The Study of Chemical Constituents and Bioactivity from the Pericarps and Seeds of *Chisocheton siamensis*

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PREFACE

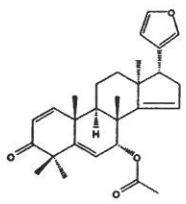
Plants have been used worldwide in traditional medicines for the treatment of diseases. It is estimated that even today approximately two-thirds to three-quarters of the world's population rely only on medicinal plants as their primary source of medicines. In Thailand, several plants have been used by the local Thai people in folk medicine for the treatment of several diseases. According to plants have been used for traditional medicines, therefore, the study of phytochemistry and biological activities are very important before using them for several purposes, for example as food supplements, cosmetics, and in agriculture and medicines. Finally, we hope that the information from our study might be helpful for other researchers who need to study on bioactive compounds and other related fields.

ACKNOWLEDEGMENT

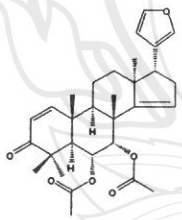
We are very grateful to Assoc. Prof. Dr. Chatchanok Karalai and Assoc. Prof. Chanita Ponglimanont, Department of Chemistry, Faculty of Science, Prince of Songkla Univeresity, for supporting some scientific materials. Special thanks are address to Assoc. Prof. Dr. Uma Prowat, Department of Chemistry, Faculty of Science and Technology, Phuket Rajabhat Univeresity for supporting NMR spectral data and Prof. Dr. Hoong-Kun Fun, X-ray Crystallography Unit, School of Physics, Universiti Sains Malaysia, Malaysia and Assoc. Prof. Dr. Suchada Chantrapromma, Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat-yai, Songkhla for structure determination by single X-ray diffraction. We also thank Mae Fah Luang University for financial support.

บทคัดย่อ

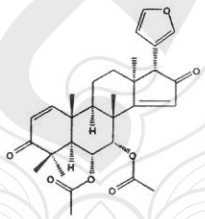
การศึกษาส่วนสกัดแอซิโตน-เฮกเซน (1:1) ของเมล็ดตาเสือใหญ่สามารถแยกสารประกอบลิโมนอยด์ใหม่ได้ 1 สารมีชื่อว่า chisosiamensin (**CS1**) และเป็นสารสารประกอบลิโมนอยด์ที่มีการรายงานแล้ว 5 สารคือ dysobinin (**CS2**), mahonin (**CS3**), azadiradione (**CS4**), epoxyazadiradione (**CS5**), และ 6α-acetoxyepoxyazadiradione (**CS6**) สำหรับการศึกษาส่วนสกัดแอซิโตน-เฮกเซน (1:1) ของเปลือกผล สามารถแยกสารประกอบได้ 2 สารคือ stigmasterol (**CS7**) และสารที่ยังระบุโครงสร้างไม่ได้อีก 1 สาร (**CS8**) โครงสร้างของสารประกอบเหล่านี้วิเคราะห์โดยอาศัยข้อมูลทาง NMR สเปกโทรสโกปี นอกจากนี้ยังยืนยันโครงสร้างของสารประกอบ **CS6** ด้วยข้อมูลทางเอกเรย์ นำสารประกอบลิโมนอยด์ **CS2-CS6** ไปทดสอบฤทธิ์ต้านเชื้อที่ก่อให้เกิดโรคมาเลเรีย (*Plasmodium falciparum*) เชื้อที่ก่อให้เกิดวัณโรค (*Mycobacterium tuberculosis*) และทดสอบความเป็นพิษกับเซลล์มะเร็ง 3 ชนิดคือ มะเร็งปอด (NCI-H187), มะเร็งในทรวงอก (MCF-7), และมะเร็งในช่องปาก (KB) จากการทดสอบพบว่าสารประกอบลิโมนอยด์ **CS2** แสดงฤทธิ์ต้านเชื้อที่ก่อให้เกิดโรคมาเลเรีย โดยมีค่า IC_{50} 2.06 μg/ml นอกจากนี้แล้วลิโมนอยด์ **CS2** ยังแสดงฤทธิ์ความเป็นพิษต่อเซลล์มะเร็งทั้ง 3 ชนิดคือ มะเร็งปอด มะเร็งในทรวงอก และมะเร็งในช่องปาก โดยมีค่า IC_{50} เท่ากับ 1.67, 3.17 and 2.15 μg/ml ตามลำดับ เฉพาะลิโมนอยด์ **CS4** เท่านั้นที่แสดงฤทธิ์ต้านเชื้อที่ก่อให้เกิดวัณโรคได้ดี โดยมีค่า MIC เท่ากับ 6.25 μg/ml



Chisosiamensin (CS1)



Dysobinin (CS2)



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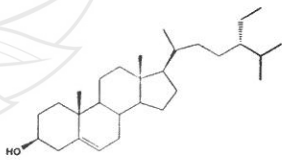
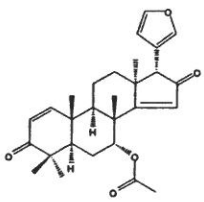
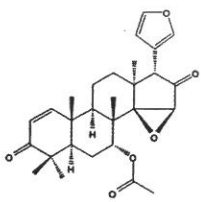


Sitisterol (CS7)



Azadiradione (CS4)



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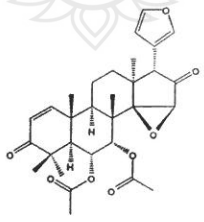
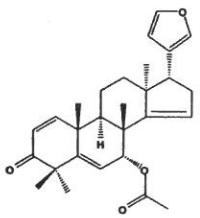


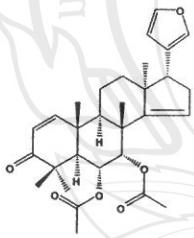
6α-Acetoxyepoxyazadiradione (CS6)

ABSTRACT

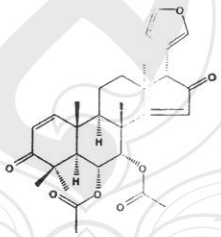
The study of acetone-hexane (1:1) extract of the seeds of *Chisocheton siamensis* led to the isolation of a novel limonoid, chisosiamensin (**CS1**), along with five known limonoids, dysobinin (**CS2**), mahonin (**CS3**), azadiradione (**CS4**), epoxyazadiradione (**CS5**), and 6α-acetoxyepoxyazadiradione (**CS6**) while the analysis of acetone-hexane (1:1) extract of the pericarps of *C. siamensis* gave sitosterol (**CS7**) and unidentify compound (**CS8**). Their structures were characterized by NMR spectroscopy. The structure of compound **CS6** was also confirmed by X-ray diffraction data. Limonoids **CS2-6** were tested for their antimalarial activity against *Plasmodium falciparum*, antimycobacterial activity against *Mycobacterium tuberculosis* and cytotoxic activity against NCI-H187 (human small cell lung cancer), KB (oral human epidermal carcinoma) and MCF-7 (breast cancer) cancer cell lines. Limonoid **CS2** showed the best inhibitory effect against *Plasmodium falciparum* with IC_{50} of 2.06 μg/ml. Also, compound **CS2** had the highest cytotoxic activity against NCI-H187, KB and MCF-7 cancer cell lines with the IC_{50} of 1.67, 3.17 and 2.15 μg/ml, respectively. Only compound **CS4** had a strong activity with the MIC value of 6.25 μg/ml.



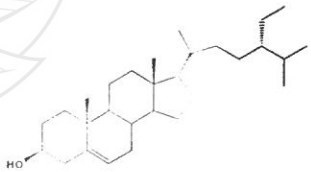
Chisosiamensin (CS1)



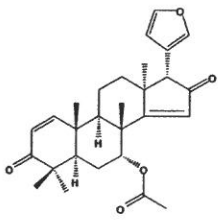
Dysobinin (CS2)



Mahonin (CS3)



Sitisterol (CS7)



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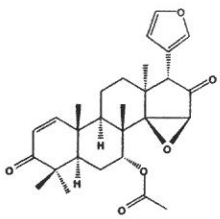

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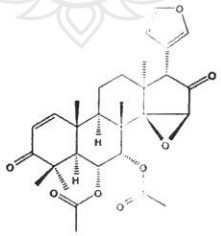

6α-Acetoxyepoxyazadiradione (CS6)

