CYTOTOXIC CONSTITUENTS OF *CYMBIDIUM FINLAYSONIANUM* AND *PAPHIOPEDILUM DIANTHUM*

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องค์ประกอบที่มีฤทธิ์เป็นพิษต่อเซลล์ของกะเรกะร่อนปากเป็ดและรองเท้านารีเชียงดาว

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นนทเลิศ เลิศนิติกุล : องค์ประกอบที่มีฤทธิ์เป็นพิษต่อเซลล์ของกะเรกะร่อนปากเป็ดและรองเท้านารีเชียงดาว. (CYTOTOXIC CONSTITUENTS OF *CYMBIDIUM FINLAYSONIANUM* AND *PAPHIOPEDILUM DIANTHUM*) อ.ที่ปรึกษาหลัก : รศ. ภก. ดร.รุทธ์ สุทธิศรี

การศึกษาองค์ประกอบทางเคมีของกล้วยไม้ 2 ชนิดซึ่งมีถิ่นกำเนิดในประเทศไทยนำไปสู่การสกัดแยกสาร 18 ชนิด โดยจากทั้งต้นกะเรกะร่อนปากเป็ด [*Cymbidium finlaysonianum* (Wall. ex Lindl)] สามารถแยกสารใหม่ในกลุ่ม dihydrophenanthrene ได้ 1 ชนิด คือ 1-(4-hydroxybenzyl)-2,7-dihydroxy-4,6-dimethoxy-9,10-dihydrophenanthrene และสารในกลุ่ม stilbenoid ที่เคยมีรายงานแล้ว 9 ชนิด ประกอบด้วย สารในกลุ่ม phenanthrenequinone 1 ชนิดคือ cymbinodin A, สารในกลุ่ม dihydrophenanthrenequinone 1 ชนิดคือ ephemeranthoquinone B, สารในกลุ่ม bibenzyl 1 ชนิดคือ batatasin III, สารในกลุ่ม phenanthrene 2 ชนิด คือ 2,4-dimethoxy-3,7-dihydroxyphenanthrene กับ 3,7-dihydroxy-2,4,6-trimethoxyphenanthrene และสารในกลุ่ม dihydrophenanthrene 4 ชนิด ได้แก่ coelonin, 6-methoxycoelonin, flavanthridin และ lusianthridin จากรากและใบรองเท้านารีเชียงดาว [*Paphiopedilum dianthum* (Tang & Wang)] สามารถแยกสารใหม่ในกลุ่ม stilbene dimer ได้ 2 ชนิด คือ paphiodianthin A กับ paphiodianthin B ร่วมด้วยสารในกลุ่ม stilbene ที่เคยมีรายงานแล้ว 3 ชนิดคือ pinosylvin monomethyl ether, 2,3'-dihydroxy-5'-methoxystilbene และ (*E*)-2,5'-dihydroxy-2'-(4-hydroxybenzyl)-3'-methoxystilbene กับสารในกลุ่ม flavonoid ที่เคยมีรายงานแล้ว 3 ชนิดคือ isalpinin, pinocembrin และ galangin พิสูจน์โครงสร้างทางเคมีของสารโดยอาศัยเทคนิคทางสเปกโทรสโกปี ได้แก่ UV, IR, MS และ NMR ร่วมด้วยการเปรียบเทียบข้อมูลกับที่เคยมีรายงานมาก่อนแล้ว สารที่แยกได้เหล่านี้ถูกนำไปทดสอบความเป็นพิษต่อเซลล์มะเร็งลำไส้ (Caco-2), มะเร็งเต้านม (MCF-7) และมะเร็งปอด (NCI-H187) รวมทั้งต่อเซลล์มะเร็งเต้านม MCF-7 ซึ่งดื้อยา 2 ชนิด คือ ดื้อต่อ doxorubicin (MCF-7/DOX) และ mitoxantrone (MCF-7/MX) ด้วยวิธีทดสอบในไมโครเพลทด้วย MTT และ resazurin ในบรรดาสารที่แยกได้จากกะเรกะร่อนปากเป็ด พบว่าสาร cymbinodin-A แสดงความเป็นพิษต่อเซลล์ระดับสูงสุดต่อเกือบทุกเซลล์มะเร็งที่นำมาทดสอบ (Caco-2, NCI-H187, MCF-7/DOX และ MCF-7/MX) ในขณะที่ coelonin เป็นสารที่มีความเป็นพิษสูงสุดต่อเซลล์มะเร็งเต้านม (MCF-7) สารชนิดอื่นจากกล้วยไม้นี้มีความเป็นพิษต่อเซลล์ในระดับปานกลาง สารจากรองเท้านารีเชียงดาวคือ สาร (*E*)-2,5'-dihydroxy-2'-(4-hydroxybenzyl)-3'-methoxystilbene กับ paphiodianthin A มีความเป็นพิษระดับสูงสุดต่อเซลล์ทุกชนิดที่ทดสอบ ขณะที่สาร pinosylvin monomethyl ether กับ paphiodianthin B เป็นพิษระดับสูงต่อเซลล์มะเร็งเต้านม (MCF-7) แต่มีพิษระดับปานกลางหรือไม่เป็นพิษต่อเซลล์ปกติ (NIH/3T3) นอกจากนี้ สาร isalpinin มีความเป็นพิษต่อเซลล์มะเร็งเต้านม MCF-7 ชนิดดื้อยา doxorubicin ได้สูงกว่าต่อเซลล์มะเร็งเต้านม MCF-7 ที่ไม่ดื้อยาและไม่มีพิษต่อเซลล์ปกติ

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Chemical investigation of two orchids native to Thailand led to the isolation of eighteen compounds. From the whole plants of *Cymbidium finlaysonianum* Wall. ex Lindl, a new dihydrophenanthrene, 1-(4-hydroxybenzyl)-2,7-dihydroxy-4,6-dimethoxy-9,10-dihydrophenan-threne, and nine known stilbenoids, including a phenanthrenequinone, cymbinodin-A, a dihydrophenanthrenequinone, ephemeranthoquinone B, a bibenzyl, batatasin III, two phenanthrenes, 2,4-dimethoxy-3,7-dihydroxyphenanthrene and 3,7-dihydroxy-2,4,6-trimethoxyphenanthrene, and four dihydrophenanthrenes i.e. coelonin, 6-methoxycoelonin, flavanthridin and lusianthridin, were obtained. From the roots and leaves of *Paphiopedilum dianthum* Tang & Wang, two new stilbene dimers, paphiodianthins A and B, were isolated along with three known stilbenes i.e. pinosylvin monomethyl ether, 2,3'-dihydroxy-5'-methoxystilbene and (*E*)-2,5'-dihydroxy-2'-(4-hydroxybenzyl)-3'-methoxystilbene, and three known flavonoids i.e. isalpinin, pinocembrin and galangin. The chemical structures of these compounds were determined by spectroscopic techniques including UV, IR, MS and NMR, as well as comparison with previously reported data. These isolated compounds were assayed for their cytotoxicity against colon (Caco-2), breast (MCF-7) and lung (NCI-H187) cancer cell lines, as well as two resistant sublines of MCF-7 cells (MCF-7/DOX and MCF-7/MX), by MTT and resazurin microplate assays. Among compounds isolated from *C. finlaysonianum*, cymbinodin-A displayed the strongest cytotoxicity against almost all cancer cell lines tested (Caco-2, NCI-H187, MCF-7/DOX and MCF-7/MX), whereas coelonin was the most cytotoxic constituent against MCF-7 cell line. Other compounds from this orchid were moderately cytotoxic. From *P. dianthum*, (*E*)-2,5'-dihydroxy-2'-(4-hydroxybenzyl)-3'-methoxystilbene and paphiodianthin A exhibited the strongest cytotoxic effect against all cell lines tested, while pinosylvin monomethyl ether and paphiodianthin B were strongly cytotoxic to MCF-7 cells, but were moderately toxic or non-toxic to normal cells (NIH/3T3). Moreover, isalpinin was more cytotoxic to MCF-7/DOX subline than to MCF-7 cell line and was non-toxic to normal cell line.

Field of Study:	Pharmacognosy	Student's Signature .....
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acetone-d_6	=	Deuterated acetone
α	=	Alpha
β	=	Beta
br s	=	Broad singlet (for NMR spectra)
°C	=	Degree Celsius
CC	=	Column chromatography
$CDCl_3$	=	Deuterated chloroform
CD_3OD	=	Deuterated methanol
CH_2Cl_2	=	Dichloromethane
cm	=	Centimeter
cm^{-1}	=	Reciprocal centimeter (unit of wave number)
^{13}C NMR	=	Carbon-13 nuclear magnetic resonance
d	=	Doublet (for NMR spectra)
dd	=	Doublet of doublets (for NMR spectra)
DMSO	=	Dimethylsulfoxide
DOX	=	Doxorubicin
δ	=	Chemical shift
ε	=	molar absorptivity
ESI-MS	=	Electrospray Ionization Mass Spectrometry
EtOAc	=	Ethyl acetate
et al.	=	et alibi (and others)
g	=	Gram
h	=	Hour
^1H-NMR	=	Proton nuclear magnetic resonance
HMBC	=	Heteronuclear multiple bond correlation

HR	=	High Resolution
HSQC	=	Heteronuclear single quantum coherence
Hz	=	Hertz
IC_{50}	=	Median inhibitory concentration
IR	=	Infrared spectrum
J	=	Coupling constant
KBr	=	Potassium bromide
Kg	=	Kilogram
L	=	Liter
λ_{max}	=	Wavelength at maximal absorption
µL	=	Microliter
µM	=	Micromolar
$[M - H]^-$	=	Pseudomolecular ion
$[M + Na]^+$	=	Sodium-adduct pseudomolecular ion
m	=	Multiplet (for NMR Spectra)
MeOH	=	Methanol
mg	=	Miligram
MHz	=	Megahertz
mM	=	Millimolar
min	=	Minute
mm	=	Millimeter
MS	=	Mass spectrometry
MTT	=	(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
MW	=	Molecular weight
MX	=	Mitoxantrone
m/z	=	Mass to charge ratio
ν_{max}	=	Wave number at maximal absorption

nm	=	Nanometer
NMR	=	Nuclear magnetic resonance
OD	=	Optical density
ppm	=	Part per million
rel int	=	Relative intensity
s	=	Singlet (for NMR spectra)
t	=	Triplet (for NMR spectra)
td	=	Triplet of doublets (for NMR spectra)
TLC	=	Thin layer chromatography
TOF	=	Time of flight
UV	=	Ultraviolet

CHAPTER I
INTRODUCTION

Cancer is a large group of diseases characterized by uncontrollable growth of abnormal cells in tissue or organ of the body. These cells will then metastasize or spread to other parts of the body by blood or lymphatic vessels and become a major cause of death from cancer. In 2018, World Health Organization (WHO) estimated that one in six deaths from around the world (about 9.6 million deaths) were from cancer. In men, the most common types of cancer are lung, prostate, colorectal, stomach and liver cancer while, in women, breast, colorectal, lung, cervical and thyroid cancer are generally found (1). Effective treatment of this fatal disease can be achieved through early identification, which will increase the patient's chance of survival, and reduce medical treatment fee (2).

There are several types of cancer treatment which can be used alone or in combination including surgery, chemotherapy, radiation therapy, immunotherapy, targeted therapy, hormone therapy, stem cell transplant and precision medicine (3). Chemotherapy, which is a type of treatment that uses cytotoxic compounds to damage cancer cells in the body by interrupting their cell cycle, is often used as a first-line therapy. The term "cytotoxic" consists of two words: "cyto" referring to cell and "toxic" which means poisonous. Therefore, even though chemotherapy drugs are designed to kill fast-growing cancer cells, they can also cause side effects by killing or slowing the growth of normal cells in the body (4).

Plants are important sources of several currently used drugs for cancer treatment (5). For example, vinblastine and vincristine were isolated from the Madagascar periwinkle, *Catharanthus roseus* (family Apocynaceae), as inhibitors of microtubule polymerization. Paclitaxel, a constituent from the bark of the Pacific yew

(*Taxus brevifolia,* family Taxaceae), interferes with the function of microtubule. Camptothecin, isolated from *Camptotheca acuminata* (family Nyssaceae), acts as inhibitor of topoisomerase-I while derivatives of epipodophyllotoxin from the roots of *Podophyllum peltatum* (family Berberidaceae) and the alkaloid ellipticine from *Ochrosia elliptica* and *Rauvolfia sandwicensis* (family Apocynaceae) are topoisomerase-II inhibitors (6, 7). Therefore, research and development with the aim of finding new lead compounds from plants to treat cancer are still necessary.

Orchidaceae is the largest monocotyledonous family of flowering plants, consisting of 750-850 genera or 25,000-35,000 species (8, 9). They are found in almost all continents in the world except in the desert areas and in Antarctica, and are especially abundant in tropical and sub-tropical areas (8). Various orchids are well-known as ornamental or economic plants; some of them are utilized as ingredients in cosmetics, perfumes and pharmaceutical products. Some orchid species have been employed in traditional medicines of China and India as remedy or aphrodisiac, and these uses have been recognized even in Europe, America, Australia and Africa as well (8). Hence, orchids can be considered as a source of interesting bioactive compounds. Various types of stilbenes, flavonoids, terpenoids and alkaloids have been isolated from orchids, and their biological activities have been studied, particularly in the field of anticancer, anti-inflammatory and neuroprotective activities (8, 9).

Cymbidium is one of orchid genera that have been used medicinally. For example, the pseudobulbs of *Cymbidium aloifolium* (boat orchid) and *C. longifolium* are used as emetic. The rhizomes of *C. ensifolium* are used to treat gonorrhea, while the juice of crushed *C. giganteum* leaves displays potent blood-clotting property (8). Thai traditional medicine has used juice from the leaves of *C. aloifolium* to cure otitis media (10). Phytochemical constituents and biological activities of some *Cymbidium* species such as *C. aloifolium* (11), *C. goeringii* (12), *C. pendulum* (13) and *Cymbidium*

Great Flower Marie Laurencin (14) have been studied. Phenanthrenes and their derivatives are major compounds found in *Cymbidium* orchids that show ability to kill or inhibit several cancer cell lines (14).

Many *Paphiopedilum* species are known as Venus slipper orchids and are widely cultivated as ornamental plants. This orchid genus belongs to subfamily Cypripedioideae, of which several *members,* such as *Cypripedium calceolus*, *C. elegans*, *C. parviflorum* and *C. pubescens,* have been used as traditional medicine by North American natives (8). Phenylpropanoids from *Phragmipedium calurum,* a member of this subfamily, were shown to be antiproliferative (15). Recently, a number of studies on the chemical constituents of *Paphiopedilum* orchids and their cytotoxic activity have been performed. Most of these compounds are flavonoids, stilbenes and *d*erivatives (16-18). Compounds obtained from the roots of *Paphiopedilum godefroyae*, *P. exul* and *P. callosum* were demonstrated to be strongly cytotoxic against human lung cancer cell lines (16-18), and some phytochemicals from *P. exul* and *P. callosum* might be able to inhibit the enzyme topoisomerase type 1 (17, 18).

However, studies on the phytochemistry and biological activities of these two orchid genera are limited in number and more research works should be done. Therefore, *Cymbidium finlaysonianum* and *Paphiopedilum dianthum* have been selected for this research in order to investigate their chemical constituents and their cytotoxic activity against human cancer cells. Phytochemical data from these two plants would be beneficial to the discovery of lead compounds for new anticancer drugs and would be useful data in the chemotaxonomic study of family Orchidaceae.

The purposes of this study were as follows:

1. To isolate and purify compounds from *Cymbidium finlaysonianum* and *Paphiopedilum dianthum*.
2. To determine chemical structures and physical properties of each pure compounds.
3. To evaluate cytotoxic activities of the isolated compounds.

CHAPTER II
LITERATURER REVIEW

2.1 Family Orchidaceae

Family Orchidaceae (orchid family), which belongs to the order Asparagales, is the largest family of monocotyledonous plants (19). Members of this group can be classified into more than 800 genera, consisting of over 30,000 known species (8). Owing to its variety, the family can be divided into five subfamilies namely Apostasioideae, Cypripedioideae, Epidendroideae, Orchidoideae and Vanilloideae (20). The word "orchid" is derived from "*orkhis*" in Greek, meaning testicle, based on the shape of its tubers which look like testicle. These plants may be terrestrial, epiphytic, lithophytic (growing on rocks) or saprophytic and can usually be found in both tropical and temperate regions. The stems of orchid can grow in two patterns: monopodial and sympodial growth. In the monopodial orchids, the stem grows from a single bud and new leaves are added from the apex. Their stems can be as long as several meters, as in genera *Vanda* and *Vanilla*. On the other hand, the sympodial orchids grow laterally from a series of adjacent shoots which grow to a particular size, then stop growing and are replaced by newer sprouts. These orchids often contain pseudobulbs or rhizomes (21, 22). Pseudobulbs are thickened bases or entire stems of sympodial epiphytic orchids which can store nutrients and water. Terrestrial orchids can have modified stems in the form of rhizome, tuber or corm, whereas the roots of some sympodial ones can be tuberous as a form of food storage. Epiphytic orchids usually have modified aerial roots that can absorb humidity. Orchid leaves are ordinarily simple with leaf sheath but without stipule. The venation is parallel except in some members of subfamily Vanilloideae. The shape of these leaves may be lanceolate, ovate or orbiculate and their phyllotaxy is alternate.

Orchid flowers are solitary or in an inflorescence; their shape is normally zygomorphic. The flowers consist of two whorls of trimerous perianths: three sepals (outer perianths) and three petals (inner perianths). The lowest and most prominent inner perianth, called labellum (lip), is more modified in shape, size or color than the other perianths and is exclusively found in orchid family. Each flower contains a column called gynostemium formed by the union of androecium and gynoecium (21, 22). Primitive orchids had three stamens, but modern orchids have only one stamen including members of subfamilies Orchidoideae, Vanilloideae and Epidendroideae. Two stamens can be found on each side of the gynostemium in genus *Apostasia* and subfamily Cypripedioideae. The ovaries are inferior and comprised of three carpels with parietal placentation. The fruits are loculicidal capsules containing numerous seeds (22). These small seeds are very lightweight and can easily be airborne for long distances (21, 22).

The first record of medicinal uses of orchids appeared in the 28th century B.C. China is the first nation that use some orchid species medicinally, as recorded in 'Materia Medica' of Chinese emperor 'Shen-nung' (or 'Shennong') who was a famous herbalist (8, 23). Afterwards, India, Europe, America, Australia and Africa also employ orchid preparation for therapy and nourishment. However, when compared to the size of the family, researches on phytochemicals and their biological functions have been performed on only a small fraction of its members. Therefore, orchid species are still important as a promising source of interesting compounds and bioactivities (23).

2.2 Subfamily Epidendroideae

Subfamily Epidendroideae is the largest orchid subfamily consisting more than 550 genera and 15,000 species. The majority of orchids in this subfamily are epiphytes,

but several are terrestrials and can commonly be found in the tropical and sub-tropical areas (24, 25). These orchids typically have pseudobulbs and contain only one fertile anther. Orchids from several genera in this subfamily including *Bletilla, Coelogyne Cymbidium, Encyclia, Dendrobium, Luisia, Malaxis, Nervilia* and *Vanda* have been used as ingredients in folk medicine all over the world (8).

In his Materia Medica which dated back to the 28th century B.C., Chinese Emperor "Shen-Nung" advised the use of medicinal herbs from *Bletilla hyacinthina* and a *Dendrobium* species. Later, in 1600 B.C., his herbal remedies were recorded in the Pun-tsae, a Chinese pharmacopoeia. *Dendrobium orchids* were also mentioned in another Chinese pharmacopoeia, the Sang Nung Pen Tsao Ching, as a source of tonic, astringent, analgesic and anti-inflammatory herbs in 200 B.C. During the North Sung Dynasty, medicinal uses of orchids (*Gastrodia elata* and *Dendrobium* sp.) were recommended in the book 'Zheng Lei Ben Cao' (A Diagnosis of Medical Herbs). Many references to the use of orchids as medicinal herbs in Ming Dynasty are also available (8, 23).

In South Asia, various kinds of Epidendroideae orchids such as salem (*Eulophia latifolia*), jewanti (*Dendrobium alpestre*), shwethuli (*Acampe papillosa*) and rasna (*Vanda tessellata*) were used as drugs in Ayurvedic system of traditional medicine. In addition, *Microstylis wallichii* is an ingredient of Astavarga, a rejuvenating herbal formulation. In ancient Sanskrit literature, the leaves of *Vanda roxburghii* were used externally in rheumatism, ear infections, fractures and diseases of nervous system. *Flickingeria macraei* was used as an astringent to the bowels, in asthma and bronchitis. This orchid, as well as several *Lissochilus* species, were used as aphrodisiacs (8).

In some areas of Malaysia, the decoction of *Nervilia aragoana* leaves in water was consumed by women after childbirth to reduce sickness. *Corymborchis longiflora*, *Tropidia curculigoides*, and *Acriopsis javanica* were also used to lower

malarial fever. Boiled water containing *Eria pannea* was considered a medicinal bath to decrease fever. The stems of *Lissochilus dilectus* were used in treatments for skin disease and the pseudobulbs of *Epidendrum bifidum* were used as anthelmintic against tapeworms and other intestinal parasites. In Indonesia, an odorous ointment made from the pseudobulbs of *Gammatophyllum scriptum* was believed to have analgesic property (8).

In Europe, a formulation made from the roots of *Epipactis gigantea* had been used as a drink to treat severe cases of mania. *E. helleborine* was valued as a remedy for gout, and the roots of *E. latifolia* were used to cure rheumatism (8).

In Africa, the dried, powdered *E. flaccida* was used to relieve pain from incision wounds. Some *Eulophia* species and *Ansellia humilis* were employed by Zulu women as a contraceptive drug, while the stems of *A. gigantea* were used as an aphrodisiac. *Cyrtorchis arcuata* was used as a cure for diabetes and skin infections, whereas *Eulophia cucullata* was taken orally as a prevention for epilepsy (8).

In some parts of America, the roots of an orchid commonly known as Bog-rose (*Arethusa bulbosa)* were used to relieve toothache. The pseudobulbs of *Grammatophyllum scriptum* were crushed and the paste applied to sores. Stems of *Corallorhiza odontorhiza* were used as a diaphoretic and an antipyretic in severe illnesses. The corms of *Bletia purpurea* were used as a tonic, stomachic and as an antidote of fish-poisoning. They were also freshly used for cuts and skin abrasions. North American natives used several *Goodyera* orchids as herbal remedy. For example, downy rattlesnake orchid (*G. pubescens)* was used as treatment when bitten by rabid dog. Hot poultice made from the leaves of *Vanda hookeriana* and *Spathoglottis plicata* were used to treat pains in the joints. Even in Australia, *Dendrobium teratifolium* and *D. discolor* were used medicinally for different ailments such as dysentery, pain and ringworm (8).

2.3 Genus *Cymbidium*

Cymbidium, commonly known as boat orchids, is a genus in the subfamily Epidendroideae. Orchids in this genus are epiphytic, lithophytic, terrestrial or leafless saprophytic herbs (*Cymbidium macrorhizon*) usually with pseudobulbs surrounded by leaf bases. Their roots are thick, spongy and fleshy (26). The inflorescence, which arises from the base of pseudobulb, is erect or pendent and bears one or up to 50 flowers. These usually large and showy flowers consist of free sepals and petals. The labellum next to the column has three lobes, the middle one often curved downward. The elongated column contains a pollinium and a sticky stigma on its underside (26). The fruit is capsule filled with numerous small seeds. There are about fifty *Cymbidium* species and their natural hybrids occurring in the wild from tropical and subtropical Asia to Australia.

There are 7 valid species of the genus *Cymbidium* found growing in Thailand as follows (27).

1. *Cymbidium bicolor* Lindl. (กะเรกะร่อนสองสี)
2. *Cymbidium findlaysonianum* Lindl. (กะเรกะร่อนปากเป็ด)
3. *Cymbdium ensifolium* (L.) Sw. (จุหลัน)
4. *Cymbidium lancifolium* Hook. (ตุ๊กตาร่อนเร่)
5. *Cymbidium lowianum* Rchb.f. (กะเรกะร่อนปากนกแก้ว)
6. *Cymbidium traceyanum* O'Brien (กะเรกะร่อนอินทนนท์)
7. *Cymbodium sinense* (Jacks.) Willd. (กะเรกะร่อนนิล)

Several *Cymbidium* orchids have been used as herbal medicine. Treatments employing *Cymbidium aloifolium* as an ingredient are popular in Asia, especially in

India, Nepal and Bangladesh (28, 29). For example, a mixture of this orchid roots with dried ginger and black pepper is taken to reduce paralysis. The juice from its pod or leaves is used as a treatment for earache and otitis (8). The flour made from its pseudobulbs is also used to cure cuts, sores, burns, eyestrain, chronic illness, and vertigo (28, 29). Juice from crushed leaves of *C. giganteum* has remarkable blood clotting property (8). In Australia, *C. madidam* and *C. canaliculatum* were used by Aborigines and early settlers as food and traditional medicine. Their pseudobulbs were chewed as a cure for dysentery, and seeds of *C. madidam* were used as oral contraceptive (8, 29). Moreover, *Cymbidium* flowers are also used as an ingredient in perfume, skin cream and antiaging cosmetics (29).

2.4 *Cymbidium finlaysonianum*

Cymbidium finlaysonianum is an epiphytic or lithophytic orchid. It can grow to about 90 cm tall. The ovoid pseudobulbs, up to 6 cm long and 3 cm broad, are covered by the leaf base. The orchid has 5-7 lanceolate, light green leaves with bi-lobed apex, 0.3-1 m in length and 2-4 cm in breadth. The inflorescence grows from the base of the pseudobulb, 0.3-1.3 m long, and carries numerous, slightly fragrant flowers with diameter of 5-6 cm. The oblong-linear perianths, about 3 cm long and 0.8 cm broad, consist of three yellow greenish sepals and three brown petals with reddish shades at the base. The tri-lobed labellum is 2 cm long and 1.4 cm broad. The oblong median lobe has purple red band and is curved at the apex. It is native to Thailand, Cambodia, Malaysia, Philippines, Indonesia and Vietnam (30, 31). In Thailand, this plant commonly grows on trees in field and forest. Thai herbalists use the juice from crushed leaves of *C. finlaysonianum* as a cure for otitis media (32).

A B

Figure 1. *Cymbidium finlaysonianum* Lindl.

A) Habit, showing whole plants and flowers, B) Flowers

2.5 Subfamily Cypripedioideae

Subfamily Cypripedioideae comprises five genera of lady's slipper orchids: *Cypripedium, Mexipedium, Paphiopedilum, Phragmipedium* and *Selenipedium* (33). Slipper orchids have unique morphological feature classified by slipper-shaped pouches (modified labellum) of their flowers which are designed to trap insects. Two fertile stamens are present at the backside of the shield-like staminode and two pieces of outer perianths are fused into pouch (synsepal) (34). Insects trapped in the pouch are forced to climb up behind the staminode and, thus, become a tool for pollination (35).

In the past, North American natives employed some *Cypripedium* species *such as C. acaule*, *C. reginae*, *C. candidum* and *C. parvifolium* as sedative and antispasmodic (36). *C. parvifolium* is the most medicinally recognized lady's slipper orchid.

Monographs of its powdered roots and fluidextracts appeared in earlier editions of the United States Pharmacopoeia (U.S.P.) as treatments of insomnia, anxiety, fever, headache, neuralgia, emotional tension, palpitations, tremors, delirium, convulsions, and irritable bowel syndrome (8). In Australia, another lady's slipper orchid from a different genus, *Selenipedium chica*, was used as vanilla substitute (8, 37).

2.6 Genus *Paphiopedilum*

Paphiopedilum, known as Venus slipper orchids, is a genus of subfamily Cypripedioideae which comprises about 80 accepted species. They are terrestrial or epiphytic herbs with short and hairy roots. Their leaves are glossy, or fleshy, and some of them are mottled in colors. The leaf arrangement is two-ranked (38). *Paphiopedilum* orchids grow sympodially, without pseudobulb to store moisture. The inflorescence is erect or pendant with one or several showy flowers. The synsepal is opposite the dorsal sepal which forms a hood over the flower. These two perianths are of various forms and colors depending on their species. The modified lip looks like a shoe. The staminal column contains an apical staminode, two anthers, and a disc-like stigma behind the staminode (38). Members of this genus are native to Southeast Asia, southern China, New Guinea and the Solomon Islands (39).

There are 14 valid species of the genus *Paphiopedilum* found growing in Thailand as follows (40).

1. *Paphiopedilum appletonianum* (Gower) Rolfe (รองเท้านารีคางกบคอแดง)
2. *Paphiopedilum bellatulum* (Rchb.f.) Stein (รองเท้านารีฝาหอย)
3. *Paphiopedilum callosum* (Rchb.f.) Stein (รองเท้านารีคางกบ)
4. *Paphiopedilum charlesworthii* (Rolfe) Pfitzer (รองเท้านารีดอยตุง)

5. *Paphiopedilum concolor* (Lindl. ex Bateman) Pfitzer (รองเท้านารีเหลืองปราจีน)

6. *Paphiopedilum dianthum* Tang & Wang (รองเท้านารีเชียงดาว)

7. *Paphiopedilum exul* (Ridl.) Rolfe (รองเท้านารีเหลืองกระบี่)

8. *Paphiopedilum godefroyae* (Godefroy) Pfitzer (รองเท้านารีเหลืองตรัง)

9. *Paphiopedilum gratrixianum* (Mast.) Guillaumin (รองเท้านารีอินทนนท์ลาว)

10. *Paphiopedilum hirsutissimum* (Lindl. ex Hook.) Stein (รองเท้านารีเหลืองเลย)

11. *Paphiopedilum niveum* (Rchb.f.) Pfitzer (รองเท้านารีขาวสตูล)

12. *Paphiopedilum parishii* (Rchb.f.) Pfitzer (รองเท้านารีหนวดฤาษี)

13. *Paphiopedilum sukhakulii* Schoser & Senghas (รองเท้านารีปีกแมลงปอ)

14. *Paphiopedilum villosum* (Lindl.) Stein (รองเท้านารีอินทนนท์)

2.7 *Paphiopedilum dianthum*

Paphiopedilum dianthum is commonly known as double flowered Paphiopedilum. It grows on rocks in shaded areas of mixed forest at 800-2,250 m above sea level (41). It is native to southern and southeastern China, Laos and Vietnam (41, 42). It can also be found in northern part of Thailand. The roots of this orchid are red, elongated and pubescent. Its stem carries 4 to 6 leathery leaves which are dark green above and light green beneath. These leaves are ligulate in shape, obliquely obtuse at the apex, 20–50 cm long and 2–5 cm wide. Peduncle of the inflorescence is up to 80 cm long and bears 2 to 5 flowers. Each flower is up to 10 cm wide and has an obcordate staminode. The dorsal sepal is white with green veining, and the synsepal is greenish to yellow-green with green veining. The striped greenish or brownish lateral petals are long and twisted with hairy warts. The labellum is helmet-shaped (41).

A

Figure 2. *Paphiopedilum dianthum* Tang & Wang

A) Habit, showing whole plants and flowers

2.8 Stilbenoids from orchids of tribe Cymbidieae and subfamily Cypripedioideae

Cymbidium is a genus in the tribe Cymbidieae of subfamily Epidendroideae. This tribe consists of 11 subtribes and approximately 200 genera. Some orchids in Cymbidieae tribe have been used as folk medicine (43). However, only ten genera from seven subtribes have been investigated phytochemically, and a number of studies on biological activities of the isolated compounds have been performed.

Genus *Cymbidium*, which belongs to subtribe Cymbidiinae, is one of these investigated genera. One reason is that the genus is a source of herbal medicine. In

particular, *Cymbidium aloifolium* is well-known in Southeast Asia as a medicinal orchid. Chemical studies of its roots revealed bibenzyls and 9,10-dihydrophenanthrenes as their major compounds. Examples of these bibenzyls include aloifol I **[80]**, aloifol II **[81]**, batatasin III **[82]** and gigantol **[87]**, and the isolated 9,10-dihydrophenanthrenes were coelonin **[30]** and 6-*O*-methylcoelonin **[51]** (44). A phenanthraquinone, named cymbinodin-A **[89]**, was later obtained from its whole plant (11). Family Orchidaceae is the most abundant source of phenanthrenes, which can also serve as chemotaxonomic markers, and many of them displayed several biological activities (9, 45, 46). One example is coelonin **[30]**, firstly isolated from the orchids *Coelogyne ochracea* and *C. elata* (47), which showed cytotoxic activity against some cancer cell lines (48) and anti-inflammatory activity in the lipopolysaccharide-induced macrophage inflammation model (49).

Two other *Cymbidium* orchids, *C. giganteum* (50) and *C. goeringii* (12), also yielded the bibenzyl (dihydrostilbene) gigantol. The compound, obtained from *C. goeringii*, displayed anti-inflammatory activity through inhibition of iNOS and COX-2 expression via NF-**K**B inactivation in RAW 264.7 macrophages (12).

Gigantol **[87]**, which was named after *C. giganteum* from which it was discovered for the first time, can also be found in *Dendrobium* orchids such as *Dendrobium nobile* (51), *D. chrysotoxum* (52), *D. gratiosissimum* (53) and *D. aurantiacum* var. *denneanum* (54). *Dendrobium* orchids belong to a different tribe (Dendrobieae) from genus *Cymbidium*, but they are in the same subfamily Epidendroideae. Multiple biological effects of gigantol have been reported including antioxidant (54), anti-cataract (52), antimutagenic (51), antispasmodic (55) and cytotoxic activities (53, 56). There are several mechanisms by which gigantol could act against cancer cells. This bibenzyl has been demonstrated to be able to suppress cancer stem

cells (57), inhibit their growth (58) and migration (59), and induce anoikis (60) and apoptosis of cancer cells (58, 61).

Batatasin III **[82]** is another bibenzyl commonly found in many orchids. In addition, it has also been reported as a constituent of dicotyledonous plants such as crowberry (*Empetrum nigrum*, family Ericaceae) (62) and water yam (*Dioscorea alata*, family Dioscoreaceae) (63). In these plants, batatasin III and its derivatives might be synthesized as a phytoalexin against fungal invasion, or a factor in the control of dormancy of yam (*Dioscorea*) bulbils (64). This bibenzyl has been considered a natural allelopathic compound which might be useful in the prevention of marine biofouling (accumulation of marine organisms such as algae and barnacles on underwater surfaces) (62). A number of beneficial health effects of batatasin III have been described e.g. antiallergic (65), α-glucosidase inhibitory (66, 67), antifungal (68), antispasmodic (55) and cytotoxic activities (56).

Investigation of another *Cymbidium* orchid, *Cymbidium pendulum,* revealed the presence of the phenanthrenes denthyrsinin **[6]** and penduline **[25]** (13). Chemical constituents of a *Cymbidium* hybrid species, called *Cymbidium* Great Flower Marie Laurencin, was studied and ten phenanthrene derivatives were isolated and identified including ephemeranthoquinone **[94]**, marylaurencinols A **[47]** and B **[48]**, marylaurencinoside A **[49]**, 3- hydroxy- 2,4,7- trimethoxy- 9,10- dihydrophenanthrene **[46]**, 3-hydroxy-2,4,7-trimethoxyphenanthrene **[21]**, penduline **[25]**, erianthridin **[41]**, flavanthridin **[43]** and 1,5,7-trimethoxy-phenanthrene-2,6-diol **[6]**. Ephemeranthoquinone **[94]** was active against *Bacillus subtilis,* with an MIC value of 4.88 μM, and human promyelocytic leukemia (HL-60) cell line, with an IC_{50} value of 2.8 μM (14).

The genus *Cyrtopodium* of terrestrial or epiphytic orchids has 47 identified species, mostly endemic to the area from Southern Florida to Central America (69).

Some *Cyrtopodium* species such as *Cyrtopodium macrobulbon* were used in traditional medicine to treat urinary infections (69). Phytochemical constituents of *Cyrtopodium macrobulbon* include aromatic compounds, phenylpropanoids and the stilbenoids 2,6-dihydroxy-1,5,7-trimethoxy-9,10-dihydrophenanthrene **[35]**, confusarin **[5]**, gigantol **[87]**, batatasin III **[82]** and flavanthridin (ephemeranthol B) **[43]**. Both gigantol **[87]** and batatasin III **[82]** isolated from this orchid showed antinociceptive activity (69). Phytochemical study of *Cyrtopodium paniculatum* whole plants yielded numerous secondary metabolites, for example, aromatic glycosides, flavonoid, phenanthrenes **1-2, 4-7, 15-16, 18** and **24**, dihydrophenanthrenes **29-31, 36-37, 41, 45-46** and **53**, dihydrostilbenes **82** and **87**, phenanthrenequinone **91**, dihydrophenanthrenequinones **93** and **94**, biphenanthrenes **96-105** and dihydrophenanthrofurans **108-110** (70, 71). Among these compounds, blestriarenes A, B and C **[96-98]** and lusidol A **[103]** displayed moderate cytotoxicity to human glioblastoma (U-87) cell line (70). Recently, a study on the chemical constituents of the pseudobulb of *Cyrtopodium glutiniferum* found that it contained the stilbenoids 2,7- dihydroxy- 1- (4- hydroxybenzoyl) -4- methoxy- 9,10- dihydrophenanthrene **[36]**, denthyrsinin **[7]** and phoyunbene D **[76]** (72).

Up to the present, five species of *Eulophia* orchids have been studied. One of these species, *Eulophia nuda,* has been used in Thai traditional medicine to treat skin rash (73). Its major compounds are stilbenoids including eulophiol **[42]** (74), nudol **[24]** (75), 1,7-dihydroxy-2,5-dimethoxy-9,10- dihydrophenanthrene **[32]**, 2,7- dihydroxy- 4 - methoxv- 9,10- dihydrophenanthrene **[30]**, 2,7-dihydroxy-1,5-dimethoxy-phenanthrene **[11],** denthyrsinin **[7],** flavanthridin **[43]**, the biphenanthrene **107** (73), 2,8-dihydroxy-1-(4'-hydroxybenzyl)-4,7-dimethoxy-9,10-dihydrophenanthrene **[39]**, 1-(4'-hydroxybenzyl)-4,8-dimethoxy-2,7-phenanthrenediol **[20]** and 3,4'-dihydroxy-3',5,5'-trimethoxyhydrostilbene **[85]** (76). Compound **32** was cytotoxic against two breast

cancer cell lines (77). Another *Eulophia* orchid that can be found growing in Thailand is *E. macrobulbon*. Extract from tubers of this orchid could inhibit the enzyme phosphodiesterase-5 (PDE5) and act as vasorelaxant. Its active compound was shown to be 1-(4'-hydroxybenzyl)-4,8-dimethoxy-2,7-phenanthrenediol **[20]** (81-83). In addition, the orchid contains other stilbenoids i.e. denthyrsinin **[7A]**, phenanthrene **11**, flavanthrinin **[17]** and dihydrophenanthrenes **30**, **32** and **33** (78-81). Almost all compounds from this orchid appeared to be active against several cancer cell lines (80).

In India, the tubers of *Eulophia ochreata* have been used for rejuvenating and aphrodisiac purposes and for treating rheumatism (82). The extract of this plant showed antioxidant activity that might be related to its rejuvenating property. Two compounds, 1,7-dihydroxy-2,5-dimethoxy-9,10-dihydrophenanthrene **[32]** and flavanthridin **[43]**, were isolated and identified from the extract. Both compounds displayed radical scavenging activity (82), while compound **32** could also act as an anti-inflammatory agent (83). The tubers of another *Eulophia* orchid, *E. herbacea*, yielded 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-1-phenanthrenecarboxylic acid methyl ester **[52]** which was cytotoxic against many human cancer cell lines (84). Furthermore, the roots of an African *Eulophia* orchid, *E. petersii*, have been phytochemically studied to afford five compounds, namely, coelonin **[30]**, 1,7-dihydroxy-2,5-dimethoxy-9,10-dihydrophenanthrene **[32],** lusianthridin **[46]**, 2,7-dihydroxy-4,8-dimethoxy-phenanthrene **[14]** and lusianthrin **[22]** (85).

Maxillaria is a large genus of mostly epiphytic orchids native to Latin American rainforest. *Maxillaria densa* is an orchid used traditionally for the treatment of gastrointestinal diseases (86). Its chemical constituents were shown to be 2,5-dihydroxy-3,4-dimethoxyphenanthrene **[9]**, 2,5-dihydroxy-3,4-dimethoxv-9,10-dihydro-phenanthrene **[34]**, erianthridin **[41]**, gymnopusin **[19]**, fimbriol A **[16]** and nudol **[24]**

(86-90). These stilbenoids displayed biological activities such as smooth muscle relaxant activity, antinociceptive and anti- inflammatory effect (87-89). In addition, gymnopusin and erianthridin exhibited phytotoxicity and ultrastructural effects against duckweed (*Lemna pausicostata*), but their moderate cytotoxicity to mammalian cells makes them unsuitable to be used as bioherbicides (90).

Erianthridin **[41]** is a 9,10-dihydrophenanthrene that has been isolated from several orchids within the same subfamily (Epidendroideae) but different tribes from genus *Cymbidium*, such as *Eria convallarioides* (91), *Ephemerantha lonchophylla* (92), *Pholidota chinensis* (93), *Dendrobium formosum* (94), *D. nobile* (95), *Liparis viridiflora* (96), *Bletilla ochracea* (97), *B. formosana* (98) and *Arundina graminifolia* (99). This stilbenoid has been shown to possess antiplatelet aggregation (77) and anti-inflammatory activities (98).

Another *Maxillaria* species, *M. picta*, was investigated, and the stilbenes phoyunbene B **[74],** phoyunbene C **[75]**, in addition to xanthones and phenolic acids, were isolated from its pseudobulbs (100). Vasorelaxant stilbenoids were also found in *M. porphyrostele.* Phytochemical study of this orchid afforded four bioactive compounds: 2,7- dihydroxy- 4,6-dimethoxy-9,10-dihydrophenanthrene **[38]**, 1,2,6,7-tetrahydroxy-4-methoxy-phenanthrene **[27]**, 3,4'-dihydroxy-3',5'-dimethoxydihydro-stilbene **[83]** and 3,4'-dihydroxy-5,5'-dimethoxydihydrostilbene **[84].** Compounds **83** and **84** showed *in vitro* vasorelaxing activity on rat aorta rings pre-treated with either phenylephrine or high K^+ (101, 102).

Peyote (*Lophophora williamsii*) is a well-known hallucinogenic cactus used by the Tarahumara Indians of northern Mexico in religious ceremonies. Sometimes, a Cymbidieae orchid, *Oncidium cebolleta,* has been used as a temporary replacement. The orchid was studied chemically, and five phenanthrene derivatives **[8, 12, 14, 24, 37]** were isolated and identified (103). Other *Oncidium* species have also been

explored phytochemically. For example, *O. baueri* yielded an admixture of triterpenoids, four flavonoid glycosides and the stilbenoids batatasin III **[82]** and moscatin **[23]** (104). Five **[23, 35, 40, 82, 95]**, four **[10, 23, 26, 88]** and four stilbenoids **[23, 46, 59, 60]** were isolated from *O. isthmi*, *O. microchilum* and *O.* Sharry Baby, respectively. All compounds displayed cytotoxic activity against human non-small cell lung cancer (NCI-H460) and human melanoma (M14) cell lines and the mechanism might involve the induction of apoptosis (105).

Other genera in the same subtribe Oncidiinae as genus *Oncidium* that have been studied include *Odontoglossum* and *Gomesa*. The dried bulb of an *Odontoglossum* hybrid called *Odontoglossum* Harvengtense ‘ Tutu’ yielded two phenanthrenes **[10, 17]**, a phenanthrenequinone **[92]** and a flavone (106), whereas phytochemical study of *Gomesa recurva* whole plants afforded four phenylpropanoids and four phenanthrenes **[3, 8-9, 27]** (107).

Genus *Catasetum* belongs to another subtribe (Catasetinae) of tribe Cymbidieae, in which there is only one report describing anti-inflammatory chemical constituents of *Catasetum barbatum*. In Paraguay, this orchid has been used as folk medicine to treat asthma, lumbago, and many other ailments. Four stilbenoids were reported from this plant including nudol **[24]**, 2,7-dihydroxy-3,4,8 trimethoxy-phenanthrene **[13]**, 2,7-dihydroxy-3,4-dimethoxy- 9,10-dihydrophenanthrene **[41]** and 3,4'-dihydroxy-5,5'- dimethoxydihydrostilbene **[84]**. Nudol **[24]** showed anti-inflammatory activity in the carrageenan-induced paw edema test and inhibitory effect on histamine-induced contraction in guinea pig ileum (108).

The last subtribe of tribe Cymbidieae that has been studied is Stanhopeinae. There is only one research on the chemical constituents of *Stanhopea lietzei* which yielded 4,4',6,6'-tetrahydroxy-2,2',7,7'-tetramethoxy-(1,1')-biphenanthrene **[106]**, 6-*O*-

methylcoelonine **[50]** and a flavonoid, isovitexin. Compound **106** was active against human cervical cancer (HeLa) cell line, with an IC_{50} of 16.9 μg/mL (109).

Currently, only a small number of phytochemical studies on slipper orchids from subfamily Cypripedioideae have been performed. Stilbenes, benzofurans and phenanthrenes are major types of stilbenoids isolated from these orchids, and many of them could inhibit the growth of several cancer cell lines.

The earliest phytochemical study on slipper orchids was performed on *Cypripedium calceolus* (110). The phrenanthrenequinone cypripedin **[90]** was reported as the skin sensitizing constituent of the orchid. Another study, on the seedlings of *C. macranthos* var. *rebunense* revealed the presence of a phrenanthrene, lusianthrin **[22]** and a dihydrophenanthrene, orchinol **[50]** (111). Lusianthrin, which highly increased following infection with symbiotic fungus, was believed to be an important antifungal compound that the orchid produced during its symbiotic germination.

Phytochemical studies have been done on two *Phragmipedium* species and one *Phragmipedium* hybrid. In the first study, *Phragmipedium calurum*, *Phragmipedium longifolium* and *Phragmipedium hybrid* var.Sorcerer's Apprentice were extracted to obtain eleven **[54-55, 57, 60-62, 66-68, 70-71]**, ten **[55, 60-62, 66-68, 71, 79, 115]**, and nine **[55-56, 59, 61-62, 65, 67, 114-115]** stilbenoids, respectively (112). A few years later, *Phragmipedium calurum* was studied again and yielded seven stilbenes **[63-64, 66-69, 22]** which showed antiproliferative activity on several cancer cell lines. Compounds **66** and **69** were markedly active (15).

Chemical constituents and biological activities of three *Paphiopedilum* orchids have been investigated; all three species are slipper orchids found in Thailand. *Paphiopedilum godefroyae* is the first one that was studied. Six stilbenes **[54-56, 63, 72, 78]** and three benzofurans **[1I-3I]** were obtained (16). Most of these stilbenoids

could inhibit the proliferation of human small cell lung cancer (NCI-H187) cell line. The benzofuran **112** was strongly cytotoxic, with an IC_{50} value of 5.10 μM (16). Next, the roots of *Paphiopedilum exul* were investigated, and six stilbenes **[56, 58 62, 67, 72, 77]** were isolated. All compounds were assayed for their cytotoxicity against three cancer cell lines (KB, MCF-7 and NCI-H187) (17). Recently, bioassay-guided extraction of the roots of *Paphiopedilum callosum* yielded four stilbenes **[55-56, 72-73]** which were cytotoxic to both MCF-7 and NCI-H187 cancer cell lines (18).

Stilbenoids from orchids of tribe Cymbidieae and subfamily Cypripedioideae are summarized in **Table 1** and their biological activities are summarized in **Table 2**. Their chemical structures are shown in **Figure 3**.

Table 1. Stilbenoids from orchids of tribe Cymbidieae and subfamily Cypripedioideae

Compounds	Sources	Plant part	Reference
A. Phenanthrenes			
Blefomin A [**1**]	*Cyrtopodium paniculatum*	pseudobulbs	(70)
Blefomin B [**2**]	*Cyrtopodium paniculatum*	pseudobulbs	(70)
Bulbophyllanthrin [**3**]	*Gomesa recurva*	whole plants	(107)

Confusaridin [**4**]	*Cyrtopodium paniculatum*	roots	(71)
Confusarin [**5**]	*Cyrtopodium macrobulbon*	pseudobulbs	(69)
	Cyrtopodium paniculatum	pseudobulbs	(70)
		roots	(71)
Cyrtopodin [**6**]	*Cyrtopodium paniculatum*	pseudobulbs	(70)
		roots	(71)
Denthyrsinin [**7**]	Cymbidium Great Flower Marie Laurencin	roots	(14)
	Cymbidium pendulu	whole plants	(13)
	Cyrtopodium glutiniferum	pseudobulbs	(72)
	Cyrtopodium paniculatum	pseudobulbs	(70)
		roots	(71)
	Eulophia macrobulbon	tubers	(80, 81)
	E. nuda	tubers	(73)

Table 1. Continued

Compounds	Sources	Plant part	Reference
2,3-Dihydroxy-4,7,8-trimethoxyphenanthrene [**8**]	*Oncidium cebolleta*	leaves	(103)
2,5-Dihydroxy-3,4-dimethoxyphenanthrene [**9**]	*Maxillaria densa*	whole plants	(86, 88)
	G. recurva	whole plants	(107)

2,5-Dihydroxy-4,9-dimethoxy phenanthrene [**10**]	Odontoglossum Harvengtense ‘Tutu’	bulbs	(106)
	Oncidium microchilum	whole plants	(105)
2,7-Dihydroxy-1,5-dimethoxyphenanthrene [**11**]	*E. macrobulbon*	tubers	(81)
	E. nuda	tubers	(73)
2,7-Dihydroxy-3,4,6-trimethoxyphenanthrene [**12**]	*Oncidium cebolleta*	leaves	(103)
2,7-Dihydroxy-3,4,8-trimethoxyphenanthrene [**13**]	*Catasetum barbatum*	aerial parts	(108)
2,7-Dihydroxy-4,8-dimethoxyphenanthrene [**14**]	*E. petersii*	roots	(85)
	Oncidium cebolleta	leaves	(103)
3,7-Dihydroxy-2,4-dimethoxyphenanthrene [**15**]	*Cyrtopodium paniculatum*	pseudobulb	(70)
		roots	(71)
Fimbriol A [**16**]	*G. recurva*	whole plants	(107)
	M. densa	whole plants	(87-89)
Flavanthrinin [**17**]	*E. macrobulbon*	tubers	(80)
	Odontoglossum Harvengtense ‘Tutu’	bulbs	(106)
Gastrodiconfusarin [**18**]	*Cyrtopodium paniculatum*	pseudobulb	(70)

Table 1. Continued

Compounds	Sources	Plant part	Reference
Gymnopusin [**19**]	*M. densa*	whole plants	(87, 88, 90)
1-(4'-Hydroxybenzyl)-4,8-dimethoxy-2,7-phenanthrenediol [**20**]	*E. macrobulbon*	tubers	(78-81)
	E. nuda	tubers	(76)

3-Hydroxy-2,4,7-trimethoxyphenanthrene [**21**]	Cymbidium Great Flower Marie Laurencin	roots	(14)
Lusianthrin [**22**]	*Cypripedium macranthos var.rebunense*	seedlings	(111)
	E. petersii	roots	(85)
Moscatin [**23**]	*Oncidium baueri*	whole plants	(104)
	O. isthmi	whole plants	(105)
	O. microchilum	whole plants	(105)
	Oncidium Sharry Baby	whole plants	(105)
Nudol [**24**]	*Catasetum barbatum*	aerial parts	(108)
	Cyrtopodium paniculatum	pseudobulbs	(70)
		roots	(71)
	E. nuda	tubers	(75)
	M. densa	whole plants	(88)
	O. cebolleta	leaves	(103)
Pendulin [**25**]	Cymbidium Great Flower Marie Laurencin	roots	(14)
	Cymbidium pendulu	whole plants	(13)

Table 1. Continued

Compounds	Sources	Plant part	Reference
Plicatol A [**26**]	*O. microchilum*	whole plants	(105)
1,2,6,7-Tetrahydroxy-4-methoxyphenanthrene [**27**]	*M. porphyrostele*	-	(102)

2,3,5-Trihydroxy-4-methoxyphenanthrene [**28**]	*G. recurva*	whole plants	(107)
B. Dihydrophenanthrenes Cephathrene B [**29**]	*Cyrtopodium paniculatum*	pseudobulbs	(70)
Coelonin [**30**]	*Cymbidium aloifolium*	roots	(44)
	Cyrtopodium paniculatum	pseudobulbs, leaves	(70)
		roots	(71)
	E. petersii	roots	(85)
	E. macrobulbon	tubers	(80)
	E. nuda	tubers	(73)
Cyrtopodinol [**31**]	*Cyrtopodium paniculatum*	roots	(71)
1,7-Dihydroxy-2,5-dimethoxy-9,10-dihydrophenanthrene [**32**]	*E. macrobulbon*	tubers	(80)
	E. nuda	tubers	(73, 77)
	E. ochreata	tubers	(82, 83)
	E. petersii	roots	(85)
1,7-Dihydroxy-4-(4'-hydroxybenzyl)-2,5-dimethoxy-9,10-dihydrophenanthrene [**33**]	*E. macrobulbon*	tubers	(81)
2,5-Dihydroxy-3,4-dimethoxv-9,10-dihydrophenanthrene [**34**]	*M. densa*	whole plants	(86)

Table 1. Continued

Compounds	Sources	Plant part	Reference
2,6-Dihydroxy-1,5,7-trimethoxy-9,10-dihydrophenanthrene [**35**]	*Cyrtopodium macrobulbon*	pseudobulbs	(69)

	O. isthmi	whole plants	(105)
2,7-Dihydroxy-1-(4-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene [**36**]	*Cyrtopodium glutiniferum*	pseudobulbs	(72)
	Cyrtopodium paniculatum	pseudobulbs	(70)
2,7-Dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene [**37**]	*Cyrtopodium paniculatum*	pseudobulbs	(70)
		roots	(71)
	O. cebolleta	leaves	(103)
2,7-Dihydroxy-4,6--dimethoxv-9,10-dihydrophenanthrene [**38**]	*M. porphyrostele*	-	(102)
2,8-Dihydroxy-1-(4'-hydroxybenzyl)-4,7-dimethoxy-9,10-dihydrophenanthrene [**39**]	*E. nuda*	tubers	(76)
4,5-Dihydroxy-2-methoxy-9,10-dihydrophenanthrene [**40**]	*O. isthmi*	whole plants	(105)
Erianthridin [**41**]	*Catasetum barbatum*	aerial parts	(108)
	Cymbidium Great Flower Marie Laurencin	roots	(14)
	Cyrtopodium paniculatum	pseudobulbs	(70)
		roots	(71)
	M. densa	whole plants	(86-90)
Eulophiol [**42**]	*E. nuda*	tubers	(74)

Table 1. Continued

Compounds	Sources	Plant part	Reference

Flavanthridin [**43**]	Cymbidium Great Flower Marie Laurencin	roots	(14)
	Cyrtopodium macrobulbon	pseudobulbs	(69)
	E. nuda	tubers	(73)
	E. ochreata	tubers	(82)
3-Hydroxy-2,4,7-trimethoxy-9,10-dihydrophenanthrene [**44**]	Cymbidium Great Flower Marie Laurencin	roots	(14)
(*S*)-9-Hydroxy-erianthridin [**45**]	*Cyrtopodium paniculatum*	pseudobulbs	(70)
Lusianthridin [**46**]	*Cyrtopodium paniculatum*	pseudobulbs, leaves	(70)
		roots	(71)
	E. petersii	roots	(85)
	Oncidium Sharry Baby	whole plants	(105)
Marylaurencinols A [**47**]	Cymbidium Great Flower Marie Laurencin	roots	(14)
Marylaurencinols B [**48**]	Cymbidium Great Flower Marie Laurencin	roots	(14)
Marylaurencinoside A [**49**]	Cymbidium Great Flower Marie Laurencin	roots	(14)

Table 1. Continued

Compounds	Sources	Plant part	Reference
Orchinol [**50**]	*Cypripedium calceolus*	-	(110)
6-*O*-methylcoelonin [**51**]	*Cymbidium aloifolium*	roots	(44)
	Stanhopea lietzei	whole plants	(109)
1,2,3,4,4a,9,10,10a-Octahydro-1,4a-dimethyl-1-phenanthrene-carboxylic acid methyl ester [**52**]	*E. herbacea*	tubers	(84)
Shancidin [**53**]	*Cyrtopodium paniculatum*	pseudobulbs, leaves	(70)
C.Stilbenes 2,3-Dihydroxy-3',5'-dimethoxystilbene [**54**]	*Phragmipedium calurum*	whole plants	(112)
	Paphiopedilum godefroyae	roots	(16)
2,3'-Dihydroxy-5'-methoxystilbene [**55**]	*Paphiopedilum callosum*	roots	(18)
	Paphiopedilum godefroyae	roots	(16)
	Phragmipedium calurum	whole plants	(112)
	Phragmipedium longifolium	whole plants	(112)
	Phragmipedium hybrid (var. Sorcerer's Apprentice)	whole plants	(112)

Table 1. Continued

Compounds	Sources	Plant part	Reference
2,3'-Dihydroxy-5,5'-dimethoxystilbene [**56**]	*Paphiopedilum callosum*	roots	(18)
	Paphiopedilum exul	roots	(17)
	Paphiopedilum godefroyae	roots	(16)
	Phragmipedium hybrid (var. Sorcerer's Apprentice)	whole plants	(112)
3',4'-Dihydroxy-5'-methoxystilbene [**57**]	*Phragmipedium calurum*	whole plants	(112)
5,6'-Dihydroxy-3,2'-dimethoxystilbene [**58**]	*Paphiopedilum exul*	roots	(17)
(*E*)-2,3-Dihydroxy-2'-(4-hydroxybenzyl)-3',5'-dimethoxystilbene [**59**]	*Phragmipedium* hybrid (var. Sorcerer's Apprentice)	whole plants	(112)
(*E*)-2,3'-Dihydroxy-2'-(4-hydroxybenzyl)-5'-methoxystilbene [**60**]	*Phragmipedium calurum*	whole plants	(112)
	Phragmipedium longifolium	whole plants	(112)
(*E*)-2,3'-Dihydroxy-2',6'-bis(4-hydroxybenzyl)-5'-methoxy-stilbene [**61**]	*Phragmipedium calurum*	whole plants	(112)
	Phragmipedium hybrid (var. Sorcerer's Apprentice)	whole plants	(112)

Table 1. Continued

Compounds	Sources	Plant part	Reference
(*E*)-2,3'-Dihydroxy-2',6'-bis(4-hydroxybenzyl)-5'-methoxy-stilbene [**61**]	*Phragmipedium longifolium*	whole plants	(112)
(*E*)-2,5'-Dihydroxy-2'-(4-hydroxybenzyl)-3'-methoxystilbene [**62**]	*Paphiopedilum exul*	roots	(17)
	Phragmipedium calurum	whole plants	(112)
	Phragmipedium hybrid (var. Sorcerer's Apprentice)	whole plants	(112)
	Phragmipedium longifolium	whole plants	(112)
2-[(1*E*)-2-(3,5-Dimethoxyphenyl)-ethenyl]-phenol [**63**]	*Paphiopedilum godefroyae*	roots	(16)
	Phragmipedium calurum	whole plants	(112)
(*E*)-2-Hydroxy-2'-(4-hydroxybenzyl)-3',5'-dimethoxystilbene [**64**]	*Phragmipedium calurum*	whole plants	(112)
(*E*)-2-Hydroxy-2'-(4-hydroxybenzyl)-5,3',5'-dimethoxystilbene [**65**]	*Phragmipedium* hybrid (var. Sorcerer's Apprentice)	whole plants	(112)
(*E*)-3'-Hydroxy-2',4'-bis(4-hydroxybenzyl)-5'-methoxystilbene [**66**]	*Phragmipedium calurum*	whole plants	(112)

Table 1. Continued

Compounds	Sources	Plant part	Reference
(*E*)-3'-Hydroxy-2',4'-bis(4-hydroxybenzyl)-5'-methoxystilbene [**66**]	*Phragmipedium calurum*	whole plants	(15)
	Phragmipedium longifolium	whole plants	(112)
(*E*)-5'-Hydroxy-2'-(4-hydroxybenzyl)-3'-methoxystilbene [**67**]	*Paphiopedilum exul*	roots	(17)
	Phragmipedium calurum	whole plants	(112)
	Phragmipedium calurum	whole plants	(15)
	Phragmipedium hybrid (var. Sorcerer's Apprentice)	whole plants	(112)
	Phragmipedium longifolium	whole plants	(112)
(*E*)-3'-Hydroxy-2'-(4-hydroxybenzyl)-5'-methoxystilbene [**68**]	*Phragmipedium calurum*	whole plants	(112)
	Phragmipedium calurum	whole plants	(15)
	Phragmipedium longifolium	whole plants	(112)
(*E*)-5'-Hydroxy-4'-(4-hydroxybenzyl)-3'-methoxystilbene [**69**]	*Phragmipedium calurum*	whole plants	(15)
2-Hydroxy-3',5'-di-methoxystilbene [**70**]	*Phragmipedium calurum*	whole plants	(112)

Table 1. Continued

Compounds	Sources	Plant part	Reference
3-Hydroxy-5-methoxystilbene [**71**]	*Phragmipedium calurum*	whole plants	(112)
	Phragmipedium longifolium	whole plants	(112)
3'-Hydroxy-2,5'-dimethoxystilbene [**72**]	*Paphiopedilum callosum*	roots	(18)
	Paphiopedilum exul	roots	(17)
	Paphiopedilum godefroyae	roots	(16)
3'-Hydroxy-2,6,5'-tri-methoxystilbene [**73**]	*Paphiopedilum callosum*	roots	(18)
Phoyunbene B [**74**]	*M. picta*	pseudobulbs	(100)
Phoyunbene C [**75**]	*M. picta*	pseudobulbs	(100)
Phoyunbene D [**76**]	*Cyrtopodium glutiniferum*	pseudobulbs	(72)
Pinosylvin monomethyl ether [**77**]	*Paphiopedilum exul*	roots	(17)
	Phragmipedium calurum	whole plants	(15)
Trans-pinostilbene [**78**]	*Paphiopedilum godefroyae*	roots	(16)
Thunalbene [**79**]	*Phragmipedium longifolium*	whole plants	(112)

Table 1. Continued

Compounds	Sources	Plant part	Reference
D. Dihydrostilbenes			
Aloifol I [**80**]	*Cymbidium aloifolium*	roots	(44)
Aloifol II [**81**]	*Cymbidium aloifolium*	roots	(44)
Batatasin III [**82**]	*Cymbidium aloifolium*	roots	(44)
	Cyrtopodium macrobulbon	pseudobulbs	(69)
	Cyrtopodium paniculatum	pseudobulbs	(70)
		roots	(71)
	O. baueri	whole plants	(104)
	O. isthmi	whole plants	(105)
	Oncidium Sharry Baby	whole plants	(105)
3,4'-Dihydroxy-3',5'-dimethoxydihydrostilbene [**83**]	*M. porphyrostele*	-	(102)
3,4'-Dihydroxy-5,5'-dimethoxydihydrostilbene [**84**]	*Catasetum barbatum*	aerial parts	(108)
	M. porphyrostele	-	(102)
3,4'-Dihydroxy-3',5,5'-trimethoxydihydrostilbene [**85**]	*E. nuda*	Tubers	(76)
3,5-Dihydroxy-3'-methoxydihydrostilbene [**86**]	*Oncidium* Sharry Baby	whole plants	(105)

Table 1. Continued

Compounds	Sources	Plant part	Reference
Gigantol [**87**]	*Cymbidium aloifolium*	roots	(44)
	Cymbidium giganteum	-	(50)
	Cymbidium goeringii	whole plants	(12)
	Cyrtopodium macrobulbon	pseudobulbs	(69)
	Cyrtopodium paniculatum	pseudobulbs	(70)
		roots	(71)
4'-Hydroxy-3,3',5,5'-tetramethoxydihydrostilbene [**88**]	*O. microchilum*	whole plants	(105)
E. Phenanthrenequinones Cymbinodin-A [**89**]	*Cymbidium aloifolium*	whole plants	(11)
Cypripedin [**90**]	*Cypripedium calceolus*	skin sensitizing	(110)
Densiflorol B [**91**]	*Cyrtopodium paniculatum*	pseudobulbs	(70)
5-Hydroxy-2,3-dimethoxy-1,4-phenanthrenequinone [**92**]	*Odontoglossum* Harvengtense 'Tutu'	bulbs	(106)
F. Dihydrophenanthrenequinones Cyrtopodinone [**93**]	*Cyrtopodium paniculatum*	roots	(71)

Table 1. Continued

Compounds	Sources	Plant part	Reference
Ephemeranthoquinone [**94**]	Cymbidium Great Flower Marie Laurencin	roots	(14)
	Cyrtopodium paniculatum	pseudobulbs, leaves	(70)
		roots	(71)
5-Hydroxy-2-methoxy-9,10-dihydrophenanthrene-1,4-dione [**95**]	*O. isthmi*	whole plants	(105)
G. Biphenanthrenes			
Blestriarene A [**96**]	*Cyrtopodium paniculatum*	pseudobulbs	(70)
		roots	(71)
Blestriarene B [**97**]	*Cyrtopodium paniculatum*	pseudobulbs	(70)
		roots	(71)
Blestriarene C [**98**]	*Cyrtopodium paniculatum*	roots	(71)
Coeludol A 3 [**99**]	*Cyrtopodium paniculatum*	roots	(71)
Coeludol B [**100**]	*Cyrtopodium paniculatum*	roots	(71)
9′,10′-Dihydro-4,5′-dimethoxy-[1,3′-biphenanthrene]-2,2′,7,7′-tetrol [**101**]	*Cyrtopodium paniculatum*	roots	(71)
Gymconopin C [**102**]	*Cyrtopodium paniculatum*	roots	(71)
Lusidol A [**103**]	*Cyrtopodium paniculatum*	pseudobulbs	(70)
		roots	(71)

Table 1. Continued

Compounds	Sources	Plant part	Reference
Lusidol B [**104**]	*Cyrtopodium paniculatum*	pseudobulbs	(70)
		roots	(71)
Monbarbatain C [**105**]	*Cyrtopodium paniculatum*	roots	(71)
4,4′,6,6'-Tetra-hydroxy 2,2′,7,7′-tetramethoxy- (1,1′)-biphenan-threne [**106**]	*Stanhopea lietzei*	whole plants	(109)
2,2′,7,7'-Tetra-hydroxy 4,4′,8,8′-tetramethoxy- (1,1′)-biphenan-threne [**107**]	*E. nuda*	tubers	(73)
H. Dihydrophenanthrofurans Cyrtonesin A [**108**]	*Cyrtopodium paniculatum*	pseudobulbs	(70)
Cyrtonesin B [**109**]	*Cyrtopodium paniculatum*	pseudobulbs	(70)
Moupilonin [**110**]	*Cyrtopodium paniculatum*	pseudobulbs	(70)
I.Benzofurans 2-(3′,5′-Dimethoxyphenyl)-6-hydroxy-5-methoxy-benzofuran [**111**]	*Paphiopedilum godefroyae*	roots	(16)
5,6-Dimethoxy-2-(3-hydroxy-5-methoxyphenyl)-benzofuran [**112**]	*Paphiopedilum godefroyae*	roots	(16)
2-(5′-Hydroxy-3′-methoxyphenyl)-6-hydroxy-5-methoxy-benzofuran [**113**]	*Paphiopedilum godefroyae*	roots	(16)

Table 1. Continued

Compounds	Sources	Plant part	Reference
J.Stilbene dimers Phragmidimer A [**114**]	*Phragmipedium* hybrid (var. Sorcerer's Apprentice)	whole plants	(112)
Phragmidimer B [**115**]	*Phragmipedium* hybrid (var. Sorcerer's Apprentice)	whole plants	(112)
	Phragmipedium longifolium	whole plants	(112)

Table 2. Bioactivities of stilbenoids from orchids of tribe Cymbidieae

Compounds	Activities	Reference
A. Phenanthrenes Confusarin [**5**]	antioxidant	(83)
Denthyrsinin [**7**]	antibacterial	(14)
	cytotoxic	(14, 80)
2,5-Dihydroxy-3,4-dimethoxyphenanthrene [**9**]	spasmolytic	(88)
2,5-Dihydroxy-4,9-dimethoxyphenanthrene [**10**]	anti-inflammatory	(106)
	cytotoxic	(105)
2,7-Dihydroxy-1,5-dimethoxyphenanthrene [**11**]	cytotoxic	(80)
2,7-Dihydroxy-3,4,8-trimethoxyphenanthrene [**13**]	anti-inflammatory	(108)

Table 2. Continued

Compounds	Activities	Reference
Fimbriol A [**16**]	antinociceptive	(87)
	spasmolytic	(88)
Flavanthrinin [**17**]	cytotoxic	(80)
Gymnopusin [**19**]	herbicide	(90)
	spasmolytic	(88)
	vasorelaxant	(89)
1-(4'-Hydroxybenzyl)-4,8-dimethoxy-2,7-phenanthrenediol [**20**]	cytotoxic	(80)
	PDE5 inhibitory	(78)
	vasorelaxant	(78, 79)
3-Hydroxy-2,4,7-trimethoxy-phenanthrene [**21**]	antibacterial, cytotoxic	(14)
Lusianthrin [**22**]	antifungal	(111)
Moscatin [**23**]	antioxidant	(105)
	antiplatelet aggregation	(85)
	cytotoxic	(105)
Nudol [**24**]	anti-inflammatory	(108)
	antioxidant	(83)
	cytotoxic	(86)
	spasmolytic	(88)
Pendulin [**25**]	antibacterial, cytotoxic	(14)
Plicatol A [**26**]	cytotoxic	(105)
1,2,6,7-Tetrahydroxy-4-methoxyphenanthrene [**27**]	cytotoxic	(102)
B. Dihydrophenanthrenes		
Coelonin [**30**]	anti-α-glucosidase	(113)
	anti-inflammatory	(49)
	cytotoxic	(80)

Table 2. Continued

Compounds	Activities	Reference
1,7-Dihydroxy-2,5-dimethoxy-9,10-dihydrophenanthrene [**32**]	anti-inflammatory	(83)
	antioxidant	(82)
	cytotoxic	(77)
1,7-Dihydroxy-4-(4'-hydroxybenzyl)-2,5-dimethoxy-9,10-dihydrophenanthrene [**33**]	PDE5 inhibitory	(81)
2,6-Dihydroxy-1,5,7-trimethoxy-9,10-dihydrophenanthrene [**35**]	cytotoxic	(102, 105)
2,7-Dihydroxy-4,6-dimethoxv-9,10-dihydrophenanthrene [**38**]	cytotoxic	(80)
2,7-Dihydroxy-4,6-dimethoxv-9,10-dihydrophenanthrene [**38**]	cytotoxic, vasorelaxant	(102)
4,5-Dihydroxy-2-methoxy-9, 10-dihydrophenanthrene [**40**]	cytotoxic	(105)
Erianthridin [**41**]	antiplatelet aggregation	(92)
	anti-inflammatory	
	antinociceptive	(87)
	cytotoxic	(14)
	spasmolytic	(88)
Flavanthridin [**43**]	antioxidant	(82)
	antibacterial, cytotoxic	(14)
3-Hydroxy-2,4,7-trimethoxy-9,10-dihydrophenanthrene [**44**]	cytotoxic	(14)
Lusianthridin [**46**]	cytotoxic	(105)
Marylaurencinols A [**47**]	antibacterial, cytotoxic	(14)
Marylaurencinols B [**48**]	antibacterial, cytotoxic	(14)
Marylaurencinoside A [**49**]	cytotoxic	(14)

