CYCLOPEPTIDE ALKALOIDS AND TRITERPENOIDS FROM *ZIZIPHUS MAURITIANA*

A THESIS

BY

PANOMWAN PANSEETA

Presented in Partial Fulfillment of the Requirements for the

Doctor of Philosophy Degree in Applied Chemistry

at Srinakharinwirot University

September 2009

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AN ABSTRACT

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Two new cyclopeptide alkaloids, named mauritine L and mauritine M, were isolated from the root of Thai *Ziziphus mauritiana* (Rhamnaceae) along with three known cyclopeptide alkaloids, nummularine B, nummularine H and hemsine A. Moreover, seven known triterpenoids were identified as lupeol, betulin, betulic acid, zizyberenalic acid, ceanothic acid, epiceanothic acid and 24-hydroxyceanothic acid. Their structures were elucidated on the basis of spectroscopic analyses, mainly NMR spectroscopic techniques. The stereochemical assignments were established by NOE experiments and comparison with other related compounds of known stereochemistry. From the X-ray diffraction technique, the stereochemistry of nummularine B was assigned to be 5*S*, 8*S*, 9*S*, 25*S* and 31*S*. Zizyberenalic acid exhibited significant *in vitro* antiplasmodial activity against the parasite *Plasmodium falciparum* with inhibitory concentration (IC_{50}) value of 3.0 μg/mL. Betulinic acid, zizyberenalic acid and 24-hydroxyceanothic acid showed antimycobacterial activity against *Mycobacterium tuberculosis* with respective MIC values of 25, 100 and 25 μg/mL.

สารประกอบไซโคลเปบไทด์อัลคาลอยด์และไตรเทอร์พีนจากพุทรา

บทคัดย่อ

ของ

พนมวรรณ ปานสีทา

เสนอต่อบัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ เพื่อเป็นส่วนหนึ่งของการศึกษา

ตามหลักสูตรปริญญาปรัชญาดุษฎีบัณฑิต สาขาวิชาเคมีประยุกต์

กันยายน 2552

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จากการศึกษาองค์ประกอบทางเคมีจากรากพุทราสามารถแยกสารประกอบไซโคลเปบไทด์อัลคาลอยด์ชนิดใหม่ได้ 2 ชนิด คือ mauritine L และ mauritine M รวมทั้งสารประกอบไซโคลเปบไทด์อัลคาลอยด์ที่เคยพบแล้ว 3 ชนิด ได้แก่ nummularine B, nummularine H และ hemsine A นอกจากนี้ยังพบสารประกอบไตรเทอร์พีน 7 ชนิด ได้แก่ lupeol, betulin, betulic acid, zizyberenalic acid, ceanothic acid, epiceanothic acid และ 24-hydroxyceanothic acid โครงสร้างของสารทราบได้จากการวิเคราะห์ข้อมูลสเปกโทรสโคปี โดยใช้เทคนิค NMR เป็นส่วนใหญ่ สำหรับการหาสเตอริโอเคมีของสารใช้เทคนิค NOE และทำการเปรียบเทียบข้อมูลกับสารประกอบอื่นที่มีโครงสร้างใกล้เคียงกัน นอกจากนี้ข้อมูล X-ray diffraction ของ nummularine B ทำให้ทราบสเตอริโอเคมีของสารเป็น 5*S*, 8*S*, 9*S*, 25*S* และ 31*S* คอนฟิกูเรชัน จากการทดสอบฤทธิ์ทางชีวภาพของสารประกอบไตรเทอร์พีน พบว่า zizyberenalic acid มีฤทธิ์ต้านเชื้อมาลาเรีย *Plasmodium falciparum* โดยมีค่าการยับยั้ง (IC_{50}) ที่ความเข้มข้น 3.0 μg/mL นอกจากนี้ betulinic acid, zizyberenalic acid และ 24-hydroxyceanothic acid แสดงฤทธิ์การต้านเชื้อวัณโรค *Mycobacterium tuberculosis* ที่ความเข้มข้น (MIC) 25, 100 และ 25 μg/mL ตามลำดับ

The dissertation titled

"Cyclopeptide alkaloids and triterpenoids from *Ziziphus mauritiana*"

by

Panomwan Panseeta

has been approved by the Graduate School as partial fulfillment of the requirements for the

Doctor of Philosophy Degree in Applied Chemistry of Srinakharinwirot University.

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Panomwan Panseeta

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CHAPTER 1

INTRODUCTION

Background

The genus *Ziziphus* (formerly known as *Zizyphus*) belongs to the family Rhamnaceae,[1] a family of 58 genera and 900 species worldwide,[2] particularly in arid regions[3] with 100 species distributed in the tropical America, Africa, the Mediterranean region, Indo-Malaysia, and Australia, and also the tropical parts of India, Nepal, Pakistan, Bhutan, Bangla Desh and Sri Lanka.[2] Only nine species of *Ziziphus* plants are found in Thailand[1] as follows:

1. *Z. angustifolia* (Miq.) Hatus. Ex Steenis or Phutsa bai liam in Thai (พุทราใบเหลี่ยม)
2. *Z. attopoensis* Pierre or Kamlang suea khrong in Thai (กำลังเสือโคร่ง)
3. *Z. calophylla* Wall. or Chin chi in Thai (ชินชี่)
4. *Z. cambodiana* Pierre or Takhrong in Thai (ตะครอง)
5. *Z. incurva* Roxb. or Ta-chu-mae in Thai (ตาฉู่แม)
6. *Z. jujuba* Mill. or Phutsa chin in Thai (พุทราจีน)
7. *Z. mauritiana* Lam. or Phutsa in Thai (พุทรา)
8. *Z. oenoplia* (L.) Mill. var. *oenoplia* or Nam lep yiao in Thai (หนามเล็บเหยี่ยว)
   Z. oenoplia (L.) Mill. var. *brunoniana* or Nam lep maeo in Thai (หนามเล็บแมว)
9. *Z. rugosa* Lam. or Ma khwat in Thai (มะควัด)

Morphology of *Ziziphus* plants

Plants of *Ziziphus* are erect trees or small to large shrubs or semi-scandent shrubs or climbers; when trees usually with a deep radical, well developed; species may be spiny or glabrous or relatively hairy. Leaf is alternate, it is simple with 3-5 nerved from the base; the leaf base is either asymmetrical, slightly asymmetrical or symmetrical. Flower is 5-merous, actinomorphic and hermaphrodite. Flower is borne sometimes solitary or 2-3 together in axillary

cymes or in umbels or racemes arranged in terminal panicles or thyrses. Inflorescence may be pedunculate or sessile. Fruit is subglobose, ovoid or oblong, usually drupe. It is 1-4 celled and 1-4 seeded but drupe mostly contains 1 seed. The flesh of the drupe is usually juicy pulp. Young fruit may be pilose.[4]

Ethnopharmacological Uses

Several *Ziziphus* species have tremendous uses in the folk medicine.[5] The fruit of *Z. mauritiana* has been used as laxative, expectorant and febrifuge,[6] the stem bark and leaf decoction are used for treatment of diarrhea, ulcers, vomiting and indigestion.[7] In Myanmar, fruit is used for anticough, the root is used for antipyretic and leaf is a poultice for skin. In traditional medicine of Korea, the seed is used as sedative.[8] The stem of *Z. cambodiana* is commonly used in Cambodian traditional medicine for treatment of fever.[9] In the Indian system of medicine, *Z. rugosa* is used for treatment of diarrhea, menorrhagia and infection of teeth.[10] In Thailand the root and stem bark of *Z. oenoplia* have been used for antidiabetic and diuretic activities.[11]

The aqueous extract of *Z. mauritiana* leaf prevents chronic alcohol-induced liver damage in albino rats by enhancing the levels of total antioxidant and inhibiting hepatic lipid peroxidation.[12] The methanol extract of *Z. jujuba* has protective effect against the carbontetrachloride-induced hepatoxicity in rats.[13] The seed of *Z. jujuba* possesses anxiolytic and sedative effect in mouse.[14] Moreover, *Z. jujuba* might exert favorable effects on improving the gastrointestinal milieu and reducing the exposure of intestinal mucosa to toxic ammonia and other detrimental compounds.[15]

From the report on the biological activities of *Ziziphus* species, it is interesting to study on chemical constituents of *Ziziphus* plants. Recently, some triterpenes have been reported by our group from the root bark of *Z. cambodiana* for the first time.[16-17] Thus, the root of *Z. mauritiana* is selected for this study to search for cyclopeptides and other constituents.

Objectives of the Study

1. To isolate and purify components from the root of *Z. mauritiana*
2. To determine chemical structure of the isolated compounds
3. To evaluate some biological activities of the isolated compounds

CHAPTER 2

REVIEW OF LITERATURES

Morphology of *Z. mauritiana*

Z. mauritiana known in Thai as Phutsa or Ma tan, other names for this plant are Ma thong, Mang-thang and Mak-kho.[1] It is a medium-sized tree, height can vary from 3-16 m or more. The bark has deep longitudinal furrows and is grayish brown or reddish in color.[4] Branch drooping, armed with stipular spines, equal or one straight, the other bent, rarely entirely unarmed. Branchlets, petioles, underside of leaves and inflorescence densely clothed with bright tawny or nearly with tomentum. Leaf is variable, from ovate-oblong to nearly orbicular, obtuse or acute, entire or serrulate, blade 0.75-2.5, petiole 0.1-0.6 inch long. Flower is greenish-yellow in short axillary nearly sessile cymes, petals unguiculate, lamina oblong, concave or hooded, disk fleshy, 10-lobed, thick, connate to middle.[18] Flower has sepals with a disk about 3 mm in diameter and a 2-celled ovary immersed in the disk. Flower tends to have an acrid smell. The fruit is a drupe varying in size and shape from 1-2 cm diameter but some oval varieties can reach 5 x 3 cm. The fruit is greenish, yellow or red when ripe. The pulp is acidic and sweet.[4] The seed is stone tuberculate, bony, irregularly furrowed, mostly 2-celled.[18]

Z. mauritiana, is called as Indian jujube, has the specific name of *jujuba* applied as *Z. jujuba* Lam. and *Z. jujuba* Gaertn. It distributes throughout the warm subtropics and tropics of South Asia.[4]

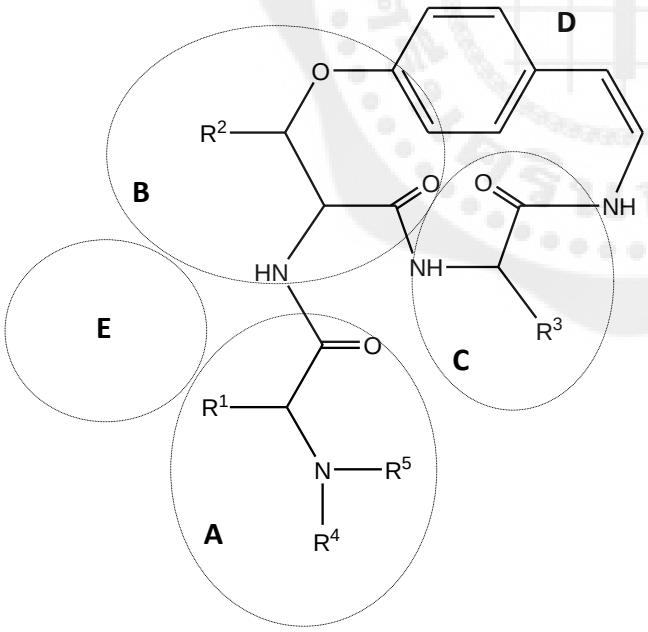
Chemical constituents of *Ziziphus* plants

Ziziphus species have been reported to possess several types of chemical constituents especially cyclopeptide alkaloids and triterpenes. In addition, pyrrolidine alkaloids, sterols, flavonoids and other miscellaneous groups were also found.

1. Cyclopeptide alkaloids and other alkaloids

Cyclopeptide alkaloids are defined as basic macromolecules with an ansa structure, in which a 10- or 12-membered peptide type bridge spans the 1,3 or 1,4 positions of benzene ring. The general structure of cyclopeptide alkaloids is represented in Figure 1. [19] The ring consists of two amino acids and a styrylamine unit.[20]

The cyclopeptide alkaloids are natural products that have been isolated from the leaf, stem bark, root bark and seed of a wide variety of plants species throughout the world.[21] They are widely distributed among plants of the Rhamnaceae family, but their occurrence has also been confirmed in representatives of Asteraceae, Celastraceae, Euphorbiaceae, Steruliaceae, Menispermaceae, Pandaceae, Rubiaceae and Urticaceae. These compounds often occur in minute amounts and as complex mixtures which its total yield from dried plant material is about 0.01-1%. [19]



A = Basic terminal (end) amino acid

B = β-hydroxy-amino acid

C = Ring-bound amino acid

D = Hydroxy-styrylamine unit

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

Figure 1 General structure of cyclopeptide alkaloids. [19]

In 1998, Gournelis *et al.* has classified the cyclopeptide alkaloids as 13-, 14- or 15-membered ring compounds, which are further divided according to the number of their units. Thus the cyclopeptides are subdivided into 4(13)-, 5(13)-, 4(14)-, 5(14)- and 4(15)-types.[19] For example, 4(13)-cylopeptide is a 13-membered ring compound with 4 units of three amino acids (A, B and C) and a styryl unit D, and 5(14)-cylopeptide is a 14-membered ring compound with 5 units of four amino acids (A, B C and E) and a styryl unit D.

Several cyclopeptide alkaloids of *Ziziphus* species in Thailand have been recorded as 4(13)-, 5(13)-, 4(14)-, 5(14)- and 4(15)-membered ring compounds. The structures of cyclopeptide alkaloids are presented in Table 1.

Cyclopeptide alkaloids of *Ziziphus* species in Thailand

Z. oenoplia

In 1963, Menard *et al.* isolated 5(13)-membered ring cyclopeptide alkaloids as zizyphine or zizyphine A (**1**) and zizyphinine or zizyphine B (**2**) from the MeOH extract of the root bark of *Z. oenoplia*.[22] Later, zizyphine A and zizyphine B were also found in the stem bark.[23]

In 1974, Cassels *et al.* studied the stem bark of *Z. oenoplia* and isolated zizyphine A(**1**) zizyphine B (**2**), abyssinine A (**3**) and abyssinine B (**4**). In addition, they found zizyphine C (**5**) containing a 13-membered ring like zizyphine A (**1**). Other two compounds, zizyphine D (**6**) and zizyphine E (**7**), have 15-membered rings like abyssinine A (**3**) and abyssinine B (**4**), with the peculiarity that one of the amino acid is 3-hydroxyisoleucine.[24] Zizyphine F (**8**) and zizyphine G (**9**) were also isolated from the bark of *Z. oenoplia*.[25]

In 1993-1994, Khokhar *et al.* succeeded in isolating a 13-membered ring, zizyphine I (**10**) and zizyphine K (**11**), from the extract of *Z. oenoplia* bark.[26-27] One year later, Maurya *et al.* also isolated mauritine D (**12**), frangufoline (**13**) and amphibine B (**14**).[28]

In 2005, Suksamrarn *et al.* found four new 13-membered ring of the 5(13)-type cyclopeptides, zizyphine N (**15**), zizyphine O (**16**), zizyphine P (**17**) and zizyphine Q (**18**) from the EtOAc extract of the root of *Z. oenoplia* var. *brunoniana*. In addition, zizyphine N and zizyphine

Q exhibited significant antiplasmodial activity against the parasite *Plasmodium falciparum*. Zizyphine N (**15**) and zizyphine Q (**18**) also demonstrated weak antimycobacterial activity against *Mycobacterium tuberculosis*.[29]

Z. rugosa

In 1981, chemical investigation of the *Z. rugosa* led to the isolation of amphibine D (**19**) by Tschesche *et al*.[30] In 1988, Pandey *et al*. isolated rugosanine A (**20**), a 13-membered *N*-formylcyclopeptide alkaloid from the C_6H_6-MeOH-NH_3 (100:1:1) extract of *Z. rugosa* stem bark.[31] Moreover, Tripathi *et al.* reported the isolation of rugosanine B (**21**), nummularine P (**22**) and sativanine H (**23**) from the same extract in 1989.[32]

Z. mauritiana

Mauritine A (**24**), mauritine B (**25**), mauritine C (**26**), mauritine D (**12**), mauritine E (**27**), mauritine F (**28**),[33-34] mauritine H (**29**),[35] amphibine B (**14**), amphibine D (**19**), amphibine F (**30**) and frangufoline (**13**)[33-34] were isolated from the alkaloids mixture of base of *Z. mauritiana* bark. Mauritine C (**26**), mauritine D (**12**), mauritine E (**27**) and mauritine F (**28**) belong to the same structural type with a 14-membered ring system configuration *trans*-3-hydroxyproline, 4-hydroxystyryl amine and one α-amino acid.[34] Mauritine J (**31**),[36] mauritine K (**32**),[37] amphibine E (**33**)[36] and sativanine K (**34**)[37] were isolated from the root bark. It had been shown that mauritine K (**32**) exhibited significant antifungal activity.[37]

***Z. jujuba* Mill.**

The 13-membered ring alkaloids; jubanine A (**35**), jubanine B (**36**), nummularine A (**37**), nummularine B (**38**), amphibine H (**39**), mucronine D or daechuine S9 (**40**),[38] zizyphine A (**1**),[39] daechuine S3 (**41**), daechuine S6 (**42**), daechuine S7 (**43**), daechuine S8-1 (**44**) and daechuine S10 (**45**)[40] and 14-membered ring alkaloids; nummularine K (**46**),[41] jubanine C (**47**), scutianine C (**48**),[39] mauritine A (**24**),[38] frangufoline (**13**)[42] and daechuine S5 (**49**)[40] have been isolated from the stem bark of Z. *jujuba*. The root bark of *Z. jujuba* afforded jubanine D (**50**)[43] frangulanine

(**51**) and adouetine X (**52**).[44] Daechucyclopeptide I (**53**) or daechuine-S26 was isolated from the MeOH extract of fruit of *Z. jujuba* var. *inermis*.[45]

Biological activities

The biological properties of cyclopeptide alkaloids had been reviewed[46] such as; frangufoline (**13**), rugosanine A (**20**), rugosanine B (**21**), nummularine B (**38**), and amphibine H (**39**) showed antibacterial activity. Frangufoline (**13**), rugosanine A (**20**), rugosanine B (**21**), mauritine K (**32**), nummularine B (**38**) and amphibine H (**39**) exhibited antifungal activity. Frangufoline (**13**) had been reported as sedative.[48] Moreover, zizyphine N (**15**) and zizyphine Q (**18**) exhibited significant antiplasmodial activity against the parasite *Plasmodium falciparum* with the inhibitory concentration (IC_{50}) values of 3.92 and 3.5 μg/mL, respectively. Zizyphine N (**15**) and zizyphine Q (**18**) also showed weak antimycobacterial activity against *Mycobacterium tuberculosis* with the same MIC value of 200 μg/mL.[29]

TABLE 1 Cyclopeptide alkaloids isolated from *Ziziphus* found in Thailand.

13-membered ring

Common name [Bioactivity]	Plant source (Parts)	R^1, R^2, R^3	stereochemistry at C8 and C9		Ref
4(13)-Nummularine-C type					
daechucyclopeptide I (**53**)	*Z. jujuba* var. *inermis* (fruit, stem bark)	R^1 = OH R^2 = Ile (side chain) R^3 = *N,N*-Me_2Phe	-	-	45
daechuine S6 (**42**)	*Z. jujuba* var. *inermis* (stem bark)	R^1 = OCH_3 R^2 = *L*-Ileu (side chain) R^3 = *L-N,N*-Me_2Phe	-	-	40
daechuine S7 (**43**)	*Z. jujuba* var. *inermis* (stem bark)	R^1 = OCH_3 R^2 = Leu (side chain) R^3 = *N,N*-Me_2Leu	-	-	40
daechuine S10 (**45**)	*Z. jujuba* var. *inermis* (stem bark)	R^1 = OCH_3 R^2 = Ile (side chain) R^3 = *N,N*-Me_2Trp	-	-	40
rugosanine B (**21**) [anti-bacterial, anti-fungi]	*Z. rugosa* (bark)	R^1 = OCH_3 R^2 = Phe (side chain) R^3 = *N,N*-Me_2Trp	-	-	32, 46

TABLE 1 (continued)

Common name [Bioactivity]	Plant source (Parts)	R^1, R^2, R^3	stereochemistry at C8 and C9		Ref
sativanine K (**34**)	*Z. mauritiana* (root bark)	R^1 = OCH_3 R^2 = Ile (side chain) R^3 = *N*-(CHO)Ile	-	-	37
5(13)-Zizyphine-A type					
amphibine H (**39**) [anti-bacterial, anti-fungi]	*Z. jujuba* (stem bark)	R^1 = OCH_3 R^2 = Phe (side chain) R^3 = *N,N*-Me_2Ala-Val	-	-	38
daechuine S3 (**41**)	*Z. jujuba* var. *inermis* (stem bark)	R^1 = OCH_3 R^2 = L-Ileu (side chain) R^3 = *L-N,N*-Me_2Ile-*L*-Ile	-	-	40
daechuine S8-1 (**44**)	*Z. jujuba* var. *inermis* (stem bark)	R^1 = OCH_3 R^2 = Leu (side chain) R^3 = *N,N*-Me_2Leu-Leu	-	-	40
jubanine A (**35**)	*Z. jujuba* (stem bark)	R^1 = OCH_3 R^2 = Ile (side chain) R^3 = *N,N*-Me_2Phe-Phe	-	-	38
jubanine B (**36**)	*Z. jujuba* (stem bark)	R^1 = OCH_3 R^2 = Phe (side chain) R^3 = *N,N*-Me_2Phe-Phe	-	-	38

TABLE 1 (continued)

Common name [Bioactivity]	Plant source (Parts)	R^1, R^2, R^3	stereochemistry at C8 and C9		Ref
jubanine D (**50**)	*Z. jujuba* (root bark)	R^1 = H R^2 = Ile (side chain) R^3 = *N,N*-Me_2Phe-Val	-	-	43
mucronine D (**40**)	*Z. jujuba* (stem bark)	R^1 = OCH_3 R^2 = Ile (side chain) R^3 = *N,N*-Me_2Phe-Leu	-	-	38
nummularine A (**37**)	*Z. jujuba* (stem bark)	R^1 = OCH_3 R^2 = Ile (side chain) R^3 = *N*-MePhe-Leu	-	-	38
nummularine B (**38**) [anti-bacterial, anti-fungi]	*Z. jujuba* (stem bark)	R^1 = OCH_3 R^2 = Phe (side chain) R^3 = *N*-MeAla-Ala	-	-	38, 46
nummularine P (**22**)	*Z. rugosa* (bark)	R^1 = OCH_3 R^2 = Leu (side chain) R^3 = *N*-MeAla-Val	-	-	32
rugosanine A (**20**) [anti-bacterial, anti-fungi]	*Z. rugosa* (stem bark)	R^1 = OCH_3 R^2 = Leu (side chain) R^3 = *N*-CHO-*N*-MeAla-Val	-	-	31, 46
sativanine H (**23**)	*Z. rugosa* (bark)	R^1 = OCH_3 R^2 = Leu (side chain) R^3 = *N,N*-Me_2Gly-Val			32

TABLE 1 (continued)

Common name [Bioactivity]	Plant source (Parts)	R^1, R^2, R^3	stereochemistry at C8 and C9		Ref
zizyphine A (**1**)	*Z. oenoplia* (root bark) (stem bark)	R^1 = OCH_3 R^2 = Pro (side chain) R^3 = *N,N*-Me_2 Ile-Ile	-	-	22-23
zizyphine B (**2**)	*Z. oenoplia* (root bark) (stem bark)	R^1 = OCH_3 R^2 = Pro (side chain) R^3 = *N*-MeIle-Ile	-	-	22-23
zizyphine C (**5**)	*Z. oenoplia* (stem bark)	R^1 = OCH_3 R^2 = Pro (side chain) R^3 = *N,N*-Me_2Phe-Ile	-	-	24
zizyphine F (**8**)	*Z. oenoplia* (bark)	R^1 = OH R^2 = Pro (side chain) R^3 = *N,N*-Me_2Ile-Ile	-	-	25
zizyphine I (**10**)	*Z. oenoplia* (bark)	R^1 = OCH_3 R^2 = Pro (side chain) R^3 = *N,N*-Me_2Ile-Phe	-	-	26
zizyphine K (**11**)	*Z. oenoplia* (stem bark)	R^1 = OH R^2 = Pro (side chain) R^3 = *N,N*-Me_2 Ile-Val	-	-	27
zizyphine N (**15**) [antiplasmodial, antimycobacterial]	*Z. oenoplia* var. *brunoniana* (root)	R^1 = OCH_3 R^2 = *L*-Pro (side chain) R^3 = *L*-N,N-Me_2Ile-*L*-Leu	*S*	*S*	29

TABLE 1 (continued)

Common name [Bioactivity]	Plant source (Parts)	R^1, R^2, R^3	stereochemistry at C8 and C9		Ref
zizyphine O (**16**)	*Z. oenoplia* var. *brunoniana* (root)	R^1 = OCH_3 R^2 = *L*-Pro (side chain) R^3 = *L*-N-Me Ile-*L*-Leu	*S*	*S*	29
zizyphine P (**17**)	*Z. oenoplia* var. *brunoniana* (root)	R^1 = OH R^2 = *L*-Pro (side chain) R^3 = *L*-N,N-Me_2 Ile-*L*-Leu	*S*	*S*	29
zizyphine Q (**18**) [antiplasmodial, antimycobacterial]	*Z. oenoplia* var. *brunoniana* (root)	R^1 = OCH_3 R^2 = *L*-Pro (side chain) R^3 = *L*-N,N-Me_2 Ile-*L*-Val	*S*	*S*	29
14-membered ring					
4(14)-Frangulanine type					
adouetine X (**52**)	*Z. jujuba* var. *inermis* (root bark)	R^1 = Ile (side chain) R^2 = *N,N*-Me_2 Leu	-	-	44
daechuine S5 (**49**)	*Z. jujuba* var. *inermis* (stem bark)	R^1 = Leu (side chain) R^2 = *N,N*-Me_2 Leu	-	-	40

TABLE 1 (continued)

Common name [Bioactivity]	Plant source (Parts)	R^1, R^2	stereochemistry at C8 and C9		Ref
frangufoline (**13**) [sedative, anti-bacterial, anti-fungi]	*Z. oenoplia* (stem bark)	R^1 = *L*-Ile (side chain) R^2 = *L*-*N*,*N*-Me_2Phe	*S*	*S*	28, 46
frangulanine (**51**)	*Z. jujuba* var. *inermis* (root bark)	R^1 = *L*-Ile (side chain) R^2 = *L*-*N*,*N*-Me_2Ile	*S*	*S*	44
nummularine K (**46**)	*Z. jujuba* (stem bark)	R^1 = Leu (side chain) R^2 = *N*,*N*-Me_2Trp	-	-	41
scutianine C (**48**)	*Z. jujuba* (stem bark)	R^1 = β-OH Phe (side chain) R^2 = COCH=CHPh	-	-	39
5(14)-Scutianine-A type					
jubanine C (**47**)	*Z. jujuba* (stem bark)	R^1 = Phe (side chain) R^2 = *N*,*N*-Me_2Ile-Pro	-	-	39
4(14)-Amphibine-F type					
amphibine F (**30**)	*Z. mauritiana* (bark)	R^1 = Phe (side chain) R^2 = *N*-MeIle	-	-	34

TABLE 1 (continued)

Common name [Bioactivity]	Plant source (Parts)	R^1, R^2	stereochemistry at C8 and C9		Ref
mauritine C (**26**)	*Z. mauritiana* (bark)	R^1 = Phe (side chain) R^2 = *N*-MeVal	-	-	34
zizyphine G (**9**)	*Z. oenoplia* (bark)	R^1 = Pro (side chain) R^2 = Ile	-	-	25
5(14)-Amphibine-B type					
amphibine D (**19**)	*Z. rugosa* *Z. mauritiana* (bark)	R^1 = Ile (side chain) R^2 = *N,N*-Me_2Phe-Ile	-	-	30, 34
amphibine B (**14**)	*Z. oenoplia* *Z. mauritiana* (bark)	R^1 = Phe (side chain) R^2 = *N,N*-Me_2Phe-Ile	-	-	28, 34
amphibine E (**33**)	*Z. mauritiana* (root bark)	R^1 = *L*-Ile (side chain) R^2 = *L*-*N,N*-Me_2Leu-*L*-Trp	*S*	*S*	36
mauritine A (**24**)	*Z. mauritiana* (bark)	R^1 = *L*-Phe (side chain) R^2 = *L*-*N,N*-Me_2Ala-*L*-Val	*S*	*S*	33
mauritine B (**25**)	*Z. mauritiana* (bark)	R^1 = Phe (side chain) R^2 = *N,N*-Me_2Ile-Val	-	-	33
mauritine D (**12**)	*Z. mauritiana* (bark)	R^1 = Ile (side chain) R^2 = *N,N*-Me_2Ile-Leu	-	-	34

TABLE 1 (continued)

Common name [Bioactivity]	Plant source (Parts)	R^1, R^2	stereochemistry at C8 and C9		Ref
mauritine E (**27**)	*Z. mauritiana* (stem bark)	R^1 = β-OHPhe (side chain) R^2 = *N,N*-Me_2Ala-Val	-	-	34
mauritine F (**28**)	*Z. mauritiana* (stem bark)	R^1 = Phe (side chain) R^2 = *N*-MeAla-Val	-	-	34
mauritine H (**29**)	*Z. mauritiana* (bark)	R^1 = Phe (side chain) R^2 = *N,N*-Me_2Ala-Leu	-	-	35
mauritine J (**31**)	*Z. mauritiana* (root bark)	R^1 = *L*-Ile (side chain) R^2 = *L*-*N*-MeLeu-*L*-Trp	*S*	*S*	36
mauritine K (**32**) [anti-fungi]	*Z. mauritiana* (root bark)	R^1 = Ile (side chain) R^2 = Ile-Ile	*S*	*S*	37
15-membered ring					
4(15)-Mucronine-A type					
abyssinine A (**3**)	*Z. oenoplia* (stem bark)	R^1 = Leu (side chain) R^2 = NHMe	-	-	24

TABLE 1 (continued)

Common name [Bioactivity]	Plant source (Parts)	R^1, R^2	stereochemistry at C8 and C9	Ref
abyssinine B (**4**)	*Z. oenoplia* (stem bark)	R^1 = Ile (side chain) R^2 = NHMe	- -	24
zizyphine D (**6**)	*Z. oenoplia* (stem bark)	R^1 = β-OHIle (side chain) R^2 = NHMe	- -	24
zizyphine E (**7**)	*Z. oenoplia* (stem bark)	R^1 = β-OHIle (side chain) R^2 = NH_2	- -	24

Other alkaloids

Deachualkaloid A (**54**),[49] a new pyrrolidine alkaloid and zizyphusine (**55**)[45] were isolated from fruit of *Z. jujuba* var. *inermis*.

deachualkaloid A (**54**)

zizyphusine (**55**)

2. Triterpenoids

Triterpenes are a large group of natural products derived from C30 precursors. Most triterpenoids are 6-6-6-5 tetracycles, 6-6-6-6-5 or 6-6-6-6-5 pentacycles.[50] Triterpenes of *Ziziphus* found in Thailand are lupane-, ceanothan-, oleanane- and ursane-type structures, as shown in Table 2.

Betulinic acid (**56**), 3-hydroxy-lup-20(29)-en-28-oic acid, which was isolated as a major components, is widely distributed pentacyclic lupane-type triterpene in *Ziziphus* plants. It was found in *Z. oenoplia*,[51-52] *Z. rugosa*,[52-55] *Z. cambodiana*,[16-17] *Z. attopoensis,*[56] *Z. jujuba*[57-59] and *Z. mauritiana*.[60]

Lupeol (**57**) was isolated from *Z. rugosa,*[52,55] *Z. cambodiana,*[16-17] *Z. attopoensis*[56] and *Z. mauritiana*.[61]

Betulin (**58**), a closely related compound with betulinic acid, was isolated from the root of *Z. jujuba* Mill var *spinosa,*[57] the root bark of *Z. rugosa*,[52-53,55] and the stem of *Z. mauritiana*.[61]

Betulinaldehyde (**59**) was isolated from *Z. rugosa,*[52,55] *Z. cambodiana,*[16-17] and the twig of *Z. attopoensis*.[56]

Alphitolic acid (**60**) contain one hydroxyl group more than betulinic acid at C-3, was found in *Z. rugosa*,[54-55] *Z. cambodiana,*[16-17] *Z. attopoensis,*[56] *Z. jujuba*[58-59] and *Z. mauritiana*.[62] 2-*O*-Protocatechuoylalphitolic acid (**61**) and 2α-hydroxypyracrenic acid (**62**) were isolated from the water-insoluble fraction of EtOH extract of *Z. jujuba* var. *spinosa* root.[63] Moreover, *p*-coumaroylates of alphitolic acid as 2-*O-trans-p*-coumaroyl alphitolic acid (**63**) was isolated from the fruit of *Z. jujuba*,[58] the root bark of *Z. rugosa*,[55] and the root bark of *Z. cambodiana*.[16-17] 3-*O-cis-p*-Coumaroyl alphitolic acid (**64**) and 3-*O-trans-p*-coumaroyl alphitolic acid (**65**) were reported to be isolated from the fruit of *Z. jujuba*.[58-59]

Betulonic acid (**66**) was isolated from the fruit of *Z. jujuba*,[59,64-65] and the stem of *Z. mauritiana*.[61]

The ceanothane-type triterpene have ring A containing five membered ring. The ceanothane-type triterpene in *Ziziphus* plants as ceanothic acid (**67**) was isolated from the root of *Z. jujuba* Mill var. *spinosa,*[57] the root bark of *Z. cambodiana*.[16-17] and *Z. rugosa*.[55]

Ceanothanolic acid (**68**), the related structure of ceanothic acid was isolated from *Z. rugosa*.[55] Two ceanothane-type derivertives, 3-*O*-protocatechuoylceanothic acid (**69**) was isolated from the root of *Z. jujuba* var. *spinosa* [63] and 3-*O*-vanillylceanothic acid (**70**) was isolated from the root bark of *Z. cambodiana*.[17]

Zizyberanalic acid (**71**) and Zizyberenalic acid (**72**) were found in the fruit of *Z. jujuba* Mill, [59,64] and the root bark of *Z. cambodiana*.[17] A-norlupa-2,20-diene-14,17-dicarboxylic acid (**73**) has been isolated from the leaf of *Z. jujuba*.[66]

The ursane-type triterpene, ursolic acid (**74**) was isolated from the root of *Z. jujuba* Mill var. *spinosa*.[57] 2α-Hydroxyursolic acid (**75**) was isolated from *Z. rugosa*[54] and the root of *Z. jujuba* Mill var. *spinosa*.[57] The fruit of *Z. jujuba* Mill. afforded ursolonic acid (**76**).[64]

The oleanane-type triterpene : oleanolic acid (**77**) was isolated from *Z. rugosa*,[54] and *Z. jujuba* Mill. fruit.[59] Oleanonic acid (**78**) obtained from the fruit of *Z. jujuba* Mill.[59,64-65] Maslinic acid (**79**) and its derivertives as 3-*O*-*cis*-*p*-coumaroyl maslinic acid (**80**) and 3-*O*-*trans*-*p*-coumaroyl maslinic acid (**81**) were also isolated from the fruit of *Z. jujuba* Mill.[59,65]

Biological activities

Betulinic acid (**56**) had been shown to inhibit tumor growth without toxic side effect,[60] it was also reported as antimycobacterial activity.[17]

Zizyberenalic acid (**72**), oleanonic acid (**78**), ursolonic acid (**76**) and betulonic acid (**66**) showed anti-viral producing hepatoma cell line MS-G2. All of these compounds displayed anti-HBsAg and anti-HBeAg activities against MS-G2 cells with EC_{50} values of 22.3 and 19.3 μM, 18.1 and 18.3 μM, 23.5 and 25.9 μM, and 4.7 and 4.5 μM, respectively. However, cytotoxic effects were found at higher concentrations with IC_{50} values of 41.9, 55.2, >100 and 71.7 μM, respectively.[64]

Oleanolic acid (**77**), 3-*O*-*cis*-*p*-coumaroyl maslinic acid (**78**) and 3-*O*-*trans*-*p*-coumaroyl maslinic acid (**81**) exhibited significant anti-complement activity with IC_{50} values of 163.4, 101.4 and 143.9 μM, respectively, whereas the ceanothane- and lupane-types triterpenes were

inactive. This suggests that the oleanane structure plays an important role in inhibiting the hemolytic activity of human serum against erythrocytes.[59]

3-*O*-vanillylceanothic acid (**70**), 2-*O*-*trans*-*p*-coumaroyl alphitolic acid (**71**) and zizyberenalic acid (**72**) exhibited significant *in vitro* antiplasmodial activity against the parasite *Plasmodium falciparum*, with IC_{50} values of 3.7, 0.9 and 3.0 μg/mL, respectively. Moreover, 3-*O*-vanillylceanothic acid (**70**), betulinaldehyde (**59**), betulinic acid (**56**), 2-*O*-*trans*-*p*-coumaroyl alphitolic acid (**63**), alphitolic acid (**60**), zizyberanalic acid (**71**) and zizyberenalic acid (**72**) showed antimycobacterial activity against *Mycobacterium tuberculosis* with respective MIC values of 25, 25, 25, 12.5, 50, 50 and 100 μg/mL.[17]

TABLE 2 Types of triterpenoids *Ziziphus* found in Thailand.

