องค์ประกอบทางเคมีและความเป็นพิษต่อเซลล์มะเร็งจากราก
พลับพลา *Microcos tomentosa* Smith. รากชะมวง *Garcinia cowa* Roxb. และรากเครือคาง
ควาย *Dalbergia velutina* Benth.

นายสุทิน แก่นนาคำ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2557

CHEMICAL CONSTITUENTS AND CYTOTOXICITY FROM THE ROOTS OF *Microcos tomentosa* Smith., *Garcinia cowa* Roxb. AND *Dalbergia velutina* Benth.

Mr. Sutin Kaennakam

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University
Academic Year 2014

Thesis Title	CHEMICAL CONSTITUENTS AND CYTOTOXICITY FROM THE ROOTS OF *Microcos tomentosa* Smith., *Garcinia cowa* Roxb. AND *Dalbergia velutina* Benth.
By	Mr. Sutin Kaennakam
Field of Study	Chemistry
Thesis Advisor	Associate Professor Santi Tip-pyang, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

.....Dean of the Faculty of Science
(Professor Supot Hannongbua, Ph.D.)

THESIS COMMITTEE

.....Chairman
(Associate Professor Vudhichai Parasuk, Ph.D.)
.....Thesis Advisor
(Associate Professor Santi Tip-pyang, Ph.D.)
.....Examiner
(Assistant Professor Worawan Bhanthumnavin, Ph.D.)
.....Examiner
(Assistant Professor Aroonsiri Shitangkoon, Ph.D.)
.....External Examiner
(Assistant Professor Jongkolnee Jongaramruong, Ph.D.)

สุทิน แก่นนาคำ : องค์ประกอบทางเคมีและความเป็นพิษต่อเซลล์มะเร็งจากรากพลับพลา *Microcos tomentosa* Smith. รากชะมวง *Garcinia cowa* Roxb. และรากเครือคางควาย *Dalbergia velutina* Benth. (CHEMICAL CONSTITUENTS AND CYTOTOXICITY FROM THE ROOTS OF *Microcos tomentosa* Smith., *Garcinia cowa* Roxb. AND *Dalbergia velutina* Benth.) อ .ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.สันติ ทิพยางค์, หน้า.

การศึกษาองค์ประกอบทางเคมีและความเป็นพิษต่อเซลล์มะเร็งจากรากพลับพลา *M. tomentosa* พบสารกลุ่ม 3*β*-*O*-vanilloyl-taraxerol ชนิดใหม่ 1 ชนิด คือ microcisin (2.1) พร้อมทั้งสารที่เคยมีการรายงานแล้ว 9 ชนิด (2.2-2.9) โดยพิสูจน์เอกลักษณ์ทางโครงสร้างของสารทั้งหมดที่แยกได้ด้วยเทคนิคทางสเปกโทรสโกปี สำหรับการทดสอบความเป็นพิษต่อเซลล์มะเร็งชนิด KB และ HeLa พบว่าสาร 2.1, 2.3 และ 2.5 มีความเป็นพิษต่อเซลล์มะเร็งชนิด KB ในระดับปานกลาง โดยมีค่า IC_{50} เท่ากับ 24.98, 28.06 และ 22.57 µM ตามลำดับ นอกจากนี้ยังพบว่าสาร 2.3 มีความเป็นพิษต่อเซลล์มะเร็งชนิด HeLa ในระดับปานกลาง โดยมีค่า IC_{50} เท่ากับ 29.38 µM

การศึกษาองค์ประกอบทางเคมีและความเป็นพิษต่อเซลล์มะเร็งจากรากพลับพลา *G. cowa* พบสารกลุ่ม xanthones ชนิดใหม่ 3 ชนิด คือ kaennacowanols A-C (3.1-3.3) พร้อมทั้งสารที่เคยมีการรายงานแล้ว 19 ชนิด (3.4-3.22) โดยพิสูจน์เอกลักษณ์ทางโครงสร้างของสารทั้งหมดที่แยกได้ด้วยเทคนิคทางสเปกโทรสโกปี สำหรับการทดสอบความเป็นพิษต่อเซลล์มะเร็งชนิด KB และ HeLa พบว่าสาร 3.17 และ 3.22 มีความเป็นพิษต่อเซลล์มะเร็งชนิด KB ในระดับดี โดยมีค่า IC_{50} เท่ากับ 7.97 และ 9.10 µM ตามลำดับ นอกจากนี้ยังพบว่าสาร 3.15 มีความเป็นพิษต่อเซลล์มะเร็งชนิด HeLa ในระดับดี โดยมีค่า IC_{50} เท่ากับ 9.34 µM

การศึกษาองค์ประกอบทางเคมีและความเป็นพิษต่อเซลล์มะเร็งจากรากพลับพลา *D. velutina* พบสารกลุ่ม pterocarpans ชนิดใหม่ 3 ชนิด คือ velucarpins A-C (4.1-4.3) สารกลุ่ม isoflavanes ชนิดใหม่ 3 ชนิด คือ kaennavelutinols A-C (5.1-5.3) และ สารกลุ่ม isoflavone glycoside ชนิดใหม่ 1 ชนิด คือ kaennavelutinose (6.1) พร้อมทั้งสารที่เคยมีการรายงานแล้ว 11 ชนิด เป็นสารในกลุ่ม pterocarpans 3 ชนิด (4.4-4.6) สารกลุ่ม isoflavanes 2 ชนิด (5.4-5.5) และกลุ่ม isoflavones 6 ชนิด (6.2-6.7) โดยพิสูจน์เอกลักษณ์ทางโครงสร้างของสารทั้งหมดที่แยกได้ด้วยเทคนิคทางสเปกโทรสโกปี สำหรับการทดสอบความเป็นพิษต่อเซลล์มะเร็งชนิด KB และ HeLa พบว่าสาร 4.3, 4.5, 5.3 และ 5.5 มีความเป็นพิษต่อเซลล์มะเร็งทั้งชนิด KB และ HeLa ในระดับดี โดยมีค่า IC_{50} เท่ากับ 8.22, 8.09, 8.29, 3.47 µM และ 5.99, 8.69, 9.54, 5.17 µM ตามลำดับ

ภาควิชา เคมี ลายมือชื่อนิสิต .....

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2557

5572849223 : MAJOR CHEMISTRY
KEYWORDS: MICROCISIN, KAENNACOWANOLS A-C, KAENNAVELUTINANS A-C, KAENNAVELUTINOLS A-C, KAENNAVELUTINOSE

SUTIN KAENNAKAM: CHEMICAL CONSTITUENTS AND CYTOTOXICITY FROM THE ROOTS OF *Microcos tomentosa* Smith., *Garcinia cowa* Roxb. AND *Dalbergia velutina* Benth.. ADVISOR: ASSOC. PROF. SANTI TIP-PYANG, Ph.D., pp.

A new 3*β*-*O*-vanilloyl-taraxerol, microcisin (2.1) and eight known compounds (2.2-2.9) were isolated from the roots of *M. tomentosa*. Their structures were determined by spectroscopic analysis. All isolated compounds were evaluated for their cytotoxicity against KB and HeLa cell lines. Compounds 2.1, 2.3 and 2.5 showed moderate cytotoxicity against KB cell lines with IC_{50} values of 24.98, 28.06 and 22.57 µM, respectively. On the other hand, compound 2.3 showed moderate cytotoxicity against HeLa cells with an IC_{50} value of 29.38 µM.

Three new xanthones, kaennacowanols A-C (3.1-3.3) together with nineteen known xanthones (3.4-3.22) were isolated from the roots of *G. cowa*. Their structures were determined by spectroscopic analysis. All isolated compounds were evaluated for their cytotoxicity against KB and HeLa cell lines. Compounds 3.17 and 3.22 showed good cytotoxicity against KB cell with IC_{50} values of 7.97 and 9.10 µM, respectively. On the other hand, compound 3.15 showed good cytotoxicity against HeLa cell with IC_{50} value of 9.34 µM.

Three new pterocarpans, velucarpins A-C (4.1-4.3), three new isoflavanes, kaennavelutinols A-C (5.1-5.3) and a new isoflavone glycoside, kaennavelutinose (6.1) together with eleven known compounds including three known pterocarpans (4.4-4.6), two known isoflavanes (5.4-5.5) and six known isoflavones (6.2-6.7) were isolated from the roots of *D. velutina*. Their structures were determined by spectroscopic analysis. All isolated compounds were evaluated for their cytotoxicity against KB and HeLa cell lines. Compounds 4.3, 4.5, 5.3 and 5.5 showed good cytotoxicity against KB and HeLa cells with IC_{50} values of 8.22, 8.09, 8.29, 3.47 µM and 5.99, 8.69, 9.54, 5.17 µM, respectively.

Department:	Chemistry	Student's Signature	.....
Field of Study:	Chemistry	Advisor's Signature	.....
Academic Year:	2014		

ACKNOWLEDGEMENTS

I would like to express my faithful gratitude to my advisor, Associate Professor Dr. Santi Tip-pyang for his assistance and encouragement in conducting this research.

I also gratefully acknowledge the committee of my thesis, Associate Professor Dr. Vudhichai Parasuk, Assistant Professor Dr. Worawan Bhanthumnavin, Assistant Professor Dr. Aroonsiri Shitangkoon and Assistant Professor Dr. Jongkolnee Jongaramruong for their discussion and guidance.

The specially thank to Dr. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University for plants identification and making the voucher specimen of plants in this research. My appreciation is also expressed to thank Dr. Pongpan Siripong and the staffs of Natural Products Research Section, Research Division, National Cancer Institute for testing on cytotoxicity against KB and HeLa cell lines experiment.

I would like to express my gratitude to the Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University for providing the chemicals and facilities throughout the course of study. A deep affectionate gratitude is acknowledged to my family for their understanding, encouragement and support throughout the education course.

My specially thank to Ms. Sangmanee Sittiwongsa, Dr. Subin Chaiya and Dr. Wisuttaya Worawalai for their technical assistance. On the other hand, I would like to thank all of my friends in the laboratory for their friendships and help during the course of my graduate research.

Finally, I would like to thank Graduate School of Chulalongkorn University for the financial supports, which is highly appreciated.

CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

LIST OF ABBREVIATIONS

^{13}C NMR	carbon 13 nuclear magnetic resonance
^{1}H NMR	proton nuclear magnetic resonance
br t	broad triplet (NMR)
COSY	correlated spectroscopy
CD	circular dichroism
c	concentration
d	doublet (NMR)
ESIMS	electrospray ionization mass spectrometry
HMBC	heteronuclear multiple bond correlation
HRESIMS	high resolution electrospray ionization mass spectrometry
HSQC	heteronuclear single quantum correlation
Hz	hertz
IC_{50}	concentration that is required for 50% inhibition in vitro
IR	infrared
J	coupling constant
m	multiplet (NMR)
M	molar
mg	milligram (s)
MHz	megahertz
min	minute
mL	milliliter (s)
NMR	nuclear magnetic resonance
q	quartet (NMR)
s	singlet (NMR)
t	triplet (NMR)
UV	ultraviolet
λmax	maximum wavelength
$[a]_D^{20}$	specific optical rotation
δ_C	chemical shift of carbon

δ_H	chemical shift of proton
μ	micro
Δε	delta epsilon

CHAPTER I
INTRODUCTION

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. These cells are born due to imbalance in the body and by correcting this imbalance. Cancer continues to be one of the major causes of death worldwide and only modest progress has been made in reducing the morbidity and mortality of this disease. Several chemo preventive agents are used to treat cancer, but they cause toxicity that restricts their usage [1].

Plants, since ancient time, are using for health benefits by all cultures as well as source of medicines. It has been estimated that about 80-90% of worldwide population depend on traditional medicines for their primarily health care needs and it is assumed that a major part of traditional therapeutics involve the use of plant extracts or their active principles. Although a lot of current investigations have been carried out for progression in the treatment and control of cancer advancement, significant work and room for development remain. The major disadvantages of synthetic drugs are the associated side effects. However natural therapies, such as the use of the plants or plant derived natural products are being useful to fight cancer. The search for anti-cancer agents from plant sources started in the 1950s when discovery and development of the vinca alkaloids (vinblastine and vincristine) and the isolation of the cytotoxic podophyllotoxins was carried out [2].

In this research, the anti-cancer agents from plants (*Microcos tomentosa* Smith., *Garcinia cowa* Roxb. and *Dalbergia velutina* Benth.) are explored by isolation and purification of compounds from the roots of these plants and evaluated the cytotoxicity of all isolated compounds.

1.1 RESEARCH BACKGROUND AND RATIONALE

1.1.1 Botanical aspect and distribution of *Microcos tomentosa* Smith.

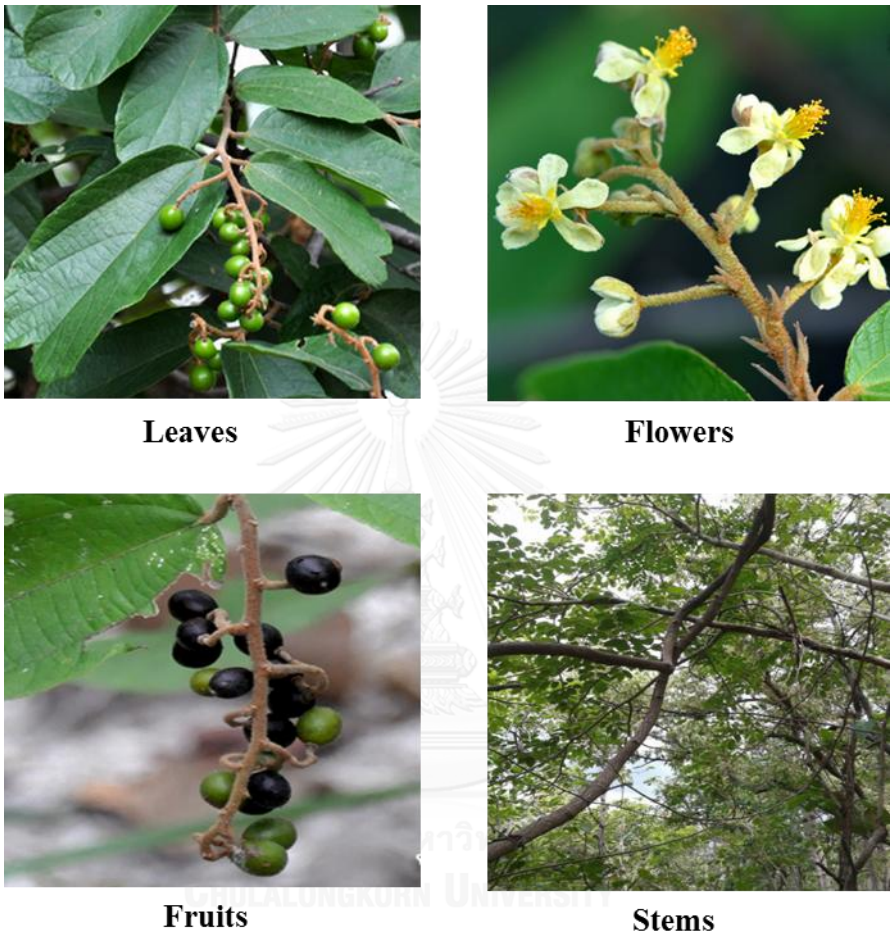


Figure 1.1 The leaves, flowers, fruits and stems of *M. tomentosa* Smith.

Scientific name: *Microcos tomentosa* Smith.

Synonym: *Grewia paniculata* DC.

Family: Tiliaceae

Genus: *Microcos*

Description: Evergreen tree up to 20 m tall; Bark pale brown, inner bark fibrous; Twigs terete, hairy; Leaves simple, alternate and distichous, with stellate hairs, elliptic or obovate, apex acute to acuminate, base rounded, margin toothed at the top; Flowers

arranged in a many-flowered inflorescence, terminal or in the upper axils, bisexual, pedicels up to 3 mm long; Fruit up to 2.5 cm long, drupe orange-red, leathery.
Distribution: Myanmar, Malaysia, Philippines, Thailand, Indochina and Laos

1.1.2 Chemical constituents from *Microcos* species and their biological activities

The EtOAc extract from the stem of *M. paniculata* was isolated to obtain five compounds, a new triterpene named methyl 3*β*-*O*-*p*-hydroxy-*E*-cinnamoyloxy-2*α*,23-dihydroxyolean-12-en-28-oate (**M1**), whose spectral data are presented for the first time, together with three known compounds, epicatechin (**M2**), 3-*trans*-feruloyl maslinic acid (**M3**) and maslinoate (**M4**) by using a normal-phase and reverse-phase silica gel column chromatography. All of the compounds were isolated from this plant for the first time. The compounds were identified by spectroscopic methods. Among them, compound **M2** displayed significant free-radical-scavenging activity which is similar to that of standard antioxidant ascorbic acid and therefore may be a promising natural antioxidant [3].

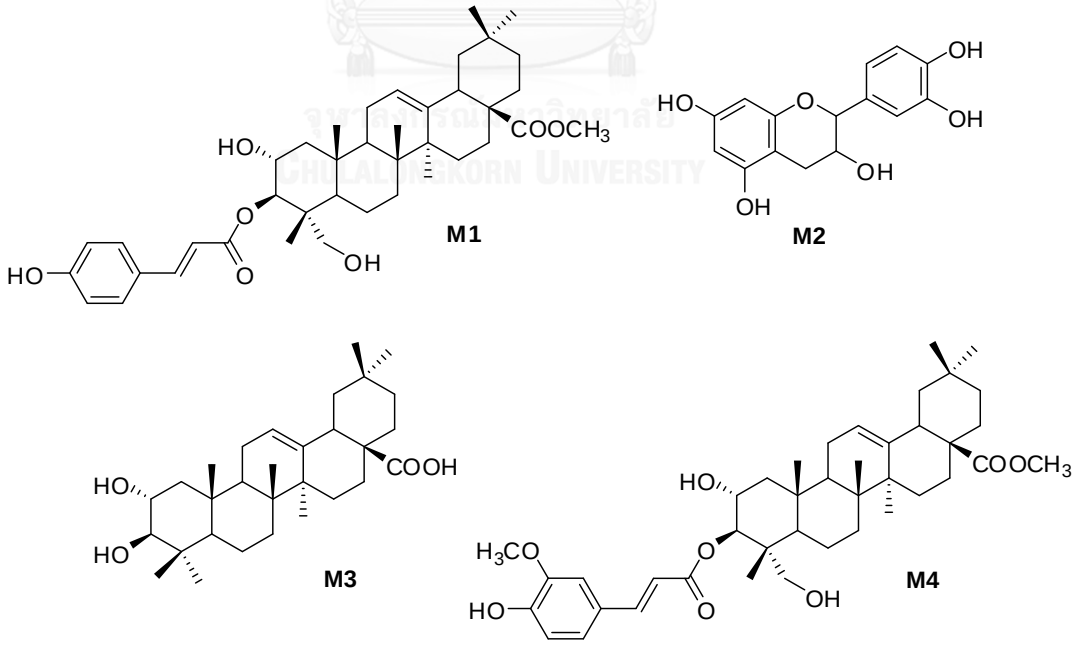


Figure 1.2 Isolated compounds from *M. paniculata.*

1.1.3 Botanical aspect and distribution of *Garcinia cowa* Roxb.

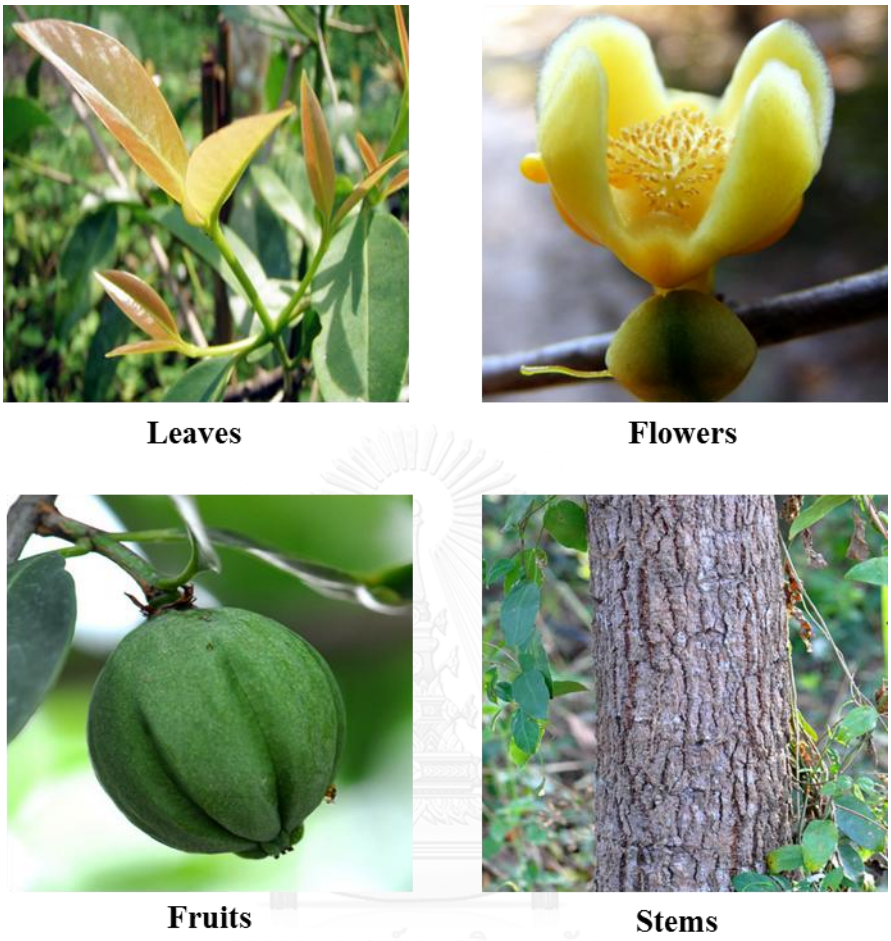


Figure 1.3 The leaves, flowers, fruits and stems of *G. cowa* Roxb.

Scientific name: *Garcinia cowa* Roxb.

Synonym: *Garcinia kydia* Roxb., *Garcinia lobulosa* Wall., *Garcinia roxburghii* Wight., *Garcinia umbellifera* Roxb.

Family: Clusiaceae

Genus: *Garcinia*

Description: Trees 8-12 m tall; Bark dark brown; Branches many, borne toward top of trunk, horizontal but usually distally pendulous, slender; Twigs dark brown, striate; Leaf blade lanceolate or oblong-lanceolate, 6-14 × 2-5 cm, papery, midvein raised abaxially, impressed adaxially; Fruit opaquely yellow-brown, ovoid-globose, oblique,

5-6 × 4-5 cm in diam, 4-8-sulcate, usually apiculate, pinkish red, looking similar to tomato; Seeds 2-4, narrow, fusiform, slightly curved, rough.

Distribution: India, Myanmar, Thailand, Cambodia, Laos, Vietnam and Malaysia

1.1.4 Chemical constituents from *Garcinia cowa* and their biological activities

Many parts of *G. cowa* have been used in traditional folk medicine. For example, the bark, latex and root have been used as an antifever agent while the fruit and leaves have been used or indigestion and improvement of blood circulation, and as an expectorant. The major compounds are xanthones and phloroglucinols. However, minor compounds, including depsidones, terpenoids, steroids and flavonoids were also observed. Currently, 78 compounds have been isolated from the twig, stem, fruit and latex. This review mainly focuses on the chemical structures and biological activities of the phytochemicals isolated from *G. cowa* and covers the literature up to April 2012. The biological activities of the extracts from various parts of *G. cowa* have been investigated, including antimicrobial, anti-inflammatory, antimalarial, antioxidant and cytotoxicity [4].

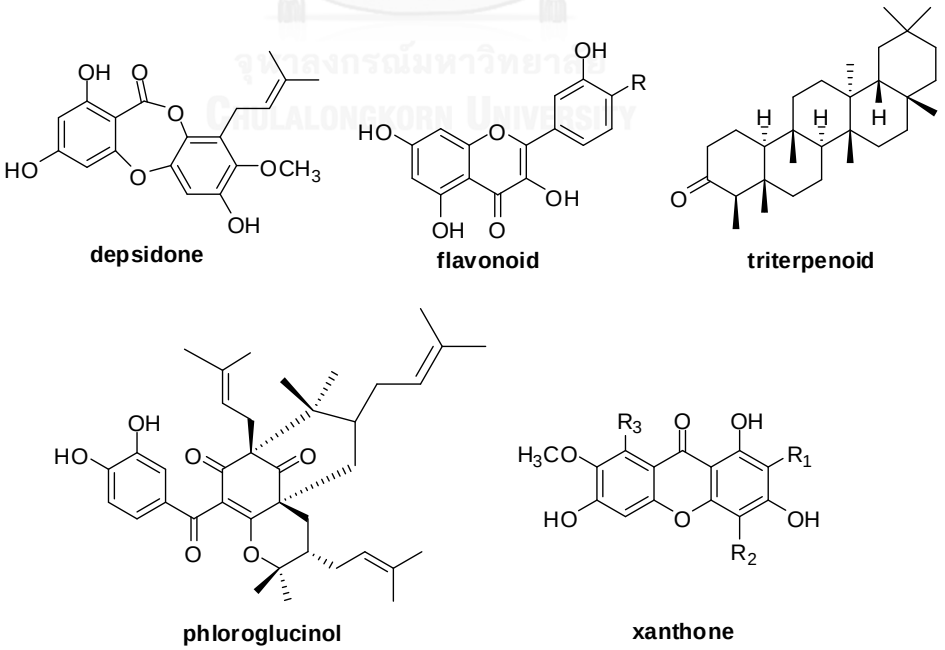


Figure 1.4 Isolated compounds from *G. cowa.*

The acetone extract from the immature fruits of *G. cowa* was isolated to obtain two new tetraoxygenated xanthones, garcicowanones A (**G1**) and B (**G2**), together with eight known tetraoxygenated xanthones (**G3-G10**). Their structures were determined by spectroscopic analysis. The antibacterial activity of the all isolated compounds is also reported. Compound **G8** was significantly active against three Gram-positive bacteria strains with MIC values 0.25-1 μg/mL and compounds **G1** and **G4** also had strong antibacterial activities against *Bacillus cereus* with the same MIC value of 0.25 μg/mL. Interestingly, an earlier study on the ripe fruit of *G. cowa* resulted in the isolation of benzoylphloroglucinols [5].

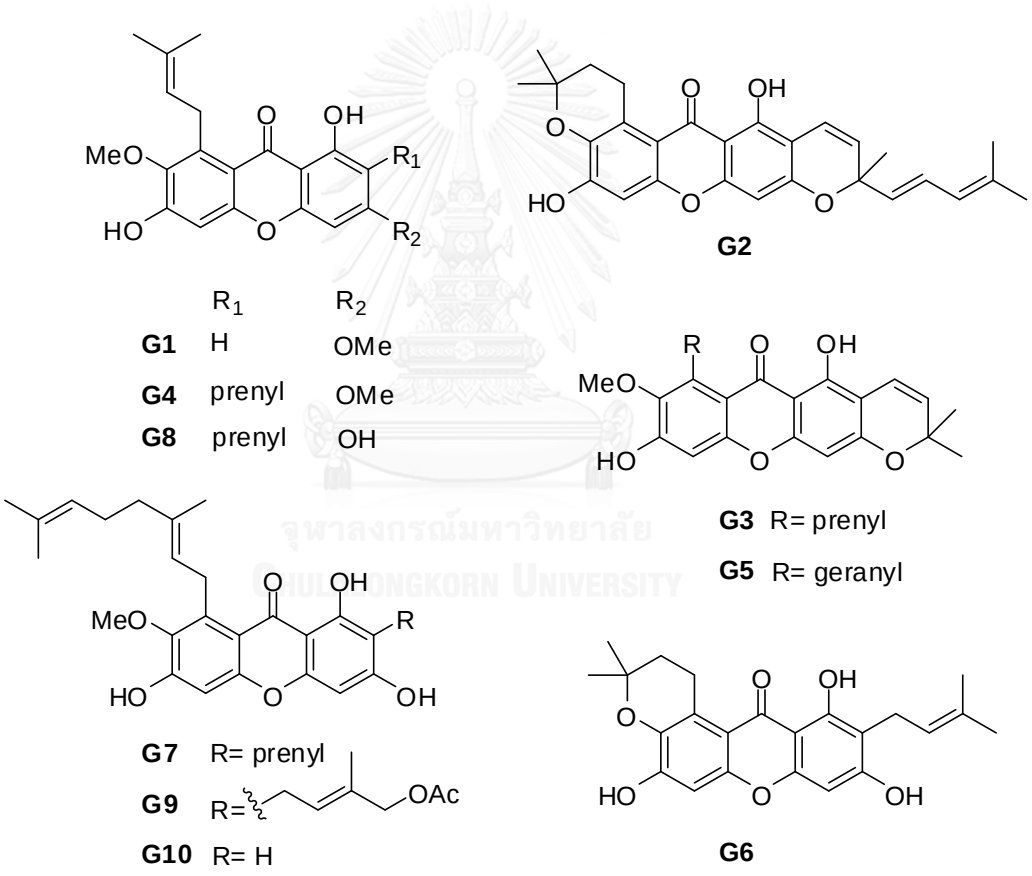


Figure 1.5 Isolated compounds from *G. cowa.*

The ripe fruits of *G. cowa* was isolated to obtain two new tetracyclo [7.3.3.3^{3,11}.0^{3,7}] tetradecane-2,12,14-trione derivatives, cowabenzophenones A (**G11**) and B (**G12**). Their structures were determined by spectroscopic methods. The

tetracyclo [7.3.3.3^{3,11}.0^{3,7}] tetradecane-2,12,14-trione skeleton from the *Garcinia* genus is reported for the first time [6].

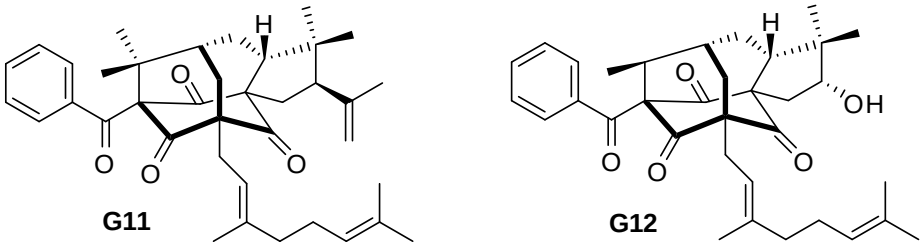


Figure 1.6 Isolated compounds from *G. cowa.*

1.1.5 Botanical aspect and distribution of *Dalbergia velutina* Benth.

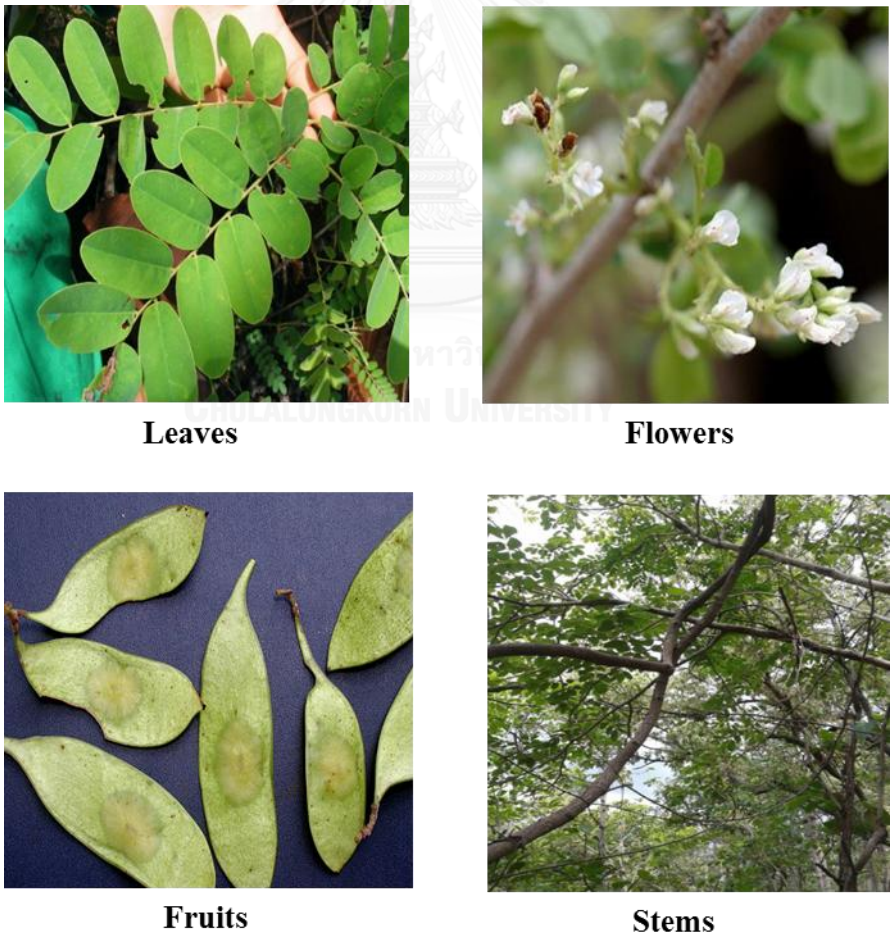


Figure 1.7 The leaves, flowers, fruits and stems of *D. velutina* Benth.

Scientific name: *Dalbergia velutina* Benth.

Synonym: *Dalbergia velutina* Var.

Family: Leguminosae

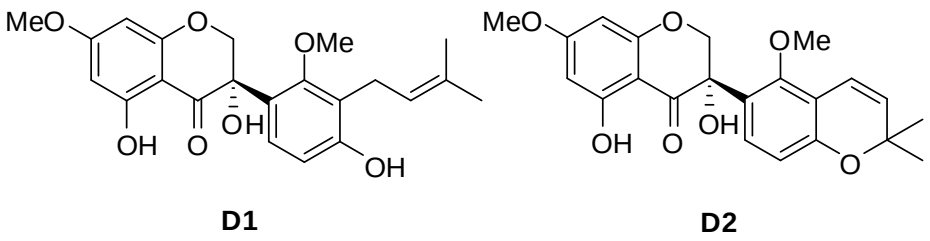
Genus: *Dalbergia*

Description: Perennial climbing shrub; Bark dark brown; Branches many, borne toward top of trunk; Twigs dark brown; Leaf compound 7.0-15.0 cm long, leaflets 9-15, 2.5-5.0 cm long, ovate or obovate or elliptic, often emarginate, glabrous, glaucous; Flower tube dull white, vexillum c. 5-10 mm long, stamens 10, in 2 groups of 5 stamens each; Fruit 5-8 cm long, narrowed at both ends, glabrous, usually 1-seeded.

Distribution: Thailand, Cambodia, Laos, Vietnam and Malaysia

1.1.6 Chemical constituents from *Dalbergia* species and their biological activities

The stem bark of *D. melanoxylon* was isolated to obtain two new 3-hydroxy isoflavanones, (S)-3,4′,5-trihydroxy-2′,7-dimethoxy-3′-prenylisoflavanone (**D1**) and (S)-3,5-dihydroxy-2′,7-dimethoxy-2″,2″-dimethylpyrano[5″,6″:3′,4′]isoflavanone(**D2**) along with two known compounds (dalbergin and formononetin). The structures were elucidated using spectroscopic techniques. Compound **G1** showed activity against Mycobacterium tuberculosis, whereas both of the new compounds were inactive against the malaria parasite plasmodium falciparum at 10 mg/mL. Docking studies showed that the new compounds **D1** and **D2** have high affinity for the M. tuberculosis drug target INHA [7].



D1 **D2**

Figure 1.8 Isolated compounds from *D. melanoxylon.*

The heartwood of *D. odorifrea* was isolated to obtain five new sesquiterpenes (**D3**–**D7**), rel-(3*R*,6*R*,7*S*)-3,7,11-trimethyl-3,7-epoxy-1,10-dodecadien-6-ol (**D3**), rel-(3*S*,6*R*,7*S*,9*E*)-3,7,11-trimethyl-3,6-epoxy-1,9,11-dodecatrien-7-ol (**D4**), rel-(3*S*,6*R*, 7*S*)-3,7,11-trimethyl-3,6-epoxy-1-dodecen-7,11-diol (**D5**), rel-(3*S*,6*R*,7*S*,10*S*)-2,6, 10-trimethyl-3,6;7,10-diepoxy-2-dodecen-11-ol (**D6**) and (3*S*,5*E*)-3,11-dimethyl-7-methy lenedodaca-1,5,10-trien-3-ol (**D7**) along with ten known compounds. Their structures were determined by spectroscopic techniques including MS, UV, IR, 1D and 2D NMR. Compound **D3** showed inhibitory effect on *C. albicans* with inhibition zone diameters of 10.86 mm [8].

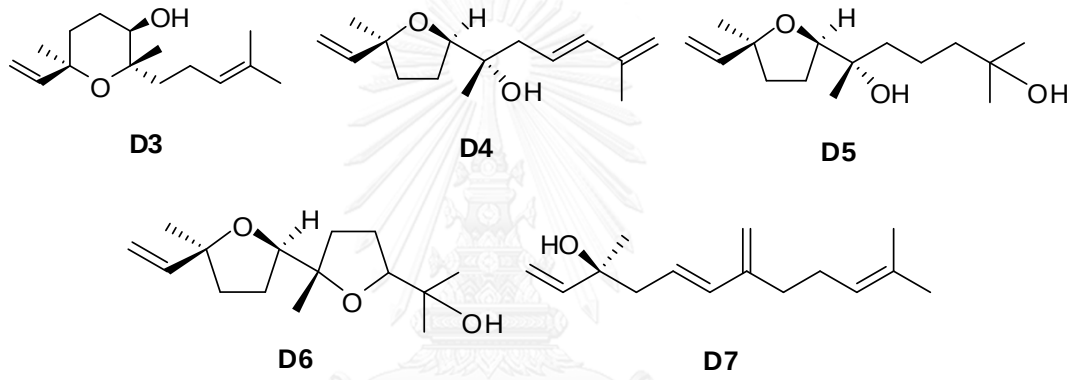


Figure 1.9 Isolated compounds from *D. odorifrea.*

The heartwood of *D. odorifera* was isolated to obtain six new phenolic compounds (**D8**–**D13**), (6a*R*,11a*R*)-6a,3,9-Trimethoxypterocarpan (**D8**), (6a*R*,11a*R*)-6a,9-Dimethoxy-3-hydroxypterocarpan (**D9**), 2-[2-(2,4-Dimethoxyphenyl)-2-oxoetho xy]-4-hydrobenzoic acid (**D10**), 2-(2,4-Dihydroxyphenyl)-1-(4-hydroxy-2-methoxy phenyl) ethanone (**D11**), 6-Methoxy-5,2′,4′-trihydroxy-3-benzoylbenzofuran (**D12**) and (3*R*)-7,30-Dihydroxy-6,2′,4′-trimethoxyisoflavanone (**D13**) along with five known phenolics (**D14**–**D18**). Their structures were determined by spectroscopic techniques including MS, UV, IR, 1D and 2D NMR. Bioassay results showed that compound **D8** exhibited cytotoxicity against SGC-7901 and BEL-7402 tumor cell lines with IC_{50} values of 15.9 and 12.7 μM, while compounds **D10**, **D12** and **D17** showed antibacterial activities against *Ralstonia solanacearum* with inhibition zone diameters of 10.55, 14.15 and 10.03 mm, respectively [9].

