องค์ประกอบทางเคมีของลำต้นข้าวหลามดง *Goniothalamus laoticus*

นางสาวยวิษฐา ลิ้มพิพัฒน์วัฒนา

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CHEMICAL CONSTITUENTS FROM THE STEMS OF *Goniothalamus laoticus*

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ยวิษฐา ลิ้มพิพัฒน์วัฒนา : องค์ประกอบทางเคมีของลำต้นข้าวหลามดง *Goniothalamus laoticus*. (CHEMICAL CONSTITUENTS FROM THE STEMS OF *Goniothalamus laoticus*) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ดร. สันติ ทิพยางค์, 46 หน้า.

ในการศึกษาสารออกฤทธิ์ยับยั้งเซลล์มะเร็งจากพืชสมุนไพรไทย จากการทดสอบเบื้องต้นพบว่าสิ่งสกัดของลำต้นข้าวหลามดง (*Goniothalamus laoticus*) ให้ฤทธิ์ที่ดี จึงเลือกสิ่งสกัดไดคลอโรมีเทน และสิ่งสกัดเมทานอลมาแยกและทำให้บริสุทธิ์ โดยวิธีทางโครมาโทกราฟี สามารถแยกสารจากสิ่งสกัดทั้งสองได้สารใหม่ 1 ชนิด เป็นสารอัลคาลอยด์ คือ laoticuzanone A (**9**) และสาร styryl-lactone ที่เคยมีรายงานจากการสังเคราะห์ คือ (-)-goniofufurone (**5**) ซึ่งเป็นสารใหม่ในธรรมชาติ พร้อมกับสารที่เคยมีรายงานมาก่อน 11 ชนิด ได้แก่ pinocembrin (**1**), altholactone (**2**), goniopypyrone (**3**), 5-hydroxy-3-amino-2-aceto-1,4-naphthoquinone (**4**), 2-*epi*-altholactone (**6**), 3-methyl-1*H*-1-azaanthracene-2,9,10-trione (**7**), griffithazanone A (**8**), methyl sinapate (**10**), 3-(4′-hydroxyphenyl)-(*E*) propenoic acid methyl ester (**11**), 2-(4-hydroxyphenyl) ethyl (E)-3-(4-hydroxyphenyl) prop-2-enoate (**12**) และ (+)goniofufurone (**13**) พิสูจน์ทราบสูตรโครงสร้างของสารทั้งหมดที่แยกได้ด้วยวิธีทางสเปกโทรสโกปี การวิเคราะห์ด้วยวิธีเอ็กซเรย์ผลึกเดี่ยว รวมทั้งเปรียบเทียบกับข้อมูลที่เคยมีรายงานมาแล้ว นำสารทั้งหมดที่แยกได้ไปทดสอบฤทธิ์ความเป็นพิษต่อเซลล์มะเร็งชนิด KB และ HeLa พบว่าสาร **1**, **9**, **4** และ **2** แสดงความเป็นพิษต่อเซลล์มะเร็งชนิด KB ที่ IC_{50} = 0.55, 0.68, 1.70 และ 2.40 μg/ml ในขณะเดียวกันสาร **9** แสดงความเป็นพิษต่อเซลล์มะเร็งชนิด HeLa สูงสุดที่ IC_{50} = 0.50 μg/ml และสาร **4**, **1**, **8**, **2** และ **7** มีความเป็นพิษต่อเซลล์มะเร็งชนิด HeLa สูงที่ IC_{50} = 1.60, 3.00, 3.00, 3.10 และ 4.00 μg/ml ตามลำดับ

ภาควิชา.....เคมี.....ลายมือชื่อนิสิต.....ยวิษฐา ลิ้มพิพัฒน์วัฒนา.....

สาขาวิชา.....เคมี.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....สันติ ทิพยางค์.....

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YAWISTHA LIMPIPATWATTANA: CHEMICAL CONSTITUENTS FROM THE STEMS OF *Goniothalamus laoticus*. THESIS PRINCIPAL ADVISOR: ASSOC. PROF. SANTI TIP-PYANG, Ph.D., 46 pp.

In phytochemical investigation for antitumor compounds from Thai medicinal plants, the extracts of *Goniothalamus laoticus* stems were found to have a promising activity on KB and HeLa cell lines in preliminary evaluation. The dichloromethane and methanolic extracts were selected for further isolation, purification and structure elucidation. The chromatographic separation of these crude extracts led to the isolation of one new alkaloid, laoticuzanone A (**9**) and a synthetically known styryl-lactone (-)-goniofufurone (**5**), new natural compound, along with eleven known compounds, pinocembrin (**1**), altholactone (**2**), goniopypyrone (**3**), 5-hydroxy-3-amino-2-aceto-1,4-naphthoquinone (**4**), 2-*epi*-altholactone (**6**), 3-methyl-1*H*-1-azaanthracene-2,9,10-trione (**7**), griffithazanone A (**8**), methyl sinapate (**10**), 3-(4′-hydroxyphenyl)-(*E*) propenoic acid methyl ester (**11**), 2-(4-hydroxyphenyl) ethyl (E)-3-(4-hydroxyphenyl) prop-2-enoate (**12**) and (+)-goniofufurone (**13**). The structures of all isolated compounds were characterized by spectroscopic method, Single-Crystal X-ray analysis as well as comparison with the previous literature data. All of isolated compounds were evaluated for cytotoxicity against KB and HeLa cells. Compound **1**, **9**, **4** and **2** showed the significantly cytotoxicity against KB cell with IC_{50} values 0.55, 0.68, 1.70 and 2.40 μg/ml, respectively. On the other hand, compound **9** also showed the highest cytotoxicity against Hela cell with IC_{50} value 0.50 μg/ml, followed by compound **4**, **1**, **8, 2** and **7** with IC_{50} values 0.50, 1.60, 3.00, 3.00, 3.10 and 4.00 μg/ml.

Department:.....Chemistry.....Student's signature.....Yawistha Limpipatwattana.....

Field of study:.....Chemistry.....Principal Advisor's signature.....Santi Tip-pyang

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CONTENTS

List of Tables

List of Figures

List of Schemes

List of Abbreviations

^{13}C NMR	carbon 13 nuclear magnetic resonance
^{1}H NMR	proton nuclear magnetic resonance
brs	broad singlet (NMR)
c	concentration
COSY	correlated spectroscopy
d	doublet (NMR)
dd	doublet of doublet (NMR)
ESIMS	electrospray ionization mass spectrometry
g	gram (s)
HMBC	heteronuclear multiple bond correlation experiment
HRESIMS	high resolution electrospray ionization mass spectrometry
HSQC	heteronuclear single quantum correlation
Hz	hertz
IC_{50}	concentration that is required for 50% inhibition in vitro
J	coupling constant
m	multiplet (NMR)
M	molar
MeOH	methanol
mg	milligram (s)
MHz	megahertz
min	minute
mL	milliliter (s)
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser enhancement spectroscopy
s	singlet (NMR)
t	triplet (NMR)
UV	ultraviolet
VLC	vacuum liquid chromatography
δ	chemical shift

δ_C	chemical shift of carbon
δ_H	chemical shift of proton
ε	molar extinction coefficient
λ_{max}	maximum wavelength
2D NMR	two dimentional nuclear magnetic resonance
$[\alpha]_D^{26}$	specific optical rotation

CHAPTER I
INTRODUCTION

It has generally been considered that tumor microenvironment influences the functional potential of immune cells by secreting immunosuppressive factors to modify the host immune responses. A number of studies raised the possibility that tumors of both mouse and human origin can evade immune surveillance by delivering apoptotic death signals to lymphocytes. Therefore, an ideal antitumor agent should possess protective effect against tumor-induced reduction of lymphocytes, and at the same time, inhibits tumor cell proliferation. Current therapies for certain malicious tumors usually take surgical removal or drug medications. However, surgical removal of certain tumor, for instance, breast carcinoma, colon carcinoma and osteogenic sarcoma, is frequently followed by rapid growth of distant metastases to the lung, liver and other organs of the host. Consequently, seeking antitumor compounds from medicinal plants are of interest in the treatment of cancer.

Medicinal plants contain various bioactive secondary metabolites. They have especially pharmacological active principle which can be used as therapeutic drugs or herbal medicine. Therefore, medicinal plants still serve as sources for scientists to be developed into new lead and more active compounds.

Medicinal plants are a vital source of medication in developing countries. Despite the wealth of human experience and folklore concerning the medicinal uses of plants, proper scientific investigation has only been applied to a small fraction of the world's plants.

Ethnobotanical uses of several species of the genus *Goniothalamus* are well known in Thailand; many of these plants have provided bioactive acetogenins[1,2], alkaloids[3-5], styryl-lactones[6-10], flavonoids[11], azaanthraquinones[12] and naphthoquinones[12]. Several compounds isolated from the plant in this genus showed cytotoxic activity against a number of human cancer cell lines. *Goniothalamus laoticus* (Finet&Gagnep) Bân or locally known as "Khao lam dong", a rare ornamental plant distributed mainly in Northeastern of Thailand, was chosen as the subject of the present investigation due to the significant cytotoxicity observed in the crude extracts against KB and HeLa cells in a preliminary biological screening procedure. It has been used as traditional medicine and there are no reports on phytochemical of this plant.

1.1 Styryl-lactones: distribution and their biosynthesis pathways

Cytotoxic styryl-lactones and their derivatives, which have been reported in almost all the *Goniothalamus* species studied, are characteristic compounds of this genus.

1.1.1 Styryl-lactones biosynthetic pathways

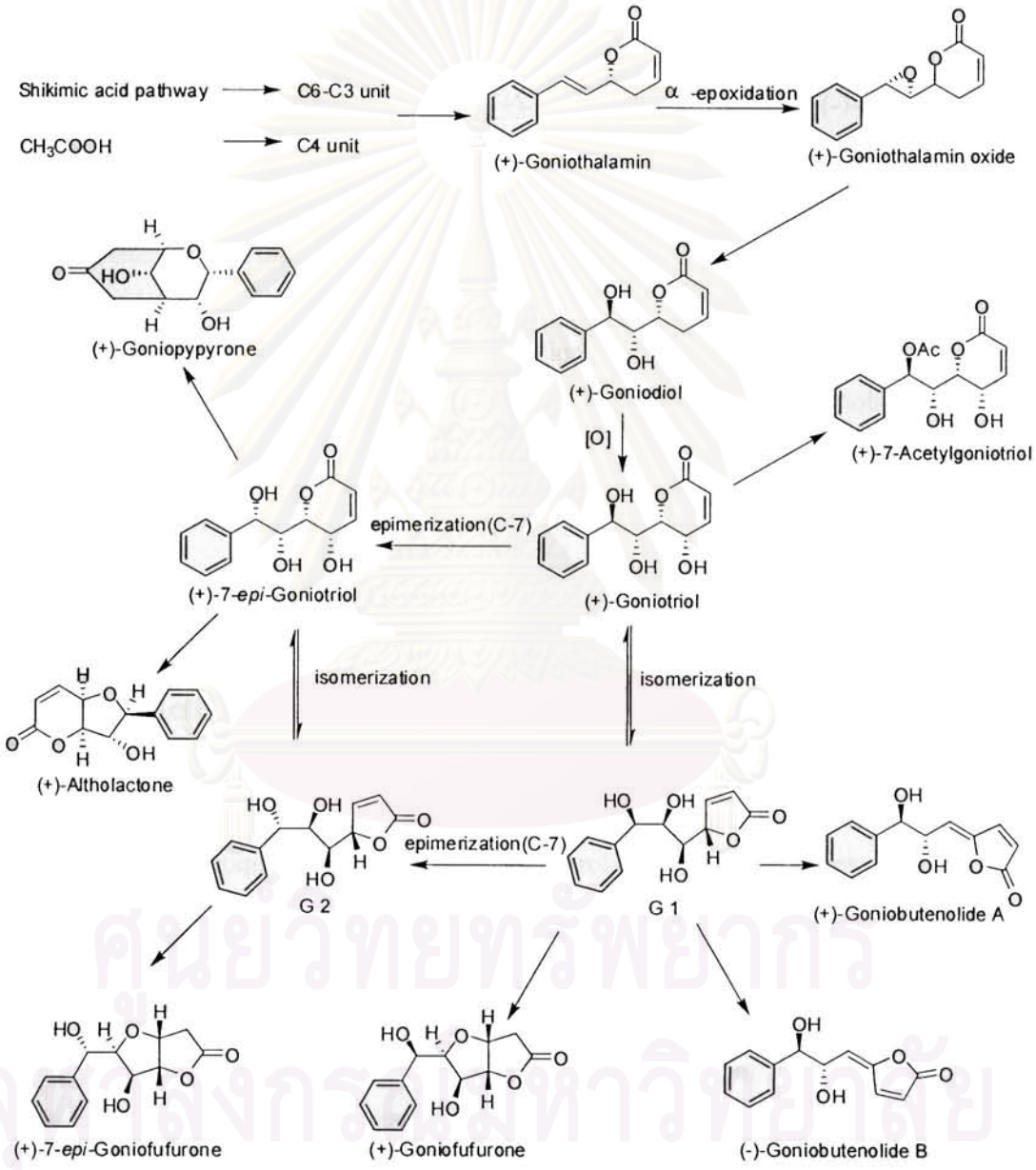


Figure 1.1 Biosynthetic pathways of styryl-lactones.