Table 2. Continued

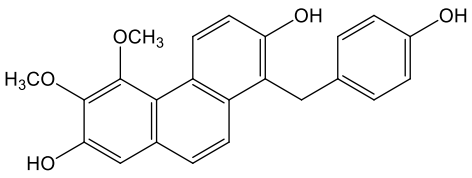
Compounds	Activities	Reference
1,2,3,4,4a,9,10,10a-Octahydro-1,4a-dimethyl-1-phenanthrenecarboxylic acid methyl ester [**52**]	cytotoxic	(84)
Shancidin [**53**]	antibacterial, anti-haemolytic	(114)
C. Dihydrostilbenes		
2,3'-Dihydroxy-5'-methoxystilbene [**55**]	cytotoxic	(16, 18)
2,3'-Dihydroxy-5,5'-dimethoxystilbene [**56**]	cytotoxic	(16-18)
5,6'-Dihydroxy-3,2'-dimethoxystilbene [**58**]	cytotoxic	(17)
(*E*)-2,5'-Dihydroxy-2'-(4-hydroxybenzyl)-3'-methoxystilbene [**62**]	cytotoxic	(112)
2-[(1*E*)-2-(3,5-Dimethoxyphenyl)-ethenyl]-phenol [**63**]	cytotoxic	(15, 16)
(*E*)-2-Hydroxy-2'-(4-hydroxybenzyl)-3',5'-dimethoxystilbene [**64**]	cytotoxic	(15)
(*E*)-3'-Hydroxy-2',4'-bis(4-hydroxybenzyl)-5'-methoxystilbene [**66**]	cytotoxic	(15)
(*E*)-5'-Hydroxy-2'-(4-hydroxybenzyl)-3'-methoxystilbene [**67**]	cytotoxic	(15, 17)
(*E*)-3'-Hydroxy-2'-(4-hydroxybenzyl)-5'-methoxystilbene [**68**]	cytotoxic	(15)
(*E*)-5'-Hydroxy-4'-(4-hydroxybenzyl)-3'-methoxystilbene [**69**]	cytotoxic	(15)

Table 2. Continued

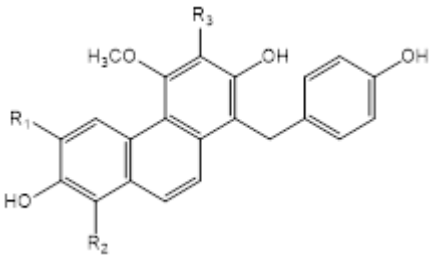
Compounds	Activities	Reference
3′-Hydroxy-2,5′-dimethoxystilbene [**71**]	cytotoxic	(16-18)
3′-Hydroxy-2,6,5′-tri-methoxystilbene [**73**]	cytotoxic	(18)
Phoyunbene B [**74**]	cytotoxic	(115)
	inhibit of nitric oxide production	(116)
Phoyunbene C [**75**]	inhibit of nitric oxide production	(116)
Phoyunbene D [**76**]	inhibit of nitric oxide production	(116)
Pinosylvin monomethyl ether [**77**]	cytotoxic	(15, 17)
Trans-pinostilbene [**78**]	cytotoxic	(16)
D. Dihydrostilbenes Aloifol II [**81**]	spasmolytic	(55)
Batatasin III [**82**]	antiallergic	(65)
	Inhibition of α-glucosidase	(66, 67)
	antifouling	(62)
	antifungal	(68)
	antinociceptive	(69)
	cytotoxic	(56, 105)
	spasmolytic	(55)
3,4'-Dihydroxy-3',5'-dimethoxydihydrostilbene [**83**]	vasorelaxant	(101, 102)
3,4'-Dihydroxy-5,5'-dimethoxydihydrostilbene [**84**]	anti-inflammatory	(108)
	vasorelaxant	(101, 102)
3,5-Dihydroxy-3'-methoxydihydrostilbene [**86**]	cytotoxic	(105)

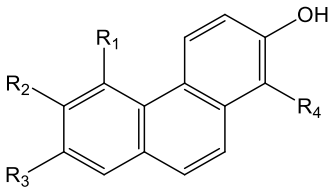
Table 2. Continued

Compounds	Activities	Reference
Gigantol [**87**]	anti-cataract	(52)
	anti-inflammatory	(12)
	antimutagenic	(51)
	antinociceptive	(69)
	antioxidant	(54)
	cytotoxic	(53, 56-61)
	spasmolytic	(55)
4'-Hydroxy-3,3',5,5'-tetra-methoxydihydrostilbene [**88**]	cytotoxic	(105)
E. Phenanthrene-quinones		
Cypripedin [**90**]	skin sensitizing	(110)
Densiflorol B [**91**]	antibacterial, anti-haemolytic	(114)
	antimalarial	(117)
	cytotoxic	(118)
F. Dihydrophenanthrenequinones		
Ephemeranthoquinone [**94**]	antibacterial, cytotoxic	(14)
5-Hydroxy-2-methoxy-9,10-dihydrophenanthrene-1,4-dione [**95**]	cytotoxic	(105)
G. **Biphenanthrenes**		
Blestriarene A [**96**]	cytotoxic	(71)
Blestriarene B [**97**]	cytotoxic	(71)
Blestriarene C [**98**]	cytotoxic	(71)



[1]





[2]: R_1 = R_2 = H, R_3 = OCH_3

[18]: R_1 = R_2 = R_3 = OCH_3

[20]: R_1 = H, R_2 = OCH_3, R_3 = H

[5]: R_1= R_2 = OCH_3, R_3 = OH, R_4 = OCH_3

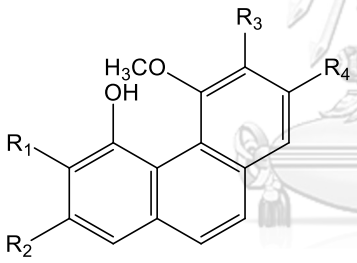
[7]: R_1= OCH_3, R_2 = OH, R_3 = R_4 = OCH_3

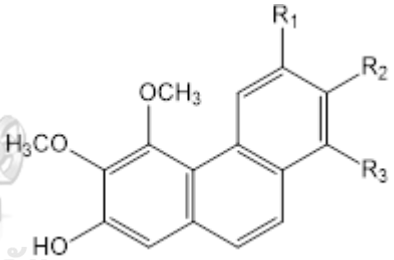
[11]: R_1= OCH_3, R_2 = H, R_3 = OH, R_4 = OCH_3

[17]: R_1= OCH_3, R_2 = H, R_3 = R_4 = OH

[22]: R_1= OH, R_2 = H, R_3 = OCH_3, R_4 = OH

[24]: R_1= R_2 = OCH_3, R_3 = R_4 = OH





[3]: R_1= OCH_3, R_2 = OH, R_3 = R_4 = H

[9]: R_1 = R_2 = H, R_3 = OCH_3, R_4 = OH

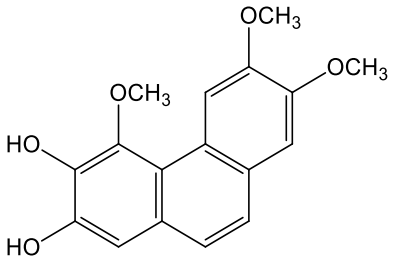
[15]: R_1= R_2 = H, R_3 = OH, R_4 = OCH_3

[4]: R_1 = R_2 = R_3 = OH

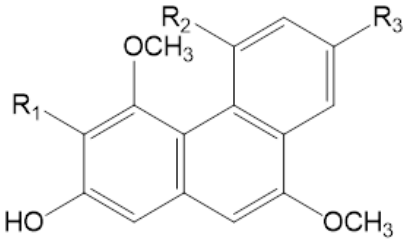
[6]: R_1 = OCH_3, R_2 = OH, R_3 = OCH_3

[12]: R_1 = R_2 = OCH_3, R_3 = OH, R_4 = H

Figure 3. Stilbenoids from orchids of tribe Cymbidieae and subfamily Cypripedioideae



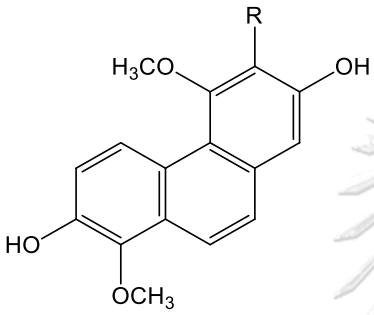
[8]



[10]: R_1 = H, R_2 = OH, R_3 = H

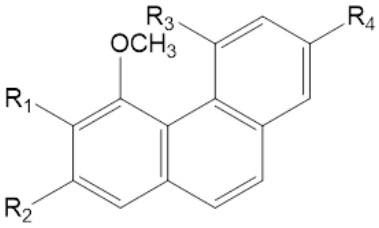
[16]: R_1 = OCH_3, R_2 = OH, R_3 = H

[19]: R_1 = OCH_3, R_2 = H, R_3 = OH



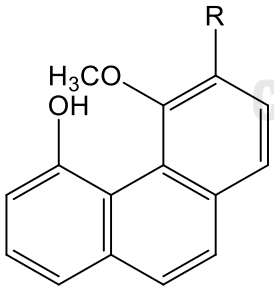
[13]: R = OCH_3

[14]: R= H



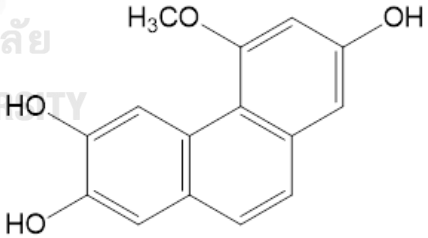
[21]: R_1 = OH, R_2 = R_3 = H, R_4 = OCH_3

[26]: R_1 = H, R_2 = R_3 = OH, R_4 = H



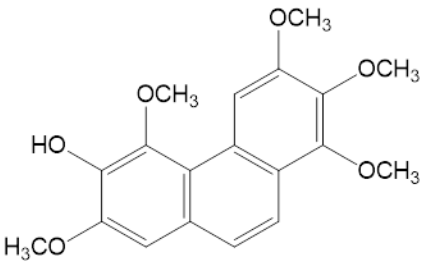
[23]: R = H

[28]: R = OH

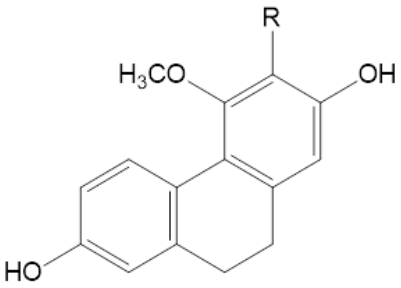


[25]

Figure 3. Continued

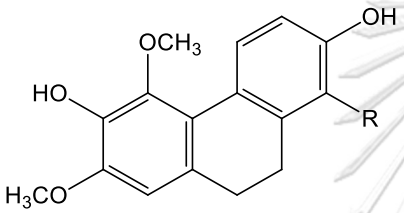


[27]



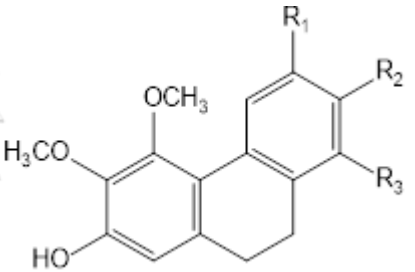
[30]: R = H

[41]: R = OCH_3



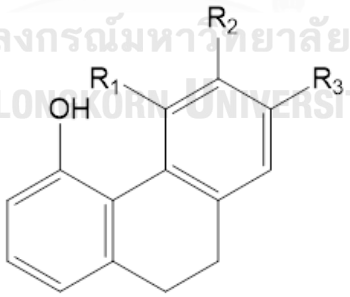
[35]: R = OCH_3

[43]: R = H



[34]: $R_1 = R_2 = OCH_3$, $R_3 = H$

[40]: $R_1 = OH$, $R_2 = H$, $R_3 = OCH_3$

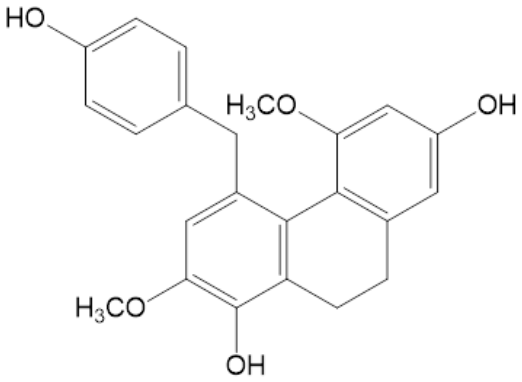


[29]: $R_1 = OCH_3$, $R_2 = OH$, $R_3 = OCH_3$

[31]: $R_1 = OH$, $R_2 = R_3 = OCH_3$

[32]: $R_1 = H$, $R_2 = OCH_3$, $R_3 = OH$

Figure 3. Continued



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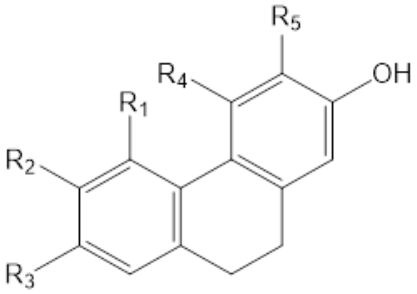


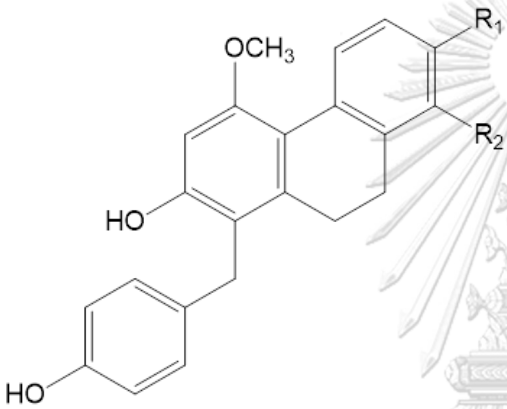
[37]: R_1 = H, R_2 = OCH_3, R_3 = OH, R_4 = R_5 = OCH_3

[38]: R_1 = OCH_3, R_2 = H, R_3 = OH, R_4 = H, R_5 = OCH_3

[46]: R_1 = OH, R_2 = H, R_3 = OCH_3, R_4 = R_5 = H

[50]: R_1 = OCH_3, R_2 = H, R_3 = OCH_3, R_4 = R_5 = H

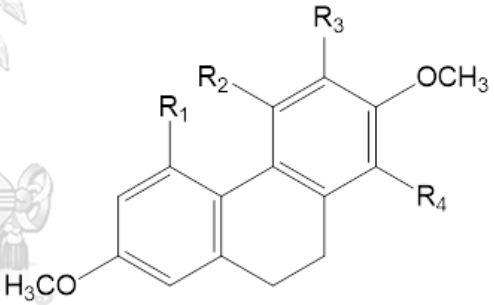
[51]: R_1 = H, R_2 = OCH_3, R_3 = OH, R_4 = OCH_3, R_5 = H



[36]: R_1 = OCH_3, R_2 = R_3 = OH, R_4 = H

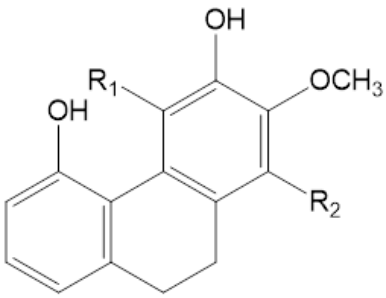
[39]: R_1 = OCH_3, R_2 = OH, R_3 = OCH_3, R_4 = OH

[53]: R_1 = OH, R_2 = H, R_3 = OH, R_4 = H



[42]: R_1 = OH, R_2 = R_3 = H, R_4 = OH

[44]: R_1 = H, R_2 = OCH_3, R_3 = OH, R_4 = H

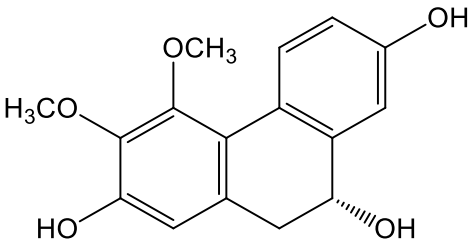


[47]: R_1 = OCH_3, R_2 = H

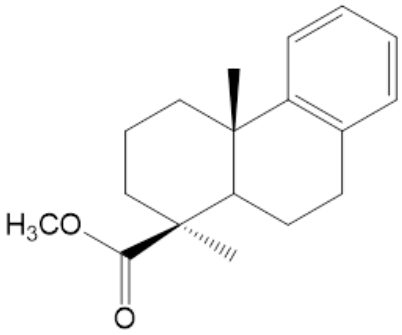
[48]: R_1 = OCH_3, R_2 = OH

[49]: R_1 = O-Glu, R_2 = H

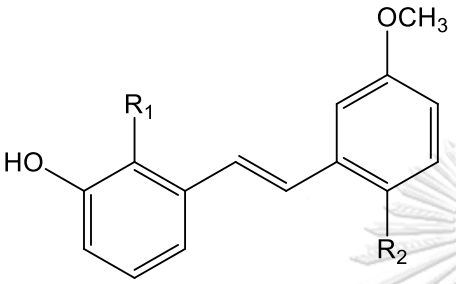
Figure 3. Continued



[45]

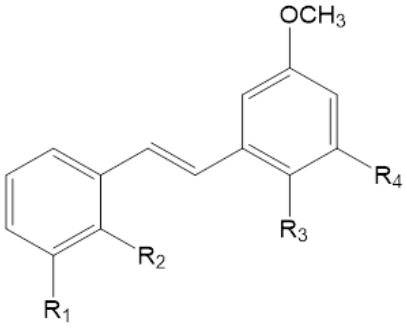


[52]



[54]: R_1 = OH, R_2 = OCH_3

[78]: R_1 = H, R_2 = OH



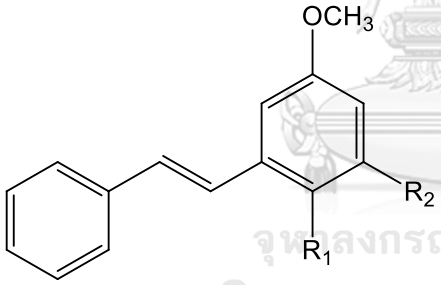
[55]: R_1 = R_2 = H, R_3 = OH, R_4 = H

[56]: R_1 = OCH_3, R_2 = H, R_3 = OH, R_4 = H

[58]: R_1 = H, R_2 = OCH_3, R_3 = H, R_4 = OH

[63]: R_1 = R_2 = H, R_3 = H, R_4 = OCH_3

[70]: R_1 = R_2 = H, R_3 = OCH_3, R_4 = H

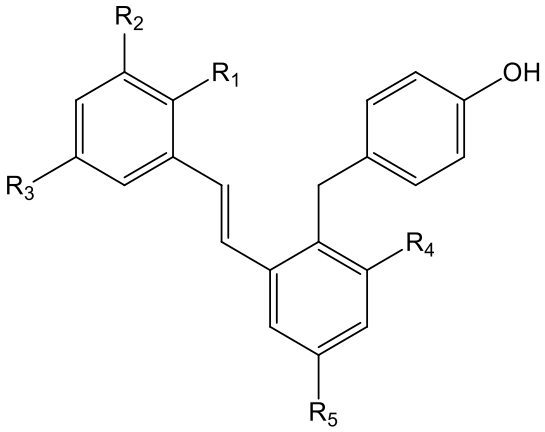


[57]: R_1 = R_2 = OH

[71]: R_1 = OH, R_2 = H

[77]: R_1 = H, R_2 = OH

Figure 3. Continued



[59]: R_1 = R_2 = OH, R_3 = H, R_4 = R_5 = OCH_3

[60]: R_1 = OH, R_2 = R_3 = H, R_4 = R_5 = OCH_3

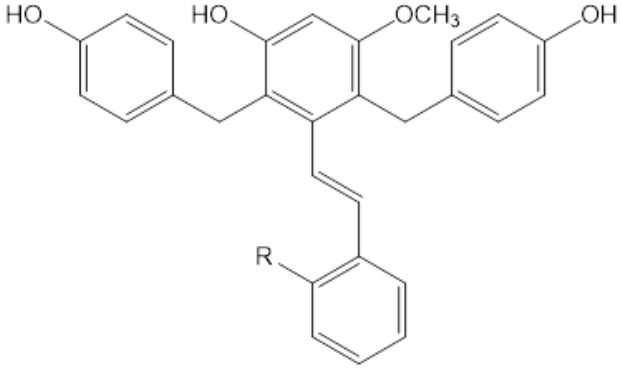
[62]: R_1 = OH, R_2 = R_3 = H, R_4 = R_5 = OH

[64]: R_1 = OH, R_2 = R_3 = R_4 = H, R_5 = OCH_3

[65]: R_1 = OH, R_2 = H, R_3 = R_4 = OCH_3, R_5 = OH

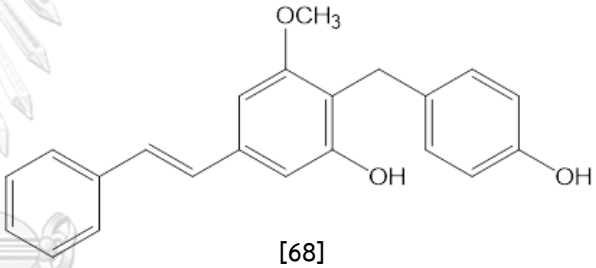
[67]: R_1 = R_2 = R_3 = H, R_4 = OCH_3, R_5 = OH

[69]: R_1 = R_2 = R_3 = H, R_4 = OH, R_5 = OCH_3

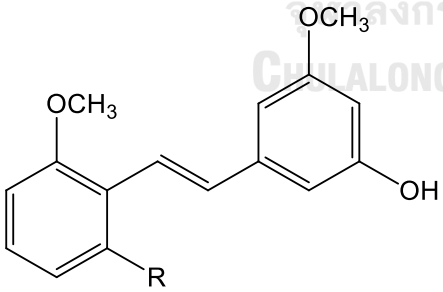


[61]: R = OH

[66]: R = H

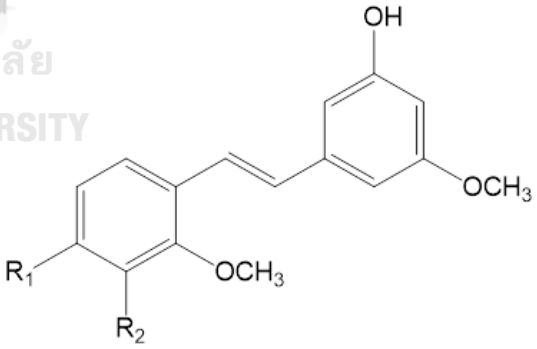


[68]



[72]: R = H

[73]: R = OCH_3

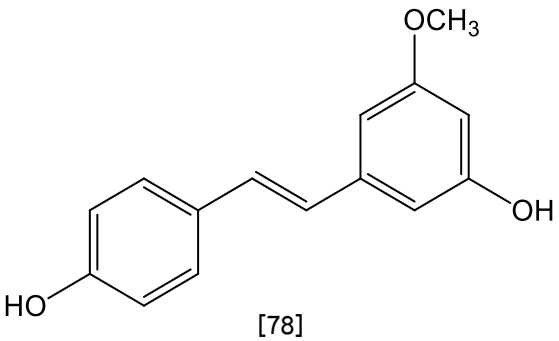


[74]: R_1 = OH, R_2 = OCH_3

[75]: R_1 = H, R_2 = OH

[76]: R_1 = H, R_2 = OCH_3

Figure 3. Continued



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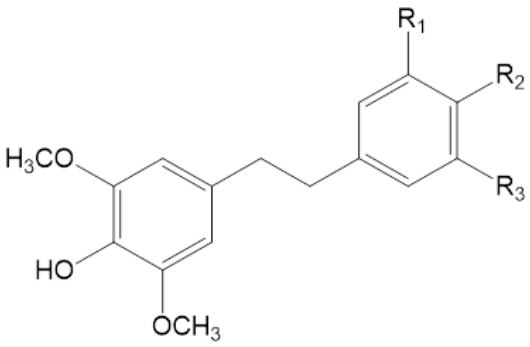
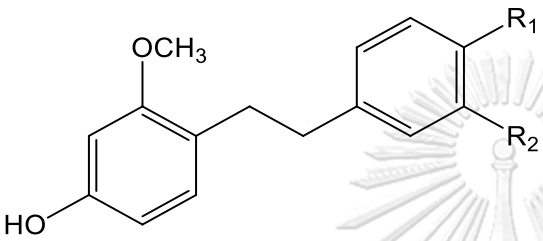


[80]: R_1 = R_2 = H, R_3 = OH

[81]: R_1 = H, R_2 = OH, R_3 = OCH_3

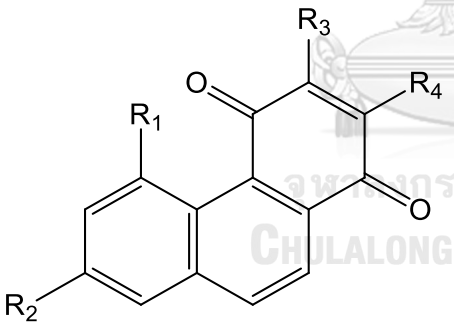
[85]: R_1 = OH, R_2 = H, R_3 = OCH_3

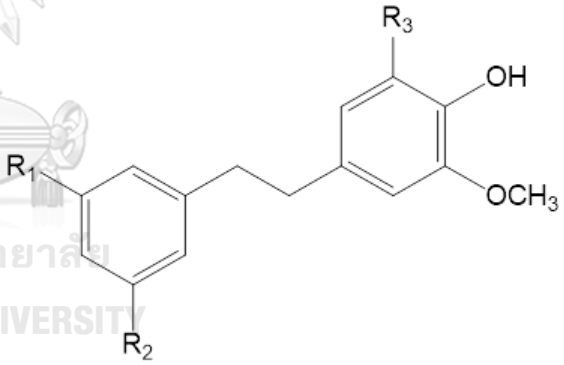
[86]: R_1 = OCH_3, R_2 = H, R_3 = OCH_3



[82]: R_1 = H, R_2 = OH

[87]: R_1 = OH, R_2 = OCH_3





[89]: R_1 = OH, R_2 = H, R_3= OCH_3, R_4 = H

[90]: R_1 = H, R_2 = OH, R_3= H, R_4 = OCH_3

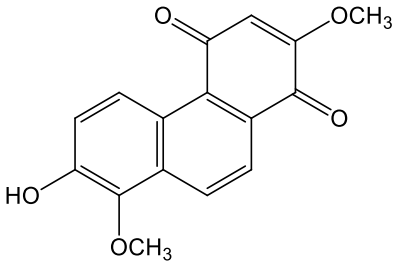
[94]: R_1 = OH, R_2 = H, R_3 = R_4 = OCH_3

[83]: R_1 = OH, R_2 = H, R_3 = OCH_3

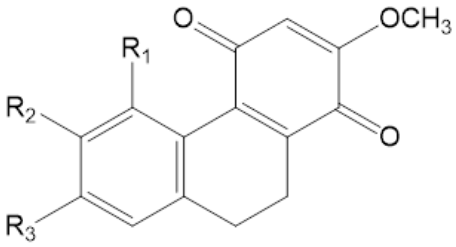
[84]: R_1 = OH, R_2 = OCH_3, R_3 = H

[88]: R_1 = R_2 = R_3 = OCH_3

Figure 3. Continued



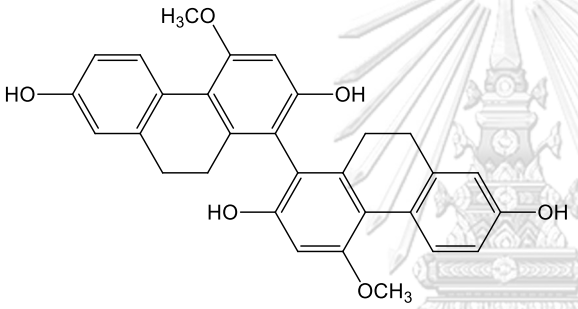
[90]



[93]: $R_1 = R_2 = OCH_3$, $R_3 = OH$

[94]: $R_1 = R_2 = H$, $R_3 = OH$

[95]: $R_1 = OH$, $R_2 = R_3 = H$



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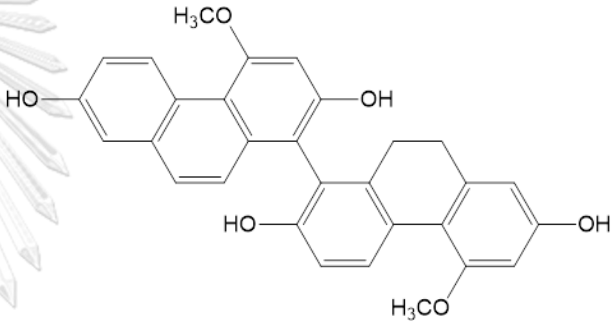
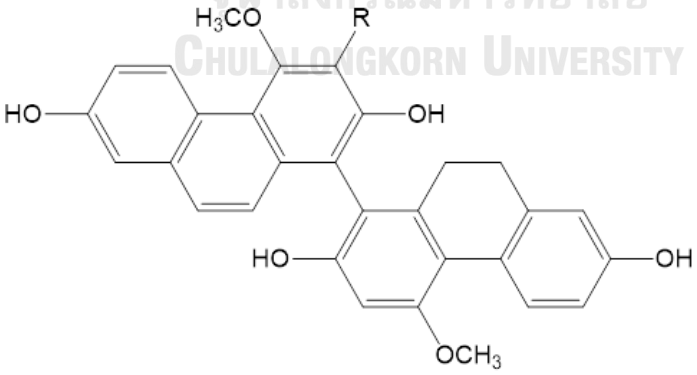


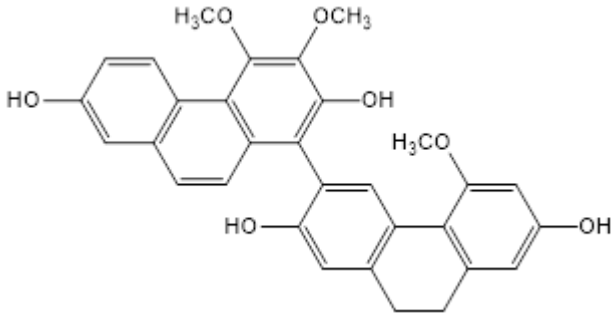
[97]



[98]: R = H

[99]: R = OCH_3

Figure 3. Continued



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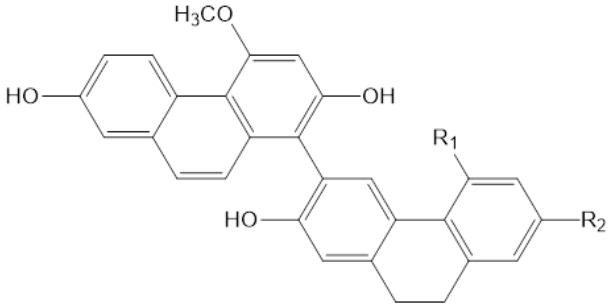
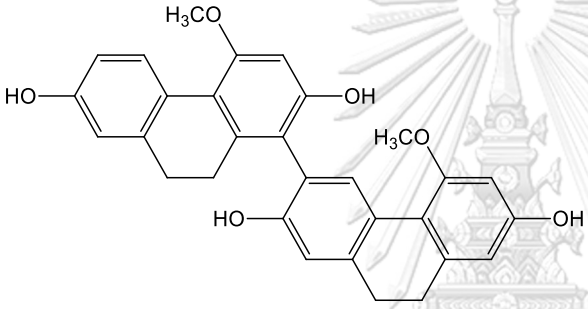
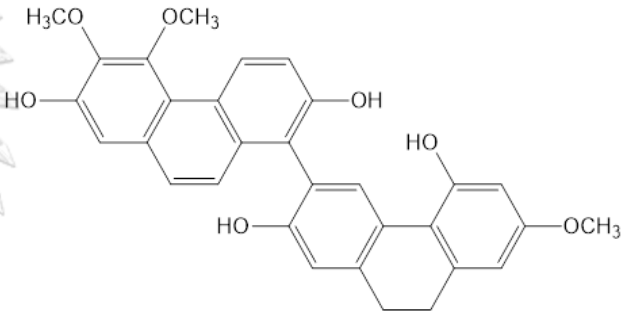


[101]: R_1 = OCH_3, R_2 = OH

[105]: R_1 = OH, R_2 = OCH_3



[102]



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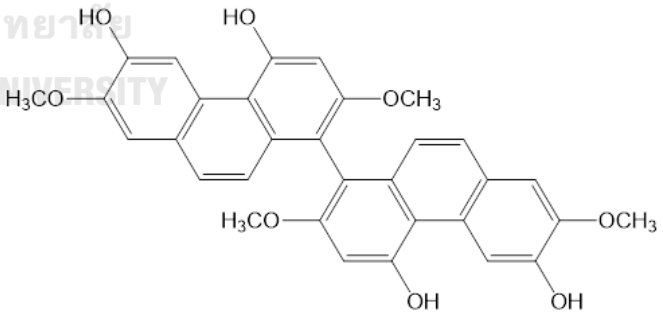
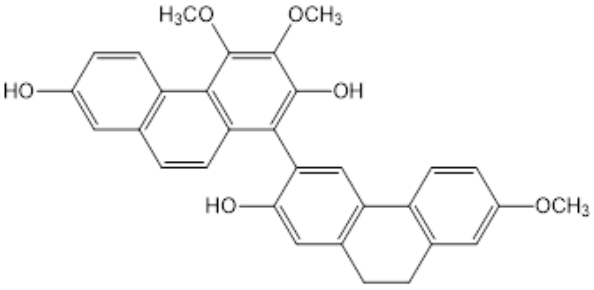
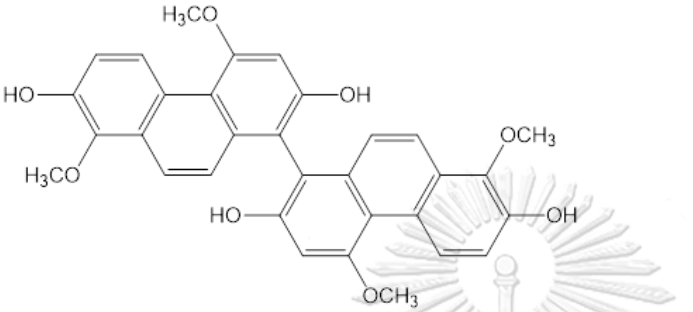




[106]

[104]

Figure 3. Continued



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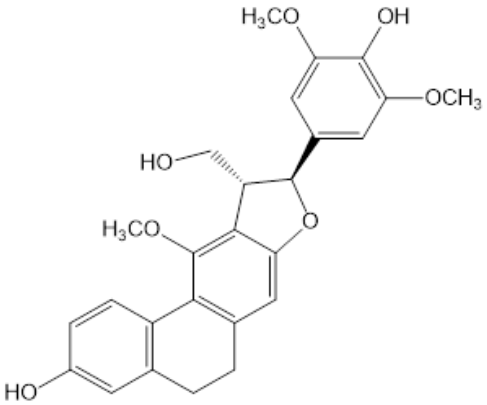

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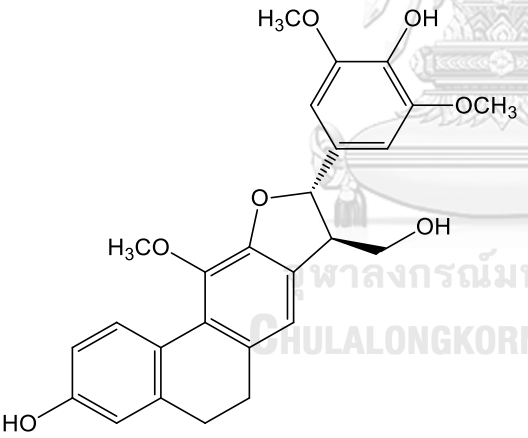

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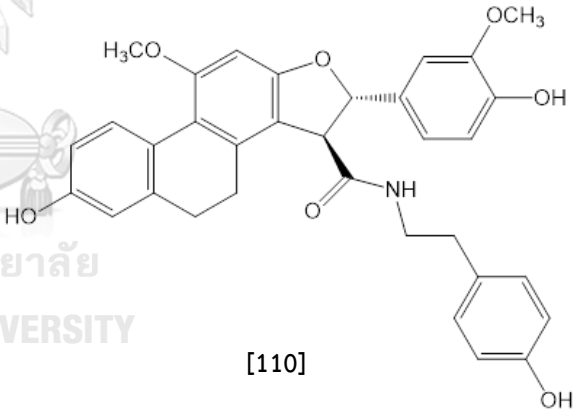

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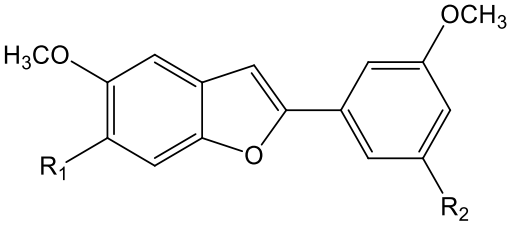
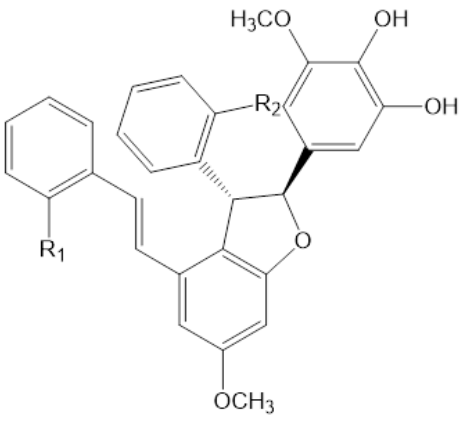


[111]: R_1 = OH, R_2 = OCH_3

[112]: R_1 = OCH_3, R_2 = OH

[113]: R_1 = R_2 = OH



[114]: R_1 = H, R_2 = OH

[115]: R_1 = OH, R_2 = H

Figure 3. Continued

2.9 Flavonoids from orchids of tribe Cymbidieae and subfamily Cypripedioideae

Flavonoids are essential secondary metabolites because they can act as antioxidant defense system in plant tissues to protect them from several different abiotic and biotic stresses. Flavonoid molecules consist of fifteen carbons (C6-C3-C6) from two benzene rings (rings A and B) linked via a propyl bridge or heterocyclic pyran ring (ring C). They are biosynthesized by phenylpropanoid pathway, coupled with three additional malonate units from polyketide pathway (119, 120). Family Orchidaceae is a natural source of flavonoids which show a variety of pharmacological activities.

Flavonoids can be found in several members of tribe Cymbidieae. For example, extraction of the flowers of *Cymbidium* Lunagrad Eternal Green have yielded, in addition to alkaloid and aromatic glucoside, three flavone glucosides, namely, ermanin **[127]**, quercetin 3-O-α-(2''-O-α-L-rhamnopyranosyl-β-D-glucopy-ranoside **[136]** and orientin **[132]** (121). Orientin is a flavone C-glycoside commonly found in several medicinal plants including holy basil (*Ocimum sanctum*, family Labiatae) and monocotyledonous plants such as bamboo (*Phyllostachys nigra*, family Gramineae) and Asiatic dayflower (*Commelina communis*, family Commelinaceae) (122). Orientin has been shown to possess several biological activities e.g. inhibition of adipogenesis (123), antioxidant (124), vasodilating (125), neuroprotective (126), radioprotective (127) and anti-inflammatory (128).

Other flavone C-glycosides have also been isolated from orchids of tribe Cymbidieae. Vitexin-7-glucoside **[140]** and isovitexin-7-glucoside **[130]** were isolated from the leaves of both *Cymbidium finlaysonianum* and *C. madidum,* while vitexin

[139] has been reported as a constituent of *C. madidum* and *Maxillaria luteoalba* (129). Furthermore, isovitexin **[129]** can be found in the leaves of *Maxillaria luteoalba* and the whole plants of *Stanhopea lietzei* (109, 129). Although more studies have been done on biological activities of vitexin, this flavone C-glucoside and its isomer, isovitexin, were demonstrated to possess many bioactivities including inhibition of advanced glycation end products formation (130), antioxidant (131), anti-inflammatory (132, 133), neuroprotective (134-136) and **α**-glucosidase inhibition (137). Vitexin was also able to inhibit adipogenesis (123) showed antinociceptive (138) and spasmolytic activities (139) and induced apoptosis in some cancer cells (140, 141).

A flavone, chrysoeriol **[124]**, has been isolated as a constituent of the pseudobulbs of *Cyrtopodium paniculatum* (70). This flavonoid has been shown to exhibit antibacterial (142), antimutagenic (143), antioxidant (144), cardioprotective (145) and anti-inflammatory activities (146). Chrysoeriol was also able to inhibit the enzymes lipase (147), tyrosine kinases VEGFR2 and c-Met (which are involved in tumorigenesis) (148), protein kinase CK2 (which takes part in many cell functions and is an interesting target in cancer research) (149) and prevent neurotoxicity in a cellular model of Parkinson's disease (150).

The pseudobulbs of another *Cyrtopodium* orchid, *C. glutiniferum,* afforded three flavonoids: dihydroformononetin **[144]**, phloridzin **[116]** and hispidulin 7-rutinoside **[8c]**) (72). Phloridzin (or phlorizin) is a dihydrochalcone found in several fruit trees and especially in apple. It has been studied as a potential treatment for type 2 diabetes since one of its principal pharmacological action is to produce renal glycosuria and block intestinal glucose absorption (151). The flavonoid appeared to have anti-aging effect on yeast (152), and could act as phytoestrogen that had double directional adjusting function of both estrogenic and antiestrogenic activities (153). Phloridzin also

displayed other interesting biological activities including anti-inflammatory (154, 155), antimicrobial (156), antioxidant (157) and hepatoprotective (158).

Flavones and flavonols which are widely found in the plant kingdom, such as apigenin **[122]**, luteolin **[131]**, kaempferol **[142]** and quercetin **[143]**, have also been obtained from the leaves of *Eulophia epidendraea* (159). Several literature reviews on their biological activities have been published over the years (160-166).

Oncidium baueri is an orchid native to Central and South America. The plant contains four flavonoid glycosides i.e. two new flavanones, oncibauerins A **[118]** and B **[119]**, acacetin-7-O-rutinoside **[121]** and pectolinarin **[134]** (104). Pectolinarin has been demonstrated to possess analgesic, anti-inflammatory and hepatoprotective activities in rats (167, 168). Another study on leaf flavonoids of the Orchidaceae (129) obtained two flavonoid glycosides, scutellarein 6-methyl ether 7-rutinoside **[137]** and pectolinarigenin 7-glucoside **[135]**, from the leaves of *O. excavatum* while the leaves of *O. sphacelatum* yielded only scutellarein 6-methyl ether 7-rutinoside **[137]**.

Another South American member of tribe Cymbidieae, *Miltonia flavescens,* has been investigated and two of its bioactive compounds were 7-*O*-rutinose-5-hydroxy-6,4'-dimethoxyflavone **[133]** and 5,7-dihydroxy-6,4'-dimethoxyflavone **[126]**. These flavone and flavone glycoside displayed antiproliferative activity against ovary sarcoma (NCI/ADR-RES) cell line (169).

Recently, a number of phytochemical studies have been done on slipper orchids of subfamily Cypripedioideae and flavonoids were also reported as their constituents. Most of them are flavonoids formerly found in another monocotyledonous family, Zingiberaceae. Pinocembrin **[120]**, a flavanone previously isolated from fingerroot (*Boesenbergia rotunda*), could be found in the roots of *Paphiopedilum godefroyae* and *P. exul* (16, 17). Pinocembrin has been shown to

protect cells from toxicity induced by advanced glycation endproducts (170), inhibit neurotoxicity induced by beta-amyloid peptide (171) and inhibit the growth of melanoma cell line (B16F10) by inducing apoptosis (172).

The roots of *P. exul* also yielded two other flavonoids, alpinetin **[117]** and galangin **[141]**. Both flavonoids have been known as constituents of plants in the family Zingiberaceae, especially greater galangal (*Alpinia galanga*). In addition, galangin was previously isolated from the dried bulb of *Odontoglossum* Harvengtense 'Tutu', a hybrid orchid of tribe Cymbidieae (106). Alpinetin exhibited anti-ageing effect on skin (173), anti-inflammatory activity by interfering with signaling pathways of inflammation (174) and antiproliferative and anti-migration activities on cancer cells (175). Galangin could also be obtained from the root of *P. callosum* (18). The flavonol was able to inhibit the growth of several cancer cell lines e.g. KB, NCI-H187 and MCF-7 cell lines (16, 17, 176) by inducing apoptosis (177) and autophagy (178). Furthermore, it exhibited antioxidant and anti-inflammatory effects (179) and could inhibit the enzymes α-glucosidase (180) and xanthine oxidase (181).

The seedlings of another lady slipper orchid, *Cypripedium macranthos* var. *rebunense* contained chrysin **[123]** as an antifungal compound which might act as a defense mechanism of the orchid in its developmental stages of germination (111). Chrysin displayed many interesting pharmacological activities such as antibacterial, anti-inflammatory, antioxidant, antiasthmatic, antiarthritic, antienteroviral, antidepressant and anticancer by inducing apoptosis of cancer cells (182).

Flavonoids from orchids belonging to tribe Cymbidieae and subfamily Cypripedioideae are summarized in **Table 3** and their bioactivities in **Table 4**. Their chemical structures are shown in **Figure 4**.

Table 3. Flavonoids from orchids of tribe Cymbidieae and subfamily Cypripedioideae

Compounds	Sources	Plant part	Reference
A. Dihydrochalcones			
Phloridzin [**116**]	*Cyrtopodium glutiniferum*	pseudobulbs	(72)
B. Flavanones			
Alpinetin [**117**]	*Paphiopedilum exul*	roots	(17)
Oncibauerin A [**118**]	*Oncidium baueri*	whole plants	(104)
Oncibauerin B [**119**]	*O. baueri*	whole plants	(104)
Pinocembrin [**120**]	*Paphiopedilum exul*	roots	(17)
	P. godefroyae	roots	(16)
C. Flavones			
Acacetin-7-*O*-rutinoside [**121**]	*O. baueri*	whole plants	(104)
Apigenin [**122**]	*Eulophia epidendraea*	leaves	(159)
Chrysin [**123**]	*Cypripedium macranthos* var. *rebunense*	seedlings	(111)
Chrysoeriol [**124**]	*Cyrtopodium paniculatum*	pseudobulbs	(70)
5,7-Dihydroxy-3-methoxyflavone [**125**]	*P. callosum*	roots	(18)
5,7-Dihydroxy-6,4'-dimethoxy-flavone [**126**]	*Miltonia flavescens*	aerial parts	(169)

Ermanin [**127**]	*Cymbidium* Lunagrad Eternal Green	flowers	(121)
Hispidulin-7-rutinoside [**128**]	*Cyrtopodium glutiniferum*	pseudobulbs	(72)
Isovitexin [**129**]	*Maxillaria luteoalba*	leaves	(109, 129)
	Stanhopea lietzei	whole plants	(109)

Table 3. Continued

Compounds	Sources	Plant part	Reference
Isovitexin-7-glucoside [**130**]	*Cymbidium finlaysonianum*	leaves	(129)
	Cymbidium madidum	leaves	(129)
Luteolin [**131**]	*E. epidendraea*	leaves	(159)
Orientin [**132**]	*Cymbidium* Lunagrad Eternal Green	flowers	(121)
7-*O*-rutinose-5-hydroxy-6,4'-dimethoxyflavone [**133**]	*Miltonia flavescens*	aerial parts	(169)
Pectolinarin [**134**]	*O. baueri*	whole plants	(104)
Pectolinarigenin-7-glucoside [**135**]	*O. excavatum*	leaves	(129)
Quercetin 3-O-α-(2''-O-α-L-rhamno-pyranosyl-β-D-glucopyranoside [**136**]	*Cymbidium* Lunagrad Eternal Green	flowers	(121)
Scutellarein-6-methyl ether 7-rutinoside [**137**]	*O. excavatum*	leaves	(129)
	O. sphacelatum	leaves	(129)
Velutin [**138**]	*Cyrtopodium paniculatum*	pseudobulbs	(70)
Vitexin [**139**]	*Cymbidium madidum*	leaves	(129)

	Maxillaria luteoalba	leaves	(129)
Vitexin-7-glucoside [**140**]	*Cymbidium finlaysonianum*	leaves	(129)
	Cymbidium madidum	leaves	(129)
D. Flavonols Galangin [**141**]	*Odontoglossum* Harvengtense 'Tutu'	bulbs	(106)
	P. callosum	roots	(18)

Table 3. Continued

Compounds	Sources	Plant part	Reference
Galangin [**141**]	*P. exul*	roots	(17)
Kaempferol [**142**]	*E. epidendraea*	leaves	(159)
Quercetin [**143**]	*E. epidendraea*	leaves	(159)
E. Isoflavanone Dihydroformononetin [**144**]	*Cyrtopodium glutiniferum*	pseudobulbs	(72)

Table 4. Bioactivities of flavonoids from orchids of tribe Cymbidieae and subfamily Cypripedioideae

Compounds	Activities	Reference
A. Dihydrochalcones Phloridzin [**116**]	antidiabetic	(151)
	anti-aging	(152)
	estrogenic, antiestrogenic	(153)
	anti-inflammatory	(154, 155)
	antimicrobial	(156)
	antioxidant	(157)
	hepatoprotective	(158)
B. Flavanones Alpinetin [**117**]	anti-ageing	(173)

	anti-inflammatory	(174)
	antiproliferation	(175)
Pinocembrin [**120**]	cytoprotective from advanced glycation endproducts	(170)
	neuroprotective	(171)
	apoptosis inducing	(172)

Table 4. Continued

Compounds	Activities	Reference
C. Flavones		
Apigenin [**122**]	anti-inflammatory, anti-angiogenic, anti-genotoxic, chemotherapeutic, hepatoprotective, muscle regeneration	(166)
Chrysin [**123**]	antibacterial, anti-inflammatory, antioxidant, antiasthmatic, antiarthritic, antienteroviral, antidepressant, anticancer	(182)
	antifungal	(111)
Chrysoeriol [**124**]	antibacterial	(142)
	antimutagenic	(143)
	antioxidant	(144)
	cardioprotective	(145)
	anti-inflammatory	(146)
	lipase inhibition	(147)
	tyrosine kinase inhibition	(148)

	protein kinase inhibition	(149)
	neuroprotective	(150)
5,7-Dihydroxy-6,4'-dimethoxyflavone [**126**]	cytotoxic	(169)
Isovitexin [**128**]	inhibition of advanced glycation endproducts formation	(130)
	α-glucosidase inhibition	(137)
	neuroprotective	(134-136)

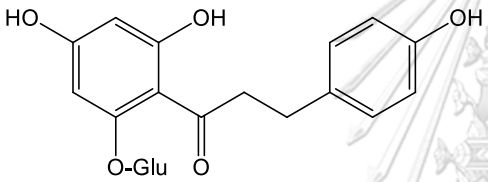
Table 4. Continued

Compounds	Activities	Reference
Isovitexin [**128**]	antioxidant	(131)
	anti-inflammatory	(132, 133)
Luteolin [**131**]	anticancer	(165)
	anti-Inflammatory, neuroprotective	(164)
	cardioprotective	(163)
Orientin [**132**]	inhibition of adipogenesis	(123)
	anti-inflammatory	(128)
	antioxidant	(124)
	radioprotective	(127)
	neuroprotective	(126)
	vasodilating	(125)
7-*O*-rutinose-5-hydroxy-6,4'-dimethoxyflavone [**133**]	cytotoxic	(169)
Pectolinarin [**134**]	Analgesic, anti-inflammatory	(168)
	hepatoprotective	(167)
Vitexin [**139**]	inhibition of adipogenesis	(123)

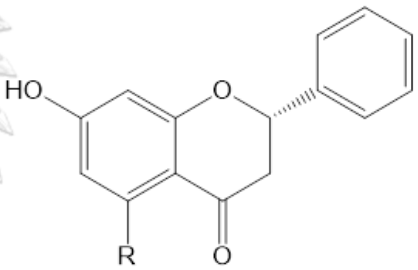
	inhibition of advanced glycation endproducts formation	(130)
	α-glucosidase inhibition	(137)
	anti-inflammatory	(132)
	anti-nociceptive	(138)
	antioxidant	(131)
	neuroprotective	(134, 136)
	spasmolytic	(139)

Table 4. Continued

Compounds	Activities	Reference
Vitexin [**139**]	apoptosis inducing	(140, 141)
D. Flavonols	α-glucosidase inhibition	(180)
Galangin [**141**]	antioxidant, anti-inflammatory	(179)
	xanthine oxidase inhibition	(181)
	apoptosis inducing	(177)
	autophagy inducing	(178)
	cytotoxic	(16, 17, 176)
kaempferol [**142**]	chemopreventive	(162)
quercetin [**143**]	anticancer	(161)
	antioxidant, antifungal, anti-carcinogenic, hepatoprotective	(160)

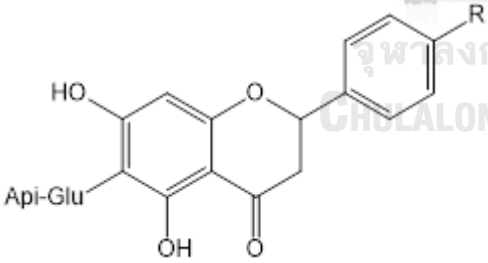


[116]



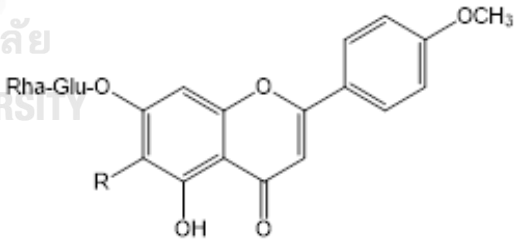
[117]: R = OCH_3

[120]: R = OH



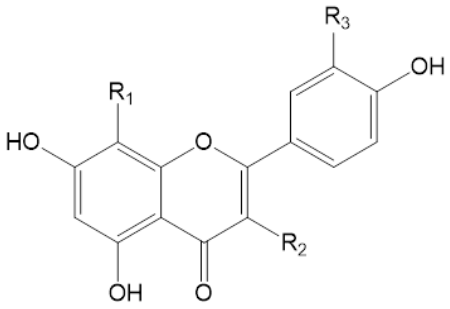
[118]: R = OH

[119]: R = OCH_3



[121]: R = H

[124]: R = OCH_3

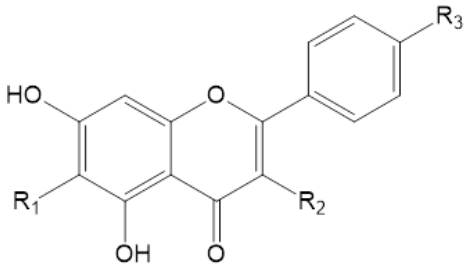


[122]: $R_1 = R_2 = R_3 = H$

[124]: $R_1 = R_2 = H$, $R_3 = OCH_3$

[131]: $R_1 = R_2 = H$, $R_3 = OH$

[132]: $R_1 = Glu$, $R_2 = H$, $R_3 = OH$



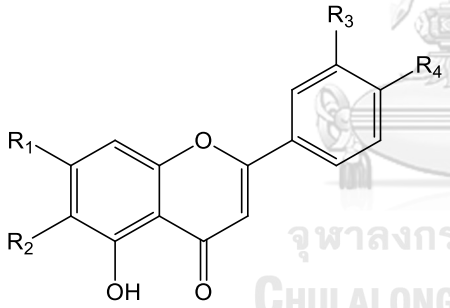
[123]: $R_1 = R_2 = R_3 = H$

[125]: $R_1 = H$, $R_2 = OCH_3$, $R_3 = H$

[126]: $R_1 = OCH_3$, $R_2 = H$, $R_3 = OCH_3$

[127]: $R_1 = H$, $R_2 = R_3 = OCH_3$

Figure 4. Flavonoids from orchids of tribe Cymbidieae and subfamily Cypripedioideae

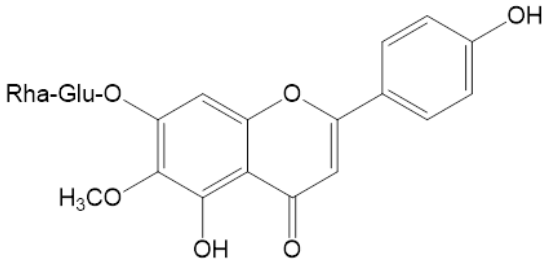


[133]: $R_1 = O\text{-Glu-Rha}$, $R_2 = R_3 = OCH_3$, $R_4 = H$

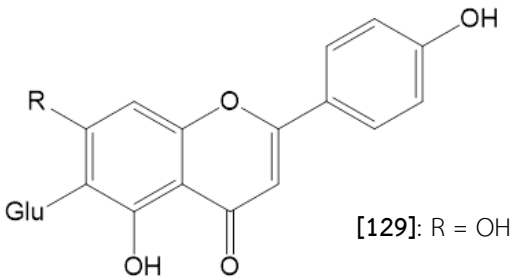
[135]: $R_1 = O\text{-Glu}$, $R_2 = OCH_3$, $R_3 = R_4 = OH$

[137]: $R_1 = O\text{-Glu}$, $R_2 = OCH_3$, $R_3 = OH$, $R_4 = H$

[138]: $R_1 = OCH_3$, $R_2 = H$, $R_3 = OH$, $R_4 = OCH_3$

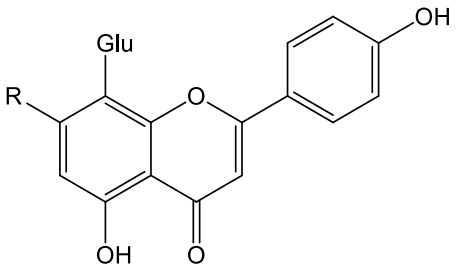


[128]



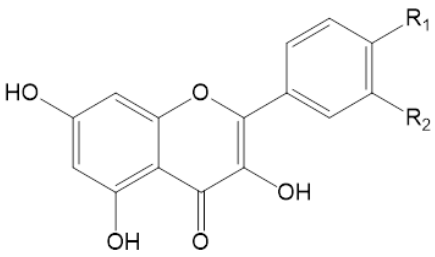
[129]: R = OH

[130]: R = O-Glu



[139]: R = OH

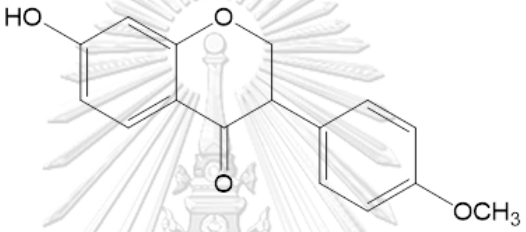
[140]: R = O-Glu



[141]: $R_1 = R_2 = H$

[142]: $R_1 = OH$, $R_2 = H$

[143]: $R_1 = R_2 = OH$



[144]

Figure 4. Continued

CHAPTER III

EXPERIMENTAL

1. Source of plant materials

The whole plants of *Cymbidium finlaysonianum* were collected from a garden in Nonthaburi in February 2016, and the whole plants of *Paphiopedilum dianthum* were collected from a garden in Chiang Mai in February 2017. Voucher specimens of both orchids have been deposited at the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

2. General techniques

2.1 Solvents

All organic solvents used in this study were of commercial grade and they were redistilled prior to use.

2.2 Analytical thin-layer chromatography (TLC)

Technique	One dimension, ascending
Adsorbent	Silica gel 60 F_{254} pre-coated plates (E. Merck, Darmstadt, Germany)
Layer thickness	0.2 mm
Distance	5 cm
Temperature	Laboratory temperature (30-35 °C)
Detection	1. Ultraviolet light (254 and 365 nm) 2. Spraying with 10% sulfuric acid, then heating at 110°C for 10 min

2.3 Column chromatography

2.3.1 Conventional column chromatography

Adsorbent	Silica gel 60 number 7734 (particle size 0.063-0.200 mm) and number 9385 (particle size 0.040-0.063 mm) (E. Merck)
Packing method	Wet packing: Silica gel was dispersed in the organic solvent, stirred into slurry, then poured into a column and allowed to settle.
Sample loading	Sample (plant extract) was dissolved in a small amount of the eluent, mixed with a small quantity of silica gel

	until dried in the air. The mixture was then placed atop the silica gel in the column.
Detection	All fractions collected were examined by TLC as described in 2.2.

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2.3.2 Vacuum liquid chromatography

Adsorbent	Silica gel 60 number 7734 (particle size 0.063-0.200 mm) (E. Merck)
Packing method	Dry packing: Silica gel was poured into a column, then the organic solvent was added onto the silica gel.
Sample loading	Sample (plant extract) was dissolved in a small amount of the eluent, mixed with a small quantity of silica gel until dried in the air. The mixture was then placed on the top face of adsorbent within the column.
Detection	All fractions collected were examined by TLC as described in 2.2.

2.3.3 Size-exclusion column chromatography

Adsorbent	Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden)
Packing method	Sephadex gel was suspended in suitable organic solvent and allowed to swell for 24 hours, then it was poured into the column and left to fully settle prior to use.
Sample loading	Sample, dissolved in a small amount of the eluent, was applied onto the top of the column.
Detection	All eluted fractions were examined by TLC as described in 2.2.

2.4 Spectroscopy

2.4.1 Ultraviolet (UV) spectra

UV spectra (in methanol) were recorded on a Shimadzu UV-160A spectrophotometer (Shimadzu, Kyoto, Japan) at Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

2.4.2 Infrared (IR) spectra

IR spectra (KBr disc) were recorded on a Perkin Elmer FT-IR 1760X spectrometer (Perkin Elmer, Massachusetts, USA) at Scientific and Technological Research Equipment Center, Chulalongkorn University.

2.4.3 Mass spectra

Mass spectra were obtained on a microTOF Bruker Daltonics mass spectrometer (Bruker, Massachusetts, USA) at Department of Chemistry, Faculty of Science, Mahidol University or a micrOTOF-Q II Bruker Daltonics mass spectrometer at Department of Chemistry, Faculty of Science, Chulalongkorn University.

2.4.4 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 recorded on a Bruker DPX-300 FT-NMR spectrometer at Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University or at Scientific and Technology Research Equipment Center, Chulalongkorn University.

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Varian INOVA-500 NMR spectrometer (Varian, Darmstadt, Germany) at Scientific and Technology Research Equipment Center, Chulalongkorn University.

2.5 Physical property

2.5.1 Optical rotations

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

3. Extraction and isolation of compounds from *Cymbidium finlaysonianum* whole plants

3.1 Extraction of *C. finlaysonianum* whole plants

One kilogram of dried *C. finlaysonianum* whole plants (without flowers) were cut into small pieces and soaked in MeOH (4 × 14 L). The MeOH extract was concentrated under reduced pressure at 45 °C using rotary evaporator. Then, it was dispersed in 500 ml of water and partitioned five times with 200 ml of ethyl acetate (EtOAc) to give a EtOAc extract (20 g, 2.0% yield) and an aqueous extract (47 g, 4.7% yield), as shown in **Figure 5.**

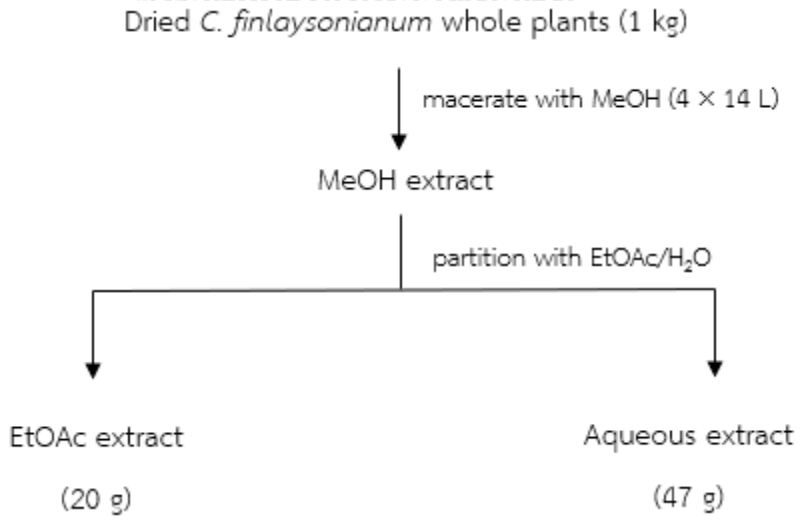


Figure 5. Extraction of *Cymbidium finlaysonianum* whole plants

3.2 Isolation of compounds from the EtOAc extract of *C. finlaysonianum*

The ethyl acetate extract (20 g) was separated on a silica gel column (600 g, 9.5 × 21 cm), eluted with a gradient mixture of *n*-hexane and EtOAc (5:2 ⟶ 0:1). Two hundred and five fractions (100 ml each) were collected and combined according to their TLC profile, using *n*-hexane-EtOAc (3:2) as the solvent system, into 6 combined fractions (A-F) shown in **Table 5**.

Table 5. Fractions obtained from the EtOAc extract of *C. finlaysonianum*

Fraction Code	Weight (g)
A	4.39
B	3.39
C	0.46
D	1.48
E	1.83
F	3.50

3.2.1 Isolation of compound CF-1 (cymbinodin A)

Fraction B (3.39 g) was purified by gel filtration through a Sephadex LH-20 column (2.5 × 80 cm) eluted with dichloromethane (CH_2Cl_2)-MeOH (1:1). Six subfractions (40 ml each) were collected, verified by TLC and pooled into two subfractions (B1-B2). Subfraction B2 (2.6 g) was separated on a silica gel column (75 g, 5 × 16.5 cm), washed down with CH_2Cl_2 into 22 subfractions, which were later combined based on their TLC pattern into 4 major subfractions (B21-B24). Subfraction B23 (0.2 g) was subjected to silica gel column chromatography (10 g, 2 × 11 cm) with *n*-hexane-CH_2Cl_2 (1:2) as the mobile phase. Twelve subfractions (2 ml each) obtained and the pooled into 3 subfractions (B231-B233). Purification of subfraction B232 (63

mg) on a Sephadex LH-20 column (2.5 × 80 cm), eluted with CH_2Cl_2-MeOH (1:1), gave compound CF-1 as dark purple needles (26 mg).

In addition, purification of subfraction B24 (0.24 g) by silica gel column chromatography, eluted with CH_2Cl_2-MeOH (1:4), afforded 3 combined subfractions (B241-B243). Subfraction B241 was further purified on a Sephadex LH-20 column (2.5 × 80 cm), washed down with CH_2Cl_2-MeOH (1:1), to give an additional amount (3 mg) of compound CF-1. Therefore, the total amount of compound CF-1 was 29 mg (0.0029% yield).

3.2.2 Isolation of compound CF-2 (ephemeranthoquinone B)

Subfraction B242 (67 mg) was repeated on a Sephadex LH-20 column (2.5 × 80 cm), using CH_2Cl_2-MeOH (1:1) as the mobile phase. The eluates were combined into 3 subfractions (B2421-B2423), and evaporation of subfraction B2423 yielded compound CF-2 as red needles (25 mg).

Fraction C (0.46 g) was separated on a Sephadex LH-20 column (2.5 × 80 cm) eluted with CH_2Cl_2-MeOH (1:1) into two subfractions (C1-C2) based on their TLC profile. Subfraction C2 (0.1 g) was chromatographed on a silica gel column (10 g, 2.5 × 10.5 cm) washed down with CH_2Cl_2. The eluates (1 ml each) were combined into 8 subfractions (C21-C28). Compound CF-2 (2 mg) was obtained from subfraction C23, bringing its total amount to 27 mg (0.0027% yield).

3.2.3 Isolation of compound CF-3 (6-methoxycoelonin)

Fraction D (1.48 g) was loaded onto a silica gel column (74 g, 2.5 × 30.5 cm) and washed down with CH_2Cl_2-acetone (40:1). Forty-four fractions (15 ml each) were collected, inspected by TLC, and then combined into four subfractions (D1-D4). Subfraction D3 (0.68 g) was further separated over a Sephadex LH-20 column (2.5 × 80 cm), eluted with CH_2Cl_2-MeOH (1:1), to give four subfractions (D31-D34). Subfraction

D33 (0.11 g) was subjected to silica gel column chromatography (10 g, 2.5 × 11 cm) eluted with CH_2Cl_2-acetone (40:1). Fifteen subfractions (2 ml each) were collected and pooled into four subfractions (D331-D334) based on their TLC pattern. Subfraction D334 was repeatedly chromatographed over silica gel columns (10 g, 2.5 × 10.5 cm), using CH_2Cl_2-acetone (40:1) as solvent system, to yield 6 subfractions (D3341-D3346). Subfraction D3341 afforded compound CF-3 as brown amorphous solid (36 mg).

Compound CF-3 (13 mg) was also obtained from subfraction E23 after evaporation of the solvent. Similarly, evaporation of subfraction E301 gave an additional amount (7 mg) of compound CF-3. Therefore, the total amount of compound CF-3 isolated was 56 mg (0.0056% yield).

3.2.4 Isolation of compound CF-4 (flavanthridin)

Subfraction D3343 was separated on a Sephadex LH-20 column (2.5 × 75 cm), using MeOH as the eluent, into 2 subfractions (D33431-D33432) Compound CF-4 was obtained from the first subfraction (D33431) as a brown amorphous solid (5 mg, 0.0005% yield).

3.2.5 Isolation of compound CF-5 (2,4-dimethoxy-3,7-dihydroxyphenanthrene)

Evaporation of subfraction D33432 to dryness afforded compound CF-5 as light brown amorphous solid (7 mg). The same compound (22 mg) was also obtained from subfraction E303 after removed of the solvent. Therefore, the total amount of compound CF-5 obtained were 29 mg (0.0029% yield).

3.2.6 Isolation of compound CF-6 (3,7-dihydroxy-2,4,6-trimethoxyphenanthrene)

Fraction E (1.83 g) was separated on a Sephadex LH-20 column (2.5 × 100) eluted with CH_2Cl_2-MeOH (2:1). Forty subfractions were collected, examined by TLC, then combined into four subfractions (E1-E4). Separation of subfraction E2 (0.6 g) on a silica gel column (30 g, 3 × 13 cm), washed down with CH_2Cl_2-acetone (40:1), gave four subfractions (E21-E24) according to their TLC profile. Evaporation of subfraction E21 yielded compound CF-6 as a brown amorphous solid (13 mg, 0.0013% yield).

3.2.7 Isolation of compound CF-7 (coelonin)

Subfraction E3 (0.9 g) was purified on a silica gel column (10 g, 2.5 × 11 cm), using CH_2Cl_2-acetone (40:1) as the solvent system. One hundred and seventy-two subfractions were collected and combined according to their TLC pattern into twelve subfractions (E301-E312). Upon evaporation of subfraction E305, compound CF-7 was obtained as a brown amorphous solid (6 mg, 0.0006% yield).

