Maldaner, G.; Marangon, P.; Ilha, V.; Balparda Caro, M. S.; Burrow, R. A.; Dalcol, I. I.; Morel, A. F. (2011). Cyclopeptide alkaloids from *Scutia buxifolia* Reiss. *Phytochemistry.* 72: 804-809.

Machado, E. A.; Filho, A. V.; & Morel, A. F. (1995). Four cyclopeptide alkaloids from Discaria longispina. *J. Nat. Prod. 58*(4): 548-553.

Memon, A. A.; Memon, N.; Luthria, D. L.; Pitafi, A. A.; Bhanger, M. I. (2012). Phenolic compounds and seed oil composition of *Ziziphus mauritiana* L. fruit. *Pol. J. Food Nutr. Sci.* 13(2): 123-128.

Menezes, A. S.; Mostardeiro, M. A.; Zanatta, N.; Morel, A. F. (1995). Scutianine J, a Cyclopeptide alkaloid isolated from *Scutia buxifolia*. *Phytochemistry*. 38: 783-786.

Michel, A. (2002). Tree, shrub and liana of west African zone. *Margraf Publishers GMBH*, Paris p. 440.

Mondal, D N.; & Kundu, A. B. (1998). A-nor-triterpenoid from *Zizyphus jujube*. *J. Indian Chem Soc*. 75(6): 384-385.

Morel, A. F.; Bravo, R. V. F.; Reis, F. D. A. M.; Ruveda, E. A. (1979). Peptide alkaloids of Scutia buxifolia. *Phytochemistry.* 18(3): 473-477.

Morel, A. F.; Machado, E. C.; Wessjohann, L. A. (1995). Cyclopeptide alkaloids of *Discaria febrifuga* (Rhamnaceae). *Phytochemistry*. 39(4): 431-434.

Nahar, N.; Das, R. N.; Shoeb, M.; Marma, M. S.; Aziz, M. A.; Mosihuzzaman, M. (1997). Four triterpenoids from bark of *Zizyphus rugosa* and *Zizyphus oenoplia*. *J. Bangladesh Acad Sci*. 21(2): 151-158.

Netsawangwicha, J. (2005). A study on chemical constituents from the root barks of *Ziziphus rugosa* Lam. [Dissertation M.Sc. Chemistry]. Bangkok: Srinakharinwirot University; p. 1-106.

Pandey, M. B.; Singh, A. K.; Singh, J. P.; Singh, V. P.; Pandey, V. B. (2008a). Three new cyclopeptide alkaloids from *Zizyphus* species. *J. Asian Nat. Prod. Res.* 10(7-8): 709-713.

Pandey, M. B.; Singh, A. K.; Singh, V. P.; Pandey, V. B. (2008b). Cyclopeptide alkaloids from *Zizyphus sativa* bark. *Nat. Prod. Res.* 22: 219-221.

Pandey, M. B.; Singh, J. P.; Singh, A. K.; Singh, V. P. (2008c). Xylopyrine-F a new cyclopeptide alkaloid from *Zizyphus xylopyra*. *J. Asian Nat. Prod. Res.* 10: 735-738.

Pandey, M. B.; Singh, S.; Malhotra, M.; Pandey, V. B.; Singh, T. D. (2012). Two new 14-membered cyclopeptide alkaloids from *Zizyphus Xylopyra. Nat. Prod. Res.* 26(9): 836-841.

Panseeta, P. (2009). Cyclopeptide alkaloids and triterpenoids from *Ziziphus mauritiana*. [Dissertation Ph.D. Applied Chemistry]. Bangkok: Srinakharinwirot University; p. 1-149.

Panseeta, P. (2004). *Ziziphus cambodiana* Pierre. [Dissertation M.Sc. Biological Chemistry]. Bangkok: Srinakharinwirot University; p. 1-74.

Panseeta, P.; Lomchoey, K.; Prabpai, 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.

Panseeta, P.; & Suksamrarn, S. (2010). Triterpenes from the root of thai *Ziziphus mauritiana*. *SWU Science and Technology Journal.* 2(4): 106-118.

Prashith, K.; Raghavendra.; & Vinayaka. (2011). Evaluation of pericarp and seed extract of *Zizyphus rugosa* Lam. for cytotoxic activity. *IJPBA.* 2(3): 887-890.

Shah, A. H.; Qureshi, S.; Tariq, M.; Ageel, A. M. (1989). Toxicity studies on six plants used in the traditional Arab system of medicine. *Phytother*. Res. 3: 25-29.

Sharma, S. C; & Kumar, R. (1982). Constituents from leaves of *Zizyphus mauritiana* Lamk . *Pharmazie*. 37(11): 809-810.

Singh, A. K.; Pandey, M. B.; Singh, V. P.; Pandey, V. B. (2008). Mauritine-K, a new antifungal cyclopeptide alkaloid from *Zizyphus mauritiana*. *ChemInform.* 39(19): 781-784.

Singh, A. K.; Pandey, M. B.; Singh, V. P.; Pandey, V. B. (2007). Xylopyrine A and xylopyrine B, two new peptide alkaloids from *Zizyphus xylopyra*. *Nat. Prod. Res*. 21(12): 1114-1120.

Singh, A. K.; Pandey, M. B.; Singh, V. P.; Pandey, V. B. (2008). Xylopyrine C, a new cyclopeptide alkaloid from *Zizyphus xylopyra*. *J. Asian Nat. Prod. Res.* 10(7-8): 725-728.

Singh, J. P.; Raghubanshi, S.; & Yadava, A. (2013). Cyclopeptide alkaloids of *Zizyphus rugosa. J. Chem. Biol. Phys.* 3(2): 994-997.

Singh, A. K.; Tripathi, M.; Singh, V. P.; & Pandey, V. B. (2002). Naturally occurring cyclopeptide alkaloids. *Oriental J. Chem*. 18: 399-404.

Singh, S.; Pandey, M. B.; Singh, J. P.; Pandey, V. B. (2006). Peptide alkaloids from *Zizyphus sativa* bark. *J. Asian Nat. Prod. Res.* 8(8): 733-739.

Smitinand, T. (2001). *Thai plant names*. Rev. ed. Bangkok: The forest herbarium, Royal Forest Department. p. 564-565.

Siriamornpun, S.; Weerapreeyakul, N.; & Barusrux, S. (2015). Bioactive compounds and health implications are better for green *jujub*e fruit than for ripe fruit. *J. Funct. Foods.* 12: 246-255.

Suksamrarn, S.; Panseeta, P.; Kunchanawatta, S.; Distaporn, T.; Ruktasing, S.; Suksamrarn, A. (2006). Ceanothane- and lupane-type triterpenes with antiplasmodial and antimycobacterial activities from *Ziziphus cambodiana*. *Chem. Pharm. Bull*. 37(32): 535-537.