The biosynthesis of the styryl-lactones was predicted to be of mixed origin **(Figure 1.1)**. The C_6-C_3 unit comes from the shikimic acid pathway, and the C_4 unit comes from two *acetyl-Coenzyme* A. Coupling of the two units followed by lactonization gives the (+)-goniothalamin as the key intermediate. α-Epoxidation of the double bond in (+)-goniothalamin give the (+)-goniothalamin oxide. Trans-opening of the epoxide at the benzylic carbon in (+)-goniothalamin oxide gives (+)-goniodiol. Allylic hydroxylation of (+)-goniodiol gives (+)-goniotriol. Esterification at the benzylic hydroxyl group gives (+)-7-acetylgoniotriol. The rearrangement of (+)-goniotriol to G1 under basic conditions found that the pyrone intermediates are the biosynthetic precursors for γ-lactones; (+)-goniofufurone, (+)-7-*epi*-goniofufurone, (+)-goniobutenolide A and (-)-goniobutenolide B. Thus, (+)-goniofufurone may derived from the rearrangement of (+)-goniotriol to butenolide G1, followed by an intramolecular Michael-type ring closure. Some styrylpyrones have the opposite stereochemistry at the benzylic carbon and this stereochemistry is expected to derive from epimerization. Thus, (+)-goniopypyrone was produced from (+)-goniotriol via epimerization at the benzylic carbon to (+)-7-*epi*-goniotriol, followed by an intramolecular Michael addition. (+)-Altholactone can be regarded as the anhydro analog of the (+)-7-*epi*-goniotriol and can be obtained via an intramolecular ring closure of (+)-7-*epi*-goniotriol with inversion at the benzylic carbon[13].

1.2 Chemical constituents from *Goniothalamus* species and their biological activities.

The chemical constituents of plants in the genus *Goniothalamus* can be classified into eleven groups namely acetogenins, alkaloids, aza-anthraquinones, benzenoids, flavoniods, naphthoquinones, styryl-lactones, sterols, styrene derivatives, terpenoids and miscellaneous compounds.

1.2.1 Styryl-lactones

Plant styryl-lactones and their derivatives isolated from *Goniothalamus* species were potential compounds for cancer chemotherapy. Styryl-lactones are low molecular weight phenolic compounds, which are essentially found in members the Annonaceae family and present a lactonic pharmacophore. The evidence currently available clearly indicate that styryl-lactones and their derivatives were toxic for several sorts of cancer

cells cultured in *vitro* including HL-60 leukemia cells, breast cancer cell line MCF-7, liver cancer cell line HepG2, PANC-1, and HeLa cell lines[14]. The cytotoxic styryl-lactones: altholactone (syn: goniothalenol), goniothalamin, goniotriol, goniopypyrone, goniofufurone, 8-acetylgoniotriol, 9-deoxygoniopypyrone, 7-*epi*-goniofufurone, goniodiol, goniobutanolides A and B and goniofupyrone have been isolated from the extracts of the *Goniothalamus giganteus* Hook. f. & Thomas[10].

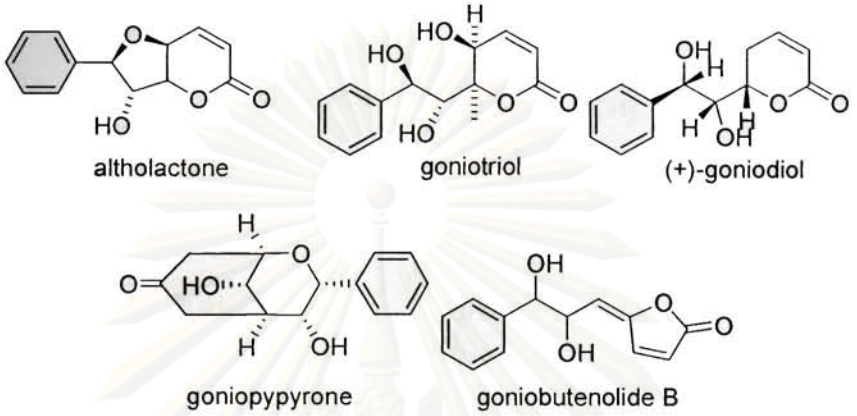


Figure 1.2 Styryl-lactones from *Goniothalamus giganteus*.

1.2.2 Acetogenins

The Annonaceous acetogenins are a class of promising anticancer, antiinfective and pesticidal natural compounds have been found in various plant species of the family Annonaceae. Since 1982, more than 250 acetogenins have been discovered. Most of previous known acetogenins belong to several classical types usually containing an unsubstitued tetrahydrofuran (THF) ring[15-18]. A previous investigation of the EtOH extract of the roots of *Goniothalamus donnaiensis* Finet et Gagnep has resulted in the isolation of several compounds: (+)-annonacin, *cis*-goniodonin and 34-*epi*-cis-goniodonin, donbutocin, donhepocin and 34-*epi*-donhepocin, donaienin, donhepocin A and 34-*epi*-donhepocin A, donhepocin B and 34-*epi*-donhepocin B, donhepocin C and34-*epi*-donhepocin C, donhepocin D and 34-*epi*-donhepocin D, donhexocin, goniodonin and 34-*epi*-goniodonin, goniothalamicin, isoannonacin and murisolin. The mixture of epimers of donnaienin A and 34-epi-donnaienin A showed potent cytotoxicity against KB (human nasopharyngeal carcinoma) (IC_{50} < 1μg/ml) and HCT-8 (human colon adenocarcinoma) cells (IC_{50} < 10μg/ml). The mixture of epimers of donnaienin B and 34-epi-donnaienin B also showed cytotoxic to KB cells (53% inhibition, 10 μg/ml)[19,20].

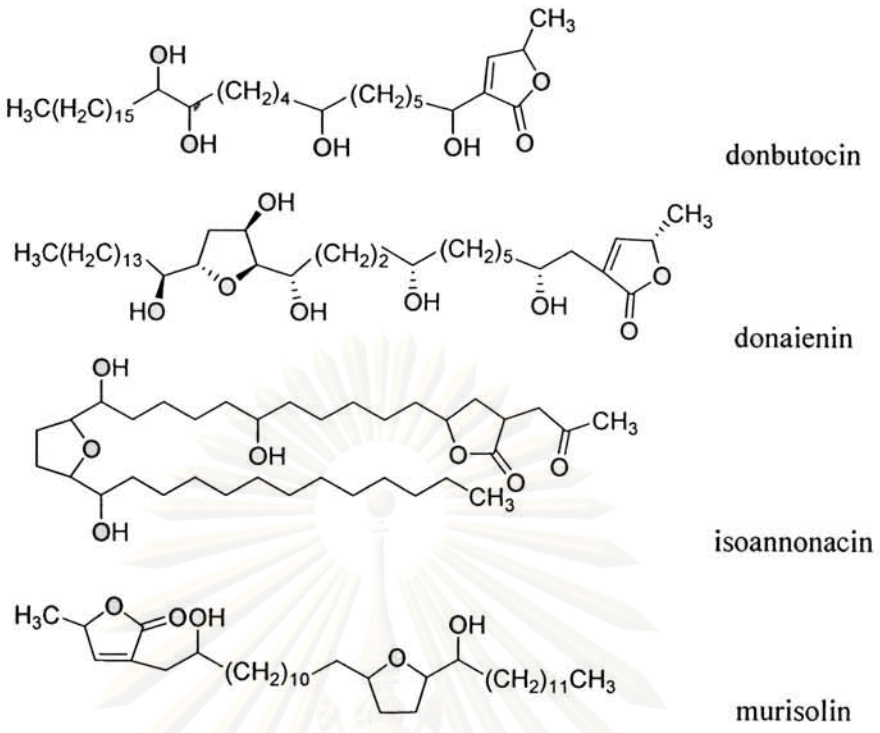


Figure 1.3 Acetogenins from *Goniothelamus donnaiensis*.

1.2.3 Alkaloids

Many of earliest isolated pure compounds with biological activity were alkaloids. An alkaloid is a plant-derived compound that is toxic or physiologically active, contains a nitrogen in a heterocyclic ring, is basic, has a complex structure, and is of limited distribution in the plant kingdom.

The alkaloids are produced by many *Goniothalamus* species, being part of the Annonaceae family, some of which are known to be biologically active[21]. Azaanthraquinone alkaloids are also found in a few members of the Annonaceae. The first 1-azaanthraquinone compounds was found in mycelium of *Pyrenocheaeta terreatris*, the fungus responsible for "pink root disease" of onions. In higher plants, the 1-azaanthraquinones were reported only from Annonaceae plants, such as cleistopholine from *Cleistopholis patens*[22], *Annona hayesii*[23] and *Meiogyne virgata*[24] and scorazanone from *Goniothalamus scortechinii*[25].

Several 1-azaanthraquinones have been isolated from the alcoholic extract of *Goniothalamus marcanii* stem bark. They were marcanine A, B, C, D, E and dielsiquinone, along with 5-hydroxy-3-amino-2-aceto-1,4-naphthoquinone. These compounds were evaluated for their cytotoxicity against a panel of human tumor cell

lines. All 1-azaanthraquinonea were comparably cytotoxic as adriamycin. Marcanine A, C and D showed cytotoxicity in all cell lines (A-549, HT-29, MCF7, RPMI and U251) with the ED_{50} in the range of 0.18 to 2.12 μM, while marcanine B and E were more active than the other macanines in A-549, MCF7 and RPMI cells, with the ED_{50} in the range of 0.04 to 0.11 μM. The 5-hydroxy-3-amino-2-aceto-1,4-naphthoquinone was less cytotoxic than the 1-azaanthraquinones[12].

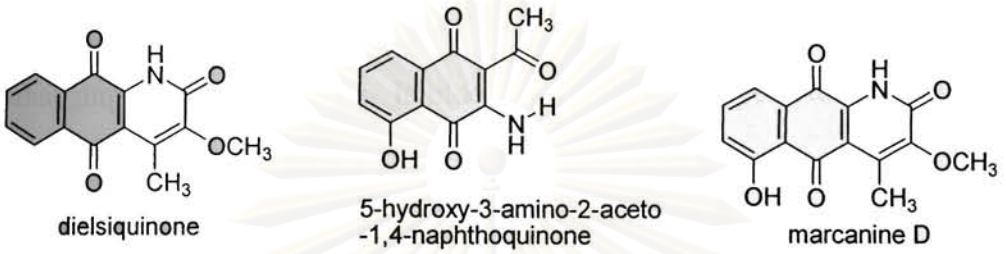


Figure 1.4 1-Azaanthraquinones and naphthoquinone from *Goniothalamus marcanii*.

1.3 Botanical aspect and distribution

Annonaceae family, also called custard apple family, is a family of flowering plants consisting of trees, shrubs or rarely woody lianas. *Goniothalamus* is a genus of plant in the Annonaceae family, consisting of 160 species of archaic shrubs and treelets which grow in the shady primary rainforest of tropical Asia. These plants can be quickly spotted in field collection by their aromatic bark and fusiform leathery flowers. A number of *Goniothalamus* species have been used for timber, as fiber sources, for ornamental and medicinal purposes. In Thailand, twenty-five species are recognized[26], for example:

G. cheliensis Hu: Pa nan yak (ปาหนันยักษ์)

G. giganteus Hook.f.&Thomson: Pa nan chang (ปาหนันช้าง)

G. laoticus (Finet&Gagnep) Bân: Khao lam dong (ข้าวหลามดง)

G. tapis Miq: Bu ngaa lam chiak (บุหงาลำเจียก)

G. tamirensis Pierre ex Finet & Gagnep (*G. marcanii* Craib): Khao lam (ข้าวหลาม)

G. undulatus Ridl.: Saa lao ton (สาเหล้าต้น)

Goniothalamus laoticus (Finet&Gagnep) Bân was originally described as a species of *Mitrephora* (Finet&Gagnepin, 1907) and, although Sinclair (1953a) suggested that its true affinities lay with *Goniothalamus*, it was not until 1974 that the new nomenclatural combination was validated (Bân, 1974). *G. laoticus* is the rarest of its kind. Its height reaches 60 ft, and has large yellow 3-5" flowers. Flowering material of *G. laoticus*, easily recognized as the outer petals, are pale yellow (rarely pale yellow-orange) with inwardly curved margins at maturity. It is very decorative, with beautiful flowers overhanging from its branches. It blooms practically all year round, but the peak of flowering is from May-July, with fruits ripening in October.

Leaves of *G. laoticus*

Bark of *G. laoticus*

Flowers of *G. laoticus*

Fruits of *G. laoticus*

Figure 1.5 Bark, flowers, fruits and leaves of *G. laoticus*

Fruiting material is equally easily determined because of the shape of the fruit, with its longitudinal ridge, although a similar ridge is observed in some other species. On the other hand, *G. laoticus* differs from the other species in having reticulate leaf venation, shorter pedicels (5-11.5 vs 10-22 in the other species collectively), and generally larger sepals (4-14.5x6-14 mm vs 5-7.5x5-10 mm) that are not reflexed and are not basally connate.

G. laoticus is distributed in Eastern, North-Eastern and Northern of Thailand (Chiang Mai, Chiang Rai, Lampang, Loei, Mae Hong Son, Mukdahan, Nakhon Ratchasima, Nan, Nong Khai, Phayao and Phetchabun provinces) and Laos [14].

1.4 Biological activities

1.4.1 Cytotoxic activity against KB and HeLa cell lines.

Cytotoxicity is the quality of being toxic to cells. Examples of toxic agents are a chemical substance or an immune cell. Cytotoxicity can be measured by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, Trypan blue (TB) assay, Sulforhodamine B (SRB) assay, 4-[3-(4-Iodophenyl)-2-(4-notrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST) assay and clonogenic assay. Test of cytotoxicity can be done in vitro or in vivo. In vitro tests are done in cells. The agent is treated with the cells in various ratio, and the effect on morphology and cell viability is studied. The Lethal dose (LD50) can also be calculated. Cytotoxicity is also a subject of heavy pharmaceutical study, particularly in the area of cancer research. Low cytotoxicity to healthy cells and high cytotoxicity to cancerous cells is the ultimate goal of many chemotherapeutic drugs. The common mechanism underlying the cytotoxicity of most antitumor agents is cell cycle arrest. Antitumor agents primarily target neoplastic cells at the surface of the cancer tumor and smaller tumors with short mass-doubling time. Several antitumor agents work by inhibiting DNA replication and terminating cell division at S phase[27]. The development of resistance by tumor cells to chemotherapeutic agents is a major problem in cancer treatments. One way to counter this is to find compounds with cytotoxic mechanisms other than those of drugs in clinical use today. The biological and chemical diversity encountered in Nature provide opportunities to discover completely new chemical classes of compounds. Some of these may represent previously unknown anticancer agents, and in some cases, novel, potentially relevant

The biological and chemical diversity encountered in Nature provide opportunities to discover completely new chemical classes of compounds. Some of these may represent previously unknown anticancer agents, and in some cases, novel, potentially relevant cytotoxic mechanisms. The selection of plants for the cytotoxic investigation was designed to cover large parts of the angiosperm system, providing a broad representation of species.

