องค์ประกอบทางเคมีของรากพะยอม *Shorea roxburghii* G. Don

นางสาววินิทรา พัชรามันต์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Shorea roxburghii* G. Don

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
Department of Chemistry
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Field of Study	Chemistry
Thesis Advisor	Associate Professor Santi Tip-pyang, Ph.D.

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วินิทรา พัชรามันต์ : องค์ประกอบทางเคมีของรากพะยอม *Shorea roxburghii* G. Don (CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Shorea roxburghii* G. Don) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร. สันติ ทิพยางค์, 55 หน้า.

การศึกษาองค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพจากสิ่งสกัดอะซิโตนของรากพะยอม *Shorea roxburghii* G. Don สามารถแยกสารในกลุ่มสติลบินอยด์ชนิดใหม่ได้ 1 ชนิด คือ roxburghiol A (**5**) พร้อมกับสารที่มีรายงานแล้ว 14 ชนิด ได้แก่ melanoxylin A (**1**), caragaphenol A (**2**), *ε*-viniferin (**3**), hopeahainanphenol (**4**), vitisinol G (**6**), vaticanol A (**7**), hopeaphenol (**8**), isohopeaphenol (**9**), apigenin 7-*O*-glucoside (**10**), *trans*-piceid (**11**), *trans*-3,5,4'-trihydroxystilbene 2-*C*-glucoside (**12**), neoisohopeaphenol A (**13**), balanocarpol (**14**) และ gnemonol K (**15**) การพิสูจน์โครงสร้างของสารทั้งหมดที่แยกได้ อาศัยสมบัติทางกายภาพและวิธีทางสเปกโทรสโกปี ร่วมกับการเปรียบเทียบกับข้อมูลที่มีรายงานแล้ว จากการทดสอบฤทธิ์ยับยั้งการเกิดออกซิเดชัน (ต้านอนุมูลอิสระ DPPH) และความเป็นพิษต่อเซลล์มะเร็งชนิด HeLa และ KB พบว่าสารส่วนใหญ่มีฤทธิ์ต้านอนุมูลอิสระ DPPH โดยมีค่า IC_{50} อยู่ในช่วง 0.23-0.41 mM และแสดงความเป็นพิษต่อเซลล์มะเร็ง KB ได้ดีกว่า HeLa สาร **8** และ **9** มีฤทธิ์ดีที่สุดสามารถยับยั้งเซลล์มะเร็ง KB ที่ IC_{50} เท่ากับ 6.47 และ 8.50 μg/mL และ HeLa ที่ IC_{50} เท่ากับ 8.66 และ 10.12 μg/mL ตามลำดับ ส่วนสาร **4**, **7**, **10** และ **11** ไม่แสดงฤทธิ์ในการยับยั้งเซลล์มะเร็งทั้งสองชนิด (IC_{50} > 80.0 μg/mL)

ภาควิชา เคมี ลายมือชื่อนิสิต วินิทรา พัชรามันต์

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก สันติ ทิพยางค์

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The investigation for chemical constituents and their biological activities from acetone crude extract of the roots of *Shorea roxburghii* G. Don led to the isolation of a new stilbenoid, roxburghiol A (**5**), along with fourteen known compounds, melanoxylin A (**1**), caragaphenol A (**2**), ε-viniferin (**3**), hopeahainanphenol (**4**), vitisinol G (**6**), vaticanol A (**7**), hopeaphenol (**8**), isohopeaphenol (**9**), apigenin 7-*O*-glucoside (**10**), *trans*-piceid (**11**), *trans*-3,5,4'-trihydroxystilbene 2-*C*-glucoside (**12**), neoisohopeaphenol A (**13**), balanocarpol (**14**) and gnemonol K (**15**). The structures of all isolated compounds were elucidated by physical properties and spectroscopic methods as well as comparison with previous literature data. The evaluation for antioxidant activity (DPPH radical scavenging) and cytotoxic activity against HeLa and KB cell lines found that most of isolated compounds showed antioxidant activity toward DPPH radical with IC_{50} values in the range of 0.23-0.41 mM, and these compounds also exhibited cytotoxicity against KB cell line more than HeLa cell line. Compounds **8** and **9** showed the most effective cytotoxicity against KB cell line with IC_{50} = 6.47 and 8.50 μg/mL, and HeLa cell line with IC_{50} = 8.66 and 10.12 μg/mL, respectively. Compounds **4, 7, 10** and **11** were inactive toward both cell lines (IC_{50} > 80.0 μg/mL).

Department : Chemistry Student's Signature : Winitra Patcharamun
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Academic Year : 2009

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^{13}C NMR	carbon 13 nuclear magnetic resonance
^{1}H NMR	proton nuclear magnetic resonance
br s	broad singlet (NMR)
c	concentration
COSY	correlated spectroscopy
d	doublet (NMR)
dd	doublet of doublet (NMR)
t	triplet (NMR)
ESIMS	electrospray ionization mass spectrometry
g	gram (s)
HMBC	heteronuclear multiple bond correlation
HRESIMS	high resolution electrospray ionization mass spectrometry
HSQC	heteronuclear single quantum correlation
Hz	hertz
IC_{50}	concentration that is required for 50% inhibition in vitro
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
q	quartet (NMR)
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
μ	micro
ε	molar extinction coefficient
λ_{max}	maximum wavelength
2D NMR	two dimentional nuclear magnetic resonance
$[\alpha]_D^{20}$	specific optical rotation

CHAPTER I

INTRODUCTION

The human lives are always related to their surrounding environment including plants. Increasing interest has been paid to primitive medicinal plants to find new substances with potentially useful biological activities. Typically red wine's health benefits initially received a great deal of attention following the reports indicating that a greater wine consumption was linked to a lower incidence of cardiovascular disease so-called "French Paradox". Wine contains a broad range of polyphenols that are present in the skin and seeds of grapes. Possible mechanisms by which these phenolic compounds exert their beneficial effects include the reactive oxygen species scavenging ability [1-4].

Recently, resveratrol, a stilbenoid-based polyphenol, widely found in medicinal plants, grape skin, peanuts, and red wine, has been found to be substantially helpful for human health owing to its significant antioxidative actions [5-7] and has been discussed in terms of cancer preventive or anticancer substances which exerts an anticarcinogenic effect in a two-stage mouse skin cancer model [8] and shows tumor growth inhibition in rat [9]. Resveratrol is therefore regarded as one of the important candidates for tumor suppressive agents and is now widely used as an additive in food, cosmetic and pharmaceutical industries [10]. Moreover, its oligomers have also received considerable chemical and biological attention because of their structural complexity as well as their array of bioactivities exhibited such as antioxidative [11], anticancer [4,12], anti-HIV [13], antibacterial [14] and antimicrobial effects [15].

Plants belonging to the Dipterocarpaceae family, most of which are distributed in Southeast Asia, have been revealed to be a rich source of stilbenoids. Studies on the metabolites from the *Shorea* genus of the Dipterocarpaceae have been carried out in previous researches [16-18]. Several structural stilbenoids have been isolated from this genus, which exhibited significant cytotoxic activities against murine leukemia cells [19] and human cultured cancer cells as well as the apoptosis-inducing effect on colon cancer cell lines [4]. Dipterocarpaceae plants are, therefore, considered to be useful sources of lead compounds for drug development.

Shorea roxburghii G. Don, an evergreen canopy species [20], is widely distributed in many parts of Thailand. Its bark and flower have been used for various medicinal purposes. Consequently, it was chosen as the subject of the present investigation due to the attractive results of preliminary screening test based on DPPH radical scavenging activity. The chemical constituents and biological activities of this plant have not been reported.

1.1 Biosynthesis pathway of stilbenoids

Stilbenoids represent a unique class of biologically active natural products produced primarily by plants. One of the most well known and widely distributed stilbenoids is the compound resveratrol (3,4',5-trihydroxystilbenoid) (**1**) - the stilbenoid nucleus is based on a 14 carbon skeleton composed of two phenyl rings joined by an ethylene bridge. The biosynthesis of **1** is dependent upon a single key enzyme known as stilbenoid synthase (STS) as part of a mixed phenylpropanoid-polyketide pathway (Figure 1.1) [21-23].

The resveratrol pathway consists of four enzymes: phenylalanine ammonia lyase (PAL), cinnamic acid 4-hydroxylase (C4H), 4-coumarate CoA ligase (4CL) and stilbenoid synthase (STS). The first two enzymes, PAL and C4H, transform phenylalanine into *p*-coumaric acid (4-coumaric acid). The third enzyme, 4CL, attaches *p*-coumaric acid to the pantetheine group of Coenzyme-A (CoA) to produce 4-coumaroyl-CoA. PAL, C4H and 4CL are members of the common phenylpropanoid pathway in plants, which synthesizes the majority of phenolic compounds found in nature, including lignins for using as cell wall components, anthocyanins as pigments and flavonols as UV protectants. The final enzyme in the pathway, STS, catalyzes the condensation of **1** from one molecule of 4-coumaroyl-CoA and three molecules of malonyl-CoA, which originate from fatty acid biosynthesis, produce **1** through a C2→C7 aldol condensation. STS is a member of the type III polyketide synthases and has extensive homology to chalcone synthase (CHS). CHS is responsible for the formation of chalcones in many higher plants - chalcones are starting molecules for all flavonoid compounds. Although CHS is ubiquitous in plants, STS is only found in species that accumulate **1** and related compounds.

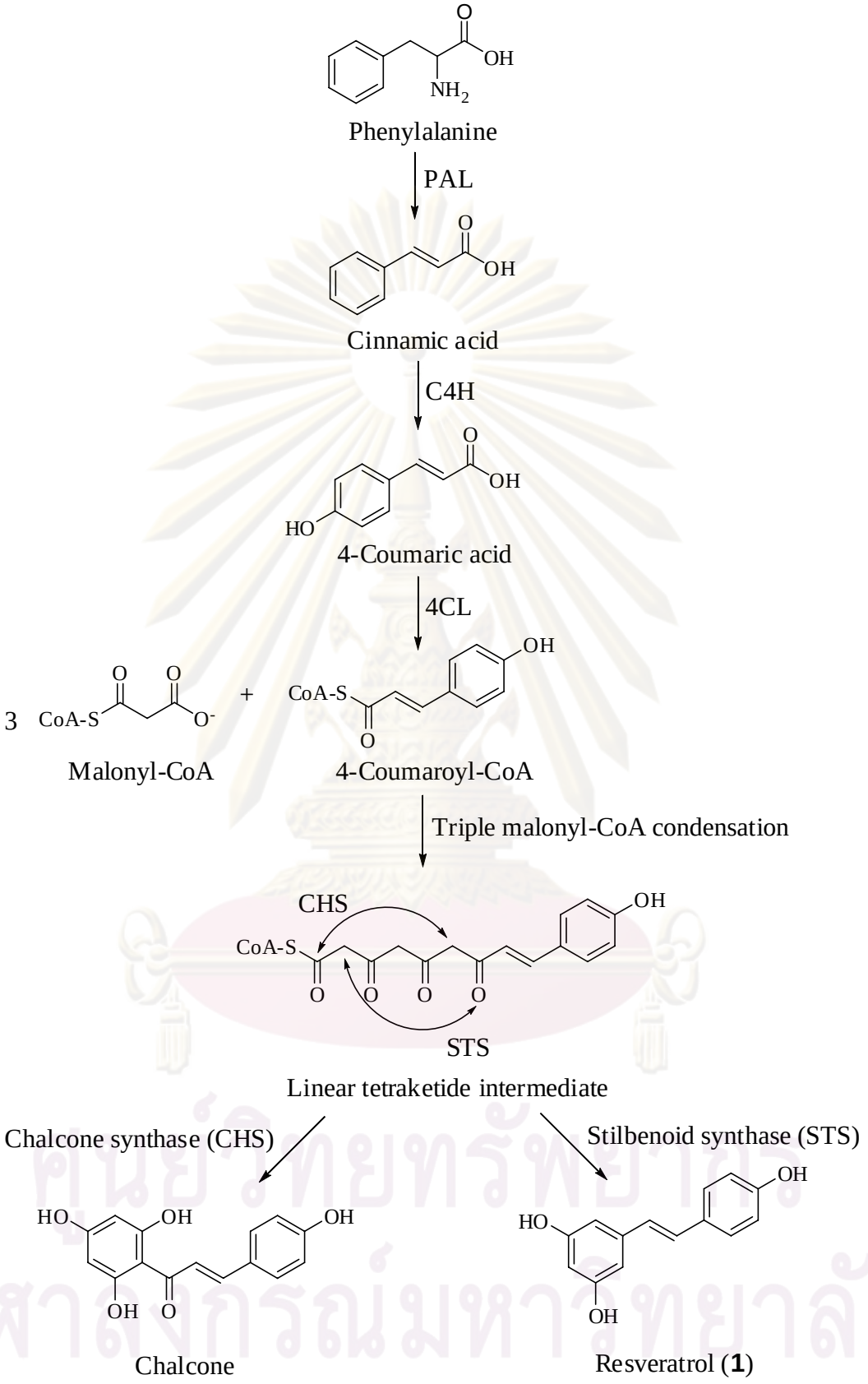


Figure 1.1 Biosynthesis pathway of resveratrol and its derivatives diverged from the flavonoid pathway after the third malonyl-CoA condensation. Cyclization of the polyketide intermediate catalyzed by stilbenoid synthase (STS) to yielde resveratrol.

In addition, many stilbenoids are believed to be the products of a successive series of oxidative couplings of resveratrol radicals. Recent efforts to identify the enzymes responsible for the biotransformation of **1** have uncovered at least two stilbenoid-metabolizing peroxidases as likely catalysts. Morales and colleagues [24] have identified a possible candidate peroxidase isozyme that has demonstrated a high affinity for **1** in an acidic medium in which it readily oxidizes **1**. The more common plant derived resveratrol dimer, *ε*-viniferin, is an example of the proposed free-radical mechanism for the formation of the stilbenoids (Figure 1.2).

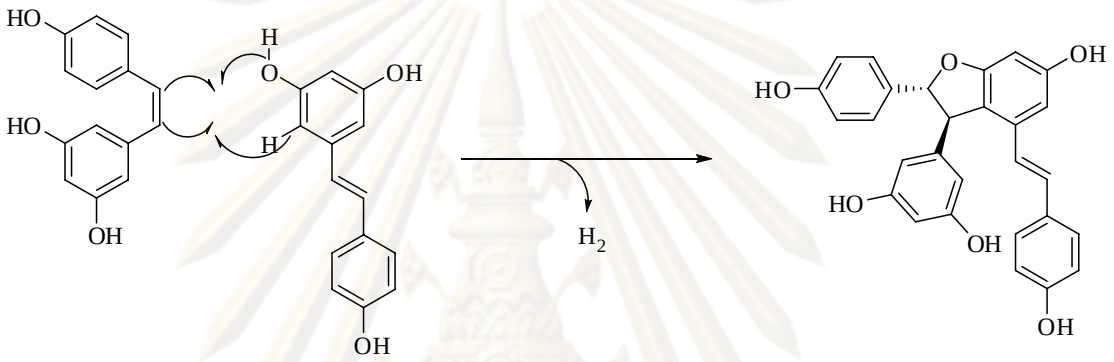


Figure 1.2 The proposed biosynthesis for the dimerization of resveratrol to *ε*-viniferin.

1.2 Stilbenoid constituents from Dipterocarpaceae species and their biological activities

In 1965, Coggon *et al*. reported the first naturally occurring resveratrol tetramer, (-)-hopeaphenol (Figure 1.3), isolated from *Hopea odorata* belonging to the Dipterocarpaceae family [25]. Based on the painstaking efforts during the past half century, the research of stilbenoids has been flowering in the recent years. The number of stilbenoids up to 1994 was about 26. However, it has increased dramatically to more than 300 [24]. Stilbenoids are commonly found in plants in the families Dipterocarpaceae [26], Vitaceae [27], Cyperaceae [28] and Gnetaceae [29].

In the phytochemical researches on Dipterocarpaceae found that stilbenoids are commonly found in *Shorea, Vatica*, *Vateria*, *Dipterocarpus* and *Hopea* [26,30] genera, and have various structures as dimer, trimer, tetramer, hexamer, heptamer and octamer stilbenoids, containing various molecular frameworks as a result of different condensation of the resveratrol monomer.

Common stilbenoid monomers, dimers, trimers and tetramers found in Dipterocarpaceae plants including resveratrol, dihydroresveratrol, ampelopsin A and F, balanocarpal, ε and α-viniferin, vaticanol A and G, and hopeaphenol (Figure 1.3).