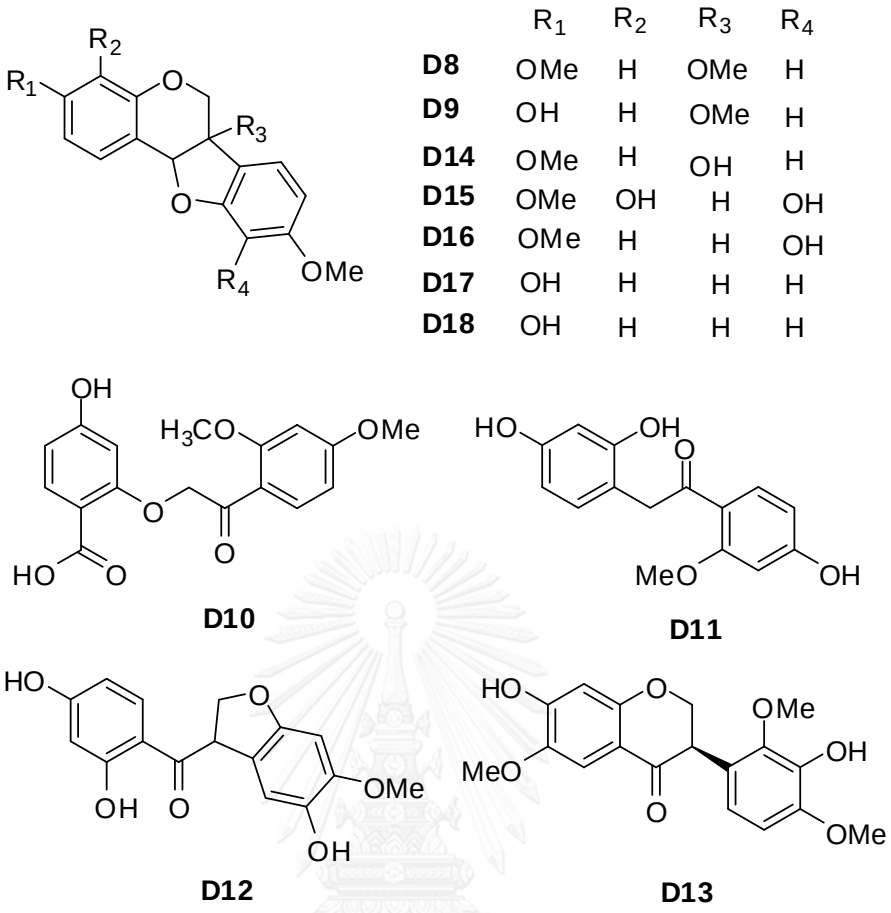


Figure 1.10 Isolated compounds from *D. odorifera.*

1.2 OBJECTIVES

The main objectives of this investigation are as follows:

1. To isolate and purify compounds from the roots of *Microcos tomentosa* Smith., *Garcinia cowa* Roxb. and *Dalbergia velutina* Benth.
2. To identify the chemical structures of all isolated compounds.
3. To evaluate the cytotoxicity against KB and HeLa cell lines of the isolated compounds.

CHAPTER II

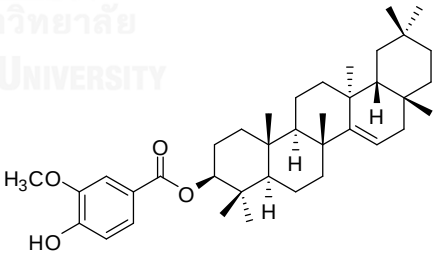
A NEW TARAXEROL DERIVATIVE FROM THE ROOTS OF *Microcos tomentosa*

Sutin Kaennakam[a], Jirapast Sichaem [a], Suttira Khumkratok[b], Pongpun Siripong[c] and Santi Tip-pyang[a,*]

[a]*Natural Products Research Unit, Department of Chemistry, Faculty of Science,Chulalongkorn University, Bangkok, 10330, Thailand*

[b]*Walai Rukhavej Botanical Research Institute, Mahasarakham University, Mahasarakham 44000, Thailand*

[c]*Natural Products Research Section, Research Division, National Cancer Institute, Bangkok 10400, Thailand*



Microcisin (2.1)

This article has been published in Natural Product Communications Year: 2013. Volume: 8. Page: 1371-1372.

2.1 ABSTRACT

A new 3*β*-*O*-vanilloyl-taraxerol, microcisin (**2.1**) and eight known compounds, 3*β*-taraxerol acetate (**2.2**), 3*β*-taraxerol (**2.3**), cholest-4-en-3-one (**2.4**), cholest-4-en-6*β*-ol-3-one (**2.5**), *β*-sitosterol (**2.6**), 7-hydroxycadalene (**2.7**), mellein (**2.8**) and vanillin (**2.9**), were isolated from the roots of *Microcos tomentosa*. The structures of the isolated compounds were determined by extensive analysis of their spectroscopic data. All isolated compounds were evaluated for their cytotoxicity against KB and HeLa cells.

Keywords: *Microcos tomentosa*, Tiliaceae, Microcisin, Cytotoxicity.

2.2 INTRODUCTION

Microcos (Tiliaceae) has approximately 60 species distributed in Asia, Africa, South America, and Australia, with 3 species found in Thailand [10]. *Microcos tomentosa* Smith, locally known as "Plub Pla" is a shrub or small tree distributed mainly in Northeastern Thailand. The dried bark, roots, fruits, and leaves of *Microcos* species have been used medicinally to treat diarrhea and fever, as forms of general tonics, and as insecticides [11]. Previous phytochemical work on the genus *Microcos* has led to reports of piperidine alkaloids [11] and triterpenoids [12] being present in their extracts. To the best of our knowledge, there have been no phytochemical investigations on this species to date. The phytochemical investigation of the CH_2Cl_2 crude extract from the roots of *M. tomentosa* afforded six triterpenoid derivatives; a new 3*β*-*O*-vanilloyl-taraxerol, microcisin (**2.1**), 3*β*-taraxerol acetate (**2.2**) [13], 3*β*-taraxerol (**2.3**) [13], cholest-4-en-3-one (**2.4**) [14], cholest-4-en-6*β*-ol-3-one (**2.5**) and *β*-sitosterol (**2.6**) [14], one sesquiterpene; 7-hydroxycadalene (**2.7**) [15] and two phenolic derivatives; mellein (**2.8**) [16] and vanillin (**2.9**) [17] (Figure 2.1). Compounds **2.1**-**2.9** were also evaluated for their cytotoxicity against KB and HeLa cells.

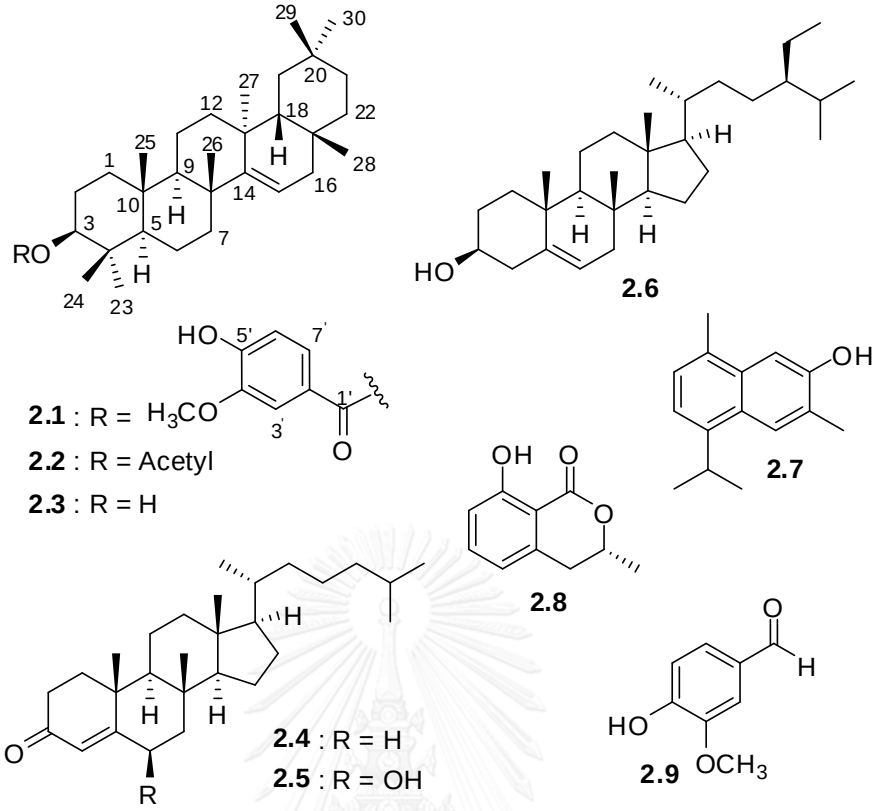


Figure 2.1 Structures of compounds **2.1**-**2.9** from the roots of *M. tomentosa*.

2.3 EXPERIMENT

2.3.1 General

1D and 2D NMR spectra were recorded on a Varian model Mercury^{+} 400 spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, a Bruker 400 AVANCE spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Melting points were determined on a Fisher-Johns Melting Point apparatus. UV–visible absorption spectra were carried out using a UV-2550 (SHIMADZU) UV–vis spectrophotometer (Shimadzu, Kyoto, Japan). Adsorbents using for chromatography were Sephadex LH-20 and silica gel (60 Merck cat. No. 7730, 7734 and 7749. IR data were obtained using a Nicolet 6700 FT-IR spectrometer using KBr disks.

2.3.2 Plant material

The roots of *Microcos tomentosa* Smith. were collected from the Kalasin Province of Thailand in October 2012 and were identified by Ms. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, where a voucher specimen (Khumkratok no.2-12) was deposited.

2.3.3 Extraction and isolation

The air-dried roots of *M. tomentosa* (2.5 kg) were extracted with CH_2Cl_2 at room temperature. The solvent was evaporated under vacuum to yield the CH_2Cl_2 (22.50 g) crude extract. The CH_2Cl_2 crude extract was subjected to silica gel column chromatography (Merck Art 7730) using hexane and CH_2Cl_2 with increasing gradient polarity to afford three fractions (M1-M3). Fraction M1 was purified using Sephadex LH-20 using MeOH/CH_2Cl_2 (1:8) as eluent to obtain compounds **2.2** (20.3 mg), **2.7** (14.2 mg) and **2.8** (10.5 mg). Fraction M2 was purified on a Chromatotron (radial chromatography) on silica gel using a stepwise gradient elution of hexane and EtOAc (increasing polarity) to afford compounds **2.1** (30.50 mg), **2.3** (25.2 mg) and **2.9** (8.60 mg). Fraction M3 was purified using Sephadex LH-20 using MeOH/CH_2Cl_2 (1:8) as eluent to give subfractions M3.1 and M3.2. Compounds **2.4** (12.10 mg) and **2.5** (14.30 mg) were obtained by separation on a Chromatotron using a stepwise gradient elution of hexane and EtOAc from subfraction M3.1. Finally, subfraction M3.1 was crystallized from CH_2Cl_2/MeOH (1:1) to yield **2.6** (35.5 mg).

Microcisin (2.1): MP: 281-282ºC ; $[\alpha]_D^{25}$+39.0 (*c* 0.20, $CHCl_3$); IR (KBr): 3424, 2927, 2860, 1696, 1280, 1106, 981 and 763 cm^{-1}; UV/Vis λ_{max} ($CHCl_3$) nm (log ε): 225 (2.81), 221 (2.47); ^1H and ^{13}C NMR: Table 1; HRESIMS: *m/z* [M + Na^+] calcd for $C_{38}H_{56}O_4Na$: 599.4078; found: 599.4076.

2.3.4 Cytotoxicity assay

All isolated compounds (**2.1-2.9**) were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma) and HeLa (human cervical carcinoma) cell lines employing the colorimetric method [18], [19]. Adriamycin was used as the

reference substance which exhibits activity against KB and HeLa cell lines. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Sigma Chemical Co., USA) was dissolved in saline to make a 5 mg/mL stock solution. Cancer cells (3×10^3 cells) suspended in 100 μg/wells of MEM medium containing 10% fetal calf serum (FCS, Gibco BRL, Life Technologies, NY, USA) were seeded onto a 96-well culture plate (Costar, Corning Incorporated, NY 14831, USA). After 24 h pre-incubation at 37 ºC in a humidified atmosphere of 5% CO_2/95% air to allow cellular attachment, various concentrations of test solution (10 μL/well) were added and these were then incubated for 48 h under the above conditions. At the end of the incubation, 10 μL of tetrazolium reagent was added into each well followed by further incubation at 37 ºC for 4 h. The supernatant was decanted, and DMSO (100 μL/well) was added to allow formosan solubilization. The optical density (OD) of each well was detected using a Microplate reader at 550 nm and for correction at 595 nm. Each determination represented the average mean of six replicates. The 50% inhibition concentration (IC_{50} value) was determined by curve fitting.

2.4 RESULTS AND DISCUSSION

Compound **2.1** was obtained as a white amorphous powder. The HRESIMS displayed a molecular ion peak at *m/z* 599.4076 $[M+Na]^+$ (calcd. 599.4078) suggesting a molecular formula of $C_{38}H_{56}O_4$, corresponding to eleven degrees of unsaturation which was in agreement with the structure elucidated using ^{1}H- and ^{13}C-NMR spectral data (Table 2.1). The IR spectrum exhibited absorption bands at 3424 cm^{-1} for hydroxyl group and 1696 cm^{-1} for carbonyl functionality. The ^{1}H-NMR spectrum of **2.1** (Table 2.1) revealed the presence of eight methyl signals attached to quaternary carbon centers at δ_H 0.76, 0.84, 0.85, 0.85, 0.89, 0.93, 0.96 and 1.04 (each 3H, s), and one doublet of doublet assigned to an oxymethine proton at δ_H 4.61 (dd, J = 5.6, 7.2 Hz) for H-3. The olefinic proton signal at δ 5.48 (dd, J = 3.2, 8.0 Hz, H-15) together with the characteristic ^{13}C-NMR signals (Table 1) at C-14 (δ_C 158.0) and C-15 (δ_C 117.0) for the double bond are suggestive of a taraxerane-type triterpenoid structure for **2.1**. The ^{13}C-NMR spectrum showed the presence of carbon centers corresponding to eight methyls, ten methylenes, four methines, one hydroxy-bearing methine, a pair of sp^2 double bond carbon centers, and six quaternary carbons for the

triterpenoid moiety. The remaining proton signals, one methoxy proton δ_H 3.87 (s), one hydroxyl proton δ_H 5.93 (s) and three methine protons attached to an aromatic ring δ_H 6.86 (d, J = 8.0), 7.49 (d, J = 1.8) and 7.56 (dd, J = 1.8, 8.0), and carbon signals at δ_C 166.1, 123.2, 111.8, 146.2, 149.8, 113.9, 123.9 and 56.1 were attributed to the presence of a vanilloyl moiety. The oxygen-bearing methine was assigned to be C-3 (δ_C 81.4). The attachment of the vanilloyl unit to C-3 of taraxerol was determined by the correlation between H-3(δ_H 4.61) and C-1′ (δ_C 166.1) in the HMBC spectrum (Figure 2.2). Analysis of the ^{1}H-^{1}H COSY spectrum indicated the presence of a homonuclear proton-proton spin system of CH-6′/CH-7′, and CH-15/CH_2-16 fragments. Significant long-range correlations were observed in the HMBC spectrum of **2.1** (Figure 2.2) notably H-3 to C-2, C-4, C-23, C-24, and C-1′; H-15 to C-14 and C-16; H-3′ to C-1′, C-4' and C-5′; H-6′ to C-4′ and C-7′; H-7′ to C-1′, C-3′ and C-5′ and O$C\underline{H}_3$ to C-4′. The β-configuration (axial) of H-3 was determined from NOESY correlations of H-3 with H-5 (Figure 2.2). Based on the above evidence the structure of **2.1** was determined as 3β-*O*-vanilloyl-taraxerol, herein given the trivial name microcisin.

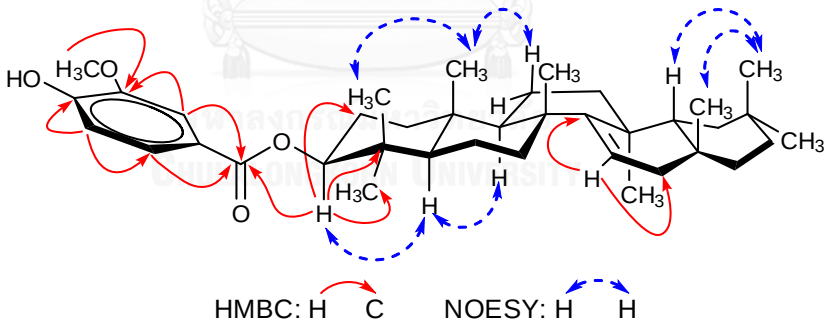


Figure 2.2 Selected HMBC and NOESY correlations of microcisin (**2.1**).

Compounds **2.1-2.9** were evaluated for their cytotoxicity against KB and HeLa cell lines, and these were determined using the MTT colorimetric assay. The results are shown in Table 2.2. Compounds **2.1**, **2.3** and **2.5** showed moderate cytotoxicity against KB cell lines with IC_{50} values of 24.98, 28.06 and 22.57 µM, respectively. On the other hand compound **2.3** showed moderate cytotoxicity against HeLa cells with

an IC_{50} value of 29.38 µM. The other compounds could be regarded as having weak activity or as inactive.

Table 2.1 ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data of **2.1** in $CDCl_3$.

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1	1.57, m ; 1.60, m	37.7	21	1.27, m ; 1.31, m	33.7
2	1.64, m ; 1.68, m	23.6	22	1.19, m ; 1.32, m	33.4
3	4.61, dd (5.6, 7.2)	81.4	23	0.85, s	28.2
4		35.8	24	0.96, s	16.9
5	0.86, m	55.7	25	0.93, s	15.5
6	1.48, m ; 1.56, m	18.7	26	0.76, s	29.8
7	1.22, m ; 1.27, m	36.7	27	1.04, s	25.9
8		39.0	28	0.84, s	29.9
9	1.42, m	49.2	29	0.85, s	21.3
10		37.6	30	0.89, s	33.1
11	1.41, m ; 1.58, m	17.5	1′		166.1
12	1.31, m ; 1.33, m	35.1	2′		123.2
13		38.1	3′	7.49, d (1.8)	111.8
14		158.0	4′		146.2
15	5.48, dd (3.2, 8.0)	117.0	5′		149.8
16	1.84, 1.88, dd (2.8, 15.6)	37.4	6′	6.86, d (8.0)	113.9
17		38.0	7′	7.56, dd (1.8, 8.0)	123.9
18	0.87, m	48.8	OH	5.93, s	
19	1.98, 2.00, d (12.8)	41.3	OCH_3	3.87, s	56.1
20		28.8			

Table 2.2 *In vitro* cytotoxicity of compounds **2.1**-**2.9** against HeLa and KB cells.

	IC_{50} (μM)			IC_{50} (μM)	
Compound	KB	HeLa	Compound	KB	HeLa
2.1	24.98	45.44	**2.5**	22.57	98.97
2.2	72.79	>100	**2.6**	60.76	>100
2.3	28.06	29.38	**2.7**	>100	>100
2.4	44.10	>100	**2.8**	95.69	>100
Adriamycin	0.09	0.31	**2.9**	>100	>100

($IC_{50} \leq 10$ = good activity, $10 < IC_{50} \leq 30$ = moderate activity, $IC_{50} > 100$ = inactive)

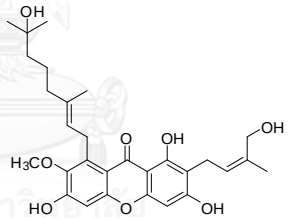
CHAPTER III

KAENNACOWANOLS A-C, THREE NEW XANTHONES AND THEIR CYTOTOXICITY FROM THE ROOTS OF *Garcinia cowa*

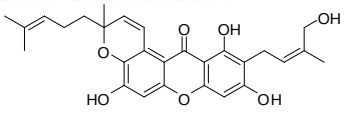
Sutin Kaennakam[a], Pongpun Siripong[b], Santi Tip-pyang[a,*]

[a]*Natural Products Research Unit, Department of Chemistry, Faculty of Science,Chulalongkorn University, Bangkok, 10330, Thailand*

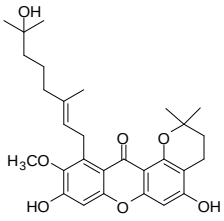
[b]*Natural Products Research Section, Research Division, National Cancer Institute, Bangkok 10400, Thailand*



Kaennacowanol A (3.1)



Kaennacowanol B (3.2)



Kaennacowanol C (3.3)

This article has been published in Fitoterapia

Year: 2015. Volume: 102. Page: 171-176.

3.1 ABSTRACT

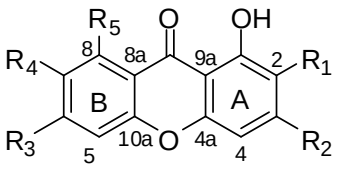
Three new xanthones, named kaennacowanols A-C (**3.1-3.3**), along with nineteen known xanthones were isolated from the roots of *Garcinia cowa* Roxb. Their structures were determined by spectroscopic analysis. All isolated compounds were evaluated for their cytotoxicity against KB and HeLa cell lines. Compounds **3.17** and **3.22** showed good cytotoxicity against KB cell with IC_{50} values of 7.97 and 9.10 µM, respectively. On the other hand, compound **3.15** showed good cytotoxicity against HeLa cell with IC_{50} value of 9.34 µM.

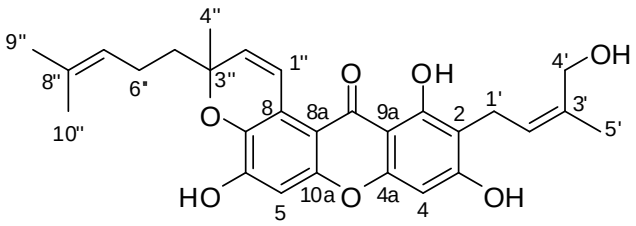
Keywords: Kaennacowanols A-C, *Garcinia cowa*, Clusiaceae, Xanthone, Cytotoxicity

3.2 INTRODUCTION

The Genus *Garcinia*, belonging to the Family Clusiaceae which comprises about 300 species that are widely distributed in the tropical and temperate regions of the world. Twenty-nine species have been observed in Thailand [4]. *Garcinia cowa*, commonly known as Cha-muang in Thai, is widely distributed throughout Thailand, Malaysia and Myanmar. This plant has been an abundant source of secondary metabolites, especially xanthones, phloroglucinols, flavonoids and terpenoids. Xanthones are well recognized as chemotaxonomic markers for plants of the *Garcinia* species [20] and many of which have interesting pharmacological activities, including antimicrobial, anti-inflammatory, antimalarial, antioxidant and cytotoxic activities [4]. Many parts of *G. cowa* have been used in traditional folk medicine. For example, the barks, latex and roots have been used as an antifever agent [21] while the fruits and leaves have been used for indigestion, improvement of blood circulation and used as an expectorant. Although, more than 86 compounds have been isolated from the twigs, stems, fruits and latex, this is the first report on chemical constituents from the roots of this plant. Therefore, the roots of this plant were attracted for further investigation. We now report the isolation of twenty-two xanthones, which are comprised of three new xanthones named kaennacowanols A-C (**3.1-3.3**) and nineteen known xanthones (**3.4-3.22**) (Figure 3.1). In addition, compounds **3.4**, **3.7**, **3.9**, **3.14**, **3.16**, **3.17** and **3.22** were isolated the first time from this plant. Their structures were

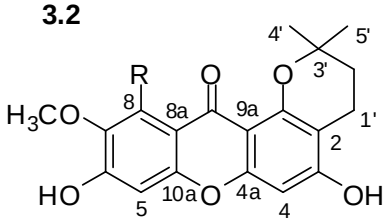
identified by interpretation of their spectroscopic data as well as comparison with those reported in the literature. Herein, we report the isolation, structural elucidation, as well as an evaluation of cytotoxicity against KB and HeLa cell lines of these xanthones**.**



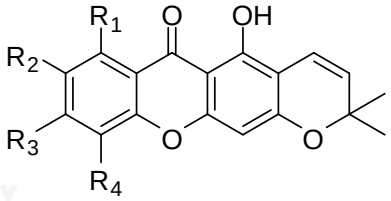


3.2

	R_1	R_2	R_3	R_4	R_5
3.1	4'-OH-Pr	OH	OH	OCH_3	8''-OH-Ge
3.4	4'-OH-Pr	OH	OH	OCH_3	3''-OH-Ge
3.5	4'-OH-Pr	OH	OH	OCH_3	Ge
3.6	Pr	OH	OH	OCH_3	Ge
3.7	Pr	OH	OH	OCH_3	3''-OH-Pr
3.8	Pr	OH	OH	OCH_3	Pr
3.9	Pr	OCH_3	OH	OCH_3	3''-OH-Pr
3.10	Pr	OCH_3	OH	OCH_3	Pr
3.11	4'-OH-Pr	OCH_3	OH	OCH_3	Pr
3.12	Pr	OCH_3	OCH_3	OCH_3	Pr
3.13	Pr	OH	OCH_3	OCH_3	Pr
3.14	H	OH	H	OH	Ge
3.15	Pr	OH	OH	OH	Ge
3.16	Ge	OH	OH	OCH_3	H



3.3 R= 8''-OH-Ge
3.17 R= 3''-OH-Pr
3.18 R= Pr



	R_1	R_2	R_3	R_4
3.19	Pr	OCH_3	OH	H
3.20	Pr	OCH_3	OCH_3	H
3.21	Ge	OCH_3	OH	H
3.22	H	H	OH	OH

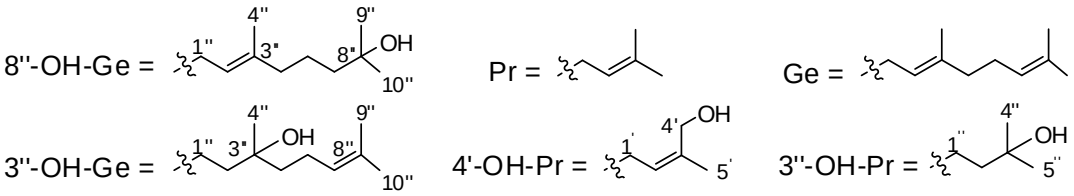


Figure 3.1 Structures of compounds **3.1-3.22** from the roots of *G. cowa.*

3.3 EXPERIMENTAL

3.3.1 General experimental procedures

1D and 2D NMR spectra were recorded on a Bruker 400 AVANCE spectrometer, and the chemical shifts were reported in parts per million (ppm) using TMS as the internal standard. Adsorbents such as silica gel 60 (Merck) were used for column chromatography and in radial chromatography (chromatotron model 7924T, Harrison Research). Merck silica gel 60F254 plates were used for TLC. HRESIMS spectra were obtained using a Bruker MICROTOF model mass spectrometer. IR data was obtained using a Nicolet 6700 FT-IR spectrometer using KBr disks. UV-visible absorption spectra were taken on a UV-2550 UV-vis spectrometer (Shimadzu, Kyoto, Japan).

3.3.2 Plant material

The roots of *G. cowa* were collected from Sahatsakhan district, Kalasin province, Thailand, in September 2014. The plant material was identified by Ms. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, and a specimen retained as a reference (Khumkratok no. 3-12).

3.3.3 Extraction and isolation

The air-dried roots of *G. cowa* (5.5 kg) were extracted with CH_2Cl_2 over a period of 3 days at room temperature (2 × 15 L). Removal of the solvent under reduced pressure provided CH_2Cl_2 crude extract (118.5 g) that was further separated by column chromatography over silica gel (Merck Art 7730) (1.5 kg) and eluted with a gradient of hexane-EtOAc (100% hexane, 80%, 60%, 40%, 20% and 100% EtOAc each 5 L, respectively) to give five fractions (A-E). Fraction A (8.5 g) was purified by Sephadex LH-20 column (100 g) with 50% CH_2Cl_2-MeOH (1 L) to afford compound **3.10** (1 .2 g) and compound **3.21** (2.5 mg). Fraction B (15.2 g) was separated by Sephadex LH-20 column (150 g) eluted with 50% CH_2Cl_2-MeOH (2 L) to give two subfractions (B1 and B2). Subfraction B1 (7.5 g) was purified by Sephadex LH-20 column (100 g) eluted with 50% CH_2Cl_2-MeOH (1 L) to afford compound **3.6** (1.4 g).

Subfraction B2 (6.5 g) was applied to Sephadex LH-20 column (100 g) eluted with 50% CH2Cl2-MeOH (1 L) to afford two subfractions (B2.1 and B2.2). Subfraction B2.1 (30 mg) was purified by radial chromatography (chromatotron) with 80% hexane-EtOAc (200 mL) to obtain compounds **3.12** (7.5 mg) and **3.13** (6.7 mg). Compounds **3.19** (7.8 mg) and **3.20** (6.5 mg) were achieved from subfraction B2.2 (25 mg) by chromatotron with 80% hexane-EtOAc (200 mL). Fraction C (18.2 g) was also applied to a Sephadex LH-20 column (150 g) using 50% CH_2Cl_2-MeOH (2 L) to provide three subfractions (C1-C3). Subfraction C1 (23 mg) was applied to chromatotron with 70% hexane-EtOAc (200 mL) to afford compounds **3.8** (6.2 mg) and **3.11** (8.4 mg). Compounds **3.14** (4.5 mg) and **3.15** (7.3 mg) were obtained from subfraction C2 (25 mg) by chromatotron with 70% hexane-EtOAc (200 mL). Subfraction C3 (26 mg) was subjected to chromatotron with 70% hexane-EtOAc (200 mL) to give **3.16** (7.2 mg). The separation of fraction D (12.4 g) by silicagel column chromatography (500 g), eluting with a gradient of EtOAc-hexane 20%, 40% and 60% (each 5 L) to provide two subfractions (D1 and D2). Subfraction D1 (6.5 g) was loaded to a Sephadex LH-20 column (500 g) using 50% CH_2Cl_2-MeOH (500 mL) to give compound **3.5** (1.3 g). Subfraction D2 (8.5 g) was fractionated by a Sephadex LH-20 column (500 g), eluted with 20% CH_2Cl_2-MeOH (500 mL) to yield three subfractions (D2.1-D2.3). Subfraction D2.1 (3.5 g) was separated by chromatographed on a Sephadex LH-20 column (25 g), eluted with 20% CH_2Cl_2-MeOH (500 mL) to afford compounds **3.2** (4.8 mg) and **3.22** (5.2 mg). Compounds **3.1** (5.8 mg) and **3.3** (4.5 mg) were obtained from subfraction D2.2 (23 mg) by a chromatotron with 60% hexane-EtOAc (200 mL). Compounds **3.9** (5.6 mg) and **3.18** (7.6 mg) were achieved from subfraction D2.3 (25 mg) by a chromatotron with 60% hexane-EtOAc (200 mL). Finally, fraction E (12.0 g) was subjected to a Sephadex LH-20 column (500 g), eluted with 100% MeOH (1 L) to give two subfractions (E1 and E2). Subfraction E1 (2.5 g) was loaded to a Sephadex LH-20 column (25 g), eluted with 100% MeOH (500 mL) to provide compound **3.17** (8.5 mg). Compounds **3.4** (12.2 mg) and **3.7** (10.5 mg) were purified from subfraction E2 (32 mg) by a chromatotron with 60% hexane-EtOAc (200 mL).

Kaennacowanol A (3.1): Yellow viscous oil; UV (MeOH) λ_{max} (log ε): 315 (4.4), 259 (4.5), 243 (4.6) nm. IR ν_{max} (KBr): 3402, 2968, 2932, 1642, 1607, 1282, 1192, 837 cm^{-1}; for ^{1}H (400 MHz, acetone-d_6) and ^{13}C NMR (100 MHz, acetone-d_6) spectroscopic data, see Table 3.1; HRESIMS *m/z* 535.2308 $[M+ Na]^+$ (calcd. for $C_{29}H_{36}O_8Na$, 535.2308).

Kaennacowanol B (3.2): Yellow viscous oil; UV (MeOH) λ_{max} (log ε): 383 (3.7), 323 (4.3), 265 (4.4), 246 (4.4) nm. IR ν_{max} (KBr): 3473, 2969, 2935, 1652, 1605, 1281, 1194, 838 cm^{-1}; for ^{1}H (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) spectroscopic data, see Table 3.1; HRESIMS *m/z* 501.1891 $[M+ Na]^+$ (calcd. for $C_{28}H_{30}O_7Na$, 501.1889).

Kaennacowanol C (3.3): Yellow viscous oil; UV (MeOH) λ_{max} (log ε): 320 (4.2), 262 (4.23), 248 (4.7) nm. IR ν_{max} (KBr): 3404, 2969, 2935, 1645, 1605, 1281, 1194, 838 cm^{-1}; for ^{1}H (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) spectroscopic data, see Table 3.1; HRESIMS *m/z* 519.2341 $[M+ Na]^+$ (calcd. for $C_{29}H_{36}O_7Na$, 519.2359).

3.3.4 Cytotoxicity assay

All isolated compounds (**3.1**-**3.22**) were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma) and HeLa (human cervical carcinoma) cell lines employing the colorimetric method [18], [19]. Adriamycin was used as the reference substance which exhibits activity against KB and HeLa cell lines. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Sigma Chemical Co., USA) was dissolved in saline to make a 5 mg/mL stock solution. Cancer cells (3×103 cells) suspended in 100 μg/wells of MEM medium containing 10% fetal calf serum (FCS, Gibco BRL, Life Technologies, NY, USA) were seeded onto a 96-well culture plate (Costar, Corning Incorporated, NY 14831, USA). After 24 h pre-incubation at 37°C in a humidified atmosphere of 5% CO_2/95% air to allow cellular attachment, various concentrations of test solution (10 μL/well) were added and these were then incubated for 48 h under the above conditions. At the end of the incubation, 10 μL of tetrazolium reagent was added into each well followed by further incubation at 37°C

for 4 h. The supernatant was decanted, and DMSO (100 μL/well) was added to allow formosan solubilization. The optical density (OD) of each well was detected using a Microplate reader at 550 nm and for correction at 595 nm. Each determination represented the average mean of six replicates. The 50% inhibition concentration (IC_{50} value) was determined by curve fitting.

3.4 RESULTS AND DISCUSSION

Phytochemical investigation of CH_2Cl_2 extract from the roots of *G. cowa* led to the isolation of three new xanthones, kaennacowanols A-C (**3.1-3.3**) together with nineteen known xanthones; fuscaxanthone I (**3.4**) [22], cowanol (**3.5**) [21], cowanin (**3.6**) [23], garcinone D (**3.7**) [24], *α*-mangostin (**3.8**) [23], pruniflorone C (**3.9**) [25], *β*-mangostin (**3.10**) [23], fuscaxanthone D (**3.11**) [26], fuscaxanthone C (**3.12**) [26], cowaxanthone B (**3.13**) [20], fuscaxanthone F (**3.14**) [26], norcowanin (**3.15**) [21], cowaxanthone (**3.16**) [21], 1-isomagostin hydrate (**3.17**) [25], 1-isomagostin (**3.18**) [25], 9-hydroxycalabaxanthone (**3.19**) [23], 5-hydroxy-8,9-dimethoxy-2,2-dimethyl-7-(3-methyl-2-butenyl)-2H,6H-pyrano[3,2-*β*]xanthen-6-one (**3.20**) [24], fuscaxanthone A (**3.21**) [23] and jacareubin (**3.22**) [27]. The structures of these isolated xanthones were elucidated using spectroscopic methods especially 1D and 2D NMR spectroscopy. The structures of the known compounds were determined and confirmed by comparison of their ^1H and ^{13}C NMR spectroscopic data with those previously published data.

Kaennacowanol A (**3.1**) was obtained as a yellow viscous oil. Its molecular formula was determined as $C_{29}H_{36}O_8$ by HRESIMS measurement through the pseudomolecular ion peak at *m/z* 535.2308 $[M+ Na]^+$ (calcd. for $C_{29}H_{36}O_8Na$, 535.2308). The UV spectrum displayed absorption bands at λ_{max} 315, 259 and 243 nm, which is typical of the xanthone chromophore[26]. The IR spectrum showed hydroxyl and carbonyl stretching bands at 3402 and 1642 cm^{-1}, respectively. The ^1H NMR spectrum had signals of two isolated aromatic protons at δ_H 6.29 (1H, s, H-4) and 6.75 (1H, s, H-5) and one methoxy at δ_H 3.73 (3H, s, 7-OCH_3). On basis of the HMBC cross peak, this methoxy group was placed at C-7 (δ_C 145.1) of ring B. The characteristic resonance of 4-hydroxy-3-methyl-2-butenyl group was appeared at δ_H 3.37 (2H, d, *J* = 7.6 Hz, H-1′), 5.29 (1H, br t, *J* = 7.6 Hz, H-2′), 4.23 (2H, s, H-4′) and

1.66 (3H, s, H-5′). Furthermore, the presence of 8-hydroxy-3,8-dimethyl-oct-2-enyl group was determinated from resonances of δ_H 4.06 (2H, d, J = 6.4 Hz, H-1″), 5.20 (1H, br t, J = 6.4 Hz, H-2″), 1.76 (3H, s, H-4″), 1.89 (2H, m, H-5″), 1.39 (2H, m, H-6″), 1.29 (2H, m, H-7″) and 1.01 (6H, s, H-9″ and H-10″). The correlation of H-1′ to C-1 (δ_C 162.3), C-2 (δ_C 110.8) and C-3 (δ_C 163.9) indicated that 4-hydroxy-3-methyl-2-butenyl unit was located at C-2 of ring A, while those H-1″ to C-7, C-8 (δ_C 139.0) and C-8a (δ_C 112.8) established that the 8-hydroxy-3,8-dimethyl-oct-2-enyl group was attached to C-8 of ring B. The three remaining hydroxyl groups on aromatic ring were placed at C-1, C-3 and C-6 (δ_C 158.1). The ^{1}H and ^{13}C NMR spectroscopic data (Table 3.1) were shown to be quite similar to those of the known xanthone, cowanol (**3.5**), except for a double bond of the geranyl chain in **3.1** was hydroxylated at C-8″ (δ_C 72.1). In the HMBC correlations of **3.1** (Figure 3.2), the methylene protons at H-7″ of hydroxyl geranyl unit showed cross peaks with C-5″ (δ_C 41.7), C-6″ (δ_C 24.0) and C-8″, and the protons of two methyl groups at H-9″ and 10″ showed cross peaks with C-6″, C-7″ (δ_C 45.0), C-8″ and one another. Thus, the complete assignment of kaennacowanol A was determinated as **3.1**.

Kaennacowanol B (**3.2**) was obtained as a yellow viscous oil. Its molecular formula was determined as $C_{28}H_{30}O_7$ by HRESIMS measurement through the pseudomolecular ion peak at *m/z* 501.1891 $[M+ Na]^+$ (calcd. for $C_{28}H_{30}O_7Na$, 501.1889). The UV spectrum displayed absorption bands at λ_{max} 383, 323, 265 and 246 nm, which is typical of the xanthone chromophore [26]. The IR spectrum showed hydroxyl and carbonyl stretching bands at 3473 and 1652 cm^{-1}, respectively. The ^{1}H and ^{13}C NMR spectral data of **3.2** (Table 3.1) were closely related to those of **3.1**. The major difference was the replacement of the ^{1}H NMR signals for the methoxy and 8-hydroxy-3,8-dimethyl-oct-2-enyl groups at C-7 (δ_C 139.6) and C-8 (δ_C 120.7), respectively, of **3.1** with a chromene ring at δ_H 8.08 (1H, d, J = 10.0 Hz, H-1″), 5.84 (1H, d, J = 10.0 Hz, H-2″), 1.45 (3H, s, H-4″), 1.75 (2H, m, H-5″), 2.16 (2H, m, H-6″), 5.13 (1H, m, H-7″), 1.58 (3H, s, H-9″) and 1.66 (3H, s, H-10″) in **3.2**. The location of a chromene ring was confirmed by HMBC (Table 3.1), in which methine proton H-1″ was correlated to C-3″ (δ_C 78.9) and C-7, while methine proton H-2″ was correlated to C-3″ and C-8. The ^{1}H and ^{13}C NMR spectroscopic data (Table 3.1) were shown to be quite similar to those of the known xanthone, cowagarcinon D [28], the

difference was found at position 4′ of prenyl chain, a proton of methyl at position 4′ of cowagarcinon D was replaced by a hydroxyl group. The HMBC correlations of **3.2** (Figure 3.2), showed that the methylene protons at δ_H 4.33 (2H, s, H-4′) of hydroxyl prenyl unit showed cross peaks with C-2′ (δ_C 124.7), C-3′ (δ_C 134.7) and C-5′ (δ_C 21.3), a methine double bond proton at δ_H 5.42 (1H, m, H-2′) showed cross peaks with C-4′ (δ_C 61.3) and C-5′ and the methyl protons at δ_H 1.77 (3H, s, H-5′) showed cross peaks with C-4′. From these data, the structure of kaennacowanol B was assigned as **3.2**.