Twenty-two species (13.7%) in the genus *Goniothalamus*, out of 160 species, have so far been phytochemically investigated[14]. Five *Goniothalamus* species are medicinal and have been used in traditional medicinal Asian system, and for a long period of time most of these in connection with abortion, childbirth and fever. In regards to the pharmacological potentials of *Goniothalamus* species, there is a massive body of evidence to suggest that this taxon has the ability to elaborate series of compounds which are cytotoxic against a broad array of cancer cells including breast, colon, kidney and pancreatic carcinoma cells.

From the literature review on the chemical constituents and their biological activities of plants in *Goniothalamus* genus, the attractive results of primary screening test are based on cytotoxic activity against KB and HeLa cells. Moreover, there is no report on chemical constituents and biological activity of *G. laoticus* (Finet&Gagnep) Bân. This plant was selected for further investigation which could be a promising sources for chemotherapeutic agents.

The objective of this research:

The main objectives in this investigation are as follows:

1. To isolate and purify compounds possessing cytotoxic activity aginst KB and HeLa cells from the stems of *G.laoticus*
2. To determine the chemical structure of all isolated compounds
3. To evaluate the cytotoxic activity aginst KB and HeLa cells of all isolated compounds

CHAPTER II
EXPERIMENTAL

2.1 Plant material

The stems of *Goniothalamus laoticus* (Finet & Gagnep) Bân were collected from Sakon Nakhon 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. 84-08) 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 and 100 MHz for ^{13}C nuclei. The chemical shift in δ (ppm) was assigned with reference to the signal from the residual protons in deuterated solvents 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 silica gel 60 Merck cat. No. 7729, 7731, and 7734 were used for quick column chromatography, open column chromatography and radial chromatography, respectively. Thin-layer chromatography (TLC) was performed on precoated Merck silica gel 60 F254 plates (0.25 mm thick layer). ESIMS data were obtained from a mass spectrometer model VG TRIO 2000. High resolution mass spectra were recorded by Micromass LCT and Bruker MICROTOF models. HPLC was conducted on Waters® 600 controller equipped with a Waters® 2996 photodiode array detector (USA). Cosmosil 5C18-ARII column (10 × 250 mm) was used for separation purposes. UV-visible apsorption spectra were recorded on UV-2552PC UV-Vis spectrometer (Shimadzu, Kyoto, Japan), Optical rotations were measured on a Jasco P-1010 polarimeter. Melting points were determined with Fisher-Johns Melting Poing Apparatus. IR data was obtained from a Nicolet 6700 FT-IR spectrometer (Thermo Electron Corporation, Madison, WI, USA) equipped with a mercury-cadmium-telluride (MCT) detector. The X-ray crystallographic data were collected on *SMART* (Siemens, 1996).

2.3 Extraction and purification

Air-dried and powdered stems of *G. laoticus* (5.5 kg) were successively extracted in a soxhlet apparatus with CH_2Cl_2, EtOAc and methanol. The CH_2Cl_2 extract was concentrated under vacuum to yield 186.0 g of crude residue. This material was fractionated by vacuum liquid chromatography (VLC) over silica gel, using hexane, EtOAc and MeOH with increasing polarity. A total of six fractions were collected. VLC fraction 4 was further fractionated by Sephadex LH-20 column chromatography to give four fractions, using CH_2Cl_2/MeOH (8:2) as eluting solvent. Sephadex fraction 3 was subjected to preparative TLC (silica gel, CH_2Cl_2/MeOH (9.5:0.5)) to obtain pinocembrin (**1**, 5.3 mg). Similarly, VLC fraction 5 was fractionated on silica gel CC, using hexane, CH_2Cl_2 and MeOH with increasing polarity, to yield seven fractions. Fraction 5 was further purified by Sephadex LH-20 column chromatography, using a gradient system of CH_2Cl_2/MeOH (8:2 and 9:1), to yield altholactone (**2**, 207.8 mg) and goniopypyrone (**3**, 11.6 mg).

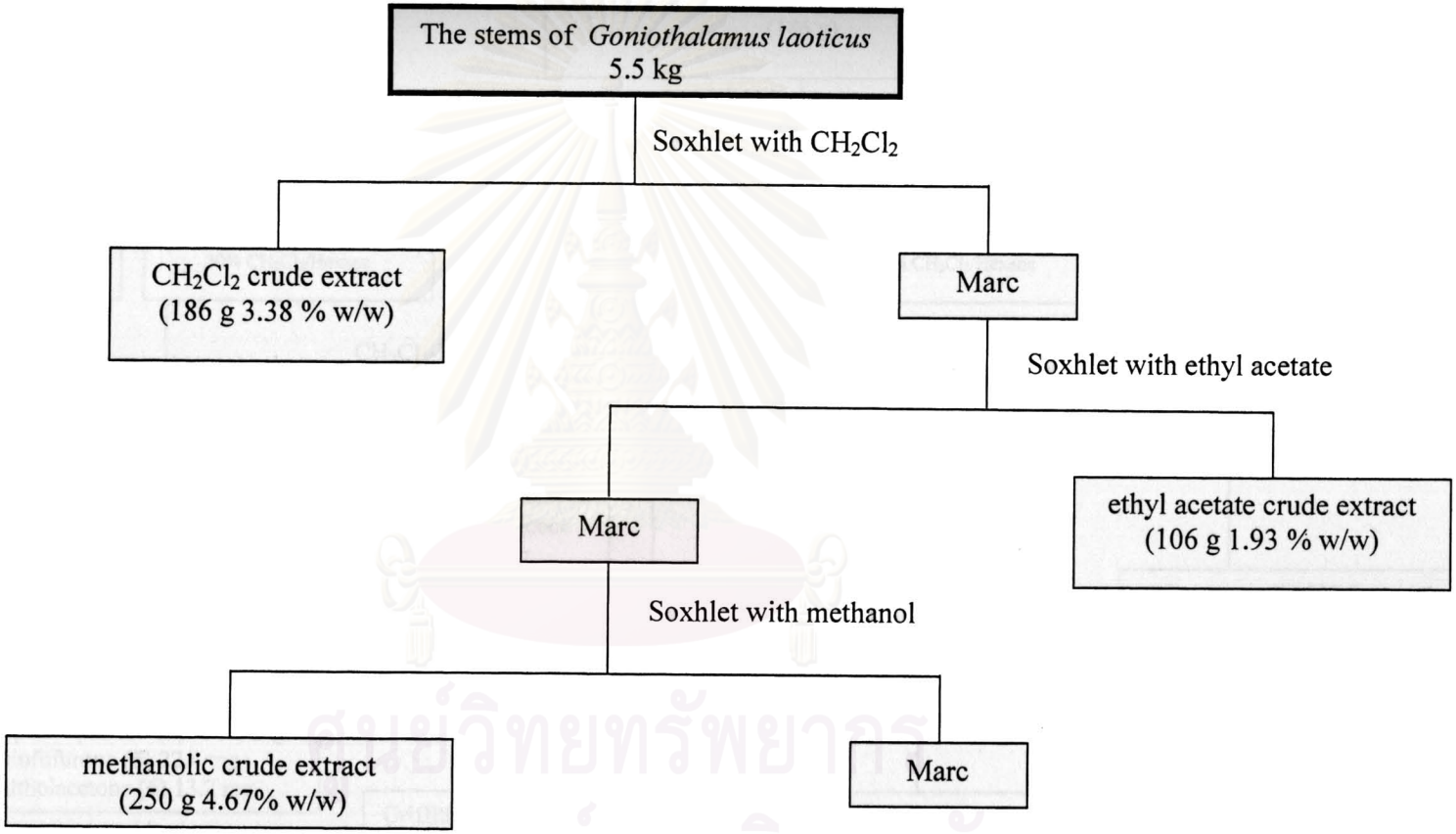
VLC fraction 2 was rechromatographed on silica gel CC, using a gradient system of hexane, CH_2Cl_2 and MeOH as eluents, followed by Sephadex LH-20 column chromatography, using CH_2Cl_2/MeOH (9.5:0.5) as eluting solvent, to yield 5-hydroxy-3-amino-2-aceto-1,4-naphthoquinone (**4**, 113.5 mg), (-)-goniofufurone (**5**, 22.4 mg) and 2-*epi*-altholactone (**6**, 13.2 mg).

Similarly, VLC fraction 3 was subjected to silica gel column eluted with a mixture of CH_2Cl_2/MeOH with increasing polarity to provide two fractions. Fraction 1 was further purified by Sephadex LH-20 column chromatography using 0.5:9.5 MeOH/CH_2Cl_2 as eluting solvent to afford 5.2 mg of 3-methyl-1*H*-1-azaanthracene-2, 9, 10-trione (**7**). Fraction 2 was further purified by Sephadex LH-20 column chromatography, using 9.5:0.5 CH_2Cl_2/MeOH as eluting solvent, followed by HPLC, using ACN/H_2O (8:2) as eluents to yield griffithazanone A (**8**, 3.5 mg) and a new alkaloid, laoticuzanone A (**9**, 4.1 mg).

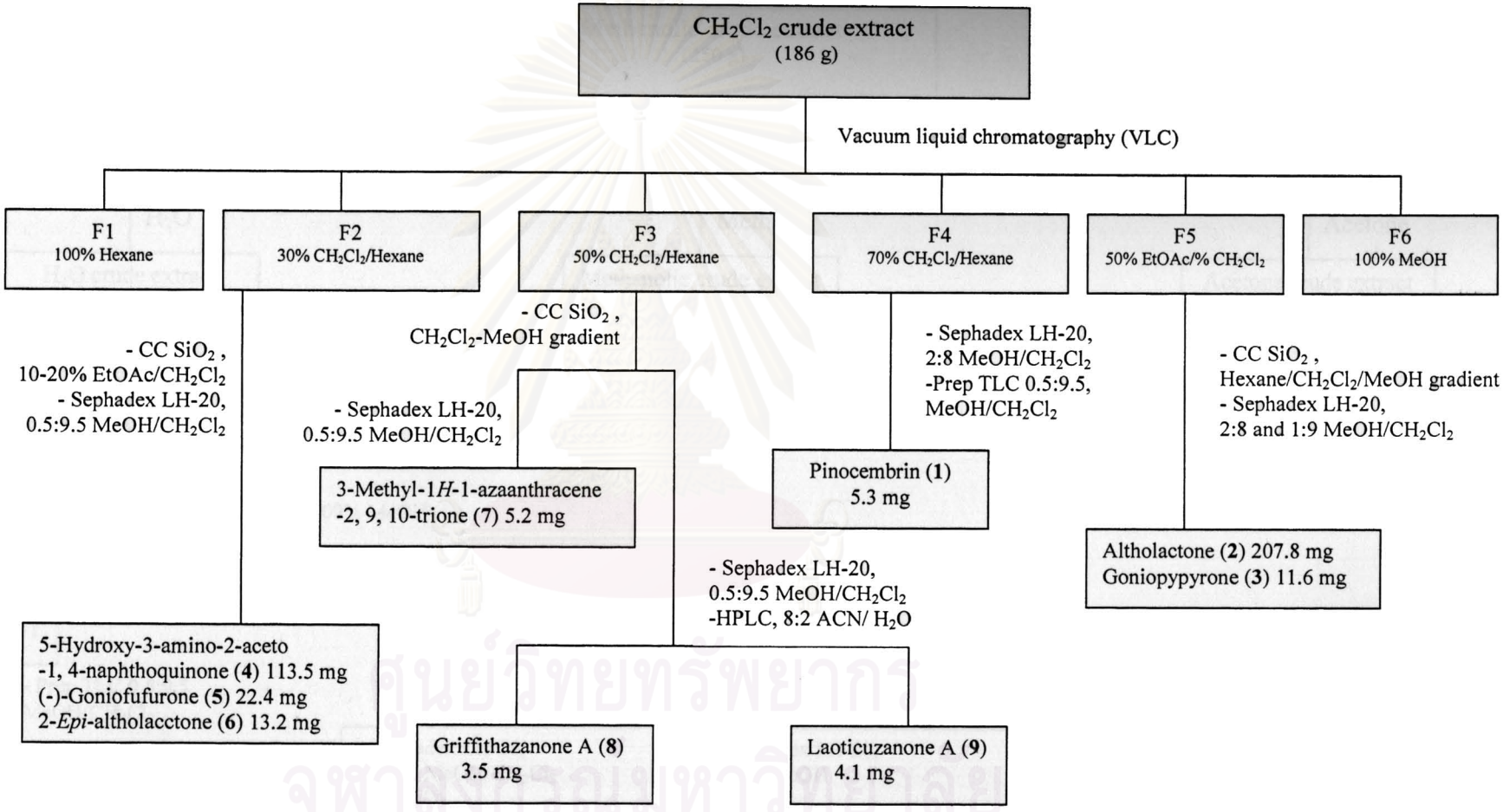
The methanolic extract was concentrated under vacuum to yield 250.0 g of crude residue. This material was fractionated by column chromatography over diaion, using H_2O, MeOH and acetone to give three soluble fractions. The methanolic soluble fraction was subjected to vacuum liquid chromatography (VLC) over silica gel, using hexane, CH_2Cl_2, EtOAc and MeOH with increasing polarity. A total of three fractions were collected. VLC fraction 1 was further fractionated by Sephadex LH-20 column

chromatography, using 100% MeOH as eluting solvent, to give two fractions, Sephadex fraction 1 was further purified by preparative TLC [silica gel, CH_2Cl_2/MeOH (9.5:0.5)] to obtain methyl sinapate (**10**, 6.7 mg). Sephadex fraction 2 was further fractionated over silica gel CC, using CH_2Cl_2 and MeOH with increasing polarity to yield three fractions (S1-S3). Fraction S1 was further purified by Sephadex LH-20 column chromatography, using 9.5:0.5 CH_2Cl_2/MeOH as eluting solvent, followed by silica gel CC, using CH_2Cl_2/MeOH with increasing polarity to afford 3-(4′-hydroxyphenyl)-(*E*) propenoic acid methyl ester (**11**, 7.3 mg). Fraction S2 was further purified by Sephadex LH-20 column chromatography, using 100% MeOH as eluting solvent, followed by silica gel CC, using a gradient system of CH_2Cl_2/MeOH, to yield (+)-goniofufurone (**13**, 12.5 mg). Fraction S3 was crystallized in a mixture of CH_2Cl_2 and MeOH to obtain 2-(4-Hydroxyphenyl) ethyl (*E*)-3-(4-hydroxyphenyl) prop-2-enoate (**12**, 6.2 mg).