3.2.8 Isolation of compound CF-8 (lusianthridin)

Subfraction E307, after evaporation to dryness, afforded compound CF-8 as a brown amorphous solid (1 mg, 0.0001% yield).

3.2.9 Isolation of compound CF-9 (batatasin III)

Evaporation of subfraction E309 gave compound CF-9 as a brown amorphous solid (4 mg, 0.0004% yield).

3.2.10 Isolation of compound CF-10 [1-(4-Hydroxybenzyl)-4,6-dimethoxy-9,10-dihydrophenanthrene-2,7-diol]

Subfraction E11 was evaporated to dryness to yield compound CF-10 as light brown amorphous powders (7 mg, 0.0007% yield).

The isolation of chemical constituents from the EtOAc extract of *C. finlaysonianum* whole plants is presented in **Figures 6-9.**

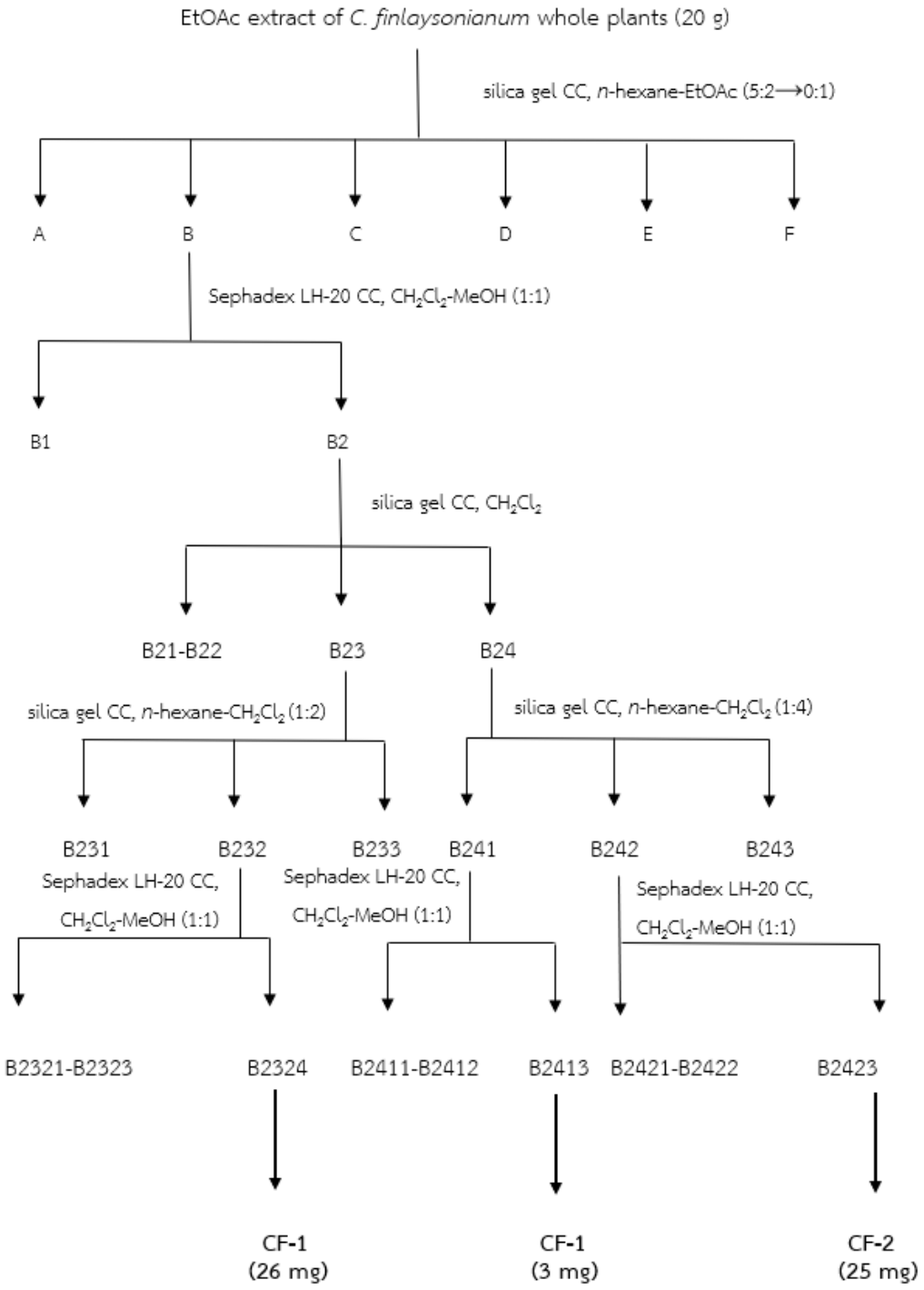


Figure 6. Isolation of compounds from fraction B of the EtOAc extract of *C. finlaysonianum*

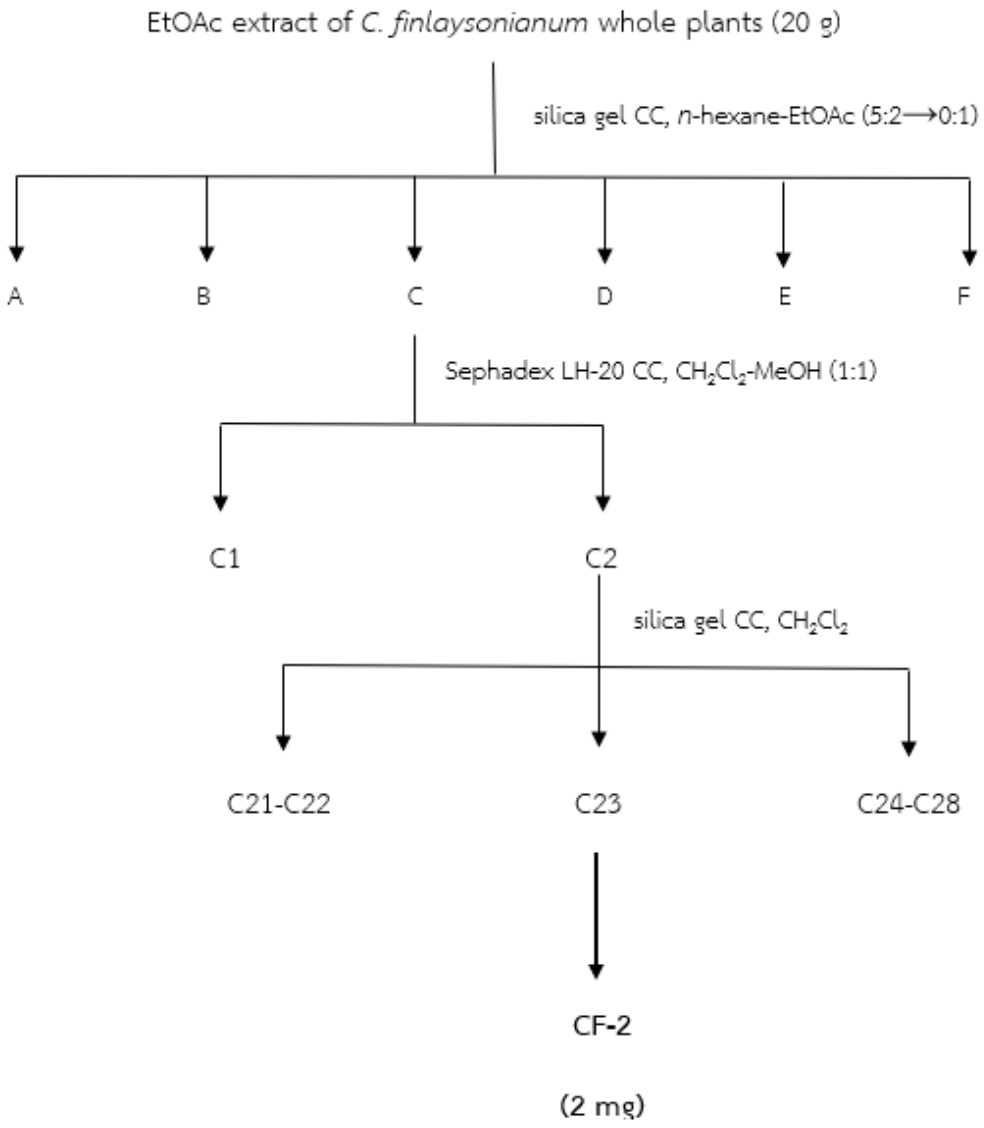


Figure 7. Isolation of compounds from fraction C of the EtOAc extract of *C. finlaysonianum*

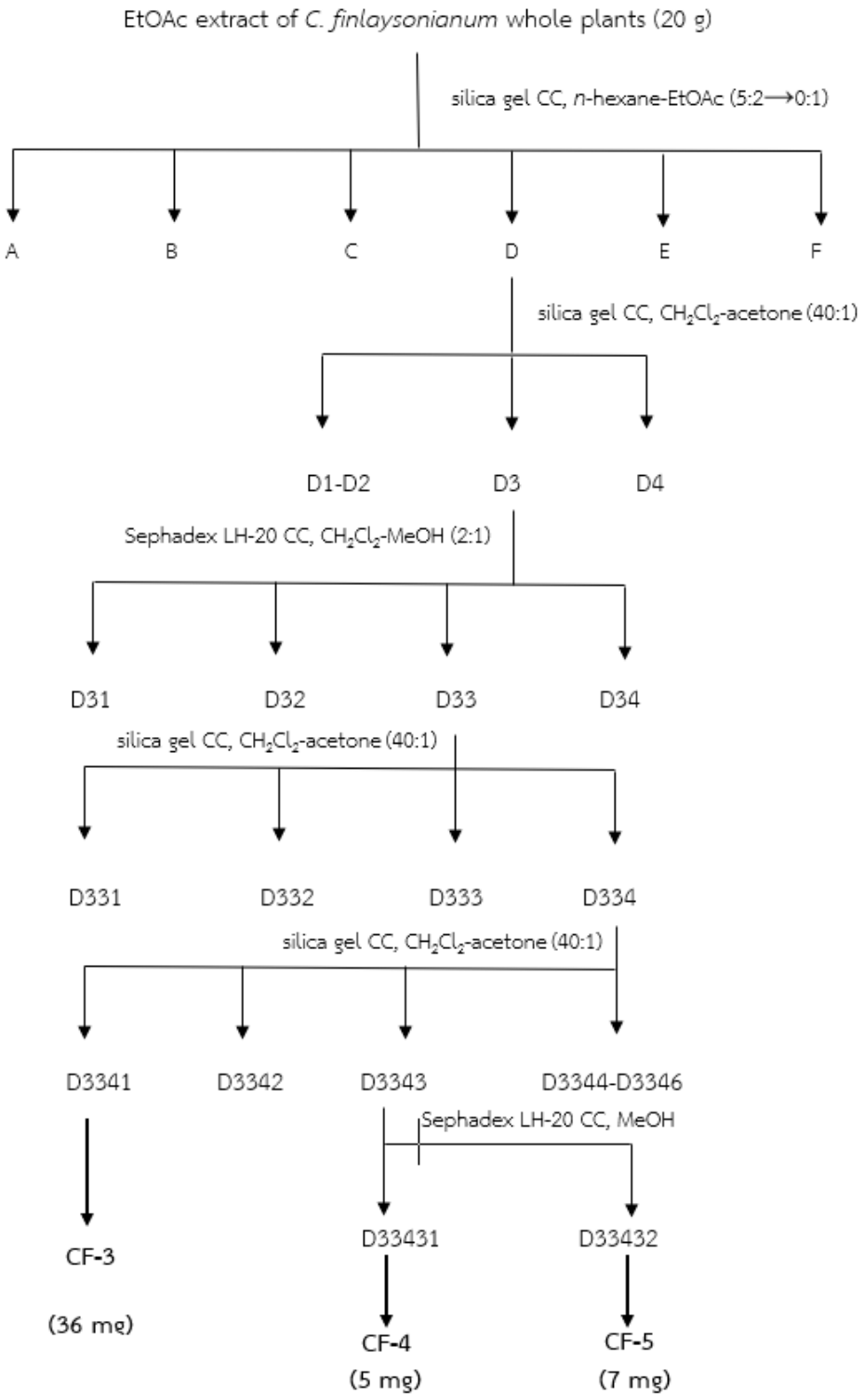


Figure 8. Isolation of compounds from fraction D of the EtOAc extract of *C. finlaysonianum*

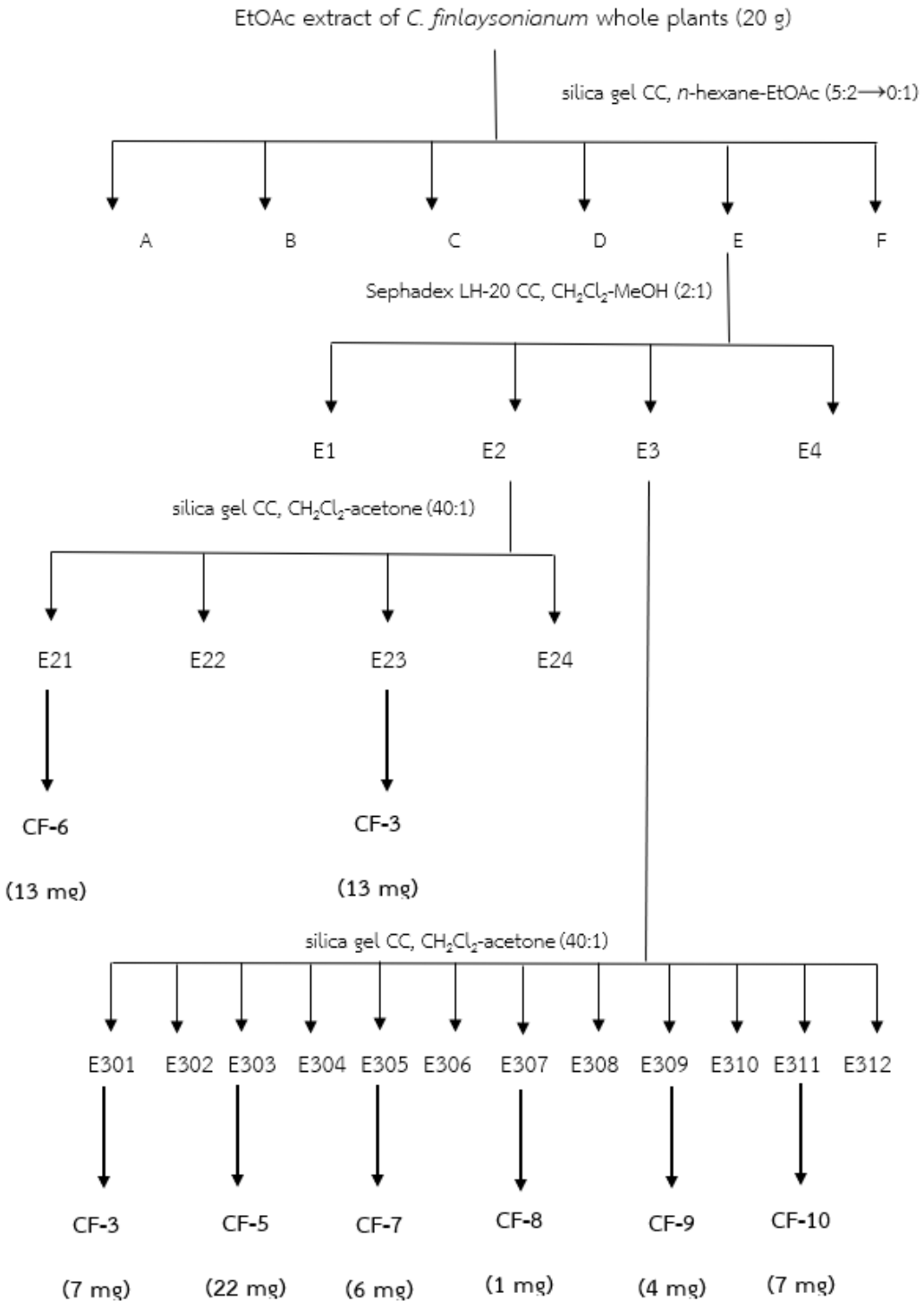


Figure 9. Isolation of compounds from fraction E of the EtOAc extract of *C. finlaysonianum*

4. Extraction and isolation of compounds from *P. dianthum* roots

4.1 Extraction of *P. dianthum* roots

Fresh roots of *P. dianthum* (800 g) were cut into small pieces and dried in hot air oven at 50°C. The dried roots (400 g) were macerated with 2 liters of MeOH three times (3 days each). The MeOH extract was concentrated at 45 °C in a rotary evaporator, then suspended in 500 ml of water and partitioned with EtOAc (5 × 200 ml) to give an EtOAc extract (61 g, 15.25% yield) and an aqueous extract (96 g, 24.0% yield), as shown in **Figure 10.**

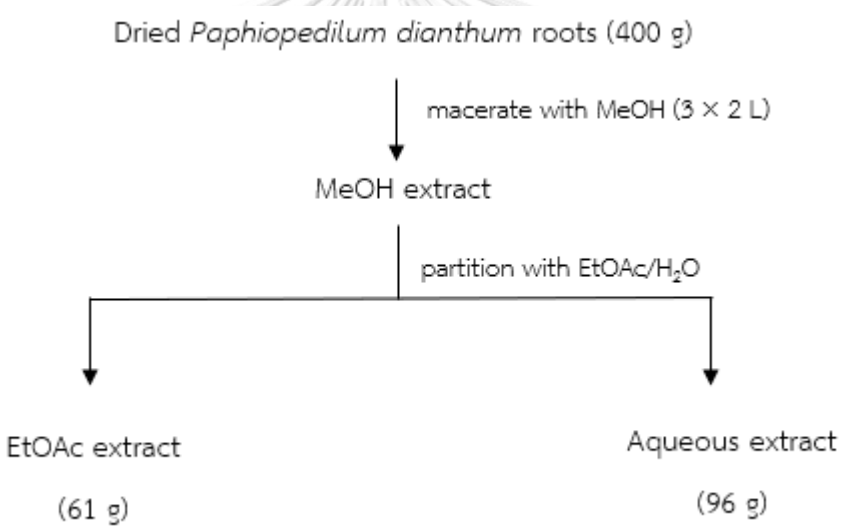


Figure 10. Extraction of *Paphiopedilum dianthum* roots

4.2 Isolation of compounds from the EtOAc extract of *P. dianthum* roots

The EtOAc extract (61 g) was separated by vacuum column chromatography (silica gel 400 g, 13 × 7 cm) eluting with gradient mixture of *n*-hexane and acetone (1:0 ⟶ 0:1). Twenty-four fractions (300 ml each) were collected and combined into five

main fractions (A-E) based on their TLC profile [solvent system: *n*-hexane and acetone (2:3)], as shown in **Table 6**.

Table 6. Fractions obtained from the EtOAc extract of *P. dianthum* roots

Fraction Code	Weight (g)
A	2.36
B	9.80
C	33.29
D	4.03
E	10.49

4.2.1 Isolation of compound PD-1 (isalpinin)

Fraction B (9.8 g) was applied to a silica gel column (160 g, 5 × 17 cm) and eluted with *n*-hexane-CH_2Cl_2 (1:1). Forty subfractions (100 ml each) were collected and combined into five subfractions (B1-B5). Subfraction B4 was purified on a Sephadex LH-20 column (2.5 × 75 cm), eluted with MeOH. Nine fractions (50 ml each) were collected and combined into 4 subfractions (B41-B44) after TLC inspection. Compound PD-1 was obtained from subfraction B44 as yellow needles (48 mg, 0.012% yield).

The isolation of chemical constituents from fraction B of the EtOAc extract of *P. dianthum* roots is shown in **Figure 11.**

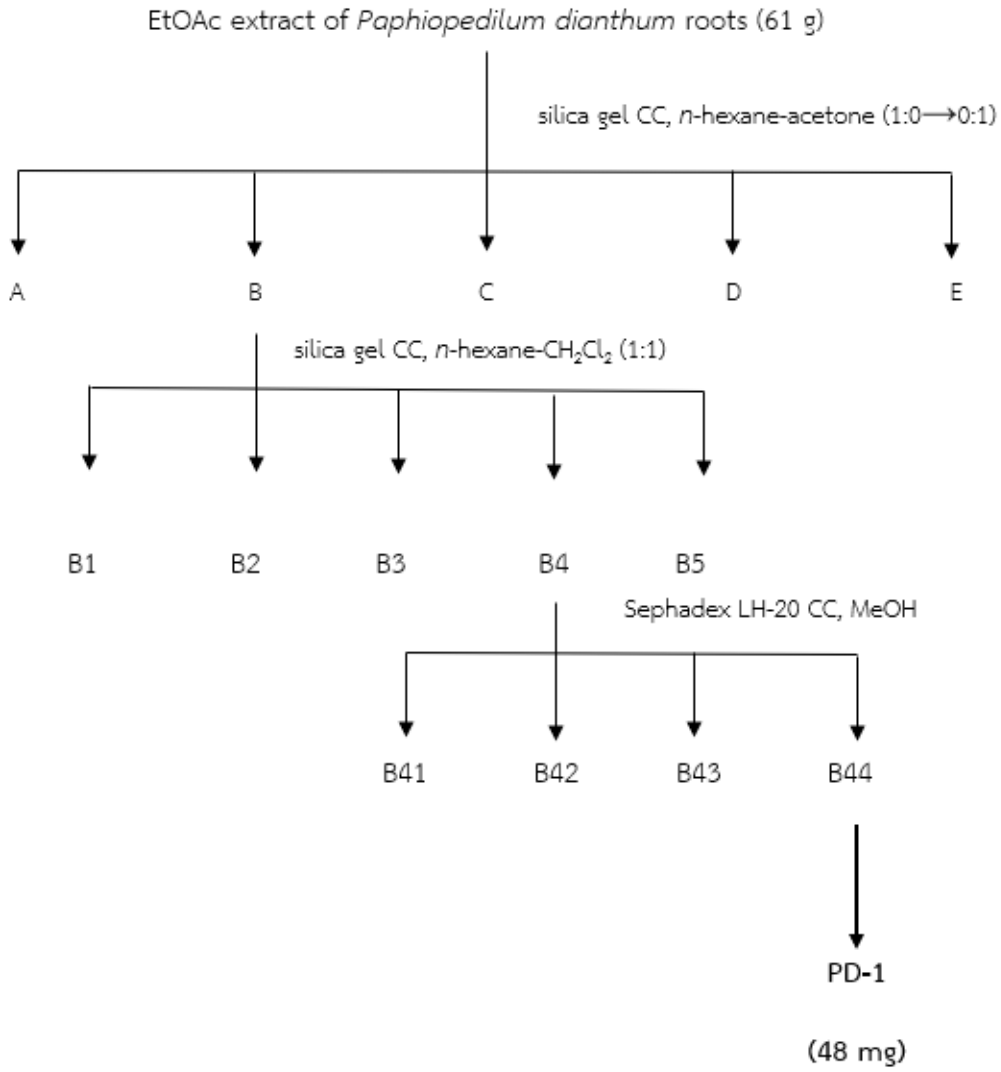


Figure 11. Isolation of compounds from fraction B of the EtOAc extract of *P. dianthum* roots

4.2.2 Isolation of compound PD-2 (pinosylvin monomethyl ether)

Fraction C (33.3 g) was separated by gel filtration on a Sephadex LH-20 column (2.5 × 75 cm), washed down with MeOH. Fifty-seven subfractions (50 ml each) were collected and pooled into five subfractions (C1-C5) based on their TLC pattern. Subfraction C1 (6.0 g) was subjected to silica gel column chromatography (300 g, 5 × 32 cm), using *n*-hexane-CH_2Cl_2 (1:1) as the mobile phase, to yield six pooled subfractions (C11-C16). Purification of subfraction C11 on a silica gel column, using hexane-CH_2Cl_2 (1:1) as the eluent, gave subfractions C111 and C112. The latter subfraction afforded compound PD-2 as a brown semisolid (1.2 g, 0.3% yield).

4.2.3 Isolation of compound PD-3 (pinocembrin)

Subfraction C13 was chromatographed over a silica gel column (80.5 g, 3 × 24 cm), eluted with *n*-hexane-CH_2Cl_2 (1:1), to yield 22 collected subfractions (20 ml each) which were combined into 5 subfractions (C131-C135). Subfraction C132 was repeated on a silica gel column (10 g, 2.5 × 10.5 cm), washed down with CH_2Cl_2, into seven subfractions (C1321-C1327). Compound PD-3 was obtained from subfraction C1324 as a yellow amorphous solid (0.5 g, 0.125% yield).

4.2.4 Isolation of compound PD-4 (galangin)

Subfraction C1326, after left drying at room temperature, gave compound PD-4 as yellow needles (0.2 g, 0.05% yield).

4.2.5 Isolation of compound PD-5 (2,3'-dihydroxy-5'-methoxystilbene)

Subfraction C2 was chromatographed on a silica gel column (230 g, 5 × 28 cm), washed down with *n*-hexane-CH_2Cl_2 (2:1). Forty subfractions (50 ml each) were collected, and examined by TLC [solvent system: *n*-hexane-CH_2Cl_2 (2:1)], and then pooled into five subfractions (C21-C25). Subfraction C22 was separated on a Sephadex LH-20 column (2.5 × 75 cm) eluted with MeOH into four subfractions (C221-C224).

Subfraction C224, after evaporation to dryness, yielded compound PD-5 as brown semisolid (1.5 g, 0.375% yield).

4.2.6 Isolation of compound PD-6 [(*E*)-2,5'-dihydroxy-2'-(4-hydroxybenzyl)-3'-methoxystilbene]

Subfraction C25 (0.65 g) was purified on a silica gel column (10 g, 2.5 × 11 cm) using CH_2Cl_2-acetone (40:1) as the eluent. Six subfractions were collected and combined into 4 subfractions (C251-C254) based on their TLC pattern. Upon evaporation of subfraction C253, compound PD-6 was obtained as a white powder (45 mg, 0.01125% yield).

4.2.7 Isolation of compound PD-7 (paphiodianthin A)

Subfraction C3 (4.03 g) was separated on a Sephadex LH-20 column (2.5 × 75 cm), using MeOH as the mobile phase, to give 5 subfractions (C31-C35). Subfraction C33 was reseparated on a Sephadex column (2.5 × 75 cm) to give three subfractions (C331-333). Subfraction C332 was evaporated to dryness to obtain compound PD-7 as brown amorphous powder (20 mg, 0.005% yield).

The isolation of chemical constituents from fraction C of the EtOAc extract of *P. dianthum* roots is shown in **Figure 12.**

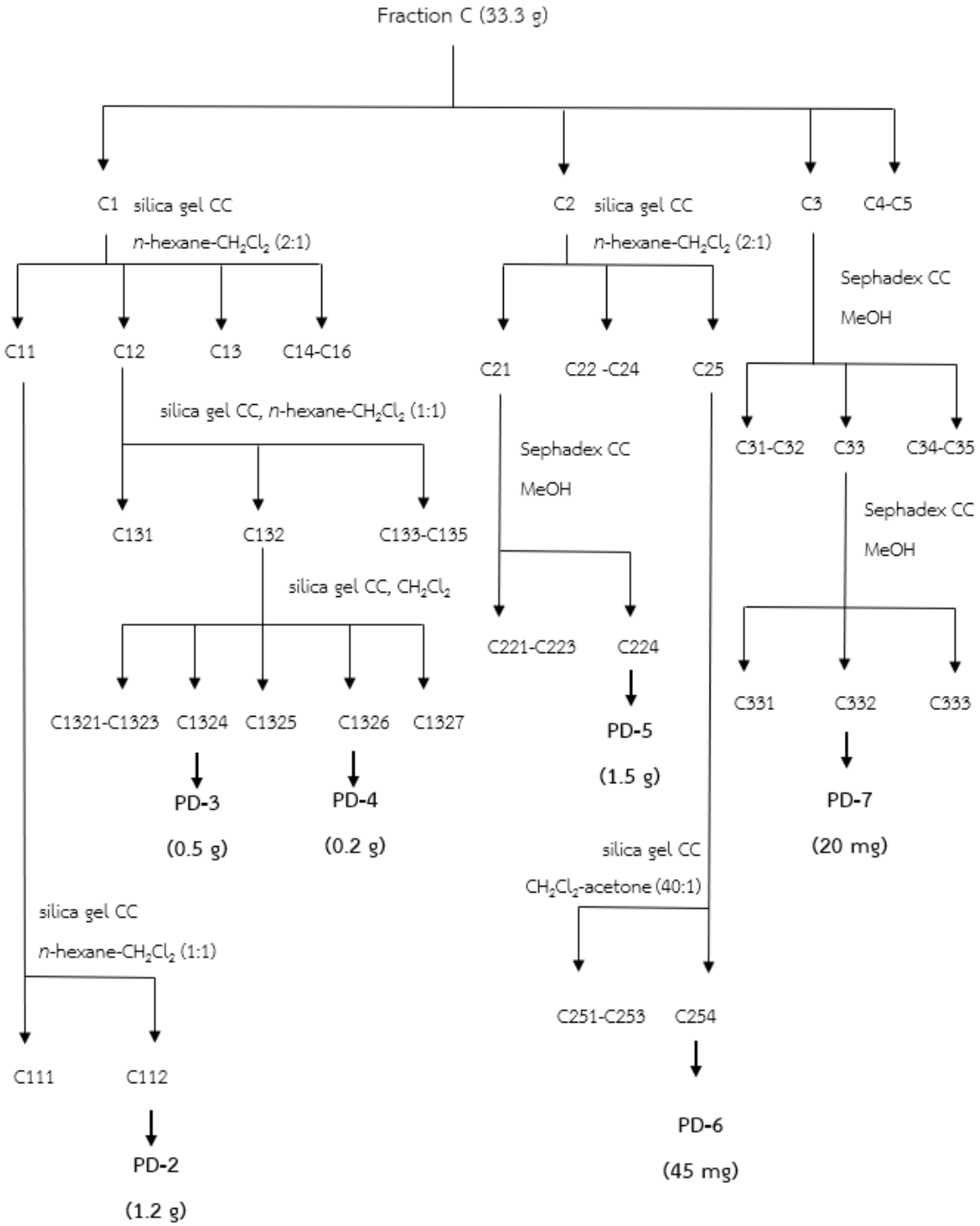


Figure 12. Isolation of compounds from fraction C of the EtOAc extract of *P. dianthum* roots

5. Extraction and isolation of compounds from *Paphiopedilum dianthum* leaves

5.1 Extraction of *P. dianthum* leaves

Cut fresh leaves of *P. dianthum* (2.3 kg) were dried by hot air oven at 50°C to obtain 600 g of dried leaves. They were macerated in MeOH (4 L) three times, and the MeOH extract was concentrated by rotary evaporation under reduced pressure at 45 °C. The concentrated extract was partitioned between EtOAc and water to give an EtOAc extract (32 g, 5.3% yield). Then, the aqueous portion was treatd with BuOH to give a BuOH extract (108 g, 18.0% yield) and an aqueous extract (109 g, 18.16% yield), as shown in **Figure 13.**

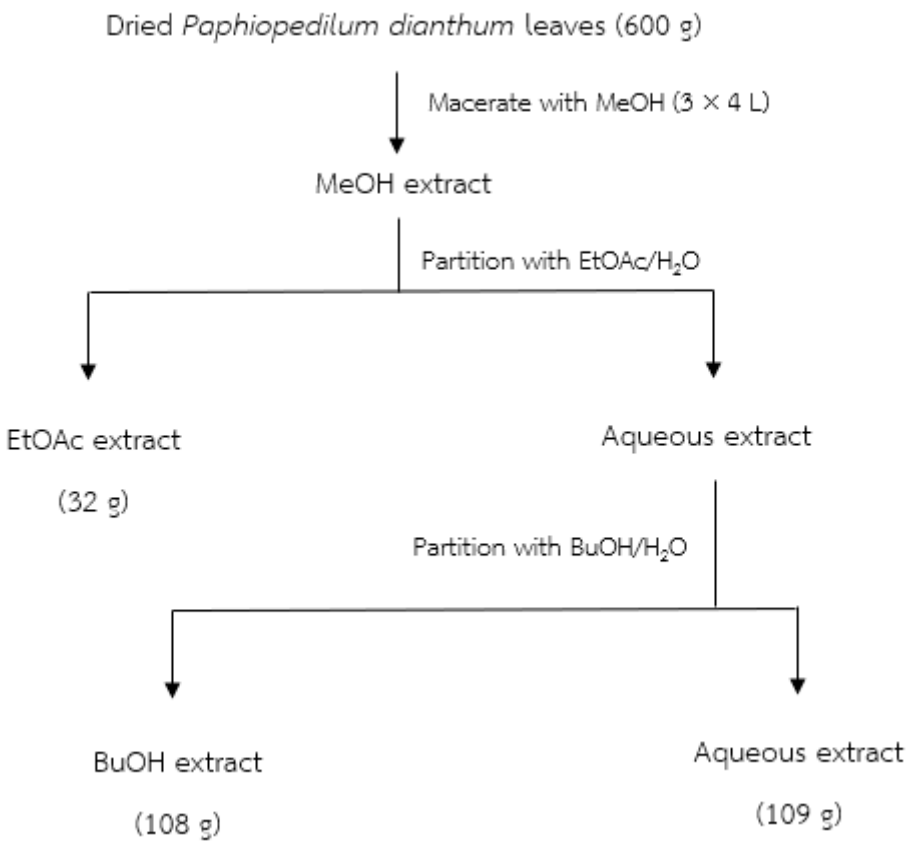


Figure 13. Extraction of *Paphiopedilum dianthum* leaves

5.2 Isolation of chemical constituents from the EtOAc extract of *P. dianthum* leaves

The EtOAc extract (32 g) was separated by vacuum column chromatography (silica gel 400 g, 13 × 9 cm), eluted with a gradient mixture of *n*-hexane-acetone (4:1 ⟶ 0:1). Twenty-three fractions (500 ml each) were collected and combined into six subfractions (A-F) according to their TLC profile [solvent system: *n*-hexane-acetone (2:3)], as shown in **Table 7**.

Table 7. Fractions obtained from the EtOAc extract of *P. dianthum* leaves

Fraction Code	Weight (g)
A	3.50
B	4.97
C	3.60
D	7.62
E	4.29
F	8.24

5.2.1 Isolation of compound PD-8 (paphiodianthin B)

Fraction C was separated by gel filtration on a Sephadex LH-20 column (2.5 × 60 cm) using acetone as the eluent. Eight subfractions (50 ml each) were collected and combined into subfractions C1-C4 based on their TLC profile. Subfraction C2 was purified on a Sephadex LH-20 column (2.5 × 100 cm), washed down with CH_2Cl_2-MeOH (1:1). Eleven subfractions (50 ml each) were collected, examined by TLC, then combined into subfractions C21-C24. Chromatography of subfraction C22 on a silica gel column (10 g, 2.5 × 11 cm), eluted with *n*-hexane-CH_2Cl_2 (1:1), yielded 3 subfractions (C221-C223). Subfraction C223 was further separated on a silica gel column

(2 g, 5 × 1 cm), washed down with *n*-hexane-acetone (7:3). Seven subfractions (1 ml each) were collected, then pooled into four subfractions (C2231-C2234). Evaporation of subfraction C2232 to dryness afforded compound PD-8 as a brown amorphous powder (3 mg, 0.0005% yield).

5.2.2 Isolation of compound PD-2 (pinosylvin monomethyl ether)

Subfraction C23 was chromatographed over a silica gel column (10 g, 2.5 × 11 cm) eluted with *n*-hexane-acetone (7:3). Nineteen subfractions (2 ml each) were collected and combined into subfractions C231-C233. Purification of subfraction C232 over a Sephadex LH-20 column (2.5 × 75 cm), using MeOH as the mobile phase, gave four subfractions (C2321-C2324). Compound PD-2 was obtained from subfraction C2322 as a brown semisolid (9 mg, 0.0015% yield).

5.2.3 Isolation of compound PD-3 (pinocembrin)

Evaporation of subfraction C2323 to dryness gave compound PD-3 as a yellow amorphous solid (3 mg, 0.0005% yield).

5.2.4 Isolation of compound PD-1 (isalpinin)

Subfraction C233 was separated on a Sephadex LH-20 column (2.5 × 75 cm) and washed down with MeOH. Twelve subfractions (10 ml each) were collected and combined into subfractions C2331 and C2332 depending on their TLC pattern. Subfraction C2332, when dried, afforded compound PD-1 as yellow needles (7 mg, 0.0012% yield).

5.2.5 Isolation of compound PD-4 (galangin)

Fraction C24 was separated by gel filtration over a Sephadex LH-20 column (2.5 × 100 cm) eluted with CH_2Cl_2-MeOH (1:1). The eluted subfractions (5 ml each) were

inspected by TLC and pooled into three subfractions (C241-C243). Compound PD-5 was obtained from subfraction C243 as yellow needles (62 mg, 0.010% yield).

The isolation of chemical constituents from the EtOAc extract of *P. dianthum* leaves is summarized in **Figures 14-15**.

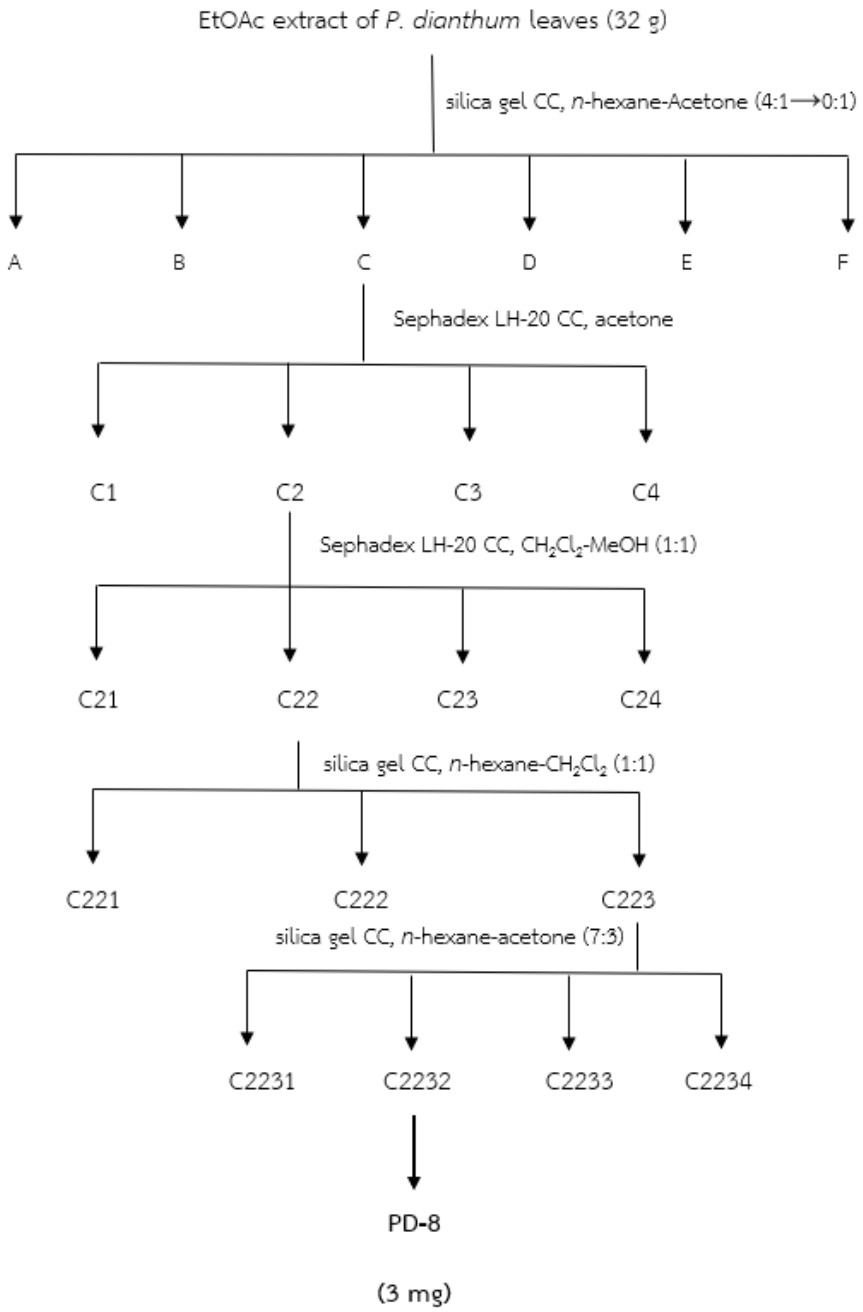


Figure 14. Isolation of compounds from fraction C of the EtOAc extract of *P. dianthum* leaves

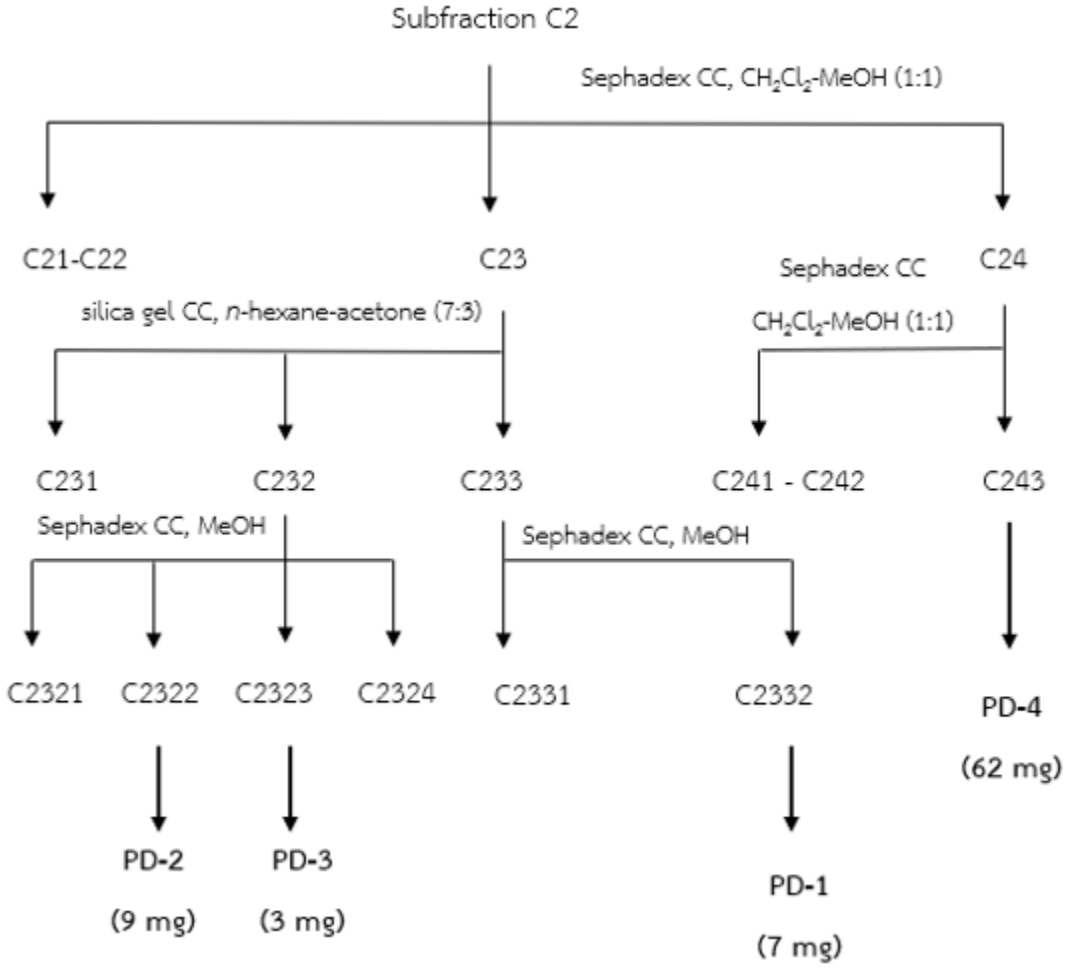


Figure 15. Isolation of compounds from fraction C of the EtOAc extract of *P. dianthum* leaves (continued)

6. Physical and spectral data of isolated compounds

6.1 Compound CF-1 (cymbinodin-A)

Compound CF-1 was obtained as dark purple needles (29 mg, 0.0029% based on dried weight of *C. finlaysonianum* whole plants). The compound is soluble in CH_2Cl_2 and acetone.

UV: λ_{max} (MeOH) nm (log ε): 216 (4.98), 304 (4.65); **Figure 16.**

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 227.0475; **Figure 17.**

IR: ν_{max} cm^{-1}: 3435, 1677, 1638, 1238, 1047, 817; **Figure 18.**

^{1}H NMR: δ ppm, 300 MHz, in $CDCl_3$; 3.98 (3H, *s*), 6.29 (1H, *s*), 7.24 (1H, *dd*, *J* = 7.8, 1.5 Hz), 7.42 (1H, *dd*, *J* = 7.8, 1.5 Hz), 7.58 (1H, *t, J* = 7.8 Hz), 8.16 (2H, *s*), 12.25 (1H, *s*); **Figure 19.**

^{13}C NMR: δ ppm, 75 MHz, in $CDCl_3$; 56.8, 111.5, 117.5, 121.0, 121.2, 121.9, 130.0, 131.0, 132.5, 137.3, 139.0, 155.6, 158.9, 180.1, 191.9; **Figure 20.**

^{1}H NMR: δ ppm, 300 MHz, in acetone-d_6; 4.06 (3H, *s*), 6.51 (1H, *s*), 7.18 (1H, *dd*, *J* = 7.7, 1.5 Hz), 7.56 (1H, *dd*, *J* = 7.7, 1.5 Hz), 7.67 (1H, *t, J* = 7.7 Hz), 8.14 (1H, *d*, *J* = 8.7 Hz), 8.35 (1H, *d*, *J* = 8.7 Hz), 12.36 (1H, *s*); **Figure 21.**

^{13}C NMR: δ ppm, 75 MHz, in acetone-d_6; 56.5, 111.5, 116.8, 120.8, 120.9, 121.6, 129.9, 130.6, 132.86, 137.1, 139.0, 155.8, 159.6, 179.6, 192.5; **Figure 22.**

6.2 Compound CF-2 (ephemeranthoquinone B)

Compound CF-2 was obtained as dark red needles (27 mg, 0.0027% based on dried weight of *C. finlaysonianum* whole plants). The compound is soluble in CH_2Cl_2 and acetone.

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 279.0635; **Figure 25**.

^{1}H NMR: δ ppm, 300 MHz, in $CDCl_3$; 2.68 (2H, *m*), 2.69 (2H, *m*), 3.90 (3H, *s*), 6.04 (1H, *s*), 6.79 (1H, *dd*, *J* = 7.2, 1.1 Hz), 6.92 (1H, *dd*, *J* = 8.3, 1.1 Hz), 7.23 (1H, *dd, J* = 8.3, 7.2 Hz), 9.82 (1H, *s*); **Figure 26**.

^{13}C NMR: δ ppm, 75 MHz, in $CDCl_3$; 21.3, 28.5, 56.6, 108.2, 117.5, 119.3, 120.3, 132.3, 139.1, 140.6, 143.2, 155.3, 158.6, 180.8, 191.5; **Figure 27**.

6.3 Compound CF-3 (6-methoxycoelonin)

Compound CF-3 was obtained as brown amorphous solid (56 mg, 0.0056% based on dried weight of *C. finlaysonianum* whole plants). The compound is soluble in CH_2Cl_2 and acetone.

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 295.0944; **Figure 32.**

^{1}H NMR: δ ppm, 300 MHz, in acetone-d_6; 2.65 (4H, *br s*), 3.84 (3H, *s*), 3.89 (3H, *s*), 5.17 (1H, *br s*), 5.62 (1H, *br s*), 6.33 (1H, *br s*), 6.40 (1H, *br s*), 6.76 (1H, *s*), 7.84 (1H, *s*); **Figure 33.**

^{13}C NMR: δ ppm, 75 MHz, in acetone-d_6; 28.9, 30.8, 55.7, 56.1, 98.2, 107.5, 111.3, 113.5, 116.6, 124.7, 131.4, 141.2, 143.5, 144.4, 154.6, 157.5; **Figure 34.**

6.4 Compound CF-4 (flavanthridin)

Compound CF-4 was obtained as brown amorphous solid (5 mg, 0.0005% based on dried weight of *C. finlaysonianum* whole plants). The compound is soluble in CH_2Cl_2 and acetone.

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 295.0948; **Figure 37.**

^{1}H NMR: **δ** ppm, 300 MHz, in $CDCl_3$; 2.70 (4H, *br s*), 3.68 (3H, *s*), 3.89 (3H, *s*), 4.90 (1H, *br*), 5.58 (1H, *br s*), 6.55 (1H, *s*), 6.70 (1H, *br s*), 6.72 (1H, *d*, *J* = 8.4 Hz), 8.14 (1H, *d*, *J* = 8.4 Hz); **Figure 38.**

^{13}C NMR: **δ** ppm, 75 MHz, in $CDCl_3$; 29.8, 30.2, 56.2, 60.1, 107.0, 113.4, 114.5, 120.2, 125.4, 128.3, 129.6, 137.6, 139.8, 144.8, 145.7, 154.1; **Figure 39.**

6.5 Compound CF-5 (2,4-dimethoxy-3,7-dihydroxyphenanthrene)

Compound CF-5 was obtained as brown amorphous solid (29 mg, 0.0029% based on dried weight of *Cymbidium finlaysonianum* whole plants). The compound is soluble in CH_2Cl_2 and acetone.

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 293.0793; **Figure 42.**

^{1}H NMR: **δ** ppm, 300 MHz, in acetone-d_6; 3.89 (3H, *s*), 3.95 (3H, *s*), 7.16 (1H, *dd*, *J* = 9.0, 2.6 Hz), 7.20 (1H, *s*), 7.22 (1H, *d*, *J* = 2.6 Hz), 7.42 (1H, *d*, *J* = 8.7 Hz), 7.57 (1H, *d*, *J* = 8.7 Hz), 9.30 (1H, *d*, *J* = 9.0 Hz); **Figure 43.**

^{13}C NMR: **δ** ppm, 75 MHz, in acetone-d_6; 56.2, 59.5, 105.8, 112.1, 117.3, 119.9, 123.6, 125.2, 126.2, 128.0, 128.9, 134.9, 141.0, 145.2, 148.4, 155.9; **Figure 44.**

6.6 Compound CF-6 (3,7-dihydroxy-2,4,6-trimethoxyphenanthrene)

Compound CF-6 was obtained as brown amorphous solid (13 mg, 0.0013% based on dried weight of *Cymbidium finlaysonianum* whole plants). The compound is soluble in CH_2Cl_2 and acetone.

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 323.0893; **Figure 48.**

^{1}H NMR: δ ppm, 300 MHz, in $CDCl_3$; 3.96 (3H, *s*), 4.02 (3H, *s*), 4.06 (3H, *s*), 5.90 (1H, *br s*), 5.95 (1H, *br s*), 7.05 (1H, *s*), 7.29 (1H, *s*), 7.47 (2H, *s*), 8.98 (1H, *s*) **Figure 49.**

^{13}C NMR: δ ppm, 75 MHz, in $CDCl_3$; 55.9, 56.1, 60.0, 104.7, 106.8, 111.1, 118.6, 123.4, 124.8, 125.0, 126.2, 127.9, 138.7, 143.6, 144.7, 146.6, 146.6; **Figure 50.**

6.7 Compound CF-7 (coelonin)

Compound CFW-7 was obtained as brown amorphous solid (6 mg, 0.0006% based on dried weight of *Cymbidium finlaysonianum* whole plants). The compound is soluble in CH_2Cl_2 and acetone.

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 265.0839; **Figure 53 .**

^{1}H NMR: δ ppm, 300 MHz, in $CDCl_3$; 2.68 (4H, *s*), 3.83 (3H, *s*), 4.90 (1H, *br s*), 5.10 (1H, *br s*), 6.32 (1H, *d*, *J* = 2.3 Hz), 6.39 (1H, *d*, *J* = 2.3 Hz), 6.68 (1H, *d*, *J* = 2.1 Hz), 6.69 (1H, *dd*, *J* = 8.1, 2.1 Hz), 8.09 (1H, *d*, *J* = 8.1 Hz); **Figure 54.**

^{13}C NMR: δ ppm, 75 MHz, in $CDCl_3$; 29.8, 30.4, 55.5, 98.1, 107.2, 112.8, 114.3, 116.4, 125.6, 129.1, 139.7, 141.1, 153.5, 154.6, 157.8; **Figure 55.**

6.8 Compound CF-8 (lusianthridin)

Compound CFW-8 was obtained as brown amorphous solid (1 mg, 0.0001% based on dried weight of *Cymbidium finlaysonianum* whole plants). The compound is soluble in CH_2Cl_2 and acetone.

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 265.0843; **Figure 59.**

^{1}H NMR: δ ppm, 300 MHz, in $CDCl_3$; 2.71 (4H, *s*), 3.78 (3H, *s*), 6.33 (1H, *d*, *J* = 2.4 Hz), 6.40 (1H, *d*, *J* = 2.4 Hz), 6.70 (1H, *dd*, *J* = 9.0, 2.0 Hz), 6.74 (1H, *d*, *J* = 2.0 Hz), 7.92 (1H, *d*, *J* = 9.0 Hz); **Figure 60.**

^{13}C NMR: δ ppm, 75 MHz, in $CDCl_3$; 29.9, 30.5, 55.3, 100.9, 106.5, 113.2, 114.7, 115.1, 125.5, 127.2, 140.2, 141.1, 153.5, 154.6, 157.8; **Figure 61.**

6.9 Compound CF-9 (batatasin III)

Compound CF-9 was obtained as brown amorphous solid (4 mg, 0.0004% based on dried weight of *Cymbidium finlaysonianum* whole plants). The compound is soluble in CH_2Cl_2 and acetone.

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 267.0995; **Figure 63.**

^{1}H NMR: δ ppm, 300 MHz, in $CDCl_3$; 2.80 (4H, *br s*), 3.73 (3H, *s*), 6.24 (2H, *br s*), 6.30 (1H, *br s*), 6.45 (1H, br *d*, *J* = 8.0 Hz), 6.63 (1H, *br s*), 6.74 (1H, *br d*, *J* = 7.5 Hz), 7.13 (1H, t, *J* = 8.0, 7.5 Hz); **Figure 64.**

^{13}C NMR: δ ppm, 75 MHz, in $CDCl_3$; 37.3, 37.7, 55.3, 99.1, 107.9, 112.9, 115.4, 120.9, 129.5, 143.6, 144.4, 155.5, 156.5, 160.8.; **Figure 65.**

6.10 Compound CF-10 [1-(4-hydroxybenzyl)-4,6-dimethoxy-9,10-dihydrophenanthrene-2,7-diol]

Compound CF-10 was obtained as light brown amorphous powder (7 mg, 0.0007% based on dried weight of *Cymbidium finlaysonianum* whole plants). The compound is soluble in CH_2Cl_2 and acetone.

UV: λ_{max} (MeOH) nm (log **ε**): 218 (1.88), 278, (1.39), 304 (1.33); **Figure 68.**

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 401.1368; **Figure 69**.

IR: ν_{max} cm^{-1}: 3435, 2920, 1627; **Figure 70.**

^1H NMR: δ ppm, 300 MHz, in $CDCl_3$; 2.57 (2H, *m*), 2.63 (2H, *m*), 3.84 (3H, *s*), 3.89 (3H, *s*), 3.97 (2H, *s*), 4.80 (1H, *br s*), 4.85 (1H, *br s*), 5.57 (1H, *br s*), 6.44 (1H, *s*), 6.70 (2H, *d*, *J* = 8.4 Hz), 6.74 (1H, *s*), 6.99 (2H, *d*, *J* = 8.4 Hz), 7.81 (1H, *s*): **Figure 71.**

^{13}C NMR: δ ppm, 75 MHz, in $CDCl_3$; 26.6, 28.8, 30.5, 56.1, 55.7, 98.6, 111.5, 113.1, 114.3, 115.4, 115.4, 116.3, 117.5, 124.9, 129.0, 129.0, 131.2, 132.0, 140.3, 143.6, 153.1, 153.9, 155.8: **Figure 72.**

6.11 Compound PD-1 (isalpinin)

Compound PD-1 was obtained as yellow needles (48 mg, 0.012% and 7 mg, 0.0012% based on dried weight of *Paphiopedilum dianthum* roots and leaves, respectively). The compound is soluble in CH_2Cl_2, acetone and MeOH.

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 307.0579; **Figure 75.**

^{1}H NMR: δ ppm, 300 MHz, in in $CDCl_3$; 3.90 (3H, *s*), 6.39 (1H, *d*, *J* = 2.1 Hz), 6.52 (1H, *d*, *J* = 2.1 Hz), 6.66 (1H, *br s*), 7.51 (2H, *m*), 7.53 (1H, *m*), 8.20 (2H, *dd*, *J* = 8.4, 1.5 Hz), 11.67 (1H, *s*); **Figure 76.**

^{13}C NMR: δ ppm, 75 MHz, in in $CDCl_3$; 55.9, 92.3, 98.0, 104.2, 127.6, 127.6, 128.6, 128.6, 130.3, 130.7, 136.6, 145.1, 157.0, 160.9, 166.0, 175.5; **Figure 77.**

6.12 Compound PD-2 (pinosylvin monomethyl ether)

Compound PD-2 was obtained as brown semisolid (1.2 g, 0.3 % and 9 mg, 0.0015 % based on dried weight of *Paphiopedilum dianthum* roots and leaves, respectively). The compound is soluble in CH_2Cl_2, acetone and MeOH.

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 249.0927; **Figure 78.**

^{1}H NMR: δ ppm, 300 MHz, in $CDCl_3$; 3.82 (3H, *s*), 6.35 (1H, *t*, *J* = 2.0 Hz), 6.61 (1H, *t*, *J* = 2.0 Hz), 6.66 (1H, *t*, *J* = 2.0 Hz), 6.99 (1H, *d*, *J* = 16.4 Hz), 7.07 (1H, *d*, *J* = 16.4 Hz), 7.27 (1H, *m*), 7.36 (2H, *td*, *J* = 7.2, 1.5 Hz), 7.50 (2H, *dd*, *J* = 7.2, 1.5 Hz); **Figure 79.**

^{13}C NMR: δ ppm, 75 MHz, in $CDCl_3$; 55.3, 101.0, 104.9, 106.1, 126.6, 126.6, 127.7, 128.2, 128.6, 128.6, 129.3, 136.9, 139.7, 156.7, 160.9; **Figure 80.**

6.13 Compound PD-3 (pinocembrin)

Compound PD-3 was obtained as yellow amorphous solid (500 mg, 0.125% and 3 mg, 0.0005% based on dried weight of *Paphiopedilum dianthum* roots and leaves, respectively). The compound is soluble in CH_2Cl_2, acetone and MeOH.

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 279.0664; **Figure 81.**

^{1}H NMR: δ ppm, 300 MHz, in CD_3OD; 2.83 (1H, *dd*, *J* = 17.3, 12.9 Hz), 3.09 (1H, *dd*, *J* = 17.3, 3.0 Hz), 5.43 (1H, *dd*, *J* = 12.9, 3.0 Hz), 6.00 (1H, *d*, *J* = 2.4 Hz), 6.01 (1H, *d*, *J* = 2.4 Hz), 7.38 (1H, *m*), 7.40 (2H, *m*), 7.47 (2H, *m*); **Figure 82.**

^{13}C NMR: δ ppm, 75 MHz, in CD_3OD; 43.3, 79.2, 95.5, 96.7, 103.2, 126.1, 126.1 128.9, 128.9, 128.9, 138.3, 164.4, 163.2, 164.5, 195.8; **Figure 83.**

6.14 Compound PD-4 (galangin)

Compound PD-4 was obtained as yellow needles (0.2 g, 0.05% and 62 mg, 0.001% based on dried weight of *Paphiopedilum dianthum* roots and leaves, respectively). The compound is soluble in CH_2Cl_2, acetone and MeOH.

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 293.0478; **Figure 84.**

^{1}H NMR: δ ppm, 300 MHz, in CD_3OD; 6.18 (1H, *d*, *J* = 2.1 Hz), 6.39 (1H, *d*, *J* = 2.1 Hz), 7.51 (2H, *m*), 7.53 (1H, *m*), 8.17 (2H, *dd*, *J* = 7.2, 1.4 Hz); **Figure 85.**

^{13}C NMR: δ ppm, 75 MHz, in in CD_3OD; 94.5, 99.4, 104.7, 128.7, 128.7, 129.4, 129.4, 130.9, 132.6, 138.5, 146.9, 158.4, 162.6, 165.9, 177.7; **Figure 86.**

6.15 Compound PD-5 (2,3'-dihydroxy-5'-methoxystilbene)

Compound PD-5 was obtained as brown semisolids (1.5 mg, 0.375% based on dried weight of *Paphiopedilum dianthum* roots). The compound is soluble in CH_2Cl_2, acetone and MeOH.

HR-ESI-MS: $[M - H]^-$ ion at *m/z* 241.0869; **Figure 87.**

^{1}H NMR: δ ppm, 300 MHz, in CD_3OD; 3.77 (3H, *s*), 6.25 (1H, *t*, *J* = 2.0 Hz), 6.58 (2H, *d*, *J* = 2.0 Hz), 6.79 (1H, *d*, *J* = 7.8 Hz), 6.81 (1H, *t*, *J* = 7.8 Hz), 7.02 (1H, *d*, *J* = 16.7 Hz), 7.06 (1H, *td*, *d*, *J* = 7.8, 1.5 Hz), 7.38 (1H, *d*, *J* = 16.7 Hz), 7.50 (1H, *dd*, *d*, *J* = 7.8, 1.5 Hz); **Figure 88.**

^{13}C NMR: δ ppm, 75 MHz, in CD_3OD; 55.7, 101.6, 104.4, 106.9, 116.6, 120.8, 125.0, 125.7, 127.5, 129.4, 129.5, 141.8, 156.3, 156.9, 162.7; **Figure 89.**

6.16 Compound PD-6 [(*E*)-2,5'-dihydroxy-2'-(4-hydroxybenzyl)-3'-methoxystilbene]

Compound PD-6 was obtained as white powder (45 mg, 0.01125% based on dried weight of roots of *Paphiopedilum dianthum*). The compound is soluble in MeOH.

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 371.1255; **Figure 90.**

^{1}H NMR: δ ppm, 300 MHz, in CD_3OD; 3.77 (3H, *s*), 3.96 (2H, *s*), 6.39 (1H, *d*, *J* = 2.1 Hz), 6.63 (2H, *d*, *J* = 8.4 Hz), 6.74 (1H, *d*, *J* = 2.1 Hz), 6.76 (1H, *t*, *J* = 7.8 Hz), 6.77 (1H, *d*, *J* = 7.8 Hz), 6.93 (2H, *d*, *J* = 8.4 Hz), 7.04 (1H, *td*, *J* = 7.8, 1.5 Hz), 7.22 (1H, *d*, *J* = 16.5 Hz), 7.32 (1H, *dd*, *J* = 7.8, 1.2 Hz), 7.36 (1H, *d*, *J* = 16.5 Hz); **Figure 91.**

^{13}C NMR: δ ppm, 75 MHz, in CD_3OD; 30.7, 56.0, 99.2, 104.9, 115.9, 115.9, 116.6, 120.3, 120.8, 126.0, 126.5, 127.6, 127.7, 129.4, 130.2, 130.2, 134.2, 140.2, 155.9, 156.1, 157.6; 160.1; **Figure 93.**

6.17 Compound PD-7 (paphiodianthin A)

Compound PD-7 was obtained as brown amorphous powder (20 mg, 0.005% based on dried weight of *Paphiopedilum dianthum* roots). The compound is soluble in CD_3OD.

$[\alpha]^{20}_{D}$: +43 ° (*c* 0.02, MeOH)

UV: λ_{max} (MeOH) nm (log **ε**): 283 (4.02); **Figure 98.**

IR: V_{max} cm^{-1}: 3432, 2936, 1618, 1519, 1480, 1461, 1307, 1235, 1200, 1092, 754; **Figure 99.**

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 537.1519; **Figure 100.**

^{1}H NMR: **δ** ppm, 300 MHz, in CD_3OD; 3.60 (6H, *s*), 3.87 (2H, *d, J* = 2.4 Hz), 5.23 (2H, *d, J* = 2.4 Hz), 5.65 (2H, *d, J* = 1.5 Hz), 6.12 (2H, *d, J* = 1.5 Hz), 6.92 (2H, *t, J* = 7.1 Hz), 6.96 (2H, *d, J* = 7.5 Hz), 7.22 (2H, *d, J* = 7.1 Hz), 7.25 (2H, *t, J* = 7.5 Hz); **Figure 101.**

^{13}C NMR: **δ** ppm, 75 MHz, in CD_3OD; 56.5, 57.0, 86.8, 101.2, 106.2, 110.4, 122.1, 126.7, 128.5, 130.3, 134.5, 134.8, 146.6, 149.4, 161.9; **Figure 102.**

6.18 Compound PD-8 (paphiodianthin B)

Compound CF-8 was obtained as brown amorphous powder (3 mg, 0.0005% based on dried weight of *Paphiopedilum dianthum* leaves). The compound is soluble in CH_2Cl_2.

$[\alpha]^{20}_{D}$: +37 ° (*c* 0.02, MeOH)

UV: λ_{max} (MeOH) nm (log **ε**): 309 (4.02); **Figure 105.**

IR: V_{max} cm^{-1}: 3426, 2930, 1613, 1583, 1453, 1361, 1318, 1197, 1135, 1094, 962, 702; **Figure 106.**

HR-ESI-MS: $[M + Na]^+$ ion at *m/z* 489.1668; **Figure 107.**

^{1}H NMR: **δ** ppm, 300 MHz, in $CDCl_3$; 3.84 (3H, *s*), 3.88 (3H, *s*), 4.60 (1H, *d, J* = 6.5 Hz), 5.45 (1H, *d, J* = 6.5 Hz), 6.47 (1H, *d, J* = 1.8 Hz), 6.51 (1H, *d, J* = 2.1

Hz), 6.75 (1H, *d, J* = 2.1 Hz), 6.56 (1H, *d, J* = 1.8 Hz), 6.62 (1H, *d, J* = 16.4 Hz), 6.88 (1H, *d, J* = 16.4 Hz), 7.10 (2H, *m*), 7.19 (1H, *t, J* = 6.9 Hz), 7.21 (2H, *d*, *J* = 7.4 Hz), 7.23 (2H, *m*), 7.26 (2H, *m*), 7.35 (2H, *d*, *J* = 7.4 Hz); **Figure 108.**

^{13}C NMR: δ ppm, 75 MHz, in $CDCl_3$; 55.6, 56.2, 56.9, 93.6, 95.5, 100.5, 102.8, 106.2, 120.2, 125.2, 126.4, 126.4, 127.2, 127.7, 127.9, 127.9, 128.5, 128.5, 129.0, 129.0, 129.9, 133.2, 134.9, 137.0, 143.0, 147.0, 161.2, 161.4; **Figure 110.**

7. Evaluation of cytotoxicity

7.1 Determination of cytotoxic activity against NCI-H187, Caco-2, MCF-7, MCF-7/DOX, MCF-7/MX and NIH/3T3 cell lines

7.1.1 Cell cultures

The fibroblast (NIH/3T3) and human colon adenocarcinoma (Caco-2) cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2.5 mM L-glutamine, 1% penicillin–streptomycin mixture, 1% non-essential amino acid and 10% fetal bovine serum (FBS) at 37 °C in a controlled atmosphere of 5% CO_2 and 90% relative humidity (183, 184).

Human breast adenocarcinoma (MCF-7) cells were maintained in Roswell Park Memorial Institute 1640 (RPMI-1640) medium containing 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere of 5% CO_2 (185).

The doxorubicin (DOX)-resistant MCF-7 cell subline (MCF-7/DOX) was developed from MCF-7 cell line by culturing the cells in RPMI-1640 complete medium containing 0.01 μM doxorubicin and the resistant cells that could grow in increasing concentrations (up to 1.5 μM) of doxorubicin were maintained. Doxorubicin was withdrawn from the cells three days prior to testing (185).

The mitoxantrone (MX)-resistant MCF-7 cell subline (MCF-7/MX) was also developed from MCF-7 cell line, but the cells were cultured in 0.01 μM mitoxantrone and the concentrations of mitoxantrone were increased up to 0.7 μM. The resistant subline was maintained in RPMI-1640 complete medium. Mitoxantrone was removed from the culture medium three days prior to the experiment (186).

7.1.2 Cytotoxicity assay

Cytotoxic activity of isolated compounds was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay (187). MTT, a yellow water-soluble dye, can be reduced by mitochondrial enzymes into its purple, water-insoluble formazan product. Therefore, the purple colour in the assay reflects the metabolic activity of the cells and the number of viable cells. Cells were seeded onto 96-well plates at the density of 5×10^3 cells/well and cultured for 24 h. Then, the cells were treated with the isolated compounds (0-100 μM) and the positive control, doxorubicin, for 72 h. After treatment, the cells were washed and further incubated in serum-free medium containing MTT reagent (0.83 mg/ml) for 3 h at 37°C. The formazan crystals were dissolved with DMSO and quantified spectrophotometrically by measuring their optical density (OD) at 570 nm with a Wallac 1420 VICTOR 3 microplate reader (PerkinElmer Inc., Massachusetts, USA). Cell viability was calculated as follows.

$$\% \text{ Cell survival} = [\text{OD test}/\text{ OD control}] \times 100$$

Half maximal inhibitory concentration (IC_{50}) values were estimated from linear regression analysis of concentration-response curve.

Resazurin assay of cytotoxicity of isolated compounds was performed by the bioassay laboratory of the National Center for Genetic Engineering and Biotechnology, Pathum Thani. Similar to MTT, resazurin solution, which is blue in colour and non-fluorescent, can be reduced to the pink-coloured, fluorescent resorufin by the activity of intracellular enzymes (188). Suspension of the cancer cells was seeded onto 384-well plates at the density of 4×10^4 cells/well and treated with solution of test compounds or doxorubicin (as a positive control). The plates were incubated at 37°C under 5% CO_2 for 72 h. Then, 12.5 µL of resazurin solution was added to each well, and the cells were further incubated at 37 °C for 4 h. Fluorescence signal was measured on a SpectraMax M5 multi-detection microplate reader (Molecular Devices, Massachusetts, USA) at excitation and emission wavelengths of 530 nm and 590 nm, respectively. The percent inhibition of cell growth was calculated as follows.

$$\% \text{ Inhibition} = [1-(FUT/FUC)] \times 100$$

FUT and FUC are the mean fluorescent units under treated and untreated conditions, respectively. Dose response curves were plotted for six concentrations of two-fold serially diluted test compounds, and the sample concentration that inhibited cell growth by 50% (IC_{50} value) was derived using SOFTMax pro software (Molecular Devices, Massachusetts, USA).

CHAPTER IV

RESULTS AND DISCUSSION

Chemical constituents from the whole plants of *Cymbidium finlaysonianum* and from the roots and leaves of *Paphiopedilum dianthum* were investigated. Ten compounds (CF-1 to CF-10) and eight compounds (PD-1 to PD-8) were isolated from *C. finlaysonianum* and *P. dianthum*, respectively, by chromatographic techniques as described in Chapter III. Their identification and structure elucidation were accomplished through spectroscopic techniques, including UV, IR, MS and NMR. Chemical constituents from both orchids were tested for their cytotoxic effects on three human cancer cell lines, two chemotherapy-resistant cancer cell sublines and one normal cell line.