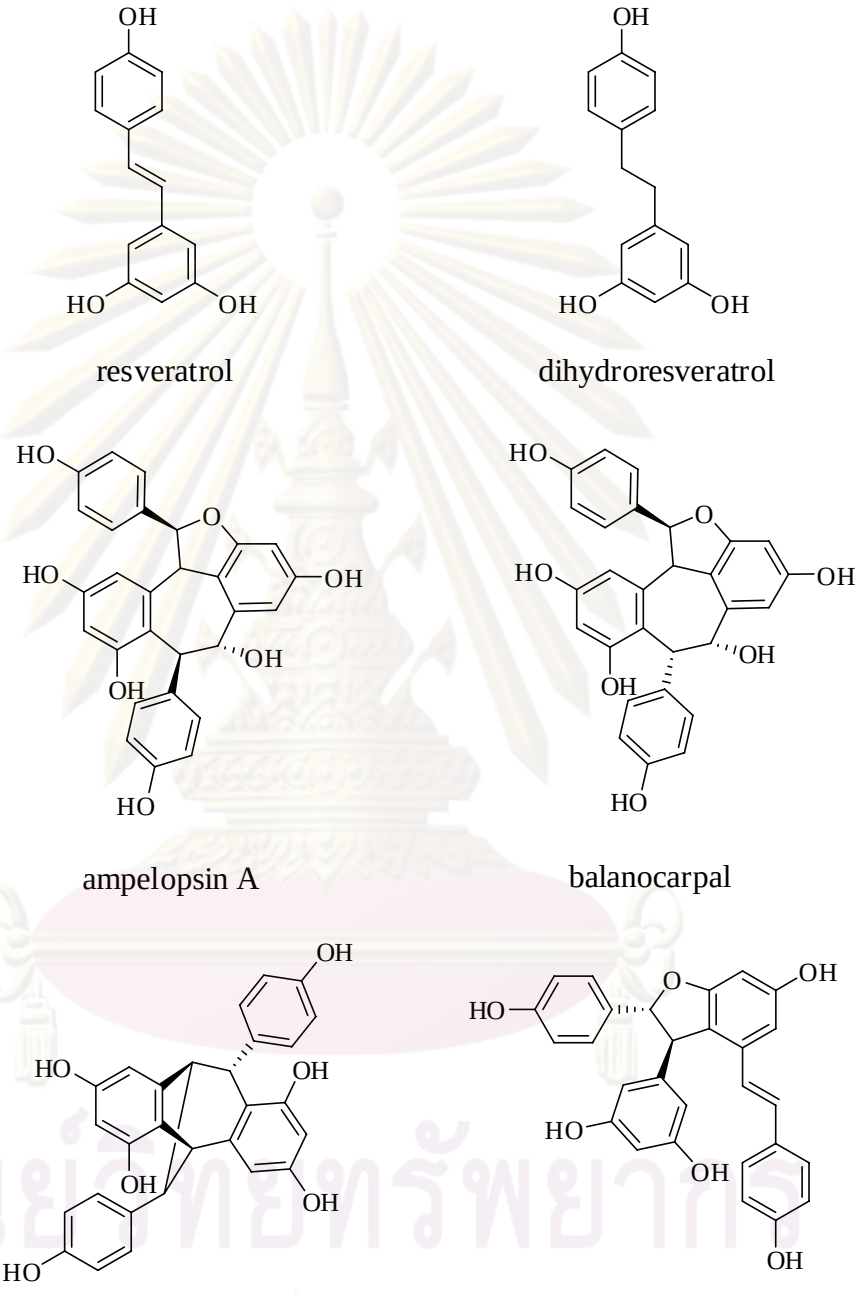
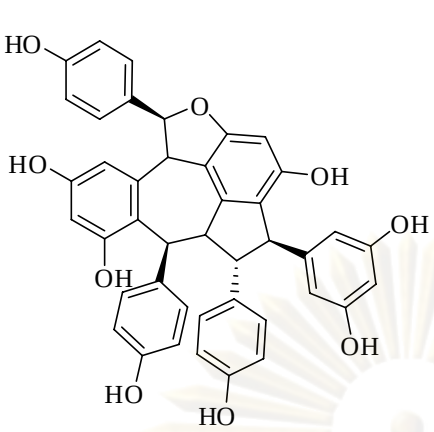
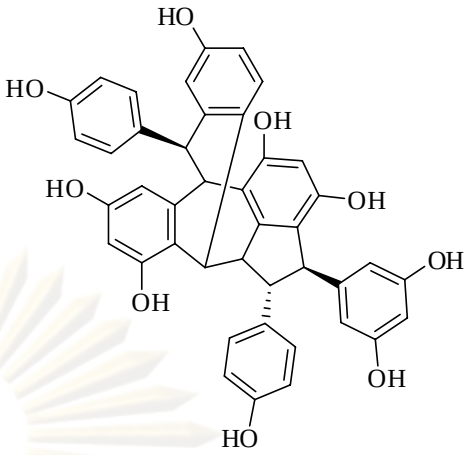


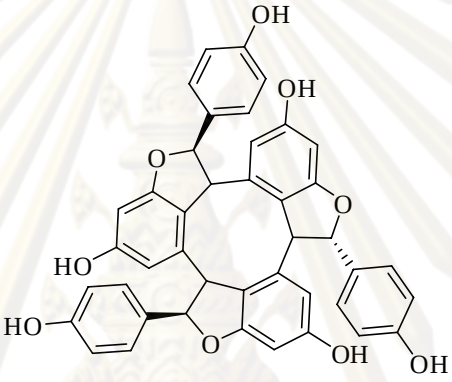
Figure 1.3 Common stilbenoids from plants in Dipterocarpaceae family.



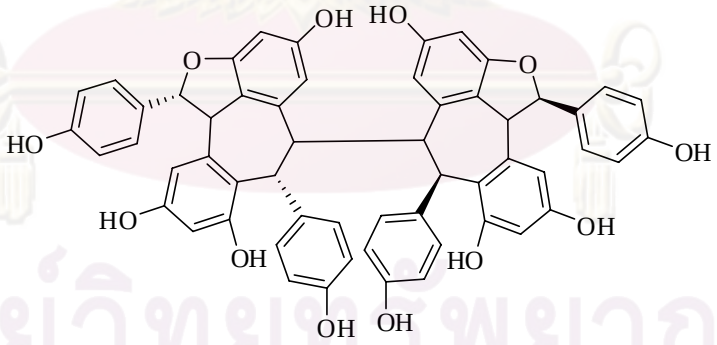
vaticanol A



vaticanol G



α-viniferin

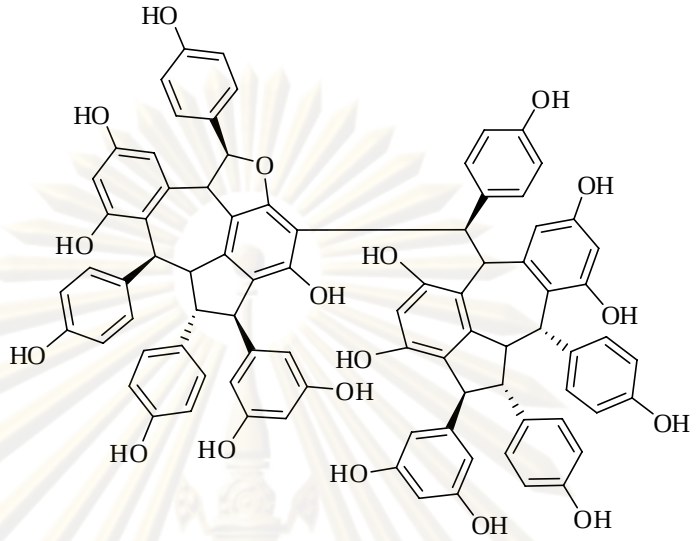


hopeaphenol

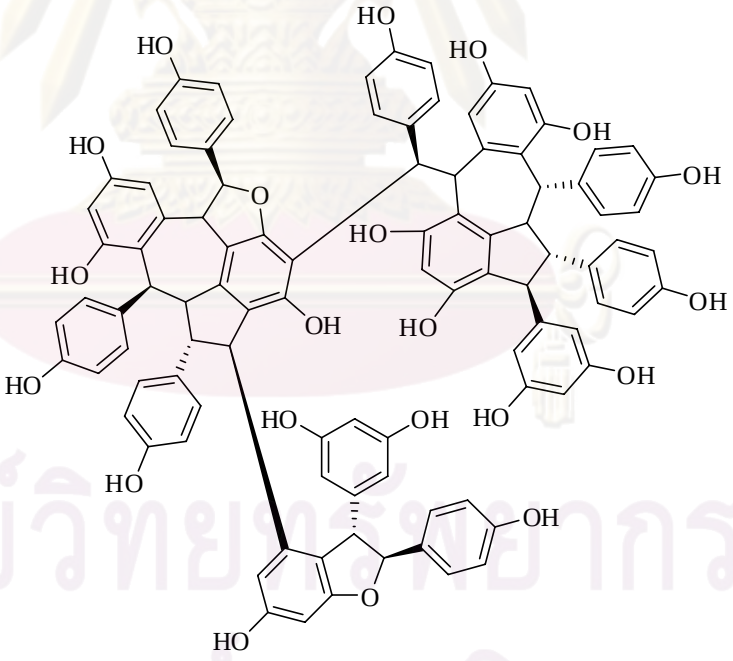
Figure 1.3 Common stilbenoids from plants in Dipterocarpaceae family. (cont.)

Among the isolations, the highest condensed oligomers were resveratrol tetramers. As part of an ongoing search for much higher condensed stilbenoids, Ito et al. reported the first naturally occurring resveratrol hexamer and heptamer, vaticanol I

and vaticanol J (Figure 1.4), isolated from stem bark of *Vatica rassak* [30], and resveratrol octamer, vateriaphenol A (Figure 1.5), isolated from stem bark of *Vateria indica* [31].



vaticanol I



vaticanol J

Figure 1.4 Structures of vaticanol I and vaticanol J.

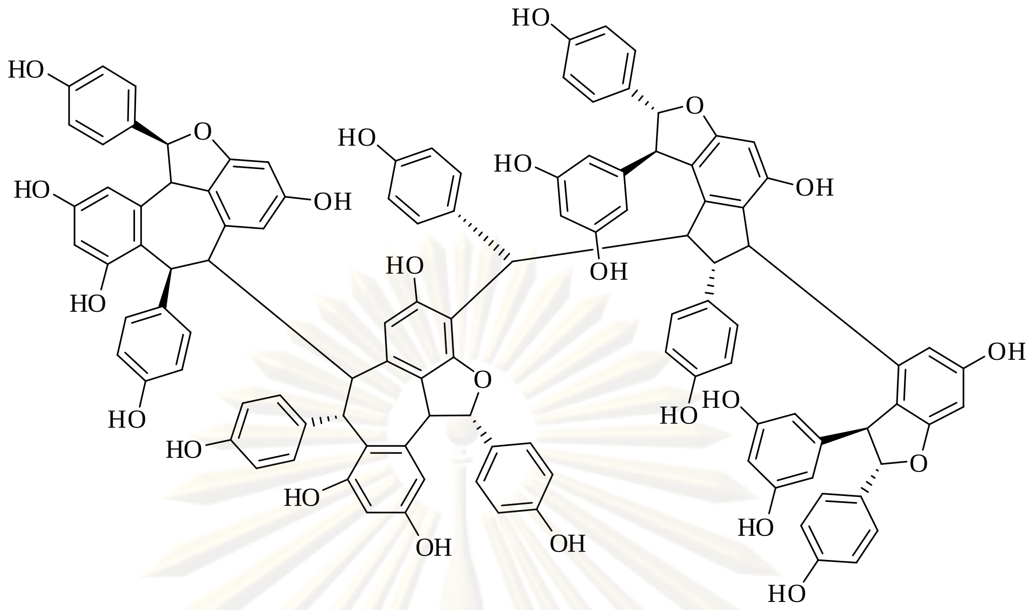
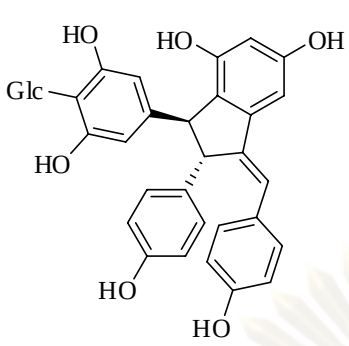


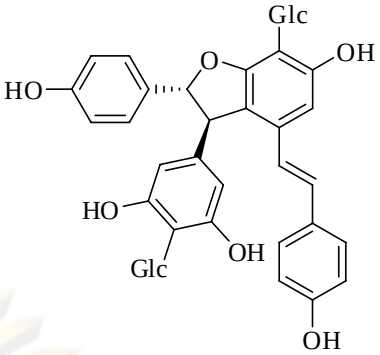
Figure 1.5 Structure of vateriaphenol A.

In addition, four new *C*-glucopyranosides of stilbenoid oligomers, hemsleyanosides A-D (Figure 1.6), are the first report of the occurrence of oligomeric stilbenoid *C*-glycosides. They were isolated from the bark of *Shorea hemsleyana* [16].

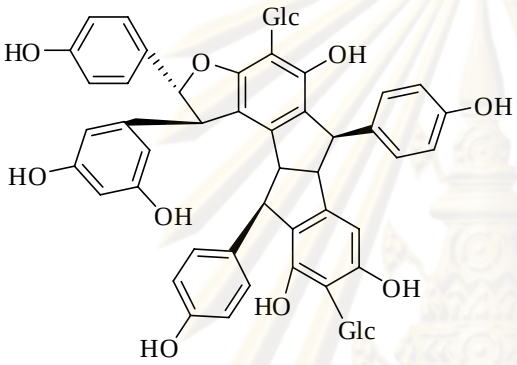
Interestingly, many of stilbenoids are commonly found in the family Dipterocarpaceae, which have demonstrated various biological activities such as ampelopsin A shown the highest cytotoxicity against murine leukemia P-388 cells [19], vaticanol A shown antibabesial activity against *Babesia gibsoni* [32], α-viniferin exhibited antiinflammatory [33] and antifungal effects [34], and hopeaphenol exhibited on murine tyrosinase [35]. Another stilbenoid, nepalensinol B (Figure 1.7), was recently isolated from the stem of *Kobresia nepalensis*. It showed a potent inhibitory effect on topoisomerase II stronger than etoposide (VP-16), a topoisomerase II inhibitor used as an anticancer drug, which exhibited the highest potent activity with an IC_{50} value of 0.02 μg/mL [36].



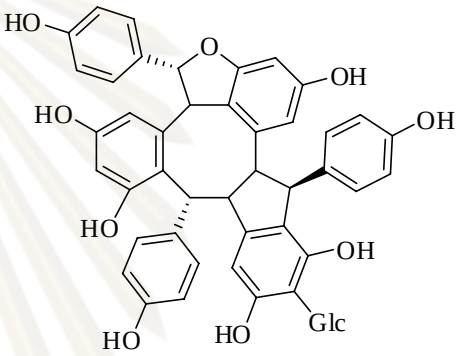
hemsleyanoside A



hemsleyanoside B



hemsleyanoside C



hemsleyanoside D

Figure 1.6 Structures of hemsleyanosides A-D.

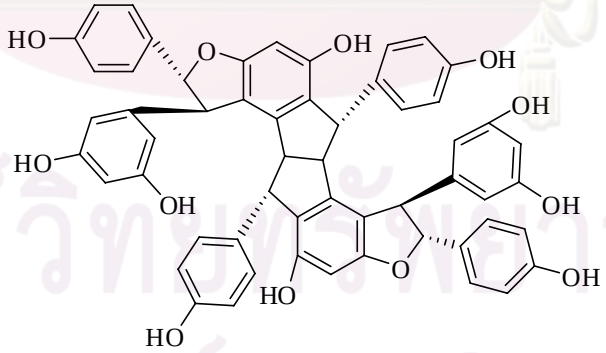


Figure 1.7 Structure of nepalensinol B.

1.3 Botanical aspect and distribution

Dipterocarpaceae is one of the largest families found in the Southeast Asian forests. The plants are widespread throughout Thailand, there for the timber of these plants are usually called "*meranti*". Most plants belonging to this family are extremely tall and have buttress roots, supporting a smooth and straight trunk. Dipterocarpaceae consists of about 16 genera and 600 species [37]. The two biggest genera of this family are *Shorea* and *Dipterocarpus* having 150 and 75 species, respectively [38].

Shorea roxburghii G. Don is one of the numerous highly prized Dipterocarpaceae species.

Distribution: It is found mainly in mixed deciduous forests or evergreen forests in Myanmar, Thailand, Lao People's Democratic Republic, Malaysia, Vietnam and the eastern coast of India [39].

Botanical Description: It is a deciduous, medium to large-sized tree that reaches from 10-25 m high. In favourable conditions, its can reach to 30 m in height and produce boles in excess of 200 cm in dbh. Buttresses may be present or absent. The sapwood and heartwood of this species are slightly differentiated by colour; the sapwood is yellowish, while the heartwood is dark yellow or reddish-brown, usually with dark-coloured lines on the surface. The wood is rather hard and heavy, with a density of 0.8-0.93. It is easy to saw and to work. The bark is 2-2.5 cm thick, gray and rather deeply fissured; the inner bark has brownish concentric bands. The twigs are slender, naked or hairy, and darkly pigmented. Leaves are simple, alternate, oblong to elliptic, with rounded or shortly pointed tips, and rounded bases. They are 18-21 cm long and 11-12 cm wide on young trees and 8-14 cm long and 4-7 cm wide on mature trees, usually naked on both surfaces, but sometimes shortly hairy below. Lateral nerves occur in 14-18 pairs, and the petiole is slender, 1.4-4 cm long. The ovate to lance-shaped stipules are 2-4 mm long, hairy, and caducous. Flowering stalks are 8-10 cm long. Flowers have are born on short pedicels and 5 lanceolate or triangular sepals that dry to a blackish hue. The flowers produce 5 white petals around 14.5 mm long and 5 mm wide at the base, and 10-15 stamens with oblong anthers that bear a linear appendage. Ovaries are glabrous and topped with a 3-lobed stigma. This obovate fruits of this species are 12 mm long and 5.5 mm wide, and bear 3 wings from 4.0-8.5 cm long and 1 cm wide. The long wing exhibit 11-14 parallel veins, and the two shorter wings reach to 4 cm long [39].