Common name	Plant sources	R^1, R^2, R^3	Ref
Lupane-type			
betulinic acid (**56**)	*Z. oenoplia*, *Z. rugosa*,	R^1 = H	16-17
	Z. cambodiana, Z. jujuba,	R^2 = H	51-61
	Z. attopoensis, Z. mauritiana	R^3 = COOH	
lupeol (**57**)	*Z. rugosa, Z. mauritiana,*	R^1 = H	16-17
	Z. cambodiana, Z. attopoensis	R^2 = H	52, 55-56
		R^3 = CH_3	61
betulin (**58**)	*Z. jujuba* Mill var *spinosa*,	R^1 = H	52-53
	Z. rugosa, *Z. mauritiana*	R^2 = H	55, 57
		R^3 = CH_2OH	61
betulinaldehyde (**59**)	*Z. rugosa, Z. cambodiana*	R^1 = H	16-17
	Z. attopoensis	R^2 = H	52, 55-56
		R^3 = CHO	
alphitolic acid (**60**)	*Z. rugosa*, *Z. jujuba,*	R^1 = OH	16-17
	Z. mauritiana,	R^2 = H	54-55
	Z. cambodiana,	R^3 = COOH	58-59
	Z. attopoensis		62

TABLE 2 (continued)

Common name	Plant sources	R^1, R^2, R^3	Ref
2-*O*-protocatechuoylalphitolic acid (**61**)	*Z. jujuba* var. *spinosa*	R^1= *O*-protocatechuoyl R^2= H R^3 = COOH	63
2α-hydroxypyracrenic acid (**62**)	*Z. jujuba* var. *spinosa*	R^1 = OH R^2 = caffeoyl R^3 = COOH	63
2-*O*-*trans*-*p*-coumaroyl alphitolic acid (**63**)	*Z. jujuba*, *Z. rugosa* *Z. cambodiana*	R^1 = *O*-*E*-*p*-coumaroyl R^2= H R^3= COOH	16-17 55, 58
3-*O*-*cis*-*p*-coumaroyl alphitolic acid (**64**)	*Z. jujuba*	R^1 = OH R^2 = *Z*-*p*-coumaroyl R^3 = COOH	58-59
3-*O*-*trans*-*p*-coumaroyl alphitolic acid (**65**)	*Z. jujuba*	R^1 = OH R^2 = *E*-*p*-coumaroyl R^3 = COOH	58-59

TABLE 2 (continued)

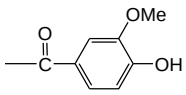
Common name	Plant sources	R^1, R^2, R^3	Ref
betulonic acid (**66**)	*Z. jujuba*, *Z. mauritiana*	R^1 = H R^2 = O R^3 = COOH	59, 61 64-65
Ceanothane-type			
ceanothic acid (**67**)	*Z. jujuba* Mill var. *spinosa,* *Z. rugosa, Z. cambodiana*	R^1 = α-COOH R^2 = β-OH	16-17 55
ceanothanolic acid (**68**)	*Z. rugosa*	R^1 = β-CH_2OH R^2 = α-OH	55
3-*O*-protocatechuoylceanothic acid (**69**)	*Z. jujuba* var. *spinosa*	R^1 = α-COOH R^2=β-*O*-protocatechuoyl	63
3-*O*-vanillylceanothic acid (**70**)	*Z. cambodiana*	R^1 = α-COOH R^2 = β-*O*-vanillyl 	17
zizyberanalic acid (**71**)	*Z. jujuba* Mill, *Z. cambodiana*	R^1 = β-CHO R^2 = α-OH	17, 59

TABLE 2 (continued)

Common name	Plant sources	R^1, R^2	Ref
zizyberenalic acid (**72**)	*Z. jujuba* Mill,	R^1 = CHO	17, 59
	Z. cambodiana	R^2 = CH_3	64
A-norlupa-2,20-diene-14,17-	*Z. jujuba*	R^1 = H	66
dicarboxylic acid (**73**)		R^2 = COOH	
Ursane-type			
ursolic acid (**74**)	*Z. jujuba* Mill var.	R^1 = H	57
	spinosa	R^2 = β-OH	
2α-hydroxyursolic acid (**75**)	*Z. jujuba* Mill var.	R^1 = α-OH	54, 57
	spinosa, *Z. rugosa*	R^2 = β-OH	
ursolonic acid (**76**)	*Z. jujuba* Mill	R^1 = H	64
		R^2 = O	

TABLE 2 (continued)

Common name	Plant sources	R^1, R^2	Ref
oleanane-types			
oleanolic acid (**77**)	*Z. rugosa*,	R^1 = H	54, 59
	Z. jujuba Mill	R^2 = OH	
oleanonic acid (**78**)	*Z. jujuba* Mill	R^1 = H	59
		R^2 = O	64-65
maslinic acid (**79**)	*Z. jujuba* Mill	R^1 = OH	59, 65
		R^2 = OH	
3-*O-cis-p*-coumaroyl maslinic	*Z. jujuba* Mill	R^1 = OH	59, 65
acid (**80**)		R^2 = *Z-p*-coumaroyl	
3-*O-trans-p*-coumaroyl maslinic	*Z. jujuba* Mill	R^1 = OH	59, 65
acid (**81**)		R^2 = *E-p*-coumaroyl	

3. Sapogenin and steroids

A new sapogenin, zizogenin (**82**) had been found from the stem of *Z. mauritiana* together with two steroids; β-sitosterol (**83**) and sitosterol acetate (**84**).[61,67]

zizogenin (**82**)

β-sitosterol (**83**): R = H

sitosterol acetate (**84**): R = Ac

4. Flavonoids and flavonoid glycosides

Rutin (**85**), quercetin (**86**), quercetin-3-rhamnoside (**87**) and quercetin-3-rhamnosylglucoside (**88**) were isolated from the leaf of *Z. jujuba*.[67] The bark showed the presence of myricetin (**89**), kaempferol (**90**) and kaempferol-7-methylether (**91**).[39] The seed of *Z. jujuba* Mill var. *spinosa* was found to be swertish (**92**), puerarin (**93**), isovitexin-2′′-*O*-β-D-glucopyranoside (**94**), apigenin-6-C-β-D-glucopyranoside (**95**), spinosin (**96**), 6′′′-feruloylspinosin (**97**), 6′′′-sinapolyspinosin (**98**), 6′′′-*p*-coumaroylspinosin (**99**)[69-70] and two novel flavonoids, isospinosin (**100**) and 6′′′-feruloylisospinosin (**101**).[70]

Investigation of the bark of *Z. rugosa* led to the isolation of quercetin-3-*O*-rhamnoside (**87**), myricetin-3-*O*-rhamnoside (**102**) and kaempferol-3-*O*-rhamnoside (**103**).[71]

The leaf of *Z. cambodiana* afforded quercetin-3-*O*-β-D-arabinosyl-(1$\rightarrow$2)-α-L-rhamnoside (**104**), quercitrin or quercetin (**86**), isoquercitrin or quercetin-3-*O*-β-D-glucoside (**105**).[72]

(-)-Epicatechin (**106**) was obtained from the EtOAc extract of the twig of *Z. attopoensis*.[56]

TABLE 3 Flavonoids and flavonoid glycosides of *Ziziphus* found in Thailand.

Common name	Plant sources (Parts)	R^1, R^2, R^3, R^4, R^5	Ref
Flavonol			
rutin (**85**)	*Z. jujuba* (leaf)	$R^1 = R^3 = H$ $R^2 = R^4 = R^5 = OH$	67
quercetin (**86**)	*Z. jujuba* *Z. cambodiana* (leaf)	$R^1 = R^2 = R^4 = R^5 = OH$ $R^3 = H$	67, 72
myricetin (**89**)	*Z. jujuba* (bark)	$R^1 = R^2 = R^3 = R^4 = R^5 = OH$	39
kaempferol (**90**)	*Z. jujuba* (bark)	$R^1 = R^3 = H$ $R^2 = R^4 = R^5 = OH$	39
Flavonol glycoside			
quercetin-3-rhamnoside (**87**)	*Z. jujuba* (leaf) *Z. rugosa*	$R^1 = R^2 = R^5 = OH$, $R^3 = H$ R^4 = *O*-rhamnose	67, 71

TABLE 3 (continued)

Common name	Plant sources (Parts)	R^1, R^2, R^3, R^4, R^5	Ref
quercetin-3-rhamnosylglucoside (**88**)	*Z. jujuba* (leaf)	$R^1 = R^2 = R^5 = OH$, $R^3 = H$ R^4 = *O*-rhamnosyl glucose	67
kaempferol-7-methylether (**91**)	*Z. jujuba* (bark)	$R^1 = R^2$= H, $R^3 = R^4 = OH$ $R^5 = OCH_3$	39
myricetin-3-*O*-rhamnoside (**102**)	*Z. rugosa* (bark)	$R^1 = R^2 = R^3 = R^5 = OH$ R^4 = *O*-rhamnose	71
kaempferol-3-*O*-rhamnoside (**103**)	*Z. rugosa* (bark)	$R^1 = R^3$ = H, $R^2 = R^5 = OH$ R^4 = *O*-rhamnose	71
quercetin-3-*O*-β-D-arabinosyl-(1→2)-α-L-rhamnoside (**104**)	*Z. cambodiana* (leaf)	$R^1 = R^2 = R^5 = OH$, $R^3 = H$ R^4 = *O*-arabinosyl rhamnose	72
quercetin-3-*O*-β-D-glucoside (**105**)	*Z. cambodiana* (leaf)	$R^1 = R^2 = R^5 = OH$, $R^3 = H$ R^4 = *O*-glucose	72
Flavone			
apigenin-6-C-β-D-glucopyranoside (**95**)	*Z. jujuba* Mill var. *spinosa* (seed)	$R^1 = -C_6H_4$-OH, $R^2 = R^6 = H$ $R^3 = OH$, $R^5 = CH_3$ R^4 =	70

TABLE 3 (continued)

Common name	Plant sources (Parts)	R^1, R^2, R^3, R^4, R^5, R^6	Ref
puerarin (**93**)	*Z. jujuba* Mill var. *spinosa* (seed)	$R^1 = R^3 = R^4 = R^5 = H$ $R^2 = -C_6H_4-OH$ $R^6 =$	70
swertish (**92**)	*Z. jujuba* Mill var. *spinosa* (seed)	$R^1 = -C_6H_4-OH$, $R^3 = OH$ $R^2 = R^6 = R^5 = H$ $R^4 =$	70
spinosin (**96**)	*Z. jujuba* Mill var. *spinosa* (seed)	$R^1 = CH_3$ $R^2 = OH$	70
isovitexin-2″-*O*-*β*-D-glucopyranoside (**94**)	*Z. jujuba* Mill var. *spinosa* (seed)	$R^1 = R^2 = H$	70

TABLE 3 (continued)

Common name	Plant sources (Parts)	R^1, R^2	Ref
6‴-feruloylspinosin (**97**)	*Z. jujuba* Mill var. *spinosa* (seed)	R^1 = CH_3 R^2 = feruloyl	69-70
6‴-sinapolyspinosin (**98**)	*Z. jujuba* Mill var. *spinosa* (seed)	R^1 = CH_3 R^2 = sinapoyl	69
6‴-*p*-coumaroylspinosin (**99**)	*Z. jujuba* Mill var. *spinosa* (seed)	R^1 = CH_3 R^2 = *p*-coumaroyl	69
isospinosin (**100**)	*Z. jujuba* Mill var. *spinosa* (seed)	R^1 = H	70
6‴-feruloylisospinosin (**101**)	*Z. jujuba* Mill var. *spinosa* (seed)	R^1 = feruloyl	70

TABLE 3 (continued)

Common name	Plant sources (Parts)	R^1, R^2	Ref
Flavanol			
(-)-epicatechin (**106**)	*Z. attopoensis* (twig)		56

5. Saponins

Rugoside A (**107**) or jujubogenin-3-O-D glucosyl-(1→3)-L-arabinopyranoside, a triterpenoid saponin[73] and zizyotin (**108**)[28] were isolated from the bark of *Z. rugosa*.

rugoside A (**107**)

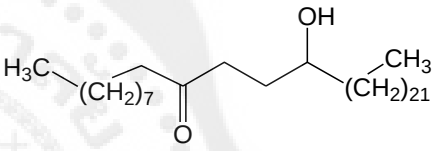
zizyotin (**108**)

6. Miscellaneous

Neo-lignan (**109**), a prostaglandin I2 inducer which is the most potent natural inhibitor of platelet aggregation, was isolated from the leaf of *Z. jujuba*.[74]

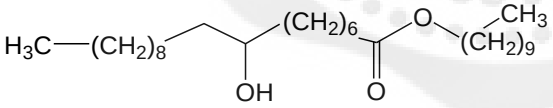
12-Hydroxy-tetratriacontan-9-one (**110**), decyl-8-hydroxy-heptadecanoate (**111**),[75] undecyl-hexadecanoate (**112**) and 12-hydroxy-hentriacontane (**113**) [76] were found from *Z. mauritiana*.

n-Nonacosane (**114**) and octacosanol (**115**) were also isolated from the stem bark of *Z. rugosa*.[77]

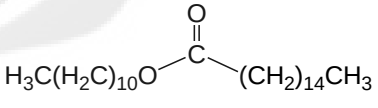


neo-lignan (**109**)

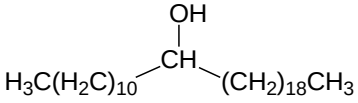
12-hydroxy-tetratriacontan-9-one (**110**)



decyl-8-hydroxy-heptadecanoate (**111**)



undecyl-hexadecanoate (**112**)



12-hydroxy-hentriacontane (**113**)

octacosanol (**114**)

n-nonacosane (**115**)

CHAPTER 3

EXPERIMENTAL

Source of plant material

The air-dried root of *Z. mauritiana* was collected from Samchuk District, Suphanburi Province, Thailand in June 2005. A voucher specimen (Jessada Netsawangwicha 002) is deposited at the Faculty of Science, Ramkhamhaeng University.

General techniques

1. Thin-layer chromatography (TLC)

Techniques : One dimension, ascending.

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

Layer Thickness : 1.25 mm

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

Detection : 1. Spots on TLC were visualized under ultraviolet light at wavelengths of 254 and 365 nm.

2. Developing reagent: anisaldehyde-sulphuric reagent (0.5% anisaldehyde in methanolic solution containing 4.5% sulphuric acid and 10% glacial acetic acid). After heating of TLC plate at 100-110 C^{o} for 2-3 minutes, the spots of organic compounds gave specific colors with this reagent.

2. Column chromatography (CC)

2.1 Liquid column chromatography

Adsorbent : 1. Silica gel 60, particle size finer than 0.063 mm (Merck 1.07729)

2. Silica gel 60, particle size 0.063-0.0200 mm (Merck 1.07734)

3. Silica gel 60 RP-18 (40-63 μm) for preparative HPLC (Merck 1.10167)

4. Alumina oxide 90 (neutral) for column chromatography

Packing method : Slurry packing method

Sample loading : The sample was dissolved in a small volume of suitable organic solvent. The solution was mixed with silica gel (particle size 0.063-0.0200 mm, Merck 1.07734). The sample was evaporated under reduced pressure and added onto the top of column.

Elution : After loading of sample onto the column, an appropriate solvent system was used as a mobile phase in the isocratic or gradient systems.

2.2 Quick column chromatography [78]

Adsorbent : Silica gel 60 GF_{254} for thin-layer chromatography (Merck 1.07730)

Packing method : Dry vacuum packing method

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

Elution : After loading of sample onto the column, an appropriate solvent system was used as a mobile phase in the gradient systems.

2.3 Size-Exclusion gel column chromatography

Adsorbent : Sephadex-LH 20

Packing method : Slurry packing method

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

Elution : The column was eluted with MeOH.

Physical properties

1. Optical rotations

Optical rotations were recorded in MeOH or $CHCl_3$ on a JASCO-1020 digital polarimeter (Department of Chemistry, Faculty of Science, Ramkhamhaeng University).

2. Melting points

Melting points were measured on a Griffin melting point apparatus in degree Celsius of temperature.

Spectroscopy

1. Infrared (IR) absorption spectra

IR spectra were recorded on a Perkin Elmer FT-IR Spectrum BX spectrophotometer by using potassium bromide (KBr) disc.

2. Ultraviolet (UV) absorption spectra

UV spectra were obtained on a Shimadzu UV-2401 PC spectrophotometer.

3. Mass spectra

Electrospray ionization mass spectra (ESMS), EIMS and HRTOFMS (APCI) were measured on a Finnigan LC-Q (Department of Chemistry, Faculty of Science, Ramkhamhaeng University) and a Bruker MicrOTOF mass spectrometers (the Chulaborn Research Institute).

4. Nuclear Magnetic Resonance (NMR) spectra

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

5. X-ray diffraction data

Crystals suitable for X-ray analysis were obtained by recrystallization from an appropriate solvent system (CH_2Cl_2-MeOH-H_2O) and crystallography data were measured on a Bruker–Nonius kappaCCD diffractometer (Department of Chemistry, Faculty of Science, Mahidol University)

Data analysis

The structures of compounds were determined using spectroscopic techniques such as Nuclear Magnetic Resonance (NMR), Mass (MS), Infrared (IR), Ultraviolet (UV) and X-ray diffraction data.

Extraction, isolation and bioassay procedures

1. Extraction of the root of *Z. mauritiana*

Pulverized, dry root (4.5 kg) of *Z. mauritiana* was successively macerated with ethyl acetate (3 x 10 L) and then with methanol (3 x 10 L) at room temperature for each 7 days. Each extract was evaporated under reduced pressure at 45 $^{\circ}$C to afford an EtOAc extract (29.6 g) and a MeOH extract (45.6 g), respectively. The extraction procedure is shown in Scheme 1.

2. Isolation of compounds from the ethyl acetate extract of the root of *Z. mauritiana*

The EtOAc extract (25.0 g) was fractionated by quick column chromatography (Merck silica gel 60 GF_{254}, 150 g), eluting with *n*-hexane, *n*-hexane-CH_2Cl_2, CH_2Cl_2, CH_2Cl_2-EtOAc, EtOAc, EtOAc-MeOH and MeOH with increasing amounts of the more polar solvent. The eluates were examined by TLC and 11 combined fractions (Fr.1-11) were obtained.

2.1 Isolation of compound ZM-E1 (lupeol, sss3194, sss3921)

Fraction 5 (0.52 g) was chromatographed on a silica gel column (finer than 0.063 mm, 16 g) eluted with *n*-hexane and *n*-hexane-EtOAc (1% increment of EtOAc, each 100 mL).

Eighty-nine fractions (7 mL per fraction) were collected and combined according to their TLC behavior to yield compound ZM-E1 (fractions 58-65) as colorless solid (26.9 mg) of lupeol (**57**).

2.2 Isolation of compounds ZM-E2 and ZM-E4 (betulin, sss3828 and zizyberenalic acid, sss3812)

Fraction 8 (2.59 g) was rechromatographed over silica gel (finer than 0.063 mm, 60 g) with *n*-hexane-EtOAc as eluting solvent to give seven subfractions. Subfraction 3 (777.2 mg) was chromatographed using *n*-hexane-CH_2Cl_2, CH_2Cl_2 and CH_2Cl_2-EtOAc as eluting solvent, with increasing amount of the more polar solvent to provide eight subfractions (fr. 3a-3h) Fraction 3b (79.8 mg) was further chromatographed, eluting with *n*-hexane-EtOAc (1% increment of EtOAc, each 100 mL), to afford compounds ZM-E2 (fractions 75-85, 37.1 mg) and ZM-E4 (fractions 96-147, 3.0 mg) as colorless solid. Compound ZM-E2 and ZM-E4 were identified as betulin (**58**) and zizyberenalic acid (**72**), respectively (see Scheme 2).

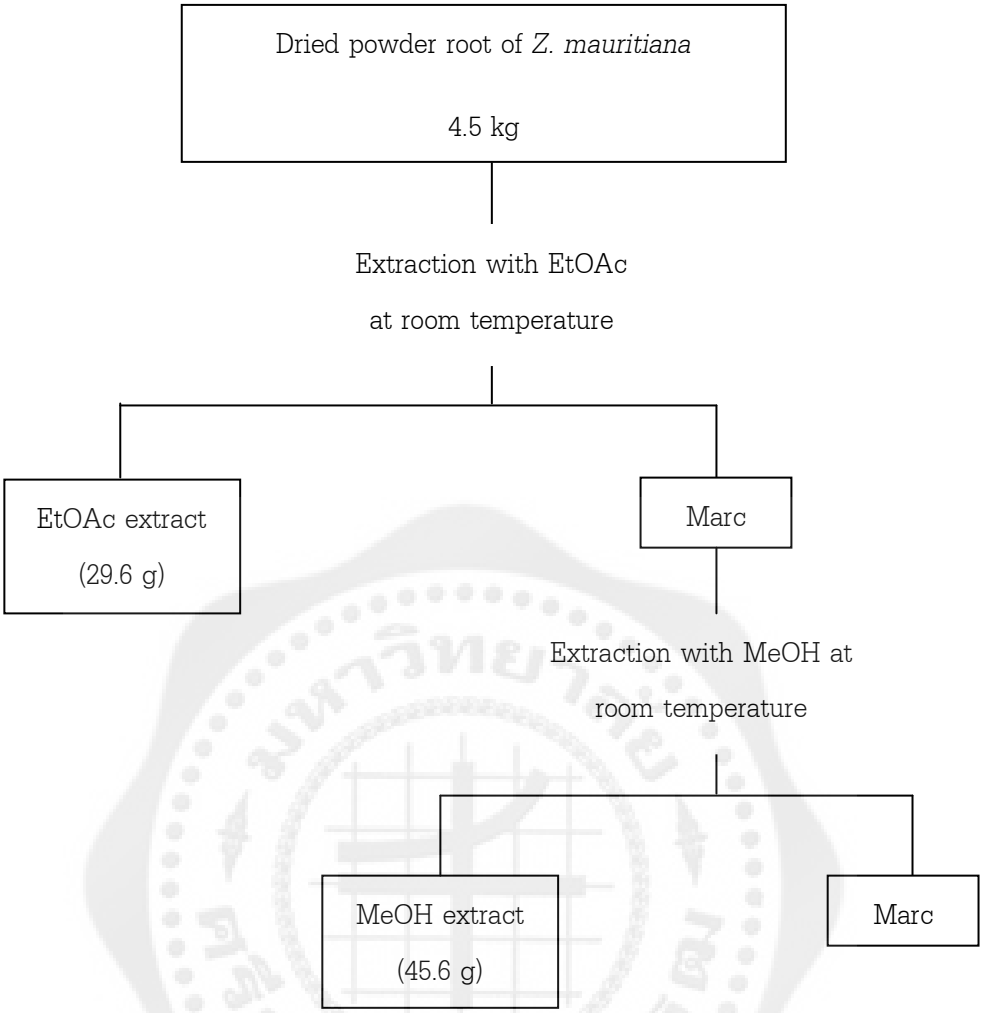
2.3 Isolation of compound ZM-E3 (betulinic acid, sss2878)

Fraction 9 (5.98 g) was recrystallized with MeOH to give compound ZM-E3 as major compound (2.38 g). This compound was a colorless solid and subsequently identified as betulinic acid (**56**) (see Scheme 2).

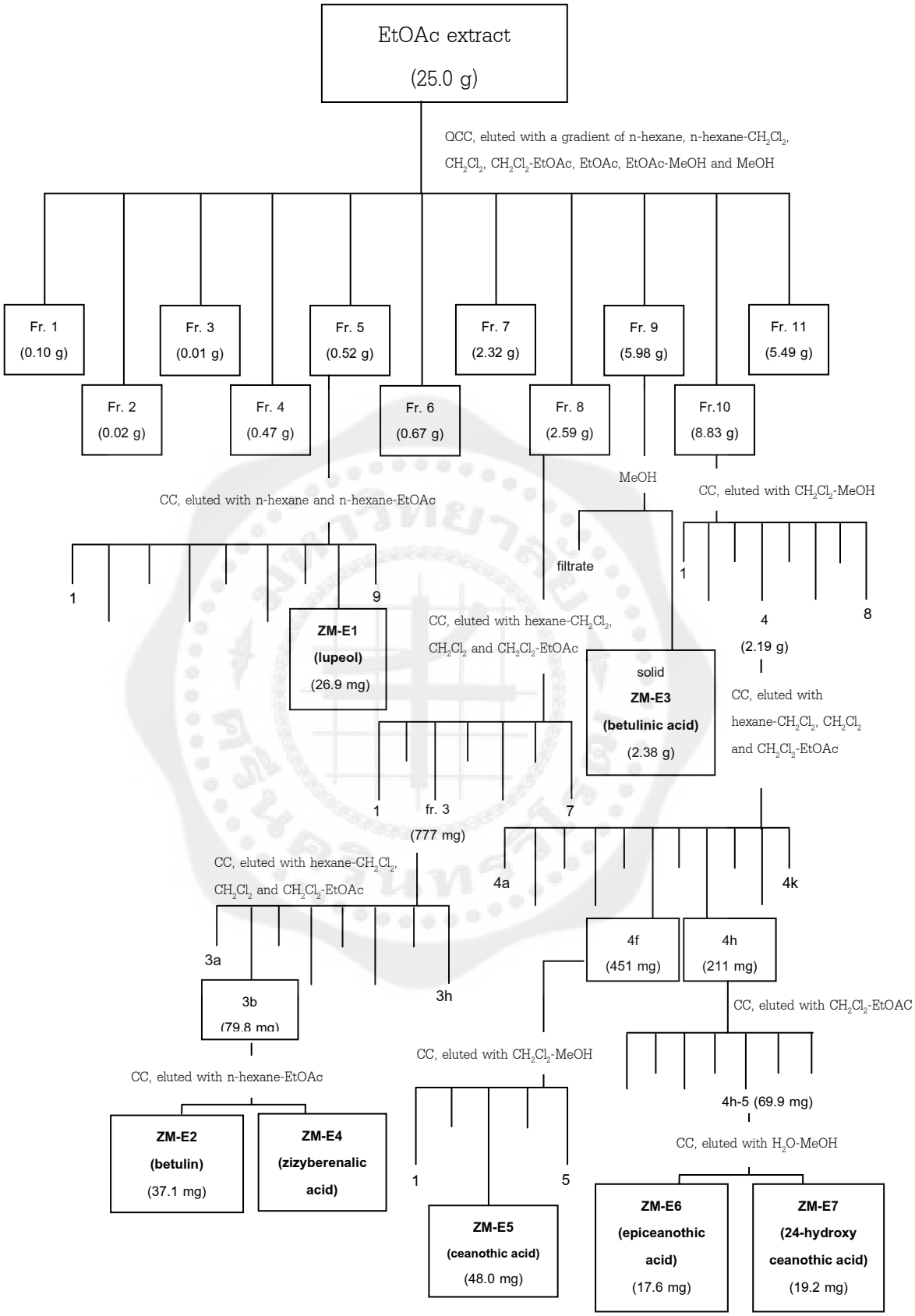
2.4 Isolation of compounds ZM-E5, ZM-E6 and ZM-E7 (ceanothic acid, sss3193; epiceanothic acid, sss3340 and 24-hydroxyceanothic acid, sss3336)

Fraction 10 (8.83 g) was chromatographed over silica gel (finer than 0.063 mm, 60 g) with CH_2Cl_2-MeOH as eluting solvent to give eight subfractions. Subfraction 4 (2.19 g) was chromatographed using *n*-hexane-CH_2Cl_2, CH_2Cl_2 and CH_2Cl_2-EtOAc as eluents, with increasing amount of the more polar solvent to give 11 fractions (fr. 4a-4k). Fraction 4f (451.9 mg) was rechromatographed over silica gel (less than 0.063 mm, 33 g) with CH_2Cl_2-MeOH (1% increment of MeOH, each 200 mL) as eluting solvent to afford compound ZM-E5 as a colorless solid (48.0 mg) of ceanothic acid (**67**).

Fraction 4h (211.5 mg) was further subjected to silica gel chromatography employing solvent gradient CH_2Cl_2-EtOAC and six fractions (fr. 4h-1 - 4h-6) were collected. Fraction 4h-5 (69.9 mg) was further separated by column chromatography (silica gel 60 RP-18, 10 g) and eluted with H_2O-MeOH of decreasing polarity (5% increment of MeOH, each 100 mL) to give compound ZM-E6 (17.6 mg) and ZM-E7 (19.2 mg) as colorless solid. Compound ZM-E6 and ZM-E7 were subsequently identified as epiceanothic acid (**116**) and 24-hydroxyceanothic acid (**119**), respectively (see Scheme 2).



Scheme 1 Extraction procedure of the root of *Z. mauritiana*



Scheme 2 Isolation of compounds from the EtOAc extract of the root of *Z. mauritiana*

3. Isolation of compounds from the methanol extract of the root of *Z. mauritiana*

The MeOH extract (30.0 g) was fractionated by quick column chromatography (Merck silica gel 60 GF_{254}, 120 g), eluting with gradient systems of CH_2Cl_2-EtOAc, EtOAc, EtOAc-MeOH, MeOH and MeOH-H_2O (5-10% increment of more polar solvent, each 300 mL). The eluates were collected and examined by TLC. Fractions (99 fractions) with similar chromatographic pattern were combined to provide six major fractions (Fr.1-6).

3.1 Isolation of compound ZM-M1 (numularine H, sss3648)

Fraction 2 (5.2 g) was subjected to silica gel chromatography (finer than 0.063 mm, 50 g), eluting with CH_2Cl_2 and CH_2Cl_2-MeOH (1% increment of MeOH, each 200 mL), to give 11 subfractions as shown in Scheme 3.

Subfraction 4 (895.3 mg) was chromatographed on a silica gel column (18 g) eluted with CH_2Cl_2 and CH_2Cl_2-EtOAc with increasing amounts of the more polar solvent to afford nine fractions (fr. 4a-4i). Fraction 4g (407.1 mg) was further separated by silica gel chromatography and eluted with CH_2Cl_2-MeOH of increasing polarity to yield 9 fractions (fr. 4g-1 to 4g-9). Repeated column chromatography of fraction 4g-6 (40.5 mg) with a Sephadex LH20 column (in MeOH), followed by silica gel column chromatography employing solvent gradient *n*-hexane-EtOAc (1% increment of EtOAc, each 200 mL) gave compound ZM-M1 as colorless solid (8.9 mg). This compound was sequently identified as nummularine H (**123**).

3.2 Isolation of compounds ZM-M2 (hemsine A, sss3346) and ZM-M3 (mauritine L, sss3170, sss3756)

Subfraction 5 (509.0 mg) was subjected to column chromatography (silica gel 60, 16 g) employing solvent gradient of CH_2Cl_2, CH_2Cl_2-EtOAc and EtOAc (5% increment of more polar solvent, each 100 mL) to provide 13 fractions (fr. 5a-5m). Recrystallization of fraction 5f (35.6 mg) with MeOH gave compound ZM-M3 as colorless solid (14.1 mg) of mauritine L (**126**).

Fraction 5j (80.5 mg) was purified by a Sephadex LH20 column (in MeOH) yielded compound ZM-M2 as colorless solid (40.7 mg) of hemsine A (**125**), as shown in Scheme 3.

3.3 Isolation of compound ZM-M4 (mauritine M, sss3100, sss3347)

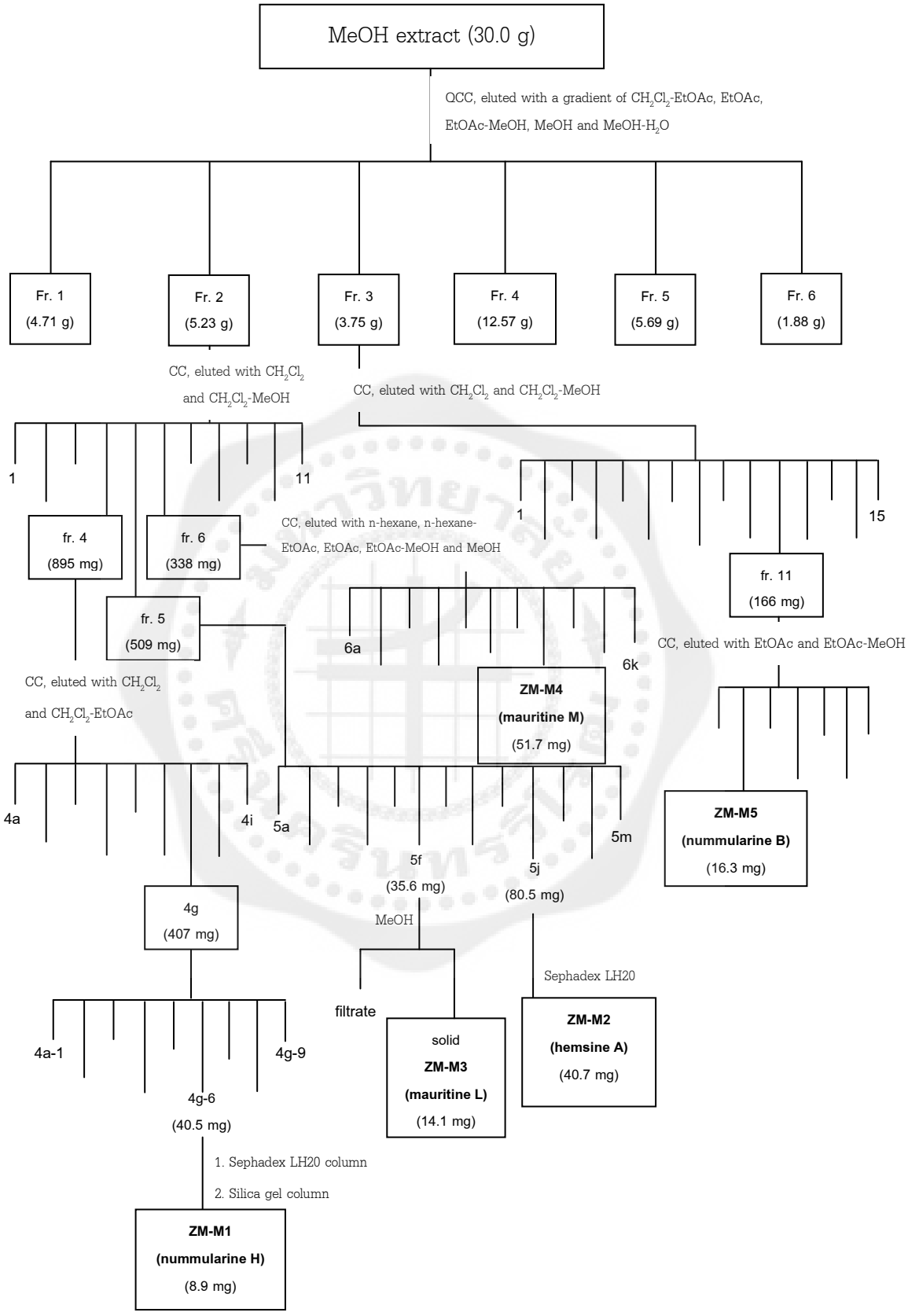
Subfraction 6 (338.2 mg) was rechromatographed (silica gel 60, finer than 0.063 mm, 16 g), eluting with *n*-hexane, *n*-hexane-EtOAc, EtOAc, EtOAc-MeOH and MeOH with increasing amount of the more polar solvent to give compound ZM-M4 (51.7 mg) as colorless solid of mauritine M (**121**), see Scheme 3.

3.4 Isolation of compound ZM-M5 (nummularine B, sss3008)

A portion of fraction 3 (3.75 g) was fractionated by silica gel chromatography (finer than 0.063 mm, 45 g) and eluted with CH_2Cl_2 and CH_2Cl_2-MeOH of increasing polarity to provide 15 subfractions (subfraction 1-15). Compound ZM-M5 (16.3 mg) was obtained after repeated silica gel column chromatography of subfraction 11 (166.0 mg), eluting with EtOAc and EtOAc-MeOH of increasing polarity). Compound ZM-M5 was identified as nummularine B (**38**).

3.5 Methylation of nummularine B (*N,N*-dimethyl nummularine B methiodide, sss3497, sss3583)

A mixture of nummularine B (40.0 mg) and MeI (1 mL) was stirred at room temperature for 48 h. Subsequent purification by column chromatography (Alumina oxide 90 (neutral), eluting with a gradient system of EtOAc-MeOH) followed by recrystallization with a mixture of H_2O-MeOH-CH_2Cl_2 to obtain *N,N*-dimethyl nummularine B methiodide (**124**) (20 mg, 48% yield) as colorless needles.



