องค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพจากรากยอทาม *Morinda pandurifolia* Kuntze และยอป่า *Morinda coreia* Ham

นาย ธนาธิป รักศิลป์

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CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES FROM THE ROOTS OF *Morinda pandurifolia* Kuntze AND *Morinda coreia* Ham

Mr. Thanatip Ruksilp

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Thesis Title	CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES FROM THE ROOTS OF *Morinda pandurifolia* Kuntze AND *Morinda coreia* Ham
By	Mr. Thanatip Ruksilp
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Thesis Advisor	Associate Professor Santi Tip-pyang, Ph.D.

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

..... Dean of the Faculty of Science
(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

..... Chairman
(Assistant Professor Warinthorn Chavasiri, Ph.D.)

..... Thesis Advisor
(Associate Professor Santi Tip-pyang, Ph.D.)

..... Examiner
(Assistant Professor Worawan Bhanthumnavin, Ph.D.)

..... Examiner
(Assistant Professor Aroonsiri Shitangkoon, Ph.D.)

..... External Examiner
(Assistant Professor Jongkolnee Jongaramruang, Ph.D.)

ธนาธิป รักศิลป์ : องค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพจากรากยอทาม *Morinda pandurifolia* Kuntze และยอป่า *Morinda coreia* Ham (CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES FROM THE ROOTS OF *Morinda pandurifolia* Kuntze AND *Morinda coreia* Ham) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร. สันติ ทิพยางค์, 102 หน้า.

การศึกษาองค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพจากสิ่งสกัดไดคลอโรมีเทน เอทิลแอซีเตต และเอ็นบิวทานอลของรากยอทาม *Morinda pandurifolia* Kuntze แยกได้สารสังเคราะห์แอนทราควิโนน ที่มีรายงานแล้ว 1 สาร คือ flavopurpurin (**77**) พร้อมสารแอนทราควิโนนที่มีรายงานแล้ว 10 สาร คือ nordamnacanthal (**16**), damnacanthal (**17**), lucidin-ω-methyl ether (**18**), phomarin (**73**), lucidin (**74**), lucidin-ω-ethyl ether (**75**), anthragallol-2,3-dimethyl ether (**76**), 1-methoxy-2-methyl anthraquinone (**78**), 3-hydroxy-1-methoxy-2-methoxymethyl anthraquinone (**79**), anthragallol (**80**) และสารอิริดอยด์ ไกลโคไซด์ที่มีรายงานแล้ว 2 สาร คือ asperulosidic acid (**43**), deacetyl asperulosidic acid (**44**) เป็นการรายงานครั้งแรกในการแยกสารและทดสอบฤทธิ์ทางชีวภาพของพืชชนิดนี้

ในการแยกสารจากสิ่งสกัดไดคลอโรมีเทน และเมทานอลของรากยอป่า *Morinda coreia* Ham แยกได้สารสังเคราะห์แอนทราควิโนนที่มีรายงานแล้ว 2 สาร คือ 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**) และ 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (**82**) สารแอนทราควิโนนไกลโคไซด์ที่มีรายงานแล้ว 1 สาร คือ 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside (lucidin-3-*O*-β-glucoside, **83**) พร้อมสารแอนทราควิโนนที่มีรายงานแล้ว 5 สาร คือ nordamnacanthal (**16**), damnacanthal (**17**), phomarin (**73**), 1-methoxy-2-methyl anthraquinone (**78**) และ anthragallol (**80**) การพิสูจน์โครงสร้างของสารทั้งหมดที่แยกได้นี้อาศัย วิธีทางกายภาพและวิธีทางสเปกโทรสโกปี ร่วมกับการเปรียบเทียบกับข้อมูลที่มีรายงานแล้ว

สารที่แยกได้ทั้งหมด (**16-18, 43-44** และ **73-83**) นำมาทดสอบฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด KB และ HeLa พบว่า สาร **16, 17** และ **73** มีฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด KB ได้ปานกลาง ที่ค่า IC_{50} 5.99 - 7.67 µg/mL และสาร **75, 77, 78** และ **80** มีฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด KB ได้ต่ำที่ค่า IC_{50} 12.06 – 21.05 µg/mL. สาร **16, 17, 73** และ **77** มีฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด HeLa ได้ต่ำที่ค่า IC_{50} 12.26 - 16.65 µg/mL ส่วนสาร **18, 43, 44, 74, 76, 79** และ **81-83** ไม่มีฤทธิ์ในการยับยั้งเซลล์มะเร็งทั้งสองชนิด เป็นการรายงานครั้งแรกของข้อมูลทางโครงสร้างอย่างสมบูรณ์ของสารสังเคราะห์ที่เคยรายงานมาแล้วสองสารคือ **77** และ **81**

ภาควิชา.....เคมี.....ลายมือชื่อนิสิต.....

สาขาวิชา.....เคมี.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

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THANATIP RUKSILP : CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES FROM THE ROOTS OF *Morinda pandurifolia* Kuntze AND *Morrinda coreia* Ham. THESIS ADVISOR : ASSOC. PROF. SANTI TIP-PYANG, Ph. D., 102 pp.

The investigation for chemical constituents and their biological activities from CH_2Cl_2, EtOAc and *n*-BuOH crude extracts of the roots of *Morinda pandurifolia* Kuntze led to the isolation of one synthetically known anthraquinone; flavopurpurin (**77**), along with ten known anthraquinones; nordamnacanthal (**16**), damnacanthal (**17**), lucidin-ω-methyl ether (**18**), phomarin (**73**), lucidin(**74**), lucidin-ω-ethyl ether (**75**), anthragallol-2,3-dimethyl ether (**76**), 1-methoxy-2-methyl anthraquinone (**78**), 3-hydroxy-1-methoxy-2-methoxymethyl anthraquinone (**79**), anthragallol (**80**) and two known iridoid glycosides; asperulosidic acid (**43**), deacetyl asperulosidic acid (**44**). This is the first report on phytochemical investigation and biological activities of this plant.

Chromatographic separation of CH_2Cl_2 and MeOH crude extracts of the roots of *Morinda coreia* Ham led to the isolation of two synthetically known anthraquinones; 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**) and 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (**82**), a known anthraquinone glycoside; 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside (lucidin-3-*O*-β-glucoside, **83**), and five known anthraquinones; nordamnacanthal (**16**), damnacanthal (**17**), phomarin (**73**), 1-methoxy-2-methyl anthraquinone (**78**) and anthragallol (**80**). The structures of all isolated compounds were elucidated by physical properties and spectroscopic methods as well as comparison with previous literature data.

All of isolated compounds (**16-18, 43-44** and **73-83**) were also evaluated for their cytotoxicity against KB and HeLa cell lines. Compounds **16, 17** and **73** showed moderate cytotoxicity against KB cells with IC_{50} values in the range of 5.99-7.67 μg/mL, and compounds **75, 77, 78** and **80** showed weak cytotoxicity against KB cells with IC_{50} values in the range of 12.06 - 21.05 μg/mL. Compounds **16, 17, 73** and **77** showed weak cytotoxicity against HeLa cells with IC_{50} values in the range of 12.26-16.65 μg/mL. In addition, compounds **18, 43, 44, 74, 76, 79** and **81-83** were inactive to both cell lines.This is also the first report giving a complete structural assignment for two synthetically known compounds **77** and **81**.

Department : Chemistry Student's Signature Thanatip Ruksilp

Field of Study : Chemistry Advisor's Signature Santi Tip-pyang

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LIST OF ABBREVIATIONS AND SYMBOLS

br s	broad singlet (NMR)
BuOH	butanol
calcd	calculated
^{13}C NMR	carbon 13 nuclear magnetic resonance
δ	chemical shift
δ_C	chemical shift of carbon
δ_H	chemical shift of proton
CC	column chromatography
c	concentration
IC_{50}	concentration that is required for 50% inhibition in vitro
COSY	correlated spectroscopy
J	coupling constant
acetone-d_6	deuterated acetone
$CDCl_3$	deuterated chloroform
DMSO-d_6	deuterated dimethyl sulfoxide
CD_3OD	deuterated methanol
CH_2Cl_2	dichloromethane
MTT	[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide]
d	doublet (NMR)
dd	doublet of doublet (NMR)
ESIMS	electrospray ionization mass spectrometry
EtOAc	ethyl acetate
g	gram (s)
Hz	hertz
HMBC	heteronuclear multiple bond correlation
HSQC	heteronuclear single quantum correlation
HRESIMS	high resolution electrospray ionization mass spectrometry
HeLa cell line	human cervical carcinoma

KB cell line	human epidermoid carcinoma
m/z	mass per charge
λ_{max}	maximum wavelength
MHz	megahertz
MeOH	methanol
μ	micro
mg	milligram (s)
mL	milliliter (s)
min	minute
M	molar
ε	molar extinction coefficient
m	multiplet (NMR)
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser enhancement spectroscopy
^{1}H NMR	proton nuclear magnetic resonance
q	quartet (NMR)
s	singlet (NMR)
TLC	thin layer chromatography
$TMSCHN_2$	trimethylsilyldiazomethane
t	triplet (NMR)
2D NMR	two dimentional nuclear magnetic resonance
UV	ultraviolet
VLC	vacuum liquid chromatography

CHAPTER I

INTRODUCTION

1.1 Introduction

Many plants belonging to *Morinda* species are valuable traditional herbs in the Austronesia and oriental countries. The roots, barks, stems, leaves, and fruits of *Morinda citrifolia* have been used traditionally as a folk medicine for the treatment of many diseases including diabetes, high blood pressure, and cancer. *M. officinalis* is another agelong herb and was considered to be an effective tonic for promoting overall health in Chinese traditional herbal medicines, but modern pharmacology validated different curative effects on lowering blood pressure, diminishing inflammation and antibacterial bioactivity. *M. parvifolia* and *M. umbellata* have displayed analgesic, hemostatic, antibacterial, and antiphlogistic activities [1].

The genus *Morinda* belongs to the Rubiaceae family and comprises approximately 80 species, distributed in all tropical regions of the world. These species may be trees, shrubs or vines; some, like *M. citrifolia*, are trees that very much resemble vines. All *Morinda* species bear aggregate or multiple fruits that can be fleshy or dry. Most species of this genus originate in the area of Borneo, New Guinea, Northern Australia, and New Caledonia. *Morinda* is derived from the Latin words *morus*, meaning "mulberry", and *indica*, meaning "of India", referring to the shape of the fruits [2].

The characteristic of plants in genus *Morinda* as follows: Flowers in peduncled capituliform, irregularly globose or ovoid inflorescences, 4-6 merous, unisexual (but seemingly bisexual), or bisexual; bracts small; calyx-tubes entirely connate or almost so; limb very short, usually truncate, rarely with 1 or 2 leaf-like lobes (calycophylly); corolla-lobes valvate in bud, keeledinside; stamens inserted in throator slightly lower, exsert; filaments short; anthers doesifixed below the middle; disk annular, glabrous; style dimorphic, glabrous; style-branches (partly stigmatic) 2, narrow; ovary 2-celled or incompletely 4-celled; ovule 1 per cell, attached near base of septum; fruit a 1-pyrenous, 1-seeded drupe, the fruits together forming a fleshy syncarp. Leaves opposite, penninerved, not rarely on upper side with numerous bacteriodomatia, on

underside in axils of midrib and nerves with pubescent acarodomatia; stipules interpetiolar. *Morinda* is a genus of tree, or erect or climbing shrubs [3].

Various species of *Morinda* have been known as dye plants since the ancient time. In India, cotton, wool and silk were colored with *Morinda* root-dyes, which are known under the name “al”, “ach”, “surangi” etc.. *M. citrifolia* L. and *M. coreia* Ham. are considered to be the chief sources of “al” dye. Some of the other species of this genus, particulary *M. bracteata* Roxb., *M. tomentosa* Heyne ex Roth and *M. umbellata* L. were also explored for dyes. For the Javanese dyeing industry, *M. citrifolia* L. is cultivated. *Morinda* species yield dyes which give permanent shades of red, purple, and chocolate which are produced on moranted cotton, silk or wool, the shades being fast to soap [4].

In Thailand, *Morinda pandurifolia* Kuntze, locally known as “Yotime”, is a shrub found scattered in the moist upper mixed deciduous forest, in the Central and Peninsula regions of the country. The leaves, bark and wood produce a yellowish-red pigments for dyeing clothes [5]. The roots, bark, stems, leaves and fruits of several *Morinda* species have been used as a traditional folk medicine for the treatment of many diseases including diabetes, hypertension and cancer [6,7]. The roots of this plant were collected from Mahasarakham province of Thailand in June 2007 and identified by Ms. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, where a voucher specimen (Khumkratok no. 103-09) is deposited.

Morinda coreia Ham. (Yo-Paa) is a tree distributed in the south-east Asia region. The bark and wood are used for anti-fever treatments, as well as an antimalarial agent in north-eastern Thai (Isarn) traditional medicine [8]. The roots of this plant were collected from Mahasarakham Province of Thailand in July 2010, and identified by Ms. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, where a voucher specimen (Khumkratok no. 1-11) is deposited.

1.2 Chemical constituents from *Morinda* genus

There are many chemical and biological investigations which have not been carried out on *Morinda* genus. The compounds isolated from *Morinda* plants are shown as follows.

M. citrifolia L., commonly called Noni or Indian mulberry, is a small evergreen tree or shrub of Polynesian origin, green to yellowish-white fruit, normally 5 to 10 cm in length with a surface covered in polygonal-shaped sections, the major chemical constituents of this plant have been found to be anthraquinones, anthraquinone glycosides [6,7,9,22,23], alcohols, esters, ketones, lactones [10], glycosides, trisaccharide fatty acid ester [11, 12], iridoids, iridoid glycosides, flavonol glycosides [13-18], lignans, flavonoids, flavonol glycosides, saccharide, fatty acid esters [19-20], benzophenones [21].

The leaves and branches of *M. coreia* Ham had been investigated and iridoid glycosides, phenolic glycoside, secoiridoid glucoside and anthraquinone glycoside were found [8].

M. elliptica Ridl is a tree of up to 25 m high, distributed in South-east Asia, the anthraquinones [24,26], iridoid, iridoid glycosides, and aromatic glycosides have been isolated from this plant [25].

M. morindoides (Baker) Milne-Redhead (syn. *Gaertnera morindoides* Bak), commonly called Nkongabululu, Kongobololo, or Nkama mesu in the Democratic Republic of Congo, is one of the most popular medicinal plant used in this African country, the investigations were found to be flavonoids, flavonoid glycosides and iridoid glycosides [27-29].

M. officinalis is a small vine that grows widely in tropical and subtropical regions, the anthraquinones, coumarin, iridoid lactone, monoterpene glycoside and iridoid glycosides have been isolated from the roots of this plant [30,31].

M. angustifolia is a resourceful perennial undershrub, and widely distributed in the southwestern mountainous areas of China, and nearby countries, such as Burma, Laos, Thailand and India. The chemical constituents have been purified to be anthraquinones and mixture of steroids [1,32].

M. parvifolia (Hong-Zhu-Teng or Bai-Yen-Teng), in Chinese folklore as herbal remedies for the treatment of human bronchitis and whooping cough. The rhizome and roots of this plant have been isolated and found to be anthraquinones [33,34].

Antraquinones were also isolated from roots and stems of *M. umbellata* [35] and the stems of *M. lucida* [36].

The chemical constituents and structures of *Morinda* genus were summarized in Table 1.1 and Figure 1.1.

Table 1.1 Chemical constituents of *Morinda* genus

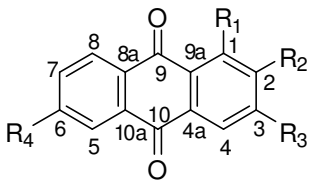
Compound	Source and plant part	Reference
1,2-Dihydroxy anthraquinone (**1**)	*M. citrifolia*, roots	[21]
1,3-Dihydroxy-2-methyl anthraquinone (**2**)	*M. citrifolia*, roots	[21]
3-Hydroxy-2-(hydroxymethyl) anthraquinone(**3**)	*M. citrifolia*, roots	[21]
1,3,6-Trihydroxy-2-methyl anthraquinone (**4**)	*M. citrifolia*, roots	[21]
6-Hydroxy-1,5-dimethoxy-2-methoxymethyl anthraquinone (1,5,15-tri-*O*-methyl morindol)(**5**)	*M. citrifolia*, fruits	[20]
1,6-Dihydroxy-5-methoxy-2-methoxymethyl anthraquinone (5,15-di-*O*-methyl morindol) (**6**)	*M. citrifolia*, fruits	[6,20]
1,3-Dihydroxy-2-methoxy anthraquinone (Anthragallol-2-methyl ether) (**7**)	*M. citrifolia*, fruits	[20]
2-Hydroxy-1-methoxy anthraquinine (Alizarin-1-methyl ether) (**8**)	*M. citrifolia*, fruits; *M. elliptica*, roots	[6,24]
2-Hydroxy-1,3-dimethoxy anthraquinone (Anthragallol-1,3-dimethyl ether) (**9**)	*M. citrifolia*, fruits	[6]
2,6-Dihydroxy-1,3-dimethoxy anthraquinone (6-Hydroxy-anthragallol-1,3-dimethyl ether) (**10**)	*M. citrifolia*, fruits	[6]
1,6-Dihydroxy-5-methoxy-2-methyl anthraquinone (Morindone-5-methyl ether) (**11**)	*M. citrifolia*, fruits; *M. elliptica*, roots	[6,24]
2-Methoxy-1,3,6-trihydroxy anthraquinone (**12**)	*M. citrifolia*, fruits	[17]
1,8-Dihydroxy-2-hydroxymethyl -5-methoxy anthraquinone (**13**)	*M. citrifolia*, fruits	[17]
2-Formyl-1-hydroxy anthraquinone (**14**)	*M. elliptica*, roots	[24]
1-Hydroxy-2-methyl anthraquinone (**15**)	*M. elliptica*, roots	[24]
2-Formyl-1,3-dihydroxy anthraquinone (Nordamnacanthal) (**16**)	*M. elliptica*, roots	[24]
2-Formyl-3-hydroxy-1-methoxy anthraquinone (Damnacanthal) (**17**)	*M. elliptica*, roots	[24]
1,3-Dihydroxy-2-methoxymethyl anthraquinone (Lucidin-ω-methyl ether) (**18**)	*M. elliptica*, roots	[24]
1,6-Dihydroxy-2-methyl anthraquinone (Soranjidiol) (**19**)	*M. elliptica*, roots	[23]
2-Methyl anthraquinone (Tectoquinone) (**20**)	*M. officinalis*, roots; *M. umbellata,* roots	[31,35]
2-Methoxy anthraquinone (**21**)	*M. officinalis*, roots; *M. umbellata,* roots and stems	[30,35]

Table 1.1 Chemical constituents of *Morinda* genus (cont.)

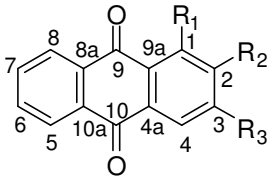
Compound	Source and plant part	Reference
2-Hydroxymethyl anthraquinone (**22**)	*M. parvifolia*, rhizome and roots	[34]
2-Hydroxy anthraquinone (**23**)	*M. umbellata,* roots and stems	[35]
1,2-Dihydroxy anthraquinone (Alizarin) (**24**)	*M. umbellata,* roots and stems	[35]
1-Hydroxy-2-methoxy anthraquinone (Alizarin-2-methyl ether) (**25**)	*M. umbellata,* roots and stems	[35]
1-Methoxy-2-methyl anthraquinone (**26**)	*M. umbellata,* roots and stems	[35]
1,2-Dioxymethylene anthraquinone (Morindaparvin-A) (**27**)	*M. parvifolia*, rhizome and roots	[34]
1-Hydroxy-2-hydroxymethyl anthraquinone (Digiferruginol) (**28**)	*M. parvifolia*, rhizome and roots	[34]
1,3-Dihydroxy-2-methyl anthraquinone (Rubiadin) (**29**)	*M. angustifolia,* roots *M. citrifolia,* roots;	[1,21]
1,8-Dihydroxy-2-methyl-3,7-dimethoxy anthraquinone (**30**)	*M. angustifolia,* roots	[1]
Digiferruginol-1-methylether-11-*O*-β-gentibioside (**31**)	*M. citrifolia,* roots	[22]
Digiferruginol-11-*O*-β-primeveroside (**32**)	*M. citrifolia,* roots	[22]
Damnacanthol-11-*O*-β-primeveroside (**33**)	*M. citrifolia,* roots	[22]
1-Methoxy-2-primeverosyloxymethyl-anthraquinone-3-olate (**34**)	*M. citrifolia,* roots	[22]
1-Hydroxy-2-primeverosyloxymethyl-anthraquinone-3-olate (**35**)	*M. citrifolia,* roots	[22]
1-Hydroxy-5,6-dimethoxy-2-methyl-7-primverosyloxy anthraquinone (**36**)	*M. citrifolia,* roots	[22]
Yopaaoside A (**37**)	*M. coreia,* leaves and branches	[8]
Yopaaoside B (**38**)	*M. coreia,* leaves and branches	[8]
Yopaaoside C (**39**)	*M. coreia,* leaves and branches	[8]
10-*O*-Acetylmonotropein (**40**)	*M. coreia,* leaves and branches	[8]
6-*O*-Acetylscandoside (**41**)	*M. coreia,* leaves and branches	[8]
Moridacin (**42**)	*M. citrifolia*, fruits	[6]
Asperulosidic acid (**43**)	*M. citrifolia*, fruits *M. coreia,* leaves and branches	[6,8]
Deacetylasperulosidic acid (**44**)	*M. citrifolia*, fruits	[6]
Asperuloside (**45**)	*M. coreia,* leaves and branches	[8]

Table 1.1 Chemical constituents of *Morinda* genus (cont.)

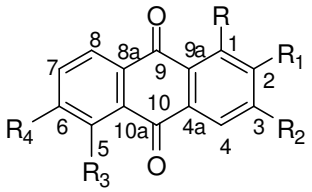
Compound	Source and plant part	Reference
Gaertneroside (**46**)	*M.morindoides*, leaves	[28]
Acetylgaertneroside (**47**)	*M.morindoides*, leaves	[28]
Dehydrogaertneroside (**48**)	*M.morindoides*, leaves	[28]
Dehydromethoxygaertneroside (**49**)	*M.morindoides*, leaves	[28]
Gaertneric acid (**50**)	*M.morindoides*, leaves	[28]
Methoxygaertneroside (**51**)	*M.morindoides*, leaves	[28]
Epoxygaertneroside (**52**)	*M.morindoides*, leaves	[28]
Epoxymethoxygaertneroside (**53**)	*M.morindoides*, leaves	[28]
Quercetin (**54**)	*M.morindoides*, leaves *M. citrifolia*, fruits	[29,19]
Quercetin-7,4'-dimethylether (**55**)	*M.morindoides*, leaves	[29]
Quercetin-3-*O*-rutinoside (**56**)	*M.morindoides*, leaves	[29]
Quercetin-3-*O*-rhamnoside (**57**)	*M.morindoides*, leaves	[29]
Kaemferol-3-*O*-rhamnoside (**58**)	*M.morindoides*, leaves	[29]
Kaemferol-3-*O*-rutinoside (**59**)	*M.morindoides*, leaves	[29]
Kaemferol-7-*O*-rhamnosylsophoroside (**60**)	*M.morindoides*, leaves	[29]
Chrysoeriol-7-*O*-neohesperidoside (**61**)	*M.morindoides*, leaves	[29]
Apigenin-7-*O*-glucoside (**62**)	*M.morindoides*, leaves	[29]
Luteolin-7-*O*-glucoside (**63**)	*M.morindoides*, leaves	[29]
Kaemferol (**64**)	*M.morindoides*, leaves *M. citrifolia*, fruits	[29,19]
Apigenin (**65**)	*M.morindoides*, leaves	[29]
Luteolin (**66**)	*M.morindoides*, leaves	[29]
(+)-3,4,3',4'-Tetrahydroxy-9,7'α-epoxylignano-7α,9'-lactone (**67**)	*M. citrifolia*, fruits	[19]
(+)-3,3'-Bisdemethyltanegool (**68**)	*M. citrifolia*, fruits	[19]
(-)-Pinoresinol (**69**)	*M. citrifolia*, fruits	[19]
(-)-3,3'-Bisdemethylpinoresinol (**70**)	*M. citrifolia*, fruits	[19]
Scopoletin (**71**)	*M. citrifolia*, fruits	[19]
Isoscopoletin (**72**)	*M. citrifolia*, fruits	[19]



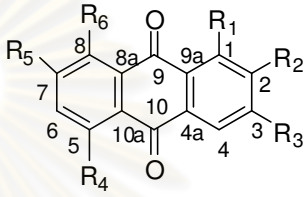
1 R_1, R_2 = OH, R_3, R_4 = H
2 R_1, R_3 = OH, R_2 = Me, R_4 = H
3 R_1, R_4 = H, R_2 = CH_2OH, R_3 = OH
4 R_1, R_3, R_4 = OH, R_2 = Me
19 R_1, R_4 = OH, R_2 = Me, R_3 = H



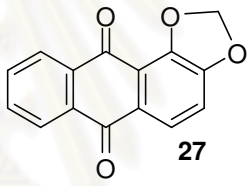
14 R_1 = OH, R_2 = CHO, R_3 = H
15 R_1 = OH, R_2 = Me, R_3 = H
16 R_1, R_3 = OH, R_2 = CHO
17 R_1 = OMe, R_2 = CHO, R_3 = OH
18 R_1, R_3 = OH, R_2 = CH_2OMe
20 R_1, R_3 = H, R_2 = Me
21 R_1, R_3 = H, R_2 = OMe
22 R_1, R_3 = H, R_2 = CH_2OH
23 R_1, R_3 = H, R_2 = OH
24 R_1, R_3 = OH, R_2 = H
25 R_1 = OH, R_2 = OMe, R_3 = H
26 R_1 = OMe, R_2 = Me, R_3 = H
28 R_1 = OH, R_2 = CH_2OH, R_3 = H
29 R_1, R_3 = OH, R_2 = Me



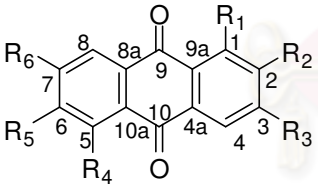
5 R, R_3 = OMe, R_1 = CH_2OMe, R_2 = H, R_4 = OH
6 R, R_4 = OH, R_1 = CH_2OMe, R_2 = H, R_3 = OMe
7 R, R_2 = OH, R_1 = OMe, R_3, R_4 = H
8 R = OMe, R_1 = OH, R_2, R_3, R_4 = H
9 R, R_2 = OMe, R_1 = OH, R_3, R_4 = H
10 R, R_2 = OMe, R_1, R_4 = OH, R_3 = H
11 R, R_4 = OH, R_1 = Me, R_2 = H, R_3 = OMe
12 R, R_2, R_4 = OH, R_1 = OMe, R_3 = H

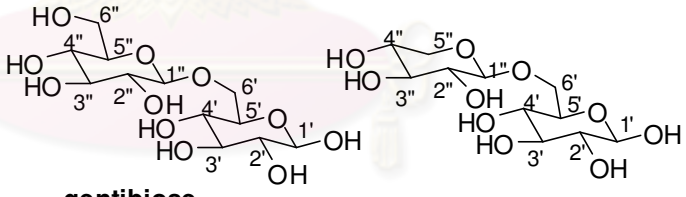


13 R_1, R_6 = OH, R_2 = CH_2OH, R_4 = OMe, R_3, R_5 = H
30 R_1, R_6 = OH, R_2 = CH_3, R_3, R_5 = OMe, R_4 = H



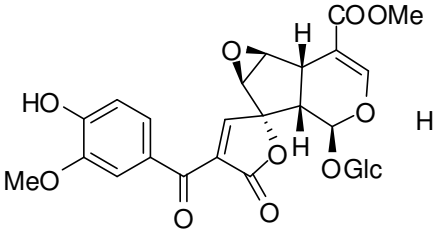
27



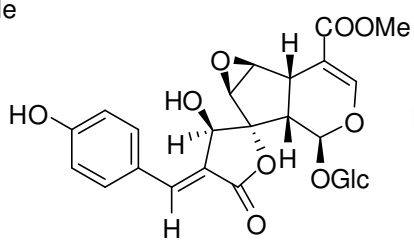


gentibiose **primeverose**

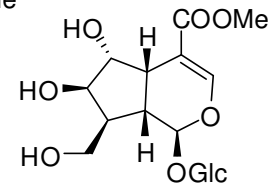
31 R_1 = OMe, R_2 = CH_2O-gentibiose, R_3, R_4, R_5, R_6 = H
32 R_1 = OH, R_2 = CH_2O-primeverose, R_3, R_4, R_5, R_6 = H
33 R_1 = OMe, R_2 = CH_2O-primeverose, R_3 = OH, R_4, R_5, R_6 = H
34 R_1 = OMe, R_2 = CH_2O-primeverose, R_3 = O- , R_4, R_5, R_6 = H
35 R_1 = OH, R_2 = CH_2O-primeverose, R_3 = O- , R_4, R_5, R_6 = H
36 R_1 = OH, R_2 = Me, R_3 = H, R_4, R_5 = OMe, R_6 = CH_2O-primeverose



37 Yopaaoside A

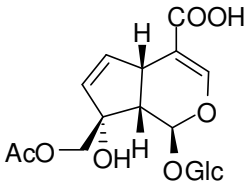


38 Yopaaoside B

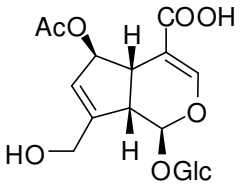


39 Yopaaoside C

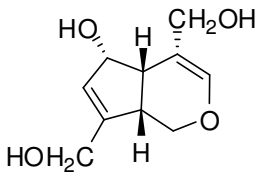
Figure 1.1 Isolated compounds from *Morinda* genus



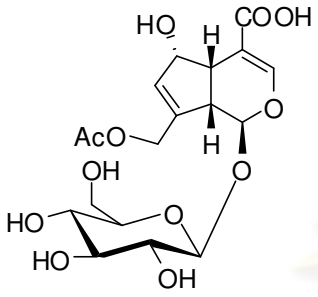
40 10-*O*-Acetylmonotropein



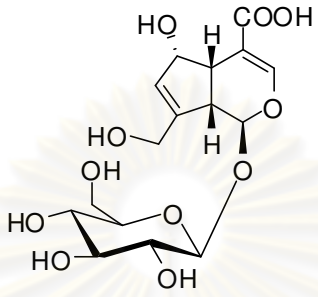
41 6-*O*-Acetylscandoside



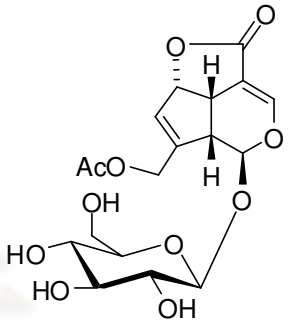
42 Moridacin



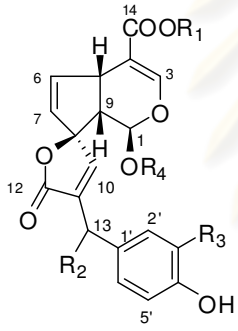
43 Asperulosidic acid

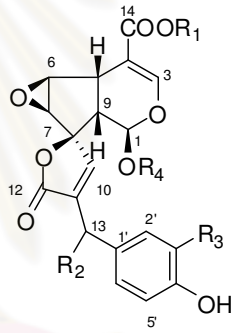


44 Deacetylasperulosidic acid

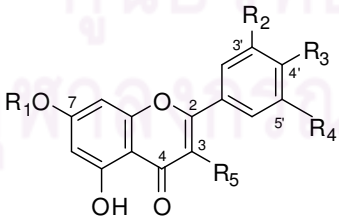


45 Asperuloside





46 R_1 = Me, R_2 = OH, R_3 = H, R_4 = glucosyl
47 R_1 = Me, R_2 = OH, R_3 = H, R_4 = 6-acetyl-glucosyl
48 R_1 = Me, R_2 = =O, R_3 = H, R_4 = glucosyl
49 R_1 = Me, R_2 = =O, R_3 = OMe, R_4 = glucosyl
50 R_1 = H, R_2 = OH, R_3 = H, R_4 = glucosyl
51 R_1 = Me, R_2 = OH, R_3 = OMe, R_4 = glucosyl
52 R_1 = Me, R_2 = OH, R_3 = H, R_4 = glucosyl
53 R_1 = Me, R_2 = OH, R_3 = OMe, R_4 = glucosyl



54 R_1 = H, R_2 = OH, R_3 = OH, R_4 = H, R_5 = OH
55 R_1 = CH_3, R_2 = OH, R_3 = CH_3, R_4 = H, R_5 = OH
56 R_1 = H, R_2 = OH, R_3 = OH, R_4 = H, R_5 = O-Rut
57 R_1 = H, R_2 = OH, R_3 = OH, R_4 = H, R_5 = O-Rha
58 R_1 = H, R_2 = H, R_3 = OH, R_4 = H, R_5 = O-Rha
59 R_1 = H, R_2 = H, R_3 = OH, R_4 = H, R_5 = O-Rut
60 R_1 = Rha-soph, R_2 = H, R_3 = H, R_4 = H, R_5 = OH
61 R_1 = Neohesp, R_2 = H, R_3 = OH, R_4 = OCH_3, R_5 = H
62 R_1 = Glc, R_2 = H, R_3 = OH, R_4 = H, R_5 = H
63 R_1 = Glc, R_2 = H, R_3 = OH, R_4 = OH, R_5 = H
64 R_1 = H, R_2 = H, R_3 = OH, R_4 = H, R_5 = H
65 R_1 = H, R_2 = H, R_3 = OH, R_4 = H, R_5 = H
66 R_1 = H, R_2 = H, R_3 = OH, R_4 = OH, R_5 = H

Figure 1.1 Isolated compounds from *Morinda* genus (cont.)

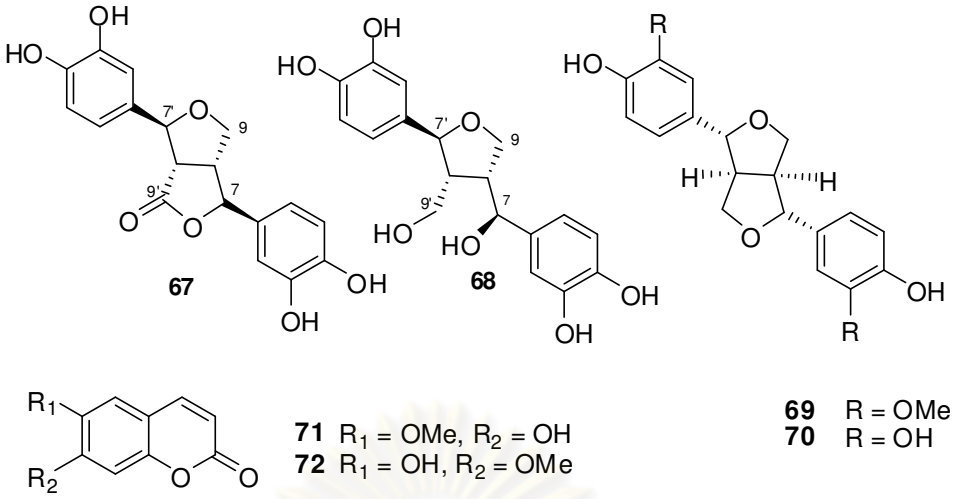


Figure 1.1 Isolated compounds from *Morinda* genus (cont.)

1.3 Biosynthesis of anthraquinones in cell cultures of the Rubiaceae

In general, quinones from higher plants are derived from various precursors and are produced via different pathways. The two main distinct biosynthetic pathways leading to anthraquinones in higher plants are the polyketide pathway and the chorismate/*o*-succinylbenzoic acid pathway [37].

1.3.1 The polyketide pathway

The polyketide pathway occurs mainly in fungi and some higher plant families as Leguminosae, Rhamnaceae and Polygonaceae [38,39]. In this pathway, anthraquinones are formed from one acetyl-CoA unit extended by seven malonyl-CoA units via an octaketide chain (Figure 1.2). These types of anthraquinones often exhibit a characteristic substitution pattern, i.e. they are substituted in both ring A and C of anthraquinone structure. Chrysophanol and emodin are typically substituted with hydroxy groups in both ring A and C and they occur in fungi and higher plants. In *Rumex* (Polygonaceae) and *Rhamnus* (Rhamnaceae), these two anthraquinones were shown to be biosynthesized via the polyketide pathway [40]. Enzyme systems, catalyzing the synthesis of polyketides, have been well documented in bacteria, but detected only rarely in higher plants [41].

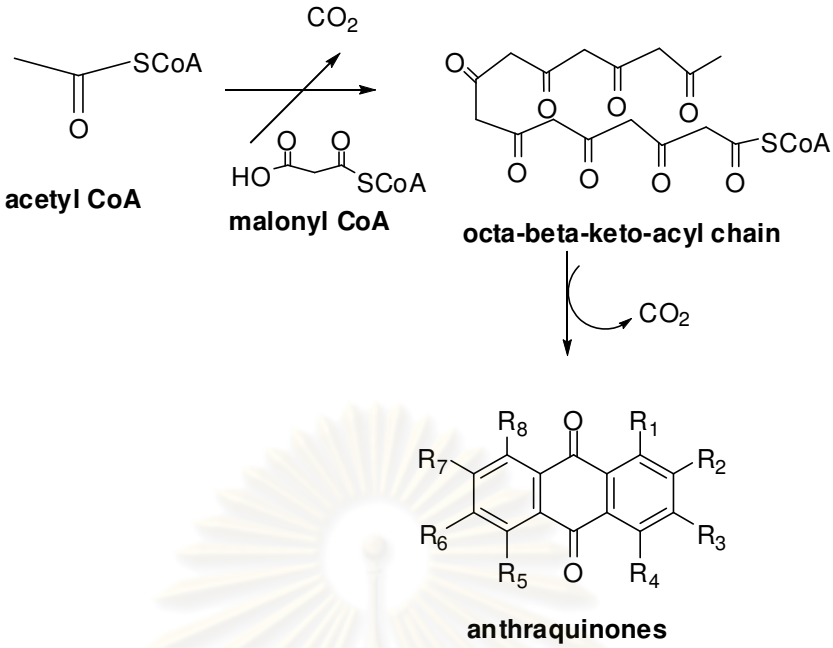


Figure 1.2 Polyketide pathway for the biosynthesis of anthraquinones

1.3.2 The chorismate/*o*-succinylbenzoic acid pathway

In the family Rubiaceae, the genera *Galium*, *Morinda, Rubia* and *Cinchona* have been extensively used for anthraquinone biosynthetic studies. Anthraquinones in the Rubiaceae are considered to be of the *Rubia* type, i.e. ring A and B are biosynthetically derived from chorismic acid and α-ketoglutarate via *o*-succinylbenzoic acid (OSB), whereas ring C is formed from isopentenyl diphosphate (IPP) via the terpenoid pathway (Figure 1.3)[42].

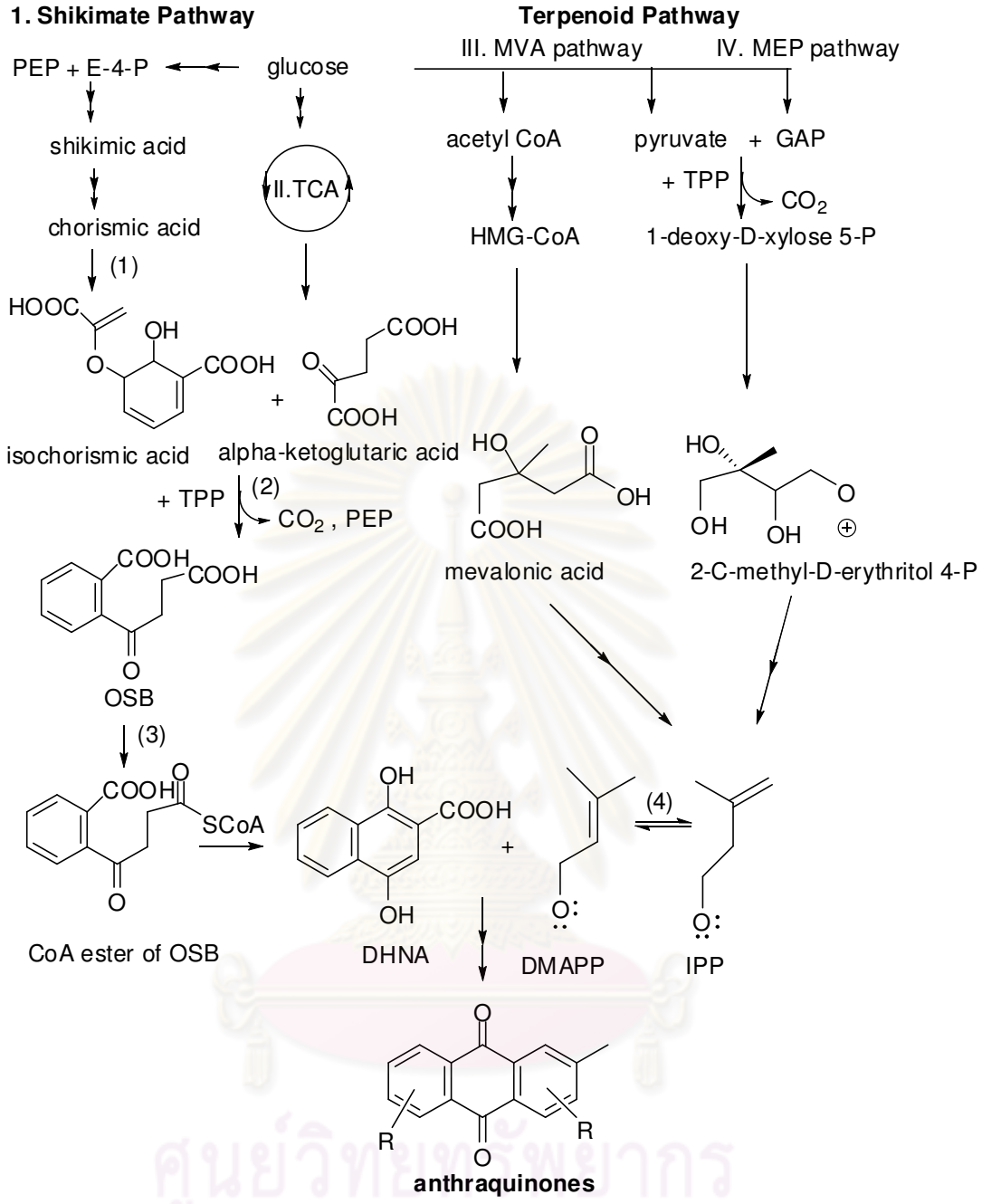


Figure 1.3 Biosynthetic pathways leading to anthraquinones in Rubiaceae

1.4 Biological activities from *Morinda* genus

1.4.1 Anti-inflammatory

The assay of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) -Induced Ear Edema Inflammation in Mice, four saccharide fatty acid esters, 2-*O*-(β-D-glucopyranosyl)-1-*O*-octanoyl-β-D-glucopyranose, 6-*O*-(β-D-glucopyranosyl)-1-*O*-hexanoyl-β-D-glucopyranose, 6-*O*-(β-D-glucopyranosyl)-1-*O*-octanoyl-β-D-glucopyranose, and 2,6-di-*O*-(β-D-glucopyranosyl)-1-*O*-octanoyl-β-D-glucopyranose, from the fruits of *M. citrifolia* exhibited potent anti-inflammatory activity, with ID_{50} values of 0.46-0.79 mg per ear [20,43].