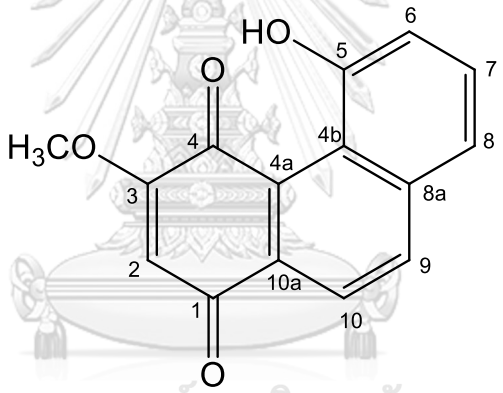
1. Identification of compound CF-1 (cymbinodin-A) and its structure revision

Compound CF-1, obtained as dark purple needles (29 mg, 0.0029% yield), appeared as a blue spot on TLC upon spraying with 10% sulfuric acid and heating. According to the peak at $[M + Na]^+$ 227.0475 *m/z* (calcd. 277.0477) in the high resolution mass spectrum (**Figure 17**), its molecular formula could be identified as $C_{15}H_{10}O_4$. Its IR spectrum displayed hydroxy absorption band at 3453 cm^{-1} and quinone carbonyl bands at 1677 and 1638 cm^{-1} (**Figure 18**). These data suggested that compound CF-1 was a 1,4-phenanthrenequinone with hydroxyl substitution.

The ^1H NMR spectrum of compound CF-1 (300 MHz, in $CDCl_3$) (**Figure 19** and **Table 8**) showed the most downfield singlet of a chelated hydroxyl proton at δ 12.36 ppm (1H, *s*, 5-OH) and a methoxy singlet at δ 3.98 ppm. Other observed signals were those of a 1,2,3-trisubstituted aromatic ring at δ 7.24 (1H, *dd*, *J* = 7.8, 1.5 Hz, H-6), 7.42 (1H, *dd*, *J* = 7.8, 1.5Hz, H-8) and 7.58 ppm (1H, *t*, *J* = 7.8 Hz, H-7), a singlet at δ 8.16 ppm integrated for two protons (H-9 and H-10) and an olefinic singlet at δ 6.29 ppm.

The ^{1}H NMR data suggested that compound CF-1 was a phenanthrenequinone with one hydrogen-bonded hydroxy group and one methoxy group.

Its ^{13}C NMR spectrum (**Figure 20** and **Table 8**) displayed two 1,4-quinone carbonyl signals at δ 180.1 (C-1) and 191.9 ppm (C-4), six methine carbon signals at δ 111.5, 117.5 (C-6), 121.0 (C-8), 121.9 (C-10), 131.0 (C-7) and 137.3 (C-9), six quaternary carbon signals (including two oxygen-substituted ones) at δ 121.2 (C-4b), 130.0 (C-10a), 132.5 (C-4a), 139.0 (C-8a), 155.6 (C-5) and 158.9 ppm, and a methoxy carbon at δ 56.8 ppm. These ^{13}C NMR data are in good agreement with those reported for the structure of cymbinodin-A, previously isolated from *Cymbidium aloifolium* (11), as shown below.



Previously proposed cymbinodin-A (11)

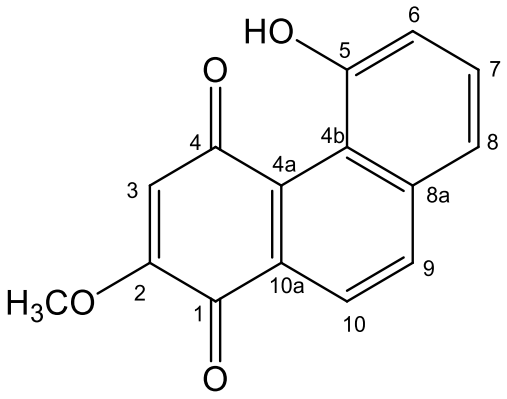
Table 8. NMR spectral data of compound CF-1 and cymbinodin-A (in $CDCl_3$)

Position	Compound CF-1		Cymbinodin-A[11]
	δ_H (mult., *J* in Hz)	δ_C	δ_C
1	-	180.1	180.1
2	6.29 (*s*)	158.9	158.7
3	-	111.5	111.4
4	-	191.9	191.7
4a	-	132.5	132.3
4b	-	121.2	120.9
5	-	155.6	155.0
6	7.24 (*dd*, 7.8, 1.5)	117.5	117.1
7	7.58 (*t*, 7.8)	131.0	130.7
8	7.42 (*dd*, 7.8, 1.5)	121.0	121.1
8a	-	139.0	138.8
9	8.16 (*s*)	137.3	137.1
10	8.16 (*s*)	121.9	121.7
10a	-	130.0	129.7
3-OCH_3	3.98 (*s*)	56.8	56.6
5-OH	12.25 (*s*)	-	-

However, the carbon chemical shift assignments of quinone carbonyls in this compound were quite different from formerly reported 3-methoxy-5-hydroxy-1,4-phenanthrenequinones such as calanquinone (48) and denbinobin (189), of which the chemical shifts of those carbonyls resonated at around δ 184–187 ppm. Their C-2

signal also appeared at a more upfield chemical shift of approximately δ 107 ppm, compared to at δ 111.5 ppm as in cymbinodin-A.

The problem in the chemical shift assignments of cymbinodin-A, which could arise from the superimposition of H-9 and H-10 signals, was solved when the NMR solvent was changed from $CDCl_3$ to acetone-d_6. In the ^1H NMR spectrum of compound CF-1 in acetone –d_6 (**Figure 21** and **Table 9**), these signals appeared separately at δ 8.14 (1H, *d*, *J* = 8.7 Hz, H-10) and 8.35 ppm (1H, d, *J* = 8.7 Hz, H-9). Their assignments were supported by three-bond heteronuclear multiple bond correlation (HMBC) (**Figure 24** and **Table 9**) from H-9 to C-4b (δ 120.9 ppm), C-8 (δ 120.8 ppm) and C-10a (δ 132.9 ppm), as well as from H-10 to C-1 (δ 179.6 ppm), C-4a (δ 129.9 ppm) and C-8a (δ 139.0 ppm). The former ^{13}C NMR assignments for C-4a and C-10a should therefore be reversed. Long-range HMBC correlations C-1 and C-4a could also be observed, indicating that methoxy substitution should be at position 2 of the quinone ring, instead of at C-3 as originally proposed for cymbinodin-A. The hydroxy substitution at C-5 was also confirmed by HMBC cross-peaks from the hydroxy proton at δ 12.36 ppm and C-4b (δ 120.9 ppm), C-5 (δ 155.8 ppm) and C-6 (δ 116.8 ppm). Therefore, compound CF-1 is identical with cymbinodin-A. The chemical structure previously assigned to cymbinodin-A should be revised to 5-hydroxy-2-methoxy-phenanthrene-1,4-dione (190).



Revised cymbinodin-A

Table 9. NMR and HMBC spectral data of compound CF-1 (in acetone-d_6)

Position	δ_H (mult., *J* in Hz)	δ_C	HMBC correlation with
1	-	179.6	-
2	-	159.6	-
3	6.51 (*s*)	111.5	C-1, C-2, C-4, C-4a
4	-	192.5	-
4a	-	129.9	-
4b	-	120.9	-
5	-	155.8	-
6	7.18 (*dd*, 7.7, 1.5)	116.8	C-4b
7	7.64 (*t*, 7.7)	130.6	C-5, C-8a
8	7.56 (*dd*, 7.7, 1.5)	120.8	C-4b, C-6, C-8a, C-9
8a	-	139.0	-
9	8.35 (*d*, 8.7)	137.1	C-4b, C-8, C-10, C-10a
10	8.14 (*d*, 8.7)	121.6	C-1, C-4a, C-8a, C-9
10a	-	132.9	-
2-OCH_3	4.06 (*s*)	56.5	C-2
5-OH	12.36 (*s*)	-	C-4b, C-5, C-6

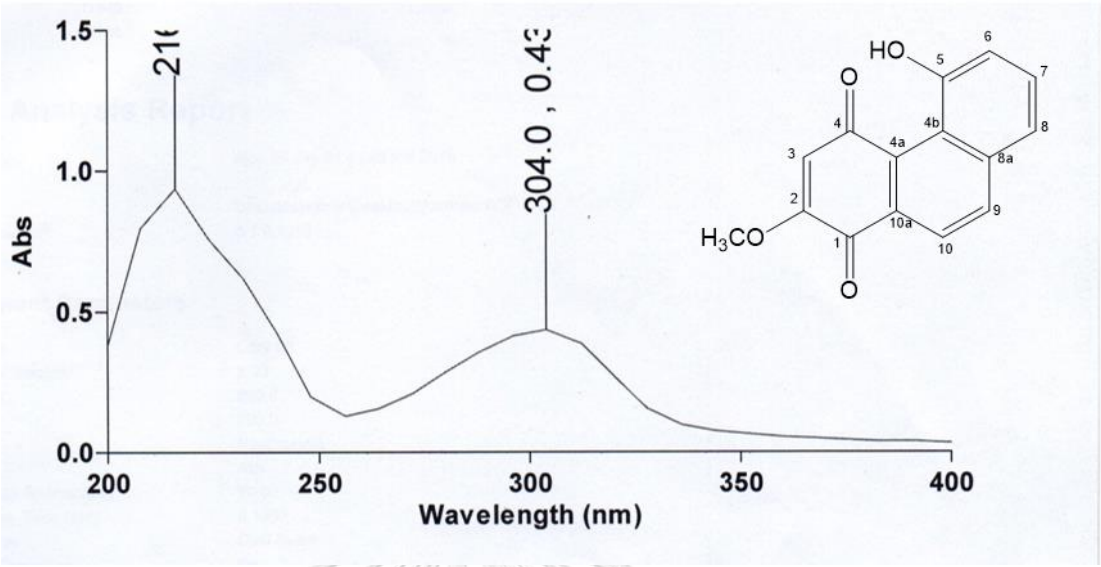


Figure 16. UV spectrum of compound CF-1

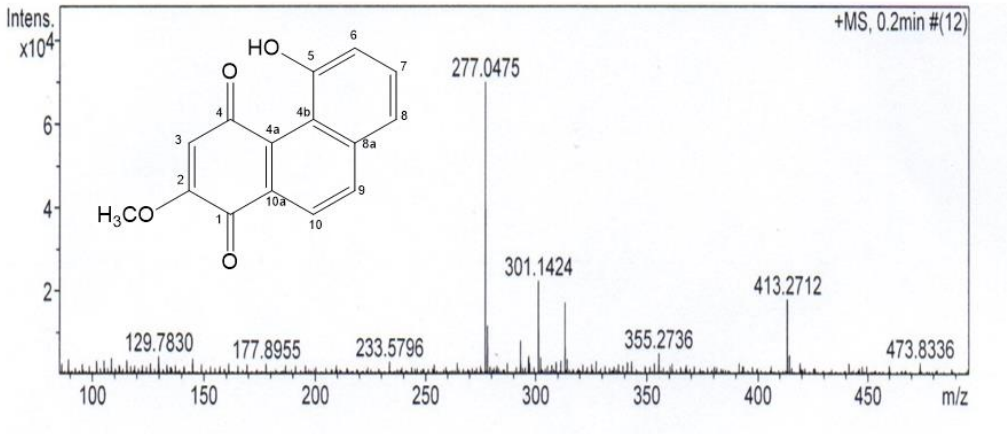


Figure 17. HR-ESI mass spectrum of compound CF-1

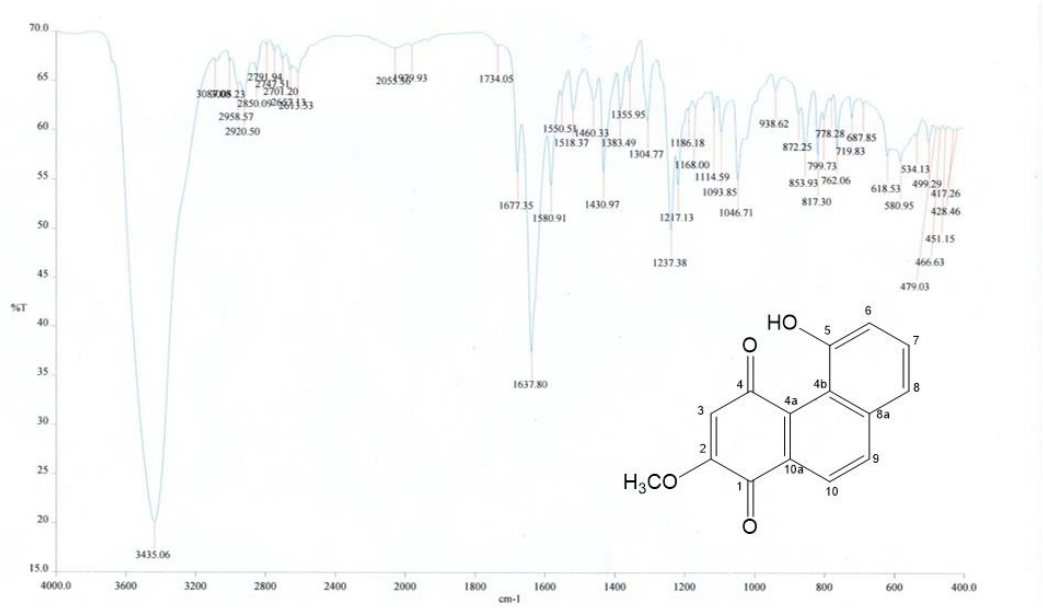


Figure 18. IR spectrum of compound CF-1

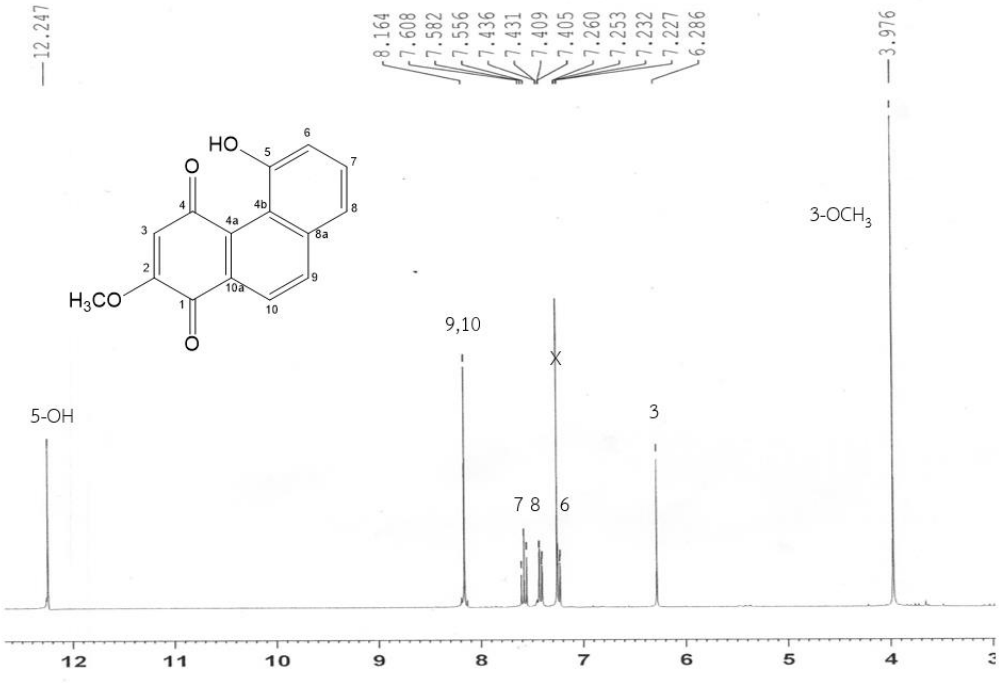


Figure 19. ^{1}H NMR spectrum of compound CF-1 (300 MHz, in $CDCl_3$)

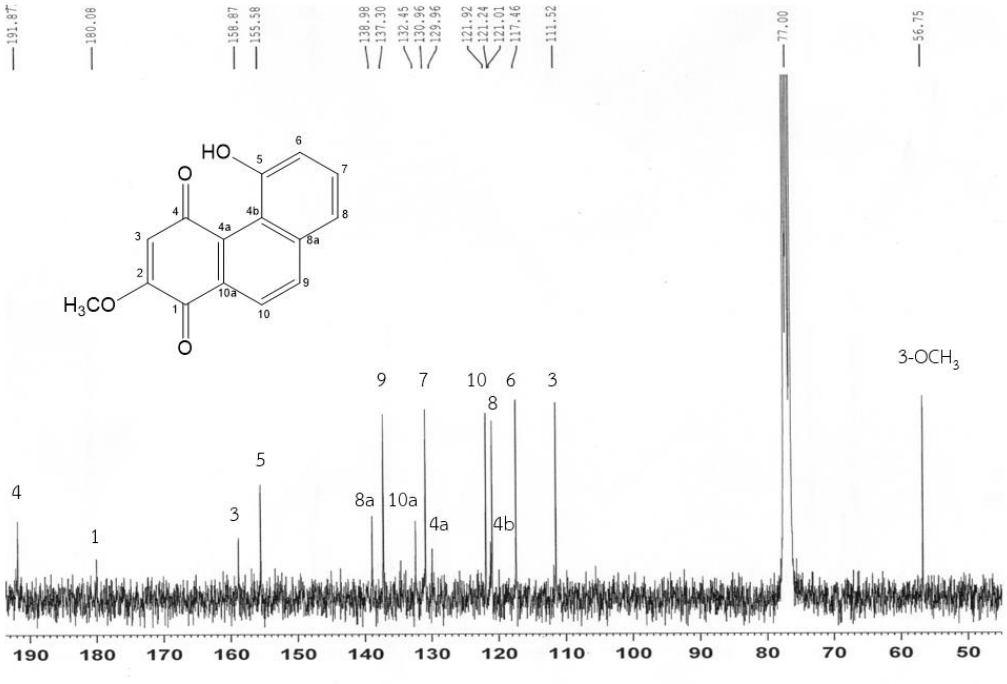


Figure 20. ^{1}H NMR spectrum of compound CF-1 (75 MHz, in $CDCl_3$)

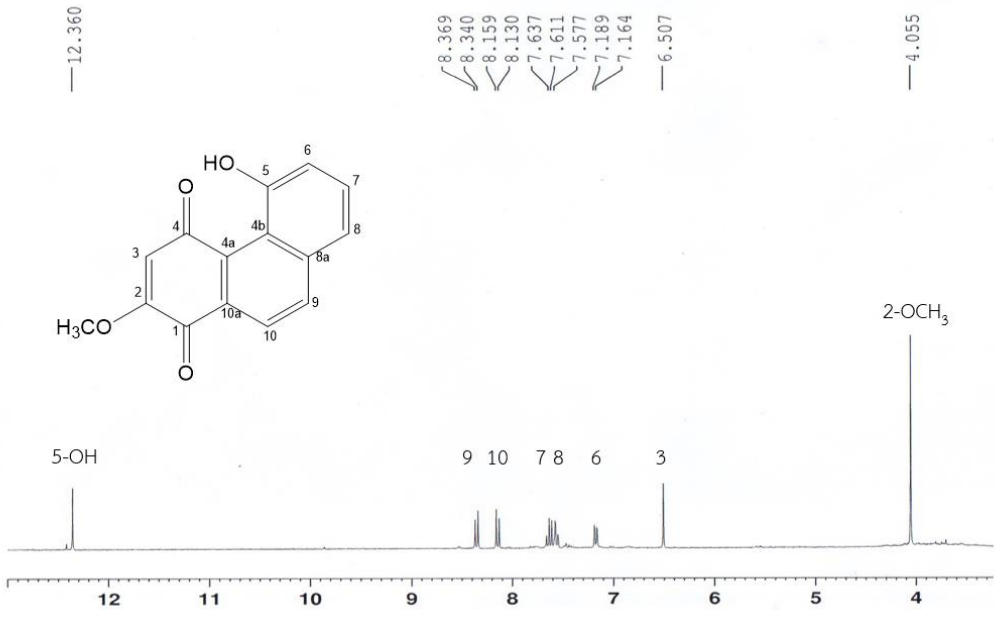


Figure 21. ^{1}H NMR spectrum of compound CF-1 (300 MHz, in acetone-d_6)

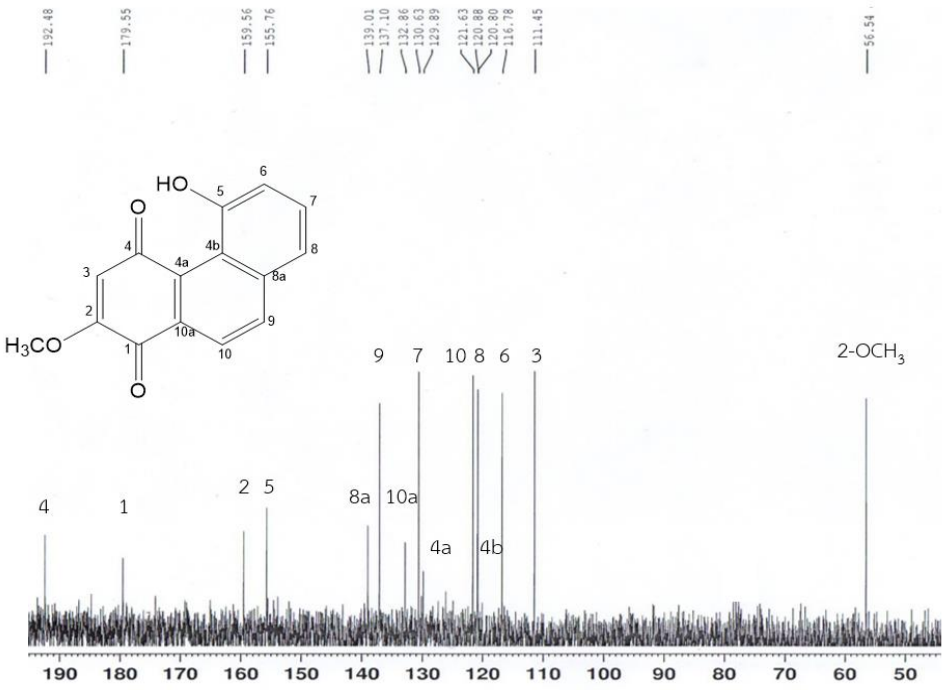


Figure 22. ^{13}C NMR spectrum of compound CF-1 (75 MHz, in acetone-d_6)

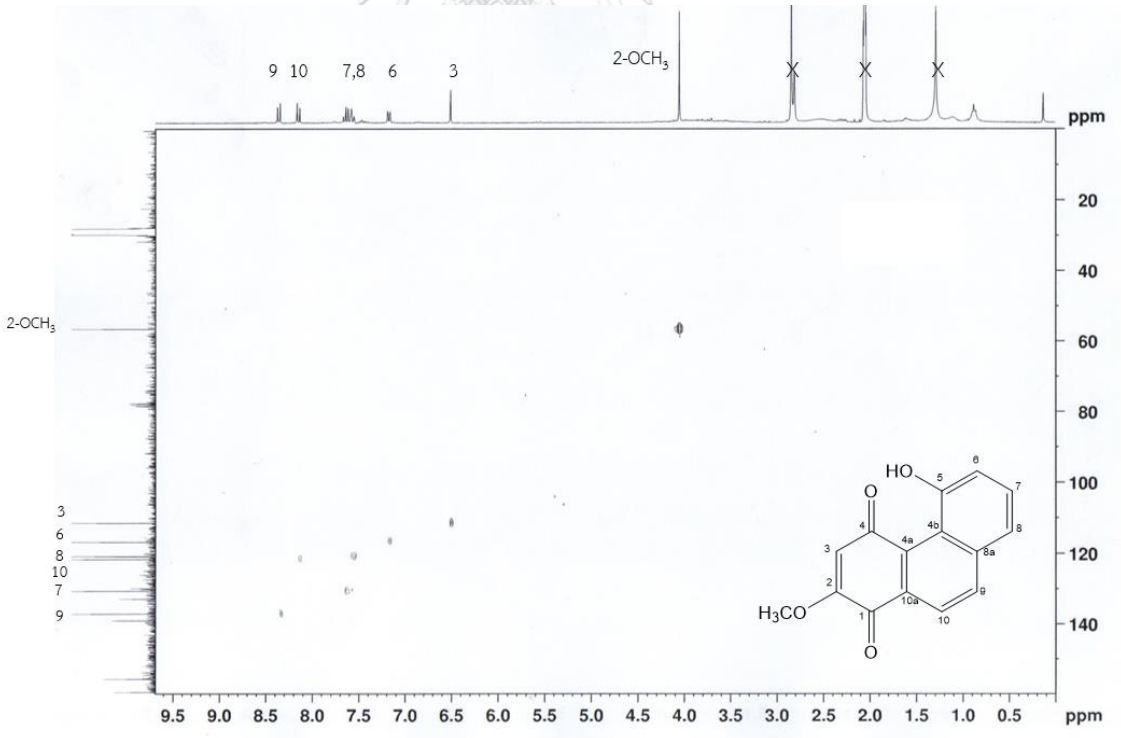


Figure 23. HSQC spectrum of compound CF-1

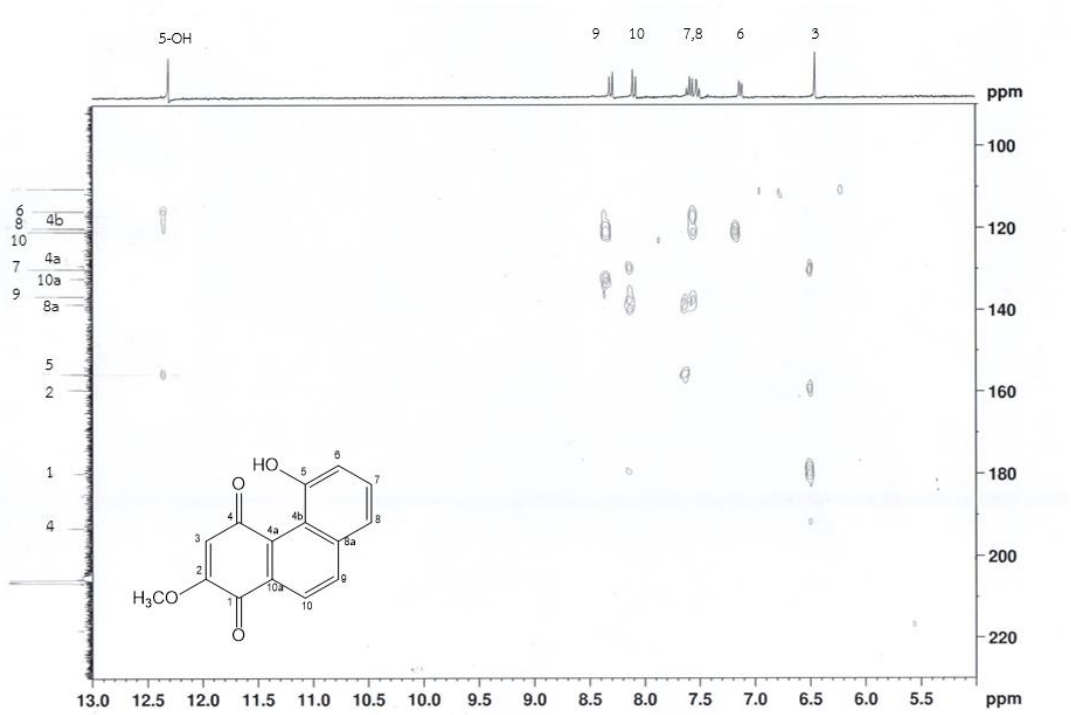


Figure 24. HMBC spectrum of compound CF-1

2. Identification of compound CF-2 (ephemeranthoquinone B)

Compound CFW-2, obtained as dark red needles (27 mg, 0.0027% yield), appeared as a blue spot on TLC upon spraying with 10% sulfuric acid and heating. According to the $[M + Na]^+$ peak at 279.0635 *m/z* (calcd. 279.0633) in the mass spectrum (**Figure 25**), its molecular formula could be determined as $C_{15}H_{12}O_4$.

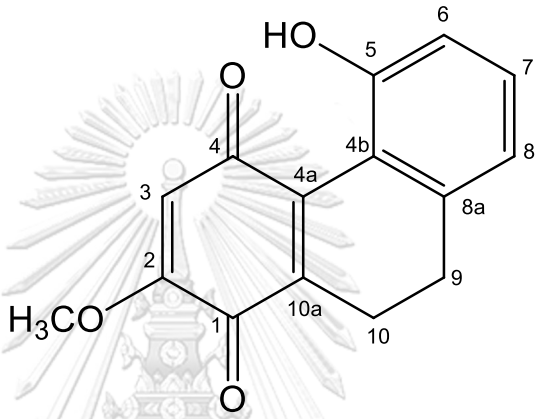
The ^1H NMR spectrum of compound CF-2 (**Figure 26** and **Table 10**) displayed aromatic signals for a 1,2,3-trisubstituted ring at δ 6.79 (1H, *dd*, *J* = 7.2, 1.1 Hz, H-8), 6.92 (1H, *dd*, *J* = 8.3, 1.1 Hz, H-6) and 7.23 ppm (1H, *dd, J* = 8.3, 7.2 Hz, H-7), a methine singlet at δ 6.04 (H-3) and signals for two pairs of methylene protons at δ 2.68 (2H, *m*, H_2-9) and 2.69 ppm (2H, *m*, H_2-10). Resonances of a methoxy group and a hydroxyl substituent appeared at δ 3.90 (3H, *s*, 2-OCH_3) and 9.82 ppm (1H, *s*, 5-OH), respectively.

Its ^{13}C NMR spectrum (**Figure 27** and **Table 10**) exhibited carbonyl resonances for a 1,4-quinone moiety at δ 180.8 (C-1) and 191.5 ppm (C-4), a methoxy signal at δ 56.6 ppm (2-OCH_3). Other carbon signals repersented four methine carbons at δ 108.2 (C-3), 119.3 (C-6), 120.3 (C-8) and 132.3 ppm (C-7), six quaternary carbons, including two oxygenated ones, at δ 117.5 (C-4b), 139.1 (C-4a), 140.6 (C-8a), 143.2 (C-10a), 155.3 (C-5) and 158.6 ppm (C-2) and two methylene carbons at δ 21.3 (C-10) and 28.5 (C-9). These NMR data were similar to those of compound CF-1, except for the presence of two methylene carbons in this compound instead of two aromatic methine carbons. Therefore, compound CF-2 should be the 9,10-dihydroderivative of CF-1.

This conclusion was confirmed by HSQC (**Figure 28**) and HMBC (**Figures 29-30** and **Table 11**) experiments. HMBC connectivities could be observed between H-8 at δ 6.79 ppm and C-9 (δ 28.5 ppm) , from H_2-9 at δ 2.68 ppm to C-4b (δ 117.5 ppm) and C-10a (δ 143.2 ppm), and between H_2-10 at δ 2.69 ppm and C-8a (δ 140.6 ppm). The presence of a hydroxy group at position 5 was supported by HMBC correlation between H-7 (δ 7.23 ppm) and C-5 (δ 155.3 ppm). Furthermore, the substitution of a methoxy group at position 2 of the 1,4-quinone moiety and the presence of an unsaturated methine proton at position 3 was confirmed by HMBC correlation observed from H-3 (δ 6.04 ppm) to C-1 (δ 180.8 ppm), C-2 (δ 158.6 ppm) and C-4a (δ 139.1 ppm). Finally, comparison of its NMR data with previously reported values (1) identified compound CF-2 as ephemeranthoquinone B.

Ephemeranthoquinone B has been reported as a constituent of a *Cymbidium* orchid hybrid, *Cymbidium* Great Flower Marie Laurencin (14), and other members in the same tribe (Cymbidieae) such as *Oncidium isthmi* (105) *and Odontioda* Marie Noel 'Velano' (hybrid of *Odontoglossum* and *Cochilida*) (191). It displayed strong antibacterial effect against *Bacillus subtilis* (14). The compound was cytotoxic against several cancer cell lines by acting as apoptosis inducer and was patented by a

Japanese company as antioxidant, antimicrobial, antitumor, and anti-inflammatory agents (192).



Ephemeranthoquinone B

Table 10. NMR spectral data of compound CF-2 and ephemeranthoquinone B (in $CDCl_3$)

Position	Compound CF-2		Ephemeranthoquinone B	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)[14]	δ_C[14]
1	-	180.8	-	180.8
2	-	158.6	-	158.6
3	6.04 (*s*)	108.2	6.04 (*s*)	108.2
4	-	191.5	-	191.5
4a	-	139.1	-	139.0
4b	-	117.5	-	117.5
5	-	155.3	-	155.3
6	6.92 (*dd*, 8.3, 1.1)	119.3	6.91 (*dd*, 8.2, 1.4)	119.3
7	7.23 (*td*, 8.3, 7.2)	132.3	7.23 (*t,* 8.2)	132.3
8	6.79 (*dd*, 7.2, 1.1)	120.3	6.78 (*dd*, 8.2, 1.4)	120.3
8a	-	140.6	-	140.6
9	2.68 (*m*)	28.5	2.71 (*m*)	28.5
10	2.69 (*m*)	21.3	2.71 (*m*)	21.3
10a	-	143.2	-	143.2
2-OCH_3	3.90 (*s*)	56.6	3.90 (*s*)	56.6
5-OH	9.82 (*s*)	-	-	-

Table 11. HMBC spectral data of compound CF-2 (in $CDCl_3$)

^{1}H signal	HMBC correlation with
H-3	C-1, C-2, C-4a
H-6	C-8
H-7	C-5, C-8a
H-8	C-6, C-9
H-9	C-4b, C-8a, C-10a
H-10	C-8a, C-9
2-OCH_3	C-2

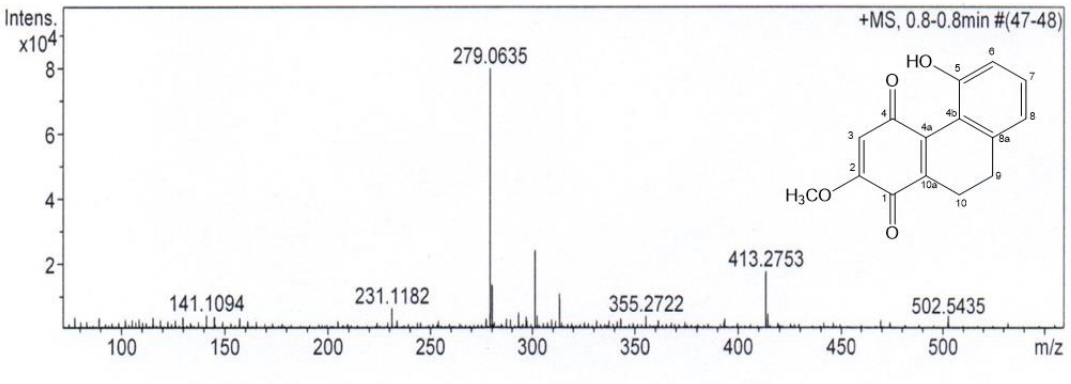


Figure 25. HR-ESI mass spectrum of compound CF-2

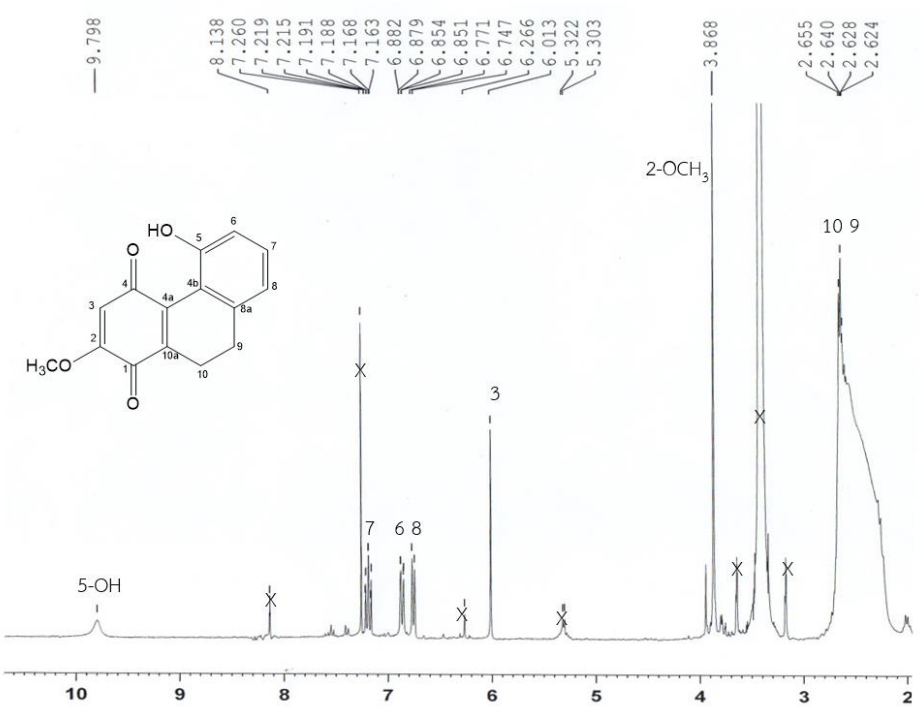


Figure 26. ^{1}H NMR spectrum of compound CF-2 (300 MHz, in $CDCl_3$)

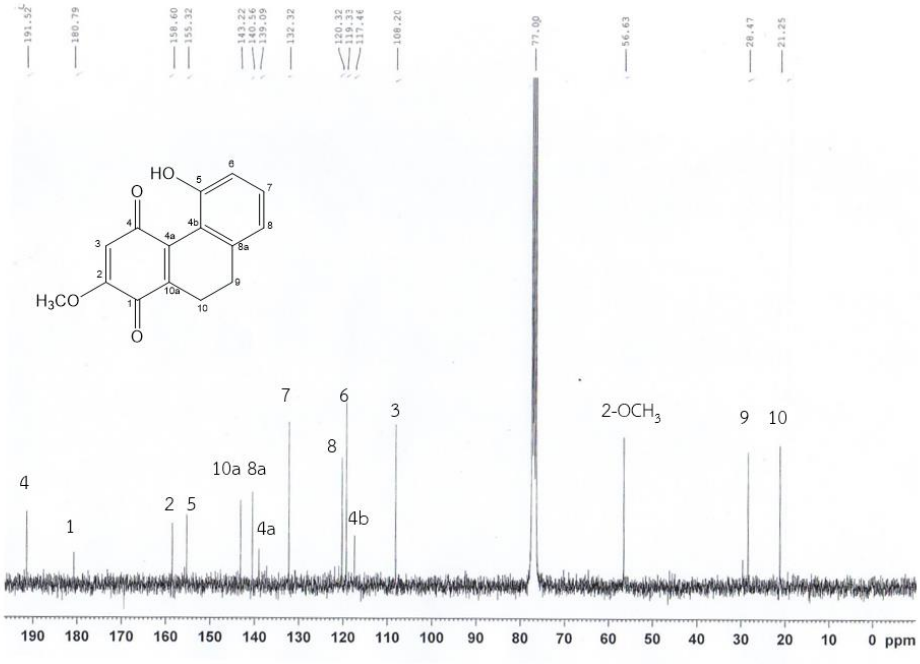


Figure 27. ^{13}C NMR spectrum of compound CF-2 (75 MHz, in $CDCl_3$)

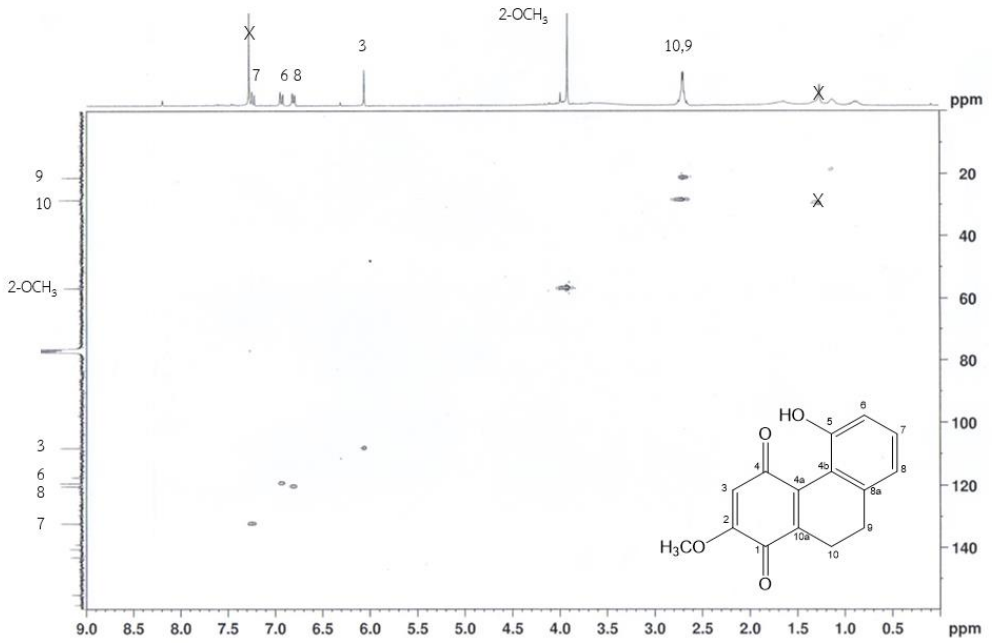


Figure 28. HSQC spectrum of compound CF-2

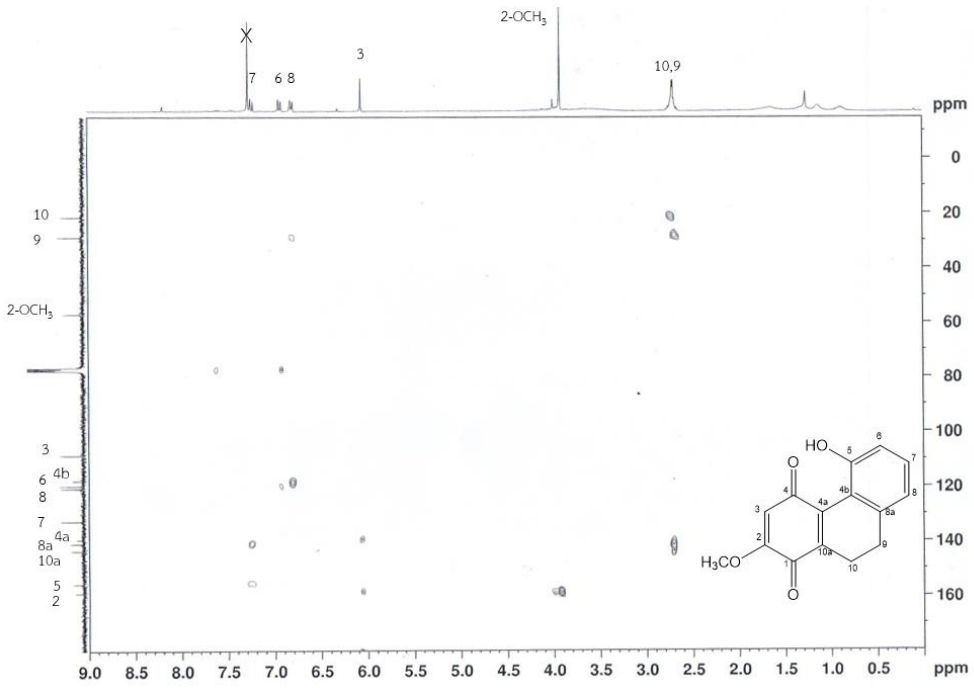


Figure 29. HMBC spectrum of compound CF-2

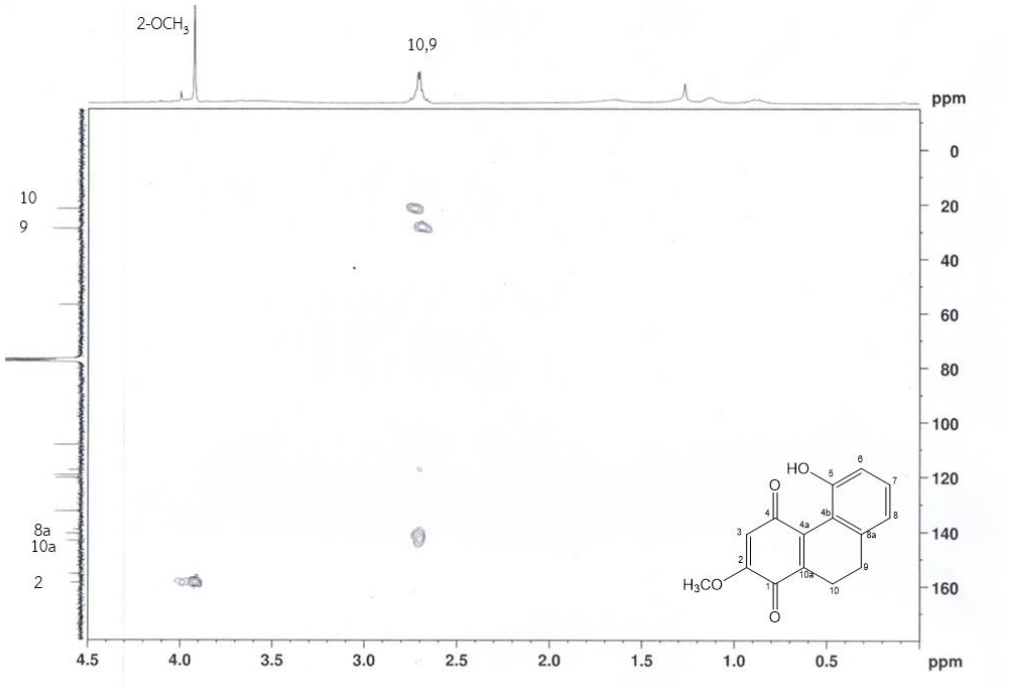


Figure 30. HMBC spectrum of compound CF-2 (expansion between δ_H 0.0-4.5, δ_C 0-180)

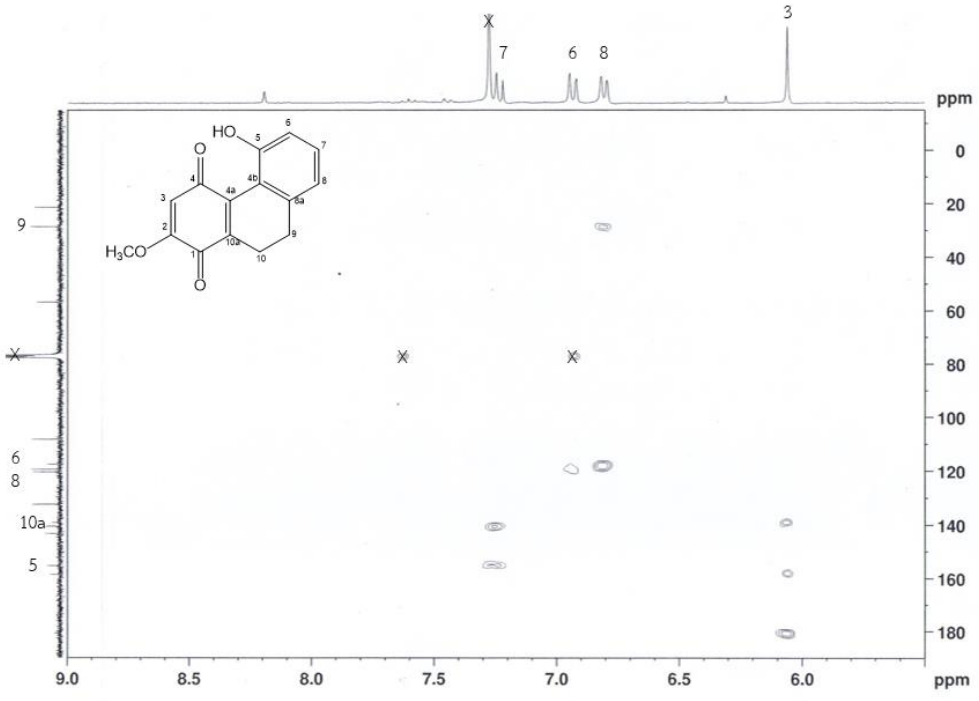


Figure 31. HMBC spectrum of compound CF-2 (expansion between δ_H 5.0-8.0, δ_C 0-190)

3. Identification of compound CF-3 (6-methoxycoelonin)

Compound CF-3 was isolated as a brown amorphous solid (56 mg, 0.0056% yield) which gave a blue spot on TLC upon spraying with 10% sulfuric acid and heating. The mass spectrum (**Figure 32**) showed a sodium-adduct molecular ion ($[M + Na]^+$) peak at *m/z* 295.0944 (calcd. 295.0946), suggesting $C_{16}H_{16}O_4$ as its molecular formula with nine degrees of unsaturation.

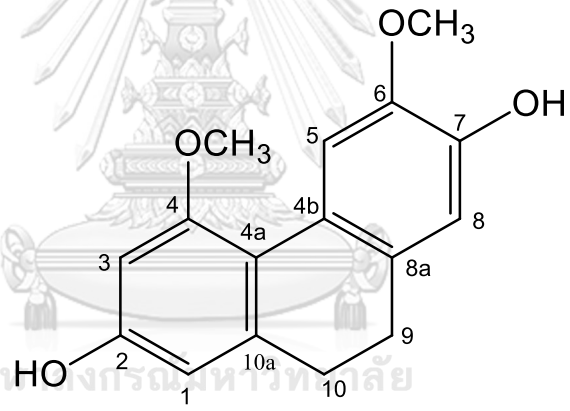
The ^1H NMR spectrum of compound CF-3 (**Figure 33** and **Table 12**) exhibited a singlet signal at δ 2.65 ppm, integrated for four protons, characteristic of 9,10-methylene protons of a 9,10-dihydrophenanthrene unsubstituted at positions 1 and 8 (1). Four substituents were represented by two methoxy signals at δ 3.84 (3H, *s,* 4-OCH_3) and 3.89 ppm (3H, *s*, 6-OCH_3) and two hydroxy broad singlets at δ 5.17 (1H, *br s*, 2-OH) and 5.62 ppm (1H, *br s*, 7-OH). The rest were resonances of four aromatic methine protons at δ 6.33 (1H, *br s*, H-1), 6.40 (1H, *br s*, H-3), 6.76 (1H, *s*, H-8) and 7.84 ppm (1H, *s*, H-5).

Its ^{13}C NMR (**Figure 34** and **Table 12**) and HSQC (**Figure 35**) spectra showed signals for two methoxy groups at δ 55.7 (4-OCH_3) and 56.1 (6-OCH_3) ppm, two aliphatic methylene carbons at δ 28.9 (C-9) and 30.8 (C-10) ppm, and twelve aromatic carbons, including four oxygenated ones at δ 143.5 (C-7), 144.4 (C-6), 154.6 (C-2) and 157.5 ppm (C-4).

From the HMBC experiment (**Figure 36** and **Table 13**), the position of one hydroxyl group could be established at C-7 from 3-bond connectivities from hydroxy proton at δ 5.62 to C-7 and C-8 (δ 113.5 ppm). Next, one methoxy group could be placed at position 6 based on HMBC correlations from both H-8 and 6-OCH_3 to C-6. The unsubstituted H-5, which resonated as a singlet at δ 7.84 ppm, showed correlations with C-4a (δ 116.6 ppm), C-4b (δ 131.4 ppm) and C-7. The other aromatic

ring is substituted by a methoxy group at C-4, as confirmed by HMBC correlations from C-4 to H-3 and 4-OCH_3. The appearance of H-1 and H-3 as broad singlets suggested that they were *meta*-coupled. Therefore, the second hydroxyl group could be placed at position 2, whose carbon gave cross-peaks with H-1 and H-3.

Therefore, the structure of compound CF-3 was identified as 2,7-dihydroxy-4,6-dimethoxy-9,10-dihydrophenanthrene or 6-methoxycoelonin, firstly isolated from the roots of *Cymbidium aloifolium* (44). This compound has also been found in other orchids such as *Agrostophyllum callosum* (193) *Appendicula reflexa* (194) and *Cephalantheropsis gracilis* (195).



6-Methoxycoelonin

Table 12. NMR spectral data of compound CF-3 (in $CDCl_3$) and 6-methoxycoelonin (in acetone-d_6)

Position	Compound CF-3		6-Methoxycoelonin[44]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)[44]	δ_C
1	6.33 (*br s*)	107.5	6.24 (*d*, 2.5)	108.5
2	-	154.6	-	157.4
3	6.40 (*br s*)	98.2	6.34 (*d*, 2.5)	99.4
4	-	157.5	-	158.7
4a	-	116.6	-	115.3
4b	-	131.4	-	131.7
5	7.84 (*s*)	111.3	7.74 (*s*)	113.4
6	-	144.4	-	146.1
7	-	143.5	-	145.3
8	6.76 (*s*)	113.5	6.55 (*s*)	114.9
8a	-	124.7	-	125.6
9	2.65 (*br s*)	28.9	2.52 (*s*)	30.1
10	2.65 (*br s*)	30.8	2.52 (*s*)	31.6
10a	-	141.2	-	141.4
4-OCH_3	3.84 (*s*)	55.7	3.72 (*s*)	55.9
6-OCH_3	3.89 (*s*)	56.1	3.75 (*s*)	56.6
2-OH	5.17 (*br s*)	-	5.83 (*br s*)	-
7-OH	5.62 (*br s*)	-	5.83 (*br s*)	-

Table 13. HMBC spectral data of compound CF-3 (in $CDCl_3$)

^{1}H signal	HMBC correlation with
H-1	C-2, C-3, C-4a, C-10
H-3	C-1, C-2, C-4, C-4a
H-5	C4a, C-4b, C-7
H-8	C-6, C-7, C-8a, C-9
H-9	C-4b, C-8, C-10, C-10a
H-10	C-1, C-4a, C-8a, C-9
4-OCH_3	C-4
6-OCH_3	C-6
7-OH	C-7, C-8

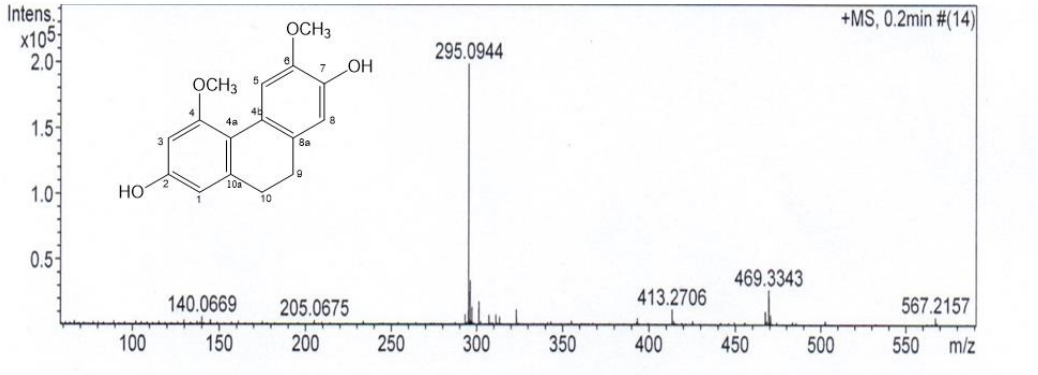


Figure 32. HR-ESI mass spectrum of compound CF-3

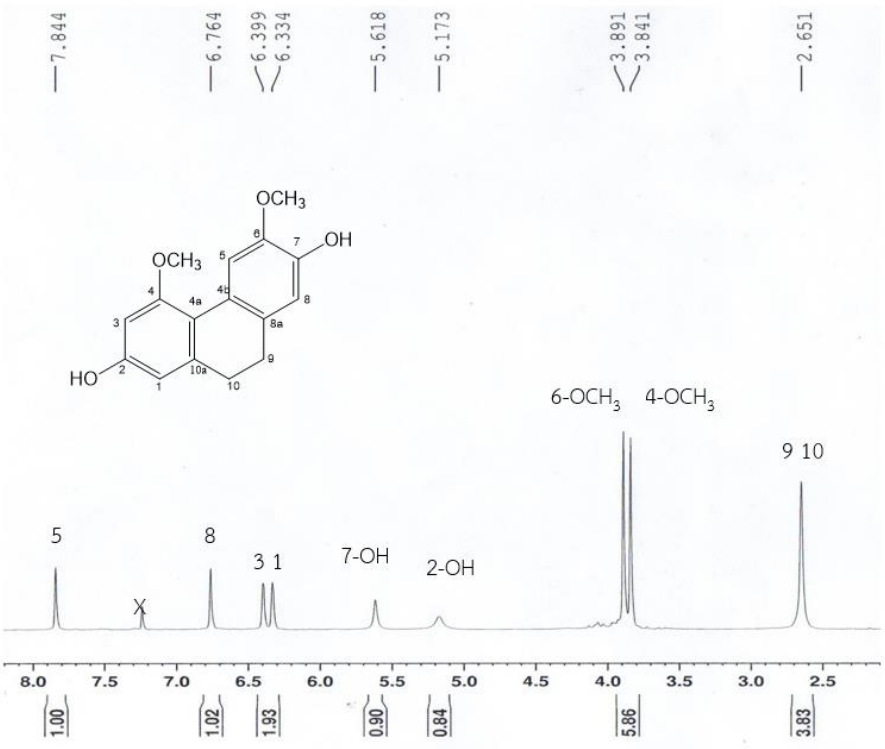


Figure 33. ^{1}H NMR spectrum of compound CF-3 (300 MHz, in $CDCl_3$)

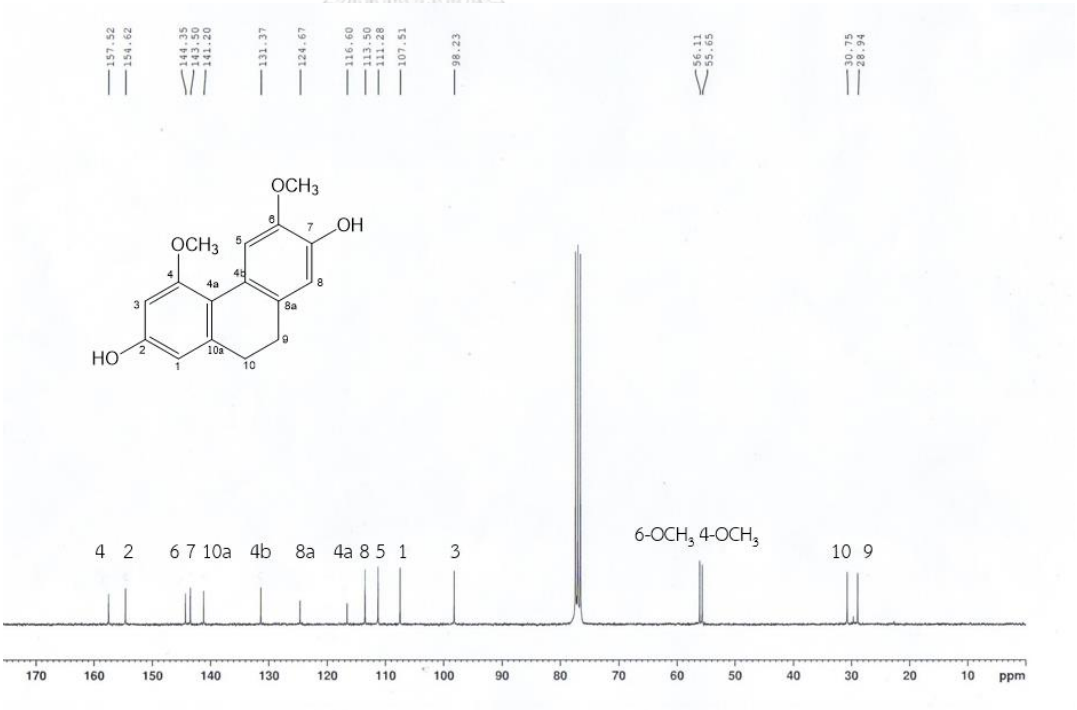


Figure 34. ^{13}C NMR spectrum of compound CF-3 (75 MHz, in $CDCl_3$)

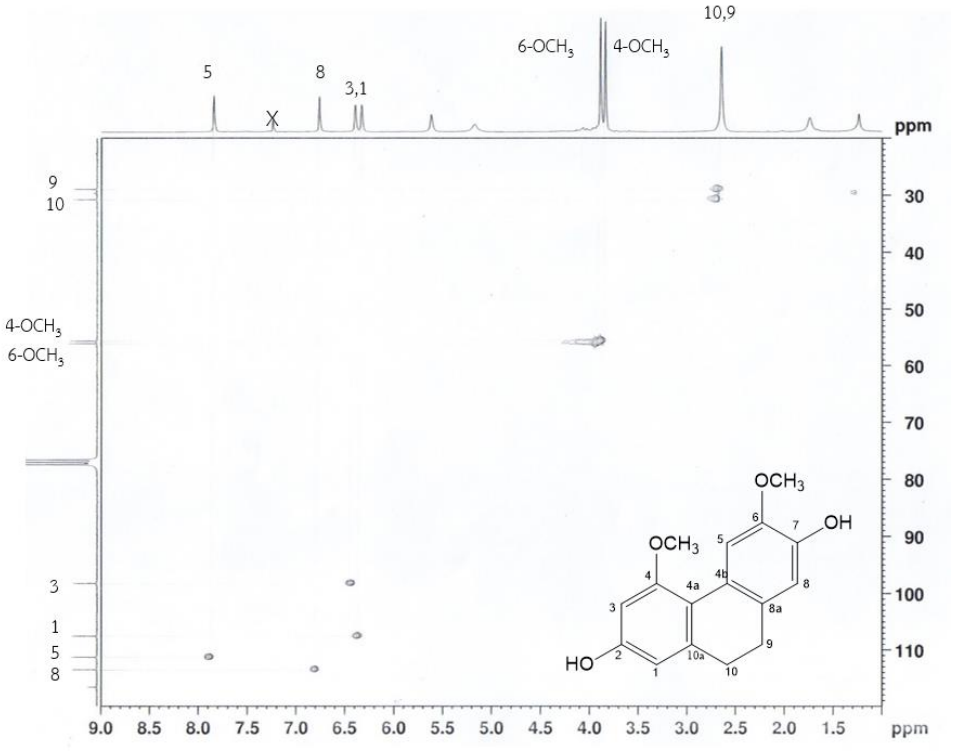


Figure 35. HSQC spectrum of compound CF-3

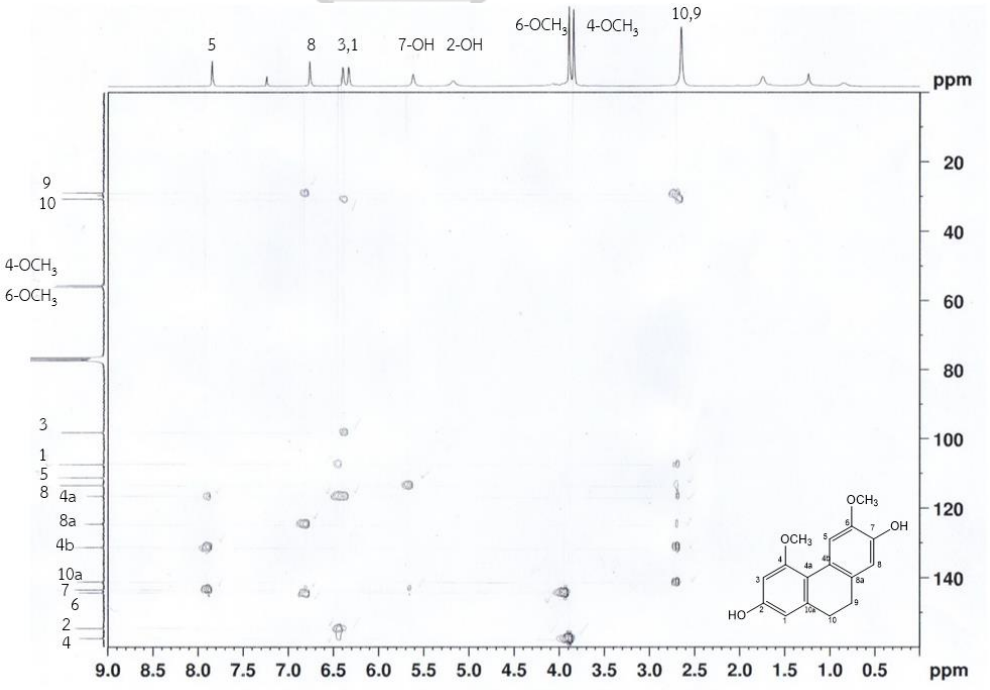


Figure 36. HMBC spectrum of compound CF-3

4. Identification of compound CF-4 (flavanthridin or ephemeranthol-B)

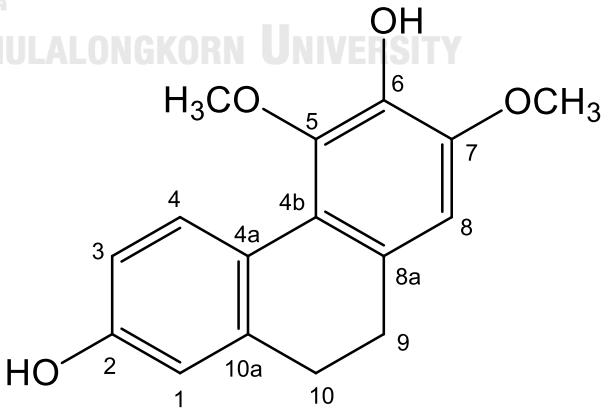
Compound CF-4, obtained as a brown amorphous solid (5 mg, 0.0005% yield), appeared as a brown spot on TLC upon spraying with 10% sulfuric acid and heating. Its $[M + Na]^+$ ion peak at *m/z* 295.0946 (calcd. 295.0946) in the HR-ESI mass spectrum (**Figure 37**) corresponded to the molecular formula $C_{16}H_{16}O_4$ (equal to nine degrees of unsaturation) and suggested that the compound was a 9,10-dihydrophenanthrene isomer of compound CF-3, containing two methoxy and two hydroxy groups.

The ^{1}H NMR spectrum of compound CF-4 (**Figure 38** and **Table 14**) displayed a broad four proton singlet signal at δ 2.70 ppm, representing H_2-9 and H_2-10 skeleton without any substitution at C-1 and C-8, similar to compound CF-3. Two methoxy signals appeared at δ 3.68 (3H, *s*, 5-OCH_3) and 3.89 ppm (3H, *s*, 7-OCH_3). A hydroxy group resonated at δ 5.58 ppm (1H, *br s*, 6-OH) while the other hydroxy group appeared as a hump at about 4.90 ppm (2-OH). Three signals, at δ 6.70 (1H, *br s*, H-1), 6.72 (1H , *d*, *J* = 8.4 Hz, H-3) and 8.14 ppm (1H, *d*, *J* = 8.4 Hz, H-4), represented the 1,2,4-trisubstituted aromatic ring on one side of the skeleton, whereas the pentasubstituted aromatic ring on the other side of the molecule was indicated by the proton singlet at δ 6.55 ppm (H-8).

Carbon resonances in its ^{13}C NMR spectrum (**Figure 39** and **Table 14**) could be differentiated, with the aid of the HSQC experiment (**Figure 40**), into those of two methoxy groups at δ 56.2 (7-OCH_3) and 60.0 ppm (5-OCH_3), two methylene carbons at δ 29.8 (C-9) and 30.2 ppm (C-10), four aromatic methine carbons at δ 107.0 (C-8), 113.4 (C-3, 114.5 (C-1 and 128.3 ppm (C-4) and eight aromatic quaternary carbons at δ 120.2 (C-4b), 125.4 (C-4a), 129.6 (C-8a), 137.6 (C-6), 139.8 (C-10a), 144.8 (C-5), 145.7 (C-7) and 154.1 ppm (C-2).

These ^{1}H and ^{13}C NMR data are in good agreement with those of flavanthridin. However, there are some revisions for its carbon assignments, which had not been confirmed by 2D experiments in the original study. In this study, H-4 was assigned at δ 8.14 ppm based on its HMBC correlations (**Figure 41** and **Table 13**) with C-3, C-4b and C-10a. Then, based on their *ortho*-coupling, the proton signal at δ 6.72 ppm should belong to H-3, which was also *meta*-coupled to H-1 at δ 6.70 ppm. HSQC cross-peaks indicated that C-1 and C-3 signals were those at δ 114.5 and 113.4 ppm, respectively. Another revision is the assignments for C-9 and C-10, which should be reversed, based on HMBC cross-peaks observed between H-1 and C-10 (at δ 30.2 ppm) and between H-8 and C-9 (at δ 29.8 ppm).

Flavanthridin has been reported for the first time as a constituent of *Eria flava* (196). However, in the following year (1991), a compound with the same chemical structure was reported from another orchid (*Ephemerantha lonchophylla*) and named ephemeranthol-B (197). Afterwards, it was also isolated from other orchids in the same subfamily Epidendroideae including *Cymbidium* Great Flower Marie Laurencin (14), *Flickingeria fimbriata* (198) and *Nidema boothii* (55).