Suksamrarn, S.; Suwannapoch, N.; Aunchai, N.; Kuno, M.; Ratananukul, P.; Haritakun, R.; Jansakulc, C.; Ruchirawat, S. (2005). Ziziphine N, O, P and Q, new antiplasmodial cyclopeptide. *Tetrahedron*. 61: 1175-1180.

Tan, N-. H.; & Zhou, J. (2006). Plant cyclopeptides. *Chem. Rev.* 106(3): 840-895.

Trevisan, G.; Maldaner, G.; Velloso, N. d. A.; Sant'Anna, G. d. S.; Ilha, V.; Velho Gewehr, C. d. C.; Rubin, M. A.; Morel, A. F.; Ferreira, J. (2009). Antinociceptive effects of 14-membered cyclopeptide alkaloids. *J. Nat. Prod.* 72: 608-612.

Tschesche, R.; & Kaussmann, R. (1975). in: ‘‘The alkaloids, chemistry and pharmacology’’ (R. H. F. Manske, ed.), Academic Press, NewYork, 15, p.165.

Tschesche, R.; Kaussmann, E. U.; & Eckhardt, G. (1973). Alkaloide aus rhamnaceen, XVI. Über die struktur des zizyphins A. *Tet. Lett.* 14(28): 2577-2580.

Waggas, A. M.; and Al-Hasani, R. H. (2009). Effect of sidr *(Zizyphus spina-christi)* fruit extract on the central nervous system in male albino rats. *Am-Euras. J. Sci. Res.* 4(4): 263-267.

Xu, R.; Fazio, G. C.; & Matsuda, S. P. T. (2004). On the origins of triterpenoid skeletal diversity. *Phytochemistry.* 65: 261-291.

Yagi, A.; Okamura, N.; Haraguchi, Y.; Noda, K.; Nishioka, I. (1978a). Studies on the constituents of *Zizyphi Fructus*. II. Structure of new *p*-coumaroylates of maslinic acid. *Chem Pharm Bull*. 26(10): 3075-3079.

Yagi, A.; Okamura, N.; Haraguchi, Y.; Noda, K.; Nishioka, I. (1978b). Studies on the constituents of *Zizyphi Fructus*. I. Structure of three new *p*-coumaroylates of alphitolic acid. *Chem Pharm Bull*. 26(6): 1798-1802.

Yayli, N.; Yildirim, N.; Usta, A.; ¨Ozkurt, S; AKG¨UN, V. (2003). Chemical constituents of *Campanula lactiflora. Turk. J. Chem.* 27: 749-755.

Zare-Zardini, H.; Tolueinia, B.; Hashemi, A.; ebrahimi, L.; Fesahat, F. (2013). Antioxidant and cholinesterase inhibitory activity of a new peptide from *Ziziphus jujuba* fruits. *AJADD.* 28(7): 702-709.

APPENDIX

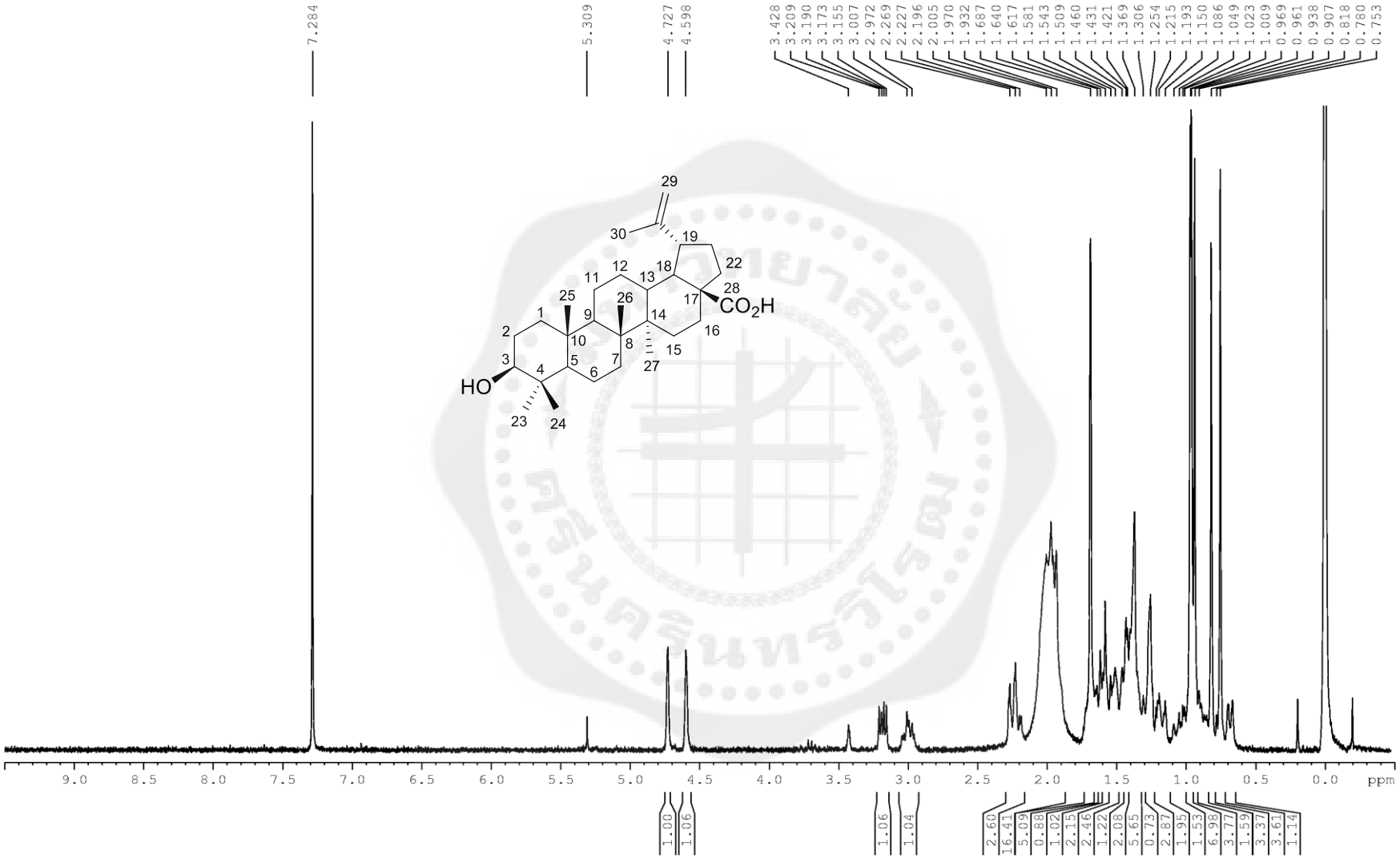

Figure 21 ^{1}H-NMR of compound **A** (**Betulinic acid**, sss5971) in $CDCl_3$+ CD_3OD 3 drops

