สารต้านอนุมูลอิสระจากเอื้องแปรงสีฟัน

นางสาว ณัฐชา ด้วงรัก

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชเวท ภาควิชาเภสัชเวทและเภสัชพฤกษศาสตร์
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2553

FREE RADICAL SCAVENGERS FROM *DENDROBIUM SECUNDUM*

Miss Natthacha Duangrak

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Pharmacognosy
Department of Pharmacognosy and Pharmaceutical Botany
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2010

Thesis Title	FREE RADICAL SCAVENGERS FROM *DENDROBIUM SECUNDUM*
By	Miss Natthacha Duangrak
Field of Study	Pharmacognosy
Thesis Advisor	Professor Kittisak Likhitwitayawuid, Ph.D.
Thesis Co-advisor	Assistant Professor Boonchoo Sritularak, Ph.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

Pintip Pongpech .....Dean of the Faculty of Pharmaceutical Sciences

(Associate Professor Pintip Pongpech, Ph.D.)

THESIS COMMITTEE

Nijsiri Ruangrungsi ..... Chairman

(Associate Professor Nijsiri Ruangrungsi, Ph.D.)

K. Likhitwitayawuid ..... Thesis Advisor

(Professor Kittisak Likhitwitayawuid, Ph.D.)

Boonchoo Sritularak .....Thesis Co-advisor

(Assistant Professor Boonchoo Sritularak, Ph.D.)

Suchada Sukrong .....Examiner

(Assistant Professor Suchada Sukrong, Ph.D.)

R Wong ..... External Examiner

(Ruchira Wangteeraprasert, Ph.D.)

ณัฐชา ด้วงรัก : สารต้านอนุมูลอิสระจากเอื้องแปรงสีฟัน. (FREE RADICAL SCAVENGERS FROM *DENDROBIUM SECUNDUM*) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ศ.ดร.กิตติศักดิ์ ลิขิตวิทยาวุฒิ, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ.ดร. บุญชู ศรีตุลารักษ์, 115 หน้า.

การศึกษาทางพฤกษเคมีของเอื้องแปรงสีฟัน สามารถแยกสารใหม่ซึ่งเป็นสารกลุ่ม bibenzyl ได้ 1 ชนิด คือ 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl พร้อมกับสารที่เคยมีรายงานมาแล้ว 4 ชนิด ได้แก่ brittonin A, moscatilin, syringaresinol และ ferulic acid สารทั้งหมดพิสูจน์โครงสร้างทางเคมีโดยเทคนิคสเปกโตรสโคปี (NMR, MS, IR, UV) ร่วมกับการเปรียบเทียบข้อมูลที่มีรายงานมาแล้ว การทดสอบฤทธิ์ในการต้านอนุมูลอิสระพบว่าสารทั้งหมดที่แยกได้ ยกเว้น brittonin A มีฤทธิ์ในการต้านอนุมูลอิสระโดยการยับยั้งอนุมูล DPPH และยังพบว่า moscatilin และ syringaresinol สามารถยับยั้งอนุมูลอิสระชนิด superoxide ได้

ภาควิชา...เภสัชเวทและเภสัชพฤกษศาสตร์...ลายมือชื่อนิสิต.....ณัฐชา ด้วงรัก.....

สาขาวิชา.....เภสัชเวท..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา .....2553..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

5276565433 : MAJOR PHARMACOGNOSY

KEYWORDS : *DENDROBIUM SECUNDUM* / FREE RADICAL SCAVENGERS

NATTHACHA DUANGRAK : FREE RADICAL SCAVENGERS FROM *DENDROBIUM SECUNDUM*. ADVISOR : PROF. KITTISAK LIKHITWITAYAWUID, Ph.D., CO-ADVISOR : ASST. PROF. BOONCHOO SRITULARAK, Ph.D., 115 pp.

Phytochemical study of the MeOH extract of *Dendrobium secundum* (Blume) Lindl. (Orchidaceae) led to the isolation of a new bibenzyl derivative named 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl, along with four known compounds which included brittonin A, moscatilin, syringaresinol and ferulic acid. Their structures were determined by spectroscopic analysis (NMR, MS, IR and UV) and by comparison with previously reported data. These compounds were evaluated for scavenging activity against DPPH (2,2-diphenyl-1-picryhydrazyl) and superoxide radicals. All compounds, except for brittonin A, exhibited moderate DPPH free radical scavenging activity. In addition, moscatilin and syringaresinol showed superoxide radical scavenging activity.

Department : ..Pharmacognosy and Pharmaceutical Botany.....
Field of Study : ....Pharmacognosy.....
Academic Year : .....2010.....

Student's Signature Natthacha Duangrak
Advisor's Signature
Co-advisor's Signature Boonchoo Sritularak

Acknowledgements

The author would like to express her deepest gratitude to her thesis advisor, Professor Dr. Kittisak Likhitwitayawuid of the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for his valuable advice, useful guidance, endless support, concern, patience and encouragement throughout the course of this study.

The author wishes to express her sincere thanks to Assistant Professor Dr. Boochoo Sritularak of the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, her thesis co-advisor, for his helpful advice, constant help and kindness.

The author wishes to express her thanks to the members of her thesis committee for their critical perusal and useful advice.

The author would like to thank the Graduate School of Chulalongkorn University for granting partial financial support to conduct this investigation.

The author would like to thank all staff members of the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for assistance in research facilities.

The author is grateful to all students of the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for unforgettable friendship and kindness.

Finally, the author wishes to express her infinite gratitude to her family for their love, understanding and encouragements.

CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

FIGURE Page

LIST OF SCHEMES

LIST OF ABBREVIATIONS

α	=	Alpha
acetone-d_6	=	Deuterated acetone
β	=	Beta
br	=	Broad (for NMR spectra)
C	=	Concentration
ºC	=	Degree Celsius
$CDCl_3$	=	Deuterated chloroform
CH_2Cl_2	=	Dichloromethane
^{13}C NMR	=	Carbon-13 Nuclear Magnetic Resonance
cm	=	Centimeter
1-D	=	One dimensional (for NMR spectra)
2-D	=	Two dimensional (for NMR spectra)
d	=	Doublet (for NMR spectra)
dd	=	Doublet of doublets (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polarization Transfer
δ	=	Chemical shift
ESIMS	=	Electrospray Ionization Mass Spectrrmetry
EtOAc	=	Ethyl acetate
CC	=	Column Chromatography
g	=	Gram
GF	=	Gel Filtration Chromatography
^1H-NMR	=	Proton Nuclear Magnetic Resonance
HMBC	=	^1H-detected Heteronuclear Multiple Bond Correlation
HMQC	=	^1H-detected Heteronuclear Multiple Quantum Coherence
Hz	=	Hertz
IR	=	Infrared
IC_{50}	=	Concentration showing 50% inhibition
J	=	Coupling constant
Kg	=	Kilogram

L	=	Liter
μl	=	Microliter
λ_{max}	=	Wavelength at maximal absorption
ε	=	Molar absorptivity
M^+	=	Molecular ion
m	=	Multiplet (for NMR spectra)
MeOH	=	Methanol
mg	=	Milligram
μg	=	Microgram
MHz	=	Mega Hertz
ml	=	Milliliter
mm	=	Millimeter
m/z	=	Mass to charge ratio
MS	=	Mass spectrum
MW	=	Molecular weight
nm	=	Nanometer
NMR	=	Nuclear Magnetic Resonance
ppm	=	Part per million
s	=	Singlet (for NMR spectra)
spp.	=	Species
t	=	Triplet (for NMR spectra)
TLC	=	Thin Layer Chromatography
UV-VIS	=	Ultraviolet and Visible spectrophotometry
VLC	=	Vacuum Liquid Chromatography
υ_{max}	=	Wave number at maximal absorption
$[\alpha]^{20}_{D}$	=	Specific rotation at Sodium D line (589 nm)

CHAPTER I

INTRODUCTION

Free radicals are atoms or molecules that contain at least one unpaired electron in atomic or molecular orbitals. There are many types of radicals, but oxygen free radicals known as reactive oxygen species (ROS) represent the most important species in living organisms (Valko *et al*., 2007), including superoxide ($O_2^{-\bullet}$), peroxyl ($ROO^{\bullet}$), alkoxyl ($RO^{\bullet}$), hydroxyl ($HO^{\bullet}$), and nitric oxide ($NO^{\bullet}$). Free radicals are produced continuously in cells as by-products of metabolism. They are unstable and highly reactive. Exposure to radiation, environmental pollutants such as tobacco smoke and automobile exhaust lead to the formation of free radicals, the excess of them can damage cell composition by attacking lipids in cell membrane, proteins, carbohydrates, and DNA (Pietta, 2000). This harm is a causative role in aging and several degenerative diseases, such as cardiovascular diseases, hypertension, neurodegenerative diseases, and cancer (Valko *et al*., 2007).

Defense mechanisms against free radical-induced oxidative stress involve preventative mechanisms, repair mechanisms, physical defenses, and antioxidant defenses (Valko *et al*., 2007). The mechanisms of antioxidant action can include enzymatic defenses, such as glutathione peroxidase, catalase, and superoxide dismutase, and nonenzymatic defenses (Pietta, 2000). Therefore, antioxidants with free radical scavenging activity may have great relevance in the prevention and therapeutics of free radical mediated diseases.

Plants of the genus *Dendrobium* are members of Orchidaceae family. They are epiphytic, lithophytic, or less often terrestrial, polymorphic, deciduous or evergreen. Their botanical characters are described by Guanghua *et al*. as follows:

Stems either: 1) rhizomatous, 2) erect and many noded, 3) erect and 1-noded or several noded from a many-noded rhizome, or 4) without a rhizome. Leaves 1 to many, alternate, apical or distichous along stem, linear, lanceolate, oblong, or ovate, sometimes subterete or terete, papery to rigid and leathery. Inflorescences lateral, generally distal, in some sections pseudoterminal, 1 to many flowered, usually racemose, erect, horizontal, or pendulous. Flowers extremely variable in color and shape, often showy, very small to large, resupinate or not resupinate, usually spreading, ephemeral or long-lived. Sepals similar, free, short to filiform; lateral

sepals adnate to elongated column foot and part of lip to form a mentum, 0.1-3 cm. Petals free, margin entire to fimbriate; lip entire to distinctly 3-lobed base joined to column foot, sometimes narrowly clawed at base, sometimes forming a closed spur with lateral sepals to which it may be joined laterally for a short distance; pollinia 4 in appressed pairs, waxy, ovate or oblong, naked (Guanghua *et al*., 2009).

Plants in genus *Dendrobium* are represented by more than 1,100 species widely distributed throughout Asia, Europe, and Australia, and there are about 150 species of *Dendrobium* in Thailand (Seidenfaden, 1985; Guanghua *et al*., 2009). The species of *Dendrobium* in Thailand according to Royal Forest Department (2001) are as follows.

Dendrobium acerosum Lindl.	**กล้วยไม้มือนาง** Kluai mai mue nang (Chumphon); เขาแพะ Khao phae (Chanthaburi).
D. acinaciforme Roxb.	เอื้องตะขาบ Ueang ta khap, **เอื้องยอดสร้อย** Ueang yot soi (Northern).
D. albosanguineum Lindl.	**เอื้องตางัว** Ueang ta ngua (Mae Hong Son); เอื้องตึง Ueang tueng (Tak); เอื้องผาเวียง (Ueang pha wiang (Bangkok).
D. aloifolium (Blume) Rchb.f.	เอื้องช้าง Ueang chang (Trat); **เอื้องมณี** Ueang mani (Bangkok).
D. anosmum Lindl.	**เอื้องสาย** Ueang sai, เอื้องสายหลวง Ueang sai luang (Chiang Mai, Peninsular).
D. aphrodite Rchb.f.	= *D. albosanguineum* Lindl.
D. aphyllum (Roxb.)	พอทุกิ Pho-thu-ki (Karen-Mae Hong Son); มอกคำเครือ Mok-kham-khruea (Shan-Mae Hong Son); เอื้องไข่เน่า Ueang khai nao, เอื้องสายไม้ Ueang sai mai (Lampang); **เอื้องงวงช้าง** Ueang nguang chang (Mae Hong Son); เอื้องย้อยไม้

	Ueang yoi mai (Northern); เอื้องล่องแล่ง Ueang long laeng (Chiang Mai).
D. bambusifolium Parish & Rchb.f.	= *D. salaccense* (Blume) Lindl.
D. bellatulum Rolfe	**เอื้องแซะภู** Ueang sae phu, เอื้องแซะดอยปุย Ueang sae doi pui (Chiang Mai).
D. bicameratum Lindl.	**เอื้องเข็ม** Ueang khem (Northern).
D. bilobulatum Seidenf.	**กล้วยไม้ก้างปลา** Kluai mai kang pla (General).
D. binoculare Rchb.f.	พอนี้โคะโพ Pho-ni-kho-pho, พอผู่ปรึย Pho-phu-prue-ya (Karen-Mae Hong Son); **เอื้องคำสาย** Ueang kham sai, เอื้องจำปา Ueang champa (Northern).
D. brymerianum Rchb.f.	**เอื้องคำฝอย** Ueang kham foi, เอื้องคำฝอยปาย Ueang kham foi pai (Northern).
D. calceolaria Carey ex Hook.	= *D. moschatum* (Buch.-Ham.) Sw.
D. capillipes Rchb.f.	**เอื้องคำกิ่ว** Ueang kham kio (Lampang,Phrae); เอื้องคำป๊อก Ueang kham pok, เอื้องคำเฮี่ย Ueang kham hia (Chiang Mai); เอื้องมิ้น Ueang min (Northern).
D. cariniferum Rchb.f.	พอมือคาพะโด่ Pho-mue-kha-do (Karen-Mae Hong Son); **เอื้องกาจก** Ueang kachok, เอื้องแซะเหลือง Ueang sae lueang (Chiang Mai); เอื้องแซะดง Ueang sae dong (Chiang Mai, Mae Hong Son); เอื้องตึง Ueang tueng (Lampang).
D. christyanum Rchb.f.	**เอื้องแซะภูกระดึง** Ueang sae phu kradueng (Loei).

D. chrysanthum Lindl.	**เอื้องสายมรกต** Ueang sai morakot (Bangkok).
D. chrysotoxum Lindl.	พอนี้โคะ Pho-ni-kho (Karen-Mae Hong Son); **เอื้องคำ** Ueang kham (Northern); เอื้องคำตา Ueang kham ta (Chiang Mai).
D. ciliatum Parish ex Hook.f.	= *D. venustum* Teijsm. & Binn.
D. ciliferum Bakh.f.	= *D. venustum* Teijsm. & Binn.
D. coelogyne Rchb.f.	= *Epigeneium amplum* (Lindl.) Summerh.
D. compactum Rolfe ex Hackett	**เอื้องข้าวตอก** Ueang khao tok (Northern).
D. concinnum Miq.	**หางเปีย** Hang pia (Narathiwat).
D. crassinode Benson & Rchb.f.	= *D. pendulum* Roxb.
D. crepidatum Lindl. & Paxton.	**เอื้องสายน้ำเขียว** Ueang sai nam khiao (General).
D. crocatum Hook.f.	**เอื้องนางนวล** Ueang nang nuan (Peninsular).
D. cruentum Rchb.f.	ปากนกแก้ว Pak nok kaeo, **เอื้องนกแก้ว** Ueang nok kaeo (Bangkok).
D. crumenatum Sw.	นกกะยาง Nok kayang (Chon buri); บวบกลางหาว Buap klang hao (Chiang Mai); แส้พระอินทร์ Sae phra in (Chanthaburi, Trat); **หวายตะมอย** Wai tamoi, เอื้องมะลิ Ueang mali (Central, Peninsular); Pigeon orchid.
D. crystallinum Rchb.f.	**เอื้องนางฟ้อน** Ueang nang fon, เอื้องนิ้วมือพระนารายณ์ Ueang nio mue phra narai (Chiang Mai); เอื้องสายสามสี Ueang saisam si (Bangkok).

D. cumulatum Lindl.	เทียนทอง Thian thong, เทียนพญาอินทร์ Thian phaya in, **เอื้องสายสี่ดอก** Ueang sai si dok (Northern, Southeastern).
D. dalhousieanum Wall.	= *D. pulchellum* Roxb. ex Lindl.
D. dantaniense Guillaumin	**เอื้องเข็ม** Ueang khem (Chiang Mai).
D. delacourii Guillaumin	= *D. venutum* Teijsm. & Binn.
D. densiflorum Lindl.	**เอื้องมอนไข่** Ueang mon khai, เอื้องมอนไข่เหลี่ยม Ueang mon khai liam, เอื้องมอนไข่เหลือง Ueang mon khai lueang (Northern); เอื้องมอนคำ Ueang mon kham (Chiang Mai).
D. devonianum Paxton	**เอื้องเมี่ยง** Ueang miang, เอื้องสายผ้าก้ัง Ueang sai pha kang, เอื้องสายพระอินทร์ Ueang sai phra in (Chiang Mai); เอื้องโรจน์เรืองแสง Ueang rot rueang saeng (Bangkok).
D. dickasonii L.O.Williams	**เอื้องเคี้ยะ** Ueang khia (Chiang Mai).
D. discolor Lindl.	**หวายกลัก** Wai klak (Bangkok).
D. dixanthum Rchb.f.	เอื้องคำปอน Ueang kham pon, เอื้องคำป่า Ueang kham pa, เอื้องคำปิว Ueang kham pio, **เอื้องเทียน** Ueang thian, เอื้องใบไผ่ Ueang bai phai, เอื้องไผ่ Ueang phai (Northern).
D. draconis Rchb.f.	พอเจ Pho-che (Karen-Mae Hong Son); **เอื้องเงิน** Ueang ngoen (Northern); เอื้องตึง Ueang tueng (Mae Hong Son).
D. ellipsophyllum Tang & Wang	**เอื้องทอง** Ueang thong (General).

D. exile Schltr. **เอื้องเสี้ยน** Ueang sian, แส้พระอินทร์ Sae phra in (General).

D. falconeri Hook. พอทุด่าง Pho-tu-dang (Karen-Mae Hong Son); เอื้องโรจน์เรืองแสง Ueang rot rueang saeng, **เอื้องสายวิสูตร** Ueang sai wisut (Bangkok); เอื้องหญ้าแพด Ueang ya phaet (Chiang Mai).

D. farmer Paxton **เอื้องมัจฉาณุ** Ueang mat chanu (Bangkok).

D. fimbriatum Hook. เอื้องคำตาดำ Ueang kham ta dam (Mae Hong Son); **เอื้องคำน้อย** Ueang kham noi (Chiang Mai); เอื้องแวมยุรา Ueang waeo mayura (Central, Nakhon Ratchasima).

D. fimbriatum Lindl. var. oculatum Hook.f. = *D. fimbriatum* Hook.

D. findlayanum Parish & Rchb.f. **พวงหยก** Phuang yok, หวายปม Wai pom (Bangkok); เอื้องข้อ Ueang kho (Chiang Mai).

D. formosum Roxb. ex Lindl. เอื้องขี้ผึ้ง Ueang khi phueng (Peninsular); **เอื้องเงินหลวง** Ueang ngoen luang, เอื้องตาเหิน Ueang ta hoen (Chiang Mai).

D. friedericksianum Rchb.f. เหลืองจันทบูร Lueang chanthabun, เอื้องนกขมิ้น Ueang nok khamin (Chanthaburi); **เอื้องเหลืองจันทบูร** Ueang lueang chanthabun (Bangkok).

var. oculatum Seidenf. & Smitinand = *D. friedericksianum* Rchb.f.

D. fuerstenbergianum Schltr. **เอื้องแซะภูกระดึง** Ueang sae phu kradueng (Loei).

D. gibsonii Lindl. พอนี้โคะโพ Pho-ni-ko-pho, พอผู่ปรึย Pho-phu-pru-ya (Karen-Mae Hong Son); เอื้อง

	คำตา Ueang kham ta, **เอื้องคำสาย** Ueang kham sai (Northern); เอื้องจำปา Ueang champa (Central).
D. grande Hook.f.	**เอื้องแผงใบใหญ่** Ueang phaeng bai yai (Peninsular).
D. gratiotissimum Rchb.f.	**เอื้องกิ่งดำ** Ueang king dam (Bangkok).
D. gregulus Seidenf.	**เอื้องมะต่อม** Ueang matom (Chiang Mai).
D. griffithianum Lindl.	**เอื้องมัจฉาณุ** Ueang matchanu, เอื้องมัจฉาเหลือง Ueang mat cha lueang (Bangkok).
D. harveyanum Rchb.f.	**เอื้องคำฝอย** Ueang kham foi, เอื้องคำฝอยอินเดีย Ueang kham foi india (Chiang Mai).
D. hendersonii Hawkes & Heller	**หวายตะมอยน้อย** Wai tamoi noi (Peninsular).
D. hercoglossum Rchb.f.	**เอื้องดอกมะเขือ** Ueang dok ma khuea (Bangkok).
D. heterocarpum Lindl.	เอื้องแซะดง Ueang sae dong, เอื้องสีจุน Ueang sichun, **เอื้องสีตาล** Ueang si tan (Chiang Mai).
D. hildebrandii Rolfe	= *D. signatum* Rchb.f.
D. indivisum (Blume) Miq. var. indivisum	**ตานเสี้ยนไม้** Tan sian mai (Chumphon).
var. lampangense Rolfe	= *D.porphyrophyllum* Guillaumin
var. pallidum Seidenf.	**ก้างปลา** Kang pla (General).
D. infundibulum Lindl.	เอื้องเงินหลวง Ueang ngoen luang (Mae Hong Son); **เอื้องตาเหิน** Ueang ta hoen (General).

D. intricatum Gagnep.	**เอื้องชมพู** Ueang chom phu (Chanthaburi).
D. jenkinsii Wall. Ex Lindl.	**เอื้องผึ้งน้อย** Ueang phueng noi (Chiang Mai).
D. kanburiense Seidenf.	**หวายเมืองกาญจน์** Wai muang kan (Kanchanaburi).
D. leonis (Lindl.) Rchb.f.	**เอื้องตะขาบใหญ่** Ueang takhap yai (General).
D. lindleyi Steud.	โพดอนแหล่ Pho-don-lae (Karen-Mae Hong Son); **เอื้องผึ้ง** Ueang phueng (Northern).
D. lituiflorum Lindl.	เอื้องครั่ง Ueang khrang (Loei); **เอื้องสายม่วง** Ueang sai muang (Bangkok, Northern).
D. lobbii Teijsm. & Binn.	= *D. villosulum* Lindl.
D. longicornu Lindl.	= *D. wattii* (Hook.f.) Rchb.f.
D. margaritaceum Finet	= *D. christyanum* Rchb.f.
D. moschatum (Buch.-Ham.) Sw.	**เอื้องจำปา** Ueang champa (Northern).
D. moulmeinense Parish ex Hook.f.	= *D. dixanthum* Rchb.f.
D. nathanielis Rchb.f.	**เกล็ดนิ่ม** Klet nim (Chanthaburi).
D. nobile Lindl.	**เอื้องเค้ากิ่ว** Ueang khao kio (Northern).
D. ochreatum Lindl.	เอื้องคำข้อ Ueang kham kho, เอื้องคำผักปราบ Ueang kham phak prap, เอื้องง่อย Ueang ngoi, **เอื้องตะขาบ** Ueang ta khap (Chiang Mai).
D. oligophyllum Gagnep.	**ข้าวตอกปราจีน** khao tok prachin (General).

D. pachyglossum C.S.P.Parish & Rchb.f. **เอื้องขนหมู** Ueang khon mu (Mae Hong Son).

D. pachyphyllum (Kuntze) Bakh.f. **เอื้องน้อย** Ueang noi, เอื้องสองใบ Ueang song bai (General).

D. palpebrae Lindl. เอื้องมัจฉา Ueang mat cha, **เอื้องมัจฉาณุ** Ueang matchanu (Bangkok).

D. parcum Rchb.f. **เอื้องก้านกิ่ว** Ueang kan kio, เอื้องไม้กวาด Ueang mai kwat (Bangkok).

D. parishii Rchb.f. **เอื้องครั่ง** Ueang khrang (Northern); เอื้องน้ำครั่ง Ueang nam khrang (Bangkok); เอื้องอัตตากริต Ueang attakrit, เอื้องอินทกริต Ueang inthakrit (Phetchabun).

D. pendulum Roxb. **เอื้องไม้เท้าฤาษี** Ueang mai thao ruesi (Bangkok, Chiang Mai).

D. pensile Ridl. หวาย Wai, หวายย้อย Wai yoi (Narathiwat).

D. porphyrophyllum Guillaumin **เอื้องลิ้น** Ueang lin (Lampang).

D. primulinum Lindl. เอื้องสายน้ำเขียว Ueang sai nam khiao (Chiang Mai); เอื้องสายน้ำผึ้ง Ueang sai nam phueng, **เอื้องสายประสาท** Ueang sai prasat, เอื้องสายเหลือง Ueang sai lueang (Bangkok).

D. pulchellum Roxb. ex Lindl. ปะแหน่มีเพ้ย Pa-nae-mi-phoei, พอมียอเอ้ะ pho-mi-yo-e (Karen-Mae Hong Son); มอกคำตาควาย Mok-kham-ta-khwai (Shan-Mae Hong Son); สบเป็ด Soppet (Loei); **เอื้องคำตาควาย** Ueang kham ta khwai, เอื้องตาควาย Ueang ta khwai (Mae

	Hong Son); เอื้องช้างน้าว Ueang chang nao (Northern).
D. pychnostachyum Lindl.	**เศวตสอดสี** Sawet sot si (Chiang Mai); เอื้องเศวตสอดสี Ueang sawet sot si (Bangkok).
D. salaccense (Blume) Lindl.	**เอื้องใบไผ่** Ueang bai phai (Chiang Mai).
D. scabrilingue Lindl.	พอดอญ่า Pho-do-ya, พอมือคา Pho-mae-kha, พอหมืนคา Pho-muen-kha, พอแหม่ และ Pho-mae-lae (Karen-Mae Hong Son); **เอื้องแซะ** Ueang sae; เอื้องแซะหลวง Ueang sae luang, เอื้องแซะหอม Ueang sae hom (Chiang Mai).
D. secundum (Blume) Lindl.	กับแกะ Kap kae (Loei); คองูเห่า Kho ngu hao (Central); **เอื้องแปรงสีฟัน** Ueang praeng si fan (Bangkok); เอื้องสีฟัน Ueang si fan, เอื้องหงอนไก่ Ueang ngon kai (Northern).
D. seidenfadenii Rchb.f.	**เอื้องเกี๊ยะ** Ueang kia (Chiang Mai).
D. senile Parish & Rchb.f.	มือชะนี Mue chani, เอื้องขนค่าง Ueang khon khang (Chiang Mai); **เอื้องชะนี** Ueang chani, เอื้องนางนี Ueang nang ni (Bangkok); เอื้องมือค่าง Ueang mue khang (Mae Hong Son); เอื้องอีฮุย Ueang i hui (Northern).
D. signatum Rchb.f.	**เอื้องเค้าก่ิว** Ueang khao kio, เอื้องตีนเป็ด Ueang tin pet (Northern), สะเมิงอาง Sa-moeng-ang (Shan-Mae Hong Son), เอื้องตีนนก Ueang tin nok (Chiang Mai).

D. stuposum Lindl.	**เอื้องสาย** Ueang sai (Chiang Mai).
D. sulcatum Lindl.	**เอื้องจำปาน่าน** Ueang champa nan (Bangkok).
D. superbiens Rchb.f.	**หวายคิง** Wai khing (Bangkok).
D. superbum Rchb.f.	= *D. anosmum* Lindl.
D. sutepense Rolfe. Ex Downie	เอื้องแซะ Ueang sae, เอื้องแซะมะลิ Ueang sae mali, **เอื้องมะลิ** Ueang mali (Chiang Mai).
D. terminale Parish & Rchb.f.	**เอื้องแผงโสภา** Ueang phaeng sopha (Peninsular).
D. thyrsiflorum Rchb.f.	กับแกะ Kap kae (Loei); พอซางดี Pho-sang-di (Karen-Mae Hong Son); มอนไข่ใบมน Ueang khai bai mon, **เอื้องมอนไข่ใบมน** Ueang mon khai bai mon (Northern).
D. tortile Lindl.	ตีนนก Tin nok (Chiang Mai); **เอื้องไม้ตึง** Ueang mai tueng (Mae Hong Son); เอื้องเค้ากิ่ว Ueang khao kio, เอื้องเค้ากิ่วแม่สะเรียง Ueang khao kio mae sarieng (Northern).
D. trigonopus Rchb.f.	เอื้องคำปากไก่ Ueang kham pak kai, เอื้องคำภู Ueang kham phu (Loei); **เอื้องคำเหลี่ยม** Ueang kham liam (Chiang Mai).
D. trinervium Ridl.	**เทียนลิง** Thian ling (Chumphon).
D. unicum Seidenf.	**เอื้องครั่งแสด** Ueang krang saet, เอื้องสายสีแสด Ueang sai si saet, เอื้องกำลังเอก Ueang kam lang ek (General).
D. uniflorum Griff.	**เอื้องทอง** Ueang thong (Pattani).

D. venustum Teijsm. & Binn.	**ข้าวเหนียวลิง** Khao niao ling, เอื้องข้าวเหนียวลิง Ueang khao niao ling (Central), เอื้องดอกขาม Ueang dok kham, เอื้องดอกมะขาม Ueang dok ma kham, เอื้องมะขาม Ueang ma kham (Phrae).
D. villosulum Lindl.	**กล้วยหญ้านา** Kluai ya na (Bangkok).
D. virgineum Rchb.f.	เอื้องนางชี Ueang nang chi, เอื้องชีปะขาว Ueang chi pa khao, **เอื้องเงินวิลาศ** Ueang ngoen wilat Northeastern).
D. wardianum Warner	พอเด่นญา Pho-den ya (Karen-Chiang Mai); **เอื้องมณีไตรรงค์** Ueang mani trai rong (Northern).
D. wattii (Hook.f.) Rchb.f.	**เอื้องแซะ** Ueang sae (Northern).
D. ypsilon Seidenf.	**เอื้องแบนปากตัด** Ueang baen pak tat (General).

Dendrobium secundum (Blume) Lindl., locally known as “Ueang praeng si fan”, “Ueang si fan”, “Ueang ngon kai”, “Kho ngu hao” or “Kap kae”, is an epiphytic herb. Stems fleshy, cylindrical, to 100 cm long (usually less); leaves to 10 by 4 cm; inflorescences from the upper nodes only, to about 12 cm long, with many closely-placed small flowers all pointing to one side, bright mauve-pink (or rarely white) with orange lip; flowers to 1.8 cm long and 0.6 cm wide; upper sepal to 7 by 4 mm.; mentum curved; petals very narrow; lip forming a long spur at the base with the column-foot (Holttum, 1957), found flowering in February to April. *D. secundum* distributed in China, Burma, Indonesia and throughout Asia, in Thailand found in North, Northeast, East, and South (Vaddhanaphuti, 2005).

The chemical constituents of this plant have not been studied previously. In this investigation, a MeOH prepared from the aerial parts of this plant was found to possess anti-oxidative potential, showing 75% DPPH reduction at the concentration of

100 μg/ml. This chemical investigation is focused on the free radical scavengers from *D. secundum.*

The main objectives in this study are as follows.

1. Isolation and purification of compounds from *D. secundum*.
2. Determination of the chemical structure of each isolated compound.
3. Evaluation of each isolated compound for its free radical scavenging activity.

Figure 1 *Dendrobium secundum* (Blume) Lindl.

CHAPTER II

HISTORICAL

1. Chemical constituents of *Dendrobium* spp.

A number of chemical constituents isolated from the genus *Dendrobium* can be classified as phenanthrenes (Table 1), bibenzyls (Table 2). In addition, other classes of natural compounds such as sesquiterpenes, sesquiterpene glycosides and miscellaneous substances have been found (Table 3).

Table 1 Distribution of phenanthrenes in *Dendrobium* spp.