1.4.2 Quinone reductase induction assay

Using Hepa IcIc7 murine hepatoma cells, quinone reductase activity (concentration to double enzyme-inducing activity, CD) were measure at 595 nm with an ELISA plate reader. 2-Hydroxy-3-(hydroxymethyl) anthraquinone, 1,3,6-trihydroxy-2-methyl anthraquinone from the roots of *M. citrifolia*, and 2-methoxy-1,3,6-trihydroxy anthraquinone from the fruits of *M. citrifolia*, showed potent quinone reductase induction activity, with concentrations required to double QR activity of 0.94, 0.56, and 0.009 μM, respectively [17,21].

1.4.3 Antioxidant activity

The isolated compounds from the fruits of *M. citrifolia* were evaluated in term of 1,1-diphenyl-2-picrylhydrazyl (DPPH), using gallic acid as positive control. The neolignan, americanin A was found to be a potent antioxidant activity, with IC_{50} value of 16.9 μM [7].

1.4.4 Antimicrobial activity

Antimicrobial activity was determined with the disk diffusion assay, using Benzylpenicillin Sodium as a control sample, 1,8-dihydroxy-2-methyl-3,7-dimethoxy anthraquinone was obtained from the whole plant of *M. angustifolia*, demonstrated significant antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Sarcina lutea*, *Candida albicans*, and *Saccharomyces sake*, with diameter of the zone of inhibition in the range of 6.3-14.0 mm [1].

1.5 Botanical aspect and distribution

In Thailand, the genus *Morinda* (Rubiaceae) consists of 14 species [44].

M. angustifolia var. *angustifolia*

M. angustifolia var. *scabridula*

M. citrifolia

M. cinnamomia

M. coreia Ham syn. *M. tinctoria* Roxb.

M. cochinchinensis

M. elliptica (Hook.f) Ridl. syn *M. citrifolia* var. *elliptica* Hook.f.

M. logifolia

M. pandurifolia Kuntze syn. *M. pericaefolia* var. *pandurifolia* Pitard in Lec.

M. scabrida

M. talmyi Pierre ex Pitard in Lec.syn. *M. persiceafolia* Ham. var. *talmyi* Pitard in Lec.*; M. nana* Craib in Kew Bull.

M. tomentosa Heyne ex Roth syn. *M. tinctoria* Roxb var *tomentosa* Hook. f.; *M. pumila* Craib in Kew Bull.

M. umbellata

M. wallichii

M. pandurifolia Kuntze (Yotime) is a shrub found scattered in the moist upper mixed deciduous forest, in the Central and Peninsula regions of the country.

Tree : Small evergreen tree

Leaf : Elliptic leaf, 3-5 cm. wide, 6-12 cm. long

Petal : The edge of petal have 4-5 rays

Fruit : Green, spherical shape or distort

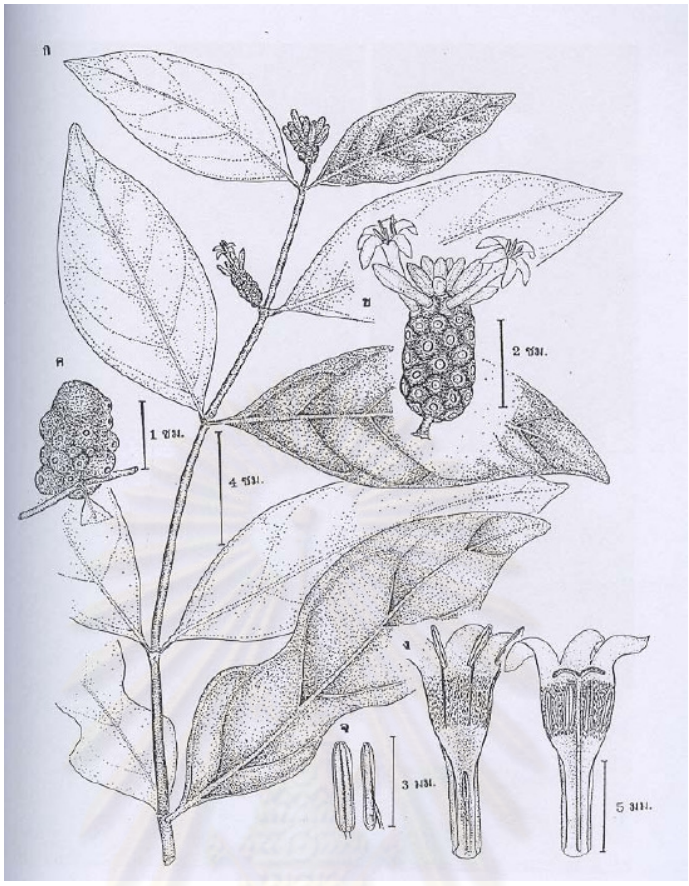
Seed : Brown, distort, 3.5-5 mm. wide, 4-7 mm. long

Flowers

Leaves and fruits

Leaves and fruits

Figure 1.4 *M. pandurifolia* Kuntze



Leaves, fruits, anthers

Figure 1.4 *M. pandurifolia* Kuntze (cont.)

M. coreia Ham (Yo-Paa) is a tree distributed in the south-east Asia region.

Tree : 15 m. tall, shrub

Leaf : Elliptic leaf, 8.5-11.5 cm. wide, 18-25.5 cm. long

Flower : Pack bouquet

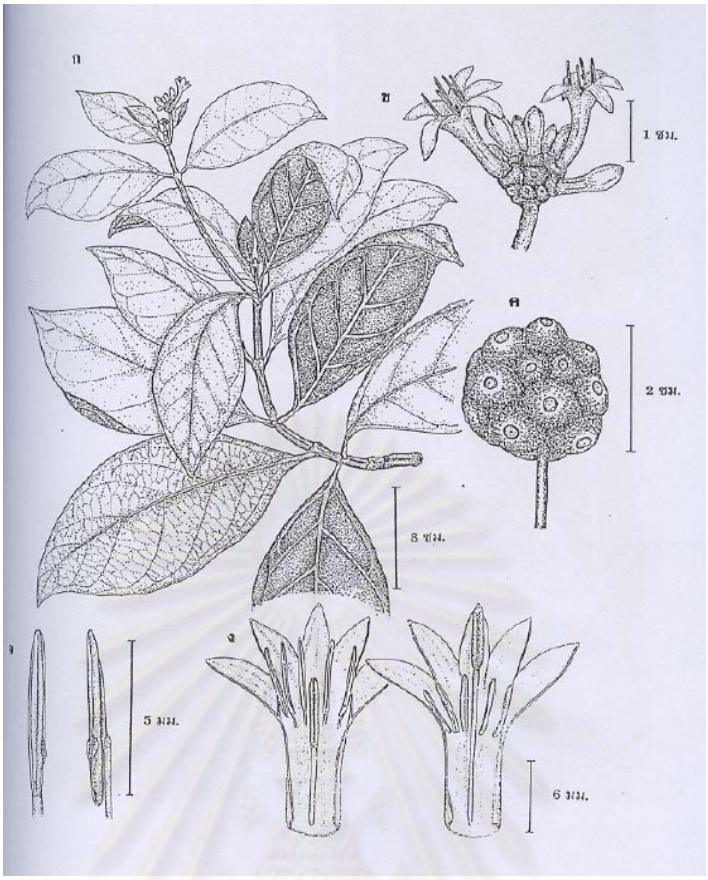
Fruit : Dark green, spherical shape or distort

Seed : Brown, distort, 3.5-5 mm. wide, 6-9.5 mm. long

Whole plant

Stem, leaves and fruits

Figure 1.5 *M. coreia* Ham



Leaves, fruits, anthers

Figure 1.5 *M. coreia* Ham (cont.)

As the literature mentioned above, there are many chemical and biological investigations which have been carried out on *Morinda* genus [1,7,8,20,21,43]. No chemical constituents and biological activities have been reported on the roots of *M. pandurifolia* Kuntze and *M. coreia* Ham. This is the first report on phytochemical investigation and biological activities of *M. pandurifolia* Kuntze.

1.6 Objectives

The main objectives in this investigation are as follows:

1. To isolate and purify compounds from the roots of *M. pandurifolia* Kuntze and *M. coreia* Ham.
2. To identify the chemical structures of all isolated compounds.
3. To evaluate the cytotoxicity against HeLa and KB cell lines of the isolated compounds.

CHAPTER II

EXPERIMENTAL

2.1 Plant material

2.1.1 *M. pandurifolia* Kuntze

The roots of *M. pandurifolia* Kuntze were collected from Mahasarakham Province of Thailand in June 2007 and identified by Ms. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, where a voucher specimen (Khumkratok no. 103-09) is deposited.

2.1.2 *M. coreia* Ham.

The roots of *M. coreia* Ham. were collected from Mahasarakham Province of Thailand in July 2010 and identified by Ms. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, where a voucher specimen (Khumkratok no. 1-11) is deposited.

2.2 General experimental procedures

NMR spectra were recorded with a Varian model Mercury^{+} 400 spectrometer operated at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR and a Bruker 400 AVANCE spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. The chemical shift in δ (ppm) was assigned with reference to the signal from the residual protons in deuterated solvent and using TMS as an internal standard in some cases. Most solvents used in this research were commercial grade and were distilled prior to use. Adsorbents such as dianion HP-20 (Sigma-Aldrich), sephadex LH-20 (Sigma-Aldrich) and silica gel (60 Merck cat. No. 7730, 7734 and 7749 were used for vacuum liquid chromatography, preparative TLC, opened column chromatography and radial thin layer chromatography (chromatotron), respectively). Thin-layer chromatography (TLC) was performed on precoated Merck silica gel 60 F_{254} plates (0.25 mm thick layer). ESIMS data were obtained from a mass spectrometer model VG TRIO 2000,

and Microflex MALDI-TOF mass spectrometer (Bruker Daltonics). High resolution mass spectra were recorded by Micromass LCT and Bruker MICROTOF models. UV-visible adsorption spectra were recorded on UV-2552PC UV-Vis spectrometer (Shimadzu, Kyoto, Japan). Melting points were determined with Fisher-Johns Melting Point Apparatus. IR data were obtained from a Nicolet 6700 FT-IR spectrometer (Thermo Electron Corporation, Madison, WI, USA) equipped with a mercury-cadmium-telluride (MCT) detector.

2.3 Extraction and purification

2.3.1 *M. pandurifolia* Kuntze

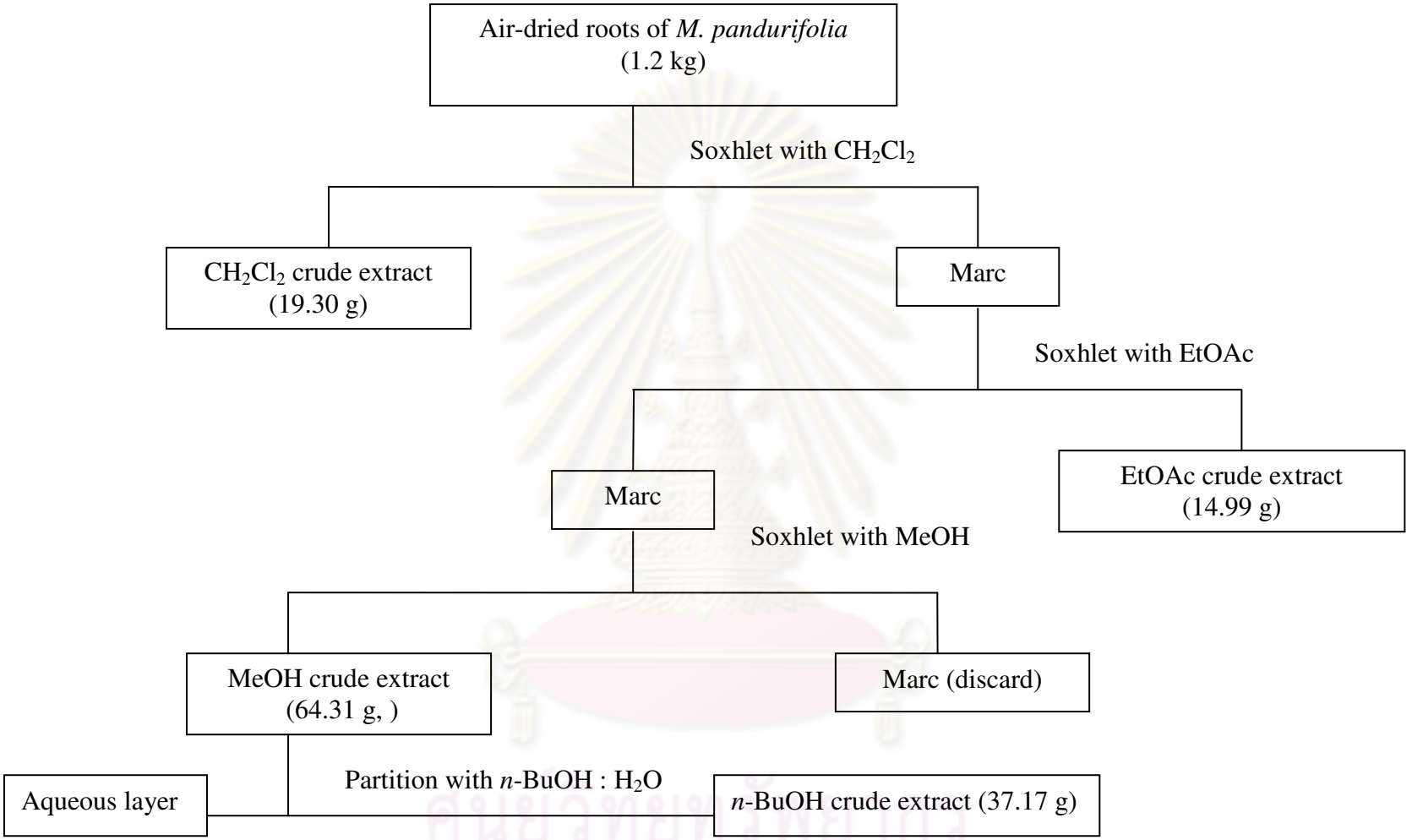
Air-dried and powdered roots of *M. pandurifolia* Kuntze (1.2 kg) were successively extracted in a soxhlet extracting apparatus with CH_2Cl_2, EtOAc, and MeOH, respectively. Removal of solvents from each extract under reduced pressure gave CH_2Cl_2 (19.30 g), EtOAc (14.99 g) and MeOH (64.31 g) crude extracts, respectively. The MeOH extract was suspended in water and partitioned successively with *n*-BuOH giving *n*-BuOH extract (37.17 g). The CH_2Cl_2 extract (19.30 g) was fractionated by vacuum liquid chromatography (VLC) over silica gel (Merck Art. 7730), eluting with *n*-hexane, CH_2Cl_2, EtOAc and MeOH with increasing polarity to provide five fractions (C1-C5). Fraction C2 was subjected to silica gel column chromatography (CC) and eluted with a gradient system of *n*-hexane-CH_2Cl_2, then CH_2Cl_2-EtOAc (from 1:0 to 0:1) to afford phomarin (**73**, 44 mg). Fraction C4 was further subjected to silica gel CC (*n*-hexane-CH_2Cl_2, 0.5:0.5) and radial chromatography (chromatotron®), using *n*-hexane-EtOAc (4:1) to yield lucidin-ω-methyl ether (**18**, 640 mg) and damnacanthal (**17**, 410 mg).

The initial EtOAc extract (14.99 g) was similarly chromatographed on silica gel VLC, eluting with CH_2Cl_2-*n*-hexane (1:2, 1:1, 3:2, 4:1 and 1:0), followed by EtOAc-CH_2Cl_2 (1:19, 1:9, 1:4 and 1:3) to yield nine fractions (E1–E9). Fraction E4 was rechromatographed on CC over silica gel and eluted with gradient mixtures of *n*-hexane, CH_2Cl_2, EtOAc and MeOH with increasing polarity followed by chromatotron®, using *n*-hexane-EtOAc (4:1) to obtain lucidin (**74**, 10 mg), lucidin-ω-ethyl ether (**75**, 8 mg) and anthragallol-2,3-dimethyl ether (**76**, 7 mg). Fraction E5 was further fractionated over silica gel CC, using CH_2Cl_2, EtOAc and MeOH with

increasing polarity and chromatotron®, using *n*-hexane-EtOAc (4:1) to furnish nordamnacanthal (**16**, 170 mg). Fraction E8 was subsequently purified on silica gel CC, using a stepwise gradient elution of CH_2Cl_2-EtOAc and EtOAc-MeOH, yielding a synthetically known anthraquinone, flavopurpurin (**77**, 10 mg). Fraction E7 was rechromatographed on silica gel CC, using CH_2Cl_2, EtOAc and MeOH with increasing polarity followed by chromatotron®, eluted with *n*-hexane-EtOAc (4:1), and then preparative TLC (100% CH_2Cl_2) to obtain 1-methoxy-2-methyl anthraquinone (**78**, 3 mg), 3-hydroxy-1-methoxy-2-methoxymethyl anthraquinone (**79**, 3 mg) and anthragallol (**80**, 5 mg).

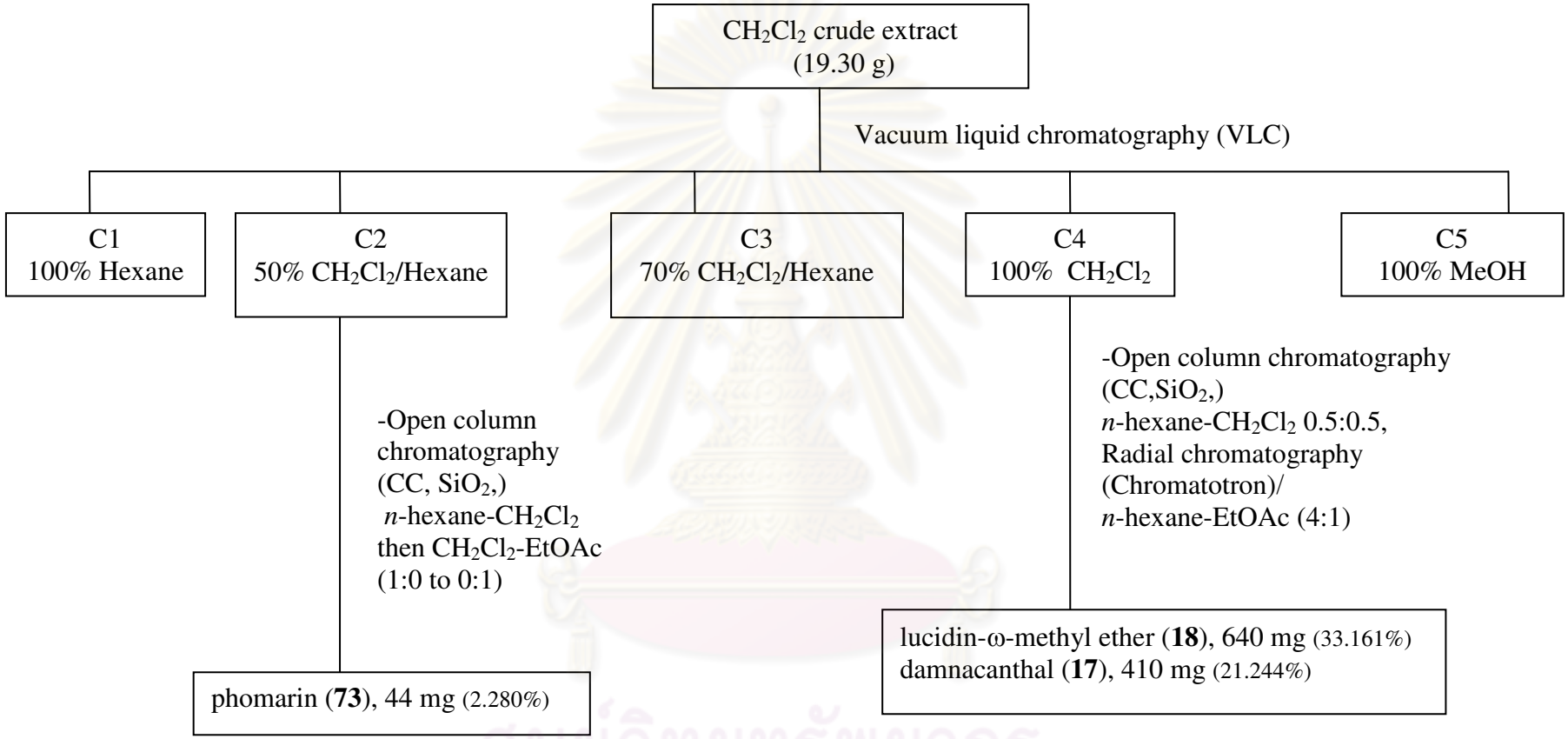
The *n*-BuOH soluble extract (37.17 g) was dissolved in water and subjected to Diaion HP-20 column and successively eluted with water, MeOH and acetone. The MeOH eluent was subjected to silica gel CC, using EtOAc-MeOH-H_2O (9:1:0, 40:10:1 and 70:30:3, respectively) to afford five fractions (B1-B5). Fraction B4 was further purified using Sephadex LH-20 column, eluted with *n*-hexane-CH_2Cl_2-MeOH (5:3:2), yielding asperulosidic acid (**43**, 5 mg) and deacetyl asperulosidic acid (**44**, 5 mg). The identification of all isolated compounds was determined by means of various spectroscopic methods including IR, MS, 1D and 2D NMR techniques as well as comparison with the literature data.

The extraction and purification of all isolated compounds from the CH_2Cl_2, EtOAc and *n*-BeOH extracts of the roots of *M. pandurifolia* Kuntze were briefly summarized in Schemes 2.1- 2.5.

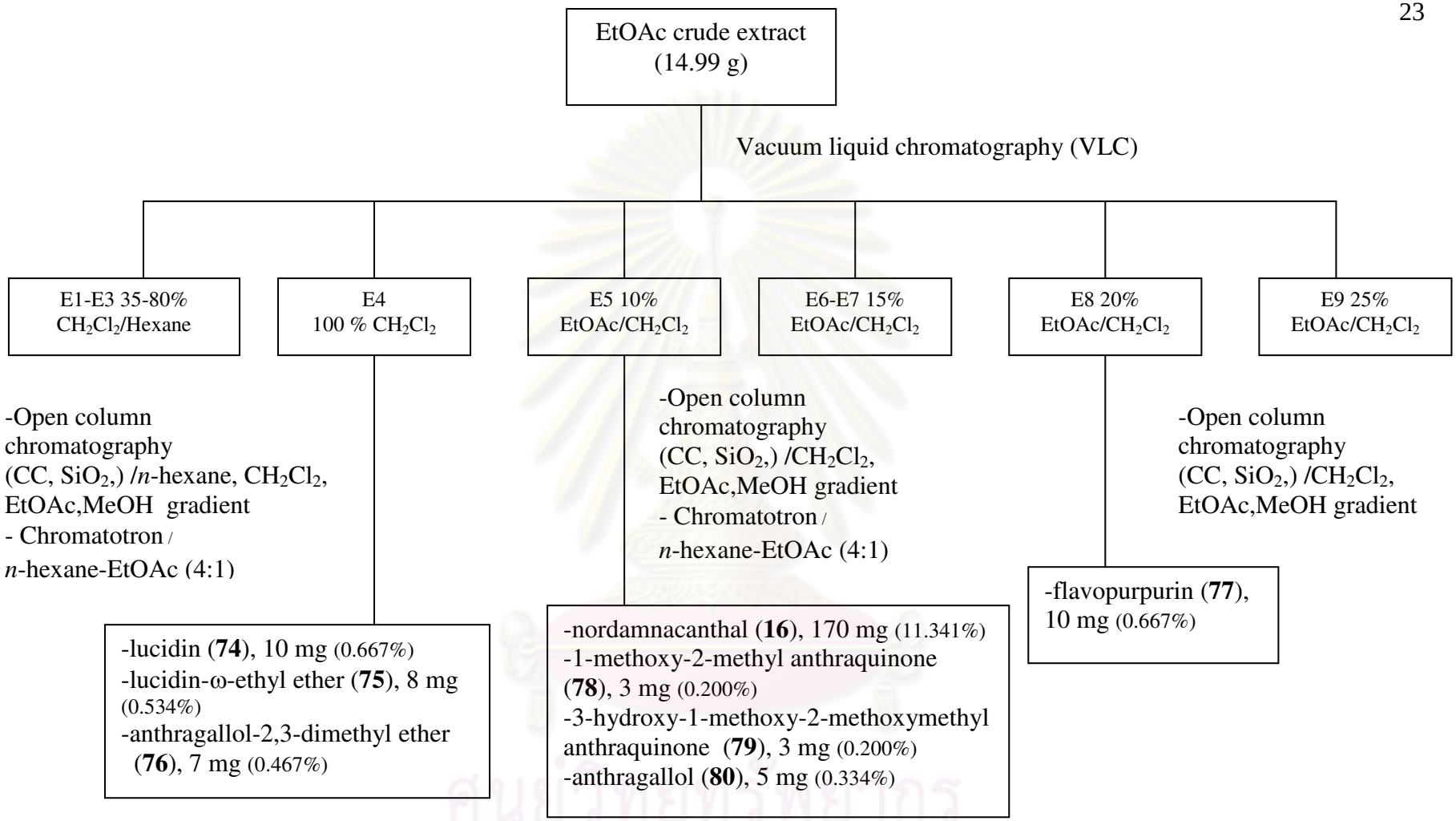


Scheme 2.1 Extraction procedure of *M. pandurifolia* roots

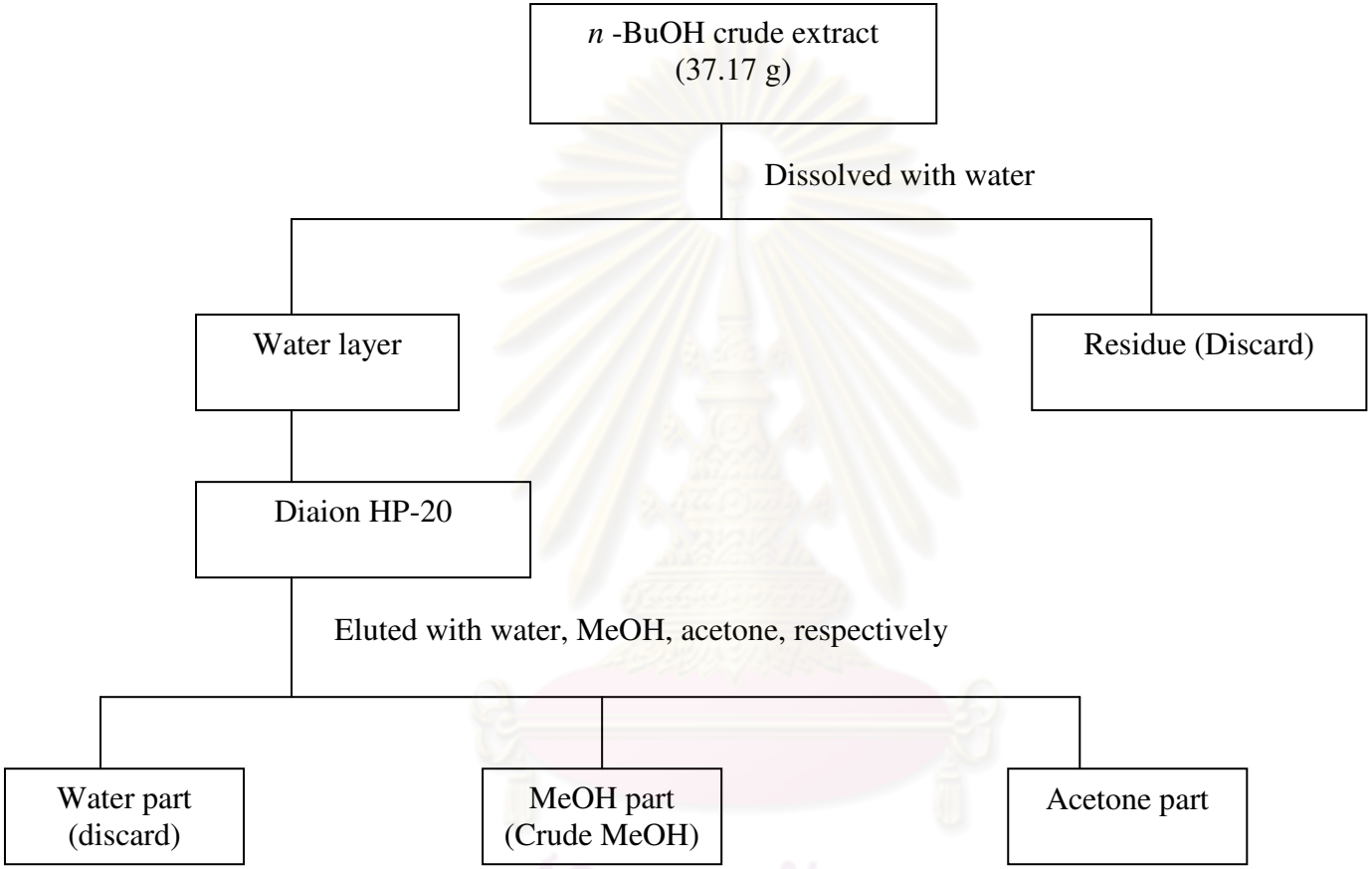
21



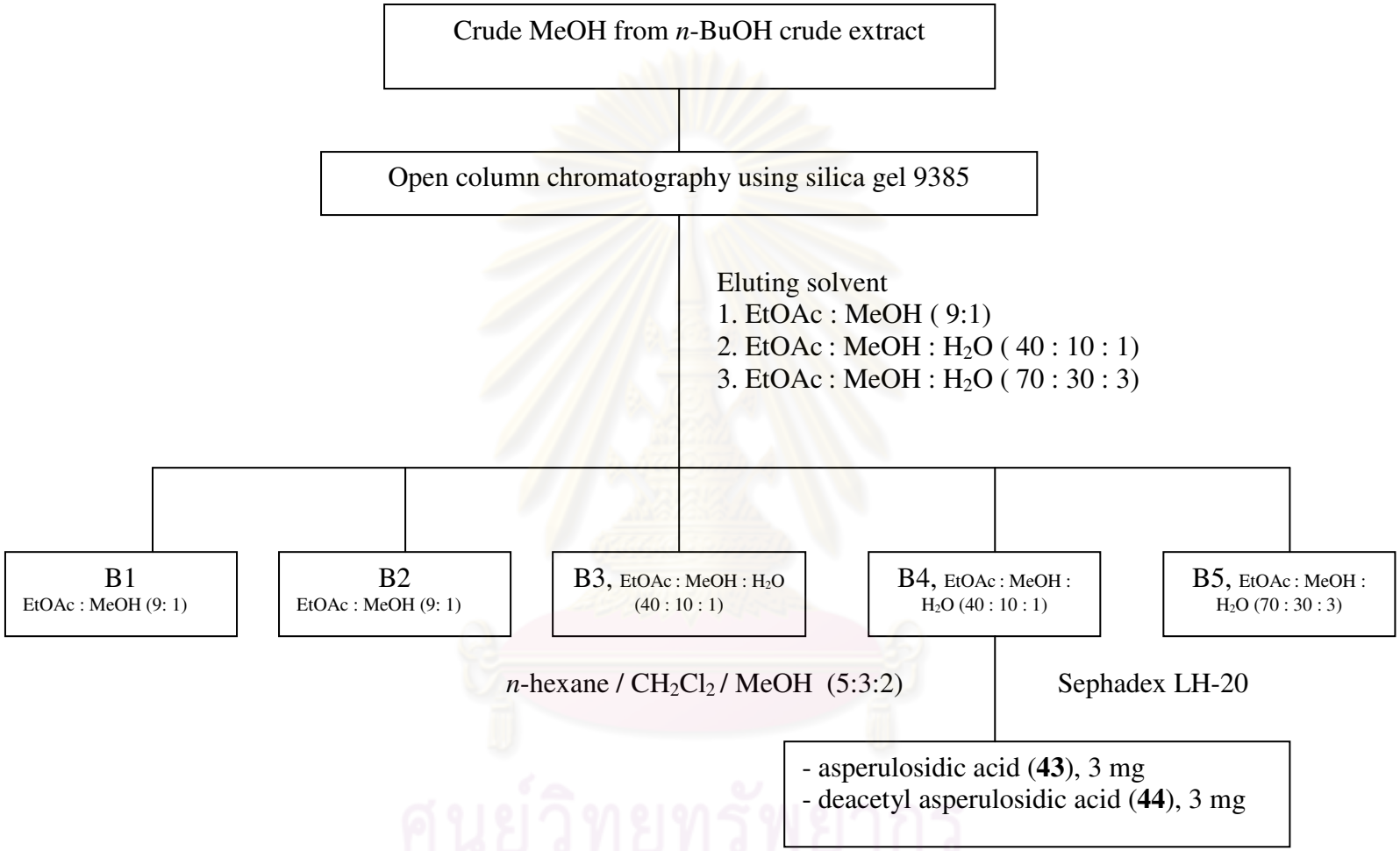
Scheme 2.2 Isolation procedure of the CH_2Cl_2 crude extract from *M. pandurifolia* roots



Scheme 2.3 Isolation procedure of the EtOAc crude extract from *M. pandurifolia* roots.



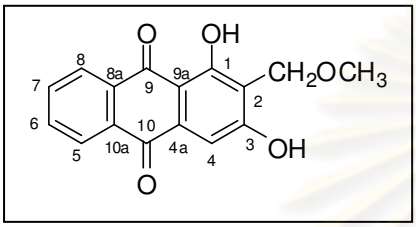
Scheme 2.4 Extraction procedure of *n*-BuOH crude extract from *M. pandurifolia* roots.



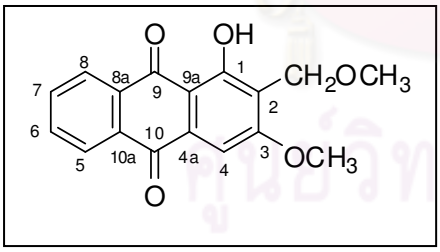
Scheme 2.5 Isolation procedure of the crude MeOH from *n*-BuOH crude extract of *M. pandurifolia* roots.

2.3.2 Preparation of methyl ethers (18a and 73a) of lucidin-ω-methyl ether (18) and phomarin (73)

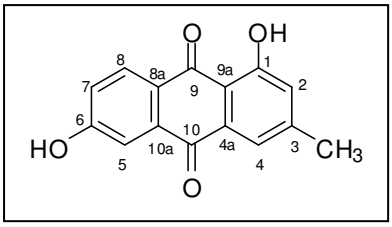
Lucidin-ω-methyl ether (**18**) or phomarin (**73**) 10.0 mg was dissolved in CH_2Cl_2 (400 μL), 6 drops of trimethylsilyldiazomethane ($TMSCHN_2$) in the presence of silica gel (10 mg) was added dropwise until yellow solution persisted. The mixture was stirred at room temperature for 1 h. The methylated product (5.0 mg) was obtained by evaporating reaction mixture until dryness.



Lucidin-ω-methyl ether (**18**) : Yellow amorphous powder; IR (KBr) ν_{max} cm^{-1} : 3300, 2922, 2850, 1674, 1626, 1580, 1556, 1368, 1277, 1159, 1097, 935; UV λ_{max} (MeOH) nm (log ε): 411 (3.66), 281 (4.27), 245 (4.31), 205 (4.31); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.51 (3H, s, 11-OC<u>H</u>$_3$), 4.87 (2H, s, 11-C<u>H</u>$_2$O-), 7.24 (1H, s, H-4), 7.71 (1H, m, H-6), 7.71 (1H, m, H-7), 8.20 (1H, m, H-5), 8.20 (1H, m, H-8), 9.32 (1H, 3-OH), 13.23 (1H, 1-OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 59.4 (C-11, -O<u>C</u>H$_3$), 69.0 (C-11, -CH_2O-), 109.0 (C-9a), 109.7 (C-4), 114.3 (C-2), 127.3 (C-8), 127.4 (C-5), 133.4 (C-10a), 133.5 (C-8a), 133.6 (C-4a), 134.1 (C-6), 134.2 (C-7), 161.8 (C-1), 164.0 (C-3), 182.0 (C-10), 186.0 (C-9).

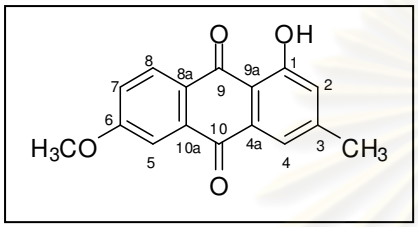


3-Methoxy lucidin-ω-methyl ether (**18a**) : Yellow amorphous powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.42 (3H, s, 11-OCH_3), 4.02 (3H, s, 3-OCH_3), 4.61 (2H, s, H-11), 7.39 (1H, s, H-4), 7.76 (1H, m, H-6), 7.76 (1H, m, H-7), 8.23 (1H, d, *J* = 8.8 Hz, H-5), 8.27 (1H, d, *J* = 8.8 Hz, H-8), 13.11 (1H, s, 1-OH).



Phomarin (1,6-dihydroxy-3-methyl-9,10-anthraquinone (**73**) : orange amorphous solid; UV λ_{max} (MeOH) nm : 215, 231, 251, 338, 356 and 441; IR(KBr) ν_{max} cm^{-1} : 3438 (OH),

2923, 1659 (C=O), 1634 (chelated C=O), 1595, 1475, 1366, 1276 and 779; ESIMS m/z 253 $[M-H]^-$; ^{1}H NMR (400 MHz, acetone-d_6) δ: 2.32 (3H, s, H-11), 7.30 (1H, d, J = 8.4 Hz, H-7), 7.59 (1H, m, H-5), 7.64 (1H, m, H-2), 7.64 (1H, m, H-4), 8.18 (1H, d, J = 8.4 Hz, H-8), 13.17 (1H, s, 1-OH) ; ^{13}C NMR (100 MHz, acetone-d_6) δ: 14.6 (C-11), 112.0 (C-4a), 112.2 (C-5), 118.1 (C-4), 120.7 (C-9a), 126.8 (C-7), 129.3 (C-8), 135.0 (C-10a), 135.5 (C-2), 136.0 (C-3), 136.2 (C-8a), 160.3 (C-1), 163.3 (C-6), 181.3 (C-10), 188.0 (C-9).



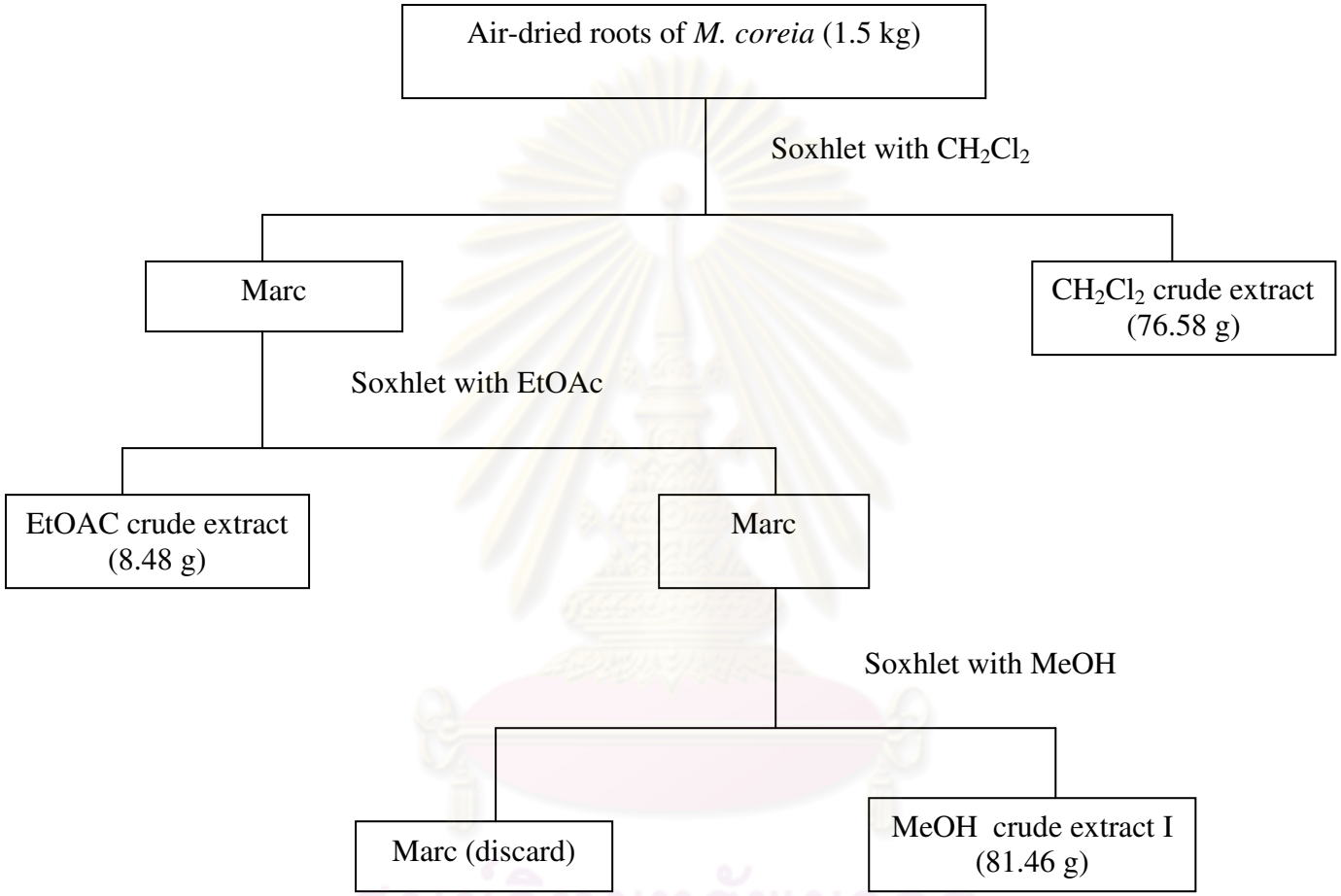
6-Methoxy phomarin (**73a**) : ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.31 (3H, s, H-11), 3.92 (3H, s, 6-OCH_3), 7.44 (1H, d, J = 8.0 Hz, H-7), 7.66 (1H, d, J = 2.4 Hz, H-2), 7.66 (1H, d, J = 2.4 Hz, H-4), 7.68 (1H, s, H-5), 8.19 (1H, d, J = 8.0 Hz, H-8), 13.04 (1H, s, 1-OH).

2.3.3 *M. coreia* Ham.