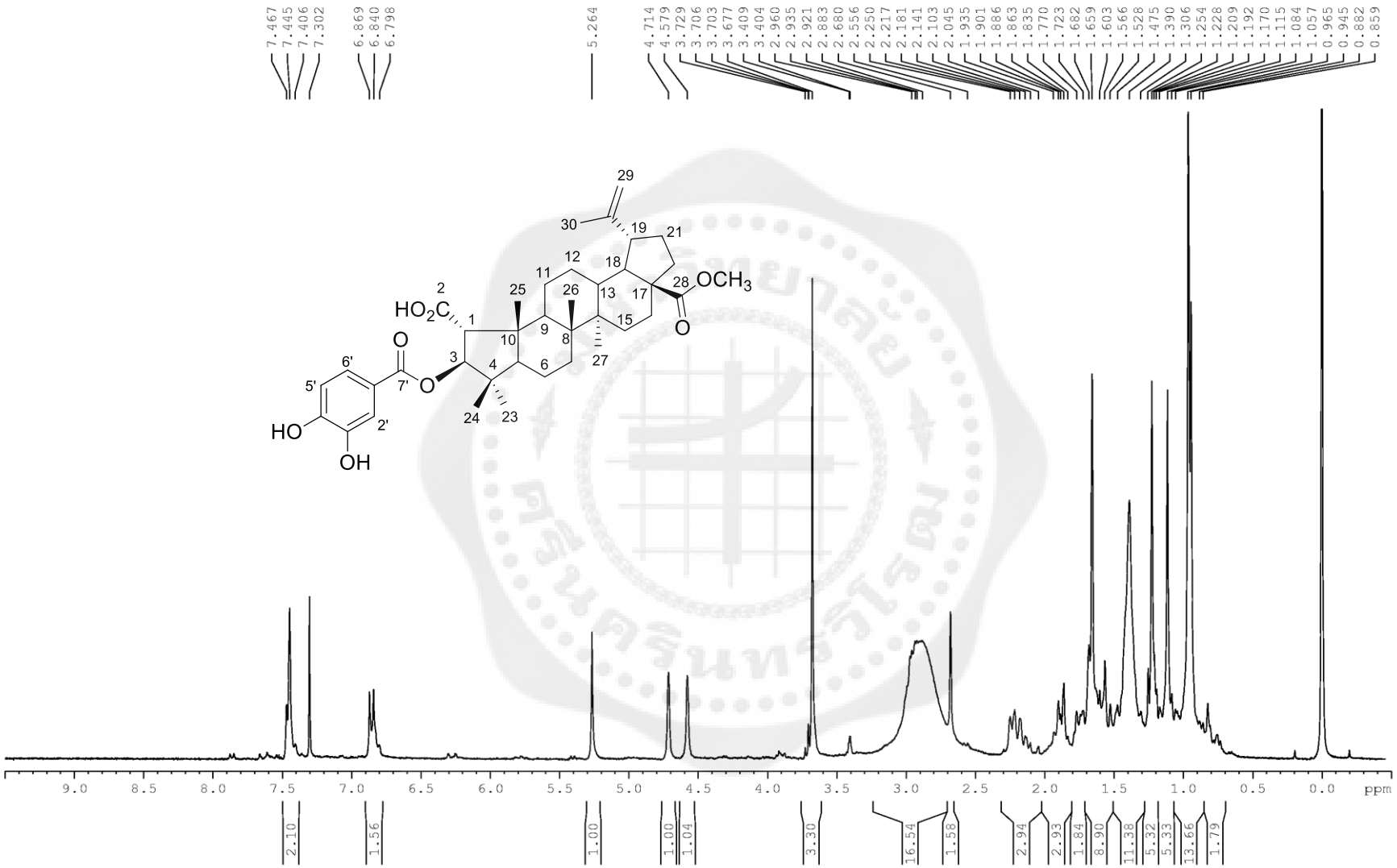

Figure 22 ^{1}H-NMR of compound **B** (**Methyl 3-*O*-protocatechuoylceanothate**, sss5998) in $CDCl_3$ + CD_3OD 2 drops

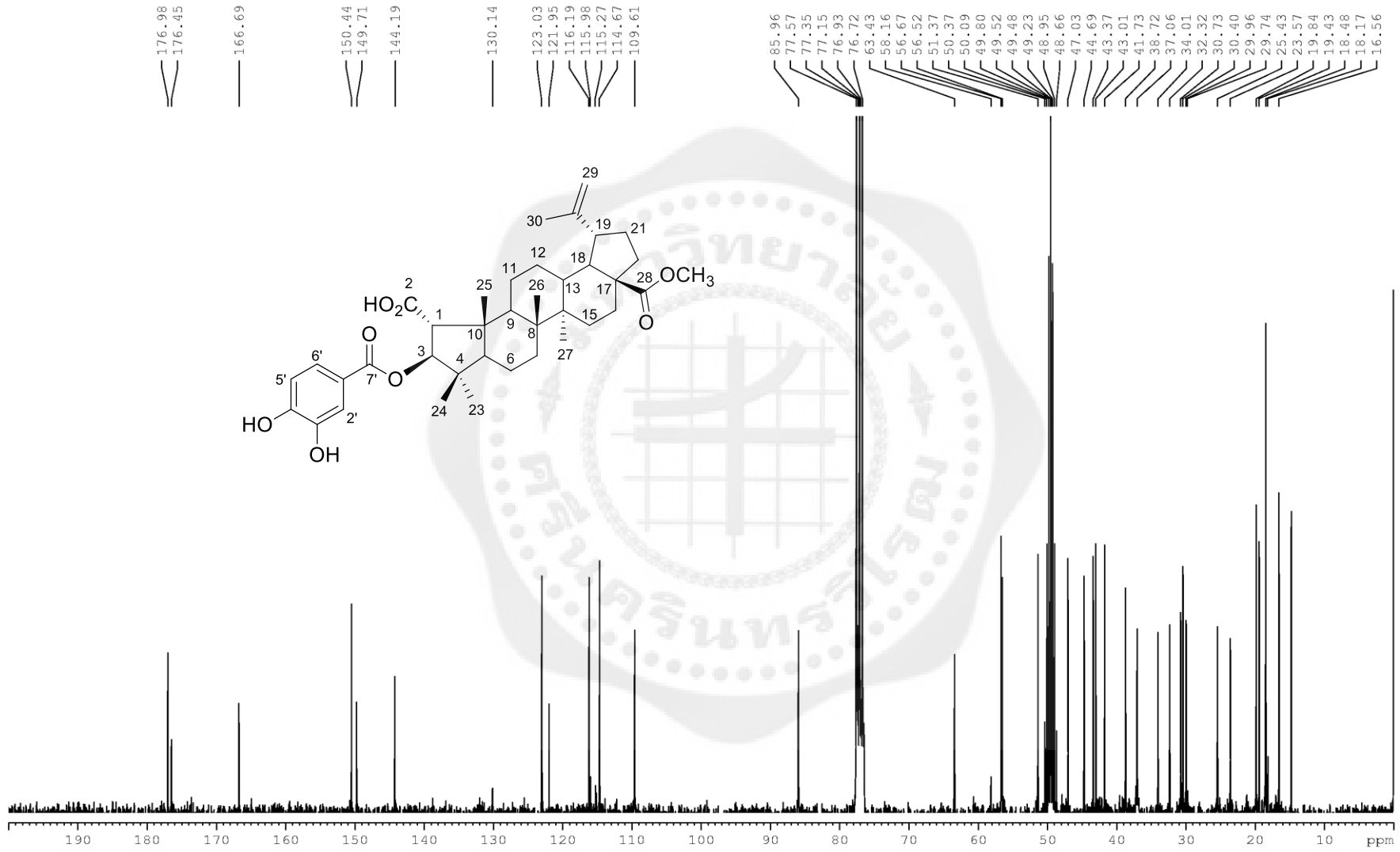

Figure 23 ^{13}C-NMR of compound **B** (**Methyl 3-*O*-protocatechuoylceanothate**, sss5998) in $CDCl_3$ + CD_3OD 2 drops