Vernacular: Pha-yom (พะยอม), Kha-yom (ขะยอม), Yom (ยอม) and Siao (เซียว)

Stems

Barks

Leaves

Flowers

Fruits

Seeds

Figure 1.8 The stems, barks, leaves, flowers, fruits and seeds of *S. roxburghii*.

1.4 Biological activities

1.4.1 Antioxidant activity

There are many methods or models to determine the antioxidative properties of the compounds. DPPH is selected in activity directed fractionation of free radical scavenging activity because this model is rapid, convenient, reliable, inexpensive, sensitive, and require little material [40]. DPPH is classified as nitrogen centered radical and stable at room temperature because it has virtual of the delocalization of the spare electron over the molecule. The radical scavenging of plant extracts against stable DPPH was determined spectrophotometrically. DPPH radical reacts with antioxidant compound which can donate hydrogen. Antioxidants scavenging DPPH radical by converting DPPH to DPPHn (2,2-diphenyl-1-picrylhydrazine). The changing of color (from deep violet to light yellow) was measured at 517 nm on a visible light spectrophotometer. Radical scavenging activity is reported in term of IC_{50} (Inhibition Concentration at 50 %) [41].

1.4.2 Cytotoxicity against KB and HeLa cell lines

Cytotoxicity assays are widely used by the pharmaceutical industry to screen for cytotoxicity in compound libraries. Researchers can either look for cytotoxic compounds, if they are interested in developing a therapeutic that targets rapidly dividing cancer cells, for instance; or they can screen "hits" from initial high-throughput drug screens for unwanted cytotoxic effects before investing in their development as a pharmaceutical [42]. Cytotoxicity can also be monitored using the MTT or MTS assay. This assay measures the reducing potential of the cell using a colorimetric reaction. Viable cells will reduce the MTS reagent to a colored formazan product. A similar redox-based assay has also been developed using the fluorescent dye, resazurin. In addition to using dyes to indicate the redox potential of cells in order to monitor their viability, researchers have developed assays that use ATP content as a marker of viability [43]. Such ATP-based assays include bioluminescent assays in which ATP is the limiting reagent for the luciferase reaction [44]. Cytotoxicity can also be measured by the sulforhodamine B (SRB) assay, WST assay and clonogenic assay. A label-free approach to follow the cytotoxic response of adherent animal cells in real-time is based on electric impedance measurements when the cells are grown on gold-film electrodes. This technology is referred to as electric

cell-substrate impedance sensing (ECIS). Label-free real-time techniques provide the kinetics of the cytotoxic response rather than just a snapshot like many colorimetric endpoint assays.

The objectives of this research:

The main objectives in this investigation are as follows:

1. To isolate and purify compounds from the acetone crude extract of the roots of *S. roxburghii*.
2. To identify the chemical structures of all isolated compounds.
3. To evaluate the DPPH radical scavenging activity and cytotoxicity against HeLa and KB cell lines of isolated compounds.

CHAPTER II

EXPERIMENTAL

2.1 Plant material

The roots of *S. roxburghii* were collected from Mahasarakham Province of Thailand in April, 2008 and identified by Ms. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, where a voucher specimen (Khumkratok no. 01-10) has been deposited.

2.2 General experimental procedures

NMR spectrum 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 TMS was used 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 Sephadex LH-20 and silica gel 60 Merck cat. No. 7730, 7734 and 7749 were used for quick column chromatography, preparative TLC, open column chromatography and centrifugal 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. High resolution mass spectra were recorded by Micromass LCT and Bruker MICROTOF models. UV-visible adsorption spectrum 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 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

The air-dried roots of *S. roxburghii* (2.0 kg) were successively extracted in a Soxhlet apparatus with CH_2Cl_2 and followed by acetone (each 500 mL, 24 h). The acetone soluble part was evaporated under vacuum to yield 105.4 g. The acetone crude extract was fractionated by vacuum liquid chromatography (VLC) over silica gel (Merck Art 7730) using hexane, CH_2Cl_2, EtOAc and MeOH with increasing polarity to afford six fractions (A-F).

The VLC fraction B was chromatographed on silica gel column using a stepwise gradient system of CH_2Cl_2, EtOAc and MeOH as eluting solvents, to provide four fractions (B1-B4). Fraction B2 was subjected to Sephadex LH-20 using a stepwise gradient elution of CH_2Cl_2 and MeOH to yield three fractions (B2-1-B2-3). Fraction B2-2 was purified by preparative TLC on silica gel using CH_2Cl_2/EtOAc/MeOH (90:5:5) as eluent, to afford melanoxylin A (**1**, 5.2 mg) and caragaphenol A (**2**, 4.5 mg). Fraction B3 was subjected to Sephadex LH-20 using a stepwise gradient elution of CH_2Cl_2 and MeOH to yield four fractions (B3-1-B3-4). Fraction B3-1 yielded ε-viniferin (**3**, 12.1 mg). Fraction B3-2 was purified by centrifugal thin layer chromatography (Chromatotron) with the eluent of CH_2Cl_2/EtOAc/MeOH (80:15:5) to give hopeahainanphenol (**4**, 8.0 mg).

Fraction B3-3 was chromatographed on Chromatotron eluted with CH_2Cl_2/EtOAc/MeOH (80:10:10) to provide two fractions (B3-3-1 and B3-3-2). Fraction B3-3-1 yielded roxburghiol A (**5**, 10.2 mg). Fraction B3-3-2 was purified by preparative TLC using CH_2Cl_2/EtOAc/MeOH (80:10:10) as eluting solvent, to yield 5.5 mg of vitisinol G (**6**).

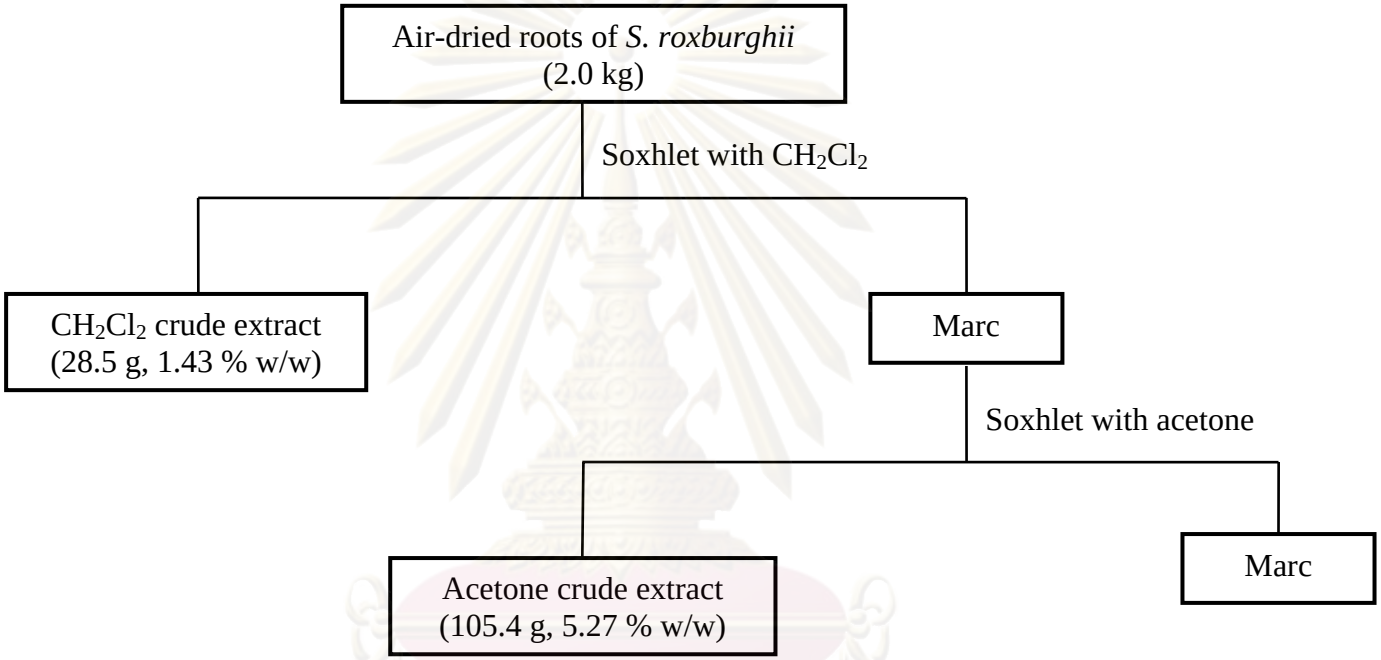
Similarly, the VLC fraction C was chromatographed on silica gel column using a stepwise gradient elution of CH_2Cl_2, EtOAc and MeOH to obtain five fractions (C1-C5). Fraction C2 was chromatographed on silica gel column using CH_2Cl_2/EtOAc/MeOH (80:10:10, 70:20:10 and 60:20:20) as elution systems, to obtain four fractions (C2-1-C2-4). Fraction C2-1 yielded vaticanol A (**7**, 43.6 mg). Fraction C2-3 was chromatographed on Chromatotron eluted with CH_2Cl_2/EtOAc/MeOH (70:20:10), and further purified by preparative TLC using CH_2Cl_2/EtOAc/MeOH (70:20:10 and 70:15:15) as eluting solvents, to give hopeaphenol (**8**, 25.5 mg) and isohopeaphenol (**9**, 8.9 mg). Fraction C3 was chromatographed on silica gel column using CH_2Cl_2/EtOAc/MeOH (70:20:10 and

60:20:20), and 100% EtOAc as elution systems, to obtain three fractions (C3-1-C3-3). Fraction C3-1 was purified by Chromatotron using a mixture of CH_2Cl_2/EtOAc/MeOH (70:20:10) as eluting solvent, to yield apigenin 7-*O*-glucoside (**10**, 13.0 mg). Fraction C3-2 was also purified by Chromatotron eluted with CH_2Cl_2/EtOAc/MeOH (70:20:10 and 70:15:15) to afford *trans*-piceid (**11**, 5.3 mg) and *trans*-3,5,4'-trihydroxystilbene 2-*C*-glucoside (**12**, 32.0 mg). Fraction C4 was subjected to Chromatotron eluted with CH_2Cl_2/EtOAc/MeOH (70:20:10), and further purified by preparative TLC using CH_2Cl_2/EtOAc/MeOH (80:10:10 and 70:15:15) as eluting solvents, to afford neoisohopeaphenol A (**13**, 14.8 mg).

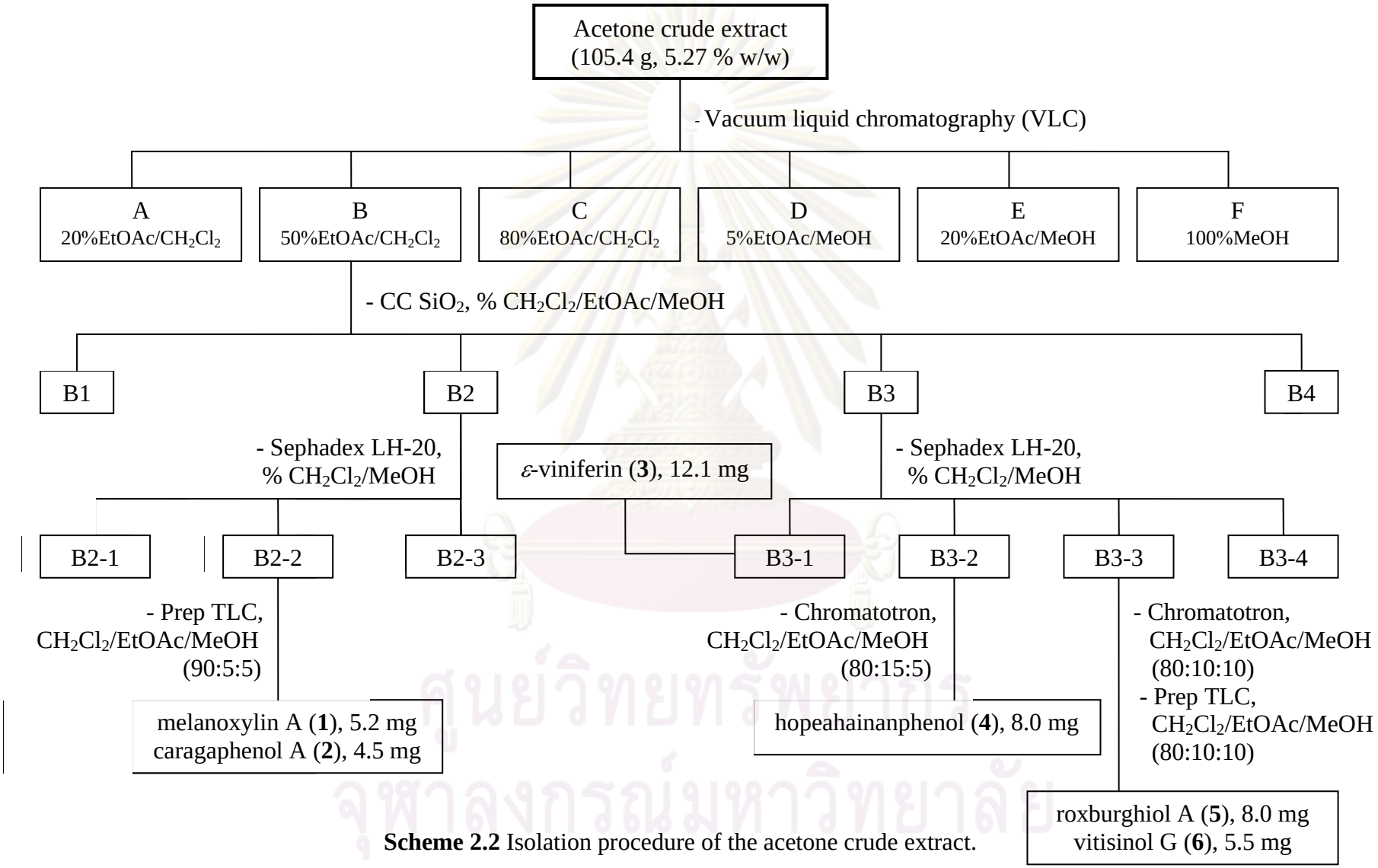
Finally, the VLC fraction D was chromatographed on silica gel column using a gradiant system of CH_2Cl_2/EtOAc/MeOH (70:20:10, 60:30:10 and 60:20:20), and 100% EtOAc to obtain three fractions (D1-D3). Fraction D1 was chromatographed on silica gel column using CH_2Cl_2/EtOAc/MeOH (70:20:10) to obtain two fractions (D1-1 and D1-2). Fraction D1-1 was further purified by preparative TLC using a gradiant system of CH_2Cl_2/EtOAc/MeOH (70:20:10, 60:30:10 and 60:20:20) to give balanocarpol (**14**, 4.8 mg). Fraction D1-2 was also purified by preparative TLC using CH_2Cl_2/EtOAc/MeOH (60:20:20 and 50:30:20) as eluting solvents, to give gnemonol K (**15**, 3.9 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 acetone crude extract of *S. roxburghii* roots were briefly summarized in Schemes 2.1 and 2.2.