Scheme 3 Isolation of compounds from the MeOH extract of the root of *Z. mauritiana*

Physical and spectral data of the isolated compounds in EtOAc extract

1. Compound ZM-E1 (lupeol, sss3194 and sss3921)

Colorless solid 26.9 mg, soluble in CH_2Cl_2

mp : 198-200 °C (lit.[79] 222-224 °C)

R_f : 0.22 (8% EtOAc-Hexane), a violet coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3342, 2944, 2867, 1638, 1465, 1453, 1380, 1043, 880

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 5, Figure 19

2. Compound ZM-E2 (betulin, sss3828)

Colorless solid 37.1 mg, soluble in CH_2Cl_2

mp : 236-237 °C (d) (lit.[51] 235-237 °C)

R_f : 0.22 (20% EtOAc-Hexane), a violet coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3380, 2945, 2875, 1644, 1438, 1425, 1375, 1037, 1009, 879

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 6, Figure 20

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 6, Figure 21

3. Compound ZM-E3 (betulinic acid, sss2878 and sss3679)

Colorless solid 2.38 g, soluble in CH_2Cl_2

mp : 280-282 °C (d) (lit.[58] 293-295 °C)

R_f : 0.36 (2% MeOH-CH_2Cl_2), a violet coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3448, 2941, 2867, 1686, 1641, 1450, 1236, 1043, 885

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 7, Figure 22

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 7, Figure 23

4. Compound ZM-E4 (zizyberenalic acid, sss3812)

Colorless solid 3.0 mg, soluble in CH_2Cl_2

R_f : 0.37 (20% EtOAc-Hexane), a purple coloration with anisaldehyde-H_2SO_4 reagent

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 8, Figure 24

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 8, Figure 25

5. Compound ZM-E5 (ceanothic acid, sss3193)

Colorless solid 48.0 mg, soluble in CH_2Cl_2-MeOH

mp : 326-329 °C (d) (lit.[80] 333-335 °C ; lit.[81] 335-338 °C)

R_f : 0.37 (6% MeOH-CH_2Cl_2), a blue coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3478, 3067, ,2939, 2867, 1693, 1640, 1455, 1376, 1312, 1206, 882

[lit.[80] 2500-3500 (COOH, OH), 1690 (C=O), 1640, 890 (C=CH_2)]

^{1}H NMR : δ ppm, 300 MHz, in pyridine-d_5; Table 9, Figure 26

^{13}C NMR : δ ppm, 75 MHz, in pyridine-d_5; Table 9, Figure 27

6. Compound ZM-E6 (epiceanothic acid, sss3340)

Colorless powder 17.6 mg, soluble in MeOH

mp : 260-261 °C (d)

(lit.[82] epiceanothic acid : mp 224–226 °C ; lit.[83] isoceanothic acid : mp 320 °C)

R_f : 0.37 (6% MeOH-CH_2Cl_2), a pale violet coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{27}$: -4.2 (c 0.35, MeOH) [lit.[82] epiceanothic acid (**116**): $[\alpha]_D^{25}$ = -5.0 (c = 0.10, $CHCl_3$);

lit.[83] isoceanothic acid (**117**): $[\alpha]_D$ = +38.2 (c 1.20, EtOH)]

UV λ_{max}^{MeOH} nm (log ε) : 244 (2.81), 250 (2.89), 255 (2.92), 261 (2.79)

IR ν_{max}^{KBr} cm^{-1}: 3505, 3409, 3077, 2955, 1706, 1642, 1463, 1377, 1238, 1207, 1188, 1058, 881

(lit.[82] epiceanothic acid: 3400, 2947, 1691, 1641; lit.[83] isoceanothic acid: 3474, 1690, 1640, 890)

ESMS (+ve) *m/z* (% rel. intensity) : 995 $[2M+Na]^+$(100) , 487 $[M+H]^+$(1)

ESMS (-ve) *m/z* (% rel. intensity) : 971 $[2M-H]^-$ (100) , 485 $[M-H]^-$ (1)

^{1}H NMR : δ ppm, 300 MHz, in pyridine-d_5; Table 11, Figure 28

^{13}C NMR : δ ppm, 75 MHz, in pyridine-d_5; Table 11, Figure 29

7. Compound ZM-E7 (24-hydroxyceanothic acid, sss3336)

Colorless solid 19.2 mg, soluble in MeOH

mp : 283-284 °C (d) (lit.[80] 24-hydroxyceanothic acid dimethyl ester : mp 237.5-238.5 °C)

R_f : 0.30 (6% MeOH-CH_2Cl_2), a blue coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{27}$: + 41.5 (c 0.30, MeOH)

[lit.[80] 24-hydroxyceanothic acid dimethyl ester : $[\alpha]_D^{24}$= +51.5 (c 1.0, $CHCl_3$)]

UV λ_{max}^{MeOH} nm (log ε) : 244 (2.84), 250 (2.92), 255 (2.96), 261 (2.82)

IR ν_{max}^{KBr} cm^{-1} : 3461, 3077, 2941, 2870, 1689, 1646, 1450, 1377, 1210, 1035, 1019, 885

(lit.[80] 3400, 2960, 1725, 1645, 1200, 1180, 1050, 890)

ESMS (+ve) *m/z* (% rel. intensity) : 503 $[M+H]^+$ (47)

ESMS (-ve) *m/z* (% rel. intensity) : 501 $[M-H]^-$ (22) , 499 (100)

^{1}H NMR : δ ppm, 300 MHz, in pyridine-d_5; Table 14, Figure 30

^{13}C NMR : δ ppm, 75 MHz, in pyridine-d_5; Table 14, Figure 31

Physical and spectral data of the isolated compounds in MeOH extract

1. Compound ZM-M1 (nummularine H, sss3648)

Colorless solid 8.9 mg, soluble in CH_2Cl_2

mp : 128-129 °C (lit.[84] 194-196 °C)

R_f : 0.41 (4% MeOH-CH_2Cl_2), a blue coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{26}$: -296.5 (c 0.22, $CHCl_3$) [lit.[84] $[\alpha]_D^{20}$: -343 (c 0.27, MeOH)]

UV λ_{max}^{MeOH} nm (log ε) : 268 (4.05), 319 (3.89) [lit.[84] UV λ_{max}^{MeOH} nm : 268 (4.05), 320 (3.65)]

IR ν_{max}^{KBr} cm^{-1} : 3402, 3338, 2959, 2927, 2878, 1668, 1642, 1508, 1453, 1221, 1032, 701

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

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

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

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 19, Figure 32

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 19, Figure 33

2. Compound ZM-M2 (hemsine A, sss3346)

Colorless solid 40.7 mg, soluble in CH_2Cl_2

mp : 108-109 °C

R_f : 0.34 (4% MeOH-CH_2Cl_2), an orange coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{27}$: -100.0 (c 0.24, MeOH) [lit.[85] $[\alpha]_D^{26}$: -64.5 (c 2.0, MeOH)]

UV λ_{max}^{MeOH} nm (log ε) : 221 (4.67), 273 (3.86), 280 (3.86), 290 (3.76)

[lit.[85] UV λ_{max}^{MeOH} nm : 224 (4.96), 272 (4.30), 280 (4.30), 290 (4.21)]

IR ν_{max}^{KBr} cm^{-1} : 3389, 3321, 2963, 2929, 2878, 1660, 1625, 1506, 1487, 1458, 1229, 1172, 1096, 1038, 866, 741 (lit.[85] 3390, 3320, 2960, 2930, 2873, 1678, 1660, 1620, 1501, 1480, 1450, 1225, 1170, 1116, 1070, 862)

ESMS (+ve) *m/z* (% rel. intensity) : 1115 $[2M+H]^+$ (100), 558 $[M+H]^+$(85)

ESMS (-ve) *m/z* (% rel. intensity) : 556 $[M-H]^-$ (18)

HRTOFMS (APCI, +ve) *m/z* : 558.3082 $[M+H]^+$ (calcd 558.3075 for $C_{32}H_{39}N_5O_4$+H)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 24, Figure 34

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 24, Figure 35

3. Compound ZM-M3 (mauritine L, sss3170, sss3756)

Colorless solid 14.1 mg, soluble in CH_2Cl_2

mp : 236-237 °C

R_f : 0.46 (8% MeOH-CH_2Cl_2), a blue coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{27}$: -56.3 (c 0.31, MeOH)

UV λ_{max}^{MeOH} nm (log ε) : end absorption : 222 (sh), 254 (sh)

IR ν_{max}^{KBr} cm^{-1} : 3543, 3291, 2964, 2937, 2878, 1679, 1633, 1535, 1508, 1237, 1007, 820, 695

ESMS (+ve) *m/z* (% rel. intensity) : 1041 $[2M+H]^+$ (100), 521 $[M+H]^+$(47)

ESMS (-ve) *m/z* (% rel. intensity) : 520 $[M-H]^-$ (100)

HRTOFMS (APCI, +ve) *m/z* : 521.3124 $[M+H]^+$ (calcd 521.3122 for $C_{30}H_{40}N_4O_4$+H)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 26, Figure 36

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 26, Figure 37

4. Compound ZM-M4 (mauritine M, sss3100 and sss3347)

Colorless solid 51.7 mg, soluble in CH_2Cl_2

mp : 188-189 °C

R_f : 0.41 (8% MeOH-CH_2Cl_2), an orange coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{27}$: -385.5 (c 0.28, MeOH)

UV λ_{max}^{MeOH} nm (log ε) : 219 (4.78), 272 (4.27), 279 (4.23), 289 (4.07), 318 (3.95)

IR ν_{max}^{KBr} cm^{-1} : 3345, 2963, 2929, 2878, 1679, 1664, 1637, 1508, 1224, 1186, 1038, 741

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

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

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

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

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 16, Figure 38

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 16, Figure 39

5. Compound ZM-M5 (nummularine B, sss3008)

Colorless solid 16.3 mg, soluble in $CHCl_3$

mp : 219-220 °C (lit.[86] 230-231 °C)

R_f : 0.30 (8% MeOH-CH_2Cl_2), a blue coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{27}$: -507.0 (c 0.29, MeOH) [lit.[86] $[\alpha]_D^{20}$: -390 (c 0.2, $CHCl_3$)]

UV λ_{max}^{MeOH} nm (log ε) : 268 (4.13), 319 (3.96) [lit.[86] UV λ_{max}^{MeOH} nm : 268 (4.05), 321 (3.9)]

IR ν_{max}^{KBr} cm^{-1} : 3409, 3325, 2966, 2929, 2870, 1693, 1651, 1642, 1528, 1514, 1421, 1369, 1227, 1182, 1041, 773, 700, 485

EIMS (+ve) *m/z* (% rel. intensity) : 592 $[M+H]^+$(9), 591 (33), 548 (78), 492 (26), 491 (96), 407 (13), 312 (34), 165 (39), 58 (100)

ESMS (+ve) *m/z* (% rel. intensity) : 1183 $[2M+H]^+$ (44), 614 $[M+Na]^+$(12), 592 $[M+H]^+$(100)

ESMS (-ve) *m/z* (% rel. intensity) : 1181 $[2M-H]^-$ (95), 590 $[M-H]^-$(92)

HRTOFMS (APCI, +ve) *m/z* : 592.3122 $[M+H]^+$ (calcd 592.3130 for $C_{32}H_{42}N_5O_6$+H)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 21, Figure 40

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 21, Figure 41

N,N-Dimethyl nummularine B methiodide (sss3497 and sss3583)

Colorless needle 20.0 mg, soluble in MeOH

mp : 190-192 °C

R_f : 0.30 (0.1 % H_2O in 9:1-CH_2Cl_2-MeOH)

a blue coloration with anisaldehyde–H_2SO_4 reagent

$[\alpha]_D^{27}$: -337.0 (c 0.31, MeOH)

UV λ_{max}^{MeOH} nm (log ε) : 269 (3.96), 319 (3.80)

IR ν_{max}^{KBr} cm^{-1} : 3468, 3393, 3276, 1676, 1638, 1528, 1510, 1461, 1417, 1219, 1104, 1025, 801, 702

ESMS (+ve) *m/z* (% rel. intensity) : 621 $[M+H]^+$(25), 620 $[M]^+$ (100)

ESMS (-ve) *m/z* (% rel. intensity) : 619 $[M-H]^-$ (10) , 618 (44)

HRTOFMS (APCI, +ve) *m/z* : 620.3428 $[M]^+$ (calcd 620.3443 for $C_{34}H_{45}N_5O_6$)

^{1}H NMR : δ ppm, 300 MHz, in methanol-d_4; Table 23

^{13}C NMR : δ ppm, 75 MHz, in methanol-d_4; Table 23

4. Bioassay procedures

4.1 Antiplasmodial activity

Antiplasmodial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug resistant strain) which was cultured continuously according to the method of Trager and Jensen.[87] Quantitative assessment of antiplasmodial activity *in vitro* was determined by means of the microculture radioisotope technique based upon the method described by Desjardins.[88] The inhibitory concentration is that which causes 50% reduction in parasite growth, as indicated by the *in vitro* uptake of [^{3}H]-hypoxanthine by *P. falciparum*. An IC_{50} value of 1 ng/mL was observed for the standard drug, artemisinin, in the same test system.

4.2 Antimycobacterial activity

The antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H_{37}Ra using the Microplate Alamar Blue Assay.[89] Briefly, initial candidate compound dilutions

were prepared in DMSO, and subsequent two-fold dilutions were performed in 0.1 mL of 7H9GC medium in the microculture plates. 100 μL of 5×10^4 CFU/mL of *M. tuberculosis* in 7H9GC-Tween was added to each well of 96-well microculture plates containing the test compound. Plates were incubated at 37 $^{\circ}$C for 7 day. To three control wells which contained drug and medium, bacteria and medium, or medium only, Alamar Blue dye solution (20 μL of Alamar Blue solution and 12.5 μL of 20% Tween) was added daily until a color change from blue to pink occurred, at which time the dye was added to all remaining wells. Plates were incubated at 37 $^{\circ}$C, and results were recorded at 24 h post-dye addition. Fluorescence was measured in a Cytofluoro Series 4000 Fluorescence Multi-Well Plate Reader (Per-Septive Biosystems, Framingham, MA, U.S.A.) in bottom-reading mode with excitation at 530 nm and emission at 590 nm. Percent inhibition was defined as (1-test well FU/mean FU of triplicate control wells)$\times$100. The lowest drug concentration effecting inhibition of 90% was considered the MIC. Experiments were usually completed within 10 day. Standard drugs rifampicin, isoniazid and kanamycin sulfate showed MIC of 0.004, 0.06 and 2.5 μg/mL, respectively.

CHAPTER 4

RESULTS AND DISCUSSION

The ethyl acetate extract of the dried root of *Z. mauritiana* was investigated by column chromatographic methods to give three known lupane-type triterpenes (**56**, **57** and **58**) and four known ceanothane-type (**67**, **72**, **116** and **119**) compounds. The structure of these compounds were determined mainly based on their NMR data, and by comparison with previously reported data.

The methanol extract of the dried root of *Z. mauritiana* was isolated by column chromatographic methods to yield five cyclopeptide alkaloids. These included the two new (**121** and **126**) and three known (**38**, **123** and **125**) cyclopeptide alkaloids. Their structures were established based on their UV, IR, MS, NMR and X-ray data and by comparison their spectroscopic data with the literature values.

Table 4 All compounds obtained from the root of *Z. mauritiana*

compound			EtOAc extract	MeOH extract
ZM-E1	sss3194	lupeol	26.9 mg (sss3921)	-
ZM-E2	sss3828	betulin	37.1 mg (sss3828)	
ZM-E3	sss2878	betulinic acid	2.38 g (sss3679)	-
ZM-E4	sss3812	zizyberenalic acid	3.0 mg (sss3812)	-
ZM-E5	sss3193	ceanothic acid	48.0 mg (sss3193)	
ZM-E6	sss3340	epiceanothic acid	17.6 mg (sss3340)	
ZM-E7	sss3336	24-hydroxyceanothic acid	19.2 mg (sss3336)	
ZM-M1	sss3648	nummularine H	-	8.9 mg (sss3648)
ZM-M2	sss3346	hemsine A	-	40.7 mg (sss3346)
ZM-M3	sss3170	mauritine L	-	14.1 mg (sss3756)
ZM-M4	sss3100	mauritine M	22.8 mg (sss3100)	51.7 mg (sss3347)
ZM-M5	sss3008	nummularine B	-	16.3 mg (sss3008)

1. Structure determination of compounds isolated from the ethyl acetate extract of *Z. mauritiana*

1.1. Structure determination of compound ZM-E1 (lupeol, sss3921)

Compound ZM-E1 was obtained as a colorless solid with the R_f value of 0.22 (8% EtOAc-Hexane) and exhibited IR absorption bands for hydroxyl (3342 cm^{-1}) and olefinic double bond ($C=CH_2$) at 1638 and 880 cm^{-1}. The ^{1}H-NMR spectrum (Table 5, Figure 19) obtained in $CDCl_3$ revealed an isopropenyl group [δ_H 4.65 (br *s*), 4.53 (br *s*) and 1.65 (*s*)], six additional singlet methyls (δ_H 0.73, 0.76, 0.80, 0.88, 0.93 and 1.00), a carbinol proton at δ 3.16 (1H, *dd*, *J* = 10.8, 5.2 Hz) and a multiplet at δ_H 1.89 including a number of methine and methylene protons suggesting that ZM-E1 was a lupane-type triterpene. From the NMR spectrum pattern and chromatographic comparison with the authentic lupeol (**57**) in several solvent systems, the structure of compound ZM-E1 was identified as lup-20(29)-en-3β-ol or lupeol (**57**).

Lupeol was commonly found in plants including *Ziziphus* such as *Z. rugosa,*[52,55] *Z. cambodiana,*[16-17] *Z. attopoensis*[56] and *Z. mauritiana*.[61] It was reported to exhibit significant anti-inflammatory and anticarcinogenic activities.[90-91]

lupeol (**57**)

Table 5 Comparison of ^{1}H NMR data of compound ZM-E1 (sss3921) with lupeol (**57**)[16] in $CDCl_3$

position	δ_H (mult., *J* in Hz)	
	lupeol	compound ZM-E1
3	3.15 (*dd*, *J* = 10.8, 5.4)	3.16 (1H, *dd*, *J* = 10.8, 5.2)
5	0.64 (*m*)	0.65 (1H, *d*, *J* = 8.9)
19	2.38 (*ddd*, *J* = 11.1, 11.1, 5.4)	2.34 (1H, *dt*, *J* = 11.0, 5.6)
23	0.93 (*s*)	0.93 (3H, *s*)
24	0.72 (*s*)	0.73 (3H, *s*)
25	0.79 (*s*)	0.80 (3H, *s*)
26	0.99 (*s*)	1.00 (3H, *s*)
27	0.91 (*s*)	0.88 (3H, *s*)
28	0.75 (*s*)	0.76 (3H, *s*)
29	4.65 (br *s*)	4.65 (1H, br *s*)
	4.53 (br *s*)	4.53 (1H, br *s*)
30	1.64 (*s*)	1.65 (3H, *s*)

1.2 Structure determination of compound ZM-E2 (betulin, sss3828)

Compound ZM-E2 was obtained as a colorless solid with more polar [R_f 0.22 (20% EtOAc-Hexane)] than lupeol. Compound ZM-E2 exhibited IR absorption bands for hydroxyl (3380 cm^{-1}) and olefinic double bond ($C=CH_2$) at 1644 and 879 cm^{-1}. The ^{13}C NMR spectrum displayed 30 carbon signals and ^{1}H-NMR spectrum (Table 6, Figure 20 and 21) revealed an isopropenyl group [δ_H 4.65 (br *s*), 4.55 (br *s*) and 1.65 (*s*)], a carbinol proton at δ_H 3.16 (1H, *dd*, *J* = 10.5, 4.6 Hz), two methine protons at δ_H 0.65 (1H, *d*, *J* = 8.8 Hz) and 2.34 (1H, *m*), an AM system of methylene proton at δ_H 3.77 (1H, *d*, *J* = 10.4 Hz) and δ_H 3.30 (1H, *d*, *J* = 10.4 Hz), and five additional singlet methyls (δ_H 0.73, 0.79, 0.94, 0.94 and 0.99). The ^{13}C NMR spectra (Table 6, Figure 21) provided 30 carbon signals including one hydroxyl methylene carbon signal at δ_C 60.5. The ^{1}H and ^{13}C NMR spectra of ZM-E2 were similar to that of lupeol except for the precence of a hydroxyl methylene unit in the former instead of a methyl singlet in the latter, suggesting that ZM-E2 was also a lupane-type triterpene containing a hydroxyl methylene group. The comparisons of the ^{1}H and ^{13}C NMR data (Table 6) and the melting point of 236-237 ^{o}C (d) with known betulin[51](mp 235-237 ^{o}C), compound ZM-E2 was characterized as betulin (**58**). Moreover, it was confirmed by chromatographic comparison with the authentic betulin in several solvent systems.

Betulin (**58**) or 3β-lup-20(29)-ene-3,28-diol was a pentacyclic triterpene of lupane type isolated from *Ziziphus* species, such as the root of *Z. jujuba* Mill var *spinosa*,[57] the root bark of *Z. rugosa*,[52-53,55] and the stem of *Z. mauritiana*.[61]

betulin (**58**)

Table 6 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-E2 (sss3828) with betulin (**58**) [51] in $CDCl_3$

position	δ_H (mult., *J* in Hz)		δ_C	
	betulin	compound ZM-E2	betulin	compound ZM-E2
1			38.7	38.6
2			27.4	27.3
3	3.16 (*dd, J* = 10.6, 5.0)	3.16 (1H, *dd, J* = 10.5, 4.6)	79.0	78.9
4			38.9	38.8
5	0.71 (*d, J* = 9.4)	0.65 (1H, *d, J* = 8.8)	55.3	55.2
6			18.3	18.2
7			34.2	34.2
8			41.0	40.8
9			50.4	50.3
10			37.3	37.1
11			20.8	20.8
12			25.2	25.1
13			37.2	37.2
14			42.7	42.6
15			27.0	27.0
16			29.2	29.1
17			47.8	47.7
18			47.8	47.7
19	2.36 (*dt, J* = 10.6, 6.0)	2.34 (1H, *m*)	48.7	48.7
20			150.5	150.4
21			29.7	29.7
22			34.0	33.9
23	0.95 (*s*)	0.94 (3H, *s*)	28.0	27.9
24	0.74 (*s*)	0.73 (3H, *s*)	15.4	15.3
25	0.80 (*s*)	0.79 (3H, *s*)	16.1	15.9
26	1.00 (*s*)	0.99 (3H, *s*)	16.0	16.0
27	0.96 (*s*)	0.94 (3H, *s*)	14.8	14.7
28	3.78 (*d, J* = 10.8)	3.77 (1H, *d, J* = 10.4)	60.6	60.5
	3.31 (*d, J* = 10.8)	3.30 (1H, *d, J* = 10.4)		
29	4.66 (br *s*)	4.65 (1H, br *s*)	109.7	109.6
	4.56 (br *s*)	4.55 (1H, br *s*)		
30	1.66 (*s*)	1.65 (3H, *s*)	19.1	19.0

1.3 Structure determination of compound ZM-E3 (betulinic acid, sss2878, sss3679)

The more polar compound ZM-E3 [R_f 0.36 (2% MeOH-CH_2Cl_2)] was obtained as major colorless solid. Its IR spectrum showed the presence of hydroxyl (3448 cm^{-1}), carboxylic acid (1686 cm^{-1}) and olefinic double bond (1641 and 885 cm^{-1}) functionalities. The ^{1}H-NMR spectrum (Table 7, Figure 22) displayed six singlet methyl protons (δ_H 0.72, 0.79, 0.90, 0.94, 0.95 and 1.66), a vinylic methylene protons at δ_H 4.71 (1H, br *s*) and 4.58 (1H, br *s*), two methine protons at δ_H 0.65 (*d, J* = 8.2 Hz) and 2.98 (1H, *m*) and a carbinol proton at δ_H 3.16 (*dd, J* = 10.9, 4.9 Hz). The ^{13}C NMR spectra (Table 7, Figure 23) provided 30 carbon signals including one carboxylic acid signal at δ_C 180.3. These spin systems suggested that compound ZM-E3 was also a lupane-type triterpene containing a carboxylic acid and a hydroxyl groups. From the spectroscopic data and chromatographic comparisons with the authentic betulinic acid in several solvent systems, compound ZM-E3 was thus identified as 3β-hydroxy-lup-20(29)-en-28-oic acid or betulinic acid (**56**).

Betulinic acid (**56**) was a widely distributed pentacyclic lupane-type triterpene in *Ziziphus* plants and commonly isolated as the major triterpene of *Z. oenoplia*,[51] *Z. rugosa*,[55] *Z. cambodiana*[16-17] and *Z. attopoensis*[56] by our group. It was also found in the root and fruit of *Z. jujuba*[57-59] and the stem bark of *Z. mauritiana*.[60]

betulinic acid (**56**)

Table 7 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-E3 (sss3679) with betulinic acid (**56**)[51] in $CDCl_3$

position	δ_H (mult., *J* in Hz)		δ_C	
	betulinic acid	compound ZM-E3	betulinic acid	compound ZM-E3
1			38.7	38.6
2			27.4	27.3
3	3.16 (*dd, J* = 10.8, 5.1)	3.16 (1H, *dd, J* = 10.9, 4.9)	79.0	79.0
4			38.9	38.8
5	0.66 (*d, J* = 9.0)	0.65 (1H, *d, J* = 8.2)	55.3	55.3
6			18.3	18.2
7			34.3	34.3
8			40.7	40.6
9			50.5	50.4
10			37.2	37.1
11			20.8	20.8
12			25.5	25.4
13			38.4	38.3
14			42.4	42.4
15			30.5	30.5
16			32.1	32.1
17			56.3	56.2
18			49.3	49.2
19	2.99 (*m*)	2.98 (1H, *m*)	46.9	46.8
20			150.4	150.3
21			29.7	29.6
22			37.0	37.0
23	0.94 (*s*)	0.94 (3H, *s*) [a]	28.0	27.9
24	0.73 (*s*)	0.72 (3H, *s*)	15.3	15.3
25	0.80 (*s*)	0.79 (3H, *s*)	16.1	16.1
26	0.91 (*s*)	0.90 (3H, *s*) [a]	16.0	16.0
27	0.95 (*s*)	0.95 (3H, *s*) [a]	14.7	14.6
28			180.0	180.3
29	4.72 (1H, br *s*)	4.71 (1H, br *s*)	109.7	109.6
	4.58 (1H, br *s*)	4.58 (1H, br *s*)		
30	1.67 (*s*)	1.66 (1H, *s*)	19.4	19.3

[a] Interchangeable within a column.

1.4 Structure determination of compound ZM-E4 (zizyberenalic acid, sss3812)

Compound ZM-E4 was obtained as a minor colorless solid with the R_f value of 0.37 (20% EtOAc-Hexane). The ^{1}H and ^{13}C NMR spectra (Table 8, Figure 24 and 25) displayed an isopropenyl group [δ_H 1.67 (*s*), 4.60 (br *s*) and 4.73 (br *s*)], five singlet methyls (δ_H 0.97, 0.97, 0.98, 1.12, 1.13 and δ_C 14.7, 16.8, 20.4, 19.0, 28.1), an α,β-unsaturated aldehyde functional group [δ_H 9.68 (1H, *s*) and δ_C 191.4, δ_H 6.54 (1H, *s*) and δ_C 163.3] and a carboxylic acid (δ_C 181.9). These data suggested compound ZM-E4 could be a lupane acid or ceanothic acid (**67**).

Comparison of ^{13}C NMR spectral data of compound ZM-E4 with those of lupane acid [eg. betulinic acid (**56**)] (Table 8) and ceanothic acid (**67**) (Table 9) isolated by us showed a similar pattern in the carbon resonances in rings B-E in these compounds but showed significant differences in the resonance signals for ring-A carbons. This indicated that ZM-E4 was a pentacyclic triterpene of the ceanothane series where ring A was five membered. Furthermore, the signals for the ring-junction carbons (C-5 at δ_C 63.0 and C-10 at δ_C 52.1) in the compound ZM-E4 were shifted accordingly from the values of these carbons in betulinic acid (**56**) (C-5 at δ_C 55.3 and C-10 at δ_C 37.1) where ring A is six membered. Thus, compound ZM-E4 appeared to be zizyberenalic acid (**72**) which containing an α,β-unsaturated aldehyde function [$\delta_{C\text{-}1}$ 157.3; $\delta_{H\text{-}1}$ 9.68 (*s*), $\delta_{C\text{-}2}$ 191.4; $\delta_{H\text{-}3}$ 6.54 (*s*), δ_C 163.3] on the five-membered ring A. The rest of the structure was the same as that of zizyberenalic acid (**72**), according to spectroscopic data[92] and chromatographic comparison with the authentic zizyberenalic acid in several solvent systems.

Zizyberenalic acid (**72**) was found in the root of *Paliurus hemsleyanus*,[92] the fruit of *Z. jujuba* Mill [59,64] and the root bark of *Z. cambodiana*.[17] It showed anti-viral producing hepatoma cell line MS-G2 which displayed anti-HBsAg and anti-HBeAg activities against MS-G2 cells with EC_{50} values of 22.3 and 19.3 μM.[64]

ceanothic acid (**67**)

betulinic acid (**56**)

zizyberenalic acid (**72**)

Table 8 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-E4 (sss3812) with betulinic acid (sss3679) and zizyberenalic acid [92]

position	δ_H (mult., *J* in Hz)			δ_C		
	betulinic acid ($CDCl_3$)	zizyberenalic acid (Pyridine-d_5)	compound ZM-E4 ($CDCl_3$)	betulinic acid ($CDCl_3$)	zizyberenalic acid (Pyridine-d_5)	compound ZM-E4 ($CDCl_3$)
1				38.6	157.9	157.3
2		9.86 (*s*)	9.68 (1H, *s*)	27.3	191.7	191.4
3	3.16 (*dd, J* = 10.9, 4.9)	6.56 (*s*)	6.54 (1H, *s*)	79.0	164.2	163.3
4				38.8	44.0	43.7
5	0.65 (*d, J* = 8.2)			55.3	63.7	63.0
6				18.2	17.2	17.6
7				34.3	35.7	35.0
8				40.6	43.5	42.5
9				50.4	48.2	47.5
10				37.1	52.7	52.1
11				20.8	24.9	24.1
12				25.4	26.0	25.1
13		2.70 (*dt, J* = 12.5, 3.5)	2.36 (1H, br *t, J* = 7.4)	38.3	38.6	38.1
14				42.4	43.1	42.9
15				30.5	30.6	30.5
16		2.61 (br *d, J* = 12.6)	2.28 (2H, br *d, J* = 12.0)	32.1	33.2	33.9
17				56.2	56.7	56.1
18		1.71 (*m*)		49.2	50.0	49.3
19	2.98 (*m*)	3.47 (*dt, J* = 11.0, 3.9)	2.99 (*dt, J* = 11.0, 4.6)	46.8	47.9	46.9
20				150.3	151.3	150.0
21				29.6	31.4	32.3
22				37.0	37.8	37.1
23	0.94 (*s*)	1.09 (*s*)	1.13 (3H, *s*)	27.9	28.3	28.1
24	0.72 (*s*)	0.88 (*s*)	0.98 (3H, *s*) [a]	15.3	20.6	20.4
25	0.79 (*s*)	1.11 (*s*)	1.12 (3H, *s*)	16.1	19.5	19.0
26	0.90 (*s*)	1.07 (*s*)	0.97 (3H, *s*) [a]	16.0	18.2	16.8
27	0.95 (*s*)	0.99 (*s*)	0.97 (3H, *s*) [a]	14.6	15.1	14.7
28				180.3	179.1	181.9
29	4.71 (br *s*) 4.58 (br *s*)	4.89 (br *s*) 4.74 (br *s*)	4.73 (1H, br *s*) 4.60 (1H, br *s*)	109.6	110.2	109.9
30	1.66 (*s*)	1.77 (*s*)	1.67 (3H, *s*)	19.3	19.7	19.2

[a] Interchangeable within a column.

1.5 Structure determination of compound ZM-E5 (ceanothic acid, sss3193)

Compound ZM-E5 was obtained as a colorless solid with high polarity [R_f 0.37 (6% MeOH-CH_2Cl_2)]. This compound exhibited IR absorption band for hydroxyl group (3478 cm^{-1}), carboxyl (1693 cm^{-1}) and olefinic double bond (C=CH_2) at 1640 and 882 cm^{-1}.

The ^{13}C NMR, DEPT, HMQC and COSY provided 30 carbon signals (including six methyls, nine methylenes, seven methines, six quaternary carbons and two carboxyl carbon signals) (Table 9, Figure 27). The ^{1}H NMR spectrum (Table 9, Figure 26) revealed an isopropenyl group [δ_H 4.83 (br *s*), 4.64 (br *s*) and 1.63 (*s*)], five singlet methyls (δ_H 1.05, 1.12, 1.26, 1.36 and 1.41), as well as the typical two correlated singlet methine protons at δ_H 4.81 and 3.19 observed in the COSY spectrum suggesting that compound ZM-E5 could be ceanothic acid (**67**), a ceanothane-type triterpene with two carboxylic acid units at C-1 and C-17. In the HMBC spectrum (Table 9), the singlet proton at δ_H 3.19 (H-1) showed correlations with C-2, C-3, C-4, C-5, C-10 and C-25, and a multiplet at δ_H 1.70 (H-18) showed correlations with C-13, C-14, C-17, C-19, C-20 and C-28 confirmed the placement of the carboxyl groups at C-1 and C-17. The carbinol proton (δ_H 4.81) at C-3 was also showed HMBC correlations with δ_C 178.2 (C-2), δ_C 43.9 (C-4), δ_C 57.1 (C-5), δ_C 49.7 (C-10), δ_C 31.5 (C-23), δ_C 20.4 (C-24). Assignments of ^{1}H and ^{13}C NMR spectra data of ZM-E5 (Table 9) were confirmed by COSY, DEPT, HMQC and HMBC experiments. From the spectroscopic methods and chromatographic comparison with the authentic ceanothic acid in several solvent systems, the structure of compound ZM-E5 was hence assigned as ceanothic acid (**67**) (Table 10). Moreover, in the NOESY spectrum (Table 10, Figure 3), the H-1 signal exhibited a cross-peak with CH_3-25, whist H-3 showed a cross-peak with CH_3-23. This evidence was consistent with the α-COOH and β-OH configurations of the 1 and 3-positions of ceanothic acid.

Ceanothic acid (**67**) 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,*[57] *Z. rugosa,*[55] *Z. cambodiana*.[16-17] and the root bark of *Ceanothus americanus*.[81] It was reported to exhibit antimicrobial activity with

MIC values of 42 μg/mL against *Actinomyces viscosus*, and 62 μg/mL against both *Porphyromonas gingivalis* and *Prevotella intermedia*.[81]

ceanothic acid (**67**)

Figure 2 Selected HMBC correlations for ZM-E5 (ceanothic acid)

Figure 3 Key NOE correlations and conformation of ZM-E5 (ceanothic acid)

Table 9 ^{1}H, ^{13}C NMR and 2D NMR data of compound ZM-E5 (sss3193) in pyridine-d_5

position	δ_H (mult., *J* in Hz)	δ_C	HMBC correlations	NOESY correlations
1	3.19 (1H, *s*)	67.0	C-2, C-3, C-4, C-5, C-10, C-25	H-25
2		178.2		
3	4.81 (1H, *s*)	84.8	C-2, C-4, C-5, C-10, C-23, C-24	H-23
4		43.9		
5	2.18 (1H, *m*)	57.1		
6	1.47 (2H, *m*)	19.1		
7	1.57 (2H, *m*)	34.8		
8		43.6		
9	2.18 (1H, *m*)	45.1		
10		49.7		
11	2.07 (1H, *m*) and 1.47 (1H, *m*)	24.3		
12	1.94 (1H, *m*) and 1.29 (1H, *m*)	26.3		
13	2.75 (1H, *dt*, *J* = 12.7, 3.0)	39.2		H-19, H-26
14		42.2		
15	1.84 (2H, *m*)	30.6		
16	2.57 (2H, br *d*, *J* = 12.8)	33.0	C-14, C-17	
17		56.7		
18	1.70 (1H, *m*)	49.8	C-13, C-14, C-17, C-19, C-20, C-28	H-27
19	3.46 (1H, *m*)	47.7	C-21	H-13, H-30
20		151.2		
21	2.18 (1H, *m*) and 1.18 (1H, *m*)	31.3		
22	2.18 (1H, *m*) and 1.49 (1H, *m*)	37.6		
23	1.41 (3H, *s*)	31.5	C-3, C-4, C-5, C-24	H-3, H-5
24	1.26 (3H, *s*)	20.4	C-3, C-4, C-5, C-23	
25	1.36 (3H, *s*)	18.9	C-1, C-5, C-9, C-10	H-1, H-24
26	1.12 (3H, *s*)	17.1	C-7, C-14	H-13
27	1.05 (3H, *s*)	15.1	C-13, C-14, C-15	H-18
28		179.0		
29	4.83 (1H, br *s*)	109.8	C-19, C-20, C-30	
	4.64 (1H, br *s*)			
30	1.63 (1H, *s*)	19.6	C-20, C-29	

Table 10 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-E5 (sss3193) with ceanothic acid (**67**) [81] in pyridine-d_5

position	δ_H (mult., *J* in Hz)		δ_C	
	ceanothic acid	compound ZM-E5	ceanothic acid	compound ZM-E5
1	3.19 (*s*)	3.19 (1H, *s*)	67.0	67.0
2			178.0	178.2
3	4.81 (*s*)	4.81 (1H, *s*)	84.7	84.8
4			43.8	43.9
5		2.18 (1H, *m*)	57.0	57.1
6		1.47 (2H, *m*)	19.1	19.1
7		1.57 (2H, *m*)	34.7	34.8
8			43.5	43.6
9		2.18 (1H, *m*)	45.1	45.1
10			49.6	49.7
11		2.07 (1H, *m*) and 1.47 (1H, *m*)	24.2	24.3
12		1.94 (1H, *m*) and 1.29 (1H, *m*)	26.2	26.3
13	2.76 (*dt*, *J* = 11.5, 2.5)	2.75 (1H, *dt*, *J* = 12.7, 3.0)	39.1	39.2
14			42.1	42.2
15		1.84 (2H, *m*)	30.5	30.6
16		2.57 (2H, br *d*, *J* = 12.8)	32.9	33.0
17			56.6	56.7
18		1.70 (1H, *m*)	49.7	49.8
19	3.49 (*m*)	3.46 (1H, *m*)	47.6	47.7
20			151.2	151.2
21		2.18 (1H, *m*) and 1.18 (1H, *m*)	31.3	31.3
22		2.18 (1H, *m*) and 1.49 (1H, *m*)	37.5	37.6
23	1.42 (*s*)	1.41 (3H, *s*)	31.5	31.5
24	1.27 (*s*)	1.26 (3H, *s*)	20.3	20.4
25	1.38 (*s*)	1.36 (3H, *s*)	18.8	18.9
26	1.15 (*s*)	1.12 (3H, *s*)	17.0	17.1
27	1.07 (*s*)	1.05 (3H, *s*)	15.1	15.1
28			178.8	179.0
29	4.85 (br *s*)	4.83 (1H, br *s*)	109.8	109.8
	4.66 (br *s*)	4.64 (1H, br *s*)		
30	1.66 (*s*)	1.63 (1H, *s*)	19.6	19.6

1.6 Structure determination of compound ZM-E6 (epiceanothic acid, sss3340)

Compound ZM-E6 was obtained as a colorless powder with equal polarity [R_f 0.37 (6% MeOH-CH_2Cl_2)] as compound ZM-E5 (ceanothic acid) and gave a pale violet coloration with anisaldehyde-H_2SO_4 reagent. Its IR spectrum showed absorption bands for hydroxyl group (3505 cm^{-1}), carboxyl (1706 cm^{-1}) and olefinic double bond (C=CH_2) at 1642 and 881 cm^{-1}. It displayed a molecular ion at m/z 487 $[M+H]^+$ in the ESMS, which afforded the formula $C_{30}H_{46}O_5$.