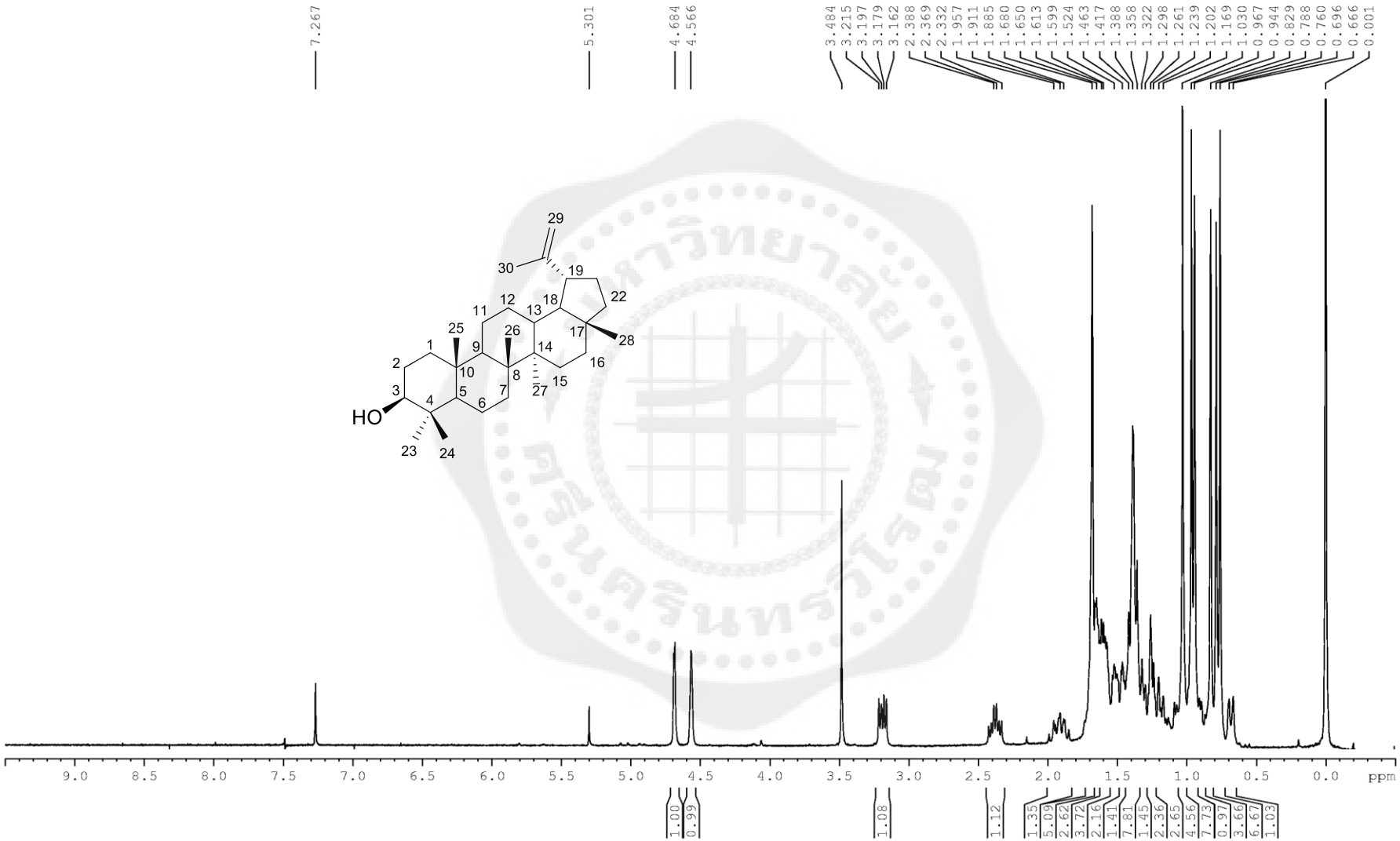

Figure 24 ^{1}H-NMR of compound **C** (**Lupeol**, sss6288) in $CDCl_3$

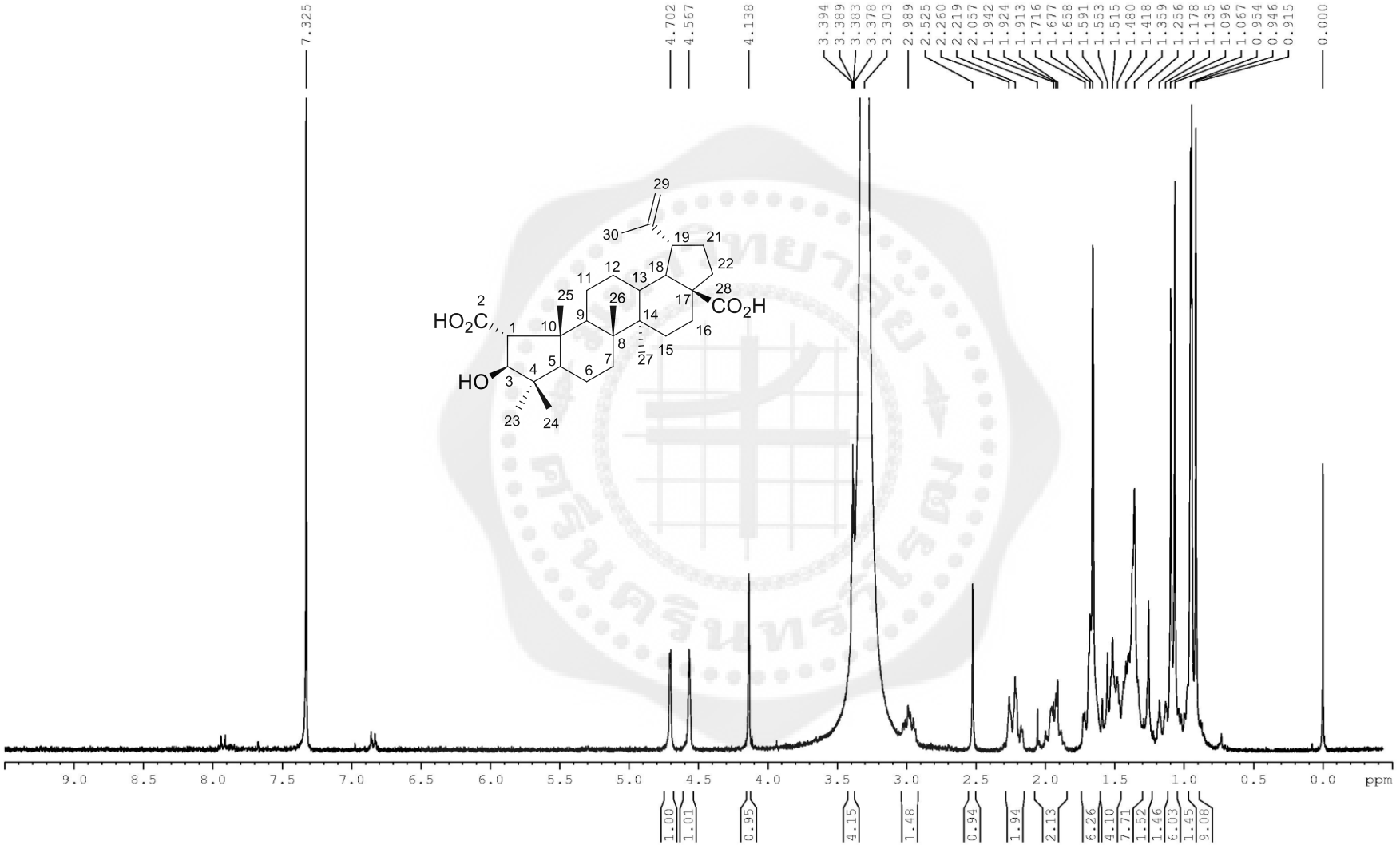

Figure 25 ^{1}H-NMR of compound **D** (**Ceanothic