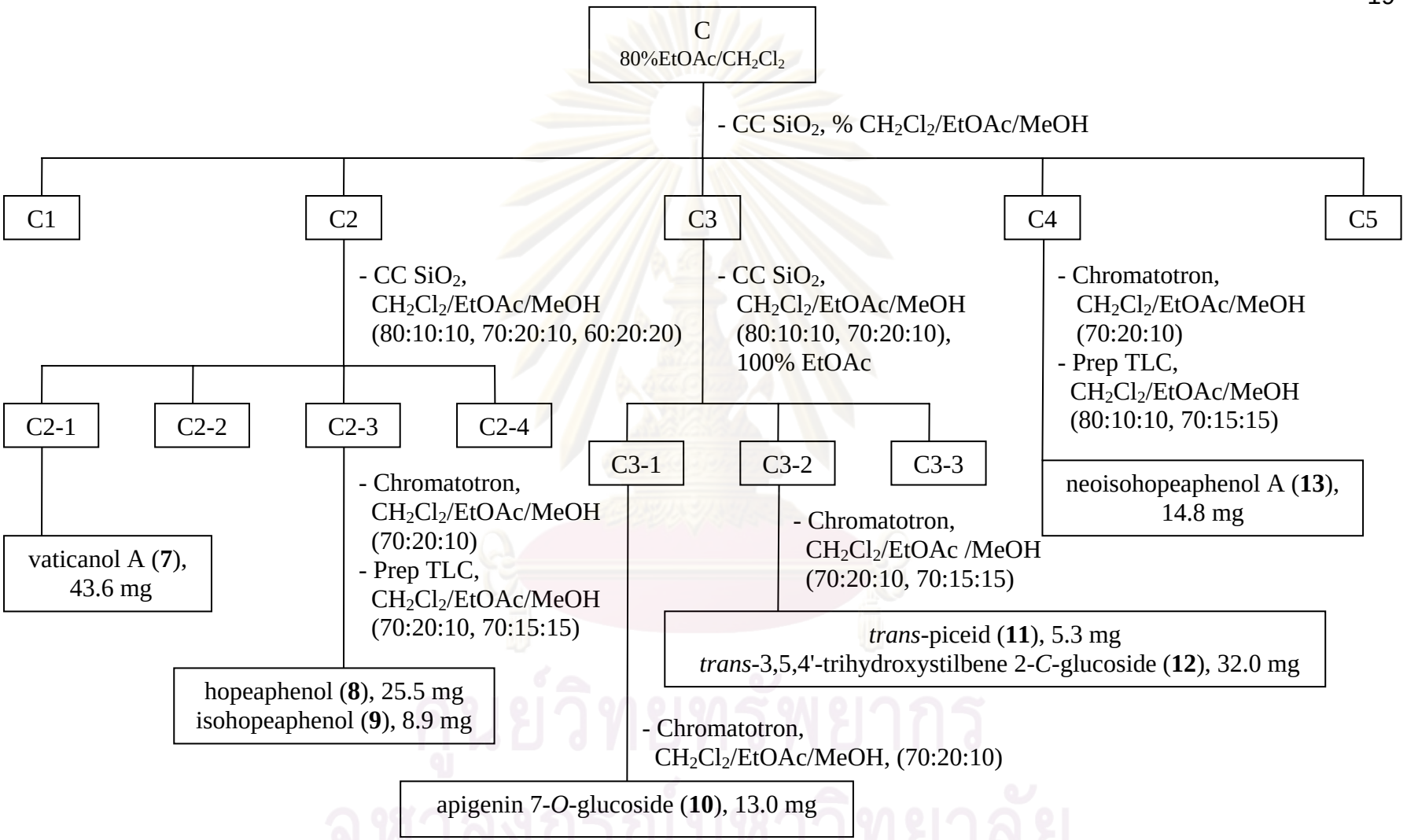


Scheme 2.1 Extraction procedure of *S. roxburghii* roots.

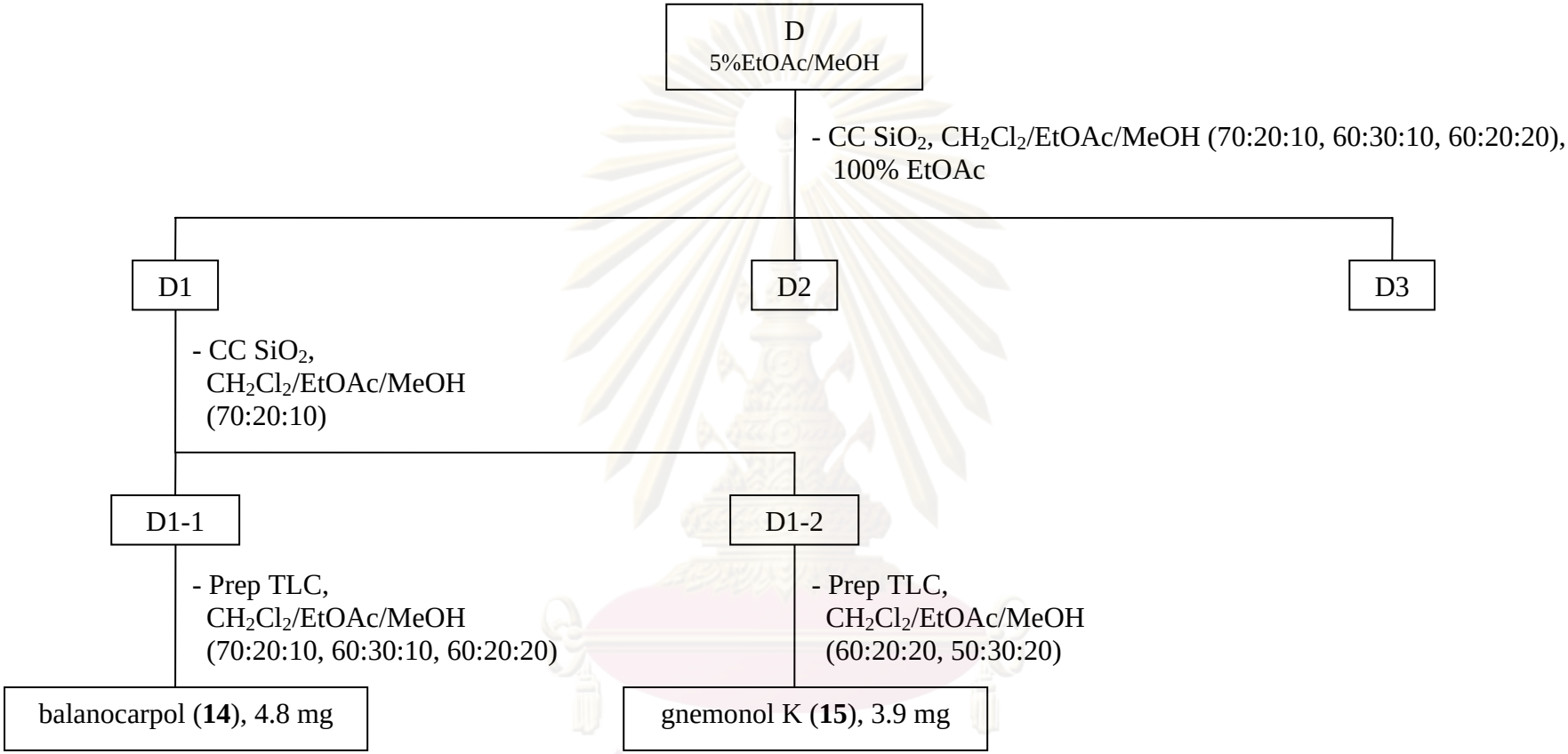


Scheme 2.2 Isolation procedure of the acetone crude extract.

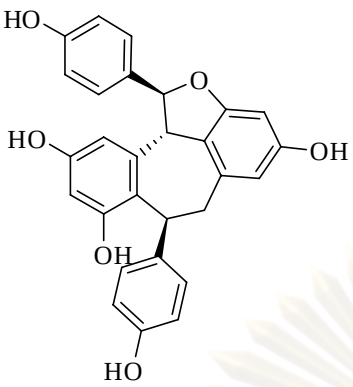
18



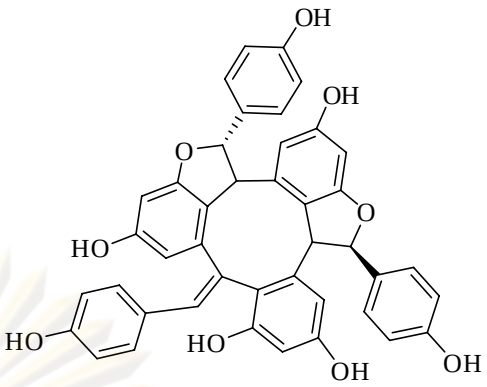
Scheme 2.2 Isolation procedure of the acetone crude extract. (cont.)



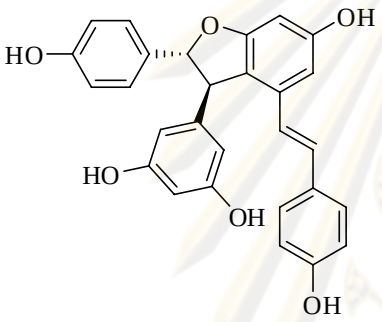
Scheme 2.2 Isolation procedure of the acetone crude extract. (cont.)



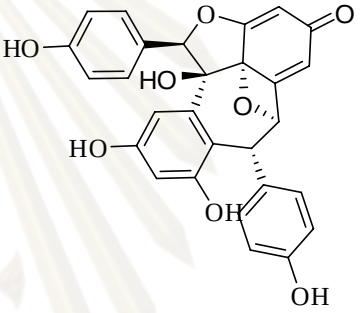
melanoxylin A (**1**)



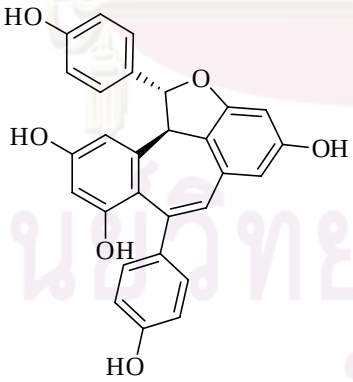
caragaphenol A (**2**)



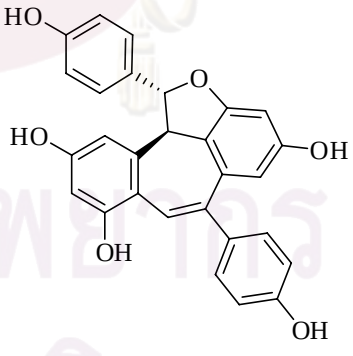
ε-viniferin (**3**)



hopeahainanphenol (**4**)

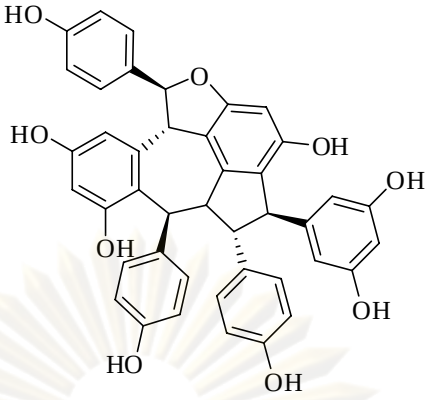


roxburghiol A (**5**)

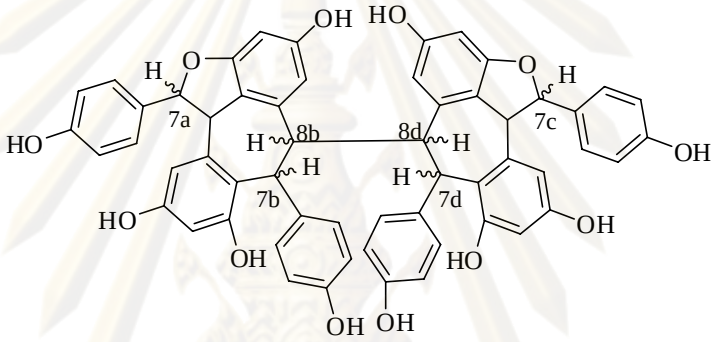


vitisinol G (**6**)

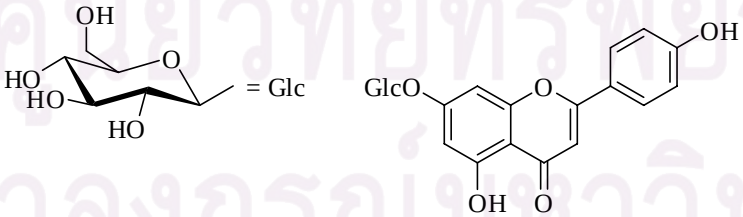
Figure 2.1 Isolated compounds from the roots of *S. roxburghii*.



vaticanol A (**7**)

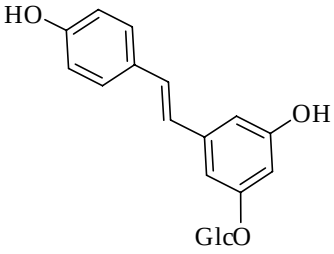


	H-7a	H-7b	H-8b	H-7c	H-7d	H-8d
hopeaphenol (**8**)	β	β	α	α	α	β
isohopeaphenol (**9**)	β	α	β	α	β	α
neoisohopeaphenol (**13**)	α	β	β	β	α	β

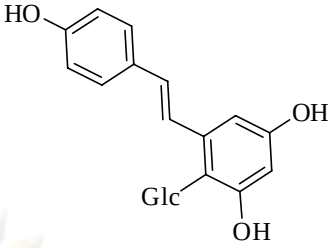


apigenin 7-*O*-glucoside (**10**)

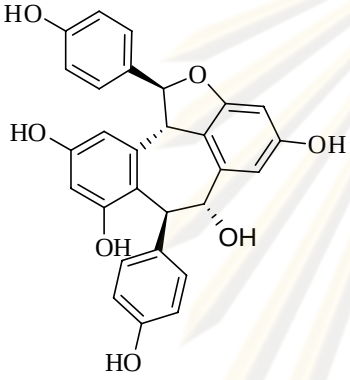
Figure 2.1 Isolated compounds from the roots of *S. roxburghii*. (cont.)



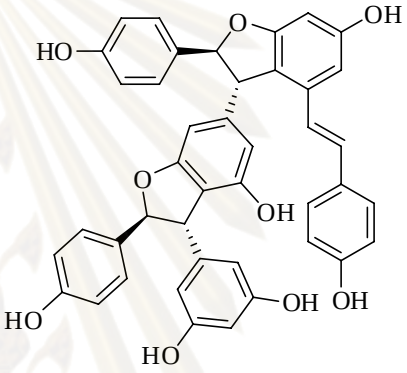
trans-piceid (**11**)



trans-3,5,4'-trihydroxystilbene 2-*C*-glucoside (**12**)



balanocarpol (**14**)



gnemonol K (**15**)

Figure 2.1 Isolated compounds from the roots of *S. roxburghii*. (cont.)

2.4 Bioassay procedures

2.4.1 DPPH radical scavenging activity

TLC autographic method

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical as a TLC spray reagent was confirmed to be well suited for the screening of antioxidants in crude plant extracts. The assay involves with spraying TLC plates with a 0.2 % DPPH solution in methanol. The plates are considered after 30 minutes of spraying. Active compounds appear as yellow spots on a purple background [45].

Spectrophotometric method

After isolation and purification, activities of pure compounds were quantified in this assay. Various concentrations of sample dissolved in methanolic solution (50 µL) were added to DPPH radical methanolic solution (0.3 mM, 200 µL). After incubation for 30 minutes at room temperature in the dark, the absorbance was measured at 517 nm with a UV-Vis spectrophotometer. All tests were run in triplicate and the data were averaged. The scavenging activity was evaluated from the decrease value of 517 nm absorption, which was calculated by the following equation.

$$\% \text{ Radical scavenging} = [1-(A_{sample}/A_{blank})] \times 100$$

The activity was shown as a IC_{50} value that donates the concentration of sample required for scavenging 50% DPPH free radicals [46].

2.4.2 The 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.* [47]. This assay was kindly performed by Natural Products Research Section, Research Division, National Cancer Institute, Thailand.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Preliminary bioassay screening results of crude extracts

Antioxidant activity of crude extracts

The dichloromethane and acetone crude extracts of *S. roxburghii* roots were preliminary evaluated using TLC autographic method for screening of antioxidants with 2,2-diphenyl-1-picrylhydrazyl (DPPH). The screening results of both crude extracts are shown in Figure 3.1.

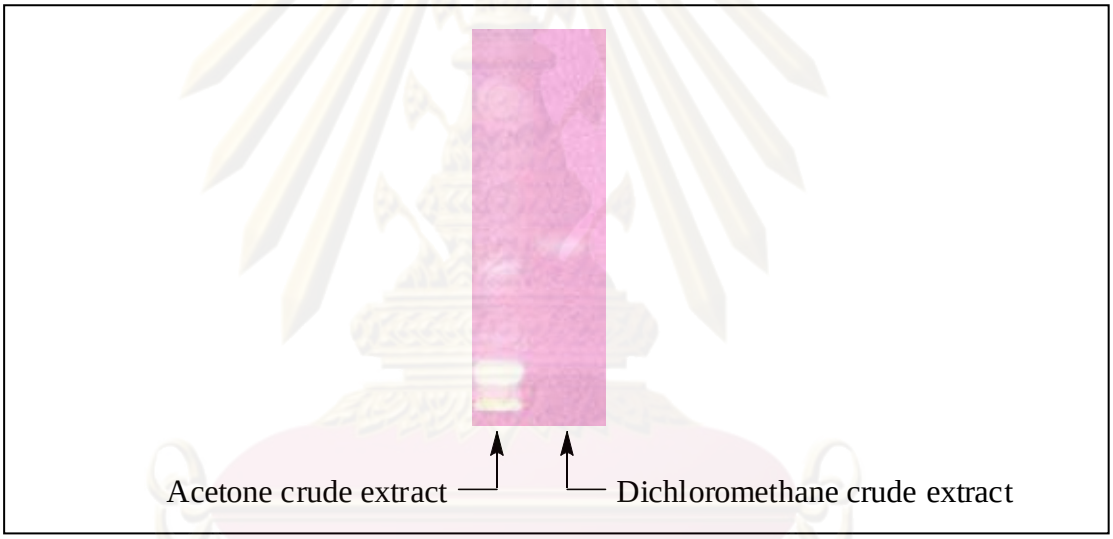


Figure 3.1 The results of screening test based on DPPH radical scavenging activity of dichloromethane and acetone crude extracts.