Kaennacowanol C (**3.3**) was obtained as a yellow viscous oil. Its molecular formula was determined as $C_{29}H_{36}O_7$ by HRESIMS measurement through the pseudomolecular ion peak at *m/z* 519.2341 $[M+ Na]^+$ (calcd. for $C_{29}H_{36}O_7Na$, 519.2359). The UV spectrum displayed absorption bands at λ_{max} 320, 262 and 248 nm, which is typical of the xanthone chromophore. The IR spectrum showed hydroxyl and carbonyl stretching bands at 3404 and 1645 cm^{-1}, respectively. The ^1H and ^{13}C NMR spectral data of **3.3** (Table 3.1) were similarly related to those of **3.1**. The major difference was the replacement of the ^1H NMR signals for the hydroxyl and 4-hydroxy-3-methyl-2-butenyl groups at C-1 (δ_C 161.6) and C-2 (δ_C 103.8), respectively, of **3.1** with a dimethylchromane ring at δ_H 2.68 (2H, m, H-1′), 1.69 (2H, m, H-2′) and 1.29 (6H, s, H-4′ and H-5′) in **3.3**. The location of a dimethyl chromane ring was confirmed by HMBC (Table 3.1), in which methylene protons H-1′ was correlated to C-2′ (δ_C 43.3), C-3′ (δ_C 71.9), C-1 and C-3 (δ_C 163.6). The ^1H and ^{13}C NMR spectroscopic data (Table 3.1) were shown to be quite similar to those of the known xanthone, fuscaxanthone G [26], except for a double bond of the geranyl chain in **3** was hydroxylated at C-8″ (δ_C 71.5). In the HMBC correlations of **3.3** (Figure 3.2), the methylene protons at δ_H 1.37 (2H, m, H-7″) of hydroxyl geranyl unit showed cross peaks with C-5″ (δ_C 41.2), C-6″ (δ_C 23.5), C-8″, C-9″ (δ_C 29.2) and C-10″ (δ_C 29.2), and the protons of two methyl groups at δ_H 1.11 (6H, s, H-9″ and H-10″) showed cross peaks with C-6″, C-8″ and one another. The structure of kaennacowanol C was therefore assigned as **3.3**.

In previous researches many xanthones showed good cytotoxicities [23], [29], [30]. Therefore, all of these compounds were in vitro evaluated for their cytotoxic potential against KB and HeLa cell lines using the modified MTT method. The in

vitro cytotoxic activities of these compounds are shown in Table 3.2. Almost of all compounds showed moderate cytotoxicity against KB and HeLa cell lines, except for compounds **3.17** and **3.22** showed good cytotoxicity against KB cell lines with IC_{50} values of 7.97 and 9.10 µM, respectively, compound **3.15** showed good cytotoxicity against HeLa cell lines with IC_{50} value of 9.34 µM and compounds **3.12**, **3.20** and **3.21** had no cytotoxic activity due to IC_{50} value over 100 µM. The SAR studied data from Figure 3.1 and Table 3.2 suggested that the geranyl group at C-8 might be improved the activity, as inferred from the comparison of their cytotoxicity from **3.1**, **3.4**, **3.5** and **3.6**-**3.8**, the hydroxyl groups at C-1, C-3 and C-5 also seem to be increased their cytotoxicity (the comparison between **3.7** and **3.18**, **3.6** and **3.21**, and **3.21** and **3.22**, respectively) [30].

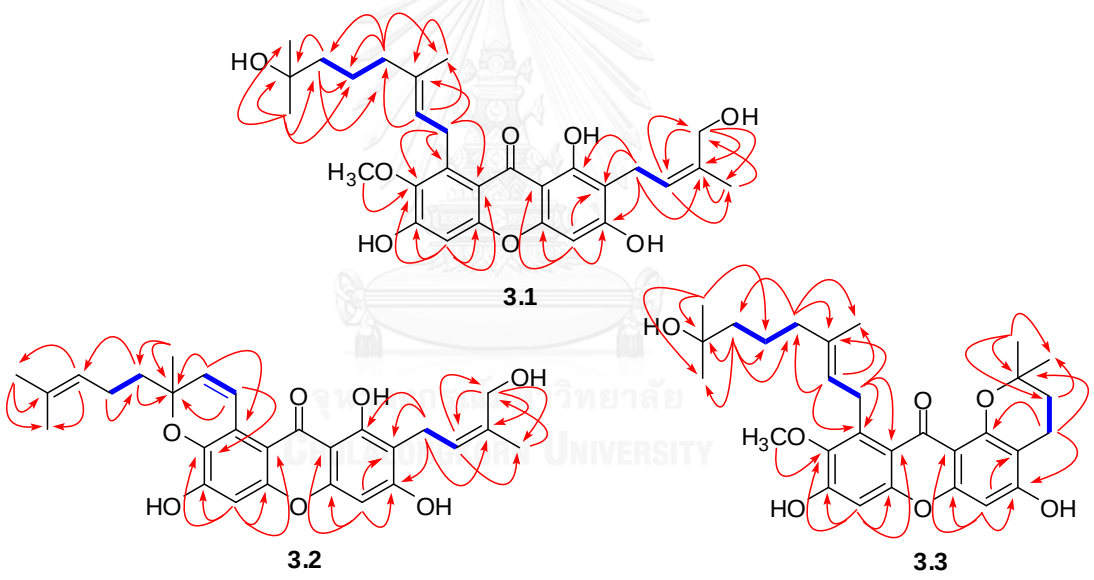


Figure 3.2 Selected HMBC (arrow curves) and COSY (bold lines) correlations in compounds **3.1**-**3.3**.

Table 3.1 NMR spectroscopic data (400 MHz, Acetone-d_6) for **3.1** (400 MHz, CD_3OD) for **3.2** and **3.3**.

Position	**3.1**		**3.2**		**3.3**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		162.3		160.9		161.6
2		110.8		110.2		103.8
3		163.9		163.1		163.6
4	6.29, s	94.2	6.29, s	92.9	6.23, s	93.3
4a		156.5		156.1		156.2
5	6.75, s	103.4	6.73, s	103.1	6.68, s	102.8
6		158.1		153.8		157.9
7		145.1		139.6		144.8
8		139.0		120.7		138.6
8a		112.8		108.3		112.3
9		183.8		183.1		183.1
9a		103.2		103.1		112.2
10a		157.2		153.4		156.7
1′	3.37, d (7.6)	22.4	3.40, d (7.6)	21.1	2.68, m	18.4
2′	5.29, br t (7.6)	126.0	5.42, br t (7.6)	124.7	1.69, m	43.3
3′		130.7		134.7		71.9
4′	4.23, s	62.5	4.33, s	61.3	1.29, s	29.0
5′	1.66, s	22.7	1.77, s	21.3	1.29, s	29.0
1″	4.06, d (6.4)	27.5	8.08, d (10.0)	122.0	4.07, d (6.0)	27.0
2″	5.20, br t (6.4)	125.3	5.84, d (10.0)	132.0	5.23, br t (6.0)	125.3
3″		136.1		78.9		135.6
4″	1.76, s	17.2	1.45, s	26.2	1.83, s	16.6
5″	1.89, m	41.7	1.75, m	41.8	1.98, m	41.2
6″	1.39, m	24.0	2.16, m	25.3	1.44, m	23.5
7″	1.29, m	45.0	5.13, m	126.1	1.37, m	44.2
8″		72.1		132		71.5
9″	1.01, s	29.4	1.58, s	25.4	1.11, s	29.2
10″	1.01, s	29.4	1.66, s	17.1	1.11, s	29.2
7-OCH_3	3.73, s	62.1			3.78, s	61.5

Table 3.2 *In vitro* cytotoxicity of compounds **3.1**-**3.22** against KB and HeLa cell lines.

	IC_{50} (μM)			IC_{50} (μM)	
Compound	KB	HeLa	Compound	KB	HeLa
3.1	20.97	16.70	**3.12**	> 100	> 100
3.2	11.01	12.23	**3.13**	12.85	14.96
3.3	32.38	32.79	**3.14**	14.50	61.13
3.4	24.43	17.20	**3.15**	10.38	9.34
3.5	11.98	12.19	**3.16**	13.59	15.75
3.6	11.96	11.68	**3.17**	7.97	10.31
3.7	22.31	22.58	**3.18**	39.67	34.04
3.8	14.14	13.69	**3.19**	13.83	21.83
3.9	32.75	39.03	**3.20**	> 100	> 100
3.10	12.05	12.78	**3.21**	> 100	> 100
3.11	15.48	33.46	**3.22**	9.10	11.43
Adriamycin	0.23	0.12			

($IC_{50} \leq 10$ = good activity, $10 < IC_{50} \leq 30$ = moderate activity, $IC_{50} > 100$ = inactive)

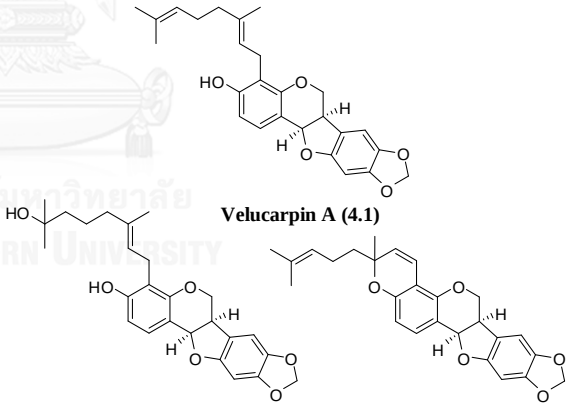
CHAPTER IV

VELUCARPINS A-C, THREE NEW PTEROCARPANS AND THEIR CYTOTOXICITY FROM THE ROOTS OF *Dalbergia velutina*

Sutin Kaennakam[a], Pongpun Siripong[b], Santi Tip-pyang[a,*]

[a]*Natural Products Research Unit, Department of Chemistry, Faculty of Science,Chulalongkorn University, Bangkok, 10330, Thailand*

[b]*Natural Products Research Section, Research Division, National Cancer Institute, Bangkok 10400, Thailand*



Velucarpin A (4.1)

Velucarpin B (4.2)

Velucarpin C (4.3)

This article has been published in Fitoterapia

Year: 2015. Volume: 105. Page: 165-168.

4.1 ABSTRACT

Three new pterocarpans, named velucarpins A-C (**4.1**-**4.3**), along with three known pterocarpans (**4.4**-**4.6**) were isolated from the roots of *Dalbergia velutina*. Their structures were determined by spectroscopic analysis. All isolated compounds were evaluated for their cytotoxicity against KB and HeLa cell lines. Compounds **4.3** and **4.5** showed good cytotoxicity against KB and HeLa cells with IC_{50} values of 8.22, 8.09 μM and 5.99, 8.69 μM, respectively.

Keywords: Velucarpins A-C, *Dalbergia velutina*, Leguminosae, Pterocarpans, Cytotoxicity

4.2 INTRODUCTION

The genus *Dalbergia*, belonging to the family Leguminosae which comprised about 275 species is widely distributed in Central and South America, Africa, Madagascar and Southern Asia [31]. This genus has been shown to possess various pharmacological activities including cancer chemopreventive activities, antimicrobial, antioxidant, anti-inflammatory, anti-analgesic, anti-pyretic, anti-diarrhoeal, antiulcerogenic, anti-giardial, antiplasmodial and anti-fertility [7]. *Dalbergia* genus has been an abundant source of secondary metabolites, especially isoflavanes, isoflavones, isoflavanones, neoflavones, anthraquinones, pterocarpans, triterpenes and cinnamyl esters [32]. *D. velutina*, commonly known as " Khruea khang khwai " in Thai, is a creeping plant mainly found in the northeastern region of Thailand. This is the first report on chemical constituents from this plant. Herein, we report the isolation, identification and cytotoxicity of three new pterocarpans, kaennavelutinans A-C (**4.1**-**4.3**) and three known pterocarpans (**4.4**-**4.6**) (Figure 4.1). Their structures were identified by interpretation of their spectroscopic data as well as comparison with those reported in the literature. All isolated compounds were evaluated for their cytotoxicity against KB and HeLa cell lines.

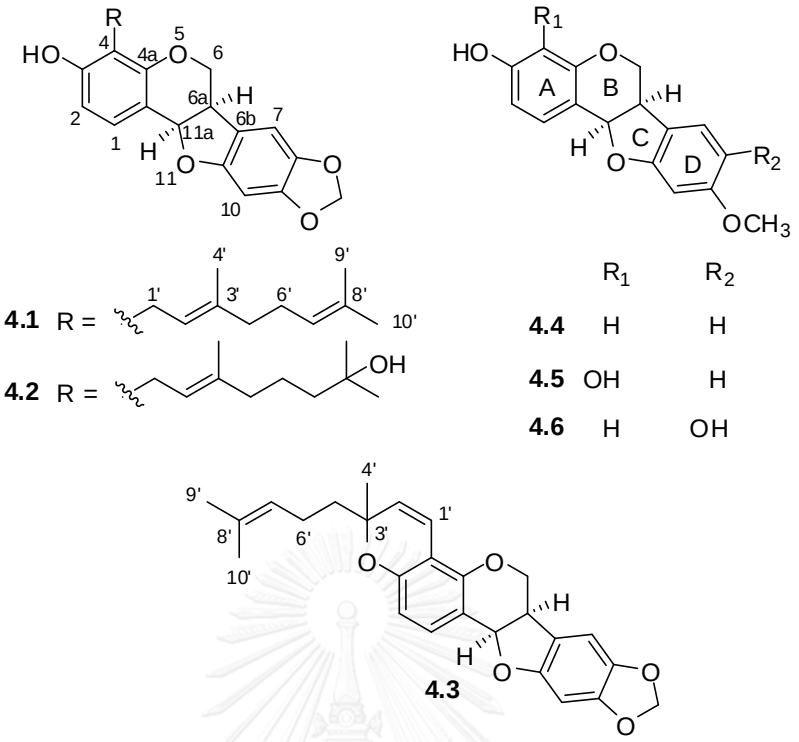


Figure 4.1 Structures of compounds **4.1-4.6** from the roots of *D. velutina.*

4.3 EXPERIMENTAL

4.3.1 General experimental procedures

1D and 2D NMR spectra were recorded on Bruker 400 AVANCE spectrometer. HRESIMS spectra were obtained using a Bruker MICROTOF model mass spectrometer. IR data was obtained using Nicolet 6700 FT-IR spectrometer using KBr disks. UV-visible absorption spectra were taken on UV-2550 UV-vis spectrometer (Shimadzu, Kyoto, Japan). Optical rotation was detected by Jasco P-1010 Polarimeter. CD spectra were recorded on Jasco J-815 Circular Dichroism (CD) spectropolarimeter.

4.3.2 Plant material

The roots of *D. velutina* were collected from Sahatsakhan district, Kalasin province, Thailand, in October 2014. The plant material was identified by Ms. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute,

Mahasarakham University, and a specimen retained as a reference (Khumkratok no. 4-12).

4.3.3 Extraction and isolation

The air-dried roots of *D. velutina* (5.5 kg) were extracted with CH_2Cl_2 over a period of 3 days at room temperature, respectively (2 × 15 L). Removal of the solvent under reduced pressure provided CH_2Cl_2 crude extract (75.5 g) that was further separated by column chromatography over silica gel (Merck Art 7734) and eluted with a gradient of Hexane-EtOAc (100% Hexane, 90%, 80%, 70%, 60%, 50% and 40% Hexane-EtOAc each 5 L, respectively) to give seven fractions (A-G). Fraction A (2.5 g) was purified by a Sephadex LH-20 column (150 g) with 80% CH_2Cl_2-MeOH (1.5 L) to afford compound **4.3** (25.5 mg). Fraction B (1.5 g) was purified by radial chromatography (chromatotron) with 80% hexane-EtOAc (200 mL) to obtain compound **4.1** (15.5 mg). Compound **4.4** (7.8 mg) was obtained from fraction D (1.1 g) by chromatotron with 80% hexane-EtOAc (200 mL). Fraction F (1.2 g) was also applied to a Sephadex LH-20 column (150 g) using 80% CH_2Cl_2-MeOH (2 L) to provide compound **4.5** (6.5 mg). Finally, fraction G (1.1 g) was subjected to chromatotron with 80% hexane-EtOAc (200 mL) to yield compound **4.2** (15.2 mg) and compound **4.6** (4.5 mg).

Velucarpin A (4.1): Yellow viscous oil; $[a]_D^{20}$-83.8 (*c* 1.2, $CHCl_3$); CD (*c* 0.1, MeOH) 247 (Δε -8.71), 292 (Δε +2.49) nm; UV ($CHCl_3$) λ_{max} (log ε): 270 (3.9), 327 (3.2) nm.; IR ν_{max} (KBr): 3645,1624,1495,1462,1145 cm^{-1}; for ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectroscopic data, see Table 4.2; HRESIMS *m/z* 443.1838 $[M+ Na]^+$ (calcd. for $C_{26}H_{28}O_5Na$, 443.1834).

Velucarpin B (4.2): Yellow viscous oil; $[a]_D^{20}$-68.7 (*c* 1.0, $CHCl_3$); CD (*c* 0.1, MeOH) 250 (Δε -6.05), 291 (Δε +0.97) nm; UV ($CHCl_3$) λ_{max} (log ε): 270 (4.3), 327 (3.5) nm.; IR ν_{max} (KBr): 3632,1622,1498,1465,1142 cm^{-1}; for ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectroscopic data, see Table 4.2; HRESIMS *m/z* 461.1945 $[M+ Na]^+$ (calcd. for $C_{26}H_{30}O_6Na$, 461.1940).

Velucarpin C (4.3):Yellow viscous oil; $[a]_D^{20}$-121.3 (*c* 1.2, $CHCl_3$); CD (*c* 0.1, MeOH) 255 (Δε -2.42), 291 (Δε +0.47) nm; UV ($CHCl_3$) λ_{max} (log ε): 270 (4.1), 328 (3.3) nm.; IR ν_{max} (KBr): 1628,1491,1463,1140 cm^{-1}; for ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectroscopic data, see Table 4.2; HRESIMS *m/z* 441.1681 $[M+ Na]^+$ (calcd. for $C_{26}H_{26}O_5Na$, 441.1678).

4.3.4 Cytotoxicity assay

All isolated compounds (**4.1**-**4.6**) were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma) and HeLa (human cervical carcinoma) cell lines employing the colorimetric method [18], [19]. Adriamycin was used as the reference substance which exhibits activity against KB and HeLa cell lines. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Sigma Chemical Co., USA) was dissolved in saline to make a 5 mg/mL stock solution. Cancer cells (3×103 cells) suspended in 100 μg/wells of MEM medium containing 10% fetal calf serum (FCS, Gibco BRL, Life Technologies, NY, USA) were seeded onto a 96-well culture plate (Costar, Corning Incorporated, NY 14831, USA). After 24 h pre-incubation at 37ºC in a humidified atmosphere of 5% CO_2/95% air to allow cellular attachment, various concentrations of test solution (10 μL/well) were added and these were then incubated for 48 h under the above conditions. At the end of the incubation, 10 μL of tetrazolium reagent was added into each well followed by further incubation at 37ºC for 4 h. The supernatant was decanted, and DMSO (100 μL/well) was added to allow formosan solubilization. The optical density (OD) of each well was detected using a Microplate reader at 550 nm and for correction at 595 nm. Each determination represented the average mean of six replicates. The 50% inhibition concentration (IC_{50} value) was determined by curve fitting.

4.4 RESULTS AND DISCUSSION

Phytochemical investigation of CH_2Cl_2 crude extract from the roots of *D. velutina* led to the isolation of three new pterocarpans, velucarpins A-C (**4.1**-**4.3**) together with three known pterocarpans, (-)-medicarpin (**4.4**) [33], 4-hydroxymedicarpin (**4.5**) [34] and (6a*R*,11a*R*)-3,8-dihydroxy-9-methoxypterocarpan (**4.6**) [35]. The structures of these isolated pterocarpans were elucidated using

spectroscopic methods especially 1D and 2D NMR spectroscopy and Circular Dichroism (CD) for the assessment of absolute configuration. The structures of the known compounds were determined and confirmed by comparison of their ^{1}H and ^{13}C NMR spectroscopic data with those previously published data.

Velucarpin A (**4.1**) was obtained as a yellow viscous oil and optically active ($[a]_D^{20}$-83.8, *c* 1.2, $CHCl_3$). Its molecular formula was determined as $C_{26}H_{28}O_5$ by HRESIMS measurement through the pseudomolecular ion peak at *m/z* 443.1838 $[M+Na]^+$ (calcd. for $C_{26}H_{28}O_5Na$, 443.1834). The UV spectrum displayed absorption bands at λ_{max} 270 and 327 nm. The IR spectrum showed O-H, C=C and C-O stretching bands at 3645, 1624 and 1145 cm^{-1}, respectively. The ^{1}H NMR spectrum showed the characteristic of a pterocarpan structure due to the splitting pattern of the protons at δ_H 5.52 (1H, d, *J* = 7.2 *Hz*, H-11a), 4.30 (1H, dd, *J* = 4.8, 10.1 *Hz*, H-6*β*), 3.66 (1H, t, *J* = 10.1 *Hz*, H-6*α*), and 3.49 (1H, m, H-6a), related to the oxymethylene protons of the heterocyclic ring B, and the bridging protons of rings B and C (H-6a and H-11a) [36]. The presence of a pterocarpan skeleton was supported by the ^{13}C NMR spectrum, which showed the corresponding carbons at δ_C 67.1 (C-6), 40.6 (C-6a) and 79.6 (C-11a). Pterocarpans are found in nature only in a 6a,11a-*cis* configuration. In agreement with this are the coupling constant between H-6a and H-11a (*J* = 7.2 *Hz*) and the comparison with the literature values (*J* = 6.6 *Hz* for *cis* and 13.4 *Hz* for *trans*) [37] and the latter proton signals were confirmed again as *cis*-orientation by NOESY experiments (Figure 4.2). The signals of three methyl singlets δ_H 1.84 (3H, s, H-4′), 1.72 (3H, s, H-10′), and 1.63 (3H, s, H-9′), two olefinic protons δ_H 5.28 (1H, t, *J* = 5.6 *Hz*, H-2′) and 5.11 (1H, t, *J* = 6.8 *Hz*, H-7′), three methylenes δ_H 3.46 (2H, d, *J* = 6.8 *Hz*, H-1′), 2.13 (2H, m, H-6′) and 2.08 (2H, m, H-5′) were recognized to geranyl moiety . A methylenedioxide (OCH_2O) as doublets centred at δ_H 5.94 and 5.92 (2H, d, *J* = 1.2 *Hz*) and δ_C 101.6. In addition, the remaining of two singlet signals of aromatic protons at δ_H 6.75 (1H, s, H-7) and 6.48 (1H, s, H-10) and ortho-coupled doublets centered at δ_H 7.27 (1H, d, *J* = 8.4 *Hz*, H-1) and 6.57 (1H, d, *J* = 8.4 *Hz*, H-2) gave clear evidence of a 3,4,8,9-tetrasubstitution pattern for the pterocarpan moiety. The HMBC correlations (Figure 4.2), the correlation of H-1′ to C-3 (δ_C 156.2), C-4 (δ_C 115.6) and C-4a (δ_C 154.6) indicated that geranyl unit was located at C-4 of A ring and two protons of methylenedioxide showed correlation

with C-8 (δ_C 142.0) and C-9 (δ_C 148.4) of ring D. The ^{1}H and ^{13}C NMR spectroscopic data (Table 4.2) were shown the same with known pterocarpan, (6a*S*,11a*S*)-nitiducol [38], except the stereochemistry at C-6a and C-11a. The specific rotation value of (6a*S*,11a*S*)-nitiducol showed positive value but compound **4.1** showed negative value (-83.8). In addition, the CD spectrum of (6a*S*,11a*S*)-nitiducol showed the positive Cotton effect at 240-270 nm and the negative Cotton effect at 270-320 nm but compound **4.1** showed the opposite Cotton effect at 240-270 nm and 270-320 nm (Figure 4.3). The absolute configuration at C-6a and C-11a was assigned as (6a*R*,11a*R*). Thus, the complete assignment of velucarpin A was determinated as **4.1**.

Velucarpin B (**4.2**) was obtained as a yellow viscous oil and optically active ($[a]_D^{20}$-68.7, *c* 1.0, $CHCl_3$). Its molecular formula was determined as $C_{26}H_{30}O_6$ by HRESIMS measurement through the pseudomolecular ion peak at *m/z* 461.1945 $[M+Na]^+$ (calcd. for $C_{26}H_{30}O_6Na$, 461.1940). The UV spectrum displayed absorption bands at λ_{max} 270 and 327 nm. The IR spectrum showed O-H, C=C and C-O stretching bands at 3632, 1622 and 1142 cm^{-1}, respectively. The ^{1}H and ^{13}C NMR spectroscopic data (Table 4.2) were closely related to those of **4.1**, except for a double bond of the geranyl chain in **4.1** was hydroxylated at C-8′ (δ_C 71.5). In the HMBC correlations of **4.2** (Figure 4.2), the methylene protons at H-7′ δ_H 1.44 (1H, m) and δ_H 1.52 (1H, m) of hydroxyl geranyl unit showed cross peaks with C-6′ (δ_C 23.4) and C-8′ and the protons of two methyl groups at H-9' and 10' δ_H 1.21 (6H, s) showed cross peaks with C-7′ (δ_C 43.3), C-8′ and one another. The similarity of the specific rotation value and CD spectrum (negative at 240-270 nm; positive at 270-320 nm) with **4.1** (Figure 4.3), the absolute configuration at C-6a and C-11a of **2** were assigned as (6a*R*,11a*R*). From these data, the structure of velucarpin B was assigned as **4.2**.

Velucarpin C (**4.3**) was obtained as a yellow viscous oil and optically active ($[a]_D^{20}$-121.3, *c* 1.2, $CHCl_3$). Its molecular formula was determined as $C_{26}H_{26}O_5$ by HRESIMS measurement through the pseudomolecular ion peak at *m/z* 441.1681 $[M+Na]^+$ (calcd. for $C_{26}H_{26}O_5Na$, 441.1678). The UV spectrum displayed absorption bands at λ_{max} 270 and 328 nm. The IR spectrum showed C=C and C-O stretching bands at 1628 and 1140 cm^{-1}, respectively. The ^{1}H and ^{13}C NMR spectroscopic data (Table 4.2) were shown to be quite similar to those of **4.1**, except for the geranyl chain at C-3' and hydroxy group at C-3 were cyclized to form dihydropyran ring in

4.1. In the HMBC correlations of **4.3** (Figure 4.2), the olefinic proton at δ_H 6.72 (1H, *J* = 9.2 *Hz*, H-1′) showed cross peaks with C-3 (δ_C 154.4), C-4 (δ_C 110.0), C-4a (δ_C 151.2) and C-3′ (δ_C 78.5) and the olefinic proton at δ_H 5.56 (1H, *J* = 10.0 *Hz*, H-2′) showed cross peaks with C-4, C-3′, C-5' (δ_C 41.3) and C-6′ (δ_C 22.8). The methyl protons at H-4′ δ_H 1.44 (3H, s) showed cross peaks with C-2′, C-3′ and C-5′. The similarity of the negative specific rotation value (-121.3) and CD spectrum (negative at 240-270 nm; positive at 270-320 nm) with **4.1** and **4.2** (Figure 4.3), helped to assign the absolute configuration at C-6a and C-11a of **4.3** as (6a*R*,11a*R*). The structure of velucarpin C was therefore assigned as **4.3**.

In a previous research pterocarpans showed good cytotoxicity [39]. Therefore, all of these compounds were *in vitro* evaluated for their cytotoxic potential against KB and HeLa cell lines using the modified MTT method. The *in vitro* cytotoxic activities of these compounds are shown in Table 4.1. Compounds **4.3** and **4.5** showed good cytotoxicity against KB and HeLa cells with IC_{50} values of 8.22, 8.09 µM and 5.99, 8.69 µM, respectively. Compounds **4.1** and **4.2** showed moderate cytotoxicity against KB and HeLa cells with IC_{50} values of 19.96, 25.24 µM and 15.77, 18.96 µM, respectively. Compound **4.4** and **4.6** had weak cytotoxic activities due to IC_{50} value over 30 µM.

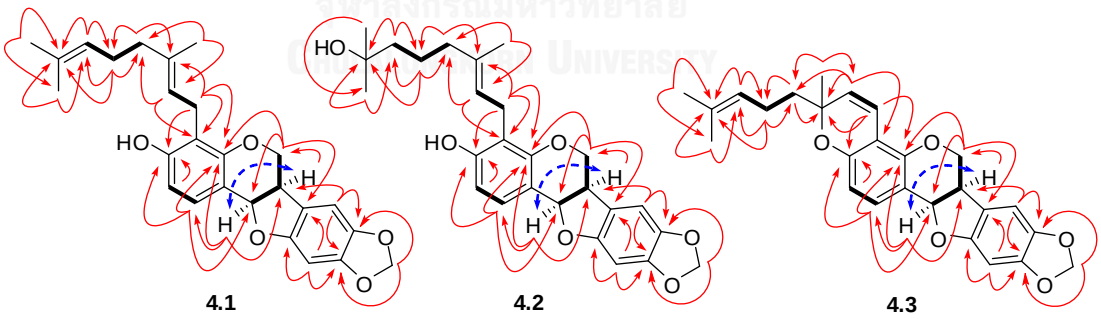


Figure 4.2 Selected HMBC (arrow curves), COSY (bold lines) and NOESY (dash curve) correlations in velucarpins A-C (**4.1-4.3**).

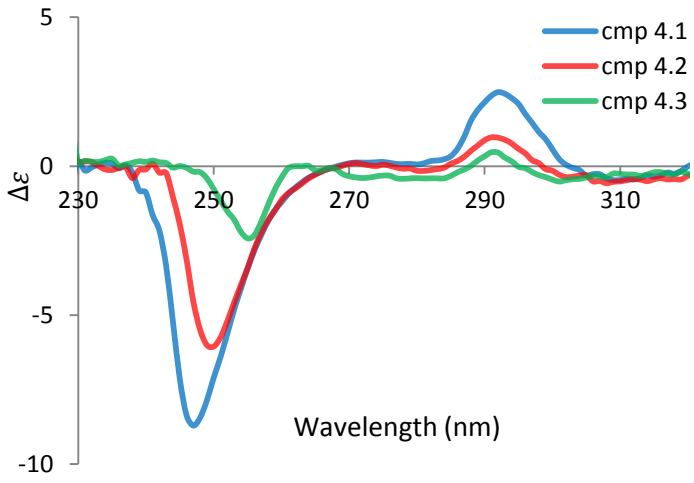


Figure 4.3 CD spectra of velucarpins A-C (**4.1-4.3**) in MeOH.

Table 4.1 *In vitro* cytotoxicity of compounds **4.1-4.6** against KB and HeLa cell lines.

	IC_{50} (μM)	
Compound	KB	HeLa
4.1	19.96	15.77
4.2	25.24	18.96
4.3	8.22	5.99
4.4	48.46	86.18
4.5	8.09	8.69
4.6	68.13	63.99
Adriamycin	0.23	0.12

($IC_{50} \leq 10$ = good activity, $10 < IC_{50} \leq 30$ = moderate activity, $IC_{50} > 100$ = inactive)

Table 4.2 NMR spectroscopic data (400 MHz, $CDCl_3$) for **4.1**, **4.2** and **4.3**.

	4.1		**4.2**		**4.3**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	7.27 (d, 8.4)	129.5	7.23 (d, 8.4)	129.0	7.27 (d, 8.4)	130.8
2	6.57 (d, 8.4)	110.3	6.56 (d, 8.4)	109.9	6.57 (d, 8.4)	110.3
3		156.2		155.8		154.4
4		115.6		115.7		110.0
4a		154.6		154.3		151.2
6α	3.66 (t, 10.1)	67.1	3.63 (t, 10.8)	66.8	3.66 (t, 10.8)	66.6
6β	4.30 (dd, 4.8, 10.1)	67.1	4.27 (dd, 4.8, 10.8)	66.8	4.27 (dd, 4.8, 10.8)	66.6
6a	3.49 (m)	40.6	3.46 (m)	40.3	3.44 (m)	40.2
6b		118.5		118.3		118.0
7	6.75 (s)	105.1	6.73 (s)	104.9	6.73 (s)	104.7
8		142.0		141.7		141.7
9		148.4		148.1		148.1
10	6.48 (s)	94.1	6.45 (s)	93.8	6.48 (s)	93.8
10a		154.4		154.2		154.2
11a	5.52 (d, 7.2)	79.6	5.50 (d, 6.8)	79.4	5.46 (d, 6.8)	78.8
11b		112.8		112.3		112.2
1′	3.46 (d, 6.8)	22.7	3.41 (d, 6.4)	22.5	6.72 (d, 10.)	117.0
2′	5.28 (t, 5.6)	122.1	5.24 (t, 6.8)	122.3	5.56 (d, 10)	128.1
3′		138.2		136.9		78.5
4′	1.84 (s)	16.5	1.79 (s)	16.2	1.44 (s)	26.4
5′	2.08 (m)	40.1	2.0 (t, 6.8)	40.0	1.79 (m)	41.3
6′	2.13 (m)	26.9	1.46 (m)	23.4	2.16 (m)	22.8
7′	5.11 (t, 6.8)	124.4	1.52 (m), 1.44 (m)	43.3	5.15 (t, 6.8)	124.2
8′		132.1		71.5		131.6
9′	1.63 (s)	18.0	1.21 (s)	29.2	1.72 (s)	25.7
10′	1.72 (s)	26.0	1.21 (s)	29.2	1.63 (s)	17.7
OCH_2O	5.94 (d, 1.2)	101.6	5.92 (d, 1.2)	101.3	5.92 (d, 1.6)	101.3
	5.92 (d, 1.2)	101.6	5.90 (d, 1.2)	101.3	5.90 (d, 1.6)	101.3

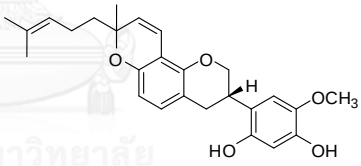
CHAPTER V

KAENNAVELUTINOLS A-C, THREE NEW ISOFLAVANES AND THEIR CYTOTOXICITY FROM THE ROOTS OF *Dalbergia velutina*

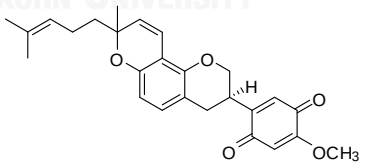
Sutin Kaennakam[a], Pongpun Siripong[b], Santi Tip-pyang[a,*]

[a]*Natural Products Research Unit, Department of Chemistry, Faculty of Science,Chulalongkorn University, Bangkok, 10330, Thailand*

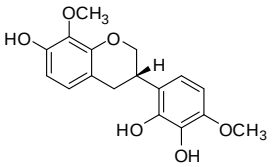
[b]*Natural Products Research Section, Research Division, National Cancer Institute, Bangkok 10400, Thailand*



Kaennavelutinol A (5.1)



Kaennavelutinol B (5.2)



Kaennavelutinol C (5.3)

This article has been submited in Phytochemistry Letters

Date: 9 June 2015

5.1 ABSTRACT

Three new isoflavanes, named kaennavelutinols A-C (**5.1-3**), along with two known isoflavanes (**5.4**-**5.5**) were isolated from the roots of *Dalbergia velutina*. Their structures were determined by spectroscopic analysis. All isolated compounds were evaluated for their cytotoxicity against KB and HeLa cell lines. Compounds **5.3** and **5.5** showed good cytotoxicity against KB and HeLa cells with IC_{50} values of 8.29, 3.47 μM and 9.54, 5.17 μM, respectively.

Keywords: Kaennavelutinols A-C, *Dalbergia velutina*, Leguminosae, Isoflavanes, Cytotoxicity

5.2 INTRODUCTION

The genus *Dalbergia*, a member of the family Leguminosae, comprises approximately 275 species distributed mainly in Central and South America, Africa, Madagascar and Southern Asia [31]. This genus have interesting pharmacological activities, including antioxidant, anticancer, anti-inflammatory, antimicrobial, antipyretic, antidiarrhoeal, antiulcerogenic, anti-giardial, antiplasmodial and anti-fertility [7]. Several isoflavones, isoflavanones, neoflavones, isoflavanes, anthraquinones, triterpenes and cinnamyl esters have been isolated in previous chemical investigation from this genus [31]. *D. velutina*, commonly known as " Khruea khang khwai " in Thai, is a creeping plant mainly found in the northeastern region of Thailand. In this paper the isolation and characterization of tree new isoflavanes (**5.1**-**5.3**) along with two known isoflavanes (**5.4** and **5.5**) (Figure 5.1) from the roots of *D. velutina* are reported. Their structures were identified by interpretation of their spectroscopic data as well as comparison with those reported in the literature. The cytotoxicity of all isolated compounds were evaluated by MTT method against KB and HeLa cell lines.

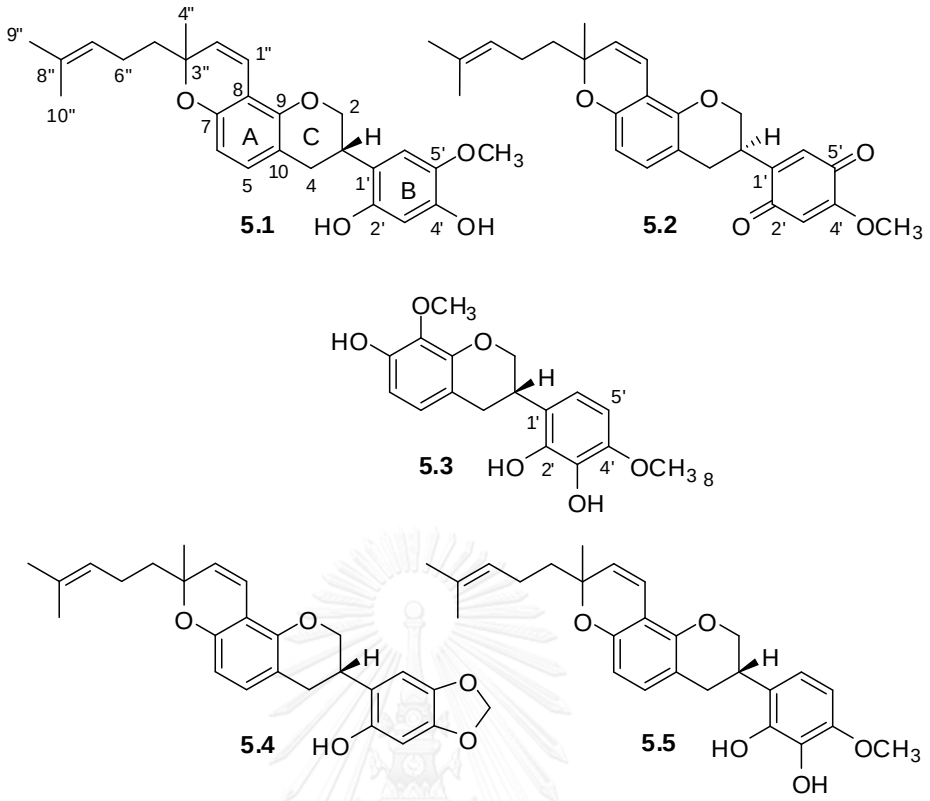


Figure 5.1 Structures of compounds **5.1-5.5** from the roots of *D. velutina.*

5.3 EXPERIMENTAL

5.3.1 General experimental procedures

1D and 2D NMR spectra were recorded on Bruker 400 AVANCE spectrometer. HRESIMS spectra were obtained using a Bruker MICROTOF model mass spectrometer. IR data was obtained using Nicolet 6700 FT-IR spectrometer using KBr disks. UV-visible absorption spectra were taken on UV-2550 UV-vis spectrometer (Shimadzu, Kyoto, Japan). Optical rotation were detected by Jasco P-1010 Polarimeter. CD spectra were recorded on Jasco J-815 Circular Dichroism (CD) spectropolarimeter.

5.3.2 Plant material

The roots of *D. velutina* were collected from Sahatsakhan district, Kalasin province, Thailand, in October 2014. The plant material was identified by Ms. Suttira

Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, and a specimen retained as a reference (Khumkratok no. 4-12).

5.3.3 Extraction and isolation

The air-dried roots of *D. velutina* (5.5 kg) were extracted with CH_2Cl_2 over a period of 3 days at room temperature, respectively (2 × 15 L). Removal of the solvent under reduced pressure provided CH_2Cl_2 crude extract (75.5 g) that was further separated by column chromatography over silica gel (Merck Art 7734) and eluted with a gradient of Hexane-EtOAc (100% Hexane, 90%, 80%, 70%, 60%, 50% and 40% Hexane-EtOAc each 5 L, respectively) to give seven fractions (A-G). Fraction B (1.5 g) was purified by Sephadex LH-20 column (150 g) with 80% CH_2Cl_2-MeOH (1.5 L) to afford compound **5.4** (15.5 mg). Fraction C (1.2 g) was purified by Sephadex LH-20 column (150 g) with 80% CH_2Cl_2-MeOH (1.5 L) to obtain compound **5.5** (17.5 mg). Compound **5.1** (7.8 mg) was achieved from fraction D (1.1 g) by radial chromatography (chromatotron) with 80% hexane-EtOAc (200 mL). Fraction E (1.3 g) was also applied to a Sephadex LH-20 column (150 g) using 80% CH_2Cl_2-MeOH (2 L) to provide compound **5.2** (16.5 mg). Finally, fraction G (1.0 g) was subjected to chromatotron with 80% hexane-EtOAc (200 mL) to yield compound **5.3** (7.2 mg).