acid**, sss6215) in $CDCl_3$ + CD_3OD 4 drops

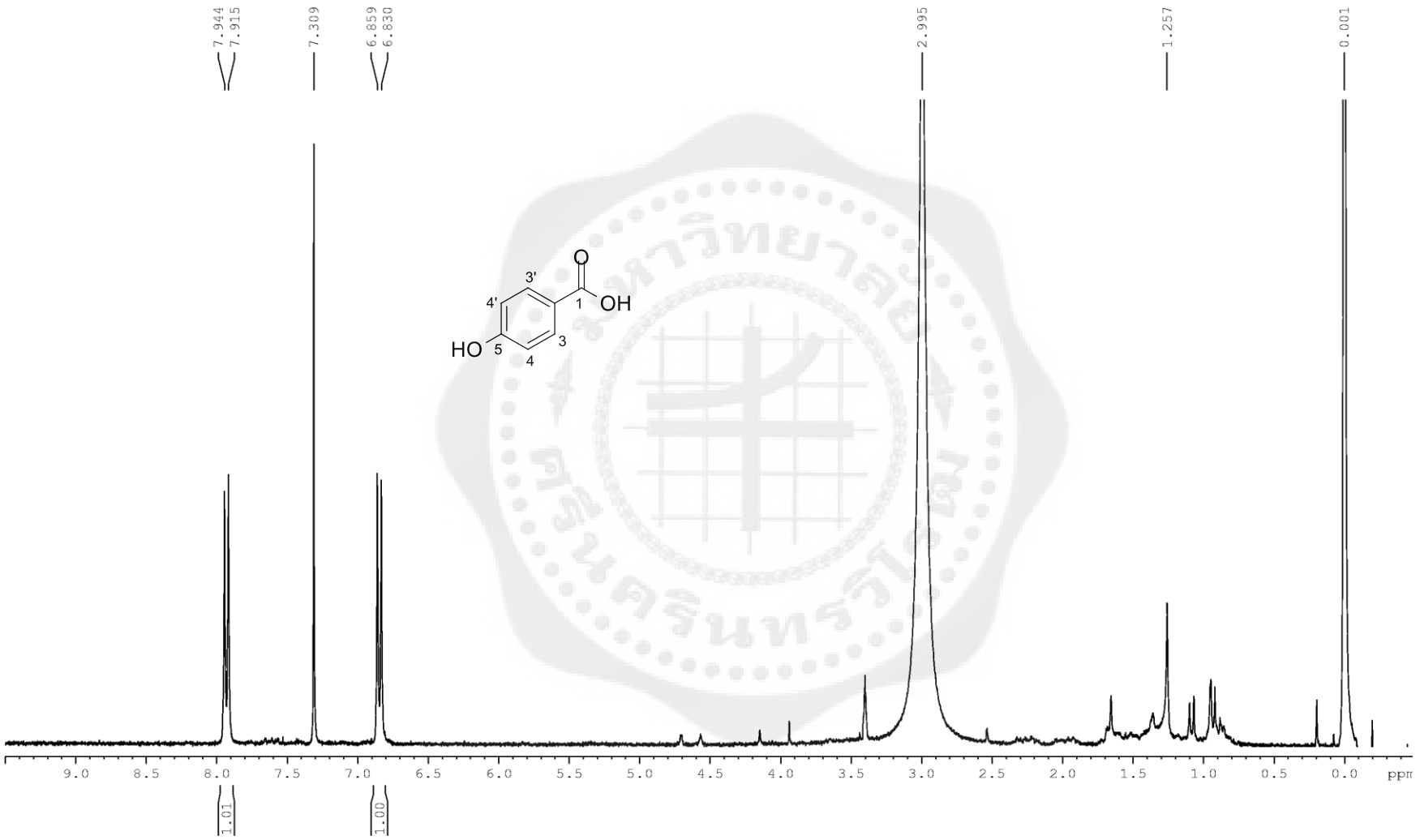

Figure 26 ^{1}H-NMR of compound **E** (***p*-Hydroxybenzoic acid**, sss6333) in $CDCl_3$ + CD_3OD 3 drops