The ^{13}C NMR (Table 11, Figure 29) and DEPT spectra provided 30 carbon signals including six methyls, nine methylenes, seven methines, and eight quaternary carbons including two carboxylic acid carbon signals. The ^{1}H and ^{13}C NMR spectra (Table 11, Figure 28) of compound ZM-E6 showed a ceanothane type triterpene pattern for an isopropenyl group [δ_H 4.85 (br *s*), 4.69 (br *s*), 1.74 (*s*) and δ_C 110.0, 151.2, 19.5] and five additional singlet methyl protons (δ_H 1.05, 1.12, 1.12, 1.20, 1.66 and δ_C 14.7, 15.0, 17.0, 20.0, 32.2) with two carboxyls at δ_C 176.2 and δ_C 179.0. NMR data of ZM-E6 was very similar to that of ceanothic acid except for the presence of two doublets signals at δ_H 2.89 (J = 7.2 Hz) and δ_H 4.66 (J = 7.2 Hz) in the former instead of two broad singlets at δ_H 3.19 and δ_H 4.81 in the latter (Table 12). In the HMBC spectrum (Table 11, Figure 4), the proton at δ_H 2.89 showed correlations with δ_C 176.2 (carboxylic carbon C-2), δ_C 83.1 (carbinol carbon C-3), δ_C 51.2 (C-9), δ_C 48.3 (C-10) and δ_C 14.7 (C-25), whilst the signal at δ_H 4.66 showed connectivity with δ_C 176.2 (C-1), δ_C 48.3 (C-10), δ_C 32.2 (C-23) and δ_C 20.0 (C-24) suggesting that ZM-E6 could be an isomer of ceanothic acid (**67**). Thus four possible stereoisomers at H-1 and H-3 in the ring A of ZM-E6 could be drawn as:

(**67a**) H-1β/H-3α	(**67b**) H-1α/H-3β	(**67c**) H-1α/H-3α	(**67d**) H-1β/H-3β
$J_{1,3}$ = 1.0 Hz[93]	$J_{1,3}$ = 9.0 Hz[83,93]	$J_{1,3}$ = 7.3 Hz[82,93]	$J_{1,3}$ = 7.0 Hz[93]

in which isomer **67a** was ceanothic acid (**67**). Isomer **67b** has been reported as isoceanothic acid (**117**), which isolated from the stem of *Z. xylopyrus*,[83] and the $J_{1,3}$ value observed as 9.0 Hz. Isomer **67c** has been isolated from the seed of *Z. jujuba* var. *spinosa*[82] as epiceanothic acid (**116**) with the $J_{1,3}$ value of 7.3 Hz, whereas isomer **67d** was a synthetic triterpene with the reported $J_{1,3}$ value of 7.0 Hz.[93] From the comparisons of ^{1}H and ^{13}C NMR data including the $J_{1,3}$ value for H-1 and H-3 protons of compound ZM-E6 with these four isomers (Table 12 and 13), compound ZM-E6 could be of H-1α and H-3α in stereochemistry. NOE interactions also observed between H-1 with H-3 and CH_3-23, whereas CH_3-24 showed a cross-peaks with CH_3-25 in the NOESY experiment, confirmed that H-1, H-3 and CH_3-23 resided on the same α-side as shown in Figure 5. Compound ZM-E6 displayed the levorotatory optical rotation value of $[\alpha]_D^{27}$ -4.2 (c 0.35, MeOH) which comparable to that of epiceanothic acid (**116**) $[\alpha]_D^{25}$= -5.0 (c = 0.10, $CHCl_3$)[82] and (H-1α/H-3α)-epiceanothic acid dimethyl ester $[\alpha]_D^{20}$ = -9.0 (c = 0.01, $CHCl_3$),[93] whereas (H-1β/H-3β)-isoceanothic acid dimethyl ester[93] and isoceanothic acid (**117**)[83] showed dextrorotation value of $[\alpha]_D^{20}$ = +37 (c = 0.01, $CHCl_3$) and $[\alpha]_D$ =+38.2 (c = 1.20, EtOH), respectively. From the spectroscopic evidence and physical data, the structure of compound ZM-E6 was concluded to be 2β-carboxy-3β-hydroxy-A(1)-norlup-20(29)-en-28-oic acid or epiceanothic acid (**116**). It has been reported isolating in seed of *Z. jujuba* var. *spinosa*.[82]

ceanothic acid (**67**)

epiceanothic acid (**116**)

isoceanothic acid (**117**)

Figure 4 Structure and selected HMBC correlations for ZM-E6 (epiceanothic acid)

Figure 5 Selected NOE correlations and conformation of ZM-E6 (epiceanothic acid)

Table 11 ^{1}H, ^{13}C NMR and 2D NMR data of compound ZM-E6 (sss3340) in pyridine-d_5

position	δ_H (mult., *J* in Hz)	δ_C	HMBC correlations	NOESY correlations
1	2.89 (1H, *d*, *J* = 7.2)	63.2	C-2, C-3, C-9, C-10, C-25	H-23
2		176.2		
3	4.66 (1H, *d*, *J* = 7.2)	83.1	C-1, C-10, C-23, C-24	H-1, H-23
4		43.0		
5	1.74 (1H, *m*)	62.7		
6	1.57 (1H, *m*) and 1.35 (1H, *m*)	18.4		
7	1.39 (2H, *m*)	34.9	C-26	
8		42.0		
9	1.86 (1H, *m*)	51.2		
10		48.3		
11	2.00 (1H, *m*) and 1.74 (1H, *m*)	24.6		
12	1.94 (2H, *m*)	25.9		
13	2.75 (1H, br *t*, *J* = 10.8)	38.6		H-19, H-26
14		43.1		
15	1.90 (1H, *m*) and 1.24 (1H, *m*)	30.5		
16	2.61 (1H, br *d*, *J* = 12.8)	33.0		
	1.53 (1H, *m*)			
17		56.6		
18	1.70 (1H, *m*)	49.8	C-13, C-17, C-19, C-28	H-27
19	3.46 (1H, *m*)	47.8		H-13
20		151.2		
21	2.20 (1H, *m*) and 1.50 (1H, *m*)	31.3		
22	2.23 (1H, *m*) and 1.48 (1H, *m*)	37.7		
23	1.12 (3H, *s*)	32.2	C-3, C-4, C-5, C-24	H-1, H-3, H-5
24	1.20 (3H, *s*)	20.0	C-3, C-4, C-5, C-23	H-25
25	1.66 (3H, *s*)	14.7	C-1, C-5, C-9, C-10	H-24
26	1.12 (3H, *s*)	17.0	C-7, C-8, C-9, C-14	H-13, H-25
27	1.05 (3H, *s*)	15.0	C-8, C-13, C-14, C-15	H-18
28		179.0		
29	4.85 (1H, br *s*)	110.0	C-19, C-30	
	4.69 (1H, br *s*)			
30	1.74 (3H, *s*) [a]	19.5	C-19, C-20, C-29	

Table 12 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-E6 (sss3340) with ceanothic acid (**67**) and isoceanothic acid (**117**)[83]

position	δ_H (mult., *J* in Hz)			δ_C		
	ceanothic acid (Pyridine-d_5)	isoceanothic acid (Pyridine-d_5)	compound ZM-E6 (Pyridine-*d*5)	ceanothic acid (Pyridine-d_5)	isoceanothic acid ($CDCl_3$+ DMSO-d_6)	compound ZM-E6 (Pyridine-d_5)
1	3.19 (*s*)	2.60 (*d*, *J* = 8.7)	2.89 (1H, *d*, *J* = 7.2)	67.0	65.6	63.2
2				178.2	176.3	176.2
3	4.81 (*s*)	3.50 (*m*)	4.66 (1H, *d*, *J* = 7.2)	84.8	83.5	83.1
4				43.9	43.9	43.0
5	2.18 (*m*)		1.74 (1H, *m*)	57.1	56.0	62.7
6			1.57 (1H, *m*)	19.1	19.5	18.4
			1.35 (1H, *m*)			
7			1.39 (2H, *m*)	34.8	33.8	34.9
8				43.6	42.7	42.0
9			1.86 (1H, *m*)	45.1	43.9	51.2
10				49.7	48.5	48.3
11			2.00 (1H, *m*)	24.3	23.2	24.6
			1.74 (1H, *m*)			
12			1.94 (2H, *m*)	26.3	25.2	25.9
13	2.75 (*dt*, *J* = 12.7, 3.0)		2.75 (1H, br *t*, *J* = 10.8)	39.2	40.9	38.6
14				42.2	42.8	43.1
15			1.90 (1H, *m*)	30.6	29.6	30.5
			1.24 (1H, *m*)			
16	2.57 (br *d*, *J* = 12.8)		2.61 (1H, br *d*, *J* = 12.8)	33.0	32.0	33.0
			1.53 (1H, *m*)			
17				56.7	55.6	56.6
18	1.70 (*m*)		1.70 (1H, *m*)	49.8	48.5	49.8
19	3.46 (*m*)	2.94 (*m*)	3.46 (1H, *m*)	47.7	46.6	47.8
20				151.2	150.4	151.2
21	2.18 (*m*)		2.20 (1H, *m*)	31.3	30.3	31.3
	1.18 (*m*)		1.50 (1H, *m*)			
22	2.18 (*m*)		2.23 (1H, *m*)	37.6	36.6	37.7
	1.49 (*m*)		1.48 (1H, *m*)			
23	1.41 (*s*)	1.43 (*s*)	1.12 (3H, *s*)	31.5	30.9	32.2
24	1.26 (*s*)	1.28 (*s*)	1.20 (3H, *s*)	20.4	18.3	20.0
25	1.36 (*s*)	1.39 (*s*)	1.66 (3H, *s*)	18.9	18.3	14.7
26	1.12 (*s*)	1.12 (*s*)	1.12 (3H, *s*)	17.1	16.3	17.0
27	1.05 (*s*)	1.06 (*s*)	1.05 (3H, *s*)	15.1	14.7	15.0
28				179.0	177.3	179.0
29	4.83 (br *s*)	4.70 (br *s*)	4.85 (1H, br *s*)	109.8	109.0	110.0
	4.64 (br *s*)	4.57 (br *s*)	4.69 (1H, br *s*)			
30	1.63 (*s*)	1.66 (*s*)	1.74 (3H, *s*)	19.6	19.2	19.5

Table 13 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-E6 (sss3340) with isoceanothic acid (**117**) [83] and epiceanothic acid (**116**) [84]

position	δ_H (mult., *J* in Hz)				δ_C			
	isoceanothic acid (Pyridine-d_5)	epiceanothic acid ($CDCl_3$)	compound ZM-E6 (5%methanol-d_4 in $CDCl_3$)	compound ZM-E6 (Pyridine-d_5)	isoceanothic acid ($CDCl_3$+ DMSO-d_6)	epiceanothic acid ($CDCl_3$)	compound ZM-E6 (5%methanol-d_4 in $CDCl_3$)	compound ZM-E6 (Pyridine-d_5)
1	2.60 (*d*, *J* = 8.7)	2.44 (*d*, *J* = 7.3)	2.31(1H, *d*, *J* = 6.9)	2.89 (1H, *d*, *J* = 7.2)	65.6	60.0	60.1	63.2
2					176.3	178.6	177.3	176.2
3	3.50 (*m*)	4.10 (*d*, *J* =*7.3*)	4.02 (1H, *d*, *J* = 6.9)	4.66 (1H, *d*, *J* = 7.2)	83.5	82.5	82.2	83.1
4				1.74 (1H, *m*)	43.9	43.3	42.9	43.0
5				1.57 (1H, *m*) 1.35 (1H, *m*)	56.0	62.1	61.9	62.7
6				1.39 (2H, *m*)	19.5	18.2	17.9	18.4
7					33.8	34.4	34.2	34.9
8				1.86 (1H, *m*)	42.7	42.0	41.6	42.0
9					43.9	50.8	49.3	51.2
10				2.00 (1H, *m*) 1.74 (1H, *m*)	48.5	49.9	49.4	48.3
11				1.94 (2H, *m*)	23.2	23.7	23.4	24.6
12				2.75 (1H, br *t*, *J* = 10.8)	25.2	25.3	25.0	25.9
13					40.9	38.9	38.3	38.6
14				1.90 (1H, *m*) 1.24 (1H, *m*)	42.8	43.1	42.7	43.1
15				2.61 (1H, br *d* *J* = 12.8) 1.53 (1H, *m*)	29.6	29.9	29.7	30.5
16					32.0	32.6	32.3	33.0
17				1.70 (1H, *m*)	55.6	56.2	56.0	56.6
18				3.46 (1H, *m*)	48.5	49.6	50.4	49.8
19	2.94 (*m*)	2.95 (*ddd*, *J* = 10.8, 10.8, 4.7)	2.90 (1H, m)		46.6	47.4	47.0	47.8
20					150.4	150.2	150.2	151.2
21				2.20 (1H, *m*) 1.50 (1H, *m*)	30.3	31.0	30.6	31.3
22				2.23 (1H, *m*) 1.48 (1H, *m*)	36.6	37.2	37.0	37.7
23	1.43 (*s*)	0.92-1.10	0.97 (3H, *s*)	1.12 (3H, *s*)	30.9	31.8	31.6	32.2
24	1.28 (*s*)	0.92-1.10	0.85 (3H, *s*)	1.20 (3H, *s*)	18.3	19.0	18.8	20.0
25	1.39 (*s*)	0.92-1.10	1.05 (3H, *s*)	1.66 (3H, *s*)	18.3	14.2	13.8	14.7
26	1.12 (*s*)	0.92-1.10	0.89 (3H, *s*)	1.12 (3H, *s*)	16.3	16.9	16.5	17.0
27	1.06 (*s*)	0.92-1.10	0.93 (3H, *s*)	1.05 (3H, *s*)	14.7	15.0	14.6	15.0
28					177.3	182.2	180.3	179.0
29	4.70 (br *s*) 4.57 (br *s*)	4.74 (br *s*) 4.63 (br *s*)	4.66 (1H, br *s*) 4.54 (1H, br *s*)	4.85 (1H, br *s*) 4.69 (1H, br *s*)	109.0	110.0	109.7	110.0
30	1.66 (*s*)	1.70 (*s*)	1.63 (3H, *s*)	1.74 (3H, *s*)	19.2	19.5	19.2	19.5

1.7 Structure determination of compound ZM-E7 (24-hydroxyceanothic acid, sss3336)

Compound ZM-E7 was the most polar triterpene obtained from this plant with the R_f value of 0.30 (6% MeOH-CH_2Cl_2) as a colorless solid, mp 283-284 °C (d) and gave a blue coloration with anisaldehyde-H_2SO_4 reagent. It exhibited IR absorption band for hydroxyl group (3461 cm^{-1}), carboxyl (1689 cm^{-1}) and olefinic double bond (C=CH_2) at 1646 and 885 cm^{-1}. Its ESMS showed the molecular ion at m/z 503 $[M+H]^+$, corresponding to the formula $C_{30}H_{46}O_6$, with one more oxygen atom than that of compound ZM-E5 (ceanothic acid).

The ^{1}H NMR spectrum (Table 14, Figure 30) of compound ZM-E7 (in pyridine-d_5) was similar to that of ceanothic acid (**67**) with only few differences were observed. The ^{1}H NMR spectrum of compound ZM-E7 displayed two singlet methine protons at δ_H 3.23 (H-1) and δ_H 4.92 (H-3), an AX methylene protons at δ_H 3.67 and 4.60 (J = 10.7), an isopropenyl group (δ_H 4.83, 4.64 and 1.63) and four additional singlet methyls (δ_H 1.02, 1.08, 1.42 and 1.77), one fewer than that of ceanothic acid (**67**). In the ^{13}C NMR and DEPT spectra, 30 carbon signals were observed, including five methyls, 10 methylenes, seven methines, and eight quaternary carbons, as well as two carboxylic carbon signals (Table 14, Figure 31). The AX coupling system of methylene proton at δ_H 3.67 and 4.60 exhibited cross-peaks with C-3 (δ_C 85.8), C-4 (δ_C 48.5) and C-5 (δ_C 57.2) in the HMBC spectrum (Table 14, Figure 6), therefore the hydroxylated position must be at either C-23 or C-24. This difference was also observed in the ^{13}C NMR spectrum (Table 15, Figure 31), in which a methyl signal at δ_C 20.4 of ceanothic acid (**67**) was replaced by a hydroxylated methylene signal at δ_C 66.7 suggesting that compound ZM-E7 was a hydroxyceanothic acid. The location of this hydroxymethylene group was assigned by the observation of NOESY correlations (Figure 7). In the NOESY spectrum, the methyl signal proton at δ_H 1.42 (CH_3-25) showed correlations with protons at δ_H 3.23 (H-1) and δ_H 4.06 (CH_2-24b) indicating they were on the same β-side. Similarly, NOE interactions were observed between CH_3-23 and H-3, and between CH_3-23 and CH_2-24a suggesting that they resided on the same α-side. Thus the relative stereochemistry of substituents on ring A of the ceanothane nucleus in compound ZM-E7 was shown in Figure 7 which was consistent with 24-hydroxyceanothic acid

dimethyl ester (**118**), a compound isolated from the EtOH extract of the root of *Paliurus ramosissimus* (Rhamnaceae).[80] It should be noted that NOE interactions also observed between H-1 and H-3 but no NOE observed between H-1 and H-24, this implied that H-1 and H-3 should reside in a suitable proximity (Figure 7). Comparison of the ^{1}H and ^{13}C NMR (recorded in 5% methanol-d_4 -$CDCl_3$) of compound ZM-E7 with those of 24-hydroxyceanothic acid dimethyl ester (**118**) ($CDCl_3$) (Table 15), and the optical rotation of both compounds ($[\alpha]_D^{27}$ + 41.58 (c 0.30, MeOH) for compound ZM-E7 and $[\alpha]_D^{24}$ = +51.5 (c 1.0, $CHCl_3$) for 24-hydroxyceanothic acid dimethyl ester[80]) thus compound ZM-E7 should have the same stereostructure as that of 24-hydroxyceanothic acid dimethyl ester (**118**). From the spectroscopic evidences and physical data observed, the structure of compound ZM-E7 was thus concluded to be 24-hydroxyceanothic acid (**119**).

24-hydroxyceanothic acid dimethyl ester (**118**)

24-hydroxyceanothic acid (**119**)

Figure 6 Selected HMBC correlations for ZM-E7 (24-hydroxyceanothic acid)

Figure 7 NOE correlations and conformation of ZM-E7 (24-hydroxyceanothic acid)

Table 14 ^{1}H, ^{13}C NMR and 2D NMR data of compound ZM-E7 (sss3336) in pyridine-d_5

position	δ_H (mult., *J* in Hz)	δ_C	HMBC correlations	NOESY correlations
1	3.23 (1H, *s*)	66.4	C-2, C-3, C-5, C-10	CH_3-25
2		177.7		
3	4.92 (1H, *s*)	85.8	C-5, C-10, C-23, C-24	H-5, CH_3-23
4		48.5		
5	2.28 (1H, *m*)	57.2		H-3, CH_3-23
6	1.51 (2H, *m*)	18.5		
7	1.45 (1H, *m*) and 1.31 (1H, *m*)	35.2		
8		43.5		
9	2.12 (1H, *m*)	45.3	C-26	
10		50.0		
11	2.06 (1H, *m*) and 1.56 (1H, *m*)	24.3		
12	1.94 (1H, *m*) and 1.32 (1H, *m*)	26.2		
13	2.74 (1H, br *t*, *J* = 10.2)	39.1	C-18	H-19, CH_3-26
14		42.0		
15	1.83 (1H, *m*) and 1.17 (1H, *m*)	30.5		
16	2.57 (1H, br *d*, *J* = 12.8)	33.0		
	1.42 (1H, *m*)			
17		56.7		
18	1.63 (1H, *m*)	49.7	C-13, C-17, C-19, C-20, C-28	CH_3-27
19	3.48 (1H, br *t*, *J* ca 10.7)	47.6	C-18, C-21	H-13
20		151.2		
21	2.22 (1H, *m*) and 1.48 (1H, *m*)	31.3	C-17, C-18	
22	2.18 (1H, *m*) and 1.48 (1H, *m*)	37.6	C-28	
23	1.77 (3H, *s*)	25.7	C-3, C-4, C-5, C-24	H-3, H-5, H-24_a
24	4.60 (1H, *d*, *J* = 10.7, H_b)	66.7	C-3, C-4, C-5, C-23	CH_3-25
	3.67 (1H, *d*, *J* = 10.7, H_a)			CH_3-23
25	1.42 (3H, *s*)	19.0	C-1, C-5, C-10	H-1, H-24_b
26	1.08 (3H, *s*)	17.1	C-7, C-8	H-13, CH_3-25
27	1.02 (3H, *s*)	15.1	C-8, C-13, C-14, C-15	H-18
28		179.1		
29	4.83 (1H, br *s*)	109.8	C-19, C-30	
	4.64 (1H, br *s*)			
30	1.63 (3H, *s*)	19.6	C-20, C-29	

Table 15 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-E7 (sss3336) with ceanothic acid (**67**) and 24-hydroxyceanothic acid dimethyl ester (**118**)[80]

position	δ_H (mult., *J* in Hz)				δ_C			
	ceanothic acid (Pyridine-d_5)	24-hydroxy ceanothic acid dimethyl ester ($CDCl_3$)	compound ZM-E7 (5%methanol-d_4 in $CDCl_3$)	compound ZM-E7 (Pyridine-d_5)	ceanothic acid (Pyridine-d_5)	24-hydroxy ceanothic acid dimethyl ester ($CDCl_3$)	compound ZM-E7 (5%methanol-d_4 in $CDCl_3$)	compound ZM-E7 (Pyridine-d_5)
1	3.19 (*s*)	2.65 (*s*)	2.52 (1H, *s*)	3.23 (1H, *s*)	67.0 178.2	64.9 174.8	65.0 177.0	66.4 177.7
2								
3	4.81 (*s*)	4.41 (*s*)	4.19 (1H, *s*)	4.92 (1H, *s*)	84.8	85.4	85.0	85.8
4					43.9	47.9	47.5	48.5
5	2.18 (*m*)			2.28 (1H, *m*)	57.1	56.9	56.6	57.2
6				1.51 (2H, *m*)	19.1	17.8	17.6	18.5
7				1.45 (1H, *m*) 1.31 (1H, *m*)	34.8	34.5	34.2	35.2
8					43.6	42.9	41.3	43.5
9				2.12 (1H, *m*)	45.1	44.9	44.5	45.3
10					49.7	49.9	49.5	50.0
11				2.06 (1H, *m*) 1.56 (1H, *m*)	24.3	23.7	23.5	24.3
12				1.94 (1H, *m*) 1.32 (1H, *m*)	26.3	25.5	25.2	26.2
13	2.75 (*dt*, *J* = 12.7, 3.0)			2.74 (1H, br *t*, *J* = 10.2)	39.2	38.7	38.4	39.1
14					42.2	41.6	42.8	42.0
15				1.83 (1H, *m*) 1.17 (1H, *m*)	30.6	29.9	29.7	30.5
16	2.57 (br *d*, *J* = 12.8)			2.57 (1H, br *d*, *J* = 12.8) 1.42 (1H, *m*)	33.0	32.3	32.2	33.0
17					56.7	56.6	56.0	56.7
18	1.70 (*m*)			1.63 (1H, *m*)	49.8	49.6	49.1	49.7
19	3.46 (*m*)	2.94 (*m*)	2.90 (1H, *m*)	3.48 (1H, br *t*, *J* ca 10.7)	47.7	47.0	46.8	47.6
20					151.2	150.3	150.4	151.2
21	2.18 (*m*) 1.18 (*m*)			2.22 (1H, *m*) 1.48 (1H, *m*)	31.3	30.7	30.5	31.3
22	2.18 (*m*) 1.49 (*m*)			2.18 (1H, *m*) 1.48 (1H, *m*)	37.6	36.9	37.0	37.6
23	1.41 (*s*)	1.29 (*s*)	1.21 (3H, *s*)	1.77 (3H, *s*)	31.5	24.5	24.5	25.7
24	1.26 (*s*)	4.18 (*d*, *J* = 10.7) 3.26 (*d*, *J* = 10.7)	4.13 (1H, *d*, *J*=11.0) 3.15 (1H, *d*, *J*=11.0)	4.60 (1H, *d*, *J* = 10.7) 3.67 (1H, *d*, *J* = 10.7)	20.4	66.6	66.4	66.7
25	1.36 (*s*)	1.14 (*s*)	1.08 (3H, *s*)	1.42 (3H, *s*)	18.9	19.0	18.5	19.0
26	1.12 (*s*)	0.87 (*s*)	0.85 (3H, *s*)	1.08 (3H, *s*)	17.1	18.4	16.3	17.1
27	1.05 (*s*)	0.87 (*s*)	0.87 (3H, *s*)	1.02 (3H, *s*)	15.1	16.5	14.5	15.1
28					179.0	176.7	179.1	179.1
29	4.83 (br *s*) 4.64 (br *s*)	4.70 (br *s*) 4.57 (br *s*)	4.63 (1H, br *s*) 4.49 (1H, br *s*)	4.83 (1H, br *s*) 4.64 (1H, br *s*)	109.8	109.5	109.3	109.8
30	1.63 (*s*)	1.64 (*s*)	1.58 (3H, *s*)	1.63 (3H, *s*)	19.6	19.4	19.2	19.6
OMe						51.1		

2. Structure determination of compounds isolated from the methanol extract of *Z. mauritiana*

2.1 Structure determination of 13-membered ring cyclopeptide alkaloids

2.1.1 Structure Determination of Compound ZM-M4 (mauritine M, sss 3100, sss3347)

Compound ZM-M4 was obtained as a colorless solid, mp 188-189 °C and gave an orange coloration with anisaldehyde-H_2SO_4 reagent. Its molecular formula was established as $C_{38}H_{50}N_6O_6$ by HRTOFMS (m/z 687.3856, $[M+H]^+$), in combination with analysis of the ^{13}C NMR spectrum that showed 38 carbon resonances. The IR spectrum of compound ZM-M4 showed absorption bands at 3345 (amino), 1679, 1664, 1637 (amide), 1508 (aromatic) and 1224 cm^{-1} (phenol ether) functions. The UV spectrum revealed absorption maxima at 219, 272, 279, 289 and 318 nm, a typical 13-membered cyclopeptide alkaloid containing a tryptophan moiety.[19, 36]

The ^1H, ^{13}C NMR (Table 16, Figure 38 and 39), DEPT and HMQC spectra of compound ZM-M4 indicated the presence of 38 carbon resonances, which provided signals for one *N*-methyl carbon, four methyls, one methoxyl, five methylenes, 17 methines and ten quaternary carbons including four carbonyl carbons. The observation of an ABX coupling for a meta-oxygenated styryl [δ_H 6.61 (*d, J* = 2.9 Hz), 6.74 (*dd, J* = 9.0, 2.9 Hz), 6.84 (*d, J* = 9.0 Hz), 5.91 (*d, J* = 9.0 Hz) and 6.91 (*dd, J* = 11.2, 9.0 Hz)] indicated that compound ZM-M4 was a 13-membered cyclopeptide[94] which corresponded to its UV and IR data. The methoxyl signal at δ_H 3.75 showed a cross-peak with a quaternary aromatic carbon signal at δ_C 151.5 (C-14) in the HMBC spectrum in addition to a strong NOE effect shown between this methoxyl group and H-15 in the NOESY experiment confirming that the methoxyl group was placed at C-14 of the 13-membered cyclopeptide feature.

Analysis of ^1H, ^{13}C NMR, DEPT and 2D-NMR (^1H-^1H COSY and ^1H-^{13}C HMQC) spectra of compound ZM-M4 together with a comparison with the literature reported value[36,94] led to the assignments for four amino acid units of isoleucine [δ_H 4.23 (*t*, H-5), 2.00 (*m*, H-17), 1.43 and 1.15 (*m*, H-18), 0.87 (*t,* H-19) and 0.96 (*d*, H-20); δ_C 60.5 (C-5), 35.4 (C-17), 24.6 (C-18), 11.7 (C-19) and 16.0 (C-20)],[36] 3-oxygenated proline [δ_H 4.38 (*d*, H-8), 5.31 (*dt*, H-9), 2.25 and 2.08 (*m*, H-21),

3.79 and 2.46 (*m*, H-22); δ_C 64.3 (C-8), 76.4 (C-9), 32.3 (C-21) and 46.2 (C-22)], [94] tryptophan [δ_H 5.09 (*ddd*, H-25), 3.21 and 3.02 (H-26), 6.76 (H-28), 8.58 (br *s,* NH-29), 7.31 (*d*, H-31), 7.15 (br *t,* H-32), 7.09 (br *t,* H-33), 7.65 (*d,* H-34); δ_C 50.3 (C-25), 29.3 (C-26), 109.3 (C-27), 122.9 (C-28), 136.0 (C-30), 111.4 (C-31), 122.1 (C-32), 119.6 (C-33), 118.4 (C-34), 127.2 (C-35)], [36] and *N*-methyl leucine [δ_H 3.06 (*t*, H-38), 1.56 and 1.41 (*m*, H-39), 1.63 (*m*, H-40), 0.90 (*d*, H-41), 0.92 (*d*, H-42), 2.37 (3H, *s*, NMe); δ_C 63.1 (C-38), 42.3 (C-39), 25.0 (C-40), 21.9 (C-41), 23.0 (C-23.0), 35.0 (NMe)]. [36]

Connections among these subgroups were provided by analysis of its HMBC and NOESY spectra (Table 16; Figures 8 and 9, respectively). The HMBC correlations were observed for styrylamine proton at δ_H 8.35 (NH-3) to C-5 (δ_C 60.5) of isoleucyl carbonyl C-4 (δ_C 167.2) together with correlations of H-2 (δ_H 6.91) to C-1, C-4 and C-13, and H-1 (δ_H 5.91) to C-2, C-12 and C-14 indicating that isoleucine amino acid was attached to the styrylamine unit. HMBC correlations from H-5, H-8 and H-9 to 3-oxygenated proline carbonyl C-7 (δ_C 170.2) confirmed the connection between the isoleucine and *β*-oxyproline amino acids. A strong NOE interactions displayed in the NOESY spectrum between the proline proton at δ_H 3.79 (H-22) and H-25 (δ_H 5.09) of trytophyl unit, but no HMBC correlation observed between these two amino acids. In addition, the latter proton H-25 showed HMBC cross peaks to the leucyl carbonyl at δ_C 174.0 (C-37) confirmed the tryptophan as the intermediate side chain amino acid connected between the leucine terminal amino acid, and the *β*-hydroxyproline of the macro molecule. Furthermore, the HMBC correlations of the leucyl proton NH-36 to C-37, and of H-38 to C-37, C-39, C-40 and *N*-methyl carbon, along with an intense peak at m/z 100 observed in its EI mass spectrum also supported the *N*-methyl leucine as the end amino acid.

The *Z*-geometry of 1,2 double bond was established on the basis of the coupling constant value of 9.0 Hz for H-1 and H-2. [29] The coupling constant value of 11.2 Hz between H-2 and NH-3 protons and a small NOE interaction observed among them indicated *trans* coplanar of these two protons. [29, 94] The NH-6 and H-5 protons showed the coupling constant of 6.0 Hz and a small NOE cross peak was observed between both protons suggesting they were in the opposite orientation. In contrast, strong NOE interaction observed between NH-6 and H-8

allowing these both protons resided on the same side. Strong NOE enhancement between H-3 and the aromatic proton H-12, and the latter with H-9 were observed in NOESY spectrum. The small vicinal coupling constant value 3.2 Hz of the methine protons H-8 and H-9 indicated a *trans* configuration together with no significant NOE observed between these two protons in its NOESY spectrum further supported the *trans* relationship between H-8 and H-9. Strong NOE correlations were observed between H-9 and H-21β but not with H-21α indicating that H-9 and H-21β were on the same side of the pyrrolidine ring. In addition, the reported CD data of paliurine G (**120**) [94] which constructed with the same amino acid units for the cyclic part, suggested the 5*S*, 8*S*, 9*S* configurations for the macrocyclic unit. The ^{1}H and ^{13}C NMR chemical shift together with the coupling pattern of H-8/H-9 and H-5/H-6 for compound ZM-M4 followed the similar values for that of paliurine G (**120**),[94] thus ZM-M4 should have the same *S*-configuration at the position 5, 8 and 9 (Table 17). ^{1}H and ^{13}C NMR data of the acyclic part of ZM-M4 which attached to the macrocyclic ring at N-22 were very similar to that of mauritine J (**31**) [36] (Table 18), a 14-membered ring cyclopeptide constructed with the same amino acid units (i.e. tryptophan as the intermediate and leucine as the end amino acids) connected to the macrocyclic ring and had L configuration. The stereochemistry of these two acyclic amino acid units in compound ZM-M4 was thus tentatively inferred to be L. Therefore, compound ZM-M4 is a new cyclopeptide and named mauritine M (**121**) as shown in Figure 9.

Compound **121** was similar to nummularine R (**122**), a 4(13)-membered ring cyclopeptide obtained from *Z. nummularia*,[42] with the only different was the absence of the leucine as terminal amino acid in compound **122**. Mauritine M (**121**) is the first 5(13)-membered ring cyclopeptide type reported containing tryptophan amino acid in the molecule.

paliurine G (**120**)

mauritine J (**31**)

nummularine R (**122**)

mauritine M (**121**)

Figure 8 Selected HMBC correlations for compound ZM-M4 (mauritine M)

Figure 9 Key NOESY correlations for compound ZM-M4 (mauritine M)

Table 16 ^{1}H, ^{13}C NMR and 2D NMR data of compound ZM-M4 (sss3100) in $CDCl_3$

position	δ_H (mult., *J* in Hz)	δ_C	HMBC correlations	NOESY correlations
1	5.91 (1H, *d, J* = 9.0)	107.2	C-2, C-12, C-14	OMe, H-2
2	6.91 (1H, *dd, J* = 11.2, 9.0)	121.3	C-1, C-4, C-13	H-1
NH-3	8.35 (1H, *d, J* = 11.2)		C-1, C-2, C-4	H-12
CO-4		167.2		
5	4.23 (1H, *t, J* = 4.5)	60.5	C-4, C-7, C-17, C-18, C-20	NH-3 (small), H-20
NH-6	7.25 (1H, *d, J* = 6.0)		C-4, C-5, C-7, C-17	H-8
CO-7		170.2		
8	4.38 (1H, *d, J* = 3.2)	64.3	C-7, C-9, C-21, C-22	NH-6
9	5.31 (1H, *dt, J* = 7.2, 3.2)	76.4	C-7, C-11, C-21	H-12, H-21β
11		150.9		
12	6.61 (1H, *d, J* = 2.9)	111.2	C-1, C-11, C-14, C-16	NH-3, H-9
13		124.1		
14		151.4		
15	6.84 (1H, *d, J* = 9.0)	113.7	C-11, C-13, C-14, C-16	OMe
16	6.74 (1H, *dd, J* = 9.0, 2.9)	117.6	C-11, C-12, C-14	
17	2.00 (1H, *m*)	35.4		
18	1.43 (1H, *m*) and 1.15 (1H, *m*)	24.6	C-19, C-20	
19	0.87 (3H, *t, J* = 7.3)	11.7	C-17, C-18	
20	0.96 (3H, *d, J* = 6.9)	16.0	C-5, C-17, C-18	
21α	2.08 (1H, *m*)	32.3	C-8, C-9, C-22	
21β	2.25 (1H, *m*)			H-9
22α	3.79 (1H, *m*)	46.2	C-9, C-21	H-21α, H-25
22β	2.46 (1H, *m*)			H-9
CO-24		171.3		
25	5.09 (1H, *ddd, J* = 8.4, 8.0, 5.1)	50.3	C-24, C-26, C-27, C-37	H-22α, H-34
26	3.21 (1H, *dd, J* = 14.0, 5.1)	29.3	C-24, C-25, C-27, C-28, C-35	
	ca 3.02 obscured signal			
27		109.3		
28	ca 6.76 obscured signal	122.9	C-26, C-27, C-30, C-35	H-29
NH-29	8.58 (1H, br *s*)		C-27, C-28, C-30, C-35	H-28, H-31
30		136.0		
31	7.31 (1H, *d, J* = 7.8)	111.4	C-33, C-35	H-29
32	7.15 (1H, *br t* ca 7.6)	122.1	C-30, C-31, C-33, C-34	
33	7.09 (1H, *br t* ca 7.3)	119.6	C-31, C-32, C-34, C-35	
34	7.65 (1H, *d, J* = 7.5)	118.4	C-27, C-30, C-32, C-35	H-25
35		127.2		
NH-36	7.86 (1H, *d, J* = 8.0)		C-25, C-37	H-25, H-38
37		174.0		
38	3.06 (1H, *t, J* = 8.5)	63.1	C-37, C-39, C-40, NMe	NH-36, H-41, H-42, NMe
39	1.56 (1H, *m*) and 1.41 (1H, *m*)	42.3	C-37, C-38, C-40, C-41, C-42	
40	1.63 (1H, *m*)	25.0	C-38, C-39, C-41, C-42	
41	0.90 (3H, *d, J* = 7.0)	21.9	C-39, C-40, C-42	
42	0.92 (3H, *d, J* = 7.0)	23.0	C-39, C-40, C-41	
NMe	2.37 (3H, *s*)	35.0	C-38	H-38
OMe	3.75 (3H, *s*)	55.9	C-14	H-1, H-15

Signals without multiplicity was assigned from COSY.

Table 17 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-M4 (sss3100) with paliurine G (**120**)[(94)] in $CDCl_3$

position	δ_H (mult., *J* in Hz)		δ_C	
	paliurine G	compound ZM-M4	paliurine G	compound ZM-M4
1	5.92 (*d*, *J* = 9.1)	5.91 (1H, *d*, *J* = 9.0)	106.6	107.2
2	6.93 (*dd*, *J* = 11.3, 9.1)	6.91 (1H, *dd*, *J* = 11.2, 9.0)	121.5	121.3
NH-3	8.45 (*d*, *J* = 11.3)	8.35 (1H, *d*, *J* = 11.2)		
CO-4			167.0	167.2
5	4.26 (*dd*, *J* = 5.0, 4.5)	4.23 (1H, *t*, *J* = 4.5)	60.3	60.5
NH-6	7.14 (*d*, *J* = 4.5)	7.25 (1H, *d*, *J* = 6.0)		
CO-7			170.2	170.2
8	4.42 (*d*, *J* = 3.2)	4.38 (1H, *d*, *J* = 3.2)	64.4	64.3
9	5.52 (*dt*, *J* = 7.4, 3.2)	5.31 (1H, *dt*, *J* = 7.2, 3.2)	76.7	76.4
11			151.0	150.9
12	6.69 (*d*, *J* = 2.9)	6.61 (1H, *d*, *J* = 2.9)	111.2	111.2
13			124.2	124.1
14			151.4	151.4
15	6.89 (*d*, *J* = 9.1)	6.84 (1H, *d*, *J* = 9.0)	113.8	113.7
16	6.82 (*dd*, *J* = 9.1, 2.9)	6.74 (1H, *dd*, *J* = 9.0, 2.9)	117.7	117.6
17	2.08	2.00 (1H, *m*)	35.1	35.4
18	1.35 and 1.10	1.43 (1H, *m*) and 1.15 (1H, *m*)	24.4	24.6
19	0.86 (*t*, *J* = 6.9)	0.87 (3H, *t*, *J* = 7.3)	11.7	11.7
20	0.94 (*d*, *J* = 6.9)	0.96 (3H, *d*, *J* = 6.9)	16.2	16.0
21α	2.10 (*m*)	2.08 (1H, *m*)	32.5	32.3
21β	2.60 (*m*)	2.25 (1H, *m*)		
22α	4.28 (*dt*, *J* = 8.8, 2.7)	3.79 (1H, *m*)	46.7	46.2
22β	3.55 (*dt*, *J* = 10.7, 6.6)	2.46 (1H, *m*)		
CO-24			171.5	171.3
25	4.49 (*dd*, *J* = 8.8, 7.7)	5.09 (1H, *ddd*, *J* = 8.4, 8.0, 5.1)	54.9	50.3
26	1.93 (*d sept*, *J* = 8.8, 6.7)	3.21 (1H, *dd*, *J* = 14.0, 5.1)	31.4	29.3
		ca 3.02 obscured signal		
27	0.83 (*d*, *J* = 6.7)		19.1	109.3
28	0.86 (*d*, *J* = 6.7)	ca 6.76 obscured signal	18.1	122.9
NH-29	7.26 (*d*, *J* = 7.7)	8.58 (1H, br *s*)		
30			172.4	136.0
31	3.29 (*dd*, *J* = 6.9, 6.3)	7.31 (1H, *d*, *J* = 7.8)	71.0	111.4
32	3.17 (*dd*, *J* = 13.9, 6.9)	7.15 (1H, *br t* ca 7.6)	32.9	122.1
	2.90 (*dd*, *J* = 13.0, 6.3)			
33		7.09 (1H, *br t* ca 7.3)	139.7	119.6
34	7.25 (*m*)	7.65 (1H, *d*, *J* = 7.5)	129.1	118.4
35	7.25 (*m*)		128.3	127.2
36	7.25 (*m*)	7.86 (1H, *d*, *J* = 8.0)	126.1	
37				174.0
38		3.06 (1H, *t*, *J* = 8.5)		63.1
39		1.56 (1H, *m*) and 1.41 (1H, *m*)		42.3
40		1.63 (1H, *m*)		25.0
41		0.90 (3H, *d*, *J* = 7.0)		21.9
42		0.92 (3H, *d*, *J* = 7.0)		23.0
NMe	2.30 (*s*)	2.37 (3H, *s*)	42.3	35.0
OMe	3.78 (*s*)	3.75 (3H, *s*)	56.0	55.9

Signals without multiplicity was assigned from COSY.