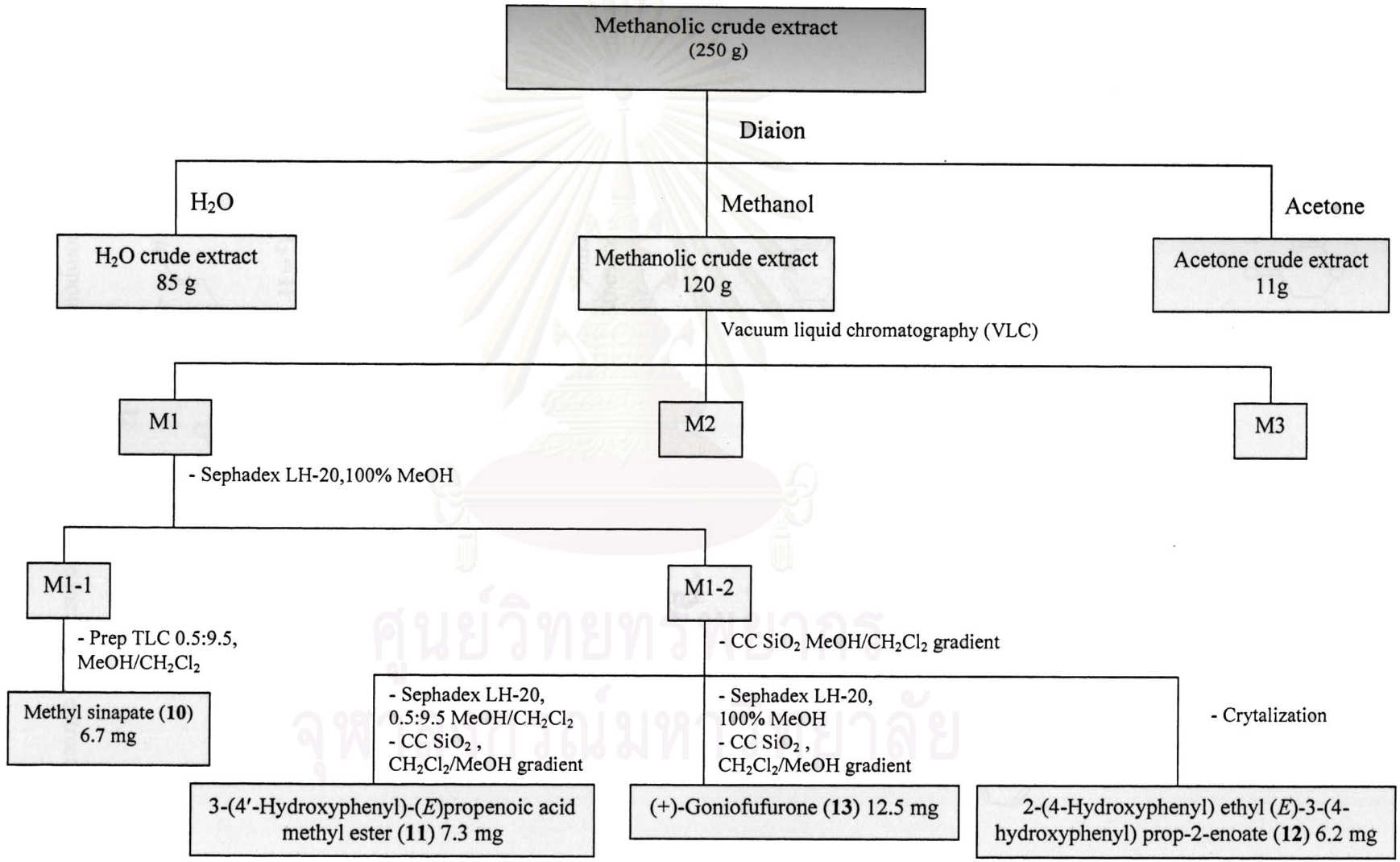
The extraction and purification of all isolated compounds from the dichloromethane and methanolic extracts from the stems of *G. laoticus* were briefly summarized in **Schemes 2.1**, **2.2** and **2.3.**



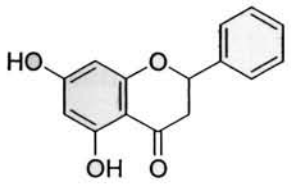
Scheme 2.1 Extraction of *G. laoticus* stems.



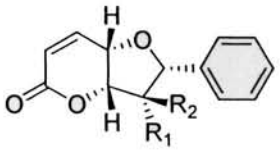
Scheme 2.2 Isolation procedure of the CH_2Cl_2 crude extract.



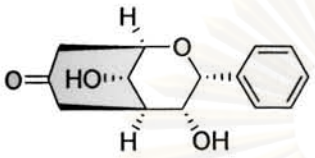
Scheme 2.3 Isolation procedure of the methanolic crude extract



1



2 R_1= H, R_2=OH
6 R_1= OH, R_2=H



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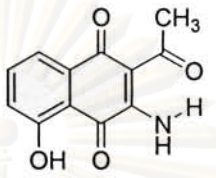

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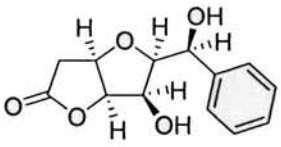

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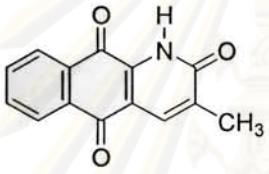

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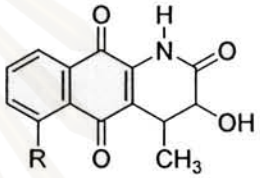
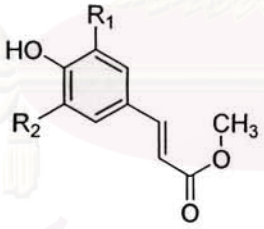
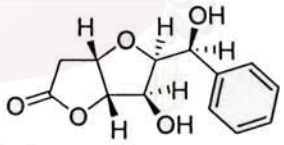


8 R = H
9 R = OH

Figure 2.1 Structure of compounds **1-9** isolated from the CH_2Cl_2 extract of *G. laoticus* stems



10 R_1, R_2 = CH_3
11 R_1, R_2 = H



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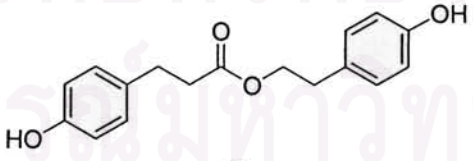

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Figure 2.2 Structure of compounds **10-13** isolated from the methanolic extract of *G. laoticus* stems

2.4 Bioassay procedure

2.4.1 The cytotoxic activity against HeLa and KB cell lines by MTT assay

All tested compounds (1 mg each) were tested for cytotoxic activity against HeLa and KB cell lines by MTT assay. This assay was kindly performed by Natural Products Research Section, Research Division, National Cancer Institute, Thailand.

CHAPTER III
RESULTS AND DISCUSSION

3.1 Primary bioassay screening results of crude extracts

3.1.1 Cytotoxic activity of crude extracts

The crude extracts of *G. laoticus* stems were preliminarily evaluated using cytotoxicity on human tumor cells assay. The dichloromethane crude extract showed the most promising activity. The cytotoxic activity results of all crude extracts are shown in **Table 3.1**.

Table 3.1 Cytotoxic Activity against KB and HeLa cell lines of crude extracts.

Crude extracts	IC_{50} (μg/mL) at λ 550 nm	
	KB	HeLa
Hexane	0.220	0.320
CH_2Cl_2	0.027	0.068
EtOAc	0.043	0.085
MeOH	0.055	0.360

Pure compound ≤ 4 μg/mL

Crude extract ≤ 30 μg/mL

KB cell line: Human epidermoid carcinoma

HeLa cell line: Human cervical carcinoma

Note: Standard agent (Adriamycin IC_{50} = 0.018 μg/mL)

3.1.2 Cytotoxic activity of main fractions

The dichloromethane crude extract was subjected on vacuum liquid chromatography (VLC) to furnish six main fractions (1-6). The cytotoxic activity of all main fractions against KB and HeLa cells were expressed as IC_{50} value (mg/mL) by MTT Colorimetric Assay. The cytotoxic activity results of all main fractions are shown in **Table 3.2**.

Table 3.2 Cytotoxic Activity against KB and HeLa cell lines of main fractions.

Main fractions	IC_{50} (µg/mL) at λ 550 nm	
	KB	HeLa
1	12.000	28.500
2	29.000	>100
3	7.700	35.000
4	4.500	4.400
5	0.120	0.120
6	0.026	0.043

Pure compound ≤ 4 µg/mL

Crude extract ≤ 30 µg/mL

KB cell line: Human epidermoid carcinoma

HeLa cell line: Human cervical carcinoma

Note: Standard agent (Adriamycin IC_{50} = 0.018 µg/mL)

3.2 Properties and structural elucidation of isolated compounds

3.2.1 Laoticuzanone A (9)

Laoticuzanone A was obtained as optically active brown amorphous powder; $[\alpha]_D^{26}$ +119.0° (*c* 0.004, MeOH) with mp 208-209 °C. Its HREIMS indicated a molecular ion $[M+H]^+$ at m/z 274.0704, compatible with a molecular formula of $C_{14}H_{11}NO_5$. The IR spectrum confirmed this evidence by showing the quinine carbonyl and the lactam carbonyl absorptions at 1463 cm^{-1} and 1632 cm^{-1}, respectively. UV absorption bands (λ_{max}= 255, 295 nm) also confirmed the presence of the conjugated quinonoid moiety.

The ^1H NMR spectrum of **9** in acetone-d_6 showed three adjacent aromatic proton signals at δ 7.34, 7.62, and 7.72. In addition, the spectrum showed a chelated hydroxyl proton at δ 12.37. Three other coupled signals in the ^1H NMR spectrum of **9** (δ 1.10, 3H, d, J=7.2 Hz; δ 3.53, 1H, m; δ 4.57, 1H, d, J=6.8 Hz) suggested the presence of a –$CH(CH_3)$-CH(OH)- moiety. The ^{13}C NMR spectrum of **9** permitted assignment of only some resonances because of the limited amount of material available. Three methane carbon signals at δ 125.1, 137.6 and 118.8 were assigned for C-6, C-7 and C-8. The NMR spectral data of **9** were similar to griffithazanone A[28]. The planar structure of **9** was depicted as in **Fig. 3.1**.

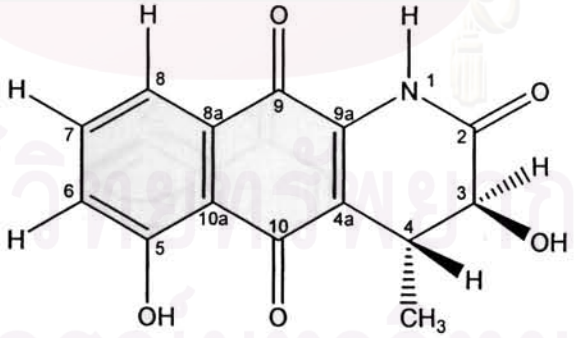


Figure 3.1 Structure of laoticuzanone A (**9**, new compound)

Based on an HMBC correlation, the methyl group was allocated to C-4. The methine proton at δ 3.53 correlated with two carbonyl groups at the δ 188.2 (C-10) and 171.2 (C-2), the oxymethine carbon at δ 68.7 (C-3), the methyl group at δ 12.0 (4-CH_3), and two quaternary carbons at δ 124.0 (C-4a) and 138.2 (C-9a). The other HMBC correlations provided the assignments of all carbon and proton signals in **9** (**Table 3.1** and **Fig.3.2**).

Thus, **9** was found to be 3,5-dihydroxy-4-methyl-3,4-dihydro-2,9,10-(2H)-1-azaanthracenetrione and this new compound was given the name as laoticuzanone A.

Table 3.3 ^{1}H, ^{13}C, HMBC and ^{1}H-^{1}H COSY NMR data of laoticuzanone A (**9**) in acetone-d_6

Position	δ_C	δ_H (mult., *J* in Hz)	HMBC	COSY
2	171.2	-	-	-
3	68.7	4.57 (d, 6.8)	C-2, C-4, 4-CH_3	H-4
4	30.5	3.53 (m)	C-2, C-3, C-4a, C-9a, C-10, 4-CH_3	H-3
4a	124.0	-	-	-
5	161.0	-	-	-
6	125.1	7.34 (d, 8.4)	C-8, C-10a	H-7
7	137.6	7.72 (t, 7.6, 6.8)	C-5, C-8a	H-6, H-8
8	118.8	7.62 (d, 7.2)	C-6, C-9, C-10a	H-7
8a	131.0	-	-	-
9	178.2	-	-	-
9a	138.2	-	-	-
10	188.2	-	-	-
10a	114.2	-	-	-
4-CH_3	12.0	1.10 (d, 7.2)	C-3, C-4, C-4a, 4-CH_3	H-4
5-OH		12.37 (s)	C-5, C-6, C-10a	-

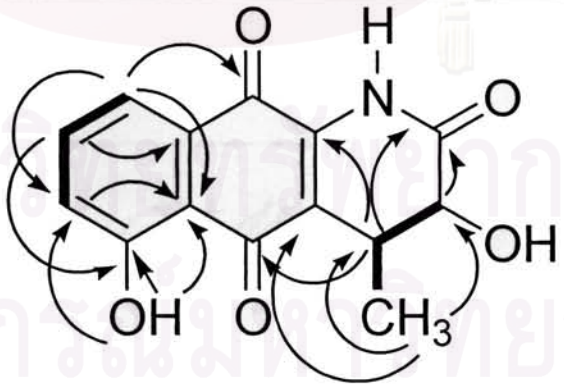


Figure 3.2 Selected HMBC (arrow curves) and COSY (bold lines) correlations of (**9**).