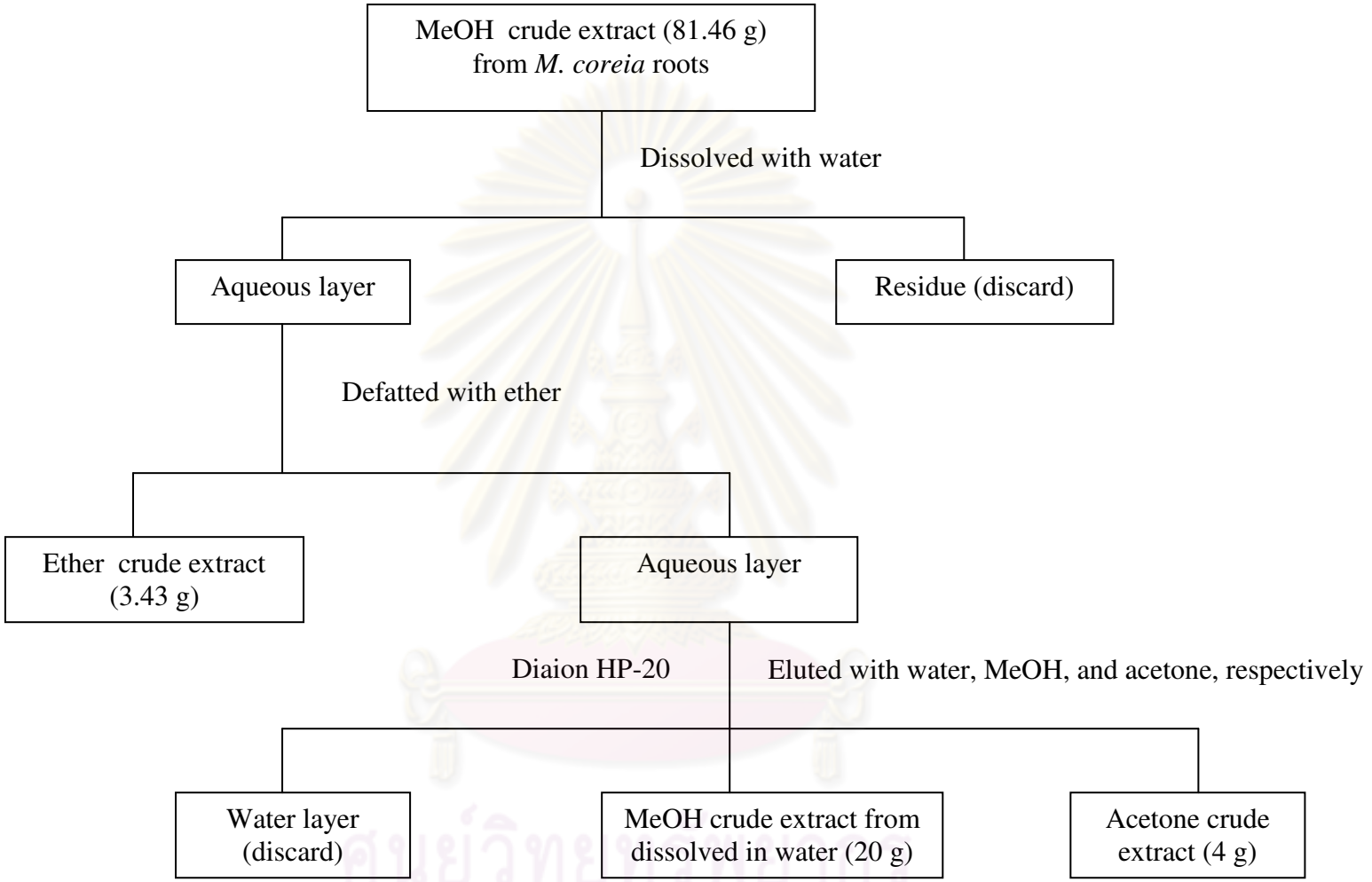
Air-dried and powdered roots of *M.coreia* (1.5 kg) were successively extracted in a soxhlet with CH_2Cl_2, EtOAc and MeOH. Removal of solvents from each extract under reduced pressure gave CH_2Cl_2 (76.58 g), EtOAc (8.48 g) and MeOH (81.46 g) crude extracts, respectively. The MeOH extract was suspended in water and the aqueous layer was defatted with ether giving ether extract (3.43 g) and aqueous layer. The CH_2Cl_2 extract (76.58 g) was fractionated by vacuum liquid chromatography (VLC) over silica gel (Merck Art. 7730), eluting with *n*-hexane, CH_2Cl_2, EtOAc and MeOH with increasing polarity to provide five fractions (DC1-DC5). Fraction DC2 was subjected to silica gel column chromatography (CC) and eluted with a gradient system of *n*-hexane-CH_2Cl_2, then CH_2Cl_2-EtOAc (from 1:0 to 0:1) to afford nordamnacanthal (**16**, 10 mg). Fraction DC4 was further subjected to silica gel CC (*n*-hexane,CH_2Cl_2, EtOAc and MeOH with gradient system) and radial chromatography (chromatotron®), using *n*-hexane-EtOAc (4:1) to yield damnacanthal (**17**, 10 mg), phomarin (**73**, 20 mg), 1-methoxy-2-methyl anthraquinone (**78**, 5 mg), anthragallol (**80**, 6 mg), 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**, 4 mg) and 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (**82**, 5 mg).

The methanolic extract was dissolved in water and the aqueous layer of *M.coreia* was subjected to Diaion HP-20 column and successively eluted with water, MeOH and acetone. The MeOH extract from dissolved in water (12.7 g) was subjected to silica gel CC, using EtOAc-MeOH-H_2O (9:1:0, 40:10:1 and 70:30:3, respectively) to afford five fractions (M1-M5). Fraction M4 was further purified using flash column chromatography, eluted with CH_2Cl_2-MeOH (4:1), yielding 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside or lucidin-3-*O*-β-glucoside (**83**, 5 mg). The identification of all isolated compounds was determined by means of various spectroscopic methods including IR, MS, 1D and 2D NMR techniques as well as comparison with the literature data.

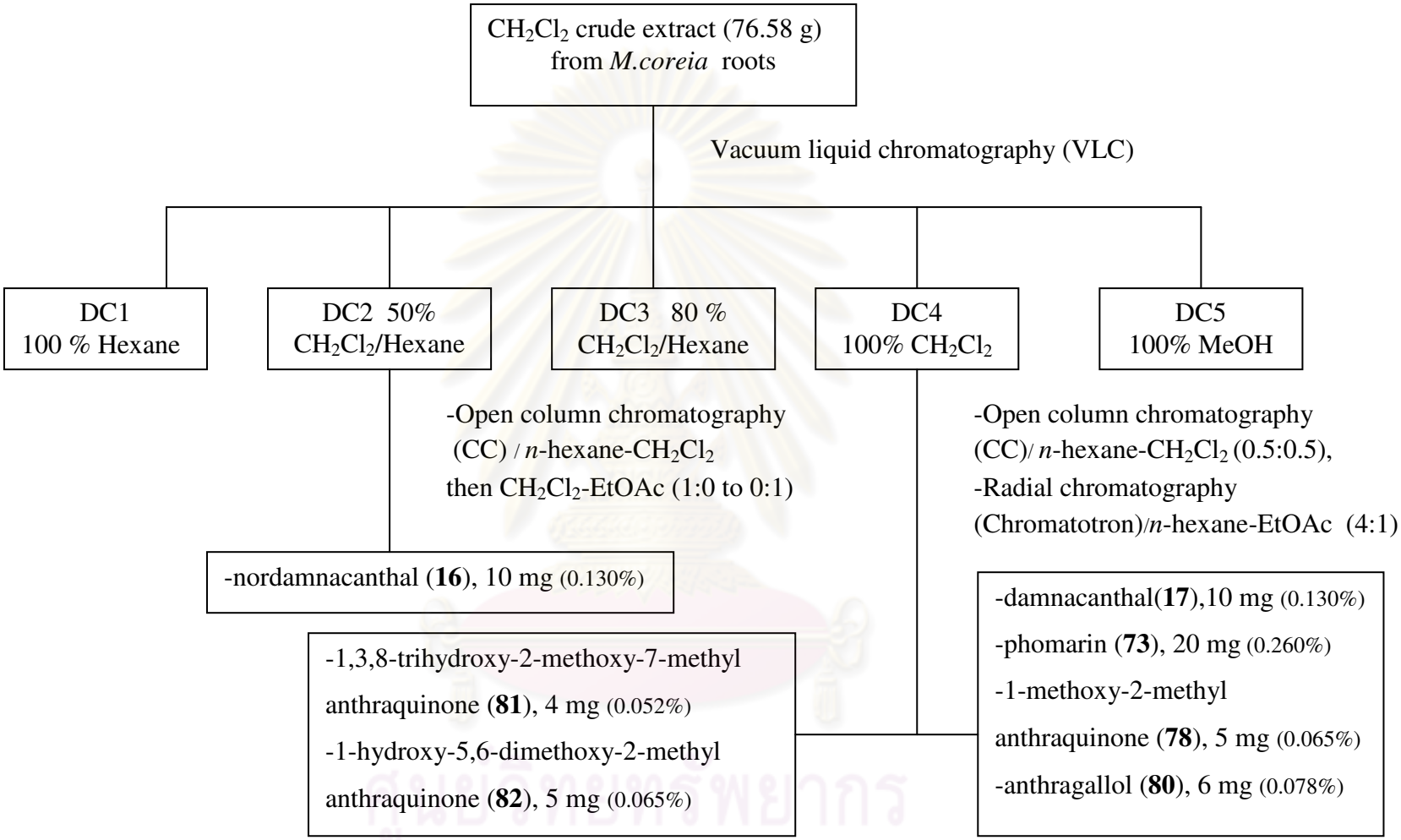
The extraction and purification of all isolated compounds from the CH_2Cl_2 and MeOH extracts of the roots of *M. coreia* were briefly summarized in Schemes 2.6-2.9.



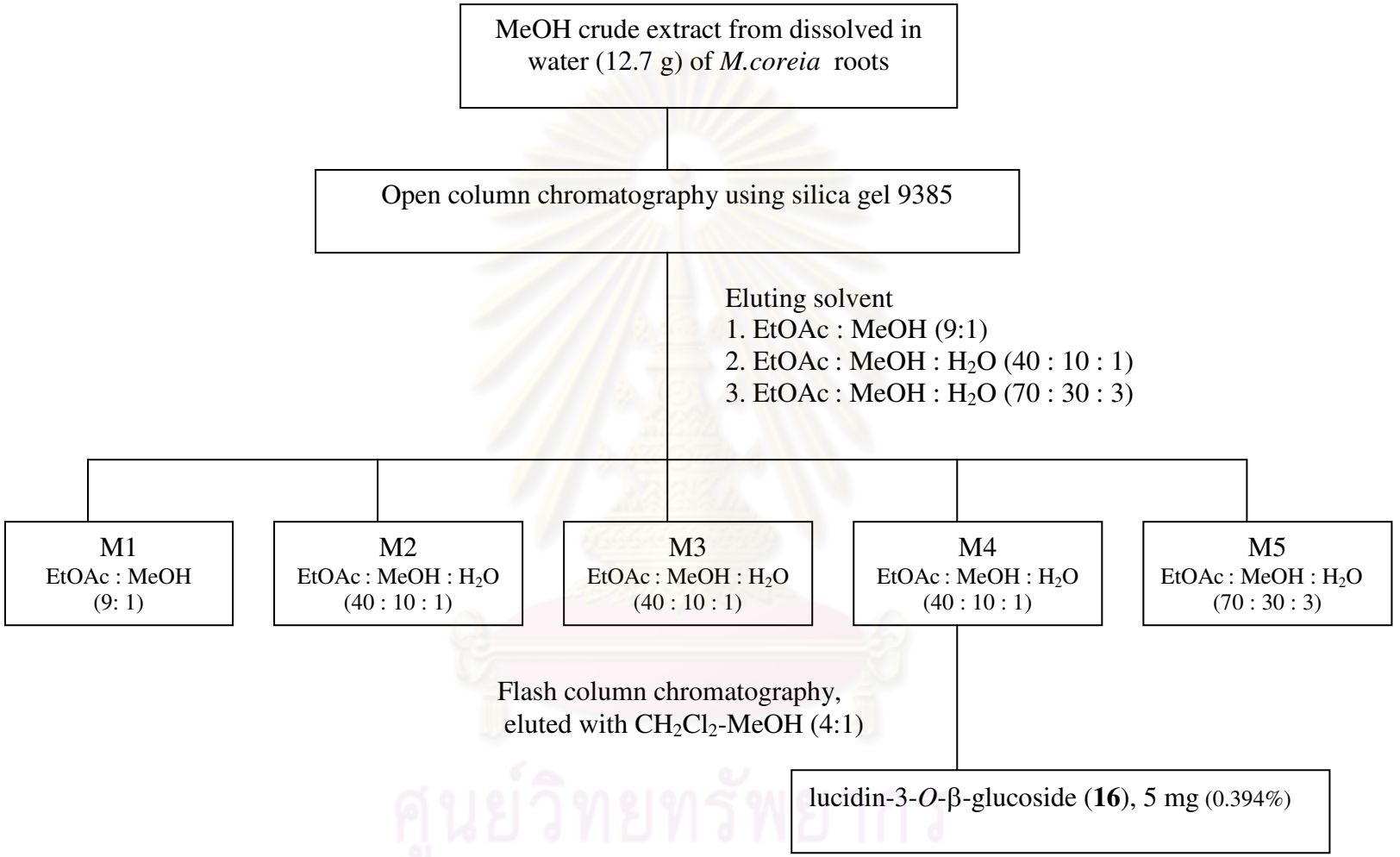
Scheme 2.6 Extraction procedure of *M. coreia* roots



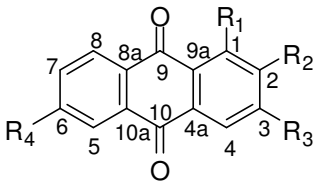
Scheme 2.7 Extraction procedure of MeOH crude extract from *M. coreia* roots



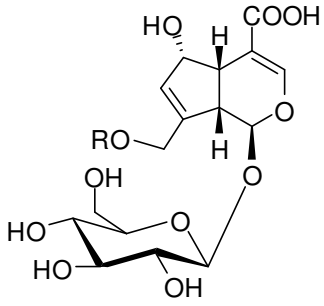
Scheme 2.8 Isolation procedure of the CH_2Cl_2 crude extract from *M. coreia* roots



Scheme 2.9 Isolation procedure of the MeOH crude extract from dissolved in water of *M. coreia* roots.

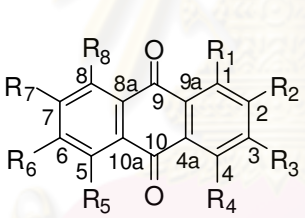


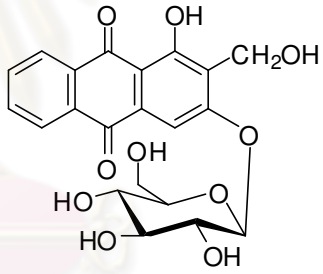
	R_1	R_2	R_3	R_4
18	OH	CH_2OCH_3	OH	H
17	OCH_3	CHO	OH	H
16	OH	CHO	OH	H
73	OH	H	CH_3	OH
74	OH	CH_2OH	OH	H
75	OH	$CH_2OCH_2CH_3$	OH	H
76	OH	OCH_3	OCH_3	H
77	OH	OH	H	OH
78	OCH_3	CH_3	H	H
79	OCH_3	CH_2OCH_3	OH	H
80	OH	OH	OH	H



43 R = Ac
44 R = H

Figure 2.1 Isolated compounds from *M. pandurifolia* roots





83

	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
17	OCH_3	CHO	OH	H	H	H	H	H
16	OH	CHO	OH	H	H	H	H	H
73	OH	H	CH_3	H	H	OH	H	H
78	OCH_3	CH_3	H	H	H	H	H	H
80	OH	OH	OH	H	H	H	H	H
81	OH	OCH_3	OH	H	H	H	CH_3	OH
82	OH	CH_3	H	H	OCH_3	OCH_3	H	H

Figure 2.2 Isolated compounds from *M. coreia* roots

2.4 Cytotoxicity against HeLa and KB cell lines by MTT colorimetric assay

All tested compounds (1 mg each) were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma) and HeLa (human cervical carcinoma) cell lines employing the MTT colorimetric assay. Adriamycin was used as standard antibiotic antitumor agent which exhibits activity against KB and HeLa cell lines according to the method of Kongkathip et al. [45]. This assay was kindly performed by Natural Products Research Section, Research Division, National Cancer Institute, Thailand.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Extraction and isolation of *M. pandurifolia* Kuntze

Air-dried and powdered roots of *M. pandurifolia* were successively extracted in a soxhlet with CH_2Cl_2, EtOAc, and MeOH. The MeOH extract was suspended in water and partitioned with *n*-BuOH, giving *n*-BuOH extract.

The CH_2Cl_2 extract was fractionated by vacuum liquid chromatography (VLC), silica gel column chromatography (CC) and radial chromatography (chromatotron®) to afford phomarin (**73**)[46]; lucidin-ω-methyl ether (**18**)[23] and damnacanthal (**17**)[23].

The EtOAc extract was similarly chromatographed on silica gel VLC, rechromatographed on CC over silica gel and followed by chromatotron®, to obtain lucidin (**74**)[23,47]; lucidin-ω-ethyl ether (**75**)[48]; anthragallol-2,3-dimethyl ether (**76**)[49]; nordamnacanthal (**16**)[23]; synthetically known anthraquinone, flavopurpurin (**77**)[50]; 1-methoxy-2-methyl anthraquinone (**78**)[51]; 3-hydroxy-1-methoxy-2-methoxymethyl anthraquinone (**79**)[23] and anthragallol (**80**)[52].

The water soluble part of *n*-BuOH extract was chromatographed, using Diaion HP-20, silica gel CC and Sephadex LH-20 column, yielding asperulosidic acid (**43**)[53] and deacetyl asperulosidic acid (**44**)[53].

Lucidin-ω-methyl ether (**18**) and phomarin (**73**) were prepared as methyl ether derivatives, using trimethylsilyldiazomethane ($TMSCHN_2$) to confirm hydroxyl group, the data and structures are shown in Tables 3.1-3.2.

Table 3.1 ^{1}H and ^{13}C NMR spectral data ($CDCl_3$) of lucidin-ω-methyl ether (**18**); and ^{1}H NMR spectral data ($CDCl_3$) of 3-methoxy lucidin-ω-methyl ether (**18a**)

Position	lucidin-ω-methyl ether (**18**)		3-methoxy lucidin-ω-methyl ether (**18a**)
	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)
1	13.23 (-OH)	161.8	13.11 (-OH)
2	-	114.3	-
3	9.32 (-OH)	164.0	4.02 ($-OCH_3$)
4	7.24	109.7	7.39
4a	-	133.6	-
5	8.20 (m)	127.4	8.23 (d, 8.8)
6	7.71 (m)	134.1	7.76 (m)
7	7.71 (m)	134.2	7.76 (m)
8	8.20 (m)	127.3	8.27 (d, 8.8)
8a	-	133.5	-
9	-	186.0	-
9a	-	109.0	-
10	-	182.0	-
10a	-	133.4	-
11	4.87 ($-C\underline{H}_2O$)	69.0($-\underline{C}H_2O$)	4.61 ($-\underline{C}H_2O$)
	3.51 ($-OC\underline{H}_3$)	59.4 ($-O\underline{C}H_3$)	3.42 ($-O\underline{C}H_3$)

Table 3.2 ^{1}H and ^{13}C NMR spectral data (acetone-d_6) of phomarin (1,6-dihydroxy-3-methyl anthraquinone) (**73**); and ^{1}H NMR spectral data ($CDCl_3$) of 6-methoxy phomarin (1-hydroxy-6-methoxy-3-methyl anthraquinone) (**73a**)

Position	phomarin (**73**)		6-methoxy phomarin (**73a**)
	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)
1	13.17 (-OH)	160.3	13.04 (-OH)
2	7.64 (m)	135.5	7.66 (d, 2.4)
3	-	136.0	-
4	7.64 (m)	118.1	7.66 (d, 2.4)
4a	-	112.0	-
5	7.59 (m)	112.2	7.68 (m)
6	-	163.3	3.92 ($-OCH_3$)
7	7.30 (d, 8.4)	126.8	7.44 (d, 8.0)
8	8.18 (d, 8.4)	129.3	8.19 (d, 8.0)
8a	-	136.2	-
9	-	188.0	-
9a	-	120.7	-
10	-	181.3	-
10a		135.0	
11	2.32 ($3-CH_3$)	14.6	2.31 ($3-CH_3$)

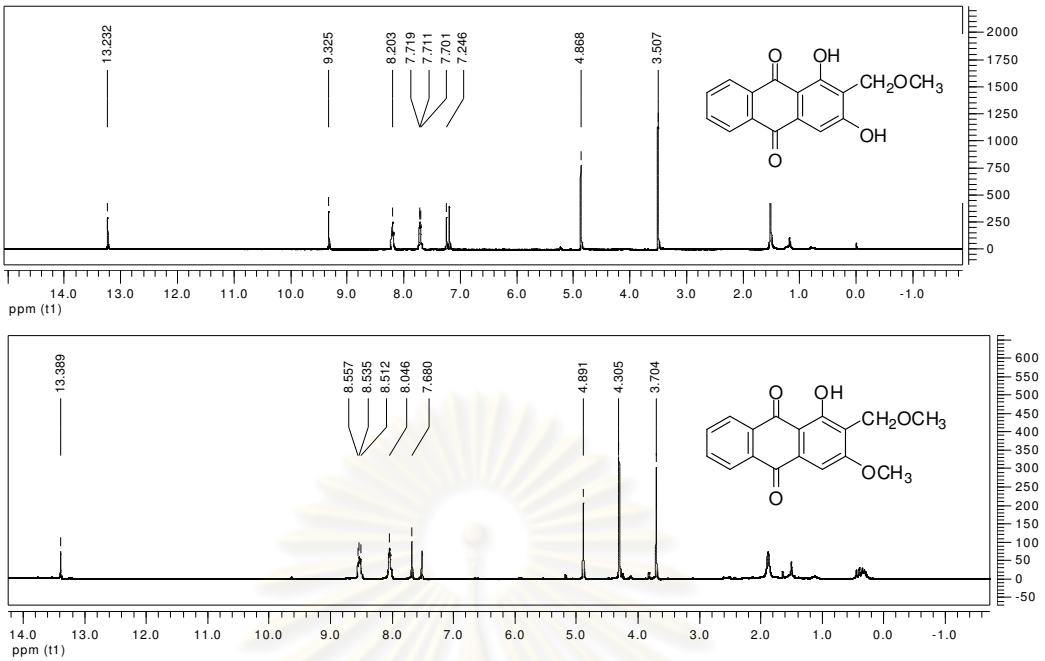


Figure 3.1 ^{1}H NMR spectrum ($CDCl_3$) of compounds **18** and **18a**

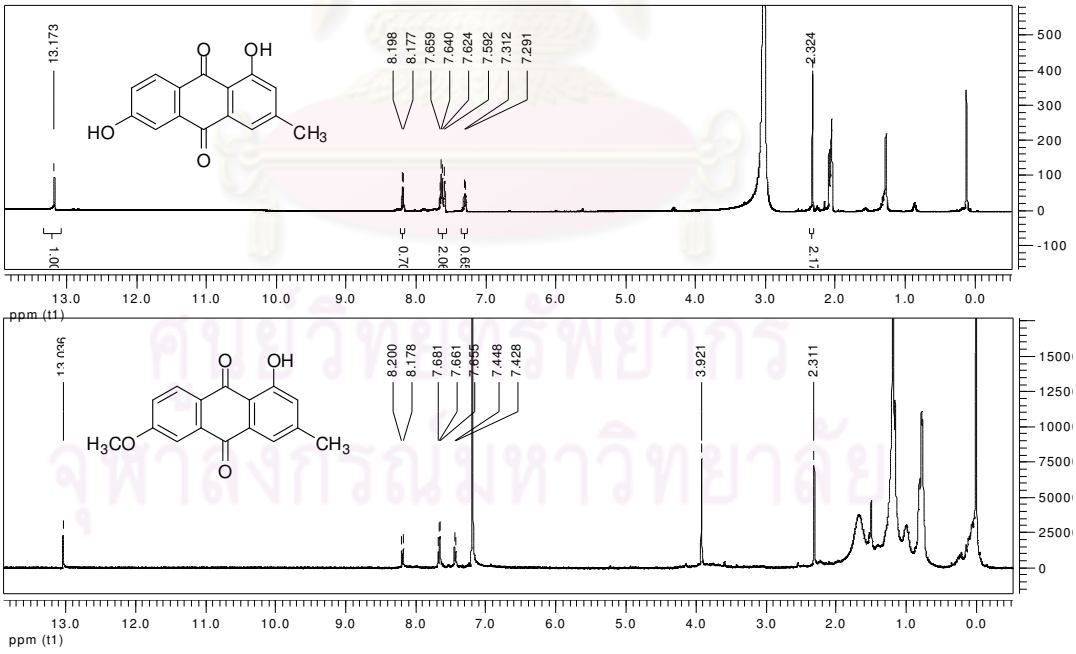


Figure 3.2 ^{1}H NMR spectrum of compounds **73** (acetone-d_6) and **73a** ($CDCl_3$)

3.2 Properties and structural elucidation of isolated compounds

3.2.1 Structure elucidation of synthetically known flavopurpurin (1,2,6-trihydroxy anthraquinone; 77)

Flavopurpurin (**77**) was obtained as a brown-yellow amorphous powder. Its molecular formula was determined to be $C_{14}H_8O_5$ and ESIMS gave a positive molecular ion at *m/z* 255.288 $[M-H]^+$, indicating eleven degrees of unsaturation. The UV spectrum of **77** exhibited absorption maxima at 228 and 273 nm, suggesting an anthraquinone chromophore as the basic structure. The IR absorption bands of **77** (Figure A-22) showed the presence of hydroxyl (3405 cm^{-1}), free carbonyl (1637cm^{-1}) and chelated carbonyl (1596 cm^{-1}) functionalities. The ^1H NMR spectrum of **8** (Figure A-23) in $CDCl_3$+CD_3OD revealed five aromatic proton signals at δ 7.12, 7.41, 7.49, 7.62 and 8.12 ppm. Two pairs of *ortho*-coupled proton signals, one at δ 7.62 and 7.41 (each 1H, d, J = 7.6 Hz), and the other at δ 8.12 and 7.12 (each 1H, d, J = 8.0 Hz) were observed. Furthermore, the spectrum showed a chelated hydroxyl proton at δ 13.10. The ^1H-^1H COSY spectrum (Figure A-25-26) displayed two pairs of proton correlations, one at δ 7.62 and 7.41, and the other at 8.12 and 7.12. The ^{13}C NMR spectrum of **77** (Figure A-24) exhibited two carbonyl groups of an anthraquinone skeleton at δ 183.2 (non-chelated), and 188.0 for the carbonyl chelated with OH group. The HMBC correlation (Fig. A-29-30) between the chelated hydroxyl group signal at δ 13.10, C-9a (δ 115.5), C-3 (δ 135.0) and C-1 (δ 160.6) was used to assign the hydroxyl group to C-1. Based on HMBC correlations, the chelated and nonchelated carbonyl groups were located on C-9 (δ 188.0) and C-10 (δ 183.2), respectively. The methine proton at δ 8.12 (C-8) correlated with the chelated carbonyl group at δ 188.0 (C-9), and signals at δ 163.2 (C-6) and 135.8 (C-7). The signals at δ 7.49 and 7.62 were assigned to the methine protons on C-5 and C-4. Both methine protons showed correlation to the non-chelated carbonyl group at δ 183.2 (C-10). The other HMBC correlations allowed unequivocal assignment of all other carbon, and proton signals in **77** (Table 3.3, Figure 3.3 and Figure 3.4). The structure of **77** was established as 1,2,6 –trihydroxy anthraquinone (flavopurpurin).

Table 3.3 ^{1}H, ^{13}C NMR, HMBC and ^{1}H-^{1}H COSY data of flavopurpurin (**77**) in $CDCl_3$+CD_3OD

Position	δ_C	δ_H (mult.,*J* in Hz)	HMBC	COSY
1	160.6	-	-	-
2	160.0	-	-	-
3	135.0	7.41 (d, 7.6)	C-2, C-4, C-4a	H-4
4	119.0	7.62 (d, 7.6)	C-3, C-9a, C-10	H-3
4a	131.0	-	-	-
5	112.9	7.49 (s)	C-8a, C-10, C-10a	-
6	163.2	-	-	-
7	135.8	7.12 (d, 8.0)	C-5, C-8a	H-8
8	130.0	8.12 (d, 8.0)	C-6, C-7, C-9	H-7
8a	125.2	-	-	-
9	188.0	-	-	-
9a	115.5	-	-	-
10	183.2	-	-	-
10a	121.2	-	-	-
1-OH	-	13.10 (s)	C-1, C-3, C-9a	-

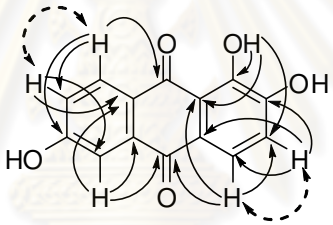


Figure 3.3 The key HMBC ( ) and COSY ( ) correlations of compound **77**

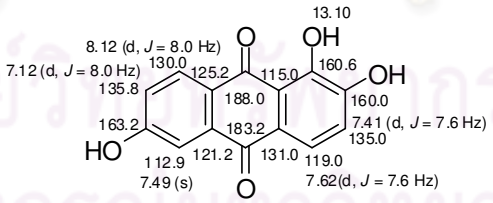
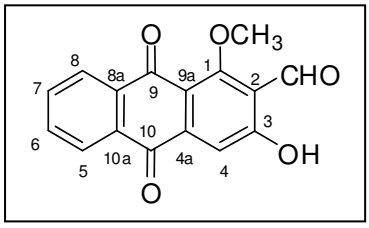
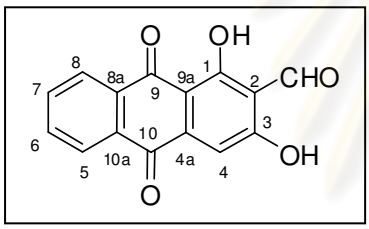


Figure 3.4 The assignments of all carbon and proton signals of compound **77**

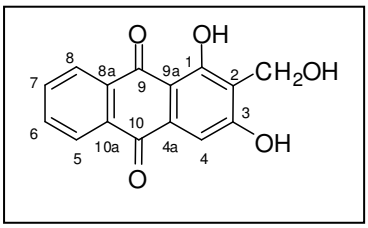
3.2.2 Properties and structural elucidation of compounds 16, 17, 43, 44, 74-76, 78-80



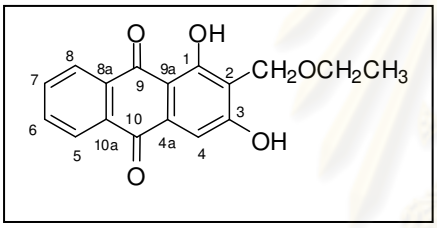
Damnacanthal (2-formyl-3-hydroxy-1-methoxy anthraquinone, **17**) : Yellow amorphous powder; IR(KBr) ν_{max} cm^{-1} : 3070, 1668, 1647, 1565, 1344, 1329, 1261, 1132, 980; UV λ_{max} (MeOH) nm (log ε) : 283 (4.23), 247 (4.26), 203 (4.12); ^{1}H NMR (400 MHz, $CDCl_3$) δ : 4.06 (3H, s, 1-OCH_3), 7.61 (1H, s, H-4), 7.71-7.77 (1H, m, H-6), 7.71-7.77 (1H, m, H-7), 8.18-8.24 (1H, m, H-5), 8.18-8.24 (1H, m, H-8), 10.41 (1H, s, 2-CHO), 12.22 (1H, s, 3-OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 64.7 (1-O<u>C</u>H_3), 113.1 (C-4), 117.6 (C-9a), 118.0 (C-2), 127.1 (C-5), 127.4 (C-8), 132.4 (C-10a), 133.6 (C-8a), 134.6 (C-6), 134.8 (C-7), 141.6 (C-4a), 166.6 (C-3), 175.5 (C-1), 180.2 (C-10), 181.9 (C-9), 195.5 (2-<u>C</u>HO).



Nordamnacanthal (2-formyl-1,3-dihydroxy anthraquinone, **16)** : Yellowish-orange needles; IR(KBr) ν_{max} cm^{-1} : 3075, 1651, 1628, 1600, 1574, 1381, 1331, 1283, 1196, 984; UV λ_{max} (MeOH) nm (log ε) : 291 (4.33), 258 (4.40), 207 (4.25); ^{1}H NMR (400 MHz, $CDCl_3$) δ : 7.26 (1H, s, H-4), 7.74-7.80 (1H, m, H-6), 7.74-7.80 (1H, m, H-7), 8.21-8.26 (1H, m, H-5), 8.21-8.26 (1H, m, H-8), 10.43 (1H, s, 2-C<u>H</u>O), 12.61 (1H, s, 3-OH), 13.91 (1H, s, 1-OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 109.4 (C-2), 109.7 (C-4), 112.1 (C-9a), 126.9 (C-8), 127.7 (C-5), 133.2 (C-7), 134.6 (C-10a), 134.7 (C-6), 134.8 (C-8a), 139.4 (C-4a), 168.1 (C-1), 169.1 (C-3), 181.4 (C-10), 186.7 (C-9), 193.9 (2-<u>C</u>HO).

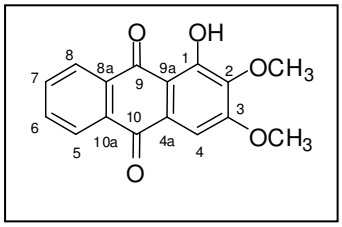


Lucidin (1,3-dihydroxy-2-hydroxymethyl anthraquinone, **74)** : Yellow amorphous powder; IR(KBr) ν_{max} cm^{-1} : 3413, 2945, 1660, 1615, 1590, 1439, 1413, 1333, 1307, 1284, 1008, 985; UV λ_{max} (MeOH) nm (log ε) : 411 (3.69), 279 (4.23), 245 (4.24), 204 (4.27); ^{1}H NMR (400 MHz, $CDCl_3$) δ : 4.91 (2H, s, 2-C<u>H</u>$_2$OH), 5.23 (1H, s, 2-CH$_2$O<u>H</u>), 7.25 (1H, s, H-4), 7.71-7.73 (1H, m, H-6), 7.71-7.73 (1H, m, H-7), 8.19-8.21 (1H, m, H-5), 8.19-8.21 (1H, m, H-8), 9.59 (1H, s, 3-OH), 13.24 (1H, s, 1-OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 66.98 (2-CH_2OH), 109.79 (C-4), 110.0 (C-9a), 115.0 (C-2), 126.7 (C-5), 127.3 (C-8), 133.0 (C-4a), 134.1 (C-8a), 134.2 (C-10a), 134.3 (C-6), 134.4 (C-7), 162.2 (C-1), 164.0 (C-3), 183.0 (C-10), 186.0 (C-9); ESI MS : $[M-H]^-$ at m/z 269.0445, for $C_{15}H_9O_5$ calculated 269.0450.

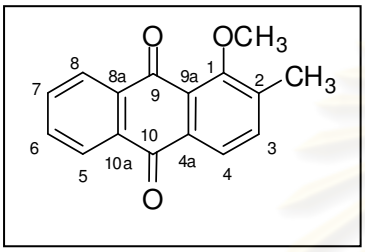


Lucidin-ω-ethyl ether (2-ethoxymethyl-1,3-dihydroxy anthraquinone, **75)** : Yellow amorphous powder; ^{1}H NMR ($CDCl_3$) δ : 1.28 (3H, t, *J* = 7.2 Hz, 2-CH$_2$OCH$_2$C<u>H</u>$_3$), 3.68 (2H, q, *J* = 7.2 Hz, 2-CH$_2$OC<u>H</u>$_2$CH$_3$), 4.91 (2H, s, 2-C<u>H</u>$_2$OCH$_2$CH$_3$), 7.25 (1H, s, H-4), 7.70-7.72 (2H, m, H-6,7), 8.19-8.21 (2H, m, H-5,8), 9.59 (1H, s, 3-OH), 13.23 (1H, s, 1-OH); ESI MS : $[M-H]^+$ at m/z 297.272

Compound **75** was reported by Itokawa, et al.[48] : UV λ_{max} (MeOH) nm (ε) : 240 (1200), 244 (11000), 406 (28000); ^{1}H NMR (DMSO-d_6) δ : 1.11 (3H, s, *J* = 7 Hz, 2-CH_3), 3.47 (2H, q, *J* = 7 Hz, 2-OCH_2), 4.40 (2H, s, 2-CH_2O), 7.14 (1H, s, H-4), 7.77-7.91 (2H, m, H-6,7), 7.95-8.13 (2H, m, H-5,8); ^{13}C NMR (DMSO-d_6) δ : 15.28 (q) (2-CH$_2$O<u>C</u>H$_2$CH$_3$), 59.21 (t) (2-CH$_2$OCH$_2$<u>C</u>H$_3$), 65.21 (t) (2-<u>C</u>H$_2$OCH$_2$CH$_3$), 107.65 (C-4), 108.86 (C-9a), 117.05 (C-2), 126.21 (C-8), 126.62 (C-5), 132.67 (C-8a), 132.84 (C-10a), 133.59 (C-4a), 134.28 (C-7), 134.46 (C-6), 163.63 (C-1), 164.03 (C-3), 181.44 (C-10), 185.89 (C-9).

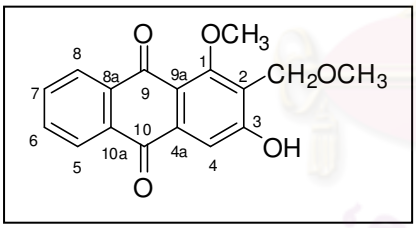


Anthragallol-2,3-dimethyl ether (1-hydroxy-2,3-dimethoxy anthraquinone, **76)** :
Yellowish-orange amorphous powder; mp 162-163 ^{0}C (absolute ethanol); IR(KBr) ν_{max} cm^{-1} : 1667, 1634, 1591, 1576, 1275; ^{1}H NMR ($CDCl_3$) δ : 3.66 (3H, s, 3-OCH_3), 4.08 (3H, s, 2-OCH_3), 7.40 (1H, s, H-4), 7.80-7.84 (2H, m, H-6,7), 8.27-8.34 (2H, m, H-5,8), 12.66 (1H, s, OH-1); ESI MS : $[M-H]^+$ at m/z 283.767.



1-Methoxy-2-methyl anthraquinone **(78)** : Yellow amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$) δ : 2.36 (3H, s, 2-CH_3), 3.86 (3H, s, 1-OCH_3), 7.14 (d, 1H, *J* = 8.4 Hz, H-3), 7.52 (m, 2H, H-6,7), 7.95 (2H, m, H-5,8), 8.14 (1H, m, H-4); ESI MS : $[M-H]^+$ at m/z 253.602.

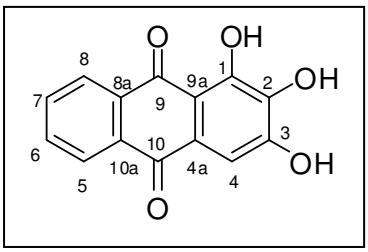
Compound **78** was reported by Zembower, et al.[51] : mp 163-164 ^{0}C, FTIR ($CHCl_3$), 1673, 1592, 1322 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ : 2.44 (3H, s, 2-CH_3), 3.95 (3H, s, 1-OCH_3), 7.62 (d, 1H, *J* = 8.0 Hz, H-3), 7.78 (m, 2H, H-6,7), 8.07 (d, 1H, *J* = 8.0 Hz, H-5), 8.27 (m, 2H, H-5,8); EIMS m/e 252.0791.



3-Hydroxy-1-methoxy-2-methoxymethyl anthraquinone **(79)** : Yellow amorphous powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ : 3.49 (3H, s, 2-$CH_2OC\underline{H}_3$), 3.85 (3H, s, 1-OCH_3), 4.91 (2H, s, 2-$C\underline{H}_2OCH_3$), 7.55 (1H, s, H-4), 7.64-7.73 (2H, m, H-6,7), 8.16-8.21 (2H, m, H-5,8); ESI MS : $[M+H]^+$ at m/z 299.595.

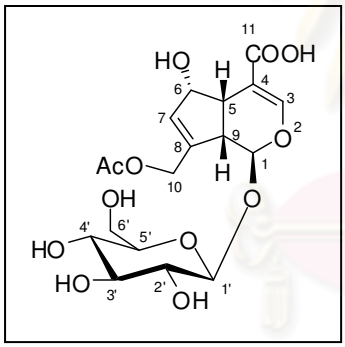
Compound **79** was reported by Kamiya, et al.[23] : IR(KBr) ν_{max} cm^{-1} : 3287, 29467, 1672, 1653, 1568, 1423, 1335, 1302, 1284, 1106, 1065, 977; UV λ_{max} (MeOH) nm (log ε) : 277 (4.52), 240 (4.29), 204 (4.42); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ : 3.29 (3H, s, 11-OCH_3), 3.81 (3H, s, 1-OCH_3), 4.43 (2H, s, H-11), 7.51 (1H, s, H-4), 7.78 (1H, td, *J* = 7.5, 1.1 Hz, H-6), 7.84 (1H, td, *J* = 7.5, 1.1 Hz, H-7), 8.05 (1H, dd, *J* = 7.5, 1.1 Hz, H-5), 8.10 (1H, dd, *J* = 7.5, 1.1 Hz, H-8); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ : 62.23 (11-OCH_3), 62.41 (1-OCH_3), 109.90 (C-4), 117.46 (C-9a), 125.23 (C-2), 126.04 (C-5), 126.60 (C-8), 131.97 (C-10a), 133.25 (C-6), 134.52 (C-

7), 134.52 (C-8a), 135.86 (C-4a), 162.33 (C-1), 162.89 (C-3), 179.77 (C-9), 182.49 (C-10).

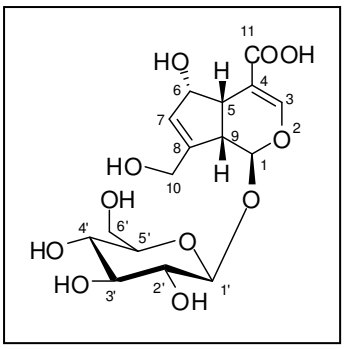


Anthragallol (1,2,3-trihydroxy anthraquinone, **80)** : Yellow amorphous powder, ^{1}H NMR (400 MHz, acetone-d_6) δ : 7.36 (1H, s, H-4), 7.91 (2H, m, H-6,7), 8.20 (1H, m, H-5), 8.28 (1H, m, H-8), 13.23 (1H, s, 1-OH); ESI MS : $[M-H]^+$ at m/z 255.334.

Compound **80** was reported by Dhananjeyan, et al.[52] : mp 282-284 ^{0}C, UV λ_{max} (MeOH) nm (log ε) : 207 (4.59), 243 (4.39), 283 (4.49), 410 (3.71) nm; ^{1}H NMR (DMSO-d_6) δ : 7.29 (1H, s, H-4), 7.91 (2H, m, H-6,H-7), 8.18 (2H, m, H-5,H-8), ^{13}C NMR (DMSO-d_6) δ : 109.52 (C-4), 111.03 (C-4a), 125.37 (C-9a), 126.94 (C-5), 127.30 (C-8), 133.77 (C-7), 133.94 (C-6), 134.79 (C-8a), 135.22 (C-10a), 139.65 (C-3), 152.48 (C-2), 152.72 (C-1), 181.67 (C-10), 187.63 (C-9); FABMS : m/z 257 (11), 256 (6), 232 (7), 209 (3); HRFAB : m/z 257.0449 $[MH^+]$.



Asperulosidic acid **(43)** : colorless oil, IR(KBr) ν_{max} cm^{-1} : 3384.7 (OH), 1730 (C=O), 1635 (C=C), 1718 (C=O); UV λ_{max} (MeOH) 235 nm, ESI-MS m/z 432 $[M]^+$, 455 $[M+Na]^+$, 887 $[2M+Na]^+$ (calc. for $C_{18}H_{24}O_{12}$); ^{1}H NMR (CD_3OD) δ : 2.09 (3H, s, -$COCH_3$), 2.62 (1H, t, H-9), 3.02 (1H, t, J = 6.5 Hz, H-5), 3.25 (1H, m, H-4′), 3.25 (1H, m, H-2′), 3.31 (1H, m, H-5′), 3.35 (1H, m, H-3′), 3.61(2H, dd, J = 12.0, 6.0 Hz, H-6a′), 3.85(2H, dd, J = 12.0, 6.0 Hz, H-6b′), 4.72 (1H, d, J = 8.0 Hz, H-1′), 4.85, 4.94 (2H, d, J = 15.0, 15.0 Hz, H-10), 4.90 (1H, s, H-6), 5.05 (1H, d, J = 9.0 Hz, H-1), 6.01 (1H, d, J = 1.0 Hz, H-7), 7.62 (1H, d, J = 1.0 Hz, H-3); ^{13}C NMR (CD_3OD) δ : 20.8 (CO**C**H_3), 46.3 (C-9), 63.8 (C-10), 71.5 (C-4′), 74.9 (C-2′), 75.4 (C-6), 77.8 (C-5′), 78.5 (C-3′), 100.5 (C-1′), 101.1 (C-1), 109.0 (C-4), 131.8 (C-7), 145.9 (C-8), 154.9 (C-3), 172.5 (C-11), 172.5 (**C**OCH_3).