Table 18 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-M4 (sss3100) with mauritine J (**31**)[36] in $CDCl_3$

position	δ_H (mult., *J* in Hz)		δ_C	
	mauritine J	compound ZM-M4	mauritine J	compound ZM-M4
1	6.30 (*d*, *J* = 7.7)	5.91 (1H, *d*, *J* = 9.0)	114.5	107.2
2	6.73 (*dd*, *J* = 10.5, 7.7)	6.91 (1H, *dd*, *J* = 11.2, 9.0)	125.4	121.3
NH-3	6.51 (*d*, *J* = 10.5)	8.35 (1H, *d*, *J* = 11.2)		
CO-4			166.9	167.2
5	4.20 (*dd*, *J* = 8.5, 3.0)	4.23 (1H, *t*, *J* = 4.5)	59.0	60.5
NH-6	6.58 (*d*, *J* = 8.5)	7.25 (1H, *d*, *J* = 6.0)		
CO-7			170.5	170.2
8	4.25 (*d*, *J* = 5.5)	4.38 (1H, *d*, *J* = 3.2)	63.9	64.3
9	5.43 (*m*)	5.31 (1H, *dt*, *J* = 7.2, 3.2)	83.4	76.4
11			157.4	150.9
12	7.18 (*m*)	6.61 (1H, *d*, *J* = 2.9)	122.6	111.2
13	7.05 (*m*)		130.1	124.1
14			132.5	151.4
15	7.09 (*m*)	6.84 (1H, *d*, *J* = 9.0)	132.4	113.7
16	7.24 (*m*)	6.74 (1H, *dd*, *J* = 9.0, 2.9)	122.3	117.6
17	2.22 (*m*)	2.00 (1H, *m*)	35.2	35.4
18	1.30 (*m*)	1.43 (1H, *m*) and 1.15 (1H, *m*)	23.7	24.6
	1.15 (*m*)			
19	0.88 (*t*, *J* = 6.5)	0.87 (3H, *t*, *J* = 7.3)	12.2	11.7
20	0.82 (*d*, *J* = 7.0)	0.96 (3H, *d*, *J* = 6.9)	16.0	16.0
21α	2.00 (*m*)	2.08 (1H, *m*)	31.8	
21β	2.32 (*m*)	2.25 (1H, *m*)		
22α	3.79 (*dd*, *J* = 11.5, 8.3)	3.79 (1H, *m*)	46.4	
22β	2.50 (*m*)	2.46 (1H, *m*)		
CO-24			171.4	171.3
25	5.00 (*ddd*, *J* = 8.5, 8.0, 6.2)	5.09 (1H, *ddd*, *J* = 8.4, 8.0, 5.1)	50.0	50.3
26	3.13 (*dd*, *J* = 14.5, 6.2)	3.21 (1H, *dd*, *J* = 14.0, 5.1)	29.3	29.3
	2.90 (*dd*, *J* = 14.5, 8.0)	ca 3.02 obscured signal		
27			110.0	109.3
28	6.76 (*d*, *J* = 2.5)	ca 6.76 obscured signal	122.7	122.9
NH-29	8.12 (br *s*)	8.58 (1H, br *s*)		
30			135.9	136.0
31	7.31 (*d*, *J* = 8.0)	7.31 (1H, *d*, *J* = 7.8)	111.2	111.4
32	7.10 (*m*)	7.15 (1H, *br t* ca 7.6)	122.8	122.1
33	7.12 (*m*)	7.09 (1H, *br t* ca 7.3)	119.8	119.6
34	7.68 (*d*, *J* = 8.0)	7.65 (1H, *d*, *J* = 7.5)	118.5	118.4
35			127.4	127.2
NH-36	7.73 (*d*, *J* = 8.5)	7.86 (1H, *d*, *J* = 8.0)		
37			174.8	174.0
38	2.98 (*dd*, *J* = 9.2, 5.0)	3.06 (1H, *t*, *J* = 8.5)	63.3	63.1
39	1.42 (*m*) and 1.25 (*m*)	1.56 (1H, *m*) and 1.41 (1H, *m*)	42.5	42.3
40	1.59 (*m*)	1.63 (1H, *m*)	25.0	25.0
41	0.87 (*d*, *J* = 6.5)	0.90 (3H, *d*, *J* = 7.0)	21.8	21.9
42	0.89 (*d*, *J* = 6.5)	0.92 (3H, *d*, *J* = 7.0)	23.1	23.0
NMe	2.38 (*s*)	2.37 (3H, *s*)	35.4	35.0
OMe		3.75 (3H, *s*)		55.9

2.1.2 Structure determination of compound ZM-M1 (nummularine H, sss 3648)

Compound ZM-M1 was a colorless solid and gave a blue coloration with anisaldehyde-H_2SO_4 reagent. The HRTOFMS displayed a pseudomolecular ion at m/z 682.3586 $[M+H]^+$ and in combination with the ^{13}C NMR data (Table 20, Figure 33) suggesting that ZM-M1 had a molecular formular of $C_{39}H_{47}N_5O_6$. Its IR absorption spectrum showed the presence of amide units at 3338 (NH stretch), 1668, 1642 (C=O stretch of 2° amide), 1221 (C-N stretch of 2° amide) and 701 cm^{-1} (NH bending). The UV absorption maxima at 268 and 319 nm suggested that compound ZM-M1 was a 13-membered-type cyclopeptide alkaloid.[29]

The ^{13}C NMR (Table 19, Figure 32 and 33) and DEPT spectra of compound ZM-M1 displayed 39 carbon signals including one *N*-methyl, one methoxyl, two methyls, five methylenes, 21 methines, nine quaternary carbons, four of which corresponded to the carbonyl groups. The meta-oxygenated styryl [δ_H 6.68 (*d, J* = 2.9 Hz), 6.88 (*d, J* = 9.1 Hz), 6.80 (*dd, J* = 9.1, 2.9 Hz), 5.93 (*d, J* = 8.9 Hz) and 6.96 (*dd, J* = 11.3, 8.9 Hz)] appeared as an ABX coupling[94] further supported that compound ZM-M1 was a 13-membered cyclopeptide which corresponded to its UV and IR data. The methoxyl signal at δ_H 3.76 showed a cross-peak with a quaternary aromatic carbon signal at δ_C 151.4 (C-14) in the HMBC spectrum in addition to a strong NOE effect shown between this methoxyl group and H-15 in the NOESY experiment confirming that the methoxyl group was placed at C-14 of the 13-membered cyclopeptide feature.

The ^1H, ^{13}C NMR, DEPT and 2D-NMR (^1H-^1H COSY and ^1H-^{13}C HMQC) spectra of compound ZM-M1 together with a comparison with the literature reported value[84,94] led to the assignments for four amino acid units of isoleucine [δ_H 4.31 (*t*, H-5), 2.07 (*m*, H-17), 1.42 and 1.18 (*m*, H-18), 0.92 (*t,* H-19) and 1.01 (*d*, H-20); δ_C 60.3, 35.4, 24.7, 11.8 and 16.1], 3-oxygenated proline [δ_H 4.41 (*d*, H-8), 5.43 (*dt*, H-9), 2.41 and 2.21 (*m*, H-21), 3.96 and 2.76 (*m*, H-22); δ_C 64.5, 76.5, 32.3 and 46.2], [94] phenylalanine [δ_H 4.98 (*m*, H-25), 2.93 (*t*, H-26), ca 7.18-7.34 (*m,* aromatic protons); δ_C 50.9, 39.4, 135.3, 129.0 and 128.7],[94] and *N*-methyl phenylalanine [δ_H 3.22 (*dd*, H-33), 2.67 and 3.12 (*dd*, H-34), 7.03-7.34 (*m*, aromatic protons) and 2.27 (3H, *s*, *N*Me); δ_C 65.8, 38.9, 137.3, 129.1, 128.7, 126.9 and 35.3]. [94]

Connecting these subgroups were provided by HMBC and NOESY experiments (Table 19, Figure 10 and 11), as follow : the styrylamine proton at δ_H 8.42 (NH-3) and isoleucyl proton at δ_H 4.31 (H-5) showed HMBC cross peaks with C-4 (δ_C 167.0) along with correlations of H-2 (δ_H 6.96) to C-13, and H-1 (δ_H 5.93) to C-2, C-12 and C-14 indicating that isoleucine amino acid was attached to the styrylamine unit. The correlations of H-5, H-8 and H-9 to 3-oxygenated proline carbonyl C-7 (δ_C 169.6) revealed the connection between the isoleucine and β-oxyproline amino acids. In the NOESY spectrum, a strong NOE interactions displayed between the proline proton at δ_H 3.96 (H-22) and H-25 (δ_H 4.98) of phenylalanyl unit, but no HMBC correlation observed between these protons. The HMBC correlation from H-26 (δ_H 2.93) to C-24, C-25, C-27, C-28 ; NH-31 (δ_H 7.78) to C-32 ; H-33 (δ_H 3.22) to C-32, C-35 and *N*Me confirmed the phenylalanine as the intermediate unit connected between the β-hydroxyproline amino acid and *N*-methyl phynylanine (as the terminal amino acid). The *Z*-geometry of 1,2 double bond was established on the basis of the coupling constant value of 8.9 Hz for H-1 and H-2.[29] The styrylamine proton NH-3 showed strong NOE effect with aromatic proton H-12. Strong NOE enhancement from H-9 to H-12 and H-21β, whist H-8 showed a cross peak with NH-6.

The ^{1}H and ^{13}C NMR data of compound ZM-M1 were closely resembled with that of nummularine H (**123**) (Table 20) in which the absolute stereochemistry was determined by its CD spectrum analysis and assigned to be 5S, 8S, 9S configuration for the cyclic unit. Compound ZM-M1 showed levorotatory optical rotation [$[\alpha]_D^{26}$ -296.5 (c 0.22, $CHCl_3$)] similar to that of nummularine H [$[\alpha]_D^{20}$ -343 (c 0.27, MeOH)],[84] thus the stereochemical structure of ZM-M1 was thus established as shown in Figure 11.

Nummularine H (**123**) has been isolated from *Z. nummularia*[84] and the stem of *Paliurus ramossisimus* (Rhamnaceae).[94]

nummularine H (**123**)

Figure 10 Selected HMBC correlations for compound ZM-M1 (nummularine H)

Figure 11 Key NOESY correlations for compound ZM-M1 (nummularine H)

Table 19 ^{1}H, ^{13}C NMR and 2D NMR data of compound ZM-M1 (sss3648) in $CDCl_3$

position	δ_H (mult., *J* in Hz)	δ_C	HMBC correlations	NOESY correlations
1	5.93 (1H, *d*, *J* = 8.9)	106.7	C-2, C-12, C-14	
2	6.96 (1H, *dd*, *J* = 11.3, 8.9)	121.4	C-13	
NH-3	8.42 (1H, *d*, *J* = 11.3)		C-4	H-12
CO-4		167.0		
5	4.31 (1H, *t*, *J* = 4.4)	60.3	C-4, C-7, C-17, C-18, C-20	H-17, H-20
NH-6	7.26			H-8
CO-7		169.6		
8	4.41 (1H, *d*, *J* = 3.1)	64.5	C-7, C-9, C-22	NH-6, H-9 (small), H-21α (small)
9	5.43 (1H, *dt*, *J* = 6.6, 3.1)	76.5	C-7, C-21	H-8 (small), H-12, H-21β
11		150.9		
12	6.68 (1H, *d*, *J* = 2.9)	111.2	C-14, C-16	NH-3, H-8 (small), H-9
13		124.3		
14		151.4		
15	6.88 (1H, *d*, *J* = 9.1)	113.7	C-11, C-13	OMe
16	6.80 (1H, *dd*, *J* = 9.1, 2.9)	117.6	C-14	
17	2.07 (1H, *m*)	35.4	C-4	
18	1.18 (1H, *m*) and 1.42 (1H, *m*)	24.7		
19	0.92 (3H, *t*, *J* = 7.2)	11.8	C-17, C-18	
20	1.01 (2H, *d*, *J* = 6.9)	16.1	C-5, C-17, C-18	
21α	2.21 (1H, *m*)	32.3		H-22α
21β	2.41 (1H, *m*)			H-9
22α	3.96 (1H, *m*)	46.2		H-8 (small), H-21α, H-25
22β	2.76 (1H, *m*)			
CO-24		170.6		
25	4.98 (1H, *m*)	50.9		H-22α, H-26, NH-31
26	2.93 (2H, *t* , *J* = 6.5)	39.4	C-24, C-25, C-27, C-28	H-25, H-31, H-36
27		135.3		
28, 28'	7.18 (2H, *m*)	129.0	C-26, C-30	
29, 29'	7.29-7.34 (*m*)	128.7	C-27	
30	7.21	127.2	C-28	
NH-31	7.78 (1H, *d*, *J* = 8.4)		C-32	H-25, H-26, H-33, H-34
CO-32		173.1		
33	3.22 (1H, *dd*, *J* = 9.2, 4.3)	65.8	C-32, C-35, NMe	H-31, NMe
34	3.12 (1H, *dd*, *J* = 13.4, 4.3)	38.9	C-32, C-33, C-35, C-37	
	2.67 (1H, *dd*, *J* = 13.4, 9.2)			
35		137.3		
36, 36'	7.03 (1H, *m*)	129.1	C-38	H-20
37, 37'	7.29-7.34 (*m*)	128.7	C-35	
38	7.21	126.9	C-36	
NMe	2.27 (3H, *s*)	35.3	C-33	H-31, H-33
OMe	3.79 (3H, *s*)	56.0	C-14	H-15

Signals without multiplicity was assigned from COSY.

Table 20 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-M1 (sss3648) with nummularine H (**123**)[(94)] in $CDCl_3$

position	δ_H (mult., *J* in Hz)		δ_C	
	nummularine H	compound ZM-M1	nummularine H	compound ZM-M1
1	5.90 (*d*, *J* = 9.1)	5.93 (1H, *d*, *J* = 8.9)	106.6	106.7
2	6.93 (*dd*, *J* = 11.3, 9.1)	6.96 (1H, *dd*, *J* = 11.3, 8.9)	121.6	121.4
NH-3	8.40 (*d*, *J* = 11.3)	8.42 (1H, *d*, *J* = 11.3)		
CO-4			167.0	167.0
5	4.29 (*t*, *J* = 4.4)	4.31 (1H, *t*, *J* = 4.4)	60.3	60.3
NH-6	7.27	7.26		
CO-7			169.8	169.6
8	4.39 (*d*, *J* = 3.1)	4.41 (1H, *d*, *J* = 3.1)	65.0	64.5
9	5.41 (*dt*, *J* = 7.3, 3.1)	5.43 (1H, *dt*, *J* = 6.6, 3.1)	76.7	76.5
11			151.2	150.9
12	6.66 (*d*, *J* = 2.9)	6.68 (1H, *d*, *J* = 2.9)	111.4	111.2
13			124.8	124.3
14			151.7	151.4
15	6.86 (*d*, *J* = 9.1)	6.88 (1H, *d*, *J* = 9.1)	114.2	113.7
16	6.77 (*dd*, *J* = 9.1, 2.9)	6.80 (1H, *dd*, *J* = 9.1, 2.9)	117.7	117.6
17	2.05 *(m)*	2.07 (1H, *m*)	35.7	35.4
18	1.12 and 1.42	1.18 (1H, *m*) and 1.42 (1H, *m*)	25.0	24.7
19	0.90 (*t*, *J* = 7.2)	0.92 (3H, *t*, *J* = 7.2)	11.8	11.8
20	0.99 (*d*, *J* = 6.9)	1.01 (2H, *d*, *J* = 6.9)	16.2	16.1
21α	2.19 *(m)*	2.21 (1H, *m*)	32.4	32.3
21β	2.41 (*dddd*, *J* = 13.6, 7.3, 6.5, 2.6)	2.41 (1H, *m*)		
22α	3.94 (*ddd*, *J* = 10.8, 8.2, 2.6)	3.96 (1H, *m*)	46.4	46.2
22β	2.73 (*dt*, *J* = 6.5, 10.8)	2.76 (1H, *m*)		
CO-24			170.9	170.6
25	4.96 (*dt*, *J* = 8.2, 6.2)	4.98 (1H, *m*)	51.1	50.9
26	2.94 (*dd*, *J* = 12.5, 6.2)	2.93 (2H, *t* , *J* = 6.5)	39.5	39.4
	2.86 (*dd*, *J* = 12.5, 8.2)			
27			135.6	135.3
28, 28'	7.16-7.32 (*m*)	7.18 (2H, *m*)	129.2	129.0
29, 29'	7.16-7.32 (*m*)	7.29-7.34 (*m*)	128.7	128.7
30	7.16-7.32 (*m*)	7.21	127.2	127.2
NH-31	7.78 (1H, *d*, *J* = 8.4)	7.78 (1H, *d*, *J* = 8.4)		
CO-32			173.1	173.1
33	3.19 (*dd*, *J* = 9.1, 4.3)	3.22 (1H, *dd*, *J* = 9.2, 4.3)	66.0	65.8
34	3.09 (*dd*, *J* = 13.7, 4.1)	3.12 (1H, *dd*, *J* = 13.4, 4.3)	39.0	38.9
	2.65 (*dd*, *J* = 13.7, 9.1)	2.67 (1H, *dd*, *J* = 13.4, 9.2)		
35			137.5	137.3
36, 36'	7.16-7.32 (*m*)	7.03 (1H, *m*)	129.1	129.1
37, 37'	7.16-7.32 (*m*)	7.29-7.34 (*m*)	128.7	128.7
38	7.16-7.32 (*m*)	7.21	126.9	126.9
NMe	2.24 (*s*)	2.27 (3H, *s*)	35.3	35.3
OMe	3.76 (*s*)	3.79 (3H, *s*)	56.2	56.0

Signals without multiplicity were assigned from COSY.

2.1.3 Structure determination of compound ZM-M5 (nummularine B, sss3008)

The major cyclopeptide, compound ZM-M5 was obtained as a colorless solid, mp 219-220 °C and also gave a blue coloration with anisaldehyde-H_2SO_4 reagent. Its IR spectrum showed absorption band for amino (3345 cm^{-1}), amide (1679 cm^{-1}), aromatic (1508 cm^{-1}) and phenol ether (1224 cm^{-1}) functional groups. UV spectrum showed absorption maxima at 268 and 319 nm, both typical of 13-membered ring cyclopeptide alkaloids.[29, 94] Its molecular formula was established as $C_{32}H_{41}N_5O_6$ by HRTOFMS (m/z 592.3122, $[M+H]^+$).

The ^{13}C NMR spectrum (Table 21, Figure 41) displayed 32 carbon signals and DEPT analysis provided signals for three methyls, one *N*-methyl, one methoxyl, three methylenes, 16 methines, eight quaternary carbons including four carbonyl carbons. Its ^1H, ^{13}C NMR, COSY, HMQC and HMBC spectra (Table 21) together with a comparison with the literature reported value[86,94] led to the assignments of compound ZM-M5 (Table 22) to have a styrylamine and four amino acid units of phenylalanine [δ_H 4.56 (*m*, H-5), 3.25 and 2.86 (H-17), ca 7.21-7.32 (*m,* aromatic protons); δ_C 56.7, 36.6, 135.7, 129.2, 128.8 and 127.2],[94] 3-oxygenated proline [δ_H 4.43 (br *s*, H-8), 5.49 (*m*, H-9), 2.54 and 2.27 (*m*, H-21), 4.11 and 3.47 (*m*, H-22); δ_C 64.2, 76.5, 32.5 and 46.5], [94] valine [δ_H 4.51 (*m*, H-25), 1.77 (H-26), 0.57 (*d*, H-27) and 0.68 (*d,* H-28) ; δ_C 54.2, 30.9, 17.2 and 19.1],[94] and *N*-methyl alanine [δ_H 3.07 (*dd*, H-31), 1.32 (*d*, H-32) and 2.42 (3H, *s*, *N*Me); δ_C 60.5, 19.8 and 35.1].[94] Compound ZM-M5 showed an ABX system [δ_H 6.67 (br *s*), 6.87 (*d, J* = 9.0 Hz) and 6.78 (*dd, J* = 9.0, 2.8 Hz)] in its ^1H NMR spectrum (Figure 40) which supported for a 13-membered ring cyclopeptide.

Constructing these moieties together by HMBC and NOESY experiments established the structure of compound ZM-M5 to be nummularine B (**38**) which has been isolated from *Z. nummularia*. HMBC correlations of the styrylamine proton at δ_H 8.41 (NH-3) and phenylalanyl proton at δ_H 4.56 (H-5) to C-4 (δ_C 167.1) indicated that phenylanine moiety was attached to the styrylamine unit. The connection between the phenylanine and β-oxyproline amino acids was confirmed by HMBC correlations of H-5, NH-6, H-8 and H-9 to 3-oxygenated proline carbonyl C-7 (δ_C 170.1). Strong NOE interactions of the proline proton at δ_H 4.11 (H-22) showed a cross

peak with H-25 (δ_H 4.51) of valine unit, but no HMBC correlation observed between these protons. The HMBC correlation from the valyl proton H-25 to C-24, C-26, C-27, C-28, C-30; NH-29 to C-25, C-30, and the alanyl proton H-31 to C-30, C-32 and *N*Me confirmed the valine residue (as the intermediate unit) connected between the β-hydroxyproline amino acid and *N*-methyl alanine (as the terminal amino acid). Complete ^{1}H and ^{13}C NMR data assignments for numularine B (**38**) were also recorded here with for the first time (Table 21, Figure 40 and 41).

The relative stereochemistry of nummularine B was determined by analysis of its NOESY interaction (Table 21, Figure 13) and comparison of ^{1}H NMR coupling constants with other related compound. The *Z*-geometry of 1,2 double bond was established on the basis of the coupling constant value of 8.9 Hz for H-1 and H-2.[29] The H-2 and NH-3 protons showed the coupling constant value of 11.0 Hz and a small NOE interaction observed among them indicated *trans* coplanar of these two protons.[29, 94] The coupling constant value of 8.9 Hz between NH-6 with H-5 protons and a small NOE cross peak was observed between both protons suggesting they were in the opposite side. In contrast, strong NOE enhancement displayed between NH-6 and H-8 both these protons resided on the same side. The NH-3 showed strong NOE effect with aromatic proton H-12, and the latter with H-9 were observed in NOESY spectrum. Moreover, strong NOE correlation was observed between H-9 and H-21β but not with H-21α indicating that H-9 and H-21β were on the same side of the pyrrolidine ring. The X-ray crystal structure analysis of compound ZM-M5, as its *N*-methylated molecule (*N,N*-dimethyl nummularine B methiodide), provided the unambiguous stereochemical assignments for 5*S*, 8*S*, 9*S*, 25*S* and 31*S* configurations as shown in Figure 14, which also showed good consistency with the NOE enhancements observed. This was the first X-ray crystal structure study of 13-membered ring Zizyphine A type cyclopeptide alkaloid.

nummularine B (**38**)

Figure 12 Selected HMBC correlations for compound ZM-M5 (nummularine B)

Figure 13 Key NOESY correlations for compound ZM-M5 (nummularine B)

N,N-dimethyl nummularine B methiodide (**124**)

(*N,N*-dimethylated product of ZM-M5)

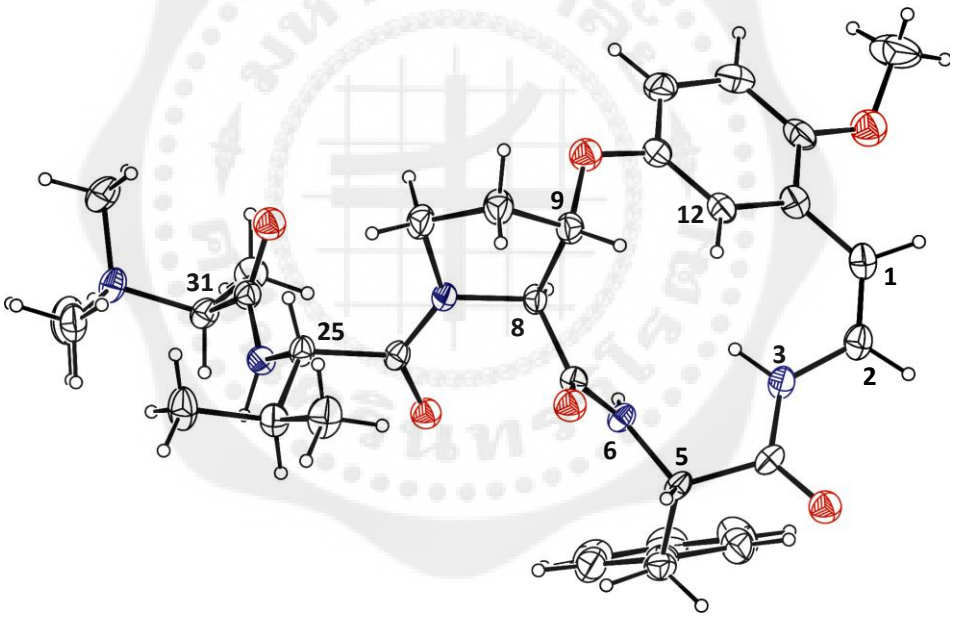


Figure 14 X-ray crystal structure of *N,N*-dimethyl nummularine B methiodide (**124**)

Table 21 ^{1}H, ^{13}C NMR and 2D NMR data of compound ZM-M5 (sss3008) in $CDCl_3$

position	δ_H (mult., *J* in Hz)	δ_C	HMBC correlations	NOESY correlations
1	5.95 (1H, *d*, *J* = 8.9)	107.2	C-2, C-12, C-14	OMe
2	6.94 (1H, *dd*, *J* = 11.0, 8.9)	121.4	C-13	
NH-3	8.41 (1H, *d*, *J* = 11.0)		C-4	H-12
CO-4		167.1		
5	4.56 (1H, *m*)	56.7	C-4, C-7, C-17, C-17a	H-17, H-18
NH-6	ca 7.30 (1H, *d*, *J*= 8.9)		C-5, C-7, C-17	H-8
CO-7		170.1		
8	4.43 (1H, br *s*)	64.2	C-7, C-9, C-22	H-12 (small), H-6
9	5.49 (1H, *m*)	76.5	C-7	H-12, H-21β
11		151.0		
12	6.67 (1H, br *s*)	111.3	C-1, C-14, C-16	NH-3, H-9
13		124.1		
14		151.5		
15	6.87 (1H, *d*, *J* = 9.0)	113.8	C-11, C-13	OMe
16	6.78 (1H, *dd*, *J* = 9.0, 2.8)	117.8	C-12, C-14	
17	3.25 (1H, *dd*, *J* = 14.0, 3.3)	36.6	C-4, C-5, C-17a, C-18, C-18'	
	2.86 (1H, *dd*, *J* = 14.0, 10.0)			
17a		135.7		
18, 18'	7.21-7.32 (*m*)	129.2	C-17a, C-19, C-19', C-20	
19, 19'	7.21-7.32 (*m*)	128.8	C-17a, C-18, C-18', C-20	
20	7.21-7.32 (*m*)	127.2	C-18, C-18', C-19, C-19'	
21α	2.27 (1H, *m*)	32.5	C-9	
21β	2.54 (1H, *m*)			H-9, H-22β
22α	4.11 (1H, br *t*, *J* = *8.7*)	46.5	C-9, C-21	H-25
22β	3.47 (1H, *m*)			
CO-24		171.3		
25	4.51 (1H, *m*)	54.2	C-24, C-26, C-27, C-28, C-30	H-22α, H-29
26	1.77 (1H, *hept*, *J* ca 6.9)	30.9	C-25, C-27, C-28	H-27, H-28
27	0.57 (3H, *d*, *J* = 6.3)	17.2	C-25, C-26, C-28	H-26
28	0.68 (3H, *d*, *J* = 6.3)	19.1	C-25, C-26, C-27	H-26
NH-29	7.59 (1H, *d*, *J* = 8.7)		C-25, C-30	H-25, H-26, H-27, H-28, H-31, H-32, NMe
CO-30		174.9		
31	3.07 (1H, *dd*, *J* ca 7.0, 6.7)	60.5	C-30, C-32, NMe	NH-29, H-32, NMe
32	1.32 (3H, *d*, *J* = 6.6)	19.8	C-30, C-31	NMe
NMe	2.42 (3H, *s*)	35.1	C-31	NH-29, H-31
OMe	3.78 (3H, *s*)	56.0	C-14	H-1, H-15

Table 22 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-M5 (sss3008) with nummularine B (**38**)[86] in $CDCl_3$.

position	δ_H (mult., *J* in Hz)		δ_C
	nummularine B	compound ZM-M5	compound ZM-M5
1	5.94 (*d*, *J* = 9)	5.95 (1H, *d*, *J* = 8.9)	107.2
2	6.8-8.6 (*m*)	6.94 (1H, *dd*, *J* = 11.0, 8.9)	121.4
NH-3	6.8-8.6 (*m*)	8.41 (1H, *d*, *J* = 11.0)	
CO-4			167.1
5		4.56 (1H, *m*)	56.7
NH-6		ca 7.30 (1H, *d*, *J*= 8.9)	
CO-7			170.1
8		4.43 (1H, br *s*)	64.2
9		5.49 (1H, *m*)	76.5
11			151.0
12		6.67 (1H, br *s*)	111.3
13			124.1
14			151.5
15		6.87 (1H, *d*, *J* = 9.0)	113.8
16		6.78 (1H, *dd*, *J* = 9.0, 2.8)	117.8
17		3.25 (1H, *dd*, *J* = 14.0, 3.3)	36.6
		2.86 (1H, *dd*, *J* = 14.0, 10.0)	
17a			135.7
18, 18'		7.21-7.32 (*m*)	129.2
19, 19'		7.21-7.32 (*m*)	128.8
20		7.21-7.32 (*m*)	127.2
21α		2.27 (1H, *m*)	32.5
21β		2.54 (1H, *m*)	
22α		4.11 (1H, br *t*, *J* = *8.7*)	46.5
22β		3.47 (1H, *m*)	
CO-24			171.3
25		4.51 (1H, *m*)	54.2
26		1.77 (1H, *hept*, *J* ca 6.9)	30.9
27	0.65 (3H, *d*, *J* = 5.5)	0.57 (3H, *d*, *J* = 6.3)	17.2
28	0.65 (3H, *d*, *J* = 5.5)	0.68 (3H, *d*, *J* = 6.3)	19.1
NH-29		7.59 (1H, *d*, *J* = 8.7)	
CO-30			174.9
31		3.07 (1H, *dd*, *J* ca 7.0, 6.7)	60.5
32	1.34 (*d*, *J* = 7)	1.32 (3H, *d*, *J* = 6.6)	19.8
NMe	2.47 (*s*)	2.42 (3H, *s*)	35.1
OMe	3.77 (*s*)	3.78 (3H, *s*)	56.0

Table 23 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-M5 (**38**) (sss3008) with *N,N*-dimethyl nummularine B methiodide (**124**) (sss3497)

position	δ_H (mult., *J* in Hz)		δ_C	
	compound ZM-M5 ($CDCl_3$)	*N,N*-dimethyl nummularine B methiodide (methanol-d_4)	compound ZM-M5 ($CDCl_3$)	*N,N*-dimethyl nummularine B methiodide (methanol-d_4)
1	5.95 (1H, *d, J* = 8.9)	6.11 (1H, *d, J* = 8.9)	107.2	110.7
2	6.94 (1H, *dd, J* = 11.0, 8.9)	ca 6.87 (1H, *d, J* = 8.9)	121.4	121.8
NH-3	8.41 (1H, *d, J* = 11.0)			
CO-4			167.1	170.4
5	4.56 (1H, *m*)	4.51 (1H, *dd, J* = 10.9, 3.6)	56.7	58.7
NH-6	ca 7.30 (1H, *d, J*= 8.9)			
CO-7			170.1	172.3
8	4.43 (1H, br *s*)	4.49 (1H, *d, J* = 3.0)	64.2	66.1
9	5.49 (1H, *m*)	5.34 (1H,br *dt, J* = 7.7, 3.0)	76.5	78.9
11			151.0	152.4
12	6.67 (1H, br *s*)	6.84 (1H, br *s*)	111.3	112.6
13			124.1	125.3
14			151.5	153.1
15	6.87 (1H, *d, J* = 9.0)	7.04 (1H, *d, J* = 9.0)	113.8	115.2
16	6.78 (1H, *dd, J* = 9.0, 2.8)	6.89 (1H, *dd, J* = 9.0, 2.8)	117.8	119.0
17	3.25 (1H, *dd, J* = 14.0, 3.3)	3.21 (1H, *dd, J* = 14.0, 3.6)	36.6	37.5
	2.86 (1H, *dd, J* = 14.0, 10.0)	2.87 (1H, *dd, J* = 14.0, 10.9)		
17a			135.7	138.2
18, 18'	7.21-7.32 (*m*)	7.29 (2H, *m*)	129.2	130.0
19, 19'	7.21-7.32 (*m*)	7.29 (2H, *m*)	128.8	129.6
20	7.21-7.32 (*m*)	7.23 (1H, *m*)	127.2	127.8
21α	2.27 (1H, *m*)	2.28 (1H, *m*)	32.5	33.7
21β	2.54 (1H, *m*)	2.61 (1H, *m*)		
22α	4.11 (1H, br *t, J = 8.7*)	4.27 (1H, *m*)	46.5	47.7
22β	3.47 (1H, *m*)	3.60 (1H, *m*)		
CO-24			171.3	171.6
25	4.51 (1H, *m*)	4.37 (1H, *d, J* = 8.3)	54.2	57.9
26	1.77 (1H, *hept, J* ca 6.9)	1.90 (1H, *hept, J* ca 7.0)	30.9	31.8
27	0.57 (3H, *d, J* = 6.3)	0.62 (3H, *d, J* = 6.6)	17.2	19.0
28	0.68 (3H, *d, J* = 6.3)	0.96 (3H, *d, J* = 6.6)	19.1	19.2
NH-29	7.59 (1H, *d, J* = 8.7)			
CO-30			174.9	168.7
31	3.07 (1H, *dd, J* ca 7.0, 6.7)	4.22 (1H, *q, J* ca 6.5)	60.5	71.2
32	1.32 (3H, *d, J* = 6.6)	1.65 (3H, *d, J* = 6.7)	19.8	13.2
NMe	2.42 (3H, *s*)	3.30 (9H, *s*, -$^{+}NMe_3$)	35.1	52.7
OMe	3.78 (3H, *s*)	3.82 (3H, *s*)	56.0	56.7

2.2 Structure determination of 14-membered ring cyclopeptide alkaloids

2.2.1 Structure determination of compound ZM-M2 (hemsine A, sss3346)

Compound ZM-M2 was obtained as a colorless solid, mp 108-109 °C and gave an orange coloration with anisaldehyde-H_2SO_4 reagent. The IR spectrum showed absorption bands at 3389 (amino), 1660 (amide), 1625 (styryl double bond), 1506, 1487, 1458 (aromatic) and 1229 cm^{-1} (phenyl ether). On the basis of its HRTOFMS ($[M+H]^+$ at *m/z* 558.3082), a molecular formula of ZM-M2 was established as $C_{32}H_{39}N_5O_4$ with support of ^{13}C NMR spectrum. The UV absorption maxima at 221, 273, 280 and 290 nm were found to belong to a 14-membered type cyclopeptide alkaloid with tryptophan moiety.[36]

^1H NMR, ^{13}C NMR, DEPT and 2D-NMR spectra data of compound ZM-M4 together with a comparison with the literature reported values[85] led to the assignments for a styrylamine unit [δ_H 6.31 (*d, J* = 7.6 Hz, H-1), 6.71 (*dd, J* = 10.4, 7.6 Hz, H-2), 6.52 (*d, J* = 10.4 Hz, NH-3), 7.24 (*m*, H-12), 7.15 (*m*, H-13), 7.07 (*m*, H-15) and 7.24 (*m*, H-16)] together with three amino acid units of isoleucine [δ_H 4.13 (*dd*, H-5), 2.10 (*m*, H-17), 1.22 and 1.08 (*m*, H-18), 0.80 (*t,* H-19), 0.59 (*d*, H-20); δ_C 58.7, 35.4, 23.8, 12.2 and 15.7], oxygenated proline [δ_H 4.26 (*d*, H-8), 5.43 (*dt*, H-9), 2.38 and 1.96 (*m*, H-21), 3.97 and 2.75 (*m*, H-22); δ_C 63.8, 83.9, 31.8 and 45.9] and *N,N*-dimethyl tryptophan [δ_H 3.64 (*t*, H-25), 3.13 (br *d*, H-26), 6.74 (H-28), 8.14 (br *s,* H-29), 7.31 (*d*, H-31), 7.13 (*m,* H-32), 7.10 (*m*, H-33), 7.56 (*d,* H-34) and 2.48 (6H, *s*, NMe_2); δ_C 65.6, 21.0, 111.7, 122.4, 136.1, 111.3, 122.8, 119.3, 118.1, 127.2 and 41.7].[36]

Connections among these subgroups were provided by analysis of its HMBC and NOESY spectra (Table 24; Figures 15 and 16, respectively). The HMBC correlations were observed for aromatic protons [δ_H 7.24 (*m*, H-12), 7.15 (*m*, H-13), 7.07 (*m*, H-15) and 7.17 (*m*, H-16)] to the oxygenated sp^2 quaternary carbon at δ_C 157.4 (C-11), and proton H-1 (δ_H 6.31), H-2 (δ_H 6.71), H-12 (δ_H 7.24), H-13 (δ_H 7.15) and H-16 (δ_H 7.17) to δ 132.5 (C-14) confirming a 1,4-disubstituted aromatic ring. The conformational constraints of 14-membered cyclopeptide alkaloid in the macrocyclic ring system induced the nonequivalence of the protons and carbon

atoms.[95] The isoleucyl proton at δ_H 4.13 (H-5) and styrylamine proton at δ_H 6.52 (NH-3) showed HMBC correlation with carbonyl C-4 (δ_C 167.1) indicating that isoleucine amino acid was attached to the styrylamine unit. The HMBC correlations from H-5 (δ_H 4.13), NH-6 (δ_H 6.70), H-8 (δ_H 4.26) and H-9 (δ_H 5.43) to 3-oxygenated proline carbonyl C-7 (δ_C 170.9) confirmed the connection between the isoleucine and β-oxyproline amino acids. The proline protons at δ_H 3.97 (H-22) and tryptophyl proton at δ_H 3.64 (H-25) showed cross peaks to the carbonyl C-24 (δ_C 171.7) in addition to a strong NOE interaction between the H-22 of proline and H-25 of trytophyl unit displayed in the NOESY spectrum confirmed connecting between the proline and tryptophan amino acids. In the HMBC experiment, H-25 showed another cross peak with a singlet methyl at δ 2.48 (6H), assigned to the protons of the *N,N*-dimethyl group also confirmed the *N,N*-dimethyl tryptophan as the end amino acid.