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ABBREVIATIONS AND SYMBOLS

s	=	*singlet*
d	=	*doublet*
t	=	*triplet*
q	=	*quartet*
m	=	*multiplet*
dd	=	*doublet of doublet*
dt	=	*doublet of triplet*
br s	=	*broad singlet*
br m	=	*broad multiplet*
g	=	gram
nm	=	nanometer
m.p.	=	melting point
cm^{-1}	=	reciprocol centimeter (wave number)
δ	=	chemical shift relative to TMS
J	=	coupling constant
$[\alpha]_D$	=	specific rotation
λ_{max}	=	maximum wavelength
ν	=	absorption frequencies
ε	=	molar extinction coefficient
m/z	=	a value of mass divided by charge
°C	=	degree Celsius
MHz	=	Mega Hertz
ppm	=	part per million
c	=	concentration
IR	=	Infrared

UV	=	Ultraviolet-Visible
MS	=	Mass Spectroscopy
NMR	=	Nuclear Magnetic Resonance
2D NMR	=	Two Dimensional Nuclear Magnetic Resonance
COSY	=	Correlation Spectroscopy
DEPT	=	Distortionless Enhancement by Polarization Transfer
HMBC	=	Heteronuclear Multiple Bond Correlation
HMQC	=	Heteronuclear Multiple Quantum Coherence
ROESY	=	Rotating from Overhause Effect Spectroscopy
CC	=	Column Chromatography
QCC	=	Quick Column Chromatography
PLC	=	Preparative Thin Layer Chromatography
TMS	=	tetramethylsilane
$CDCl_3$	=	deuterochlroform

CHAPTER 1
INTRODUCTION

1.1 Statement and significance of the problem

Synthesis of many important drugs makes use of natural product starting materials. Researches are conducted in order to find major constituents with biological activity to be used as drugs or in synthesis of analog or derivatives. Pure compounds extracted from many plants and many parts of the plants are explored and tested for biological activities. However, elucidation of chemical constituents from natural products and biological activity testing are only the initial step in the process of study to find new compounds and acquire basic knowledge of biological activity against fungi, malaria, AIDS, inflammation and cytotoxic activity. The important process is the application of the knowledge in pharmacology and medicine.

1.2 Objectives

The objectives of this project are involved:

1. To isolate and characterize compounds from the seeds and pericarps of *C. siamensis*.
2. To test biological activity of pure compounds

1.3 Scope of study

1. Extraction and isolation of secondary metabolite from the seeds and pericarps of *C. siamensis*.

2. Characterization of all isolates by spectroscopic methods, including UV, IR, NMR and MS.

1.4 Benefit

1. Some compounds, which were isolated the seeds and pericarps of *C. siamensis*, might be showed significant biological activity.

2. Some active compounds might be applied into the related field i.e. pharmacy, cosmetics and agriculture.

3. Acquire basic knowledge of chemical compounds and biological activity.

4. This work might be published in international journals.

1.5 Review of Literatures

Chisocheton siamensis (Figure 1) or "Ta suea" in local Thai name belongs to the Meliaceae family, which was found in northern part of Thailand. This genus were found in 7 species, including *C. ceramicus, C. macrophyllus* King subsp. *fulvescens, C. patens, C. penduliflorus, C. pentandrus* Merr. subsp. *paucijugus, C. siamensis* (*C.* cumingianus Harms subsp. *balansae*) and *C. tomentosus* (*C. rubiginosus*) (Smitinand, 2001), in Thailand. The characteristics of *C. siamensis* are summarized below.

Barks: dark brown or grayish, shallowly cracked, inner bark cream.

Leaves: 35-70 cm, pinnate, 5-9 pairs of opposite or rarely alternate leaflets, often with a bud at the end rather than a leaflet, 12-28 × 4.5-7 cm, oblong or lanceolate with tapering tip & asymmetric base, no teeth. Young shoots densely brow-hairy, mature leaves smooth or with scattered brownish hairs on stalks & veins below. 10-15 pairs of side veins, joined at margin. Leaflet stalks 0.3-0.6 cm, main stalk 7.10 cm.

Twigs: stout with large leaf scars.

Flowers: ≤ 0.5 cm, white or yellow, in narrow branched clusters on long drooping stalks in or slightly above upper leaf axils, 20-50 cm, individual stalks 0.2 cm. buds narrowly tubular, calyx cup-shaped, 4-6 free petals in 1-2 rows, fused to stamen tube at base, slightly hairy near tips. Stamen tube cylindrical, as long as corolla, with 6 tiny teeth & anthers in-between, anthers with long hairs, style 17 mm, ovary 1 mm with indistinct disc.

Fruits: 5-8 cm, bright red or dull pinkish-yellow, hanging in very long-stalked clusters up to 70 cm, globose, splitting into 2-5 sections each with 1 (2) glossy black seeds partly covered with an orange coating (aril).

A B C

Figure 1 Leaves, young fruits and ripe fruits of *C. siamensis* (**A-C**)

Almost of chemical compounds isolated from *Chisocheton* genus were limonoids and summarized in Table 1.

Table 1 Compounds isolated from *Chisocheton* genus

Plant	Part	Compound	Bibliography
C. erythrocarpus	Barks	Erythrocarpine A, **1**	Awang, *et al.*, 2007
		Erythrocarpine B, **2**	
		Erythrocarpine C, **3**	
		Erythrocarpine D, **4**	
		Erythrocarpine E, **5**	
C. microcarpus	Leaves	Betulonic acid, **12**	Inada, *et al.*, 1993
		Moronic acid, **13**	
		24-Hydroxydammara-20,25-dien-3-one, **16**	

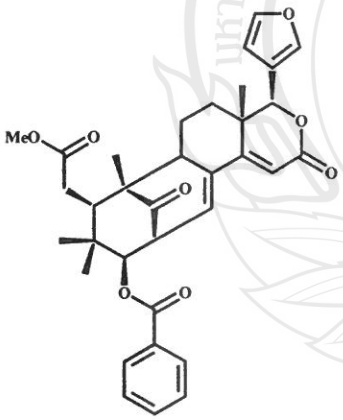
Table 1 (continued)

Plant	Part	Compound	Bibliography
C. macrophyllus	Leaves	Chisocheton F, **14**	Gunning, *et al.*, 1994
		7-Hydroxy-4,4,8-trimethyl-3-oxo-carda-1,14-dienolide, **15**	
		Moronic acid, **13**	Innada, *et al.*, 1993
		Chisocheton F, **14**	
		24-Hydroxydammara-20,25-dien-3-one, **23**	
C. paniculatus	Seeds	Dysobinin, **17**	Chatterjee, *et al.*, 1989
		Mahonin, **19**	
		6α-Acetoxyepoxyazadirone, **24**	
		17β-Hydroxy-6α-acetoxyazadiradione, **25**	
	Fruits	Dysobinin, **17**	Saikia, *et al.*, 1979
		Mahonin, **19**	
		6α,7α-Dihydroxymaliaca-1,14,20,22-tetraene-3,14-dione, **21**	
		Dyobinin, **17**	Manobjyoti, *et al.*, 1993
		Deacetylmeldenin, **18**	
		Mahonin, **19**	
		Meldenin acetate, **20**	
		Dysobinin, **17**	Bordoloi, *et al.*, 1993
		Mahonin, **19**	
		(-)-β-Sitosterol, **22**	

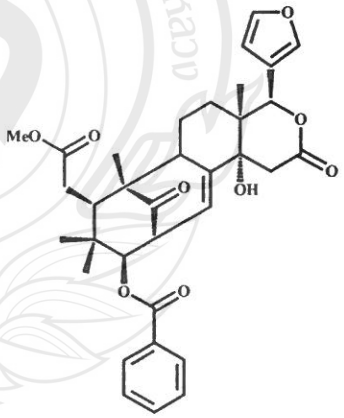
Table 1 (continued)

Plant	Part	Compound	Bibliography
C. paniculatus	Fruits	Deacetylmeldenin, **18**	Bordoloi, *et al.*, 1993
		Meldenin acetate, **20**	
		Arunachalin, **6**	Bhattacharyya, *et al.*, 2004
		Paniculatin B, **7**	
		Paniculatin C, **8**	
		Paniculatin D, **9**	
		Paniculatin G, **10**	
		Paniculatin H, **11**	
	Root-wood	Paniculatin B, **7**	Yadav, *et al.*, 1999
		Paniculatin C, **8**	
		Paniculatin D, **9**	
		Paniculatin G, **10**	
		Paniculatin H, **11**	