The acetone crude extract showed the promising activity rather than dichloromethane crude extract (Figure 3.1). Therefore, the acetone crude extract was selected for further investigation.

3.2 Properties and structural elucidation of isolated compounds

3.2.1 Roxburghiol A (5)

Roxburghiol A was obtained as an optically active brown amorphous powder, $[\alpha]_D^{20}$ -66.0° (*c* 0.20, MeOH). The UV spectrum (λ_{max} 315 nm) revealed the presence of a highly conjugated system in the molecule. The molecular formula of $C_{28}H_{20}O_6$ was deduced from the HRESIMS ion at *m*/*z* 453.1364 $[M+H]^+$ (calcd for $C_{28}H_{20}O_6$, 453.1370) and NMR data.

The ^{1}H NMR spectrum exhibited the signals of two sets of *ortho*-coupled protons in the A_2B_2 system on the *para*-substituted phenyl moieties (rings A_1 and B_1) at δ 7.28 (2H, d, *J* = 8.4 Hz, H-2a, 6a)/6.81 (2H, d, *J* = 8.4 Hz, H-3a, 5a) and δ 7.23 (2H, d, *J* = 8.4 Hz, H-2b, 6b)/6.75 (2H, d, *J* = 8.4 Hz, H-3b, 5b), and two sets of *meta*-coupled protons in the AB system on tetrasubstituted benzene rings (rings A_2 and B_2) at δ 6.23 (1H, d, *J* = 2.0 Hz, H-12a)/6.73 (1H, d, *J* = 2.0 Hz, H-14a) and δ 6.25 (1H, d, *J* = 1.6 Hz, H-12b)/6.37 (1H, d, *J* = 1.6 Hz, H-14b). A set of mutually coupled aliphatic methine protons at δ 6.38 (1H, d, *J* = 4.4 Hz, H-7a)/4.05 (1H, d, *J* = 4.4 Hz, H-8a), a olefinic methine proton at δ 7.09 (1H, br s, H-8b), and five phenolic hydroxyl protons at δ 8.50 (1H, br s, OH-4a), 7.39 (1H, br s, OH-11a), 8.55 (1H, br s, OH-13a) and 8.38 (2H, br s, OH-4b, 13b) were also observed in the spectrum.

The molecular formula ($C_{28}H_{20}O_6$) and NMR (^{1}H and ^{13}C) spectral data revealed that **5** was composed of two resveratrol units. The correlations of all protons to the respective carbons were clarified with the help of HMQC and HMBC spectrum. The HMBC correlations (Figure 3.3) between H-7a/C-2a(6a), H-7a/C-11b, H-8a/C-10a, H-8a/C-9b, H-12a/C-10a, H-14a/C-10a, H-8b/C-10a, H-8b/C-1b, H-8b/C-10b, H-8b/C-14b, H-2b(6b)/C-7b revealed the connection between C-1a/C-7a, C-8a/C-9a, C-8a/C-10b, C-1b/C-7b, C-7b/C-10a and C-8b/C-9b. The signals at H-7a (δ 6.38) and H-8a (δ 4.05) with their HMQC correlated at C-7a (δ 85.0) and C-8a (δ 53.2) showed the characteristics of a resveratrol derivative containing a 1,2-diaryl-dihydrobenzofuran moiety [48], which the conformation of this 1,2-diaryl-dihydrobenzofuran was assigned to be *trans*-oriented by NOE experiments.

The absolute configuration of **5** was assigned based on the circular dichroism (CD) spectroscopic evidence. The CD spectra of **5** (Figure 3.4) exhibited the Cotton

signal at 232 nm ($\Delta\varepsilon$ -23.8 (*c* 20.0 μM, MeOH)); the sign and wavelength maxima are consistent with similar one, (-)-ε-viniferin (CD (*c* 32.8 μM, MeOH) nm ($\Delta\varepsilon$): 236 (-24.8)), two chiral centers of which have been assigned as absolute *R* configurations [48]. The structure of **5** has the same absolute configuration as (-)-ε-viniferin. Thus, this new stilbenoid was named as roxburghiol A (Figure 3.2).

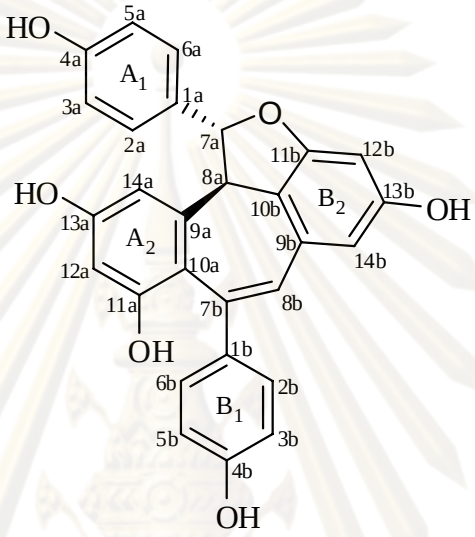


Figure 3.2 Structure of roxburghiol A (**5**, new compound).

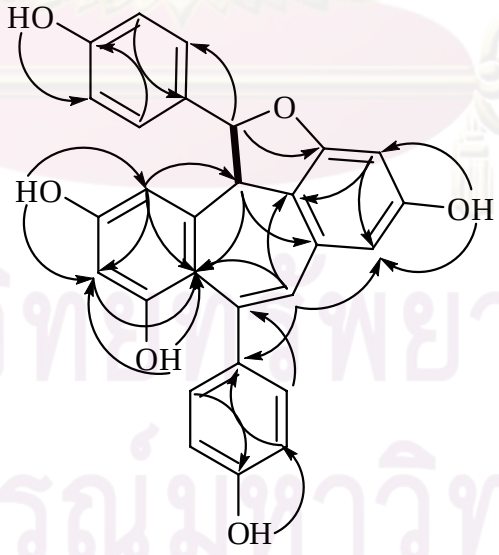


Figure 3.3 Selected HMBC (arrow curves) and COSY (bold lines) correlations of **5**.

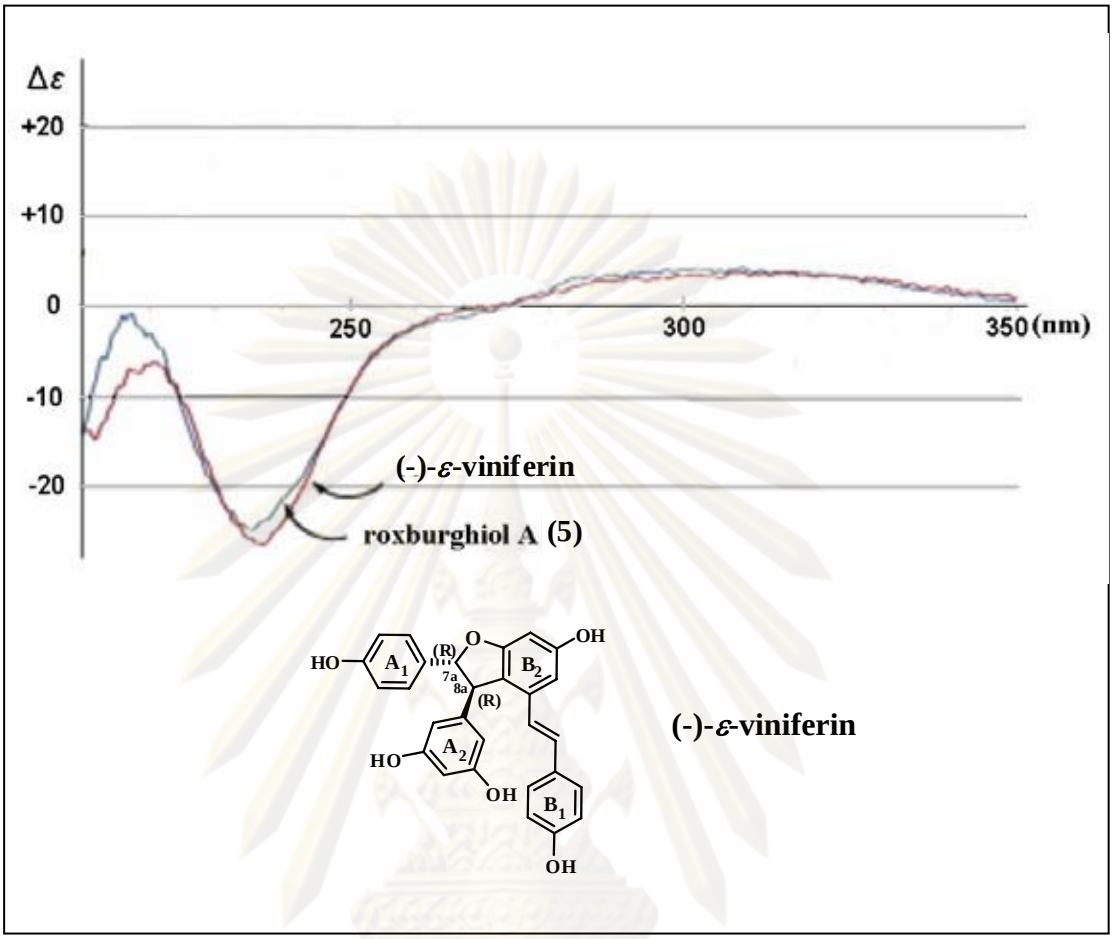


Figure 3.4 The CD spectra of roxburghiol A (**5**) and (-)-ε-viniferin.

Table 3.1 NMR data of **5** in CD_3COCD_3 (400 MHz for ^1H, 100 MHz for ^{13}C).

Position	δ_C	δ_H (mult, *J* in Hz)	HMBC
1a	133.7	-	-
2a, 6a	126.9	7.28 (2H, d, *J*=8.4 Hz)	C-4a, C-7a
3a, 5a	115.4	6.81 (2H, d, *J*=8.4 Hz)	C-1a
4a-OH	157.4	8.50 (1H, s)	C-3a, 5a
7a	85.0	6.38 (1H, d, *J*=4.4 Hz)	C-2a, 6a, C-11b
8a	53.2	4.05 (1H, d, *J*=4.4 Hz)	C-10a, C-9b
9a	134.0	-	-
10a	115.0[a]	-	-
11a-OH	157.0	7.39 (1H, s)	C-10a, C-12a
12a	101.3[b]	6.23 (1H, d, *J*=2.0 Hz)	C-10a, C-14a
13a-OH	158.9	8.55 (1H, s)	C-12a, C-14a
14a	101.2[b]	6.73 (1H, d, *J*=2.0 Hz)	C-8a, C-10a, C-12a
1b	135.9	-	-
2b, 6b	127.6	7.23 (2H, d, *J*=8.4 Hz)	C-4b, C-7b
3b, 5b	115.0[a]	6.75 (2H, d, *J*=8.4 Hz)	C-1b
4b-OH	156.6	8.38[c] (1H, s)	C-3a, 5a
7b	141.8	-	-
8b	127.1	7.09 (1H, br s)	C-10a, C-1b, C-10b, C-14b
9b	145.2	-	-
10b	122.0	-	-
11b	157.7	-	-
12b	95.6	6.25 (1H, d, *J*=1.6 Hz)	C-10b, C-14b
13b-OH	156.9	8.38[c] (1H, s)	C-12b, C-14b
14b	105.0	6.37 (1H, d, *J*=1.6 Hz)	C-10b

[a-c] Signals were overlapped

Melanoxylin A (1) [49]: yellow amorphous powder; ^1H NMR (CD_3COCD_3, 400 MHz): δ 6.97 (2H, d, *J* = 8.8 Hz, H-2a, 6a), 6.64 (2H, d, *J* = 8.8 Hz, H-3a, 5a), 5.60 (1H, brs, H-7a), 4.05 (1H, brs, H-8a), 6.30 (1H, brs, H-12a), 6.10 (1H, brs, H-14a), 6.81 (2H, d, *J* = 8.4 Hz, H-2b, 6b), 6.52 (2H, d, *J* = 8.8 Hz, H-3b, 5b), 5.09 (1H, brt, H-7b), 3.20 (1H, dd, *J* = 3.0, 15.2 Hz, H-8bα), 3.60 (1H, dd, *J* = 3.4, 15.2 Hz, H-8bβ), 5.93 (1H, brs, H-12b), 6.21 (1H, brs, H-14b), 8.48 (1H, s, OH-4a), 8.15 (1H, s, OH-11a), 8.52 (1H, s, OH-13a), 8.20 (1H, s, OH-4b), 8.13 (1H, s, OH-13b). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 130.1 (C-1a), 129.1 (C-2a, 6a), 115.1 (C-3a, 5a), 156.0 (C-4a), 87.4 (C-7a), 48.4 (C-8a), 141.7 (C-9a), 121.9 (C-10a), 159.4 (C-11a), 100.6 (C-12a), 158.5 (C-13a), 104.5 (C-14a), 133.6 (C-1b), 127.7 (C-2b, 6b), 114.6 (C-3b, 5b), 157.2 (C-4b), 35.0 (C-7b), 32.9 (C-8b), 142.6 (C-9b), 118.1 (C-10b), 158.7 (C-11b), 94.79 (C-12b), 154.3 (C-13b), 108.1 (C-14b).

Caragaphenol A (2) [50]: reddish amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 6.89 (2H, d, *J* = 8.4 Hz, H-2a, 6a), 6.55 (2H, d, *J* = 8.4 Hz, H-3a, 5a), 5.72 (1H, d, *J* = 3.5 Hz, H-7a), 4.33 (1H, d, *J* = 3.5 Hz, H-8a), 5.89 (1H, d, *J* = 2.0 Hz, H-12a), 6.05 (1H, d, *J* = 2.0 Hz, H-14a), 6.74 (2H, d, *J* = 8.4 Hz, H-2b, 6b), 6.60 (2H, d, *J* = 8.4 Hz, H-3b, 5b), 4.41 (1H, d, *J* = 11.0 Hz, H-7b), 4.33 (1H, d, *J* = 11.2 Hz, H-8b), 6.21 (1H, d, *J* = 2.5 Hz, H-12b), 5.47 (1H, d, *J* = 2.5 Hz, H-14b), 6.80 (2H, d, *J* = 8.4 Hz, H-2c, 6c), 6.60 (2H, d, *J* = 8.4 Hz, H-3c, 5c), 6.26 (1H, s, H-7c), 6.12 (1H, d, *J* = 1.5 Hz, H-12c), 6.40 (1H, d, *J* = 1.5 Hz, H-14c). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 131.1 (C-1a), 126.9 (C-2a, 6a), 114.7 (C-3a, 5a), 158.4 (C-4a), 89.5 (C-7a), 58.0 (C-8a), 142.0 (C-9a), 117.0 (C-10a), 159.2 (C-11a), 94.5 (C-12a), 158.9 (C-13a), 103.8 (C-14a), 134.2 (C-1b), 129.0 (C-2b, 6b), 115.1 (C-3b, 5b), 148.6 (C-4b), 90.5 (C-7b), 58.0 (C-8b), 137.0 (C-9b), 115.4 (C-10b), 156.9 (C-11b), 101.9 (C-12b), 156.5 (C-13b), 110.3 (C-14b), 128.8 (C-1c), 130.6 (C-2c, 6c), 115.2 (C-3c, 5c), 155.7 (C-4c), 131.7 (C-7c), 135.5 (C-8c), 140.6 (C-9c), 119.8 (C-10c), 159.1 (C-11c), 95.8 (C-12c), 153.4 (C-13c), 109.5 (C-14c).

***ε*-viniferin (3) [48]:** brown amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 7.07 (2H, d, *J* = 8.8 Hz, H-2a, 6a), 6.70 (2H, d, *J* = 8.8 Hz, H-3a, 5a), 5.29 (1H, d, *J* = 5.2 Hz, H-7a), 4.34 (1H, d, *J* = 5.6 Hz, H-8a), 6.11 (3H, brs, H-10a, 12a, 14a), 7.04 (2H, d, *J* = 8.4 Hz, H-2b, 6b), 6.60 (2H, d, *J* = 8.4 Hz, H-3b, 5b), 6.78 (1H, d, *J* = 16.4 Hz, H-7b), 6.58 (1H, d, *J* = 16.2 Hz, H-8b), 6.19 (1H, d, *J* = 1.6 Hz, H-12b), 6.59 (1H, d, *J* = 1.2 Hz, H-14b), 8.40 (1H, s, OH-4a), 8.16 (2H, s, OH-11a, 13a), 8.44 (1H, s, OH-4b), 8.36 (1H, s, OH-13b). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 132.9 (C-1a), 127.0 (C-2a, 6a), 115.2 (C-3a, 5a), 157.3 (C-4a), 92.9 (C-7a), 56.2 (C-8a), 146.5 (C-9a), 106.0 (C-10a, 14a), 158.9 (C-11a, 13a), 101.2 (C-12a), 135.5 (C-1b), 127.8 (C-2b, 6b), 115.4 (C-3b, 5b), 157.3 (C-4b), 129.1 (C-7b), 122.5 (C-8b), 128.9 (C-9b), 118.9 (C-10b), 161.5 (C-11b), 95.9 (C-12b), 158.7 (C-13b), 103.2 (C-14b).