Flavanthridin (ephemeranthol-B)

Table 14. NMR spectral data of compound CF-4 and flavanthridin (in $CDCl_3$)

Position	Compound CF-4		Flavanthridin	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz) [196]	δ_C [196]
1	6.70 (*br s*)	114.5	6.69 (*br s*)	113.4
2	-	154.1	-	154.0
3	6.72 (*d*, 8.4)	113.4	8.11 (*d*, 8.0)	114.4
4	8.14 (*d*, 8.4)	128.3	6.69 (*d*, 8.0)	128.2
4a	-	125.4	-	125.4
4b	-	120.2	-	120.2
5	-	144.8	-	144.8
6	-	137.6	-	137.5
7	-	145.7	-	145.7
8	6.55 (*s*)	107.0	6.52 (*s*)	106.9
8a	-	129.6	-	129.5
9	2.70 (*br s*)	29.8	2.67 (*s*)	30.1
10	2.70 (*br s*)	30.2	2.67 (*s*)	29.8
10a	-	139.8	-	139.7
5-OCH_3	3.68 (*s*)	60.1	3.86 (*s*)	59.9
7-OCH_3	3.89 (*s*)	56.2	3.65 (*s*)	56.1
2-OH	4.90 (*br*)	-	4.81 (*s*)	-
6-OH	5.58 (*br s*)	-	5.56 (*s*)	-

Table 15. HMBC spectral data of compound CF-4 (300 MHz, in $CDCl_3$)

^{1}H signal	HMBC correlation with
H-1	C-3, C-4a, C-10
H-3	C-1, C-4a
H-4	C-2, C-4b, C-10a
H-8	C-4b, C-6, C-7, C-9
H-9	C-4a, C-8, C-8a, C-10, C-10a
H-10	C-4a, C-8a, C-9, C-10a
5-OCH_3	C-5
7-OCH_3	C-7

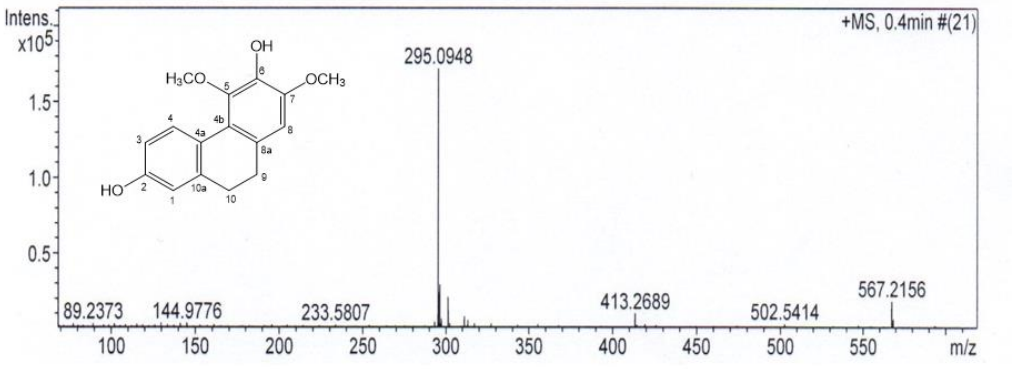


Figure 37. HR-ESI mass spectrum of compound CF-4

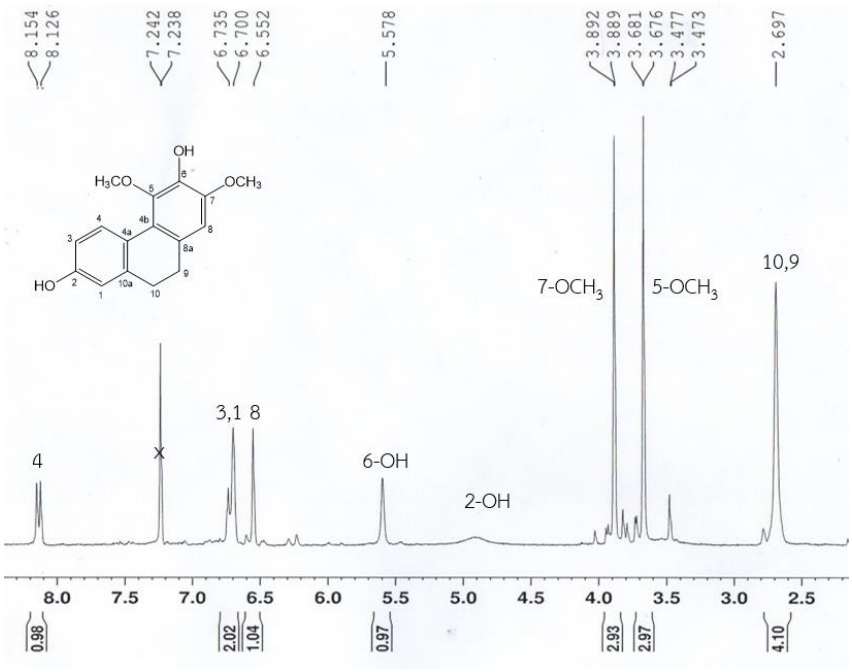


Figure 38. ^{1}H NMR spectrum of compound CF-4 (300 MHz, in $CDCl_3$)

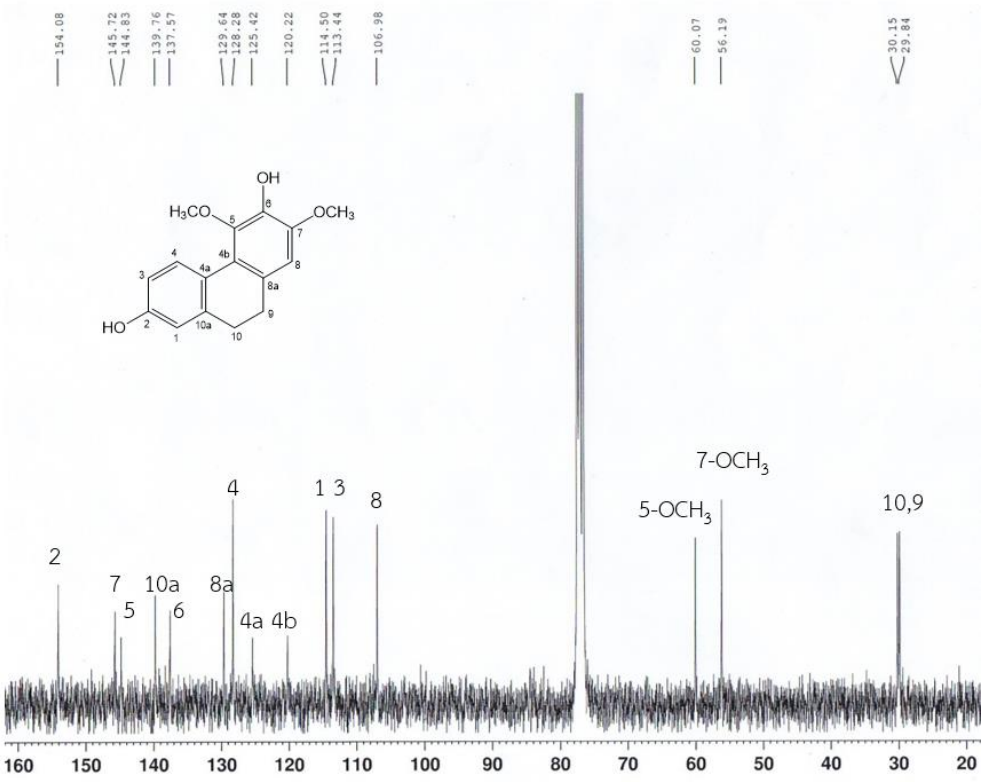


Figure 39. ^{13}C NMR spectrum of compound CF-4 (75 MHz, in $CDCl_3$)

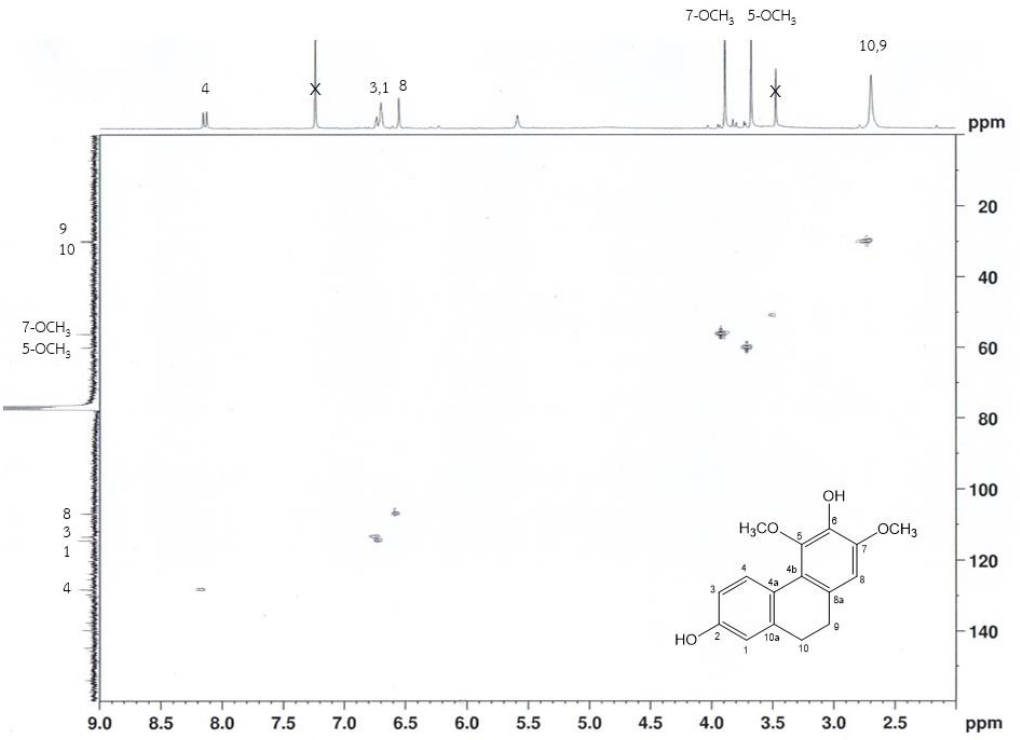


Figure 40. HSQC spectrum of compound CF-4

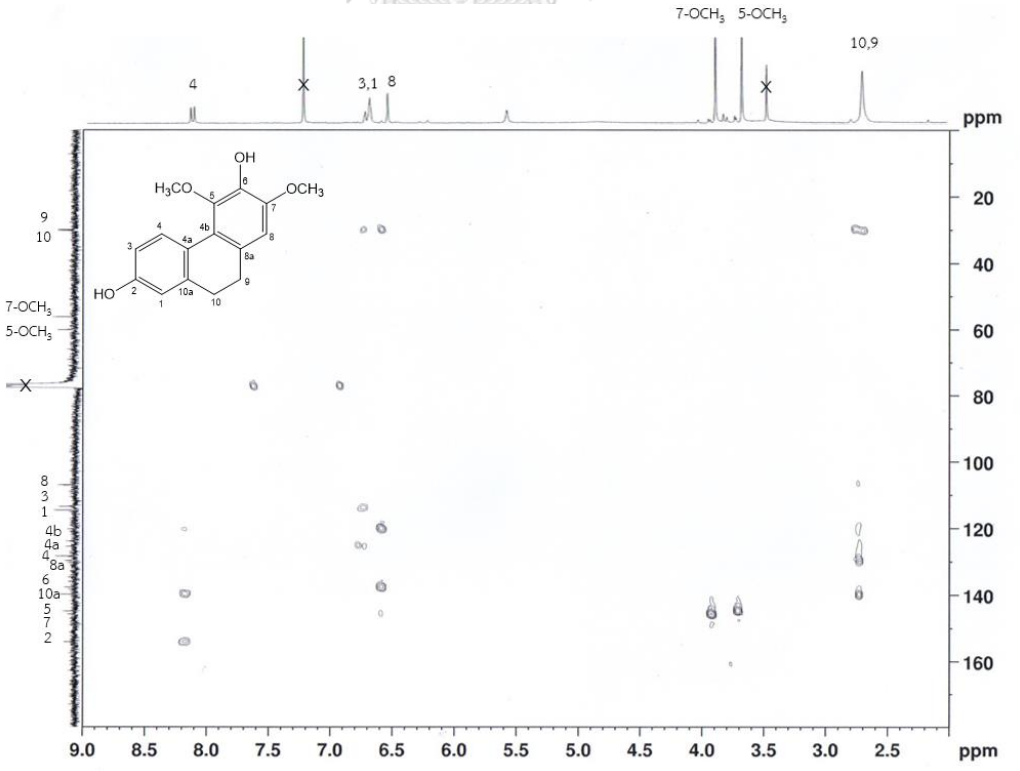


Figure 41. HMBC spectrum of compound CF-4

5. Identification of compound CF-5 (2,4-dimethoxy-3,7-dihydroxyphenanthrene or 2,4-dimethoxyphenanthrene-3,7-diol)

Compound CF-5 was isolated as a brown amorphous solid (29 mg, 0.0029% yield) which gave a blue spot on TLC upon spraying with 10% sulfuric acid and heating. The mass spectrum (**Figure 42**) showed $[M + Na]^+$ ion peak at *m/z* 293.0793 (calcd. 293.0790), suggesting its molecular formula as $C_{16}H_{14}O_4$ (equal to ten degrees of unsaturation). It has two protons less than compound CF-4, indicating that it should be a phenanthrene with the same types and same number (four) of substitutions.

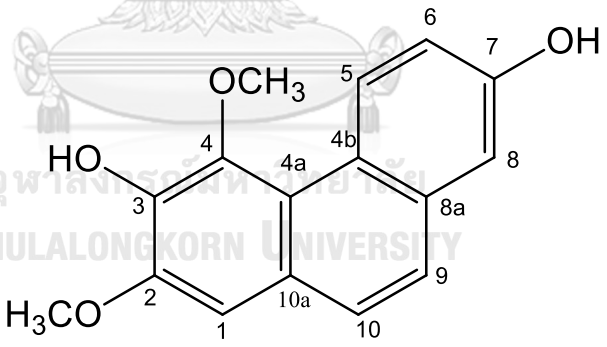
The ^{1}H NMR spectrum of compound CF-5 (**Figure 43** and **Table 16**) showed signals for two methoxy groups at δ 3.89 (3H, *s*, 4-OCH_3) and 3.95 ppm (3H, *s*, 2-OCH_3) and six aromatic protons including those of a 1,2,4-trisubstituted aromatic ring at δ 7.16 (1H, *dd*, *J* = 9.0, 2.6 Hz, H-6), 7.22 (1H, *d*, *J* = 2.6 Hz, H-8) and 9.30 ppm (1H, *d*, *J* = 9.0 Hz, H-5), a pair of *ortho*-coupled doublets at δ 7.42 (1H, *d*, *J* = 8.7 Hz, H-9) and 7.57 ppm (1H, *d*, *J* = 8.7 Hz, H-10), and a singlet at δ 7.20 ppm (1H, *s*, H-1).

The ^{13}C NMR spectrum of compound CF-5 (**Figure 44** and **Table 16**) and its HSQC spectrum (**Figure 45**) exhibited two methoxy peaks at δ 56.2 (2-OCH_3) and 59.5 ppm (4-OCH_3), and resonances of six methine carbons and eight quaternary carbons including four oxygenated ones at δ 141.0 (C-3), 145.2 (C-4), 148.4 (C-2) and 155.9 ppm (C-7). These data indicated that compound CF-5 was a phenanthrene with two hydroxy and two methoxy substitutions, similar to compound CF-4.

Its HMBC spectrum (**Figures 46-47** and **Table 17**) showed 3-bond long-range coupling between from H-9 (at δ 7.42 ppm) and C-4b (δ 123.6 ppm), C-8 (δ 112.1 ppm) and C-10a (δ 126.2 ppm), whereas H-10 (at δ 7.57 ppm) displayed three-bond correlations with C-1 (δ 105.8 ppm) and C-8a (δ 134.9 ppm). Methoxy protons at positions 2 and 4 displayed HMBC correlation with C-2 and C-4, respectively. H-1 (at δ

7.20 ppm) exhibited cross-peaks with C-2 and the hydroxy-substituted C-3. The second hydroxy group should therefore be located at position 7. Therefore, compound CF-5 was characterized as the known compound 2,4-dimethoxy-3,7-dihydroxyphenanthrene (2) (or 2,4-dimethoxyphenanthrene-3,7-diol (1)).

This phenanthrene derivative was originally obtained by chemical transformation of flavanthridin (compound CF-4) from the orchid *Eria flava* (196). Later, it was found in other orchids such as *Bulbophyllum vaginatum* (199), *Dendrobium plicatile* (200), *Ephemerantha lonchophylla* (92) *and Scaphyglottis livida* (201). Moreover, it can be isolated from a plant in other monocotyledonous family i.e. from the rhizomes of *Tamus communis* (family Dioscoreaceae). The compound was cytotoxic to cervical cancer (HeLa) cell line (202) and exhibited significant anti-platelet aggregation activity against arachidonic acid-induced aggregation (92).



2,4-Dimethoxy-3,7-dihydroxyphenanthrene

Table 16. NMR spectral data of compound CF-5 (in acetone-d_6) and 2,4-dimethoxy-3,7-dihydroxyphenanthrene (in $CDCl_3$)

Position	Compound CF-5		2,4-Dimethoxy-3,7-dihydroxyphenanthrene[199]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	7.20 (*s*)	105.8	7.07 (*s*)	104.9
2	-	148.4	-	146.7
3	-	141.0	-	139.2
4	-	145.2	-	143.9
4a	-	119.9	-	119.0
4b	-	123.6	-	123.7
5	9.30 (*d*, 9.0)	128.9	9.33 (*d*, 9.1)	127.4
6	7.16 (*dd*, 9.0, 2.6)	117.3	7.18 (*dd*, 9.1, 2.8)	116.3
7	-	155.9	-	153.2
8	7.22 (*d*, 2.6)	112.1	7.21 (*d*, 2.8)	111.6
8a	-	134.9	-	133.8
9	7.42 (*d*, 8.7)	125.2	7.47 (*d*, 8.8)	124.9
10	7.57 (*d*, 8.7)	128.0	7.56 (*d*, 8.8)	128.5
10a	-	126.2	-	126.0
2-OCH_3	3.95 (*s*)	56.2	4.04 (*s*)	56.1
4-OCH_3	3.89 (*s*)	59.5	3.95 (*s*)	59.8
3-OH	-	-	5.98 (*s*)	-
7-OH	-	-	5.08 (*s*)	-

Table 17. HMBC spectral data of compound CF-5 (300 MHz, in acetone-d_6)

^{1}H signal	HMBC correlation with
H-1	C-2, C-3, C-4a
H-5	C-4a, C-7, C-8a
H-6	C-4b, C-8
H-8	C-6, C-7, C-9
H-9	C-4b, C-8, C-8a, C-10a
H-10	C-1, C-8a, C-9
2-OCH_3	C-2
4-OCH_3	C-4

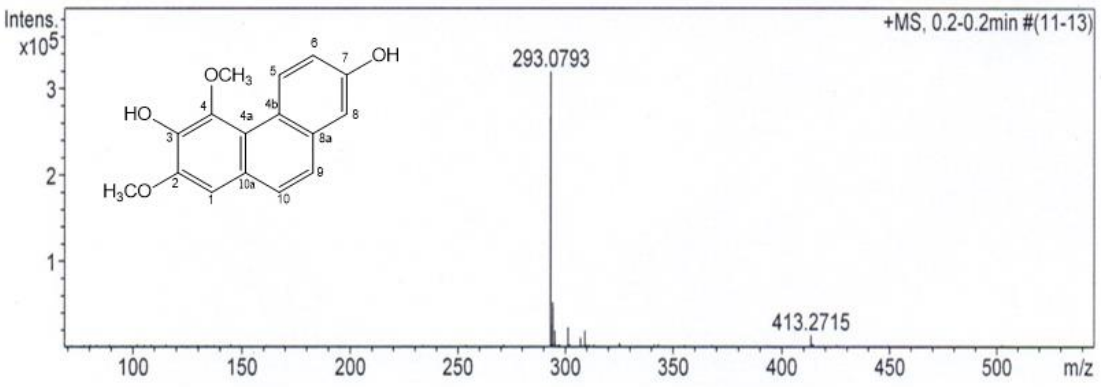


Figure 42. HR-ESI mass spectrum of compound CF-5

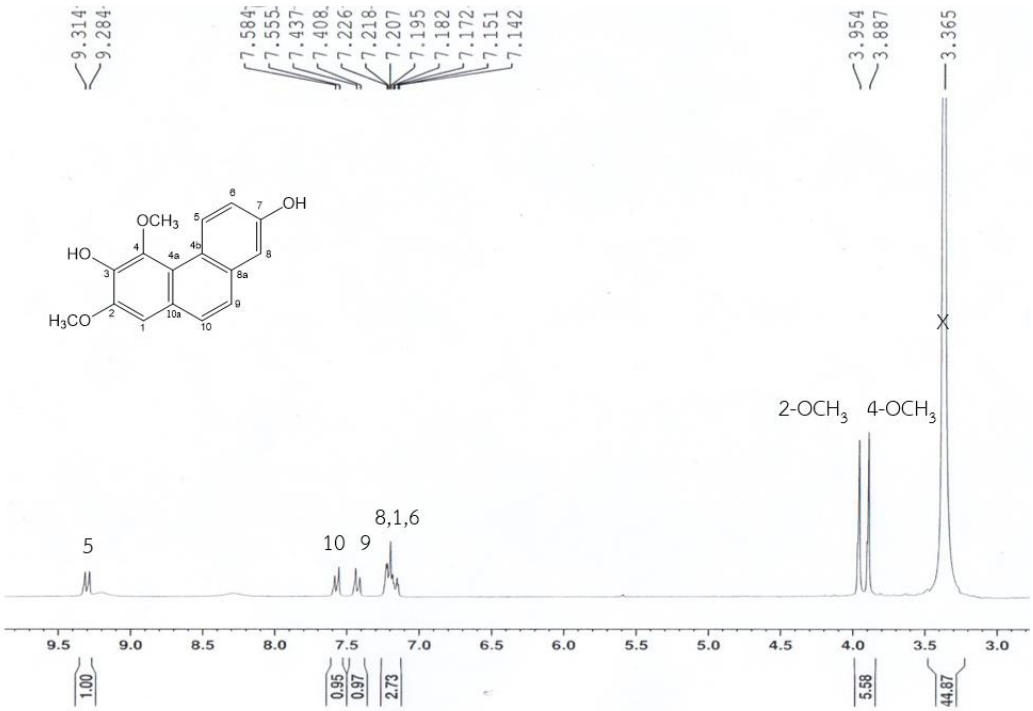


Figure 43. ^{1}H NMR spectrum of compound CF-5 (300 MHz, in acetone-d_6)

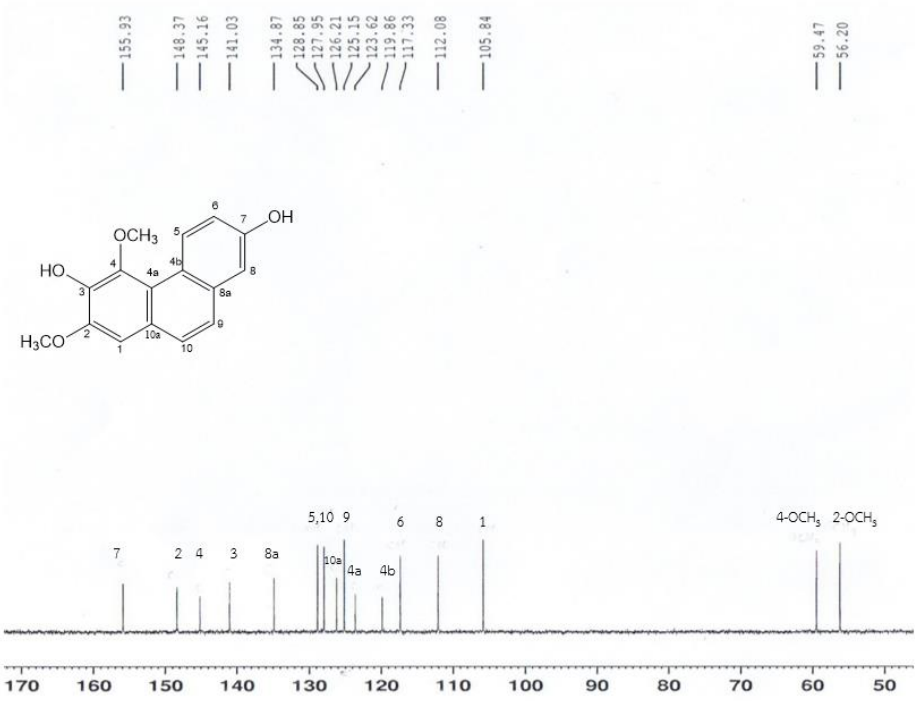


Figure 44. ^{13}C NMR spectrum of compound CF-5 (75 MHz, in acetone-d_6)

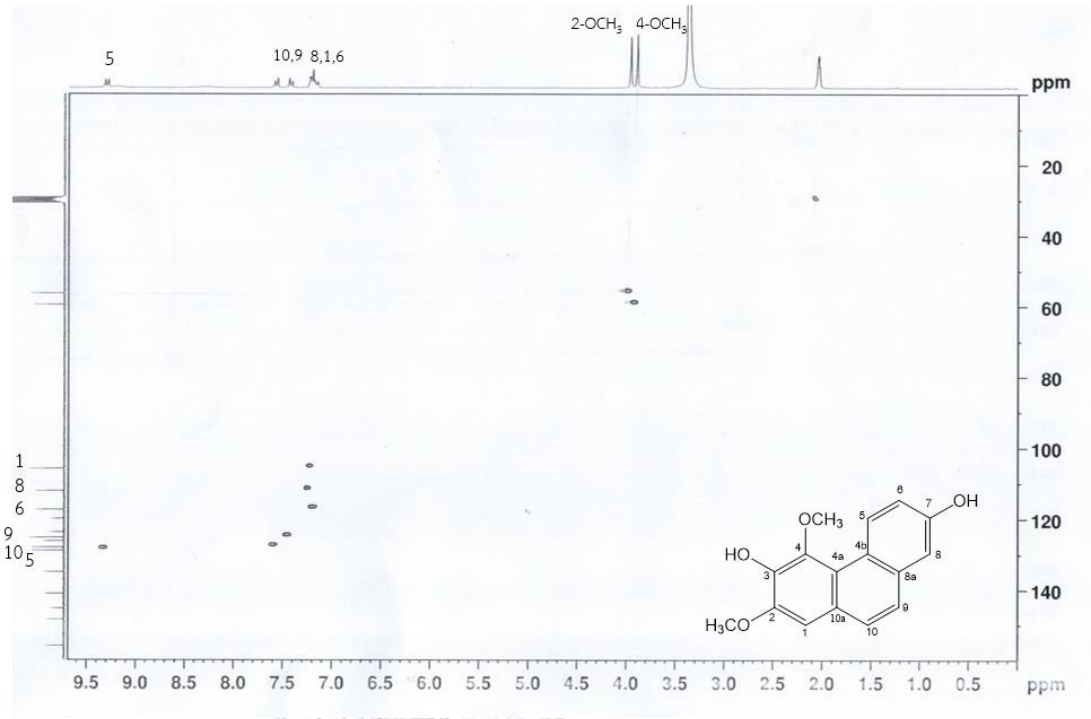


Figure 45. HSQC spectrum of compound CF-5

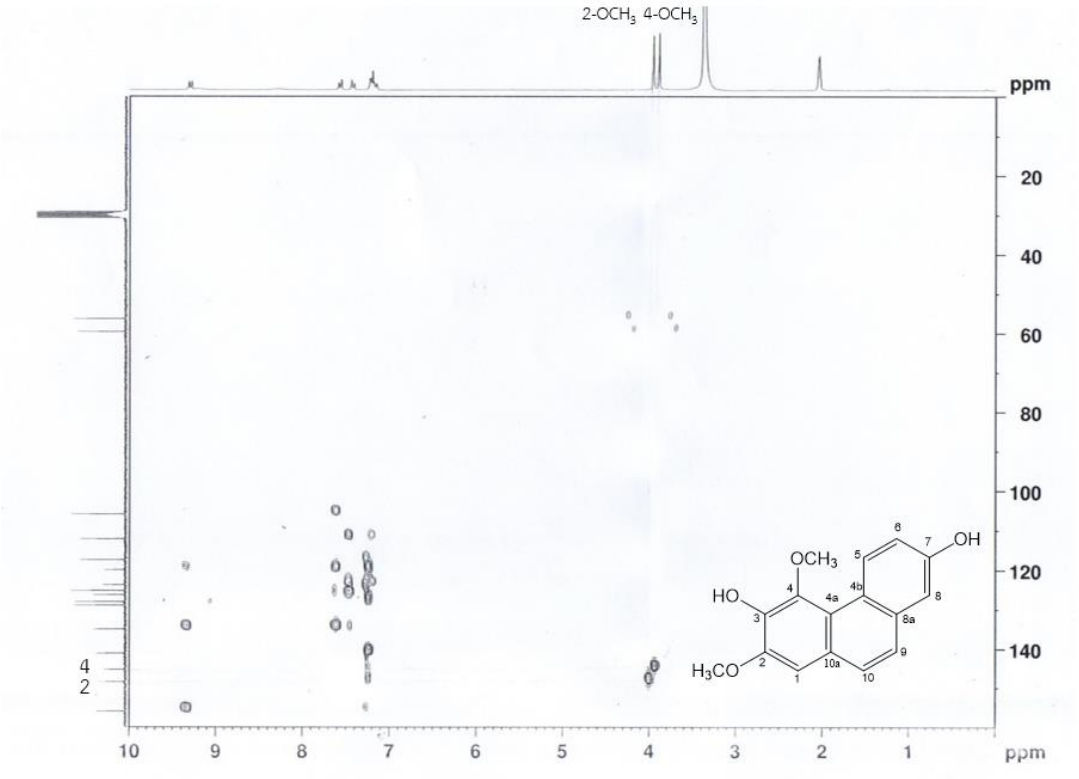


Figure 46. HMBC spectrum of compound CF-5

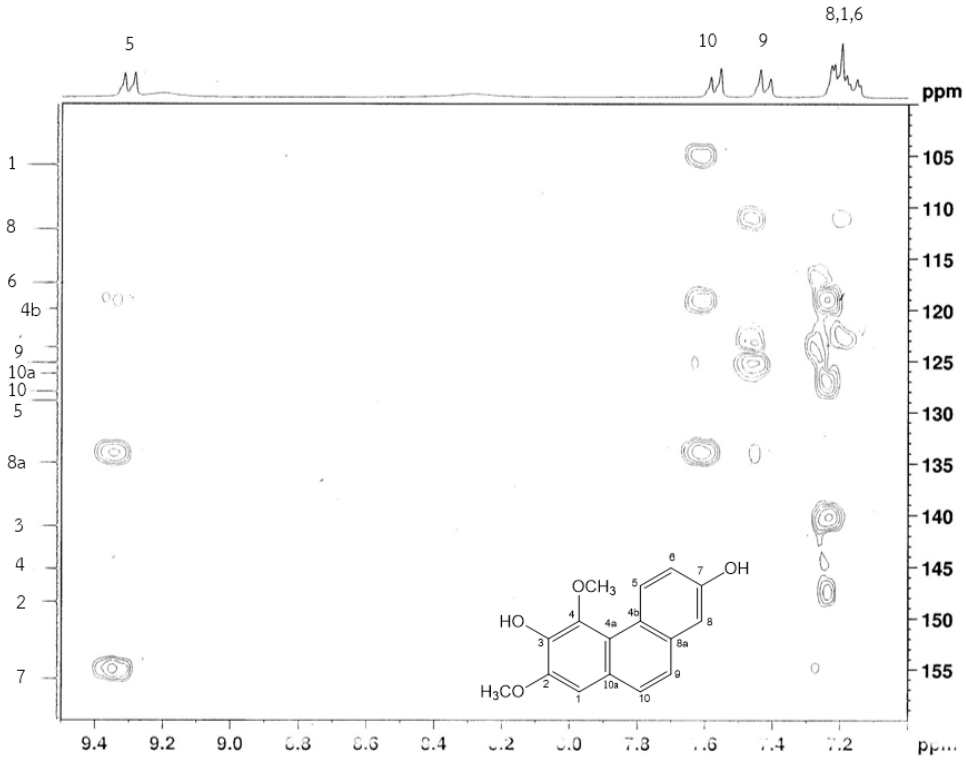


Figure 47. HMBC spectrum of compound CF-5 (expansion between δ_H 7.0-9.5, δ_C 100-160)

6. Identification of compound CF-6 (3,7-dihydroxy-2,4,6-trimethoxyphenanthrene)

Compound CF-6, obtained as a brown amorphous solid (13 mg, 0.0013% yield), appeared as a gray spot on TLC upon spraying with 10% sulfuric acid and heating. Based on the $[M + Na]^+$ ion peak at *m/z* 323.0893 in the mass spectrum (**Figure 48**), its molecular formula was established as $C_{17}H_{16}O_5$ (calcd. 323.0895). Ten degrees of unsaturation, calculated from its molecular formula, was suggestive of a phenanthrene skeleton with five substituents.

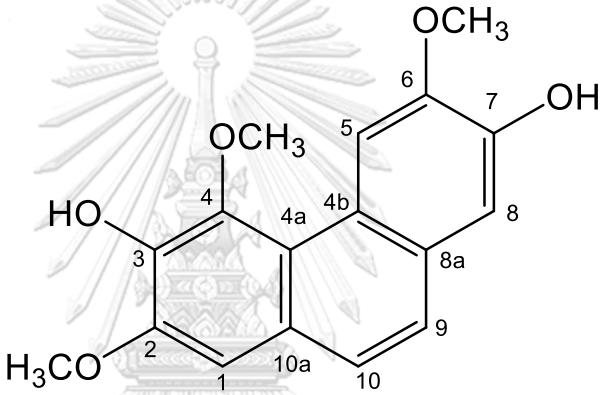
The signals for these substituents appeared in its ^{1}H NMR spectrum (**Figure 49** and **Table 18**) as three methoxy singlets at δ 3.96 (3H, *s*, 4-OCH_3), 4.02 (3H, *s*, 2-OCH_3) and 4.06 ppm (3H, *s*, 6-OCH_3) and two hydroxy broad singlets at δ 5.90 (1H, *br s*, 7-OH)

and 5.95 ppm (1H, *br s*, 3-OH). Other proton resonances were those of three unconnected aromatic protons at δ 7.05 (1H, *s*, H-1), 7.29 (1H, *s*, H-8) and 8.98 ppm (1H, *s*, H-5). The most deshielded signal at δ 8.98 ppm was characteristic of H-5 of a phenanthrene derivative (203). A two-proton singlet, resonating at δ 7.47 ppm, represented H-9 and H-10 of the phenanthrene nucleus and reflected their similar environment. These data indicated that compound CF-6 was a phenanthrene with five functional groups (three methoxy and two hydroxy) at positions 2, 3, 4, 6 and 7.

The ^{13}C NMR (**Figure 50** and **Table 18**) and HSQC spectra (**Figure 51**) of compound CF-6 exhibited three methoxy carbon signals at δ 55.9 (6-OCH_3), 56.1 (2-OCH_3) and 60.0 ppm (4-OCH_3), five methine carbon signals at δ 104.7 (C-1), 106.8 (C-5), 111.1 (C-8), 125.0 (C-10) and 124.8 ppm (C-9) and nine quaternary carbon signals, including five oxygen-substituted ones, at δ 118.6 (C-4a), 123.4 (C-4b), 126.2 (C-10a), 127.9 (C-8a), 138.7 (C-3), 143.6 (C-4), 144.7 (C-7), 146.6 (C-2) and 146.6 ppm (C-6).

Comparison of these NMR data with phenanthrenes with similar substructure, e.g. 4,6-dimethoxyphenanthrene-2,3,7-triol (199) and 3,7-dihydroxy-2,4,6-trimethoxyphenanthrene (203), indicated that on one side of the phenanthrene skeleton a methoxy group and a hydroxy group should be placed at C-6 and C-7, respectively. From HMBC spectrum (**Figure 52** and **Table 19**), three-bond correlation peaks could be observed between H-8 at δ 7.29 ppm and C-4b, C-6 and C-9, whereas H-1 at δ 7.05 ppm showed long-range cross-peaks with C-3, C-4a and C-10. H-5 (at δ 8.98 ppm) displayed three-bond correlations with C-4a, C-7 and C-8a. A methoxy group could be located at C-4, based on its HMBC correlation with 4-OCH_3 protons and no other aromatic proton in the structure. Finally, comparison of these NMR data with previously reported values identified compound CF-6 as 3,7-dihydroxy-2,4,6-trimethoxyphenanthrene.

3,7-Dihydroxy-2,4,6-trimethoxyphenanthrene was firstly found as a constituent of the orchid *Bulbophyllum odoratissimum* (203). In addition, it was also isolated from other plants i.e. from the stems and leaves of *Dioscorea nipponica* Makino (family Dioscoreaceae) (204) and from a dicotyledonous plant i.e. from the leaves of *Combretum apiculatum* (family Combretaceae) (205).



3,7-Dihydroxy-2,4,6-trimethoxyphenanthrene

Table 18. NMR spectral data of compound CF-6 (in acetone-d_6) and 3,7-dihydroxy-2,4,6-trimethoxyphenanthrene (in $CDCl_3$)

Position	Compound CF-6		3,7-Dihydroxy-2,4,6-trimethoxyphenanthrene[203]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	7.05 (*s*)	104.7	6.97 (*s*)	103.6
2	-	146.6	-	146.5
3	-	138.7	-	138.3
4	-	143.6	-	143.0
4a	-	118.6	-	117.4
4b	-	123.4	-	122.2
5	8.98 (*s*)	106.8	8.95 (*s*)	106.1
6	-	146.6	-	146.6
7	-	144.7	-	144.3
8	7.29 (*s*)	111.1	7.19 (*s*)	110.4
8a	-	127.9	-	126.9
9	7.47 (*s*)	124.8	7.31 (*s*)	122.8
10	7.47 (*s*)	125.0	7.31 (*s*)	123.7
10a	-	126.2	-	124.9
2-OCH_3	4.02 (*s*)	56.1	3.88 (*s*)	54.0
4-OCH_3	3.96 (*s*)	60.0	3.85 (*s*)	57.8
6-OCH_3	4.06 (*s*)	55.9	3.97 (*s*)	53.8
3-OH	5.95[a] (*br s*)	-	-	-
7-OH	5.90[a] (*br s*)	-	-	-

[a] interchangeable

Table 19. HMBC spectral data of compound CF-6 (300 MHz in acetone-d_6)

^{1}H signal	HMBC correlation with
H-1	C-2, C-3, C-4a, C-10
H-5	C-4a, C-6, C-7, C-8a
H-8	C-4b, C-6, C-7, C-9
H-9	C-4b, C-8, C-10a
H-10	C-1, C-4a, C-8a
2-OCH_3	C-2
4-OCH_3	C-4
6-OCH_3	C-6

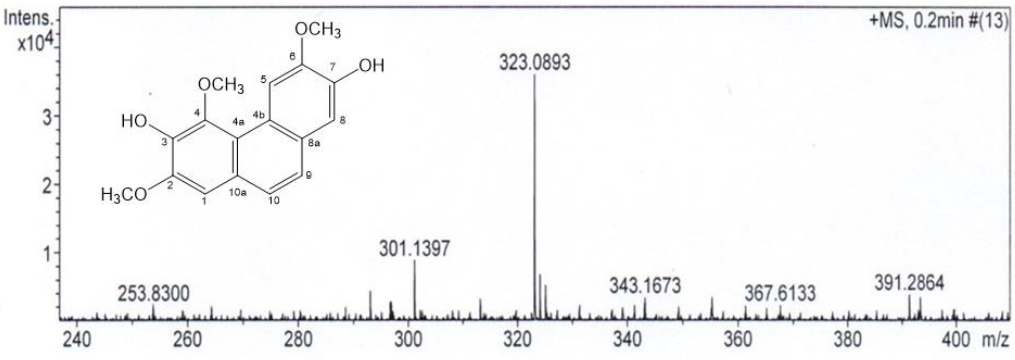


Figure 48. HR-ESI mass spectrum of compound CF-6

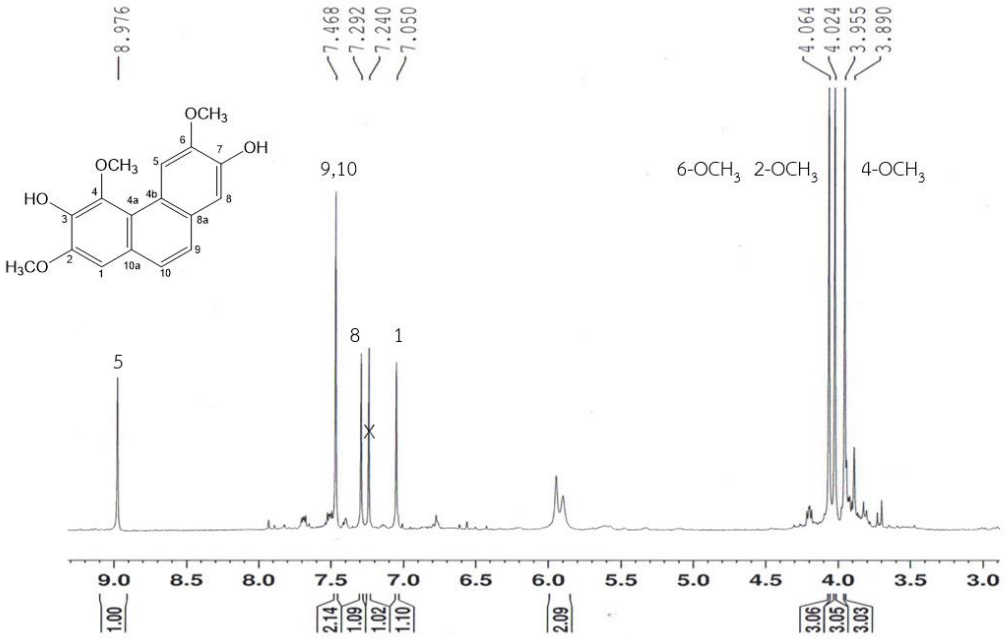


Figure 49. ^{1}H NMR spectrum of compound CF-6 (300 MHz, in acetone-d_6)

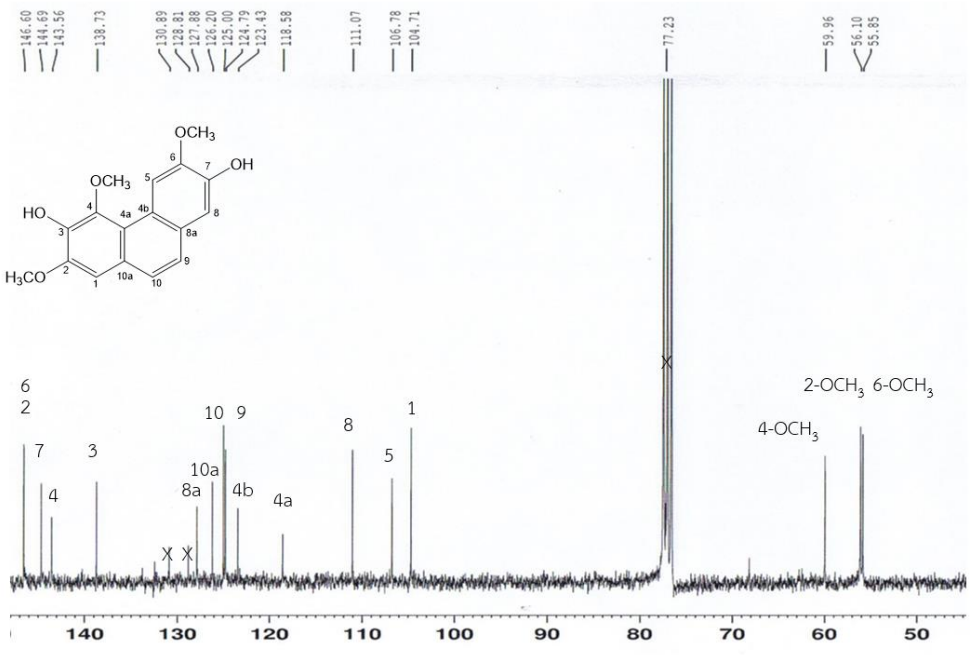


Figure 50. ^{13}C NMR spectrum of compound CF-6 (75 MHz, in acetone-d_6)

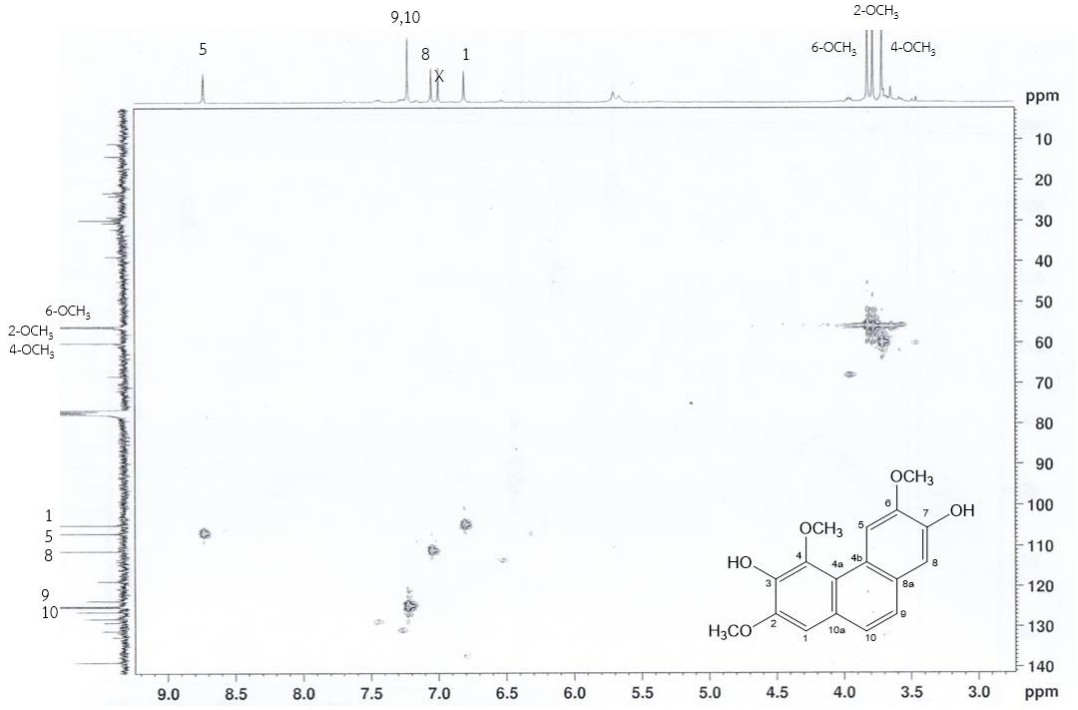


Figure 51. HSQC spectrum of compound CF-6

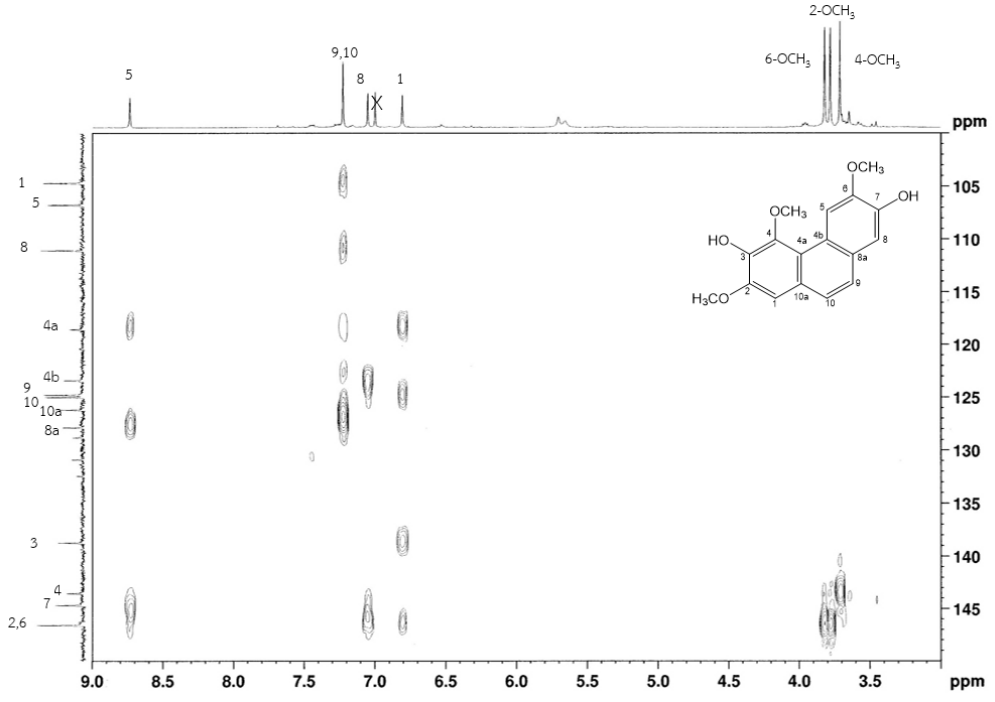


Figure 52. HMBC spectrum of compound CF-5 (expansion between δ_H 3.0-9.0, δ_C 100-150)

7. Identification of compound CF-7 (coelonin)

Compound CFW-7 was obtained as a brown amorphous solid (6 mg, 0.0006% yield) which gave a gray spot on TLC upon spraying with 10% sulfuric acid and heating. The mass spectrum (**Figure 53**) showed sodium-adductmolecular ion peak at *m/z* 265.0839 $[M + Na]^+$, suggesting its molecular formula as $C_{15}H_{14}O_3$.

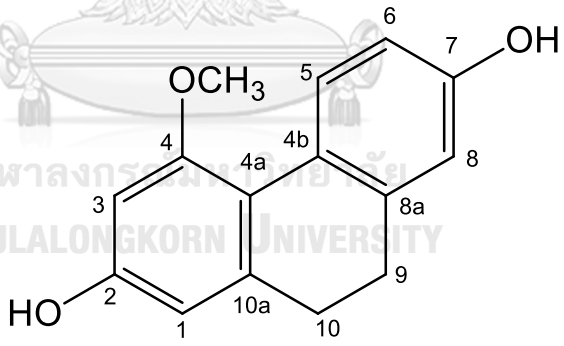
The ^1H NMR spectrum of compound CF-7 (**Figure 54** and **Table 20**) exhibited a methoxy singlet at δ 3.83 ppm (3H, *s*, 4-OCH_3) and two hydroxy broad singlets at δ 4.90 (1H, *br s*, 7-OH) and 5.01 ppm (1H, *br s*, 2-OH). A four-proton singlet, characteristic of 9,10-dihydrophenanthrene nucleus, resonated at δ 2.68 ppm (4H, *s*, H_2-9 and H_2-10). Other signals could be viewed as two sets of coupled aromatic protons: a pair of *meta*-coupling protons (*J* = 2.3 Hz) at δ 6.32 (1H, *d*, H-1) and 6.39 ppm (1H, *d*, H-3), and three protons of a 1,2,4-trisubstituted benzene ring at δ 6.68 (1H, *d*, *J* = 2.1 Hz, H-8), 6.69 (1H, *dd*, *J* = 8.1, 2.1 Hz, H-6) and 8.09 ppm (1H, *d*, *J* = 8.1 Hz, H-5).

From the ^{13}C NMR spectrum (**Figure 55** and **Table 20**) and HSQC experiment (**Figures 56-57**) of compound CF-7, fifteen carbon signals could be differentiated as those of a methoxy carbon at δ 55.5 ppm (4-OCH_3), two aliphatic methylene carbons at δ 29.8 (C-9) and 30.4 ppm (C-10), five aromatic methide carbons at δ 98.1 (C-3), 107.2 (C-1), 112.8 (C-8), 114.3 (C-6) and 129.1 ppm (C-5) and seven quaternary carbon signals at δ 116.4 (C-4a), 125.6 (C-4b), 139.7 (C-8a), 141.0 (C-10a), 153.5 (C-7), 154.6 (C-2) and 157.8 ppm (C-4). These data suggested compound CF-7 as a 9,10-dihydrophenanthrene containing two hydroxy and one methoxy groups.

Partial assignments made by comparison with an aromatic ring on one side of compound CF-3 (6-methoxycoelonin) (44) suggested identical substitutions at positions 2 and 4. Therefore, the other hydroxy group could be located on the second aromatic ring of compound CF-7.

Confirmation was obtained by HMBC examination of the spectrum (**Figure 58** and **Table 21**), in which three-bond cross-peaks were observed between H-9 (at δ 2.68 ppm) and C-4b, C-8 and C-10a, and between H-5 (at (δ 8.09 ppm) and C-4a, C-7 and C-8a. Therefore, compound CF-7 was identified as 2,7-dihydroxy-4-methoxy-9,10-dihydrophenanthrene or coelonin. Its spectroscopic data are in good agreement with literature values (44, 203).

Coelonin was originally found as a constituent of the orchids *Coelogyne ochracea* and *C. elata* (47). Since then, it has been isolated from other orchids including *Bletilla striata* (49), *Cymbidium aloifolium* (44), *Dendrobium scabrilingue* (113), *Eulophia nuda* (73) and *E. macrobulbon* (80). The compound has been shown to possess several biological activities such as anti-inflammatory (49), α-glucosidase inhibitory (113) and cytotoxic activities (80).



Coelonin

Table 20. NMR spectral data of compound CF-7 (in $CDCl_3$) and coelonin (in CD_3OD)

Position	Compound CF-7		Coelonin[203]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	6.32 (*d*, 2.3)	107.2	6.30 (*d*, 2.3)	107.9
2	-	154.6	-	155.5
3	6.39 (*d*, 2.3)	98.1	6.39 (*d*, 2.3)	98.9
4	-	157.8	-	158.6
4a	-	116.4	-	116.3
4b	-	125.6	-	125.7
5	8.09 (*d*, 8.1)	129.1	7.99 (*d*, 7.4)	129.5
6	6.69 (*dd*, 8.1, 2.1)	114.3	6.62 (*dd*, 7.4, 2.5)	114.6
7	-	153.5	-	156.9
8	6.68 (*d*, 2.1)	112.8	6.62 (*d*, 2.5)	113.1
8a	-	139.7	-	140.0
9	2.68 (*s*)	29.8	2.66 (*s*)	31.3
10	2.68 (*s*)	30.4	2.66 (*s*)	30.7
10a	-	141.0	-	141.4
4-OCH_3	3.83 (*s*)	55.5	3.80 (*s*)	55.4
2-OH	5.01[a] (*br s*)	-	-	-
7-OH	4.90[a] (*br s*)	-	-	-

[a] interchangeable

Table 21. HMBC spectral data of compound CF-7 (300 MHz in $CDCl_3$)

^{1}H signal	HMBC correlation with
H-1	C-3, C-4a, C-10
H-3	C-1, C-2, C-4a
H-5	C-4a, C-7, C-8a
H-6	C-4b, C-8
H-8	C-4b, C-6, C-9
H-9	C-4b, C-8, C-10, C-10a
H-10	C-1, C-4a, C-9
4-OCH_3	C-4

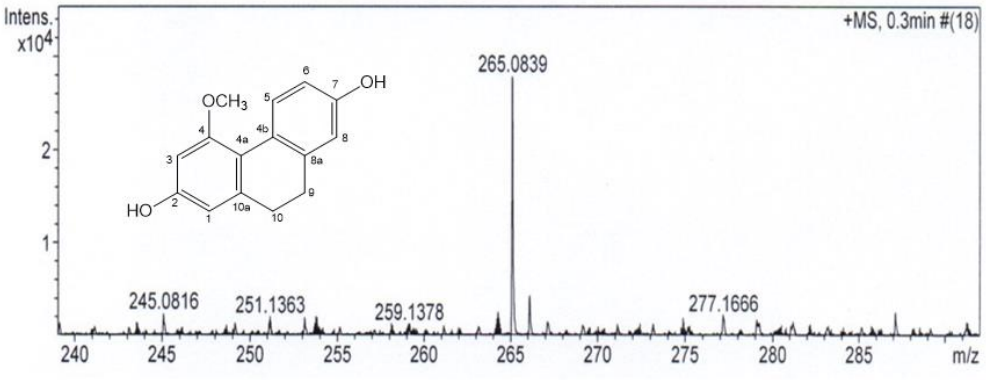


Figure 53. HR-ESI mass spectrum of compound CF-7

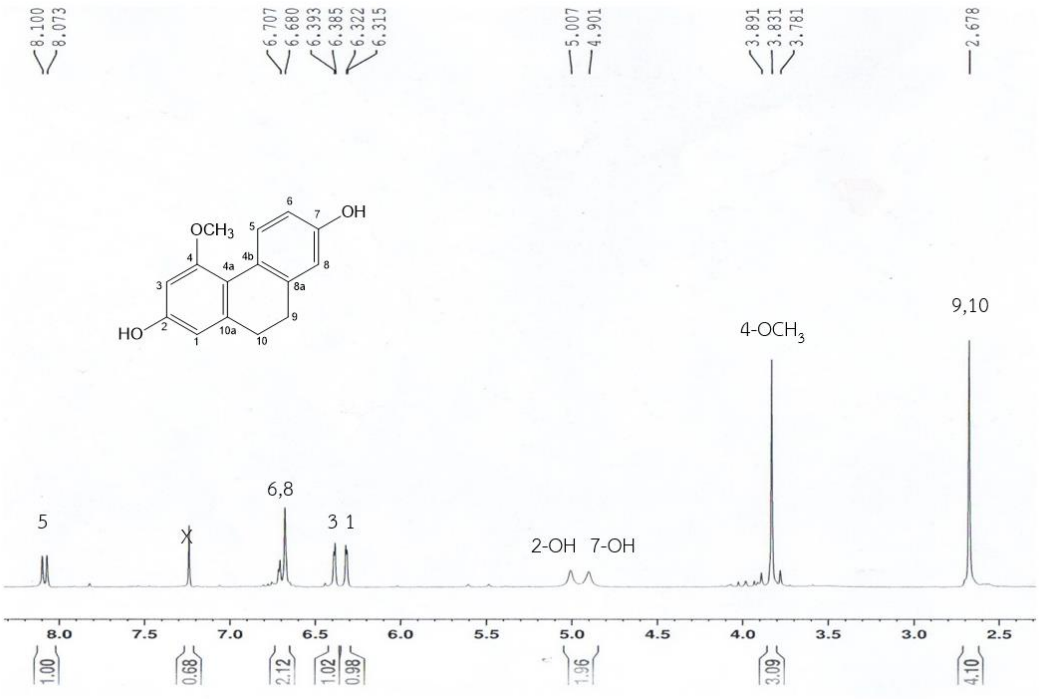


Figure 54. ^{1}H NMR spectrum of compound CF-7 (300 MHz, in $CDCl_3$)

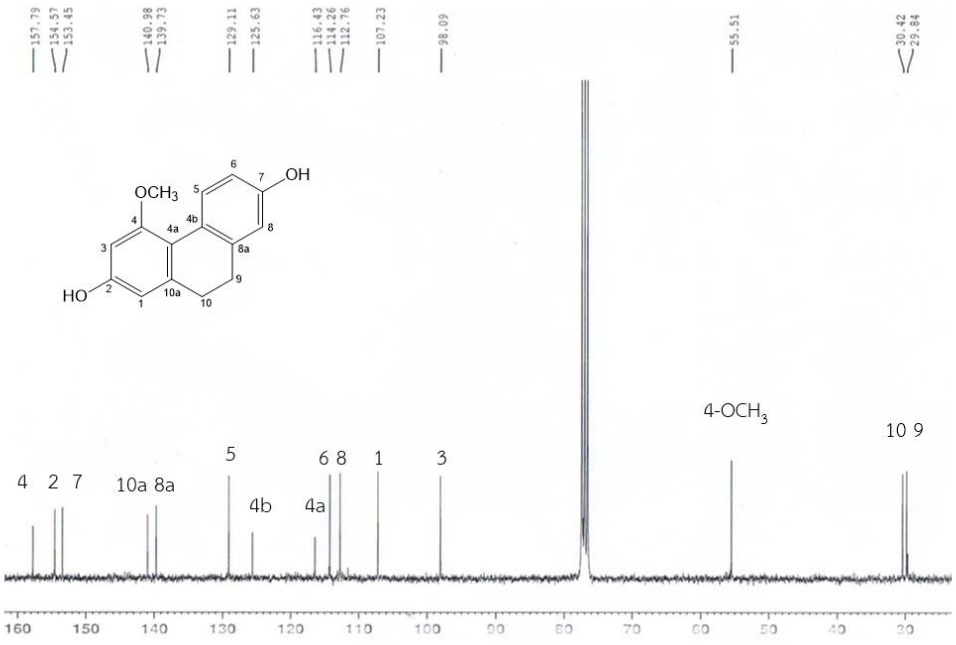


Figure 55. ^{13}C NMR spectrum of compound CF-7 (75 MHz, in $CDCl_3$)

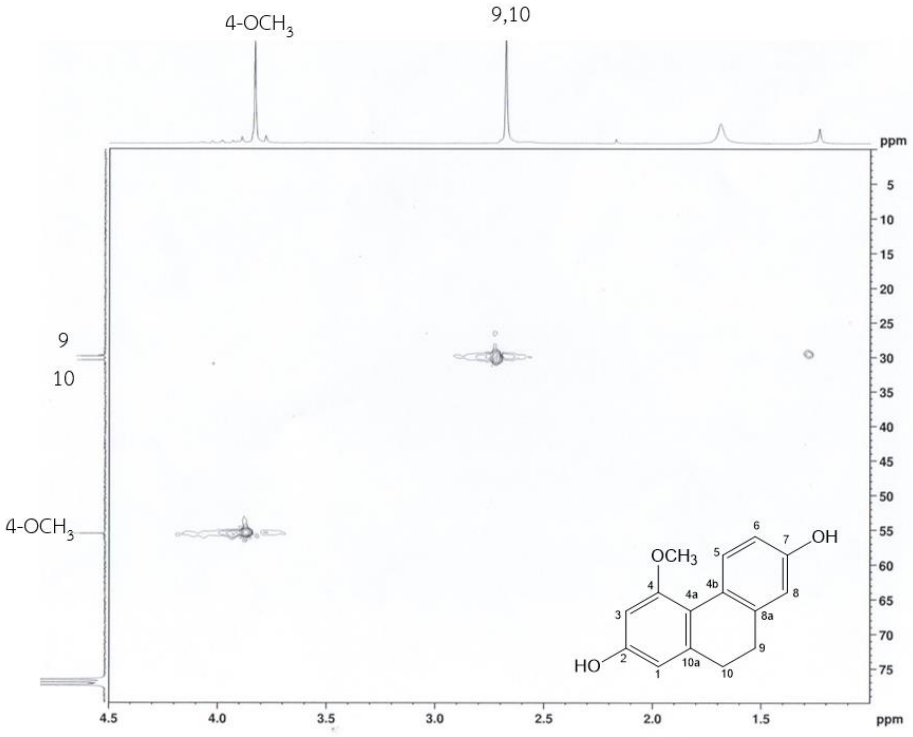


Figure 56. HSQC spectrum of compound CF-7 (expansion between δ_H 0.0-4.5, δ_C 0-80)

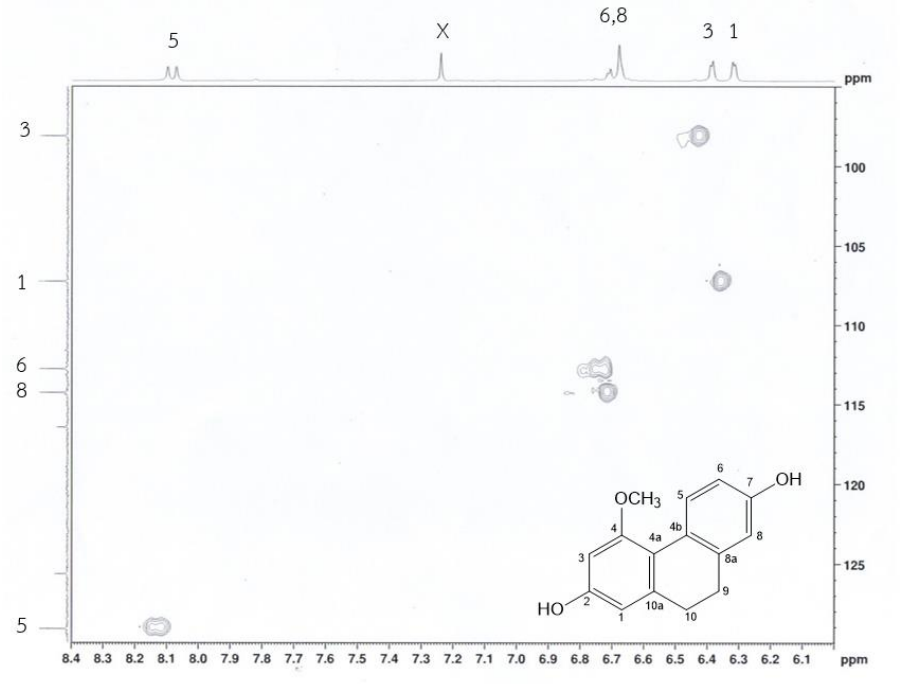


Figure 57. HSQC spectrum of compound CF-7 (expansion between δ_H 6.0-8.4, δ_C 90-130)

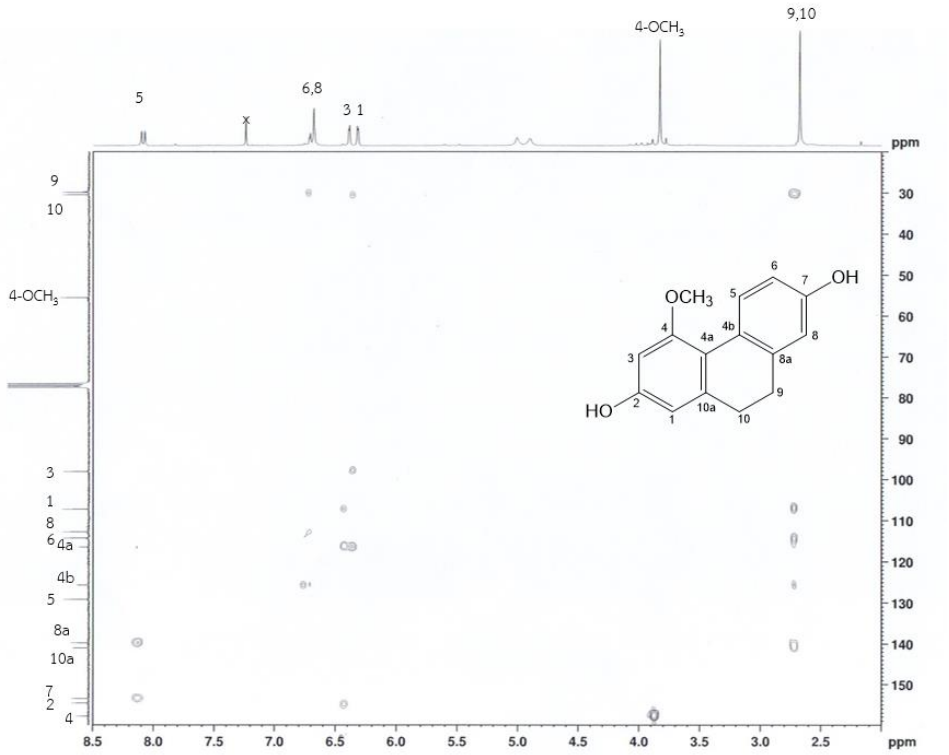


Figure 58. HMBC spectrum of compound CF-7

8. Identification of compound CF-8 (lusianthridin)

Compound CF-8 was isolated as a yellow amorphous solid (1 mg, 0.0001% yield) which produced a yellow spot on TLC upon spraying with 10% sulfuric acid and heating. Its molecular formula was determined as $C_{15}H_{14}O_3$ from its quasi-molecular $[M + Na]^+$ ion peak at *m/z* 265.0843 (**Figure 59**). This compound was thus an isomer of compound CF-7.

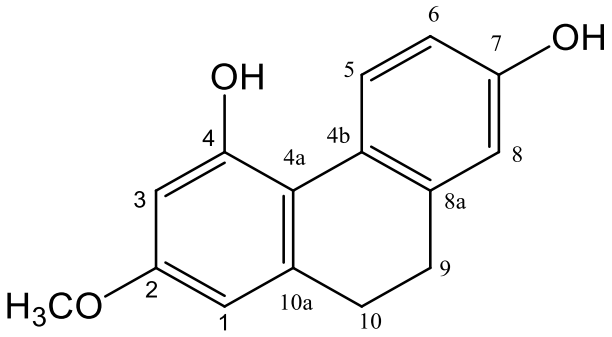
The ^1H NMR spectrum of compound CF-8 (**Figure 60** and **Table 22**), exhibited a four-proton methylene singlet signifying its dihydrophenanthrene nature at δ 2.71 ppm (4H, *s*, H_2-9 and H_2-10), a pair of *meta*-coupling doublets at δ 6.33 (1H, *d*, *J* = 2.4 Hz, H-1) and 6.40 ppm (1H, *d*, *J* = 2.4 Hz, H-3), a set of resonances at δ 6.70 (1H, *dd*, *J* = 9.0, 2.0 Hz, H-6), 6.74 (1H, *d*, *J* = 2.0 Hz, H-8) and 7.92 ppm (1H, *d*, *J* = 9.0 Hz, H-5),

suggesting an ABX system of a 1,2,4-trisubstituted benzene ring, and a methoxy singlet at δ 3.78 ppm (3H, *s*, 2-OCH_3).

Its ^{13}C NMR spectrum (**Figure 61** and **Table 22**) exhibited fifteen signals which could be differentiated by the HSQC experiment (**Figure 62**) into those of one methoxy carbon at δ 55.3 ppm (2-OCH_3), two aliphatic methylene carbons at δ 29.9 (C-9) and 30.5 ppm (C-10), five aromatic methide carbons at δ 100.9 (C-3), 106.5 (C-1), 113.2 (C-6), 115.1 (C-8) and 127.2 ppm (C-5) and seven aromatic quaternary carbons at δ 114.7 (C-4a), 125.4 (C-4b), 140.2 (C-8a), 141.1 (C-10a), 153.3 (C-4), 153.7 (C-7) and 158.7 ppm (C-2).

These ^{1}H and ^{13}C NMR spectra were very similar to those of compound CF-7, especially the assignments on one side of the molecule with a lone hydroxy substitution at position 7. Therefore, the difference from the previous compound should be the switching of positions between hydroxy and methoxy groups on the other aromatic ring. Finally, comparison of these NMR data with literatures (196, 206, 207) confirmed that compound CF-8 was 4,6-dihydroxy-2-methoxy-9,10-dihydrophenanthrene or the known stilbenoid lusianthridin.

Lusianthridin was firstly described (under the name 4,6-dihydroxy-2-methoxy-9,10-dihydrophenanthrene) as a constituent of the tuber of *Bletilla striata*, a medicinal orchid used in traditional Chinese medicine (206). However, seven years later it was described as a new compound, called lusianthridin, isolated from another orchid, *Luisia indivisa* (196). Since then, it has been isolated from several other orchids including *Dendrobium brymerianum* (207), *Eulophia petersii* (85) and *Pholidota chinensis* (93, 196, 206). It could inhibit lung cancer stem cells by suppression of Src-STAT3 signaling pathway involved in multidrug resistance of cancer cell (208).