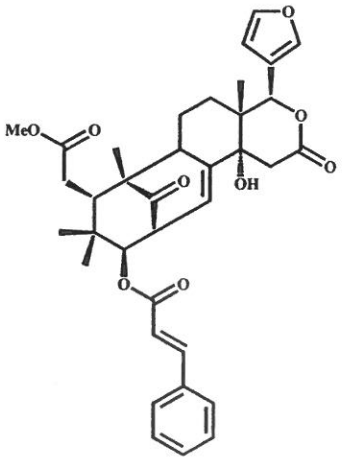
Structures



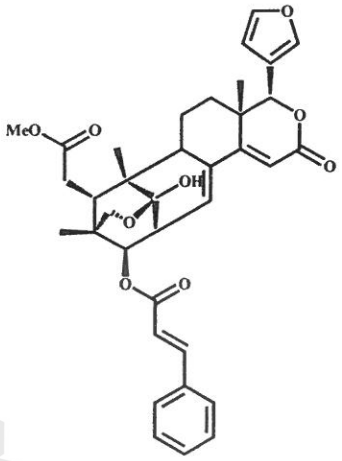
Erythrocarpine A, **1**



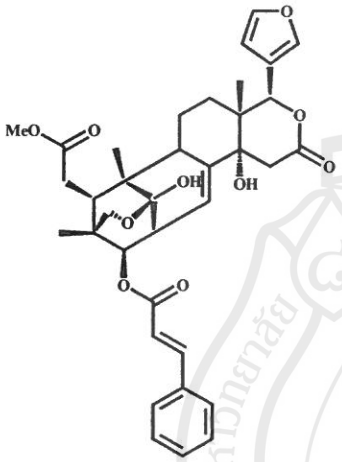
Erythrocarpine B, **2**



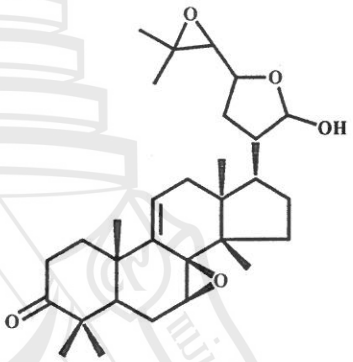
Erythrocarpine C, **3**



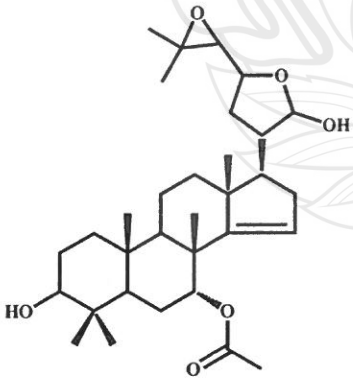
Erythrocarpine D, **4**



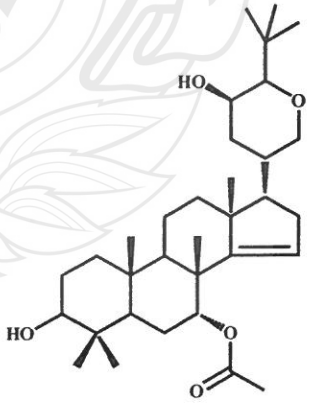
Erythrocarpine E, **5**



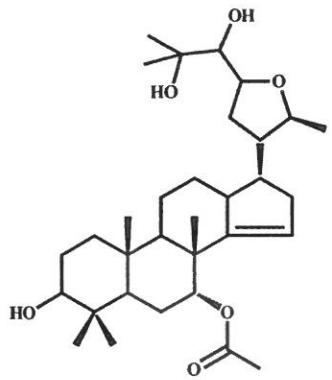
Arunachalin, **6**



Paniculatin B, **7**



Paniculatin C, **8**



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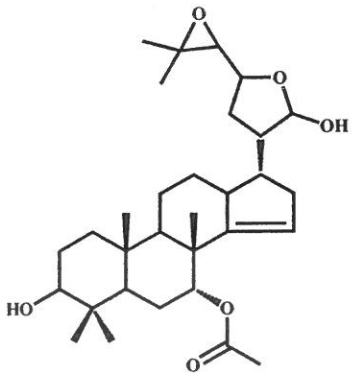

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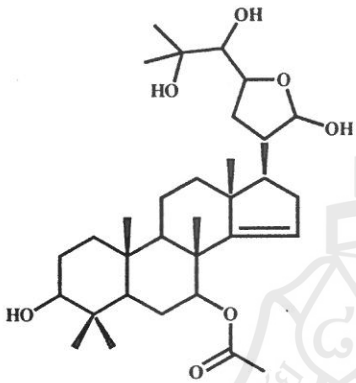
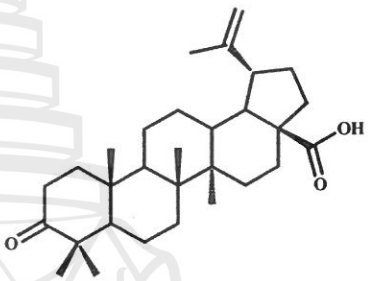
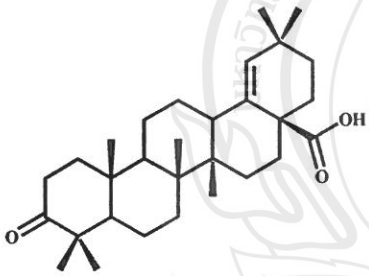


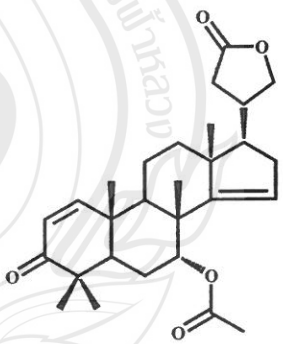
Paniculatin H, **11**



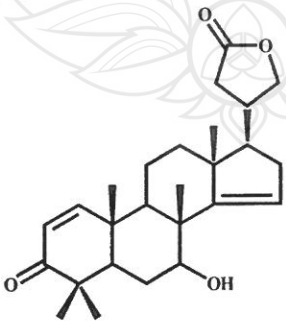
Betulonic acid, **12**



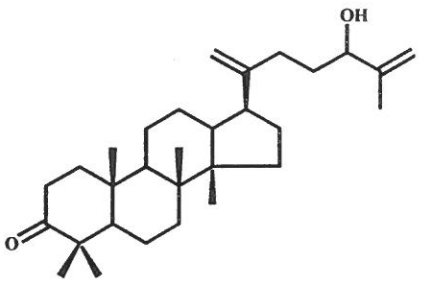
Moronic acid, **13**



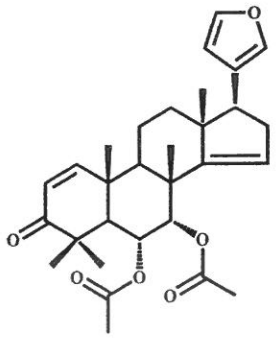
Chisocheton F, **14**



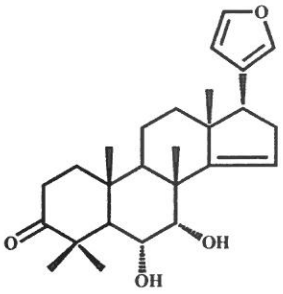
7-Hydroxy-4,4,8-trimethyl-3-oxo-carda-1,14-dienolide, **15**



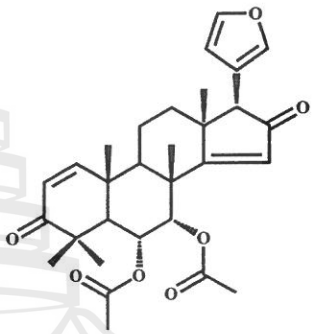
24-Hydroxydammara-20,25-dien-3-one, **16**



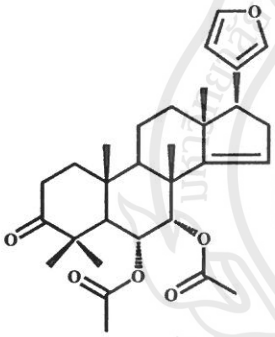
Dysobinin, **17**



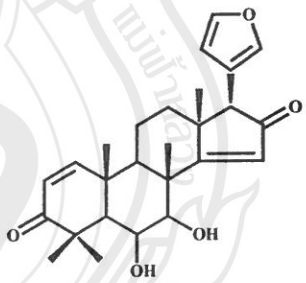
Deacetylmeldenin, **18**



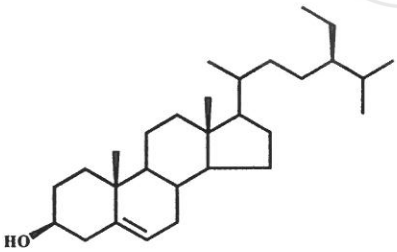
Mahonin, **19**



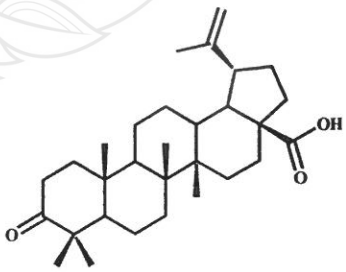
Meldenin acetate, **20**



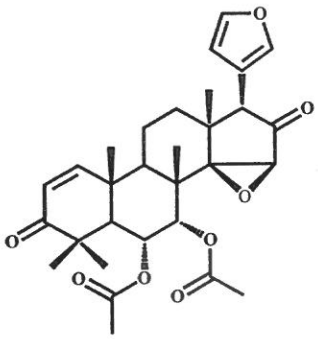
6α,7α-Dihydroxymaliaca-1,14,20,22-tetraene-3,14-dione, **21**