Hopeahainanphenol (4) [12]: brown amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 7.52 (2H, d, *J* = 8.8 Hz, H-2a, 6a), 6.84 (2H, d, *J* = 8.8 Hz, H-3a, 5a), 5.70 (1H, brs, H-7a), 6.47 (1H, brs, H-12a), 6.25 (1H, brs, H-14a), 6.92 (2H, d, *J* = 8.8 Hz, H-2b, 6b), 6.52 (2H, d, *J* = 8.8 Hz, H-3b, 5b), 5.12 (1H, brs, H-7b), 4.69 (1H, brs, H-8b), 5.33 (1H, brs, H-12b), 6.15 (1H, brs, H-14b), 8.77 (1H, s, OH-4a), 8.72 (1H, s,

OH-11a), 8.38 (1H, s, OH-13a), 8.72 (1H, s, OH-4b), 8.01 (1H, s, OH-13b). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 126.3 (C-1a), 131.2 (C-2a, 6a), 115.8 (C-3a, 5a), 158.9 (C-4a), 87.4 (C-7a), 74.0 (C-8a), 128.7 (C-9a), 119.2 (C-10a), 156.1 (C-11a), 104.4 (C-12a), 156.5 (C-13a), 110.3 (C-14a), 130.1 (C-1b), 129.3 (C-2b, 6b), 114.5 (C-3b, 5b), 155.8 (C-4b), 47.5 (C-7b), 71.5 (C-8b), 149.2 (C-9b), 62.6 (C-10b), 170.0 (C-11b), 102.1 (C-12b), 186.1 (C-13b), 129.5 (C-14b).

Roxburghiol A (5): brown amorphous powder; mp 234-236 °C; $[\alpha]_D^{20}$ -66.0° (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 315 (4.8), 279 (2.9) nm; IR bands (KBr): 3399, 2925, 2856, 1606, 1512, 1444, 1384, 1254, 1111, 830 cm^{-1}; positive ion HRESIMS *m/z*: $[M+H]^+$ 453.1364 (calcd for $C_{28}H_{20}O_6$, 453.1370); ^{1}H NMR (CD_3COCD_3, 400 MHz) and ^{13}C NMR (CD_3COCD_3, 100 MHz) are shown in Table 3.1.

Vitisinol G (6) [51]: brown amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 7.20 (2H, d, *J* = 8.0 Hz, H-2a, 6a), 6.72 (2H, d, *J* = 8.0 Hz, H-3a, 5a), 6.26 (1H, d, *J* = 3.6 Hz, H-7a), 4.01 (1H, d, *J* = 3.6 Hz, H-8a), 6.55 (1H, brs, H-12a), 6.26 (1H, brs, H-14a), 7.20 (2H, d, *J* = 8.0 Hz, H-2b, 6b), 6.74 (2H, d, *J* = 8.0 Hz, H-3b, 5b), 7.20 (1H, brs, H-7b), 6.22 (1H, brs, H-12b), 5.97 (1H, brs, H-14b), 8.45 (1H, s, OH-4a), 8.46 (1H, s, OH-11a, 13a), 8.67 (1H, s, OH-4b), 8.11 (1H, s, OH-13b). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 138.4 (C-1a), 126.9 (C-2a, 6a), 115.3 (C-3a, 5a), 157.4 (C-4a), 85.0 (C-7a), 51.9 (C-8a), 133.6 (C-9a), 114.7 (C-10a), 156.8 (C-11a, 13a), 101.9 (C-12a), 104.9 (C-14a), 34.1 (C-1b), 124.5 (C-2b, 6b), 114.9 (C-3b, 5b), 157.0 (C-4b), 130.2 (C-7b), 138.2 (C-8b), 141.8 (C-9b), 120.2 (C-10b), 158.6 (C-11b), 96.2 (C-12b), 157.8 (C-13b), 106.0 (C-14b).

Vaticanol A (7) [52]: yellow amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 7.14 (2H, d, *J* = 8.8 Hz, H-2a, 6a), 6.69 (2H, d, *J* = 8.8 Hz, H-3a, 5a), 6.04 (1H, d, *J* = 3.9 Hz, H-7a), 4.37 (1H, d, *J* = 3.9 Hz, H-8a), 5.94 (1H, d, *J* = 2.0 Hz, H-12a), 6.34 (1H, d, *J* = 2.0 Hz, H-14a), 6.46 (2H, d, *J* = 8.8 Hz, H-2b, 6b), 6.90 (2H, d, *J* = 8.8 Hz, H-3b, 5b), 5.02 (1H, brs, H-7b), 4.37 (1H, brs, H-8b), 6.08 (1H, brs, H-12b), 6.41 (2H, d, *J* = 8.4 Hz, H-2c, 6c), 6.23 (2H, d, *J* = 8.4 Hz, H-3c, 5c), 3.50 (1H, brs, H-7c), 4.05 (1H, brs, H-8c), 6.13 (2H, brs, H-10c, 14c), 6.06 (1H, brs, H-12c). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 133.6 (C-1a), 127.2 (C-2a, 6a), 115.3 (C-3a, 5a),

157.8 (C-4a), 85.8 (C-7a), 49.5 (C-8a), 143.9 (C-9a), 118.5 (C-10a), 157.6 (C-11a), 100.5 (C-12a), 156.1 (C-13a), 102.4 (C-14a), 137.9 (C-1b), 128.4 (C-2b, 6b), 114.2 (C-3b, 5b), 155.7 (C-4b), 35.3 (C-7b), 47.9 (C-8b), 144.1 (C-9b), 117.8 (C-10b), 159.6 (C-11b), 94.5 (C-12b), 156.3 (C-13b), 121.5 (C-14b), 134.9 (C-1c), 128.8 (C-2c, 6c), 114.2 (C-3c, 5c), 153.6 (C-4c), 63.6 (C-7c), 56.7 (C-8c), 146.8 (C-9c), 105.9 (C-10c, 14c), 153.0 (C-11c, 13c), 100.6 (C-12c).

Hopeaphenol (8) [17]: brown amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 7.01 (4H, d, *J* = 8.8 Hz, H-2a, 6a, 2c, 6c), 6.66 (4H, d, *J* = 8.8 Hz, H-3a, 5a, 3c, 5c), 5.63 (2H, d, *J* = 4.0 Hz, H-7a, 7c), 4.10 (2H, d, *J* = 3.8 Hz, H-8a, 8c), 6.42 (2H, d, *J* = 1.2 Hz, H-12a, 12c), 6.16 (2H, d, *J* = 1.2 Hz, H-14a, 14c), 6.78 (4H, d, *J* = 8.8 Hz, H-2b, 6b, 2d, 6d), 6.43 (4H, d, *J* = 8.8 Hz, H-3b, 5b, 3d, 5d), 5.67 (2H, brs, H-7b, 7d), 3.81 (2H, brs, H-8b, 8d), 5.60 (2H, brs, H-12b, 12d), 5.04 (2H, brs, H-14b, 14d). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 130.0 (C-1a, 1c), 129.4 (C-2a, 6a, 2c, 6c), 115.1 (C-3a, 5a, 3c, 5c), 157.6 (C-4a, 4c), 87.3 (C-7a, 7c), 48.8 (C-8a, 8c), 141.5 (C-9a, 9c), 120.2 (C-10a, 10c), 157.9 (C-11a, 11c), 100.2 (C-12a, 12c), 156.2 (C-13a, 13c), 105.4 (C-14a, 14c), 134.3 (C-1b, 1d), 128.4 (C-2b, 6b, 2d, 6d), 114.3 (C-3b, 5b, 3d, 5d), 154.7 (C-4b, 4d), 40.3 (C-7b, 7d), 47.3 (C-8b, 8d), 139.5 (C-9b, 9d), 117.7 (C-10b, 10d), 158.3 (C-11b, 11d), 94.3 (C-12b, 12d), 156.3 (C-13b, 13d), 110.3 (C-14b, 14d).

Isohopeaphenol (9) [53]: brown amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 7.42 (4H, d, *J* = 8.8 Hz, H-2a, 6a, 2c, 6c), 6.89 (4H, d, *J* = 8.8 Hz, H-3a, 5a, 3c, 5c), 5.53 (2H, d, *J* = 8.0 Hz, H-7a, 7c), 5.33 (2H, d, *J* = 8.0 Hz, H-8a, 8c), 6.28 (2H, d, *J* = 2.2 Hz, H-12a, 12c), 6.17 (2H, d, *J* = 2.2 Hz, H-14a, 14c), 6.28 (4H, d, *J* = 8.8 Hz, H-2b, 6b, 2d, 6d), 6.22 (4H, d, *J* = 8.8 Hz, H-3b, 5b, 3d, 5d), 5.04 (2H, brs, H-7b, 7d), 3.33 (2H, brs, H-8b, 8d), 5.73 (2H, d, *J* = 2.0 Hz, H-12b, 12d), 5.39 (2H, d, *J* = 2.0 Hz, H-14b, 14d). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 131.2 (C-1a, 1c), 129.7 (C-2a, 6a, 2c, 6c), 114.3 (C-3a, 5a, 3c, 5c), 154.7 (C-4a, 4c), 91.9 (C-7a, 7c), 51.5 (C-8a, 8c), 138.4 (C-9a, 9c), 116.3 (C-10a, 10c), 155.2 (C-11a, 11c), 100.2 (C-12a, 12c), 153.8 (C-13a, 13c), 104.9 (C-14a, 14c), 135.5 (C-1b, 1d), 127.8 (C-2b, 6b, 2d, 6d), 112.3 (C-3b, 5b, 3d, 5d), 152.1 (C-4b, 4d), 41.5 (C-7b, 7d), 50.7 (C-8b, 8d), 139.6 (C-9b, 9d), 115.1 (C-10b, 10d), 157.6 (C-11b, 11d), 92.7 (C-12b, 12d), 154.5 (C-13b, 13d), 107.6 (C-14b, 14d).

Apigenin 7-*O*-glucoside (10) [54]: brown amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 7.25 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.74 (2H, d, *J* = 8.8 Hz, H-3', 5'), 7.42 (1H, brs, H-3), 6.96 (1H, brs, H-6), 6.83 (1H, brs, H-8), 4.82 (1H, d, *J* = 9.5 Hz, H-1''), 3.63 (1H, m, H-2''), 3.43 (1H, m, H-3''), 3.48 (1H, m, H-4''), 3.28 (1H, m, H-5''), 3.72 (1H, m, H-6''), 3.78 (1H, m, H-6''). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 130.2 (C-1'), 127.8 (C-2', 6'), 115.5 (C-3', 5'), 157.4 (C-4'), 165.0 (C-2), 106.3 (C-3), 184.2 (C-4), 158.6 (C-5), 103.1 (C-6), 159.4 (C-7), 105.6 (C-8), 76.8 (C-1''), 74.5 (C-2''), 79.7 (C-3''), 71.3 (C-4''), 82.3 (C-5''), 62.1 (C-6'').

***Trans*-piceid (11) [12]:** brown amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 7.30 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.71 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.96 (1H, d, *J* = 16.0 Hz, H-α), 6.78 (1H, d, *J* = 16.0 Hz, H-β), 6.54 (1H, brs, H-2), 6.35 (1H, brs, H-4), 6.68 (1H, brs, H-6), 4.82 (1H, d, *J* = 9.5 Hz, H-1''), 3.63 (1H, m, H-2''), 3.43 (1H, m, H-3''), 3.48 (1H, m, H-4''), 3.28 (1H, m, H-5''), 3.72 (1H, m, H-6''), 3.78 (1H, m, H-6''). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 130.2 (C-1'), 127.8 (C-2', 6'), 115.5 (C-3', 5'), 157.4 (C-4'), 129.3 (C-α), 125.6 (C-β), 139.9 (C-1), 107.3 (C-2), 158.6 (C-3), 103.1 (C-4), 159.4 (C-5), 105.6 (C-6), 76.8 (C-1''), 74.5 (C-2''), 79.7 (C-3''), 71.3 (C-4''), 82.3 (C-5''), 62.1 (C-6'').

***Trans*-3,5,4'-trihydroxystilbene 2-*C*-glucoside (12) [54]:** brown amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 7.27 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.70 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.69 (1H, d, *J* = 15.4 Hz, H-α), 6.73 (1H, d, *J* = 15.4 Hz, H-β), 6.47 (1H, brs, H-4), 6.12 (1H, brs, H-6), 4.71 (1H, d, *J* = 9.8 Hz, H-1''), 3.87 (1H, m, H-2''), 3.45 (1H, m, H-3''), 3.50 (1H, m, H-4''), 3.36 (1H, m, H-5''), 3.71 (1H, m, H-6''), 3.81 (1H, m, H-6''). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 129.9 (C-1'), 127.7 (C-2', 6'), 115.4 (C-3', 5'), 157.3 (C-4'), 129.4 (C-α), 125.1 (C-β), 139.8 (C-1), 104.6 (C-6), 157.7 (C-5), 103.0 (C-4), 157.6 (C-3), 113.8 (C-2), 77.0 (C-1''), 73.5 (C-2''), 79.6 (C-3''), 70.9 (C-4''), 82.1 (C-5''), 62.4 (C-6'').

Neoisohopeaphenol A (13) [12]: brown amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 7.09 (2H, d, *J* = 8.8 Hz, H-2a, 6a), 6.64 (2H, d, *J* = 8.8 Hz, H-3a, 5a), 5.64 (1H, d, *J* = 3.2 Hz, H-7a), 4.28 (1H, d, *J* = 3.2 Hz, H-8a), 6.24 (1H, brs, H-12a), 5.98 (1H, brs, H-14a), 6.80 (2H, d, *J* = 8.8 Hz, H-2b, 6b), 6.39 (2H, d, *J* = 8.8 Hz, H-

3b, 5b), 5.15 (1H, brs, H-7b), 3.27 (1H, brs, H-8b), 6.09 (1H, brs, H-12b), 6.63 (1H, brs, H-14b), 6.93 (2H, d, *J* = 8.8 Hz, H-2c, 6c), 6.69 (2H, d, *J* = 8.8 Hz, H-3c, 5c), 4.79 (1H, d, *J* = 4.0 Hz, H-7c), 3.38 (1H, d, *J* = 4.0 Hz, H-8c), 5.95 (1H, brs, H-12c), 5.20 (1H, brs, H-14c), 6.57 (2H, d, *J* = 8.8 Hz, H-2d, 6d), 6.34 (2H, d, *J* = 8.8 Hz, H-3d, 5d), 4.41 (1H, brs, H-7d), 3.75 (1H, brs, H-8d), 5.88 (1H, brs, H-12d), 5.20 (1H, brs, H-14d). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 129.9 (C-1a), 129.2 (C-2a, 6a), 115.2 (C-3a, 5a), 156.5 (C-4a), 89.6 (C-7a), 47.9 (C-8a), 140.8 (C-9a), 123.8 (C-10a), 154.9 (C-11a), 100.8 (C-12a), 155.3 (C-13a), 104.8 (C-14a), 132.6 (C-1b), 129.9 (C-2b, 6b), 114.7 (C-3b, 5b), 154.2 (C-4b), 36.4 (C-7b), 54.5 (C-8b), 142.2 (C-9b), 112.4 (C-10b), 156.9 (C-11b), 94.9 (C-12b), 155.7 (C-13b), 104.9 (C-14b), 133.9 (C-1c), 127.1 (C-2c, 6c), 115.3 (C-3c, 5c), 156.4 (C-4c), 93.5 (C-7c), 54.5 (C-8c), 147.1 (C-9c), 124.0 (C-10c), 155.8 (C-11c), 101.3 (C-12c), 156.4 (C-13c), 105.5 (C-14c), 131.6 (C-1d), 128.4 (C-2d, 6d), 115.3 (C-3d, 5d), 156.1 (C-4d), 53.1 (C-7d), 56.4 (C-8d), 140.1 (C-9d), 120.6 (C-10d), 157.4 (C-11d), 95.6 (C-12d), 156.2 (C-13d), 104.8 (C-14d).

Balanocarpol (14) [55]: brown amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 7.38 (2H, d, *J* = 8.0 Hz, H-2a, 6a), 6.84 (2H, d, *J* = 8.2 Hz, H-3a, 5a), 5.59 (1H, brs, H-7a), 5.05 (1H, brs, H-8a), 6.15 (1H, brs, H-12a), 6.07 (1H, brs, H-14a), 6.64 (2H, d, *J* = 8.2 Hz, H-2b, 6b), 6.31 (2H, d, *J* = 8.2 Hz, H-3b, 5b), 4.80 (1H, *J* = 9.2 Hz, H-7b), 5.28 (1H, *J* = 9.2 Hz, H-8b), 5.85 (1H, brs, H-12b), 5.99 (1H, brs, H-14b). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 132.9 (C-1a), 129.5 (C-2a, 6a), 115.5 (C-3a, 5a), 157.6 (C-4a), 92.5 (C-7a), 51.4 (C-8a), 139.8 (C-9a), 119.5 (C-10a), 156.4 (C-11a), 101.1 (C-12a), 155.9 (C-13a), 105.8 (C-14a), 132.5 (C-1b), 130.5 (C-2b, 6b), 113.2 (C-3b, 5b), 154.8 (C-4b), 49.3 (C-7b), 72.2 (C-8b), 141.8 (C-9b), 112.8 (C-10b), 158.7 (C-11b), 94.1 (C-12b), 158.2 (C-13b), 103.4 (C-14b).