Kaennavelutinol A (5.1): Yellow viscous oil; $[a]_D^{20}$+ 39.2 (*c* 1.0, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 270 (4.3), 327 (3.5) nm.; IR ν_{max} (KBr): 3635,1620, 1141 cm^{-1}; for ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectroscopic data, see Table 5.2; HRESIMS *m/z* 445.2016 $[M+ Na]^+$ (calcd. for $C_{26}H_{30}O_5Na$, 445.1991).

Kaennavelutinol B (5.2): Yellow viscous oil; $[a]_D^{20}$+ 87.7 (*c* 0.7, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 270 (4.3), 328 (3.4) nm.; IR ν_{max} (KBr): 1734,1621, 1138 cm^{-1}; for ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectroscopic data, see Table 5.2; HRESIMS *m/z* 443.1855 $[M+ Na]^+$ (calcd. for $C_{26}H_{28}O_5Na$, 443.1834).

Kaennavelutinol C (5.3): Yellow viscous oil; $[a]_D^{20}$+ 34.5 (*c* 0.6, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 270 (4.2), 327 (3.5) nm.; IR ν_{max} (KBr): 3642, 1627,1143 cm^{-1}; for ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectroscopic data, see Table 5.2; HRESIMS *m/z* 341.1013 $[M+ Na]^+$ (calcd. for $C_{17}H_{18}O_6Na$, 341.1001).

5.3.4 Cytotoxicity assay

All isolated compounds (**5.1**-**5.5**) were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma) and HeLa (human cervical carcinoma) cell lines employing the colorimetric method [18], [19]. Adriamycin was used as the reference substance which exhibits activity against KB and HeLa cell lines. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Sigma Chemical Co., USA) was dissolved in saline to make a 5 mg/mL stock solution. Cancer cells (3×103 cells) suspended in 100 μg/wells of MEM medium containing 10% fetal calf serum (FCS, Gibco BRL, Life Technologies, NY, USA) were seeded onto a 96-well culture plate (Costar, Corning Incorporated, NY 14831, USA). After 24 h pre-incubation at 37ºC in a humidified atmosphere of 5% CO_2/95% air to allow cellular attachment, various concentrations of test solution (10 μL/well) were added and these were then incubated for 48 h under the above conditions. At the end of the incubation, 10 μL of tetrazolium reagent was added into each well followed by further incubation at 37ºC for 4 h. The supernatant was decanted, and DMSO (100 μL/well) was added to allow formosan solubilization. The optical density (OD) of each well was detected using a Microplate reader at 550 nm and for correction at 595 nm. Each determination represented the average mean of six replicates. The 50% inhibition concentration (IC_{50} value) was determined by curve fitting.

5.4 RESULTS AND DISCUSSION

Phytochemical investigation of CH_2Cl_2 crude extract from the roots of *D. velutina* led to the isolation of three new isoflavanes, kaennavelutinols A-C (**5.1**-**5.3**) together with two known isoflavanes, nitidulan (**5.4**) and nitidulin (**5.5**) [38]. The structures of these isolated isoflavanes were elucidated using spectroscopic methods especially 1D and 2D NMR spectroscopy and Circular Dichroism (CD) (Figure 5.3) for the assessment of absolute configuration.

Kaennavelutinol A (**5.1**) was obtained as a yellow viscous oil and optically active ($[\alpha]_D^{20}$+ 39.2, *c* 1.0, $CHCl_3$). Its molecular formula was determined as $C_{26}H_{30}O_5$ by HRESIMS measurement through the pseudomolecular ion peak at *m/z* 445.1991 $[M+ Na]^+$ (calcd. for $C_{26}H_{30}O_5Na$, 445.2016). The UV spectrum displayed absorption bands at λ_{max} 270 and 327 nm. The IR spectrum showed O-H, C=C and C-O stretching bands at 3635, 1620 and 1141 cm^{-1}, respectively. The ^1H NMR data (Table 5.2) showed the characteristic of the isoflavane structure due to the splitting pattern of the protons at δ_H 4.36 (1H, dd, *J* = 1.6 and 10.0 *Hz*, H-2α), 4.04 (1H, t, *J* = 10.0 *Hz*, H-2β), 3.51 (1H, m, H-3), 2.96 (1H, dd, *J* = 10.4 and 15.6 *Hz*, H-4α) and 2.89 (1H, dd, *J* = 6.0 and 15.6 *Hz*, H-4β) related to the oxymethylene protons of the heterocyclic ring C (H-2), the methylene protons of ring C (H-4) and the bridging protons of rings C and B (H-3). The signals of two olefinic protons δ_H 6.68 (1H, d, *J* = 10.0 *Hz*, H-1″) and 5.52 (1H, d, *J* = 10.0 *Hz*, H-2″) and one methyl singlet δ_H 1.38 (3H, s, H-4″) of dihydropyran ring, one olefinic proton δ_H 5.10 (1H, t, *J* = 6.8 *Hz*, H-7″), two methylenes protons δ_H 2.11 (2H, m, H-6″) and 1.72 (2H, m, H-5″) and two methyl protons δ_H 1.67 (3H, s, H-9″) and 1.59 (3H, s, H-10″) were recognized to prenyl moiety. In addition, the remaining of two singlet signals of aromatic protons ring B at δ_H 6.62 (1H, s, H-6′) and 6.43 (1H, s, H-3′) and ortho-coupled doublets centered ring A at δ_H 6.81 (1H, d, *J* = 7.2 *Hz*, H-5) and 6.36 (1H, d, *J* = 7.2 *Hz*, H-6) gave clear evidence of a 7,8,1′,2′,4′,5′-hexasubstitution pattern for the isoflavane moiety. The presence of a isoflavane skeleton was supported by the ^{13}C NMR data (Table 5.2), which showed the corresponding carbons at δ_C 70.1 (C-2), 32.1 (C-3) and 30.7 (C-4). The HMBC correlations (Figure 5.2), the correlation of H-3 to C-1′ (δ_C 118.6), C-2′ (δ_C 148.0) and C-6′ (δ_C 110.8) indicated that ring C was located at C-1′ of ring B, the correlation of H-1″ to C-7 (δ_C 152.3), C-8 (δ_C 109.9) and C-9 (δ_C 149.9) indicated that dihydropyran ring was connected at C-7 and C-8 of ring A and the methyl protons δ_H 3.78 (3H, s, OCH_3) of methoxy unit showed cross peak with C-5′ (δ_C 141.1) of ring B and also was confirmed the proton and carbon assignments of ring B by comparison ^1H and ^{13}C NMR data with known isoflavane, lespedezol G_1 [40]. The absolute configuration at C-3 of the isoflavans were assigned by CD spectrum (for *S* negative at 270-320 nm; for *R* positive at 270-320 nm) [41], compound **5.1 s**howed the negative Cotton effect at 270-320 nm (Figure 5.3). The absolute configuration at C-3

was assigned as 3*S*. Thus, the complete assignment of kaennavelutinol A was determinated as **5.1**.

Kaennavelutinol B (**5.2**) was obtained as a yellow viscous oil and optically active ($[a]_D^{20}$+ 87.7, *c* 0.7, $CHCl_3$). Its molecular formula was determined as $C_{26}H_{28}O_5$ by HRESIMS measurement through the pseudomolecular ion peak at *m/z* 443.1855 $[M+ Na]^+$ (calcd. for $C_{26}H_{28}O_5Na$, 443.1834). The UV spectrum displayed absorption bands at λ_{max} 270 and 328 nm. The IR spectrum showed C=C and C-O stretching bands at 1621 and 1138 cm^{-1}, respectively. The ^1H and ^{13}C NMR spectroscopic data (Table 5.2) were closely related to those of **5.1**, except for ring B of **5.1** was replaced to 2-methoxy-1,4-benzoquinone. In the HMBC correlations of **5.2** (Figure 5.2), the olefinic proton at δ_H 6.48 (1H, s, H-6′) showed cross peaks with C-3 (δ_C 30.8), C-1′ (δ_C 149.0), C-2′ (δ_C 186.3) and C-4′ (δ_C 158.3). On the other hand, the olefinic proton at δ_H 5.95 (1H, s, H-3′) showed cross peaks with C-1′, C-2′, C-4′ and C-5′ (δ_C 181.7). The methyl protons δ_H 3.80 (3H, s, OCH_3) of methoxy unit showed cross peak with C-4′. The absolute configuration at C-3 was assigned by CD spectrum (Figure 5.3), compound **5.2 s**howed the positive Cotton effect at 270-320 nm. The absolute configuration at C-3 was assigned as 3*R*. From these data, the structure of kaennavelutinol B was assigned as **5.2**.

Kaennavelutinol C (**5.3**) was obtained as a yellow viscous oil and optically active ($[a]_D^{20}$+ 34.5, *c* 0.6, $CHCl_3$). Its molecular formula was determined as $C_{17}H_{18}O_6$ by HRESIMS measurement through the pseudomolecular ion peak at *m/z* 341.1013 $[M+ Na]^+$ (calcd. for $C_{17}H_{18}O_6Na$, 341.1001). The UV spectrum displayed absorption bands at λ_{max} 270 and 327 nm. The IR spectrum showed O-H, C=C and C-O stretching bands at 3642, 1627 and 1143 cm^{-1}, respectively. The ^1H and ^{13}C NMR spectroscopic data (Table 5.2) were shown to be quite similar to known isoflavane, nitidulin (**5.5**), except for dihydropyran ring of **5.5** was replaced to a hydroxyl at C-7 (δ_C 152.9) and a methoxy group at C-8 (δ_C 135.0). In the HMBC correlations of **5.3** (Figure 5.2), the methoxy protons at δ_H 3.91 (3H, s, OCH_3) showed cross peak with C-8 and the olefinic proton at δ_H 6.50 (1H, d, *J* = 8.4 *Hz*, H-6) showed cross peaks with C-8 and C-10 (δ_C 115.6). The absolute configuration at C-3 was assigned by CD spectrum (Figure 5.3), compound **5.3** showed the negative Cotton effect at 270-320

nm. The absolute configuration at C-3 was assigned as 3*S*. Thus, the complete assignment of kaennavelutinol C was determinated as **5.3**.

In a previous research isoflavanes showed good cytotoxicities [42]. Therefore, all of these compounds were *in vitro* evaluated for their cytotoxic potential against KB and HeLa cell lines using the modified MTT method. The *in vitro* cytotoxic activities of these compounds are shown in Table 5.1. Compounds **5.3** and **5.5** showed good cytotoxicity against KB and HeLa cells with IC_{50} values of 8.29, 3.47 µM and 9.54, 5.17 µM, respectively. Compounds **5.1**, **5.2** and **5.4** showed moderate cytotoxicity against KB and HeLa cells with IC_{50} values of 12.74, 29.20, 17.64 µM and 15.37, 27.54, 14.89 µM, respectively.

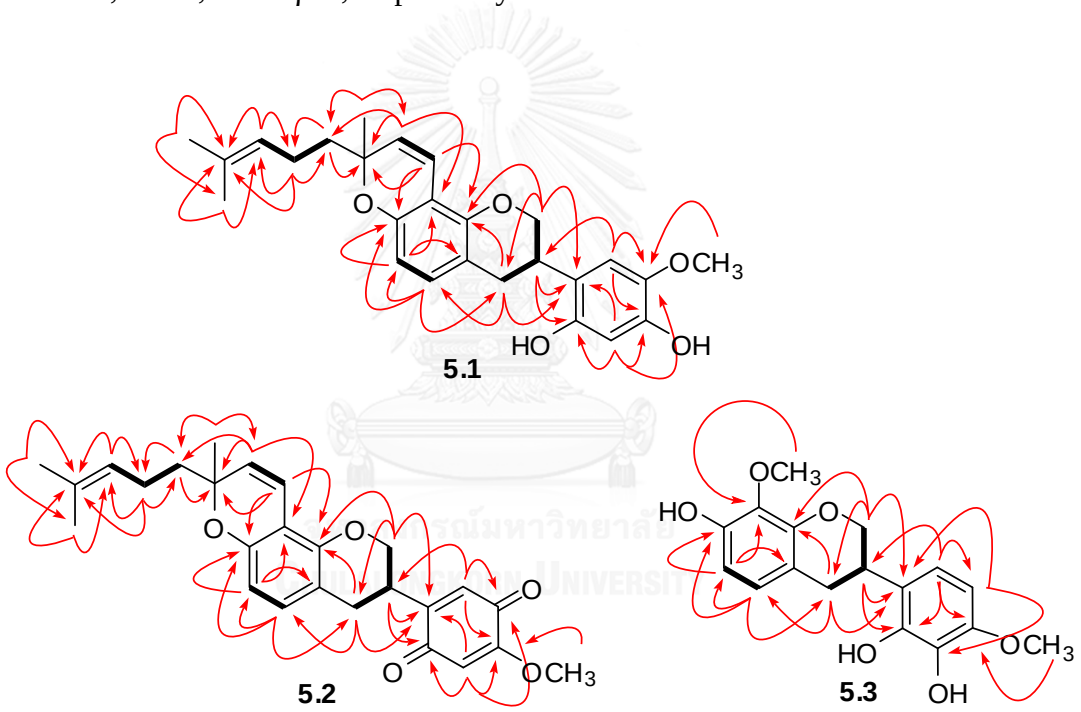


Figure 5.2 Selected HMBC (arrow curves), COSY (bold lines) in kaennavelutinols A-C (**5.1**-**5.3**).

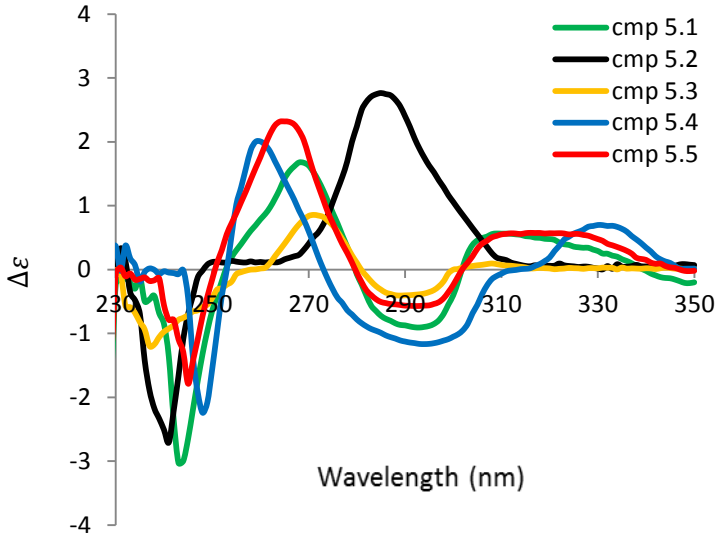


Figure 5.3 CD spectra of 5.1-5.5 in MeOH.

Table 5.1 *In vitro* cytotoxicity of compounds **5.1-5.5** against KB and HeLa cell lines.

	IC_{50} (μM)	
Compound	KB	HeLa
5.1	12.74	15.37
5.2	29.20	27.54
5.3	8.29	9.54
5.4	17.64	14.89
5.5	3.47	5.17
Adriamycin	0.23	0.12

($IC_{50} \leq 10$ = good activity, $10 < IC_{50} \leq 30$ = moderate activity, $IC_{50} > 100$ = inactive)

Table 5.2 NMR spectroscopic data (400 MHz, $CDCl_3$) for **5.1**, **5.2** and **5.3**

position	**5.1**		**5.2**		**5.3**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
2α	4.36 (dd, 1.6, 10.0)	70.1	4.04 (dd, 6.4, 10.4)	68.2	4.45 (dd, 2.0, 10.4)	70.0
2β	4.04 (t, 10.0)	70.1	4.27 (dd, 2.0, 10.4)	68.2	4.10 (t, 10.4)	70.0
3	3.51 (m)	32.1	3.42 (m)	30.8	3.56 (m)	32.2
4α	2.96 (dd, 10.4, 15.6)	30.7	2.69 (dd, 7.2, 16.0)	29.0	3.02 (dd, 10.8, 16.0)	30.5
4β	2.89 (dd, 6.0, 15.6)	30.7	2.97 (dd, 5.6, 16.0)	29.0	2.90 (dd, 9.6, 16.0)	30.5
5	6.81 (d, 7.2)	129.3	6.76 (d, 8.4)	128.8	6.71 (d, 8.4)	124.4
6	6.36 (d, 7.2)	108.7	6.34 (d, 8.4)	109.1	6.50 (d, 8.4)	107.1
7		152.3		152.2		152.9
8		109.9		109.6		135.0
9		149.9		149.0		147.6
10		114.2		111.6		115.6
1′		118.6		149.0		121.0
2′		148.0		186.3		142.4
3′	6.43 (s)	103.3	5.95 (s)	107.7		132.4
4′		145.0		158.3		145.9
5′		141.1		181.7	6.44 (d, 8.8)	102.9
6′	6.62 (s)	110.8	6.48 (s)	130.6	6.61 (d, 8.8)	117.9
1″	6.68 (d, 10.0)	117.5	6.59 (d, 10.0)	116.8		
2″	5.52 (d, 10.0)	128.0	5.49 (d, 10.0)	128.0		
3″		78.1		77.8		
4″	1.38 (s)	26.2	1.34 (s)	25.9		
5″	1.72 (m)	41.1	1.72 (m)	40.8		
6″	2.11 (m)	22.9	2.08 (m)	22.5		
7″	5.10 (t, 6.8)	124.4	5.08 (t, 6.8)	124.0		
8″		131.7		131.3		
9″	1.67 (s)	25.8	1.64 (s)	25.4		
10″	1.59 (s)	17.7	1.56 (s)	17.4		
8-OCH3					3.91 (s)	61.1
2′-OCH3						
4′-OCH3			3.80 (s)	56.0	3.87 (s)	56.3
5′-OCH3	3.78 (s)	56.8				

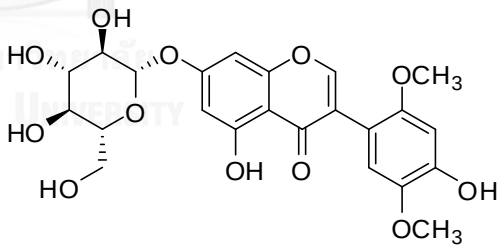
CHAPTER VI

KAENNAVELUTINOSE, A NEW ISOFLAVONES GLYCOSIDE WITH CYTOTOXICITY FROM THE ROOTS OF *Dalbergia velutina*

Sutin Kaennakam[a], Pongpun Siripong[b], Santi Tip-pyang[a,*]

[a]*Natural Products Research Unit, Department of Chemistry, Faculty of Science,Chulalongkorn University, Bangkok, 10330, Thailand*

[b]*Natural Products Research Section, Research Division, National Cancer Institute, Bangkok 10400, Thailand*



Kaennavelutinose (6.1)

This article has been submited in Natural Product Research

Date: 23 June 2015

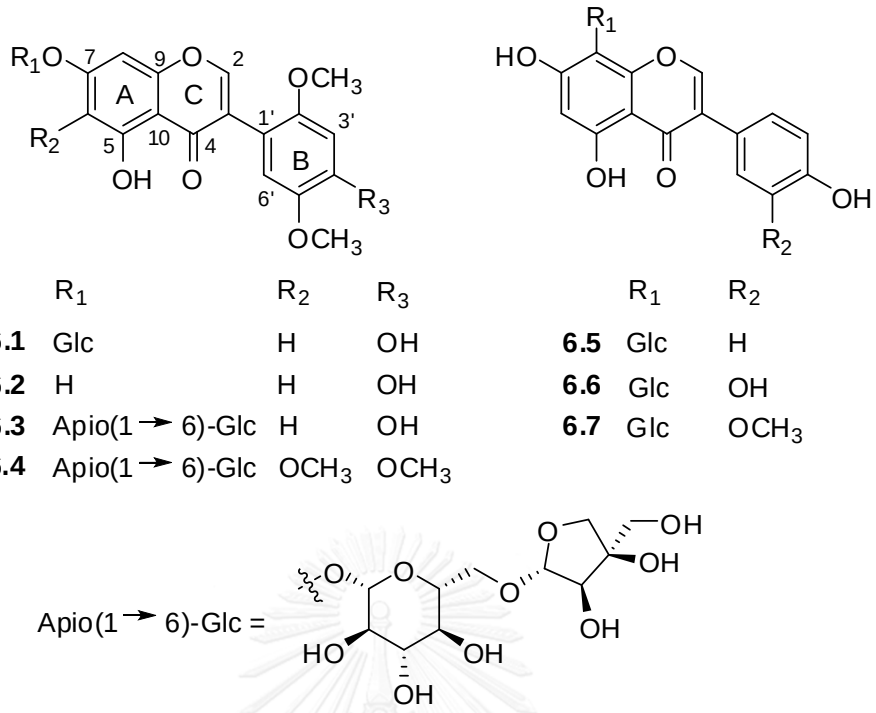
6.1 ABSTRACT

A new isoflavone glycoside, kaennavelutinose (**6.1**), together with one known isoflavone (**6.2**) and five known isoflavone glycosides (**6.3**-**6.7**) were isolated from the roots of *Dalbergia velutina*. Their structures were determined by spectroscopic analysis. All isolated compounds were evaluated for their cytotoxicity against KB and HeLa cell lines. Compounds **6.2** and **6.4** showed weak cytotoxicity against KB and HeLa cells with IC_{50} values of 48.06, 63.77 µM and 59.82, 86.36 µM, respectively.

Keywords: Kaennavelutinose; *Dalbergia velutina*; Isoflavone glycoside; Cytotoxicity

6.2 INTRODUCTION

The genus *Dalbergia* belongs to the family Leguminosae and comprises about 30 species in Thailand. *D. velutina*, locally known as " Khruea khang khwai ", is a creeping plant distributed mainly in the northeastern of Thailand. Ethnobotanical use of several species of the genus *Dalbergia* are well known that many of these plants have provided bioactive isoflavones, isoflavanones, neoflavones, anthraquinones, triterpenes and cinnamyl esters [32]. Several isolated compounds from the plants in this genus showed cancer chemopreventive activities, antimicrobial, antioxidant, anti-inflammatory, analgesic, antipy-retic, antidiarrhoeal, antiulcerogenic, anti-giardial, antiplasmodial and anti-fertility [7]. During our continuing phytochemical investigation of *D. velutina*, a new isoflavone glycoside, Kaennavelutinose (**6.1**) along with six known as isoflavone, olibergin A (**6.2**) [26] and isoflavone glycoside, 2′,5′-dimethoxy-genistein 7-O-*β*-D-apiofuranosyl-(1‴→6″)-O-*β*-D-glucopyranoside (**6.3**) [43], caviunin 7-O-*β*-D-apiofuranosyl-(1‴→6″)-*β*-D-glucopyranoside (**6.4**) [44], genistein-8-C-*β*-D-glucopyranoside(**6.5**) [45], orobol-8-C-*β*-D-glucopyranoside (**6.6**) [45] and dalpanitin (**6.7**) [46] (Figure 6.1) were isolated and elucidated. In addition, cytotoxicity result against KB and HeLa cells of all isolated compounds are also reported. Their structures were identified by interpretation of their spectroscopic data as well as comparison with those reported in the literature.



	R_1	R_2	R_3
6.1	Glc	H	OH
6.2	H	H	OH
6.3	Apio(1 → 6)-Glc	H	OH
6.4	Apio(1 → 6)-Glc	OCH_3	OCH_3

	R_1	R_2
6.5	Glc	H
6.6	Glc	OH
6.7	Glc	OCH_3

Figure 6.1 Structures of compounds **6.1-6.7** from the roots of *D.velutina.*

6.3 EXPERIMENTAL

6.3.1 General experimental procedures

1D and 2D NMR spectra were recorded on a Bruker 400 AVANCE spectrometer. Melting points, Fisher-Johns Melting Point apparatus. HRESIMS spectra were obtained using a Bruker MICROTOF model mass spectrometer. IR data was obtained using a Nicolet 6700 FT-IR spectrometer using KBr disks. UV-visible absorption spectra were taken on a UV-2550 UV-vis spectrometer (Shimadzu, Kyoto, Japan). Optical rotation, Jasco P-1010 Polarimeter.

6.3.2 Plant material

The roots of *D. velutina* were collected from Sahatsakhan district, Kalasin province, Thailand, in October 2014. The plant material was identified by Ms. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, and a specimen retained as a reference (Khumkratok no. 4-12).

6.3.3 Extraction and isolation

The air-dried roots of *D. velutina* (5.5 kg) were extracted with CH_2Cl_2 and MeOH over a period of 3 days at room temperature, respectively (3 × 15 L). Removal of the solvent under reduced pressure provided CH_2Cl_2 (75.5 g) and MeOH (80.5 g) crude extracts. The MeOH crude extract was further separated by column chromatography over silica gel (Merck Art 7730) and eluted with a gradient of EtOAc-MeOH (100% EtOAc, 90%, 80% and 70% EtOAc-MeOH each 5 L, respectively) to give five fractions (A-E). Fraction A (4.5 g) was purified by Sephadex LH-20 column (150 g) with 100% MeOH (1.5 L) to afford compound **6.1** (12.5 mg) and compound **6.2** (20.5 mg). Fraction B (6.5 g) was separated by Sephadex LH-20 column (150 g) eluted with 100% MeOH (1.5 L) to give compound **6.5** (8.5 mg), compound **6.6** (10.5 mg) and compound **6.7** (14.5 mg). Finally, fraction C (5.0 g) was subjected to a Sephadex LH-20 column (150 g) eluted with 100% MeOH (1 L) to yield compound **6.3** (15.0 mg) and compound **6.4** (16.5 mg).

Kaennavelutinose (6.1): White amorphous powder; m.p. 218.0–219.0 ^{o}C; $[\alpha]_D^{20}$ -62.8 (*c* 1.0, DMSO); UV λ_{max} (DMSO) 244, 262, 309 nm; IR ν_{max} (KBr): 3410, 1665, 1540, 1500 cm^{-1}; ^{1}H NMR (400 MHz, in DMSO-d_6) and ^{13}C NMR (100 MHz, in DMSO-d_6) see in Table 6.1; HRESIMS *m/z*: 515.1161 [M+ Na]+ (calcd. for $C_{23}H_{24}O_{12}Na$, 515.1165).

6.3.4 Acidic hydrolysis

A solution of Kaennavelutinose **6.1** (1 mg) in 1% HCl (0.1 mL) was heated at reflux for 1 h. The reaction mixture was neutralized with an equal volume of 1% Na_2SO_2 and extracted with 10% MeOH/CH_2Cl_2 (2 × 1 mL). Glucose was identified as the sugar moity by co-TLC analysis (EtOAC: MeOH: H_2O, 1:8:1) of the aqueous solution compared with an authentic glucose sample.

6.3.5 Cytotoxicity assay

All isolated compounds (**6.1**-**6.7**) were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma) and HeLa (human cervical carcinoma) cell lines employing the colorimetric method [18], [19] Adriamycin was used as the

reference substance which exhibits activity against KB and HeLa cell lines. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Sigma Chemical Co., USA) was dissolved in saline to make a 5 mg/mL stock solution. Cancer cells (3×103 cells) suspended in 100 μg/wells of MEM medium containing 10% fetal calf serum (FCS, Gibco BRL, Life Technologies, NY, USA) were seeded onto a 96-well culture plate (Costar, Corning Incorporated, NY 14831, USA). After 24 h pre-incubation at 37°C in a humidified atmosphere of 5% CO_2/95% air to allow cellular attachment, various concentrations of test solution (10 μL/well) were added and these were then incubated for 48 h under the above conditions. At the end of the incubation, 10 μL of tetrazolium reagent was added into each well followed by further incubation at 37°C for 4 h. The supernatant was decanted, and DMSO (100 μL/well) was added to allow formosan solubilization. The optical density (OD) of each well was detected using a Microplate reader at 550 nm and for correction at 595 nm. Each determination represented the average mean of six replicates. The 50% inhibition concentration (IC_{50} value) was determined by curve fitting.

6.4 RESULTS AND DISCUSSION

The MeOH extract from the roots of *D. velutina* was separated by silica gel (Merck Art 7730) and Sephadex LH-20 column chromatography to obtain the compounds **6.1**-**6.7**. Their structures were elucidated on the basis of detailed spectroscopic analysis including 1D NMR, 2D NMR and mass spectroscopic techniques.

Kaennavelutinose (**6.1**) was obtained as a white amorphous powder, $[\alpha]_D^{20}$ -62.8 (*c* 1.0, DMSO). Its molecular formula was determined as $C_{23}H_{24}O_{12}$ by HRESIMS measurement through the pseudomolecular ion peak at *m/z* 515.1161 $[M+Na]^+$ (calcd. for $C_{23}H_{24}O_{12}Na$, 515.1165). The UV spectrum displayed absorption bands at λ_{max} 244, 262 and 309 nm. The IR spectrum showed absorption bands for hydroxyl groups (3410 cm^{-1}), a carbonyl group (1665 cm^{-1}) and aromatic moieties (1540 and 1500 cm^{-1}). The ^1H NMR spectrum had signals of four aromatic protons at δ_H 6.47 (1H, d, *J* = 2.0 Hz, H-6), 6.59 (1H, s, H-3′), 6.72 (1H, d, *J* = 2.4 Hz, H-8), 6.88 (1H, s, H-6′), two phenolic protons at δ_H 9.25 (1H, s, OH-4′) and 12.92 (1H, s, OH-5, chelating to the carbonyl) which were consistent with a 5,7-disubstituted

aromatic ring A and a 2,4,5-trisubstituted aromatic ring B. The four hydroxyl groups of sugar unit at δ_H 4.61 (1H, t, J = 5.4 Hz, OH-6″), 5.06 (1H, d, J = 5.6 Hz, OH-3″), 5.13 (1H, d, J = 4.4 Hz, OH-4″), 5.41 (1H,d, J = 4.8 Hz, OH-2″), and two methoxy at δ_H 3.64 (3H, s, OCH_3-2′) and 3.72 (3H, s, OCH_3-5′). In addition, one olefinic proton at δ_H 8.28 (1H, s, H-2) is the characteristic of isoflavone skeleton. The ^{13}C NMR spectra showed 23 signals comprising two methyl, one methylene, ten methine and ten quaternary carbons. Compound **6.1** was isoflavone glucoside with anomeric proton signal at δ_H 5.06 (1H, d, J = 7.2 Hz, H-1″) and anomeric carbon resonance at δ_C 99.7 (C-1″) of glucose indicated the β-configuration. In the HMBC correlations of **6.1** (Figure 6.2), the anomeric proton at H-1″ showed cross peaks with C-3″ (δ_C 77.0) and C-7 (δ_C 162.7) indicated that one glucoside unit was located at C-7 of ring A, while those H-2 to C-3 (δ_C 120.4), C-4 (δ_C 180.1), C-9 (δ_C 157.0) and C-1′ (δ_C 108.8) established that ring C was attached to C-1′ of ring B and two methoxyl groups connected to C-2′ and C-5′ showed cross peak with δ_C 151.8 and 140.9, respectively. In addition, a hydroxyl group at position 4′ related to C-3′ (δ_C 100.7), C-4′ (δ_C 147.7) and C-5′ (δ_C 140.9). The ^{1}H and ^{13}C NMR spectroscopic data were shown to be quite similar to those of the known isoflavone glycoside, 2′,5′-dimethoxy-genistein 7-O-β-D-apiofuranosyl-(1‴→6″)-O-β-D-glucopyranoside (**6.3**), except for compound **6.1** comprised only one glucose unit. Thus, the complete assignment of kaennavelutinose **6.1** was determinated as 2′,5′-dimethoxy-genistein 7-O-β-D-glucopyranoside.

The MeOH crude extract from the roots of *D. velutina* comprise of one new kaennavelutinose (**6.1**), one known isoflavone (**6.2**) and five known isoflavone glycosides (**6.3**-**6.7**). The *in vitro* cytotoxic activities of these compounds are shown in Table 6.2. Compound **6.3** was isolated the first time from this genus. Compounds **6.2** and **6.4** showed weak cytotoxicity against KB and HeLa cells with IC_{50} values of 48.06, 63.77 µM and 59.82, 86.36 µM, respectively. There for, we believe that this plant is an important source for the diverse structure of isoflavone glycosides and should be further investigated for other biological activities.

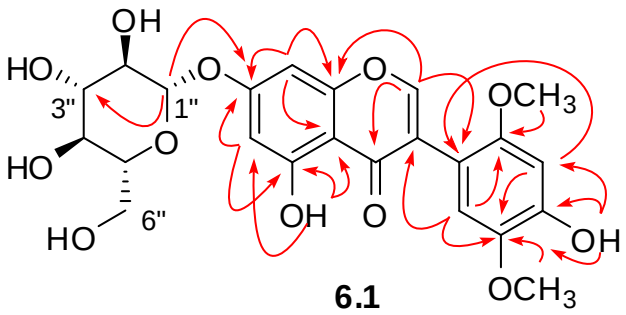


Figure 6.2 Key HMBC correlations of compound **6.1**.

Table 6.1 NMR spectroscopic data (400 MHz, DMSO-d_6) for **6.1**.

Position	δ_H (J in Hz)	δ_C	Position	δ_H (J in Hz)	δ_C
2	8.28, s	155.7	1″	5.06, d (7.2)	99.7
3		120.4	2″	3.29, m	72.9
4		180.1	3″	3.45, m	77.0
5		161.3	4″	3.18, m	69.4
6	6.47, d (2.0)	99.3	5″	3.32, m	76.2
7		162.7	6″	3.7, m; 3.46, m	60.4
8	6.72, s	94.4	2′-OCH3	3.64, s	55.8
9		157.0	5′-OCH3	3.72, s	56.5
10		105.7	5-OH	12.92, s	
1′		108.8	4′-OH	9.25, s	
2′		152.0	2″-OH	5.41, d (4.8)	
3′	6.59, s	100.7	3″-OH	5.07, d (5.6)	
4′		147.7	4″-OH	5.13, d (4.4)	
5′		141.0	6″-OH	4.61, t (5.4)	
6′	6.88, s	116.4			

Table 6.2 *In vitro* cytotoxicity of compounds **6.1**-**6.7** against KB and HeLa cell lines.

Compound	IC_{50} (μM)		Compound	IC_{50} (μM)	
	KB	HeLa		KB	HeLa
6.1	>100	>100	**6.4**	63.77	86.36
6.2	48.06	59.82	**6.5**	>100	>100
6.3	>100	>100	**6.6**	>100	>100
Adriamycin	0.22	0.10	**6.7**	>100	>100

(IC50 ≤ 10 = good activity, 10 < IC50 ≤ 30 = moderate activity, IC50 > 100 = inactive)

CHAPTER VII
CONCLUSIONS

The investigation for chemical constituents and their cytotoxicity from the CH_2Cl_2 crude extract of the roots of *M. tomentosa* led to the isolation of a new 3*β*-*O*-vanilloyl-taraxerol, microcisin (**2.1**) and eight known compounds, 3*β*-taraxerol acetate (**2.2**), 3*β*-taraxerol (**2.3**), cholest-4-en-3-one (**2.4**), cholest-4-en-6*β*-ol-3-one (**2.5**), *β*-sitosterol (**2.6**), 7-hydroxycadalene (**2.7**), mellein (**2.8**) and vanillin (**2.9**) (Figure 7.1). All isolated compounds were evaluated for their cytotoxicity against KB and HeLa cells. Compounds **2.1**, **2.3** and **2.5** showed moderate cytotoxicity against KB cell lines with IC_{50} values of 24.98, 28.06 and 22.57 µM, respectively. On the other hand, compound **2.3** showed moderate cytotoxicity against HeLa cells with an IC_{50} value of 29.38 µM.

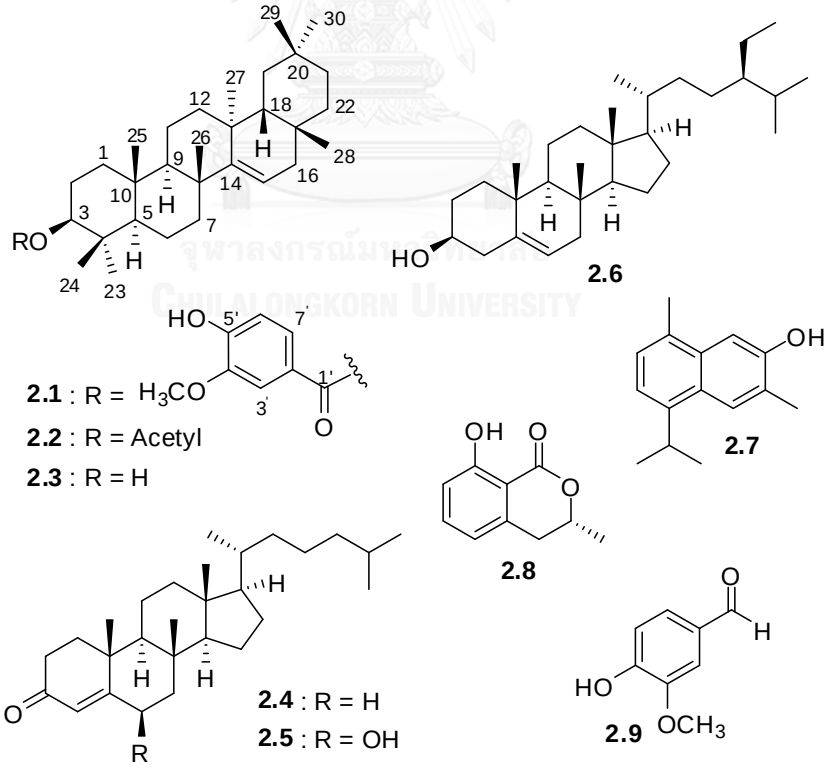


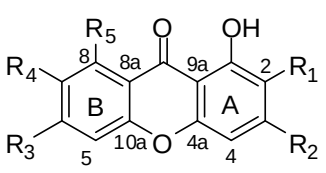
Figure 7.1 Isolated compounds **2.1**-**2.9** from the roots of *M. tomentosa*.

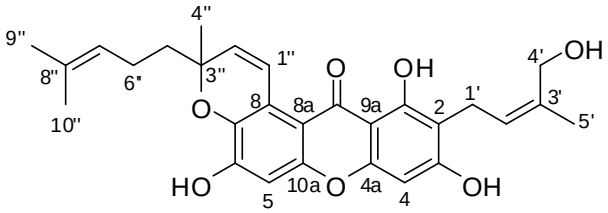
The investigation for chemical constituents and their cytotoxicity from the CH_2Cl_2 extract from the roots of *G. cowa* led to the isolation of three new xanthones, kaennacowanols A-C (**3.1**-**3.3**) together with nineteen known xanthones; fuscaxanthone I (**3.4**), cowanol (**3.5**), cowanin (**3.6**), garcinone D (**3.7**), *α*-mangostin (**3.8**), pruniflorone C (**3.9**), *β*-mangostin (**3.10**), fuscaxanthone D (**3.11**), fuscaxanthone C (**3.12**), cowaxanthone B (**3.13**), fuscaxanthone F (**3.14**), norcowanin (**3.15**), cowaxanthone (**3.16**), 1-isomagostin hydrate (**3.17**), 1-isomagostin (**3.18**), 9-hydroxycalabaxanthone (**3.19**), 5-hydroxy-8,9-dimethoxy-2,2-dimethyl-7-(3-methyl-2-butenyl)-2H,6H-pyrano[3,2-b]xanthen-6-one (**3.20**), fuscaxanthone A (**3.21**) and jacareubin (**3.22**) (Figure 7.2). All isolated compounds were evaluated for their cytotoxicity against KB and HeLa cells. Compounds **3.17** and **3.22** showed good cytotoxicity against KB cell with IC_{50} values of 7.97 and 9.10 µM, respectively. On the other hand, compound **3.15** showed good cytotoxicity against HeLa cell with IC_{50} value of 9.34 µM.

The investigation for chemical constituents and their cytotoxicity from the CH_2Cl_2 and MeOH extract from the roots of *D. velutina* led to the isolation of three new pterocarpans, velucarpins A-C (**4.1**-**4.3**), three new isoflavanes, kaennavelutinols A-C (**5.1**-**5.3**) and a new isoflavone glycoside, Kaennavelutinose (**6.1**) together with three known pterocarpans, (-)-medicarpin (**4.4**), 4-hydroxy medicarpin (**4.5**) and (6a*R*,11a*R*)-3,8-dihydroxy-9-methoxypterocarpan (**4.6**), two known isoflavanes, nitidulan (**5.4**) and nitidulin (**5.5**) and one known isoflavone, olibergin A (**6.2**) and five known isoflavone glycosides, 2′,5′-dimethoxy-genistein 7-O-*β*-D-apio furanosyl-(1‴→6″)-O-*β*-D-glucopyranoside (**6.3**), caviunin 7-O-*β*-D-apiofuranosyl-(1‴→6″)-*β*-D-glucopyranoside (**6.4**), genistein-8-C-*β*-D-glucopyranoside (**6.5**), orobol-8-C-*β*-D-glucopyranoside (**6.6**) and dalpanitin (**6.7**) (Figure 7.3). All isolated compounds were evaluated for their cytotoxicity against KB and HeLa cell lines. Compounds **4.3**, **4.5**, **5.3** and **5.5** showed good cytotoxicity against KB and HeLa cells with IC_{50} values of 8.22, 8.09, 8.29, 3.47 µM and 5.99, 8.69, 9.54, 5.17 µM, respectively.