Plant and compound	Plant part	Reference
Dendrobium amoenum		
Amoenumin [**1**]	Whole plant	Veerraju *et al*., 1989
Flaccidin (Amoenumin) [**1**]	Whole plant	Majumder *et al*., 1999
Dendrobium aphyllum		
Coelonin [**2**]	Whole plant	Chen *et al*., 2008a
Flavanthrin [**3**]	Whole plant	Chen *et al*., 2008a
Lusianthridin [**4**]	Whole plant	Chen *et al*., 2008a
Moscatin [**5**]	Whole plant	Chen *et al*., 2008a
Dendrobium cariniferum		
Dendronone [**6**]	Whole plant	Chen *et al*., 2008d
Dendrobium chrysanthum		
Dendrochrysanene [**7**]	Whole plant	Yang *et al*., 2006a
Moscatin [**5**]	Whole plant	Yang *et al*., 2006a

Table 1 (continued)

Plant and compound	Plant part	Reference
Dendrobium chrysotoxum		
2,7-Dihydroxy-8-methoxyphenanthro [4,5,*bcd*]pyran-5-(5*H*)-one [**8**]	Whole plant	Yang *et al*., 2004
Crystalltone [**9**]	Stem	Wang *et al*., 2009
Dendrobium densiflorum		
Cypripedin [**10**]	Stem	Fan *et al*., 2001
Densiflorol B [**11**]	Stem	Fan *et al*., 2001
2,6-Dihydroxy-1,5,7-trimethoxyphenanthrene [**12**]	Stem	Fan *et al*., 2001
4,7-Dihydroxy-2-methoxy-9,10-dihydrophenanthrene [**13**]	Stem	Fan *et al*., 2001
Moscatin [**5**]	Stem	Fan *et al*., 2001
Dendrobium loddigesii	Stem	Ito *et al*., 2010
Hircinol [**14**]		
5-Hydroxy-2,4-dimethoxyphenanthrene [**15**]	Stem	Ito *et al*., 2010
Loddigesiinol A [**16**]	Stem	Ito *et al*., 2010
Loddigesiinol B [**17**]	Stem	Ito *et al*., 2010
Lusianthridin [**4**]	Stem	Ito *et al*., 2010
Moscatin [**5**]	Stem	Chen, 1994; Ito *et al*., 2010
Rotundatin [**18**]	Stem	Ito *et al*., 2010
Dendrobium longicornu		
5-Hydroxy-7-methoxy-9,10-dihydrophenanthrene-1,4-dione [**19**]	Stem	Hu *et al*., 2008a

Table 1 (continued)

Plant and compound	Plant part	Reference
Dendrobium longicornu		
7-Methoxy-9,10-dihydrophenanthrene-2,4,5-triol [**20**]	Stem	Hu *et al*., 2008a
Dendrobium moniliforme		
Denbinobin [**21**]	Stem	Lin *et al*., 2001
Moniliformin [**22**]	Stem	Lin *et al*., 2001
Dendrobium nobile		
Bulbophyllanthrin [**23**]	Stem	Yang, Sung, and Kim, 2007
Coelonin [**2**]	Stem	Yang *et al*., 2007; Hwang *et al*., 2010
Confusarin [**24**]	Stem	Zhang *et al*., 2008c
Denbinobin [**21**]	Stem	Yang *et al*., 2007
3,7-Dihydroxy-2,4-dimethoxyphenanthrene [**25**]	Stem	Zhang *et al*., 2008c
4,5-Dihydroxy-2-methoxy-9,10-dihydrophenanthrene [**26**]	Stem	Yang *et al*., 2007
2,2′-Dihydroxy-3,3′,4,4′,7,7′-hexamethoxy-9,9′,10,10′-tetrahydro-1,1′-biphenanthrene [**27**]	Stem	Yang *et al*., 2007
2,8-Dihydroxy-3,4,7-trimethoxy-9,10-dihydrophenanthrene [**28**]	Stem	Yang *et al*., 2007
2,8-Dihydroxy-3,4,7-trimethoxyphenanthrene [**29**]	Stem	Yang *et al*., 2007
2,5-Dihydroxy-3,4-dimethoxyphenanthrene [**30**]	Stem	Yang *et al*., 2007
4,5-Dihydroxy-3,7-dimethoxydihydrophenanthrene [**31**]	Stem	Ye and Zhao, 2002

Table 1 (continued)

Plant and compound	Plant part	Reference
Dendrobium nobile		
2,5-Dihydroxy-4,9-dimethoxyphenanthrene [**32**]	Stem	Zhang *et al*., 2008c
5,7-Dimethoxyphenanthrene-2,6-diol [**33**]	Stem	Hwang *et al*., 2010
Ephemeranthol A [**34**]	Stem	Yang *et al*., 2007; Hwang *et al*., 2010
Ephemeranthol C [**35**]	Stem	Hwang *et al*., 2010
Erianthridin [**36**]	Stem	Yang *et al*., 2007; Hwang *et al*., 2010
Fimbriatone [**37**]	Stem	Zhang *et al*., 2008c
Fimbriol B [**38**]	Stem	Yang *et al*., 2007; Hwang *et al*., 2010
Flavanthridin [**39**]	Stem	Hwang *et al*., 2010
Flavanthrinin [**40**]	Stem	Zhang *et al*., 2008c
Hircinol [**14**]	Stem	Hwang *et al*., 2010
3-Hydroxy-2,4,7-trimethoxy-9,10-dihydrophenanthrene [**41**]	Stem	Yang *et al*., 2007
3-Hydroxy-2,4,7-trimethoxyphenanthrene [**42**]	Stem	Yang *et al*., 2007
2-Hydroxy-3,4,7-trimethoxy-9,10-dihydrophenanthrene [**43**]	Stem	Yang *et al*., 2007
2-Hydroxy-4,7-dimethoxy-9,10-dihydrophenanthrene [**44**]	Stem	Yang *et al*., 2007
Lusianthridin [**4**]	Stem	Yang *et al*., 2007; Hwang *et al*., 2010
Nudol [**45**]	Stem	Yang *et al*., 2007

Table 1 (continued)

Plant and compound	Plant part	Reference
Dendrobium nobile		
Plicatol A [**46**]	Stem	Yang *et al*., 2007
2,3,5-Trihydroxy-4,9-dimethoxyphenanthrene [**47**]	Stem	Yang *et al*., 2007
3,4,8-Trimethoxyphenanthrene-2,5-diol [**48**]	Stem	Hwang *et al*., 2010
Dendrobium plicatile		
2,2′-Dimethoxy-4,4′-7,7′-tetrahydroxy-9,9′,10,10′-tetrahydro-1,1′-biphenanthrene [**49**]	Stem	Yamaki and Honda, 1996
Ephemeranthoquinone [**50**]	Stem	Yamaki and Honda, 1996
Epheranthol B [**51**]	Stem	Yamaki and Honda, 1996
Erianthridin [**36**]	Stem	Yamaki and Honda, 1996
Lusianthridin [**4**]	Stem	Yamaki and Honda, 1996
Plicatol A [**46**]	Stem	Honda and Yamaki, 2000
Plicatol B (Moscatin) [**5**]	Stem	Honda and Yamaki, 2000
Plicatol C (Rotundatin) [**18**]	Stem	Honda and Yamaki, 2000

Table 1 (continued)

Plant and compound	Plant part	Reference
Dendrobium rotundatum		
2,7-Dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene [**52**]	Whole plant	Majumder and Pal, 1992
2,7-Dihydroxy-3,4,6-trimethoxyphenanthrene [**53**]	Whole plant	Majumder and Pal, 1992
Moscatin [**5**]	Whole plant	Majumder and Pal, 1992
Nudol [**45**]	Whole plant	Majumder and Pal, 1992
Rotundatin [**18**]	Whole plant	Majumder and Pal, 1992
Dendrobium thyrsiflorum		
Denthyrsinin [**54**]	Stem	Zhang *et al*., 2005
Denthyrsinol [**55**]	Stem	Zhang *et al*., 2005
Denthyrsinone [**56**]	Stem	Zhang *et al*., 2005
Dendrobium trigonopus		
Hircinol [**14**]	Stem	Hu *et al*., 2008b
Moscatin [**5**]	Stem	Hu *et al*., 2008b

Table 2 Distribution of bibenzyls in *Dendrobium* spp.

Plant and compound	Plant part	Reference
Dendrobium amoenum		
Amoenylin [**57**]	Whole plant	Majumder *et al*., 1999
3,4′-Dihydroxy-5-methoxybibenzyl [**58**]	Whole plant	Majumder *et al*., 1999
Isoamoenylin [**59**]	Whole plant	Majumder *et al*., 1999
Moscatilin [**60**]	Whole plant	Majumder *et al*., 1999
Dendrobium aphyllum		
Batatasin III [**61**]	Whole plant	Chen *et al*., 2008a
Gigantol [**62**]	Whole plant	Chen *et al*., 2008a
Dendrobium aurantiacum		
Chrysotobibenzyl [**63**]	Stem	Yang, Wang, and Xu, 2006b
Chrysotoxin [**64**]	Stem	Yang *et al*., 2006b
Gigantol [**62**]	Stem	Yang *et al*., 2006b
Moscatilin [**60**]	Stem	Yang *et al*., 2006b
Dendrobium candidum		
Dendrocandin A [**65**]	Stem	Li *et al*., 2008
Dendrocandin B [**66**]	Stem	Li *et al*., 2008
Dendrocandin C [**67**]	Stem	Li *et al*., 2009a
Dendrocandin D [**68**]	Stem	Li *et al*., 2009a
Dendrocandin E [**69**]	Stem	Li *et al*., 2009a
Dendrocandin F [**70**]	Stem	Li *et al*., 2009b
Dendrocandin G [**71**]	Stem	Li *et al*., 2009b

Table 2 (continued)

Plant and compound	Plant part	Reference
Dendrobium candidum		
Dendrocandin H [**72**]	Stem	Li *et al*., 2009b
Dendrocandin I [**73**]	Stem	Li *et al*., 2009b
Dendrophenol [**74**]	Stem	Li *et al*., 2008
4,4′-Dihydroxy-3,5-dimethoxybibenzyl [**75**]	Stem	Li *et al*., 2008
3,4-Dihydroxy-5,4′-dimethoxybibenzyl [**76**]	Stem	Li *et al*., 2008
Gigantol [**62**]	Stem	Li *et al*., 2008
3-*O*-Methylgigantol [**77**]	Stem	Li *et al*., 2008
Dendrobium cariniferum		
Batatasin III [**61**]	Whole plant	Chen *et al*., 2008d
Gigantol [**62**]	Whole plant	Chen *et al*., 2008d
Dendrobium chrysanthum		
Chrysotobibenzyl [**63**]	Whole plant	Yang *et al*., 2006a
Chrysotoxin [**64**]	Whole plant	Yang *et al*., 2006a
Crepidatin [**78**]	Whole plant	Yang *et al*., 2006a
4,4′-Dihydroxy-3,3′,5-trimethoxybibenzyl [**79**]	Whole plant	Tanaka *et al*., 1987
Gigantol [**62**]	Whole plant	Yang *et al*., 2006a
Moscatilin [**60**]	Whole plant	Yang *et al*., 2006a
Dendrobium crepidatum		
Crepidatin [**78**]	Whole plant	Majumder and Chatterjee, 1989

Table 2 (continued)

Plant and compound	Plant part	Reference
Dendrobium crystallinum		
Dencryol A [**80**]	Stem	Wang *et al*., 2009
Dencryol B [**81**]	Stem	Wang *et al*., 2009
Dendrobium cumulatum		
Cumulatin [**82**]	Whole plant	Majumder and Pal, 1993
Dendrobium densiflorum		
Densiflorol A [**83**]	Stem	Fan *et al*., 2001
Gigantol [**62**]	Stem	Fan *et al*., 2001
Moscatilin [**60**]	Stem	Fan *et al*., 2001
Tristin [**84**]	Stem	Fan *et al*., 2001
Dendrobium falconeri		
Dendrofalconerol A (Dendrocandin F) [**70**]	Stem	Sritularak and Likhitwitayawuid, 2009
Dendrofalconerol B [**85**]	Stem	Sritularak and Likhitwitayawuid, 2009
Dendrobium gratiosissimun		
Batatasin III [**61**]	Stem	Zhang *et al*., 2008a
Dengraol A [**86**]	Stem	Zhang *et al*., 2008a
Dengraol B [**87**]	Stem	Zhang *et al*., 2008a
3,4-Dihydroxy-5,4′-dimethoxybibenzyl [**76**]	Stem	Zhang *et al*., 2008a
3,4′-Dihydroxy-5-methoxybibenzyl [**58**]	Stem	Zhang *et al*., 2008a
Gigantol [**62**]	Stem	Zhang *et al*., 2008a
Moscatilin [**60**]	Stem	Zhang *et al*., 2008a

Table 2 (continued)

Plant and compound	Plant part	Reference
Dendrobium gratiosissimun		
3,5,4′-Trihydroxybibenzyl [**88**]	Stem	Zhang *et al*., 2008a
Tristin [**84**]	Stem	Zhang *et al*., 2008a
Dendrobium loddigesii		
Batatasin III [**61**]	Stem	Ito *et al*., 2010
Gigantol [**62**]	Stem	Ito *et al*., 2010
Loddigesiinol C [**89**]	Stem	Ito *et al*., 2010
Loddigesiinol D [**90**]	Stem	Ito *et al*., 2010
Moscatilin [**60**]	Stem	Chen, 1994; Ito *et al*., 2010
Dendrobium longicornu		
Aloifol I [**91**]	Stem	Hu *et al*., 2008a
Batatasin [**92**]	Stem	Hu *et al*., 2008a
Gigantol [**62**]	Stem	Hu *et al*., 2008a
4-[2-(3-Hydroxyphenol)-1-methoxyethyl]-2,6-dimethoxyphenol [**93**]	Stem	Hu *et al*., 2008a
Longicornuol A [**94**]	Stem	Hu *et al*., 2008a
Moscatilin [**60**]	Stem	Hu *et al*., 2008a
3,3′,4-Trihydroxybibenzyl [**95**]	Stem	Hu *et al*., 2008a
Tristin [**84**]	Stem	Hu *et al*., 2008a
Dendrobium moniliforme		
Dendromoniliside E [**96**]	Stem	Zhao *et al*., 2003

Table 2 (continued)

Plant and compound	Plant part	Reference
Dendrobium moscatum		
Moscatilin [**60**]	Whole plant	Majumder and Sen, 1987
Dendrobium nobile		
Chrysotobibenzyl [**63**]	Stem	Zhang *et al*., 2007a
Chrysotoxin [**64**]	Stem	Zhang *et al*., 2007a
Crepidatin [**78**]	Stem	Zhang *et al*., 2007a
Dendrobin A [**97**]	Stem	Zhang *et al*., 2007a
4,5-Dihydroxy-3,3′-dimethoxybibenzyl [**98**]	Stem	Ye and Zhao, 2002
3,4′-Dihydroxy-5,5′-dimethoxydihydrostilbene [**99**]	Stem	Hwang *et al*., 2010
Gigantol [**62**]	Stem	Zhang *et al*., 2007a
4-Hydroxy-3,3′,5-trimethoxybibenzyl [**100**]	Stem	Ye and Zhao, 2002
3-*O*-Methylgigantol [**77**]	Stem	Hwang *et al*., 2010
Moscatilin [**60**]	Stem	Yang *et al*., 2007; Hwang *et al*., 2010
Nobilin A [**101**]	Stem	Zhang *et al*., 2006
Nobilin B [**102**]	Stem	Zhang *et al*., 2006
Nobilin C [**103**]	Stem	Zhang *et al*., 2006
Nobilin D [**104**]	Stem	Zhang *et al*., 2007a
Nobilin E [**105**]	Stem	Zhang *et al*., 2007a
Dendrobium plicatile		
Batatasin [**92**]	Stem	Yamaki and Honda, 1996

Table 2 (continued)

Plant and compound	Plant part	Reference
Dendrobium plicatile		
3-*O*-Methylgigantol [**77**]	Stem	Yamaki and Honda, 1996
Dendrobium rotundatum		
Batatasin III [**61**]	Whole plant	Majumder and Pal, 1992
Dendrobium trigonopus		
Gigantol [**62**]	Stem	Hu *et al*., 2008b
Trigonopol A [**106**]	Stem	Hu *et al*., 2008b
Trigonopol B [**107**]	Stem	Hu *et al*., 2008b
Tristin [**84**]	Stem	Hu *et al*., 2008b

Table 3 Distribution of miscellaneous compounds in *Dendrobium* spp.

Plant and compound	Category	Plant part	Reference
Dendrobium aduncum			
Aduncin [**108**]	Sesquiterpene	Plant	Gawell and Leander, 1976
Dendrobium amoenum			
Amoenin [**109**]	Sesquiterpene	Whole plant	Dahmen and Leander, 1978; Majumder, Guha, and Sen, 1999
Amotin [**110**]	Sesquiterpene	Whole plant	Dahmen and Leander, 1978; Majumder *et al*., 1999
Dendrobium aphyllum			
Dibutyl phthalate [**111**]	Benzoic acid ester	Whole plant	Chen *et al*., 2008a
Diisobutyl phthalate [**112**]	Benzoic acid ester	Whole plant	Chen *et al*., 2008a
p-Hydroxyphenylpropionic methyl ester [**113**]	Phenylpro-panoid	Whole plant	Chen *et al*., 2008a
Dendrobium aurantiacum			
Coumarin [**114**]	Coumarin	Stem	Yang *et al*., 2006b
Defuscin [**115**]	Phenylpro-panoid	Stem	Yang *et al*., 2006b
Dendroflorin [**116**]	Fluorenone	Stem	Yang *et al*., 2006b

Table 3 (continued)

Plant and compound	Category	Plant part	Reference
Dendrobium aurantiacum			
Dengibsin [**117**]	Fluorenone	Stem	Yang *et al*., 2006b
Kaempferol [**118**]	Flavone	Stem	Yang *et al*., 2006b
Naringenin [**119**]	Flavanone	Stem	Yang *et al*., 2006b
n-Octacosyl ferulate [**120**]	Phenylpro-panoid	Stem	Yang *et al*., 2006b
Taraxerol [**121**]	Triterpene	Stem	Yang *et al*., 2006b
Dendrobium chrysanthum			
Dengibsin [**117**]	Fluorenone	Whole plant	Yang *et al*., 2006a
Dendrobium chrysotoxum			
Denchrysan A [**122**]	Fluorenone	Whole plant	Chen *et al*., 2008c
Dendroflorin [**116**]	Fluorenone	Whole plant	Chen *et al*., 2008c
(9*R*)-4-Methoxy-9*H*-fluorene-2,5,9-triol [**123**]	Fluorenol	Whole plant	Yang *et al*., 2004
1,4,5-Trihydroxy-7-methoxy-9*H*-fluoren-9-one [**124**]	Fluorenone	Whole plant	Chen *et al*., 2008c
Dendrobium crystallinum			
Apigenin [**125**]	Flavone	Stem	Wang *et al*., 2009
Crystallinin [**126**]	Sesquiterpene	Stem	Wang *et al*., 2009
Dendronobilin B [**127**]	Sesquiterpene	Stem	Wang *et al*., 2009

Table 3 (continued)

Plant and compound	Category	Plant part	Reference
Dendrobium crystallinum			
6‴-Glucosyl-vitexin [**128**]	Flavone glycoside	Stem	Wang *et al*., 2009
3-Hydroxy-2-methoxy-5,6-dimethylbenzoic acid [**129**]	Hydroxybenzoic acid	Stem	Wang *et al*., 2009
Isoviolanthin [**130**]	Flavone glycoside	Stem	Wang *et al*., 2009
Palmarumycin JC2 [**131**]	Dioxane	Stem	Wang *et al*., 2009
Syringic acid [**132**]	Hydroxybenzoic acid	Stem	Wang *et al*., 2009
Dendrobium densiflorum			
Ayapin [**133**]	Coumarin	Stem	Fan *et al*., 2001
Dengibsin [**117**]	Fluorenone	Stem	Fan *et al*., 2001
Homoeriodictyol [**134**]	Flavanone	Stem	Fan *et al*., 2001
Naringenin [**119**]	Flavanone	Stem	Fan *et al*., 2001
Scoparone [**135**]	Coumarin	Stem	Fan *et al*., 2001
Scopoletin [**136**]	Coumarin	Stem	Fan *et al*., 2001
1,4,7-Trihydroxy-5-methoxy-9*H*-fluoren-9-one [**137**]	Fluorenone	Stem	Fan *et al*., 2001
Dendrobium falconeri			
Docosanoyl (*E*)-ferulate [**138**]	Cinnamic acid ester	Stem	Sritularak and Likhitwitayawuid, 2009

Table 3 (continued)

Plant and compound	Category	Plant part	Reference
Dendrobium falconeri			
p-Hydroxybenzaldehyde [**139**]	Benzaldehyde	Stem	Sritularak and Likhitwitayawuid, 2009
p-Hydroxybenzoic acid [**140**]	Hydroxybenzoic acid	Stem	Sritularak and Likhitwitayawuid, 2009
2-(*p*-Hydroxyphenyl) ethyl-*p*-coumarate [**141**]	Phenylpropa-noid	Stem	Sritularak and Likhitwitayawuid, 2009
Tetracosyl (*E*)-*p*-coumarate [**142**]	Phenylpropa-noid	Stem	Sritularak and Likhitwitayawuid, 2009
Tetracosyl (*Z*)-*p*-coumarate [**143**]	Phenylpropa-noid	Stem	Sritularak and Likhitwitayawuid, 2009
Dendrobium fimbriatum			
Defuscin [**115**]	Phenylpropa-noid	Whole plant	Talapatra, Bhaumik, and Talapatra, 1992
Denfigenin [**144**]	Steroid	Whole plant	Talapatra *et al*., 1992
Diosgenin [**145**]	Steroid	Whole plant	Talapatra *et al*., 1992

Table 3 (continued)

Plant and compound	Category	Plant part	Reference
Dendrobium fuscescens			
Defuscin [**115**]	Phenylpropa-noid	Whole plant	Talapatra, Das, and Talapatra, 1989
(-)-Shikimic acid [**146**]	Aliphatic acid	Whole plant	Talapatra *et al*., 1989
Dendrobium huoshanense			
6-*C*-(*α*-Arabinopyranosyl)-8-*C*-[(2-*O*-*α*-rhamnopyranosyl)-*β*-galactopyranosyl]apigenin [**147**]	Flavone glycoside	Leaf and stem	Chang *et al*., 2010
6-*C*-(*α*-Arabinopyranosyl)-8-*C*-[(2-*O*-*α*-rhamnopyranosyl)-*β*-glucopyranosyl]apigenin [**148**]	Flavone glycoside	Leaf and stem	Chang *et al*., 2010
Dimethyl malate [**149**]	Aliphatic acid ester	Leaf and stem	Chang *et al*., 2010
Isopentyl butyrate [**150**]	Aliphatic acid ester	Leaf and stem	Chang *et al*., 2010
Isoschaftoside [**151**]	Flavone glycoside	Leaf and stem	Chang *et al*., 2010
Malic acid [**152**]	Aliphatic acid	Leaf and stem	Chang *et al*., 2010
Phenylacetamide [**153**]	Benzene acetamide	Leaf and stem	Chang *et al*., 2010

Table 3 (continued)

Plant and compound	Category	Plant part	Reference
Dendrobium huoshanense			
6-*C*-[(2-O-α-Rhamnopyranosyl)-β-glucopyranosyl]-8-*C*-(α-arabinopyranosyl)apigenin [**154**]	Flavone glycoside	Leaf and stem	Chang *et al*., 2010
Salicylic acid [**155**]	Hydroxy-benzoic acid	Leaf and stem	Chang *et al*., 2010
6-*C*-(β-Xylopyranosyl)-8-*C*-[(2-*O*-α-rhamnopyranosyl)-β-glucopyranosyl]apigenin [**156**]	Flavone glycoside	Leaf and stem	Chang *et al*., 2010
Dendrobium loddigesii			
Dehydrovomifoliol [**157**]	Cyclohexanone	Stem	Ito *et al*., 2010
(-)-Medioresinol [**158**]	Lignan	Stem	Ito *et al*., 2010
(-)-Pinoresinol [**159**]	Lignan	Stem	Ito *et al*., 2010
Sitostenone [**160**]	Steroid	Stem	Ito *et al*., 2010
β-Sitosterol [**161**]	Steroid	Stem	Ito *et al*., 2010
Stigmasterol [**162**]	Steroid	Stem	Ito *et al*., 2010
Dendrobium longicornu			
Episyringaresinol [**163**]	Lignan	Stem	Hu *et al*., 2008a
Episyringaresinol 4″-*O*-β-D-glucopyranoside [**164**]	Lignan glycoside	Stem	Hu *et al*., 2008a
Erythro-1-(4-*O*-β-D-glucopyranosyl-3-methoxyphenyl)-2-[4-(3-hydroxy propyl)2,6-dimethoxyphenoxy]-1,3-propanediol [**165**]	Lignan glycoside	Stem	Hu *et al*., 2008a

Table 3 (continued)

Plant and compound	Category	Plant part	Reference
Dendrobium longicornu			
Eugenyl-*O*-*β*-D-glucopyranoside [**166**]	Phenylpropa-noid	Stem	Hu *et al*., 2008a
3-(3-Methoxy,4-hydroxyphenyl)-1-propanol [**167**]	Phenylpropa-noid	Stem	Hu *et al*., 2008a
Methyl *β*-orsellinate [**168**]	Phenolic compound	Stem	Hu *et al*., 2008a
Naringenin [**119**]	Flavanone	Stem	Hu *et al*., 2008a
9-*β*-D-Ribofuranosyl-9*H*-purin-6-amine [**169**]	Purine nucleotide	Stem	Hu *et al*., 2008a
(3*S*,4*S*,5*R*)-3,4,5-Trihydroxy-1-cyclohexene carboxylic acid ((-)-Shikimic acid) [**146**]	Aliphatic acid	Stem	Hu *et al*., 2008a
Dendrobium moniliforme			
Acanthoside B [**170**]	Lignan glycoside	Stem	Zhao *et al*., 2003
Dendromoniliside A [**171**]	Sesquiterpene glycoside	Stem	Zhao *et al*., 2003
Dendromoniliside B [**172**]	Sesquiterpene glycoside	Stem	Zhao *et al*., 2003
Dendromoniliside C [**173**]	Sesquiterpene glycoside	Stem	Zhao *et al*., 2003
Dendromoniliside D [**174**]	Sesquiterpene glycoside	Stem	Zhao *et al*., 2003
Dendroside A [**175**]	Sesquiterpene glycoside	Stem	Zhao *et al*., 2003

Table 3 (continued)

Plant and compound	Category	Plant part	Reference
Dendrobium moniliforme			
Dendroside C [**176**]	Sesquiterpene glycoside	Stem	Zhao *et al*., 2003
Dendroside F [**177**]	Sesquiterpene glycoside	Stem	Zhao *et al*., 2003
Vanilloloside [**178**]	Phenolic glycoside	Stem	Zhao *et al*., 2003
Dendrobium nobile			
Dendrobane A [**179**]	Sesquiterpene	Stem	Ye and Zhao, 2002
Dendrobine [**180**]	Sesquiterpene alkaloid	Stem	Wang, Zhao, and Che, 1985; Ye and Zhao, 2002
Dendroflorin [**116**]	Fluorenone	Stem	Zhang *et al*., 2007a
Dendronobilin A [**181**]	Sesquiterpene	Stem	Zhang *et al*., 2007a
Dendronobilin B [**127**]	Sesquiterpene	Stem	Zhang *et al*., 2007a
Dendronobilin C [**182**]	Sesquiterpene	Stem	Zhang *et al*., 2007a
Dendronobilin D [**183**]	Sesquiterpene	Stem	Zhang *et al*., 2007a
Dendronobilin E [**184**]	Sesquiterpene	Stem	Zhang *et al*., 2007a
Dendronobilin F [**185**]	Sesquiterpene	Stem	Zhang *et al*., 2007a
Dendronobilin G [**186**]	Sesquiterpene	Stem	Zhang *et al*., 2007a
Dendronobilin H [**187**]	Sesquiterpene	Stem	Zhang *et al*., 2007a
Dendronobilin I [**188**]	Sesquiterpene	Stem	Zhang *et al*., 2007a
Dendronobilin J [**189**]	Sesquiterpene	Stem	Zhang *et al*., 2007b
Dendronobilin K [**190**]	Sesquiterpene	Stem	Zhang *et al*., 2008b

Table 3 (continued)

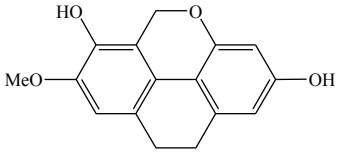
Plant and compound	Category	Plant part	Reference
Dendrobium nobile			
Dendronobilin L [**191**]	Sesquiterpene	Stem	Zhang *et al*., 2008b
Dendronobilin M [**192**]	Sesquiterpene	Stem	Zhang *et al*., 2008b
Dendronobilin N [**193**]	Sesquiterpene	Stem	Zhang *et al*., 2008b
Dendronobiloside A [**194**]	Sesquiterpene glycoside	Stem	Zhao *et al*., 2001; Ye and Zhao, 2002
Dendronobiloside B [**195**]	Sesquiterpene glycoside	Stem	Zhao *et al*., 2001; Ye and Zhao, 2002
Dendronobiloside C [**196**]	Sesquiterpene glycoside	Stem	Ye and Zhao, 2002
Dendronobiloside D [**197**]	Sesquiterpene glycoside	Stem	Ye and Zhao, 2002
Dendronobiloside E [**198**]	Sesquiterpene glycoside	Stem	Ye and Zhao, 2002
Dendroside A [**175**]	Sesquiterpene glycoside	Stem	Zhao *et al*., 2001; Ye and Zhao, 2002
Dendroside B [**199**]	Sesquiterpene glycoside	Stem	Ye and Zhao, 2002
Dendroside C [**176**]	Sesquiterpene glycoside	Stem	Ye and Zhao, 2002
Dendroside D [**200**]	Sesquiterpene glycoside	Stem	Ye, Qin, and Zhao, 2002
Dendroside E [**201**]	Sesquiterpene glycoside	Stem	Ye *et al*., 2002

Table 3 (continued)

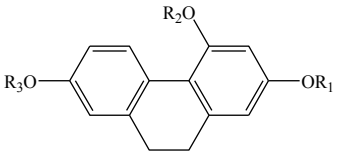
Plant and compound	Category	Plant part	Reference
Dendrobium nobile			
Dendroside F [**177**]	Sesquiterpene glycoside	Stem	Ye *et al*., 2002
Dendroside G [**202**]	Sesquiterpene glycoside	Stem	Ye *et al*., 2002
7,12-Dihydroxy-5-hydroxymethyl-11-isopropyl-6-methyl-9-oxatricyclo[6.2.1.$0^{2,6}$]undecan-10-one-15-*O*-β-D-glucopyranoside [**203**]	Sesquiterpene glycoside	Stem	Shu, Zhang, and Guo, 2004
3-Hydroxy-2-oxodendrobine [**204**]	Alkaloid	Stem	Wang *et al*., 1985
Lirioresinol A [**205**]	Lignan	Stem	Zhang *et al*., 2008c
Medioresinol [**206**]	Lignan	Stem	Zhang *et al*., 2008c
Nobilone [**207**]	Fluorenone	Stem	Zhang *et al*., 2007a
Pinoresinol [**208**]	Lignan	Stem	Zhang *et al*., 2008c
Protocatechuic acid [**209**]	Benzoic acid	Stem	Ye and Zhao, 2002
Syringaresinol [**210**]	Lignan	Stem	Zhang *et al*., 2008c
10β,12,14-Trihydroxyalloaromadendrane [**211**]	Sesquiterpene	Stem	Ye and Zhao, 2002
Dendrobium ochreatum			
Dendrosteroside [**212**]	Steroid glycoside	Plant	Behr and Leander, 1976
Epiochreasteroside [**213**]	Steroid glycoside	Plant	Behr and Leander, 1976
Ochreasteroside [**214**]	Steroid glycoside	Plant	Behr and Leander, 1976

Table 3 (continued)

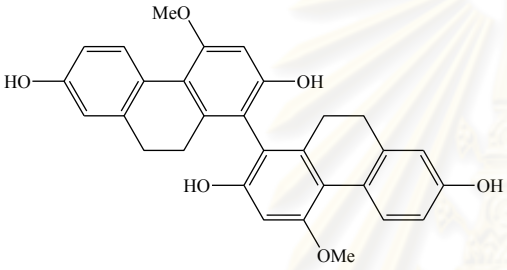
Plant and compound	Category	Plant part	Reference
Dendrobium thyrsiflorum			
Chrysophanol [**215**]	Anthraquinone	Stem	Zhang *et al*., 2005
Daucosterol [**216**]	Steroid glycoside	Stem	Zhang *et al*., 2005
Denthyrsin [**217**]	Coumarin	Stem	Zhang *et al*., 2005
Emodin [**218**]	Anthraquinone	Stem	Zhang *et al*., 2005
Physcion [**219**]	Anthraquinone	Stem	Zhang *et al*., 2005
Scoparone [**135**]	Coumarin	Stem	Zhang *et al*., 2005
β-Sitosterol [**161**]	Steroid	Stem	Zhang *et al*., 2005
Dendrobium trigonopus			
3-(4-Hydroxy-3-methoxyphenyl)-2-propen-1-ol [**220**]	Phenylpropa-noid	Stem	Hu *et al*., 2008b
Naringenin [**119**]	Flavanone	Stem	Hu *et al*., 2008b
(-)-Syringaresinol [**221**]	Lignan	Stem	Hu *et al*., 2008b



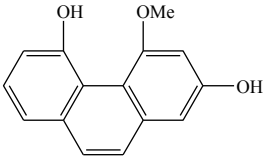
[**1**] Amoenumin (Flaccidin)



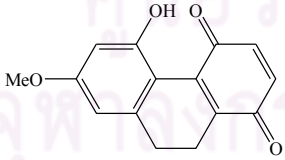
	R_1	R_2	R_3
Coelonin [**2**]	H	Me	Me
Lusianthridin [**4**]	Me	H	H



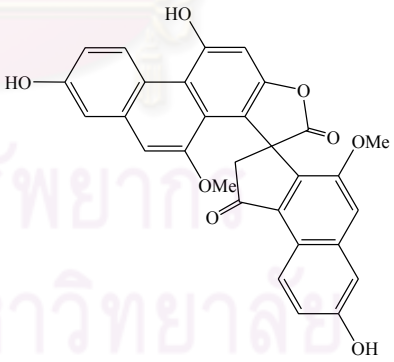
Flavanthrin [**3**]



Moscatin [**5**]

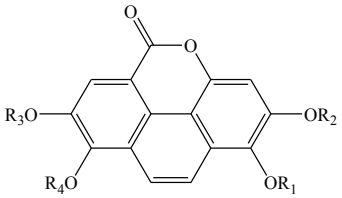


Dendronone [**6**]

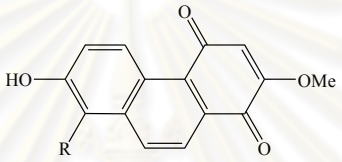


Dendrochrysanene [**7**]

Figure 2 Structure of phenanthrenes isolated from *Dendrobium* spp.