Gnemonol K (15) [56]: brown amorphous powder; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 7.25 (2H, d, *J* = 8.8 Hz, H-2a, 6a), 6.76 (2H, d, *J* = 8.8 Hz, H-3a, 5a), 5.56 (1H, d, *J* = 5.0 Hz, H-7a), 4.48 (1H, d, *J* = 5.0 Hz, H-8a), 6.24 (1H, brs, H-10a), 6.31 (1H, brs, H-12a), 6.25 (1H, brs, H-14a), 7.26 (2H, d, *J* = 8.4 Hz, H-2b, 6b), 6.80 (2H, d, *J* = 8.4 Hz, H-3b, 5b), 5.54 (1H, d, *J* = 4.5 Hz, H-7b), 4.64 (1H, d, *J* = 4.5 Hz, H-8b), 6.62 (1H, brs, H-10b), 6.21 (1H, brs, H-14b), 7.25 (2H, d, *J* = 8.4 Hz, H-2c, 6c), 6.84 (2H, d, *J* = 8.4 Hz, H-3c, 5c), 6.95 (1H, d, *J* = 16.0 Hz, H-7c), 6.73 (1H, d, *J* =

16.0 Hz, H-8c), 6.32 (1H, d, *J* = 2.0 Hz, H-12c), 6.73 (1H, d, *J* = 1.8 Hz, H-14c). ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 132.9 (C-1a), 127.4 (C-2a, 6a), 115.9 (C-3a, 5a), 158.1 (C-4a), 93.2 (C-7a), 53.7 (C-8a), 144.8 (C-9a), 104.6 (C-10a), 159.3 (C-11a), 102.5 (C-12a), 159.1 (C-13a), 104.4 (C-14a), 133.2 (C-1b), 127.8 (C-2b, 6b), 115.6 (C-3b, 5b), 156.5 (C-4b), 92.9 (C-7b), 58.2 (C-8b), 148.8 (C-9b), 101.2 (C-10b), 159.8 (C-11b), 113.0 (C-12b), 156.2 (C-13b), 108.4 (C-14b), 129.9 (C-1c), 128.6 (C-2c, 6c), 115.9 (C-3c, 5c), 155.8 (C-4c), 131.2 (C-7c), 126.7 (C-8c), 133.2 (C-9c), 119.2 (C-10c), 161.0 (C-11c), 95.8 (C-12c), 158.6 (C-13c), 103.8 (C-14c).

3.3 Bioassay activity of isolated compounds

3.3.1 Antioxidant activity of isolated compounds

The isolation and purification of acetone crude extract from *S. roxburghii* roots yielded fifteen compounds including a new stilbenoid (**5**). The antioxidant activity of isolated compounds was evaluated for inhibitory activity toward DPPH radical scavenging activity. Their activity was expressed as IC_{50} (mM). The biological activity results of all tested compounds are shown in Table 3.2.

Table 3.2 Antioxidant activity of isolated compounds.

Isolated compounds	**IC_{50} (mM)**
	DPPH scavenging
Melanoxylin A (**1**)	-
Caragaphenol A (**2**)	-
ε-viniferin (**3**)	0.36 ± 0.04
Hopeahainanphenol (**4**)	0.28 ± 0.02
Roxburghiol A (**5**)	0.38 ± 0.01
Vitisinol G (**6**)	0.32 ± 0.01
Vaticanol A (**7**)	0.41 ± 0.02
Hopeaphenol (**8**)	0.31 ± 0.01
Isohopeaphenol (**9**)	0.23 ± 0.00
Apigenin 7-*O*-glucoside (**10**)	0.30 ± 0.01
Trans-piceid (**11**)	0.34 ± 0.00
Trans-3,5,4'-trihydroxystilbene 2-*C*-glucoside (**12**)	0.36 ± 0.01
Neoisohopeaphenol A (**13**)	0.30 ± 0.03
Balanocarpol (**14**)	0.31 ± 0.01
Gnemonol K (**15**)	-
Ascorbic acid*	0.02 ± 0.00

* Standard antioxidant

-: Not determined

Antioxidant activity of isolated compounds was tested against various radical sources by UV-Vis spectroscopy. Anti-radical property of flavonoid glycoside and stilbenoids was examined with DPPH, which is widely used for assessing the ability of polyphenol to transfer labile H-atoms to radicals. The greater effectiveness of compounds was possible due to the presence *ortho*-dihydroxy groups (catechol) which upon donating hydrogen radicals will give higher stability to their radical forms [57].

DPPH radical scavenging activity results in Table 3.2 showed that most of tested compounds had IC_{50} values in the range of 0.23-0.41 mM. They were non catecholic compounds, which showed antioxidant activity less than ascorbic acid, a positive control (IC_{50} = 0.02 mM).

3.3.2 Cytotoxicity against KB and HeLa cell lines of isolated compounds

The cytotoxicity against HeLa and KB cell lines of isolated compounds were determined using MTT colorimetric assay and the results were shown in Table 3.3.

Table 3.3 *In vitro* cytotoxicity of isolated compounds against HeLa and KB cells.

Isolated compounds	IC_{50} (µg/mL)	
	HeLa cell	**KB cell**
Melanoxylin A (**1**)	93.52	50.14
Caragaphenol A (**2**)	>100	48.12
ε-viniferin (**3**)	67.15	32.0
Hopeahainanphenol (**4**)	>100	>100
Roxburghiol A (**5**)	57.38	17.15
Vitisinol G (**6**)	68.42	15.56
Vaticanol A (**7**)	95.29	>100
Hopeaphenol (**8**)	8.66	6.47
Isohopeaphenol (**9**)	10.12	8.50
Apigenin 7-*O*-glucoside (**10**)	>100	80.0
Trans-piceid (**11**)	>100	>100
Trans-3,5,4'-trihydroxystilbene 2-*C*-glucoside (**12**)	>100	42.05
Neoisohopeaphenol A (**13**)	-	-
Balanocarpol (**14**)	-	-
Gnemonol K (**15**)	-	-
Adriamycin*	2.48	2.16

* Standard agent

-: Not determined

HeLa cell line: Human cervical carcinoma

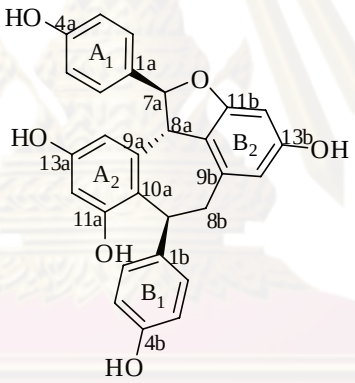
KB cell line: Human epidermoid carcinoma

The cytotoxicity results (Table 3.3) showed that most of tested compounds exhibited cytotoxic activity against KB cell line more than HeLa cell line. Compounds **8** and **9** showed modest cytotoxic activity against KB cell line with IC_{50} = 6.47 and 8.50 μg/mL, and against HeLa cell line with IC_{50} = 8.66 and 10.12 μg/mL, respectively. Compounds **3**, **5** and **6** showed mild cytotoxic activity against KB cell line with IC_{50} = 32.0, 17.15 and 15.56 μg/mL, and against HeLa cell line with IC_{50} = 67.15, 57.38 and 68.42 μg/mL, respectively. In addition, compounds **2** and **12** showed specific activity against only KB cell line (IC_{50} = 48.12 and 42.05 µg/mL). Compounds **4**, **7**, **10** and **11** could be regarded as inactive toward both cell lines.

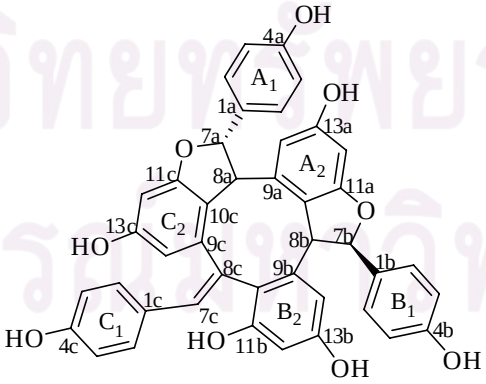
CHAPTER IV

CONCLUSION

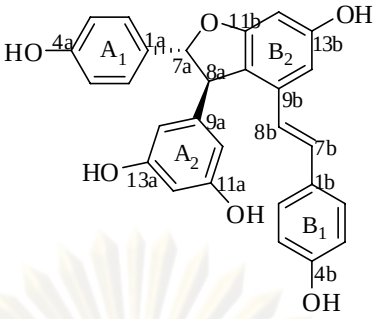
In conclusion, the isolation and purification of the acetone crude extract from the roots of *S. roxburghii* afforded a new stilbenoid, roxburghiol A (**5**), along with fourteen known compounds, melanoxylin A (**1**), caragaphenol A (**2**), ε-viniferin (**3**), hopeahainanphenol (**4**), vitisinol G (**6**), vaticanol A (**7**), hopeaphenol (**8**), isohopeaphenol (**9**), apigenin 7-*O*-glucoside (**10**), *trans*-piceid (**11**), *trans*-3,5,4'-trihydroxystilbene 2-*C*-glucoside (**12**), neoisohopeaphenol A (**13**), balanocarpol (**14**) and gnemonol K (**15**). The chemical structures of all isolated compounds were characterized by means of spectral analysis as well as comparison with the previous literature data.



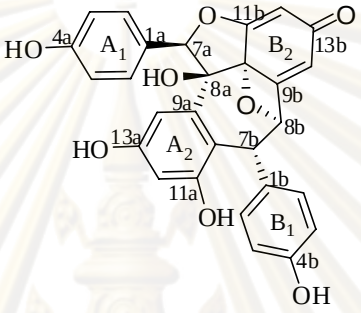
melanoxylin A (**1**)



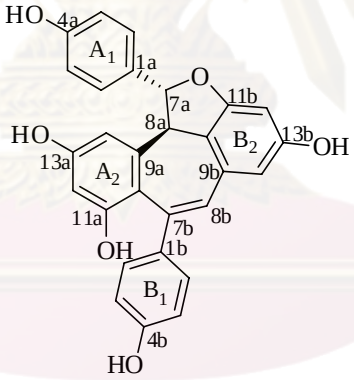
caragaphenol A (**2**)



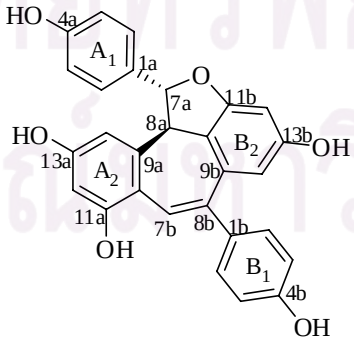
ε-viniferin (**3**)



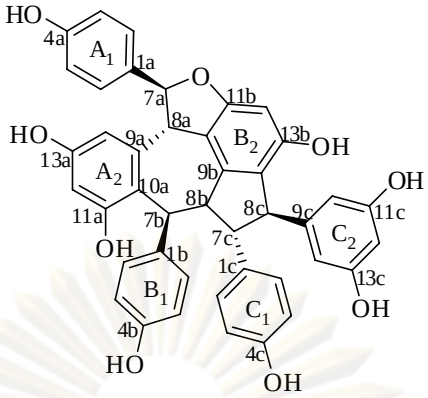
hopeahainanphenol (**4**)



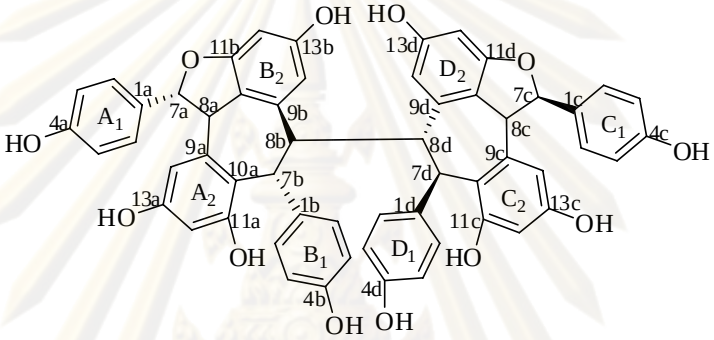
roxburghiol A (**5,** new compound)



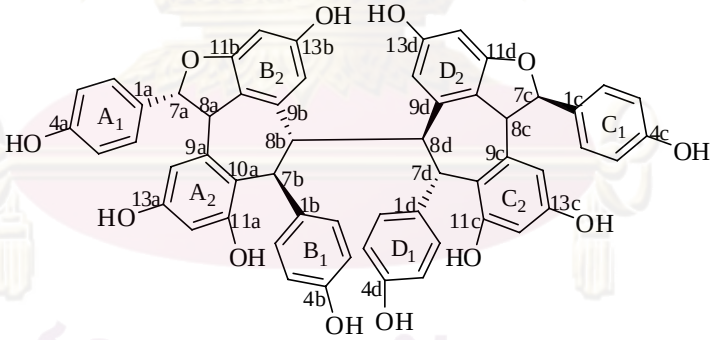
vitisinol G (**6**)



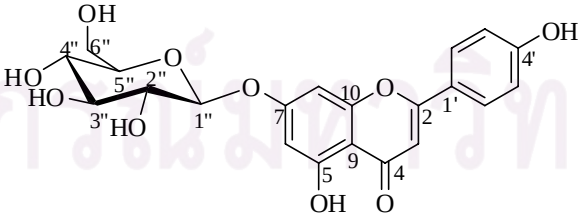
vaticanol A (**7**)



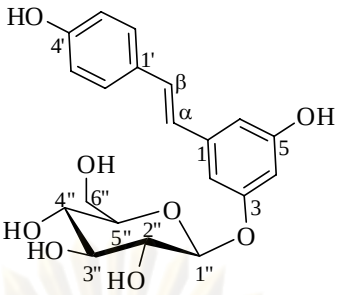
hopeaphenol (**8**)



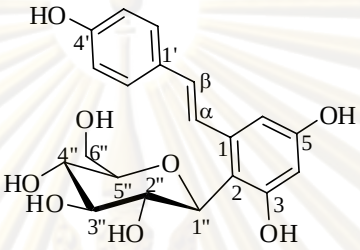
isohopeaphenol (**9**)



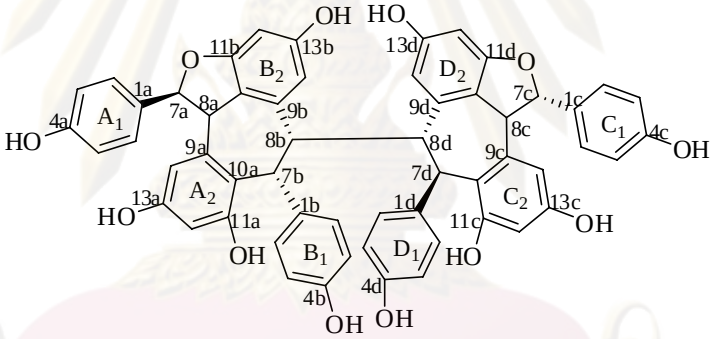
apigenin 7-*O*-glucoside (**10**)



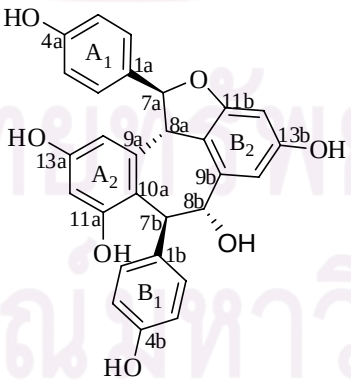
trans-piceid (**11**)



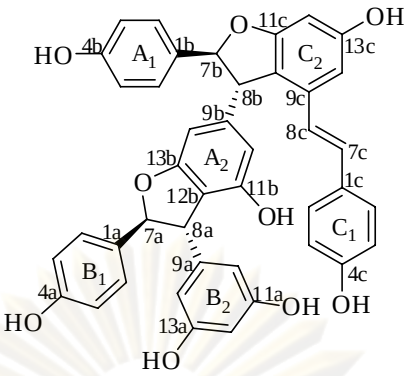
trans-3,5,4'-trihydroxystilbene 2-*C*-glucoside (**12**)



neoisohopeaphenol A (**13**)



balanocarpol (**14**)



gnemonol K (**15**)

The DPPH radical scavenging activity indicated that the IC_{50} of all tested compounds showed moderate antioxidant activity (IC_{50} = 0.23-0.41 mM).

The investigation for cytotoxic activity against HeLa and KB cell lines of isolated compounds indicated that most of the tested compounds exhibited cytotoxic activity against KB cell line rather than HeLa cell line. Compounds **8** and **9** showed the highest effective on cytotoxic activity against KB cell line with IC_{50} = 6.47 and 8.50 μg/mL, and against HeLa cell line with IC_{50} = 8.66 and 10.12 μg/mL, respectively.