3.2.2 5-Hydroxy-3-amino-2-aceto-1,4-naphthoquinone (4)

5-Hydroxy-3-amino-2-aceto-1,4-naphthoquinone was obtained as yellow crystals. It was proposed as an intermediate in the biosynthesis of the hydroxylated 1-azaanthraquinones by undergoing the formation of the pyridine ring via incorporation of one acetate unit[12]. The structure of **4** were elucidated by ^{1}H, ^{13}C, 2D NMR and Single-Crystal X-ray analysis. The X-ray crystallographic data of 4 (**Fig. 3.3-3.4** and **Table 3.2**) has not been previously reported.

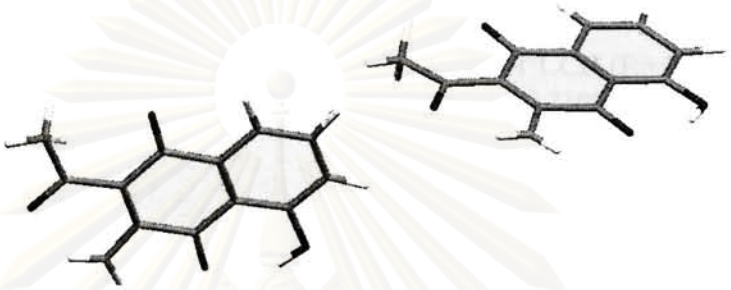


Figure 3.3 X-ray ORTEP diagram of compound **4**.

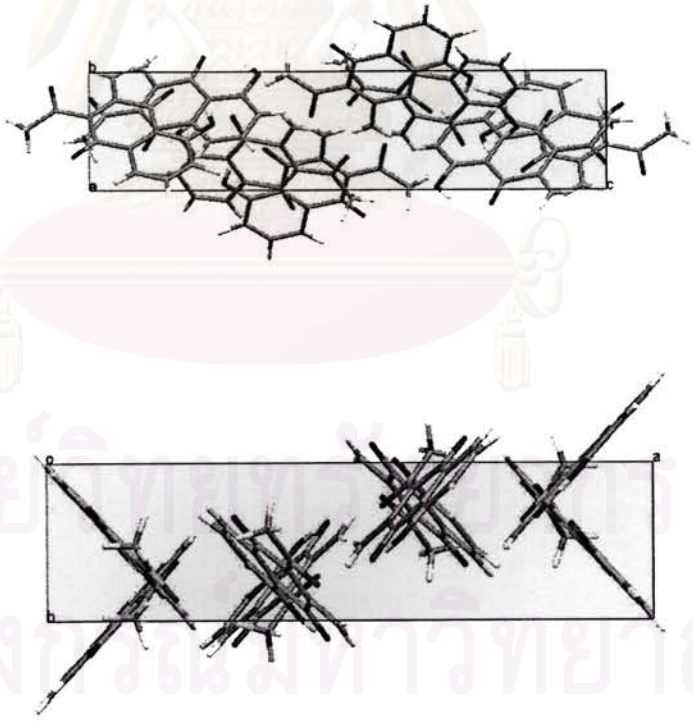


Figure 3.4 X-ray packing diagrams of compound **4**.

Table 3.4 Crystal Data and Data Collection Parameters of compound **4**.

Chemical formula	$2(C_{12}H_9NO_4)$
Chemical formula weight	231.2
Crystal habit, color	Needle, light yellow
Crystal size (mm^3)	$0.1 \times 0.1 \times 0.2$
Cell system, space group	Monoclinic, $P2_1/c$
a, b, c (Å)	19.292(2), 4.9814(5), 21.430(2)
β (°)	93.316(2)
V (Å^3)	2056.0(4)
Z	4
D_x (g cm^{-3})	1.494
μ (mm^{-1})	0.11
F(000)	960
Diffractometer	SMART CCD (Bruker)
Radiation type, wavelength (Å)	Mo$K\alpha$, 0.71073
Temperature (°C)	20
θ range (°)	1.06–30.42
Resolution (Å)	0.7
Reflections measured/independent/observed $[F^2 > 2\sigma(F^2)]$	8,714/4,138/1,224
R_{int}	0.084
Range of h, k, l	$-27 \rightarrow h \rightarrow 0$ $-6 \rightarrow k \rightarrow 5$ $-27 \rightarrow l \rightarrow 0$
Structure solution	Direct methods (SHELXS-97)
Refinement method	Full-matrix least-squares on F^2
Weighting scheme	$w = [S^2(F_o^2) + (0.0791P)^2 + 0.0000P]^{-1}$, where $P = (F_o^2 + 2F_c^2)/3$
Data/parameters	4,138/331
R $[F^2 > 2\sigma(F^2)]$	$R^a = 0.066$, $wR^b = 0.133$
R (all data)	$R^a = 0.207$, $wR^b = 0.267$
Goodness of fit	0.902
Highest peak/deepest hole (e Å^{-3})	0.20/–0.23

[a] $R = \sum||F_o| - |F_c||/\sum|F_o|$.

[b] $wR = \sum\{w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2\}^{1/2}$.

3.2.3 (-)-Goniofufurone (5)

(-)-Goniofufurone, obtained as colorless crystals, is one of four diastereoisomers of goniofufurone which contain a furanofurone bicyclic structure and show significant cytotoxic activities against several human tumor cell lines. (-)-goniofufurone has been reported from the synthesis of four diastereoisomers of goniofufurone from commercially available tartaric acids[29]. From our knowledge, this is the first report of (-)-goniofufurone from natural resources. The spectroscopic data of (-)-goniofufurone are in accord with (+)-Goniofufurone. The NOESY spectrum of (-)-goniofufurone indicates that H-4 and H-5 must be *cis* to each other. Similarly, H-7 and H-8 are also *cis* to each other.

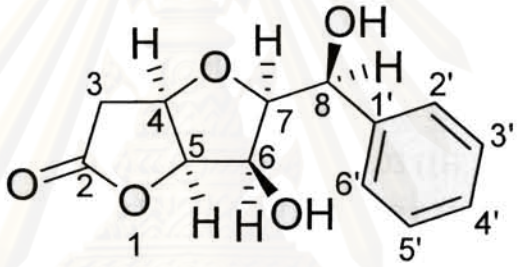


Figure 3.5 Structure of (-)-goniofufurone (**5**, new natural compound).

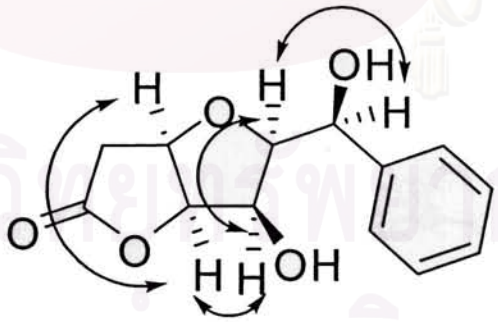


Figure 3.6 Key NOESY correlations for (-)-giniofufurone (**5**).

Pinocembrin (1): white amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.04 (1H, s, OH-5), 7.44-7.45 (5H, m, H-2′,3′,4′,5′, 6′), 6.01 (2H, s, H-6, 8), 5.43 (1H, dd, *J* = 2.4, 12.8 Hz, H-1), 3.09 (1H, dd, *J* = 13.2,16.8 Hz, H-2b), 2.83 (1H, dd, *J* = 2.8, 17.2 Hz, H-2a). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 195.92 (C-3), 164.76 (C-7), 164.32 (C-5), 163.14 (C-9), 138.13 (C-1′), 128.73 (C-3′, 4′, 5′), 126.02 (C-2′, 6′), 103.05 (C-4), 96.58 (C-6), 95.32 (C-8), 79.25 (C-1), 43.34 (C-2).

Altholactone (2): yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.30 (5H, m, H-2′,3′,4′,5′, 6′), 6.98 (1H, dd, *J* = 4.8,10 Hz, H-4), 6.19 (1H, d, *J* = 10 Hz, H-3), 4.87 (1H, dd, *J* = 2.4, 5.2 Hz, H-6), 4.71 (1H, d, *J* = 5.6 Hz, H-8), 4.59 (1H, t, *J* = 5.2, 5.2 Hz, H-5), 4.40 (1H, dd, *J* = 1.6, 5.2 Hz, H-7). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 161.90 (C-2), 140.17 (C-4), 138.17 (C-1′), 128.61 (C-4′), 128.29 (C-3′, 5′), 126.10 (C-2′, 6′), 123.51 (C- 3), 86.67 (C-6), 86.05 (C-8), 83.48 (C-7), 68.14 (C-5).

Goniopypyrone (3): colorless crystals; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.44-7.45 (5H, m, H-2′,3′,4′,5′, 6′), 5.02 (1H, s, H-7), 4.81 (1H, t, *J* = 2.4, 3.6 Hz, H-1), 4.47 (1H, d, *J* = 2.4 Hz, H-5), 4.11 (1H, d, *J* = 10.4 Hz, H-8), 4.03 (1H, d, *J* = 8.4, H-9), 3.06 (1H, dd, *J* = 1.6, 19.6 Hz, H-4b), 3.02 (1H, dd, *J* = 5.2, 19.6 Hz, H-4a), 2.2 (1H, s, OH-8). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 167.90 (C-3), 135.90 (C-1′), 129.00 (C-3′, 5′), 128.70 (C-4′), 126.20 (C-2′, 6′), 72.60 (C-1), 70.90 (C-5), 70.30 (C-7), 70.10 (C-8), 64.50 (C-9), 35.2 (C-4). The Single-crystal X-ray analysis of goniopypyrone was shown in **Figure.3.7-3.8**.

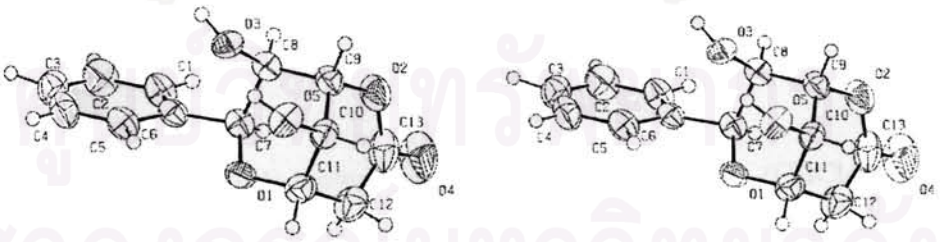


Figure 3.7 X-ray ORTEP diagrams of goniopypyrone (**3**).

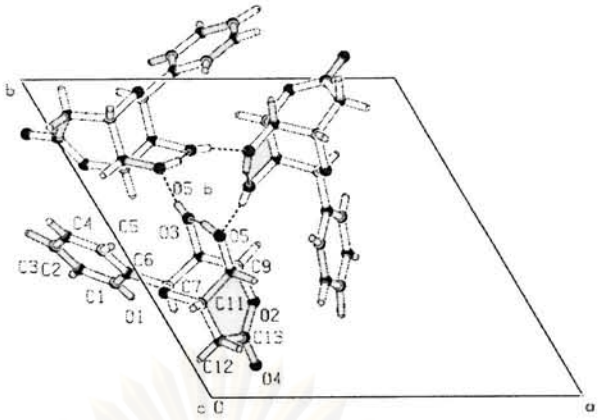


Figure 3.8 X-ray packing diagram of goniopypyrone (**3**).

5-Hydroxy-3-amino-2-aceto-1,4-naphthoquinone (4): yellow crystals; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.73 (1H, d, J = 7.2 Hz, H-8), 7.71 (1H, t, J = 7.2, 7.6 Hz, H-7), 7.19 (1H, dd, J = 2, 7.2 Hz, H-6), 7.12, 10.69 (2H, br s, NH_2-3), 2.71 (3H, s, CH_3-10). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 202.18 (C-9), 184.75 (C-4), 180.40 (C-1), 161.81 (C-5), 152.52 (C-3), 139.05 (C-7), 133.61 (C-8a), 127.24 (C-2), 122.16 (C-6), 119.68 (C-8), 113.97 (C-4a), 33.17 (CH_3-10). The Single-crystal X-ray analysis of **4** was shown in **Figure.3.3-3.4** and **Table 3.2.**

(-)-Goniofufurone (5): colorless crystals; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 7.46 (2H, m, H-2′, 6′), 7.33 (2H, m, H-3′, 5′), 7.26 (1H, m, H-4′), 4.99 (1H, s, H-8), 4.97 (1H, s, H-4), 4.91 (1H, d, J = 4 Hz, H-5), 4.46 (1H, s, H-6), 4.00 (1H, dd, J = 2.8, 7.2 Hz, H-7), 2.8 (1H, dd, J = 6.4, 18.4 Hz, H-3a), 2.34 (1H, d, J =18.8 Hz, H-3b). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 175.24 (C-2), 142.71 (C-1′), 127.94 (C-3′, 5′), 127.28 (C-4′), 126.82 (C-2′, 6′), 87.67 (C-5), 84.12 (C-7), 77.18 (C-4), 73.87 (C-6), 71.35 (C-8), 35.60 (C-3). Key NOESY correlations of (-)-giniofufurone was shown in **Figure 3.6.**

2-*Epi*-altholactone (6): brown amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.36-7.41 (5H, m, H-2′,3′,4′,5′, 6′), 7.00 (1H, dd, J = 10 Hz, H-4), 6.20 (1H, d, J = 10, H-3), 5.35 (1H, d, J = 2.4, H-8), 5.09 (1H, d, J = 4 Hz, H-6), 4.89 (1H, t, J = 4.8, 4.8 Hz, H-5), 4.51 (1H, s, H-7). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.05 (C-2), 139.60 (C-4), 130.00 (C-1′), 127.85 (C-3′, 5′), 127.58 (C-4′), 125.60 (C-2′, 6′), 122.04 (C- 3), 83.24 (C-6), 82.53 (C-8), 76.85 (C-7), 67.09 (C-5).