The NMR data together with a comparison with the reported values (Table 24 and 25) led to the structure assignment for compound ZM-M2 as hemsine A (**125**), a 4(14)-Amphibine F-type cyclopeptide alkaloid, hemsine A has been isolated from the root of *Paliurus hemsleyanus*.[85] The relative stereochemistry of ZM-M2 was determined by the aid of NOESY experiments, as follow : the H-9 proton showed cross-peaks with H-8 (weak), H-16 (strong) and H-21β (strong), but did not show a cross-peak with H-21α; the H-8 proton exhibited cross-peaks with NH-6 (weak) and H-12 (weak), the NH-3 proton showed a cross-peaks with the H-5 (weak), and the H-25 proton exhibited cross-peaks with H-22α, H-28, H-34 and NMe_2. The stereostructure of hemsine A (**125**) [85] was assigned as 5*S*, 8*S*, 9*S* configuration by its CD spectra analysis shown in the Figure 16, with *N,N*-dimethyl L-tryptophan as terminal amino acid unit. Compound ZM-M2 showed a levorotatory optical rotation, $[\alpha]_D^{27}$ -100.0 (c 0.24, MeOH), similar to that of hemsine A, $[\alpha]_D^{26}$ -64.5 (c 2.0, MeOH)[85] thus both of them should have a similar configuration for the acyclic unit. Therefore stereostructure of compound ZM-M2 should be the same as those for hemsine A (**125**).

hemsine A (**125**)

Figure 15 Selected HMBC correlations for compound ZM-M2 (hemsine A)

Figure 16 Key NOESY correlations for compound ZM-M2 (hemsine A)

Table 24 ^{1}H, ^{13}C NMR and 2D NMR data of compound ZM-M2 (sss3346) in $CDCl_3$

position	δ_H (mult., *J* in Hz)	δ_C	HMBC correlations	NOESY correlations
1	6.31 (1H, *d*, *J* = 7.6)	114.4	C-2, C-13, C-14, C-15	
2	6.71 (1H, *dd*, *J* = 10.4, 7.6)	125.4	C-1, C-14	
NH-3	6.52 (1H,*d*, *J* = 10.4)		C-1, C-4	
CO-4		167.1		
5	4.13 (1H, *dd*, *J* = 8.5, 2.7)	58.7	C-4, C-7, C-17, C-18, C-20	
NH-6	ca 6.70 obscured signal		C-5, C-7	H-8 (small)
CO-7		170.9		
8	4.26 (1H, *d*, *J* = 5.2)	63.8	C-7, C-9, C-22, C-24	H-6 (small), H-9 (small)
9	5.43 (1H, br *dt*, *J* = 6.8, 5.2)	83.9	C-7	H-12, H-21β
11		157.4		
12	7.24 (1H, *m*)[a]	122.6[b]	C-11, C-14, C-16	H-9
13	7.15 (1H, *m*)	130.1	C-11, C-14, C-15	
14		132.5		
15	7.07 (1H, *m*)	132.4	C-1, C-11, C-12, C-13	
16	7.17 (1H, *m*)[a]	122.0[b]	C-11, C-12, C-14, C-15	
17	2.10 (1H, *m*)	35.4	C-4	
18	1.22 (1H, *m*)	23.8	C-5, C-17, C-19, C-20	
	1.08 (1H, *m*)			
19	0.80 (3H, *t*, *J* = 7.2)	12.2	C-17, C-18	
20	0.59 (3H, *d*, *J* = 6.9)	15.7	C-5, C-17, C-18	H-5, H-17
21α	1.96 (1H, *m*)	31.8	C-8, C-9, C-22	
21β	2.38 (1H, *m*)			H-9
22α	3.97 (1H, *m*)	45.9	C-8, C-9, C-21, C-24	H-25
22β	2.75 (1H, *ddd*, *J* = 12.6, 11.7, 4.9)			H-9
CO-24		171.7		
25	3.64 (1H, *t*, *J* = 6.4)	65.6	C-24, C-26, C-27, NMe_2	H-22α, H-28, H-34, NMe_2
26	3.13 (2H, br *d*, *J* = 6.5)	21.0	C-24, C-25, C-27, C-28, C-35	H-25, H-34
27		111.7		
28	6.74 (1H, *br s*)	122.4	C-27, C-30, C-35	
NH-29	8.14 (1H, *br s*)		C-27, C-30, C-35	
30		136.1		
31	7.31 (1H, *d*, *J* = 7.9)	111.3	C-33, C-35	
32	7.13 (1H, *m*)	122.8	C-30, C-31, C-34	
33	7.10 (1H, *m*)	119.3	C-31, C-35	
34	7.56 (1H, *d*, *J* = 7.6)	118.1	C-27, C-30, C-32	H-25
35		127.2		
NMe_2	2.48 (6H, *s*)	41.7	C-25	H-25

Signals without multiplicity were assigned from COSY.

[a,b] Interchangeable within a column.

Table 25 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-M2 (sss3346) with hemsine A (**125**) [85] in $CDCl_3$

position	δ_H (mult., *J* in Hz)		δ_C	
	hemsine A	compound ZM-M2	hemsine A	compound ZM-M2
1	6.29 (*d*, *J* = 7.8)	6.31 (1H, *d*, *J* = 7.6)	114.4	114.4
2	6.71 (*dd*, *J* = 10.2, 7.8)	6.71 (1H, *dd*, *J* = 10.4, 7.6)	125.5	125.4
NH-3	6.52 (*d*, *J* = 10.2)	6.52 (1H,*d*, *J* = 10.4)		
CO-4			166.9	167.1
5	4.13 (*dd*, *J* = 10.4, 8.7)	4.13 (1H, *dd*, *J* = 8.5, 2.7)	59.0	58.7
NH-6	6.55 (*d*, *J* = 8.7)	ca 6.70 obscured signal		
CO-7			170.3	170.9
8	4.24 (*d*, *J* = 5.6)	4.26 (1H, *d*, *J* = 5.2)	63.9	63.8
9	5.43 (*ddd*, *J* = 9.3, 7.1, 6.4)	5.43 (1H, br *dt*, *J* = 6.8, 5.2)	83.4	83.9
11			157.4	157.4
12	7.18 (*m*)	7.24 (1H, *m*)[a]	122.8	122.6[b]
13	7.07 (*m*)	7.15 (1H, *m*)	130.2	130.1
14			132.6	132.5
15	7.07 (*m*)	7.07 (1H, *m*)	132.5	132.4
16	7.18 (*m*)	7.17 (1H, *m*) [a]	122.7	122.0[b]
17	2.14 (*m*)	2.10 (1H, *m*)	35.3	35.4
18	1.25 (*m*)	1.22 (1H, *m*)	23.8	23.8
	1.08 (*m*)	1.08 (1H, *m*)		
19	0.78 (*t*, *J* = 7.4)	0.80 (3H, *t*, *J* = 7.2)	12.2	12.2
20	0.57 (*d*, *J* = 7.0)	0.59 (3H, *d*, *J* = 6.9)	16.0	15.7
21α	2.03 (*m*)	1.96 (1H, *m*)	31.9	31.8
21β	2.36 (*dt*, *J* = 9.4, 5.6)	2.38 (1H, *m*)		
22α	3.95 (*dd*, *J* = 10.5, 8.2)	3.97 (1H, *m*)	46.4	45.9
22β	2.73 (*ddd*, *J* = 12.1, 11.6, 5.4)	2.75 (1H, *ddd*, *J* = 12.6, 11.7, 4.9)		
CO-24			171.7	171.7
25	3.63 (*dd*, *J* = 7.5, 5.6)	3.64 (1H, *t*, *J* = 6.4)	65.6	65.6
26	3.11 (*m*)	3.13 (2H, br *d*, *J* = 6.5)	21.2	21.0
27			111.8	111.7
28	6.73 (*d*, *J* = 1.6)	6.74 (1H, *br s*)	122.1	122.4
NH-29	8.08 (br *s*)	8.14 (1H, *br s*)		
30			136.1	136.1
31	7.29 (br *d*, *J* = 7.2)	7.31 (1H, *d*, *J* = 7.9)	111.3	111.3
32	7.17 (*m*)	7.13 (1H, *m*)	122.4	122.8
33	7.09 (*m*)	7.10 (1H, *m*)	119.4	119.3
34	7.54 (br *d*, *J* = 7.8)	7.56 (1H, *d*, *J* = 7.6)	118.1	118.1
35			127.3	127.2
NMe_2	2.46 (*s*)	2.48 (6H, *s*)	41.8	41.7

Signals without multiplicity were assigned from COSY.

[a,b] Interchangeable within a column.

2.2.2 Structure determination of compound ZM-M3 (mauritine L, sss3170, sss3756)

Compound ZM-M3 was obtained as a colorless solid, mp 236-237 °C and showed a pseudomolecular ion at *m/z* 521.3124 $[M+H]^+$ in the HRTOFMS spectrum, which corresponded to a molecular formula of $C_{30}H_{40}N_4O_4$. Compound ZM-M3 gave a blue coloration with anisaldehyde-H_2SO_4.[29] The IR spectrum exhibited absorption bands for amino (3291 cm^{-1}), amide (1679 cm^{-1}), styryl double bond (1633 cm^{-1}) and phenol ether (1237 cm^{-1}) functionalities. The end absorption in its UV spectrum showed the characteristic of 14-membered cyclopeptide alkaloids.[19]

The ^{13}C NMR (Figure 37) and DEPT spectra displayed 30 carbon signals including one *N*-methyl, four methyls, two methylenes, 17 methines and six quaternary carbons, three of which corresponded to the carbonyl groups. Analysis of ^1H, ^{13}C NMR, DEPT, COSY, HMQC and HMBC spectra and comparison with the literature reported value[85,96] led to the assignments for a *para*-oxygenated styrylamine unit [δ_H 6.36 (*d, J* = 7.3 Hz, H-1), 6.71 (*dd, J* = 9.7, 7.3 Hz, H-2), 6.57 (*d, J* = 9.7 Hz, NH-3), 7.34 (*m*, H-12), 7.12 (br *t*, H-13), 7.12 (br *t*, H-15) and 7.34 (*m*, H-16)] in addition to three amino acid units of isoleucine unit [δ_H 4.03 (*dd*, H-5), 2.15 (*m*, H-17), 1.61 and 0.95 (*m*, H-18), 0.81 (*t,* H-19) and 0.66 (*d*, H-20); δ_C 59.6, 35.0, 24.0, 12.1 and 16.0], β-phenylserine moiety [δ_H 4.64 (*dd*, H-8), 6.17 (*d*, H-9), ca 7.38-7.50 (aromatic protons); δ_C 56.4, 81.4, 137.2, 127.5, 128.9 and 128.7], and *N*-methyl isoleucine [δ_H 2.52 (*d*, H-27), 1.36 (*m*, H-28), 0.51 and 0.75 (*m*, H-29), 0.65 (*t,* H-30), 0.62 (*d*, H-31) and 2.05 (3H, *s*, NMe); δ_C 69.6, 37.5, 23.7, 11.7, 15.7 and 36.5].

An upfield shift observed in NH-3 proton (δ_H 6.57) of the 14-membered ring compound ZM-M3, when compared with the δ_H 8.42 ppm for NH-3 of the 13-membered ring compound ZM-M1 (nummularine H) could be due to an anisotropic effect of the aromatic ring in the cyclic system. An anisotropic effect observed for H-9 (δ_H 6.17) in compound ZM-M3, compared with corresponding H-9 (δ_H 5.43) in compound ZM-M2 (hemsine A) also indicated the presence of phenylserine amino acid.

Connections among these subgroups were provided by analysis of its HMBC and NOESY spectra (Table 26). The HMBC correlations were observed for the aromatic proton H-13 and H-15 to the oxygenated sp^2 quaternary carbon at δ_C 155.1 (C-11); the aromatic protons [δ_H 7.34 (*m*, H-12), 7.12 (br *t*, H-13), 7.12 (br *t*, H-15) and 7.34 (*m*, H-16)] and styryl proton H-2 to δ_C 132.2 (C-14) confirming a 14-membered cyclopeptide alkaloid in the macrocyclic ring system.[95] The styrylamine proton at δ_H 6.57 (NH-3) showed HMBC cross peaks with carbonyl C-4 (δ_C 167.1), in addition, a small NOE interactions between the NH-3 and isoleucyl proton H-5 (δ_H 4.03) displayed in the NOESY spectrum suggesting that isoleucine amino acid was attached to the styrylamine unit. The HMBC correlations of proton at δ_H 6.17 (H-9) showed cross peak with carbonyl C-7 (δ_C 171.5), though the absence of HMBC coupling between NH-6 and CO-7, however H-8 showed NOE correlation with NH-6 in NOESY spectrum confirming isoleucine unit connect to phenylserine amino acid. The proton at δ_H 4.64 (H-8) displayed NOE interactions with NH-25 (δ_H 7.43) in the NOESY spectrum, but no HMBC interaction observed between both amino acids. The latter proton NH-25 showed NOE cross peaks to the *N*-methyl isoleucyl proton at δ_H 2.52 (H-27) confirming the connection between the β-phenylserine of the macro molecule and the *N*-methyl isoleucine, the terminal amino acid.

The complete ^1H and ^{13}C NMR data (Table 26) of compound ZM-M3 were assigned as a 4(14)-Integerrine type cyclopeptide, named mauritine L (**126**), by the analysis of DEPT, COSY, HMQC and HMBC experiments, together with comparison spectroscopic data (Table 27) with the known cyclopeptide alkaloids, scutianine M (**127**)[96] and hemsine D (**128**).[85]

The relative stereochemistry of compound ZM-M3 was determined by analysis of ^1H NMR coupling constants and NOSEY interactions, as follow: the H-9 proton showed cross-peaks with H-8 (weak), H-22 and H-22' (strong); the H-8 proton exhibited cross-peaks with NH-6 (strong), H-9 (weak) and NH-25 (strong), H-5 proton showed cross-peaks with NH-3 (weak), NH-6 (weak) and H-17 (strong), and the NH-3 proton showed a cross-peaks with the H-5 (weak). ^1H and ^{13}C NMR data of ZM-M3 were similar to those of hemsine D (**128**),[85] a structure related alkaloid with the presence of the *N*-methyl valine instead of an *N*-methyl isoleucine as terminal

amino acid in ZM-M3. The CD data of hemsine D (**128**) suggested all amino acid residues to be L together with compound ZM-M3 showed the levorotatory optical rotation, $[\alpha]_D^{27}$ -56.3 (c 0.31, MeOH), similar to that of hemsine D, $[\alpha]_D^{26}$ -573.3 (c 0.75, $CHCl_3$),[85] thus the stereostructure of compound ZM-M3 should be the same, as shown in Figure 18.

mauritine L (**126**)

scutianine M (**127**)

hemsine D (**128**)

Figure 17 Selected HMBC correlations for compound ZM-M3 (mauritine L)

Figure 18 Key NOESY correlations for compound ZM-M3 (mauritine L)

Table 26 ^{1}H, ^{13}C NMR and 2D NMR data of compound ZM-M3 (sss3756) in $CDCl_3$

position	δ_H (mult., *J* in Hz)	δ_C	HMBC correlations	NOESY correlations
1	6.36 (1H, *d*, *J* = 7.3)	115.5	C-2	H-2 (small)
2	6.71 (1H, *dd*, *J* = 9.7, 7.3)	125.5	C-14	H-1 (small)
NH-3	6.57 (1H,*d*, *J* = 9.7)		C-4	H-5 (small)
CO-4		167.1		
5	4.03 (1H, *dd*, *J* = 7.8, 2.8)	59.6		NH-3 (small)
NH-6	6.42 (1H, *d*, *J* = 7.8)			H-6 (small), H-17, H-8
CO-7		171.5		
8	4.64 (1H, *dd*, *J* = 8.4, 6.3)	56.4	C-9	NH-6, H-9 (small), NH-25
9	6.17 (1H, *d*, *J* = 6.3)	81.4	C-7, C-21, C-22, C-22'	
11		155.1		
12	7.34 (*m*)	123.5	C-14, C-15	
13	7.12 (1H, br *t*, *J* ca 7.8)	130.1[a]	C-11, C-14, C-15	
14		132.2		
15	7.12 (1H, br *t*, *J* ca 7.8)	132.2[a]	C-11, C-13, C-14	
16	7.34 (*m*)	123.6	C-14, C-15	
17	2.15 (1H, *m*)	35.0		H-5
18	1.61 (1H, *m*) and 0.95 (1H, *m*)	24.0		
19	0.81 (3H, *t*, *J* = 7.2)	12.1	C-17, C-18	
20	0.66 (3H, *d*, *J* = 6.4)	16.0	C-5, C-17, C-18	
21		137.2		
22, 22'	7.50 (2H, br *d*, *J* ca 7.4)	127.5	C-23, C-23', C-24	
23, 23'	7.40 (*m*)	128.9	C-21, C-24	
24	7.40 (*m*)	128.7	C-21, C-23, C-23'	
NH-25	ca 7.43			H-8, H-27
CO-26		173.5		
27	2.52 (3H, *d*, *J* = 4.1)	69.6	C-26, C-28, C-29	H-28, NMe
28	1.36 (1H, *m*)	37.5		
29	0.75 (1H, *m*) and 0.51 (1H, *m*)	23.7		
30	0.65 (3H, *t*, *J* = 6.4)	11.7	C-28, C-29	
31	0.62 (3H, *d*, *J* = 6.8)	15.7	C-27, C-28, C-29	
NMe	2.05 (3H, *s*)	36.5	C-27	H-27

Signal without multiplicity were assigned from COSY.

[a] Interchangeable within a column.

Table 27 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-M3 (sss3756) with scutianine M (**127**)[96]

position	δ_H (mult., *J* in Hz)		δ_C	
	scutianine M (DMSO-d_6)	compound ZM-M3 ($CDCl_3$)	scutianine M (DMSO-d_6)	compound ZM-M3 ($CDCl_3$)
1	6.41 (*d*, *J* = 7.0)	6.36 (1H, *d*, *J* = 7.3)	116.20	115.5
2	6.72 (*dd*, *J* = 10.0, 7.0)	6.71 (1H, *dd*, *J* = 9.7, 7.3)	125.54	125.5
NH-3	6.60 (*d*, *J* = 10.0)	6.57 (1H,*d*, *J* = 9.7)		
CO-4			167.08	167.1
5	4.08 (*dd*, *J* = 8.2, 3.8)	4.03 (1H, *dd*, *J* = 7.8, 2.8)	59.52	59.6
NH-6	6.36 (*d*, *J* = 8.2)	6.42 (1H, *d*, *J* = 7.8)		
CO-7			171.39	171.5
8	4.70 (*dd*, *J* = 9.0, 7.7)	4.64 (1H, *dd*, *J* = 8.4, 6.3)	56.39	56.4
9	6.16 (*d*, *J* = 7.7)	6.17 (1H, *d*, *J* = 6.3)	81.40	81.4
11			155.21	155.1
12	7.00-7.60	7.34 (*m*)		123.5
13	7.00-7.60	7.12 (1H, br *t*, *J* ca 7.8)		130.1 [a]
14				132.2
15	7.00-7.60	7.12 (1H, br *t*, *J* ca 7.8)		132.2 [a]
16	7.00-7.60	7.34 (*m*)		123.6
17	2.18 (1H, *m*)	2.15 (1H, *m*)	35.03	35.0
18	1.24 (*m*) and 1.00 (*m*)	1.61 (1H, *m*) and 0.95 (1H, *m*)	23.80	24.0
19	0.83 (*t*, *J* = 7.0)	0.81 (3H, *t*, *J* = 7.2)	15.24	12.1
20	0.70 (*d*, *J* = 7.0)	0.66 (3H, *d*, *J* = 6.4)	12.02	16.0
21	7.00-7.60			137.2
22, 22'	7.00-7.60	7.50 (2H, br *d*, *J* ca 7.4)		127.5
23, 23'	7.00-7.60	7.40 (*m*)		128.9
24	7.00-7.60	7.40 (*m*)		128.7
NH-25	7.40	ca 7.43		
CO-26			173.24	173.5
27	2.90 (*dd*, *J* = 9.4, 3.6)	2.52 (3H, *d*, *J* = 4.1)	65.82	69.6
28	2.78 (*dd*, *J* = 9.4, 14.0)	1.36 (1H, *m*)	37.96	37.5
	1.58 (*dd*, *J* = 3.6, 14.0)			
29, 29'		0.75 (1H, *m*) and 0.51 (1H, *m*)		23.7
30	7.00-7.60	0.65 (3H, *t*, *J* = 6.4)		11.7
31	7.00-7.60	0.62 (3H, *d*, *J* = 6.8)		15.7
32	7.00-7.60			
NMe	1.90 (*s*)	2.05 (3H, *s*)	35.28	36.5

Signal without multiplicity were assigned from COSY.

[a] Interchangeable within a column.

Table 28 Comparison of ^{1}H and ^{13}C NMR data of compound ZM-M3 (sss3756) with hemsine D (**128**) [85]

position	δ_H (mult., *J* in Hz)		δ_C	
	hemsine D (pyridine-d_5)	compound ZM-M3 ($CDCl_3$)	hemsine D (pyridine-d_5)	compound ZM-M3 ($CDCl_3$)
1	6.84 (*d*, *J* = 7.3)	6.36 (1H, *d*, *J* = 7.3)	129.4	115.5
2	6.56 (*dd*, *J* = 7.3, 3.0)	6.71 (1H, *dd*, *J* = 9.7, 7.3)	127.6	125.5
NH-3	9.24 (*d*, *J* = 3.0)	6.57 (1H,*d*, *J* = 9.7)		
CO-4			170.2	167.1
5	4.64 (*t like*, *J* = 9.0)	4.03 (1H, *dd*, *J* = 7.8, 2.8)	58.5	59.6
NH-6	9.29 (*d*, *J* = 9.0)	6.42 (1H, *d*, *J* = 7.8)		
CO-7			172.0	171.5
8	5.67 (*dd*, *J* = 9.7, 8.7)	4.64 (1H, *dd*, *J* = 8.4, 6.3)	57.5	56.4
9	6.43 (*d*, *J* = 8.7)	6.17 (1H, *d*, *J* = 6.3)	81.9	81.4
11			156.2	155.1
12	7.58 (*m*)	7.34 (*m*)	118.3	123.5
13	7.18 (*m*)	7.12 (1H, br *t*, *J* ca 7.8)	131.0	130.1[a]
14			132.2	132.2
15	7.30 (*m*)	7.12 (1H, br *t*, *J* ca 7.8)	130.3	132.2 [a]
16	7.03 (*dd*, *J* = 8.2, 2.0)	7.34 (*m*)	121.4	123.6
17	2.00 (1H, *m*)	2.15 (1H, *m*)	36.9	35.0
18	1.58 (*m*) and 1.17 (*m*)	1.61 (1H, *m*) and 0.95 (1H, *m*)	25.0	24.0
19	0.70 (*t*, *J* = 7.4)	0.81 (3H, *t*, *J* = 7.2)	11.0	12.1
20	0.91 (*d*, *J* = 6.7)	0.66 (3H, *d*, *J* = 6.4)	15.8	16.0
21			139.3	137.2
22, 22'	7.72 (br *d*, *J* = 7.3)	7.50 (2H, br *d*, *J* ca 7.4)	129.2	127.5
23, 23'	7.37 (*t like*, *J* = 7.3)	7.40 (*m*)	128.5	128.9
24	7.30 (*m*)	7.40 (*m*)	128.6	128.7
NH-25	8.63 (*d*, *J* = 9.7)	ca 7.43		
CO-26			172.8	173.5
27	2.73 (*d*, *J* = 5.2)	2.52 (3H, *d*, *J* = 4.1)	70.7	69.6
28	1.80 (*m*)	1.36 (1H, *m*)	31.9	37.5
29	0.76 (*d*, *J* = 6.8)	0.75 (1H, *m*) and 0.51 (1H, *m*)	19.9	23.7
30	0.71 (*d*, *J* = 6.8)	0.65 (3H, *t*, *J* = 6.4)	18.1	11.7
31		0.62 (3H, *d*, *J* = 6.8)		15.7
NMe	2.07 (*s*)	2.05 (3H, *s*)	35.7	36.5

Signal without multiplicity were assigned from COSY.

[a] Interchangeable within a column.

3. Antiplasmodial and antimycobacterial activities of the isolated compounds from *Z. mauritiana*

The isolated triterpenoids were tested *in vitro* for antiplasmodial activity against *P. falciparum* and antimycobacterial activity against *M. tuberculosis*. Only zizyberenalic acid (**72**), a ceanothane-type triterpene, gave positive result to the antiplasmodial assay with the IC_{50} value of 3.0 μg/mL. Betulinic acid (**56**), zizyberenalic acid (**72**) and 24-hydroxyceanothic acid (**119**) exhibited antimycobacterial activities against *M. tuberculosis* with the MIC values of 25, 100 and 25 μg/mL, respectively.

Double bond in ring A of zizyberenalic acid (**72**) and hydroxyl group of 24-hydroxyceanothic acid (**119**) which belong to the ceanothane-type triterpene highly increased the inhibitory activity in the antimycobacterial assay as compared to ceanothic acid (**67**).[17]

Table 29 IC_{50} Values for antiplasmodial and MIC values for antimycobacterial activities of triterpenes from *Z. mauritiana*

Compound	IC_{50} (μg/mL)	MIC (μg/mL)
ZM-E1 (lupeol) [a]	inactive [b]	inactive [c]
ZM-E2 (betulin) [a]	NT	NT
ZM-E3 (betulinic acid) [a]	inactive [b]	25
ZM-E4 (zizyberenalic acid) [a]	3.0	100
ZM-E5 (ceanothic acid) [a]	inactive [b]	inactive [c]
ZM-E6 (epiceanothic acid)	inactive [b]	inactive [c]
ZM-E7 (24-hydroxyceanothic acid)	inactive [b]	25

[a] previously reported by our group[17]

[b] inactive at IC_{50} 10 μg/mL

[c] inactive at MIC $>$ 200 μg/mL

NT = not tested

CHAPTER 5

CONCLUSION

Investigation of the chemical constituents of the root of *Z. mauritiana* led to the isolation of two new cyclopeptide alkaloids, mauritian L (**126**) as the 4(14)-Integerrine type and mauritian M (**121**) as the 5(13)-Zizyphine-A type together with three known cyclopeptide alkaloids, nummularine H (**123**), hemsine A (**125**) and nummularine B (**38**). In addition, seven known triterpenes; three lupane-type, lupeol (**57**), betulin (**58**) and betulinic acid (**56**), and four ceanothane-type, zizyberenalic acid (**72**), ceanothic acid (**67**), epiceanothic acid (**116**) and 24-hydroxyceanothic acid (**119**) were also isolated from EtOAc extract of *Z. mauritiana*. The structures of two new cyclopeptide alkaloids were elucidated by spectroscopic techniques, whilst the known compounds were identified by comparisons of spectroscopic data with those of reported values and chromatographic comparison with an authentic sample in several solvent systems.

The biological activity testings of triterpenoids from the root of *Z. mauritiana* showed that zizyberenalic acid (**72**) containing double bond in ring A showed the most active antiplasmodial activity against *P. falciparum*, whilst ceanothic acid (**67**) and its isomer, epiceanothic acid (**116**) were found inactive in the same test. Moreover, betulinic acid (**56**), zizyberenalic acid (**72**) and 24-hydroxyceanothic acid (**119**) exhibited the antimycobacterial activities against *M. tuberculosis*.

betulinic acid (**56**) ceanothic acid (**67**) zizyberenalic acid (**72**) 24-hydroxyceanothic acid (**119**)

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APPENDIX

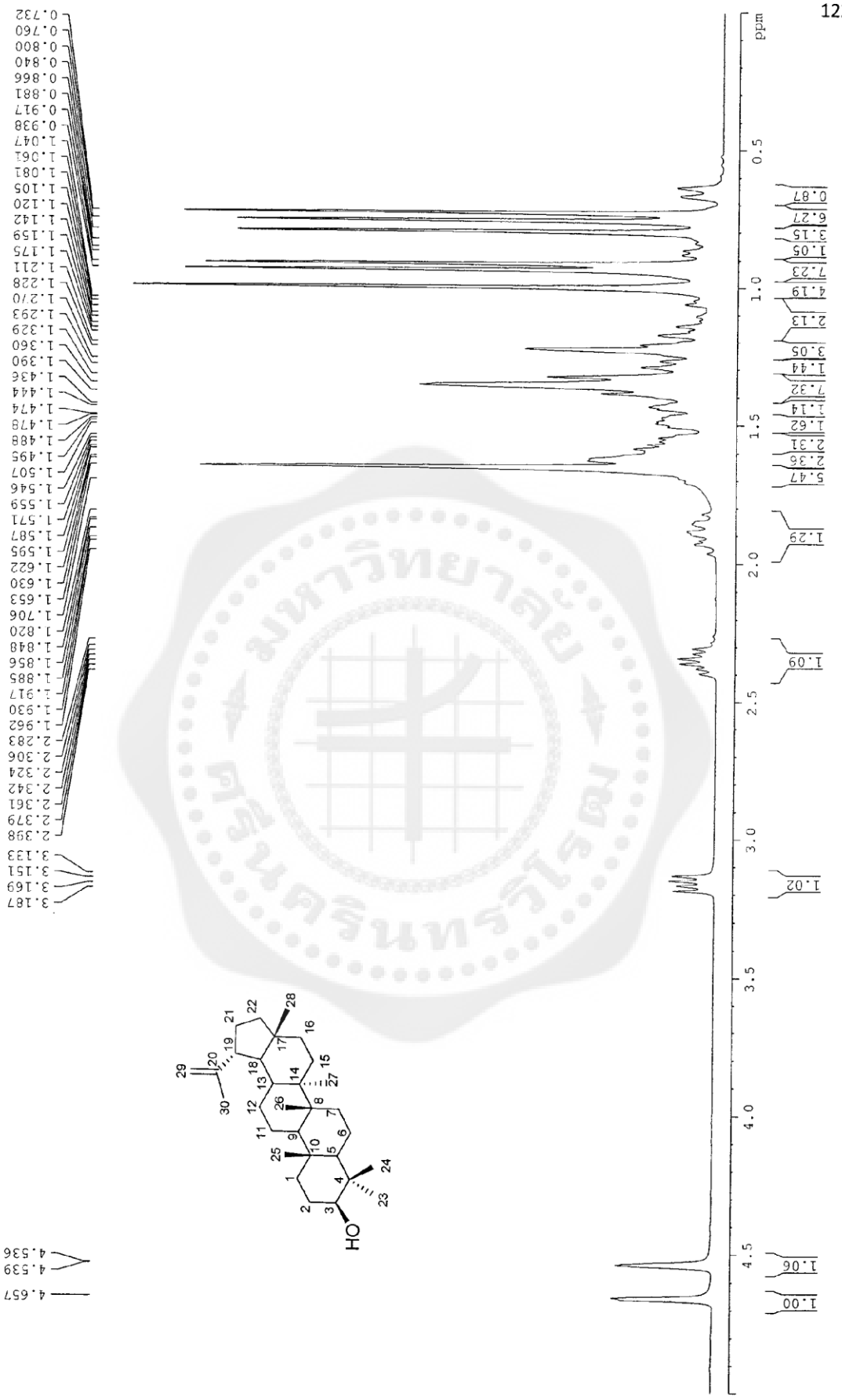

Figure 19 ^{1}H NMR spectrum of compound ZM-E1 (lupeol, sss3921) in $CDCl_3$

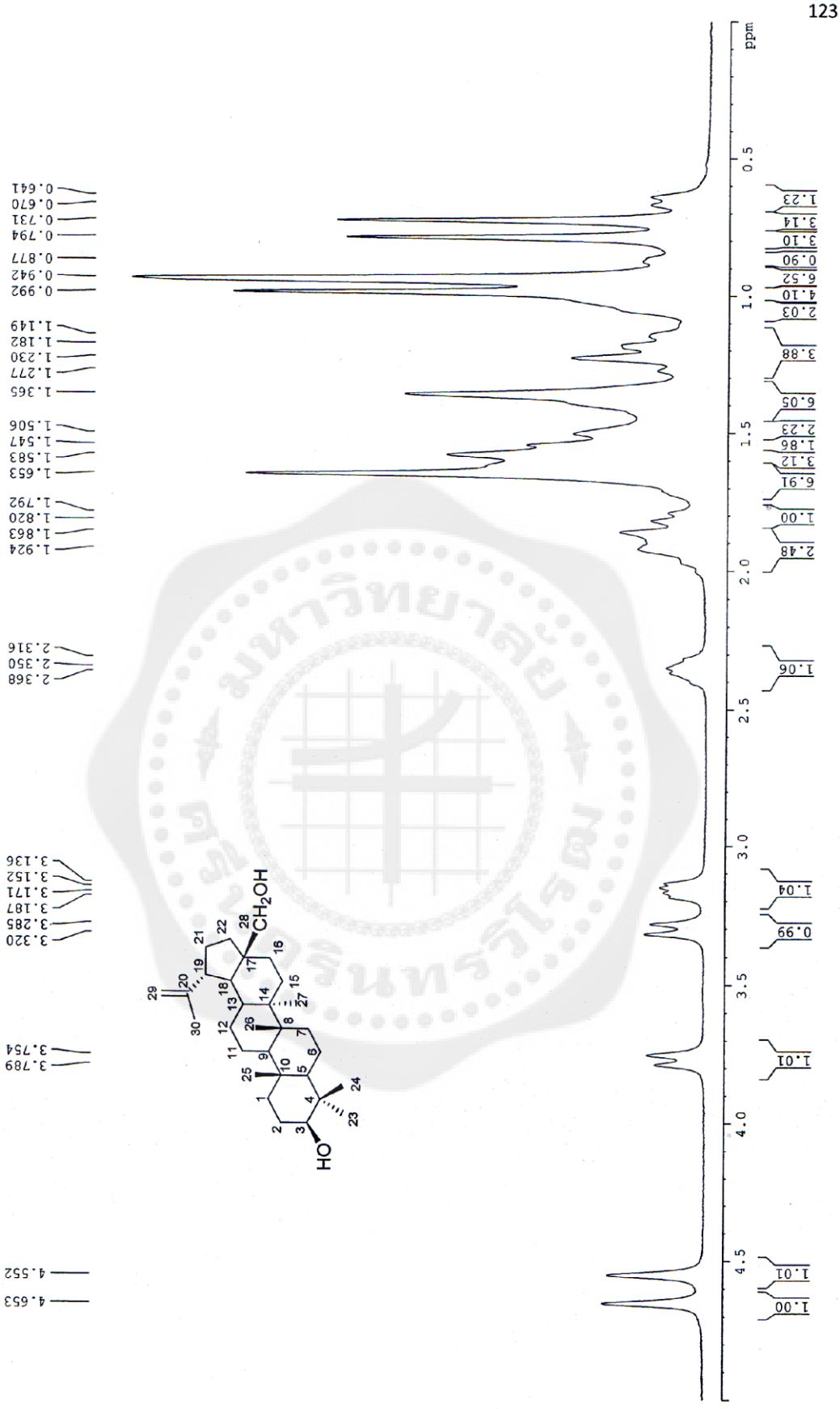

Figure 20 ^{1}H NMR spectrum of compound ZM-E2 (betulin, sss3828) in $CDCl_3$

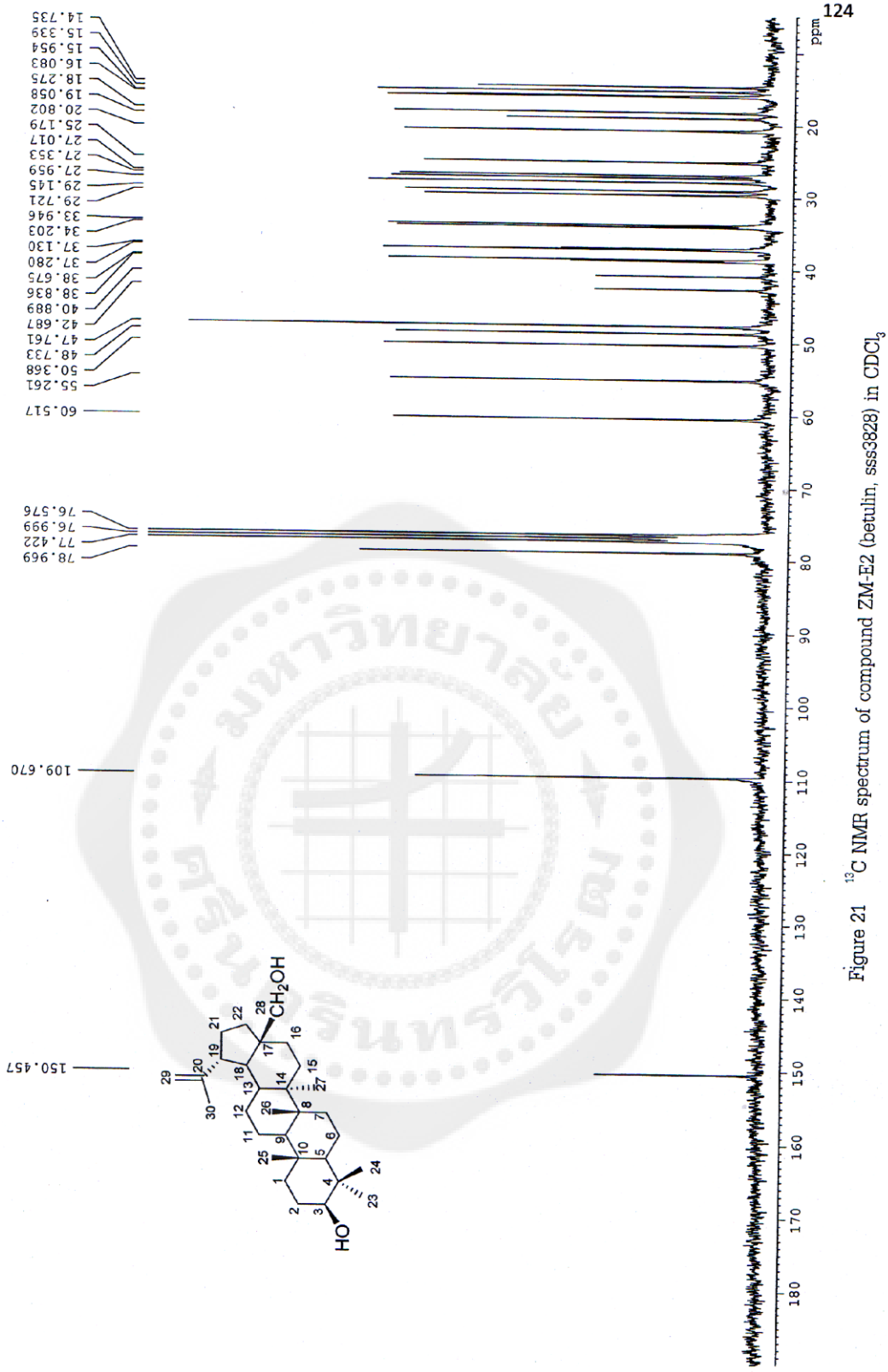

Figure 21 ^{13}C NMR spectrum of compound ZM-E2 (betulin, sss3828) in $CDCl_3$

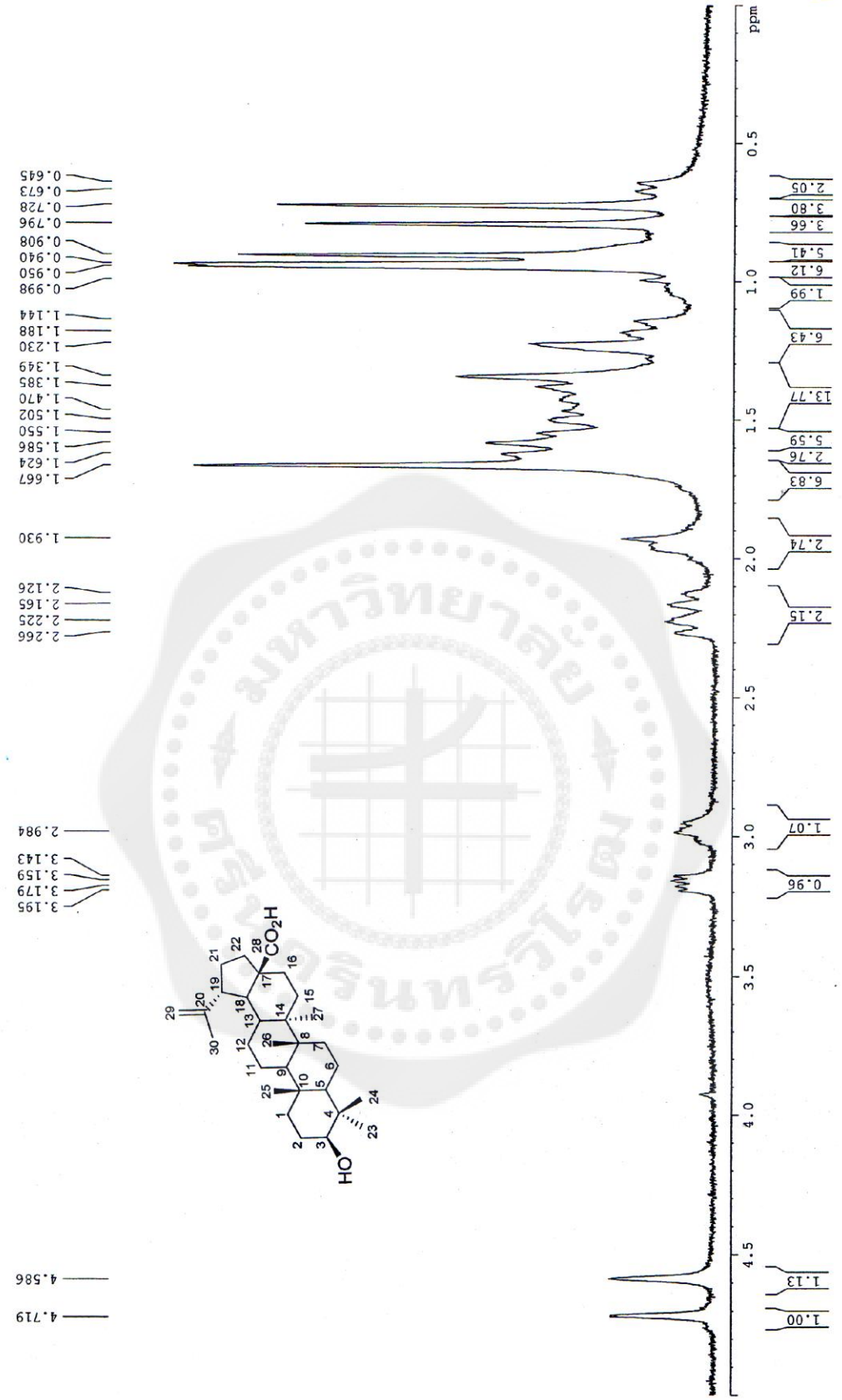

Figure 22 ^{1}H NMR spectrum of compound ZM-E3 (betulinic acid, sss3679) in $CDCl_3$