Deacetylasperulosidic acid **(44)** : colorless oil, IR(KBr) ν_{max} cm^{-1} : 3550 (OH), 1700 (C=O), 1650 (C=C); UV λ_{max} (MeOH) 234 nm, ESI-MS m/z 390 $[M]^+$, (calc. for $C_{16}H_{22}O_{11}$); ^1H NMR (CD_3OD) δ : 2.54 (1H, t, *J* = 8.0 Hz, H-9), 3.01 (1H, t, *J* = 6.5 Hz, H-5), 3.21 (1H, m, H-2′), 3.26 (1H, m, H-3′), 3.26 (1H, m, H-4′), 3.37 (1H, m, H-5′), 3.61(2H, dd, *J* = 12.0, 5.5 Hz, H-6a′), 3.84 (2H, dd, *J* = 12.0, 1.5 Hz, H-6b′), 4.21 (2H, d, *J* = 15.0 Hz, H-10), 4.45 (2H, dd, *J* = 15.5, 1.0 Hz, H-10), 4.71 (1H, d, *J* = 8.0 Hz, H-1′), 4.82 (1H, H-6), 5.03 (1H, d, *J* = 9.0 Hz, H-1), 6.0 (1H, d, *J* = 1.5 Hz, H-7), 7.58 (1H, H-3); ^{13}C NMR (CD_3OD) δ : 43.2 (C-5), 46.1 (C-9), 61.8 (C-10), 62.8 (C-6′), 71.7 (C-4′), 75.0 (C-2′), 75.6 (C-6), 77.8 (C-5′), 78.5 (C-3′), 100.4 (C-1′), 101.3 (C-1), 110.3 (C-4), 129.8 (C-7), 151.5 (C-8), 154.2 (C-3), 172.5 (C-11).

3.3 Extraction and isolation of *M. coreia* Ham

Air-dried and powdered roots of *M. coreia* were successively extracted in a soxhlet with CH_2Cl_2, EtOAc and MeOH. The MeOH extract was suspended in water and the aqueous layer was defatted with ether, giving ether extract and aqueous layer.

The CH_2Cl_2 extract was fractionated by vacuum liquid chromatography (VLC) and radial chromatography (chromatotron®) to afford nordamnacanthal (**16**)[23], damnacanthal (**17**)[23], phomarin (**73**)[46], 1-methoxy-2-methyl anthraquinone (**78**)[51], anthragallol (**80**)[52], synthetically known 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**) and synthetically known 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (**82**)[54].

The aqueous layer was subjected to diaion HP-20 column and successively eluted with water, MeOH and acetone. The MeOH extract was subjected to silica gel CC, flash column chromatography, yielding 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside or lucidin-3-*O*-β-glucoside (**83**)[55].

3.4 Properties and structural elucidation of isolated compounds

3.4.1 Structure elucidation of synthetically known 1,3,8-trihydroxy-2-methoxy -7-methyl anthraquinone (81)

1,3,8-Trihydroxy-2-methoxy-7-methyl anthraquinone (**81**) was isolated as yellow amorphous powder. The molecular formula was established to be $C_{16}H_{12}O_6$ based on the $[M+H]^+$ ion peak at *m/z* 301.632.

The ^{1}H NMR spectrum (Figure A-48) revealed the two singlet resonances of chelated hydroxyl protons (δ_H 13.20 (OH-1), 12.92 (OH-8)); δ_H 4.08, 2.30 were assigned as OCH_3-2 and CH_3-7. The signals of protons in aromatic region, δ_H 7.39 (1H, s, H-4), 7.45 (1H, d, *J* = 8.0 Hz, H-6), 7.65 (1H, d, *J* = 8.0 Hz, H-5). The ^{1}H-^{1}H COSY spectra (Figure A-50-51) displayed a pair of proton correlations at δ_H 7.65 and 7.45. The ^{13}C NMR spectrum (Figure A-49) showed 16 signals, and exhibited two carbonyl groups of an anthraquinone skeleton at δ 187.0 (non-chelated), and 180.0 for the carbonyl chelated with OH group.

The overall structure of **81** was deduced based on HMBC data. The HMBC correlation (Figure A-54-56) between the chelated hydroxyl group signal (OH-1) at δ 13.20, C-9a (δ 112.0), C-1 (δ 156.0) and C-2 (δ 139.0) were used to assign the hydroxyl group to C-1. The other chelated hydroxyl group (OH-9) at δ 12.92 correlated C-7 (δ 136.0), C-8 (δ 160.5) and C-8a (δ 115.0). Based on HMBC correlations, the nonchelated carbonyl groups were located on C-4 (δ 106.5). The methine proton at δ 7.39 (C-4) correlated with the non-chelated carbonyl group at δ 187.0 (C-10), and signals at δ 155.0 (C-3), 139.1 (C-4a) and 112.0 (C-9a). The signals at δ 7.45 and 7.65 were assigned to the methine protons on C-6 and C-5. For methine protons at δ 7.65 (C-5) showed correlation to C-7 at δ 136.0 and C-8a at δ 115.0, respectively. The other HMBC correlations allowed unequivocal assignment of all other carbon, and proton signals in **81** (Table 3.4, Figure 3.5, and Figure 3.6). The structure of **81** was established as 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone.

Table 3.4 ^{1}H, ^{13}C NMR, HMBC and ^{1}H-^{1}H COSY data of 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**) in $CDCl_3$

Position	δ_C	δ_H (mult.,*J* in Hz)	HMBC	COSY
1	156.0	-	-	-
2	139.0	-	-	-
3	155.0	-	-	-
4	106.5	7.39 (s)	C-3, C-4a, C-9a, C-10	-
4a	139.1	-	-	-
5	117.9	7.65 (d, 8.0)	C-7, C-8a	H-6
6	137.0	7.45 (d, 8.0)	C-8, C-10a	H-5
7	136.0	-	-	-
8	160.5	-	-	-
8a	115.0	-	-	-
9	180.0	-	-	-
9a	112.0	-	-	-
10	187.0	-	-	-
10a	132.0	-	-	-
1-OH	-	13.20 (s)	C-1, C-2, C-9a	-
8-OH	-	12.92 (s)	C-7, C-8, C-8a	-
2-OCH_3	60.2	4.08 (s)	C-2	-
7-CH_3	15.2	2.30 (s)	C-6, C-7, C-8	-

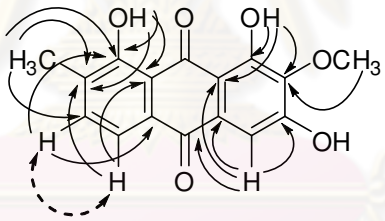


Figure 3.5 The key HMBC ( ) and COSY ( ) correlations of **81**

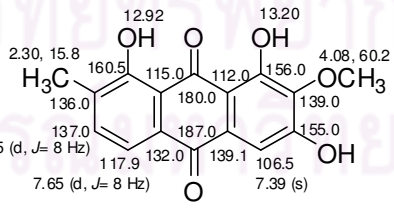


Figure 3.6 The assignments of all carbon and proton signals in **81**

3.4.2 Structure elucidation of synthetically known 1-hydroxy-5,6-dimethoxy -2-methyl anthraquinone (morindone dimethyl ether, 82)

1-Hydroxy-5,6-dimethoxy -2-methyl anthraquinone (morindone dimethyl ether, **82**) was isolated as yellow amorphous powder. The molecular formula was established to be $C_{17}H_{14}O_5$ based on the $[M-H]^+$ ion peak at *m/z* 297.420. The IR spectrum (Figure A-58) exhibited absorption bands for hydroxyl (3444.2 cm^{-1}), 1630.1 cm^{-1} (unchelated CO), 1259.3 cm^{-1}, and 1108.4 cm^{-1}

^1H NMR spectrum (Figure A-59) revealed the singlet resonance of chelated hydroxyl protons (δ_H 13.03); δ_H 4.02, 3.82, 2.37 were assigned as OCH_3-5, OCH_3-6 and CH_3-2, respectively. The four signals of protons in aromatic region, δ_H 7.34 (1H, d, *J* = 8.4 Hz), 8.13 (1H, d, *J* = 8.4 Hz), 7.51 (1H, d, *J* = 7.6 Hz) and.7.69 (1H, d, *J* = 7.6 Hz), were assigned as H-7, H-8, H-3 and H-4. The ^1H-^1H COSY spectra (Figure A-61-62) displayed two pair of proton correlations at δ_H 7.34 and 8.13, the other at δ_H 7.51 and 7.69. The ^{13}C NMR spectrum (Figure A-60) showed 17 signals, and exhibited two carbonyl groups of an anthraquinone skeleton at δ 180.0 (non-chelated), and 187.0 for the carbonyl chelated with OH group.

The overall structure of **82** was deduced based on HMBC data. The HMBC correlation (Figure A-64-67) between the chelated hydroxyl group signal (OH-1) at δ 13.03, C-9a (δ 114.5), C-1 (δ 160.5) and C-2 (δ 134.5) were used to assign the hydroxyl group to C-1. Based on HMBC correlations, the nonchelated carbonyl groups were located on C-4 (δ 117.9). The methine proton at δ 7.69 (C-4) correlated with the non-chelated carbonyl group at δ 180.0 (C-10), and signals at δ 134.5 (C-2) and 114.5 (C-9a). The methine protons at δ 8.13 (C-8) showed correlation to chelated carbonyl group (C-9) at δ 187.0, C-10a (δ 126.0) and C-6 (δ 155.0), respectively.
The other HMBC correlations allowed unequivocal assignment of all other carbon, and proton signals in **82** (Table 3.5, Figure 3.7 and Figure 3.8). The structure of **82** was established as 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (morindone dimethyl ether).

Table 3.5 ^{1}H, ^{13}C NMR, HMBC and ^{1}H-^{1}H COSY data of 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (morindone dimethyl ether); (**82**) in $CDCl_3$

Position	δ_C	δ_H (mult.,*J* in Hz)	HMBC	COSY
1	160.5	-	-	-
2	134.5	-	-	-
3	135.9	7.51 (d, 7.6)	C-2, C-4a, (CH_3-2)	H-4
4	117.9	7.69 (d, 7.6)	C-2, C-9a, C-10	H-3
4a	130.0	-	-	-
5	126.0	-	-	-
6	155.0	-	-	-
7	118.9	7.34 (d, 8.4)	C-5, C-8a	H-8
8	124.5	8.13 (d, 8.4)	C-6, C-9, C-10a	H-7
8a	127.0	-	-	-
9	187.0	-	-	-
9a	114.5	-	-	-
10	180.0	-	-	-
10a	146.0	-	-	-
1-OH	-	13.03 (s)	C-1, C-2, C-9a	-
2-CH_3	17.0	2.37 (s)	C-1, C-2, C-3	-
5-OCH_3	54.7	4.02 (s)	-	-
6-OCH_3	60.6	3.82 (s)	-	-

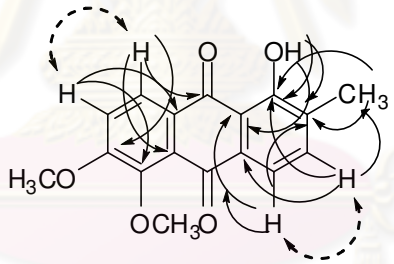


Figure 3.7 The key HMBC ( ) and COSY ( ) correlations of compound **82**

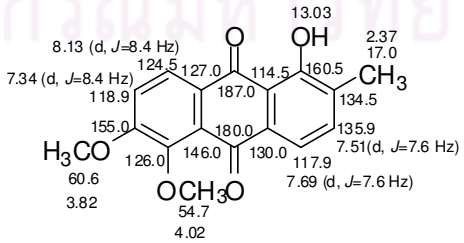


Figure 3.8 The assignments of all carbon and proton signals of compound **82**

3.4.3 Properties and structural elucidation of 1,3-dihydroxy-2-hydroxy methyl anthraquinone 3-*O*-β-glucopyranoside (lucidin-3-*O*-β-glucoside, 83)

Compound **83** was obtained as yellow crystals. A molecular formula of $C_{21}H_{20}O_{10}$ was deduced from its $[M-H]^+$ ion at *m/z* 431.135. The UV absorptions showed at 289.2, 301.6, 404.2 nm. The IR spectrum (Figure A-69) exhibited absorption bands for hydroxyl (3448.9 cm^{-1}), 1664.2 cm^{-1} (unchelated CO), 1617.7 cm^{-1} (chelated CO), and 1081.2 cm^{-1}.

^1H NMR spectrum (Figure A-70) revealed the singlet resonance of chelated hydroxyl protons (δ_H 13.13) and displayed a set of four protons in aromatic ring at δ_H 7.57 (s, 1H), 8.03 (m, 2H), 8.26 (m, 1H), 8.33 (m, 1H) were assigned as H-4, H-6, H-7, H-5 and H-8, respectively. The ^1H-^1H COSY spectra (Figure A-72-73) displayed two pair of proton correlations at δ_H 8.03 and 8.26, the other at δ_H 8.03 and 8.33. The ^{13}C NMR spectrum (Figure A-71) showed two signal of C=O at δ_C 187.1, 181.4 were assigned as C-9, C-10 and the signal of two C-O at δ_C 162.1, 161.8 were assigned as C-3, C-1.

The overall structure of **83** was deduced based on HMBC data. The HMBC correlation (Figure A-75-77) between the chelated hydroxyl group signal (OH-1) at δ 13.13, C-9a (δ 111.3), C-1 (δ 162.1) and C-2 (δ 123.6) were used to assign the hydroxyl group to C-1. Based on HMBC correlations, the nonchelated carbonyl groups were located on C-4 (δ_C 106.6), C-5 (δ_C 126.8). The methine proton at δ 7.57 (C-4) correlated with the non-chelated carbonyl group at δ_C 181.4 (C-10), and signals at 123.6 (C-2), 161.8 (C-3), 134.0 (C-4a), 111.3 (C-9a). The methine protons at δ 8.33 (C-8) showed correlation to chelated carbonyl group (C-9) at δ 187.1, C-8a (δ 132.0) and C-7 (δ 134.8), respectively. The other HMBC correlations allowed unequivocal assignment of all other carbon, and proton signals in **83** (Table 3.6, Figure 3.9 and Figure 3.10). The structure of **83** was established as 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside (lucidin-3-*O*-β-glucoside).

Compound **83** (lucidin-3-*O*-β-glucoside) has been isolated from the whole plants of *Rhynchotechum vestitum* [55]. The ^1H and ^{13}C NMR spectral data are presented in table 3.6.

Table 3.6 ^{1}H and ^{13}C NMR spectral data for 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside or lucidin-3-*O*-β-glucoside (**83**).

Position	δ_H (mult.,*J* in Hz)	δ_C	COSY	HMBC
1	13.13 (1H,s, OH-1)	161.8	-	C-1, C-2, C-9a
2	-	123.6	-	-
3	-	162.1	-	-
4	7.57 s	106.6	-	C-2, C-3, C-4a, C-9a, C-10
4a		134.0	-	-
5	8.26	126.8	H-6	C-6, C-10, C-10a
6	8.03	134.8	H-5	-
7	8.03	134.8	H-8	C-8, C-8a
8	8.33	126.5	H-7	C-7, C-8a, C-9
8a	-	132.0	-	-
9	-	187.1	-	-
9a	-	111.3	-	-
10	-	181.4	-	-
10a	-	132.0	-	_
2-CH_2OH	4.65, 4.75	50.9	-	C-1, C-2
1′	5.20	100.9	-	C-3
2′	3.45	73.5	-	-
3′	3.55	77.4	-	-
4′	3.30	69.4	-	-
5′	3.40	76.0	-	-
6′	3.60, 3.80	60.4	-	-

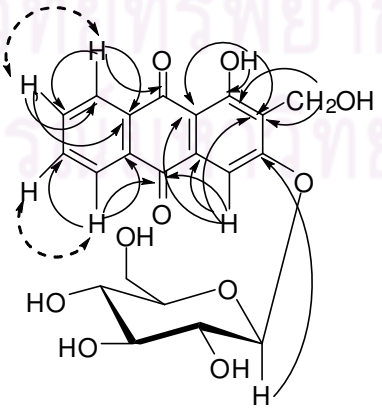


Figure 3.9 The key HMBC ( ) and COSY ( ) correlations of compound **83**

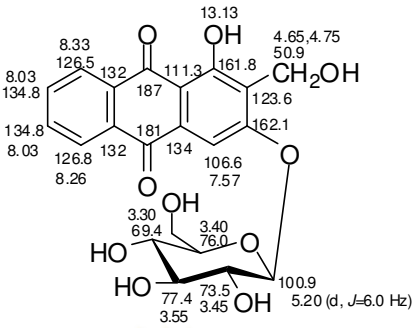


Figure 3.10 The assignments of all carbon and proton signals of compound **83**

3.5 Cytotoxicity against HeLa and KB cell lines of isolated compounds

The cytotoxicity against HeLa and KB cell lines of isolated compounds were determined using MTT colorimetric assays and the results were shown in Table 3.7.

Table 3.7 ***In vitro*** **cytotoxic activity of compounds 16-18, 43-44, 73-83 against KB and HeLa cell lines**

Isolated compounds	IC_{50} (μg/mL)	
	KB cell line	**HeLa cell line**
Lucidin-ω-methyl ether (**18**)	42.85	72.50
Damnacanthal (**17**)	6.35	13.37
Nordamnacanthal (**16**)	5.99	12.26
Phomarin (**73**)	7.67	16.65
Lucidin (**74**)	>100	>100
Lucidin-ω-ethyl ether (**75**)	21.05	>100
Anthragallol-2,3-dimethyl ether (**76**)	>100	>100
Flavopurpurin (**77**)	20.75	16.19
1-Methoxy-2-methyl anthraquinone (**78**)	12.06	>100
3-Hydroxy-1-methoxy-2-methoxymethyl anthraquinone (**79**)	99.11	91.62
Anthragallol (**80**)	13.83	64.56
Asperulosidic acid (**43**)	46.35	>100
Deacetylasperulosidic acid (**44**)	>100	>100
1,3,8-Trihydroxy-2-methoxy-7-methyl anthraquinone (**81**)	>100	>100
1-Hydroxy-5,6-dimethoxy-2-methyl anthraquinone (**82**)	>100	>100
Lucidin-3-*O*-β-glucoside (**83**)	>100	>100
Adriamycin (standard)	0.033	0.33

Compounds **16-18, 43-44** and **73-83** were tested for their cytotoxic activity against KB and HeLa cell lines and the results are shown in Table 3.7. Compounds **16, 17** and **73** showed moderate cytotoxicity against KB cells with IC_{50} values in the range of 5.99 - 7.67 μg/mL, and compounds **75, 77, 78** and **80** showed weak cytotoxicity against KB cells with IC_{50} values in the range of 12.06 - 21.05 μg/mL. Compounds **16, 17, 73** and **77** showed weak cytotoxicity against HeLa cells with IC_{50}

values in the range of 12.26 - 16.65 μg/mL. In addition, compounds **18, 74, 76, 79, 43, 44** and **81**-**83** were inactive to both cell lines, showing little inherent cytotoxicity.

CHAPTER IV

CONCLUSION

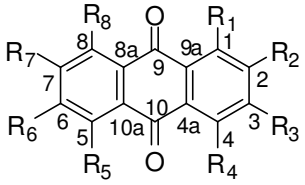
In summary, the isolation and purification of the CH_2Cl_2, EtOAc and *n*-BuOH crude extracts from the roots of *M. pandurifolia* Kuntze led to the isolation of one synthetically known anthraquinone, flavopurpurin (**77**), along with ten known anthraquinones; lucidin-ω-methyl ether (**18**), damnacanthal (**17**), nordamnacanthal (**16**), phomarin (**73**), lucidin(**74**), lucidin-ω-ethyl ether (**75**), anthragallol-2,3-dimethyl ether (**76**), 1-methoxy-2-methyl anthraquinone (**78**), 3-hydroxy-1-methoxy-2-methoxymethyl anthraquinone (**79**), anthragallol (**80**) and two known iridoid glycosides; asperulosidic acid (**43**), deacetyl asperulosidic acid (**44**).

Methyl ether derivatives of lucidin-ω-methyl ether (**18**) and phomarin (**73**) were prepared, using trimethylsilyldiazomethane ($TMSCHN_2$) to confirm hydroxyl group. 3-Methoxy lucidin-ω-methyl ether (1-hydroxy-3-methoxy-2-methoxymethyl anthraquinone; **18a**) and 6-methoxy phomarin (1-hydroxy-6-methoxy-3-methyl anthraquinone; **73a**) were obtained.

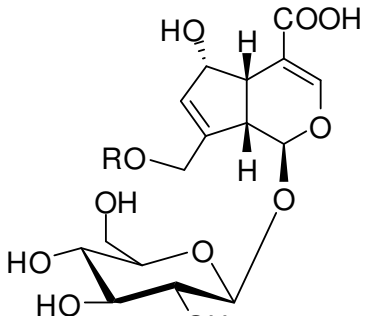
Chromatographic separation of CH_2Cl_2 and MeOH extracts of the roots of *M. coreia* Ham led to the isolation of eight compounds. Two synthetically known anthraquinones, 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**) and 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (**82**). A known anthraquinone glycoside, 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside (lucidin-3-*O*-β-glucoside, **83**). Five known anthraquinones, damnacanthal (**17**), nordamnacanthal (**16**), phomarin (**73**), 1-methoxy-2-methyl anthraquinone (**78**) and anthragallol (**80**).

The structures of all isolated compounds and its derivatives from the roots of *M. pandurifolia* Kuntze and *M. coreia* Ham were characterized according to means of spectral analysis as well as comparison with the previous literature data, and were summarized in table 4.1 and figure 4.1.

Table 4.1 The structure of isolated compounds from the roots of *M. pandurifolia* Kuntze and *M. coreia* Ham

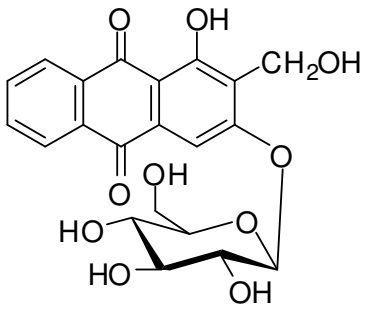


Compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
(**18**) Lucidin-ω-methyl ether	OH	CH_2OCH_3	OH	H	H	H	H	H
(**18a**) 3-Methoxy lucidin-ω-methyl ether	OH	CH_2OCH_3	OCH_3	H	H	H	H	H
(**17**) Damnacanthal	OCH_3	CHO	OH	H	H	H	H	H
(**16**) Nordamnacanthal	OH	CHO	OH	H	H	H	H	H
(**73**) Phomarin	OH	H	CH_3	H	H	OH	H	H
(**73a**) 6-Methoxy phomarin	OH	H	CH_3	H	H	OCH_3	H	H
(**74**) Lucidin	OH	CH_2OH	OH	H	H	H	H	H
(**75**) Lucidin-ω-ethyl ether	OH	$CH_2OCH_2CH_3$	OH	H	H	H	H	H
(**76**) Anthragallol-2,3-dimethyl ether	OH	OCH_3	OCH_3	H	H	H	H	H
(**77**) Flavopurpurin	OH	OH	H	H	H	OH	H	H
(**78**) 1-Methoxy-2-methyl anthraquinone	OCH_3	CH_3	H	H	H	H	H	H
(**79**) 3-Hydroxy-1-methoxy-2-methoxymethyl anthraquinone	OCH_3	CH_2OCH_3	OH	H	H	H	H	H
(**80**) Anthragallol	OH	OH	OH	H	H	H	H	H
(**81**)1,3,8-Trihydroxy-2-methoxy-7-methyl anthraquinone	OH	OCH_3	OH	H	H	H	CH_3	OH
(**82**)1-Hydroxy-5,6-dimethoxy-2-methyl anthraquinone	OH	CH_3	H	H	OCH_3	OCH_3	H	H



Asperulosidic acid (**43**) R = Ac

Deacetylasperulosidic acid (**44**) R = H



Lucidin-3-*O*-β-glucoside (**83**)

Figure 4.1 The structure of isolated compounds from the roots of *M. pandurifolia* Kuntze and *M. coreia* Ham

The evaluation for cytotoxicity against KB and HeLa cell lines of all isolated compounds (**16-18, 43-44** and **73-83**) showed that compounds **16, 17** and **73** showed moderate cytotoxicity against KB cells with IC_{50} values in the range of 5.99-7.67 μg/mL, and compounds **75, 77, 78** and **80** showed weak cytotoxicity against KB cells with IC_{50} values in the range of 12.06- 21.05 μg/mL. Compounds **16, 17, 73** and **77** showed weak cytotoxicity against HeLa cells with IC_{50} values in the range of 12.26-16.65 μg/mL. In addition, compounds **18, 74, 76, 79, 43, 44** and **81**-**83** were inactive to both cell lines.

Future work may involve the synthesis of isolated compounds for increasing quantity and biological activity that could be developed into new drugs. Novel active compounds will be the target for future synthesis and structure activity relationship studies as well. This will lead to better understanding on the interaction between active compounds and diseases.

REFERENCES

[1] Xiang, W., Song, Q., Zhang, H., and Guo, S. Antimicrobial anthraquinones from *Morinda angustifolia. Fitoterapia* 79 (2008) : 501-504.

[2] Wikipedia, the free encyclopedia. Morinda. [Online]. 2011. Available from : http://en.wikipedia.org/wiki/Morinda [2011, February 15]

[3] Backer, C.A., and Bakhuizen, R.C. Flora of Java. N.V.P. Noordhoff Groningen, Netherland. 3(1965).

[4] Bhuyan, R., Saikia, D.C., and Saikia, D.N. Isolation of colour component from the roots of *Morinda angustifolia* Roxb. And evaluation of its dyeing characteristics. *Ind. J.Fibre and Textile Res*. 27(2002) : 429-433.

[5] Chayamarit, K. Key characters of plant families. Agriculture co-operative press, Bangkok. 2005: 80-81.

[6] Kamiya, K., Tanaka, Y., Endang, H., Umar, M., and Satake, T. New anthraquinone and iridoid from the fruits of *Morinda citrifolia*. *Chem. Pharm.Bull*. 53 (2005) : 1597-1599.

[7] Su, B., et al. Chemical constituents of the fruits of *Morinda citrifolia* (noni) and their antioxidant activity. *J. Nat. Prod.* 68 (2005) : 592-595.

[8] Kanchanapoom, T., Kasai, R., and Yamasaki, K. Iridoid and phenolic glycosides from *Morinda coreia. Phytochemistry* 9 (2002) : 551-556.

[9] Inoue, K., Nayeshiro, H., Inouye, H., and Zenk, M. Anthraquinones in cell suspension cultures of *Morinda citrifolia. Phytochemistry* 20 (1981) : 1693-1700.

[10] Farine, J., Legal, L., Moreteau, B., and Quere, J. Volatile components of ripe fruits of *Morinda citrifolia* and their effects on *Drosophila. Phytochemistry* 41 (1996) : 433-438.

[11] Wang, M., et al. Novel trisaccharide fatty acid ester identified from the fruits of *Morinda citrifolia* (Noni). *J. Agric. Food Chem*. 47 (1999) : 4880-4882.

[12] Wang, M., et al. Novel glycosides from noni (*Morinda citrifolia*). *J. Nat. Prod.* 63 (2000) : 1182-1183.

[13] Sang, S., et al. Citrifolin A, a new unusual iridoid with inhibition of activator protein-1(AP-1) from the leaves of noni (*Morinda citrifolia* L.). *Tetrahedron Lett.* 42 (2001) : 1823-1825.

[14] Sang, S., et al. A New unusual iridoid with inhibition of activator protein-1 (AP-1) from the leaves of *Morinda citrifolia* L. *Org. Lett.* 3 (2001) : 1307-1309.

[15] Sang, S., et al. Iridoid glycosides from the leaves of *Morinda citrifolia*. *J. Nat. Prod.* 64 (2001) : 799-800.

[16] Sang, S., et al. Flavonol glycosides and novel iridoid glycoside from the leaves of *Morinda citrifolia*. *J. Agric. Food Chem*. 49 (2001) : 4478-4481.

[17] Pawlus, A., Su, B., Keller, W., and Kinghorn, A. An anthraquinone with potent quinone reductase-inducing activity and other constituents of the fruits of *Morinda citrifolia* (noni). *J. Nat. Prod.* 68 (2005) : 1720-1722.

[18] Samoylenko, V., et al. New constituents from noni (*Morinda citrifolia*) fruit juice. *J. Agric. Food Chem*. 54 (2006) : 6398-6402.

[19] Deng, S., et al. Lipoxygenase inhibitory constituents of the fruits of noni (*Morinda citrifolia*) collected in Tahiti. *J. Nat. Prod.* 70 (2007) : 859-862.

[20] Akihisa, T., et al. Anti-inflammatory and potential cancer chemopreventive constituents of the fruits of *Morinda citrifolia* (noni). *J. Nat. Prod.* 70 (2007) : 754-757.

[21] Deng, Y., Chin, Y., Chai, H., Keller, W., and Kinghorn, A. Anthraquinones with quinone reductase-inducing activity and benzophenones from *Morinda citrifolia* (noni) Roots. *J.Nat. Prod.* 70 (2007) : 2049-2052.

[22] Kamiya, K., Hamabe, W., Tokuyama, S., and Satake, T. New anthraqunone glycosides from the roots of *Morinda citrifolia*. *Fitoterapia* 80 (2009) : 196-199.

[23] Kamiya, K., et al. Inhibitory effect of anthraqunones isolated from the noni (*Morinda citrifolia*) root on animal A-, B- and Y-famillies of DNA polymerases and human cancer cell proliferation. *Food Chem.* 118 (2010) : 725-730.

[24] Ismail, N., et al. Anthraquinones from *Morinda elliptica. Phytochemistry* 45 (1997) :1723-1725.

[25] Noiarsa, P., Ruchirawat, S., Otsuka, H., and Kanchanapoom, T. A new iridoid glucoside from the Thai medicinal plant, *Morinda elliptica* Ridl. *J. Nat. Med.* 60 (2006) : 322-324.

[26] Duangjinda, D. Chemical constituents from the stems of *Morinda. elliptica* and biological activity. Master's Thesis, Department of Chemistry, Faculty of Science, Prince of Songkla Univesity, 2007.

[27] Cimanga, K., et al. Flavonoid *o*-glycosides from the leaves of *Morinda morindoides*. *Phytochemistry* 38 (1995) : 1301-1303.

[28] Cimanga, K., et al. Complement-inhibiting iridoids from *Morinda morindoides*. *J. Nat. Prod.* 66 (2003) : 97-102.

[29] Cimanga, K., et al. Cytotoxicity and *in vitro* susceptibility of *Entamoeba histolytica* to *Morinda morindoides* leaf extracts and its isolated constituents. *J. Ethnopharmacol.* 107 (2006) : 83-90.

[30] Wu, Y., et al. Antisteoporotic activity of anthraquinones from *Morinda officinalis* on osteoblasts and osteoclasts. *Molecules* 14 (2009) : 573-583.

[31] Yoshikawa, M., Yamaguchi, S., Nishisaka, H., Yamahara, J., and Murakami, N. Chemical constituents of Chinese natural medicine, Morindae Radix, the dried roots of *Morinda officinalis* How.: Structure of morindolide and morofficinaloside. *Chem. Pharm. Bull.* 43 (1995) : 1462-1465.

[32] Booranachad, P. Phytochemical study of the roots of *Morinda angustifolia* Roxb. Master's Thesis, Department of Pharmaceutical Botany, Faculty of Phamaceutical Science, Chulalongkorn Univesity, 2003.

[33] Chang, P., Lee, K., Shingu, T., Hirayama, T., and Hall, I. Antitumor agents 50, morindaparvin-A, a new antileukemic anthraquinones, and alizarin-1-methyl ether from *Morinda parvifolia*, and the antileukemic activity of the related derivatives. *J. Nat. Prod.* 45 (1982) : 206-210.

[34] Chang, P., and Lee, K. Cytotoxic antileukemic anthraquinones from *Morinda parvifolia. Phytochemistry* 23 (1984) : 1733-1736.

[35] Burnett, A., and Thomson, R. Anthraquinones in *Morinda umbellate* L. *Phytochemistry* 7 (1968) : 1421-1422.

[36] Adesogan, E. Oruwacin, A New Iridoid ferulate from *Morinda lucida. Phytochemistry* 18 (1979) : 175-176.

[37] Han, Y., Heijden, R., and Verpoorte, R. Biosynthesis of anthraquinones in cell cultures of the Rubiaceae. *Plant Cell Tissue Organ Cult.* 67 (2001) : 201- 220.

[38] Simpson, T. The biosynthesis of polyketides. *Nat. Prod. Rep.* 4 (1987) : 339-376.

[39] Inouye, H., and Leistner, E. Biosynthesis of quinones, the chemistry of quinonoid compounds. John Wiley & Sons Ltd., New York, (2) 1988 : 1293-1349.

[40] Leistner, E. A second pathway leading to anthraquinones in higher plants. *Phytochemistry* 10 (1971) : 3015-3020.

[41] Bernard, J. Biosynthesis of polyketides (other than actinomycete macrolides). *Nat. Prod. Rep.* 16 (1999) : 425-484.

[42] Leistner, E. Biosynthesis of plant quinones, the biochemistry of plants. Academic Press, London, 7 (1981) : 403-423.

[43] Akihisa, T., et al. Azaphilones, furannoisophthalides, and amino acids from the extracts of *Monascus pilosus*-fermented rice (red-mold rice) and their chemopreventive effects. *J. Agric. Food Chem*. 53 (2005) : 562-565.

[44] Kasonbua, W. The genus *Morinda* L. in Thailand. Master's Thesis, Department of Chemistry, Faculty of Science, Khon Kaen Univesity, 2004.

[45] Kongkathip, N., et al. Potent antitumor activity of synthetic 1,2-naphthoquinones and 1,4-naphthoquinones. *Bioorg. Med. Chem.* 11(2003) : 3179-3191.

[46] Borges, W., and Pupo, M. Novel anthraquinone derivatives produced by *Phoma sorghina*, an endophyte found in association with the medicinal plant *Tithonia diversifolia* (asteraceae). *J. Braz. Chem. Soc.* 17 (2006) : 929-934.

[47] Jegorov, A., et al. Crystal structures of mollugin and lucidin. *J. Chem. Crystallogr.* 35 (2005) : 621-627.

[48] Itokawa, H., Mihara, K., and Takeya, K. Studies on a novel anthraquinone and its glycosides isolated from *Rubia cordifolia* and *R. akane*. *Chem. Pharm. Bull.* 31(1983) : 2353-2358.

[49] Roberge, G., and Brassard, P. Reactions of ketene acetals, 13, synthesis of contiguously trihydroxylated naphtha- and anthraquinones. *J. Org. Chem.* 46 (1981) : 4161-4166.

[50] Auerbach, G., and Crookes, W. Anthracene: Its constitution, properties, manufacture, and derivatives, including artificial alizarin, anthrapurpurin, etc (1877). Kessinger Publishing, Montana. 2008 : 204.

[51] Zembower, D., Kam, C., Powers, J., and Zalkow, L. Novel anthraquinone inhibitors of human leukocyte elastase and cathepsin G. *J. Med. Chem.* 35 (1992) : 1597-1605.

[52] Dhananjeyan, M., Milev, Y., Kron, M., and Nair, M. Synthesis and activity of substituted anthraquinones against a human filarial parasite, *Brugia malayi. J. Med. Chem.* 48 (2005) : 2822-2830.

[53] Demirezer, L., Gurbuz, F., Guvenalp, Z., Stroch, K., and Zeeck, A. Iridoids, flavonoids and monoterpene glycosides from *Galium verum* subsp. *verum. Turk. J. Chem.* 30 (2006) : 525-534.

[54] Savard, J., and Brassard, P. Reactions of ketene acetals-14, the use of simple mixed vinylketene acetals in the annulation of quinones. *Tetrahedron* 40 (1984) : 3455-3464.

[55] Lu, Y., Xu, P., Chen, Z., and Liu, G. The anthraquinoes of *Rhynchotechum vestitum. Phytochemistry* 47 (1998) : 315-317.