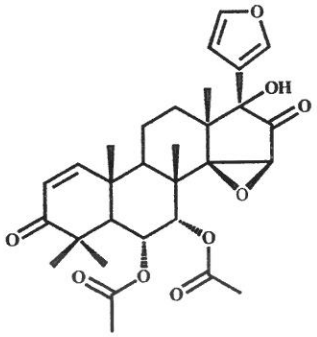
(-)-β-Sitosterol, **22**



24-Hydroxydammara-20,25-dien-3-one, **23**



6α-Acetoxyepoxyazadirone, **24**



17β-Hydroxy-6α-acetoxyazadiradione, **25**

Although several limonoids exhibit antimalarial (Lee et al., 2007, Saewan et al., 2006), cytotoxic (Awang et al., 2007, Takeya et al., 1996), antiprotozoal (Khalid et al., 1998), and antifeedant (Koul et al., 2003, Nihei et al., 2002) activities but almost of limonoids, which isolated from *Chisocheton* genus have not been tested for their biological activity. The tested biological activity of compounds isolated from *Chisocheton* genus was summarized in Table 2.

Table 2 Biological activity of some compounds isolated from *Chisocheton* genus

Compound	Activity	Bibliography
1	Cytotoxicity	Awang, *et al.*, 2007
2	Cytotoxicity	
3	Cytotoxicity	
4	Cytotoxicity	
5	Cytotoxicity	
13	Anti-HIV	Ito et al. 2001
17	Anti-inflamatory	Singh, *et al.* 1976

According to no phytochemical and biological activity study on the seeds and pericarps of *C. siamensis*, this prompted us to investigate its chemical constituents and their biological activity in order to providing additional information of the plant. Therefore, the objectives of this project are to study chemical compounds and their biological activity from the seeds of *C. siamensis*.

CHAPTER 2
MATERIALS AND METHODS

2.1 Instruments and Chemicals

Melting points were determined using a Fisher-John melting point apparatus. The optical rotation $[\alpha]_D$ values were determined with a JASCO P-1020 polarimeter. UV spectra were measured with UV-160A spectrophotometers (Shimadzu). The IR spectra were measured with a Perkin-Elmer FTS FT-IR spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded using 400 and/ or 300 MHz Bruker FTNMR Ultra Shield spectrometers. Chemical shifts were recorded in parts per million (δ) in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. The EIMS was obtained from a MAT 95 XL mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 F_{254} (Merck, 230-400 Mesh ASTM) and silica gel 100 (Merck, 70-230 Mesh ASTM), respectively. Precoated plates of silica gel 60 F_{254} were used for analytical purposes.

2.2 Plant materials

The fruits of *C. siamensis* were collected at Queen Sirikit Garden, Mae rim district, Chiang Mai province, northern of Thailand, in February 2006. The identification was made by Dr. Prachaya Srisanga and the plant voucher specimen has been deposited at Queen Sirikit Garden, Mae rim district, Chiang Mai province, northern of Thailand.

2.3 Biological Assays

Anti-malaria assay

Antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K_1, mutidrug resistant), using the method of Trager and Jensen (1976). Quantitative assessment of *in vitro* malarial activity was determined by means of the microculture radioisotope technique based on the method described by Desjardins et al. (1979). The inhibitory concentration (IC_{50}) represented the concentration that caused 50% reduction in parasite growth which was indicated by the *in vitro* uptake of

[^{3}H]-hypoxanthine by *P. falciparum*. The standard compound was dihydroartemisinin (IC_{50} 4.4 nM).

Antimycobacterial assay

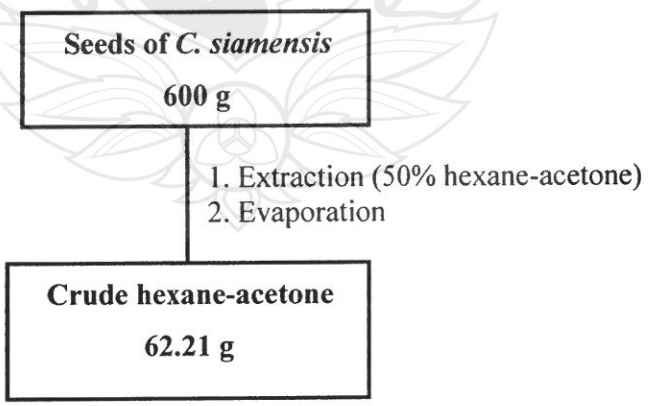
Antimycobacterial activity was evaluated against *M. tuberculosis* $H_{37}Ra$ employing the Microplate Alamar Blue Assay (MABA) (Collins and Franzblau 1997). The reference drugs were rifampicin, kanamycin and isoniazid and the minimum inhibitory concentration (MIC) values were summarized in Table 9.

Cytotoxic assay

The procedures for cytotoxic assay were performed by sulphorhodamine B (SRB) assay (anti-KB and MCF-7) and colorimetric method (anti-NCI-H187) as described by Skehan et al. (1990). In this study, three cancer cell lines, MCF-7 (breast cancer), NCI-H187 (human, small cell lung cancer) and KB (oral human epidermal carcinoma) were used. Ellipticin and doxorubicin were the reference substances in this study and the IC_{50} values are summarized in Table 9.

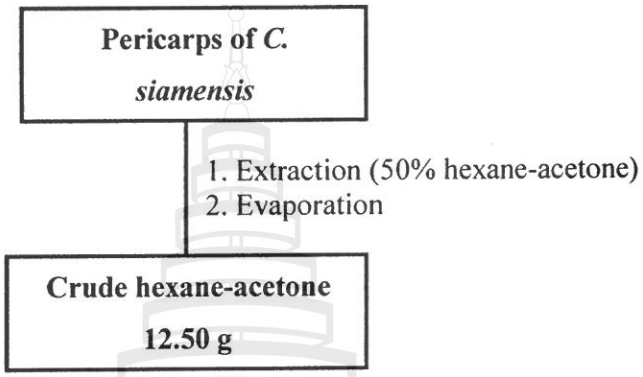
2.4 Extraction

The seeds of *C. siamensis* (600 g) were extracted with hexane-acetone (1:1) over the period of 3 days at room temperature, and evaporated under reduced pressure to give crude hexane-acetone extract (62.21 g).



Extraction of the seeds of *C. siamensis*

The pericarps of *C. siamensis* (200 g) were extracted with hexane-acetone (1:1) over the period of 3 days at room temperature, and evaporated under reduced pressure to give crude hexane-acetone extract (12.50 g).

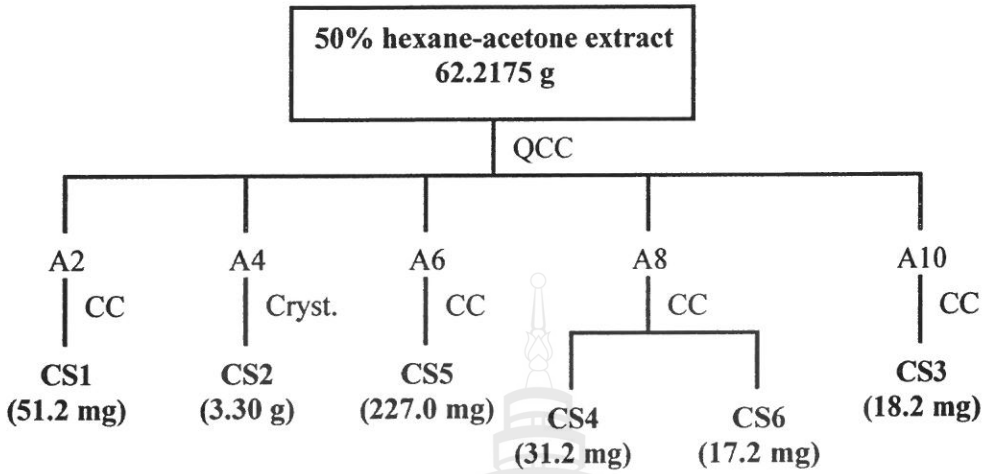


Extraction of the pericarps of *C. siamensis*

2.4 Isolation

The crude hexane-acetone extract (62.21 g) of seeds of *C. siamensis* was subjected to QCC over silica gel and eluted with a gradient of 5% EtOAc-hexane to 20% acetone-EtOAc afforded 13 factions (A1-A13). Fraction A2 (435.9 mg) was subjected to CC using 15% EtOAc-hexane to give compound **CS1** (51.2 mg). Fraction A4 (4 g) was crystallized from EtOAc-hexane to afford compound **CS2** (3.30 g). Fraction A6 (576.9 mg) was purified by CC with 25% EtOAc-hexane to yield compound **CS5** (227.0 mg). Purification of fraction A8 (1.0107 g) was also performed by CC with 25% EtOAc-hexane gave compounds **CS4** (31.2 mg) and **CS6** (17.2 mg). Compound **CS3** (18.2 mg) was obtained from fraction A10 (65.5 mg) by repeated CC using 5% EtOAc-DCM as eluent.