The future work may involve the isolation of interesting plants and the synthesis of isolated compounds for increasing quantity and biological activity that could be developed into lead compounds or new drugs. New active compounds will afford the target for future synthesis and structure activity relationship (SAR) studies

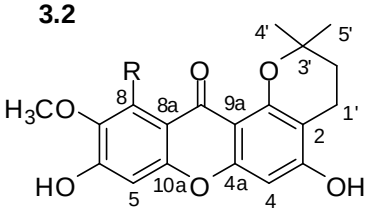
as well. This will lead to better understanding on the interaction between active compounds and cancer cells. Moreover, other cell lines will be tested for their cytotoxicity.



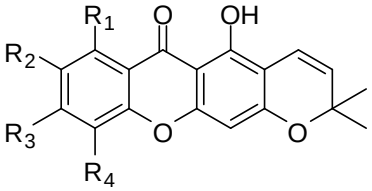


	R_1	R_2	R_3	R_4	R_5
3.1	4'-OH-Pr	OH	OH	OCH_3	8''-OH-Ge
3.4	4'-OH-Pr	OH	OH	OCH_3	3''-OH-Ge
3.5	4'-OH-Pr	OH	OH	OCH_3	Ge
3.6	Pr	OH	OH	OCH_3	Ge
3.7	Pr	OH	OH	OCH_3	3''-OH-Pr
3.8	Pr	OH	OH	OCH_3	Pr
3.9	Pr	OCH_3	OH	OCH_3	3''-OH-Pr
3.10	Pr	OCH_3	OH	OCH_3	Pr
3.11	4'-OH-Pr	OCH_3	OH	OCH_3	Pr
3.12	Pr	OCH_3	OCH_3	OCH_3	Pr
3.13	Pr	OH	OCH_3	OCH_3	Pr
3.14	H	OH	H	OH	Ge
3.15	Pr	OH	OH	OH	Ge
3.16	Ge	OH	OH	OCH_3	H

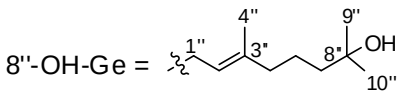
3.2

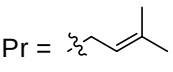


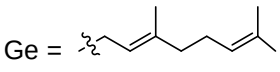
3.3 R= 8''-OH-Ge
3.17 R= 3''-OH-Pr
3.18 R= Pr

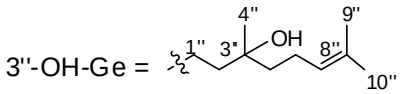


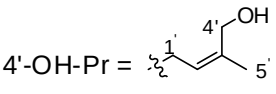
	R_1	R_2	R_3	R_4
3.19	Pr	OCH_3	OH	H
3.20	Pr	OCH_3	OCH_3	H
3.21	Ge	OCH_3	OH	H
3.22	H	H	OH	OH











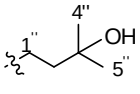


Figure 7.2 Isolated compounds **3.1-3.22** from the roots of *G. cowa.*

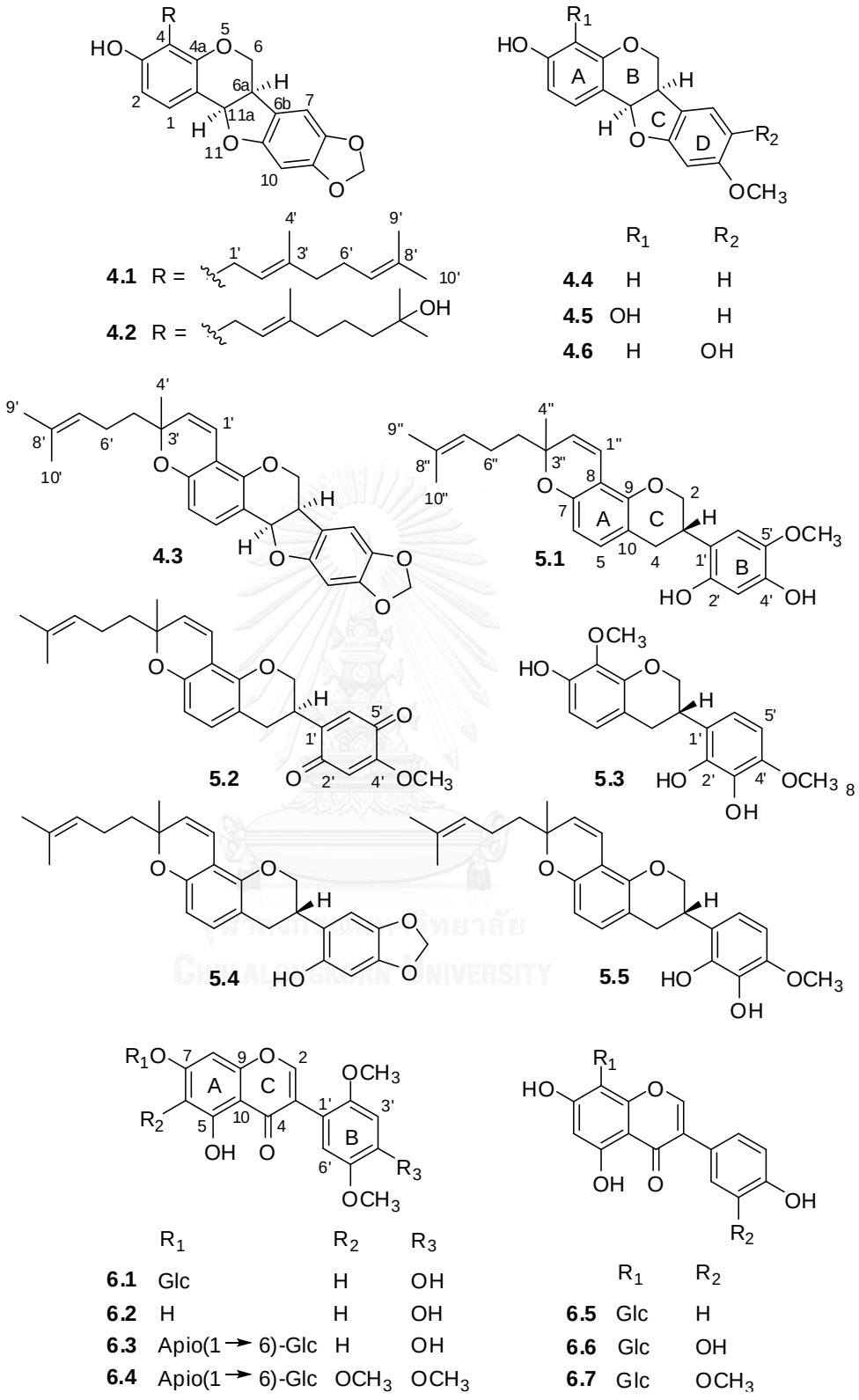


Figure 7.3 Isolated compounds **4.1-4.6**, **5.1-5.5** and **6.1-6.7** from the roots of *D. velutin.*

REFERENCES

[1] Bhanot, A., Sharma, R., and Noolvi, M.N. Natural sources as potential anti-cancer agents: A review. International Journal of Phytomedicine 3(1) (2011): 9-26.

[2] Cragg, G.M. and Newman, D.J. Plants as a source of anti-cancer agents. Journal of Ethnopharmacology 100(1-2) (2005): 72-79.

[3] Fan, H., et al. Chemical constituents with free-radical-scavenging activities from the stem of *Microcos paniculata.* Molecules 15(8) (2010): 5547-5560.

[4] Ritthiwigrom, T., Laphookhieo, S., and Pyne, S.G. Chemical constituents and biological activities of *Garcinia cowa* Roxb. Maejo International Journal of Science and Technology 7 (2013): 212-231.

[5] Auranwiwat, C., Trisuwan, K., Saiai, A., Pyne, S.G., and Ritthiwigrom, T. Antibacterial tetraoxygenated xanthones from the immature fruits of *Garcinia cowa.* Fitoterapia 98 (2014): 179-183.

[6] Sriyatep, T., et al. Cowabenzophenones A and B, two new tetracyclo[7.3.3.$3^{3,11}$.$0^{3,7}$]tetradecane-2,12,14-trione derivatives, from ripe fruits of *Garcinia cowa.* Fitoterapia 92 (2014): 285-289.

[7] Mutai, P., Heydenreich, M., Thoithi, G., Mugumbate, G., Chibale, K., and Yenesew, A. 3-Hydroxyisoflavanones from the stem bark of *Dalbergia melanoxylon*: Isolation, antimycobacterial evaluation and molecular docking studies. Phytochemistry Letters 6(4) (2013): 671-675.

[8] Wang, H., et al. Five new sesquiterpenoids from *Dalbergia odorifera.* Fitoterapia 95 (2014): 16-21.

[9] Wang, H., et al. Phenolic compounds from *Dalbergia odorifera.* Phytochemistry Letters 9 (2014): 168-173.

[10] Smitinand, T. and Larsen, K. Flora of Thailand. Bangkok. The Rumthai Press 6 (1993): 1-3.

[11] Still, P.C., et al. Alkaloids from *Microcos paniculata* with cytotoxic and nicotinic receptor antagonistic activities. Journal of Natural Products 76(2) (2013): 243-249.

[12] Fan, H., et al. Chemical constituents with free-radical-scavenging activities from the stem of *Microcos paniculata.* Molecules 15(8) (2010): 5547.

[13] Li, D.-L., Li, X.-M., and Wang, B.-G. Pentacyclic triterpenoids from the mangrove plant *Rhizophora stylosa.* Natural Product Research 22(9) (2008): 808-813.

[14] Georges, P., Sylvestre, M., Ruegger, H., and Bourgeois, P. Ketosteroids and hydroxyketosteroids, minor metabolites of sugarcane wax. Steroids 71(8) (2006): 647-652.

[15] Burden, R.S. and Kemp, M.S. Sesquiterpene phytoalexins from *Ulmus glabra.* Phytochemistry 23(2) (1984): 383-385.

[16] Schmeda-Hirschmann, G., Hormazabal, E., Astudillo, L., Rodriguez, J., and Theoduloz, C. Secondary metabolites from endophytic fungi isolated from the Chilean gymnosperm *Prumnopitys andina* (Lleuque). World Journal of Microbiology and Biotechnology 21(1) (2005): 27-32.

[17] Beckers, J.L. The determination of vanillin in a vanilla extract: An analytical undergraduate experiment. Journal of Chemical Education 82(4) (2005): 604.

[18] Skehan, P., et al. New colorimetric cytotoxicity assay for anticancer-drug screening. Journal of the National Cancer Institute 82(13) (1990): 1107-1112.

[19] Kongkathip, N., et al. Potent antitumor activity of synthetic 1, 2-naphthoquinones and 1, 4-naphthoquinones. Bioorganic & Medicinal Chemistry 11(14) (2003): 3179-3191.

[20] Panthong, K., Pongcharoen, W., Phongpaichit, S., and Taylor, W.C. Tetraoxygenated xanthones from the fruits of *Garcinia cowa.* Phytochemistry 67(10) (2006): 999-1004.

[21] Thongtheeraparp, W., Wiriyachitra, P., and Taylor, W.C. Xanthones of *Garcinia cowa.* Planta Medica 60(4) (1994): 365-368.

[22] Nontakham, J., Charoenram, N., Upamai, W., Taweechotipatr, M., and Suksamrarn, S. Anti-*Helicobacter pylori* xanthones of *Garcinia fusca.* Archives of Pharmacal Research 37(8) (2014): 972-977.

[23] Cheenpracha, S., Phakhodee, W., Ritthiwigrom, T., Prawat, U., and Laphookhieo, S. A new depsidone from the twigs of *Garcinia cowa.* Heterocycles 83 (2011): 1139-1144.

[24] Sen, A.K., Sarkar, K.K., Majumder, P.C., Banerji, N., and Garcinone, D. A new xanthone from *Garcinia mangostana* Linn. Indian Journal of Chemistry 25(11) (1986): 1157-1158.

[25] Boonnak, N., et al. Bioactive prenylated xanthones and anthraquinones from *Cratoxylum formosum* ssp. pruniflorum. Tetrahedron 62(37) (2006): 8850-8859.

[26] Ito, C., et al. Chemical constituents of *Garcinia fusca*: Structure elucidation of eight new xanthones and their cancer chemopreventive activity 1. Journal of Natural Products 66(2) (2003): 200-205.

[27] King, F., King, T., and Manning, L. The chemistry of extractives from hardwoods. Part XIV. The constitution of jacareubin, a pyranoxanthone from *Calophyllum brasiliense.* Journal of the Chemical Society (Resumed) (1953): 3932-3937.

[28] Mahabusarakam, W., Chairerk, P., and Taylor, W. Xanthones from *Garcinia cowa* Roxb. latex. Phytochemistry 66(10) (2005): 1148-1153.

[29] Tian, Z., et al. Dulxanthone A induces cell cycle arrest and apoptosis via up regulation of p53 through mitochondrial pathway in HepG2 cells. International Journal of Cancer 122(1) (2008): 31-38.

[30] Wang, Y.-H., Hou, A.-J., Zhu, G.-F., Chen, D.-F., and Sun, H.-D. Cytotoxic and antifungal isoprenylated xanthones and flavonoids from *Cudrania fruticosa*. Planta Medica 71(3) (2005): 273-274.

[31] Lianhe, Z., Xing, H., Li, W., and Zhengxing, C. Physicochemical properties, chemical composition and antioxidant activity of *Dalbergia odorifera* T. Chen seed oil. Journal of the American Oil Chemists' Society 89(5) (2012): 883-890.

[32] Vasudeva, N., Vats, M., Sharma, S., and Sardana, S. Chemistry and biological activities of the genus *Dalbergia*-A review. Pharmacognosy Reviews 3(6) (2009): 307.

[33] Bandara, B.R., Kumar, N.S., and Samaranayake, K.S. An antifungal constituent from the stem bark of *Butea monosperma*. Journal of Ethnopharmacology 25(1) (1989): 73-75.

[34] Ingham, J.L. Fungal modification of pterocarpan phytoalexins from *Melilotus alba* and *Trifolium pratense*. Phytochemistry 15(10) (1976): 1489-1495.

[35] Bezuidenhoudt, B.C., Brandt, E.V., and Ferreira, D. Flavonoid analogues from *Pterocarpus* species. Phytochemistry 26(2) (1987): 531-535.

[36] Woo, H.S., et al. Potent inhibition of bacterial neuraminidase activity by pterocarpans isolated from the roots of *Lespedeza bicolor*. Bioorganic & Medicinal Chemistry Letters 21(20) (2011): 6100-6103.

[37] Kiss, L., Kurtan, T., Antus, S., and Benyei, A. Chiroptical properties and synthesis of enantiopure *cis* and *trans* pterocarpan skeleton. Chirality 15(6) (2003): 558-563.

[38] Van Heerden, F.R., Brandt, E.V., and Roux, D.G. Structure and synthesis of some complex pyranoisoflavonoids from the bark of *Dalbergia nitidula* Welw. ex Bak. Journal of the Chemical Society, Perkin Transactions 1 (2) (1978): 137-145.

[39] Niu, D.-y., Li, Y.-k., Wu, X.-x., Shi, Y.-d., Hu, Q.-f., and Gao, X.-m. Pterocarpan derivatives from *Clinopodium urticifolium* and their Cytotoxicity. Asian Journal of Chemistry 25(17) (2013): 9672.

[40] Miyase, T., Sano, M., Yoshino, K., and Nonaka, K. Antioxidants from *Lespedeza homoloba* (II). Phytochemistry 52(2) (1999): 311-319.

[41] Slade, D., Ferreira, D., and Marais, J.P. Circular dichroism, a powerful tool for the assessment of absolute configuration of flavonoids. Phytochemistry 66(18) (2005): 2177-2215.

[42] Gutierrez, R.M.P. and Baez, E.G. Cytotoxic activity of isoflavan-cinnamylphenols from *Dalbergia congestiflora* on HeLa cells. Journal of Medicinal Plants Research 7(40) (2013): 2992-2998.

[43] Marzouk, M.S., Ibrahim, M.T., El-Gindi, O.R., and Bakr, M.S.A. Isoflavonoid glycosides and rotenoids from *Pongamia pinnata* leaves. Zeitschrift für Naturforschung C 63(1-2) (2008): 1-7.

[44] Dixit, P., et al. Constituents of *Dalbergia sissoo* Roxb. leaves with osteogenic activity. Bioorganic & Medicinal Chemistry Letters 22(2) (2012): 890-897.

[45] Zapesochnaya, G. and Laman, N. The structures of isoflavone C-glycosides from *Lupinus luteus.* Chemistry of Natural Compounds 13(6) (1977): 729-730.

[46] Adinarayana, D. and Rao, J.R. Isoflavonoid glycosides of *Dalbergia paniculata*: The constitutions of dalpanitin and dalpatin. Tetrahedron 28(21) (1972): 5377-5384.

APPENDIX

Supplementary supporting

A new taraxerol triterpene from the roots of *Microcos tomentosa*

Sutin Kaennakam[a], Jirapast Sichaem[a], Suttira Khumkratok[b], Pongpun Siripong[c] and Santi Tip-pyang[a,*]

[a]*Natural Products Research Unit, Department of Chemistry, Faculty of Science,Chulalongkorn University, Bangkok, 10330, Thailand*

[b]*Walai Rukhavej Botanical Research Institute, Mahasarakham University, Mahasarakham 44000, Thailand*

[c]*Natural Products Research Section, Research Division, National Cancer Institute, Bangkok 10400, Thailand*

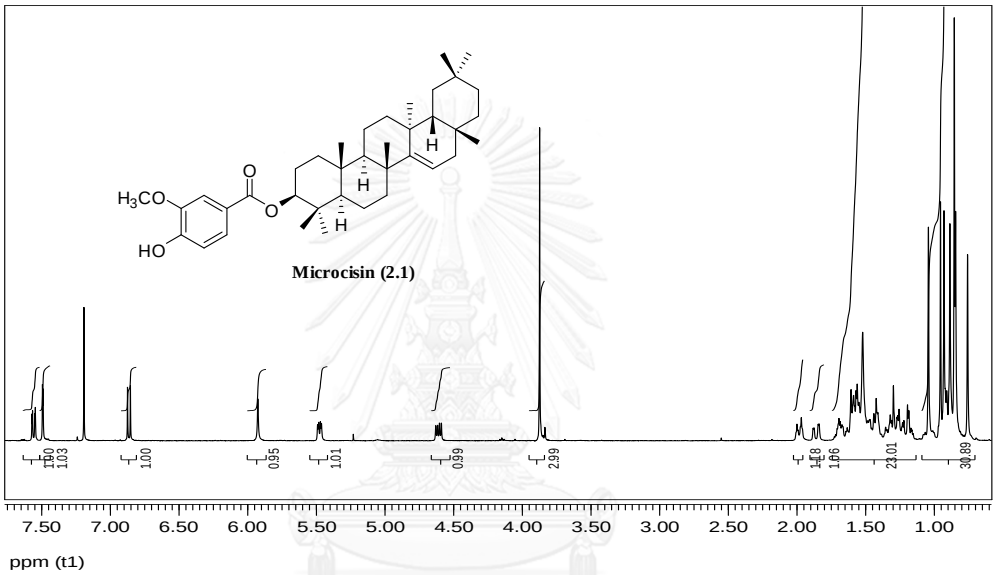


Figure A1 ^{1}H NMR spectrum of **2.1** in $CDCl_3$.

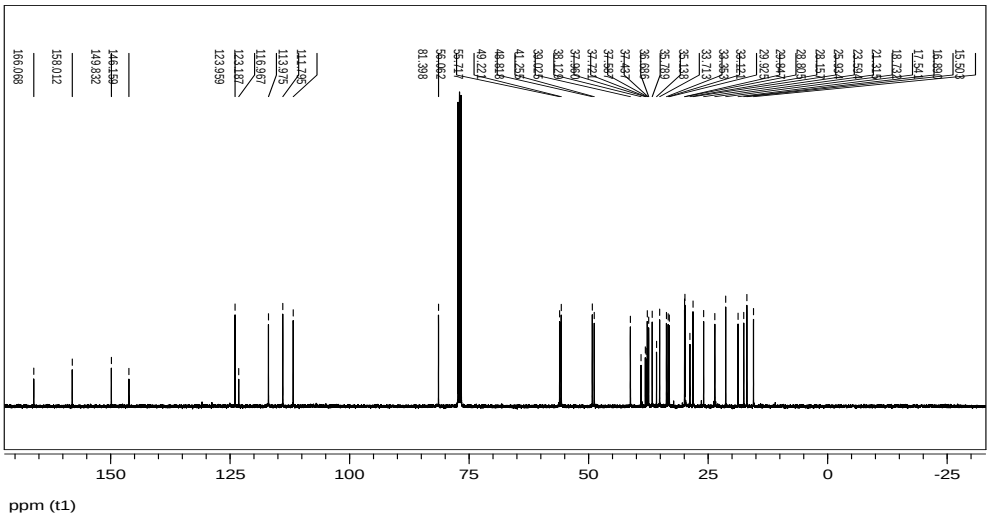


Figure A2 ^{13}C NMR spectrum of **2.1** in $CDCl_3$.

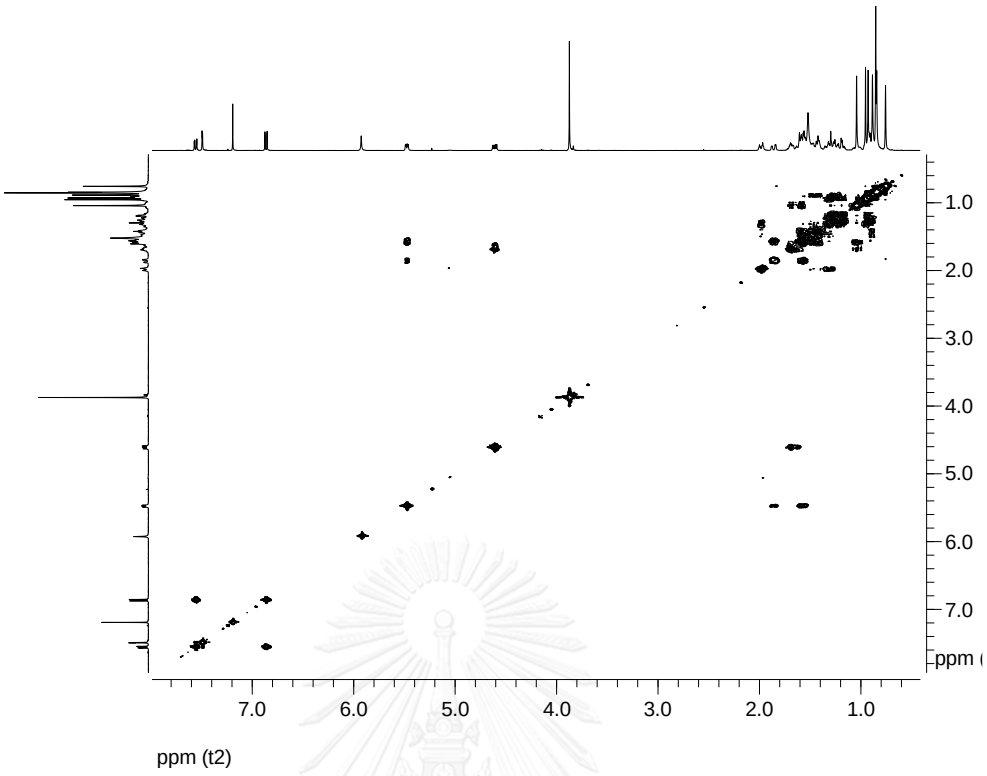


Figure A3 COSY NMR spectrum of **2.1** in $CDCl_3$.

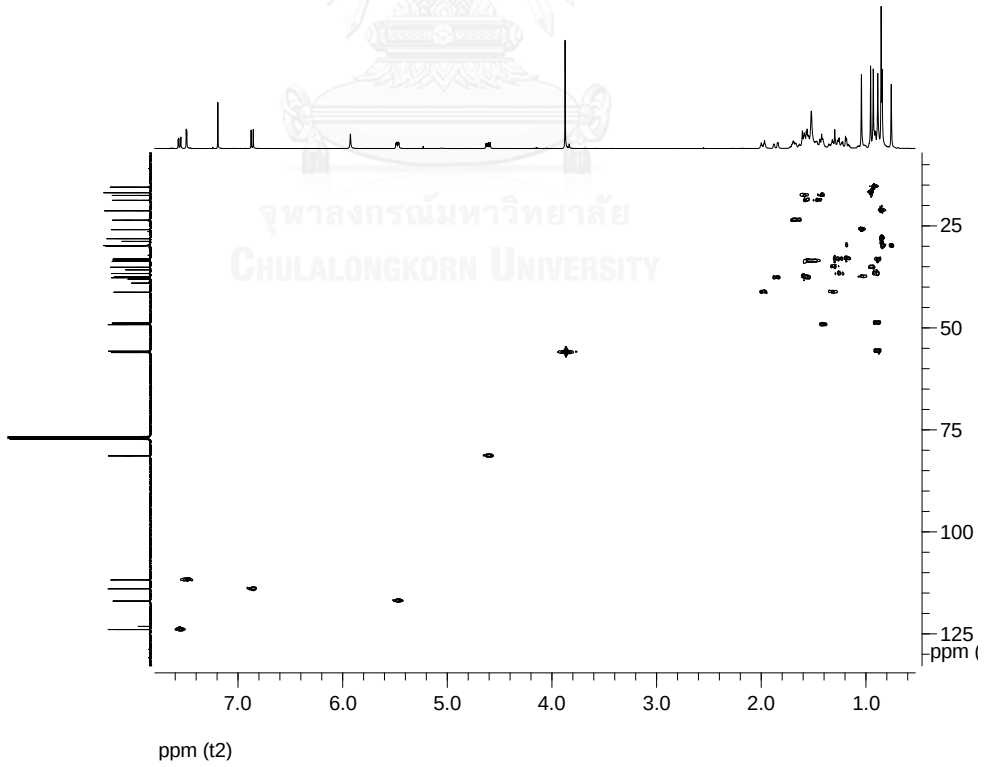


Figure A4 HSQC NMR spectrum of **2.1** in $CDCl_3$.

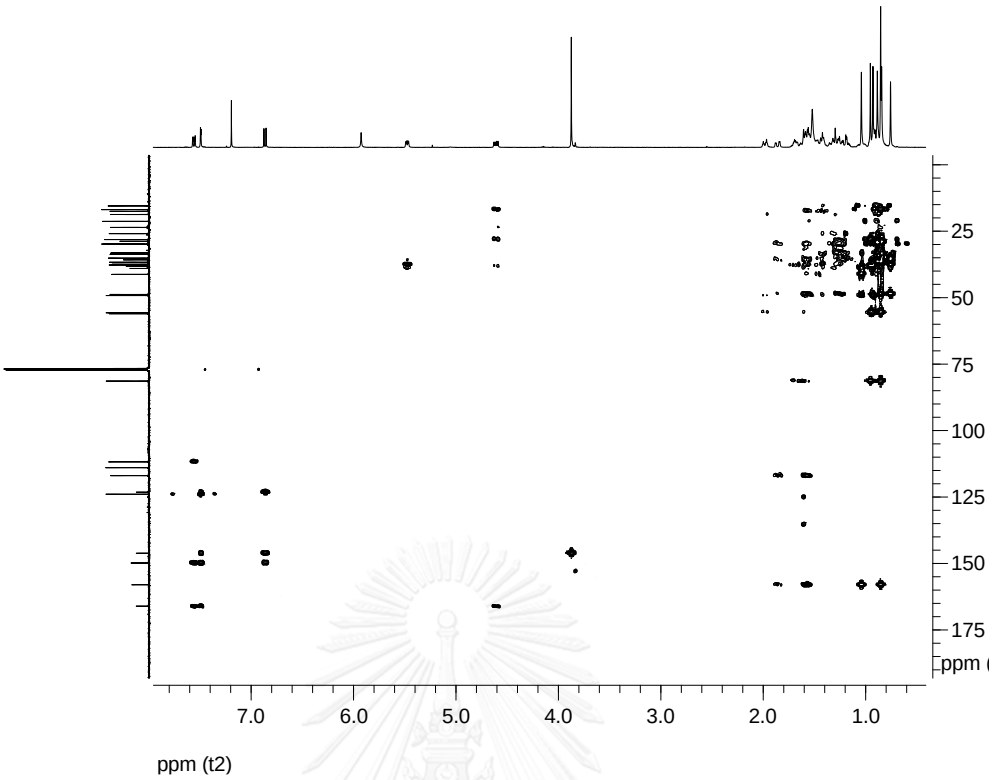


Figure A5 HMBC NMR spectrum of **2.1** in $CDCl_3$.

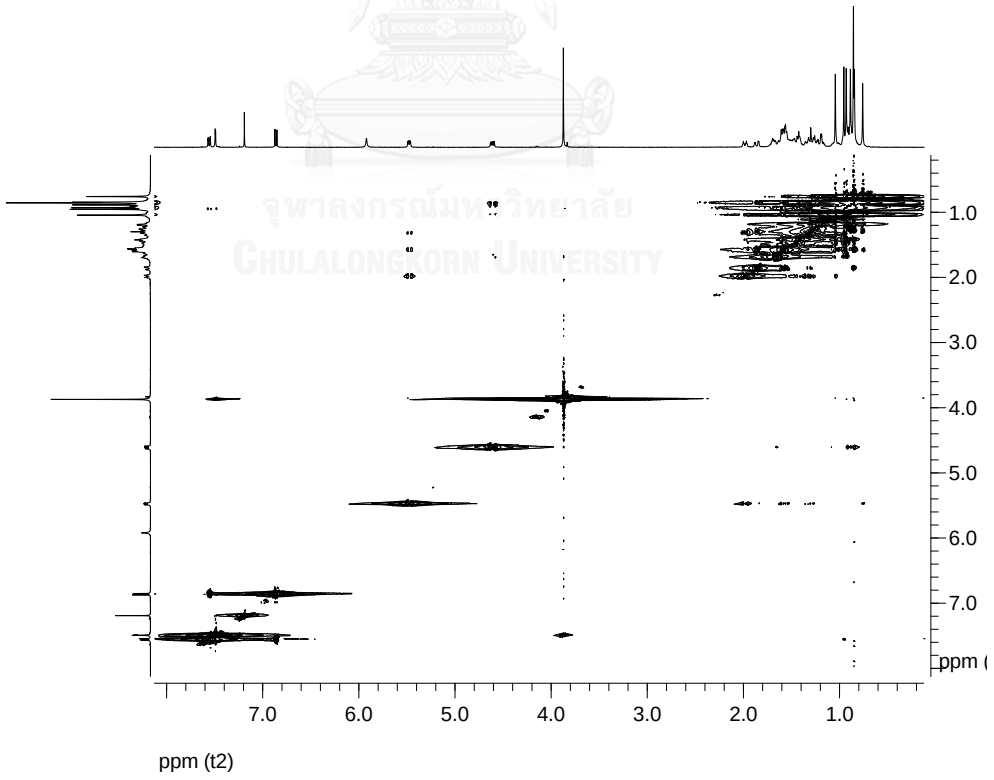


Figure A6 NOESY NMR spectrum of **2.1** in $CDCl_3$.

Supplementary supporting

Kaennacowanol A-C, three new xanthones and their cytotoxicity from the roots of *Garcinia cowa*

Sutin Kaennakam[a], Pongpun Siripong[b], Santi Tip-pyang[a],*

[a]*Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand*

[a]*Natural Products Research Section, Research Division, National Cancer Institute, Bangkok 10400, Thailan*

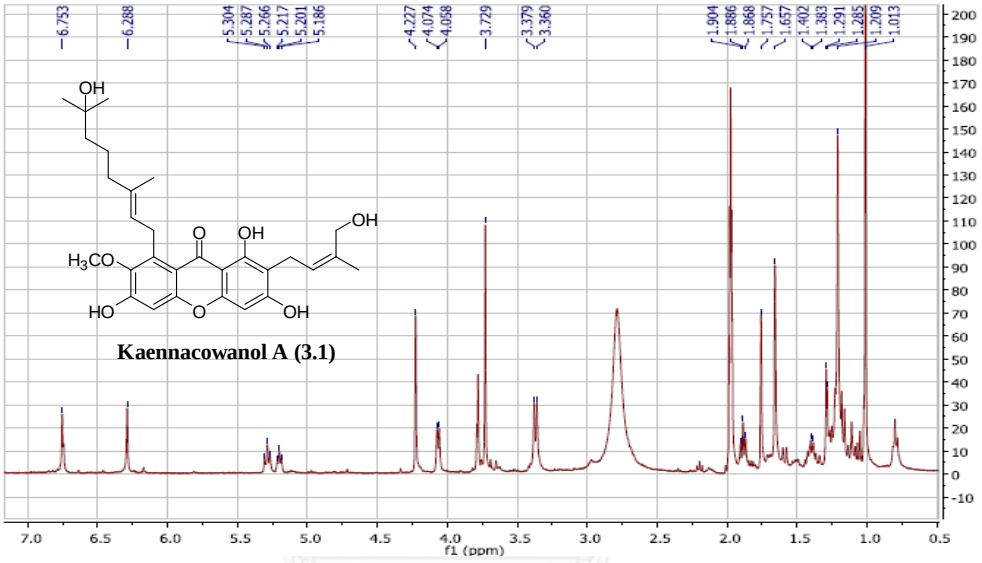


Figure A7 ^{1}H NMR spectrum of **3.1** in Acetone-d_6.

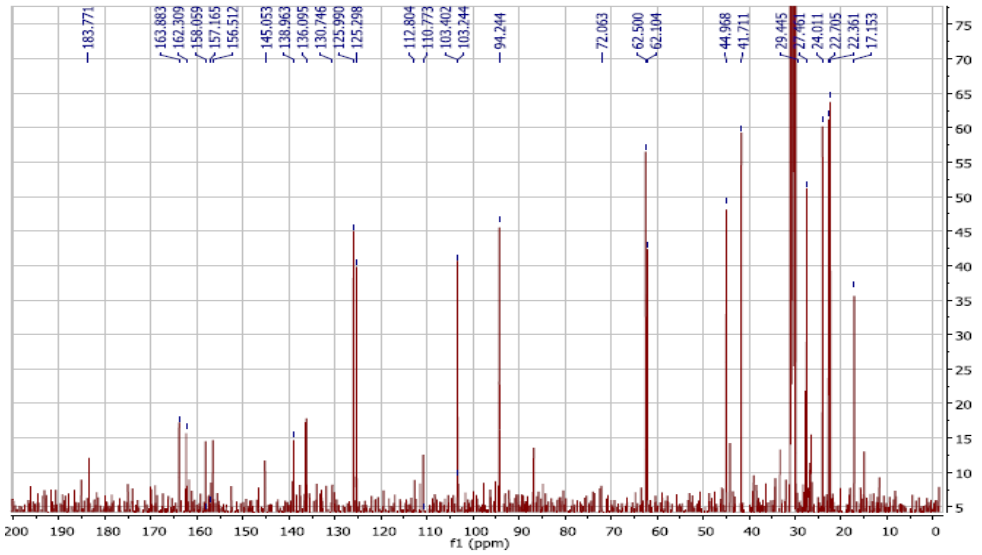


Figure A8 ^{13}C NMR spectrum of **3.1** in Acetone-d_6.

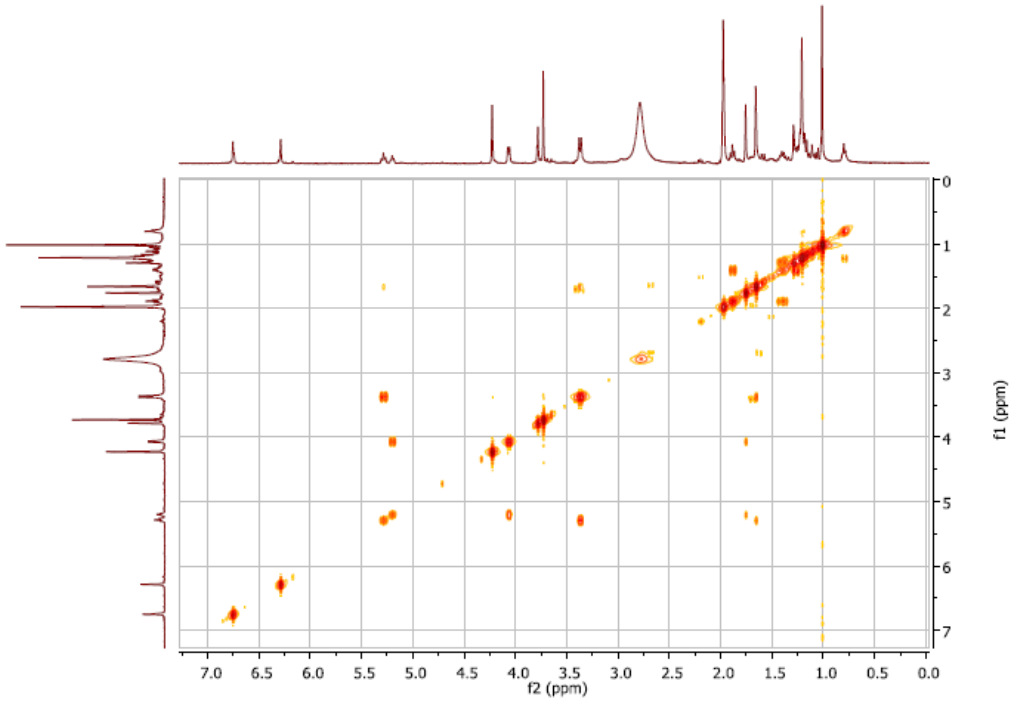


Figure A9 COSY NMR spectrum of **3.1** in Acetone-d_6.

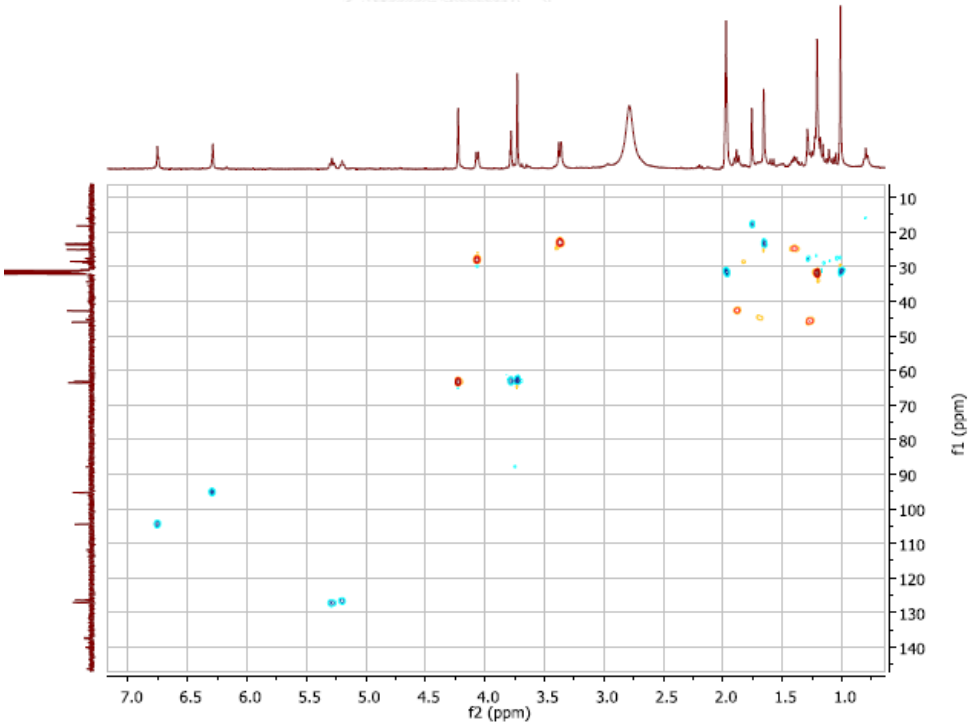


Figure A10 HSQC NMR spectrum of **3.1** in Acetone-d_6.