3-Methyl-1*H*-1-azaanthracene-2,9,10-trione (7): yellow amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.72 (1H, br s, NH-1), 8.19 (1H, d, J = 4 Hz, H-8), 8.14 (1H, d, J = 5.2 Hz, H-5), 7.69 (1H, d, J = 3.2 Hz, H-7), 7.68 (1H, d, J = 1.6 Hz, H-6), 6.93 (1H, s, H-4), 2.45 (3H, s, CH_3-3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.80 (C-9), 176.50 (C-2), 175.70 (C-10), 134.60 (C-8a), 134.50 (C-10a), 133.40 (C-6), 132.80 (C-7), 132.60 (C-9a), 126.80 (C-8), 126.20 (C-5), 125.10 (C-3), 124.80 (C-4), 123.10 (C-4a), 11.4 (CH_3-3).

Griffithazanone A (8): brown amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.34 (1H, s, OH-3), 8.14 (1H, m, H-5), 8.10 (1H, m, H-8), 7.79 (1H, m, H-6), 7.74 (1H, m, H-7), 4.47 (1H, t, J = 2.8, 3.2 Hz, H-3), 3.70 (1H, dd, J = 3.6, 7.2 Hz, H-4), 1.12 (3H, dd, J = 2.8, 4 Hz, CH_3-4). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.80 (C-10), 178.70 (C-9), 171.20 (C-2), 136.90 (C-9a), 135.00 (C-6), 133.50 (C-7), 132.20 (C-10a), 130.10 (C-8a), 126.80 (C-5), 126.40 (C-8), 125.50 (C-4a), 69.20 (C-3), 30.4 (C-4), 11.2 (CH_3-4).

Laoticuzanone A (9): brown amorphous powder; $[\alpha]_D^{26}$ +119° (c 0.004, MeOH); UV (MeOH) λ_{max} (log ε): 255 (3.8), 295 (3.9) nm; positive ion ESIMS *m/z*: 273.59 $[M+H]^+$; positive ion HRESIMS *m/z*: $[M+H]^+$ 274.0704 (calcd for $C_{14}H_{11}O_5$, 274.0715); ^{1}H NMR (CD_3COCD_3, 400 MHz) and ^{13}C NMR (CD_3COCD_3, 100 MHz) are shown in **Table 3.1**.

Methyl sinapate (10): brown oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.59 (1H, d, J = 16 Hz, H-3), 6.76 (2H, s, H-2′, 6′), 6.29 (1H, d, J = 15.6 Hz, H-2), 3.91 (6H, s, OMe-3′, 5′), 3.79 (3H, s, OMe-1). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 167.58 (C-1), 147.15 (C-3′, 5′), 145.12 (C-3), 137.06 (C-4′), 125.82 (C-1′), 115.50 (C-2), 104.97 (C-2′, 6′), 51.60 (OMe-1).

3-(4′-Hydroxyphenyl)-(*E*)propenoic acid methyl ester (11): brown oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.64 (1H, d, J = 6 Hz, H-3), 7.41 (2H, d, J = 8.4 Hz, H-3′, 5′), 6.85 (2H, d, J = 8 Hz, H-2′, 6′), 6.30 (1H, d, J = 16 Hz, H-2), 3.8 (3H, s, OMe-1). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 168.35 (C-1), 158.02 (C-4′), 144.98 (C-3), 130.03 (C-3′, 5′), 126.91 (C-1′), 115.90 (C-2′, 6′), 114.86 (C-2), 51.81 (OMe-1).

2-(4-Hydroxyphenyl)ethyl (*E*)-3-(4-hydroxyphenyl)prop-2-enoate (12): white crystals;^{1}H NMR (CD_3OD, 400 MHz): δ 7.43 (1H, d, *J* = 15.6 Hz, H-3), 7.39 (2H, d, *J* = 8.8 Hz, H-2′, 6′), 7.05 (2H, d, *J* = 8.4 Hz, H-2‴, 6‴), 6.78 (2H, d, *J* = 8.4 Hz, H-3′, 5′), 6.71 (2H, d, *J* = 8.4 Hz, H-3‴, 5‴), 6.37 (1H, d, *J* = 15.6 Hz, H-2), 3.45 (1H, t, *J* = 7.2, 7.6 Hz, H-1″), 2.74 (1H, t, *J* = 7.2, 7.6Hz, H-2″). ^{13}C NMR (CD_3OD, 100 MHz): δ 167.80 (C-1), 159.09 (C-4′), 155.49 (C-4‴), 140.35 (C-3), 129.86 (C-1‴), 129.31 (C-2‴, 6‴), 129.13 (C-2′, 6′), 126.25 (C-1′), 116.92 (C-2), 115.27 (C-3′, 5′), 114.81 (C-3‴, 5‴), 41.15 (C-1″), 34.39 (C-2″).

(+)-Goniofufurone (13): brown oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.40 (2H, m, H-2′, 6′), 7.38 (2H, m, H-3′, 5′), 7.34 (1H, m, H-4′), 5.16 (1H, d, J = 5.2 Hz, H-8), 5.07 (1H, t, J = 4.4, 5.2 Hz, H-4), 4.86 (1H, d, *J* = 4 Hz, H-5), 4.42 (1H, s, H-6), 4.07 (1H, dd, *J* = 2.8, 4.8 Hz, H-7), 2.70 (2H, m, H-3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 175.80 (C-2), 139.11 (C-1′), 128.78(C-3′, 5′), 128.43 (C-4′), 125.97 (C-2′, 6′), 87.56 (C-5), 82.93 (C-7), 77.28 (C-4), 74.43 (C-6), 73.32 (C-8), 36.08 (C-3).

3.3 Bioassay activity of isolated compounds

3.3.1 Cytotoxic activity against KB and HeLa cell lines of isolated compounds

The cytotoxic activity against HeLa and KB cell lines of all isolated compounds were determined using MTT assay and the result was shown in **Table 3.5**.

Table 3.5 Cytotoxic Activity Against HeLa and KB Cell lines of Isolated Compounds.

Isolated compounds	IC_{50} (μg/mL)	
	KB cell line	**HeLa cell line**
Pinocembrin (**1**)	0.55	3.00
Altholactone (**2**)	2.40	3.10
Goniopypyrone (**3**)	23.00	20.00
5-Hydroxy-3-amino-2-aceto-1,4-naphthoquinone (**4**)	1.70	1.60
(-)-Goniofufurone (**5**)	22.00	20.50
2-*Epi*-altholactone (**6**)	5.00	5.10
3-Methyl-1*H*-1-azaanthracene-2,9,10-trione (7)	5.50	4.00
Griffithazanone A (**8**)	5.20	3.00
Laoticuzanone A (**9**)	0.68	0.50
Methyl sinapate (**10**)	79.00	52.00
3-(4′-Hydroxyphenyl)-(*E*) propenoic acid methyl ester (**11**)	57.00	37.00
2-(4-Hydroxyphenyl)ethyl (*E*)-3-(4-hydroxyphenyl) prop-2-enoate (**12**)	69.00	80.00
(+)-Goniofufurone (**13**)	6.90	4.50

KB cell line: Human epidermoid carcinoma

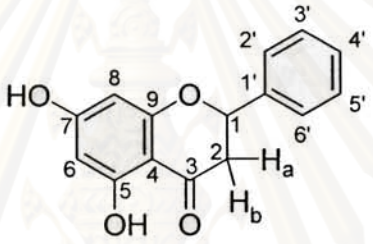
HeLa cell line: Human cervical carcinoma

Note: Standard agent (Adriamycin IC_{50} = 0.018 μg/mL)

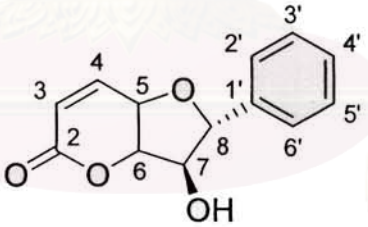
From **Table 3.5**, all of isolated compounds were examined for cytotoxicity against KB and HeLa cells. Compounds **1**, **9**, **4**, and **2** showed significant cytotoxicity against KB cell with IC_{50} values 0.55, 0.68, 1.70, and 2.40 μg/ml, respectively. On the other hand, compound **9** showed the highest cytotoxicity against HeLa cell with IC_{50} value 0.50 μg/ml, followed by compounds **4**, **1**, **8**, **2**, and **7** with IC_{50} values 1.60, 3.00, 3.00, 3.10, and 4.00 μg/ml.

CHAPTER IV
CONCLUSION

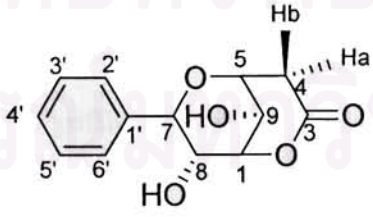
In conclusion, the chromatographic separation of dichloromethane and methanoic crude extracts led to the isolation of one new alkaloid, laoticuzanone A (**9**) and a synthetically known styryl-lactone, (-)-goniofufurone (**5**), new natural compound, along with eleven known compounds, pinocembrin (**1**), altholactone (**2**), goniopypyrone (**3**), 5-hydroxy-3-amino-2-aceto-1,4-naphthoquinone (**4**), 2-*epi*-altholactone (**6**), 3-methyl-1*H*-1-azaanthracene-2,9,10-trione (**7**), griffithazanone A (**8**), methyl sinapate (**10**), 3-(4′-hydroxyphenyl)-(*E*) propenoic acid methyl ester (**11**), 2-(4-hydroxyphenyl) ethyl (*E*)-3-(4-hydroxyphenyl) prop-2-enoate (**12**) and (+)-goniofufurone (**13**). The structures of all isolated compounds were characterized by spectroscopic method, Single-Crystal X-ray analysis as well as comparison with the previous literature data.



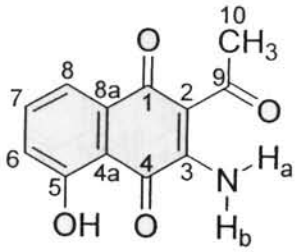
pinocembrin (**1**)



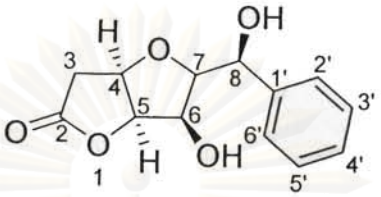
altholactone (**2**)



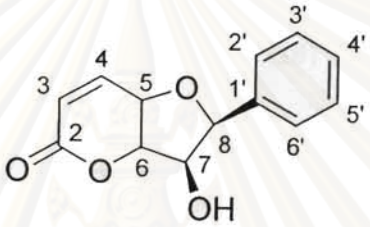
goniopypyrone (**3**)



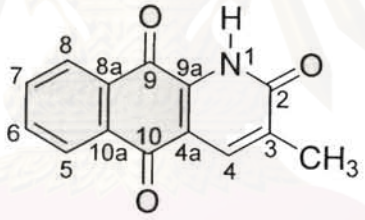
5-hydroxy-3-amino-2-aceto-1,4-naphthoquinone (**4**)



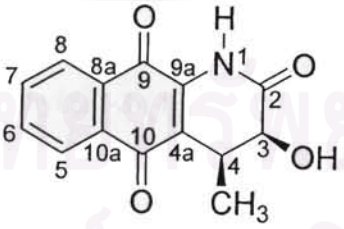
(-)-goniofufurone (**5**, new natural compound)



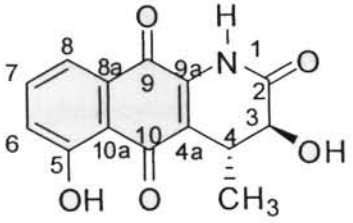
2-*epi*-altholactone (**6**)



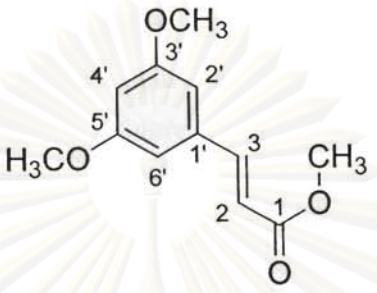
3-methyl-1*H*-1-azaanthracene-2,9,10-trione (**7**)



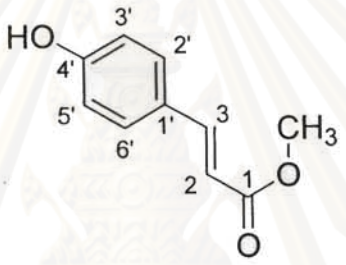
griffithazanone A (**8**)



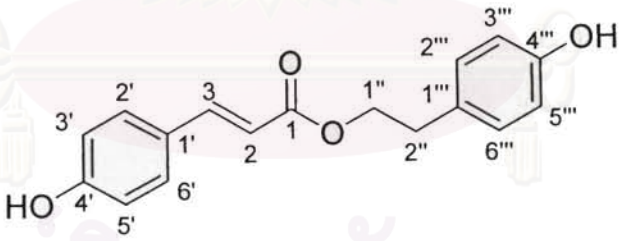
laoticuzanone A (**9**, New compound)



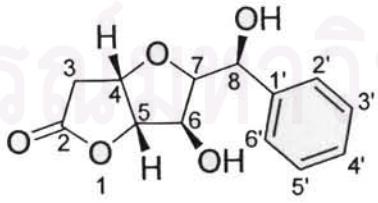
methyl sinapate (**10**)



3-(4′-hydroxyphenyl)-(*E*) propenoic acid methyl ester (**11**)



2-(4-hydroxyphenyl) ethyl (*E*)-3-(4-hydroxyphenyl) prop-2-enoate (**12**)



(+)-goniofufurone (**13**)

The evaluation for cytotoxic activity against KB and HeLa cell lines was found that compound **1** showed the highest cytotoxicity against KB cell with IC_{50} value 0.55 μg/ml, followed by compound **9**, **4**, and **2** with IC_{50} values 0.68, 1.70, and 2.40 μg/ml, respectively. On the other hand, compound **9** showed the highest cytotoxicity against HeLa cell with IC_{50} value 0.50 μg/ml, followed by compound **4**, **1**, **8**, **2**, and 7 with IC_{50} values 1.60, 3.00, 3.00, 3.10, and 4.00 μg/ml.