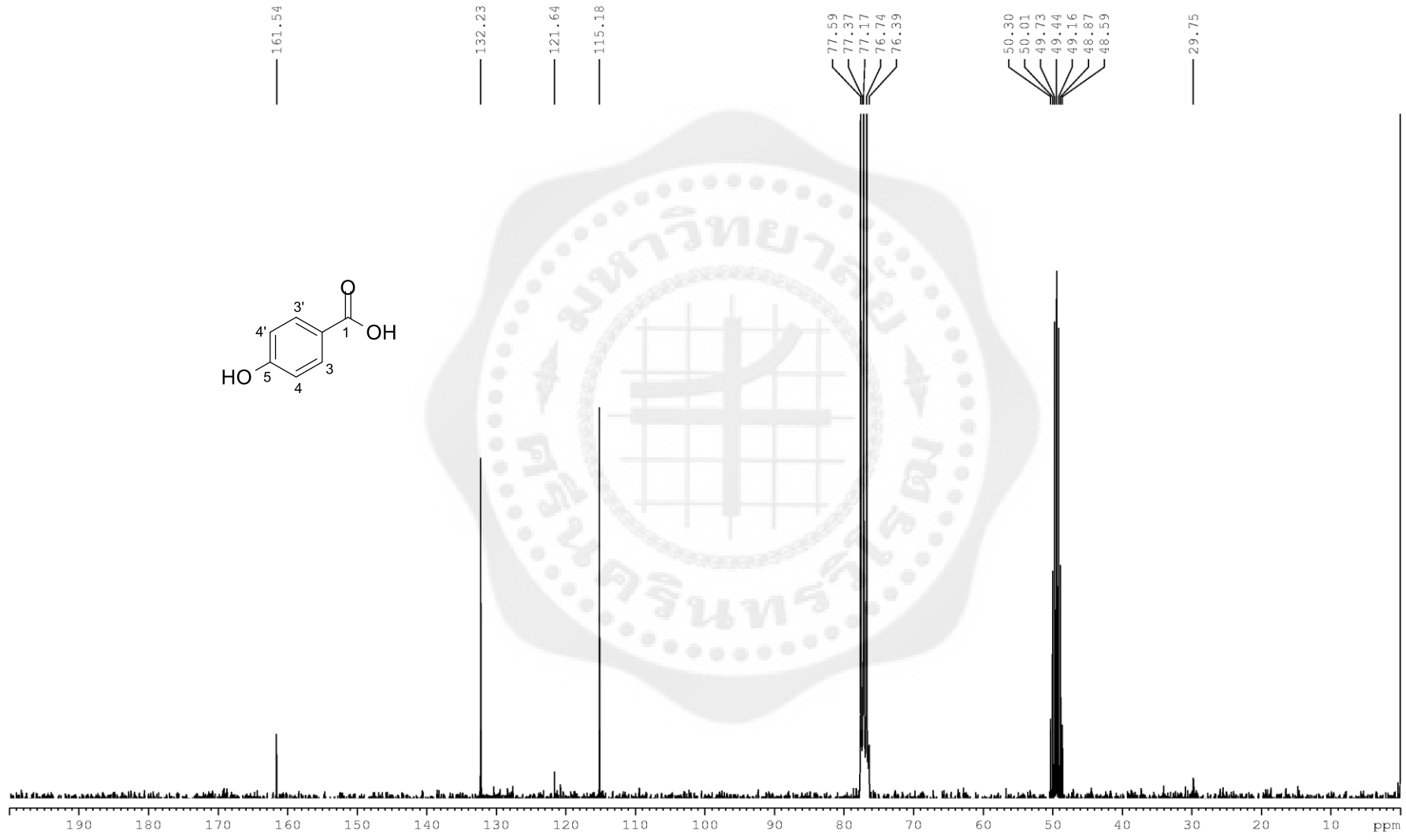

Figure 27 ^{13}C-NMR of compound **E** (***p*-Hydroxybenzoic acid**, sss6333) in $CDCl_3$ + CD_3OD 3 drops

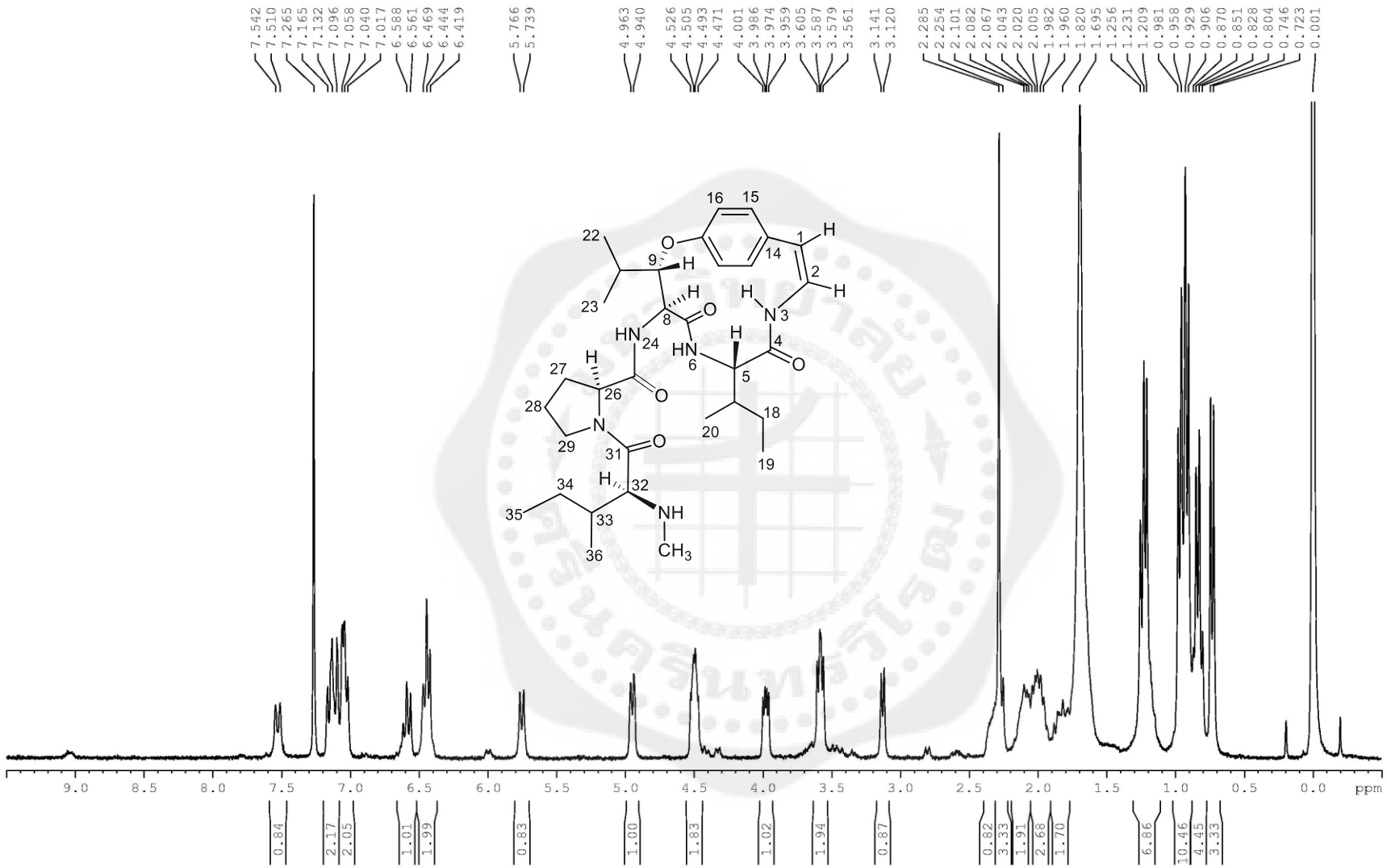

Figure 28 ^{1}H-NMR of compound **F** (**Cambodianine**, sss5780) in $CDCl_3$ + CD_3OD 2 drops