APPENDIX

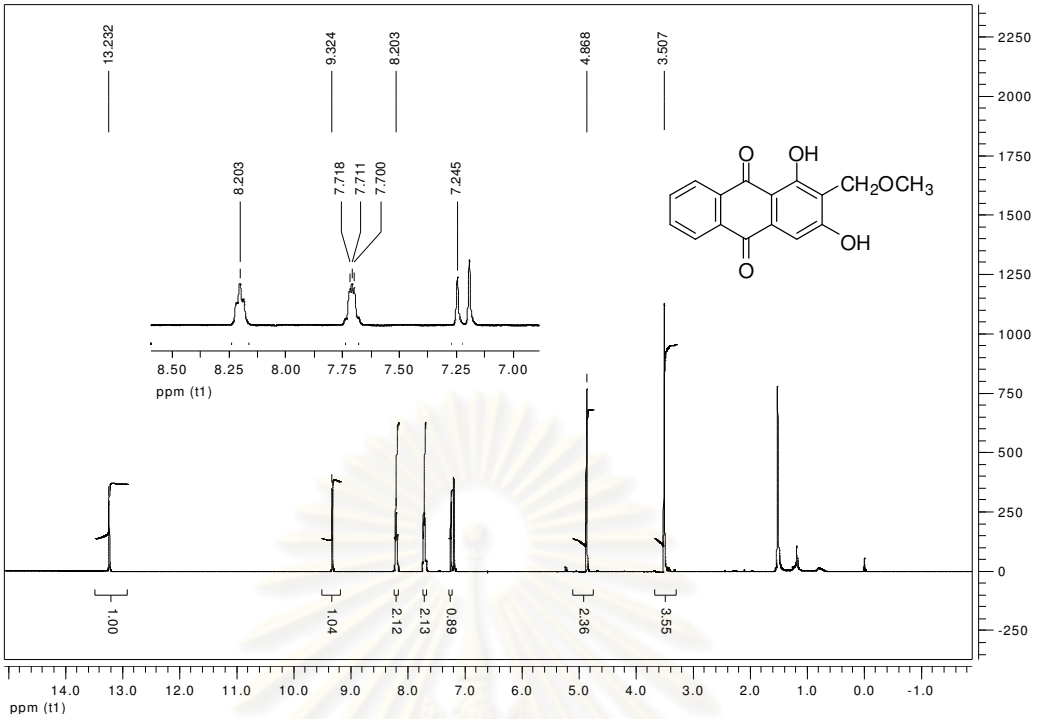


Figure A-1 ^{1}H NMR spectrum ($CDCl_3$) of lucidin-ω-methyl ether **18**

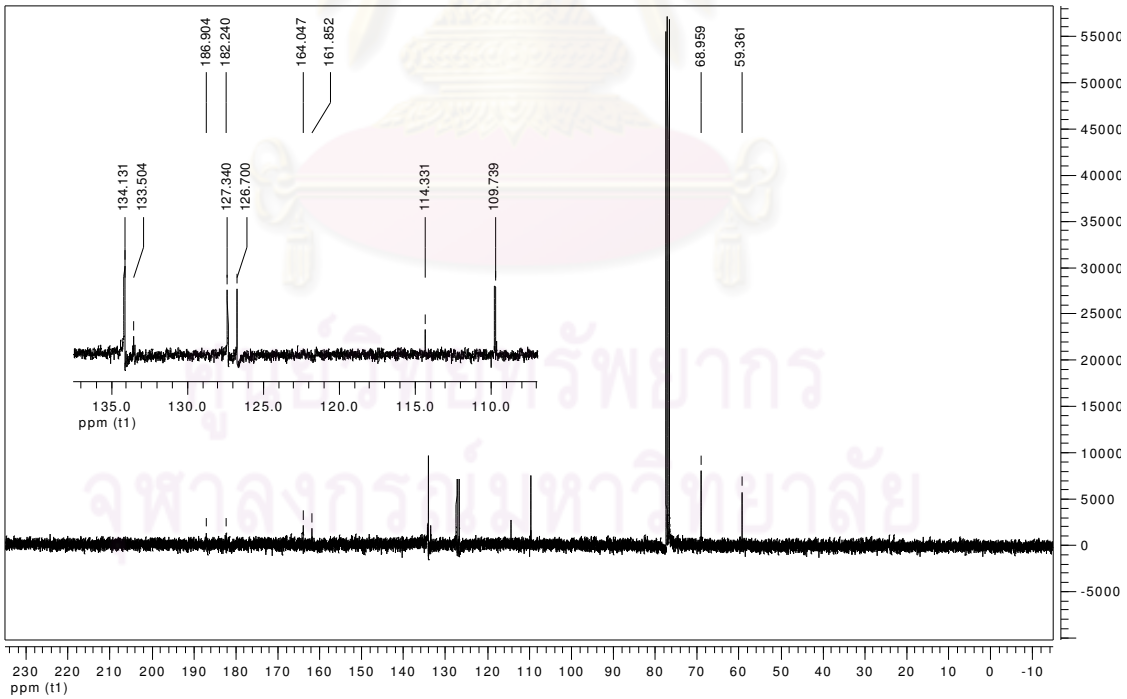


Figure A-2 ^{13}C NMR spectrum ($CDCl_3$) of lucidin-ω-methyl ether **18**

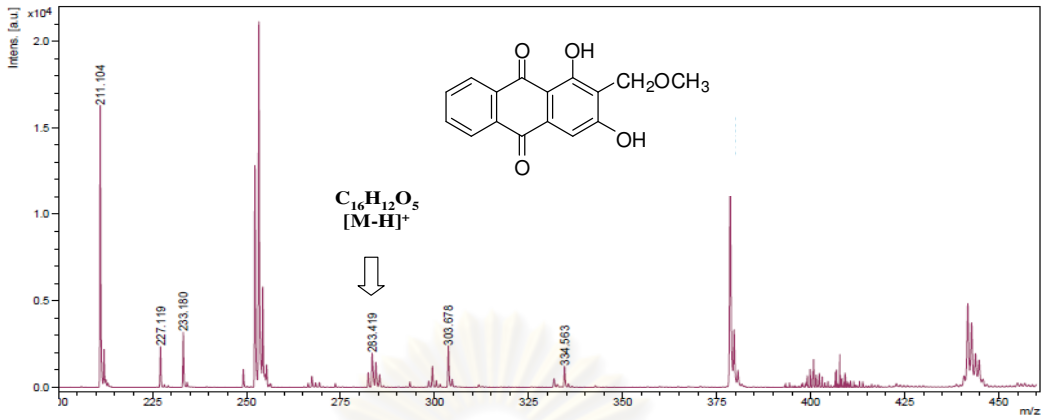


Figure A-3 Mass spectrum of lucidin-ω-methyl ether **18**

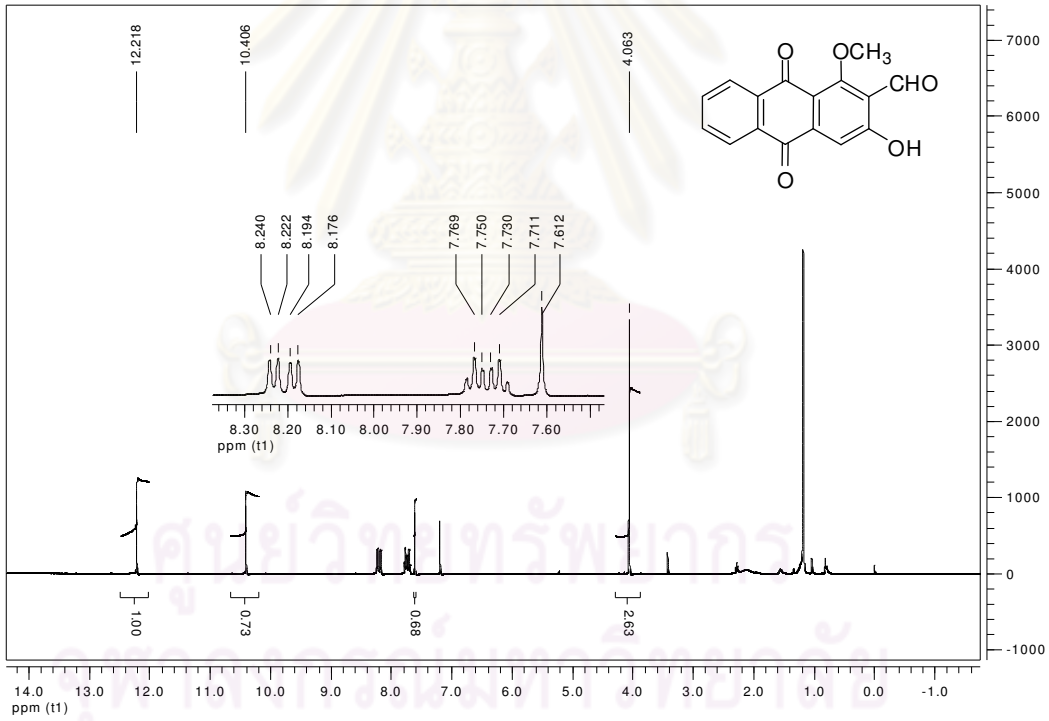


Figure A-4 ^{1}H NMR spectrum ($CDCl_3$) of damnacanthal **17**

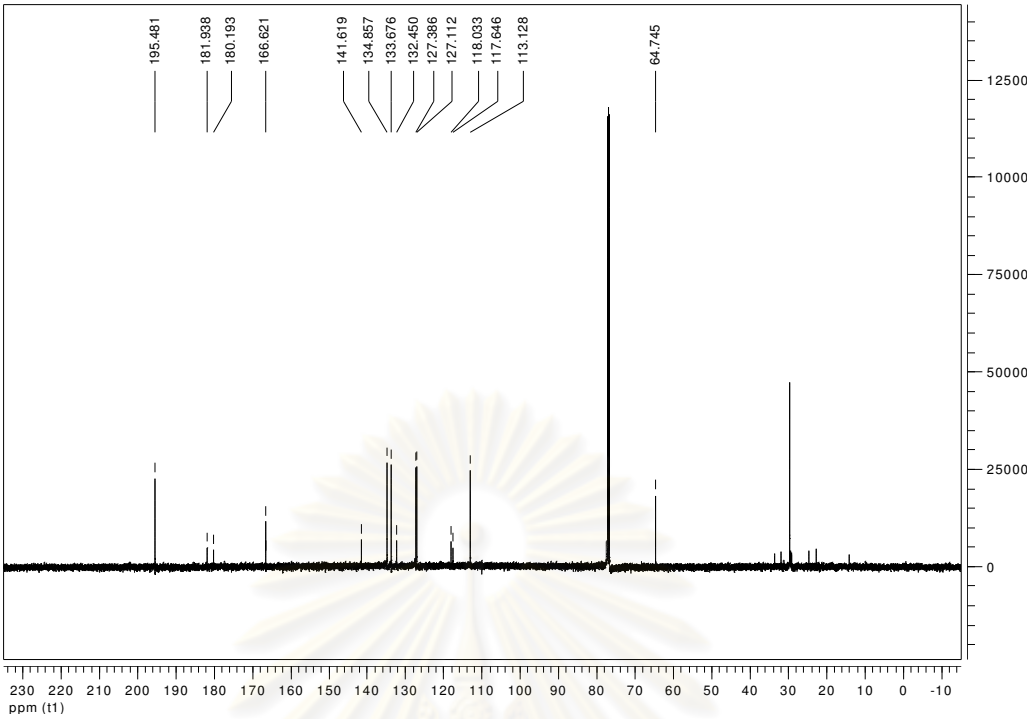


Figure A-5 ^{13}C NMR spectrum ($CDCl_3$) of damnacanthal **17**

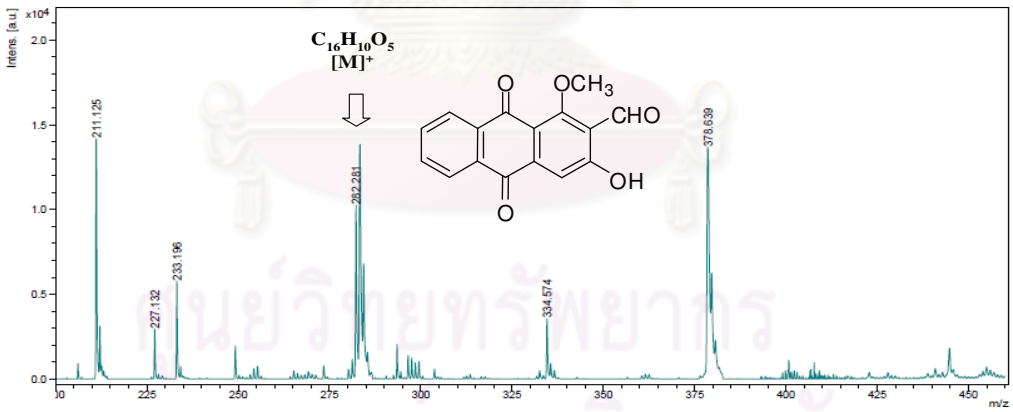


Figure A-6 Mass spectrum of damnacanthal **17**

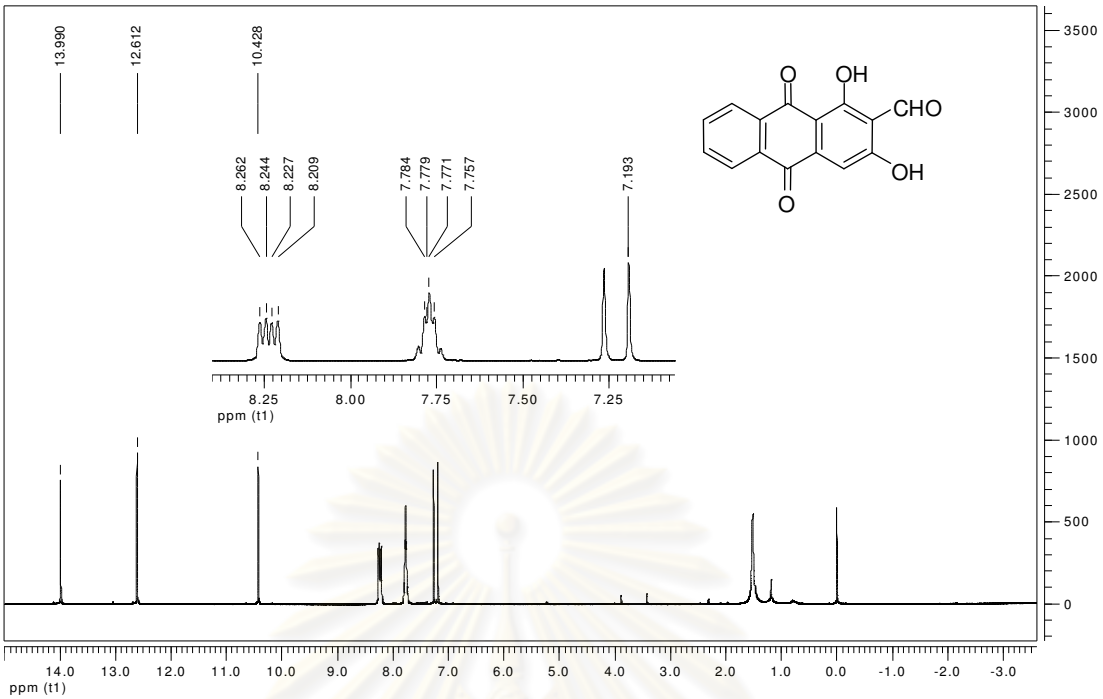


Figure A-7 ^{1}H NMR spectrum ($CDCl_3$) of nordamnacanthal **16**

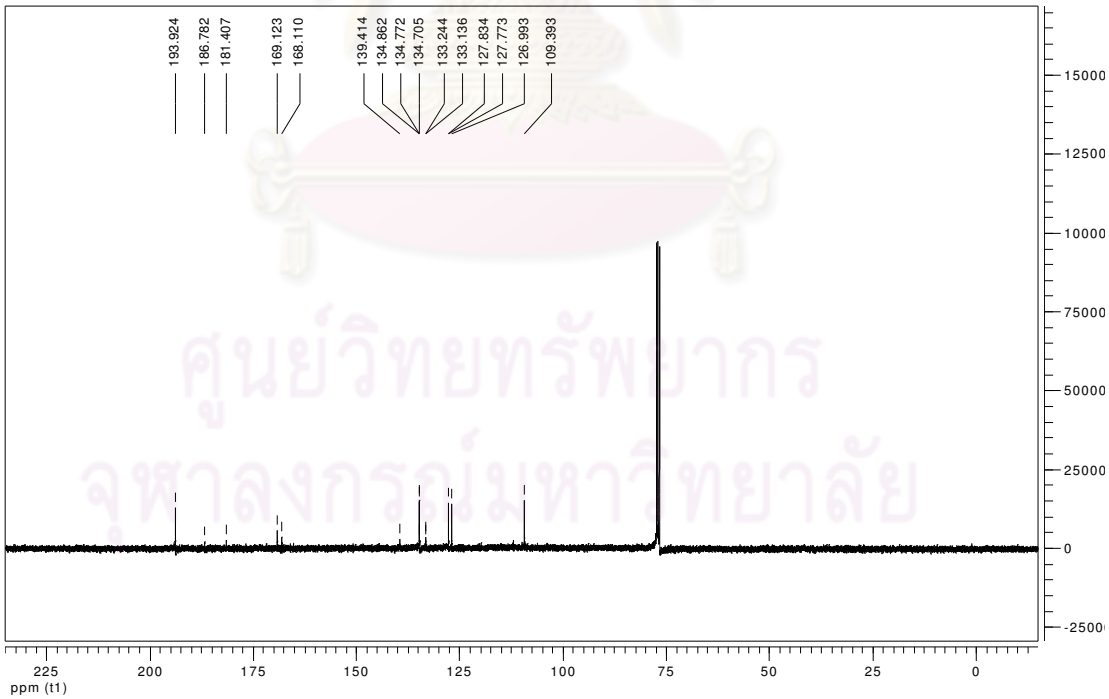


Figure A-8 ^{13}C NMR spectrum ($CDCl_3$) of nordamnacanthal **16**

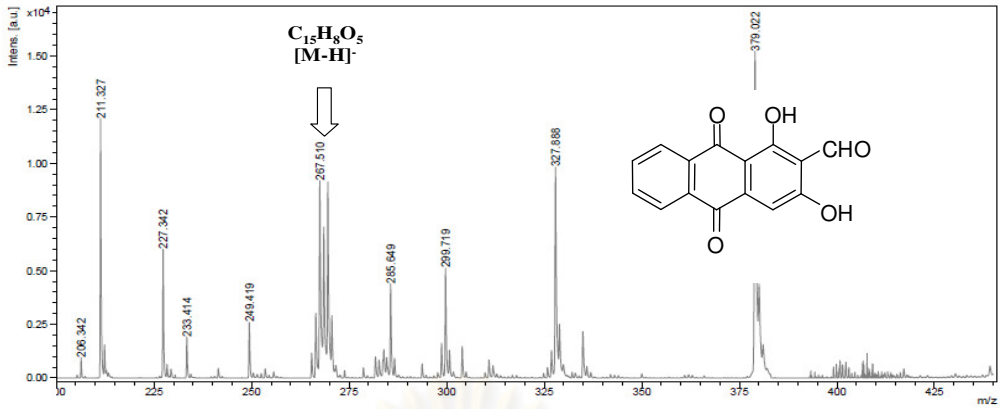


Figure A-9 Mass spectrum of nordamnacanthal **16**

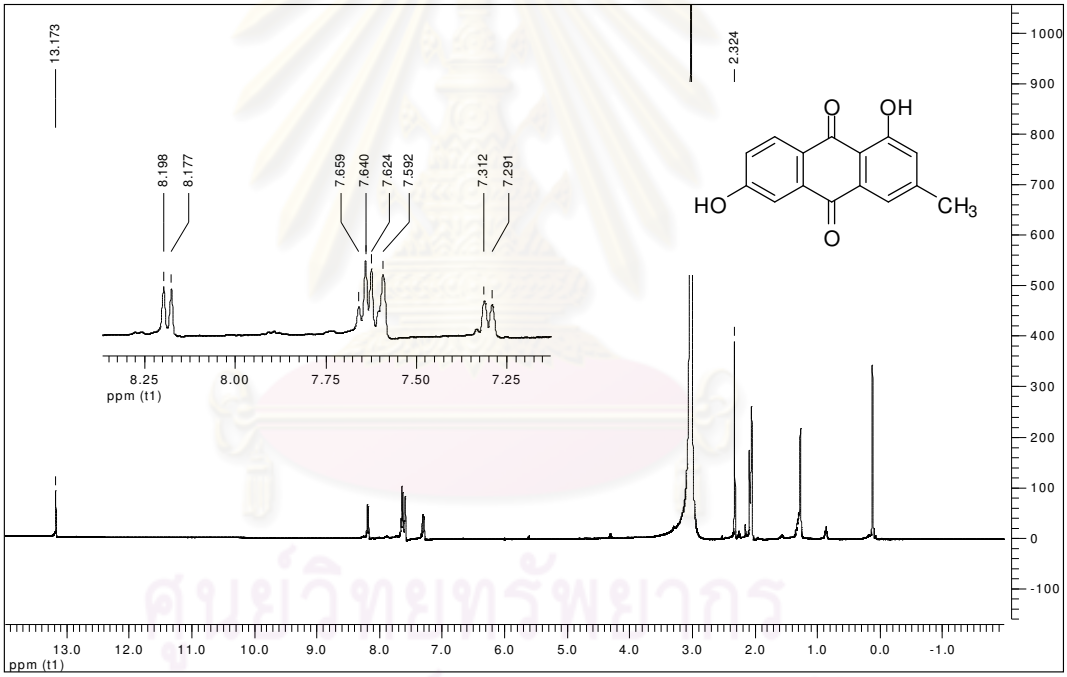


Figure A-10 ^{1}H NMR spectrum (acetone-d_6) of phomarin **73**

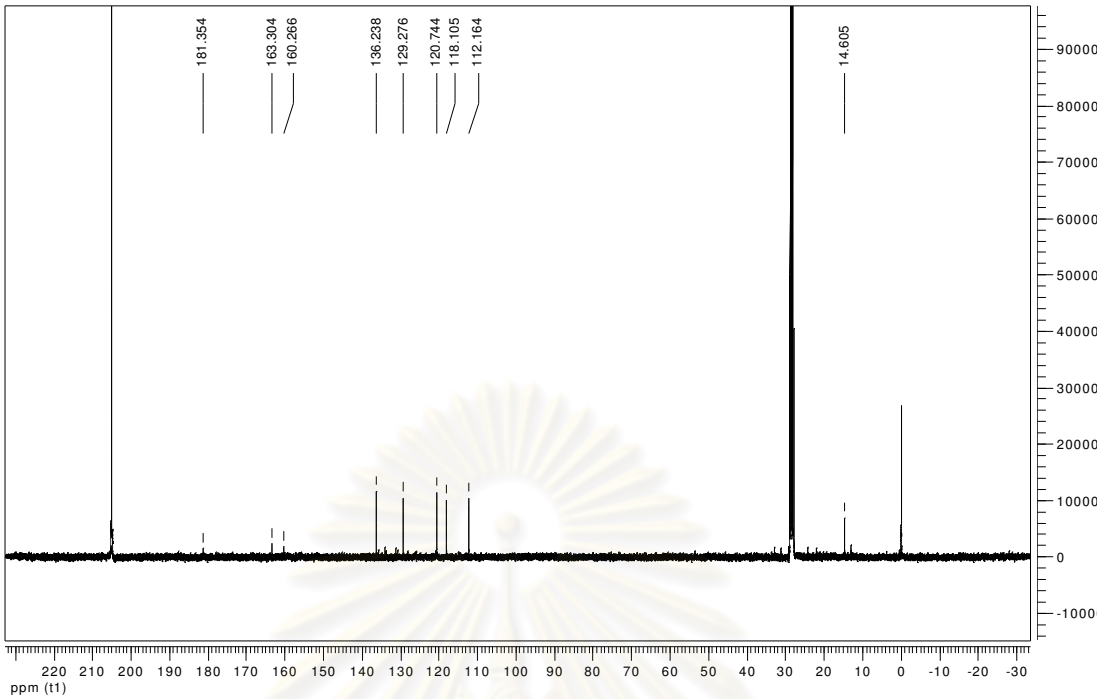


Figure A-11 ^{13}C NMR spectrum (acetone-d_6) of phomarin **73**

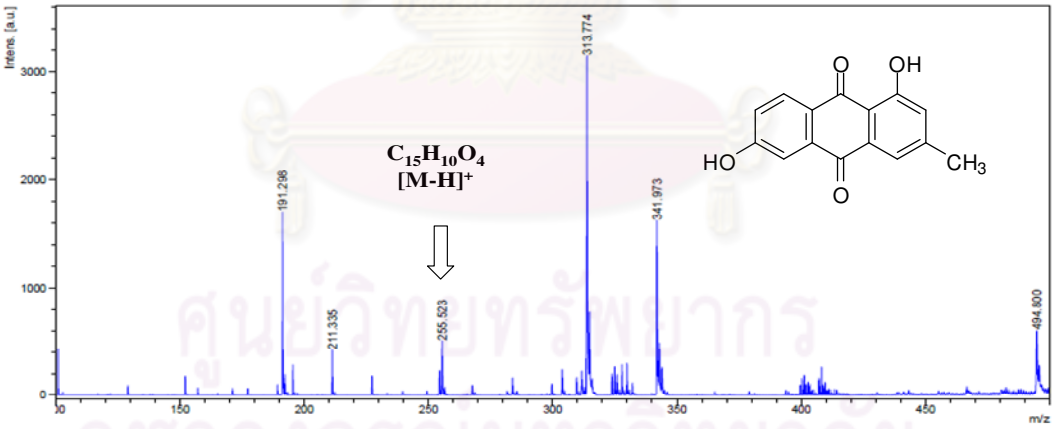


Figure A-12 Mass spectrum of phomarin **73**

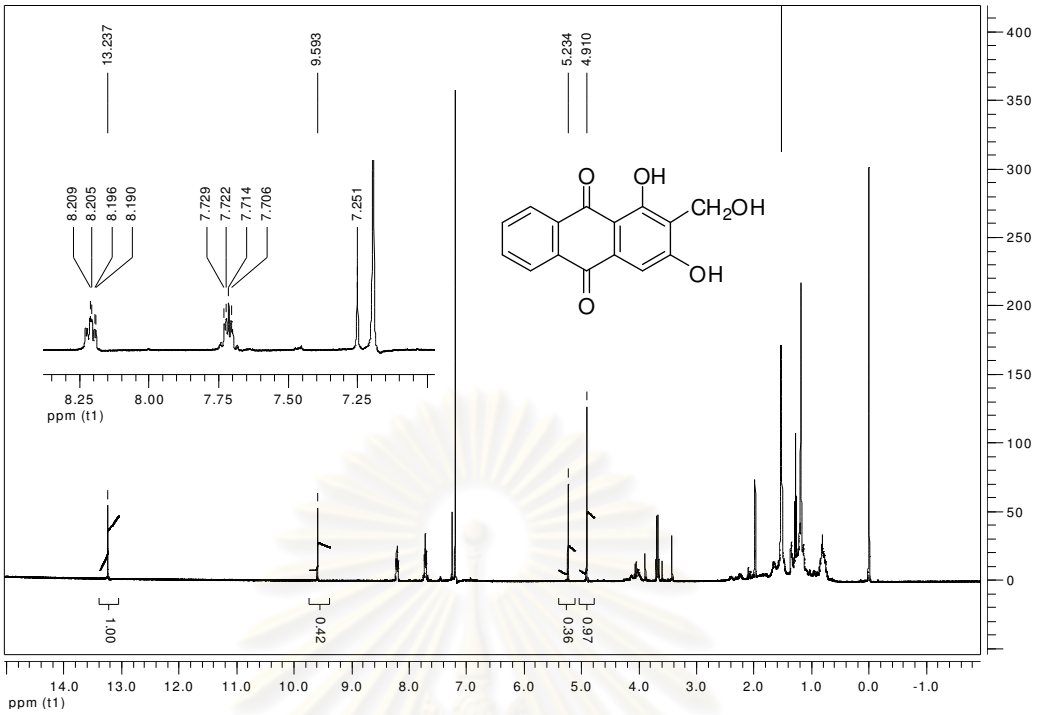


Figure A-13 ^{1}H NMR spectrum ($CDCl_3$) of lucidin **74**

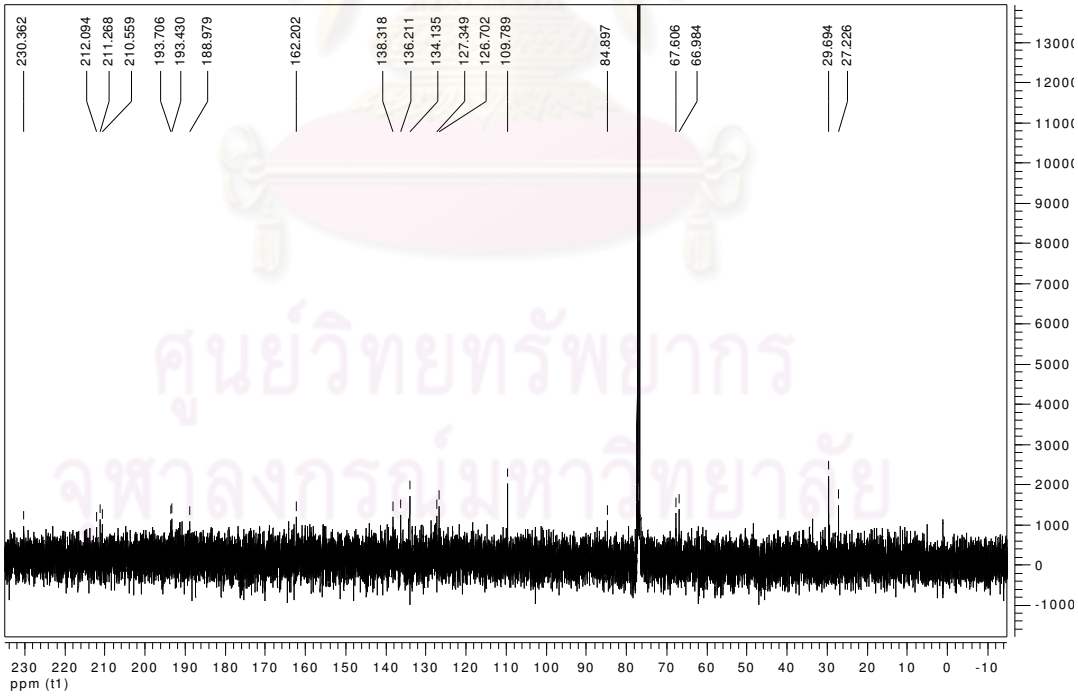


Figure A-14 ^{13}C NMR spectrum ($CDCl_3$) of lucidin **74**

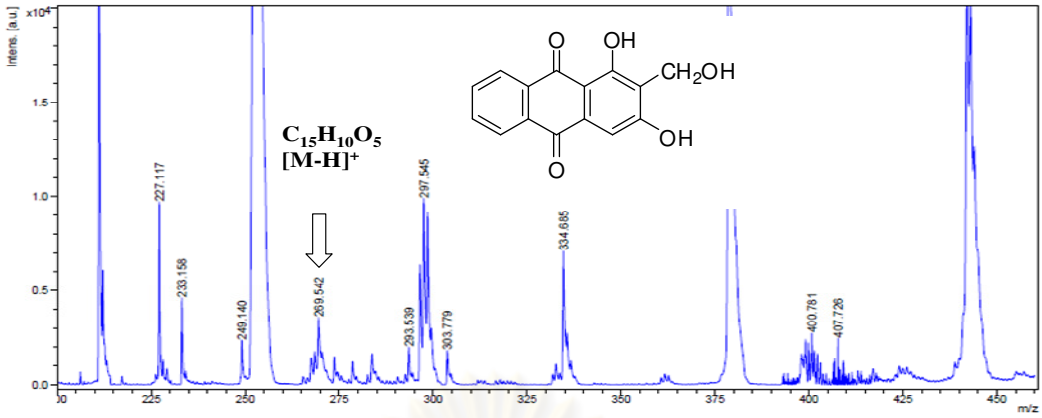


Figure A-15 Mass spectrum of lucidin **74**

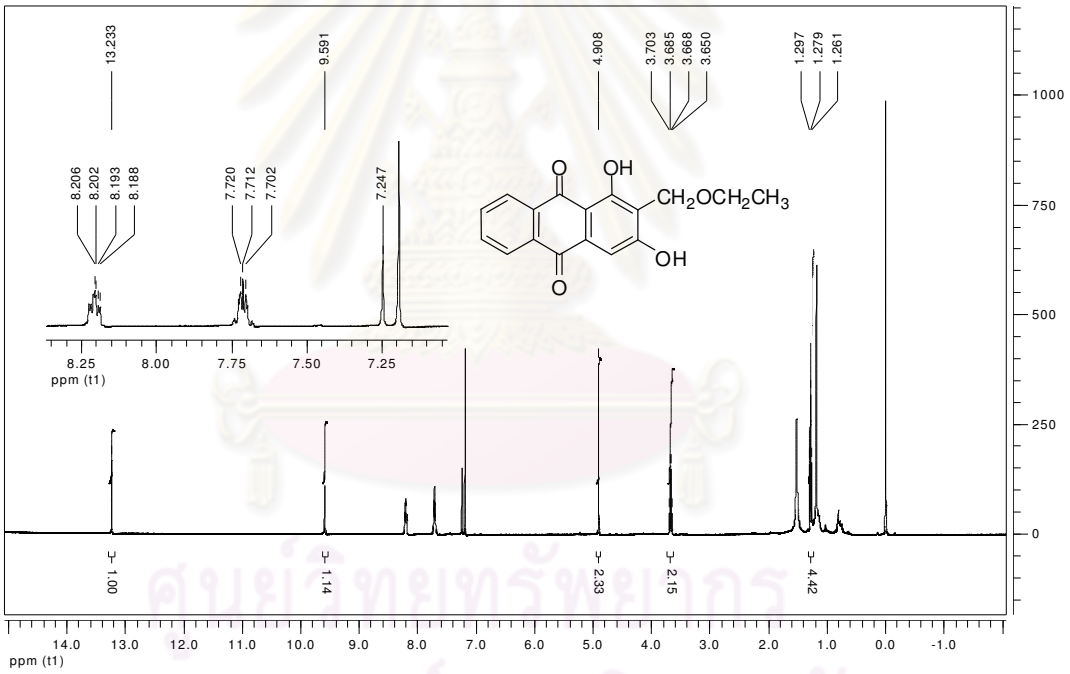


Figure A-16 ^{1}H NMR spectrum ($CDCl_3$) of lucidin-ω-ethyl ether **75**

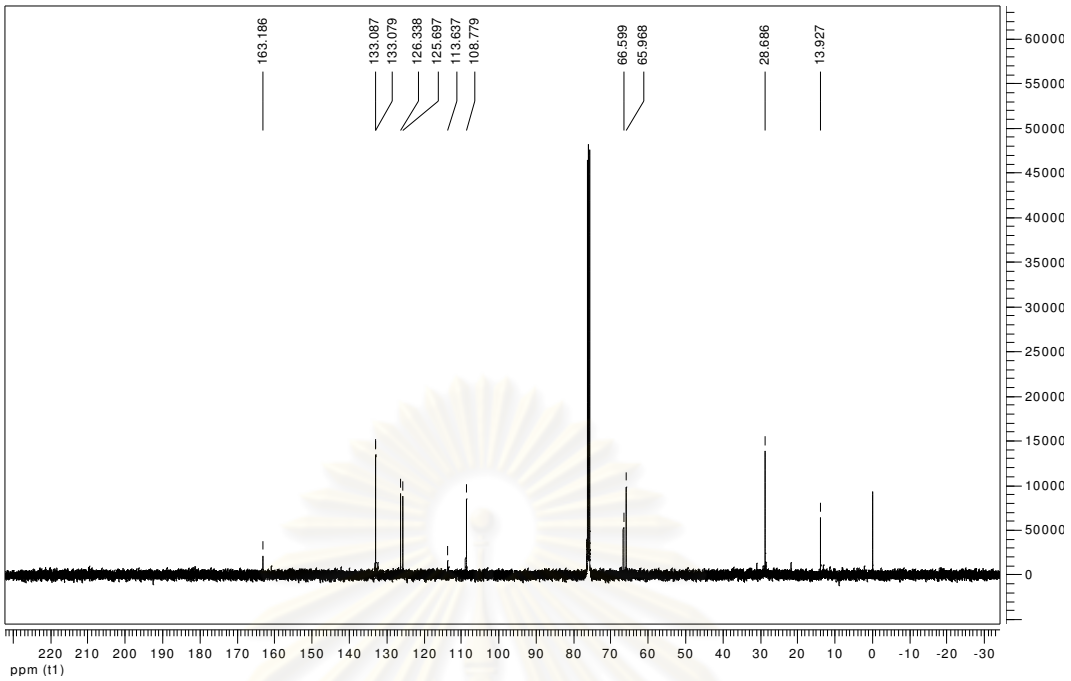


Figure A-17 ^{13}C NMR spectrum ($CDCl_3$) of lucidin-ω-ethyl ether **75**

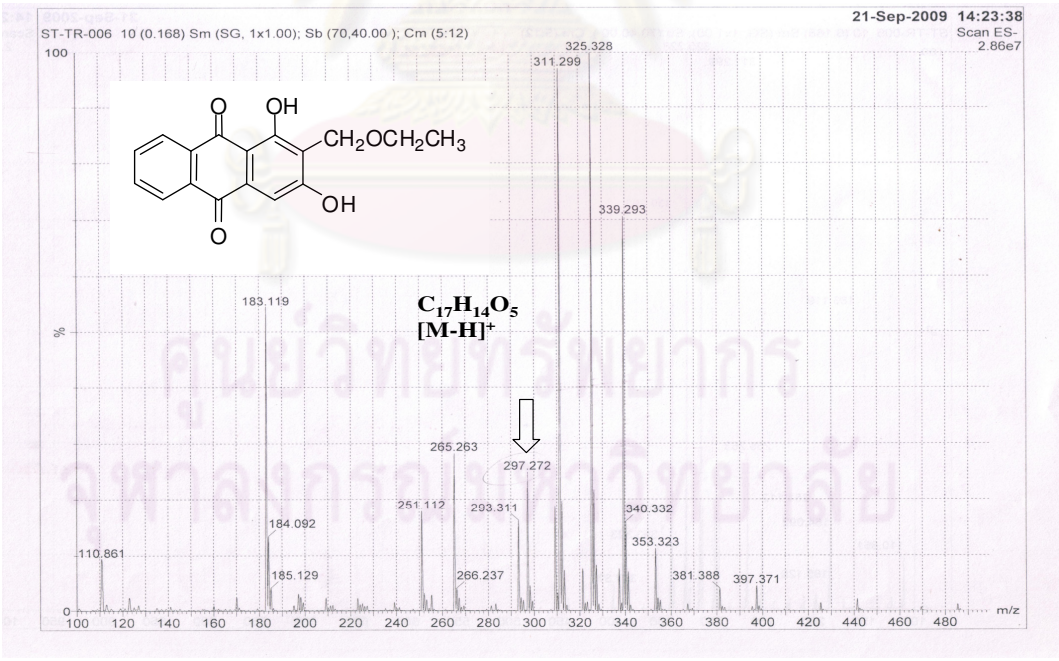


Figure A-18 Mass spectrum of lucidin-ω-ethyl ether **75**

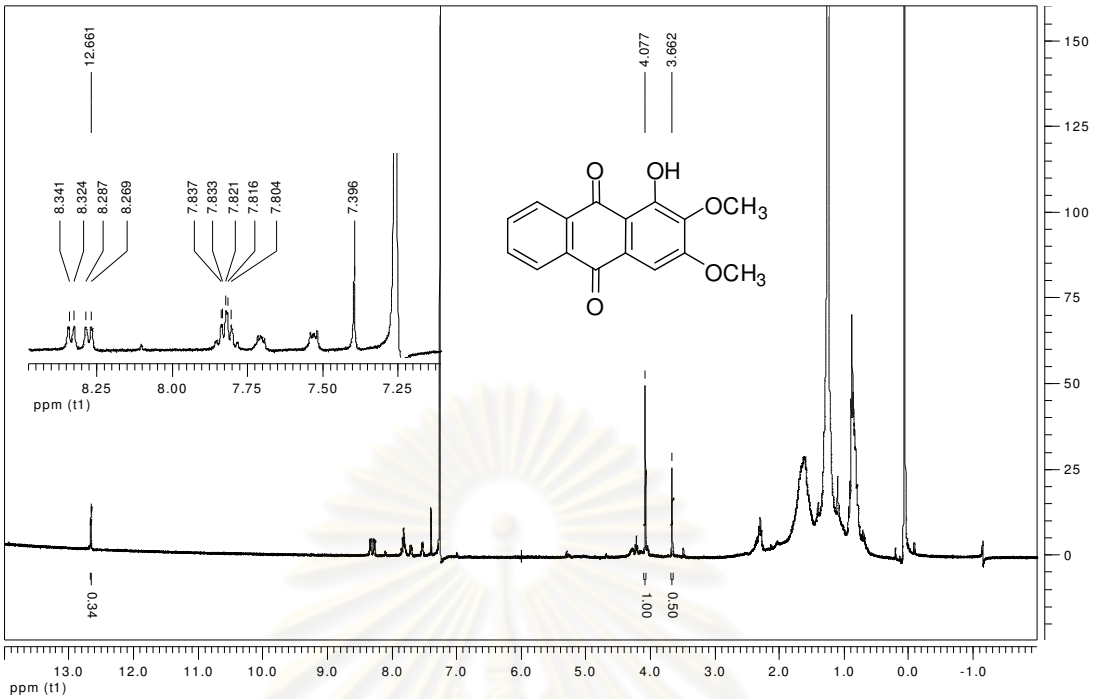


Figure A-19 ^{1}H NMR spectrum ($CDCl_3$) of anthragallol-2,3-dimethyl ether **76**

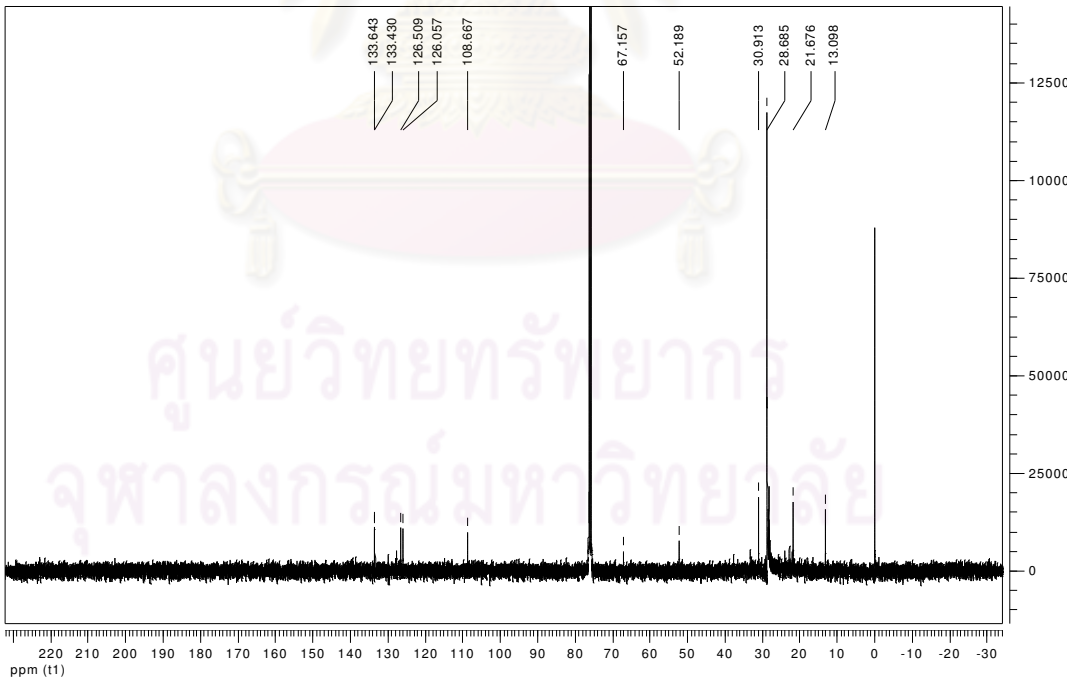


Figure A-20 ^{13}C NMR spectrum ($CDCl_3$) of anthragallol-2,3-dimethyl ether **76**