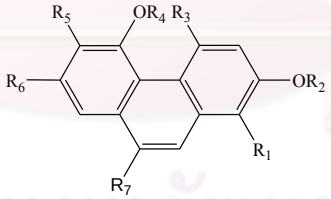


	R_1	R_2	R_3	R_4
[**8**] 2,7-Dihydroxy-8-methoxyphenanthro [4,5,*bcd*]pyran-5-(5*H*)-one	H	OH	OH	OMe
[**9**] Crystalltone	OH	OEt	OMe	H



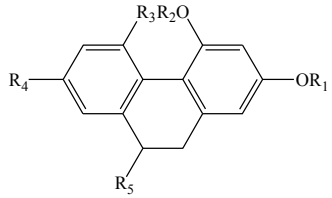
[**10**] Cypripedin R = OMe

[**11**] Densiflorol B R = H

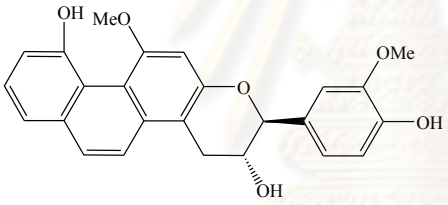


	R_1	R_2	R_3	R_4	R_5	R_6	R_7
[**12**] 2,6-Dihydroxy-1,5,7-trimethoxyphenanthrene	OMe	H	H	Me	OH	OMe	H
[**15**] 5-Hydroxy-2,4-dimethoxyphenanthrene	H	Me	OMe	H	H	H	H
[**16**] Loddigesiinol A	H	H	OMe	Me	H	H	OH

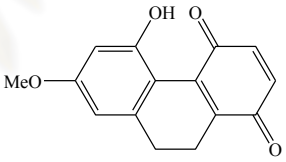
Figure 2 Structure of phenanthrenes isolated from *Dendrobium* spp. (continued)



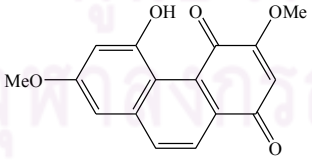
	R_1	R_2	R_3	R_4	R_5
[**13**] 4,7-Dihydroxy-2-methoxy-9,10-dihydrophenanthrene	Me	H	H	OH	H
[**14**] Hircinol	H	Me	OH	H	H
[**18**] Rotundatin	H	Me	OH	H	OH
[**20**] 7-Methoxy-9,10-dihydrophenanthrene-2,4,5-triol	H	Me	OH	OH	H



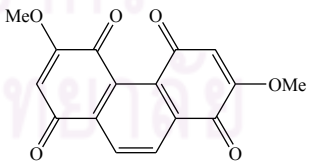
[**17**] Loddigesiinol B



[**19**] 5-Hydroxy-7-methoxy-9,10-dihydrophenanthrene-1,4-dione

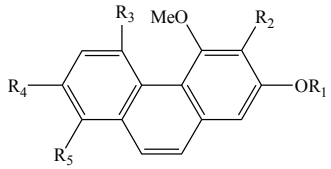


[**21**] Denbinobin

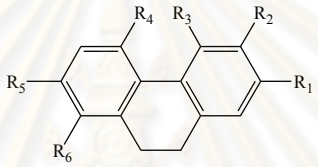


[**22**] Moniliformin

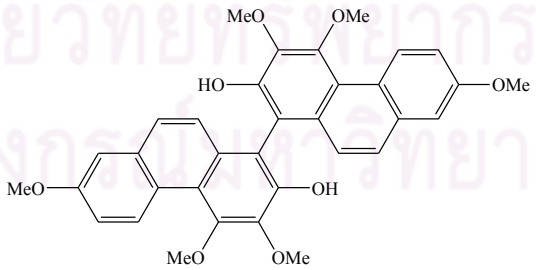
Figure 2 Structure of phenanthrenes isolated from *Dendrobium* spp. (continued)



	R_1	R_2	R_3	R_4	R_5
[**23**] Bulbophyllanthrin	Me	OH	OH	H	H
[**24**] Confusarin	H	OMe	H	OH	OMe
[**25**] 3,7-Dihydroxy-2,4-dimethoxyphenanthrene	Me	OH	H	OH	H
[**29**] 2,8-Dihydroxy-3,4,7-trimethoxyphenanthrene	H	OMe	H	OMe	OH
[**30**] 2,5-Dihydroxy-3,4-dimethoxyphenanthrene	H	OMe	OH	H	H

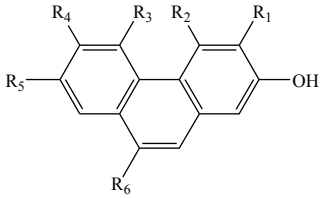


	R_1	R_2	R_3	R_4	R_5	R_6
[**26**] 4,5-Dihydroxy-2-methoxy-9,10-dihydrophenanthrene	OMe	H	OH	OH	H	H
[**28**] 2,8-Dihydroxy-3,4,7-trimethoxy-9,10-dihydrophenanthrene	OH	OMe	OMe	H	OMe	OH
[**31**] 4,5-Dihydroxy-3,7-dimethoxy dihydrophenanthrene	H	OMe	OH	OH	OMe	H

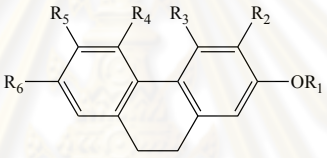


[**27**] 2,2′-Dihydroxy-3,3′,4,4′,7,7′-hexamethoxy -9,9′,10,10′-tetrahydro-1,1′-biphenanthrene

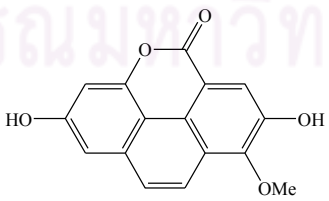
Figure 2 Structure of phenanthrenes isolated from *Dendrobium* spp. (continued)



	R_1	R_2	R_3	R	R_6	R_7
[**32**] 2,5-Dihydroxy-4,9-dimethoxyphenanthrene	H	OMe	OH	H	H	OMe
[**33**] 5,7-Dimethoxyphenanthrene-2,6-diol	H	H	OMe	OH	OMe	H
[**38**] Fimbriol B	OH	OMe	OH	H	H	H
[**40**] Flavanthrinin	H	OMe	H	H	OH	H

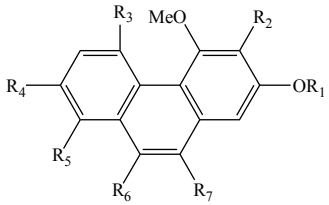


	R_1	R_2	R_3	R_4	R_5	R_6
[**34**] Ephemeranthol A	H	H	H	OH	OMe	OMe
[**35**] Ephemeranthol C	H	OH	OMe	OH	H	H
[**36**] Erianthridin	H	OMe	OMe	H	H	OH
[**39**] Flavanthridin	H	H	H	OMe	OH	OMe
[**41**] 3-Hydroxy-2,4,7-trimethoxy-9,10-dihydrophenanthrene	Me	OH	OMe	H	H	OMe

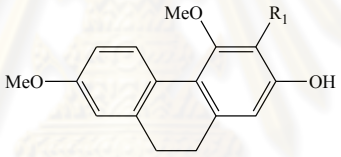


[**37**] Fimbriatone

Figure 2 Structure of phenanthrenes isolated from *Dendrobium* spp. (continued)

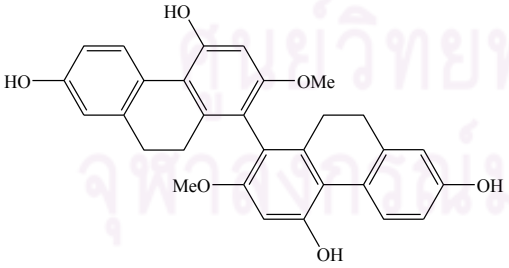


	R_1	R_2	R_3	R_4	R_5	R_6	R_7
[**42**] 3-Hydroxy-2,4,7-trimethoxyphenanthrene	Me	OH	H	OMe	H	H	H
[**45**] Nudol	H	OMe	H	OH	H	H	H
[**46**] Plicatol A	H	H	OH	H	H	OMe	OMe
[**47**] 2,3,5-Trihydroxy-4,9-dimethoxyphenanthrene	H	OH	OH	H	H	OMe	H
[**48**] 3,4,8-Trimethoxy phenanthrene-2,5-diol	H	OMe	OH	H	OMe	H	H

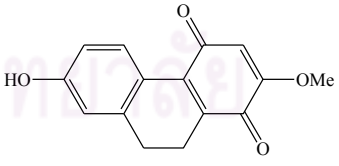


[**43**] 2-Hydroxy-3,4,7-trimethoxy-9,10-dihydrophenanthrene — R_1 = OMe

[**44**] 2-Hydroxy-4,7-dimethoxy-9,10-dihydrophenanthrene — R_1 = H

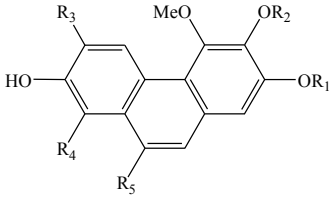


[**49**] 2,2′-Dimethoxy-4,4′-7,7′-tetrahydroxy-9,9′,10,10′-tetrahydro-1,1′-biphenanthrene

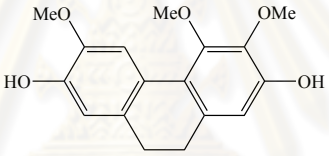


[**50**] Ephemeranthoquinone

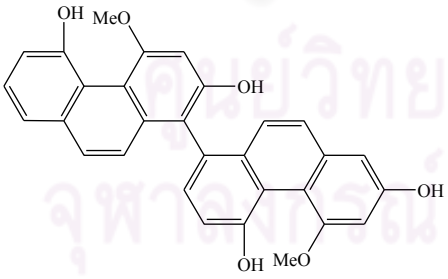
Figure 2 Structure of phenanthrenes isolated from *Dendrobium* spp. (continued)



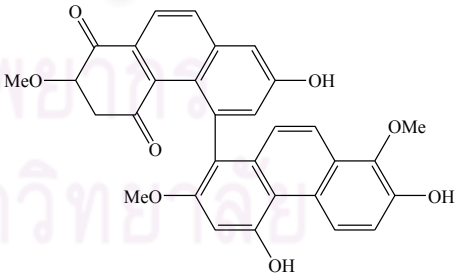
	R_1	R_2	R_3	R_4
[**51**] Epheranthol B	Me	H	H	H
[**53**] 2,7-Dihydroxy-3,4,6-trimethoxyphenanthrene	H	Me	OMe	H
[**54**] Denthyrsinin	Me	H	H	OMe



[**52**] 2,7-Dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene

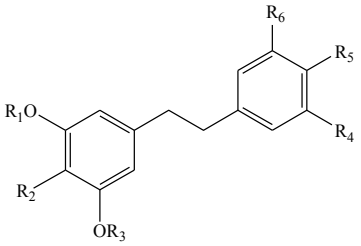


Denthyrsinol [**55**]

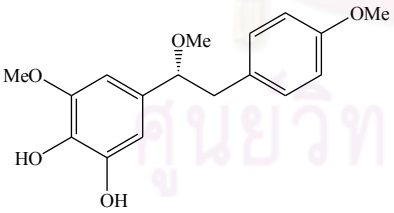


Denthyrsinone [**56**]

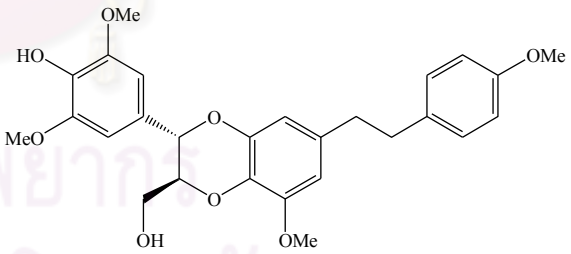
Figure 2 Structure of phenanthrenes isolated from *Dendrobium* spp. (continued)



	R_1	R_2	R_3	R_4	R_5	R_6
[**57**] Amoenylin	Me	OH	Me	H	OMe	H
[**58**] 3,4′-Dihydroxy-5-methoxybibenzyl	H	H	Me	H	OH	H
[**59**] Isoamoenylin	Me	OMe	Me	H	H	OH
[**60**] Moscatilin	Me	OH	Me	H	OH	OMe
[**61**] Batatasin III	Me	H	H	H	H	OH
[**62**] Gigantol	Me	H	H	H	OH	OMe
[**63**] Chrysotobibenzyl	Me	OMe	Me	OMe	OMe	H
[**64**] Chrysotoxin	Me	OH	Me	OMe	OMe	H

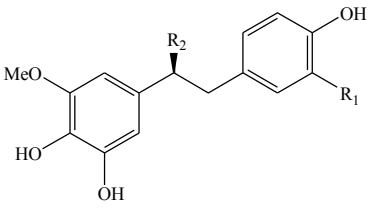


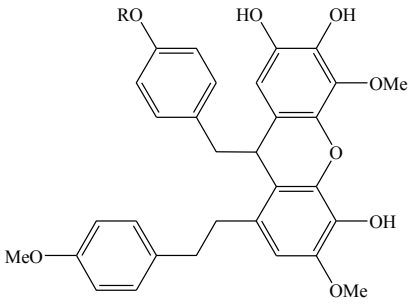
[**65**] Dendrocandin A



[**66**] Dendrocandin B

Figure 3 Structure of bibenzyls isolated from *Dendrobium* spp.

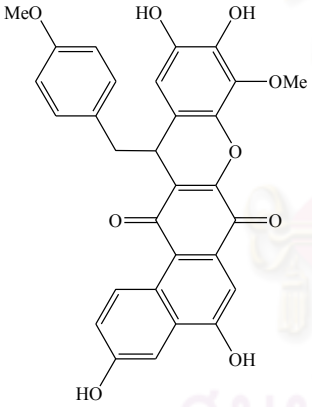




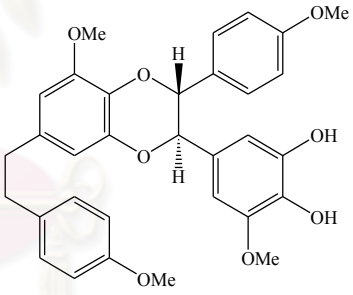
	R_1	R_2
[**67**] Dendrocandin C	H	OMe
[**68**] Dendrocandin D	H	OEt
[**69**] Dendrocandin E	OH	H

[**70**] Dendrocandin F R = Me
(Dendrofalconerol A)

[**71**] Dendrocandin G R = H

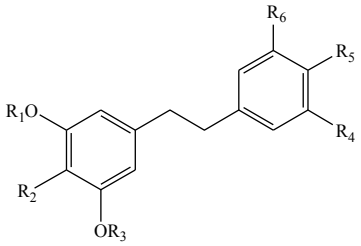


[**72**] Dendrocandin H

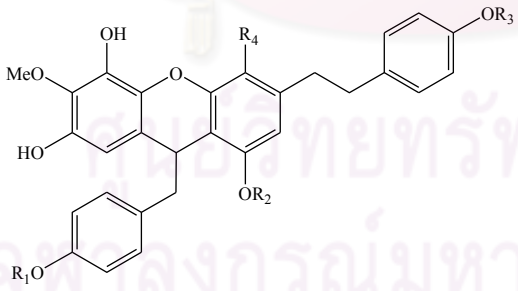


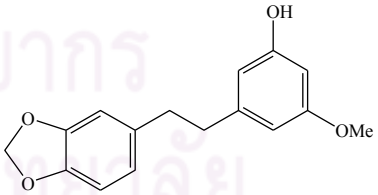
[**73**] Dendrocandin I

Figure 3 Structure of bibenzyls isolated from *Dendrobium* spp. (continued)



	R_1	R_2	R_3	R_4	R_5	R_6
[**74**] Dendrophenol	Me	OH	Me	OH	OH	H
[**75**] 4,4′-Dihydroxy-3,5-dimethoxybibenzyl	Me	OH	Me	H	OH	H
[**76**] 3,4-Dihydroxy-5,4′-dimethoxybibenzyl	H	OH	Me	H	OMe	H
[**77**] 3-*O*-Methylgigantol	H	H	Me	OMe	OMe	H
[**78**] Crepidatin	Me	OMe	Me	OMe	OH	H
[**79**] 4,4′-Dihydroxy-3,3′,5-trimethoxybibenzyl	Me	OH	Me	H	OH	OMe
[**82**] Cumulatin	H	OMe	Me	OH	OMe	OMe
[**84**] Tristin	H	H	H	H	OH	OMe

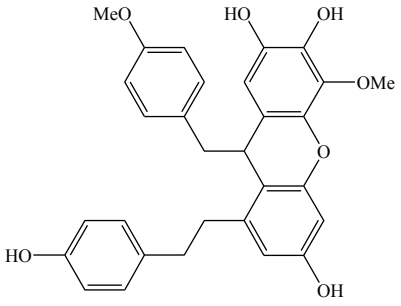




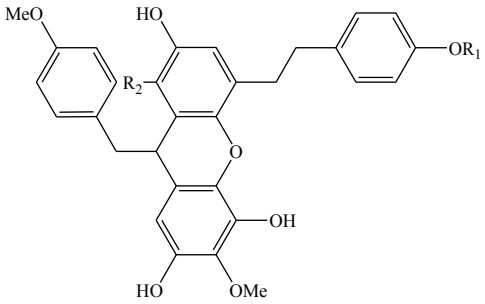
	R_1	R_2	R_3	R_4
[**80**] Dencryol A	Me	H	H	H
[**81**] Dencryol B	H	Me	Me	OH

[**83**] Densiflorol A

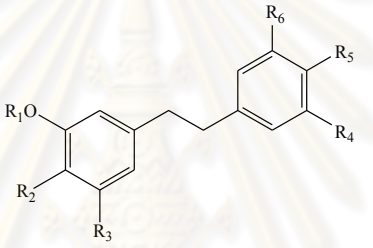
Figure 3 Structure of bibenzyls isolated from *Dendrobium* spp. (continued)



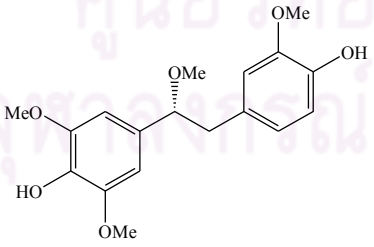
[**85**] Dendrofalconerol B



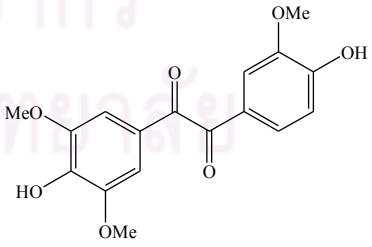
	R_1	R_2
[**86**] Dengraol A	H	H
[**87**] Dengraol B	Me	OMe



	R_1	R_2	R_3	R_4	R_5	R_6
[**88**] 3,5,4′-Trihydroxybibenzyl	H	H	OH	H	OH	H
[**91**] Aloifol I	Me	OH	OMe	OH	H	H
[**92**] Batatasin	Me	H	H	OH	H	OH

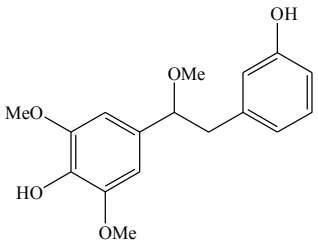


[**89**] Loddigesiinol C

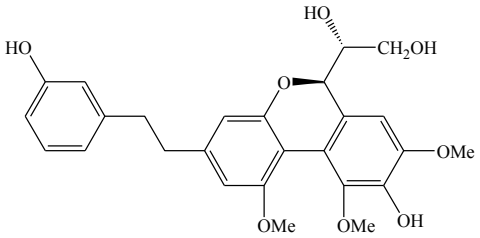


[**90**] Loddigesiinol D

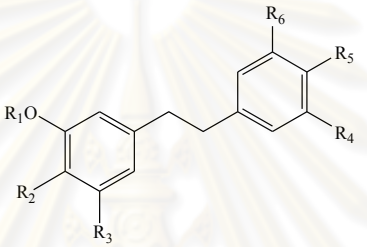
Figure 3 Structure of bibenzyls isolated from *Dendrobium* spp. (continued)



[**93**] 4-[2-(3-Hydroxyphenol)-1-methoxyethyl]-2,6-dimethoxyphenol

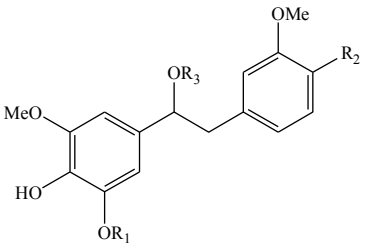


[**94**] Longicornuol A

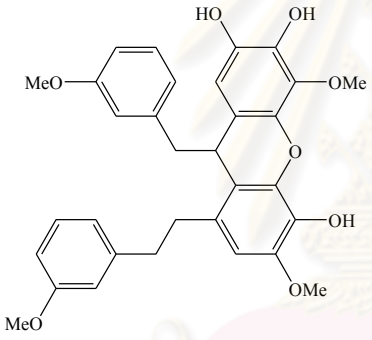


	R_1	R_2	R_3	R_4	R_5	R_6
[**95**] 3,3′,4-Trihydroxybibenzyl	H	OH	H	H	H	OH
[**96**] Dendromoniliside E	Glc	OGlc	OMe	H	OMe	H
[**97**] Dendrobin A	H	OH	OMe	H	H	OMe
[**98**] 4,5-Dihydroxy-3,3′-dimethoxybibenzyl	Me	OH	OH	H	H	OMe
[**99**] 3,4′-Dihydroxy-5,5′-dimethoxydihydrostilbene	H	H	OMe	OMe	OH	H
[**100**] 4-Hydroxy-3,3′,5-trimethoxybibenzyl	Me	OH	OMe	H	H	OMe

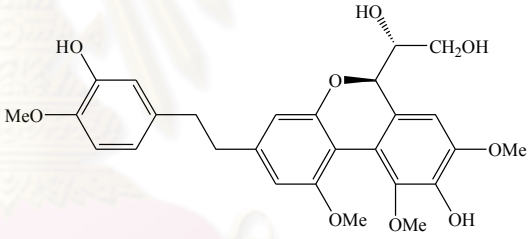
Figure 3 Structure of bibenzyls isolated from *Dendrobium* spp. (continued)



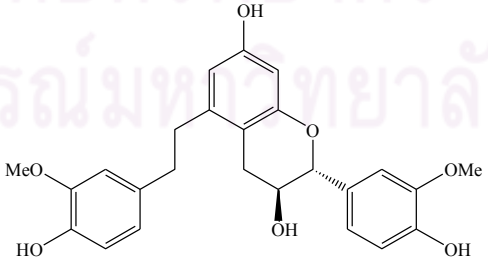
	R_1	R_2	R_3
[**101**] Nobilin A	H	H	Me
[**102**] Nobilin B	Me	OH	Me
[**103**] Nobilin C	Me	OMe	Me
[**104**] Nobilin D	Me	OH	H



[**105**] Nobilin E

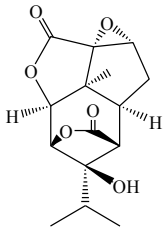


[**106**] Trigonopol A

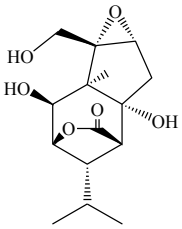


[**107**] Trigonopol B

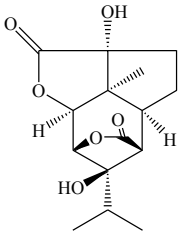
Figure 3 Structure of bibenzyls isolated from *Dendrobium* spp. (continued)



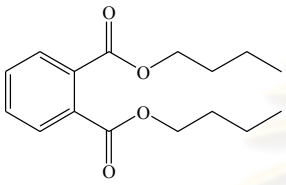
[**108**] Aduncin



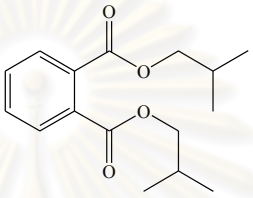
[**109**] Amoenin



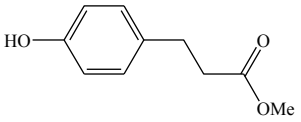
[**110**] Amotin



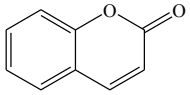
[**111**] Dibutyl phthalate



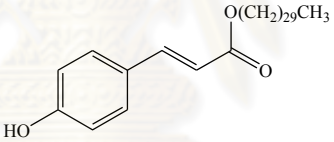
[**112**] Diisobutyl phthalate



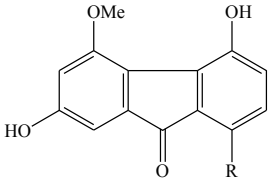
[**113**] *p*-Hydroxyphenyl propionic methyl ester



[**114**] Coumarin

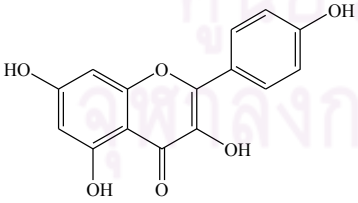


[**115**] Defuscin

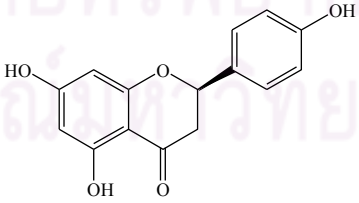


[**116**] Dendroflorin R = OH

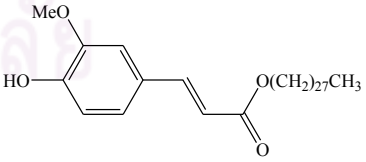
[**117**] Dengibsen R = H



[**118**] Kaempferol

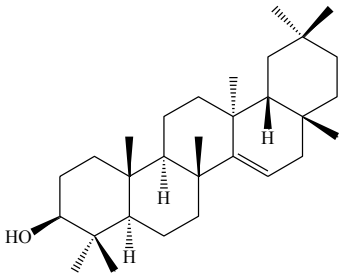


[**119**] Naringenin

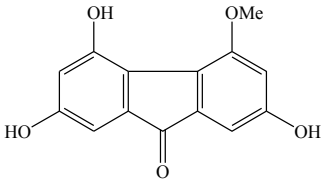


[**120**] *n*-Octacosyl ferulate

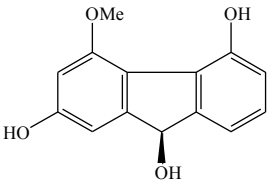
Figure 4 Structure of miscellaneous compounds isolated from *Dendrobium* spp.



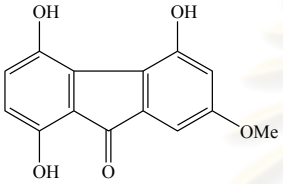
[**121**] Taraxerol



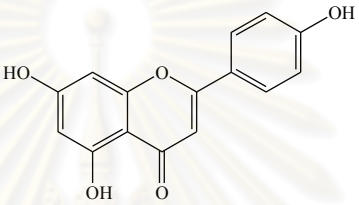
[**122**] Denchrysan A



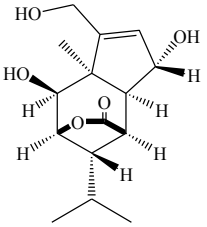
[**123**] (9*R*)-4-Methoxy-9*H*-fluorene-2,5,9-triol



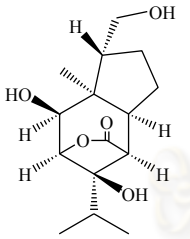
[**124**] 1,4,5-Trihydroxy-7-methoxy-9*H*-fluoren-9-one



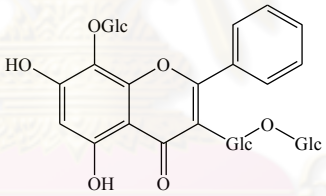
[**125**] Apigenin



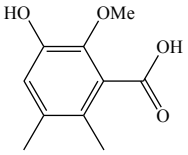
[**126**] Crystallinin



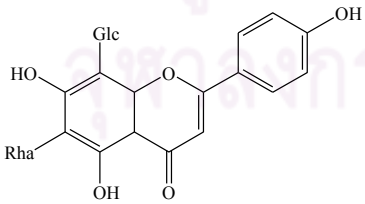
[**127**] Dendronobilin B



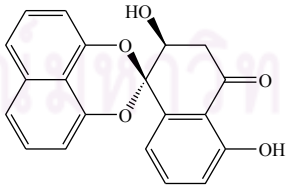
[**128**] 6‴-Glucosyl-vitexin



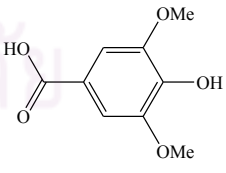
[**129**] 3-Hydroxy-2-methoxy -5,6-dimethylbenzoic acid



[**130**] Isoviolanthin

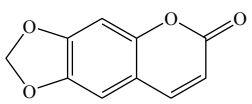


[**131**] Palmarumycin JC2

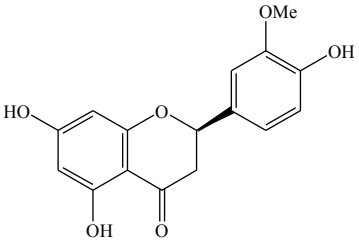


[**132**] Syringic acid

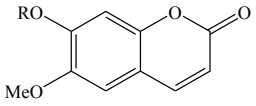
Figure 4 Structure of miscellaneous compounds isolated from *Dendrobium* spp. (continued)



[**133**] Ayapin

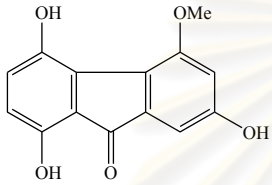


[**134**] Homoeriodictyol

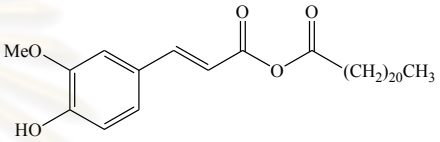


[**135**] Scoparone R = Me

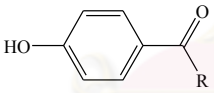
[**136**] Scopoletin R = H



[**137**] 1,4,7-Trihydroxy-5-methoxy-9*H*-fluoren- 9-one

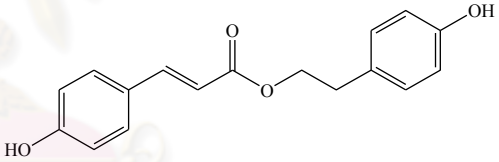


[**138**] Docosanoyl (*E*)-ferulate

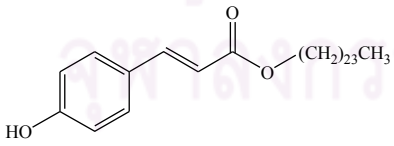


[**139**] *p*-Hydroxybenz-aldehyde R = H

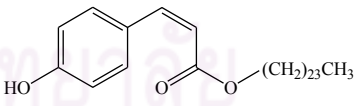
[**140**] *p*-Hydroxybenzoic acid R = OH



[**141**] 2-(*p*-Hydroxyphenyl) ethyl-*p*-coumarate

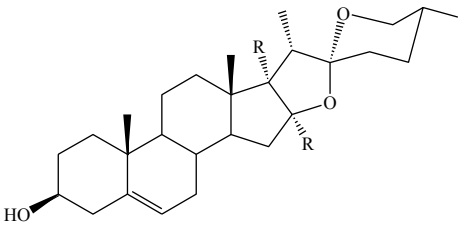


[**142**] Tetracosyl (*E*)-*p*-coumarate



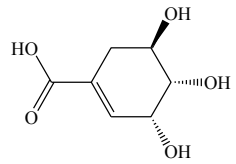
[**143**] Tetracosyl (*Z*)-*p*-coumarate

Figure 4 Structure of miscellaneous compounds isolated from *Dendrobium* spp. (continued)

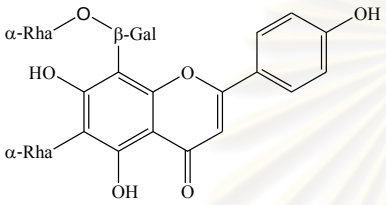


[**144**] Denfigenin R = OH

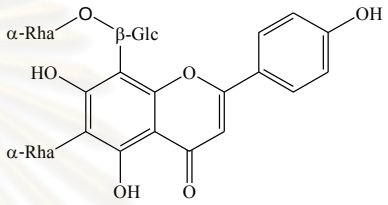
[**145**] Diosgenin R =H



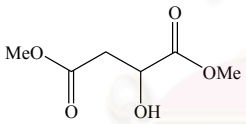
[**146**] (-)-Shikimic acid



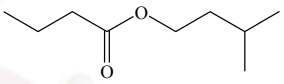
[**147**] 6-*C*-(*α*-Arabinopyranosyl)-8-*C*-[(2-*O*-*α*-rhamnopyranosyl)-*β*-galactopyranosyl]apigenin



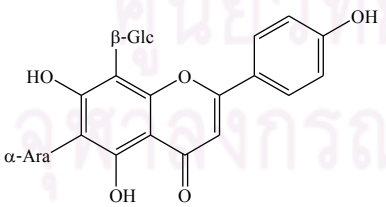
[**148**] 6-*C*-(*α*-Arabinopyranosyl)-8-*C*-[(2-*O*-*α*-rhamnopyranosyl)-*β*-glucopyranosyl]apigenin



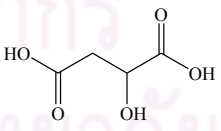
[**149**] Dimethyl malate



[**150**] Isopentyl butyrate

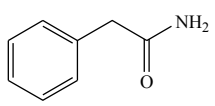


[**151**] Isoschaftoside

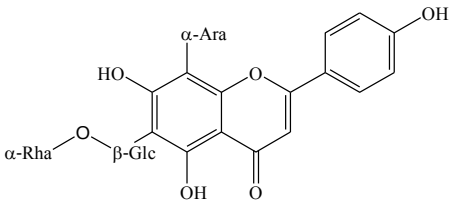


[**152**] Malic acid

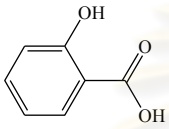
Figure 4 Structure of miscellaneous compounds isolated from *Dendrobium* spp. (continued)



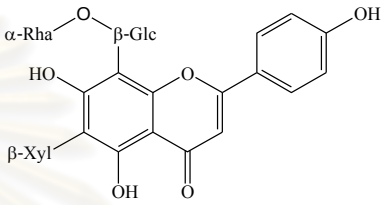
[**153**] Phenylacetamide



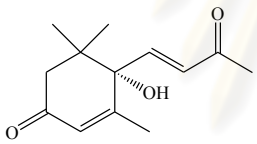
[**154**] 6-*C*-[(2-O-α-Rhamnopyranosyl)-β-glucopyranosyl]-8-*C*-(α-arabinopyranosyl)apigenin



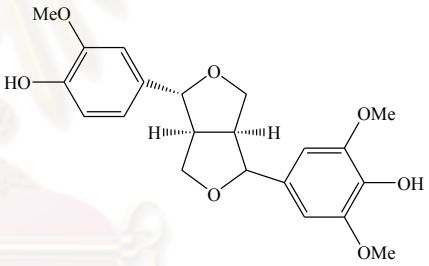
[**155**] Salicylic acid



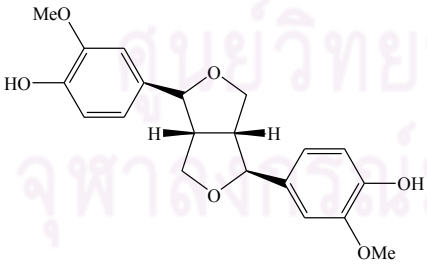
[**156**] 6-*C*-(β-Xylopyranosyl)-8-*C*-[(2-*O*-α-rhamnopyranosyl)-β-glucopyranosyl]apigenin



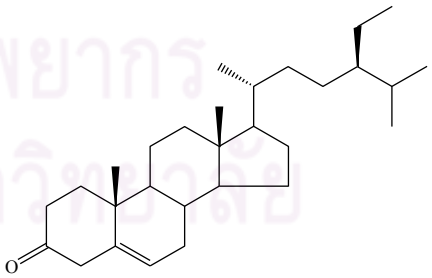
[**157**] Dehydrovomifoliol



[**158**] (-)-Medioresinol

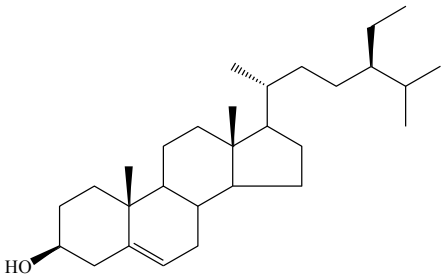


[**159**] (-)-Pinoresinol

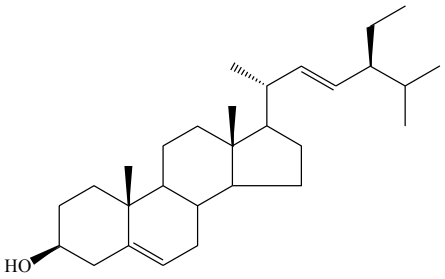


[**160**] Sitostenone

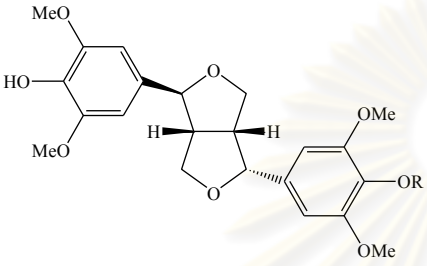
Figure 4 Structure of miscellaneous compounds isolated from *Dendrobium* spp. (continued)