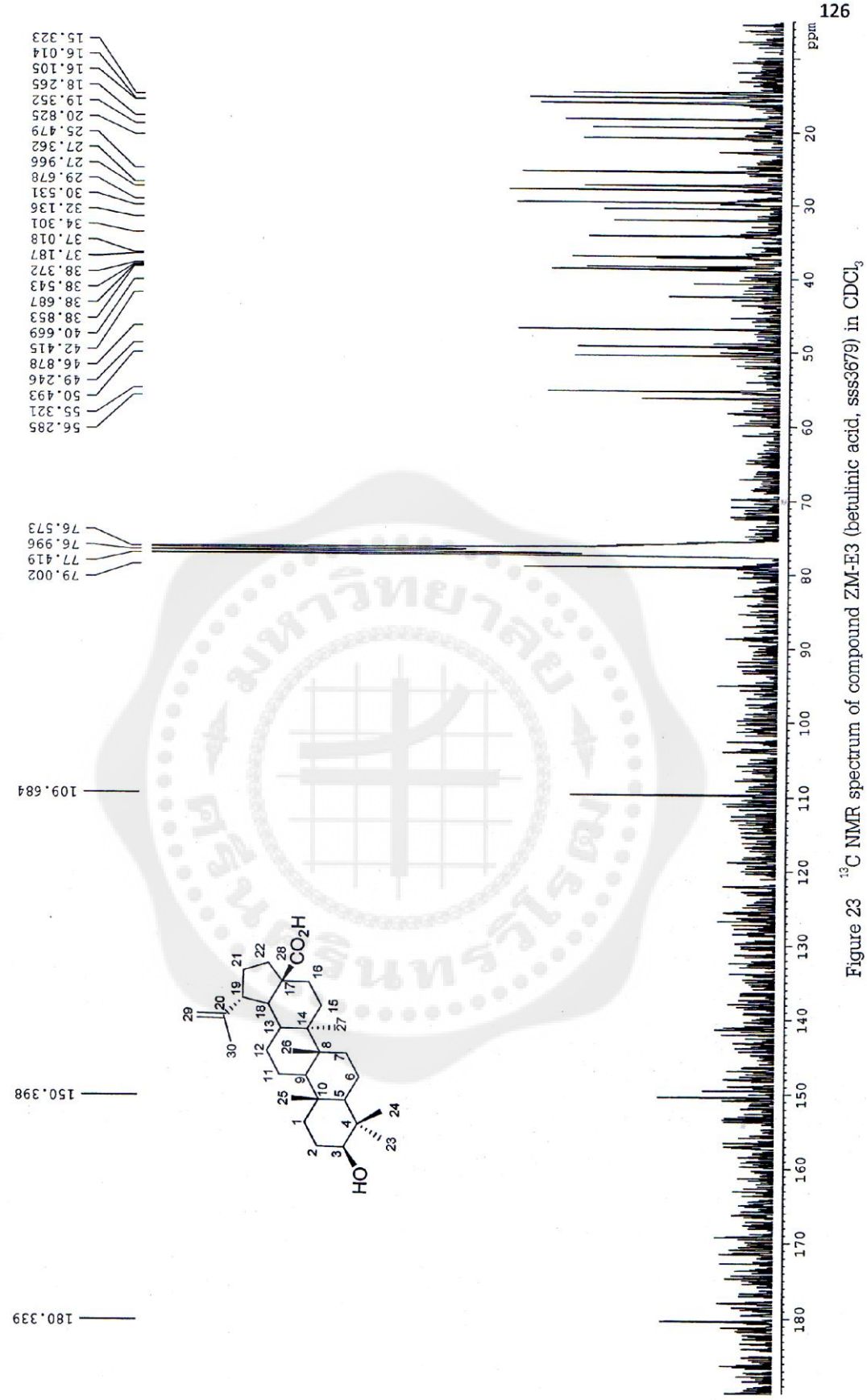

Figure 23 ^{13}C NMR spectrum of compound ZM-E3 (betulinic acid, sss3679) in $CDCl_3$

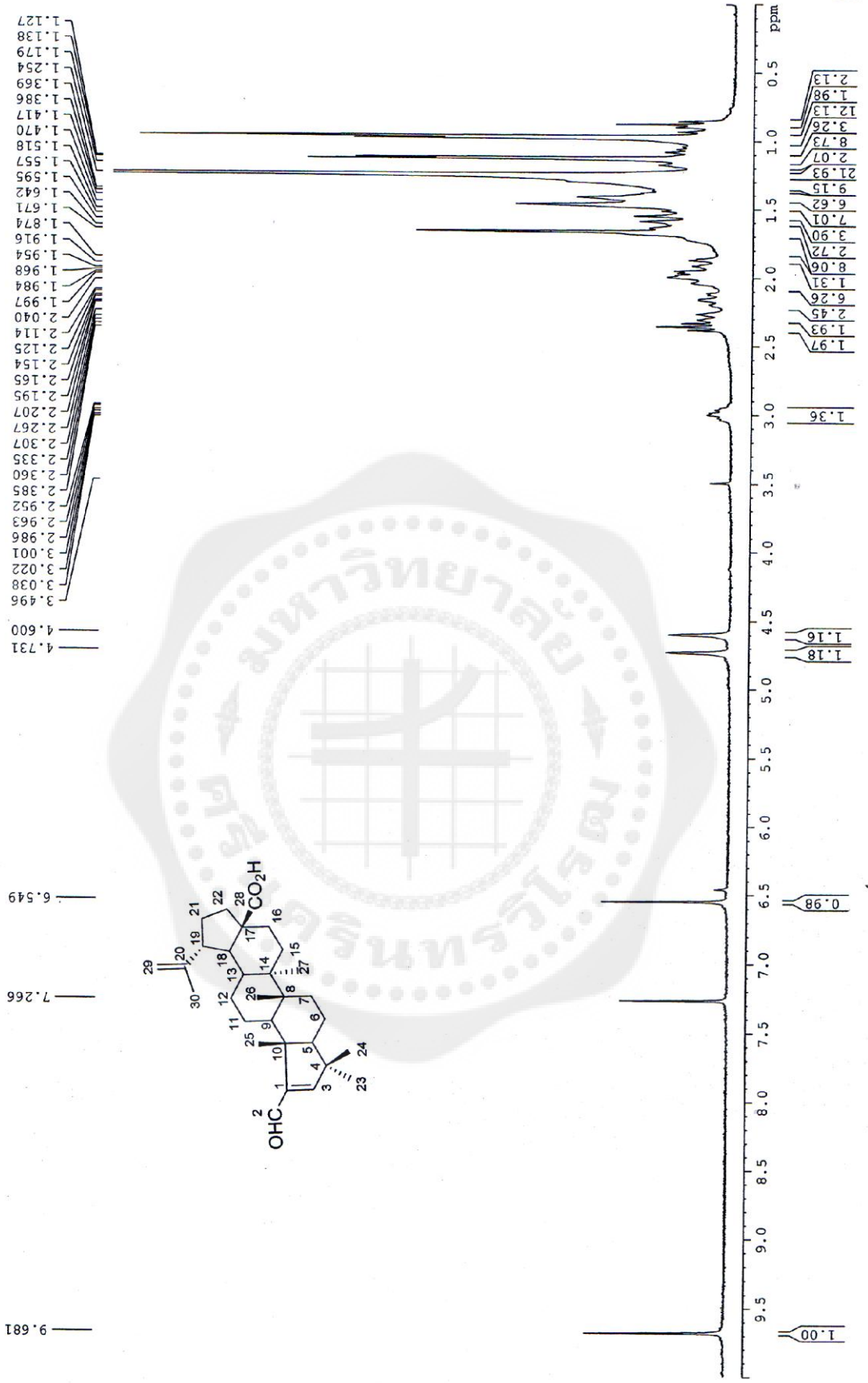

Figure 24 ^{1}H NMR spectrum of compound ZM-E4 (zizyberenalic acid, sss3812) in $CDCl_3$

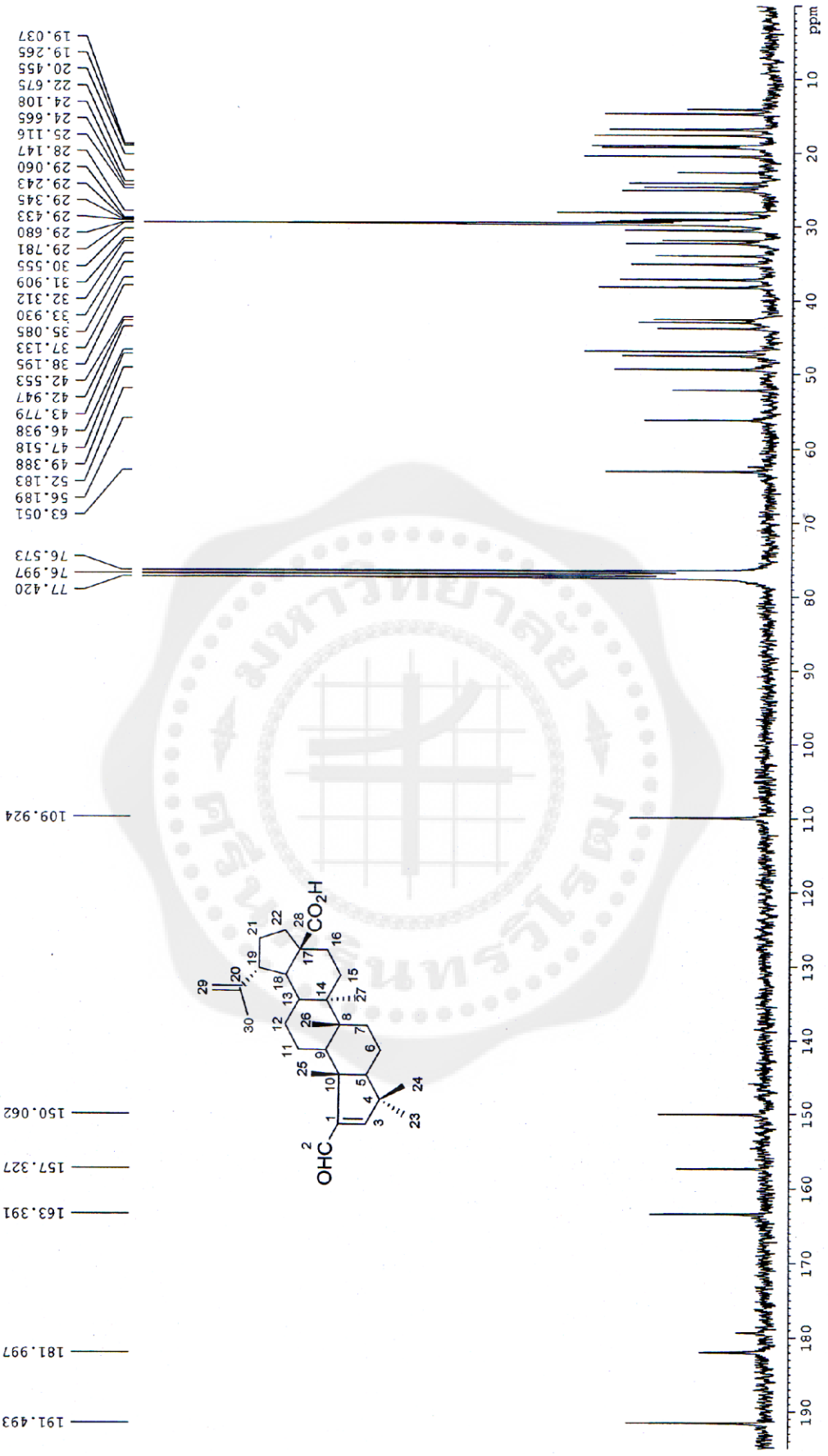

Figure 25 ^{13}C NMR spectrum of compound ZM-E4 (zizyberenalic acid, sss3812) in $CDCl_3$

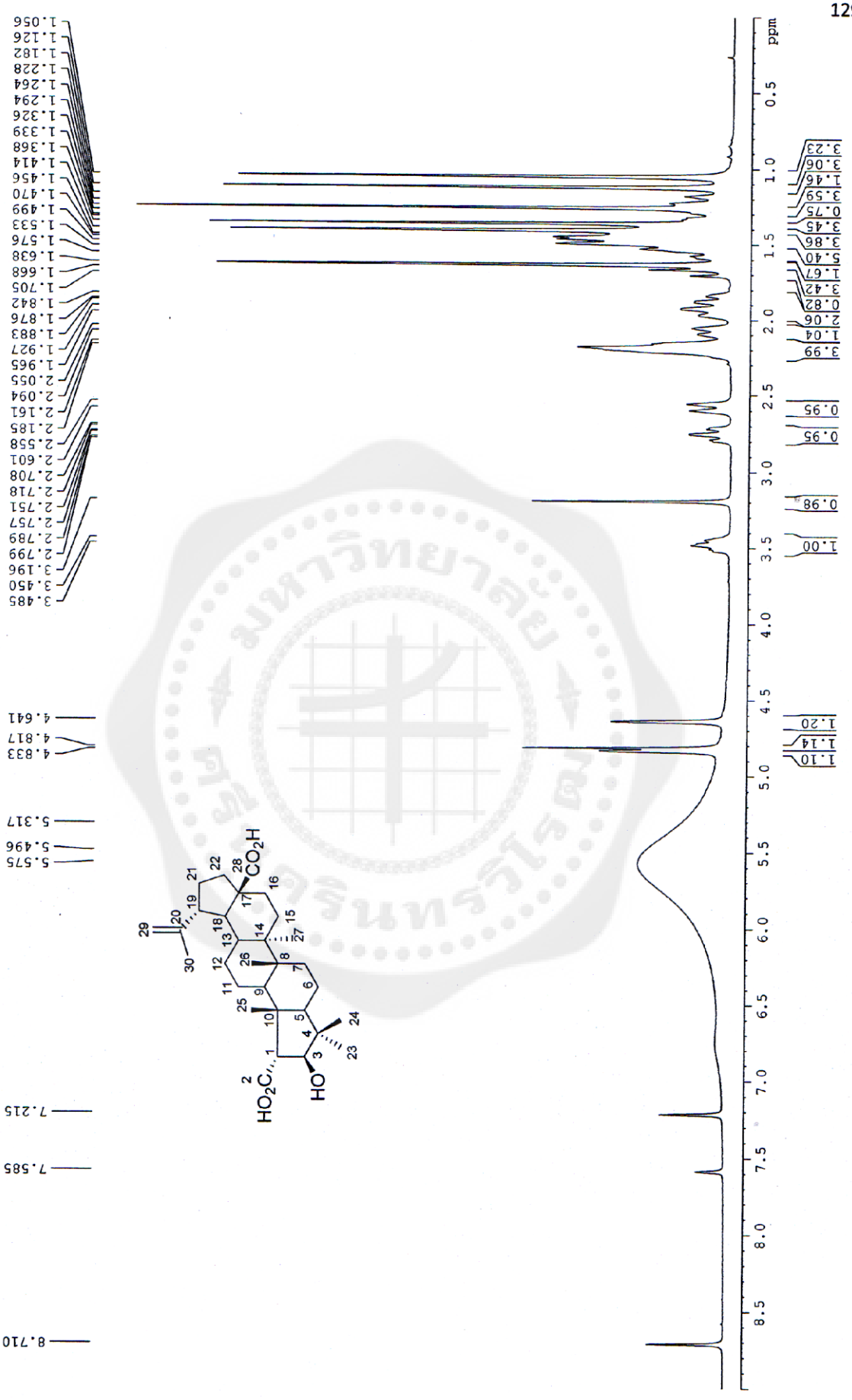

Figure 26 ^{1}H NMR spectrum of compound ZM-E5 (ceanothic acid, sss3193) in pyridine-d_5

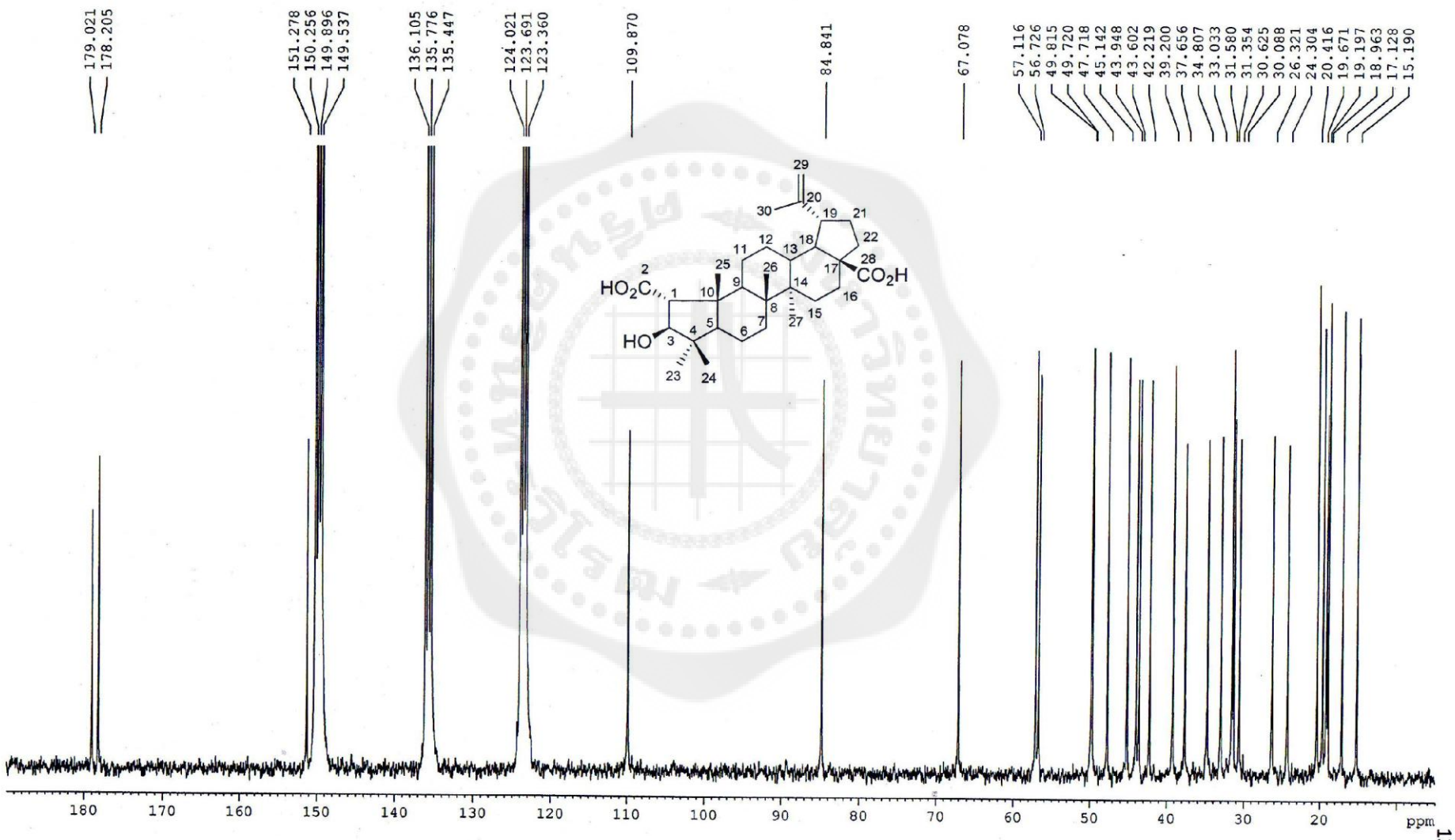

Figure 27 ^{13}C NMR spectrum of compound ZM-E5 (ceanothic acid, sss3193) in pyridine-d_5

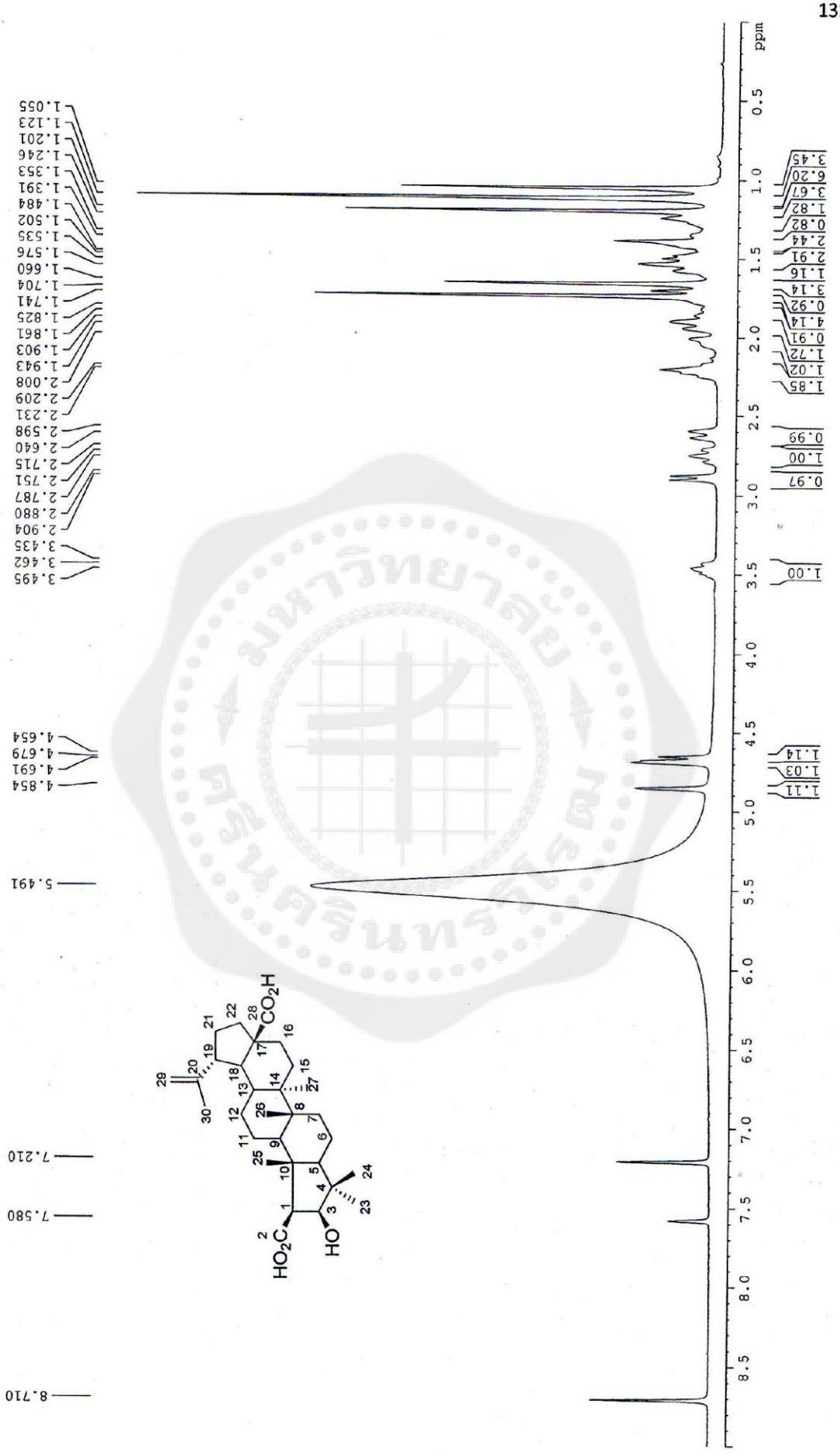

Figure 28 ^{1}H NMR spectrum of compound ZM-E6 (epiceanothic acid, sss3340) in pyridine-d_5

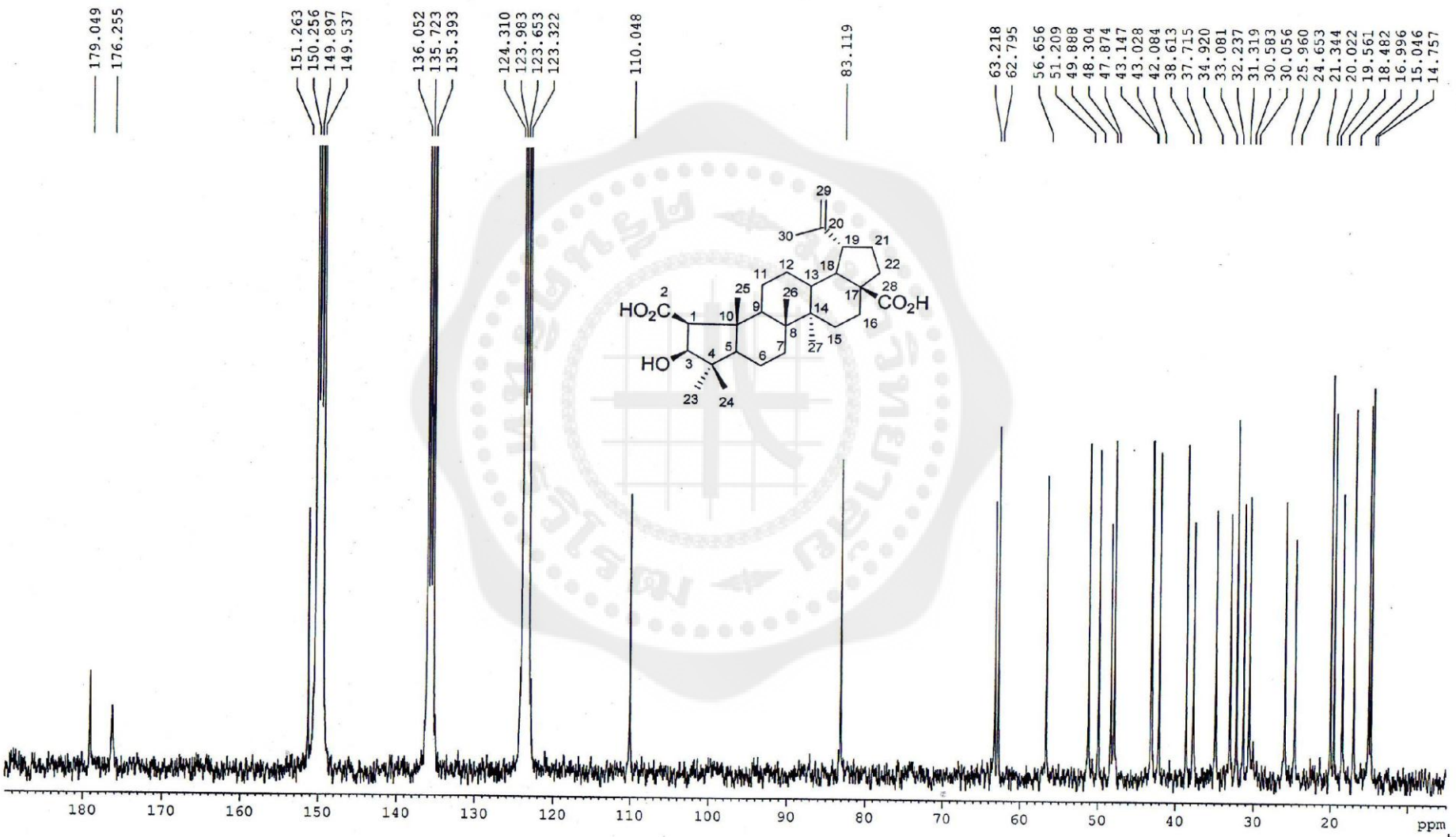

Figure 29 ^{13}C NMR spectrum of compound ZM-E6 (epiceanothic acid, sss3340) in pyridine-d_5

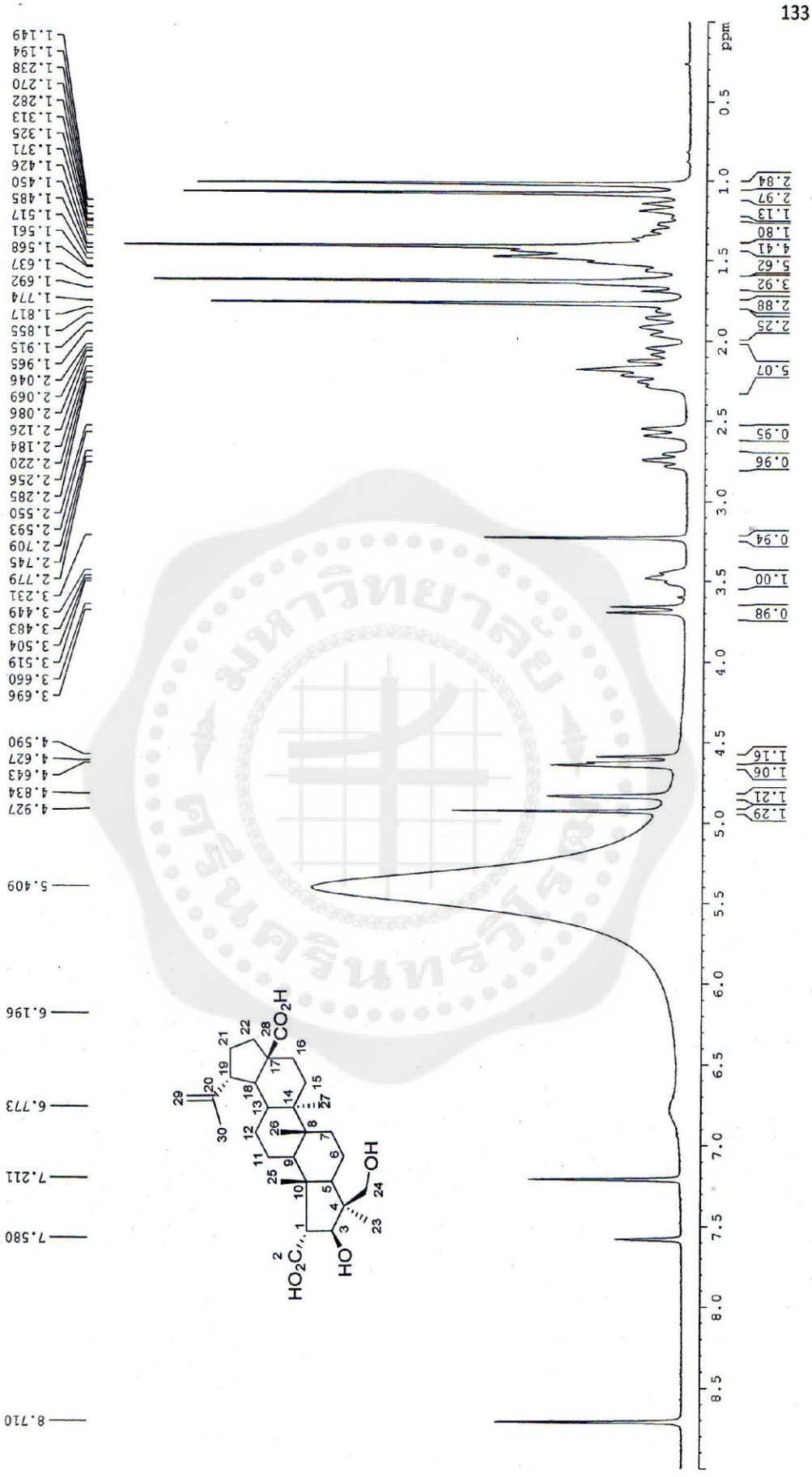

Figure 30 ^{1}H NMR spectrum of compound ZM-E7 (24-hydroxyceanothic acid, sss3336) in pyridine-d_5

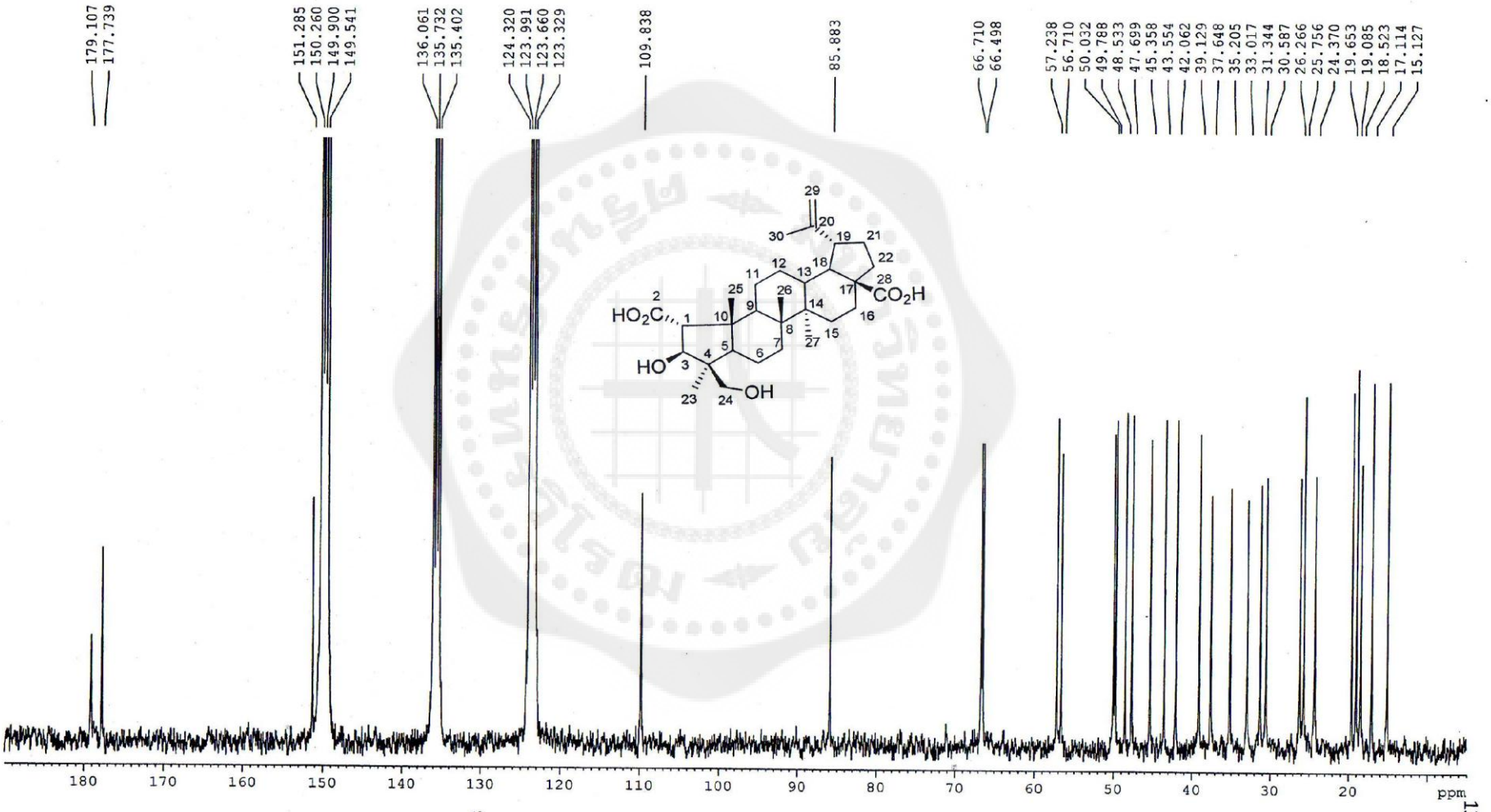

Figure 31 ^{13}C NMR spectrum of compound ZM-E7 (24-hydroxyceanothic acid, sss3336) in pyridine-d_5

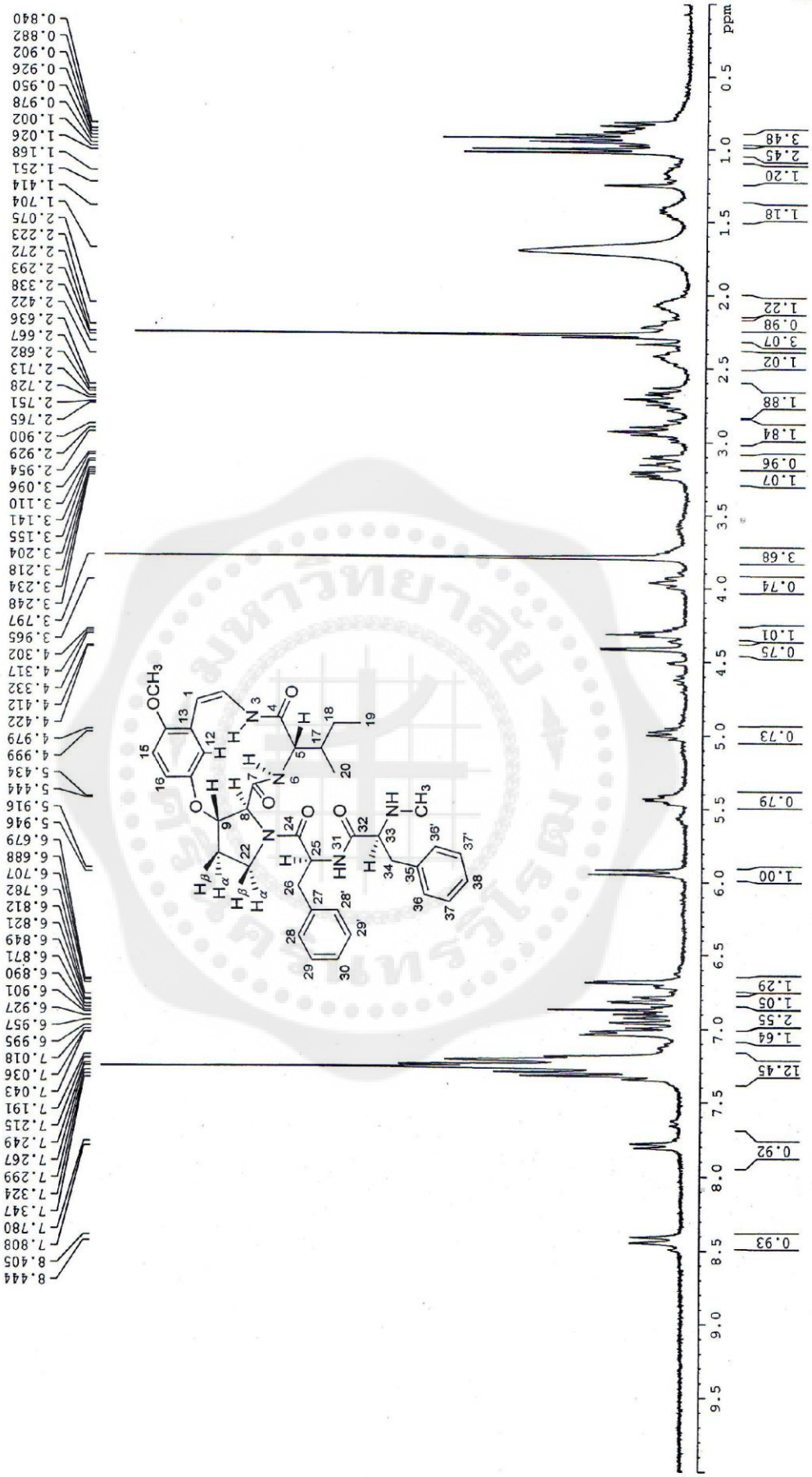

Figure 32 ^{1}H NMR spectrum of compound ZM-M1 (nummularine H, sss3648) in $CDCl_3$