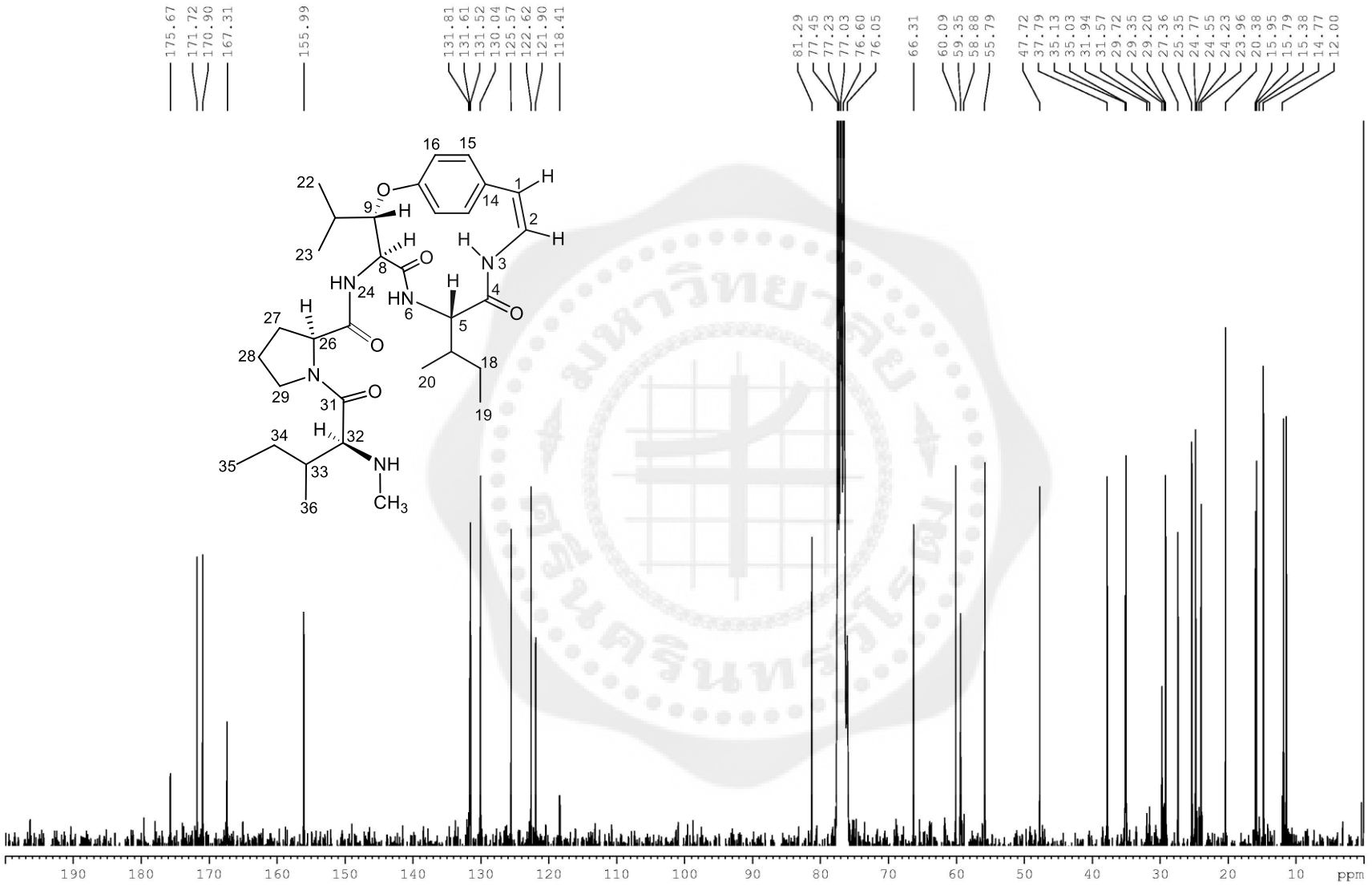

Figure 29 ^{13}C-NMR of compound **F** (**Cambodianine**, sss5780) in $CDCl_3$ + CD_3OD 2 drops