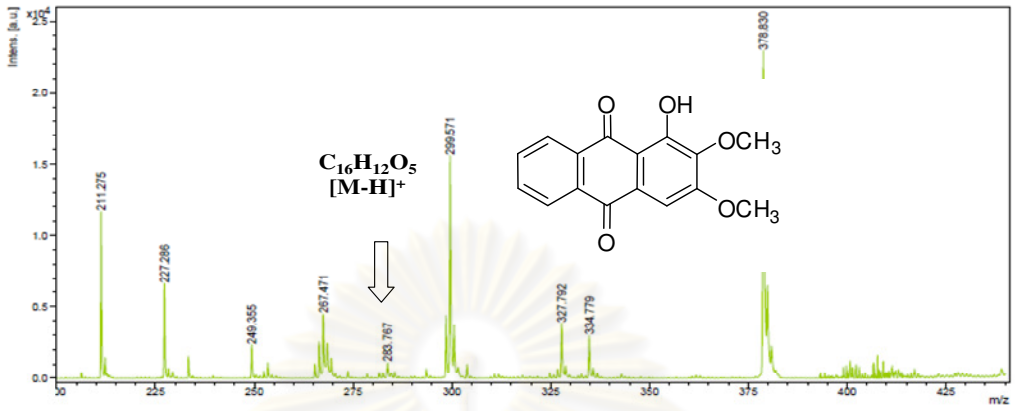


Figure A-21 Mass spectrum of anthragallol-2,3-dimethyl ether **76**

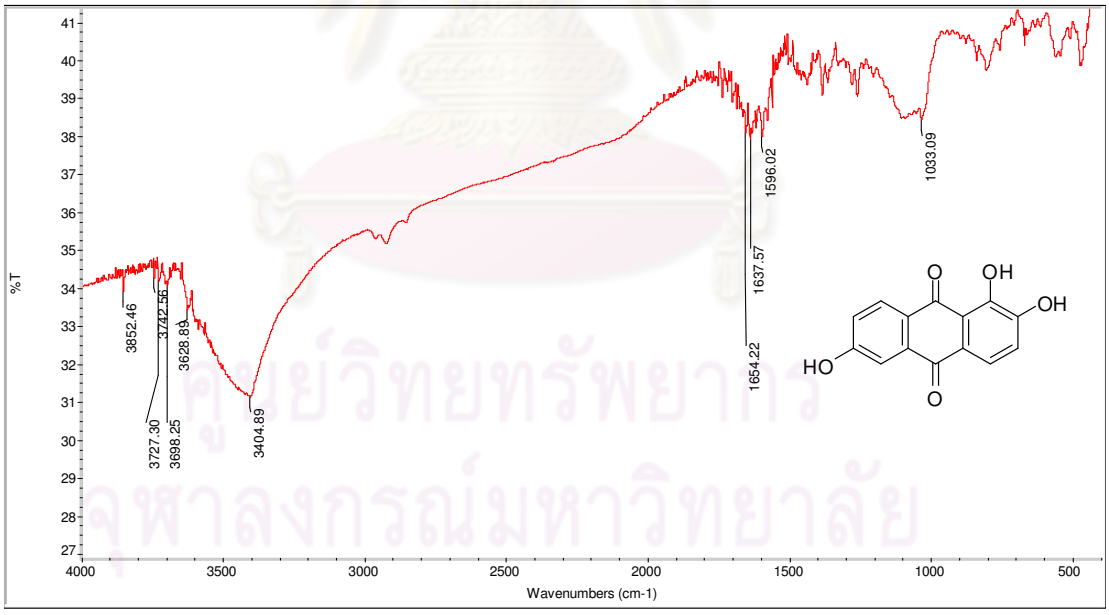


Figure A-22 IR spectrum of flavopurpurin **77**

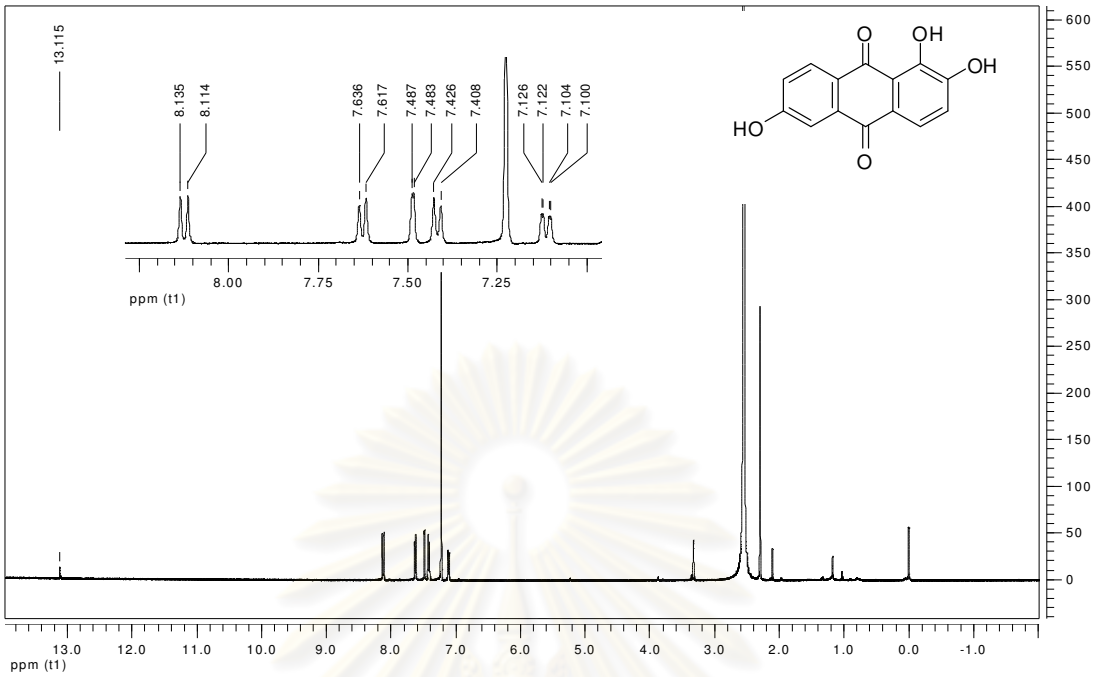


Figure A-23 ^{1}H NMR spectrum ($CDCl_3$+CD_3OD) of flavopurpurin **77**

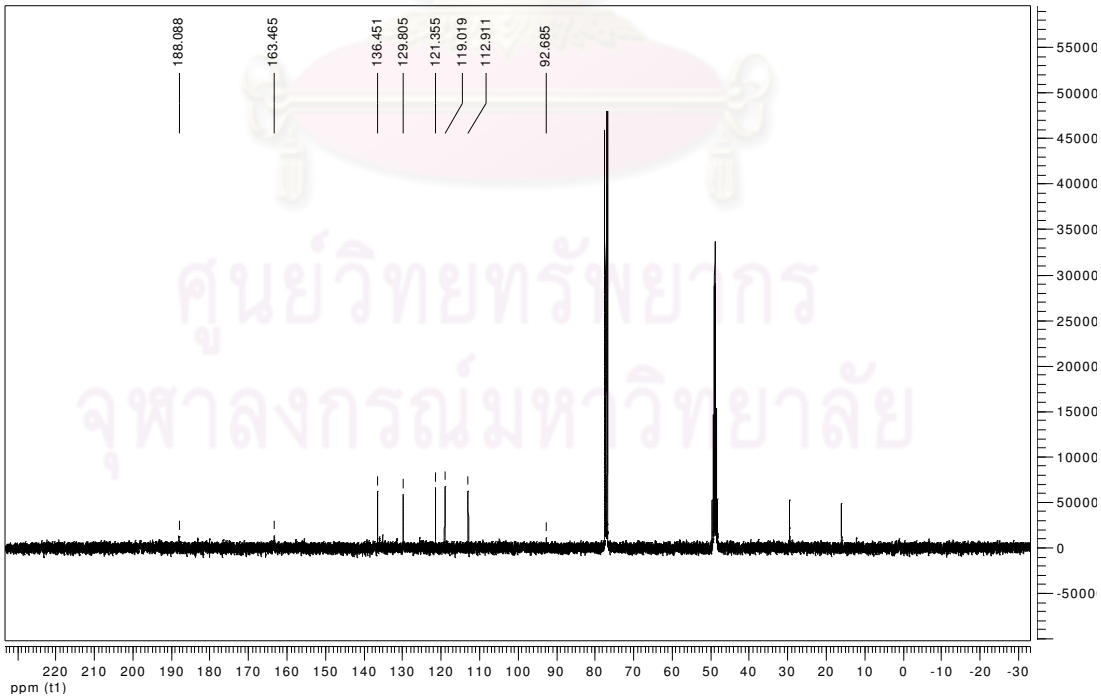


Figure A-24 ^{13}C NMR spectrum ($CDCl_3$+CD_3OD) of flavopurpurin **77**

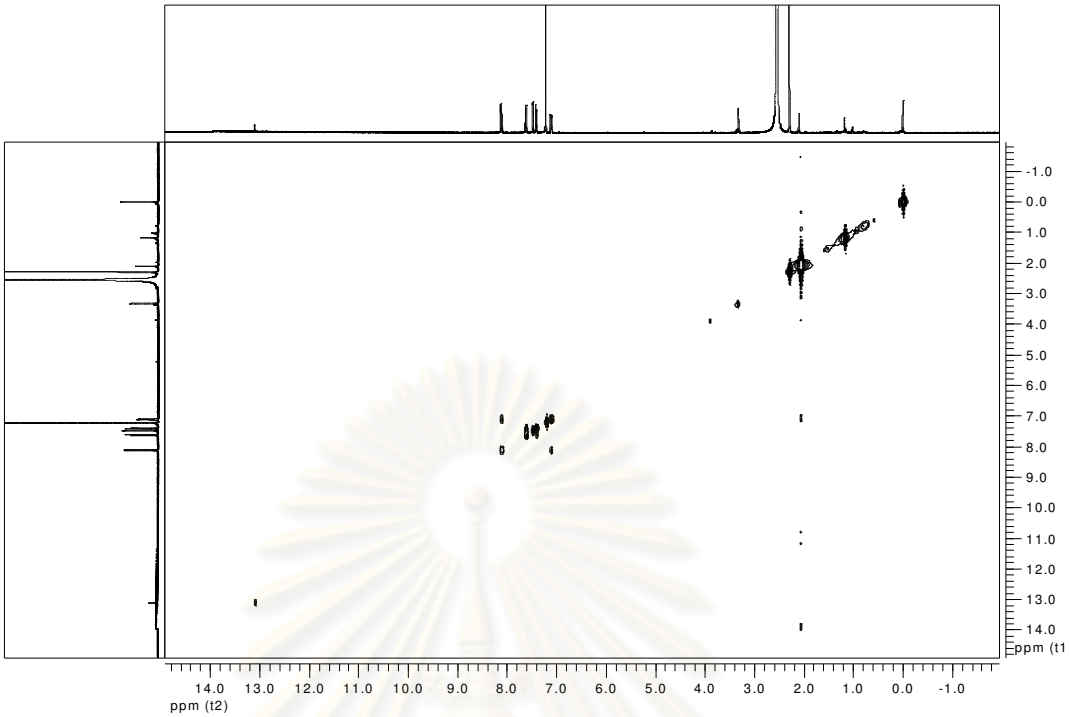


Figure A-25 COSY spectrum ($CDCl_3$+CD_3OD) of flavopurpurin **77**

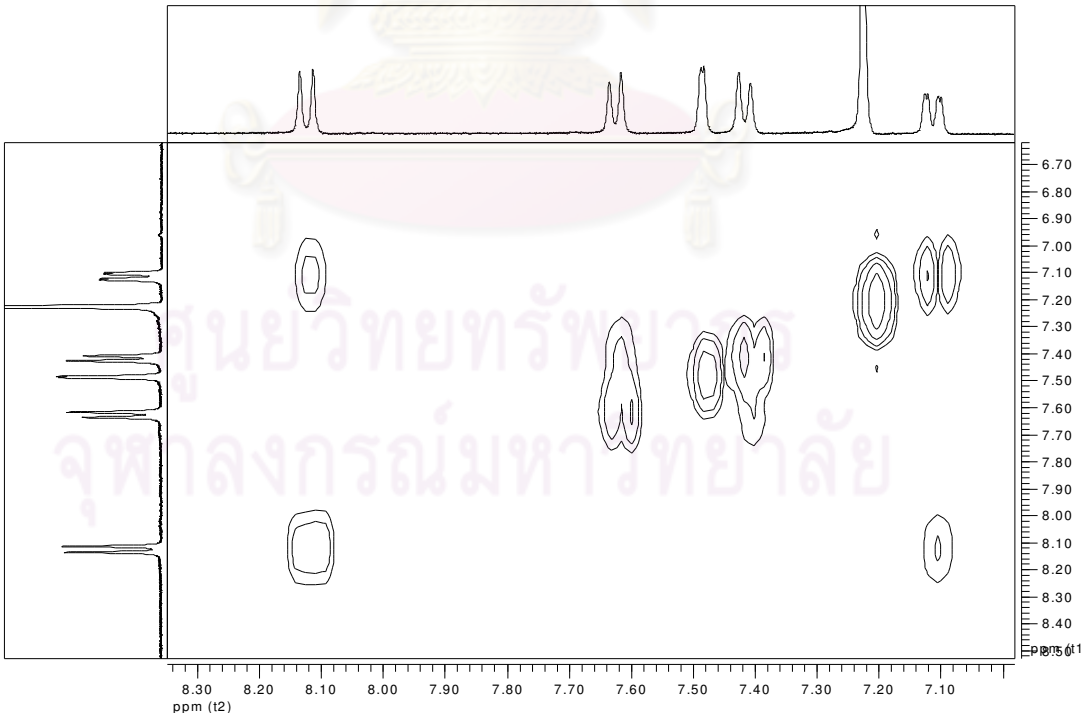


Figure A-26 COSY spectrum ($CDCl_3$+CD_3OD) of flavopurpurin **77**

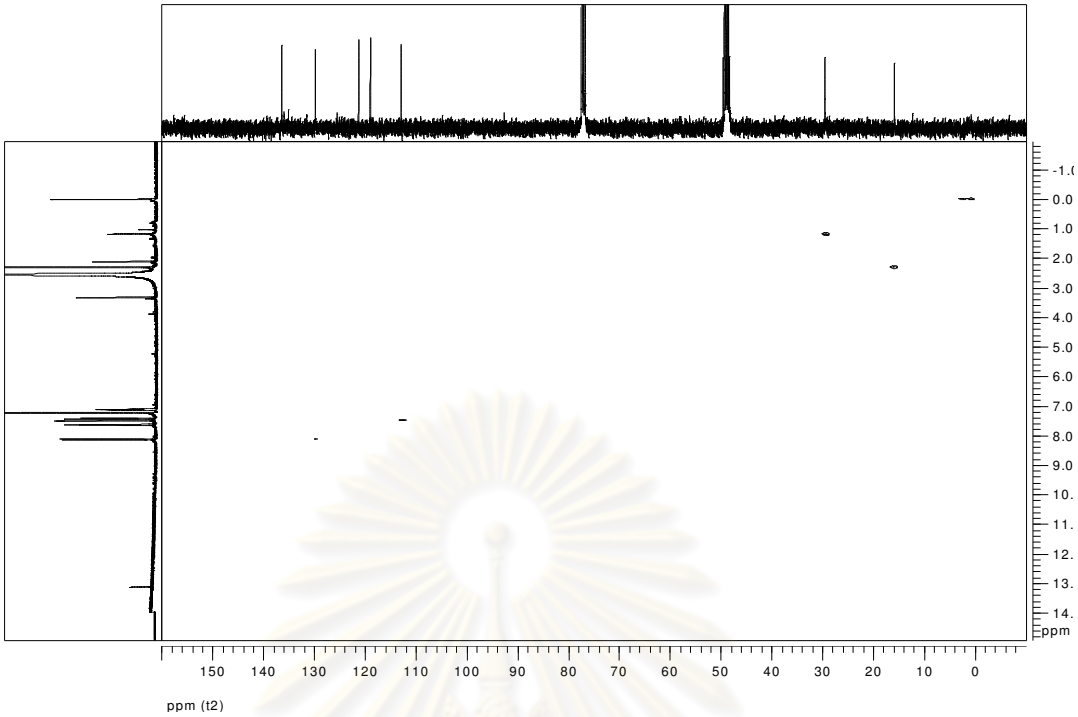


Figure A-27 HSQC spectrum ($CDCl_3$+CD_3OD) of flavopurpurin **77**

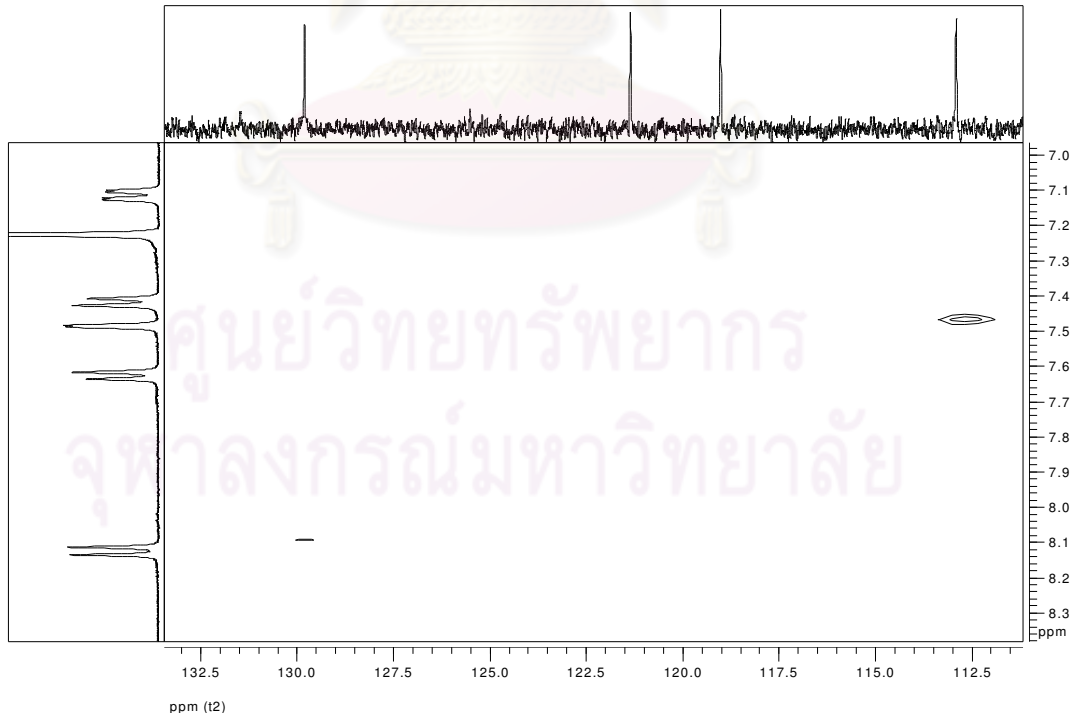


Figure A-28 HSQC spectrum ($CDCl_3$+CD_3OD) of flavopurpurin **77**

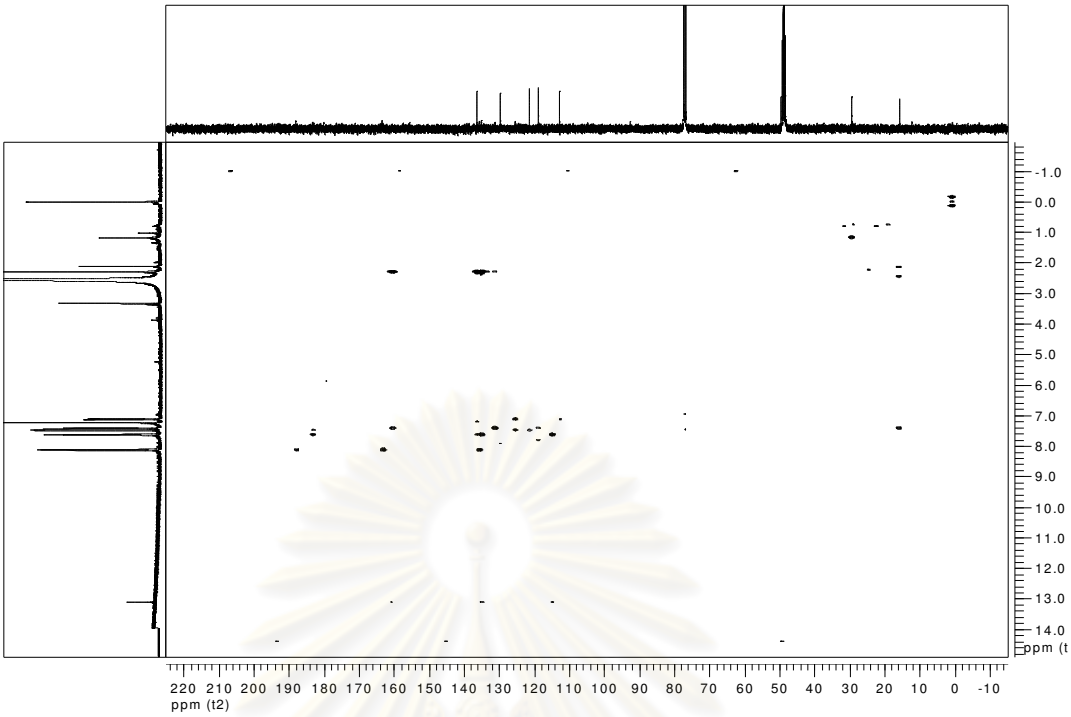


Figure A-29 HMBC spectrum ($CDCl_3$+CD_3OD) of flavopurpurin **77**

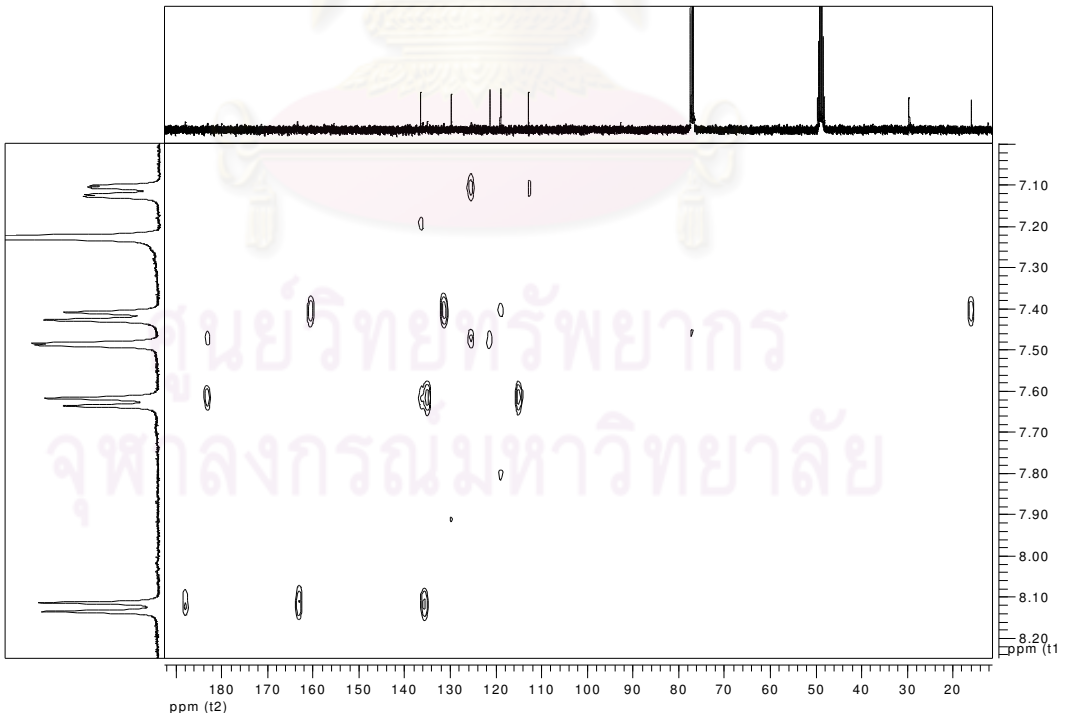


Figure A-30 HMBC spectrum ($CDCl_3$+CD_3OD) of flavopurpurin **77**

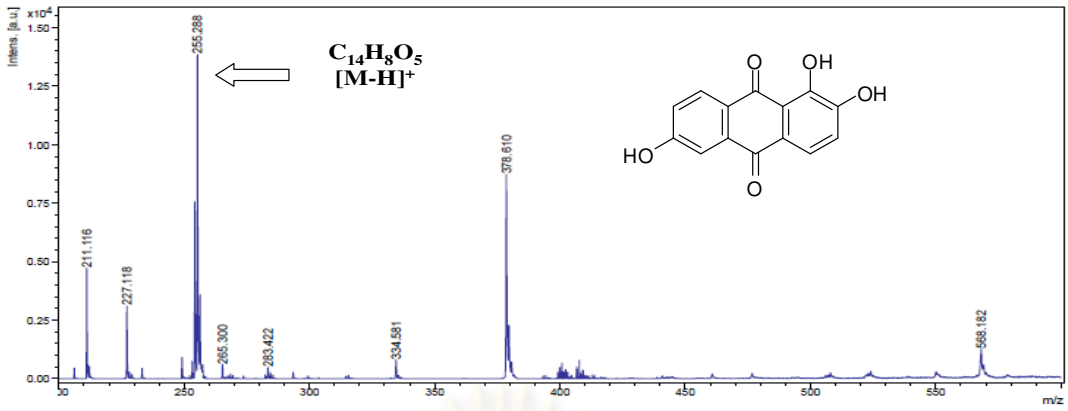


Figure A-31 Mass spectrum of flavopurpurin **77**

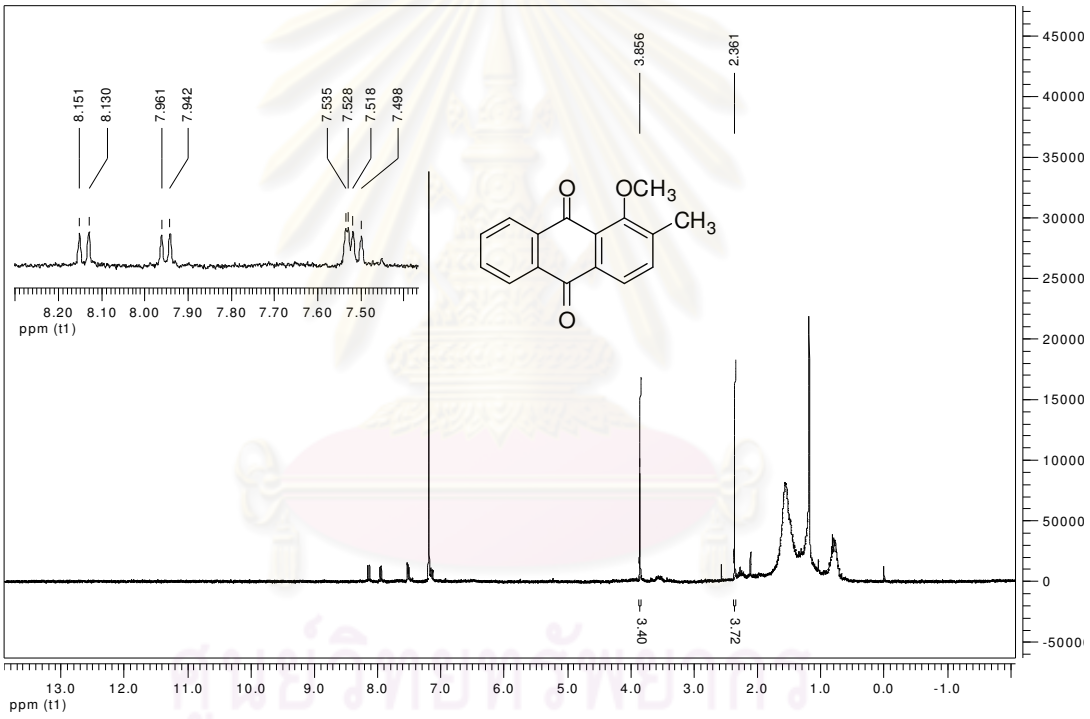


Figure A-32 ^{1}H NMR spectrum ($CDCl_3$) of 1-methoxy-2-methyl anthraquinone **78**

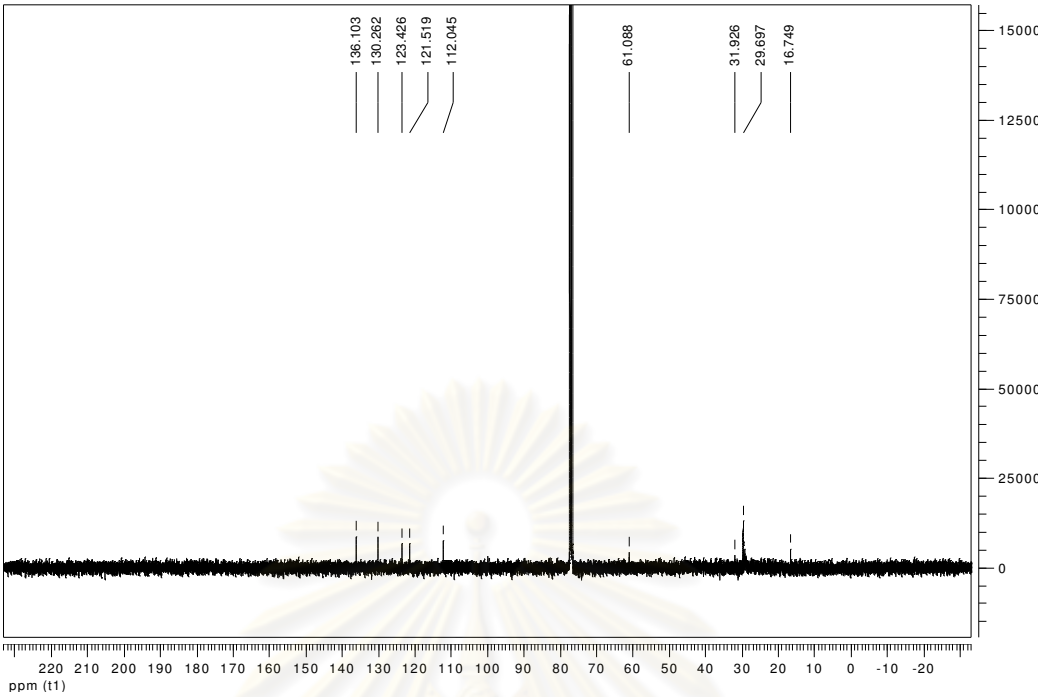


Figure A-33 ^{13}C NMR spectrum ($CDCl_3$) of 1-methoxy-2-methyl anthraquinone **78**

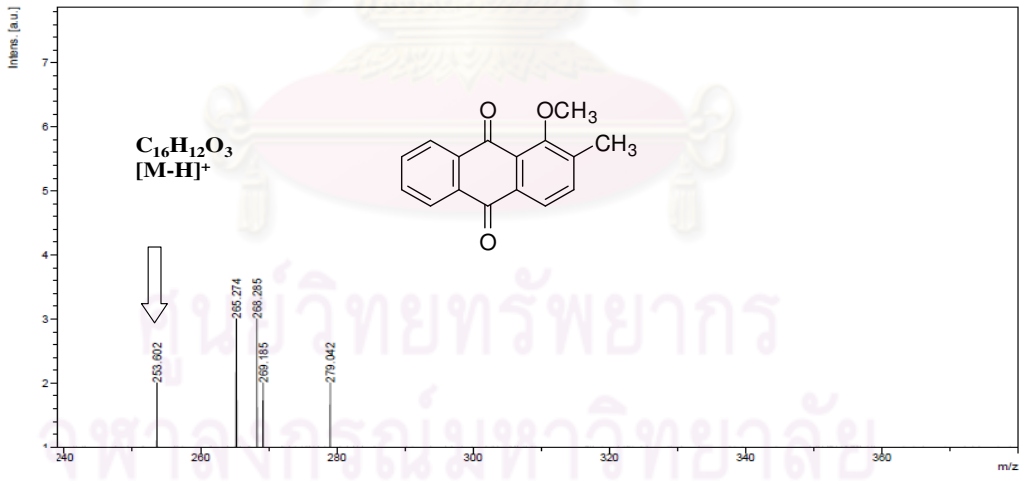


Figure A-34 Mass spectrum of 1-methoxy-2-methyl anthraquinone **78**

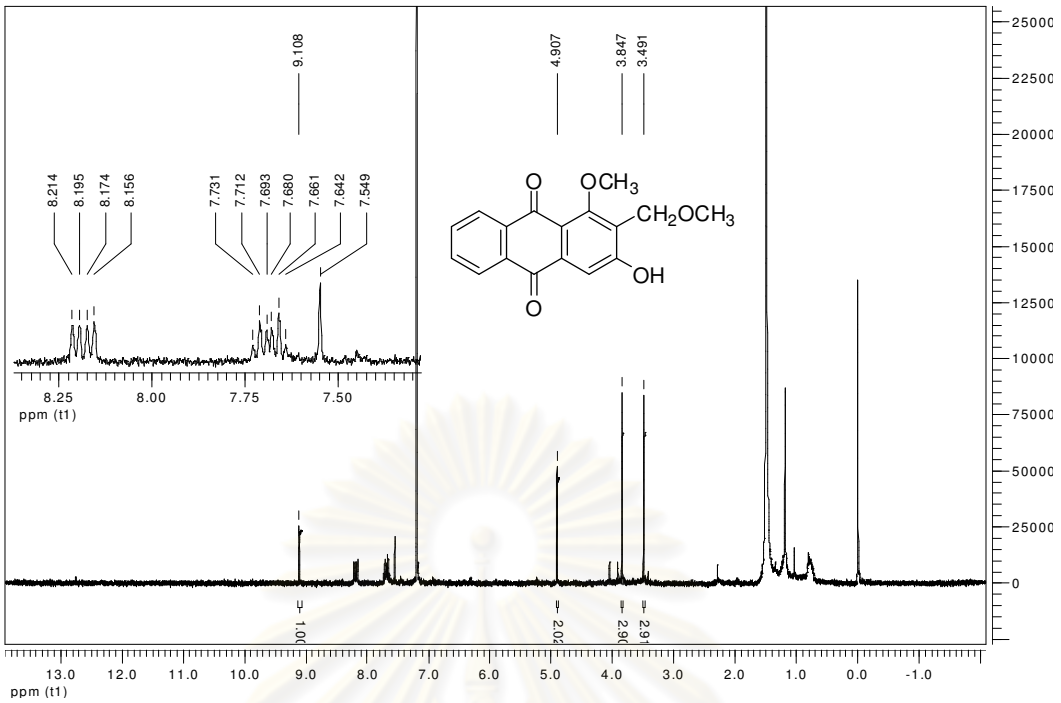


Figure A-35 ^{1}H NMR spectrum ($CDCl_3$) of 3-hydroxy-1-methoxy-2-methoxymethyl anthraquinone **79**

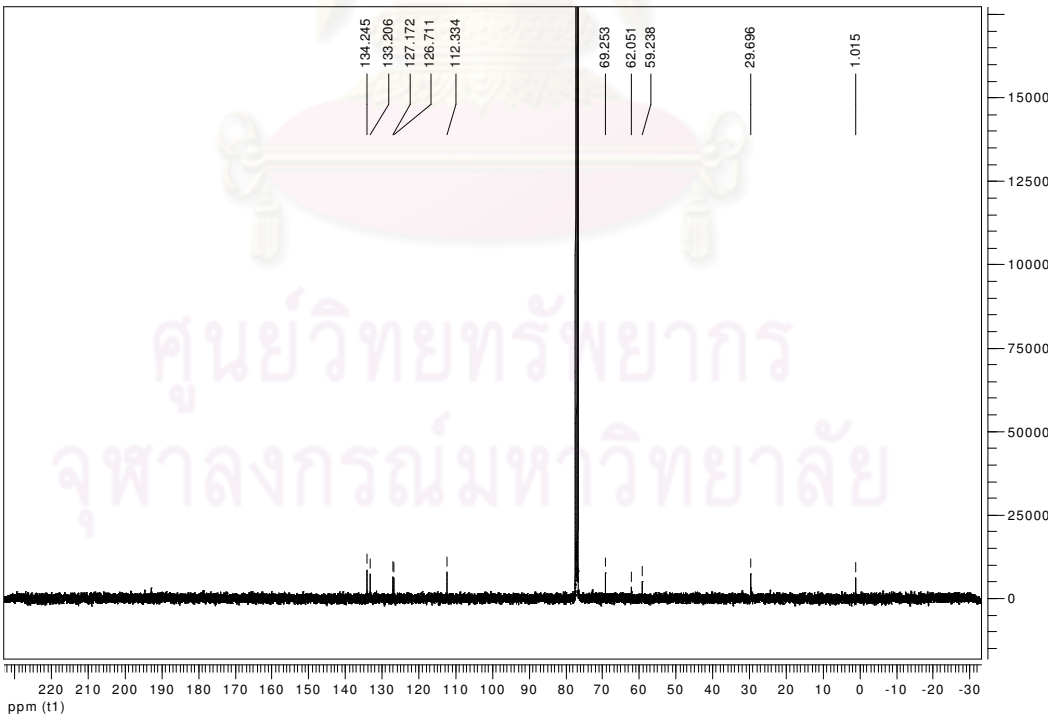


Figure A-36 ^{13}C NMR spectrum ($CDCl_3$) of 3-hydroxy-1-methoxy-2-methoxymethyl anthraquinone **79**

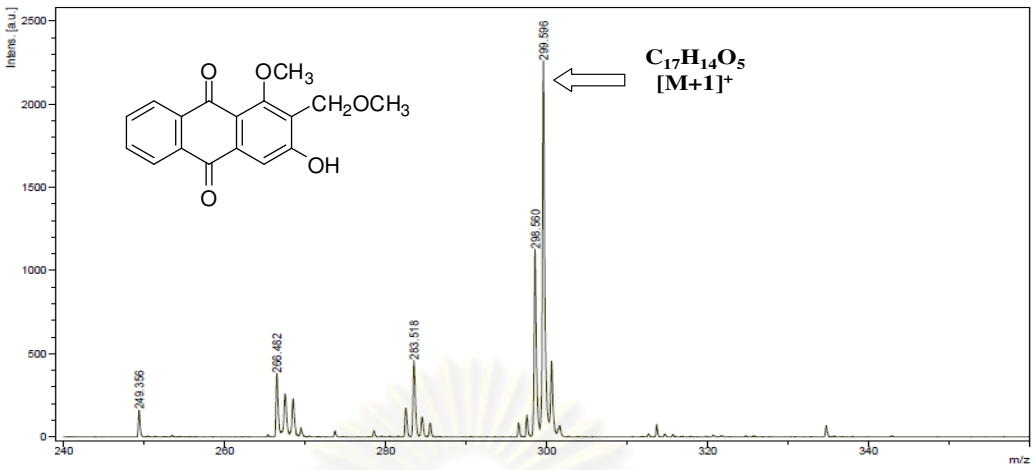


Figure A-37 Mass spectrum of 3-hydroxy-1-methoxy-2-methoxymethyl anthraquinone **79**

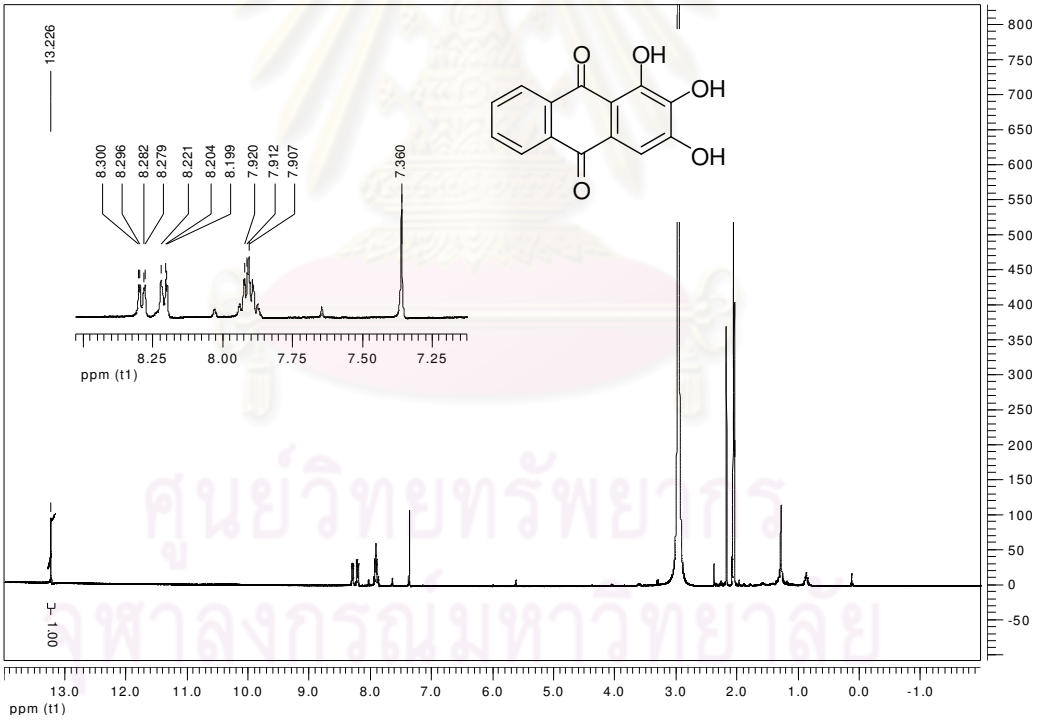


Figure A-38 ^{1}H NMR spectrum (acetone-d_6) of anthragallol (1,2,3-trihydroxy anthraquinone, **80**)

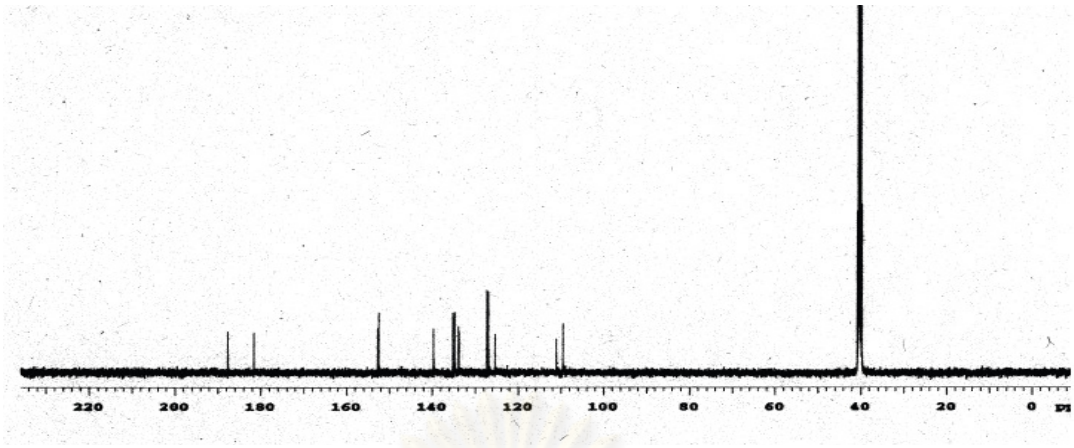


Figure A-39 ^{13}C NMR spectrum (acetone-d_6) of anthragallol (1,2,3-trihydroxy anthraquinone, **80**)

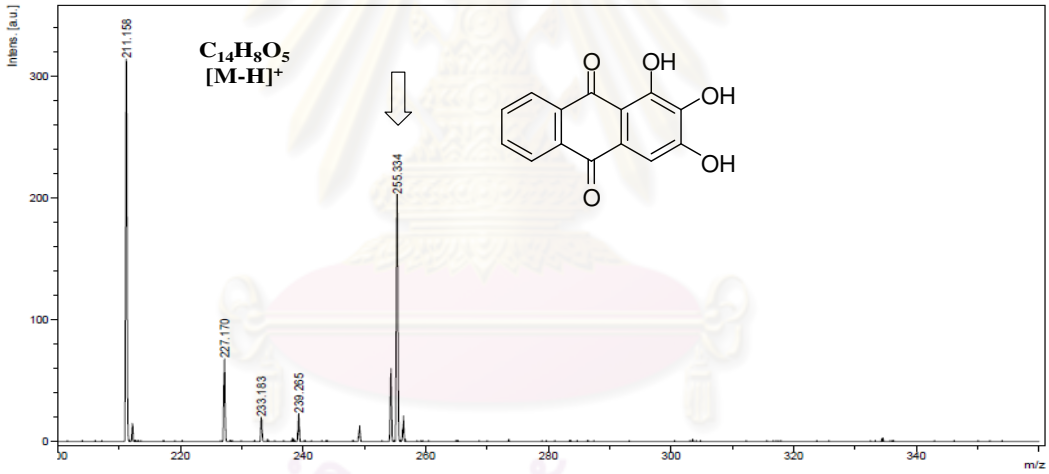


Figure A-40 Mass spectrum of anthragallol (1,2,3-trihydroxy anthraquinone , **80**)