The crude hexane-acetone extract (12.50 g) of pericarps of *C. siamensis* was separated by the same methods as the crude of the seeds to yield **CS7** (1 mg) and **CS8** (13 mg).



Compound CS1: $C_{28}H_{34}O_4$, colorless viscous oil, $[\alpha]_D^{25}$ +49.0° (*c* 0.04, MeOH). UV λ_{max}^{MeOH} (log ε): 218 (4.37). IR (neat) ν_{max}: 1750, 1680 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz), ^{13}C NMR (75 MHz, $CDCl_3$), and DEPT spectra, see Table 3. HREIMS *m/z* 435.4526 $[M+H]^+$).

Compound CS2: $C_{30}H_{38}O_6$, white solid; ^1H NMR ($CDCl_3$, 300 MHz), ^{13}C NMR ($CDCl_3$, 75 MHz) spectra, see Table 4.

Compound CS3: $C_{30}H_{36}O_7$, yellow oil; ^1H NMR ($CDCl_3$, 300 MHz), ^{13}C NMR ($CDCl_3$, 75 MHz) spectra, see Table 5.

Compound CS4: $C_{28}H_{34}O_5$, yellowish oil; ^1H NMR ($CDCl_3$, 300 MHz), ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 6.

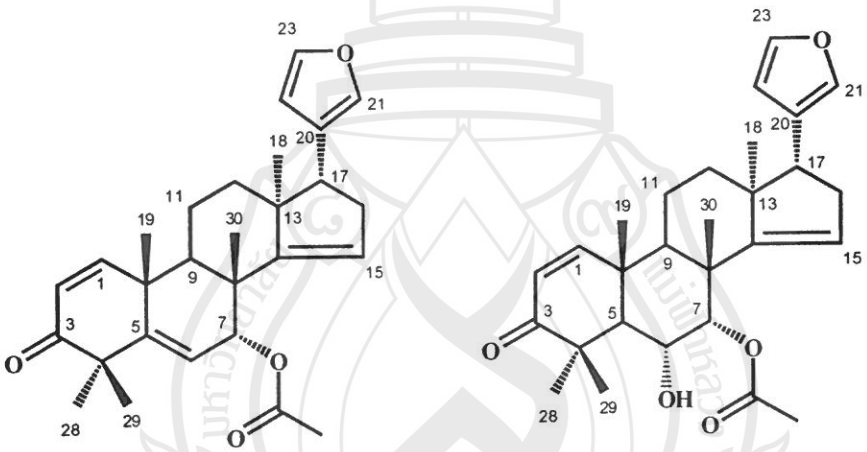
Compound CS5: $C_{28}H_{34}O_6$, yellow oil; ^1H NMR ($CDCl_3$, 300 MHz), ^{13}C NMR ($CDCl_3$, 75 MHz) spectra, see Table 7.

Compound CS6: $C_{30}H_{36}O_8$, colorless solid; m.p. 273 °C; ^1H NMR ($CDCl_3$, 300 MHz), ^{13}C NMR ($CDCl_3$, 75 MHz) spectra, see Table 8.

CHAPTER 3
RESULTS AND DISCUSSION

The acetone-hexane extract of the seeds and pericarps of *C. siamensis* were separated and purified by chromatographic techniques and/or recrystallization to give compounds **CS1** (51.2 mg), **CS2** (3.30 g), **CS3** (18.2 mg), **CS4** (31.2 mg), **CS5** (227.0 mg), **CS6** (17.2 mg), **CS7** (1 mg) and **CS8** (13 mg). The structure elucidation of all compounds was determined by using spectroscopic methods. In addition, the structure of **CS6** was confirmed by X-ray diffraction.

3.1. Compound CS1 (Chisosiamensin)



Nimonol

Chisosiamensin (**1**) was isolated as colorless viscous oil with the molecular ion peak at *m/z* 435.4526 $[M+H]^+$ in HREI-MS, corresponding to the molecular formula $C_{28}H_{34}O_4$. The UV spectrum indicated the presence of α, β-unsaturated ketone at 218 nm, while the IR spectrum of this compound showed two carbonyl functionalities at 1750 and 1680 cm^{-1}. The ^{13}C NMR and DEPT spectral data of **CS1** (Table 3) revealed 28 carbons, including 6 methyls (δ 19.0, 20.6, 21.1, 21.2, 27.0 and 27.3), 3 methylenes (δ 16.5, 33.0 and 34.2), 10 methines (δ 38.6, 51.6, 74.5, 111.0, 119.6, 125.4, 129.7, 139.6, 142.5, 158.1), and 9 quaternary carbons (δ 39.9, 42.8, 44.1, 47.1, 124.5, 158.2, 158.8, 170.1 and 204.6). Compound **CS1** was classified as tetranortriterpenoidal

limonoid by the presence of five methyl singlets at δ 0.78 (Me-19), 1.07 (2 x Me, Me-18 and Me-29), 1.19 (Me-30) and 1.22 (Me-28) and a β-furan moiety at δ 6.27 (br s, H-22), 7.23 (s, H-21) and 7.37 (s, H-23), in ^{1}H NMR spectrum (Siddiqui, *et al.*, 2000; Suresh *et al.*, 1997). The ^{1}H- and ^{13}C-NMR spectral data of **1** (Table 3) were similar to nimonol (**7**), isolated from *Azadirachta indica* (Suresh *et al.*, 1997), except that a compound **1** showed an olefinic proton at δ 5.33 (*m*), which was connected to sp^2 carbon at δ 129.7 (C-6) in the HMQC experiment, instead of two methine protons (H-5 and H-6) of nimonol (Table 3). These results indicated that compound **1** consisted of a double at C-5/6. Therefore, chisosiamensin was identified as **1**, a dehydrated derivative of nimonol.

Table 3 Comparison of ^{1}H and ^{13}C spectral data of chisosiamensin (**1**) and nimonol (**7**)

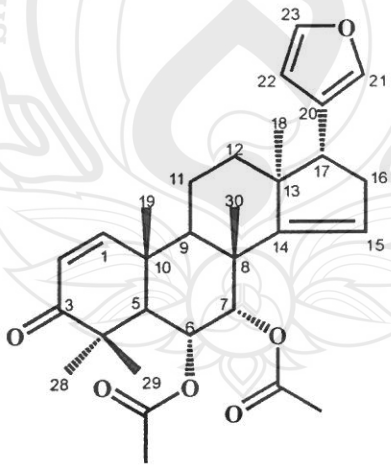
Position	**Chisosiamensin**		**Nimonol**		**DEPT**[a]
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	
1	158.1	7.15 (*d*, *J* = 10.2 Hz)	157.40	7.12 (*d*, *J* = 10.06 Hz)	CH
2	125.4	5.84 (*d*, *J* = 10.2 Hz)	126.14	5.90 (*d*, *J* = 10.06 Hz)	CH
3	204.6	-	205.96	-	C
4	39.9	-	40.51	-	C
5	158.2	-	49.88	2.21 (*d*, *J* = 11.65 Hz)	C
6	129.7	5.37 (*br s*)	68.08	4.38 (*dd*, *J* = 11.65, 2.37 Hz)	CH
7	74.5	5.27 (*br s*)	79.08	5.36 (*d*, *J* = 2.37 Hz)	CH
8	44.1	-	45.43	-	C
9	38.6	2.28 (*m*)	37.13	2.21 (*m*)	CH
10	42.8	-	43.11	-	C
11	16.5	1.75 (*m*); 2.03 (*m*)	16.39	1.82 (*m*)	CH_2
12	33.0	1.68 (*m*); 1.95 (*m*)	32.72	1.82 (*m*)	CH_2
13	47.1	-	47.08	-	C
14	158.8	-	158.53	-	C
15	119.6	5.33 (*br t*)	119.55	5.42 (*dd*, *J* = 1.82, 2.84 Hz)	CH
16	34.3	2.35 (*m*)	34.30	1.7 (*m*); 2.4 (*m*)	CH_2
17	51.6	2.80 (*dd*, *J* = 7.8, 10.5 Hz)	51.12	2.83 (*m*)	CH

Table 3 (Continued)

Position	Chisosiamensin		Nimonol		DEPT[a]
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	
18	27.0	1.07 (*s*)	27.08	1.14 (*s*)	CH_3
19	19.0	0.78 (*s*)	20.84	0.82 (*s*)	CH_3
20	124.5	-	124.37	-	C
21	139.6	7.23 (*br s*)	139.64	7.26 (*m*)	CH
22	111.0	6.27 (*br m*)	110.94	6.29 (*m*)	CH
23	142.5	7.37 (*br m*)	142.57	7.39 (*m*)	CH
28	27.3	1.22 (*s*)	31.87	1.31 (*s*)	CH_3
29	20.6	1.07 (*s*)	20.79	1.41 (*s*)	CH_3
30	21.1	1.19 (*s*)	20.23	1.27 (*s*)	CH_3
7-O*CO*CH_3	170.1	-	172.32	-	C
7-OCO*CH*$_3$	21.2	1.95 (*s*)	21.16	2.05 (*s*)	CH_3

[a] The data of chisosiamensin was analyzed by DEPT 90° and 135°.