Lusianthridin

Table 22. NMR spectral data of compound CF-8 (in $CDCl_3$) and lusianthridin (in acetone-d_6)

Position	Compound CF-8		Lusianthridin[207]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	6.33 (*d*, 2.4)	106.5	6.36 (*d*, 1.5)	105.8
2	-	157.8	-	159.2
3	6.40 (*d*, 2.4)	100.9	6.42 (*d*, 1.5)	101.5
4	-	153.5	-	155.8
4a	-	114.7	-	115.7
4b	-	125.5	-	125.8
5	7.92 (*d* ,9.0)	127.2	8.22 (*d*, 9.0)	129.8
6	6.70 (*dd*, 9.0, 2.0)	115.1	6.67 (*dd*, 9.0, 2.5)	113.4
7	-	154.6	-	155.9
8	6.74 (*d*, 2.0)	113.2	6.70 (*d*, 2.5)	114.9
8a	-	140.2	-	139.7
9	2.71 (*s*)	29.9	n.s.	30.6
10	2.71 (*s*)	30.5	n.s.	31.4
10a	-	141.1	-	141.3
2-OCH_3	3.78 (*s*)	55.3	3.72 (*s*)	55.2

n.s. = not stated

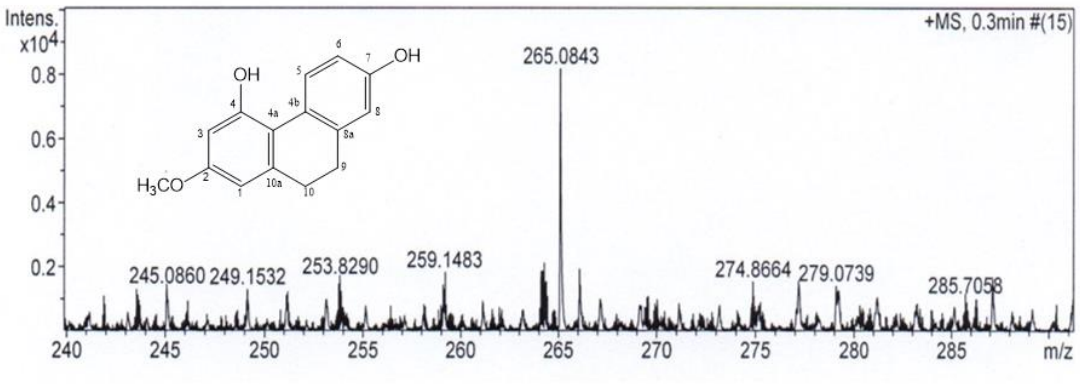


Figure 59. HR-ESI mass spectrum of compound CF-8

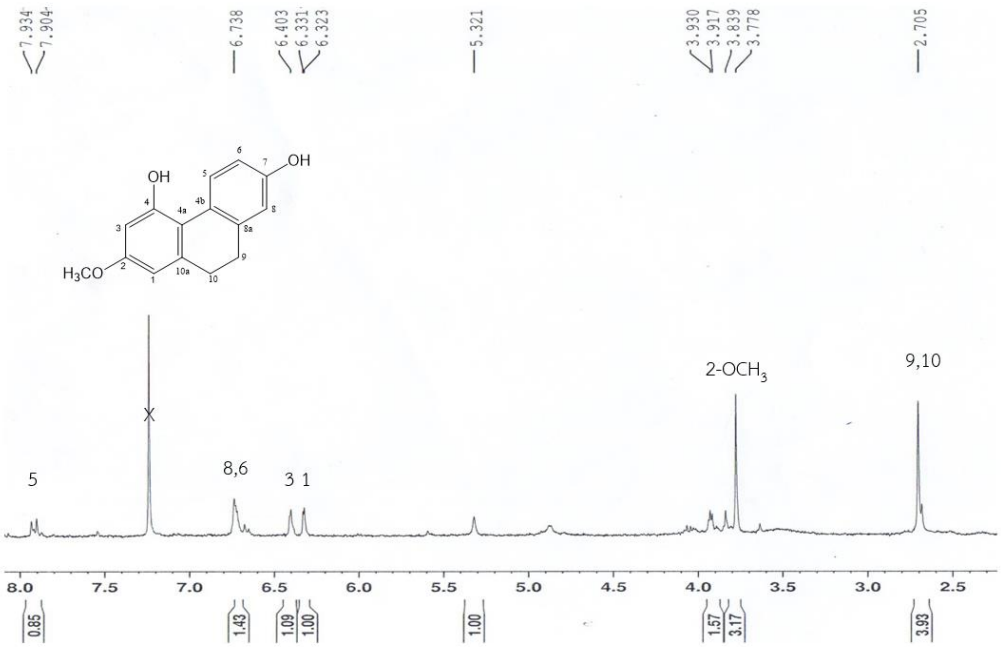


Figure 60. ^{1}H NMR (MHz) spectrum of compound CF-8

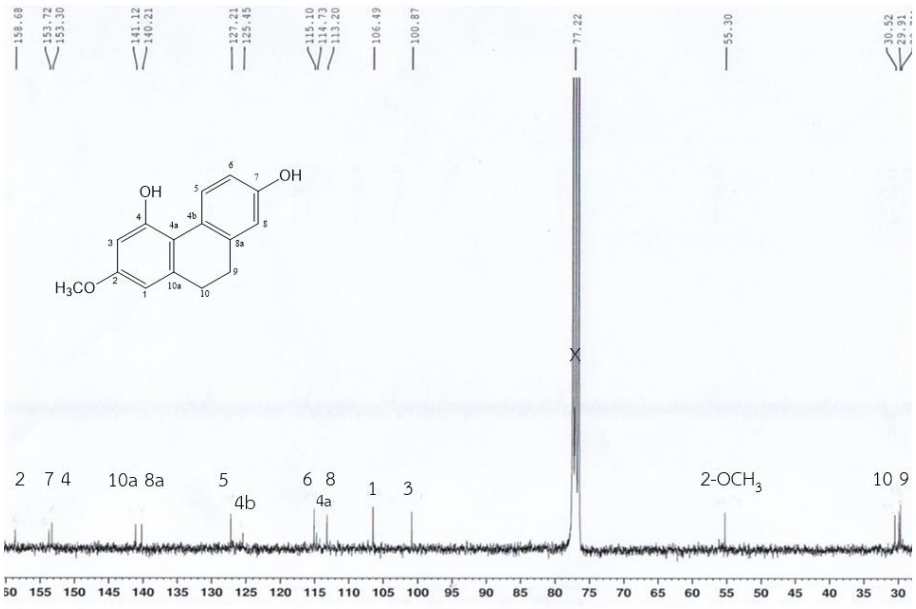


Figure 61. ^{13}C NMR (MHz) spectrum of compound CF-8

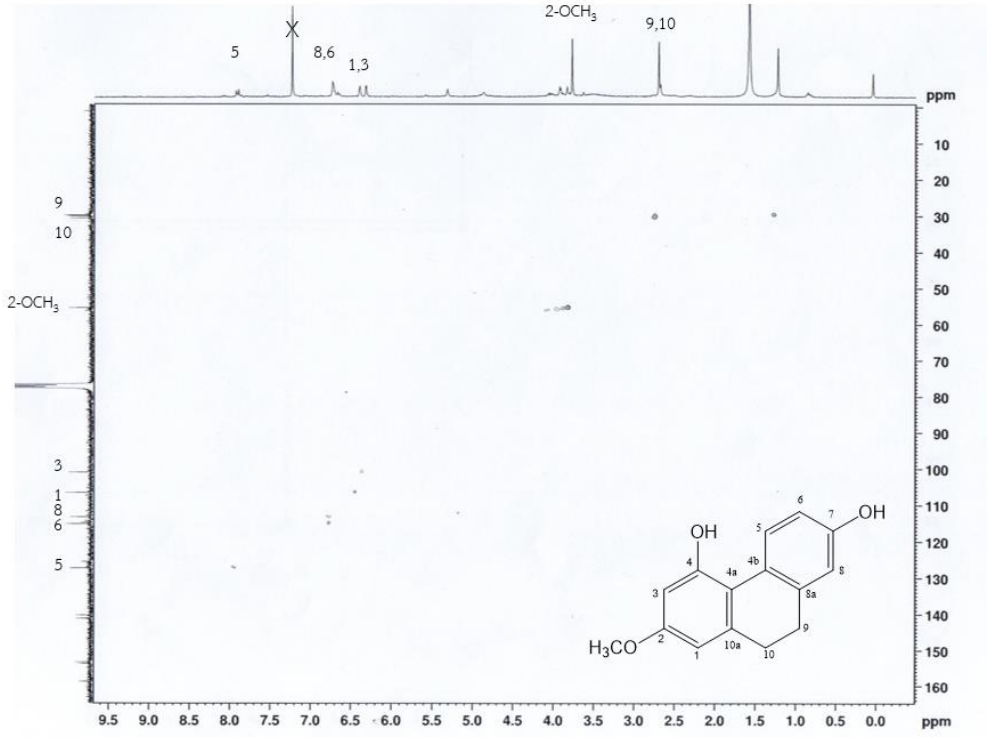


Figure 62. HSQC spectrum of compound CF-8

9. Identification of compound CF-9 (batatasin III)

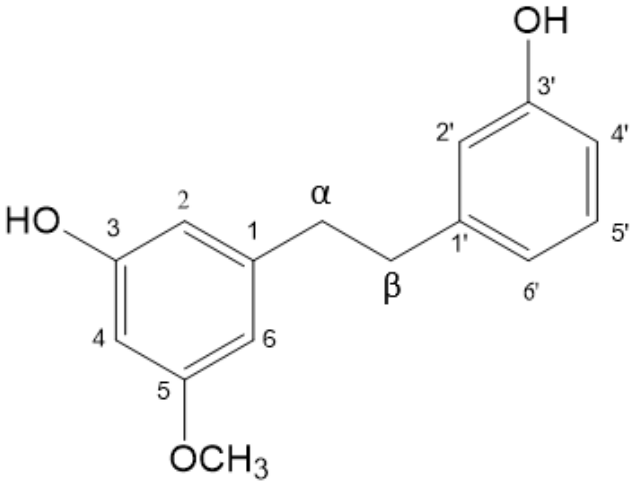
Compound CF-9 was isolated as a brown amorphous solid (4 mg, 0.0004% yield) which gave a purple spot on TLC upon spraying with 10% sulfuric acid and heating. According to its $[M + Na]^+$ ion peak at *m/z* 267.0995 in the mass spectrum (**Figure 63**), its molecular formula could be determined as $C_{15}H_{16}O_3$ (calcd. 267.0997). Eight degrees of unsaturation, calculated from its molecular formula, was suggestive of a bibenzyl (dihydrostilbene) skeleton.

The ^{1}H NMR spectrum of compound CF-9 (**Figure 64** and **Table 23**) showed a methoxy singlet at δ 3.73 ppm (3H, *s*, 5-OCH_3), a multiplet at δ 2.80 ppm integrated for four methylene protons (H_2-α and H_2-β) of a bibenzyl. Other seven aromatic proton signals were those of one tri-substituted and one di-substituted benzene ring. Protons of the first ring, which resonated at δ 6.24 (2H, *br s*, H-2 and H-4) and 6.30 ppm (1H, *br s*, H-6), represented a 1,3,5-trisubstituted aromatic ring, whereas those at δ 6.45 (1H, *br d*, *J* = 8.0 Hz, H-4'), 6.63 (1H, *br s*, H-2'), 6.74 (1H, br d, J = 8.0 Hz, H-6') and 7.13 ppm (1H, *t*, *J* = 8.0 Hz, H-5') represented a 1,3-disubstituted benzene ring. Based on its molecular formula and NMR data, this compound should have one methoxy and two hydroxy substituents.

The ^{13}C NMR (**Figure 65** and **Table 23**) and HSQC spectra (**Figure 66**) of compound CF-9 displayed resonances of one methoxy carbon at δ 55.3 ppm (5-OCH_3), two methylene carbons at δ 37.7 (C-α) and 37.3 ppm (C-β), and twelve aromatic carbons of two benzene rings including seven methine carbons at δ 99.1 (C-4), 106.8 (C-6), 107.9 (C-2), 112.9 (C-4'), 115.4 (C-2'), 120.9 (C-6') and 129.5 (C-5') ppm and five quaternary carbons at δ 143.6 (C-1'), 144.4 (C-1), 155.5 (C-3'), 129.5 (C-5'), 156.5 (C-3) and 160.8 ppm (C-5).

Its HMBC spectrum (**Figure 67** and **Table 24**) displayed a cross-peak between methoxy protons (δ 3.73 ppm) and the most downfield oxygenated quaternary carbon at δ 160.8 ppm. A three-bond HMBC correlation was observed between H-5' at δ 7.13 ppm and the oxygen-substituted quaternary carbon at δ 155.5 ppm (C-5'), indicating that the substituent on this 1,3-disubstituted benzene ring should be a hydroxy group and, therefore, the other hydroxy group and a methoxy group should be located at positions 3 and 5, respectively, of the second benzene ring. These assignments were further confirmed by comparison with previously reported values (44, 118). Compound CF-9 was thus identified as the known compound 3,3'-dihydroxy-5-methoxybibenzyl or batatasin III.

Batatasin III has been found in another *Cymbidium* orchid, namely, *Cymbidium aloifolium* (44). The bibenzyl was later obtained from several orchid species such as *Bulbophyllum odoratissimum* (118), *Gymnadenia conopsea* (65), *Dendrobium nobile* (68) and *Malaxis acuminata* (56), as well as from some monocotyledonous plants e.g. Chinese yam (*Dioscorea opposita*, family Dioscoreaceae) (67) and dicotyledonous plants e.g. black crowberry (*Empetrum hermaphroditum*, family Ericaceae) (62). Batatasin III exhibited many biological activities including antiallergic (65), α-glucosidase inhibitory (67), antifungal (68) and cytotoxic activities (56).



Batatasin III

Table 23. NMR spectral data of compound CF-9 and batatasin III (in $CDCl_3$)

Position	Compound CF-9		Batatasin III[118]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	-	144.4	-	144.4
2	6.24 (*br s*)	107.9	6.27 (*dd*, 1.4, 1.4)	108.2
3	-	156.5	-	156.4
4	6.24 (*br s*)	99.1	6.29 (*dd*, 1.4, 1.4)	99.3
5	-	160.8	-	160.7
6	6.30 (*br s*)	106.8	6.34 (*dd*, 1.4, 1.4)	106.9
α	2.80 (*m*)	37.7	2.80 (*m*)	37.3
β	2.80 (*m*)	37.3	2.81 (*m*)	36.9
1'	-	143.6	-	143.4
2'	6.63 (*br s*)	115.4	6.64 (*dd*, 2.4, 2.4)	115.4
3'	-	155.5	-	155.4
4'	6.45 (*br d*, 8.0)	112.9	6.67 (*dd*, 8.0, 2.4)	112.9
5'	7.13 (*t*, 8.0)	129.5	7.12 (*dd*, 8.0, 8.0)	129.3
6'	6.74 (*br d*, 8.0)	120.9	6.74 (*d*, 8.0)	120.8
5-OCH_3	3.73 (*s*)	55.3	3.73 (*s*)	55.2

Table 24. HMBC spectral data of compound CF-9 (in $CDCl_3$)

^{1}H signal	HMBC correlation with
H-2	C-α, C-3, C-6
H-4	C-3, C-6
H-6	C-2
H-α	C-β
H-β	C-α, C-2', C-6'
H-3'	C-1', C-5'
H-5'	C-1', C-3'
H-6'	C-2', C-4'
5-OCH_3	C-5

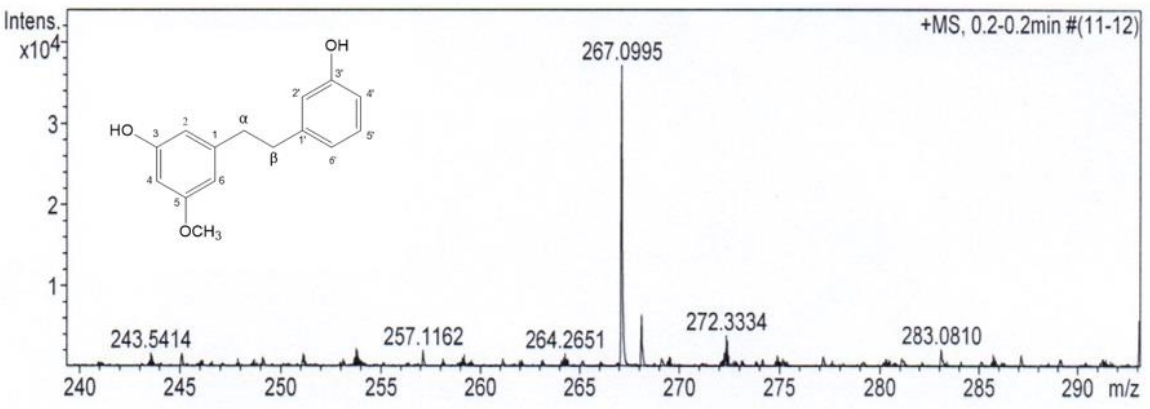


Figure 63. HR-ESI mass spectrum of compound CF-9

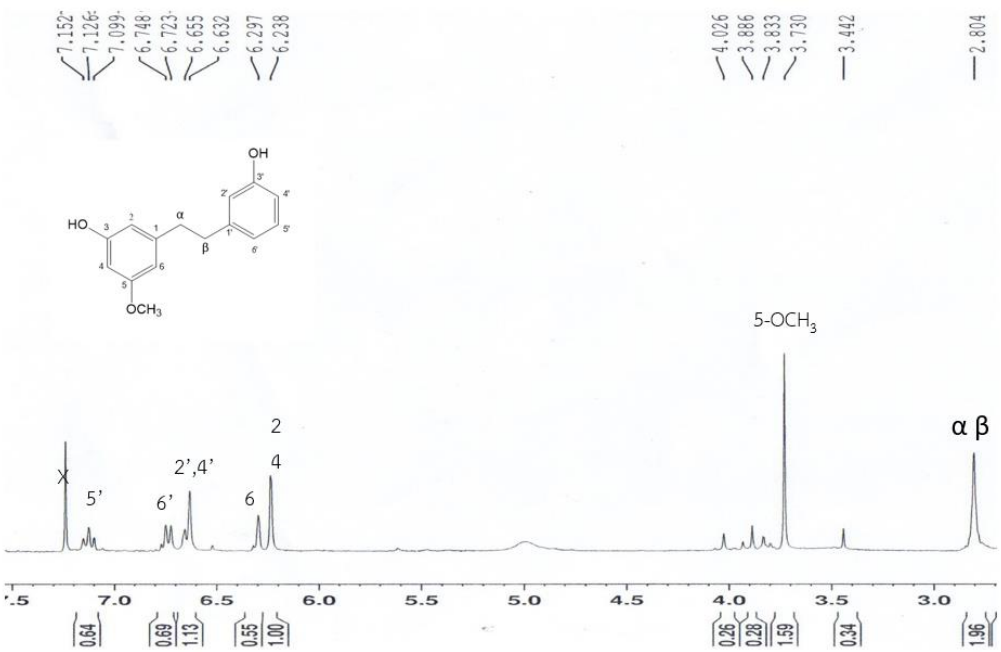


Figure 64. ^{1}H NMR spectrum of compound CF-9 (300 MHz, in $CDCl_3$)

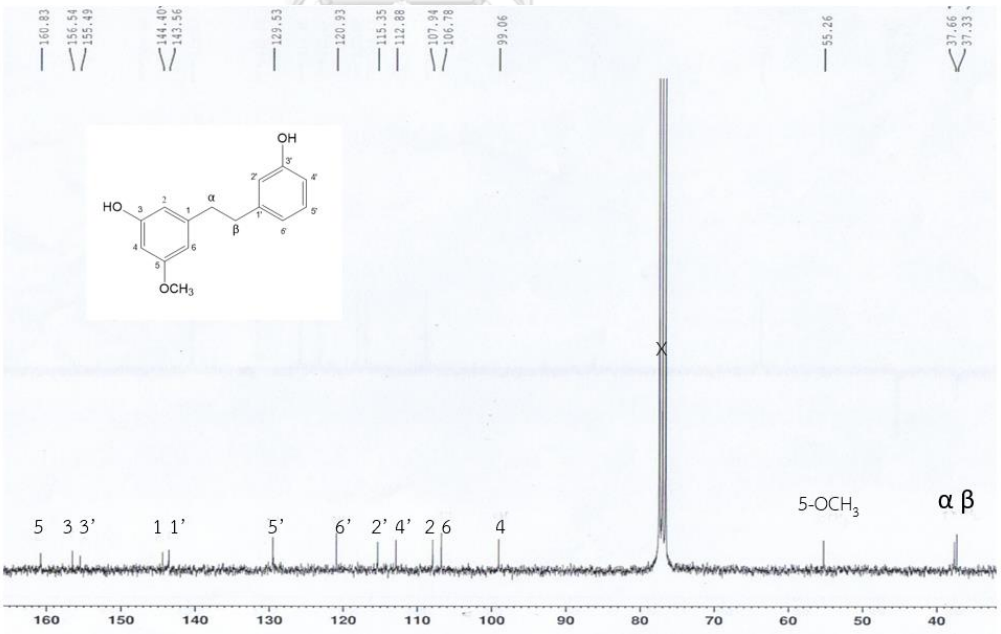


Figure 65. ^{13}C NMR spectrum of compound CF-9 (75 MHz, in $CDCl_3$)

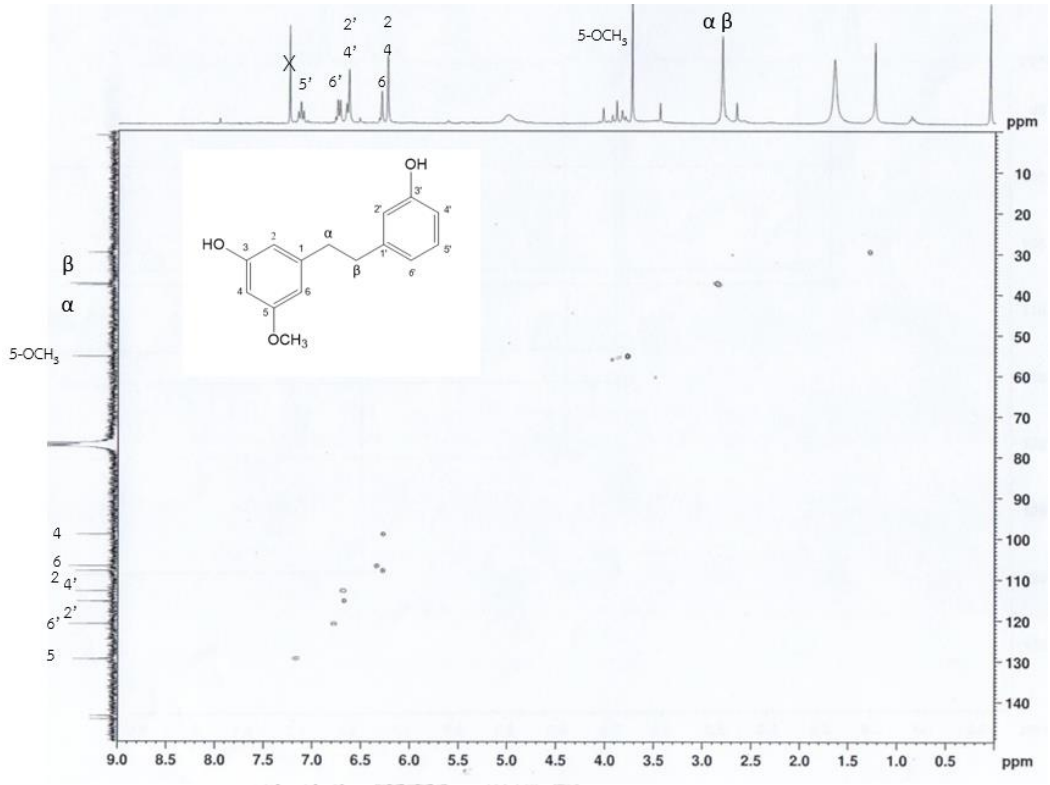


Figure 66. HSQC spectrum of compound CF-9

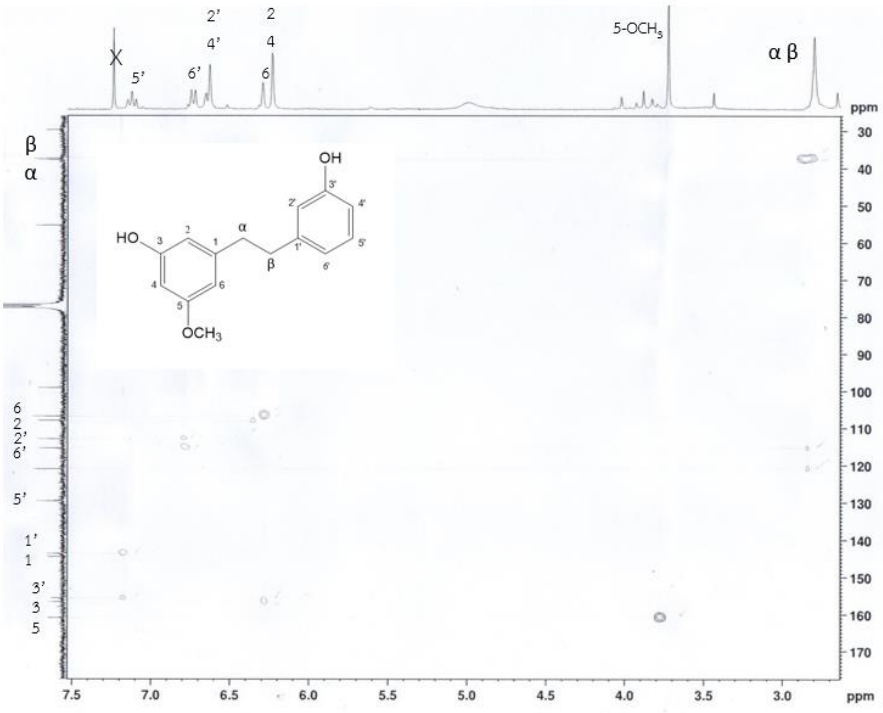


Figure 67. HMBC spectrum of compound CF-9

10. Structure elucidation of compound CF-10 [1-(4-hydroxybenzyl)-4,6-dimethoxy-9,10-dihydrophenanthrene-2,7-diol]

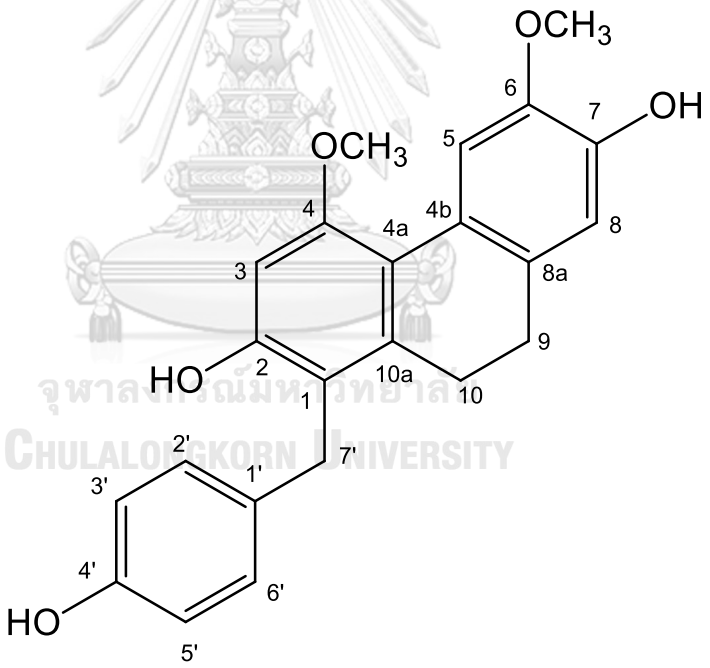
Compound CF-10 was isolated as a light brown amorphous powder (7 mg, 0.0007% yield) which gave a brown spot on TLC upon spraying with 10% sulfuric acid and heating. Its high-resolution ESI mass spectrum (**Figure 69**) showed $[M + Na]^+$ ion peak at *m/z* 401.1368 (calcd. 401.1365), suggesting its molecular formula as $C_{23}H_{22}O_5$. Its IR spectrum (**Figure 70**) displayed an absorption band of hydroxyl group at 3435 cm^{-1}.

The ^1H NMR spectrum of compound CF-10 (**Figure 71** and **Table 25**) exhibited three isolated aromatic singlets at δ 6.44 (1H, *s*, H-3), 6.74 (1H, *s*, H-8) and 7.81 ppm (1H, *s*, H-5), two characteristic methylene multiplets of a 9,10-dihydrophenanthrene nucleus at δ 2.57 (2H, *m*, H_2-9) and 2.63 ppm (2H, *m*, H_2-10), two methoxy singlets at δ 3.84 (3H, *s*, 4-OCH_3) and 3.89 ppm (3H, *s*, 6-OCH_3) and three hydroxy broad singlets at δ 4.80, 4.85 (2-OH and 4'-OH, interchangeable assignments) and 5.57 ppm (7-OH). The remaining proton signals at δ 3.97 (2H, *s*, H_2-7'), 6.70 (2H, *d*, *J* = 8.4 Hz, H-3' and H-5') and 6.99 ppm (2H, *d*, *J* = 8.4 Hz, H-2' and H-6') represented a *para*-hydroxybenzyl moiety (1).

Detailed analysis of its ^{13}C NMR (**Figure 72** and **Table 25**) and HSQC spectra (**Figure 73**) indicated the presence of two methoxy carbons at δ 55.7 (4-OCH_3) and 56.1 ppm (6-OCH_3), three methylene carbons at δ 26.6 (C-10), 28.8 (C-9) and 30.5 ppm (C-7'), seven aromatic methide carbons at δ 98.6 (C-3), 111.5 (C-5), 113.1 (C-8), 115.4 (C-3'/C-5') and 129.0 ppm (C-2'/C-6') and eleven aromatic quaternary carbons at δ 116.3 (C-1), 117.5 (C-4a), 124.9 (C-8a), 131.2 (C-4b), 132.0 (C-1'), 140.3 (C-10a), 143.6 (C-7), 144.3 (C-6), 153.1 (C-2), 153.9 (C-4') and 155.8 ppm (C-4). These NMR data are similar to those of compound CF-3 (6-methoxycoelonin), except for the presence of additional

resonances due to the *p*-hydroxybenzyl unit and the absence of H-1 signal while H-3 appeared as an uncoupled singlet.

Attachment of the *p*-hydroxybenzyl moiety to C-1 was supported by the HMBC correlations (**Figure 74** and **Table 25**) observed from H_2-7' (at δ 3.97 ppm) to C-1, C-2 and C-10a. HMBC cross-peaks from 4-OCH_3 protons at δ 3.84 ppm to C-4 and from 6-OCH_3 protons at δ 3.89 ppm to C-6 confirmed these methoxy positions to be the same as those in compound CF-3. Therefore, compound CF-10 was determined as a new dihydrophenanthrene,1-(4-hydroxybenzyl)-4,6-dimethoxy-9,10-dihydro-phenanthrene-2,7-diol. A similar compound, without substitution at position 6, has been isolated from another orchid, *Bletilla formosana* (209).



1-(4-Hydroxybenzyl)-4,6-dimethoxy-9,10-dihydrophenanthrene-2,7-diol

Table 25. NMR spectral data of compound CF-10 (in $CDCl_3$)

Position	δ_H (mult., *J* in Hz)	δ_C	HMBC correlation with
1	-	116.3	-
2	-	153.1	-
3	6.44 (*s*)	98.6	C-1, C-2, C-4, C-4a
4	-	155.8	-
4a	-	117.5	-
4b	-	131.2	-
5	7.81 (*s*)	111.5	C-4a, C-4b, C-7
6	-	144.3	-
7	-	143.6	-
8	6.74 (*s*)	113.1	C-6, C-8a, C-9
8a	-	124.9	-
9	2.57 (*m*)	28.8	C-10, C-10a
10	2.63 (*m*)	26.6	C-9
10a	-	140.3	-
1'	-	132.0	-
2'	6.99 (*d*, 8.4)	129.0	C-4', C-6', C-7'
3'	6.70 (*d*, 8.4)	115.4	C-1'
4'	-	153.9	-
5'	6.70 (*d*, 8.4)	115.4	C-1'
6'	6.99 (*d*, 8.4)	129.0	C-2', C-4', C-7'
7'	3.97 (*s*)	30.5	C-1, C-2, C-10a, C-1', C-2', C-6'

Position	δ_H (mult., *J* in Hz)	δ_C	HMBC correlation with
4-OCH_3	3.84 (*s*)	55.7	C-4
6-OCH_3	3.89 (*s*)	56.1	C-6
2-OH	4.85[a] (*br s*)	-	-
7-OH	5.57 (*br s*)	-	-
4'-OH	4.80[a] (*br s*)	-	-

[a] interchangeable

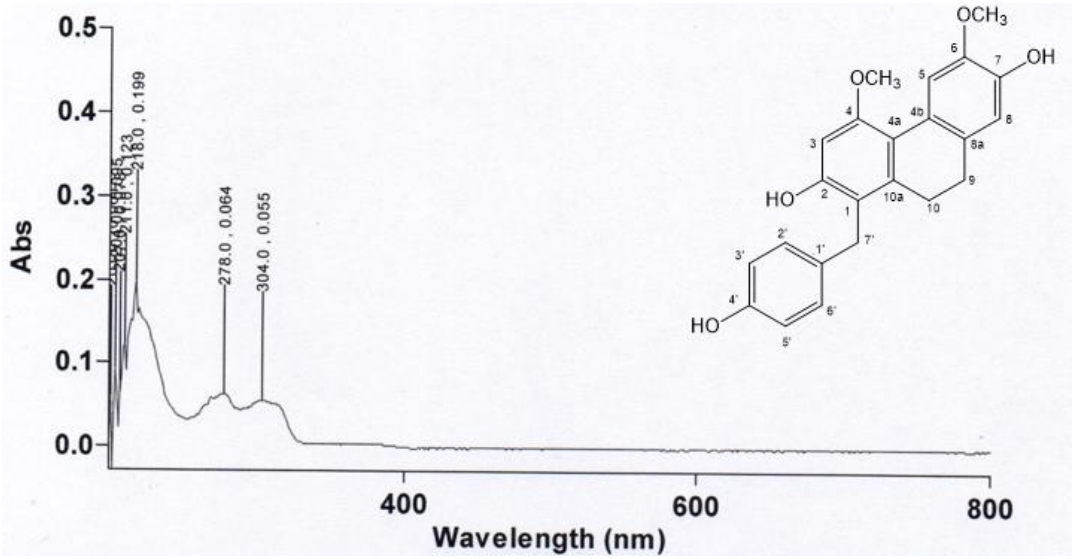


Figure 68. UV spectrum of compound CF-10

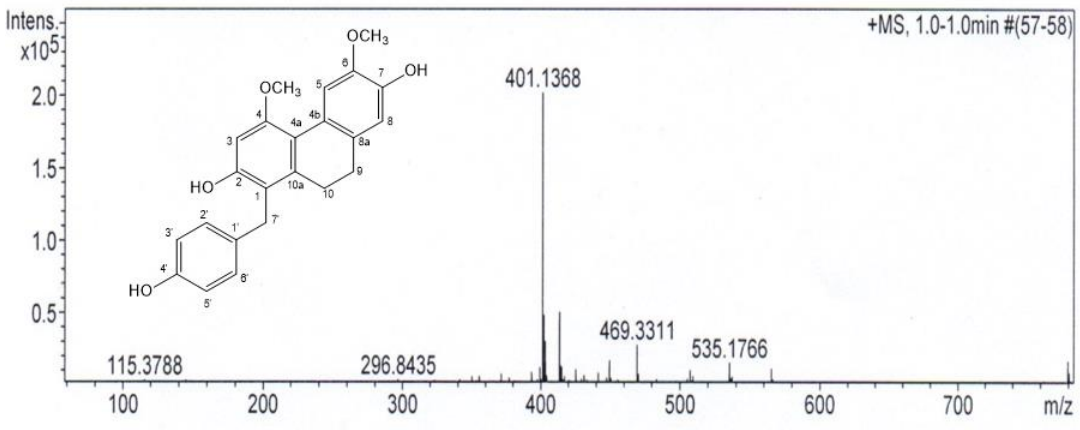


Figure 69. HR-ESI mass spectrum of compound CF-10

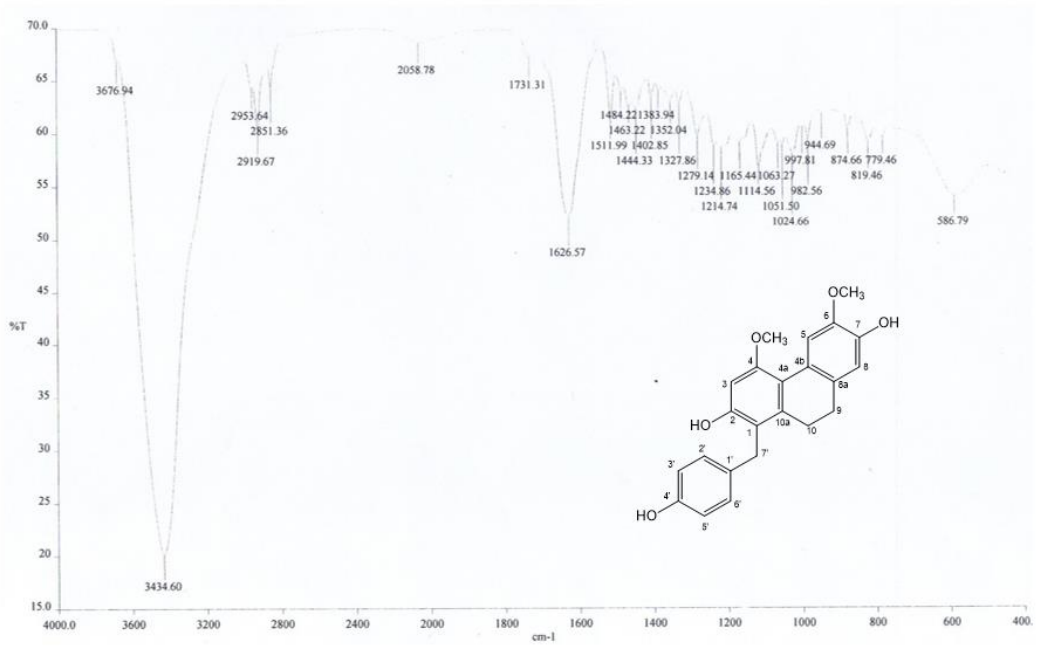


Figure 70. IR spectrum of compound CF-10

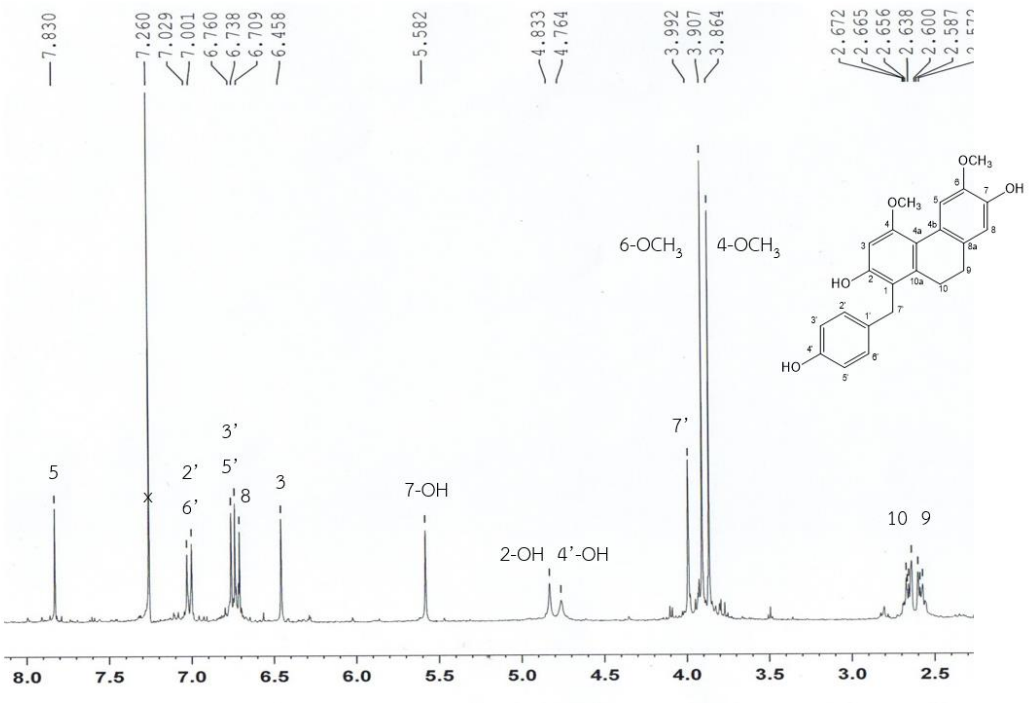


Figure 71. ^{1}H NMR spectrum of compound CF-10 (300 MHz, in $CDCl_3$)

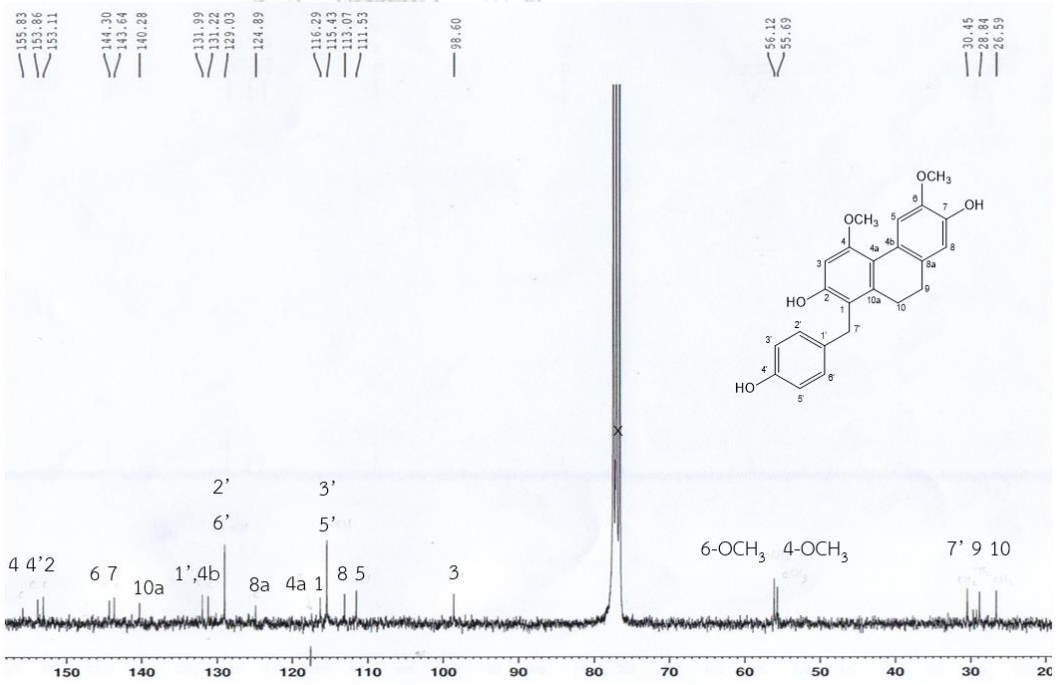


Figure 72. ^{13}C NMR spectrum of compound CF-10 (75 MHz, in $CDCl_3$)

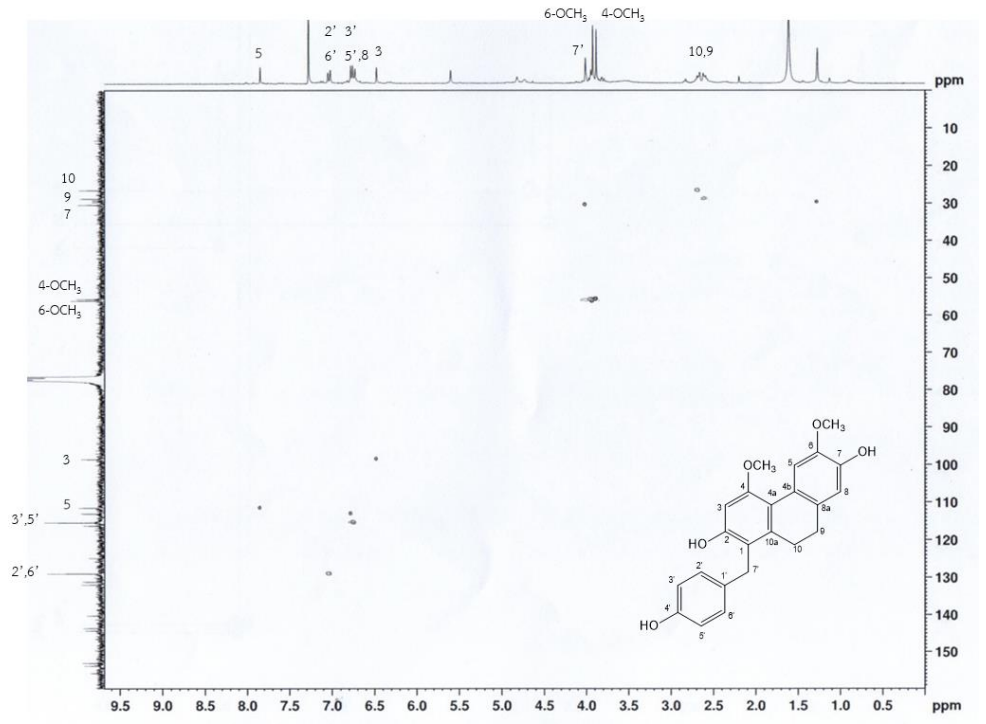


Figure 73. HSQC spectrum of compound CF-10

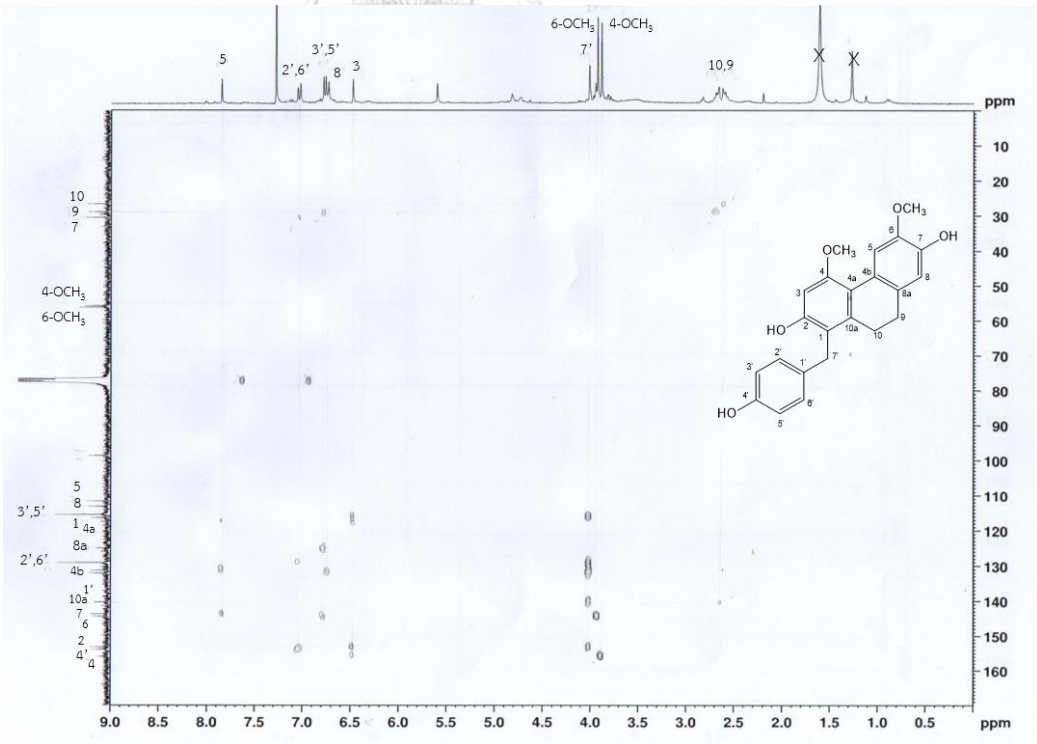


Figure 74. HMBC spectrum of compound CF-10

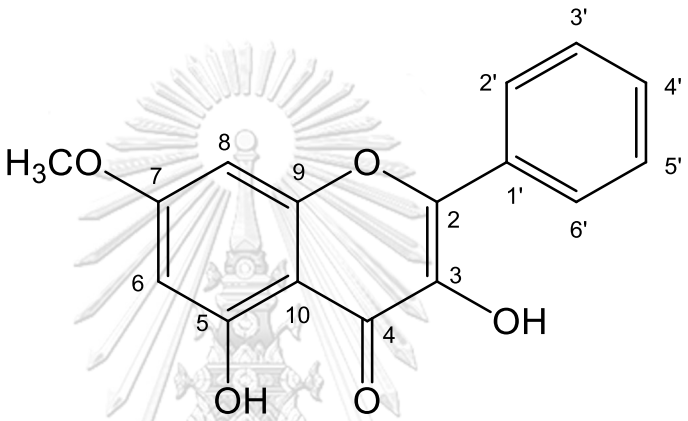
11. Identification of compound PD-1 (isalpinin)

Compound PD-1, obtained as yellow needles (48 mg, 0.012% yield from the dried roots and 7 mg, 0.0012% yield from the dried leaves of *Paphiopedilum dianthum*), appeared as a yellow spot on TLC upon spraying with 10% sulfuric acid and heating, suggesting this compound could be a flavonoid. Its molecular formula of $C_{16}H_{12}O_5$ was analyzed from the $[M + Na]^+$ ion peak at 307.0579 *m/z* (calcd. 307.0582) in its high resolution ESI-TOF mass spectrum (**Figure 75**).

The ^{1}H NMR spectrum of compound PD-1 (**Figure 76** and **Table 26**) showed a methoxy signal at δ 3.90 ppm (3H, *s*, 7-OCH_3) and two broad hydroxy singlets at δ 6.66 (1H, *br s*, 3-OH) and δ 11.67 ppm (1H, *s*, 5-OH). The downfield shift of the latter signal indicated the position of one hydroxy group at position 5 of flavonoid nucleus. A pair of doublets at δ 6.39 (1H, *d*, *J* = 2.1 Hz) and 6.52 ppm (1H, *d*, *J* = 2.1 Hz) represented the *meta*-coupled aromatic protons of positions 6 and 8, respectively, on ring A of a flavonoid. Three aromatic proton resonances, integrated for five protons, at δ 7.51 (2H, *m*, H-3'/H-5'), 7.53 (1H, *m*, H-4') and 8.20 ppm (2H, *dd*, *J* = 8.4, 1.5 Hz, H-2'/H-6') were characteristic of the unsubstituted ring B of flavonoids. Absence of other proton signals, especially those of positions 2 and 3 was suggestive of a flavonol structure. Therefore, compound PD-1 should be a flavonol with one hydroxy substitution at C-5 and one methoxy substitution at C-7.

The ^{13}C spectrum of compound PD-1 (**Figure 77** and **Table 26**) displayed sixteen carbon resonances including those of a methoxy carbon at δ 55.9 ppm (7-OCH_3), a carbonyl carbon at δ 175.5 ppm (C-4) and an unsubstituted ring B at δ 127.6 (C-2'/C-6'), 128.6 (C-3'/C-5'), 130.3 (C-4') and 130.7 ppm (C-1'). Two quaternary carbon signals at δ 145.1 (C-2), 136.6 ppm (C-3) and the carbonyl C-4 peak at δ 175.5 ppm were supportive of its flavonol nature (4). Comparison of these NMR data with

previously reported values (210) led to the identification of compound PD-1 as isalpinin (5-hydroxy-7-methoxyflavonol). This flavonoid has been found in propolis (royal jelly) from several sources (210-212). In addition, it could be extracted from plants such as *Polygonum hydropiper* (Family Polygonaceae) (213), *Chromolaena leivensis* and *C. tacotana* (family Asteraceae) (214, 215).



Isalpinin

Table 26. NMR spectral data of compound PD-1 and isalpinin (in $CDCl_3$)

Position	Compound PD-1		Isalpinin[210]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
2	-	145.1	-	145.0
3	-	136.6	-	136.0
4	-	175.5	-	176.1
5	-	160.9	-	160.8
6	6.39 (*d*, 2.1)	98.0	6.35 (*d*, 2.0)	97.8
7	-	166.0	-	164.3
8	6.52 (*d*, 2.1)	92.3	6.45 (*d*, 2.0)	92.0
9	-	157.0	-	156.5
10	-	104.2	-	103.2
1'	-	130.7	-	130.9
2'	8.20 (*dd*, 8.4, 1.5)	127.6	8.15 (*dd*, 8.0, 1.5)	126.9
3'	7.51 (*m*)	128.6	7.50 (*m*)	128.0
4'	7.53 (*m*)	130.3	7.52 (*m*)	130.0
5'	7.51 (*m*)	128.6	7.50 (*m*)	128.0
6'	8.20 (*dd*, 8.4, 1.5)	127.6	8.15 (*dd*, 8.0, 1.5)	126.9
7-OCH_3	3.90 (*s*)	55.9	3.75 (*s*)	55.2
3-OH	6.66 (*br s*)	-	-	-
5-OH	11.67 (*s*)	-	11.61 (*s*)	-

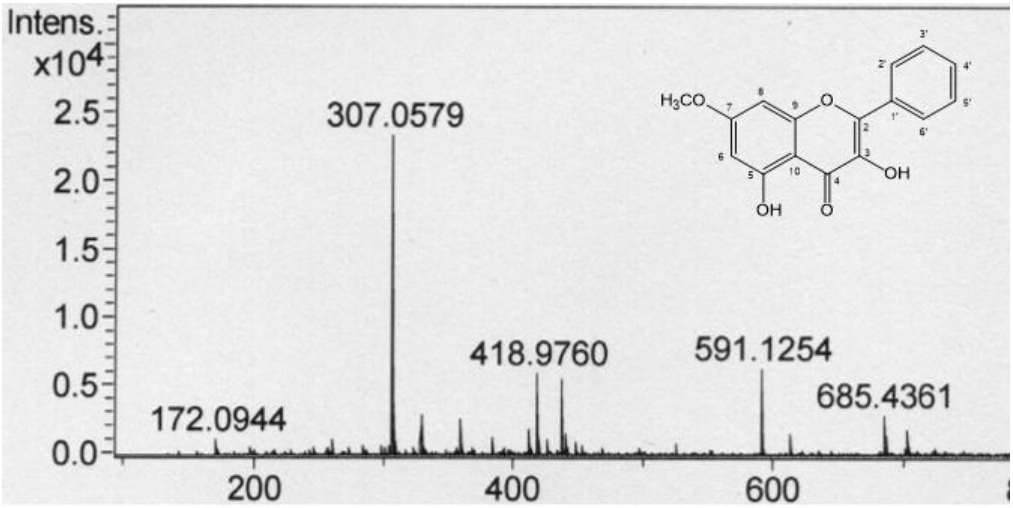


Figure 75. HR-ESI mass spectrum of compound PD-1

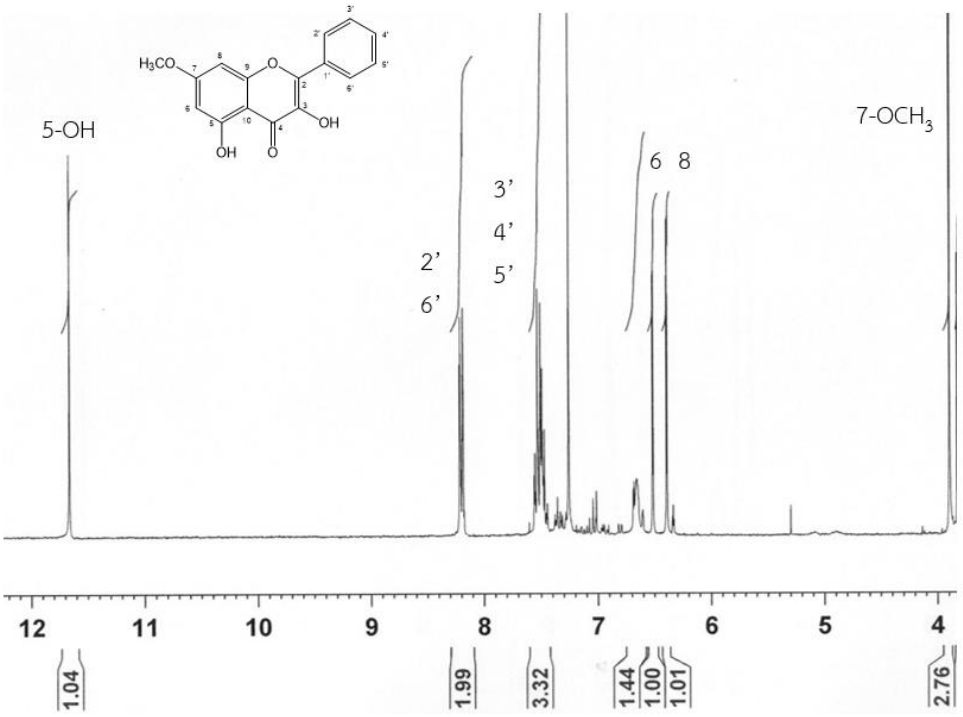


Figure 76. ^{1}H NMR spectrum of compound PD-1 (300 MHz, in $CDCl_3$)

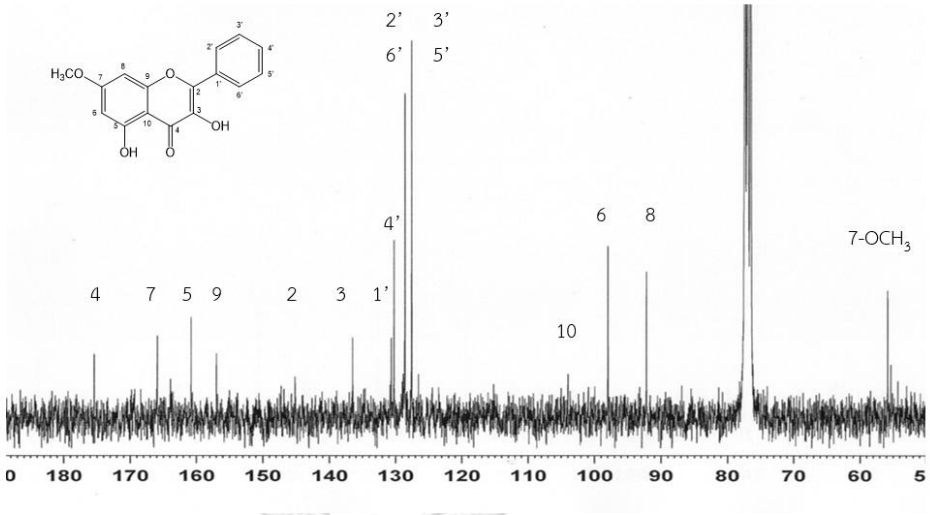


Figure 77. ^{13}C NMR spectrum of compound PD-1 (75 MHz, in $CDCl_3$)

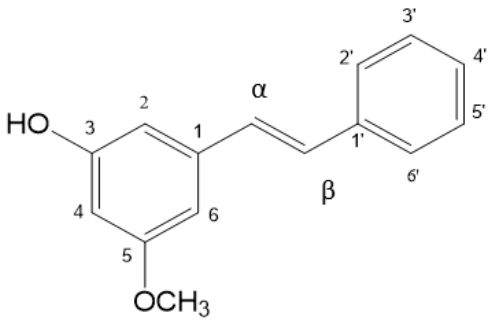
12. Identification of compound PD-2 (pinosylvin monomethyl ether)

Compound PD-2, which was obtained as a brown semisolid from dried roots (1.2 g, 0.3% yield) and dried leaves (9 mg, 0.0015 % yield) of *Paphiopedilum dianthum*, appeared as a brown spot on TLC upon spraying with 10% sulfuric acid and heating. Its molecular formula was determined as $C_{15}H_{14}O_2$ based on the $[M + Na]^+$ ion peak at *m/z* 279.0664 in the ESI mass spectrum (**Figure** 78). Nine degrees of unsaturation, calculated from its molecular formula, was suggestive of a stilbene skeleton.

The ^1H spectrum of compound PD-2 (**Figure 79** and **Table 26**) revealed the presence of two aromatic ring systems. Three proton signals, integrated for five protons, at δ 7.27 (1H, *m*, H-4'), 7.36 (2H, *td*, *J* = 7.2, 1.5 Hz, H-3'/H-5') and 7.50 ppm (2H, *dd*, *J* = 7.2, 1.5 Hz, H-2'/H-6') represented a mono-substituted benzene ring**,** whereas three *meta*-coupled triplets at δ 6.35 (1H, *t*, *J* = 2.0 Hz, H-4), 6.61 (1H, *t*, *J* = 2.0 Hz, H-2) and 6.66 ppm (1H, *t*, *J* = 2.0 Hz, H-6) could be assigned to those of a 1,3,5-trisubstituted aromatic ring. The ^1H NMR spectrum also displayed a methoxy resonance at δ 3.82 ppm (5-OCH_3) and two *trans*-coupled olefinic proton signals at δ 6.99 (1H, *d*, *J* = 16.4

Hz, H-α) and 7.07 ppm (1H, *d*, *J* = 16.4 Hz, H-β) of the ethylene bridge at the center of a *trans*-stilbene derivative. Based on these NMR data and its molecular weight, compound PD-2 should have a hydroxy group and a methoxy group on one of its two aromatic rings. Its ^{13}C NMR spectrum (**Figure 80** and **Table 26**) showed thirteen carbon signals including those of seven aromatic methide carbons at δ 101.0 (C-4), 104.9 (C-2), 106.1 (C-6), 126.6 (C-2'/C-6'), 127.7 (C-4') and 128.6 ppm (C-3'/C-5'), two olefinic methine carbons at δ 128.2 (C-α) and 129.3 ppm (C-β), four aromatic quaternary carbons at δ 136.9 (C-1'), 139.7 (C-1), 156.7 (C-5) and 160.9 ppm (C-3), and one methoxy carbon at δ 55.3 ppm (5-OCH_3). Therefore, compound PD-2 was identified as (*E*)-3-hydroxy-5-methoxystilbene or pinosylvin monomethyl ether, and its chemical structure was confirmed by comparison of spectral data with previous report (216).

Pinosylvin monomethyl ether has been found in lady slipper orchids such as *Paphiopedilum exul* (17) and *Phragmipedium calurum* (15). Furthermore, it has been reported as a constituent in other monocot plants such as *Alpinia katsumadai* (family Zingiberaceae) (216) and dicot plants such as *Swartzia apetala* var. *glabra* (family Fabaceae) (217). Pinosylvin monomethyl ether has been shown to possess biological activities including antifungal (217) and cytotoxicity (15).



Pinosylvin monomethyl ether

Table 27. NMR spectral data of compound PD-2 and pinosylvin monomethyl ether (in $CDCl_3$)

Position	Compound PD-2		Pinosylvin monomethyl ether[216]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	-	139.7	-	139.6
2	6.61 (*t*, 2.0)	104.9	6.60 (*s*)	104.7
3	-	160.9	-	160.9
4	6.35 (*t*, 2.0)	101.0	6.37 (*s*)	101.2
5	-	156.7	-	157.3
6	6.66 (*t*, 2.0)	106.1	6.62 (*s*)	106.3
α	6.99 (*d*, 16.4)	128.2	6.93 (*d*, 16.3)	128.4
β	7.07 (*d*, 16.4)	129.3	6.99 (*d*, 16.3)	129.2
1'	-	136.9	-	137.1
2'	7.50 (*dd*, 7.2, 1.5)	126.6	7.42 (*d*, 7.5)	126.6
3'	7.36 (*td*, 7.2, 1.5)	128.6	7.30 (*t*, 7.5)	128.6
4'	7.27 (*m*)	127.7	7.21 (*t*, 7.5)	127.7
5'	7.36 (*td*, 7.2, 1.5)	128.6	7.30 (*t*, 7.5)	128.6
6'	7.50 (*dd*, 7.2, 1.5)	126.6	7.42 (*d*, 7.5)	126.6
5-OCH_3	3.82 (*s*)	55.3	3.74 (*s*)	55.3

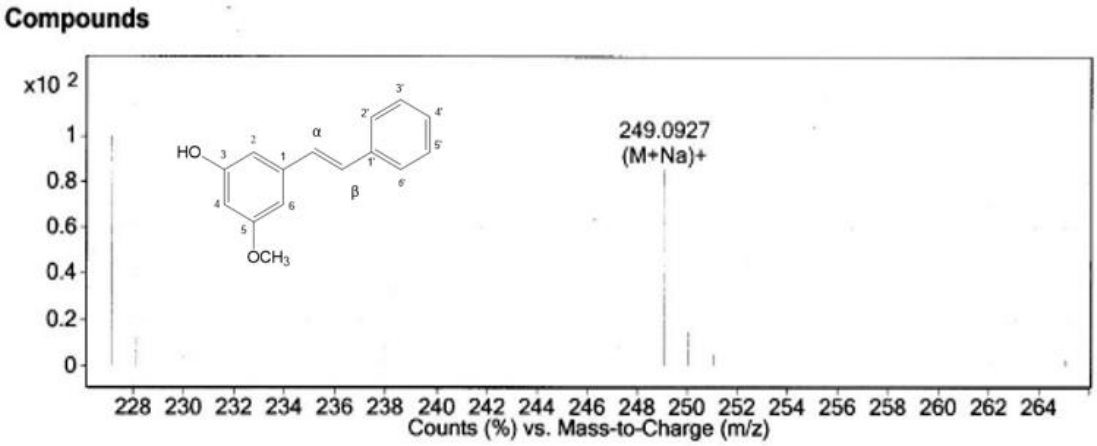


Figure 78. HR-ESI mass spectrum of compound PD-2

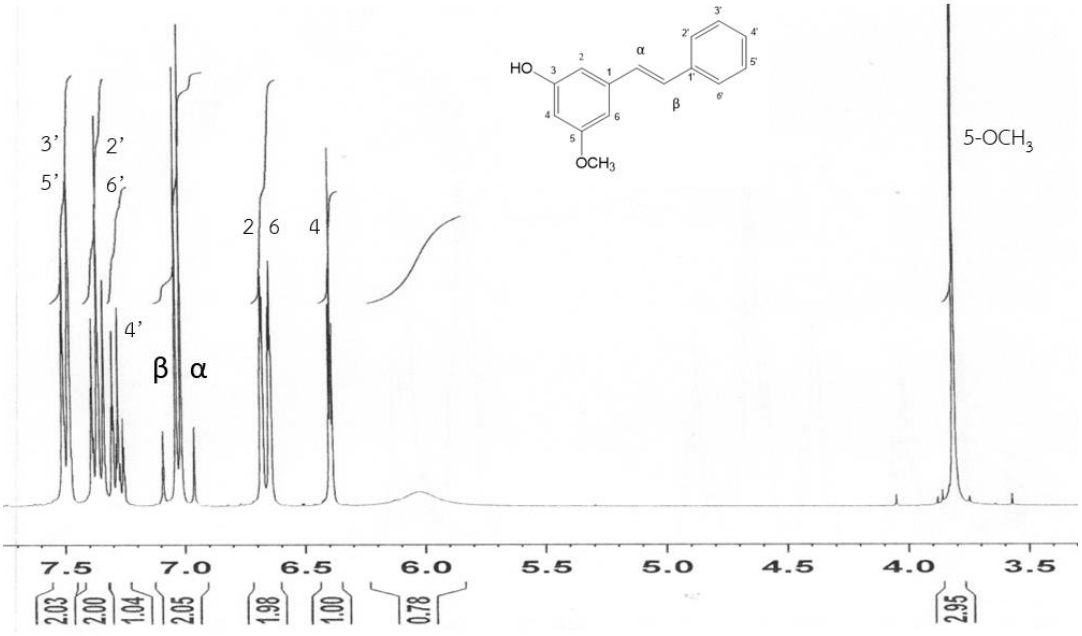


Figure 79. ^{1}H NMR spectrum of compound PD-2 (300 MHz, in $CDCl_3$)

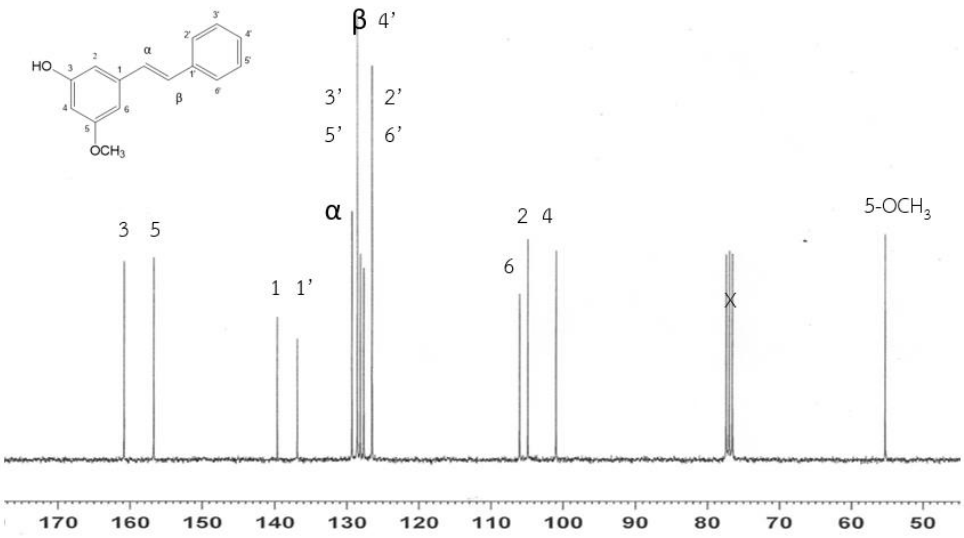


Figure 80. ^{13}C NMR spectrum of compound PD-2 (75 MHz, in $CDCl_3$)

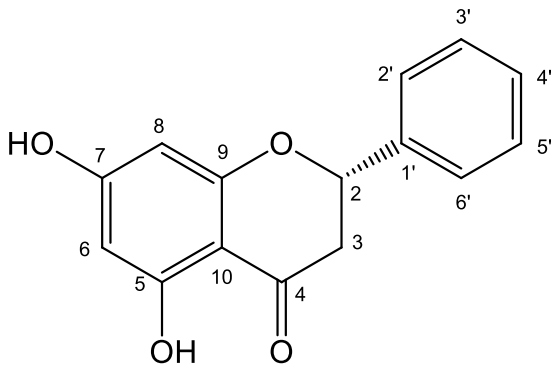
13. Identification of compound PD-3 (pinocembrin)

Compound PD-3 was obtained as a yellow amorphous solid at a much better yield (500 mg, 0.125% yield) from the dried roots than from the dried leaves (3 mg, 0.0005% yield) of *Paphiopedilum dianthum.* It appeared as a yellow spot on TLC upon spraying with 10% sulfuric acid and heating, suggesting that the compound could be a flavonoid. According to the sodium-adduct molecular ion ($[M + Na]^+$) peak at *m/z* 279.0664 (calcd. 279.0633) in the mass spectrum (**Figure 81**), its molecular formula was identified as $C_{15}H_{12}O_4$.

Its ^1H NMR spectrum (**Figure 82** and **Table 28**) exhibited characteristic aliphatic proton signals of a flavanone skeleton at δ 2.83 (1H, *dd*, *J* = 17.3, 12.9 Hz, H-3a), 3.09 (1H, *dd*, *J* = 17.3, 3.0 Hz, H-3b) and 5.43 ppm (1H, *dd*, *J* = 12.9, 3.0 Hz, H-2). Similar to compound PD-1, PD-3 also displayed a set of three multiplets, representing the unsubstituted ring B, which resonated at δ 7.38 (1H*, m*, H-4'), 7.40 (2H*, m*, H-3' and H-5') and 7.47 ppm (2H*, m*, H-2' and H-6'). The most downfield signal at δ 12.05 ppm could be assigned to the hydroxy substituent at position 5 which was hydrogen-

bonded to the carbonyl group at position 4. A pair of doublets at δ 6.00 (1H, *d*, *J* = 2.4 Hz) and 6.01 ppm (1H, *d*, *J* = 2.4 Hz) represented the *meta*-coupled H-6 and H-8, respectively, on ring A of this flavanone. Based on the molecular formula and NMR data, compound PD-3 should have another hydroxy substituent at position 7. The ^{13}C NMR spectrum (**Figure 83** and **Table 28**) showed twelve peaks for fifteen carbons of this flavanone. The most downfield carbon signal at δ 195.8 ppm was typical of keto carbonyl (C-4) of a flavanone. Two aliphatic carbon signals at δ 46.3 and 79.2 ppm were also supportive of C-3 and C-2, respectively, of a flavanone nucleus. Carbons of the unsubstituted ring B resonated as three peaks at δ 126.1 (C-2'/C-6'), 128.9 (C-3'/C-4'/C-5') and 138.3 ppm (C-1'). Finally, compound PD-3 was identified as 5,7-dihydroxyflavanone (pinocembrin) by comparison of its ^{1}H and ^{13}C NMR spectral data with a previous report (218).

Pinocembrin has previously been found in two other *Paphiopedilum* species: *P. godefroyae* (16) and *P. exul* (17). It was isolated from several flowering plants from families Asteraceae, Lauraceae, Piperaceae and Zingiberaceae (219). In addition, it could be found in some gymnosperms such as *Ginkgo biloba* (family Ginkgoaceae) (220) and *Pinus massoniana* (family Pinaceae) (221). The flavanone displayed several biological activities, including antimicrobial, anti-inflammatory, anticancer and neuroprotection (219).