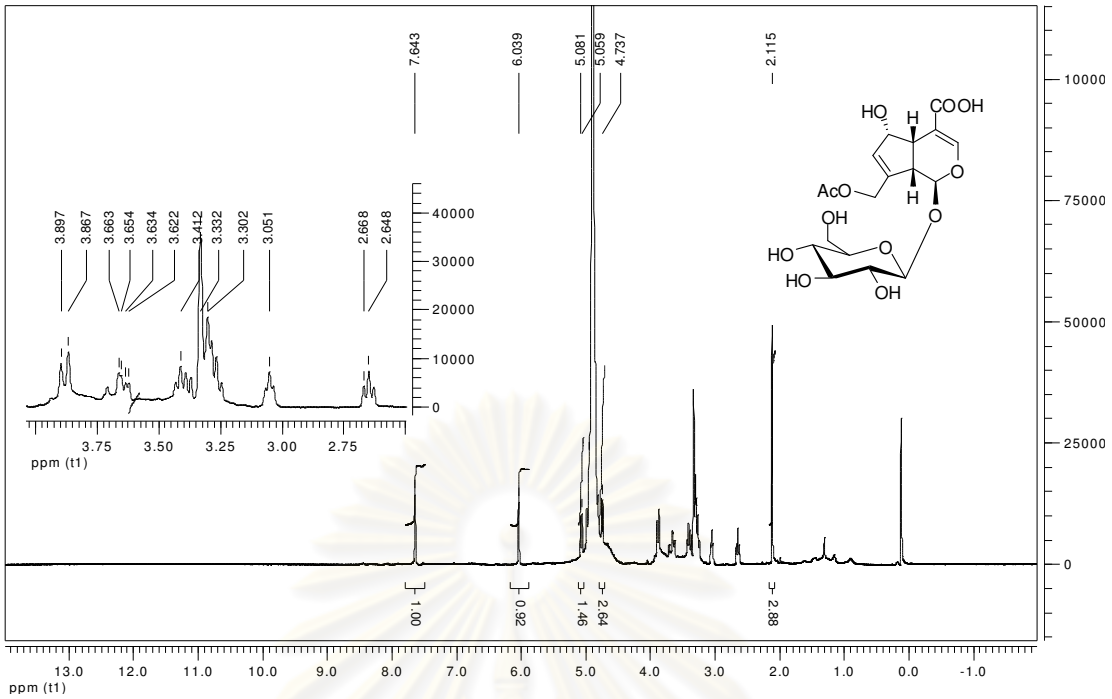


Figure A-41 ^{1}H NMR spectrum (CD_3OD) of asperulosidic acid **43**

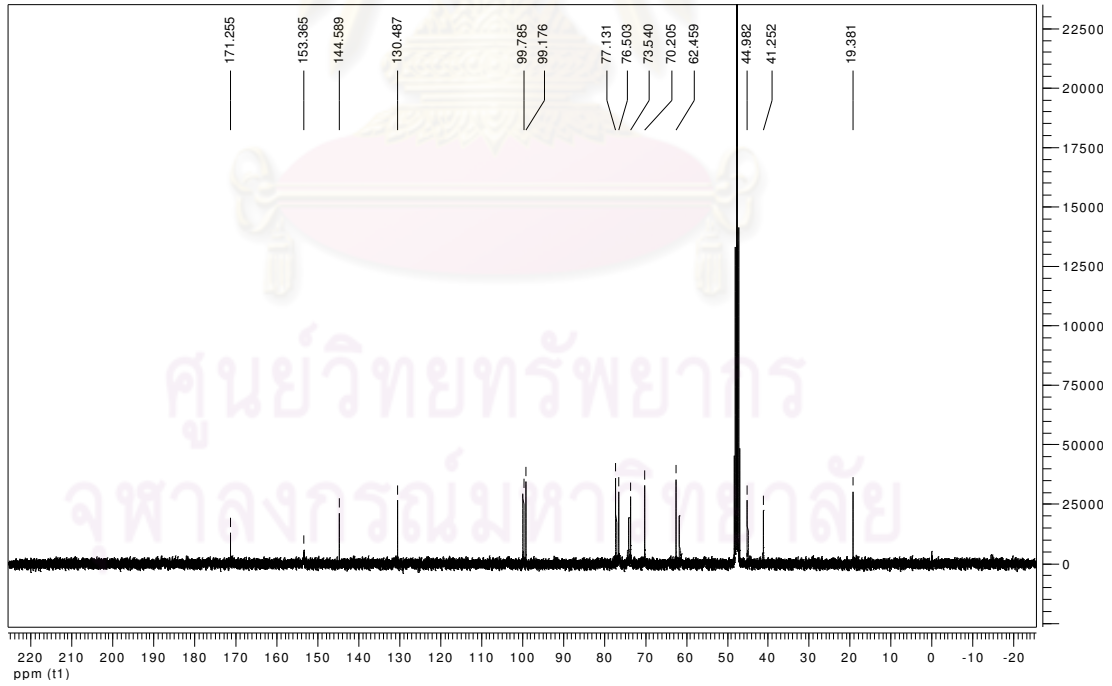


Figure A-42 ^{13}C NMR spectrum (CD_3OD) of asperulosidic acid **43**

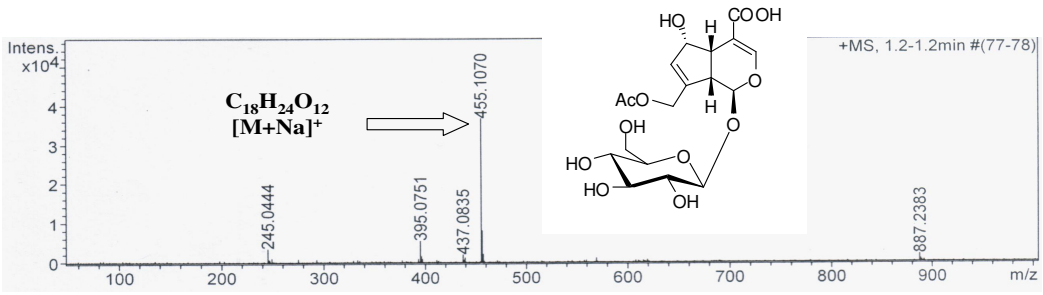


Figure A-43 High resolution mass spectrum of asperulosidic acid **43**

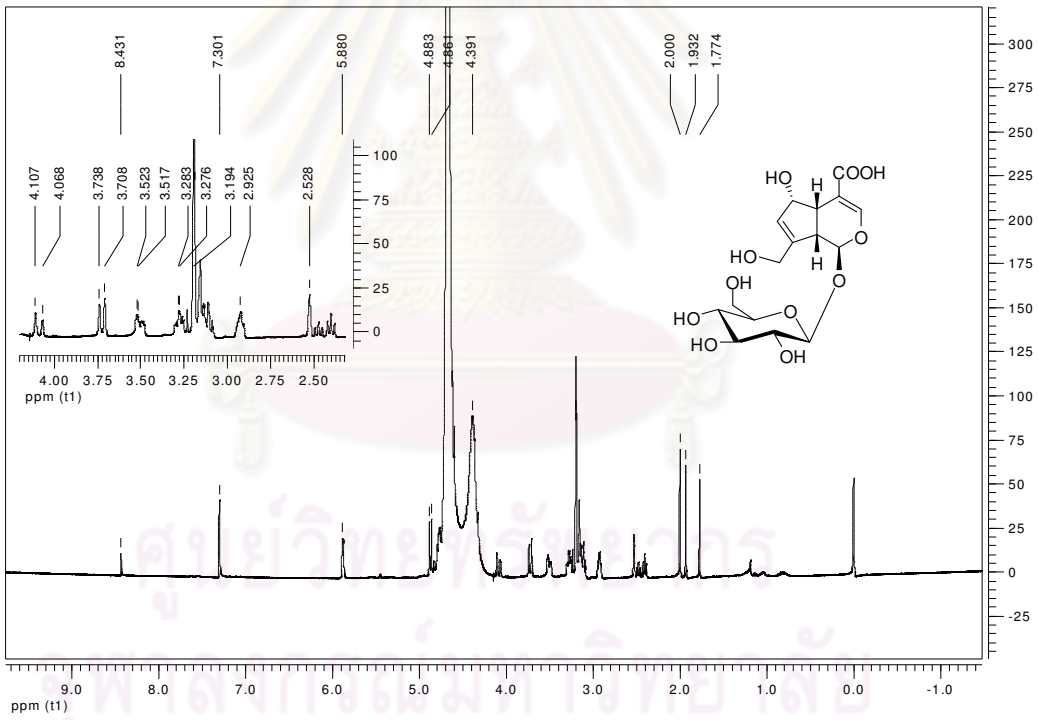


Figure A-44 ^{1}H NMR spectrum (CD_3OD) of deacetylasperulosidic acid **44**

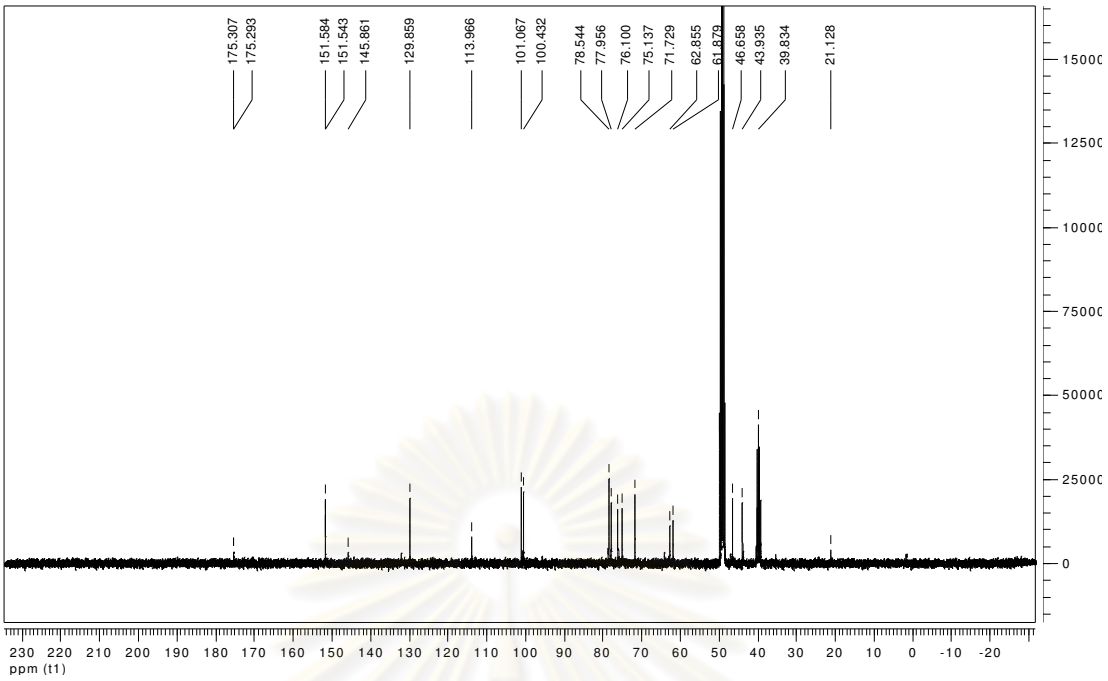


Figure A-45 ^{13}C NMR spectrum (CD_3OD) of deacetylasperulosidic acid **44**

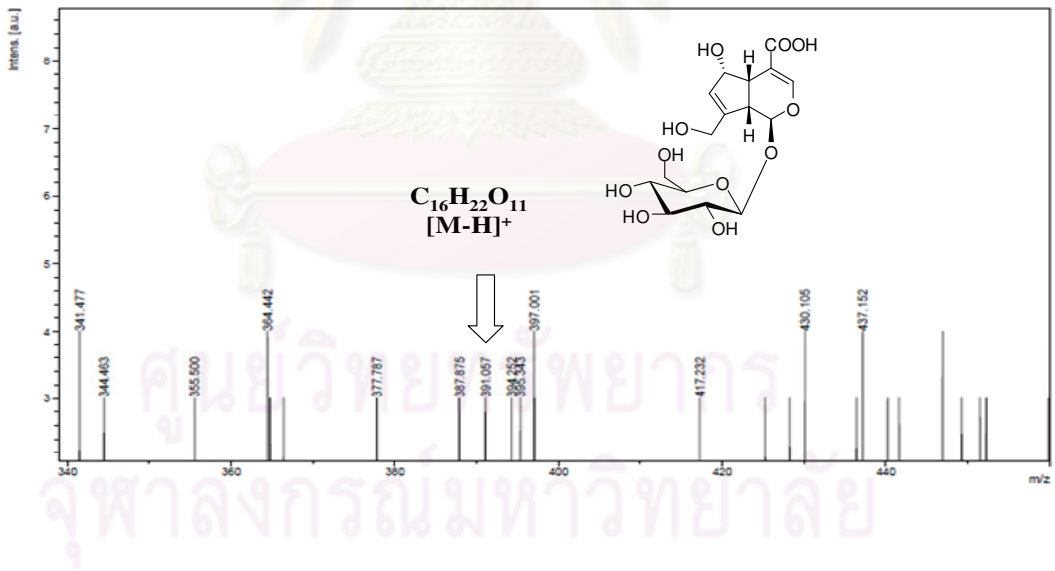


Figure A-46 Mass spectrum of deacetylasperulosidic acid **44**

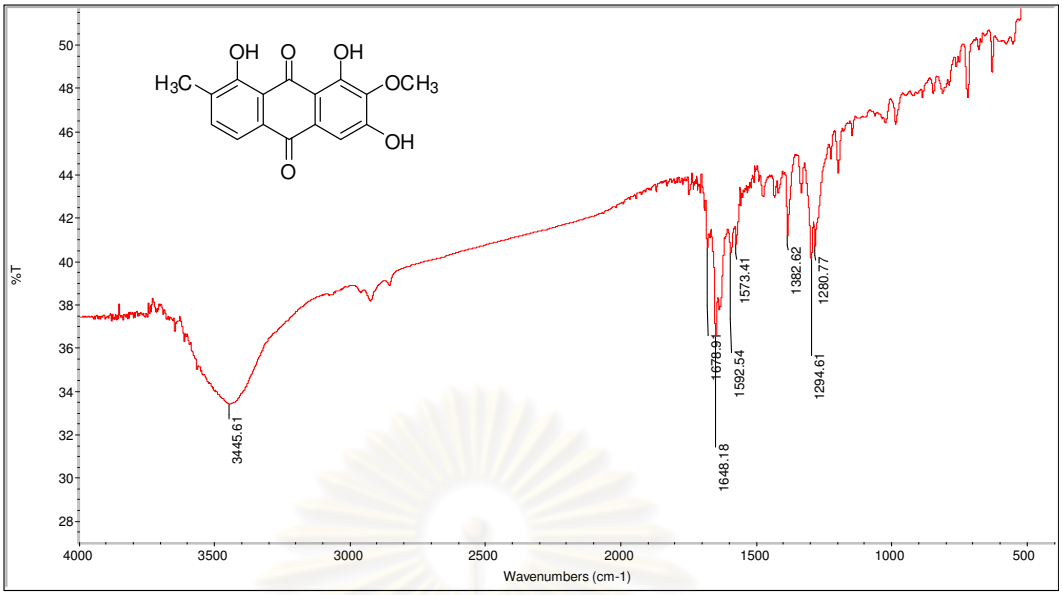


Figure A-47 IR spectrum of 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**)

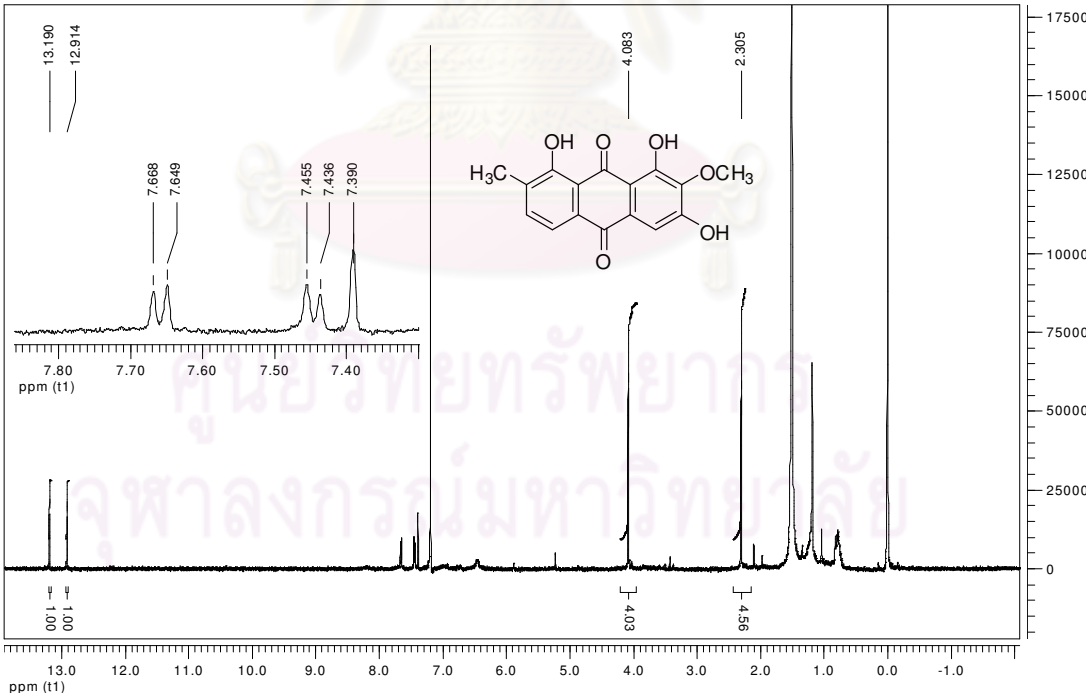


Figure A-48 ^{1}H NMR spectrum ($CDCl_3$) of 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**)

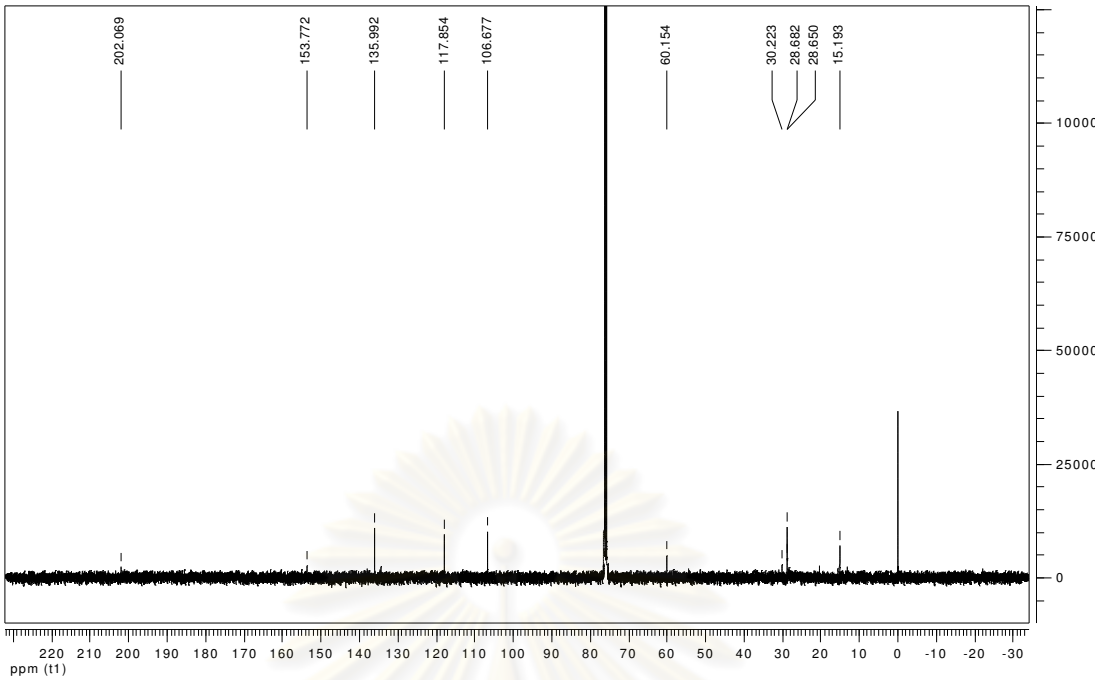


Figure A-49 ^{13}C NMR spectrum ($CDCl_3$) of 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**)

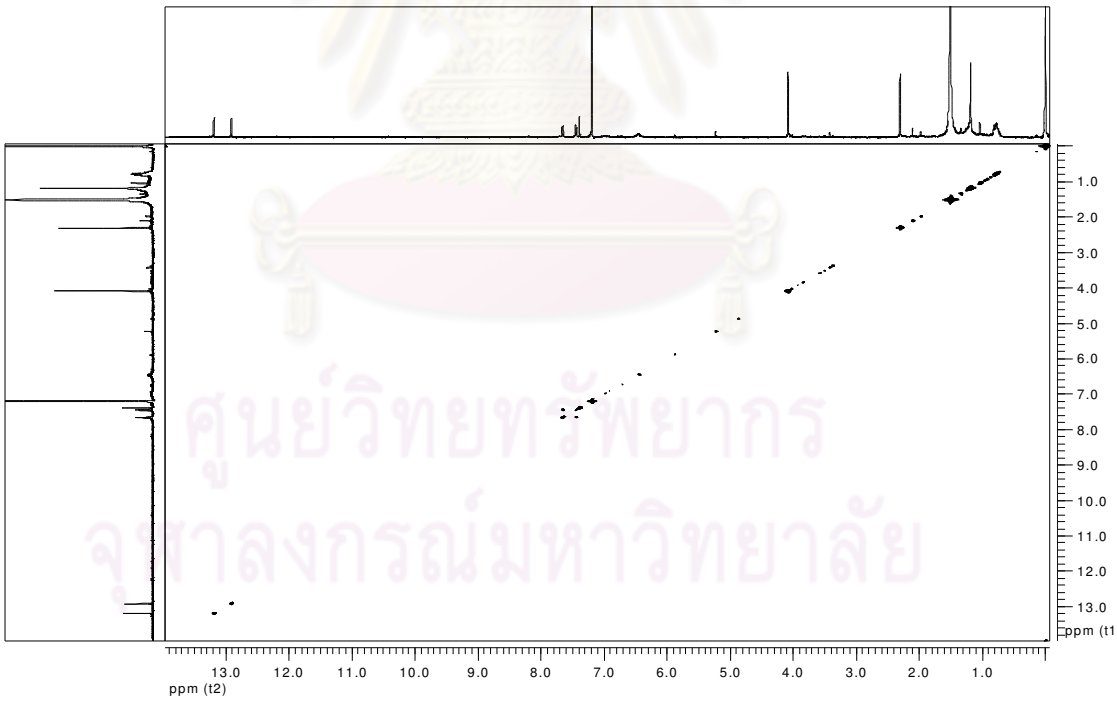


Figure A-50 COSY spectrum ($CDCl_3$) of 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**)

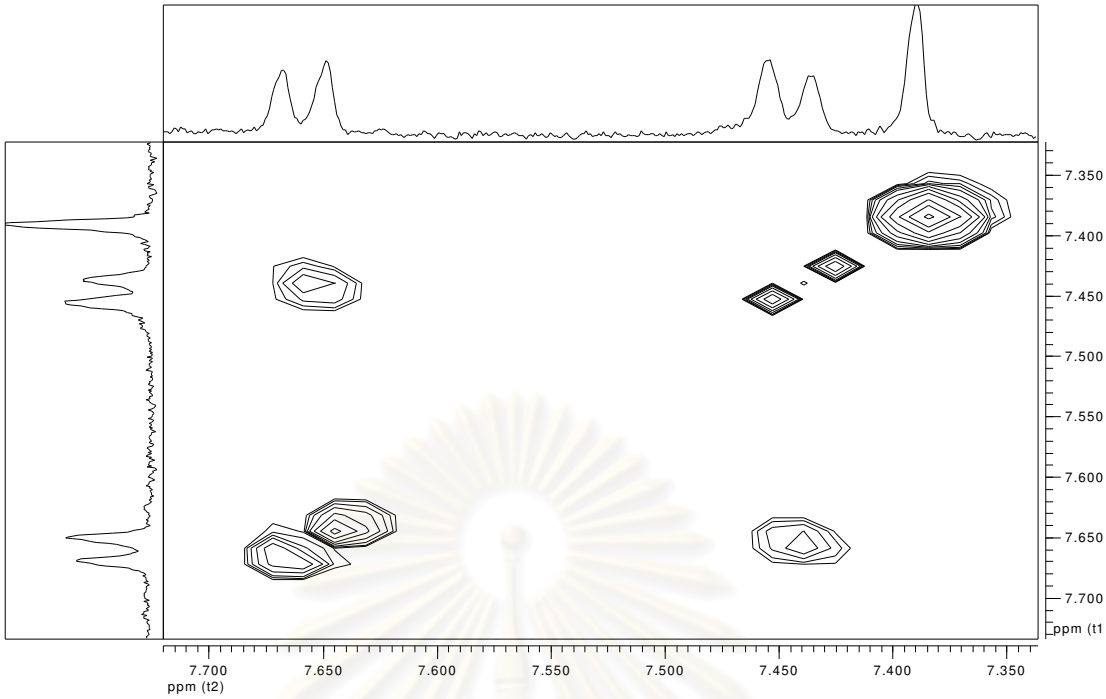


Figure A-51 COSY spectrum ($CDCl_3$) of 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**)

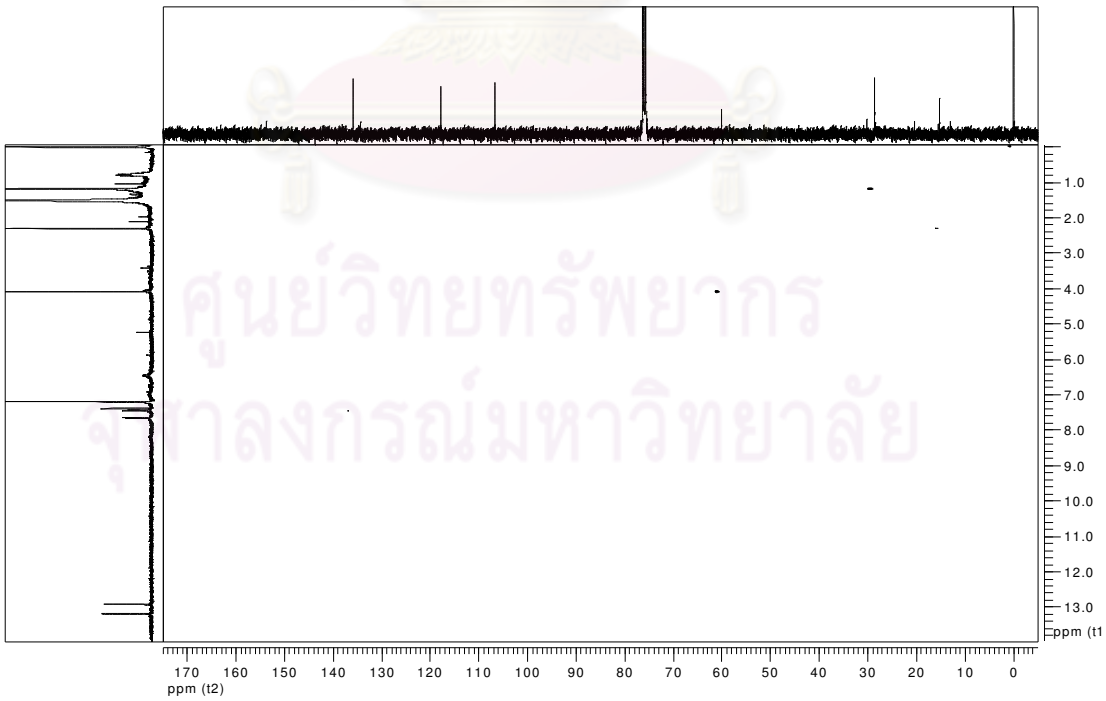


Figure A-52 HSQC spectrum ($CDCl_3$) of 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**)

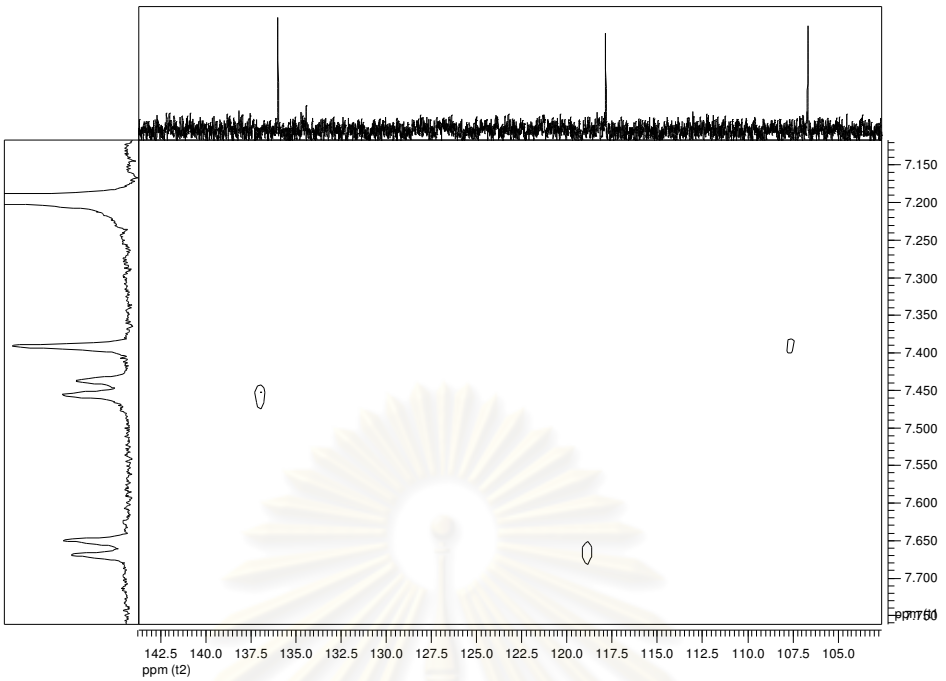


Figure A-53 HSQC spectrum ($CDCl_3$) of 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**)

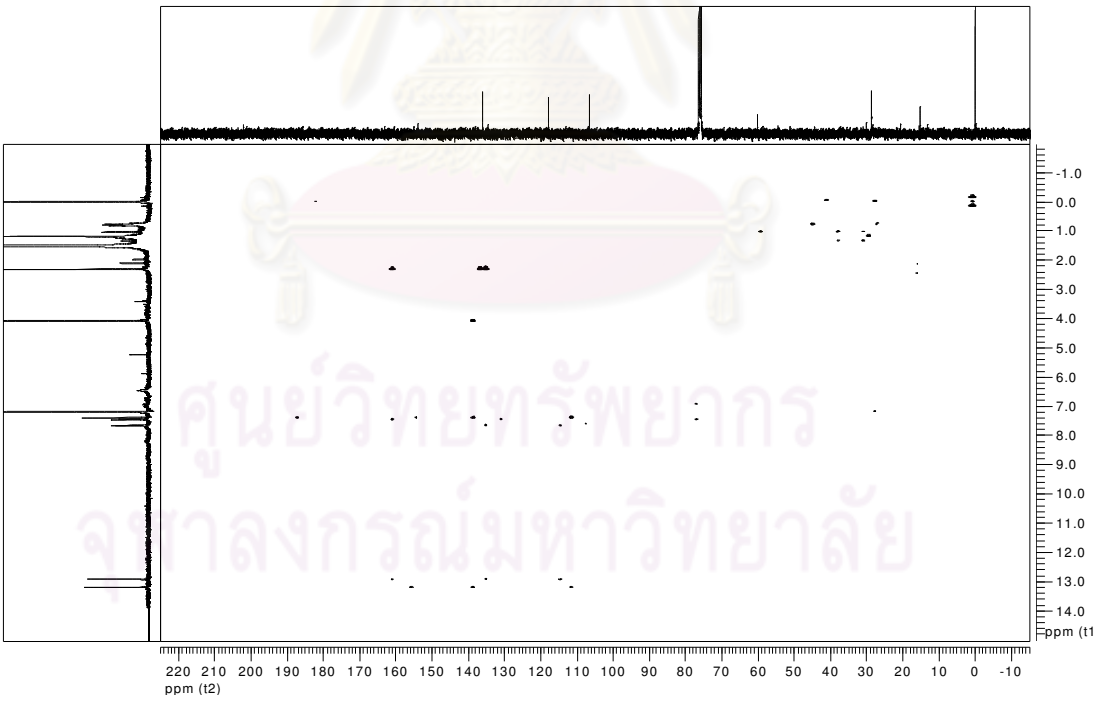


Figure A-54 HMBC spectrum ($CDCl_3$) of 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**)

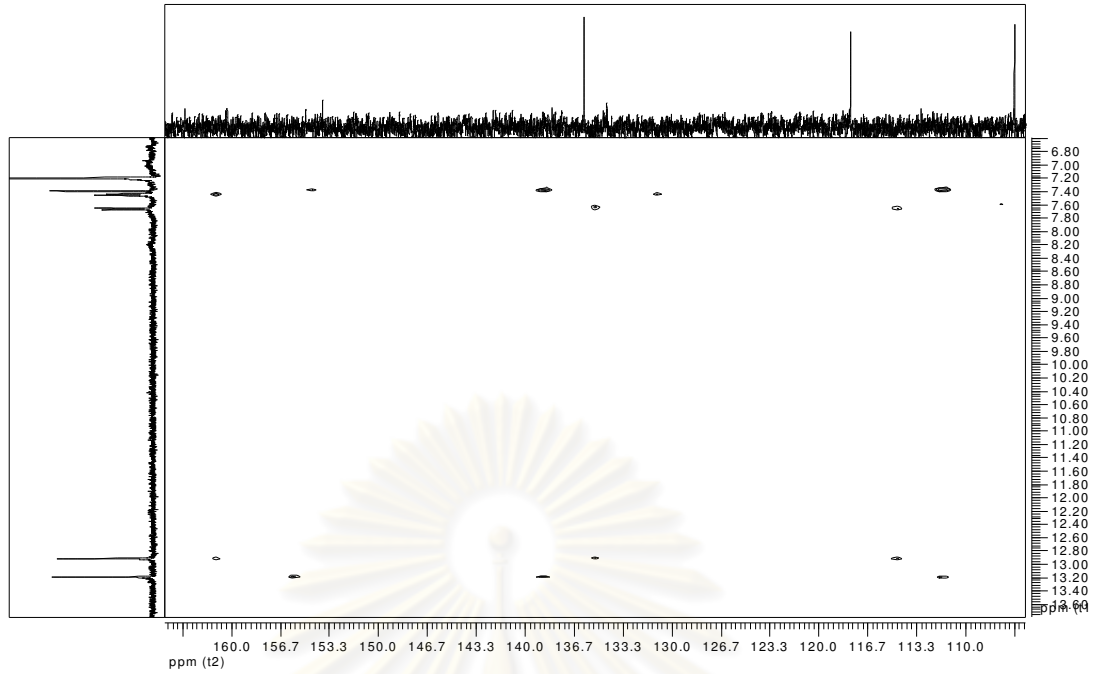


Figure A-55 HMBC spectrum ($CDCl_3$) of 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**)

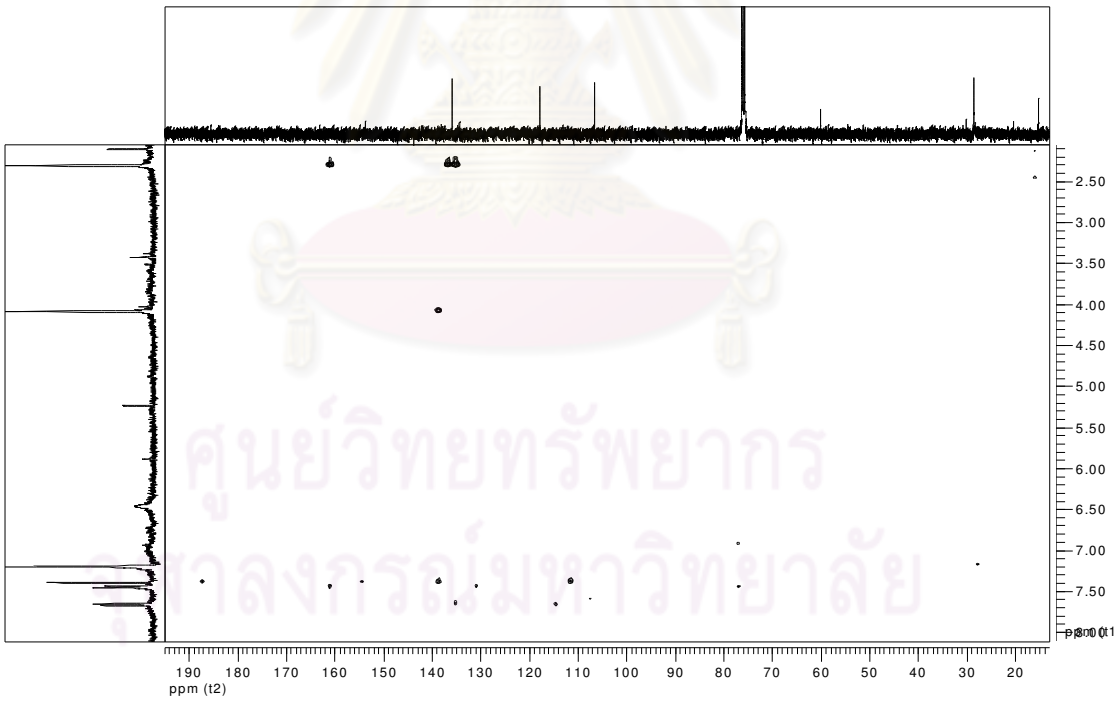


Figure A-56 HMBC spectrum ($CDCl_3$) of 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**)

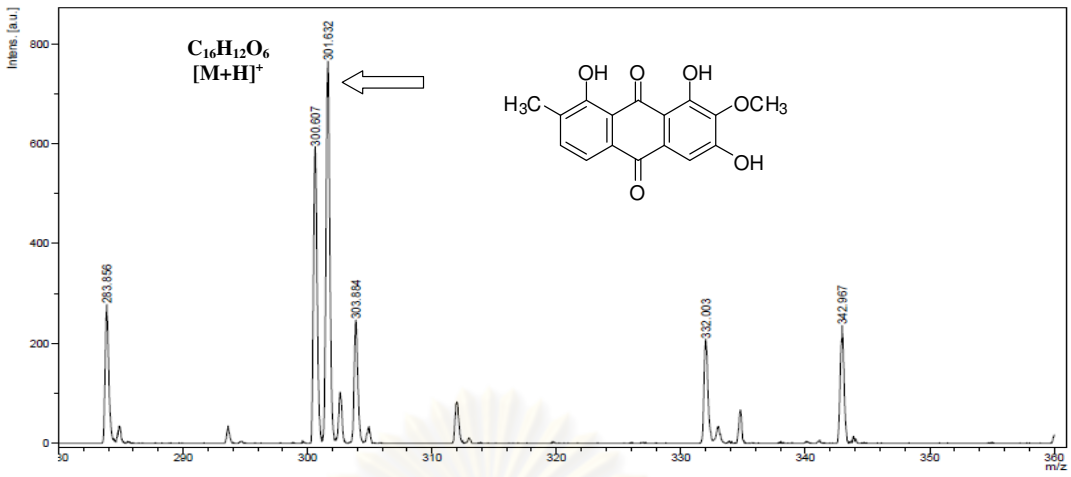


Figure A-57 Mass spectrum of 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone (**81**)

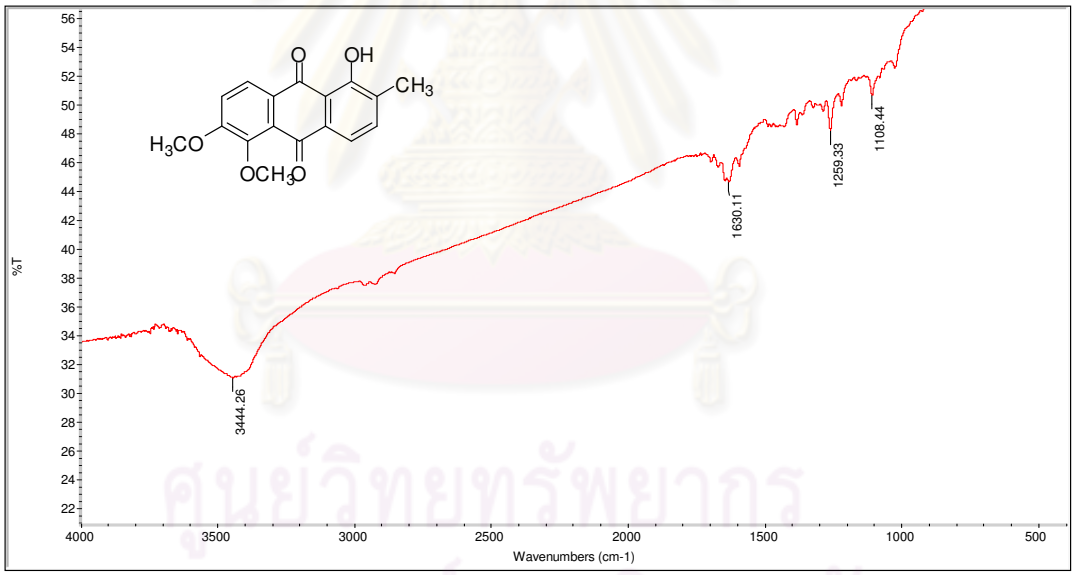


Figure A-58 IR spectrum of 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (morindone dimethyl ether) (**82**)

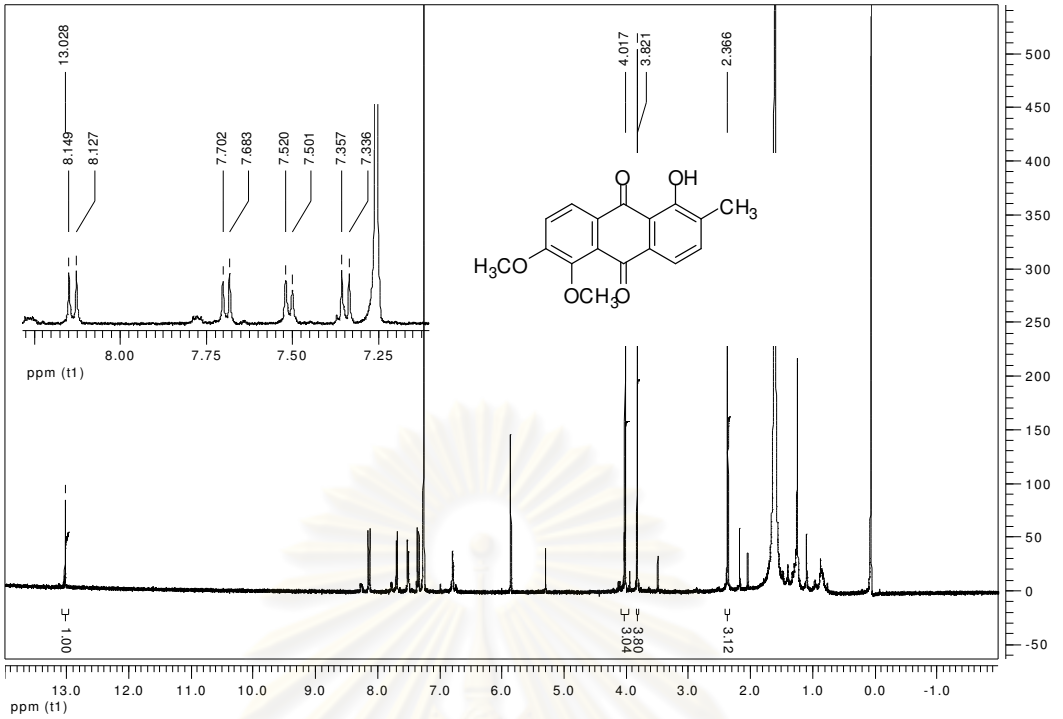


Figure A-59 ^{1}H NMR spectrum ($CDCl_3$) of 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (morindone dimethyl ether) (**82**)

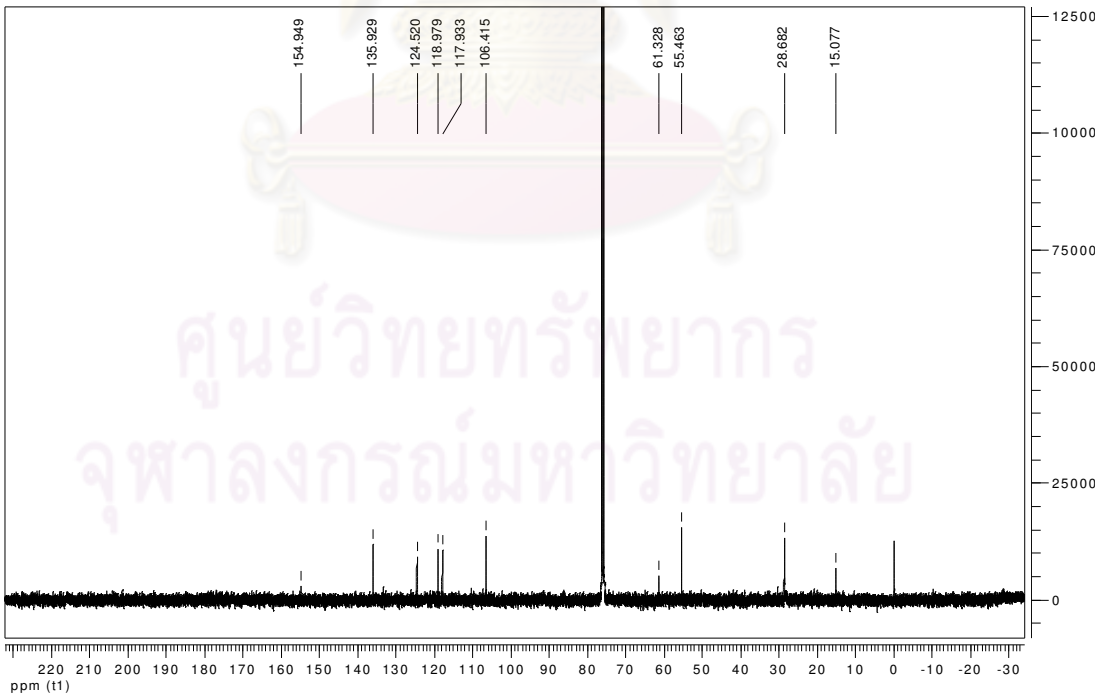


Figure A-60 ^{13}C NMR spectrum ($CDCl_3$) of 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (morindone dimethyl ether) (**82**)

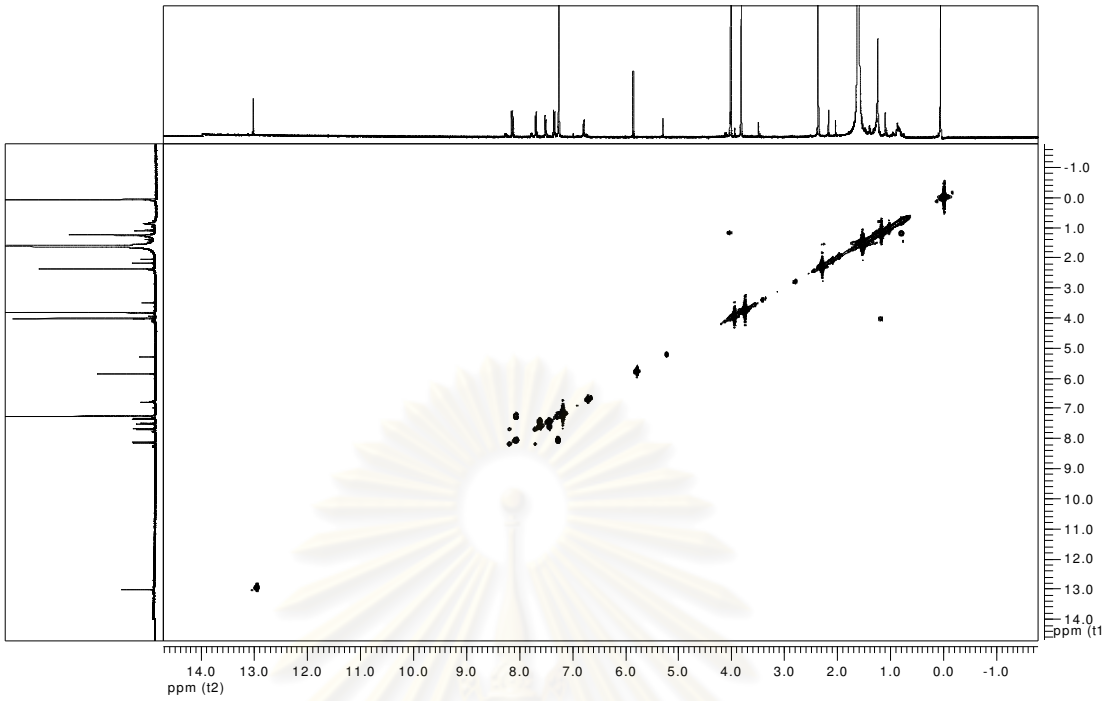


Figure A-61 COSY spectrum ($CDCl_3$) of 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (morindone dimethyl ether) (**82**)