3.2. Compound CS2 (Dysobinin)



Compound **CS2** was isolated as a white solid. The ^{13}C NMR and DEPT spectra (Table 4) revealed 30 carbons, including 7 methyls (δ 20.4, 20.7, 20.7, 21.3, 21.9, 26.7 and 31.6), 3 methylenes (δ 16.4, 31.4 and 34.3), 11 methines (δ 37.2, 47.9,

51.6, 69.6, 74.5, 110.9, 119.7, 126.6, 139.7, 142.6 and 157.2), and 9 quaternary carbons (δ 40.7, 42.9, 44.9, 47.0, 124.4, 170.0, 170.2, 158.1 and 204.5). The ^{1}H spectrum of **CS2** (Table 4) was closely related to those of **CS1**, expect that **CS2** showed an additional of acetoxy substituent at C-6 [δ 5.31 (*m*, H-6) and 2.06 (*s*, 6-$OCOCH_3$)] instead of an olefinic proton H-6 (δ 5.33) in **CS1**. Therefore, compound **CS2** was determined as dysobinin (Suresh, *et al.*, 1997).

Table 4 ^{1}H-NMR (300 MHz), ^{13}C-NMR (75 MHz) and DEPT spectral data of **CS2** in $CDCl_3$

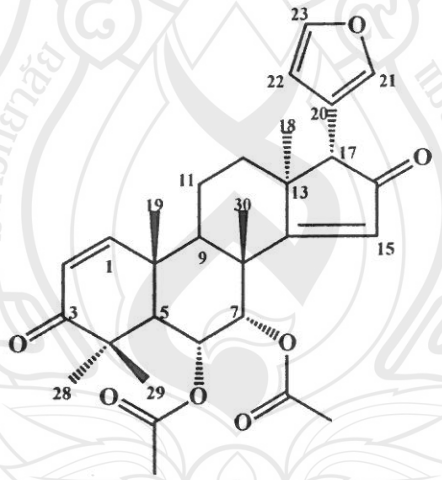
Position	δ_C	δ_H	**DEPT**
1	157.2	7.01 (*d*, *J* = 10.2)	CH
2	126.6	5.78 (*d*, *J* = 10.2)	CH
3	204.5	–	C
4	40.7	–	C
5	47.9	2.38 (*d*, *J* = 9.6 Hz)	CH
6	69.6	5.31 (*br d*, *J* = 9.6 Hz)	CH
7	74.5	5.20 (*br s*)	CH
8	44.9	–	C
9	37.2	*	CH
10	42.9	–	C
11	16.4	*	CH_2
12	31.4	*	CH_2
13	47.0	–	C
14	158.1	–	C
15	119.7	5.33 (*s*)	CH
16	34.3	2.33 (*m*)	CH_2
17	51.6	2,68 (*dd*, *J* = 6.1, 6.1)	CH
18	26.7	1.20 (*s*)	CH_3
19	20.7	0.68 (*s*)	CH_3
20	124.4	–	C
21	139.7	7.25 (*br s*)	CH

Table 4 (Continued)

Position	δ_C	δ_H (mult., *J* in Hz)	DEPT
22	110.9	7.39 (*br s*)	CH
23	142.6	6.28 (*br s*)	CH
28	31.6	1.13 (*s*)	CH_3
29	20.4	1.06 (*s*)	CH_3
30	20.7	1.06 (*s*)	CH_3
6-OCO*CH*$_3$	21.3	2.06 (*s*)	CH_3
6-O*CO*CH$_3$	170.0	–	C
7-OCO*CH*$_3$	21.9	2.01 (*s*)	CH_3
7-O*CO*CH$_3$	170.2	–	C

* Not assigned

3. 3. Compound CS3 (Mahonin)



Compound **CS3** was isolated as yellow oil. The ^{13}C NMR and DEPT spectra (Table 5) revealed 30 carbons, including 7 methyls (δ20.4, 20.7, 20.7, 21.2, 25.6, 26.7 and 31.5), 2 methylenes (δ15.7 and 29.6), 11 methines (δ36.8, 47.9, 60.8, 69.2, 73.3, 111.0, 123.5, 126.6, 141.6, 142.7 and 155.8), and 10 quaternary carbons (δ40.7, 44.7, 44.8, 47.7, 118.2, 155.8, 169.2, 170.1, 204.0 and 204.6). The ^1H and ^{13}C NMR spectral of **CS3** (Table 5) were similar to those of **CS2**, expect that **CS3** showed α

proton of α,β-unsaturated ketone at H-15 (δ 5.87, *s*). From this information, **CS3** should be determined as mahonin (Siddiqui, *et al.*, 2000; Kadota *et al.* 1990).

Table 5 ^{1}H-NMR (300 MHz), ^{13}C-NMR (75 MHz) and DEPT spectral data of **CS3** in $CDCl_3$

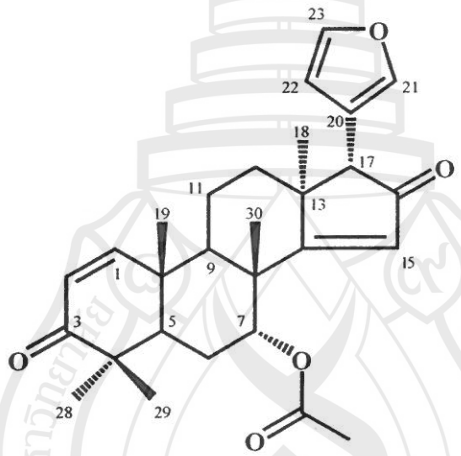
Position	δ_C	δ_H	**DEPT**
1	155.8	7.12 (*d*, *J* = 9.9)	CH
2	126.6	5.95 (*d*, *J* = 9.9)	CH
3	204.6	–	C
4	40.7	–	C
5	47.9	2.53 (*d*, *J* = 12.3)	CH
6	69.2	5.47 (*dd*, *J* = 12.3, 2.1)	CH
7	73.3	5.56 (*d*, *J* = 1.8)	CH
8	44.8	–	C
9	36.8	*	CH
10	44.7	–	C
11	15.7	*	CH_2
12	29.6	*	CH_2
13	47.7	–	C
14	155.8	–	C
15	123.5	5.87 (*s*)	CH
16	204.0	–	C
17	60.8	3.42 (*s*)	CH
18	26.7	1.02	CH_3
19	20.7	1.24 (*s*)	CH_3
20	118.2	–	C
21	141.6	7.42 (*br s*)	CH
22	111.0	6.26 (*br s*)	CH
23	142.7	7.47 (*br s*)	CH
28	31.5	1.27 (*s*)	CH_3
29	20.4	1.19 (*s*)	CH_3
30	25.6	1.43 (*s*)	CH_3

Table 5 (Continued)

Position	δ_C	δ_H	DEPT
6-OCO*CH*$_3$	21.2	1.99 (*s*)	CH_3
6-O*CO*CH$_3$	169.2	–	C
7-OCO*CH*$_3$	20.7	2.05 (*s*)	CH_3
7-O*CO*CH$_3$	170.1	–	C

* Not assigned

3.4. Compound CS4 (Azadiradione)



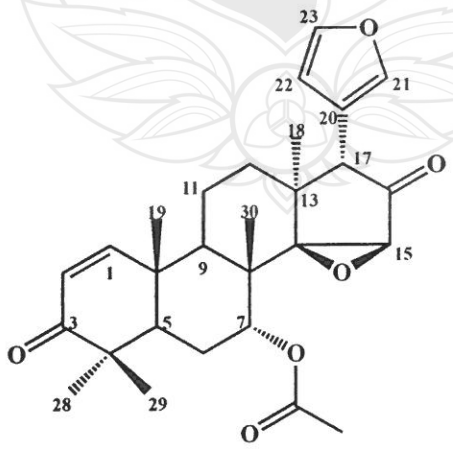
Compound **CS4** was isolated as yellowish oil. The ^{13}C NMR and DEPT spectra (Table 6) revealed 28 carbons, including 6 methyls (δ17.2, 17.9, 19.4, 20.6 21.0 and 26.5), 3 methylenes (δ 14.4, 29.1 and 31.4), 10 methines (δ 39.0, 45.5, 56.3, 72.7, 109.3, 119.9, 119.9, 140.6, 142.5 and 156.4) and 9 quaternary carbons (δ 39.5, 42.1, 42.1, 43.5, 125.4, 156.4, 169.3, 203.3 and 203.4). The ^{1}H and ^{13}C spectral data of **CS4** (Table 6) were almost identical to that of **CS3**, except that **CS4** appeared an acetoxyl group at C-7, while **CS3** showed two acetoxyl group at C-6 and C-7. Therefore, **CS4** should be identified as azadiradione (Lavie, *et al.*, 1971).