[**161**] β-Sitosterol

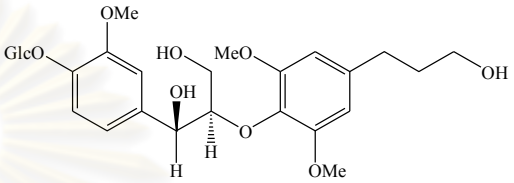


[**162**] Stigmasterol

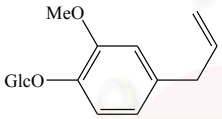


[**163**] Episyringaresinol R = H

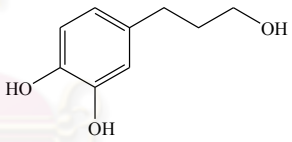
[**164**] Episyringaresinol 4′′-*O*-β -D-glucopyranoside R = β -D-Glc



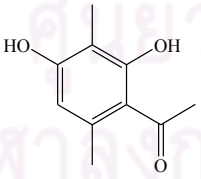
[**165**] Erythro-1-(4-*O*-β-D-glucopyranosyl-3-methoxyphenyl)-2-[4-(3-hydroxypropyl) 2,6-dimethoxyphenoxy]-1,3-propanediol



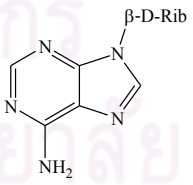
[**166**] Eugenyl-*O*-β-D-glucopyranoside



[**167**] 3-(3-Methoxy,4-hydroxyphenyl)-1-propanol

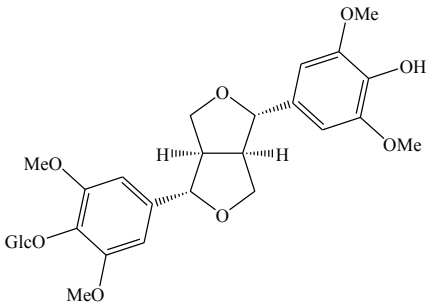


[**168**] Methyl β-orsellinate

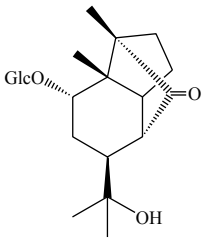


[**169**] 9-β-D-Ribofuranosyl-9*H*-purin-6-amine

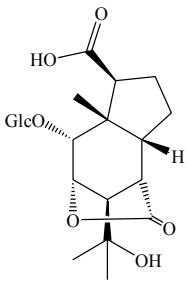
Figure 4 Structure of miscellaneous compounds isolated from *Dendrobium* spp. (continued)



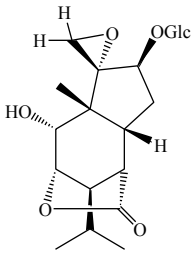
[**170**] Acanthoside B



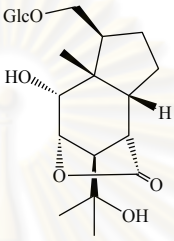
[**171**] Dendromoniliside A



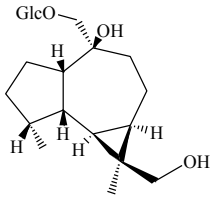
[**172**] Dendromoniliside B



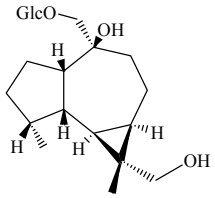
[**173**] Dendromoniliside C



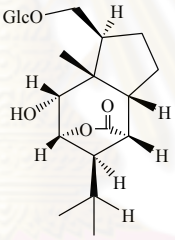
[**174**] Dendromoniliside D



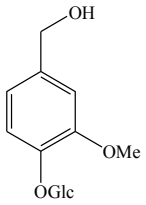
[**175**] Dendroside A



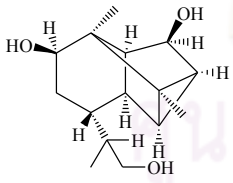
[**176**] Dendroside C



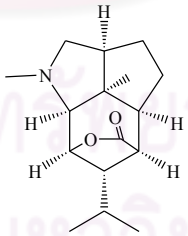
[**177**] Dendroside F



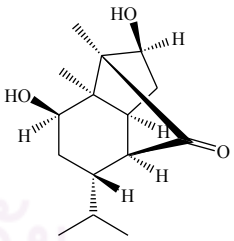
[**178**] Vanilloloside



[**179**] Dendrobane A

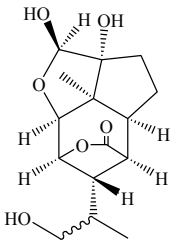


[**180**] Dendrobine

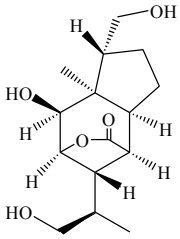


[**181**] Dendronobilin A

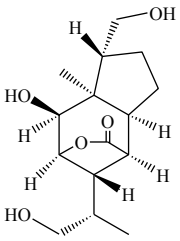
Figure 4 Structure of miscellaneous compounds isolated from *Dendrobium* spp. (continued)



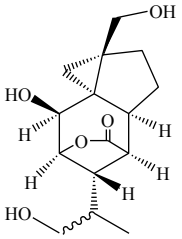
[**182**] Dendronobilin C



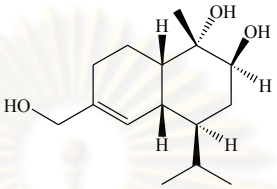
[**183**] Dendronobilin D



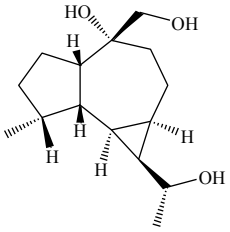
[**184**] Dendronobilin E



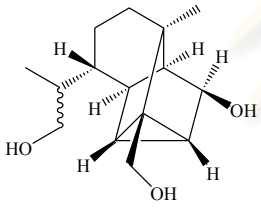
[**185**] Dendronobilin F



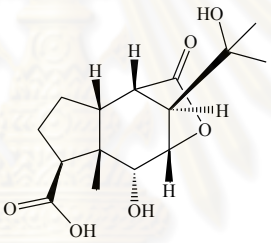
[**186**] Dendronobilin G



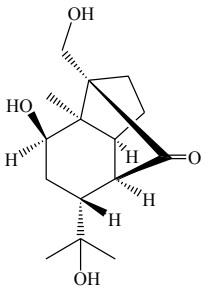
[**187**] Dendronobilin H



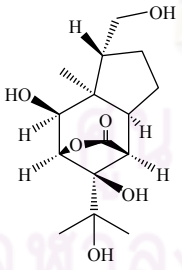
[**188**] Dendronobilin I



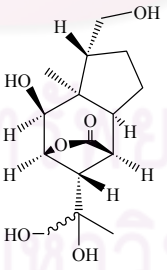
[**189**] Dendronobilin J



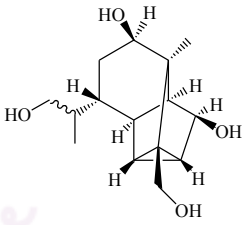
[**190**] Dendronobilin K



[**191**] Dendronobilin L

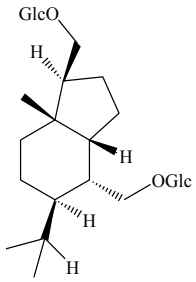


[**192**] Dendronobilin M

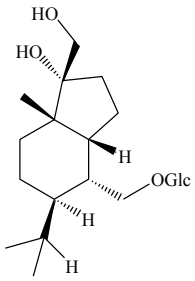


[**193**] Dendronobilin N

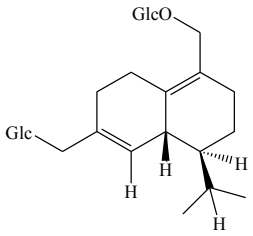
Figure 4 Structure of miscellaneous compounds isolated from *Dendrobium* spp. (continued)



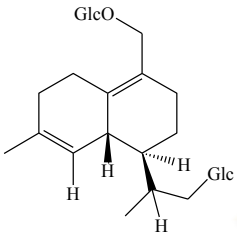
[**194**] Dendronobiloside A



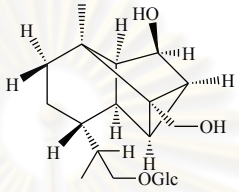
[**195**] Dendronobiloside B



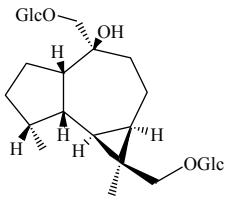
[**196**] Dendronobiloside C



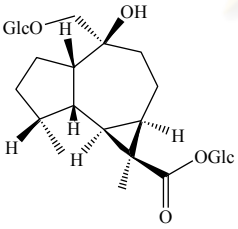
[**197**] Dendronobiloside D



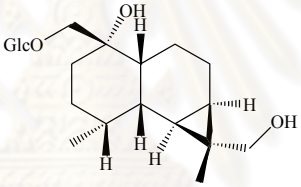
[**198**] Dendronobiloside E



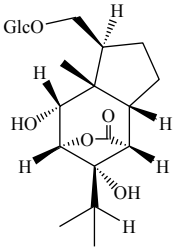
[**199**] Dendroside B



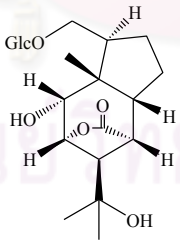
[**200**] Dendroside D



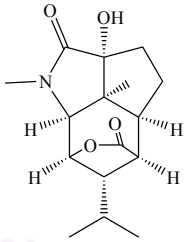
[**201**] Dendroside E



[**202**] Dendroside G

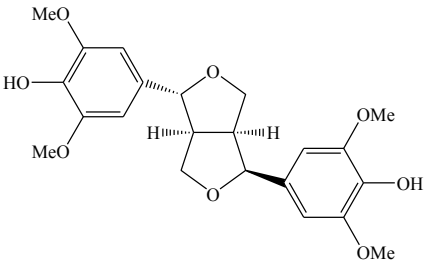


[**203**] 7,12-Dihydroxy-5-hydroxymethyl-11-isopropyl-6-methyl-9-oxatricyclo[6.2.1.$0^{2,6}$]undecan-10-one-15-*O*-β-D-glucopyranoside

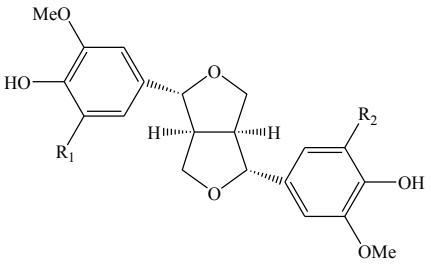


[**204**] 3-Hydroxy-2-oxodendrobine

Figure 4 Structure of miscellaneous compounds isolated from *Dendrobium* spp. (continued)



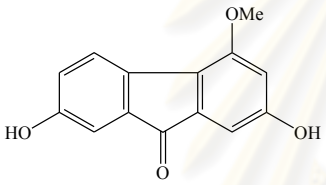
[**205**] Lirioresinol A



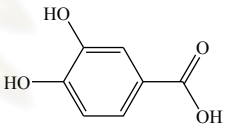
[**206**] Medioresinol $R_1 = H, R_2 = OMe$

[**208**] Pinoresinol $R_1 = R_2 = H$

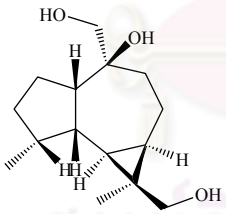
[**210**] Syringaresinol $R_1 = R_2 = OMe$



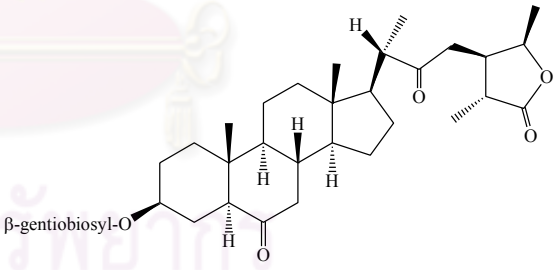
[**207**] Nobilone



[**209**] Protocatechuic acid

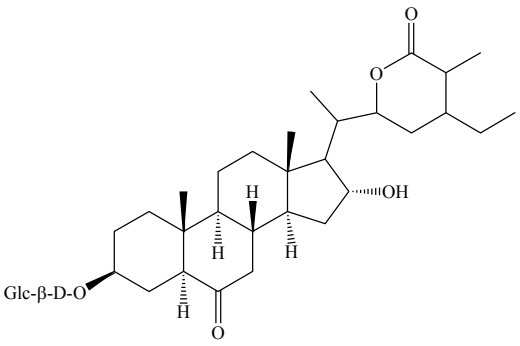


[**211**] 10*β*,12,14-Trihydroxyalloaromadendrane

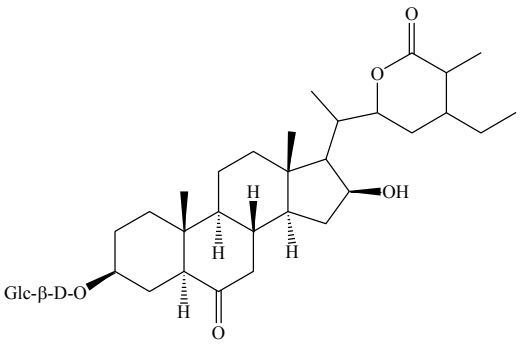


[**212**] Dendrosteroside

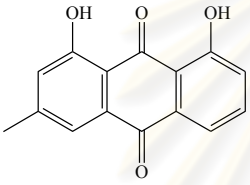
Figure 4 Structure of miscellaneous compounds isolated from *Dendrobium* spp. (continued)



[**213**] Epiochreasteroside



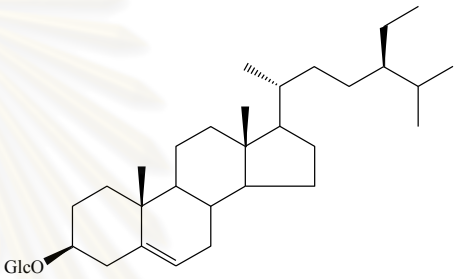
[214] Ochreasteroside



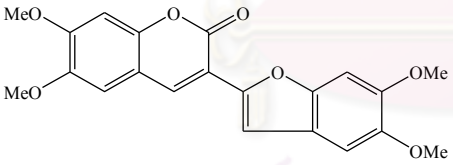
[**215**] Chrysophanol R = H

[**218**] Emodin R =OH

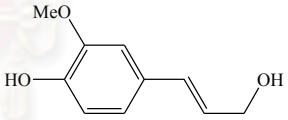
[**219**] Physcion R = OMe



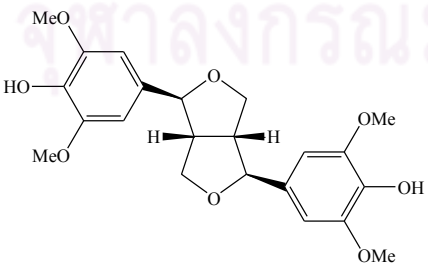
[**216**] Daucosterol



[**217**] Denthyrsin



[**220**] 3-(4-Hydroxy-3-methoxyphenyl)-2-propen-1-ol



[**221**] (-)-Syringaresinol

Figure 4 Structure of miscellaneous compounds isolated from *Dendrobium* spp. (continued)

2. Traditional uses and biological activities of *Dendrobium* spp.

The stems of several *Dendrobium* species are widely used in traditional Chinese medicine as "Shi-Hu" (Herba Dendrobii). Shi-Hu are used as a Yin tonic to nourish the stomach, promote the production of body fluid and reduce fever (Bensky and Gamble, 1993).

A series of chemical components including bibenzyls, phenanthrenes, sesquiterpenes and sesquiterpene glycosides have been previously identified from *Dendrobium* species, and some of them were found to possess antioxidant, anti-inflammatory, immunomodulatory, antiplatelet aggregation activities, cytotoxicity and antitumor activity.

Bibenzyl derivatives [**60**, **64**, **78**, **104**, **105**], a phenanthrenes [**24**], a lignan [**210**] and a fluorenone [**116**] were isolated from *D. nobile*, and exhibited significant antioxidant activity in the DPPH assay with IC_{50} values of 21.8, 14.0, 14.5, 19.9, 21.0, 12.9, 9.8 and 16.2 μM, respectively (Zhang *et al*., 2007a; Zhang *et al*., 2008c).

Phenanthrenes isolated from *D. nobile*, including erianthridin [**36**], ephemeranthol A [**34**], coelonin [**2**], ephemeranthol C [**35**] and lusianthridin [**4**] exhibited significant inhibitory effects on nitric oxide production in macrophage cells with IC_{50} values of 19.5, 12.0, 10.2, 17.6, 9.6 μM, respectively (Hwang *et al*., 2010).

Previous reports demonstrated that several compounds from this genus possess potent cytotoxic effect against many cancer cell lines. Denthyrsin [**217**], denthyrsinol [**55**], denthyrsinone [**56**] and denthyrsinin [**54**] from *D. thyrsiflorum* showed significant cytotoxicity against Hela, K-562 and MCF-7 cells (Zhang *et al*., 2005). Furthermore, four bibenzyl derivatives, moscatilin [**60**], gigantol [**62**], dengraol A [**86**] and dengraol B [**87**] from *D. gratiosissimum*, inhibited proliferation of HL-60 cells with IC_{50} values of 0.082, 10.6, 2.1, 6.4 μM, respectively (Zhang *et al*., 2008a). Three fluorenones from *D. chrysotoxum* including dendroflorin [**116**], denchrysan A [**122**] and 1,4,5-trihydroxy-7-methoxy-9-fluorenones [**124**] displayed selective cytotoxicity against human hepatoma (BEL-7402) with IC_{50} values of 0.97, 1.38 and 1.49 μg/ml, respectively (Chen *et al*., 2008c).

Moscatilin [**60**], a bibenzyl found in several *Dendrobium* species, exerted potent cytotoxic effect against lung and stomach cancer cells (Ho and Chen, 2003), induced apoptosis of human colorectal cancer cells through tubulin depolymerization

and DNA damage (Chen *et al*., 2008a), and suppressed tumor angiogenesis and growth *in vitro* and *in vivo* (Tsai *et al*., 2010).

Sesquiterpene glycosides from *D. nobile*, dendroside A [**175**] and dendrosides D-G [**200**, **201**, **177**, **202**], were reported to stimulate significantly the proliferation of mouse T and/or B lymphocytes *in vitro* (Zhao *et al*., 2001 and Ye *et al*., 2002).

Moscatilin [**60**], gigantol [**62**], homoeriodictyol [**134**] and scoparone [**135**] from *D. densiflorum* were found to exhibit antiplatelet aggregation activity on rat platelet in preliminary pharmacological tests *in vitro* (Fan *et al*., 2001).

CHAPTER III

EXPERIMENTAL

1. Source of plant materials

The fresh stems of *Dendrobium secundum* (Blume) Lindl. were purchased from Jatujak market, Bangkok, in September 2009. Authentication of the plant was performed by comparison with a herbarium specimen (BKF No. 110498) at the National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment and identified by Asso. *Thatree Phadungcharoen* (Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University). A voucher specimen (DS/BS-092552) is deposit at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

2. General techniques

2.1 Analytical thin-layer chromatography (TLC)

Technique	:	One dimension, ascending
Absorbent	:	Silica gel 60 F_{254} (E. Merck) precoated plate
Layer thickness	:	0.2 mm
Distance	:	6.5 cm
Temperature	:	Laboratory temperature (30-35ºC)
Detection	:	1. Ultraviolet light at wavelengths of 254 and 365 nm 2. Anisaldehyde and heating at 105°C for 10 min

2.2 Column chromatography

2.2.1 Vacuum liquid chromatography

Adsorbent	:	Silica gel 60 (0.063-0.200 mm) No.07734 (E. Merck)
Packing method	:	Dry packing
Sample loading	:	The sample was dissolved in a small amount of organic solvent, mixed with a small quantity of adsorbent, triturated, dried and then placed gently on top of the column.

Detection : Fractions were examined by TLC under UV light at the wavelengths of 254 and 365 nm

2.2.2 Flash column chromatography

Adsorbent : Silica gel 60 (0.040-0.063 mm) No.09385 (E. Merck)

Packing method : Wet packing

Sample loading : The sample was dissolved in a small amount eluent and then applied gently on top of the column.

Detection : Fractions were examined in the same way as described in section 2.2.1

2.2.3 Gel filtration chromatography

Adsorbent : Sephadex LH 20 (Pharmacia)

Packing method : Gel filter was suspended in the eluent and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tightly.

Sample loading : The sample was dissolved in a small amount eluent and then applied gently on top of the column.

2.3 Spectroscopy

2.3.1 Ultraviolet (UV) absorption spectra

UV (in methanol) spectra were obtained on a Shimadzu UV-160A UV/VIS spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.3.2 Infrared spectra

Infrared spectra were obtained on a Perkin-Elmer FT-IR 1760X spectrophotometer. (Scientific and Technological Research Equipment Center, Chulalongkorn University).

2.3.3 Mass spectra

Mass spectra were recorded on a Bruker microTOF mass spectrometer (National Center for Genetic Engineering and Biotechnology).

2.3.4 Proton and carbon-13 nuclear magnetic resonance (^{1}H and ^{13}C-NMR) spectra

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained with a Bruker Avance DPX-300 FT-NMR spectrometer (Faculty of Pharmaceutical Sciences, Chulalongkorn University).

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were obtained with a JEOL JMN-A 500 NMR spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University).

Solvents for NMR spectra were deuterated chloroform (chloroform-*d*) and deuterated acetone (acetone-d_6). Chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal.

2.4 Physical property

2.4.1 Optical rotation

Optical rotations were measured on a Perkin Elmer Polarimeter 341 (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.5 Solvents

All organic solvents employed throughout this work were of commercial grade and were redistilled prior to use.

3. Extraction and isolation

3.1 Extraction

The dried stems (1.6 kg) were chopped, ground and then macerated with MeOH (3x10 L) to give, after removal of the solvent, a MeOH extract (206 g).

3.2 Separation of methanol extract

The MeOH extract (206 g) was separated by vacuum liquid chromatography (VLC) using a sintered glass filter column of silica gel (No.07734, 650 g). The methanol extract was dissolved in a small amount of CH_2Cl_2, triturated with silica gel (No.07734) and dried under vacuum. Elution was performed in a polarity gradient manner with *n*-hexane-EtOAc (100:0 to 0:100) and CH_2Cl_2-MeOH (95:5 to 0:100).

The eluates were collected 500 ml per fraction and examined by TLC (silica gel, *n*-hexane-EtOAc 7:3) to yield forty four fractions. Fractions with similar chromatographic patterns were combined to yield eight fractions: A (176.3 mg), B (5.63 g), C (3.56 g), D (4.52 g), E (1.63 g), F (2.29 g), G (16.82 g), H (73.70 g).

3.2.1 Isolation of compound DS1 (brittonin A)

Fraction F (2.29 g) was separated by flash column chromatography (FCC) on a silica gel (No. 09385) column. Elution was performed in a polarity gradient manner with mixtures of *n*-hexane and EtOAc (8:2). Fractions showing similar chromatographic patterns were combined (TLC, silica gel, *n*-hexane-EtOAc 1:1) to yield eleven fractions: F1 (21.6 mg), F2 (243.5 mg), F3 (229.9 mg), F4 (207.8 mg), F5 (380.9 mg), F6 (117.7 mg), F7 (237.9 mg), F8 (298.2 mg), F9 (41.1 mg), F10 (145.6 mg), F11 (39.2 mg).

Fraction F3 (229.9 mg) was further separated on a Sephadex LH20 column (CH_2Cl_2-MeOH, 1:1) to give five fractions: F3A (25.7 mg), F3B (183.7 mg), F3C (4.3 mg), F3D (2.3 mg), F3E (5.6 mg). Separation of fraction F3B (183.7 mg) was performed on a silica gel, CH_2Cl_2-MeOH, 99.5:0.5) to afford compound DS1 as white needles (110.0 mg, R_f 0.5, silica gel, CH_2Cl_2-MeOH, 99:1). It was identified as brittonin A.

3.2.2 Isolation of compound DS2 (moscatilin)

Fraction G (16.82 g) was separated by VLC over silica gel, eluted with CH_2Cl_2-MeOH gradient (99:1 to 0:100) to give six fractions: G1 (535.5 mg), G2 (522.6 mg), G3 (2.74 g), G4 (1.31 g), G5 (1.32 g), G6 (7.55 g).

Fraction G3 (2.74 g) was separated by FCC over silica gel, (*n*-hexane-EtOAc, 7:3) to give seven fractions: G3A (21.7 mg), G3B (609.8 mg), G3C (785.3 mg), G3D (167.3 mg), G3E (121.7 mg), G3F (437.0 mg), G3G (37.1 mg). Fraction G3D (167.3 mg) was further separated on Sephadex LH 20 (MeOH) to give seven fractions: G3D1 (37.1 mg), G3D2 (20.4 mg), G3D3 (87.9 mg), G3D4 (3.0 mg), G3D5 (2.0 mg), G3D6 (2.8 mg), G3D7 (2.7 mg). Fraction G3D3 (87.9 mg) was subjected to repeated FCC over silica gel, eluted with CH_2Cl_2 to yield compound DS2 as a yellow amorphous solid (37.0 mg, R_f 0.3, silica gel, CH_2Cl_2-MeOH, 98:2). It was identified as moscatilin.

3.2.3 Isolation of compound DS3 (4,5,4′-trihydroxy-3,3′-dimethoxy bibenzyl)

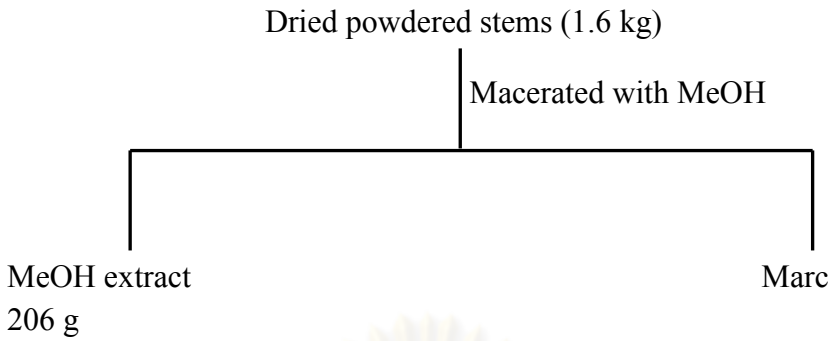
Fraction G4 (1.31 g) was separated by FCC (silica gel; CH_2Cl_2-acetone, 97:3) to give eleven fractions: G4A (9.6 mg), G4B (7.2 mg), G4C (4.9 mg), G4D (33.0 mg), G4E (57.7 mg), G4F (177.7 mg), G4G (587.0 mg), G4H (154.9 mg), G4I (89.4 mg), G4J (139.1 mg), G4K (62.4 mg). Fraction G4E (57.7 mg) was subjected to repeated FCC over silica gel, eluted with *n*-hexane-EtOAc (4:1) to afford compound DS3 as a yellow amorphous solid (5.1 mg, R_f 0.29, silica gel, *n*-hexane-EtOAc, 1:1). It was characterized as a new compound named 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl.

3.2.4 Isolation of compound DS4 (syringaresinol)

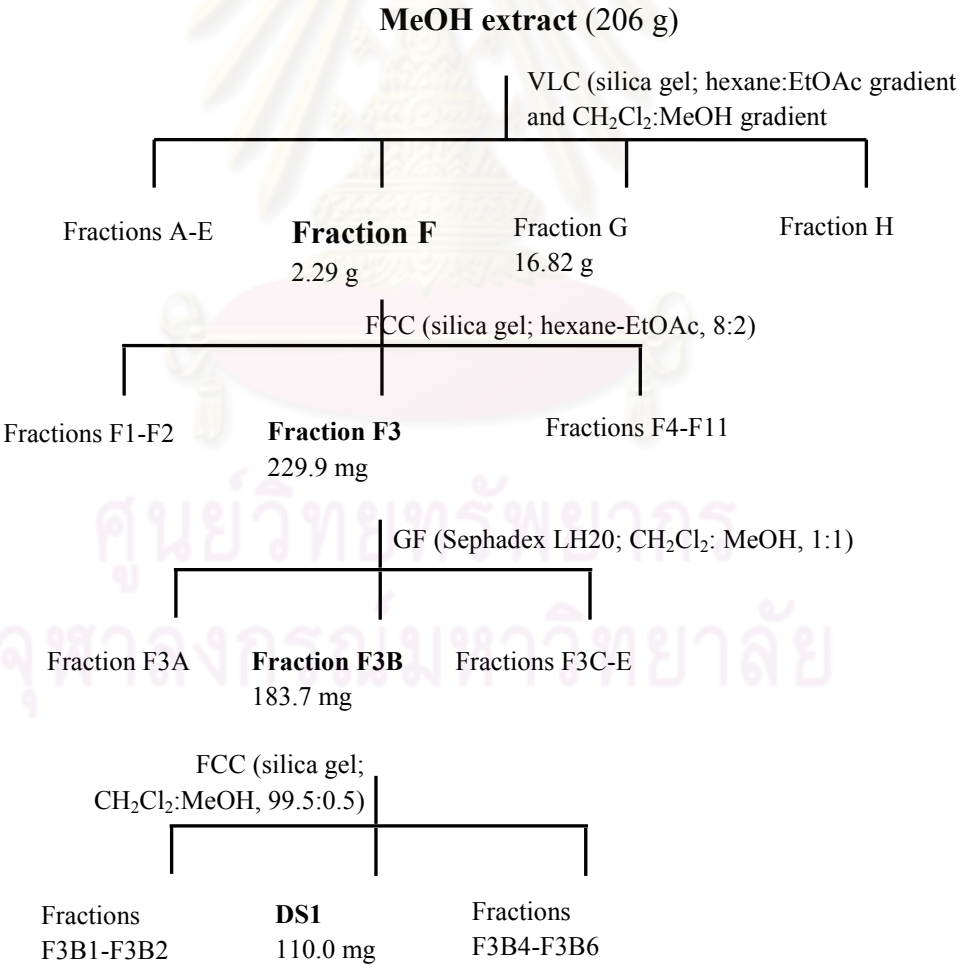
Fraction G4G (587.0 mg) was subjected to Sephadex LH 20 (MeOH) gel filtration to give seven fractions: G4G1 (76.3 mg), G4G2 (232.9 mg), G4G3 (101.6 mg), G4G4 (144.1 mg), G4G5 (20.4 mg), G4G6 (14.1 mg), G4G7 (2.0 mg). Fraction G4G3 (101.6 mg) was subjected to FCC (silica gel; *n*-hexane-EtOAc, 7:3) to give six fractions: G4G3A (4.1 mg), G4G3B (6.1 mg), G4G3C (12.7 mg), G4G3D (47.3 mg), G4G3E (11.2 mg), G4G3F (11.7 mg). Fraction G4G3D (47.3 mg) was subjected to repeated FCC over silica gel, eluted with CH_2Cl_2-MeOH (99.6:0.4) to afford compound DS4 as white needles (22.2 mg, R_f 0.4, silica gel, CH_2Cl_2-MeOH, 95:5). It was identified as syringaresinol.

3.2.5 Isolation of compound DS5 (ferulic acid)

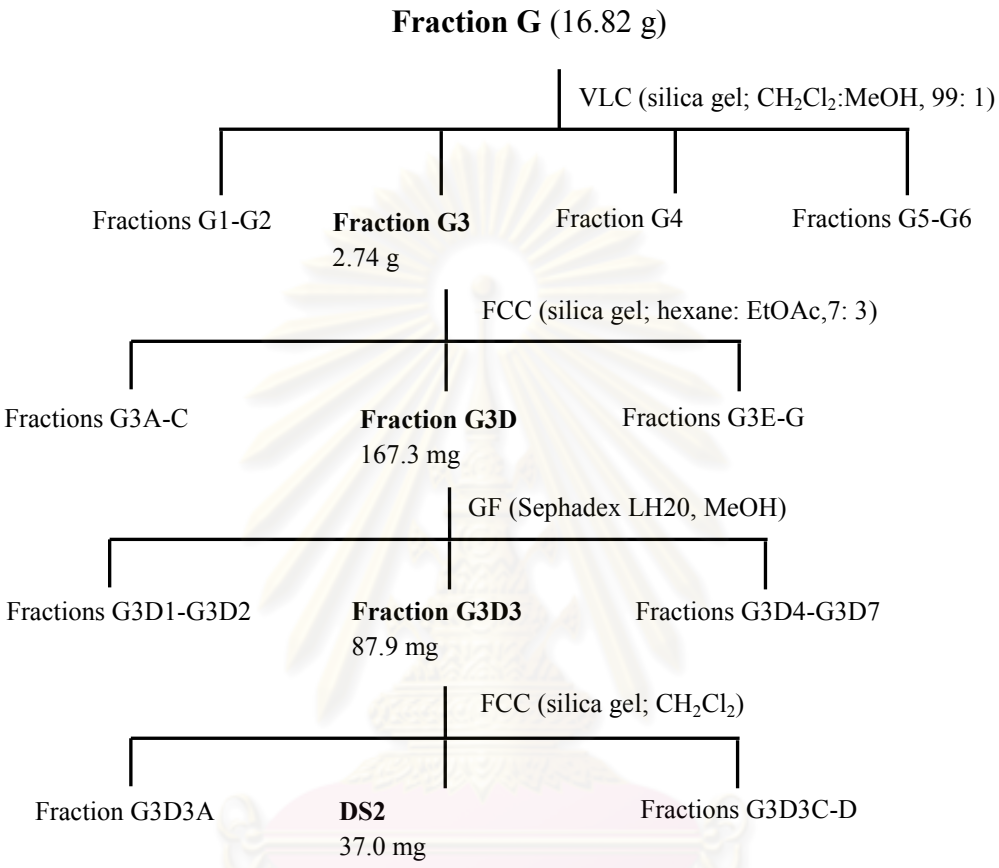
Fraction G4I (89.4 mg) was separated on Sephadex LH 20 (MeOH) to give five fractions: G4I1 (18.5 mg), G4I2 (30.1 mg), G4I3 (10.8 mg), G4I4 (20.7 mg), G4I5 (3.8 mg). Fraction G4I4 (20.7 mg) was subjected to repeated FCC over silica gel, eluted with CH_2Cl_2-MeOH (98.5:1.5) to afford compound DS5 as a white amorphous solid (7.4 mg, R_f 0.36, silica gel, CH_2Cl_2-MeOH, 9:1). It was identified as ferulic acid.