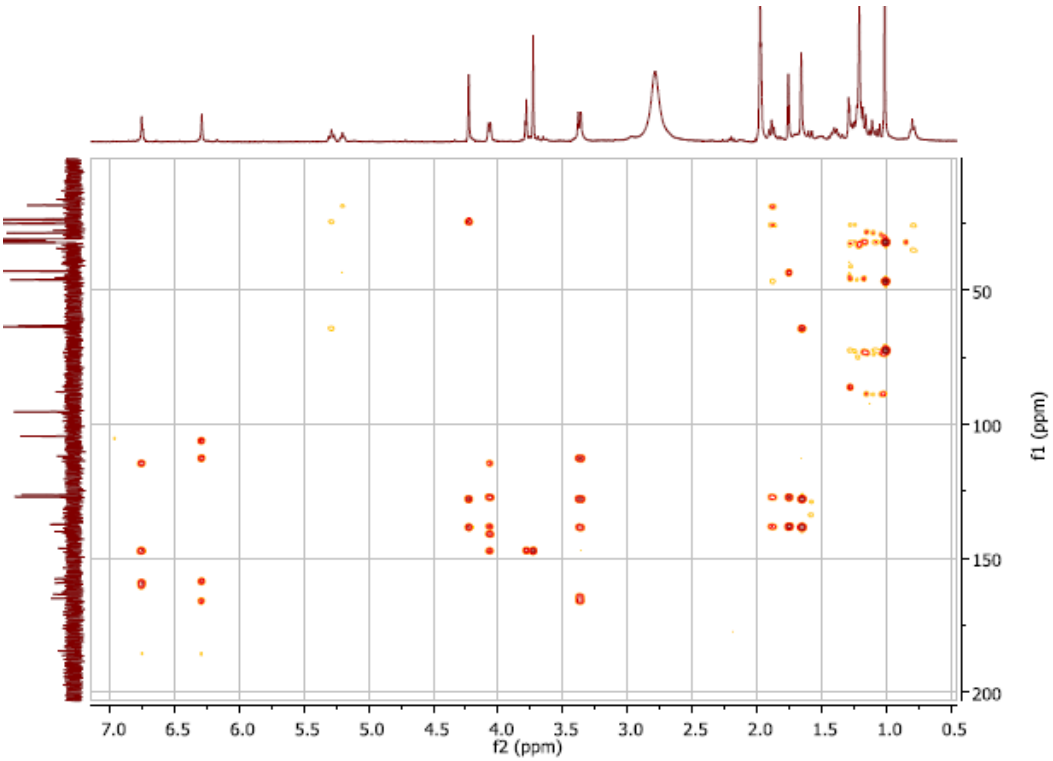


Figure A11 HMBC NMR spectrum of **3.1** in Acetone-d_6.

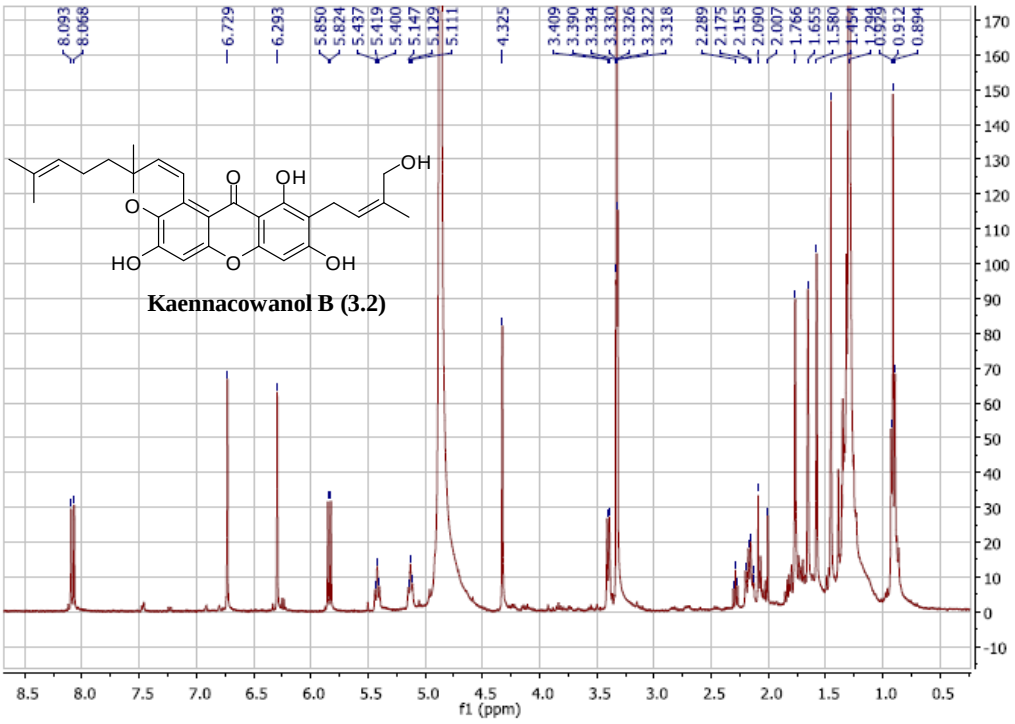


Figure A12 ^{1}H NMR spectrum of **3.2** in CD_3OD.

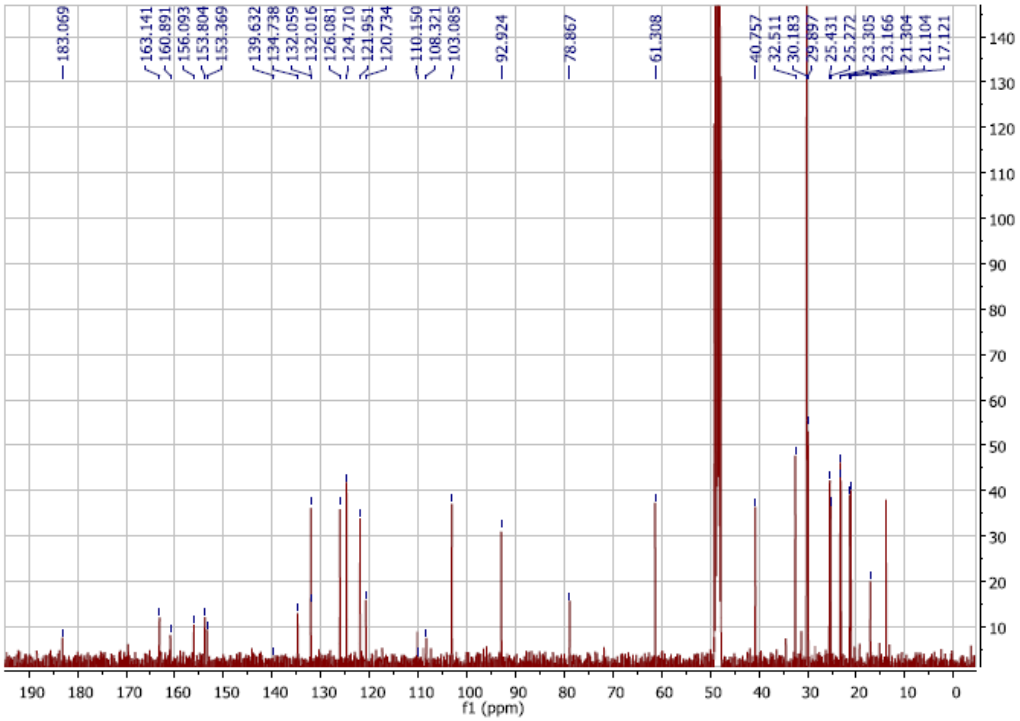


Figure A13 ^{13}C NMR spectrum of **3.2** in CD_3OD.

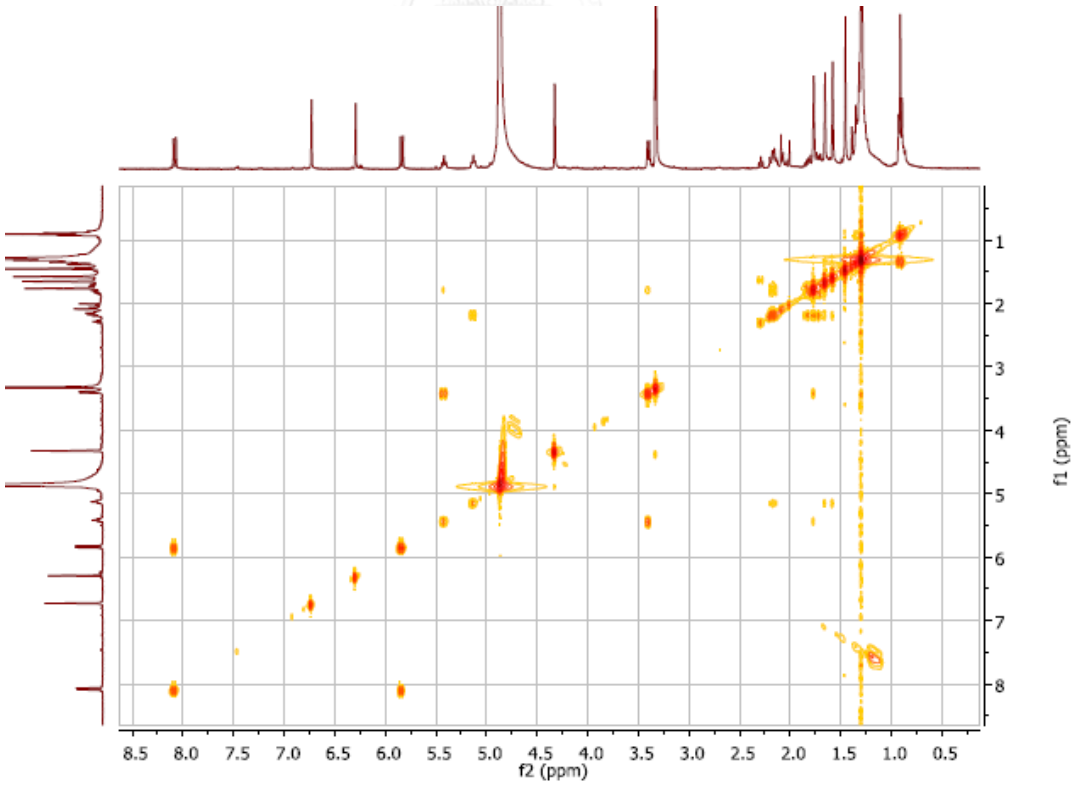


Figure A14 COSY NMR spectrum of **3.2** in CD_3OD.

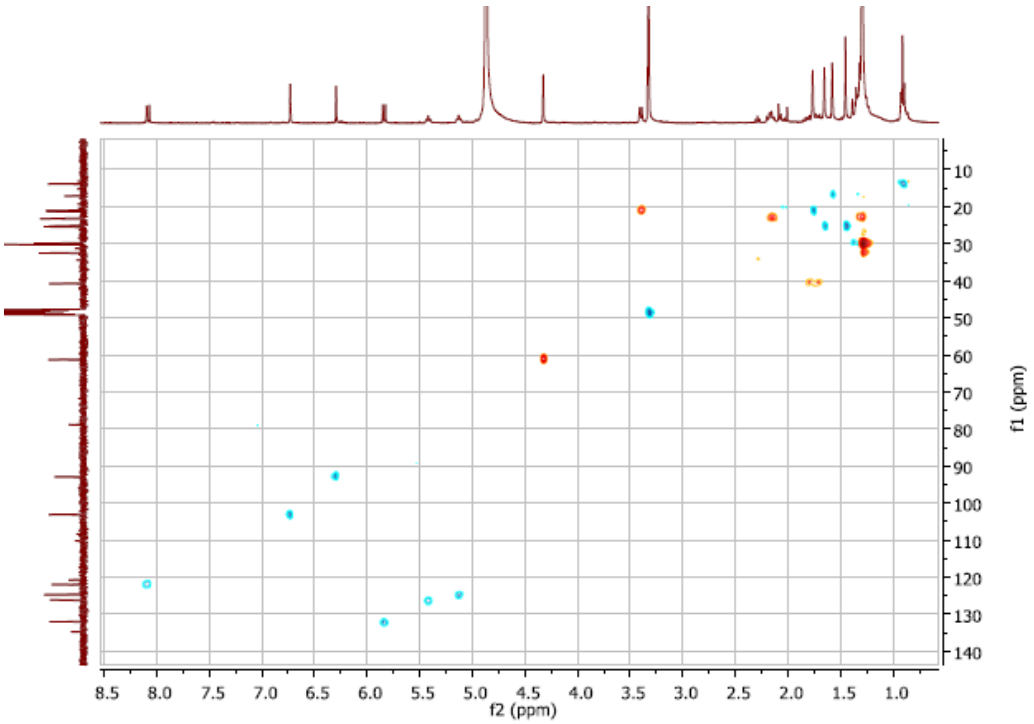


Figure A15 HSQC NMR spectrum of **3.2** in CD_3OD.

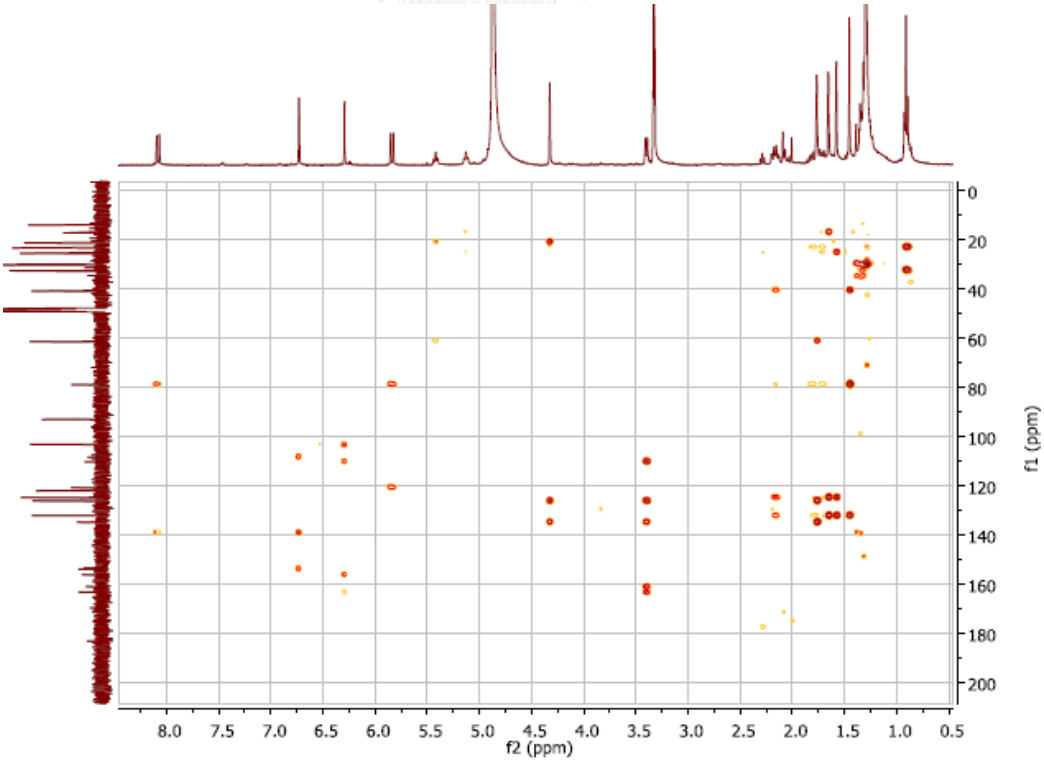


Figure A16 HMBC NMR spectrum of **3.2** in CD_3OD.

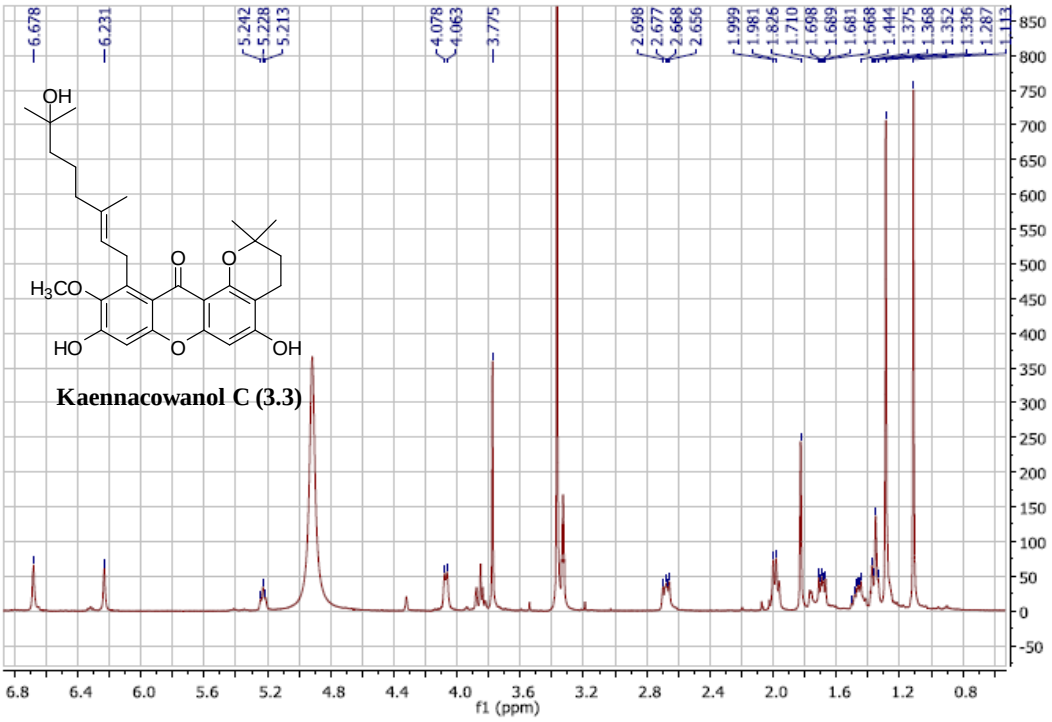


Figure A17 ^{1}H NMR spectrum of **3.3** in CD_3OD.

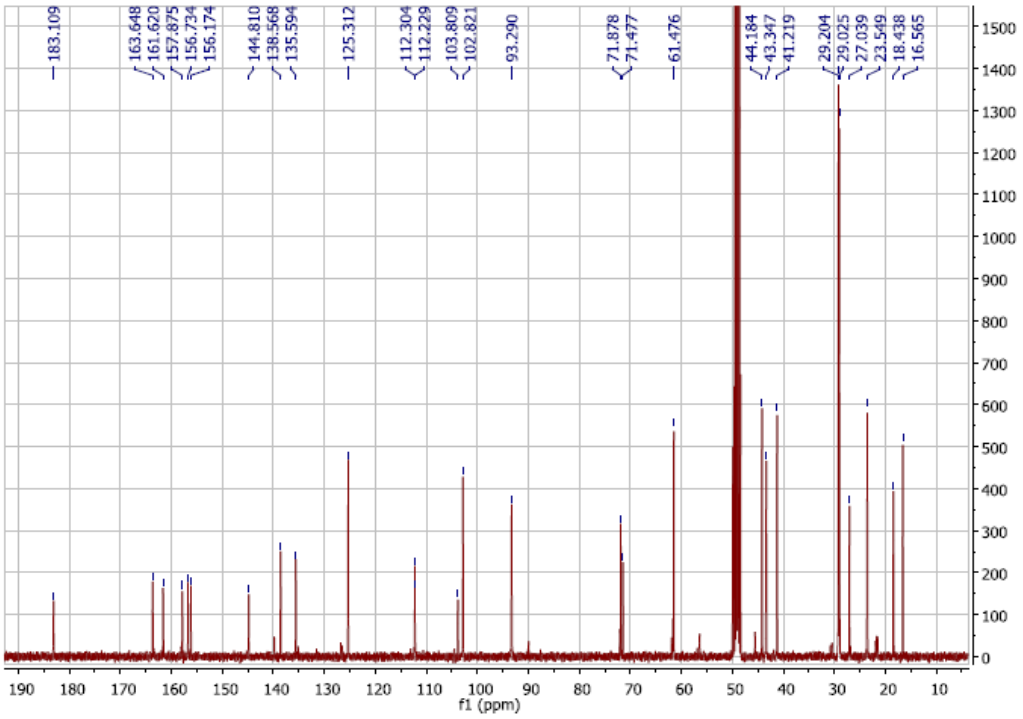


Figure A18 ^{13}C NMR spectrum of **3.3** in CD_3OD.

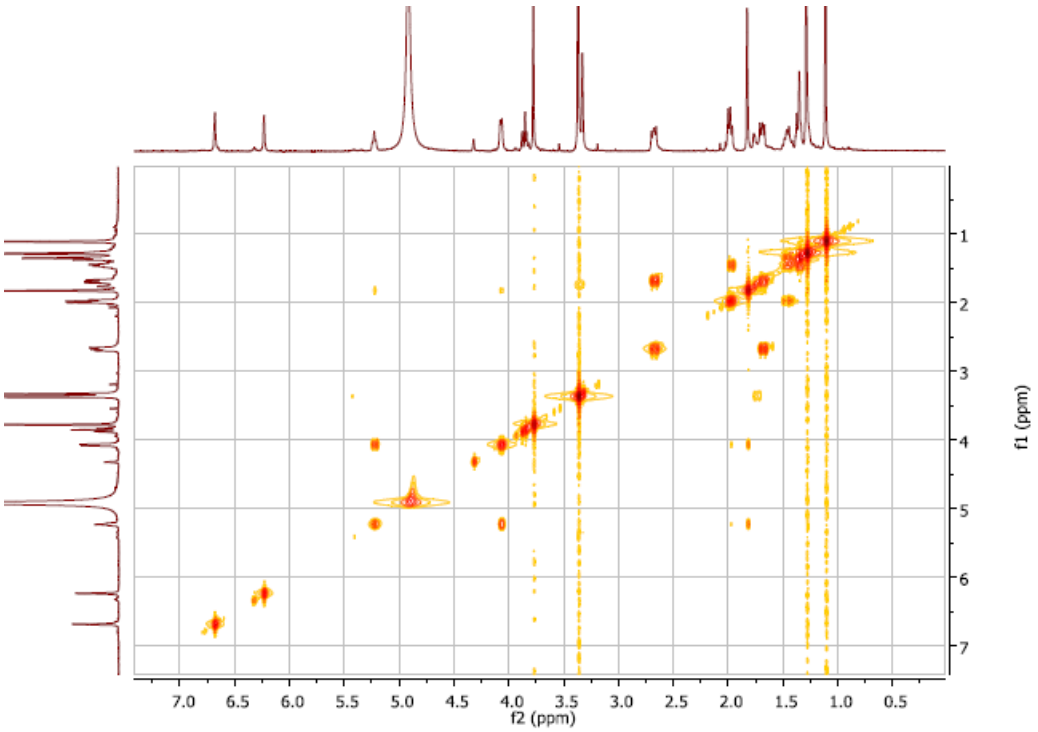


Figure A19 COSY NMR spectrum of **3.3** in CD_3OD.

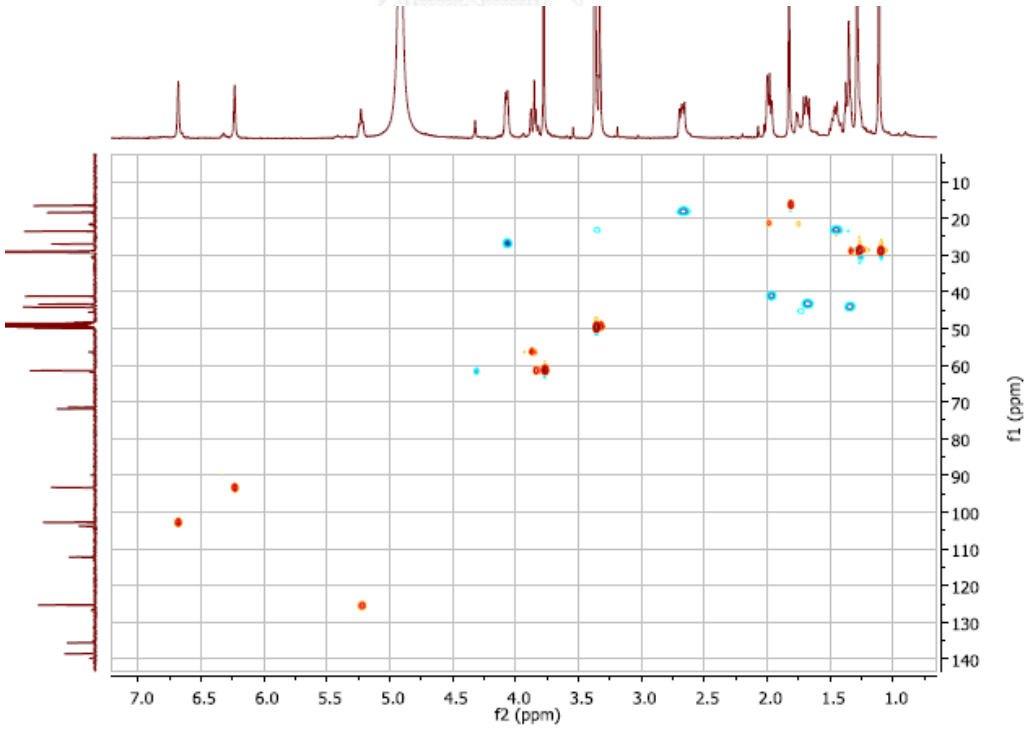


Figure A20 HSQC NMR spectrum of **3.3** in CD_3OD.

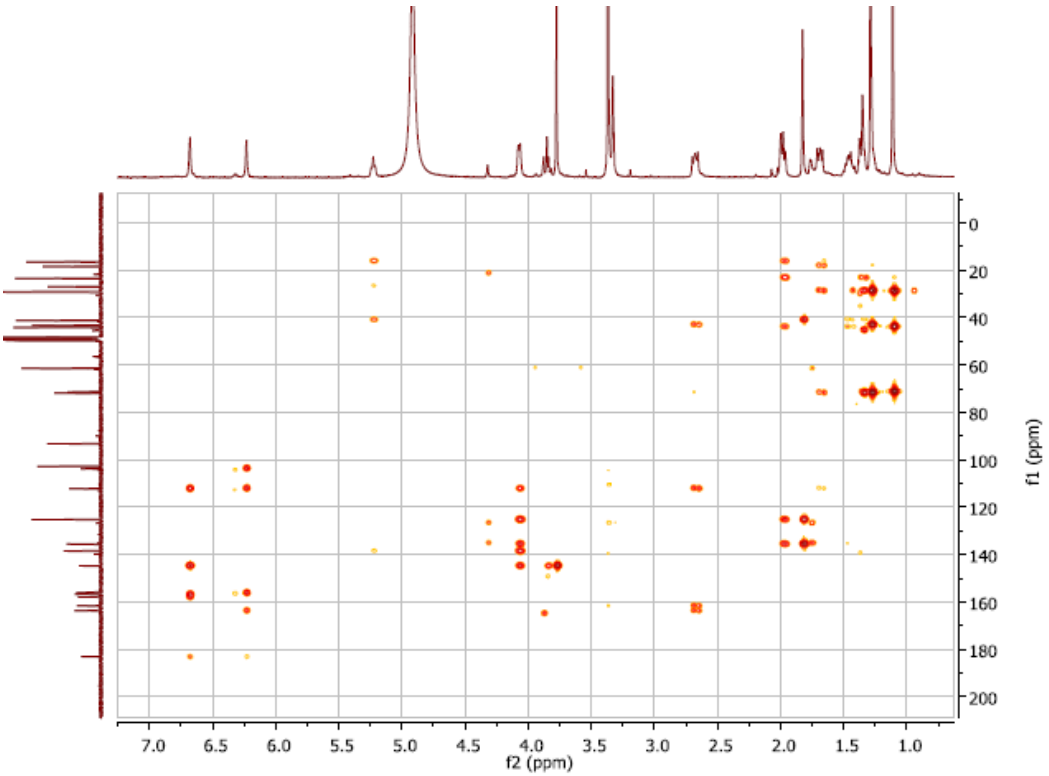


Figure A21 HMBC NMR spectrum of **3.3** in CD_3OD.

Supplementary supporting

Kaennavelutinans A-C, three new pterocarpans and their cytotoxicity from the roots of *Dalbergia velutina*

Sutin Kaennakam[a], Pongpun Siripong[b], Santi Tip-pyang[a,*]

[a]*Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand*

[b]*Natural Products Research Section, Research Division, National Cancer Institute, Bangkok 10400, Thailand*

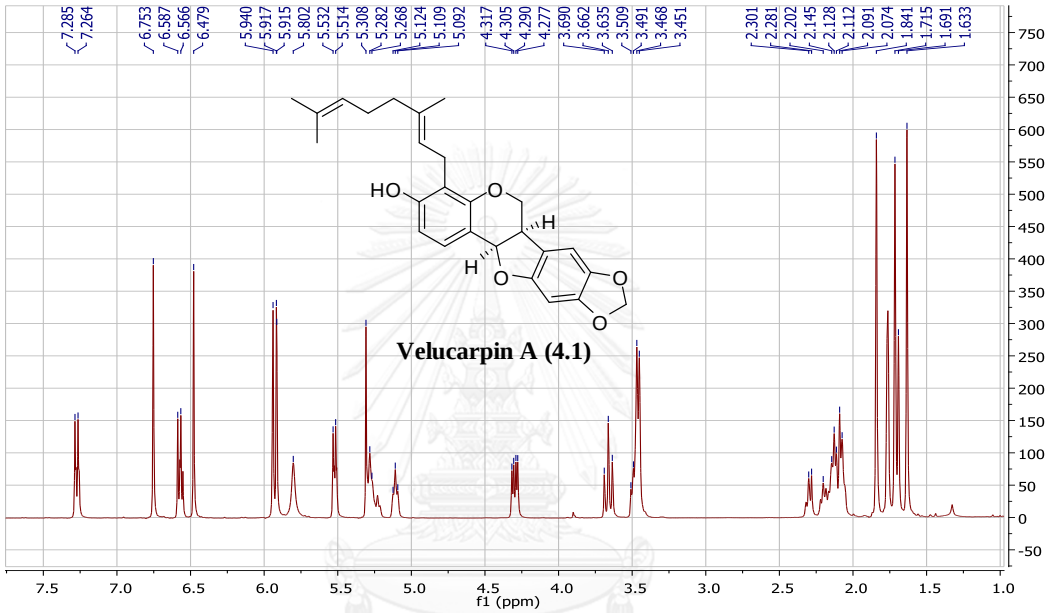


Figure A22 ^{1}H NMR spectrum of **4.1** in $CDCl_3$.

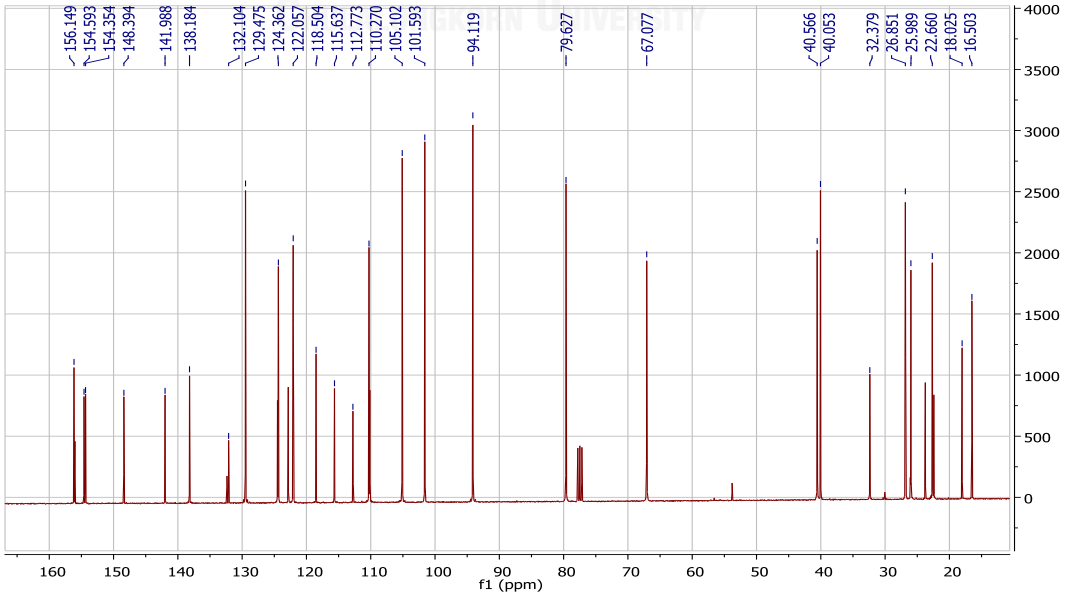


Figure A23 ^{13}C NMR spectrum of **4.1** in $CDCl_3$.

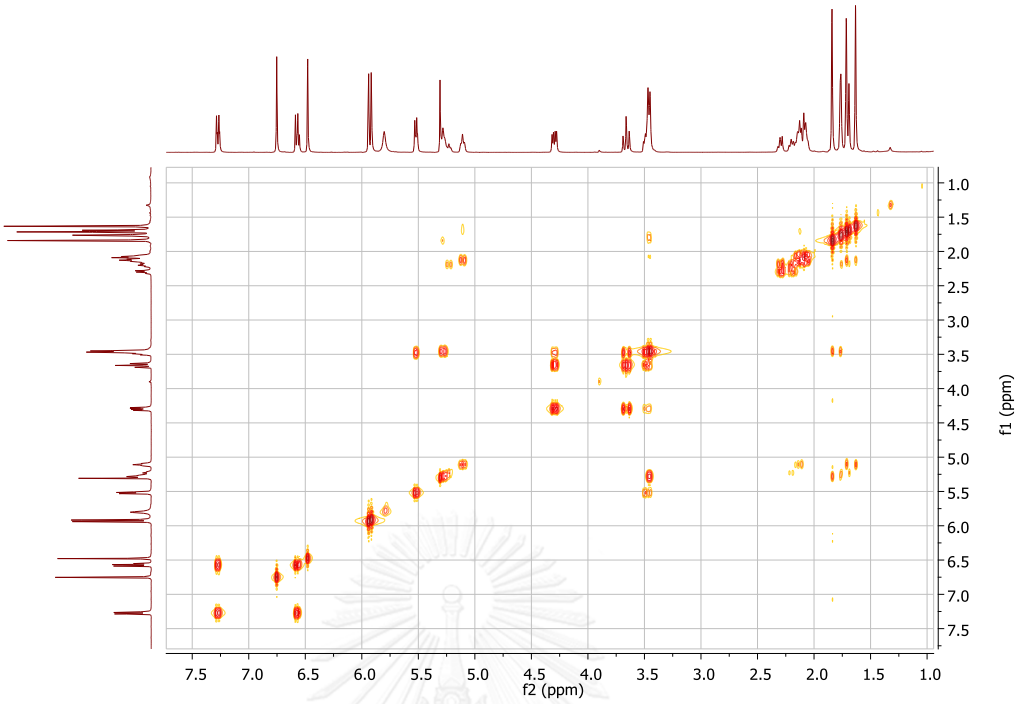


Figure A24 COSY NMR spectrum of **4.1** in $CDCl_3$.

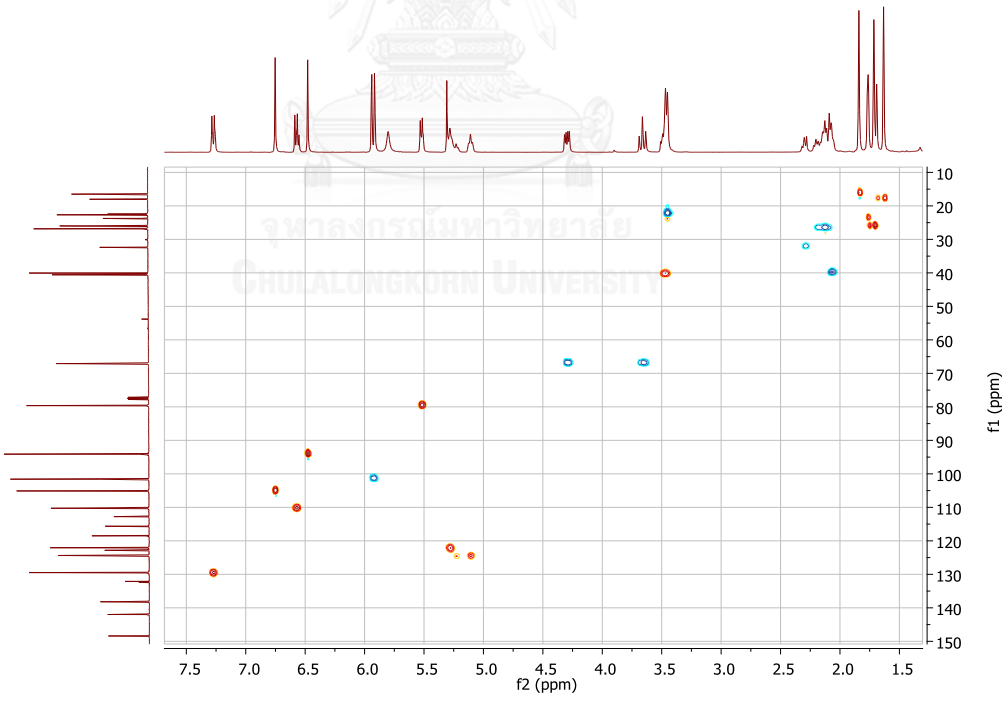


Figure A25 HSQC NMR spectrum of **4.1** in $CDCl_3$.

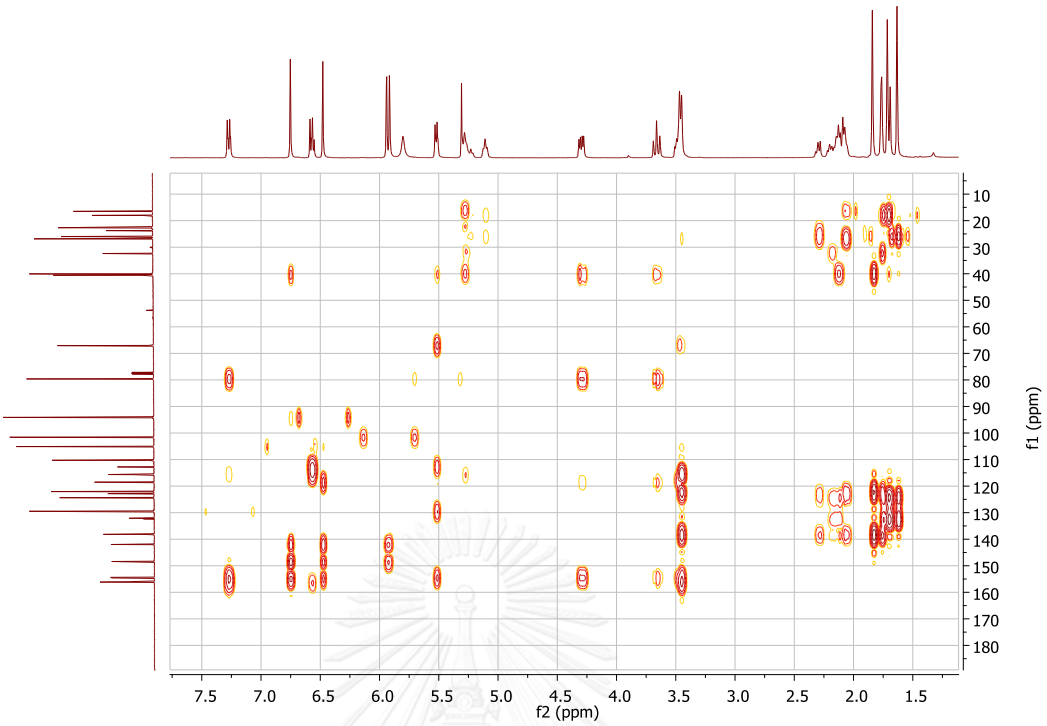


Figure A26 HMBC NMR spectrum of **4.1** in $CDCl_3$.