Table 6 ^{1}H-NMR (300 MHz), ^{13}C-NMR (75 MHz) and DEPT spectral data of **CS4** in $CDCl_3$

Position	δ_C	δ_H	DEPT	Position	δ_C	δ_H	DEPT
1	156.4	7.10 (*d*, *J* = 10.2)	CH	15	119.9	5.60 (*s*)	CH
2	119.9	5.93 (*d*, *J* = 10.2)	CH	16	203.3	–	C
3	203.4	–	C	17	56.3	3.51 (*s*)	CH
4	39.5	–	C	18	26.6	1.05 (*s*)	CH_3
5	45.5	*	CH	19	17.2	1.23 (*s*)	CH_3
6	29.1	*	CH_2	20	125.4	–	C
7	72.7	4.54 (*br s*)	CH	21	140.6	7.04 (*br s*)	CH
8	42.1	–	C	22	109.3	6.33 (*br s*)	CH
9	39.0	2.48 (*dd*, *J* = 12.0, 6.3 Hz)	CH	23	142.5	7.04 (*br s*)	CH
10	42.1	–	C	28	21.0	1.21 (*s*)	CH_3
11	14.4	*	CH_2	29	17.9	1.14 (*s*)	CH_3
12	31.4	*	CH_2	30	19.4	1.23 (*s*)	CH_3
13	43.5	–	C	7-OCO*C*H_3	20.6	2.09 (*s*)	CH_3
14	156.4	–	C	7-O*C*OCH$_3$	169.3	–	C

*Not assigned

3.5. Compound CS5 (Epoxyazadiradione)



Compound **CS5** was isolated as yellow oil, showed a molecular formula $C_{28}H_{34}O_6$. The ^{13}C NMR and DEPT spectra (Table 7) revealed 28 carbons, including 6 methyls (δ 19.3, 20.0, 20.9, 21.2, 26.9 and 29.0), 3 methylenes (δ 16.0, 29.6 and 29.6), 10 methines (δ 39.8, 46.4, 50.8, 57.1, 73.5, 110.8, 125.6, 141.4, 142.3 and 157.4) and 9 quaternary carbons (δ 42.4, 43.0, 44.1, 46.6, 72.4, 125.6, 169.6, 204.1 and 208.3). The ^1H and ^{13}C spectral data of **CS5** (Table 7) were similar correlation with **CS4** except **CS4** appeared the epoxy proton H-15 at δ 3.86 instead of an olefinic proton H-15 at δ 5.60. Therefore, compound **CS5** should be identified as epoxyazadiradione (Singh, *et al.*, 1976).

Table 7 ^1H-NMR (300 MHz), ^{13}C-NMR (75 MHz) and DEPT spectral data of **CS5** in $CDCl_3$

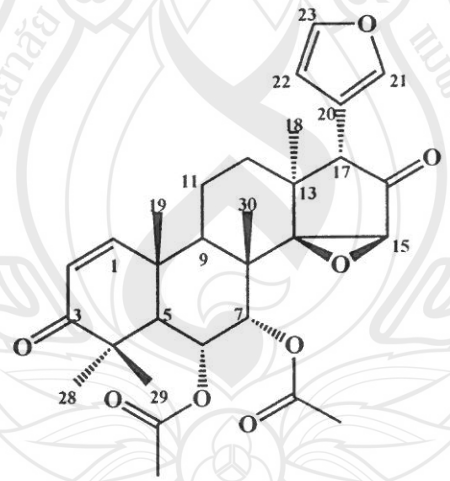
Position	δ_C	δ_H	**DEPT**
1	157.4	7.15 (*d*, *J* = 10.2)	CH
2	125.6	5.75 (*d*, *J* = 10.2)	CH
3	204.1	–	C
4	42.4	–	C
5	46.4	*	CH
6	29.6	*	CH_2
7	73.5	4.70 (*br s*)	CH
8	43.0	–	C
9	39.8	2.60 (*dd*, 4.2, 12.6 Hz)	CH
10	44.1	–	C
11	16.0	*	CH_2
12	29.6	*	CH_2
13	46.6	–	C
14	72.4	–	C
15	57.1	3.86 (*s*)	CH
16	208.3	–	C
17	50.8	3.38 (*s*)	CH
18	29.0	1.05 (*s*)	CH_3

Table 7 (Continued)

Position	δ_C	δ_H	DEPT
19	20.0	1.19 (*s*)	CH_3
20	125.6	–	C
21	141.4	7.37 (*br s*)	CH
22	110.8	6.21 (*br s*)	CH
23	142.3	7.53 (*br s*)	CH
28	26.9	1.01 (*s*)	CH_3
29	21.2	1.05 (*s*)	CH_3
30	19.3	1.23 (*s*)	CH_3
7-OCO*CH*$_3$	20.9	2.00 (*s*)	CH_3
7-O*CO*CH$_3$	169.6	–	C

*Not assigned

3.6. Compound CS6 (6α-Acetoxyepoxyazadiradione)



Compound CS6 was isolated as yellow oil, showed a molecular formula $C_{30}H_{36}O_8$. The ^{13}C NMR and DEPT spectra data (Table 8) revealed 30 carbons, including 7 methyls (δ 18.9, 20.1, 21.0, 21.1, 21.4, 24.6 and 31.5), 2 methylenes (δ 16.1 and 28.6), 11 methines (δ 38.4, 48.5, 50.7, 57.0, 69.8, 72.8, 110.8, 126.4, 141.6, 142.4 and 156.6) and 10 quaternary carbons (δ 40.4, 42.4, 43.2, 45.1, 73.0, 126.4,

169.8, 169.8 204.2 and 207.8,). The ^{1}H and ^{13}C NMR spectra exhibited signals similar to those of **CS5** except **CS6** appeared an additional acetoxy unit at C-6 in ^{1}H NMR spectrum (Table 8). Thus, **CS6** was characterized to be 6α-acetoxyepoxyazadiradione (Kadota, *et al.*, 1990). This compound was also confirmed by X-ray crystallography (Figure 2) (Wisanu, *et al.*, 2007).

Table 8 ^{1}H-NMR (300 MHz), ^{13}C-NMR (75 MHz) and DEPT spectral data of **CS6** in $CDCl_3$

Position	δ_C	δ_H	**DEPT**
1	156.6	7.12 (*d*, *J* = 10.2)	CH
2	126.4	5.95 (*d*, *J* = 10.2)	CH
3	204.2	–	C
4	40.4	–	C
5	48.5	2.52 (*d*, *J* = 12.3)	CH
6	69.8	5.36 (*dd*, *J* = 12.3, 2.1)	CH
7	72.8	5.02 (*d*, *J* = 2.1)	CH
8	43.2	–	C
9	38.4	2.68 (*dd*, 12.0, 4.2 Hz)	CH
10	42.4	–	C
11	16.1	*	CH_2
12	28.6	*	CH_2
13	45.1	–	C
14	73.0	–	C
15	57.0	3.88 (*s*)	CH
16	207.8	–	C
17	50.7	3.42 (*s*)	CH
18	24.6	1.03 (*s*)	CH_3
19	18.9	1.20 (*s*)	CH_3
20	126.4	–	C
21	141.6	7.39 (*br s*)	CH
22	110.8	6.22 (*br s*)	CH
23	142.4	7.55 (*br s*)	CH

Table 8 (Continued)