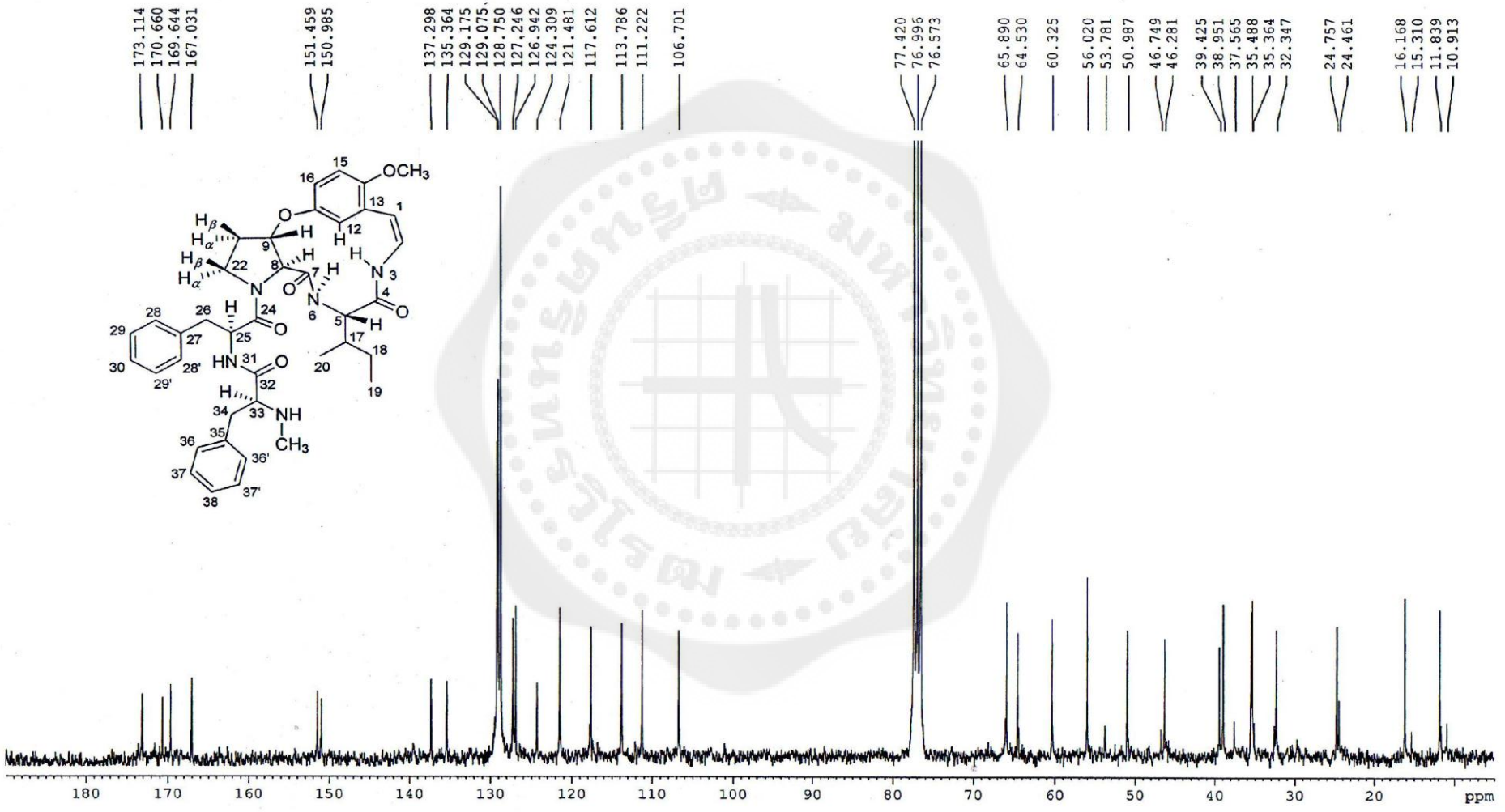

Figure 33 ^{13}C NMR spectrum of compound ZM-M1 (nummularine H, sss3648) in $CDCl_3$

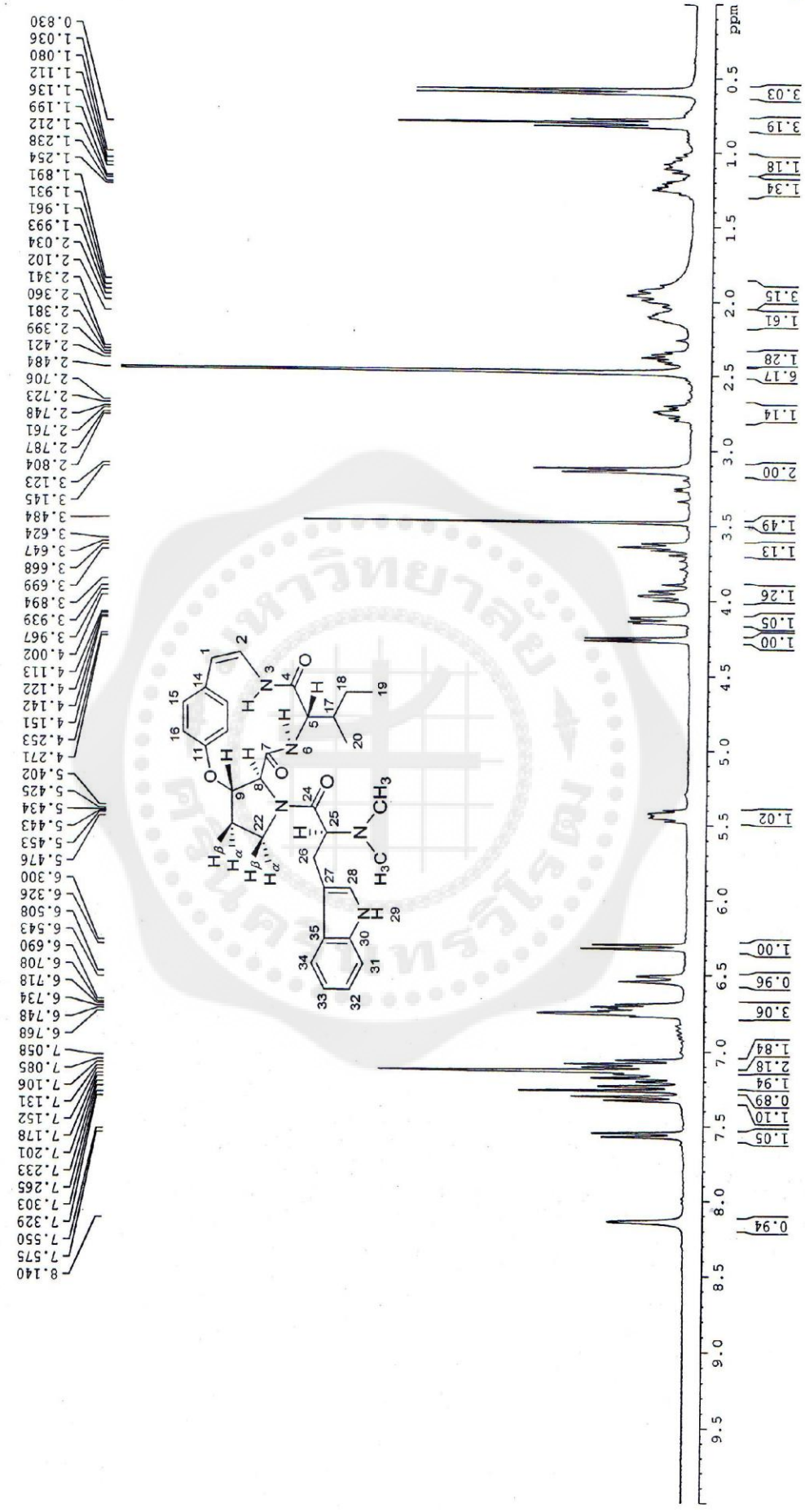

Figure 34 ^{1}H NMR spectrum of compound ZM-M2 (hemsine A, sss3346) in $CDCl_3$

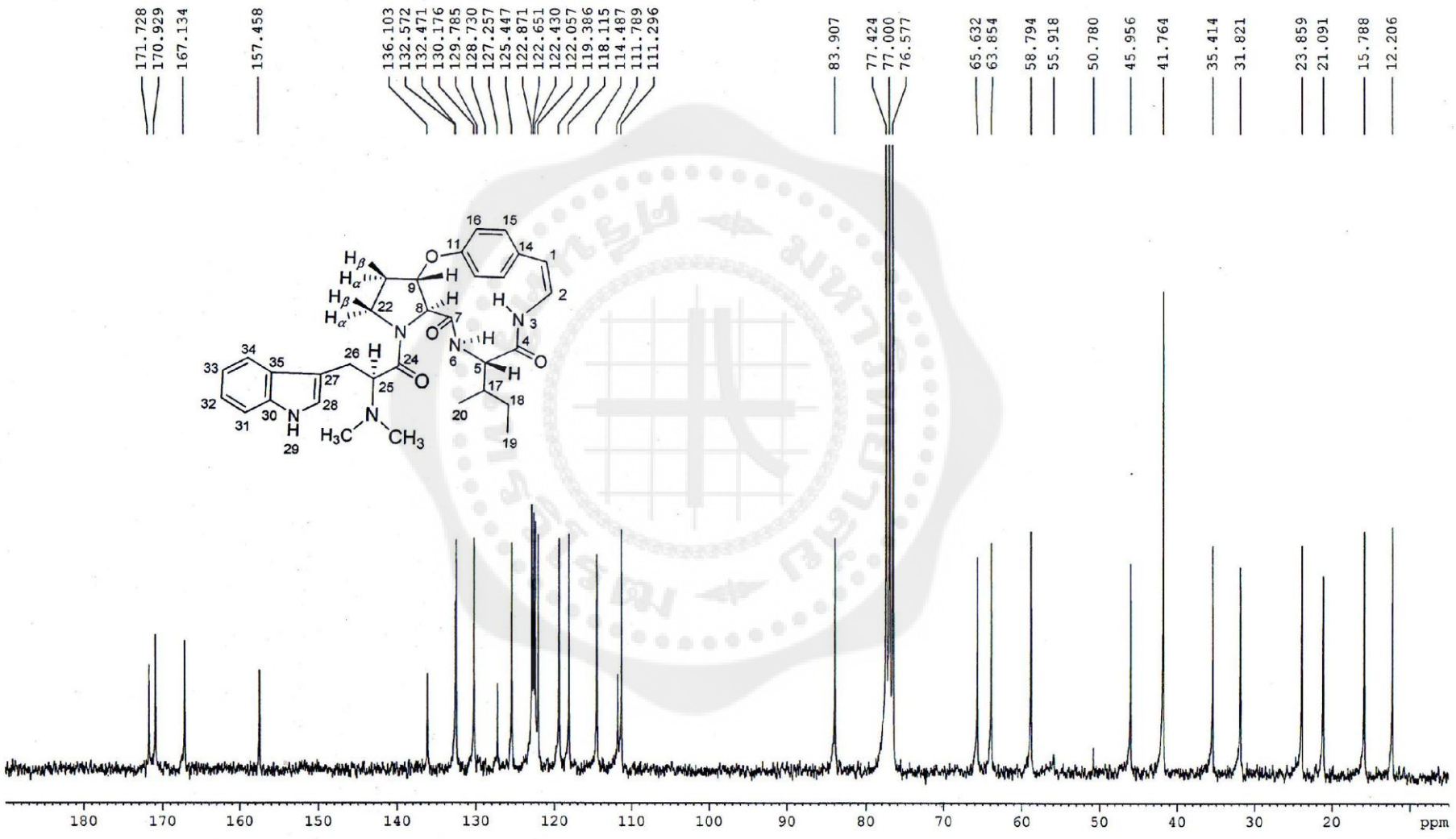

Figure 35 ^{13}C NMR spectrum of compound ZM-M2 (hemsine A, sss3346) in $CDCl_3$

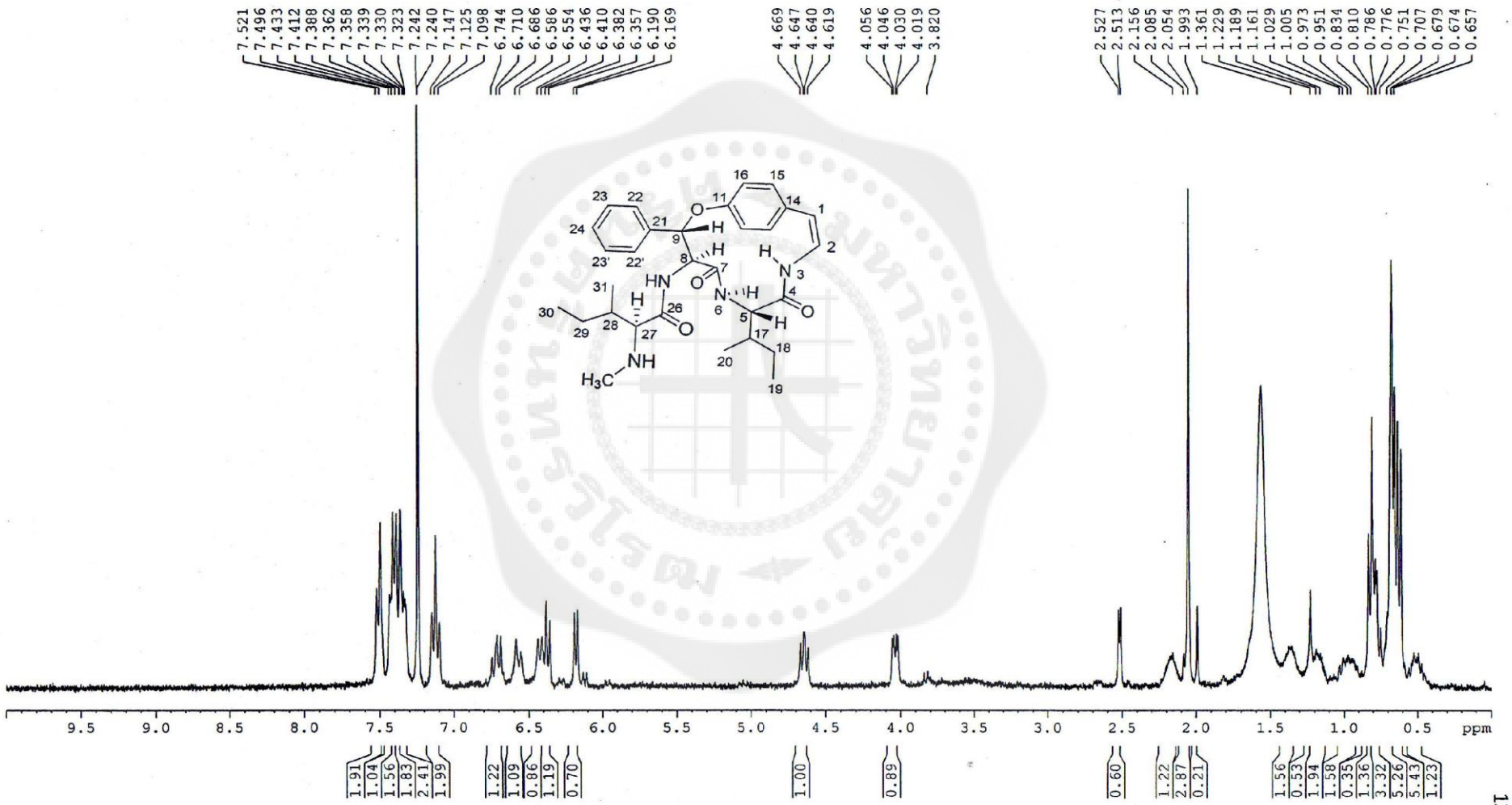

Figure 36 ^{1}H NMR spectrum of compound ZM-M3 (mauritine L, sss3756) in $CDCl_3$

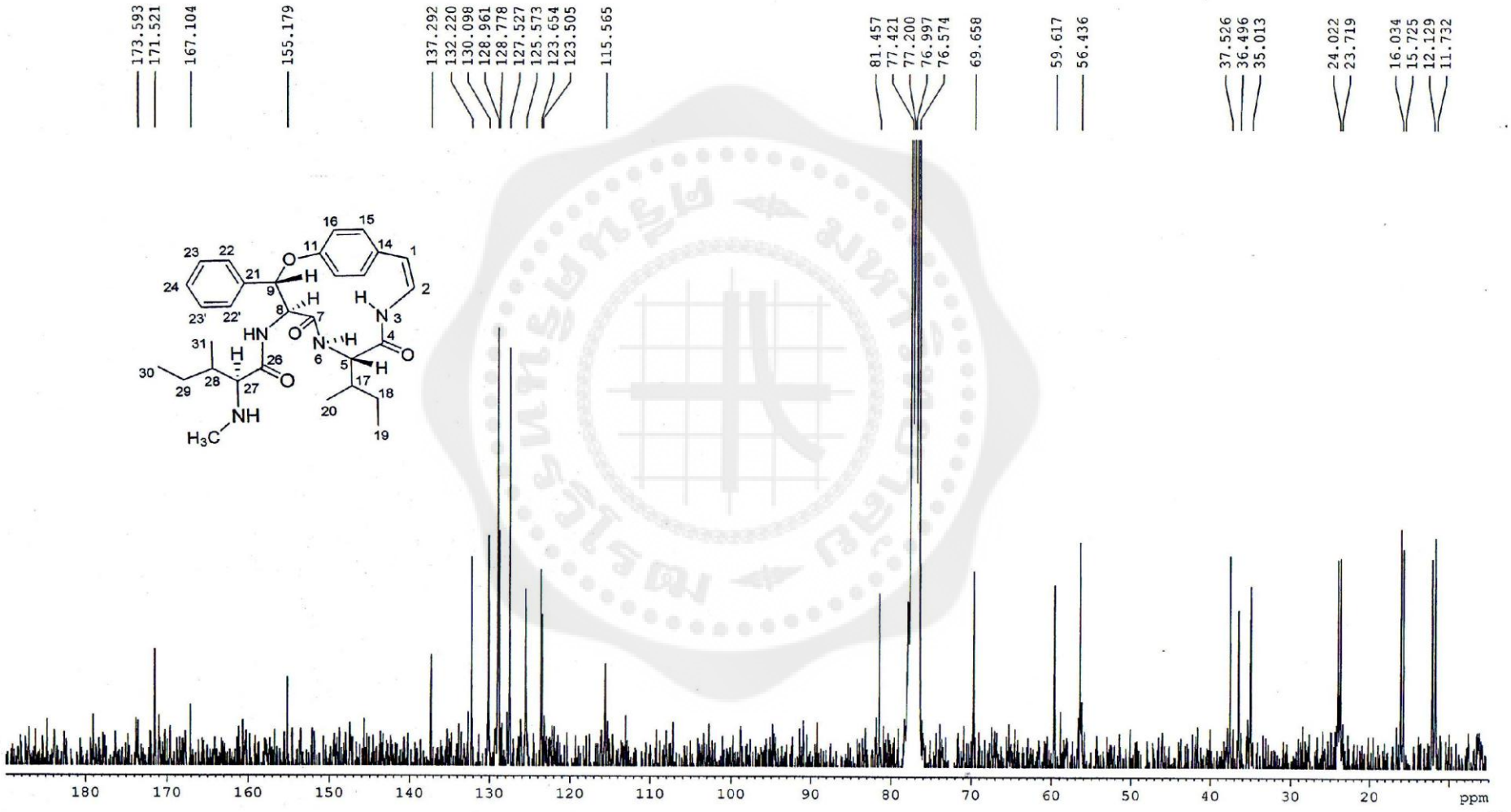

Figure 37 ^{13}C NMR spectrum of compound ZM-M3 (mauritine L, sss3756) in $CDCl_3$

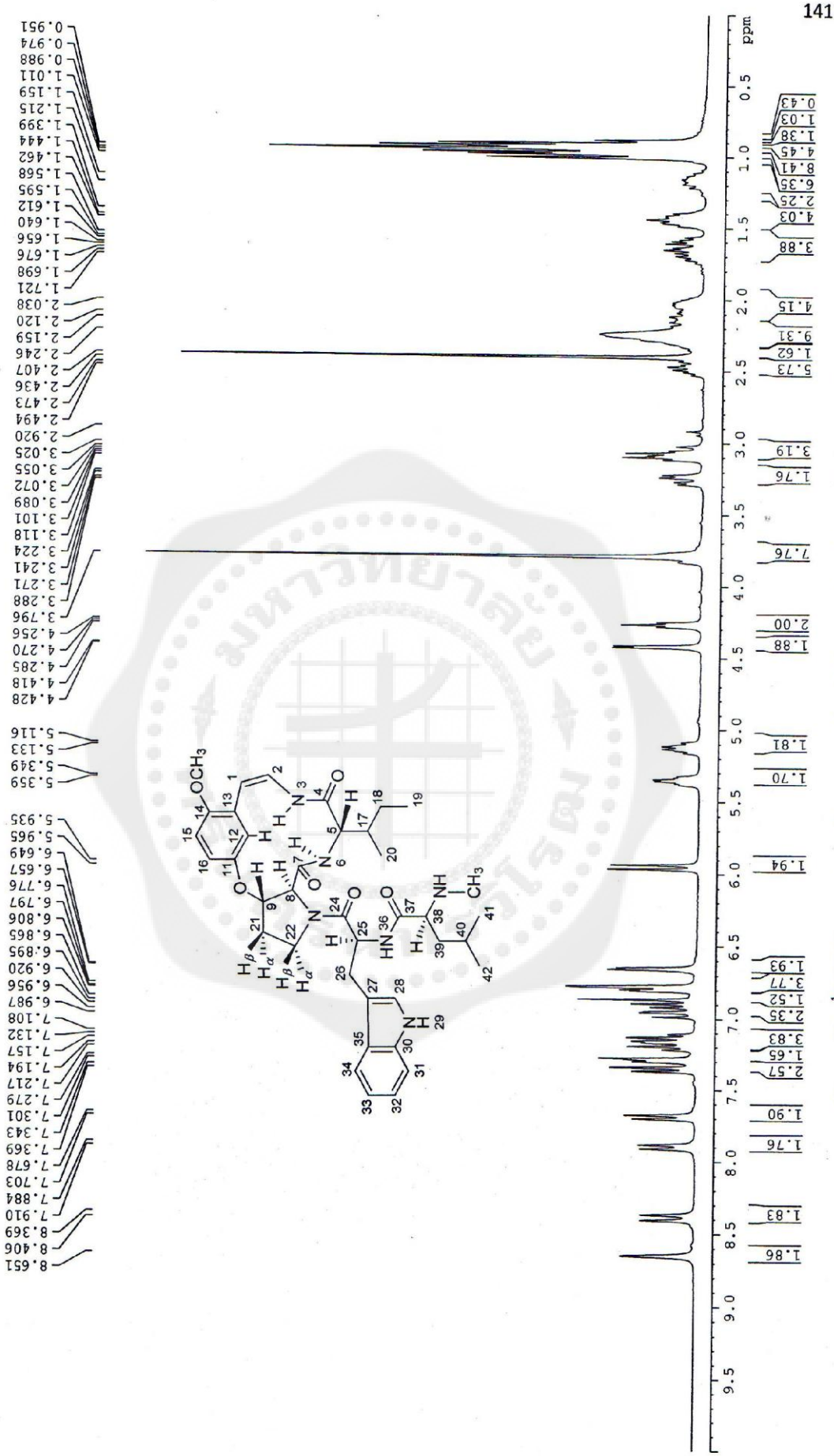

Figure 38 ^{1}H NMR spectrum of compound ZM-M4 (mauritine M, sss3100) in $CDCl_3$

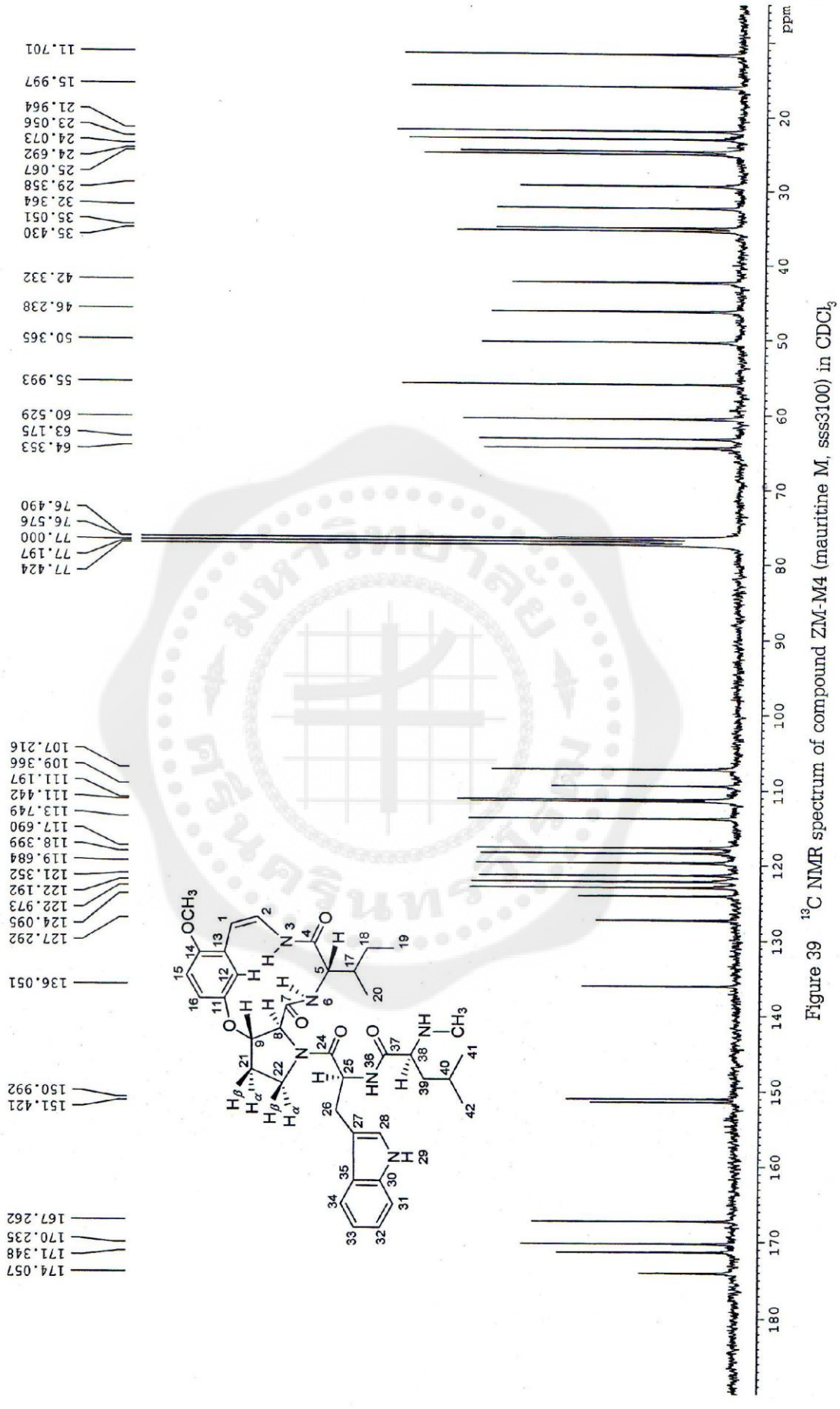

Figure 39 ^{13}C NMR spectrum of compound ZM-M4 (mauritine M, sss3100) in $CDCl_3$

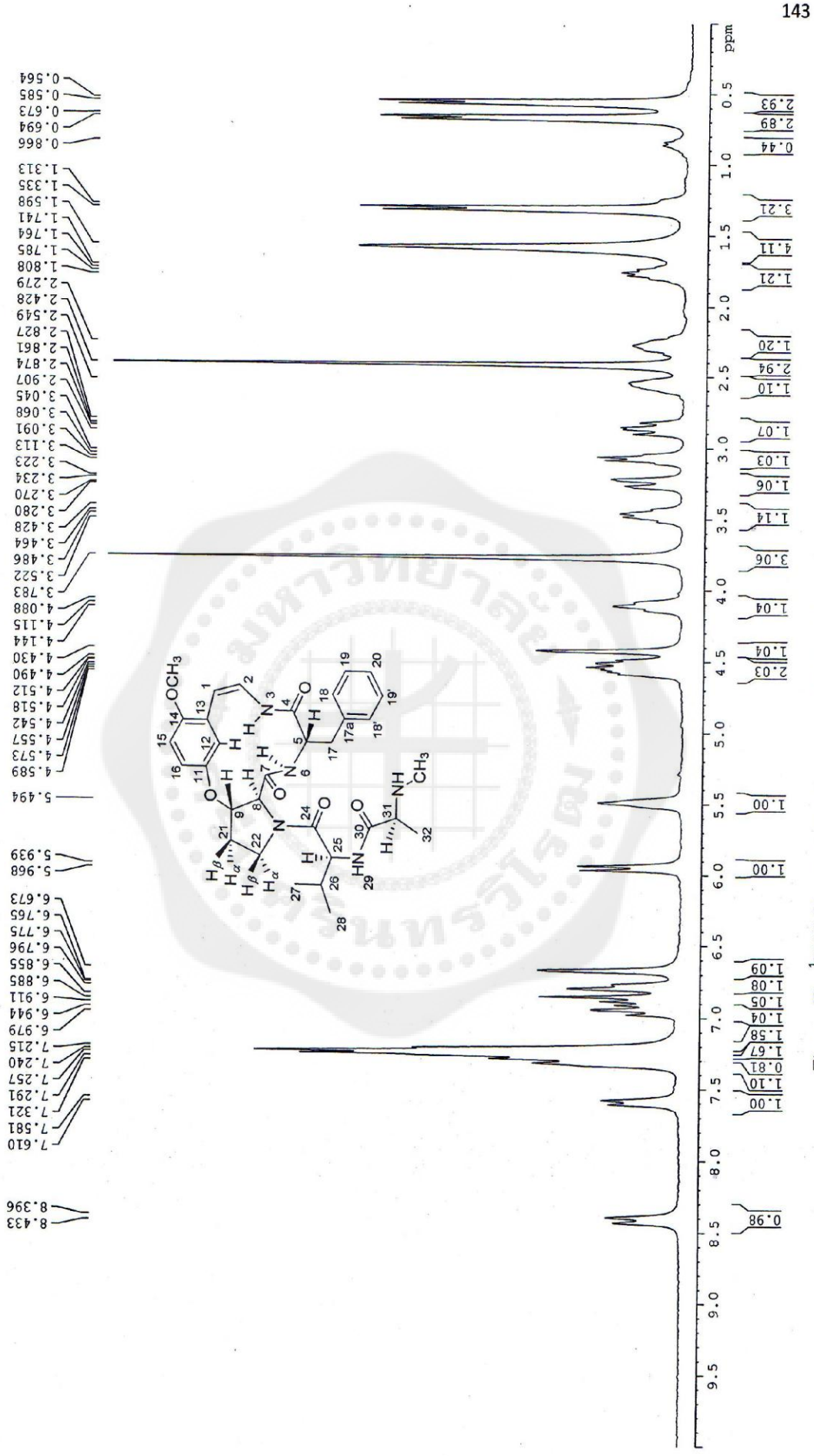

Figure 40 ^{1}H NMR spectrum of compound ZM-M5 (nummularine B, sss3008) in $CDCl_3$

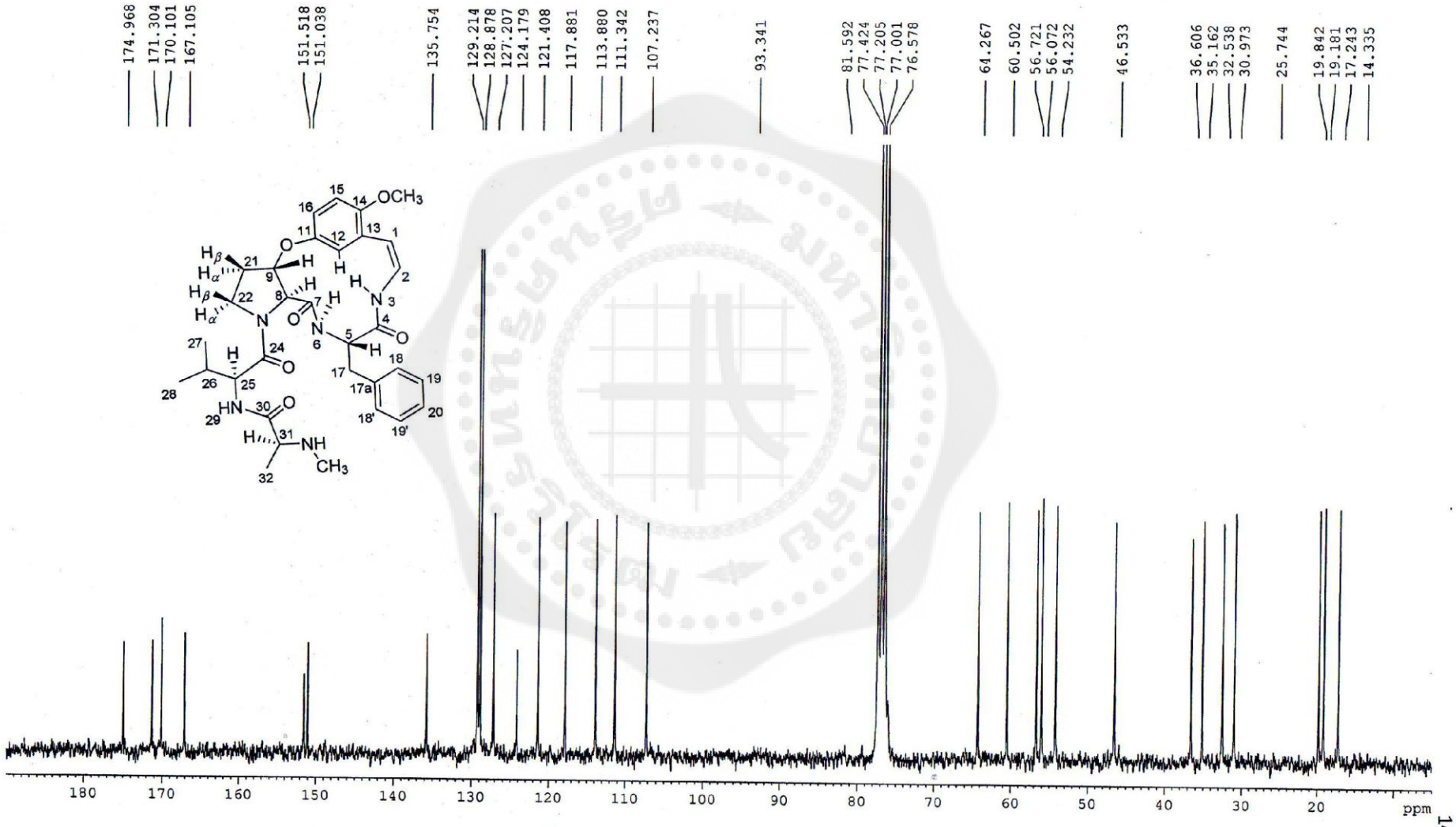

Figure 41 ^{13}C NMR spectrum of compound ZM-M5 (nummularine B, sss3008) in $CDCl_3$

GLOSSARY

LIST OF ABBREVIATIONS AND SYMBOLS

α	=	Alpha
$[\alpha]_D^{27.0}$	=	Specific rotation at 27.0° and sodium D line
β	=	Beta
br *d*	=	Broad doublet (for NMR data)
br *t*	=	Broad triplet (for NMR data)
br *s*	=	Broad singlet (for NMR data)
$CDCl_3$	=	Deuterated chloroform
$CHCl_3$	=	Chloroform
CH_2Cl_2	=	Dichloromethane
°C	=	Degree Celsius
^{13}C NMR	=	Carbon-13 Nuclear Magnetic Resonance
calcd	=	Calculated
cm	=	Centimeter
cm^{-1}	=	Reciprocal centimeter (unit of wave number)
d	=	Doublet (for NMR data)
dd	=	Doublet of doublets (for NMR data)
ddd	=	Double doublet of doublets (for NMR data)
dt	=	Doublet of triplets (for NMR data)
DEPT	=	Distortionless Enhancement by Polarization Transfer
δ	=	Chemical shift (for NMR data)
ESMS	=	Electrospray ionization Mass Spectrometry
EIMS	=	Electron-Ionization Mass Spectrometry
EtOAc	=	Ethyl acetate
g	=	Gram
h	=	Hour
^{1}H-^{1}H COSY	=	Homonuclear (Proton-Proton) Correlation Spectroscopy
^{1}H NMR	=	Proton Nuclear Magnetic Resonance
HMBC	=	^{1}H-Detected Heteronuclear Multiple Bond Coherence
HMQC	=	^{1}H-Detected Heteronuclear Multiple Quantum Coherence

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

HRTOFMS	=	High Resolution Time of Flight Mass Spectrometry
Hz	=	Hertz
IC_{50}	=	50% Inhibitory Concentration
IR	=	Infrared Spectrum
J	=	Coupling constant
KBr	=	Potassium bromide
Kg	=	Kilogram
L	=	Liter
λ_{max}	=	Wavelength at maximal absorption
ε	=	Molar absorptivity
MeOH	=	Methanol
MIC	=	Minimum Inhibitory Concentration
$[M+H]^+$	=	Protonated molecular ion
m	=	Multiplet (for NMR data)
mg	=	Milligram
m/z	=	Mass to charge ratio
μl	=	Microliter
NMR	=	Nuclear Magnetic Resonance Spectroscopy
NOESY	=	Nuclear Overhauser Effect Spectroscopy
nm	=	Nanometer
ν_{max}	=	Wave number at maximal absorption
s	=	Singlet (for NMR data)
sh	=	shoulder
TLC	=	Thin Layer Chromatography
t	=	Triplet (for NMR data)
UV	=	Ultraviolet

CURRICULUM VITAE

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