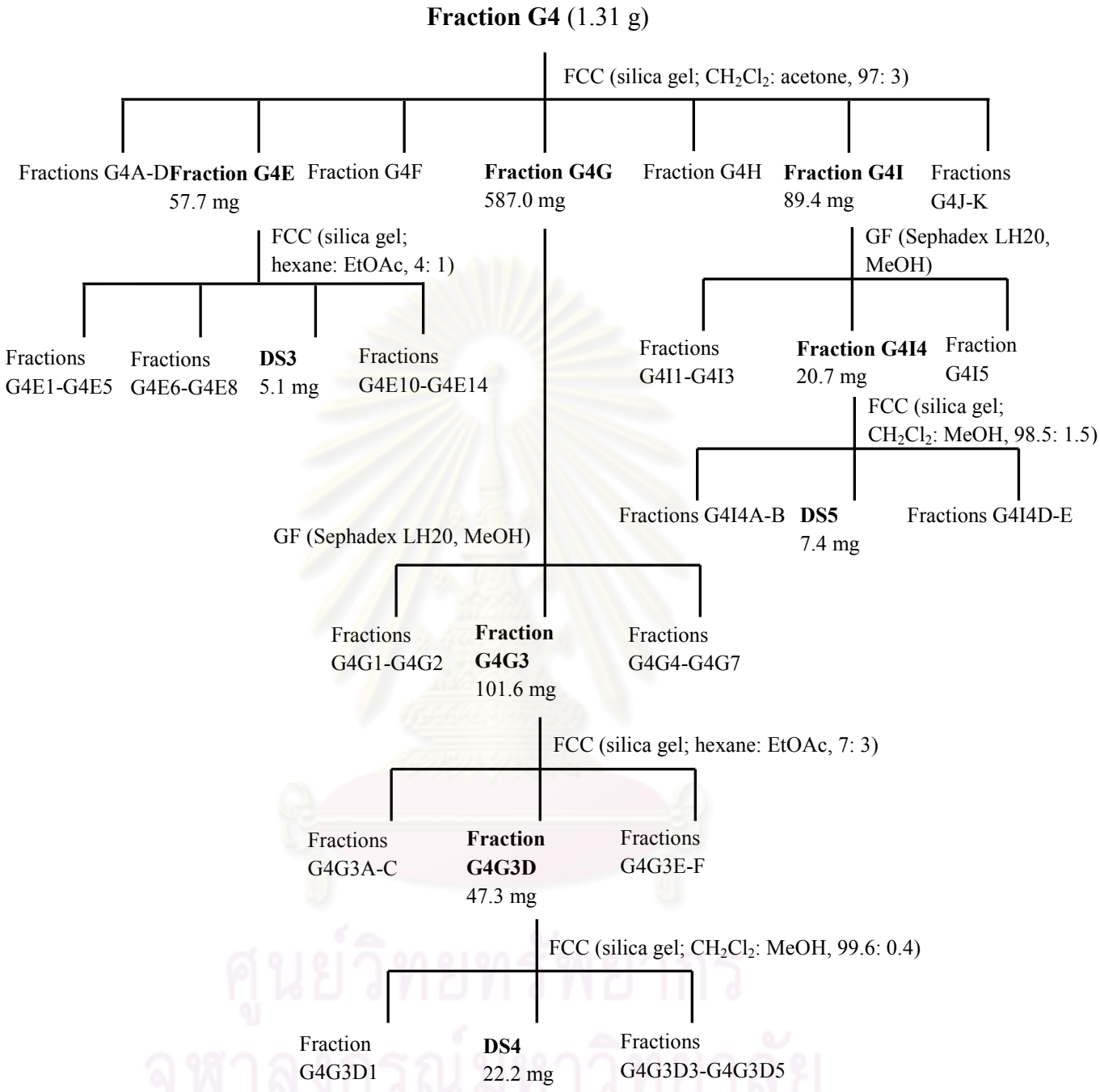
Scheme 1 Extraction of *Dendrobium secundum*



Scheme 2 Separation of fraction F of the MeOH extract



Scheme 3 Separation of fraction G of the MeOH extract



Scheme 4 Separation of fraction G of the MeOH extract (continued)

4. Physical and spectral data of isolated compounds

4.1 Compound DS1 (brittonin A)

Compound DS1 was obtained as white needles, soluble in CH_2Cl_2 (110.0 mg, 68.75 x 10^{-4} % based on dried weight of stems).

ESI-MS : $[M+H]^+$ ion at *m/z* 363.18; Figure 5

FT-IR : 1592, 1506, 1469, 1423, 1127 cm^{-1}; Figure 6

UV : λ_{max} nm (log ε), in methanol; Figure 7
219 (4.23), 269 (2.94)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; see Table 4, Figure 8

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; see Figure 9

4.2 Compound DS2 (moscatilin)

Compound DS2 was obtained as a yellow amorphous solid, soluble in CH_2Cl_2 (37.0 mg, 23.12 x 10^{-4} % based on dried weight of stems)

ESI-MS : $[M+H]^+$ ion at *m/z* 305.14; Figure 10

FT-IR : 3446, 2938, 1614, 1515, 1463, 1456, 1214 cm^{-1}; Figure 11

UV : λ_{max} nm (log ε), in methanol; Figure 12
219 (4.2), 281 (3.6)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; see Table 5, Figure 13

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; see Table 5, Figure 14

4.3 Compound DS3 (4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl)

Compound DS3 was obtained as a yellow amorphous solid, soluble in CH_2Cl_2 (5.1 mg, 3.19 x 10^{-4} % based on dried weight of stems).

HR-ESI-MS : $[M+Na]^+$ ion at *m/z* 313.1049; Figure 17

IR : 3419, 2934, 1721, 1609, 1515, 1463, 1455, 1202 cm^{-1}; Figure 18

UV : λ_{max} nm (log ε), in methanol; Figure 19
219 (4.1), 281 (3.4)

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; see Table 6, Figure 20

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; see Table 6, Figure 21

4.4 Compound DS4 (syringaresinol)

Compound DS4 was obtained as white needles, soluble in CH_2Cl_2 (22.2 mg, 13.88 x 10^{-4}% based on dried weight of stems).

ESI-MS : $[M+Na]^+$ ion at *m/z* 441.15; Figure 25

FT-IR : 3418, 1614, 1519, 1456 cm^{-1}; Figure 26

UV : λ_{max} nm (log ε), in methanol; Figure 27
218 (4.26), 272 (3.33)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; see Table 7, Figure 28

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; see Table 7, Figure 29

$[\alpha]^{20}_D$: -73.4 ° (*c* 0.000346; MeOH)

4.5 Compound DS5 (ferulic acid)

Compound DS5 was obtained as a white solid, soluble in acetone (7.4 mg, 4.62 x 10^{-4} % based on dried weight of stems).

ESI-MS : $[M+Na]^+$ ion at *m/z* 217.05; Figure 32

FT-IR : 3436, 1683, 1622, 1600, 1515, 1433 cm^{-1}; Figure 33

UV : λ_{max} nm (log ε), in methanol; Figure 34
220 (3.91), 233 (3.83), 321 (3.96)

^{1}H NMR : δ ppm, 300 MHz, in acetone-d_6; see Table 8, Figure 35

^{13}C NMR : δ ppm, 75 MHz, in acetone-d_6; see Table 8, Figure 36

5. Determination of free radical scavenging activity

5.1 DPPH radical scavenging activity assay

5.1.1 Preparation of test sample

The assay was performed according to an established protocol (Braca *et al*., 2002). The test compound (1.0 mg) was dissolved in 1 ml methanol (or suitable solvent) and diluted with methanol until a suitable range of concentration (mg/ml) was obtained. The concentration was expressed as μM in final concentration. For example, DS1 (MW 362) at 1.0 mg/1ml was equal to 2762 μM (1.0 mg/1ml / 362). For each well, 20 μl of test solution was added to the reaction mixture to furnish the total volume of 200 μl. The final concentration was calculated by the formula below.

$$N_1V_1 = N_2V_2$$

N_1 = Beginning concentration (μM)

V_1 = Beginning volume (μl)

N_2 = Final concentration (μM)

V_2 = Final volume (μl)

Thus, the final concentration of DS1 solution = 2762 μM × 20 μl / 200 μl

= 276.2 μM

5.1.2 Preparation of DPPH solution (100 μM)

DPPH (2 mg) was dissolved in 100 ml of methanol, and the solution was stirred for 30 min.

5.1.3 Measurement of activity

The test sample (20 μl) was added to 180 μl DPPH solution (100 μM) in 96-well plate. The solution mixture was incubated at 37ºC for 30 min and then the absorbance of each well was measured at 510 nm. The DPPH solution (180 μl) mixed with methanol (20 μl) was used as negative control. Quercetin and Trolox® were used as reference compounds.

5.1.4 Calculation of percent inhibition of DPPH radical scavenging activity

The percentage of DPPH reduction was calculated as follows.

$$\% \text{ DPPH reduction} = (A-B) \times 100 / A$$

A = The absorbance of DPPH solution after incubation at 510 nm

B = The absorbance of the reaction mixture after incubation at 510 nm

For IC_{50} evaluation of pure compounds, a graph showing concentration versus % DPPH reduction was plotted. The IC_{50} was calculated from the graph.

5.2 Superoxide radical scavenging activity assay

5.2.1 Preparation of test sample

The assay was conducted according to a reparted method (Dasgupta and De, 2004). The test compound (0.25 mg) was dissolved in 1 ml of 30% methanol in 50 mM sodium phosphate buffer, pH 7.8. The concentration was expressed as μg/ml in final concentration. For each well, 80 μl of test solution (250 μg/ml) was added to the reaction mixture to furnish the total volume of 200 μl. The final concentration was calculated by the formula below.

$$N_1V_1 = N_2V_2$$

N_1 = Beginning concentration (μg/ml)

V_1 = Beginning volume (μl)

N_2 = Final concentration (μg/ml)

V_2 = Final volume (μl)

Thus, the final concentration of test solution = 250 μg/ml × 80 μl / 200 μl

= 100 μg/ml

5.2.2 Preparation of nitroblue tetrazolium (NBT) solution (750 μM)

NBT (6.1 mg) was dissolved in 10 ml of 50 mM sodium phosphate buffer, pH 7.8.

5.2.3 Preparation of methionine solution (130 mM)

Methionine (0.194 g) was dissolved in 10 ml of 50 mM sodium phosphate buffer, pH 7.8.

5.2.4 Preparation of riboflavin solution (20 μM)

Riboflavin (0.753 mg) was dissolved in 10 ml of 50 mM sodium phosphate buffer, pH 7.8.

5.2.5 Preparation of EDTA solution (1 mM)

EDTA (3.722 mg) was dissolved in 10 ml of 50 mM sodium phosphate buffer, pH 7.8.

5.2.6 Measurement of activity

The test sample (80 µl) was added to a mixture containing 50 mM sodium phosphate buffer, pH 7.8 (40 µl), 130 mM methionine (20µl), 20 µM riboflavin (20µl), 1 mM EDTA (20µl), and 750 µM NBT (20µl) in a 96-well plate. The production of blue formazan was followed by monitoring the increase in absorbance at 570 nm after a 10-min illumination from a fluorescent lamp. The entire reaction assembly was enclosed in a box lined with aluminium foil. The reaction mixtures that were kept in the dark served as blank. The 30 % methanol solution in 50 mM sodium phosphate buffer, pH 7.8 (80 µl) mixed with reaction mixture was used as negative control. Trolox® was used as a reference compound.

5.2.7 Calculation of percent inhibition of superoxide radical scavenging activity

The percentage of inhibition was calculated as follows.

$$\% \text{ Inhibition} = (A-B) \times 100 / A$$

A = The absorbance of the negative control at 570 nm

B = The absorbance of the reaction mixture at 570 nm

CHAPTER IV

RESULTS AND DISCUSSION

The dried powdered *Dendrobium secundum* stems (1.6 kg) were macerated with MeOH. The MeOH extract (206 g) was separated using several chromatographic techniques to afford five pure compounds (DS1-DS5). The structure determinations of all of these isolates were carried out by interpretation of their UV, IR, MS and NMR data, and further comfirmed by comparison with literature values.

1. Structure determination of isolated compounds

1.1 Structure determination of compound DS1

Compound DS1 was obtained as white needles. The ESI-MS (Figure 5) showed an $[M+H]^+$ ion at *m/z* 363.18, suggesting the molecular formula $C_{20}H_{26}O_6$.

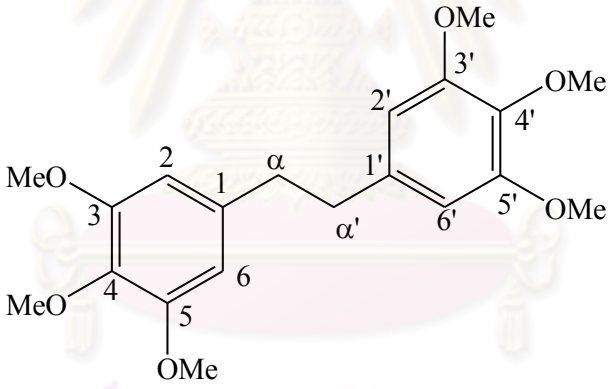
The IR spectrum (Figure 6) showed absorption bands at 1592, 1506, 1469, 1423 cm^{-1} for aromatic groups. The UV absorptions (Figure 7) at 219 and 269 nm were indicative of a bibenzyl derivative (Zhang *et al*., 2007a).

The ^1H NMR spectrum (Figure 8 and Table 4) of compound DS1 showed a singlet (18H) at δ 3.84 representing six aromatic methoxyl groups, a broad singlet at δ 2.86 (4H, H_2-α and H_2-α′) for four methylene protons of a bibenzyl derivative. The spectrum also revealed signals for four aromatic protons, appearing as a four-proton broad singlet at δ 6.38 (H-2, H-6, H-2′ and H-6′).

The ^{13}C NMR spectrum (Figures 9) exhibited twenty carbon signals and showed a signal for two methylene carbons at δ 38.4 (C-α and C-α′). Through comparison of its ^1H NMR, MS, IR and UV data with reported values (Asakawa, Tanikawa, and Aratani, 1976), DS1 was identified as brittonin A.

Table 4 NMR Spectral data of compound DS1 ($CDCl_3$) and brittonin A ($CDCl_3$)

Position	Compound DS1		Brittonin A
	δ_H	δ_C	δ_H
1, 1′	-	137.3	-
2,6,2′,6′	6.38 brs	105.6	6.33 s
3,5,3′,5′	-	153.0	-
4,4′	-	137.3	-
α, α′	2.86 s	38.4	2.80 s
4,4′-OMe	3.84 s	60.8	3.83 s
3,5,3′,5′-OMe	3.84 s	56.1	3.83 s



brittonin A [**222**]

1.2 Structure determination of compound DS2

Compound DS2 was obtained as a yellow amorphous solid. The ESI-MS (Figure 10) showed an $[M+H]^+$ ion at *m/z* 305.14, suggesting the molecular formula $C_{17}H_{20}O_5$.

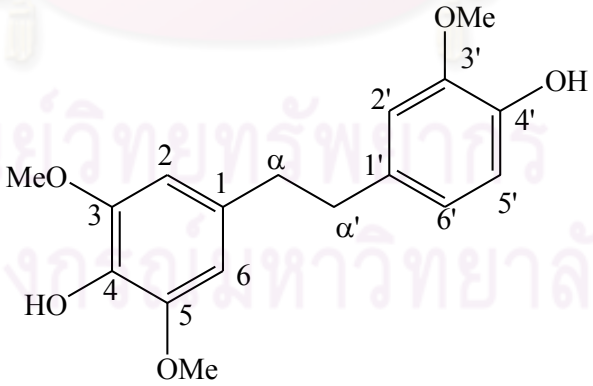
The IR spectrum (Figure 11) showed an absorption band at 3446 cm^{-1} for hydroxyl groups and bands at 1614, 1515, 1463, 1456 cm^{-1} for aromatic groups. The UV absorptions (Figure 12) at 219 and 281 nm were similar to those of DS1. The ^1H NMR spectrum (Figure 13 and Table 5) of compound DS2 showed a singlet (9H) at δ 3.86 for three aromatic methoxyl groups, a singlet (4H) at δ 2.84 (H_2-α and H_2-α′) for four benzylic methylene protons of a bibenzyl derivative. The spectrum also revealed signals for five aromatic protons, two of which appeared as a two-protons broad singlet at δ 6.38 (H-2 and H-6). The remaining three protons resonated at δ 6.63 (1H, brs, H-2′), δ 6.86 (1H, d, *J* = 8.1 Hz, H-5′), and δ 6.70 (1H, brd, *J* = 8.1Hz, H-6′)

The ^{13}C NMR, DEPT 90, and DEPT 135 spectra (Figures 14-15 and Table 5) exhibited seventeen carbon signals, corresponding to three methyls, two methylenes, five methines and seven quaternary carbons. The spectrum showed the two methylene carbon signals appeared at δ 37.8 (C-α) and δ 38.3 (C-α′). The NOESY (figure 16) interaction between H-2′ and the methoxyl protons at δ 3.86 suggested the location of this methoxyl group at C-3′.

Through comparison of its ^1H and ^{13}C NMR, MS, IR and UV data with reported values (Majumder and Sen, 1987), DS2 was identified as moscatilin [**60**].

Table 5 NMR Spectral data of compound DS2 ($CDCl_3$) and moscatilin ($CDCl_3$)

Position	Compound DS2		Moscatilin	
	δ_H	δ_C	δ_H	δ_C
1	-	132.9	-	132.8
2,6	6.38 brs	105.3	6.30 s	105.2
3,5	-	146.8	-	146.8
4	-	133.6	-	133.5
1′	-	132.8	-	132.8
2′	6.63 brs	111.3	6.60 d (*J* = 2.0 Hz)	111.2
3′	-	146.2	-	146.1
4′	-	143.5	-	143.7
5′	6.86 d (*J* = 8.1 Hz)	114.1	6.77 d (*J* = 8.0 Hz)	114.1
6′	6.70 brd (*J* = 8.1Hz)	121.0	6.74 dd (*J* = 8.0, 2.0 Hz)	121.0
α	2.84 s	37.8	2.79	37.8
α′	2.84 s	38.3	2.79	38.3
3,5-OMe	3.86 s	55.8	3.81 s	56.2
3′-OMe	3.86 s	55.2	3.81 s	55.8



moscatilin [**60**]

1.3 Structure determination of compound DS3

Compound DS3 was obtained as a yellow amorphous solid. The positive HR-ESI-MS (Figure 17) exhibited an $[M+Na]^+$ ion at *m/z* 313.1049 (calcd. for 313.1052; $C_{16}H_{18}NaO_5$), suggesting the molecular formula $C_{16}H_{18}O_5$.

The IR spectrum (Figure 18) showed absorption bands for hydroxyl (3419 cm^{-1}) and aromatic (1609, 1463 cm^{-1}) groups. The UV absorptions (Figure 19) at 219 and 281 nm were similar to those of DS2.

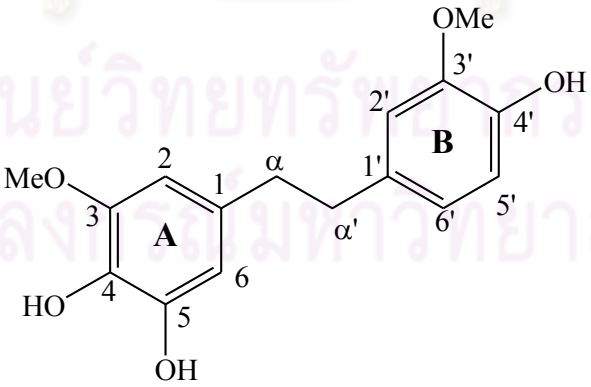
The ^1H NMR spectrum (Figure 20 and Table 6) of compound DS3 exhibited a pair of two-proton multiplets at δ 2.75 (H_2-α) and δ 2.78 (H_2-α′). The ^1H-^1H COSY spectrum of compound DS4 showed an ABM coupling system representing three aromatic protons at δ 6.60 (1H, d, *J* = 2.0 Hz, H-2′), δ 6.65 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′) and δ 6.80 (1H, d, *J* = 8.0 Hz, H-5′). The ^{13}C NMR spectrum (Figure 21 and Table 6) displayed sixteen carbon signals, corresponding to two methoxyls, two methylenes, five methines and seven quaternary carbons. The methylene protons were correlated to two methylene carbon signals at δ 38.2 (C-α) and δ 37.7 (C-α′) in the HSQC spectrum (Figure 22). The NOESY spectrum (Figure 23a, 23b) exhibited a cross-peak between H-2′ and the methoxyl protons at δ 3.83. These ^1H NMR spectral properties indicated that compound DS3 had a B-ring substitution pattern similar to that of compound DS2 (moscatilin). The fact that the molecular weight of compound DS3 was 14 amu lower than that of compound DS2 suggested that ring A of compound DS3 had only one methoxyl group. This methoxyl group should be located at C-3 of ring A, as supported by the NOESY interaction between H-2 (δ 6.21) and MeO-3 protons (δ 3.80). This was confirmed by the HMBC correlations (Figure 24) from C-α to H-2 and H-6, and from C-α′ to H-2′ and H-6′.

Based on above spectral evidence, compound DS3 was characterized as 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl. This structure was unknown prior to this study.

Table 6 NMR Spectral data of compound DS3 ($CDCl_3$)

Position	δ_H	δ_C	HMBC (correlation with ^1H)
1	-	130.4	2*, 6*, α′
2	6.21 d (*J* = 2.0 Hz)	103.5	6, α
3	-	146.6	2*, 3-OMe
4	-	133.7	2
5	-	143.7	6*
6	6.42 d (*J* = 2.0 Hz)	108.6	2, α
1′	-	133.8	2′*, 5′, α, α′*
2′	6.60 d (*J* = 2.0 Hz)	111.2	6′, α′
3′	-	146.2	2′*, 5′, 3′-OMe
4′	-	143.7	2′, 5′*, 6′
5′	6.80 d (*J* = 8.0 Hz)	114.1	6′*
6′	6.65 dd (*J* = 8.0, 2.0 Hz)	121.0	2′, 5′*, α′
α	2.75 m	38.2	2, 6, α′*
α′	2.78 m	37.7	2′, 6′, α*
3-OMe	3.80 s	56.1	-
3′-OMe	3.83 s	55.9	-

* Two-bond coupling



4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl [**223**]

1.4 Structure determination of compound DS4

Compound DS4 was obtained as white needles, $[\alpha]^{20}_{D}$ -73.4 (*c* 0.000346, MeOH). The ESI-MS (Figure 25) showed an $[M+Na]^+$ ion at *m/z* 441.15, suggesting the molecular formula $C_{22}H_{26}O_8$.

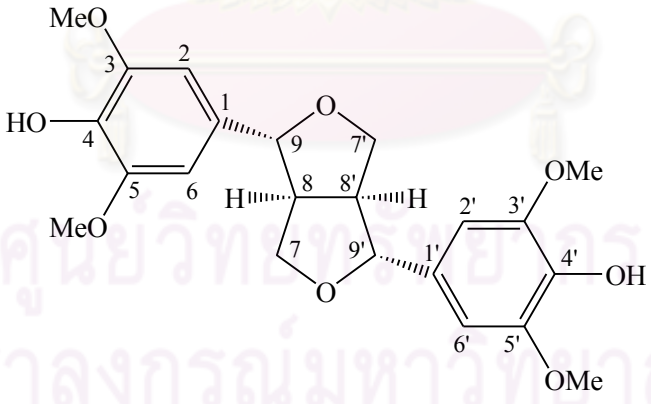
The IR spectrum (Figure 26) showed absorption bands for hydroxyl groups (3418 cm^{-1}) and aromatic groups (1614, 1519, 1456 cm^{-1}). The UV spectrum (Figure 27) showed absorptions at 218 and 272 nm.

The ^1H NMR spectrum (Figure 28 and Table 7) of compound DS4 showed a singlet (12H) at δ 3.91 for four aromatic methoxyl groups. The spectrum also revealed signals for four aromatic protons, appearing as a broad singlet (4H) at δ 6.60 (H-2, H-6, H-2′and H-6′).

The ^{13}C NMR, DEPT 90, DEPT 135 and HMQC spectra (Figures 29-31 and Table 7) exhibited 22 carbon signals, corresponding to four methyls, two methylenes, eight methines and eight quaternary carbons. Through comparison of its ^1H and ^{13}C NMR, MS, IR and UV data with reported values (Zhang *et al*., 2008c), DS4 was identified as syringaresinol [**210**].

Table 7 NMR Spectral data of compound DS4 ($CDCl_3$) and syringaresinol ($CDCl_3$)

Position	Compound DS4		Syringaresinol	
	δ_H	δ_C	δ_H	δ_C
1,1′	-	132.0	-	132.1
2,2′	6.60 brs	102.7	6.58 s	102.7
3,3′	-	147.1	-	147.2
4,4′	-	134.3	-	134.3
5,5′	-	147.1	-	147.2
6,6′	6.60 brs	102.7	6.58 s	102.7
7,7′	4.74 d (*J* = 3.3 Hz)	86.0	4.73 brd (*J* = 4.3 Hz)	86.1
8,8′	3.11 m	54.2	3.09 m	54.3
9,9′	4.30 m	71.7	4.28 m	71.8
3,5-OMe	3.91 s	56.3	3.90 s	56.4
3′,5′-OMe	3.91 s	56.3	3.90 s	56.4
4,4′-OH	5.57 brs	-	5.50	-



syringaresinol [**210**]

1.5 Structure determination of compound DS5

Compound DS5 was obtained as a white solid. The ESI-MS (Figure 32) showed an $[M+Na]^+$ ion at *m/z* 217.05, suggesting the molecular formula $C_{10}H_{10}O_4$.

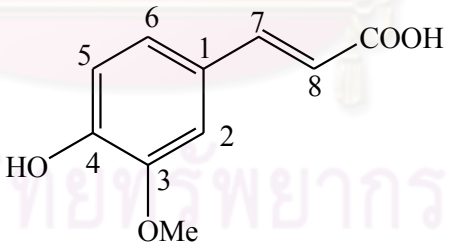
The IR spectrum (Figure 33) showed absorption bands at 3436 cm^{-1} for phenolic hydroxyl group, at 1683 cm^{-1} for conjugated double bond, and at 1600, 1515, 1433 cm^{-1} for aromatic groups. The UV spectrum (Figure 34) showed absorptions at 220, 233 and 321 nm.

The ^1H NMR spectrum (Figure 35 and Table 8) of compound DS5 showed a three-proton singlet at δ 3.91 for one aromatic methoxyl group. The spectrum showed the presence a pair of downfield doublets at δ 7.58 (1H, d, *J* = 15.9 Hz, H-7) and δ 6.36 (1H, d, *J* = 15.9 Hz, H-8), suggesting the presence of a double bond with a *trans*-configuration. The aromatic protons were observed at δ 7.32 (1H, brs, H-2), δ 7.13 (1H, brd, *J* = 7.2 Hz, H-6) and δ 6.86 (1H, d, *J* = 8.1 Hz, H-5).

The ^{13}C NMR spectrum (Figures 36 and Table 8) exhibited ten carbon signals, corresponding to one methyl, five methines and four quaternary carbons. Through comparison of its ^1H and ^{13}C NMR, MS, IR and UV data with reported values (Dobhal *et al*., 1999), DS5 was identified as ferulic acid.

Table 8 NMR Spectral data of compound DS5 (acetone-d_6) and ferulic acid ($CDCl_3$)

Position	Compound DS5		Ferulic acid	
	δ_H	δ_C	δ_H	δ_C
1	-	127.3	-	127.1
2	7.32 brs	111.1	7.01 d	109.4
3	-	148.5	-	146.8
4	-	149.7	-	147.9
5	6.86 d (J = 8.1 Hz)	115.8	6.90 d (J = 8.0 Hz)	115.8
6	7.13 brd (J = 7.2 Hz)	123.5	7.05 dd (J = 8.0, 2.0 Hz)	122.9
7	7.58 d (J = 15.9 Hz)	145.6	7.59 d (J = 16.0 Hz)	144.4
8	6.36 d (J = 15.9 Hz)	115.8	6.28 d (J = 16.0 Hz)	115.8
C=O	-	167.8	-	167.7
3-OMe	3.91 s	56.1	3.90 s	55.9



ferulic acid [**224**]

2. Free radical scavenging activity

The MeOH extract of *D. secundum* was found to possess anti-oxidative potential, showing 75% DPPH reduction at 100 μg/ml. The free radical scavenging effect of the samples was assessed by two *in vitro* models, DPPH radical scavenging activity assay as previously described (Braca *et al*., 2002) and superoxide radical scavenging activity assay (Dasgupta and De, 2004). DPPH test is based on the ability to decolor a methanolic solution of 1,1,-diphenyl-2-picrylhydrazyl radical (DPPH, Sigma). Superoxide radical scavenging activity assay is based on the capacity of the samples to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) in the riboflavin-light-NBT system. Each compound was first tested at the concentration of 100 μg/ml. An IC_{50} value was determined if the compound showed more than 50% inhibition. Quercetin (Sigma) and Trolox® were used as positive controls. The results are summarized in Table 9.

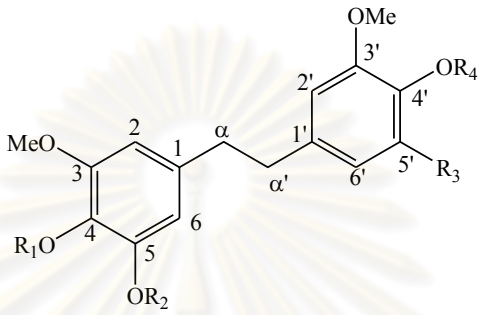
Table 9 Free radical scavenging activity of compounds isolated from *D. secundum*

Compounds	**% DPPH reduction at 100 μg/ml**	**IC_{50} (μM) Mean ± SD**	**% NBT reduction at 100 μg/ml**
MeOH extract	75	nd	nd
DS1 [**222**]	12.53	nd	4.08
DS2 [**60**]	89.71	5.14 ± 0.18	34.28
DS3 [**223**]	78.81	15.87 ±1.48	nd
DS4 [**210**]	92.09	11.38 ± 0.24	67.42
DS5 [**224**]	93.82	37.52 ± 0.47	nd
Quercetin	94.24	2.47 ± 0.08	nd
Trolox®	97.77	11.68 ± 0.44	82.94

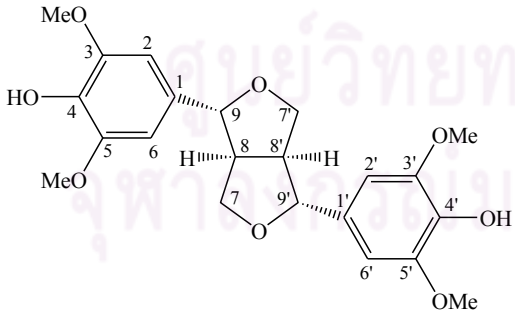
nd = not determined

From Table 9, five pure compounds were evaluated for DPPH radical scavenging activity and superoxide radical scavenging activity. Compounds DS2-DS5 exhibited recognizable DPPH radical scavenging activity with IC_{50} values 5.14, 15.87, 11.38, 37.52 μM, respectively, as compared with quercetin and Trolox® (IC_{50} values 2.47 and 11.68 μM, respectively). Compounds DS2 and DS4 also exhibited superoxide radical scavenging activity at 100 μg/ml. Compound DS1, without any

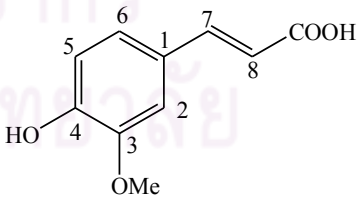
phenolic hydroxyl group, was inactive both in the DPPH and superoxide radical scavenging activity assay. This was in accordance with the previous report that indicated that the phenolic hydroxyl group played an important role in antioxidant activity (Zhang *et al*., 2007a).



	R_1	R_2	R_3	R_4
DS1 [**222**]	Me	Me	OMe	Me
DS2 [**60**]	H	Me	H	H
DS3 [**223**]	H	H	H	H



DS4 [**210**]



DS5 [**224**]

CHAPTER V

CONCLUSION

In this study, a new bibenzyl named 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl and four known compounds which included brittonin A, moscatilin [**60**], syringaresinol [**210**] and ferulic acid were isolated from the MeOH extract of *Dendrobium secundum* (Blume) Lindl. (Orchidaceae). These isolated compounds were also evaluated for free radical scavenging activity. All compounds, except for brittonin A, exhibited appreciable DPPH free radical scavenging activity. In addition, moscatilin [**60**] and syringaresinol [**210**] were found to show weak superoxide radical scavenging activity. The results obtained in this study indicated that *D. secundum* is a potential source of natural antioxidants.

References

Asakawa, Y., Tanikawa, K., and Aratani, T. 1976. New substituted bibenzyls of *Frullania brittoniae* subsp. truncatifolia. Phytochemistry 15 : 1057-1059.

Behr, D., and Leander, K. 1976. Three steroid glycosides of the stigmastane type from *Dendrobium ochreatum*. Phytochemistry 15 : 1403-1406.

Bensky, D., and Gamble, A. 1993. Chinese Herbal Medicine: Materia Medica. Revised edition. United States of America: Eastland Press, Incorporated.

Braca, A., Sortino, C., Politi, M., Morelli, I., and Mendez, J. 2002. Antioxidant activity of flavonoids from *Licania licaniaeflora*. Journal of Ethnopharmacology 79 : 379-381.

Chang, C.C., *et al*. 2010. 6,8-Di-*C*-glycosyl flavonoids from *Dendrobium huoshanense*. Journal of Natural Products 73 : 229-232.

Chen, C.C. 1994. Antiplatelet aggregation principles of *Dendrobium loddigesii*. Journal of Natural Products 57 : 1271-1274.