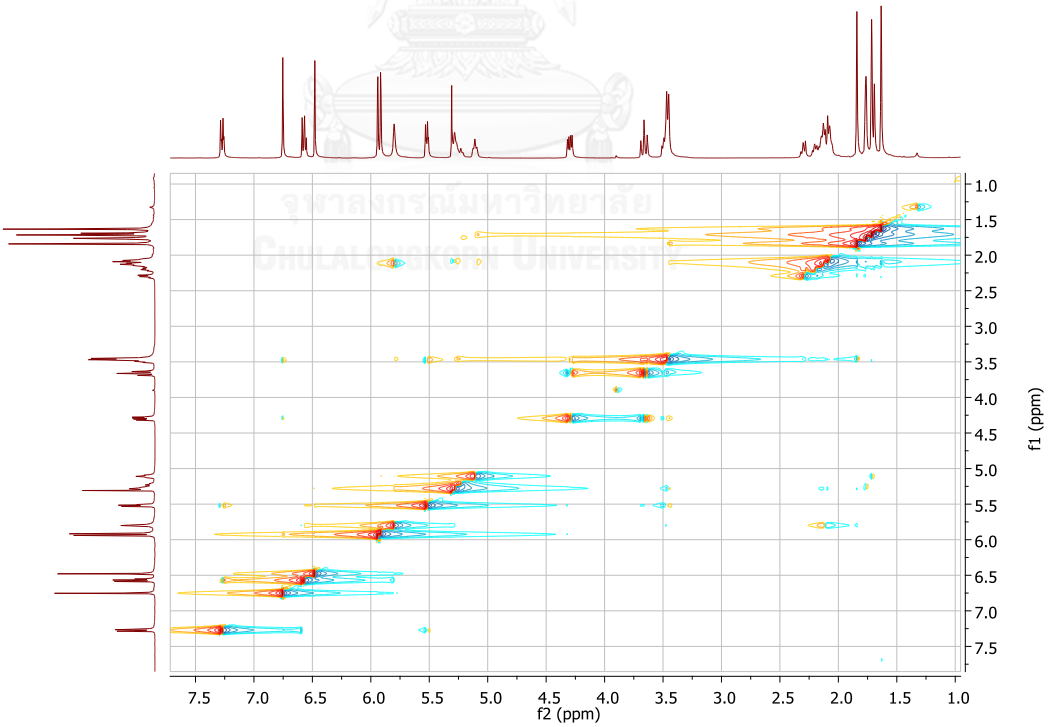


Figure A27 NOESY NMR spectrum of **4.1** in $CDCl_3$.

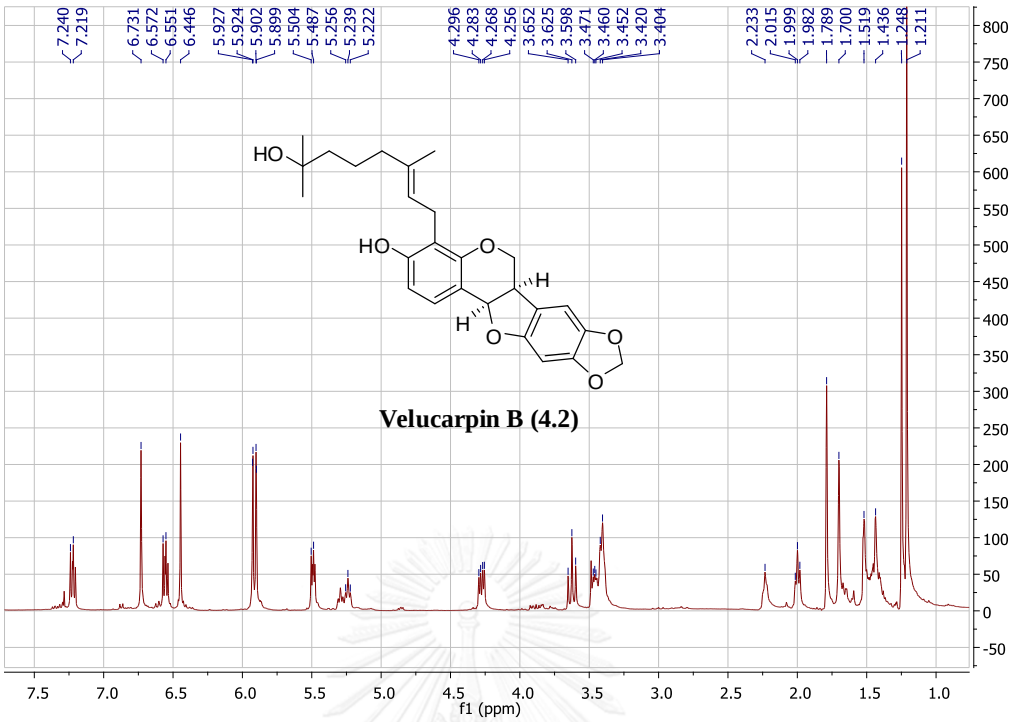


Figure A28 ^{1}H NMR spectrum of **4.2** in $CDCl_3$.

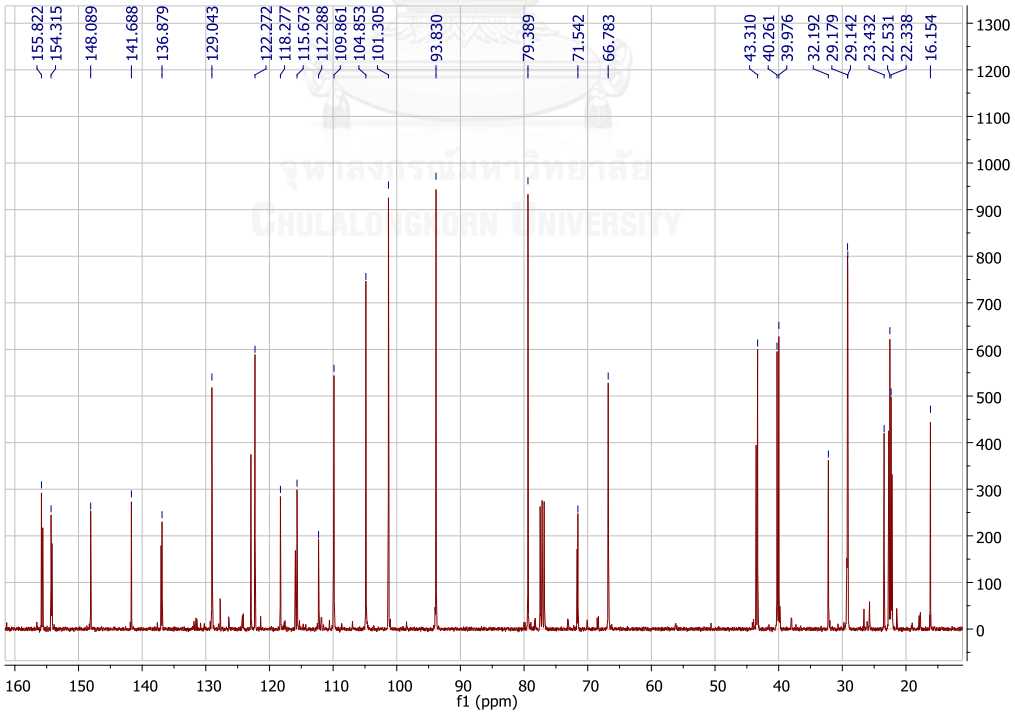


Figure A29 ^{13}C NMR spectrum of **4.2** in $CDCl_3$.

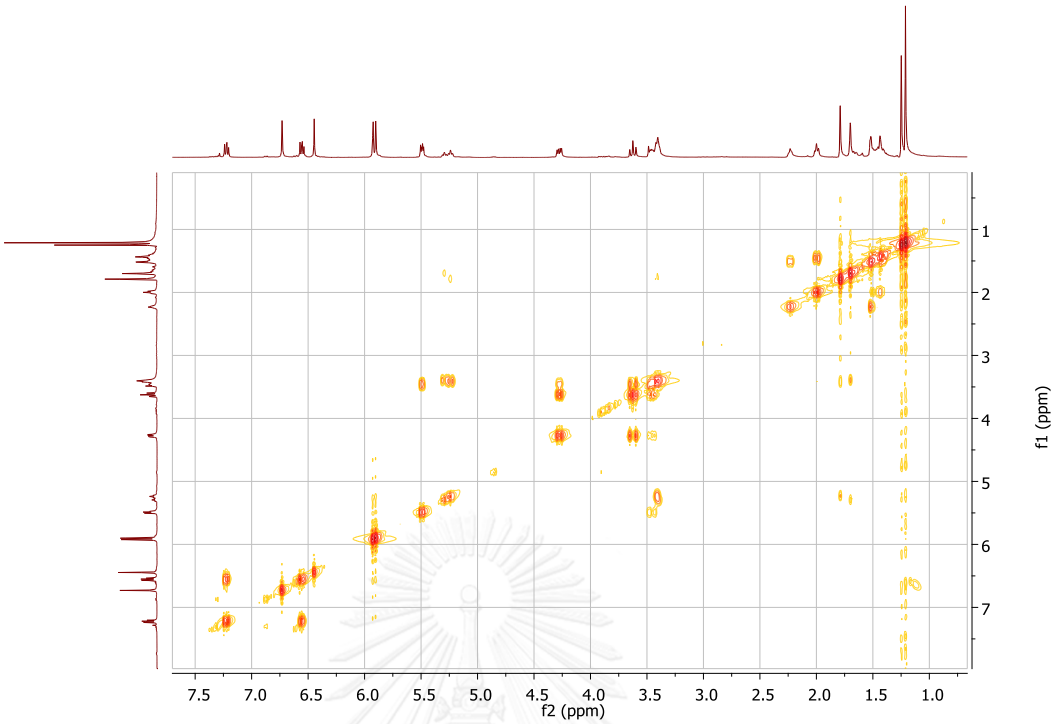


Figure A30 COSY NMR spectrum of **4.2** in $CDCl_3$.

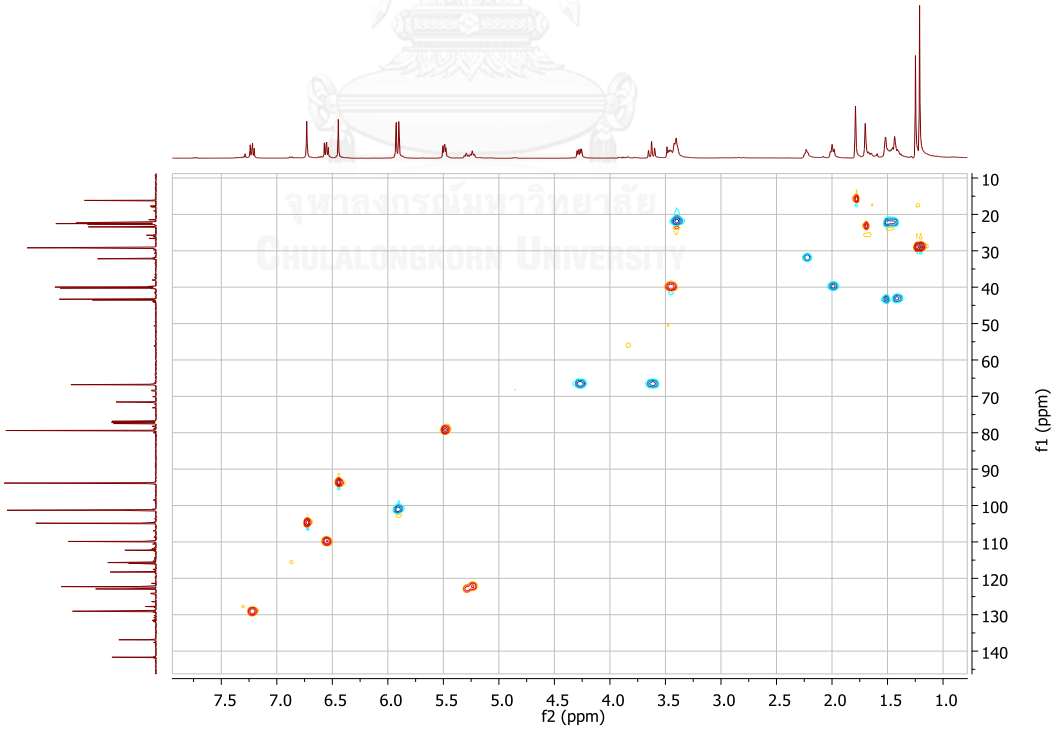


Figure A31 HSQC NMR spectrum of **4.2** in $CDCl_3$.

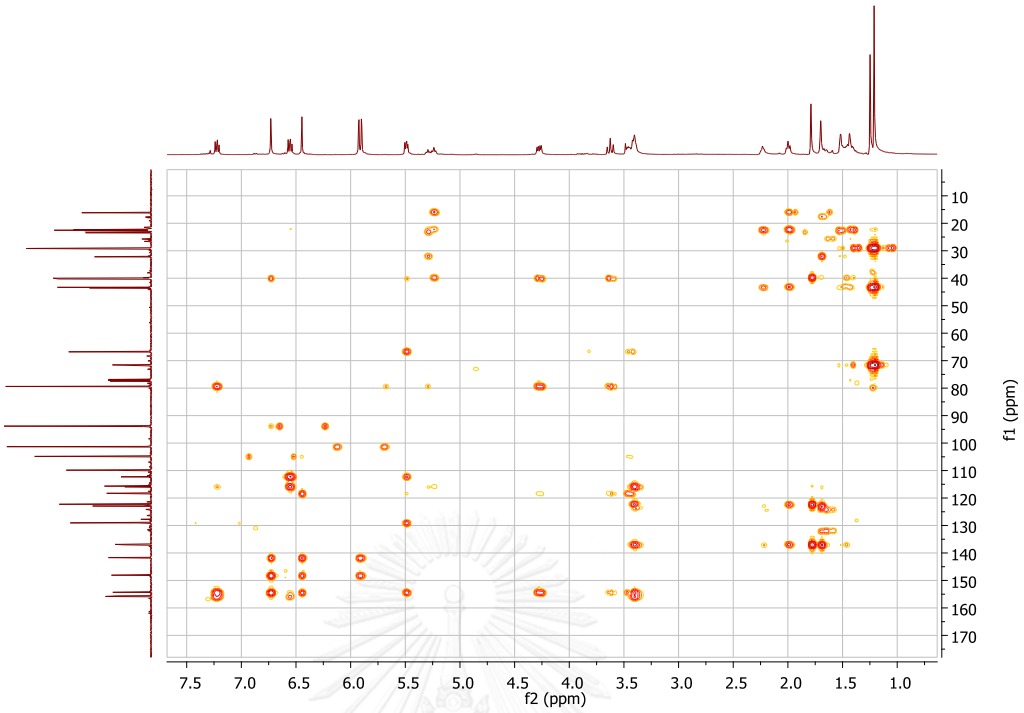


Figure A32 HMBC NMR spectrum of **4.2** in $CDCl_3$.

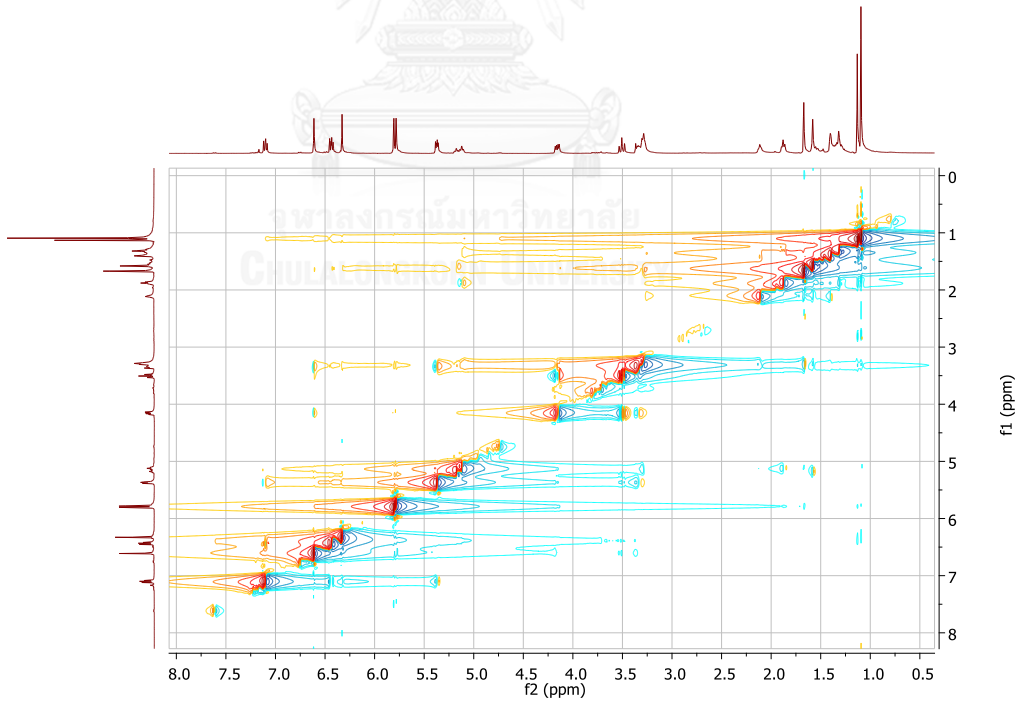


Figure A33 NOESY NMR spectrum of **4.2** in $CDCl_3$.

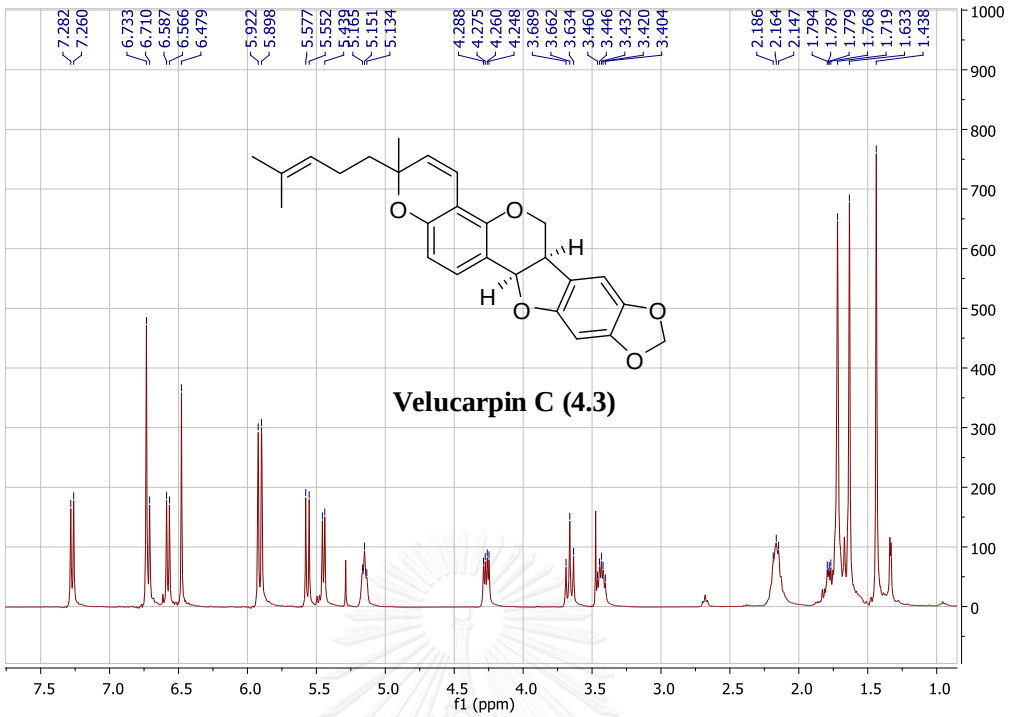


Figure A34 ^{1}H NMR spectrum of **4.3** in $CDCl_3$.

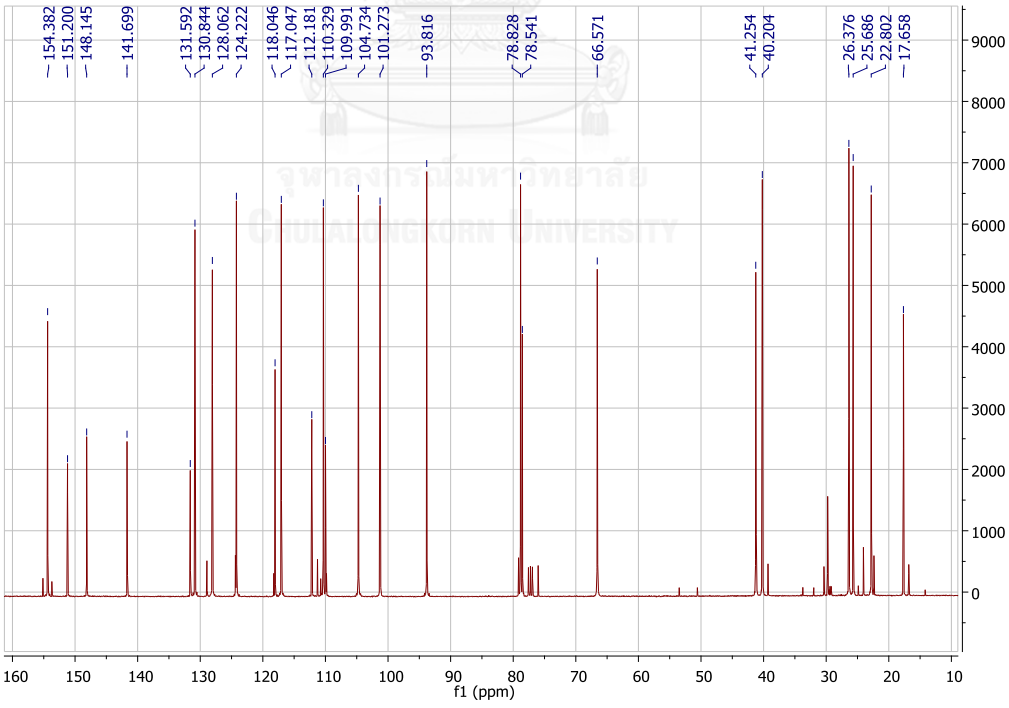


Figure A35 ^{13}C NMR spectrum of **4.3** in $CDCl_3$.

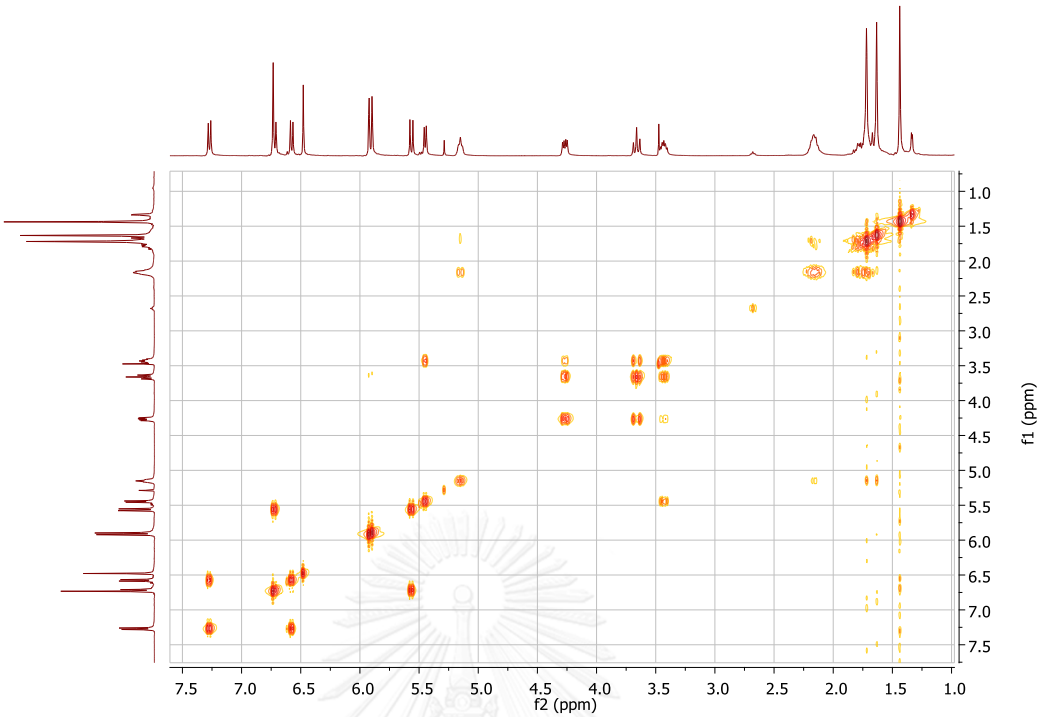


Figure A36 COSY NMR spectrum of **4.3** in $CDCl_3$.

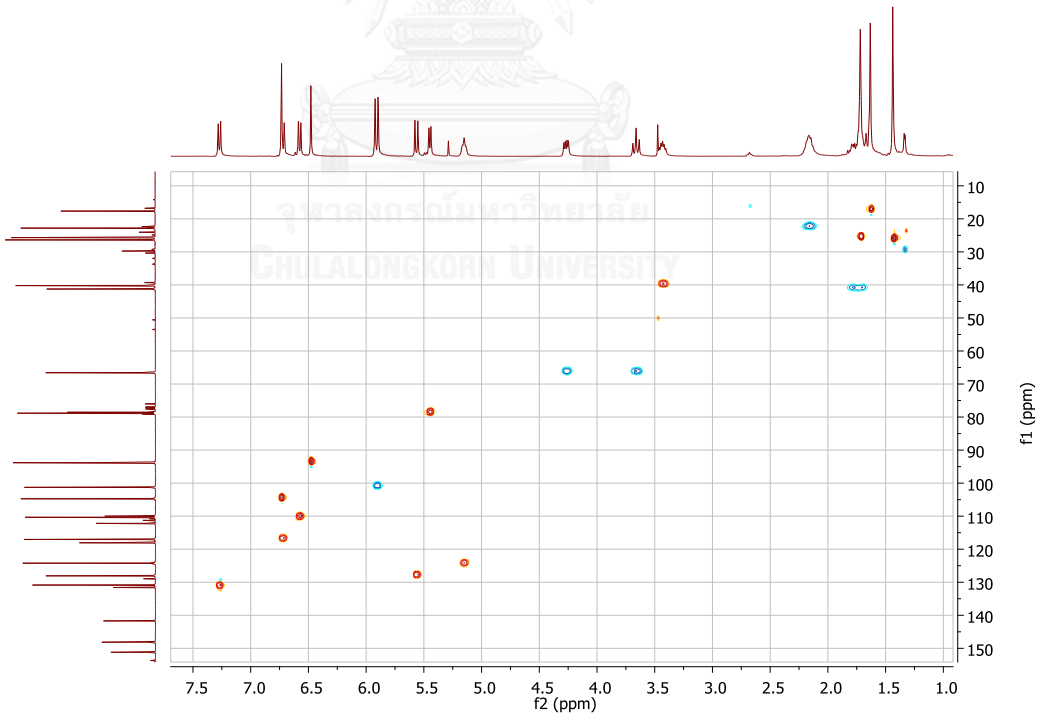


Figure A37 NSQC NMR spectrum of **4.3** in $CDCl_3$.

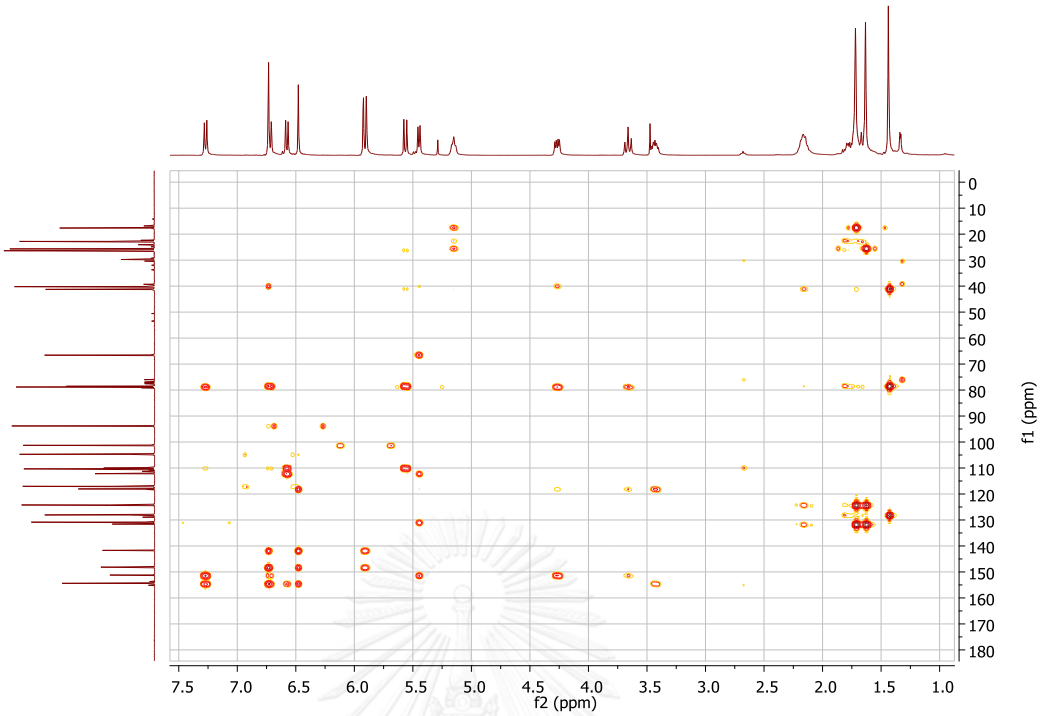


Figure A38 HMBC NMR spectrum of **4.3** in $CDCl_3$.

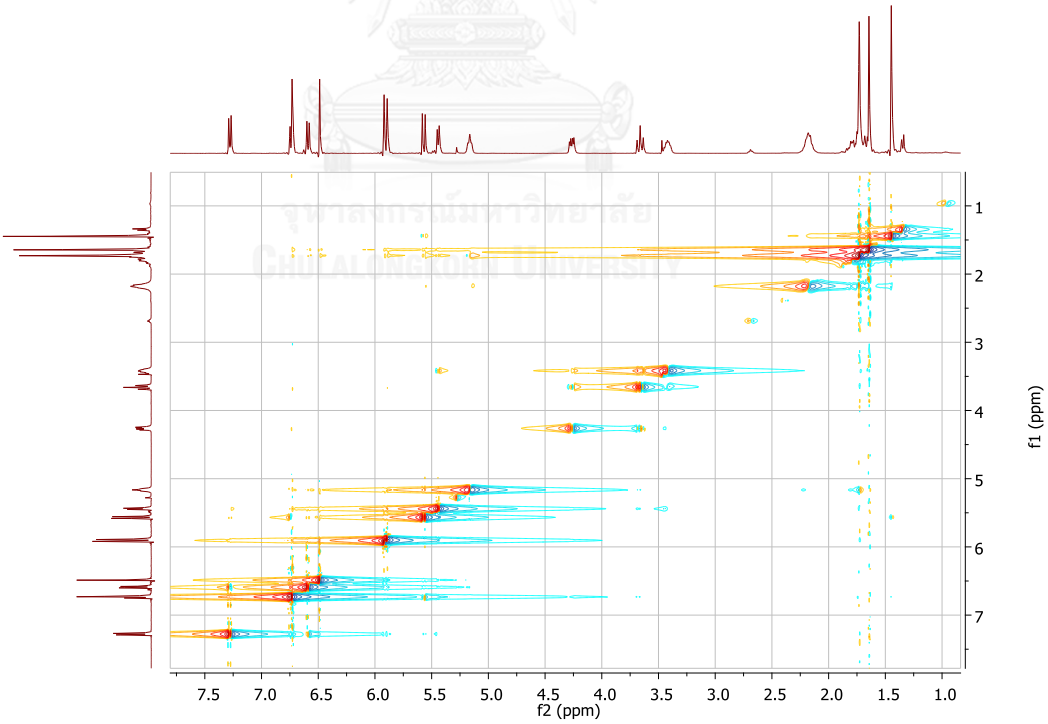


Figure A39 NOESY NMR spectrum of **4.3** in $CDCl_3$.

Supplementary supporting

Kaennavelutinols A-C, three new isoflavanes and their cytotoxicity from the roots of *Dalbergia velutina*

Sutin Kaennakam[a], Pongpun Siripong[b], Santi Tip-pyang[a,*]

[a]*Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand*

[b]*Natural Products Research Section, Research Division, National Cancer Institute, Bangkok 10400, Thailand*

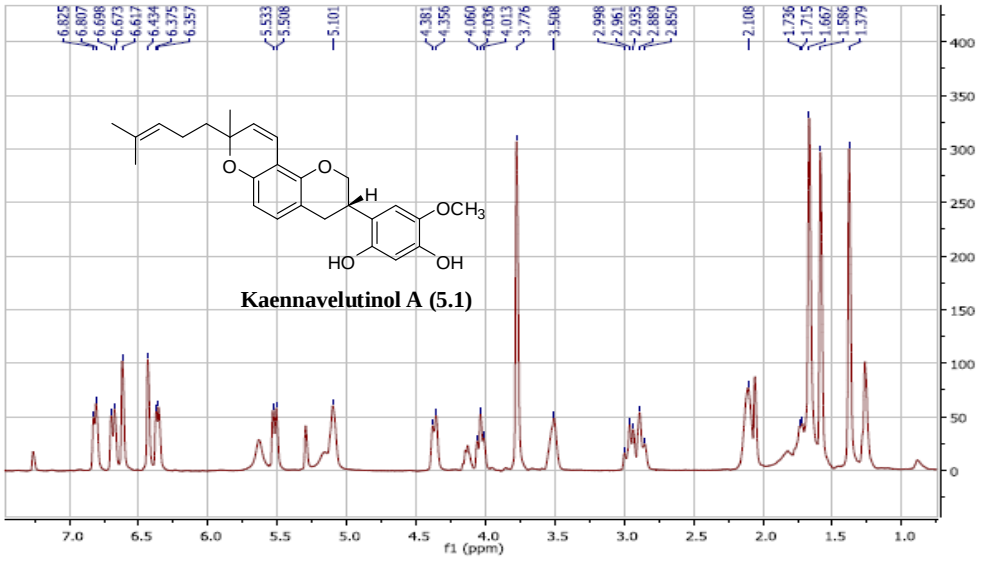


Figure A40 ^{1}H NMR spectrum of **5.1** in $CDCl_3$.

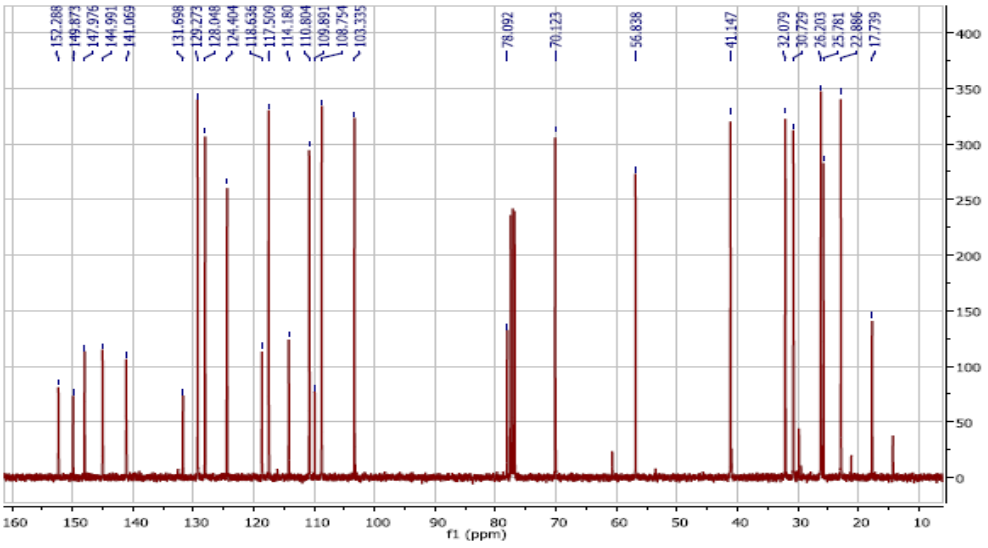


Figure A41 ^{13}C NMR spectrum of **5.1** in $CDCl_3$.

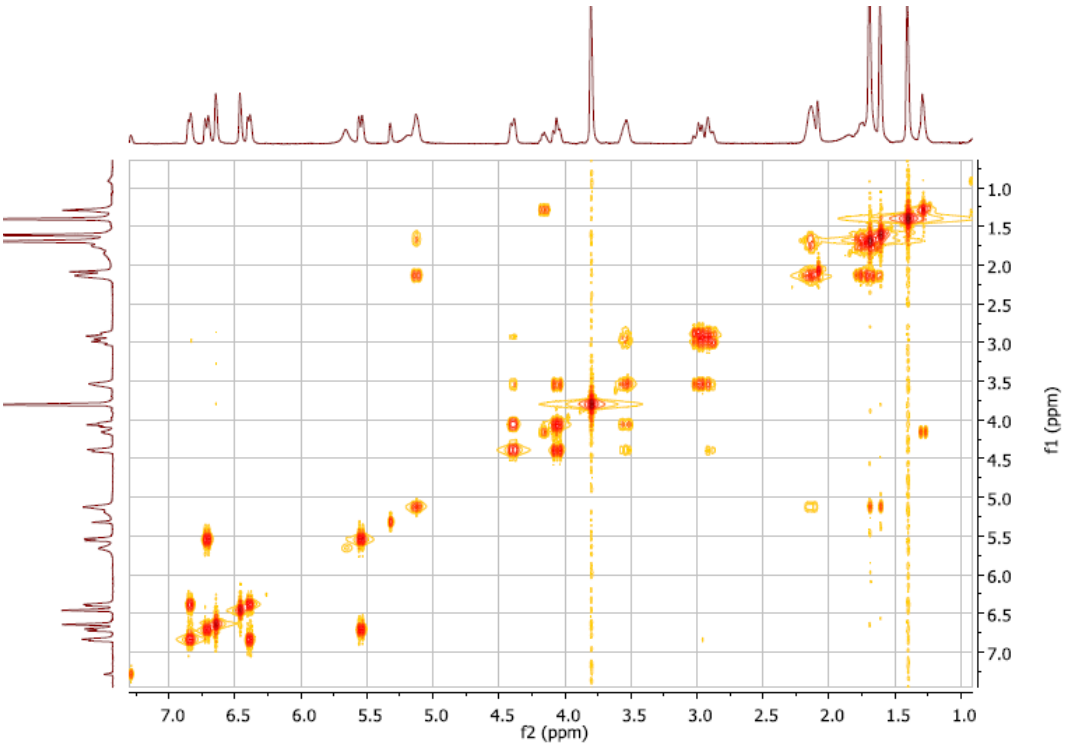


Figure A42 COSY NMR spectrum of **5.1** in $CDCl_3$.

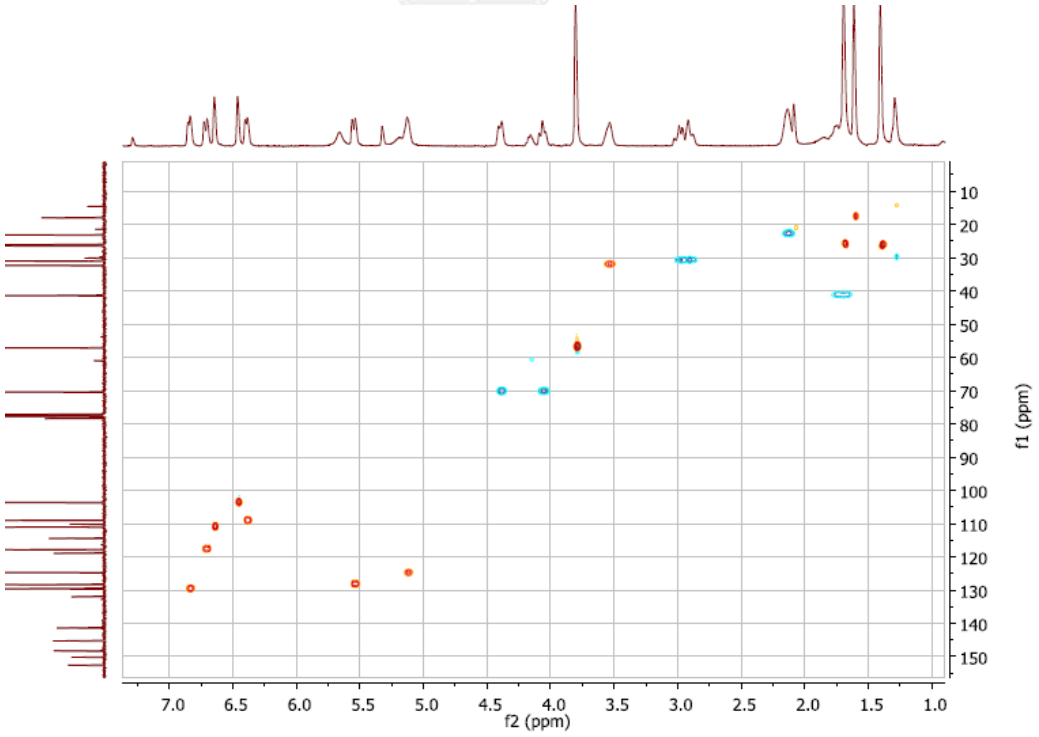


Figure A43 HSQC NMR spectrum of **5.1** in $CDCl_3$.