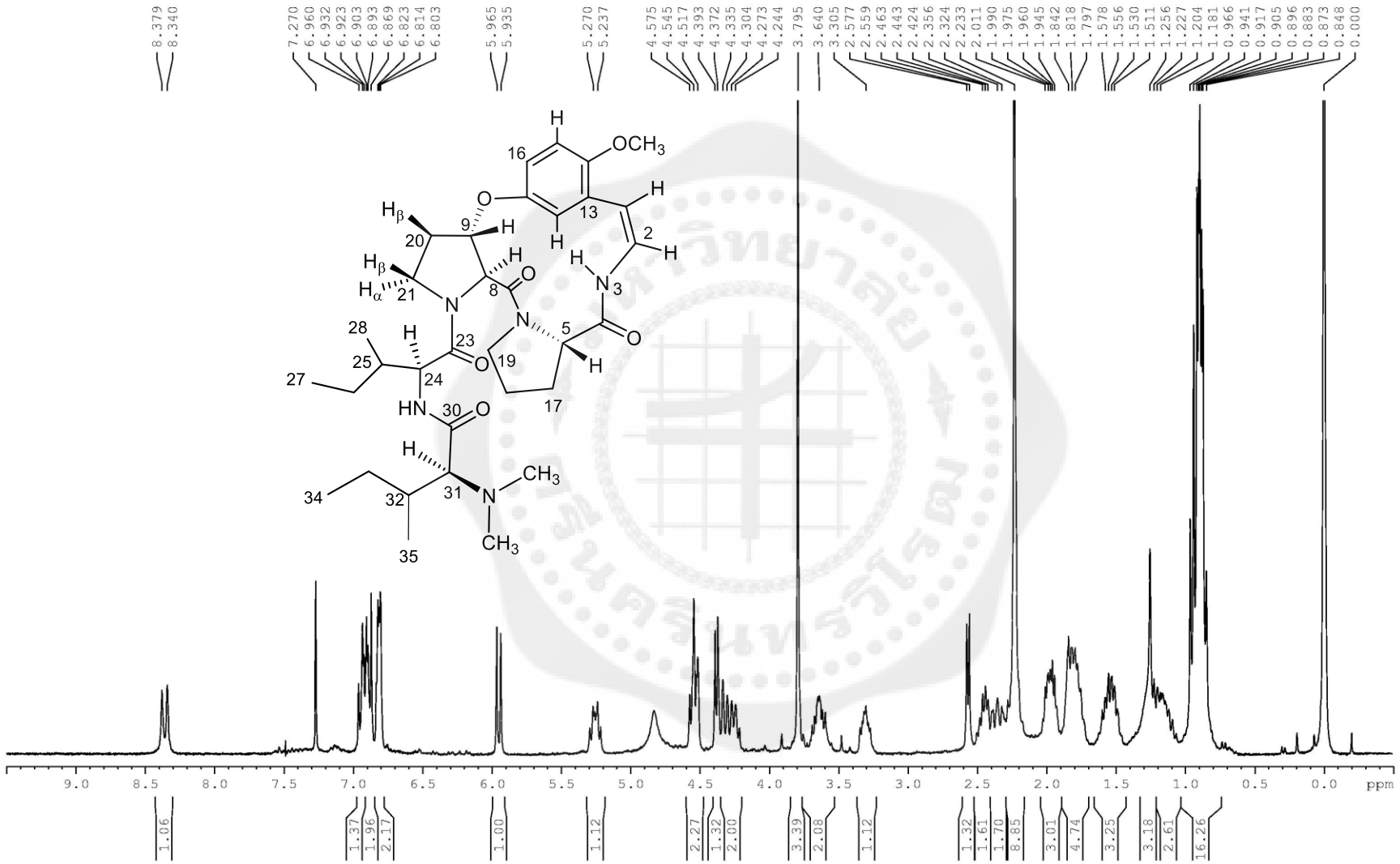

Figure 30 ^{1}H-NMR of compound **G** (**Ziziphine A**, sss6114) in $CDCl_3$

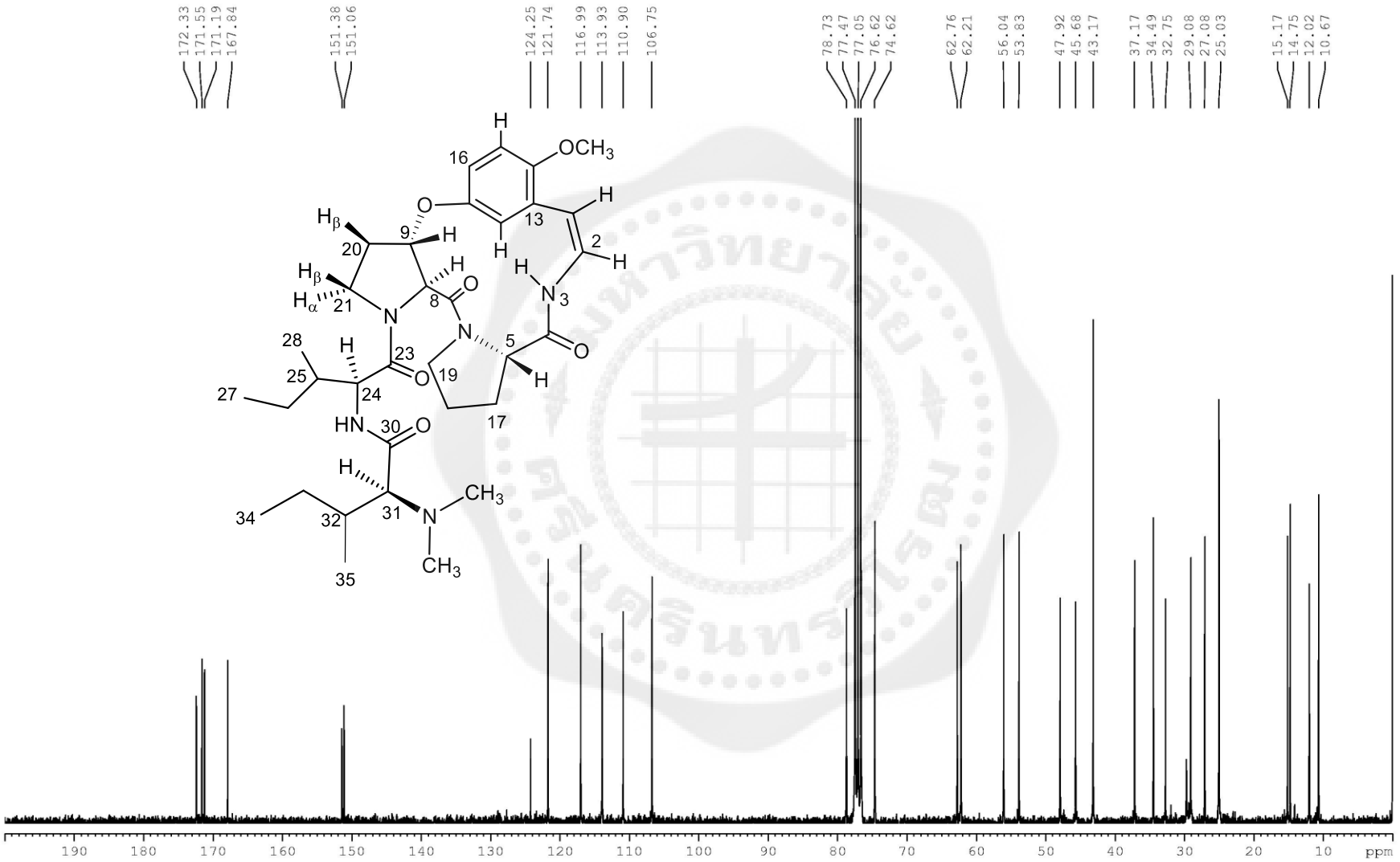

Figure 31 ^{13}C-NMR of compound **G** (**Ziziphine A**, sss6114) in $CDCl_3$

GLOSSARY

LIST OF ABBREVIATIONS AND SYMBOLS

$[\alpha]_D^{27}$

Specific rotation at 27° C and sodium D line

$[\alpha]_D^{29}$

Specific rotation at 29° C and sodium D line

$[\alpha]_D^{30}$

Specific rotation at 30° 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

$[M-H]^-$

Deprotonated 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

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

br d

Broad doublet (for NMR data)

br dd

Broad doublet of doublet (for NMR data)

br s

Broad singlet (for NMR data)

br t

Broad triplet (for NMR data)

calcd

Calculated

C_6H_6

benzene

CC

Column chromatography

$CDCl_3$

Deuterated chloroform

CD3OD

Deuterated methanol

CH_2Cl_2 or DCM

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)

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

DEPT

Distortionless Enhancement by Polarization Transfer

dq

Doublet of quartets (for NMR data)

dt

Doublet of triplets (for NMR data)

ESIMS

Electrospray Ionization Mass Spectrometry

EtOAc

Ethyl acetate

g

Gram

Glc

Glucoside

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

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

kg

Kilogram

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

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

s

Singlet (for NMR data)

sh

shoulder

t

Triplet (for NMR data)

TLC

Thin Layer Chromatography

UV

Ultraviolet

α

Alpha

β

Beta

VITAE

VITAE

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Saenkham, A.; Lomchoey, K.; Nontakham, J.; Taweechotipatr, M.; & Suksamrarn, S. (2015). Lupane- and ceanothane-type triterpenes of *Ziziphus cambodiana* Pierre stem barks with anti-*Helicobacter pylori* activity. *KKU Science Journal.* 3(43).

Scholarships

2012-2013 The Center of Excellence for Innovation in Chemistry (PERCH-CIC)