Pinocembrin

Table 28. NMR spectral data of compound PD-3 and pinocembrin (in CD_3OD)

Position	Compound PD-3		Pinocembrin[218]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
2	5.43 (*dd*, 12.9, 3.0)	79.2	5.44 (*dd*, 12.8, 3.1)	80.5
3	a 2.83 (*dd*, 17.3, 3.0)	43.3	a 2.76 (*dd*, 17.0. 3.1)	41.2
	b 3.09 (*dd*, 17.3, 12.9)		b 3.07 (*dd*, 17.0, 12.8)	
4	-	195.8	-	197.3
5	-	164.4	-	165.5
6	6.00 (*d*, 2.4)	96.7	5.90 (*d*, 2.2)	97.2
7	-	164.5	-	168.4
8	6.01 (*d*, 2.4)	95.5	5.93 (*d*, 2.2)	96.2
9	-	163.2	-	164.7
10	-	103.2	-	103.4
1'	-	138.3	-	140.4
2'	7.47 (*m*)	126.1	7.48 (*m*)	127.4
3'	7.40 (*m*)	128.9	7.41 (*m*)	129.7
4'	7.38 (*m*)	128.9	7.36 (*m*)	129.6
5'	7.40 (*m*)	128.9	7.41 (*m*)	129.7
6'	7.47 (*m*)	126.1	7.48 (*m*)	127.4
5-OH	12.05 (*s*)	-	-	-

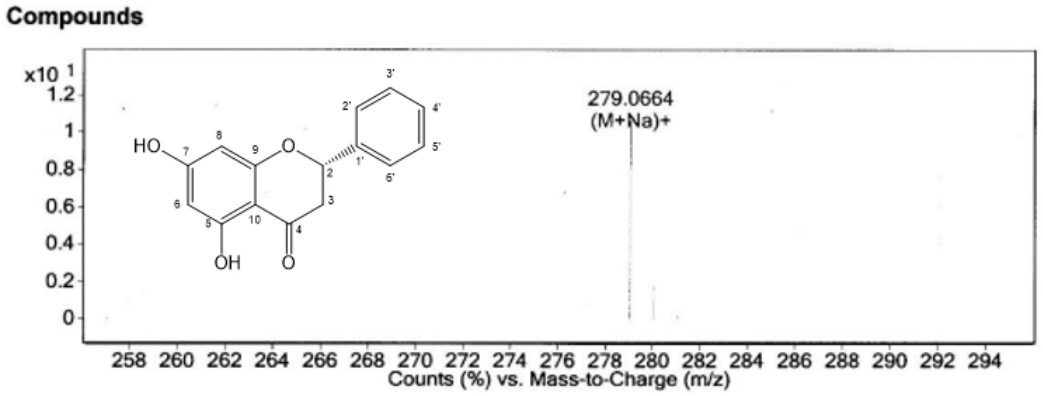


Figure 81. HR-ESI mass spectrum of compound PD-3

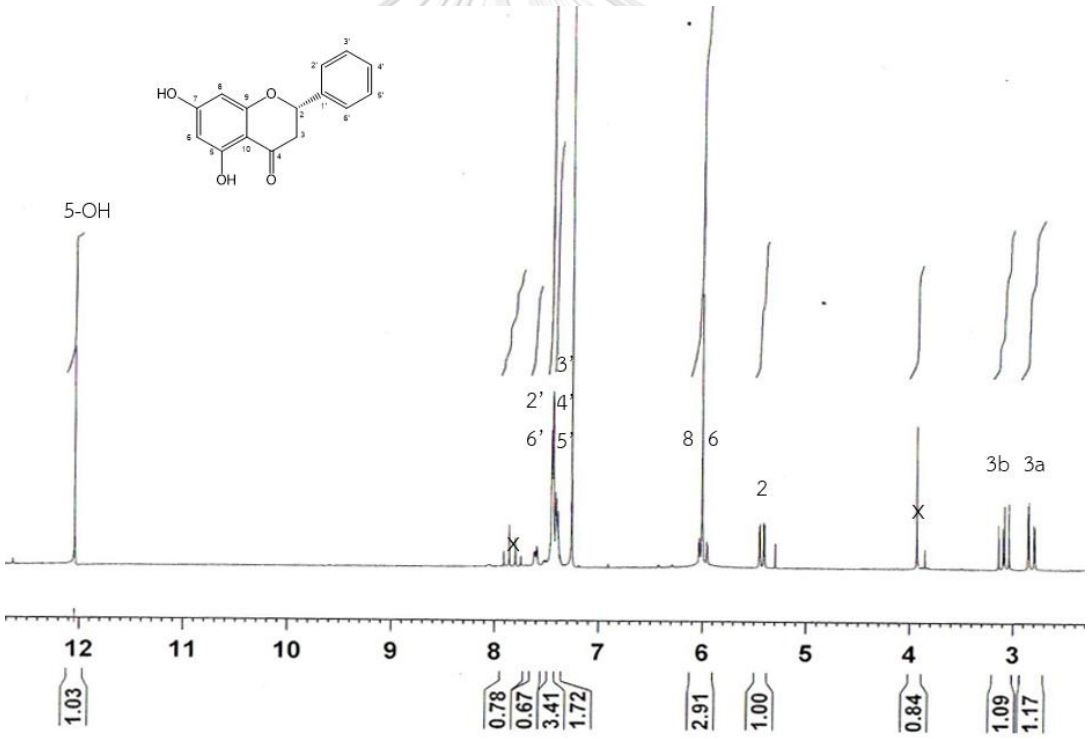


Figure 82. ^{1}H NMR spectrum of compound PD-3 (300 MHz, in CD_3OD)

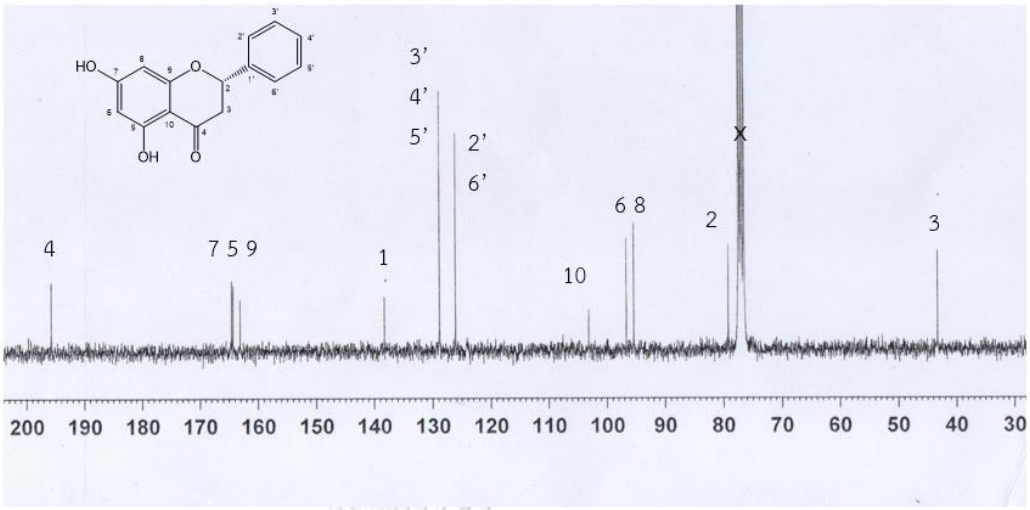


Figure 83. ^{13}C NMR spectrum of compound PD-3 (75 MHz, in CD_3OD)

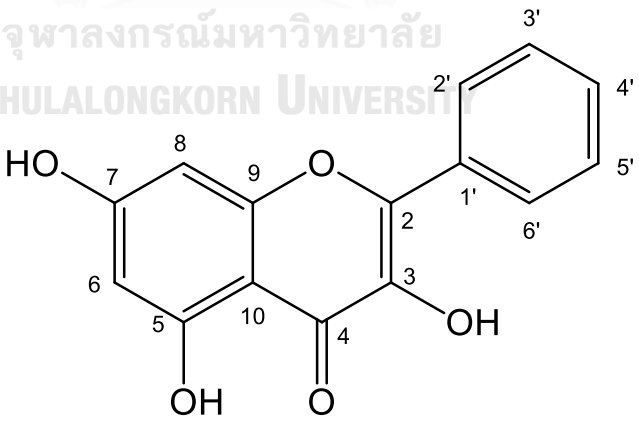
14. Identification of compound PD-4 (galangin)

Compound PD-4 was obtained as yellow needles from dried roots (0.2 g, 0.05% yield) and dried leaves (62 mg, 0.0010% yield) of *Paphiopedilum dianthum.* When sprayed with 10% sulfuric acid and heating, it appeared as a yellow spot on TLC, suggesting that the compound could be a flavonoid. Its molecular formula of $C_{15}H_{10}O_5$ was determined based on the $[M + Na]^+$ ion peak at *m/z* 293.0478 in the mass spectrum (**Figure 84**).

The ^1H NMR spectrum of compound PD-4 (**Figure 85** and **Table 29**) was similar to that of compound PD-1 (the flavonol isalpinin), except for the absence of methoxy singlet. Signals representing the unsubstituted ring B appeared at δ 7.51 (2H*, m*, H-3'/H-5'), 7.53 (1H*, m*, H-4') and 8.17 ppm (2H, *dd*, *J* = 7.2, 1.4 Hz, H-2'/H-6'), while the meta-coupled protons on ring A resonated as a pair of doublets at δ 6.18 (1H, *d*, *J* = 2.1 Hz, H-6) and 6.39 ppm (1H, *d*, *J* = 2.1 Hz, H-8). These data and its molecular formula suggested that compound PD-4 might be a flavonol with two hydroxy substituents on ring A.

The ^{13}C spectrum of compound PD-4 (**Figure 86** and **Table 29**) showed thirteen carbon peaks (including two double peaks) in agreement with fifteen carbons in its basic flavonol nucleus. The most downfield carbon resonance at δ 177.7 ppm (C-4) was supportive of its being a flavonol (215). Comparison of these ^{1}H and ^{13}C NMR data with literature vaules (222) (**Table 29**) led to the identification of compound PD-4 as 5,7-dihydroxyflavonol or galangin.

Galangin has previously been reported as a constituent of *Paphiopedilum exul* roots (17). The flavonol can also be found in many natural sources such as honey and propolis (222), medicinal plants e.g. in the rhizomes of lesser galangal (*Alpinia officinarum,* family Zingiberaceae) (223), and in the aerial parts of golden everlasting (*Helichrysum aureonitens*, family Asteraceae) (224). Galangin displayed various biological activities including antioxidant, antimicrobial, anti-inflammatory, antiviral and anticancer (223, 224).



Galangin

Table 29. NMR spectral data of compound PD-4 (in CD_3OD) and galangin (in DMSO-d_4)

Position	Compound PD-4		Galangin[222]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
2	-	146.9	-	146.1
3	-	138.5	-	137.5
4	-	177.7	-	176.7
5	-	162.6	-	161.2
6	6.18 (*d*, 2.1)	99.4	6.16 (*d*, 2.0)	98.7
7	-	165.9	-	164.7
8	6.39 (*d*, 2.1)	94.5	6.40 (*d*, 2.0)	94.0
9	-	158.4	-	156.8
10	-	104.7	-	103.7
1'	-	132.6	-	131.4
2'	8.17 (*dd*, 7.2, 1.4)	129.4	8.08 (*d*, 8.8)	128.0
3'	7.51 (*m*)	128.7	7.44 (*m*)	128.9
4'	7.53 (*m*)	130.9	7.44 (*m*)	130.3
5'	7.51 (*m*)	128.7	7.44 (*m*)	128.9
6'	8.17 (*dd*, 7.2, 1.4)	129.4	8.08 (*d*, 8.8)	128.0
3-OH	-	-	9.59 (*s*)	-
5-OH	-	-	12.31 (*s*)	-
7-OH	-	-	10.59 (*s*)	-

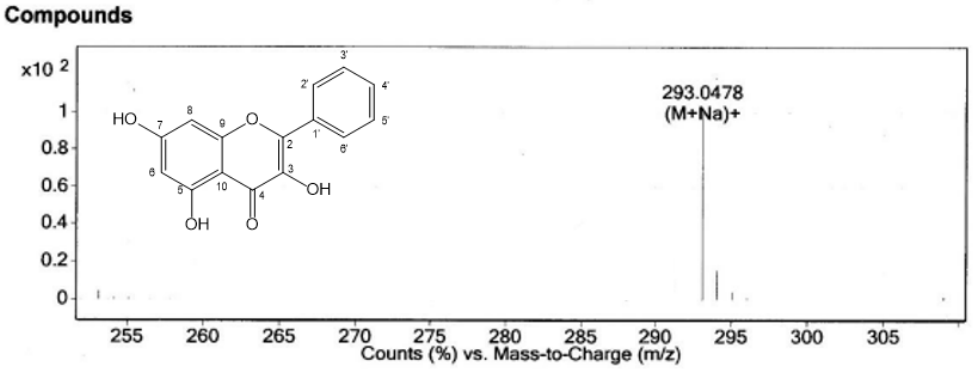


Figure 84. HR-ESI mass spectrum of compound PD-4

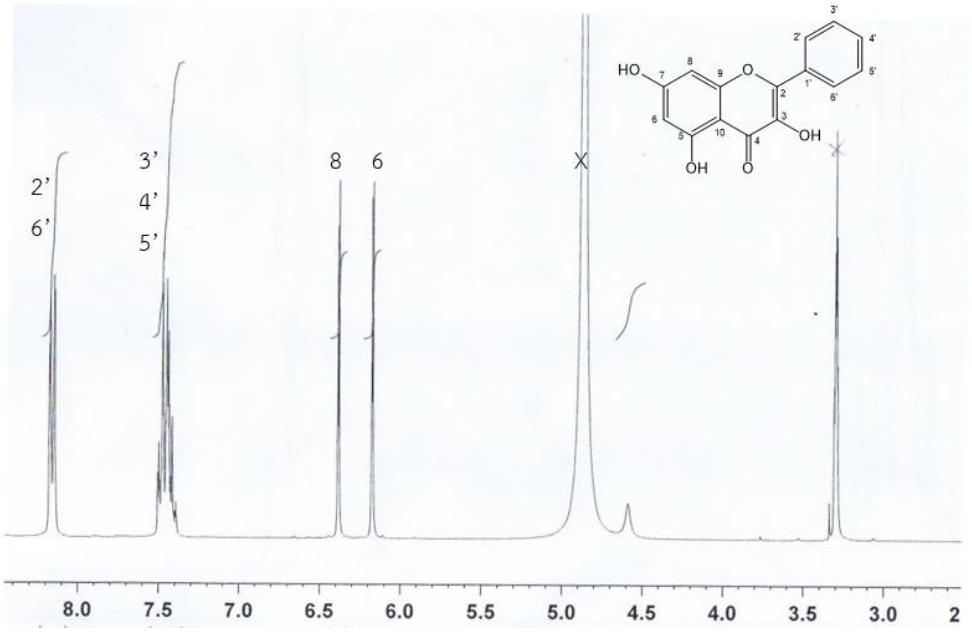


Figure 85. ^{1}H NMR spectrum of compound PD-4 (300 MHz, in CD_3OD)

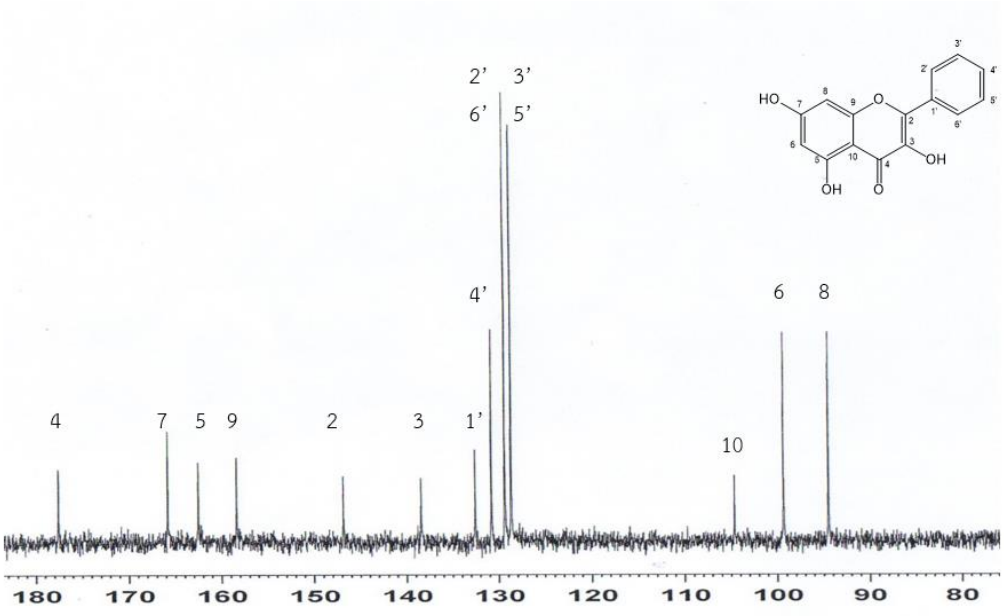


Figure 86. ^{13}C NMR spectrum of compound PD-4 (75 MHz, in CD_3OD)

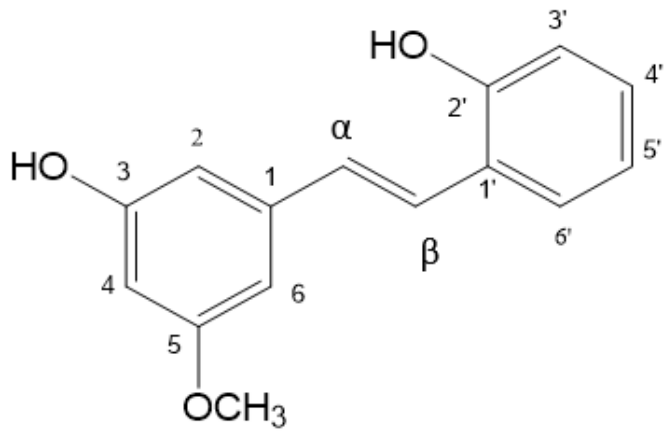
15. Identification of compound PD-5 (2,3'-dihydroxy-5'-methoxystilbene)

Compound PD-5 was obtained as a brown semisolid (1.5 g, 0.375% yield). According to its $[M - H]^-$ deprotonated ion peak in the ESI mass spectrum at *m/z* 241.0869 (**Figure** 87)**,** its molecular formula was established as $C_{15}H_{14}O_3$.

The ^1H spectrum of compound PD-5 (**Figure 88** and **Table 30**) exhibited resonances of one methoxy group at δ 3.77 ppm (5-OCH_3), two olefinic protons of a *trans*-double bond (*J* = 16.7 Hz) at δ 7.02 (H-α) and 7.38 ppm (H-β) and three *meta*-coupled aromatic protons of a 1,3,5-trisubstituted benzene ring at δ 6.25 (1H, *t*, *J* = 2.0 Hz, H-4) and 6.58 (2H, *d*, *J* = 2.0 Hz, H-2/H-6). These data were similar to those of compound PD-2 (pinosylvin monomethyl ether). The difference was the presence of other signals assignable to those of a 1,2-disubstituted aromatic ring at δ 6.79 (1H, *d*, *J* = 7.8 Hz, H-3'), 6.81 (1H, *t*, *J* = 7.8 Hz, H-5'), 7.06 (1H, *td*, *d*, *J* = 7.8, 1.5 Hz, H-4') and 7.50 ppm (1H, *dd*, *d*, *J* = 7.8, 1.5 Hz, H-6') in place of a mono-substituted ring of compound PD-2. Its molecular weight, which was 16 mass unit higher than that of

compound PD-2, suggested the presence of an additional hydroxy group. Therefore, this compound should be a *trans*-stilbene containing one methoxy and one hydroxy group on one aromatic ring, and another hydroxy group on the other ring. The ^{13}C NMR spectrum of compound PD-5 (**Figure 89** and **Table 30**) showed signals assignable to a methoxy carbon at δ 55.7 ppm (5-OCH_3), two olefinic carbons of the *trans*-ethylene bridge at δ 129.4 (C-α) and 125.0 ppm (C-β), and methide and quaternary carbons of two aromatic rings. Therefore, compound PDR-5 was identified as 2,3'-dihydroxy-5'-methoxystilbene by comparison of its NMR data with literature values (**Table 30**).

Up to the present, 2,3'-dihydroxy-5'-methoxystilbene has been found only in orchids of subfamily Cypripedioideae such as *Phragmipedium calurum* (112), *Paphiopedilum godefroyae* (16) and *Paphiopedilum callosum* (18). This stilbene has been demonstrated to be cytotoxic to small cell lung carcinoma (NCI-H187) cell line (16, 18).



2,3'-Dihydroxy-5'-methoxystilbene

Table 30. NMR spectral data of compound PD-5 and 2,3'-dihydroxy-5'-methoxystilbene (in CD_3OD)

Position	Compound PD-5		2,3'-Dihydroxy-5'-methoxystilbene	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	-	141.8	-	141.2
2	6.58 (*d*, 2.0)	104.4	6.59 (*br s*)	104.4
3	-	n.d.	-	158.0
4	6.25 (*t*, 2.0)	101.6	6.26 (*br s*)	101.5
5	-	162.7	-	162.0
6	6.58 (*d*, 2.0)	106.9	6.59 (*br s*)	106.7
α	7.02 (*d*, 16.7)	129.4	7.03 (*d*, 16.5)	129.7
β	7.38 (*d*, 16.7)	125.0	7.39 (*d*, 16.5)	125.4
1'	-	125.7	-	n.d.
2'	-	156.3	-	155.6
3'	6.79 (*d*, 7.8)	116.6	6.81 (*m*)	116.9
4'	7.06 (*td*, 7.8, 1.5)	129.5	7.06 (*t*, 6.8)	129.5
5'	6.81 (*t*, 7.8)	120.8	6.81 (*m*)	120.7
6'	7.50 (*dd*, 7.8, 1.5)	127.5	7.51 (d, 7.5)	127.5
5-OCH_3	3.77 (*s*)	55.7	3.78 (*s*)	55.5

n.d. = not detected

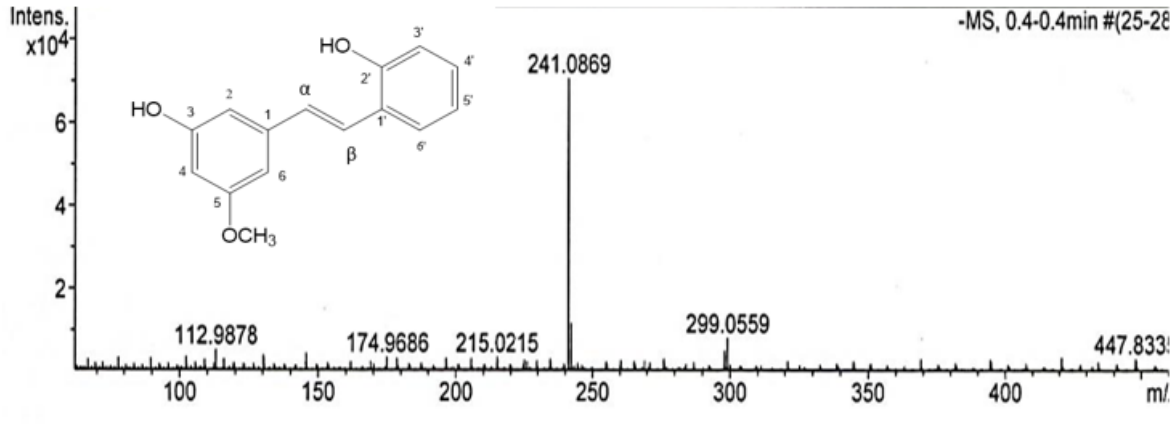


Figure 87. HR-ESI mass spectrum of compound PD-5

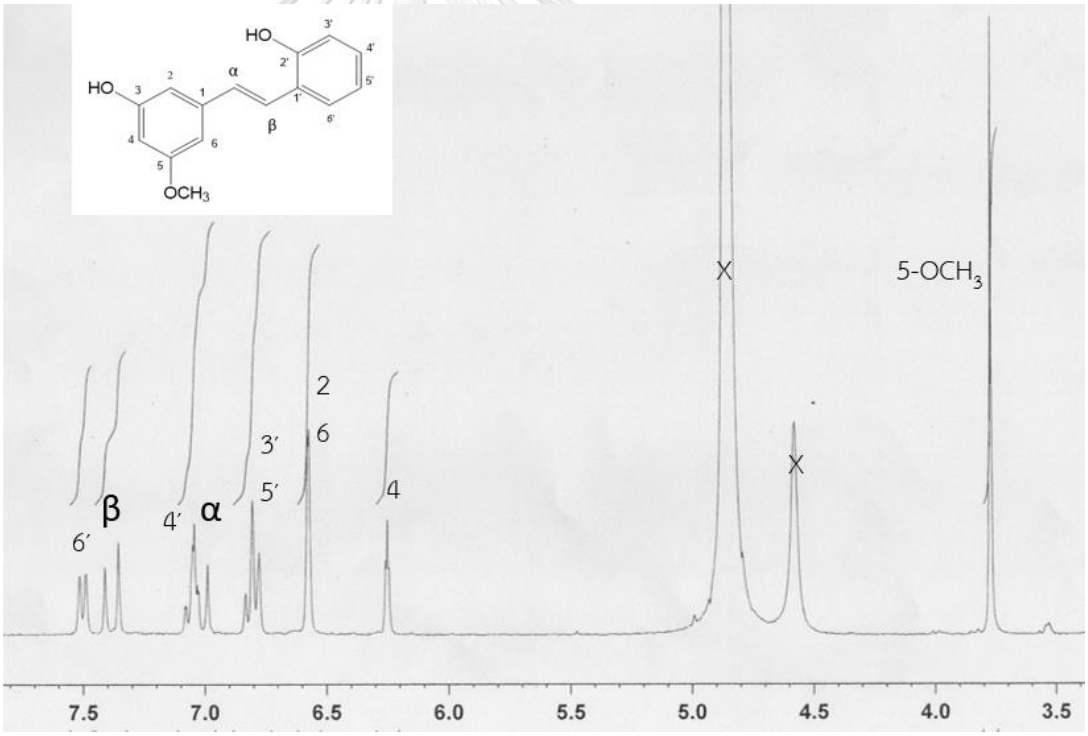


Figure 88. ^{1}H NMR spectrum of compound PD-5 (300 MHz, in CD_3OD)

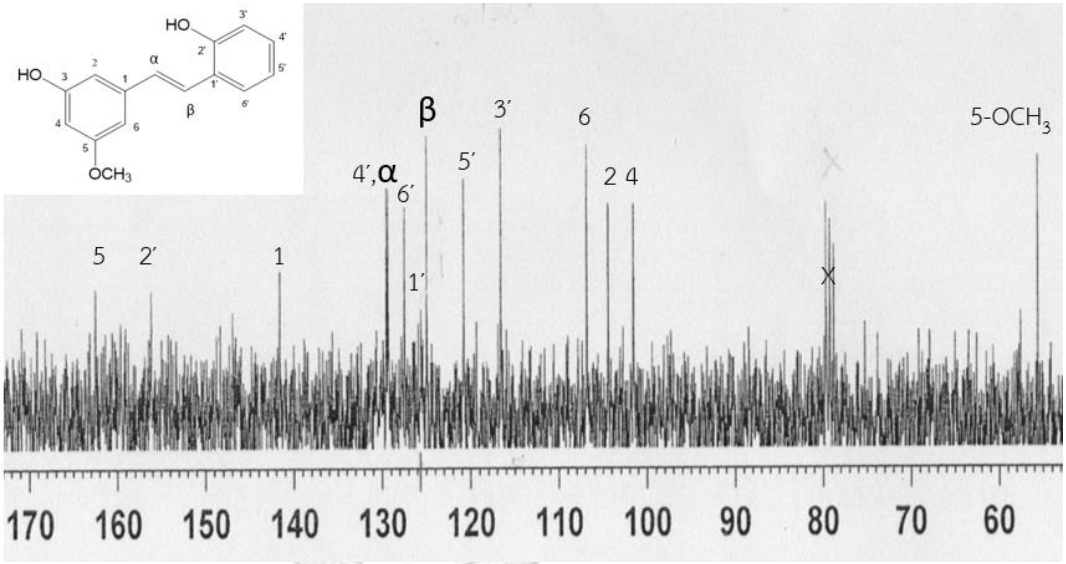


Figure 89. ^{13}C NMR spectrum of compound PD-5 (75 MHz, in CD_3OD)

16. Identification of compound PDR-6 [(*E*)-2,5'-dihydroxy-2'-(4-hydroxybenzyl)-3'-methoxystilbene]

Compound PD-6, obtained as a white powder (45 mg, 0.01125% yield), appeared as a brown spot on TLC upon spraying with 10% sulfuric acid and heating. Based on the sodium- adduct molecular ion peak ($[M + Na]^+$) at *m/z* 371.1255 in the mass spectrum (**Figure 90**), its molecular formula was determined as $C_{22}H_{20}O_4$.

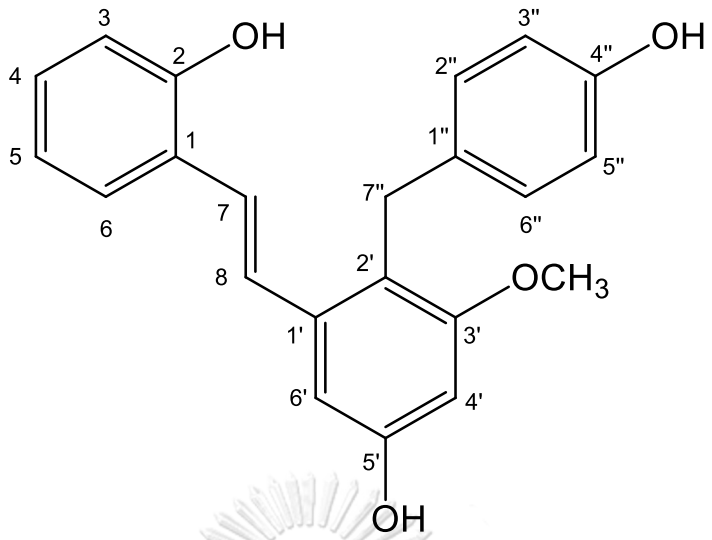
The ^{1}H NMR spectrum of compound PD-6 (**Figures 91-92** and **Table 31**) exhibited several signals similar to those of compound PD-5 including one methoxy singlet at δ 3.77 ppm (3H, *s*, 3'-OCH_3), a pair of *trans*-coupled doublets at δ 7.22 (1H, *d*, *J* = 16.5 Hz, H-7) and 7.36 ppm (1H, *d*, *J* = 16.5 Hz, H-8) and four aromatic proton resonances of a 1,2-disubstituted benzene ring at δ 6.76 (1H, *t*, *J* = 7.8 Hz, H-5), 6.77 (1H, *d*, *J* = 7.8 Hz, H-3), 7.04 (1H, *td*, *J* = 7.8, 1.5 Hz, H-4) and 7.32 ppm (1H, *dd*, *J* = 7.8, 1.2 Hz, H-6). These data were indicative of a stilbene nucleus with substitution pattern on one aromatic ring similar to previous compound. Other signals were due to changes

occurring on the second aromatic ring of the stilbene, which appeared to be tetra-substituted, and its two remaining *meta*-coupled protons resonated at δ 6.39 (1H, *d*, *J* = 2.1 Hz, H-4') and 6.74 ppm (1H, *d*, *J* = 2.1 Hz, H-6'). In addition, resonances of a *para*-hydroxybenzyl moiety appeared at δ 3.96 (2H, s, H_2-7''), 6.63 (2H, *d*, *J* = 8.4 Hz, H-3''/H-5'') and 6.93 ppm (2H, *d*, *J* = 8.4 Hz, H-2''/H-6'').

The ^{13}C NMR (**Figures 93-94** and **Table 31**) and HSQC spectra (**Figure 65**) of compound PD-6 exhibited signals of one methoxy carbon at δ 56.0 ppm (3'-OCH_3), one methylene carbon at δ 30.7 ppm (C-7''), two olefinic carbons at δ 126.5 (C-7) and 127.7 ppm (C-8), ten aromatic methine carbons at δ 99.2 (C-4'), 104.9 (C-6'), 115.9 (C-3''/C-5''), 116.6 (C-3), 120.8 (C-5), 127.6 (C-6), 129.4 (C-4), and 130.2 ppm (C-2''/C-6''), and eight aromatic quaternary carbons, including four oxygen-substituted ones, at δ 120.3 (C-2'), 126.0 (C-1), 134.2 (C-1''), 140.2 (C-1'), 155.9 (C-4''), 156.1 (C-2), 157.6 (C-5') and 160.1 ppm (C-3').

The HMBC experiment (**Figures 96-97** and **Table 32**) was perfprmed to help confirm the location of the *para*-hydroxybenzyl group at position 2'. HMBC cross-peaks were observed between H-7'' methylene protons (at δ 3.96 ppm) and carbons at δ 140.2 (C-1'), 120.3 (C-2'), 160.1 (C-3'), 134.2 (C-1''), and 130.2 ppm (C-2''/C-6''). Methoxy substitution at position 3' was also confirmed by HMBC correlations from methoxy protons (at δ 3.77 ppm) and H-4' (at δ 6.39 ppm) to C-3' at δ 160.1 ppm. From these spectral data and comparison with a previous report (112), compound PD-6 was identified as (*E*)-2,5'-dihydroxy-2'-(4-hydroxybenzyl)-3'-methoxystilbene.

This stilbene was firstly reported as a constituent of three *Phragmipedium* species (112). It was later isolated from *Paphiopedilum exul* roots (17).



(*E*)-2,5'-dihydroxy-2'-(4-hydroxybenzyl)-3'-methoxystilbene

Table 31. NMR spectral data of compound PD-6 and (*E*)-2,5'-dihydroxy-2'-(4-hydroxybenzyl)-3'-methoxystilbene (in CD_3OD)

Position	Compound PD-6		(*E*)-2,5'-Dihydroxy-2'-(4-hydroxy-benzyl)-3'-methoxystilbene[112]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	-	126.0	-	126.7
2	-	156.1	-	156.3
3	6.77 (*d*, 7.8)	116.6	6.77 (*m*)	116.8
4	7.04 (*td*, 7.8, 1.5)	129.4	7.04 (*td*, 7.4)	129.6
5	6.76 (*t*, 7.8)	120.8	6.77 (*m*)	120.9
6	7.32 (*dd*, 8.1, 1.2)	127.6	7.32 (*br d*, 7.4)	127.7
7	7.22 (*d*, 16.5)	126.5	7.22 (*d*, 16.2)	126.7

Position	Compound PD-6		(*E*)-2,5'-Dihydroxy-2'-(4-hydroxy-benzyl)-3'-methoxystilbene[112]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
8	7.36 (*d*, 16.5)	127.7	7.35 (*d*, 16.2)	127.9
1'	-	140.2	-	140.4
2'	-	120.3	-	120.5
3'	-	160.1	-	160.3
4'	6.39 (*d*, 2.1)	99.2	6.40 (*d*, 2.1)	99.4
5'	-	157.6	-	157.7
6'	6.74 (*d*, 2.1)	104.9	6.74 (*d*, 2.1)	105.1
1''	-	134.2	-	134.3
2''	6.93 (*d*, 8.4)	130.2	6.96 (*d*, 8.4)	130.3
3''	6.63 (*d*, 8.4)	115.9	6.63 (*d*, 8.4)	116.0
4''	-	155.9	-	156.1
5''	6.63 (*d*, 8.4)	115.9	6.63 (*d*, 8.4)	116.0
6''	6.93 (*d*, 8.4)	130.2	6.96 (*d*, 8.4)	130.3
7''	3.96 (*s*)	30.7	3.96 (*s*)	30.8
3'-OCH_3	3.77 (*s*)	56.0	3.78 (*s*)	56.2

Table 32. HMBC spectral data of compound PD-5 (in CD_3OD)

^{1}H signal	HMBC correlation with
H-3	C-1, C-2, C-5
H-4	C-2, C-3, C-6
H-5	C-1, C-3, C-4, C-6
H-6	C-1, C-2, C-4, C-5, C-7
H-7	C-2, C-8, C-1'
H-8	C-7, C-1', C-6'
H-4'	C-2', C-3', C-5'
H-6'	C-8, C-4', C-5'
H-2"	C-1", C-3", C-4", C-6"
H-3"	C-1", C-4", C-5"
H-5"	C-1", C-3", C-4"
H-6"	C-1", C-2", C-3", C-4"
H-7"	C-1', C-2', C-3", C-1", C-2", C-6"
3'-OCH_3	C-3'

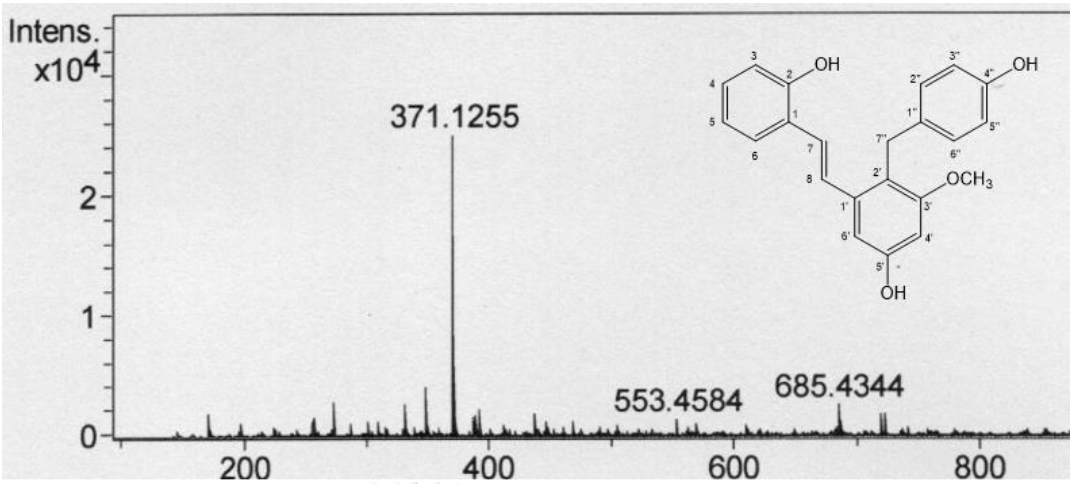


Figure 90. HR-ESI mass spectrum of compound PD-6

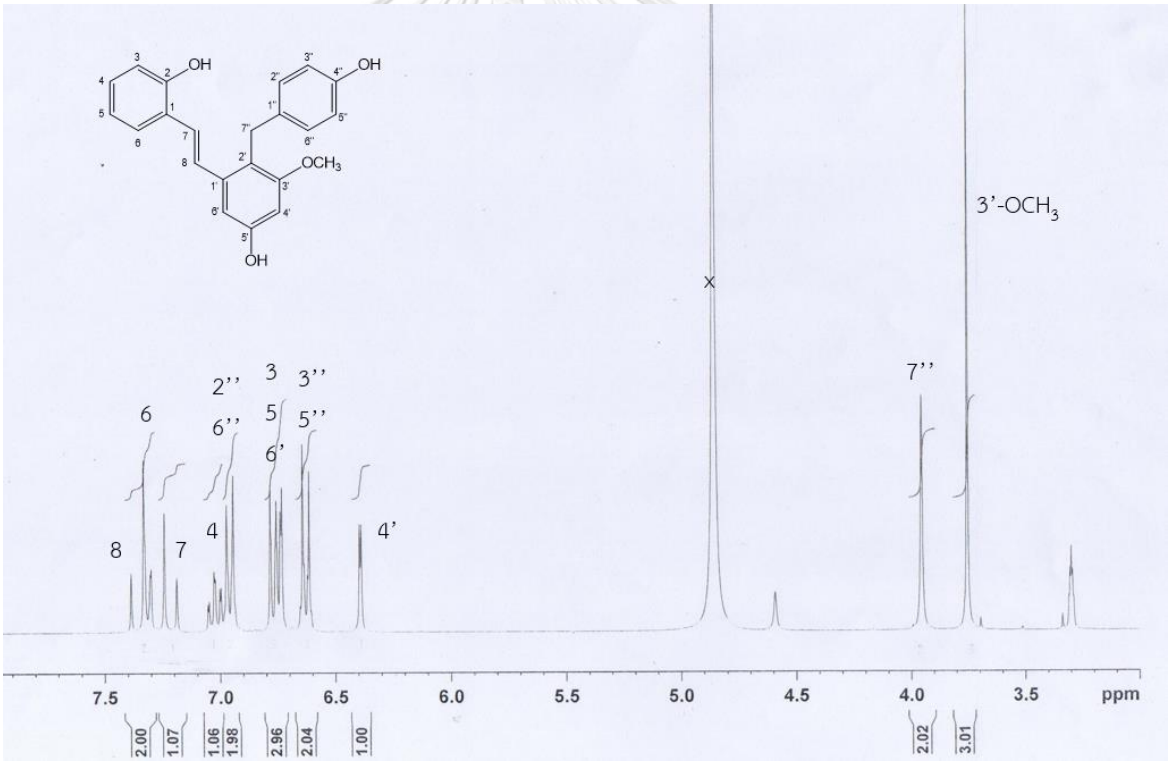


Figure 91. ^{1}H NMR spectrum of compound PD-6 (300 MHz, in CD_3OD)

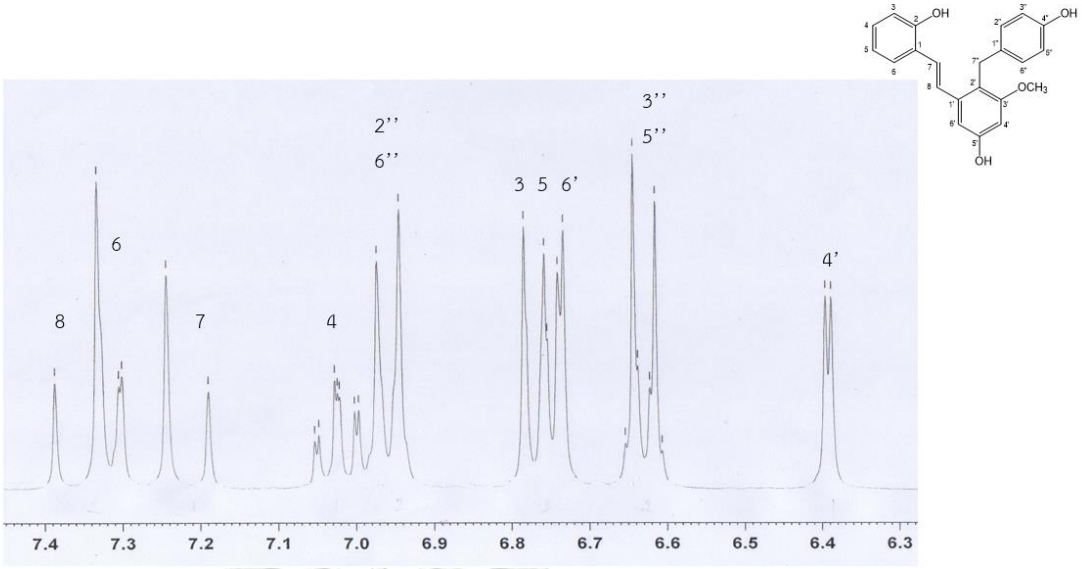


Figure 92. ^{1}H NMR spectrum of compound PD-6 (300 MHz, in CD_3OD, expansion between δ_H 6.3-7.5 ppm)

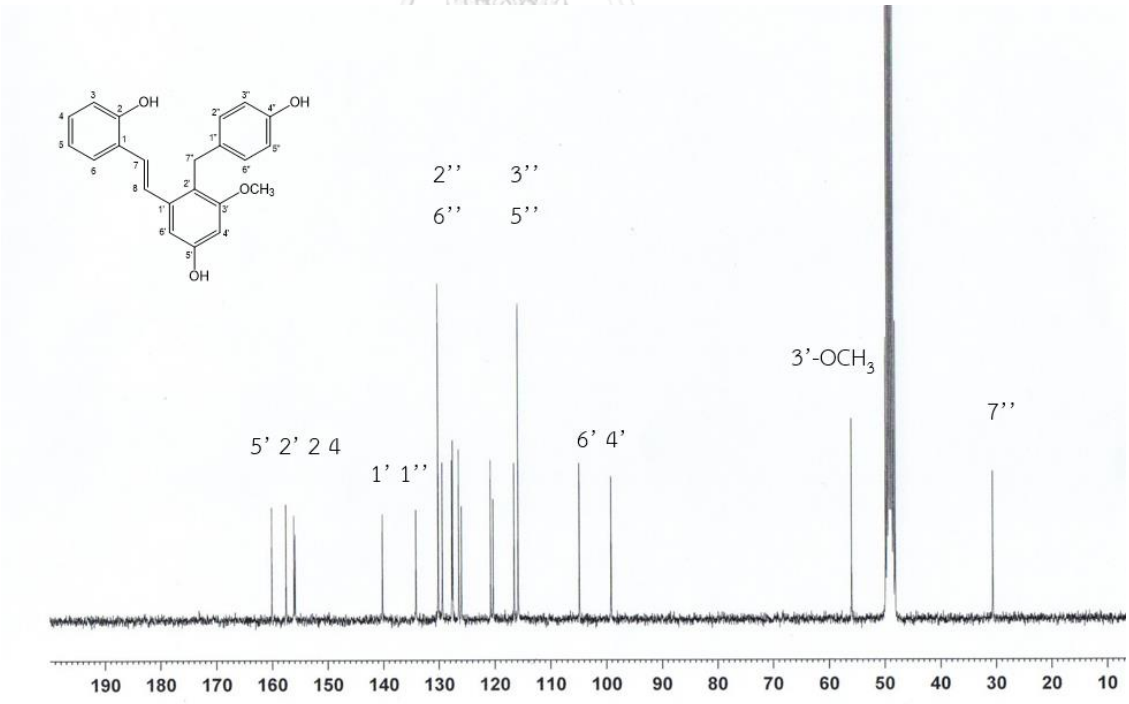


Figure 93. ^{13}C NMR spectrum of compound PD-6 (75 MHz, in CD_3OD)

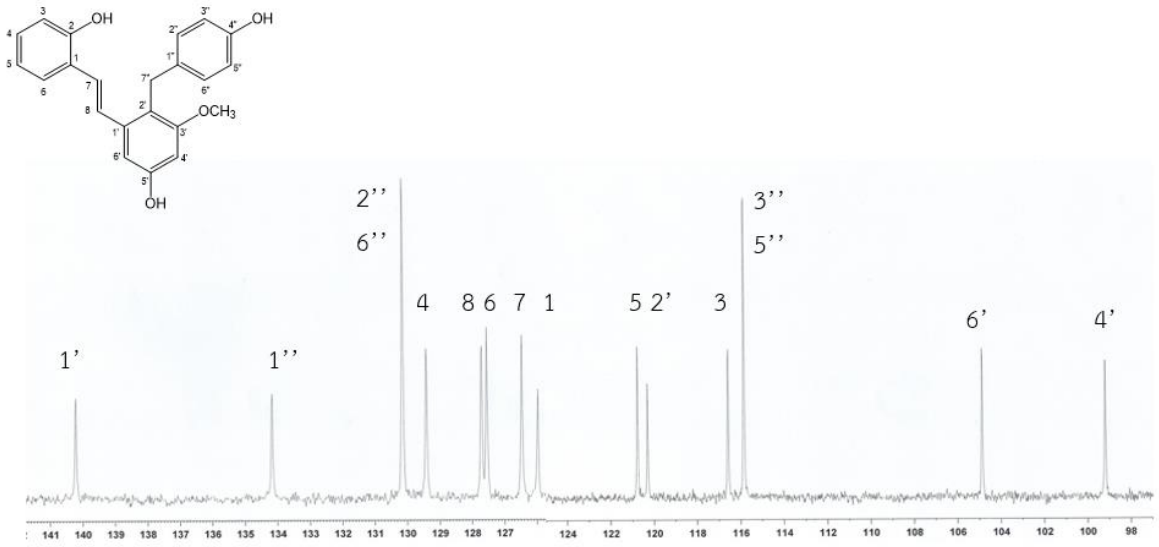


Figure 94. ^{13}C NMR spectrum of compound PD-6 (75 MHz, in CD_3OD, expansion between δ_C 97.0-142.0 ppm)

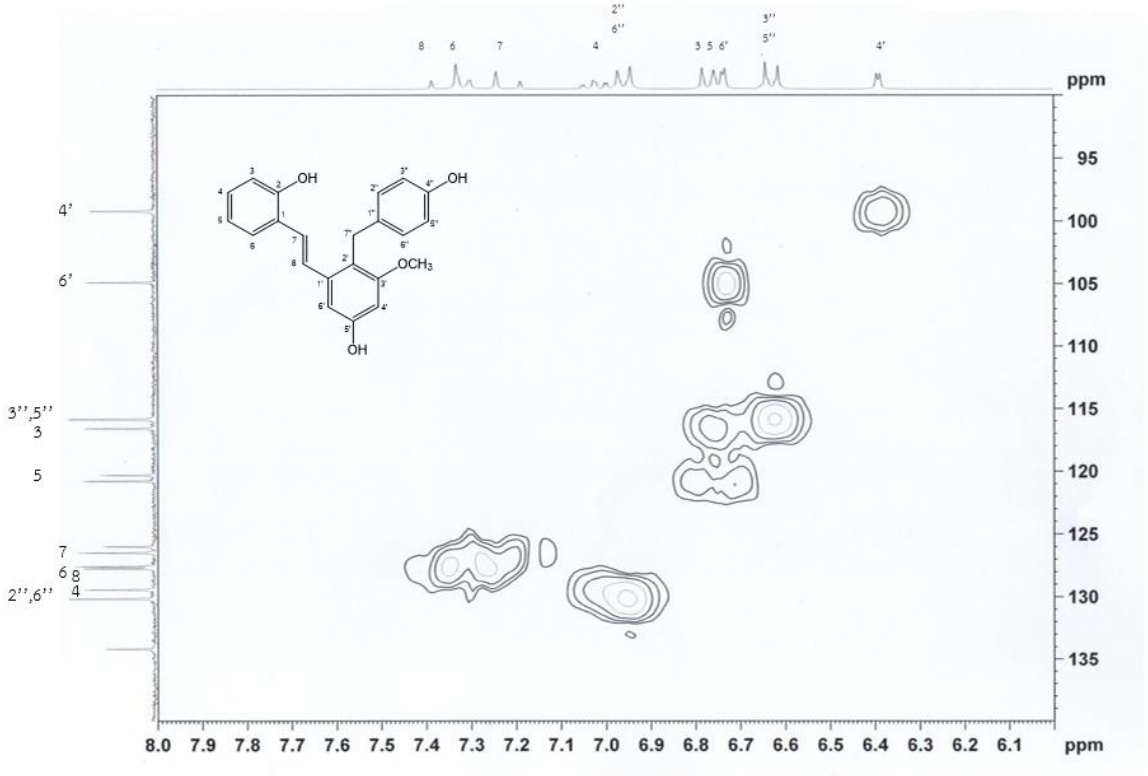


Figure 95. HSQC spectrum of compound PD-6 (expansion between δ_H 6.0-8.0 ppm, δ_C 90-140 ppm)

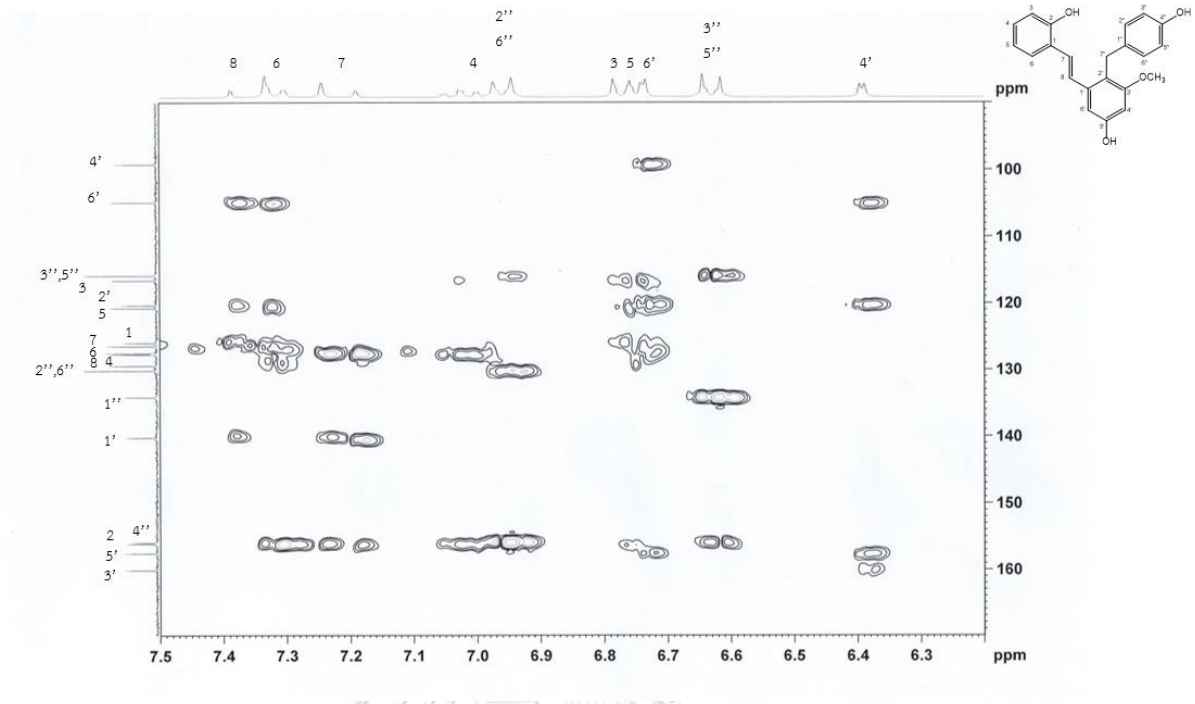


Figure 96. HMBC spectrum of compound PD-6 (expansion between δ_H 6.2-7.5 ppm, δ_C 100-170 ppm)

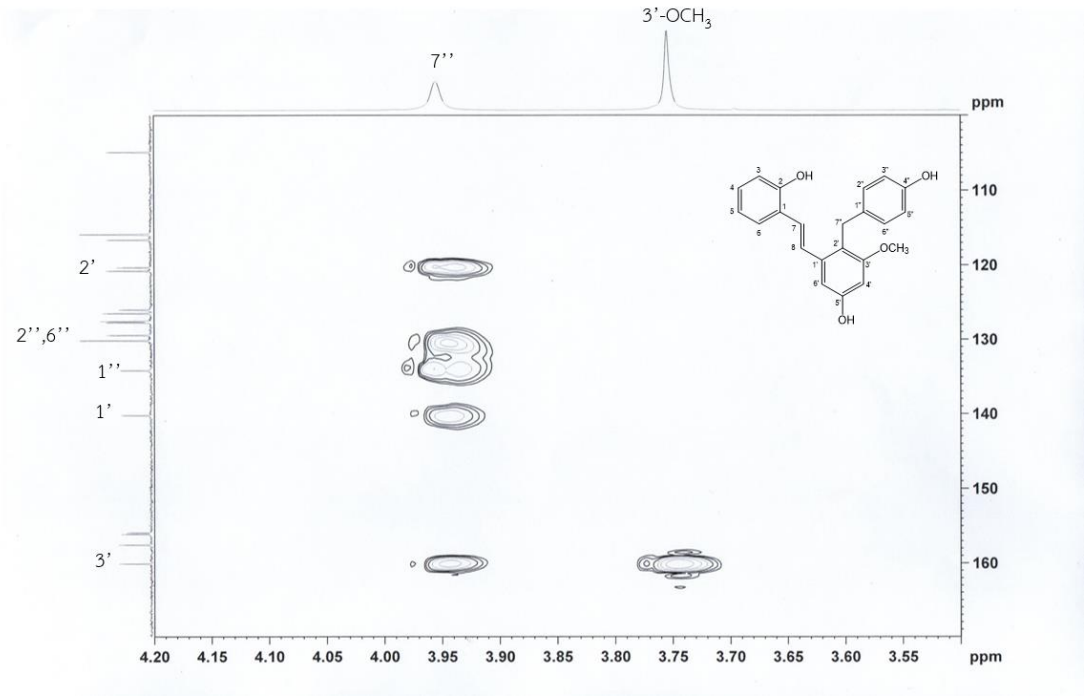


Figure 97. HMBC spectrum of compound PD-6(expansion between δ_H 3.5-4.2 ppm, δ_C 100-170 ppm)

17. Structure elucidation of compound PD-7 (paphiodianthin A)

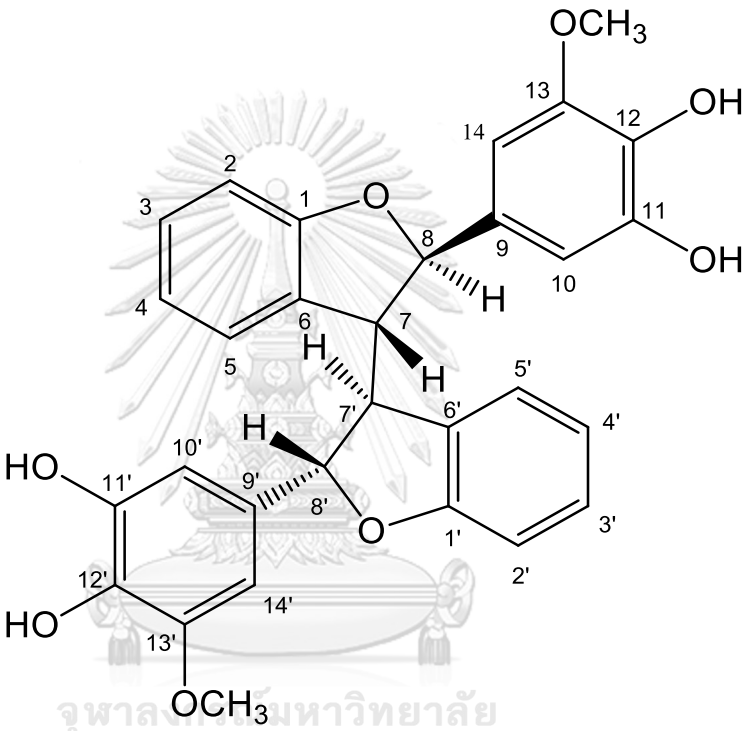
Compound PD-7, obtained as a brown amorphous powder (20 mg, 0.005% yield), appeared as a brown spot on TLC after being sprayed with 10% sulfuric acid and heating. IR absorption bands at 3432 and 1235 cm^{-1} (**Figure 99**) indicated the presence of hydroxyl groups and ether of a furan ring, respectively. According to the sodium-adduct molecular ion peak ($[M + Na]^+$) at *m/z* 537.1519 in the high resolution mass spectrum (**Figure 100**), its molecular formula was determined as $C_{30}H_{26}O_8$ (calculated $C_{30}H_{26}O_8Na$, 537.1525). Eighteen degrees of unsaturation, calculated from its molecular formula, was rather high, whereas the number of carbon peaks in its ^{13}C NMR spectrum (**Figure 102**) was fifteen only (including one methoxy carbon peak). These data indicated that compound PD-7 could be a symmetrical dimer of stilbenoid, with one methoxy group on each monomer

The ^1H NMR spectrum of compound PD-7 (**Figure 101** and **Table 33**) showed a methoxy singlet at δ 3.60 ppm (13-OCH_3/13'-OCH_3), a pair of doublets at δ 5.65 (2H, *J* = 1.5 Hz, H-14/H-14') and 6.12 ppm (2H, *J* = 1.5 Hz, H-10/H-10') for 1,3,4,5-tetrasubstituted phenyl moieties, four coupled proton signals of a 1,2-disubstituted aromatic ring at δ 6.96 (2H, *d, J* = 7.5 Hz, H-2/H-2'), 7.25 (2H, *t, J* = 7.5 Hz, H-3/H-3'), 6.92 (2H, *t*, *J* = 7.1 Hz, H-4/H-4') and 7.22 ppm (2H, *d, J* = 7.1 Hz, H-5/H-5') and two vicinal-coupled doublets at δ 3.87 (2H, *d, J* = 2.4 Hz, H-7/H-7') and 5.23 ppm (2H, *d, J* = 2.4 Hz, H-8/H-8') characteristic of disubstituted dihydrobenzofuran moieties. Therefore, the basic skeleton of each monomer of this compound should be a phenyldihydrobenzofuran with no substituent on the aromatic ring of the benzofuran moiety, whereas, based on its molecular weight, the phenyl moiety should be substituted with one methoxy and two hydroxy groups.

The ^{13}C NMR (**Figure 102** and **Table 33**) and HSQC spectra (**Figure 103**) of compound PD-7 displayed fifteen carbon peaks in total, including those of methoxy carbons at δ 56.5 ppm (13-OCH_3/13'-OCH_3), four aliphatic methine carbons at δ 57.0 (C-7/C-7') and 86.8 ppm (C-8/C-8'), twelve aromatic methine carbons at δ 101.2 (C-14/C-14'), 106.2 (C-10/C-10'), 110.4 (C-2/C-2'), 122.1 (C-4/C-4'), 126.7 (C-5/C-5') and 130.3 ppm (C-3/C-3') and twelve aromatic quaternary carbons at δ 128.5 (C-6/C-6'), 134.5 (C-12/C-12'), 134.8 (C-9/C-9'), 146.6 (C-11/C-11'), 149.4 (C-13/C-13') and 161.9 ppm (C-1/C-1').

The position of methoxy substituent at positions 13/13' on the phenyl moieties was established by the HMBC experiment (**Figure 104** and **Table 33**) in which C-13/C-13' (at δ 149.4 ppm) showed cross-peaks with H-14/H-14' (at δ 5.65 ppm) and methoxy protons (at δ 3.60 ppm). One hydroxy group could be located at C-11/C-11', based on HMBC cross-peak between H-10/H-10' (at δ 6.12 ppm) and the carbon at δ 146.6 ppm. Therefore, the second hydroxy group could be placed at positions 12/12'. The connection between the two benzofuran units was confirmed by HMBC correlations observed between H-7 (at δ 3.87 ppm) with C-1, C-6/C-6', C-8, C-9 and C-7'. Long-range HMBC correlations between H-8/H-8' (at δ 5.23 ppm) and carbons C-1/C-1', C-6/C-6', C-10/C-10' and C-14/C-14' also confirmed the connection of phenyl ring to the dihydrobenzofuran nucleus at positions 8/8'. The relative configuration of H-7/H-7' and H-8/H-8' was deduced to be *trans* based on comparison of their coupling constant to previously reported values from similar compounds (112). In addition, relative configuration between H-7 and H-7' was also determined to be *trans*, based on this coupling constant, which would be larger (around 7 Hz) if these two protons were *cis*-oriented (225).

Therefore, compound PD-7 was elucidated as (2*R*,2'*R*,3*R*,3'*R*)-*rel*-2,2'-*bis*(3,4-dihydroxy-5-methoxyphenyl)-2,2',3,3'-tetrahydro-3-3'-bibenzofuran, and was given the trivial name paphiodianthin A.