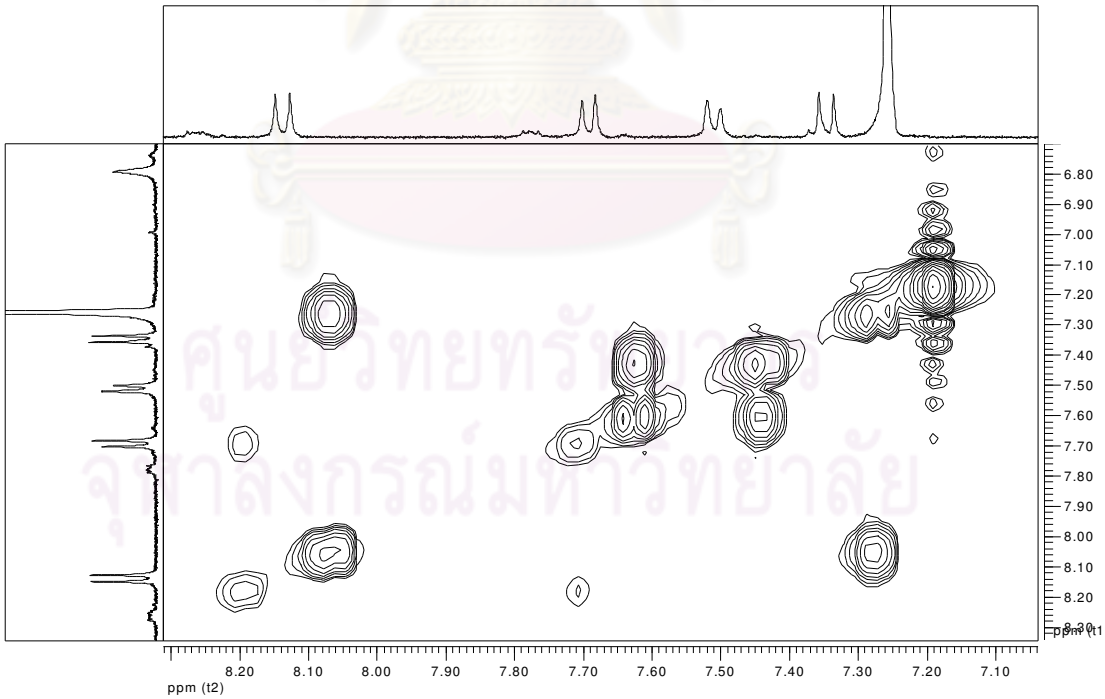


Figure A-62 COSY spectrum ($CDCl_3$) of 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (morindone dimethyl ether) (**82**)

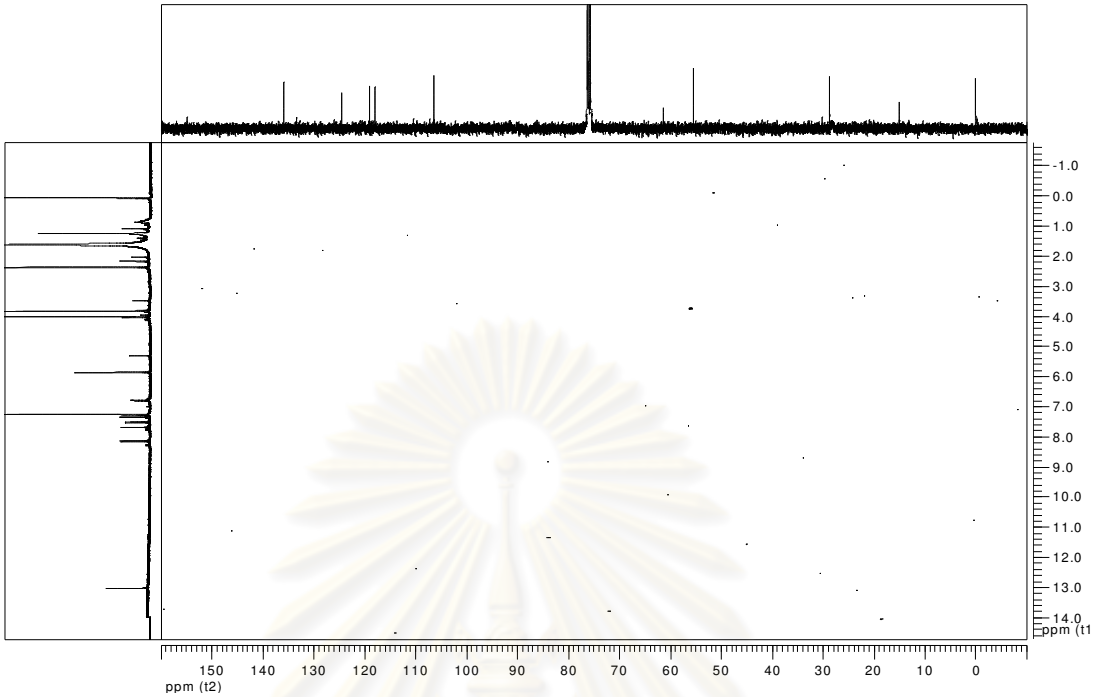


Figure A-63 HSQC spectrum ($CDCl_3$) of 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (morindone dimethyl ether) (**82**)

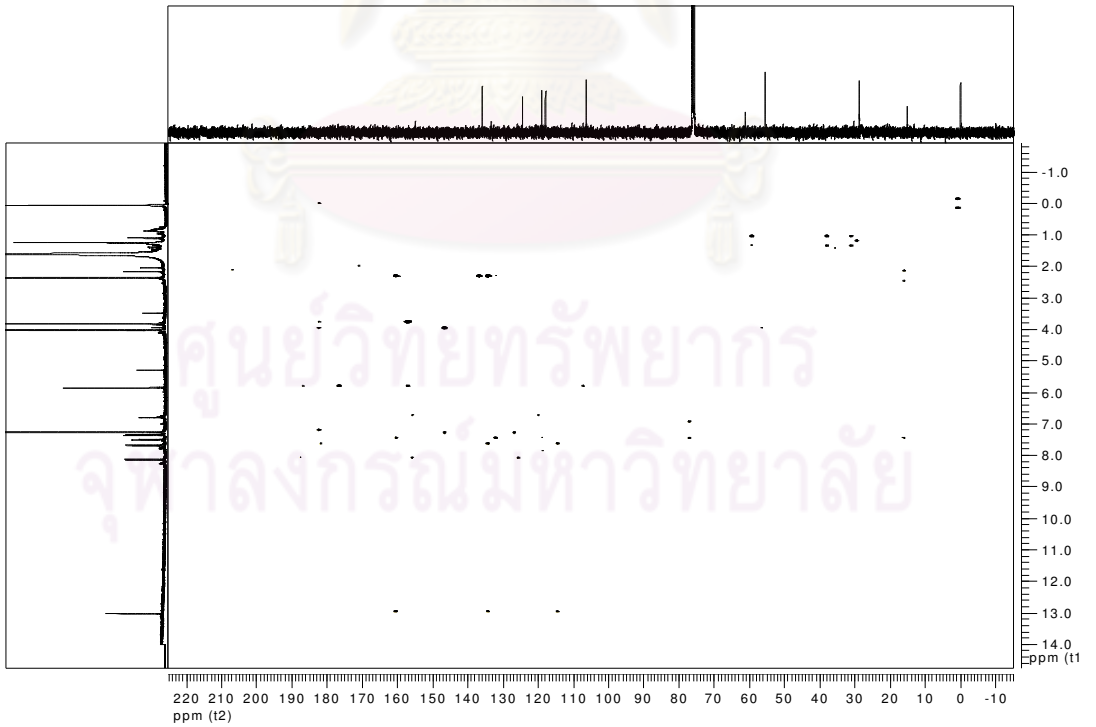


Figure A-64 HMBC spectrum ($CDCl_3$) of 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (morindone dimethyl ether) (**82**)

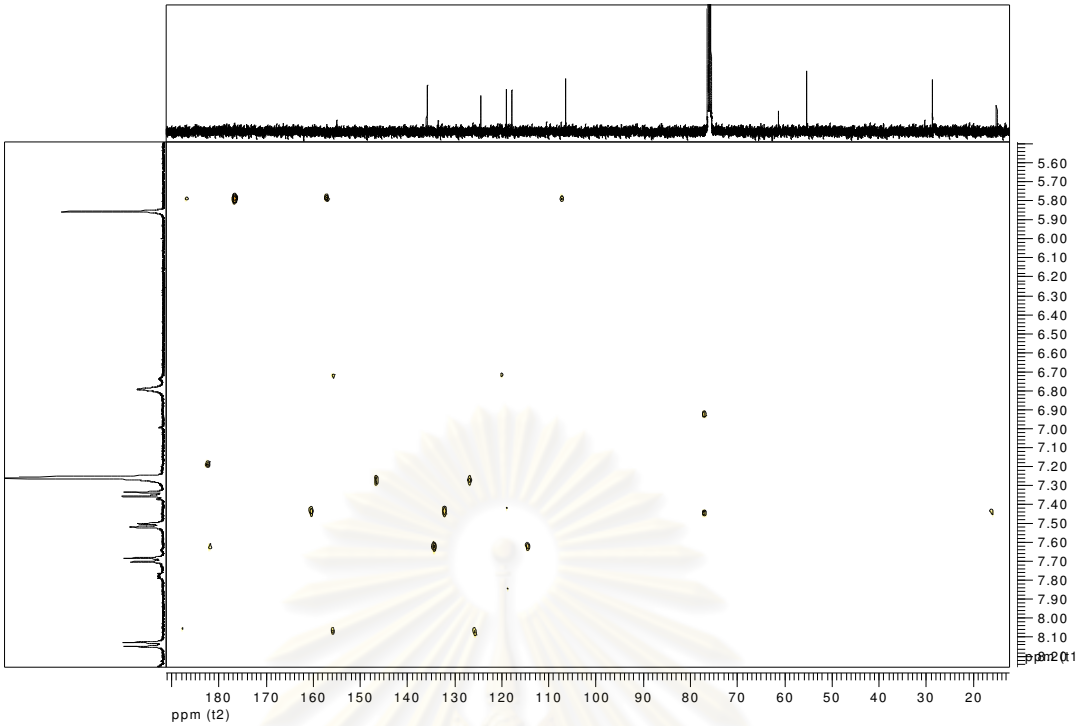


Figure A-65 HMBC spectrum ($CDCl_3$) of 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (morindone dimethyl ether) (**82**)

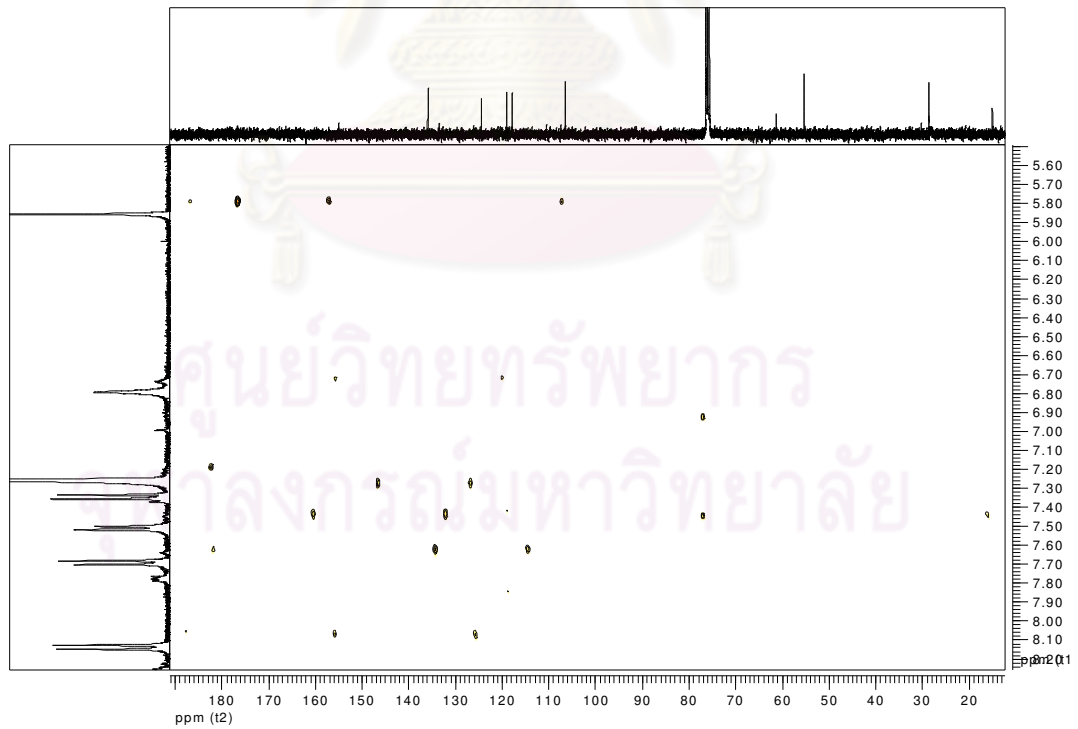


Figure A-66 HMBC spectrum ($CDCl_3$) of 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (morindone dimethyl ether) (**82**)

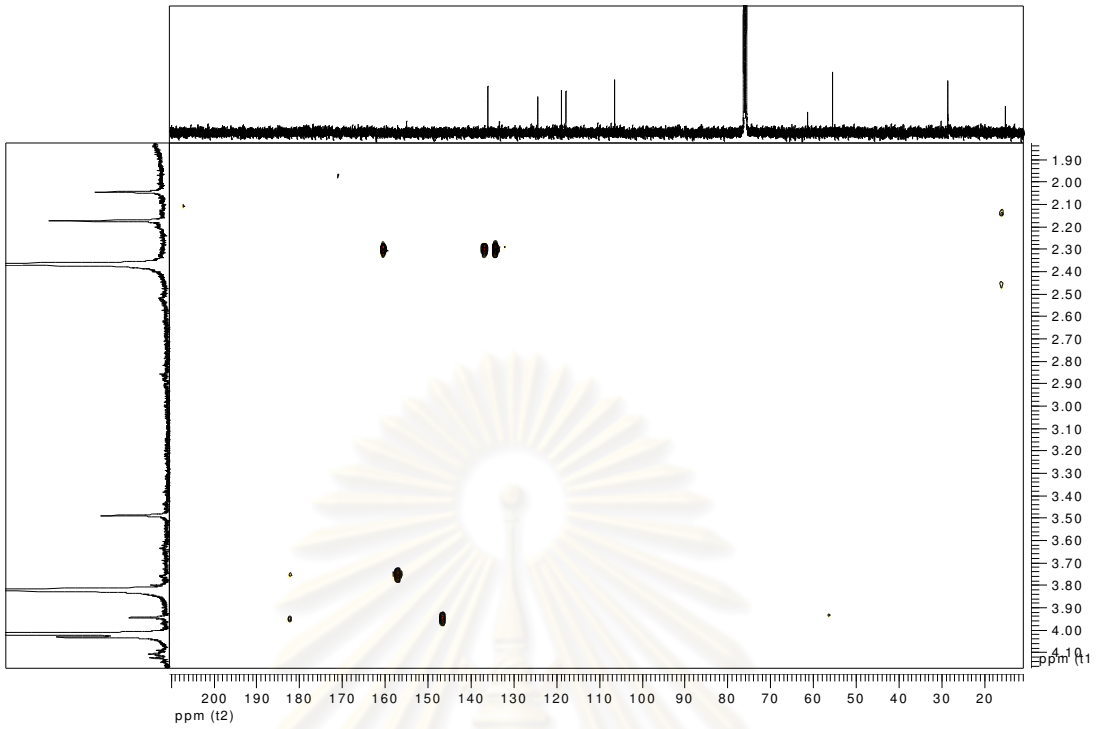


Figure A-67 HMBC spectrum ($CDCl_3$) of 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (morindone dimethyl ether) (**82**)

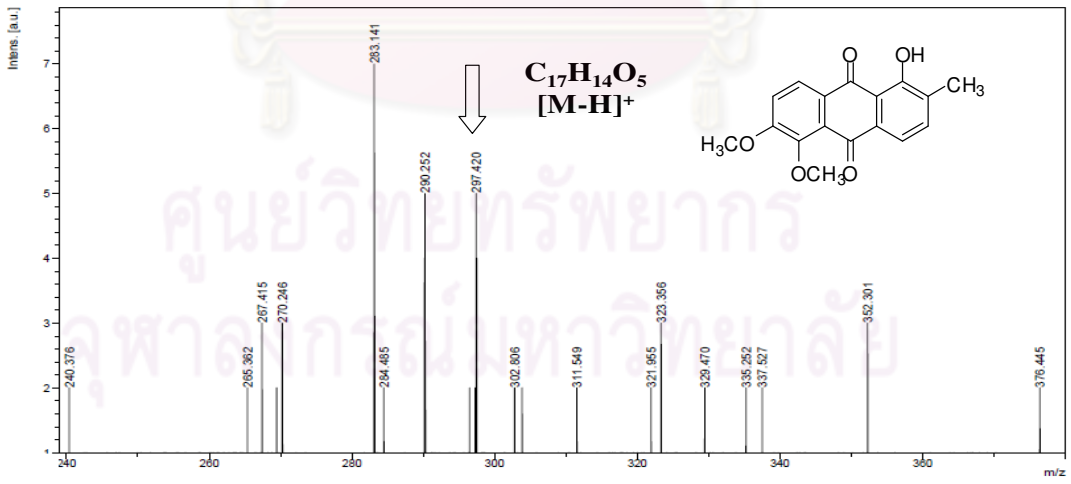


Figure A-68 Mass spectrum of 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone (morindone dimethyl ether) (**82**)

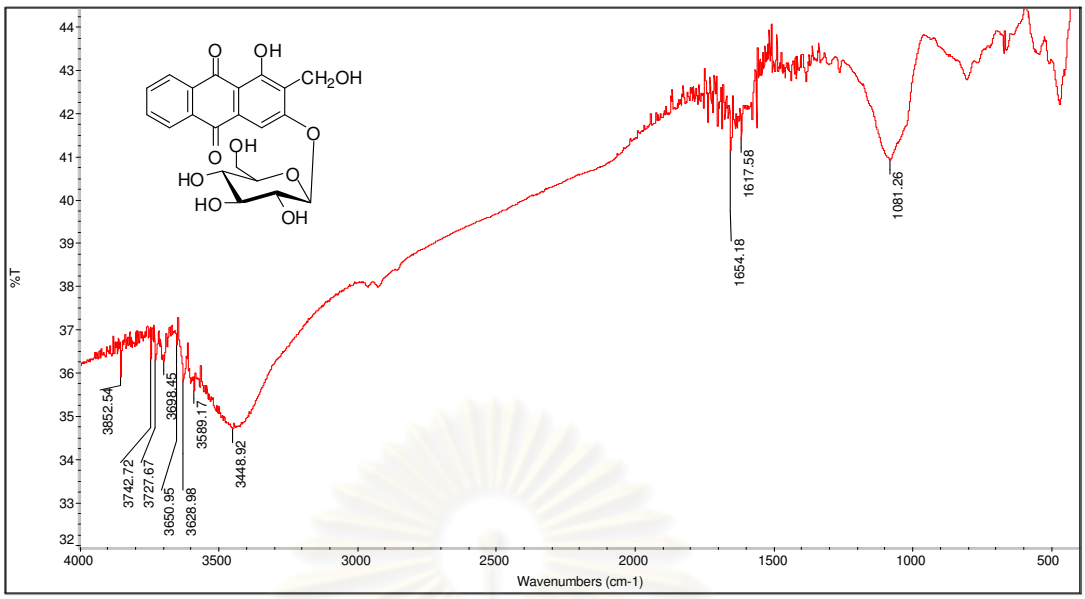


Figure A-69 IR spectrum of 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside or lucidin-3-*O*-β-glucoside (**83**)

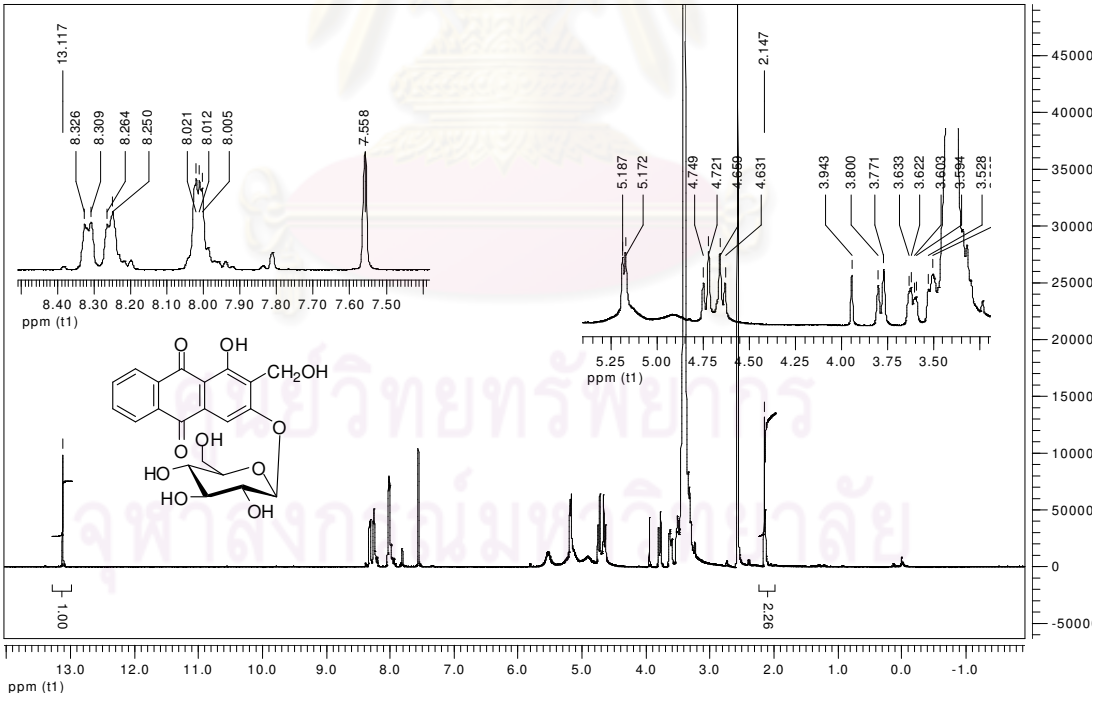


Figure A-70 ^{1}H NMR spectrum (DMSO-d_6) of 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside or lucidin-3-*O*-β-glucoside (**83**)

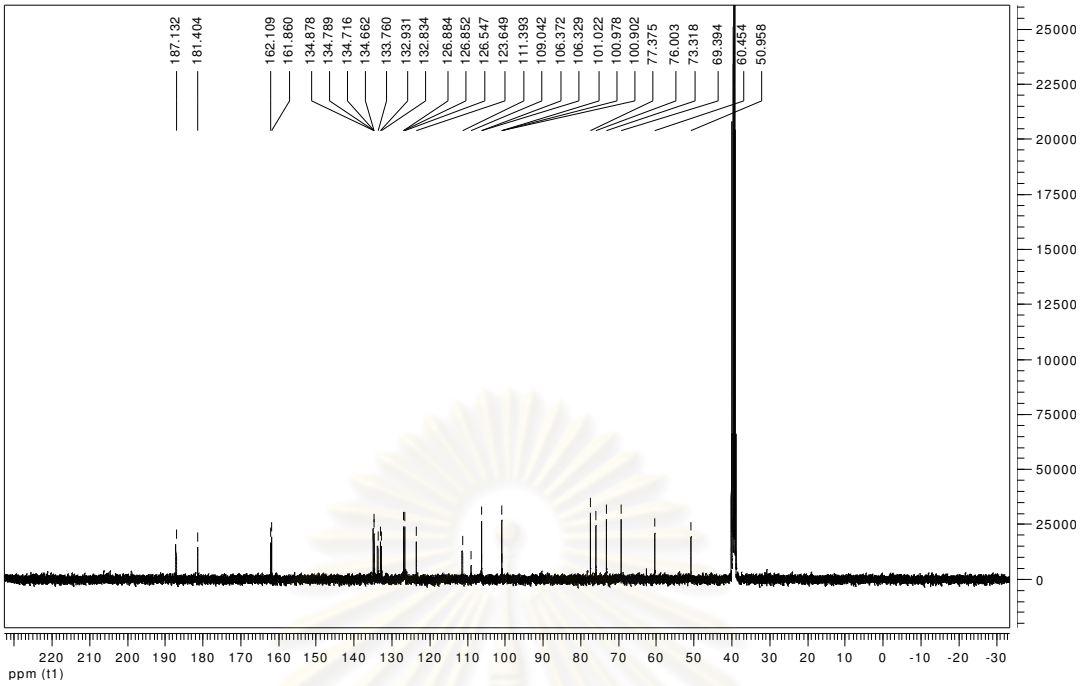


Figure A-71 ^{13}C NMR spectrum (DMSO-d_6)of 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside or lucidin-3-*O*-β-glucoside (**83**)

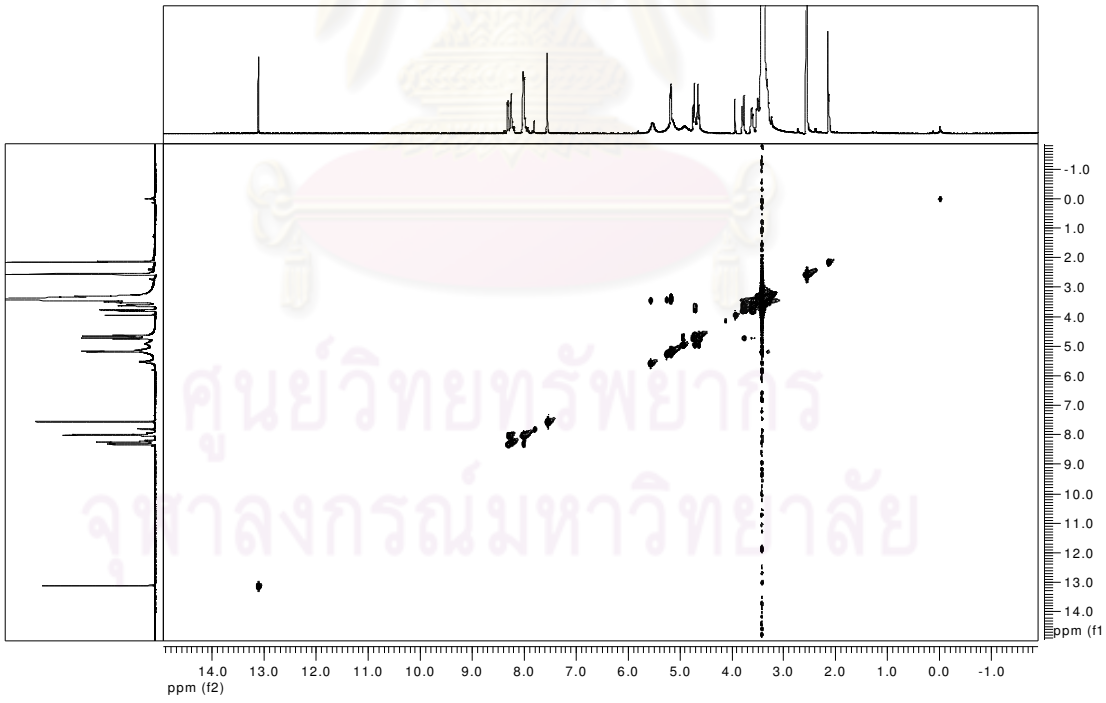


Figure A-72 COSY spectrum (DMSO-d_6)of 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside or lucidin-3-*O*-β-glucoside (**83**)

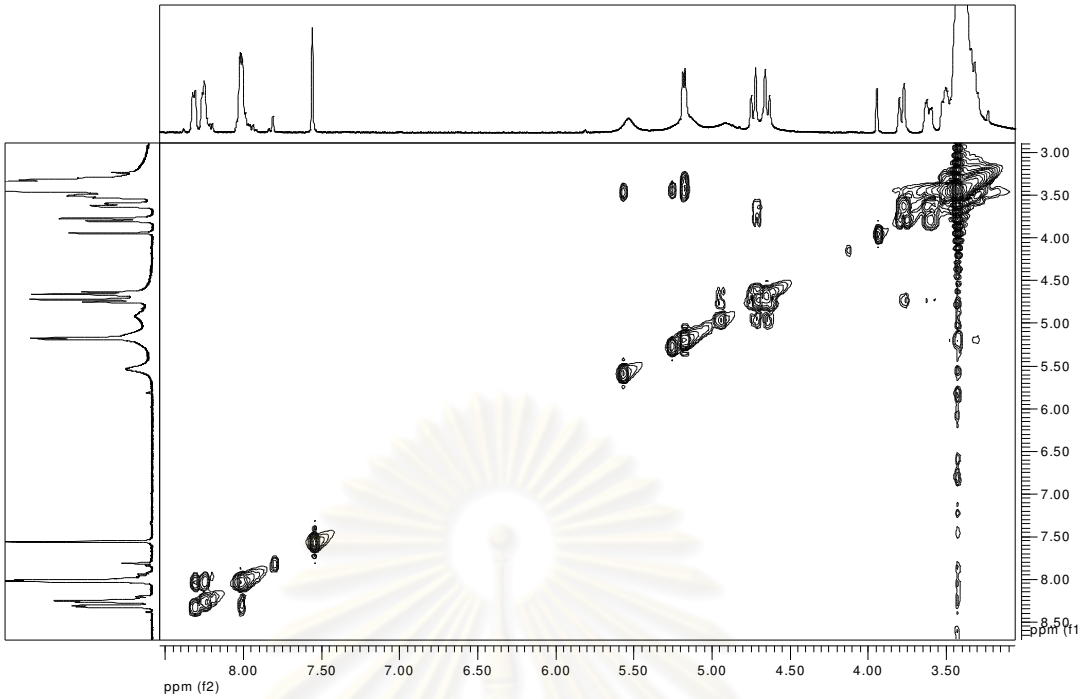


Figure A-73 COSY spectrum (DMSO-d_6)of 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside or lucidin-3-*O*-β-glucoside (**83**)

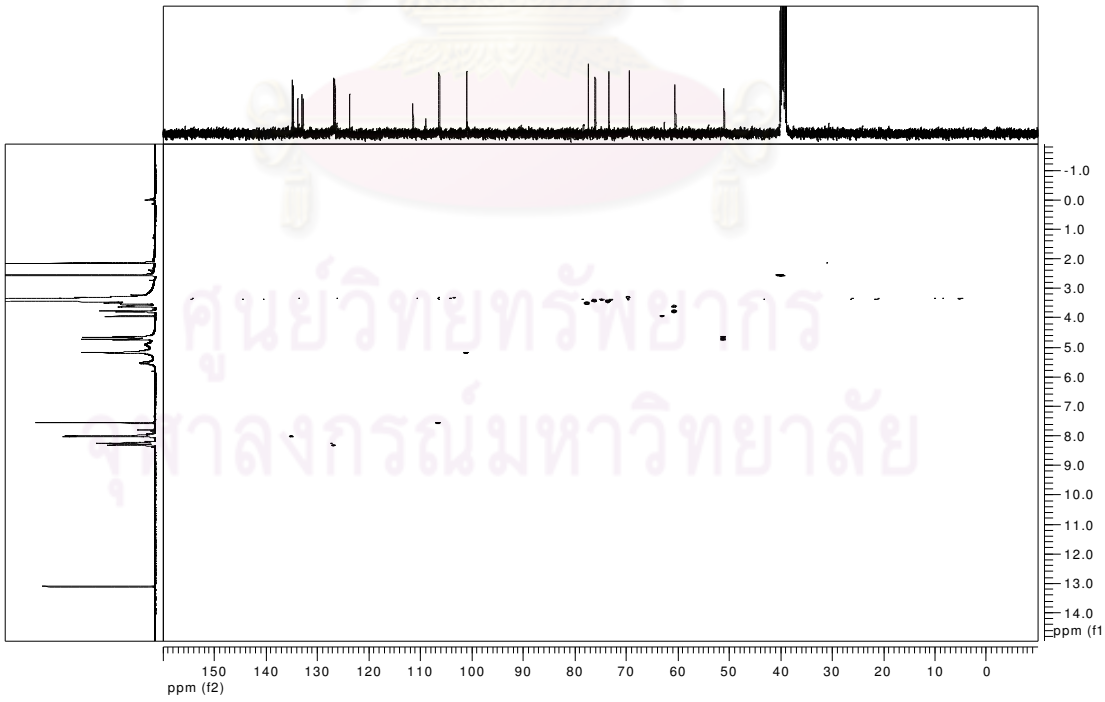


Figure A-74 HSQC spectrum (DMSO-d_6)of 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside or lucidin-3-*O*-β-glucoside (**83**)

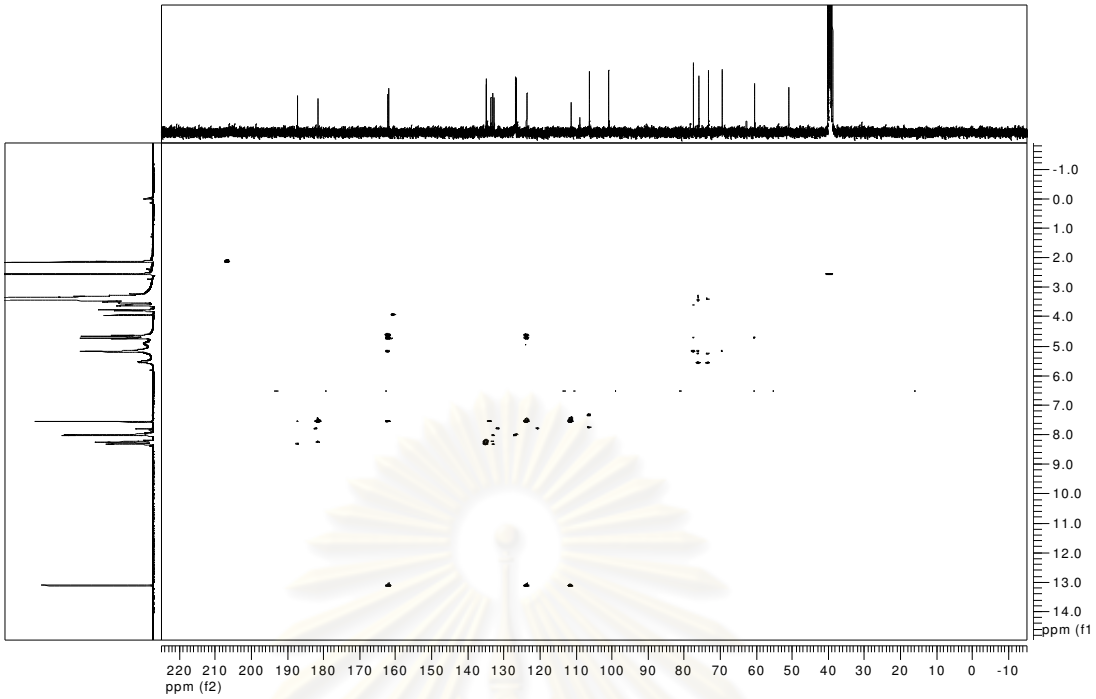


Figure A-75 HMBC spectrum (DMSO-d_6)of 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside or lucidin-3-*O*-β-glucoside (**83**)

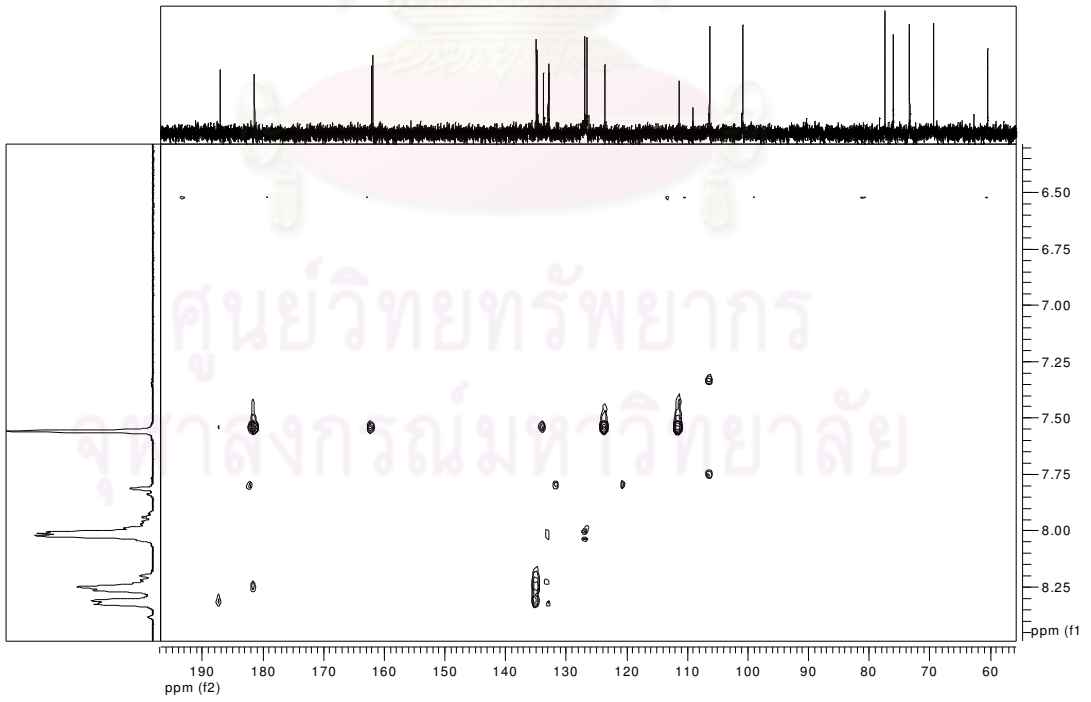


Figure A-76 HMBC spectrum (DMSO-d_6)of 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside or lucidin-3-*O*-β-glucoside (**83**)

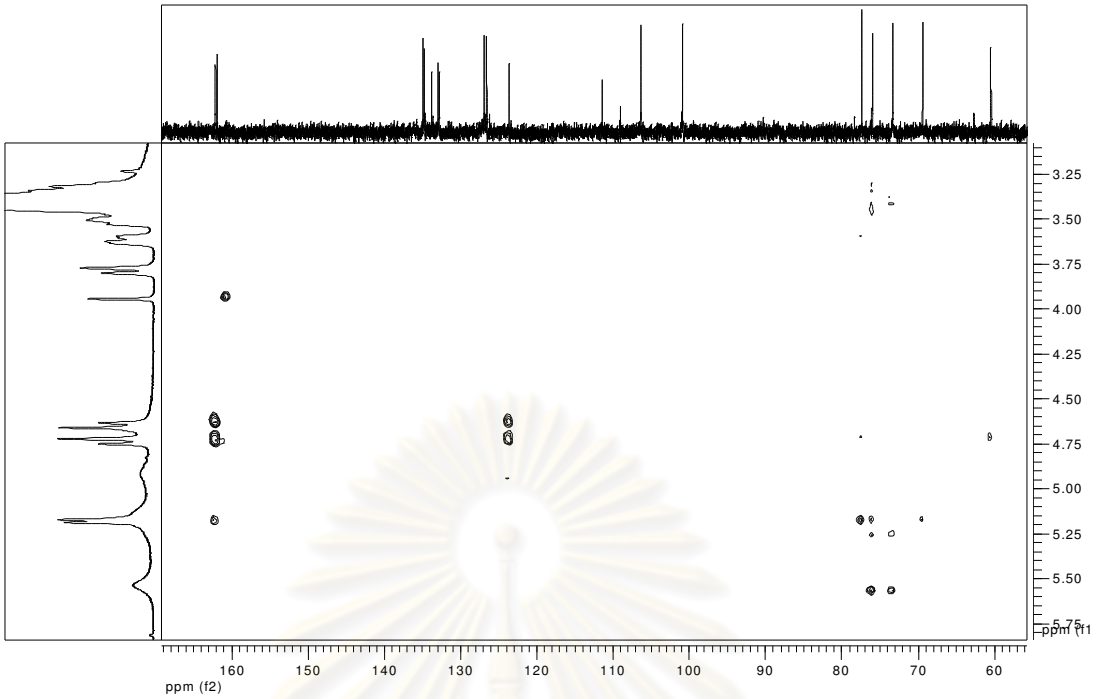


Figure A-77 HMBC spectrum (DMSO-d_6)of 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside or lucidin-3-*O*-β-glucoside (**83**)

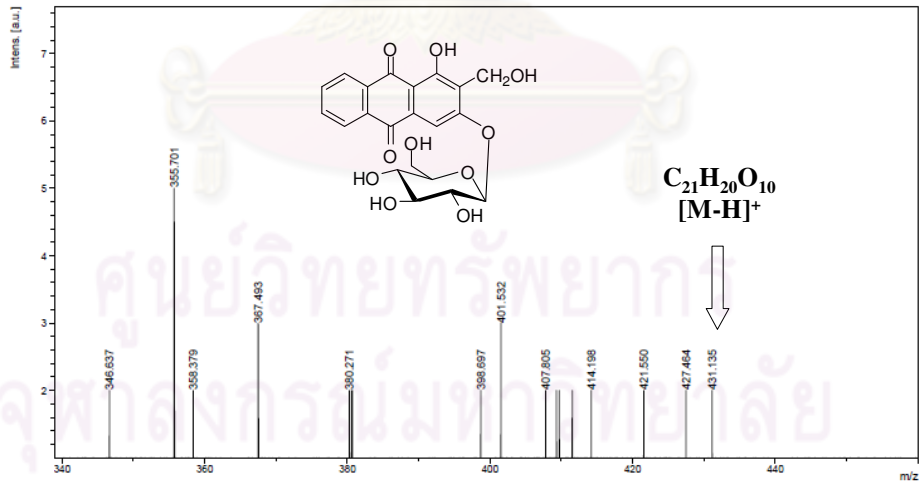


Figure A-78 Mass spectrum of 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranoside or lucidin-3-*O*-β-glucoside (**83**)

VITA

Mr. Thanatip Ruksilp was born on May 2, 1962 in Udonthani, Thailand. He graduated with Bachelor Degree of Science in Chemistry from Faculty of Science, Khon Kaen University, in 1985 and graduated with Master Degree of Science in Chemistry from Faculty of Science, Chulalongkorn University, in 1995. During his studies towards the Doctoral's degree in Department of Chemistry, he received a financial support for the Doctoral program from Loei Rajabhat University and a research grant for his dissertation from Graduate School, Chulalongkorn University.

His present address is 234 Loei-Chaingkhan road, Amphoe Muang, Loei, Thailand, 42000, Tel : 0895638226.