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APPENDICES

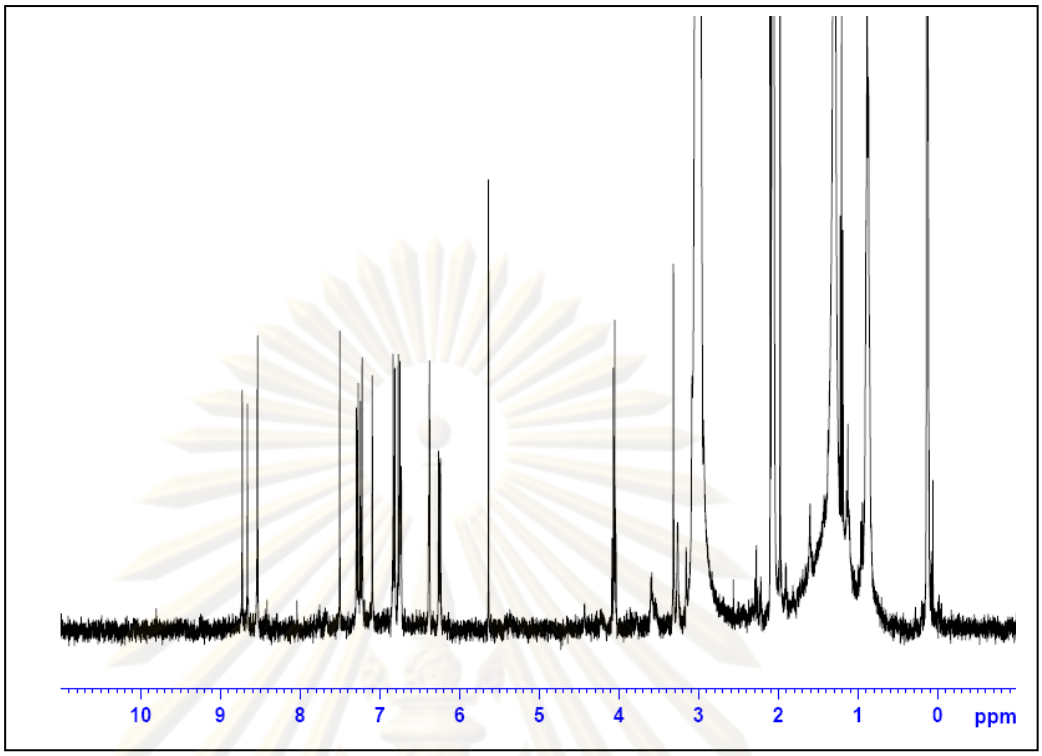

Figure A-1 ^{1}H NMR spectrum (CD_3COCD_3) of roxburghiol A (**5**).

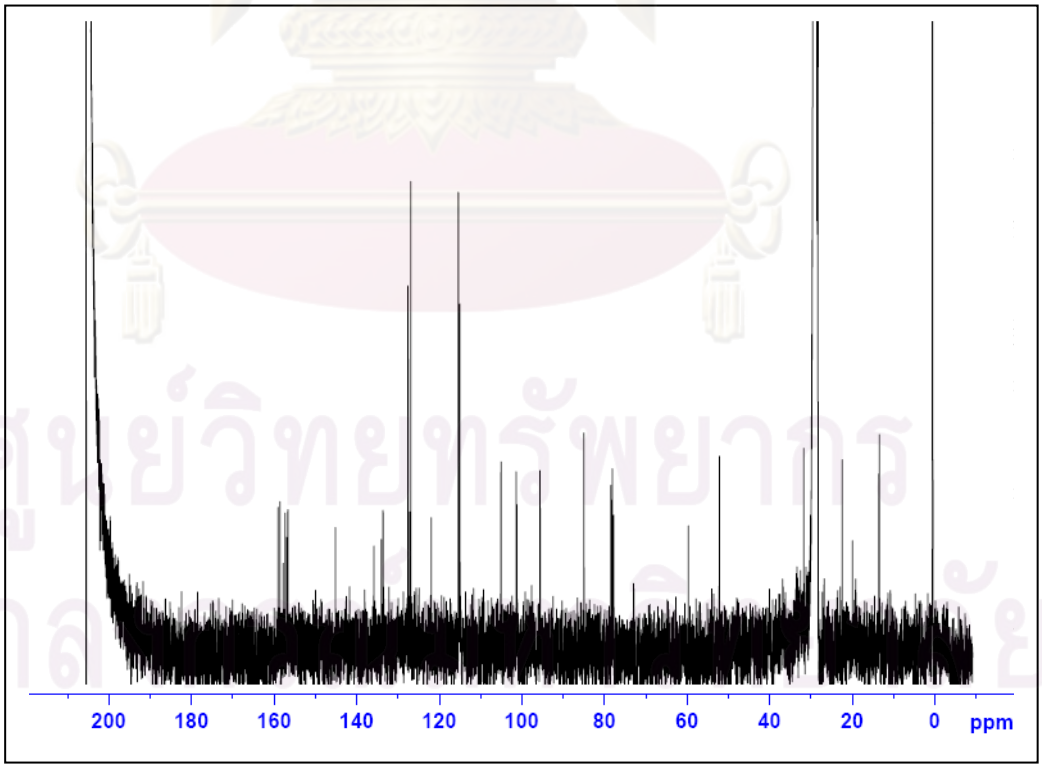

Figure A-2 ^{13}C NMR spectrum (CD_3COCD_3) of roxburghiol A (**5**).

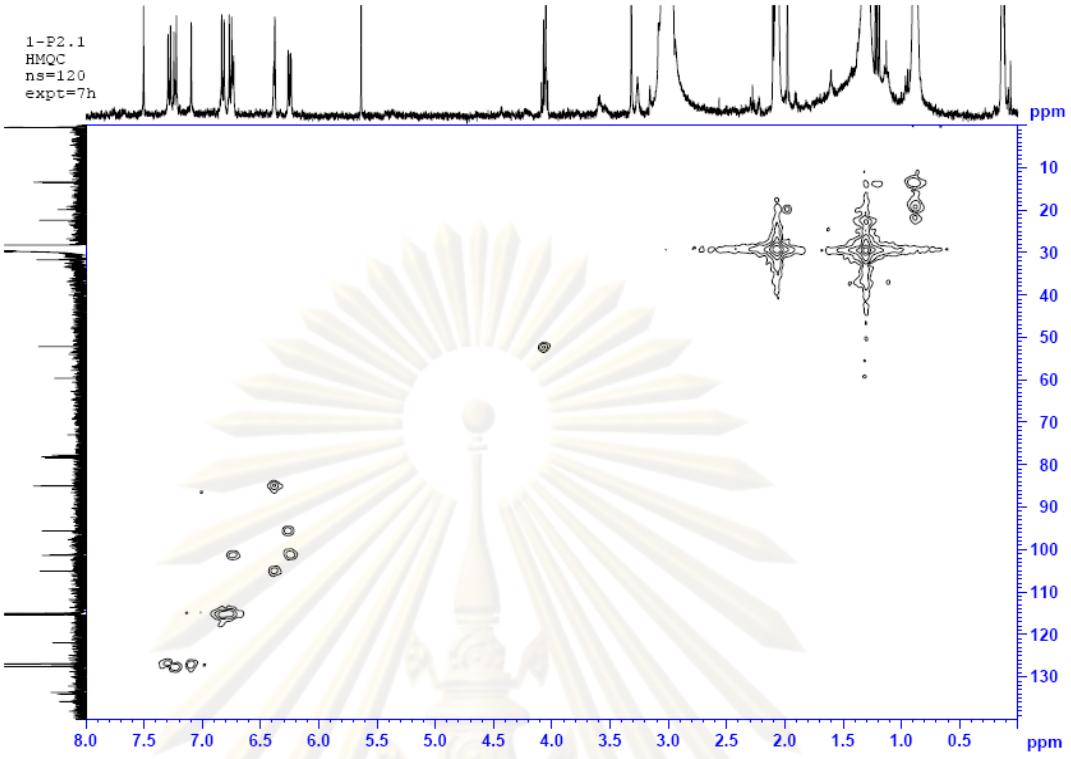

Figure A-3 HSQC spectrum (CD_3COCD_3) of roxburghiol A (**5**).

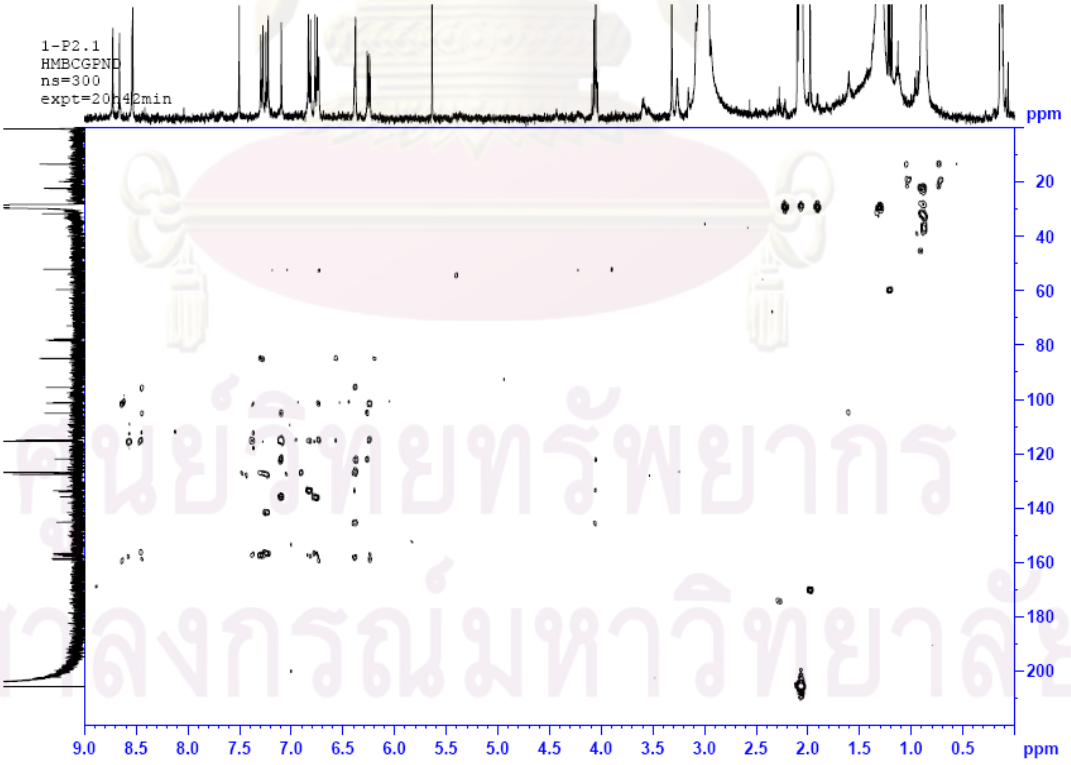

Figure A-4 HMBC spectrum (CD_3COCD_3) of roxburghiol A (**5**).

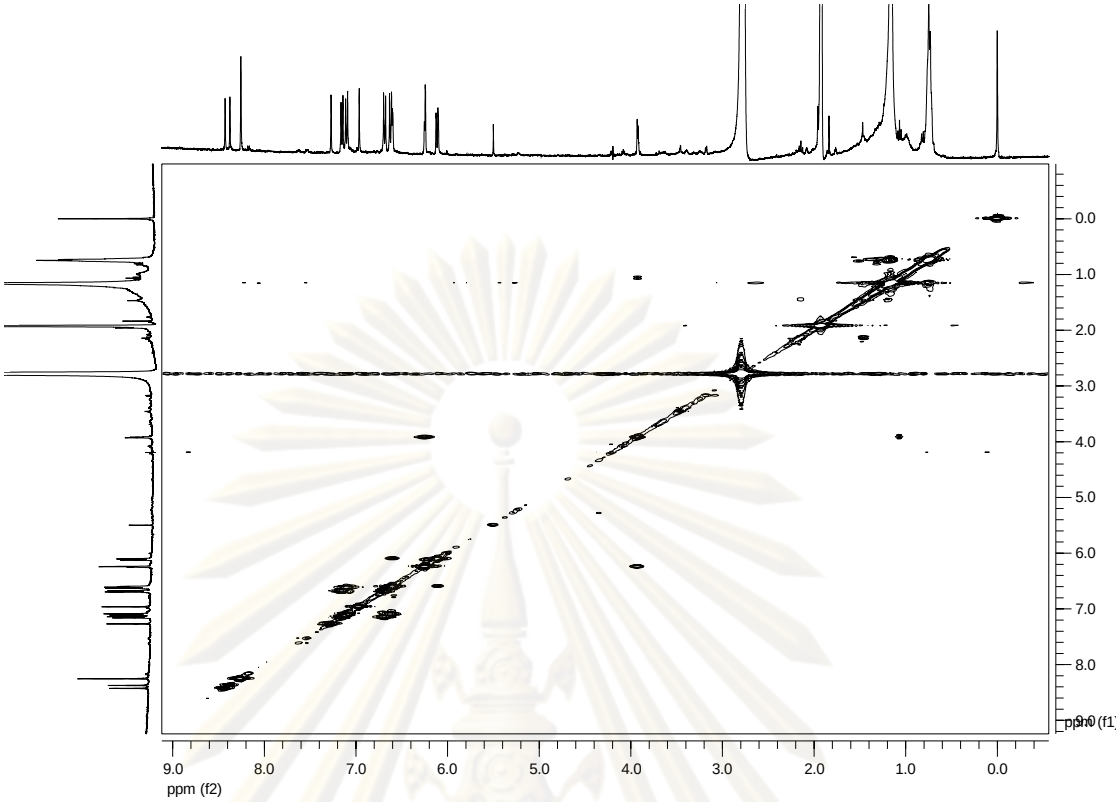

Figure A-5 COSY spectrum (CD_3COCD_3) of roxburghiol A (**5**).

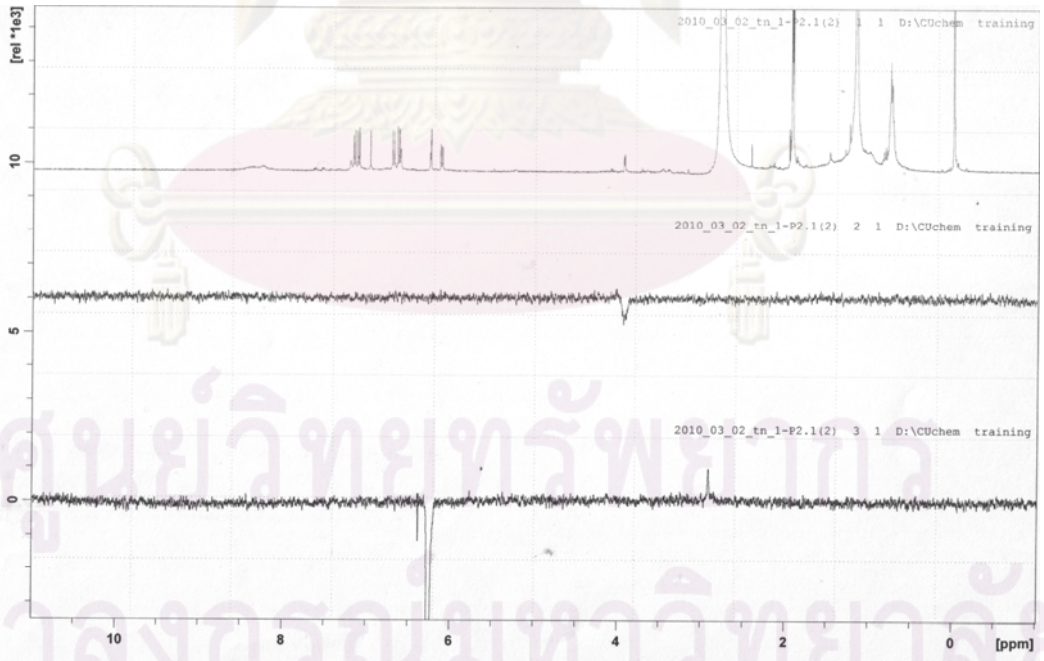

Figure A-6 NOE spectrum (CD_3COCD_3) of roxburghiol A (**5**).

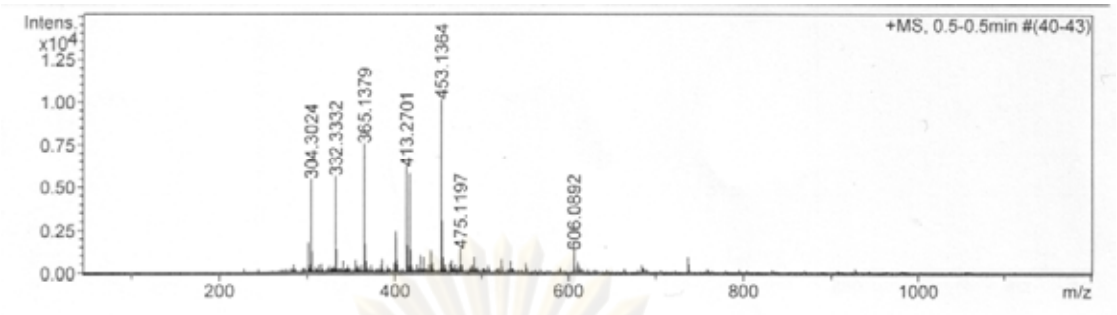

Figure A-7 High resolution mass spectrum of roxburghiol A (**5**).

VITA

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