Paphiodianthin A

Table 33. NMR spectral data of compound PD-7 (in CD_3OD)

Position	δ_H (mult., *J* in Hz)	δ_C	HMBC correlation with
1	-	161.9	-
2	6.96 (*d*, 7.5)	110.4	C-1, C-4, C-6
3	7.25 (*t*, 7.5)	130.3	C-1, C-2, C-5
4	6.92 (*t*, 7.1)	122.1	C-2, C-6
5	7.22 (*d*, 7.1)	126.7	C-1, C-3, C-7
6	-	128.5	-
7	3.87 (*d*, 2.4)	57.0	C-1, C-6, C-8, C-9, C-6', C-7'
8	5.23 (*d*, 2.4)	86.8	C-1, C-6, C-7, C-9, C-10, C-14
9	-	134.8	-
10	6.12 (*d*, 1.5)	106.2	C-8, C-9, C-11, C-14
11	-	146.6	-
12	-	134.5	-
13	-	149.4	-
14	5.65 (*d*, 1.5)	101.2	C-9, C-10, C-13
1'	-	161.9	-
2'	6.96 (*d*, 7.5)	110.4	C-1', C-4', C-6'
3'	7.25 (*t*, 7.5)	130.3	C-1', C-2', C-5'
4'	6.92 (*t*, 7.1)	122.1	C-2', C-6'
5'	7.22 (*d*, 7.1)	126.7	C-1', C-3', C-7'
6'	-	128.5	-
7'	3.87 (*d*, 2.4)	57.0	C-6, C-7, C-1', C-6', C-8', C-9'
8'	5.23 (*d*, 2.4)	86.8	C-1', C-6', C-7', C-9', C-10', C-14'

Position	δ_H (mult., *J* in Hz)	δ_C	HMBC correlation with
9'	-	134.8	-
10'	6.12 (*d*, 1.5)	106.2	C-8', C-9', C-11', C-14'
11'	-	146.6	-
12'	-	134.5	-
13'	-	149.4	-
14'	5.65 (*d*, 1.5)	101.2	C-9', C-10', C-13'
13-OCH_3	3.60 (*s*)	56.5	C-13
13''-OCH_3	3.60 (*s*)	56.5	C-13''

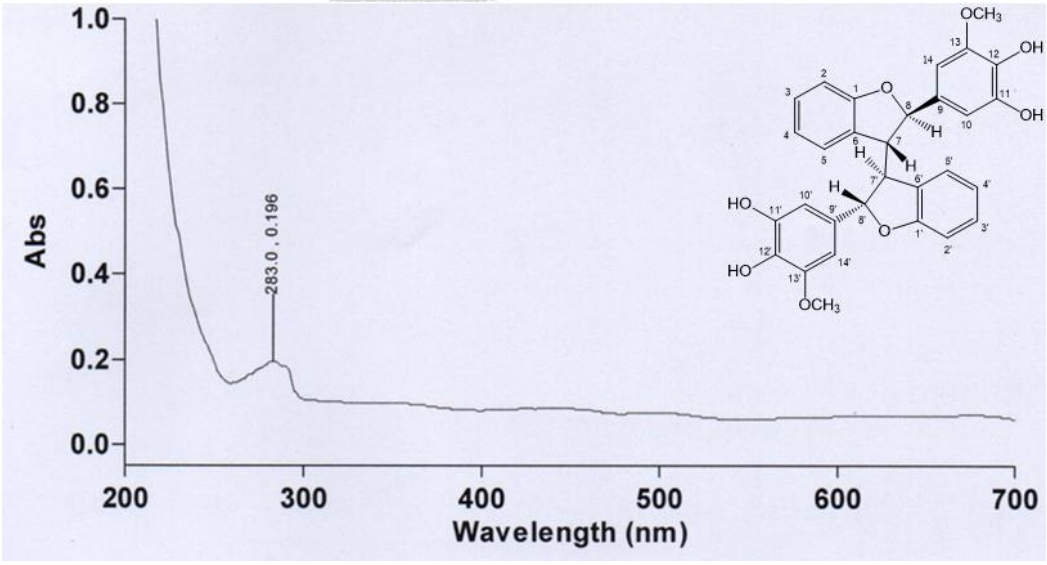


Figure 98. UV spectrum of compound PR-7

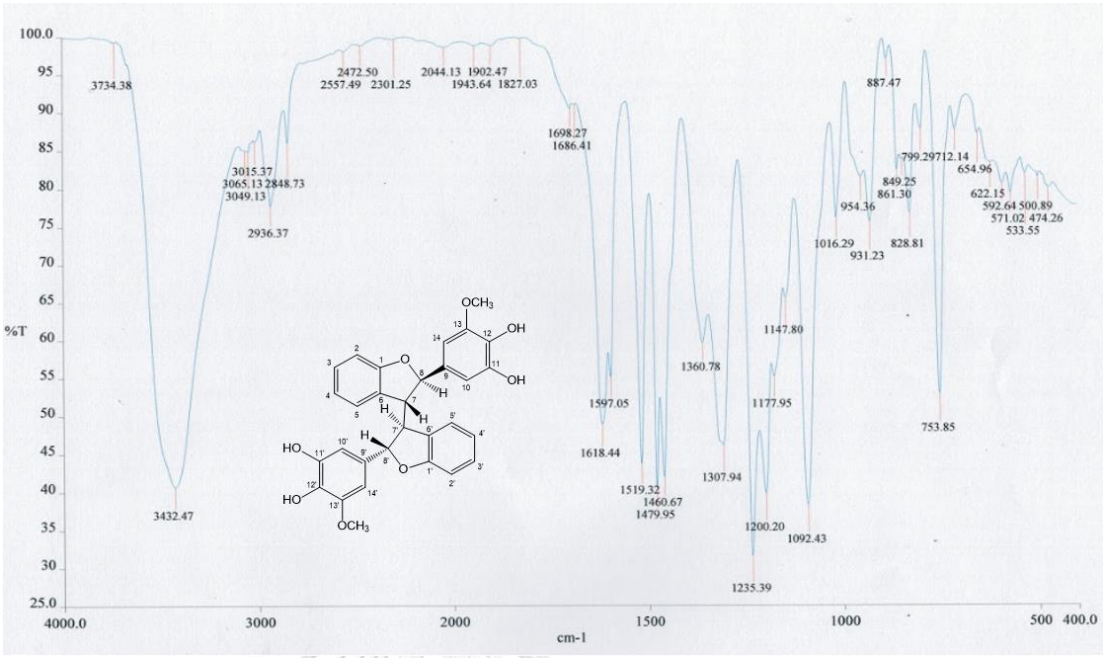


Figure 99. IR spectrum of compound PD-7

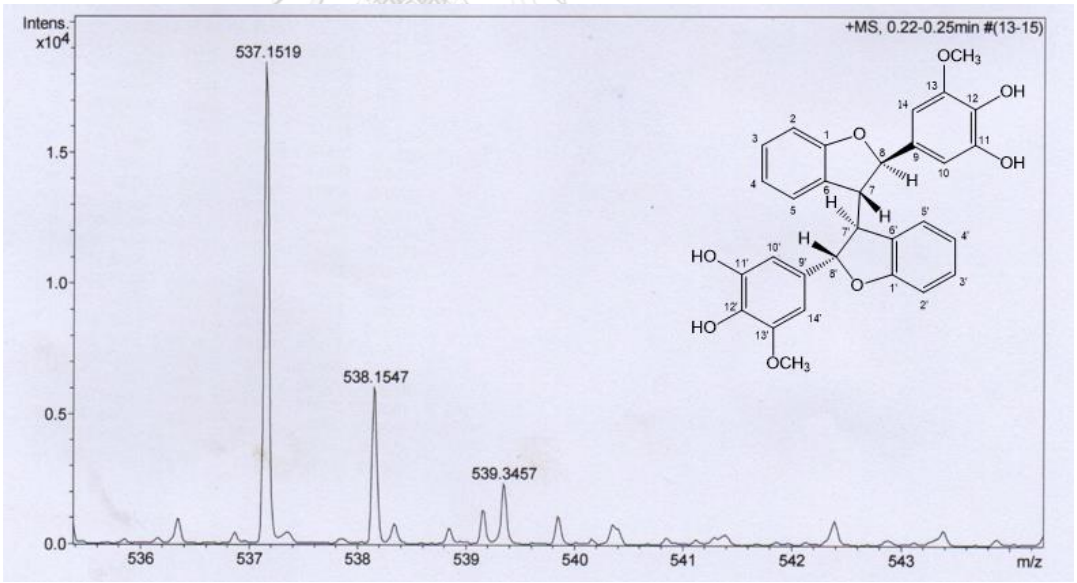


Figure 100. HR-ESI-MS spectrum of compound PR-7

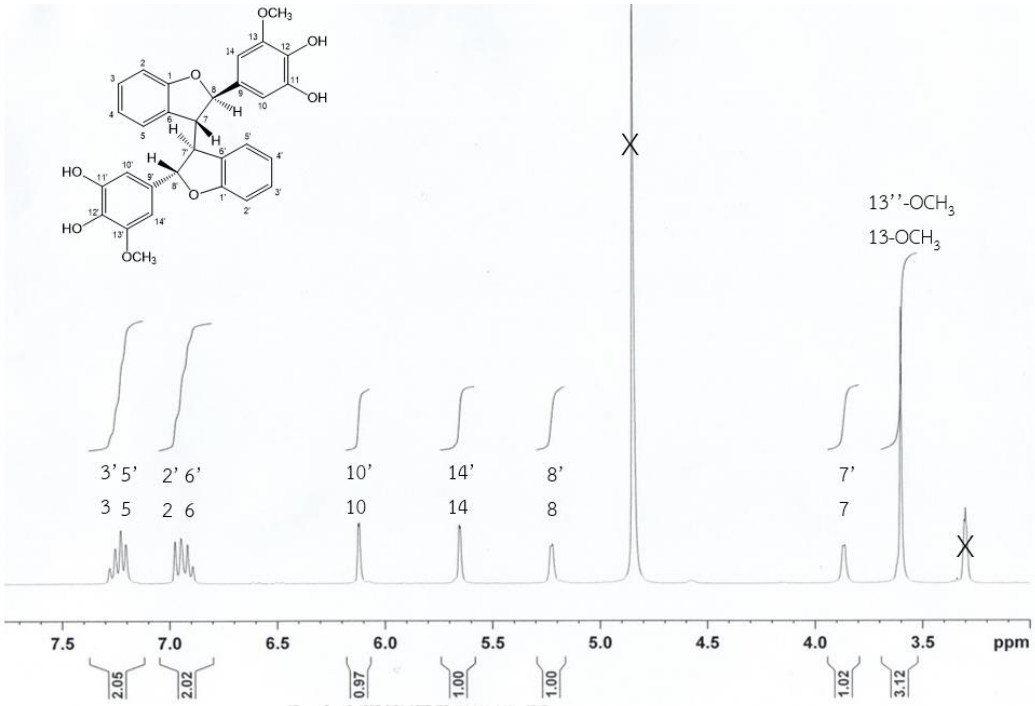


Figure 101. ^{1}H NMR spectrum of compound PD-7 (300 MHz, in CD_3OD)

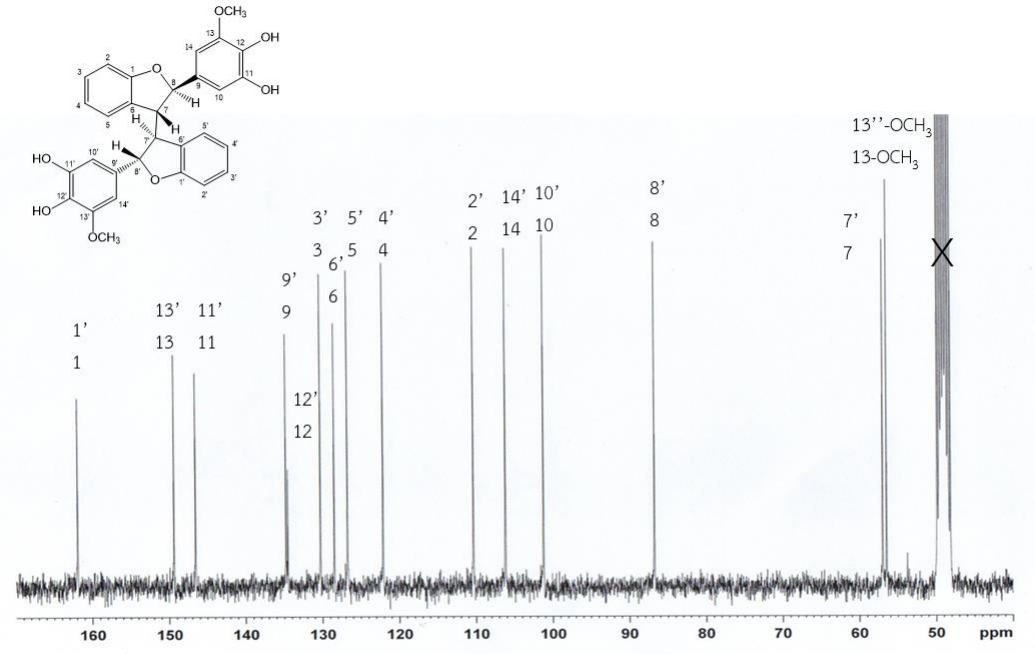


Figure 102. ^{13}C NMR spectrum of compound PD-7 (75 MHz, in CD_3OD)

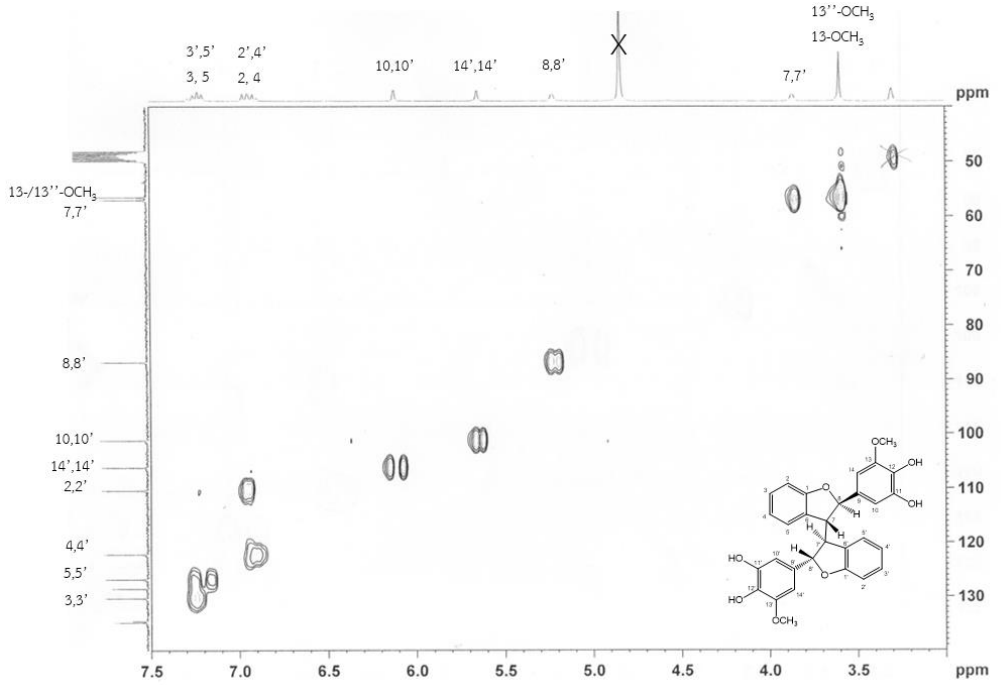


Figure 103. HSQC spectrum of compound PD-7

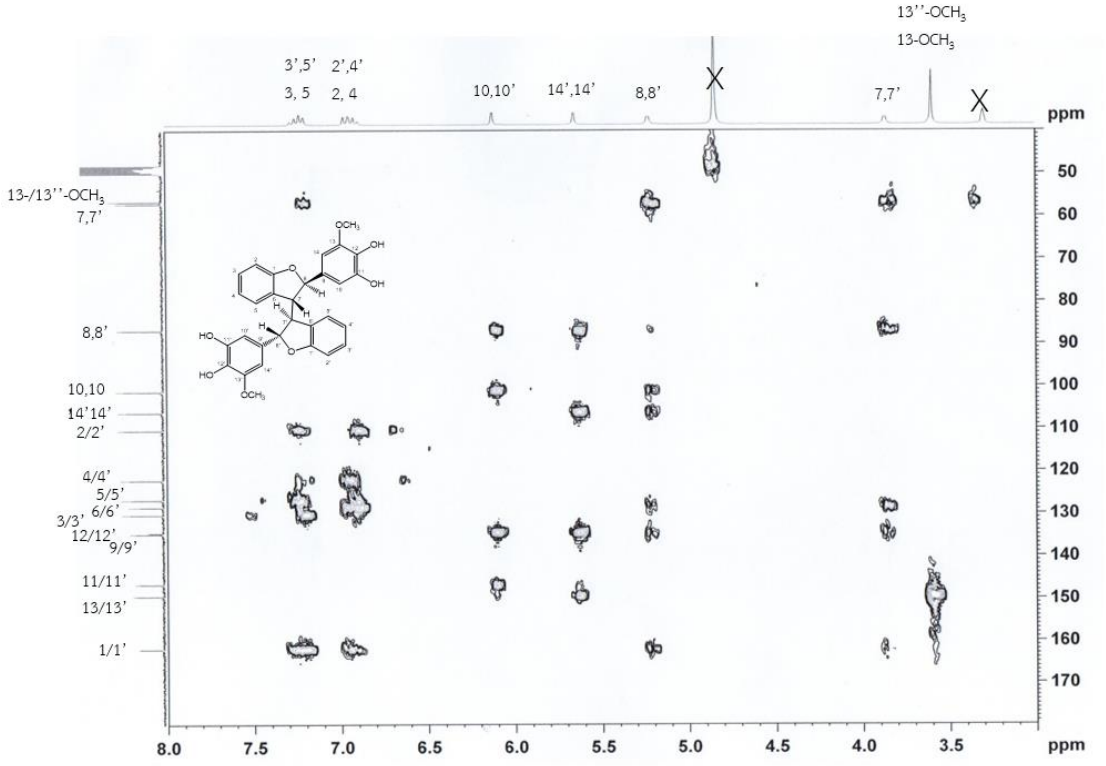


Figure 104. HMBC spectrum of compound PD-7

18. Structure elucidation of compound PD-8 (paphiodianthin B)

Compound PD-8, obtained as a brown amorphous powder (3 mg, 0.0005% yield), appeared as a brown spot on TLC upon spraying with 10% sulfuric acid and heating. Its IR spectrum (**Figure 106)** showed absorption bands of hydroxy groups at 3426 cm^{-1} and ether function of furan ring at 1197 cm^{-1}. Its molecular formula was determined as $C_{30}H_{26}O_5$ based on the $[M + Na]^+$ ion peak in the high resolution mass spectrum (**Figure 107**) at *m/z* 489.1668 (calcd. for $C_{30}H_{26}O_5Na$, 489.1678).

The ^1H NMR spectrum of compound PD-8 (**Figures 108-109** and **Table 33**) displayed two methoxy singlets at δ 3.88 (13-OCH_3) and 3.84 ppm (13'-OCH_3), two doublets of a *trans*-double bond at δ 6.62 (1H, *d, J* = 16.4 Hz, H-8) and 6.88 ppm (1H, *d, J* = 16.4 Hz, H-7), two coupled doublets at δ 4.60 (1H, *d, J* = 6.5 Hz, H-7') and 5.45 ppm (1H, *d, J* = 6.5 Hz, H-8') characteristic of a disubstituted dihydrobenzofuran moiety. Two pairs of *meta*-coupled protons from two 1,3,4,5-tetrasubstituted aromatic rings could also be observed: one at δ 6.51 (1H, *d, J* = 2.1 Hz, H-12) and 6.75 ppm (1H, *d, J* = 2.1 Hz, H-14), and the other at δ 6.47 (1H, *d, J* = 1.8 Hz, H-14') and 6.56 ppm (1H, *d, J* = 1.8 Hz, H-10'). The rest of these proton resonances were assignable to two unsubstituted phenyl moieties. Five protons from one ring resonated at δ 7.10 (2H, *m*, H-2/H-6), 7.19 (1H, *t, J* = 6.9 Hz, H-4) and 7.23 ppm (2H, *m*, H-3/H-5), while protons from another phenyl ring resonated at δ 7.21 (2H, *d*, *J* = 7.4 Hz, H-2'/H-6'), 7.26 (1H, *m*, H-4'), 7.35 ppm (1H, *d*, *J* = 7.4 Hz, H-3'/H-5'). These NMR data and its molecular formula corresponded to a stilbene dimer substituted with two hydroxy and two methoxy groups. A part of this molecule should be dihydrobenzofuran, whereas one partial structure (an unsubstituted phenyl ring connected to *trans*-double bond) would be similar to pinosylvin monomethyl ether (compound PD-2).

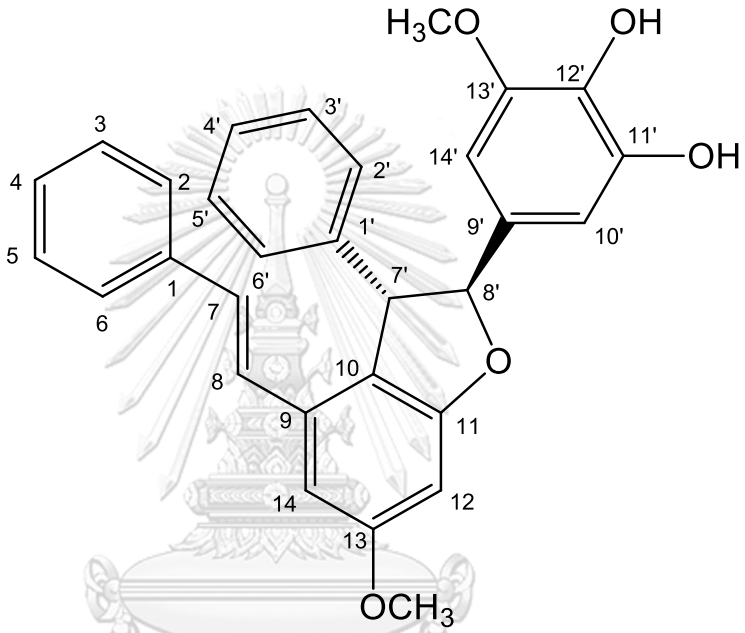
All thirty carbons peaks in the ^{13}C NMR spectrum of compound PD-8 (**Figures 110-111** and **Table 33**), could be differentiated, using the HSQC experiment (**Figures**

112-113), into those of ten quaternary carbons at δ 120.2 (C-10), 132.2 (C-12'), 133.2 (C-9'), 134.9 (C-9), 137.0 (C-1), 143.0 (C-1'), 143.9 (C-11'), 147.0 (C-13'), 161.2 (C-13) and 161.4 ppm (C-11), fourteen aromatic methine carbons at δ 95.5 (C-12), 100.5 (C-14'), 102.8 (C-14), 106.2 ppm (C-10'), 126.4 (C-2/C-6), 127.2 (C-4'), 127.7 (C-4), 127.9 (C-2'/C-6'), 128.5 (C-3/C-5) and 129.0 (C-3'/C-5'), two aliphatic methine carbons at δ 56.9 (C-7') and 93.6 ppm (C-8'), two olefinic methine carbons at δ 125.2 (C-8) and 129.9 ppm (C-7), and two methoxy carbons at δ 55.6 (13-OCH_3) and 56.2 ppm (13'-OCH_3).

The HMBC spectrum (**Figures 114-117**) displayed long-range cross-peaks between H-7 and C-9 at δ 134.9 ppm, as well as between H-8 and C-10 (at δ 120.2 ppm) and C-14 (at δ 102.8 ppm), which confirmed the connection of the phenylethylene moiety to the tetrasubstituted aromatic ring at position 9. This aromatic ring is a part of the benzofuran nucleus, as confirmed by HMBC cross-peaks from H-7' to C-10 and C-11 (at δ 161.4 ppm), and also from H-8' to C-11. A methoxy group could be located at position 13, based on HMBC correlations from H-12, H-14 and 13-OCH_3 to C-13 at δ 161.2 ppm. On the dihydrofuran ring, one phenyl group could be connected to C-7', based on cross-peaks between this carbon (at δ 56.9 ppm) and H-2'/H-6', and between H-7' and C-1' at δ 143.0 ppm. Another aromatic ring, substituted with one methoxy and two hydroxy groups, was located at C-8' based on the HMBC correlations between this carbon at δ 93.6 ppm and H-14' and between the signals of H-7' and C-9' at δ 133.2 ppm. The position of methoxy group at C-13' on this ring was determined from correlations between the methoxy proton and H-14' to this carbon at δ 147.0 ppm. The relative configuration of H-7' and H-8' was determined as *trans* based on comparison of their coupling constant with a similar stilbene dimer, phragmidimer B (112).

Therefore, compound PD-8 was elucidated as a new stilbene dimer with benzofuran nucleus and was given the trivial name paphiodianthin B. Closely similar

stilbene dimers have previously been found in some *Phragmipedium* orchids (112), which are members of the same subfamily (Cypripedioideae) as *Paphiopedilum dianthum*.



Chemical structure of paphiodianthin B

Table 34. NMR spectral data of compound PD-8 (in $CDCl_3$)

Position	δ_H (mult., *J* in Hz)	δ_C	HMBC correlation with
1	-	137.0	-
2	7.10 (*m*)	126.4	C-1, C-4, C-7
3	7.23 (*m*)	128.5	C-1
4	7.19 (*t,* 6.9)	127.7	-
5	7.23 (*m*)	128.5	C-1
6	7.10 (*m*)	126.4	C-1, C-4, C-7
7	6.88 (*d*, 16.4)	129.9	C-2, C-6, C-9
8	6.62 (*d*, 16.4)	125.2	C-1, C-10, C-14
9	-	134.9	-
10	-	120.2	-
11	-	161.4	-
12	6.61 (*d*, 2.1)	95.5	C-10, C-11, C-13, C-14
13	-	161.2	-
14	6.75 (*d*, 2.1)	102.8	C-8, C-10, C-12, C-13
1'	-	143.0	-
2'	7.21 (*d*, 7.4)	127.9	C-4', C-6', C-7'
3'	7.35 (*t,* 7.4)	129.0	C-1', C-5'
4'	7.26 (*m*)	127.2	-
5'	7.35 (*t,* 7.4)	129.0	C-1', C-3'
6'	7.21 (*d*, 7.4)	127.9	C-2', C-4', C-7'
7'	4.60 (*d*, 6.5)	56.9	C-10, C-11, C-1', C-8', C-9'

Position	δ_H (mult., *J* in Hz)	δ_C	HMBC correlation with

8'	5.45 (*d*, 6.5)	93.6	C-11, C-1', C-7', C-9', C-14'
9'	-	133.2	-
10'	6.56 (*d*, 1.8)	106.2	C-12', C-14'
11'	-	143.9	-
12'	-	132.2	-
13'	-	147.0	-
14'	6.47 (*d*, 1.8)	100.5	C-8', C-10', C-12', C-13'
13-OCH_3	3.88 (*s*)	55.6	C-13
13''-OCH_3	3.84 (*s*)	56.2	C-13''

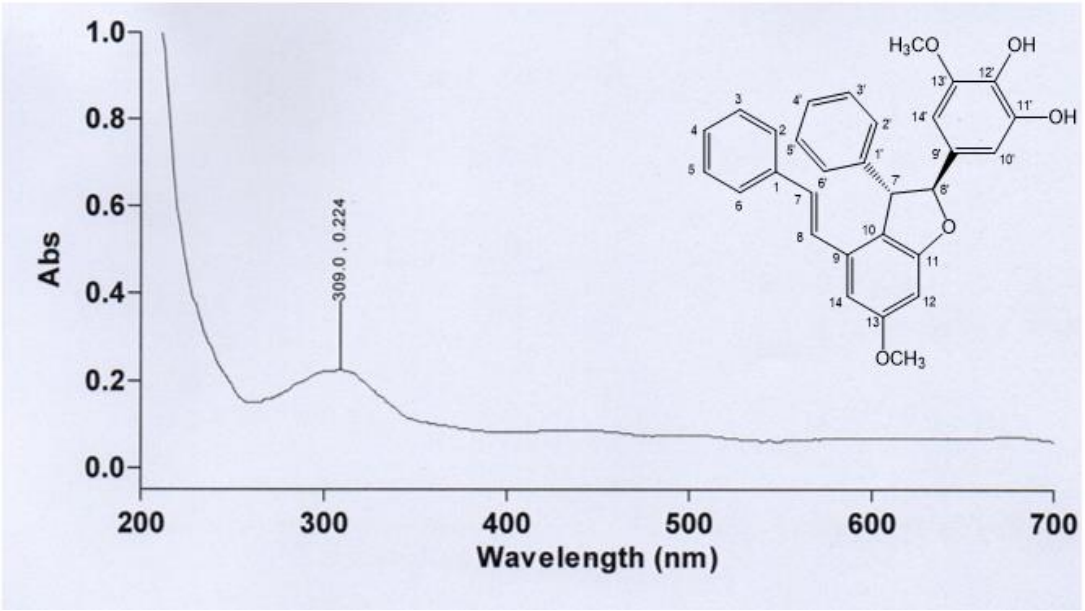


Figure 105. UV spectrum of compound PD-8

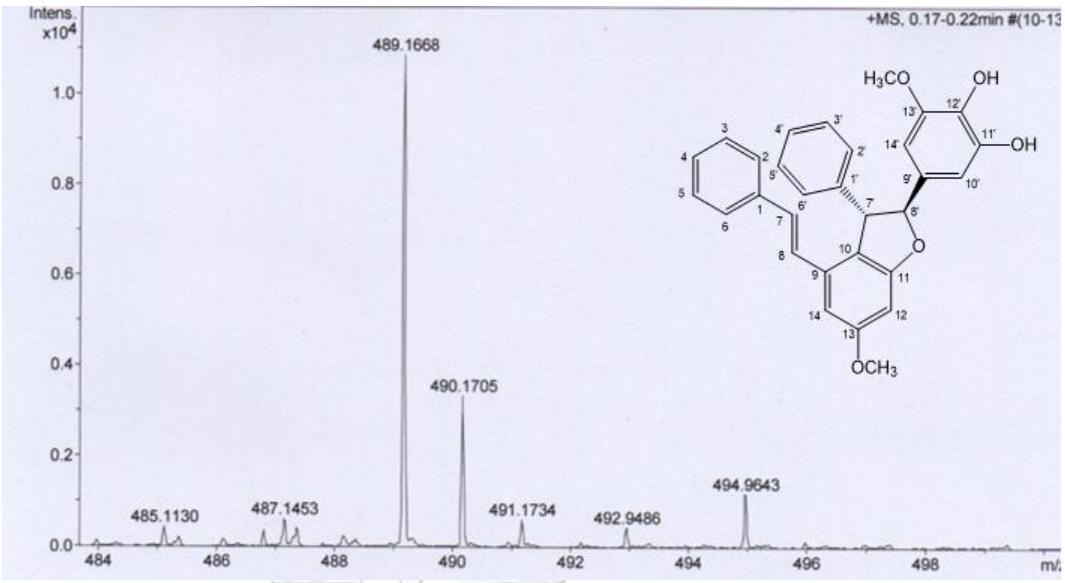


Figure 106. HR-ESI mass spectrum of compound PD-8

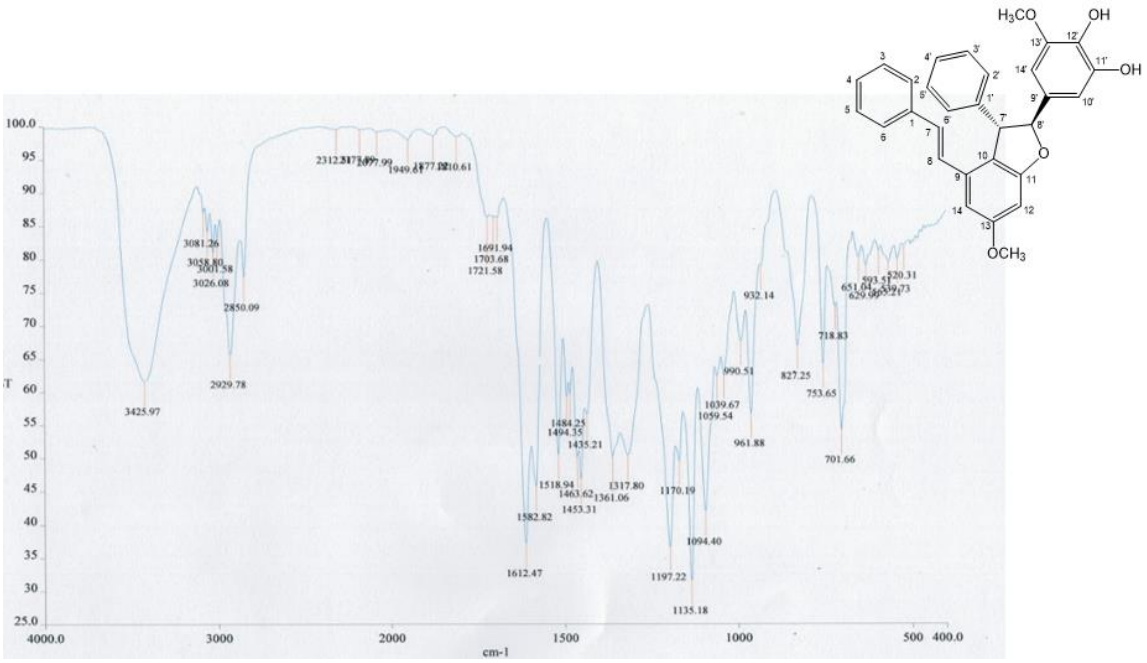


Figure 107. IR spectrum of compound PD-8

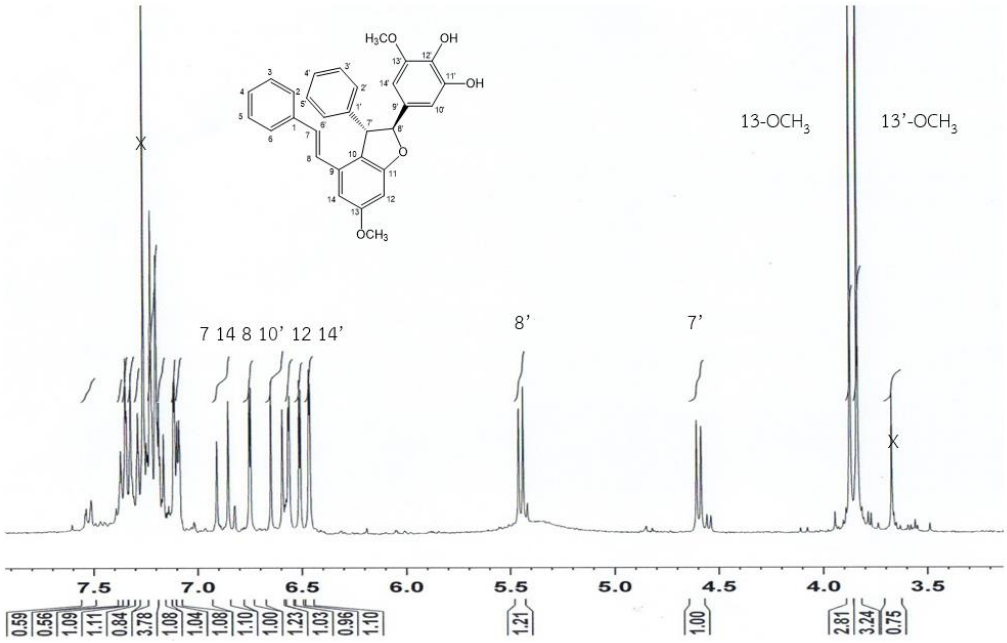


Figure 108. ^{1}H NMR spectrum of compound PD-8 (300 MHz, in $CDCl_3$)

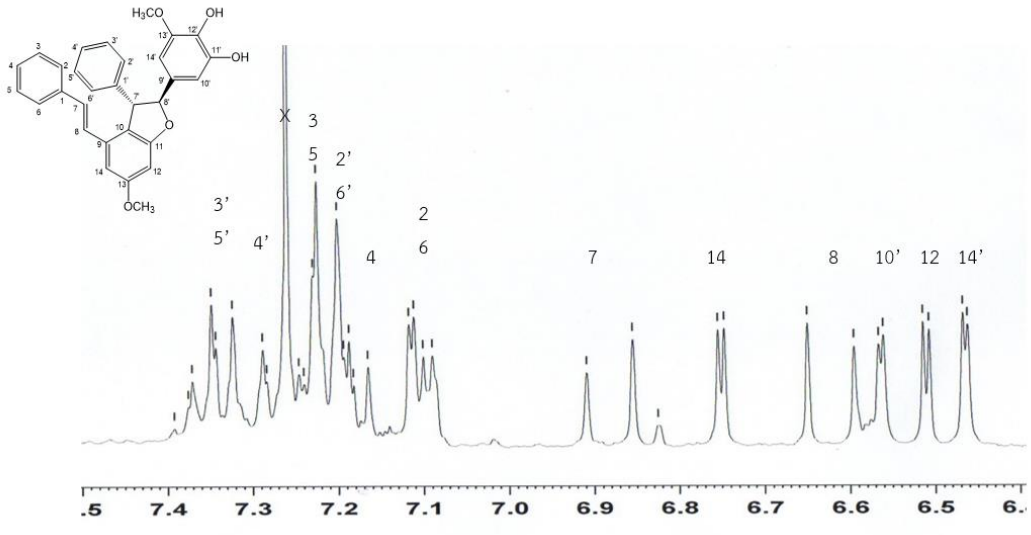


Figure 109. 1H NMR spectrum of compound PD-8 (300 MHz, in CDCl3, expansion between δH 6.4-7.5 ppm)

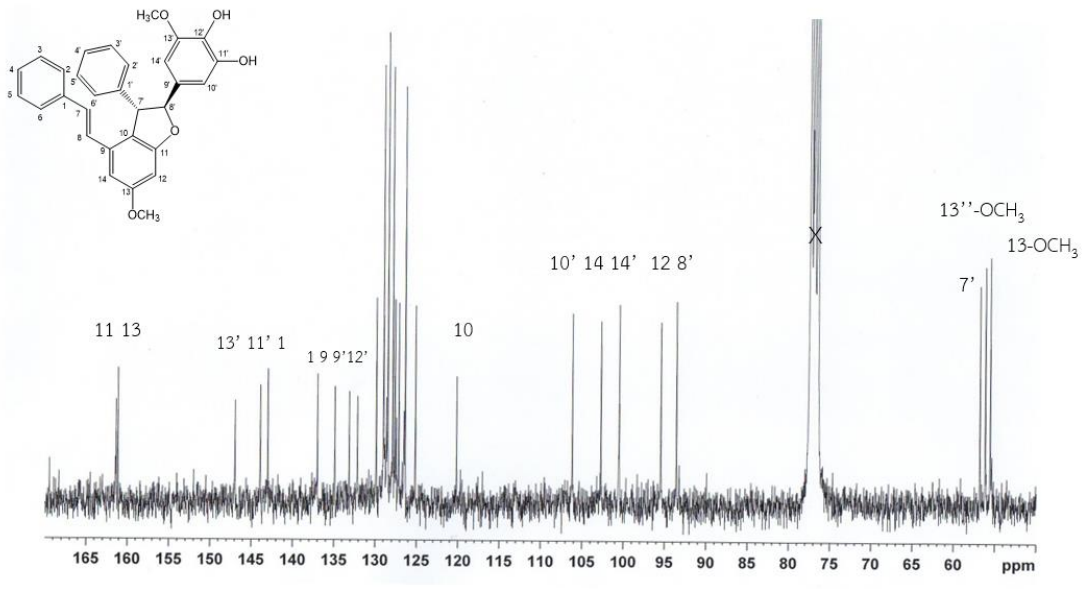


Figure 110. ^{13}C NMR spectrum of compound PD-8 (75 MHz, in $CDCl_3$)

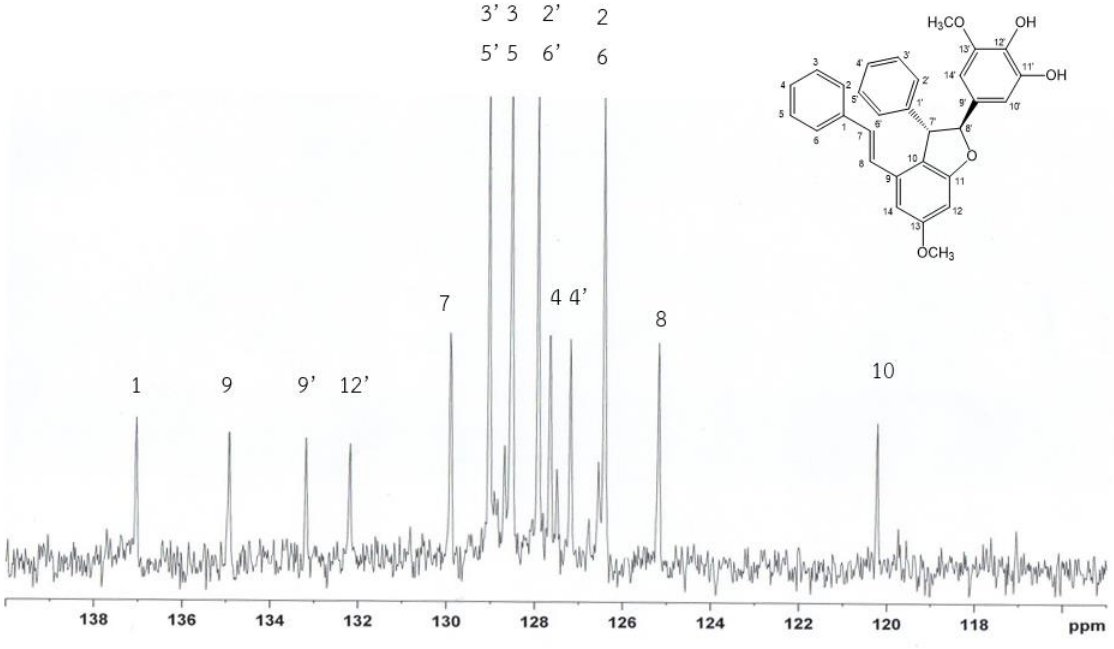


Figure 111. ^{13}C NMR spectrum of compound PD-8 (75 MHz, in $CDCl_3$, expansion between δ_C 118-139 ppm)

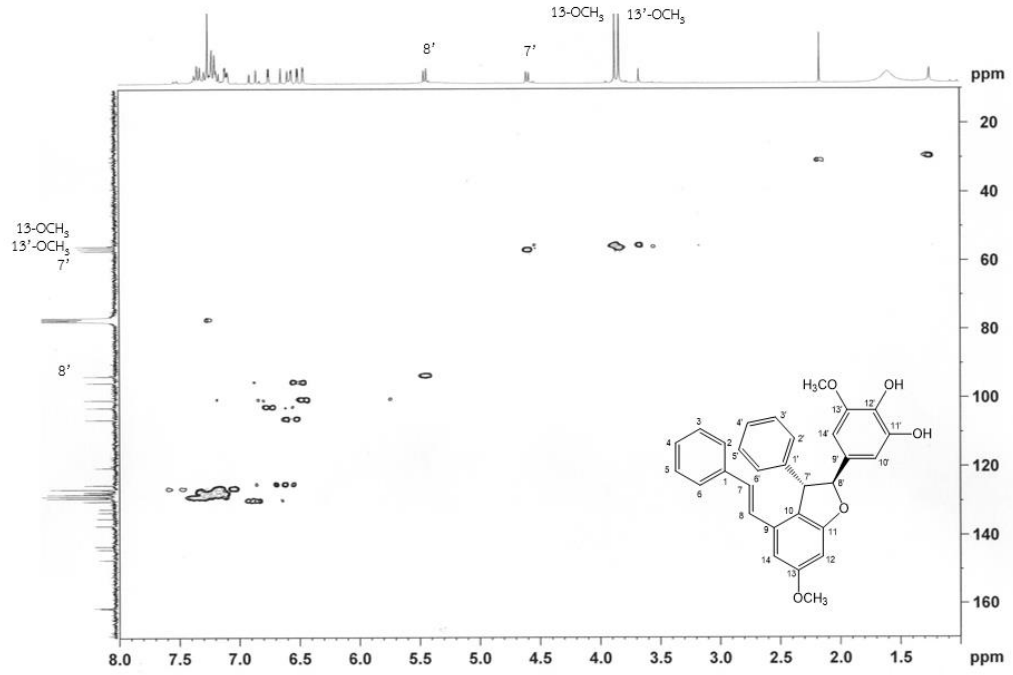


Figure 112. HSQC spectrum of compound PD-8

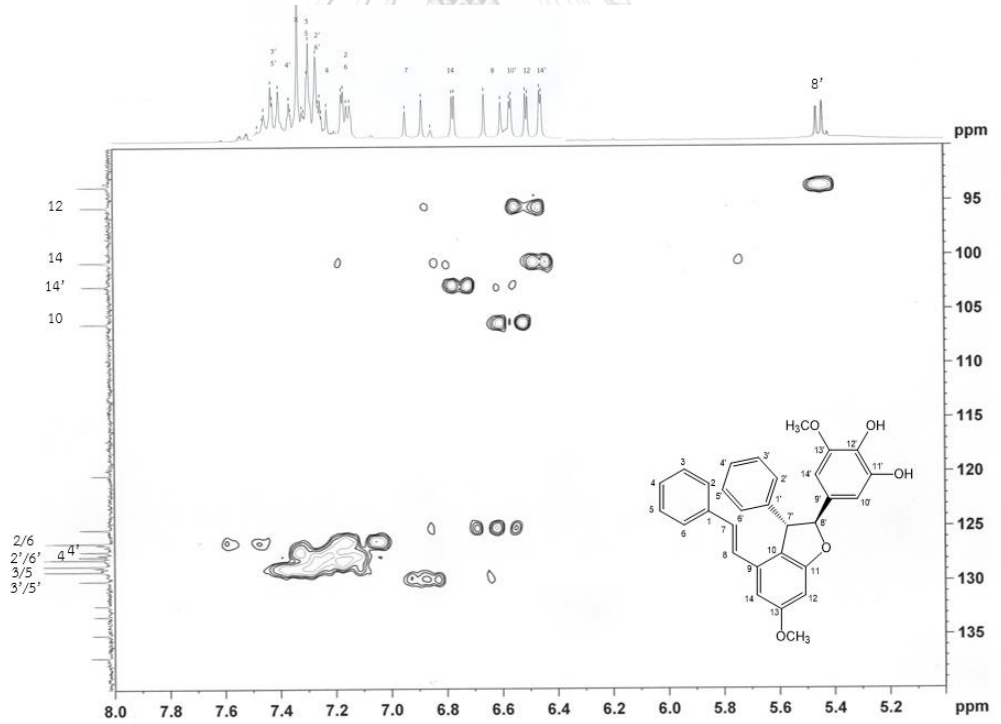


Figure 113. HSQC spectrum of compound PD-8 (expansion between δ_H 5.1-8.0 ppm, δ_C 90-140 ppm)

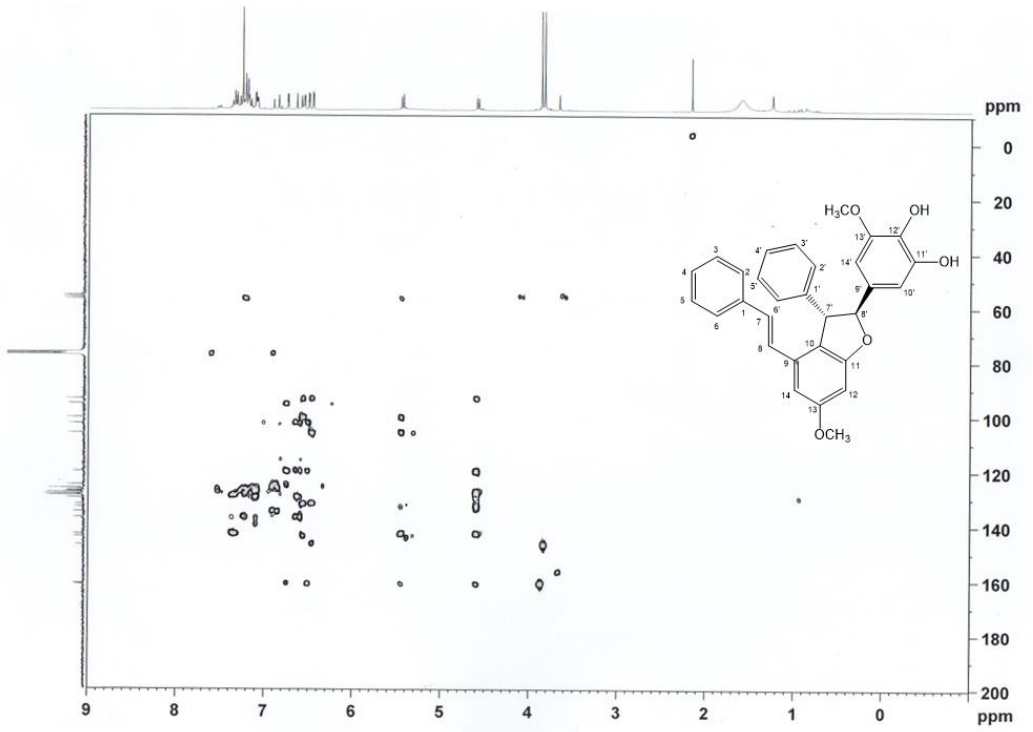


Figure 114. HMBC spectrum of compound PD-8

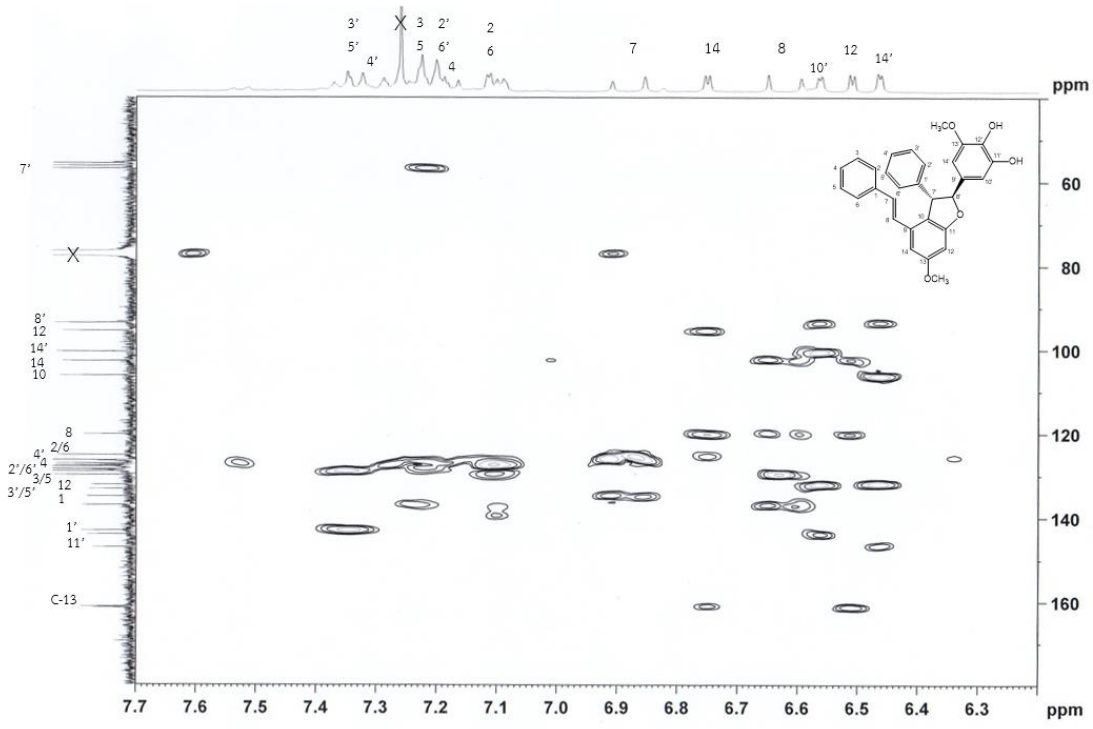


Figure 115. HMBC spectrum of compound PD-8 (expansion between δ_H 6.2-7.7 ppm, δ_C 50-180 ppm)

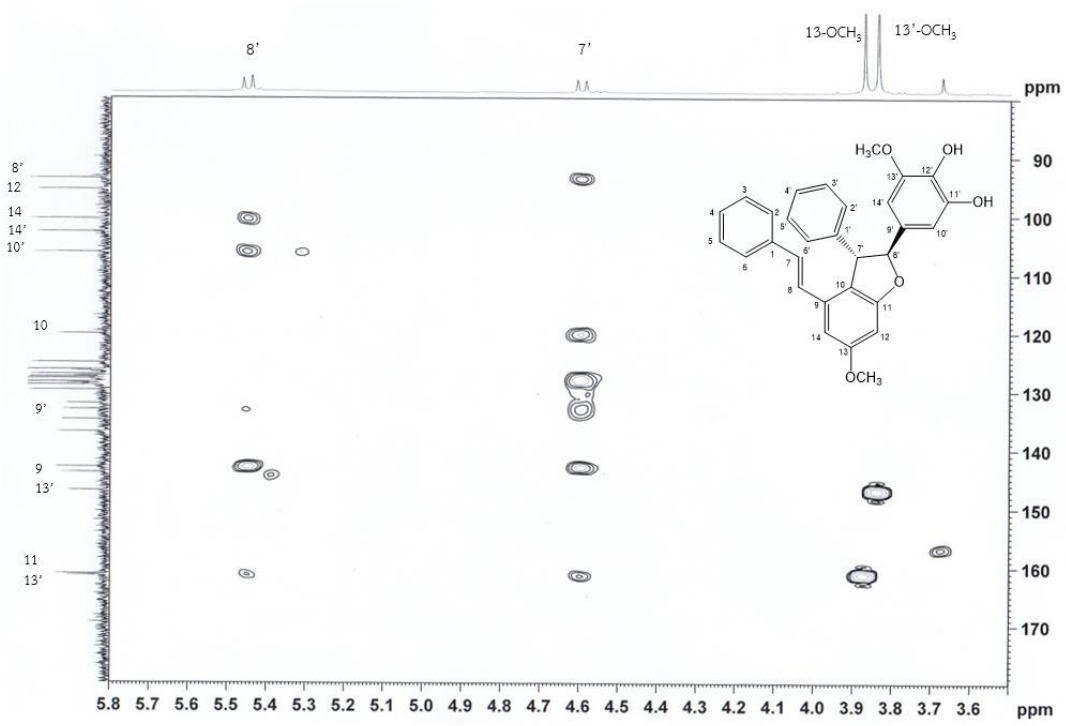


Figure 116. HMBC spectrum of compound PD-8 (expansion between δ_H 3.5-5.8 ppm, δ_C 80-180 ppm)

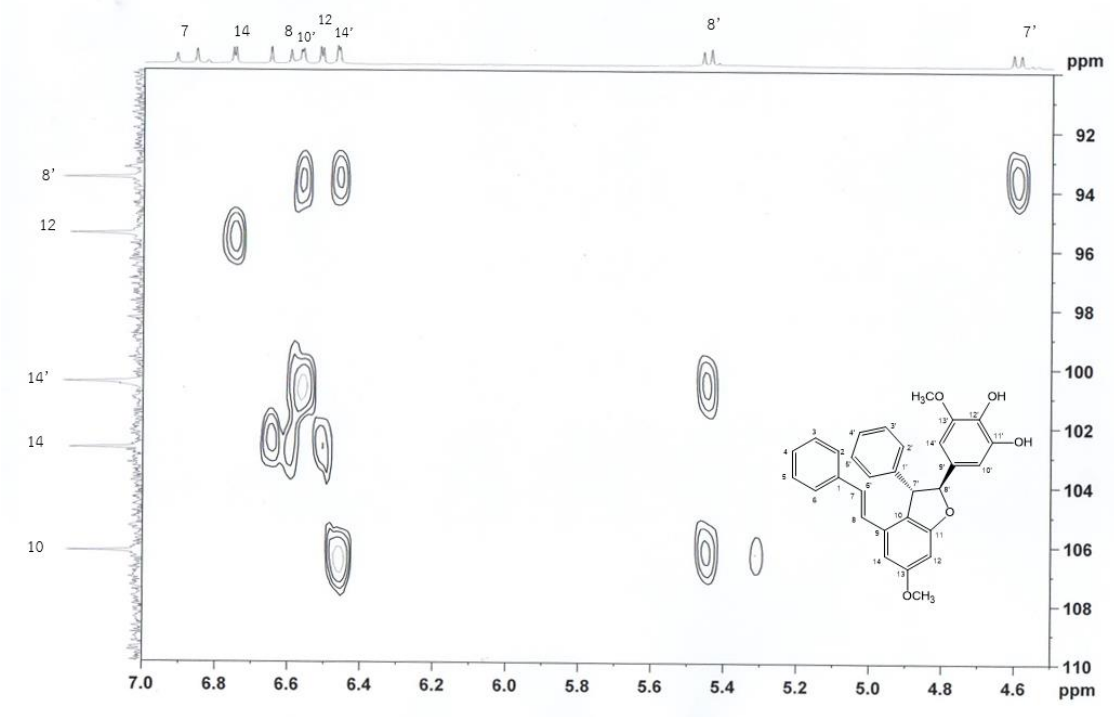


Figure 117. HMBC spectrum of compound PDL-1 (expansion between δ_H 4.5-7.0 ppm, δ_C 90-110 ppm)

19. Chemotaxonomic significance

At the present, although more than 450 natural phenanthrene derivatives have been discovered (46), only a small number of phenanthrenequinones have been reported. Most phenanthrenequinones have been found in orchids, for example, in some *Dendrobium* (226, 227) and *Pleione* species (228, 229). These are orchids from the largest subfamily Epidendroideae. An exception was a mention of a skin-sensitizing phenanthrenequinone from a lady's slipper orchid, *Cypripedium calceolus,* which belongs to a different subfamily i.e. Cypripedioideae (110). In this study, two known phenanthrenequinones (compounds CF-1 and CF-2) have been isolated from *Cymbidium finlaysonianum*. The genus *Cymbidium* is a member of tribe Cymbidieae within subfamily Epidendroideae. Other genera within the same tribe that have been reported to contain phenanthrenequinones or dihydrophenanthrenequinones are *Cyrtopodium* (70), *Odontoglossum* (106) and *Oncidium* (105). There are eleven subtribes within tribe Cymbidieae and genus *Cymbidium* belongs to subtribe Cymbidiinae. Currently, there is no report of phenanthrenequinone derivatives from other genera in this subtribe. However, nearly all (three out of four) *Cymbidium* species and *Cymbidium* hybrids chemically investigated thus far contained at least one phrenanthrenequinone (11, 14).

Recently, studies on the chemical constituents of *Paphiopedilum* orchids have been done (16-18) and, although only three *Paphiopedilum* species have been explored, some interesting observations could be made. Genus *Paphiopedilum* belongs to different subfamily (Cypripedioideae) from that of genus *Cymbidium*. The pattern of compounds isolated was, thus, rather different from that of *Cymbidium finlaysonianum*. In this study, a number of stilbenoids were isolated from *Paphiopedilum dianthum*. However, none of them were phenanthrene derivatives or bibenzyls, as found in the first plant. Instead, two constituents of this orchid with the highest yields (compounds PD-2 and PD-5) were *trans*-stilbenes. Some of the isolated stilbenes, e.g. compounds PD-5 and PD-6, contains 2-hydroxyphenyl moiety which

facilitates cyclization of the central ethylene chain of stilbene nucleus into the furan ring of 2-phenylbenzofuran stilbenes previously obtained from *Paphiopedilum godefroyae* (16), as well as the dimeric compound PD-7 isolated from this orchid. The occurrence of these phenylbenzofurans appears to be rather limited to a few members of subfamily Cypripedioideae, including another genus (*Phragmipedium*) (15, 112), and might be a good chemotaxonomic marker.

Three flavonoids (galangin, isalpinin and pinocembrin) were also obtained from *P. dianthum* in adequate yields. These flavonoids share the same characteristic ring B with no substituent. Although they can be found in many natural sources, one notable source of these flavonoids are several medicinal plants in the monocotyledonous family Zingiberaceae.

20. Cytotoxicity of isolated compounds

Fourteen stilbenoids from *C. finlaysonianum* and *P. dianthum*, as well as three flavonoids from *P. dianthum*, were assayed for their cytotoxic effect against two cancer cell lines (wild type MCF-7 and Caco-2), two chemotherapy-resistant cancer cell sublines (doxorubicin-resistant MCF-7 and mitoxantrone-resistant MCF-7) and one normal fibroblast cell line (NIH/3T3). All ten compounds from the first orchids were also tested against another cancer cell line, NCI-H187. Doxorubicin was used as a positive control and tested compounds showing the IC_{50} value of less than 10 μM were considered to be strongly active, whereas the IC_{50} value of more than 100 μM was considered to be inactive. Due to its very poor yield, compound CF-8 was submitted to cytotoxicity assay against NCI-H187 cell line only and found to be nearly inactive. Results of these assays are presented in **Tables 35** and **36**.

All ten compounds obtained from *C. finlaysonianum* are stilbenoids. They can be divided into five subgroups: one phenanthrenequinone (compound CF-1), one 9,10-dihydrophenanthrenequinone (compound CF-2), two phenanthrenes (compounds CF-

5 and CF-6), five dihydrophenanthrenes (compounds CF-3, CF-4, CF-7, CF-8 and CF-10) and one bibenzyl (compound CF-9). Compound CF-1 displayed the strongest cytotoxic activity against NCI-H187 cell line. Although it was not as active as doxorubicin, it was more cytotoxic toward this cancer cell line than ellipticine, which was another positive control used only in the resazurin assay. Saturation of the 9,10-double bond, as in compound CF-2, led to decreasing of cytotoxic activity to moderate level. Other stilbenoids from this orchid were even less active against this cell line, and some of them (compounds CF-8 and CF-9) were inactive or nearly inactive. The quinone nature of both compounds CF-1 and CF-2 should be noted, since some anticancer agents e.g. doxorubicin and mitoxantrone are also quinone compounds. Furthermore, a previous study on natural and semi-synthetic phenanthrenequinones had proposed that generation of intracellular reactive oxygen species (ROS) was the underlying mechanism for their cytotoxicity (230).

When these stilbenoids were tested against wild type MCF-7 cell line, both quinonoids CF-1 and CF-2 were also strongly cytotoxic to this cell line, but the dihydrophenanthrene CF-7 appeared to be the most active compound isolated, exhibiting an almost equal IC_{50} value to that of doxorubicin. Two other dihydrophenanthrenes that were strongly active, though less active than compound CF-7, were compounds CF-3 and CF-10. The chemical structures of these dihydrophenanthrenes are similar, with an addition of a methoxy group at C-6 in compound CF-3 and additions of a methoxy group at C-6 and a *para*-hydroxybenzyl group at C-1 in compound CF-10. Therefore, it could be inferred that these additions led to slightly decreased cytotoxicity against this cell line. Other stilbenoids were moderately cytotoxic to MCF-7 cells. It is interesting to note that all isolated compounds from *C. finlaysonianum* (**Figure 118**) (except compound CF-8 which was not tested) were less cytotoxic than doxorubicin to normal cells (NIH/3T3).

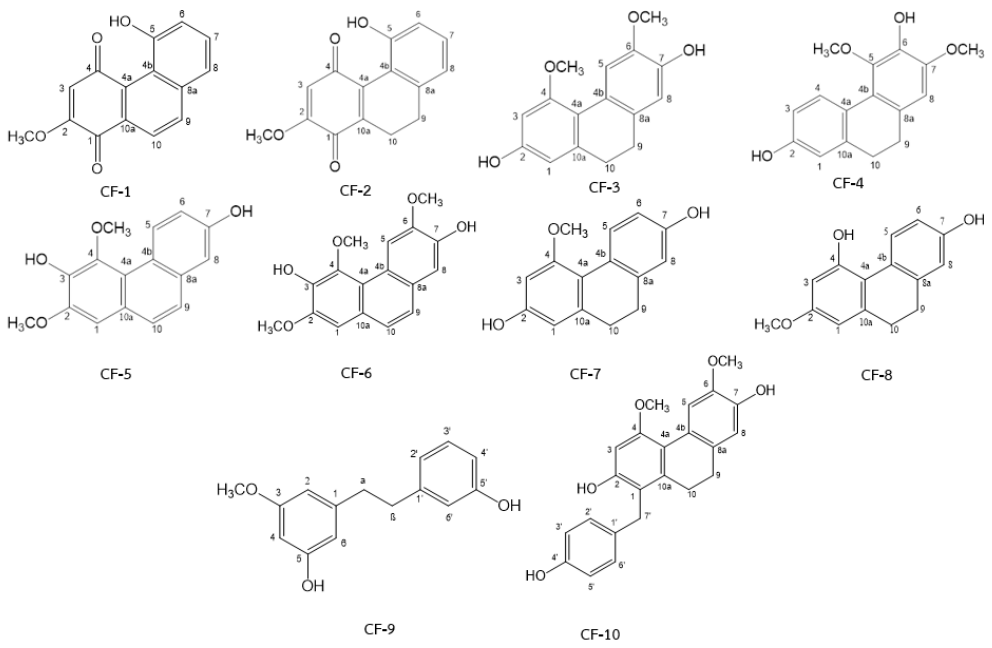


Figure 118. The isolated compounds from *Cymbidium finlaysonianum*

These stilbenoids were also assayed for their cytotoxicity against doxorubicin-resistant MCF-7 cell subline (MCF-7/DOX) and mitoxantrone-resistant MCF-7 cell subline (MCF-7/MX). As could be expected, compound CF-1 was less active, but still borderline strongly active, against MCF-7/DOX cells. The cytotoxic effect of compound CF-7 also decreased to moderate level, but about 2 times higher than that of doxorubicin. Other compounds were moderately active against these resistant cells. Similar results were obtained when they were assayed against MCF/MX cells; the isolated compounds were either moderately active or inactive. Comparison between assay results of compounds CF-3 and CF-10 indicated that the addition of a *para*-hydroxybenzyl group at C-1 in compound CF-10 led to about 2 times higher cytotoxicity to these mitoxantrone-resistant cancer cells.

The results of cytotoxic assay of *C. finlaysonianum* chemical constituents against Caco-2 cell line were similar to their effects against wild type MCF-7 cells, except in this case compound CF-7 exhibited only moderate activity, while both

quinonoids CF-1 and CF-2 were the most active ones, with an IC_{50} value of compound CF-1, nearly equal to that of doxorubicin. Other six stilbenoids tested were moderately cytotoxic to Caco-2 cell line. These stilbenoids isolated from the first orchid were mostly moderately cytotoxic to normal fibroblast cell line (NIH/3T3), while one compound (CF-4) was non-toxic.

Table 35. Cytotoxicity of compounds isolated from *Cymbidium finlaysonianum*

Compound	Cytotoxicity (IC_{50} in μM)					
	NCI-H187	MCF-7	MCF-7/DOX	MCF-7/MX	Caco-2	NIH/3T3
CF-1	3.73	1.73 ± 1.94	9.58 ± 1.71	13.36 ± 0.87	2.47 ± 1.04	11.97 ± 2.07
CF-2	14.40	1.99 ± 1.94	13.24 ± 0.95	29.55 ± 1.13	3.28 ± 1.01	14.53 ± 1.29
CF-3	52.48	7.18 ± 1.37	32.35 ± 0.96	34.42 ± 2.78	21.89 ± 1.05	18.95 ± 1.21
CF-4	24.38	39.25 ± 1.14	77.27 ± 0.59	Inactive	60.66 ± 0.75	Inactive
CF-5	30.34	34.57 ± 1.44	73.39 ± 0.49	Inactive	52.30 ± 0.97	58.53 ± 1.04
CF-6	59.67	52.27 ± 1.45	70.42 ± 0.73	66.04 ± 1.04	44.48 ± 0.90	45.51 ± 1.10
CF-7	71.94	0.91 ± 1.11	10.32 ± 0.97	49.14 ± 1.43	41.64 ± 1.06	10.28 ± 0.93
CF-8	98.65	ND	ND	ND	ND	ND
CF-9	Inactive	19.14 ± 1.56	33.43 ± 0.95	32.47 ± 1.92	22.01 ± 1.19	33.96 ± 1.64
CF-10	49.89	5.41 ± 4.02	68.57 ± 1.14	16.28 ± 1.81	65.03 ± 1.60	27.48 ± 1.13
Doxorubicin	0.24	0.90 ± 2.37	25.25 ± 2.27	6.55 ± 2.59	2.45 ± 0.58	3.33 ± 1.01

ND = not determined

Eight compounds obtained from *P. dianthum* (**Figure 119**) can be separated into two major groups: stilbenoids (compounds PD-2, PD-5 to PD-8) and flavonoids (compounds PD-1, PD-3 and PD-4). The stilbenes PD-2 and PD-5 were obtained in relatively high yields of around 0.3-0.4% each. When assayed against wild type MCF-7 cell line, the stilbenoid compound PD-6 exhibited potent cytotoxic effect, comparable to doxorubicin. Other strongly cytotoxic compounds were also stilbenoids, including compounds PD-2, PD-7 and PD-8. Only one stilbene, compound PD-5, was moderately

cytotoxic, whereas all flavonoids tested were also moderately toxic to MCF-7 cells. Both dimeric stilbenoids PD-7 and PD-8 were strongly cytotoxic. Compound PD-7 is a phenylbenzofuran dimer, while compound PD-8 can also be considered a phenylbenzofuran. Interestingly, compounds PD-2 and PD-5, which are different only in an additional hydroxy group at C-2' in compound PD-5, exhibited different levels of cytotoxicity: PD-2 was strongly cytotoxic, whereas PD-5 was moderately cytotoxic. Therefore, the presence of this hydroxy substituent might lessen the cytotoxic effect. However, further addition of a *para*-hydroxybenzyl group to position 2' of compound PD-5, as in compound PD-6, appeared to restore the cytotoxicity toward this cell line to strong level.

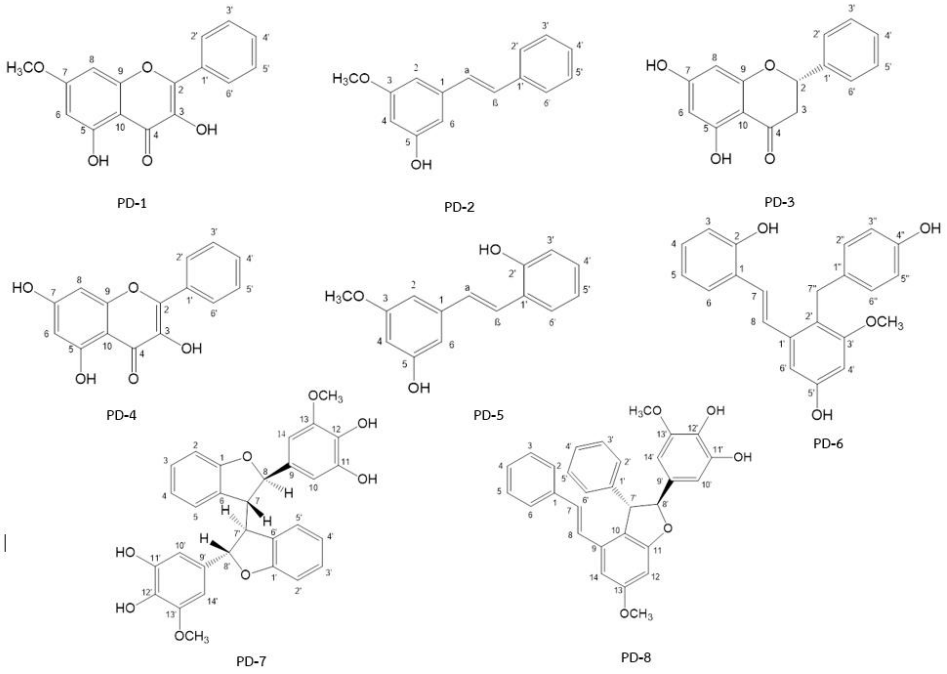


Figure 119. The isolated compounds from *Paphiopedilum dianthum*

When these chemical constituents of *P. dianthum* were tested against the doxorubicin-resistant and mitoxantrone-resistant sublines of MCF-7 cells, both compounds PD-6 and PD-7 retained their strong cytotoxicity and were even more active than doxorubicin against both cell sublines. It should be noted that the flavonoid PD-1, although moderately active, was more cytotoxic (exhibiting lower IC_{50} value) to the doxorubicin-resistant cell subline than to its wild type cell line.

All stilbenoids with larger molecules, e.g. compounds PD-6, PD-7 and PD-8, were strongly cytotoxic to Caco-2 cell line. Again, compound PD-6 exhibited the strongest cytotoxicity, which was about 4-5 times higher than that of doxorubicin. All three flavonoids tested were either moderately cytotoxic or non-toxic.

All of the isolated flavonoids were inactive against normal cell line (NIH/3T3), but the strongly cytotoxic compounds PD-6 and PD-7 were also toxic to these fibroblasts and might limit their usefulness. However, compound PD-6 appeared to be more potent than doxorubicin in all cell lines tested and, thus, should be further studied. Another worthy candidate to be developed as anticancer agent might be compound PD-8, which displayed strong cytotoxic activity against both MCF-7 and Caco-2 cancer cell lines but was non-toxic to normal cells. Interesting results were also obtained for compound PD-2, which was isolated in good yield from the orchid and exhibited strong cytotoxicity to MCF-7 cells but its toxic effect on normal cells was moderate.

The most potently cytotoxic compound isolated in this study is the stilbene compound PD-6. This result is in accordance with current interest in the role of natural stilbenes as anticancer agents. Several recent review articles on this topic have been published (231-233).

Table 36. Cytotoxicity of compounds isolated from *Paphiopedilum dianthum*

Compound	Cytotoxicity (IC_{50} in μM)				
	MCF-7	MCF-7/DOX	MCF-7/MX	Caco-2	NIH/3T3
PD-1	76.24 ± 1.46	24.33 ± 1.10	Inactive	32.09 ± 1.24	Inactive
PD-2	6.20 ± 1.24	24.45 ± 0.77	45.79 ± 1.42	12.74 ± 1.69	30.75 ± 0.54
PD-3	17.43 ± 0.68	41.09 ± 1.33	Inactive	Inactive	Inactive
PD-4	22.44 ± 1.56	Inactive	Inactive	54.15 ± 1.02	Inactive
PD-5	19.31 ± 0.50	47.66 ± 1.20	56.27 ± 1.29	18.38 ± 1.05	31.78 ± 0.81
PD-6	0.50 ± 1.87	0.55 ± 0.57	0.57 ± 1.04	0.55 ± 0.97	1.57 ± 2.58
PD-7	2.08 ± 3.10	1.80 ± 0.98	2.19 ± 1.30	3.44 ± 1.07	0.93 ± 1.51
PD-8	6.24 ± 1.32	Inactive	75.12 ± 1.71	6.74 ± 0.95	Inactive
Doxorubicin	0.90 ± 2.37	25.25 ± 2.27	6.55 ± 2.59	2.45 ± 0.58	3.33 ± 1.01

CHAPTER V

CONCLUSION

Phytochemical study of the whole plants of *Cymbidium finlaysonianum*, an epiphytic orchid native to Thailand, led to the isolation of nine known and one new stilbenoids. The new compound was determined as 1-(4-Hydroxybenzyl)-2,7-dihydroxy-4,6-dimethoxy-9,10-dihydrophenanthrene (CF-10), while other compounds were one phenanthrenequinone, cymbinodin A (CF-1), one 9,10-dihydrophenanthrenequinone, ephemeranthoquinone B (CF-2), one bibenzyl, batatasin III (CF-9), two phenanthrenes, 2,4-dimethoxy-3,7-dihydroxyphenanthrene (CF-5) and 3,7-dihydroxy-2,4,6-trimethoxyphenanthrene (CF-6) and four dihydrophenanthrenes including coelonin (CF-7), 6-methoxycoelonin (CF-3), flavanthridin (CF-4) and lusianthridin (CF-8). Revision of the chemical structure of cymbinodin A has been proposed. The occurrence of phenanthrenequinone derivatives in orchids is quite rare. However, most *Cymbidium* orchids investigated up to the present contain at least one phenanthrenequinone, and, hence, this type of constituents might be a chemotaxonomic marker of the genus *Cymbidium*. These isolated compounds were assayed for their cytotoxicity against lung (NCI-H187), colon (Caco-2) and breast cancer cell lines (MCF-7), plus two resistant MCF-7 sublines (MCF-7/DOX and MCF-7/MX). Cymbinodin A was the most cytotoxic compound isolated from this orchid. It exhibited the strongest cytotoxicity against almost all cancer cell lines tested, except in the case of wild type MCF-7 cell line, in which coelonin was more cytotoxic. Other compounds from *C. finlaysonianum* were mostly moderately cytotoxic to the cancer cell lines and a representative normal cell line (the fibroblast NIH/3T3).

Similar investigation of the chemical constituents of the roots and leaves of *Paphiopedilum dianthum*, a lady's slipper orchid native to northern Thailand, afforded

two new stilbene dimers, paphiodianthins A (PD-7) and B (PD-8), three known stilbenes i.e. pinosylvin monomethyl ether (PD-2), 2,3'-dihydroxy-5'-methoxystilbene (PD-5) and (*E*)-2,5'-dihydroxy-2'-(4-hydroxybenzyl)-3'-methoxystilbene (PD-6), and three known flavonoids i.e. isalpinin (PD-1), pinocembrin (PD-3) and galangin (PD-4). *Trans*-stilbenes were found in abundance in the roots of this orchid, some of which contain the characteristic 2-hydroxyphenyl moiety that could facilitate the cyclization of these stilbenes into phenylbenzofurans. The limited distribution of phenylbenzofuran derivatives within a few members of subfamily Cypripedioideae might be an important chemotaxonomic information on the subfamily. When tested against MCF-7, MCF-7/DOX, MCF-7/MX and Caco-2 cancer cell lines, (*E*)-2,5'-dihydroxy-2'-(4-hydroxybenzyl)-3'-methoxystilbene and paphiodianthin A were the most cytotoxic constituents from this orchid, but they were also cytotoxic to normal cell line. Promising results were also obtained from pinosylvin monomethyl ether and paphiodianthin B. Both were strongly cytotoxic to MCF-7 cells, but their effect on normal cells was only moderate or non-toxic. The flavonol, isalpinin, although not very active against wild type MCF-7 cell line, was more active against its doxorubicin-resistant cell subline MCF-7/DOX. Further study should be done on its effect on transport proteins involved in the resistance mechanism of the cancer cells.

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PUBLICATION	1. Lertnitikul, N., Jittham, P., Khankhampoch, L., Pattamadilok, C., Sukrong S., and Suttisri, R. (2016). Cytotoxic stilbenes from the roots of Paphiopedilum godefroyae. Journal of Asian Natural Products Research, 18: 1143-1150. 2. Pooreecharurot, N., Lertnitikul, N., Suttisri, R., and Sukrong, S. (2016). Stilbenes from Paphiopedilum exul roots. Thai Journal of Pharmaceutical Sciences. 40: 116-119. 3. Lertnitikul, N., Pattamadilok, C., Chansriniyom, C. and Suttisri, R. (2020). A new dihydrophenanthrene from Cymbidium finlaysonianum and structure revision of cymbinodin-A. Journal of Asian Natural Products Research. 22(1): 83-90.
AWARD RECEIVED	1. Outstanding Senior Project Presentation, 2014, Chulalongkorn University, Thailand 2. Outstanding Graduate Student Award, 2019, Chulalongkorn University, Thailand