Position	δ_C	δ_H	DEPT
28	31.5	1.24 (*s*)	CH_3
29	20.1	1.16 (*s*)	CH_3
30	21.4	1.32 (*s*)	CH_3
6-OCO*CH*$_3$	21.1	2.11 (*s*)	CH_3
6-O*CO*CH$_3$	169.8	–	C
7-OCO*CH*$_3$	21.0	2.01 (*s*)	CH_3
7-O*CO*CH$_3$	169.8	–	C

* Not assigned

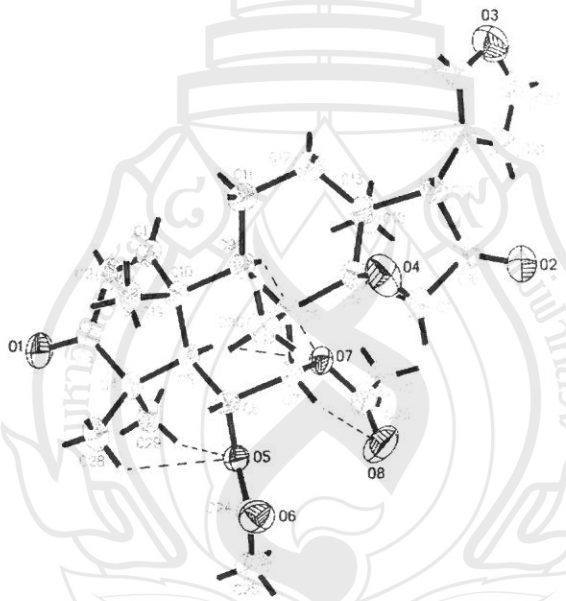
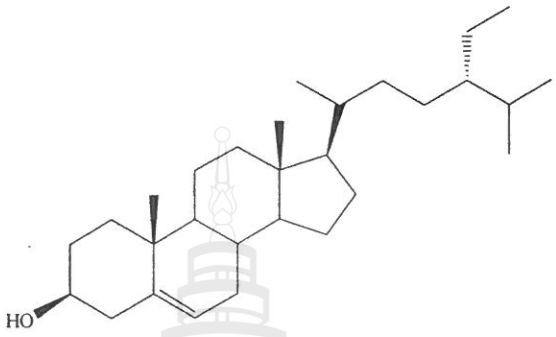


Figure 2 X-ray ORTEP of **CS6**

3.7 Compound CS7



Compound **CS7** was isolated as a white solid, mp = 128-130 °C. The IR spectrum showed absorption band at 3415 cm^{-1} (O-H stretching). The ^1H NMR spectrum of this compound was identical to those of β-sitosterol. Thus compound **CS7** was identified as β-sitosterol.

3.8 Compound CS8

The structural elucidation of compound **CS8** is under investigation. Some interesting ^1H NMR of this compound are summarized below:

Six olefinic protons resonated at δ 6.89 (1H, m), 6.80 (2H, m), 6.77 (1H, m), 5.75 (2H, m) and 5.49 (2H, d). Four oxymethines protons resonated at δ 4.32 (2H, s) and 4.20 (2H, s) and seven methyls groups resonated δ 1.6-1.9.

3.9 Biological activity evaluation

As summarized in Table 9, the crude extract and pure limonoids **(2-6)** were evaluated for their antimalarial activity against *P. falciparum*, antimycobacterial activity against *M. tuberculosis* and cytotoxic activity against NCI-H187 (human lung cancer), KB (oral human epidermal carcinoma) MCF-7 (breast cancer) cancer cell lines. The crude extract showed strong inhibitory effect against *P. falciparum* with IC_{50} 0.784 μg/ml and weak inhibitory effect against *M. tuberculosis* with MIC 100 μg/ml. Also, the crude extract exhibited strong cytotoxic activity against NCI-H187,

KB, MCF-7 cancer cell lines with IC_{50} 2.78, 5.43 and 5.33 μg/ml, respectively. In case of pure compounds, limonoids **2-6** exhibited moderate inhibitory effect against *P. falciparum* with the IC_{50} of 2.06, 2.91, 2.92, 3.18 and 6.31 μg/ml, respectively (Table 9). All compounds were also found to be active with antimycobacterial activity against *M. tuberculosis* and only azadiradione (**4**) showed strong inhibitory effect with the MIC value of 6.25 μg/ml. In case of cytotoxic activity, all limonoids showed inhibitory effects against all three cancer cell lines, except 6α-acetoxyepoxyazadiradione (**6**) was found to be inactive with all three cancer cell lines (Table 9). Dysobinin (**2**) exhibited strong active against NCI-H187, KB and MCF-7 cancer cell lines with the IC_{50} values of 3.17, 1.67 and 2.15 μg/ml, respectively.

Table 9 Biological activity of crude extract and compounds **CS2-CS6**

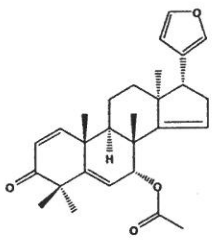
Compound	Cytotoxic activity (IC_{50}, μg/ml)			Antimalarial activity[d]	Antimycobacterial activity[f]
	KB[a]	NCI-H187[b]	MCF7[c]	(IC_{50}, μg/ml)	(MIC, μg/ml)
CSA	5.43	2.78	5.33	0.784	100.00
2	3.17	1.67	2.15	2.06	200.00
3	In active	15.61	18.42	2.92	50.00
4	9.38	6.44	7.13	2.91	6.25
5	12.87	7.54	4.68	3.18	25.00
6	In active	In active	In active	6.31	200.00
Elliticine	0.217	0.592	0.738	-	-
Doxorubicin	0.096	0.023	0.149	-	-
Kanamycin	-	-	-	-	1.25
Rifampicin	-	-	-	-	0.019
Isoniazid	-	-	-	-	0.052
Dihydroartemisinine	-	-	-	e	-

CSA = Crude extract of the seeds of *Chisocheton siamensis*; [a]KB = Oral human epidermal carcinoma; [b]NCI-H187 = human small cell lung cancer; [c]MCF-7 = Breast cancer; [d]Against *Plasmodium falciparum*; [e]IC_{50} of dihydroartemisinine = 4.4 nM; [f]Against *Mycobacterium tuberculosis*.

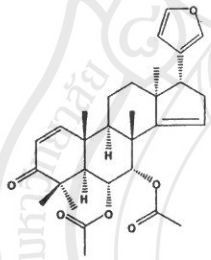
CHAPTER 4

CONCLUSION

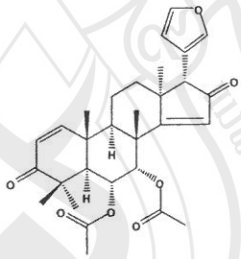
Eight compounds have been isolated from the seeds (**CS1-CS6**) and pericarps (**CS7** and **CS8**) of *Chisocheton siamensis*. One of them was new compounds namely chisosiamensin (**CS1**). Compounds **CS2-6** were tested for their antimalarial activity against *Plasmodium falciparum*, antimycobacterial activity against *Mycobacterium tuberculosis* and cytotoxic activity against NCI-H187 (human small cell lung cancer), KB (oral human epidermal carcinoma) and MCF-7 (breast cancer) cancer cell lines. Limonoid **CS2** showed the best inhibitory effect against *Plasmodium falciprarum* with IC_{50} of 2.06 μg/ml. Also, compound **CS2** had the highest cytotoxic activity against NCI-H187, KB and MCF-7 cancer cell lines with the IC_{50} of 1.67, 3.17 and 2.15 μg/ml, respectively. Only compound **CS4** had a strong activity with the MIC value of 6.25 μg/ml.



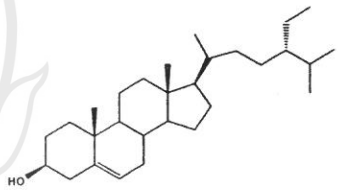
Chisosiamensin (CS1)



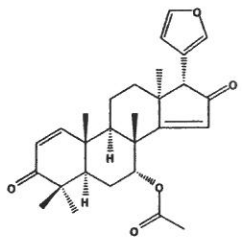
Dysobinin (CS2)



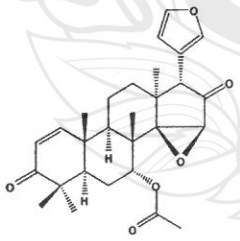
Mahonin (CS3)



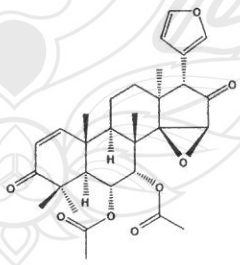
Sitisterol (CS7)



Azadiradione (CS4)



Epoxyazadiradione (CS5)



6α-Acetoxyepoxyazadiradione (CS6)

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