The 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 afford 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.

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APPENDICES

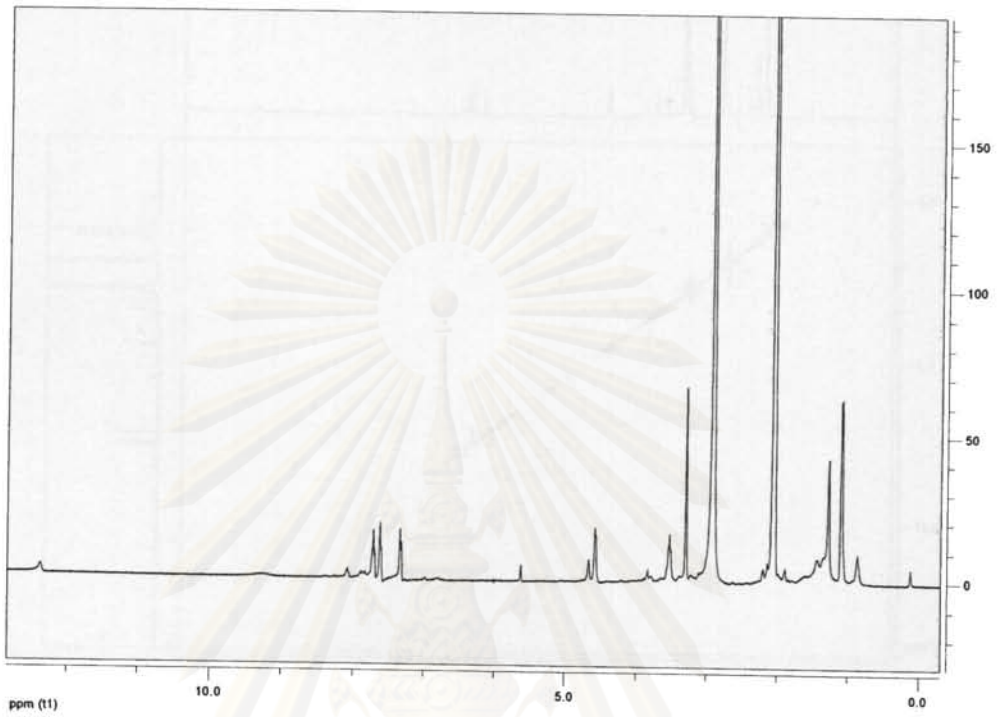


Figure A-1.1 ^{1}H NMR spectrum (acetone-d_6) of laoticuzanone A (9).

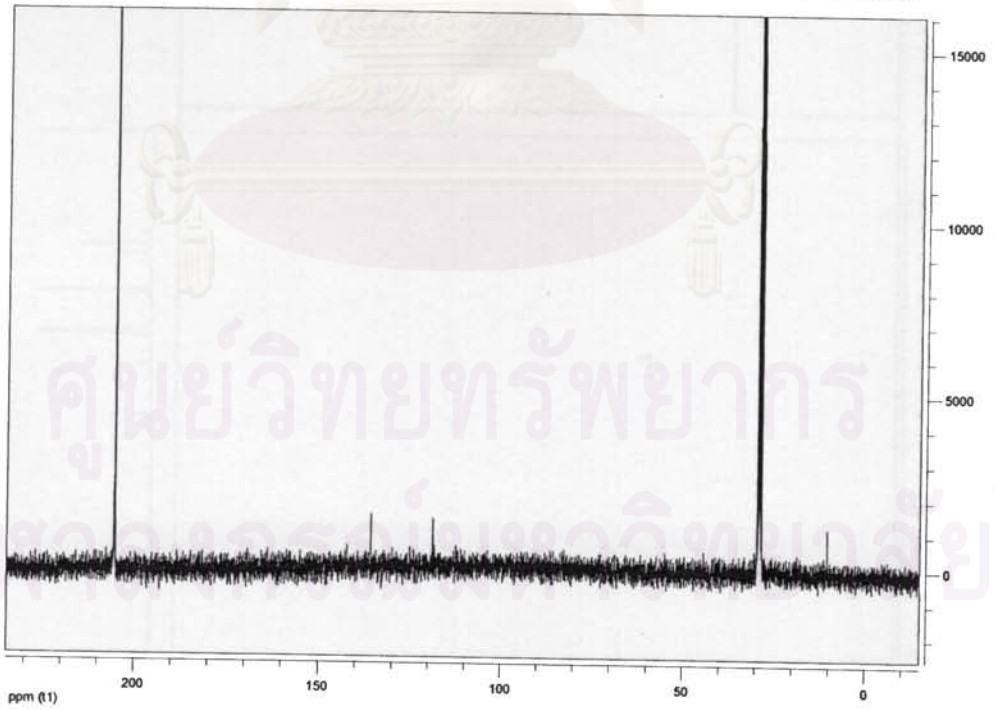


Figure A-1.2 ^{13}C NMR spectrum (acetone-d_6) of laoticuzanone A (9).

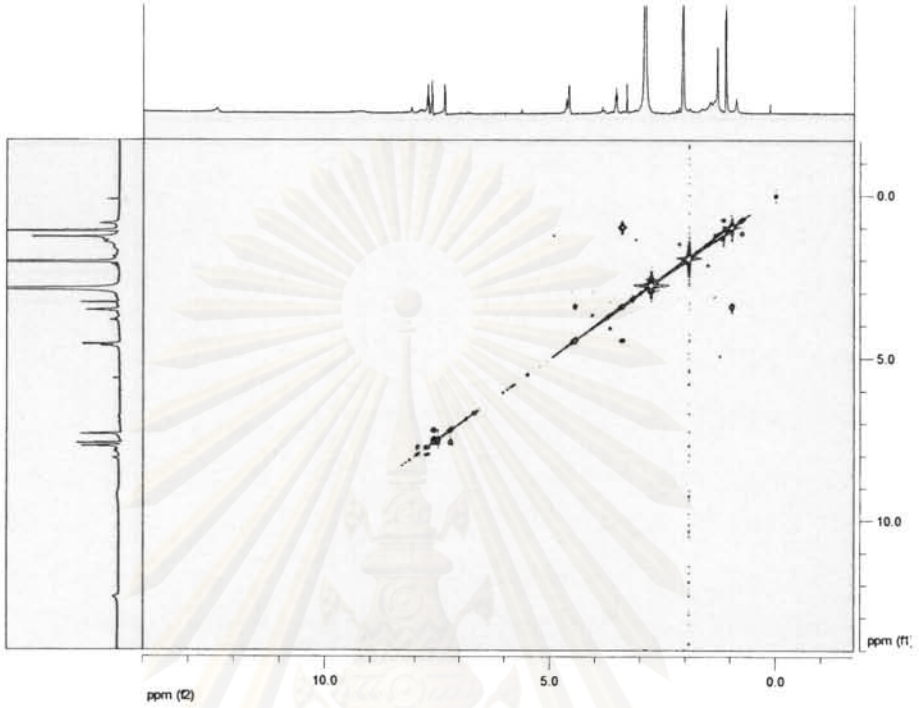


Figure A-1.3 COSY spectrum (acetone-d_6) of laoticuzanone A (9).

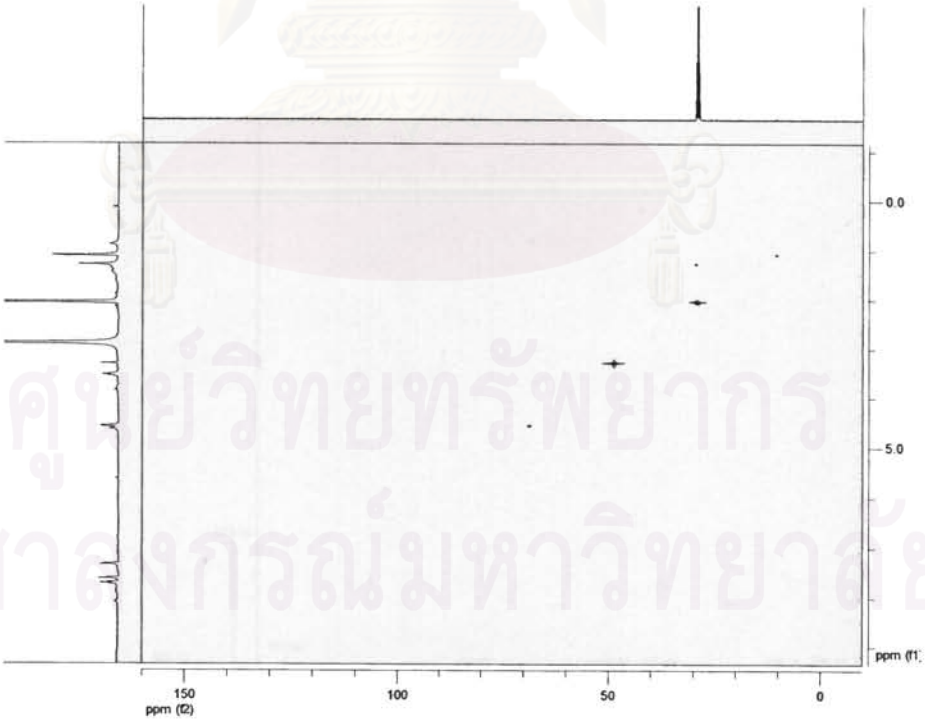


Figure A-1.4 HSQC spectrum (acetone-d_6) of laoticuzanone A (9).

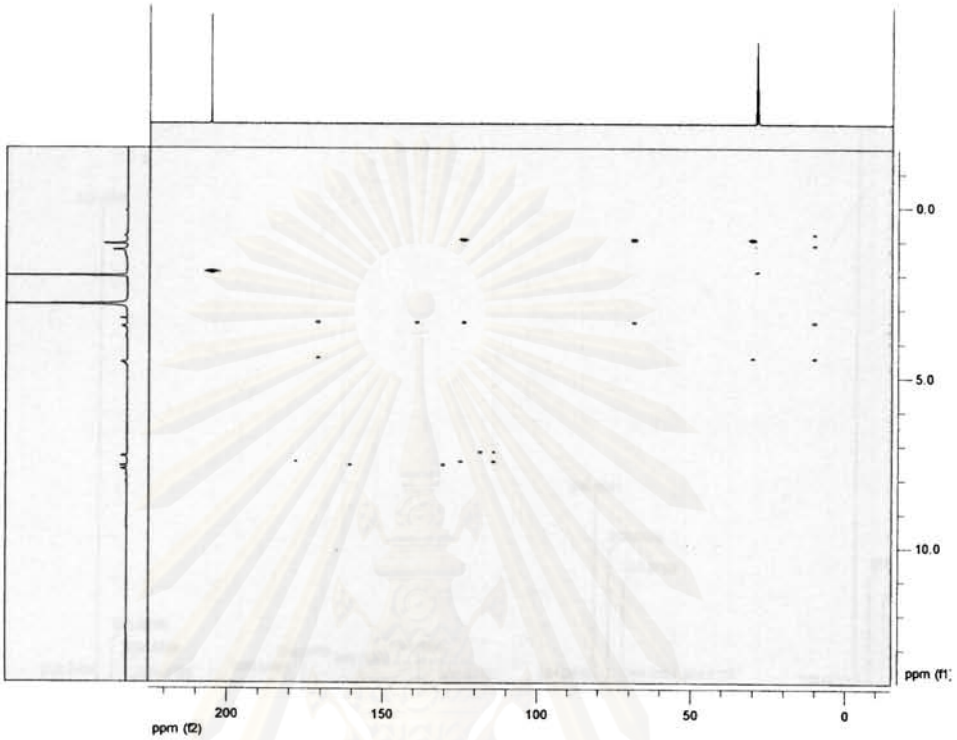


Figure A-1.5 HMBC spectrum (acetone-d_6) of laoticuzanone A (9).

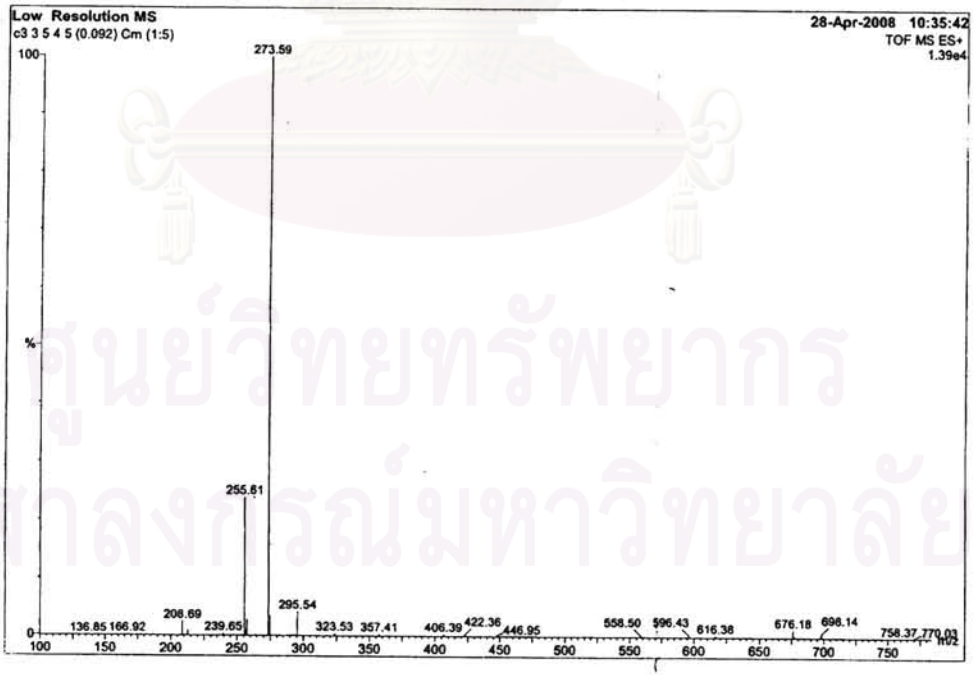
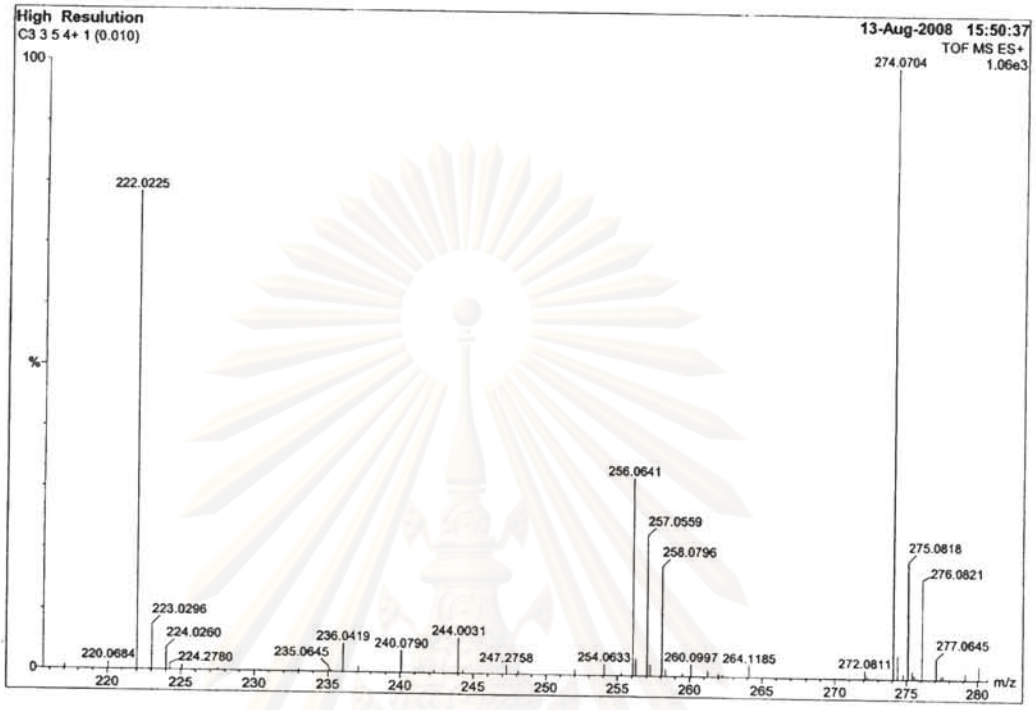