Chen, T.H. *et al*. 2008a. Moscatilin induces apoptosis in human colorectal cancer cells: a crucial role of c-Jun NH_2-terminal protein kinase activation caused by tubulin depolymerization and DNA damage. Clinical Cancer Research 14 : 4250-4257.

Chen, Y. *et al*. 2008c. 1,4,5-Trihydroxy-7-methoxy-9*H*-fluoren-9-one, a new cytotoxic compound from *Dendrobium chrysotoxum*. Food Chemistry 108 : 973-976.

Chen, Y., Li, J., Wang, L., and Liu, Y. 2008b. Aromatic compounds from *Dendrobium aphyllum*. Biochemical Systematics and Ecology 36 : 458-460.

Chen, Y., Liu, Y., Jiang, J., Zhang, Y., and Yin, B. 2008d. Dendronone, a new phenanthrenequinone from *Dendrobium cariniferum*. Food Chemistry 111 : 11-12.

Dahmen, J., and Leander, K. 1978. Amotin and amoenin, two sesquiterpenes of the picrotoxane group from *Dendrobium amoemum*. Phytochemistry 17 : 1949-1952.

Dasguta, N., and De, B. 2004. Antioxidant activity of *Piper betle* L. leaf extract in vitro. Food Chemistry 88 : 219-224.

Dobhal, M.P., Hasan, A.M., Sharma, M.C., and Joshi, B.C. 1999. Ferulic acid esters from *Plumeria bicolor*. Phytochemistry 51 : 319-321.

Fan, C., Wang, W., Wang, Y., Qin, G., and Zhao, W. 2001. Chemical constituents from *Dendrobium densiflorum*. Phytochemistry 57 : 1255-1258.

Gawell, L., and Leander, K. 1976. The constitution of Aduncin, a sesquiterpene related to picrotoxinin, found in *Dendrobium acundum*. Phytochemistry 15 : 1991-1992.

Guanghua, Z., Zhanhe, J., Wood, J.J., and Wood, H.P. 2009. *Dendrobium* Swartz. Flora of China 25 : 367.

Ho, C.K., and Chen, C.C. 2003. Moscatilin from the orchid *Dendrobium loddigesii* is a potential anticancer agent. Cancer Investigation 21 : 729-736.

Holtum, R.E. 1957. Orchids of Malaya. Flora of Malaya Volume I second edition. Singapore : Authority Government printing office.

Honda, C., and Yamaki, M. 2000. Phenanthrenes from *Dendrobium plicatile*. Phytochemistry 53 : 987-990.

Hu, J.M., Chen, J.J., Yu, H., Zhao, Y.X., and Zhou, J. 2008a. Five new compounds from *Dendrobium longicornu*. Planta Medica 74 : 535-539.

Hu, J.M., Chen, J.J., Yu, H., Zhao, Y.X., and Zhou, J. 2008b. Two novel bibenzyls from *Dendrobium trigonopus*. Journal of Asian Natural Products Research 10 : 647-651.

Hwang, J.S. *et al*. 2010. Phenanthrenes from *Dendrobium nobile* and their inhibition of the LPS-induced production of nitric oxide in macrophage RAW 264.7 cells. Bioorganic & Medicinal Chemistry Letters 20 : 3785-3787.

Ito, M. *et al*. 2010. New phenanthrenes and stilbenes from *Dendrobium loddigesii*. Chemical and Pharmaceutical Bulletin 58 : 628-633.

Li, Y. *et al*. 2009a. Three new bibenzyl derivatives from *Dendrobium candidum*. Chemical and Pharmaceutical Bulletin 57 : 218-219.

Li, Y. *et al*. 2009b. Four new bibenzyl derivatives from *Dendrobium candidum*. Chemical and Pharmaceutical Bulletin 57 : 997-999.

Li, Y., Wang, C.L., Guo, S.X., Yang, J.S., and Xiao, P.G. 2008. Two new compounds from *Dendrobium candidum*. Chemical and Pharmaceutical Bulletin 56 : 1477-1479.

Likhitwitayawuid, K., Klongsiriwet, C., Jongbunprasert, V., Sritularak, B., and Wongseripipatana, S. 2006. Flavones with free radical scavenging activity from *Goniothalamus tenuifolius*. Archives of Pharmacal Research 29 : 199-202.

Lin, T.H., Chang, H.J., Chen, C.C., Wang, J.P., and Tsao, L.T. 2001. Two phenantraquinones from *Dendrobium moniliforme*. Journal of Natural Products 64 : 1084-1086.

Majumder, P.L., and Chatterjee, S. 1989. Crepidatin, a bibenzyl derivative from the orchid *Dendrobium crepidatum*. Phytochemistry 28 : 1986-1988.

Majumder, P.L., and Pal, S. 1992. Rotundatin, a new 9,10-dihydrophenanthrene derivative from *Dendrobium rotundatum*. Phytochemistry 31 : 3225-3228.

Majumder, P.L., and Pal, S. 1993. Cumulatin and tristin, two bibenzyl derivatives from the orchids *Dendrobium cumulatum* and *Bulbophyllum triste*. Phytochemistry 32 : 1561-1565.

Majumder, P.L., and Sen, R.C. 1987. Moscatilin, a bibenzyl derivative from the orchid *Dendrobium moscatum*. Phytochemistry 26 : 2121-2124.

Majumder, P.L., Guha, S., and Sen, S. 1999. Bibenzyl derivatives from the orchid *Dendrobium amoenum*. Phytochemistry 52 : 1365-1369.

Min, Z.D., Tanaka, T., Iinuma, M., and Mizuno, M. 1987. A new dihydrostilbene in *Dendrobium chrysanthum*. Journal of Natural Products 50 : 1189.

Pietta, P.G. 2000. Flavonoids as antioxidants. Journal of Natural Products 63 : 1035-1042.

Royal Forest Department, The Forest Herbarium. 2001. Thai plant names Tem Smitinand revised edition. Bangkok : Prachachon.

Seidenfaden, G. 1985. Orchid genera in Thailand XII. *Dendrobium* Sw. Opera Botanica 83 : 1-295.

Shu, Y., Zhang, D.M., and Guo, S.X. 2004. A new sesquiterpene glycoside from *Dendrobium nobile* Lindl. Journal of Asian Natural Products Research 6 : 311-314.

Sritularak, B., and Likhitwitayawuid, K. 2009. New bisbibenzyls from *Dendrobium falconeri*. Helvetica Chemica Acta 92 : 740-744.

Talapatra, B., Das, A.K., and Talapatra, S.K. 1989. Defuscin, a new phenolic ester from *Dendrobium fuscescens*: conformation of shikimic acid. Phytochemistry 28 : 290-292.

Talapatra, S.K., Bhaumik, A., and Talapatra, B. 1992. Denfigenin, a diosgenin derivative from *Dendrobium fimbriatum*. Phytochemistry 31 : 2431-2434.

Tanaka, T., Iinuma, M., and Mizuno, M. 1987. A new dihydrostilbene in *Dendrobium chrysanthum*. Journal of Natural Products 50 : 1189.

Tsai, A.C. *et al*. 2010. Moscatilin, a bibenzyl derivative from the India orchid *Dendrobium loddigesii*, suppresses tumor angiogenesis and growth *in vitro* and *in vivo*. Cancer Letters 292 : 163-170.

Vaddhanaphuti, N. 2005. A Field Guide to the Wild Orchids of Thailand. Fourth and expanded edition. Bangkok : O.S. Printing House.

Valko, M. *et al*. 2007. Free radicals and antioxidants in normal physiological functions and human disease. Journal of Biochemistry & Cell Biology 36 : 44-84.

Veerraju, P., Prakasa, N.S., Jaganmohana, L., Jagannadha, K.V., and Mohana, P.R. 1989. Amoenumin, a 9,10-dihydro-5H-phenanthro-(4,5-*b,c,d*)-pyran from *Dendrobium amoenum*. Phytochemistry 28 : 950-951.

Wang, H., Zhao, T., and Che, C.T. 1985. Dendrobine and 3-hydroxy-2-oxodendrobine from *Dendrobium nobile*. Journal of Natural Products 48 : 796-801.

Wang, L., Zhang, C.F., Wang, Z.T., Zhang, M., and Xu, L.S. 2009. Five new compounds from *Dendrobium crystallinum*. Journal of Asian Natural Products Research 11 : 903-911.

Wang, Q., Gong, Q., Wu, Q., and Shi, J. 2010. Neuroprotective effects of *Dendrobium* alkaloids on rat cortical neurons injured by oxygen-glucose deprivation and reperfusion. Phytomedicine 17 : 108-115.

Yamaki, M., and Honda, C. 1996. The stilbenoids from *Dendrobium plicatile*. Phytochemistry 43 : 207-208.

Yang, H. *et al*. 2004. Two new compounds from *Dendrobium chrysotoxum*. Helvetica Chemica Acta 87 : 394-399.

Yang, H., Sung, S.H., and Kim, Y.C. 2007. Antifibrotic phenanthrenes of *Dendrobium nobile* stems. Journal of Natural Products 70 : 1925-1929.

Yang, L. *et al*. 2006a. A new phenanthrene with a spirolactone from *Dendrobium chrysanthum* and its anti-inflammatory activities. Bioorganic & Medicinal Chemistry 14 : 3496-3501.

Yang, L., Wang, Z., and Xu, L. 2006b. Phenols and a triterpene from *Dendrobium aurantiacum* var. *denneanum* (Orchidaceae). Biochemical Systematics and Ecology 34 : 658-660.

Ye, Q., and Zhao, W. 2002. New alloaromadendrane, cadinene and cyclocopacamphane type sesquiterppene derivatives and bibenzyls from *Dendrobium nobile*. Planta Medica 68 : 723-729.

Ye, Q., Qin, G., and Zhao, W. 2002. Immunomodulatory sesquiterpene glycosides from *Dendrobium nobile*. Phytochemistry 61 : 885-890.

Zhang, C.F., *et al*. 2008a. Chemical constituents of *Dendrobium gratiosissimum* and their cytotoxic activities. Indian Journal of Chemistry 47B : 952-956.

Zhang, G.N., *et al*. 2005. Bi-bicyclic and bitricyclic compounds from *Dendrobium thyrsiflorum*. Phytochemistry 66 : 1113-1120.

Zhang, X., *et al*. 2007a. Bioactive bibenzyl derivatives and fluorenones from *Dendrobium nobile*. Journal of Natural Products 70 : 24-28.

Zhang, X., *et al*. 2007b. Sesquiterpenes from *Dendrobium nobile*. Zhongcaoyao 38 : 1771-1774.

Zhang, X., *et al*. 2008b. Copacamphane, picrotoxane and cyclocopacamphane sesquiterpenes from *Dendrobium nobile*. Chemical and Pharmaceutical Bulletin 56 : 854-857.

Zhang, X., Gao, H., Wang, N.L., and Yao, X.S. 2006. Three new bibenzyl derivatives from *Dendrobium nobile*. Journal of Asian Natural Products Research 8 : 113-118.

Zhang, X., Xu, J.K., Wang, N.L., Kurihara, H., and Yao, X.S. 2008c. Antioxidant phenanthrenes and lignans from *Dendrobium nobile*. Journal of Chinese Pharmaceutical Sciences 17 : 314-318.

Zhao, C., *et al*. 2003. Copacamphane, picrotoxane, and alloaromadendrane sesquiterpene glycosides and phenolic glycosides from *Dendrobium moniliforme*. Journal of Natural Products 66 : 1140-1143.

Zhao, W., *et al*. 2001. Three new sesquiterpene glycosides from *Dendrobium nobile* with immunomodulatory activity. Journal of Natural Products 64 : 1196-1200.

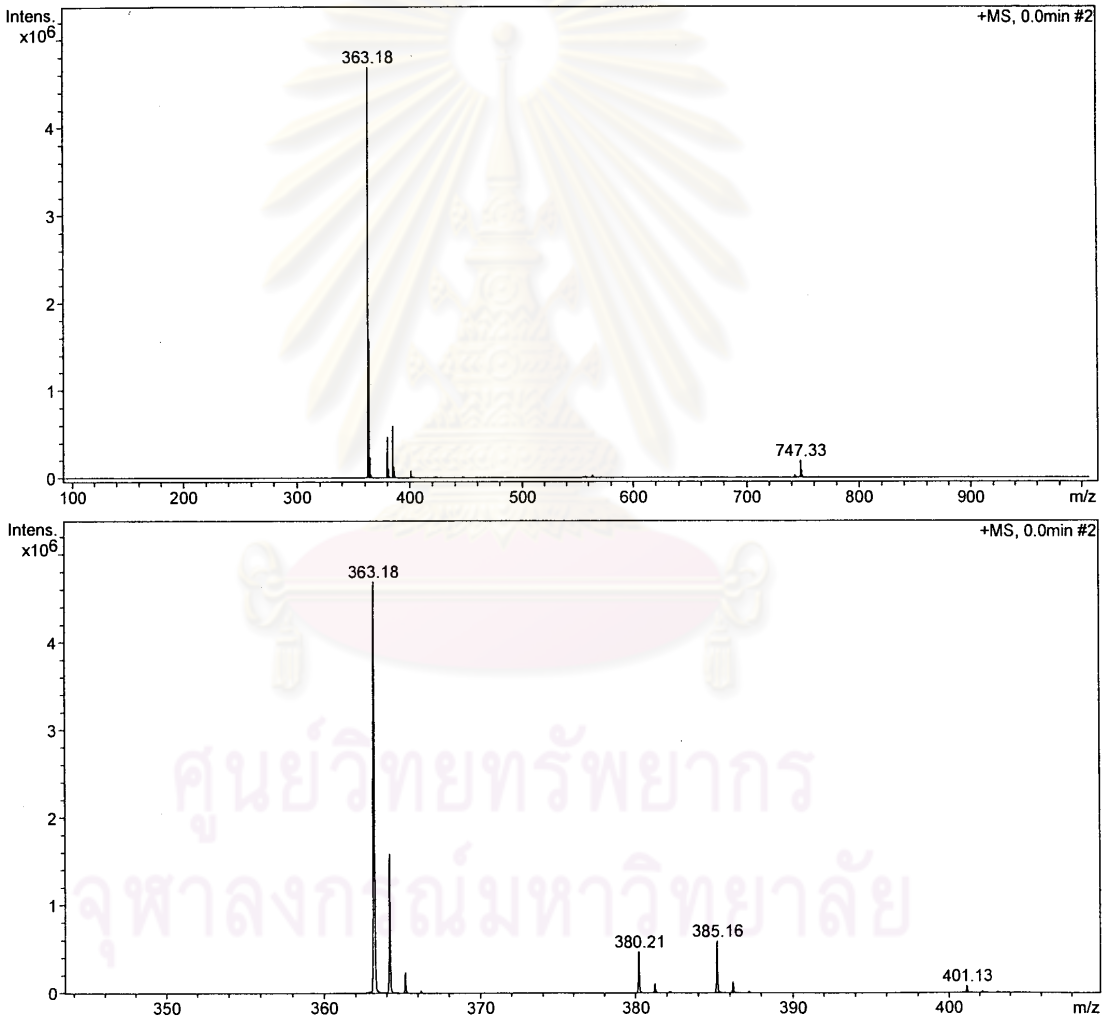
APPENDIX

Low resolution report

		Acquisition Date	10/11/2010 1:40:11 PM
Analysis Name	D:\Data\customer\DS17.d		
Method	NaFormate_pos_infusion.m	Operator	Sutichai Ext: 3560
Sample Name	DS17	Instrument	micrOTOF Bruker

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Not active			Set Dry Heater	150 °C
Scan Begin	100 m/z	Set Capillary	4000 V	Set Dry Gas	6.0 l/min
Scan End	1000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



Bruker Daltonics DataAnalysis 3.4 printed: 10/11/2010 1:50:34 PM Page 1 of 1

Figure 5 Mass spectrum of compound DS1

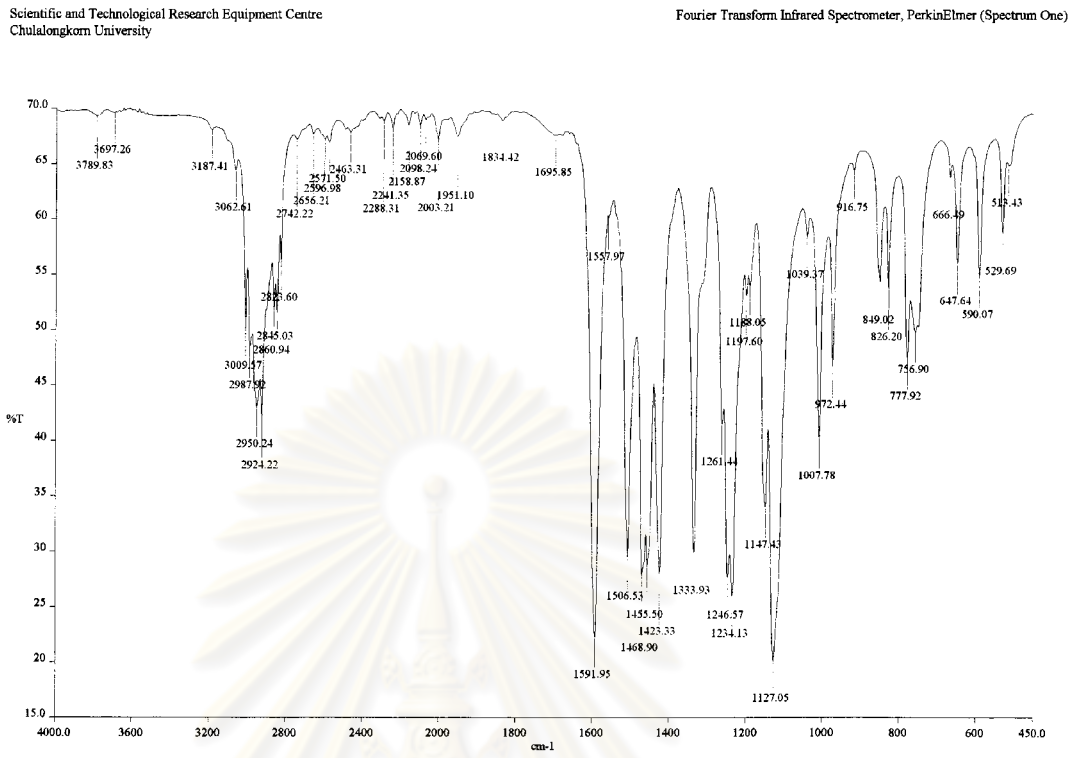


Figure 6 IR spectrum of compound DS1

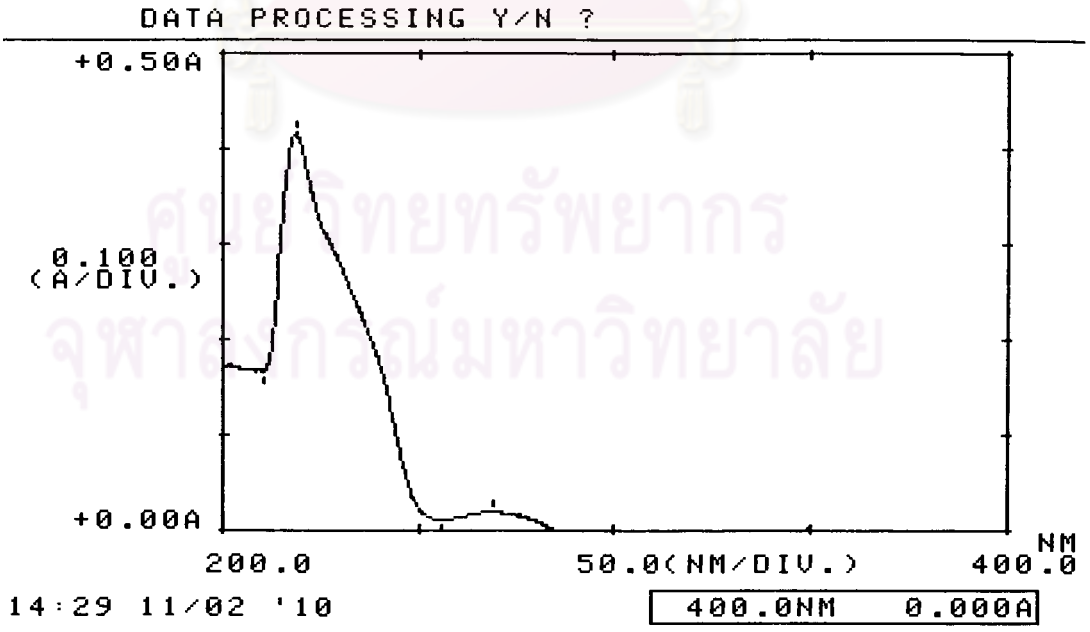


Figure 7 UV spectrum of compound DS1

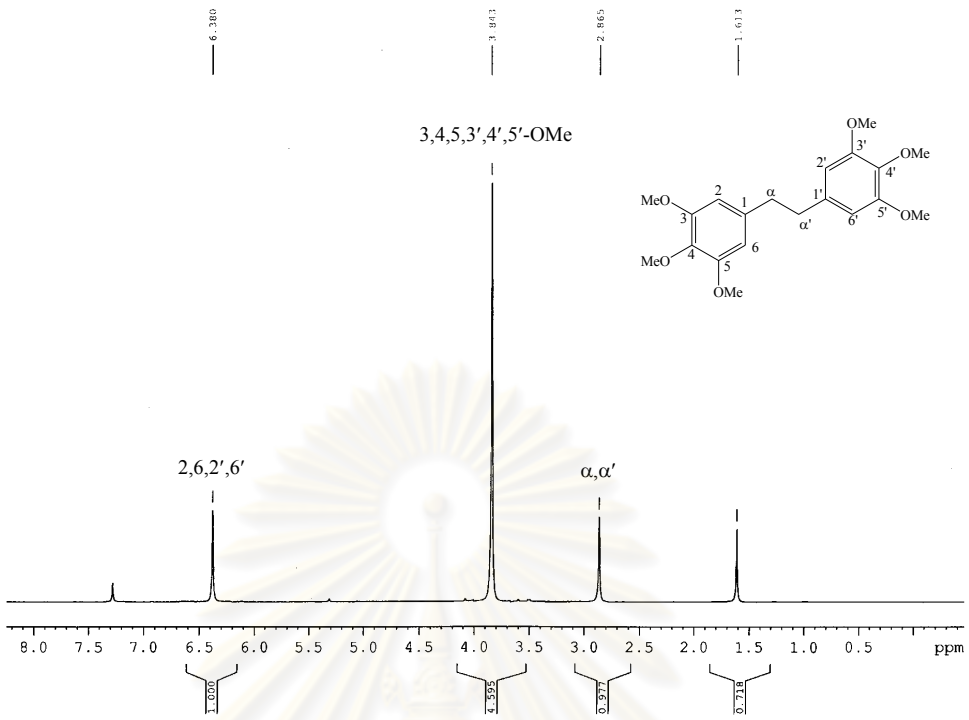


Figure 8 ^{1}H-NMR (300 MHz) spectrum of compound DS1 ($CDCl_3$)

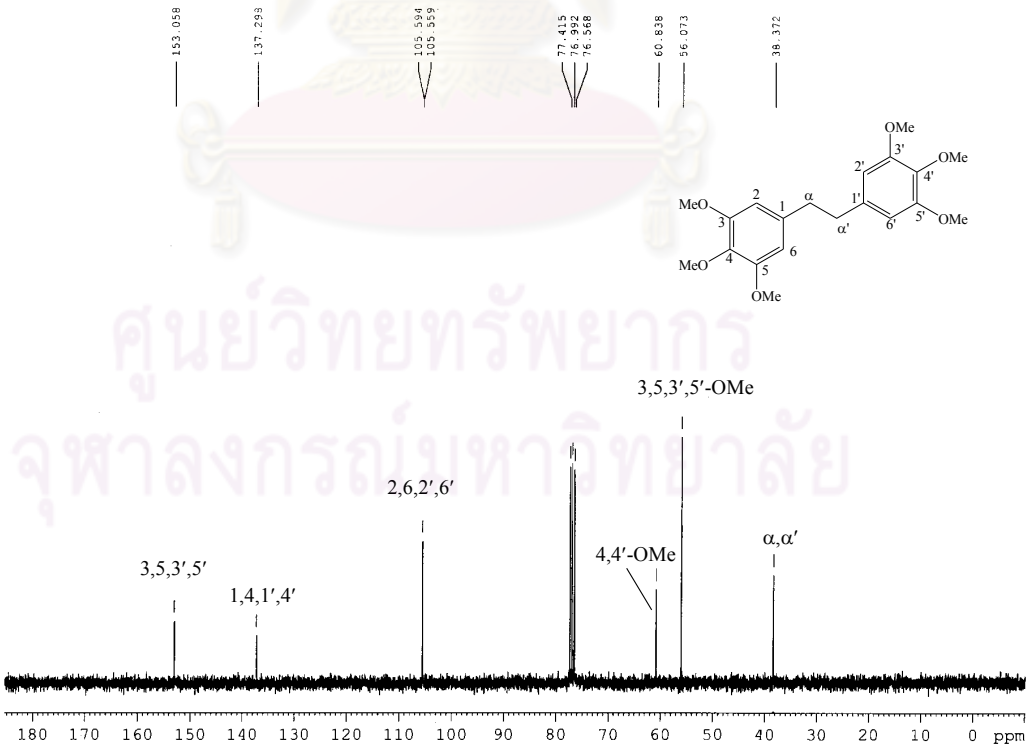


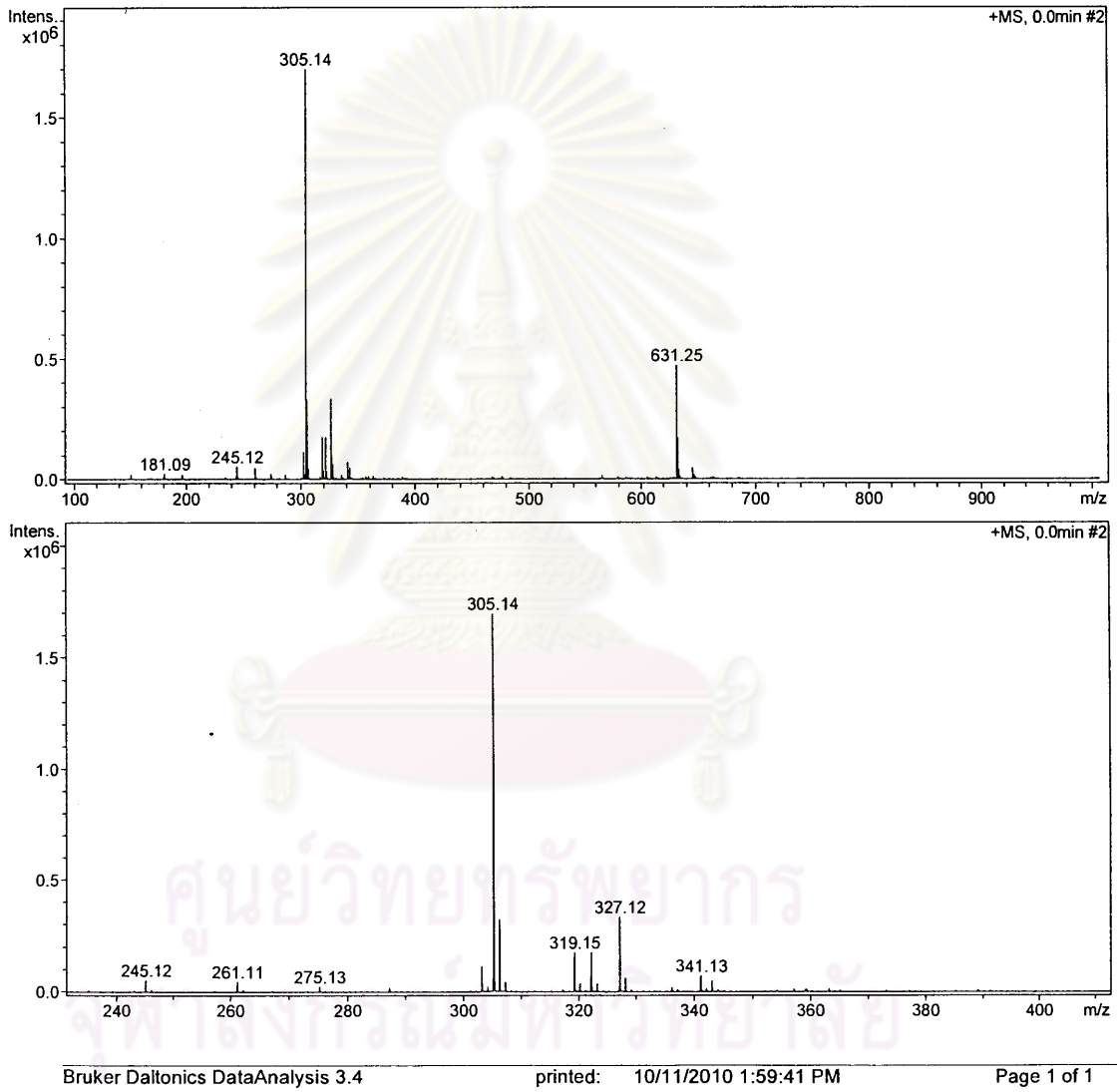
Figure 9 ^{13}C-NMR (75 MHz) spectrum of compound DS1 ($CDCl_3$)

Acquisition Date 10/11/2010 1:57:19 PM

Analysis Name	D:\Data\customer\DS3.d				
Method	NaFormate_pos_infusion.m			Operator	Sutichai Ext: 3560
Sample Name	DS3			Instrument	micrOTOF Bruker

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Not active			Set Dry Heater	150 °C
Scan Begin	100 m/z	Set Capillary	4000 V	Set Dry Gas	6.0 l/min
Scan End	1000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



Bruker Daltonics DataAnalysis 3.4 printed: 10/11/2010 1:59:41 PM Page 1 of 1

Figure 10 Mass spectrum of compound DS2

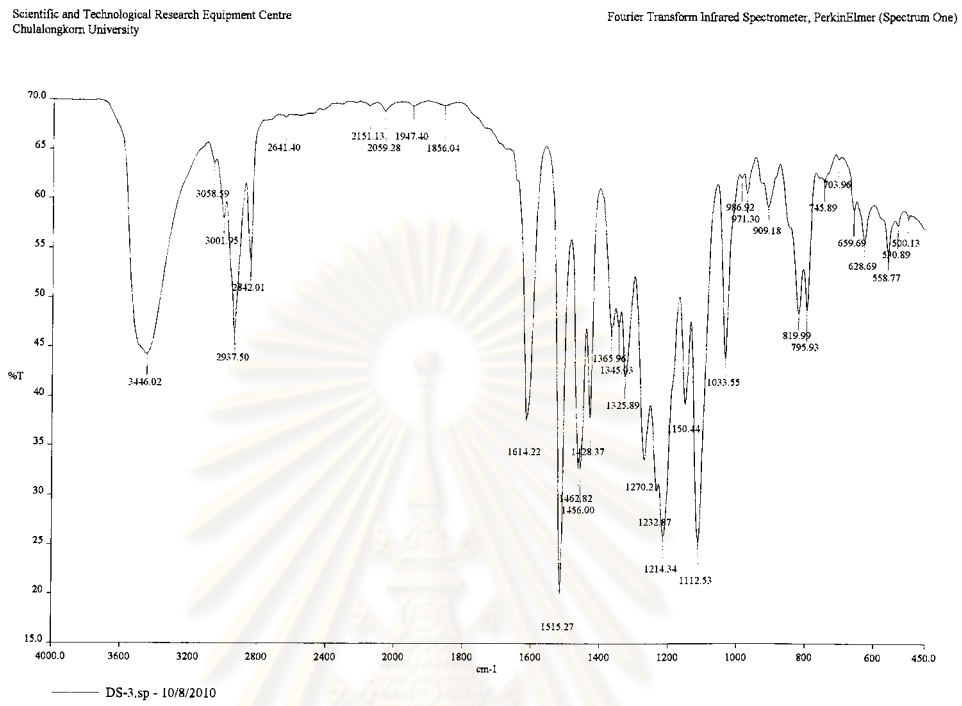


Figure 11 IR spectrum of compound DS2

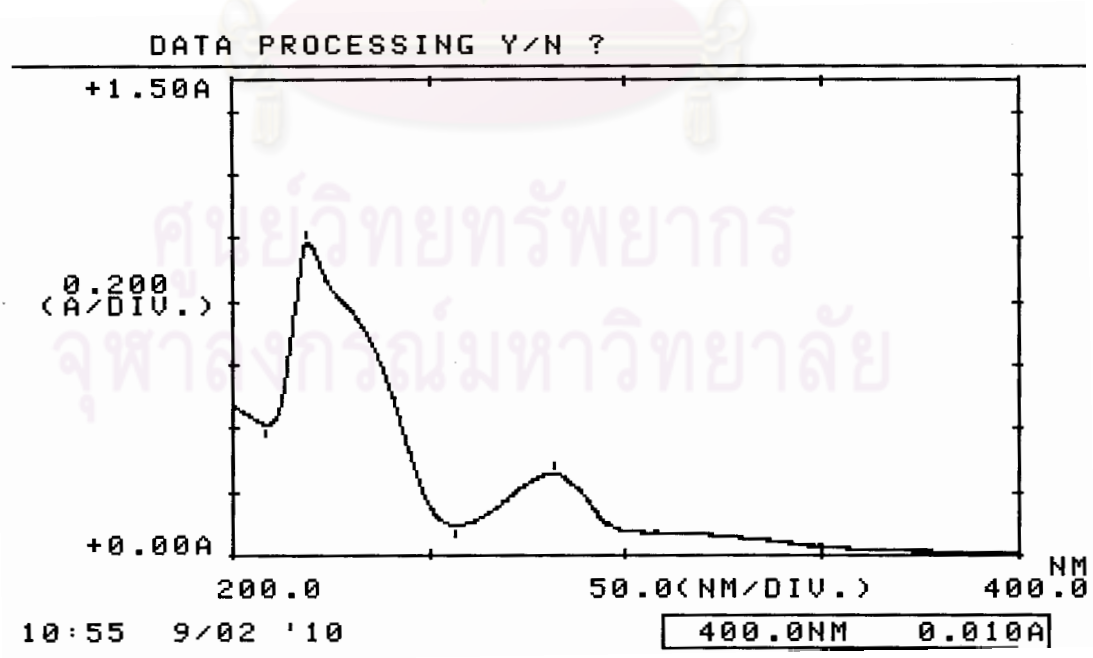


Figure 12 UV spectrum of compound DS2

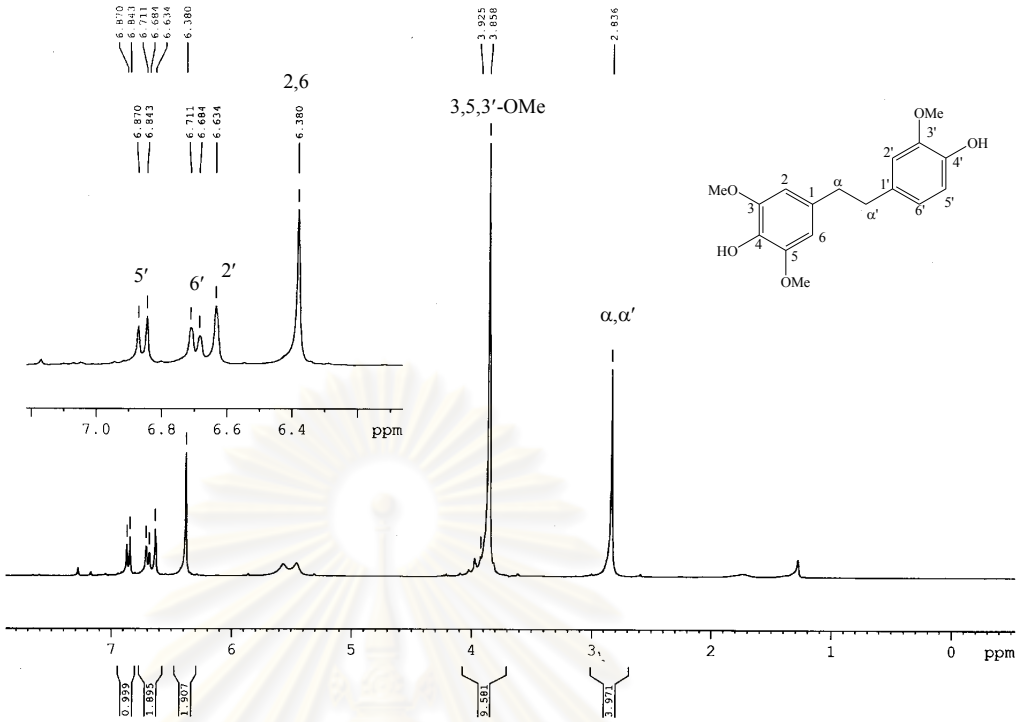


Figure 13 ^{1}H-NMR (300 MHz) spectrum of compound DS2 ($CDCl_3$)