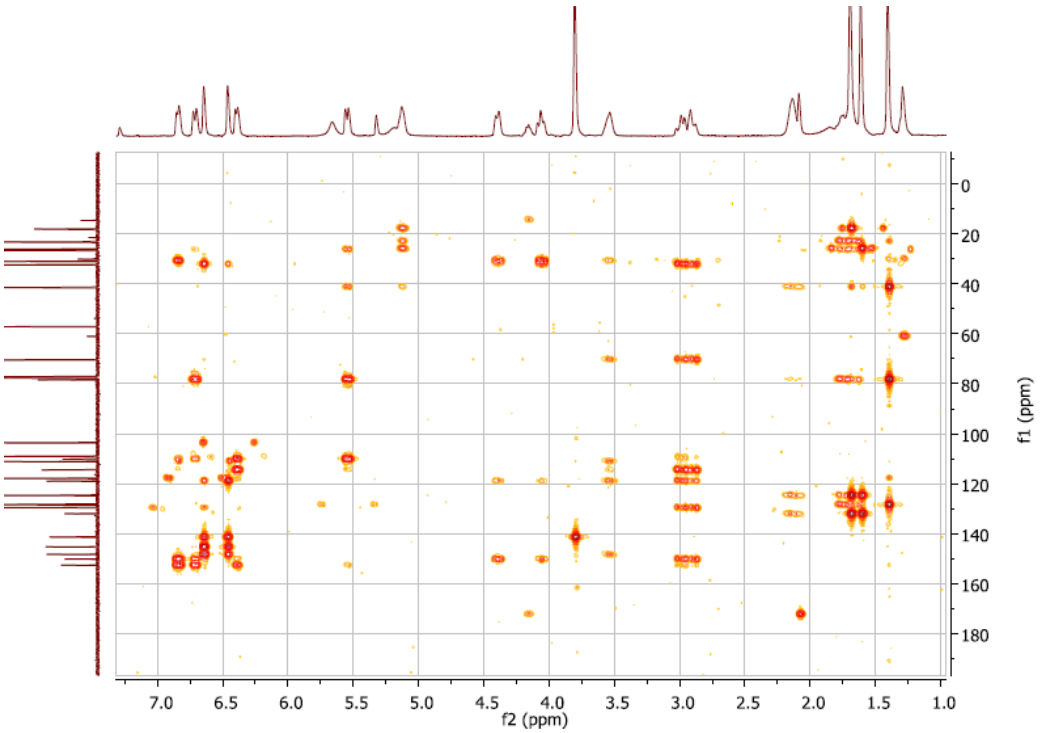


Figure A44 HMBC NMR spectrum of **5.1** in $CDCl_3$.

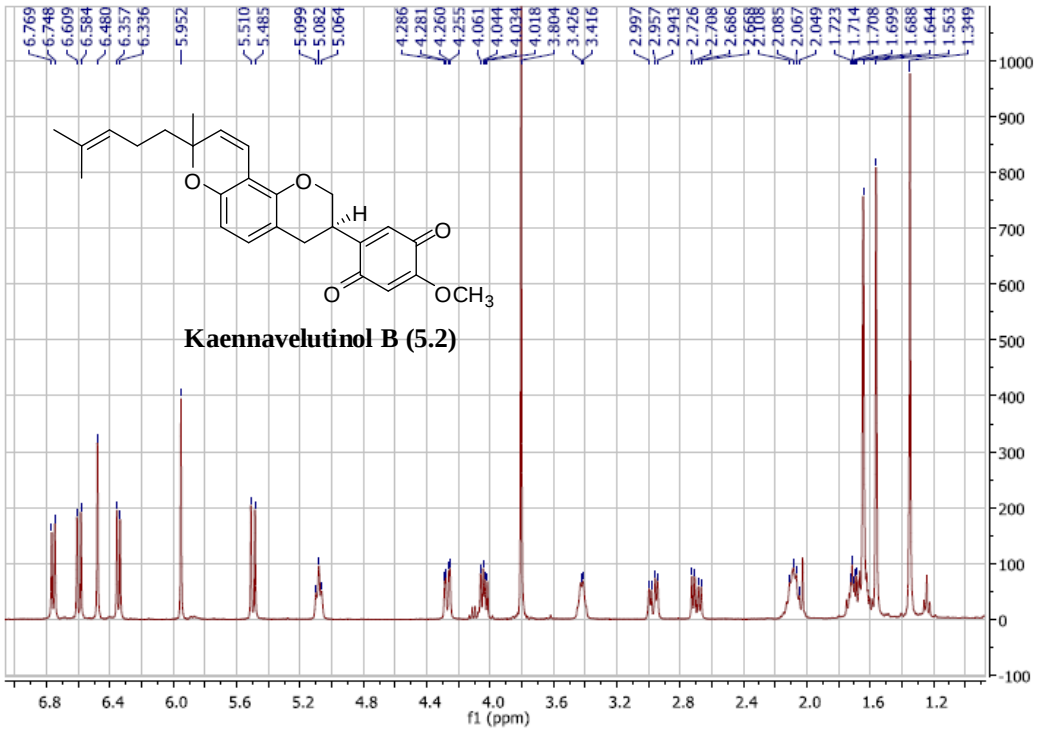


Figure A45 ^{1}H NMR spectrum of **5.2** in $CDCl_3$.

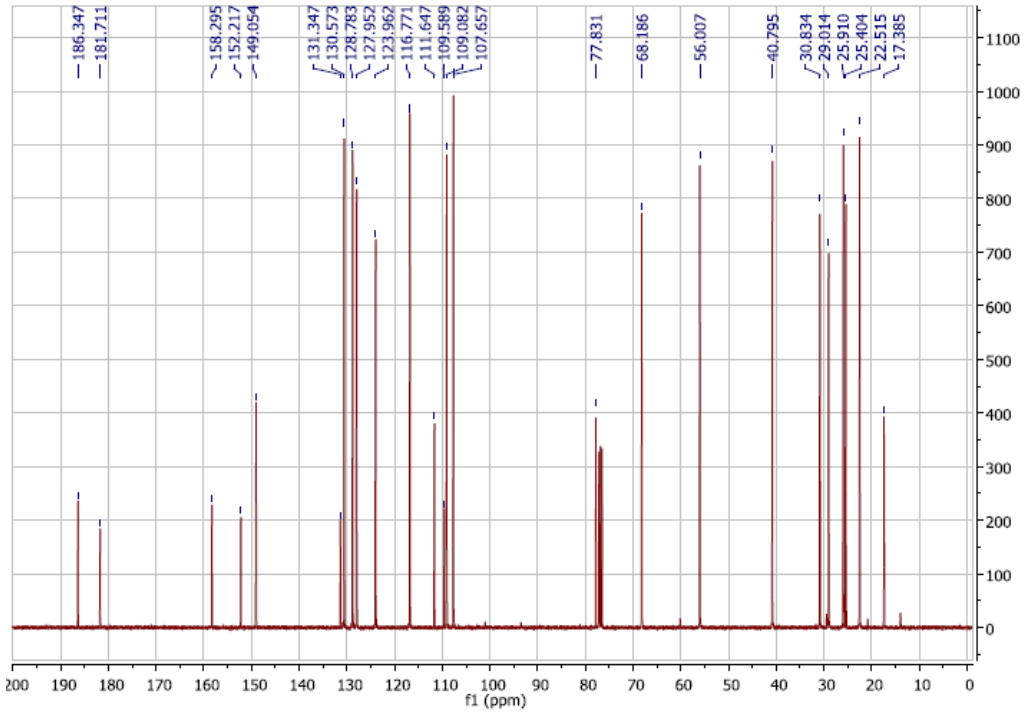


Figure A46 ^{13}C NMR spectrum of **5.2** in $CDCl_3$.

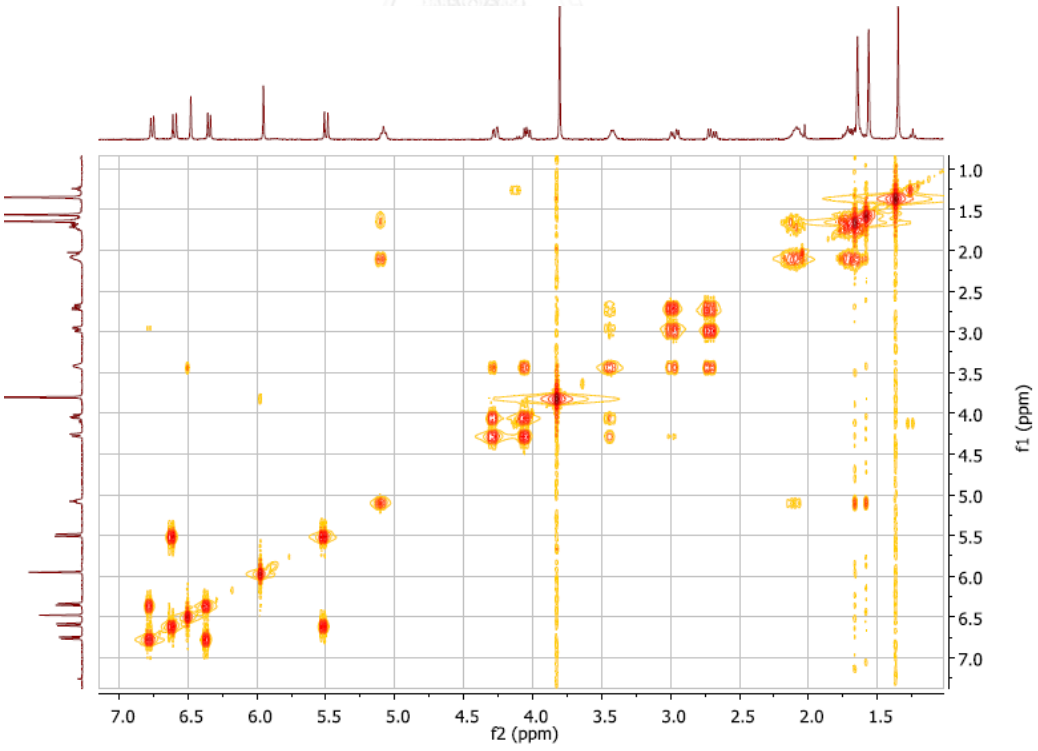


Figure A47 COSY NMR spectrum of **5.2** in $CDCl_3$.

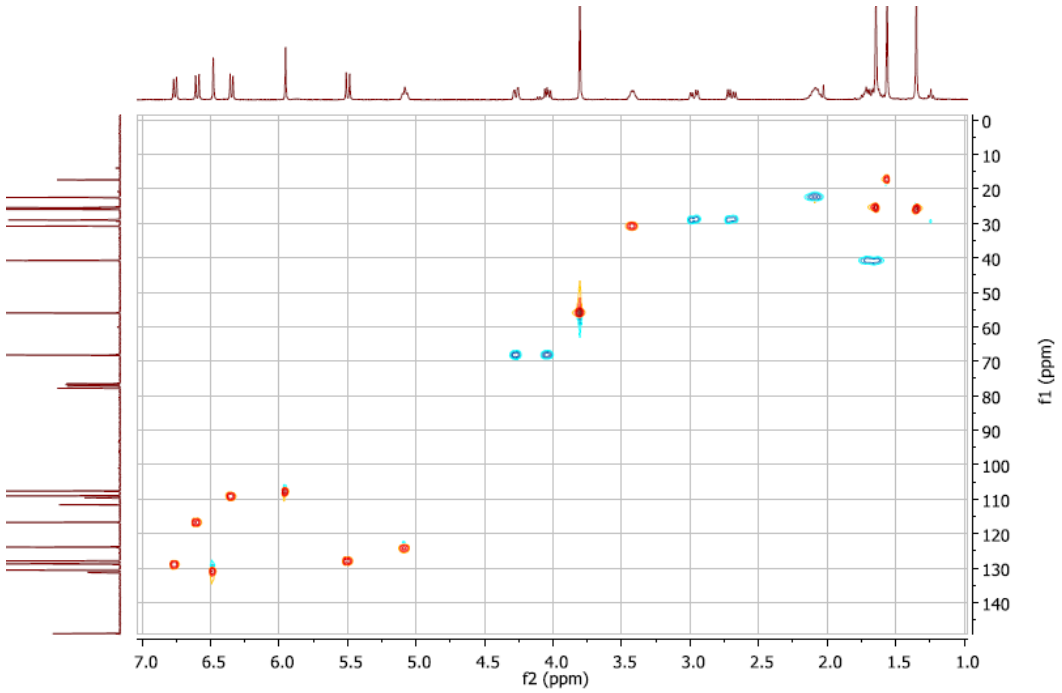


Figure A48 HSQC NMR spectrum of **5.2** in $CDCl_3$.

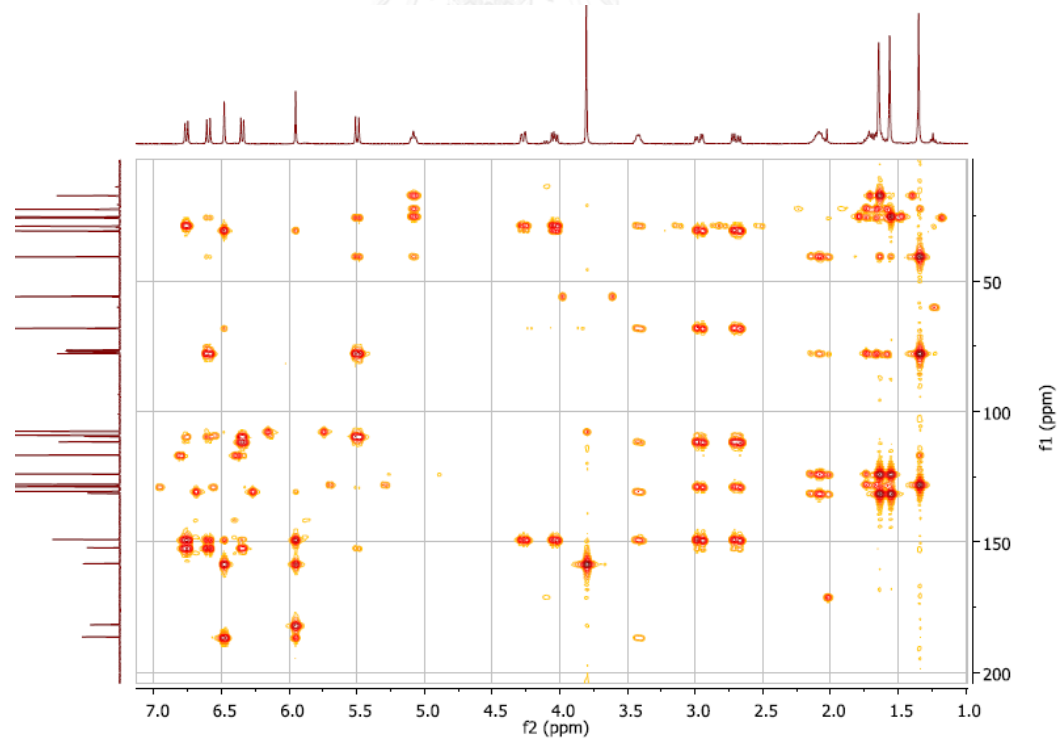


Figure A49 HMBC NMR spectrum of **5.2** in $CDCl_3$.

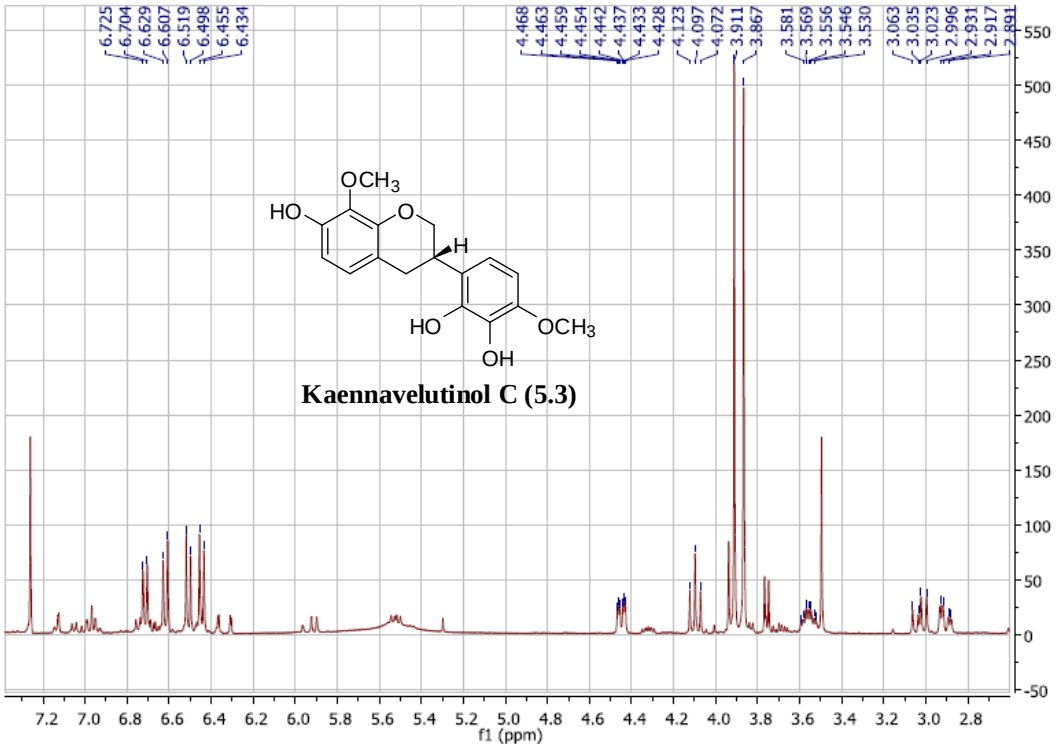


Figure A50 ^{1}H NMR spectrum of **5.3** in $CDCl_3$.

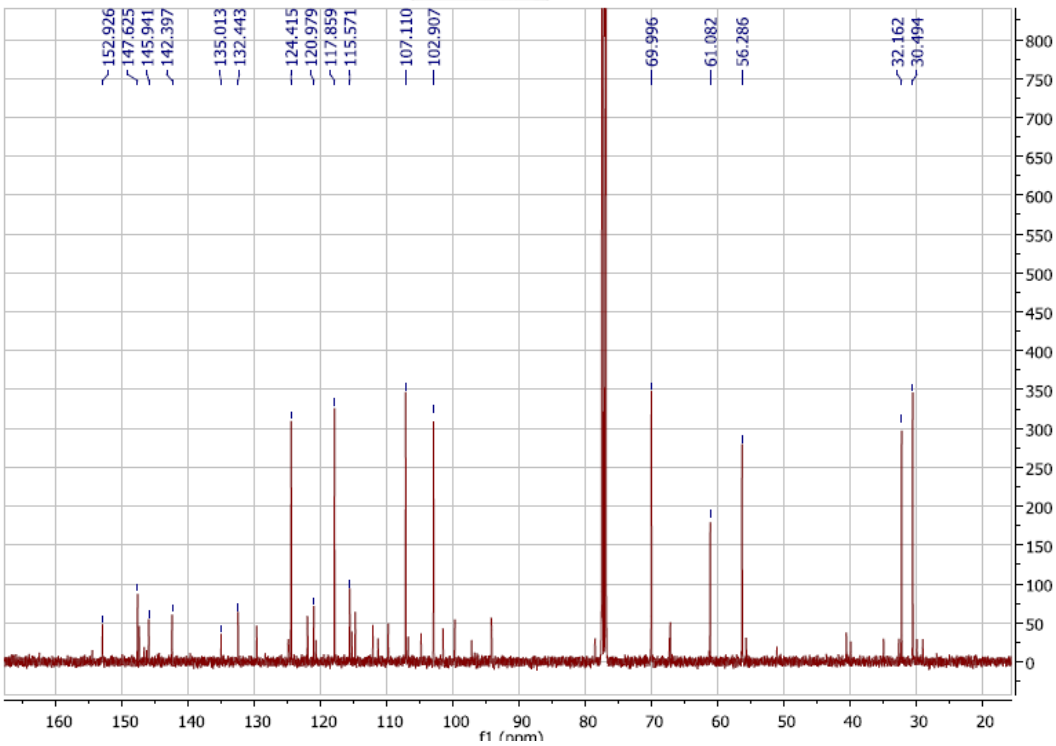


Figure A51 ^{13}C NMR spectrum of **5.3** in $CDCl_3$.

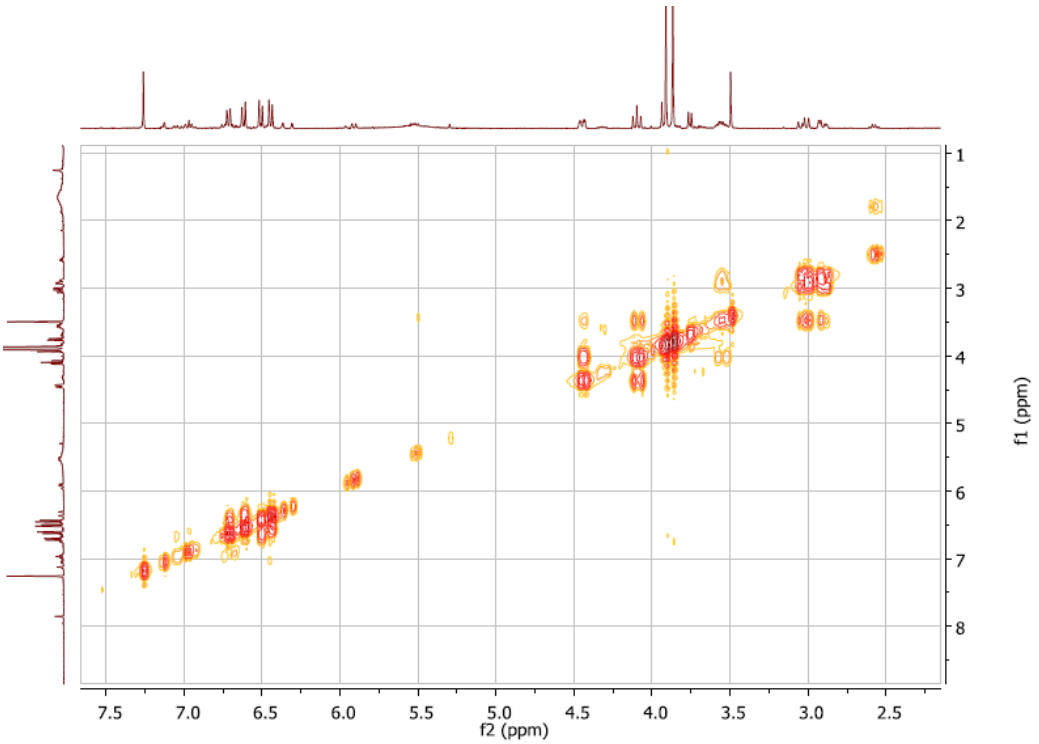


Figure A52 COSY NMR spectrum of **5.3** in $CDCl_3$.

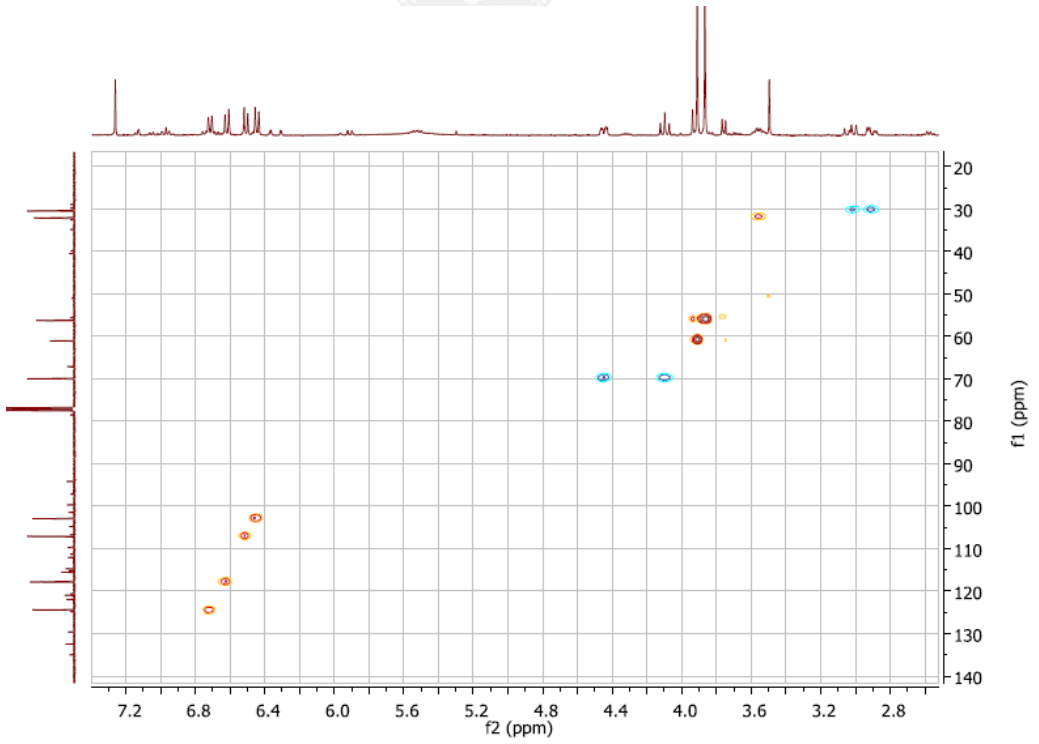


Figure A53 HSQC NMR spectrum of **5.3** in $CDCl_3$.

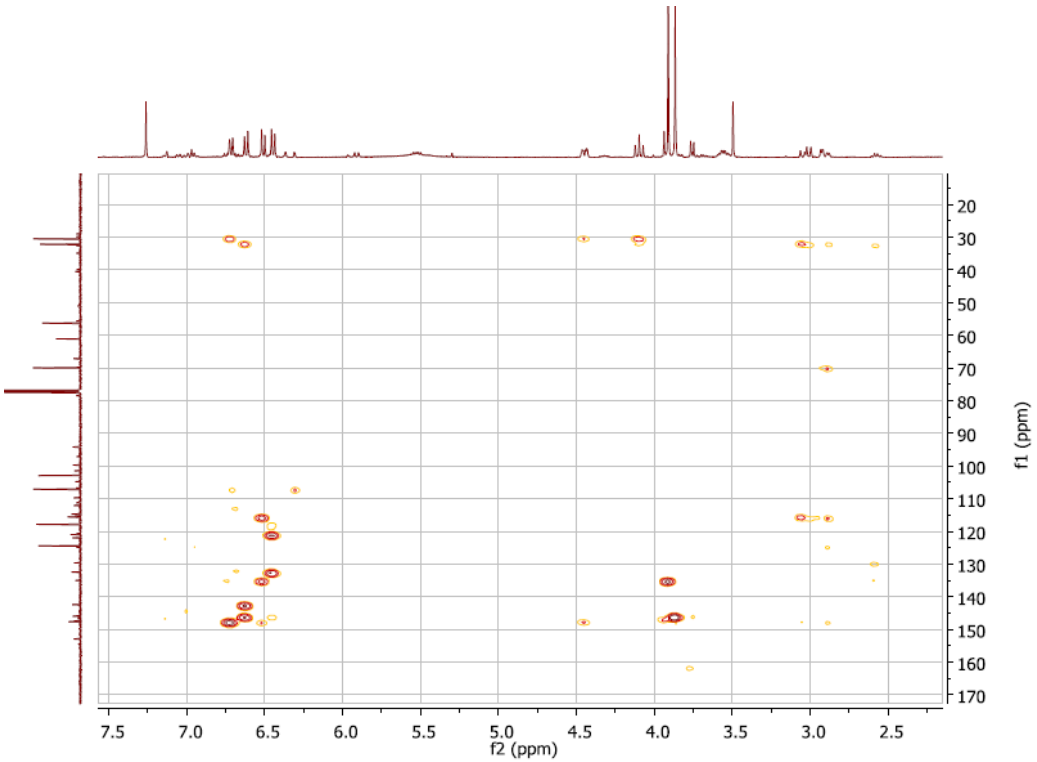


Figure A54 HMBC NMR spectrum of **5.3** in $CDCl_3$.

Supplementary supporting

Kaennavelutinose, a new isoflavone glycoside and their cytotoxicity from the roots of *Dalbergia velutina*

Sutin Kaennakam[a], Pongpun Siripong[b], Santi Tip-pyang[a,*]

[a]*Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand*

[b]*Natural Products Research Section, Research Division, National Cancer Institute, Bangkok 10400, Thailand*

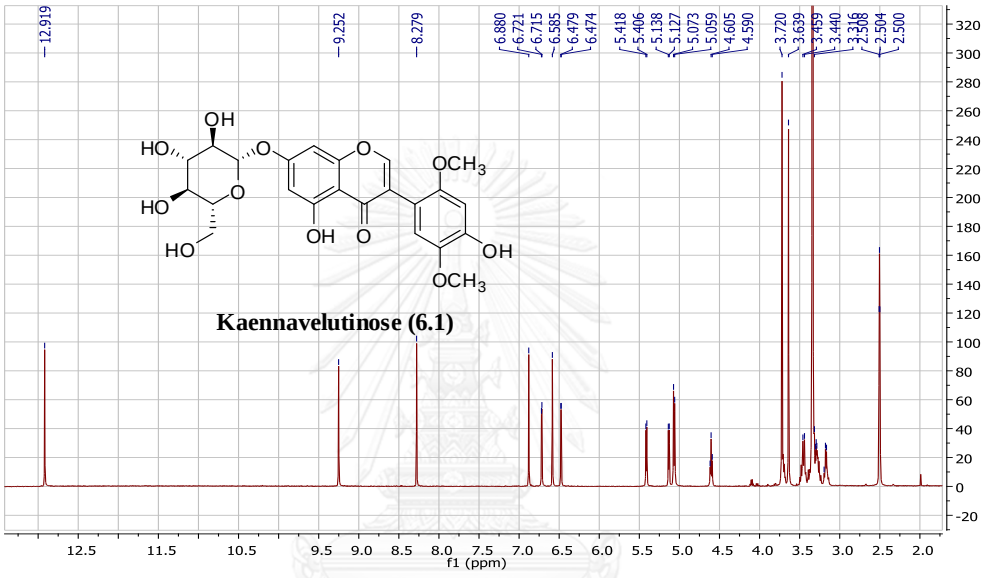


Figure A55 ^{1}H NMR spectrum of **6.1** in DMSO-d_6.

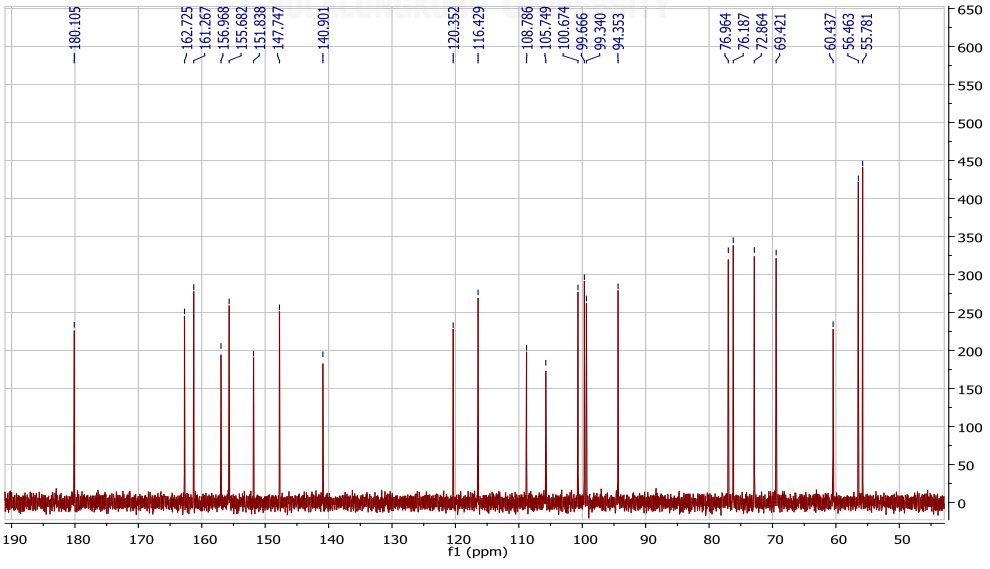


Figure A56 ^{13}C NMR spectrum of **6.1** in DMSO-d_6.

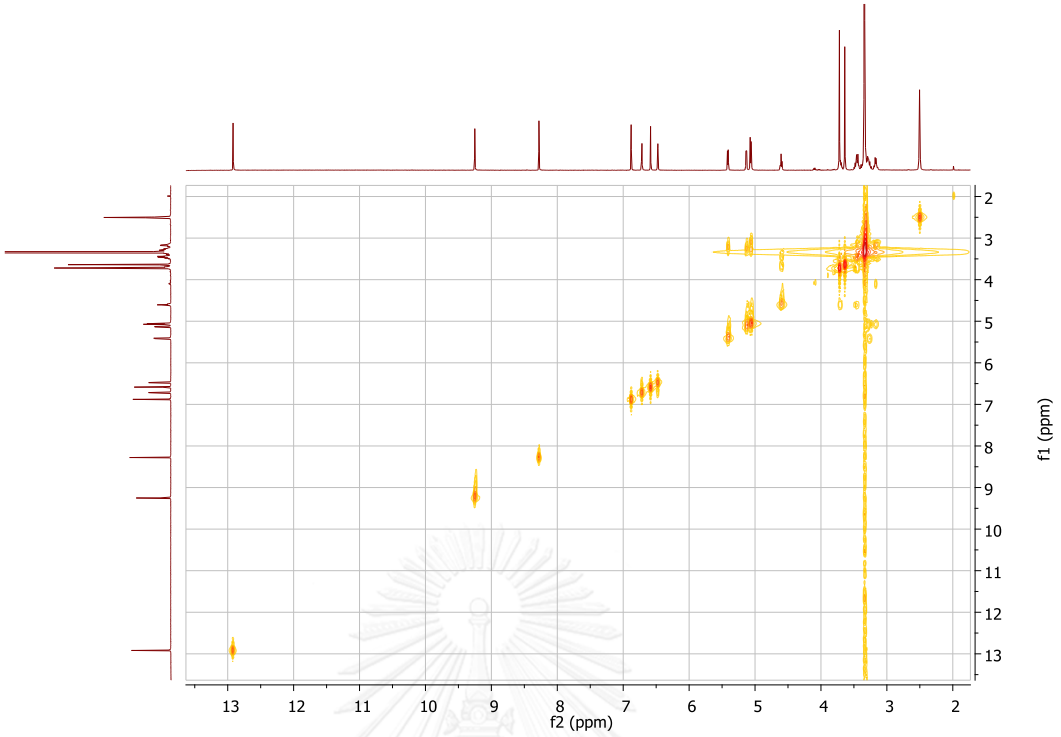


Figure A57 COSY NMR spectrum of **6.1** in DMSO-d_6.

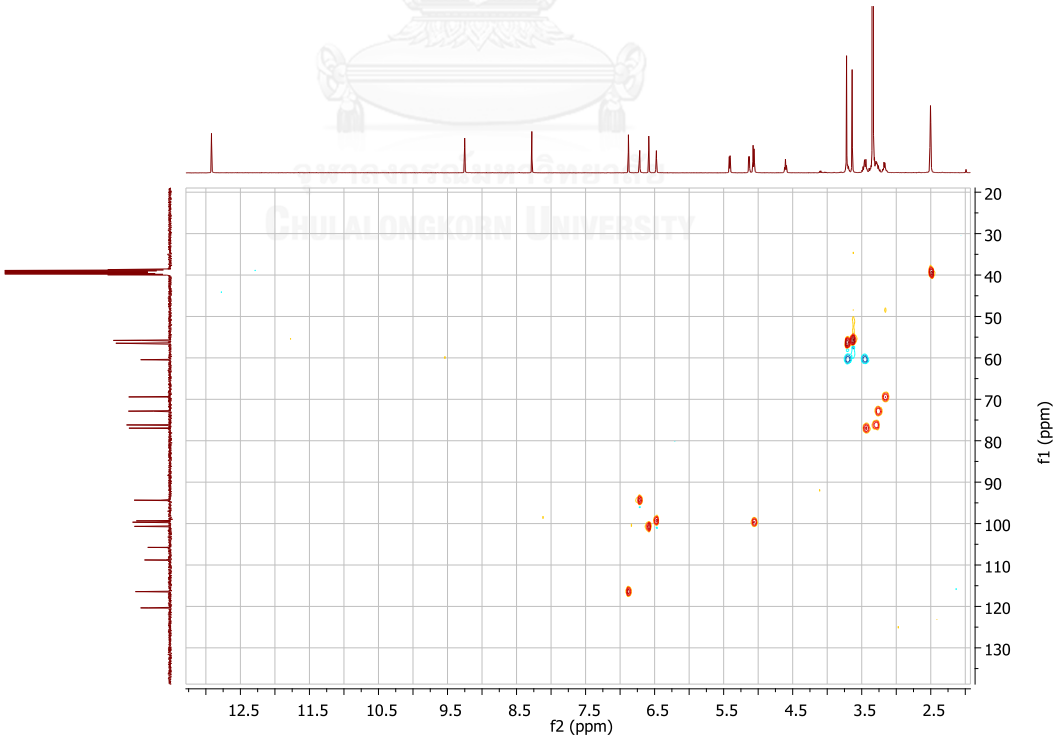


Figure A58 HSQC NMR spectrum of **6.1** in DMSO-d_6.

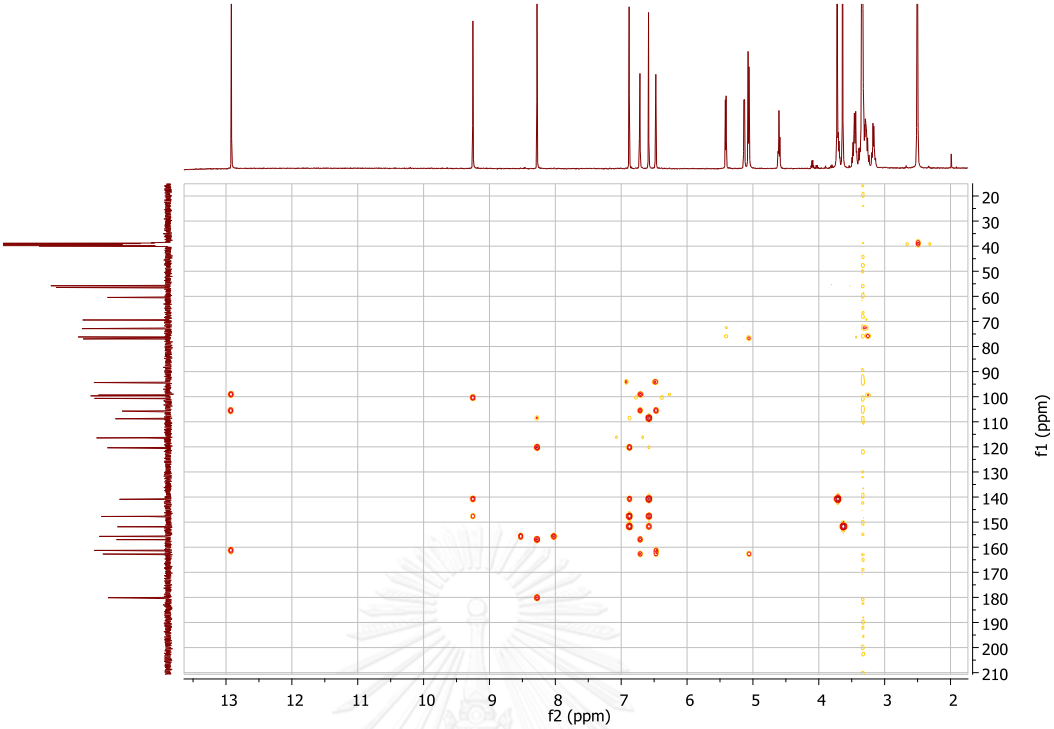


Figure A59 HMBC NMR spectrum of **6.1** in DMSO-d_6.

VITA

Mr. Sutin Khaennakam was born on January 3, 1987 in Kalasin Province, Thailand. He graduated with Bachelor's Degree of Science, major in Chemistry from Faculty of Science, Khon Kaen University, in 2009 and graduated with Master Degree of Science, major in Chemistry from Faculty of Science, Chulalongkorn University, in 2011. He then continued his graduate degree at the Department of Chemistry, Chulalongkorn University. During the time he was studying in the Docter of Philosophy of Science program at the Department of Chemistry.

His present address is 117 Moo 5, Sahatsakhan district, Kalasin Province, Thailand, 46140, E-mail: n-s-k-@hotmail.com, Tel: 091-414-2346.