Figure A-1.6 Low resolution mass spectrum of laoticuzanone A (9).



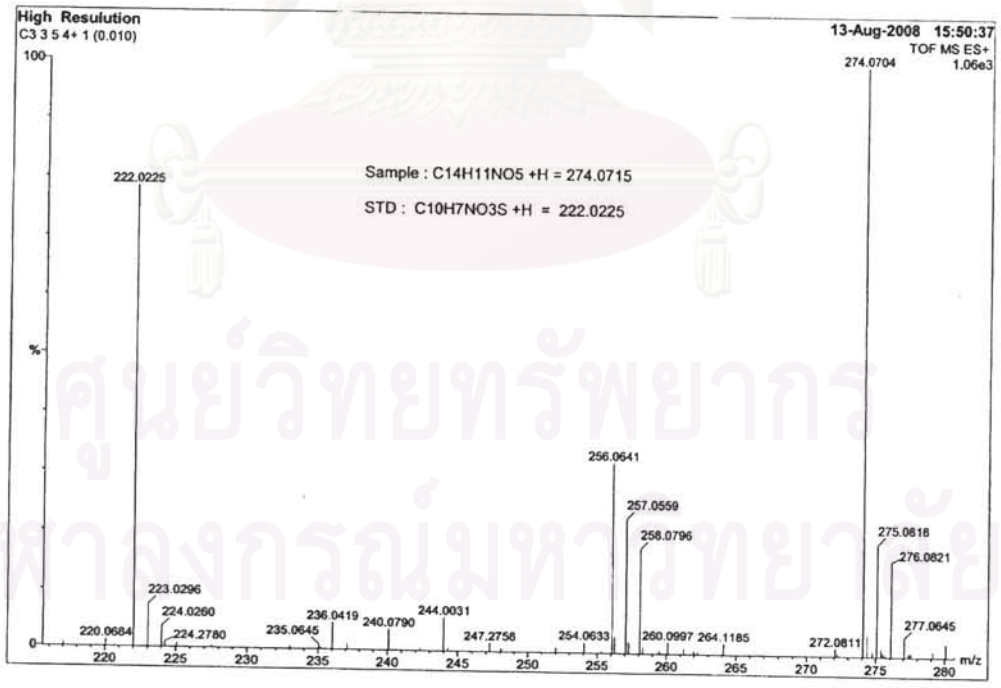


Figure A-1.7 High resolution mass spectrum of laoticuzanone A (9).

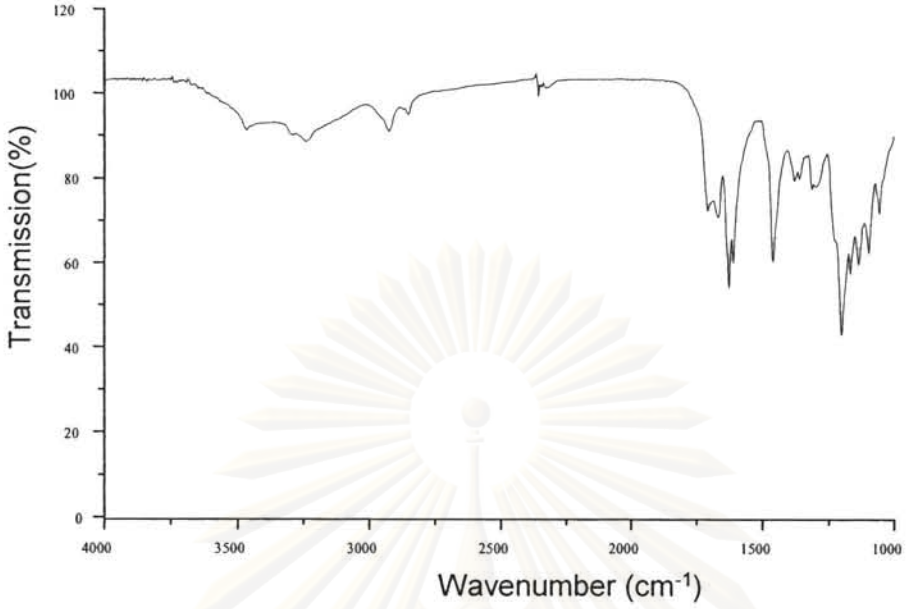


Figure A-1.8 IR spectrum of laoticuzanone A (9).

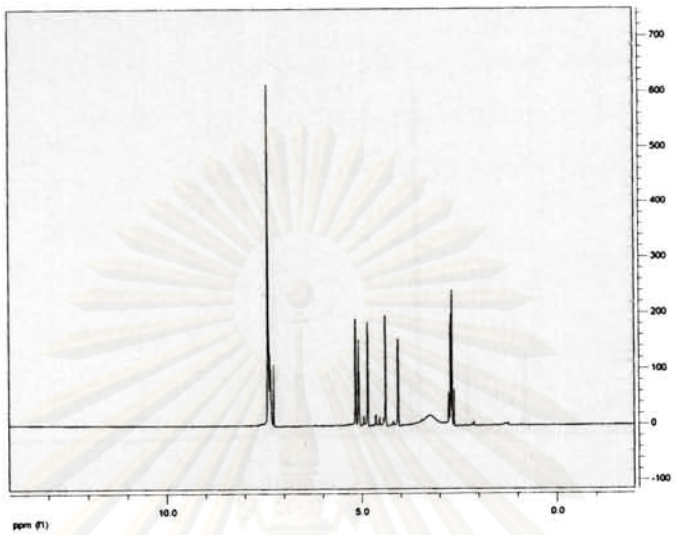


Figure A-2.1 ^{1}H NMR spectrum ($CDCl_3$) of (-)-goniofufurone (5).

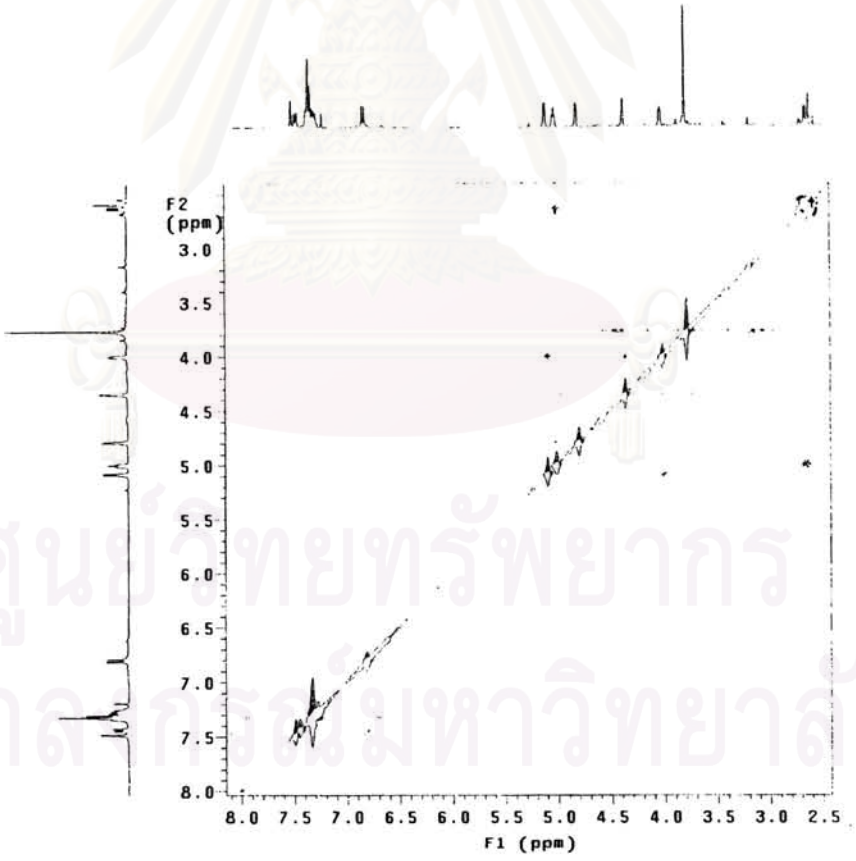


Figure A-2.2 NOESY spectrum ($CDCl_3$) of (-)-goniofufurone (5).

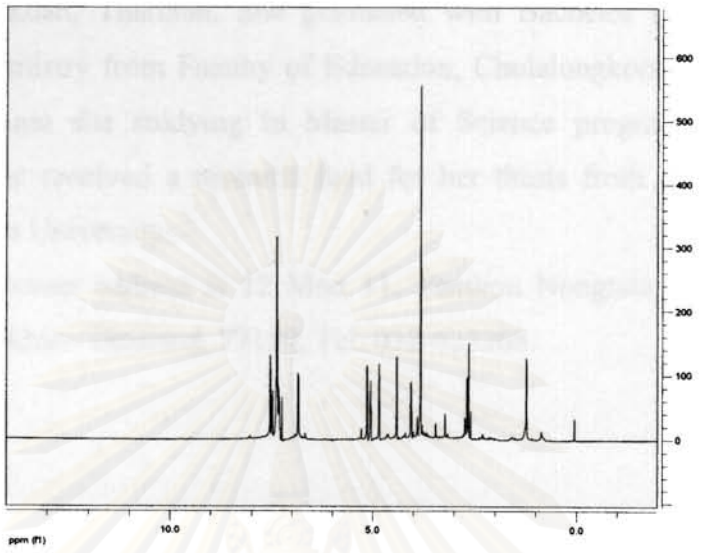


Figure A-3.1 ^{1}H NMR spectrum ($CDCl_3$) of (+)-goniofufurone (13).

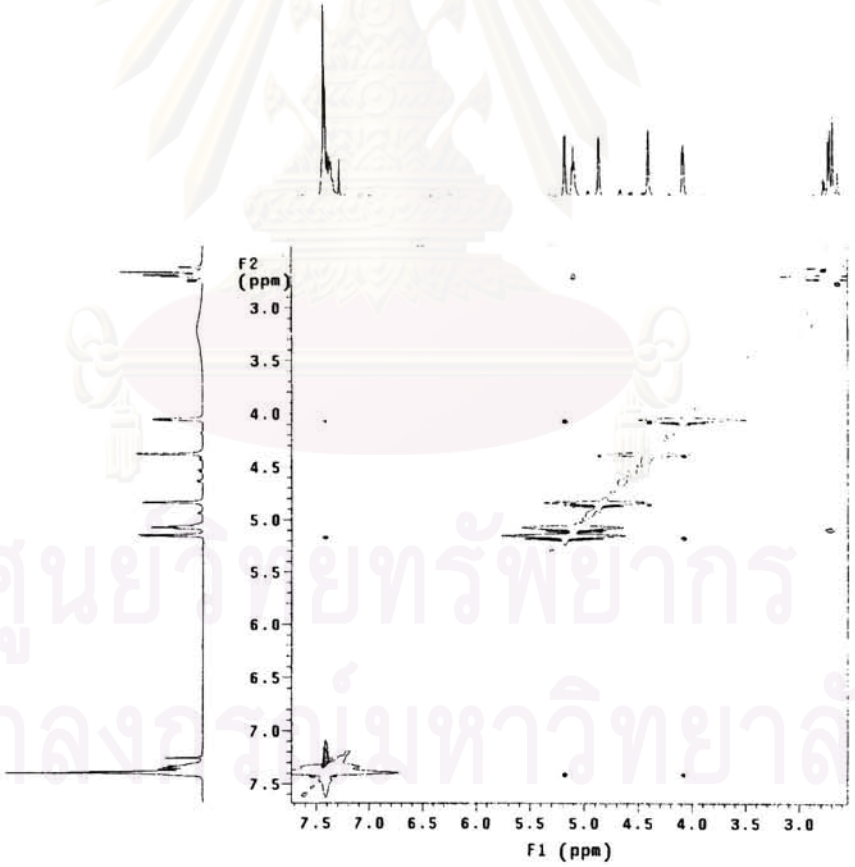


Figure A-3.2 NOESY spectrum ($CDCl_3$) of (+)-goniofufurone (13).

VITA

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