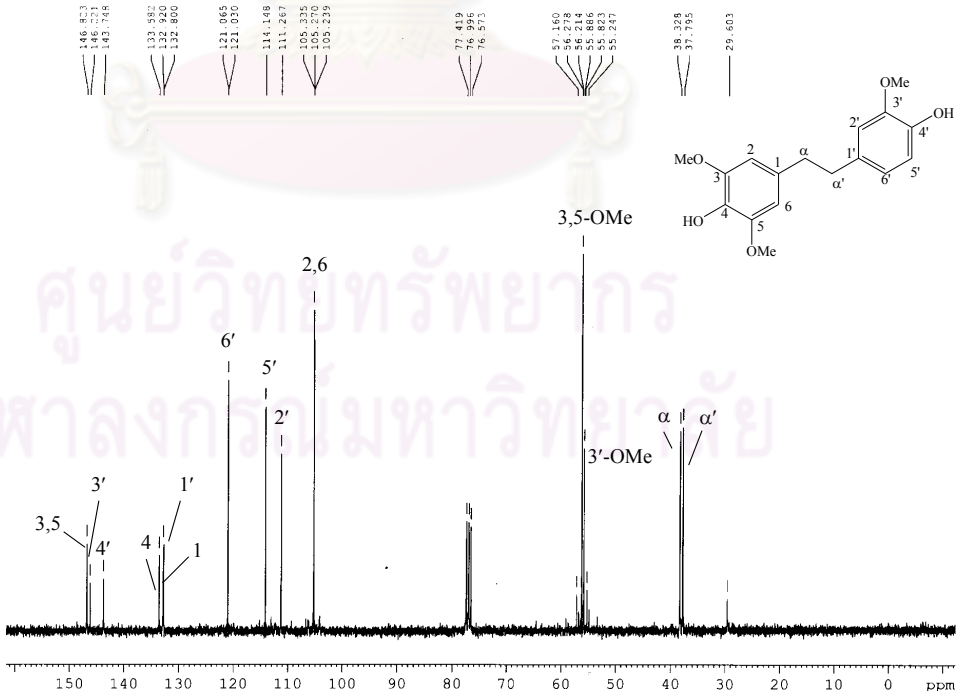


Figure 14 ^{13}C-NMR (75 MHz) spectrum of compound DS2 ($CDCl_3$)

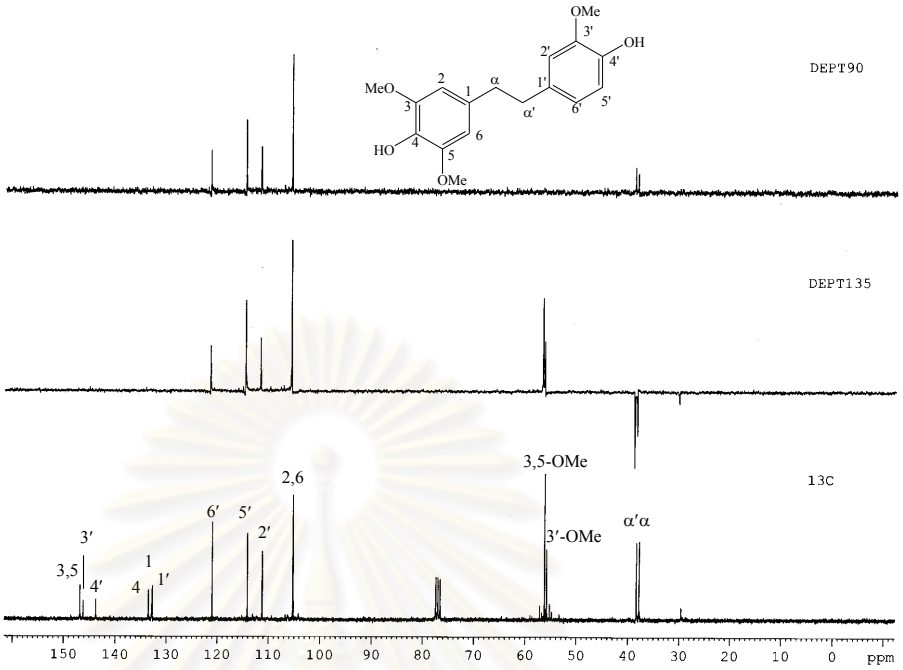


Figure 15 ^{13}C-NMR and DEPT spectra of compound DS2 ($CDCl_3$)

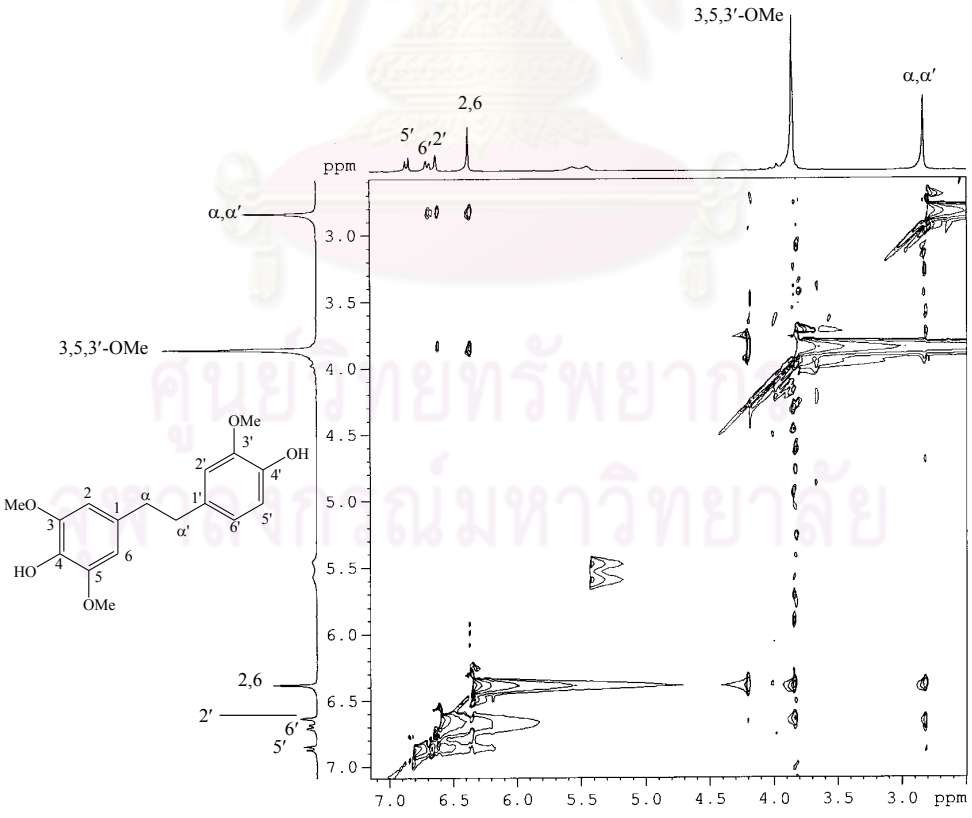


Figure 16 NOESY spectrum of compound DS2 ($CDCl_3$)

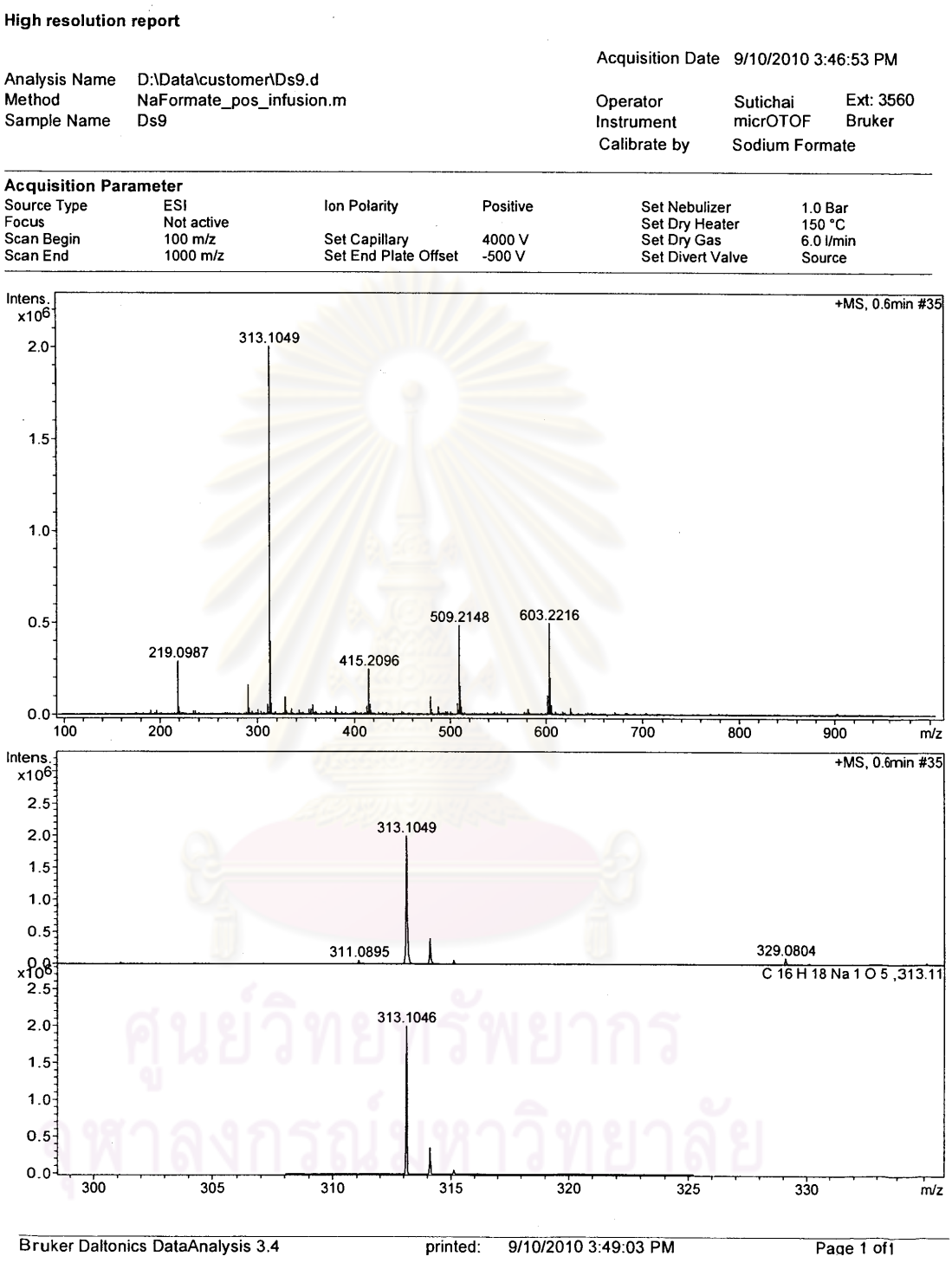


Figure 17 Mass spectrum of compound DS3

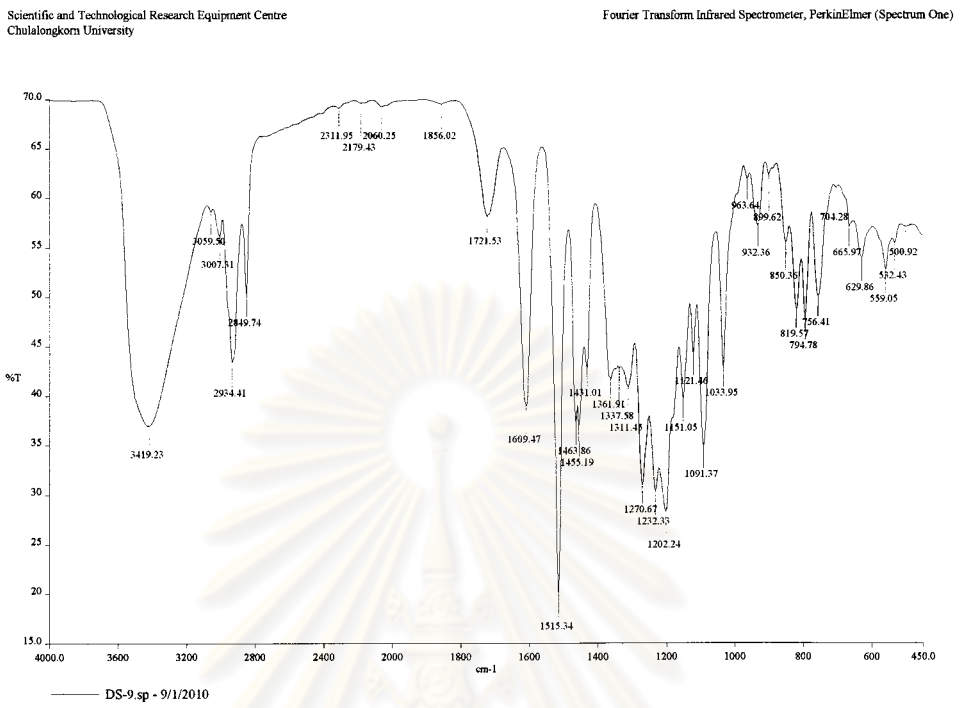


Figure 18 IR spectrum of compound DS3

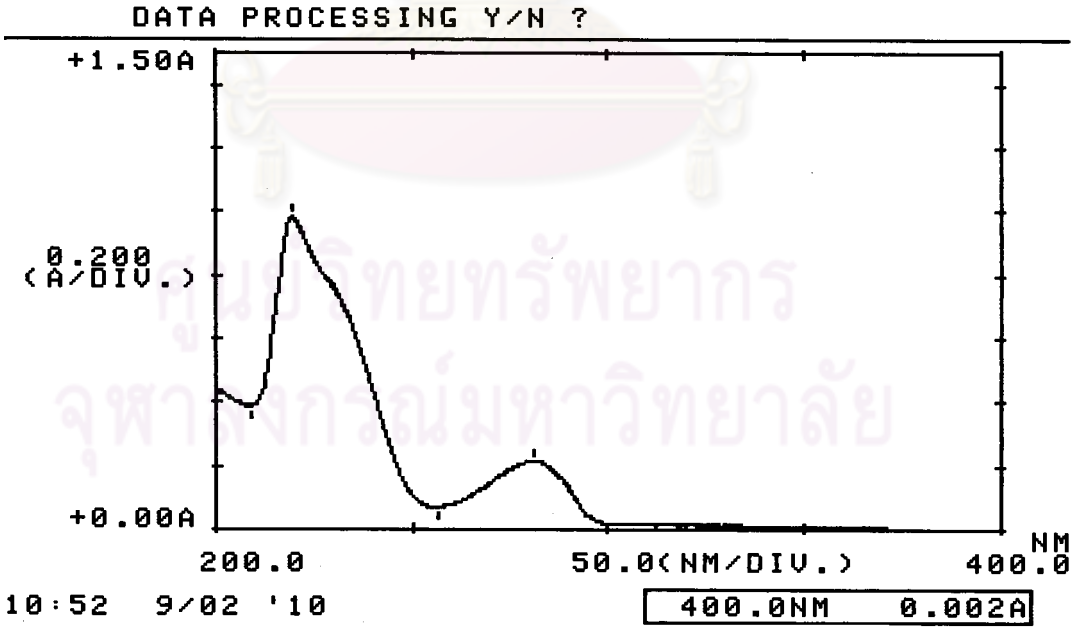


Figure 19 UV spectrum of compound DS3

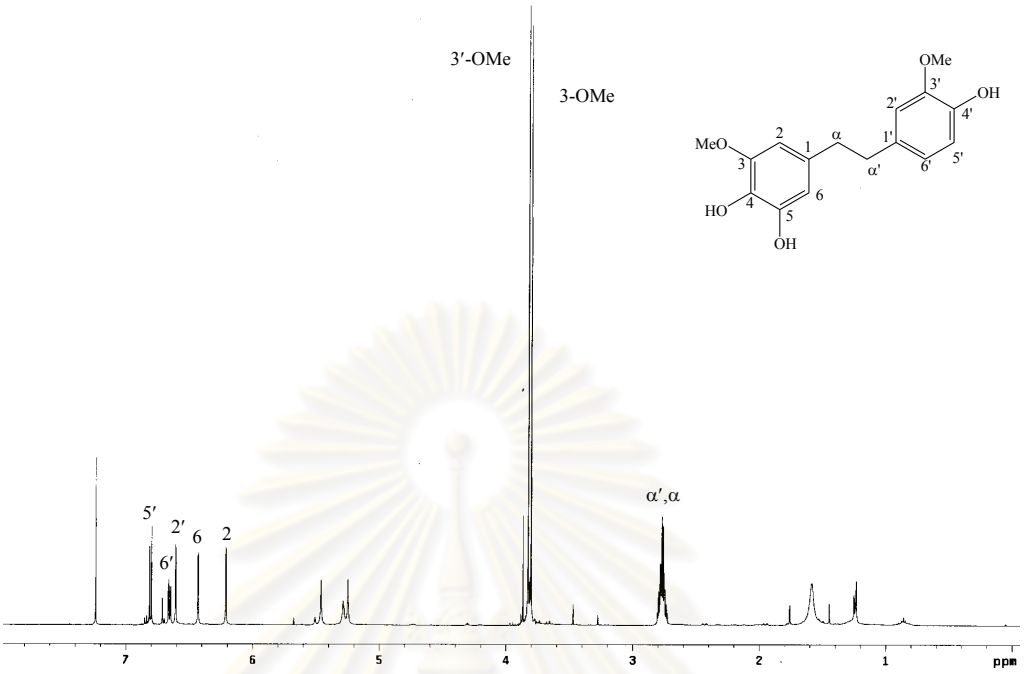


Figure 20 ^{1}H-NMR (500 MHz) spectrum of compound DS3 ($CDCl_3$)

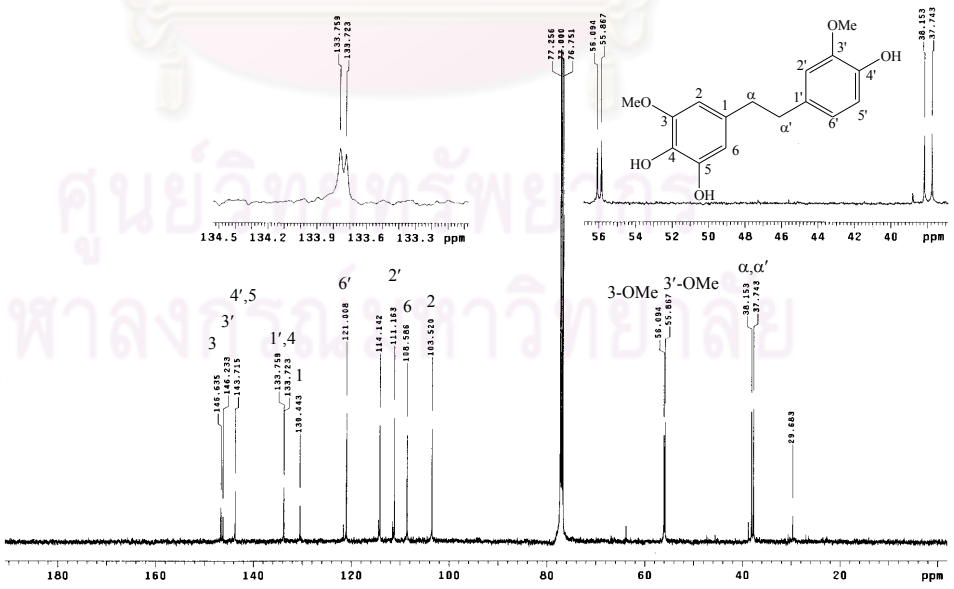


Figure 21 ^{13}C-NMR (125 MHz) spectrum of compound DS3 ($CDCl_3$)

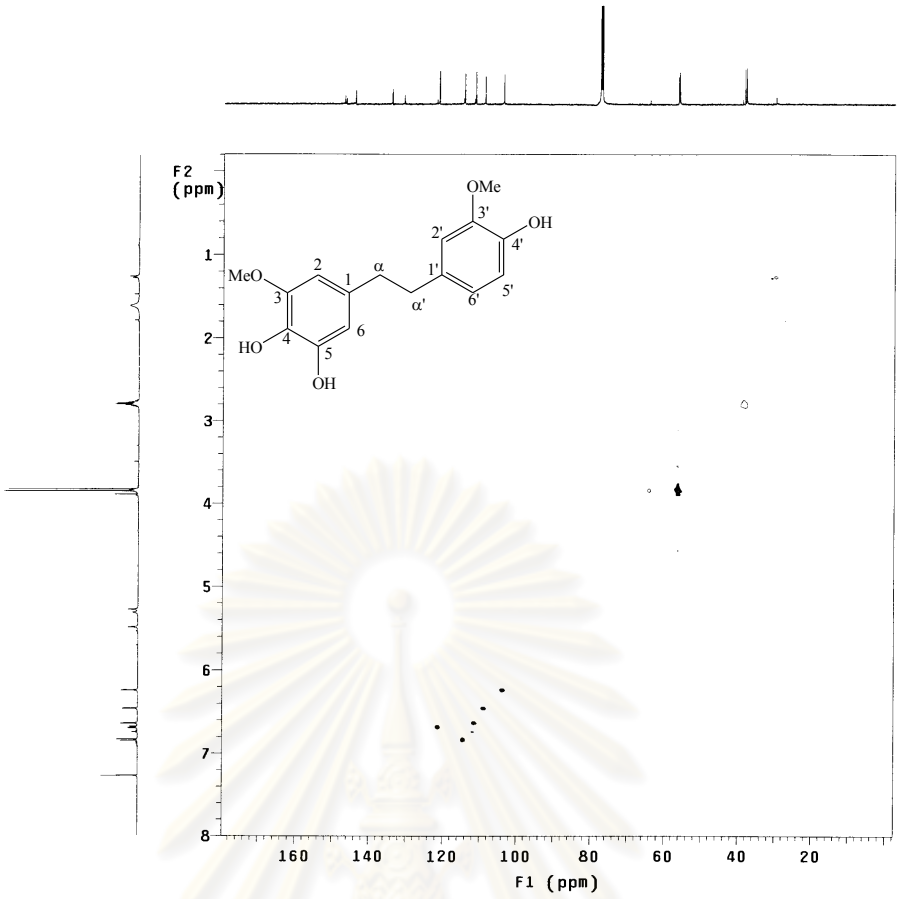


Figure 22a HSQC spectrum of compound DS3 ($CDCl_3$)

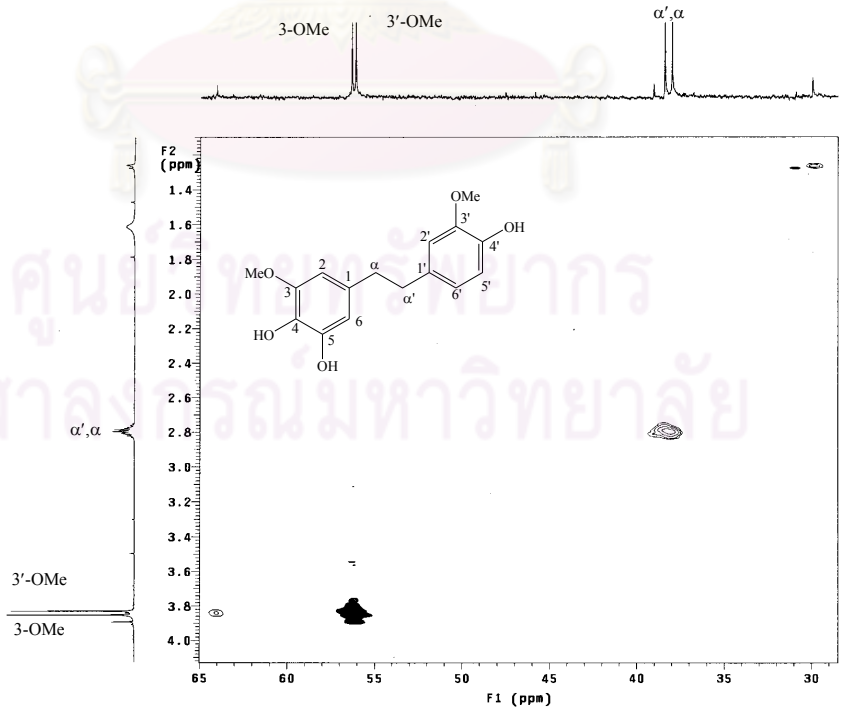


Figure 22b HSQC spectrum of compound DS3 ($CDCl_3$)

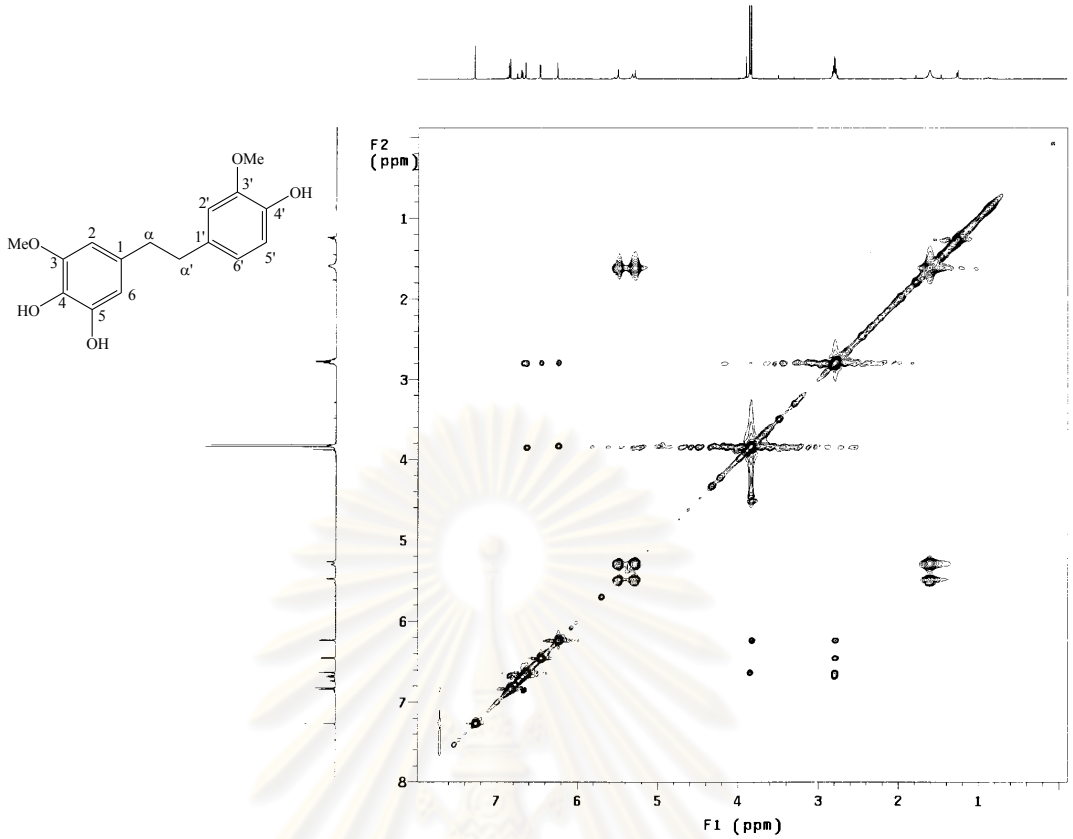


Figure 23a NOESY spectrum of compound DS3 ($CDCl_3$)

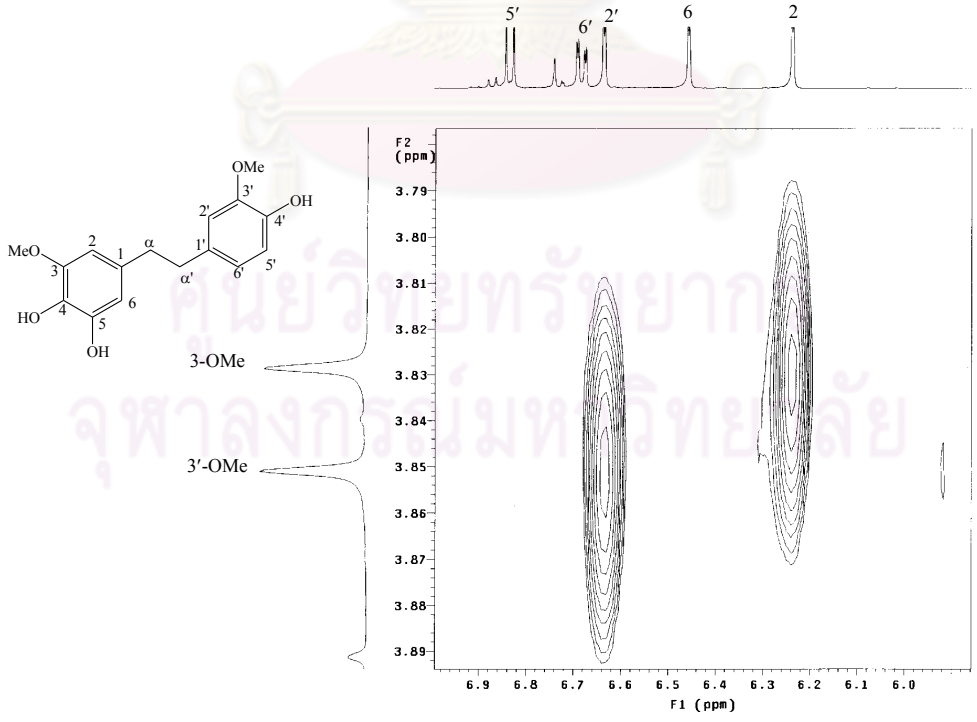


Figure 23b NOESY spectrum of compound DS3 ($CDCl_3$)

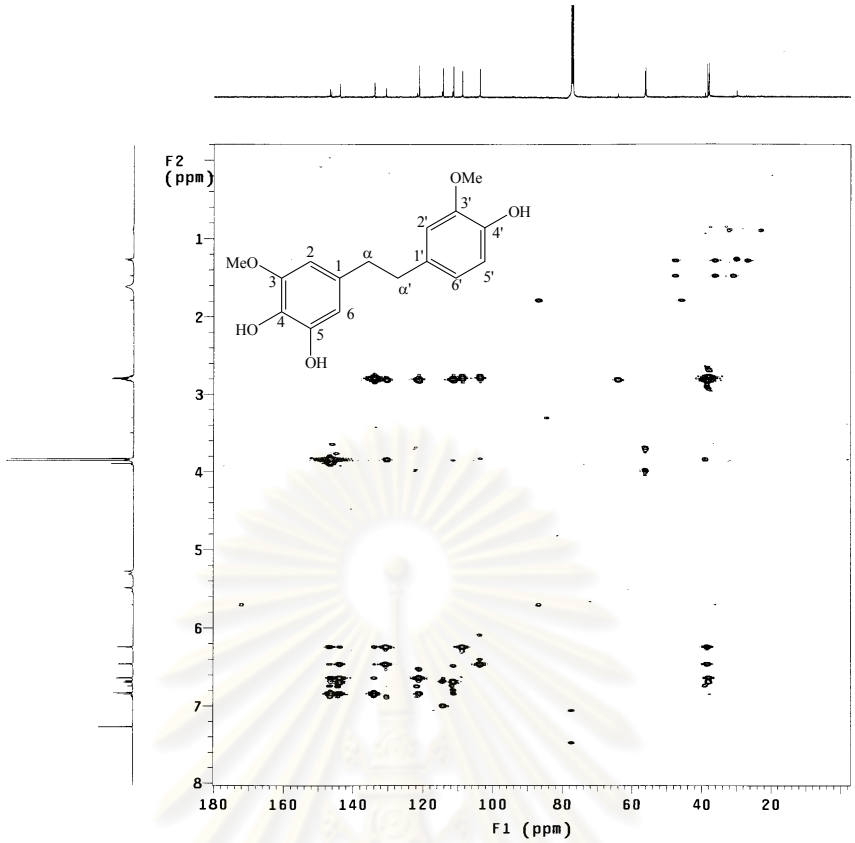


Figure 24a HMBC spectrum of compound DS3 ($CDCl_3$)

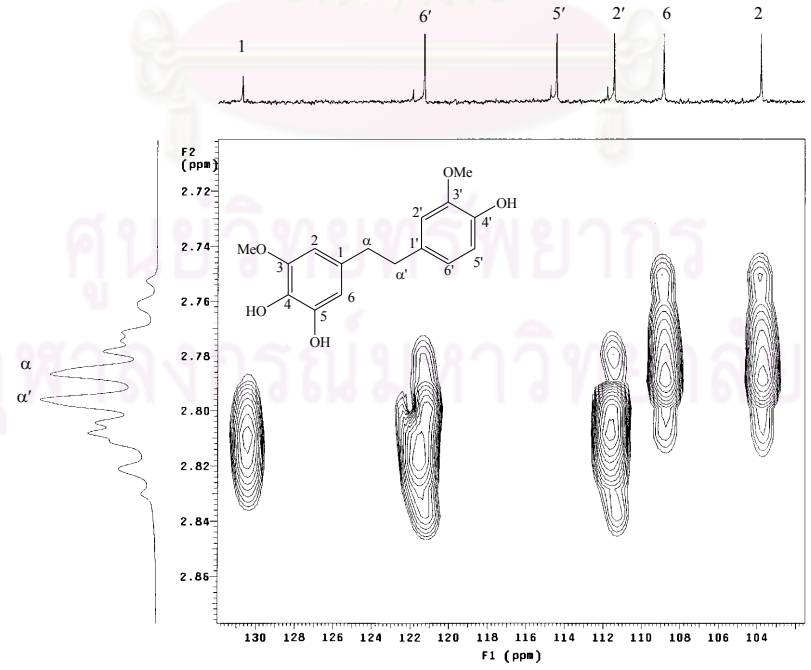


Figure 24b HMBC spectrum of compound DS3 ($CDCl_3$)

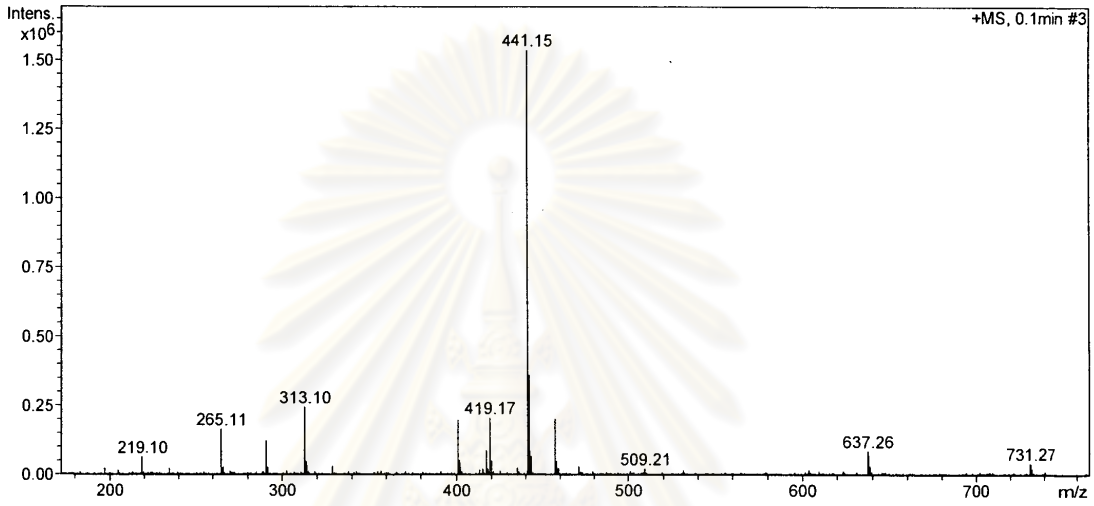
Acquisition Date 9/10/2010 3:49:54 PM

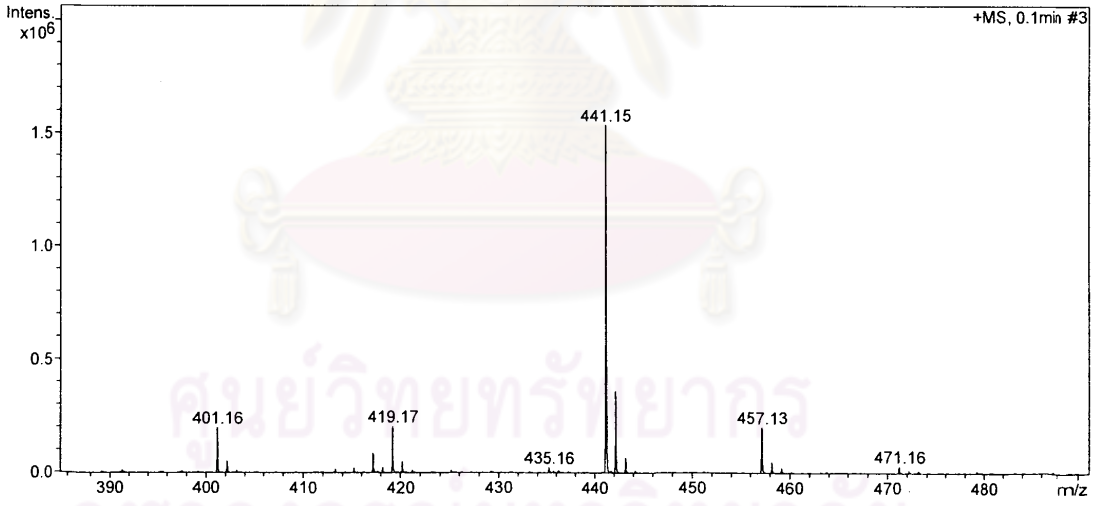
Analysis Name D:\Data\customer\Ds13.d
Method NaFormate_pos_infusion.m
Sample Name Ds13

Operator Sutichai Ext: 3560
Instrument micrOTOF Bruker

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Not active			Set Dry Heater	150 °C
Scan Begin	100 m/z	Set Capillary	4000 V	Set Dry Gas	6.0 l/min
Scan End	1000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source





Bruker Daltonics DataAnalysis 3.4 printed: 9/10/2010 3:51:15 PM Page 1 of 1

Figure 25 Mass spectrum of compound DS4

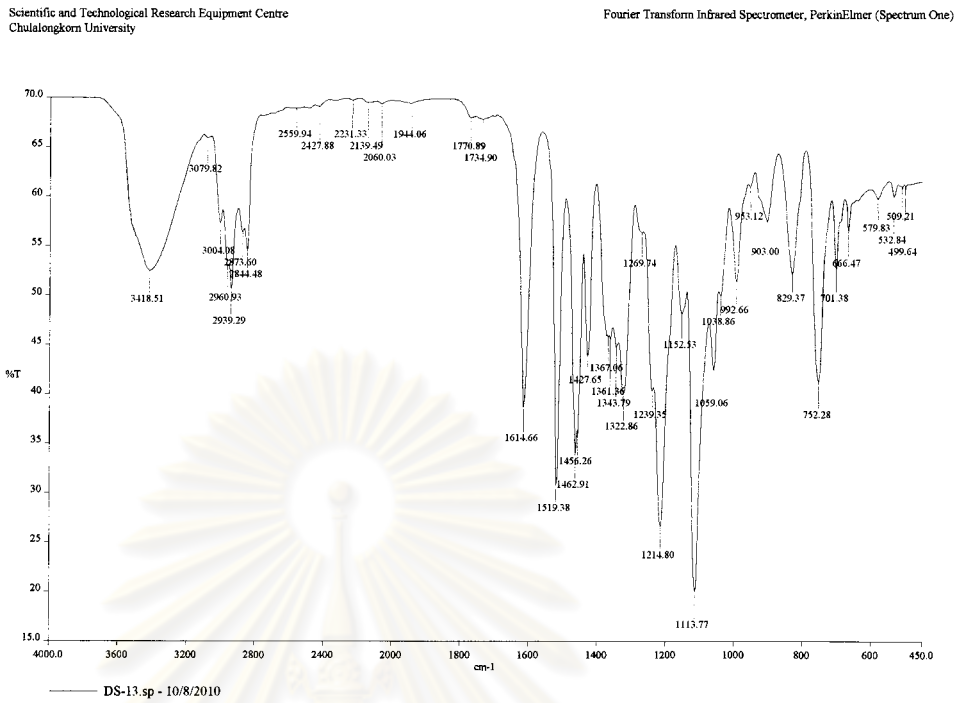


Figure 26 IR spectrum of compound DS4

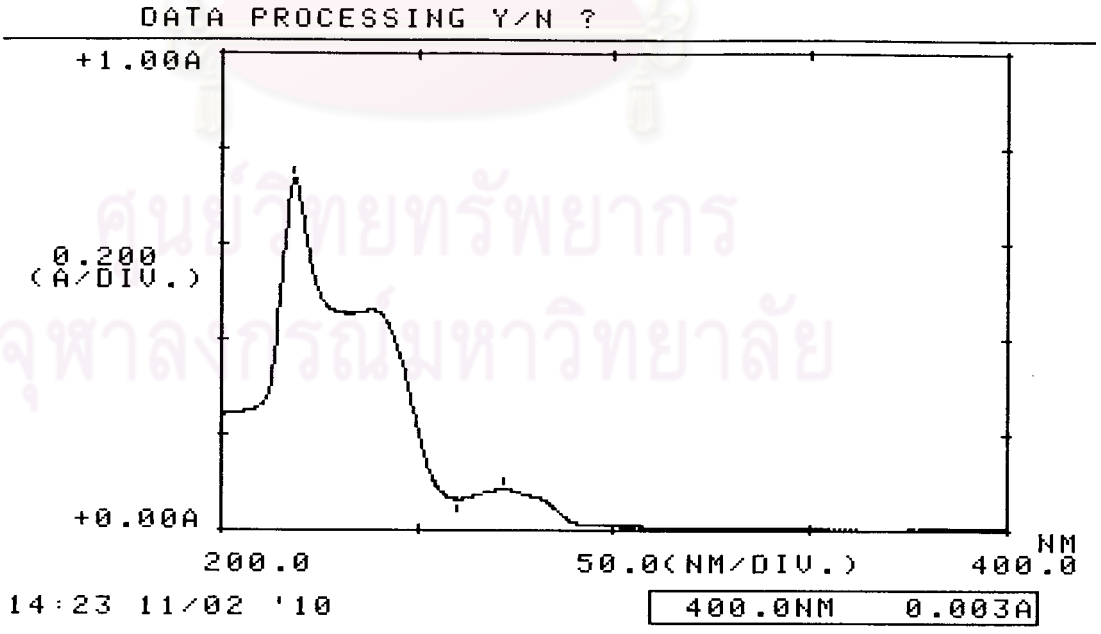


Figure 27 UV spectrum of compound DS4

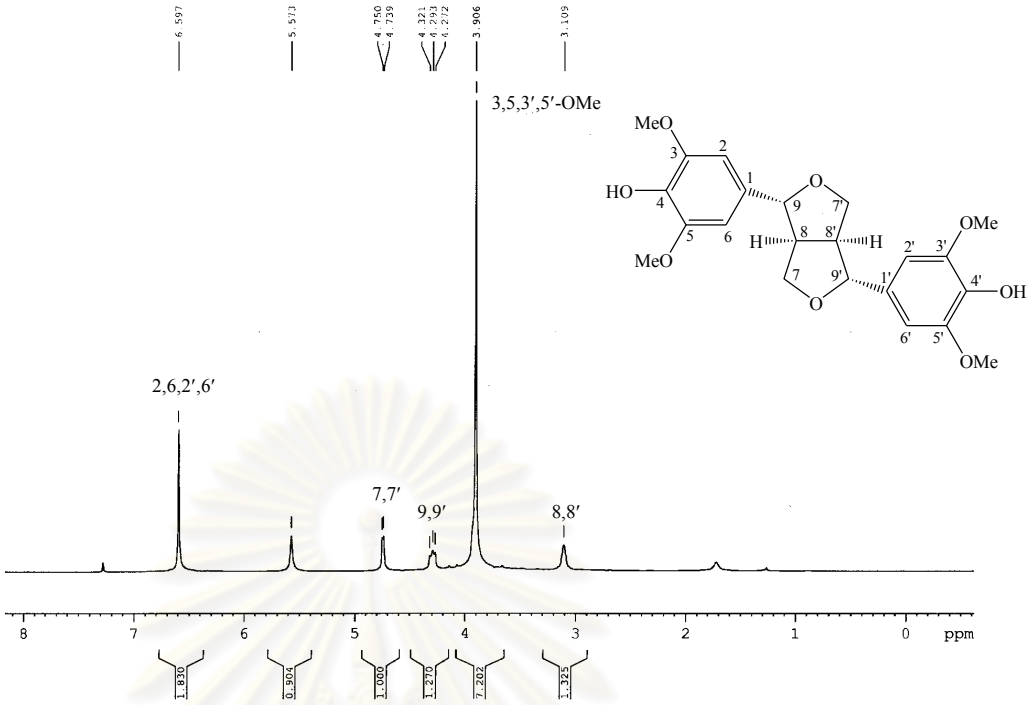


Figure 28 ^{1}H-NMR (300 MHz) spectrum of compound DS4 ($CDCl_3$)

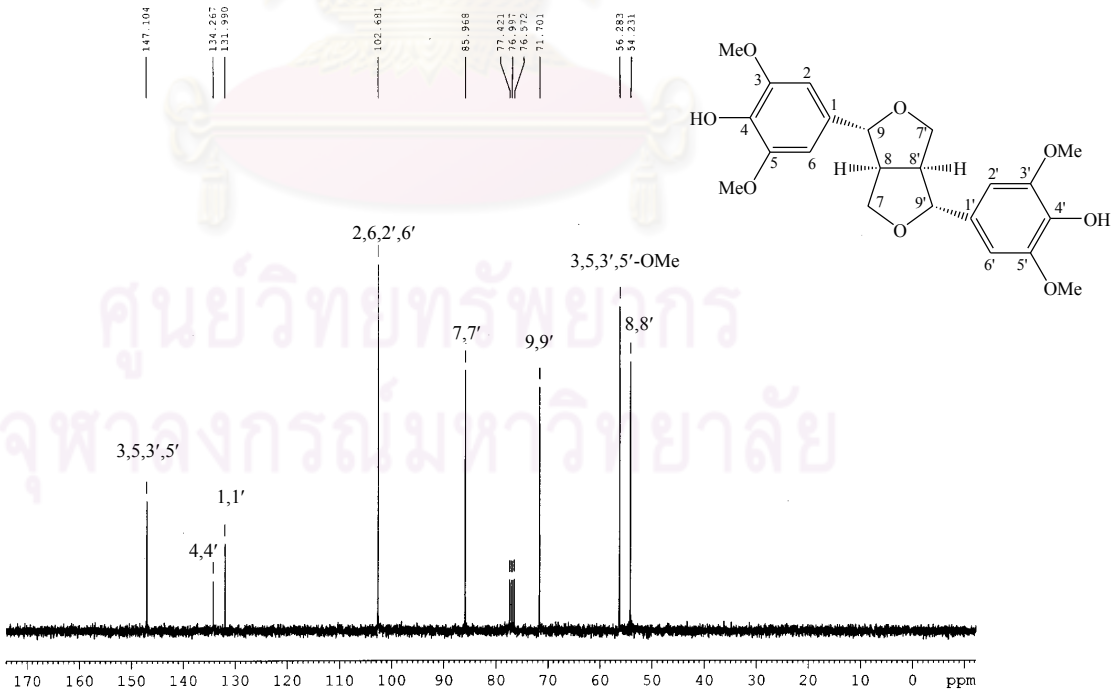


Figure 29 ^{13}C-NMR (75 MHz) spectrum of compound DS4 ($CDCl_3$)

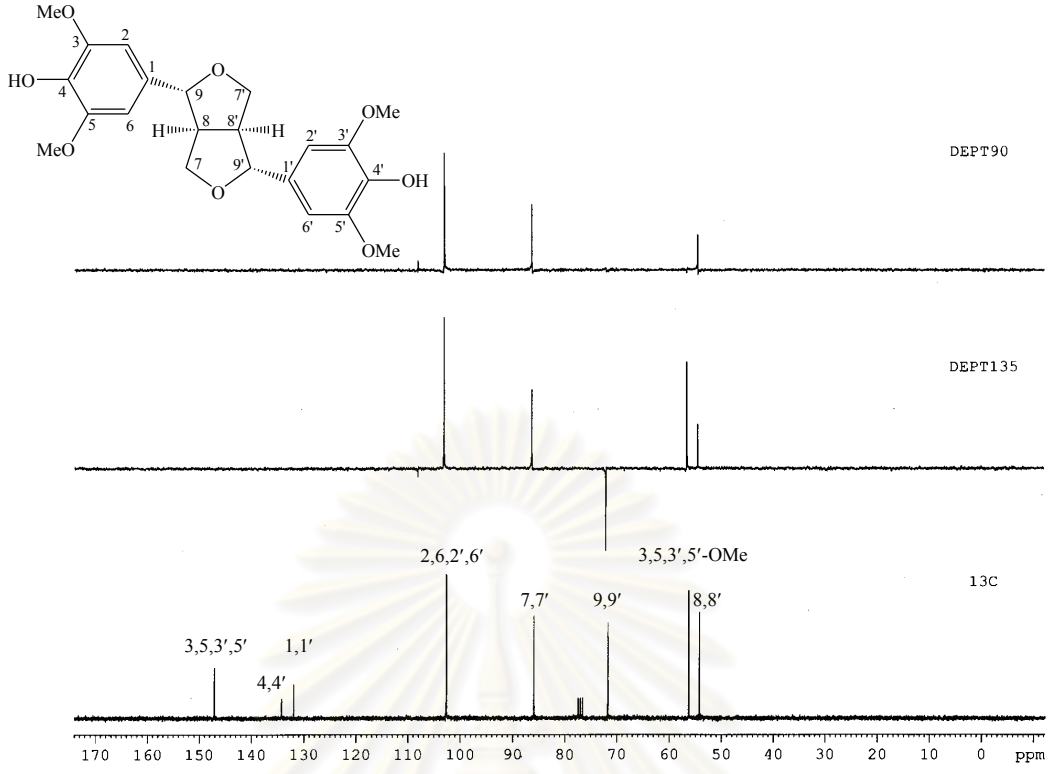


Figure 30 ^{13}C-NMR (75 MHz) and DEPT spectra of compound DS4 ($CDCl_3$)

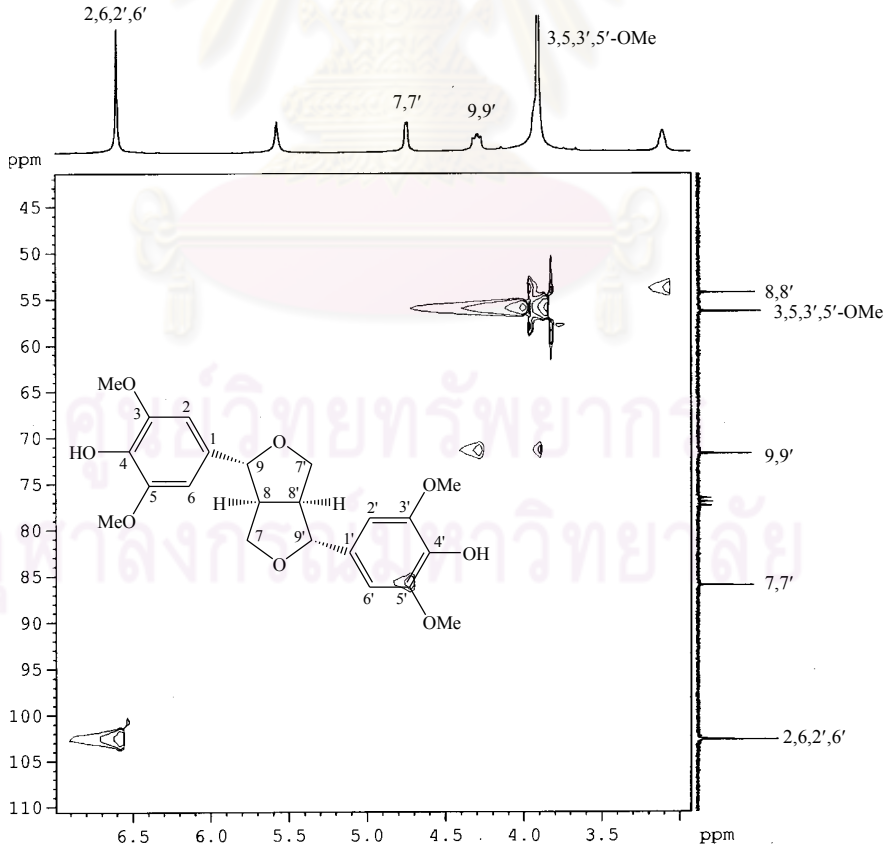


Figure 31 HMQC spectrum of compound DS4 ($CDCl_3$)

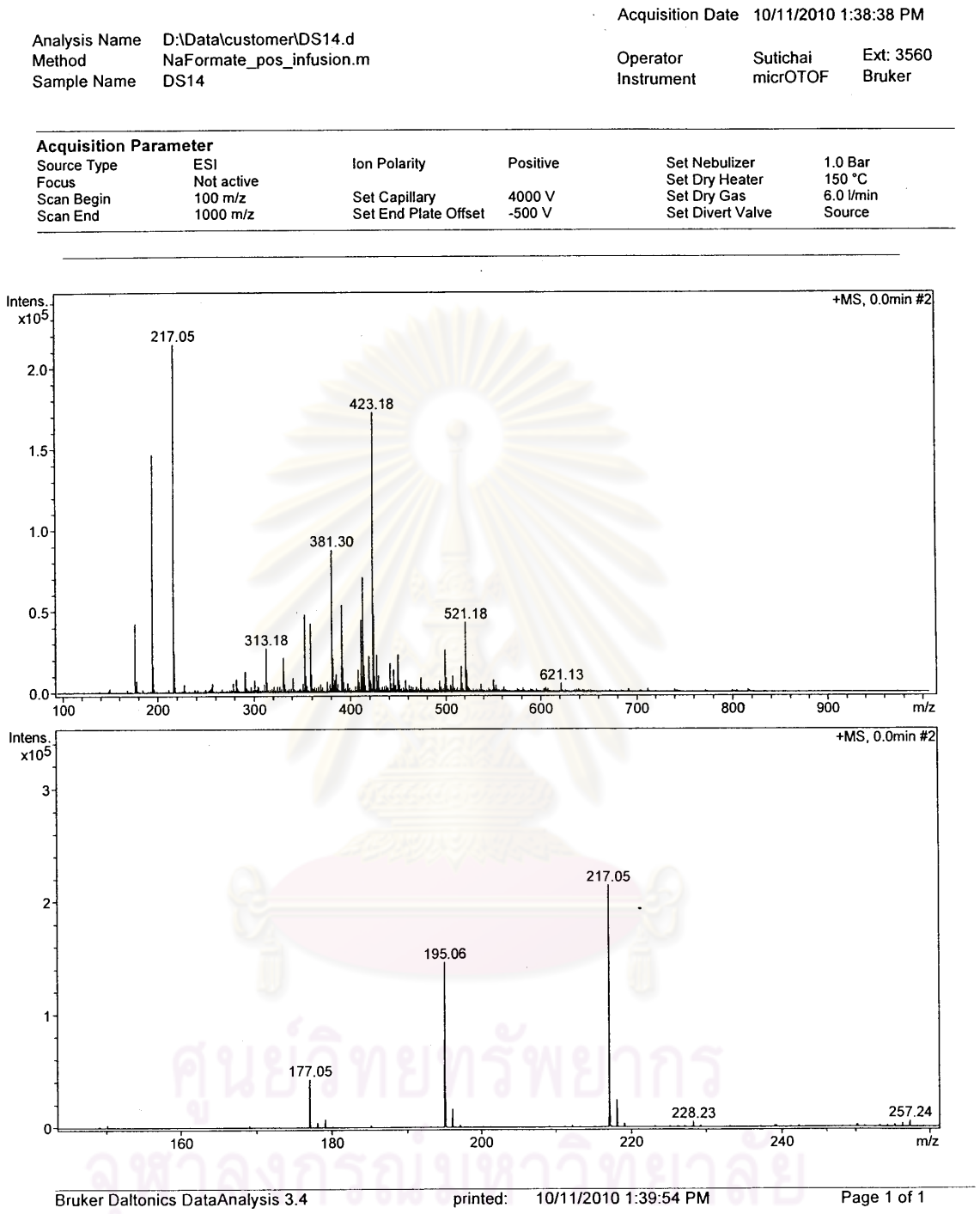


Figure 32 Mass spectrum of compound DS5 (acetone-d_6)

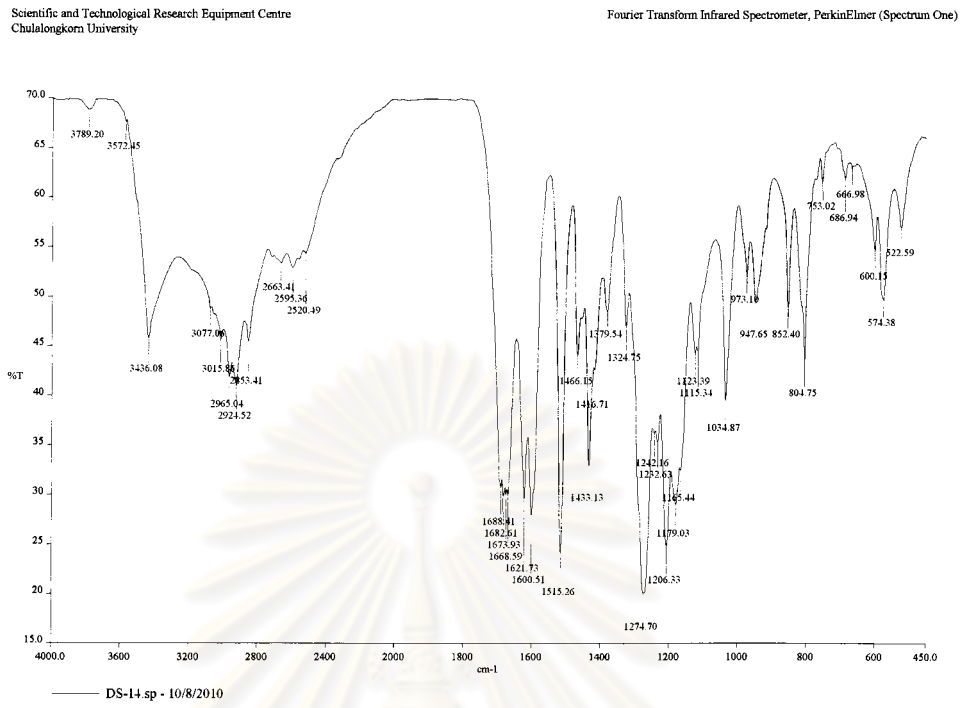


Figure 33 IR spectrum of compound DS5 (acetone-d_6)

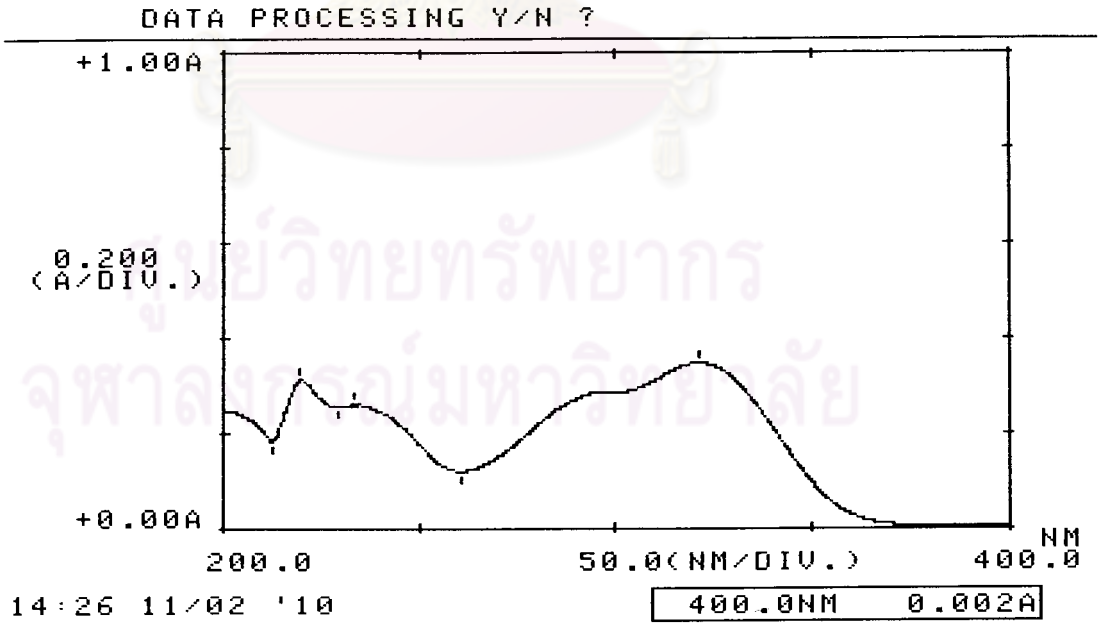


Figure 34 UV spectrum of compound DS5 (acetone-d_6)

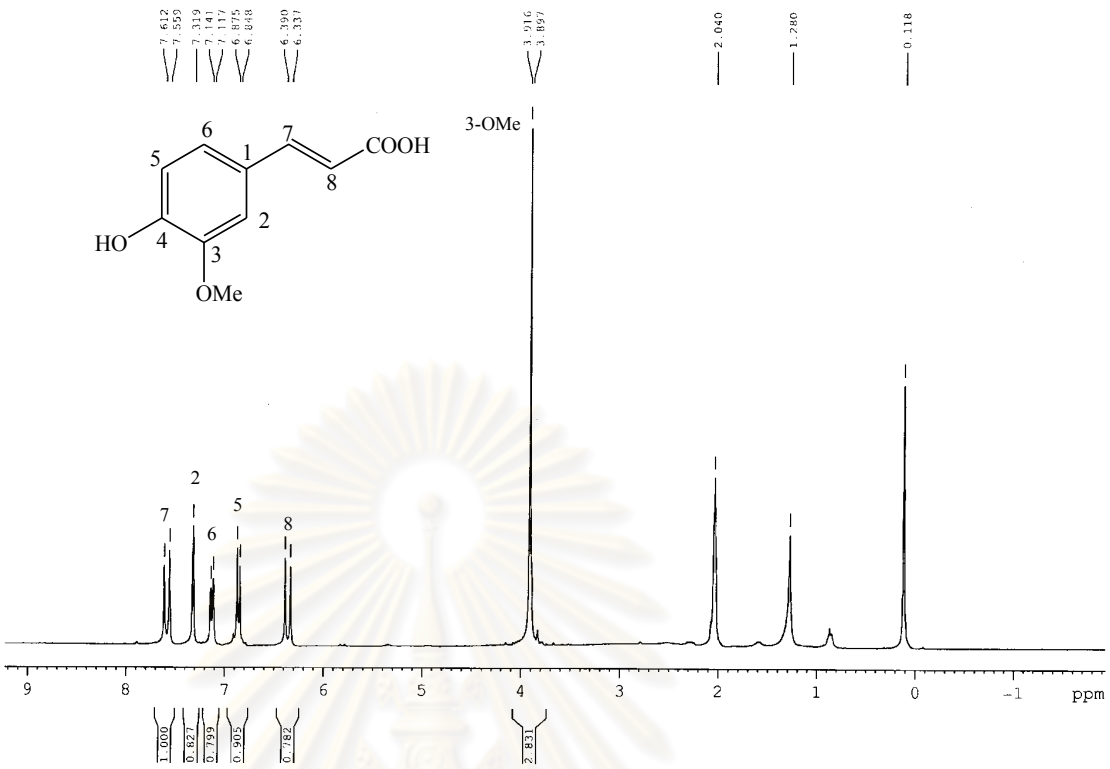


Figure 35 ^{1}H-NMR (300 MHz) spectrum of compound DS5 (acetone-d_6)

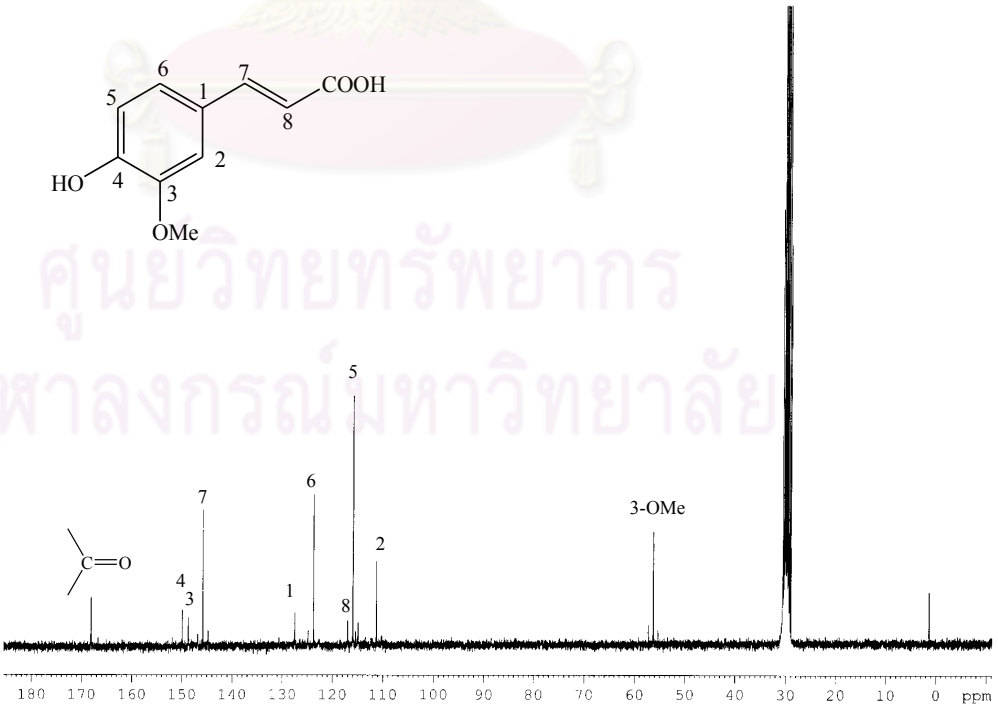


Figure 36 ^{13}C-NMR (75 MHz) spectrum of compound DS5 (acetone-d_6)

VITA

Miss Natthacha Duangrak was born on December 17, 1982 in Trang, Thailand. She received B. Sc. in Pharm. in 2006 from the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. She works at Regional Medical Sciences Center, Trang, Department of Medical Sciences, Ministry of Public Health.

Publications

1. Sritularak, B., Duangrak, N., and Likhitwitayawuid, K. 2011. A New Bibenzyl from *Dendrobium secundum*. Zeitschrift für Naturforschung C (in press).

2. Duangrak, N., Sritularak, B., and Likhitwitayawuid, K. DPPH Radical Scavengers from *Dendrobium secundum*. Abstract of The 9th NRCT-JSPS Joint Seminar, December 8-9, 2010. Faculty of Pharmaceutical Sciences Chulalongkorn University, Bangkok, Thailand. p.111-112.